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Short communication

On-line coupling of solid-phase extraction and capillary electrophoresis for the determination of cefoperazone and ceftiofur in plasma

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Abstract

We present a method for determining two cephalosporins (cefoperazone and ceftiofur) in plasma by on-line solid-phase extraction (SPE)—capillary zone electrophoresis (CZE) with a T-split interface. Using this interface, a part of the SPE elution plug containing the cephalosporins is injected while the rest of the sample is flushed to waste. SPE was carried out using a C_{18} micro-precolumn and the cephalosporins presented good retention properties with breakthrough volumes above 1 ml. Using a desorption volume of 426 nl of acetonitrile, recoveries were 75 and 90%, for cefoperazone and ceftiofur, respectively. The resulting elution volume was about 1.8 μl. A deproteinization step was included prior to SPE for the analysis of plasma samples with recoveries of 90 and 57% for cefoperazone and ceftiofur, respectively. With UV detection at 254 nm, linear relationships between the injected concentration and peak area was measured between 10 and 500 ng ml^{-1} for standards, and 200 and 1500 ng ml^{-1} for plasma samples. Intra-day ($n = 5$) and inter-day ($n = 5$) peak area repeatability were lower than 12% RSD. The detection limits obtained for spiked plasma (100 ng ml^{-1} cefoperazone and ceftiofur) are sufficient for applying the method to pharmacokinetic studies.
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1. Introduction

Cephalosporins are β-lactam antibiotics that are closely related to penicillins in chemical structure and antibacterial action. Cephalosporins are the most prescribed antibiotics because they are among the safest and the most effective broad-spectrum antimicrobial agents [1]. Cefoperazone and ceftiofur, which are two of the most important third-generation cephalosporins, are used for the treatment of infections caused by Gram-positive and Gram-negative bacteria and have activity against β-lactamases. In veterinary medicine, cephalosporins are widely used to treat and prevent various infectious diseases. However, this can be a potential risk because drug residues may be present in biological fluids and tissues. Therefore, sensitive determination of cephalosporins in biological samples is required in various fields such as therapeutic drug monitoring and analytical and forensic toxicology.

Liquid chromatography (LC) has played a key role in the determination of cephalosporins in pharmaceutical preparations and in biological fluids [2–5]. This versatile technique has been commonly used to analyze cephalosporin residues in biological samples such as milk [2,3], plasma [4], serum [1,2,5], cerebrospinal fluid [5], urine [1,5] and synovial fluid [4]. Some papers have been published the determination of cefoperazone [6] or ceftiofur [4,7–9] in plasma by HPLC and two different plasma pretreatments were used: a simple deproteinization, (using organic solvent or acidic media) [6,7], and a derivatization step followed by SPE [4,8,9]. Detection limits ranged from 1000 [6,7] to 150 ng ml^{-1} [4,9], for the deproteinization and the derivatization pretreatment, respectively.

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However, while in the first pretreatment only the parent compound ceftiofur is determined, in the second one, also their metabolites could be quantified. Although higher sensitivity is obtained by the derivatization method, the procedure is considerably longer and more time-consuming than the protein precipitation.

In the last few years, capillary electrophoresis (CE) has also been used as an alternative to HPLC to analyze cephalosporins in different matrices [10–17]. Generally, the electroseparation modes used for these compounds are capillary zone electrophoresis (CZE) [10–13] and micellar electrokinetic capillary electrophoresis (MEKC) [13–17]. The detection limits for the CE analysis of cephalosporins reported were in the range of 0.2–2 $\mu g\,ml^{-1}$ [7–11].

CE offers high separation efficiency but its routine application in bioanalysis is still limited due to a relatively low sensitivity when UV absorbance detection is used. This is caused by the small injection volumes and the short detection path length, leading to concentration limits of detection which often are higher than analyte concentrations in biological matrices. A straightforward way of improving detection limits in capillary electrophoresis is sample stacking [18,19], which is based on conductivity differences between the sample and the electrolyte solution. However, stacking can be problematic when complex sample matrices, such as biological samples, have to be analyzed because they often contain high concentrations of salts and proteins. Solid-phase extraction (SPE) is frequently used as sample preparation technique because it can simultaneously enrich the trace analytes and remove salts and a wide variety of other compounds that can interfere with the subsequent CE separation [20–26]. The coupling of SPE and CE for the analysis of biological samples would preferably be carried out in an on-line mode because of short total analysis times, the minimum of sample handling, and the possibility of automation [21–23].

