*See also:* **II/Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Layers; Modes of Development: Conventional; Modes of Development: Forced Flow, Over Pressured; Spray Reagents. **Extraction:** Analytical Extractions; Solid-Phase Extraction; **III/Herbicides:** Gas Chromatography; Solid-Phase Extraction. **Impregnation Techniques: Thin-Layer (Planar) Chromatography. Pesticides:** Gas Chromatography; Thin-Layer (Planar) Chromatography.

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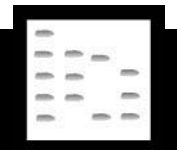
# HEROIN: LIQUID CHROMATOGRAPHY AND CAPILLARY ELECTROPHORESIS



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## Introduction

The separation and quantitative determination of opiates is required for a wide variety of purposes and applications. These include therapeutic drug monitoring, metabolism and pharmacokinetic studies and forensic investigations, as well as the detection and control of drug abuse. The determination of opiates in human urine is of considerable analytical interest, particularly in the context of detecting the consumption of heroin; it is in this context that the present article is written.

The first step in the establishment of such presumptive consumption of heroin is usually by enzyme immunoassay. This has the merit of low detection limit and large sample throughput, making it very suitable for large screening programmes. The immunoassay technique, however, is nonselective with respect to individual opiates and a positive result in such a screen for legal purposes must be followed by identification of individual opiates present. The purpose of this is usually to confirm or refute the hypothesis that heroin has been consumed. The short metabolic half-life of heroin complicates its confirmation, so that its consumption is inferred by detection of its metabolites. One generally sought metabolite is morphine owing to its relatively long half-life. This approach has the disadvantage that morphine is also produced as a metabolite of codeine, so that heroin consumption is presumed or not on the basis of notional codeine-to-morphine ratios. The detection of the first metabolite of morphine, 6-monoacetylmorphine, is now taken as an unequivocal indicator of heroin consumption.

The detection of heroin consumption is further complicated by the quite general inclusion of legal opiates such as codeine, pholcodine and dihydrocodeine in commonly available medicines. This results in numbers of subjects being screened as positive for opiate consumption who are not confirmed by alternative methods as having consumed heroin or morphine.

To confirm the presence of individual opiates, techniques are required that are more selective than immunoassay. These are usually based on established chromatographic techniques. Several thin-layer systems have been used but in general these have

insufficiently low limits of detection to confirm adequately results obtained by immunoassay. This is a particular problem since immunoassay detects total opiates and, following separation, individual concentrations may be much lower. The most generally accepted confirmation is by combined gas chromatography–mass spectrometry (GC-MS), owing to the selectivity of the capillary gas chromatography separation coupled with the unequivocal identification by electron impact ionization mass spectrometry. The technique of high performance liquid chromatography (HPLC) has been extensively applied to heroin and some of its metabolites as well as individual related opiates for a variety of purposes associated with drug abuse. No liquid chromatography (LC) method has specifically addressed the problem of confirmation of results obtained from immunoassay. Work in this area has concentrated on developing LC methods capable of quantifying morphine and codeine individually since, as indicated above, the codeine-to-morphine ratio has been a major criterion in establishing heroin consumption. The emerging technique of capillary electrophoresis (CE) is potentially attractive as a separation technique because of its capability for high resolution and peak capacity. As yet little has appeared in the literature concerned with the capability of this technique to determine low concentrations of opiates in biological matrices.

The literature on the determination of drugs of abuse and screening procedures using GC-MS is extensive and will not be discussed here. The present article describes both normal and reversed phase LC systems for the separation and quantification of a set of opiates comprising heroin, 6-monoacetylmorphine, morphine, codeine, dihydrocodeine and pholcodine, and discusses their potential in acting as an intermediate test in determining opiate abuse. Such a test may eliminate so-called false positives arising from urine samples screened as containing opiate that is subsequently found not to originate from consumption of heroin. A method based on CE is also described and its potential in relation to the LC methods discussed. Heroin is rapidly metabolized and cannot be detected in urine. This drug is included in this set, however, as the separation with respect to legal opiates may be of relevance in forensic applications.

## Reversed-Phase LC System

Several reversed-phase separations of selected groups of opiates have been reported in the literature using column switching, gradient elution and ion pairing techniques. From our experience in the analysis of basic drugs in biological fluids, the most general approach to ion pairing is the use of mobile-phase additives. An anionic surfactant such as sodium dodecyl sulfate (SDS) or alkylsulfonic acid, which is adsorbed onto a $C_{18}$ surface, is incorporated in the mobile phase to increase retention and thus separation. The inclusion, in addition, of a less hydrophobic species of similar charge to the analyte, such as a tetraalkylammonium bromide, has been found to reduce tailing of such basic compounds and also in some cases to alter selectivity. In using such systems the pH of the mobile phase is made acid, thus protonating the basic analytes.

