

CONTENTS

| | Page |
|--|-------------|
| CONTENTS | vii |
| LIST OF TABLES | xi |
| LIST OF FIGURES | xiv |
| CHAPTER | |
| 1 INTRODUCTION | 1 |
| 1.1 Background and Rationale | 1 |
| 1.2 Review of Literatures | 2 |
| 1.2.1 β -agonists | 2 |
| 1.2.2 Salbutamol | 5 |
| 1.2.3 Measurement of salbutamol residue | 8 |
| 1.2.4 Sample pretreatment | 9 |
| 1.2.5 Sample extraction and clean-up | 9 |
| 1.2.6 Detection of salbutamol | 12 |
| 1.3 Objectives | 22 |
| 2 EXPERIMENTAL | 23 |
| 2.1 Chemicals and materials | 23 |
| 2.1.1 Standard chemicals | 23 |
| 2.1.2 Ion pair reagent | 23 |
| 2.1.3 Other chemicals | 23 |
| 2.1.4 Solid Phase Extraction (SPE) | 24 |
| 2.2 Instruments and apparatus | 24 |
| 2.2.1 Spectrofluorometer | 24 |
| 2.2.2 High Performance Liquid Chromatograph- Fluorescence Detector (HPLC-FLD) | 24 |
| 2.2.3 Apparatus | 24 |
| 2.3 Analysis system | 25 |
| 2.4 Preparation of salbutamol standard solutions | 27 |
| 2.5 Determination of salbutamol by spectrofluorometer | 27 |
| | vii |

CONTENTS (CONTINUED)

| | Page |
|---|-------------|
| 2.5.1 Diluting solution, 8% (v/v) acetonitrile, pH 2.5 | 27 |
| 2.5.2 Salbutamol standard working solution | 27 |
| 2.5.3 Excitation (λ_{ex}) and emission (λ_{em}) wavelengths | 28 |
| 2.5.4 Limit of detection (LOD) | 28 |
| 2.5.5 Linear range | 28 |
| 2.6 Determination of salbutamol by ion-pair chromatography with fluorescence detection (IPC-FLD) | 29 |
| 2.6.1 Bamethan internal standard (IS) stock solution | 29 |
| 2.6.2 Salbutamol standard working solution | 29 |
| 2.6.3 Degassing of mobile phase | 29 |
| 2.6.4 Optimization of IPC-FLD conditions | 30 |
| 2.6.5 System performance of IPC-FLD | 35 |
| 2.7 Sample preparation | 36 |
| 2.7.1 Preparation of salbutamol standard fortification solution | 36 |
| 2.7.2 Fortified sample | 36 |
| 2.7.3 Pretreatment of octadecyl (C ₁₈) packing material | 36 |
| 2.7.4 Optimization of matrix solid-phase dispersion (MSPD) extraction | 37 |
| 2.7.5 Optimization of Solid phase extraction (SPE), clean up | 39 |
| 2.7.6 Matrices interferences | 41 |
| 2.8 Method validation | 43 |
| 2.8.1 Selectivity | 43 |
| 2.8.2 Accuracy, Precision and Recovery | 43 |
| 2.8.3 Calibration curve (Matrix-based calibration curve) | 44 |
| 2.8.4 Limit of Quantification (LOQ) | 44 |
| 2.9 Qualitative and quantitative analysis of lean meat sample | 44 |
| 2.9.1 Qualitative analysis of IPC-FLD technique for salbutamol analysis | 44 |
| 2.9.2 Quantitative analysis | 45 |
| 2.9.3 Standard addition | 45 |

CONTENTS (CONTINUED)

| | Page |
|--|-------------|
| 3 RESULTS AND DISCUSSION | 46 |
| 3.1 Determination of salbutamol by spectrofluorometer | 46 |
| 3.1.1 Excitation and emission wavelength | 46 |
| 3.1.2 Limit of detection | 47 |
| 3.1.3 Linear range | 48 |
| 3.2 Determination of salbutamol by HPLC-FLD | 51 |
| 3.2.1 Excitation and emission wavelengths | 52 |
| 3.2.2 Percentage of acetic acid (pH effect) | 55 |
| 3.2.3 Concentration of ion-pair reagent | 57 |
| 3.2.4 Percentage of methanol (Solvent-strength effect) | 59 |
| 3.2.5 Temperature | 63 |
| 3.2.6 Flow rate | 65 |
| 3.3 System performance of IPC-FLD | 71 |
| 3.3.1 Repeatability | 71 |
| 3.3.2 Limit of detection (LOD) | 72 |
| 3.3.3 Linear range | 72 |
| 3.3.4 Internal standard curve | 74 |
| 3.4 Sample preparation | 77 |
| 3.4.1 Matrix solid phase dispersion (MSPD) | 77 |
| 3.4.2 Solid phase extraction (SPE), clean up | 87 |
| 3.4.3 Matrices interferences | 94 |
| 3.5 Method validation | 98 |
| 3.5.1 Selectivity | 98 |
| 3.5.2 Accuracy, precision and recovery | 100 |
| 3.5.3 Calibration/Standard curve | 100 |
| 3.5.4 Limit of Quantification (LOQ) | 105 |
| 3.6 Qualitative and quantitative analysis of lean meat sample | 105 |
| 3.6.1 Qualitative analysis | 106 |

