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Contributed Paper

# Mixed Micelle-mediated Cloud Point Extraction Coupled to Spectrophotometry for Fast Screening of Salbutamol in Wastewater, Pig Feed and Pork Samples

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## ABSTRACT

A simple and rapid screening method for spectrophotometric determination of salbutamol based on mixed micelle-cloud point extraction (MM-CPE) coupled to derivatization with 4-aminoantipyrine reagent has been presented. A MM-CPE has been developed for the analysis of salbutamol in wastewater, pig feed and pork samples using Triton X-114 (TX-114) and cetyl trimethylammonium bromide (CTAB) as the mixed micellar extractant. The optimum MM-CPE conditions were: 10 mmol L-1 phosphate buffer pH 6.4, 5 mmol $L^{-1}$ CTAB, 0.25% (w/v) TX-114, 10% (w/v) sodium chloride and 6 min equilibration time at 30 °C. And the optimum for derivatization were: 0.05 mol $L^{-1}$ 4-aminoantipyrine, 0.12 mol $L^{-1}$ potassium hexacyanoferrate (III), 0.05 mol $L^{-1}$ sodium carbonate and 1 min reaction time at room temperature. Under the selected condition, the proposed method gave linear calibrations in the range of 0.05 – 10 mg $L^{-1}$. Limit of detection and limit of quantitation were 0.01 mg $L^{-1}$ and 0.03 mg $L^{-1}$, respectively. The proposed method has shown to be of high potential for analysis of salbutamol residues in wastewater, pig feed, and pork samples. The results of the proposed method agreed well with those obtained from HPLC (t-test, P=0.05).

**Keywords:** salbutamol, mixed micelle-mediated cloud point extraction, 4-aminoantipyrine, UV-visible spectrophotometry

## 1. INTRODUCTION

Salbutamol (2-(tert.-butylamino)-1-(4-hydroxy-3-(hydroxymethyl)phenyl)ethanol) is one $\beta_2$-agonist drug and is therefore widely used for the treatment of pulmonary diseases in animals and humans [1]. It is also used as growth-promoting agent in various animals to increase feeding efficiency and carcass leanness [2, 3]. However, $\beta_2$-agonists accumulated in animal tissues can cause acute poisoning when consumed by humans, with symptoms of muscular tremor, cardiac palpitation,

nervousness, headache, muscular pain, dizziness, nausea, vomiting, fever, and chills [4]. The world anti-doping agency (WADA) has prohibited the oral use of salbutamol [5]. In Thailand some farmers use beta-agonists such as salbutamol, clenbuterol, and ractopamine as feed additives to improve product performance and reduce carcass fat accretion. To ensure food safety, many countries have been established standard/regulation of maximum residue limit of salbutamol in food products. Therefore, a simple, sensitive, convenient and reliable method for analyzing the salbutamol residues in the samples is still required.

Several analytical methods have been developed for the detection of salbutamol, including spectrophotometry [6], flow injection analysis [7], high-performance liquid chromatography [8, 9], capillary electrophoresis [10], TLC-spectrodensitometric [11], electrogenerated chemiluminescence [12], electrochemical sensors [13, 14] and spectrophotometry is a simple and less expensive method which reported for determination of salbutamol based on forming of colour complexes [15]. The reagents namely 4-aminoantipyrine [7], Folin–Ciocalteau reagent [15], 2,6-dichloroquinone chlorimide [6], and 7,7,8,8-tetracyanoquinodimethane [6] have been widely used as coupling reagents for the determination of salbutamol in different matrices. These methods are rather non-selective and, moreover, they are influenced by the presence of other substances containing a redox group [7]. To improve the sensitivity of the detection in various complex samples, effective sample preparation methods are still required before analysis. Solid-phase extraction has been commonly employed for salbutamol analysis [7]. Some disadvantages of SPE are time-consuming, organic-solvent wasting, and quite expensive. Liquid-liquid extraction has been applied for β-agonists [16–18] and uses toxic organic solvents. The development of liquid-liquid extraction method using surfactants, known as cloud-point extraction (CPE), is an alternative approach that using surfactant as the extractant and required a temperature higher than its critical temperature for each surfactant is needed for clouding and phase separation. Their unique micro-heterogeneous structures capable of selective interaction with different solute molecules can strongly modify solubility, chemical equilibrium, kinetic and spectroscopic properties of analytes and reagents [19]. CPE presents important advantages such as its environmentally friendly, simplicity, good extraction efficiency [20], and high preconcentration factor [21] because a relatively small volume of surfactant-rich phase (SRP) is gained compared to that of the original aqueous solution (AQ) [22].

