ELSEVIER

Journal of Chromatography B, 662 (1994) 61–70

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Automated column liquid chromatographic determination of amoxicillin and cefadroxil in bovine serum and muscle tissue using on-line dialysis for sample preparation

N. Snippe[a], N.C. van de Merbel[a,*], F.P.M. Ruiter[a], O.M. Steijger[b], H. Lingeman[a], U.A.Th. Brinkman[a]

[a]*Department of Analytical Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam, Netherlands*
[b]*Institute for Veterinarian Research, P.O. Box 81, 8090 AB Wezep, Netherlands*

First received 25 May 1994; revised manuscript received 17 August 1994

Abstract

A fully automated method is described for the determination of amoxicillin and cefadroxil in bovine serum and muscle tissue. The method is based on the on-line combination of dialysis and solid-phase extraction for sample preparation, and column liquid chromatography with ultraviolet detection. In order to enhance the UV detectability of the analytes, post-column addition of 0.1 *M* sodium hydroxide is performed. The method shows good linearity and repeatability for both analytes in serum as well as in muscle tissue; the limits of detection in these samples are 0.05 μg/ml and 0.2 μg/g, respectively. The method has a sample throughput of 30 samples per 24 h.

1. Introduction

Amoxicillin, an α-aminopenicillin, is a broad-spectrum antibiotic which is widely used in human and veterinary medicine, while cefadroxil, a cephalosporin, is only used in human medicine for patients which are over-sensitive to penicillin derivatives. Since some of these antibiotics are also used as supplements in veterinary food [1], residual levels may well be present in edible animal tissues. These may cause the development of resistance in human pathogens [2] or lead to reactions in people allergic to penicillins [3]. Consequently, establishing residue profiles after drug administration to an animal, both in edible tissues – to define withdrawal times – and in plasma or serum – to obtain pharmacokinetic data – is an important item for the registration of these compounds by veterinary pharmaceutical companies. Today, by far most of the analytical methods used for the determination of both penicillins and cephalosporins are based on immunological or microbiological techniques [1]. However, as these techniques can not discriminate between different penicillins or different cephalosporins, the use of chromatographic techniques, which can, is receiving increasing attention.

In the chromatographic analysis of biological samples, sample preparation generally is the

* Corresponding author.

0378-4347/94/$07.00
SSDI 0378-4347(94)00377-7

bottleneck: it often involves several manual steps and can easily become a time-consuming process. Many chromatographic methods used for amoxicillin [4–11] and cefadroxil [12–15] also require extensive manual sample preparation. Since pharmacokinetic studies normally generate a large number of samples, the application of an automated sample-preparation method will be advantageous, because of an increased sample throughput. One of the most important goals of sample preparation is the removal of macromolecules, which makes the application of on-line dialysis an interesting approach. This technique allows the automated removal of proteins from a variety of complex samples in a simple and flexible way [16]: only low-molecular-mass compounds can diffuse through the pores of a dialysis membrane and are introduced into the chromatographic system, whereas macromolecular material is effectively retained.

There are two factors, however, which limit the applicability of dialysis. Firstly, dialysis usually causes dilution of the sample and subsequent reconcentration of the analytes on a solid-phase extraction column (precolumn) is therefore necessary. Obviously, in the case of highly polar analytes, such as amoxicillin and cefadroxil, the application of on-line dialysis and solid-phase extraction will not be really straightforward, because of the lack of retention of the analytes on most types of precolumns, and the optimization of the sample preparation will need considerable attention. A second complicating factor is the low selectivity of dialysis, because the separation is only based on differences in molecular size. The trace-level determination in complex biological samples will, therefore, be relatively difficult for compounds without selective detection characteristics such as, again, the present analytes.

In this paper an automated method for the determination of amoxicillin and cefadroxil in bovine serum and muscle tissue is described, based on the on-line combination of dialysis, solid-phase extraction and column liquid chromatography (LC) with UV detection. Special attention will be paid to the optimization of the crucial steps mentioned above: the (re)concentration and detection of the analytes.

