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A rapid g.l.c. procedure for the determination of codeine and norcodeine in biological fluids based on micro-phase extraction techniques

WILLEM J. SERFONTEIN*, DEO BOTHA** AND LOUIS DE VILLIERS

*Department of Chemical Pathology and Department of Pharmacology***, *University of Pretoria, South Africa*

Therapeutic serum concentrations of codeine can be virtually completely extracted and obtained sufficiently concentrated for g.c. measurement without distillation, by acid extraction of the drug from the initial crude organic extracts of alkalinized serum followed by a micro-phase back extraction step. The procedure has simplicity, rapidity and eliminates concentration by distillation and background interference. The method can be readily adapted for the separate measurement of codeine and norcodeine and it can also be used for similar analyses of other body fluids.

Pharmacokinetic studies of the opium alkaloids have been greatly facilitated by recent analytical techniques, many of which are based on g.l.c. separations. (Brochmann-Hanssen & Furuya, 1964; Schmerzler, Yu & others, 1966; Yoshimura, Mori & others, 1970; Yeh & Woods, 1971). Artifacts in the g.l.c. of codeine and norcodeine have been reported which were found to be related to the extraction solvent used (Yeh & Woods, 1971). Yoshimura & others (1970) could not separate codeine and norcodeine by g.l.c. and an extraction procedure to separate the drug and its metabolites in urine was needed. Similar problems were experienced by Schmerzler & others (1966).

We present a simple and rapid analytical method, based on the micro-phase extraction for the determination of codeine in biological material. The micro-phase extraction of drugs was introduced by Ramsay & Campbell (1971) who showed that a number of lipophilic drugs can be effectively extracted from aqueous medium by using small volumes of chloroform (0·05–0·1 ml) at the appropriate pH values. The method has since been modified to include a wide variety of drugs and its usefulness greatly increased by the use of low density organic solvents and "micro-phases" consisting of small volumes of aqueous buffers at different pH values for the extraction of different classes of drugs from organic solvents into the buffer "micro-phases" (Serfontein, de Villiers & Botha, 1975). The principal advantage of the method is the elimination of distillation. Additionally, "cleaner" extracts and therefore greatly reduced background interference in chromatographic separations are obtained. An important potential disadvantage associated with the procedure is that a substantial portion of drug (depending on its lipophilic character) may not be extracted by the small volumes of organic phase used. However, with codeine, preliminary experiments showed that after saturating the aqueous phase (5 ml containing 10 μg of drug)

* To whom all correspondence should be addressed.

with sodium chloride and sodium bicarbonate, one extraction with chloroform–2-propanol (9:1) solvent removed more than 95% of the drug and no remaining codeine could be demonstrated in the aqueous phase by further extractions with organic solvents (Fig 1a, b). The method can be readily adapted to incorporate selective acylation as a means of separating codeine and norcodeine according to Schmerzler & others (1966).

MATERIALS AND METHODS

Phosphate buffer, pH 8·5. K_2HPO_4 (272 g) was dissolved in 900 ml of distilled water, the pH adjusted to 8·5 with 10 N NaOH and the solution diluted to 1000 ml.

Codeine butyrate internal standard. Codeine phosphate (14·17 mg) was dissolved in 5 ml of distilled water, 1·0 ml of phosphate buffer pH 8·5 added and the solution saturated with respect to NaCl and $NaHCO_3$ (finely ground solids) and finally extracted with ethyl acetate (5 ml + 5 ml, 5 min). All of the ethyl acetate phase was collected, dried briefly over anhydrous sodium sulphate and evaporated to dryness. The residue was dissolved in 0·2 ml of butyric acid anhydride and 0·3 ml of anhydrous pyridine. The residue was dissolved by swirling the solution and then heated for 60 min at 50° in a waterbath and diluted to 50 ml with chloroform–2-propanol (9:1). This solution contained 200 μg of codeine (as codeine butyrate) ml^{-1}.

Codeine standard solutions. Codeine phosphate (14·17 mg) was dissolved in distilled water and diluted to 500 ml (20 μg of codeine ml^{-1}). Standards were prepared by adding 0·0 to 2·5 ml of the above codeine solution (0–25 μg of codeine) to 5 ml portions of serum and analysed as below. A standard curve was drawn by plotting peak area ratios against concentration in the usual manner.

