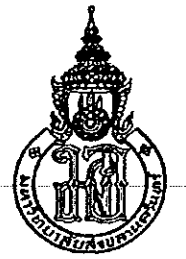

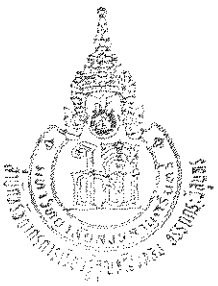

Preparation and Stability Study of Extemporaneous Clonidine Hydrochloride Syrups and Suspensions

Pranee Bocam

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

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ชื่อวิทยานิพนธ์	การเตรียมและศึกษาความคงตัวของยาเตรียมเฉพาะตราว โคลนิดีนไฮโดรคลอไรด์รูปแบบยาน้ำเชื่อมและยาน้ำแขวนตะกอน
ผู้เขียน	นางปราณี บ่อดำ
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ปีการศึกษา	2552

บทคัดย่อ

โคลนิดีนไฮโดรคลอไรด์เป็นยาลดความดันโลหิตสูง รักษาอาการปวด รักษาอาการถอนพิษยาเฮโรอีน เนื่องจากยาโคลนิดีนไฮโดรคลอไรด์ไม่มีจำหน่ายในรูปแบบของเหลวสำหรับรับประทานซึ่งจำเป็นต่อผู้ป่วยบางกลุ่ม จึงศึกษาการเตรียมยาเตรียมเฉพาะตราวยาน้ำเชื่อมโคลนิดีนไฮโดรคลอไรด์โดยเตรียมจากผงยาโคลนิดีนไฮโดรคลอไรด์และยาเตรียมเฉพาะตราว ยาน้ำแขวนตะกอนโคลนิดีนไฮโดรคลอไรด์โดยเตรียมจากยาเม็ดที่มีจำหน่ายในท้องตลาด ยาเตรียมทั้งสองชนิดมีโคลนิดีนไฮโดรคลอไรด์ในขนาด 0.15 มิลลิกรัม/ 5 มิลลิลิตร น้ำกระสายยาที่ใช้มีสองชนิดคือน้ำกระสายยาที่มีน้ำตาลและน้ำกระสายยาที่ปราศจากน้ำตาลเป็นส่วนประกอบ ยาเตรียมตำรับละ 3 ตัวอย่างถูกบรรจุในขวดแก้วใส เก็บให้พ้นแสงที่สามสภาวะคือในตู้เย็น อุณหภูมิห้องและที่อุณหภูมิ 45 องศาเซลเซียส ศึกษาความคงตัวทางกายภาพและทางเคมีของยาเตรียมหลังจากเตรียมตำรับเสร็จใหม่ๆและเมื่อเก็บไว้เป็นเวลา 7, 14, 30, 60, 90, 180, 203 และ 240 วัน ศึกษาความคงตัวทางจุลชีววิทยาในตำรับที่มีความคงตัวทางกายภาพและทางเคมี ในตำรับยาน้ำเชื่อมสังเกตการเปลี่ยนแปลงของความใส สีและกลิ่น วัดการเปลี่ยนแปลงความเป็นกรดด่าง ส่วนตำรับยาน้ำแขวนตะกอนสังเกตการเปลี่ยนแปลงของสีและกลิ่น วัดการเปลี่ยนแปลงของปริมาตรสารนอนก้น สภาพกระจายได้อีก ความเป็นกรดด่างและความหนืด วิเคราะห์ปริมาณยาโคลนิดีนไฮโดรคลอไรด์ในตำรับด้วยเครื่องโครมาโตกราฟีของเหลวสมรรถนะสูง ผลการศึกษาพบว่ายาน้ำเชื่อมโคลนิดีนไฮโดรคลอไรด์ในน้ำกระสายยาทั้งสองชนิดมีความคงตัวทางกายภาพไม่พบตะกอน ไม่มีการเปลี่ยนแปลงสี แต่พบว่ากลิ่นมีการเปลี่ยนแปลงในยาน้ำเชื่อมโคลนิดีนไฮโดรคลอไรด์ในน้ำกระสายยาที่มีน้ำตาลเป็นส่วนประกอบ เมื่อเก็บไว้ที่ 45 องศาเซลเซียส เป็นเวลา 60 วัน และยาน้ำเชื่อมโคลนิดีนไฮโดรคลอไรด์ในน้ำกระสายยาที่ปราศจากน้ำตาลเมื่อเก็บไว้ที่อุณหภูมิห้อง เป็นเวลา 203 วัน และเก็บไว้ที่ 45 องศาเซลเซียส เป็นเวลา 60 วัน ค่าความเป็นกรดด่างมีการเปลี่ยนแปลงในช่วง 0.08-1.21 หน่วย ปริมาณโคลนิดีนไฮโดรคลอไรด์คงเหลือมากกว่าร้อยละ 90 ในช่วงเวลา 240, 60 และ 30 วัน เมื่อเก็บไว้ในตู้เย็น (6.6 $\pm$ 1.82 องศาเซลเซียส),

อุณหภูมิห้อง (29.33 ± 0.98 องศาเซลเซียส) และ 45 องศาเซลเซียส ตามลำดับ ตำรับมีความคงตัวทางจุลชีววิทยา สำหรับยาน้ำแขวนตะกอนโคลนิดีนไฮโดรคลอไรด์ในน้ำกระสายยาทั้งสองชนิด มีความคงตัวทางกายภาพ ไม่มีการเปลี่ยนแปลงสี กลิ่นและความหนืด ปริมาตรสารนอนก้นต่อปริมาตรทั้งหมด มีค่าประมาณ 0.1 ตะกอนกระจายตัวได้ดีเมื่อเขย่า ค่าความเป็นกรดด่างมีการเปลี่ยนแปลงในช่วง 0.02-0.26 หน่วย ปริมาณโคลนิดีนไฮโดรคลอไรด์คงเหลือมากกว่าร้อยละ 90 ของทั้งสองตำรับอยู่ในช่วงเวลา 30 วัน เมื่อเก็บไว้ในตู้เย็น (3.75 ± 0.5 องศาเซลเซียส) และอุณหภูมิห้อง (29.13 ± 0.25 องศา-เซลเซียส) สำหรับตำรับที่มีน้ำตาลเป็นส่วนประกอบ อยู่ในช่วงเวลา 30 วัน และตำรับที่ปราศจากน้ำตาล อยู่ในช่วงเวลา 14 วัน เมื่อเก็บที่ 45 องศาเซลเซียส ตำรับมีความคงตัวทางจุลชีววิทยา เมื่อนำยาเตรียมที่มีความคงตัวหลังเก็บไว้เป็นเวลา 90 วันมาศึกษาความคงสภาพแบบเร่งที่อุณหภูมิ 45, 60 และ 70 องศาเซลเซียส เพื่อทำนายอายุยา และประเมินความพึงพอใจของตำรับที่มีความคงตัวอย่างน้อย 30 วัน พบว่ายาน้ำเชื่อมโคลนิดีนไฮโดรคลอไรด์ในน้ำกระสายยาที่มีน้ำตาลเป็นส่วนประกอบมีจลนศาสตร์การเสื่อมสลายตัวเป็นปฏิกิริยาอันดับสอง ค่าความร้อนแห่งการกระตุ้น 10.62 กิโลแคลอรีต่อโมล อายุยาที่ระดับความเชื่อมั่นร้อยละ 95 จากการทำนายเมื่อเก็บที่ 6 องศาเซลเซียสและ 29 องศาเซลเซียสเท่ากับ 248 และ 70 วัน ส่วนยาน้ำเชื่อมโคลนิดีนไฮโดรคลอไรด์ในน้ำกระสายยาที่ปราศจากน้ำตาลมีจลนศาสตร์การเสื่อมสลายเป็นปฏิกิริยาอันดับหนึ่ง ค่าความร้อนแห่งการกระตุ้น 18.45 กิโลแคลอรีต่อโมล มีอายุยาที่ระดับความเชื่อมั่นร้อยละ 95 จากการทำนายเมื่อเก็บที่ 6 องศาเซลเซียสและ 29 องศาเซลเซียส เท่ากับ 915 และ 105 วัน เมื่อประเมินความพึงพอใจของตำรับในอาสาสมัครสุขภาพดีพบว่าความพึงพอใจต่อยาน้ำเชื่อมโคลนิดีนไฮโดรคลอไรด์มากกว่ายาน้ำแขวนตะกอนโคลนิดีนไฮโดรคลอไรด์

Thesis Title	Preparation and Stability Study of Extemporaneous Clonidine Hydrochloride Syrups and Suspensions
Author	Mrs Pranee Bocam
Major Program	Pharmaceutical Sciences
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ABSTRACT

Clonidine hydrochloride is used as hypotensive agent, treatment of pain and opioid detoxification. It is not commercially available in oral liquid dosage forms that should be advantage to specific patients. Extemporaneous clonidine hydrochloride syrups using clonidine hydrochloride powder and extemporaneous clonidine hydrochloride suspensions using clonidine hydrochloride commercial tablets in the concentration of 0.15 mg/5 mL were prepared in both sugar vehicle and sugar-free vehicle. Three samples of each preparation were stored in glass bottles protected from light in a refrigerator, at room temperature and 45°C. The physical and chemical stability were determined immediately after preparation and at 7, 14, 30, 60, 90, 180, 203 and 240 days. Microbiological stability was determined after the preparation was physical and chemical stable. The syrups were observed for clarity, color and odor changes. The pH of each sample was determined. The suspensions were observed for color and odor changes. Sedimentation volume and redispersibility were evaluated. The pH and viscosity were measured. The concentration of clonidine hydrochloride was assayed in triplicate by high performance liquid chromatography. The results demonstrated that clonidine hydrochloride syrups in two vehicles were not detectable changed in precipitate and color but the odor was changed in clonidine hydrochloride syrups in sugar vehicle after storage at 45°C for 60 days and in clonidine hydrochloride syrups in sugar-free vehicle after storage at room temperature (29.33 $\pm$ 0.98°C) for 203 days and 45°C for 60 days, respectively. The pH value was changed in the range of 0.08-1.21 unit. At least 90% of the initial clonidine hydrochloride in both formulations remained throughout 240, 60 and 30 days after storage in the refrigerator (6.6 $\pm$ 1.82°C), at room temperature (29.33 $\pm$ 0.98°C) and 45°C, respectively. Microbiological stability was maintained. Clonidine hydrochloride suspensions in two vehicles were not appreciably changed in color, odor and

viscosity. Sedimentation volume was about 0.1 but the suspensions can be redispersed. The pH value was changed in the range of 0.02-0.26 unit. The suspensions were chemical stable at least 30 days after storage in the refrigerator (3.75 $\pm$ 0.5 °C) and at room temperature (29.13 $\pm$ 0.25°C), respectively but the suspension in a sugar vehicle and in a sugar-free vehicle were chemical stable at least 30 and 14 days after storage at 45 °C, respectively. Microbiological stability was maintained. Clonidine hydrochloride syrups both formulation which stable for 90 days were selected to determine the shelf life by accelerated testing technique at 45°C, 60°C and 70°C. The palatability of all preparations which stable at least 30 days was evaluated. Accelerated stability study indicated that the kinetic decomposition of extemporaneous clonidine hydrochloride syrup in a sugar vehicle was a second order reaction; the activation energy was 10.62 kcal/mol and the estimated shelf life at lower 95% confident limit days at 6°C and 29°C by Arrhenius equation was 248 and 70 days, respectively. The kinetic decomposition of extemporaneous clonidine hydrochloride syrup in a sugar-free vehicle was a first order reaction, the activation energy was 18.45 kcal/mol and the estimated shelf life at lower 95% confident limit days at 6°C and 29°C by Arrhenius equation was 915 and 105 days, respectively. The palatability of clonidine hydrochloride syrups was preferred over clonidine hydrochloride suspensions in healthy adult volunteers.

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Pranee Bocam

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LIST OF ABBREVIATIONS AND SYMBOLS

%	percent
µg	microgram
µL	microliter
µm	micrometer
cfu	colony forming unit
cps	centipoises
d	day
eq	equation
gm	gram
HCl	hydrochloride
K	Kelvin
Kcal	Kilocalories
min	minute
mg	milligram
mg/tab	milligram per tablet
mL	milliliter
mL/min	milliliter per minute
mm	millimeter
mmole	millimole
MW	molecular weight
nm	nanometer
N	normal
q.s.	a sufficient quantity
^{o}C	degree Celcius

rpm	revolution per minute
RSD	relative standard deviation
SCMC	Sodium carboxymethylcellulose
SD	standard deviation
tab	tablet
v/v	volume by volume
vs	versus
w/w	weight by weight

CHAPTER 1

INTRODUCTION

Introduction

Clonidine hydrochloride is an imidazoleine derivative. It acts through the central nervous system and the peripheral system by stimulating alpha-2 adrenergic receptors. In the central nervous system, it produces a reduction in sympathetic tone, decreases blood pressure as well as heart rate. In the peripheral system, it produces transient vasoconstriction (Abounassif, 1992). It is also used as a hypotensive agent, an analgesic drug and a treatment for attention deficit hyperactivity disorder (Sweetman, 2005). In addition, it is also used in opioid detoxification (National drug committee, 2551; Gold *et al.*, 1980; Sweetman, 2005; Tiucksuban *et al.*, 1995; Washton, 1981) and neonatal narcotic abstinence syndrome (Levinson *et al.*, 1992) because it is an effective, non-opioid drug, non-narcotic drug, used in short duration and has a low cost. The dose for treatment opioid withdrawal syndrome in adult is 10 to 17 μg/kg per day in divided doses (Gilman, 1991). While the dosage for neonate is 3 to 5 μg/kg per day, giving every four to six hours in divided doses (Levinson *et al.*, 1992). Its chemical structure is shown in Figure 1-1.

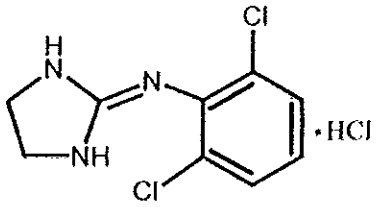


Figure 1-1 Chemical structure of clonidine hydrochloride

Clonidine hydrochloride utility is limited to commercial dosage forms. Commercial dosage forms of clonidine hydrochloride are typically available in tablets for oral administration, the transdermal patch or injectable form to be given epidurally, directly to the central nervous system. It is not commercially available in an oral liquid dosage form. In Thailand, the clonidine hydrochloride products are commercially available only in tablets

(Romano, 2008). Clonidine hydrochloride tablets are not suitable for some patients for examples pediatric patients, elderly patients, patients who are unable to swallow solid dosage forms, patients who just prefer the liquid form and non-compliant patients in solid form. A liquid formulation would be advantageous for patients especially pediatric and adult patients who are unable to swallow tablets or who must receive medications by nasogastric or gastrostomy tube (Johnson *et al.*, 2003) or patients who just prefer the liquid form and non-compliant patients. Extemporaneous clonidine hydrochloride in liquid form would be essential for those types of patients and allows the dose to be easily adjusted and administered. Otherwise, it will increase patient compliant. However, clonidine hydrochloride oral liquid form does not reveal in Pharmacopoeial formula. In addition, there are few data existing on the stability of clonidine hydrochloride in oral liquid preparations. There was extemporaneous clonidine hydrochloride oral solution in the concentration of 5 μg/mL. It was prepared from clonidine hydrochloride injection (150 μg/mL) and adjusted to volume with water for irrigation. The product was stored in amber glass bottles. The stability of this preparation is 10 days when storage in the refrigerator. Levinson *et al.* (1992) prepared an extemporaneous clonidine hydrochloride oral liquid dosage form by levigating clonidine hydrochloride tablets or powder with a small amount of purified water and adjusted to final volume with Simple Syrup NF and storage in amber glass bottles in the dark at 4°C. The stability study revealed that on day 28, the mean percentages of the initial clonidine hydrochloride concentrations remaining were 92.4% in the suspension and 93.7% in the solution. So, an extemporaneously compounded oral liquid preparation of clonidine hydrochloride in Simple Syrup NF was stable under the conditions studied for up to 28 days. However, commercial products that used in these studies are not available in Thailand. It is available only in the form of local commercial tablet. The different commercial tablets may consist of different excipients. Unknown excipients may cause problems in formulation and stability of products (Freed *et al.*, 2005). The kind and amount of excipients in difference commercial tablets gave difference physical characteristics in extemporaneous preparation (Boonme *et al.*, 2000). In addition, the study of Levinson *et al.* (1992) showed only chemical stability and physical stability but not microbiological stability study, therefore the product might be contaminated with microbes such as molds, yeast or bacteria. High tires of microorganisms may be dangerous to one's health and may cause product instability. Furthermore, Simple Syrup

NF is not suitable for patients who require controlling sugar or patients who always have dental caries. In this case, a study in a sugar-free vehicle is suitable. Moreover, there is no stability data of clonidine hydrochloride in other vehicles. Because oral administration of clonidine hydrochloride is well absorbed about 95% from gastrointestinal tract with the peak plasma concentration observed after about 3-5 hours (Sweetman, 2005) and it is soluble in water (1:13) at 20°C (Abounassif, 1992), syrup dosage form is one of the most appropriate dosage forms. Clonidine hydrochloride syrup can be prepared from clonidine hydrochloride powder or clonidine hydrochloride extracted from tablets. Clonidine hydrochloride powder was used in the formulation because it is the first choice for preparing an extemporaneous oral liquid preparation (Allen, 2009) and the potency of the product is assured. In the case powdered drug is not available, it can be prepared from clonidine hydrochloride extracted from tablets because drug is water soluble. In addition, a suspension dosage form prepared from local commercial tablets is also appropriate because they are generally available. The objectives of this study were to formulate extemporaneous clonidine hydrochloride syrups from clonidine hydrochloride powder and from clonidine hydrochloride extracted from local commercial tablets, and to formulate extemporaneous clonidine hydrochloride suspensions from local commercial tablets. The two oral liquid vehicles; a sugar vehicle and a sugar-free vehicle were used in these formulations. The extemporaneous formulation should possess palatable, acceptable appearance, and stable for at least 30 days. The physical, chemical and microbiological stability of these formulations kept in glass bottle protected from light at three temperatures, in the refrigerator, at ambient temperature and 45°C, were evaluated immediately after preparation and at 7, 14, 30, 60, 90 days. The formulation that was stable in the period of study was continuously studied and predicted shelf life by accelerated testing method. Then, the formulation that gave physical, chemical and microbiological stability for at least 30 days were selected to evaluate for satisfaction by sensory method.

Objectives

1. To develop and prepare extemporaneous clonidine hydrochloride syrups and suspensions.
2. To evaluate the stability of extemporaneous clonidine hydrochloride syrups and suspensions
3. To predict shelf life of extemporaneous clonidine hydrochloride syrups and suspensions

Review of literatures

1. Clonidine hydrochloride

Clonidine hydrochloride is used in hypertension, either alone or in combination with other hypertensive agents. It also used for continuous epidural administration as adjunctive with intraspinal opiates for treatment of cancer pain in patients tolerant to or unresponsive to intraspinal opiates. It is also used in opioid detoxification (National drug committee, 2551; Gold *et al.*, 1980; Sweetman, 2005; Tiucksuban *et al.*, 1995; Washton, 1981) and neonatal narcotic abstinence syndrome.

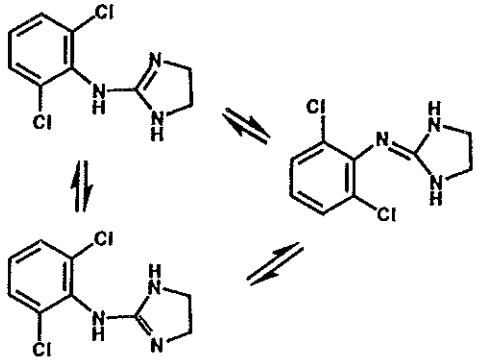
Physical and chemical characteristics

Clonidine hydrochloride [2-(2,6-Dichlorophenylamino)-2-imidazoline hydrochloride] is synthesized by the condensation of 1-acetyl-2-imidazolinone and 2,6-dichloroaniline (Kostecka *et al.*, 1998). It is a white or almost white crystalline powder that has a bitter taste. A crystal is columnar shape, 0.2 x 0.2 x 0.6 mm. It is stable in light, air and room temperature. It has a molecular weight of 266.6 gm/mole, a melting point of 305°C and a pKa of 8.2. The molecular and conformational structure of clonidine hydrochloride exists predominately in the imino form (Figure 1-2) in solution and at the receptor site (Szasz and Budvari-Barany, 1998). It is soluble in 13 parts of water (20°C), soluble in absolute ethanol, slightly soluble in chloroform. It absorbs ultraviolet light. The UV spectra of clonidine hydrochloride exhibited maxima at 213 nm and some at 271 nm and 302 nm (Abounassif, 1992).

Mechanism of action

For epidural administration, clonidine hydrochloride is used for analgesia by preventing pain-signal transmission to the brain at presynaptic and postjunctional alpha 2-adrenoceptors in the spinal cord. This effect is dose dependent and is not antagonized by opiate antagonists. For hypertension, it stimulates alpha-2 adrenergic agonist in the brain that causes the

brain to reduce its signals to the adrenal medulla and catecholamine production is decrease. The result is lower heart rate and blood pressure. For reducing opioid withdrawal syndrome, it inhibits alpha-adrenergic activity in locus ceruleus of the brain (Anonymous, 2008).



amino forms imino form

Figure 1-2 The structure of clonidine in amino and imino form (Szasz and Budvari-Barany, 1998).

Pharmacokinetics

Absorption

Clonidine hydrochloride is well absorbed from the gastro-intestinal tract. Its effects appear in about 30-60 min when given by mouth. An absorption time is 2 to 4 hours as lasting up to 8 hours. Peak plasma concentration from oral administration occurs in approximately 3-5 hours. It is well absorbed through the skin by topical application of a transdermal system to the arm or chest. It may be absorbed when applied topically to the eye (Abounassif, 1992).

Distribution

Clonidine hydrochloride is distributed into body tissue, the drug concentration in tissue are higher than that in plasma. For oral administration, the highest concentrations of the drug are found in the kidneys, liver, spleen and gastrointestinal tract. High concentration of

clonidine hydrochloride also appears in the lacrimal and paratid glands. Clonidine hydrochloride is concentrated in the choroid of the eye and is also distributed into the heart, lungs, testes, adrenal glands, fat and muscle. The lowest concentration occurs in the brain. Clonidine hydrochloride is distributes in cerebrospinal fluid. It is not known whether the drug crosses the placanta. Clonidine hydrochloride is distributed into milk (Abounassif, 1992).

Elimination

The plasma half-life of clonidine hydrochloride in patients with normal renal function is 6-20 hours. The half-life in patients with impaired renal function has been reported to range from 8-41 hours. Clonidine hydrochloride elimination half-life may be dose dependent, increasing with increasing dose. Clonidine hydrochloride is metabolized in the liver. In humans, 4-metabolites have been detected but only one, the inactive p-hydroxylated derivative, has been identified.

In human, 65% of administered dose of clonidine hydrochloride is excreted by the kidneys, 32% as unchanged drug and the remainder as inactive metabolites. Approximately 20 % of dose is excreted in feces. Approximately 85% of a single dose is excreted with 72 hours and excretion is complete after 5 days (Abounassif, 1992).

Uses and administration

Clonidine hydrochloride is used in the treatment of hypertension. The usual initial dose of clonidine hydrochloride is 50 to 100 μg orally three times daily increased every second or third day according to the response of the patient. The usual maintenance dose is 0.3 to 1.2 mg daily but doses of 1.8 mg or more daily may be sometimes required. It may be given with thiazide diuretic but used with a beta blocking agent should be avoided where possible. Clonidine hydrochloride may also be given by a transdermal delivery system that is applied once weekly and delivers 100 to 300 μg daily at a constant rate. It may be given by slow intravenous injection over 10 to 15 min in hypertensive crises usually in dose of 150 to 300 μg. The effect usually

appears within 10 min, reaches a maximum about 30 to 60 min after administration and the duration is about 3 to 7 hours.

In the treatment of severe cancer pain, clonidine hydrochloride may be given by continuous epidural infusion with an opioid, in an initial dose of 30 μg/hour, adjusted according to response (Sweetman, 2005).

In controlling withdrawal symptom from discontinuation of opioid, the dose in adult is 10 to 17 μg/kg daily in divided doses (Gilman, 1991).

In the management of neonatal abstinence syndrome, an initial clonidine hydrochloride dose of 0.5 to 1 μg/kg by mouth, increased over 1 to 2 days to 3 to 5 μg/kg daily in divided doses. Total length of treatment ranged from 6 to 17 days (Sweetman, 2005).

Adverse effects and treatment

Serious adverse effects occurring rarely in cardiovascular are atrioventricular block. Common adverse effect in cardiovascular is bradyarrhythmia (oral, 0.5%; epidural, common), hypotension (epidural, 44.8%), orthostatic hypotension (oral, 3%; epidural, 31.6%), rebound hypertension (epidural, common). Other adverse effects in gastrointestinal tract are constipation (oral, 10%; epidural, 2.6%), nausea (oral, 5%; epidural, 13.2%), vomiting (oral, 5%; epidural, 10.5%), xerostomia (oral, 40%; epidural 13%). Neurologic adverse effects are confusion (epidural, 13.2%), dizziness (oral, 16%; epidural, 13.2%), sedated (oral, 10%), somnolence (oral, 33%; epidural, 13.2%) (Anonymous, 2008). Rashes and pruritus may commonly occur with the use of transdermal delivery system (Abounassif, 1992). The most frequently adverse effect for orally therapy is dry mouth, drowsiness and sedation, and constipation. Dizziness, headache, fatigue, and weakness have also been reported. These adverse effects tend to diminish with continued therapy or may be relieved by a reduction in dosage (McEVOY, 1999).

Symptoms of over dosage include transient hypotension or profound hypotension, bradycardia, sedation, miosis, respiratory depression, and coma. Sign and symptom of clonidine hydrochloride over dosage usually occur within 30 to 60 min after ingestion and may persist for 36 to 48 hours. Symptomatic and supportive treatment should be initiated. All transdermal therapy should be removed in patients receiving transdermal systems. In acute over dosage with oral clonidine hydrochloride, the stomach should be emptied immediately by emesis or by lavage followed by administration of activated charcoal slurry and a saline cathartic. Intravenous of dopamine may be useful for severe, persistent hypotension. Intravenous of atropine sulfate may be useful in symptomatic bradycardia. An alpha-adrenergic blocking agent such as phetolamine or naloxone may be given if necessary (McEVOY, 1999; Abounassif, 1992).

Precautions

Clonidine hydrochloride should be used with caution in patients with cerebrovascular disease, ischaemic heart disease including myocardial infarction, renal impairment, occlusive peripheral vascular disorders such as Raynaud's disease, or those with a history of depression. Clonidine hydrochloride causes drowsiness and patients should not drive or operate machinery because loss of attention could be dangerous. Systemic effects also occur following epidural administration and patients should be closely monitored, particularly during the first few days of therapy. Intravenous injections of clonidine hydrochloride should be given slowly to avoid a possible transient increase blood pressure especially in patients already receiving other antihypertensives such as guanethidine or reserpine (Sweetman, 2005).

Interactions

The hypotensive effect of clonidine hydrochloride may be enhanced by diuretics, other antihypertensives, and drugs that cause hypotension. However, beta blockers may exacerbate rebound hypertension following clonidine hydrochloride withdrawal and tricyclic antidepressants may antagonise the hypotensive effect. The sedative effect of clonidine hydrochloride may be enhanced by central nervous system depressants (Sweetman, 2005).

Preparations

Clonidine hydrochloride preparations are clonidine hydrochloride injection 0.1 and 0.5 mg/mL, clonidine hydrochloride tablets 0.1, 0.2 and 0.3 mg and clonidine hydrochloride transdermal 0.1, 0.2 and 0.3 mg. In Thailand, it is commercially available only local tablets 0.075 and 0.15 mg (Romano, 2008).

Studies of extemporaneous clonidine hydrochloride preparations

There were few studies of extemporaneous preparation of clonidine hydrochloride. One of these was clonidine hydrochloride oral solution in the concentration of 5 µg/mL. It was prepared from clonidine hydrochloride injection 150 µg/mL and adjusts to volume with water for irrigation. The product was stored in amber glass bottles. The stability of this preparation was 10 days after storage in the refrigerator (Anonymous, 2009a). Another extemporaneous preparation of clonidine hydrochloride was clonidine hydrochloride 1 mg/gm in VanPen cream. It was prepared from clonidine hydrochloride powder that was dissolved in the purified water. Then incorporated into the VanPen cream and mixed thoroughly. It was stored in tight, light-resistant container. There is no specific stability study in this preparation (Anonymous, 2000). The other extemporaneous preparation is clonidine hydrochloride 0.2%, gabapentin 6%, and ketamine hydrochloride 10% in a pluronic lecithin organogel. The product was stored in tight, light-resistant container. There is no specific stability study in this preparation (Anonymous, 2002).

Stability of admixture clonidine hydrochloride and other drug was studied. The admixture contained morphine sulfate, bupivacaine hydrochloride and clonidine hydrochloride in an implantable infusion system. All drugs in the mixture were stable at 37°C for 90 days (Classen, 2004). It can assume that clonidine hydrochloride should be stable at room temperature.

Stability of clonidine hydrochloride injection containing 100 µg/mL clonidine hydrochloride and 9 mg/mL sodium chloride in water for injection was studied. The formulation

of a stable parenteral product; clonidine hydrochloride injection does not require protection from light, oxygen and freezing. The product showed acceptable stability within pH 4-8 (Kostecka, 1998).

From these studies, it can be assumed that clonidine hydrochloride solution is stable at room temperature. The appropriate pH is 4-8.

2. Extemporaneous oral liquid preparation

Extemporaneous preparation is a small scale of preparations that occasionally prepare for individual patients because they are necessary for patients but products are not commercially available. Oral liquid dosage forms are generally suitable for administration to newborns, infants, children and elderly patients. Sometimes they were prepared for some patients who cannot swallow solid dosage forms, patients who are on enteral feeding methods (Glass, 2006), patients who are often administered oral liquids to prevent them from placing tablets/capsules under tongue and not swallowing them and patients who require special treatments.

Preparation

Extemporaneous oral liquid preparations can be prepared from drug powder (Pathmanathan *et al.*, 2004) and other commercially available dosage forms such as crushing tablets (Hutchinson *et al.*, 2009), contents of capsules (Dupuis *et al.*, 2009) and injectables (Yamreudeewong *et al.*, 1995). The first choice for extemporaneous preparation is using drug powder because the potency of the product is assured. Normally, it is prepared from commercially available dosage forms because they are generally available. However, unknown excipients in the preparation may cause problems in formulation and stability of products (Freed *et al.*, 2005; Haywood *et al.*, 2005).

The oral liquid pharmaceutical dosage forms, syrups (Webster, 1997), elixirs (Peterson *et al.*, 1994; Brook *et al.*, 1973), suspensions (Burnett and Balkin, 2006; Hutchinson *et al.*, 2009; Olguin, 2008), solutions (Preechagoon *et al.*, 2005) or mixtures (Haywood *et al.*, 2005) can be formulated depending on physiochemical properties of active drug such as concentration, solubility, pKa, pH, taste, odor, and stability of active drugs. Syrups can be prepared from water-soluble active drug. Whereas, elixirs and suspensions are suitable dosage forms for drug that soluble in water–alcohol-glycerin and insoluble drug, respectively.

The general method for preparing extemporaneous oral liquid is dissolving or dispersing drugs in suitable base or vehicle. Then, adding excipients such as suspending agents, flavorants, colorants, or preservative. Other agents sometimes were added; including alternative solvents such as ethanol, particularly when the drug is poorly soluble in water. Buffer system is used to provide the optimum pH for drug stability and preservative is used to inhibit microbial growth.

Stability of extemporaneous preparation

There are five general types of stability defined by the United States Pharmacopeia (USP 30). Firstly, physical: the original physical properties, including appearance, palatability, uniformity, dissolution, and suspend ability are retained. Secondly, chemical: each active ingredient retains its chemical integrity and labeled potency, within the specified limits. The remaining concentration of active drug must not less than 90% of the initial concentration (Hutchinson *et al.*, 2009). Thirdly, microbiological: sterility or resistance to microbial growth is retained according to the specified requirement (Salgado *et al.*, 2005; Preechagoon *et al.*, 2005). Antimicrobial agents that are present retain effectiveness within the specified limits. Fourthly, therapeutic: the therapeutic effect remains unchanged. The last factor is toxicological: no significant increase in toxicity occurs (Fawcett *et al.*, 1997; Olguin *et al.*, 2008). It is not easy to evaluate all five types of stability but the compounder must concern in the chemical stability, physical stability and microbiological stability.

Many factors effecting drug stability should be considered. They are physicochemical property of drugs, particle size, pH, excipient/vehicle; microbial contamination, container properties and the environment including temperature, light, air, oxygen, and humidity (Anonymous, 2009b). All factors require careful consideration to ensure that the product is optimum quality, efficacy and safety throughout its period storage and use.

Beyond use date

Beyond use date is a date after compounding that the preparation should not be used. The date may be assigned base on drug stability information and literature when available, nature of the drug, its degradation mechanism, the container (Gupta and Singh, 2007), the expected storage condition and intended duration of therapy. In the absence of drug stability information, the USP recommend beyond use date for nonsterile compounded preparations that are packaged in tight, light resistance container and stored at controlled room temperature unless otherwise indicate. For water-containing formulations (prepared from ingredients in solid form), the beyond use date is not later than 14 days for liquid preparations when storage at cool temperatures between 2°C and 8°C (USP 30, 2007).

Management of oral liquid preparation (Glass *et al.*, 2006)

If oral liquid preparation is recommended for specific patient, the pharmacist can be managed by using a flow chart that is presented in Figure 1-3 and discussed below.

Step 1 Commercial product

Commercially available products should be considered. If there is not a liquid dosage form, consider alternative available drug products such as transdermal patch or dispersible tablets or the injection.

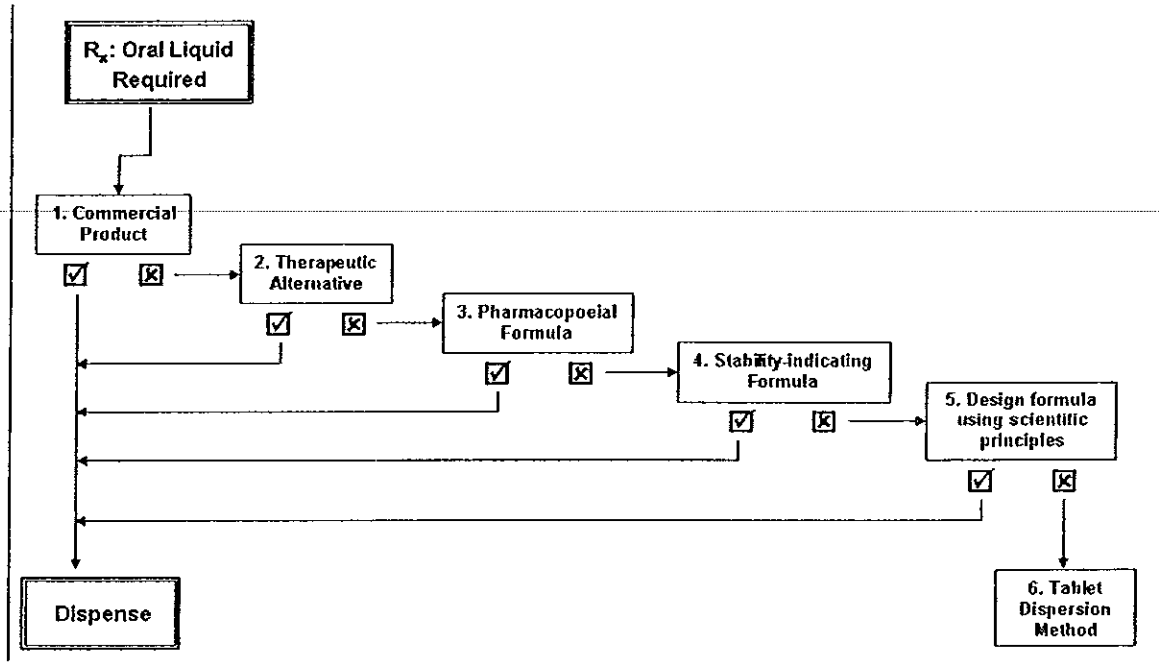


Figure 1-3 Management of oral liquid preparation in practice (Glass *et al.*, 2006)

Step 2 Therapeutic alternatives

If there is not an appropriate commercial product, alternatives therapeutic that is available in suitable dosage form must be consulted to the physician.

Step 3 Pharmacopoeial formula

If no suitable therapeutic alternative, consult the information that reveal in pharmacopoeia formula such as British Pharmacopoeia, United States Pharmacopeia and Martindale: The Complete Drug Reference.

Step 4 Stability indicating formula

If there is no data in Pharmacopoeia formula, a suitable stability indicating formula should be sought in the literature. A formulation should be prepared according to published data-bases and follow the condition as closely as possible. Modification to publish information can be preformed if there is no effect on stability.

Step 5 Design formula using scientific principles

If no appropriate formula in a published literature, the formula must be designed base on scientific principles. The study should be considered on (i) potential degradation such as oxidation, hydrolysis, photolysis and thermolysis, (ii) storage and packaging, (iii) a suitable shelf life, and (iv) interaction with excipients.

Step 6 Tablet dispersion methods

If the stability data has not been carried out, the tablet dispersion method would be instructed by placing tablets in a beaker or cup of water, swirling beaker or cup until they have dispersed, and then immediately administering to the patients. However, this method has disadvantages. The medication is bitter or unpalatable. The preparation must be prepared at the time of administration. Some solid dosage form should not be crushed such as sublingual or buccal preparations, enteric coated, sustained release formulation, products with carcinogenic potential since aerosolisation of particles will expose healthcare workers who handle these products, and products that contain drugs that are extremely bitter, irritate the oral mucosa, or contain dyes or inherently could stain teeth and mucosal tissue.

3. Syrups

Syrups are liquid oral solutions, containing high concentrations of a sugar or sugar-substitute with or without flavors and medical agent. Syrups are classified into two types; flavored syrups (nonmedicated syrups) and medicated syrups. Flavored syrups or nonmedicated syrups are syrups with flavoring agent but not medicinal substance. There are many commercial flavoring syrups such as Simple Syrup (USP), Cherry Syrup (USP), Orange Syrup (BP), Cocoa Syrup (USP), Lemon Syrup (BP), Ora-sweet (Paddock Laboratories) and Ora-sweet SF (Paddock Laboratories). Most of them were acidic vehicle that use for drugs stable in acidic medium. These syrups always provide good taste so they are used as a vehicle for the standard formulation of

medical preparation or in the extemporaneous compounding. Medicated syrups are syrups contain medicament or therapeutic agents that are used for medical treatment.

Syrups are advantage for a number of reasons. They are a palatable liquid preparation so they can increase compliance in children, the elder and the patients who have a problem with oral solid-dosage forms. Since they are homogeneous mixtures, the medication is uniformly distributed throughout the preparation. The dose can be easily adjusted as fractional doses by dilution to meet the needs of the patients. The drug in solution is immediately available absorb from the gastrointestinal tract and is more rapidly and efficiently absorbed than the same amount of drug administered in an aqueous suspension or a tablet or capsule. However, they have limitation. Drug substance generally is less stable in liquid media than in the solid-dosage form. Poorly soluble drugs are requiring special techniques for increasing solubility. Masking the taste of very bitter drugs is sometimes difficult. They are not appropriate for potent drug with a low therapeutic index because the patients could be made dosage measurement errors (Woznicki, 1994).

Components of medicated syrups (Swinyard, 1990; Woznicki and Schoneker, 1994)

Medicated syrups generally consists of

1. Active ingredient
2. Sweetening agent
3. Antimicrobial preservative
4. Flavoring agents or flavorants
5. Coloring agents or colorants
6. Purified water
7. Others

Active ingredient

An active ingredient is an active pharmaceutical ingredient that has pharmacological effect in medicine or formulation.

Sweetening agent

Sweeteners, sugar and sugar substitute, are additive that have a sweet taste. They are employed to mask bitter or unacceptable tastes of substance. Sugar such as sucrose is the most commonly used in syrups. It is a crystalline powder, soluble in water. A sucrose concentration above 65% w/w can inhibit the growth of microorganisms in solution by reducing water-activity coefficient (Woznicki and Schoneker, 1994) or increasing osmotic pressure in microbe's cell. It is chemically and physically stable in the pH range of 4.0 to 8.0. Official Simple Syrup USP is a solution of 85% w/v sucrose in water. High concentration of sucrose can induce the sucrose crystallizes on the threads of the bottle cap and trouble with opening. For reducing this problem, it is used in conjunction with other sweetener such as glycerin, sorbitol or mannitol. A sugar substitute is a non-sugar sweetener that has the taste like sugar but usually has less food energy. Some of them are natural and some are synthetic that are called artificial sweeteners. Examples for common use in liquid medication are glycerin, sorbitol, mannitol, xylitol, saccharin and aspartame.

Glycerin is used as a sugar substitute, co-solvent or humectants. It is about 60% as sweet as sucrose. The usual concentration is less than 20% (Wade and Weller, 1994).

Sorbitol solution 70%w/w is a nutritive sweetener because it provides dietary energy less than carbohydrate. The sweetness is less than sucrose. It is effective in preventing crystallization around the cap of bottles. The usual concentration is 20-35% (Wade and Weller, 1994).

