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ROUTINE DETERMINATION OF EIGHT COMMON ANTI-EPILEPTIC DRUGS AND METABOLITES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING A COLUMN-SWITCHING SYSTEM FOR DIRECT INJECTION OF SERUM SAMPLES

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SUMMARY

A simple and rapid high-performance liquid chromatographic method for determining eight common anti-epileptic drugs and metabolites in serum is described. A column-switching system including one analytical column and two precolumns for sample enrichment offers the possibility of directly injecting patients' sera without any pretreatment. The two precolumns are alternately switched over to avoid time loss in analysis due to the sample washing step. The samples are flushed with dilute phosphoric acid, as the purge liquid, onto the precolumns which consist of very short cartridges (length 0.5 cm) filled with spherical ODS silica gel (particle size 30 μm). The retained substances are carried over, after purification, onto the analytical column in the same direction of flow as in the flushing step. A mixture of acetonitrile and phosphoric acid—sodium phosphate buffer solution is thereby used as solvent for the gradient elution. The separation was carried out using an analytical column, which was filled with ODS material of particle size 5 μm.

INTRODUCTION

Many authors have in the last decade been concerned with the analysis of anti-epileptic drugs (AEDs) in biological fluids. More than 80 original papers and short communications dealing with the determination of AEDs by high-performance liquid chromatography (HPLC) — valproic acid and the benzodiazepines are not included — in human plasma and serum have been published. For reasons of space not all these papers can be quoted.

In 21 papers protein precipitation using acetonitrile is described [1—4]. In only two laboratories acetone was used for protein precipitation [5, 6]. Extraction of the samples using a solvent which is immiscible with water was

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carried out eleven times with dichloromethane [7—9], twelve times with chloroform [3, 10, 11], nine times with diethyl ether [12—14], seven times with ethyl acetate [15, 16] and eight times using a mixture of different solvents [17, 18]. In ten laboratories the analysed AEDs were first adsorbed onto solid material such as activated charcoal [19, 20], kieselguhr [21] or RP-18 material [22—24] in order to elute them after purification with a suitable purge liquid. One study describes protein precipitation using perchloric acid, but recovery of the analysed AEDs was relatively low [25].

To the best of our knowledge only one of the publications dealing with the HPLC analysis of AEDs describes the direct injection of plasma [26]. However, the separation, using an anion-exchange column, took a very long time and is not comparable with the quality of present-day HPLC separations.

Today on-line column switching techniques offer a better possibility for the direct injection of serum. These have, in the last few years, increasingly been used instead of extraction procedures in sample pretreatment of biological fluids and other complex matrices for HPLC analyses. The substances to be analysed are adsorbed and purified on precolumns, which are filled with ion exchangers [27—29], silica gel [30, 31], CPG (controlled pore glass) [32], styrene—divinylbenzene copolymers [31, 33] and, in the large majority, with reversed-phase material [34—49].

The adaption of the automated column switching technique to the problems encountered in routine drug monitoring in the analytical laboratory of an