To preconcentrate of hydrophilic analytes such as salbutamol using CPE, some modified condition s required. CPE method using of mixed micelle exhibits synergism compared to a single surfactant due to higher surface activity, as well as showing co-stabilizing and co-sensitizing properties [23]. Mixed surfactants of different charges are used in order to achieve both ideal hydrophobic and non-ideal electrostatic interactions within the same extraction system [24]. The addition of small amount of ionic surfactants too non-ionic surfactants could be resulting in ideal hydrophobic and non-ideal electrostatic interactions within the same extraction system [25]. The combined use of cationic surfactant (such as cetyltrimethylammonium bromide (CTAB)) with nonionic surfactant (such as Triton X-114) has been documented to facilitate an increase in the extraction efficiency of polar organic compounds [26]. To the best of our knowledge, the applications of CPE have never reported for preconcentration of salbutamol.

This study is aimed to study mixed micelle-cloud point extraction combined with 4-aminoantipyrine derivatization for the determination of salbutamol residue in real samples. Cetyltrimethylammonium bromide (CTAB) and Triton X-114 were selected as an extraction solvent. The conditions for both derivatization and extraction are studied before applying to the spectrophotometric determination of salbutamol residue. The applicability of this

proposed method was evaluated by screening of salbutamol residue in wastewater, pig feed, and pork samples.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Materials

All reagents were of the analytical grade or higher. Salbutamol and clenbuterol standards of highest purity were purchased from Sigma-Aldrich (China), and ractopamine standard of highest purity was purchased from Fluka (Germany). The stock standard solutions of salbutamol, clenbuterol, and ractopamine (1000 μg $mL^{-1}$) were prepared by dissolving salbutamol standard in methanol. Working standard solutions were prepared by diluting the stock solution with water. Deionized water obtained from RiOs™ Type I Simplicity 185 (Millipore Waters, USA) with the resistivity of 18.2 MΩ.cm was used throughout the experiments. 4-aminoantipyrine was obtained from New Jersey (USA). Potassium hexacyanoferrate (III) ($K_3[Fe(CN)_6]$) was obtained from Merck (Germany). Sodium carbonate, sodium hydrogen phosphate, sodium dihydrogen phosphate, and sodium chloride were purchased from Ajax Finechem (Australia). Cetyltrimethylammonium bromide (CTAB) was purchased from Calbiochem (Germany), and Triton X-114 was obtained from Acros (USA).

### 2.2 Instrumentation

Absorbance measurements and spectra recordings were performed on a UV-Vis Spectrophotometer (Cary 60 series, USA). A 1-cm quartz cell was used throughout the experiments. A vortex mixer (Touch mixer model 232, USA) was used to increase mass transfer. Centrifuge instrument (NF 200 Santrifuj, Turkey) was used for phase separation. A pH meter model 713 from Metrohm (Metrohm, Switzerland) was employed to check pH of the buffer. The chromatographic instruments consisted of a Waters 1525 binary HPLC pump (USA), a Rheodyne injector with a sample loop of 20 μL, and a photodiode array detector. The Empower software 3 (Waters) was used for data acquisition. An Atlantis dC18 column (4.6×150 mm, 5.0 μm) from Waters (Ireland) was used.

### 2.3 4-Aminoantipyrine Derivatization Procedure

Sample solutions were sequentially performed at room temperature by adding of, 0.05 mol $L^{-1}$ of sodium carbonate and 0.12 mol $L^{-1}$ of potassium hexacyanoferrate (III), the solution was homogeneously mixed for 30 sec using a vortex mixer. Then, 0.05 of 4-aminoantipyrine was added. The solution was diluted with deionized water to the mark of 10.00 mL volumetric flask before detection. A blank reagent solution was performed in the same manner without salbutamol spiking. Absorbance was evaluated at the maximum absorption wavelength of 500 nm. All the experiments were consecutively studied in triplicate.

### 2.4 Mixed Micelle Cloud Point Extraction

Schematic diagram of the proposed extraction method was shown in Figure 1. Sample solutions were mixed with phosphate buffer (pH 6.4) and CTAB and left for 5 min. Afterward, NaCl and Triton X-114 solutions were added, and made up to a final volume of 10.00 mL with water. The mixture solution was transferred to a test tube, equilibrated at 30 °C for 10 min, and then centrifuged at 4000 rpm for 20 min. The solution was kept in an ice bath for 10 min. The surfactant-rich phase (upper part) was collected by a 1-mL syringe and then diluted with methanol 500 μL. An aqueous solution was then derivatized with 4-aminoantipyrine reagent (see Section 4-Aminoantipyrine derivatization procedure).

