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Column liquid chromatography of cefadroxil on poly(styrene–divinylbenzene)

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ABSTRACT

Isocratic column liquid chromatography on a poly(styrene–divinylbenzene) stationary phase (PLRP-S, 25 cm × 0.46 cm I.D.) at 50°C allowed the separation of cefadroxil from related substances. The mobile phase was acetonitrile–0.02 *M* sodium 1-octanesulphonate–0.2 *M* phosphoric acid–water (10.5:20:5:up to 100, v/v). The flow-rate was 1.0 ml/min and UV detection was performed at 254 nm. Official standards were compared and a number of commercial bulk samples and specialities were analysed.

INTRODUCTION

Cefadroxil is a semi-synthetic β-lactam antibiotic from the group of the cephalosporins. Although column liquid chromatography (LC) of cefadroxil has been discussed in several papers, the separation of cefadroxil from its potential impurities has not been reported. Some papers have described the determination of cefadroxil in biological samples [1–4], and mainly considered to the separation of the antibiotic from the biological matrix. The separation of cefadroxil from a mixture of cephalosporins has also been reported [5,6]. Tsuji *et al.* [7] studied the degradation kinetics of cefadroxil by means of LC techniques. LC has also been used for pharmacokinetic studies of cefadroxil [8]. The separation of cefadroxil from excipients of pharmaceuticals has also been reported [9]. The European Pharmacopoeia (Ph. Eur.) proposed an LC method for the assay of cefadroxil [10]. Nearly the same LC method is prescribed by the United States Pharmacopeia XXII (USP) for the assay of cefadroxil [11]. This method was examined in our laboratory and it was found that it did not allow the complete separation of cefadroxil from its potential impurities; these results will be reported later. The Ph. Eur. and the USP prescribe the use of reversed-phase materials based on silica, for which the poor reproducibility of the selectivity towards cephalosporins has been reported earlier [5].

In this paper, an isocratic method using poly(styrene–divinylbenzene) (PS–DVB) as the stationary phase is described. It permits the complete separation of cefadroxil from known related substances and performs equally well on different available brands of PS–DVB. The method has been used to compare official standards and to analyse a number of commercial samples of different origin.

EXPERIMENTAL

Reference substances and samples

The United States Pharmacopeia Reference Standard (USP-RS; Lot H; 929 μg/mg) and the European Pharmacopoeia Chemical Reference Standard (Ph. Eur.-CRS; 94.2%) were used.

Bulk samples were obtained from Italy, India and

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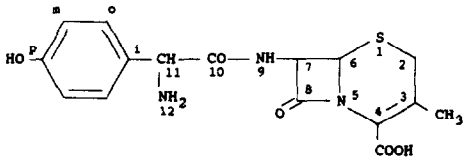
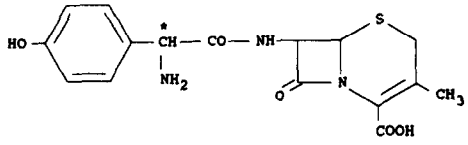
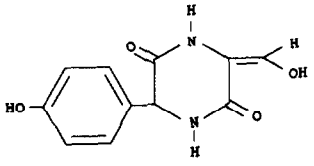


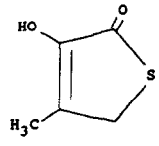
I D-Cefadroxil



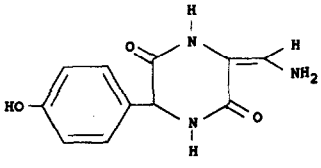
II L-Cefadroxil **



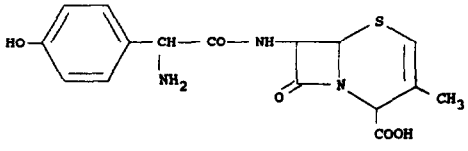
III 3-Hydroxymethylene-6-(4-hydroxyphenyl)-piperazine-2,5-dione *



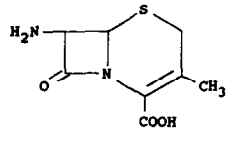
IV 3-Hydroxy-4-methyl-2(5H)-thiophenone *



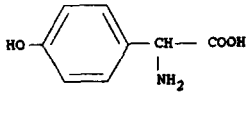
V 3-Aminomethylene-6-(4-hydroxyphenyl)-piperazine-2,5-dione *