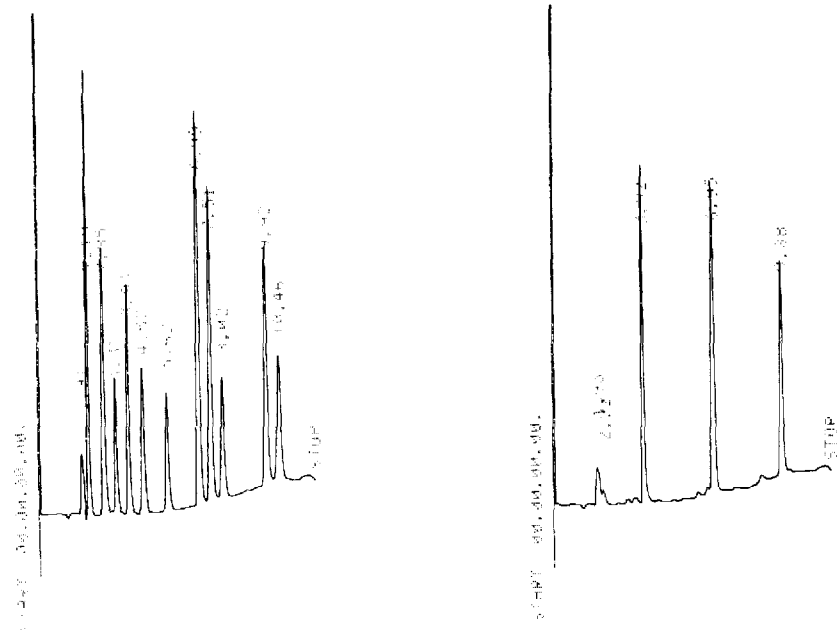

Fig. 1. Chromatogram of a calibration sample. Retention time (min), drug/MB, concentration (μg/ml): 2.19 THE 10, 2.85 CAF 10, 3.37 PE 12.5, 3.93 ET 50, 4.57 PR 12.5, 5.62 DIOL 5, 6.99 PB 25, 7.51 DM 25, 8.02 C-EP 5, 9.92 PT 12.5, 10.46 CBZ 7.5.

Fig. 2. Chromatogram of a serum sample from a patient on ethosuximide, phenobarbital and phenytoin medication. Retention time (min), AED/MB, concentration (μg/ml): 3.92 ET 74.3, 6.95 PB 18.5, 9.88 PT 10.1.

epilepsy centre is discussed in this paper. The following AEDs and their clinically relevant metabolites (MBs) were chromatographically separated: phenobarbital (PB), phenytoin (PT), ethosuximide (ET), carbamazepine (CBZ), carbamazepine-10,11-epoxide (C-EP), primidone (PR), 2-ethyl-2-phenylmalonediamide (PE), and N-desmethylmethsuximide (DM). In addition, the chromatographic conditions must be so chosen that there is no interference in the determination of PE by caffeine (CAF) or theophylline (THE), and of PR by the second main CBZ metabolite 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine (DIOL), (see Figs. 1—4).

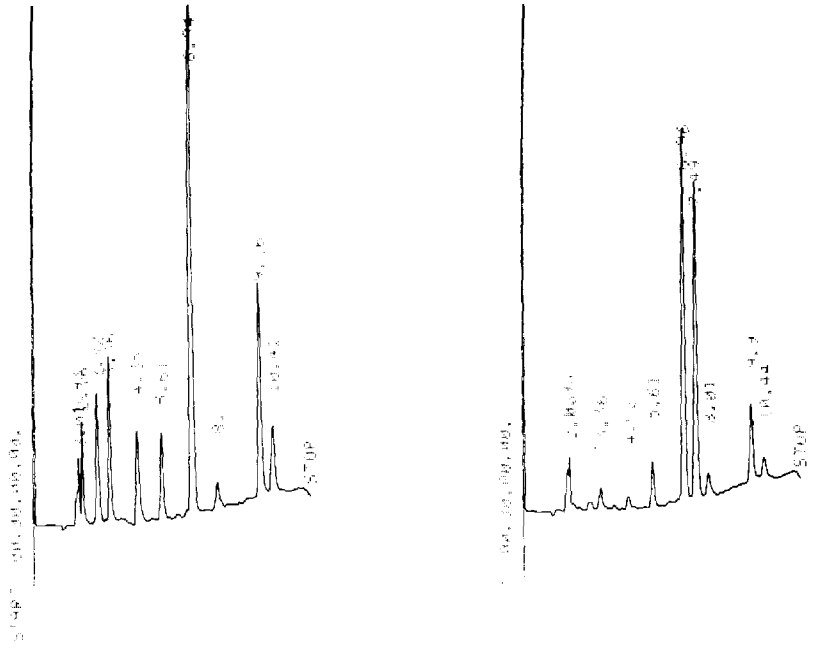

Fig. 3. Chromatogram of a pool serum of patients on primidone medication (inc. other co-medication). Retention time (min), AED/MB, concentration (μg/ml): 2.82 CAF 4.5, 3.36 PE 14.0, 4.55 PR 8.4, 5.61 DIOL 7.1, 6.94 PB 34.6, 8.00 C-EP 2.0, 9.86 PT 10.6, 10.41 CBZ 3.6.

