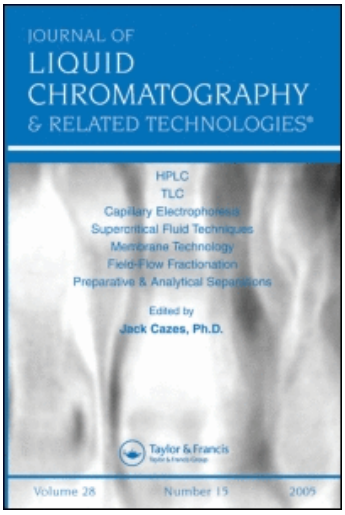

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Quantitative Analysis of Cefalexin by Liquid Chromatography on Poly(styrene-divinylbenzene)

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QUANTITATIVE ANALYSIS OF CEFALEXIN BY LIQUID CHROMATOGRAPHY ON POLY(STYRENE-DIVINYLBENZENE)

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ABSTRACT

A method for analysis of cefalexin by isocratic liquid chromatography is described. Cefalexin is separated from its related substances on a poly(styrene-divinylbenzene) stationary phase (PLRP-S, 250 x 4.6 mm I.D.) at 50 °C. The mobile phase contains acetonitrile, 0.02 M sodium 1-octanesulphonate, 0.2 M phosphoric acid and water (15.5:10:5:up to 100). The flow rate is 1.0 ml/min, 30 µg samples are injected and UV-detection is at 254 nm. Results for official reference substances, a number of bulk samples and pharmaceutical dosage forms are reported.

INTRODUCTION

Cefalexin is a semi-synthetic ß-lactam antibiotic which belongs to the group of the cephalosporin antibiotics. The complex degradation pathways of this

relatively unstable molecule explain the need for a discriminating liquid chromatographic (LC) method.

A number of papers on LC of cephalosporins described the determination of cefalexin in biological samples [1-11]. These methods emphasize the separation of the antibiotic from the biological matrix. The separation of cefalexin from a mixture of cephalosporins by liquid chromatographic methods was also reported frequently [12-21]. The main application of these methods is mostly identification of the antibiotics, without considering the quantitative aspects. The separation of cefalexin from excipients of pharmaceuticals was described by Das Gupta et al [22]. A few articles dealt with the determination of cefalexin as an impurity in cefradine [23-25]. LC-techniques were also used in pharmacokinetical studies [26] and in studies about kinetics and degradation [27]. Fabregas et al described the LC-separation of cefalexin and lysine for the analysis of the lysine salt of cefalexin [28]. The United States Pharmacopeia XXII, first supplement, prescribes an isocratic LC-method for the assay of cefalexin and a LC-method using gradient elution for the determination of related substances in cefalexin [29]. The European Pharmacopoeia also prescribes a LC-method for the assay of cefalexin [30]. These pharmacopoeial methods were examined in our laboratory and did not show complete separation of cefalexin from its potential impurities. These results will be reported elsewhere.

Generally, in literature little attention is paid to the separation of cefalexin from its related substances. A few exceptions are the separation of cefalexin and 7-aminodesacetoxycephalosporanic acid (7-ADCA) [20,31], the separation of the 7α- and 7ß-epimers of cefalexin [32], the separation between Δ^2-cefalexin and cefalexin [33] and between cefalexin and L-cefalexin [34]. Mason and Tranter

described the separation of cefalexin from L-cefalexin, 7α-cefalexin, Δ^2-cefalexin, 7-ADCA, fenyl-glycin and 3-hydroxy-4-methyl-2(5H)-thiophenone using ion-paired chromatography on C_8 [31].

Except for the last cited publication, none of the LC-methods described in literature has been examined thoroughly on its suitability to separate cefalexin from its known decomposition products and side-products, arising from the semi-synthetic preparation. The reproducibility of the method of Mason and Tranter [31] on C_8-columns of different origin was not investigated. A formerly published comparison of different reversed-phase packing materials [18] indicated that C_8 and C_{18} stationary phases did not show very good reproducibility of the selectivity towards cephalosporins.

This paper describes a LC-method for both qualitative and quantitative analysis of cefalexin, using poly(styrene-divinylbenzene) (PSDVB) as stationary phase. This method is able to separate cefalexin from all its known related substances and has been used to compare official standards and to analyse a number of commercial samples of different origin. The method is performing well on different brands of PSDVB available on the market.

EXPERIMENTAL

Reference Substances and Samples

The United States Pharmacopeia Reference Standard (Lot H; 950 μg/mg) (USP-RS), the European Pharmacopoeia Chemical Reference Standard (93.4 %) (Ph. Eur.-CRS) and the British Pharmacopoeia Chemical Reference Standard (Batch No. 1291; 94.2 %) (BP-CRS) were available.