For the opiates morphine, pholcodine, dihydrocodeine and codeine the best anionic mobile phase additive is pentanesulfonic acid used in conjunction with tetraethylammonium bromide. At concentrations of 1 mmol $L^{-1}$ and 100 mmol $L^{-1}$, respectively, these additives in methanol/aqueous buffer (10 : 90 by vol.) containing disodium hydrogen phosphate at pH 2.5 give excellent separation of these four opiates in 15 min when dissolved in water. This system allows the inclusion of nalorphine as an internal standard, which elutes between pholcodine and dihydrocodeine. This optimum separation is shown in **Figure 1**. Both heroin and 6-monoacetylmorphine elute much later than codeine and therefore cannot be determined at sensitivities comparable with the other compounds. The separation of these four opiates is accompanied by excellent individual linearity of response of peak height ratios versus concentration over the range 1–5 $\mu g\,cm^{-3}$ using UV detection at 285 nm.

A standard solid-phase extraction procedure was applied to urine spiked with the four opiates and the internal standard. Bond-Elut Certify extraction cartridges containing a mixed stationary phase of nonpolar C8 and strong cation exchanger (SCX) were used. The extraction consisted of cartridge conditioning with methanol, water and acetate buffer followed by sample addition and washing with acetate buffer, water and methanol. After drying, the analytes were eluted with dichloromethane/isopropyl alcohol (80 : 20 by vol.) mixture containing 2% (v/v) ammonia. The resultant solution was dried at 40°C under nitrogen, reconstituted in 250 μL of ethyl acetate, and again dried under nitrogen. Immediately prior to injection, the sample was redissolved in 500 μL of mobile phase. This procedure gave percentage recoveries in excess of 80% for all the compounds other than morphine, for which the recovery was 71%, presumably as a result of its known propensity for being adsorbed onto glass surfaces.

This extraction procedure gives a large solvent peak in this reversed-phase solvent system. At the

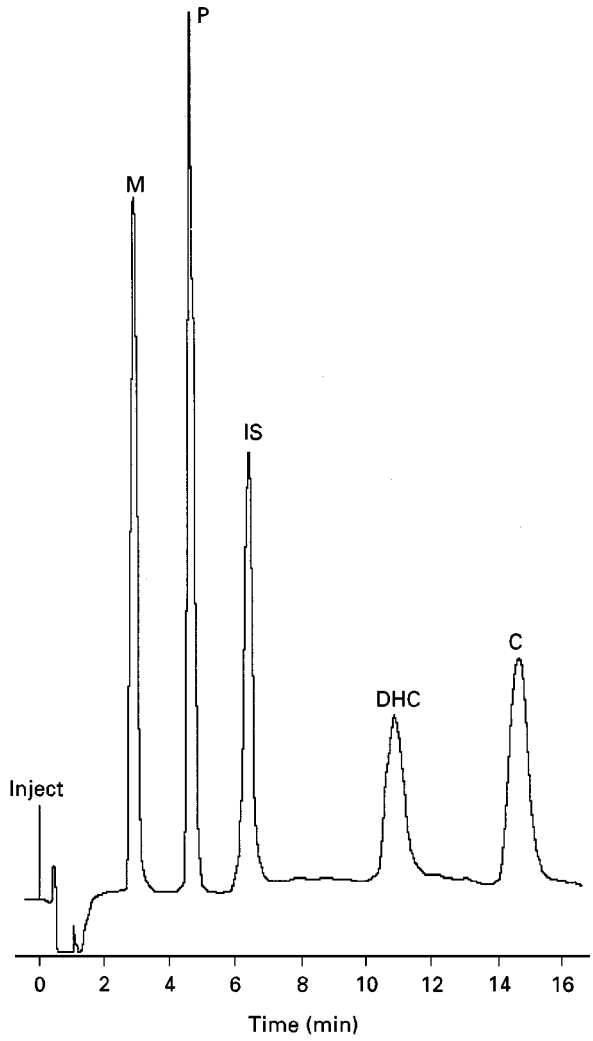


**Figure 1** Representative reversed-phase separation of morphine (M), pholcodine (P), dihydrocodeine (DHC), codeine (C) and nalorphine (IS) using 10% methanol/90% water, containing 50 mmol $L^{-1}$ disodium hydrogen orthophosphate, 1 mmol $L^{-1}$ pentanesulfonic acid and 100 mmol $L^{-1}$ tetraethylammonium bromide as mobile phase at a flow rate of 0.4 mL $min^{-1}$, column 100 × 2 mm i.d. packed with 3 μm microporous octadecylsilica, detection by UV at 280 nm.

detector sensitivity required for 500 ng $cm^{-3}$ opiate concentrations, it is not possible to quantify morphine or pholcodine since they are not separated from the solvent front. A specimen chromatogram of urine spiked (500 ng $cm^{-3}$) with the four opiates and nalorphine is shown in **Figure 2**.

