Journal of Chromatography, 419 (1987) 233–242
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3668

LIQUID CHROMATOGRAPHIC ANALYSIS OF ALIZAPRIDE AND METOCLOPRAMIDE IN HUMAN PLASMA AND URINE USING SOLID-PHASE EXTRACTION

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(First received December 5th, 1986; revised manuscript received February 19th, 1987)

SUMMARY

A universal rapid, sensitive and selective high-performance liquid chromatographic method with UV detection at 230 nm has been developed for the determination of benzamide drugs in human plasma and urine. Sample pretreatment is carried out using solid-phase extraction columns, resulting in very high extraction recoveries of the compounds investigated (alizapride, metoclopramide, alpiropride, amisulpride). The detector response is linear from 25 to 10 000 ng/ml, and the detection limit is 3 ng/ml for alizapride and 10 ng/ml for metoclopramide. The proposed method is highly suitable for pharmacokinetic studies and for drug monitoring.

INTRODUCTION

Nausea and vomiting are well known adverse effects associated with cancer chemotherapy. Drugs such as cisplatinum and cyclophosphamide are particularly emetogenic. Anti-emetic drugs such as the antihistamines or the phenothiazines, offer limited protection against nausea and vomiting evoked by chemotherapeutic agents or produce extrapyramidal side-effects [1]. Recent clinical studies have established that high doses of metoclopramide, a benzamide drug, effectively reduce the gastrointestinal side-effects of cancer chemotherapeutics [2,3]. Alizapride, a relatively new benzamide analogue, has properties similar to those of metoclopramide. In animal studies, alizapride displayed greater anti-emetic effects than metoclopramide [4,5].

A variety of chromatographic methods has been developed for the determination of metoclopramide in body fluids. Both gas chromatographic methods using electron-capture detection [6] or nitrogen–phosphorus-specific detection [7] and high-performance liquid chromatographic (HPLC) methods using UV detection

0378-4347/87/$03.50

have been described [8–13]. Alizapride has been determined in plasma and urine by HPLC with fluorimetric detection [14,15].

All these methods employ a liquid extraction step, which is labour-intensive and time-consuming, and likely to lead to the formation of emulsions. An attractive alternative to liquid extraction is based on solid-phase extraction (SPE). Sample preparation can be accelerated and even automated by the use of SPE columns.

In this paper we present a rapid and sensitive HPLC method for the determination of metoclopramide and alizapride, in which sample clean-up was carried out by SPE.

EXPERIMENTAL

Apparatus

A Perkin-Elmer Series 2 high-performance liquid chromatopgraph (Perkin-Elmer, Gouda, The Netherlands) equipped with a Rheodyne 7105 septumless 175-μl loop injector and a 25 cm × 4.6 mm I.D. stainless-steel column, packed with LiChrosorb RP-8 (particle size 10 μm; Chrompack, Middelburg, The Netherlands) was used. The analytical column was protected by a 10-cm guard column packed with reversed-phase pellicular material (Chrompack). Substituted benzamides were detected at 230 nm with a Perkin-Elmer LC-75 variable-wavelength detector, connected to a Waters 740 data module. The solvent flow-rate was 2.0 ml/min, at ca. 15 MPa.

SPE was carried out with 3-ml disposable columns with octylsilane-bonded silica gel (C_8) (Analytichem, Betron Scientific, Rotterdam, The Netherlands), ion-exchange quaternary aminesilicane-bonded silica gel (N^+) and carboxylic acid-bonded silica gel (COOH) (both from J.T. Baker, Deventer, The Netherlands), respectively, using a Baker-10 extraction manifold (J.T. Baker).

The acid–base composition of the HPLC mobile phase was controlled using a calibrated Metrohm 632 pH meter (Metrohm, Herisau, Switzerland).

Reagents and standards

Water was purified by a Milli RO4/Milli Q system (Millipore, Bedford, MA, U.S.A.). Alizapride [4,5-azimido-N-(1-allyl-2-pyrrolidinyl)methyl-2-methoxybenzamide] hydrochloride, metoclopramide [4-amino-5-chloro-N-2-(diethylamino)ethyl-2-methoxybenzamide], alpiropride [5-(methylaminosulphonyl)-N-(1-allyl-2-pyrrolidinyl)methyl-2-methoxy-4-aminobenzamide] and amisulpride [4-amino-5-ethylsulphonyl-N-(1-allyl-2-pyrrolidinyl)methyl-2-methoxybenzamide] were obtained from Delagrange (Paris, France). Other chemicals were obtained from E. Merck (Amsterdam, Netherlands).