Recently, we have developed two on-line SPE–CE systems, in which the SPE and CE steps can be optimized and conducted independently [20,21,27]. In the present study, one of the systems [20] has been applied to determine cephalosporins in cow plasma. The on-line SPE–CE approach is based on a Tee-split interface, which is required for coupling both techniques and to allow an injection volume that is suitable for CE analysis because the SPE elution volume is considerably larger than the maximum volume that can be injected into the CE capillary. Using this interface, a part of the SPE elution plug is injected while the rest of the sample is flushed to waste. As plasma is a relatively complex sample, the introduction of a pretreatment step prior to injection was necessary to prevent clogging of the SPE column. Attention has been paid to the optimization of the sample pretreatment in order to achieve a fast, easy and effective sample preparation procedure.

The aim of this study was to develop a simple and rapid method for the analysis of ceftiofur and cefoperazone in plasma samples. In order to improve the poor sensitivity of the CE, an SPE step on-line coupled to CE has been included. We also evaluated the suitability of a plasma pretreatment procedure.

2. Materials and methods

2.1. Chemicals

Sodium hydroxide, potassium hydroxide and ammonium acetate were purchased from Merck (Darmstadt, Germany) and acetonitrile from Biosolve BV (Valkenswaard, The Netherlands). Ammonium hydroxide was obtained from Fluka Chemie (Buchs SG, Switzerland) and perchloric acid 70% was from Acros Organics (Morris Plains, NJ, USA). Cefoperazone was supplied by Sigma (St. Louis, MO, USA) and ceftiofur was kindly donated by Pfizer (Madrid, Spain). Buffer and sample solutions were made with deionized water purified with a Milli-Q system (Millipore, Bedford, MA, USA). Cow plasma, treated with citrate, was from abattoir Van Kooten (Montfoort, The Netherlands). As a routine procedure, acetonitrile and buffer solutions were sonicated for 5 min before use.

2.2. Apparatus

A schematic diagram of the SPE–CE setup used for the experiments is shown in Fig. 1. The system is similar to that used in previous work [21]. The preconcentration system consists of a pump, a SPE column and 2 six-port switching valves. A Shimadzu LC10ADVP pump (Shimadzu, Kyoto, Japan) supplied the mobile phase to the PepMap C_{18} SPE column (5 mm × 0.5 mm i.d., 5 μm particles, 100 Å pores) (LC Packings-A Dionex Company, Amsterdam, The Netherlands). Valve 1 in Fig. 1 was a Rheodyne 7010 valve (Rhonert Park, CA, USA) (50 or 250 μl loop) and allowed the injection of the sample in the system. The introduction of the elution solvent was performed with a VICI Cheminert C2-1006 valve (valve 2) (VICI Ag/Valco International, Schenkon, Switzerland) (426 nl loop). For the study of the retention of the compounds on the trapping column, a UV detector (model K-2501 detector; Knauer

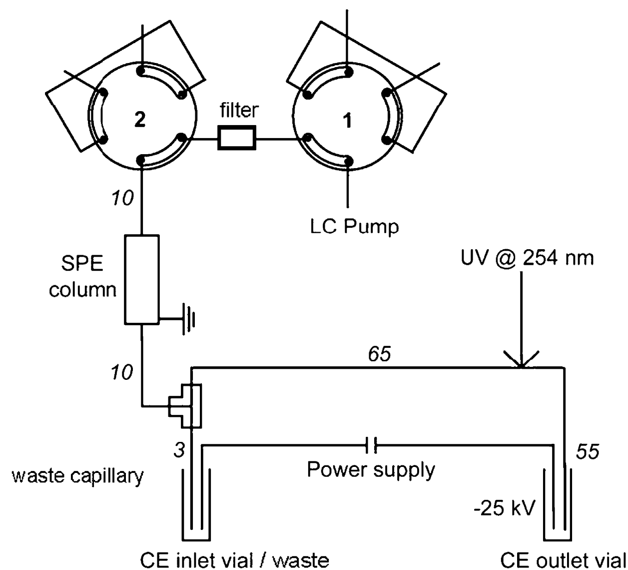

Fig. 1. Schematic diagram of the on-line SPE–CE system with the Tee-split interface. System components and dimensions are described in Section 2.2.

