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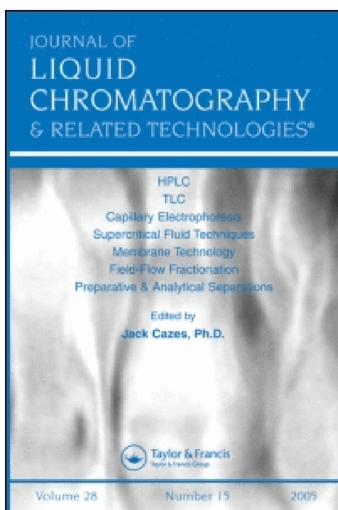


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# Comparative Study of Different Solid-Phase Extraction Cartridges in the Simultaneous RP-HPLC Analysis of Morphine and Codeine in Biological Fluids

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JOURNAL OF LIQUID CHROMATOGRAPHY, 16(14), 3107-3040 (1993)

# COMPARATIVE STUDY OF DIFFERENT SOLID-PHASE EXTRACTION CARTRIDGES IN THE SIMULTANEOUS RP-HPLC ANALYSIS OF *MORPHINE* AND *CODEINE* IN BIOLOGICAL FLUIDS

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## ABSTRACT

A comparative study of different Solid-Phase Extraction (SPE) Cartridges for the extraction and purification of morphine and codeine in plasma and urine samples is presented.

A rapid and sensitive Reversed-Phase isocratic High-Performance Liquid Chromatographic (RP-HPLC) method has been developed for the simultaneous determination of morphine and codeine in plasma and urine samples, using quinine as internal standard. The drugs were extracted from the sample matrices by using the SPE technique and a series of cartridges from different manufacturers and with different sorbents were tested. The best recovery from plasma was achieved with Alltech C18 cartridges and the best recovery from urine with Bakerbond C18 cartridges. The HPLC analysis was performed with an Adsorbosphere HS C18, ODS, 5 μm, 250 x 4.6 mm I.D., column and UV detection at 241 nm and it was completed within approximately 10 min. The mobile phase was a mixture of methanol-acetonitrile 0.1 M ammonium acetate (40 : 25 : 35 v/v) and the flow rate was 0.80 ml/min. The detection limits were found to be 2.0 ng on-column for morphine and 1.0 ng on-column for codeine.

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## INTRODUCTION

Simultaneous determination of morphine and codeine in biological samples is a common practice in many laboratories involved in forensic and clinical toxicology. Therefore, there is a growing need for the development of sensitive and rapid methods for the analysis of these drugs.

The analysis of morphine and codeine aim to the monitoring of therapeutic levels in patients and of drug concentrations in pharmacokinetic studies as well as to the investigation of heroin abuse in cases of intoxication or death.

The main source of morphine and codeine is the metabolism of heroin which undergoes rapid enzymatic deacylation to the 6-acetylmorphine conjugate, mainly catalysed by blood esterases and then the 6-acetylmorphine is converted to morphine presumably in the liver [1, 2].

On the other hand, morphine is the active metabolite of codeine itself and is excreted in urine. Therefore, with only urine as evidence the interpretation of the results can be confusing. If both drugs are found to be present, it is difficult to say whether single or multiple opiate use has occurred. Although blood samples are definitive about which opiate has been used, urine samples seem to be preferably sumbitted for analysis by some law enforcement officials. Analysis of urine samples has revealed that after 20 to 40 hours of codeine ingestion for therapeutic reasons, the concentration of its major metabolite morphine increases with respect to codeine and surpasses that of the parent drug [3]. Therefore, extreme care should be taken before drawing conclusions from analysis of only urine specimens collected over a short period of time.

Radioimmunoassays (RIA) have been used for screening purposes in urine and blood [2-4], but codeine cross-reacts with the morphine specific-antibody and the results can only be given as positive or negative for total opiate use. Positive screens need to be further evaluated by other methods of analyses in which quantitation of morphine and codeine is performed.

There is a considerable number of publications using various kinds of chromatographic techniques for either the single analysis of morphine or codeine or for their simultaneous analysis or finally for the analysis of these opiates and their metabolites in various matrices.

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Thus Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) with ultra-violet (UV) [2-9], fluorescence [10] or electrochemical detection [11], reversed-phase ion-pair HPLC with UV detection [12-15], normal-phase HPLC with UV detection [16, 17], Gas Chromatography (GC) - Mass Fragmentography (MF) [18], GC - Mass Spectrometry (MS) [19,20] GC with Electron Capture Detection (ECD) [21], Supercritical Fluid Chromatography (SFC) [22] and Thin - Layer Chromatography with Flame Ionization Detection (TLC-FID) [23] have been used for the analysis of the opiates.

Among the chromatographic techniques commonly used, TLC is simple but usually lacks sensitivity and specificity, while GC though more specific and reliable is time-consuming, because it usually requires derivatization of the opiates. Therefore, HPLC is the techniques usually preferred because of its specificity, sensitivity and reliability.

Most of the chromatographic techniques used require sample preparation which usually consists of extraction (liquid -liquid or SPE). SPE is gaining a wider acceptance because it is less time - consuming than liquid - liquid extraction and requires small volumes of solvents.

Only a few of the above mentioned publications using HPLC, deal with the simultaneous analysis of morphine and codeine in blood and/or urine [2-4, 8, 10, 15] and only two of them [2,15] make use of SPE.

The present paper describes a RP-HPLC method for the simultaneous determination of morphine and codeine in 100 μl of blood plasma and urine using SPE for the removal of matrix interferences. The cartridges used for extraction of the drugs were carefully selected after a comparison study between cartridges from various manufactures and with different sorbents. The proposed method offers rapidity, very good separation of the drugs, satisfactory sensitivity for UV detection and good precision and accuracy. The retention times were 4.49 min for morphine 6.25 min for codeine and 8.65 min for quinine. As little as 2.0 ng of morphine and 1.0 ng of codeine could be detected and the Relative Standard Deviations (RSD) of eight replicate analyses of three standards ranged from 1.7 to 3.6% for morphine concentrations between 0.887 and 14.2 ng/μl and from 1.9 to 5.3% for codeine concentrations between 1.0 and 16.0 ng/μl.

