Talanta 81 (2010) 486–492

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Mixed micelle-cloud point extraction for the analysis of penicillin residues in bovine milk by high performance liquid chromatography

Chunyapuk Kukusamude[a], Apichai Santalad[a], Suthasinee Boonchiangma[a], Rodjana Burakham[a], Supalax Srijaranai[a,*], Orawon Chailapakul[b]

[a] Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[b] Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

ARTICLE INFO

Article history:
Received 23 October 2009
Received in revised form
16 December 2009
Accepted 16 December 2009
Available online 28 December 2009

Keywords:
Antibiotics
Penicillins
Mixed micelle-cloud point extraction
High performance liquid chromatography
Milk

ABSTRACT

A mixed micelle-cloud point extraction (MM-CPE) has been developed for the analysis of penicillin antibiotics (ampicillin, penicillin G, oxacillin, and cloxacillin) in milk samples using Triton X-114 (TX-114) and cethyl trimethylammonium bromide (CTAB) as the mixed micellar extractant. The parameters affecting the MM-CPE that were investigated including solution pH, CTAB concentration, TX-114 concentration, electrolyte salt, equilibration temperature and incubation time. The optimum MM-CPE conditions were: 10 mmol L^{-1} phosphate buffer pH 8, 0.06% (w/v) CTAB, 1.5% (w/v) TX-114, and 7% (w/v) Na_2SO_4, and 5 min equilibration at 40 °C. The separation of penicillins was achieved within 8 min under the HPLC conditions: a Vydac C_{18} column, isocratic elution of 5 mmol L^{-1} phosphate buffer (pH 6.6) and methanol (55:45, v/v), and a flow rate of 1 mL min^{-1}, with photodiode array detection at 215 and 244 nm. Under the selected condition, the proposed method gave linear calibrations in the range 0.002–10 μg mL^{-1} with correlation coefficients greater than 0.999. Limits of detection (LOD) were 2–3 ng mL^{-1}, and 15–40-fold enhancement compared to that without preconcentration. Good reproducibility was achieved with relative standard deviation <5% for peak area and <3% for retention time. High accuracy, with recoveries higher than 80%, was obtained. The proposed mixed micelle-CPE-HPLC method has shown to be of high potential for the analysis of penicillin residues in milk with LOD comparable to the established maximum residue limits (4–30 ng mL^{-1}).

1. Introduction

Penicillins are β-lactam antibiotics which are widely used in livestock for treatment of bacterial infections caused by Gram-positive and Gram-negative organisms, promote growth and maintain animal health [1]. They include cloxacillin (CLO), ampicillin (AMP), oxacillin (OXA), and penicillin G (PEN-G). CLO is formulated for use in cows at the point of milk production to treat existing mastitis and to protect further infections during the dry period [1]. AMP and CLO are used against both Gram-positive and Gram-negative organisms, especially caused by penicillinase-producing staphylococci [1–3]. OXA is the penicillinase-resistant penicillin, while PEN-G is the only natural penicillin available in the market and is one of the most commonly misused drugs in steers and dairy cows [1]. The systematic use of antibiotics may leave residues in derived, food including milk. The antibiotic residues can have undesirable effects on consumer health such as allergies and the appearance of drug-resistant strains [4]. To protect the health of consumers, the maximum residue limits (MRLs) of antibiotics in milk have been regulated by authorities including the Food and Drug Administration (FDA) and European Council Regulation (ECC) 2377/90 [5,6]. The MRLs for antibiotic residues in milk and muscle are in the range of 4–125 ng mL^{-1} depending on the specific type of antibiotic. Thus, the analytical methods with the following characteristics are in demand: accurate, ease to use, economical in terms of cost and time, and capable of detecting the residues below MRLs.

Several analytical methods have been developed for the analysis of antibiotic residues analysis in dairy products such as microbial assays based on inhibition of microbial growth [7,8], liquid chromatography–mass spectrometry (LC–MS) [9–11], and capillary electrophoresis (CE) [9,12]. However, microbial assays fail to identify and quantify of individual residues, while LC–MS is very expensive and CE when equipped with UV detection is low sensitivity because of its short optical path length. High performance liquid chromatography (HPLC) with photodiode array (PDA) or UV detection is widely used as a sensitive method for the analysis of antibiotics and metabolites in biomatrices [5–9,13–17]. To obtain the reliability and accuracy of the detection in complex matrices, suitable sample preparation methods are required before instru-

* Corresponding author. Tel.: +66 4320 2222 41x12243; fax: +66 4320 2373.
E-mail address: supalax@kku.ac.th (S. Srijaranai).

