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Note

Rapid high-performance liquid chromatographic method for the determination of dapsone and monoacetyldapsone in biological fluids

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Dapsone (DDS, diaminodiphenylsulfone) is an important drug in the treatment of leprosy (Fig. 1). In humans it is mainly metabolised to monoacetyldapsone (MADDS). The determination of the MADDS:DDS ratio has been found to be

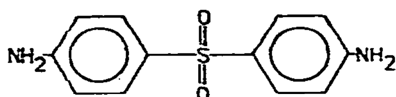

Fig. 1. Chemical structure of dapsone.

useful for the characterisation of the acetylator phenotype [1]. For this purpose, for therapy control and for biopharmaceutical studies, a rapid and reliable determination of DDS and MADDS in biological fluids is needed.

Until the nineteen-seventies blood concentrations were measured with spectrophotometric methods. A well-known colorimetric method for aromatic amines was published in 1939 by Bratton and Marshall [2]. This method is frequently used for the determination of sulfones and many minor modifications of it have been described [3, 4]. Fluorimetric methods have been described since 1968 [5, 6]. They require prior extraction with, for example,

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ethyl acetate or dichloroethane. These methods are seldom specific and are more erratic than modern chromatographic methods. Thin-layer and paper chromatography gave the first impetus in this field [7]. A gas chromatographic method with electron-capture detection has been described by Burchfield and co-workers [8, 9]. The method is laborious since derivatisation appeared to be necessary. Much easier and more quick to perform are the high-performance liquid chromatographic (HPLC) methods. Ion-exchange HPLC methods have been described by Murray and co-workers [10, 11], Orzech et al. [12] and Ribi et al. [13]. Reversed-phase methods have been reported by Mannan et al. [14] and recently by Carr et al. [1]. UV absorption and fluorimetry were used for the detection.

All HPLC methods were preceded by an extraction procedure. In this paper we present a rapid, non-extractive absolute method as a modification of the method of Carr et al. Proteins are removed by precipitation with perchloric acid. The supernatant is neutralised by potassium carbonate. Excess perchloric acid is thus precipitated as potassium perchlorate.

MATERIALS AND METHODS

A 0.5-ml serum sample was pipetted into a tube containing 50 μl of 70% perchloric acid and mixed for 30 sec on a whirl-mixer. Then 50 μl of a saturated potassium carbonate solution and 400 μl of a mixture containing 5% acetic acid, 65% acetonitrile and finally aqueous solutions of standards were added and mixed. The water:acetonitrile ratio of the sample equalled that in the mobile phase. The potassium perchlorate was precipitated by centrifuging for 10 min. An aliquot (100 μl) of the supernatant was injected onto the column with a syringe.

Analyses were performed using a Waters Assoc. (Milford, Mass., U.S.A.) Model M-6000 A pump and Model 440 absorbance detector. A reversed-phase system was used, consisting of a μBondapak C_{18} column (30 cm × 4 mm I.D.) with a particle size of 10 μm (Waters Assoc.). The mobile phase solvent system, acetonitrile—1.5% (v/v) acetic acid (26:74), was delivered at a rate of 2 ml/min at room temperature. Absorbance was monitored at 280 nm. The detector was operated at a sensitivity of 0.05 a.u.f.s. Peak heights were used for quantitation.

The method was compared with a non-extractive internal standard method. As an internal standard monopropionyldapsone (MPD) was used as recommended by Carr et al. [1]. MPD was synthesised, without using dapsone as an intermediate, by reacting 4′-amino-4-nitrodiphenylsulfone with propionylchloride in pyridine and hydrogenation. Before use, purity and identity were checked by HPLC, NMR and infrared spectroscopy. No measurable amounts of dapsone were present. MPD was dissolved in water and added to the serum. Then acetic acid and acetonitrile were added. The MPD concentration in the sample was then 2 μg/ml. Apart from the internal standard the procedure was the same as that described above.

The method was also compared with the extractive, internal standard method described by Carr et al. [1].