Acetonitrile and methanol were of LiChrosolv quality, while the remaining chemicals were of pro analyse grade. Triethylamine was glass-distilled before use. Drugs used for drug interference studies were kindly provided by the Pharmacy Department, Academic Hospital of the Free University (Amsterdam, The Netherlands).

Stock solutions of the benzamides (1 mg/ml) were prepared in 0.01 *M* hydro-

chloric acid. These solutions were stable at 2–8 °C for at least one month. Working solutions were freshly prepared daily by diluting stock solutions with 0.01 *M* hydrochloric acid. The plasma standards containing 25–10 000 μg/l alizapride or metoclopramide were prepared by adding working solution to pooled, drug-free blood bank plasma (CLB, Amsterdam, Netherlands). Urine standards containing 25–10 000 μg/l were prepared by adding working solutions to fresh urine of healthy male volunteers. In routine assays 50–100 μl of internal standard (I.S.) solution containing 10 μg/ml amisulpride and 10 μg/ml alpiropride were added to each sample.

Mobile phase

The mobile phase was prepared by adding 10 ml of triethylamine to 760 ml of water. The solution was brought to pH 6.8 by addition of acetic acid (ca. 4.2 ml). Subsequently 160 ml of acetonitrile and 80 ml of methanol were added to the solution. The solution was mixed and filtered over a 0.45-μm HVLP membrane filter (Millipore, Etten-Leur, Netherlands). Addition of small amounts of either triethylamine or acetic acid was sometimes necessary to obtain complete resolution of the two internal standards alpiropride and amisulpride.

Solutions used for plasma and urine assay

Solution A consisted of 10 ml of triethylamine added to 1000 ml of water; the pH was adjusted to 7.00 with acetic acid. Solution B was 800 ml of water mixed with 200 ml of methanol. Solution C was made by adding 10 ml of triethylamine and 7 ml of acetic acid to 1000 ml of methanol. Solution D was composed of 2.10 ml of hydrochloric acid (37%) mixed with 250 ml of methanol (0.1 *M*).

Procedures

Plasma sample preparation. Transfer 1 ml of plasma into a 5-ml glass test-tube. Add 100 μl of I.S. solution and 1 ml of solution A and mix. Place C_8-columns on the top of the extraction manifold. Pass one volume (2.7 ml) of methanol and one volume of solution A through each column. Do not allow the columns to dry out. Place the plasma samples on top of each column and slowly pass them through the column by connecting the vacuum. Rinse the test-tube with 1 ml of solution A and pass the rinsing liquid through the column. Wash the column with one volume of water and 2 ml of solvent B. Dry the column for 1 min, then wash it with 200 μl of acetone to remove remaining water. Dry for 30 s. Elute the benzamides with 1 ml of solution C. Add 50 μl of solution D to eluate and evaporate to dryness under a gentle stream of air. Reconstitute the residue in 200 μl of mobile phase by sonication for 1 min and inject a suitable amount into the HPLC system.

Urine sample preparation. Transfer 1 ml of urine into a 5-ml glass test-tube. Add 1 ml of water and 100 μl of I.S. solution and mix. Affix COOH columns to the extraction manifold. Connect N^+ columns to the top of the COOH columns by means of an adaptor. Pass one volume of solution D through each stack of columns. Rinse with one volume of water, one volume of methanol and again with one volume of water. Transfer the urine samples to the top of each N^+ column. Rinse the test-tube with 2 ml of water and transfer this rinsing liquid to the top

of the column stack. Let the samples slowly pass through the system by connecting the vacuum. Wash the N^+ column with 1 ml of water and then remove the N^+ column. Wash the COOH column with one volume of water and two volumes of methanol and dry for 1 min. Elute the benzamides from the column with 1 ml of solution D. Evaporate the eluate at 45 °C under a gentle stream of air. Reconstitute the residue into 200 μl of mobile phase by sonication for 1 min and inject a suitable amount into the HPLC system.

