Journal of Chromatography, 120 (1976) 491–495

CHROM. 8949

Note

Quantitative morphine assay by means of gas–liquid chromatography and on-column silylation

KNUT E. RASMUSSEN

Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Oslo, Blindern, Oslo 3 (Norway)

(Received November 26th, 1975)

It is known that the polarity associated with phenolic alkaloids such as morphine causes adsorption on the gas chromatographic column leading to non-linear and variable quantitative results at low concentration. This problem can be partly overcome by careful deactivation of the solid support[1], and it can be eliminated completely if the drug is converted into a suitable non-polar derivative before entering the column.

The acetyl, methyl and the trimethylsilyl derivatives are used extensively for gas chromatography. These derivatives are formed either off-column or on-column. The technique of on-column derivatization was introduced by Anders and Mannering[2,3]. The acetyl derivative of morphine (heroine) was formed by injecting the drug solution and acetic anhydride into the gas chromatograph[4]. The trimethylsilyl derivative was formed by injecting a mixture of bis(trimethylsilyl)acetamide (BSA) and the drug[5]. Trimethylanilinium hydroxide was used as the reagent for flash heater methylation of morphine to form codeine[6]. Except for the flash heater methylation technique, on-column derivatization methods have been used primarily to provide additional data for drug identification while off-column derivatization methods have been used most widely in quantitative analysis.

The purpose of the present investigation is to show that on-column silylation methods may successfully be used in quantitative analysis. A method for on-column silylation of morphine has been developed. Bis(O-trimethylsilyl)morphine was formed quantitatively and reproducibly by injecting the silylating reagent, trimethylsilylimidazole, and the drug solution simultaneously into the gas chromatograph.

EXPERIMENTAL

Chemicals

Ethyl acetate (E. Merck, Darmstadt, G.F.R.) was used for the extraction of morphine and to prepare the test solutions. *n*-tetracosane (C_{24}) (Koch-Light, Colnbrook, Great Britain) was used as internal standard for the determination of bis(O-trimethylsilyl)morphine (M-TMS). Trimethylsilylimidazole (Supelco, Bellefonte, Pa., U.S.A.) was used as silylating reagent. Morphine hydrochloride was obtained from Norsk Medisinaldepot (Oslo, Norway).

GC–MS analysis

Gas chromatography–mass spectrometry (GC–MS) analysis was carried out using a Varian Aerograph 1400 gas chromatograph connected to a Varian MAT CH7 mass spectrometer. The column was a glass coil (6 ft. × 1/8 in.) filled with 3% SE-30 on 80–100 mesh Gas-Chrom Q. The column temperature was 260° and the injector temperature was 275°. Two test solutions containing 1 mg/ml morphine and 1 mg/ml codeine, respectively, were separately studied. 1 μl of test solution was drawn into the syringe followed by 2 μl of trimethylsilylimidazole and injected into the gas chromatograph.

Gas-liquid chromatographic (GLC) analysis

A Fractovap 2300 gas chromatograph (Carlo Erba, Milan, Italy) with a flame ionization detector (FID) was used for the quantitative morphine assays. The column was a glass coil (2 m × 2 mm I.D.) filled with 3% Dexil 300 on 100–120 mesh Supelcoport. Nitrogen was used as carrier gas at a flow-rate of 30 ml/min. The sensitivity setting was 10 × 128. The flash heater temperature was 275° and the column temperature 250°.

Reproducibility of quantitative analysis after derivatization

A test solution containing 1 mg/ml morphine and 0.5 mg/ml C_{24} was prepared. 1 μl of this solution was drawn into a syringe (Hamilton 701-N) followed by 2 μl of trimethylsilylimidazole. The mixture was injected into the gas chromatograph and the peak height ratio M-TMS: C_{24} was calculated. Ten such analyses were carried out and the coefficient of variation was calculated.

Procedure for the analysis of morphine in extracts of opium

200 μl of drug solution were accurately transferred to a glass-stoppered centrifuge tube by means of a Hamilton syringe 750-N. 100 μl of 1 *N* sodium carbonate solution and 5.00 ml of ethyl acetate containing 1.5 mg C_{24} as internal standard was added to the tube. The tube was rotated for 30 min using a rotary mixer (Cenco Instruments). The organic phase was filtered into another centrifuge tube through a funnel containing anhydrous sodium sulphate. The filter was washed with ethyl acetate (2 × 1 ml) and the combined ethyl acetate extracts were evaporated to dryness under a stream of nitrogen at 60°. The residue was dissolved in 1 ml ethyl acetate, and 1 μl was drawn into the syringe followed by 2 μl trimethylsilylimidazole and injected into the gas chromatograph. The peak height ratio M-TMS: C_{24} was calculated and the concentration of morphine determined by using a calibration graph. The calibration graph was obtained by treating 200 μl of standard solutions containing 2–15 mg/ml morphine hydrochloride in water as described above. The accuracy of the procedure was checked by analysing ten samples of a standard extract which had been preanalysed in three laboratories by a gravimetric method[7] and were known to contain 14.9 mg/ml morphine.

RESULTS AND DISCUSSION

By using on-column silylation less time is needed for forming derivatives and the reagent cost is greatly reduced since only 2 μl of the reagent is used for each

analysis. Furthermore, there is no possibility of hydrolysis of the derivatives which may occur in damp atmospheres, necessitating the use of special precautions to avoid moisture. To verify the formation of the silyl derivatives of morphine and codeine a GC–MS investigation was carried out. The investigation showed that bis(O-trimethylsilyl)morphine and O-trimethylsilylcodeine was formed. No morphine and codeine could be detected and it was concluded that the reaction was complete. The average peak height ratio M-TMS: C_{24} was 0.994, and the coefficient of variation was 1.3%. This result shows that the derivatization method is well suited for quality control in pharmaceutical analysis. A coefficient of variation of a similar magnitude has been found by Rasmussen[8] for on-column silylation of cannabinoids.

