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IMPROVED SELECTIVE ION MONITORING MASS-SPECTROMETRIC ASSAY FOR THE DETERMINATION OF N,N-DIMETHYLTRYPTAMINE IN HUMAN BLOOD UTILIZING CAPILLARY COLUMN GAS CHROMATOGRAPHY

R.W. WALKER and L.R. MANDEL

Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, N.J. 07065 (U.S.A.)

J.E. KLEINMAN, J.C. GILLIN and R.J. WYATT

National Institute of Mental Health, St. Elizabeth's Hospital, Washington, D.C. 20032 (U.S.A.)

and

W.J.A. VANDENHEUVEL

Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, N.J. 07065 (U.S.A.)

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SUMMARY

A gas—liquid chromatographic—mass spectrometric procedure is described for the assay of dimethyltryptamine (DMT) in whole blood. The use of a glass capillary column in combination with selective ion monitoring results in an assay with a high degree of specificity and sensitivity. 5-Methoxy-DMT is used as an internal standard and carrier in the isolation procedure. The superior resolving characteristics of the capillary column (as compared to previously employed packed columns) allows monitoring of the intense *m/e* 58 ion arising from the DMT side-chain. A sensitivity limit of 10 pg/ml blood is realized with a 10-ml blood sample.

INTRODUCTION

Gas—liquid chromatography—mass spectrometry (GLC—MS) is a specific, sensitive analytical technique for determining concentrations of drugs [1, 2] and endogenous compounds [3] in various states of health and disease [4]. The use of GLC—MS in psychiatric disorders is particularly important as endogenous compounds of interest are frequently present in low concentrations and specificity is of considerable concern. This is particularly true for dimethyl-

tryptamine (DMT) and its potential role in schizophrenia. The indoleamine DMT is a known psychotomimetic agent [5—7], and has been reported to be present in human blood and urine [8—12]. Moreover, an enzyme system capable of forming DMT has been demonstrated to be present in man [13—16].

In 1973 we reported on a GLC—MS isotope dilution assay with a sensitivity of 1 ng/ml for measuring DMT in human plasma [12]. Using this assay, we found no differences in DMT plasma concentrations between groups of normals, chronic schizophrenics, acute schizophrenics, and psychotic depressives [17]. In fact, we were unable to detect DMT in plasma at all except in one normal and one psychotic depressive. Subsequent studies by Bidder et al. [18] and Lipinski et al. [19] reported finding DMT in blood and urine in a limited number of patients with psychotic illnesses. Kaplan et al. [20] observed that DMT blood concentrations following administration of a psychoactive DMT dose (0.7 mg/kg) to human subjects were low, peaking at less than 100 ng/ml at ten minutes post-dose (the time of the greatest subjective response) and dropping off rapidly thereafter.

One possible explanation for the failure to find a correlation between blood concentrations of DMT and schizophrenia may be that endogenous DMT concentrations are exceedingly small. Any differences in DMT concentrations between normals and schizophrenics would be missed if the higher level were still below the detection limit of the assay employed. Several refinements in the original assay procedure improved the detection limit to 0.05 ng/ml whole blood. Using this modified assay procedure Angrist et al. [21] found no statistically significant differences in DMT concentrations between psychiatric patients and control subjects. In this study 50% of the patients and 40% of the controls gave measurable DMT concentrations. In the work described here, the assay procedure has been modified extensively, resulting in a practical sensitivity limit for DMT of 10 pg/ml whole blood.

EXPERIMENTAL

Internal standard

5-Methoxy-N,N-dimethyltryptamine (5-OCH_3-DMT, 99% pure; Aldrich, Milwaukee, Wisc., U.S.A.) was used as an internal standard. A solution containing 100 ng of 5-OCH_3-DMT per 10 μl methanol was prepared for introduction of the internal standard.