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## EXPERIMENTAL

### Apparatus

A Spectra Physics, Model SP8800 (California, USA) ternary gradient pump, a Rheodyne 7125 (California, USA) injection valve with a 10-μl loop and a Spectra Physics, Model Spectra Chrom 100, variable - wavelength detector were employed for analysis. The detector was connected to a Spectra Physics, Model 4290, integrator for the recording of results and evaluation of peak areas and retention times.

The reversed-phase chromatographic column used was an Adsorbosphere HS C18, ODS, 5 μm, 250 x 4.6 mm I.D. stainless-steel column which was obtained from Alltech Associates Inc. (Illinois, USA).

A Glass-col, Terre Haute In 47802 small vortexer and a Hermle centrifuge, Model Z230 (B. Hermle AG, Gosheim, Germany), were employed for the treatment of blood plasma and urine samples prior to SPE.

The SPE cartridges were positioned in a Vac-Elut system, having a 10-column capacity, which was obtained from Analytichem International, a division of Varian (Harbor City, USA).

The cartridges used for comparison study of the extraction efficiency (percent recovery) of the drugs were the following: Bond Elut C18 (200 mg/3ml) and C8 (200 mg/3 ml) obtained from Analytichem International a division of Varian, C18 (100 mg/3 ml) obtained from Alltech Associates Inc., Bakerbond C18 (200 and 500 mg/3ml) obtained from J.T. Baker Inc. (Gross Gerau, Germany), Separcol C18 and C18 RPS as well as Separcol C8 and C1 all obtained from Anapron spol Sr.O (Bratislava, Slovakia) and Polymer Institute, Slovak Academy of Sciences, (Bratislava, Slovakia).

A glass vacuum solvent-filtration apparatus obtained from Alltech Associates Inc., was employed for the filtration of mobile phase using 0.2 μm Anodisc 47 mm I.D. filters obtained also from Alltech.

Computations and statistical treatment of data were performed using a PC Vip 312.

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## Reagents

Codeine free base and morphine hydrochloride were supplied by the Greek State Laboratory. Strychnine was obtained from the Toxicological Laboratory of the University of Thessaloniki. Analytical-reagent grade caffeine (anhydrous powder) was obtained from Sigma Chemical Co., USA, and quinine sulphate, $(C_{20}H_{24}N_2O_2) \cdot H_2SO_4 \cdot 2H_2O$, from BDH Chemicals Ltd (Poole, UK). All stock solutions of these compounds were prepared by dissolving the appropriate amounts in HPLC-grade methanol and kept refrigerated.

The borate buffer pH 9.2 was prepared by mixing 250 ml of 0.025 M sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) and 18 ml of 0.1 M sodium hydroxide. Both components were of analytical-reagent grade obtained from Merck (Darmstadt, Germany).

The ammonium acetate stock solution was prepared by dissolving the appropriate quantity of the analytical-reagent grade compound obtained from Merck in double-deionized water.

Different compositions of mobile phase, always consisting of a mixture of methanol, acetonitrile and different concentrations of ammonium acetate solution, were tested. The mobile phase was always filtered through a 0.2 μm filter and degassed in an ultra-sonic bath prior to use.

## Chromatographic Conditions

The chromatographic analysis was performed under isocratic conditions and at ambient temperatures, 22°C, with the detector operating at 241 nm and with a sensitivity setting of 0.002 AUFS. The mobile phase was a mixture of methanol - acetonitrile - 0.1 M ammonium acetate (40 : 25 : 35 v/v). The flow rate was 0.80 ml/min with a pressure of 1900 psi. The above mentioned composition and flow rate of the mobile phase as well as quinine sulphate, as internal standard, were selected among several ones tested as can be seen in Table 1.

## Calibration Curves for the Simultaneous Determination of Morphine and Codeine in Standard Solutions

Eight working standards of morphine and codeine, all containing the same fixed amount of internal standard, quinine, were prepared in mobile phase. A stock

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**TABLE 1**
**Isocratic High - Performance Liquid Chromatographic Conditions Examined in the Present Study**

| Eluent | Flow Rate (ml/min) | Retention Time (min) | | | | |
|---|---|---|---|---|---|---|
| | | Morphine (3.55ng/μl) | Codeine (4.0ng/μl) | Caffeine (IS) (4.0ng/μl) | Quinine (IS) (2.4ng/μl) | Strychnine (IS) (4.0ng/μl) |
| $CH_3OH$:$CH_3CN$:0.006M $CH_3COONH_4$ | | | | | | |
| 30 : 35 : 35 | 0.80 | 4.89 | 8.10 | 4.20 | 43.0 | ND |
| $CH_3OH$:$CH_3CN$: 0.013M $CH_3COONH_4$ | | | | | | |
| 25 : 20 : 55 | 0.80 | 5.49 | 9.80 | 4.94 | 27.0 | NT |
| 30 : 30 : 40 | 0.80 | 4.82 | 6.80 | 4.23 | 12.15 | NT |
| 30 : 35 : 35 | 0.80 | 4.73 | 6.37 | 4.18 | 8.39 | NT |
| $CH_3OH$:$CH_3CN$: 0.026M $CH_3COONH_4$ | | | | | | |
| 20 : 30 : 50 | 0.80 | 4.74 | 7.05 | 4.41 | 11.89 | 15.24 |
| 25 : 25 : 50 | 0.80 | 4.93 | 8.18 | NT | 18.16 | NT |
| 25 : 40 : 35 | 0.90 | 3.96 | 5.27 | 3.67 | 7.13 | 18.16 |
| 32.5: 32.5: 35 | 0.90 | 4.11 | 5.75 | 3.93 | 8.49 | ND |
| 40 : 25 : 35 | 0.90 | 4.20 | 5.80 | 3.86 | 8.55 | 20.74 |
| $CH_3OH$:$CH_3CN$: 0.052M $CH_3COONH_4$ | | | | | | |
| 20 : 20 : 60 | 0.80 | 5.20 | 9.20 | 4.42 | NT | NT |
| 25 : 25 : 50 | 0.80 | 4.64 | 7.05 | NT | 13.25 | NT |
| 25 : 40 : 35 | 0.80 | 3.86 | 4.96 | NT | 6.28 | NT |
| 32.5: 32.5: 35 | 0.80 | 3.96 | 5.19 | NT | 6.95 | NT |
| 35 : 20 : 45 | 0.80 | 4.30 | 6.51 | 4.25 | 11.80 | NT |
| 40 : 25 : 35 | 0.80 | 4.05 | 5.41 | NT | 7.36 | NT |
| 45 : 10 : 45 | 0.80 | 5.24 | 8.12 | 5.20 | NT | NT |
| $CH_3OH$:$CH_3CN$: 0.078M $CH_3COONH_4$ | | | | | | |
| 30 : 35 : 35 | 0.80 | 4.20 | 4.25 | 4.17 | 8.60 | NT |
| $CH_3OH$:$CH_3CN$: 0.10M $CH_3COONH_4$ | | | | | | |
| 32.5: 32.5: 35 | 0.90 | 4.97 | 6.60 | 3.75 | NT | NT |
| 36 : 22.5: 41.5 | 0.90 | 6.60 | 10.47 | 4.52 | NT | NT |
| 40 : 25 : 35 | 0.80 | 4.49 | 6.25 | NT | 8.65 | NT |
| 40 : 35 : 25 | 0.70 | 4.48 | 7.53 | 3.99 | NT | NT |
| 45 : 20 : 35 | 0.70 | 5.91 | 7.97 | 4.38 | NT | NT |