Saccharin is a synthetic sweetening agent. It has about 500 times the sweetening power of sucrose. It is used for diabetics, the obese, and others who do not ingest sucrose. The usual concentration is 0.02-0.05% (สุธี เวคะวากยานนท์, 2531)

Antimicrobial preservative

Liquid oral preparation is nonsterile preparation that could be contaminated by microorganisms. The product includes of water, sugars and other excipients that can support the growth of microbial. The preparations in the market are multidose form, strengthening the risk of exposure to microbes. Therefore, appropriate preservative is essential to prevent microbiological contamination. Preservatives must be safe and lack of toxicity. Moreover, it must be soluble, stable, microbiologically active and compatible with other substances in the formulation. The typical antimicrobial preservatives in liquid oral preparation are presented in Table 1-1.

Table 1-1 Preservatives used in liquid oral preparation (นัฏฐา แก้วนพรัตน์, 2546)

Preservative	Usual concentration (%)
Alcohol	15-20%
Benzoic acid	0.1-0.3%
Butylparaben (BP)	0.02%
Propylparaben (PP)	0.05%
Methylparaben (MP)	0.1%
Sodium benzoate	0.1-0.3%
Sorbic acid	0.05-0.2%
Combination of MP+PP+BP	maximum about 0.1%
Paraben concentrate*	1.0%

* Paraben concentrate consists of methylparaben 10 gm, propylparaben 2 gm, and propylene glycol to 100 mL.

Flavoring agents or flavorants

The flavoring agent is useful in oral liquid product as it can mask the disagreeable taste of drugs and provide pleasant odor. The choice must be based on the taste of active ingredient, usage time, age or gender of patients, enhance therapeutic effect, stable and non-toxic.

Coloring agents or colorants

The coloring agent is not has therapeutic benefit but afforded the psychological effect. It can be attractive medication and enhance patient acceptation. The coloring agents in liquid formulation should be water-soluble, stable, and approved by the Food and Drug Administration. Color is usually chosen according to the flavor of the products. Most liquid drug products have color in the range of 0.0005-0.001% depending upon the colorant and the depth of color desired (Swinyard, 1990).

Purified water

Purified water is water from any source that is removed impurities. It can be purified by reverse osmosis, carbon filtration, ultraviolet oxidation, distillation or ultra filtration.

Others

Other excipients that may be used in syrups are buffer, antioxidant or viscosity inducing agent. Buffer is used to stabilize pH of preparation such as citric acid and a salt. Antioxidant is used to block an oxidation chain reaction such as ethylenediaminetetraacetic acid (EDTA). Viscosity inducing agent is used to increase the viscosity of a liquid such as methylcellulose.

4. Suspensions

Suspensions are the preparation that consists of low solubility solid drug particles suspends or disperses in the liquid vehicle. They are coarse dispersion. The size of individual particles is more than 0.5 μm. whereas, the particle size of aggregated particle is about or more than 50 μm. Ideal suspensions should have the following properties. The particle size should not change after storage. The dispersed particles should slowly settle and should redispersed immediately after shaking. The particle should not form a cake on settling. The viscosity of the preparation should viscose on shelf to suspend the particles and should reduce after shaking in order to pour the preparation easily. It should be physical, chemical, microbiological stable and palatable (Swarbrick, 2005).

Components of suspensions

1. Active ingredient
2. Vehicle
3. Wetting agent (as necessary)
4. Flocculating agent (as necessary)
5. Suspending agent
6. Antimicrobial preservative
7. Flavoring agents or flavorants
8. Coloring agent or colorants
9. Others

Active ingredient

An active ingredient is an active pharmaceutical ingredient that has pharmacological effect in medicine or formulation.

Wetting agent

Wetting agent is used to wetting hydrophobic solid particles in order to be easily dispersed in the aqueous vehicle. Alcohol, glycerin and propylene glycol are example of wetting agents that can be used to remove adsorbed air from the surface of particles. In addition, the surfactants such as sodium lauryl sulfate and polysorbate are used to reduce the interfacial tension between particles and the vehicle to improve the wettibility. Examples of wetting agent are glycerin, polysorbate, acacia, tragacanth, bentonite, xanthan gum and cellulose derivatives. Excessive amount of wetting agent can cause foaming or undesirable taste and odor.

Flocculating agent

Flocculating agent is added into the preparation in order to produce flocculated particles. Particles form loose aggregate and from a network-like structure. Rate of sedimentation is high. They rapidly sediment, loosely packed, provide high sedimentation and do not form a hard cake. The supernatant is clear and they are easy to redisperse. Examples of flocculating agent are electrolyte that has charge opposite to particle charge and polymer that has long chain and act as interparticle bridges.

Suspending agent

Suspending agent is used to increase viscosity and help sediment easily redisperse. The general suspending agents are natural plant hydrocolloids such as acacia, tragacanth, cellulose derivatives such as methylcellulose, sodium carboxymethylcellulose, Clays such as bentonite, veegum, hectorite, and synthetic polymers such as carbomer, polyvinyl alcohol.

Others

The purpose of using antimicrobial preservatives, flavorants and colorants is the same as other oral liquid preparations that were mention previously. The general amount of

colorant and flavoring agent that is used in the suspension is 0.001-0.005%, 0.01-0.02%, respectively (สุธี เวคะวากยานนท์, 2531).

5. High performance liquid chromatography (HPLC) (USP 30, 2007)

High performance liquid chromatography or high pressure liquid chromatography is a procedure or technique that solutes are separated by a solid stationary phase and a liquid mobile phase. The principle of separations is based on the difference in adsorption, solubility, partition, ion exchange processes and depending upon the type of stationary phase and mobile phase used. The solutes distribute between two phases, a solid stationary phase and a liquid mobile phase. The solid stationary phase such as activated alumina or silica gel absorbs the solute. The liquid mobile phase transfers the solute through the medium by partitioning effects until it separated from other solutes that are eluted earlier or latter.

Apparatus

A liquid chromatography consists of a solvent reservoir, a pump, an injector, a chromatography column, a detector and data collection device.

A reservoir is used to contain mobile phase. It is generally a glass container that is acid-base resistance.

Pumping systems deliver metered amounts of mobile phase from the solvent reservoirs through the column at high pressure. Modern systems consist of one or more computer controlled metering pumps that can be programmed to vary the ratio of mobile phase components, as is required for gradient chromatography, or to mix isocratic mobile phases. (i.e. mobile phase having a fixed ratio of solvents. However, the proportion of ingredients in premixed isocratic mobile phases can be more accurately controlled than in those delivered by most pumping system. Pumps used for quantitative analysis should be constructed of material inert to corrosive

mobile phase component and can be delivered mobile phase at a constant rate with minimum fluctuations over extended periods of time.

Injector is a device that is used to inject sample into the mobile phase. There are two types of injector, manual injector and automatic injector. Syringe or loop injectors are manual injector that can be used for manual injection of samples through the septum. The test solution is transferred to the column in mobile phase. The other systems, the test solution is transferred to a cavity by syringe and then switch to the mobile phase. Automatic injector is used for automatically injection. It consists of rack to hold sample vials with septum or stopper in tops and injector device to transfer samples from the vials to a loop. Some auto samplers can be programmed to control sample volume, the number of injections and loop rinse cycles and interval between injections.

Columns used for analytical separation usually have internal diameters of 2 to 5 mm. Larger diameter columns are used for preparative chromatography. They contain stationary phase. System consists of polar stationary phase and nonpolar mobile phase is called normal phase, while the opposite arrangement, nonpolar stationary phase and polar mobile phase is described as reverse phase chromatography. Stationary phase of reverse phase chromatography consists of an organic compound chemically bond to silica or other materials. Particle diameter generally about 3 to 10 μm but size may range up to 50 μm or more for preparative column. Column polarity depends on the polarity of the bound functional groups. The range relative from nonpolar to polar is octadecylsilane (C18), octylsilane (C8), nitril ($CNCH_3$) or cyanopropyl, diol (OH) and aminopropyl. Stationary phase must be presaturated with mobile phase in order to prevent stripping of the stationary phase from the column.

Detector is used to detect a compound that elute from the column. It can be spectrophotometric detector, differential refractometer detector, fluorometric detector, potentiometric detector, voltameric and polarographic electrochemical detector.

Validation of analytical method (USP 30, 2007; ICH, 1996)

Method validation is a procedure to verify that the result from the analytical method is accurate and precise as required. Method should be validated in many cases such as new analytical method, the method is changed from original scope; instrument, laboratory and modification of drug composition or the analytical procedure.

The general characteristics for evaluation an analytical method are determined specificity, linearity, range, accuracy, precision, limit of detection and limit of quantitation. Analytical performance characteristics are determined only a part or all of them depending on a particular type of the analytical method that can be seen in Table 1-2.

Table 1-2 Validation characteristics according to the United States Pharmacopeia (2007)

Characteristics	Category				
	I	II		III	IV
		Quantitative tests	Limiting tests		
Specificity	Yes	Yes	Yes	-	Yes
Linearity	Yes	Yes	No	-	No
Range	Yes	Yes	-	-	No
Accuracy	Yes	Yes	-	-	No
Precision	Yes	Yes	No	Yes	No
Limit of detection	No	No	Yes	-	No
Limit of quantitation	No	Yes	No	-	No

Notes: Yes = usually studied, No = usually not studied, - = may be required (depending on a specific test nature)

Category I: Analytical procedure for the content of main components of bulk drug substance or active ingredient in finished pharmaceutical products.

Category II: Analytical procedure for determination of impurities in bulk drug substance or decomposition products in finished pharmaceutical products

Category III: Analytical procedure for determination of performance characteristics (e.g., dissolution, drug release).

Category IV: Identification test.

Characteristics for analytical method validation

Specificity

Specificity is an ability to distinguish between a studied analyte and impurities or degradation products or other excipients in the preparation. The procedures used to determine specificity will be identification, assay and impurities test which depend on intended objectives of the analytical procedure. The discrimination of the identification test may be confirmed by comparison sample containing the analyte and sample does not contain the analyte. In the case of the assay, it can be done by spiking pure substance (drug substance or drug product) with appropriate level of impurities or excipients and demonstrating that the assay result is unaffected by the presence of these extraneous materials by comparison with the assay result obtained on unspiked samples. For the impurity tests, specificity may be determined by spiking drug substance or drug product with appropriate level of impurities and demonstrating the separation of these impurities individually and/or from other components in the samples matrix. If impurity or degradation product standard is unavailable, specificity may be demonstrated by stress condition (e.g. light, heat, humidity, hydrolysis, oxidation, acid/base). The test results of samples containing impurity or degradation product are compared with a second well-characterized procedure. For the assay, the two results should be compared. For the impurity tests, the impurity profiles should be compared. Peak purity tests using diode array or mass spectrometry may be useful to show that the analyte chromatographic peak is not attributable to more than one component (USP 30, 2007; ICH, 1996).

Linearity and range

Linearity is a directly relationship between visual signal and analyte concentration in appropriate range. It can be preformed by diluting standard stock solution into at least five concentrations. The analyte concentration should be within a given range. It should be

linear relationship. The correlation coefficient should be more than 0.999. The range of the analytical method is a minimum and maximum of concentration that have acceptable linearity, precision and accuracy. For assay of drug substance, specific range should be 80% to 120% of the test concentration (USP 30, 2007; ICH, 1996).

Accuracy

The accuracy of the analytical method means the result from the method is closely to the true result. For the assay of drug in the formulated product, accuracy may be determined by analyzing a sample with known concentration and comparing the measured value with the true value as supplied. For ready-to-use drugs, the accuracy is evaluated through the analysis of standard additives at an amount of 50, 80, 100, 120 and 150% of its expected content in the analyzed solution and placebo or sample matrix. The placebo is prepared separately and then spiked with a known concentration by weight or volume. Accuracy should be assessed over a minimum of three different concentrations covering the specific range in three replicates. Accuracy should be reported as the percent recovery that can be determined by comparing the obtained data with a known content of additive. The percent recovery should be 80-110% as an active ingredient concentration at 0.001% (AOAC, 1993).

Precision

Precision is a variation of the individual test result to the mean value of multiple injections of homogeneous samples. The precision is measured in three categories: repeatability, intermediate precision and reproducibility. Repeatability is determined for the same sample preparation by the same analyst using the same instrument during a short period time. Intermediate precision is evaluated for different days or different analyst or different equipment with the same sample and laboratory. Reproducibility is the same as intermediate precision but in different laboratories. Repeatability should be assessed over a minimum of three different concentrations covering the specific range in three replicates or using a minimum of six determinations at 100% of the test concentration. The precision is evaluated in the terms of

standard deviation (SD) or the relative standard deviation (% RSD). The acceptance criteria for pharmaceutical quality control precision should less than 2%. (ดวงสมร ลิมปิติ, 2545)

6. Stability study

Stability of product is defined that physical, chemical, microbiological, therapeutic and toxicological of the product remain within specifications throughout the period of storage and used. The period of time that the product remains stable at the recommend storage condition is called shelf life. Shelf life labels as expiration date for manufacture products or beyond use date for extemporaneous compounding. Physical and chemical stability are the common parameters that are used to indicate shelf life. In physical stability study, the product should have good appearance that means color; odor and uniformity are not changed. Moreover, the pH of the product should be stable. For chemical stability study, the remaining concentration of active drug must not less than 90% of the initial concentration (USP 30, 2007).

Rates and orders of reactions

The assessment of the shelf life involves kinetics of drug degradation that will be discussed in rates and orders of reactions. The rate of a reaction can be expressed as –d[D]/dt or d[P]/dt, where d[D] is the decrease of any reacting substance concentration over a time interval (dt), or d[P] is the increase of the degradation product concentration over a time interval (dt). Order of reaction is used to define the rate of reaction that is depending on the molar concentration of the reactants. For example, the reaction of substance A and B that has the number of molecule a and b, respectively can be expressed as

$$aA+bB \text{ ----> } P \qquad \text{(eq. 1)}$$

the rate of reaction is

$$\text{rate} = -\frac{1}{a}\frac{d[A]}{dt} = -\frac{1}{b}\frac{d[B]}{dt} = k[A]^m[B]^n \qquad \text{(eq. 2)}$$

where –d[A]/dt or –d[B]/dt is the velocity with which the concentration of a decrease (the minus signal indicates a decrease), k is a rate constant, m+n will be the order of reaction

The order of reaction can follow zero-, first- or second-order reaction.

Zero-order reaction, degradation is independent of the concentration of drug remaining and the speed of reaction is seen to be constant. The rate reaction is the change of substance with time.

$$\text{rate} = -\frac{d[A]}{dt} = k_0 \qquad \text{(eq. 3)}$$

The rate equation may be integrated between the initial concentration $[A]_0$ at t=0 and the remaining concentration $[A]_t$ after t hours.

$$[A]_t = [A]_0 - k_0 t \qquad \text{(eq. 4)}$$

From this reaction, the plot of percent drug remaining against time is linear, with slope of $-k_0$ (Figure 1-4). The unit of k_0 is concentration/time. The half life is the time that one half of the substance disappears and the shelf life is the time that the substance remains at least 90 percent or 10 percent of substance disappears.

$$T_{1/2} = 0.5[A]_0/k_0 \qquad \text{(eq. 5)}$$

$$T_{90} = 0.1[A]_0/k_0 \qquad \text{(eq. 6)}$$

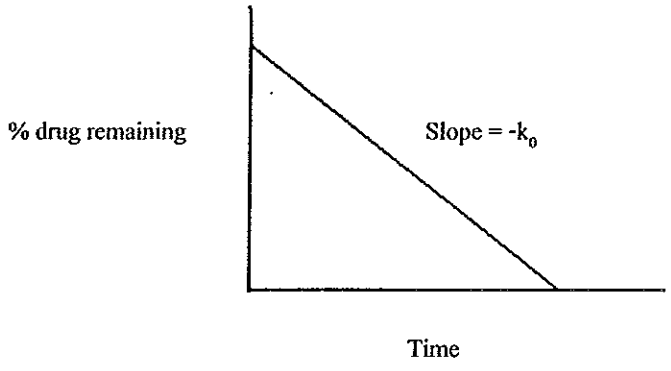


Figure 1-4 A plot of percent drug remaining against time

First order reaction, degradation is depending on the concentration of substance and the rate constant. The rate equation is written as

$$\text{rate} = -\frac{d[A]}{dt} = k_1[A]_t \qquad \text{(eq. 7)}$$

in which $[A]_t$ is the remaining concentration of substance at time t, and k_1 is the first order rate constant. The rate equation may be integrated between the initial concentration $[A]_0$ at t = 0 and the remaining concentration $[A]_t$ after t hours. The rate equation is obtained:

$$[A]_t = [A]_0 e^{-kt} \quad \text{(eq. 8)}$$

Alternative forms of this equation are

$$\ln [A]_t = \ln [A]_0 - k_1 t \quad \text{(eq. 9)}$$

or

$$\log [A]_t = \log [A]_0 - kt/2.303 \quad \text{(eq. 10)}$$

or

$$k = \frac{2.303}{t} \log \frac{[A]_0}{[A]_t} \quad \text{(eq. 11)}$$

A plot of natural logarithm of percent drug remaining against time will be linear with the slope of $-k_1$, yielding the rate constant (Figure 1-5). The unit of k_1 is 1/time. An equation for the half life and shelf life are

$$T_{1/2} = 0.693/k_1 \quad \text{(eq. 12)}$$

and

$$T_{90} = 0.105/k_1 \quad \text{(eq. 13)}$$

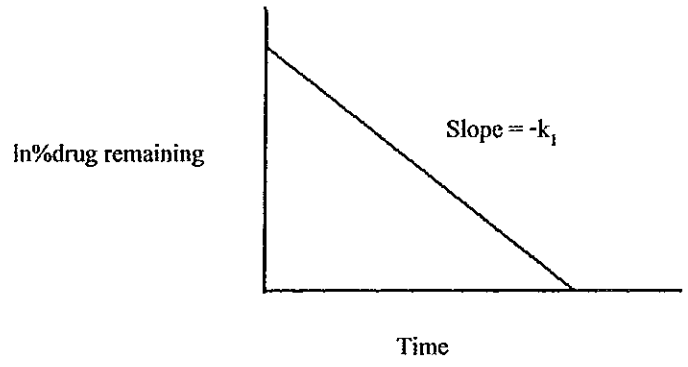


Figure 1-5 A plot of natural logarithm of percent drug remaining against time

Second order reaction, the rate of decomposition involves of two-molecule come together.

$$2A \text{ ----> products}$$

The rate equation is

$$\text{rate} = -\frac{1}{2}\frac{d[A]}{dt} = k_2[A]^2 \quad \text{(eq. 14)}$$

The integration equation is $\frac{1}{[A]_t} - \frac{1}{[A]_0} = 2k_2 t \rightarrow \frac{1}{[A]_0} - \frac{1}{[A]_t} = -kt$ (eq. 15)

A plot of the reciprocal of percent drug remaining against time will be linear with the slope equation to k, yielding the rate constant (Figure 1-6). The unit of k is 1/conc.time. An equation for the half life and shelf life are

$$T_{1/2} = 1/k_2 [A]_0 \quad \text{(eq. 16)}$$

and

$$T_{90} = 1/9[A]_0 k_2 \quad \text{(eq. 17)}$$

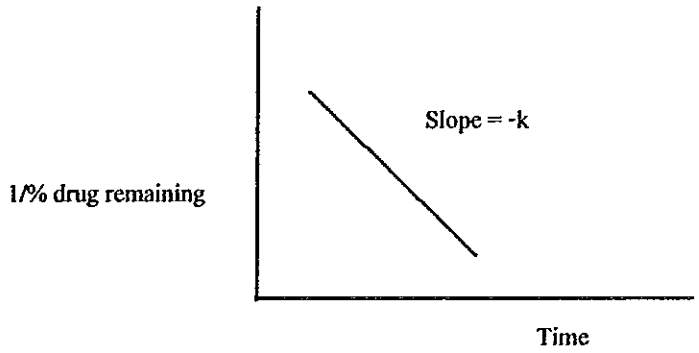


Figure 1-6 A plot of the reciprocal of percent drug remaining against time

Assessing shelf life

The stability study for shelf life can be determined in two types: real time stability test and accelerated stability test.

Real time stability test or long-term stability test, a product is stored at actual storage condition and monitor until it fails the specification. It is used to determine true shelf life of the product at actual storage condition. It is always done parallel to an accelerated stability test to confirm the shelf life prediction. This method was time consuming and uneconomical.

Accelerated stability test is used to evaluate transient shelf life of products at recommend storage condition. The product is stored in elevated stress conditions (such as

temperature, humidity and pH). Degradation at the recommended storage conditions can be predicted using known relationships between the acceleration factor and the degradation rate.

Temperature is the most common acceleration factor used for an accelerated stability test. The speed of many reactions increases about two or three times with each 10°C rise in temperature. The test is suitable for substance that can be degraded by temperature. The effect of temperature on reaction rate is given by the Arrhenius equation.

$$k = Ae^{(-Ea/RT)} \qquad \text{(eq. 18)}$$

where k is the reaction rate constant of any order

A is a constant factor

Ea is the activation energy of the chemical reaction.

R is the gas constant, 1.987 calories/degree mole

T is the absolute temperature (Kelvin, °C +273.14)

This equation can be written in several equations such as

$$\ln k = -\frac{Ea}{RT} + \ln A \qquad \text{(eq. 19)}$$

$$\log k = -\frac{Ea}{2.303RT} + \log A \qquad \text{(eq. 20)}$$

$$\log \frac{k_2}{k_1} = -\frac{Ea}{2.303R}\frac{(T_2 - T_1)}{T_2T_1} \qquad \text{(eq. 21)}$$

where k_2 and k_1 are the rate constant at temperature T_2 and T_1, respectively.

High temperature that used for accelerated stability test should not less than three points. The study at each temperature gives each reaction rate constant. Then, natural logarithm (or logarithm) of reaction rate constant against the reciprocal of absolute temperature was plotted. An arrhenius plot was showed in Figure 1-7. The slope of the line is -Ea/R or -Ea/2.303R and the intercept on the vertical axis is ln A or log A as shown in equation 19 or 20. From this plot Ea and A can be obtained. Ea can be used to predict the rate constant (k) at room

temperature from equation 19 or 20. The obtained k is used to estimate the shelf life. Moreover, the linear graph of the Arrhenius plot can be used to predict the rate constant (k) at room temperature or at any storage temperature by extrapolation. The predicted k is used to estimate the shelf life under ordinary shelf conditions.

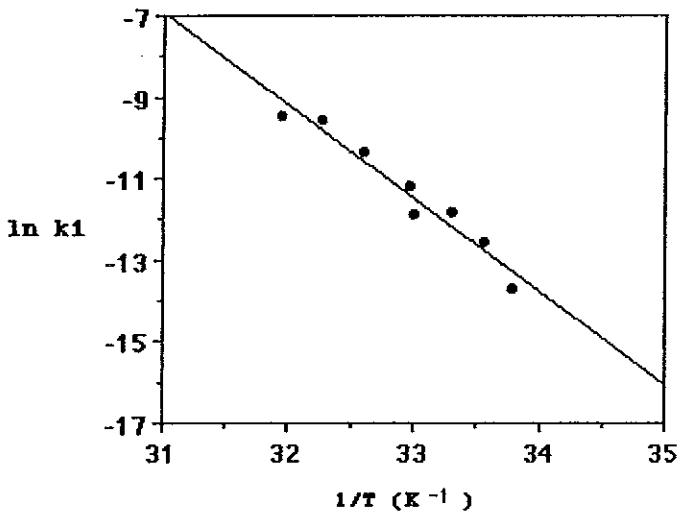


Figure 1-7 A plot of natural logarithm of reaction rate constant against the reciprocal of absolute temperature

CHAPTER 2

MATERIALS AND METHODS

Materials

1. Banana Flavor (Lot no. 8850543891427, Great hill, Thailand)

2. Clonidine hydrochloride powder, analytical grade (Lot no. SL07043, Sigma, Thailand)

3. Clonidine hydrochloride powder (Batch no. 20070301, Central Poly Trading, Thailand)

4. Clonidine hydrochloride tablets (Lot no. K21036, Central Poly Trading, Thailand)

5. Glycerin (Lot no. FPG-G-22907-S1617A, S. Tong Chemicals, Thailand)

6. Lactose broth (Lot no. 310012, Lab Scan, Thailand)

7. Mac Conkey agar (Lot no. VM929865, Merck, Germany)

8. Methanol, analytical grade (Lot no. 08081003, Lab Scan, Thailand)

9. Methyl paraben (Lot no. HF 111, Srichand United Dispensary, Thailand)

10. Orthophosphoric acid (Lot no. B07810, J.T. Baker, USA)

11. Polysorbate 20 (Lot no. 709557, Sri Jun Sahaosut, Thailand)

12. Propyl paraben (Lot no. BH2311, P.C. Drug Center, Thailand)

13. Propylene glycol (Lot no. 7608922088, P.C. Drug Center, Thailand)

14. Sabouraud 4% Glucose agar (Lot no. VM715638, Merck, Germany)

15. Saccharin sodium (P.C. Drug Center, Thailand)

16. Sodium carboxymethylcellulose (high viscosity) 1500 cps (Lot no. 500619, Srichand United Dispensary, Thailand)

17. Sodium chloride (Lot no. 1239, P.C. Drug Center, Thailand)

18. Sorbitol (Lot no. 03840506, P.C. Drug Center, Thailand)

19. Tartrazine (Lot no. 286063/1, Fluka, Switzerland)

20. Triethylamine (Lot no. 436068/1, Fluka, Switzerland)

21. Tryptic soy agar (Lot no. VM803358, Merck, Germany)

22. Tryptic soy broth (Lot no. VM276159, Merck, Germany)

Equipments

1. Analytical column (Vertisep GES C18 4.6x250 mm 5 µm P/N:03AA-E521 QC 51285 Vertical Chromatography, Bangkok, Thailand)
2. Autoclave (Hirayama HV 110 HICL, Hirayama Manufacturing Corporation, Japan)
3. Centrifuge (Model 2323K, Herttle, Germany)
4. High performance liquid chromatography (HPLC) system (Algilent 1100 Series isocratic pump). Pump (G1310A, Germany) Injector (ALSG1313A, Germany) Detector (VWM G1314A, Germany) Diode array Detector (G1315, USA)
5. Hot air oven (Model ED400/E2 II, Binder, Germany)
6. pH meter (Seven Easy S-20, K.P.S. Octatech, Thailand)
7. Sonicator (Model no. 575HTAE, Crest Ultrasonic, Malaysia)
8. Vortex (VX 100, S0K00-230V, Labnet)
9. Viscometer (model RVT, Brookfield, USA)
10. Analytical Balance (Type 310-9403/G PJ500C, Precisa Junior 500C, Switzerland)

Experimental methods

1. HPLC method for the quantitative determination of clonidine hydrochloride in clonidine hydrochloride syrups and suspensions

1.1 HPLC conditions

The high pressure liquid chromatographic technique was used for the analysis of clonidine hydrochloride concentration in the preparation. The technique was modified based on the method of Wojtulewicz and Sosnowska (1986). HPLC conditions were

Analytical column	:	Vertisep GES C18 4.6x250 mm 5 µm P/N:03AA-E521 QC 51285 Vertical Chromatography Co., Ltd., Bangkok, Thailand.
Mobile phase	:	Methanol: Water: Triethylamine (40: 60: 0.1) adjusted to pH 6.8 with phosphoric acid.
Detector wavelength:		208 nm
Flow rate	:	1.0 mL/min
Injection volume	:	20 µl

1.2 Preparation of mobile phase

Triethylamine 1.0 mL was added to purified water 20 mL and adjusted to 600 mL with purified water. Then, phosphoric acid was added to adjust a pH to 6.8. Methanol 400 mL was added. The mobile phase was filtered through a 0.45 µm membrane filter, and degassed by sonication for 30 min.

1.3 Preparation of standard solution

Standard clonidine hydrochloride stock solution was prepared by dissolving an accurately weighed of clonidine hydrochloride, 0.002 gm, in deionized water and adjusted quantitatively to volume in 10 mL volumetric flask, and mixed. The concentration was 200 µg/mL. The solution was prepared on each day of sample analysis.

Standard solution was prepared by pipetting 1.0 mL of standard clonidine hydrochloride stock solution to a 10 mL volumetric flask, diluted with mobile phase to volume. The concentration of this solution is 20 µg/mL. Then, this solution was pipette 0.5 mL to a 10 mL volumetric flask, 1, 2 and 3 mL to a 5 mL volumetric flask. The solutions were diluted with mobile phase to yield concentrations of clonidine hydrochloride to 1, 4, 8 and 12 µg/mL and determined by HPLC. The standard curve was constructed by plotting the peak area of clonidine hydrochloride against the clonidine hydrochloride concentrations and was used to calculate the drug concentrations in samples.

1.4 Preparation of sample solution

Preparation of sample solution from extemporaneous clonidine hydrochloride syrup

Extemporaneous clonidine hydrochloride syrup was shaken by shaker for 15 min. Then, accurately measured 1 mL of clonidine hydrochloride syrup into a 5-mL volumetric flask and adjusted to volume with mobile phase. The solution was filtered through a 0.45 µm membrane filter and determined for clonidine hydrochloride by HPLC.

Preparation of sample solution from extemporaneous clonidine hydrochloride suspension

Extemporaneous clonidine hydrochloride suspension was shaken by shaker for 30 min. Then, accurately measured 1 mL of clonidine hydrochloride suspension into a 5-mL volumetric flask and adjusted to volume with mobile phase. Then, the mixture was centrifuged at

5,500 rpm for 30 min at 25°C. The supernatant was filtered through a 0.45 μm membrane filter and determined for clonidine hydrochloride by HPLC.

1.5 Method validation (ความสมร ลิมปิติ, 2545; International Conference on Harmonization steering committee, 1996; AOAC, 1993)

Specificity

Under the chromatographic conditions, the peak of other pharmaceutical components in the preparation must not interfere with the peak of clonidine hydrochloride. Clonidine hydrochloride standard solution, clonidine hydrochloride in a sugar vehicle, clonidine hydrochloride in a sugar-free vehicle, clonidine hydrochloride in a sugar suspending vehicle, clonidine hydrochloride in a sugar-free suspending vehicle, a sugar vehicle, a sugar-free vehicle, a sugar suspending vehicle, a sugar-free suspending vehicle and clonidine hydrochloride tablet were assayed by HPLC. Chromatograms of these preparations were evaluated by comparing with chromatogram of clonidine hydrochloride standard solution.

The stability-indicating capability of the method was determined by performing degradation of clonidine hydrochloride under acid hydrolysis, basic hydrolysis, oxidation and heat. The aqueous solution of standard clonidine hydrochloride, 200 μg/mL, was forced by adjusting the pH to pH 2 with 1 N sulfuric acid, to pH 12 with 1 N sodium hydroxide or adding 4 drops of 3% hydrogen peroxide. Then, it was heated to 100°C for 3 hours. The samples were assayed after dilution to an expected concentration of 8 μg/mL. Clonidine hydrochloride suspensions in a sugar suspending vehicle and a sugar-free suspending vehicle was incubated at 45°C for 45 days and assayed by HPLC. Degradation product peak must not interfere with the clonidine hydrochloride peak (Levinson *et al.*, 1992). The purity of clonidine hydrochloride peak was determined from diode array HPLC detector.

Linearity

Three sets of clonidine hydrochloride standard solutions in the concentration range of 1, 4, 8, 12 and 20 µg/mL were prepared and analyzed by HPLC. Each of the standard solution was injected three times. Linear regression analysis of the means peak area versus their concentrations was performed. The linear regression coefficient (r^2) $\geq$ 0.999 is the acceptable criteria (ดวงสมร ลิมปิติ, 2545).

Accuracy

Three sets of clonidine hydrochloride solution were spiked into a sugar vehicle, and a sugar-free vehicle to give 20, 30 and 40 µg/mL of clonidine hydrochloride in the syrup. The samples were analyzed by this method. Each individual sample was injected three times. Percent recovery of each sample was calculated by comparing amounts of drug found and the amount of drug added. The percent mean recovery needs to be 98-102% (AOAC, 1993).

Three sets of clonidine hydrochloride solution were spiked into a sugar suspending vehicle and a sugar-free suspending vehicle to give 20, 30 and 40 µg/mL of clonidine hydrochloride in the suspension. The samples were analyzed by this method. Each individual sample was injected three times. Percent recovery of each sample was calculated by comparing amounts of drug found and the amount of drug added. The percent mean recovery needs to be 98-102% (AOAC, 1993).

Precision

a) Within run precision

Three sets of clonidine hydrochloride samples in each vehicle in the concentration of 20, 30 and 40 µg/mL were prepared and analyzed by this method within one day. Each of the sample solution was injected three times. The standard deviation and percent relative standard deviation (%RSD) of peak area were calculated. The %RSD should not be more than 2.0%.

b) Between run precision

Clonidine hydrochloride samples in each vehicle in the concentration of 20, 30 and 40 µg/mL were prepared and analyzed by this method on three different days. Each of the sample solution was injected three times. The standard deviation and percent relative standard deviation (%RSD) of peak area were calculated. The %RSD should not be more than 2.0%.

System Suitability

Clonidine hydrochloride standard solution (6 µg/mL) was prepared and injected onto the HPLC six times. The standard deviation and percent relative standard deviation (%RSD) of peak area were calculated. The %RSD should not be more than 2.0%.

2. Extraction of clonidine hydrochloride from clonidine hydrochloride tablets

The extraction of clonidine hydrochloride from clonidine hydrochloride commercial tablets (0.15 mg/tab) was prepared by two methods as follows;

Method 1 Three tablets of clonidine hydrochloride were added to 5 mL of purified water. The mixture was disintegrated for 5 min. Then, it was sonicated for 5 min, shaken for 30 min and filtered through filter paper (Whatman paper No. 1). The quantity of clonidine hydrochloride in solution was determined by HPLC.

Method 2 Three tablets of clonidine hydrochloride were added to various amount of purified water (5 mL, 7.5 mL and 15 mL). The mixture was disintegrated for 5 min. Then, it was sonicated 5 min, shaken for 30 min and centrifuged at 5,500 rpm for 30 min at 25°C. The supernatant was collected and determined for clonidine hydrochloride by HPLC.

3. Formulation of extemporaneous clonidine hydrochloride syrups in a sugar vehicle and a sugar-free vehicle.

Extemporaneous clonidine hydrochloride syrups prepared from clonidine hydrochloride powder in a sugar vehicle and a sugar-free vehicle were formulated (Table 2-1). The concentration of clonidine hydrochloride in each formulation was 0.15 mg/5 mL. Since clonidine hydrochloride has a bitter taste therefore glycerin and Syrup USP as sweetening agents and sodium chloride were selected to mask undesirable taste of drug in a sugar vehicle. Whereas, glycerin, sorbitol solution 70%w/w, saccharin sodium and sodium chloride were selected to mask a bitter taste of drug in a sugar-free vehicle. The amount of each sweetening agent and sodium chloride were evaluated for palatable preparations by using zero to five point scales. The formulation that gave palatability was selected and further formulated by adding preservative; 1% of paraben concentrate, coloring agent; 0.0005% of tartrazine and flavoring agent; 0.1% of banana flavor.

Table 2-1 Formula of a sugar vehicle and a sugar-free vehicle for extemporaneous clonidine hydrochloride syrups

Ingredients	A sugar vehicle	A sugar-free vehicle
Syrup USP (mL)	42	-
Glycerin (mL)	3.6	4.5
Sodium chloride (gm)	0.3	0.45
Sorbitol solution 70%w/w (mL)	-	24.0
Saccharin sodium (gm)	-	0.06
Paraben concentrate[a] (mL)	0.9	0.9
1% Tartrazine solution (mL)	0.045	0.045
Banana flavor (mL)	0.09	0.09
Purified water to (mL)	90	90

[a] Paraben concentrate consists of methylparaben 10 gm, propylparaben 2 gm, and propylene glycol to 100 mL.

4. Formulation of a sugar suspending vehicle and a sugar-free suspending vehicle for extemporaneous clonidine hydrochloride suspensions

The sugar vehicle and the sugar-free vehicle for extemporaneous clonidine hydrochloride suspensions in the concentration of 0.15 mg/5 mL were formulated by using the sugar vehicle and the sugar-free vehicle that used in extemporaneous clonidine hydrochloride syrups (Table 2-1). To increase the viscosity of these vehicles, the suspending agent; methylcellulose or sodium carboxymethylcellulose was added. The different amount of suspending agents was added in the preparations. The viscosity, redispersibility and aggregation of prepared formulation were determined. The viscosity was determined by using zero to five point scales. The redispersibility was determined by recording times of rotating bottle by hand. The aggregation was determined by visual observation.

5. Preparation of extemporaneous clonidine hydrochloride syrups

Extemporaneous clonidine hydrochloride syrups in the concentration of 0.15 mg/5 mL using clonidine hydrochloride powder were prepared according to formula in Table 2-2.

Extemporaneous Clonidine hydrochloride syrup in a sugar vehicle (Formulation 1)

Clonidine hydrochloride powder and sodium chloride were dissolved in 30 mL of purified water. Then, glycerin, Syrup USP, paraben concentrate, 1% tartrazine solution and banana flavor were added and thoroughly mixed. The final volume was adjusted to 90 mL with purified water. The preparation was prepared in triplicate.

Extemporaneous Clonidine hydrochloride syrup in a sugar-free vehicle (Formulation 2)

Clonidine hydrochloride powder, sodium chloride and saccharin sodium were dissolved in 30 mL of purified water. Then, glycerin, sorbitol solution, paraben concentrate, 1% tartrazine solution and banana flavor were added and thoroughly mixed. The final volume was adjusted to 90 mL with purified water. The preparation was prepared in triplicate.

Table 2-2 Formula for the preparation of extemporaneous clonidine hydrochloride syrups

Ingredients	Amount Used	
	Formulation 1	Formulation 2
Clonidine hydrochloride powder (mg)	2.7	2.7
Syrup USP pH 5 (mL)	42	-
Glycerin (mL)	3.6	4.5
Sodium chloride (gm)	0.3	0.45
Sorbitol solution 70% w/w (mL)	-	24.0
Saccharin sodium (gm)	-	0.06
Paraben concentrate[a] (mL)	0.9	0.9
1% Tartrazine solution (mL)	0.045	0.045
Banana Flavor (mL)	0.09	0.09
Purified water to (mL)	90	90

[a] Paraben concentrate consists of methylparaben 10 gm, propylparaben 2 gm, and propylene glycol to 100 mL.

6. Preparation of a sugar suspending vehicle and a sugar-free suspending vehicle

A sugar suspending vehicle and a sugar-free suspending vehicle were prepared according to Table 2-3.

A sugar suspending vehicle was prepared by dissolving sodium carboxymethylcellulose in 35 mL of hot water (80-90°C). Sodium chloride was dissolved in 5 mL purified water and added to sodium carboxymethylcellulose solution. Then, glycerin, Syrup USP, paraben concentrate, 1% tartrazine solution and banana flavor were added and thoroughly mixed. The final volume was adjusted to 90 mL with purified water.

A sugar-free suspending vehicle was prepared by dissolving sodium carboxymethylcellulose in 50 mL of hot water (80-90°C). Sodium chloride and saccharin sodium was dissolved in 5 mL purified water and added to sodium carboxymethylcellulose solution.

Then, glycerin, sorbitol solution, paraben concentrate, 1% tartrazine solution and banana flavor were added and thoroughly mixed. The final volume was adjusted to 90 mL with purified water.

Table 2-3 Formula for the preparation of vehicle for extemporaneous clonidine hydrochloride suspensions

Ingredients	Amount Used	
	A sugar suspending vehicle	A sugar-free suspending vehicle
Syrup USP (mL)	42	-
Glycerin (mL)	3.6	4.5
Sodium chloride (gm)	0.3	0.45
Sorbitol solution 70%w/w (mL)	-	24.0
Saccharin sodium (gm)	-	0.06
SCMC high viscosity (gm)	0.45	0.63
Paraben concentrate[a] (mL)	0.9	0.9
1% Tartrazine solution (mL)	0.045	0.045
Banana flavor (mL)	0.09	0.09
Purified water to (mL)	90	90

[a] Paraben concentrate consists of methylparaben 10 gm, propylparaben 2 gm, and propylene glycol to 100 mL.

7. Preparation of extemporaneous clonidine hydrochloride suspensions

Extemporaneous clonidine hydrochloride suspensions in the concentration of 0.15 mg/5 mL using clonidine hydrochloride commercial tablets were prepared according to the formula in Table 2-4.

Eighteen tablets of clonidine hydrochloride, 0.15 mg/tab, were ground in glass mortar for 5 min. Then, a small amount of a sugar suspending vehicle or a sugar-free suspending vehicle was added and triturated until smooth paste obtained. The ¾ part of a sugar suspending

vehicle or a sugar-free suspending vehicle was added and thoroughly mixed. The final volume was adjusted to volume with a sugar suspending vehicle or a sugar-free suspending vehicle. The preparation was prepared in triplicate.

Table 2-4 Formula for the preparation of extemporaneous clonidine hydrochloride suspensions

Ingredients	Amount Used	
	Formulation 3	Formulation 4
Clonidine hydrochloride tablet (0.15 mg)	18 tab	18 tab
A sugar suspending vehicle q.s. to	90 mL	-
A sugar-free suspending vehicle q.s. to	-	90 mL

8. Stability study

All formulations were stored in 60-mL glass bottles, protected from light at three temperatures; refrigerator, room temperature and 45°C. Physical, chemical and microbial stability were evaluated.

Physical stability of extemporaneous clonidine hydrochloride syrups and suspensions

Extemporaneous clonidine hydrochloride syrups were evaluated in general appearance such as clarity (visual precipitation), odor and color. The pH of samples was measured by using a pH meter. The preparation in a sugar vehicle (Formulation 1) storage in the refrigerator was determined for crystal of sucrose under an optical microscope. Samples were carried out at 0, 7, 14, 30, 60, 90, 180, 203 and 240 days.

