

Determination of pholcodine together with other opiates in urine and blood samples by gas chromatography/mass spectrometry (GC/MS)

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Abstract

A GC-MS method for simultaneous determination of pholcodine, morphine, codeine, 6-acetylmorphine, dihydrocodeine and ethylmorphine in urine and blood is described. The method employs propionic anhydride in the presence of triethylamine to propionylate free hydroxyl groups of the opiates. Confirmation is achieved using gas chromatography/ mass spectrometry in the electron impact mode.

Introduction

Pholcodine is widely used as an antitussive. It is also classified like, other opiates, as a drug with adverse effects on driving ability. The cough mixture containing pholcodine (Tuxi) can be defined over-the-counter in the pharmacies without physicians prescription. The cough mixture is also marked with red triangle in Finland, which means that this medicine may have detrimental impact on road side performance.

Pholcodines chemical structure is 3-O-morpholinethylmorphine. Seven metabolites have been identified in urine besides the unchanged pholcodine, among them morphine in traces (1). Pholcodine is metabolised and eliminated slowly.

Pholcodine can give positive result in immunoassay for opiates in urine screening for several weeks after ingestion of therapeutic oral dose (2). The widespread use of immunological screening methods has introduced the need for specific and sensitive confirmation methods to distinguish illegal from legal use of opiates.

Several methods for analysing pholcodine have been described. Johansen etc. have used high-performance liquid chromatographic detection with florescence and electrochemical detection for analysing pholcodine (3). She has used also enzyme multiplied immunoassay technique (EMIT) and capillary gas chromatography (2,4) for analysing pholcodine and its metabolite morphine. Maurer etc. has used immunoassay, fluorescence polarisation immunoassay, entzyme immunoassay and gas chromatography-mass spectrometry for pholcodine analysis (1,5)

In spite the fact that pholcodine is known to interfere immunoassay of opiates, it is still poorly detected. For the interpretation of positive opiate screening results the simply and sensitive identification method for pholcodine together with other opiates is needed.

Methods

Chemicals and reagents

Morphine sulphate was obtained from Sigma, ethylmorphine hydrochloride from University Pharmacy, codeine phosphate from Leiras, dihydrocodeine hydrochloride from RBI and 6-acetyl morphine hydrochloride from UNDCP (United Nations Drug Control Program). Pholcodine and nalorphine hydrochloride were obtained from Medical Bureau of Finland. Propionic anhydride and triethylamine were purchased from Fluka. β -Glucuronidase (Helix pomatia) was purchased from Sigma. All chemicals were analytical reagent grade.

Instrumentation

Immunoassay. EMIT-dau system of Syva (VIVA).

Gas chromatography-mass spectrometry. The analysis was performed with a Hewlett Packard GC-MS HP-5973A instrument equipped with an autosampler. HP-5 (30m x 0.32 mm, 0.25 μ m film thickness) capillary column was used for identification. The carrier gas was helium at the constant flow 1.5 ml/min and the operation conditions were as follows: The initial temperature was 100 °C for one min, which was then increased with the rate of 15 °C per min to 320°C which temperature was held constant for 4 min.

Experimental

Hydrolysis

Enzymatic hydrolysis with β - glucuronidase was employed for urine. 1 ml of urine was mixed with 200 μ l of 0.1 M acetate buffer (pH 4.5) and 40 μ l of β - glucuronidase. The samples were incubated at 37 °C for overnight.

Extraction of opiates

The urine samples were analysed without and with enzymatic hydrolysis. Blood samples were analysed without hydrolysis.

Urine standards: 0.5, 0.25 and 0.05 mg/l for 6-monoacetylmorphine and 2.0, 1.0, 0.1 mg/l for other opiates.

Blood standards: 1.0, 0.5, 0.05 mg/l

Extraction

1ml of 0.5 M $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$ buffer was added to 1 ml of (urine/blood) standards, controls, blank and samples. The opiates were extracted with 5 ml of n-butylacetate (nalorphine 0.1 ng/ml in extraction solvent, as internal standard). After centrifugation, the organic layer was transferred to a clean test tube and evaporated to dryness.

Derivatization. Triethylamine (100 μ l) and propionic anhydride (100 μ l) were added to the residue and mixed. Tubes were tightly capped and heated at 80 °C for 30 minutes (6).

Extraction of derivated substances.

After cooling the derivated substances were again extracted by mixing with 2 ml of 0.5 M $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$ and 5 ml of organic solvent. After centrifugation, the organic layer was evaporated to dryness.

The residue was redissolved in organic solvent (100 μ l) and 1 μ l of that was injected into the GC-MS.

Results and discussion

A good separation of the compounds in urine and blood was achieved.

Derivated substances were analysed with GC-MS-instrument. Example of the total ion chromatogram is shown in Figure 1.