### 2.5 Sample Preparations

#### 2.5.1 Wastewater samples

Wastewater samples were obtained near pigsty in Maha Sarakham province, Northeast of Thailand. The samples were mixed well before the representative 250 mL of each sample was

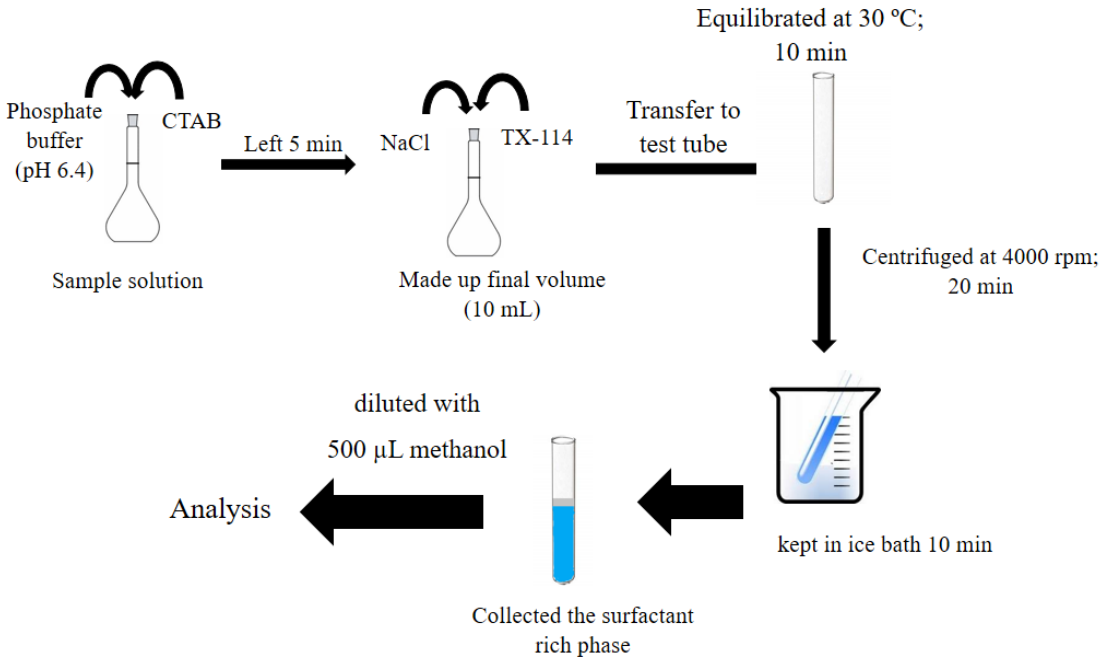


**Figure 1.** Schematic diagram of proposed extraction method.

filtered through a Whatman No.1 filter paper. Then 5.00 mL of wastewater samples were directly extracted by mixed micelle cloud point extraction (see Section Mixed micelle cloud point extraction). The collected surfactant-rich phase was then derivatized with the 4-aminoantipyrine reagent (as in Section 2.3. 4-Aminoantipyrine derivatization procedure). For the preparation of fortified samples, the samples were spiked with 0.50, 1.00, 2.00 mg $L^{-1}$ salbutamol before the extraction. All analyses were performed in triplicate under the optimum conditions of both spectrometry and HPLC. The solutions were filtered through 0.45 µm membrane filter before injecting into HPLC system.

### 2.5.2 Animal feed samples

Animal feed samples were obtained from a feed producer, which were gently pulverized, then the accurate amount (2.00 g) of each powder sample was sonicated with 8 mL ethanol and 2 mL water for 2 h. Then the sample solution was filtered through 0.22 µm filter twice. Then 5.00 mL of animal feed samples were directly extracted by mixed micelle cloud point extraction (see Section 2.4. Mixed micelle cloud point extraction). The collected surfactant-rich phase was then derivatized with the 4-aminoantipyrine reagent (as in Section 2.3. 4-Aminoantipyrine derivatization procedure). For the preparation of fortified samples, the samples were spiked with 0.50, 1.00, 2.00 mg $kg^{-1}$ salbutamol before the extraction. All analyses were performed in triplicate under the optimum conditions of both spectrometry and HPLC. The solutions were filtered through 0.45 µm membrane filter before injecting into HPLC system.