Wellchrom, Berling, Germany) was placed just after the SPE column. All tubing used in the SPE system was red PEEK (1/16 in. o.d., 0.005 in. i.d., Upchurch Scientific, Oak Harbor, WA, USA) except for the tubing between valve 2 and the CE apparatus, which was fused silica capillary of 75 μm i.d., 375 μm o.d. and the loop in valve 2 which was a fused silica capillary of 50 μm i.d., 375 μm o.d. All capillaries in the system were from Polymicro (Polymicro Technologies, Phoenix, AZ, USA).

The interface between the SPE and CE systems was a micro-Tee (model P-775; Upchurch Scientific, Oak Harbor, WA, USA) that splits the flow in two parts depending on the length of the capillaries after the Tee-piece. Unless stated otherwise, the CE system consisted of a waste capillary of 3 cm and a separation capillary of 120 cm full length (both 75 μm i.d., 375 μm o.d.), so the ratio between the part of the flow going to the waste and the part that goes to the separation capillary is 1–40. The detection window in the separation capillary was placed at 65 cm from the interface.

The CE instrument was either an Agilent CE system (model G1600Ax; Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector for detection at a wavelength of 254 nm, or a PrinCE CE system (model 560; PrinCE Technologies, Emmen, The Netherlands) with the aforementioned Knauer detector set at a wavelength of 200 nm. The PrinCE system was used for the study of the retention of the analytes on the SPE column and for the method calibration for standards, while the Agilent system was used for the calibration of biological samples and for determining the detection limits of both methods.

2.3. Procedures

2.3.1. SPE–CE system

The procedure used for the on-line SPE–CE is summarized in Table 1. First, the sample was introduced by pumping mobile phase, consisting of 25 mM ammonium acetate at pH 7.5, with a flow rate of 50 μl min^{-1}. At 11 min, the sample was loaded and washed with the mobile phase, and the flow was changed to 3 μl min^{-1}. Thirty seconds later, the analytes were eluted with a 426-nl plug of acetonitrile introduced via valve 2 and the vial in which the waste capillary was placed, was changed to the CE inlet vial. At 13 min, the analytes were injected into the separation capillary, and the flow in the SPE system was stopped. Finally CE analysis was started (see below). The background electrolyte (BGE) for the CE separation was the same as the mobile phase used for SPE, i.e. 25 mM ammonium acetate at pH 7.5. In order to avoid introduction of sample matrix into the separation capillary, BGE was flushed from the cathodic outlet vial of the CE system until the elution step at 11 min. The separation was started after the SPE step at 13 min by applying voltage. As the inlet of the capillary was grounded, negative polarity (−25 kV) was applied at the outlet of the capillary during analysis.

On each day, the SPE sorbent was conditioned by pumping, water, acetonitrile, water and mobile phase each for 5 min. At the end of the day, the SPE system was cleaned for 10 min with water. Unless otherwise stated, the flow was 50 μl min^{-1}. The CE system was conditioned daily flushing with 0.1 M NaOH, water and background electrolyte for 5 min. Between runs, the capillary was rinsed with 0.1 M NaOH, water and BGE each for 1 min.

Table 1
Procedure used for the on-line SPE–CE

Time (min)	Flow (μl min^{-1})	Valve 1	Valve 2	
	50	Load	Load	Fill loop of valve 1
0	50	Inject	Load	Injection of sample
11	3	Inject	Load	Change pump flow Fill loop of valve 2
11.5	3	Inject	Inject	Injection of elution solvent
13	0	Inject	Inject	Start CE analysis (−25 kV)

2.3.2 Sample pretreatment

Cow plasma was obtained from animals which did not receive any medication. The plasma was centrifuged three times for 5 min at 1886 × *g*, while after each step the supernatant was taken, and finally stored at −18 °C until use.

