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High-performance liquid chromatographic determination of furosemide in plasma and urine and its use in bioavailability studies

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Abstract

A sensitive, selective and efficient reversed-phase high-performance liquid chromatographic (HPLC) method is reported for the determination of furosemide in human plasma and urine. The method has a sensitivity limit of 5 ng/ml in plasma, with acceptable within- and between-day reproducibilities and good linearity (r^2>0.99) over a concentration range from 0.05 to 2.00 μg/ml. The one-step extract of furosemide and the internal standard (warfarin) from acidified plasma or urine was eluted through a μBondapak C_{18} column with a mobile phase composed of 0.01 *M* potassium dihydrogenphosphate and acetonitrile (62:38, v/v) adjusted to pH 3.0. Within-day coefficients of variation (C.V.s) ranged from 1.08 to 8.63% for plasma and from 2.52 to 3.10% for urine, whereas between-day C.V.s ranged from 4.25 to 10.77% for plasma and from 5.15 to 6.81% for urine at three different concentrations. The minimum quantifiable concentration of furosemide was determined to be 5 ng/ml. The HPLC method described has the capability of rapid and reproducible measurement of low levels of furosemide in small amounts of plasma and urine. This method was utilized in bioavailability/pharmacokinetic studies for the routine monitoring of furosemide levels in adults, children and neonate patients.

Keywords: Furosemide

1. Introduction

Furosemide is a potent and widely used diuretic in the treatment of edematous states associated with cardiac, chronic renal failure [1,2], hypertension, congestive heart failure [3,4] and cirrhosis of the liver [5]. The large degree of variability in the pharmacokinetic behavior of furosemide can be attributed to the differences in organ function, the types of patients treated with furosemide and from differences within- and between subjects and study protocols [6]. The site of action of furosemide is believed to be at the lumen surfaces [7]. Because it is highly protein bound [8], access of furosemide to this site occurs through active secretion into the tubule lumen via the nonspecific organic acid of the proximal tubule [9].

The bioavailability of furosemide from oral dosage forms is highly variable. Drug information sources generally report values in the 60 to 65% range for

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the extent of absorption [10,11]. The half life reported for furosemide in healthy subjects generally falls in the range of 30 to 120 min, and is influenced by the underlying disease process, while its half-life reported in patients with congestive heart failure is in the range of 50–327 min (mean=122 min) [12].

Several analytical methods for the determination of furosemide in plasma and urine have been reported, including spectrophotometric [12–14] and spectrofluorometric methods [15–18]. These methods suffer from a lack of sensitivity and selectivity and are not suitable for routine determination of furosemide. Although gas chromatographic methods [19,20] are more sensitive, they require a laborious extraction procedure and derivatization of furosemide prior to analysis. Therefore, the analysis time may exceed 1 h per sample. Four methods were reported that allow the determination of furosemide in plasma using thin-layer chromatography [21–24]. These methods require complicated preparative steps before quantitation of the drug can be carried out.

A variety of high-performance liquid chromatography (HPLC) techniques [25–44] were recently developed. Some of these [25–27,29,30,38] required 1–2 ml of plasma. Other methods required lengthy sample extraction and have very long elution times for the drug and the internal standard [29,31–33,36]. Farthing et al. [41] used an external standard and solid-phase extraction, while Reeuwijk et al. [42] used the lengthy and tedious technique of reversed-phase ion pair chromatography. On the other hand, Saugy et al. [43] used gas chromatography–mass spectrometry with different types of ionization to confirm the occurrence of furosemide after permethylation of the extract eluted by HPLC. Most of the HPLC methods have not achieved the complete separation of furosemide from endogenous substances with a minimum detectable concentration of less than 20 ng/ml, which would allow their application to the measurement of low furosemide concentrations in biological fluids. Most of the previously reported methods published the precision of the calibration data but usually omitted the accuracy for concentrations at the lower end of the concentration range found in pharmacokinetic and bioavailability/bioequivalence studies. Furthermore, the minimum quantifiable concentrations are rarely reported. A summary report of analytical methods validation [45], sponsored by the U.S. Food and Drug Administration, the American Association of Pharmaceutical Scientists and other agencies, outlined the acceptable standards for documenting and validating analytical methods and procedures to yield reliable results in bioavailability, bioequivalence and pharmacokinetic studies that can be satisfactorily interpreted. Most of the HPLC assays for furosemide analysis do not meet all the criteria suggested by the summary report. Interference from commonly administered drugs is also not uncommon in those methods [29,30,33]. Potential interference from the major furosemide metabolite, furosemide glucuronide, and the hydrolytic product 4-chloro-5-sulfamoyl anthranilic acid (CSA) was also not reported for the majority of these methods. These disadvantages may limit their use in the therapeutic drug monitoring of furosemide levels in patients as well as in pharmacokinetic bioequivalence studies of furosemide.