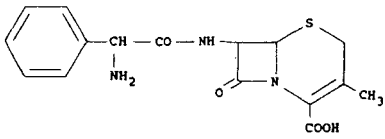
Several bulk samples were of known origin (The Netherlands, India, Belgium) and most, obtained from wholesalers, were from unknown origin. Dosage forms containing cefalexin were obtained from the Belgium market.

Related Substances

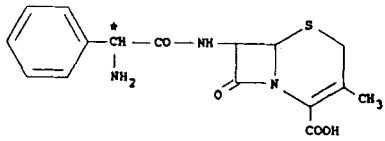
Related substances present as impurities in cefalexin, can originate from the semi-synthesis and from degradation. Figure 1 shows the structures of D-cefalexin and its potential impurities. **VII** and **VIII**, which are the basic constituents of the cefalexin molecule, are commercially available. **II**,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

Solvents and reagents

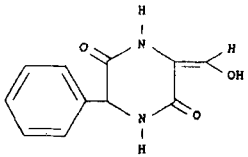
Acetonitrile 99 % (Janssen Chimica, Beerse, Belgium) and methanol (N.V. Roland, Brussels, Belgium) were distilled before use. 2-Methyl-2-propanol 99.5 % (Janssen Chimica) was used as such. Phosphoric acid 85 % and potassium dihydrogen phosphate were from Merck (Darmstadt, Germany). Sodium 1-octanesulphonate (NaOS) was from Janssen Chimica. Water was distilled twice.



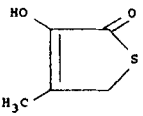
I D-Cefalexin



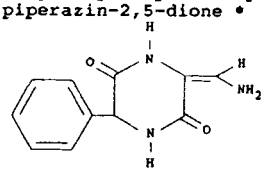
II L-Cefalexin **



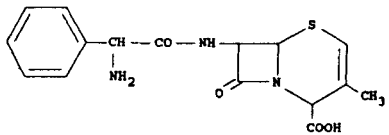
III 3-Hydroxymethylene-6-phenyl-piperazin-2,5-dione *



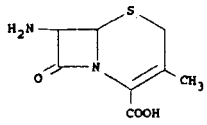
IV 3-Hydroxy-4-methyl-2(5H)-thiophenone *



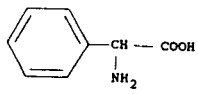
V 3-Aminomethylene-6-phenyl-piperazin-2,5-dione *



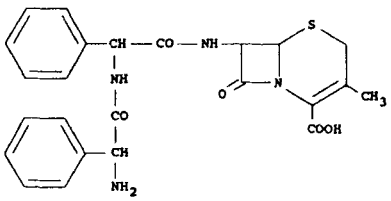
VI Δ^2-Cefalexin **



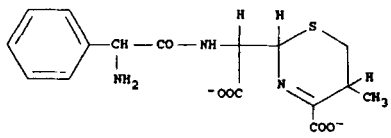
VII 7-Aminodesacetoxycephalosporanic acid (7-ADCA) ***



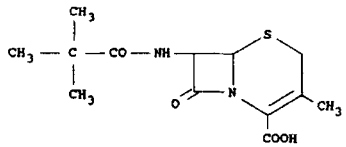
VIII Phenylglycin ***



IX Phenylglycylcefalexin **



X Cefalexin Δ^4-cephalosporoates *



XI Pivalamide of 7-ADCA **

FIGURE 1. Structures of cefalexin and its related substances.
* degradation product
** side-product of the synthesis
*** start product of the synthesis

LC Apparatus and Operating Conditions

Isocratic elution was used throughout the study. The equipment consisted of a L-6200 pump (Merck-Hitachi, Darmstadt, Germany), a 20 µl loop injector Model CV-6-UHPa-N60 (Valco, Houston, TX, USA), a 250 x 4.6 mm I.D. column, packed with 8 µm PLRP-S 100 Å (Polymer Laboratories, Church Stretton, Shropshire, UK), a 254 nm fixed wavelength UV monitor D (LDC/Milton Roy, Riviera Beach, Fl, USA) and an integrator Model 3396 A (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 GMBH, Seelbach, Germany). The selectivity of the method was also tested on other PSDVB 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 by a photodiode array detector Model 990 (Waters Assoc., Milford, MA, USA). A Marathon autosampler with sample-cooling (Spark Holland, The Netherlands) equipped with a fixed 20 µl loop and a cryomat Julabo C and F10 was used for the quantitative determinations.