Extemporaneous clonidine hydrochloride suspensions were studied in general appearance such as color, flavor. Sedimentation volume (ratios of final volume of sediment and initial volume of suspension) was determined. The pH of samples was measured with a pH meter. The viscosity of the preparation was measured at 250 rpm, using spindle SC4-31 by Brookfield Rheometer Model DV-III. Redispersibility of the products was studied by recording times of redisperse on shaking. The preparation in a sugar suspending vehicle (Formulation 3) storage in the refrigerator was determined for crystal of sucrose under an optical microscope. Samples were carried out at 0, 7, 14, 30, 60 and 90 days.

Chemical stability of extemporaneous clonidine hydrochloride syrups and suspensions

Clonidine hydrochloride concentration was determined by HPLC immediately after preparation and at 7, 14, 30, 60, 90, 180, 203 and 240 days. Samples stored in refrigerator and 45°C were left to room temperature before analysis. Standard solutions of clonidine hydrochloride were prepared on each day of sample analysis by diluting a stock solution with mobile phase to the concentration of 1 to 20 µg/mL. The standard curve was constructed by plotting the peak area of clonidine hydrochloride against the clonidine hydrochloride concentration. Drug concentration remaining was determined by comparing with the standard curve.

Microbial stability of extemporaneous clonidine hydrochloride syrups and suspensions

The microbial contaminations were determined by total microbial count tests immediately and after storage at 30, 60 and 90 days or until the preparations were physical and chemical stable. Testing was performed using aseptic technique by the plate method according to the method described in USP 30.

Validity of the test

Escherichia coli was inoculated in 10 mL of sterile tryptic soy broth and incubated at 35-37°C for 24-48 hours. Six sterile tubes were prepared. 10 mL of tryptic soy broth was added into one of sterile tube to serve as the control. 9 mL of sterile medium that consist of tryptic soy broth and polysorbate 20, 4.0%, 6.0%, 8.0%, 10.0% and 12.0% was added to five sterile tubes. Then, 1 mL of clonidine hydrochloride syrups or suspensions was added into each tube and mixed. 10 µl of *Escherichia coli* was added into each tube and mixed. 1 mL of the mixture was pipetted and transferred onto each of two sterile petri dishes. Promptly 15-20 mL of tryptic soy agar medium that has been steriled, melted and cooled to approximately 45°C was added to each dish. Covered the petri dishes, mixed the sample with the agar by rotating the dishes, and allowed the contents to solidify at room temperature. Inverted the petri dishes, and incubated at 35-37°C for 24-48 hours. The plate was examined for growth of *Escherichia coli*. If the result showed that *Escherichia coli* was found on the plate, it means that polysorbate 20 can neutralize antimicrobial substances present in the sample.

Total microbial count

9 mL of sterile medium that consisted of tryptic soy borth and polysorbate 20, 4.0% was added into a tube. Pipetted 1 mL of the clonidine hydrochloride syrups or suspensions and transferred into a tube and mixed. Then, the mixture was diluted to obtain 1:100. 1 mL of each dilution was pipetted and transferred onto each of two sterile petri dishes. Promptly, 15-20 mL of tryptic soy agar medium that has been steriled, melted and cooled to approximately

45°C was added to each dish. Covered the petri dishes, mixed the sample with the agar by rotating the dishes, and allowed the contents to solidify at room temperature. Inverted the petri dishes, and incubated at 35-37°C for 24-48 hours. The plates were examined for microbial growth. The plate that has higher number of colonies was calculated the number of colonies in terms of the number of colonies per mL of specimen. An acceptance criteria for total aerobic microbial count of nonsterile aqueous preparation for oral use is 100 cfu/mL. The maximum acceptable count is 200 cfu/mL.

Total yeasts and molds count

9 mL of sterile medium that consisted of tryptic soy broth and polysorbate 20, 4.0% was transferred into a tube. Pipetted 1 mL of the clonidine hydrochloride syrups or suspensions and transferred into a tube and mixed. Then, the mixture was diluted to obtain 1:100. 1 mL of each dilution was pipetted and transferred onto each of two sterile petri dishes. Promptly 15-20 mL of sabouraud dextrose agar medium that has been sterilized, melted and cooled to approximately 45°C was added to each dish. Covered the petri dishes, mixed the sample with the agar by rotating the dishes, and allowed the contents to solidify at room temperature. Inverted the petri dishes, and incubated at 20-25°C for 5-7 days. The plates were examined for microbial growth. The plate that has higher number of colonies was calculated the number of colonies in terms of the number of colonies per mL of specimen. An acceptance criterion for total combined yeasts/molds count of nonsterile aqueous preparation for oral use is 10 cfu/mL. The maximum acceptable count is 20 cfu/mL.

Test for *Escherichia coli*

Sample 1 mL was placed into 10 mL of sterile lactose broth and polysorbate 20, 4.0%, mixed and incubated at 35-37°C for 48 hours. An inoculating loop was used for streak portions from media on the surface of MacConkey Agar Medium. Covered and inverted the dishes, and incubated at 35-37°C for 24-48 hours. If none of the colonies conforms to Brick-red, the test present for absence of *Escherichia coli*. If Brick-red colonies were found, proceeded the

further identification. An acceptance criterion for specified microorganisms of nonsterile aqueous preparation for oral use is absence of *Escherichia coli* (1 gm or 1 mL).

9. Determination of shelf life

The formulation that was stable for 90 days was chosen for prediction of shelf life by accelerated studies at elevated temperatures. Three sets of formulation were incubated at 45°C, 60°C and 70°C. The samples were taken at suitable time intervals and analyzed for the remaining of clonidine hydrochloride. The order of reaction and rate of reaction at each temperature were determined. Arrhenius plot and /or Arrhenius equation was used to determine the rate of degradation and predict the shelf life (t_{90}) of clonidine hydrochloride at actual storage condition.

10. Evaluation the palatability of the preparations (Chamber and Wolf, 1996)

The extemporaneous clonidine hydrochloride syrups or suspensions that gave physical, chemical and microbiological stability for at least 30 days were selected to evaluate for palatability.

The study was established at the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hatyai, Songkhla. Subjects were healthy volunteers, 30 persons. The selected preparations were determined by administering a double-blind taste test to 30 healthy volunteers. Exclusion criteria were person with hypersensitivity to drugs or chemicals, or person with hypotension or person who had problem in tasting or person who use other medication. Subjects were suggested to avoid using perfumed cosmetics or lipstick or eating highly spiced foods at the meal before they were tested. No food, drink, smokes and chew a gum was allowed for 1 hour before the study. Each subject was given 2 mL of each preparation in a plastic medicine teaspoon by the investigator. The solution was kept in the mouth for 30 seconds, then spilt the liquid and rinsed the mouth with water. Between tasting of each preparation, subjects were asked to rinse their mouths with water at least 30 min to remove any residual taste from the previous

preparation. Immediately after each testing, subjects received a questionnaire asking about the degree of their satisfaction of preparation. The questionnaire asked about appearance, color, flavor, taste and overall. It was five-choice of satisfaction level with the statement, i.e. dislike (score = 1), dislike slightly (score = 2), neither like nor dislike (score= 3), like (score = 4), and like extremely (score = 5).

10. Data analysis

The initial concentration of clonidine hydrochloride was defined as 100%. The mean concentrations of clonidine hydrochloride for each time were determined and converted to percentage of initial drug remaining. The percentage remaining of drug in each preparation was compared to its initial concentration. The stability was defined as the remaining not less than 90% of the original concentration of the active drug. The significance of percent recovery of extracted clonidine hydrochloride from tablets between method 1 and method 2 was evaluated by a Student's t-test (α = 0.05). Palatability of preparations was analyzed using mean score and standard deviations. Statistical significance was set at $p<0.05$. The analysis was performed by using the Statistical Package for the Social Sciences (SPSS).

CHAPTER 3

RESULTS AND DISCUSSION

1. HPLC method for the quantitative determination of clonidine hydrochloride in clonidine hydrochloride syrups and suspensions

Clonidine hydrochloride concentration in the preparations was determined by reversed phase high performance liquid chromatography modified from the method of Wojtulewicz and Sosnowska (1986). A C_{18} reversed phase column was used for the analysis. The C18 stationary phase has residual acidic silanol groups on the surface of silica. The interaction of positively charge amine in the structure of clonidine hydrochloride with silanol groups causes peak tailing. This problem was corrected by adding triethylamine to the mobile phase. Triethylamine interacts strongly with the silanol groups and inhibits them from interacting with amines (ดวงสมร ลิมปิติ, 2545). Therefore, the mobile phase was 40%(v/v) methanol in purified water containing 0.1% of triethylamine and adequate amount of 10% phosphoric acid to adjusted the pH to 6.8. The flow rate was 1 mL/min. Detection wavelength was 208 nm.

2. Method Validation

Validation of this method was performed because it is changed from original scope. Since this procedure is used to analyze the content of active ingredient in finished pharmaceutical products, it was validated in terms of specificity, linearity, range, accuracy and precision (USP 30, 2007).

Specificity

Specificity is an ability to discriminate between clonidine hydrochloride and impurities or degradation products or other excipients in the preparation. The specificity of this

analytical method was validated by two procedures. Firstly, comparing HPLC chromatogram of standard clonidine hydrochloride (Figure 3-1) with HPLC chromatogram of clonidine hydrochloride in a sugar vehicle (Figure 3-2), a sugar vehicle (Figure 3-3), clonidine hydrochloride in a sugar-free vehicle (Figure 3-4), a sugar-free vehicle (Figure 3-5), clonidine hydrochloride in a sugar suspending vehicle (Figure 3-6), a sugar suspending vehicle (Figure 3-7), clonidine hydrochloride in a sugar-free suspending vehicle (Figure 3-8), a sugar-free suspending vehicle (Figure 3-9) and clonidine hydrochloride tablet (Figure 3-10). The result shows that the peaks of other pharmaceutical components in these vehicles and tablet do not interfere with the peak of clonidine hydrochloride.

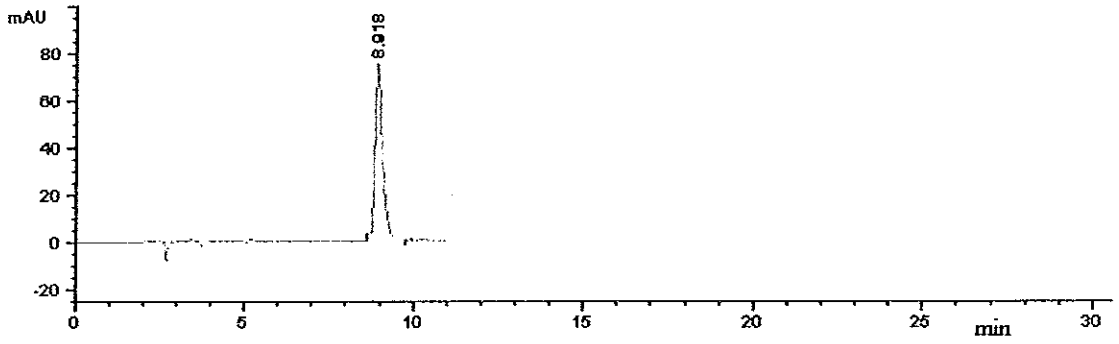


Figure 3-1 HPLC chromatogram of clonidine hydrochloride standard solution

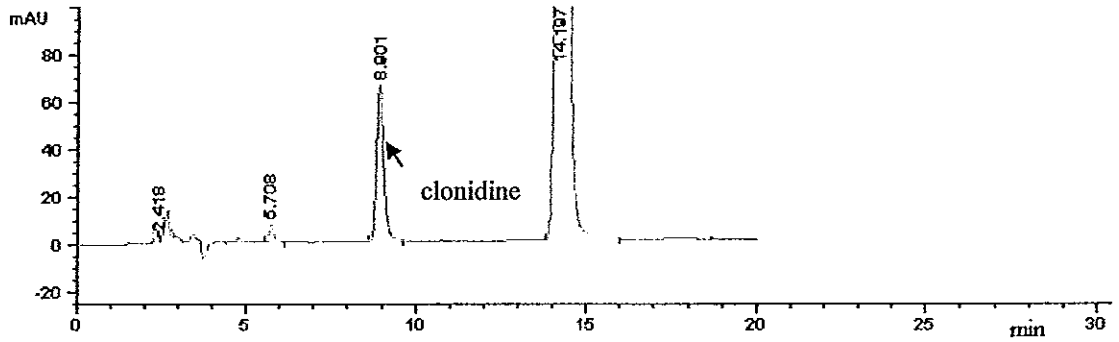


Figure 3-2 HPLC chromatogram of clonidine hydrochloride in a sugar vehicle

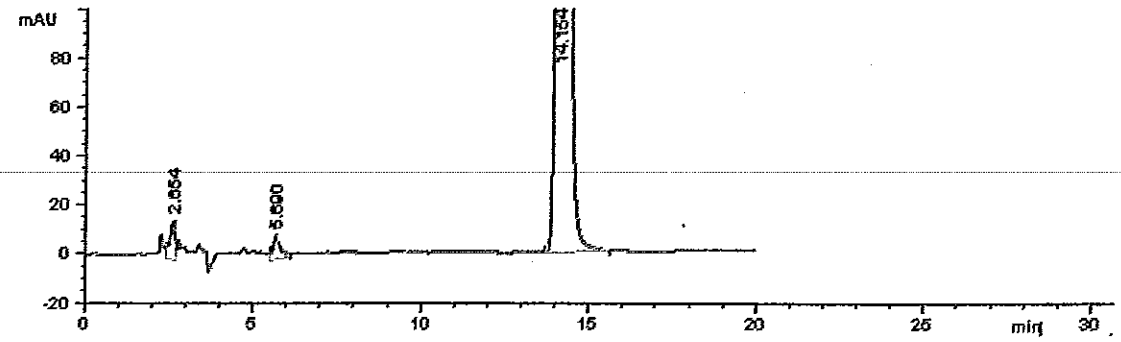


Figure 3-3 HPLC chromatogram of a sugar vehicle

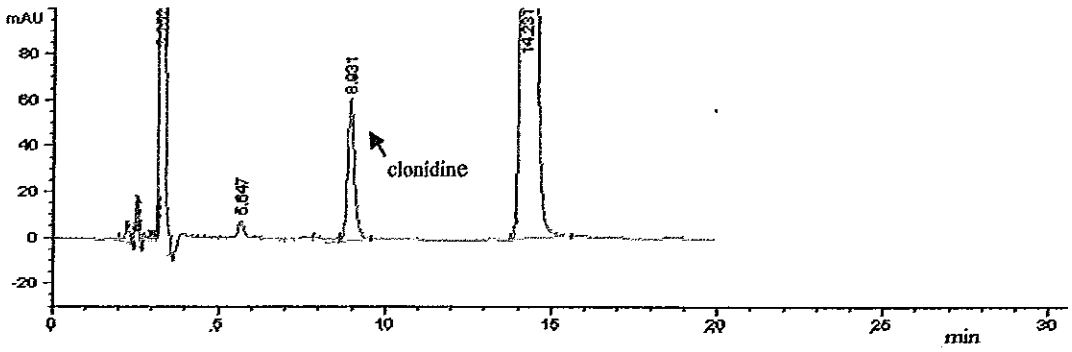


Figure 3-4 HPLC chromatogram of clonidine hydrochloride in a sugar-free vehicle

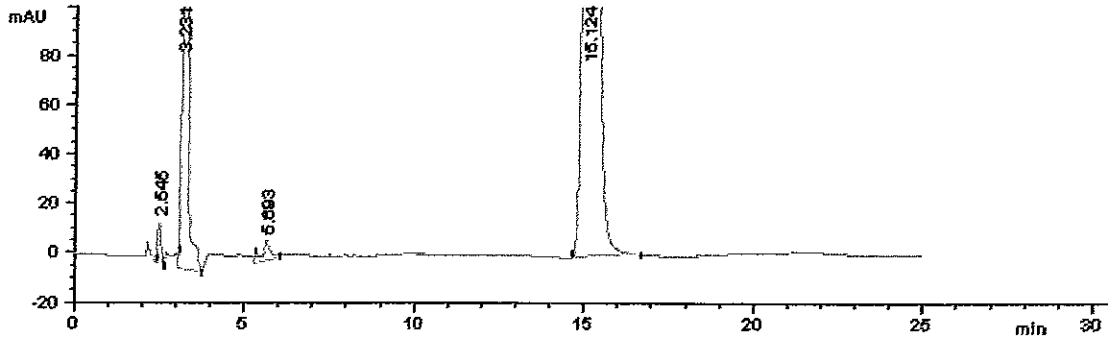


Figure 3-5 HPLC chromatogram of a sugar-free vehicle

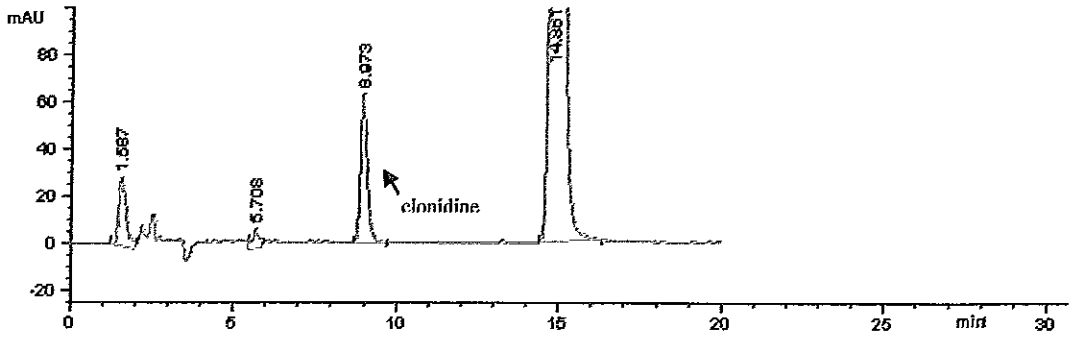


Figure 3-6 HPLC chromatogram of clonidine hydrochloride in a sugar suspending vehicle

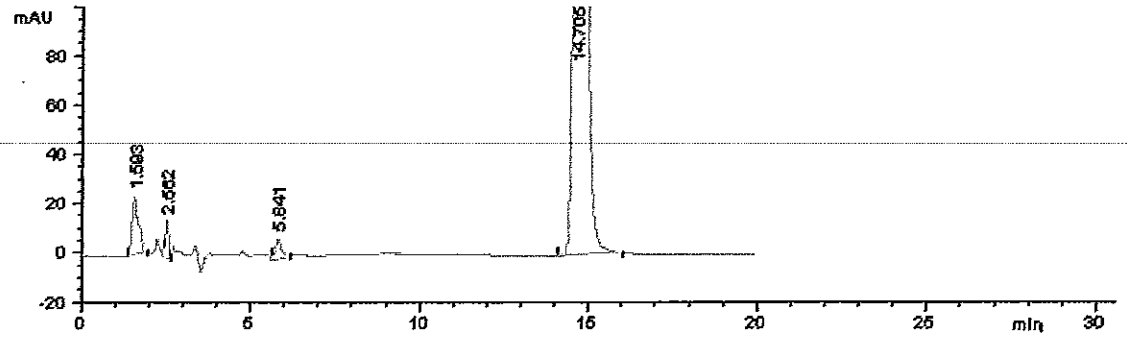


Figure 3-7 HPLC chromatogram of a sugar suspending vehicle

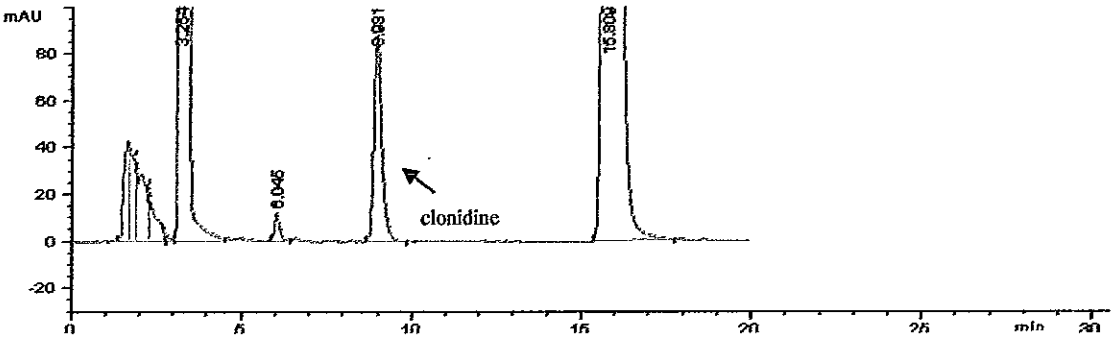


Figure 3-8 HPLC chromatogram of clonidine hydrochloride in a sugar-free suspending vehicle

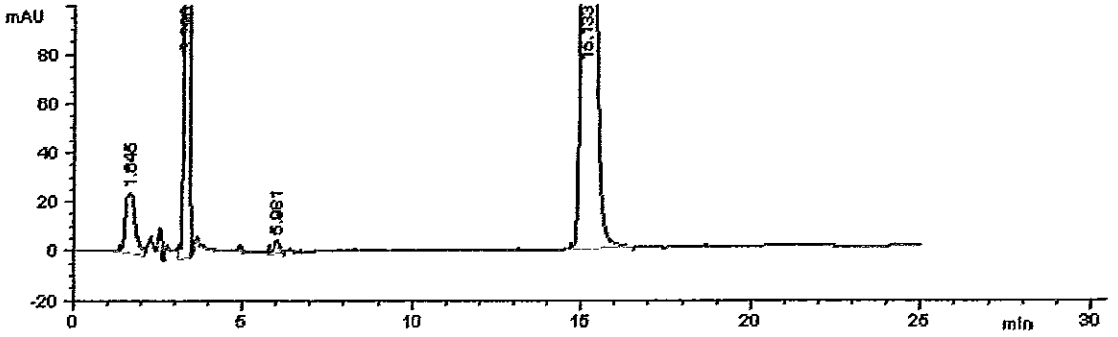


Figure 3-9 HPLC chromatogram of a sugar-free suspending vehicle

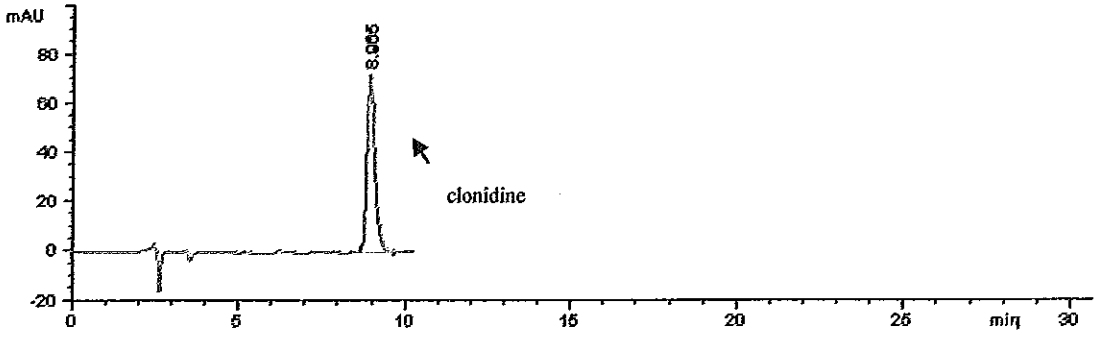


Figure 3-10 HPLC chromatogram of clonidine hydrochloride in tablet

Secondly, the stability-indicating capability of the method was evaluated from forced degradation of clonidine hydrochloride under acid hydrolysis, basic hydrolysis, oxidation and heat. Figure 3-11 shows chromatogram of acid degradation of clonidine hydrochloride in 1 N sulfuric acid at 100 °C for 3 hours. The peak of degradation products were not observed at the retention time of clonidine hydrochloride. In contrast, the peak of unknown product was observed under basic condition and was elute in less than 4 min after adjusting the pH of standard clonidine hydrochloride solution, 200 µg/mL, to pH 12 with 1 N sodium hydroxide and heating to 100°C. (Figure 3-12). In addition, peak of degradation products was observed with oxidation after heating the standard clonidine hydrochloride solution with 4 drops of 3% hydrogen peroxide for 3 hours. The peak of degradation products were eluted in less than 3 min and more than 9 min (Figure 3-13). Figure 3-14 and Figure 3-15 show the chromatogram of clonidine hydrochloride in a sugar suspending vehicle and a sugar-free suspending vehicle, respectively after heating at 45°C for 45 days. The peak purity was evaluted by using diode array detector. The UV-absorption spectra at three points of the peaks, the middle of left and right of peak and the top of the peak, were compared. The results show that clonidine hydrochloride peak was not attributable to more than one component (Figure 3-16, Figure 3-17, Figure 3-18, Figure 3-19 and Figure 3-20). This indicated that analytical method is specific for clonidine hydrochloride.

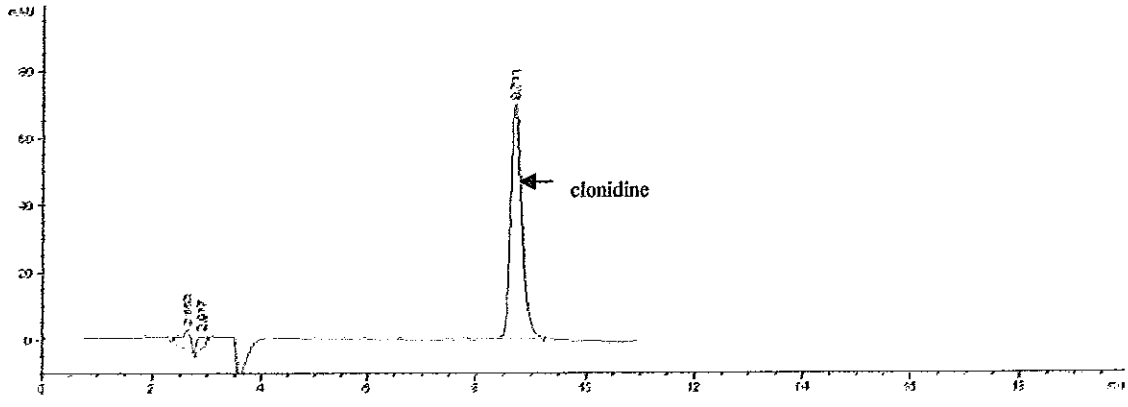


Figure 3-11 HPLC chromatogram of clonidine hydrochloride in 1N sulfuric acid (pH 2) after heating at 100 °C for 3 hours

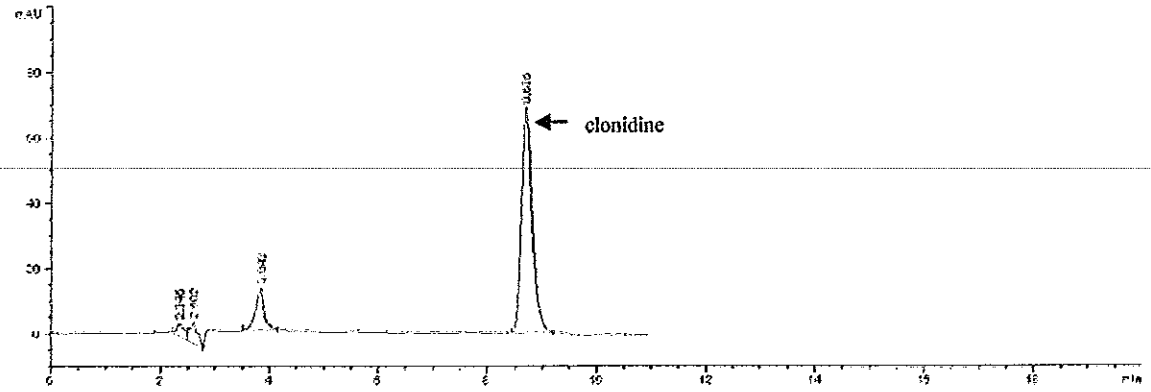


Figure 3-12 HPLC chromatogram of clonidine hydrochloride in 1N sodium hydroxide (pH 12) after heating at 100 °C for 3 hours

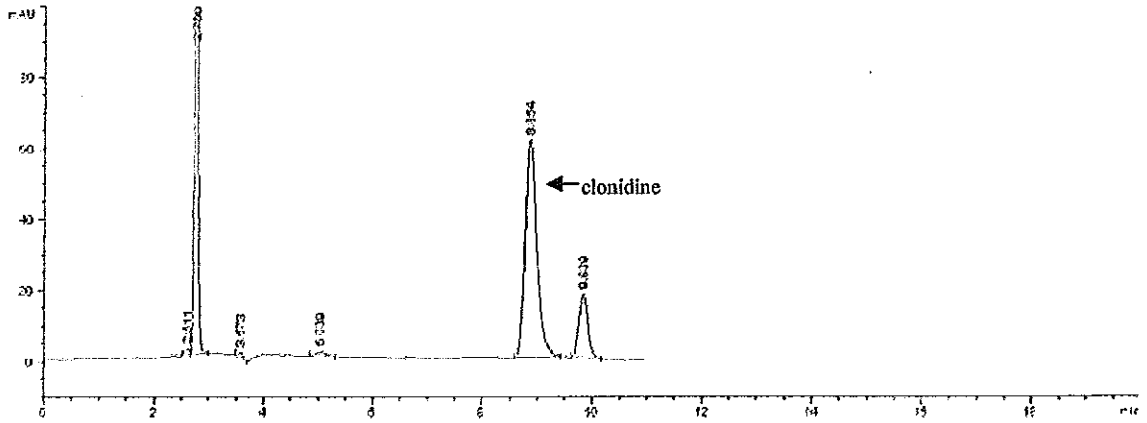


Figure 3-13 HPLC chromatogram of clonidine hydrochloride in 4 drops of 3% hydrogen peroxide after heating at 100 °C for 3 hours

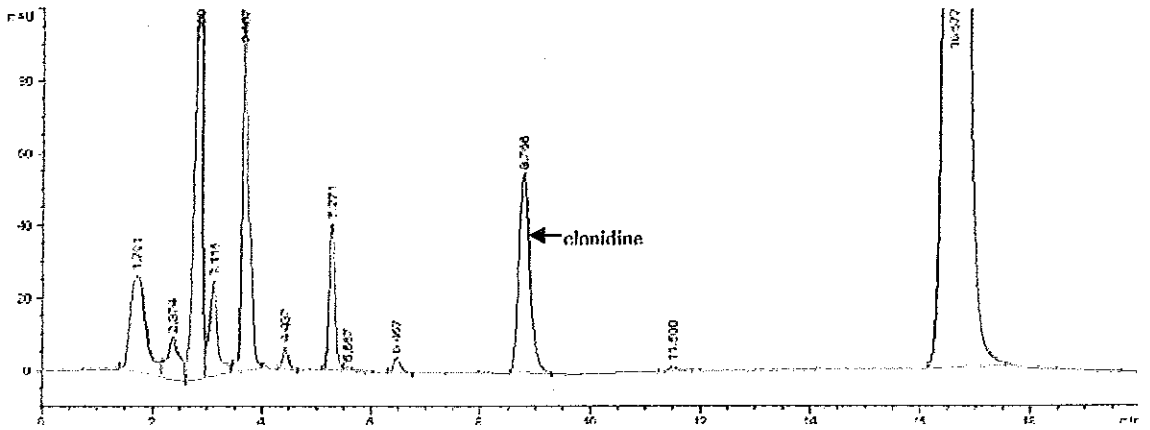


Figure 3-14 HPLC chromatogram of clonidine hydrochloride in a sugar suspending vehicle at 45°C for 45 days

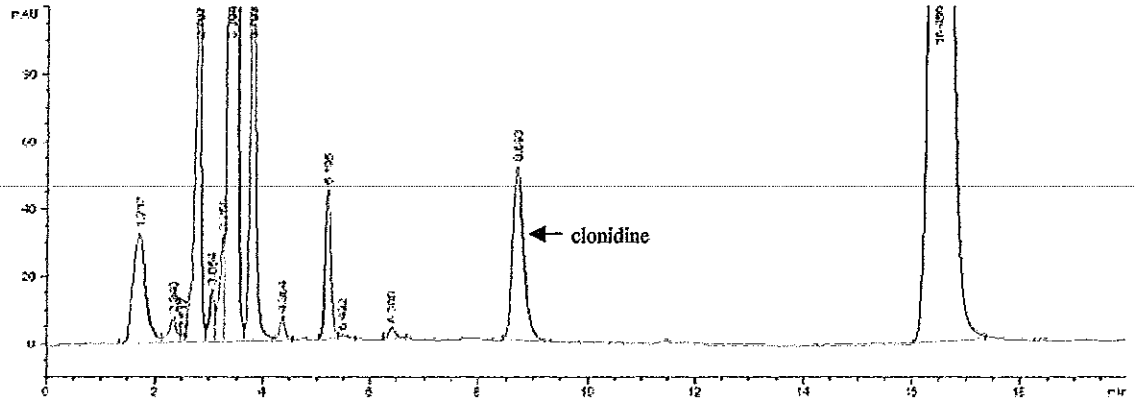


Figure 3-15 HPLC chromatogram of clonidine hydrochloride in a sugar-free suspending vehicle at 45°C for 45 days

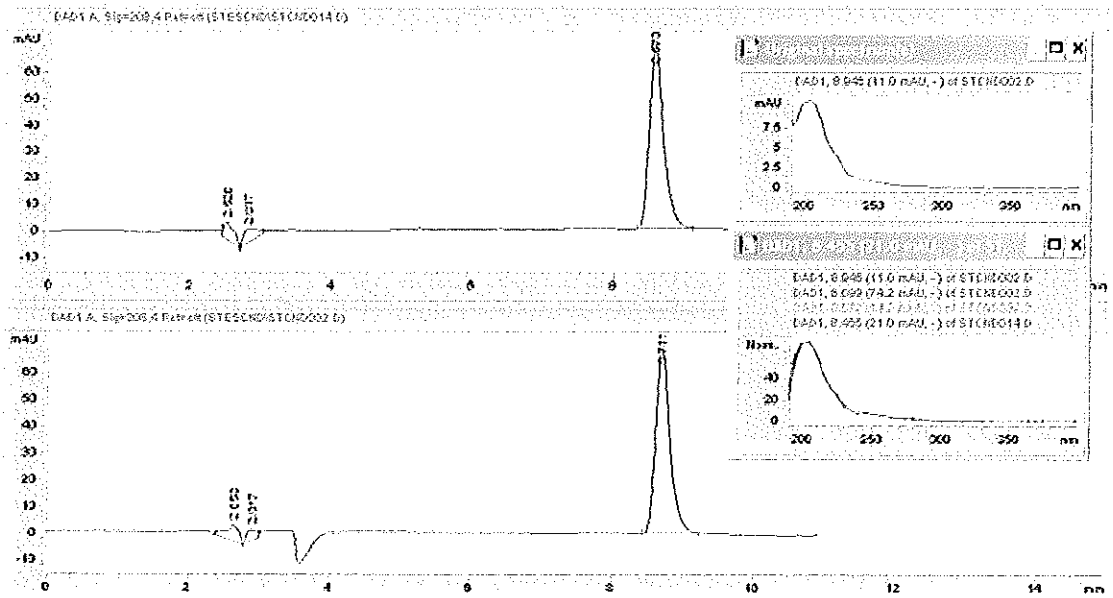


Figure 3-16 HPLC chromatogram and UV Spectra of clonidine hydrochloride in standard solution and in 1N sulfuric acid (pH 2) after heating at 100 °C for 3 hours

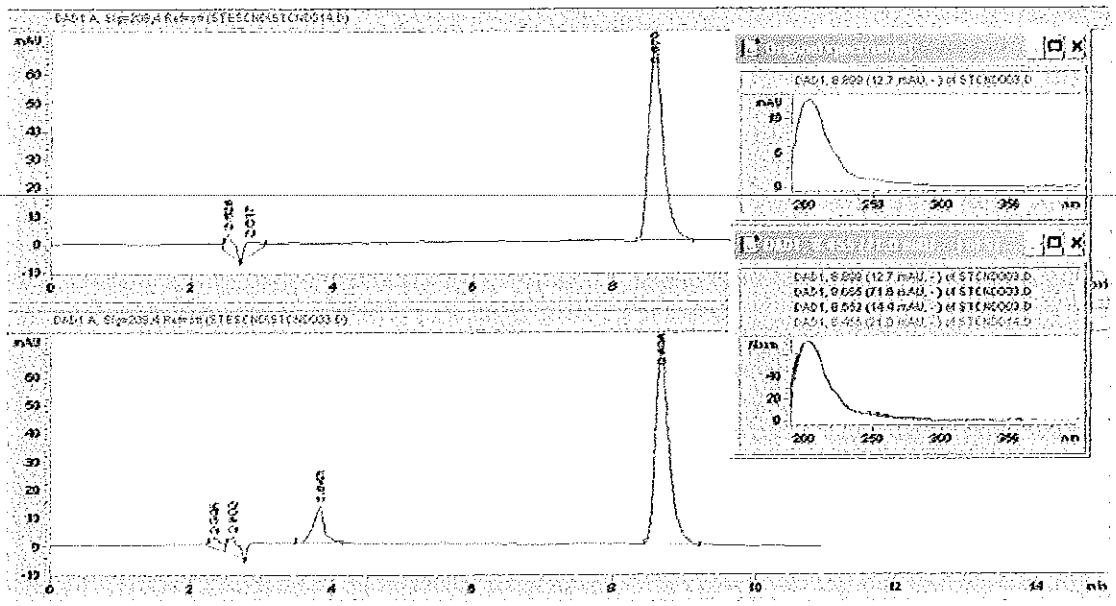


Figure 3-17 HPLC chromatogram and UV Spectra of clonidine hydrochloride in standard solution and in 1N sodium hydroxide (pH 12) after heating at 100 °C for 3 hours

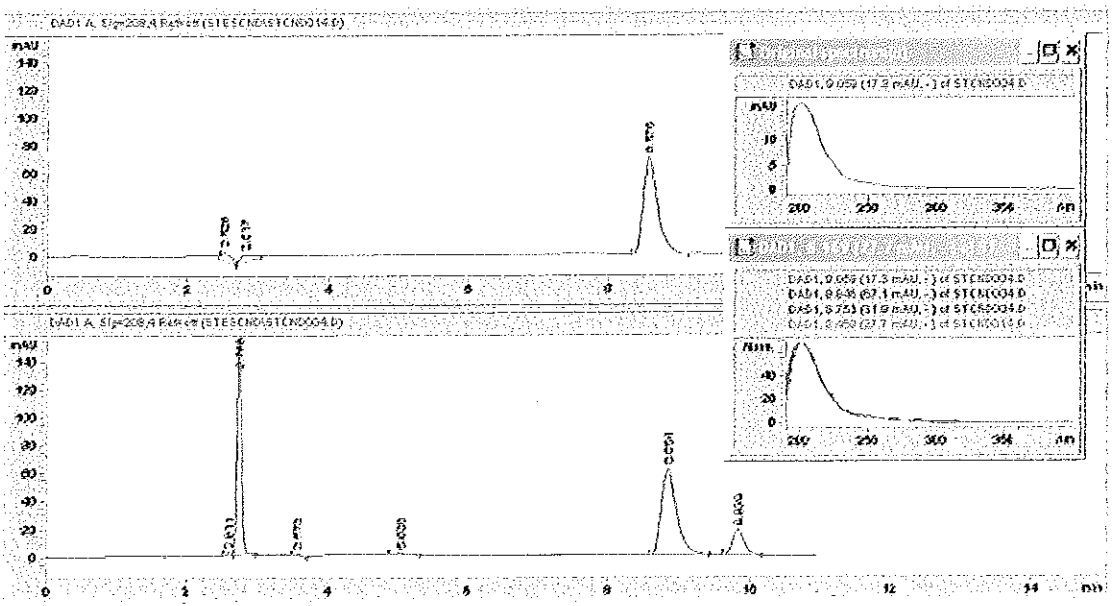


Figure 3-18 HPLC chromatogram and UV Spectra of clonidine hydrochloride in standard solution and in 4 drops of 3% hydrogen peroxide after heating at 100 °C for 3 hours

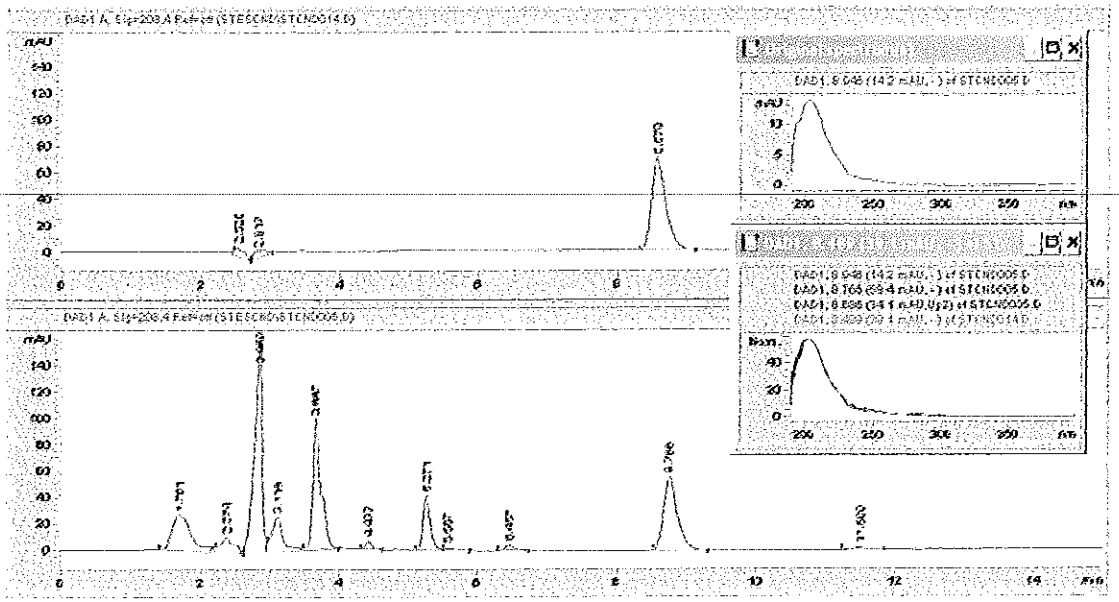


Figure 3-19 HPLC chromatogram and UV Spectra of clonidine hydrochloride in standard solution and in a sugar suspending vehicle at 45°C for 45 days

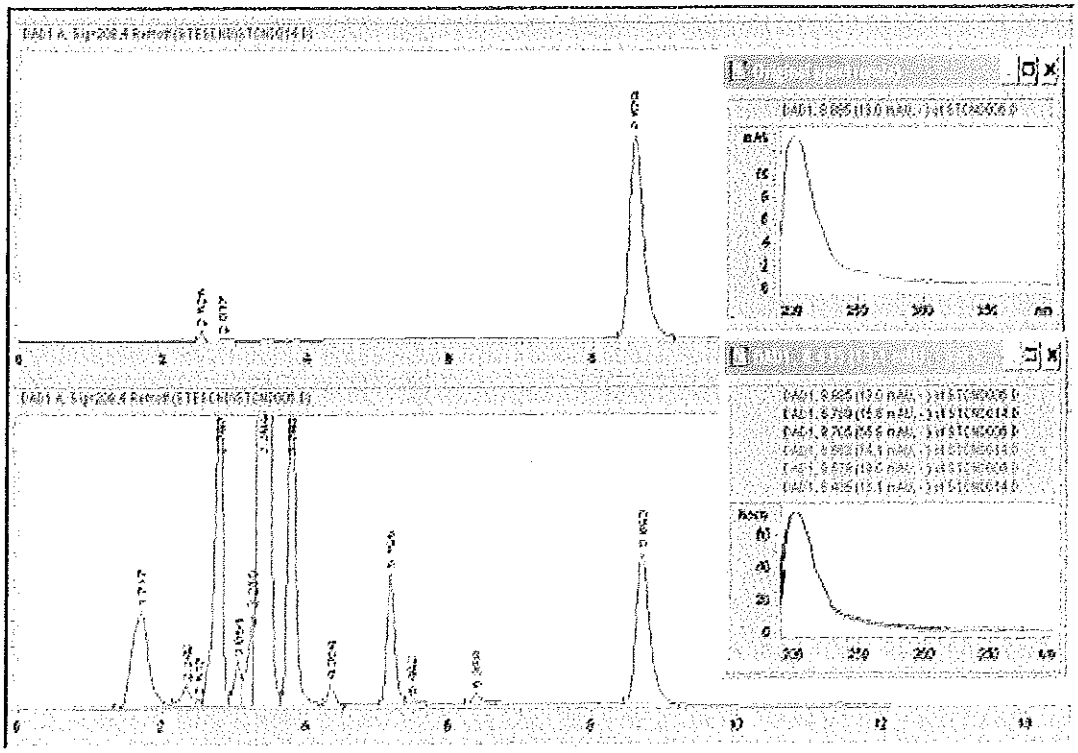


Figure 3-20 HPLC chromatogram and UV Spectra of clonidine hydrochloride in standard solution and in a sugar-free suspending vehicle at 45°C for 45 days

Linearity and range

Linearity was preformed by diluting three sets of standard clonidine hydrochloride into five concentrations and analyzed by HPLC. Each of the standard solutions was injected three times. Data of standard curve of clonidine hydrochloride in mobile phase is shown in Table 3-1. The standard curve of clonidine hydrochloride was performed by plotting concentration of clonidine hydrochloride against mean peak area (Figure 3-21). Linear regression analysis was presented by a coefficient of determination (r^2) in 0.9998 (y = 137.89x-11.357, r^2 = 0.9998). The curve exhibited linearity over the concentration range of 1.0 to 20.0 μg/mL. According to ดวงสมร ลิมปีติ (2545), a correlation coefficient should be more than 0.999. Therefore, the method exhibited acceptable linear relationship between concentrations of clonidine hydrochloride and peak area in the range of 1.0 to 20.0 μg/mL.

