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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

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Abstract

The use of a monolithic column (Chromolith®, SpeedROD RP-18e, by Merck) was studied on the determination of cephalosporin antibiotics. Results were compared with those from a previously developed analytical method using conventional silica-based analytical column. A rapid, accurate and sensitive method has been developed and validated for the quantitative simultaneous determination of four cephalosporins: Cephalexine and Cephadroxil (first generation), Cefaclor (second generation) and Cefotaxim (third generation) in pharmaceuticals as well as in human blood serum and urine. Hydroflumethiazide (HFM) (3,4-dihydro-6(trifluoromethyl)-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide) was used as an internal standard at a concentration of 1.5 ng/μL. A rectilinear relationship was observed up to 5 ng/μL for the four compounds. Analysis time was less than 4 min. The statistical evaluation of the method was examined by means of within-day repeatability ($n = 8$) and day-to-day precision ($n = 8$) and was found to be satisfactory with high accuracy and precision results. The method was applied to the determination of the cephalosporins in commercial pharmaceuticals and in biological fluids: human blood serum after solid phase extraction and urine simply after filtration and dilution. Recovery of analytes in spiked serum samples was in the range from 88.7 to 107.8%, while for urine samples recovery was from 98.0 to 105.6%. By comparing the figures of merit for the monolithic column and the silica-based one, regarding the determination of the four cephalosporins investigated in the present study, the outstanding efficiency of the monolithic column can be noticed.

Keywords: Monolithic columns; Cephalosporins; Cephadroxil; Cefaclor; Cefotaxime; Cephalexine

1. Introduction

Speed of analysis has become of paramount importance in many application areas of HPLC, such as in pharmaceutical and clinical analysis, in order to increase throughput and reduce costs [1].

The recently invented monolithic columns offer new practical possibilities for decreasing retention times and/or increasing column efficiencies, while escaping the pressure constraint to a certain extent. Monolithic columns can potentially provide high performance close to that of UHPLC or CE using common HPLC instrumentation [2,3].

Different research groups have studied monolithic materials for use in HPLC. Based upon the nature of their construction materials, monolithic columns can be classified as organic polymer- or silica-based columns. The first monolithic columns were based upon organic polymers. Polymeric monolithic columns offer excellent biocompatibility, a wide pH range and the ability to be cleaned with caustic mobile phases. A disadvantage is their lack of mechanical stability as most polymers swell or shrink in organic solvents. Silica monoliths can overcome the drawbacks of polymer monoliths. Monolithic silica gel in rod form such as that commercially available by Merck KGaA, Darmstadt, Germany,

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is the suitable material to fulfill the requirements of high throughput analysis [3–5].

The topical manufacturing of these monolithic columns is based on a sol–gel process, which results in a single piece of solid silica adsorbent that possesses an interconnected skeleton and interconnected flow path through this skeleton. The result is a biporous structure consisting of through-pores (macropores with diameters of approximately 2 μm) and mesopores (with diameters of approximately 13 nm) [6].

The through-pores provide the channels for bulk convective flow, while the mesopores are located in the silica skeletons and the surface of the mesopores provides the internal surface area of the monolith on which adsorption of adsorbate molecules occurs. The surface area created by these mesopores is approximately 300 m^2/g. Because the total porosity of the monolithic silica matrix is greater than 80%, users can perform chromatography using a much lower backpressure than they could with conventional packed particles that have total porosities of approximately 65%. Higher flow rates such as 9 mL/min can be also used. This capability is the result of accelerated adsorption and desorption kinetics of the substances separated at the silica–gel surface. Retention times achieved are much shorter than those by conventional reversed-phase columns, resulting in faster separations. An important issue is that the equivalent selectivity enables easy transfer of existing methods from particulate to monolithic columns [7,8].

Cephalosporins are β-lactam antibiotics with the same fundamental structural requirements as penicillin. They are used for the treatment of infections caused by gram-positive and gram-negative bacteria. They act by inhibiting the synthesis of essential structural components of bacterial cell wall. They are among the safest and the most effective broad-spectrum bactericidal antimicrobial agents therefore they are the most prescribed of all antibiotics [9].

