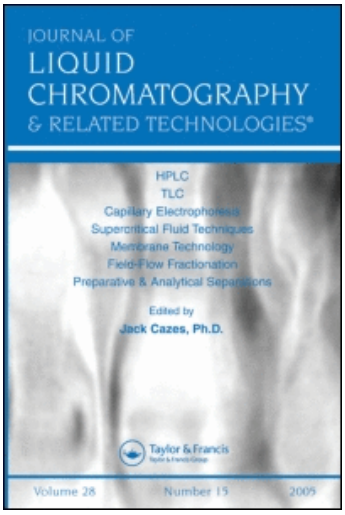

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Determination of Cefacetrile and Cefuroxime Residues in Milk by Thin-Layer Chromatography

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Abstract: A simple thin-layer chromatography screening method was developed for the determination of two cephalosporins (cefacetrile and cefuroxime) in milk. Only two developments of TLC plates with concentrating zones are required: pre-development with hexane, as a clean-up procedure to remove lipids from milk samples, and a proper development with methanol-toluene-ethyl acetate-98% formic acid (5:20:65:10) phase. The video camera was used to detect spots on chromatograms. The recoveries of both antibiotics in milk were calculated over five days from the preparation of the samples. The best results, obtained on the second day of the experiment, were 97.66% for cefacetrile and 86.13% for cefuroxime.

Keywords: Cefacetrile, Cefuroxime, Cephalosporins, Milk, Residues, TLC

INTRODUCTION

Cephalosporins are the group of ß-lactam antibiotics widely used both in human and veterinary medicine. These compounds are divided into four generations based on their antimicrobial properties and time of their discovery.[1] Cefacetrile, a first generation cephalosporin, exerts high activity against Gram-positive bacteria but less activity against Gram-negative ones.[2] Cefuroxime, a second generation cephalosporin, is highly active

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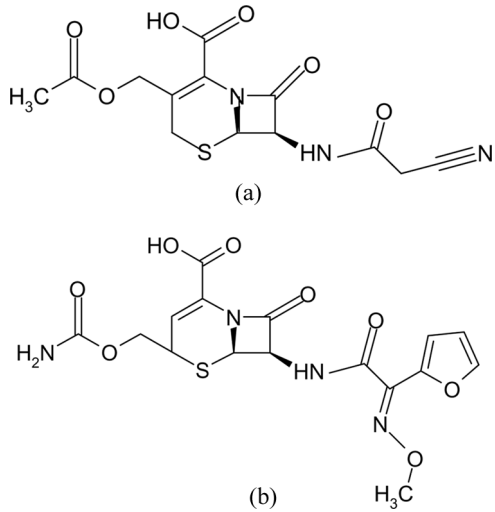


Figure 1. Chemical structure of (a) cefacetrile and (b) cefuroxime.

against a range of Gram-positive and Gram-negative bacteria.[3] The chemical structures of the compounds analyzed are presented in Figure 1.

Common use of cefacetrile and cefuroxime for the treatment of clinical mastitis in cows has made it compulsory to monitor the concentration of these agents in bovine milk and meat. Consumption of products containing antibiotic residues can cause allergic reactions in humans and the emergence of antibiotic-resistant bacteria.[4] The use of veterinary drugs is precisely established by appropriate regulations in the European Union countries.

Various chromatographic methods have been reported for the determination of cephlosporins in different matrices such as animal tissues,[5–6] milk[6–11] or biological fluids.[12–18] Among them, only several thin-layer chromatography (TLC) methods have been described.

This paper presents a simple TLC method for the determination of cefacetrile and cefuroxime in milk.

EXPERIMENTAL

Equipment and Reagents

DS chambers were obtained from Chromdes, Lublin, Poland. Pre-coated TLC plates Si60 F_{254} 10 cm × 20 cm, with concentrating zone 2.5 cm × 10 cm were purchased from Merck (Darmstadt, Germany). The areas of the spots were obtained via measurement of the absorbance at

254 nm using the Camag Reprostar 3 Video Camera (Muttenz, Switzerland). Plate images were documented by DigiStore 2 Documentation System (Camag). The data was processed with winCATS software, version 1.4.1 (Camag).

Cefacetrile was purchased from Sigma Chemicals (St. Louis, MO) while cefuroxime was obtained from GlaxoSmithKline (Poznań, Poland). Methanol, hexane, ethyl acetate, toluene and 98% formic acid were supplied from POCH (Gliwice, Poland).

