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Note

Rapid gas chromatographic determination of dimethyl sulphoxide and its metabolite dimethyl sulphone in plasma and urine

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Recent interest in the potential medical use of dimethyl sulphoxide (DMSO) has emphasized the need for a simple and reliable method for its determination in biological fluids. Several pathological conditions have been reported to respond to DMSO [1]. It is indicated in a variety of painful conditions such as post-surgical pain. It is also used as a vehicle for drugs in determatological products. Since its use is still in the experimental stage, there is no well defined therapeutic range for DMSO. The usual practice in the treatment of post-operative hemiplegia is to administer DMSO intravenously, 1 g/kg per day for four to five days.

Few methods have been reported for the determination of DMSO in biological fluids. They are based on gas chromatographic (GC) separation on a Carbowax column and detection by a flame ionization detector. They involve either direct injection of sample [2] or require sample preparation step such as solvent extraction [3] or protein precipitation by perchloric acid [4] or methanol [5]. Because of the high polarity of DMSO, the solvent extraction

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procedure does not seem to be appropriate for its isolation from biological media. It could lead to a variable recovery. The direct injection of sample onto the chromatograph is not advisable either since there is a rapid build-up of protein in the column. None of the above procedures is properly validated except the perchloric acid precipitation procedure [4]; however, in this procedure the acid needs to be neutralized before sample injection, which makes the method more manipulative and time-consuming. This paper describes a simple GC procedure for the determination of DMSO and its metabolite dimethyl sulphone ($DMSO_2$) in plasma and urine. It involves protein precipitation by acetone followed by separation on a Porapak Q column. Results obtained on patient samples following intravenous DMSO therapy are also presented.

EXPERIMENTAL

Chemicals and reagents

Analytical-reagent-grade acetone, toluene and DMSO were purchased from BDH (Poole, U.K.) and $DMSO_2$ was purchased from Sigma (Poole, U.K.).

Apparatus

A Model 8310 gas chromatograph (Perkin-Elmer, Beaconsfield, U.K.) equipped with flame ionization detector and a glass column (2 m × 3 mm I.D.) packed with Porapak Q was used in conjunction with a Model BD 40 recorder (Kipp & Zonen, Delft, The Netherlands). The following GC conditions were used: column, 240°C; injection port, 250°C; detector, 275°C; nitrogen flow-rate, 25 ml/min; hydrogen and air flow-rates, 33 and 390 ml/min, respectively.

Procedure

To a stoppered centrifuge tube 0.2 ml plasma or urine sample, 0.8 ml internal standard (0.02% toluene in acetone, v/v) were added. The contents were vortexed for 30 s followed by centrifugation at 750 *g* for 5 min. A 1-μl volume of the supernatant solution was then injected onto the chromatograph.

Standard solutions of DMSO and $DMSO_2$ ranging from 0.5 to 5.0 g/l ($n = 5$) were prepared in drug-free plasma and 2.0—20.0 g/l DMSO and 0.5—5.0 g/l $DMSO_2$ ($n = 5$) were prepared in drug-free urine. Each standard (0.2 ml) was assayed according to the procedure described. The peak-height ratio of drug or metabolite to internal standard was then plotted against concentration.

RESULTS AND DISCUSSION

Porapak Q is widely used in GC for the analysis of solvents. It has the ability to accommodate large sample loads and polar compounds are readily eluted to give symmetrical peaks. Since DMSO is a polar solvent, we chose Porapak Q for its GC assay. This approach also gave a good separation of $DMSO_2$. Chromatograms of standards and samples are shown in Figs. 1 and 2. The calibration graphs were linear within the above mentioned concentration ranges. The regression equations for the calibration graphs are as follows: DMSO in plasma,

$y = 0.208x - 0.0062$; $DMSO_2$ in plasma, $y = 0.196x + 0.017$; DMSO in urine, $y = 0.211x - 0.13$; $DMSO_2$ in urine, $y = 0.188x - 0.02$; for all equations $r = 0.999$.

Good reproducibility of the method was indicated when ten separate determinations of plasma sample containing 0.5 g/l DMSO and 0.5 g/l $DMSO_2$ gave within-day coefficients of variation (C.V.) of 3.5 and 7.7% and day-to-day C.V. values of 8.0 and 8.2%, respectively. Similarly ten separate urine samples each containing 2.0 g/l DMSO and 1.0 g/l $DMSO_2$ gave within-day C.V. values of 5.2 and 6.5% and day-to-day C.V. values of 6.6 and 6.8%, respectively. The lower limit of determination for each compound was found to be 0.1 g/l. Recoveries (%) of each compound were calculated by comparing the peak-height ratios of samples (plasma and urine) with those of aqueous solutions containing the same amount of these compounds. The concentrations of DMSO in recovery experiments were 0.5 and 5.0 g/l in plasma and 2.2 and 8.8 g/l in urine. For $DMSO_2$ the concentrations were 0.5 and 5.0 g/l in plasma and 0.5 and 2.0 g/l in urine. The recoveries of these compounds from plasma and urine were nearly 100%. This is not unexpected since no sample manipulation (solvent extraction, sample transfer, etc.) is involved and the compounds are not co-precipitated with protein. The use of acetone serves two purposes,

