volunteers, the mean concentrations of FAEE in the sodium citrate tube, the heparin tube, and the anticoagulant-free tube were similar. However, the EDTA vacuum tube showed a lower FAEE concentration than the others. We speculate that the extensive chelation of calcium by EDTA contributed to this observation in some way. The mean FAEE concentrations in plasma in the sodium citrate and heparin tubes, and in serum in the anticoagulant-free tubes, were more than twice the mean plasma FAEE concentration in the EDTA tubes. The variability was least for sodium citrate tubes. This higher precision suggests the use of 32 g/L sodium citrate as the preferred anticoagulant.

Storage time and temperature also affected FAEE concentrations in serum. The FAEE concentration increased over time when incubated as whole blood at 25 and 37 °C. As shown in Fig. 1, there was a rapid increase in FAEE concentrations at 37 °C from 2273 ± 507 nmol/L (mean ± SE) at time 0 to 4826 ± 245 nmol/L (mean ± SE) 48 h after blood collection. At 25 °C, there was no change in the FAEE concentration after up to 4 h of incubation: 1712 ± 385 nmol/L at time 0 and 1766 ± 486 nmol/L at 4 h (mean ± SE for both). However, there was an increase to 4750 ± 875 nmol/L (mean ± SE) with storage at 25 °C for 48 h. There was less variability, in general, for the samples stored at 37 °C, but this higher precision is not likely to have a biological basis.

The in vitro formation of FAEEs, which is fastest at 37 °C, is most likely the result of enzyme-mediated FAEE synthesis using the residual ethanol in the specimen collection tubes. Ethanol remains in the blood for up to 6 h. White blood cells, platelets, and to a lesser extent, red blood cells, have been shown to have FAEE synthase activity (11). A previously published study from our laboratory with plasma and serum samples from individuals in a clinical trial involving ethanol ingestion showed no changes in FAEE concentration after 2 days of storage at −4 or −80 °C (12). Taken together, the results of the current storage experiments and our previously reported findings indicate a need for removal of the plasma or serum from the cells within 4 h at room temperature, with freezing of plasma or serum at −4 °C or −80 °C if longer periods of time are required before analysis of the specimen. In addition, samples should not be collected for FAEE analysis in EDTA-containing vacuum tubes.

Improved Gas Chromatography–Mass Spectrometry Method for Simultaneous Identification and Quantification of Opiates in Urine as Propionyl and Oxime Derivatives, Larry A. Broussard, Lance C. Presley, Mike Tanous, and Cecelia Queen (1 Department of Clinical Laboratory Sciences, Louisiana State University Health Sciences Center, New Orleans, LA 70112-2262; 2 LabOne, Inc., Lenexa, KS 66219-9752; 3 LabCorp, Memphis, TN 38118; *author for correspondence: fax 504-568-6761, e-mail lbrous@lsuhsc.edu)

Several authors have reviewed existing methods (1–9) or presented new techniques (6–11) for the analysis and separation of codeine, morphine, and the keto-opiates

Table 1. Relative amounts of FAEE detected in different blood collection tubes.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Contents</th>
<th>FAEE (mean ± SE) nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>No anticoagulant</td>
<td>1712 ± 385</td>
</tr>
<tr>
<td>Purple</td>
<td>EDTA</td>
<td>624 ± 60</td>
</tr>
<tr>
<td>Green</td>
<td>Heparin</td>
<td>2033 ± 1064</td>
</tr>
<tr>
<td>Blue</td>
<td>Sodium citrate</td>
<td>1994 ± 33</td>
</tr>
</tbody>
</table>

* n = 4.
hydrocodone, hydromorphone, oxycodone, and oxymorphone. We present a modification of previously published procedures (6, 10) that incorporates the use of methoxyamine after enzymatic hydrolysis to form methoxime derivatives of the keto-opiates, which are then extracted using solid-phase columns and derivatized with propionic anhydride/pyridine.