2. Experimental

2.1. Chemicals

Amoxicillin and cefadroxil were obtained from Sigma (St. Louis, MO, USA). Acetonitrile, potassium phosphate (mono- and dibasic), and sodium hydroxide were obtained from J.T. Baker (Deventer, Netherlands). Methanol was obtained from Rathburn (Walkerburn, UK) and hexadecyltrimethylammonium chloride (HTACl) from Kodak (Rochester, NY, USA). Triton X-100 was purchased from Merck (Darmstadt, Germany) and water was purified using a Milli-Q system (Millipore, Milford, MA, USA). Standard stock solutions contained 400 μg/ml of amoxicillin and cefadroxil in 10 m*M* phosphate buffer (pH 4.7). Aliquots of 2 ml could be stored at −20°C for up to one month and working solutions were freshly prepared every day by diluting the stock solution with the same buffer.

2.2. Instrumentation

A Gilson (Villiers-le-Bel, France) ASTED system for sample preparation was on-line combined with an LC system and UV detection (Fig. 1). The ASTED consisted of a Model 231 autosampling injector and a Model 401 dilutor equipped with a 1-ml syringe. The Model 30 sample rack, with a capacity for 60 sample vials, was kept at 4°C by means of a Savant Instru-

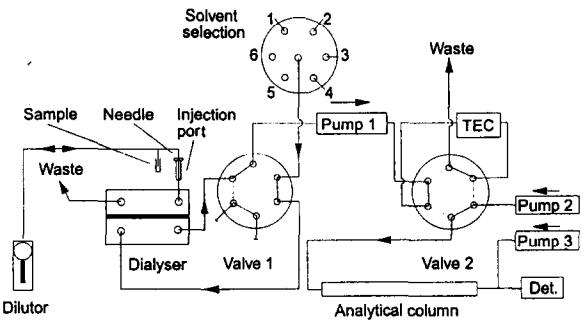

Fig. 1. Schematic representation of the on-line dialysis LC-UV system. The dialyser consists of two blocks coupled in series. Four solvents are connected to the solvent selection valve: (1) methanol; (2) water; (3) 100 m*M* phosphate buffer (pH 8.5) containing 0.5 m*M* HTACl; (4) 100 m*M* phosphate buffer (pH 8.5). TEC = trace-enrichment column.

ments (Hicksville, NY, USA) cryostat. Two flat-bed dialysis blocks with donor volumes of 110 μl and 125 μl, respectively, and fitted with Cuprophan membranes with a molecular weight cut-off value of 15 kDa, were coupled in series. Dialysis was performed at ambient temperature. For preconcentration of the dialysate two automatic six-port switching valves (Nos. 1 and 2 in Fig. 1) and a solvent selection valve were used, mounted on either a Must (Spark Holland, Emmen, Netherlands) or a Prospekt (Spark Holland) system. The stainless-steel precolumn (20 × 4.6 mm I.D.), inserted in a home-made holder, was slurry-packed with 10 μm C_{18}-bonded silica (J.T. Baker) and fitted with stainless-steel screens (pore size: 8 μm). The precolumn was repacked every 3–5 days. A Model 300 LC pump (No. 1; Separations, Hendrik Ido Ambacht, Netherlands) was used to pump the acceptor phase or the washing solutions to the precolumn. The LC system consisted of an LC pump Model SF-400 (No. 2; Kratos, Ramsey, NJ, USA) and a 150 × 4.6 mm I.D. analytical column (Type ABZ, Supelco, Leusden, Netherlands). The eluent was acetonitrile–phosphate buffer (10 m*M*, pH 7.0) (13:87, v/v) containing 0.5 m*M* HTACl and was delivered at a flow-rate of 1.0 ml/min at ambient temperature. Post-column reagent addition was performed by a Model 2150 LC pump (No. 3; Pharmacia LKB, Uppsala, Sweden) with titanium pumpheads. A UV detector (Applied Biosystems, Foster City, CA, USA) set at 260 nm was used for detection and the signals were recorded with a Model 101 recorder (Pharmacia LKB).