CONTENTS (CONTINUED)

| | Page |
|-----------------------------|-------------|
| 3.6.2 Quantitative analysis | 106 |
| 4 CONCLUSIONS | 120 |
| REFERENCES | 123 |
| VITAE | 135 |

LIST OF TABLES

| Table | Page |
|--|------|
| 1 Food poisoning from β -agonist compound. | 5 |
| 2 The physical properties of salbutamol and bamethan (IS). | 7 |
| 3 Summary of determination of salbutamol by HPLC-FLD. | 19 |
| 4 Response of salbutamol by spectrofluorometer at various concentrations. | 49 |
| 5 Response of salbutamol and bamethan (IS) at different percentage of acetic acid. | 56 |
| 6 Response, retention factor and analysis time of salbutamol and bamethan (IS) at various concentration of hexanesulfonate. | 58 |
| 7 Response, capacity factor (k), separation factor (α) and analysis time of salbutamol and bamethan (IS) at various percentage of methanol. | 60 |
| 8 Response, capacity factor (k), separation factor (α) and analysis time of salbutamol and bamethan (IS) at various column temperature. | 64 |
| 9 Contribution of different band-broadening processes to column plate height H . | 66 |
| 10 Plate counts (N) and plate height ($HETP$) of salbutamol and bamethan (IS) at various mobile phase flow rate. | 69 |
| 11 Optimum conditions of chromatographic conditions. | 70 |
| 12 %RSD of retention time and peak area of five repetition injections of mixed solution 20 ng mL^{-1} of salbutamol and bamethan (IS). | 72 |
| 13 Response at various concentrations of salbutamol. | 73 |
| 14 Relationship between the concentration of salbutamol and the ratio of peak area of salbutamol to bamethan (IS). | 76 |
| 15 Response of salbutamol at each type of sorbent. | 78 |
| 16 Response of salbutamol at various type of washing solvent. | 80 |
| 17 Response of salbutamol at each washing solvent volume. | 81 |
| 18 The response of salbutamol at each type of eluting solvent. | 83 |

LIST OF TABLES (CONTINUED)

| Table | Page |
|--|-------------|
| 19 Response of salbutamol at each various eluent flow rate. | 84 |
| 20 Response of salbutamol at various volume of eluting solvent. | 86 |
| 21 Response of salbutamol at various flow rate of sample solution. | 87 |
| 22 Response of salbutamol at various type of eluting solvent. | 89 |
| 23 Response of salbutamol at various flow rate of eluting solvent. | 90 |
| 24 Response of salbutamol at each collected fractions. | 92 |
| 25 Optimum conditions of sample preparation procedure. | 93 |
| 26 Response of standard salbutamol and fortified porcine lean meat samples at various salbutamol concentrations. | 95 |
| 27 Response of standard salbutamol and fortified bovine lean meat samples at various salbutamol concentrations. | 96 |
| 28 Statistical test result by two-way ANOVA in R program. | 97 |
| 29 The recovery of salbutamol at three concentrations. | 100 |
| 30 Relationship between the fortified concentrations of salbutamol in porcine lean meat and the ratio of peak area of salbutamol to bamethan (IS). | 102 |
| 31 Relationship between the fortified concentrations of salbutamol in bovine lean meat and the ratio of peak area of salbutamol to bamethan (IS). | 103 |
| 32 Standard addition calibration curve of salbutamol in porcine sample 1. | 107 |
| 33 Standard addition calibration curve of salbutamol in porcine sample 2. | 108 |
| 34 Standard addition calibration curve of salbutamol in porcine sample 3. | 109 |
| 35 Standard addition calibration curve of salbutamol in porcine sample 4. | 110 |

LIST OF TABLES (CONTINUED)

| Table | Page |
|--|-------------|
| 36 Standard addition calibration curve of salbutamol in porcine sample 5. | 111 |
| 37 Standard addition calibration curve of salbutamol in porcine sample 6. | 112 |
| 38 Standard addition calibration curve of salbutamol in bovine sample 1. | 113 |
| 39 Standard addition calibration curve of salbutamol in bovine sample 2. | 114 |
| 40 Standard addition calibration curve of salbutamol in bovine sample 3. | 115 |
| 41 Standard addition calibration curve of salbutamol in bovine sample 4. | 116 |
| 42 Standard addition calibration curve of salbutamol in bovine sample 5. | 117 |
| 43 Standard addition calibration curve of salbutamol in bovine sample 6. | 118 |
| 44 The salbutamol concentrations in porcine and bovine samples by standard addition. | 119 |