ND = Not Detected NT = Not Tested IS = Internal Standard

standard solution was separately prepared for each of the opiates and for quinine sulphate in methanol, to give concentrations of 1064 ng/µl for morphine, 1000 ng/µl for codeine, both in terms of free base and 240 ng/µl for quinine sulphate. The stock solutions were serially diluted, with methanol, by a factor of ten and of these dilute solutions appropriate volumes of morphine and codeine were added together in 50-ml volumetric flasks for the preparation of the working standards. A 5-ml volume of a 24 ng/µl quinine sulphate methanolic solution was always added to the working solutions during dilution to give a final concentration of quinine sulphate equal to 2.4 ng/µl. The solutions were made up to the final volume with mobile phase. The resulting concentrations of the solutions were 0.177, 0.443, 0.887, 1.77, 3.55, 7.09, 14.2 and 21.3 ng/µl with respect to morphine free base and 0.20, 0.50, 1.0, 2.0, 4.0, 8.0, 16.0 and 24.0 ng/µl with respect to codeine free base, respectively. Five replicate injections of each of these standard mixtures were made. The mean values of the peak area ratios of morphine and codeine to quinine were plotted separately as a function of the corresponding concentration of each compound.

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## Determination of Morphine and Codeine in Human Blood Plasma and Urine

A new set of calibration curves was constructed for the blood plasma and urine samples using the standard additions method. The samples were spiked with mixtures of morphine and codeine at five different concentration levels with quinine as internal standard. After the appropriate treatment, i.e. precipitation of proteins and SPE as a clean-up step, the extracted samples were subjected to HPLC analysis. Six replicate injections of each concentration level were made and the peak area ratios of morphine and codeine were plotted against the corresponding concentration of the drugs.

## Sample Preparation and Solid-Phase Extraction

A 100-µl volume of 0.443/0.5, 0.887/1.0, 1.77/2.0, 3.55/4.0 and 7.09/8.0 ng/µl morphine/codeine standard solutions in mobile phase, with quinine sulphate at a concentration of 2.4 ng/µl and a 200-µl volume of acetonitrile for protein precipitation were added to 100-µl aliquots of the **plasma** sample. The mixtures were vortex-mixed for 60 sec and then centrifuged for 15 min at 3500 rpm. The supernatants were subjected directly to SPE using C18 (100 mg) cartridges obtained

from Alltech. Just prior to sample application the cartridges were conditioned by passing 1 x 3 ml of methanol and then 2 x 3 ml of deionized water. A 2-ml volume of borate buffer pH 9.2 was applied onto the cartridges and then immediately the supernatant was applied and slowly forced through the cartridges. A washing step with 2 x 3 ml water followed and then the drugs were eluted with 1 x 2 ml of methanol. The methanolic solutions obtained were evaporated to dryness in a water bath at 45°C, using a stream of nitrogen and the residues were reconstituted with 100 μl of mobile phase. Aliquots of 10 μl of the resulting solutions were repeatedly injected onto the HPLC column.

It should be pointed out that no evaporation of the acetonitrile and methanol contained in the supernatants was needed before their application onto the cartridges, when the pH 9.2 buffer was introduced immediately before. In this way a time-consuming step was avoided. It was experimentally confirmed that no preliminary drug elution due to the organic solvents present in the supernatants occured. The fractions collected after application of the buffer plus supernatant and washing of the preconditioned cartridges, showed no trace of morphine and/or codeine on the HPLC column.

For **urine** assay the same procedure as outlined for plasma was adopted with the exception of the cartridges which were C18 Bakerbond cartridges (200 mg) and the washing step, after application of the sample, which was carried out with 3 x 3 ml of water.

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## RESULTS AND DISCUSSION

With the selected composition and flow rate of the mobile phase the analysis was completed within approximately 10 min and the separation between the three peaks was very good as can be seen in Fig. 1. The Resolution Factors, $R_s$, between adjacent peaks were calculated and found to be 2.20 between morphine and codeine and 2.18 between codeine and the internal standard quinine.

