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Note

Gas chromatographic determination of tris(hydroxymethyl)aminomethane in pharmaceutical preparations after silylation

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Tris(hydroxymethyl)aminomethane (Tris) is frequently used for therapeutic purposes as an alkylinizing buffer and in clinical acid–base disturbances, and is used for the treatment of respiratory, metabolic and diabetic acidosis[1]. The buffer properties of this compound are interesting and have been applied in many chemical reactions[2].

Several methods have been developed for Tris determination. Non-chromatographic methods include a radioactive assay[3] using Tris labelled with ^{14}C, various colorimetric methods based on oxidation of Tris by potassium dichromate[4,5], by alkaline periodate with release of ammonia followed by the use of a Conway cell[6] and by periodic acid and determination of the formaldehyde formed with chromotropic acid[7], a time-consuming diazotation reaction[8] and other colorimetric methods[9,10].

Chromatographic methods include a high-performance thin-layer chromatographic method[11] and a gas chromatographic (GC) method using benzoylation of the three hydroxy groups and of the primary amine group[12].

The method proposed here is based on trimethylsilylation of the hydroxy groups of Tris. The silylated product is then analysed by GC with flame-ionization detection. The quantitative study was completed by the structural analysis of the silyl derivative of Tris by mass and NMR spectrometry.

EXPERIMENTAL

Reagents

The reagent for silylation, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), was obtained from Pierce. A standard solution of THAM was supplied by Lederlé (Oullins, France). The internal standard was dimethyl phthalate. The pharmaceutical form studied was a 13.5 mg/ml injectable aqueous solution of Tris.

Apparatus

A Model 5710 A gas chromatograph (Hewlett-Packard) equipped with a

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flame-ionization detector was used. The glass column (2 m × 2 mm I.D.) was packed with 3% OV-17 on Chromosorb W AW DMCS (100–120 mesh). The column was conditioned at 250°C for 16 h with nitrogen at a flow-rate of 50 ml/min. The results were calculated using a Hewlett-Packard 3385 A electronic integrator. Separations were performed with oven temperature programming from 120 to 160°C at 5°C/min. The other operating conditions were as follows: injection port temperature, 250°C; detector temperature, 250°C; carrier gas (nitrogen) flow-rate, 40 ml/min; hydrogen flow-rate, 30 ml/min; air flow-rate, 250 ml/min. Mass spectra were obtained using a VG Micromass 70-70 F mass spectrometer in the electron impact (EI) mode (70 eV) coupled to a Perkin Elmer Sigma 3B gas chromatograph (GC–MS).

Samples of the silyl derivative were analysed using a direct introduction probe on the above mass spectrometer working in the chemical ionization (CI) mode with ammonia as reagent gas and a trap current set at 200 μA.

NMR analysis were carried out on a Bruker spectrometer operating at room temperature and 80 MHz. Samples were dissolved in deuteriomethanol.

Preparation of products for analysis

After evaporation to dryness of 5 ml of an aqueous solution of Tris, 5 ml of the solution of an internal standard (1.65 ml of dimethyl phthalate in 10 ml of methanol) were added. The solution was dried over anhydrous sodium sulphate and filtered using a Whatman 42 filter.

A 0.2-ml volume of the solution was introduced into a conical silylation tube and 1 ml of BSTFA was added rapidly. The reaction of silylation is immediate at room temperature. The silyl derivative is fairly stable and can be stored at 4°C until taken for GC analysis. A 0.5-μl portion of the silylated product is injected into the gas chromatograph.

Structural analysis of derivatives

CI and EI mass spectra of the silyl derivative of Tris are shown in Figs. 1 and 2, respectively. The first spectrum shows a peak at *m/z* 338 which corresponds to the quasi-molecular ion $(M + H)^+$. The structure of this derivative corresponds to a compound containing three trimethylsilyl groups.

According to this ionization process, the two main fragment ions are recorded at *m/z* 194 and 266, which is the base peak. These two ions are formed from the trimethylsilylated molecule and correspond to the loss of one and two TMS groups, respectively. From each of the ions the loss of the fragment [CH_2–OTMS + H] (104 daltons) was observed, giving rise to ions at *m/z* 234 (*m/z* 338 − 104), 162 (*m/z* 266 − 104) and 90 (*m/z* 194 − 104).

The mass spectrum of the derivative using EI ionization shows a base peak at *m/z* 234, which corresponds to the disilylated fragment ion. The molecular ion at *m/z* 337 cannot be seen with this ionization mode.

The NMR spectra confirm the presence of three trimethylsilyl groups in the molecule. Fig. 3a showd the NMR spectrum of Tris with tetramethylsilane (TMS) as internal standard, and Fig. 3b the spectrum of the silyl derivative recorded without TMS in order to avoid interferences with the silyl groups. The integration curve between 0 and 0.030 ppm shows the presence of 27 protons, corresponding to three trimethylsilyl groups.