In this paper, we report a rapid, sensitive and selective reversed-phase HPLC assay that meets the acceptable criteria for analytical method validation and that is suitable for the processing of a large number of furosemide samples.

2. Experimental

2.1. Chemicals and reagents

All chemicals and reagents were of analytical grade and were used as received. The organic solvents used for extraction and in the mobile phase were of HPLC grade (BDH, Poole, UK). Furosemide was obtained from Dumex and the internal standard, warfarin, was obtained from Sigma (St. Louis, MO, USA).

2.2. Apparatus

The HPLC system consisted of a single-piston solvent delivery pump, Model 501; an automatic injector, Model WISP 710B; a scanning fluorescence detector, Model 470, and a data module integrator, Model 746. Analytical separation was accomplished using a μBondapak C_{18} reversed-phase column (15 cm×3.9 mm I.D., 10 μm particle size) for plasma and a resolve spherical C_{18} column (15 cm× 3.9 mm I.D., 5 μm particle size) for urine. All of the above

were supplied by Waters Associates (Milford, MA, USA).

2.3. Chromatographic conditions

A mobile phase containing 0.01 *M* potassium dihydrogen (62:38, v/v) adjusted to pH 3.0 with phosphoric acid (1:3, v/v) was used. The mixture was filtered through a 0.22-μm membrane filter (Millipore, Bedford, MA, USA) under vacuum. The flow-rate was 1.5 ml/min. The eluents from the column were detected at excitation and emission wavelengths of 225 and 389 nm, respectively. The separation was carried out at ambient temperature.

2.4. Standard solutions

The standard stock solution of furosemide (200 μg/ml) was prepared by dissolving and diluting 10 mg of furosemide in a 50-ml volumetric flask with methanol. The internal standard solution was prepared by dissolving 500 mg of warfarin in a 50-ml volumetric flask (10 mg/ml) using methanol as the diluting solvent. For plasma, the internal standard solution was further diluted by ten-folds with methanol. Both the standard stock solution and the internal standard solution were protected from light by covering them with aluminum foil and they were stored at 4°C. Both solutions were stable for at least three months.

Calibration curves, ranging from 0.05 to 2.00 μg/ml for plasma and 0.50 to 50.00 μg/ml for urine, were constructed by adding appropriate volumes of the stock solutions of furosemide to 0.5 ml aliquots of drug-free plasma and 200 μl of drug-free urine specimens in each run of the assay. The volumes of internal standard (10 mg/ml warfarin) added to each tube were 30 μl for plasma and 40 μl for urine, to account for any variability in recovery through extraction. A weighted least squares regression analysis was the best-fit regression line. Data were presented as mean±S.D.

2.5. Analytical procedure

2.5.1. Plasma

To a 10-ml stoppered glass tube, 0.5 ml of plasma specimen, 30 μl of internal standard (10 mg/ml warfarin), 50 μl of 6 *M* hydrochloric acid and 3 ml of HPLC-grade diethyl ether were added. The tube was closed tightly and vortex-mixed for 30 s and centrifuged at a speed of 2000 *g* for 10 min. The ether layer was quantitatively transferred to another clean centrifuge tube. On a water bath adjusted at 45°C, the ether was allowed to evaporate under a stream of nitrogen gas. The residue was reconstituted in 100 μl of methanol and a 20-μl aliquot was injected onto the column.