LIST OF FIGURES

| Figure | Page |
|--|------|
| 1 General structure of β -agonists. | 3 |
| 2 Structure of salbutamol. | 6 |
| 3 Structure of bamethan. | 7 |
| 4 Structure of the bis-MBA derivative. | 15 |
| 5 Block diagram showing the components of IPC-FLD. | 26 |
| 6 Retention of protonated salbutamol during IPC. | 26 |
| 7 Illustration of HPLC parameters. | 31 |
| 8 Sample extraction by matrix solid phase dispersion. | 38 |
| 9 Summary of the sample preparation procedure. | 42 |
| 10 Spectrum of salbutamol standard $0.5 \mu\text{g mL}^{-1}$ from spectrofluorometer. | 47 |
| 11 Detector response curve. | 48 |
| 12 Response of salbutamol by spectrofluorometry. | 50 |
| 13 Spectrum of mixed standard salbutamol and bamethan (IS) 20 ng mL^{-1} by IPC-FLD system. | 53 |
| 14 3D plot of mixed standard salbutamol and bamethan (IS) 20 ng mL^{-1} by HP Chemstation program in IPC-FLD system. | 54 |
| 15 Chemical structures of salbutamol and cationic salbutamol under acidic condition. | 55 |
| 16 Response of salbutamol and bamethan (IS) at different percentage of acetic acid. | 56 |
| 17 Retention factor of salbutamol and bamethan (IS) at various concentration of ion-pair reagent. | 58 |
| 18 Response of salbutamol and bamethan (IS) at various concentration of ion-pair reagent. | 59 |
| 19 Retention factor of salbutamol and bamethan (IS) at various percentage of methanol. | 61 |

LIST OF FIGURES (CONTINUED)

| Figure | Page |
|--|------|
| 20 Separation factor (or selectivity) of salbutamol and bamethan (IS) at various percentage of methanol . | 61 |
| 21 Response of salbutamol and bamethan (IS) at various percentage of methanol. | 62 |
| 22 Chromatogram of sample extractant at 33% and 30% methanol. | 62 |
| 23 Capacity factor of salbutamol and bamethan (IS) at various column temperatures. | 65 |
| 24 Effect of mobile-phase flow rate on plate height for liquid chromatography and gas chromatography . | 68 |
| 25 van Deemter plot of salbuamol and bamethan (IS). | 70 |
| 26 Chromatogram of mixed standard salbutamol and bamethan (IS) at 20 ng mL ⁻¹ under optimum IPC-FLD conditions. | 71 |
| 27 Linear range of salbutamol by IPC-FLD system. | 74 |
| 28 Internal standard curve: salbutamol/bamethan (IS) peak area ratio <i>versus</i> salbutamol concentrations. | 76 |
| 29 Response of salbutamol at each type of sorbent. | 79 |
| 30 Response of salbutamol at various type of washing solvent. | 80 |
| 31 Response of salbutamol at each volume of hexane:diethyl ether (60:40). | 82 |
| 32 Response of salbutamol at each type of eluting solvent. | 83 |
| 33 Response of salbutamol at various eluent flow rate. | 85 |
| 34 Response of salbutamol at various volume of eluting solvent. | 86 |
| 35 Response of salbutamol at various flow rate of sample solution. | 88 |
| 36 Response of salbutamol at various type of eluting solvent. | 89 |
| 37 Response of salbutamol at various flow rate of eluting solvent. | 91 |
| 38 Eluting profile of salbutamol from Strata X SPE cartridge. | 92 |
| 39 Matrix (porcine) curve compare with standard curve. | 95 |

LIST OF FIGURES (CONTINUED)

| Figure | Page |
|--|------|
| 40 Matrix (bovine) curve compare with standard curve. | 96 |
| 41 HPLC chromatogram of six blank porcine lean meat. | 99 |
| 42 Matrix-based calibration from fortified porcine lean meat. | 102 |
| 43 Matrix-based calibration from fortified bovine lean meat. | 103 |
| 44 The HPLC chromatogram of mixed standard solution, blank porcine sample and fortified porcine sample. | 104 |
| 45 The HPLC chromatogram of mixed standard solution, blank porcine sample and fortified bovine sample. | 105 |
| 46 Standard addition calibration curve of salbutamol in porcine sample 1. | 107 |
| 47 Standard addition calibration curve of salbutamol in porcine sample 2. | 108 |
| 48 Standard addition calibration curve of salbutamol in porcine sample 3. | 109 |
| 49 Standard addition calibration curve of salbutamol in porcine sample 4. | 110 |
| 50 Standard addition calibration curve of salbutamol in porcine sample 5. | 111 |
| 51 Standard addition calibration curve of salbutamol in porcine sample 6. | 112 |
| 52 Standard addition calibration curve of salbutamol in bovine sample 1. | 113 |
| 53 Standard addition calibration curve of salbutamol in bovine sample 2. | 114 |
| 54 Standard addition calibration curve of salbutamol in bovine sample 3. | 115 |
| 55 Standard addition calibration curve of salbutamol in bovine sample 4. | 116 |

LIST OF FIGURES (CONTINUED)

| Figure | Page |
|--|-------------|
| 56 Standard addition calibration curve of salbutamol in bovine sample 5. | 117 |
| 57 Standard addition calibration curve of salbutamol in bovine sample 6. | 118 |