Mobile Phase

The mobile phase finally used was prepared by mixing 500 ml of water, 155 ml of acetonitrile, 100 ml of 0.02 M sodium 1-octanesulphonate 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 cefalexin into a 20 ml volumetric flask. Mobile phase containing 20 % of the 0.02 M solution of sodium 1-octanesulphonate was

used as solvent. For the tablets the mixture of powder and solvent was ultrasonicated and centrifuged, the supernatant was analysed. Other dosage forms were soluble in the mobile phase at the concentration required.

The chemical reference substances were dissolved the same way as the samples.

RESULTS AND DISCUSSION

Development of the Chromatographic Method

Poly(styrene-divinylbenzene) (PSDVB) was used as stationary phase because it is a very stable material, even at extreme pH conditions and high temperatures [35]. From the beginning, the stationary phase was heated to enhance the mass-transfer and to reduce the backpressure. A temperature of 60 °C was chosen to start with.

First, mixtures of methanol, phosphate buffer (pH 7 to 9) and water were used as mobile phase. The addition of tetrabutylammonium as ion-pairing reagent improved the separation. Due to decomposition of the sample during analysis, the higher pH range had to be abandoned and because of the better acid stability of cefalexin, the phosphate buffer was replaced by phosphoric acid. As a consequence, another ion-pairing reagent, sodium 1-octanesulphonate (NaOS) was used. The separation of cefalexin from its potential impurities was much improved when acetonitrile, instead of methanol, was used as organic modifier. Methanol generally showed a very low efficiency, which was already observed earlier during LC-analysis of tetracycline [36] and erythromycin [37] on PSDVB. Thus, a mobile phase containing acetonitrile - 0.2 M phosphoric acid (pH 1.4) - 0.02 M NaOS - water, was further evaluated by systematic examination of its components.

The pH of the mobile phase was altered by using 0.2 M phosphate buffers pH 3.0 or 4.0. For each pH, the amount of organic modifier was varied to optimise the separation (Fig. 2). The retention of **III**, **IV** and **V** was not affected by the pH. **III** and **IV** had no positively charged amino group like the other related substances and therefore did not interact with the ion-pairing reagent. **III** and **V** were almost never separated. **V** was probably hydrolysed by acid mobile phases and transformed into **III**. This degradation was confirmed using photodiode array detection. At pH 1.4 a very good selectivity between cefalexin and its related substances was achieved, using 14.0 % of acetonitrile. At pH 3.0 the elution order of the products was changed. **IV** was now eluted after **VI** and even after cefalexin, except for the slow elution with 10.0 % of acetonitrile, which gave a good selectivity. However, by increasing the pH, the efficiency decreased. At pH 4.0 no good separation was obtained due to the very low efficiency. The theoretical plate number was only 700 N/m at pH 4.0, while at pH 1.4 3200 N/m was achieved. As a result, phosphoric acid was chosen to regulate the pH of the mobile phase. For the three pH-values the retention time of **IX** was at least three times that of cefalexin, and because its separation caused no problem, **IX** was not included anymore in the following experiments to reduce the total analysis time.

Mobile phases with 2-methyl-2-propanol were also examined. This organic modifier proved to be very suitable in LC-analysis of tetracycline [36] or erythromycin [37] on PSDVB. In figure 3 the capacity factors obtained with different concentrations of 2-methyl-2-propanol are shown. The order of elution was altered in comparison with acetonitrile. The Δ^2-isomer **VI** now eluted after cefalexin. **VI** and **XI** eluted on the tail of the cefalexin peak, which could cause difficulties for the

