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Article

# Determination of Buprenorphine, Fentanyl and LSD in Whole Blood by UPLC–MS-MS

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**A sensitive ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS-MS) method has been developed and validated for the quantification of buprenorphine, fentanyl and lysergic acid diethylamide (LSD) in whole blood. Sample preparation was performed by liquid–liquid extraction (LLE) with methyl *tert*-butyl ether. UPLC–MS-MS analysis was performed with a mobile phase consisting of ammonium formate (pH 10.2) and methanol. Positive electrospray ionization MS-MS detection was performed with two multiple reaction monitoring transitions for each of the analytes and the deuterium labeled internal standards.**

**Limit of detection values of buprenorphine, fentanyl and LSD were 0.28, 0.044 and 0.0097 ng/mL and limit of quantification values were 0.94, 0.14 and 0.036 ng/mL, respectively. Most phospholipids were removed during LLE. No or only minor matrix effects were observed. The method has been routinely used at the Norwegian Institute of Public Health since September 2011 for qualitative and quantitative detections of buprenorphine, fentanyl and/or LSD in more than 400 whole blood samples with two replicates per sample.**

## Introduction

Buprenorphine, fentanyl and lysergic acid diethylamide (LSD) are highly potent basic drugs. Buprenorphine is a lipid-soluble base and a potent semi-synthetic opioid that acts as a partial agonist binding at the μ-opioid receptor in the human brain. It is used to treat opioid addiction and to treat or control acute pain. As an analgesic, buprenorphine in low doses is several times more potent than morphine, whereas at higher doses the agonist effects flatten and it acts more like an antagonist (1–3). It is metabolized in the liver to the active metabolite norbuprenorphine and/or by glucuronidation to buprenorphine-glucuronid and norbuprenorphine-glucuronide (4, 5). Fentanyl is a highly potent synthetic opioid analgesic and a strong agonist to the μ-opioid receptor. It is used to treat or control pain, but may also be abused. The high lipid solubility facilitates its transfer across the blood-brain barrier and fentanyl is much more potent than morphine (6, 7). Fentanyl is primarily metabolized in the liver by *N*-dealkylation to norfentanyl. Minor metabolite pathways are amide hydrolysis to despropionylfentanyl and alkyl hydroxylization to hydroxyfentanyl, the latter further metabolized by *N*-dealkylation to hydroxynorfentanyl (8). LSD is a highly potent semi-synthetic hallucinogen that is illegally used worldwide. It affects the brain through interactions between the drug and both the serotoninergic and dopaminergic systems (9). In humans, LSD is metabolized to structural similar metabolites, like lysergic acid ethylamide (LAE), 2-oxo-LSD, 2-oxo-3-OH-LSD, trioxylated LSD, dihydroxy-LSD and 13- and 14-hydroxyl-LSD as glucuronides (10–12).

