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Short Communication

Measurement of dothiepin and its major metabolites in plasma by high-performance liquid chromatography

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ABSTRACT

This paper describes a reversed-phase high-performance liquid chromatographic method which will simultaneously measure dothiepin and its three major metabolites (northiaden, northiaden-S-oxide and dothiepin-S-oxide) in plasma using trimipramine as internal standard. Sample preparation involved a basic extraction using diethyl ether followed by an acid back-extraction. The method we report is linear over the range 50–1000 ng/ml (r = 0.999), for all analytes. Total imprecision is less than 11% (coefficient of variation) and accuracy is greater than 94% (n = 20). Recovery of analytes varied considerably from 51.7% for northiaden-S-oxide to 90.2% for dothiepin-S-oxide.

INTRODUCTION

Dothiepin is a tricyclic antidepressant resembling amitriptyline in structure and is prescribed to treat major depressive disorders, particularly in the elderly or where there is underlying heart disease [1]. While patients on dothiepin therapy report fewer and less serious side-effects than those on other antidepressants [1], dothiepin remains a serious concern in patients who overdose on the drug [2].

Dothiepin is metabolised extensively in animals and man [3] and together with its three major metabolites, northiaden, northiaden-S-oxide and dothiepin-S-oxide, it is present in measurable concentrations in plasma after oral dosing [4]. It has been suggested that these metabolites are also pharmacologically active and therefore should be monitored with dothiepin in clinical studies [5].

Relatively few assay methods have been re-

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ported in the literature for dothiepin and its metabolites in serum or plasma. Some require the use of mass spectrometry [5], some have complex extraction procedures [4], some are not designed for complex matrices and low concentrations required for serum assays [6]. Brodie *et al.* [7] reported a high-performance liquid chromatographic (HPLC) method which involved a basic diethyl ether extraction which was suitable for the measurement of only dothiepin and northiaden in serum and plasma. Kawahara *et al.* [8] reported an HPLC method for the analysis of four dothiepin metabolites, however, the method is restricted to urine analysis and is unsuitable for determination of the parent compound. None of these methods enables simultaneous assay of dothiepin and all three of its metabolites. We report here an HPLC method which enables the simultaneous determination of dothiepin and its three major metabolites.

EXPERIMENTAL

Chromatographic system

The HPLC system (Millipore, Waters Chromatography Division) consisted of a Model 590 pump, a 710B autoinjector, a Model 490 variable-wavelength UV detector, a temperature control module with column oven, and a Model 730 data module. The HPLC column was a cyano column (250 mm × 4.6 mm I.D., 5 μm, Regis, Morton Grove, IL, USA), maintained at 50°C. The mobile phase consisted of 500 ml of acetonitrile, 300 ml of buffer (0.01 *M* dipotasium hydrogen orthophosphate, pH 7.0) and 200 ml of methanol delivered at 1.7 ml/min. The eluent was monitored at a wavelength of 240 nm.

Materials

All reagents used were of AR grade and all solvents of HPLC grade, with the exception of diethyl ether which was nanograde. Reagent-grade deionised water was obtained from a Milli-Q system (Millipore, Milford, MA, USA). Dothiepin, dothiepin-S-oxide, northiaden and northiaden-S-oxide were all generously supplied by Boots (Nottingham, UK).

Internal standard

The internal standard was trimipramine maleate, prepared as a stock solution in methanol of 7 mg/50 ml concentration. A working internal standard solution was prepared by dilution (1:100) of the stock in deionised water just prior to commencing extraction of each batch of samples.

Methods

To extract dothiepin and metabolites, 1 ml of plasma from standard, control or sample was pipetted into respective 15-ml conical glass centrifuge tubes. Working internal standard (0.5 ml), 1 *M* NaOH (1 ml), and diethyl ether (10 ml) were added. PTFE-lined screw caps were fitted and the tube contents mechanically rotated for 20 min to effect mixing. After centrifugation (5 min, 850 *g*) to separate the phases, the upper (organic) layer in each tube was transferred to respective tubes containing 200 μl each of 0.01 *M* HCl. The contents of the tubes were vortex-mixed for 1 min and then separated by centrifugation (5 min, 850 *g*) into organic and aqueous phases. The lower (acid) phase was transferred to respective vials and 100-μl volumes were injected into the HPLC system.