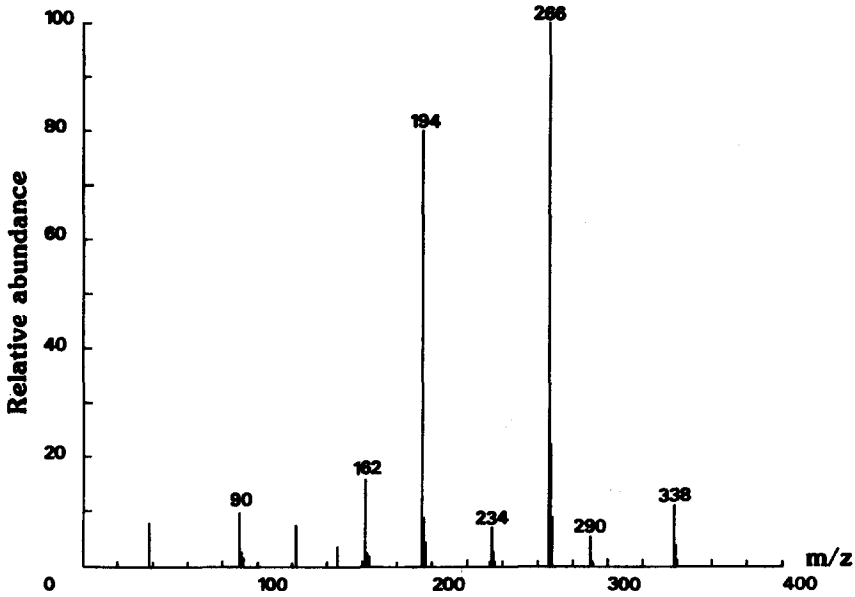

Fig. 1. Mass spectrum of the silyl derivative of Tris under CI conditions.

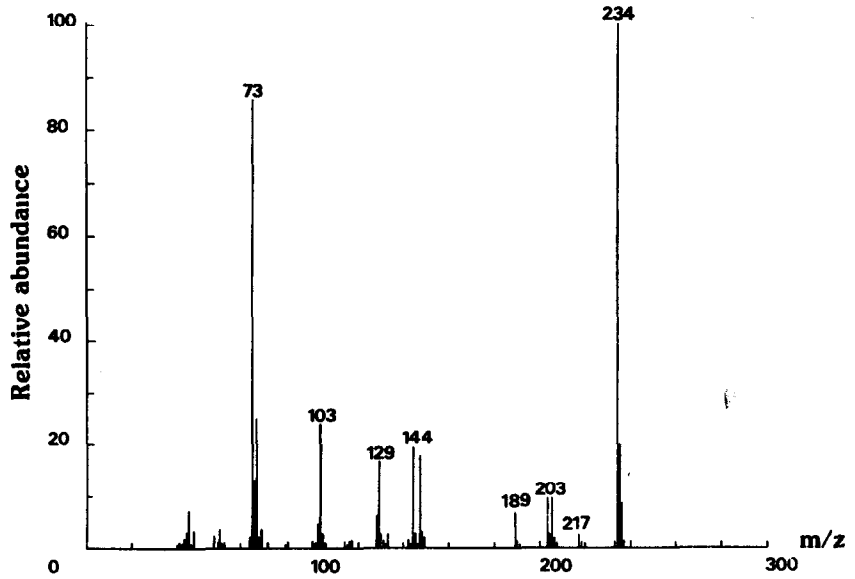

Fig. 2. Mass spectrum of the silyl derivative of Tris under EI conditions.

In the range 3.35–3.60 ppm in both spectra, a signal corresponding to six methylene protons can be observed. For the native molecule (Fig. 3a) the zone from 4.7 to 4.9 ppm shows the presence of five protons (3 hydroxy groups and one amino group).