The low concentrations of highly potent drugs in biological samples make the identification and quantification challenging. Liquid chromatography–tandem mass spectrometry (LC–MS-MS) is commonly used due to the high selectivity and sensitivity of this technique. Several papers describe LC–MS-MS analyses of buprenorphine, fentanyl and/or LSD in biological samples, in whole blood (10, 13–21), urine (10, 14–17, 21–23), plasma (10, 22–26) or oral fluid (27–29). Ultra-performance liquid chromatography (UPLC) or ultra-high-pressure liquid chromatography (UHPLC) are used in some of these LC–MS-MS methods (14, 18, 20, 21, 27, 28). The use of UPLC or UHPLC has recently increased, due to the high chromatographic resolving power in these systems (30–33). Many UPLC and UHPLC columns are compatible with both high pH and low pH mobile phases, but usually acidic mobile phases are used. However, high pH mobile phases have been shown to increase retention, improve peak shape and increase the analyte responses of many basic compounds in reversed-phase (RP) LC–MS-MS analyses (20, 21, 34–38). When using positive electrospray ionization ($ESI^+$) LC–MS-MS, it is important to be aware of ion suppression and ion enhancement effects, and that analytes present in low concentrations will be even more exposed to these effects. There are many ways to reduce the possibility for ion suppression, e.g., to reduce sample injection volume, to optimize sample preparation and/or to optimize chromatographic separation (39–42). When whole blood samples are analyzed, it is important to remove phospholipids, because they are present at high concentrations and may cause ion suppression, reduce the lifetime of a column and pollute the cone and other parts of the MS-MS. Phospholipids are major components in cell membranes. They are divided into two structural classes: glycerophospholipids and sphingomyelins (SM). The glycerophospholipids are classified into several different classes, such as phosphatidylcholine (PC). PC accounts for approximately 60–70% of the total phospholipids in human plasma (43). A subclass of PCs is lysophosphatidylcoline (lyso-PC). In RP-LC–MS-MS, the lyso-PCs often elute near the analytes, whereas the other phospholipids elute later and after most analytes. A parent ion scan of $m/z$ 184 and/or MS-MS analysis in multiple reaction monitoring (MRM) mode of $m/z$ 184/184 will reveal PC, SM and lyso-PC phospolipids (44, 45). Several sample preparation filters exist that remove phospholipids, but none of these were investigated in the present study. If phospholipids are not removed during sample preparation, an adequate washing step at the end of the LC gradient with 90–100% organic ensures that phospholipids are eluted and not accumulated on the column. This may take several minutes, depending on column length, type of organic modifier and mobile phase flow rate.

The aim of this study was to develop a sensitive UPLC–MS-MS method for the qualitative and quantitative confirmation

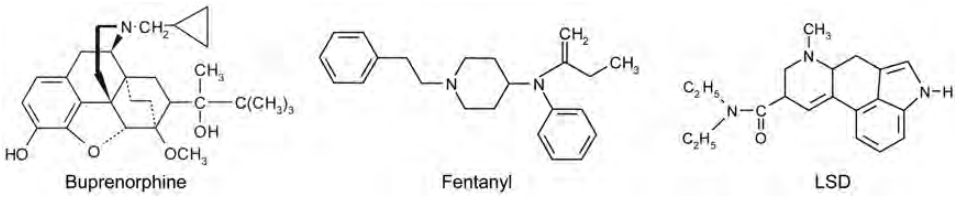


**Figure 1.** Molecular structures of buprenorphine, fentanyl and LSD.

of buprenorphine, fentanyl and LSD in whole blood. Sample preparation was performed by liquid–liquid extraction (LLE) with methyl *tert*-butyl ether (MTBE). Compared to using protein precipitation (PPT) only, sample preparation using LLE with MTBE reduced the phospholipid background by approximately 90%. More importantly, the lyso-PCs that co-eluted with buprenorphine were removed. UPLC–MS-MS analyses were performed with a mobile phase consisting of ammonium formate (pH 10.2) and methanol (MeOH). Deuterium labeled internal standards were used to compensate for different extraction recoveries and to correct for possible ion suppression or enhancement effects. MS-MS detection was performed using two MRM ions for each analyte and each internal standard. Figure 1 shows the molecular structures of buprenorphine, fentanyl and LSD. Norway recently established legislative blood concentration limits for impairment and graded sanctions for driving under the influence of 20 non-alcohol drugs (46). Two of the compounds in the developed method, buprenorphine and LSD, are among these drugs. This is the first time a high pH mobile phase UPLC–MS-MS method has been developed and validated for the determination of these three compounds. However, Verplaetze and Tytgat have recently investigated both low and high pH mobile phases for the UPLC–MS-MS analyses of buprenorphine, fentanyl and other opioides in urine and whole blood (20, 38).