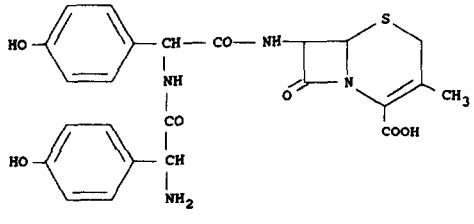
VI Δ^2-Cefadroxil **



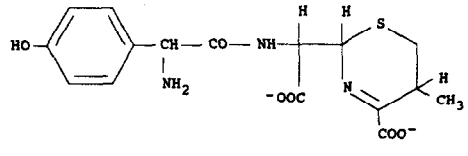
VII 7-Aminodesacetoxycephalosporanic acid (7-ADCA) ***



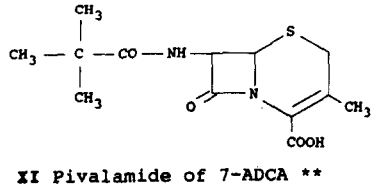
VIII 4-Hydroxyphenylglycine ***



IX 4-Hydroxyphenylglycylcefadroxil **



X Cefadroxil Δ^4-cephalosporoates *



XI Pivalamide of 7-ADCA **

Fig. 1. Structures of cefadroxil and related substances. * Degradation product. ** Side-product of the synthesis. *** Starting product of the synthesis.

Belgium. Dosage forms containing cefadroxil (Duracef, Moxacef, Bristol) were obtained commercially in Belgium.

Related substances

Related substances present as impurities in cefadroxil can originate from the semi-synthesis and from degradation. Fig. 1 shows the structures of D-cefadroxil and its potential impurities. Compounds **VII** and **VIII**, which are the basic constituents of the cefadroxil molecule, are commercially available (**VII**, Gist-Brocades, Delft, Netherlands; **VIII**, Janssen Chimica, Beerse, Belgium). Compounds **II**, **VI**, **IX** and **XI** can arise from the semi-synthesis of cefadroxil. Compounds **II**, **VI** and **IX** were prepared in the laboratory and **XI** was provided by a manufacturer. The other related substances are decomposition products formed in acidic (**III**, **IV**), neutral (**III**, **V**) and alkaline (**X**) media. Compounds **III**, **IV**, **V** and **X** were prepared in the laboratory. The preparation of these products will be described elsewhere. Compound **X** was never isolated but was prepared *in situ* by dissolving cefadroxil in 0.1 *M* NaOH (1 mg/ml) and storing the solution at room temperature for 10 min.

Solvents and reagents

Acetonitrile (99%) (Janssen Chimica) and methanol (Roland, Brussels, Belgium) were distilled before use. 2-Methyl-2-propanol (99.5%) (Janssen Chimica) was used as received. Phosphoric acid (85%) and potassium dihydrogen phosphate (analytical-reagent grade) were purchased from Merck (Darmstadt, Germany) and sodium 1-octanesulphonate (NaOS) from Janssen Chimica. Water was distilled twice.

LC apparatus and operating conditions

Isocratic elution (1 ml min^{-1}) was used throughout. The equipment consisted of an L-6200 pump (Merck–Hitachi, Darmstadt, Germany), a CV-6-UHPa-N60 20-μl loop injector (Valco, Houston, TX, USA), a 25 cm × 0.46 cm I.D. column, packed with 8-μm PLRP-S 100 Å (Polymer Labs., Church Stretton, Shropshire, UK), a Model D 254-nm fixed-wavelength UV monitor (LDC/Milton Roy, Riviera Beach, FL, USA) and a Model 3396 A integrator (Hewlett-Packard, Avondale, PA, USA). The column temperature was maintained at 50°C by means of a water-bath heated by a Julabo EM thermostat (Julabo Labortechnik, Seelbach, Germany). The selectivity of the method was also tested on other PS–DVB stationary phases [PRP-1 (10 μm) and PRP-1 (7–9 μm); Hamilton, Reno, NV, USA]. For the examination of peak homogeneity the UV detector was replaced with a Model 990 photodiode-array detector (Waters Assoc., Milford, MA, USA). A Marathon autosampler with sample cooling (Spark Holland, Emmen, Netherlands) equipped with a fixed 20-μl loop and a Julabo C and F10 cryomat was used for quantitative analysis.

Mobile phase

The mobile phase finally used was prepared by mixing 500 ml of water, 105 ml of acetonitrile, 200 ml of 0.02 *M* NaOS and 50 ml of 0.2 *M* phosphoric acid. The mixture was diluted to 1000 ml with water and degassed by ultrasonication before use.