2.3. Set-up and mode of dialysis

The ASTED system was slightly modified: normally a dilutor is used to transport the dialysate from the dialysis block to the precolumn. This configuration, however, only operates reliably at relatively low back pressures (< ca. 2 bar). In order to optimize the concentration step and to be able to use e.g. longer precolumns, smaller particles and/or higher flow-rates, a conventional LC pump, which can handle much higher back pressures, was used instead. Prior to the actual dialysis, the precolumn was conditioned with 5 ml of methanol, 5 ml of water and 5 ml of a 100 m*M* phosphate buffer (pH 8.5) containing 0.5 m*M* HTACl. Dialysis was performed in the pulsed mode [17]: 750-μl samples were dialysed in three 250-μl pulses, of which only 235 μl had direct contact with the membrane. Each aliquot was held static for 6.67 min while the acceptor phase (100 m*M* phosphate buffer, pH 8.5) was continuously pumped through the acceptor compartment at a flow-rate of 0.2 ml/min. After 20 min the dialysis was stopped by switching valve 1. In order to remove possibly interfering compounds the precolumn was washed with 2 ml of 100 m*M* phosphate buffer (pH 8.5) containing 0.5 m*M* HTACl. By switching valve 2, the analytes were backflushed to the analytical column. Finally, the donor channel was washed with a 100-μl plug of 0.01% Triton X-100 and flushed with 5 ml of water. Simultaneously, the acceptor channel was flushed with 5 ml of 100 m*M* phosphate buffer (pH 8.5). During the LC separation of the sample, the next sample was injected and dialysed. Details of the total procedure are summarized in Table 1.

2.4. Sample preparation of blood

Whole blood samples (9 ml) were buffered at pH 4.7 by adding 1 ml of a 1 *M* phosphate buffer (pH 2.6). After centrifugation (4000 *g*, 20 min) 70 μl of the internal standard (cefadroxil in the case of amoxicillin determinations and vice versa) were added to 1330-μl aliquots, which were stored at −20°C for up to one week or at −70°C for up to one year.

2.5. Sample preparation of meat

To 25 g of sliced muscle tissue, 25 g of phosphate buffer (100 m*M*, pH 4.7) were added, followed by homogenization using a blender; 50 μg of the internal standard solution were added to aliquots of 10 g homogenate, which were centrifuged (4000 *g*, 20 min). The supernatant (final pH 5.5) was stored at −20°C for up to one week. For longer periods, up to one year, the samples were stored at −70°C [18].

Table 1
Typical cycle used for the determination of amoxicillin and cefadroxil in serum and muscle tissue

Time (min)	Event	Valve[a]			Total acceptor volume (ml)
		1	2	SSV	
0.00	Conditioning of precolumn (with methanol)[b]	....	–	1	0
5.00	Idem (with water)			2	
10.00	Idem (with buffer plus HTACl)			3	
15.00	Dialysis of first 250-μl pulse[c]	–	–	4	1.33
21.67	Dialysis of second 250-μl pulse				2.67
28.33	Dialysis of third 250-μl pulse				4.00
35.00	End of dialysis				
37.00	Washing of precolumn[b] Removal of sample from donor channel	....	–	3	6.00
39.00	Start of analysis	....	....		
41.00	Flushing of donor and acceptor channels[b]	–	....	4	
46.00	End of run; start of new run				

[a] Valve positions are those shown in Fig. 1; SSV = solvent selection valve.
[b] Conditioning and washing of precolumn and flushing of dialysis block performed at 1.0 ml/min.
[c] Acceptor phase flow-rate during dialysis, 0.2 ml/min.

3. Results and discussion

3.1. Trace enrichment

When using on-line dialysis in combination with a solid-phase extraction precolumn, it is essential to have a sufficiently large breakthrough volume of the analytes on this column (preferably at least ca. 5 ml), in order to be able to optimize the dialysis step and maximize the analyte recovery. In the present study, several packing materials were tested, first of all without the addition of an ion-pairing reagent. Reversed-phase sorbents such as C_{18}-bonded silica as well as the more hydrophobic styrene–divinylbenzene copolymer PLRP-S, however, provided insufficient retention for the two analytes of interest, even when the particle size was reduced from 40 μm to 10 μm and the precolumn dimensions were increased from 10 × 2 mm to 20 × 4.6 mm I.D. Very small breakthrough volumes of less than 1 ml were found for both analytes in all cases. Next, an ion-pairing reagent was added to the acceptor phase and this option was tested in combination with a 20 × 4.6 mm I.D. precolumn containing 10 μm C_{18}-bonded silica. As both analytes are zwitter-ionic, with pK_a values of ca. 2.5 for the carboxylic acid and ca. 7.5 for the amine group, the alternatives are the addition of an anionic counter-ion at a low pH or a cationic counter-ion at a high(er) pH. Since penicillins and cephalosporins are known to be rather unstable at low pH values, the use of cationic ion-pair reagents was preferred. When using tetrabutylammonium salts in concentrations of up to 50 mM, the breakthrough volumes of both analytes were acceptable (ca. 5 ml). However, the addition of up to 50 mM of these reagents to the LC eluent did not result in sufficient retention on the analytical column. Only the more hydrophobic hexadecyltrimethylammonium ion (in a concentration of 0.5 mM) gave a breakthrough volume larger than 5 ml for both analytes on the precolumn as well as satisfactory