## Experimental

### *Reagents and standards*

Ammonia (25%) and MTBE were purchased from Merck (Darmstadt, Germany). Acetonitrile (ACN) and MeOH were purchased from LabScan (Dublin, Ireland). Ammonium formate was purchased from BDH (Poole, England) and formic acid was obtained from BDH Prolabo (Briare, France). Buprenorphine was purchased from Lipomed (Arlesheim, Switzerland) and fentanyl was purchased from Sigma-Aldrich (St. Louis, MO). LSD, buprenorphine-$D_4$, fentanyl-$D_5$ and LSD-$D_3$ were purchased from Cerilliant (Round Rock, TX). Type 1 water (18.2 MΩ) was obtained from an in-house Milli-Q Biocel from Millipore with an Ultrapore Quantum Organex cartridge.

### *Sample specimen*

Whole blood samples screened positive for buprenorphine, fentanyl and/or LSD were analyzed by the developed UPLC–MS-MS method. Blood samples received for analyses at Norwegian Institute of Public Health (NIPH) are from different case categories such as forensic autopsies, medical cases, social medicine and persons suspected driving under the influence of drugs. Blood samples are received in 4 mL BD Vacutainer Plus plastic blood collection tubes containing 10 mg of sodium fluoride and 8 mg of potassium oxalate from BD (Franklin Lake, NJ) and/or in 25 mL Sterilin tubes containing 200 mg of potassium fluoride from Sterilin (Caerphilly, UK). An amount of 0.50 mL blood is transferred to 5 mL polypropylene tubes from Sarstedt AG (Rommelsdorf, Germany) before analysis. Blank whole blood containing 2 g of sodium fluoride, 6 mL of heparin and 10 mL of water per 450 mL blood, used for calibrants, control samples and blank samples, was obtained from the Blood Bank at Ullevaal University Hospital (Oslo, Norway).

### *Preparation of solutions and samples*

Each assay contained calibrants, control samples and blank samples in addition to the unknown samples, all utilizing 0.50 mL of whole blood. Stock solutions of each analyte were prepared in MeOH in glass volumetric flasks. Working solutions were made in type 1 water by appropriate dilution of the stock solutions. Calibrants with buprenorphine concentrations in the range of 0.94–19 ng/mL, fentanyl concentrations in the range of 0.67–13 ng/mL and LSD concentrations in the range of 0.13–2.6 ng/mL, all in blank whole blood, were prepared by appropriate dilution of the working solutions. Control working solutions were made in the same way as the calibrants by appropriate dilutions of the stock solutions. Each assay contained three control samples with buprenorphine concentrations of 0.94, 1.9 and 9.4 ng/mL, fentanyl concentrations of 0.67, 1.3 and 6.7 ng/mL and LSD concentrations of 0.13, 0.26 and 1.3 ng/mL. An internal standard working solution containing buprenorphine-$D_4$, fentanyl-$D_5$ and LSD-$D_3$ was made in type 1 water.

### *Sample preparation*

Sample preparation was performed by adding 0.050 mL of internal standard working solution to each sample, calibrants, control samples, blank samples and unknown whole blood samples, before LLE with 2 mL of MTBE. Buprenorphine-$D_4$, fentanyl-$D_5$ and LSD-$D_3$ concentrations in all samples were 19, 6.8 and 1.6 ng/mL, respectively. After the samples were tilted for 10 min, they were centrifuged for 10 min at 3,000 rpm in a Megafuge 2.0R sentrifuge from Hereas (Hanau, Germany). The organic layers were then transferred to 5 mL glass tubes and the samples were evaporated at 50°C with nitrogen gas, reconstituted in 0.060 mL of MeOH–ammonium formate buffer, 315 μg/mL, pH 3.1 (10/90) and transferred to autosampler vials. Two microliters of the extracted samples were analyzed by UPLC–MS-MS. Validation samples were prepared in the same way as the calibrants.