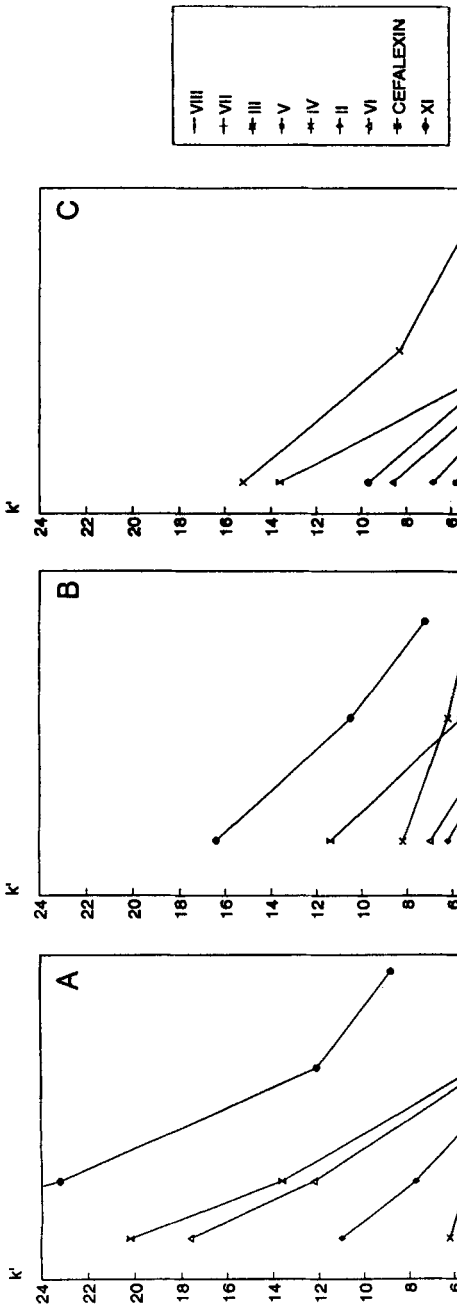


FIGURE 2. Influence of the concentration of acetonitrile on the separation of cefalexin and related substances at pH 1.4 (A), pH 3.0 (B) and pH 4.0 (C) (symbols: see fig. 1). Stationary phase: PLRP-S 100 Å 8 µm. Mobile phase: CH_3CN - 0.02 M NaOS - 0.2 M H_3PO_4 or 0.2 M phosphate buffer pH 3.0 or 4.0 - H_2O (x:10:5:up to 100).

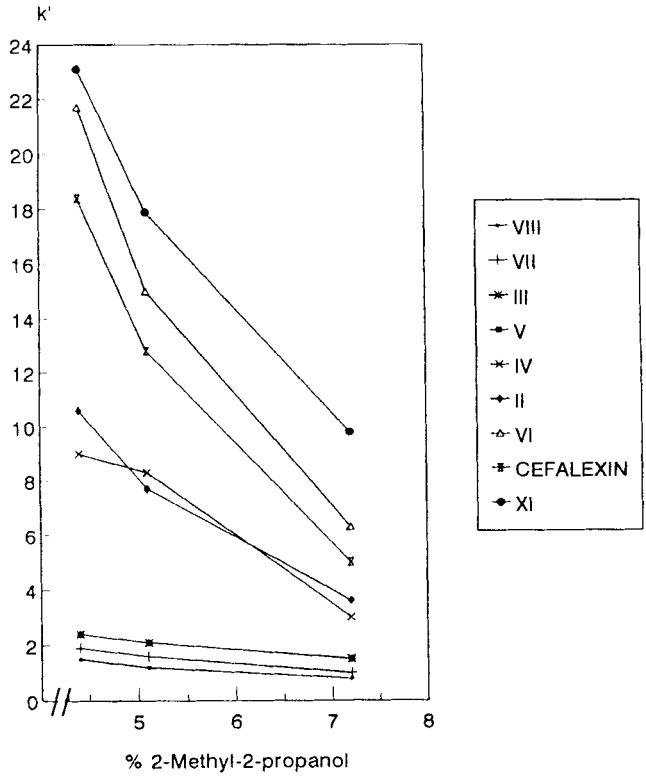


FIGURE 3. Influence of the concentration of 2-methyl-2-propanol as organic modifier on the separation of cefalexin and related substances (symbols: see Fig. 1). Stationary phase: PLRP-S 100 Å 8 μm. Mobile phase: 2-methyl-2-propanol - 0.02 M NaOS - 0.2 M H_3PO_4 - H_2O (x:10:5:up to 100).

detection and integration of small amounts of these products. The mobile phase with acetonitrile as organic modifier not only gave better selectivity but also better efficiency.

Figure 4 shows the influence of the concentration of the ion-pairing reagent sodium 1-octanesulphonate (NaOS). Doubling the concentration in the mobile phase to 20 % of