Imprecision of the assay was assessed using drug-free plasma supplemented with 50 and 1000 ng/ml dothiepin and metabolites. These samples were assayed in quadruplicate on each of five days, and between-day and within-day imprecision were assessed using a one-way analysis of variance approach in which all the data were used to estimate variability [9].

An estimate of accuracy was gained by comparing the mean assayed concentrations from the imprecision experiment with their target values as determined by the weighed in amount of each analyte. These samples for imprecision determination had been prepared independently of the standards, using plasma from a different source and independent weighings for all of the analytes. Recoveries were assessed at 50 ng/ml by comparing the peak height of injections of stock compounds in 0.01 *M* HCl with peak heights obtained from analysis of each of five replicates of

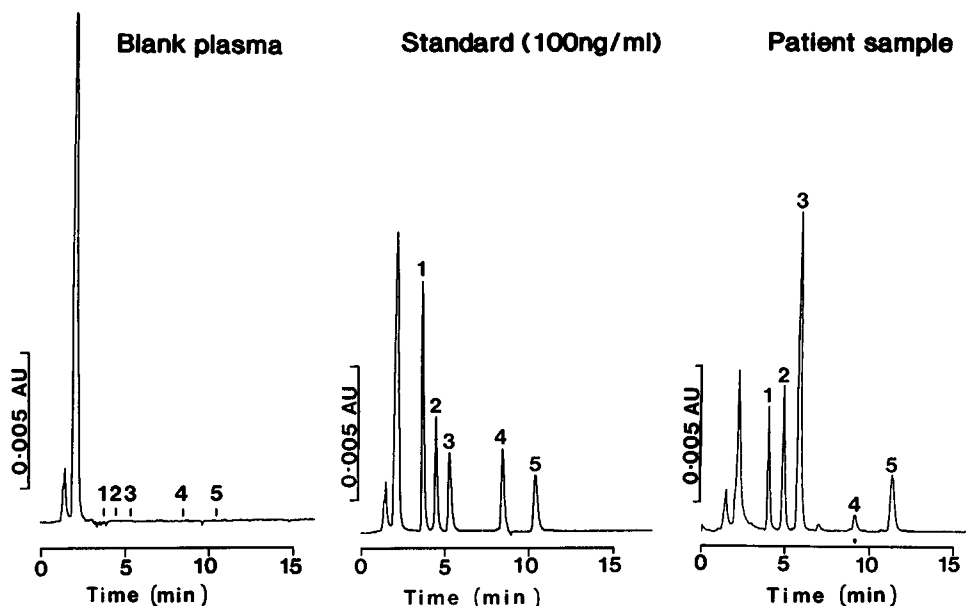

Fig. 1. Typical chromatograms of blank plasma, a 100 ng/ml standard and a patient sample. Peaks: 1 = trimipramine (internal standard); 2 = dothiepin; 3 = dothiepin-S-oxide; 4 = northiaden; 5 = northiaden-S-oxide. Retention times are 3.8, 4.6, 5.5, 8.8 and 10.7 min, respectively. Analyte concentrations in patient sample of 256 ng/ml for peak 2, 837 ng/ml for peak 3, 41 ng/ml for peak 4 and 203 ng/ml for peak 5.

prepared plasma standards of dothiepin and its metabolites.

Linearity of assay response was determined by least-squares regression of the peak-height ratio of analyte to internal standard on weighed in concentration of analyte in plasma, from 50 to 1000 ng/ml.