Table 3-1 Data of standard curve of clonidine hydrochloride in mobile phase

Clonidine HCl concentration (μg/mL)	Peak area			Mean ± SD
	No. 1	No. 2	No. 3	
1	143.42335	138.65706	139.01923	140.36655 ± 2.60
4	547.40938	545.35781	532.80824	541.85848 ± 7.42
8	1082.92651	1085.92546	1058.26815	1075.70671 ± 13.64
12	1654.25224	1641.51485	1598.05237	1631.27315 ± 25.61
20	2777.94132	2747.8667	2751.49015	2759.09939 ± 15.46

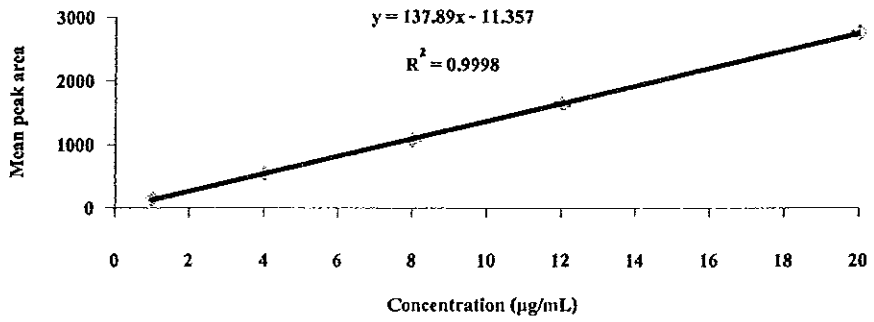


Figure 3-21 The standard curve of clonidine hydrochloride

Accuracy

Percent recovery of clonidine hydrochloride in syrups at the concentration of 20, 30 and 40 μg/mL is shown in Table 3-2 and Table 3-3. Percent recovery of clonidine hydrochloride in a sugar vehicle at the concentration of 20, 30 and 40 μg/mL were 98.62 ± 1.20, 99.88 ± 0.68 and 99.10 ± 0.68, respectively with the overall mean of 99.03± 1.13 and a %RSD of 1.14. Percent recovery of clonidine hydrochloride in a sugar-free vehicle were 98.92 ± 1.39, 99.38 ± 0.74 and 99.72 ± 0.72, respectively with the overall mean of 99.34 ± 1.02 and a %RSD of 1.02. Percent recovery of clonidine hydrochloride in each suspension vehicle is shown in Table 3-4 and Table 3-5. Percent recoveries of clonidine hydrochloride in a sugar suspending vehicle at the concentration of 20, 30 and 40 μg/mL were 98.42 ± 1.26, 99.21 ± 0.82 and 99.05 ± 0.87, respectively with the overall mean of 98.89 ± 1.03, a % RSD of 1.04 and clonidine hydrochloride in a sugar-free suspending vehicle were 98.19 ± 1.25, 98.86 ± 0.86 and 99.41 ± 0.81, respectively with the overall mean of 98.82 ± 1.08, a % RSD of 1.10, respectively. According to AOAC, (1993), this method is accurate for the determination of clonidine hydrochloride in these preparations.

Table 3-2 Percent recovery of clonidine hydrochloride in a sugar vehicle

Amount added (μg/mL)	Amount found (μg/mL)	Percent recovery (Mean ± SD, n=3)	% RSD*
20	19.62	98.62 ± 1.20	1.23
30	29.96	99.88 ± 0.68	0.69
40	39.64	99.10 ± 0.68	0.68

*% RSD = (SD/mean)*100

$\bar{X}$ = 99.03%

SD = 1.13

% RSD = 1.14

Table 3-3 Percent recovery of clonidine hydrochloride in a sugar-free vehicle

Amount added (μg/mL)	Amount found (μg/mL)	Percent recovery (Mean ± SD, n=3)	% RSD*
20	19.78	98.92 ± 1.39	1.41
30	29.81	99.38 ± 0.74	0.74
40	39.88	99.72 ± 0.72	0.72

*% RSD = (SD/mean)*100

$\bar{X}$ = 99.34%

SD = 1.02

% RSD = 1.02

Table 3-4 Percent recovery of clonidine hydrochloride in a sugar suspending vehicle

Amount added (μg/mL)	Amount found (μg/mL)	Percent recovery (Mean ± SD, n=3)	% RSD*
20	19.68	98.42 ± 1.26	1.28
30	29.76	99.21 ± 0.82	0.83
40	39.62	99.05 ± 0.87	0.88

*% RSD = (SD/mean)*100

$\bar{X}$ = 98.89%

SD = 1.03

% RSD = 1.04

Table 3-5 Percent recovery of clonidine hydrochloride in a sugar-free suspending vehicle

Amount added (μg/mL)	Amount found (μg/mL)	Percent recovery (Mean ± SD, n=3)	% RSD*
20	19.64	98.19 ± 1.25	1.28
30	29.66	98.86 ± 0.86	0.87
40	39.76	99.41 ± 0.81	0.82

*% RSD = (SD/mean)*100

$\bar{X}$ = 98.82%

SD = 1.08

% RSD = 1.10

Precision

a) Within run precision (Repeatability, intra-day)

Three sets of clonidine hydrochloride samples in each vehicle in the concentration of 20, 30 and 40 μg/mL were prepared and analyzed within one day. The standard deviation and percent relative standard deviation are shown in Table 3-6. The precision was evaluated in the terms of the relative standard deviation (% RSD). The % RSD was less than 2% at these concentrations.

Table 3-6 Within run precision data (Repeatability, intra-day)

Vehicle	Amount added (μg/mL)	Amount found (μg/mL)			Mean ± SD	% RSD*
		Sample 1	Sample 2	Sample 3		
a sugar vehicle	20	19.66	19.68	19.53	19.62 ± 0.24	1.23
	30	30.04	29.88	29.98	29.96 ± 0.21	0.69
	40	39.64	39.63	39.63	39.64 ± 0.27	0.68
a sugar-free vehicle	20	19.70	19.87	19.77	19.78 ± 0.28	1.41
	30	29.83	29.82	29.79	29.81 ± 0.22	0.74
	40	39.95	39.79	39.91	39.88 ± 0.29	0.72
a sugar suspending vehicle	20	19.56	19.65	19.84	19.68 ± 0.25	1.28
	30	29.93	29.79	29.57	29.76 ± 0.25	0.83
	40	39.44	39.58	39.83	39.62 ± 0.35	0.88
a sugar-free suspending vehicle	20	19.53	19.59	19.79	19.64 ± 0.25	1.28
	30	29.86	29.60	29.51	29.66 ± 0.26	0.87
	40	39.74	39.87	39.68	39.76 ± 0.32	0.82

*% RSD = (SD/mean)*100

b) Between run precision (intermediate precision, inter-day)

Three sets of clonidine hydrochloride samples in each vehicle at the concentration of 20, 30 and 40 μg/mL were prepared and analyzed on different three days. The

standard deviation and percent relative standard deviation were exhibited in Table 3-7. The precision is evaluated in the terms of the relative standard deviation (% RSD). The % RSD was less than 2% at these concentrations.

Table 3-7 Between run precision data (intermediate precision)

Vehicle	Amount added (µg/mL)	Amount found (µg/mL)			Mean ± SD	% RSD*
		Day 1	Day 2	Day 3		
a sugar vehicle	20	19.62	19.84	20.08	19.85 ± 0.28	1.42
	30	29.96	29.80	30.16	29.97 ± 0.32	1.07
	40	39.64	39.16	40.16	39.65 ± 0.46	1.15
a sugar-free vehicle	20	19.78	19.80	19.86	19.81 ± 0.34	1.71
	30	29.81	29.68	29.94	29.81 ± 0.25	0.85
	40	39.88	39.28	39.84	39.67 ± 0.44	1.10
a sugar suspending vehicle	20	19.68	19.66	19.63	19.66 ± 0.29	1.48
	30	29.76	29.73	29.81	29.77 ± 0.30	1.02
	40	39.62	39.49	39.85	39.65 ± 0.41	1.03
a sugar-free suspending vehicle	20	19.64	19.84	19.86	19.78 ± 033	1.65
	30	29.66	29.55	29.76	29.66 ± 0.31	1.04
	40	39.76	39.67	39.63	39.69 ± 0.43	1.08

% RSD = (SD/mean)*100

According to ดวงสมร ลิมปีติ (2545), repeatability or intra-day precision and intermediate precision or inter-day precision was less than 2% at these concentrations. It means a variation of each test is low. This method is precise.

System Suitability

Clonidine hydrochloride standard solution (6 µg/mL) was prepared and injected six times. The concentration and standard deviation (%RSD) were 6.03 ± 0.08 µg/mL and percent relative standard deviation (%RSD) was 0.01%. The %RSD was less than 2.0%.

3. Extraction of clonidine hydrochloride solution from clonidine hydrochloride tablets

Clonidine hydrochloride syrup can be prepared from raw material or clonidine hydrochloride powder. It also can be prepared by using extracted solution from tablets (Cisternino *et al.*, 2003) because clonidine hydrochloride is soluble in water (1:13) at 20°C. Clonidine hydrochloride clear solution may be extracted from tablets by dissolving clonidine hydrochloride local commercial tablets in water. Then, insoluble excipients were separated. In this study, two methods were used for separation of soluble substance from insoluble substance. The first one (Method 1) is using filter paper (Whatman paper No. 1) and the second one (Method 2) is using centrifugation. The obtained clear solution was determined for clonidine hydrochloride by HPLC. The result is presented in Table 3-8. Percent recovery of these two methods was less than 80%. Percent recovery of extracted clonidine hydrochloride by filtering with filter paper was less than extracted clonidine hydrochloride by centrifugation. The percent recovery of two methods was significantly different (p value < 0.05). Percent recovery of Method 1 was very low because the drug was adsorbed by filter paper. Therefore, Method 1 was not suitable for extraction clonidine hydrochloride. To increase the percent recovery of clonidine hydrochloride by method 2, ¾ part of purified water in the formulation (5 mL), total amount of purified water in the formulation (7.5 mL) and total amount of vehicle in the formulation (15 mL) were studied. The result is presented in Table 3-9. The highest percent recovery of extracted clonidine hydrochloride from tablets is extracted 3 tablets of clonidine hydrochloride with 15 mL of purified water. It means 90 mL of purified water should be used to extract 18 tablets of clonidine hydrochloride. High amount of purified water for giving higher percent recovery was the total vehicle in the formulation so it was not suitable for preparing clonidine hydrochloride syrups from extracted clonidine hydrochloride tablets in this study (Formula in Table 2-2).

Table 3-8 Percent recovery of extracted clonidine hydrochloride from method 1 and method 2

	Method		*p*-value
	1 (Filtered with filter paper)	2 (clear solution from centrifuge)	
% Recovery (Mean ± SD, n=3)	69.77 ± 3.71	79.45 ± 1.91	0.025

Table 3-9 Percent recovery of extracted clonidine hydrochloride from method 2 using various amount of purified water

Extracted clonidine hydrochloride	% Recovery (Mean ± SD, n=3)
Clonidine hydrochloride 3 tabs+ purified water 5 mL	79.45 ± 1.91
Clonidine hydrochloride 3 tabs+ purified water 7.5 mL	86.76 ± 0.60
Clonidine hydrochloride 3 tabs+ purified water 15 mL	98.71 ± 0.73

5 mL=¾ part of purified water in the formulation, 7.5 mL= total amount of purified water in the formulation and 15 mL=total amount of vehicle in the formulation

4. Formulation of extemporaneous clonidine hydrochloride syrups in a sugar vehicle and a sugar-free vehicle.

4.1 Formulation of extemporaneous clonidine hydrochloride syrup in a sugar vehicle (Formulation 1)

Extemporaneous clonidine hydrochloride syrup in the concentration of 0.15 mg/5 mL in a sugar vehicle was studied. Clonidine hydrochloride is soluble in water. 0.9 mg of clonidine hydrochloride powder dissolved in purified water 0.2 mL provide a clear, colorless, odorless solution but it obtain bitter taste because chemical structure of clonidine hydrochloride (Figure 1-1) is an amine, salts of organic compound, nitrogen-containing compound and high molecular weight salts (MW: 266.55 gm/mol). Some additive substances are used to mask a bitter taste of the drug. Sweetening agents such as Syrup USP and glycerin, are selected because they are viscous liquid that can coat taste bud, preventing drug contact with the tongue and they have sweet taste that can improve the taste of liquid preparation. The bitterness and sweetness were

evaluated by using zero to five point scales as shown in Table 3-10. The result is shown in Table 3-11. The selected formulation (Formulation 1.3) consisted of glycerin 1.2 mL and Syrup USP 14 mL because the taste of product was least bitter and sweet (just right).

Table 3-10 The zero to five point scales

	Scale					
	0	1	2	3	4	5
Bitterness	*Not bitter*	Least bitter	Less bitter	Bitter	More bitter	Most bitter
Sweetness	Not sweet	Least sweet	Less sweet	*Sweet* (just right)	More sweet	Most sweet
Saltiness	*Not salty*	*Least salty*	Less salty	Salty	More salty	Most salty
Viscosity	Not viscous	Least viscous	Less viscous	*Viscous* (just right)	More viscous	Most viscous

The taste of the preparation should be non bitter, sweet (just right), not salty or least salty and viscous.

Table 3-11 The amount of sweetening agent and the palatability for Formulation 1

Formulation/ Components	1.1	1.2	1.3	1.4	1.5
Clonidine HCl powder (mg)	0.9	0.9	0.9	0.9	0.9
Glycerin (mL)	-	*0.6*	*1.2*	*1.8*	*2.4*
Syrup USP (mL)	-	*12*	*14*	*16*	*18*
Purified water q.s. to (mL)	30	30	30	30	30
Result					
Bitterness	3	2	1	1	0
Sweetness	0	2	3	3	4

The selected formulation (Formulation 1.3) is still least bitter taste. Therefore, sodium chloride was included as flavoring agent to overcome this problem. Sodium chloride can reduce a bitterness of bitter compounds (Murray, 2004). The salty was evaluated by using zero to

five point scales as shown in Table 3-10. Table 3-12 shows small amount of sodium chloride, 0.05-0.2 gm. is used in the formulation. The selected formulation (Formulation 1.8) consisted of sodium chloride 0.1 gm because the taste of product is non bitter, sweet (just right) and least salty. Although, sodium chloride can increase blood pressure, Kurtz (1987) reported that oral administration of sodium chloride in men for seven days, 240 mmole/day (5.52 gm of sodium per days) induced significant in systolic and diastolic blood pressure and sodium chloride should consume less than 6 g daily in the hypertension patient (Weibert, 1996). Adult patient who take the selected preparation (Formulation 1.8) 5 mL four times a day will receive sodium chloride 0.067 gm/day therefore it does not effect to blood pressure.

Table 3-12 The study of amount of sodium chloride and the palatability for Formulation 1

Formulation/components	1.6	1.7	1.8	1.9	1.10
Clonidine HCl powder (mg)	0.9	0.9	0.9	0.9	0.9
Glycerin (mL)	1.2	1.2	1.2	1.2	1.2
Syrup USP (mL)	14	14	14	14	14
Sodium chloride (gm)	-	*0.05*	*0.1*	*0.15*	*0.2*
Purified water q.s. to (mL)	30	30	30	30	30
Result					
Bitterness	1	1	0	0	0
Sweetness	3	3	3	3	4
Saltiness	0	1	1	2	2

Other inactive ingredients were added. Paraben concentrate as preservative in the concentration of 1%. 0.0005% of tartrazine was selected for coloring agent. 0.1% of banana flavor was selected for flavoring agent. The final formulation consists of 0.9 mg of clonidine hydrochloride, 14 mL of Syrup USP, 1.2 mL of glycerin, 0.1 gm of sodium chloride, 0.3 mL of paraben concentrate, 0.015 mL of 1% tartrazine solution, 0.03 mL of banana flavor and purified water a sufficient quantity to 30 mL.

4.2 Formulation of extemporaneous clonidine hydrochloride syrup in a sugar-free vehicle (Formulation 2)

Extemporaneous clonidine hydrochloride syrup in the concentration of 0.15 mg/5 mL in a sugar-free vehicle was studied. Glycerin, sorbitol solution 70%w/w and saccharin sodium, sweetening agents, were used to give palatable preparations. The bitterness and sweetness were evaluated by using zero to five point scales as shown in Table 3-10. The result is shown in Table 3-13. The Formulation 2.3 was selected that was comprised of glycerin 1.5 mL, sorbitol solution 70%w/w 8 mL and saccharin sodium 0.02 gm because the taste of product is least bitter and sweet (just right).

Sodium chloride also is used to mask a bitter taste. Table 3-14 shows the amount of sodium chloride that is used to improve a taste. The selected formulation (Formulation 2.9) consisted of sodium chloride 0.15 gm because the taste of product is non-bitter, sweet (just right) and least salty.

Table 3-13 The amount of sweetening agent and the palatability for Formulation 2

Formulation/Components	2.1	2.2	2.3	2.4	2.5
Clonidine HCl powder (mg)	0.9	0.9	0.9	0.9	0.9
Glycerin (mL)	-	*1.0*	*1.5*	*2.0*	*2.5*
Sorbitol solution70%w/w (mL)	-	*6.0*	*8.0*	*10.0*	*12.0*
Saccharin sodium (gm)	-	0.01	0.02	0.03	0.04
Purified water q.s. to (mL)	30	30	30	30	30
Result					
Bitterness	3	2	1	0	0
Sweetness	0	2	3	4	5

Table 3-14 The amount of sodium chloride and the palatability for Formulation 2

Formulation/Components	2.6	2.7	2.8	2.9	2.10
Clonidine HCl powder (mg)	0.9	0.9	0.9	0.9	0.9
Glycerin (mL)	1.5	1.5	1.5	1.5	1.5
Sorbitol solution 70%w/w (mL)	8.0	8.0	8.0	8.0	8.0
Saccharin sodium (gm)	0.02	0.02	0.02	0.02	0.02
Sodium chloride (gm)	-	*0.05*	*0.1*	*0.15*	*0.2*
Purified water q.s. to (mL)	30	30	30	30	30
Result					
Bitterness	1	1	1	0	0
Sweetness	3	3	3	3	4
Saltiness	0	1	1	1	2

The other excipients; 1% of paraben concentrate, 0.0005% tartrazine, 0.1% of banana flavor was added. The final formulation consists of 0.9 mg of clonidine hydrochloride, 1.5 mL of glycerin, 0.15 gm of sodium chloride, 8 mL of sorbitol solution 70%w/w, 0.02 gm of saccharin sodium, 0.3 mL of paraben concentrate, 0.015 mL of 1% tartrazine solution, 0.03 mL of banana flavor and purified water a sufficient quantity to 30 mL.

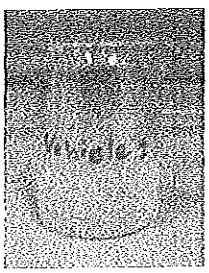
5. Formulation of a sugar suspending vehicle and a sugar-free suspending vehicle for extemporaneous clonidine hydrochloride suspensions

A sugar suspending vehicle and a sugar-free suspending vehicle for extemporaneous clonidine hydrochloride suspensions in the concentration of 0.15 mg/5 mL using commercial tablets were evaluated. Methylcellulose and sodium carboxymethylcellulose were used as suspending agent to increase the viscosity that can suspend insoluble substance from tablets. When 1% of methylcellulose solution was added into each vehicle, the precipitate was observed as shown in Figure 3-22. This may be due to the interaction or the incompatibility between sorbitol solution or paraben concentrate and methylcellulose. Therefore, methylcellulose

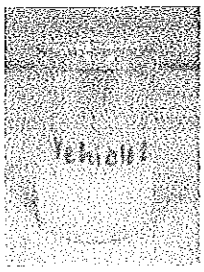
is not suitable for increase viscosity in these vehicles. In contrast, both suspending vehicles using sodium carboxymethylcellulose as suspending agent gave the clear solution. Therefore, only sodium carboxymethylcellulose was selected as suspending agent. The viscosity was evaluated by using zero to five point scales as show in Table 3-10. Redispersibility of the products was studied by recording times of redisperse on shaking after storage for 30 days. The aggregation was visual observed. The results are shown in Table 3-15 and Table 3-16. The sugar suspending vehicle Formulation 3.3 and the sugar-free suspending vehicle Formulation 4.4, which consist of sodium carboxymethylcelluose 0.5% and 0.7%, respectively were selected for providing preparations just right viscosity, no aggregation and can redisperse.

The final sugar suspending vehicle consists of 14 mL of Syrup USP, 1.2 mL of glycerin, 0.1 gm of sodium chloride, 0.15 gm of sodium carboxymethylcellulose, 0.3 mL of paraben concentrate, 0.015 mL of 1% tartrazine solution, 0.03 mL of banana flavor and purified water a sufficient quantity to 30 mL.

The final sugar-free suspending vehicle consists of 1.5 mL of glycerin, 0.15 gm of sodium chloride, 8 mL of sorbitol solution 70%w/w, 0.02 gm of saccharin sodium, 0.21 gm of sodium carboxymethylcellulose, 0.3 mL of paraben concentrate, 0.015 mL of 1% tartrazine solution, 0.03 mL of banana flavor and purified water a sufficient quantity to 30 mL.



a sugar suspending vehicle



a sugar-free suspending vehicle

Figure 3-22 Methylcellulose in a sugar suspending vehicle and a sugar-free suspending vehicle

Table 3-15 The study of sodium carboxymethylcellulose for a sugar suspending vehicle

A sugar suspending vehicle/ Components	3.1	3.2	3.3	3.4	3.5
Glycerin (mL)	1.2	1.2	1.2	1.2	1.2
Syrup USP (mL)	14	14	14	14	14
Sodium chloride (gm)	0.1	0.1	0.1	0.1	0.1
Paraben concentrate[a] (mL)	0.3	0.3	0.3	0.3	0.3
1%Tartrazine (mL)	0.015	0.015	0.015	0.015	0.015
Banana flavor (mL)	0.03	0.03	0.03	0.03	0.03
SCMC high viscosity (%)	-	*0.3*	*0.5*	*0.7*	*1.0*
Purified water q.s. to (mL)	30	30	30	30	30
Result					
Viscosity	0	2	3	4	5
Redispersibility*	3	4	6	7	8
Aggregation*	No	No	No	No	No

[a] Paraben concentrate consists of methylparaben 10 gm, propylparaben 2 gm, and propylene glycol to 100 mL.

* After storage for 30 days

Table 3-16 The study of sodium carboxymethylcellulose for a sugar-free suspending vehicle

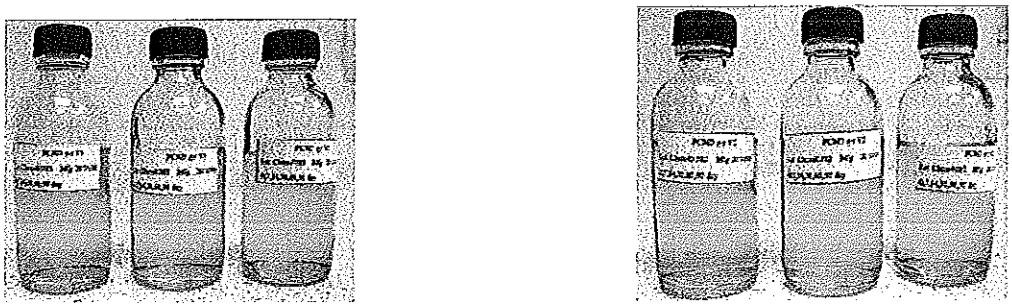
A sugar-free suspending vehicle /Components	4.1	4.2	4.3	4.4	4.5
Glycerin (mL)	1.5	1.5	1.5	1.5	1.5
Sorbitol solution 70%w/w (mL)	8.0	8.0	8.0	8.0	8.0
Saccharin sodium (gm)	0.02	0.02	0.02	0.02	0.02
Sodium chloride (gm)	0.15	0.15	0.15	0.15	0.15
Paraben concentrate[a] (mL)	0.3	0.3	0.3	0.3	0.3
1%Tartrazine (mL)	0.015	0.015	0.015	0.015	0.015
Banana flavor (mL)	0.3	0.3	0.3	0.3	0.3
SCMC high viscosity (%)	-	*0.3*	*0.5*	*0.7*	*1*
Purified water q.s. to (mL)	30	30	30	30	30
Result					
Viscosity	0	1	2	3	4
Redispersibility*	2	4	6	6	8
Aggregation*	No	No	No	No	No

[a] Paraben concentrate consists of methylparaben 10 gm, propylparaben 2 gm, and propylene glycol to 100 mL.

*After storage for 30 days

6. Preparation of extemporaneous clonidine hydrochloride syrups

Extemporaneous clonidine hydrochloride syrups in the concentration of 0.15 mg/5 mL using clonidine hydrochloride powder were prepared according to Table 2-2. Both formulations were clear pale yellow solution, had sweet taste and banana flavor (Figure 3-23).

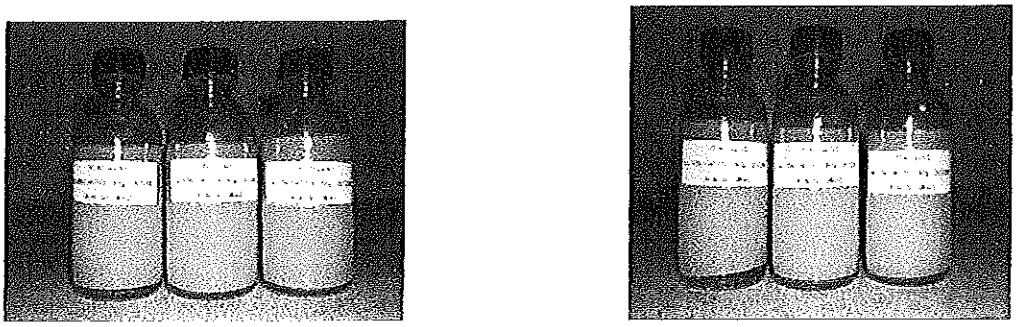


A B

Figure 3-23 Clonidine hydrochloride syrups Formulation 1 (in a sugar vehicle) (A) and clonidine hydrochloride syrups Formulation 2 (in a sugar-free vehicle) (B)

7. Preparation of extemporaneous clonidine hydrochloride suspensions

Extemporaneous clonidine hydrochloride suspensions in the concentration of 0.15 mg/5 mL using commercial clonidine hydrochloride tablets were prepared according to Table 2-3 and Table 2-4. Both formulations were turbid pale yellow, had sweet taste and banana flavor (Figure 3-24).



C D

Figure 3-24 Clonidine hydrochloride suspension Formulation 3 (in a sugar suspending vehicle) (C) and clonidine hydrochloride suspension Formulation 4 (in a sugar-free suspending vehicle) (D)

8. Stability study

All formulations were stored in 60-mL glass bottles protected from light in the refrigerator, at room temperature and 45°C. Physical, chemical and microbial stability of samples were performed in triplicate.

8.1 Stability study of extemporaneous clonidine hydrochloride syrups

Physical stability

The extemporaneous clonidine hydrochloride syrups prepared from powder were observed in general appearance such as precipitation, color and odor. The pH of samples were measured and recorded with a pH meter. Samples were carried out at 0, 7, 14, 30, 60, 90, 180, 203 and 240 days. The results are shown in Table 3-17. The initial pH of clonidine hydrochloride syrups in Formulation 1 and Formulation 2 was 6.49 ± 0.04. The final pH value of Formulation 1 after storage in the refrigerator (6.6 ± 1.82°C), at room temperature (29.33 ± 0.98°C), and 45°C for 240, 203 and 240 days was 5.84 ± 0.06, 5.84 ± 0.06, and 5.28 ± 0.09, respectively and the final pH value of Formulation 2 was 6.40 ± 0.02, 6.41 ± 0.01 and 5.52 ± 0.01, respectively. The pH value was decreased in 0.08-1.21 units. No appreciable change in clarity and color in all samples during the study period. The odor was changed in Formulation 1 after storage at 45°C for 60 days and in Formulation 2 after storage at room temperature (29.33 ± 0.98°C) for 203 days and 45°C for 60 days, respectively. This may due to isoamyl acetate that is the important substance in banana flavor can be hydrolyzed back into alcohol and acetic acid at elevated temperature (Anonymous, 2010).

The crystals of sucrose in a sugar vehicle (Formulation 1) were determined under an optical microscope because needle-shaped crystals of sucrose were found under microscope during freeze-thaw condition (Boonme *et al.*, 2008). In this study, sucrose crystals in the preparations cannot determine because all substances in the formulation has crystal form as shown in Appendix-B. So, sucrose crystals, from Syrup USP and 14 mL of Syrup USP in purified

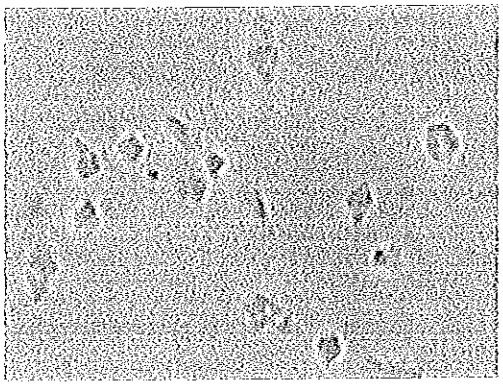
water 30 mL that was used in the preparation, was evaluated after storage at room temperature and in the refrigerator for 7 days,. The sucrose crystal shape from Syrup USP and Syrup USP that was used in the preparation were not difference when storage at room temperature (29°C) and in the refrigerator (2-8°C) for 7 days. The needle-shaped crystals were not found in Formulation 1 when storage at room temperature and the refrigerator for 240 days. The pictures are presented in Figure 3-25.

Table 3-17 Physical stability of extemporaneous clonidine hydrochloride syrups

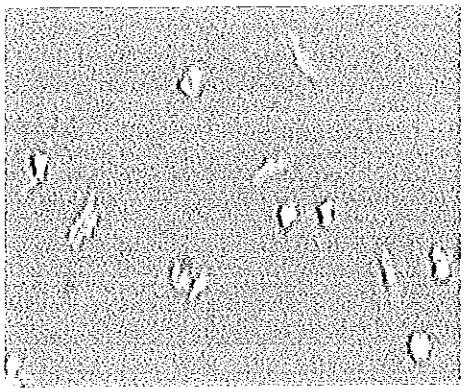
Day / Temp.	pH (Mean ± SD; n=3)					
	Formulation 1			Formulation 2		
	6.6 ± 1.8°C	29.33 ± 0.98°C	45°C	6.6 ± 1.8°C	29.33 ± 0.98°C	45°C
0	6.49 ± 0.04	6.49 ± 0.04	6.49 ± 0.04	6.49 ± 0.04	6.49 ± 0.04	6.49 ± 0.04
7	6.41 ± 0.02	6.47 ± 0.03	6.56 ± 0.04	6.52 ± 0.06	6.47 ± 0.03	6.51 ± 0.08
14	6.56 ± 0.09	6.54 ± 0.05	6.62 ± 0.01	6.58 ± 0.03	6.60 ± 0.04	6.62 ± 0.07
30	6.78 ± 0.03	6.73 ± 0.05	6.84 ± 0.05	6.81 ± 0.02	6.87 ± 0.03	6.81 ± 0.04
60	6.45 ± 0.03	6.47 ± 0.04	6.31 ± 0.05	6.51 ± 0.05	6.57 ± 0.03	6.39 ± 0.04
90	6.80 ± 0.06	6.86 ± 0.02	6.52 ± 0.09	6.95 ± 0.03	6.96 ± 0.04	6.56 ± 0.09
180	5.84 ± 0.05	5.92 ± 0.05	5.92 ± 0.06	6.46 ± 0.05	6.50 ± 0.05	6.16 ± 0.06
203	5.76 ± 0.05	5.84 ± 0.06	-	6.41 ± 0.03	6.41 ± 0.01	-
240	5.84 ± 0.06	-	5.28 ± 0.09	6.40 ± 0.02	-	5.52 ± 0.06
	Color/Precipitation (n=3)					
0	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP
7	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP
14	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP
30	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP
60/90	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP
180/203/240	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP	Y/NP
	Odor (n=3)					
0	G	G	G	G	G	G
7/14/30	G	G	G	G	G	G
60/90/180	G	G	B	G	G	B
203	G	G	-	G	B	-
240	G	-	B	G	-	B

Y= Yellow, P= Precipitate, NP= no precipitate, G= good, B=bad, - = Not tested

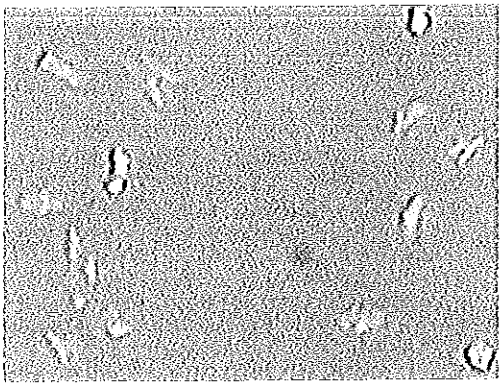
Figure 3-25 Crystals of Syrup USP, 14 mL of Syrup USP in purified water 30 mL and clonidine hydrochloride syrup Formulation 1 storage at room temperature (29°C) and in the refrigerator (2-8°C) for 7, 7 and 240 days (x20)



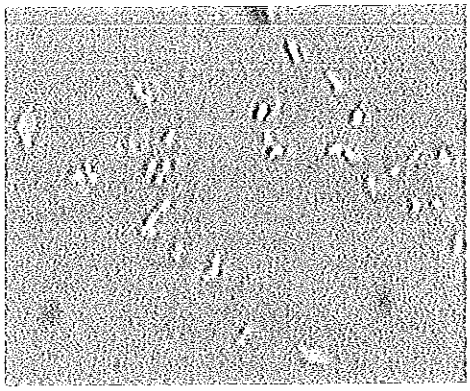
Syrup USP storage at room temperature for 7 days



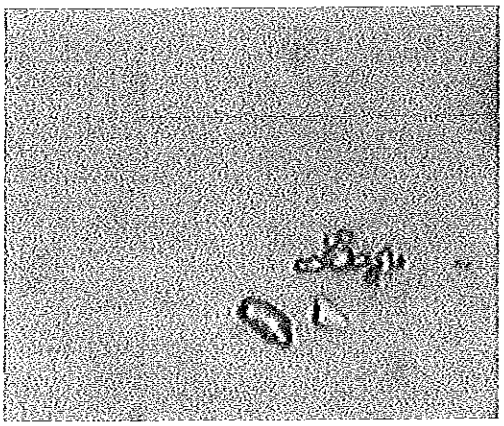
Syrup USP storage in the refrigerator for 7 days



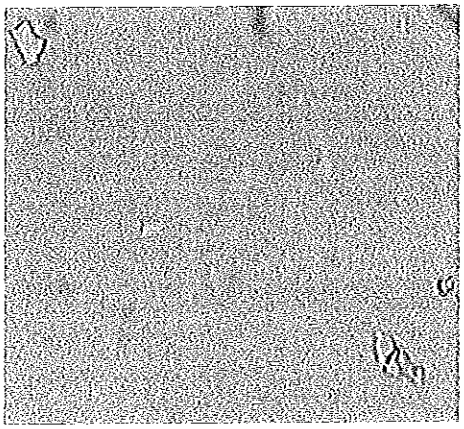
14 mL of Syrup USP in purified water 30 mL storage at room temperature for 7 days



14 mL of Syrup USP in purified water 30 mL storage in the refrigerator for 7 days



Clonidine hydrochloride syrup Formulation 1 storage at room temperature 240 days



Clonidine hydrochloride syrup Formulation 1 storage in the refrigerator 240 days

The amount of sucrose crystals, from Syrup USP and 14 mL of Syrup USP in purified water 30 mL, were counted by hemocytometer. The method was described in Appendix-B. The amount of sucrose crystals, from Syrup USP and Syrup USP that was used in the preparation storage at room temperature (29°C) and in the refrigerator (2-8°C) for 7 days, were not difference. That means solubility of sucrose dose not change when storage in the refrigerator. Thus, Syrup USP and the preparations which containing Syrup USP can be storage in the refrigerator.

Chemical stability

Clonidine hydrochloride concentration was determined by HPLC after various storage times. Samples storage in refrigerator and at 45°C were left to room temperature before analysis. Percent drug remaining was determined. Samples were carried out at 0, 7, 14, 30, 60, 90, 180, 203 and 240 days. Chemical stability of two formulations of clonidine hydrochloride syrups are shown in Table 3-18, Figure 3-26 and Figure 3-27. Stability was defined as not less than 90% of initial concentration remaining in the preparation. The percent initial concentration of clonidine hydrochloride in Formulation 1 was 90.92 ± 0.73%, 98.06 ± 3.58%, 99.94 ± 3.64% after storage at refrigerator (6.6 ± 1.82°C) for 240 days, room temperature (29.33 ± 0.98°C) for 60 days and 45°C for 30 days, respectively and percent initial concentration of clonidine hydrochloride in Formulation 2 was 90.53 ± 0.86%, 97.34 ± 3.01% and 97.40 ± 0.84% after storage at refrigerator (6.6 ± 1.82°C) for 240 days, room temperature (29.33 ± 0.98°C) for 60 days and 45°C for 30 days, respectively. The Formulation 1 and Formulation 2 retained more than 90% of initial clonidine hydrochloride concentration for 240 days at refrigerator (6.6 ± 1.82°C), 60 days at room temperature (29.33 ± 0.98°C) and 30 days at 45°C, respectively. Both formulations gave the similar result. Clonidine hydrochloride syrups Formulation 1 and Formulation 2 were chemical stable for 240 days at refrigerator (6.6 ± 1.82°C), 60 days at room temperature (29.33 ± 0.98°C) and 30 days at 45°C, respectively when storage in glass bottle protected from light.

