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Short Communication

Sensitive method for the quantitation of nortriptyline and 10-hydroxynortriptyline in human plasma by capillary gas chromatography with electron-capture detection

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ABSTRACT

A method for the determination of nortriptyline and 10-hydroxynortriptyline concentrations in human plasma by capillary gas chromatography with electron-capture detection is described. The procedure requires 1.0 ml of plasma and uses maprotiline as an internal standard. The compounds are extracted from alkalinized plasma with hexane–2-butanol (98:2) and back-extracted into hydrochloric acid. The acid solution is then made basic and the compounds are re-extracted into *n*-butyl chloride. The extract is evaporated to dryness, derivatized with heptafluorobutyric anhydride, and analyzed by gas chromatography on a fused-silica capillary column coated with phenylmethyl silicone. The calibration curves for nortriptyline and 10-hydroxynortriptyline are linear in the ranges 3–40 and 7–90 μg/l, respectively, with coefficients of variation for within-day and between-day precision of less than 12%. The quantitation limits for nortriptyline and 10-hydroxynortriptyline are 1 and 3 μg/l, respectively. This procedure was used to analyze more than 1400 samples following sub-therapeutic doses of nortriptyline in human subjects. The assay was sufficiently sensitive for use in pharmacokinetic analysis.

INTRODUCTION

Nortriptyline is a tricyclic drug that is widely prescribed for the treatment of depression. It is extensively metabolized in humans to both *cis*- and *trans*-10-hydroxynortriptyline. A great deal of controversy exists regarding the routine monitoring of patient plasma concentrations in clinical practice. This is due to considerable variability in the steady state plasma concentrations of patients treated with a fixed dose [1]. However, sensitive assays are still required to obtain pharmacokinetic data and to evaluate drug formulations.

A wide variety of procedures have been developed for the determination of nortriptyline in plasma including immunoassay [2–8] and chromatographic [9–16] methods. The majority of these methods are designed to measure therapeutic (15–500 μg/l) [10] and/or toxic concentrations of nortriptyline. However, a few methods have been reported that have adequate sensitivity for quantitation of sub-therapeutic concentrations of nortriptyline for use in the determination of pharmacokinetic parameters. The most sensitive assays employ gas chromatography (GC) with detection by use of either chemical ionization mass spectrometry (MS) [17] or by use of a nitrogen–phosphorus selective detection (NPD). The method of Jones *et al.* [18] uses GC–NPD to achieve a detection limit of less than 0.5 ng nortriptyline using a 1-ml plasma sample. Their method involves an extraction of alkalinized plasma with hexane–2-butanol (98:2) followed by

back-extraction with 0.001 *M* hydrochloric acid. The aqueous layer is again alkalinized and the drug is extracted into butyl acetate and analyzed on a capillary GC–NPD system. An internal standard (protriptyline) is used in the quantitation of nortriptyline and its hydroxy metabolites. Although this procedure gives excellent chromatography, experience with this method indicates that the degree of sensitivity described by the authors is difficult to maintain on a day-to-day basis, presumably because of instability of the NPD system.

This paper describes a sensitive and robust method to determine plasma concentrations of nortriptyline and 10-hydroxynortriptyline following sub-therapeutic dosing. The method uses capillary GC with electron-capture detection (ECD). This detector is easier to use and less expensive than the chemical ionization mass spectrometer, yet offers a high degree of sensitivity and day-to-day reproducibility. The method has been used to analyze about 1400 human plasma samples from a bioequivalence study of Aventyl HCl (nortriptyline hydrochloride, Eli Lilly & Co.) formulations. A representative mean plasma curve obtained from this study is presented.

EXPERIMENTAL

Chemicals and reagents

Nortriptyline hydrochloride was obtained from Lilly Research Laboratories (Indianapolis, IN, USA) and 10-hydroxynortriptyline hydrogen maleate was obtained from Merck, Sharp and Dohme Research Labs. (West Point, PA, USA). The internal standard, maprotiline hydrochloride, was purchased from CIBA Pharmaceutical (Summit, NJ, USA). Heptafluorobutyric anhydride was purchased from Pierce (Rockford, IL, USA). The extraction solvents *n*-hexane and *n*-butyl chloride were distilled-in-glass (Burdick and Jackson, Muskegon, MI, USA). Reagent-grade hydrochloric acid and sodium hydroxide solutions were purchased from Fisher Scientific (Pittsburgh, PA, USA). Reagent-grade 2-butanol was obtained from Eastman Kodak (Kingsport, TN, USA). Distilled, deionized water was used in the preparation of all aqueous solutions.