At the retention times of morphine, codeine and quinine no interferences from endogenous compounds were found in chromatograms of extracted blood serum and urine samples as can be seen in Figs 2-5. Additionally, the selection of quinine as internal standard, which comes after the analytes and within reasonable time, eliminated the problem of poor separation from the matrix without losing in

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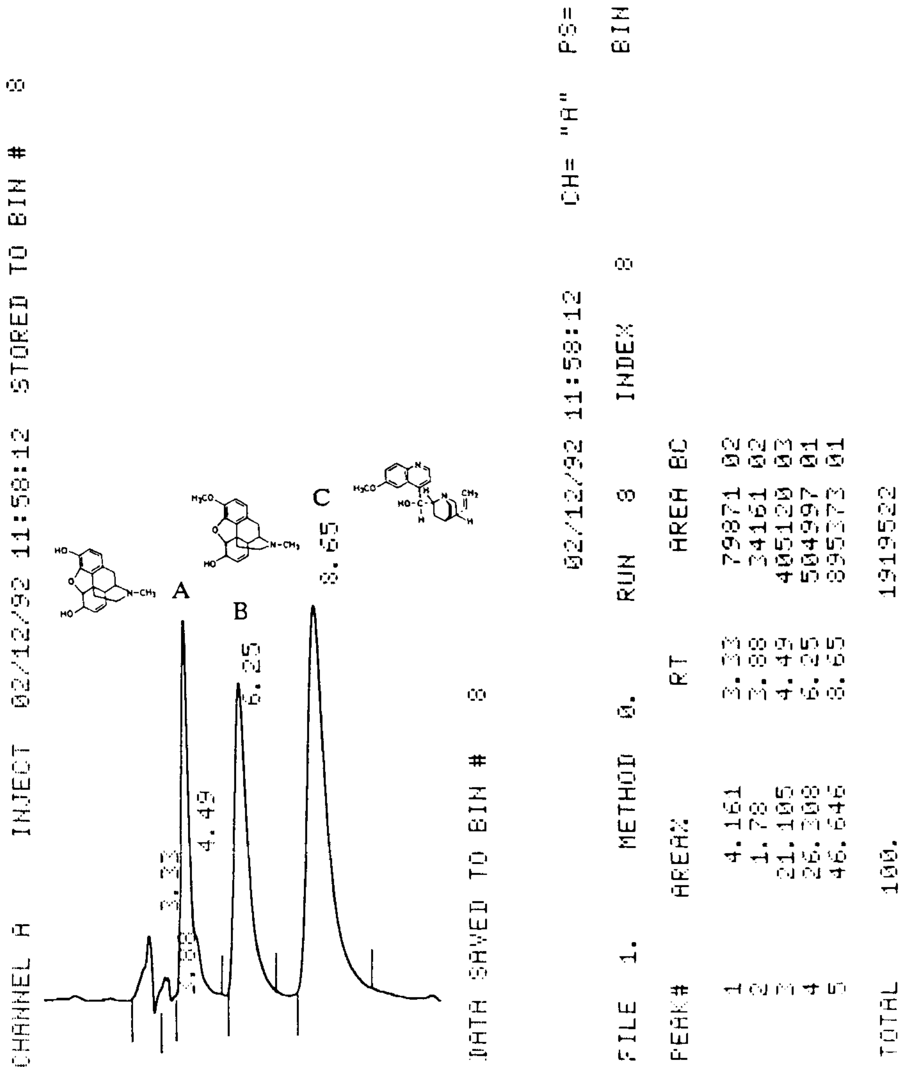
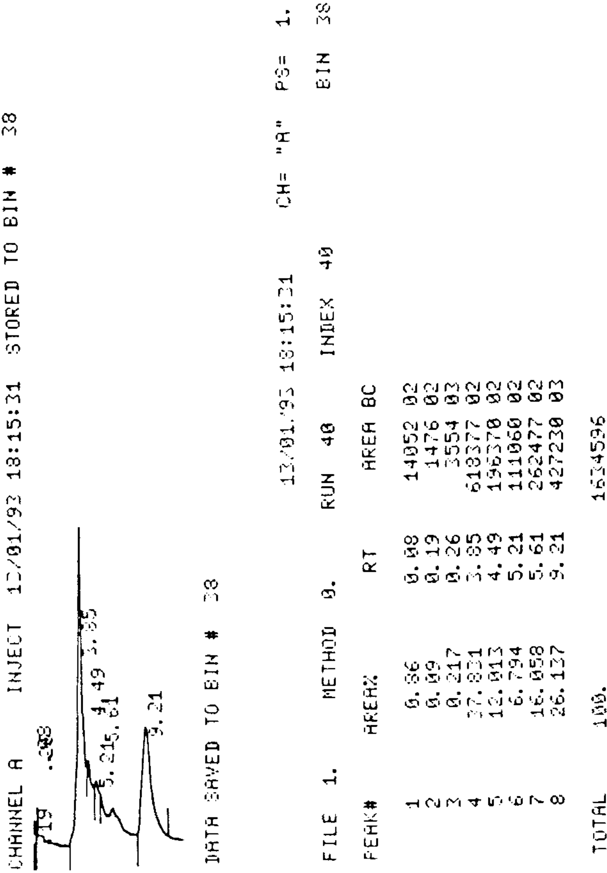



**Figure 1:** High-Performance Liquid Chromatogram of Morphine and Codeine standard solution with Quinine as Internal Standard. Chromatographic conditions as described in Experimental. Chart speed: 0.5 cm/min. Peaks: A (4.49 min) = Morphine (3.55 ng/μl), B(6.25 min) = Codeine (4.0 ng/μl) and C (8.65 min) = Quinine Sulphate (2.4 ng/μl).




| PEAK# | AREA% | RT | AREA | BC |
|---|---|---|---|---|
| 1 | 0.86 | 0.08 | 14052 | 02 |
| 2 | 0.09 | 0.19 | 1476 | 02 |
| 3 | 0.217 | 0.26 | 3554 | 03 |
| 4 | 37.831 | 3.85 | 618377 | 02 |
| 5 | 12.013 | 4.49 | 196370 | 02 |
| 6 | 6.794 | 5.21 | 111060 | 02 |
| 7 | 16.058 | 5.61 | 262477 | 02 |
| 8 | 26.137 | 9.21 | 427230 | 03 |
| TOTAL | 100. | | 1634596 | |

**Figure 2:** High-Performance Liquid Chromatogram of Blank Plasma extracted on an Alltech C18 (100 mg/3ml) cartridge with Quinine as Recovery Internal Standard. Chromatographic conditions as described in Experimental. Chart Speed: 0.2 cm/min. Peaks: 3.85 = unknown, 9.21 = Quinine Sulphate (2.4 ng/μl).

