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Analysis of Isomeric Opioids in Urine using LC-TIMS-TOF MS

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Abstract

In the present work, a fast separation, identification and quantification workflow based on liquid chromatography coupled to trapped ion mobility in tandem with mass spectrometry (LC-TIMS-MS) is described for the analysis of common isomeric drugs of abuse and their metabolites in human urine. In particular, the analytical performance of LC-TIMS-MS is shown for identification based on retention time, collision cross section and accurate mass for three sets of common isomeric opioids and their deuterated analogs in urine. The LC-TIMS-MS analysis provided limits of detection of 1.4 - 35.2 ng/mL with demonstrated linearity up to 500 ng/mL, enabling discovery and targeted monitoring (DTM) of opioids in urine, with high precision in retention times (RT) (<0.3%), collision cross sections (CCS) (<0.6%) and mass accuracy (<1 ppm) across multiple measurements using external calibration. A good

agreement was observed between theoretical and experimental CCS from candidate structures optimized at the DFT/B3LYP level. The need for complementary liquid and mobility separations prior to mass analysis is shown for the analysis of complex mixtures, with mobility resolving power of 80-130. The reproducibility and high speed of LC-TIMS-MS analysis provides a powerful platform for drug and metabolite screening in biological matrices with higher precision and confidence than traditional LC-multiple reaction monitoring (MRM) approaches.

Keywords: liquid chromatography; trapped ion mobility spectrometry; mass spectrometry, drugs of abuse; isomeric separation; opioids.

Introduction

An opioid epidemic has existed in the United States for almost twenty years; however, the rate of ongoing drug abuse continues to increase. Since 2000, deaths from drug overdose have virtually tripled and deaths involving opioids (including opioid painkillers and heroin) have increased nearly 200% [1]. In 2015 ~62% of the ca. 50000 deaths related to drug overdose are associated to opioid use, involving both illicit and legally prescribed drugs [2-5]. This ever-increasing incidence of drug-related mortalities translates into a clear and present need for more sensitive techniques for drug detection and identification [6, 7]. Low therapeutic and abuse concentrations pose a challenge for screening and quantification of illicit drugs, analytical methods with high selectivity and sensitivity are need as monitoring tools for opioids to aid health care providers in their assessment for addiction treatment compliance and misuse [8, 9].

Urine testing is a common first step when caring for opioid addicts or individuals using drugs for pain management purposes [10-13]. Preliminary drug testing in urine typically includes the use of immunoassays, which provide qualitative results allowing the analyst to confirm the presence of broad

drug classes [14-17]. Although immunoassays provide rapid results, they typically fail to identify specific drugs types and lack sensitivity (cut-off concentrations ~300 ng/mL) and are also prone to cross-reactivity, increasing the possibility of false results [14-17]. In comparison, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) provides specific drug identifications based on retention time, intact mass and fragmentation patterns, and is becoming the gold standard for the detection of drugs of abuse and their metabolites in human fluids [14, 18-21]. The use of LC-MS/MS significantly decreases the rate of false results and is traditionally employed following a positive immunoassay test as a confirmatory tool [14, 16, 22, 23]. Identification, confirmation, and quantification of opioids in biological fluids, including urine and plasma, have been accomplished with LC-MS/MS, typically using triple-quadrupole instruments operating under multiple reaction monitoring (MRM) scan mode [9, 14-16]. Chromatography methods range from 6-35 minutes in length and report cut-off concentrations, or limits of detection (LODs) significantly lower than those of immunoassays ranging from 0.1 to 126 ng/mL in urine [14].

Ion mobility spectrometry coupled to mass spectrometry (IMS-MS) has been used for detection and separation of opioid compounds [18, 24-32]. Previous studies have reported mobility values for codeine, morphine, normorphine, norcodeine, acetylcodeine, O⁶-monoacetylmorphine, heroin and several other drugs using drift tube ion mobility spectrometers (DT-IMS) [18, 25, 30, 32]. In a more recent opioid analysis using high-field asymmetric wave-form ion mobility spectrometry (FAIMS), the separation of various isomeric opioids was shown with limits of detection (LODs) in urine for morphine and codeine of 60 ng/mL and 20 ng/mL, respectively [26, 28, 29, 31]. With the recent advent of higher resolving powers (R up to 400 [33]) and more sensitive ion mobility analyzers (e.g., Trapped Ion Mobility Spectrometers [34-36]) there is a need to further develop complementary separations based on mass spectrometry for the study and characterization of complex biological samples [37-39]. In particular, liquid chromatography and trapped ion mobility separation techniques have proven useful for the analysis of single components in biological matrices [37].

In the present study, for the first time, LC is coupled to TIMS in tandem with high resolution MS to provide a cohesive, multidimensional method to achieve high throughput analysis of isomeric opioids in urine. As a proof of concept, three sets of common isomeric opioids and their corresponding deuterated analogs are detected at trace levels in human urine after a “dilute-and-shoot” strategy. The compounds are identified based on their retention time, collisional cross section (CCS) and accurate mass, providing detection levels similar to those obtained with LC-MS/MS applications. With the additional selectivity provided by the TIMS separation much higher selectivity is afforded (decreased false positives). In this method, because detection is not limited to a few MRM transitions the discovery of new targets or metabolites and/or data back-interrogation is enabled.