2.5.2. Urine

To a 10-ml stoppered glass tube, 200 μl of urine, 20 μl of 6 *M* hydrochloric acid, 40 μl of the internal standard solution (10 mg/ml warfarin) and 8 ml of HPLC-grade diethyl ether were added. The contents of the tube were vortex-mixed for 30 s. The ether layer was quantitatively transferred to another clean centrifuge tube, and evaporated to dryness on a water bath adjusted to 45°C under a stream of nitrogen gas. The residue was reconstituted in 100 μl of mobile phase and a 20-μl aliquot of the solution was injected onto the column.

2.5.3. Clinical study

Three formulations of furosemide were administered to six healthy male volunteers in a crossover design experiment, separated by a one week washout period. The volunteers fasted overnight for at least 10 h before the administration of drug (40 mg tablet) and they continued fasting until 3 h post dose. The participants did not take any other medication for at least two weeks prior to and during the day of the study. Venous blood samples were drawn into heparinized tubes before drug administration and at 0.25, 0.5, 0.75, 1.0, 1.5., 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 10.0 and 12.0 h after the drug was given. All blood samples were taken via an indwelling catheter. The blood samples were centrifuged at 1100 *g* for 15 min and the plasma samples were frozen at −20°C pending analysis.

During each study day, urine was collected immediately before drug administration and at the following intervals: 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–8, 8–12 and 12–24 h after drug administration. Urine volumes were recorded and an aliquot was frozen at −20°C until it was assayed.

3. Results and discussion

The determination of drug concentrations in plasma and urine and pharmacokinetic parameters in bioavailability/bioequivalence studies require both precise and accurate data. Several published furosemide assays failed to report the possible interferences from furosemide metabolites and degradation products. This lack of specificity may result in an overestimation of actual concentrations, leading to erroneous bioavailability/bioequivalence decisions. The composition and pH of the mobile phase used in the present assay provided good separation of furosemide and warfarin and gave sharp peaks with no interferences from endogenous components in either plasma or urine. Fig. 1 shows representative chromatograms of blank plasma and a subject's plasma sample obtained by the above described method. The chromatograms showed excellent resolution between furosemide and the internal standard, appearing at 3.0 and 9.0 min, respectively. No interference was observed during the chromatographic run of the plasma and the urine sample in the area where furosemide or the internal standard peak appears. The retention time of the hydrolytic product CSA is less than 2 min and, thus, will not interfere with furosemide or the internal standard. This method allows the determination of the concentration of furosemide within 10 min.

Quantitation of furosemide in plasma and urine samples was carried out by determining the slope of the calibration curve, constructed using the peak-area ratio for furosemide and the internal standard (warfarin), obtained for the calibration standards. The calibration curves of furosemide was typically described by $Y=0.0005(\pm0.017)+1.157(\pm0.119)X$, ($r^2=0.994$; $n=10$), where Y corresponds to the peak-area ratio of furosemide to the internal standard and X to the concentration of furosemide added over a concentration range of 0.5 to 50 μg/ml. The linear least-squares equation was $Y=0.012(\pm0.086)+0.042(\pm0.002)X$, $r^2=0.998$; $n=9$.

Standard curves for furosemide were constructed on different days to determine the variability of the slopes and intercepts. The results showed little day-to-day variability of slopes and intercepts and gave acceptable linearity ($r>0.996$) over the plasma and urine concentration ranges studied. The coefficient of variation for the slopes was 10.32% for the plasma assay and 5.14% for the urine assay.