### *Instrumentation*

An Acquity UPLC with a sample manager and a binary solvent manager was used, coupled to a Quattro Premiere Xe tandem mass spectrometer from Waters (Milford, MA). Chromatographic separation was performed at 60°C on an Acquity UPLC BEH C18 column (2.1 mm i.d. × 50 mm, 1.7 μm particles) from Waters (Wexford, Ireland). A column inline filter was used in front of the column. The mobile phase for the validated method consisted of 315 μg/mL ammonium formate, pH 10.2 (solvent A), and MeOH (solvent B). The flow rate of the mobile phase was 0.400 mL/min. The gradient profile (percentage of B) was; 5% B in 0.0–0.15 min, 5–30% B in 0.15–0.30 min, 30–50% B in 0.30–2.70 min, 50–90% B in 2.70–3.80 min, 90% B in 3.80–4.70 min, 90–98% B in 4.70–4.80, 98% B in 4.80–6.30, 98–5% B in 6.30–6.40 min, 5% B in 6.40–6.90 min. The total post-injection equilibration time was 1.2 min, including a 0.7 min injection time and 0.5 min at the end of the gradient. ESI-MS-MS detection was performed in MRM mode. The desolvation gas temperature was 500°C and the flow was 900 mL/h. The capillary voltage was 1 kV. Table I shows the analyte and internal standard transition ions, mass spectrometric parameters, cone voltage and collision energy.

### *Method validation*

Validation of the method included accuracy, precision, extraction recovery, limit of detection (LOD), limit of quantification (LOQ), retention time stability, matrix effects, linear range and stability.

#### *Accuracy and precision*

Accuracies were determined as the average differences in the percent between found and theoretical concentrations of validation samples at four different concentrations of 10 successive assays. Within-assay precisions were determined as relative standard deviation (RSD) values of found concentrations of 10 replicate analyses of validation samples. Between-assay precisions were determined as RSD values of found concentrations of validation samples analyzed in 10 successive assays.

#### *Extraction recovery*

The recovery was investigated by comparing the LC–MS-MS concentrations of validation samples spiked with the three analytes before extraction with the LC–MS-MS concentrations of validation samples spiked with the analytes after extraction. Internal standards were added after extraction in all cases. Only 1.8 mL of the 2.5 mL organic phase was transferred during sample preparation, which was compensated for during the calculation.

**Table I**
Transition Ions, Cone Voltages and Collision Energies

| Compound | Time window (min) | $MH^+$ | Daughter ion | Cone voltage (V) | Collision energy (V) |
|---|---|---|---|---|---|
| Buprenorphine | 4.0–4.8 | 468.31 | 396.22 | 65 | 35 |
| | | 468.31 | 414.26 | 65 | 35 |
| Fentanyl | 3.7–4.8 | 337.23 | 105.07 | 30 | 25 |
| | | 337.32 | 188.14 | 30 | 15 |
| LSD | 2.4–3.6 | 324.21 | 208.08 | 30 | 35 |
| | | 324.21 | 223.12 | 35 | 30 |
| Buprenorphine-$D_4$ | 4.0–4.8 | 472.34 | 400.24 | 65 | 35 |
| | | 472.34 | 414.26 | 65 | 35 |
| Fentanyl-$D_5$ | 3.7–4.8 | 342.26 | 105.07 | 30 | 25 |
| | | 342.26 | 188.14 | 30 | 15 |
| LSD-$D_3$ | 2.4–3.6 | 327.23 | 211.10 | 35 | 35 |
| | | 327.23 | 226.14 | 35 | 30 |

#### *LOD and LOQ*

The LOD and LOQ were determined by UPLC–MS-MS analysis of extracted blank whole blood samples and extracted validation samples with low analyte concentrations from 10 successive assays. The validation samples were prepared in blank whole blood by appropriate dilutions of the working solutions. In the 10 successive assays, blank whole blood samples from six different persons were used. The LOD and LOQ were determined by Equations 1 and 2, respectively:

$$\text{LOD} = \text{Mean concentration of blank} + 3 \times \text{SD Validation sample} \quad (1)$$

$$\text{LOQ} = \text{Mean concentration of blank} + 10 \times \text{SD Validation sample} \quad (2)$$

where SD is the standard deviation. The validation sample contained buprenorphine, fentanyl and LSD with concentrations of 0.23, 0.17 and 0.032 ng/mL, respectively.

