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1 Quantification of methadone, buprenorphine, naloxone, opioids, and their derivatives in whole
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3 involvement in fatal forensic cases.

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1 **Abstract**

2 Opioids represent a broad family of compounds that can be used in several indications:
3 analgesics, antitussives, opioid substitution therapy (e.g. methadone, buprenorphine...).

4 When these products are misused, they are often addictive. Thus, we aimed to develop an
5 analytical method able to rapidly quantify several opiates and opioids (6-
6 monoacetylmorphine, buprenorphine, codeine, dihydrocodeine, 2-ethyl-1,5-dimethyl-3,3-
7 diphenylpyrrolidine, ethylmorphine, heroin, methadone, morphine, nalbuphine, naloxone,
8 norbuprenorphine, norcodeine, norpropoxyphene, oxycodone and propoxyphene) in whole
9 blood by ultra-high performance liquid chromatography combined with high resolution
10 mass spectrometry (UHPLC-HRMS). The validated assay requires only 100 μL of the
11 blood sample. The sample is prepared by a rapid liquid-liquid extraction using 5% zinc
12 sulfate (W/V), methanol and acetonitrile. Calibration curves range from 0.98 to 1000 $\mu\text{g/L}$,
13 except for buprenorphine (0.39-100 $\mu\text{g/L}$) and norbuprenorphine (0.20-100 $\mu\text{g/L}$). Inter-
14 and intra-analytical accuracy was less than 15%. Therefore, we describe the development
15 and full validation of an accurate, sensitive and precise assay using UHPLC-HRMS for the
16 analysis of opioids in whole blood. After validation, this new assay is successfully applied
17 on a routine laboratory application basis.

18
19 **Keywords:** high-resolution mass spectrometry, opioids, toxicological analysis, fatal forensic
20 cases analysis.
21

1 **1. Introduction**

2
3
4 Opioids represent a large family of compounds that can be used in several indications:
5 analgesics, antitussives, opioid substitution therapy (e. g. methadone, buprenorphine...).

6 When these molecules are misused, it is often addictive. In the case of overuse, the clinical
7 consequences associated with opioid intoxication or overdose will be observed: respiratory
8 depression, reduced heart rate, drowsiness, myosis, coma, and possible death. For the past
9 15 years, the United States and Europe have been confronted with an ever-increasing
10 opiate crisis [1–3]. The number of deaths due to opioid overdose in the United States has
11 continued to increase, with more than 50,000 deaths reported in 2016 [4]. In Europe,
12 opioids are present in 81% of cases of fatal overdose [3]. Heroin or its metabolites are
13 implicated in the majority of declared fatal overdoses, frequently in combination with other
14 substances. Other opioids, mainly methadone and high-dose buprenorphine, are also often
15 reported in toxicological reports of fatal overdoses [1–3].

16 According to the most up-to-date data, methadone-related deaths have exceeded heroin-
17 related deaths in Croatia, Denmark, France, and Ireland [3]. In response to this global
18 public health crisis, many countries have been working on their health policies, mainly to
19 improve prescription regulation and limit the misuse of these substances. The
20 quantification of opioids and their derivatives in biological fluids is of considerable
21 importance in clinical and forensic toxicology in many situations such as driving under the
22 influence, understanding the cause of death, research into doping agents or chemical
23 submission [5].

24
25
26
27 Different biological matrices can be used for the analysis of opioids and derivatives [6–8].

28 Whole blood is the matrix of choice for the quantification of illicit drugs [5,7]. As blood

1 concentrations can be very low, analytical methods must be very sensitive. The
2 recommended quantitation limits are between 5 and 10 µg/L for all these types of analytes
3 in the blood [5]. In the 1990s, opioid quantification was performed by gas chromatography
4 coupled with mass spectrometry (GC-MS) [9]. In recent years, thanks to the improvement
5 of technology in terms of specificity, precision, and sensitivity, liquid chromatography
6 coupled with mass spectrometry has replaced the "old gold standard", GC-MS [10]. LC-
7 MS is widely used in clinical and forensic toxicology for screening and quantification of
8 drugs in different complex biological matrices [11–24]. In recent years, a new physical and
9 analytical approach using high-resolution mass spectrometry (HRMS) has emerged in
10 toxicology laboratories [25–31]. Thanks to this technology, the identification, and
11 quantification of compounds were based on the precise measurement of mass and fragment
12 ions. The objective of this study was, therefore, to develop and validate a rapid, sensitive
13 and quantitative method for the analysis of 16 opioids and derivatives: 6-
14 monoacetylmorphine (6-MAM), buprenorphine, codeine, dihydrocodeine, 2-ethyl-1,5-
15 dimethyl-3,3-diphenylpyrrolidine (EDDP), ethylmorphine, heroin, methadone, morphine,
16 nalbuphine, naloxone, norbuprenorphine, norcodeine, norpoxyphene, oxycodone and
17 propoxyphene in high-performance liquid chromatography coupled with high-resolution
18 mass spectrometry (UHPLC- HRMS).

19

20 **2. Materials and methods**

21

22

23 **2.1. LC-MS analysis**

24

25

26 **2.1.1 Chemicals**

27

28

29 All reference standards and their internal standards (IS) were purchased from Cerilliant
30 (Round Rock, Texas, USA). Acetonitrile, methanol, formic acid, and water, all hypergrade

1 LC-MS for mobile phase, were obtained from Biosolve (Dieuze, France). Zinc sulfate and
2 ammonium formate were obtained from Sigma-Aldrich (Saint-Louis, Missouri, USA).
3 Whole blood from healthy donors was purchased from the Etablissement Français du Sang
4 (EFS, Reims, France).

5

6 **2.1.2 Chromatographic and mass-spectrometric conditions**

7

8 An ultra-high performance liquid chromatography system with a Dionex Ultimate 3000
9 high-pressure pump (ThermoFisher Scientific, San Jose, USA) coupled with a quadrupole
10 hybrid mass spectrometer QExactive Orbitrap (ThermoFisher Scientific, San Jose, USA)
11 was used to develop and validate this method.

12 Chromatographic separation was achieved using a UHPLC Waters Acquity HSS T3 1.8
13 μm (2.1 \times 50 mm) column (Waters Corp; Milford, MA, USA), maintained at 50°C. The
14 mobile phases were composed of water + formic acid 0.1% (V/V) (mobile phase A) and
15 acetonitrile + formic acid 0.1% (V/V) (mobile phase B). A programmed mobile-phase
16 gradient was used at a flow rate of 0.5 mL/ min during 5 min of run: 0 min, 3% B,
17 following concave curve; 3 min, 95% B; 4 min, 95% B, following concave curve; 4.3 min,
18 3% B following linear curve. The analysis and acquisition time were 5.10 minutes.

