Journal of Chromatography, 428 (1988) 265–274
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4207

SIMULTANEOUS QUANTITATION OF BUSPIRONE AND 1-(2-PYRIMIDINYL)PIPERAZINE IN HUMAN PLASMA AND URINE BY CAPILLARY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

M.A. SCIACCA*, G.F. DUNCAN, J.P. SHEA, H.C. FAULKNER, III, R.H. FARMEN and K.A. PITTMAN

Department of Metabolism and Pharmacokinetics, Pharmaceutical Research and Development Division, Bristol-Myers Company, Syracuse, NY 13221 (U.S.A.)

(First received January 8th, 1988; revised manuscript received March 14th, 1988)

SUMMARY

Buspirone and a buspirone metabolite, 1-(2-pyrimidinyl)piperazine (1-PP), are extracted from matrix using C_{18} extraction columns. The metabolite and its internal standard (d4-1-PP) are derivatized with pentafluorobenzoyl chloride to the corresponding amides. The 1-PP derivatives, buspirone and the buspirone internal standard (5-fluorobuspirone) are co-chromatographed. Chromatography and detection are performed using capillary gas chromatography with a fused-silica column and selected-ion monitoring–mass spectrometry. Linear range of the standard curves in plasma is 0.1–14 ng/ml for buspirone and 0.2–25 ng/ml for 1-PP with lower limits of quantitation of 0.1 and 0.2 ng/ml, respectively. In urine the linear range of the standard curves is 0.2–14 ng/ml for buspirone and 8–500 ng/ml for 1-PP with lower limits of quantitation of 0.2 and 8.0 ng/ml, respectively. Intra-assay accuracies were within 14% for buspirone and 1-PP in plasma and urine. Intra-assay precision was within 12% for both compounds in both matrices.

INTRODUCTION

Buspirone, 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione, is a new anti-anxiety drug that is marketed under the trade name Buspar®. 1-(2-Pyrimidinyl)piperazine (1-PP) is a major metabolite of buspirone. The structures of buspirone and the pentafluorobenzamide of 1-PP are shown in Figs. 1 and 3, respectively. Typical doses of buspirone are 5 or 10 mg orally. Plasma concentrations of buspirone are expected to reach a maximum of only 10 ng/ml. 1-PP concentrations are expected to be approximately double those of buspirone [1].

Bioanalytical assays using either high-performance liquid chromatography (HPLC) or gas chromatography (GC) could not offer the necessary sensitivity

0378-4347/88/$03.50

to quantitate these low concentrations of analyte. Radioimmunoassay (RIA) could be used, however, it was difficult to obtain large amounts of antiserum [2]. Gammans and co-workers [3,4] reported gas chromatographic—mass spectrometric (GC–MS) methods for separately quantitating both buspirone and 1-PP in the low ng/ml range. Sample processing was extensive and analytes were not simultaneously chromatographed. Our work involved the simultaneous extraction and chromatography of buspirone, 1-PP and their respective internal standards 8-[4-[4-(5-fluoro-2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione(5-fluorobuspirone) and 1-(2-pyrimidinyl)piperazine(2,2,3,3-d4) (d4-1-PP) from human plasma and urine. Isotopic purity of the d4-1-PP was 94.3%. The primary advantage of this assay over previous compound-specific methodologies is the simultaneous quantitation of buspirone and 1-PP, which saves both analyst and (GC–MS) time, with no sacrifice in the assay performance. Plasma sample volume requirements were also reduced from 4 to 1.5 ml.

