Journal of Chromatography, 105 (1975) 377–381

CHROM. 8059

RAPID AND SENSITIVE GAS CHROMATOGRAPHIC DETERMINATION OF DIACETYLMORPHINE AND ITS METABOLITE MONOACETYLMORPHINE IN BLOOD USING A NITROGEN DETECTOR

D. A. SMITH and W. J. COLE

University Department of Anaesthetics, University Hospital of South Manchester, Manchester M20 8LR (Great Britain)

(Received September 13th, 1974)

SUMMARY

A quantitative gas chromatographic method for the determination of plasma concentrations of diacetylmorphine and its metabolite monoacetylmorphine using an alkali flame detector (nitrogen detector) is described. Plasma samples (pH 9.0) containing ethylmorphine acetate as internal standard are extracted with benzene. The dried benzene extracts are analysed as their corresponding acetylated derivatives following treatment with trifluoroacetic anhydride–benzene (1 : 5). The nitrogen detector permits quantitation of narcotic levels down to 100 ng/ml with detection as low as 20 ng/ml. The higher sensitivity and selectivity of the nitrogen detector are compared to those obtained in flame ionization detection. Species differences in the rate of conversion of diacetylmorphine to monoacetylmorphine *in vitro* in blood are also presented.

INTRODUCTION

Diacetylmorphine (heroin, diamorphine) is rapidly deacetylated in most species to 6-monoacetylmorphine (MAM). Enzymes responsible for this hydrolysis are found in most tissues and blood[1]. Owing to its high potency, diacetylmorphine is administered only in low doses. Conventional gas chromatographic (GC) systems with a flame ionization detector (FID) lack the sensitivity required for the determination of low plasma concentrations of diacetylmorphine and its metabolites. The nitrogen detector (alkali FID) affords an increase in both selectivity and sensitivity over conventional flame ionization detection of nitrogen-containing compounds[2]. The use of this detector in the analysis of the barbiturates thiopentone[3] and hexobarbital[4], caffeine[5], and the analgesic pentazocine[6] has been reported.

This paper reports a method for the determination of diacetylmorphine and MAM in blood.

MATERIALS AND METHODS

Materials

Diacetylmorphine·HCl was obtained from May and Baker (Dagenham, Essex, Great Britain). Ethylmorphine·HCl (Macfarlane-Smith, Edinburgh, Great Britain) was converted to the corresponding acetate by reaction with acetic anhydride–pyridine (1:1) (BDH, Poole, Dorset, Great Britain) in excess for 2 h at 70°; the acetate was recrystallized from ethyl alcohol. Authentic MAM was obtained as a gift from Dr. S. J. Mulé (Narcotic Addiction Control Commission, New York, N.Y., U.S.A.). MAM was converted to the corresponding trifluoroacetate standard (MAMTFA) by heating for 1 h at 50° with excess trifluoroacetic anhydride (Pierce, Rockford, Ill., U.S.A.)–benzene (1:5). Analytical grade benzene and ethyl acetate (BDH) were further purified by redistillation.

Extraction procedure and sample preparation

Blood (1.0 ml) was pipetted into a centrifuge tube containing ethylmorphine acetate as internal standard (normally 0.5–3.0 μg), and adjusted to pH 9.0 by addition of 0.1 *M* glycine–NaOH buffer (1.0 ml). After addition of benzene (2.0 ml) the mixture was well shaken, separated by centrifugation (3000 *g* for 5 min) ,and the upper layer transferred to a 3.0-ml screw-top vial (Reacti-Vial, Pierce). Following evaporation to dryness at 50° in a stream of nitrogen, the extract was reacted with trifluoroacetic anhydride (10 μl) and benzene (50 μl) for 1 h at 50°. The reaction mixture (2-μl aliquots) or, if necessar y,ethyl acetate dilutions were then injected into the gas chromatograph.

