*Journal of Chromatography*, 422 (1987) 322–327
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3844

## Note

# Dapsone and monoacetyldapsone determined in serum and saliva by a sensitive high-performance liquid chromatographic method with a single extraction step

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(First received May 8th, 1987; revised manuscript received June 19th, 1987)

Dapsone (diaminodiphenylsulphone; DDS) is widely used to treat leprosy patients [1]. Several other dermatological disorders also respond well to DDS [2]. After administration of DDS, acetylation leads to the formation of monoacetyldapsone (MADDS), which can be detected in blood with the parent compound. The extent of acetylation, reflected in the ratio between the serum concentrations of MADDS and DDS, depends on the acetylation status of a subject, which is a genetically determined characteristic [3,4].

Several high-performance liquid chromatographic (HPLC) methods have been described to determine DDS and MADDS in serum [3,5–9]. However, these methods are all rather laborious and sometimes lack sensitivity and reproducibility.

In this paper a new, rapid and sensitive HPLC method is described for simultaneous analysis of DDS and MADDS in serum. With a slight modification the method is also applicable to the determination of DDS in saliva.

## EXPERIMENTAL

### *Chemicals*

The following materials were used for the determination method: 1,2-dichloroethane (Rathburn, U.K.; HPLC grade), *n*-butyl acetate (Merck, F.R.G.; analytical grade), water (glass-distilled), phosphate buffer (1.4 *M*, pH 7.5).

DDS (Roussel, France) and MADDS (obtained as described previously [10]) were used as references.

0378-4347/87/$03.50 

*Instrumentation*

The system consisted of a Waters Model 6000A HPLC pump, a WISP 710B autosampler, a Chromspher Si column (100×3 mm I.D.; particle size 5 μm; Chrompack, Middelburg, The Netherlands), a Baird Atomic Fluoricord spectrofluorimeter and a Spectra-Physics SP 4100 integrator.

The mobile phase was 1,2-dichloroethane–50% water-saturated *n*-butyl acetate (1:9, v/v). The flow-rate was 1 ml/min. The excitation wavelength was 290 nm and the emission wavelength 380 nm. The concentrations of DDS and MADDS were determined by comparing peak heights of samples and standards.

*Extraction procedure and standards*

After addition of 500 μl of the mobile phase to serum samples of 200 μl, the mixture was well shaken for 10 min and centrifuged at 2400 *g* for 5 min. The organic layer was transferred to a WISP insert vial, and 20 μl were injected into the HPLC system.

Saliva samples of 200 μl were diluted with 200 μl of phosphate buffer. The mixture was treated as the serum samples.

Standards were obtained by diluting 20 μl of aqueous solutions of 2.5, 5, 7.5, 10, 20, 30 and 40 mg/l DDS and MADDS with 180 μl of blank serum or saliva. Standards were treated similarly to the samples.

*Evaluation of the HPLC method*

The day-to-day and within-run coefficients of variation (C.V.) of the determination of DDS and MADDS in serum were calculated at concentrations of 0.1, 0.5 and 1 mg/l using a calibration curve of 0, 0.25, 0.5, 0.75 and 1 mg/l, and at concentrations of 1, 2 and 4 mg/l using a calibration curve of 0, 1, 2, 3 and 4 mg/l. The accuracy of the method followed from comparison of the results with the actual, known amounts of DDS and MADDS present. The extraction efficiency was determined by comparing the chromatographic peak heights after extraction with the peak heights obtained after direct injection of standard solutions of DDS and MADDS in mobile phase. The limit of detection was the serum concentration at a signal-to-noise ratio of 3.

Other parameters that were calculated were the capacity factor ($k' = (t_R - t_{R0})/t_{R0}$), the selectivity ($\alpha = k'_{MADDS}/k'_{DDS}$) and the resolution ($R = (t_{R,MADDS} - t_{R,DDS})/0.5(w_{MADDS} + w_{DDS})$), in which $t_R$ represents the retention time and $w$ the peak width.

