CHAPTER 4

CONCLUSIONS

Lean meat, both porcine and bovine, are the main edible tissue largely consumed in Thailand. There is concern that the presence of salbutamol in lean meat would affect human health, thus, the detection becomes an important issue in food safety analysis. This thesis investigated an alternative method for routine laboratory analysis to determine salbutamol in lean meat.

The first part of this work was the study of two fluorescence detection systems, *i.e.*, spectroflurometry and ion-pair chromatography with fluorescence detection (IPC-FLD). In spectrofluorometry, salbutamol was determined at the optimum excitation and emission wavelengths, 227 and 310 nm, respectively. This system provided a detection limit of 100 ng mL^{-1} and linearity from 100 to 10,000 ng mL^{-1} (R^2 ≥0.99). However, these values were not sensitive enough to directly determine trace concentration of salbutamol in lean meat.

IPC-FLD was then developed to enhance the sensitivity and selectivity of the analysis method. The analysis was done using an Alltima HP C_{18}, 3μm, 150×4.6 mm column. Optimum conditions were excitation and emission wavelengths, 227 and 310 nm; mobile phase 3 mM hexanesulfonate (containing 1.5% acetic acid) and methanol (70:30 v/v); flow rate 0.4 mL min^{-1}. This IPC-FLD system provided a detection limit of 0.5 ng mL^{-1} and the linear range from 0.5 to 10,000 ng mL^{-1} (R^2 ≥0.99). An internal standard (IS), bamethan, was used to increase the accuracy and precision of the extraction step. The calibration curve of internal standard for salbutamol, 1 to 50 ng mL^{-1}, provided a coefficient of determination (R^2) greater than 0.99 and relative standard deviation (%RSD) of less than 4%.

The second part was to investigate the optimum conditions for sample preparation step. Matrix solid phase dispersion (MSPD) combined with solid phase extraction (SPE) was used to extract and clean-up salbutamol from lean meat. A 0.5 g of lean meat sample was blended with 2 g of C_{18} sorbent and transferred to a column.

The column was washed with 8 mL of hexane-diethyl ether (60:40) and dried, then 8 mL of methanol was used to elute salbutamol from the column at a flow rate of 0.3 mL min^{-1}. For clean up step by SPE, the extractant was transferred to the SPE cartridge at a flow rate of 4 mL min^{-1} and eluted with 0.8 mL of methanol. The dried substance was reconstituted with 2 % acetic acid and filtered through a 0.2 μm Polyvinylidene fluoride (PVDF) disposable syringe filter before being injected to the optimum IPC-FLD system.

The proposed analysis method for salbutamol in lean meat was validated according to US-FDA bioanalytical method validation guidance and the Commission Decision 2002/657/EC. This analysis method provided low limit of quantification (LOQ) (1 ng g^{-1}) good selectivity and recovery (82-88 %) with acceptable precision (%RSD less than 15).

The effect of matrices interference was also studied in both porcine and bovine lean meat, the results showed that the slope of matrix curves were different from the standard curve, therefore, matrix-based calibration curve were used for quantitative analysis.

For qualitative and quantitative analysis of salbutamol in real sample, porcine and bovine lean meat, were purchased from fresh market and supermarket. Twelve samples, six porcine and six bovine, were extracted and analyzed using the proposed method. The concentrations of salbutamol were trace level and lower than the limit of quantification. These were confirmed by standard addition method where the result showed the concentration in the range of not detectable to 0.7 ng g^{-1}.

The proposed MSPD procedure has many advantages over the LLE reported by Kaewklapanyachareon (2001), *i.e.*,

(i) Simplicity
(ii) Small sample used (0.5 g *versus* 4 g)
(iii) Less solvent usage (16.5 mL *versus* 170 mL)
(iv) Lower quantification limit (1 ng g^{-1} *versus* 10 ng g^{-1})

In conclusion, the proposed analysis method in this thesis successfully combined a rapid extraction technique with highly effective method for analysis. This

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method is, therefore, practicable for routine monitoring analysis in food safety policy. In addition, a reduction in the use of solvent is achieved and this would help to reduce the risk of related to its use for both the operator's health and the environment.