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Note

On-column acylation, heart-cutting and electron-capture detection of opiates

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Previous reports on on-column derivatization have shown that the method can be used successfully in quantitative drug analysis using flame-ionization detection[1–3]. The analysis of drugs in biological material, however, often requires electron-capture detection and derivatization reagents which make the compounds more sensitive for the electron-capture detector (ECD). It was the purpose of this investigation to develop a procedure for on-column perfluoroacylation of opiates and electron-capture detection of the drug derivatives. When on-column derivatization techniques are applied it has been shown that the use of perfluoroacetylated imidazoles offers considerable advantages[3]. The acylimidazoles produce stable volatile derivatives ideal for gas chromatography (GC) and with excellent electron-capture sensitivity[4,5]. N-(Heptafluorobutyryl)imidazole (HFBI) was used as the derivatization reagent in this investigation.

When working with on-column derivatization and electron-capture detection, the excess of reagent must be removed prior to entering the ECD otherwise it will depress the standing current and detector response long enough to interfere with the quantitation of the peaks of interest. Removal of excess of reagent is possible with a pre-column venting system. Pre-column venting systems of different constructions have earlier been used in GC to permit the injection of large sample volumes and to permit the direct introduction of the reaction mixture[6–10].

In this study a simple venting system was made which had a constant flow of carrier gas through the detector during the chromatographic run, which is especially important in GC–ECD. Codeine, morphine and ethylmorphine were used as test substances. A calibration graph was set up for codeine using ethylmorphine as the internal standard, a reproducibility test was carried out and the minimal detectable amounts of codeine and morphine were evaluated.

MATERIALS AND METHODS

Reagents

Morphine was supplied by Weiders Farmasøytiske A/S (Oslo, Norway). Codeine was of pharmacopoeial grade and supplied by Norsk Medisinaldepot (Oslo, Norway). Ethylmorphine, used as the internal standard, was extracted with diethyl ether from an aqueous alkaline solution of ethylmorphine chloride (pharmacopoeial

grade from Norsk Medisinaldepot). Analytical-reagent grade ethyl acetate and diethyl ether were obtained from E. Merck (Darmstadt, G.F.R.). HFBI in 1-ml ampoules was purchased from Regis (Chicago, Ill., U.S.A.). After they had been opened, the ampoules were kept tightly capped in an desiccator at 4°; the same ampoules could then be used for several weeks. No decomposition or contamination of the reagent was observed. Stock solutions containing codeine, morphine and ethylmorphine of concentration 1 mg/ml in ethyl acetate were prepared.

Gas chromatography

A Fractovap 2300 gas chromatograph (Carlo Erba, Milan, Italy) equipped with a ^{63}Ni ECD was used and modified as shown in Fig. 1. The glass pre-column (0.32 m × 3 mm I.D.) and the glass analytical column (1.3 m × 3 mm I.D.) were packed with 3% SE-30 on Supelcoport (80–100 mesh). Nitrogen was used as the carrier gas at a flow-rate of 50 ml/min through the pre-column and 40 ml/min through the analytical column when the valves were opened. The flow-rate through the system was 40 ml/min when the valves were closed. The injector temperature was 225° and the pre-column and the analytical column temperature was 200°. The detector temperature was 250°. The sensitivity setting was 128 × 10.

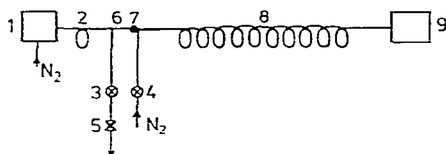


Fig. 1. Schematic diagram of gas chromatographic system. 1 = Injector block; 2 = pre-column; 3, 4 = toggle valves; 5 = needle valve; 6 = splitter union consisting of a stainless-steel tube with a glass tube inside and (7) a stainless-steel frit (2-μm pores); 8 = analytical column; 9 = detector. The sample was introduced on to the pre-column and the reagent was vented to the atmosphere through valves 3 and 5. Valves 3 and 4 were closed before the main fraction arrived at the splitter, permitting it to pass into the analytical column. Valves 3 and 4 were re-opened in order to vent the remaining reagent to the atmosphere.

Gas chromatography–mass spectrometry

GC–mass spectrometry (MS) was carried out using a Varian Model 112 combined gas chromatograph–mass spectrometer (Varian-MAT, Bremen, G.F.R.). The gas chromatograph was a Varian Model 1400 (Varian, Walnut Creek, Calif., U.S.A.) and the glass capillary column (LKB, Stockholm, Sweden) was wall-coated with SE-30 (12 m × 0.28 mm I.D.). A 1-μl volume of HFBI was drawn into the syringe (Hamilton 701 N), followed by 0.5 μl of the test solution, and the mixture was injected into the gas chromatograph.

Calibration graph

A calibration graph in the concentration range 1–10 μg/ml of ethyl acetate was constructed for codeine. The concentration of the internal standard was 10.0 μg/ml. A 1-μl volume of HFBI was drawn into a syringe (Hamilton 701 N), followed by 1 μl of the test solution, and the mixture was injected into the gas chromatograph; 110 sec after the injection, valves 3 and 4 were closed for 60 sec. The peak-height ratios

(codeine derivative to internal standard derivative) were plotted against codeine concentration in micrograms per millilitre. Five assays on each solution were carried out and the regression line and correlation coefficient were calculated.

