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Application of the Combined Use of Fused Silica Capillary Columns and NPD for the Toxicological Determination of Codeine and Ethylmorphine in a Human Overdose Case

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Abstract

The applicability of capillary gas chromatography to the toxicological analysis of extracts of human tissue samples was investigated for codeine and ethylmorphine in a case of drug overdose. After column extraction, the samples were injected onto a fused silica capillary column, using a direct injection technique. The nitrogen-phosphorus detector provided excellent stability and sensitivity without the need for extensive clean-up procedures.

Introduction

A 25-year-old male was found dead one month after his heroin addiction had been arrested. Since the age of 19, the man had been repeatedly convicted of drug posession, including amphetamine, norephedrine and cannabis. Police investigation suggested drug overdose as the cause of death, but no traces of recent injections were observed. When the toxicological analysis was started, no other autopsy findings or anatomo-pathological data were available. Using radioimmunoassays (RIA) or EMIT techniques, a preliminary screening detected positive results for phenobarbital and morphine; no benzodiazepines, tricyclic antidepressants, cocaine, methadone, methaqualone, or cannabinoids were detected. A gas chromatographic (GC) analysis for alcohol was also negative. In order to confirm the presence of morphine, 5 mL urine was treated with β-glucuronidase, extracted at pH 8.5 ± 0.2, and analysed qualitatively on the capillary Sil 8 column. In the chromatogram, no peak was present corresponding to morphine. However, two peaks appeared corresponding to codeine and ethylmorphine, respectively. Identity of these compounds was confirmed by GC/mass spectrometry (GC/MS). It was decided to investigate the relevancy of the capillary system for quantitation of both compounds in the tissue extracts, using hydrocodone as the internal standard (IS). Figure 1 illustrates the structural relationship between codeine, ethylmorphine, and hydrocodone, respectively.

Reagents and Materials

Instrumentation

A 25 m × 0.32 mm i.d. fused silica capillary column, coated with a CP-Sil 8 liquid phase film 0.61 μm (Chrompack) was installed in a Perkin-Elmer Sigma 2 GC, equipped with a nitrogen-phosphorus detector (NPD). Column connections have been previously described (1). A direct injection technique was applied, using a 1.0 μL Hamilton syringe. The carrier gas was purified helium at a pressure of 0.8 bar. A Hewlett-Packard 3380S Integrator/Recorder was used.

GC Parameters

Injector and detector temperatures were set at 300°C. A temperature program was initiated at 220°C for 2 minutes, increased 5°C/min to 260°C (held for 2 min), then increased 10°C/min to 300°C. This temperature was held isothermally for 14 minutes. Sensitivity was set at range 1, attenuation 256.

Reagents

Analytical grade solvents and β-glucuronidase were obtained from Merck. Codeine, ethylmorphine and hydrocodone were obtained from Bios-Coutelier and standard stock solutions of 1 mg free base/mL were prepared in methanol. Custom-made quartz conical test tubes were obtained from Vel and the Ex-

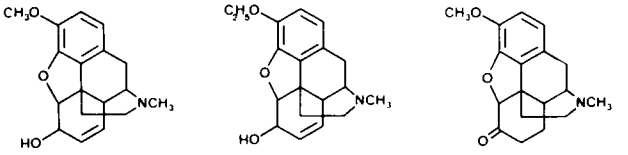

Figure 1. Chemical structures of codeine, ethylmorphine, and hydrocodone (IS).

tube Clin Elut extraction columns (No. 1003) were obtained from Analytichem.

Sample Preparation and Extraction

To the Clin Elut columns, a 3-mL sample (blood, urine, or stomach content) and 12 μg IS were added according to the product instructions. For complete elution, two 6-mL aliquots of chloroform were added; the second aliquot being added after 5 minutes. The eluant was collected in quartz conical test tubes, evaporated to dryness under reduced pressure (100 mm Hg) using a Buchler Vortex-Evaporator, reconstituted with 100 μL methanol, and 0.1 μL was injected onto the GC column. Tissue samples (3 g) were homogenized in 6 mL water using an Ultra-Turrax mixer. To the extraction tube, 4 μg IS and 3 g of the homogenized tissue sample (corresponding to 1 g of tissue) were added. The dried extract was redissolved in 33 μL of methanol.

Preparation of the Standard Calibration Curve

A standard calibration curve, evaluated for urine, yielded excellent results in the 0.5 to 10 μg/mL range for both codeine and ethylmorphine, using an IS concentration of 4 μg/mL (Figure 2). However, once the concentration in the urine samples exceeded 10 μg/mL, no further linearity could be obtained. This might be due to "column overload", since a sample size of 0.1 μL, corresponding to 30 ng or more for one component, approximates the maximum sample capacity of a small or medium bore WCOT-column. Therefore, if the peak area ratio, calculated by means of the integrator, for one of the samples was higher than that of the 8 μg/mL standard calibration urine, the extraction was repeated on a three-fold diluted sample, and the concentration calculated from a corrected equation.

