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Simultaneous identification and quantification of several opiates and derivatives by capillary gas chromatography and nitrogen selective detection

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Summary. A capillary column gas chromatographic method is described for the simultaneous determination of morphine, codeine, heroin, 3- and 6-monoacetylmorphine, nalorphine, naloxone, ethylmorphine, and naltrexone. The drugs were extracted from 2 ml plasma, urine, or other biological samples, including tissue under alkaline conditions in chloroform-isopropanol-*n*-heptane (50:17:33, v/v), with levallorphan as an internal standard. The drugs were extracted into acid and then reextracted into chloroform after the acid had been alkalinized. After derivatization with trifluoroacetic anhydride, an aliquot was injected into a 25 m capillary column equipped with a nitrogen phosphorus detector. The lower limits of detectability, extraction recovery, and the within-run and day-to-day precision of results were determined for each drug. Our results indicate that the procedure is suitable for use in overdose screening and therapeutic drug monitoring.

Key words: Capillary gas chromatography, opiates – Drug screening, determination of capillary gas chromatography

Zusammenfassung. Es wird eine Capillargaschromatographische Methode zur simultanen Bestimmung von Morphin, Codein, Heroin, 3- und 6-Monoacetylmorphin, Nalorphin, Naloxone, Naltrexon und Ethylmorphin beschrieben. Von 2 ml Plasma, Urin oder anderen biologischen Proben (z.B. Gewebepräparate) wurden die Substanzen in alkalischen Milieu durch Chloroform: isopropylalkohol: *n*-heptan extrahiert, anschließend in Chloroform rückextrahiert. Nach Derivatiesierung mittels Trifluoressigsäureanhydrid wurden die aliquoten Teile an einer 25 m Capillarsäule eingesetzt. Die Detektion erfolgte durch einen Nitrogen-Phosphorous Detektor, und die Nachweisgrenzen und Wiederfindungsraten wurden ebenfalls ermittelt.

Schlüsselwörter: Capillargaschromatographie, Opiatnachweis – Drogenscreening, Capillargaschromatographie

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Introduction

Many different gas chromatography (GC) methods using packed or capillary columns are available for the quantitative analysis of opiate narcotic analgesics, which control pain by a CNS depressant effect. In most instances only one drug or parent drug has been detected, requiring time-consuming multiple extractions to achieve global screening of the nonlegal substances.

Due to the polarity of morphine, the molecule is adsorbed onto silicious column support materials, thus giving rise to poor peak shapes, low sensitivities, and long retention times. Consequently, in order to chromatograph morphine using GC, a suitable derivative must be prepared [1]. The derivative most frequently prepared is that of the silylether, by using either *N,O*-bis-trimethylsilylacetamide [2, 3] or bis-trimethylsilyltrifluoroacetamide [4]. Since silylation methods employ pyridine, acetonitrile, or tetrahydrofuran, which are solvents containing a nitrogen atom, they were unsuitable for use with a nitrogen phosphorous detector (NPD), causing background noise. Consequently, alternative derivatization procedures were investigated. Methods employing fluorinated anhydrides have been developed for electron capture detection and found to be compatible with NPD. These methods have included acetylation with acetic anhydride [5, 6] and trifluoroacetic anhydride [6, 7], or acetylation with pentafluoropentanoic anhydride [8, 9] and heptafluorobutyric anhydride [6, 10]. Since the acetylation of morphine by acetic anhydride leads to the formation of heroin, the latter procedure appears to be useless in forensic medicine, where powders sometimes have to be characterized, particularly heroin.

Opiate addiction is usually treated by nalorphine, naloxone, or naltrexone. Their determination is suitable in cases of therapeutic drug monitoring. In spite of the data already published [11, 12], no capillary GC method seems to be available.

In this paper, we present our results in the simultaneous capillary GC determination of opiates and therapeutic derivatives.

Materials and methods

Chemicals and reagents

Morphine hydrochloride, codeine hydrochloride, 3-monoacetylmorphine base, 6-monoacetylmorphine base, and diacetylmorphine base were generous gifts from Francopia (France); nalorphine hydrochloride and naltrexone hydrochloride were provided by Sanofi (France) and Du Pont de Nemours (France), respectively. Naloxone hydrochloride, ethylmorphine hydrochloride, and levallorphan bitartrate were purchased from the Theta Corporation (USA). Trifluoroacetic anhydride (TFA) and β-glucuronidase were supplied by Sigma (USA). The chloroform, isopropanol, *n*-heptane and methanol were HPLC grade (Merck). All other chemicals were analytical grade.