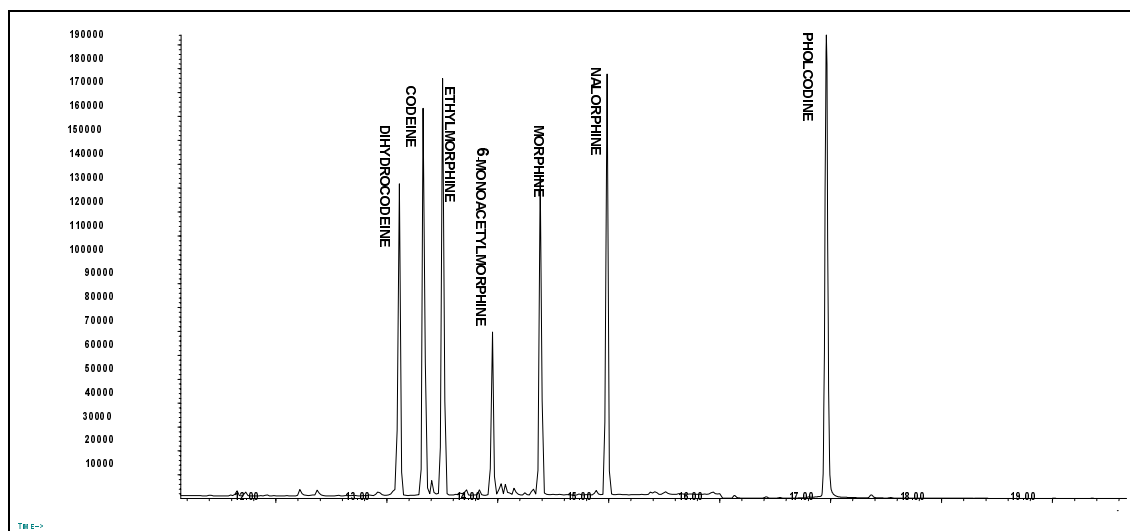


Figure 1. Chromatogram of urine standards at the concentration of 2.0 mg/l

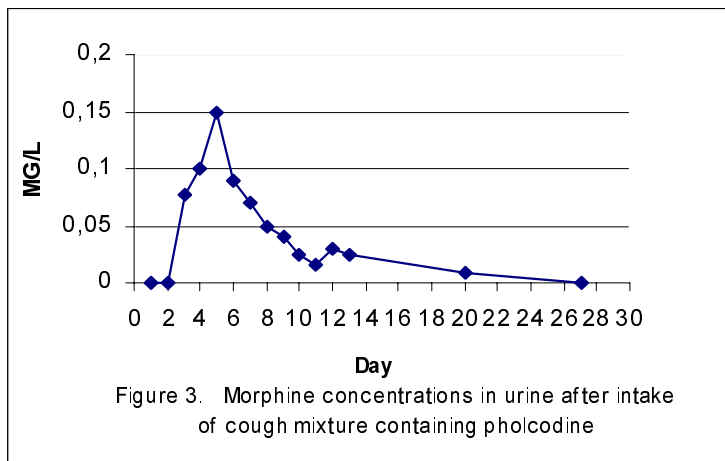
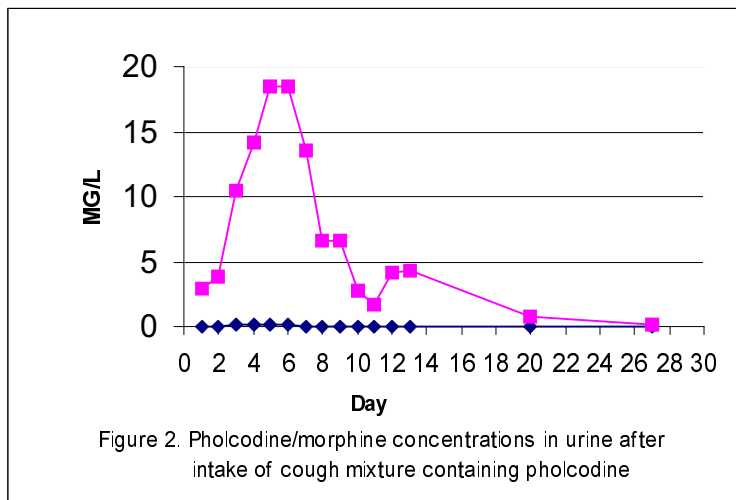
Validation of method

The quantitation limit varied from 0.01mg/l to 0.025 mg/l in blood and 0.02 – 0.15 mg/l in urine. The linearity was 0.01-10 mg/l for blood samples and 0.02- 10 mg/l for the urine samples. The intraday relative standard deviation (RSD) varied from 7.2 - 10% at the level of 0.5 mg/l for blood and urine samples.

Pholcodine metabolism

We made a case study of pholcodine and morphine concentrations after intake of cough mixture containing pholcodine. The results we obtained by analysing urine samples collected up to 28 days after the first day of 5 days intake. The taken amount of cough mixture containing 2 mg/ml pholcodine was 10 ml four times a day, which resulted 80 mg of pholcodine per day. The urine samples were collected on days 1-14, 21 and 28.

Figures 2.and 3. show pholcodine and morphine concentrations during one month after the first day of intake of cough mixture (creatinine correction has not been made).



From the figures can be seen that both pholcodine and morphine can be measured from urine samples several weeks after intake of pholcodine containing cough mixture. The urine specimens were also still positive with immulogical screening three weeks after cessation of cough mixture.

In this study the blood concentration was not measured.

Also Johansen et al (2) have measured that after ingestion of therapeutic oral dose of pholcodine, a positive opiate response in urine can be find for 2-6 weeks by the immulogical screening methods. Also pholcodine blood, urine and plasma concentrations due the time have been measured by Chen *et al* (7). Chen didn't find morphine as a metabolite of pholcodine.

Laboratory analysis results

During the year 1999 in our laboratory has been confirmed and quantitated over 200 opiate positive drug and driving samples with the developed method. Six urine samples have found to be pholcodine positive. The urine pholcodine concentrations varied from 0.13 mg/l to 3.7 mg/l. Five sample contained also morphine at the level of 0.07 to 0.36 mg/l. One sample contained also codeine 2.8 mg/l.

Two urine pholcodine positive samples were also positive in blood quantitation. The measured concentrations were 0,01 and 0,11 mg/l

If no proper pholcodine confirmation had been done those cases, which contained also trace amounts of morphine as a metabolite of pholcodine, the drivers could have been labelled as morphine abusers.

Journal Articles

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