19

20 Detector coupling was performed using a heated electrospray ionization source (HESI)
21 operating in positive ionization mode, with the following settings: sheath gas at 45
22 arbitrary units (AU), auxiliary gas at 15 AU, sweep gas flow rate at 1 AU, spray voltage at
23 3.50 kV, ion transfer capillary temperature at 300 °C, S lens RF level at 70 AU and heating
24 temperature at 350 °C. The acquisition was performed using a parallel reaction monitoring
25 mode (PRM). The QExactive Orbitrap mass spectrometer can acquire masse range from 50
26 to 2000 m/z. The execution acquisition was from 0 to 5.25 min. The settings for the
27 acquisition were as follows: high resolution from 17,500 (resolution was calculated at full

1 width at maximum mid-height at m/z 200); target 1^{e5} of the automatic gain control (AGC);
2 maximum injection time (IT) 50 ms. Collision energies have been optimized for each
3 analyte. Two PRM transitions were selected: the first for quantification and the second for
4 analytes confirmation (Table 1).

5 Mass calibration was performed once a week in a positive and negative mode according to
6 the manufacturer's recommendations, using an external calibration solution (Pierce®,
7 ThermoScientific, San Jose, USA).

8 TraceFinder Forensic 3.3 was used for LC-MS monitoring, library management,
9 acquisition, and processing.

10

2.2 Preparation of stock solutions, calibration standards and quality control samples

12

13 These solutions were stored for one month at $-20\text{ }^{\circ}\text{C}$. To prepare the calibration standards,
14 a working solution (higher calibrator) was prepared with an appropriate volume of
15 methanol and then diluted in series by half. Calibration standards ranged from 0.98 to 1000
16 $\mu\text{g/L}$ except for buprenorphine (0.39-100 $\mu\text{g/L}$) and norbuprenorphine (0.20-100 $\mu\text{g/L}$).
17 Ten microlitres of each dilution were then added to 100 μL of whole blank blood and
18 treated as a patient. Quality controls (QC) were prepared in our laboratory at different
19 concentrations corresponding to 3 times LLOQ (low level, QCL), 40% (medium level,
20 MCQ) and 80% (high level, QCH) of the highest standards. These quality control samples
21 were freshly prepared before analysis with stock solutions different from those used for
22 standard preparation. Besides, four internal quality controls (BTMF A, BTMF B, BTMF
23 C, and STM A) were purchased from ACQ Science (Rottenburg-Hailfingen, Germany)
24 (supplemental table 1). Internal quality controls were prepared in accordance with the
25 manufacturer's instructions.

26

2.3 Sample processing

1
2
3 A stock solution was prepared in acetonitrile, containing all analytes at a final
4 concentration of 10 mg/L, except buprenorphine and norbuprenorphine at 1 mg/L. An
5 internal standard solution was prepared under the same conditions to obtain a final
6 concentration of 1 mg/L each. Ten microlitres of the internal standard solution were added
7 to 100 μ L of the whole blood sample. Deproteinization with 100 μ L of 5% zinc sulfate
8 (w/v), 100 μ L of methanol and 200 μ L of acetonitrile was performed. After 60 seconds of
9 vortex mixing, the sample was centrifuged at 10,000 g for 5 minutes. The supernatant (150
10 μ L) was evaporated under nitrogen at 40°C. The dry extract was reconstituted with 100 μ L
11 of water (LC-MS hypergrade) containing 0.1% (V/V) formic acid. Twenty microlitres of
12 this extract were injected into the UHPLC-HRMS system.

13

2.4 Validation procedure

15
16
17 Validation was conducted in accordance with international recommendations [32–34].
18 Linearity, precision, accuracy, selectivity, matrix effect (ME), carry-over and stability were
19 assessed as previously described [35].

20

2.4.1 Linearity

22

23

24 Following the guidelines, the lower limit of quantification (LLOQ) was determined for
25 each analyte as the lowest concentration for which the accuracy was between 80% and
26 120% and the precision below 20% (n = 6). The upper limit of quantification (ULOQ) was
27 also determined as the high concentration with an accuracy between 80 % and 120 % and
28 precision below 20%. Different weighting functions were tested in 10 calibration ranges
29 for each analyte to select the regression calibration (linear, 1 / X, 1 / X², 1 / Y ...). The
30 accuracy of quality controls, the analysis of the distribution of deviations from the

1 prediction of regression or residual value (Gaussian distribution verified by the D'Agostino
2 Pearson test) and the accuracy of the standards to $\pm 15\%$ ($\pm 20\%$ at LLOQ) of the nominal
3 concentration were criteria for determining the regression model [35].

4 5 **2.4.2 Precision and accuracy**

6
7
8 Three QC prepared in our laboratory (QCL, QCM, QCH) and four levels of internal
9 quality controls (BTMF A, BTMF B, BTMF C and STM A purchased from ACQ Science,
10 Supplemental Table 1) were used to evaluate precision and accuracy of the method.
11 Within-run and between-run accuracy and precision were assessed by analyzing 10
12 samples per level of control. Precision was expressed as the relative standard deviation and
13 had to be within $\pm 15\%$. For accuracy, the mean concentration had to be within $\pm 15\%$ from
14 target concentration at each tested level of control.

15 16 **2.4.3 Selectivity**

17
18
19 Six whole blood samples from donors were pre-treated and analyzed individually as blanks
20 to investigate interferences. As recommended, the absence of interfering components was
21 accepted when the blanks' responses were lower than 20% of the LLOQ for the analytes
22 and 5 % for the corresponding internal standard [32,33].

23 24 **2.4.4 Matrix effect**

25
26 The matrix effect (ME) was evaluated with six whole blood samples from different
27 sources, according to Matuszewsky *et al.* [34]. Two sets of the matrices were spiked after
28 extraction with internal standards and all the analytes at a low or a high concentration: 2.94
29 $\mu\text{g/L}$ or 800 $\mu\text{g/L}$ respectively for all analytes, except for buprenorphine (1.17 $\mu\text{g/L}$ or 80
30 $\mu\text{g/L}$) and norbuprenorphine (0.6 $\mu\text{g/L}$ or 80 $\mu\text{g/L}$).

31 The matrix effect factor was calculated by comparing the area under the peak derived from

1 the matrix spiked after extraction and the area under the peak of a pure solution at the same
2 concentration. The normalized factor of the matrix effect is defined for each matrix and
3 analyte by comparing the matrix factor of the analyte and the matrix factor of the
4 appropriate internal standard. The relative standard deviation of the normalized factors
5 must be less than 20%.

6 7 **2.4.5 Stability**

8
9
10 The stability of illicit drugs in whole blood has been described in the literature [5,6,36–38].
11 Opiates, morphine and codeine are stable at -20°C even for several years [36]. Under the
12 same storage conditions, 6-MAM degrades more quickly [36].

13 The stability of the solution of the calibration standards and the IS solution was evaluated
14 by comparing 1-month solutions and freshly prepared solutions.

15 The stability of ACQ science internal controls was evaluated over 3 months by comparing
16 controls stored at -20°C for 3 months and freshly reconstituted control.

17 Post-preparation stability was assessed by keeping the treated samples (STDs) placed in
18 glass vials in the autosampler (+10°C) for 24 hours.

19 20 **2.4.6 Carry-over effects**

21
22 The carry-over was evaluated by analyzing blank samples (n=6) immediately after the
23 highest concentration standard. As recommended, the signal should be less than 20% of
24 the LLOQ and less than 5% of the internal standard.

25 26 **2.5 Forensic fatal cases review**

27
28
29 A two-year review of fatal forensic cases was conducted in our laboratory. The samples
30 were analyzed using this analytical method. The involvement of opioids or their

1 derivatives has been studied. Other compounds that may increase toxicity have also been
2 reported.