Gas—liquid chromatography—mass spectrometry

A Finnigan Model 3200 GC—MS instrument equipped with a 6110 computer system was used. The chromatographic conditions were as follows: glass capillary column, 18 m × 0.33 mm, coated with SE-30; oven temperature, 200°; injection port temperature, 260°; carrier gas (helium) flow-rate ca. 2 ml/min; retention time of trimethylsilyl (TMS) derivative of DMT, 6.8 min, and that of 5-OCH_3-DMT TMS derivative, 13.7 min. The mass spectrometer was operated using the following conditions: ionizing potential, 70 eV; emission current, 0.8 mA; electron multiplier, 1800 V. Measurements were performed by selective ion monitoring, focusing the spectrometer upon the ions *m/e* 58 for DMT and *m/e* 232 for 5-OCH_3-DMT.

Extraction procedure and derivative formation

The internal standard (100 ng) was added to 10 ml of whole blood and equilibrated with a Vibra-stirrer. Extraction with chloroform (2 × 15 ml) was then carried out after pH adjustment with 2.5 ml of 1 *N* ammonium hydroxide. The combined chloroform phases were filtered (Whatman No. 2 paper), reduced to 0.5 ml with a stream of nitrogen, and extracted with 1 ml of 0.5 *N* HCl. The aqueous phase was then washed with chloroform (3 × 1 ml), made alkaline with 0.4 ml of 2.5 *N* sodium hydroxide, and extracted with chloroform (2 × 1.0 ml). The chloroform was evaporated and the residue transferred with methanol to a micro derivatization vessel (Pierce micro reacti-vial or equivalent). Derivatization was effected by treating the isolate with 10 μl of bis-trimethylsilyltrifluoroacetamide (BSTFA) and pyridine (4:1, v/v) at 70° for 10 min.

Calibration standards

Calibration mixtures were prepared, each containing 100 ng of 5-OCH_3-DMT and DMT levels up to 1000 pg. A working curve was constructed by plotting the ratio of the peak height intensities of the two ions of interest, I_{58}/I_{232}, vs. the amount of DMT present in the mixture.

RESULTS AND DISCUSSION

The mass spectrum of DMT-TMS (Fig. 1) is dominated by the base peak, $CH_2=\overset{+}{N}(CH_3)_2$, *m/e* 58. Other pertinent but much less intense ions are M—58 (*m/e* 202) and M (*m/e* 260). The internal standard for this assay is 5-OCH_3-DMT, which has a retention time (as the TMS derivative) twice that of DMT-TMS. The mass spectrum of the internal standard (Fig. 2) is characterized by an intense *m/e* 58 ion (base peak) and ions at *m/e* 232 (M—58) and 290 (M). The internal standard is added in relatively large amount (100 ng) to serve also as a carrier in the isolation procedure. Monitoring of the *m/e* 58 ion for both DMT and the internal standard is not possible, as the effective dynamic range of the computer is not sufficiently great. Rather, the *m/e* 232 (M—58)

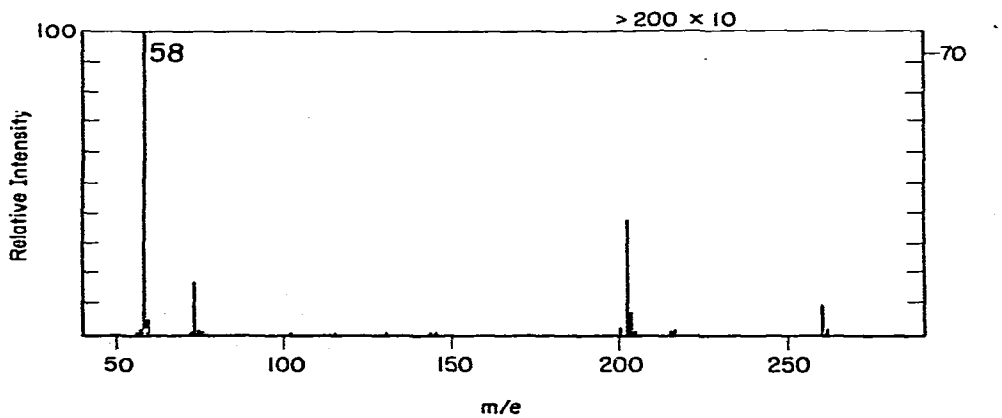

Fig. 1. Mass spectrum of DMT-TMS.