The chromatograms obtained from the 2 μg/mL standard calibration urine sample and extracts of stomach and small intestinal contents, respectively, are shown in Figure 3, with the order of elution for codeine, ethylmorphine, and hydrocodone noted in the caption.

Results

Comparison of chromatograms of extracts from spiked urine and liver samples with those of methanolic standard mixtures showed no discrimination and almost quantitative recoveries. In order to evaluate the reproducibility of the procedure, a blank

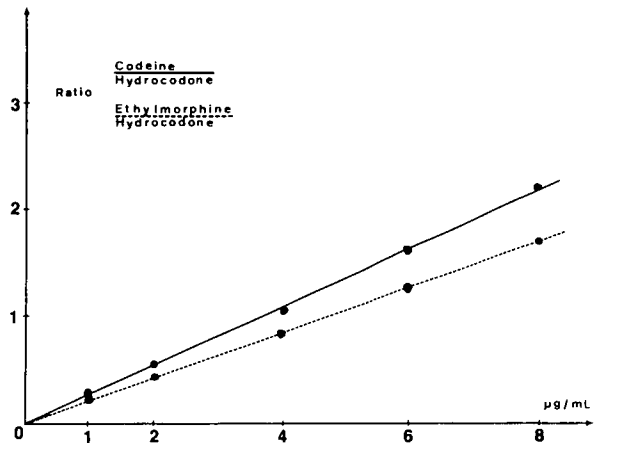

Figure 2. Standard calibration curves for urine. Regression lines drawn from a best-fit equation.

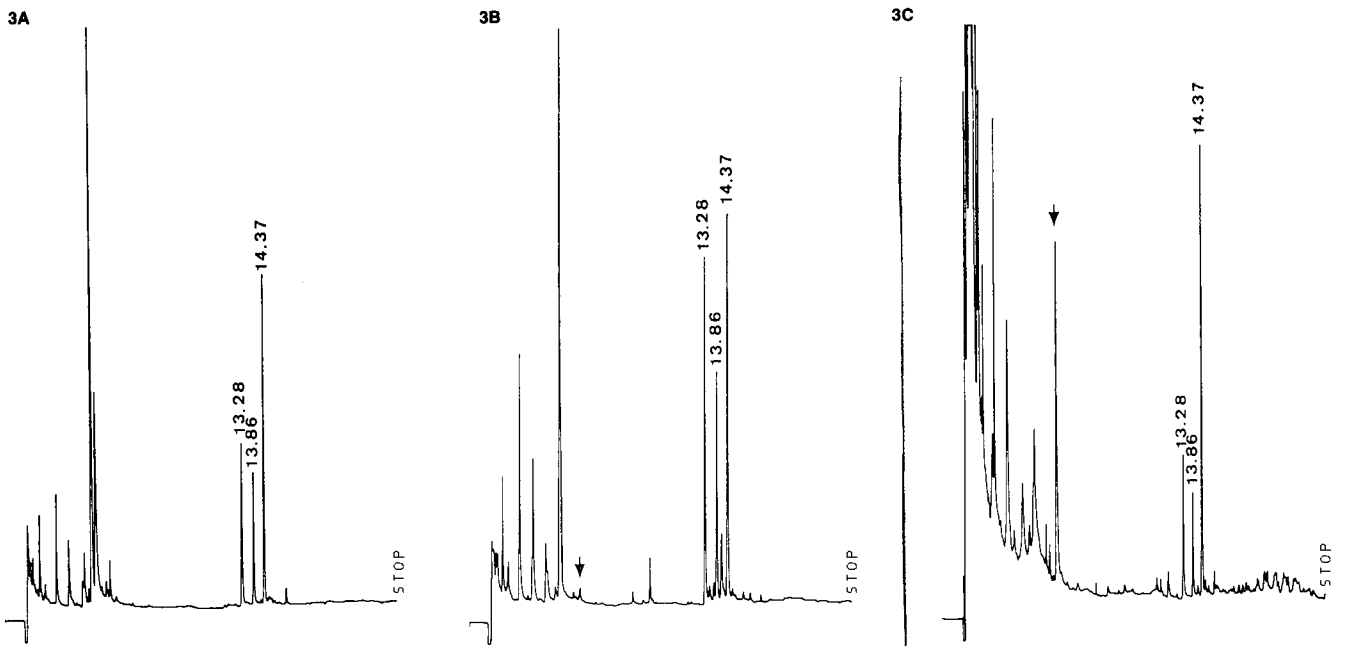

Figure 3. Chromatograms of A) 2 μg/mL standard calibration urine sample, B) extracts of stomach contents, and C) contents of small intestine. The order of elution was: codeine, 13.28 min; ethylmorphine, 13.86 min; and hydrocodone, 14.37 min. Arrows on B and C indicate phenobarbital. Note that the morphine free base under these conditions eluted after 14.21 min, just preceding the IS.