EXPERIMENTAL

Materials

All four standards, buspirone, 5-fluorobuspirone, 1-PP and d4-1-PP, were obtained as hydrochloride salts from the Bristol-Myers Company. Toluene, methanol and acetonitrile were all obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.) and were HPLC grade or better. Ethanol was absolute, U.S.P. from U.S. Industrial Chemicals (Houston, TX, U.S.A.). Deionized water was obtained from a Milli-Q® filtering system (Millipore, Milford, MA, U.S.A.). Hydrochloric acid and Tris (hydroxyaminomethane) were ACS reagent grade from Fisher Scientific. Human plasma was obtained from Interstate Blood Bank (Memphis, TN, U.S.A.). The plasma was obtained from EDTA-treated blood. Human urine was obtained from male volunteers. Physiological saline was from Travenol Labs. (Deerfield, IL, U.S.A.). Pentafluorobenzoyl chloride (98%) was from Aldrich (Milwaukee, WI, U.S.A.). Pyridine (silylation grade) was from Pierce (Rockford, IL, U.S.A.). N,N-Bis(2-hydroxyethyl)glycine (bicine) and triethylamine (TEA) were from Eastman Kodak (Rochester, NY, U.S.A.).

Plasma and urine were extracted using 1-ml C_{18} Bond Elut® columns and Vac Elut® apparatus from Analytichem International (Harbor City, CA, U.S.A.). Solvent was removed with nitrogen evaporators from Organomation Assoc. (Northborough, MA, U.S.A.). The temperature of the water in the nitrogen evaporators was $40 \pm 5\,^{\circ}C$. Labindustries repipet dispensers from American Scientific Products (McGraw Park, IL, U.S.A.) were used for solvent and buffer additions during the extraction. Limited-volume inserts for the autosampler were from Sunbrokers (Wilmington, NC, U.S.A.).

Instrumentation

All analyses were performed with a Hewlett-Packard (HP) Model 5970B mass spectrometer and 5890 gas chromatograph (Palo Alto, CA, U.S.A.) An HP 7673A autosampler was used to introduce sample to the gas chromatograph. The MS and GC parameters and data integration were controlled by HP chemstation soft-

ware. The electronic transfer of data from the chemstation to an HP-1000E computer was accomplished through a direct link between the computers.

The chromatographic column was a fused-silica capillary column, 15 m×0.25 mm I.D., DB-5 bonded phase at 0.25 μm film thickness from J & W Scientific (Folsom, CA, U.S.A.). Helium was used for a carrier gas.

GC–MS conditions

Tuning the mass spectrometer was accomplished by analyzing the mass spectral quality control compound perfluorotributylamine according to the chemstation program "autotune". For 1-PP sample analyte determination two selected masses were repetitively monitored. For buspirone and its internal standard one ion was monitored. The masses that were selectively monitored were 358 for 1-PP, 362 for d4-1-PP and 277 for buspirone and 5-fluorobuspirone. During the analysis of plasma samples the electron multiplier voltage was set at autotune value (approximately 2000 V) and increased to 2600 V after 1-PP eluted. During urine sample analysis the electron multiplier voltage was set at 1400 V until 1-PP eluted, then increased to 2600 V. Detector tuning was only necessary after about 200 injections had been made.

The effluent from the chromatographic column was routed directly to the ion source. The interface between the GC oven and mass spectrometer was set at 270°C. Samples were introduced to the capillary column through a splitless injector that was set at 270°C. Following injection of a 5-μl sample the purge valve was closed for 1.5 min. While this volume is uncharacteristically large for splitless injection it had no adverse effects on the chromatography. The large volume was required to obtain the necessary sensitivity. The only problem that was encountered due to the large injection volume was that the system purge line needed to be cleaned occasionally. Initially, the oven temperature was held at 150°C for 1.5 min and brought up to 310°C at 25°C/min.

Reagents and standards

Tris buffer was prepared at 0.01 *M* and pH 7.2. Bicine buffer, 2 *M*, was prepared at pH 9.3 ± 0.1 using 6 *M* sodium hydroxide. The derivatization solution was prepared by diluting pentafluorobenzoyl chloride 1.5:2000 (v/v) with toluene. Solutions were prepared fresh monthly and refrigerated when not in use. The 0.11% (v/v) TEA in toluene solution was prepared by diluting TEA with toluene. Plasma and urine standards were prepared fresh daily using ethanolic solutions of each compound. Matrix standards were prepared in 13 mm×100 mm glass tubes.