Table 3-18 Chemical stability of extemporaneous clonidine hydrochloride syrups

Day	% Initial Concentration Remaining (Mean ± SD; N=3)					
	Formulation 1			Formulation 2		
	Storage temperature			Storage temperature		
	6.6 ± 1.82°C	29.33 ± 0.98°C	45 °C	6.6 ± 1.82°C	29.33 ± 0.98°C	45°C
0	100	100	100	100	100	100
7	100.27 ± 2.66	100.22 ± 2.68	100.82 ± 2.61	100.95 ± 1.49	99.02 ± 0.71	98.91 ± 0.45
14	101.51 ± 1.06	101.57 ± 3.88	102.21 ± 3.52	101.81 ± 1.33	100.82 ± 1.47	101.94 ± 1.84
30	103.56 ± 2.69	101.93 ± 3.15	99.94 ± 3.64	102.04 ± 1.41	103.00 ± 0.97	97.40 ± 0.84
60	97.06 ± 3.18	98.06 ± 3.58	87.07 ± 2.53	98.18 ± 0.59	97.34 ± 3.01	84.99 ± 0.94
90	94.35 ± 4.23	89.21 ± 1.58	81.01 ± 2.81	91.73 ± 0.98	88.70 ± 1.49	73.86 ± 0.98
180	93.28 ± 3.34	-	69.92 ± 3.05	92.66 ± 1.40	-	65.50 ± 2.81
203	92.31 ± 3.43	-	-	92.62 ± 0.77	-	-
240	90.92 ± 0.73	-	53.45 ± 2.65	90.53 ± 0.86	-	51.27 ± 1.78

Actual Initial Concentration of Formulation 1 was 0.163 ± 0.005 mg/ 5mL

Actual Initial Concentration of Formulation 2 was 0.161 ± 0.003 mg/ 5mL

- = Not tested

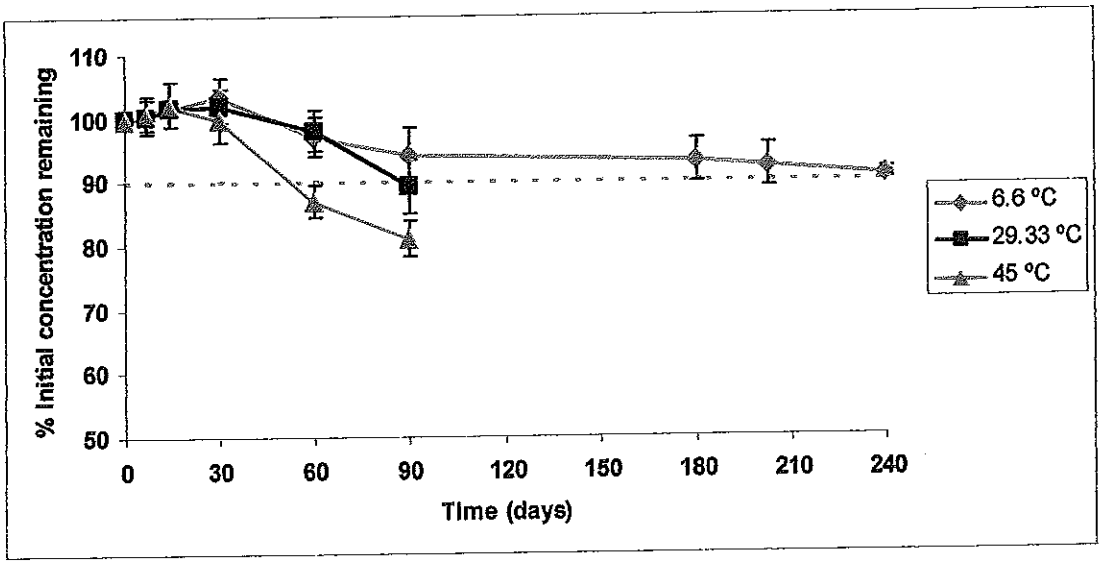


Figure 3-26 Percent initial concentration remaining of clonidine hydrochloride in Formulation 1 after storage in the refrigerator (6.6 ± 1.82°C), at room temperature (29.33 ± 0.98°C) and 45°C

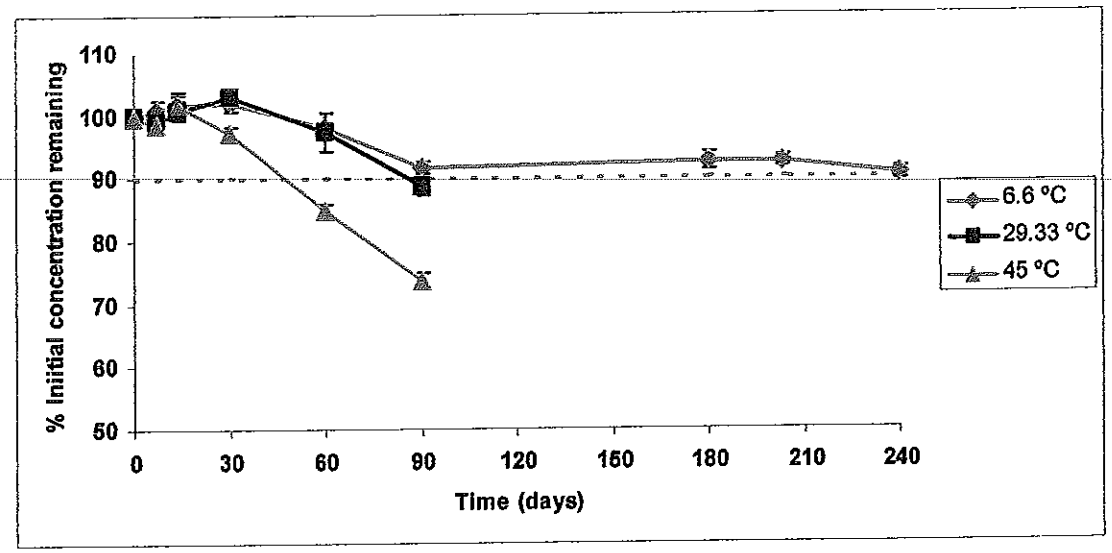


Figure 3-27 Percent initial concentration remaining of clonidine hydrochloride in Formulation 2 after storage in the refrigerator (6.6 ± 1.82°C), at room temperature (29.33 ± 0.98°C) and 45°C

Microbial stability

The microbial contamination was determined by total microbial count, total yeasts and molds count and Test for *Escherichia coli*. The result is shown in Table 3-19. Total microbial count of the samples in Formulation 1 and Formulation 2 storage at refrigerator (6.6 ± 1.82°C) for 240 days, room temperature (29.33 ± 0.98°C) for 60 days and 45°C for 60 days was not found. Yeasts and molds were not found in Formulation 1 but in Formulation 2, they were found 2, 1 and 2 cfu/mL after storage at refrigerator (6.6 ± 1.82°C) for 240 days, room temperature (29.33 ± 0.98°C) for 60 days and 45°C for 30 days, respectively. *Escherichia coli* was not found in any formulations during the study period. According to the USP 30 for nonsterile aqueous preparation for oral use, total aerobic microbial count is 100-200 cfu/mL. Total combined yeasts/molds count is 10-20 cfu/mL. *Escherichia coli* are absence (1 g or 1 mL). Microbial contamination was still within Pharmacopoeial specification. Therefore, these formulations were microbiological stable during the period of the study.

Table 3-19 Microbial contaminations of extemporaneous clonidine hydrochloride syrups

Day	Dilution	Total microbial count (cfu/mL)					
		Formulation 1			Formulation 2		
		6.6 ± 1.82°C	29.33 ± 0.98°C	45 °C	6.6 ± 1.82°C	29.33 ± 0.98°C	45 °C
0	1	NF	NF	NF	NF	NF	NF
	1:10	10	10	10	10	10	10
	1:100	NF	NF	NF	NF	NF	NF
30	1	7	8	5	7	7	5
	1:10	NF	NF	NF	NF	NF	NF
	1:100	NF	NF	NF	NF	NF	NF
60	1	NF	NF	NF	NF	NF	NF
	1:10	NF	NF	NF	NF	NF	NF
	1:100	NF	NF	NF	NF	NF	NF
240	1	NF	-	-	NF	-	-
	1:10	NF	-	-	NF	-	-
	1:100	NF	-	-	NF	-	-
		Total yeasts and molds count (cfu/mL)					
0	1	NF	NF	NF	NF	NF	NF
	1:10	NF	NF	NF	NF	NF	NF
	1:100	NF	NF	NF	NF	NF	NF
30	1	NF	NF	NF	2	2	2
	1:10	NF	NF	NF	NF	NF	NF
	1:100	NF	NF	NF	NF	NF	NF
60	1	NF	NF	NF	NF	1	NF
	1:10	NF	NF	NF	NF	NF	NF
	1:100	NF	NF	NF	NF	NF	NF
240	1	NF	-	-	2	-	-
	1:10	NF	-	-	NF	-	-
	1:100	NF	-	-	NF	-	-

NF= Not found

8.2 Stability study of extemporaneous clonidine hydrochloride suspensions

Physical stability

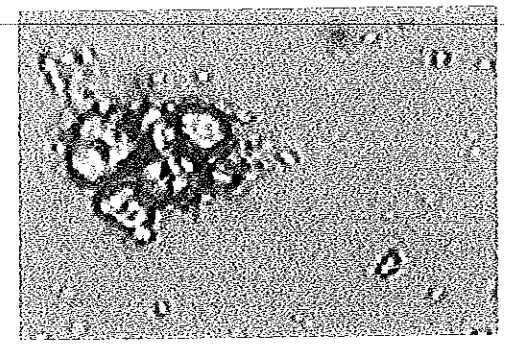
Clonidine hydrochloride suspensions were studied in general appearance such as color and flavor. Sedimentation volume (ratio of final volume of sediment and initial volume of suspension) was determined. The pH of samples were measured and recorded with a pH meter. The viscosity of the preparation was measured at 250 rpm, using spindle SC4-31 by Brookfield Rheometer Model DV-III. Redispersibility of the products was studied by recording times of redisperse on shaking. The preparations in a sugar suspending vehicle (Formulation 3) which storage at refrigerator was determined for crystal of sucrose under an optical microscope. Sample was carried out at 0, 7, 14, 30 and 60 days. The results were shown in Table 3-20. The initial pH of clonidine hydrochloride suspensions in Formulation 3 and Formulation 4 was 6.84 $\pm$ 0.16 and 7.17 $\pm$ 0.03, respectively. The final pH value of Formulation 3 after storage in the refrigerator (3.75 $\pm$ 0.5 °C), at room temperature (29.13 $\pm$ 0.25°C) and 45°C for 60 days was 6.78 $\pm$ 0.06, 6.86 $\pm$ 0.01, and 6.73 $\pm$ 0.02 and the final pH of Formulation 4 was 7.06 $\pm$ 0.04, 7.09 $\pm$ 0.01 and 6.91 $\pm$ 0.05, respectively. The pH value in Formulation 3 and Formulation 4 was changed in 0.02-0.11 units and 0.08-0.26 units, respectively. The pH of both clonidine hydrochloride suspensions was higher than the pH of clonidine hydrochloride syrups. It may be due to unknown excipients in tablets that increase pH of the solution because clonidine hydrochloride tablet that dissolves in water has pH about 7.4. No appreciable change in color and odor in all samples during the study period. Sedimentation volume of clonidine hydrochloride suspension in Formulation 3 and Formulation 4 was about 0.1 in all samples during the study period. Redispersibility of clonidine hydrochloride suspension Formulation 3 was about 8 times under storage study for 60 days. Whereas, redispersibility of clonidine hydrochloride suspension Formulation 4 when storage at refrigerator (3.75 $\pm$ 0.5 °C), room temperature (29.13 $\pm$ 0.25°C) and 45°C for 60 days was about 8, 9 and 12, respectively. Viscosity was not markedly change in all samples under storage study for 60 days. The needle-shaped crystals in Formulation 3 were not found after storage at room temperature (29.13 $\pm$ 0.25°C) and the refrigerator (3.75 $\pm$ 0.5 °C) for 60 days (Figure 3-28).

Table 3-20 Physical stability of extemporaneous clonidine hydrochloride suspensions

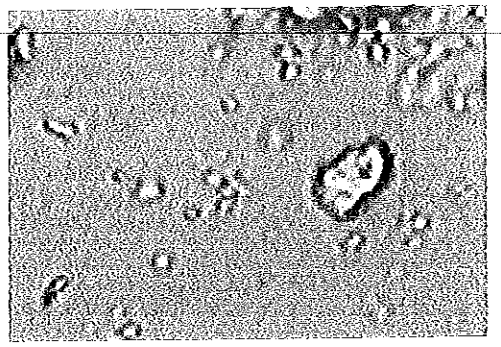
Day	pH (Mean ± SD; n=3)					
	Formulation 3			Formulation 4		
	3.75 ± 0.5°C	29.13 ± 0.25°C	45°C	3.75 ± 0.5°C	29.13 ± 0.25°C	45°C
0	6.84 ± 0.16	6.84 ± 0.16	6.84 ± 0.16	7.17 ± 0.03	7.17 ± 0.03	7.17 ± 0.03
7	6.91 ± 0.03	6.66 ± 0.17	6.67 ± 0.2	7.18 ± 0.02	7.1 ± 0.05	7.05 ± 0.02
14	6.88 ± 0.06	6.93 ± 0.04	6.88 ± 0.03	7.12 ± 0.04	7.14 ± 0.03	7.09 ± 0.03
30	6.75 ± 0.12	6.87 ± 0.02	6.8 ± 0.02	7.06 ± 0.04	7.06 ± 0.03	7.00 ± 0.03
60	6.78 ± 0.06	6.86 ± 0.01	6.73 ± 0.02	7.06 ± 0.04	7.09 ± 0.01	6.91 ± 0.05
	Color/odor (n=3)					
0/7	PY/G	PY/G	PY/G	PY/G	PY/G	PY/G
14	PY/G	PY/G	PY/G	PY/G	PY/G	PY/G
30	PY/G	PY/G	PY/G	PY/G	PY/G	PY/G
60	PY/G	PY/G	PY/G	PY/G	PY/G	PY/G
	Sedimentation volume (n=3)					
0	1	1	1	1	1	1
7	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.00	0.13 ± 0.02
14	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.00	0.13 ± 0.01	0.12 ± 0.00	0.13 ± 0.02
30	0.14 ± 0.00	0.13 ± 0.01	0.12 ± 0.00	0.15 ± 0.01	0.12 ± 0.00	0.11 + 0.02
60	0.14 ± 0.00	0.12 ± 0.01	0.1 ± 0.00	0.14 ± 0.01	0.12 ± 0.00	0.11 ± 0.01
	Redispersibility (times, n=3)					
0	-	-	-	-	-	-
7	9.67 ± 1.15	8.67 ± 0.58	8.67 ± 0.58	10 ± 1.73	8.67 ± 1.15	9.67 ± 0.58
14	9.33 ± 0.58	8.67 ± 0.58	8.33 ± 0.58	9.33 ± 0.58	9 ± 1.73	10.7 ± 0.58
30	6.33 ± 0.58	8	8	7.67 ± 1.15	9 ± 1	11.67 ± 2.89
60	8.33 ± 0.58	8	8.33 ± 0.58	7.67 ± 0.58	8.67 ± 1.15	12 ± 1.73
	Viscosity (cps, n=3)					
0	107.98 ± 7.69	107.98 ± 7.69	107.98 ± 7.69	127.73 ± 6.15	127.73 ± 6.15	127.73 ± 6.15
7	118.07 ± 6.83	113.77 ± 6.49	112.27 ± 5.88	131.54 ± 1.28	131.78 ± 0.56	131.39 ± 1.72
14	108.42 ± 7.07	111.76 ± 6.11	111.98 ± 6.01	131.36 ± 1.75	131.96 ± 0.04	131.39 ± 1.72
30	108.42 ± 7.07	111.76 ± 6.11	111.98 ± 6.01	131.36 ± 1.75	131.96 ± 0.04	131.2 ± 2.23
60	104.58 ± 6.95	101.58 ± 6.90	96.82 ± 5.97	126.42 ± 6.54	121.13 ± 6.52	112.92 ± 6.88

PY= Pale Yellow, G= good, B=bad

Figure 3-28 Crystal shape of clonidine hydrochloride suspension Formulation 3 storage at room temperature (29.13 ± 0.25°C) and in the refrigerator (3.75 ± 0.5 °C) for 60 days (x20)



Crystal shape of clonidine hydrochloride Suspension Formulation 3 at room temperature



Crystal shape of clonidine hydrochloride suspension Formulation 3 in the refrigerator

Chemical stability

The concentration of clonidine hydrochloride was determined by using HPLC system after various storage times. Samples storage in refrigerator and 45°C were left to room temperature before analysis. Percent drug remaining was determined. Sample was carried out at 0, 7, 14, 30 and 60 days. Chemical stability of two formulations of clonidine hydrochloride suspensions are shown in Table 3-21, Figure 3-29 and Figure 3-30. Stability was defined as not less than 90% of initial concentration remaining in the preparation. The percent initial concentration remaining of clonidine hydrochloride in Formulation 3 after storage at refrigerator (3.75 ± 0.5 °C), room temperature (29.13 ± 0.25°C) and 45°C for 30 days was 96.00 ± 2.20%, 96.06 ± 2.45%, and 90.27 ± 3.85%, respectively and in Formulation 4 was 92.41 ± 3.66%, 90.58 ± 1.49% and 87.33 ± 1.62%, respectively. The results indicate that clonidine hydrochloride suspensions Formulation 3 and Formulation 4 were chemical stable for 30 days when storage in glass bottle protected from light at refrigerator (3.75 ± 0.5 °C) and room temperature (29.13 ± 0.25°C), respectively.

In this study, the chemical stability of clonidine hydrochloride syrups in two formulations was stable for 240, 60 and 30 days when storage at refrigerator (6.6 ± 1.82°C), room

temperature (29°C) and 45°C, respectively. Clonidine hydrochloride suspension Formulation 3 was stable for 30 days after storage at refrigerator (3.75 ± 0.5 °C), room temperature (29.13 ± 0.25°C) and 45°C, respectively and Formulation 4 was stable for 30, 30 and 14 days after storage at refrigerator (3.75 ± 0.5 °C), room temperature (29.13 ± 0.25°C) and 45°C, respectively. Clonidine hydrochloride syrups prepared from clonidine hydrochloride powder in two formulations were more stable than clonidine hydrochloride suspensions prepared from clonidine hydrochloride commercial tablets in two formulations. The reason may be due to an anionic sodium carboxymethylcellulose that only consist in suspension dosage form might cause product instability. This was due to the possible charge interaction between cationic drug and anionic polymer. However, there are many extemporaneous preparations that comprise of cationic drug and anionic sodium carboxymethylcellulose remains chemical stability more than 30 days. (Trissel *et al.*, 2006; Johnson *et al.*, 2005; VandenBussche *et al.*, 2002; Dentinger *et al.*, 2000; Alaxander *et al.*, 1997; Nahata *et al.*, 1993). Otherwise, excipients in tablets may affect to preparations instability. Similarly the previous study, fludrocortisone acetate oral solutions 40 µg/mL prepared from powder and tablets in ethanol 17%v/v were stable at least 60 days and 19 days, respectively, when storage at +4°C. Fludrocortisone acetate oral solutions 40 µg/mL prepared from powder more stable than those prepared tablets (Cisternino *et al.*, 2003). Sulfadiazine 200 mg/mL oral liquids prepared from powder and tablets in sterile water for irrigation were stable 3 days and 2 days storage at 4°C, respectively (Pathmanathan *et al.*, 2004). On the other hand, the study of stability of naltrexone oral liquid prepared from powder and tablets shown the rates of decomposition of formulations prepared from powder (3.9 ± 0.6 µg/mL/d) and unfiltered tablets (3.6 ± 0.3 µg/mL/d) at 70°C were not significantly different and were unaffected by the naltrexone concentration (Fawcett *et al.*, 1997). Furthermore, higher pH of the suspension preparation may affect product instability.

Table 3-21 Chemical stability of extemporaneous clonidine hydrochloride suspensions

Day	% Initial Concentration Remaining (Mean ± SD; N=3)					
	Formulation 3			Formulation 4		
	Storage temperature			Storage temperature		
	3.75 ± 0.5°C	29.13 ± 0.25°C	45 °C	3.75 ± 0.5°C	29.13 ± 0.25°C	45°C
0	100	100	100	100	100	100
7	103.40 ± 3.53	100.47 ± 2.89	103.40 ± 1.96	107.22 ± 1.76	105.78 ± 1.52	104.08 ± 2.53
14	95.52 ± 2.09	97.68 ± 2.5	96.48 ± 2.15	97.58 ± 4.98	95.35 ± 2.01	97.91 ± 3.77
30	96.00 ± 2.20	96.06 ± 2.45	90.27 ± 3.85	92.41 ± 3.66	90.58 ± 1.49	87.33 ± 1.62
60	80.99 ± 1.81	80.70 ± 2.75	78.36± 1.68	84.44 ± 3.66	80.52 ± 1.48	76.02 ± 1.63

Actual Initial Concentration of Formulation 3 was 0.181 ± 0.007 mg/ 5mL

Actual Initial Concentration of Formulation 4 was 0.173 ± 0.007 mg/ 5mL

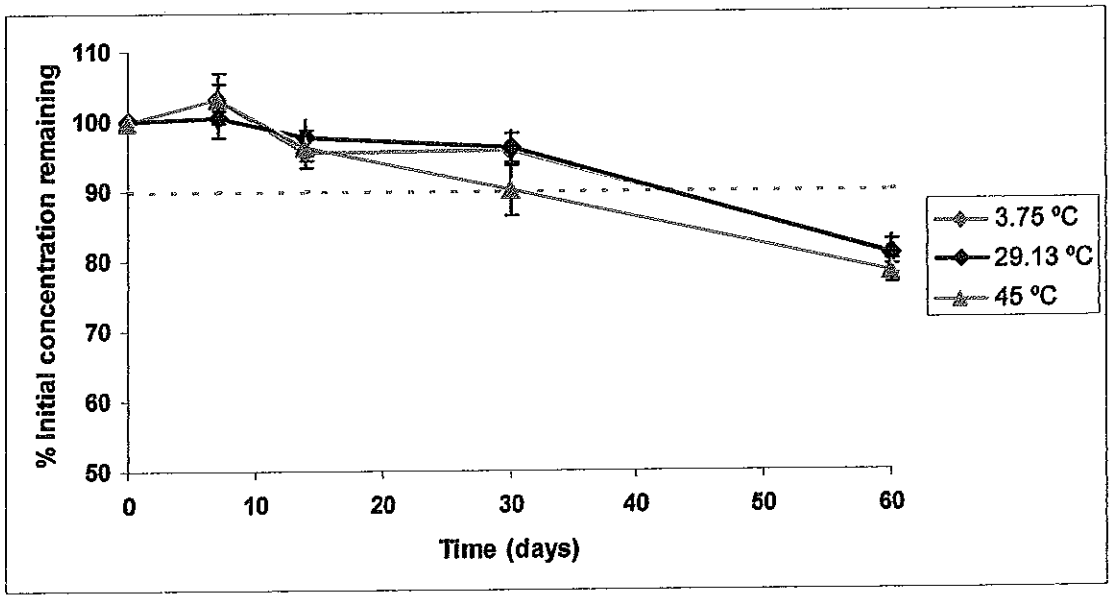


Figure 3-29 Percent initial concentration remaining of clonidine hydrochloride in Formulation 3 after storage in the refrigerator (3.75 ± 0.5 °C), at room temperature (29.13 ± 0.25°C) and 45°C

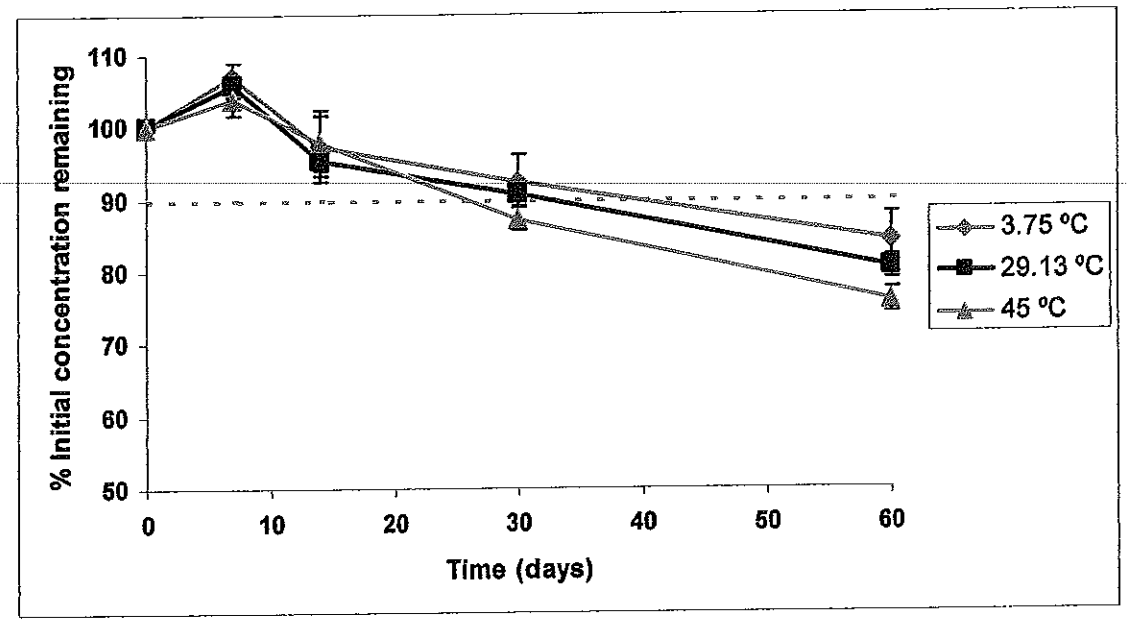


Figure 3-30 Percent initial concentration remaining of clonidine hydrochloride in Formulation 4 after storage in the refrigerator (3.75 ± 0.5 °C), at room temperature (29.13 ± 0.25°C) and 45°C

Microbial stability

The microbial contamination was determined by total microbial count, total yeasts and molds count and test for *Escherichia coli*. The result is presented in Table 3-22. Total microbial count of the samples in Formulation 3 and Formulation 4 storage at refrigerator (3.75±0.5°C) and 45°C for 30 days was not found but it was found 1 cfu/mL when storage at room temperature (29.13 ± 0.25°C). Yeasts and molds count in two Formulations when storage at refrigerator (3.75±0.5°C) was not found but they were found 10 cfu/mL after storage at room temperature (29.13 ± 0.25°C) for 30 days. Only Formulation 3, they were found 10 cfu/mL after storage at 45°C for 30 days. *Escherichia coli* were not found in any formulations during the study period. According to the USP 30 for nonsterile aqueous preparation for oral use, total aerobic microbial count is 100-200 cfu/mL. Total combined yeasts/molds count is 10- 20 cfu/mL. *Escherichia coli* is absence (1 gm or 1 mL). Microbial contamination was still within Pharmacopoeial specification. Therefore, these formulations were microbiological stable during the period of the study.

Table 3-22 Microbial contaminations of extemporaneous clonidine hydrochloride suspensions

Day	Dilution	Total microbial count (cfu/mL)					
		Formulation 3			Formulation 4		
		3.75 ± 0.5°C	29.13 ± 0.25°C	45 °C	3.75 ± 0.5°C	29.13 ± 0.25°C	45 °C
0	1	3	3	3	7	7	7
	1:10	NF	NF	NF	NF	NF	NF
	1:100	NF	NF	NF	NF	NF	NF
30	1	NF	1	NF	NF	1	NF
	1:10	NF	NF	NF	NF	NF	NF
	1:100	NF	NF	NF	NF	NF	NF
		Total yeasts and molds count (cfu/mL)					
0	1	9	9	9	3	3	3
	1:10	10	10	10	NF	NF	NF
	1:100	NF	NF	NF	NF	NF	NF
30	1	NF	4	3	NF	NF	NF
	1:10	NF	10	10	NF	10	NF
	1:100	NF	NF	NF	NF	NF	NF

NF=Not found

9. Determination of shelf life

Extemporaneous clonidine hydrochloride syrups Formulation 1 and Formulation 2 which were stable within 90 days were chosen for prediction of shelf life by accelerated studies at elevated temperature. Three sets of formulation were incubated at 45°C, 60°C and 70°C. The first samples that were taken at zero time were referred to as 100 percent of initial concentration. The percent remaining in later time were calculated as percent of initial concentration. Table 3-23 and Table 3-24, respectively show stability data of clonidine hydrochloride syrups Formulation 1 and Formulation 2, respectively after storage at 45°C, 60°C and 70°C. The pH of samples was decreased. For clonidine hydrochloride Formulation 1, the pH was decreased from 6.24 to 2.74 and 6.24 to 2.34 after storage at 60°C and 70°C for 60 days, respectively. This might be due to

unknown degradation product of clonidine hydrochloride or sucrose that only consists in Formulation 1. The acidic degradation product of sucrose on heating by Maillard reaction is 2-furoic acid (Yuan and Chen, 1999). Moreover, sucrose is hydrolyzed by diluted aqueous acid; it yields equal amounts of D-glucose and fructose. In aqueous solution, D-glucose is converted via D-glucose (open ring) into an equilibrium mixture. D-glucose (open ring) was oxidized by hydrogen ion in the solution to form dicarboxylic acid resulted in decrease pH of the solution. The Figure 3-31 was shown as follows: (Morrison and Boyd, 1987).

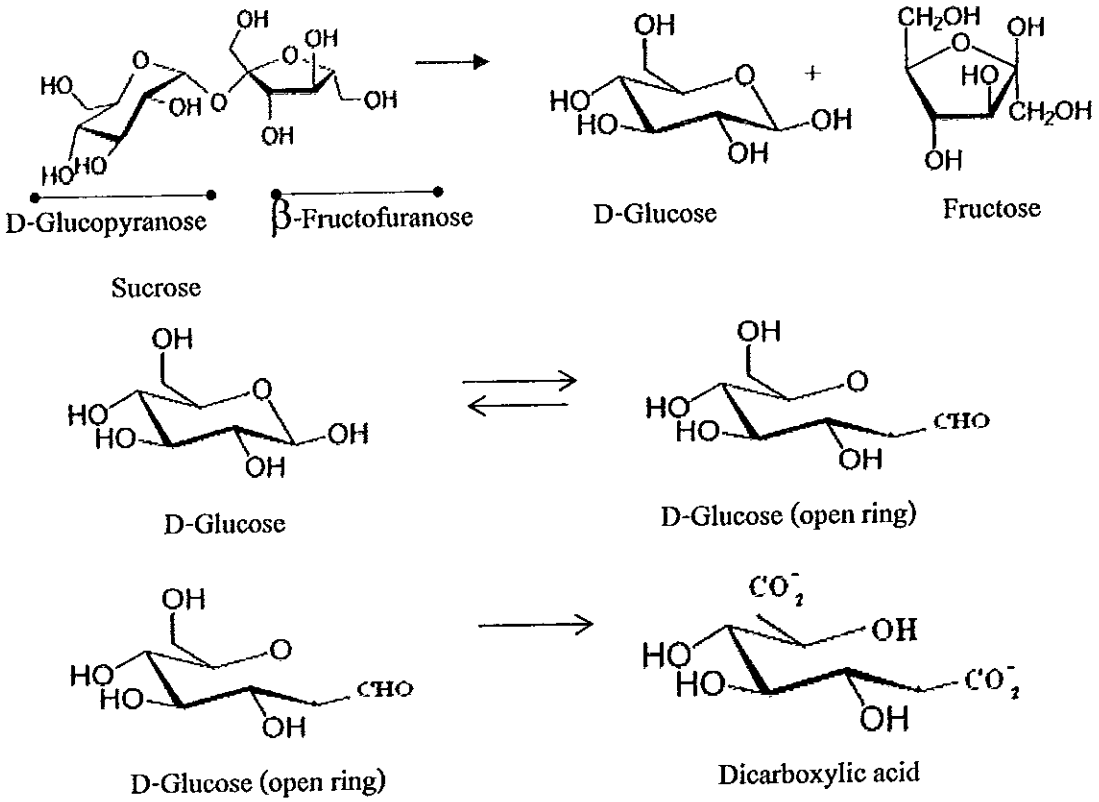


Figure 3-31 Hydrolysis and oxidation of sucrose

Furthermore, the color of clonidine hydrochloride syrup Formulation 1 was changed from yellow to brown and dark brown after storage at 70°C but the color of clonidine hydrochloride syrup Formulation 2 did not change in all storage conditions. Therefore, Syrup USP in clonidine hydrochloride syrup Formulation 1 caused changing in color (Formula in Table 2-2). Degradation products of sucrose on heating by Maillard reaction are 5-hydroxymethylfurfural, 2,5-dimethyl-4-hydroxy-3-(2H)-furanose, furfural, 2-furoic acid, 2-acthylfuran and furfuryl alcohol (Yuan and Chen, 1999). The colorless compounds from

degradation product of glucose were identified as 5-hydroxymethylfurfural and 2,5-dimethyl-4-hydroxy-3-(2H)-furanose (Ames, Balley and Mann, 1999) but furan group generated brown color (Hofman, Bors and stettmaier, 1999, Apriyantono *et al*, 2002).

Table 3-23 Stability data of extemporaneous clonidine hydrochloride syrup Formulation 1 storage at 45°C, 60°C and 70°C

Time (Day)	pH	Color	Flavor	% Initial concentration remaining			
				No. 1	No. 2	No. 3	Mean ± SD
45°C							
0	6.49 ± 0.04	Yellow	Good	100	100	100	100
14	6.62 ± 0.01	Yellow	Good	104.56	104.47	97.59	102.21 ± 3.52
30	6.84 ± 0.05	Yellow	Good	102.44	102.19	95.20	99.94 ± 3.64
60	6.31 ± 0.05	Yellow	Bad	87.66	89.53	84.02	87.07 ± 2.53
90	6.52 ± 0.09	Yellow	Bad	79.39	84.60	79.03	81.01 ± 2.81
180	5.92 ± 0.06	Yellow	Bad	71.89	69.96	67.91	69.92 ± 3.05
240	5.28 ± 0.09	Yellow	Bad	55.38	54.93	50.03	53.45 ± 2.65
60°C							
0	6.24 ± 0.11	Yellow	Good	100	100	100	100
14	5.7 ± 0.05	Yellow	Good	91.59	88.48	90.63	88.90 ± 3.36
28	4.0 ± 0.16	Yellow	Bad	82.18	75.97	80.96	79.70 ± 2.87
39	3.09 ± 0.04	Yellow	Bad	78.03	73.30	78.70	76.67 ± 2.62
49	2.85 ± 0.18	Yellow	Bad	76.41	69.46	76.56	74.14 ± 3.52
60	2.74 ± 0.08	Yellow	Bad	74.17	67.61	73.68	71.82 ± 3.17
70°C							
0	6.24 ± 0.11	Yellow	Good	100	100	100	100
14	3.48 ± 0.2	Yellow	Bad	83.48	77.82	84.38	81.89 ± 3.16
28	2.69 ± 0.09	Brown	Bad	74.69	70.41	76.31	73.81 ± 2.67
39	2.55 ± 0.06	Brown	Bad	71.34	67.39	71.55	70.09 ± 2.34
49	2.34 ± 0.04	DB	Bad	67.40	66.70	68.27	67.46 ± 1.95
60	2.34 ± 0.02	DB	Bad	61.15	58.24	61.83	60.41 ± 1.86

DB= dark brown

Table 3-24 Stability data of extemporaneous clonidine hydrochloride syrup Formulation 2 storage at 45°C, 60°C and 70°C

Time	pH	Color	Flavor	% Initial concentration remaining			
(Day)				No.1	No.2	No.3	Mean ± SD
45°C							
0	6.49 ± 0.04	Yellow	Good	100	100	100	100
14	6.62 ± 0.07	Yellow	Good	100.25	102.68	102.90	101.94 ± 1.84
30	6.81 ± 0.04	Yellow	Good	98.02	97.11	97.06	97.40 ± 0.83
60	6.39 ± 0.04	Yellow	Good	85.40	84.06	85.54	84.99 ± 0.94
90	6.56 ± 0.10	Yellow	Good	75.04	72.92	73.61	73.86 ± 0.98
180	6.15 ± 0.06	Yellow	Bad	68.84	62.70	64.95	65.50 ± 2.81
240	5.52 ± 0.06	Yellow	Bad	53.17	49.82	49.89	51.27 ± 1.78
60°C							
0	6.58 ± 0.05	Yellow	Good	100	100	100	100
14	5.76 ± 0.02	Yellow	Good	84.11	84.17	85.86	85.86 ± 2.78
28	5.14 ± 0.08	Yellow	Bad	67.03	66.31	67.91	67.42 ± 1.37
39	4.85 ± 0.04	Yellow	Bad	58.59	57.19	54.69	56.83 ± 1.99
49	4.82 ± 0.07	Yellow	Bad	51.05	49.01	52.03	50.80 ± 1.2.6
60	4.80 ± 0.09	Yellow	Bad	45.17	42.66	41.72	43.18 ± 1.82
70°C							
0	6.58 ± 0.05	Yellow	Good	100	100	100	100
14	4.89 ± 0.26	Yellow	Bad	72.22	69.75	70.01	70.01 ± 1.90
28	4.57 ± 0.07	Yellow	Bad	46.34	42.70	44.12	44.39 ± 1.67
39	4.56 ± 0.02	Yellow	Bad	38.12	34.99	32.92	35.35 ± 2.67
49	4.51 ± 0.05	Yellow	Bad	33.91	29.94	31.91	31.92 ± 1.88
60	4.43 ± 0.03	Yellow	Bad	30.51	25.75	26.01	27.42 ± 2.37

DB= dark brown

Determination of the order of reaction and the specific rate constant

The reaction kinetic is zero-order if a plot of percent initial concentration remaining vs. time is a straight line. The reaction kinetic is first-order when a plot of ln (percent initial concentration remaining) vs. time gives a straight line whereas the second-order is the result of the straight line of the plot of 1/ (percent initial concentration remaining) vs. time. The order of reaction was determined by plotting percent initial concentration remaining vs. time (Appendix-C: Figure C-1, C-4, C-7, C-10, C-13 and C-16), ln (percent initial concentration remaining) vs. time (Appendix-C: Figure C-2, C-5, C-8, C-11, C-14 and C-17) and 1/ (percent initial concentration remaining) vs. time (Appendix-C: Figure C-3, C-6, C-9, C-12, C-15 and C-18). Linear regression was performed for the correlation coefficients (r) at each temperature that were then comparing for the order of reaction at each temperature. The order of reaction of drug was determined by comparing the order of reaction of three temperatures. If the order of reaction at three temperatures is first order, the order of reaction is first order (จุไรรัตน์ รักวาทิน, 2538). Table 3-25 summarized stability data and correlation coefficient (r) of clonidine hydrochloride syrups Formulation 1 storage at 45°C, 60°C and 70°C. The highest correlation coefficient (r) values of the regression lines were 0.9694, 0.9645 and 0.9760 at 45°C, 60°C and 70°C, respectively. The result indicated that it was zero-order reaction at 45°C; second-order reaction at 60°C and 70°C. The most order reaction at three temperatures was second order reaction so the order of reaction was second order reaction. Thus, decomposition of clonidine hydrochloride syrup Formulation 1 is second-order reaction and this order reaction was used to determine the shelf life of the formulation. Table 3-26 shows stability data and correlation coefficient (r) of clonidine hydrochloride syrup Formulation 2 storage at 45°C, 60°C and 70°C. The highest correlation coefficient (r) values of the regression lines were 0.9684, 0.9964 and 0.9923 at 45°C, 60°C and 70°C, respectively. The result indicated that it was first-order reaction at 45°C and 60°C; second-order reaction at 70°C. The most order reaction at three temperatures was first order reaction so the order reaction of drug was first order reaction. Thus, decomposition is first-order reaction and this order reaction was used to determine the shelf life of clonidine hydrochloride syrup Formulation 2. From the result, these mean the order reaction may be different at difference temperature (จุไรรัตน์ รักวาทิน, 2538; Lachman, 1986).

The order of reaction of extemporaneous clonidine hydrochloride syrup Formulation 1 was second-order reaction. In contrast, the order of reaction of extemporaneous clonidine hydrochloride syrup Formulation 2 was first-order reaction. From this study, it means that the same drug in difference formulation may provide difference order of reaction. On the other hand, there are other factors that can affect to the order of reaction. For examples, the different in amount of each composition in the same formulation and substances that buy from difference manufacturer. Therefore, shelf life should be revised, if a formulation or a quantity of substances in the formulation or a producer is changed (จุไรรัตน์ รักวาทิน, 2538).