It appears that for such a set of opiates, which vary widely in hydrophobicity, the reversed-phase system is of minimal use for the purpose of detecting heroin consumption by urine analysis when coupled with UV detection. Neither heroin nor 6-monoacetylmorphine interfere with the quantification of the legal opiates. However, the retention time of the 6-monoacetyl metabolite is excessive for quantification and the codeine-to-morphine ratio cannot be established because of the masking of the morphine peak at levels below 500 ng $cm^{-3}$ by endogenous compounds in the solvent front when using UV detection. The inherent high resolution of reversed-phase chromatography is not helpful in the quantification of this widely differing range of analytes. The obvious choice of gradient elution, by analogy with GC, would result in compression of the timescale for elution of the complete range of compounds. However, the potential improvement in detection limits may not be wholly achievable owing to increased baseline noise. The use

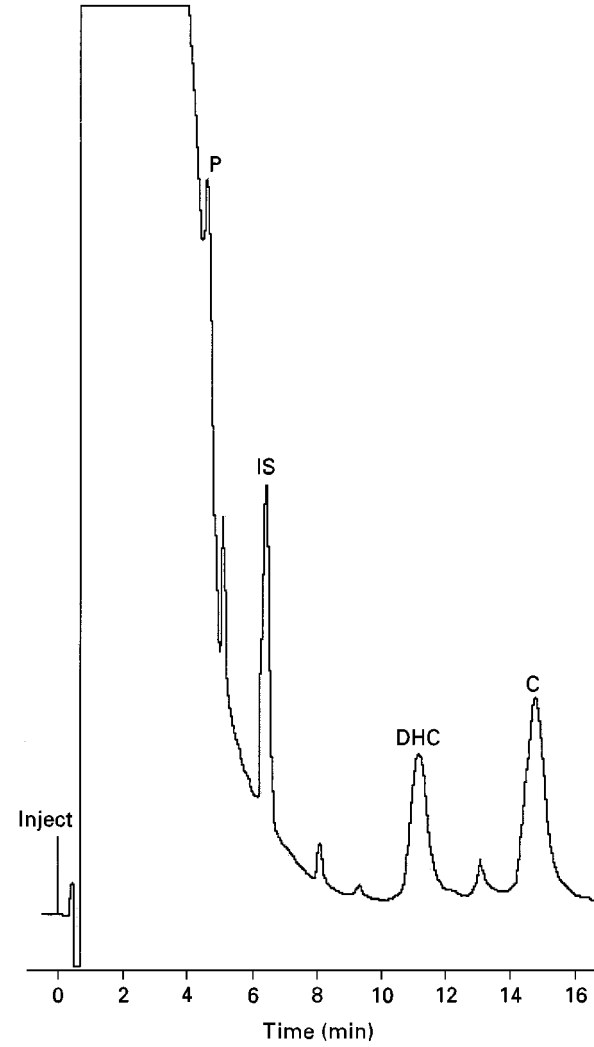


**Figure 2** Representative chromatogram of a urine sample spiked with morphine (M), pholcodine (P), dihydrocodeine (DHC) and codeine (C) at a concentration of 500 ng $cm^{-3}$ and incorporating nalorphine (IS) as internal standard after solid-phase extraction using 10% methanol/90% water containing 50 mmol $L^{-1}$ disodium hydrogen orthophosphate, 1 mmol $L^{-1}$ pentanesulfonic acid and 100 mmol $L^{-1}$ tetraethylammonium bromide as mobile phase at a flow rate of 0.4 mL $min^{-1}$, column 100 × 2 mm i.d. packed with 3 μm microporous octadecylsilica, detection by UV at 280 nm.

of electrochemical detection, while it has been shown to be highly sensitive in the detection of opiates, would not overcome the long retention time required to elute 6-monoacetylmorphine. Its characteristics were not examined using this separation system.

## Normal-Phase LC System

In common with most drug separations by LC, reversed-phase methods predominate in the literature for the separation and quantification of opiates. Normal-phase solvent systems have been reported for the quantitative determination of selected groups of opiates. None of these, however, is suitable for the purpose of determining heroin consumption, either by identification of 6-monoacetylmorphine or by quantitative estimation of the concentration of morphine relation to other legal opiates present.