3

4 **2.6. Statistical analysis**

5

6

7 GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) was used for statistical
8 analysis.

9

10 **3. Results and discussion**

11

12

13 **3.1. Optimization of the method**

14

15

16 In a previous study, extraction procedure was performed with chloroform: ethyl acetate
17 50/50 (v/v) and 20 μ L of sodium carbonate buffer solution (20%, w/v) at pH 11, and
18 optimized to ensure enough signals for opioids and in particular morphine [13]. This
19 extraction was then assayed for the determination of other opioids and was not enough for
20 buprenorphine, methadone and their metabolites. Deproteinization using 100 μ L of 5%
21 zinc sulfate (w/v), 100 μ L of methanol and 200 μ L of acetonitrile was performed. This
22 procedure was chosen because it allowed obtaining satisfactory signals for all compounds.
23 Column choice, curvilinear gradient and chromatographic parameters were optimized as
24 described [13]. After that, different mass spectrometry acquisition modes were tested: full
25 scan and parallel reaction monitoring (PRM). Full scan mode was unable to discriminate
26 isomeric compounds (morphine/norcodeine and 6-MAM/naloxone), so PRM mode was
27 chosen. For each compound, the energy of collision was optimized, and two fragment ions
28 were selected, the first one for the quantification and the second one for the confirmation
29 of the analytes (Table 1). Figure 1 depicts the reconstructed chromatogram of standard 6
30 for all analytes.

1
2
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4 **3.2. Validation of the method**

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6
7 **3.2.1. Linearity, precision and accuracy**

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9
10 For all analytes, quadratic regression ($Y = ax^2 + bx$) without weighting satisfied all the
11 predefined criteria [35]. Within the considered concentration range, regression coefficient
12 (r^2) of the calibration curves were always higher than 0.997 ($n = 10$) with back-calculated
13 concentrations for the calibration samples within $\pm 15\%$ ($\pm 20\%$ at LLOQ) of nominal
14 concentration.

15 The precision and accuracy ($n = 6$) of the LLOQ and ULOQ for each analyte were within
16 the recommended limits (Table 2). The relative standard deviations of quality controls
17 (commercial QC Table 3, home- made QC table 4) were comprised between 0.87 and
18 14.98% for both intra- and inter-assay precision ($n = 10$) and were within acceptance
19 criteria [38,39]. Evaluation of the accuracy of the quality controls showed a relative
20 standard deviation ($n = 10$) less than $\pm 15\%$ (85.01% - 109.96%) from target concentration
21 at each tested level (Table 3, 4) except for 6-MAM and EDDP in commercial quality
22 control. For both compounds, accuracy for intra- and inter- assay was less than 85%, but the
23 result was still in the confidence range of the quality control with exact precision.

24
25 **3.2.2. Specificity and selectivity**

26
27
28 Analysis of 6 different blank whole blood samples did not show any interference at the
29 retention time windows for each specified PRM. For each sample, the response was less
30 than 20% of the lower limit of quantification for analytes and 5% for internal standards.
31 Supplemental figure 1 represents overlapped chromatograms of LLOQ and blank samples
32 for all compounds.

1
2 **3.2.3. Matrix effect**

3
4
5 Matrix factor (MF) and the internal standard normalized MF ranged from 0.8 to 1.85
6 (Supplemental Table 2). As recommended, the relative standard deviation of normalized
7 matrix factors of each analyte was below 15% (n = 6) [32].

8
9 **3.2.5. Stability**

10
11
12 All analytes were stable ($\pm 15\%$, n = 6) in the processed samples (all standards) stored for
13 24 h in the autosampler (+10°C).

14 Long-term storage at -20°C, for 3 months, did not show any degradation of analytes in the
15 stock solution or the ACQ quality controls ($\pm 15\%$, n = 6).

16
17 **3.2.6. Carry-over effects**

18
19
20 The signals observed for all analytes in the blank samples processed immediately after the
21 highest standard was less than 20% of the signals of a LLOQ sample and less than 5% of
22 the signal of the corresponding internal standard.

23 These results confirm the absence of contamination and therefore, the choice of
24 appropriate chromatographic conditions.

25
26
27 **3.3 Applicability**

28
29
30 This method has been applied for the quantification of opioids and their derivatives in
31 whole blood for clinical and forensic toxicology purposes. Due to a complex metabolism
32 and pharmacokinetic parameters of each substance, it was essential to propose a method to
33 quantify these metabolites [5,6]. For example, heroin is the most commonly used illicit
34 opioid. Still, considering its very short half-life (3-9 min in blood), it will only be detectable

1 in blood for a few minutes after administration. On the contrary, its metabolites, 6-MAM
2 and mainly morphine, with longer half-lives, will be detectable for 1-2 h and for more than
3 8 h, respectively. Considering pharmacokinetics parameters, heroin will only be detectable
4 in the blood for a few minutes, 6-MAM 1 to 2 hours, while morphine and codeine will be
5 detectable more than 8 hours after intake. Other synthetic opioids such as methadone and
6 buprenorphine are also being misused [3]. Methadone and buprenorphine, synthetic opioids
7 used in substitution treatment, are metabolized to EDDP and norbuprenorphine,
8 respectively [5,6]. It therefore seemed appropriate to develop a method to quantify these
9 various opioids and their metabolites.

10 Figure 2 illustrates an example of positive results obtained on a patient sample:
11 chromatograms of the quantification ion, the confirmation ion, and the internal standard are
12 presented, as well as the corresponding calibration curves. The sample was positive for
13 methadone (296 µg/L), EDDP (14 µg/L), morphine (32 µg/L) and codeine (6 µg/L). The
14 supplemental figure 2 illustrates another positive sample analysis for morphine (279 µg/L),
15 6-MAM (20 µg/L) and codeine (19 µg/L).

16 A two-year review of the fatal forensic cases analyzed at the toxicology laboratory of the
17 Reims hospital was carried out. Opioids and their derivatives were implicated in 16.2 % of
18 fatal cases (n=186). Morphine was the most commonly detected opioid (41 %), followed
19 by methadone (26 %), codeine (21 %), 6-MAM and buprenorphine (both 6 %). In 86 % of
20 cases, the opiates detected were associated with other substances: benzodiazepines (50 %),
21 THC (15.5 %), cocaine (7.7 %), or with each other. Opioids were also associated with
22 opioid substitution therapies, methadone (23 %) and buprenorphine (3.8 %). The median,
23 first and third quartiles concentrations for the different compounds were as follows: 6-
24 MAM (3 µg/L, 3-8), buprenorphine (2.8 µg/L, 2.7-2.9), codeine (12 µg/L, 5-88.25), EDDP
25 (18 µg/L, 9.2-30.5), methadone (139 µg/L, 128.15-188.7), morphine (22.4 µg/L, 6.3-

1 129.5), norbuprenorphine (5.3 µg/L, 3.15-5.45) and norcodeine (29 µg/L, 13.25-52.25).

3 **3.4 Comparison with reported methods**

4
5
6 Ultra-high-performance liquid chromatography coupled with tandem mass spectrometry
7 (UHPLC-MS/MS) has become a well-established analytical technique for the
8 determination of drugs of abuse in clinical and forensic toxicology laboratories in whole
9 blood and serum. The first application using high-resolution mass spectrometry for the
10 quantification of opioids and their derivatives was performed with a Time of Flight (TOF)
11 mass spectrometer [11,12].