retention on the analytical column. Due to its low UV absorbance, the chloride salt (HTACl) was selected for further experiments.

It is obvious that a proper choice of the pH value is essential when working with zwitter-ionic analytes. Fig. 2 shows the breakthrough volume of the more polar analyte (cefadroxil) versus the pH of the acceptor phase (10 m*M* phosphate) in the presence of HTACl. Although the carboxylic acid group is completely ionized at pH > ca. 4, increasing breakthrough volumes were observed when the pH was increased from 5.0 to 8.0. Apparently, not only the presence of a negative charge is a prerequisite for ion-pair formation with the cationic counter-ion, but also the absence of a positive charge on the amino group of the analyte. The largest breakthrough volumes will, therefore, be found for analytes with a full negative charge, i.e. at pH > ca. 9.5. However, because both the analytes and the precolumn material are known to be unstable under alkaline conditions, no experiments were performed at pH > 8.5. Surprisingly, an increase in breakthrough volume was also encountered for pH < 5. This can not be attributed to ion-pair formation with HTACl; presumably some form of ion-exchange interaction of the positively charged analytes with the packing material is responsible for this effect.

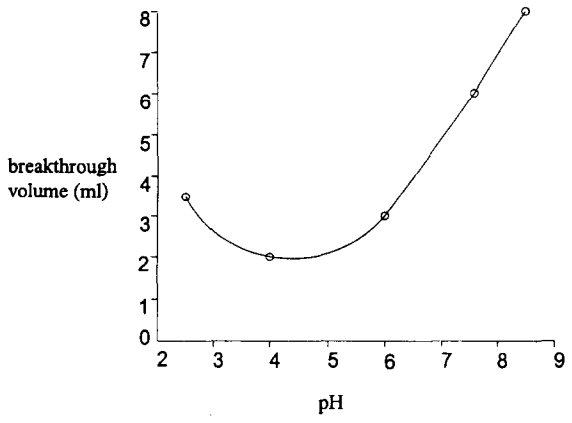

Fig. 2. Plot of breakthrough volume vs. pH of the acceptor phase (10 m*M* phosphate, containing 0.5 m*M* HTACl) for cefadroxil. Precolumn dimensions, 20 × 4.6 mm I.D. Acceptor phase flow-rate, 0.2 ml/min.

Using an acceptor phase of pH 8.0 (10 m*M* phosphate buffer) and 0.5 m*M* HTACl as the counter-ion, dialysis of aqueous standards and spiked serum samples (pH 7.4) could be performed without any problems by pumping 4 ml of dialysate over the precolumn. However, a complication arose when serum samples buffered at pH 4.7 (100 m*M* phosphate) were analysed. In this case, the breakthrough volumes of both amoxicillin and cefadroxil decreased sharply. When this phenomenon was studied in more detail, it appeared that, apart from the pH, the ionic strength and also the flow-rate of the acceptor phase had a distinct influence on the analyte breakthrough volumes. From the data presented in Table 2, several interesting conclusions can be drawn.