Sample processing

Standard curves were prepared fresh daily and consisted of seven non-zero concentration points over the range 0.1–14 ng/ml for buspirone and 0.2–25 ng/ml for 1-PP in plasma. Urine standard curves consisted of six non-zero concentration points ranging from 0.1 to 14 ng/ml for buspirone and from 8 to 500 ng/ml for 1-PP. Plasma samples were transferred in 1.5-ml volumes to 13 mm×100 mm glass tubes. Urine samples were transferred in 2.0-ml volumes. Internal standard, 50 μl, was added as an ethanolic solution followed by vortexing for 30 s.

Physiological saline, 0.5 ml, was added and the samples were vortexed again for 30 s. Extraction columns were conditioned with 2 column volumes of methanol followed by 2 column volumes of Tris buffer. The vacuum was diverted to keep the columns from drying out and the samples were transferred to the columns. Vacuum was again applied allowing the samples to pass completely through. Each column was then washed with 2 column volumes of Tris buffer and 1 column volume of methanol–water (50:50) and dried under vacuum for 1 min. The vacuum was diverted, the manifold needles were wiped dry and a collection tray equipped with 10 mm × 75 mm glass tubes was inserted into the Vac Elut. Compounds were eluted with 1 ml of 1% (v/v) TEA in acetonitrile. The TEA–acetonitrile was evaporated with nitrogen and the 1-PP and d4-1-PP were derivatized using 50 μl of the derivatization solution and 0.5 μl of pyridine. The samples were vortexed for 30 s, kept at room temperature for about 5 min and evaporated to dryness. The samples were reconstituted in 20 μl of 0.11% (v/v) TEA in toluene, vortexed for 1 min and transferred to autosampler vials equipped with limited-volume inserts for automatic injection.

Urine samples were processed using the same procedure with the following minor exceptions: 0.4 ml of bicine buffer was added in place of the saline and 1% (v/v) TEA in methanol rather than TEA–acetonitrile was used to elute the compounds.

Validation procedures

The accuracy and precision of the method were assessed by analyzing replicates of quality control samples spiked at three different concentrations of each compound in each matrix. Quality control samples were prepared at concentrations in the upper and lower quartiles of the standard curves and at the lower limits of quantitation (LLQ) for each curve. The accuracy and precision at the LLQs were determined by spiking volumes of matrix from ten individuals (1.5 ml for plasma and 2.0 ml for urine) at the desired LLQ and analyzing them versus a standard curve. Intra-assay precision was determined by analyzing replicates of plasma and urine samples at two concentrations of each compound. Inter-assay precision and accuracy was determined by analyzing replicates of plasma and urine samples at two concentrations on three different days.

Stability of the compounds in the reconstituted residues was demonstrated by processing samples, combining the reconstituted residues and then injecting from the pool, in replicates of four, at six different time points.

RESULTS AND DISCUSSION

The electron-impact mass spectra for buspirone, 5-fluorobuspirone and the pentafluorobenzamides of 1-PP and d4-1-PP are shown in Figs. 1–4. The choice of which ions to selectively monitor was based on intensity of the ions, background noise and possible interfering peaks. The 277 a.m.u. ion was chosen for buspirone and 5-fluorobuspirone; the 358 a.m.u. ion was selected for derivatized 1-PP and the 362 a.m.u. ion was selected for d4-1-PP. The common ion at *m/z*

