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Journal of Pharmaceutical and Biomedical Analysis
15 (1997) 759–764

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Determination of morphine and codeine in plasma by HPLC following solid phase extraction

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Received 25 March 1996; accepted 17 June 1996

Abstract

A cheap simple and rapid extraction procedure followed by a UV high performance liquid chromatography (HPLC) assay is described for the simultaneous determination of morphine (M) and codeine (C) in plasma. The method is based on extraction of these opiates from plasma using reversed phase (solid phase) extractions columns followed by HPLC with UV detection at 240 nm. The extraction step provides, respectively, 85 and 80% recovery for M and C. The response of the detection system is linear for both molecules in the studied range from 50 to 750 ng ml^{-1}. No other drugs have been found to interfere with the assay. This method offers a quick, cost effective and reliable procedure for specifically determining M and C, from a small sample volume. © 1997 Elsevier Science B.V.

Keywords: Morphine analysis; Codeine analysis; HPLC of opiates; Solid phase extraction; UV detection

1. Introduction

Opiates and their derivatives are the most potent analgesics available today. Recently, the increasing use of morphine (M) and codeine (C), and the multiplication, in France, of methadone centers, set up to medicaly support heroin users, has led to the development of procedures for the determination of M and C, in order to maintain acceptable plasma levels of these drugs [1], or to detect their presence/absence.

Many techniques aiming at quantifying M and C in plasma and/or in urine are already available. Most of them use solid phase extraction (SPE) followed by GC-MS [2–5] or HPLC [1,6–11] analysis. GC-MS is often used because of its sensitivity, but the necessity of sample derivatization and the cost of the technique itself are restricting its applicability. HPLC methods are often based on a dual-electrochemical/UV or fluorescence detection which in fact decrease the practicability and increase the cost of an analysis.

This paper describes the development of a new solid phase extraction procedure based on the simplification of already existing methods [1,9] for a cheap reliable and rapid assay to determine M and C in plasma using reversed phase HPLC coupled with UV detection at 240 nm.