### 2.5.3 Pork samples

Pork samples were purchased from a local market and a supermarket in Maha Sarakham province, Northeast of Thailand. Briefly, the 1.5 g of pork was treated with 0.5 g of anhydrous sodium sulfate and 10.00 mL of 1% (v/v) acetic

acid in acetonitrile were added, vortexed, and centrifuged at 3,500 rpm for five minutes for fat and protein precipitation. The supernatants were passed through Whatman filter paper No. 1 and diluted with water to 10.00 mL. The 100 mL of acetic acid was added, and the samples were centrifuged again to further induce fat and protein precipitation. The resulting clear solutions were subjected to both spectrometry and HPLC. The solutions were filtered through 0.45 μm membrane filter before injecting into HPLC system. For recovery studies, samples were fortified with the analytes at concentrations of 0.50, 1.00, and 2.00 mg $kg^{-1}$ before fat and protein precipitation. All experiments were performed in triplicate.

## 3. RESULTS AND DISCUSSION

### 3.1 Optimum Condition for 4-aminoantipyrine Derivatization

Figure 2 shows the proposed reaction mechanism of salbutamol and 4-aminoantipyrine reagent in the presence of hexacyanoferrate (III) in alkaline medium. Base on the proposed method, salbutamol oxidizes with sodium carbonate and $K_3[Fe(CN)_6]$ to obtained a newly ligand that reacts with 4-aminoantipyrine to produce high-intensity red colour complex at room temperature. The water-soluble dye is stable and estimated colorimetrically with maximum absorption at 500 nm. To obtained high yield of diazotization, effects of $K_3[Fe(CN)_6]$, $Na_2CO_3$, and 4-aminoantipyrine concentration on the derivatization of salbutamol (1.0 mg $L^{-1}$) were tested. The concentration of $K_3[Fe(CN)_6]$ (0.015 – 0.30 mol $L^{-1}$), $Na_2CO_3$ (0.02 – 0.6 mol $L^{-1}$), and 4-aminoantipyrine (0.02 – 0.5 mol $L^{-1}$) were studied. It was observed that the highest response was obtained at the concentration of 0.12 mol $L^{-1}$ $K_3[Fe(CN)_6]$, 0.05 mol $L^{-1}$ $Na_2CO_3$ and 0.05 mol $L^{-1}$ 4-aminoantipyrine. The results were shown in Figure 3- 5. The results indicate that salbutamol could be determined by 4-aminoantipyrine reagent in the presence of hexacyanoferrate (III) in alkaline medium and the absorbance of derivatization was higher than that of a blank solution.

Under the selected derivatization conditions for salbutamol, other $\beta_2$-agonist drug (1.0 mg $L^{-1}$), including clenbuterol and ractopamine, were also tested with the derivatizing reagents. It was found that clenbuterol and ractopamine could also be derivatized with 4-aminoantipyrine reagent with high thermal reaction and high reaction time. Therefore, the azo compound formed between other $\beta_2$-agonist drug and 4-aminoantipyrine is slow and low sensitivity than that occur with salbutamol. In addition, because of the difference of steric hindrance, the most $\beta_2$-agonist drug could not be formed azo compounds with 4-aminoantipyrine. As can be seen that salbutamol gave the highest sensitivity compared with the other $\beta_2$-agonist drugs tested. Therefore, salbutamol could be used as the representative of the $\beta_2$-agonist drugs in screening methodology.

### 3.2 Optimization of the Mixed Micelle Cloud Point Extraction Conditions

Since the studied salbutamol is quite polar and pH-sensitive, therefore, the cationic ion-pair reagent, CTAB, was chosen in this study forms the ion-pair of salbutamol-CTAB before extraction with CPE. The salbutamol-CTAB ion-pair can transfer effectively into the aggregates of Triton X-114 compared to the original polar forms, leading to increase the extraction efficiency. Consequently, the pH of the CPE mixture and the concentration of the CTAB are the important parameters to study. The other parameters, including surfactant concentration, salt addition, equilibration temperature and time, were also optimized in subsequent experiments.

#### 3.2.1 Effect of pH

The pH of the sample solution is the first parameter to extract the target analyte because it affects the existing forms of analyte and its partitioning in the micellar phase [25]. When using mixed cationic and non-ionic surfactant as the extraction solvent, the maximum extraction efficiency is achieved at the pH value where the