Stocked solutions of all narcotis (1 mg/ml, free base) were prepared in methanol and stored at 4°C. Levallorphan was used as an internal standard (IS). Phosphate buffer was prepared with a saturated solution of K_2HPO_4, 40% diluted with deionized water and adjusted to pH 9.2.

Chromatographic conditions

Gas chromatography was performed on a vitreous silica capillary column, bonded phase BP 10 (SGE) 25 m × 0.22 mm i.d. The flow of carrier gas (helium) through the column was 3.2 ml/min and the head pressure on the column was maintened at 18 psi.

The GC system consisted of a Perkin-Elmer (8500) chromatograph with a nitrogen phosphorous detector and a Perkin-Elmer data collector (Sigma 15). The operating conditions were as follows: column, injector port, and detector temperatures were 240°, 290°, and 300°C, respectively. A split of 10:1 was used to inject all samples into the column.

Quantification was done for narcotics by plotting peak area ratios (drug/IS) against the concentration of standards to produce standard curves and by comparing the results for the case samples with the curves.

Extraction procedure

Plasma (2 ml) was pipetted into a 15-ml Pyrex centrifuge tube and followed by 50 µl 4 *N* NaOH, 2 ml phosphate buffer (40%, pH 9.2), 20 µl levallorphan (10 µg/ml), and 10 ml chloroform-isopropanol-*n*-heptane (50:17:33, v/v).

After agitation and centrifugation, the organic phase was purified by an additional acidic extraction (5 ml 0.2 *N* HCl). Then, the aqueous layer was reextracted after addition of 2 ml phosphate buffer (40%, pH 9.2), 0.5 ml concentrated ammonia solution, and 5 ml chloroform. After agitation and centrifugation, the organic phase was taken off and evaporated to dryness at 45°C in a Speed Vac concentrator (Savant Instruments). TFA (100 µl) was added to the dry extract, which was then stoppered and incubated at 60°C for 45 min. Excess reagent was completely evaporated to dryness. The products were dissolved in 25 µl ethylacetate and 1 µl was injected into the column.

Enzymatic hydrolysis prior to urine and bile extraction was necessary in order to cleave the opiate-glucuronic acid linkage. Into a 15-ml Pyrex centrifuge tube, urine or bile (2 ml) was pipetted followed by 0.5 ml 1.1 *M* sodium acetate buffer (pH 5.2) and 0.25 ml β-glucuronidase (20,000 UI/ml). Tubes were capped and heated for 12 h at 56°C. After hydrolysis, samples were treated in the same manner as plasma.

Results and discussion

Following extraction and trifluoroacetylation, plasma samples spiked with one of the drugs were found to contain only one chromatographic peak. No other byproducts or impurities were observed. This was particularly the case for morphine, whose only trifluoroacetylation product was 3,6-diTFA-morphine. 3-TFA-morphine was not characterized. The TFA derivatives showed good peak symmetry with shortened retention times. Once the samples were derivatized and evaporated to dryness, they were stable for a few hours.

The optimum derivatization conditions were evaluated by incubating samples containing equivalent amounts of the eight drugs for various times (0 to 90 min) and various temperatures (20°, 37°, 60°, and 80°C). A significant difference in response was found throughout the range of reactions studied; derivatization assays at 37° and 80°C showed large interferences in chromatographed samples and did not allow a correct analysis. No additional conversions were observed after 45 min at 60°C.

Two chromatograms for patient samples are shown in Fig. 1. Chromatogram A shows a urine extract after hydrolysis in a case involving heroin overdose. Morphine and 6-monoacetylmorphine are detected. Chromatogram B shows a plasma extract from a subject being treated with nalorphine.