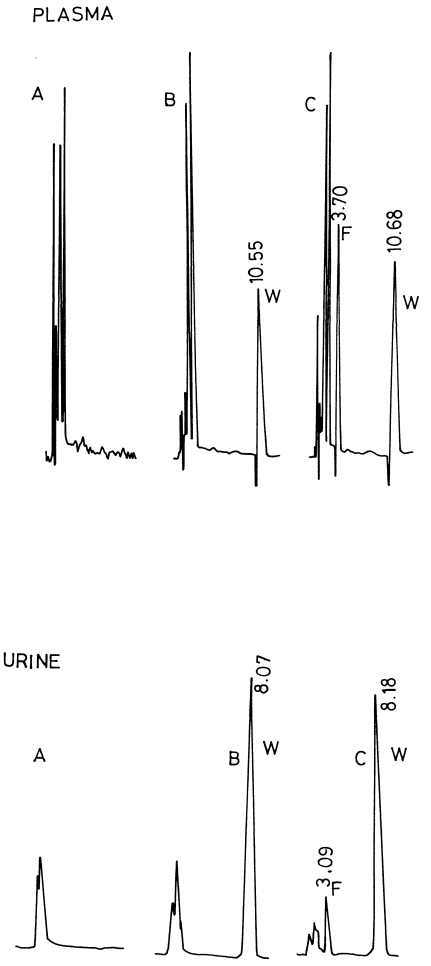

Fig. 1. Liquid chromatograms of: (I) Plasma samples, (A) blank plasma, (B) blank plasma spiked with the internal standard, (C) plasma extract from a healthy subject following oral administration of a furosemide tablet (furosemide concentration=0.60 μg/ml). (II) Urine samples, (A) blank urine, (B) blank urine spiked with the internal standard, (C) urine extract from a healthy subject following oral administration of a furosemide tablet (furosemide concentration=8.03 μg/ml); W=warfarin (internal standard).

To evaluate the precision of the method, the drug-free plasma and the urine were spiked with three different known concentrations (low, medium, high) of drug. The spiked samples were analyzed both on the same day and on different days to determine the variability. The within-day precision showed a coefficient of variation (C.V.) of 1.08 to 8.63% and a

Table 1
Within-day and day-to-day accuracy and precision of furosemide in plasma

Added concentration (μg/ml)	Within-day[a]			Day-to-day[b]		
	Measured concentration (μg/ml)	Accuracy (%)	Precision (%)	Measured concentration (μg/ml)	Accuracy (%)	Precision (%)
0.08	0.077±0.0066 $n=11$	−3.75	8.63	0.084±0.009 $n=11$	5.0	10.77
0.4	0.368±0.0054 $n=9$	−8.0	1.47	0.42±0.027 $n=10$	5.5	6.40
1.8	1.57±0.017 $n=9$	−12.78	1.08	1.79±0.76 $n=12$	−0.56	4.25

[a] Mean value represents different plasma samples for each concentration.
[b] Day-to-day reproducibility was determined from different runs over a six-week period for the three concentrations.

between-day precision of 4.25 to 10.77% for plasma (Table 1). For urine, within-day precision showed a C.V. of 2.52 to 3.10% and a between-day precision of 5.15 to 6.81% (Table 2). The minimum quantifiable concentration of furosemide was determined to be 5 ng/ml. However, the minimum detectable concentration was in the order of 2–3 ng/ml using a signal-to-noise ratio of three.

The absolute and relative recoveries of furosemide in plasma and urine were determined at three different concentration levels by comparing extracted versus unextracted samples. Tables 3 and 4 show the

Table 2
Within-day and day-to-day accuracy and precision of furosemide in urine

Added concentration (μg/ml) precision (%)	Within-day[a]			Day-to-day[b]		
	Measured concentration (%)	Accuracy (μg/ml)	Precision (%)	Measured concentration (%)	Accuracy (μg/ml)	Precision (%)
7.50	8.07±0.25 $n=10$	7.60	3.10	7.80±0.44 $n=12$	4.00	5.64
25	25.36±0.71 $n=12$	1.44	2.80	26.04±1.34 $n=12$	4.16	5.15
45	43.70±1.10 $n=10$	−2.89	2.52	46.27±3.15 $n=11$	2.84	6.81

[a]Mean value represents different plasma samples for each concentration.
[b]Day-to-day reproducibility was determined from different runs over a six-week period for the three concentrations.

Table 3
Analytical recovery of furosemide from plasma[a]

Concentration (μg/ml)	Mean peak area ratio		Absolute recovery (%)	Relative recovery (%)	
	Aqueous	Plasma		Mean	Range
0.080	0.09	0.064	71.11	95.75	82.69–109.0
0.40	0.405	0.39	96.30	91.9	89.55–93.92
1.80	2.038	2.098	102.91	87.31	85.96–88.85

[a] Eight replicate injections of each concentration.