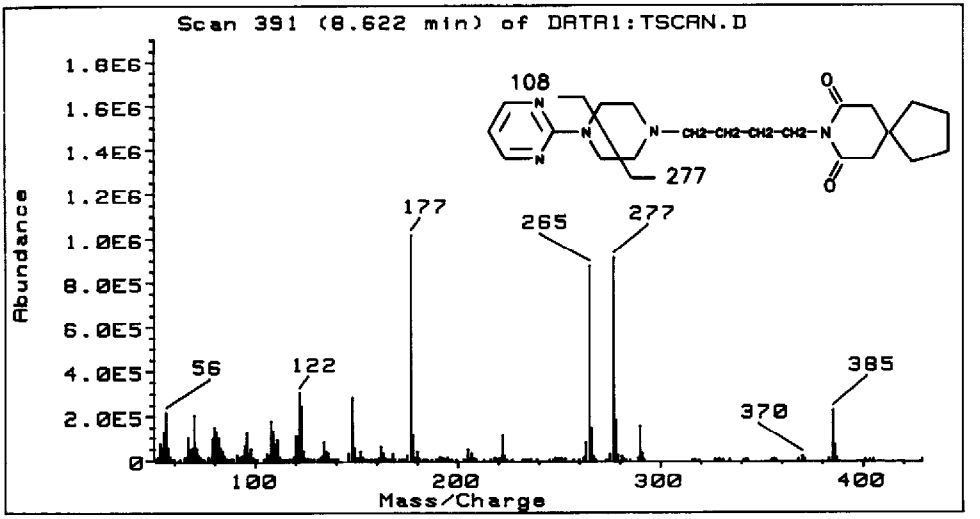

Fig. 1. Electron-impact mass spectrum and structure of buspirone.

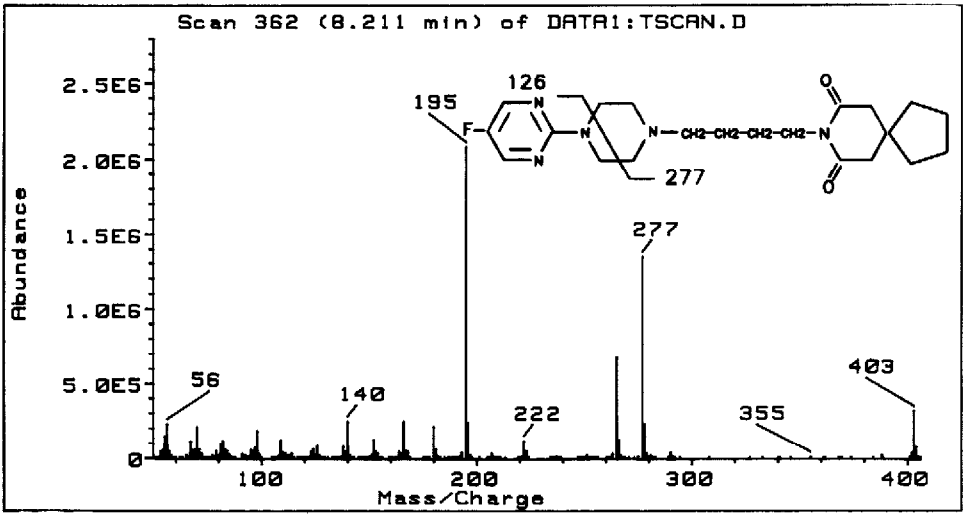

Fig. 2. Electron-impact mass spectrum and structure of 5-fluorobuspirone.

277 results from fragmentation as shown in Figs. 1 and 2. The *m/z* 358 and *m/z* 362 ions are the parent ions of derivatized 1-PP and d4-1-PP, respectively.

Representative chromatograms of processed plasma samples are shown in Figs. 5–7. No interferences from endogenous plasma constituents were present in the retention areas of the compounds of interest at the selected ions. The chromatography was also consistent, such that peak tailing did not become a problem until more than 200 injections had been made. To correct the tailing problem, when it did occur, approximately 30–60 cm of column was clipped from the inlet end of the gas chromatograph.

