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Gas chromatography with surface ionization detection: a highly sensitive method for determining underivatized codeine and dihydrocodeine in body fluids

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Abstract

Underivatized codeine and dihydrocodeine in human plasma and urine have been determined with a high degree of accuracy by capillary gas chromatography (GC) with surface ionization detection (SID). The drugs were extracted with the aid of Sep-Pak C_{18} cartridges. Recovery of both drugs was ≥90%. The calibration curves obtained with dimemorfan as an internal standard showed linearity in the range 4.5–72.3 and 3.0–75.5 ng/ml of plasma for codeine and dihydrocodeine, respectively. The detection limit was about 100 pg on column (2.5 ng/ml sample). Codeine was determined quantitatively in plasma and urine obtained from a volunteer who had received 10 mg codeine phosphate orally 3 h before the sampling: the levels were found to be 14.1 and 142 ng/ml, respectively. The present GC–SID method has been compared carefully with GC–NPD (nitrogen–phosphorus detection) using the same extracts; the sensitivity of GC–SID was more than ten times greater than that of GC–NPD, with background noise correspondingly lower.

1. Introduction

Codeine and dihydrocodeine are widely used narcotic antitussives, and are one of the most important targets in forensic analysis [1–4]. Moreover, the 1% powder of these drugs is not controlled in Japan, and can easily be obtained for abuse.

In 1985, Fujii and Arimoto first introduced surface ionization detection (SID) for gas chromatography (GC) [5]; they reported that tertiary amino compounds, such as tributylamine and triethylamine, could be detected and quantified with a high degree of accuracy by SID. As studies on GC–SID have progressed, success has been reported in the identification and quantification of a number of medicolegally important substances [6]. In this paper, we present data to show that underivatized codeine and

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dihydrocodeine in human body fluids can also be detected and quantified by GC–SID.

2. Experimental

2.1. Materials

The chemical structures of codeine, dihydrocodeine and dimemorfan (internal standard, I.S.) are shown in Fig. 1. Codeine phosphate and dihydrocodeine phosphate were obtained from Shionogi (Osaka, Japan); dimemorfan phosphate from Yamanouchi Pharmaceutical (Tokyo, Japan). Sep-Pak C_{18} cartridges were purchased from Waters Associates (Milford, MA, USA). Other common chemicals used were of the highest quality commercially available. Plasma and urine were obtained from healthy subjects.

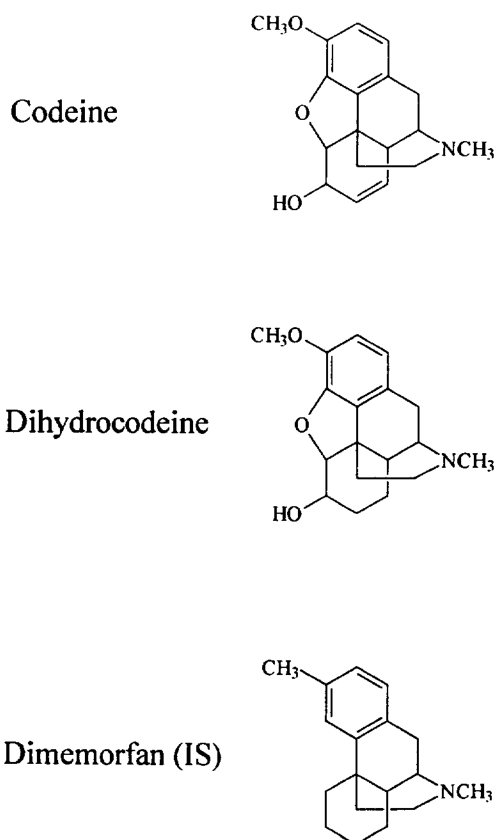


Fig. 1. Chemical structures of codeine, dihydrocodeine and dimemorfan (I.S.).

2.2. Administration of codeine

A 29-year-old healthy male subject volunteered to take part in this study; informed consent was obtained from this subject. He received an oral dose of 10 mg codeine phosphate. Blood and urine samples were obtained 3 h after the codeine administration.

2.3. Sample preparation with Sep-Pak C_{18} cartridges

The Sep-Pak C_{18} cartridges were pretreated by washing with 10 ml of distilled water, 10 ml of methanol and then 10 ml of distilled water.