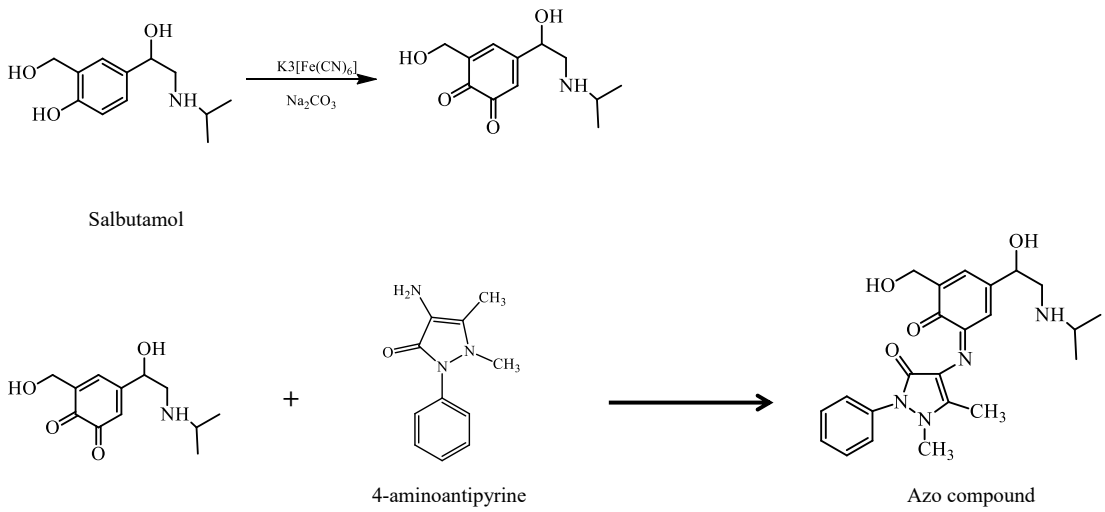


**Figure 2.** Proposed reaction mechanism of salbutamol with 4-aminoantipyrine by means of diazotization reaction.

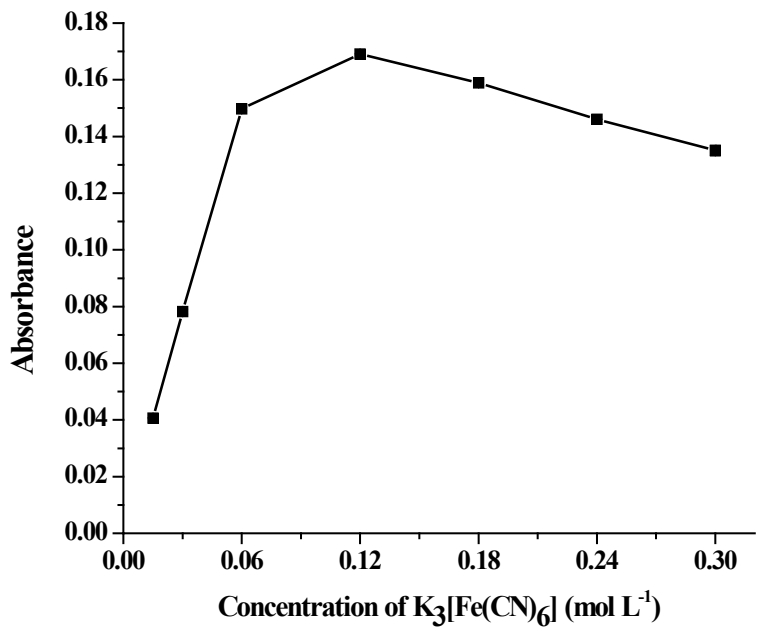


**Figure 3.** Effect of $K_3[Fe(CN)_6]$ concentration for derivatization of salbutamol.

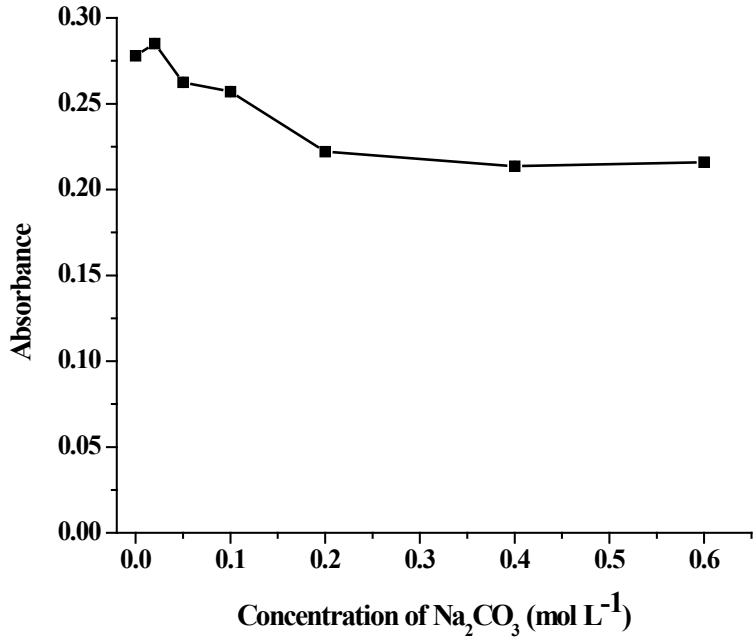


**Figure 4.** Effect of $Na_2CO_3$ concentration for derivatization of salbutamol.

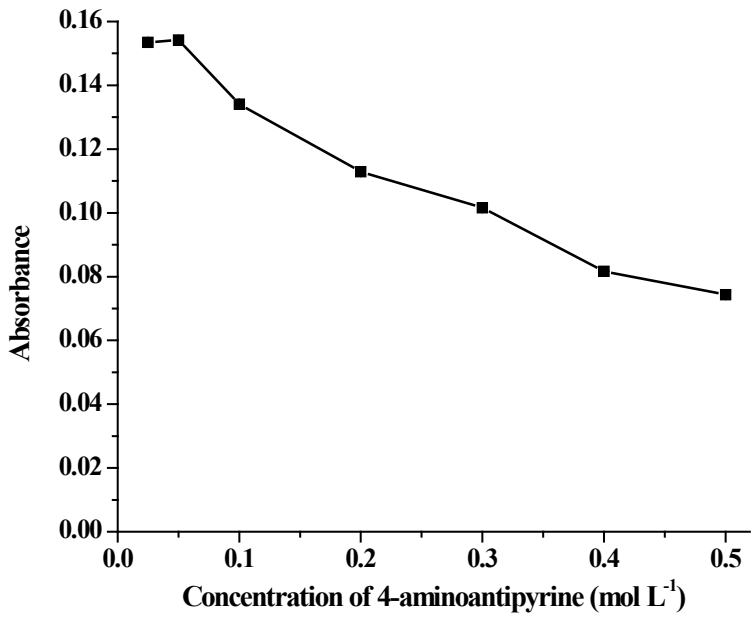


**Figure 5.** Effect of 4-aminoantipyrine concentration for derivatization of salbutamol.

anionic charge forms of the target analyte prevail [22]. In this study, the effect of pH was investigated in the range of 4.0 – 8.0, using 10 mmol $L^{-1}$ acetate buffer (data not shown). It was found that phase separation could not occur when the pH less than 5.8. The absorbance of salbutamol increases when the solution pH was increased which the highest absorbance was obtained up to pH 5.8. Therefore, the pH of 5.8 was selected for further studies.

**3.2.2 Effect of concentration of CTAB**

The effects of CTAB concentration on the extraction and determination of salbutamol was investigated in the range of 0.05 – 2.50 mmol $L^{-1}$ (data not shown). It was found that the absorbance of the surfactant-rich phase increased with increasing CTAB concentration up to 0.50 mmol $L^{-1}$ and slightly decreased at higher CTAB concentrations. Therefore, 0.05 mmol $L^{-1}$ of CTAB was chosen for the following experiments.