First of all, the lower pH of the buffered serum samples caused the dialysate pH to decrease as well. As has been outlined above, this directly influences the breakthrough volumes of the analytes. By increasing the molarity of the acceptor phase buffer from 10 to 200 m*M* (keeping its pH at 8.0), this problem could be circumvented. However, only the breakthrough volume of amoxicillin was found to improve in this case. Apparently, next to the pH there is another factor that influences the behaviour of cefadroxil. Most probably, its low breakthrough volume can be ascribed to the increased ionic strength of the acceptor phase, for when the molarity of the acceptor phase buffer was reduced from 200 to 100 m*M*, and the pH was increased to 8.5 to keep the pH of the dialysate at 7.5, the breakthrough volume of cefadroxil markedly improved. Possibly, the relatively large amounts of inorganic anions in the acceptor phase compete with the analytes for the counter-ion [19]. This would also explain why cefadroxil, the more polar analyte, was more strongly affected by an increase of the ionic strength than amoxicillin. In addition, it was found that the breakthrough volume of cefadroxil could be increased by reducing the flow-rate of the acceptor phase from 1.5 to 0.2 ml/min. Obviously, the binding of cefadroxil and HTACl takes place relatively slowly. This means that, due to a kinetic limitation, a smaller percentage of the analytes will

Table 2
Influence of the ionic strength, pH and flow-rate of the acceptor phase on the breakthrough volume of cefadroxil and amoxicillin

Sample	Acceptor phase		pH of dialysate	Flow-rate of dialysate (ml/min)	Breakthrough volume (ml)	
	Phosphate buffer concentration (m*M*)	pH			Cefadroxil	Amoxicillin
Unbuffered serum, pH 7.4	10	8.0	7.5	1.5	4	>6
Buffered serum[a], pH 4.7	10	8.0	6.0	1.5	1	<1
Buffered serum, pH 4.7	200	8.0	7.5	1.5	1	>6
Buffered serum, pH 4.7	100	8.5	7.5	1.5	4	>4
Buffered serum, pH 4.7	100	8.5	7.5	0.2	6	>6

[a] Adjusted with 100 m*M* phosphate buffer.

bind to the counter-ion at a high flow-rate or, in other words, breakthrough will occur earlier.

Using a 100-m*M* phosphate buffer of pH 8.5, at a flow-rate of 0.2 ml/min, as the acceptor phase, the breakthrough volumes for both analytes were at least 6 ml, both for serum samples buffered at pH 4.7 with 100 m*M* phosphate and for muscle tissue samples buffered at pH 5.5 with 50 m*M* phosphate. It should be noted that the analytes have to be sufficiently stable in the acceptor phase for the duration of the dialysis step (30 min at pH 8.5 and ambient temperature). It was found that both amoxicillin and cefadroxil showed negligible degradation (<2%) under these conditions. In addition, it has already been reported that the analytes can be stored for more than 16 h at pH 4.7 and 4°C [18], which is adequate for overnight analysis.

3.2. Dialysis

Initially, the ion-pairing reagent was added to the acceptor phase and, as such, pumped through the dialysis block. Unfortunately, it was found to adsorb strongly to the membrane, which resulted in severe memory effects and highly irreproducible results. Any contact of the ion-pairing reagent with the membrane had, therefore, to be prevented. As an alternative, the precolumn was loaded with HTACl prior to dialysis. In this way, the analytes, which were now collected in an acceptor phase without ion-pairing reagent, could also be conveniently trapped. It was, however, necessary to include an additional switching valve in the system to bypass the dialysis block.

In general, for the dialysis of samples larger than the donor compartment volume–as was the situation encountered–two different modes are available: the sample is pumped through the dialysis block either continuously or in pulses [16]. Typical values for analyte recovery and dialysis time are 10–20% and 10 min for continuous dialysis, compared to 30–40% and 20 min for pulsed dialysis [20]. For the present application, where sensitivity is more important than speed, the pulsed mode was the logical choice and the 750-μl samples were divided into three 250-μl pulses, which were consecutively dialysed. As the acceptor phase flow-rate was set at 0.2 ml/min (in order to create a sufficiently large breakthrough volume of cefadroxil) and 4.0

ml of acceptor phase had to be handled, dialysis took 20 min. Although ca. 20% of amoxicillin is bound to proteins, analyte recoveries in aqueous standards and in serum and muscle tissue were essentially equal [1]. Recoveries of 32% (±2% R.S.D.) and 34% (±2% R.S.D.) were found for amoxicillin and cefadroxil, respectively, which agrees rather well with the typical data mentioned earlier.