*Volunteer study*

Two healthy male volunteers, 23 and 29 years old and both weighing 80 kg, ingested a tablet of 100 mg DDS (OPG, The Netherlands). Blood and unstimulated saliva samples were taken before and 3, 8, 24 and 48 h after administration. The blood samples were centrifuged after clotting to obtain serum. All samples were frozen at −20°C pending analysis. The elimination half-life of DDS was derived from the slope of the DDS serum concentration–time curve in the elimination phase, using linear regression. The acetylation status of both subjects was

determined using the standards for the ratio between the DDS and MADDS serum concentration (M/D ratio) according to Philip et al. [11].

## RESULTS

### *HPLC method*

Fig. 1 shows representative chromatograms of a blank serum sample and of serum samples containing 0.1, 1 and 4 mg/l DDS and MADDS.

DDS and MADDS were perfectly separated, with retention times of 0.92 and 1.72 min, corresponding to $k'$ values of 0.59 and 1.97 for DDS and MADDS, respectively. The selectivity was 3.34 and the resolution 4.26. All these parameters were derived from the 1 mg/l samples, but no important differences were observed with the other concentrations.

The day-to-day and within-run C.V. and accuracy of the method are presented in Table I. The method is linear in the concentration range of this study, as follows from the linear regression coefficient found for the calibration curves, being 0.999 or higher in all the assays. A limit of detection of 5 μg/l for DDS in serum and of 10 μg/l for MADDS in serum was found. This corresponds to absolute amounts of 0.04 ng of DDS and 0.8 ng of MADDS measured. The extraction ratio was 100% for DDS and 69% for MADDS.

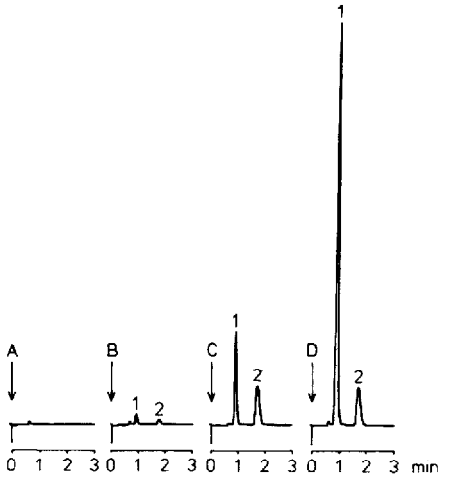


Fig. 1. Representative chromatograms of a blank serum (A) and of serum samples from healthy volunteers containing 0.11 mg/l DDS and 0.08 mg/l MADDS (B), 0.85 mg/l DDS and 0.98 mg/l MADDS (C) and 3.64 mg/l DDS and 0.97 mg/l MADDS (D). Peaks: 1 = DDS; 2 = MADDS. Chromatograms B and C are from rapid acetylators, D from a slow acetylator.

### *Volunteer study*

After ingestion of DDS, the drug and its main metabolite in blood can be rapidly detected. The individual results are presented in Table II, while Fig. 2 provides a graphic presentation of the results from subject 1. Both subjects were classified as rapid acetylators. Saliva and serum DDS concentrations were highly

TABLE I

WITHIN-RUN AND DAY-TO-DAY COEFFICIENTS OF VARIATION AND ACCURACY OF THE DDS AND MADDS DETERMINATION IN SERUM

In all cases, $n = 6$.

| Concentration (mg/l) | Within-run | | | | Day-to-day | | | |
|---|---|---|---|---|---|---|---|---|
| | DDS | | MADDS | | DDS | | MADDS | |
| | C.V. (%) | Accuracy (%) | C.V. (%) | Accuracy (%) | C.V. (%) | Accuracy (%) | C.V. (%) | Accuracy (%) |
| 0.1 | 11.2 | −3.6 | 10.6 | +13.7 | 5.6 | +6.0 | 6.5 | +7.0 |
| 0.5 | 1.5 | 0 | 6.9 | +1.4 | 2.7 | +5.0 | 3.5 | +2.2 |
| 1.0* | 1.8 | +0.2 | 3.8 | +2.1 | 1.3 | +2.5 | 3.6 | +3.4 |
| 1.0** | 2.1 | −2.6 | 2.7 | −5.6 | 2.0 | +1.5 | 2.7 | +1.9 |
| 2.0 | 1.8 | −1.1 | 1.9 | +3.0 | 3.6 | +0.8 | 2.6 | −0.5 |
| 4.0 | 1.6 | +0.4 | 4.4 | +9.5 | 3.1 | +0.4 | 2.4 | +1.9 |

*Using a calibration curve from 0 to 1.0 mg/l.
**Using a calibration curve from 0 to 4.0 mg/l.