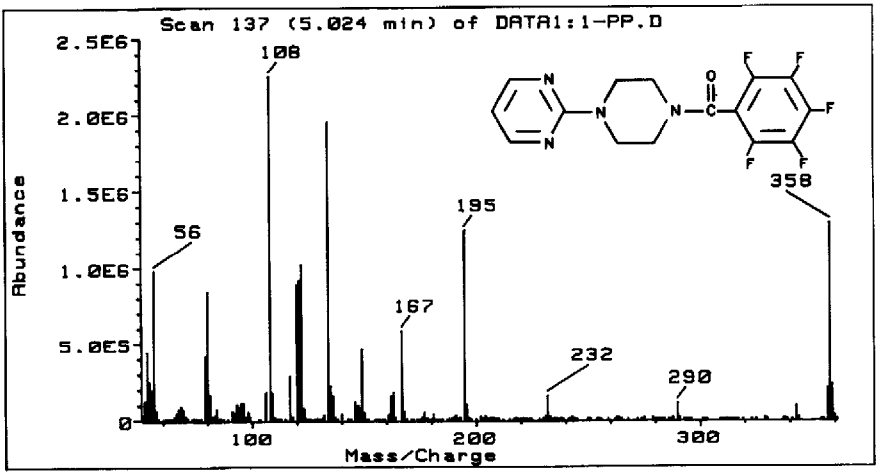

Fig. 3. Electron-impact mass spectrum and structure of derivatized 1-PP.

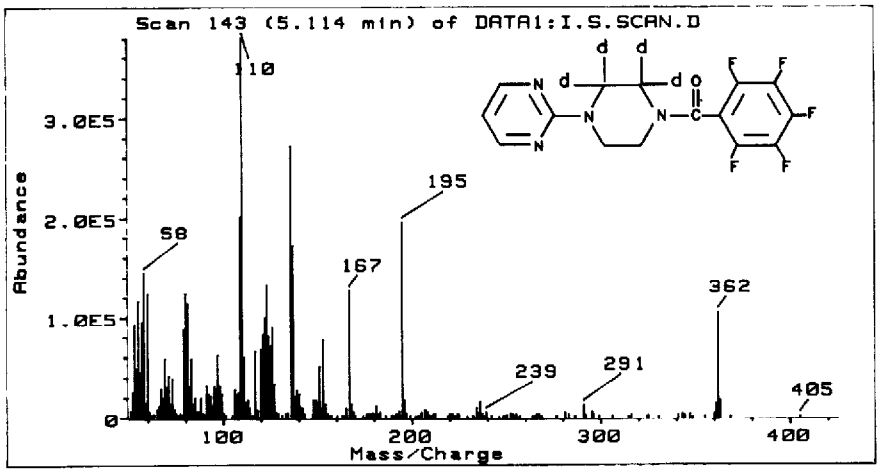

Fig. 4. Electron-impact mass spectrum and structure of derivatized d4-1-PP.

Concentrations of 1-PP in urine samples after administration of a 10-mg oral dose of buspirone are expected to reach approximately 1 μg/ml [1]. Thus, the linear range of the 1-PP standard curve was extended to 500 ng/ml in human urine. In order to prevent column and/or detector overload of the 1-PP derivatives it was necessary to incompletely derivatize the 1-PP and d4-1-PP and to run at a less than optimum electron multiplier voltage. Incomplete derivatization was accomplished by adding enough pentafluorobenzoyl chloride to derivatize only a small portion of the 1-PP and d4-1-PP that were present. Derivatization rates for the 1-PP compounds are equivalent making it possible to obtain a linear standard curve from 8 to 500 ng/ml based on the area ratio between the two derivatized compounds.