Using a 200 mm × 2.1 mm column packed with 3 μm Hypersil it has been found possible to resolve completely morphine, dihydrocodeine, pholcodine, codeine, 6-monoacetylmorphine, nalorphine (as internal standard) and heroin. The optimized mobile phase consists of dichloromethane/pentane/methanol (29.8 : 65 : 5.2 by vol.) containing 0.026% (v/v) diethylamine. **Figure 3** shows a representative chromatogram of this set of opiates. The resolutions between individual pairs of opiates are less than in the reversed-phase system, although still in excess of one. This system results in complete elution of all components of interest in approximately 16 min. This separation appears to be more advantageous for the detection of heroin consumption than the reversed-phase system in that the order of elution is reversed and heroin and 6-monoacetylmorphine are eluted fairly early in the chromatogram, thus allowing maximum sensitivity of detection of the latter.

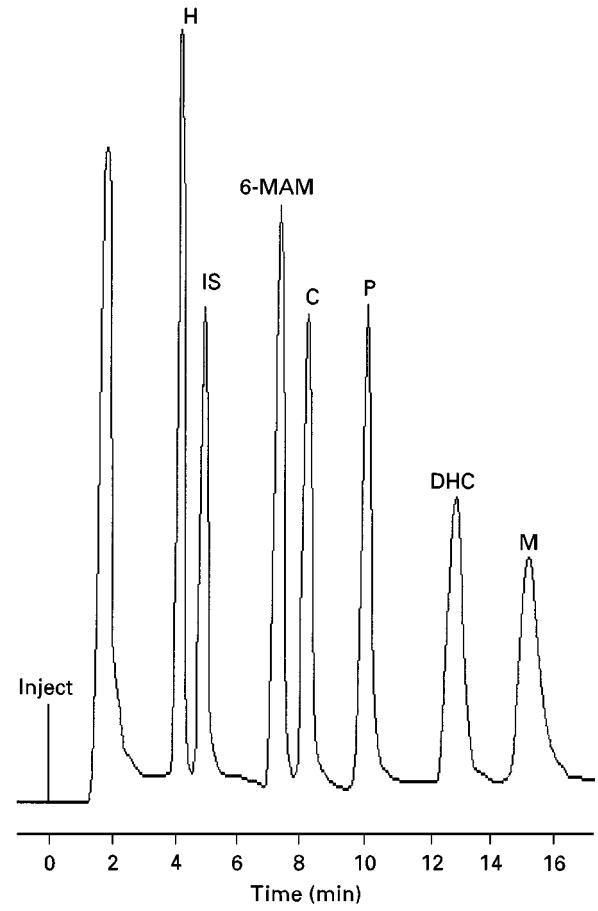


**Figure 3** Representative chromatogram showing the separation of a pentane solution of morphine (M), dihydrocodeine (DHC), pholcodine (P), codeine (C), 6-monoacetylmorphine (6-MAM), nalorphine (IS) and heroin (H) (column 200 × 2 mm i.d. packed with 3 μm microporous siilca mobile phase–dichloromethane/pentane/methanol 29.8 : 65 : 5.2 by volume containing 0.026% v/v diethylamine at a volumetric flow rate of 0.4 mL $min^{-1}$, detection by UV at 280 nm).

When a solid-phase extraction procedure identical to that described above is used, it is found that the choice of solvent is critical to redissolve the dried analytes completely while preserving peak shape on injection. The final injection solvent consists of dichloromethane/pentane (10 : 90 by vol.). This ensures complete solution of the extracted analytes and allows injection in a solvent chromatographically weaker than the mobile phase, which maintains peak sharpness. **Figure 4** shows a chromatogram of the six opiates together with the internal standard, nalorphine, after extraction from a spiked urine sample. In contrast to the reversed-phase system, all the analytes of interest are completely resolved from the solvent front and can thus be readily quantified using UV absorption at 280 nm. The main quantitative characteristics of analysis for these six opiates are shown in **Table 1**. The separation is adequately rugged with respect to the retention times as a result of the inclusion of nalorphine as an internal standard, even allowing for the relatively volatile solvents used in the mobile phase. The selectivity aspect of the separation is excellent with respect to both endogenous materials and the opiates of interest. The retention times of commonly ingested drugs, opiate metabolites and other compounds relative to nalorphine are listed in **Table 2**.