0039-9140/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2009.12.029

mental analysis. The most popular techniques for the extraction of antibiotics from their original matrices are liquid–liquid extraction or solvent extraction [9,18,19], solid-phase extraction (SPE) [10–12,20–22], dispersive solid-phase extraction (DSPE) [23,24] and pressurized liquid extraction (PLE) [6,9]. Recently, extraction method using surfactants, known as cloud point extraction (CPE), is a promising new separation and preconcentration technique that is being applied to a range of analytes in different sample matrices [25–29]. The advantages of CPE over traditional solvent extraction are good extraction efficiency, low cost, and use of non-toxic reagents, and less toxic organic solvents [30,31]. In addition, CPE shows good compatibility between the surfactant and the hydro-organic mobile phase in the analysis using HPLC [30,31]. CPE provides high extraction efficiency and a large preconcentration factor because a relatively small volume of surfactant-rich phase (SRP) is obtained compared to that of the original aqueous solution (AQ). This methodology is most effective for hydrophobic solutes [25,32].

To apply CPE for hydrophilic analytes such as penicillin antibiotics, a modified condition is needed. Mixed micelle-cloud point extraction has been possible by the addition of a small amount of the cationic cethyl trimethylammonium bromide (CTAB) or anionic sodium dodecyl sulphate (SDS) surfactants into the original non-ionic surfactant CPE system. Mixed surfactants of different charges are used in order to achieve both ideal hydrophobic and non-ideal electrostatic interactions within the same extraction system [33]. Mixed micelle-CPE or mixed micelle mediated extraction has been used for preconcentration of organic compounds [33–35] and metal cations [33,36]. The combined use of cationic surfactant with non-ionic surfactant has been documented to facilitate an increase in the extraction efficiency of polar organic compounds [30,37]. Until now, the application of CPE has never been reported for preconcentration of β-lactam antibiotics.

The propose of this study is to develop CPE based on mixed micellar extractants of Triton X-114 and CTAB for extraction and preconcentration of penicillin antibiotics (ampicillin, penicillin G, oxacillin and cloxacillin) in milk samples prior to analysis by HPLC. The parameters affecting CPE include pH of sample solution, concentration of reagents (CTAB, Triton X-114 and electrolyte salt), equilibration temperature and incubation time, have been optimized.

2. Experimental

2.1. Chemicals and reagents

All the penicillin standards were purchased from Fluka, including the penicillin G sodium salt (Austria), ampicillin trihydrate (Belgium), oxacillin sodium salt (India), and cloxacillin (USA). The stock solutions of the penicillin standards (1000 μg mL^{-1}) were prepared by dissolving each in an appropriate amount in deionized water and stored at 4 °C. The working solutions were freshly prepared by diluting the stock solutions with water. Triton X-114 was purchased from ACROS Organics (USA). The stock solution of Triton X-114 (25%, w/v) was prepared by dissolving it in deionized water. Cethyl trimethylammonium bromide (CTAB) was obtained from Fluka (Denmark). The stock solution of CTAB (2%, w/v) was prepared in deionized water. Table 1 shows the chemical structures and physical properties of the studied penicillins and surfactants. Sodium chloride (NaCl) (APS Fine Chem, Australia) and sodium sulfate anhydrous (anhydrous Na_2SO_4) (Fluka, Switzerland) were also used, along with disodium hydrogen phosphate (Na_2HPO_4) from Merck (Germany). Deionized water with the resistivity of 18.2 MΩ cm from RiO_s^{TM} Type I Simplicity 185 (Millipore water, USA) was used throughout. Acetonitrile (ACN) and methanol (MeOH) were of HPLC grade (Lab-Scan Asia, Co., Ltd, Thailand).

2.2. Instrumentation

The HPLC system consisted of a Waters 600E Multi-solvent Delivery System, a Waters In-Line Degasser AF, a Rheodyne injector with sample loop of 20 μL, and a Waters 2996 Photodiode Array Detector. Empower Software was used for data acquisition and analysis. A Vydac C_{18} reversed-phase column (250 mm × 4.6 mm, 5 μm) from Dionex (USA) coupled to a guard column was used. A centrifuge (Kokusan Type H-11N, Biomed Group Co. Ltd., Japan) was used for phase separation.

2.3. Mixed micelle cloud point extraction

Sample or standard solutions (3.00 mL) were mixed with phosphate buffer (pH 8) and CTAB and left for 5 min. Afterward, Na_2SO_4 and Triton X-114 solution 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 40 °C for 5 min, and then centrifuged at 3000 rpm for 20 min. The solution was kept in an ice bath for 10 min. The aqueous phase (lower phase) was then removed by a long-needled syringe. A surfactant-rich phase (600 μL) was mixed with 300 μL of MeOH and ACN (1:1, v/v) to reduce the viscosity before injecting into HPLC. The concentrations of the reagents used were optimized.

2.4. Analysis of milk samples

Commercial milk samples were obtained from a local market in Khon Kaen province, northeastern Thailand, and fresh milk samples were obtained from the dairy shelf of the store at the Faculty of Agriculture, Khon Kaen University. The proteins and fats in 3.00 mL samples were precipitated by shaking vigorously with 6 mL of a mixture of acetone and acetonitrile (5:1, v/v). The solution was then centrifuged at 4000 rpm for 20 min. The supernatant was evaporated in a nitrogen atmosphere to eliminate organic solvents. The residue was dissolved with phosphate buffer to a final volume of 3.00 mL and finally extracted by mixed micelle-cloud point extraction (see Section 2.3).

