

## Chapter 4

### Conclusions

Nitrofurans were compounds that used widely in animal agriculture. This thesis demonstrated the method for determination of these compounds by focusing on three nitrofurans; nitrofurazone (NZ), nitrofurantoin (NF) and furazolidone (FZ) which were used in Thailand. The analysis was performed by high-performance liquid chromatography with UV detector (HPLC-UV) and UV-Vis spectrophotometry.

Optimization process of HPLC-UV conditions was carried out to obtain the best and high efficiency responses. Nitrofurans were separated by Ultra Aqueous C-18 column (250mm × 4.6 mm I.D., 5 μm). Optimum conditions for HPLC-UV technique were; mobile phase flow rate 1.0 mL min<sup>-1</sup>; composition of mobile phase 35% acetonitrile in water. In the part of UV detector, the optimum wavelength was 365 nm. These optimum conditions provided an analysis time of 10 minutes, low detection limit (5 μg L<sup>-1</sup>) and wide linear dynamic ranges, *i.e.* NZ : 0.01-500 μg mL<sup>-1</sup>, NF : 0.01-500 μg mL<sup>-1</sup> and FZ : 0.01-900 μg mL<sup>-1</sup>, with a coefficient of determination, R<sup>2</sup>, greater than 0.999.

For UV-Vis spectrophotometric optimization, nitrofurans were reacted with methanolic potassium hydroxide at optimum concentration of 1.5 M. The optimum time of color forming was 15 seconds. The optimum wavelength of nitrofurans were; nitrofurazone (NZ) 530 nm, nitrofurantoin (NF) 430 nm, furazolidone (FZ) 560 nm, nitrofurazone-nitrofurantoin (NZ-NF) 520 nm, nitrofurazone-furazolidone (NZ-FZ) 535 nm, nitrofurantoin-furazolidone (NF-FZ) 515 nm and nitrofurazone-nitrofuramntoin-furazolidone (NZ-NF-FZ) 525 nm. Detection limits of nitrofurans at these optimum conditions were 0.15, 0.5, 0.15, 0.2, 0.2, 0.5 and 0.25 μg mL<sup>-1</sup>, respectively. The linearity of UV-Vis spectrophotometry which obeyed Beer's law were 0.1-15 μg mL<sup>-1</sup> (NZ), 0.3-25 μg mL<sup>-1</sup> (NF), 0.1-20 μg mL<sup>-1</sup> (FZ), 0.1-15 μg mL<sup>-1</sup> (NZ-NF), 0.1-10 μg mL<sup>-1</sup> (NZ-FZ), 0.1-20 μg mL<sup>-1</sup> (NF-FZ) and 0.1-13 μg mL<sup>-1</sup> (NZ-NF-FZ). In addition, this work evaluated the possible

change of maximum wavelength of nitrofurans mixtures at various concentration ratios. The results found that different concentration ratios did not provide any different from the 1:1:1 ratio of NZ:NF:FZ.

In sample preparation, nitrofurans were extracted by solvent extraction technique which used N, N-Dimethylformamide as extraction solvent. The optimum extraction and shaking times were 15 seconds and 1 hour, respectively. The sample was clean up by using a glass column 20 mm I.D. × 300 mm length, which was packed with alumina (70-230 mesh). The analyte was washed by dimethylformamide. These optimum conditions of sample preparation process gave high percentage recovery of each compound in the samples; *i.e.*, NZ: 93.7±1.1%, NF: 97.3±1.0% and FZ: 89.6±1.2%

Real samples, *i.e.*, chicken feed, pig feed and shrimp feed were sampling from the local animal feed stores. Nitrofurans were analyzed by standard addition method and were not detected in the samples.

In summary, this research could be applied for the determination of nitrofurans residues in animal feed products. These techniques have many advantages *i.e.* spectrophotometric method is simple, rapid and inexpensive for qualification and quantification of these antibiotics in samples and can be used for screening test these drug residues in farms. In the part of HPLC-UV method it provides low detection limits, using small volume of sample and can be used for routine analysis.

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