Table 2 shows that the selectivity with respect to the more commonly ingested drugs such as aspirin and caffeine, and also to several of the established opiate metabolites, is good in that retention times are appreciably different from the compounds of interest. Paracetamol, however, elutes between codeine and pholcodine and is incompletely resolved from the latter. At the wavelength of 280 nm used, the sensitivity for paracetamol is very low and

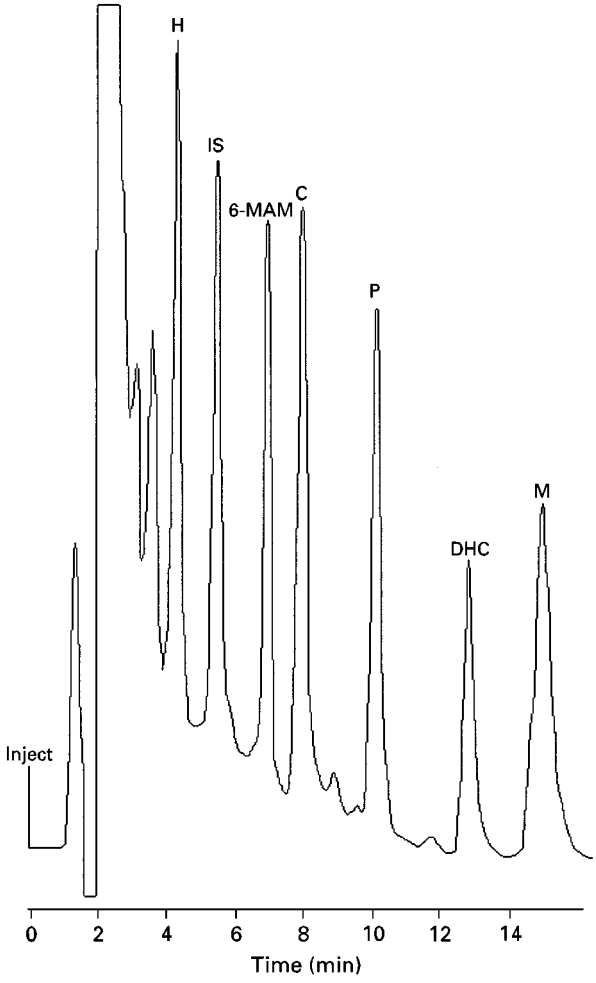


**Figure 4** Representative chromatogram of a urine sample spiked with 500 ng $cm^{-3}$ morphine (M), dihydrocodeine (DHC), pholcodine (P), codeine (C), 6-monoacetylmorphine (6-MAM), nalorphine (IS) and heroin (H) following solid-phase extraction on subsequent reconstitution in 10% dichloromethane/90% pentane and injected (column 200 × 2 mm i.d. packed with 3 μm microporous silica mobile phase dichloromethane/pentane/methanol 29.8 : 65 : 5.2 by volume containing 0.026% v/v diethylamine at a volumetric flow rate of 0.4 mL $min^{-1}$, detection by UV at 280 nm).

there would be minimal interference from paracetamol in the determination of any pholcodine present. The linearity and limits of quantification show that quantification after extraction and separation is adequate to confirm the presence of 6-monoacetylmorphine. The relative amounts of legal opiates as well as morphine detectable in urine are at levels corresponding to those that would result in the detection of opiate by immunoassay; the cutoff value generally used is 300 ng $mL^{-1}$.

An advantage of the normal-phase separation is the facility of coupling this with MS detection via an appropriate interface. The system described has been successfully linked via a particle beam interface with some loss of resolution. Electron impact ionization allows unequivocal identification of all of the opiates.

## Capillary Electrophoresis

Separation of charged analytes by utilizing their differential migration rates in an electric field has been achievable for many decades. The high theoretical plate numbers readily realized by using narrow capillaries, and the relatively recent (compared to LC) availability of reliable microprocessor-controlled equipment, have resulted in increased interest in the theory and application of this technique. The high efficiency can potentially be exploited to achieve resolution and also to accommodate higher peak capacity than is readily or conveniently achieved in LC. Both of these aspects are relevant to the identification and quantification of this group of opiates. However, because only a small sample volume can be injected onto a capillary and only a short pathlength is available when direct UV detection is used, the concentration sensitivity of capillary electrophoresis with UV detection suffers in comparison with LC methods when hydrodynamic injection is used. Within the extensive literature that now exists on CE methods, there are relatively few reports on applications concerning the determination of drugs in biological fluids such as plasma or urine, in which the analyte is generally at low concentration levels. Most applications in the literature reporting quantification of drugs at the ng $mL^{-1}$ concentration level in biological fluids have used sample pretreatment methods that involve a preconcentration step in order to reach the required limits of detection, and thus exploit the undoubted separational advantages of the technique.

Samples can also be introduced into the capillary electrokinetically, i.e. by application of a relatively low voltage with the capillary end immersed in the solution of analyte. It has been established both theoretically and practically that the amount of analyte of a particular charge introduced electrokinetically can be dramatically increased if the solution from which the sample is injected is of low ionic strength compared with that of the buffer used for the electrophoretic separation. While this injection method is subject to bias (not all analytes will be introduced into the capillary in this way), this can be a positive advantage when quantifying particular analytes in a given matrix.

