Journal of Chromatography, 345 (1985) 447—452
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2812

Note

Rapid and sensitive liquid chromatographic method for the determination of dapsone and monoacetyldapsone in plasma and urine

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(First received May 20th, 1985; revised manuscript received July 8th, 1985)

Dapsone, 4,4′-diaminodiphenyl sulphone (DDS), is widely used for the treatment of leprosy [1]. This drug has also been used as a model drug for determining the acetylation phenotype [2, 3]. Phenotypic differentiation by this drug has been proved to agree with that using the elimination half-life of isoniazid or the urinary excretion ratio of acetylsulphamethazine to sulphamethazine [2]. Based upon these reported data where no serious side-effect was seen during the phenotyping test and a single-point measurement of the drug level in the blood (plasma or serum) is sufficient to detect phenotyping status [2, 3], DDS has been often selected for assessing the acetylator phenotype as a safe and convenient test agent [4—6]. The plasma ratio of monoacetyldapsone (MADDS) to DDS at 3 h after a single oral dose of 100 mg of DDS is commonly used and clinically acceptable [3].

Analyses of DDS and MADDS in plasma have already been developed by several investigators [7—10] using a high-performance liquid chromatographic (HPLC) method. However, to our knowledge, there is no report describing urinary analyses of DDS and MADDS by a simple HPLC method. The determination of DDS and MADDS in urine samples could be a useful tool for assessing acetylation polymorphism as a non-invasive procedure. In addition, a mass screening test to identify acetylation polymorphism in a selected population would become more feasible and convenient if the ratio of MADDS to DDS obtained from urine samples could be correlated with that from the blood samples.

The purpose of the present study is to develop a rapid, sensitive and reliable HPLC assay method for determining DDS and MADDS simultaneously in

0378-4347/85/$03.30

plasma or urine. Our sample preparation is considered to be simple as compared to the previous HPLC method which seems to be associated with a complex sample preparation [3].

EXPERIMENTAL

Chemicals and reagents

DDS and MADDS were donated by Yoshitomi (Osaka, Japan) and by Warner-Lambert (Ann Arbor, MI, U.S.A.). *m*-Aminophenyl sulphone (m-APS) as an internal standard was purchased from Aldrich (Milwaukee, WI, U.S.A.). The chemical structures of these compounds are illustrated in Fig. 1. Acetonitrile was of HPLC grade, and dichloromethane, acetic acid and sodium hydroxide were of reagent grade. All of these chemicals were purchased from Wako (Osaka, Japan) and used without further purification. Standard stock solutions were prepared in methanol (HPLC grade). All calibration curves were made by diluting stock solution for the required concentration of each compound.

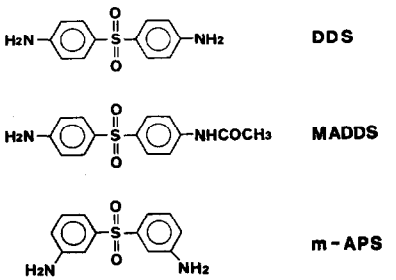

Fig. 1. Chemical structures of dapsone (DDS), monoacetyldapsone (MADDS) and the internal standard, *m*-aminophenyl sulphone (m-APS).

Instrumentation

The HPLC analysis was performed by a Hitachi liquid chromatograph, Model 635 (Hitachi, Tokyo, Japan) equipped with a Shimadzu variable-wavelength spectrophotometric detector, SPD-2A (Shimadzu, Kyoto, Japan), a Rheodyne 7125 sample injector fitted with a 20-μl loop (Rheodyne, Cotati, CA, U.S.A.) and a Hitachi recorder, Model 056 (Hitachi, Tokyo, Japan). The UV wavelength was set at 250 nm. The current HPLC separation was carried out with a Hibar LiChrosorb RP-18 column (250 mm × 4 mm I.D.), 5-μm particle size (Merck, Darmstadt, F.R.G.). The mobile phase consisted of water—acetonitrile—acetic acid (730:250:20) supplied at a flow-rate of 1.3 ml/min at 40°C.

Sample preparation

Plasma (0.5 ml) or urine (0.1—0.5 ml) in a 10-ml PTFE-lined screw-capped tube was mixed with 50 μl of the internal standard (m-APS) solution (1 μg), 100 μl of 1 *M* sodium hydroxide and 350 μl of water. The sample mixture, plus 3 ml of dichloromethane, was capped and shaken manually by vortex mixer for 1 min. After centrifugation at 950 *g* for 10 min, the upper aqueous layer was discarded by aspiration. Dichloromethane was transferred and

evaporated to dryness with a gentle stream of nitrogen gas at 35°C. The residue was reconstituted with 30—50 μl of mobile phase and 10—15 μl of this solution were injected onto the chromatograph.

RESULTS

As can be seen in Fig. 2, an appropriate UV wavelength selection was required for the analytes. Since the UV absorbance for m-APS was extremely low compared to those of DDS and MADDS at a UV range of more than 260 nm, we adopted 250 nm for determining DDS and MADDS.