Sample preparation

Samples for quantitative analysis were prepared by weighing an amount corresponding to 30 mg of cefadroxil into a 20-ml volumetric flask. Mobile phase containing 30% of 0.02 *M* NaOS solution was used as the solvent. For the specialities a mixture of sample powder and solvent was ultrasonicated and centrifuged and the supernatant was analysed. The reference substances were dissolved the same way as the samples.

RESULTS AND DISCUSSION

Development of the chromatographic method

PS–DVB was used as the stationary phase because of its good stability, even at extreme pH conditions and high temperatures [12]. The stationary phase was heated at 60°C to enhance the mass transfer and to reduce the back-pressure. The influence of the column temperature is discussed below.

The mobile phase, developed earlier for the chromatography of cefalexin [13], was examined for its suitability to separate cefadroxil from its potential impurities. It consisted of acetonitrile–0.02 *M* NaOS–0.2 *M* phosphoric acid (pH 1.4)–water (10:5:10:up to 100, v/v). Using this mobile phase, cefadroxil was separated from the related substances, but some substances (**II** and **VI**) were eluted as split peaks. The composition of the mobile phase

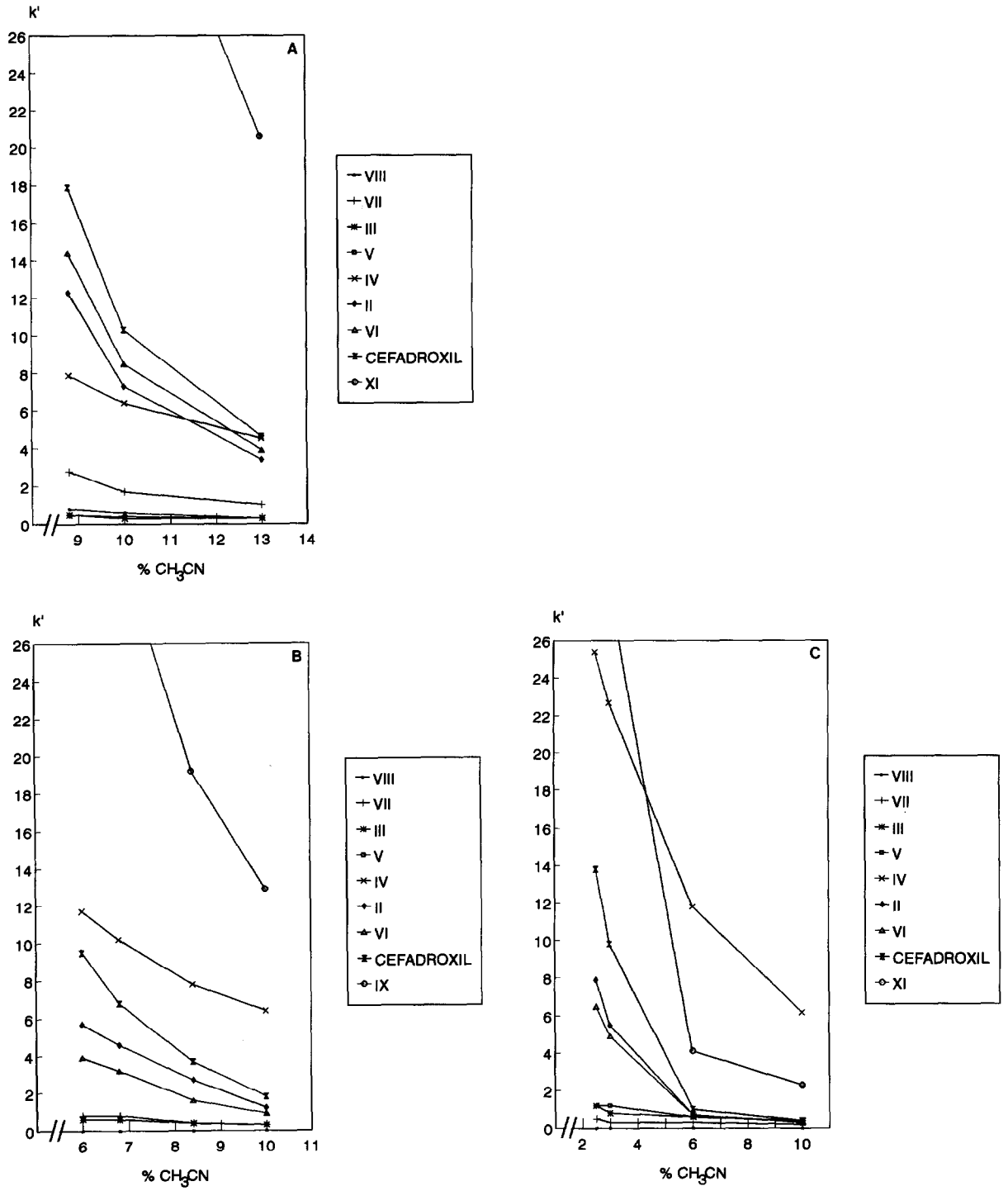


Fig. 2. Influence of the concentration of acetonitrile on the separation of cefadroxil and related substances at (A) pH 1.4, (B) pH 3.0 and (C) pH 4.0. Stationary phase: PLRP-S 100 Å (8 μm). Mobile phase: acetonitrile–0.02 *M* NaOS–0.2 *M* H_3PO_4 or 0.2 *M* phosphate buffer (pH 3.0 or 4.0)–water [*x*:10:5:up to 100, v/v].

obviously needed some modification. Therefore, the mobile phase was further evaluated by systematic examination of its components.