3. Results and discussion

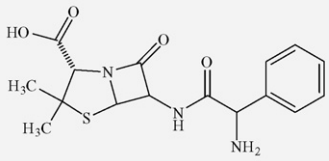
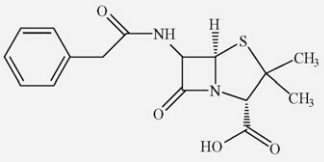
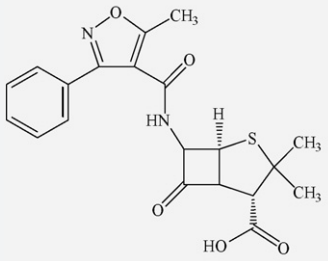
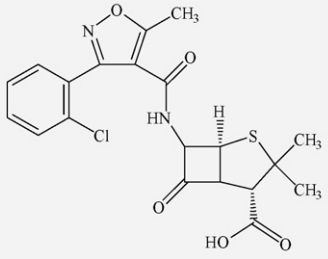
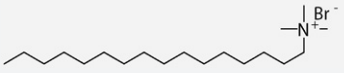
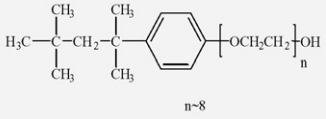
3.1. HPLC conditions for analysis of penicillins

The separation of four penicillins including ampicillin (AMP), penicillin G (PEN-G), oxacillin (OXA) and cloxacillin (CLO) was carried out using isocratic elution of 5 mmol L^{-1} phosphate buffer (pH 6.6) and MeOH (55:45, v/v) at a flow rate of 1.0 mL min^{-1}. The photodiode array detection was accomplished at 215 nm for AMP and PEN-G and 244 nm for OXA and CLO. Experiments were performed at ambient temperature. Under the optimum conditions, the studied penicillins were separated within 8 min with the elution order of AMP, PEN-G, OXA, and CLO, respectively (see Fig. 1). The resolution of all the studied penicillins was greater than 2.3.

3.2. Optimization of mixed micelle cloud point extraction

Mixed micelle-cloud point extraction was chosen for extraction of the target analytes since the studied penicillin antibiotics are quite polar and pH-sensitive. The pK_{a1} (–COOH) of the studied penicillins is about 2.4 [40]. Under normal conditions, the studied analytes are anions and highly soluble in aqueous solution, leading to poor extraction efficiency in CPE. Consequently, the cationic ion-pair reagent, CTAB, was used in this study forms the ion-pair of penicillin-CTAB before CPE. The penicillin-CTAB ion-pair can transfer effectively into the aggregates of Triton X-114 compared to the original polar forms, leading to higher extraction efficiency. Thus,

Table 1
Chemical and physical properties of the studied penicillins and surfactants.

Name	Abbreviation	Structure	pK_a	Molecular weight (g mol^{-1})	cmc[a] (mmol L^{-1})
Ampicillin	AMP		2.4	349.46	
Penicillin G	PEN-G		2.5	333.37	
Oxacillin	OXA		2.4	400.40	
Cloxacillin	CLO		2.4	434.90	
Cethyl trimethyl ammonium bromide	CTAB			364.46	0.92[b]
Triton X-114	TX-114			558.75	0.265[c]

[a] Critical micelle concentration.
[b] 0.034% (w/v) [38].
[c] 0.015% (w/v) [39].

the pH of the CPE mixture and the concentration of the CTAB are the first parameters to investigate. The other parameters, including surfactant concentration, salt, equilibration temperature and time, were optimized in subsequent experiments. The results are expressed as peak area detected at 215 or 244 nm, depending on the antibiotics.

3.2.1. Effect of solution pH

In case of ionizable organic analytes, pH influences the partition of the target analytes in the micelle phase [25]. When using mixed cationic and non-ionic surfactant as the extractants, the maximum extraction efficiency is achieved at the pH value where the anionic charged form of the target analyte prevails [30]. In this study, the effect of pH was studied over the range 5–10, using 10 mmol L^{-1} phosphate buffer. The results demonstrate that pH of solution had strong effect on the extraction of the studied penicillins (see Fig. 2a). The peak area of all analytes increased when the solution pH was increased which was the highest peak area obtained up to pH 7. To ensure extraction of the analytes with low sensitivity (AMP and PEN-G) and to avoid the critical dependence on pH for OXA and CLO at pH 7, the pH of 8 was selected for further studies.