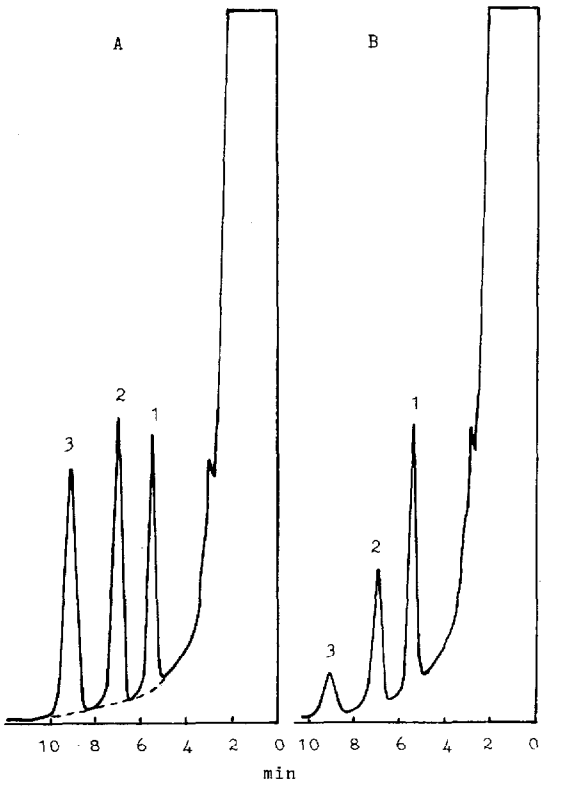

Fig. 1. (A) Chromatogram of plasma standard containing (1) 0.02% toluene (internal standard), (2) 5.0 g/l DMSO and (3) 5.0 g/l $DMSO_2$. The broken line shows a trace from blank plasma. (B) Chromatogram of a patient's plasma sample containing (1) 0.02% toluene as an internal standard, (2) 2.491 g/l DMSO and (3) 0.690 g/l $DMSO_2$.

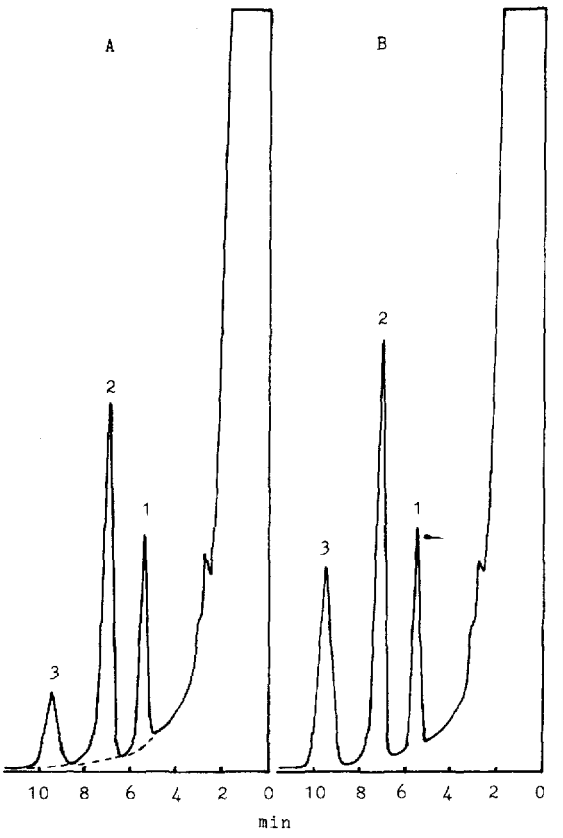

Fig. 2. (A) Chromatogram of urine standard containing (1) 0.02% toluene (internal standard), (2) 8.80 g/l DMSO and (3) 2.0 g/l $DMSO_2$. The broken line shows a trace from blank urine. (B) Chromatogram of a patient's urine sample containing (1) 0.02% toluene as an internal standard, (2) 10.14 g/l DMSO and (3) 5.052 g/l $DMSO_2$.

TABLE I

DMSO AND $DMSO_2$ CONCENTRATIONS IN PATIENTS DURING AN INTRAVENOUS DMSO THERAPY

Dosing regimen: 1.5 g/kg over 8 h, 0.8 g/kg over the next 16 h, followed by 1 g/kg per 24 h for four days or until resolution of hemiplegia.

Patient	Sample	DMSO (g/l)	$DMSO_2$ (g/l)
1	Plasma (24 h)	3.282	0.470
1	Plasma (48 h)	2.920	0.540
1	Plasma (72 h)	2.491	0.690
2	Plasma (24 h)	3.165	1.837
2	Urine (0—24 h)	18.17	1.879
3	Urine (0—24 h)	10.14	5.052
3	Urine (24—48 h)	5.952	8.210
3	Urine (48—72 h)	0.904	9.895

i.e. it acts as a solvent for internal standard and also as a protein precipitant. We also observed that acetone gave less tailing peak in comparison to other organic protein precipitants such as ethanol, methanol or acetonitrile, thus facilitating the separation of compounds near the baseline. No peaks were observed at the retention times of toluene, DMSO or $DMSO_2$ from the blank plasma or urine nor from valproic acid which could be co-administered with DMSO. When stored at −20°C, the samples were stable for at least six weeks.

Table I shows plasma and urine levels of DMSO and $DMSO_2$ in patients who received intravenous DMSO for treatment of post-operative hemiplegia. No biological interpretation of results can be made since the study involved few patients and limited data have been gathered. However, the results demonstrate the usefulness of this method for the rapid determination of DMSO and $DMSO_2$ in plasma and urine. We believe that the proposed method, because of its simplicity, should be beneficial to workers involved in studying the pharmacokinetics of DMSO in man.

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