RESULTS

Chromatographic separation

Fig. 1 shows chromatograms of blank plasma and urine, and of plasma and urine samples spiked with 25 ng/ml each of the four benzamides investigated. The various substances are well separated without any serious interference. The retention times of alizapride, amisulpride, alpiropride and metoclopramide were 3.8, 4.9, 6.0 and 7.1 min, respectively. Fig. 2 shows typical chromatograms of plasma and urine samples obtained from patients who had been treated with alizapride or metoclopramide.

Recovery

Extraction recoveries were determined from drug-free human plasma and pooled human urine. Detector responses of plasma and urine samples spiked with the benzamide drugs were compared with detector responses of directly injected aqueous solutions that had identical concentrations of the compounds investigated (Table I).

Linearity and sensitivity

Linearity was checked by measuring nine concentrations of alizapride and metoclopramide in the range 25–10 000 ng/ml. From the detector responses calibration lines were calculated using the linear least-squares method for peak detector responses ratio (drug/I.S.) versus concentration. The equations of the regression lines are tabulated in Table II. All regression lines were linear in the concentration range stated. Under these conditions limits of detection (defined as a signal-to-noise ratio of 2) of 3 and 10 ng/ml could be reached in plasma for alizapride and metoclopramide, respectively. In urine the detection limits for these compounds were 5 and 10 ng/ml, respectively.

Accuracy and precision

Accuracy and precision data were obtained by analyzing plasma and urine samples containing different concentrations of metoclopramide and alizapride. The results are listed in Tables III–V. The data are calculated using both amisulpride and alpiropride as an I.S.: in the plasma assay both gave equal values; in the urine assay alpiropride gave significantly higher results than amisulpride.