Fig. 4. Chromatogram of a pool serum of patients on methsuximide medication (inc. other co-medications). Retention time (min), AED/MB, concentration (μg/ml): 3.36 PE 2.5, 4.52 PR 1.0, 5.61 DIOL 3.6, 6.96 PB 23.2, 7.49 DM 25.2, 8.01 C-EP 1.9, 9.90 PT 4.0, 10.44 CBZ 1.0.

EXPERIMENTAL

Chemicals

Chemicals were obtained from the following firms: PR, ET, PT, CBZ, and C-EP from Desitin-Werk/Carl Klinke, Hamburg (F.R.G.), PE and DM from EGA-Chemie, Steinheim (F.R.G.), PB from Bayer, Leverkusen (F.R.G.). We are grateful to Ciba-Geigy, Basle (Switzerland), for providing DIOL. Acetonitrile and water "for use in HPLC" were from Baker Chemicals, Deventer (The Netherlands). All other chemicals were of analytical reagent grade and were obtained from E. Merck, Darmstadt (F.R.G.).

Apparatus

The equipment was obtained from the following firms: HPLC low-pressure gradient-former 2500, two HPLC constant flow pumps 600/200, column switching module SE-2, spectrophotometer SP-4, Shimadzu printer-plotter integrator C-R 1B from Gynkotek, Munich (F.R.G.), autosampler WISP 710 B from Waters, Koenigstein/Taunus (F.R.G.), HPLC thermostat from Barkey Labortechnik, Bielefeld (F.R.G.), analytical column Shandon-ODS Hypersil (5 μm, 250 mm × 4.6 mm I.D.) and precolumn cartridges (5 mm × 4.6 mm I.D.) filled with Nucleosil 30 C_{18} (30 μm) from Bischoff-Analysentechnik, Leonberg (F.R.G.), on-line filter with 0.5-μm frits from DuPont, Frankfurt/Main (F.R.G.). Materials for the dry filling of the precolumns (40 mm × 4.6 mm I.D.) were: Nucleosil 30 C_{18} (30 μm) from Macherey-Nagel, Düren (F.R.G.), Perisorb RP-18 (30—40 μm) from Merck, and Vydac 201-RP (30—40 μm) from Gynkotek.

Chromatographic parameters

Temperature of the column: 70°C. Injection volume: 50 μl. Detection wavelength: 205 nm. Precolumns: purge liquid 0.01 vol.% phosphoric acid, flow-rate 0.3 ml/min, duration of washing 300 sec. Analytical column: buffer pH 4 (0.01 vol.% phosphoric acid buffered with saturated disodium hydrogen phosphate solution to pH 4). Gradient elution mixtures: A = buffer pH 4—acetonitrile (9:1, v/v), B = buffer pH 4—acetonitrile (4:6, v/v), flow-rate 1.5 ml/min.

The gradient programme is given in Table I.

TABLE I

GRADIENT ELUTION PROGRAMME

Step	A (vol.%)	B (vol.%)	Acetonitrile (vol.%)	Time (min)
1	085	015	17.5	00.00
2	085	015	17.5	01.00
3	050	050	35.0	06.00
4	050	050	35.0	02.00
5	085	015	17.5	00.00
6	085	015	17.5	End

Calibration and control samples

A 10 ml quantity of a stock solution (for calibration) containing 25 mg PE, 100 mg ET, 25 mg PR, 50 mg PB, 50 mg DM, 10 mg C-EP, 25 mg PT, and 15 mg CBZ in 100 ml acetonitrile and another 10 ml quantity of a stock solution with 20 mg THE, 20 mg CAF and 10 mg DIOL (for test purposes) were mixed and made up to 200 ml with HPLC water. The same quantity of this standard solution as for the patient samples (50 μl) was injected, so that the amount of AEDs in μg/ml is given by the integrator on using the external standard mode.