The solution of phosphoric acid was replaced with 0.2 *M* phosphate buffers of pH 3.0 or 4.0 in order to examine the influence of the pH on the separation. For each pH, the amount of organic modifier was varied to optimize the separation (Fig. 2). All the products eluted much faster at higher pH, except for **III**, **IV** and **V**, which were not affected by the pH. Compounds **III** and **IV** had no positively charged amino group like the other related substances and consequently did not interact with the ion-pairing reagent. Compounds **III** and **V** were almost never separated at the investigated pH values. Compound **V** was probably hydrolysed by acidic mobile phases and converted into **III**. This was confirmed by photodiode-array detection. Compound **XI** was eluted very late compared with the other substances, except at pH 4.0. At pH 1.4

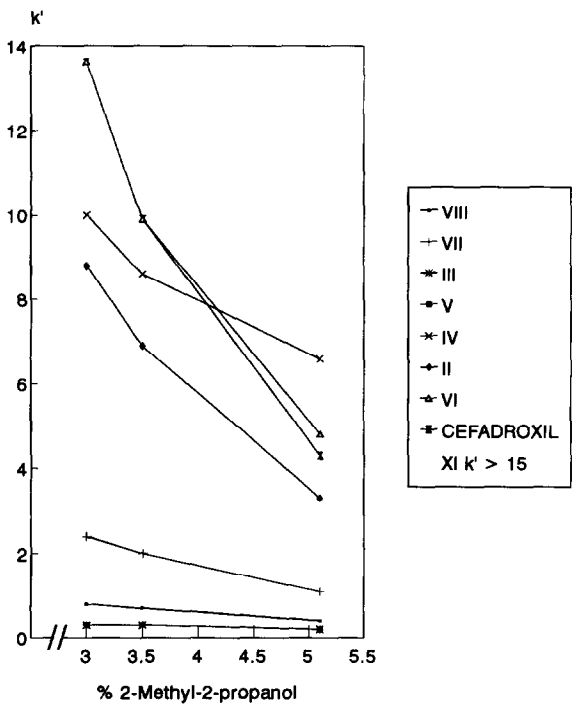


Fig. 3. Influence of the concentration of 2-methyl-2-propanol as organic modifier on the separation of cefadroxil and related substances. Stationary phase: PLRP-S 100 Å (8 μm). Mobile phase: 2-methyl-2-propanol–0.02 *M* NaOS–0.2 *M* H_3PO_4–water [*x*:10:5:(85 − *x*), w/v/v/v].

complete separation between cefadroxil and related substances was achieved when less than 12% of acetonitrile was used. A pH of 1.4 gave an average plate number of 3000/m. At pH 3.0 the order of elution was changed, **IV** being eluted after cefadroxil. Using about 7% of acetonitrile, all the related substances were separated from cefadroxil. The efficiency was distinctly lower, however, than at pH 1.4 (1600 plates/m). At pH 4.0 a very low efficiency was obtained (400 plates/m). Only very slow elution with 3% or less of acetonitrile present allowed the complete separation of cefadroxil. In conclusion, phosphoric acid was chosen to adjust the pH of the mobile phase. At this pH the retention time of **IX** was at least four times that of cefadroxil, and because its separation caused no problem, **IX** was omitted from subsequent experiments.

