Journal of Chromatography, 224 (1981) 116—121
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 846

Note

Analysis of trimethobenzamide in human saliva by gas chromatography—mass spectrometry

T.A. ROBERT*, A.N. HAGARDORN and E.A. DAIGNEAULT

Department of Pharmacology, East Tennessee State University, Johnson City, TN 37614 (U.S.A.)

and

R.D. BROWN

Department of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130 (U.S.A.)

(First received November 12th, 1980; revised manuscript received January 26th, 1981)

In recent years there has been an increasing interest in the analysis of drugs in saliva. This interest has been inspired by the ease with which such non-invasively obtained samples can be collected and by the observation that many drugs are distributed in the saliva compartment in direct proportion to serum concentration [1—4]. For those drugs which do exhibit a proportional distribution between saliva and serum (and ultimately a relevant physiological compartment), analysis of their pharmacokinetic parameters allows a basis for comparing observed drug levels to therapeutic concentrations.

We report here a gas chromatography—mass spectrometry (GC—MS) analysis for an antiemetic drug, trimethobenzamide (TMB; N-[*p*-(2-dimethylamino-ethoxy)benzyl]-3,4,5-trimethoxybenzamide; 554-92-7; Tigan®, Beecham Laboratories, Bristol, TN, U.S.A.), in human mixed saliva specimens. In addition, we report upon the observed pharmacokinetics of this drug in the saliva of five healthy volunteers following an intramuscular dose of trimethobenzamide.

EXPERIMENTAL

Subjects

Five normal healthy adults volunteered to partake in the experiment, and informed consent was obtained from each. The subjects fasted from midnight the previous day until 2 h post medication, late the next morning. The experiment was initiated at time zero with an intramuscular injection of 200 mg TMB (Thera-Ject™ disposable syringes). Free flowing saliva specimens of approximately 2 ml were collected at time intervals of 0, 0.67, 1, 1.5, 2, 4, 6, and 8 h post drug injection. Saliva samples were stored at −60°C until analyzed. After the 2-h sampling period, all subjects were allowed to eat and drink.

0378-4347/81/0000—0000/$02.50

Assay

Pure TMB·HCl for use as an analytical standard was a generous gift from Beecham Laboratories. Thioridazine·HCl (THZ), used as an internal standard, was obtained from Sandoz (Basle, Switzerland). Other reagents and spectroscopic grade solvents were purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.). Initially, GC—MS analytical conditions were established by using pure TMB and THZ dissolved in methanol. Analyses were performed using a Finnigan 4000 gas chromatograph—mass spectrometer with Incos data system. The chromatographic analysis was done on a 1.2 m × 2 mm I.D. glass column packed with 3% SE-30 on 100—120 mesh Supelcoport. The column oven was isothermal at 250°C and the injection port temperature was maintained at 260°C. Ultra-pure helium was used as the carrier gas at a flow-rate of 30 ml/min. The glass jet separator interface was held at 270°C. Electron impact (EI) mass spectra were recorded by multiscan monitoring of the GC effluent. The MS acquisition parameters were as follows: ion source temperature, 250°C; emission current, 0.25 mA; electron multiplier voltage, —1210 V; electron energy, 70 V. Multiscan parameters were set for masses 50—400 in a period of 1.95 sec with a hold time of 0.05 sec. The retention times of the test compounds were recorded and the base peak of each compound was chosen to determine the ion peak ratios used for TMB quantitation in saliva samples. The mass spectrometer was tuned using the calibration standard perfluorotributylamine (FC43, *m/e* peaks 69, 131, 219 and 264).

The following extraction procedure was employed for both unknown samples and saliva standards prepared with known amounts of TMB. To 1.0 ml saliva in a screw-capped PTFE-lined 15 × 120 mm glass tube were added 300 μl THZ internal standard (10 mg THZ base per l water). After the contents were mixed, 1 ml extraction buffer (1 *M* boric acid containing 1 *M* potassium chloride, titrated to pH 9.0 with 1 *M* sodium carbonate) was added and the tube contents were remixed. Diethyl ether (5 ml) was then added and the contents vigorously shaken for 2 min. The tubes were centrifuged at 1000 *g* for 5 min to separate phases. The organic phase was pipetted into a clean 50-ml glass centrifuge tube and set aside. The aqueous phase was re-extracted with a second 5 ml ether as before and the organic phase was added to that from the first extraction. The pooled organic phases were then evaporated to dryness under a stream of Zero Grade nitrogen (Air Products, Tamaqua, PA, U.S.A.) in a 60°C water bath. The dried residue was reconstituted with 25 μl methanol by vigorous vortexing for 30 sec. A 2-μl aliquot was then taken for injection into the gas chromatograph.