Calibration curves

Normal plasma and serum were spiked with known amounts by mixing concentrated standard solutions with serum (5:95) of DDS and MADDS over the range 0.2—5 µg/ml and determined by the three methods described above. To define the standard curves for HPLC measurements, the peak heights, or the ratios of the DDS or MADDS peak heights to the heights of the internal standard peaks, were plotted against the DDS or MADDS concentrations. Regression coefficients and y-intercepts could be calculated by linear regression (least-squares method).

RESULTS

A representative chromatogram of spiked serum is shown in Fig. 2. The retention times for DDS, MADDS and MPD were 4.8—4.9, 5.6—5.7 and 9.4—9.5 min, respectively. Results of the calibration curves are given in Table I.

The within-run variation of the non-extractive absolute method could be determined by repetitive injection of samples of 1 and 3 µg/ml and is presented as the variation coefficient in Table II. The between-run variation was determined by injecting, on 30 consecutive days, spiked standard serum samples of 1, 3 and 4 µg/ml (freshly prepared daily). The results are presented in Table III. The within-run variation makes up the error of the method, the

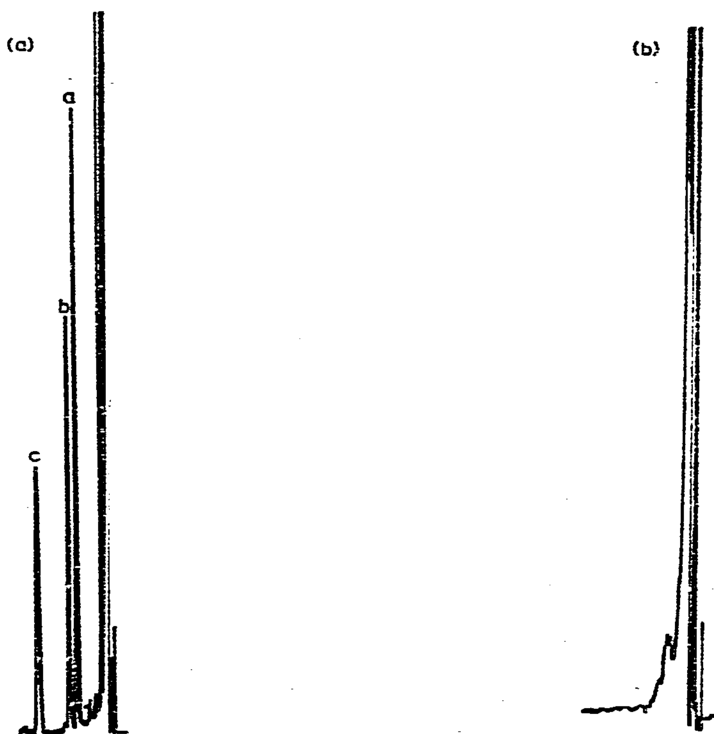

Fig. 2. (a) A representative HPLC chromatogram of human serum containing: a, dapsone (DDS), 4 µg/ml; b, monoacetyldapsone (MADDS), 4 µg/ml; c, internal standard (MPD), 2 µg/ml. (b) A representative blank sample.

TABLE I

COMPARISON OF THE METHODS BY REGRESSION COEFFICIENTS AND y-INTERCEPTS

The results represent an average of m serum standard curves, obtained from serum or plasma from different volunteers. Each curve was calculated from n points, representing different concentrations.