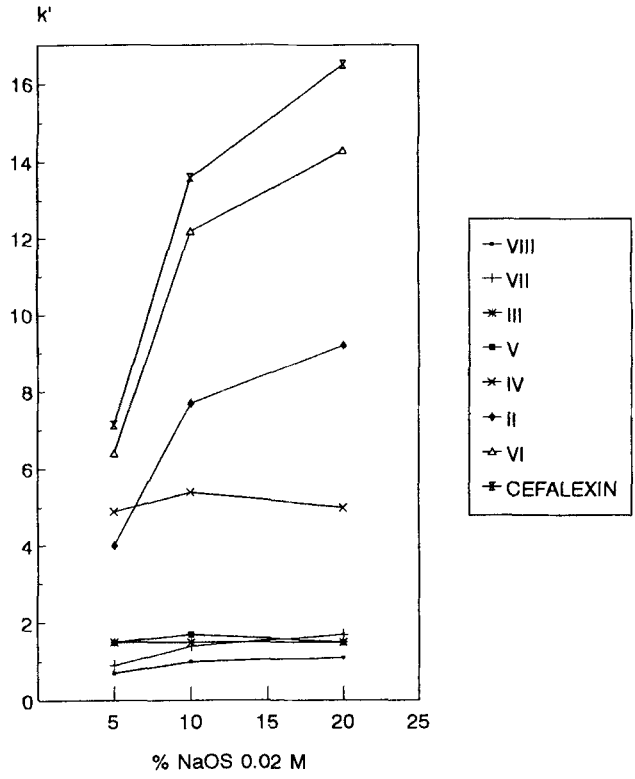


FIGURE 4. Influence of the concentration of sodium 1-octanesulphonate (NaOS) in the mobile phase on the separation of cefalexin and related substances (symbols: see Fig. 1). Stationary phase: PLRP-S 100 Å 8 μm. Mobile phase: CH_3CN - 0.02 M NaOS - 0.2 M H_3PO_4 - H_2O (14:x:5:up to 100). k' **XI** > 19.

the 0.02 M solution caused an increase of the retention times, except for **III**, **IV** and **V**. Decreasing the amount of NaOS to half the original concentration decreased the separation of cefalexin and **VI**. Moreover, the concentration of NaOS had to be maintained at a certain level to prevent splitting of the peaks after injection

of more concentrated solutions of cefalexin. Formation of double peaks can occur when insufficient amounts of ion-pairing reagent are available. For further experiments 10 % of a 0.02 M solution of NaOS was considered to be suitable.

Changing the amount (5%) of 0.2 M phosphoric acid in the mobile phase to 2.5 % or 7.5 % did not influence the elution pattern, nor the quality of the separation. Therefore the initial concentration of 5 % was maintained.

The mobile phase finally chosen for further use throughout the study was : acetonitrile - 0.2 M phosphoric acid - 0.02 M NaOS - water (15.5:5:10:up to 100, v/v).

For further validation of the selectivity of the method, cefalexin was degraded in alkaline or acid medium and the chromatograms were recorded by means of photodiode array detection. A solution of cefalexin in 0.1 N NaOH was stored at room temperature for 10 minutes, neutralised and analysed (1 mg/ml). Figure 5 shows the chromatogram. The cefalexin content was already decreased by 55 %. The UV-spectra of the left slope, the maximum and the right slope of the cefalexin peak were examined. The cefalexin peak was found to be homogeneous, notwithstanding the high level of degradation. The peak eluting after cefalexin showed a UV-spectrum similar to that of cefalexin. This decomposition product was not identified. It is possibly 7-epicefalexin, which arises in alkaline medium. A solution of cefalexin in 0.1 N HCl (1 mg/ml) stored at 60 °C during 5 hours was examined the same way. The cefalexin peak remained homogeneous (Fig. 6). The small peaks at 6.4 and 15.0 min were identified as respectively **III** and **IV** by comparison of their retention times and UV-spectra with those of the pure substances. The other peaks were not identified.

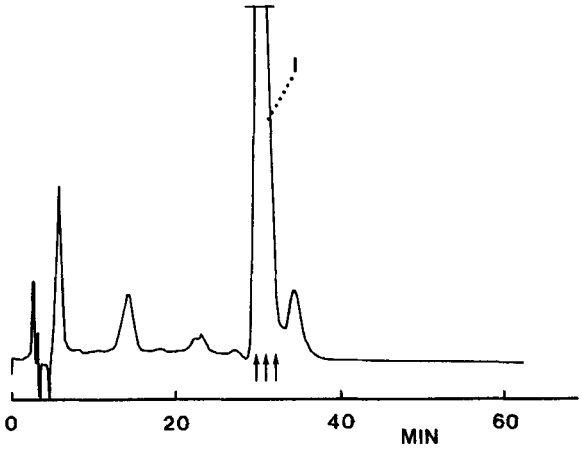


FIGURE 5. Chromatogram of a solution of cefalexin in 0.1 N NaOH (1 mg/ml), stored at room temperature for 10 min. Stationary phase: PLRP-S 100 Å 8 μm. Mobile phase: CH_3CN - 0.02 M NaOS - 0.2 M H_3PO_4 - H_2O (14:10:5:up to 100).