Mobile phases with other organic modifiers were also examined. 2-Methyl-2-propanol had already been demonstrated to be very suitable for the LC of tetracycline [14] or erythromycin [15] on PSDVB. Fig. 3 shows the capacity factors obtained with different concentrations of 2-methyl-2-propanol. None of the investigated mobile phases was able to separate cefadroxil and its Δ^2-isomer **VI**, although the efficiency (3500 plates/m) was better than with acetonitrile. In Fig. 4 the elution pattern obtained with different concentrations of methanol as organic modifier is shown. Also owing to the low efficiency (1700 plates/m), cefadroxil was not separated from **IV** and **VI**. As a result, acetonitrile was preferred as the organic modifier.

The concentration of 0.2 *M* phosphoric acid did not affect the selectivity in the range examined (2.5–7.5%, v/v). Therefore, the initial concentration of 5% was maintained.

The influence of the ion-pairing reagent NaOS is shown in Fig. 5. With 5% of the 0.02 *M* NaOS solution most substances were eluted too fast and the elution order had changed, the non-protonated **IV** now being eluted between cefadroxil and **VI**. Doubling the original concentration of 0.02 *M* NaOS to 20% gave a remarkable improvement. The peaks corresponding to **II** and **VI** were symmetrical and the separation between **II** and **IV** had improved. In further experiments, 20% of 0.02 *M* NaOS solution in the mobile phase was adopted.

The mobile phase finally selected for further use throughout the study was acetonitrile–0.02 *M*

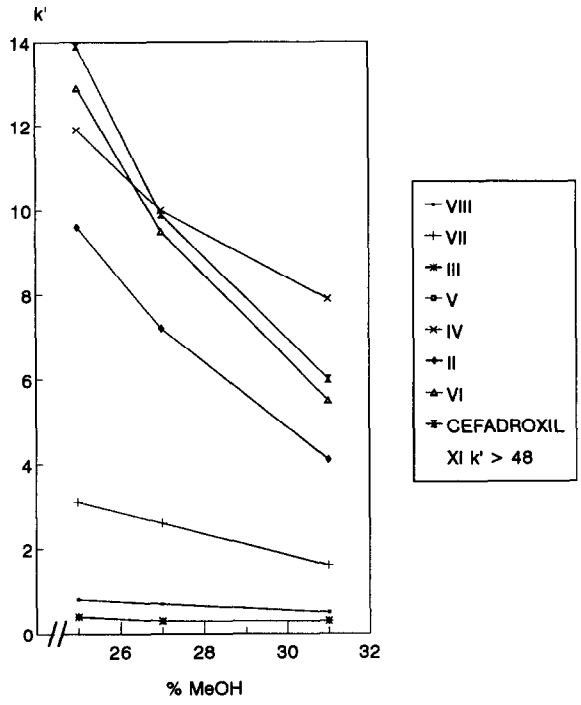


Fig. 4. Influence of the concentration of methanol as organic modifier on the separation of cefadroxil and related substances. Stationary phase: PLRP-S 100 Å (8 μm). Mobile phase: methanol–0.02 *M* NaOS–0.2 *M* H_3PO_4–water [*x*:10:5:up to 100, v/v].

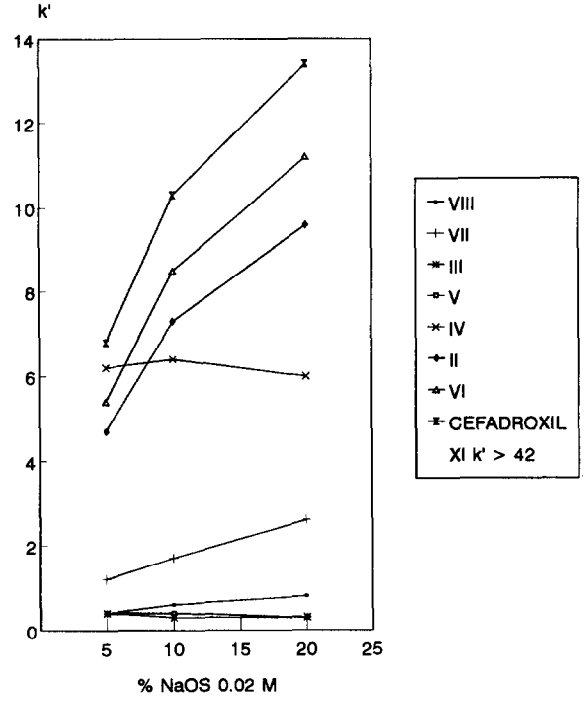


Fig. 5. Influence of the concentration of NaOS in the mobile phase on the separation of cefadroxil and related substances. Stationary phase: PLRP-S 100 Å (8 μm). Mobile phase: acetonitrile–0.02 *M* NaOS–0.2 *M* H_3PO_4–water [10:*x*:5:up to 100, v/v)].