Reproducibility and quantitative analysis after derivatization

Test solutions containing 2.5 and 10.0 μg/ml of codeine and 10.0 μg/ml of internal standard were injected into the gas chromatograph as described above and the peak-height ratios were calculated. The mean and the relative standard deviations (R.S.D.) of then assays were calculated.

Analysis of morphine

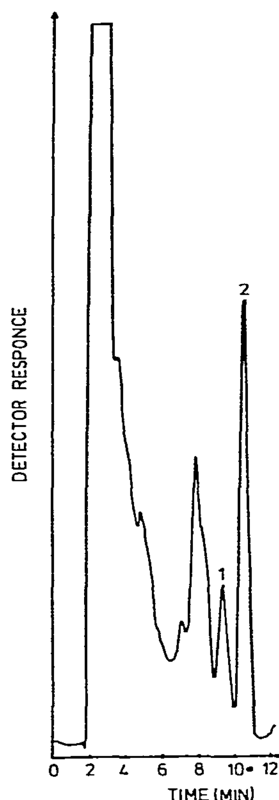
Test solutions of morphine were analysed as earlier described, and the detector response was compared with that for codeine.

RESULTS AND DISCUSSION

The principle of the venting system shown in Fig. 1 is similar to that described by Gyllenhaal *et al.*[8]. The venting programme was worked out by using a flame-ionization detector instead of the ECD. The use of a pre-column and heart-cutting showed that most of the derivatization reagent was removed, which made on-column derivatization possible without depressing the standing current and detector response. Baseline disturbances and "ghost" peaks were avoided by adjusting the pressure controllers in such a way that there was a minimum of pressure change when turning the valves. The carrier gas flow-rate through the detector was constant irrespective of the direction of the pre-column flow.

The on-column derivatization system was tested by using codeine, ethylmorphine and morphine as model substances. The identity of the drug derivatives was first checked by GC–MS. This investigation showed that O,O-bis(heptafluorobutyryl)-morphine ($M^+ = 677$), O-heptafluorobutyrylcodeine ($M^+ = 495$) and O-heptafluorobutyrylethylmorphine ($M^+ = 509$) were formed. No codeine, ethylmorphine, morphine or monoheptafluorobutyrylmorphine could be detected and it was concluded that the reaction was complete. This is in good agreement with earlier findings where the same HFB derivatives were formed by using heptafluorobutyric acid anhydride as the reagent[11]. The optimal amount of HFBI reagent required in order to obtain complete reaction was found to be 1 μl. No change in the peak-height ratio was observed when the sample volume injected was increased to 4 μl. This was the largest sample volume that was tested and the analysis of biological samples often requires volumes of this size to be injected into the gas chromatograph.

Fig. 2 shows a typical chromatogram obtained after on-column acylation and venting when 1 ng of codeine and 10 ng of ethylmorphine were injected. Fig. 3 shows a chromatogram obtained when 1 μl of morphine test solution containing 100 pg of morphine was injected. The minimal detectable amount of codeine was found to be about 100 pg and that of morphine about 20 pg. A blank run showed no interfering peaks with codeine, ethylmorphine or morphine. In a previous report, calibration graphs were set up for codeine, morphine, amphetamine and ephedrine using flame-ionization detection[3]. In order to check the linearity of the method using electron-capture detection, a calibration graph was set up for codeine. The codeine calibration



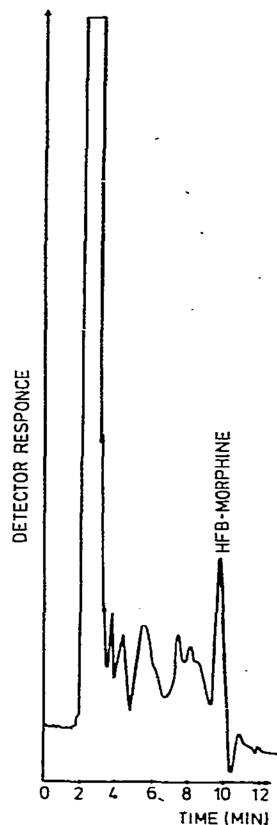


Fig. 2. Chromatogram of 1 ng of HFB-codeine (peak 1) and 10 ng of ethylmorphine (peak 2) after on-column acylation, heart cutting and electron-capture detection. Chromatographic conditions, see text.

Fig. 3. Chromatogram of 100 pg of HFB-morphine after on-column acylation, heart cutting and electron-capture detection. Chromatographic conditions, see text.

graph was calculated according to the method of least squares, relating y, the peak-height ratio of the codeine derivative to the internal standard, to x (μg/ml), the concentration of the drug solution. The calibration graph for the concentration range 1–10 μg/ml was $y = 0.128x + 0.116$, with a correlation coefficient of 0.998. The data obtained from the reproducibility test showed that with 2.5 μg/ml the R.S.D. was 5.3% and with 10.0 μg/ml the R.S.D. was 2.3%. These results are considered to be satisfactory for quantitative use of the method. During the analysis a standard solution containing codeine and ethylmorphine was injected every day and the peak-height ratio was calculated in order to test the venting system.

On the basis of these results and earlier findings[3], it should be possible to use on-column derivatization and heart cutting in combination with electron-capture detection for the analysis of drugs in pharmaceutical preparations and biological materials. By using this method less time is needed for forming the derivative, only a small amount of reagent is required and tedious extraction steps for removal of excess of reagent are unnecessary.

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