Two sample pretreatments before SPE step were studied: dilution and deproteinization. When the sample was diluted four times, 375 μl of plasma was spiked with a 100 μg ml^{-1} standard solution and diluted to a final volume of 1.5 ml. In the 10 times dilution, the same procedure was used except that 150 μl of plasma was spiked and diluted to 1.5 ml. For deproteinization of the sample, 1000 μl plasma was transferred into a vial and spiked with a standard solution of 100 μg ml^{-1}. Then, 0.215 μl of a 10% perchloric acid solution was added and the sample was diluted to a final volume of 1.5 ml. The sample was mixed for 15 s using a vortex and centrifuged at 12,754 × *g* for 5 min. The supernatant was injected into the SPE–CE system.

3. Results and discussion

3.1. Optimization and performance of the SPE–CE method

For CE a BGE consisting of 25 mM ammonium acetate (pH 7.5) was selected because a good separation of cephalosporins was obtained with this BGE in a previous work [28]. Here, a short study showed that under the chosen conditions (capillary 75 μm i.d., total capillary length 123 cm and capillary length to detector 65 cm) cefoperazone and ceftiofur could be completely separated in about 11 min.

Before coupling the SPE and CE, the appropriate SPE conditions for trapping and eluting the test compounds were investigated. The breakthrough volumes, desorption efficiency and desorption volume were studied. A solution of each analyte with a concentration of 25 μg ml^{-1} was passed through the SPE column and the column effluent was monitored at 200 nm to examine retention of the cephalosporin on the SPE material. Analyte breakthrough was observed at 23 min for cefoperazone and 25 min for ceftiofur using a flow rate of 50 μl min^{-1}. This means that the breakthrough volumes were 1150 and 1250 μl for cefoperazone and ceftiofur, respectively. A sample volume of 250 μl was chosen for subsequent experiments because this

Table 2
Electrophoretic peak data for different split ratios in on-line SPE–CE system

Split ratio		Cefoperazone		Ceftiofur
1:20	Area (mAU·s)	23.7		60.8
	Height (mAU)	2.6		6.4
	Resolution		1.4	
1:40	Area (mAU·s)	14.6		36.5
	Height (mAU)	2.1		5.0
	Resolution		1.6	

volume allowed the trapping of the compounds and additional washing. To study the desorption efficiency, a desorption volume of 426 nl acetonitrile was tested, and this volume provided elution efficiencies of 75 and 90% for cefoperazone and ceftiofur, respectively. These values were considered to be acceptable for both cephalosporins. When a desorption volume of 426 nl was used, the SPE elution volume was 1.8 μl for both cephalosporins.

The SPE–CE system is depicted in Fig. 1. Because the elution volume was too large to be completely injected in the electrophoretic system, a micro-Tee piece was necessary as interface between SPE and CE. This interface splits the volume: one part is directed to the waste capillary, the other part to the separation capillary. Because the internal diameters of the waste and separation capillaries were equal, the split ratio was inversely proportional to the length ratio of those capillaries. We studied 1:20 and 1:40 length ratios of the capillaries (see Table 2). Peak areas increased almost proportionally when a smaller split ratio was used to introduce a larger volume into the CE. Peak heights for the two split ratios differed less and the resolution between cefoperazone and ceftiofur was higher when a larger split ratio was used. In further experiments, the 1:40 split ratio was chosen because of the better resolution. In this way 2.5% of the elution volume was injected in the CE capillary. Fig. 2 shows an example of the analysis of cefoperazone and ceftiofur at a concentration of 10 ng ml^{-1} with 1:40 split ratio. The cephalosporins were baseline separated within 11 min. The negative signal at around 8 min is due to the acetonitrile plug used for desorption.