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PII S0731-7085(96)01911-5

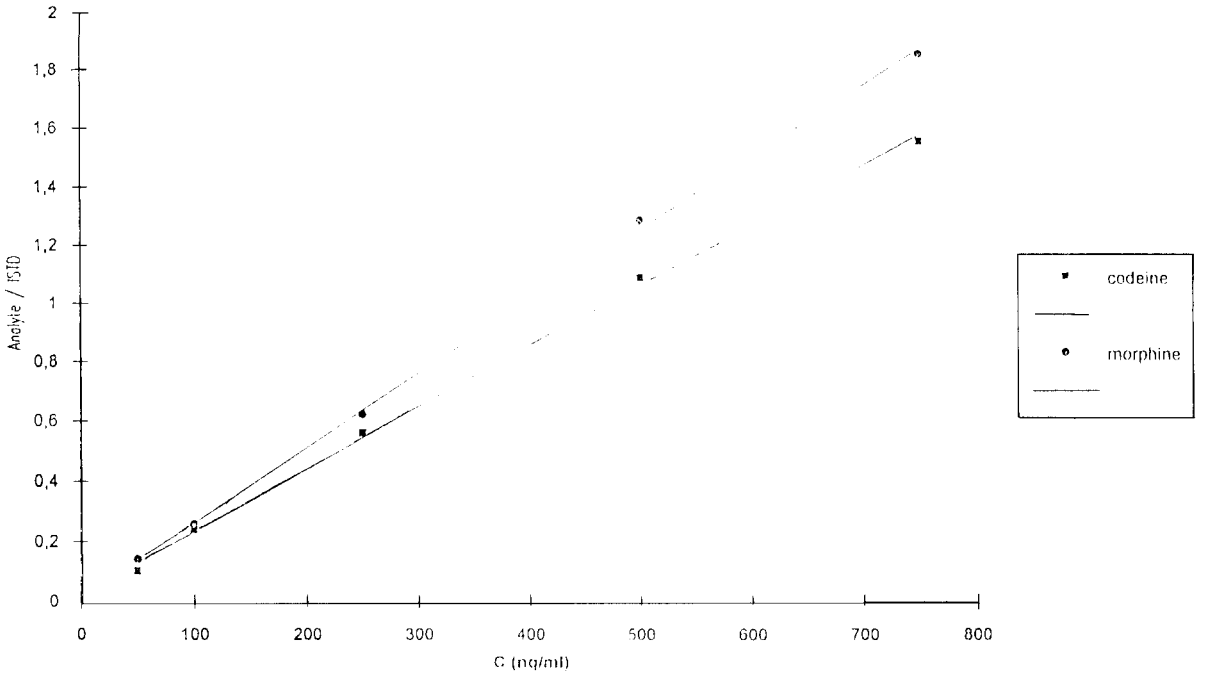

Fig. 1. Standard concentration curves for morphine and codeine in plasma. Morphine: $y = 2.5 \times 10^{-3}x + 14.5 \times 10^{-3}$ and codeine: $y = 2.1 \times 10^{-3}x + 26 \times 10^{-3}$.

2. Materials and methods

2.1. Chemicals and reagents

Morphine was purchased from Sanofi (94, Gentilly, France), codeine from Rhone-Poulenc (France), and nalorphine (internal standard) from Sigma (Sigma Aldrich Chimie SARL, L'Isle-d'Abeau-Chesnes, 38, Saint-Quentin-Fallavier, France) as well as trishydroxymethylaminomethane (Tris), sodium azide and sodium heptane sulfonate. Triethylamine was obtained from Fluka Chemika (38, Saint-Quentin-Fallavier, France). Methanol, HPLC grade, glacial acetic acid and hydrochloric acid (HCl), analytical grade, were obtained from Merck (D 6100 Darmstadt, Germany). Acetonitrile, HPLC grade, was purchased from Prolabo (Centre d'Activités de la Poudrette, 69, Vaulx-en-Velin, France). In order to study extraction, normal human plasma was purchased from a Blood Transfusion Center (01, Beynost, France). Stock solutions, of M, C and nalorphine were performed by dissolving 20 mg of each product into 100 ml of a 0.05% sodium azide solution in water. These solutions can be kept refrigerated 4 weeks. Suitable dilution (one dilution for each analysis) of these solutions were prepared to spike drug free plasma with 50, 100, 250, 500, 750 ng ml^{-1} for M and C assay, and 600 ng ml^{-1} for nalorphine (internal standard). Tris buffer pH 7.5 was obtained by dissolving 6.1 g of Tris in 1 l of water, then 250 ml were removed and buffered to pH 7.5 with 0.1 N HCl and then completed to 1 l with deionized water.

2.2. Chromatography

The HPLC system consists of: membrane degasser (Thermo Separation Products, 91, Les Ulis, France); P 1000 XR gradient pump (Thermo Separation Products, 91, Les Ulis, France); Kontron 360 autosampler (Kontron, 78, Saint-Quentin-en-Yvelines, France); home made guard column (packed with Novapack C_{18}, 35–45 μm phase, 25 × 2 mm i.d; Waters S.A., 78, Saint-Quentin-en-Yvelines, France); Waters Symmetry analytical

Table 1
Within-day precision ($n = 10$)

	50 ng ml^{-1}	250 ng ml^{-1}	750 ng ml^{-1}
Morphine R.S.D%	6.8	3.7	2.7
Codeine R.S.D%	9	1	1.5

column (C_{18}, 150 × 3.9 mm i.d., 5 μm; Waters S.A., 78, Saint-Quentin-en-Yvelines, France); UV 2000 spectrophotometer (Thermoseparation Products, 91, Les Ulis, France) set at 240 nm and Spectra Physics SP 4400 integrator (Spectra Physics, CA, USA). The mobile phase consisted of 215 ml of an aqueous solution of sodium heptane sulfonate at 3 mM l^{-1}, 85 ml of methanol, 3 ml of glacial acetic acid and 120 μl of triethylamine.

The flow rate was 0.8 ml min^{-1} and the injection volume was 50 μl.