12 Concerning sample pre-treatment, solid-phase extraction (SPE) is the most common in the
13 literature [11,12,14–20,24]. Many procedures use SPE columns for whole blood analysis,
14 despite the risk of obstruction of the SPE column. To avoid this problem, some authors
15 propose to work at low temperatures [18] or to add a step of sonication of the sample by
16 centrifugation before loading the column with the supernatant. [12,16]. Feliu *et al.*
17 propose a liquid-liquid extraction of whole blood with chloroform and ethyl acetate [13].
18 Moreno-Vicente *et al.* [21], and Sartori *et al.* [22] propose methanol plasma precipitation.
19 In this work, we describe the precipitation of whole blood using acetonitrile, zinc sulfate
20 and methanol.

21 For the mass spectrometer, many procedures referenced in the literature use a triple-
22 quadrupole mass spectrometer with multiple-reaction-monitoring (MRM) mode. Due to its
23 high sensitivity and specificity, MRM is considered as a reference for quantitative analysis
24 [11,13–22]. Rosano *et al.* proposed a multi-drug and metabolite quantification by liquid
25 chromatography-high-resolution mass spectrometry in whole blood using a UHPLC–
26 MS^E/TOF[12]. Quantification was performed using precursor ion data obtained with a mass
27 extraction window of ±5 ppm. Fragment and residual precursor ion acquisitions at ramped

1 collision energies were evaluated as additional analyte identifiers. In parallel, UHPLC–
2 MS/MS analysis showed comparable precision and bias. Viaene *et al.* also compared a
3 triple-quadrupole and a quadrupole time-of-flight mass analyzer to quantify 16 opioids in
4 human plasma [11]. They concluded that the most efficient system depends on the
5 compound and even the concentration and that the best calibration model often differs by
6 compounds and method.

7 8 **4. Conclusion**

9
10 In this work, we describe the development and the full validation of a precise, sensitive
11 and accurate UHPLC-HRMS method which can simultaneously quantify 6-MAM,
12 buprenorphine, codeine, norcodeine, dihydrocodeine, EDDP, ethylmorphine, heroin,
13 methadone, morphine, nalbuphine, naloxone, norbuprenorphine, oxycodone,
14 norpropoxyphene, and propoxyphene. The assay only requires a small volume of whole
15 blood and a simple pre-treatment. After validation, this new assay was routinely applied
16 for the analysis of clinical and forensic cases.

18

19 **Bibliography**

20

- 21 [1] L. Rutkow, J.S. Vernick, Emergency Legal Authority and the Opioid Crisis, *N. Engl. J.*
22 *Med.* 377 (2017) 2512–2514. <https://doi.org/10.1056/NEJMp1710862>.
23 [2] K.L. Koenig, The Opioid Crisis in America: Too much, too little, too late, *West. J.*
24 *Emerg. Med.* 19 (2018) 557–558. <https://doi.org/10.5811/westjem.2018.2.38087>.
25 [3] European Drug Report 2019: Trends and Developments | www.emcdda.europa.eu, (n.d.).
26 <http://www.emcdda.europa.eu/publications/edr/trends-developments/2019> (accessed
27 June 10, 2019).
28 [4] National Institute on Drug, Overdose Death Rates, (2017).
29 <https://www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates>
30 (accessed May 25, 2018).
31 [5] P. Mura, P. Kintz, eds., *Drogues et accidentalité*, EDP sciences, DL 2011, Les Ulis,
32 France, 2011.
33 [6] R.C. Baselt, *Disposition of toxic drugs and chemicals in man*, Biomedical Publications,
34 Foster City (Calif.), Etats-Unis, 2008.
35 [7] C. Feliu, A. Fouley, H. Millart, C. Gozalo, H. Marty, Z. Djerada, Clinical and analytical
36 toxicology of opiate, cocaine and amphetamine, *Ann. Biol. Clin. (Paris)*. 73 (2015) 54–

69. <https://doi.org/10.1684/abc.2014.1009>.
- [8] V. Samanidou, L. Kovatsi, D. Fragou, K. Rentifis, Novel strategies for sample preparation in forensic toxicology, *Bioanalysis*. 3 (2011) 2019–2046. <https://doi.org/10.4155/bio.11.168>.
- [9] Y. Gaillard, G. Pepin, P. Marquet, P. Kintz, M. DEVEAUX, P. MURA, Identification and quantification of benzoylecgonine, cocaine, methylecgonine-ester, codeine, morphine and 6-acetylmorphine in whole blood, *Toxicorama*. (1996).
- [10] F. PETERS, Recent advances of liquid chromatography-(tandem) mass spectrometry in clinical and forensic toxicology, *Clin. Biochem*. 44 (2011) 54–65. <https://doi.org/10.1016/j.clinbiochem.2010.08.008>.
- [11] J. Viaene, K. Lanckmans, B. Dejaegher, D. Mangelings, Y. Vander Heyden, Comparison of a triple-quadrupole and a quadrupole time-of-flight mass analyzer to quantify 16 opioids in human plasma, *J. Pharm. Biomed. Anal.* 127 (2016) 49–59. <https://doi.org/10.1016/j.jpba.2015.12.055>.
- [12] T.G. Rosano, S. Na, K. Ihenetu, T.A. Swift, M. Wood, Multi-drug and metabolite quantification in postmortem blood by liquid chromatography-high-resolution mass spectrometry: comparison with nominal mass technology, *J. Anal. Toxicol.* 38 (2014) 495–506. <https://doi.org/10.1093/jat/bku066>.
- [13] C. Feliu, H. Millart, H. Guillemin, D. Vautier, L. Binet, A. Fouley, Z. Djerada, Validation of a fast UPLC-MS/MS method for quantitative analysis of opioids, cocaine, amphetamines (and their derivatives) in human whole blood, *Bioanalysis*. 7 (2015) 2685–2700. <https://doi.org/10.4155/bio.15.157>.
- [14] M.J. Ahsman, B.C. van der Nagel, R.A. Mathot, Quantification of midazolam, morphine and metabolites in plasma using 96-well solid-phase extraction and ultra-performance liquid chromatography-tandem mass spectrometry, *Biomed. Chromatogr. BMC*. 24 (2010) 969–976. <https://doi.org/10.1002/bmc.1394>.
- [15] A.I. Al-Asmari, R.A. Anderson, Method for quantification of opioids and their metabolites in autopsy blood by liquid chromatography-tandem mass spectrometry, *J. Anal. Toxicol.* 31 (2007) 394–408.
- [16] M.K. Bjørk, M.K.K. Nielsen, L.Ø. Markussen, H.B. Klinke, K. Linnet, Determination of 19 drugs of abuse and metabolites in whole blood by high-performance liquid chromatography-tandem mass spectrometry, *Anal. Bioanal. Chem.* 396 (2010) 2393–2401. <https://doi.org/10.1007/s00216-009-3268-9>.
- [17] D.J. Christoffersen, C. Brasch-Andersen, J.L. Thomsen, M. Worm-Leonhard, P. Damkier, K. Brøsen, Quantification of morphine, morphine 6-glucuronide, buprenorphine, and the enantiomers of methadone by enantioselective mass spectrometric chromatography in whole blood, *Forensic Sci. Med. Pathol.* 11 (2015) 193–201. <https://doi.org/10.1007/s12024-015-9673-9>.
- [18] R. Coles, M.M. Kushnir, G.J. Nelson, G.A. McMillin, F.M. Urry, Simultaneous determination of codeine, morphine, hydrocodone, hydromorphone, oxycodone, and 6-acetylmorphine in urine, serum, plasma, whole blood, and meconium by LC-MS-MS, *J. Anal. Toxicol.* 31 (2007) 1–14.
- [19] K. Eckart, J. Röhrich, D. Breitmeier, M. Ferner, R. Laufenberg-Feldmann, R. Urban, Development of a new multi-analyte assay for the simultaneous detection of opioids in serum and other body fluids using liquid chromatography-tandem mass spectrometry, *J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci.* 1001 (2015) 1–8. <https://doi.org/10.1016/j.jchromb.2015.06.028>.
- [20] M. Fernandez, S. Wille, N. Kummer, V. Di Fazio, E. Ruysinckx, N. Samyn, Quantitative analysis of 26 opioids, cocaine, and their metabolites in human blood by ultra performance liquid chromatography-tandem mass spectrometry, *Ther. Drug Monit.*