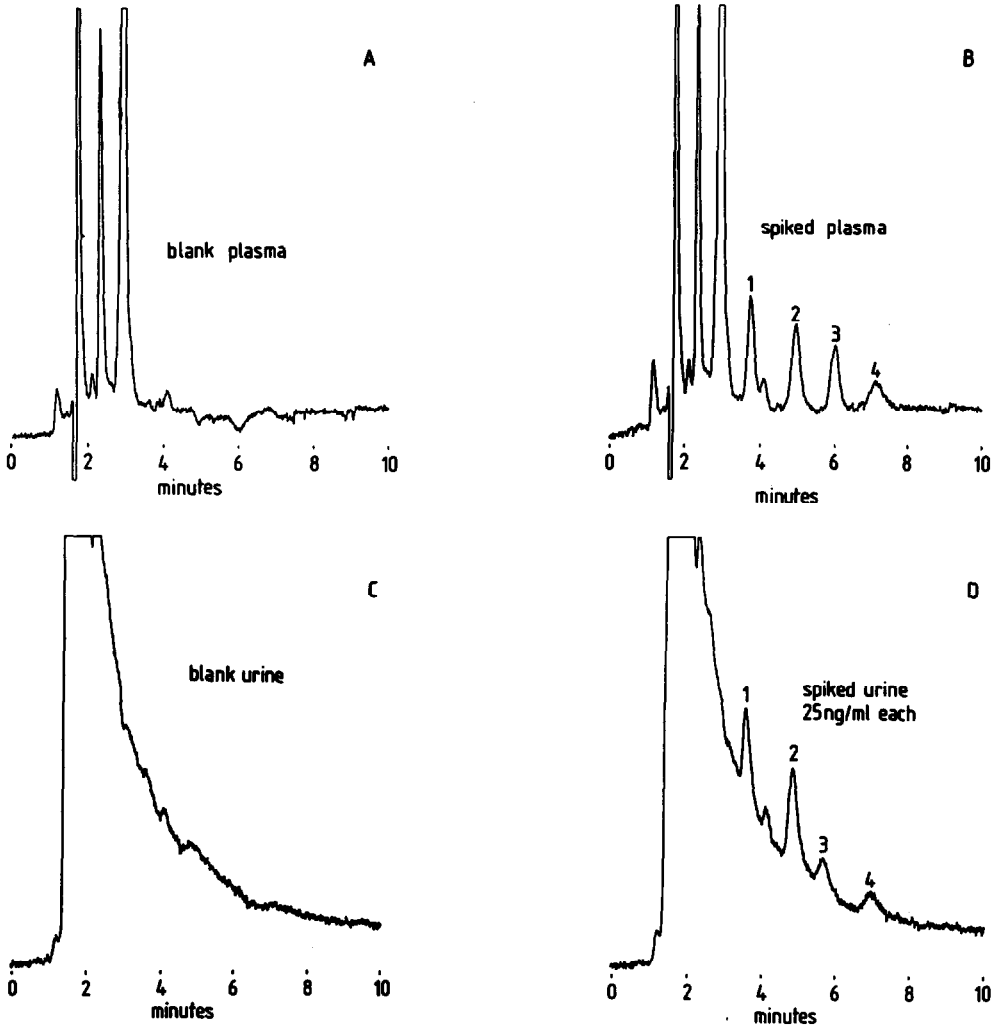

Fig. 1. Typical chromatograms obtained after injection on a LiChrosorb RP-8 (10 μm) column of 100 μl of blank (A) and spiked (B) plasma extracts and of blank (C) and spiked (D) urine extracts. Peaks: 1 = alizapride; 2 = amisulpride; 3 = alpiropride; 4 = metoclopramide.

Drug interference studies

The HPLC assay was assessed for interference by other drugs by determination of retention times of solutions containing 100 μg/ml of one of the drugs listed in Table VI. Most of the compounds that were investigated did not disturb the benzamide assay, since they were either well separated or undetected. Only peaks from isosorbide-5-mononitrate (IS-5-MN), codeine and nitrofurantoin could possibly coincide with the benzamide peaks in the chromatogram. Therefore, plasma and urine samples containing 20 μg/ml of these drugs were put through the complete sample clean-up. In the urine assay only codeine was eluted from the SPE system, with a recovery of 75%. In the chromatogram the codeine peak coincided with the amisulpride peak, so that alizapride and metoclopramide con-