Apparatus

Chromatographic separations were accomplished with a Hewlett-Packard Model 5890 gas chromatograph equipped with a split–splitless inlet, a ^{63}Ni electron-capture detector and an automatic sample injector (HP7673A). The fused-silica capillary column used was either a Supelco SP2250 (30 m × 0.25 mm I.D., 0.20 μm film thickness) or a Hewlett-Packard HP-17 (25 m × 0.20 mm I.D., 0.17 mm film thickness). The liquid stationary phase was cross-linked 50% phenylmethyl silicone. The carrier gas and make-up gas to the detector were argon–methane (95:5). The column head pressure and make-up gas were maintained at 100 kPa and 60 ml/min, respectively. The GC system was operated in the splitless mode (the purge was turned off at the time of injection and turned on at 0.4 min). The inlet and detector temperatures were maintained at 250 and 340°C, respectively. A temperature gradient was employed to elute the analytes. The gradient began at a column temperature of 200°C and was increased to 275°C over a 22-min period.

Calibration standards

Prepare a solution of nortriptyline in water to give 3.0 mg/l free base. Prepare a solution of the maleate salt of 10-hydroxynortriptyline in water to give 7.1 mg/l free base. Combine aliquots of these solutions and dilute with drug-free human plasma to prepare standards that contain 3–30 μg/l nortriptyline and 7–70 μg/l 10-hydroxynortriptyline. Store 1.0-ml aliquots of the plasma standards at −20°C until use. Prepare the internal standard solution by dissolving 1.0 mg of maprotiline hydrochloride in 10 ml of methanol. Dilute an aliquot of this solution with water to give an internal standard solution that contains 88 mg/l maprotiline base.

Sample preparation procedure

Transfer 1.0-ml aliquots of human plasma, samples or standards, into 15-ml silanized disposable glass screw-cap centrifuge tubes and add 100 μl of internal standard solution. Add 1 ml of 4 *M* sodium hydroxide and 8 ml of 2-butanol in hexane (2:98, v/v) to each tube. Cap the tubes tightly and shake vigorously for 2 min. Centrifuge at 1000 *g* for 5 min to separate the layers.

Transfer the organic layers to a second set of silanized 15-ml screw-cap centrifuge tubes and add 1.0 ml of 0.1 *M* hydrochloric acid. Cap the tubes and shake for 2 min. Centrifuge at 1000 *g* for 5 min. Aspirate the organic layers to waste. Add 0.5 ml of 4 *M* sodium hydroxide and 4 ml of *n*-butyl chloride to each tube. Cap the tubes and shake for 2 min. Centrifuge and transfer the *n*-butyl chloride layer to a clean set of silanized glass centrifuge tubes. Repeat the extraction with a second 4-ml aliquot of *n*-butyl chloride. Combine the *n*-butyl chloride extracts and evaporate the solvent to dryness under nitrogen. Dissolve the residue in 1 ml of hexane. Add 20 μl of heptafluorobutyric anhydride to each tube, cap tightly and place in a heating block (Tecam dri-block DB-3) at 65°C for 30 min. Evaporate the solvent to dryness under nitrogen and reconstitute in 100 μl of hexane. Inject 1 μl of each solution onto the GC column.

Calculations

A least-squares calibration curve was obtained by plotting concentration *versus* peak-height ratio (nortriptyline/internal standard or 10-hydroxynortriptyline/internal standard). The concentration of the compounds in each sample was determined from the peak-height ratios and the least-squares lines.

Clinical samples

Blood samples were obtained from volunteer subjects following a single 50-mg oral dose of Aventyl HCl (pulvules or liquid). The samples were collected by venipuncture over a 144-h period. Blood plasma was separated by centrifugation, and all plasma samples were stored at −20°C until analysis. Samples were analyzed in batches of twenty per day. Two plasma curves were prepared on each assay date. One set of calibration standards was injected prior to and one set was injected after the samples. Two well characterized control samples spiked with known amounts of nortriptyline and 10-hydroxynortriptyline were also assayed with the samples.

RESULTS

Fig. 1 shows a chromatogram for a human plasma sample that contains 22.4 μg/l nortriptyline and 78.6 μg/l 10-hydroxynortriptyline. The retention times for nortriptyline, 10-hydroxynortriptyline and the internal standard were 920, 990 and 1120 s, respectively.