- 35 (2013) 510–521. <https://doi.org/10.1097/FTD.0b013e31828e7e6b>.
- [21] R. Moreno-Vicente, Z. Fernández-Nieva, A. Navarro, I. Gascón-Crespí, M. Farré-Albaladejo, M. Igartua, R.M. Hernández, J.L. Pedraz, Development and validation of a bioanalytical method for the simultaneous determination of heroin, its main metabolites, naloxone and naltrexone by LC-MS/MS in human plasma samples: Application to a clinical trial of oral administration of a heroin/naloxone formulation, *J. Pharm. Biomed. Anal.* 114 (2015) 105–112. <https://doi.org/10.1016/j.jpba.2015.04.044>.
- [22] D. Sartori, T. Lewis, A. Breaud, W. Clarke, The development of a high-performance liquid chromatography-tandem mass spectrometric method for simultaneous quantification of morphine, morphine-3- β -glucuronide, morphine-6- β -glucuronide, hydromorphone, and normorphine in serum, *Clin. Biochem.* 48 (2015) 1283–1290. <https://doi.org/10.1016/j.clinbiochem.2015.05.023>.
- [23] M. Dawson, B. Fryirs, T. Kelly, J. Keegan, L.E. Mather, A rapid and sensitive high-performance liquid chromatography-electrospray ionization-triple quadrupole mass spectrometry method for the quantitation of oxycodone in human plasma, *J. Chromatogr. Sci.* 40 (2002) 40–44.
- [24] S. Ghassabian, S.M. Moosavi, Y.G. Valero, K. Shekar, J.F. Fraser, M.T. Smith, High-throughput assay for simultaneous quantification of the plasma concentrations of morphine, fentanyl, midazolam and their major metabolites using automated SPE coupled to LC-MS/MS, *J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci.* 903 (2012) 126–133. <https://doi.org/10.1016/j.jchromb.2012.07.005>.
- [25] H.H. Maurer, Perspectives of liquid chromatography coupled to low- and high-resolution mass spectrometry for screening, identification, and quantification of drugs in clinical and forensic toxicology, *Ther. Drug Monit.* 32 (2010) 324–327. <https://doi.org/10.1097/FTD.0b013e3181dca295>.
- [26] J.-L.H. Jiwan, P. Wallemacq, M.-F. Hérent, HPLC-high resolution mass spectrometry in clinical laboratory?, *Clin. Biochem.* 44 (2011) 136–147. <https://doi.org/10.1016/j.clinbiochem.2010.08.018>.
- [27] S. Ojanperä, A. Pelander, M. Pelzing, I. Krebs, E. Vuori, I. Ojanperä, Isotopic pattern and accurate mass determination in urine drug screening by liquid chromatography/time-of-flight mass spectrometry, *Rapid Commun. Mass Spectrom. RCM.* 20 (2006) 1161–1167. <https://doi.org/10.1002/rcm.2429>.
- [28] A.H. Wu, R. Gerona, P. Armenian, D. French, M. Petrie, K.L. Lynch, Role of liquid chromatography-high-resolution mass spectrometry (LC-HR/MS) in clinical toxicology, *Clin. Toxicol. Phila. Pa.* 50 (2012) 733–742. <https://doi.org/10.3109/15563650.2012.713108>.
- [29] H.H. Maurer, What is the future of (ultra) high performance liquid chromatography coupled to low and high resolution mass spectrometry for toxicological drug screening?, *J. Chromatogr. A.* 1292 (2013) 19–24. <https://doi.org/10.1016/j.chroma.2012.08.069>.
- [30] M.R. Meyer, A.G. Helfer, H.H. Maurer, Current position of high-resolution MS for drug quantification in clinical & forensic toxicology, *Bioanalysis.* 6 (2014) 2275–2284. <https://doi.org/10.4155/bio.14.164>.
- [31] H.H. Maurer, M.R. Meyer, High-resolution mass spectrometry in toxicology: current status and future perspectives, *Arch. Toxicol.* 90 (2016) 2161–2172. <https://doi.org/10.1007/s00204-016-1764-1>.
- [32] European Medicines Agency (EMA), Guideline on bioanalytical method validation. 21 July 2011. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf. (accessed June 2013)., (n.d.). http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/