Table 4
Analytical recovery of furosemide from urine[a]

Concentration (μg/ml)	Mean peak area ratio		Absolute recovery (%)	Relative recovery (%)	
	Aqueous	Plasma		Mean	Range
7.5	0.292	0.313	107.19	107.60	01.87–111.87
25	1.078	1.005	93.23	101.4	98.08–106.20
45	1.947	1.750	89.88	87.31	93.60–101.84

[a] Eight replicate injections of each concentration.

recovery of furosemide in plasma and urine. The data obtained showed satisfactory recovery for furosemide in plasma as well as in urine and the extraction provided adequate sensitivity to process the samples.

To determine the specificity of the assay, blank plasma samples, collected from healthy adult male volunteers, were analyzed using the reported procedure. Chromatograms were inspected for the presence of interfering peaks. In addition, other commonly administered drugs were tested for their possible interference under the same chromatographic conditions. Table 5 lists the retention times for some of the frequently coadministered drugs. In addition, no interfering peaks were observed for furosemide's metabolite or its hydrolytic product.

Blank plasma samples were spiked with known amounts of furosemide at three different concen-

Table 5
Retention times for commonly used drugs using the current method

Drug	Retention time (min)
Carbamazepine	N.D.
Cimitedine	N.D.
Diazepam	N.D.
Disopyramide	N.D.
Fluvoxamine	N.D.
Meclofenamate	N.D.
Metoclopramide	N.D.
Phenobarbital	N.D.
Phenylbutazone	N.D.
Phenytoin	N.D.
Quinidine	1.80
Ranitidine	N.D.
Sulfamethoxazole	2.00
Theophylline	N.D.
Trimethoprim	N.D.

N.D., not detected.

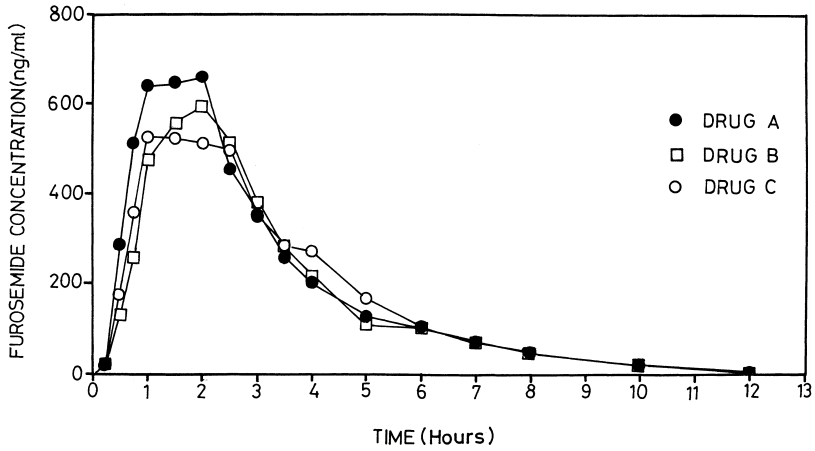

Fig. 2. Mean furosemide plasma concentrations following oral administration of three tablet formulations of furosemide (40 mg) to six health adult males.

Table 6
Pharmacokinetic parameters of furosemide (mean±S.D.) following oral administration (40 mg) of three commercially available formulations to six subjects

Parameter	Brand A	Brand B	Brand C
$AUC_{0-\infty}$ (ng·h/ml)	2216.2±146.9	2038.5±466.9	2101.9±642.7
C_{max} (ng/ml)	1058.0±188.1	763.5±242.9	858.2±433.9
T_{max} (h)	1.38±0.54	1.60±0.58	2.2±1.0
K_{el} (h^{-1})	0.39±0.06	0.42±0.05	0.42±0.08
$t_{1/2}$ (h)	1.84±0.31	1.66±0.21	1.71±0.41
$AU_{0.24}$ (mg)	11.18±2.0	11.67±3.57	11.58±2.69

$AUC_{0-\infty}$, area under the plasma concentration–time curve from time 0 to infinity.
C_{max}, peak plasma concentration.
T_{max}, time of peak concentration.
K_{el}, elimination rate constant.
$t_{1/2}$, elimination half life.
AU_{0-24}, cumulative amount excreted unchanged in urine in 24 h.

trations; 15, 120 and 360 ng/ml, and were subsequently stored in a freezer at −20°C. To determine the stability of the drug in human plasma, the above frozen samples were analyzed over a ten-week period. The results demonstrated that furosemide was stable in the frozen plasma for at least ten weeks without degradation.