**3.2.3 Effect of concentration of Triton X-114**

The effect of surfactant concentration on cloud point extraction is considered to be very important because there is a narrow range within which easy phase separation, high extraction efficiency and increase analytical signal are accomplished [27]. In the proposed method, the effect of Triton X-114 concentration was studied in the range of 0.125 – 2.500% (w/v) (data not shown). It was found that phase separation has occurred with 0.25% (w/v) of Triton X-114. Therefore, 0.25% (w/v) of Triton X-114 was selected as the optimum value.

**3.2.4 Effect of salt addition**

The cloud point extraction of mixed surfactant systems decreased with the addition of a small amount of inorganic salts [25]. The cloud point depends on the kind and concentration of the salt added and the concentration of the surfactant used. The influence of cation and anion of salts on the decrease in the cloud point could be separately explained. The presence of the $Na^+$ cation may decrease the cloud point due to the dehydration of the polyoxyethylene chain, while anions ($Cl^-$ and $SO_4^{2-}$) likely cause a decrease in the self-association of water molecules. Thus, the hydration of the polyoxyethylene chain is decreased, and the surfactant solubility in water is diminished, causing a decrease in cloud point. In this study, NaCl, $Na_2CO_3$, $CH_3COONa$, and $Na_2SO_4$ were studied (data not shown). The results reveal that $Na_2SO_4$, $Na_2CO_3$, $CH_3COONa$ could not complete phase separation. Because NaCl is the inorganic salt used to make the mixed surfactant cloud point temperature reach room temperature [28]. Therefore, NaCl was further investigated in the weight range 1–15% (w/v) (data not shown). The maximum absorbance for salbutamol was obtained by 10% (w/v). Therefore, concentration of NaCl of 10% (w/v) was used.