- 1 WC500109686.pdf.
- 2 [33] US Food and Drug Administration, Guidance for industry. Bioanalytical method
3 validation; May 2001. <http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf>.
4 (accessed July 2010), (n.d.).
5 <http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf> (accessed April 2,
6 2013).
- 7 [34] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of
8 matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS, *Anal.*
9 *Chem.* 75 (2003) 3019–3030.
- 10 [35] Z. Djerada, C. Feliu, C. Tournois, D. Vautier, L. Binet, A. Robinet, H. Marty, C. Gozalo,
11 D. Lamiable, H. Millart, Validation of a fast method for quantitative analysis of
12 elvitegravir, raltegravir, maraviroc, etravirine, tenofovir, boceprevir and 10 other
13 antiretroviral agents in human plasma samples with a new UPLC-MS/MS technology, *J.*
14 *Pharm. Biomed. Anal.* 86 (2013) 100–111. <https://doi.org/10.1016/j.jpba.2013.08.002>.
- 15 [36] G. Høiseth, B. Fjeld, M.L. Burns, D.H. Strand, V. Vindenes, Long-term stability of
16 morphine, codeine, and 6-acetylmorphine in real-life whole blood samples, stored at -
17 20°C, *Forensic Sci. Int.* 239 (2014) 6–10. <https://doi.org/10.1016/j.forsciint.2014.03.008>.
- 18 [37] R. Karinen, W. Andresen, A. Smith-Kielland, J. Mørland, Long-Term Storage of
19 Authentic Postmortem Forensic Blood Samples at -20°C: Measured Concentrations of
20 Benzodiazepines, Central Stimulants, Opioids and Certain Medicinal Drugs Before and
21 After Storage for 16-18 Years, *J. Anal. Toxicol.* (2014).
22 <https://doi.org/10.1093/jat/bku080>.
- 23 [38] O.H. Drummer, Postmortem toxicology of drugs of abuse, *Forensic Sci. Int.* 142 (2004)
24 101–113. <https://doi.org/10.1016/j.forsciint.2004.02.013>.
- 25 [39] A. Makarov, Mass spectrometer; 1999 US Patent, 5886346., n.d.
- 26 [40] Q. Hu, R.J. Noll, H. Li, A. Makarov, M. Hardman, R. Graham Cooks, The Orbitrap: a
27 new mass spectrometer, *J. Mass Spectrom. JMS.* 40 (2005) 430–443.
28 <https://doi.org/10.1002/jms.856>.
- 29 [41] A.G. Helfer, J.A. Michely, A.A. Weber, M.R. Meyer, H.H. Maurer, Orbitrap technology
30 for comprehensive metabolite-based liquid chromatographic-high resolution-tandem
31 mass spectrometric urine drug screening - exemplified for cardiovascular drugs, *Anal.*
32 *Chim. Acta.* 891 (2015) 221–233. <https://doi.org/10.1016/j.aca.2015.08.018>.
- 33 [42] L. Roche, J. Pinguet, P. Herviou, F. Libert, C. Chenaf, A. Eschalier, N. Authier, D.
34 Richard, Fully automated semi-quantitative toxicological screening in three biological
35 matrices using turbulent flow chromatography/high resolution mass spectrometry, *Clin.*
36 *Chim. Acta Int. J. Clin. Chem.* 455 (2016) 46–54.
37 <https://doi.org/10.1016/j.cca.2016.01.017>.
- 38 [43] A.G. Helfer, J.A. Michely, A.A. Weber, M.R. Meyer, H.H. Maurer, Liquid
39 chromatography-high resolution-tandem mass spectrometry using Orbitrap technology
40 for comprehensive screening to detect drugs and their metabolites in blood plasma, *Anal.*
41 *Chim. Acta.* 965 (2017) 83–95. <https://doi.org/10.1016/j.aca.2017.03.002>.
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Conflict of interest

None to declare.

Table legends:

Table 1: Retention time (RT), transition of precursors and fragments ions, mass spectrometry parameters for 16 opioids and their respective internal standards.

Table 2: Limit of detection (LOD), lower limit of quantification (LLOQ), upper limit of quantification (ULOQ), precision (relative standard deviation (RSD)) and accuracy of LLOQ and ULOQ ($n = 6$).

Table 3: Intra- and inter-assay precision and accuracy for 8 opiates and derivates determined on ACQ science quality controls ($n = 15$).

Table 4: Intra- and inter-assay precision and accuracy for 16 opioids and derivatives ($n = 15$) at three levels of concentration (3xLLOQ, 40 % and 80 % of ULOQ).

Supplemental table 1: Target concentrations and confidence range of ACQ science internal quality controls

Supplemental table 2: Matrix effect factor at two levels of concentration determined in 6 different blank whole blood samples

Figure legends:

Figure 1: Reconstructed chromatograms for all analytes (A) of the standard 6 and their internal standard (B). Minimal signal intensity was observed for buprenorphine ($1.49e6$) and the maximum signal intensity was observed for methadone ($8.71e8$).

Figure 2 : Example of result: chromatograms and calibration curve obtained for a sample positive for methadone (A), EDDP (B), codeine (C) and morphine (D).

Supplemental Figure 1: Overlapping chromatograms of LLOQ and blank samples extracted for 6-MAM (A), buprenorphine (B), codeine (C), dihydrocodeine (D), EDDP

1 (E), ethylmorphine (F), heroin (G), methadone (H), morphine (I), nalbuphine (J), naloxone
2 (K), norbuprenorphine (L), norcodeine (M), norpropoxyphene (N), oxycodone (O) and
3 propoxyphene (P).

4 **Supplemental figure 2:** Example of result: chromatograms and calibration curve obtained
5 for a sample positive for morphine, 6-MAM and codeine.

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7 **Acknowledgements:**

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9 This work was supported by Reims University Hospital, France.
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Table 1: Retention time (RT), transition of precursors and fragments ions, mass spectrometry parameters for 16 opioids.

Compound	Internal standard	Precursor ion (m/z) Theoretical values	Collision energy (eV)	Quantification fragment ion (m/z) Measured values			Confirmation fragment ion (m/z) Measured values			Retention time (min)
				measured	theoretical	fragment	measured	theoretical	fragment	
6-monoacetylmorphine	6-monoacetylmorphine-D3	328,15433	35	211,07518	211,07590	C14 H11 O2	165,06974	165,07043	C13 H9	2,43
buprenorphine	buprenorphine-D4	468,31084	35	414,26303	414,26443	C25 H36 N O4	396,21281	396,21748	C24 H30 N O4	3,48
codeine	codeine-D3	300,15942	25	215,10667	215,10720	C14 H15 O2	243,10133	243,10212	C15 H15 O3	2,11
dihydrocodeine	dihydrocodeine-D3	302,17507	30	245,11722	245,11642665 245,11777	C13 H15 N3 O2 C15 H17 O3	202,09387	202,0868	C12 H12 N O2 ???	2,05
EDDP	EDDP-D3	278,19033	25	249,15085	248,14392	C18 H18 N	234,12741	234,12827	C17 H16 N	3,53
ethylmorphine	morphine-D3	314,17507	25	229,12213	229,12151	C13 H15 N3 O	257,11703	257,11643	C14 H15 N3 O2	2,63
heroin	heroin-D9	370,1649	25	268,1327	268,13375	C17 H18 N O2	328,15423	328,15488	C19 H22 N O4	3,12
methadone	methadone-D3	310,21654	15	265,15823	265,15924023 265,15790	C19 H21 O C17 H19 N3	105,03381	105,03404	C7 H5 O	3,59
morphine	morphine-D3	286,14377	25	201,09094	201,09155	C13 H13 O2	229,0861	229,08647	C14 H13 O3	1,09
nalbuphine	morphine-D3	358,20128	25	340,19045	340,19127	C21 H26 N O3	254,11728	254,1181	C16 H16 N O2	2,7
naloxone	morphine-D3	328,15433	25	310,14352	310,14432	C19 H20 N O3	253,10949	253,11028	C16 H15 N O2	2,13
norbuprenorphine	norbuprenorphine-D3	414,26389	35	101,09634	101,09664	C6 H13 O	396,25326	396,25387	C25 H34 N O3	3,3
norcodeine	morphine-D3	286,14377	25	268,1366	286,14432	C17 H20 N O3	225,09099	225,09155	C15 H13 O2	2
norpropoxyphene	propoxyphene-D5	326,21146	10	308,20089	308,20144	C21 H26 N O	100,07619	100,07624	C5 H10 N O	3,59
oxycodone	oxycodone-D6	316,15433	35	241,10925	241,11028	C15 H15 N O2	256,1331	256,13375	C16 H18 N O2	316
propoxyphene	propoxyphene-D5	340,22711	15	58,06603	58,06567	C3 H8 N	266,19032	266,19087	C19 H24 N	3,57

Table 2 : Limit of detection (LOD), lower limit of quantification (LLOQ), upper limit of quantification (ULOQ), precision (relative standard deviation (RSD)) and accuracy of LLOQ and ULOQ ($n = 6$).