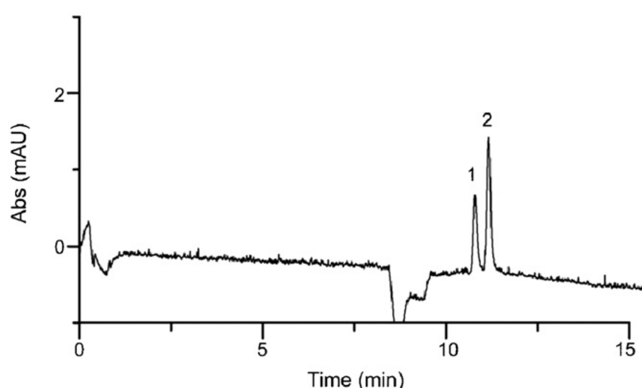

Fig. 2. Electropherogram of a standard mixture of cefoperazone (1) and ceftiofur (2) at a concentration of 10 ng ml^{-1}. CE conditions: 25 mM ammonium acetate at pH 7.5; applied voltage, −25 kV. Capillary, uncoated fused-silica, 123 cm (effective length, 65 cm) and 75 μm i.d.; temperature, 25 °C; detection wavelength, 254 nm. SPE conditions: sample volume: 250 μl. Other conditions in Table 1.

Detection limits (S/N = 3) were 5 ng ml^{-1} for both compounds at a detection wavelength of 254 nm using the Agilent CE system. Comparing with other CE systems in the literature for standards, cefoperazone presents lower detection limits with the proposed method than with the CZE method developed by Gàspàr et al., who could detect the cephalosporin down to 1.61 μg ml^{-1} [29]. Two electrophoretic methods based on microemulsion electrokinetic chromatography at high and low pH were also used for the analysis of cefoperazone [18]. The sensitivity was improved by using the reversed electrode polarity stacking mode (REPSM) and a reverse migrating pseudostationary phase (SRPM) obtaining detection limits of 30 and 40 ng ml^{-1}. On the other hand, for ceftiofur, better sensitivity was obtained in an in-line SPE–CE method (l.o.d. of 0.25 ng ml^{-1}) [12] in which the ratio between the sample and elution volumes in the SPE step was higher than in the present on-line SPE–CE method.

3.2. Plasma analysis

The applicability of the method was evaluated by the analysis of spiked plasma samples. As plasma has a complex composition, the sample volume was reduced from 250 to 50 μl, and a filter was included just after the sample loop (valve 1) in order to avoid clogging of SPE column. Nonetheless, direct injections of non-pretreated plasma samples led to rapid increase of the backpressure of the SPE column. Therefore, pretreatment of the plasma samples was necessary prior to introduction into the SPE system. First, a dilution of the sample with water was tested because this appeared to be the simplest procedure. Blank plasma samples were diluted in two different ratios (1:4 and 1:10). The diluted plasma samples were directly injected into the system. Initially, good results were obtained for both dilution ratios when a plasma sample was spiked with cefoperazone and ceftiofur at a concentration of 1000 ng ml^{-1}. Sensitivity is slightly reduced when using diluted plasma, in essence only a factor of 2 taking the sample volume into account. Unfortunately, the backpressure of the system increased considerably after a few runs, presumably because of protein adsorption in the SPE column. Therefore, another pretreatment procedure based on protein precipitation was tested [30]. In this case, 10% perchloric acid was added to precipitate the plasma proteins. Protein precipitation with an acidic solution was preferred over precipitation with organic solvents, such as acetonitrile, because these solvents reduce the breakthrough volume on the C_{18} SPE column. Moreover, evaporation of the organic solvent and reconstitution of the sample in an aqueous solvent would increase total analysis time. The electropherograms of Fig. 3 show a satisfactory separation of cefoperazone and ceftiofur for a concentration of 300 ng ml^{-1} (Fig. 3B), and the absence of interfering compounds in the blank sample (Fig. 3A).

Loading of larger volumes of acid-deproteinized plasma samples (100 and 250 μl) were also tested, but these resulted in a gradual increase of the backpressure of the system. Therefore, a volume of 50 μl deproteinizated plasma was used for further SPE–CE experiments.