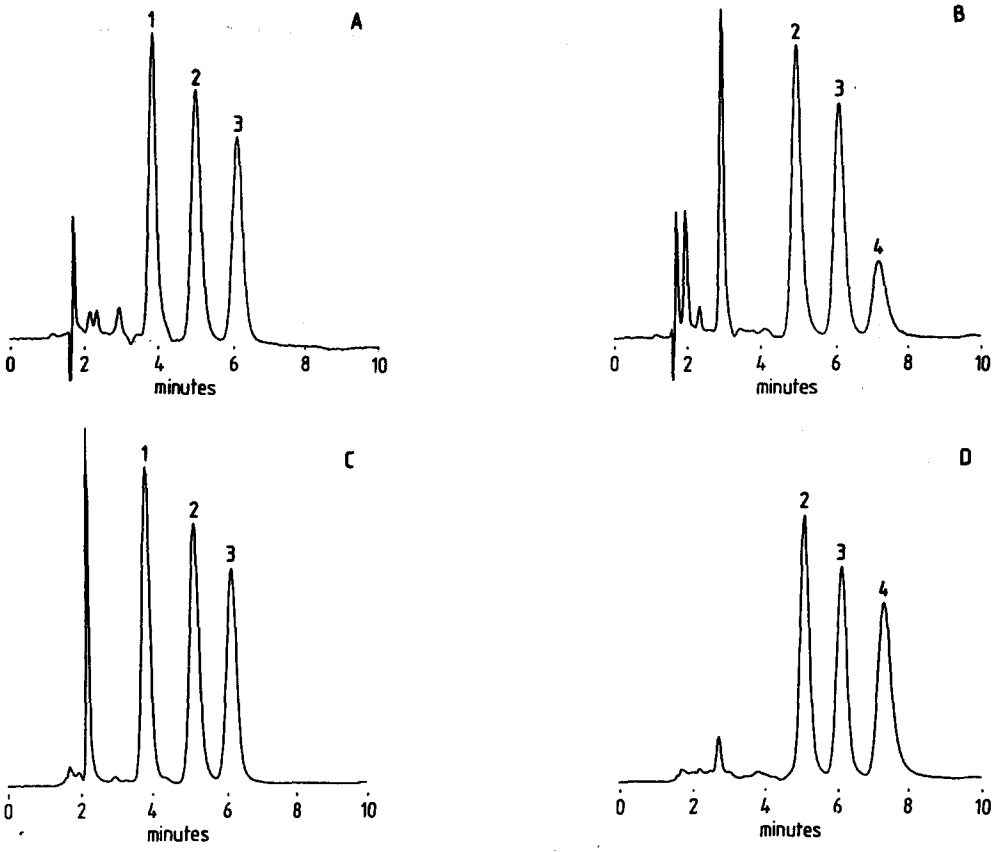

Fig. 2. Typical chromatograms obtained after injection on a LiChrosorb RP-8 (10 μm) column of 25 μl of plasma (A and B) and urine (C and D) extracts of patients treated with alizapride or metoclopramide. Peaks: 1 = alizapride; 2 = amisulpride; 3 = alpiropride; 4 = metoclopramide.

centrations could still be determined using alpiropride as I.S. In the plasma assay all three drugs were eluted from the SPE system, although in the cases of nitrofurantoin and IS-5-MN the recoveries were low (0.7 and 9.2%, respectively). Recovery of codeine was rather high: 87%. In the HPLC assay IS-5-MN co-eluted with alizapride so that IS-5-MN, when present in very high concentrations, might

TABLE I

EXTRACTION RECOVERIES FROM PLASMA AND URINE

Compound	Concentration (ng/ml)	Recovery (%)	
		Plasma ($n=10$)	Urine ($n=10$)
Alizapride	508.5	95.2 ± 5.3	95.3 ± 2.9
Metoclopramide	507.0	101.9 ± 3.9	93.9 ± 4.7
Amisulpride	1009	98.1 ± 5.2	90.1 ± 3.3
Alpiropride	1013	102.6 ± 5.2	82.1 ± 3.4

TABLE II

TYPICAL CALIBRATION GRAPHS OF ASSAYS IN PLASMA AND URINE

Test compound	Concentration range (ng/ml)	Internal standard	Plasma equation	Urine equation
Alizapride	25.4-10 170	Amisulpride	$y=1052.98x-3.77$ $r=0.9999$	$y=997.94x+20.87$ $r=0.9999$
Alizapride	25.4-10 170	Alpiropride	$y=1020.36x-4.00$ $r=0.9999$	$y=786.35x-43.46$ $r=0.9995$
Metoclopramide	25.4-10 140	Amisulpride	$y=1916.69x-17.43$ $r=0.9999$	$y=1993.24x+81.12$ $r=0.9998$
Metoclopramide	25.4-10 140	Alpiropide	$y=1857.16x-17.01$ $r=0.9999$	$y=1571.84x+20.39$ $r=0.9998$

TABLE III

ACCURACY AND PRECISION OF BENZAMIDE ASSAY IN PLASMA: WITHIN RUN

Concentration added (ng/ml)	n	Amisulpride as I.S.		Alpiropride as I.S.	
		Concentration found (ng/ml)	C.V. (%)	Concentration found (ng/ml)	C.V. (%)
Alizapride					
508.5	5	525.3 ± 6.3	1.2	510.0 ± 5.2	1.0
1017	5	989 ± 13.9	1.4	999.6 ± 16.5	1.6
2543	5	2472.9 ± 61.4	2.4	2508.3 ± 49.4	1.9
Metoclopramide					
507	5	522.5 ± 10.8	2.1	513.8 ± 7.7	1.5
1014	5	1033.5 ± 29.6	2.9	1023.5 ± 26.9	2.7
2535	5	2686.4 ± 63.8	2.5	2612.0 ± 74.3	2.9

TABLE IV

ACCURACY AND PRECISION OF BENZAMIDE ASSAY IN PLASMA: WITHIN DAY

Concentration added (ng/ml)	n	Amisulpride as I.S.		Alpiropride as I.S.	
		Concentration found (ng/ml)	C.V. (%)	Concentration found (ng/ml)	C.V. (%)
Alizapride					
101.7	8	106.9 ± 5.9	5.5	122.3 ± 5.5	4.5
1017	8	1029.0 ± 50.4	4.9	1026.8 ± 43.1	4.2
10170	8	10787 ± 743	6.9	10480 ± 440	4.2
Metoclopramide					
101.4	9	107.8 ± 17.4	16.1	113.4 ± 15.5	13.7
1014	9	994.2 ± 53.1	5.3	981.1 ± 36.2	3.7
10140	9	10377 ± 487	4.7	10199 ± 179	1.8