Samples (1 ml) of either plasma or urine, some with drugs added and some with no drug addition, were each mixed with 4 ml of 1 *M* sodium bicarbonate and 2 ml distilled water. In all cases the sample solution was loaded onto a pretreated Sep-Pak cartridge at a rate not exceeding 5 ml/min. The cartridge was washed with 20 ml of distilled water. Finally, 3 ml chloroform–ethanol (9:1) was passed through it to elute the drugs. The upper aqueous phase of the eluate was discarded by aspiration with a Pasteur pipette, and the lower organic phase was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 50 μl methanol, and 2-μl aliquots of this solution were used for the GC analyses.

2.4. GC conditions

GC was carried out on a Shimadzu GC-15A instrument with SID and on a Hewlett-Packard HP5890A gas chromatograph with nitrogen–phosphorus detection (NPD). A DB-17 fused-silica capillary column (30 m × 0.25 mm I.D., film thickness 0.25 μm) (J&W Scientific, Folsom, CA, USA) and split–splitless injectors were used for both GC instruments. The GC conditions for both instruments were: column temperature 130°C (hold for 2 min) to 300°C at 8°C/min; injection temperature 240°C; detector tempera-

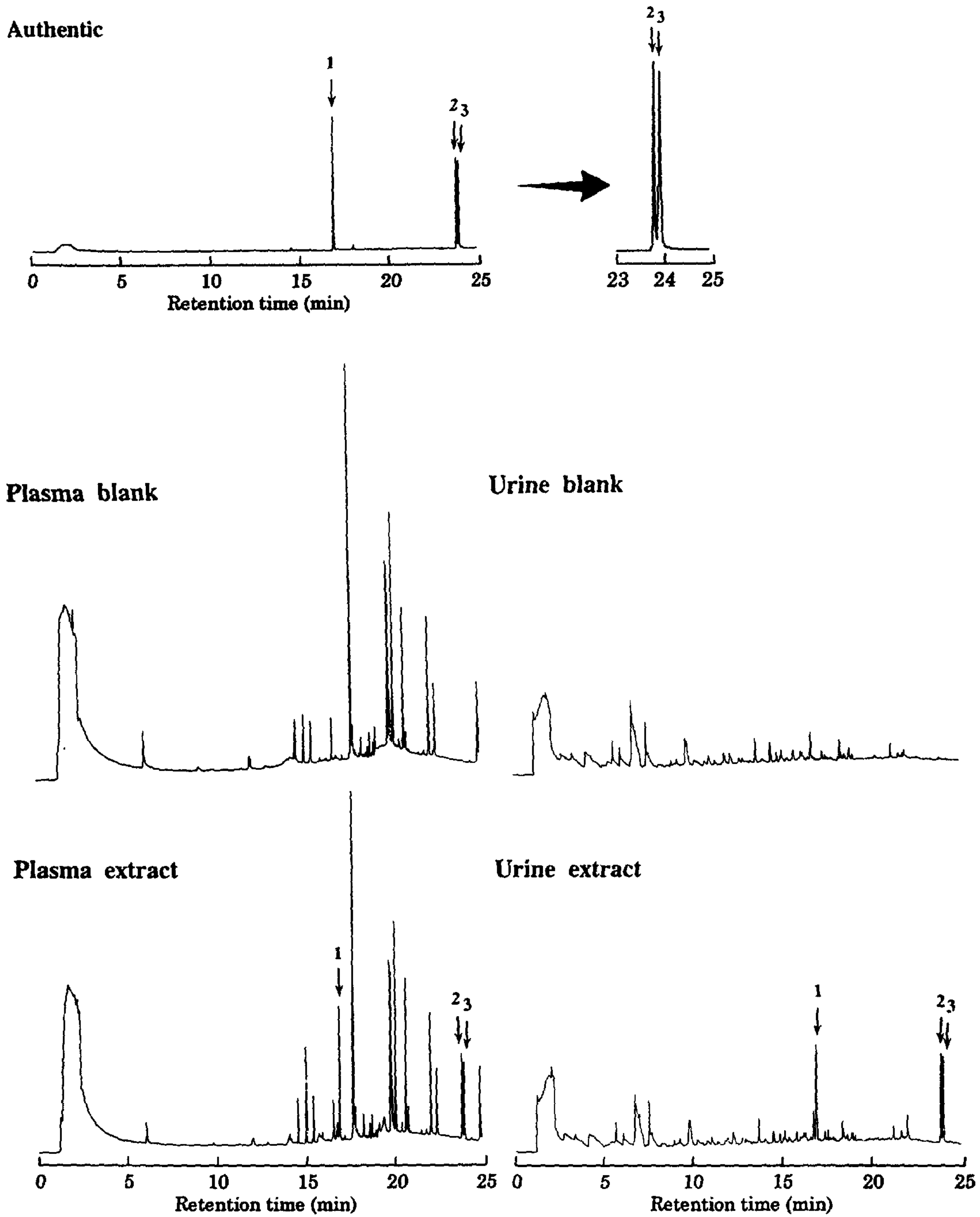


Fig. 2. Capillary GC–SID for codeine (peak 3), dihydrocodeine (peak 2) and I.S. (peak 1) extracted from plasma and urine, and their backgrounds. Samples were 1-ml extracts from plasma or urine to which 26.5 ng codeine, 22.6 ng dihydrocodeine, and 20 ng I.S. had been added; 3–5 replications, typical chromatograms shown. Authentic: chromatogram of 26.5 ng codeine/22.6 ng dihydrocodeine/20 ng I.S. per vial.

ture 280°C; helium flow-rate 22 cm/s. The SID conditions were: heating current through the platinum emitter 2.2 A; emitter temperature ca. 600°C; ring electrode bias voltage +200 V with respect to the collector electrode. The samples were injected in the splitless mode and the splitter was opened after 1 min.

3. Results