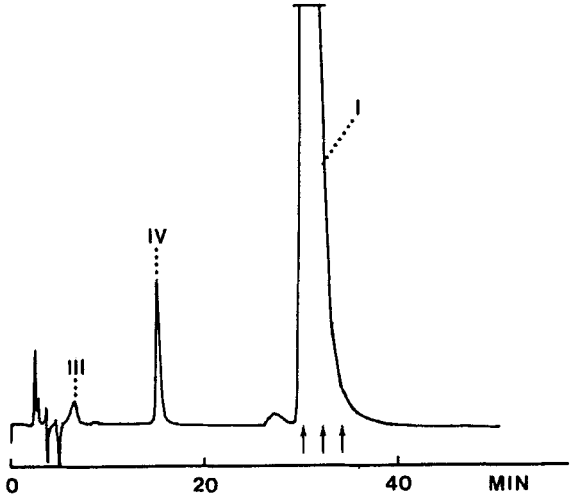


FIGURE 6. Chromatogram of a solution of cefalexin in 0.1 N HCl (1 mg/ml), stored at 60 °C for 5 hours. Stationary phase: PLRP-S 100 Å 8 μm. Mobile phase: CH_3CN - 0.02 M NaOS - 0.2 M H_3PO_4 - H_2O (14:10:5:up to 100).

TABLE 1

Characteristics of the PSDVB-columns used, with the respective concentrations of acetonitrile in the mobile phase.

Column	Age (years)	Stationary phase (250 x 4.6 mm I.D.)	Batch	% CH_3CN
A	6	PLRP-S 100 Å 8 µm	RPS-1-14	14.0
B	5	PLRP-S 100 Å 8 µm	(10-12-85)B	14.0
C	2	PLRP-S 100 Å 8 µm	35	13.8
D	1	PLRP-S 100 Å 8 µm	35	13.6
E	new	PLRP-S 100 Å 8 µm	8M-RPS-1-64	14.0
F	7	PRP-1 10 µm	79400	14.0
G	6	PRP-1 10 µm	79400	14.0
H	new	PRP-1 7-9 µm	457	14.0

The selectivity of different PSDVB columns was also checked using the mobile phase described in the experimental section. The content of acetonitrile was adapted for each column to produce comparable retention times. Table 1 shows an overview of the characteristics of the columns. As can be seen in figure 7, the separation pattern is the same for each column. This advantage of the use of PSDVB as stationary phase ensures an improvement of the reproducibility (between-laboratory precision) of the method.

The influence of the column temperature on the separation was investigated at 50 °C, 60 °C and 70 °C. The products were eluted faster at higher temperature, but the elution order and the separation were similar at the three temperatures. The stability of a solution of cefalexin in mobile phase stored at these three temperatures was investigated. The solutions stored at 60 or 70 °C showed decomposition after 45 minutes storage

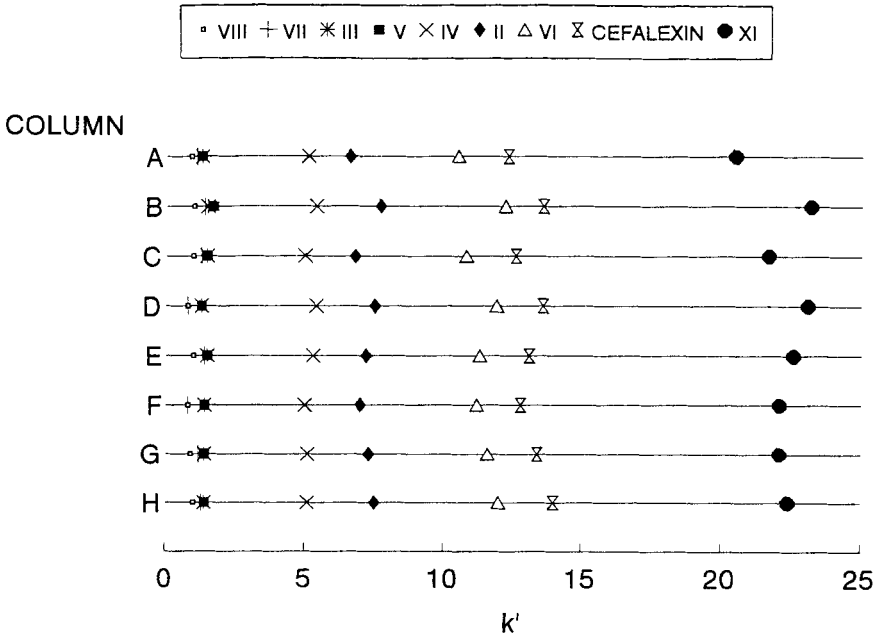


FIGURE 7. Elution order of cefalexin and its related substances (symbols: see Fig. 1) on different PSDVB columns (see table 1 for characteristics). Mobile phase: CH_3CN - 0.02 M NaOS - 0.2 M H_3PO_4 - H_2O (x:10:5:up to 100), x: see table 1.