Cephalosporin antibiotics are divided into four generations: first, second, third and recently fourth generation compounds. Classification is according to the extent of their effect against gram-negative activity. Third generation cephalosporins are characterized by a broader antibacterial spectrum in comparison with the first and second generation agents as they are resistant to β-lactamases. The most recent fourth generation includes molecules with a spectrum similar to the third, but with markedly increased stability to hydrolysis by β-lactamase [10].

Various methods and reviews have been published covering the analysis of cephalosporins in biological matrices and pharmaceuticals. Among the published methods of determination of cephalosporins TLC, GC, HPLC, CE, and microbiological assays are available. HPLC methods have been described for the determination of cephalosporins in biological fluids using different stationary phases, mobile phases with different buffer systems (mostly phosphates or ion pairing agents), detection mode, e.g. UV and electrochemical and sample preparation procedures [11–21].

The authors in a previous study had developed a simple, rapid assay method for the simultaneous determination of Cephalexine (CPL), Cephadroxil (CPD), Cefaclor (CFC) and Cefotaxim (CFT), in pharmaceutical formulations and biological fluids (blood serum and urine) requiring small volumes of biological fluids [22]. The method gave good separation, sensitivity and linearity over a wide concentration range.

In the present study a monolithic column was used to improve the performance characteristics of the chromatographic system. Results from the two analytical columns are presented comparatively.

2. Experimental

2.1. Instrumentation and chromatography

A Shimadzu (Kyoto, Japan) LC-10AD pump was used to deliver the mobile phase to the monolithic analytical column, Chromolith®, SpeedROD RP-18e 50 mm × 4.6 mm (Merck, Darmstadt, Germany). Sample injection was performed via a Rheodyne 7125 injection valve (Rheodyne, Cotati California, USA) with a 20-μL loop. Detection was achieved by an SSI 500 UV–vis detector (SSI, State College, PA, USA) at a wavelength of 265 nm and a sensitivity setting of 0.002 AUFS. A Hewlett-Packard (Avondale, PA, USA) HP3396 Series II integrator was used for quantitative determination of eluted peaks. A glass vacuum-filtration apparatus obtained from Alltech Associates was employed for the filtration of the buffer solution, using 0.2 μm membrane filters obtained from Schleicher and Schuell (Dassel, Germany). Degassing of solvents was achieved by helium sparging before use. Dissolution of compounds was enhanced by sonication in a Transonic 460/H Ultrasonic bath (Elma, Germany). A Glass-col, Terre Haute 47802 small vortexer and a Hermle centrifuge, model Z 230 (B. Hermle, Gosheim, Germany) were employed for the sample pre-treatment.

All evaporations were performed with a 9-port Reacti-Vap evaporator (Pierce, Rockford, IL, USA).

The mobile phase was prepared by mixing sodium acetate-acetic acid buffer solution (pH 4.0) and methanol in a ratio of 90:10 (v/v). Inlet pressure observed at a flow rate of 1.8 mL/min was 18 kg/cm^2. The injection volume was 20 μL. Hydroflumethiazide (HFM) was used as internal standard at a concentration of 1.5 ng/μL.

2.2. Chemicals and reagents

Cefotaxime sodium salt and Cephalexine hydrate were purchased from Fluka Chemie GmbH (Buchs, Switzerland). Cefaclor, Cephadroxil and hydroflumethiazide (internal standard) were supplied from Sigma (St. Louis, MO, USA). HPLC grade methanol, acetonitrile and acetic acid were supplied from Merck (Darmstadt, Germany), while sodium acetate was from Riedel-de-Haën (AG, Seelze, Germany).

Diol BondElut SPE cartridges were purchased from Analytichem, a division of Varian (Harbor City, CA, USA). Other SPE cartridges examined are LC18 by Supelco (Bellefonte, PA, USA), OASIS by Waters (Waters Corporation, MA, USA), NEXUS by Varian and STRATA-X by Phenomenex (Torrance CA, USA).