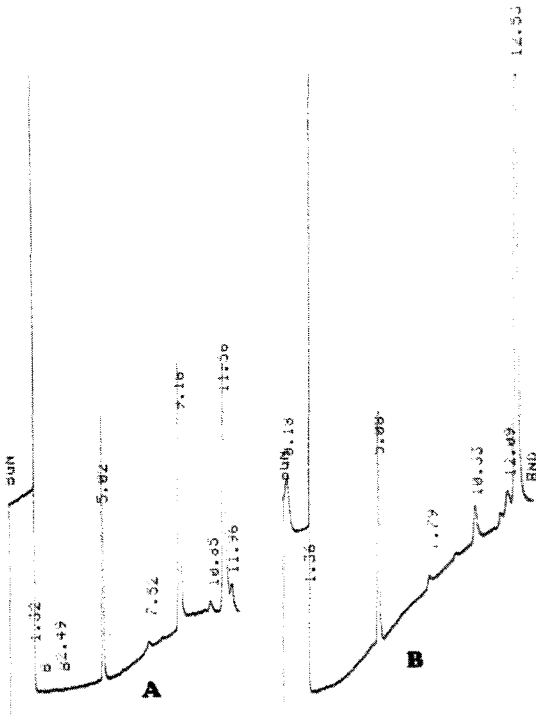

Fig. 1A, B. Chromatograms show **A** a urine extract after hydrolysis from a heroin overdose (5.02 min, levallorphan, internal standard; 9.16 min morphine 212 ng/ml; 11.56 min, 6-monoacetylmorphine 77 ng/ml) and **B** a plasma extract from a subject treated with nalorphine (12.53 min, 314 ng/ml)

Table 1. GC retention times for the opiates tested

Compound	Retention time (min)
Levallorphan	5.02
Morphine	9.16
Codeine	9.90
Ethylmorphine	10.67
6-Monoacetylmorphine	11.56
Heroin	12.12
Nalorphine	12.53
3-Monoacetylmorphine	15.33
Naltrexone	18.06
Naloxone	24.91

Retention times for the narcotics tested are presented in Table 1. They were reproducible as long as the gas flow and the split ratio remained constant. The retention time of the internal standard varied by less than 0.12 min over a 4-month period.

Standard curves for all drugs analyzed were linear in the range of 50–2,000 ng/ml with correlation coefficients greater than 0.98. For each drug, we determined the extraction recovery, the within-run coefficient of variation ($n = 6$),

Table 2. Data for opiates

Compound	Extraction recovery (%)	Within run precision (%)	Limit of detection (ng/ml)
Morphine	81.2 ± 4.6	6.4	8.0
Codeine	76.8 ± 7.1	5.9	30.0
Ethylmorphine	79.6 ± 5.9	6.2	9.0
6-Monoacetylmorphine	81.6 ± 5.7	6.1	6.0
Heroin	65.5 ± 9.8	6.9	7.0
Nalorphine	76.7 ± 4.7	5.7	8.0
3-Monoacetylmorphine	80.7 ± 6.4	5.8	6.0
Naltrexone	79.8 ± 5.8	5.1	6.0
Naloxone	81.5 ± 3.9	6.0	10.0

and the limit of detection (Table 2). These parameters were studied at 100 ng/ml of each components.

Levallorphan, chosen as the internal standard, was clearly separated from all narcotics tested. Traditionally, authors [4–6, 8–12] have used nalorphine as an internal standard. This choice was not retained, since nalorphine is sometimes present in plasma samples, especially in addicted subjects.

After an intravenous injection, heroin is rapidly metabolized to 6-monoacetylmorphine, then to morphine [13]. Since codeine is partially metabolized to morphine, even in postmortem samples [14], heroin abuse cannot be confirmed simply by the detection and identification of morphine. It has been previously demonstrated that evidence for heroin abuse can be obtained by the simultaneous detection of morphine and 6-monoacetylmorphine, since there is no claim in the literature that 6-monoacetylmorphine can be formed by the *in vivo* metabolic acetylation of morphine [8]. This is the reason why 6-monoacetylmorphine was included in the present report.

Data for ethylmorphine were investigated, since the drug has been shown to be associated with codeine and/or morphine in several cases [15]. Naloxone, at first a pure opiate antagonist but inactive when taken orally [16], and naltrexone, an orally effective antagonist with a prolonged course of action [17], were determined in this study due to their increasing use in detoxification treatment.

In conclusion, the capillary gas chromatography column allows excellent separation; it has good sensitive in the detection of opiates and their derivatives. This method can be used for screening and/or confirmation purposes in biological fluids and tissue samples. The analyses of more than 600 clinical and forensic specimens has been possible with this method without column reconditioning.

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