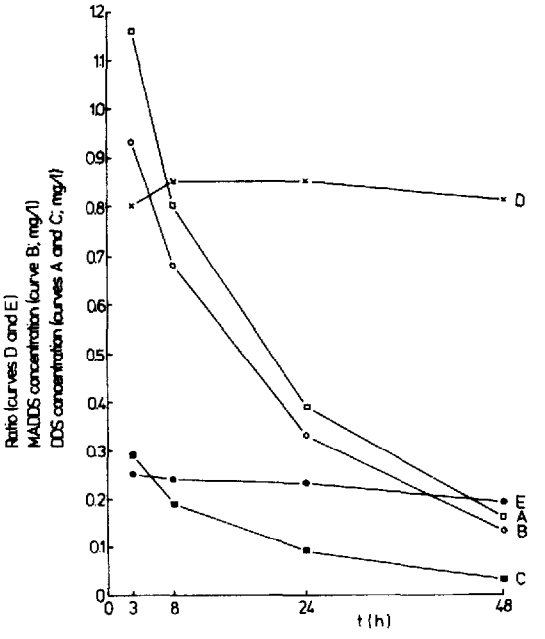


Fig. 2. Experimental results in subject 1 after oral ingestion of 100 mg of DDS. Time course of (A) DDS serum concentration (mg/l), (B) MADDS serum concentration (mg/l), (C) DDS saliva concentration (mg/l), (D) MADDS/DDS serum concentration ratio and (E) saliva/serum DDS concentration ratio.

TABLE II

INDIVIDUAL RESULTS OF THE VOLUNTEER STUDY

$t_{1/2}$ DDS = DDS elimination half-life; $t_{1/2}$ MADDS = MADDS elimination half-life; M/D ratio = MADDS/DDS concentration ratio.

| | Subject 1 | Subject 2 |
|---|---|---|
| $t_{1/2}$ DDS (serum; h) | 16.2 | 16.3 |
| $t_{1/2}$ DDS (saliva; h) | 15.6 | 13.9 |
| $t_{1/2}$ MADDS (serum; h) | 16.1 | 15.6 |
| Mean M/D ratio (serum) | 0.83 | 0.48 |
| Mean saliva/serum ratio | 0.23 | 0.23 |

correlated ($r = 0.999$, $n = 8$), the ratio between the two remaining between 0.19 and 0.26 in both subjects throughout the study. This ratio was independent of the serum DDS concentration in this study.

## DISCUSSION

The use of fluorometric instead of UV detection improves the sensitivity of this new HPLC method, so that compared with other methods only small samples were required. The quality of the chromatograms benefits from the fact that the mobile phase is used as the extracting medium, without any further additives. The detection limit for DDS of 5 $\mu$g/l is quite satisfactory with regard to the serum concentrations of 0.1 to 5 mg/l, which are considered to be the limits of the therapeutic index

The analysis time is also markedly improved. Only a single extraction step is performed, without time-consuming evaporation procedures. No internal standard is needed. The run time of each analysis is only 3 min, and 100 or more assays can be performed per day if an autosampler is available.

The pharmacokinetics of DDS observed in this study are in agreement with earlier findings as far as elimination half-life, serum and saliva concentrations and linearity in the elimination phase are concerned [8,12,13]. The saliva DDS concentration reflects the protein non-bound fraction of the drug in serum, which has been reported to be ca. 20–25% of the total DDS serum concentration [12,14]. No significant amounts of MADDS were detectable in saliva, which is in agreement with the very high serum protein binding of the compound [14].

It appears that this HPLC method for the determination of DDS and MADDS in serum and saliva is a rapid and sensitive alternative to previously published methods.

## ACKNOWLEDGEMENTS

Mr. H.A.C. Titulaer and Mr. P. Stekkinger are gratefully acknowledged for their skilled technical assistance.

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