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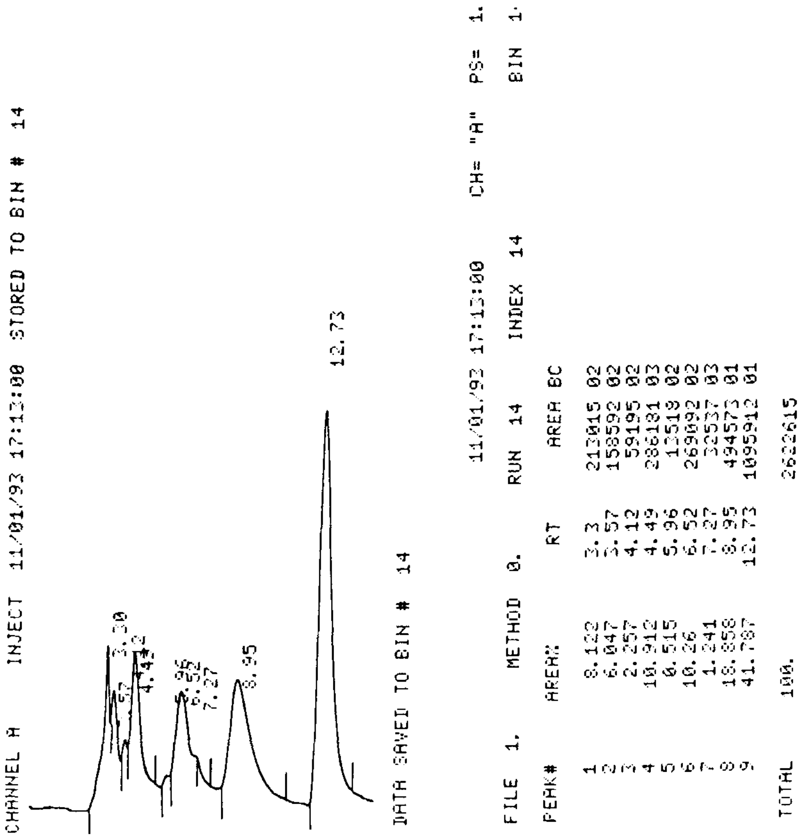


**Figure 3**: High-Performance Liquid Chromatogram of Morphine and Codeine extracted from Spiked Plasma on a Alltech C18 (100mg/3ml) cartridge with Quinine as Recovery Internal Standard. Chromatographic conditions as described in Experimental. Chart speed: 0.5 cm/min. Peaks: 3.30, 3.57, 4.12 = unknown, 4.49 = Morphine (3.55 ng/µl) 6.52 = Codeine (4.0 ng/µl), 8.95 = Quinine Sulphate (2.4 ng/µl) and 12.73 = unknown.

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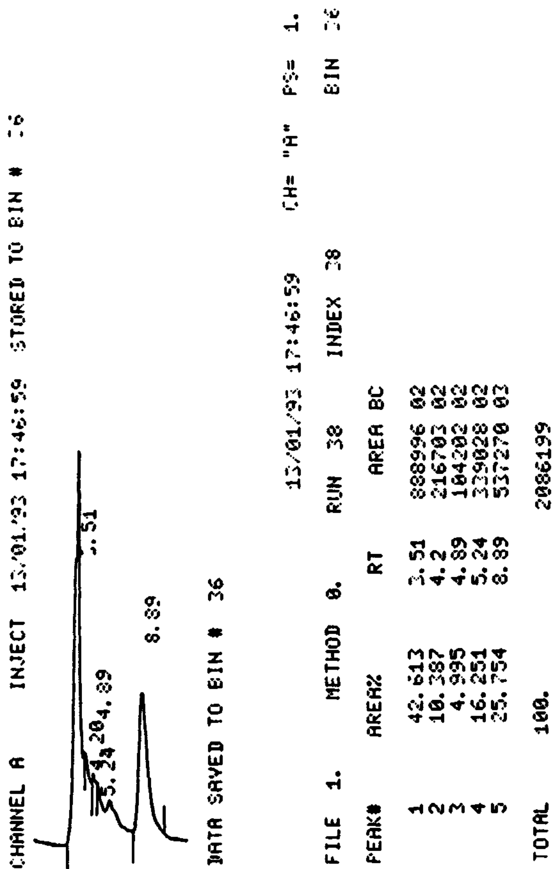


**Figure 4:** High-Performance Liquid Chromatogram of Blank Urine extracted on a Bakerbond C18 (200 mg/3ml) cartridge with Quinine as Recovery Internal Standard. Chromatographic conditions as described in Experimental. Chart speed: 0.2 cm.min$^{-1}$. Peaks: 3.51 = unkown, 8.89 = Quinine Sulphate (2.4 ng/µl).

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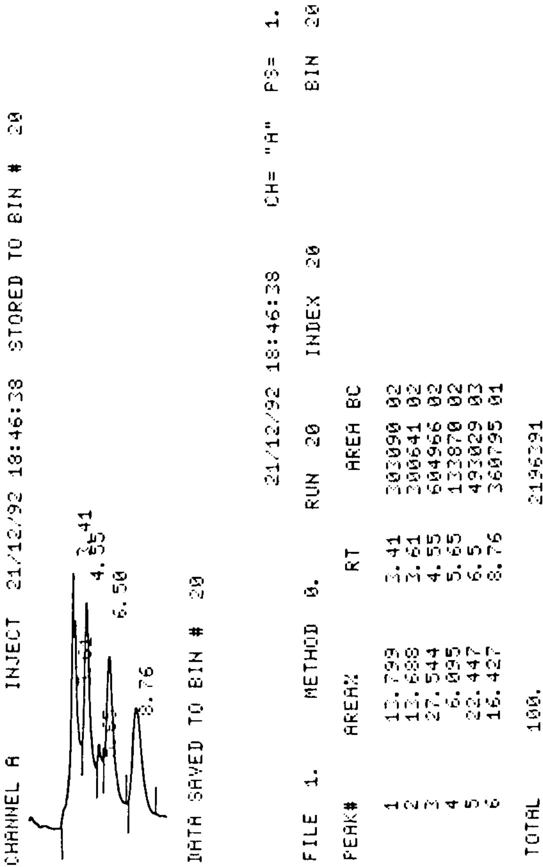


**Figure 5:** High-Performance Liquid Chromatogram of Morphine and Codeine extracted from Spiked Urine on a Bakerbond C18 (200 mg/3 ml) cartridge with Quinine as Recovery Internal Standard. Chromatographic conditions as described in Experimental. Chart speed: 0.2 $cm.min^{-1}$. Peaks: 3.41, 3.61 unknown, 4.55 = Morphine (3.55 ng/µl), 6.50 = Codeine (4.0 ng/µl) and 8.76 = Quinine Sulphate (2.4 ng/µl).

