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SIMULTANEOUS ANALYSIS OF IMIPRAMINE AND ITS METABOLITE DESIPRAMINE IN BIOLOGICAL FLUIDS

J. CYMERMAN CRAIG*, LARRY D. GRUENKE and TRONG-LANG NGUYEN
Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94143 (U.S.A.)

SUMMARY

A method for the simultaneous quantitation of imipramine and its N-demethylated metabolite desipramine at the nanogram level in a single gas chromatograph peak is presented, utilizing gas chromatography–mass spectrometry with selected ion recording. The assay is specific and quantitation is achieved using [$^2H_4$]imipramine as the internal standard.

The method involves the *in situ* methylation of desipramine with [$^2H_2$]formaldehyde and sodium borohydride to give [$^2H_2$]imipramine. Quantitation is then achieved by selected ion recording at m/z 280, 282 and 284, whence the ratio of the ion currents at 280/284 and at 282/284 gives the quantities of imipramine and desipramine respectively.

INTRODUCTION

The four most widely used tricyclic antidepressants are imipramine and amitriptyline and their N-demethylated analogues (and metabolites) desipramine and nortriptyline, and recent studies indicate a relationship between blood levels of these drugs and therapeutic response[1]. Because of wide interpatient differences in the hepatic elimination of tricyclic antidepressants, the lack of response observed in about 30–40 % of patients[2,3] may be due to non-therapeutic plasma levels, and sensitive and specific analytical methods for both the tertiary drugs and their secondary metabolites are required in order to measure their low (nanogram range) plasma or cerebrospinal fluid concentrations for pharmacokinetic studies with single doses or for clinical evaluation.

A number of drug assay procedures have been developed using thin-layer chromatography[4], spectrophotometry with fluorometric techniques[5–7], radioisotopic derivatization methods[8,9], and radioimmunoassay[10,11]. Gas chromatography (GC) with electron-capture or nitrogen-specific flame-ionization detectors has been used[12–14], as has high-performance liquid chromatography[15,16], but (like most of the other methods) is prone to problems of sensitivity and–or lack of specificity, mainly due to interference from coeluting compounds. The use of combined GC–mass spectrometry (MS) made it possible to obtain the required specificity by monitoring the identity of the peaks as they eluted from the gas chromatograph, and selected ion

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recording brought the sensitivity into the 5–10 ng/ml range using *e.g.* promazine as the internal standard for quantitation[17,18]. A further improvement was achieved by the use of the deuterated drugs as internal standards, whereby the internal standard was the actual drug itself in the form of an isotopic variant[19–22]. For maximum sensitivity and reproducibility of the assay, the secondary amine drugs had to be acylated to the N-acetyl, N-trifluoroacetyl, or N-heptafluorobutyryl derivatives[19–24].

This report describes a simple and convenient method for the simultaneous measurement of imipramine and desipramine in a *single* GC peak. The assay involves the *in situ* methylation of desipramine with [$^2H_2$]formaldehyde and sodium borohydride to give [$^2H_2$]imipramine. Quantitation is then achieved by selected ion recording at the molecular ions of imipramine and [$^2H_2$]imipramine, using [$^2H_4$]imipramine as an internal standard in a single GC peak. Imipramine N-oxide, a known metabolite of imipramine[25,26] is not reduced to imipramine by this methylation procedure. The same method has been applied successfully to the conversion of nortriptyline to amitriptyline to achieve the simultaneous measurement of these two drugs in a single GC peak[27].

EXPERIMENTAL

Standard solutions and reagents

Standard solution A (5.92 nmoles [$^2H_4$]imipramine per μl) was prepared by dissolving 9.5 mg of [$^2H_4$]imipramine · HCl (KOR Isotopes; 98% $^2H_4$, 2% $^2H_3$ by electron impact MS analysis of the molecular ion) in 5.00 ml of degassed water. This solution was stored in an amber hypovial (Pierce) under nitrogen for several months at 4°C without noticeable decomposition.

Standard solution B (1.022 nmoles per μl of imipramine (Ciba-Geigy) and 1.093 nmoles per μl of desipramine (Geigy Pharmaceuticals) was made by dissolving 32.4 mg of imipramine · HCl and 33.1 mg of freshly recrystallized desipramine · HCl in 100.0 ml of degassed water. This solution was stored in a standard hypovial under nitrogen at 4°C. After one month there was a noticeable loss (about 5%) of desipramine.