Representative chromatograms obtained from plasma and urine extracts are shown in Fig. 3. The retention times for DDS, MADDS and m-APS were 4.5, 5.3 and 6.2 min, respectively. Although there were a few unknown peaks from plasma or urine, the analyses were not affected by the unknowns.

The absolute recoveries of the three analytes from plasma and urine were assessed by comparing peak heights obtained from the standard stock solutions of the drugs and drug-free plasma or urine spiked with the respective drugs. The extraction recoveries from plasma averaged 95% or more for DDS and MADDS at concentrations of 0.1 and 1.0 μg/ml, and 92.3% for m-APS at a concentration of 0.1 μg/ml. The recoveries from urine were fairly similar to those from plasma as given above.

The calibration curves were made by plotting the peak-height ratios of DDS and MADDS to m-APS with six different concentrations expected from DDS dose size used for the phenotyping test. The regression lines were linear over the concentrations examined (0.05—2 μg/ml for DDS and 0.054—2.16 μg/ml for MADDS). The respective correlation coefficients of the calibration curves ranged between 0.9992 and 0.9999.

To assess the precision of this analytical procedure, reproducibilities for

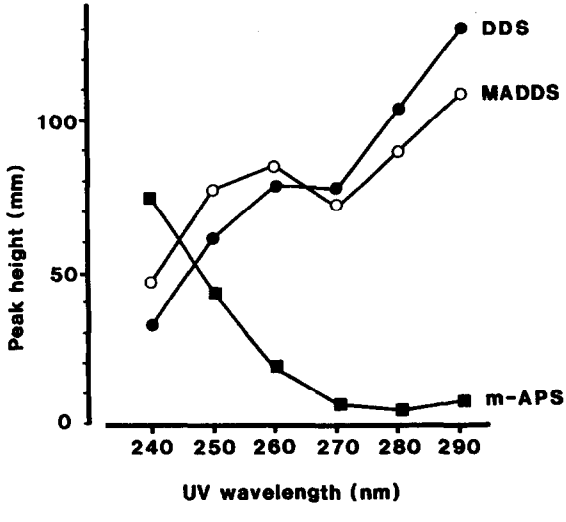

Fig. 2. Relationship between UV wavelength and peak-height value of three interested compounds. Each peak height was determined by injecting the mixture solution consisting of 100 ng of each of the compounds onto the chromatograph.

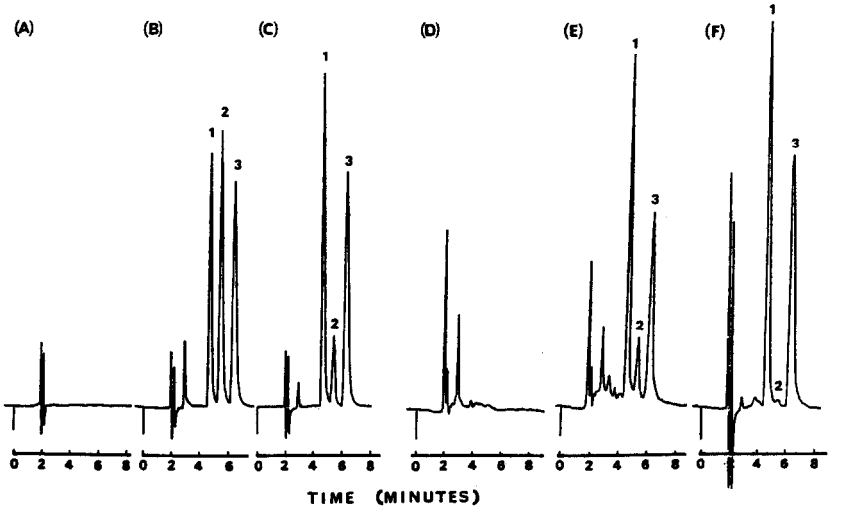

Fig. 3. Chromatograms of extracts obtained from (A) drug-free plasma; (B) plasma of a rapid acetylator, containing 1.58 μg/ml dapsone (DDS) and 1.55 μg/ml monoacetyldapsone (MADDS); (C) plasma of a slow acetylator, containing 2.00 μg/ml DDS and 0.35 μg/ml MADDS; (D) drug-free urine; (E) urine of a rapid acetylator, containing 1.23 μg/ml DDS and 0.18 μg/ml MADDS; (F) urine of a slow acetylator, containing 10.47 μg/ml DDS and 0.11 μg/ml MADDS. Peaks: 1 = DDS; 2 = MADDS; 3 = the internal standard, *m*-aminophenyl sulphone (m-APS). All analyses were carried out using the 0.5-ml volume of plasma or urine sample, except that the 0.1-ml volume of urine sample was used in the slow acetylator.