2.3. Extraction procedure

For the extraction procedure, 50 μl of a nalorphine solution (12 mg l^{-1}), 50 μl of an appropriate dilution of M and C stock solutions, and 5 ml of Tris buffer were added to 1 ml of drug free plasma. For unknown samples, only 50 μl of nalorphine solution (12 mg l^{-1}) were added to 1 ml of plasma. Extractions were performed using C 18 Bond Elut (3 ml, 200 mg) solid phase extraction cartridges (Varian, CA, USA), whith an Alltech Vacuum manifold (Alltech, CA, USA). Cartridges were conditioned with 2 ml of methanol, followed by water (2 ml). The sample was then applied to the cartridge and washed with 2 × 1 ml of deionized water followed by 2 × 0.5 ml of a 40% acetonitrile solution in water. The compounds of interest were eluted in 5 ml polypropylene tubes with 3 × 0.5 ml of a 0.05 M HCl solution at 10% in acetonitrile. The eluate was evaporated under a stream of nitrogen at 40°C, then 100 μl of mobile phase were added and 50 μl were injected onto the column.

3. Results and discussion

3.1. Linearity studies

The linearity of the extracted M and C was determined by spiking blank plasma with known amounts of each analyte, in order to obtain concentrations of 50, 100, 250, 500, 750 ng ml^{-1}. The spiked plasma were extracted in the described manner. As shown in Fig. 1, Standard concentration curves for M and C ($y = 2.5 \times 10^{-3}x + 14.5 \times 10^{-3}$ and $y = 2.1 \times 10^{-3}x + 26 \times 10^{-3}$; established on the basis of ten calibration curves—mean value) were linear in the range evaluated. The correlation coefficients exceeded 0.999 in any case.

These curves were obtained by plotting drug to internal standard peak height ratios versus drug concentration.

3.2. Reproducibility studies

The within run relative standard deviations (%R.S.D.) calculated from the peak height ratios, of M and C to internal standard at analyte concentrations of 50, 250 and 750 ng ml^{-1} (ten samples assayed for each concentration), are shown in Table 1.

The between run R.S.D. for M and C were calculated while extracting all the plasma standards (50, 100, 250, 500, 750 ng ml^{-1}) obtained

Table 2
Between-day precision ($n = 10$)

	50 ng ml^{-1}	100 ng ml^{-1}	250 ng ml^{-1}	500 ng ml^{-1}	750 ng ml^{-1}
Morphine R.S.D%	9.4	8.2	3.4	5.5	7
Codeine R.S.D%	9.2	7.3	2.7	3.5	3