We used a gas chromatography–mass spectrometry system composed of a model 5890 gas chromatograph with splitless injection, a model 5970 mass-selective detector (both from Hewlett Packard), and a DB-5 capillary column [15 m × 0.25 mm (i.d.); 0.25 μm film thickness; J&W Scientific]; helium (flow rate, 0.7 mL/min; linear velocity, 38 cm/s) was used as the carrier gas. The temperature program was as follows: initial temperature, 185 °C; ramp at 25 °C/min to 240 °C; hold for 0.5 min; ramp at 5 °C/min to 250 °C, then 40 °C/min to 290 °C; hold for 1.0 min. The injection temperature was 260 °C, and the transfer line temperature was 290 °C.

The following were obtained from Radian Corporation: (a) codeine, morphine, hydromorphone, and oxycodone, which were used to prepare calibrators; (b) deuterated codeine, morphine, hydromorphone, and hydromorphone, which were used as internal standards; and (c) oxymorphone and norcodeine, which were used for interference studies. Methoxyamine hydrochloride, 2,4-dimethylaninopyridine, and propionic anhydride were obtained from Sigma. All other solvents and reagents were of reagent- or HPLC-grade quality.

A 2.0-mL urine sample was combined with 100 μL of a 6 mg/L internal standard solution and 1.0 mL of a 0.1 mol/L acetate buffer (pH 4.0) in an appropriately labeled 16 × 100 mm screw-cap tube. After mixing, conjugates were hydrolyzed by the addition of 150 μL of β-glucuronidase solution (99.2 U/L; type L-II Patella vulgata; Sigma) to all tubes and incubation at 60 °C for 2 h. After hydrolysis, the keto-opiates were derivatized by the addition of 350 μL of 6 mol/L HCl and 250 μL of 100 g/L methoxyamine hydrochloride to each tube, followed by vortex mixing and incubation for 15 min at room temperature. Two milliliters of 1.5 mol/L phosphate buffer (pH 7.0) and 200 μL of 10 mol/L NaOH were added to each tube. After mixing and centrifugation, each supernatant was placed on a Bond Elute Certify™ extraction column that had been activated by the sequential addition and elution of 2 mL of methanol and 2 mL of deionized water. Columns were then washed by sequential addition and elution of 2 mL of water, 2 mL of 0.1 mol/L acetate buffer (pH 4.0), and 6 mL of methanol. After the columns were dried under reduced pressure, analytes were eluted with 2 mL of freshly prepared dichloromethane/isopropyl alcohol/ammonium hydroxide (79:19:2 by volume). The eluates were dried under nitrogen at 40 °C, the extracted residues were reconstituted with 100 μL of 1 g/L propionic anhydride in pyridine, and the capped tubes were heated for 20 min at 70 °C. After cooling, 200 μL of methanol was added to each tube, followed by evaporation to dryness under nitrogen at 40 °C. The residue was reconstituted with 300 μL of ethyl acetate and transferred to a correspondingly labeled autoinjector vial; 1 μL was then injected into the system with a HP 6890 Automatic Liquid Sampler (Hewlett Packard).

Using the selected-ion monitoring mode and a HP DOS Chemstation™ data system (Hewlett Packard), we monitored the following ions (quantitative ions in parentheses) for the derivatized analytes: codeine, (m/z 355), 282, and 356; codeine-d₃, (m/z 358) and 359; morphine (m/z 341), 397, and 268; morphine-d₉, (m/z 344) and 400; hydromorphone, (m/z 328), 297, and 329; hydrocodeine-d₉, (m/z 331) and 332; hydromorphone, (m/z 314), 370, and 283; hydrocodeine-d₃, (m/z 317) and 373; oxycodone (m/z 400), 343, and 230. Quantification was based on an extracted calibrator (300 μg/L; ElSohly Laboratories, Inc.) for all five opiates. For expediency and economy, hydrocodeine-d₉ was used as the internal standard for the quantification of oxycodone as well as hydromorphone. Results using this common internal standard are acceptable, but use of oxycodone-d₉ would be preferable.