Commercial cephadroxil, cephalexine, cefotaxime and cefaclor pharmaceuticals were purchased from a local market. Ceclor, a product by Eli Lilly & Co., Indianapolis, USA, in tablets and oral suspension, was manufactured and packed by VIANEX S.A. Patras, Greece. Tablets contained 500 mg of cefaclor as monohydrate, while suspension, after dilution with water, provided a solution of 250 mg/5 mL. Claforan a product of Hoechst Marion Roussel Rommainville France, is formulated as dry powder to be diluted in 2 mL of water, containing 0.524 g cefotaxime sodium salt corresponding to 0.5 g IM/IV of cefotaxime. It is manufactured and packed by VIANEX S.A. Metamorfosi-Attikis, Greece. Moxacef suspension containing 500 mg/5 mL of cephadroxil monohydrate, a product of Bristol-Myers Squibb Co., USA, is manufactured in Greece.

Serum samples were kindly provided from the Blood Donation Unit of a state hospital.

Urine samples for calibration curve construction and recovery assay were taken from healthy volunteers. Urine samples from a patient were analysed in certain time intervals after administration of cefaclor drug.

2.3. Standard solutions

Stock standard solutions of each cephalosporin were prepared by dissolving an accurate weight of 10 mg for each compound in 100 mL water. Working standard solutions were freshly prepared in deionized water at concentrations ranging from 0.001 to 5 ng/μL.

A 20-μL aliquot was injected onto the column. Quantitative analysis was based on peak area measurements as ratios towards the peak area of internal standard.

Pooled human drug-free serum obtained from healthy volunteers was spiked from the stock cephalosporin solutions to attain concentrations of 0.5, 1.0, 2.0, 3.0, and 4.0 ng/μL.

Pooled human drug-free urine sample was diluted 30-fold with acetate buffer (pH 4) and filtered using 0.2 μm filters. Aliquots from this sample were spiked from the stock cephalosporin solutions to attain concentrations of 0.5, 1.0, 2.0, 3.0, and 4.0 ng/μL.

2.4. Method validation

Method validation was performed in terms of sensitivity and specificity, precision and accuracy, and linearity. Stability was studied previously [22].

2.4.1. Selectivity

The interference from endogenous compounds was investigated by the analysis of six different blank matrices.

2.4.2. Precision and accuracy

Method validation regarding reproducibility was achieved by replicate injections of standard solutions at low, medium and high concentration levels, where peak areas were measured in comparison to the peak area of the internal standard.

An inter-assay precision study (day-to-day reproducibility) was conducted during routine operation of the system over a period of eight consecutive days. Statistical evaluation revealed relative standard deviations at different values for six injections.

Intra-assay precision was studied by eight replicate measurements at three concentration levels.

2.5. Pharmaceuticals sample preparation

2.5.1. Capsules

A number of commercial capsules were finely powdered in a porcelain mortar and an accurately weighed portion of the pooled sample equivalent to the antibiotic content of one capsule 0.5738 g, was quantitatively transferred to a volumetric flask, dissolved and diluted to volume with water. Further dilution provided solutions containing the following concentrations 1.0, 2.0 and 3.0 ng/μL.

2.5.2. Oral suspension

Granules were diluted to 60 mL providing a concentration of 250 mg/5 mL for Ceclor and 500 mg/5 mL for Moxacef. Aliquots of the prepared suspensions were suitably diluted with water to provide three solutions at 1.0, 2.0 and 3.0 ng/μL.

2.5.3. Sterile powder for injection

The total content of one package of the injection in dosage form of sterile powder was transferred into a 1000 mL volumetric flask, dissolved and diluted to volume with water. Aliquots of the prepared solution were suitably diluted to provide three solutions at 2.0, 3.0 and 5.0 ng/μL.

All working solutions originated from pharmaceutical formulations contained the internal standard HFM at a concentration of 1.5 ng/μL. Aliquots of 20 μL were injected onto the HPLC analytical column.

2.6. Sample extraction procedure

Five different sorbents were assayed for the extraction of the investigated cephalosporins: Nexus by Varian, LC18 by Supelco, OASIS by Waters, C-18 Merck and 2-OH Bondelut by Varian. Up to twenty different extraction protocols were assayed using different washing and eluting solvents. Optimum extraction protocol in terms of highest extraction efficiency includes conditioning of the cartridge with 1 mL buffer–MeOH 10:90 (v/v). After sample application, elution was performed by 1 mL of a mixture CAN–MeOH 1:1 (v/v). No washing step was used as a significant analytes loss was noticed during this stage.