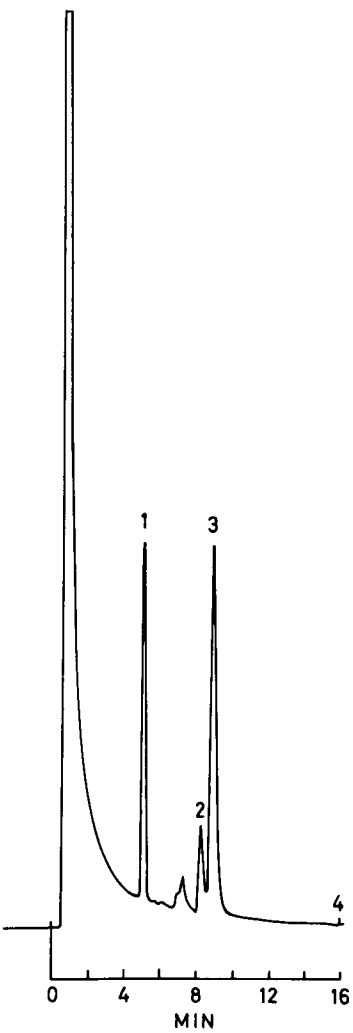

Fig. 1. Gas chromatogram of alkaloids in opium after on-column silylation. 1 = Tetracosane (internal standard); 2 = O-trimethylsilylcodeine; 3 = bis(O-trimethylsilyl)morphine; 4 = retention time of thebaine (present as a minor constituent in opium).

A Dexil 300 column was used for the quantitative analysis of morphine in extracts of opium. On this column bis(O-trimethylsilyl)morphine, O-trimethylsilylcodeine and thebaine were well separated and the alkaloids eluted as narrow peaks without tailing (see Fig. 1). Bis(O-trimethylsilyl)morphine and thebaine eluted as one single peak on an SE-30 column and bis(O-trimethylsilyl)morphine and O-trimethylsilylcodeine were difficult to separate completely on an OV-17 column. Fig. 2 shows the calibration curve for the determination of morphine in extracts containing 2–15 mg/ml morphine. The calibration curve was a straight line passing through the origin. Analysis of a standard extract of morphine (14.9 mg/ml) gave an average content of 15.0 mg/ml and a coefficient of variation of 1.4%. On the basis of these results it was concluded that GC and on-column silylation is a rapid and reliable method for the analysis of morphine in pharmaceutical preparations.

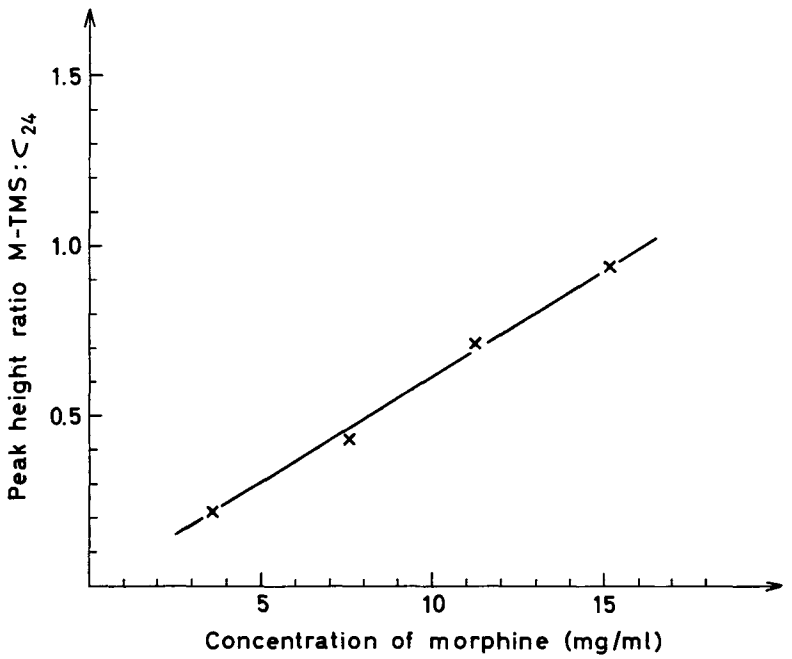

Fig. 2. Calibration curve for the determination of morphine in extracts of opium containing 2–15 mg/ml morphine.

ACKNOWLEDGEMENT

The author acknowledges Mr. Per E. Helland, Department of Clinical Biochemistry, Rikshospitalet, Oslo, Norway for obtaining the mass spectra.

REFERENCES

1 H. V. Street, *J. Chromatogr.*, 37 (1968) 162.
2 M. W. Anders and G. J. Mannering, *Anal. Chem.*, 34 (1962) 731.

3 M. W. Anders and G. J. Mannering, in A. Stolman (Editor), *Progress in Chemical Toxicology*, Vol. 3, Academic Press, London, New York, 1967.
4 S. J. Mulé, *Anal. Chem.*, 36 (1964) 1907.
5 H. V. Street, *J. Chromatogr.*, 41 (1969) 358.
6 E. Brockmann-Hanssen and T. O. Oke, *J. Pharm. Sci.*, 58 (1969) 371.
7 *Pharmacopoea Nordica*, Vol. 1, Universitetsforlaget, Oslo, 1963, p. 122.
8 K. E. Rasmussen, *J. Chromatogr.*, 114 (1975) 250.