Gas chromatography

For GC of samples a Pye Series 104 chromatograph equipped with a Pye heated nitrogen detector head unit was used. The glass column (213 cm × 0.4 cm I.D.) was packed with 2% OV-17 (Phase Separations, Queensferry, Flints., Great Britain) coated onto Diatomite C-AW 100–120 mesh (Pye Unicam, Cambridge, Great Britain). The column temperature was maintained at 250° and the detector temperature at 300°. The nitrogen carrier gas flow-rate was 65 ml/min and the air flow-rate 240 ml/min. The hydrogen flow-rate (approximately 24 ml/min) was optimized each day.

RESULTS

GC sensitivity and selectivity

Prior to the use of an alkali FID the hydrogen flow-rate needs to be optimized for maximum sensitivity. The adjustment, which was carried out daily, involves stepwise increases in hydrogen flow-rate whilst monitoring sensitivity (peak height) for standard solutions of narcotics. A typical calibration graph for hydrogen flow-rate *versus* sensitivity is presented in Fig. 1 for standard injections of 80 ng diacetylmorphine and 68 ng ethylmorphine acetate. MAMTFA shows a similar profile to diacetylmorphine and ethylmorphine acetate.

The detector response to ethylmorphine acetate, diacetylmorphine and MAMTFA was linear over the range illustrated in Fig. 2 once the detector was optimized. The retention times in our system for MAMTFA, ethylmorphine acetate and diacetylmorphine were 2.1, 4.1, and 6.7 min, respectively.

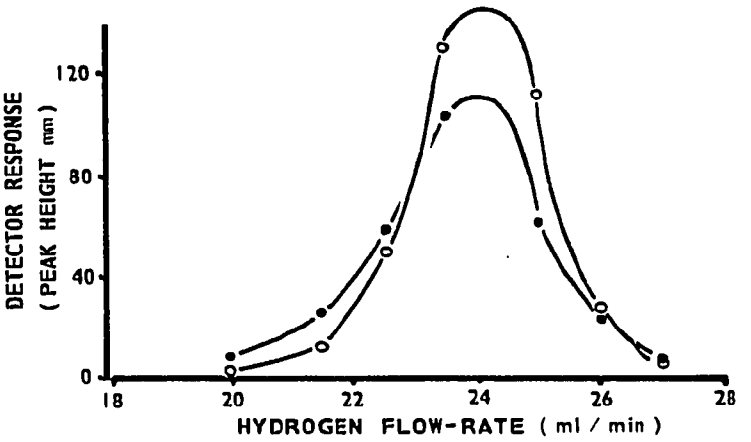

Fig. 1. Influence of hydrogen flow-rate on detector response for diacetylmorphine (●—●) and ethylmorphine acetate (○—○).

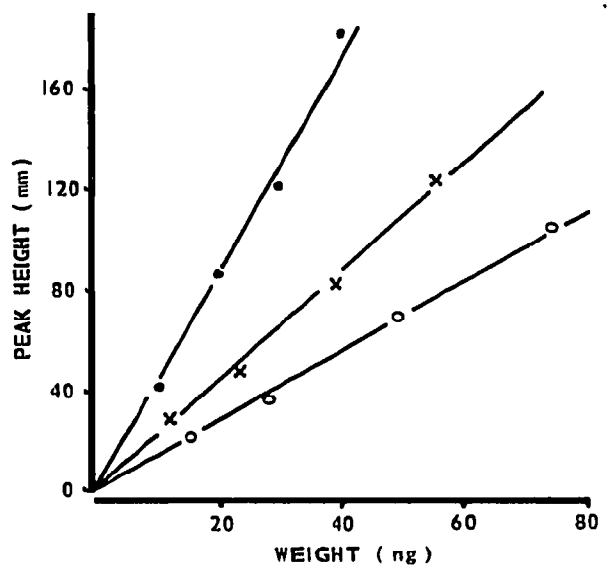

Fig. 2. Nitrogen detector response to diacetylmorphine (○—○), ethylmorphine acetate (×—×) and MAMTFA (●—●).

When the nitrogen detector was not in actual use longer crystal life and faster equilibrium upon lighting the flame were obtained by removing the crystal tip from the holder and storing it in the heated GC oven.