A 37% solution of [$^2H_2$]formaldehyde-d_2 in ^2H_2O was prepared by placing 195 mg of perdeuterioparaformaldehyde (98% ^2H, Stohler Isotope Chemicals) and 500 μl of ^2H_2O (99.8% D. Stohler Isotope Chemicals) in a 1-ml reaction vial and heating at 100°C for 3 h.

Extraction procedure

To a 2 ml of sample of human plasma in a silanized (dimethylchlorosilane) 8-ml glass culture tube fitted with a Teflon® lined screw cap was added 5.00 μl of standard solution A (27.6 nmoles of [$^2H_4$]imipramine), 4 drops of 1 *N* aqueous sodium hydroxide (to adjust the plasma to pH 10) and 3 ml of 1.5% isoamyl alcohol (aldehyde free) in *n*-pentane. The sample was extracted by Vortex mixing for 10 min. The organic layer was removed and a second extraction of the plasma with 3 ml of isoamyl alcohol–pentane (1.5:98.5) was carried out. The combined organic extracts were evaporated to dryness at room temperature under a stream of nitrogen and the residue was submitted to the methylation procedure described below.

Methylation procedure

To the above residue was added 250 μl of methanol and 3 μl of [$^2H_2$]formaldehyde solution (37% in ^2H_2O, 35 μmoles). After 5 min at room temperature, 3.2 mg of sodium borohydride (100 μmoles) in 150 μl of water was added. The mixture was allowed to stand for 15 min and was then extracted with 2 ml of dichloromethane. The dichloromethane extract was shaken for 2 min with 1.5 ml of 0.1 *N* sulfuric acid in order to back-extract the organic bases. The acidic aqueous layer was removed, brought to pH 10 with 10 drops of 15% KOH, and extracted with 1 ml of dichloromethane. The sample was reduced to 10 μl under a stream of nitrogen and was then ready for GC–MS analysis.

Extent of conversion of desipramine to imipramine

A mixture containing 27.5 μg (86.9 nmoles) of imipramine · HCl and 28.1 μg (29.9 nmoles) of desipramine · HCl was subjected to the methylation procedure described above and the product was analyzed by direct insertion electron impact MS. The peak height ratios of the ion currents at *m/z* 282 and *m/z* 280 were determined by five repetitive scans, and when corrected for the (M + 2) isotopic abundance from natural imipramine, gave the yield for the conversion of desipramine to [$^2H_2$]imipramine. Five separate methylation experiments were carried out in this manner and gave values of 95.5 ± 1% for the conversion of desipramine to [$^2H_2$]imipramine.

Extent of conversion of imipramine N-oxide to imipramine

To each of four tubes containing 1 ml of water was added 25 μl of standard solution A (148 nmoles of [$^2H_4$]imipramine) and 32.5 μg (97.6 nmoles) of imipramine N-oxide · HCl (Dumex) in 25 μl of water. The solutions were made alkaline to pH 10 with 2 drops of 1 *N* sodium hydroxide, extracted with 1 ml of dichloromethane, and the extracts were evaporated at room temperature under a stream of nitrogen. Samples 1 and 2 were immediately subjected to thin-layer chromatography (Analtech silica gel GF plates) using benzene–isopropanol–conc. aq. ammonium hydroxide (90:10:1) as solvent, while samples 3 and 4 were subjected to the methylation procedure described above and then to thin layer chromatography. The imipramine bands (UV visualization) were scraped off from each plate (R_F value 0.65 for imipramine and 0.04 for imipramine N-oxide) and extracted with methanol–dichloromethane (1:1). The extracts were reduced to 50 μl volume under a stream of nitrogen at room temperature and the ratio of imipramine to [$^2H_4$]imipramine determined by GC–MS by monitoring the ions at *m/z* 280 and *m/z* 284. Samples 3 and 4 showed a ratio 1.55 ± 0.08% higher than samples 1 and 2, indicating that 98% of the imipramine N-oxide had remained unaffected by the methylation procedure.

Instrumentation

An Infotronics 2400 gas chromatograph coupled to an AEI (Kratos) MS-12 mass spectrometer via a single-stage ceramic frit molecular separator was used for all GC–MS work. A PDP 8I computer using the DS-30 software was used to record magnetically scanned mass spectra and a specially constructed accelerating voltage alternator was used to obtain selected ion records[28]. For imipramine ions at *m/z* 284, *m/z* 282, and *m/z* 280 were recorded using either a 1.3 m × 2 mm I.D. glass column

with 2% Dexyl 400 on Gas-Chrom Q (100–120 mesh) at 235°C (retention time 4 min) or a 2 m × 2 mm I.D. glass column with 1% SP 2250 on Supelcoport (100–120 mesh) at 235°C (retention time 3.5 min). The mass spectrometer was operated at a nominal accelerating voltage of 8 kV with 500 μA of trap current, an ionization potential of 50 eV and a source temperature of 250°C.