2.7. Serum sample preparation

Aliquots of 50 μL human blood serum were spiked with 200 μL of cephalosporin solutions at different concentration levels and treated with 300 μL of acetone in order to precipitate proteins. After vortex mixing for 2 min, the samples were centrifuged at 800 × *g* for 15 min and the supernatants were diluted with water up to 2 mL. Subsequently the samples were quantitatively transferred and slowly applied to the solid phase cartridge, which was preconditioned with 1 mL methanol and 1 mL buffer–MeOH 10:90 (v/v). The samples were subsequently treated according to the procedure described in Fig. 1.

2.8. Urine sample preparation

Urine samples were initially diluted using acetate buffer solution (pH 4) at a volume ratio of 1:30 and filtered using 0.2 μm filters. Drug-free urine spiked samples were prepared by adding the appropriate concentrations of standard solutions containing the internal standard. Aliquots from these samples were injected into the HPLC, analyzed and chromatographed under the conditions described above.

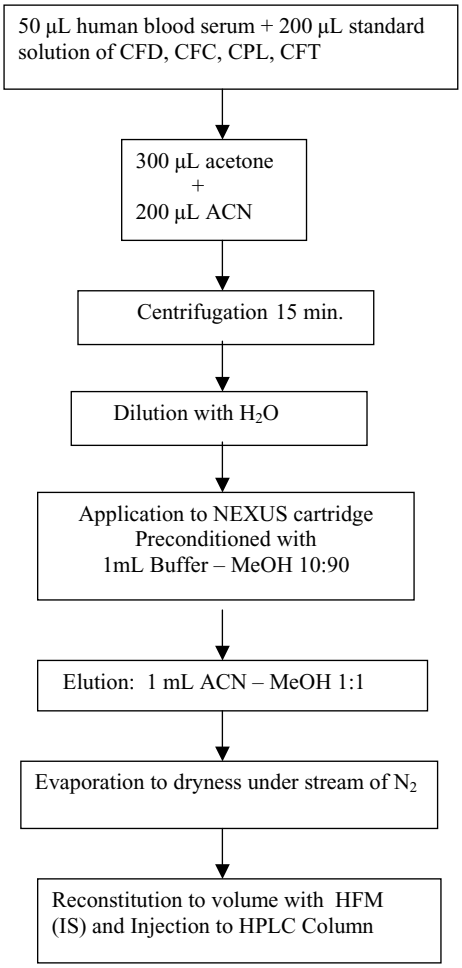

Fig. 1. Solid phase extraction protocol for the isolation of cephalosporins from serum.

3. Results and discussion

3.1. Chromatography

A chromatogram obtained using the developed method conditions is illustrated in Fig. 2. Resolution factors ranged from 0.8 to 1.5 indicating satisfactory separation of resolved analytes.