3.3. Selectivity

As mentioned above, dialysis is a powerful tool for preparing complex samples for chromatographic analysis, as far as removal of macromolecules is concerned. Selectivity, however, can only be obtained in the chromatographic and/or detection part of the system. At pH 7, amoxicillin and cefadroxil both have their UV absorbance maximum at 230 nm and in the chromatograms of bovine serum recorded at this relatively non-selective wavelength a large number of interfering matrix components was observed, which obscured the analyte peaks. Introducing a washing step of the precolumn (2 ml of a buffer containing HTACl) improved the situation only slightly; it was, therefore, attempted to apply a more selective detection mode. As amoxicillin and cefadroxil both contain a phenol group (pK_a value ca. 9.5), under alkaline conditions (pH > 11) their UV absorbance maximum will shift to ca. 260 nm due to the abstraction of the phenolic proton [21]. Although the pH shift caused a moderate decrease of the sensitivity, viz. of 25% and 10% for amoxicillin and cefadroxil, respectively, the contemplated increase in selectivity was achieved. By simple post-column addition of 0.1 *M* sodium hydroxide (flow-rate, 0.1 ml/min) and subsequent detection at 260 nm a considerable reduction of the matrix background was observed. A limit of detection of 0.05 μg/ml was obtained for both analytes in serum, which is sufficient for pharmacokinetic studies after the administration of commonly used drug doses. A typical chromatogram of bovine serum, spiked with cefadroxil and amoxicillin at the 2 μg/ml level, is shown in Fig. 3A.

Next, other, more complex sample types were studied, viz. bovine muscle tissue, kidney and liver. As can be seen in Fig. 3, the number of interferences strongly varies with the type of sample. Although muscle tissue contains much more interfering endogenous compounds than serum (Fig. 3B), an acceptable limit of detection was found with the present method (0.2 μg/g). The presence of interfering sample components is even more striking in the chromatograms

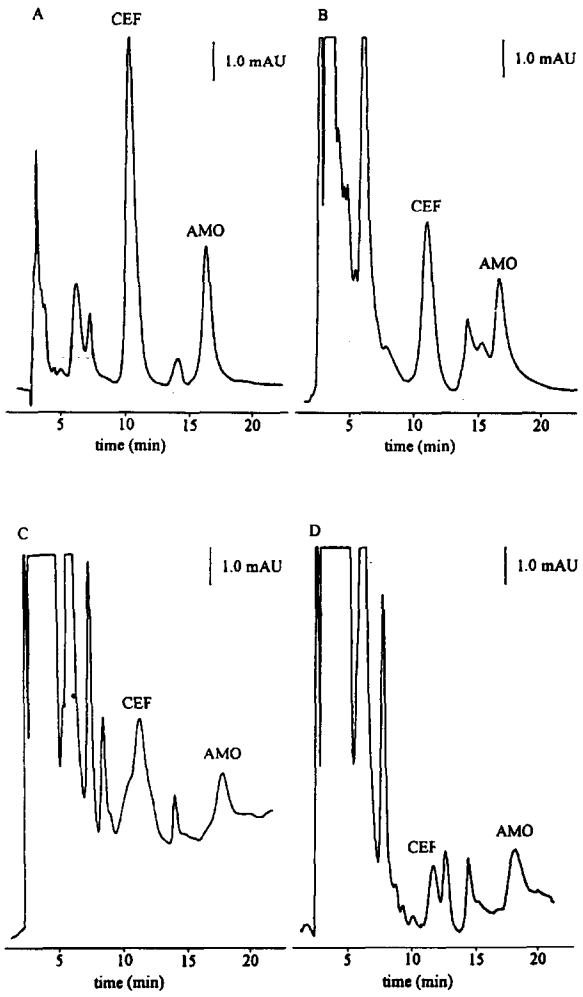

Fig. 3. On-line dialysis LC-UV (260 nm) chromatograms of bovine (A) serum, (B) muscle tissue, (C) kidney and (D) liver. The samples were spiked with amoxicillin (amo) and cefadroxil (cef), at (A) 2 μg/ml, (B) 2 μg/g, and (C and D) 1 μg/g. As samples (B)–(D) were two-fold diluted prior to dialysis, the analyte concentrations during analysis were (B) 1 μg/g and (C and D) 0.5 μg/g.

obtained for kidney (Fig. 3C) and liver (Fig. 3D) homogenates. In fact, for these sample types the method is not sufficiently selective to be of practical value and future work will have to be aimed at introducing more selectivity into the system.