The precision and accuracy of the method were established by analyzing five plasma pools containing different concentrations of nortriptyline and 10-hydroxynortriptyline (four or five replicates per day) on each of three days. The results are summarized in Table I. Plasma pools containing nortriptyline and 10-hydroxynortriptyline at concentrations of 1.2 and 2.8 ng/ml, respectively, have coefficients of varation (C.V.) less than 30% and are considered to be the quantitation limits of the assay.

DISCUSSION

The assay described here uses heptafluorobutyric acid derivatization and GC–ECD in order to achieve a low quantitation limit of nortriptyline and 10-hydroxynortriptyline. The 10-hydroxynortriptyline compound is actually detected as 10,11-dehydronortriptyline because loss of water occurs under the derivatization conditions [19].

The precision of the assay is comparable to that of other GC procedures for nortriptyline and 10-hydroxynortriptyline. The lower quantitation limits for the method are comparable to other ultra-sensitive assays which employ GC–MS [17] and GC–NPD [18]. The GC–ECD method described here has the advantage of ruggedness over the GC–NPD procedure and employs less expensive equipment than GC–MS.

The utility of the method was demonstrated by the results obtained from the analysis of plasma samples from volunteer subjects following single 50-mg oral doses (pulvules or liquid) of nortriptyline. Representative pharmacokinetic curves for mean (n = 19) plasma concentrations of nortriptyline and 10-hydroxynortriptyline from one solid dosage form are shown in Fig. 2. Mean peak plasma concentrations observed in the study were approximately 25 and 40 μg/l for nortriptyline and 10-hydroxynortriptyline, respectively.

The selectivity of the assay was excellent as, to

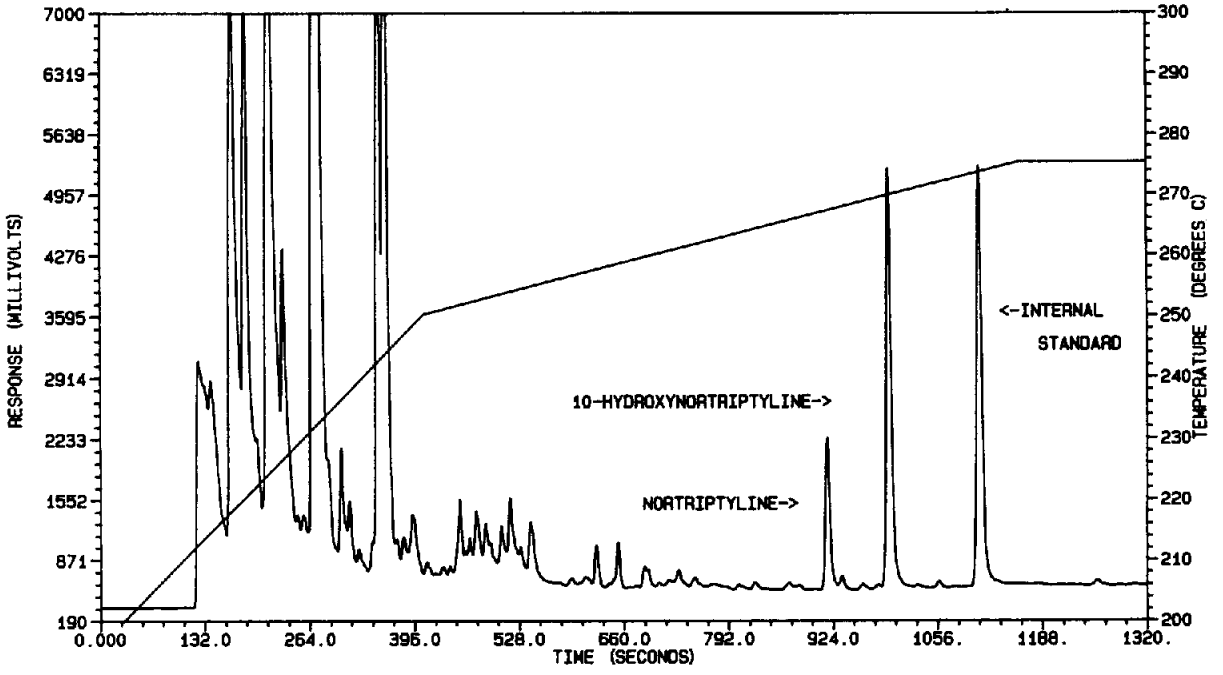

Fig. 1. Chromatogram of a plasma sample drawn 5 h after a single 50-mg dose of Aventyl HCl. Concentrations of nortriptyline and 10-hydroxynortriptyline are 22.4 and 78.6 μg/l, respectively.