Method*	Regression coefficient (mean ± var. coeff.)	y-Intercept (mean ± var. coeff.)	Correlation coefficient (mean ± S.D.)		
DDS:					
n.e. abs.	35.4 (± 3.1%)	7.0 (± 21.4%)	0.999 ± 0.000	n = 5, m = 4	P<0.01
	35.3	7.3	0.998	n =20, m = 1	P<0.01
n.e. + i.s.	0.559 (±3.6%)	0.121 (± 22.3%)	0.998 ± 0.001	n = 5, m = 4	P<0.01
	0.573	0.055	0.976	n =20, m = 1	P<0.01
e + i.s.	0.501 (± 7.4%)	0.425 (± 33.4%)	0.995 ± 0.004	n = 6, m = 4	P<0.01
	0.517	0.396	0.983	n =24, m = 1	P<0.01
MADDS:					
n.e. abs.	26.5 (± 5.3%)	0.82 (± 89.0%)	0.999 ± 0.000	n = 4, m = 4	P<0.01
	26.3	0.8	0.996	n =16, m = 1	P<0.01
n.e. + i.s.	0.425 (± 1.7%)	0.019 (± 94.7%)	0.999 ± 0.001	n = 4, m = 4	P<0.01
	0.423	0.215	0.999	n =16, m = 1	P<0.01
e. + i.s.	0.391 (± 3.8%)	−0.000	0.997 ± 0.003	n = 4, m = 6	P<0.01
	0.396	−0.001	0.997	n =25, m = 1	P<0.01

*n.e. abs. = non-extractive absolute method; n.e. + i.s. = non-extractive method with internal standard; e. + i.s. extractive method with internal standard.

TABLE II

COMPARISON OF THE METHODS BY THE WITHIN-RUN VARIATION

n = 10. Abbreviations as in Table I.

Conc. (μg/ml)	n.e. abs.		n.e. + i.s.		e. + i.s.	
	DDS	MADDS	DDS	MADDS	DDS	MADDS
1	4.8%	8.8%	3.5%	7.3%	17.3%	2.2%
3	3.4%	5.8%	3.4%	5.7%	3.7%	2.7%

TABLE III

BETWEEN-RUN VARIATION OF THE NON-EXTRACTIVE ABSOLUTE METHOD

(μg/ml)	DDS	MADDS	*n*
1	12.0%	11.3%	61
3	7.8%	10.2%	26
4	10.6%	8.4%	25

between-run variation makes up both the error of the method and the "spike error".

The recovery of the extraction procedure could be calculated from a plot of the absolute method against an extractive method with external standard, being the regression coefficient. The recovery with diethyl ether was essentially complete (r.c. = 1.03). The standard deviation of the regression line was S_r = 0.36 and the standard deviation of the regression coefficient was S_a = 0.12. It can be calculated that at a level of $P \leq 0.05$ the recovery of 100% was significantly different from recovery of 85% and lower.

DISCUSSION

This HPLC method is easy and quick to perform; it involves no extraction and can be successfully performed with 0.5 ml of serum, or even smaller samples.

We could demonstrate that the results of both DDS and MADDS determinations with the three methods are well-fitted by a straight line ($P<0.01$) over the range 0.2–5 μg/ml. The extraction method with internal standard has been published by Carr et al. [1]. The authors did not mention a *y*-intercept. We found a large *y*-intercept in all of the calibration curves using this method, so we cannot confirm their results. The mean *y*-intercept was significantly different from zero ($P<0.01$) and significantly different (larger) from the mean *y*-intercept as calculated for the non-extractive method with internal standard ($P<0.01$). Thus the advantages of the non-extractive DDS determination methods are a smaller *y*-intercept, a better precision and time gained by deleting the extraction procedure.

There is no difference in the precision and *y*-intercept between the non-extractive methods with or without internal standard as compared by the variation coefficients and *y*-intercept versus regression coefficient ratio.

The variation coefficient of the regression coefficient makes up the error of the method and the "spike error". The within-run variation of the non-extractive DDS determination methods was smaller than that of the extraction method (Table II). The difference between the non-extractive internal standard method and the absolute method was not significant. Advantages of the absolute method are the performance time (a simpler calculation), and the fact that no interference can occur with an internal standard peak.

The MADDS regression lines are quite accurate. The *y*-intercepts are not significantly different from zero ($P<0.01$). The precision of the three methods is good and they are not significantly different from each other.

Thus, from comparing the non-extractive methods and the extractive method, it can be concluded that the non-extractive methods are to be preferred for the determination of DDS in terms of precision, accuracy, chance of interfering peaks and performance time. For the determination of MADDS this can only be said with respect to the chance of interfering peaks and the performance time. The gain in time for twenty samples was about 3 h.

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