Table 3-25 The stability and correlation coefficient (r) of extemporaneous clonidine hydrochloride syrup Formulation 1 storage at 45°C, 60°C and 70°C

45°C			60°C			70°C		
Day	%conc*	ln%conc* 1/%conc*	Day	%conc*	ln%conc* 1/%conc*	Day	%conc*	ln%conc* 1/%conc*
0	100	4.6052 0.001	0	100	4.6052 0.01	0	100	4.6052 0.01
14	102.21	4.6270 0.00978	14	88.9	4.4875 0.01125	14	81.89	4.4054 0.01221
30	99.94	4.6046 0.01001	28	79.7	4.3783 0.01255	28	73.81	4.3015 0.01355
60	87.07	4.4667 0.01149	39	76.67	4.3395 0.01304	39	70.09	4.2498 0.01427
90	81.01	4.3946 0.01235	48	74.14	4.3060 0.01349	48	67.46	4.2115 0.01482
180	69.92	4.2474 0.01430	60	71.82	4.2742 0.01392	60	60.41	4.1012 0.01655
240	53.45	3.9788 0.01871						
Zero-order								
% conc* = -0.1984x+102.2,			% conc* = -0.4648x+96.513,			% conc* = -0.6041x+94.639,		
r^2 = 0.9694			r^2 = 0.9282			r^2 = 0.9266		
First-order								
ln%conc* = -0.0026x+4.4667,			ln%conc* = -0.0055x+4.572,			ln%conc* = -0.0077x+4.5561,		
r^2 = 0.9680			r^2 = 0.9497			r^2 = 0.9577		
Second-order								
/%conc* = -0.00003x+0.0093,			1/%conc* = -0.00007x+0.0103,			1/%conc* = -0.0001x+0.0104,		
r^2 = 0.9468			**r^2 = 0.9645**			**r^2 = 0.9760**		

*% initial concentration remaining, Conclusion: Decomposition is second-order reaction

Table 3-26 The correlation coefficient (r) of extemporaneous clonidine hydrochloride syrup Formulation 2 storage at 45°C, 60°C and 70°C

45°C			60°C			70°C		
Day	%conc*	ln%conc* 1/conc*	Day	%conc*	ln%conc* 1/conc*	Day	%conc*	ln%conc* 1/conc*
0	100	4.6052 0.01	0	100	4.6052 0.01	0	100	4.6052 0.01
14	101.94	4.6244 0.00981	14	85.86	4.4527 0.01165	14	70.01	4.2486 0.01428
30	97.40	4.5788 0.01027	28	67.42	4.2109 0.01483	28	44.39	3.7930 0.02253
60	84.99	4.4425 0.01177	39	56.83	4.0401 0.01759	39	35.35	3.5653 0.02829
90	73.86	4.3022 0.01354	48	50.8	3.9279 0.01969	48	31.92	3.4632 0.03133
180	65.50	4.1821 0.01527	60	43.18	3.7654 0.02316	60	27.42	3.3113 0.03647
240	51.27	3.9371 0.01950						
Zero-order:								
% conc* = -0.2093x+100.94			% conc* = -0.9763x+98.103			% conc* = -1.2036x+89.514		
$r^2 = 0.9524$			$r^2 = 0.9829$			$r^2 = 0.8987$		
First-order								
ln%conc* = -0.0028x+4.6271			ln%conc* = -0.0144x+4.6206			ln%conc* = -0.0222x+4.5306		
$r^2 = 0.9684$			**$r^2 = 0.9964$**			$r^2 = 0.9661$		
Second-order:								
1/%conc* = -0.00004x+0.0095			1/% conc* = -0.0002x+0.00914			1/%conc* = -0.0005x+0.0094		
$r^2 = 0.9641$			$r^2 = 0.9845$			**$r^2 = 0.9923$**		

*% initial concentration remaining, Conclusion: Decomposition is first-order

The specific rate constants (k) were determined from the slope values of second order reaction of extemporaneous clonidine hydrochloride syrup Formulation 1 and first order reaction of extemporaneous clonidine hydrochloride syrup Formulation 2 at three temperatures by statistic regression analysis. The standard error of slope was preformed by using regression statistic that was shown in Appendix-C (Table C-1, C-2, C-3, C-4, C-5 and C-6). Table 3-27 shows the specific rate constants (k) of extemporaneous clonidine hydrochloride syrup Formulation 1 storage at 45°C, 60°C and 70°C. These were 0.00003 $\pm$ 0.000004 day^{-1}, 0.00007 $\pm$ 0.000006 day^{-1} and 0.0001 $\pm$ 0.000008 day^{-1} for 45°C, 60°C and 70°C, respectively.

Table 3-28 shows the specific rate constants (k) of extemporaneous clonidine hydrochloride syrup Formulation 2. These were 0.0028 ± 0.000226 day^{-1}, 0.0144 ± 0.000435 day^{-1} and 0.0222 ± 0.002080 day^{-1} for 45°C, 60°C and 70°C, respectively.

Table 3-27 The specific rate constants (k) of 2° order reaction of extemporaneous clonidine hydrochloride syrup Formulation 1 storage at 45°C, 60°C and 70°C

°C	n	2° order reaction	$k \pm S_k$
45°C	7	1/%Conc*=-0.00003time + 0.0093, r^2=0.9468	0.00003 ± 0.000004
60°C	6	1/%Conc* =-0.00007time + 0.0103, r^2=0.9645	0.00007 ± 0.000006
70°C	6	1/%Conc* =-0.0001time + 0.0104, r^2=0.9760	0.0001 ± 0.000008

*% initial concentration remaining

Table 3-28 The specific rate constants (k) of 1° order reaction of extemporaneous clonidine hydrochloride syrup Formulation 2 storage at 45°C, 60°C and 70°C

°C	n	1° order reaction	$k \pm S_k$
45°C	7	ln %Conc*=-0.0028time + 4.6271, r^2=0.9684	0.0028 ± 0.000226
60°C	6	ln %Conc*=-0.0144time + 4.6206, r^2=0.9964	0.0144 ± 0.000435
70°C	6	ln %Conc*=-0.0222time + 4.5036, r^2=0.9661	0.0222 ± 0.002080

*% initial concentration remaining

Arrhenius relationship

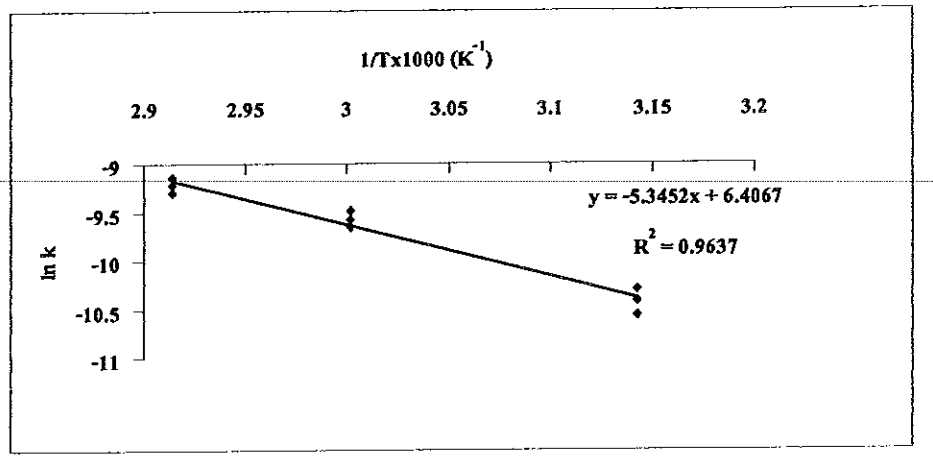
The specific rate constants of second-order reaction of extemporaneous clonidine hydrochloride Formulation 1 and first-order reaction of extemporaneous clonidine hydrochloride Formulation 2 at 45°C, 60°C and 70°C (Table 3-29) were plotted according to Arrhenius relationship. The Arrhenius plot of natural logarithm of specific rate constants (ln k) against the reciprocals of the degree Kelvin (1/T) for clonidine hydrochloride syrup Formulation 1 and clonidine hydrochloride syrup Formulation 2 exhibited linearity as shown in Figure 3-32 (r=0.9637) and Figure 3-33 (r=0.9528). Arrhenius Equation of clonidine hydrochloride syrup Formulation 1 and Formulation 2 were ln k = -5.3452(1/T)+6.4067 and

ln k = -9.283(1/T)+23.389, respectively. The standard error of estimated regression line ($S_{y/x}$) was preformed by using regression statistic that was shown in Appendix-C (Table C-7 and C-8). These were 0.110989 and 0.207343 for regression line of extemporaneous clonidine hydrochloride syrup Formulation 1 and Formulation 2, respectively.

Table 3-29 Arrhenius relation of clonidine hydrochloride degradation of extemporaneous clonidine hydrochloride syrup Formulation 1

Formulation	Temperature			Specific rate constant (mg $mL^{-1}day^{-1}$)	
	°C	T(Kelvin)	1/Tx1000K	k	ln k
1	45	318.15	3.143	0.00003	-10.4143
				0.000034	-10.2892
				0.000026	-10.5574
	60	333.15	3.002	0.00007	-9.56702
				0.000076	-9.48478
				0.000064	-9.65663
	70	343.15	2.914	0.0001	-9.21034
				0.000108	-9.13338
				0.000092	-9.29372
2	45	318.15	3.143	0.0028	-5.87814
				0.003026	-5.80051
				0.002574	-5.96229
	60	333.15	3.002	0.0144	-4.24053
				0.014835	-4.21077
				0.014174	-4.25635
	70	343.15	2.914	0.0222	-3.80766
				0.02428	-3.7181
				0.02012	-3.90604

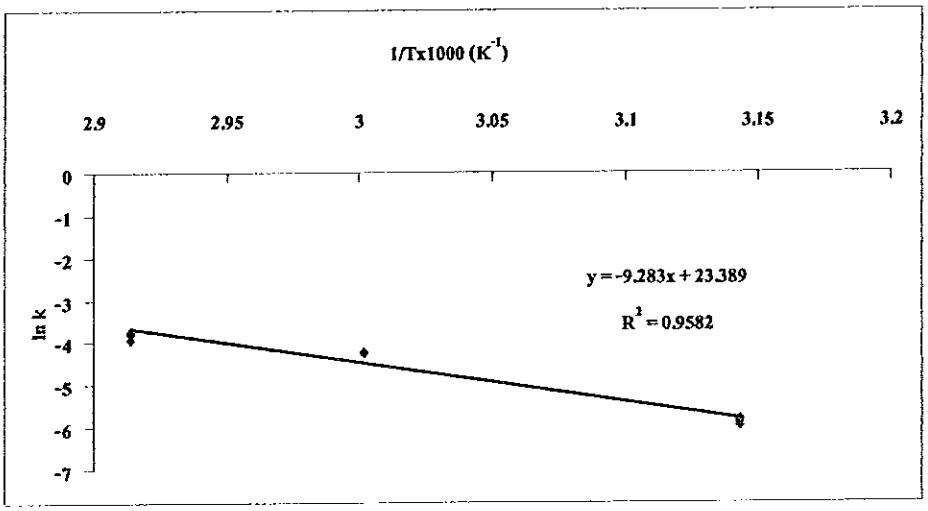
K = °C +273.15



Arrhenius Equation ln k = -5.3452(1/T) + 6.4067 $S_{y/x}$ = 0.110989

Heat of activation (kcal/mol) $Ea_{(95\%cl)}$ = 10.62 ± 0.79 Kcal/mole

Figure 3-32 Arrhenius plot of the natural logarithm of specific rate constant (k) vs. the reciprocal of the absolute temperature (degree Kelvin) (1/T) of clonidine hydrochloride in extemporaneous clonidine hydrochloride syrup Formulation 1



Arrhenius Equation ln k = -9.283(1/T) + 23.389 $S_{y/x}$ = 0.207343

Heat of activation (kcal/mol) $Ea_{(95\%cl)}$ = 18.45 ± 2.76 Kcal/mole

Figure 3-33 Arrhenius plot of the natural logarithm of specific rate constant (k) vs. the reciprocal of the absolute temperature (degree Kelvin) (1/T) of clonidine hydrochloride in extemporaneous clonidine hydrochloride syrup Formulation 2

The linearity of Arrhenius plot is obtained, so it could be used to predict the specific rate constant at lower temperature by extrapolation. Predicted specific rate constant from Arrhenius equation; ln k = -5.3452(1/T)+6.4067 is used for formulation 1 and ln k = -9.283(1/T) + 23.389 is used for Formulation 2. The predicted specific rate constant at 29°C of extemporaneous clonidine hydrochloride syrup Formulation 1 and extemporaneous clonidine hydrochloride syrup Formulation 2 were 0.000012575 $(mg/ml)(day^{-1})$ and 0.00065294556 $(mg/ml)(day^{-1})$, respectively. While, The predicted specific rate constant at 6°C of extemporaneous clonidine hydrochloride syrup Formulation 1 and extemporaneous clonidine hydrochloride syrup Formulation 2 were 0.000002927 $(mg/ml)(day^{-1})$ and 0.000051936387 $(mg/ml)(day^{-1})$, respectively. The calculation was shown in Appendix-C (Table C-9 and C-10).

Activation energy

The activation energy (Ea) of extemporaneous clonidine hydrochloride syrup Formulation 1 and extemporaneous clonidine hydrochloride syrup Formulation 2 was calculated from the slope of the Arrhenius plot (Ea = slope x 1.987 Kcal/mole). The activation energy of extemporaneous clonidine hydrochloride syrup Formulation 1 was 10.62 $\pm$ 0.79 Kcal/mole. While the activation energy of extemporaneous clonidine hydrochloride syrup Formulation 2 was 18.45 $\pm$ 2.76 Kcal/mole. The calculation was shown in Appendix-C (Table C-9 and C-10).

Activation energy is the energy that needed to initiate the reaction. It has been used in identification of reaction mechanisms. For instance, the degradation in solution had activation energy in the range of 10-30 Kcal/mole. Formulation was degraded through solvolytic process. This activation energy supported accelerated temperature studied and determined shelf life by Arrhenius equation since there was marked increase in reaction rates at elevated temperatures (จุไรรัตน์ รักวาทิน, 2538). In this study, extemporaneous clonidine hydrochloride syrup Formulation 1 and extemporaneous clonidine hydrochloride syrup Formulation 2 had activation energy of 10.62 $\pm$ 0.79 and 18.45 $\pm$ 2.76 Kcal/mole, respectively. As a result, the reaction rate obtained at elevated temperature could be used to estimate the reaction rate at any temperature for prediction of product stability.

Shelf life

Shelf life (t_{90}) is the time that the remaining concentration of clonidine hydrochloride in the preparation must not less than 90% of the initial concentration. In this study, the shelf life was calculated by using Arrhenius equation to predicted specific rate constant at room temperature. Then, this predicted specific rate constant was used to predict shelf life of the formulation. The degradation of clonidine hydrochloride in extemporaneous clonidine hydrochloride syrup Formulation 1 was assumed to be a second-order. Therefore, the shelf life of extemporaneous clonidine hydrochloride syrup Formulation 1 at 6°C and 29°C could be calculated from the second order reaction rate (equation 17). While, the shelf life of extemporaneous clonidine hydrochloride syrup Formulation 2 at 6°C and 29°C could be calculated from the first order reaction rate (equation 13). The predicted shelf life of extemporaneous clonidine hydrochloride syrup Formulation 1 and extemporaneous clonidine hydrochloride syrup Formulation 2 at 6°C and 29°C is presented in Table 3-30 and the calculation was shown in Appendix-C (Table C-9 and C-10). The predicted shelf life and lower 95% confident limit of extemporaneous clonidine hydrochloride syrup Formulation 1(sugar vehicle) at 6°C was about 379 and 248 days and 29°C was about 88 and 70 days, respectively. However, the actual shelf life at 6°C and at 29°C was 240 and 60 days, respectively. For clonidine hydrochloride syrup Formulation 2 (sugar-free vehicle), the predicted shelf life and lower 95% confident limit at 6 °C was about 2,021 and 915 days and at 29°C was about 160 and 105 days, respectively. However, the actual shelf life at 6°C and 29°C was 240 and 60 days, respectively. The predicted shelf life to lower 95% confident limit of extemporaneous clonidine hydrochloride syrup Formulation 1 at 6°C and 29°C was nearly the real time shelf life, but the predicted shelf life to lower 95% confident limit of extemporaneous clonidine hydrochloride syrup Formulation 2 at 6°C and 29°C was more than the real time shelf life. The possible reasons could be due to the limitation of using higher temperature rates of degradation for predicting room temperature stability of drug products. Higher temperature may evaporate solvents, thus producing unequal moisture concentrations and drug concentrations at different temperature. Degradation mechanisms at different temperature may predominate (Lachwan *et al.*, 1986). In addition, the order of reaction of clonidine hydrochloride syrups in Formulation 2 may not be treated as zero-

order, first-order or second-order. Clonidine hydrochloride in solution exists in amino and imino form in reversible structure (Figure 1-2) so it may degrade by complicated mechanism which involve more than one step or elementary reaction. Therefore, the study for predicted shelf life should be done parallel to the real time testing at storage condition (Lachwan *et al.*, 1986, Martin, 1993).

Table 3-30 The predicted shelf life (t_{90}) of extemporaneous clonidine hydrochloride syrup Formulation 1 and extemporaneous clonidine hydrochloride syrup Formulation 2 at 6°C and 29°C

Formulation	Temperature (°C)	Predicted shelf life		
		Days	Lower 95% confidence limit days	upper 95% confidence limit days
1	6.0	379.61	248.39	580.02
	29.0	88.35	70.44	110.83
2	6.0	2,021.70	915.58	4464.13
	29.0	160.81	105.31	245.56

10. Evaluation the palatability of the preparations

The double blind study was conducted in the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hatyai, Songkhla. Four preparations were determined; extemporaneous clonidine hydrochloride syrup Formulation 1, extemporaneous clonidine hydrochloride syrup Formulation 2, extemporaneous clonidine hydrochloride suspension Formulation 3 and extemporaneous clonidine hydrochloride suspension Formulation 4. The bottles of each preparation were labeled with the letter A, B, C and D, respectively. 28 healthy volunteers evaluated each preparations in a plastic medicine unit, and graded each on five-choice of satisfaction level with the statement, i.e. dislike (score = 1), dislike slightly (score = 2), Neither like or dislike (score= 3), like (score = 4), and like extremely (score = 5) for the categories of appearance, color, flavor, taste and overall.

The result is presented in Table D-1 (Appendix D). A total of 28 health volunteers were enrolled in the study. About forty-six (46.4%) were male and fifty-three (53.6%) were female. The mean age was 23.61 years (SD = 2.69) with an age range from 21 to 33 years. Table 3-31 shows mean score ± standard deviations and statistically significant difference for extemporaneous clonidine hydrochloride preparation. Over all mean score of extemporaneous clonidine hydrochloride syrup Formulation 1 and extemporaneous clonidine hydrochloride syrup Formulation 2 was about 4. It means healthy volunteers like both syrup preparations. Mean scores of extemporaneous clonidine hydrochloride syrup Formulation 1 and extemporaneous clonidine hydrochloride syrup Formulation 2 were not statistically significant difference in all categories. Over all mean score of extemporaneous clonidine hydrochloride suspension Formulation 3 and extemporaneous clonidine hydrochloride suspension Formulation 4 was about 3. This indicated that healthy volunteers neither like nor dislike both suspension preparations. Mean scores of extemporaneous clonidine hydrochloride syuspension Formulation 3 and extemporaneous clonidine hydrochloride suspension Formulation 4 were not statistically significant difference in appearance, color, flavor and over all but the taste was statistically significant difference (p-value < 0.05). Extemporaneous clonidine hydrochloride syrup Formulation 1 and extemporaneous clonidine hydrochloride syrup Formulation 2 had a higher mean score than clonidine hydrochloride suspension Formulation 1 and clonidine hydrochloride suspension Formulation 2 in all categories. Mean scores of extemporaneous clonidine hydrochloride syrups both formulations and extemporaneous clonidine hydrochloride suspensions both formulations were statistically significant difference in appearance, color, flavor and over all but the taste between extemporaneous clonidine hydrochloride syrups both formulations and extemporaneous clonidine hydrochloride suspension Formulation 3 was not statistically significant difference. In short, clonidine hydrochloride syrups prepared from clonidine hydrochloride powder were preferred over clonidine hydrochloride suspensions prepared from clonidine hydrochloride commercial tablets in all categories among healthy adult volunteers. The result is similarly the previous study, Lucas-Bouwman *et al.*, (2001) compared prednisolone oral solution (5 mg/mL), mixed with banana essence and sorbitol (prepared by the hospital pharmacy), and with prednisolone crushed tablets, mixed with lemonade or custard. They found that the oral solution received significantly better taste scores than the crushed tablets in children 3 months to 8 years old. These indicate that

the clear oral solution is more favorably than oral suspensions in adult or children and either the same or difference vehicle. The reason may result from a clear solution of syrups give a better appearance than suspensions. This result was supported by the mean score appearance of syrups that showed significantly difference compared with suspensions. Moreover, tablet excipients may affect flavor in the preparation which was shown in the mean score flavor of syrups significantly difference with suspensions. From the result of this study suggest that the vehicle for preparing oral solution or syrup from powder should not be applied to the preparation prepared from tablets. No adverse effects were reported in any of volunteers from investigation of the four preparations during the study.

Table 3-31 Mean score ± standard deviations and statistically significant differences for extemporaneous clonidine hydrochloride preparation*

Preparations	Appearance	Color	Flavor	Taste	Overall
A	3.82 ± 0.67	3.57 ± 0.84	4.11 ± 0.83	4.00 ± 1.02	3.93 ± 0.66
B	3.79 ± 0.69	3.57 ± 0.79	4.14 ± 0.85	3.71 ± 0.71	3.71 ± 0.54
C	2.82 ± 0.91	2.89 ± 0.96	3.43 ± 0.84	3.64 ± 1.02	3.25 ± 0.84
D	2.89 ± 0.83	2.79 ± 1.07	3.29 ± 1.07	3.07 ± 0.86	3.25 ± 0.80
p-value A*B	NS	NS	NS	NS	NS
p-value A*C	< 0.05	< 0.05	< 0.05	NS	< 0.05
p-value A*D	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
p-value B*C	< 0.05	< 0.05	< 0.05	NS	< 0.05
p-value B*D	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
p-value C*D	NS	NS	NS	< 0.05	NS

* n=28, A = extemporaneous clonidine hydrochloride syrup Formulation 1, B = extemporaneous clonidine hydrochloride syrup Formulation 2, C = extemporaneous clonidine hydrochloride suspension Formulation 3 and D = extemporaneous clonidine hydrochloride suspension Formulation 4

NS=No significant

CHAPTER 4

CONCLUSIONS

Extemporaneous clonidine hydrochloride syrups in the concentration of 0.15 mg/5 mL were prepared from clonidine hydrochloride powder in a sugar vehicle (Formulation 1) and a sugar-free vehicle (Formulation 2). The sugar vehicle comprised of Syrup USP, glycerin, sodium chloride, paraben concentrate, tartrazine, banana flavor and purified water. The sugar-free vehicle consisted of glycerin, sodium chloride, sorbitol solution 70%w/w, saccharin sodium, paraben concentrate, tartrazine, banana flavor and purified water. They were physically, chemically and microbiologically stable in the period of 240, 60 and 30 days after storage in glass bottle protected from light at refrigerator (6.6 $\pm$ 1.82°C), room temperature (29.33 $\pm$ 0.98°C) and 45°C, respectively.

Extemporaneous clonidine hydrochloride suspensions in the concentration of 0.15 mg/ 5 mL were prepared from clonidine hydrochloride commercial tablets in a sugar suspending vehicle (Formulation 3) and a sugar-free suspending vehicle (Formulation 4). The sugar suspending vehicle comprised of Syrup USP, glycerin, sodium chloride, sodium carboxymethylcellulose, paraben concentrate, tartrazine, banana flavor and purified water. The sugar-free suspending vehicle consisted of glycerin, sodium chloride, sorbitol solution 70%w/w, saccharin sodium, sodium carboxymethylcellulose, paraben concentrate, tartrazine, banana flavor and purified water. They were physically, chemically and microbiologically stable in the period of 30 days after storage in glass bottle protected from light at refrigerator (3.75 $\pm$ 0.5°C) and room temperature (29.13 $\pm$ 0.25°C). The physical and chemical stability of extemporaneous clonidine hydrochloride suspension Formulation 3 and extemporaneous clonidine hydrochloride suspension Formulation 4 were 30 and 14 days after storage at 45°C, respectively.

In accelerated stability study at 45°C, 60°C and 70°C, the kinetic decomposition of extemporaneous clonidine hydrochloride syrup Formulation 1 and extemporaneous clonidine

hydrochloride syrup Formulation 2 was a second order reaction and a first order reaction, respectively.

In healthy adult volunteers, extemporaneous clonidine hydrochloride syrups were more palatable than extemporaneous clonidine hydrochloride suspensions. These preparations were be an alternative to the administration of tablets for specific patients.

Suggestion for future study

Stability study for determine shelf life of clonidine hydrochloride syrups and suspensions containing buffering agent.

Stability study for determine shelf life of clonidine hydrochloride suspensions containing sodium carboxymethylcellulose compare with clonidine hydrochloride suspensions without sodium carboxymethylcellulose.

Controlled room temperature should be used in the study to reduce the variation of the study.

Clonidine hydrochloride tablets should be standardized before extraction clonidine hydrochloride from tablets.

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ภาคผนวก

ภาคผนวก ก

เอกสารรับรองจากคณะกรรมการพิจารณาจริยธรรมการวิจัยในคน

ที่ ศธ 0521.1.07/1467

คณะเภสัชศาสตร์
มหาวิทยาลัยสงขลานครินทร์
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หนังสือฉบับนี้ให้ไว้เพื่อรับรองว่า

โครงการวิจัยเรื่อง : การประเมินความพึงพอใจของยาเตรียมเฉพาะคราวยาน้ำเชื่อมและยาน้ำแขวนตะกอนโคลนิดีนไฮโดรคลอไรด์

คณะผู้ดำเนินการศึกษาวิจัย : 1. นางปราณี บ่อคำ นักศึกษาสาขาวิชาเภสัชศาสตร์
2. รศ.นัฏฐา แก้วนพรัตน์ อาจารย์ที่ปรึกษา

ได้ผ่านการพิจารณาและได้รับความเห็นชอบจากคณะกรรมการ Ethics Committee ซึ่งเป็นคณะกรรมการพิจารณาศึกษาวิจัยในคนของคณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ แล้ว

ให้ไว้ ณ วันที่ 29 กรกฎาคม 2552

..... ประธานกรรมการ
(ผู้ช่วยศาสตราจารย์ ดร.สิริรัศมิ์ ปิ่นสุวรรณ)
รักษาการในตำแหน่งรองคณบดีฝ่ายวิจัยและบริการวิชาการ ปฏิบัติราชการแทน
รักษาการในตำแหน่งคณบดีคณะเภสัชศาสตร์

ภาคผนวก ข

ใบเชิญชวน

ข้อมูลเชิญชวนเข้าร่วมโครงการวิจัย

ชื่อโครงการวิจัย : การประเมินความพึงพอใจของยาเตรียมเฉพาะตราวยาน้ำเชื่อมและยาน้ำแขวนตะกอนโคลนิดีนไฮโดรคลอไรด์ ในอาสาสมัครสุขภาพดี

คณะผู้วิจัยและสังกัด : นางปราณี บ่อคำ นักศึกษาปริญญาโทสาขาเภสัชศาสตร์
รองศาสตราจารย์นัฏฐา แก้วนพรัตน์ ภาควิชาเทคโนโลยีเภสัชกรรม
คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่
จังหวัดสงขลา

เรียน ท่านผู้อ่านที่นับถือ

ข้าพเจ้า นางปราณี บ่อคำ หัวหน้าโครงการวิจัย ขอเชิญชวนท่านเข้าร่วมในโครงการการประเมินความพึงพอใจของยาเตรียมเฉพาะตราวยาน้ำเชื่อมและยาน้ำแขวนตะกอนโคลนิดีนไฮโดรคลอไรด์ โดยมีรายละเอียดเกี่ยวกับโครงการวิจัยดังจะกล่าวต่อไป เพื่อเป็นข้อมูลประกอบในการตัดสินใจ กรุณาอ่านและทำความเข้าใจ หากไม่เข้าใจหรือมีข้อสงสัยประการใด กรุณาซักถาม และหากท่านสนใจเข้าร่วมโครงการ สามารถกรอกรายละเอียดในใบสมัครเข้าร่วมโครงการพร้อมลงลายมือชื่อ

ในการวิจัยครั้งนี้ทำการพัฒนาและเตรียมยาน้ำโคลนิดีนไฮโดรคลอไรด์ ที่เตรียมผงยาโคลนิดีนไฮโดรคลอไรด์ ยาน้ำโคลนิดีนไฮโดรคลอไรด์ ที่สกัดยาโคลนิดีนไฮโดรคลอไรด์จากยาเม็ดที่จำหน่ายในประเทศไทย และยาน้ำแขวนตะกอนโคลนิดีนไฮโดรคลอไรด์ จากยาเม็ดที่มีจำหน่ายในประเทศไทย โดยใช้น้ำกระสายยาทั้งที่มีส่วนผสมของน้ำตาล และปราศจากน้ำตาล และทำการศึกษาความคงตัวทั้งทางด้านกายภาพ ความคงตัวทางเคมี และความคงตัวทางจุลชีววิทยา รวมถึงคัดเลือกยาเตรียมที่มีความคงตัวมา ประเมินความพึงพอใจต่อผลิตภัณฑ์ เพื่อให้ได้ยาเตรียมที่มีประสิทธิภาพ มีข้อมูลความคงตัว รสชาติดี และมีความปลอดภัยต่อผู้ใช้ยา

Clonidine hydrochloride เป็นอนุพันธ์ imidazoleine มีฤทธิ์ทางเภสัชวิทยาที่หลากหลาย ใช้เป็นยาลดความดันโลหิตสูง อาการปวดจากเส้นประสาท attention deficit hyperactivity disorder (ADHD) ความผิดปกติในการนอน (Florey, 1992) ในบัญชียาหลักแห่งชาติ ปี 2551 ระบุ ให้ใช้สำหรับถอนพิษยาเฮโรอีน ขนาดยาในผู้ใหญ่ 10 ถึง 17 ไมโครกรัมต่อกิโลกรัมต่อวัน โดยแบ่งให้ อาการไม่พึงประสงค์ที่อาจพบจากการรับประทานยา คืออัตราการเต้นของหัวใจช้าลง(0.5%) ผู้ป่วยอาจเกิดอาการหน้ามืดจากการเปลี่ยนอิริยาบทอย่างรวดเร็ว (Orthostatic hypotension) (3%) ท้องผูก(10%) คลื่นไส้(5%) อาเจียน(5%) ปากแห้ง (40%) มึนงง(16%) สงบ (10%) และง่วงนอน(33%) แต่เนื่องจากปริมาณยาโคลนิดีนไฮโดรคลอไรด์ที่ใช้ในการศึกษา 0.06 มิลลิกรัมและอาสาสมัครต้องบ้วนยาทิ้งหลังจากอมไว้ในปาก 30 วินาทีและบ้วนปากซ้ำด้วยน้ำสะอาด จึงมีโอกาสน้อยที่จะเกิดอาการดังกล่าว

Clonidine hydrochloride เป็นยาที่มีประสิทธิภาพ ไม่เสพติดเมื่อใช้เป็นเวลานาน แต่ รูปแบบยาที่มีจำหน่ายในประเทศไทย มีเฉพาะรูปแบบยาเม็ด จึงไม่เหมาะกับผู้ป่วยบางประเภท เช่น ผู้ป่วยเด็ก ผู้ป่วยที่กลืนยายาก หรือมีปัญหาในการกลืน ผู้ป่วยที่คุ้นเคยต่อยาน้ำในการรักษา โดยเฉพาะผู้ป่วยที่เคยได้รับการถอนพิษยาเฮโรอีนด้วยยาน้ำเมธาโดน และผู้ป่วยที่ไม่ให้ความร่วมมือในการใช้ยาเม็ด ยาเตรียมเฉพาะคราวโคลนิดีนไฮโดรคลอไรด์ ในรูปแบบยาน้ำชนิดรับประทาน จึงเหมาะสมต่อผู้ป่วยเหล่านี้ เนื่องจากสามารถบริหารยาและการปรับขนาดยาได้ง่าย และเพิ่มความร่วมมือในการใช้ยาของผู้ป่วย

อาสาสมัครที่สมัครเข้าร่วมโครงการ จะต้องมีอายุ 20 – 35 ปี สุขภาพดี ไม่มีโรคประจำตัว ไม่มีประวัติแพ้ยาหรือสารเคมี ไม่รับประทานยาใดๆก่อนและระหว่างการศึกษา ไม่มีปัญหาการรับรส มีความดันโลหิตปกติ (120/80 –130/89 มม. ปรอท) อัตราการเต้นของหัวใจปกติ (70-90 ครั้งต่อนาที) มีความสนใจจะเข้าร่วมโครงการ ยินดีทำตามเงื่อนไขของการวิจัย และสามารถเข้าร่วมวิจัยในช่วงศึกษา

อาสาสมัครจะได้รับการวัดความดันโลหิต และอัตราการเต้นของหัวใจก่อนการศึกษา อาสาสมัครจะได้รับยาน้ำ ครั้งละ 2 มิลลิลิตร ในท่านั่ง ให้อมยาไว้ในปาก 30 วินาทีแล้วบ้วนทิ้งและบ้วนปากซ้ำด้วยน้ำสะอาดซึ่ง อาสาสมัครต้องประเมินยาน้ำทั้งหมด 4 ตำรับ โดยมีระยะห่างแต่ละ ตำรับ 30 นาที มีการเตรียมยานพาหนะให้พร้อมเพื่อนำส่งอาสาสมัครไปโรงพยาบาลกรณีที่เกิดเหตุฉุกเฉิน กรณีอาสาสมัครมีอาการผิดปกติ ให้หยุดการชิมยาทันที และแจ้ง

ให้ผู้วิจัยทราบเพื่อ ติดตามอาการของอาสาสมัคร หากอาการไม่ดีขึ้นหลังจากหยุดยา อาสาสมัครจะถูกนำไปพบแพทย์ซึ่งผู้วิจัยเป็นผู้รับผิดชอบค่าใช้จ่ายในการรักษาทั้งหมด

เมื่อเสร็จสิ้นการศึกษา ท่านก็จะได้รับค่าตอบแทน 100 บาท ข้อมูลที่ได้ถูกนำมารวบรวมสรุปและนำเสนอผลการศึกษาโดยไม่มีการจะไม่ระบุถึงชื่อหรือข้อมูลส่วนตัวของอาสาสมัครที่เข้าร่วมวิจัย

อาสาสมัครที่มีข้อสงสัยเกี่ยวกับการศึกษาวิจัย มีสิทธิ์ซักถามหัวหน้าโครงการวิจัยและคณะวิจัยได้ในระหว่างการศึกษาหรือสอบถามทางโทรศัพท์หมายเลข 08-6967-3435

หากการกระทำของคณะผู้วิจัยผิดพลาดจากข้อตกลง ท่านมีสิทธิ์แจ้งต่อคณบดีคณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ โทรศัพท์หมายเลย 0-7421-2824 หรือประธานคณะกรรมการจริยธรรมการวิจัย คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ โทรศัพท์หมายเลข 0-7421-3057 กรณีท่านเปลี่ยนใจไม่ต้องการที่จะเข้าร่วมในโครงการต่อไป ท่านมีสิทธิ์ที่จะถอนตัวจากการเข้าร่วมในโครงการ โดยแจ้งให้ผู้วิจัยทราบ ในการถอนตัวนี้จะไม่มีผลกระทบใดๆ ยกเว้นท่านจะไม่ได้รับค่าตอบแทน

ขอแสดงความนับถือ

ลงชื่อ.....หัวหน้าโครงการวิจัย

(นางปราณี บ่อคำ)

...../...../.....

ภาคผนวก ค

แบบฟอร์มใบสมัครเข้าร่วมโครงการ

ชื่อโครงการวิจัย: การประเมินความพึงพอใจของยาเตรียมเฉพาะคราวยาน้ำเชื่อมและยาน้ำแขวนตะกอนโคลนิดีนไฮโดรคลอไรด์ ในอาสาสมัครสุขภาพดี

วันที่ลงชื่อสมัครใจ.....

ข้าพเจ้า (ชื่อ/สกุล ตัวบรรจง).....
ได้อ่านเอกสารใบเชิญชวนข้างต้น และได้รับคำอธิบายด้วยวาจาจนข้าพเจ้าทราบถึงวัตถุประสงค์ของโครงการ วิธีการศึกษา ประโยชน์จากการเข้าร่วมโครงการ ความรับผิดชอบของผู้วิจัยที่มีต่อข้าพเจ้า สิทธิของข้าพเจ้าในฐานะเป็นอาสาสมัคร รวมถึงในกรณีที่ข้าพเจ้าเกิดผลข้างเคียงจากการชิมยา ผู้วิจัยจะเป็นผู้รับผิดชอบค่าใช้จ่ายทั้งหมด ทั้งนี้ ข้าพเจ้าได้มีโอกาสซักถามผู้วิจัย/ผู้ให้ข้อมูลจนเป็นที่พอใจและเข้าใจดีแล้วโดยมีพยานรับรู้

ข้าพเจ้าได้แจ้งผู้วิจัยแล้วว่า ข้าพเจ้าไม่มีโรคประจำตัวและไม่ได้ใช้ยาใดๆ ไม่มีประวัติแพ้ยาหรือสารเคมีใด ไม่มีปัญหาการรับรส ข้าพเจ้าเข้าใจดีว่าเมื่อข้าพเจ้าสมัครเข้าร่วมโครงการ ข้าพเจ้าจะต้องอมยาน้ำ จำนวน 4 ตำรับ และประเมินรสชาติ ในท่านั่งโดยปฏิบัติ 4 ครั้ง แต่ละครั้งเว้นระยะห่างกันประมาณ 30 นาที

ข้าพเจ้ามีความประสงค์สมัครเข้าร่วมโครงการวิจัย ยินดีปฏิบัติตามเงื่อนไขของการวิจัย และสามารถเข้าร่วมวิจัยในช่วงเดือน กรกฎาคม 2552 ถึง กันยายน 2552

ทั้งนี้ผู้วิจัย/ผู้ให้ข้อมูล ได้ให้ใบเชิญชวนและสำเนาใบสมัครนี้ไว้กับข้าพเจ้าอย่างละ 1 ฉบับ

ลงชื่อ.....ผู้สมัครใจ

(.....)

...../...../.....

เบอร์โทรศัพท์ที่สามารถติดต่อได้.....

ลงชื่อ.....หัวหน้าโครงการวิจัย ลงชื่อ.....พยาน

(นางปราณี บ่อคำ) (.....)

...../...../..... ...../...../.....

ภาคผนวก ง

แบบประเมินความพึงพอใจผลิตภัณฑ์ .....

เพศ ☐ ชาย ☐ หญิง อายุ.....

โปรดใส่เครื่องหมายถูก (/) ลงในช่องที่ตรงความจริงมากที่สุด

1 = ไม่พึงพอใจ 2 = พึงพอใจน้อย 3 = ปานกลาง

4 = พึงพอใจ 5 = พึงพอใจมาก

ประเด็นประเมิน	คะแนนความพึงพอใจ				
	1	2	3	4	5
ความชอบต่อลักษณะภายนอก					
ความชอบต่อสี					
ความชอบต่อกลิ่น					
ความชอบต่อรส					
ความชอบโดยรวม					
ข้อเสนอแนะ สี กลิ่น รส อื่นๆ	....				

APPENDIX

APPENDIX-A

Details of substances in preparations

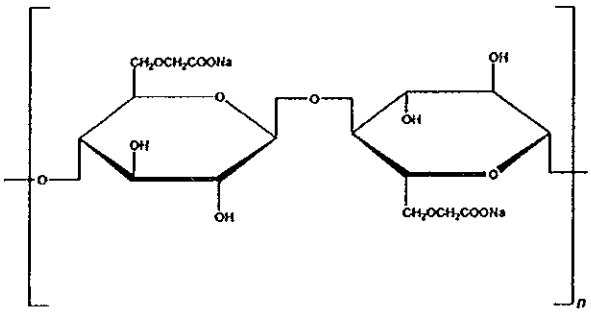
Carboxymethylcellulose sodium

Synonyms: cellulose gum; CMC sodium; E466; SCMC; sodium carboxymethylcellulose; sodium cellulose glycolate; sodium CMC

Chemical name: Cellulose, carboxymethyl ether, sodium salt

Molecular weight: 90,000-700,000

Structural Formula:



Description: Carboxymethylcellulose sodium occurs as a white to almost white, odorless, granular powder.

Typical Properties:

Density (bulk): 0.52 g/cm^3

Density (tapped): 0.78 g/cm^3

Dissociation constant pKa : 4.30

Melting point: browns at approximately 227°C, and chars at approximately 252°C.

Solubility: practically insoluble in acetone, ethanol, ether, and toluene. It easily dispersed in water at all temperatures, forming clear, colloidal solutions. The aqueous solubility varies with the degree of substitution.

Viscosity: Aqueous 1% w/v solutions with viscosities of 5–13 000 mPa s (5–13 000 cP) may be obtained. An increase in concentration results in an increase in aqueous solution viscosity.

Prolonged heating at high temperatures will depolymerize the gum and permanently decrease the viscosity. The viscosity of sodium carboxymethylcellulose solutions is fairly stable over a pH range of 4–10. The optimum pH range is neutral.

Safty : LD_{50}(guinea pig, oral): 16 g/kg , LD_{50}(rat, oral): 27 g/kg

Functional category: Coating agent; tablet and capsule disintegrant; tablet binder; stabilizing agent; suspending agent; viscosity-increasing agent; water-absorbing agent.

Applications in Pharmaceutical Formulation or technology: Carboxymethylcellulose sodium is widely used in oral and topical pharmaceutical formulations, primarily for its viscosity-increasing properties. Viscous aqueous solutions are used to suspend powders intended for either topical application or oral and parenteral administration. Carboxymethylcellulose sodium may also be used as a tablet binder and disintegrant, and to stabilize emulsions.

Incompatibilities: Carboxymethylcellulose sodium is incompatible with strongly acidic solutions and with the soluble salts of iron and some other metals, such as aluminum, mercury, and zinc. Precipitation may occur at pH <2, and also when it is mixed with ethanol (95%). Carboxymethylcellulose sodium forms complex coacervates with gelatin and pectin. It also forms a complex with collagen and is capable of precipitating certain positively charged proteins.

Stability and Storage condition: Aqueous solutions are stable at pH 2–10; precipitation can occur below pH 2, and solution viscosity decreases rapidly above pH 10. Generally, solutions exhibit maximum viscosity and stability at pH 7–9. Aqueous solutions may similarly be sterilized by heating, although this also results in some reduction in viscosity. After autoclaving, viscosity is reduced by about 25%, but this reduction is less marked than for solutions prepared from material sterilized in the dry state. The bulk material should be stored in a well-closed container in a cool, dry place.