RESULTS AND DISCUSSION

Fig. 1 shows typical chromatograms obtained with this method and indicates the lack of interference form endogenous compounds in plasma and the adequate separation of the compounds of interest. Satisfactory assay reproducibility is

TABLE I

IMPRECISION OF THE ASSAY

Between-day (B), within-day (W) and total (T) coefficients of variation for dothiepin and its metabolites at spiked concentrations of 50 and 1000 ng/ml are given. Partitioning of variances was performed as per Krouwer and Rabinowitz [9] on samples assayed in quadruplicate on each of five days.

Compound	Concentration spiked (ng/ml)	Coefficient of variation (%)		
		B	W	T
Dothiepin	50	2.0	2.7	3.4
	1000	1.0	1.7	1.9
Dothiepin-S-oxide	50	3.7	4.5	5.8
	1000	5.7	4.0	7.0
Northiaden	50	1.5	3.3	3.6
	1000	5.5	2.7	6.1
Northiaden-S-oxide	50	8.6	5.2	10.1
	1000	5.1	4.4	6.8

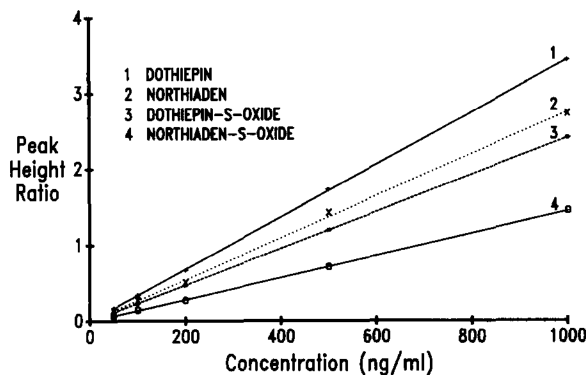

Fig. 2. Typical standard curves. Slope, intercept and correlation coefficient for dothiepin and each of its metabolites are as follows: dothiepin, 0.00343, −0.00428, 0.999; dothiepin-S-oxide, 0.00242, −0.00747, 0.999; northiaden, 0.00276, −0.00668, 0.999; northiaden-S-oxide, 0.00145, −0.00812, 0.999.

TABLE II

ACCURACY OF THE ASSAY

Mean results of twenty assays of dothiepin and its metabolites at spiked concentrations of 50 and 1000 ng/ml are given. Values in parentheses are concentrations expressed as a percentage of the spiked concentrations.

Compound	Concentration found (ng/ml)	
	50 ng/ml	1000 ng/ml
Dothiepin	49.95 (99.9)	978.5 (97.9)
Dothiepin-S-oxide	47.79 (95.6)	943.2 (94.3)
Northiaden	49.69 (99.4)	962.4 (96.2)
Northiaden-S-oxide	49.76 (99.5)	956.9 (95.7)

demonstrated by the data in Table I where between-day, within-day and total coefficients of variation (C.V.) are presented for plasma concentrations of 50 and 1000 ng/ml for each compound. The total C.V.s in all cases are equal to or less than 7% with the exception of northiaden-S-oxide at 50 ng/ml which has a total C.V. of 10.1%.

Standard curves across the 50–1000 ng/ml range for dothiepin and each of its metabolites are presented in Fig. 2 and indicate excellent linearity with correlation coefficients of 0.999, in all cases. Accuracy of the assay of dothiepin and its metabolites is also satisfactory with mean results of 94.3–99.9% across a twenty-fold plasma concentration range (Table II).

The extraction of dothiepin-S-oxide with diethyl ether from alkalinized plasma was more efficient than for the remaining analytes. Mean ± S.D. recoveries (n = 5, 50 ng/ml) for dothiepin, dothiepin-S-oxide, northiaden and northiaden-S-oxide were 63.1 ± 9.5, 90.2 ± 6.6, 58.8 ± 13.3 and 51.7 ± 9.4%, respectively.

The method described provides a selective, accurate and precise means of assaying dothiepin and its metabolites in plasma. The assay is valuable for gaining a further insight into the relationship between therapeutic response and plasma concentrations of dothiepin and its metabolites [10,11].

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