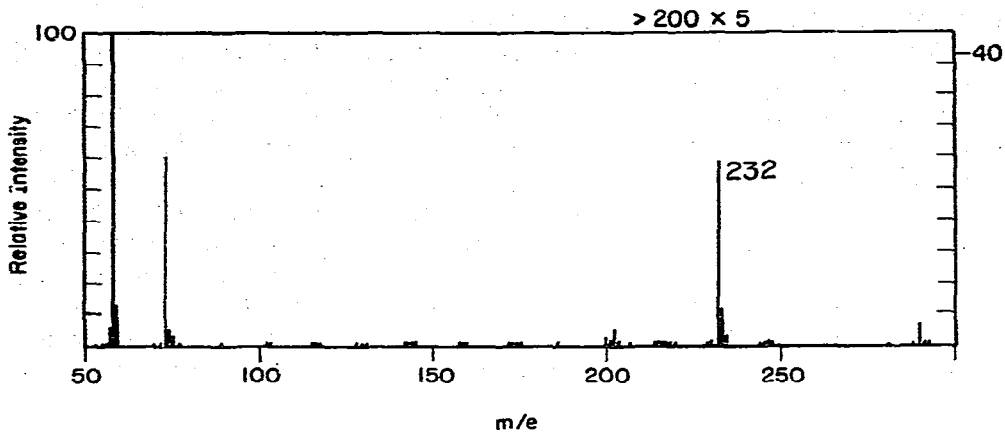

Fig. 2. Mass spectrum of 5-OCH_3-DMT-TMS.

ion from 5-OCH_3-DMT is used for this purpose. We found that, unlike packed column chromatography, adsorption losses with capillary columns are minimal. As little as 4 pg of DMT can be detected as its TMS derivative when monitoring the *m/e* 58 ion, wheras with packed column injection, at least 50 pg is required. Splitless injection is used for sample application to the capillary column so that the entire sample is analyzed. Aliquots of derivatized isolates containing approximately 5—10 ng of internal standard are injected into the capillary column for each measurement; a 30—40% total isolation efficiency allows multiple injections of each isolate for replicate analyses.

Ion response plots for the internal standard and a mixture of DMT and internal standard are shown in Fig. 3. The *m/e* 58 ion response is monitored