Buspirone and 1-PP intra-assay precision (relative standard deviation) and

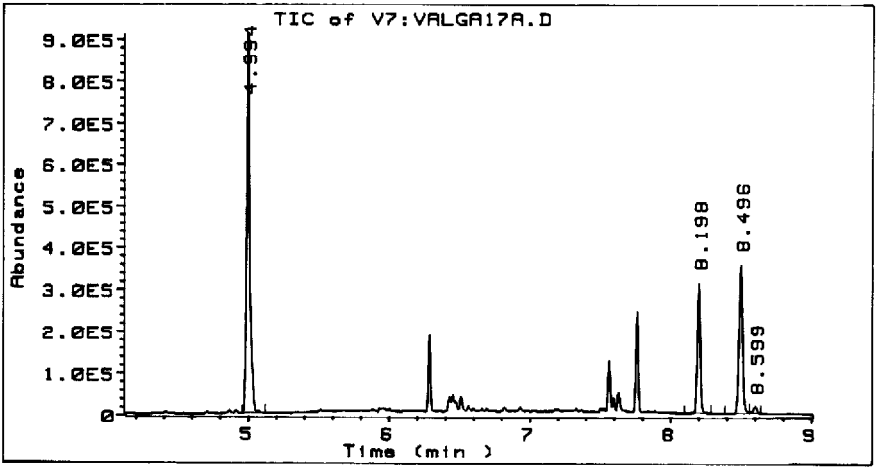

Fig. 5. Ion chromatogram of processed plasma standard spiked with 3 ng/ml d4-1-PP, 25 ng/ml 1-PP, 7 ng/ml 5-fluorobuspirone and 14 ng/ml buspirone. Peaks: 4.99 min = 1-PP and d4-1-PP; 8.19 min = 5-fluorobuspirone; 8.49 min = buspirone.

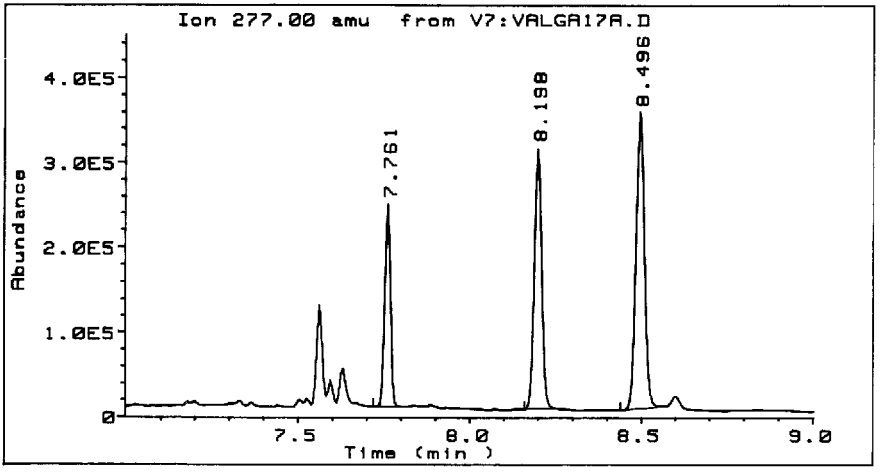

Fig. 6. Selected-ion chromatogram of plasma extract, spiked with 7 ng/ml internal standard and 14 ng/ml buspirone. Peaks. 8.19 min = 5-fluorobuspirone, internal standard; 8.49 min = buspirone

accuracies are shown in Table I, the corresponding data for urine are given in Table II.

Stability of buspirone in the derivatization reaction was addressed by determining the stability of the compounds in the reconstitution residues. The data in Fig. 8 show that the compounds are stable in urine and plasma residues for a minimum of 48 h.

During development of this method, a liquid–solid extraction was evaluated due to several advantages it would have over the existing liquid–liquid extraction procedures. The liquid–liquid plasma procedure involves several extractions, back extractions and wash steps followed by several reconstitution steps. Overall, the procedure requires approximately one to two days to process sixty samples. These procedures also require the use of potentially hazardous solvents, such as ben-