**3.2.5 Effect of incubation time and equilibration temperature**

The incubation time and equilibration temperature are necessary to complete the reaction and to achieve easy phase separation and preconcentration as efficient as possible. It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature, which compromises completion of the reaction and efficient separation of phases. Triton X-114 was used in subsequent experiments because it has low cloud-point temperature (≈ 22 to 23 °C) [19]. Therefore, this study was undertaken at room temperature. The dependence of absorbance upon equilibration time was also studied within the range of 2 – 8 min (data not shown). It was found that 6 min was chosen as optimal time for equilibration and incubation time.

**3.3 Analytical Performance of the Method**

Under the selected conditions of derivatization condition and/or MM-CPE method, the linearity range of calibration method for determination of the salbutamol studied is obtained in the range of

0.1 – 10.0 mg $L^{-1}$. The linear equation is y=0.055x + 0.0051; $R^2$ = 0.9957, when Y is absorbance value and X is concentration of salbutamol (mg $L^{-1}$). LOD (average + 3SD) and LOQ (average + 10SD) of with the method were 0.10 and 0.30 mg $L^{-1}$, respectively where average represented a concentration of salbutamol in blank solution and SD was the standard deviation of the blank using seven experiments. Figure 6 shows absorption spectra of salbutamol and salbutamol with derivatization and MM-CPE method. Enrichment factor was 8, calculated by the slope of calibration graph with preconcentration/the slope of calibration graph without preconcentration. Precision of the developed method investigation at 1.0 mg $L^{-1}$ of salbutamol using n = 7 was good at 1.34%. Reproducibility of the proposed method was 4.71%. for 3.0 mg $L^{-1}$ of salbutamol for 7 days

### 3.4 Application to Real Samples

The proposed method was applied to determine salbutamol in wastewater, pig feed, and pork samples. Salbutamol residues were detected in all studied samples as summarized in Table 1. It can be seen that the contamination was in range 017 – 1.10 mg $L^{-1}$ in wastewater samples, 1.26 mg $kg^{-1}$ in pig feed, and 1.56 mg $kg^{-1}$ in pork samples.

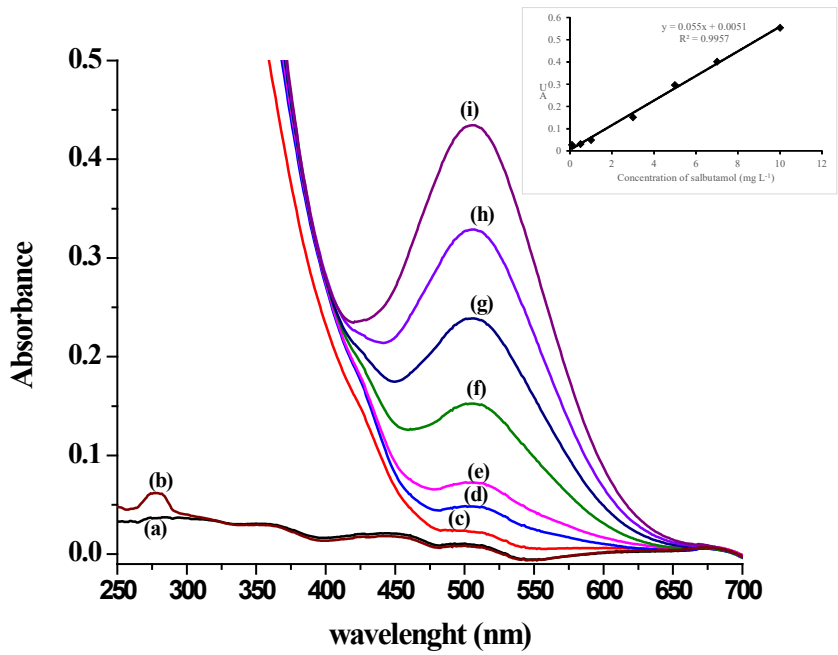


**Figure 6.** Absorption spectra of (a) reagent blank, (b) salbutamol 3 mg $L^{-1}$, (c) salbutamol 0.1 mg $L^{-1}$ with 4-aminoantipyrine by means of diazotization reaction, (d) salbutamol 0.5 mg $L^{-1}$ with 4-aminoantipyrine by means of diazotization reaction, (e) salbutamol 1.0 mg $L^{-1}$ with 4-aminoantipyrine by means of diazotization reaction, (f) salbutamol 3.0 mg $L^{-1}$ with 4-aminoantipyrine by means of diazotization reaction, (g) salbutamol 5.0 mg $L^{-1}$ with 4-aminoantipyrine by means of diazotization reaction, (h) salbutamol 7.0 mg $L^{-1}$ with 4-aminoantipyrine by means of diazotization reaction, (i) salbutamol 10.0 mg $L^{-1}$ with 4-aminoantipyrine by means of diazotization reaction.