The present method was applied to the determination of several pharmacokinetic studies, including testing the bioavailability and bioequivalence of drug formulations. Fig. 2 shows the mean plasma concentration profiles of furosemide after oral administration of a 40–mg tablet from three formulations to six healthy male volunteers. The calculated pharmacokinetic parameters ($AUC_{0-\infty}$, C_{max}, T_{max}, K_{el} and $t_{1/2}$) of the three brands are shown in Table 6.

Fig. 3 shows the cumulative amounts of unchanged furosemide in urine.

In conclusion, a rapid, sensitive and selective reversed-phase HPLC analysis of furosemide is reported. The method improves upon previously reported methods for accurate measurement of furosemide concentrations in biological samples and allows the determination of the drug within 10 min. The simplicity of this method for the determination of furosemide in human plasma and urine, coupled

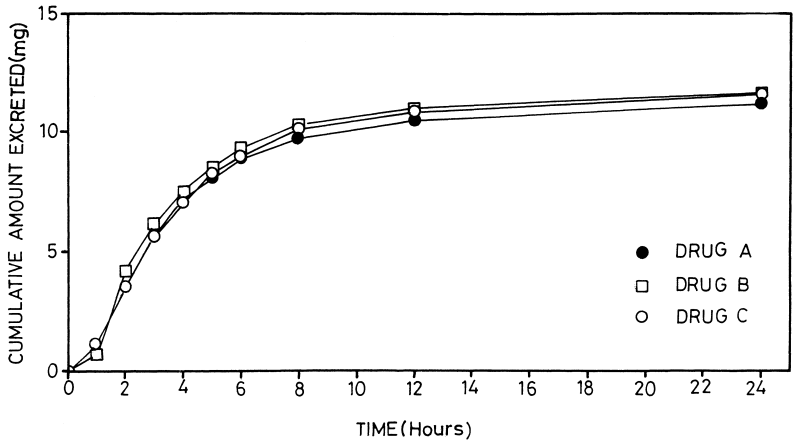

Fig. 3. Mean cumulative amounts of furosemide excreted uncharged in urine following the oral administration of three tablet formulations of furosemide (40 g) to six healthy adult males.

with its high specificity and sensitivity, provides a particularly suitable analytical method for pharmacokinetic studies and for monitoring the bioavailability/bioequivalence of pharmaceutical formulations.