Table 3
Linearity, calibration curves, relative standard deviation (RSD), and detection limits (LODs) of the on-line SPE–CE method for plasma samples after deproteinization with 10% perchloric acid

Compound	Range ($ng\,ml^{-1}$)	Calibration curves	R^2	Intraday precision[a] (RSD)	Interday precision[a] (RSD)	LOD ($ng\,ml^{-1}$)
Cefoperazone	200–1500	$y = 0.0173x + 1.761$	0.966	7.0	11.9	100
Ceftiofur	200–1500	$y = 0.0159x + 1.666$	0.976	3.6	8.1	100

Other conditions, see Section 2.
[a] 300 $ng\,ml^{-1}$ ($n = 5$).

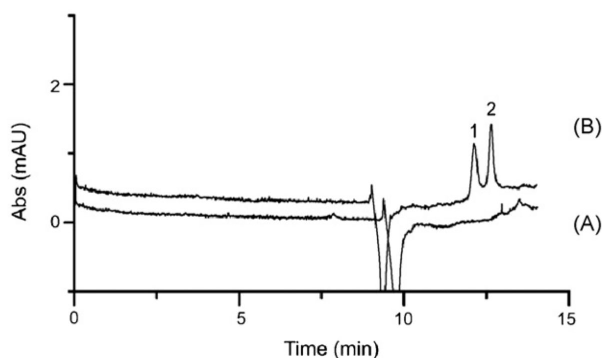

Fig. 3. Electropherograms obtained by on-line SPE–CE analysis of plasma samples after deproteinization with 10% perchloric acid (A) blank plasma and (B) plasma sample spiked with 300 $ng\,ml^{-1}$ of cefoperazone and ceftiofur. SPE conditions: sample volume: 50 μl. Other conditions as in Fig. 2.

The recovery factor of the pretreatment of the sample by precipitation with 10% of perchloric acid was studied by spiking plasma samples at 300 $ng\,ml^{-1}$ and analyzing three replicates. The recoveries were 90% for cefoperazone and 57% for ceftiofur compared to the standard injections. These dissimilar peak area recoveries can be attributed to differences in retention on the SPE column. In more detail, at a pH below pK_a ceftiofur (2.62) cefoperazone is neutral and the retention on the C_{18} sorbent is maximum, while ceftiofur is then positively charged and, thus, its retention is lower and can results in a loss of analyte mass.

The method was validated for biological samples (see Table 3) by spiking drug-free plasma samples with five known concentrations of the compounds. The samples were deproteinizated and 50 μl was loaded on the SPE column. The linear range was studied between 200 and 1500 $ng\,ml^{-1}$ and adequate precision was observed at the 300 $ng\,ml^{-1}$ level. Both intraday and interday precision were slightly higher than when standards were analyzed because of the additional deproteinization step. The detection limits were in the range of 50–100 $ng\,ml^{-1}$ for the cephalosporins. Ceftiofur has been analyzed in plasma samples by HPLC-UV [4,7–9,31] with detection limits in the 150 $ng\,ml^{-1}$ range. So, the SPE–CE results presented here are comparable to those reported LC–UV methods.

4. Conclusions

A simple and sensitive CE method is presented for the simultaneous determination of ceftiofur and cefoperazone in plasma. The method developed for standards was adapted for the analysis of cephalosporins in plasma samples in the $ng\,ml^{-1}$ range. The plasma samples were deproteinizated, which avoided clogging of the SPE column. The simple pretreatment step for plasma samples, the high sensitivity (50–100 $ng\,ml^{-1}$ for 50 μl plasma) and the short overall analysis times are the main advantages of the presented approach. The validation data for analysis of the two cephalosporins are comparable to those reported in the literature for HPLC and show that the on-line SPE–CE method can be suitable for pharmacokinetic studies.