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rapidity. On the basis of selectivity and rapidity caffeine and strychnine which were also tested as potential internal standards were rejected.

With the 0.1 M concentration of ammonium acetate better peak shapes were obtained compared to those taken at lower concentrations. Low acidities of the mobile phase were also tested by using a phosphate buffer at pHs of 5.0, 3.5 and 3.0, but the retention times were too short and overlapping peaks occurred. The lower the pH the shorter the retention times became.

The peak area ratios of the analytes to internal standard quinine were linearly related to concentrations between 0.177-21.3 ng/μl (or 1.77-212.8 ng injected on-column) of morphine free base and between 0.20-24.0 ng/μl (2.0-240 ng injected on-column) of codeine free base.

The detection limits, i.e., those quantities producing a signal of a peak height twice the size of background noise, were found to be 2.0 ng injected on-column for morphine and 1.0 ng injected on-column for codeine.

The linear regression equations with their confidence limits at a 95% confidence level, as well as the correlation coefficients, of morphine and codeine in standard solutions and in spiked plasma and urine samples were the following:

**STATISTICAL EVALUATION**

| **Samples** | **Regression Equation** $Y=(a\pm t_{\alpha}\cdot S_a)+(b\pm t_{\alpha}\cdot S_b)X$ | **Correlation Coefficient** |
|---|---|---|
| **Standard Solutions in mobile phase** | | |
| Morphine | $Y=(0.02988\pm0.05772)+(0.1175\pm0.0061)X$ | 0.9987 |
| Codeine | $Y=(-0.02244\pm0.08444)+(0.1427\pm0.0079)X$ | 0.9985 |
| **Blood Plasma** | | |
| Morphine | $Y=(-0.001171\pm0.03600)+(0.1372\pm0.0098)X$ | 0.9992 |
| Codeine | $Y=(0.03747\pm0.02077)+(0.1199\pm0.0050)X$ | 0.9997 |
| **Urine** | | |
| Morphine | $Y=(0.2610\pm0.0971)+(0.3128\pm0.0265)X$ | 0.9989 |
| Codeine | $Y=(0.2010\pm0.0307)+(0.2306\pm0.0074)X$ | 0.9998 |

Y=Peak area ratios of morphine and codeine to quinine, X=Concentrations in ng/μl, a=intercept, b=slope, Sa,Sb=Standard deviations of intercept and slope respectively, $t_{\alpha}$=Student's t-test for n-2 degrees of freedom and 95% confidence level.

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Eight replicate injections of each of three standard morphine and codeine mixtures were made during a day's time and the peak area ratios of the analytes to internal standard quinine are given in Table 2. The results were treated statistically to assess the within-day precision and accuracy of the method which is given in Table 3.

The day - to - day precision and accuracy of the method was assessed by the repeated analyses of standard solutions of morphine and codeine over ten days. Each day's representative peak area ratio for the three concentration levels tested was the mean value of five replicate injections. The results are presented in Table 4.

The SPE technique was used as a clean-up step during sample preparation. A study of the variations between manufacturer - to - manufacturer, phase - to - phase and different amounts of sorbent was carried out in order to find the maximum percent extraction recovery of morphine and codeine. This study was repeated for both standard solutions and for spiked plasma and urine samples. Similar studies have been made before [24-27] in an attempt to see how polar interactions between drugs and sorbents of different origins, i.e., differences in content and availability of polar groups on silica, as well as hydrophobic properties of the drugs and the silica modifiers, i.e., non - polar interactions, affect the extraction and elution of the drugs.

In the present paper apart from the variety of cartridges which have been examined, different elution solvents have been also tested with cartridges from different vendors and with different sorbent. The study of the effect of different elution solvents on the SPE of morphine and codeine was carried out only in standard solutions of the drugs and the elution volume was always kept to 2 ml. The examined elution solvents and the results observed were the following: 1) Ethanol, tested with Alltech C18 and Bond Elut C18 and C8 cartridges. In all cases the recovery was poor. 2) Mixture of methanol-acetonitrile (1:1), tested with Bakerbond C18 (200 - and 500 mg/3 ml), Separcol C18, C18 RPS and C1 cartridges. In all cases the recovery was poor. 3) Mixture of phosphate buffer pH 2.4 - methanol (1:1), tested with Alltech C18 and Bond Elut C18 and C8 cartridges. The separation of the drugs on the HPLC column was bad, though the optimum chromatographic conditions were used. This could be attributed to the low pH of the eluted solution,

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TABLE 2

Peak Area Ratios of Morphine and Codeine to Internal Standard Quinine in Standard Mixtures

| Concentration (ng/μl) | Equivalent Quantity injected (ng) | Peak Area Ratio | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Mean Value | SD | RSD (%) |
| Morphine | | | | | | | | | | | | |
| 0.887 | 8.87 | 0.1340 | 0.1212 | 0.1313 | 0.1249 | 0.1266 | 0.1223 | 0.1318 | 0.1290 | 0.1276 | 0.0046 | 3.6 |
| 3.55 | 35.5 | 0.4894 | 0.4380 | 0.4750 | 0.4957 | 0.4321 | 0.4485 | 0.4650 | 0.4720 | 0.4645 | 0.0232 | 5.0 |
| 14.2 | 141.9 | 1.751 | 1.697 | 1.727 | 1.761 | 1.757 | 1.738 | 1.800 | 1.730 | 1.745 | 0.0301 | 1.7 |
| Codeine | | | | | | | | | | | | |
| 1.0 | 10.0 | 0.1303 | 0.1421 | 0.1257 | 0.1318 | 0.1461 | 0.1264 | 0.1345 | 0.1355 | 0.1341 | 0.0072 | 5.3 |
| 4.0 | 40.0 | 0.5020 | 0.5018 | 0.5584 | 0.5669 | 0.5329 | 0.4983 | 0.5669 | 0.5373 | 0.5331 | 0.0295 | 5.5 |
| 16.0 | 160.0 | 2.252 | 2.315 | 2.210 | 2.250 | 2.325 | 2.329 | 2.283 | 2.320 | 2.286 | 0.0441 | 1.9 |