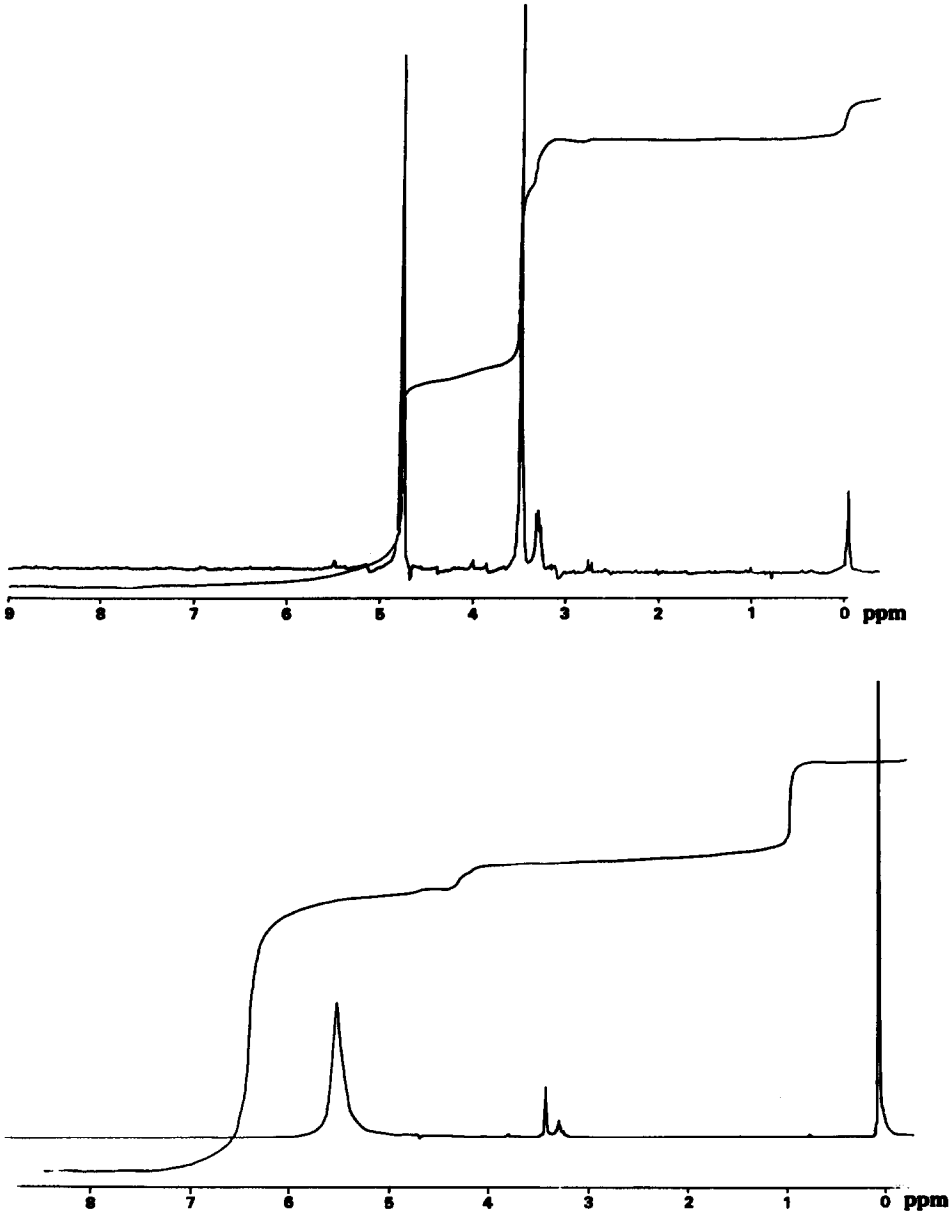

Fig. 3. (a) NMR spectrum of Tris (TMS as internal standard; (b) NMR spectrum of the silyl derivative of Tris (without internal standard).

It can be concluded that the compound determined by GLC after silylation corresponds to a tris(trimethylsilyl) derivative of Tris.

Chromatographic analysis

The retention times for the trimethylsilylated product and dimethylphthalate (internal standard) were 3.64 and 8.67 min, respectively. Blanks extracted by the same procedure and injected into the gas chromatograph did no show any interfering peak at the corresponding retention times (Fig. 4).

Linearity

The linearity of the method was checked by injecting five samples of Tris at concentrations of 5, 10, 13, 15 and 20 mg/ml, each sample being injected five times. Standardization was performed using the internal standard method. Linear regression analysis of peak-height ratios *versus* concentration indicated a good linear fit of the data (Fig. 5): slope 0.074, intercept -0.0208, $r = 0.9979$. The same procedure carried out with the same concentrations of Tris from injectable preparations gave the following results: slope 0.078, intercept -0.051, r: 0.9982.

Precision

The precision of the method was defined by its repeatability and reproducibility. In order to determine the repeatability of the assay the coefficient of variation was calculated by injecting a sample containing 13.5 mg/ml ten times, and was found to be 2.73%.

The reproducibility was determined over 3 days by injecting three times each morning and evening a solution containing 13.5 mg/ml of THAM. Analysis of variances was carried out, giving the results in Table I.

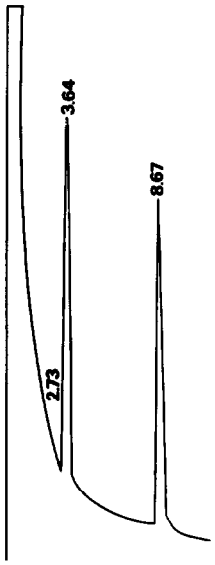

Fig. 4. Chromatographic separation of Tris (retention time = 3.64 min) and dimethylphthalate (retention time = 8.67 min).