3.2.2. Effect of concentration of CTAB and reaction time

The effects of CTAB concentrations in the range 0.00–0.07% (w/v) and reaction times (2–10 min) on the extraction of penicillins were investigated. As the results in Fig. 2b, illustrate clearly that the concentration of CTAB strongly affects the extraction efficiency. The peak areas of all analytes increased sharply with increasing CTAB concentration up to 0.01% (w/v). Beyond this point, the peak area was only slightly affected by the CTAB concentration, so the high value of 0.06% (w/v) of CTAB was chosen for the following experiments.

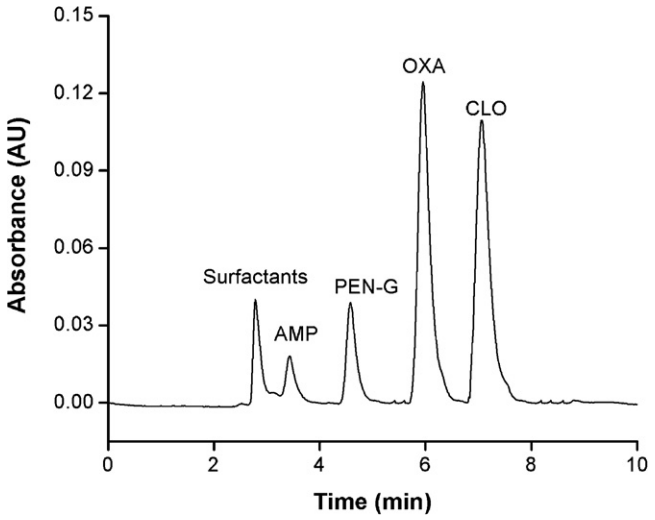


Fig. 1. Chromatogram of standard antibiotics (5 μg mL^{-1} each) under the optimum conditions of mixed micelle-CPE and HPLC. CPE conditions: a mixture solution (10 mmol L^{-1} phosphate pH 8) of 0.06% (w/v) CTAB, 1.5% (w/v) TX-114, and 7% (w/v) Na_2SO_4, and 5 min equilibrated at 40 °C. HPLC conditions: a Vydac C_{18} column, isocratic elution of 5 mmol L^{-1} phosphate buffer (pH 6.6) and methanol (55:45, v/v), sample injection of 20 μL, and a flow rate of 1 mL min^{-1}. Photodiode array detection was set at 215 nm.

The results of reaction time experiments (data not shown) reveal that it had little effect on the extraction of AMP, PEN-G and CLO, but OXA extraction depended strongly on reaction time, with the maximum peak area at 5 min. However, the peak area of the studied penicillins decreased after 5 min, especially for OXA, so a 5-min reaction time was used throughout.

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, maximum extraction efficiency and analytical signal detection are accomplished [25]. In this study, the effect of Triton X-114 concentration was investigated in the range 0.025–2.5% (w/v). Fig. 2c demonstrates that the peak area of all studied penicillins increased with the increasing of Triton X-114 concentration. Increasing of the concentration in the range 0.025–1.5%, resulted in the significant increases in peak area for AMP and OXA, but there were only slight increases for PEN-G and CLO. Therefore, 1.5% (w/v) Triton X-114 was selected as the optimum value.

3.2.4. *Effect of electrolyte salt*

It was reported that the cloud point (CP) of a mixed non-ionic and ionic surfactants decreased with the addition of a small amount of inorganic salts [41]. The CP depends on the nature and concentration of the salt added and the concentration of the surfactant used [42]. The influence of cation and anion of salts on the decrease in

