Journal of Chromatography, 634 (1993) 257-261
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 805

Comparative study of liquid chromatographic methods for the determination of cefadroxil

C. Hendrix, Yongxin Zhu, C. Wijsen, E. Roets and J. Hoogmartens*

Katholieke Universiteit Leuven, Laboratorium voor Farmaceutische Chemie, Instituut voor Farmaceutische Wetenschappen, Van Evenstraat 4, B-3000 Leuven (Belgium)

(First received September 22nd, 1992; revised manuscript received December 8th, 1992)

ABSTRACT

A comparative study of two isocratic liquid chromatographic methods for the determination of cefadroxil is described. The first method, prescribed in the monograph of the European Pharmacopoeia for the assay of cefadroxil, uses a classical alkyl-bonded phase (C_n) as the stationary phase. This method is very similar to that prescribed by the United States Pharmacopeia. The other method uses poly(styrene–divinylbenzene). Poor reproducibility of the selectivity towards cefadroxil and related substances was observed when the first method was examined on different C_{18} columns. Copolymer columns, on the other hand, gave the same elution order on stationary phases from different manufacturers and of different age. Four bulk samples of cefadroxil were analysed following both methods and the results were compared.

INTRODUCTION

It has been described that classical alkyl-bonded phases (C_n) can suffer from poor reproducibility of the selectivity during liquid chromatography (LC) of cephalosporins [1]. This type of reversed phase is, nevertheless, widely prescribed in pharmacopoeial methods. For the assay of cefadroxil, the European Pharmacopoeia (Ph. Eur.) prescribes an LC method using a C_{18} stationary phase [2]. The selectivity of this method for cefadroxil and related substances was examined on six stationary phases. The United States Pharmacopeia (USP) XXII precribes nearly the same method for the assay of cefadroxil [3]; only the pH and concentration of the buffer of the mobile phase are slightly different. The influence of these differences on the selectivity was examined.

On four columns, four bulk samples were analysed following the Ph. Eur. method. The results were compared with those obtained by an isocratic LC method using poly(styrene–divinylbenzene) (PS-DVB) as stationary phase. This PS-DVB method was proved to give very reproducible selectivity [4].

EXPERIMENTAL

Reference substances and samples

The European Pharmacopoeia Chemical Reference Standard (Ph. Eur. CRS; 94.2%) was used as the standard.

Bulk samples of different origin and age were chosen in order to have samples of variable purity.

Related substances

Related substances present as impurities in cefadroxil can originate from the semi-synthesis and from degradation. The structures and origin of potential impurities of cefadroxil have been given previously [4]. 7-Aminodesacetoxycephalosporanic acid (VII) and D-4-hydroxyphenylglycine (VIII) are the basic constituents of the

* Corresponding author.

0021-9673/93/$06.00

cefadroxil molecule. L-Cefadroxil (II), Δ^2-cefadroxil (VI), 4-hydroxyphenylglycylcefadroxil (IX) and the pivalamide of 7-ADCA (XI) can arise from the semi-synthesis of cefadroxil. The other related substances are decomposition products. 3-Hydroxymethylene-6-(4-hydroxyphenyl)piperazine-2,5-dione (III) and 3-hydroxy-4-methyl-2(5*H*)-thiophenone (IV) are formed in acidic medium. III and 3-aminomethylene-6-(4-hydroxyphenyl)piperazine-2,5-dione (V) are formed in neutral medium and the cefadroxil Δ^4-cephalosporoates (X) are formed in alkaline medium. 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, Beerse, Belgium) was distilled before use. Phosphoric acid (85 %) and potassium dihydrogenphosphate (analytical-reagent grade) were obtained from Merck (Darmstadt, Germany) and sodium 1-octanesulphonate (NaOS) from Janssen Chimica. Water was distilled twice.