An optimized separation of the six opiates and levallorphan (as internal standard) by CE is shown in **Figure 5**. The parameters affecting separation are buffer concentration and pH. As is general, increase of buffer ionic strength over the range 20 to 140 mmol $L^{-1}$ increases retention and resolu-

**Table 1** Analytical characteristics of opiate determination in urine following normal-phase separation

| | *Heroin* | *6-MAM* | *Codeine* | *Pholcodine* | *Dihydrocodeine* | *Morphine* |
|---|---|---|---|---|---|---|
| Relative retention drug/IS (%RSD) | 0.785 (6.1) | 1.25 (6.1) | 1.51 (5.2) | 1.99 (5.6) | 2.57 (3.2) | 3.04 (3.2) |
| Recovery (%) | 89.1 | 82.7 | 82 | 88.4 | 79.7 | 79.3 |
| Calibration correlation coefficient | 0.9987 | 0.9901 | 0.9900 | 0.9980 | 0.9909 | 0.9964 |
| Slope of calibration line $1 \times 10^3$ ( $\pm$ SD) | 12.6 (0.26) | 9.5 (0.51) | 3.8 (0.32) | 3.1 (0.11) | 2.2 (0.17) | 7.8 (0.04) |
| Limit of quantification at S/N = 4 (ng cm$^{-3}$) | 3.0 | 5.4 | 4.2 | 6.3 | 6.0 | 15 |
| Within day precision as %RSD at 200 ng cm$^{-3}$ | 5.03 | 4.45 | 4.34 | 6.38 | 4.29 | 5.85 |
| Day to day precision as %RSD at 200 ng cm$^{-3}$ | 5.43 | 4.92 | 5.11 | 7.22 | 6.1 | 8.07 |

IS, internal standard; 6-MAM, 6-monoacetylmorphine; RSD, relative standard deviation; SD, standard deviation; S/N, signal-to-noise ratio. Reprinted in part from Low and Taylor (1995) with permission from Elsevier Scientific.

tion. Increase of buffer pH from 4 to 8 decreases the retention as a consequence of increased electroosmotic flow. Figure 5 shows that all the opiates of interest are eluted in 12 min with resolution between individual pairs well in excess of that achieved by normal-phase LC. What is equally significant is that all seven compounds are eluted within a narrow timescale of 9–12 min, so that there is much less disparity between the relative amounts of peak broadening than is the case in either of the LC methods described earlier.

A urine extraction procedure based on the method developed for LC has been developed in which a 0.5 cm$^3$ urine sample is extracted as previously but reconstituted in 1 cm$^3$ of a solvent consisting of water/methanol (9 : 1). The resultant electropherogram obtained from urine spiked at approximately 300 ng cm$^{-3}$ subsequent to extraction and electrokinetic injection is shown in **Figure 6**A. Figure 6B shows the corresponding electropherogram of a blank urine sample. In contrast to the LC methods there are very few endogenous compounds brought through the extraction and electrokinetic injection procedures. Only one of these is a potential interference as it elutes close to pholcodine.

The separational abilities of the CE method are clearly superior to those of either of the liquid LC methods, taking into account both the selectivity with respect to individual opiates and also the separation from endogenous urine components. The quantitative aspects of the CE method are also significant. It has been found that reconstitution of extracted and dried

**Table 2** Retention times relative to nalorphine of some drugs that may interfere with normal-phase LC determination of opiates

| *Drug* | *Relative retention time* |
|---|---|
| Chlodiazepoxide | < 0.39 |
| Papaverine | 0.43 |
| Diazepam | 0.48 |
| Lignocaine | 0.54 |
| Methadone | 0.58 |
| Naloxone | 0.62 |
| Theophylline | 0.65 |
| Diphenylhydramine | 0.74 |
| Ephedrine | 1.33 |
| Hydrocodone | 1.76 |
| Paracetamol | 1.84 |
| Dextropropoxyphene | 2.09 |
| Quinine | 2.28 |
| Norcodeine | 3.29 |
| Caffeine | 4.84 |
| Normorphine | 5.62 |
| Acetylsalicylic acid | > 8 |
| Procaine | > 8 |
| Theobromine | > 8 |