(respectively 0.6 % and 4.3 %), while at 50 °C the area of the cefalexin peak was equal to that of a freshly prepared solution. In conclusion the column temperature was decreased to 50 °C in order to guarantee stability of the samples during analysis.

A solution of cefalexin in water gave several disturbing system peaks. Using mobile phase as the solvent, some system peaks disappeared. After systematic variation of the composition of this solvent, mobile phase containing 20 % instead of 10 % 0.02 M NaOS was

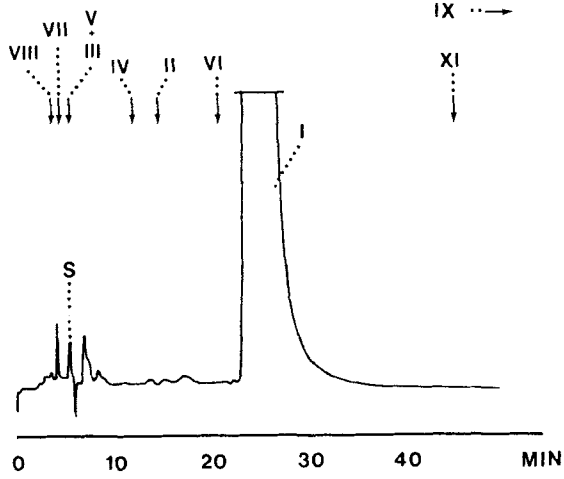


FIGURE 8. Typical chromatogram of cefalexin (30 μg), the position of the related substances is indicated. Stationary phase: PLRP-S 100 Å 8 μm, 50 °C. Mobile phase: CH_3CN - 0.02 M NaOS - 0.2 M H_3PO_4 - H_2O (15.5:10:5:up to 100). S = system peak.

chosen as the solvent for the samples. Unfortunately, one small system peak was still present.

Fig. 8 shows a typical chromatogram of cefalexin with the selected chromatographic conditions. The positions of the related substances are indicated.

Finally, a resolution test was developed for this LC-method. The most critical separation was that between cefalexin and **VI**. Since **VI** was not commercially available, a solution of 10 mg of cefalexin and 15 mg of cefradine in 100 ml of water was used for the calculation of the resolution. A mixture of cefalexin and a small amount of **VI** was analysed for visual evaluation of the separation. These solutions were repeatedly analysed on each column listed in Table 1 while the amount of organic modifier was varied. It was concluded that a resolution

of 4.0 between cefalexin and cefradine guaranteed sufficient separation of cefalexin from **VI**.

Quantitative aspects of the LC method

The loadability of the column was found to be 100 μg of cefalexin. Above this amount, a shoulder due to splitting of the main peak appeared on the ascending part of the cefalexin peak. It was decided to use a 30 μg amount for qualitative analysis of cefalexin samples. For this quantity the limit of detection (LOD), expressed as cefalexin, was 0.025 %. The limit of quantitation (LOQ) was 0.05 % [n = 10, relative standard deviation (RSD) = 15.5 %]. The repeatability was checked by analysing 11 times the same solution of cefalexin (30 μg) (RSD = 0.23 %, calculated on the area of the cefalexin peak) and by analysing subsequently 6 freshly prepared solutions of cefalexin (30 μg) (RSD = 0.27 %, calculated on the area of the cefalexin peak). At about 6 °C, solutions of cefalexin 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 = 9520 x - 3865, $S_{y,x}$ (standard error of estimate) = 1190, r (correlation coefficient) = 0.999, range of x covered in the experiments = 24 - 36 μg.

Comparison of cefalexin standards

The newly developed LC method was used to compare the USP-RS, the BP-CRS and the Ph. Eur.-CRS. The cefalexin content of the USP-RS and the BP-CRS was calculated by comparison with the Ph. Eur.-CRS, which has an assigned cefalexin content of 93.4 % on "as is" [38]. 30 μg samples of each standard were analysed. Because the standards contained only small amounts of impurities, the total content of impurities was calculated by comparison

TABLE 2

Composition of cefalexin standards.
Values in percent (m/m); RSD values are given in parentheses; ND = not determined due to limited amount of sample.