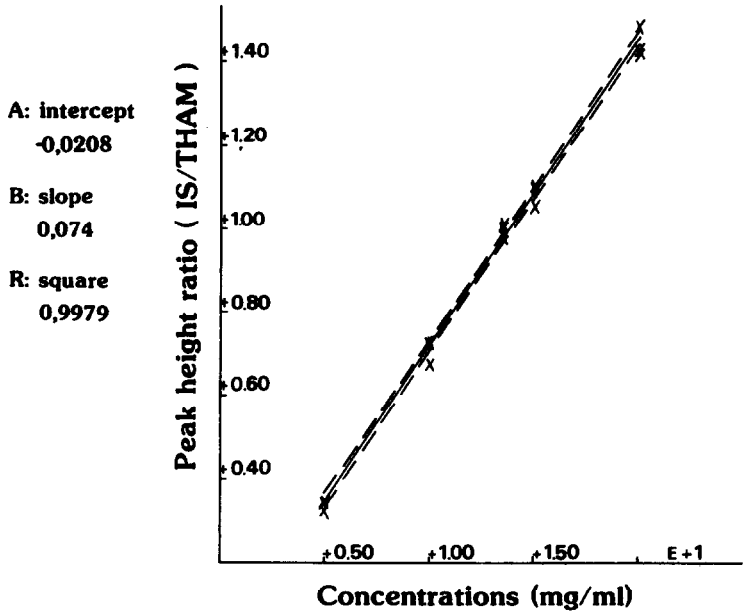

Fig. 5. Calibration line with confidence interval for Tris determination.

Using the Snedecor test the tabulated value for $F(4,10)$ was 3.48 whereas the calculated value was 1.5. This result indicates no significant difference between the different injections, if the reproducibility is good.

Accuracy

The accuracy of the method was determined by injecting solutions containing theoretical concentrations of THAM of 5, 10, 15 and 20 mg/ml five times. Each result calculated from the calibration graph was compared with the true value. The inaccuracy is given by the equation $I = (\Delta x/x)100$, the accuracy (A) being $A = 100 - I$. The mean accuracy was 98.04%.

Confidence interval of assay

Ten commercial aqueous solution solutions of Tris containing 13.5 mg/ml were each injected twice into the gas chromatograph. The results obtained were compared

TABLE I

PRECISION: RESULTS OF ANALYSIS OF VARIANCE

Source	*Sum of squares*	*Degrees of freedom*	*F**
Inter-column	$1.08 \cdot 10^{-2}$	4	
Residual	$1.80 \cdot 10^{-2}$	10	1.5
Overall	$2.88 \cdot 10^{-2}$	14	

* F = Snedecor's test.

with those given by a standard solution at 13.497 mg/ml. A *t*-test was carried out in order to compare the mean experimental results with the reference value for the standard solution. The values obtained were t (calculated) = 0.3225 and t (tabulated) = 2.093. There was no significant difference between the reference value of the standard solution and the mean result for the ten commercial solutions.

CONCLUSION

The GC method described here provides a sensitive quantitative assay for Tris in pharmaceutical preparations. The derivatization of the hydroxy groups is complete and produces a derivative containing three trimethylsilyl groups. The method is linear, specific and precise and is suitable for the analytical control of Tris solutions.

REFERENCES

1 G. G. Nahas, *Pharmacol. Rev.*, 14 (1962) 447.
2 R. Bates and V. Bower, *Anal. Chem.*, 28 (1956) 1322.
3 G. G. Nahas and M. H. Holmdahl, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 20 (1961) 75.
4 H. Rosen, *Ann. N.Y. Acad. Sci.*, 92 (1961) 414.
5 J. Strauss, K. Bernath and S. A. Kaplan, *Proc. Soc. Exp. Biol. Med.*, 113 (1963) 414.
6 S. Linn and H. Roberts, *Ann. N.Y. Acad. Sci.*, 92 (1961) 419.
7 B. Zaar and A. Grönwall, *Scand. J. Clin. Lab. Invest.*, 13 (1961) 588.
8 A. Kanarek and M. Tal, *Anal. Biochem.*, 57 (1974) 78.
9 S. R. Chapman, L. M. Smith and D. H. Simmons, *J. Lab. Clin. Med.*, 66 (1965) 698.
10 I. M. Beaumont and A. C. Mehta, *Proc. Anal. Div. Chem. Soc.*, 15 (1978) 339.
11 G. Andermann and C. Andremann, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 36.
12 G. Hulshoff and H. B. Kostenbauder, *J. Chromatogr.*, 145 (1978) 155.