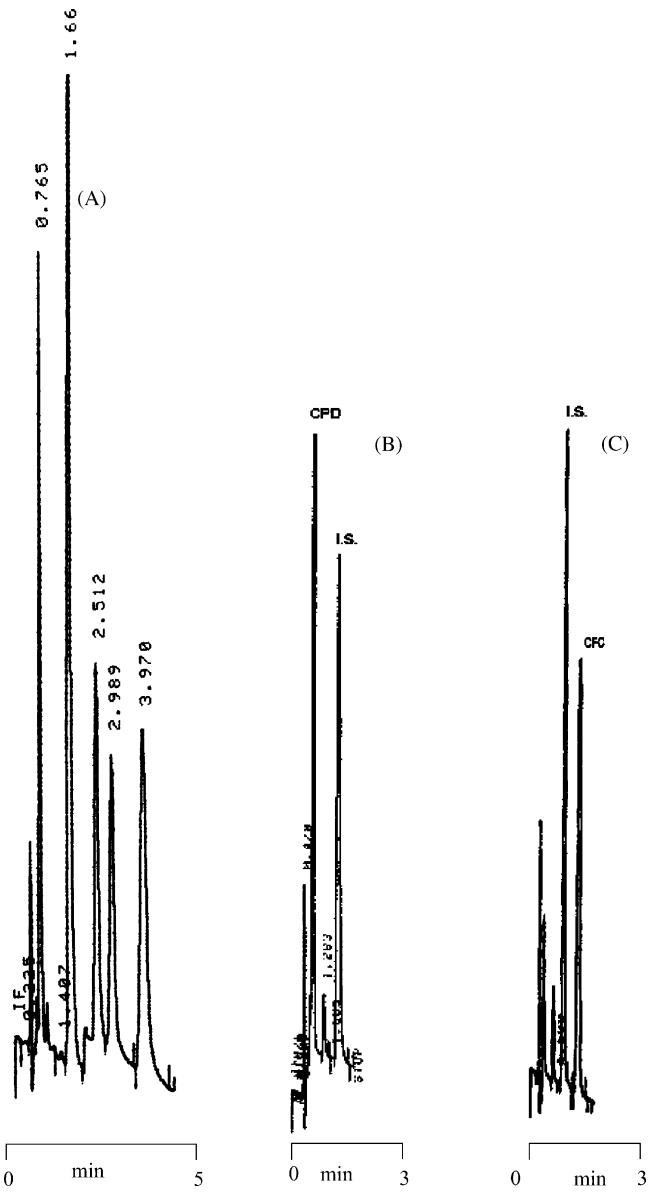

Fig. 2. (A) Typical HPLC chromatogram of examined cephalosporins using a monolithic column: CPD: 0.765 min, HFM (IS): 1.667 min, CFC: 2.512 min, CPL: 2.989 min and CFT:3.970 min. (B) Cephadroxil determination in commercial suspension Moxacef. (C) Cefaclor determination in commercial Ceclor capsules. Chromatographic conditions are described in text.

Table 1
Linearity and sensitivity data of monolithic column application to cephalosporins determination in standard solutions and biological fluids (Y = peak area ratio and X = ng/μL)

Compound	Standard solutions	R	Serum sample	R	Urine sample	R	LOD (ng/μL) (S/N = 3)	LOQ (ng/μL) (S/N = 10)
CPD	$Y = 0.3425X + 0.214$	0.998	$Y = 0.4079X - 0.0693$	0.998	$Y = 0.9827X + 0.9615$	0.998	0.001	0.003
CFC	$Y = 0.3569X + 0.0464$	0.993	$Y = 0.0708X + 0.0055$	0.994	$Y = 0.8934X - 0.4925$	0.9991	0.025	0.075
CPL	$Y = 0.2766X + 0.1115$	0.992	$Y = 0.1614X - 0.0773$	0.9920	$Y = 0.9122X - 0.4842$	0.9991	0.025	0.075
CFT	$Y = 0.5255X + 0.0695$	0.9993	$Y = 0.3895X + 0.2725$	0.9993	$Y = 1.3604X - 0.7082$	0.9994	0.025	0.075

Retention time precision for a 10-day period revealed R.S.D. values <0.1.

3.2. Method validation

3.2.1. Linearity and sensitivity

Calibration curves were obtained by least-squares linear regression analysis of the peak area ratio of analyte/internal standard versus analyte concentration. The method was linear up to 5 ng/μL. Correlation coefficients ranged from 0.9984 to 0.9993.

Table 1 summarizes the results of the method validation regarding accuracy, within-day and day-to-day precision assays. The measured concentrations had R.S.D. values <1%, with almost zero relative error (inaccuracy).

The LOD was defined as the compound concentration that produced a signal-to-noise ratio greater than three. Respective LOD values for each compound based upon this criterion are shown in Table 1. These values were observed for ten samples. The limit of quantitation of the assay was evaluated as the absolute amount of drug equal to ten times the value of the signal-to-noise ratio and found to be 1.5 ng for all compounds except for cephadroxil, which was 0.06 ng.

3.2.2. Precision and accuracy

The precision of the method based on within-day repeatability was performed, by replicate injections ($n = 8$) of three standard solutions covering different concentration levels: low, medium and high, where peak areas were measured, in comparison to the peak area of the internal standard. Statistical evaluation revealed relative standard deviations, at different values. Results are shown in Table 2.