NaOS–0.2 *M* phosphoric acid–water (10.5:20:5:up to 100, v/v).

The selectivity of the method was further validated by examination of the peak homogeneity of the cefadroxil peak after decomposition of a sample in alkaline or acidic medium. For this purpose, the chromatograms were recorded by means of photodiode-array detection. A solution of cefadroxil in 0.1 *M* NaOH (1 mg/ml) was stored at room temperature for 10 min, neutralized and analysed. The cefadroxil content had already decreased by 50%. After examination of the UV spectra of the left-hand slope, the maximum and the right-hand slope of the cefadroxil peak, it was found to be homogeneous. A solution of cefadroxil in 0.1 *M* HCl (1 mg/ml) stored at 60°C for 5 h was examined in the same way. The cefadroxil content was reduced by about 5% but the peak was homogeneous.

The reproducibility of the selectivity on other PS–DVB stationary phases was checked using the same mobile phase. The content of acetonitrile was adapted for each column to obtain comparable retention times. The characteristics of the columns are listed in Table I. The separation pattern, shown in Fig. 6, is the same for each column. This reproducible selectivity is an advantage with regard to the use of reversed-phase stationary phases based on silica and ensures an improvement of the between-laboratory precision.

The influence of the column temperature on the separation was investigated at 50, 60 and 70°C. The products were eluted faster at higher temperature, but the selectivity was similar at all three temperatures. The stability of a solution of cefadroxil in the mobile phase stored at these three temperatures was also examined. The solutions stored at 60 or 70°C showed decomposition after 45 minutes (0.3% and 3.7%, respectively), whereas at 50°C no decomposition was observed. In order to guarantee stability of the samples during analysis, the column temperature was decreased to 50°C.

Analysis of a solution of cefadroxil in water gave

TABLE I

CHARACTERISTICS OF THE PS–DVB COLUMNS USED, WITH THE RESPECTIVE CONCENTRATIONS OF ACETONITRILE IN THE MOBILE PHASE

Column	Age (years)	Stationary phase (250 × 4.6 mm I.D.)	Batch	Acetonitrile (%)
A	5	PLRP-S 100 Å (8 μm)	(10-12-85)B	11.0
B	5	PLRP-S 100 Å (8 μm)	(10-12-85)B	11.0
C	2	PLRP-S 100 Å (8 μm)	35	10.5
D	1	PLRP-S 100 Å (8 μm)	35	11.0
E	1	PLRP-S 100 Å (8 μm)	35	11.5
F	New	PLRP-S 100 Å (8 μm)	8M-RPS-1-64	10.5
G	6	PRP-1 (10 μm)	79400	11.5
H	New	PRP-1 (7–9 μm)	457	11.0

several disturbing system peaks. Using the mobile phase as the solvent, some system peaks disappeared. After systematic variation of the composition of this solvent, a mobile phase containing 30% of 0.02 *M* NaOS was chosen as the solvent for the samples. Unfortunately, one small system peak was still present.

Fig. 7 shows a typical chromatrogram of cefadroxil obtained using the selected chromatographic conditions. The positions of the related substances are indicated.

Finally, a resolution test for this LC method was developed. The products that eluted closest to cefadroxil were **IV**, **II** and **VI**. As these related substances were not commercially available, a solution of 5 mg of cefadroxil and 40 mg of amoxicillin trihydrate in 50 ml of water was used to calculate the resolution. Amoxicillin was chosen because its structure is closely related to that of cefadroxil. As its specific absorption is much lower than that of cefadroxil, the concentration was adjusted in order to obtain peaks of equal height. A mixture of ce-