#### *Retention time stability*

The retention time stability was investigated for the internal standards because these are present in all samples. The retention times of buprenorphine-$D_4$, fentanyl-$D_5$ and LSD-$D_3$ from six series (including standard samples, control samples, blank samples and unknown samples) were used to calculate percentage RSD values of retention times. In total, more than 200 samples were analyzed in the six series that were analyzed over a time period of approximately five weeks.

#### *Matrix effects*

The matrix effect (ME) was investigated by using a procedure similar to that described by Matuzewski *et al.* (47). Two sets of samples were analyzed. In Set 1, the analytes and internal standards were spiked in autosampler vials containing extracted whole blood samples from eight different sources. In Set 2, the analytes and internal standards were spiked into empty autosampler vials. The final sample solvent volume in both Set 1 and Set 2 was 0.060 mL MeOH–ammonium formate (pH 3.1), 315 μg/mL (10:90). The MEs of each analyte were calculated by using Equation 3:

$$\text{ME} = (PA_{Set1}/PA_{Set2}) \times 100 \quad (3)$$

where $PA_{Set1}$ and $PA_{Set2}$ were peak areas from Sets 1 and 2.

ME = 100 indicates no matrix effects. ME > 100 indicates possible matrix enhancement, and ME < 100 indicates possible matrix suppression.

#### *Linear range*

Linear ranges were investigated by UPLC–MS-MS analysis of six extracted validation samples with buprenorphine concentrations from 0.94 to 75 ng/mL, fentanyl concentrations from 0.67 to 54 ng/mL, and LSD concentrations from 0.13 to 10 ng/mL. Deviations of more than 20% from linearity indicated that the maximum linear concentration was reached.

*Stability*

The stability of the analytes in the autosampler vials was investigated by UPLC–MS-MS analyses of three extracted validation samples, three extracted control samples and two extracted blank samples. The samples were analyzed twice: once the day they were extracted and a second time after storage at 4°C for 12 days.

## Results and Discussion

### *Sample preparation*

Previously at NIPH, the sample preparation of buprenorphine and fentanyl in whole blood was performed by PPT with ACN–MeOH (85:15) and PPT with ACN, respectively. LSD has not been previously analyzed in whole blood at NIPH. When developing a new sample preparation method, it was important to remove phospholipids, especially the lyso-PCs because they had similar retention to buprenorphine. The background of PC, SM and lyso-PC phospholipids was investigated by parent ion scans of $m/z$ 184 of whole blood samples prepared by LLE or PPT. Figure 2 shows the phospholipid background of a whole blood sample prepared by the developed LLE method compared to the background of a sample of the same whole blood prepared by PPT with ACN–MeOH (85:15). The figure shows that approximately 90% of the phospholipids were removed by using LLE with MTBE compared to using PPT with ACN–MeOH (85:15). The figure also shows that the lyso-PCs, eluting between 4.3 and 4.8 min, were removed when LLE with MTBE was used.

### *Chromatographic separation*

A high pH mobile phase was chosen because increased retention, improved peak shape and increased analyte response have been observed in RP-LC–MS-MS analyses of basic compounds (20, 21, 34–38). A mobile phase with high pH also ensured that the retention of compounds in the developed method was different than the retention of the same compounds in the UPLC–MS-MS screening method that uses a mobile phase with low pH (18). As the aqueous part of the mobile phase, ammonium formate (pH 10.2) and ammonium bicarbonate buffer (pH 10.2) were considered. Both solvents lead to similar retention times, but ammonium formate was chosen because salt precipitation on the cone has been observed when ammonium bicarbonate buffer is used. The chosen column and gradient profile were similar to an earlier published study in which opiates in urine were analyzed by UPLC–MS-MS (34). In the gradient profile, a washing step with ≥90% B from 3.8 to 6.3 min ensured that remaining phospholipids were eluted and did not accumulate on the column. Figure 3 shows MRM chromatograms of buprenorphine, fentanyl and LSD and their respective internal standards. The figure shows narrow and symmetric peaks and satisfactory signal-to-noise (S/N) values, even at the low concentrations of analytes used in this test.