LC apparatus and operating conditions

Isocratic elution was always used. The equipment consisted of an L-6200 pump (Merck–Hitachi, Darmstadt, Germany), 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). For the examination of peak homogeneity the UV detector was replaced with a Model 990 photodiode-array detector (Waters, Milford, MA, USA). The samples were injected by a Marathon autosampler (Spark Holland, Emmen, Netherlands) with sample cooling (6°C) equipped with a fixed 20-μl loop and a Julabo C and F10 Cryomat (Julabo Labortechnik, Seelbach, Germany). The columns (250 x 4.6 mm I.D.) were packed in the laboratory with (A) Hypersil ODS, 5 μm (Shandon, Runcorn, UK), (B) Partisil ODS-3, 10 μm (Whatman, Clifton, NJ, USA), (C) Spherisorb ODS-1, 10 μm (Phase Separations, Queensferry, UK), (D) RSIL C_{18} I-IL, 10 μm (Bio-Rad, Eke, Belgium), (E) Nucleosil 100 C_{18}, 5 μm (Macherey-Nagel, Düren, Germany), (F) Bio-Sil C_{18} LL, 90 A, 5 μm (Bio-Rad), (G) PLRP-S, 100 A, 8 μm (Polymer Labs., Church Stretton, Shropshire, UK) or (H) PRP-1, 7-9 μm (Hamilton, Reno, NV, USA). The columns were immersed in a water-bath heated by a Julabo EM thermostat. The column temperature was 30°C for the alkyl-bonded phases and 50°C for the PS-DVB phases. For both methods the flow-rate was 1 ml/min.

Mobile phase

The Ph. Eur. method prescibes acetonitrile-0.272% (w/v) KH_2PO_4 solution (4:96, v/v). The USP XXII method prescribes acetonitrile-buffer (pH 5.0) (4:96, v/v). The buffer is prepared by dissolving 13.6 g of KH_2PO_4 in water to make 2000 ml. The pH of the solution is then adjusted to 5.0 with 10 *M* KOH. The PS-DVB method uses acetonitrile-0.02 *M* sodium 1-octanesulphonate-0.2 *M* phosphoric acid-water (12.5:20:5:up to 100, v/v).

Mobile phases were degassed by ultrasonication before use.

Sample preparation

Samples for quantitative analysis following the Ph. Eur. method were prepared by weighing 50 mg of cefadroxil into a 100-ml volumetric flask. Mobile phase was used as the solvent. For the PS-DVB method 30 mg of cefadroxil were weighed into a 20-ml volumetric flask and mobile phase containing 30% of 0.02 *M* sodium 1-octanesulphonate solution was used as the solvent.

The chemical reference substance was dissolved in the same way as the samples.

RESULTS AND DISCUSSION

Examination of the selectivity of the LC methods

In the USP method the pH of the mobile phase is controlled by phosphate buffer (pH 5.0). The Ph. Eur. method uses a phosphate solution at pH 4.7. These very similar mobile phases were compared on the same C_{18} column by analysing a mixture of cefadroxil and related substances. The small difference in pH did not influence the selectivity, so it was decided that

both mobile phases gave the same result. A shortcoming of the USP method was the lack of a resolution test. The capacity factor and the number of plates of the cefadroxil peak were used as criteria for the adjustment of the mobile phase. These criteria are obviously not indicative for the selectivity of a stationary phase. The USP method was not considered further in the experiments because of its similarity with the Ph. Eur. method.

The selectivity of the Ph. Eur. method was examined on six C_{18} columns (A-F) by the determination of the capacity factors of cefadroxil and related substances. For each column the composition of the mobile phase was adapted to obtain the required resolution of at least 5.0 between cefadroxil and amoxicillin. Table I shows that only two of the columns examined (A and E) complied with this requirement. The resolution on the other columns was insufficient, even after complete elimination of the organic modifier from the mobile phase. Nevertheless, the selectivity was investigated on each column.

TABLE I

COMPOSITION OF THE MOBILE PHASE FOR EACH COLUMN FOLLOWING THE PH. EUR. METHOD, WITH THE CORRESPONDING RESOLUTION BETWEEN CEFADROXIL AND AMOXICILLIN

Column	Mobile phase composition		Resolution
	CH_3CN (vol.)	0.272% KH_2PO_4 (vol.)	
A	4	96	5.4"
	1.5	98.5	10.1"
B	3	97	1.8
	0	loo	3.2
C	4	96	1.5
	0.6	99.4	3.1
	0	100	3.7
D	3	97	2.4
	0.6	99.4	3.6"
	0	100	4.0
E	4	96	5.4"
	0.9	99.1	9.6
F	0.3	99.7	2.9"

[a] Mobile phases used for quantitative analysis.