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References

[1] V.F. Samanidou, E.A. Hapeshi, I.N. Papadoyannis, J. Chromatogr. B 788 (2003) 147.
[2] K.L. Tyczkowska, R.D. Voyksner, K.L. Anderson, A.L. Aronson, J. Chromatogr. 614 (1993) 123.
[3] J. Keever, R.D. Voyksner, K.L. Tyczkowska, J. Chromatogr. A 794 (1998) 57.
[4] S. De Baere, F. Pille, S. Croubels, L. Ceelen, P. De Backer, Anal. Chim. Acta 512 (2004) 75.
[5] F.J. Jiménez, M. Callejón, J.C. Jiménez, M.A. Bello, A. Guiráum, Chromatographia 62 (2005) 355.
[6] S.A. Signs, T.M. File, J.S. Tan, Antimicrob. Agents Chemother. 26 (1984) 652.
[7] C.B. Navarre, L. Zhang, G. Sunkarra, S.H. Duran, U.B. Kompella, J. Vet. Pharmaco. Ther. 22 (1999) 13.
[8] F. Pille, S. Baere, L. Ceelen, J. Dewulf, S. Croubels, F. Gasthuys, P. Backer, A. Martens, Vet. Surg. 34 (2005) 610.
[9] G.A. Jacobson, S. Martinod, C.P. Cunningham, J. Pharm. Biomed. Anal. 40 (2006) 1249.
[10] Y. Mrestani, R. Neubert, A. Härtl, J. Wohlrab, Anal. Chim. Acta 349 (1997) 207.
[11] Y. Mrestani, R. Neubert, J. Schiewe, A. Härtl, J. Chromatogr. B 690 (1997) 321.
[12] P. Puig, F. Borrull, M. Calull, F. Benavente, V. Sanz-Nebot, J. Barbosa, C. Aguilar, Anal. Chim. Acta 587 (2007) 208.
[13] G. Castaneda, M. Kelly, H. Maillols, H. Fabre, Anal. Chem. 69 (1997) 1364.
[14] S.H. Tseng, Y.H. Yang, Y.R. Chen, S.H. Chen, Electrophoresis 25 (2004) 1641.
[15] B.X. Mayer, U. Hollenstein, M. Brunner, H.G. Eichler, M. Müller, Electrophoresis 21 (2000) 1558.
[16] T. Kitahashi, T. Furuta, J. Pharm. Biomed. Anal. 34 (2004) 409.

[17] H.H. Yeh, Y.H. Yang, Y.W. Chou, J.Y. Ko, C.A. Chou, S.H. Chen, Electrophoresis 26 (2005) 927.
[18] P. Puig, F. Borrull, M. Calull, C. Aguilar, Chromatographia 62 (2005) 603.
[19] P. Puig, F. Borrull, C. Aguilar, M. Calull, J. Chromatogr. B 831 (2006) 196.
[20] F.W.A. Tempels, G. Wiese, W.J.M. Underberg, G.W. Somsen, G.J. Jong, J. Chromatogr. B 839 (2006) 30.
[21] F.W.A. Tempels, J. Teeuwsen, I. Kyriakou, G. Theodoridis, W.J.M. Underberg, G.W. Somsen, G.J. Jong, J. Chromatogr. A 1053 (2004) 263.
[22] N.F.C. Visser, M. Harmelen, H. Lingeman, H. Irth, J. Pharm. Biomed. Anal. 33 (2003) 451.
[23] T. Stroink, E. Paarlberg, J.C.M. Waterval, A. Bult, W.J.M. Underberg, Electrophoresis 22 (2001) 2375.
[24] J.R. Veraart, H. Lingeman, U.A.T. Brinkman, J. Chromatogr. A 856 (1999) 483.
[25] M. Petersson, K.G. Wahlund, S. Nilsson, J. Chromatogr. A 841 (1999) 249.
[26] N.A. Guzman, Electrophoresis 24 (2003) 3718.
[27] F.W.A. Tempels, W.J.M. Underberg, G.W. Somsen, G.J. Jong, Anal. Chem. 76 (2004) 4432.
[28] P. Puig, F. Borrull, C. Aguilar, M. Calull, Chromatographia 65 (2006) 501.
[29] A. Gáspár, M. Andrási, S. Kardos, J. Chromatogr. B 775 (2002) 239.
[30] S. Sentellas, L. Puignou, M.T. Galceran, J. Sep. Sci. 25 (2002) 975.
[31] P.S. Jaglan, B.L. Cox, T.S. Arnold, M.F. Kubicek, D.J. Stuart, T.J. Gilbertson, J. Assoc. Off. Anal. Chem. 73 (1990) 26.