Drug	LOD (µg/L)	LLOQ (µg/L)	Precision (RSD %) of LLOQ	Accuracy (%) of LLOQ	ULOQ (µg/L)	Precision (RSD %) of ULOQ	Accuracy (%) of ULOQ
6-monoacetylmorphine	0.5	0.98	6.28	96.77	1000	6.64	91.24
buprenorphine	0.15	0.39	19.80	82.48	100	7.76	103.06
codeine	0.5	0.98	6.03	100.43	1000	5.70	92.73
dihydrocodeine	0.5	0.98	7.88	99.47	1000	10.00	85.48
EDDP	0.5	0.98	5.07	115.82	1000	5.70	99.35
ethylmorphine	0.5	0.98	5.64	100.15	1000	7.90	109.64
heroin	0.5	0.98	4.78	102.96	1000	5.30	96.94
methadone	0.5	0.98	4.87	117.33	1000	3.51	96.01
morphine	0.5	0.98	3.16	81.16	1000	0.84	99.42
nalbuphine	0.5	0.98	2.63	106.51	1000	6.18	104.45
naloxone	0.5	0.98	3.67	110.27	1000	3.19	102.39
norbuprenorphine	0.1	0.20	14.26	106.75	100	7.59	90.19
norcodeine	0.5	0.98	4.51	88.03	1000	3.02	103.46
norpropoxyphene	0.5	0.98	10.72	88.06	1000	10.38	111.64
oxycodone	0.5	0.98	8.64	103.25	1000	6.59	110.10
propoxyphene	0.5	0.98	4.62	95.68	1000	6.39	108.50

Table 3: Intra- and inter-assay precision and accuracy for 8 opiates and derivates determined on ACQ science quality controls ($n = 10$).

RSD: relative standard deviation (%)

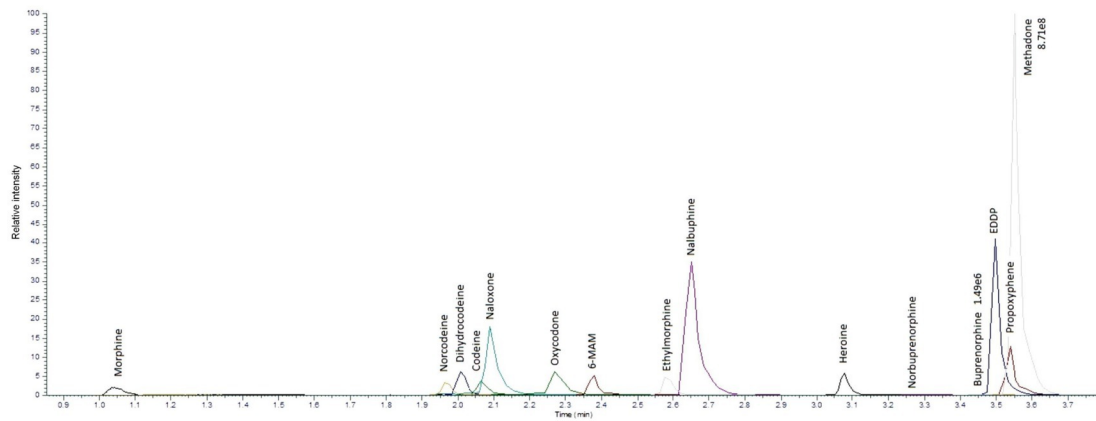
	BTMF A				BTMF B				BTMF C				STM A			
	Intra-assay		Inter-assay		Intra-assay		Inter-assay		Intra-assay		Inter-assay		Intra-assay		Inter-assay	
	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)
6-monoacetylmorphine	2.78	77.41	6.58	75.55	0.87	85.30	7.14	86.94	Not available							
buprenorphine	Not available												10.64	92.22	12.93	89.79
codeine	3.44	85.08	5.12	90.04	3.41	86.40	6.65	91.47	1.86	97.01	5.87	93.88	Not available			
dihydrocodeine	5.92	87.39	14.84	93.67	8.52	92.28	14.49	101.02	4.09	90.66	9.36	98.6	Not available			
EDDP	Not available												4.77	80.22	6.82	84.17
methadone	Not available												4.27	85.01	3.13	87.8
morphine	2.04	85.13	6.09	87.94	1.58	97.57	5.82	109.37	1.51	90.6	7.28	104.12	Not available			
norbuprenorphine	Not available												4.72	85.08	13.44	97.06

Table 4 Intra- and inter-assay precision and accuracy for 16 opiates and derivatives ($n = 10$) at three levels of concentrations (3xLLOQ, 40% and 80% of ULOQ) ($n=10$).

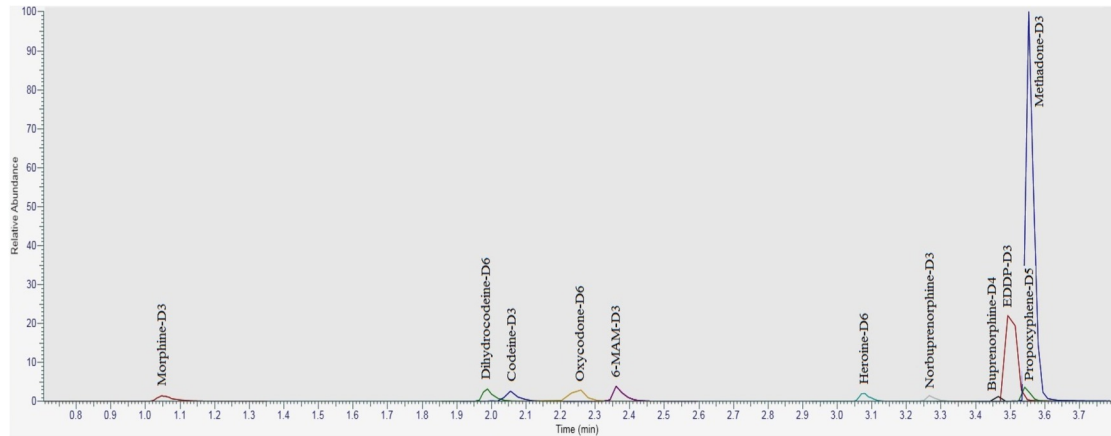
RSD (%): relative standard deviation.