The total-ion chromatographs of the propionyl derivatives of codeine and morphine, the propionyl-oxime derivatives of hydromorphone and oxycodone, and the methoxime derivative of hydrocodeine are shown in Fig. 1. The chromatographic peaks are gaussian-shaped and demonstrate near-baseline resolution with all peaks eluted within 6.5 min. This resolution remains for the life of the column, which is ~2000 injections.

Criteria for linearity included quantification within 20% of target concentration (12 calibrators; 60–10 000 μg/L for each opiate) and acceptable ion ratios (± 20%). The upper limit of linearity was 5000 μg/L for codeine, 8000 μg/L for morphine, 6000 μg/L for hydrocodeine, and 3000 μg/L for hydromorphone and oxycodone.

Between-run precision (n = 31–52) around the 300
$\mu g/L$ cutoff was determined monthly using data obtained from daily analysis of samples with target concentrations of 240 and 360 $\mu g/L$. Representative (most recent) CVs at these concentrations were as follows: 3.8% and 3.1% for codeine, 3.9% and 3.2% for morphine, 3.3% and 3.6% for hydrocodone, 3.2% and 3.9% for hydromorphone, and 4.4% and 4.5% for oxycodone, respectively.

Possible cross-interference with the quantification of codeine, morphine, hydrocodone, hydromorphone, and oxycodone was assessed by supplementing urine samples with 5000 $\mu g/L$ oxymorphone and norcodeine (the two most likely interfering substances) in the presence of codeine, morphine, hydrocode, hydromorphone, and oxycodone at 300 and 120 $\mu g/L$. In all cases, quantification of the analyte of interest was not affected by the presence of the potential interfering substance. Additionally, no cross-interference between the five opiates of interest at concentrations up to 10,000 $\mu g/L$ was observed. Large concentrations of oxymorphone can interfere with the m/z 268 qualifier ion of morphine, but this does not interfere with the quantification of morphine.

The limit of detection (LOD) and limit of quantification (LOQ) determined by duplicate analysis of serially diluted samples were equal for each of the five analytes. The LODs and LOQs were 60 $\mu g/L$ for codeine, hydrocodeone, and hydromorphone; 90 $\mu g/L$ for oxycodone; and 120 $\mu g/L$ for morphine. The criteria for both LOD and LOQ included acceptable chromatography and acceptable ion ratios. Quantification within 20% of the target concentration was required for the LOQ but not the LOD.

In conclusion, we present a method that allows the simultaneous quantification of codeine, morphine, hydrocodeone, hydromorphone, and oxycodone at concentrations from a minimum of 60–120 $\mu g/L$ (LOQ for each opiate) to a maximum of 5000 $\mu g/L$ for codeine, 8000 $\mu g/L$ for morphine, 6000 $\mu g/L$ for hydrocodeone, and 3000 $\mu g/L$ for hydromorphone and oxycodone. The method demonstrates acceptable precision and lack of cross-interference and interference from other opiates, uses a relatively small sample volume (2.0 mL), and has an analysis time of ~6.5 min. This method has been used in the laboratory (Memphis, TN) for the analysis of >3500 samples in a 4-month period and has been found to be reliable as demonstrated by calibrator and control reproducibility and the absence of interference.


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Previously reported clinical trials have shown that measurement of the different forms of prostate-specific antigen (PSA) (1) is useful in the differentiation of prostate cancer from benign prostatic conditions. Men with prostate cancer tend to have lower percent free PSA (%FPSA) values than men with benign disease (2–4); %FPSA is the ratio of free PSA to total PSA times 100%.

Beckman Coulter, Inc. (Fullerton, CA) has developed the Access Hybritech PSA and Hybritech PSA assays for use on the automated Access Immunoassay System. The assays are two-site immunoenzymatic (“sandwich”) assays that use mouse monoclonal antibody in alkaline phosphatase conjugate and paramagnetic particles coated with a second mouse monoclonal antibody. After unbound particles are removed by washing, a chemiluminescent substrate, Lumi-Phos 530,6 is added to produce

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6 Lumi-Phos 530 is a trademark of Lumigen, Inc.