	Ph. Eur. - CRS 93.4 %	BP-CRS 94.2 %	USP-RS 950 µg/mg
Number of analyses	13	20	20
Number of solutions analysed	6	4	4
Number of days	2	2	2
Cefalexin	93.4 [38] (0.3)	94.06 (0.2)	94.76 (0.2)
Impurities	0.2 [38]	0.15 (34)	0.24 (32)
Solvents	0.09 [38]	ND	ND
Water determined	6.2	ND	ND
Water declared	6.3 [38]	6.1 [39]	5.21 [40]
Total	100.0	100.3	100.2

with the peak area obtained for 0.3 µg of cefalexin Ph. Eur.-CRS (1% of the main peak). Results obtained for the standards are shown in Table 2. The RSD values, given in parentheses below the assay values, are within acceptable limits. The USP-RS and the BP-CRS are very pure samples. For both standards, the LC-results are close to the declared content. Due to the limited amount available of these standards, the content of water and solvents was not determined.

Analysis of commercial samples

Table 3 shows the results obtained for a number of bulk samples using the Ph. Eur.-CRS. The RSD on the cefalexin content mostly did not exceed 1.0 %. The total mass was not entirely explained by addition of the

TABLE 3

Composition of bulk samples of cefalexin. Values in percent (m/m); RSD is given in parentheses; water was determined by Karl Fischer titration; n = number of analyses.

Origin	Sample number	LC Cefalexin (n = 4) A	LC Impurities (n = 4) B	Water (n = 4) C	Total A + B + C	Total C + D	Non aqueous titration (n = 4) D
A	1	92.72 (0.6)	0.36 (3.9)	6.35 (1.4)	99.43	99.67	93.32 (0.1)
A	2	92.87 (0.2)	0.24 (6.7)	6.25 (1.6)	99.36	99.38	93.13 (0.2)
A	3	92.94 (0.7)	0.40 (4.3)	6.37 (1.7)	99.71	99.36	92.99 (0.2)
A	4	92.53 (0.3)	0.34 (4.4)	6.51 (2.2)	99.38	100.05	93.54 (0.1)
B	5	93.42 (1.5)	0.26 (4.9)	6.12 (2.0)	99.80	100.34	94.22 (0.1)
C	6	90.10 (0.5)	1.63 (3.0)	6.50 (2.5)	98.23	99.27	92.77 (0.2)
C	7	81.33 (1.2)	5.35 (4.8)	6.06 (1.3)	92.74	96.42	90.36 (0.1)
C	8	88.76 (0.3)	0.83 (6.7)	6.31 (2.2)	95.90	98.98	92.67 (0.0)
D	9	92.84 (0.8)	0.44 (9.9)	6.15 (1.1)	99.43	100.09	93.94 (0.2)
D	10	90.49 (1.3)	0.36 (9.9)	6.09 (3.4)	96.94	100.06	93.97 (0.2)

TABLE 4

Cefalexine content of pharmaceuticals as a percentage (m/m) of label claim.

Sample number and origin	Form	Mean content (n = 4)	RSD (%)
1 A	Tablets	106.0	0.3
2 B		102.8	0.6
3 C		105.5	0.8
4 C		99.1	0.7
5 A	Powder for suspension	109.5	0.6
6 D		100.9	0.1
7 B	Powder for oral drops	104.9	0.8

content of cefalexin, impurities and water. This may be due to the fact that some of the decomposition products have a lower UV-absorbance at 254 nm than cefalexin.

The base content of the bulk samples was determined by non aqueous titration and the results were compared with LC-figures. Cefalexin (250 mg) was dissolved in 40 ml of acetic acid and titrated with $HClO_4$ 0.1 N using potentiometric endpoint detection. Each sample was titrated four times. The results are also shown in table 3. For pure samples the total figures should theoretically give the same result. However, the total of the titration and water (C and D) is usually higher than the sum of LC-cefalexin, LC-impurities and water (A, B and C). When related substances or other impurities, carrying no basic function, are present, titration figures do not allow a correct estimate of the total non volatile mass. This can explain the deviation for C + D from the theoretical value in sample 7. The presence of residual organic solvents was not examined. All the

samples except 7 and 8 complied with Ph. Eur. requirements for content (95.0 % on anhydrous) and water (4.0 - 8.0 %).

A few pharmaceutical preparations were analysed using the same LC method and the Ph. Eur.-CRS as the reference material. The content was expressed as a percentage of label claim. The results are shown in Table 4.

It can be concluded that the method described is suitable for purity control and assay of bulk samples and different types of pharmaceuticals containing cefalexin. Major advantages of the method are its selectivity and its applicability on poly(styrene-divinylbenzene) stationary phases from different manufacturers and of different age.

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