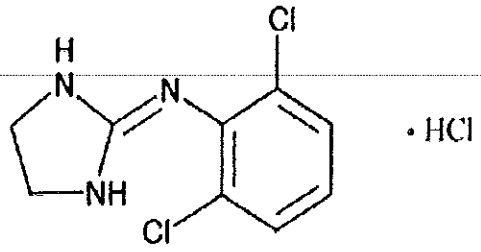
Clonidine hydrochloride

Chemical name: N-(2,6-dichlorophenyl)-4,5-dihydro-1H-imidazol-2-amine

Empirical formular: $C_9H_9Cl_2N_3$ HCL

Molecular weight: 266.55 g/mol

Structural Formula:



Description: A white or almost white crystalline powder which has a bitter taste.

Typical Properties:

Acidity/alkalinity: pH = 4.0–5.0 for a 5% solution in water(Ph. Eur 5)

Density (obs) : 1.543 g/cm^3

Melting point: 305 °C, 300 °C with decomposition

Solubility: Soluble in 13 parts of water : 1 g soluble in 6 ml H_2O (60 °C)

About 13 ml H_2O (20 °C)

: Soluble in absolute alcohol : about 5.8 ml CH_3OH

: slightly soluble in choroform about 5000 ml $CHCL_3$

: and practically insoluble in ether.

Polymorphism: no

pka : The drug has a pKa of 8.2

Log P : 1.59

Safety : LD_{50}(mouse, oral): 328 mg/kg LD_{50}(mouse, IV): 18 mg/kg ,

LD_{50} (rat, oral) : 270 mg/kg LD_{50}(rat, IV): 29 mg/kg,

LD_{50} (rabbit, oral) : 80 mg/kg LD_{50}(rabbit, IV): 45 mg/kg,

LD50 (dog, oral) : 30-100 mg/kg LD50(dog, IV): 6 mg/kg,

LD50(monkey, oral): 150-267 mg/kg

Absorption: 95 %

Storage condition: The drug should be kept in well-closed container and protect from sun light.

Glycerin

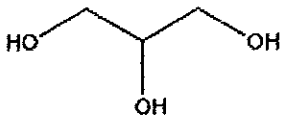
Synonyms : Croderol; E422; glycerine; Glycon G-100; *Kemstrene*; Optim; Pricerine; 1,2,3-propanetriol; trihydroxypropane glycerol.

Chemical name: Propane-1,2,3-triol

Empirical formula: $C_3H_8O_3$

Molecular weight: 92.09

Structural Formula:



Description: Glycerin is a clear, colorless, odorless, viscous, hygroscopic liquid; it has a sweet taste, approximately 0.6 times as sweet as sucrose.

Typical Properties:

Density: 1.2656 g/cm^3 at 15°C, 1.2636 g/cm^3 at 20°C, 1.2620 g/cm^3 at 25°C

Melting point: 17.8 °C

Solubility : Soluble in water, ethanol 95% and methanol. Slightly soluble in acetone. Practically insoluble in choroform and benzene.

Safety: LD_{50}(guinea pig, oral): 7.75 g/kg, LD_{50}(mouse, oral): 4.1 g/kg

LD_{50}(rat, oral): 12.6 g/kg,

Functional category : Antimicrobial preservative; emollient; humectant; plasticizer; solvent; sweetening agent; tonicity agent.

Applications in Pharmaceutical Formulation or technology: Glycerin is used in a wide variety of pharmaceutical formulations including oral, otic, ophthalmic, topical, and parenteral preparations. In oral solutions, glycerin is used as a solvent, sweetening agent, antimicrobial preservative, and viscosity-increasing agent. It is also used as a plasticizer and in film coatings

Incompatibilities: Glycerin may explode if mixed with strong oxidizing agents such as chromium trioxide, potassium chlorate, or potassium permanganate. In dilute solution, the reaction proceeds at a slower rate with several oxidation products being formed. Black discoloration of glycerin

occurs in the presence of light, or on contact with zinc oxide or basic bismuth nitrate. An iron contaminant in glycerin is responsible for the darkening in color of mixtures containing phenols, salicylates, and tannin. Glycerin forms a boric acid complex, glyceroboric acid that is a stronger acid than boric acid.

Stability and storage condition: Glycerin is hygroscopic. Pure glycerin is not prone to oxidation by the atmosphere under ordinary storage conditions but it decomposes on heating, with the evolution of toxic acrolein. Mixtures of glycerin with water, ethanol, and propylene glycol are chemically stable. Glycerin may crystallize if stored at low temperatures; the crystals do not melt until warmed to 20°C. Glycerin should be stored in an airtight container, in a cool, dry place.

Methylparaben

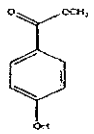
Synonyms: E218; 4-hydroxybenzoic acid methyl ester; methyl *p*-hydroxybenzoate; *Nipagin M*; Uniphen P-23.

Chemical name: Methyl-4-hydroxybenzoate

Empirical formula: $C_8H_8O_3$

Molecular weight: 152.15

Structural Formula:



Description: Methylparaben occurs as colorless crystals or a white crystalline powder. It is odorless or almost odorless and has a slight burning taste.

Typical Properties: Methylparaben exhibits antimicrobial activity of pH 4–8. Preservative efficacy decreases with increasing pH owing to the formation of the phenolate anion. Parabens are more active against yeasts and molds than against bacteria. They are also more active against Gram-positive bacteria than against Gram-negative bacteria. Methylparaben is the least active of the parabens; antimicrobial activity increases with increasing chain length of the alkyl moiety. Activity may be improved by using combinations of parabens as synergistic effects occur. Therefore, combinations of methyl-, ethyl-, propyl-, and butylparaben are often used together.

Activity has also been reported to be enhanced by the addition of other excipients such as: propylene glycol (2–5%); phenylethyl alcohol; and edetic acid. Activity may also be enhanced owing to synergistic effects by using combinations of parabens with other antimicrobial preservatives such as imidurea. The hydrolysis product *p*-hydroxybenzoic acid has practically no antimicrobial activity.

Density (true): 1.352 g/cm^3

Dissociation constant: pK_a = 8.4 at 22°C

Melting point: 125-128 °C

Solubility : Soluble 1 in 400 of water, 1 in 50 of water at 50°C, 1 in 30 of water at 30°C and 1 in 5 of propylene glycol, 1 in 60 of glycerin, 1 in 2 of ethanol and 1 in 6 of ethanol 50 %

Safety: Methylparaben and other parabens are widely used as antimicrobial preservatives in cosmetics and oral and topical pharmaceutical formulations. Although parabens have also been used as preservatives in injections and ophthalmic preparations, they are now generally regarded as being unsuitable for these types of formulations owing to the irritant potential of the parabens. These experiences may depend on immune responses to enzymatically formed metabolites of the parabens in the skin. Parabens are nonmutagenic, nonteratogenic, and noncarcinogenic. Sensitization to the parabens is rare, and these compounds do not exhibit significant levels of photocontact sensitization or phototoxicity. Hypersensitivity reactions to parabens, generally of the delaved type and appearing as contact dermatitis. have been reported. However. given the widespread use of parabens as preservatives, such reactions are relatively uncommon; the classification of parabens in some sources as high-rate sensitizers may be overstated. Immediate hypersensitivity reactions following injection of preparations containing parabens have also been reported. Delayed-contact dermatitis occurs more frequently when parabens are used topically, but has also been reported to occur after oral administration. Unexpectedly, preparations containing parabens may be used by patients who have reacted previously with contact dermatitis provided they are applied to another, unaffected, site. This has been termed the paraben paradox. Concern has been expressed over the use of methylparaben in infant parenteral products because bilirubin binding may be affected, which is potentially hazardous in hyperbilirubinemic neonates. The WHO has set an estimated total acceptable daily intake for methyl-, ethyl-, and propylparabens at up to 10 mg/kg body-weight.

LD_{50} (mouse, IP): 0.96 g/kg , LD_{50}(dog, oral): 3 g/kg

LD_{50} (mouse, Sc): 1.20 g/kg

Functional category: Antimicrobial preservative.

Applications in Pharmaceutical Formulation or technology: Methylparaben is widely used as an antimicrobial preservative in cosmetics, food products, and pharmaceutical formulations. It may be used either alone or in combination with other parabens or with other antimicrobial agents. In cosmetics, methylparaben is the most frequently used antimicrobial preservative. The parabens are effective over a wide pH range and have a broad spectrum of antimicrobial activity, although they are most effective against yeasts and molds. Antimicrobial activity increases as the chain length of the alkyl moiety is increased, but aqueous solubility decreases; therefore a mixture of parabens is frequently used to provide effective preservation. Preservative efficacy is also improved by the addition of propylene glycol (2–5%), or by using parabens in combination with other antimicrobial agents such as imidurea. Owing to the poor solubility of the parabens, paraben salts (particularly the sodium salt) are more frequently used in formulations. However, this raises the pH of poorly buffered formulations. Methylparaben (0.18%) together with propylparaben (0.02%) has been used for the preservation of various parenteral pharmaceutical formulations.

Incompatibilities: The antimicrobial activity of methylparaben and other parabens is considerably reduced in the presence of nonionic surfactants, such as polysorbate 80, as a result of micellization. However, propylene glycol (10%) has been shown to potentiate the antimicrobial activity of the parabens in the presence of nonionic surfactants and prevents the interaction between methylparaben and polysorbate 80. Incompatibilities with other substances, such as bentonite, magnesium trisilicate, talc, tragacanth, sodium alginate, essential oils, sorbitol, and atropine, have been reported. It also reacts with various sugars and related sugar alcohols. Absorption of methylparaben by plastics has also been reported; the amount absorbed is dependent upon the type of plastic and the vehicle. It has been claimed that low-density and high-density polyethylene bottles do not absorb methylparaben. Methylparaben is discolored in the presence of iron and is subject to hydrolysis by weak alkalis and strong acids.

Storage condition: Aqueous solutions of methylparaben at pH 3–6 may be sterilized by autoclaving at 120°C for 20 minutes, without decomposition. Aqueous solutions at pH 3–6 are

stable (less than 10% decomposition) for up to about 4 years at room temperature, while aqueous solutions at pH 8 or above are subject to rapid hydrolysis (10% or more after about 60 days storage at room temperature. Methylparaben should be stored in a well-closed container in a cool, dry place.

Propylparaben

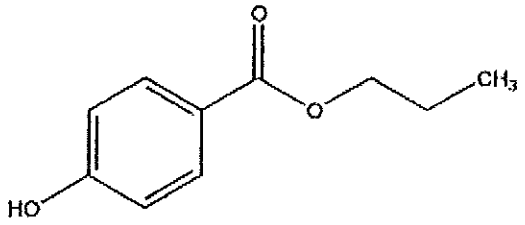
Synonyms: E216; 4-hydroxybenzoic acid propyl ester; Nipasol M; propagin; propyl *p*-hydroxybenzoate; Propylparasept, Solbrol P; uniphen P-23

Chemical name: Propyl 4-hydroxybenzoate

Empirical formula: $C_{10}H_{12}O_3$

Molecular weight: 182.20

Structural Formula:



Description: Propylparaben occurs as a white, crystalline, odorless, and tasteless powder.

Typical Properties: Propylparaben exhibits antimicrobial activity between pH 4–8. Preservative efficacy decreases with increasing pH owing to the formation of the phenolate anion. Parabens are more active against yeasts and molds than against bacteria. They are also more active against Gram-positive than against Gram-negative bacteria. The activity of the parabens increases with increasing chain length of the alkyl moiety; however, solubility decreases. Activity may be improved by using combinations of parabens, as additive effects occur. Propylparaben has been used with methylparaben in parenteral preparations, and is used in combination with other parabens in topical and oral formulations. Activity has also been reported to be improved by the addition of other excipients

Density (bulk): 0.426 g/cm^3

Density (tapped): 0.706 g/cm^3

Density (true): 1.288 g/cm3

Boiling point: 295 °C

Solubility : Soluble 1 in 2500 of water, 1 in 3.9 of propylene glycol, 1 in 1.1 of alcohol; and 1 in 250 of glycerin.

Safety: Propylparaben and other parabens are widely used as antimicrobial preservatives in cosmetics, food products, oral and topical pharmaceutical formulations. Propylparaben and methylparaben have been used as preservatives in injections and ophthalmic preparations; however they are now generally regarded as being unsuitable for these types of formulations owing to the irritant potential of the parabens. Systemically, no adverse reactions to parabens have been reported, although they have been associated with hypersensitivity reactions. The WHO has set an estimated acceptable total daily intake for methyl, ethyl, and propyl parabens at up to 10 mg/kg body-weight.

LD_{50}(mouse, IP): 0.2 g/kg , LD_{50}(mouse, oral): 6.33 g/kg

LD_{50} (mouse, Sc) : 1.65 g/kg

Functional category: Antimicrobial preservative.

Applications in Pharmaceutical Formulation or technology: Propylparaben is widely used as an antimicrobial preservative in cosmetics, food products, and pharmaceutical formulations. It may be used alone, in combination with other paraben esters, or with other antimicrobial agents. It is one of the most frequently used preservatives in cosmetics. The parabens are effective over a wide pH range and have a broad spectrum of antimicrobial activity, although they are most effective against yeasts and molds. Propylparaben (0.02% w/v) together with methylparaben (0.18% w/v) has been used for the preservation of various parenteral pharmaceutical formulations.

Incompatibilities: The antimicrobial activity of propylparaben is reduced considerably in the presence of nonionic surfactants as a result of micellization. Absorption of propylparaben by plastics has been reported, with the amount absorbed dependent upon the type of plastic and the vehicle. Magnesium aluminum silicate, magnesium trisilicate, yellow iron oxide, and ultramarine blue have also been reported to absorb propylparaben, thereby reducing preservative efficacyPropylparaben is discolored in the presence of iron and is subject to hydrolysis by weak alkalis and strong acids.

Storage condition: Aqueous propylparaben solutions at pH 3–6 can be sterilized by autoclaving, without decomposition. At pH 3–6, aqueous solutions are stable (less than 10% decomposition) for up to about 4 years at room temperature, while solutions at pH 8 or above are subject to rapid hydrolysis (10% or more after about 60 days at room temperature. Propylparaben should be stored in a well-closed container in a cool, dry place.

Saccharin sodium

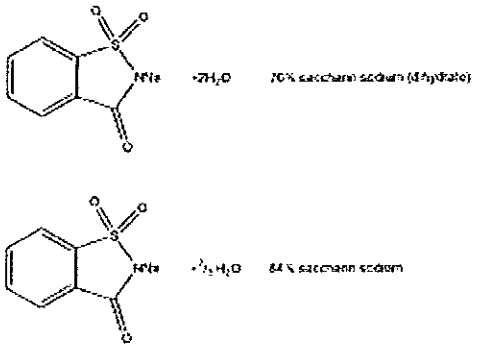
Synonyms: 1,2-Benzisothiazolin-3-one 1,1-dioxide, sodium salt; *Crystallose*; E954; sodium *o*-benzosulfimide; soluble gluside; soluble saccharin; *Sucaryl Sodium*.

Chemical name: 1,2-Benzisothiazol-3(2 *H*)-one 1,1-dioxide, sodium salt

Empirical formula: $C_7H_4NNaO_3S$, $C_7H_4NNaO_3S \cdot 2/3H_2O$ (84%), $C_7H_4NNaO_3S \cdot 2H_2O$ (76%)

Molecular weight: 205.16, 217.24, 241.19

Structural Formula:



Description: Saccharin sodium occurs as a white, odorless or faintly aromatic, efflorescent, crystalline powder. It has an intensely sweet taste, with a metallic aftertaste that at normal levels of use can be detected by approximately 25% of the population. Saccharin sodium can contain variable amounts of water.

Typical Properties:

Acidity/alkalinity: pH = 6.6 (10% w/v aqueous solution)

Density (bulk) : 0.8–1.1 g/cm^3 (76% saccharin sodium), 0.86 g/cm^3 (84% saccharin sodium)

Density (Particle) : 1.70 g/cm^3 (84% saccharin sodium)

Density (tapped) : 0.9–1.2 g/cm^3 (76% saccharin sodium), 0.96 g/cm^3 (84% saccharin sodium)

Melting point: decomposes upon heating.

Solubility : Soluble 1 in 1.2 of water, Soluble 1 in 50 of ethanol 95% ,Soluble 1 in 3.50 of propylene glycol.

Safety: The WHO has set a temporary acceptable daily intake of up to 2.5 mg/kg body-weight for saccharin, including its salts. In the UK, the Committee on Toxicity of Chemicals in Food, Consumer Products, and the Environment (COT) has set an acceptable daily intake for saccharin and its salts (expressed as saccharin sodium) at up to 5 mg/kg body-weight

LD_{50} (mouse, oral): 17.5 g/kg LD_{50} (rat, IP): 7.1 g/kg

LD_{50} (rat, oral): 14.2 g/kg

Functional category: Sweetening agent.

Applications in Pharmaceutical Formulation or technology : Saccharin sodium is an intense sweetening agent used in beverages, food products, table-top sweeteners, and pharmaceutical formulations such as tablets, powders, medicated confectionery, gels, suspensions, liquids, and mouthwashes. It was used 0.04-0.25% in oral syup and 0.075-0.6% in oral solution. It is also used in vitamin preparations. Its sweetening power is approximately 300 times that of sucrose. Saccharin sodium enhances flavor systems and may be used to mask some unpleasant taste characteristics.

Incompatibilities: -

Stability and storage condition: saccharin sodium is stable under the normal range of conditions employed in formulations. Only when it is exposed to a high temperature (125°C) at a low pH (pH 2) for over 1 hour does significant decomposition occur. The 84% grade is the most stable form of saccharin sodium since the 76% form will dry further under ambient conditions. Saccharin sodium should be stored in a well-closed container in a cool, dry place.

Sodium chloride

Synonyms **:** chlorure de sodium; common salt; dendritis; hopper salt; murinate of sada; natural halite; rock salt; saline; salt; sea salt; table salt.

Chemical name: Sodium chloride

Empirical formula: NaCl Molecular weight 58.44

Structural Formula: NaCl

Description: Sodium chloride occurs as a white crystalline powder or colorless crystals; it has a saline taste. The crystal lattice is a face-centered cubic structure. Solid sodium chloride contains no water of crystallization although, below 0 °C, salt may crystallize as a dihydrate.

Typical Properties:

Acidity/alkalinity: pH = 6.7–7.3 (saturated aqueous solution)

Density: 2.17 g/cm^3, 1.20 g/cm^3 for saturated aqueous solution

Density (bulk): 0.93 g/cm^3,

Density (tapped): 1.09 g/cm^3,

Dielectric constant: 5.9 at 1 MH

Boiling point: 1413 °C

Solubility : Soluble 1 in 2.8 of water, Soluble 1 in 2.6 of water at 100°C Soluble 1 in 250 of ethanol 95% ,slightly soluble in ethanol, Soluble 1 in 10 in glycerin.

Safety: Sodium chloride is the most important salt in the body for maintaining the osmotic tension of blood and tissues. About 5–12 g of sodium chloride is consumed daily, in the normal adult diet, and a corresponding amount is excreted in the urine. As an excipient, sodium chloride may be regarded as an essentially nontoxic and nonirritant material. However, toxic effects following the oral ingestion of 0.5–1.0 g/kg body-weight in adults may occur. The oral ingestion of larger quantities of sodium chloride, e.g., 1000 g in 600 mL of water, is harmful and can induce irritation of the gastrointestinal tract, vomiting, hypernatremia, respiratory distress, convulsions, or death

LD_{50}(mouse, IP): 6.61 g/kg, LD_{50}(mouse, IV): 0.65 g/kg,

LD_{50}(mouse, oral): 4 g/kg, LD_{50} (rat, oral) : 3 g/kg

LD_{50} (mouse, Sc) : 3.0 g/kg

Functional category: Tablet and capsule diluent; tonicity agent.

Applications in Pharmaceutical Formulation or technology : Sodium chloride is widely used in a variety of parenteral and nonparenteral pharmaceutical formulations, where the primary use is to produce isotonic solutions. Sodium chloride has been used as a lubricant and diluent in capsules and direct-compression tablet formulations in the past, although this practice is no longer common. Sodium chloride has also been used as a channeling agent and as an osmotic agent in the cores of controlled-release tablets. It has been used as a porosity modifier in tablet coatings, and to control drug release from microcapsules. The addition of sodium chloride to aqueous spray-coating solutions containing hydroxypropyl cellulose or hypromellose suppresses the agglomeration of crystalline cellulose particles. Sodium chloride can also be used to modify drug release from gels and from multiple emulsions. It can be used to control micelle size, and to adjust the viscosity of polymer dispersions by altering the ionic character of a formulation.

Incompatibilities: Aqueous sodium chloride solutions are corrosive to iron. They also react to form precipitates with silver, lead, and mercury salts. Strong oxidizing agents liberate chlorine from acidified solutions of sodium chloride. The solubility of the antimicrobial preservative methylparaben is decreased in aqueous sodium chloride solutions and the viscosity of carbomer gels and solutions of hydroxyethyl cellulose or hydroxypropyl cellulose is reduced by the addition of sodium chloride.

Stability and Storage condition: Aqueous sodium chloride solutions are stable but may cause the separation of glass particles from certain types of glass containers. Aqueous solutions may be sterilized by autoclaving or filtration. The solid material is stable and should be stored in a well-closed container, in a cool, dry place. It has been shown that the compaction characteristics and the mechanical properties of tablets are influenced by the relative humidity of the storage conditions under which sodium chloride was stored.

Sorbitol

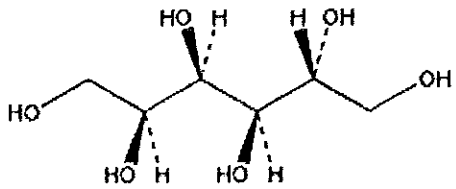
Synonyms **:** 1,2,3,4,5,6-hexanehexol; Lipo; Lipomic 76-NC; Meritol; Neosorb; Sorbifin; sorbite; D-sorbital; sorbitol instant; sorbogem

Chemical name: D-Glucitol

Empirical formula: $C_6H_{14}O_6$

Molecular weight: 182.17

Structural Formula:



Description: Sorbitol is D-glucitol. It is a hexahydric alcohol related to mannose and is isomeric with mannitol. Sorbitol occurs as an odorless, white or almost colorless, crystalline, hygroscopic powder.

Typical Properties:

Acidity/alkalinity: pH = 4.5–7.0 for a 10% w/v aqueous solution

Density: 1.49 g/cm^3

Density (bulk): 0.448 g/cm^3

Density (tapped): 0.400 g/cm^3

Density (true): 1.507 g/cm3

Melting point: Anhydrous form: 110–112 °C, Gamma polymorph: 97.7 °C,

Metastable form: 93 °C

Solubility: Soluble 1 in 0.5 of water and 1 in 25 of alcohol; practically insoluble in choroform and ether

Sorbital solution 70 % w/v : a clear, colorless and odorless, viscous liquid, density at 25 ° C 1.293 g/cm ^{3}, viscosity at 25 ° C 110 mPas

Safety: LD_{50} (mouse, IV): 9.48 g/kg , LD_{50}(mouse, oral): 17.8 g/kg

LD_{50} (rat, IV): 7.1 g/kg, LD_{50} (rat, Sc) : 29.6 g/kg

Functional category: Humectant; plasticizer; sweetening agent; tablet and capsule diluent.

Applications in Pharmaceutical Formulation or technology: Sorbitol is widely used as an excipient in pharmaceutical formulations. In liquid preparations, sorbitol is used as a vehicle in sugar-free formulations and as a stabilizer for drug.

Incompatibilities: Sorbitol will form water-soluble chelates with many divalent and trivalent metal ions in strongly acidic and alkaline conditions. Addition of liquid polyethylene glycols to sorbitol solution, with vigorous agitation, produces a waxy, water-soluble gel with a melting point of 35–40°C. Sorbitol solutions also react with iron oxide to become discolored. Sorbitol increases the degradation rate of penicillins in neutral and aqueous solutions.

Storage condition: airtight container in a cool, dry place.

Sucrose

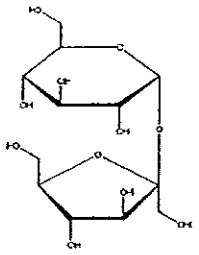
Synonyms **:** Beet sugar; cane sugar; α-D-glucopyranosyl-β-D-fructofuranoside; refined sugar; saccharose; sugar.

Chemical name: β-D-fructofuranosyl-α-D-glucopyranoside

Empirical formula: $C_{12}H_{22}O_{11}$

Molecular weight: 342.30

Structural Formula:



Description**:** Sucrose occurs as colorless crystals, as crystalline masses or blocks, or as a white crystalline powder; it is odorless and has a sweet taste.

Typical Properties:

Acidity/alkalinity: pH = 4.5–7.0 for a 10% w/v aqueous solution

Density: 1.49 g/cm^3

Density (bulk): 0.93 g/cm^3 (crystalline sucrose), 0.60 g/cm^3 (powdered sucrose)

Density (tapped): 1.03 g/cm^3 (crystalline sucrose), 0.82 g/cm^3 (powdered sucrose)

Density (true): 1.6 g/cm^3

Melting point: 160–186°C (with decomposition)

Solubility : Soluble 1 in 0.5 of water and 1 in 400 of ethanol; 1 in 170 of ethanol(95%) practically insoluble in chloroform.

Safety: LD_{50}(mouse, IP): 14 g/kg , LD_{50}(rat, oral): 29.7 g/kg

Functional category: Base for medicated confectionery; granulating agent; sugar coating adjunct; suspending agent; sweetening agent; tablet and capsule diluent; viscosity-increasing agent

Applications in Pharmaceutical Formulation or technology: Sucrose is widely used in oral pharmaceutical formulations. Sucrose syrup, containing 50–67% w/w sucrose, is used in tableting as a binding agent for wet granulation. In the powdered form, sucrose serves as a dry binder (2–20% w/w) or as a bulking agent and sweetener in chewable tablets and lozenges. Tablets that contain large amounts of sucrose may harden to give poor disintegration. Sucrose syrups are used as tablet-coating agents at concentrations between 50% and 67% w/w. With higher concentrations, partial inversion of sucrose occurs, which makes sugar coating difficult. Sucrose syrups are also widely used as vehicles in oral liquid-dosage forms to enhance palatability or to increase viscosity. Because sucrose is nontoxic, biodegradable, and has good emulsifying properties, esters of sucrose have been used increasingly in cosmetic formulations.

Incompatibilities: Powdered sucrose may be contaminated with traces of heavy metals, which can lead to incompatibility with active ingredients, e.g., ascorbic acid. Sucrose may also be contaminated with sulfite from the refining process. With high sulfite content, color changes can occur in sugar-coated tablets; for certain colors used in sugar-coating the maximum limit for sulfite content, calculated as sulfur, is 1 ppm. In the presence of dilute or concentrated acids, sucrose is hydrolyzed or inverted to dextrose and fructose (invert sugar). Sucrose may attack aluminum closures.

Stability and Storage condition: Sucrose has good stability at room temperature and at moderate relative humidity. It absorbs up to 1% moisture, which is released upon heating at 90°C. Sucrose caramelizes when heated to temperatures above 160°C. Dilute sucrose solutions are liable to fermentation by microorganisms but resist decomposition at higher concentrations, e.g., above

60% w/w concentration. Aqueous solutions may be sterilized by autoclaving or filtration. The bulk material should be stored in a well-closed container in a cool, dry place.

Tartrazine

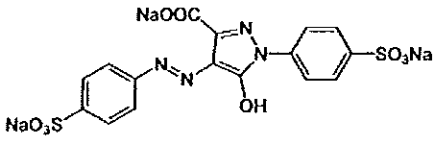
Synonyms : FD&C Yellow 5

Chemical name: Trisodium-5-hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-H-pyrazole-3-carboxylate

Empirical formula: $C_{16}H_9N_4Na_3O_9S_2$

Molecular weight: 534.3 gm/mol

Structural Formula:



Description: Yellow or orange-yellow powder. Aqueous solutions are yellow colored.

Typical Properties:

Solubility : Soluble in water (1 in 5 at 25°C), propylene glycol 50% (1 in 5), glycerin (1 in 5.6), ethanol 75% (1 in 91) and practically insoluble in acetone.

Safety: It can cause the most allergic and intolerance reactions of all the azo dyes, particularly among asthmatics and those with an aspirin intolerance. Symptoms from tartrazine sensitivity can occur by either ingestion or cutaneous exposure to a substance containing tartrazine. A variety of immunologic responses have been attributed to tartrazine ingestion, including anxiety, migraines, clinical depression, blurred vision, itching, general weakness, heatwaves, feeling of suffocation, purple skin patches, and sleep disturbance. In children, it may be increase in irritability, restlessness, and sleep disturbance after ingesting tartrazine. LD50 (mouse, oral): 12.75 gm/kg.

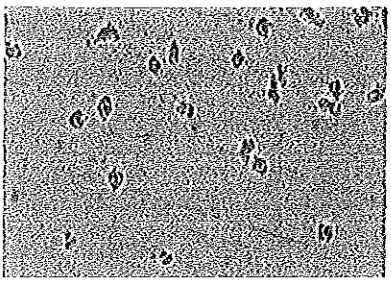
Functional category: It is used as coloring agent in food and medical products.

Incompatibilities: poorly compatible with citric acid solution, Incompatible with ascorbic acid, lactose, 10% glucose solution, and saturated aqueous sodium bicarbonate solution. Gelatin accelerates the fading of the color.

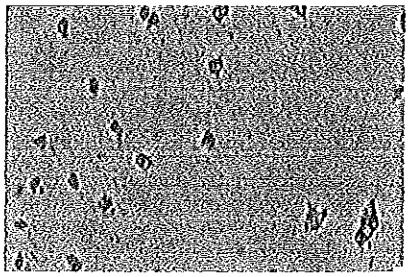
APPENDIX-B

Study of crystals in preparations

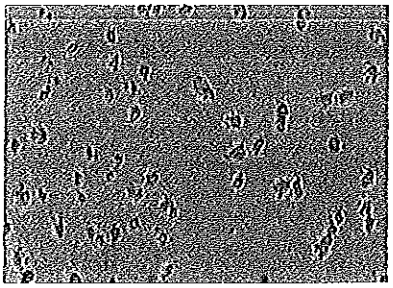
Figure B-1 Crystals of 0.03 mL of banana flavor, 0.3 mL of paraben concentrate, 1.5 mL of glycerin, 0.02 gm of saccharin sodium, 0.15 gm of sodium chloride, 8 mL of sorbitol solution, 14 mL of Syrup USP and 0.015 mL of 1% tartrazine solution in purified water 30 mL (x20)



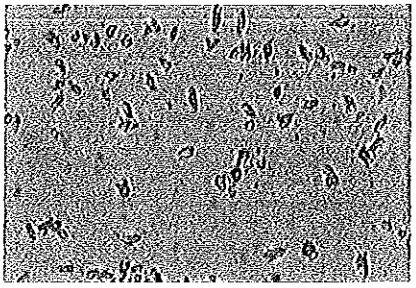
Banana flavor



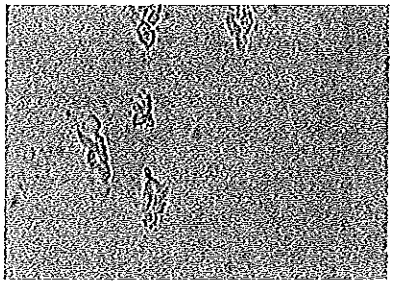
Paraben concentrate



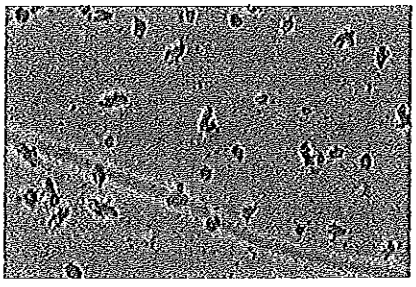
Glycerin



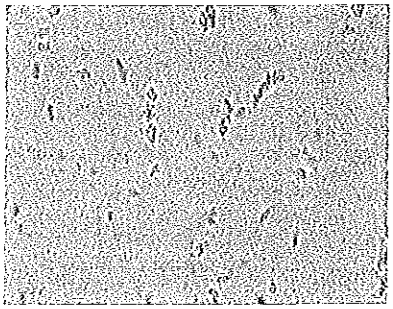
Saccharin sodium



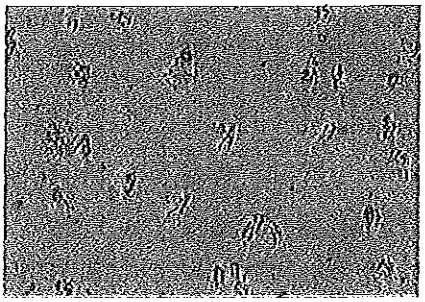
Sodium chloride



Sorbitol

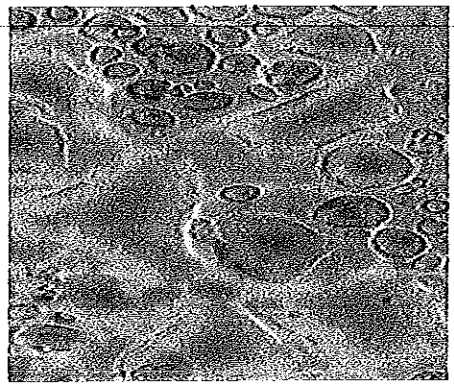


Syrup USP



Tartrazine

Figure B-2 Crystals of sodium carboxymethyl cellulose 0.21 gm in purified water 30 mL (x20)



Sucrose crystals were counted by hemocytometer which consists of a thick glass plate and a grooved calibrated grid on the surface (Figure B-3). A sucrose solution is put onto the grid by touching the end of the tip containing the sucrose solution at the edge of a cover slip placed on the upper surface of the hemocytometer. The sucrose crystals are then counted in a standard volume (usually 5 x 0.1 μL) as defined by the area of the grid (Figure B-4). A hand-held tally counter helps in counting. The result was shown in Table B-1.

Figure B-3 Hemocytometer

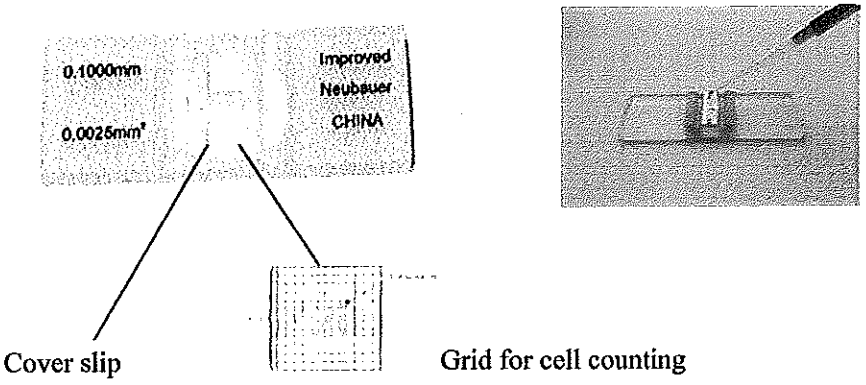
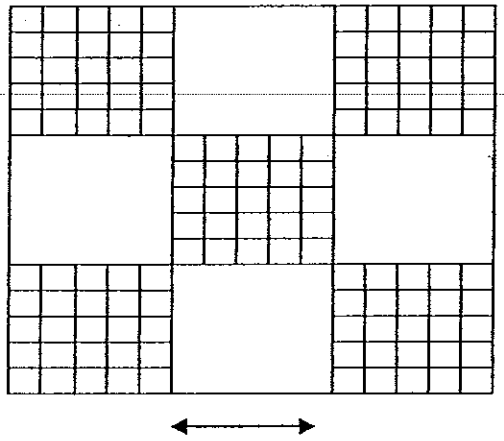


Figure B-4 Cell counting by hemocytometer



5 large grid each 0.1 μL

1 mm^2 square grid 0.1 mm depth

Table B-1 The amount of sucrose crystals, from Syrup USP and Syrup USP that was used in the preparation storage at room temperature (29°C) and in the refrigerator (2-8°C) for 7 days

	The amount of sucrose crystals (crystal/0.5μl)		
	Freshly prepared	Storage at room temperature	Storage at the refrigerator
Syrup USP	18	18	19
Syrup USP that was used in the preparation	5	5	6

APPENDIX-C

Stability study of clonidine hydrochloride

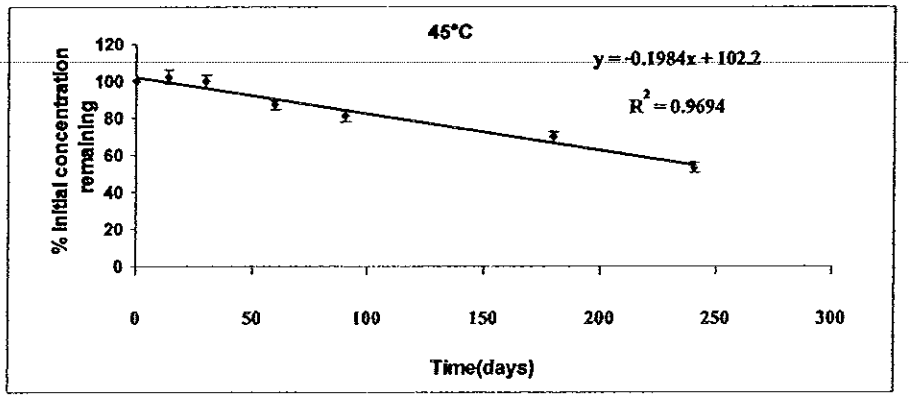


Figure C-1 Linear plot of percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 45°C

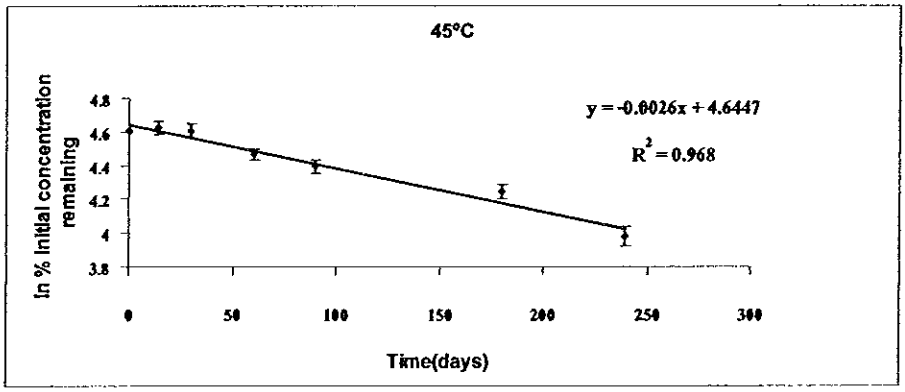


Figure C-2 Linear plot of ln percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 45°C

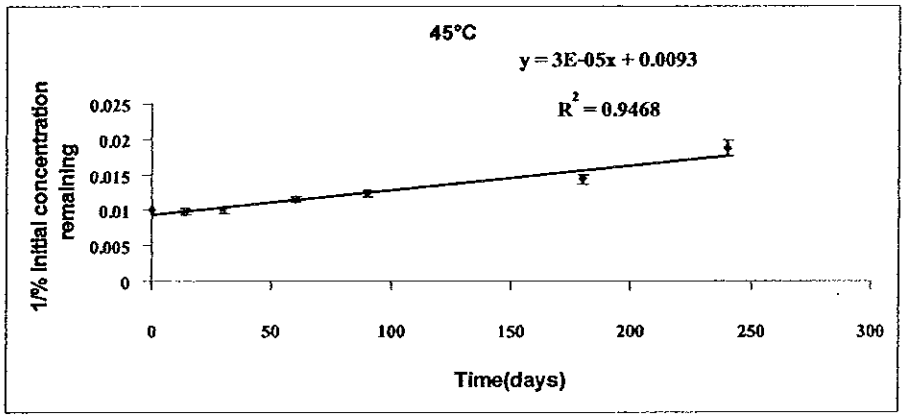


Figure C-3 Linear plot of 1/percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 45°C

Table C-1 Regression statistics of 1/ percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 45°C

Regression Statistics	
Multiple R	0.973057214
R Square	0.946840341
Adjusted R Square	0.936208409
Standard Error	0.000815781
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	5.9267E-05	5.9E-05	89.0563	0.0002256
Residual	5	3.3275E-06	6.7E-07		
Total	6	6.2594E-05			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.00932329	0.00044687	20.8635	4.7E-06	0.0081746	0.01047	0.008175	0.010472
X Variable 1	3.47998E-05	3.6876E-06	9.43696	0.00023	2.532E-05	4.4E-05	2.53E-05	4.43E-05

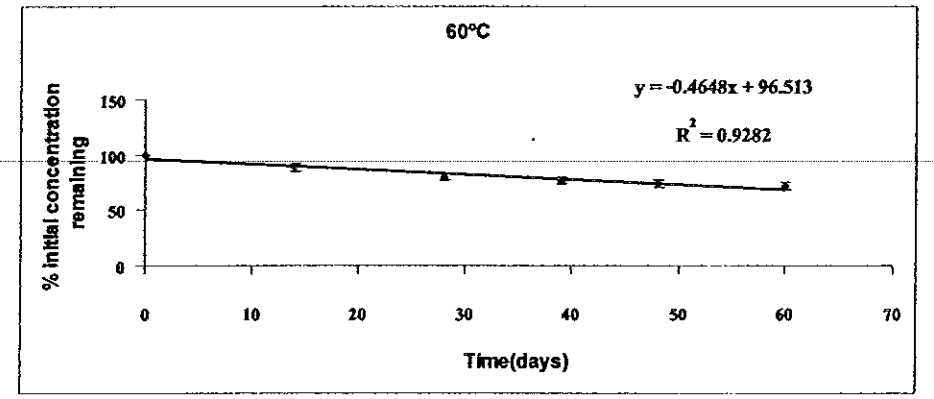


Figure C-4 Linear plot of percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 60°C