RESULTS

Standards

The EI mass spectrum of pure TMB is shown in Fig. 1. The base peak of *m/e* 58 was selected as the fragment for use in subsequent quantitative analyses. An example of a saliva standard reconstructed ion current (RIC) plot is shown in Fig. 2. THZ as internal standard exhibited a retention time of approximately 4.5 min and a base peak of *m/e* 98 [5], which was used in determining peak area ratios for TMB quantitation. The observed retention time for TMB was 3.2 min.

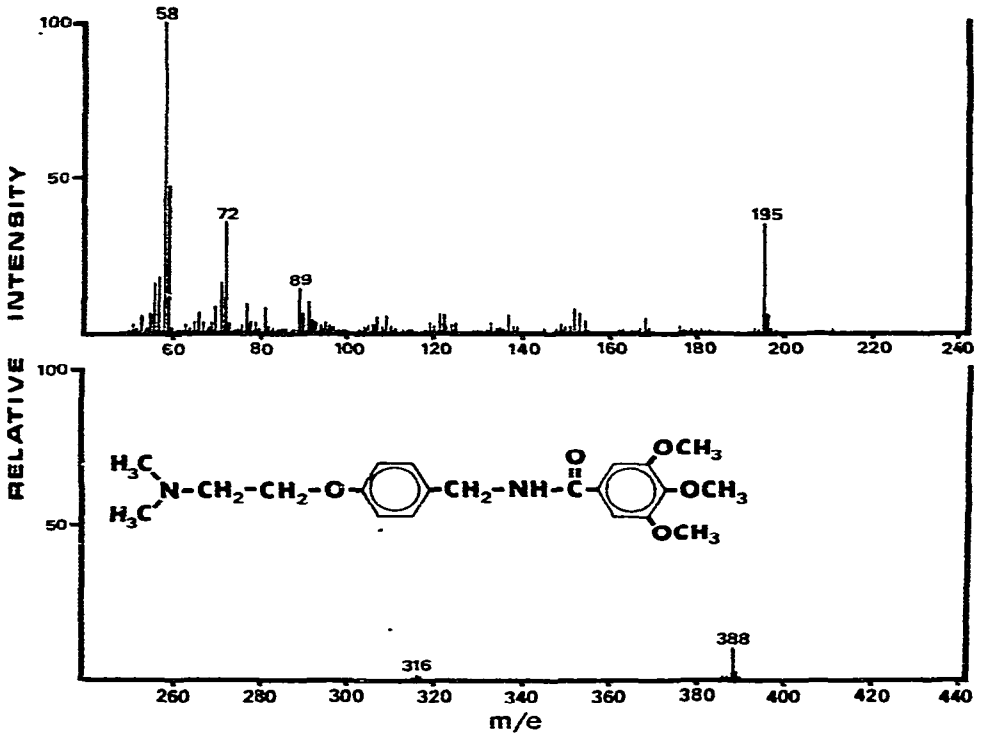


Fig. 1. EI mass spectrum of trimethobenzamide. The parent ion occurs at *m/e* 388, while the base peak at *m/e* 58 was used in quantitative analysis.