As control sera, lyophilized samples from the firms Biotrol, Fisher and Merck were dissolved in distilled water. Without further pretreatment 50 μl of these solutions were likewise injected.

Lifetime of the columns

At first the method as described here with 4 cm precolumns, filled with Nucleosil 30 C_{18}, was carried out using the back-flush technique. This method has been employed in routine analysis for about half a year. The introduction of very short precolumns (0.5 cm) made it possible to concentrate and purify the drugs, using the purge liquid, as well as subsequently to elute onto the analytical column in the same direction of flow. Thus, blockage which in the back-flush technique occurred mainly at the head of the separation column, could be avoided. As additional protection an on-line filter holder for replaceable filter frits of 0.5 μm pore diameter was built in in front of the analytical column. The frits were replaced after every 200—250 injections of serum. Under the chosen chromatographic and technical conditions the precolumn cartridges had a lifetime of 300—400 serum injections and the analytical column up to about 1400 serum injections.

RESULTS AND DISCUSSION

Recovery of the drugs and metabolites determined

At the very beginning of the development of the routine method described above a suitable precolumn material was sought. This material had to adsorb fully all the analysed AEDs and MBs but not lead to blockage on direct injection of serum. Therefore 4 cm columns were first of all filled with a material consisting of a type of porous layer beads (PLB) such as those used by Roth et al. [42] and Beschke et al. [45]. Unfortunately the recovery rate using both Perisorb RP-18 (30—40 μm) and Vydac 201-RP (30—40 μm) was poor for nearly all the analysed AEDs (see Table II). Both requirements mentioned above (long lifetime and complete recovery) were complied with using Nucleosil 30 C_{18} (30 μm, spherical) which was recommended by Kronbach et al. [47] (see Table II).

TABLE II

RECOVERY FROM THE DIFFERENT PRECOLUMN MATERIALS TESTED

Recovery is expressed as a percentage of the values achieved by direct injection of a calibration sample onto the analytical column.

	Perisorb RP-18 40 mm*	Vydac 201-RP 40 mm*	Nucleosil 30 C_{18}	
			40 mm*	5 mm
THE	3.6	1.5	98.3	99.7
CAF	93.1	29.8	101.1	99.2
PE	0	0	98.1	97.6
ET	0	0	103.1	96.8
PR	7.5	0	100.1	99.0
DIOL	100.6	0	95.9	100.0
PB	24.4	3.7	100.4	103.2
DM	66.5	14.9	100.2	104.1
C-EP	95.6	86.8	100.5	105.3
PT	100.4	62.4	98.7	106.3
CBZ	99.8	103.5	98.9	106.3

*Determination in the back-flush mode.

The recovery rate was determined by repeatedly injecting a calibration sample onto the analytical column and then onto each of the precolumns. The values for direct injection onto the analytical column were taken as 100%. The values obtained following precolumn enrichment were compared with these values and likewise expressed as a percentage (Table II).

A comparison of recovery, as described, could not be carried out with serum samples since the analytical column (5 μm) would already be blocked after one direct injection of serum. For this reason other methods were employed in order to test the quality of the method described here. This was initially checked by analysing commercially obtained control sera. The results using direct injection were in very good agreement with those given by the producers. Because the clinically relevant metabolites PE and DM were not present in the control sera, we carried out a comparative study with some hundred patient samples. These were analysed using gas chromatography (GC) [50] and HPLC with ethyl acetate extraction (HPLC—EE) [51] as sample pretreatment and in addition by direct serum injection, in part as double determinations. Double determination in this case means that each serum was injected once onto precolumn A and once onto precolumn B. For purposes of calibration, the mean values of the double injections of the standard samples were used. The agreement of the double determinations is given in Table III.