TABLE V

ACCURACY AND PRECISION OF BENZAMIDE ASSAY IN URINE

Drug	Concentration added (ng/ml)	n	Amisulpride as I.S.		Alpiropride as I.S.	
			Concentration found (ng/ml)	C.V. (%)	Concentration found (ng/ml)	C.V. (%)
Within run						
Alizapride	508.5	10	502.3 ± 11.6	2.3	556.6 ± 22.7	4.5
Metoclopramide	507.0	10	480.1 ± 9.0	1.8	517.4 ± 23.0	4.5
Within day						
Alizapride	508.5	3	502.5 ± 27.1	5.4	563.2 ± 11.7	2.1
Metoclopramide	507.0	3	478.9 ± 28.7	6.0	520.6 ± 13.1	2.5

interfere with the alizapride assay. Nitrofurantoin and codeine peaks coincided with the amisulpride peak.

DISCUSSION

Numerous HPLC methods have been described for the determination of metoclopramide in body fluids [8–13]. A critical appraisal of the merits of earlier methods has been given by Riley [11]. SPE by means of Extrelut columns has been applied by Block and Pingoud [8] and by Bishop-Freudling and Vergin [9]. In Block's procedure large sample volumes (10 ml) are used, and the need to subject the dried eluate to an extra chloroform extraction makes the method rather labour-intensive. In Bishop-Freudling's method smaller sample volumes were applied and the liquid extraction step was deleted. A disadvantage of the latter method was the use of elevated temperatures (50°C) in the assay method.

With respect to alizapride, only HPLC methods have been described using liquid extraction [14,15]. Although high alizapride recoveries were reported by Houin

TABLE VI

DRUG INTERFERENCE STUDIES

N.D. = not detectable

Drug	Retention time (min)	Drug	Retention time (min)
Acetylsalicylic acid	2.5	Nitrazepam	44.5
Theophylline	2.5	Clonazepam	59
Paracetamol	2.6	Indomethacine	N.D.
Caffeine	2.9	Orfenadrine	N.D.
Isosorbide-5-mononitrate	3.6	Furosemide	N.D.
Nitrofurantoin	4.2	Cisplatin	N.D.
Codeine	5.0	Amitryptiline	N.D.
Acenocoumarol	21.8	Isosorbide dinitrate	N.D.
Carbamazepine	33.5	Propranolol	N.D.

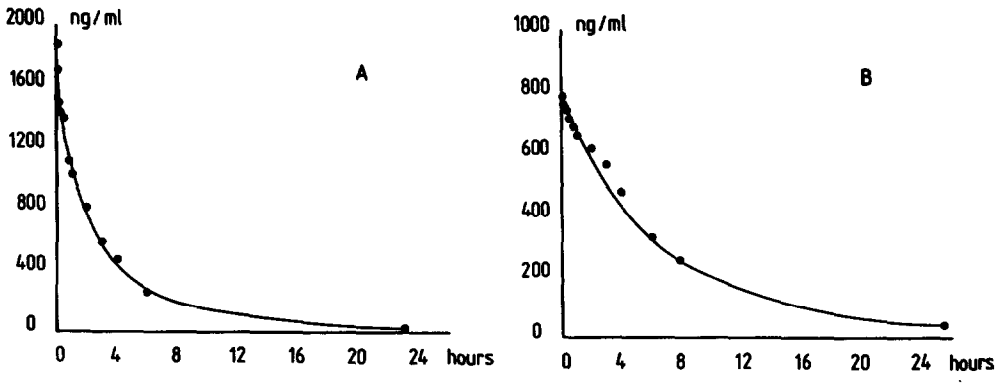

Fig. 3. Plasma concentration–time profiles of alizapride (A) and metoclopramide (B). Patients were receiving either 15 mg/kg alizapride or 6 mg/kg metoclopramide by intravenous infusion over 24 h, when treatment was stopped. The time profiles show the decline of alizapride and metoclopramide plasma levels from the moment that treatment was discontinued.

et al. [14], based on the use of external calibration curves obtained from drug-spiked plasma, this procedure gave rather poor extraction recoveries (71.7%) as was shown by Coulais et al. [15]. In our hands Houin's procedure gave extraction recoveries of 65%.