The reproducibility (day-to-day variation) of the method was established using the same concentration range as above. A triplicate determination of each concentration was conducted during routine operation of the system over a period of nine consecutive days. Reproducibility results are illustrated in Table 2.

Accuracy was determined by replicate analysis of three different levels (1.0, 2.0 and 3.0 ng/μL). Expressed as relative error of measurement can be calculated by the equation:

$$\text{Relative error (\%)} = \frac{[\text{mean determined value} - \text{theoretical (added amount)}]}{\text{theoretical}} \times 100$$

Table 2
Accuracy and precision data of monolithic column application to the cephalosporins determination

Intra-day				Inter-day ($n = 5$)			
Added (ng)	Found (ng) ± S.D.	R.S.D.	Relative error (%)	Added (ng)	Found (ng) ± S.D.	R.S.D.	Relative error (%)
CPD				CFD			
20	20.0 ± 0.2	1.0	0.0	20	20 ± 0.2	1.0	0.0
40	42.0 ± 0.5	1.2	5.0	40	44 ± 0.3	0.7	10.0
60	60.0 ± 0.3	0.5	0.0	60	62 ± 0.3	0.5	3.3
CFC				CFC			
20	20.0 ± 0.1	0.5	0.0	20	20.0 ± 0.1	0.5	0.0
40	42.0 ± 0.1	0.2	5.0	40	42.0 ± 0.1	0.2	5.0
60	58.0 ± 0.5	0.9	−3.3	60	58.0 ± 0.1	0.2	−3.3
CPL				CPL			
20	20.0 ± 0.2	1.0	0.0	20	20.0 ± 0.1	0.5	0.0
40	40.0 ± 0.1	0.3	0.0	40	40.0 ± 0.1	0.3	0.0
60	60.0 ± 0.1	0.2	0.0	60	60.0 ± 0.1	0.2	0.0
CFT				CFT			
20	20.0 ± 0.1	0.5	0.0	20	20.0 ± 0.2	1.0	0.0
40	40 ± 0.1	0.3	0.0	40	40.0 ± 0.1	0.3	0.0
60	58 ± 0.1	0.2	−3.3	60	60.0 ± 0.3	0.5	0.0

Table 3
Results from the application of monolithic column to the analysis of pharmaceuticals

Commercial preparation	Active ingredient concentration units	Concentration	
		Labeled	Found ± S.D.
Moxacef suspension	CFD mg/5 mL	500	550 ± 4.0
Ceclor suspension	CFC mg/5 mL	250	313 ± 2.0
Ceclor capsules	CFC mg	500	589 ± 20
Êeflex suspension	CPL mg/5 mL	250	258 ± 5.5
Claforan injection solution IM, IV	CFT g/vial	0.5	0.6 ± 0.03

3.3. Analysis of pharmaceuticals

High performance liquid chromatograms of cephalosporins in pharmaceutical formulations are shown in Fig. 1. The experimental results from these analyses are given in Table 3.

3.4. Solid phase extraction

NEXUS cartridges provided higher recovery rates in cephalosporins' extraction. Elution was achieved by 1 mL of a 1:1 mixture of acetonitrile and methanol. Recovery rates determined by comparing observed analyte concentration in extracted sample to those of non-processed standard solutions, ranged from 98.9 to 106.8%.

3.4.1. Biological fluids: human blood serum and urine

The precision and accuracy studies of SPE of cephalosporins from biological samples were conducted by spiking blood serum samples, at three known concentrations of the compounds and then by comparing obtained results, with those as calculated from regression equations of SPE processed samples. Calibration curves were constructed using the same sample matrix and sample processing procedure. Regression equations revealed correlation coefficients ranging between 0.9920 and 0.9994. Chromatograms of blank and spiked human blood serum are illustrated in Fig. 3. Relative recovery for serum was determined at three different concentrations by comparing the peak area ratios for extracted cephalosporins from serum and the respective values derived from the serum calibration curve. Results of recovery studies for serum samples are given in Table 4. Each value represents the mean of six measurements carried out.