TABLE III

REPRODUCIBILITY OF DOUBLE INJECTIONS

n = number of duplicates, $\overline{x}$ = mean value (μg/ml), r = coefficient of correlation, $\overline{d}$ = mean value of the absolute differences of the duplicates = $x_1 - x_2$, $\overline{d}_x$ (%) = mean deviation from the mean value expressed as a percentage = $100(x_1 - x_2)/(x_1 + x_2)$.

	n	$\overline{x}_1$	$\overline{x}_2$	r	$\overline{d}$	$\overline{d}_x$ (%)
PE	135	8.15	8.17	0.990	0.34	2.25
ET	74	50.11	51.17	0.994	1.69	1.80
PR	135	6.86	6.83	0.991	0.26	1.79
PB	309	26.29	26.30	0.997	0.51	1.07
DM	68	23.06	23.00	0.994	0.55	1.27
PT	244	9.51	9.52	0.998	0.19	1.07
CBZ	203	4.68	4.67	0.995	0.14	1.66

The results in Table III show that for some substances (PB, DM, PT) the agreement is better than for the other compounds. As exactly the same sample had been injected onto both precolumns and as the autosampler used has a good reproducibility, it is apparently — at the time of writing — not possible for the manufacturer to produce two precolumn cartridges which are absolutely identical. As shown in Table III, each substance reacts specifically to the variations in the filling of the cartridges.

Agreement of direct serum injection with the results of the reference methods GC and HPLC—EE

We have recently reported on a study comparing three basic methods of sample pretreatment for HPLC analysis of AEDs [51] with the results obtained from routine GC analysis [50]. GC and HPLC—EE were likewise chosen for

controlling the results obtained from direct serum injection (DI) using a column-switching technique. The results were evaluated, as described in ref. 51, using the statistical procedure of linear regression analysis [52, 53] as given in Table IV.

TABLE IV

STATISTICAL EVALUATION OF THE DIRECT INJECTION HPLC METHOD USING GC AND HPLC—EE AS REFERENCE METHODS

R = reference method, DI = direct injection, n = number of samples, $\overline{x}$ and $\overline{y}$ = mean values (μg/ml), b = slope of the regression line expressing the systematic deviation from the reference values as a percentage, a = intercept of the ordinate by the regression line as a measure of a constant and systematic deviation, r = coefficient of correlation.

	n	$\overline{x}$ (R)	$\overline{y}$ (DI)	b	a	r
PE (GC)	105	7.23	8.58	1.05	1.02	0.972
PE (HPLC—EE)	133	8.07	8.20	1.03	−0.15	0.983
ET (GC)	69	49.71	50.05	0.84	8.47	0.968
ET (HPLC—EE)	73	52.03	50.67	0.88	4.88	0.983
PR (GC)	105	6.59	7.01	0.92	0.98	0.961
PR (HPLC—EE)	133	6.62	6.81	0.99	0.23	0.974
PB (GC)	246	25.97	25.51	0.99	0.85	0.988
PB (HPLC—EE)	298	25.74	26.24	1.01	0.15	0.987
DM (GC)	63	23.26	22.90	0.95	0.79	0.986
DM (HPLC—EE)	67	23.36	22.92	0.98	−0.06	0.988
PT (GC)	193	9.14	9.38	0.98	0.43	0.990
PT (HPLC—EE)	235	9.31	9.44	0.98	0.27	0.991
CBZ (GC)	156	4.56	4.66	0.95	0.30	0.971
CBZ (HPLC—EE)	197	4.66	4.70	1.01	0.01	0.989

As seen from Table IV, the method of direct injection agrees sufficiently with both reference methods (with two exceptions the coefficient of correlation (r) for all AEDs and MBs is greater than 0.970). Whilst the agreement for PB, DM and PT with both reference methods is equally good, the correlation in the case of the other substances between DI and the HPLC—EE is higher than between DI and GC.