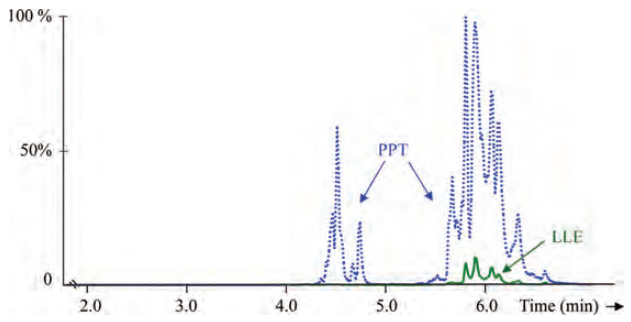


**Figure 2.** Phospolipid background (parent ion scan of *m/z* 184) of whole blood sample extracted as in the validated method (solid line) and whole blood sample extracted by PPT with ACN/MeOH, 85:15 (dashed line). Both extracts were reconstituted in 60μL MeOH-ammonium formate pH 3.1 (10/90) before UPLC-MS/MS analysis. The gradient profile was similar to the validated method. An exception was that the time period with 90–98% MeOH at the end of the gradient program was increased to last from 3.8 to 7.3 min to be sure all phospholipids were eluted.

### *Method validation*

*Accuracy, precision, recovery, LOD and LOQ*

Table II shows the between-assay accuracies, the between-assay precisions (RSD) and recovery, and Table III shows the LOD, LOQ and cut-off values for the three analytes.

Table II shows that the between-assay accuracy and precision values of fentanyl were within ± 9% and within ± 13% for LSD. The between-assay accuracy and precision values of buprenorphine at the three highest concentrations were within ± 20%. The recoveries of fentanyl and LSD were 80–100%, and the recovery of buprenorphine was 40–50%. Tables II and III show that both lower accuracy and precision values, and higher LOD and LOQ values, were observed for buprenorphine, however, the reason has not been investigated.

*Retention time stability*

The retention time stability was examined for the internal standards because these are present in all samples. The RSD values of retention times for buprenorphine-$D_4$, fentanyl-$D_5$ and LSD-$D_3$ were 0.05, 0.05 and 0.23%, respectively. The values are based on 200 samples analyzed in six series over a period of five weeks. The RSD values show that the retention times are stable.

*Matrix effects*

The ME was investigated in a procedure similar to that described by Matuzewski *et al.* (47). Table IV shows the ME values for the analytes and internal standards, and that no or only minor MEs were observed.

*Linear ranges*

The calibration curves of buprenorphine, fentanyl and LSD were linear within 0.94–75, 0.67–54 and 0.13–10 ng/mL, respectively. The calibration curves were linear up to the maximum investigated concentration levels.

*Stability*

The stability of extracted samples (calibrators, control samples, blank samples and unknown samples) was investigated by UPLC–MS-MS analyses on the day of extraction and after 12 days of storage at 4°C. The internal standard peak responses from the two UPLC–MS-MS analyses were within ± 60% for buprenorphine-$D_4$, fentanyl-$D_5$ and LSD-$D_3$. In general, internal