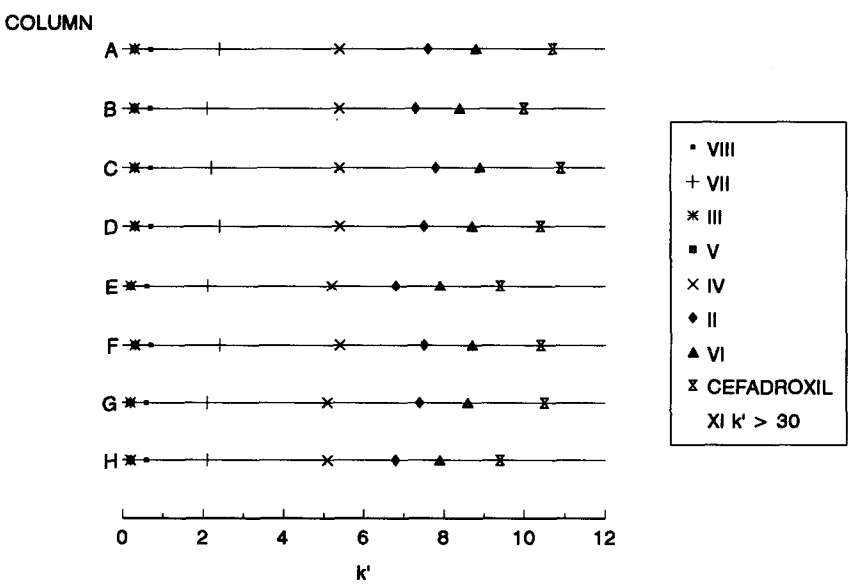


Fig. 6. Capacity factors of cefadroxil and related substances on different PS–DVB columns (see Table I for characteristics). Mobile phase: acetonitrile–0.02 *M* NaOS–0.2 *M* H_3PO_4–water [*x*:20:5:up to 100, v/v]; for *x*, see Table I.

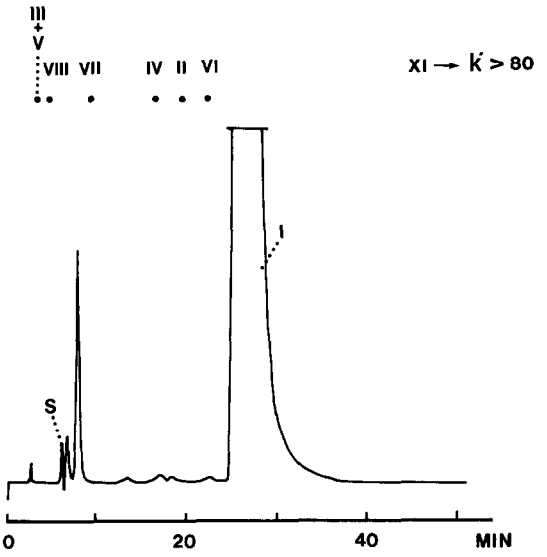


Fig. 7. Typical chromatogram of cefadroxil (30 μg). The positions of the related substances are indicated. Stationary phase: PLRP-S 100 Å (8 μm), 50°C. Mobile phase: acetonitrile–0.02 *M* NaOS–0.2 *M* H_3PO_4–water (10.5:20:5:up to 100, v/v). S = System peak.

fadroxil and small amounts of **IV**, **II** and **VI** was analysed for visual evaluation of the separation. These solutions were repeatedly analysed on the columns listed in Table I, while the amount of organic modifier was increased. It was concluded that a resolution of 4.0, calculated for the peaks of cefadroxil and amoxicillin, guaranteed sufficient separation of cefadroxil from **IV**, **II** and **VI**.

Quantitative aspects of the LC method

The loadability was not only limited by the capacity of the column or the composition of the mobile phase, but also by the equipment used. Analysis of more than 100 μg of cefadroxil gave a chromatogram with an incompletely integrated cefadroxil peak. It was decided to use a 30-μg amount for quantitative analysis of cefadroxil samples. For this amount the limit of detection (LOD) (S/N = 3) was 0.04%, expressed as cefadroxil. The limit of quantification (LOQ) was 0.08% [n = 8, relative standard deviation (R.S.D.) = 16%]. The repeatability was checked by analysing six times the same solution of cefadroxil (30 μg) (R.S.D. = 0.04%, calculated on the area of the cefadroxil peak) and by analysing subsequently six freshly prepared solutions of cefadroxil (30 μg) (R.S.D. = 0.3%, calculated on the area of the cefadroxil peak). At about 6°C, solutions of cefadroxil in the mobile phase remained stable for at least 24 h. Linearity tests were performed (y = peak area/1000, x = amount injected in μg): $y = 9969x - 2709$, standard error of estimate $S_{y,x}$ = 609, correlation coefficient r = 0.999 (n = 9), range of x covered in the experiments = 24–36 μg.