urine sample was spiked with codeine (3 μg/mL) and ethylmorphine (7 μg/mL) and subjected to ten determinations. The first five determinations were carried out on the same day. Determinations 6-10 were done on five consecutive days. The results are listed in Table I. Table II shows the distribution of codeine and ethylmorphine and the concentrations found in different organs.

Discussion

The gas chromatographic separation of methadone, codeine, morphine, and heroin on a 20 m SE-30 glass capillary column following derivatisation has been described previously (2). More recently (1-3), experience with the relatively new fused silica capillary columns has been reported, which provide improved inertness. The use of this type of column, permanently deactivated with a polysiloxane at a high temperature and a NPD, allowed the determination of morphine and some structurally related products in the low nanogram range without derivatisation. Independently, Caddy *et al.* (4) mentioned the separation of some underivatised narcotics on a 12 m quartz capillary column, fitted with a NPD. As a consequence of this earlier work, it was considered worthwhile to investigate whether fused silica capillary columns could be applied for the separation and identification of a comprehensive group of products structurally related to morphine and cross-reacting in the RIA test, without preceding derivatisation procedures. The final purpose was to see if the combination of this type of column and NPD could be used for confirmation of RIA-morphine positive results [100 ng morphine equiv/mL or more, as recommended as an appropriate cutoff level (5)]. This research is still in progress, but the described case already illustrates the advantages of a capillary system.

Most vitreous silica capillary columns are coated with a liquid phase film of about 0.2 μm, and therefore can provide excellent efficiency. In order to inject *raw* tissue extracts by a direct injection technique, it was decided to use a column with a thicker liquid loading (0.61 μm), allowing larger sample sizes.

The Clin Elut extraction procedure was based on the liquid-liquid extraction principle. The aqueous sample is poured into the Clin Elut column, which is prepacked with an inert matrix of large surface area. The organic solvent added interfaces with the sample. Water and impurities are retained in the matrix, while the organic solvent with the components of interest pass freely through. Compared to classical back-extraction procedures (6), the Clin Elut extraction offers the advantage of extracts pure enough for direct analysis, with few interfering peaks, good recovery, and reproducibility.

Since only non-ionized molecules can be considered for diffusion in the process of resorption of orally administered drugs, weak bases are absorbed preferably from the intestine (pH 6.6). Therefore, the content of the small intestine was included in the analysis. On the other hand, some important specimens, such as brain and kidney, were not available for this toxicological examination. It was concluded that death in this case was most probably attributed to the combination of codeine, ethylmorphine, and phenobarbital. Total amounts of codeine and ethylmorphine in the stomach amounted to 1.1 and 0.9 mg, respectively. Phenobarbital concentrations, determined by RIA, were 6 and 4.5 μg/mL in blood and urine, respectively.

Table I. Reproducibility Data: Within-Run and Day-to-Day Precision

	Sample Number	Concentration Found (μg/mL) Codeine	Ethylmorphine
Within-Run	1	3.1	6.9
	2	3.2	7.2
	3	3.2	7.5
	4	3.0	7.0
	5	3.3	7.7
Day-to-Day	6	3.3	7.3
	7	3.1	7.1
	8	3.1	7.0
	9	3.0	6.6
	10	3.1	6.8
Average		3.1	7.1
SD		0.1	0.3
CV (%)		3.23	4.23

Table II. Distribution of Codeine and Ethylmorphine

Organ	Codeine	Ethylmorphine
Blood (μg/mL)	0.4	0.5
Urine (μg/mL)	13.7	9.4
Stomach content (μg/g)	3.7	3.1
Small intestine (μg/g)	1.4	1.1
Liver (μg/g)	0.2	0.2

Conclusion

Column extraction, followed by capillary GC was described for quantitation of the structurally related compounds (codeine and ethylmorphine) in biological samples in an overdose case due to codeine, ethylmorphine and phenobarbital. The described method was fast and reliable. One disadvantage with the use of the capillary column was that only in the low concentration range was a linear response obtained. This problem was evaded by simple dilution of too concentrated samples.

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