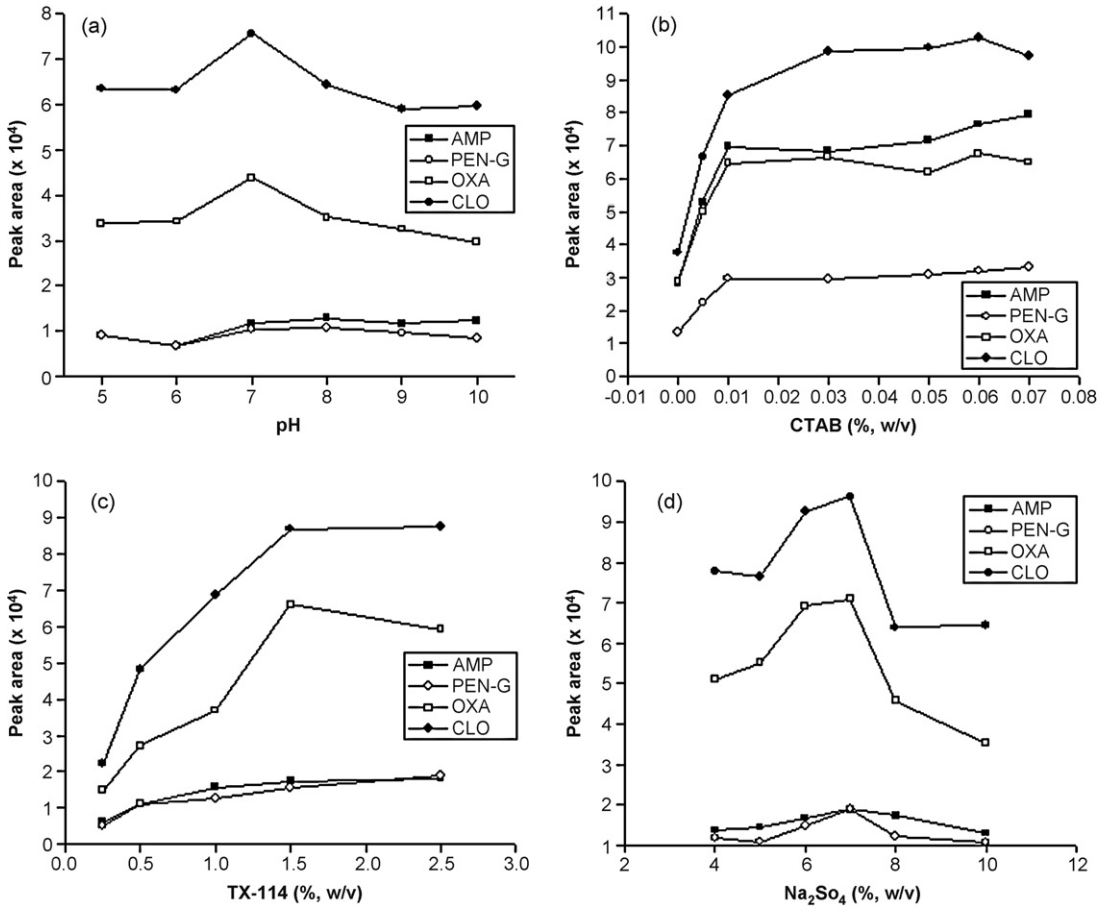


Fig. 2. Influence of (a) pH, (b) CTAB concentration, (c) Triton X-114 concentration, and (d) amount of Na_2SO_4 on the extraction efficiency of the studied penicillins (0.5 μg mL^{-1} each). Conditions: (a) 10 mmol L^{-1} phosphate buffers containing 0.01% (w/v) CTAB, 10% (w/v) NaCl, and 0.5% (w/v) Triton X-114, equilibrated at 30 °C for 5 min; (b) 10 mmol L^{-1} phosphate buffer pH 8, 10% (w/v) Na_2SO_4, and 0.5% (w/v) Triton X-114, equilibrated at 40 °C for 5 min; (c) solution was mixed with 10 mmol L^{-1} phosphate buffer pH 8, 0.01% (w/v) CTAB, 7% (w/v) Na_2SO_4, equilibrated at 30 °C for 5 min; (d) 10 mmol L^{-1} phosphate buffer pH 8, 0.01% (w/v) CTAB, 0.5% (w/v) Triton X-114, and equilibrated at 30 °C for 5 min.

Table 2
Analytical features of MM-CPE-HPLC method compared with direct HPLC method for the standard antibiotics.

Antibiotic	Linear range ($\mu g\,mL^{-1}$)	Linear equation	r^2	LOD ($ng\,mL^{-1}$)	LOQ ($ng\,mL^{-1}$)	MRL[b] ($ng\,mL^{-1}$)
Ampicillin	0.002–10 (0.08–10)[a]	$y = 32296x$ ($y = 26542x$)	0.9993 (0.9992)	2 (80)	7 (270)	4
Penicillin G	0.002–10 (0.08–10)	$y = 90356x$ ($y = 25906x$)	0.9996 (0.9990)	2 (80)	7 (270)	4
Oxacillin	0.002–10 (0.03–10)	$y = 164049x$ ($y = 21094x$)	0.9994 (0.9994)	2 (30)	7 (100)	30
Cloxacillin	0.003–10 (0.1–10)	$y = 90978x$ ($y = 11453x$)	0.9993 (0.9986)	3 (100)	10 (330)	30

[a] The results in parenthesis obtained from direct HPLC method (without MM-CPE).
[b] Maximum residue limits (MRL) in milk samples established by EU [12,45].

the CP can be separately explained. The presence of the Na^+ cation may decrease the CP due to the dehydration of the polyoxyethylene chain, while anions (Cl^- and SO_4^{2-}) likely cause a decrease in 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 CP [43]. In this study, NaCl and Na_2SO_4 were investigated at 10% (w/v) salts (data not shown). The results reveal that Na_2SO_4 produced higher extraction efficiency than NaCl at the same concentration. So, Na_2SO_4 was further investigated in the weight range 4–10% (w/v) as the results shown in Fig. 2d. The maximum peak area for each of the analytes was achieved at 7% (w/v) salt. Beyond this point, the peak area decreased sharply for CLO, OXA and PEN-G, while AMP was only slightly affected. Na_2SO_4 7% (w/v) was selected as optimum.