Fig. 2 shows gas chromatograms obtained via SID of 36 ng of codeine phosphate (free form: 26.5 ng), 30 ng of dihydrocodeine phosphate (free form: 22.6 ng), and 20 ng of I.S., which have been added to 1 ml of plasma or urine and extracted with Sep-Pak C_{18} cartridges. Impurity peaks from plasma or urine samples did not interfere with the drug peaks under our conditions. The retention times were 23.9, 23.7 and 16.1 min for codeine, dihydrocodeine and dimemorfan (I.S.), respectively. The recoveries and coefficients of variation (C.V.) for both drugs as obtained from plasma and urine extracts are listed in Table 1.

For comparison the same extracts as used for the above addition tests were submitted to GC–NPD under the same conditions (Fig. 3). Both plasma and urine samples gave rise to many large impurity peaks, one of which interfered with the I.S. peak. The intensities of the drug peaks were very low compared with those of the impurity peaks. The baseline rose slightly with increasing column temperature. The sensitivity of GC–NPD was only about one-tenth of that of GC–SID, mainly owing to the signal-to-noise ratio.

The calibration curves obtained by GC–SID for codeine and dihydrocodeine which had been added to, and extracted from, 1 ml of plasma were measured against 20 ng of the I.S. The curves were linear in the range 4.5–72.3 ng/ml (0.18–2.89 ng on column assuming 100% recovery for codeine), and 3.0–75.5 ng/ml (0.12–3.02 ng on column assuming 100% recovery for dihydrocodeine). The equation and r values for the curves were: $y = 0.015x + 0.158$ and $r = 0.9958$ for codeine; $y = 0.019x + 0.117$ and $r = 0.9933$ for dihydrocodeine. The detection limit for both compounds was about 100 pg on column (2.5 ng/ml sample).

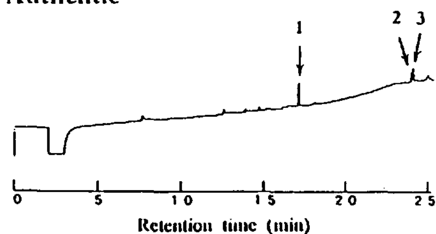
Fig. 4 shows GC–SID chromatograms for plasma and urine obtained from a 29-year-old healthy volunteer 3 h after oral administration of 10 mg of codeine phosphate. I.S. (20 ng) was added at the start of the initial extraction step. The levels of codeine were 14.1 and 142 ng/ml for plasma and urine, respectively.