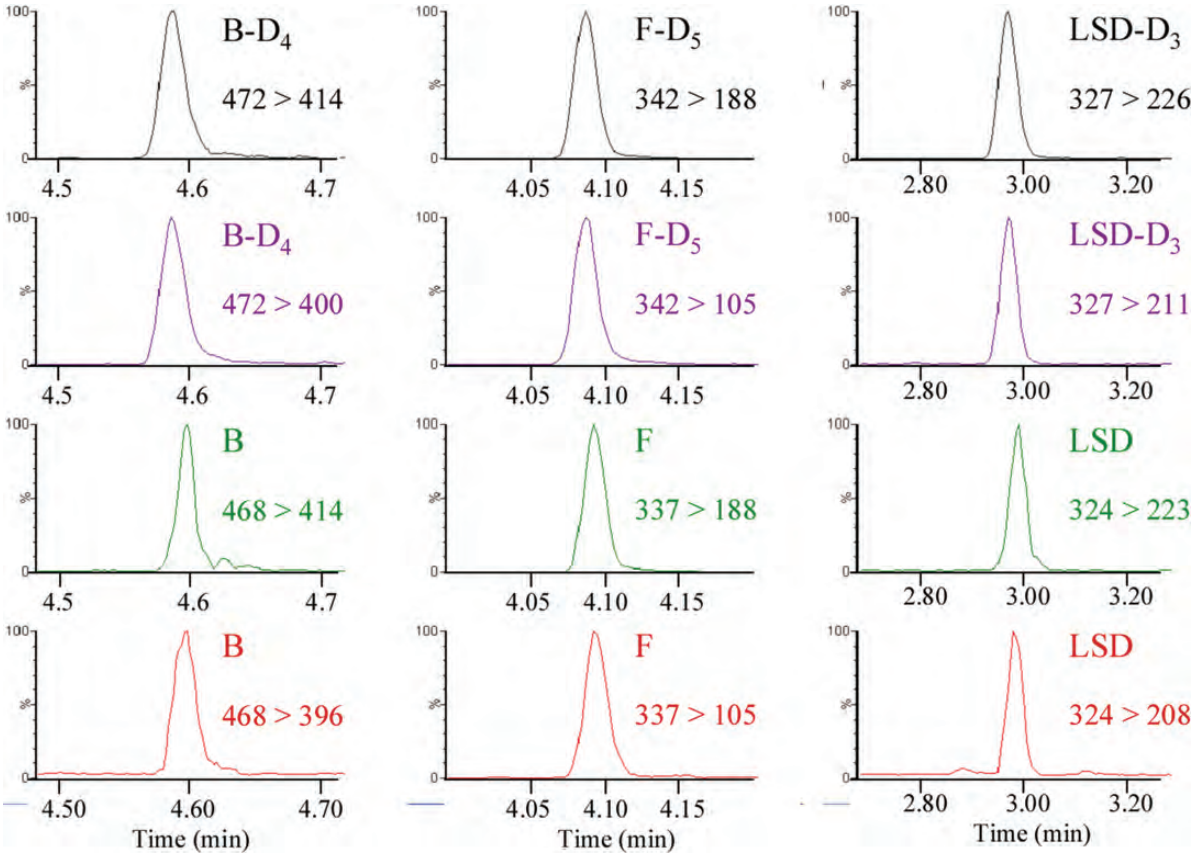


**Figure 3.** MRM chromatograms of analytes and internal standards. UPLC–MS-MS analysis of an extracted standard sample with buprenorphine, fentanyl and LSD concentrations 0.94, 0.67 and 0.13 ng/mL, respectively. UPLC–MS-MS conditions are the same as in the validated method; buprenorphine (B), fentanyl (F).