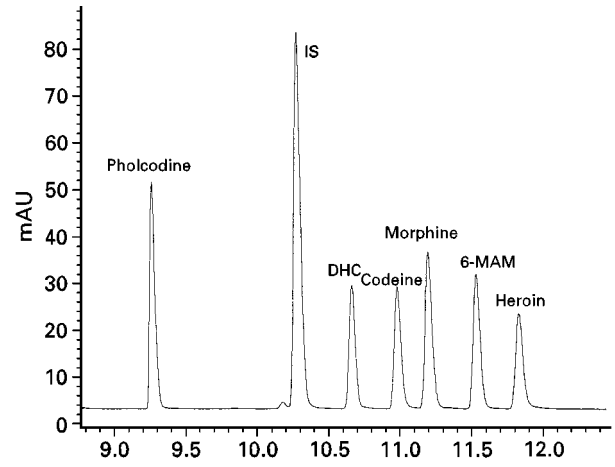


**Figure 5** Representative separation of morphine, pholcodine, heroin, codeine, 6-monoacetylmorphine (6-MAM), dihydrocodeine (DHC) and levallorphan (IS) as internal standard in aqueous solutions using capillary electrophoresis (capillary 50 μm i.d. × 500 mm effective length, running buffer 100 mmol L$^{-1}$ disodium hydrogen phosphate at pH 6, applied voltage 20 kV, electrokinetic injection for 10 s at 5 kV, UV detection at 200 nm). (Reprinted in modified form from Taylor *et al.,* 1996, with permission from Elsevier Scientific.)

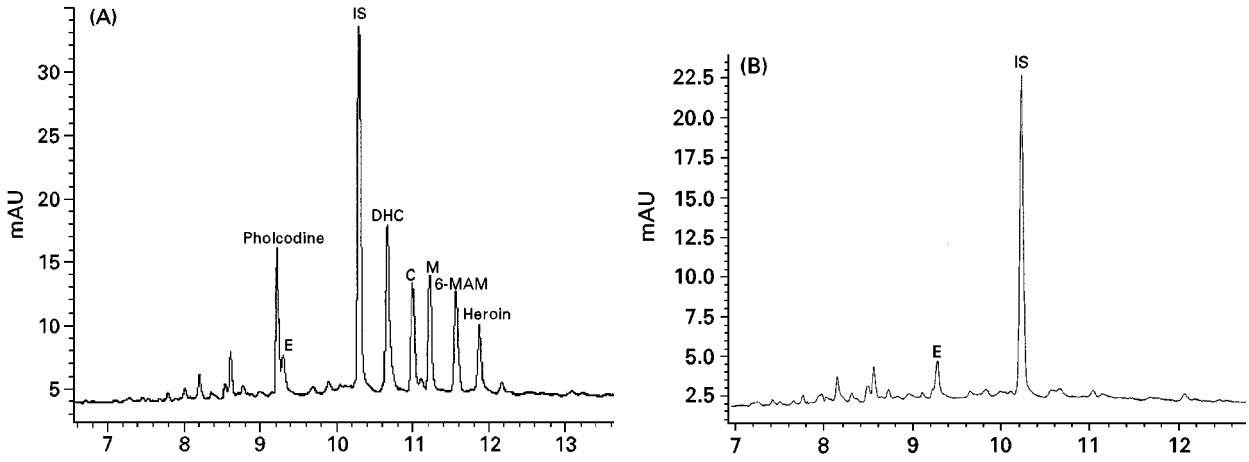


**Figure 6** (A) Representative electropherogram of a urine sample spiked with 300.8 ng $cm^{-3}$ pholcodine, 805 ng $cm^{-3}$ levallorphan (IS), 241 ng $cm^{-3}$ dihydrocodeine (DHC), 274 ng $cm^{-3}$ codeine (C), 304.8 ng $cm^{-3}$ morphine (M), 288.3 ng $cm^{-3}$ 6-monoacetylmorphine (6-MAM) and 284.4 ng $cm^{-3}$ heroin after solid-phase extraction. (E) designates an endogenous component in urine. (B) Representative electropherogram of a blank urine sample spiked with 805 ng $cm^{-3}$ levallorphan after solid-phase extraction (capillary 50 μm i.d. × 500 mm effective length, running buffer 100 mmol $L^{-1}$ disodium hydrogen phosphate at pH6, applied voltage 20 kV, electrokinetic injection for 10 s at 5 kV, UV detection at 200 nm). (Reprinted in modified form from Taylor *et al.,* 1996, with permission from Elsevier Science.)

analytes in aqueous methanol allows the field-amplified sample injection technique to be applied extremely effectively. Indeed, in the method development it was found that the increase of peak areas when comparing electrokinetic injection from water with hydrodynamic injection ranged form 90 in the case of heroin to 160 for 6-monoacetylmorphine and pholcodine. This, coupled with the use of 200 nm as the wavelength of detection, allows limits of detection well below the cutoff level for immunoassay to be achieved. The electrokinetic injection technique for the enhancement of concentration sensitivity applied to this assay procedure is capable of overcoming the perceived limitation of CE as technique for determining these compounds is urine. **Table 3** summarizes the main validation parameters of the assay of these drugs based on the CE procedure described.