Methods

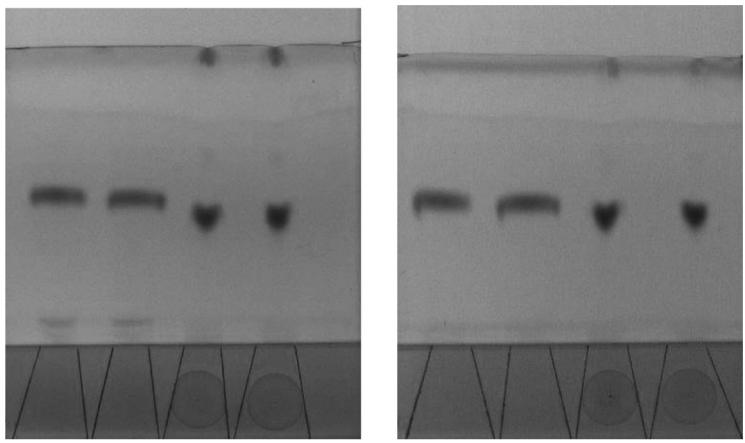
The standard stock solutions of cefacetrile and cefuroxime were prepared at a concentration of $1.0\,mg\,mL^{-1}$ by dissolving the pure substances in methanol. Working standard solutions at a concentration of $0.1\,mg \cdot mL^{-1}$ were prepared by appropriate dilution with methanol. Milk samples containing $1.0\,mg\,mL^{-1}$ of cefacetrile or cefuroxime were prepared by fortification of 1 mL of 2% fat milk with 1 mg of one of the antibiotic. Milk samples containing $0.1\,mg\,mL^{-1}$ of cefacetrile or cefuroxime were prepared by fortification of 0.9 mL of 2% fat milk with 0.1 mL of an appropriate standard solution at $1.0\,mg\,mL^{-1}$.

Two 10 μL samples of standard solutions and two 10 μL samples of milk spiked at the same concentration level were applied on the TLC plates Si60 F_{254} in the middle of trapezoidal-shaped regions created by the incision in the plate's concentrating zones. The plates were set into DS sandwich chambers and pre-developed with hexane to remove lipids from milk samples. After air-drying, the plates were developed to a distance of 9.5 cm with a proper solvent system, i.e., methanol-toluene-ethyl acetate-98% formic acid (5:20:65:10). The measurement of each spot area was carried out at 254 nm using the video camera and then recoveries were calculated. The TLC experiments were repeated twice for each of antibiotics at a given level on first, second, third and fifth day after preparation of milk samples.

RESULTS AND DISCUSSION

The milk samples were applied into the middle of trapezoidal-shaped regions prepared by incision in the plate's concentrating zone in order to obtain a regular front of developing solvent (see Figure 2). This idea was taken from the paper of I. Choma concerning flumequine and doxycycline[19] and worked well in this experiment too.

It has appeared necessary to defat the samples to prevent tailing of the spots. Thus, pre-development with hexane had been applied before



(a) (b)

Figure 2. TLC chromatogram of: (a) (1,2) cefacetrile methanol standard ($1.0\,mg \cdot mL^{-1}$); (3,4) milk spiked with cefacetrile at $1.0\,mg \cdot mL^{-1}$ ($10\,\mu g$ of the antibiotic in the spot); (b) (1,2) cefuroxime methanol standard ($1.0\,mg \cdot mL^{-1}$); (3,4) milk spiked with cefuroxime at $1.0\,mg \cdot mL^{-1}$ ($10\,\mu g$ of the antibiotic in the spot). The plate was pre-developed with hexane. Solvent system: methanol-toluene-ethyl acetate-98% formic acid (5:20:65:10).

the development with methanol-toluene-ethyl acetate-98% formic acid (5:20:65:10) solvent system. The proteins of milk left at the concentrating zone while the antibiotics, free of the matrix, migrated along the plate.