**Table II**
Accuracy, Precision and Recovery

| Analyte | Theoretical concentration (ng/mL) | Between-assay accuracy and precision ($n = 10$) | | | Recovery ($n = 6$) | |
|---|---|---|---|---|---|---|
| | | Mean (ng/mL) | Accuracy (%) | RSD (%) | Recovery (%) | RSD (%) |
| Buprenorphine | 0.23 | 0.173 | −26 | 54 | | |
| | 0.94 | 0.753 | −20 | 17 | 42 | 3.3 |
| | 2.34 | 2.081 | −11 | 11 | | |
| | 9.35 | 8.562 | −8.5 | 8.4 | 51 | 5.0 |
| Fentanyl | 0.17 | 0.169 | 0.4 | 8.0 | | |
| | 0.67 | 0.652 | −3.1 | 8.8 | 103 | 6.0 |
| | 1.68 | 1.656 | −1.5 | 6.2 | | |
| | 6.73 | 6.286 | −6.6 | 5.1 | 101 | 2.1 |
| LSD | 0.032 | 0.028 | −12 | 12 | | |
| | 0.13 | 0.125 | −3.4 | 13 | 85 | 11 |
| | 0.32 | 0.337 | −4.3 | 5.1 | | |
| | 1.29 | 1.301 | −0.6 | 3.6 | 101 | 2.7 |

standard responses were higher in the second analyses, probably because of the evaporation of solvent during storage. The quantified values from the two UPLC–MS-MS analyses were found to be within ± 25% for buprenorphine, fentanyl and LSD at concentrations ≥ LOQ, indicating that the internal standards compensate for possible loss or gain of the analytes.

**Table III**
LOD, LOQ and Cut-Off Concentrations

| Analyte | LOD* (ng/mL) | LOQ† (ng/mL) | Cut-off‡ (ng/mL) |
|---|---|---|---|
| Buprenorphine | 0.28 | 0.94 | 0.94 |
| Fentanyl | 0.044 | 0.14 | 1.01 |
| LSD | 0.0097 | 0.036 | 0.65 |

*LOD is calculated by the following formula: LOD = mean concentration of blank + 3 × SD validation sample.
†LOQ is calculated by the following formula: LOQ = mean concentration of blank + 10 × SD validation sample.
‡Cut-off is the detection limit chosen to be used at NIPH.

### *Specificity*

No specificity tests were performed. However, the retention times of approximately 100 compounds were previously determined under similar UPLC–MS-MS conditions using the same gradient profile, column and the mobile phase composition as in the validated method (34). None of the compounds in this test had similar $MH^+$ ions or similar retention times as buprenorphine, fentanyl or LSD. Two MRM ions for all analytes and internal standards were used for improved qualitative and quantitative detections.

**Table IV**
Matrix Effects ($n = 8$)*

| Compound | Concentration† (ng/mL) | ME | RSD (%) |
|---|---|---|---|
| Buprenorphine | 7.8 | 99 | 7.4 |
| | 62.3 | 109 | 3.9 |
| Fentanyl | 5.6 | 102 | 9.2 |
| | 44.9 | 102 | 3.2 |
| LSD | 1.1 | 101 | 14 |
| | 8.6 | 100 | 3.5 |
| Buprenorphine-$D_4$ | 62.9 | 107 | 11 |
| Fentanyl-$D_5$ | 22.7 | 98 | 11 |
| LSD-$D_3$ | 5.4 | 100 | 9.3 |

*Eight whole blood samples were analyzed: four samples obtained from living persons and four postmortem samples.
†Concentration in autosampler vial (60 μL extract).

## Conclusion

A sensitive UPLC–MS-MS method was developed and validated for the qualitative and quantitative determination of buprenorphine, fentanyl and LSD in whole blood. Sample preparation was performed using LLE with MTBE. Most phospholipids were removed during sample preparation and the lyso-PCs that co-eluted with buprenorphine were almost completely removed. A mobile phase with high pH consisting of ammonium formate (pH 10.2) and MeOH provided narrow and symmetrical peaks and repeatable retention times. LOD values of buprenorphine, fentanyl and LSD were 0.28, 0.044 and 0.0097 ng/mL, and LOQ values were 0.94, 0.14 and 0.036 ng/mL, respectively. No or only minor matrix effects were observed. Deuterium labeled internal standards were used to compensate for different extraction recoveries and to correct for possible ion suppression/enhancement effects. The method has been routinely used at NIPH since September of 2011 for qualitative and quantitative detections of buprenorphine, fentanyl and/or LSD in more than 400 whole blood samples with two replicates of each sample.

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