Within-day and day-to-day precision

In order to check on the precision of the method described, three pooled sera containing various AEDs and a control serum were analysed several times in a day and daily over the course of several weeks. The results are summarized in Tables V and VI.

The within-day precision is for all AEDs and MBs, with one exception, very much the greater the higher the concentration of the substances concerned in the serum. This was to be expected. Only in the case of ET is there a greater range of fluctuation for the control serum than for the pool serum A. This could be due to a small quantity of interfering compounds which are present in the control serum and which are eluted in the retention range of ET. The coefficients of variation (C.V.) for all AEDs and MBs lie in the within-day control, with one exception, below 5%. Only in pool serum C, CBZ with a very low concentration of 1.1 μg/ml has a C.V. of 7.25% (see Table V).

TABLE V

WITHIN-DAY PRECISION OF DIRECT SERUM INJECTION

x_{max} = maximum value, x_{min} = minimum value, $\overline{x}$ = mean value (μg/ml), m = median, S.D. standard deviation, C.V. = coefficient of variation.

	n	x_{max}	x_{min}	$\overline{x}$	m	S.D.	C.V. (%)
Control sample							
ET	14	99.5	86.3	93.76	93.45	3.39	3.61
PR	14	19.6	18.1	18.79	18.80	0.41	2.20
PB	14	31.8	30.6	31.00	31.00	0.30	0.96
PT	14	31.7	30.4	30.79	30.75	0.33	1.07
CBZ	14	10.7	10.2	10.35	10.30	0.13	1.24
Pool serum A							
PE	18	6.5	5.6	6.06	6.10	0.26	4.24
PR	18	4.8	4.3	4.50	4.50	0.11	2.53
PB	18	22.1	20.5	21.03	20.95	0.44	2.08
PT	18	5.4	4.9	5.11	5.10	0.13	2.64
CBZ	18	2.2	2.0	2.13	2.10	0.06	2.78
Pool serum B							
ET	18	55.0	51.6	53.53	53.45	1.07	2.00
PB	18	19.2	17.6	18.35	18.35	0.49	2.66
PT	18	2.7	2.5	2.61	2.60	0.08	3.08
CBZ	18	3.3	2.8	3.03	3.00	0.12	4.08
Pool serum C							
PB	18	24.2	23.2	23.72	23.70	0.25	1.04
DM	18	26.8	25.7	26.34	26.30	0.30	1.13
PT	18	4.0	3.7	3.85	3.80	0.08	2.04
CBZ	18	1.3	1.0	1.08	1.10	0.08	7.25

TABLE VI

DAY-TO-DAY PRECISION OF DIRECT SERUM INJECTION

	n	x_{max}	x_{min}	$\overline{x}$	m	S.D.	C.V. (%)
Pool serum A							
PE	20	6.9	5.8	6.32	6.30	0.26	4.13
PR	20	6.1	4.5	5.41	5.45	0.39	7.26
PB	20	23.8	21.4	22.67	22.60	0.65	2.88
PT	20	6.0	5.2	5.72	5.75	0.21	3.65
CBZ	20	2.8	2.2	2.49	2.55	0.14	5.61
Pool serum B							
ET	20	60.1	52.3	55.12	54.85	2.09	3.79
PB	20	19.9	18.1	18.65	18.65	0.42	2.23
PT	20	3.0	2.7	2.76	2.75	0.09	3.38
CBZ	20	3.4	3.1	3.26	3.25	0.10	3.11
Pool serum C							
PB	23	24.4	22.1	23.27	23.25	0.69	2.96
DM	23	27.0	23.2	25.16	25.30	1.03	4.10
PT	23	4.2	3.7	3.90	3.90	0.14	3.48
CBZ	23	1.2	0.9	1.04	1.05	0.08	7.55

The coefficients of variation for the day-to-day control of precision lay, with three exceptions, always below 5% (see Table VI). For the very low CBZ in pool serum C, already mentioned above, the C.V. is 7.55%. Likewise, for the relatively low CBZ concentration (mean value 2.5 μg/ml) of the pool serum A the C.V. is 5.61% and for the PR (mean concentration 5.4 μg/ml) in the same serum is 7.26%.