Experimental Methods

Materials and Reagents

All solvents were purchased from Fisher Scientific (Pittsburg, PA) and were of LC-MS quality or better. Opioid compounds and deuterated standards were purchased from Cerilliant (Round Rock, TX). Eight opioid compounds and their deuterated analogs were analyzed: 6-acetylmorphine (A-009), 6-acetylmorphine-D₃ (A-006), naloxone (N-004), naloxone-D₅ (N-063), codeine (C-006), codeine-D₆ (C-040), hydrocodone (H-003), hydrocodone-D₃ (H-005), morphine (M-005), morphine-D₃ (M-003), hydromorphone (H-004), hydromorphone-D₃ (H-006), norcodeine (N-005), norcodeine-D₃ (N-082), norhydrocodone (N-053) and norhydrocodone-D₃ (N-054). Human urine was purchased from Innovative Research (Novi, MI, USA) and supplied by opioid-free volunteers.

Human Urine “dilute-and-shoot” Sample Preparation

Calibration curves were prepared by adding a known amount of a mixture of the Cerilliant standards in human urine or water and spiking with 50 μ L of deuterated internal standard (IS) mix. The curves consisted of seven calibration points ranging from 0.1 - 500 ng/mL with a constant 50 ng/mL of deuterated IS mix. The spiked samples were diluted with water with 10% methanol for a final sample volume of 300 μ L. No further extraction or preparation procedures were performed prior to analysis.

Limits of detection (LODs) were determined using the linear regression method, where the lowest detectable signal is calculated from the intercept and standard error of the regression line calculated; limits of quantification (LOQs) are reported as 5-times the LOD. Matrix effect experiments were performed using ten opioid-free urine samples spiked at low (75 ng/mL) and high (400 ng/mL) concentrations with 50 ng/mL of IS. Matrix effects were calculated by comparing the ratios of the spiked matrix samples to the average of six matrix -free water samples to obtain a matrix factor (MF).

LC- TIMS-MS Analysis

The LC-TIMS-TOF MS analysis was performed using a custom-built TIMS-TOF MS based on the maXis impact Q-ToF MS (Bruker Daltonics Inc, Billerica, MA). Sample injection (50 μ L) and LC separation was performed on a Shimadzu Prominence HPLC system consisting of two 20AD pumps, a SIL-20AC auto-sampler and a CTO 20-A column oven held at 40° C (Kyoto, Japan). An Onyx Monolithic C18 HPLC column (100 x 4.6 mm) was used protected by an Onyx guard column (5 x 4.6 mm), both from Phenomenex (Torrance, CA, USA). The mobile phase A composition consisted of 50 mM ammonium acetate in water and the mobile phase B consisted of 50 mM ammonium acetate in 96:4 methanol:water v:v. Mobile phase composition was changed as follows: sample injection at 0% B and hold for 1.5 minutes. From 1.5 to 2.5 minutes increase to 99% B and hold until 4.25 minutes. Decrease to 0% B at 4.5 minutes and hold until 6 minutes for column re-equilibration at a flow rate of 2 mL/min.

Samples were ionized using an ionBooster ESI source (Bruker Daltonics Inc, Billerica, MA) in positive ion mode. Typical ionBooster operating conditions were 1000 V capillary voltage, 400 V end plate offset, 300 V charging voltage, 4.1 bar nebulizer pressure, 3.0 L/min dry gas, 250 °C dry heater, and 375 °C vaporizer.

A detailed overview of the TIMS analyzer and its operation can be found elsewhere [34-36]. The nitrogen bath gas flow is defined by the pressure difference between entrance funnel $P_1 = 3.0$ mbar and the exit funnel $P_2 = 0.9$ mbar at *ca.* 300 K (see Figure S1). The TIMS separation depends on the gas flow velocity (v_g), ramp voltage (V_{ramp}), base voltage (V_{out}) and ramp time ($t_{\text{ramp}} = \text{number of steps} \times \text{TOF time}$). The scan rate ($Sr = \Delta V_{\text{ramp}}/t_{\text{ramp}}$) is directly related to the resolving power of the TIMS analyzer.