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TABLE 3

Experimental Results for the Simultaneous Determination of Morphine and Codeine in Standard Solutions using Quinine as Internal Standard

| Drug | Quantity injected (ng) | Found[a], $\bar{x}$ (ng) | SD | RSD (%) |
|---|---|---|---|---|
| Morphine | 8.87 | 8.33 | 0.39 | 4.7 |
| | 35.5 | 36.96 | 1.97 | 5.3 |
| | 141.9 | 145.82 | 2.56 | 1.8 |
| Codeine | 10.0 | 10.97 | 0.50 | 4.6 |
| | 40.0 | 38.92 | 2.07 | 5.3 |
| | 160.0 | 161.70 | 3.09 | 1.9 |

[a] obtained from the linear regression equation for standard solutions, $\bar{x}$ = mean value of n=8 determinations.

TABLE 4

Day - to - day Precision and Accuracy of Morphine and Codeine Determination in Standard Solutions using Quinine as Internal Standard.

| Drug | Quantity injected (ng) | Found[a], $\bar{x}$ (ng) | SD | RSD (%) |
|---|---|---|---|---|
| Morphine | 8.87 | 8.29 | 0.42 | 5.0 |
| | 35.5 | 35.70 | 1.90 | 5.3 |
| | 141.9 | 144.10 | 3.14 | 2.2 |
| Codeine | 10.0 | 10.80 | 0.34 | 3.1 |
| | 40.0 | 40.49 | 2.29 | 5.6 |
| | 160.0 | 161.57 | 3.49 | 2.2 |

[a] obtained from the linear regression equation for standard solutions, $\bar{x}$ = mean value for n=10 determinations.

which due to its small volume could not be adjusted. With the chromatographic conditions chosen, at low pHs the retention times of the opiates decrease and the resolution deteriorates. 4) The mobile phase chosen for chromatographic analysis, tested with all the cartridges available in this study. The recovery was satisfactory. 5) Methanol, tested also with all the cartridges available. The recovery achieved was the best, therefore methanol was the elution solvent preferred in this study.

Using methanol, the elution volume was examined by eluting also twice with 2 ml of methanol. Each eluted fraction was collected separately, evaporated to dryness, reconstituted with mobile phase and injected on-column. No trace of morphine and/or codeine was found in the second fraction. Therefore a 1 x 2 ml volume of methanol was used for elution throughout this study.

The percent extraction recovery of morphine and codeine was calculated by comparing the peak area ratios of the extracted drugs to internal standard quinine with the peak area ratios of unextracted standards of the drugs at the same concentration level, measured the same day. For the evaluation of extraction recoveries quinine was used as a chromatographic internal standard, i.e. added just prior to the injection on the HPLC column. The residue obtained after evaporation of the eluted solution was reconstituted with 100 μl of mobile phase containing 2.4 ng/μl of quinine sulphate.

The extraction of morphine and codeine from standard solutions containing 3.55 ng/μl of morphine and 4.0 ng/μl of codeine and from plasma and urine samples spiked with the above concentrations of the drugs was evaluated for a series of cartridges from different vendors and packed with different sorbents or different amounts of sorbent. The results are presented in Table 5 and are based on triplicate HPLC determinations.

The differences in recoveries for the same drug and from the same cartridge as well as between same type of sorbent from different manufactures of between type of corbent from different manufactures or between different types of sorbents, observed in Table 5, are attributed to interactions between matrix-sorbent, matrix-isolate and sorbent-isolate. Thus, higher recoveries of the drugs in spiked plasma or urine samples than in the standard, using the same cartridge, might be attributed to matrix-sorbent interactions, i.e. endogens from matrix facilitate the elution of the drugs by occupying polar groups of the silica surface thus reducing the possibility

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TABLE 5

Percent Ectraction Recoveries of Morphine (3.55 ng/μl) and Codeine (4.0 ng/μl) from Standard Solution and Spiked Plasma and Urine Samples using Different SPE Cartridges with Quinine as Chromatographic Internal Standard

| Cartridge | Recoveries[a] (%) | | | | | |
|---|---|---|---|---|---|---|
| | Morphine | | | Codeine | | |
| | Standard Solution | Plasma | Urine | Standard Solution | Plasma | Urine |
| 1. Bond Elut C18 (200 mg/3 ml) | 37.0 ± 9.7 | 23.2 ± 2.9 | NT | 37.6 ± 7.6 | 27.1 ± 3.0 | NT |
| 2. Bakerbond C18 (200 mg/3 ml) | 122.4 ± 13.0 | 48.0[b] | 88.3 ± 7.0 | 41.7 ± 11.9 | 51.0[b] | 89.4 ± 7.3 |
| 3. Bakerbond C18 (500 mg/3 ml) | 76.9 ± 3.6 | 70.0[b] | 128.8 ± 14.6 | 28.5 ± 0.01 | 37.0[b] | 81.6 ± 12.6 |
| 4. Alltech C18 (100 mg/3 ml) | 29.3 ± 2.2 | 87.6 ± 8.2 | NT | 47.0 ± 4.4 | 87.0 ± 6.6 | NT |
| 5. Separcol C18 | 31.4 ± 10.3 | 27.2 ± 6.6 | NT | 33.6 ± 1.9 | 17.2 ± 1.3 | NT |
| 6. Separcol C18 RPS | 94.3 ± 13.2 | 97.4[b] | NT | 40.4 ± 12.7 | 57.0[b] | NT |
| 7. Bond Elut C8 (200 mg/3 ml) | 46.5 ± 1.9 | 27.1 ± 1.8 | 102.6 ± 21.7 | 46.6 ± 1.1 | 30.1 ± 0.01 | 112.9 ± 30.7 |
| 8. Separcol C8 | 51.6 ± 5.0 | 55.2 ± 1.4 | 41.0 ± 8.9 | 56.9 ± 8.8 | 38.1 ± 7.7 | 49.9 ± 6.8 |
| 9. Separcol C1 | 109.4 ± 10.7 | 108.5[b] | 116.1 ± 8.6 | 55.2 ± 0.0 | 62.0[b] | 102.5 ± 1.2 |