TABLE I

PRECISION DATA

Drug	Concentration (μg/l)			*n*	C.V. (%)
	Added	Mean measured	S.D.		
Within-run					
Nortriptyline	1.2	1.1	0.3	4	24.6
	6.0	5.8	0.2	4	3.7
	18.0	17.8	0.4	5	2.3
	37.5	33.6	1.1	4	3.2
10-Hydroxynortriptyline	2.8	2.5	0.6	4	23.7
	14.2	13.3	1.4	4	10.8
	42.5	42.6	2.0	5	4.6
	88.5	84.2	2.4	4	2.8
Overall[a]					
Nortriptyline	1.2	1.0	0.3	13	27.0
	6.0	6.0	0.6	14	10.7
	18.0	17.8	1.2	15	6.9
	37.5	32.8	2.6	14	7.8
10-Hydroxynortriptyline	2.8	2.8	0.8	13	28.5
	14.2	13.2	1.5	14	11.6
	42.5	41.6	2.3	15	5.6
	88.5	81.8	4.9	11	6.0

[a] Three-day characterization.

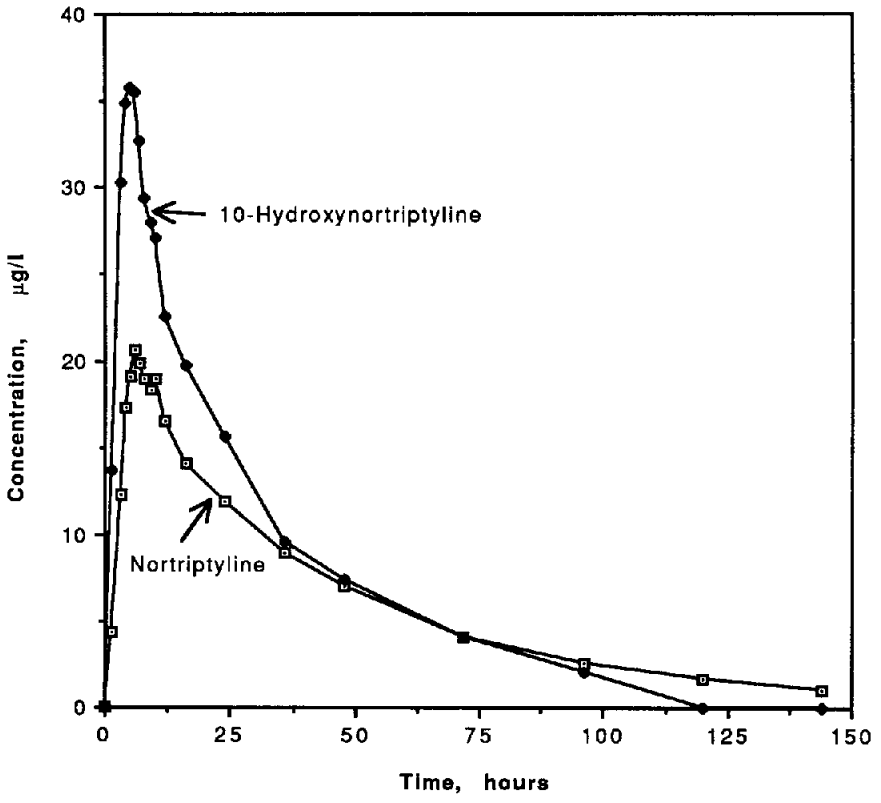

Fig. 2. Pharmacokinetic curves for mean (n = 19) plasma concentrations of nortriptyline and 10-hydroxynortriptyline following single 50-mg doses of Aventyl HCl pulvules.

our knowledge, no endogenous components or other drugs interfere with the assay (including amitriptyline and doxepin). The selectivity resulted from the highly specific combination of heptafluorobutytic acid derivatization and ECD.

In conclusion, the present assay is shown to be sufficiently sensitive for use in pharmacokinetic studies following single sub-therapeutic oral doses of nortriptyline. The method is relatively simple and rugged. In addition, electron-capture detectors require little maintenance and, unlike GC–chemical ionization MS, a high degree of operator training is not required.

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