RESULTS AND DISCUSSION

In the mild reductive methylation procedure, desipramine is first treated with [$^2H_2$] formaldehyde and subsequently (after 5 min) sodium borohydride is added (when the order of addition was reversed, the unreduced secondary amine was recovered). Using imipramine as internal standard, the time course of the reaction was followed by electron impact MS at the molecular ions and showed that the reductive methylation was essentially complete in 5 min; additional samples taken at 10, 15 and 30 min showed no further changes in the *m/z* 282 to *m/z* 280 ratio. The conversion to [$^2H_2$]imipramine proceeded to 95.5 ± 1% completion in trials. It was also found that the reaction could be carried out with the same efficiency by using sodium cyanoborohydride as reducing agent and methyl cyanide as solvent under the same experimental conditions.

In contrast, imipramine N-oxide remained unaffected by the methylation procedure, only <2% of imipramine N-oxide being converted to imipramine in duplicate experiments.

Table I gives the results for the standard durve obtained for the analysis of desipramine and imipramine in human plasma over the therapeutic range of concentrations (0–200 ng/ml). [$^2H_4$]Imipramine is used as the internal standard for both drugs and the analysis is thus carried out on a single GC peak resulting from a single injection. A least-squares analysis shows excellent linearity of the data with slopes close to unity and correlation coefficients of 0.997 and 0.998.

TABLE I

STANDARD CURVE DATA FOR THE SIMULTANEOUS ANALYSIS OF IMIPRAMINE AND DESIPRAMINE IN PLASMA

Mole ratio (%) (Concentration in ng/ml)		*Ratio (%) of the ion currents**	
Desipramine [$^2H_2$]/[$^2H_4$]	*Imipramine* [$^2H_0$]/[$^2H_4$]	*At m/z 282 to m/z 284***	*At m/z 280 to m/z 284****
0.00 (0.0)	0.00 (0.0)	1.38 ± 0.01	0.49 ± 0.01
0.37 (14)	0.34 (14)	1.64 ± 0.11	0.96 ± 0.12
0.91 (36)	0.85 (36)	2.20 ± 0.06	1.43 ± 0.09
2.74 (108)	2.56 (106)	4.43 ± 0.04	3.46 ± 0.06
5.48 (216)	5.12 (213)	6.99 ± 0.27	5.98 ± 0.42

* Average observed value (triplicate injections) obtained from 3 samples ± standard deviation between samples.

** Calculation of a regression line gives $y = 1.34 + 1.048x$ with $r^2 = 0.997$.

*** Calculation of a regression line gives $y = 0.56 + 1.072x$ with $r^2 = 0.998$.

TABLE II

STANDARD CURVE DATA FOR THE SIMULTANEOUS ANALYSIS OF IMIPRAMINE AND DESIPRAMINE, USING LABELED AND UNLABELED DERIVATIZATION*

Mole ratio (%) vs [$^2H_4$]-impramine		*Ratio (%) of the ion currents***		*Ratio of the ion currents**** (%)
Desipramine	*Imipramine*	*At m/z 282 to m/z 284 §, desipramine §§*	*At m/z 280 to m/z 284 §, imipramine §§§*	*At m/z 280 to m/z 284 §, total drug †*
0.00	0.00	2.10 ± 0.15	0.86 ± 0.10	0.83 ± 0.07
1.34	1.26	3.81 ± 0.28	2.54 ± 0.17	3.83 ± 0.12
3.36	3.14	6.27 ± 0.33	5.26 ± 0.11	8.81 ± 0.12
14.8	13.8	19.1 ± 0.75	19.7 ± 0.21	36.5 ± 0.46
36.9	34.5	47.3 ± 3.3	47.4 ± 0.51	92.9 ± 0.81

* Using mixtures of standard solutions containing 42 μg [$^2H_4$]imipramine as internal standard.
** Experiment using [$^2H_2$]formaldehyde in the methylation step.
*** Experiment using unlabeled formaldehyde in the methylation step.
§ Average value (triplicate injections) obtained from 3 samples ± standard deviation between samples.
§§ Calculation of a regression line gives $y = 1.96 + 1.220x$ with $r^2 = 0.9993$.
§§§ Calculation of a regression line gives $y = 0.94 + 1.349x$ with $r^2 = 0.9999$.
† Calculation of a regression line gives $y = 0.41 + 2.672x$ with $r^2 = 0.9998$.