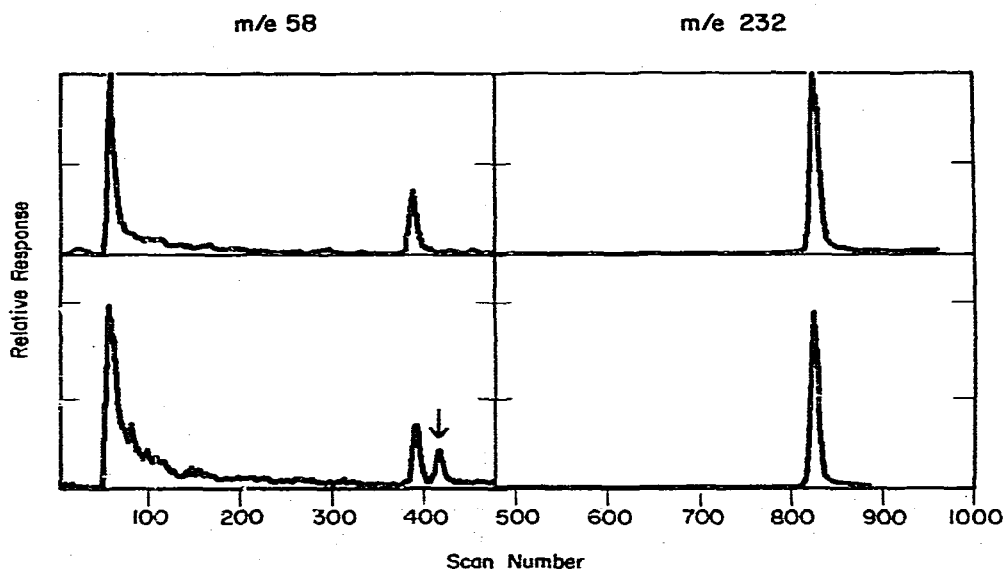

Fig. 3. Ion response plots of internal standard (top panel) and a 500 pg/100 ng mixture of DMT and internal standard (bottom panel).

during the first half of the analysis (0—8 min). The computer then switches to the *m/e* 232 ion for the latter half of the analysis to record the response from the internal standard. The retention time for DMT-TMS (6.8 min) corresponds to scan number 415; for the internal standard the corresponding values are 13.7 min and scan number 820. The response at scan number 390 observed with these standard mixtures results from a reagent component which does not interfere with the analysis. A linear response was obtained for up to 1000 pg DMT with the prepared standards (see Experimental section). Replicate values on the 50-pg standard gave a coefficient of variation of 16.2% (n = 5).

To evaluate the possibility of interfering substances yielding the *m/e* 58 ion, several 10-ml aliquots of control blood were carried through the isolation procedure and analyzed for DMT. Essentially no response was observed at the DMT retention time (Fig. 4). Similar experiments with control blood spiked with DMT gave positive results (see Fig. 5). In a related experiment, 40 ml of control blood were spiked with 50 pg DMT per ml blood. The assay was carried out on four 10-ml aliquots. Results are shown in Table I.

The assay procedure has been used to evaluate DMT levels in whole blood (both arterial and venous) from schizophrenic patients. The results of this study for arterial and venous blood from the same patients are listed in Table

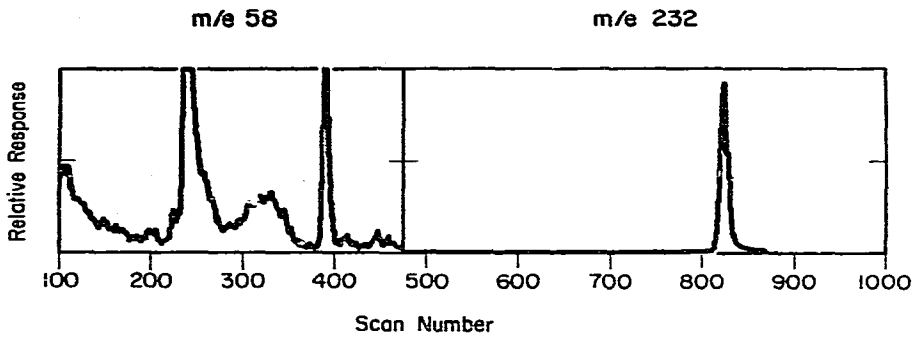

Fig. 4. Ion response plot of control blood isolate.

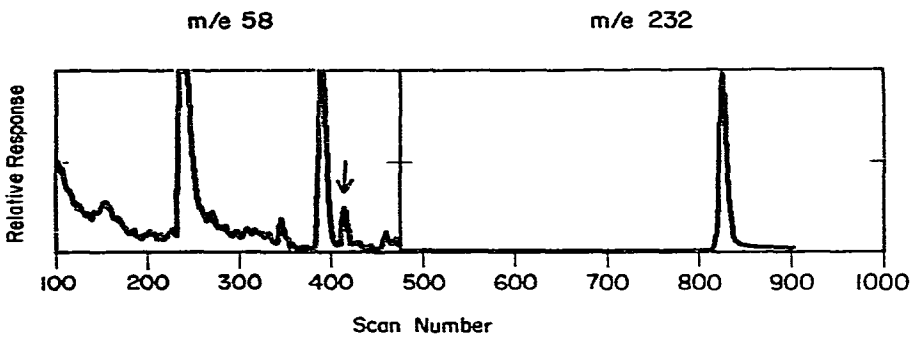

Fig. 5. Ion response plot of an isolate of control blood spiked with 50 pg DMT per ml blood; the response corresponds to 42 pg/ml blood.