3.2.5. *Effect of equilibration temperature and incubation time*

Temperature and incubation time in the cloud point extraction (CPE) play important roles, especially when dealing with inert species. It has been shown that increased temperature and prolonged reaction time result in more satisfactory extraction [25]. Thus, it is necessary to examine the effect of temperature on MM-CPE performance. In general, CPE methodology is achieved at the optimum equilibrium temperature where it is 15–20 °C higher than the cloud point temperature (T_C) of the surfactant [26,44]. In this study, equilibration temperatures were investigated in the range 30–60 °C (T_C of Triton X-114 is about 23–25 °C) [26,27]. It was found (data not shown) that peak area of most analytes (except AMP) decreased when the temperature was higher than 40 °C. It was clearly observed for OXA and CLO which exhibited sharp decreases in peak area. This may due to the decomposition of the studied penicillins. The incubation time (2–10 min) was also studied (data not shown). It was found that peak area of the studied penicillins was highest value at 5 min and remained constant for AMP and PEN-G, but the area decreased for OXA and CLO. Thus, the MM-CPE was carried out at 40 °C for 5 min.

To summarize, the optimum conditions of MME-CPE were: 10 mmol L^{-1} phosphate buffer pH 8, 0.06% (w/v) CTAB, 1.5% (w/v) TX-114, and 7% (w/v) Na_2SO_4 in 10.00 mL solution, and 5 min equilibration at 40 °C.

3.3. *Analytical feature and method validation*

Using the optimum conditions of CPE and HPLC, the analytical performance of the proposed method including linearity, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated, along with precision in terms of intra-day and inter-day behavior. Accuracy (% recovery) was investigated to verify the capability of the proposed method in real samples.

The calibration curves of peak area versus concentration of the studied penicillins after MM-CPE were linear over the concentration range of 0.002–10 $\mu g\,mL^{-1}$ (13 concentrations) with the coefficient of correlation (r^2) greater than 0.999. The analytical parameters of working calibration curves for the studied analytes are listed in Table 2.

LOD and LOQ are used to illustrate the sensitivity of the proposed method in milk sample matrices. The LOD was evaluated as the concentration giving a response signal three times the noise level (S/N = 3), while the LOQ used S/N = 10. The results are summarized in Table 2. It is shown that the proposed mixed micelle-CPE gave low detection limits in the range of 2–3 ng mL^{-1} which is lower than the maximum residue limits (MRLs) established by several control authorities at 30 ng mL^{-1} (for OXA and CLO), while that of AMP and PEN-G were set at 4 ng mL^{-1} [12,45]. Enhancement factor (EF) is defined as the ratio of LOD obtained from MM-CPE preconcentration method and without MM-CPE. A high EF in the range of 15-fold (OXA) to 40-fold (AMP and PEN-G) was obtained.

To verify the precisions of the proposed method, intra-day ($n = 5$) and inter-day ($n = 3$ days $\times$ 5) were evaluated in terms of relative standard deviation (%RSD) of retention time (t_R) and peak area obtained by replicate injections of the standard mixture of 0.5, 2, and 5 $\mu g\,mL^{-1}$. The results (Table 2) demonstrate that good precisions of overall intra-day and inter-day measurements were obtained with RSD lower than 3% (t_R) and 5% (peak area).

3.4. *Analysis of antibiotics in milk samples*

The proposed mixed micelle-CPE method was used to analyze the residual antibiotics in 12 brands of commercial and fresh milk samples. No contamination of the studied penicillin antibiotics was observed in any of the milk samples studied. Typical chromatograms of milk sample blank and with spiked the studied penicillins after the CPE are demonstrated in Fig. 3.

The accuracy of the proposed method was evaluated using recovery index (%recovery). Three levels of standard mixture of the studied penicillins (0.5, 2, and 5 $\mu g\,mL^{-1}$) were fortified into milk samples prior to mixed micelle-CPE, followed by five replicate analyses. The recoveries of each level and mean recovery were calculated and are summarized in Table 3. It shows high recoveries (greater than 80% on average) which are in the acceptable

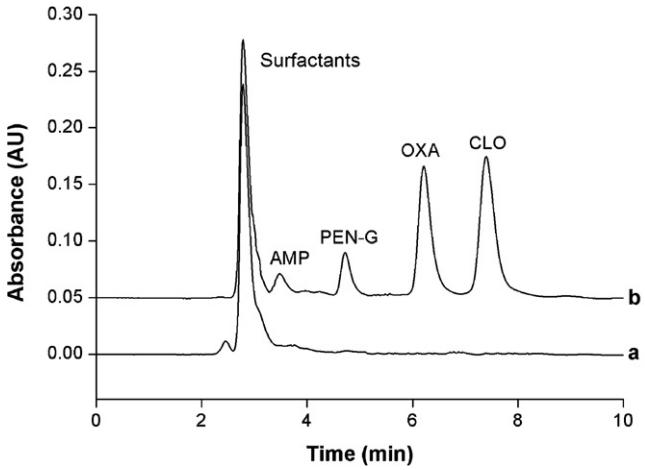


Fig. 3. Overlaid chromatograms of (a) bovine milk sample and (b) spiked milk sample at 5 $\mu g\,mL^{-1}$. The conditions are described in Fig. 1.