TABLE I

ANALYTICAL PRECISION IN THE DETERMINATION OF DDS AND MADDS FROM SPIKED PLASMA SAMPLES

DDS concentration given (μg/ml)	Peak-height ratio (DDS/m-APS) (mean ± S.D.)	Coefficient of variation (%)	MADDS concentration given (μg/ml)	Peak-height ratio (MADDS/m-APS) (mean ± S.D.)	Coefficient of variation (%)
Within-day variation (n = 5)					
0.05	0.0708 ± 0.0057	8.0	0.054	0.0871 ± 0.0069	7.9
0.1	0.141 ± 0.0034	2.4	0.108	0.173 ± 0.0055	3.2
0.2	0.291 ± 0.0143	4.9	0.216	0.339 ± 0.0173	5.1
0.5	0.700 ± 0.0311	4.4	0.54	0.818 ± 0.0390	4.8
1.0	1.446 ± 0.0304	2.1	1.08	1.685 ± 0.0539	3.2
2.0	2.909 ± 0.1035	3.6	2.16	3.397 ± 0.1588	4.7
Day-to-day variation (n = 6)					
0.05	0.0699 ± 0.0059	8.4	0.054	0.0879 ± 0.0075	8.5
0.2	0.284 ± 0.0172	6.1	0.216	0.335 ± 0.0214	6.4
1.0	1.421 ± 0.0356	2.5	1.08	1.637 ± 0.0651	4.0

within-day and day-to-day variations were determined. As given in Table I, the coefficients of variation for six different concentrations in the within-day study varied between 2.1 and 8.0%, whereas those in the day-to-day study ranged between 2.5 and 8.5%.

The accuracy was assessed by comparing the actual amounts of analytes with those estimated. The estimated amounts were in good agreement with the actual amounts: the relative errors ranged from −0.4 to 4.0% for DDS and from −2.9 to 9.3% for MADDS in the respective concentration ranges as given Table I.

Detection limits were determined using diluted working solutions. Both compounds can be detected in concentrations as low as 0.01 μg/ml (signal-to-noise ratio > 4) in plasma or urine using the 0.5-ml samples.

DISCUSSION

The polymorphic acetylation of DDS was first observed by Peters et al. [11] with the higher plasma ratio of MADDS to DDS in rapid rather than in slow acetylators. Subsequently, Reidenberg et al. [3] showed that rapid acetylators have a plasma MADDS/DDS ratio of > 0.35 and that the ratio of < 0.30 indicates slow acetylators. Since then, DDS has been used to identify acetylator phenotype in an individual [4—8].

The present report described a rapid, sensitive and reliable method for the determination of DDS and MADDS in biological fluids by HPLC. The analytical procedures consisted of a simple basic extraction, evaporation of the organic phase and injection of the reconstituted residue onto the chromatograph.

Although various methods, including the non-specific Bratton-Marshall technique [12] and the fluorometric detection with a complex extraction procedure [13, 14], were introduced into the analyses of DDS and MADDS in plasma, nowadays the HPLC technique is considered to be the most effective method for the simultaneous determination of these analytes in plasma [7—10]. However, to our knowledge, there appears to have been no report on the urinary analyses of DDS and MADDS using an HPLC method with a simple or uncomplicated sample preparation.

We chose an internal standard method to correct possible errors in handling pipettes and syringes, and erratic extraction efficiencies. Because β-hydroxyethyltheophylline [4] was eluted too early under the present HPLC conditions and monopropionyl DDS [7] was required synthetized, potential structural analogues of DDS and MADDS were considered for use as suitable internal standards. *m*-Aminophenyl sulphone (m-APS), which has an ideal retention time and a good extraction recovery, was adopted as the internal standard, despite the fact that the UV absorbance of m-APS at 290 nm, where the absorbances of both DDS and MADDS gave the highest peaks, was very low (Fig. 2). UV wavelength, therefore, was adopted at 250 nm. However, under this condition, the assay sensitivity was still sufficiently enough to detect MADDS in plasma of a slow acetylator after the administration of 100 mg of DDS (Fig. 3). With the 0.5-ml volume of samples (plasma or urine) used in the present study, the sensitivity was such that the compounds can be detected in plasma or urine in concentrations as low as 0.01 μg/ml. In addition, the present method can be applied for the analyses of these compounds with the fixed UV wavelength detector usually set at 254 nm. The same UV wavelength was also selected in a sensitive ion-pair chromatographic assay for DDS and MADDS as recently reported by Edstein [10].

Extraction of blank plasma or urine by dichloromethane instead of diethyl ether [7] gave a chromatogram that was consistently free of undesired peaks in the areas corresponding to the retention times of three interested compounds. This extraction procedure was reproducible (Table I) and the recoveries

exceeded 90% for all analytes. The retention times for DDS, MADDS and the internal standard were less than 8 min and comparable to those of the method reported by Philip et al. [8]. The simple extraction and relatively short retention times for the three compounds measured in our assay method allow the analyses of more than 30 samples per day.

For population studies related to an acetylation phenotyping assessment, a simple method with a single, non-toxic and reliable test drug, which requires only a single, hopefully non-invasive, sample collection after the test drug, is desired. DDS seems to be the drug having all the above-mentioned criteria.

ACKNOWLEDGEMENTS

The authors are thankful to Miss Kaori Narita for her secretarial assistance, and to Yoshitomi Pharmaceutical Industries, Ltd. (Osaka, Japan) and Warner-Lambert Company (Ann Arbor, MI, U.S.A.) for their generous supply of DDS and MADDS used in this study.

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