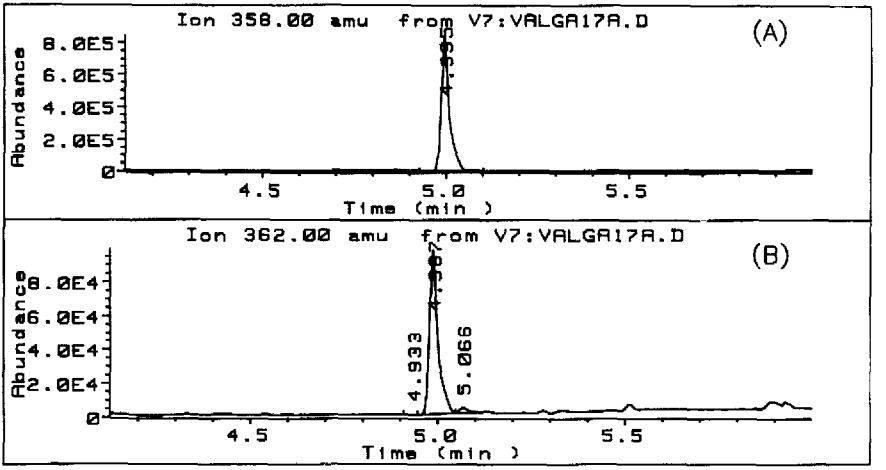

Fig. 7. Selected-ion chromatograms of plasma extract spiked with 3 ng/ml d4-1-PP and 25 ng/ml 1-PP. (A) Ion trace of *m/z* 358. (B) Ion trace of *m/z* 362. Peaks: 4.995 min = 1-PP; 4.987 min = d4-1-PP.

TABLE I

PRECISION AND ACCURACY OF PLASMA ASSAY

Test*	*n***	Buspirone			1-PP		
		Concentration (ng/ml)		R.S.D. (%)	Concentration (ng/ml)		R.S.D. (%)
		Nominal	Found		Nominal	Found	
Intra-assay	10	0.11	0.11	18.3	0.20	0.20	17.6
Intra-assay	9	1.11	0.96	11.5	4.07	3.55	2.7
Intra-assay	9	11.06	10.96	5.9	20.35	20.00	7.2
Inter-assay	9	1.11	0.96	11.6	4.07	3.55	2.7
	9	1 11	0.89	11.3	4.07	3.66	3.8
	9	1.11	0.91	9.5	4.07	3.39	15.0
	27(3)***	1.11	0.92	11.0	4.07	3.53	8.8
	9	11.06	10.96	5.9	20.35	20.00	7.2
	9	11.06	10.41	8 9	20.35	20.82	10.1
	9	11.06	10.39	4 1	20.35	20.07	6.0
	27(3)***	11.06	10.59	6.6	20.35	20.30	8.0

*See text for description of tests.
**Number of data points
***Combination of the three days above.

zene, toluene and diethyl ether. Solid-phase extraction reduces sample preparation time and limits exposure to organic aromatic solvents. A one-step liquid–solid extraction also reduces the possibilities of experimental error.

Originally an ion-exchange mechanism was chosen to extract the compounds from the matrices. Both buspirone and 1-PP contain tertiary amines, suggesting that the compounds should be retained on cation-exchange resins. While this

TABLE II

PRECISION AND ACCURACY OF URINE ASSAY

Test*	n**	Buspirone			1-PP		
		Concentration (ng/ml)		R.S.D. (%)	Concentration (ng/ml)		R.S.D. (%)
		Nominal	Found		Nominal	Found	
Intra-assay	10	0.22	0.17	31.4	8.01	7.68	12.1
Intra-assay	10	1.14	1.16	9.3	41.04	44.09	3.6
Intra-assay	10	12.21	13.56	3.3	440.45	451.32	2.6
Inter-assay	10	1.14	1.16	9.3	41.05	44.09	3 6
	10	1.14	1.11	6.3	41.05	43.16	2.1
	10	1.14	1.06	10.2	41.05	41.86	1.2
	30(3)***	1.14	1.11	9.12	41.05	42.36	2.8
	10	12.21	13.56	3.3	440.45	451.32	2.4
	10	12.21	12.98	3.3	440.45	448.34	2.4
	10	12.21	12.65	6.2	440.45	454.42	1.6
	30(3)***	12.21	13.06	4.4	440.45	453.28	2.3

*See text for description of tests.
**Number of data points.
***Combination of the three days above.