Urine samples were directly analyzed without SPE. The samples were simply diluted and filtered before analysis. Calibration curves were constructed by spiking pooled drug-free urine sample. Correlation coefficients ranged between 0.9982 and 0.9994. Chromatograms of a blank and a spiked urine sample are illustrated in Fig. 3. Results of recovery studies for urine samples are given in Table 4.

No endogeneous interference was noticed for both biological matrices. This is a significant advantage towards the previously developed method where the quantitation of cefadroxil in urine was impaired by interferences caused by an unidentified metabolite.

3.4.2. Application to urine samples after drug administration

Urine samples were analyzed for cefaclor after oral administration to a patient in certain time intervals. Concentrations found are summarized in Table 5.

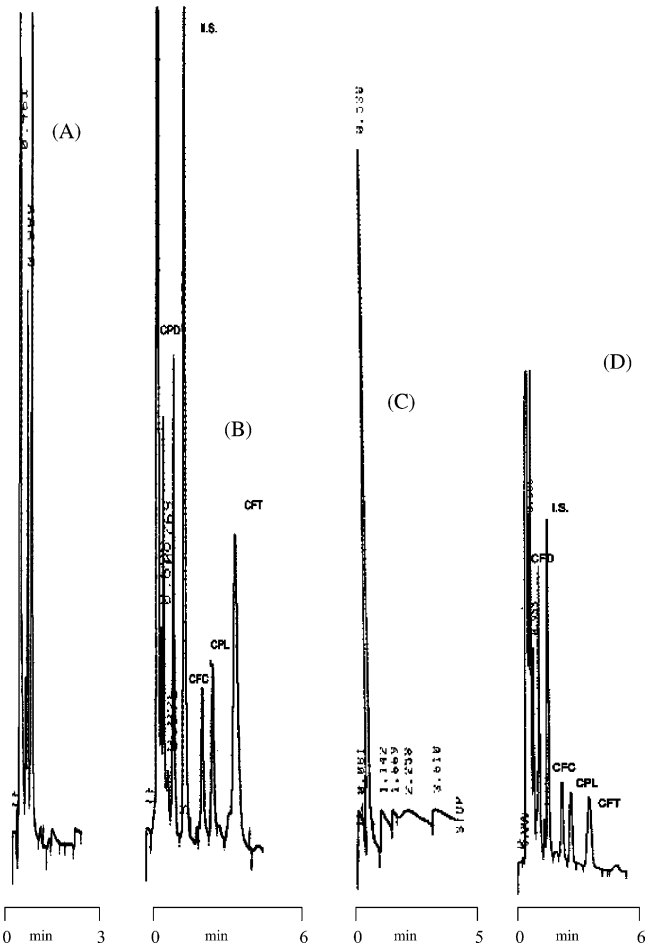

Fig. 3. Typical chromatograms of human blood serum and urine analysis for the determination of cephalosporins using a monolithic column: (A) blank serum sample; (B) spiked serum sample; (C) blank urine sample; (D) spiked urine sample.

Table 4
Cephalosporins recovery rates from serum and urine samples chromatographed on a monolithic column

Serum				Urine			
Added (ng)	Found (ng) ± S.D.	R.S.D.	Recovery (%)	Added (ng)	Found (ng) ± S.D.	R.S.D.	Recovery (%)
CPD				CPD			
20	22.0 ± 0.0	0.0	110.0	40	42.0 ± 0.0	0.0	105.0
40	42.0 ± 0.1	0.2	105.0	60	62.0 ± 0.1	0.2	103.3
60	54.0 ± 0.7	1.3	90.0	80	80.0 ± 0.0	0.0	100.0
CFC				CFC			
20	20 ± 0.1	0.5	100.0	40	42.0 ± 0.1	0.2	105.0
40	42 ± 0.1	0.2	105.0	60	64.0 ± 0.1	0.2	106.7
60	64 ± 0.0	0.0	106.7	80	88.0 ± 0.6	0.7	110.0
CPD				CPL			
20	22.0 ± 0.1	0.5	110.0	40	42.0 ± 0.0	0.0	105.0
40	40.0 ± 0.1	0.3	100.0	60	64.0 ± 0.0	0.0	106.7
60	50.0 ± 0.1	0.2	83.3	80	88.0 ± 0.6	0.7	110.0
CFT				CFT			
20	18.0 ± 0.0	0.0	90.0	40	42.0 ± 0.0	0.0	105.0
40	42.0 ± 0.1	0.2	105.0	60	64.0 ± 0.1	0.2	110.0
60	64.0 ± 0.0	0.0	106.7	80	88.0 ± 0.6	0.7	110.0