Performance of analytical procedure

The assay procedure was studied by recovery experiments utilising blood sample blanks. To these known amounts of diacetylmorphine and MAM were added. The samples were processed as outlined above, and the peak heights plotted against

those of the internal standard. Benzene was chosen as a solvent for these relatively lipophilic narcotics owing to the high extraction ratio of drug to interfering compound from blood. Ethylmorphine acetate was used as internal standard following assessment of a range of narcotic and narcotic derivative compounds. The pH of 9 was found to be optimal for extraction of the narcotics under investigation. The reproducibility of results obtained for diacetylmorphine and MAM over the range of concentrations expected in blood (0–3 μg/ml) was $\pm$ 5% and $\pm$ 7%, respectively; reproducibility was higher with higher blood levels. Derivatization of MAM to its trifluoroacetate was necessary as absorption of this compound occurred in the gas chromatograph when the free hydroxyl group was present, thus interfering with analysis.

Our studies have shown hydrolysis rates of diacetylmorphine to MAM by blood *in vitro* to be appreciable (rabbit 20–40 nmoles/ml/min, human 8–15 nmoles/ml/min, dog 2–4 nmoles/ml/min). The extraction procedure is therefore designed so that upon withdrawal of blood, extraction can proceed very quickly and minimize *in vitro* heroin decomposition. The narcotics are stable when in solution in benzene.

Diacetylmorphine and MAM levels in a dog following intravenous injection

We have studied the blood levels of diacetylmorphine and MAM in a dog (Irish greyhound, 25 kg) following intravenous (i.v.) injection of diacetylmorphine (16 mg) utilizing the assay outlined above. The results are reproduced as Fig. 3. Diacetylmorphine shows a very rapid decline as expected from *in vitro* experiments. There is no diacetylmorphine detectable in the blood after 3 min. MAM will presumably show a rise in level before our first sample time (1 min). The metabolite is detectable for 8 min, showing a slower rate of disappearance than the parent compound.

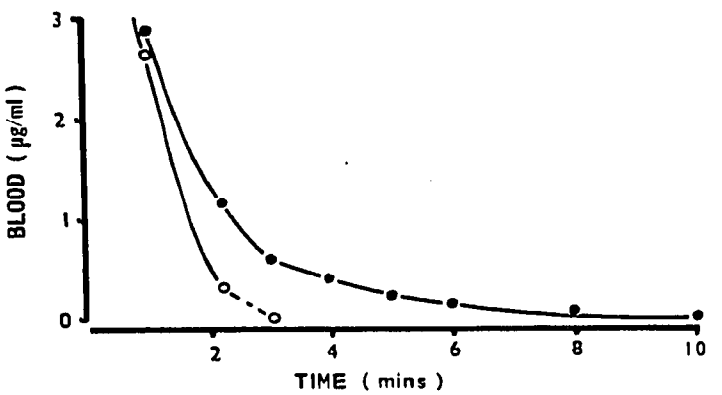

Fig. 3. Levels (*in vivo*) of diacetylmorphine (○—○) and MAM (●—●) in canine blood following i.v. injection of diacetylmorphine.

DISCUSSION

The method offers a rapid and accurate method for the determination of heroin and MAM in blood. To our knowledge, this is the first quantitative assay of MAM in blood. We have used the technique *in vivo* and *in vitro* for

some time with good results. Detection limits with conventional FIDs are reported as 0.05 mg/ml from illicit preparations for diacetylmorphine[7]; the nitrogen detector can quantitate levels of 100 ng/ml in blood and detection limits could be as low as 20 ng/ml for illicit preparations. Continuous infusion in man of diacetylmorphine has failed to show heroin or metabolites present in blood when monitored by an FID-equipped gas chromatograph[8].

While we have outlined the method for blood samples, a similar method could be used for quantitation of tissue concentration. The higher sensitivity afforded by the nitrogen detector towards narcotics could be of great value in forensic science, particularly in the case of diacetylmorphine.

ACKNOWLEDGEMENTS

The work was supported by grants from the Medical Research Council (W.J.C.) and Imperial Chemical Industries Ltd. (D.A.S.). We are indebted to Pye Unicam Ltd., Cambridge, for the extensive loan of an alkali FID. The technical assistance of Miss S. Prestwood and the secretarial work of Mrs. E. M. McCreery are highly appreciated.

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