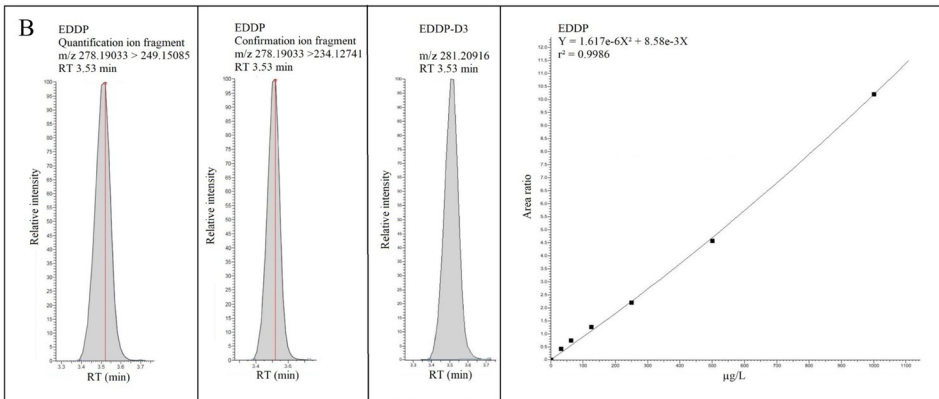
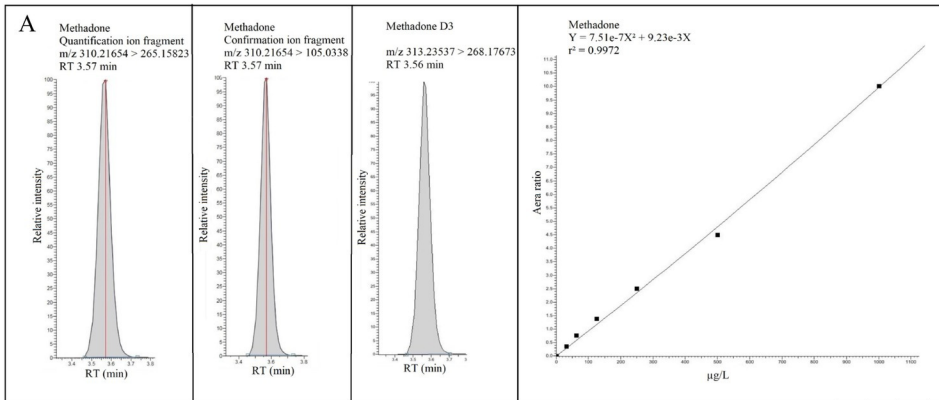
Compounds	QCL				QCM				QCH			
	Intra-assay		Inter-assay		Intra-assay		Inter-assay		Intra-assay		Inter-assay	
	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)
6-monoacetylmorphine	5.53	88.82	10.41	92.92	5.11	92.38	6.47	92.24	2.42	93.46	8.63	95.83
Buprenorphine	6.51	86.8	8.51	101.65	8.93	90.67	13.99	94.32	7.38	94.60	12.95	96.65
Codeine	4.18	101.60	7.26	97.32	3.97	102.55	6.87	94.22	4.96	105.89	6.74	99.83
Dihydrocodeine	6.59	113.23	11.61	97.57	3.29	109.07	5.03	93.24	4.43	102.79	7.70	93.60
EDDP	5.61	93.43	10.38	92.08	8.55	89.17	11.86	97.02	8.31	88.96	13.65	100.94
Ethylmorphine	7.87	95.00	5.15	96.25	13.05	86.82	9.01	91.64	5.81	91.34	5.39	95.09
Heroin	9.40	92.50	9.92	88.93	7.08	88.09	6.34	89.30	4.59	92.30	9.86	96.21
Methadone	4.56	94.14	3.10	87.06	4.87	93.35	10.76	92.01	4.63	99.36	8.79	99.28
Morphine	3.94	85.94	9.24	105.77	2.48	95.31	6.31	93.96	2.65	99.60	6.48	101.92
Nalbuphine	4.99	100.10	11.22	105.6	7.19	97.09	6.78	96.92	7.03	104.24	6.52	101.12
Naloxone	3.20	104.89	8.20	99.52	7.17	98.91	4.24	89.77	1.31	108.06	9.25	100.63
Norbuprenorphine	5.57	86.80	9.95	87.04	2.96	95.40	8.10	93.35	3.77	95.84	10.25	98.23
Norcodeine	5.78	85.01	9.42	86.80	3.70	92.58	5.72	90.78	2.20	106.50	6.22	97.97
Norpropoxyphene	5.02	92.06	14.71	86.52	8.75	91.90	14.74	89.31	11.21	98.59	13.66	87.50
Oxycodone	6.17	96.30	14.98	109.96	2.76	97.45	6.75	98.30	3.50	100.15	6.96	101.20
Propoxyphene	3.09	92.04	10.74	101.94	1.81	94.71	8.15	97.24	4.38	95.35	10.74	96.63

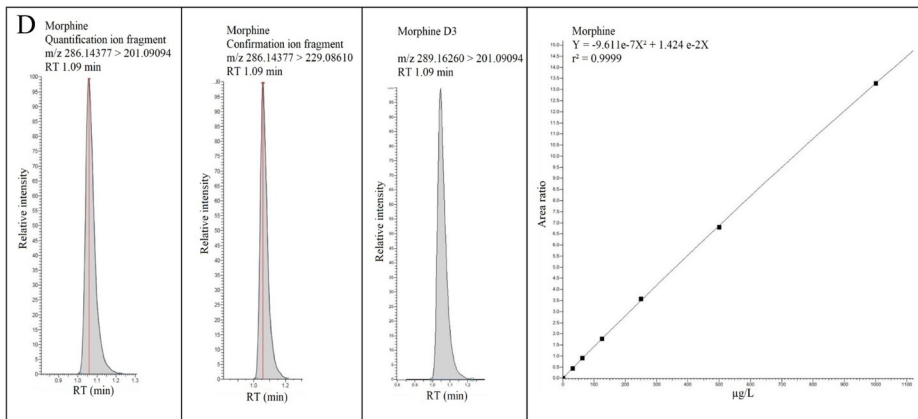
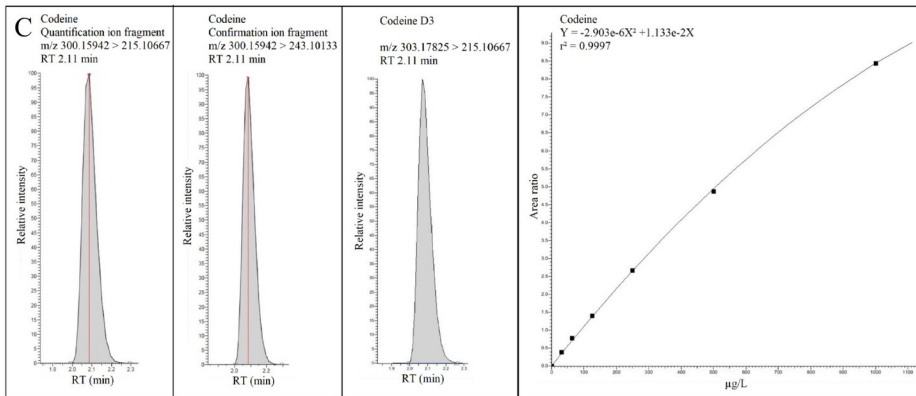
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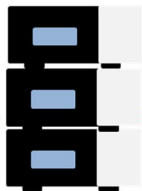




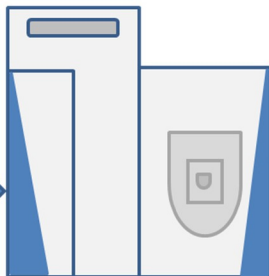
Whole blood or plasma sample



Work-up sample :
- Precipitation
- Centrifugation
- Evaporation of organic phase
- Reconstitution of dry extract in mobil phase



Chromatographic separation



High resolution mass spectrometry