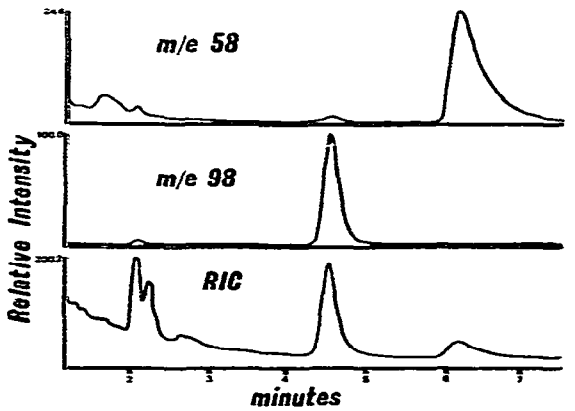
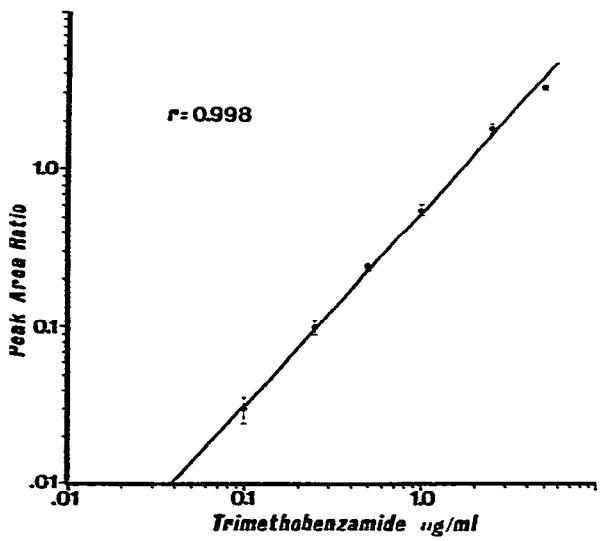


Fig. 2. Reconstructed ion current (RIC) plot and mass chromatograms of a 1 μg/ml TMB saliva standard. In the RIC tracing, the peak at 4.5 min retention time corresponds to the internal standard, THZ. The smaller peak with a retention time of 6.2 min is TMB. All peaks on the RIC plot represent all ions detected within the scanning range of 50—400 *m/e*. The two upper tracings show the *m/e* 58 (TMB) and *m/e* 98 (THZ) ion chromatograms, respectively, which were used in peak area ratio calculations.

A standard curve of base peak area ratios vs. known concentrations of TMB was prepared prior to analyses of the unknown specimens. Fig. 3 shows the curve used to quantitate TMB concentrations; the brackets indicate standard error of the mean from individual standards run each day the analysis was performed. Day-to-day precision of the 1 μg/ml saliva standard exhibited a coefficient of variation of 8% within observed base peak area ratios. Double ether extractions were used to increase the amount of TMB extracted from 40.5% with a single extraction to 60.6% of total TMB with two successive extractions. Ultimate sensitivity of the assay for TMB was not determined. However, for the lowest standard concentration (0.1 μg per ml TMB in saliva) the signal-to-noise ratio of the *m/e* 58 peak was typically greater than 63. The stability of TMB in solution and in normal saliva was checked and no deterioration of the drug was detected after one month at −60°C.

Pharmacokinetics

Saliva samples were analyzed and the observed concentrations were subjected to pharmacokinetic data analysis with the ESTRIP program of Brown and Manno [6]. Computerized fitting of the observed mean drug concentrations with sampling time interval produced the curve shown in Fig. 4. A two-compartment open model with linear absorption provides the polyexponential curve that best fits the data (r^2 = 0.956). Analysis of the function describing this curve provides the characteristic pharmacokinetic coefficients and ex-



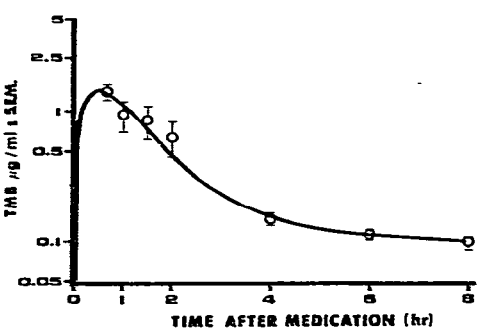


Fig. 3. TMB standard curve. The graph represents mean values ± S.E.M. of observed area ratios from known concentrations of TMB in saliva. The correlation coefficient (r = 0.998) indicates a high degree of linearity in the curve over the concentration range studied.

Fig. 4. Mean saliva TMB concentrations at each sampling interval. The open circles and associated brackets represent the mean ± S.E.M. saliva concentration of all subjects (n = 5). The curve through the concentration data points is the computer-generated polyexponential equation that best fits these mean concentration data.

ponents necessary for determining the drug distribution parameters. These calculated factors and the derived salivary half-lives of TMB are summarized in Table I. The elimination half-life for TMB from saliva appears to be 12.2 h.