Because columns A and E complied with the resolution test when using a fast-eluting mobile phase, the selectivity was also checked using a mobile phase with a lower content of acetonitrile. The results are shown in Fig. 1. X, which is a complex mixture of diastereoisomers, is not shown. This polar mixture eluted close to the dead volume of the chromatogram. IV was eluted at k' close to 10 or more. IX and XI were always eluted much later than cefadroxil ($k' > 30$). Using a mobile phase containing 4% of acetonitrile columns A and E complied with the resolution test (R_s = 5.4); nevertheless, cefadroxil was not separated from all related substances. Complete separation was achieved using columns A (1.5% CH_3CN), D (0.6% CH_3CN), column E (0.9% CH_3CN) and F (0.3% CH_3CN). The resolution test gave R_s = 10.1, 3.6, 9.6 and 2.9, respectively. Obviously, there is insufficient relationship between this resolution test and the

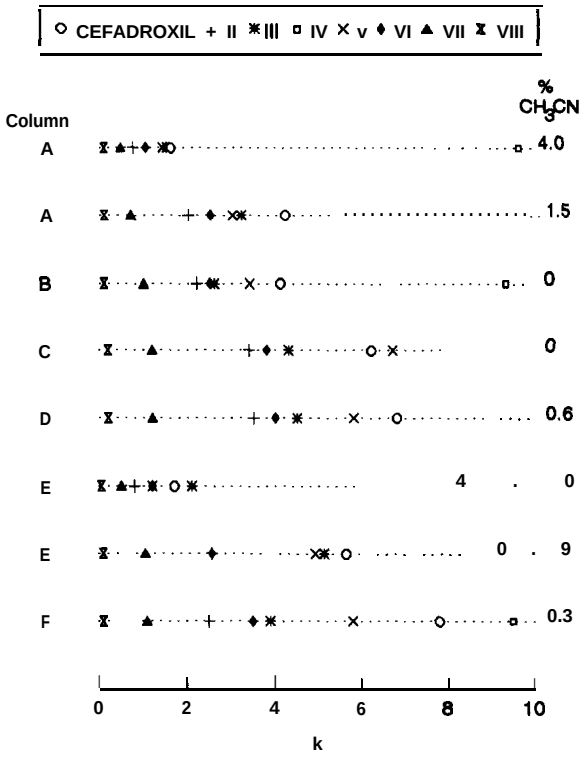

Fig. 1. Capacity factors of cefadroxil and related substances on different C_{18} columns following the Ph. Eur. method. Mobile phase: CH,CN-0.272% KH_2PO_4 [x:(100 − x), v/v), where x is given on the right of the figure.

selectivity of the LC method when applied to different columns. Also, differences in elution order can be observed for the different columns, e.g., the elution order of cefadroxil and V is column dependent. The separation of cefadroxil from decomposition products formed in alkaline medium was examined by analysis of an *in situ* prepared solution of X under the best LC conditions for each column. The homogeneity of the cefadroxil peak was examined by normalization at 230 nm of the UV spectra taken at the left slope, the maximum and the right slope. Only columns D (0.6% CH_3CN) and F (0.3% CH_3CN) gave a homogeneous cefadroxil peak. It can be concluded that the selectivity is column dependent and that the method does not guarantee a selective determination of cefadroxil.

The selectivity of the PS-DVB method has already been examined and reported previously [4]. Tbis method is able to separate cefadraxil from all known related substances and performs equally well on different commercially available brands of PS-DVB. The age and the history (former use) of the columns were observed to have a limited influence on the selectivity. These characteristics are an important improvement compared with the pharmacopoeia1 method.

Quantitative analysis of bulk samples by two LC methods

Four bulk samples were analysed following the pharmacopoeial method and the PS-DVB method. The Ph. Eur. CRS for cefadroxil was used as the standard.

The analyses following the Ph. Eur. were performed on four C_{18} columns, A, D, E and F. The mobile phase compositions were those marked with superscript *a* in Table I. Columns A and E were used under both non-separating (4% CH_3CN) and separating (1.5 and 0.9% CH_3CN) conditions. The results are given in Table II. The relative standard deviation (R.S.D.) calculated on the peak area of six subsequent injections of cefadroxil was well below the prescribed limit of 1.0% [2]. Each sample was analysed four times. For all the LC conditions nearly identical results were obtained. The R.S.D. did not exceed 1 .0%.