Time of the antibiotics dissolution in milk seemed to be essential. Thus, portions of milk with appropriate amounts of cefacetrile or cefuroxime were sonificated for three different periods of time. In case of cefuroxime, time of antibiotic dissolution in milk did not appear to be significant, whereas recoveries of cefacetrile in milk samples sonificated for 10, 20 or 30 minutes were significantly different. Figure 3 shows the dependence between the time of cefacetrile dissolution in milk and recoveries of the antibiotic from the samples. As can be seen, the best results were acquired when the sample was sonificated for 30 minutes. From that moment all experiments were done for milk samples spiked in this way.

The percentage recoveries of cefacetrile and cefuroxime from milk were controlled on first, second, third and fifth day after preparation of the samples. Table 1 presents the mean recoveries and the standard deviations of both antibiotics isolated from milk samples spiked at two different concentration levels. The highest results were obtained on the second day of the experiment.

The lower recoveries on the first day of the experiment seemed to be related to insufficient homogenization of the milk samples directly after

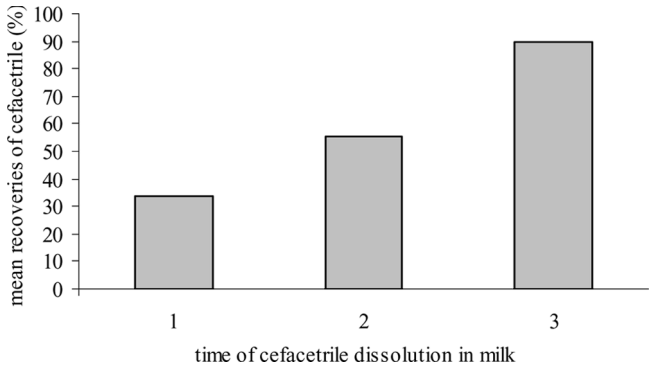


Figure 3. The dependence between the time of cefacetrile dissolution in milk and the recoveries of cefacetrile from the milk samples. Dissolution in milk: (1) 10 minutes; (2) 20 minutes; (3) 30 minutes.

spiking. Nevertheless, the results in Table 1 pointed to the drop in the concentrations of both antibiotics in analyzed milk samples in the course of time. This can be related to a partial decomposition of the antibiotics in milk matrix probably related to milk enzymatic system. As it was checked earlier methanol standards are stable in the fridge at least for one month. The highest recoveries were equal to 94.80% and 97.66%

Table 1. The mean recoveries of cefacetrile and cefuroxime isolated from milk

Analyte	Concentration ($mg \cdot mL^{-1}$)	The day of the experiment	Mean recoveries (%)	Standard deviation (n = 4)
Cefacetrile	1.0	1	89.52	4.61
		2	94.80	2.49
		3	92.91	4.01
		5	86.97	6.29
	0.1	1	72.47	8.17
		2	97.66	7.50
		3	46.99	5.57
		5	37.84	10.40
Cefuroxime	1.0	1	85.04	1.19
		2	86.13	2.97
		3	83.84	2.79
		5	72.00	3.30
	0.1	1	75.38	5.15
		2	63.20	9.62
		3	58.15	5.07
		5	47.99	5.10

for cefacetrile at concentration levels 1.0 mg mL^{-1} and 0.1 mg mL^{-1} respectively. For cefuroxime the highest recoveries were 86.13% and 75.38% at concentration levels 1.0 mg mL^{-1} and 0.1 mg mL^{-1} respectively.

CONCLUSIONS

The purpose of this work was to develop a simple thin-layer chromatography screening method for the determination of cefacetrile and cefuroxime in milk. The method described herein was found to be simple, rapid and cheap. Especially important was: to obtain regular front of a solvent system, to defat the sample, to find adequate time of the antibiotics dissolution in milk and to receive the highest recoveries of cefacetrile and cefuroxime. Only two developments of the plate with concentrating zone are required: pre-development with hexane, in order to remove lipids from the samples and a proper analysis. The percentage recoveries of both antibiotics added to milk were controlled on first, second, third and fifth day after preparation of the samples. Time of cefacetrile dissolution in milk, significant in this experiment, would not be considered when the method is applied to natural milk samples. Very essential is the information on the drop in the recoveries during storage of the milk samples related to the decomposition of the antibiotics in the milk matrix.