TABLE I

MEAN SALIVARY PHARMACOKINETIC PARAMETERS OF TRIMETHOBENZAMIDE IN FIVE VOLUNTEERS AFTER A SINGLE 200-mg INTRAMUSCULAR INJECTION*

Subscript	Coeff. (A)	Exp. (B)	$t_{1/2}$**	Data points used***
1	0.157	0.057	12.2	2
2	3.941	1.231	0.56	4
3	−4.098	3.249	0.21	2
r^2 =0.956§				

*Based upon the following triexponential equation: $C_t = A_3 \exp(-B_3 t) + A_2 \exp(-B_2 t) + A_1 \exp(-B_1 t)$ ($B_1 = \beta; B_2 = \alpha; B_3 = k_{abs}$).

**Half-lives expressed in hours.

***Number of data pairs used in calculation of particular subscripted coefficient and exponent (counted backward beginning with last data).

§The r^2 value (squared correlation coefficient) provides an estimate of the fit between the calculated polyexponential curve and observed mean concentration—time values.

DISCUSSION

The current literature contains very few references [7—9] for the analysis of TMB. Indeed, there are no published accounts for the quantitative analysis of this drug in biological fluids. Since there are so little analytical data available regarding this clinically useful drug, our approach to its analysis by GC—MS, using the most fundamental methodology, was considered to be generally most useful. The use of THZ as an internal standard appears to be appropriate in this assay. While deuterated derivatives may possess some advantages as internal standards in GC—MS analyses, their lack of general availability can be a problem for other analysts. A phenothiazine, THZ, was selected for use since it is readily available and the likelihood of its concomitant use with TMB is small. Use of multiscan EI data acquisition in this assay is suitable because the therapeutic drug levels observed are sufficiently high to be easily detected by this type of GC—MS analysis and, because of the possibility of encountering this drug in cases of general drug screening (where EI is generally the instrumental mode employed), the spectrum and retention time reported may be of greatest interest. Derivatization of samples was unnecessary as the chromatographic analysis described was adequate for underivatized drugs, and drug screening techniques usually do not incorporate derivatizing steps. It must be pointed out, however, that our reported analytical technique is probably unsuitable for serum specimens since cholesterol and THZ have approximately equal retention times with our chromatographic conditions and therefore profound interference may be expected with quantitation based upon this internal standard. Some preliminary experiments in this laboratory indicate

that methoxypromazine may be a suitable internal standard for both serum and saliva samples. Using the assay conditions described here, we have found no interfering peaks due to the following clinically used antiemetics: prochlorperazine (Compazine®), thiethylperazine (Torecan®), trifluprομazine (Vesprin®), chlorpromazine (Thorazine®).

The pharmacokinetic analysis of mean saliva levels provides an estimate of the disposition of TMB in humans. Our reported analyses, in terms of both biological samples examined and kinetics, were far from exhaustive but were intended, rather, to provide some basic preliminary information regarding this drug. Extensive derivation of the more useful pharmacokinetic parameters awaits a more detailed and comprehensive examination of TMB disposition. There is a clear need for further study of TMB and we anticipate more fully characterizing the relationship between salivary and serum TMB kinetics at a later date.

ACKNOWLEDGEMENT

This work was supported in part by Beecham Laboratories, Bristol, TN, U.S.A.

REFERENCES

1 J.C. Mucklow, M.R. Bending, G.C. Kahn and C.T. Dolley, Clin. Pharmacol. Ther., 24 (1978) 563.
2 P.R.A. May, T. Van Patten, D.J. Jenden and A.K. Cho, Arch. Gen. Psychiat., 35 (1978) 1091.
3 G.J. DiGregorio, A.J. Pirano and E. Ruch, Clin. Pharmacol. Ther., 24 (1978) 720.
4 K.E.L. McColl, B. Whiting, M.R. Moore and A. Goldberg, Clin. Sci., 56 (1979) 283.
5 R. Ryhage and H. Brandenberger, Biomed. Mass Spectrom., 5 (1978) 615.
6 R.D. Brown and J.E. Manno, J. Pharm. Sci., 67 (1978) 1687.
7 K.W. Blessel, B.C. Budy and B.Z. Senkowski, in K. Florey (Editor), Analytical Profiles of Drug Substances, Vol. 2, Academic Press, New York, 1973, p. 551.
8 J.A. McIntyre, M. Black, M.E. McIntosh and J.K. Lynn, Clin. Chem., 24 (1978) 171.
9 H.G. Nowicki, J. Forensic Sci., 21 (1976) 154.