Table 3
Intra- and inter-day precisions and recovery of the standards spiked in bovine milk samples.

Antibiotic	Spiked level ($\mu g\,mL^{-1}$)	Precision, RSD (%)				Recovery (%) (mean ± SD) ($n = 5$)
		Intra-day ($n = 5$)		Inter-day ($n = 3 \times 5$)		
		t_R	Peak area	t_R	Peak area	
Ampicillin	0.5	1.3	4.5	2.4	4.5	84.4 ± 5.5
	2	0.9	1.4	2.7	1.6	78.7 ± 2.6
	5	1.4	1.8	2.8	2.3	82.8 ± 3.8
Mean						81.9 ± 3.6
Penicillin G	0.5	0.6	2.2	2.4	3.1	94.0 ± 3.7
	2	0.8	1.9	2.5	2.5	98.6 ± 3.5
	5	1.3	2.1	2.6	2.7	93.9 ± 4.0
Mean						95.5 ± 2.7
Oxacillin	0.5	0.2	1.3	1.9	2.3	97.6 ± 2.2
	2	0.7	3.7	2.3	4.4	91.4 ± 4.5
	5	0.5	0.9	1.3	1.1	97.0 ± 2.8
Mean						95.4 ± 3.4
Cloxacillin	0.5	0.3	2.8	1.7	3.5	97.2 ± 1.8
	2	0.4	1.3	2.0	1.5	91.3 ± 1.6
	5	0.6	1.7	1.1	2.3	98.0 ± 3.5
Mean						95.5 ± 3.7

recoveries for trace analysis established by the Association of Official Agricultural Chemists (AOAC) and European commission ($\geq$70% and $\leq$110%) [46]. Therefore, this method has been proven to be suitable for the determination of antibiotic residues in milk. It is expected that this method will be effective for multi-residues analysis in other matrices as well.

4. Conclusion

Mixed micelle-cloud point extraction using Triton X-114 nonionic and CTAB cationic surfactants coupled with HPLC has been proven to be an effective preconcentration method that is simple, rapid, and reliable for the analysis of penicillin antibiotics and potentially for other highly polar compounds. The present method gave high enhancement factors of (15–40-fold) as well as low limits of detection (in the range of 2–3 $ng\,mL^{-1}$ which are below the acceptable MRLs) for the studied penicillins in milk samples. The method has excellent potential for the determination of penicillin antibiotic residues in milk samples.

Acknowledgements

Financial support from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education is gratefully acknowledged. We are also grateful to the Commission on the Higher Education through the CHE-RES-RG project and the National Research Council of Thailand (NRCT) through the High throughput Screening/Analysis: Tool for Drug Discovery, Disease diagnosis and Health safety project for financial support. Prof. Dr. Richard L. Deming (Department of Chemistry and Biochemistry, California State University, Fullerton, CA) is gratefully acknowledged for his helpful discussion.