[a] Percent Recoveries ± Standard Deviation based on triplicate determinations
[b] Based only on one determination. Bad peak shapes and poor resolution from the matrix
NT = Not Tested

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TABLE 6

Experimental Results for the Simultaneous Determination of Morphine and Codeine Extracted from Spiked Plasma with Quinine as Recovery Internal Standard

| Drug | Quantity added (ng) | Found[a], $\bar{x}$ (ng) | SD | RSD (%) |
|---|---|---|---|---|
| Morphine | 8.87 | 8.60 | 0.29 | 3.3 |
| | 17.7 | 17.39 | 0.23 | 1.3 |
| | 35.5 | 34.30 | 0.37 | 1.1 |
| Codeine | 10.0 | 10.26 | 0.33 | 3.2 |
| | 20.0 | 20.70 | 0.69 | 3.4 |
| | 40.0 | 40.78 | 0.65 | 1.6 |

[a] obtained from the linear regression equation for spiked plasma, $\bar{x}$ = mean value of n=6 determinations.

TABLE 7

Experimental Results for the Simultaneous Determination of Morphine and Codeine Extracted from Spiked Urine with Quinine as Recovery Internal Standard

| Drug | Quantity added (ng) | Found[a], $\bar{x}$ (ng) | SD | RSD (%) |
|---|---|---|---|---|
| Morphine | 8.87 | 8.91 | 0.08 | 0.84 |
| | 17.7 | 18.15 | 0.61 | 3.4 |
| | 35.5 | 34.97 | 0.75 | 2.1 |
| Codeine | 10.0 | 9.56 | 0.50 | 5.2 |
| | 20.0 | 19.78 | 0.42 | 2.1 |
| | 40.0 | 39.43 | 0.66 | 1.7 |

[a] obtained from the linear regression equation for spiked urine, $\bar{x}$ = mean value for n=6 determinations

TABLE 8

**Overall Precision and Accuracy of Morphine and Codeine Determination in Spiked Plasma and Urine using Quinine as Recovery Internal Standard**

| Drug | Cartridge | Extracted from Plasma | Extracted from Urine |
|---|---|---|---|
| | Alltech C18 | Found[a], x | Found[b], x |
| Morphine Added 35.5 ng | 1 | 39.03 | 33.12 |
| | 2 | 36.70 | 35.96 |
| | 3 | 34.42 | 36.54 |
| | 4 | 35.43 | 34.94 |
| | 5 | 34.01 | 35.00 |
| | | ------------- | ----------- |
| | | Mean = 35.92 | Mean = 35.11 |
| | | SD = 2.03 | SD = 1.30 |
| | | RSD = 5.6% | RSD = 3.7% |
| | | Recovery = 101.2% | Recovery = 98.9% |
| | Bakerbond C18 | | |
| Codeine Added 40.0 ng | 1 | 41.69 | 39.07 |
| | 2 | 40.17 | 39.20 |
| | 3 | 40.77 | 39.46 |
| | 4 | 42.43 | 38.94 |
| | 5 | 40.91 | 39.85 |
| | | ------------- | ----------- |
| | | Mean = 41.19 | Mean = 39.30 |
| | | SD = 0.88 | SD = 0.36 |
| | | RSD = 2.1% | RSD = 0.92% |
| | | Recovery = 102.9% | Recovery = 98.2% |

[a] Mean value of n=3 determinations, obtained from the linear regression equation for plasma.

[b] Mean value of n=3 determinations, obtained from the linear regression equation for urine.

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of polar interactions between isolate-sorbent. Lower recoveries in spiked samples than in the standard might be due to isolate-matrix interactions. Variations in recoveries using the same type of sorbent but from different manufacturers are due to polar interactions between isolate-sorbent.

As can be seen in Table 5 the best recovery of morphine and codeine from plasma was achieved by using the Alltech C18 (100 mg/3 ml) cartridges. The best recovery of the drugs from urine was achieved with the Bakerbond C18 (200 mg/3 ml) cartridges. Therefore, these cartridges were chosen for the SPE of the drugs in this study.

The cartridges selected were also tested for the extraction of the internal standard quinine from spiked plasma and urine. In this case, the plasma and urine samples were spiked with a mixture of the drugs and the internal standard and were processed by SPE. The absolute recovery of the three compounds, i.e. the ratio of the peak areas of the extracted to the peak areas of the unextracted compound was more or less the same, signifying that quinine could be used as a recovery internal standard as well.

The calibration curves of morphine and codeine for plasma and urine samples were constructed using the standard additions method as described in Experimental, with quinine as recovery internal standard.

The plasma and urine samples were spiked with morphine and codeine mixtures at three different concentration levels, with quinine as recovery internal standard. Each sample was injected six replicate times on the HPLC column. The results were treated statistically and are given in Tables 6 and 7.

The precision and accurary of the overall analytical procedure for the determination of morphine and codeine in plasma and urine samples was assessed by processing spiked plasma and urine samples, five times each by SPE. Quinine was added as a recovery internal standard. The eluate resulting from each SPE was analyzed three times by HPLC. The results are given in Table 8.

## CONCLUSION

A comparative study of different SPE cartridges from different manufacturers and with different sorbents was carried out.

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The proposed reversed-phase HPLC method is suitable for the simultaneous determination of morphine and codeine in very small volumes of plasma and urine samples (100 μl). Quinine can be used either as a recovery or as a chromatographic internal standard. The analysis is completed within approximately 10 min which is a reasonably short time for routine analysis. The sensitivity, precision and accuracy of the method are satisfactory. SPE has been used as a clean-up step of the plasma and urine matrices. From a variety of cartridges tested, the octadecyl (C18) sorbent was found to be the most suitable for the extraction of morphine and codeine from the sample matrix, though the best extraction recovery of the drugs was achieved with C18 cartridges from different manufacturers for the plasma and urine samples.

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