In the case of serum or plasma samples, published methods typically yield detection limits of 0.5–1.0 μg/ml both for amoxicillin [4,6] and cefadroxil [12,13,15] when direct UV detection at ca. 230 nm is used. This can be improved to 0.1 μg/ml when a more complicated sample clean-up is performed [9]. In order to improve the detectability of the analytes in kidney and liver samples, derivatization in combination with fluorescence detection is a rather logical option. If one wishes to perform the analyses on-line and introduce full automation–as we do–the utilization of post-column derivatization (with fluorescamine [10,14,18]) is more attractive than that of pre-column derivatization (which involves a laborious procedure using mercury(II) chloride [5,7]). However, the low detection limits of 0.01–0.1 μg/ml reported in the quoted literature, are for serum and plasma samples only. So, in view of the results shown in Fig. 3, it is to be expected that much work will have to be performed to optimize the experimental conditions for kidney and liver samples.

3.4. Performance of the total on-line system

Based on the above results, a method for the determination of amoxicillin and cefadroxil was set up for bovine serum and muscle tissue. Samples (750-μl) were dialysed in three 250-μl pulses. An acceptor phase of 100 m*M* phosphate (pH 8.5) was pumped at 0.2 ml/min through a C_{18}-bonded silica packed precolumn, which had been loaded with the counter-ion HTACl. After preconcentration of the analytes, the precolumn was washed with 2 ml of the same phosphate buffer, containing 0.5 m*M* HTACl. After valve switching, the analytes were desorbed and separated by LC, using post-column addition of 0.1 *M* sodium hydroxide prior to UV detection at 260 nm. The dialysis time was 20 min; the total time for sample preparation, including the steps for conditioning of the precolumn and flushing of the dialysis block, was 46 min. Since a chromatographic run took ca. 30 min and sample preparation and separation were performed in parallel, about 30 samples can be processed per 24 h. Relevant data on the analysis are summarized in Table 3. Calibration curves were constructed for both analytes in serum in two concentration ranges: 0.05–1.0 μg/ml and 1.0–10 μg/ml, respectively. In muscle tissue only one calibration curve was made, ranging from 0.2–10 μg/g. The limit of detection was 0.05 μg/ml for both analytes in serum and, because of the presence of more matrix interferences, 0.2 μg/g in muscle tissue (cf. above). Inter-assay precision was determined at two levels in both serum and muscle tissue. The R.S.D. values ranged from ca. 2–5% at a concentration of 2 μg/ml to ca. 4–10% at twice the detection limit, which is fully satisfactory.

4. Conclusions

The routine determination of amoxicillin and cefadroxil in serum and muscle-tissue samples can be carried out using an automated system, which is based on the on-line combination of dialysis, solid-phase extraction and liquid chromatography with UV detection. As the analytes are highly polar and lack selective detection characteristics, optimization of the preconcentration and detection system is of utmost importance. By using an ion-pairing reagent and properly choosing the acceptor phase pH, ionic strength and flow-rate, a (sufficiently large) breakthrough volume of 6 ml for both analytes can be obtained. Post-column addition of 0.1 *M* sodium hydroxide to the column effluent and detection at 260 nm instead of 230 nm increase the selectivity of the method. The method allows complete automation of the sample-preparation procedure and, compared with literature studies using UV detection, a 10-fold increase in sensitivity is obtained for serum samples. In addition, the method can be used for the determination of the analytes in muscle-tissue samples,

Table 3
Analytical data on the automated determination of amoxicillin and cefadroxil in bovine serum and muscle tissue using on-line dialysis LC-UV