The validation parameters in Table 3 show that the quantitative aspects are very comparable with those quoted for LC. The electrokinetic introduction of sample is in general more variable than hydrodynamic introduction but incorporation of an internal standard, which is required to accommodate variable extraction efficiencies, results in within day and day to day precision better than that achieved by normal-phase LC. The limits of quantification quoted are indicative only, since time of sampling could be considerably increased over the 10 s at 5 kV used. The significance is that there is more than adequate sensitivity to allow detection of these particular opiates at the required levels for elimination of common opiates that have resulted in so-called false positives during immunoassay screening. The electrokinetic method of sample introduction, however, does require that the solution for injection into the capillary be of low ionic strength. Consideration must be given to the overall sample matrix pretreatment to ensure this. In addition, at very low concentrations, there is considerable sample depletion from the small sample volumes used and care has to be taken to limit the number of successive injections from a given sample vial.

## Conclusions

There are many opiates for which a multitude of assays have been determined in a variety of matrices for often very widely different purposes. In screening for drugs of abuse, chromatographic methods are extensively used to increase the information gained by rapid immunoassay. The method of choice for unequivocal identification of particular opiates is likely to continue to be GC-MS on the basis of the mass spectral information obtainable. The present article indicates that, at least for the specified relevant group of opiates chosen, separations in the liquid phase are capable of achieving adequate separations and that these can be applied with variable success to the determination of the specified compounds in urine. The results quoted for the reversed-phase LC method show an inherent limitation of this approach when determining a number of compounds differing widely in hydrophobicity. Such methods, however, can usually be manipulated to allow good resolution and quantification for more restricted mixtures of compounds. The widely

**Table 3** Analytical characteristics of opiate determination in urine by CE

| | *Pholcodine* | *Levorphanol* | *Dihydrocodeine* | *Codeine* | *Morphine* | *6-MAM* | *Heroin* |
|---|---|---|---|---|---|---|---|
| Migration time (min) | 9.18 | 10.18 | 10.68 | 10.99 | 11.21 | 11.55 | 11.87 |
| (%RSD) | (1.1) | (0.80) | (0.71) | (0.77) | (0.79) | (0.79) | (0.74) |
| Resolution | 13.4 | 4.7 | 3.7 | 2.3 | 3.9 | 3.4 | |
| Peak efficiency $\times 10^{-5}$ | 2.5 | 2.7 | 2.8 | 2.6 | 2.2 | 2.2 | 2.8 |
| Recovery (%) | 94.8 | | 90.7 | 92.4 | 88.5 | 91.8 | 96.4 |
| Calibration correlation coefficient | 0.9917 | | 0.9924 | 0.9944 | 0.9966 | 0.9967 | 0.9980 |
| Slope of calibration line $\times 10^{3}$ | 1.3 | | 1.6 | 1.5 | 1.4 | 1.5 | 1.2 |
| (%RSD) | (5.1) | | (5.2) | (4.2) | (3.4) | (3.2) | (2.6) |
| Limit of quantification at $S/N = 6$ (ng cm$^{-3}$) | 6.0 | | 16 | 14 | 16 | 18 | 16 |
| Within day precision as %RSD at 300 ng cm$^{-3}$ | 3.8 | | 1.8 | 1.4 | 2.1 | 0.6 | 3.4 |
| Day to day precision as %RSD at 300 ng cm$^{-3}$ | 2.9n | | 2.2 | 2.1 | 2.5 | 1.4 | 2.8 |

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differing retention times for codeine and morphine tends to make reliable quantification of the ratio of these compounds difficult. The normal-phase method offers a viable alternative to GC as a separation procedure and is capable of quantifying the codeine-to-morphine ratio and of detecting 6-monoacetylmorphine at useful concentration levels. More importantly, the normal-phase method is capable of detecting and quantifying the commonly ingested legal opiates that can obscure or delay the final results of an immunoassay opiate screen. The separation achieved by CE arguably offers the most realistic alternative to GC. Method development is rapid and the plate numbers are comparable with those achievable by capillary GC. The underlying difficulty of lack of concentration sensitivity can be overcome by electrokinetic injection techniques if suitable sample pretreatment methods are developed with appropriate sample solvent composition. The eventual linking of CE with MS on a commercial basis should result in a combined technique capable of challenging GC-MS in this area of drug analysis.

**See Colour Plate 86.**

*See also:* **III/Alkaloids:** Gas Chromatography; Liquid Chromatography. **Drugs of Abuse: Solid-Phase Extraction.**

## Further Reading

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# HOT-PRESSURIZED WATER: EXTRACTION

*See* **III / SUPERCRITICAL FLUID EXTRACTION-SUPERCRITICAL FLUID CHROMATOGRAPHY**