TABLE I

ANALYSIS OF CONTROL BLOOD SPIKED WITH DMT (50 pg/ml)

The DMT found is the average result from two injections in each case.

Sample No.	DMT found (pg/ml)
Control (unspiked)	0*
1	48
2	48
3	44
4	56
Mean	49
σ	5.0
C.V. (%)	10.3

*Found < 10 pg/ml.

II. Absolute values ranged from a high of 118 pg/ml to undetectable amounts (< 10 pg/ml) in three patients. Entry 9-A shows the results obtained with blood samples from patient number 9 to which DMT had been added at 60 pg/ml (blind spike). The observed values are in excellent agreement with theory. Fig. 6 illustrates a positive result obtained with this study; for this patient a DMT concentration of 18 pg/ml was found.

The increased sensitivity (10 pg/ml using a 10-ml blood sample) of this assay compared with the sensitivities (1 ng/ml and 50 pg/ml) using 10-ml blood samples) of those previously published [12,21] arises from a number of factors. Use of a capillary column with its inherently greater resolving power when compared with that of a packed column reduces the possibility of im-

TABLE II

ANALYSIS FOR DMT IN BLOOD FROM SCHIZOPHRENIC PATIENTS

DMT found is the average result from two injections in each case.

Patient No.	DMT found (pg/ml)	
	Arterial	Venous
1	24	40
2	84	70
3	118	103
4	0*	0
5	24	21
6	29	0
7	23	18
8	0	0
9	0	0
9-A**	61	65

*Found <10 pg/ml.
**Blood from patient No. 9 to which DMT was added (blind spike) at 60 pg/ml.

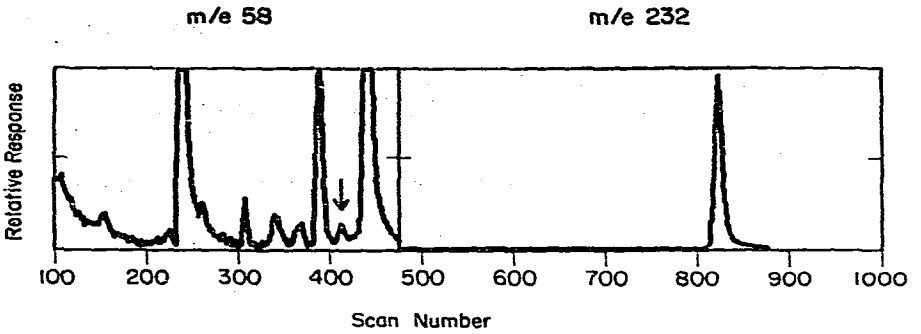

Fig. 6. Ion response plot of an isolate from a schizophrenic patient; the response corresponds to 18 pg/ml blood.

purity interference. Suñol and Gelpi [22], for example, have employed capillary column GLC in the analysis of several indoles. A factor of 100 is observed between the intensities of the base peak (*m/e* 58) and the molecular ion (*m/e* 260) in the mass spectrum of DMT-TMS (Fig. 1). The former is thus, from a sensitivity standpoint, a much more attractive ion than the latter, which is the ion monitored in our earlier packed column assays [12, 21]. The greater separating power of a capillary column allows the monitoring with confidence of the intense *m/e* 53 ion (arising from the DMT sidechain fragmentation), even though this low mass ion could arise from a variety of contaminants. GLC peaks from a capillary column are sharper because of greater column efficiency; increased sensitivity is thus realized with this greater peak height per unit mass. The combination of all these factors results in a practical sensitivity of up to 100 times greater than those assays previously reported [12, 21].

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