Table 5
Urine samples analysed after Cefaclor administration

Urine sample	Time after administration (min)	CFC concentration (ng/μL) ± S.D.
1	30	2.3 ± 0.2
2	45	9.3 ± 0.8
3	120	16.2 ± 1.1
4	180	18.8 ± 1.3

3.5. Comparison of column performance towards a conventional silica-based column

As previously stated the objective of this study was to compare a monolithic column towards a conventional silica based with regards to cephalosporins analysis of pharmaceuticals and biological fluids. Table 6 summarizes the performance characteristics of the monolithic and the conventional silica-based analytical columns. By comparing the figures of merit for the two columns, regarding the determination

Table 6
Comparison of monolithic column performance towards a conventional silica-based reversed-phase HPLC column with regards to cephalosporins analysis

Parameter	Chromolith®, SpeedROD RP-18e	Spherisorb-ODS-2
Mobile phase	CH_3COOH/CH_3COONa–MeOH 90:10	CH_3COOH/CH_3COONa–MeOH 78: 22
Flow rate (mL/min)	1.8	1.2
Linear velocity (cm/min)	22.2	24.0
Number of theoretical plates (m)	31080[a]	15410[a]
Pressure (kg/cm^2)	18	203
Analysis time (min)	3.9	6.6
LOD (ng/μL)	CPD: 0.001 CFC: 0.025 CPL: 0.025 CFT: 0025	CPD: 0.01 CFC: 0.005 CPL: 0.01 CFT: 0.005
LOQ (ng/μL)	CPD: 0.003 CFC: 0.075 CPL: 0.075 CFT: 0.075	CPD: 0.03 CFC: 0.015 CPL: 0.03 CFT: 0.015
Upper limit (ng/μL)	5.0	CPD: 8, CFC: 12, CPL: 35, CFT: 5
Intra-day (relative error, %)	−3.3 to +5.0	−8.5 to + 3.0
Intra-day R.S.D. (%)	0.2 to 1.2	0.6 to 5.1
Inter-day (relative error, %)	−3.3 to +10.0	−6.6 to +4.2
Inter-day R.S.D. (%)	0.2 to 1.0	0.2 to 6.9

[a] Cefaclor is used as test compound.

of the four cephalosporins investigated in the present study, we can notice the outstanding efficiency of the monolithic column. At almost the same linear velocity, the number of theoretical plates per meter is twofold in the case of the monolithic column, using cefaclor as test compound. Additionally an extremely high precision and accuracy is noticed, at a significantly lower analysis time.

4. Conclusions

Monitoring of the antibiotics concentration in biological fluids such as serum and urine is important in pharmacokinetic studies. The aim of this study was to investigate the use of a monolithic column for the simultaneous determination of four cephalosporin antibiotic agents in pharmaceuticals and biological fluids. This was achieved with the development of a simple, fast and sensitive method, improving the efficiency of a previously reported one from the authors. Though simultaneous dosing of the four compounds is unlikely and formulations usually contain only one as active ingredient, the simultaneous determination is useful for routine application in the clinical laboratory as with a single sample preparation protocol the analyst can monitor any of the studied analytes.

The monolithic column presented an outstanding efficiency with extremely high precision and accuracy as well as the low retention times achieved by applying higher flow rates. The method described herein is an improved validated assay that can readily be used in any laboratory for the quantitative determination of cephalosporins: cephadroxil, cefaclor, cefotaxime and cephalexine in less than 4 min. Sample preparation time for the urine is minimal since no SPE is required. For serum samples, the sample pre-treatment step using SPE can be accelerated by using a SPE manifold.

No endogeneous interference was noticed for both biological matrices. This is a significant advantage since in the previous method the quantitation of cefadroxil in urine was impaired by interferences caused by an unidentified metabolite.

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