**Table 1.** Concentration of salbutamol residue in the studied samples measured by the proposed method and HPLC-UV method.

| Sample | Concentration of salbutamol (mg $L^{-1}$ for wastewater, mg $kg^{-1}$ for pig feed and pork) ± SD (n=3) | |
|---|---|---|
| | Proposed method | HPLC-UV |
| Wastewater I | 1.10 ± 0.05 | 1.05 ± 0.04 |
| Wastewater II | 1.07 ± 0.02 | 1.01 ± 0.02 |
| Wastewater III | 1.08 ± 0.07 | 1.00 ± 0.05 |
| Pig feed | 1.26 ± 0.11 | 1.34 ± 0.29 |
| Pork | 1.56 ± 0.19 | 1.64 ± 0.04 |

The accuracy of the proposed method was evaluated using the recovery index (%recovery). Three levels of standard mixture of the studied penicillins (0.10, 0.50, and 1.00 mg $L^{-1}$) were fortified into milk samples prior to mixed micelle-CPE, followed by three replicate analyses. The recoveries of each level and mean recovery were calculated and are summarized in Table 2. Therefore, this method has been proven to be suitable for the determination of salbutamol residues in wastewater, pig feed, and pork samples. It shows high recoveries (greater than 90% on average), which are in the acceptable recoveries for trace analysis. Therefore, this method has been proven to be suitable for the determination of salbutamol in samples. It is expected that this method will be effective for multi-residues analysis in other matrices as well.

**Table 2.** Recovery studied of the proposed method.

| Sample | Concentration of salbutamol (mg $L^{-1}$ for wastewater, mg $kg^{-1}$ for pig feed and pork) | | Recovery (%) ± SD (n=3) |
|---|---|---|---|
| | Spiked | Found | |
| Wastewater I | 0.00 | 1.10 | - |
| | 0.10 | 1.20 | 100.00 ± 1.70 |
| | 0.50 | 1.66 | 111.50 ± 2.37 |
| | 1.00 | 2.12 | 101.69 ± 5.35 |
| Wastewater II | 0.00 | 1.07 | - |
| | 0.10 | 1.19 | 120.00 ± 2.30 |
| | 0.50 | 1.58 | 102.00 ± 4.30 |
| | 1.00 | 1.99 | 92.00 ± 3.20 |
| Wastewater III | 0.00 | 1.08 | - |
| | 0.10 | 1.20 | 120.00 ± 2.80 |
| | 0.50 | 1.43 | 70.00 ± 1.34 |
| | 1.00 | 2.00 | 92.00 ± 2.30 |

**Table 2.** Recovery studied of the proposed method. (Continued)

| Sample | Concentration of salbutamol (mg $L^{-1}$ for wastewater, mg $kg^{-1}$ for pig feed and pork) | | Recovery (%) ± SD (n=3) |
|---|---|---|---|
| | Spiked | Found | |
| Pig feed | 0.00 | 1.26 | - |
| | 0.10 | 1.37 | 110.00 ± 1.90 |
| | 0.50 | 1.78 | 104.00 ± 2.90 |
| | 1.00 | 2.21 | 95.00 ± 2.45 |
| Pork | 0.00 | 1.56 | - |
| | 0.10 | 1.65 | 90.00 ± 1.21 |
| | 0.50 | 2.12 | 111.26 ±1.91 |
| | 1.00 | 2.34 | 78.18 ± 0.70 |

## 4. CONCLUSIONS

Mixed micelle-mediated cloud point extraction coupled to spectrophotometric method has been demonstrated to be a powerful method for determination of salbutamol in wastewater, animal feed and pork samples. 4-aminoantipyrine reagent in the presence of hexacyanoferrate (III) in alkaline medium has been chosen as a derivatizing agent. The proposed method was compared to other sample preparation methods for the analysis of salbutamol residues. The proposed method is superior to the others in terms of high analytical performance, short analysis time and environmentally friendly since it required just a low cost. The sensitivity of the proposed method in term of LOD is almost comparable to that obtained from another microextraction method. The presented method achieves low LODs, which are below the MRLs of salbutamol residues in various samples. The result obtained by the developed method were in good agreement with those obtained by HPLC (*t*-test, P=0.05). This method shows simple, rapid and reliable for applying to screening of salbutamol residues in wastewater, animal feed, and pork samples and possible to be a test kit for routine analysis.

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