REFERENCES

1. El-Shaboury, S.R.; Saleh, G.A.; Mohamed, F.A.; Rageh, A.H. Analysis of cephalosporin antibiotics. J. Pharm. Biomed. Anal. **2007**, *45*, 1–19.
2. The European Agency for the Evaluation of Medical Products Paper, No EMEA/MRL/754/00-FINAL July 2000.
3. Lewicki, J.; Arnold, D. FAO Food and Nutrition Paper, No 41/16.
4. Dajan, A.D. Vet. Microbiol. **1993**, *35*, 213.
5. Mastovska, K.; Lightfield, A.R. StreamLining methodology for the multiresidue analysis of β-lactam antibiotics in bovine kidney using liquid chromatography–tandem mass spectrometry. J. Chromatogr. A **2008**, *1202*, 118–123.
6. Becker, M.; Zittlau, E.; Petz, M. Residue analysis of 15 penicillins and cephalosporins in bovine muscle, kidney and milk by liquid chromatography–tandem mass spectrometry. Anal. Chim. Acta **2004**, *520*, 19–32.
7. Oliveira, R.V.; De Pietro, A.C.; Cass, Q.B. Quantification of cephalexin as residue levels in bovine milk by high-performance liquid chromatography with on-line sample cleanup. Talanta **2007**, *71*, 1233–1238.
8. Suhren, G.; Knappstein, K. Detection of cefquinome in milk by liquid chromatography and screening methods. Anal. Chim. Acta **2003**, *483*, 363–372.

9. Sørensen, L.K.; Snor, L.K. Determination of cephalosporins in raw bovine milk by high-performance liquid chromatography. J. Chromatogr. A **2000**, *882*, 145–151.
10. Keever, J.; Voyksner, R.D.; Tyczkowska, K.L. Quantitative determination of ceftiofur in milk by liquid chromatography–electrospray mass spectrometry. J. Chromatogr. A **1998**, *794*, 57–62.
11. Choma, I. M.; Kowalski, C.; Lodkowski, R.; Burmańczuk, A.; Komaniecka, I. TLC-DB as an alternative to the HPLC method in the determination of cefacetril residues in cow's milk. J. Liq. Chromatogr. Rel. Technol. **2008**, *31*, 1903–1912.
12. Denooz, R.; Charlier, C. Simultaneous determination of five β-lactam antibiotics (cefepim, ceftazidim, cefuroxim, meropenem and piperacillin) in human plasma by high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B **2008**, *864*, 161–167.
13. Vera López, K.J.; Faria Bertoluci, D.; Vicente, K.M.; Dell'Aquilla, A.M.; Jorge Santos, S.R.C. Simultaneous determination of cefepime, vancomycin and imipenem in human plasma of burn patients by high-performance liquid chromatography. J. Chromatogr. B **2007**, *860*, 241–245.
14. Aleksić, M.M.; Kapetanović, V.; Atanacković, J.; Jocić, B.; Zečević, M. Simultaneous determination of cefotaxime and desacetylcefotaxime in real urine sample using voltammetric and high-performance liquid chromatographic methods. Talanta **2008**, *77*, 131–137.
15. Can, N.Ö.; Altiokka, G.; Aboul-Enein, H.Y. Determination of cefuroxime axetil in tablets and biological fluids using liquid chromatography and flow injection analysis. Anal. Chim. Acta **2006**, *576*, 246–252.
16. Jacobson, G.A.; Martinod, S.; Cunningham, C.P. Determination of ceftiofur in bovine plasma by HPLC-DAD. J. Pharm. Biomed. Anal. **2006**, *40*, 1249–1252.
17. De Baere, S.; Pille, F.; Croubels, S.; Ceelen, L.; De Backer, P. High-performance liquid chromatographic-UV detection analysis of ceftiofur and its active metabolite desfuroylceftiofur in horse plasma and synovial fluid after regional intravenous perfusion and systemic intravenous injection of ceftiofur sodium. Anal. Chim. Acta **2004**, *512*, 75–84.
18. Samanidou, V.F.; Ioannou, A. S.; Papadoyannis, I.N. The use of a monolithic column to improve the simultaneous determination of four cephalosporin antibiotics in pharmaceuticals and body fluids by HPLC after solid phase extraction–a comparison with a conventional reversed-phase silica-based column. J. Chromatogr. B **2004**, *809*, 175–182.
19. Choma, I.; Grenda, D.; Malinowska, I.; Suprynowicz, Z. Determination of flumequine and doxycycline in milk by a simple thin-layer chromatographic method. J. Chromatogr. B **1999**, *734*, 7–14.

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