TABLE II

COMPOSITION OF CEFADROXIL STANDARDS

Values in % (w/w); R.S.D. values (%) are given in parentheses.

Parameter	Ph. Eur.-CRS (94.2%)	USP-RS (929 μg/mg)
Number of analyses	9	9
Number of solutions analysed	3	3
Cefadroxil	94.24[a] (0.8)	93.92 (0.4)
Impurities	0.31[a]	0.48 (9.5)
Residual solvents	0.29[a]	<0.1[b], ND[c]
Water	5.16[a]	5.21[b], ND[c]
Total	100.0	99.6

[a] Following ref. 16.
[b] Following ref. 17.
[c] ND = Not determined owing to limited amount of sample.

TABLE III

COMPOSITION OF BULK SAMPLES OF CEFADROXIL

Values in % (w/w); R.S.D. (%) is given in parentheses. Water was determined by Karl Fischer titration. N = Number of analyses.

Origin	Sample No.	LC: Cefadroxil (n = 5) (A)	LC: Impurities (n = 5) (B)	Water (n = 4) (C)	Total ($A + B + C$)	Total ($C + D$)	Non-aqueous titration (n = 4) (D)
A	1	94.01 (0.4)	0.30 (10.0)	5.52 (2.3)	99.83	100.03	94.51 (0.1)
A	2	92.47 (0.3)	1.06 (11.2)	5.50 (2.3)	99.03	100.10	94.60 (0.2)
B	3	93.98 (0.6)	0.59 (12.6)	5.65 (2.0)	100.22	101.06	95.41 (0.4)
C	4	93.72 (0.2)	0.58 (7.8)	5.68 (2.4)	99.98	100.46	94.78 (0.3)
C	5	94.21 (0.5)	0.44 (11.0)	5.16 (1.5)	99.81	100.45	95.29 (0.0)

Comparison of cefadroxil standards

The USP-RS and the Ph. Eur.-CRS were compared by means of the proposed LC method. The cefadroxil content of the USP-RS was calculated by comparison with the Ph. Eur.-CRS, which has an assigned cefadroxil content of 94.2% on "as is" [16]. Samples of 30 μg of each standard were analysed. Because the standards contained only small amounts of impurities, the total content of impurities was calculated by comparison with the peak area obtained for 0.3 μg of cefadroxil Ph. Eur.-CRS (1% of the main peak). The results are given in Table II. The R.S.D. values, given in parentheses, are within acceptable limits. The LC result for the USP-RS seems to be higher than the declared content. The latter, however, is expressed in μg of activity per mg and not in μg of mass per mg. The sum of cefadroxil, impurities, residual solvents and water for the USP-RS is close to 100%. Owing to the limited amount of this standard available, the contents of water and residual solvents were not determined.

Analysis of commercial samples

A number of bulk samples of cefadroxil were analysed against the Ph. Eur.-CRS and the results are given in Table III. The R.S.D. on the cefadroxil content did not exceed 1.0%. The total mass was well explained by addition of the content of cefadroxil, impurities and water, except for sample 2, which has the lowest content of cefadroxil. This may be because some of the decomposition products have a lower UV absorbance than cefadroxil at 254 nm.

For comparison, the base content of the bulk samples was determined by non-aqueous titration. Cefadroxil (250 mg) was dissolved in 40 ml of acetic

TABLE IV

CEFADROXIL CONTENT OF PHARMACEUTICALS AS A PERCENTAGE OF LABEL CLAIM

Sample No.	Form	Mean content (%, w/w) (n = 4)	R.S.D. (%)
1	Capsules	106.78	0.9
2		104.92	0.4
3	Powder for suspension	111.18	0.8
4		120.90	1.0
5		101.57	0.8
6		111.08	1.3

acid and titrated with 0.1 *M* perchloric acid using potentiometric end-point detection. Each sample was titrated four times. The results are given in Table III. The total figures are higher for the titration results, which might be due to the presence of impurities carrying a basic function. The content of residual organic solvents was not determined. All the samples complied with Ph. Eur. requirements for content (95.0% on an anhydrous basis) and water (3.0–6.0%).

A few pharmaceutical preparations of the same origin were analysed following the same method. The content was expressed as a percentage of the label claim. The results are given in Table IV.

CONCLUSIONS

The results show that the described LC method is suitable for purity control and assay of bulk samples and of preparations of cefadroxil. Important advantages of the method are its reproducible selectivity and the applicability on PS–DVB stationary phases of different origins and ages.

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