Compared with earlier HPLC procedures for the assay of benzamides in body fluids our method has several advantages. The main one is that our method combines the assets of SPE with a versatile analytical method for quantitating benzamides. All four benzamides investigated in this study can be analysed by the same method and using a single detection system, without loss of sensitivity. The detection limit for alizapride of 3 ng/ml is comparable with that (5 ng/ml) of the fluorimetric method [14], owing to the very clean extractions made possible by the SPE method.

Our method is also quicker than earlier HPLC methods based on liquid extraction. The SPE procedure takes ca. 45 min for ten samples, and the chromatography run-time is only 8 min for plasma samples and 15 min for urine samples (owing to coeluting peaks).

An additional pleasant surprise was that the SPE columns used for plasma extraction could be regenerated simply by washing out with methanol. Even after ten regenerations the columns were still showing the same retention and elution properties. Regrettably, the SPE columns used for urine extraction were not reusable.

In this method we used two internal standards. We consider this to be beneficial in the analysis of plasma and urine samples from patients, when unexpected substances, such as metabolites or other prescribed drugs, e.g. codeine, may interfere. Moreover, the peak-area ratio of the two internal standards provides a useful parameter to check the proper functioning of the analytical method.

In this paper we have presented an HPLC procedure for the assay of four benzamides in body fluids. The method can probably be used more universally. In an earlier contribution we described how this HPLC method is able to separate eight

therapeutically used benzamide drugs [16]. Preliminary experiments have shown that the method is also applicable to the assay of sultopride and veralipride in biofluids, and we have used it very satisfactorily in a comparative pharmacokinetic study of alizapride and metoclopramide (results to be published), as can be seen from the plasma concentration–time curves for alizapride and metoclopramide presented in Fig. 3.

ACKNOWLEDGEMENTS

The authors thank Delagrange International (Paris, France) for their generous gift of the substituted benzamides, and Mrs. J. Kuyper for typing the manuscript.

REFERENCES

1 J. Laszlo, Drugs, 25 (Suppl. 1) (1983) 1.
2 R.J. Gralla, L.M. Itri, S.E. Pisko, A.F. Squillante, D.P. Kelsen, D.W. Braun, L.A. Bordin, T.J. Braun and C.W. Young, N. Engl. J. Med., 305 (1981) 905.
3 R.J. Gralla, Drugs, 25 (Suppl. 1) (1983) 63.
4 C.J. Laville and J. Margarit, Sem. Hôp. Paris, 58 (1982) 323.
5 J. Perrot, G. Nahas and C. Laville, in D.S. Poster, J.S. Penta and S. Bruno (Editors), Treatment of Cancer Chemotherapy-Induced Nausea and Vomiting, Masson Publishing U.S.A., New York, 1981, p. 195.
6 Y.K. Tam, J.E. Axelson and R. Ongley, J. Pharm. Sci., 68 (1979) 1254.
7 R. Saller, D. Hellenbrecht, L. Briemann, A. Hellstern, H. Hess, P. Mitrou, M. Hodgson, G. Achtert, P. Brockmann and H.J. Hausleiter, Clin. Pharmacol. Ther., 37 (1985) 43.
8 W. Block and A. Pingoud, Fresenius' Z. Anal. Chem., 301 (1980) 109.
9 G.B. Bishop-Freudling and H. Vergin, J. Chromatogr., 273 (1983) 453.
10 S.M. Bryson, E.M. McGovern and L.M. Gilbert, Clin. J. Hosp. Pharm., 9 (1984) 263.
11 C.M. Riley, J. Pharm. Biomed. Anal., 2 (1984) 81.
12 G. Nygard, L.J. Lovett and S.K. Wahba Khalil, J. Liq. Chromatogr., 9 (1986) 157.
13 L. Slørdal, P.S. Prytz, U. Aasebo, and J. Aarbakke, Acta Pharmacol. Toxicol., 58 (1986) 240.
14 G. Houin, F. Bree, N. Lerumeur and J.P. Tillement, J. Pharm. Sci., 72 (1983) 71.
15 Y. Coulais, G. Campistron, C. Caillard and G. Houin, J. Chromatogr., 374 (1986) 425.
16 J. Bron, A.J. Wittebrood, A.P. de Jong and W.M. du Chatinier, J. Pharm. Belg., 42 (1987) 47.