Parameter	Serum		Muscle	
	Amoxicillin	Cefadroxil	Amoxicillin	Cefadroxil
LOD (μg/ml)	0.05	0.05	0.20	0.20
Linearity[a]				
Range (μg/ml)	0.05–1.00	0.05–1.00	0.20–10.0	0.20–10.0
Slope (± S.D.)	43 (± 1.8)	124 (± 2.5)	44 (± 1.0)	99 (± 2.0)
Intercept (± S.D.)	0.7 (± 1.0)	−1.4 (± 1.3)	−13 (± 5)	−21 (± 9)
R^2	0.9707	0.9930	0.9897	0.9954
Range (μg/ml)	1.00–10.0	1.00–10.0		
Slope (± S.D.)	46 (± 0.8)	108 (± 1.6)		
Intercept (± S.D.)	−4.4 (± 3.2)	−2.0 (± 6.2)		
R^2	0.9925	0.9935		
Precision (R.S.D.%)[b]				
Level, 0.10 μg/ml	10	8.0	n.d[c]	n.d
Level, 0.40 μg/lml	n.d	n.d	4.3	4.8
Level, 2.00 μg/ml	2.8	1.4	3.1	5.2

[a] Seven data points in triplicate.
[b] $n = 8$.
[c] Not determined.

which is here reported for the first time. Since kidney and liver samples contain too many interfering endogenous compounds to allow proper analysis with the present procedure it is recommended to improve selectivity by post-column labelling with fluorescamine. With the present fully automated set-up up to 30 samples can be handled per day. The method has been used for several months for pharmacokinetic studies of amoxicillin in bovine serum samples using cefadroxil as internal standard.

Acknowledgements

The authors wish to express their thanks to Mr. J. Brands (Supelco) for the gift of the ABZ column. This work was supported by the Dutch Foundation for Technical Science, Grant No. 349-2119.

References

[1] J.O. Boison, *J. Chromatogr.*, 624 (1992) 171.
[2] D. Fein, G. Barton, R. Tsutakawa and D. Blenden, *J. Infect. Disease.*, 130 (1974) 274.
[3] L.C. Altman and L.S. Tompkins, *Postgrad. Med.*, 64 (1978) 157.
[4] M. Foulstone and C. Reading, *Antimicrob. Agents Chemother.*, 22 (1982) 753.
[5] K. Miyazaki, K. Ohtani, K. Sunada and T. Arita, *J. Chromatogr.*, 276 (1983) 478.
[6] T.L. Lee and M.A. Brook, *J. Chromatogr.*, 306 (1984) 429.
[7] J. Haginaka and J. Wakai, *Analyst*, 110 (1985) 1277.
[8] H. Mascher and C. Kikuta, *J. Chromatogr.*, 506 (1990) 417.
[9] H.J. Nelis, J. Vandenbranden, A. de Kruif and A.P. de Leenheer, *Antimicrob. Agents Chemother.*, 36 (1992) 1859.
[10] P. Leroy, C. Gavriloff, A. Nicolas, P. Archimbault and G. Ambroggi, *Intern. J. Pharm.*, 82 (1992) 157.
[11] W.J.J. Krauwinkel, N.J. Volkers-Kamermans and J. van Zijtveld, *J. Chromatogr.*, 617 (1993) 334.
[12] J.A. McAteer, M.F. Hiltke, B.M. Silker and R.D. Faulkner, *Clin. Chem.*, 33 (1987) 1788.

[13] K. Lindren, *J. Chromatogr.*, 413 (1987) 347.
[14] M.D. Blanchin, H. Fabre and B. Mandrou, *J. Liq. Chromatogr.*, 11 (1988) 2993.
[15] M.C. Nahata and D.S. Jackson, *J. Liq. Chromatogr.*, 13 (1990) 1651.
[16] N.C. van de Merbel, J.J. Hageman and U.A.Th. Brinkman, *J. Chromatogr.*, 634 (1993) 1.
[17] M.M.L. Aerts, W.M.J. Beek and U.A.Th. Brinkman, *J. Chromatogr.*, 500 (1990) 453.
[18] J. Carlqvist and D. Westerlund, *J. Chromatogr.*, 344 (1985) 285.
[19] E.R. Brouwer, J. Slobodnik, H. Lingeman and U.A.Th. Brinkman, *Analusis*, 20 (1992) 121.
[20] N.C. van de Merbel and U.A.Th. Brinkman, *Trends Anal. Chem.*, 12 (1993) 249.
[21] P.K. Bhattacharyya and W.M. Cost, in K. Floreg (Editor), *Analytical Profiles of Drug Substances*, Vol. 7, Academic Press, New York, NY, 1978, pp. 19–41.