References

[1] V.F. Samanidou, D.E. Giannakis, A. Papadaki, J. Sep. Sci. 32 (2009) 1302–1311.
[2] M.A. Bacigalupa, G. Meroni, F. Secundo, R. Lelli, Talanta 77 (2008) 126–130.
[3] V. Kumar, H. Bhutani, S. Singh, J. Pharm. Biomed. Anal. 43 (2007) 769–773.
[4] M.A. García-Mayor, R.M. Garcinuño, P. Fernández-Hernando, J.S. Durand-Alegría, J. Chromatogr. A 1122 (2006) 76–83.
[5] R.V. Oliveira, A.C. De Pietro, Q.B. Cass, Talanta 71 (2007) 1233–1238.
[6] E. Benito-Peña, J.L. Urraca, M.C. Moreno-Bondi, J. Pharm. Biomed. Anal. 49 (2009) 289–294.
[7] W.A. Moats, R.D. Romanowski, J. Chromatogr. A 812 (1998) 237–247.
[8] T.F.H. Bovee, M.G. Pikkemaat, J. Chromatogr. A 1216 (2009) 8035–8050.
[9] L. Kantiani, M. Farré, D. Barceló, Trends Anal. Chem. 28 (2009) 729–744.
[10] S.B. Turnipseed, W.C. Andersen, C.M. Karbiwnyk, M.R. Madson, K.E. Miller, Rapid Commun. Mass Spectrom. 22 (2008) 1467–1480.
[11] L. Kantiani, M. Farré, M. Sibum, C. Postigo, M. López de Alda, D. Barceló, Anal. Chem. 48 (2009) 4285–4295.
[12] S.M. Santos, M. Henriques, A.C. Duarte, V.I. Esteves, Talanta 71 (2007) 731–737.
[13] C.G. Smyrniotakis, H.A. Archontaki, J. Pharm. Biomed. Anal. 43 (2007) 506–514.
[14] M.D. Marazuela, M.C. Moreno-Bondi, J. Chromatogr. A 1034 (2004) 25–32.
[15] B.F. Spisso, A.L. de Oliveira e Jesus, M.A.G. de Araújo Júnior, M.A. Monteiro, Anal. Chim. Acta 581 (2007) 108–117.
[16] S. De Baere, P. De Backer, Anal. Chim. Acta 586 (2007) 319–325.
[17] V. Gamba, C. terzano, L. Fioroni, S. Moretti, G. Dusi, R. Galarini, Anal. Chim. Acta 637 (2009) 18–23.
[18] M.J. Gramse, P.E. Jacobson, J. AOAC Int. 88 (2005) 679–683.
[19] M. Dousa, R. Hosamnova, J. Pharm. Biomed. Anal. 37 (2005) 273–279.
[20] S. Makeswaran, I. Patterson, J. Points, Anal. Chim. Acta 529 (2005) 151–157.
[21] A.A.M. Stolker, P. Rutgers, E. Oosterink, J.J.P. Lasaroms, R.J.B. Peters, J.A. Van Rhijn, M.W.F. Nielen, Anal. Bioanal. Chem. 391 (2008) 2309–2322.
[22] M. Becker, E. Zittlau, M. Petz, Anal. Chim. Acta 520 (2004) 19–32.
[23] W.H. Tsai, T.C. Huang, J.J. Huang, Y.H. Hsue, H.Y. Chuang, J. Chromatogr. A 1216 (2009) 2263–2269.
[24] N. Fontanals, R.M. Marcïe, F. Borrull, J. Chromatogr. A 1152 (2007) 14–31.
[25] E.K. Paleologos, D.L. Giokas, M.I. Karayannis, Trends Anal. Chem. 24 (2005) 426–436.
[26] A. Santalad, S. Srijaranai, R. Burakham, J.D. Glennon, R.L. Deming, Anal. Bioanal. Chem. 394 (2009) 1307–1317.
[27] Z.-M. Zhou, D.-Y. Zhao, J. Wang, W.-J. Zhao, M.-M. Yang, J. Chromatogr. A 1216 (2009) 30–35.
[28] N. Pourreza, M. Zareian, J. Hazard. Mater. 165 (2009) 1124–1127.
[29] J. Zhou, J. Chen, Y. Cheng, D. Li, F. Hu, H. Li, Talanta 79 (2009) 189–193.
[30] J.C.A. de Wuilloud, R.G. Wuilloud, B.B.M. Sadi, J.A. Caruso, Analyst 128 (2003) 453–458.
[31] A. Afkhami, M. Bahram, S. Gholami, Z. Zand, Anal. Biochem. 336 (2005) 295–299.
[32] R.P. Paradkar, R.R. Williams, Anal. Chem. 66 (1994) 2752–2756.
[33] T. Madrakian, A. Afkhami, A. Mousavi, Talanta 71 (2007) 610–614.
[34] E.J. Kim, D.O. Shah, Langmuir 18 (2002) 10105–10108.
[35] J.W. Kang, G. De Reymaeker, A.V. Schepdael, E. Roets, J. Hoogmartens, Electrophoresis 22 (2001) 1356–1362.
[36] E.K. Paleologos, A.G. Vlessidis, M.I. Karayannis, N.P. Evmiridis, Anal. Chim. Acta 477 (2003) 223–231.
[37] F.H. Quina, W.L. Hinze, Ind. Eng. Chem. Res. 38 (1999) 4150–4168.
[38] A. Chattopadhyay, E. London, Anal. Biochem. 139 (1984) 408–412.
[39] I. Xiarchos, D. Doulia, J. Hazard. Mater. 136 (2006) 882–888.
[40] M.I. Bailón-Pérez, A.M. García-Campaña, C. Cruces-Blanco, M. del Olmo Iruela, Electrophoresis 28 (2007) 4082–4090.

[41] T. Gu, P.A. Galera-Gómez, Colloids Surf. A: Physicochem. Eng. Aspects 104 (1995) 307–312.
[42] S. Kumar, D. Sharma, Kabir-ud-Din, Langmuir 16 (2000) 6821–6824.
[43] E. Tatara, K. Materna, A. Schaadt, Hans-Jrg Bart, J. Szymanowski, Environ. Sci. Technol. 39 (2005) 3110–3115.
[44] X. Liu, X.-H. Chen, Y.-Y. Zhang, W.-T. Liu, K.-S. Bi, J. Chromatogr. B 856 (2007) 273–277.
[45] Commission Regulation (EC) 508/1999, Off. J. Eur. Commun. 4 March 1999, L 60, 16.
[46] C.M. Stobba-Wiley, R.S. Readnour, J. AOAC Int. 83 (2000) 555–562.