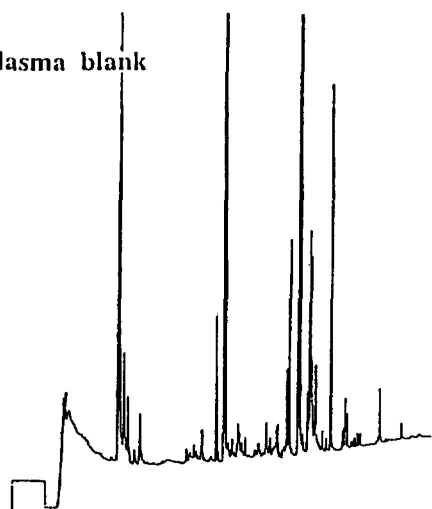
4. Discussion

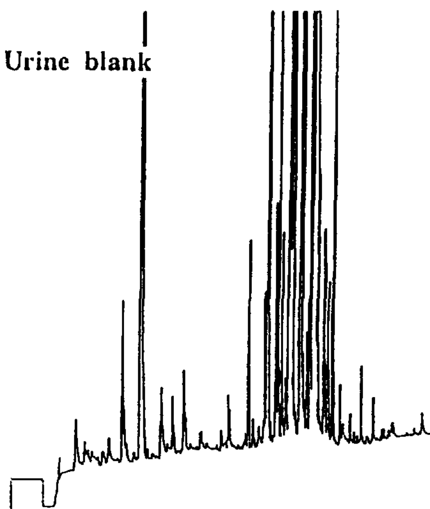
This is the first report to show that codeine and dihydrocodeine can be detected by GC–SID with high sensitivity; our response using GC–SID is ten times greater than with GC–NPD (compare Figs. 2 and 3). According to previous reports, analysis for and quantification of these drugs have usually been carried out by GC after derivatization [7–12], because they are relatively

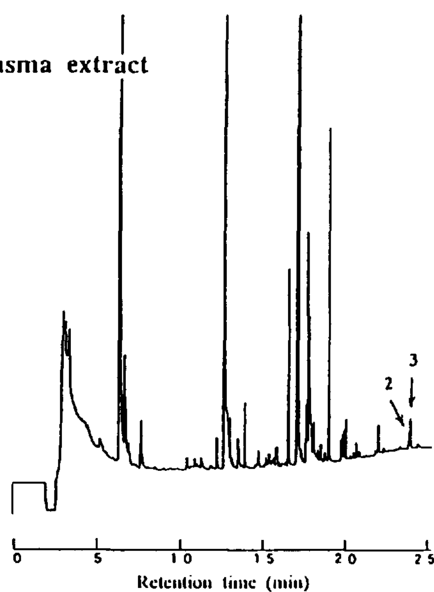
Table 1
Recoveries and coefficients of variation (C.V. values) for codeine and dihydrocodeine determined with spiked plasma and urine samples ($n = 5$)

Compound	Concentration added (ng/ml)	Plasma		Urine	
		Recovery (%)	C.V. (%)	Recovery (%)	C.V. (%)
Codeine	17.7	90.6	7.86	92.6	9.60
	53.0	95.4	10.8	98.0	6.08
Dihydrocodeine	15.1	91.0	6.78	98.5	6.21
	60.4	92.0	7.40	90.9	6.88









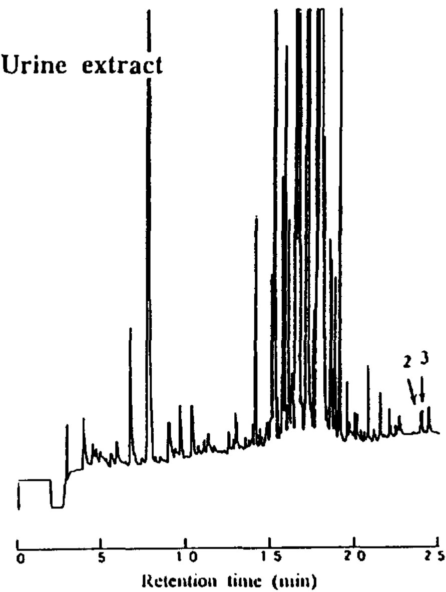
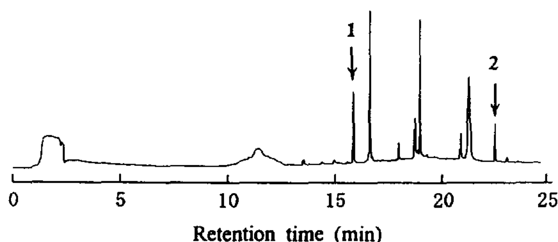