This shows that PR as well as to a less extent PE and ET, all three of which lie in the early eluted range of the chromatogram, are exposed to disturbing influences to a somewhat greater degree than are the substances that are eluted later.

CONCLUSION

A column-switching technique with purification and enrichment of the samples enables sera of epileptic patients to be injected directly. A large number of possible sources of error in the otherwise necessary extraction, enrichment and evaporation steps can thereby be eliminated in the pretreatment of samples for HPLC analysis. Under the technical and chromatographic conditions described in the experimental section more than 100 samples can be analysed in 24 h. Direct injection of serum is especially suitable for the analysis of small quantities of sample (less than 50 μl). In routine analysis there is normally enough serum to enable 50 μl to be injected. Thereby the reproducibility of the results is improved.

Considering the values given in Table V for within-day precision and those in Table VI for day-to-day precision, the method described is sufficiently suitable for purposes of routine analysis. The avoidance of pretreatment of the samples results in a great saving of costs of reagents and laboratory staff.

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REFERENCES

1 S.J. Soldin and J.G. Hill, Clin. Chem., 22 (1976) 856.
2 P.M. Kabra, B.E. Stafford and L.J. Marton, Clin. Chem., 23 (1977) 1284.
3 P.M. Kabra, D.M. McDonald and L.J. Marton, J. Anal. Toxicol., 2 (1978) 127.
4 H.M. Neels, J.A. Totte, R.M. Verkerk, A.J. Vlietinck and S.L. Scharpe, J. Clin. Chem. Clin. Biochem., 21 (1983) 295.
5 M. Riedmann, B. Rambeck and J.W.A. Meijer, Ther. Drug Monit., 3 (1981) 397.
6 G.K. Szabo and T.R. Browne, Clin. Chem., 28 (1982) 100.
7 M. Eichelbaum and L. Bertilsson, J. Chromatogr., 103 (1975) 135.
8 S.H. Atwell, V.A. Green and W.G. Haney, J. Pharm. Sci., 64 (1975) 806.
9 B. Salvesen and E. Lyngbakken, Medd. Nor. Farm. Selsk., 43 (1981) 45.
10 S. Kitazawa and T. Komuro, Clin. Chim. Acta, 73 (1976) 31.
11 R.F. Adams, G.J. Schmidt and F.L. Vandemark, J. Chromatogr., 145 (1978) 275.
12 J.E. Evans, Anal. Chem., 45 (1973) 2428.