Table II gives data for two standard curves run under identical conditions except that unlabeled formaldehyde was used in the methylation step for one standard curve while [$^2H_2$]formaldehyde was used for the other. It is possible to calculate from the known weight ratio of desipramine to imipramine the expected increase in the slope of the line obtained for imipramine alone when total drug is measured. The slope of the regression line for the last column in Table II is in fact 96% of the theoretical value. Thus the use of the methylation procedure to obtain total drug by a single GC measurement from a single injection is not likely to fall short of the true value by more than a few percent. It would be possible to avoid this error entirely by carrying out a GC measurement of imipramine *before* and *after* methylation, using the appropriate standard curves.

In conclusion, the method described in this paper allows the simultaneous determination of imipramine and desipramine in a single GC peak, using a simple and rapid *in situ* methylation reaction.

REFERENCES

1 L. F. Gram, *Clin. Pharmacokinetics*, 2 (1977) 237; and references cited therein.
2 A. H. Glassman, M. Shostak, S. J. Kantor and J. M. Perel, *Psychopharmacol. Bull.*, 14 (1975) 27.
3 A. H. Glassman, J. M. Perel, M. Shostak, S. J. Kantor and J. L. Fleiss, *Arch. Gen. Psychiatry*, 34 (1977) 197.
4 A. Nagy and L. Treiber, *J. Pharm., Pharmacol.*, 18 (1973) 599.
5 J. V. Dingell, F. Sulser and J. R. Gillette, *J. Pharmacol. Exp. Ther.*, 143 (1964) 14.
6 J. P. Moody, A. C. Tait and A. Todrick, *Br. J. Psychol.*, 113 (1967) 183.
7 J. P. Moody, S. F. Whyte and G. J. Naylor, *Clin. Chim. Acta*, 43 (1973) 355.
8 W. M. Hammer and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, 157 (1967) 503.

9 J. E. Wallace, H. E. Hamilton, L. K. Goggin and K. Blum, *Anal. Chem.*, 47 (1975) 1516.
10 S. Spector, N. L. Spector and M. P. Almeida, *Psychopharmacol. Communications*, 1 (1975) 421.
11 K. P. Maguire, G. D. Burrows, T. R. Norman and B. A. Scoggins, *Clin. Chem.*, 24 (1978) 549.
12 R. A. Braithwaite and B. Widdop, *Clin. Chim. Acta*, 35 (1971) 461.
13 O. Borgå and M. Garle, *J. Chromatogr.*, 68 (1972) 77.
14 N. Bailey and P. I. Jatlow, *Clin. Chem.*, 22 (1976) 1697.
15 I. D. Watson and M. J. Stewart, *J. Chromatogr.*, 132 (1977) 155; 134 (1977) 182.
16 F. L. Vandemark, R. F. Adams and G. J. Schmidt, *Clin. Chem.*, 24 (1978) 87.
17 A. Frigerio, G. Belvedere, F. De Nadai, R. Fanelli, C. Pantarotto, E. Riva and P. L. Morselli, *J. Chromatogr.*, 74 (1972) 201.
18 G. Belvedere, L. Burti, A. Frigerio and C. Pantarotto, *J. Chromatogr.*, 111 (1975) 313.
19 W. A. Garland, *J. Pharm. Sci.*, 66 (1977) 77.
20 M. Claeys, G. Muscettola and S. P. Markey, *Biomed. Mass Spectrom.*, 3 (1976) 110.
21 H. A. Heck, N. W. Flynn, S. E. Butrill, R. L. Dyer and M. Anbar, *Biomed. Mass Spectrom.*, 5 (1978) 250.
22 J. T. Biggs, W. H. Holland, S. Chang, P. P. Hipps and W. R. Sherman, *J. Pharm. Sci.*, 65 (1976) 261.
23 O. Borga, L. Palmer, A. Linnarson and B. Holmstedt, *Anal. Lett.*, 4 (1971) 837.
24 J. P. Dubois, W. Kund, W. Theobald and B. Wirz, *Clin. Chem.*, 22 (1976) 892.
25 A. Nagy and T. Hansen, *Acta Pharmacol. Toxicol.*, 42 (1978) 58.
26 W. Rapp, B. Noren and F. Pedersen, *Acta Psychiat. Scand.*, 49 (1973) 77.
27 L. D. Gruenke and J. C. Craig, in preparation.
28 L. D. Gruenke, J. C. Craig and D. M. Bier, *Biomed. Mass Spectrom.*, 7 (1980) 381.