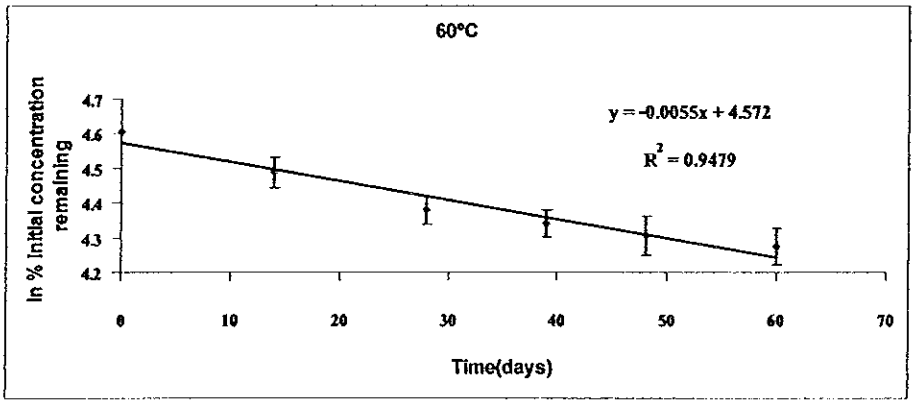


Figure C-5 Linear plot of ln percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 60°C

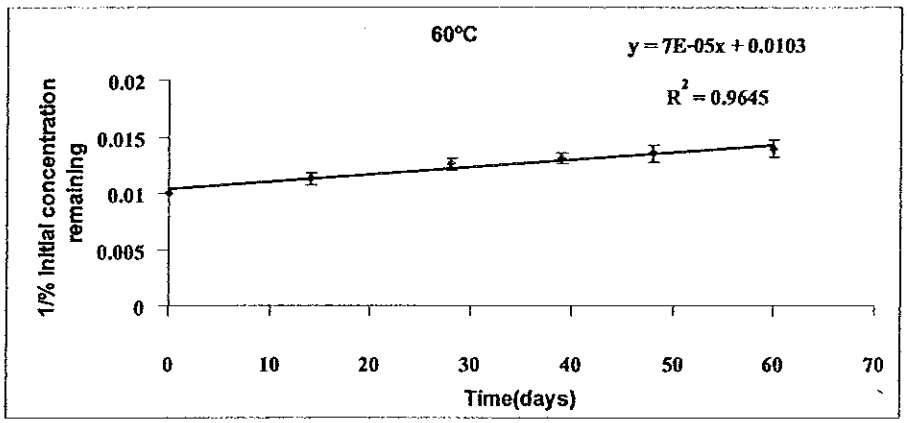


Figure C-6 Linear plot of 1/ percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 60°C

Table C-2 Regression statistics of 1/percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 60°C

Regression Statistics	
Multiple R	0.9820695
R Square	0.9644605
Adjusted R Square	0.9555757
Standard Error	0.0003129
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.06E-05	1.06E-05	108.5509	0.0004794
Residual	4	3.92E-07	9.79E-08		
Total	5	1.1E-05			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.0103007	0.000237	43.54399	1.66E-06	0.0096439	0.010958	0.009644	0.010958
X Variable 1	6.585E-05	6.32E-06	10.41878	0.000479	4.83E-05	8.34E-05	4.83E-05	8.34E-05

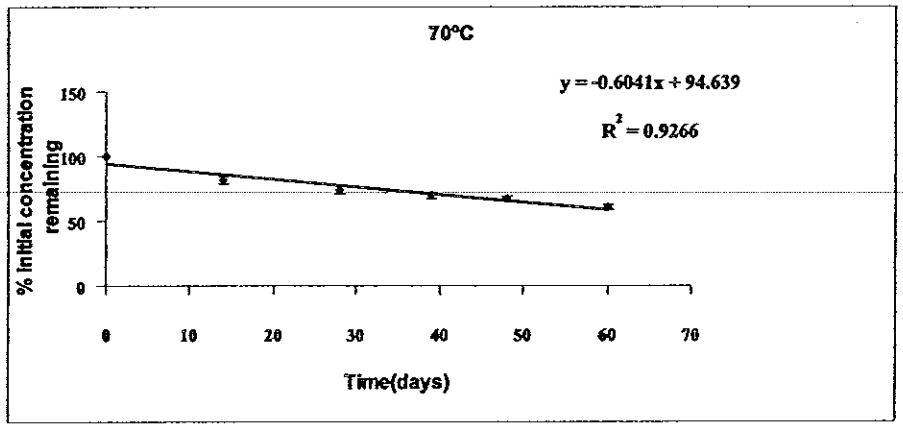


Figure C-7 Linear plot of percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 70°C

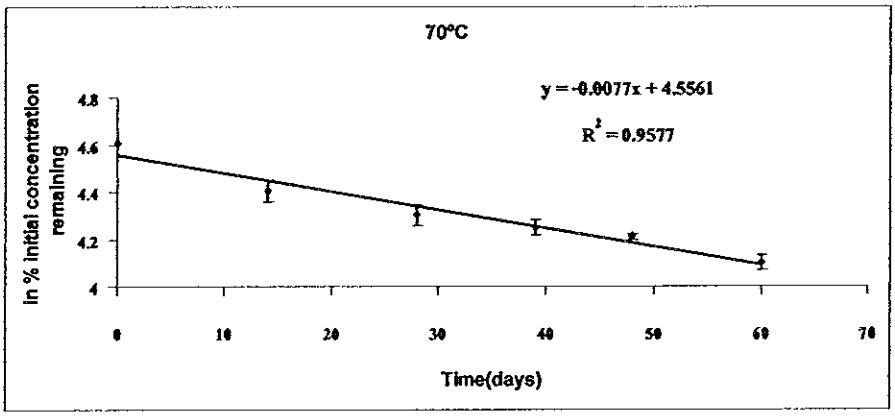


Figure C-8 Linear plot of ln percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 70°C

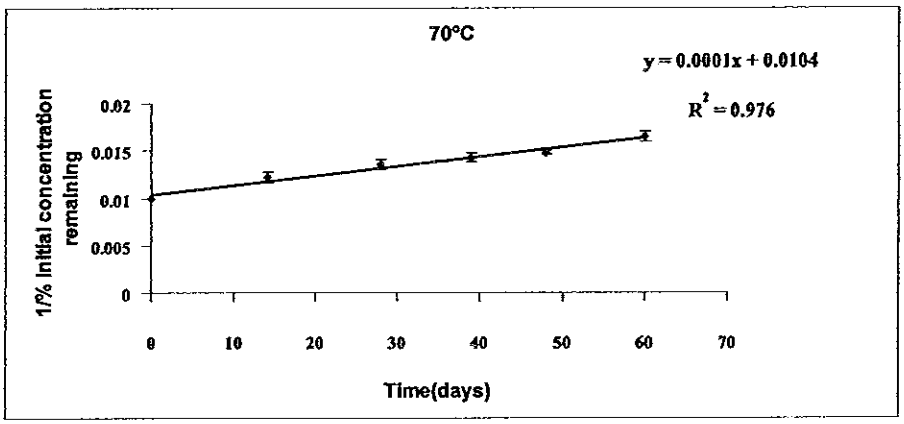


Figure C-9 Linear plot of 1/percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 70°C

Table C-3 Regression statistics of of 1/ percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 1 storage at 70°C

Regression Statistics	
Multiple R	0.9879242
R Square	0.9759942
Adjusted R Square	0.9699928
Standard Error	0.0003916
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.49E-05	2.49E-05	162.626	0.000218
Residual	4	6.13E-07	1.53E-07		
Total	5	2.56E-05			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.0103904	0.000296	35.10179	3.9E-06	0.009569	0.011212	0.009569	0.011212
X Variable 1	0.0001009	7.91E-06	12.75251	0.00022	7.89E-05	0.000123	7.89E-05	0.000123

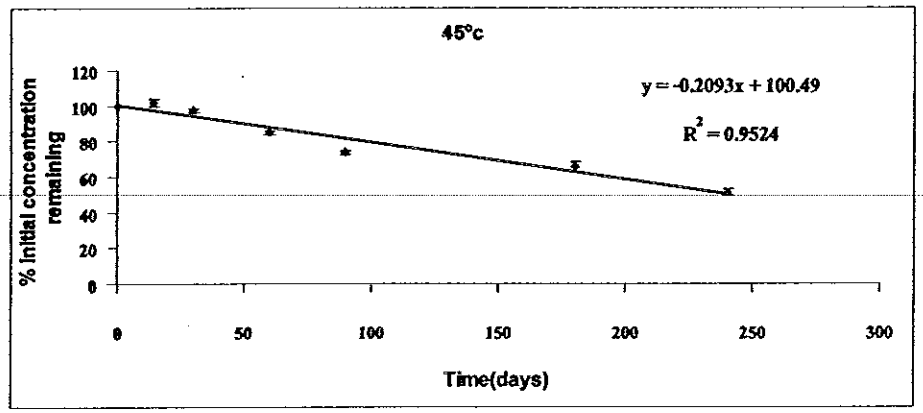


Figure C-10 Linear plot of percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 45°C

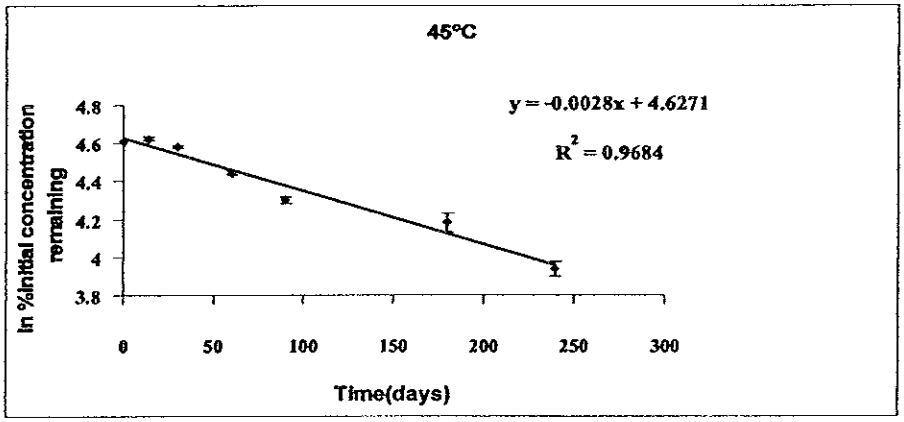


Figure C-11 Linear plot of ln percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 45°C

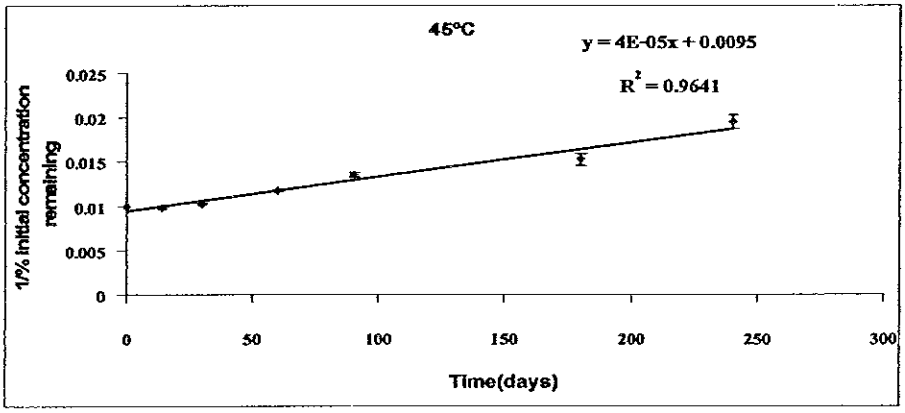


Figure C-12 Linear plot of 1/percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 45°C

Table C-4 Regression statistics of of ln percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 45°C

Regression Statistics	
Multiple R	0.984086459
R Square	0.968426158
Adjusted R Square	0.96211139
Standard Error	0.049958661
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.382763636	0.382764	153.3589	6.08E-05
Residual	5	0.012479339	0.002496		
Total	6	0.395242975			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	4.627053718	0.027366574	169.0768	1.37E-10	4.556706	4.697402	4.55670581	4.697401622
X Variable 1	-0.002796636	0.00022583	-12.3838	6.08E-05	-0.00338	-0.00222	-0.0033771	-0.002216122

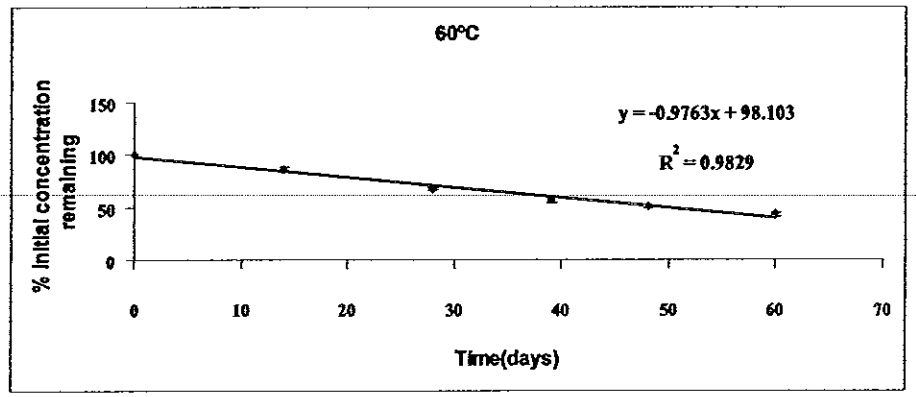


Figure C-13 Linear plot of percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 60°C

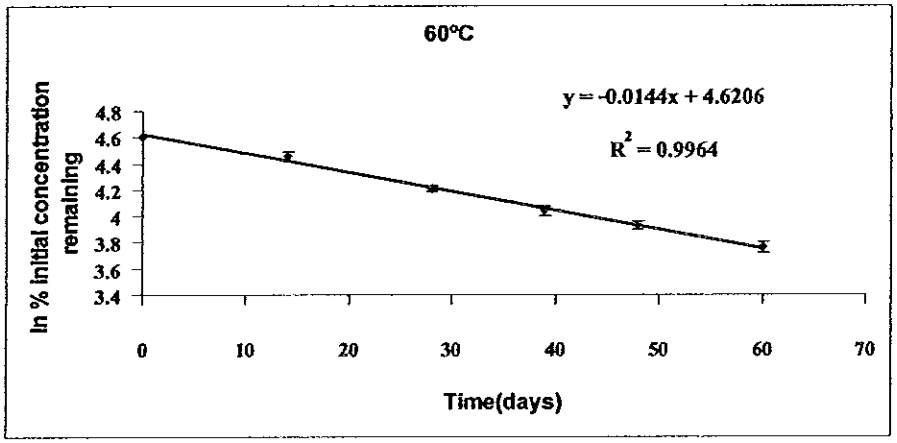


Figure C-14 Linear plot of ln percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 60°C

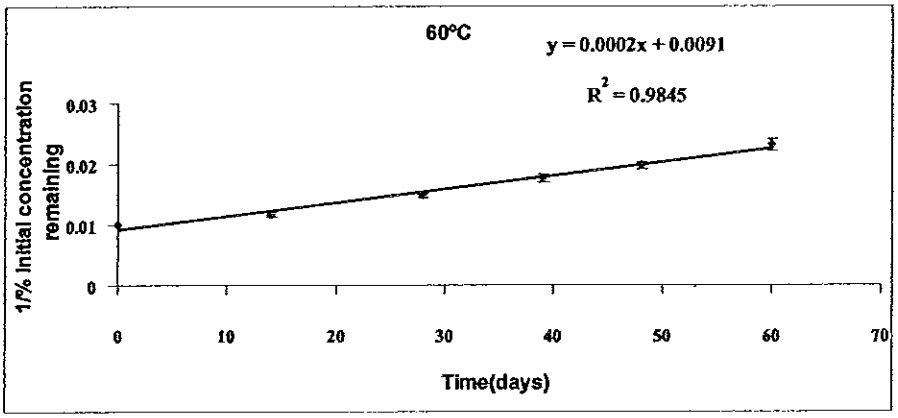


Figure C-15 Linear plot of 1/percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 60°C

Table C-5 Regression statistics of ln percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 60°C

Regression Statistics	
Multiple R	0.998180664
R Square	0.996364639
Adjusted R Square	0.995455798
Standard Error	0.021532261
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.508288143	0.508288109	6.303	4.96196E-06
Residual	4	0.001854553	0.000464		
Total	5	0.510142696			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	4.620603522	0.016276721	283.8789	2.4E-10	4.575412007	4.665795	4.57541201	4.665795038
X Variable 1	-0.014399222	0.000434884	-33.1105	4.96E-06	-0.015606657	-0.01319	-0.0156067	-0.01319179

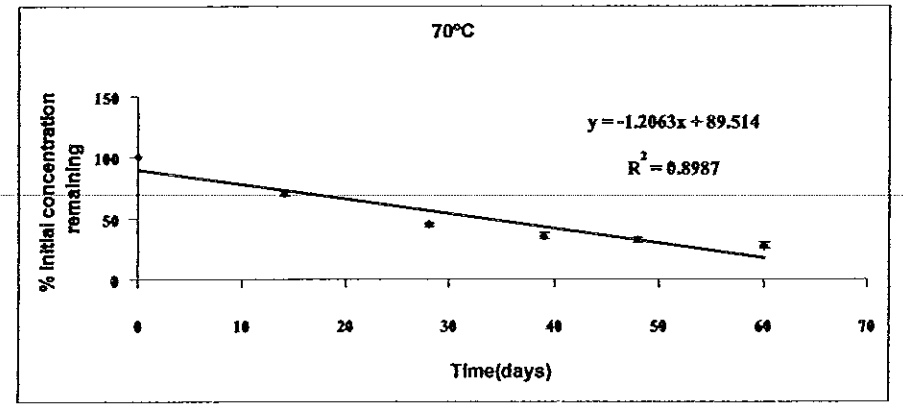


Figure C-16 Linear plot of percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 70°C

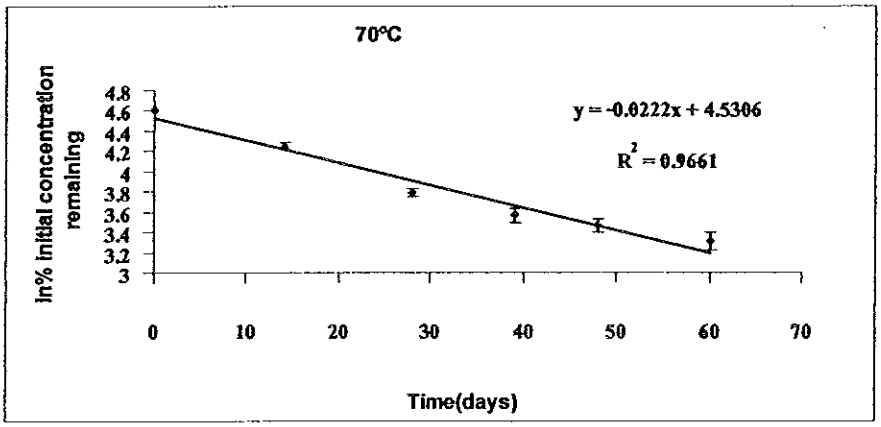


Figure C-17 Linear plot of ln percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 70°C

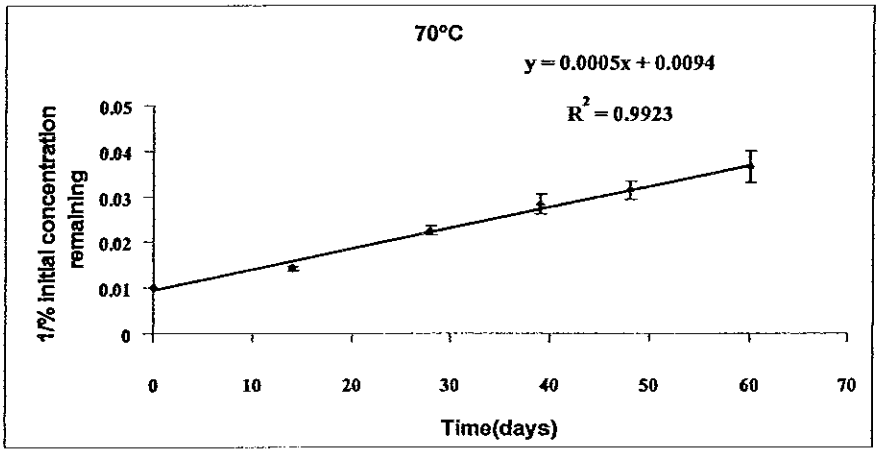


Figure C-18 Linear plot of 1/percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 70°C

Table C-6 Regression statistics of ln percent initial concentration remaining vs. time of clonidine hydrochloride in Formulation 2 storage at 70°C

Regression Statistics	
Multiple R	0.982892108
R Square	0.966076896
Adjusted R Square	0.95759612
Standard Error	0.103009206
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.208726889	1.208726889	113.9137	0.000436516
Residual	4	0.042443586	0.010610897		
Total	5	1.251170476			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	4.530557646	0.077866979	58.18329801	5.23E-07	4.314363805	4.746751486	4.314363805	4.746751486
X Variable 1	-0.022204865	0.002080463	-10.67303788	0.000437	-0.02798117	-0.01643	-0.02798117	-0.016428561

Table C-7 Regression statistics of Arrhenius relation of extemporaneous clonidine hydrochloride syrup Formulation 1

Regression Statistics	
Multiple R	0.981669149
R Square	0.963674319
Adjusted R Square	0.958484936
Standard Error	0.110989462
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.287589256	2.287589	185.7011	2.69719E-06
Residual	7	0.086230625	0.012319		
Total	8	2.373819881			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	6.406677265	1.185027525	5.406353	0.001002	3.604534446	9.20882	3.604534446	9.208820084
X Variable 1	-5.34521229	0.392245238	-13.6272	2.7E-06	-6.272724225	-4.4177	-6.272724225	-4.417700348

Table C-8 Regression statistics of Arrhenius relation of extemporaneous clonidine hydrochloride syrup Formulation 2

Regression Statistics	
Multiple R	0.9788802
R Square	0.95820645
Adjusted R Square	0.95223594
Standard Error	0.20734293
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	6.899637416	6.899637	160.4899	4.4157E-06
Residual	7	0.300937629	0.042991		
Total	8	7.200575045			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	23.3893465	2.213787441	10.56531	1.49E-05	18.15457482	28.62412	18.1545748	28.62411828
X Variable 1	-9.2830153	0.732765749	-12.6685	4.42E-06	-11.01572976	-7.5503	-11.01573	-7.550300922

Table C-9 Arrhenius relation of extemporaneous clonidine hydrochloride syrup Formulation 1

°C	1/Tx1000 (k^{-1})	k	ln k	$(1/Ti \times 1000 - 1/\bar{T}x1000)^2$
45	3.143	0.00003	-10.4143	0.015203
	3.143	0.000034	-10.2892	0.015203
	3.143	0.000026	-10.5574	0.015203
60	3.002	0.00007	-9.56702	0.000313
	3.002	0.000076	-9.48478	0.000313
	3.002	0.000064	-9.65663	0.000313
70	2.914	0.0001	-9.21034	0.011172
	2.914	0.000108	-9.13338	0.011172
	2.914	0.000092	-9.29372	0.011172
	Mean = 3.0197			$\sum$ 0.080064

Arrhenius Equation $\ln k = -5.3452(1/T)+6.4067$ $S_{y/x} = 0.110989$

Heat of activation Ea = slope x 1.987 Kilocalories/mole

$$S_k = \frac{S_{y/x}}{\sqrt{\sum(1/Tix1000 - 1/\bar{T}x1000)^2}} = \frac{0.110989}{\sqrt{0.080064}} = 0.208651$$

$$Ea_{(95\%cl)} = (\text{slope} \pm S_k x\, t_{0.05,df=7})x1.987 = (5.3452 \pm (0.208651x1.895))x1.987$$

$$= 10.62 \pm 0.79 \text{ Kilocalories/mole}$$

Predict 29°C (1/Tx1000) = 3.3096 --> $\ln k_{29} = -11.283774$ --> antiln k 0.000012575

t_{90} of second-order reaction

$$t_{90} = 1/9[A]_0k = 1/(9 \text{ x}100\text{x } 0.000012575) = 88.35 \text{ days}$$

$$\ln k_{29,(95\%cl)} = \ln k_{29} \pm S_{y/x} \text{ x } t_{0.05,df=7} \text{ x } \sqrt{\frac{1}{n} + \frac{(1/Tsx1000 - 1/\bar{T}x1000)^2}{\sum(1/Tix1000 - 1/\bar{T}x1000)^2}}$$

$$= -11.283774 \pm 0.110989x1.895 \text{ x } \sqrt{\frac{1}{9} + \frac{(3.3096 - 3.0197)^2}{0.080064}}$$

$$= -11.283774 \pm 0.226604 = -11.0571702, -11.510378$$

$$k_{29,(95\%cl)} = 0.000015773642, 0.000010025507$$

$$t_{90,(95\%cl)} = 1/9[A]_0k = 1/(9 \text{ x}100\text{x } 0.000015773642) = 70.44 \text{ days}$$

$$t_{90,(95\%cl)} = 1/9[A]_0k = 1/(9 \text{ x}100\text{x } 0.000010025507) = 110.83 \text{ days}$$

Predict 6°C (1/Tx1000) = 3.5823 --> ln k_6 = -12.74142825 --> antiln k 0.000002927

t_{90} of second-order reaction

$$t_{90} = 1/9[A]_0k = 1/(9 \times 100 \times 0.000002927) = 379.61 \text{ days}$$

$$\ln k_{6,(95\%cl)} = \ln k_6 \pm S_{y/x} \times t_{0.05,df=7} \times \sqrt{\frac{1}{n} + \frac{(1/Tsx1000 - \overline{1/Tx1000})^2}{\sum(1/Tix1000 - \overline{1/Tx1000})^2}}$$

$$= -12.74142825 \pm 0.110989 \times 1.895 \times \sqrt{\frac{1}{9} + \frac{(3.5823 - 3.0197)^2}{0.080064}}$$

$$= -12.74142825 \pm 0.424022726 = -12.31740552, -13.16545098$$

$k_{6,(95\%cl)}$ = 0.0000044732044, 0.000001915649

$t_{90,(95\%cl)}$ = $1/9[A]_0k = 1/(9 \times 100 \times 0.0000044732044) = 248.39$ days

$t_{90,(95\%cl)}$ = $1/9[A]_0k = 1/(9 \times 100 \times 0.000001915649) = 580.02$ days

Table C-10 Arrhenius relation of extemporaneous clonidine hydrochloride syrup Formulation 2

°C	1/Tx1000 (k^{-1})	k	ln k	$(1/Ti \times 1000 - 1/Tx1000)^2$
45	3.143	0.0028	-5.87814	0.015203
	3.143	0.003026	-5.80051	0.015203
	3.143	0.002574	-5.96229	0.015203
60	3.002	0.0144	-4.24053	0.000313
	3.002	0.014835	-4.21077	0.000313
	3.002	0.014174	-4.25635	0.000313
70	2.914	0.0222	-3.80766	0.011172
	2.914	0.02428	-3.7181	0.011172
	2.914	0.02012	-3.90604	0.011172
	Mean = 3.0197			Σ 0.080064

Arrhenius Equation ln k = -9.283(1/T)+23.389 $S_{y/x}$ = 0.207343

Heat of activation Ea = slope x 1.987 Kilocalories/mole

$$S_k = \frac{S_{y/x}}{\sqrt{\sum(1/Tix1000 - \overline{1/Tx1000})^2}} = \frac{0.207343}{\sqrt{0.080064}} = 0.732775$$

$$Ea_{(95\%cl)} = (\text{slope} \pm S_b \times t_{0.05,df=7}) \times 1.987 = (9.283 \pm (0.732775 \times 1.895)) \times 1.987$$

$$= 18.45 \pm 2.76 \text{ Kilocalories/mole}$$

Predict 29°C (1/Tx1000) = 3.3096 --> $\ln k_{29} = -7.3340168$ --> antiln k 0.00065294556

t_{90} of first-order reaction

$$t_{90} = \frac{0.105}{k} = \frac{0.105}{0.00065294556} = 160.81 \text{ days}$$

$$\ln k_{29,(95\%cl)} = \ln k_{29} \pm S_{y/x} \times t_{0.05,df=7} \times \sqrt{\frac{1}{n} + \frac{(1/Tsx1000 - \overline{1/Tx1000})^2}{\sum(1/Tix1000 - \overline{1/Tx1000})^2}}$$

$$= -7.3340168 \pm 0.207343 \times 1.895 \times \sqrt{\frac{1}{9} + \frac{(3.3096 - 3.0197)^2}{0.080064}}$$

$$= -7.3340168 \pm 0.423327649 = -6.910689151, -7.757344449$$

$$k_{29,(95\%cl)} = 0.00099707042, 0.00042759056$$

$$t_{90,(95\%cl)} = 0.105/ 0.00099707042 = 105.31 \text{days}$$

$$t_{90,(95\%cl)} = 0.105/ 0.00042759056 = 245.56 \text{ days}$$

Predict 6°C (1/Tx1000) = 3.5823 --> $\ln k_6 = -9.8654909$ --> antilnk 0.000051936387

t_{90} of first-order reaction

$$t_{90} = \frac{0.105}{k} = \frac{0.105}{0.000051936387} = 2021.70 \text{ days}$$

$$\ln k_{6,(95\%cl)} = \ln k_6 \pm S_{y/x} \times t_{0.05,df=7} \times \sqrt{\frac{1}{n} + \frac{(1/Tsx1000 - \overline{1/Tx1000})^2}{\sum(1/Tix1000 - \overline{1/Tx1000})^2}}$$

$$= -9.8654909 \pm 0.207343 \times 1.895 \times \sqrt{\frac{1}{9} + \frac{(3.5823 - 3.0197)^2}{0.080064}}$$

$$= -9.8654909 \pm 0.792133853 = -9.073357047, -10.65762475$$

$$k_{6,(95\%cl)} = 0.0001146809, 0.000023520815$$

$$t_{90,(95\%cl)} = 0.105/ 0.0001146809 = 915.58 \text{ days}$$

$$t_{90,(95\%cl)} = 0.105/0.000023520815 = 4464.13 \text{ days}$$

APPENDIX-D

Statistically data of palatability study of clonidine hydrochloride

Table D-1 Demographics of healthy volunteers

Characteristics		No. (%)
Sex	Male	13(46.4)
	Female	15(53.6)
	Total	28(100)
Age (Year)*	21	5(17.9)
	22	7(25)
	23	7(25)
	24	1(3.6)
	26	5(17.9)
	27	2(17.1)
	33	1(3.6)

* mean $\pm$ SD = 23.61 $\pm$ 2.69 years

Two-way ANOVA was used for comparing the palatability in various variables and performed by using SPSS® 13 programs. A null hypothesis, there is no difference in the mean of palatability in four preparations, was tested against an alternative hypothesis, at least one pair of the mean of palatability is not equal (a significant level, $\alpha = 0.05$)

The p-value is always related to a hypothesis test. If the p-value was more than 0.05, then the null hypothesis was accepted and the difference in the mean of palatability were said to be statistically insignificant. If the p-value was less than 0.05, the null hypothesis was rejected and the alternative hypothesis was accepted that means at least one pair of the mean of palatability in four preparations was not equal. The post hoc and Turkey's test were used to determine which pairs were different.

Table D-2 Test of appearance in four preparations

Tests of Between-Subjects Effects

Dependent Variable: appearan

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	1242.223	1	1242.223	1313.915	.000
	Error	25.527	27	.945[a]		
product	Hypothesis	25.170	3	8.390	16.955	.000
	Error	40.080	81	.495[b]		
sub	Hypothesis	25.527	27	.945	1.911	.014
	Error	40.080	81	.495[b]		

a. MS(sub)

b. MS(Error)

Expected Mean Squares [a,b]

Source	Variance Component		
	Var(sub)	Var(Error)	Quadratic Term
Intercept	4.000	1.000	Intercept, product
product	.000	1.000	product
sub	4.000	1.000	
Error	.000	1.000	

a. For each source, the expected mean square equals the sum of the coefficients in the cells times the variance components, plus a quadratic term involving effects in the Quadratic Term cell.

b. Expected Mean Squares are based on the Type III Sums of Squares.

Table D-3 The post hoc and Turkey's test for appearance in four preparations

Multiple Comparisons

Dependent Variable: appearan

Tukey HSD

(I) product	(J) product	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.04	.188	.998	-.46	.53
	3	1.00*	.188	.000	.51	1.49
	4	.93*	.188	.000	.44	1.42
2	1	-.04	.188	.998	-.53	.46
	3	.96*	.188	.000	.47	1.46
	4	.89*	.188	.000	.40	1.39
3	1	-1.00*	.188	.000	-1.49	-.51
	2	-.96*	.188	.000	-1.46	-.47
	4	-.07	.188	.981	-.56	.42
4	1	-.93*	.188	.000	-1.42	-.44
	2	-.89*	.188	.000	-1.39	-.40
	3	.07	.188	.981	-.42	.56

Based on observed means.

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

appearance

Tukey HSD

product	N	Subset	
		1	2
3	28	2.82	
4	28	2.89	
2	28		3.79
1	28		3.82
Sig.		.981	.998

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .495.

a Uses Harmonic Mean Sample Size = 28.000.

b Alpha = .05.

Table D-4 Test of color in four preparations

Tests of Between-Subjects Effects

Dependent Variable: color

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	1150.723	1	1150.723	827.929	.000
	Error	37.527	27	1.390(a)		
product	Hypothesis	15.170	3	5.057	7.644	.000
	Error	53.580	81	.661(b)		
sub	Hypothesis	37.527	27	1.390	2.101	.006
	Error	53.580	81	.661(b)		

a MS(sub)
b MS(Error)

Expected Mean Squares(a,b)

Source	Variance Component		
	Var(sub)	Var(Error)	Quadratic Term
Intercept	4.000	1.000	Intercept, product
product	.000	1.000	product
sub	4.000	1.000	
Error	.000	1.000	

a For each source, the expected mean square equals the sum of the coefficients in the cells times the variance components, plus a quadratic term involving effects in the Quadratic Term cell.
b Expected Mean Squares are based on the Type III Sums of Squares.

Table D-5 The post hoc and Turkey's test for color in four preparations

Multiple Comparisons

Dependent Variable: color

Tukey HSD

(I) product	(J) product	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.00	.217	1.000	-.57	.57
	3	.68*	.217	.013	.11	1.25
	4	.79*	.217	.003	.22	1.36
2	1	.00	.217	1.000	-.57	.57
	3	.68*	.217	.013	.11	1.25
	4	.79*	.217	.003	.22	1.36
3	1	-.68*	.217	.013	-1.25	-.11
	2	-.68*	.217	.013	-1.25	-.11
	4	.11	.217	.960	-.46	.68
4	1	-.79*	.217	.003	-1.36	-.22
	2	-.79*	.217	.003	-1.36	-.22
	3	-.11	.217	.960	-.68	.46

Based on observed means.

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

color

Tukey HSD[a,b]

product	N	Subset 1	Subset 2
4	28	2.79	
3	28	2.89	
1	28		3.57
2	28		3.57
Sig.		.960	1.000

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .661.

a. Uses Harmonic Mean Sample Size = 28.000.

b. Alpha = .05.

Table D-6 Test of flavor in four preparations

Tests of Between-Subjects Effects

Dependent Variable: smell

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	1567.509	1	1567.509	1136.453	.000
	Error	37.241	27	1.379[a]		
product	Hypothesis	16.813	3	5.604	10.955	.000
	Error	41.438	81	.512[b]		
sub	Hypothesis	37.241	27	1.379	2.696	.000
	Error	41.438	81	.512[b]		

a. MS(sub)

b. MS(Error)

Expected Mean Squares[a,b]

Source	Variance Component		
	Var(sub)	Var(Error)	Quadratic Term
Intercept	4.000	1.000	Intercept, product
product	.000	1.000	product
sub	4.000	1.000	
Error	.000	1.000	

a. For each source, the expected mean square equals the sum of the coefficients in the cells times the variance components, plus a quadratic term involving effects in the Quadratic Term cell.

b. Expected Mean Squares are based on the Type III Sums of Squares.

Table D-7 The post hoc and Turkey's test for flavor in four preparations

Multiple Comparisons

Dependent Variable: smell

Tukey HSD

(I) product	(J) product	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.04	.191	.998	-.54	.47
	3	.68*	.191	.004	.18	1.18
	4	.82*	.191	.000	.32	1.32
2	1	.04	.191	.998	-.47	.54
	3	.71*	.191	.002	.21	1.22
	4	.86*	.191	.000	.36	1.36
3	1	-.68*	.191	.004	-1.18	-.18
	2	-.71*	.191	.002	-1.22	-.21
	4	.14	.191	.878	-.36	.64
4	1	-.82*	.191	.000	-1.32	-.32
	2	-.86*	.191	.000	-1.36	-.36
	3	-.14	.191	.878	-.64	.36

Based on observed means.

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

smell

Tukey HSD[a,b]

product	N	Subset	
		1	2
4	28	3.29	
3	28	3.43	
1	28		4.11
2	28		4.14
Sig.		.878	.998

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .512.

a. Uses Harmonic Mean Sample Size = 28.000.

b. Alpha = .05.

Table D-8 Test of taste in four preparations

Tests of Between-Subjects Effects

Dependent Variable: taste

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	1457.286	1	1457.286	1086.497	.000
	Error	36.214	27	1.341[a]		
product	Hypothesis	12.714	3	4.238	6.382	.001
	Error	53.786	81	.664[b]		
sub	Hypothesis	36.214	27	1.341	2.020	.008
	Error	53.786	81	.664[b]		

a. MS(sub)

b. MS(Error)

Expected Mean Squares[a,b]

Source	Variance Component		
	Var(sub)	Var(Error)	Quadratic Term
Intercept	4.000	1.000	Intercept, product
product	.000	1.000	product
sub	4.000	1.000	
Error	.000	1.000	

a. For each source, the expected mean square equals the sum of the coefficients in the cells times the variance components, plus a quadratic term involving effects in the Quadratic Term cell.

b. Expected Mean Squares are based on the Type III Sums of Squares.

Table D-9 The post hoc and Turkey's test for taste in four preparations

Multiple Comparisons

Dependent Variable: taste

Tukey HSD

(I) product	(J) product	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.29	.218	.558	-.29	.86
	3	.36	.218	.362	-.21	.93
	4	.93*	.218	.000	.36	1.50
2	1	-.29	.218	.558	-.86	.29
	3	.07	.218	.988	-.50	.64
	4	.64*	.218	.021	.07	1.21
3	1	-.36	.218	.362	-.93	.21
	2	-.07	.218	.988	-.64	.50
	4	.57*	.218	.050	.00	1.14
4	1	-.93*	.218	.000	-1.50	-.36
	2	-.64*	.218	.021	-1.21	-.07
	3	-.57*	.218	.050	-1.14	.00

Based on observed means.

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

taste

Tukey HSD[a,b]

product	N	Subset	
		1	2
4	28	3.07	
3	28		3.64
2	28		3.71
1	28		4.00
Sig.		1.000	.362

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .664.

a. Uses Harmonic Mean Sample Size = 28.000.

b. Alpha = .05.

Table D-10 Test of overall in four preparations

Tests of Between-Subjects Effects

Dependent Variable: overall

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	1400.143	1	1400.143	1729.588	.000
	Error	21.857	27	.810[a]		
product	Hypothesis	9.786	3	3.262	7.722	.000
	Error	34.214	81	.422[b]		
sub	Hypothesis	21.857	27	.810	1.916	.013
	Error	34.214	81	.422[b]		

a. MS(sub)

b. MS(Error)

Expected Mean Squares[a,b]

Source	Variance Component		
	Var(sub)	Var(Error)	Quadratic Term
Intercept	4.000	1.000	Intercept, product
product	.000	1.000	product
sub	4.000	1.000	
Error	.000	1.000	

a. For each source, the expected mean square equals the sum of the coefficients in the cells times the variance components, plus a quadratic term involving effects in the Quadratic Term cell.

b. Expected Mean Squares are based on the Type III Sums of Squares.

Table D-11 The post hoc and Turkey's test for overall in four preparations

Multiple Comparisons

Dependent Variable: overall

Tukey HSD

(I) product	(J) product	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.21	.174	.607	-.24	.67
	3	.68*	.174	.001	.22	1.13
	4	.68*	.174	.001	.22	1.13
2	1	-.21	.174	.607	-.67	.24
	3	.46*	.174	.044	.01	.92
	4	.46*	.174	.044	.01	.92
3	1	-.68*	.174	.001	-1.13	-.22
	2	-.46*	.174	.044	-.92	-.01
	4	.00	.174	1.000	-.46	.46
4	1	-.68*	.174	.001	-1.13	-.22
	2	-.46*	.174	.044	-.92	-.01
	3	.00	.174	1.000	-.46	.46

Based on observed means.

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

overall

Tukey HSD[a,b]

product	N	Subset 1	Subset 2
3	28	3.25	
4	28	3.25	
2	28		3.71
1	28		3.93
Sig.		1.000	.607

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .422.

a. Uses Harmonic Mean Sample Size = 28.000.

b. Alpha = .05.

VITAE

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List of Publication and Proceeding

Bocam, P., Kaewnopparat, N., Sirikatitham, A. and Kaewnopparat, S. (2009), Stability of extemporaneous clonidine hydrochloride syrups in two vehicles, Poster presentation at 4th BUU Grad Research Conference, 13-14 March 2009, Burapha University, Thailand.