Gas chromatography. A Becker–Packard model 417 gas chromatograph equipped with a flame ionization detector and a 1 m silylated glass column (internal diameter 3 mm) packed with 3% OV17 on Supelcoport 100/120 mesh was used. Inlet block temp., 290°; detector temp. 290°; column temp. (isothermal) 250°; nitrogen, 40 ml min^{-1}, hydrogen, 20 ml min^{-1}, air, 300 ml min^{-1}.

Procedure for serum. One drop of an N NaOH solution and 2 ml of phosphate buffer solution (pH 8·5) were added to 5 ml serum sample (or standard). The solution was saturated with respect to finely powdered NaCl and $NaHCO_3$ and extracted once with 15 ml of chloroform–2-propanol (9:1). After centrifugation 10 ml of the chloroform phase was extracted with 4·0 ml of 0·05 N H_2SO_4 solution (10 min). The mixture was centrifuged and the acid phase transferred to a 10 ml ground glass stoppered tube, 0·3 ml of a N NaOH solution and 1·0 ml of phosphate buffer (pH 8·5) added and the solution saturated with respect to $NaHCO_3$ and NaCl (finely powdered solids). The mixture was centrifuged and exactly 3·0 ml of the clear supernatent transferred to a 10 ml nipple tube (ground glass stoppered tube with drawn out bottom portion). Fifty μl of internal standard-containing chloroform–2-propanol (9:1) were added and the mixture shaken on a Vortex mixer for 2 min with dispersion of the chloroform phase. After centrifugation, the chloroform phase which had collected in the lower, drawn out portion of the nipple tube, was transferred to a second, dry nipple tube and the solvent removed by evaporation on a rotary evaporator or in a nitrogen stream at 50°. The residue was dissolved in 10 μl of propionic anhydride and 20 μl of dry pyridine. After heating the solution for 40 min in a water bath at 40°, 3 μl were injected into the gas chromatograph.

RESULTS AND DISCUSSION

Fig. 1A illustrates a typical gas chromatogram obtained by analysing a 5 ml serum sample containing 2 μg of codeine ml^{-1}. Figure 1B was obtained by carrying out a second micro-phase extraction (50 μl of chloroform–2-propanol (9:1)) on the sample from which it can be seen that virtually all of the codeine was removed by a single extraction.

Recoveries of known quantities of codeine added to 5 ml serum samples were 94% ± 5% (average of 5 experiments).

The procedure can be readily adapted to determine codeine and norcodeine separately according to Schmerzler & others (1966). For serum analyses, it is convenient to remove norcodeine in the initial chloroform extract (before extracting the bases with 0·05 N sulphuric acid and after drying over anhydrous sodium sulphate) by mild acetylation as decribed by these authors. In this case pure chloroform (15 ml) was used for the initial extraction and a 2% solution (v/v) of acetic anhydride in

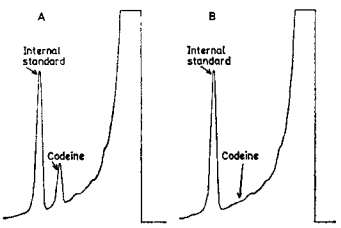

FIG. 1A. Gas chromatogram obtained during the analysis of a 5 ml serum sample containing 2 μg of codeine ml^{-1}.
B. Gas chromatogram obtained after a second micro-phase extraction of the sample described in Fig. 1a.

ethyl acetate added to the sodium sulphate dried chloroform solution using 0·05 ml of this solution ml^{-1} of chloroform extract. After 5 min at room temperature the solution was extracted with 4·0 ml of 0·05 N H_2SO_4 solution and analysed as described above.

The low level of background interference due to normal serum and urine compounds was reflected in a low and constant base line in the g.c. analyses. This allows maximal use of the sensitivity of the apparatus thus improving the overall sensitivity and precision of the method compared with older methods.

The method can be readily used for the analysis of codeine in other biological samples such as urine and tissue homogenates with obvious alterations in the procedure.

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