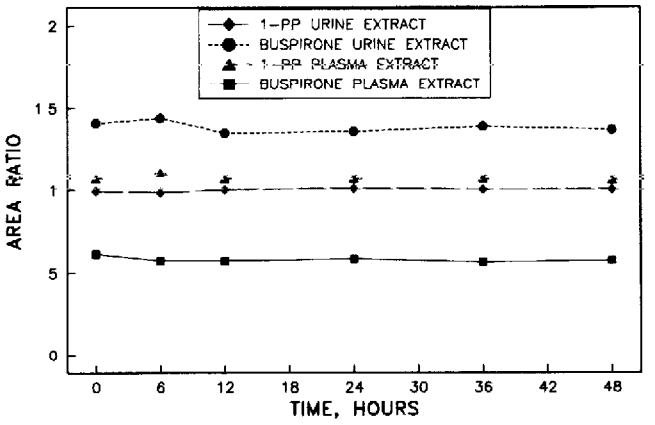

Fig. 8. Stability data for buspirone and 1-PP urine and plasma extraction residues.

type of extraction was possible for both compounds on separate resins, it was not possible using one resin. Extraction on C_{18} extraction columns was shown to retain both compounds exceptionally well. For efficient elution of both compounds, it was necessary to add 1% TEA to the elution solvent (acetonitrile, methanol). This led to the belief that this was not a true reversed-phase extraction, but more likely due to ion-exchange interactions between residual silanols on the unreacted surface of the silica support material and the compounds [5,6]. This type of interaction has been reported for a number of lipophilic amines [7].

Hydrochloric acid is produced from the derivatization of 1-PP. The hydrochlo-

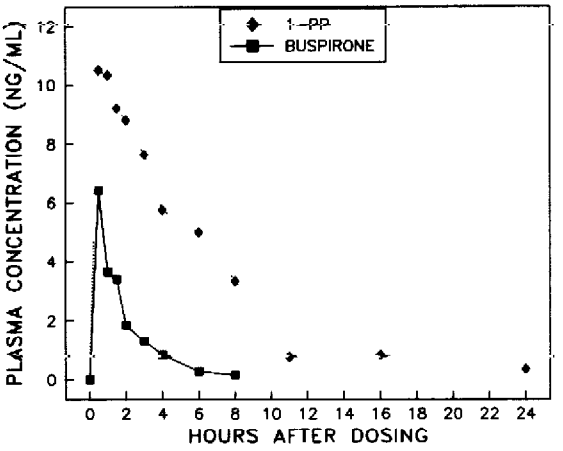

Fig. 9. Buspirone and 1-PP kinetics in a subject given a 20-mg Buspar oral tablet.

ric acid protonates the buspirone compounds making them insoluble in toluene. A 10% molar excess of TEA to residual hydrochloric acid was added to the reconstitution solvent to overcome the solubility problem.

The plasma method has been used to analyze clinical pharmacokinetic study samples. An example of time versus concentration data for one subject is shown in Fig. 9.

CONCLUSIONS

In conclusion, the GC–MS method has been shown to be reproducible, sensitive, precise, accurate and specific. It maintains the sensitivity of the previous compound-specific assays with considerable savings in plasma sample, analyst time and instrument time. The development and validation of a simultaneous assay for buspirone and 1-PP in human plasma and urine was accomplished.

REFERENCES

1 R.E. Gammans, E.H. Kerns, R.F. Mayol and J.A. LaBudde, Am. J. Med., 80 (1985) 41.
2 S. Caccia, M. Muglia, A. Mancinelli and S. Garatini, Xenobiotica, 13 (1983) 147.
3 R.E. Gammans, E.H. Kerns and W.W. Bullen, J. Chromatogr., 345 (1985) 285.
4 E.H. Kerns, W.W. Bullen and R.E. Gammans, unpublished results.
5 E. Papp and G. Vigh, J. Chromatogr., 259 (1983) 49.
6 R.N. Nikolov, J. Chromatogr., 286 (1984) 147.
7 B.A. Bidlingmeyer, J.K. Del Rios and J. Korpl, Anal. Chem., 54 (1982) 442.