13 K. Kushida, K. Chiba and T. Ishizaki, Ther. Drug Monit., 5 (1983) 127.
14 D.C. Turnell, S.C. Trevor and J.D.H. Cooper, Ann. Clin. Biochem., 20 (1983) 37.
15 R. Farinotti and G. Mahuzier, J. Liquid Chromatogr., 2 (1979) 345.
16 J.A. Christofides and D.E. Fry, Clin. Chem., 26 (1980) 499.
17 P. Draper, D. Shapcott and B. Lemieux, Clin. Biochem., 12 (1979) 52.
18 J.W. Dolan, S. van der Wal, S.J. Bannister and L.R. Snyder, Clin. Chem., 26 (1980) 871.
19 R.F. Adams and F.L. Vandemark, Clin. Chem., 22 (1976) 25.
20 B. Kinberger, P. Wahrgren and A. Holmen, Anal. Lett., 14 (1981) 1419.
21 P.J. Helmsing, J. van der Woude and O.M. van Eupen, Clin. Chim. Acta, 89 (1978) 301.
22 R.C. Williams and J.L. Viola, J. Chromatogr., 185 (1979) 505.
23 R.C. George, Clin. Chem., 27 (1981) 198.
24 P.M. Kabra, M.A. Nelson and L.J. Marton, Clin. Chem., 29 (1983) 473.
25 J.C. Kraak, F. Smedes and J.W.A. Meijer, Chromatographia, 13 (1980) 673.
26 K.S. Albert, M.R. Hallmark, M.E. Carroll and J.G. Wagner, Res. Commun. Chem. Pathol. Pharmacol., 6 (1973) 845.
27 F.F. Cantwell, Anal. Chem., 48 (1976) 1854.
28 H.Y. Mohammed and F.F. Cantwell, Anal. Chem., 50 (1978) 491.
29 R.A. Hux, H.Y. Mohammed and F.F. Cantwell, Anal. Chem., 54 (1982) 113.
30 R.J. Dolphin, F.W. Willmott, A.D. Mills and L.P.J. Hoogeveen, J. Chromatogr., 122 (1976) 259.
31 E.L. Johnson, R. Gloor and R.E. Majors, J. Chromatogr., 149 (1978) 571.
32 F. Erni and R.W. Frei, J. Chromatogr., 149 (1978) 561.
33 D. Ishii, K. Hibi, K. Asai, M. Nagaya, K. Mochizuki and Y. Mochida, J. Chromatogr., 156 (1978) 173.
34 J. Lankelma and H. Poppe, J. Chromatogr., 149 (1978) 587.
35 H.P.M. van Vliet, T.C. Bootsman, R.W. Frei and U.A.T. Brinkman, J. Chromatogr., 185 (1979) 483.
36 G.C. Davis and P.T. Kissinger, Anal. Chem., 51 (1979) 1960.
37 H. Hulpke and U. Werthmann, Chromatographia, 12 (1979) 390.
38 G.J. de Jong, J. Chromatogr., 183 (1980) 203.
39 J.C. Gfeller and M. Stockmeyer, J. Chromatogr., 198 (1980) 162.
40 D.D. Koch and P.T. Kissinger, Anal. Chem., 52 (1980) 27.
41 F. Erni, H.P. Keller, C. Morin and M. Schmitt, J. Chromatogr., 204 (1981) 65.
42 W. Roth, K. Beschke, R. Jauch, A. Zimmer and F.W. Koss, J. Chromatogr., 222 (1981) 13.
43 T.V. Alfredson, J. Chromatogr., 218 (1981) 715.
44 W. Voelter, T. Kronbach, K. Zech and R. Huber, J. Chromatogr., 239 (1982) 475.
45 K. Beschke, R. Jauch, W. Roth, A. Zimmer and F.W. Koss, GIT Lab.-Med., 5 (1982) 357.
46 C.J. Little, D.J. Tompkins, O. Stahel, R.W. Frei and C.E. Werkhoven-Goewie, J. Chromatogr., 264 (1983) 183.
47 T. Kronbach, W. Voelter and K. Zech, poster presentation at the VIIth International Symposium on Column Liquid Chromatography, Baden-Baden, F.R.G., May, 1983.
48 C.E. Werkhoven-Goewie, C. de Ruiter, U.A.T. Brinkman, R.W. Frei, G.J. de Jong, C.J. Little and O. Stahel, J. Chromatogr., 255 (1983) 79.
49 U. Juergens, J. Chromatogr., 275 (1983) 335.
50 B. Rambeck and J.W.A. Meijer, Arzneim.-Forsch., 29 (1979) 99.
51 U. Juergens, T. May, K. Hillenkoetter and B. Rambeck, Ther. Drug Monit., in press.
52 J.O. Westgard and M.R. Hunt, Clin. Chem., 19 (1973) 49.
53 R.B. Davis, J.E. Thompson and H.L. Pardue, Clin. Chem., 24 (1978) 611.