The analyses by the LC method developed on PS-DVB were performed on columns G and H. Using the mobile phase described under Ex-

TABLE II

RESULTS OF THE ASSAY FOLLOWING THE PH. EUR. METHOD

Values in % (w/w) with R.S.D. values (%) in parentheses (n = 4).

Column	CH_3CN in mobile phase (%)	Sample no.			
		1	2	3	4
A	4.0	93.32 (0.3)	93.94 (0.4)	90.36 (0.4)	90.93 (1.1)
	1.5	93.45 (0.2)	94.16 (0.2)	90.19 (0.8)	90.77 (0.3)
D	0.6	93.28 (0.5)	94.28 (0.1)	90.13 (0.5)	90.15 (0.2)
E	4.0	93.71 (0.2)	94.29 (0.4)	90.59 (0.3)	91.00 (0.4)
	0.9	93.73 (0.5)	93.85 (0.6)	90.46 (0.3)	91.09 (0.2)
F	0.3	93.70 (0.2)	94.49 (0.1)	90.84 (0.2)	90.77 (0.8)
Mean		93.53 (0.4) (n = 24)	94.17 (0.4) (n = 24)	90.46 (0.5) (n = 24)	90.78 (0.5) (n = 24)

TABLE III

RESULTS OF THE ASSAY FOLLOWING THE PS-DVB METHOD

Values in % (w/w) with R.S.D. values (%) in parentheses and number of analyses (n).

Column	Sample no.			
	1	2	3	4
G	93.72 (0.2) (n = 7)	94.21 (0.5) (*n* = 6)	90.50 (0.4) (*n* = 5)	91.13 (0.5) (*n* = 6)
H	93.43 (0.4) (*n* = 4)	93.96 (0.3) (n = 4)	90.11 (0.2) (n = 4)	90.21 (0.2) (*n* = 4)
Mean	93.62 (0.3) (*n* = 11)	94.11 (0.4) (n = 10)	90.31 (0.3) (*n* = 9)	90.67 (0.7) (n = 10)

perimental, a resolution of 4.3 for column G and 4.7 for column H was obtained between cefadroxil and amoxicillin, which is better than the required resolution of 4.0 [4]. The results are given in Table III. Both columns gave nearly identical results.

The results of the assay of four bulk samples by the pharmacopoeia1 method using four different C_{18} columns and by the LC method using two different PS-DVB columns were compared. The test of significance of differences of means [5] was performed using the grand means of both methods. The resulting figures were less than the tabulated limits ($t_{0.80}$), so the difference was not significant even at the 20% level.

CONCLUSIONS

The results might lead to the conclusion that both methods are equivalent. However, this can be confirmed only for the samples examined, which apparently did not contain impurities that were co-eluted with cefadroxil in the pharmacopoeial method. It should also be mentioned that the resolution test prescribed by the pharmacopoeial method did not guarantee the complete separation of cefadroxil from related substances. Even when complying with this resolution test, the mobile phase still needed adaptation in order to obtain complete separation.

In general, it can be concluded that the poor reproducibility of the selectivity of the classical alkyl-bonded phases was again demonstrated here. Therefore, these methods are less suitable as official methods. The PS-DVB method, on the other hand, offers more reliable results because of its reproducible selectivity.

ACKNOWLEDGEMENTS

The National Fund for Scientific Research (Belgium) is acknowledged for financial support. The gifts of samples by the Belgian Ministry of Health and by different manufacturers are gratefully acknowledged. The authors thank A. Decoux and I. Quintens for editorial assistance.

REFERENCES

1 I. Wouters, S. Hendrickx, E. Roets, J. Hoogmartens and H. Vanderhaeghe, *J. Chromutogr., 291* (1984) 59.
2 Cefadroxilum, *Pharmeuropa, 2* (1990) 184.
3 *United States Pharmacopeia XXII,* 3rd Suppl., United States Pharmacopeiai Convention, Rockville, MD, 1989.
4 C. Hendrix, C. Wijsen, Li Ming Yun, E. Roets and J. Hoogmartens, *J. Chromatogr., 628 (1993) 49.*
5 *Theory and Problems of Statistics in SI Units,* McGraw-Hill, New York, 1st ed., 1972, p. 189.