References

[1] L.L.B. Laura, D.S. Ronald, Clin. Pharmacokinet. 18 (1990) 81.
[2] F. Cantarovich, C. Galli, L. Benedetti, Br. Med. J. 4 (1973) 449.
[3] H.S. Frazier, H. Yager, N. Engl. J. Med. 288 (1973) 246.
[4] K. Dikshit, J.K. Vyden, J.S. Forrester, K. Chatterjee, R. Parkash, H.J.C. Swan, N. Engl. J. Med. 288 (1973) 1087.
[5] F. Sadikal, Br. J. Clin. Pract. 27 (1973) 222.
[6] P. Chennavasiu, R.A. Johnson, D.C. Brater, J. Pharmacokinet. Biopharm. 9 (1981) 623.
[7] M.B. Burg, L. Stoner, J. Cardinal, N. Green, Am. J. Physiol. 225 (1973) 119.
[8] R. Zini, P. d'Athis, A. Hoareau, J.P. Tillement, Eur. Clin. Pharmacol. 10 (1976) 139.
[9] J.B. Hook, H.E. Williamson, J. Pharmacol. Exp. Ther. 149 (1965) 404.
[10] A.G. Gilman, L.S. Goodman, A. Gilman, The Pharmacological Basis of Therapeutics, 8th ed., Macmillan, New York, 1991.
[11] G.K. McEvoy, G.M. McQuarrie and H.J. DiPietro, American Hospital Formulary Service Monograph, Furosemide USP (40:28), American Hospital Formulary Service, American Society of Hospital Pharmacists, Bethesda, MD, 1987, pp. 1355–1359.
[12] D.C. Brater, R. Seiwell, S. Anderson, Kidney Int. 22 (1982) 171.
[13] A. Haussler, V.P. Hadjue, Arzneimittelforsche 14 (1964) 710.
[14] F. Andreasen, P. Jacobsen, Acta Pharmacol. Toxicol. 35 (1974) 49.
[15] A.W. Forrey, B. Kimpel, A.D. Blair, R.E. Cutler, Clin. Chem. 20 (1974) 152.
[16] V.P. Hadju, A. Haussler, Arzneimittelforsche 14 (1964) 7.
[17] R.E. Cutler, A.W. Forrey, T.C. Christopher, B.M. Kimpel, Clin. Pharmacol. Ther. 15 (1974) 588.
[18] M.R. Kelly, R.E. Cutler, A.W. Forrey, B. Kimpel, Clin. Pharmacol. Ther. 15 (1974) 178.
[19] B. Lindstrom, M. Molander, J. Chromatogr. 101 (1974) 219.
[20] J. Perez, D.S. Sitar, R.I. Oglivie, Drug Metab. Dispos. 7 (1979) 383.
[21] M. Schafer, H.E. Geissler, E. Mutschler, J. Chromatogr. 143 (1977) 636.
[22] G. Yakatan, D.D. Maness, J. Scholler, W.J. Novick, J.T. Doluisio, J. Pharm. Sci. 65 (1976) 1456.
[23] E. Mikkelsen, F. Andreasen, Acta Pharmacol. Toxicol. 41 (1977) 254.
[24] B. Wesley-Hadzija, A.M. Mattocks, J. Chromatogr. 229 (1982) 425.
[25] A.D. Blair, A.W. Forrey, B.T. Meijsen, R.E. Cutler, Pharm. Sci. 64 (1975) 1334.
[26] B. Lindstrom, J. Chromatogr. 100 (1974) 189.
[27] M.L. MacDougall, D.W. Shoeman, D.L. Azarnoff, Res. Commun. Pathol. Pharmacol. 10 (1975) 285.
[28] H. Roseboom, R.H.A. Sorel, Anal. Lett. 16 (1978) 46.
[29] K. Carr, A. Rane, J. Frolich, J. Chromatogr. 145 (1978) 41.
[30] S.E. Swezey, P.J. Meffin, T.F. Blaschke, J. Chromatogr. 17 (1979) 469.
[31] E.T. Lin, D.E. Smith, L.Z. Benet, B.A. Hoener, J. Chromatogr. 163 (1979) 315.
[32] W. Snedden, J.N. Sharma, P.G. Fernandez, Ther. Drug Monit. 4 (1982) 381.
[33] K. Uchino, S. Isozaki, Y. Saitoh, F. Nakagawa, Z. Tamura, J. Chromatogr. 308 (1984) 241.
[34] R.L. Nation, G.W. Peng, W.L. Chiou, J. Chromatogr. 162 (1979) 88.
[35] R.S. Rapaka, J. Roth, C.T. Viswanathan, T.J. Goehl, V. Prasad, B.E. Cabana, J. Chromatogr. 227 (1982) 463.
[36] A.L.M. Kerremans, Y. Tan, C.A.M. Van Ginneken, W.J. Gribnau, J. Chromatogr. 229 (1982) 129.
[37] L.J. Lovett, G. Nygard, P. Dura, S.K.W. Khalil, J. Liq. Chromatogr. 8 (1985) 611.
[38] M.T. Bauza, C.L. Lesser, J.T. Johnston, R.V. Smith, J. Pharm. Biomed. Anal. 3 (1985) 459.
[39] S.P. Sood, V.I. Green, Z.M. Norton, Ther. Drug Monit. 9 (1987) 484.
[40] T.B. Vree, M. van der Biggelaar Martea, C.P. Verwey van Wissen, J. Chromatogr. B 655 (1994) 53.
[41] D. Farthing, T. Karnes, T.W. Gehr, C. March, I. Fakhry, D.A. Sica, J. Pharm. Sci. 81 (1992) 569.
[42] H.J. Reeuwijk, U.R. Tjaden, J. van der Greef, J. Chromatogr. 575 (1992) 269.
[43] M. Saugy, P. Neuwly, A. Munafo, L. Rvier, J. Chromatogr. 564 (1991) 567.
[44] J.S. Sidhu, B.G. Charles, J. Chromatogr. 612 (1993) 161.
[45] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, Pharm. Res. 9 (1992) 588.