Fig. 3. Capillary GC–NPD for codeine (peak 3), dihydrocodeine (peak 2) and I.S. (peak 1) extracted from plasma and urine, and their backgrounds. Samples as for Fig. 2; 3–5 replications, typical chromatograms shown. Authentic: see Fig. 2.



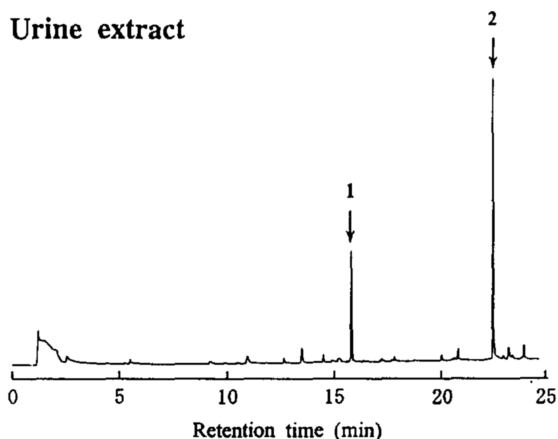


Fig. 4. Capillary GC–SID for the extracts of plasma and urine obtained from a 29-year-old healthy volunteer who had received an oral dose of 10 mg of codeine phosphate 3 h before sampling. Peak 1 = I.S. (20 ng in 1 ml sample); Peak 2 = codeine.

polar and thus show poor chromatographic properties especially with packed columns. Moore [7] reported that the detection limit was 80 pg for codeine with GC–electron capture detection (ECD) after derivatization with heptafluorobutyric anhydride; Edlund [8] reported that it was 5 ng/ml of plasma for codeine with GC–ECD after acylation with pentafluoropropionic anhydride. In the present study, we have been able to detect underivatized codeine and dihydrocodeine by GC–SID at a sensitivity comparable to that attainable with GC–ECD after derivatization.

We have used chloroform–ethanol (9:1) for elution of the drugs from Sep-Pak C_{18} cartridges. There is one report dealing with extraction of dihydrocodeine by use of Sep-Pak C_{18} cartridges, in which methanol was used for elution of the drug from urine samples [13]. Aqueous solutions of methanol or acetonitrile are usually employed to elute compounds from Sep-Pak C_{18} cartridges according to the manufacturer's manual. The use of the chloroform mixture as the elution solvent has reduced evaporation times and background noise markedly [14].

Codeine is known to be metabolized in the liver by O-demethylation to form morphine, by N-demethylation to form norcodeine, and by conjugation to form glucuronides and sulfates of both the original (unchanged) drug and its metabolites. The plasma half-life of codeine has been reported to be 2–4 h; unchanged drug accounts for about 10% of the dose in urine 24 h after administration [15]. The metabolism of dihydrocodeine is similar to that of codeine; a plasma half-life of 4 h has been reported [16]. No interconversion between codeine and dihydrocodeine has been reported.

Peak plasma concentrations of 67.8 to 147 ng/ml of codeine 0.5–1.5 h after 60 mg oral administration of codeine phosphate [17,18], and 71.8 ng/ml of dihydrocodeine 1.6 h after 30 mg ingestion of dihydrocodeine bitartrate [19], have been reported. The detection limit by our method is sufficiently low for measurement to be performed at the respective therapeutic levels of the drugs; actual detection in plasma and urine after oral administration of 10 mg codeine phosphate proved possible (Fig. 4).

GC–SID has been found to be sufficiently sensitive to codeine and dihydrocodeine even without derivatization due to its specific response to their tertiary amino groups. It seems likely, therefore, that the present method will prove useful for the quantitative analysis of a number of drugs in the fields of forensic toxicology, clinical toxicology and clinical pharmacology.

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