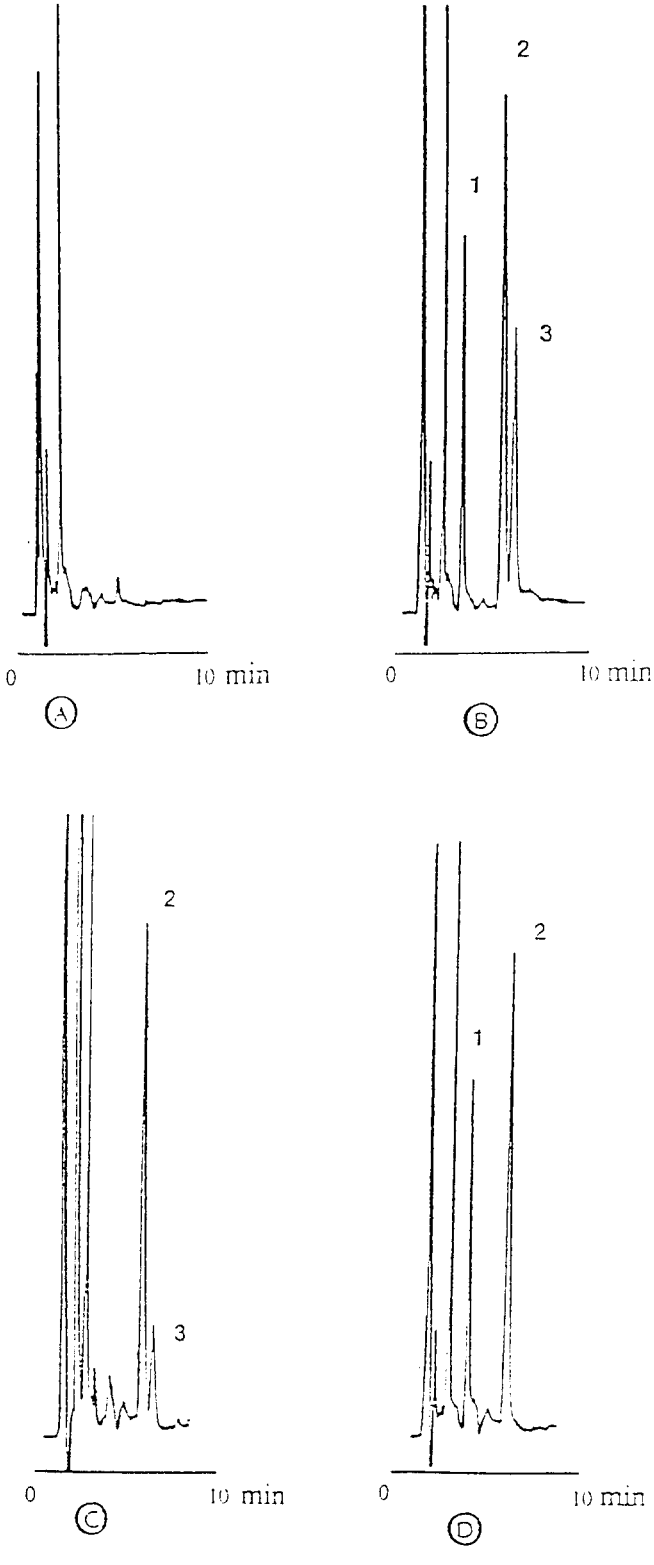

Fig. 2.

from independently prepared standards dilutions, twice a day during 5 days (see Table 2). These data demonstrate adequate reproducibility for routine laboratory use.

3.3. Recovery studies

The results of the recovery study for C, M and nalorphine were 80% (range 77–83%), 85% (range 83–87%) and 86%.

3.4. Limits of quantitation

The Limits of Quantitation (LOQ) is based on the minimum reliably integrable peak height with our equipment and sample volume of 1 ml of plasma. The LOQ is of 50 ng ml^{-1} for M and C. Further studies are conducted to improve these results.

3.5. Interferences

As discussed above, we have found no interferences from endogenous compounds, neither from currently administrated drugs: heparin, tranquilizers, benzodiazepines, buprenorphine, acetaminophen, other opiates derivatives. Only nalbuphine presents nearly the same extraction profile but does not interfere with other peaks during the analysis.

3.6. Applications

Fig. 2 shows chromatograms of blank plasma and of plasma spiked with 250 ng ml^{-1} of M and C. It also shows chromatograms of extracted plasma from two differents patients treated for chronic pain at hospital. The determination was performed 3 and 1 h, respectively after oral M and C administration (unknown doses).

The extraction of M and C from plasma on solid phase cartridges is shown to be reliable and efficient for their analysis by HPLC with UV detection, provided that the eluates are collected in polypropylene tubes, otherwise erratic drug losses can occur. This procedure yields clean extracts with good drug recovery. Furthermore, this procedure is attractive since it involves single step rather than several manipulations typical of liquid–liquid extraction. This method, easy to implement, can be applied in many clinical laboratories because it uses standard apparatus and has the advantages of being fast an economic, as only 10 min are necessary for a run, and only one cartridge is used per extraction.

Recirculation of the mobile phases may significantly reduce the cost of an analysis.

4. Conclusion

The method presented here for the analysis of morphine and codeine in plasma is rapid, sensitive, linear and can be used for the detection of these opiates in plasma, or to monitor long term treatments in chronic pain patients receiving slow-release or intrathecal M, as well as in children receiving oral M and/or C preoperatively.

Acknowledgements

We are grateful to L. Chevallet and G. Freiermuth for their technical collaboration.

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Fig. 2. Chromatograms of morphine and codeine under assay conditions. Column: Waters 'Symmetry' (15 × 0.39 cm i.d., 5 μm); flow rate: 0.8 ml min^{-1}; mobile phase was sodium heptane sulfonate 3 mM l^{-1}, methanol, glacial acetic acid, triethylamine 215 ml/85 ml/3 ml/120 μl. The samples were extracted according to the procedure described above. (A) Blank plasma, (B) plasma spiked with 250 ng ml^{-1} of codeine and morphine, (C) plasma from patient at 67 ng ml^{-1} (codeine) and < 50 ng/mL (morphine), and (D) plasma from patient at 257 ng ml^{-1} (morphine). 1: morphine; 2: nalorphine; and 3: codeine.

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