Each isomer emerges at a characteristic voltage (V_{elution}):

$$K_0 = v_g/E \approx A/(V_{\text{elution}} - V_{\text{out}}) \quad (1)$$

where A is a calibration constant that can be determined using standards of known mobilities (*i.e.*, Tuning Mix calibration standard m/z 322, $K_0 = 1.376 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ and m/z 622, $K_0 = 1.013 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) [36]. The TIMS cell was operated using a fill/ramp sequence of 10ms/100ms for ~10% duty cycle and the TOF analyzer was operated at 10 kHz (m/z 100-2500). Typical values were $V_{\text{deflector}} = 180$, $V_{\text{capillary}} = 150$, $V_{\text{funnel in}} = 90\text{V}$, $V_{\text{ramp}} = -175 - 20$, $V_{\text{out}} = 60\text{V}$, and a 250 Vpp at 880 kHz rf. A typical scan rate of $Sr = 1.95$ V/ms was used, or lower as needed to increase the mobility resolution. All voltages were controlled using custom software in LabView (National Instruments) synchronized with the MS platform controls. The data was segmented in LC frames over 10 analysis cycles yielding an LC-TIMS-TOF MS step size of ~2 s. The TIMS operation was controlled using in-house software, written in National Instruments Lab VIEW, and synchronized with the maXis Impact Q-ToF acquisition program [34].

Reduced mobility values (K_0) were correlated with collisional cross section (Ω) using the equation:

$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{z}{(k_B T)^{1/2}} \left[\frac{1}{m_i} + \frac{1}{m_b} \right]^{1/2} \frac{1}{K_0} \frac{1}{N^*} \quad (2)$$

where z is the charge of the ion, k_B is the Boltzmann constant, N^* is the number density of the bath gas, and m_i and m_b refer to the masses of the ion and bath gas, respectively [40]. LC-TIMS-TOF MS data were processed using Data Analysis software v. 5.0 (Bruker Daltonics Inc, Billerica, MA).

Theoretical calculations

A pool of candidate structures was proposed for all molecules of interest. Final structures were optimized at the DFT/B3LYP/6-311G(d,p) level using Gaussian software [41]. Vibrational frequencies were calculated to guarantee that the optimized structures correspond to actual minima in the energy space, and zero-point energy corrections were applied to calculate the relative stability between the structures. Theoretical ion-neutral collision cross sections were calculated using MOBCAL [42, 43] software for nitrogen as a bath gas at ca. 300K. Partial atomic charges were calculated using the Merz-Singh-Kollman scheme constrained to the molecular dipole moment [44, 45].

Results and Discussion

Ion mobility profiles of isomeric opioid compounds (6-acetylmorphine (6-AM) and naloxone; codeine and hydrocodone; morphine, hydromorphone, norcodeine and norhydrocodone; and their respective deuterated analogs) show a single band for each of the protonated molecules $[M+H]^+$ (Figure 1) with small differences in ion-neutral collision cross section values in nitrogen ($^{TIMS}CCS_{N_2}$): 6-AM and naloxone (176.7 and 171.1 \AA^2 , ~3%), codeine and hydrocodone (168.2 and 167.8 \AA^2 , <1%) and morphine, hydromorphone, norcodeine, and norhydrocodone (162.9, 163.2, 167.9 and 167.4 \AA^2 , <1-3%) (see Table 1). These CCS values agree (Table 1) with theoretically calculated CCS (<5%) and previous studies that measured reduced mobilities using drift tube ion mobility spectrometry (DT-IMS) [18, 24, 25, 27, 32, 46]. Upon review of the proposed candidate structures, visual similarities and differences in the size and shape, and, therefore, the theoretical CCS, are observed between opioid isomers (Figure 2). For example, major differences in the orientation of the nitrogen group as well as the methyl group on the oxygen atom are observed between 6-AM and naloxone (as highlighted in Figure 2). These differences are also observed in the measured experimental and theoretical CCS, which allow isomer separation, even at fast scanning rates (Table 1 and Figure 1). The candidate structures of codeine and hydrocodone, vary by the presence or absence of a carbonyl group on a six-membered ring. This difference results in minimal changes in size; that is, the CCS values only slightly differ from each other (Figure 2). Morphine,

hydromorphone, norcodeine and norhydrocodone differ in structure at the nitrogen, depending on whether a secondary (norcodeine and norhydrocodone) or tertiary amine (morphine and hydromorphone) is present in the compound. The difference in orientation of the amine group alters the theoretically calculated and experimentally measured CCS (Figure 2). Specifically, the similar amine group orientations of morphine and hydromorphone mean that the compounds cannot be separated based on CCS. Conversely, morphine/norcodeine and hydromorphone/norhydrocodone have different amine orientations can be baseline separated in their mobility profiles (see Figures 2 and 3).

While mobility separation was observed using fast scan rates ($Sr = 0.5-1.5$ V/ms); it is noteworthy that baseline mobility separations are observed between 6-AM and naloxone, hydromorphone and norhydrocodone and morphine and norcodeine using slower scan rates ($Sr = 0.2$ V/ms) with resolving power in excess of 100 (see Figure 3). The ability to obtain baseline separation between these isomeric opioids can be attributed to the size and shape of the individual compounds, based the reported candidate structures (Figure 2). Previous mobility analyses using drift tube IMS report resolving powers of about 70 for codeine and morphine, which are not isomers [30]. Despite the high resolving power of the TIMS analyzer, complete separation for all the isomers considered was not obtained (e.g., codeine and hydrocodone, morphine and hydromorphone, nor norcodeine and norhydrocodone), due to the marginal structural differences leading to minimal variations in CCS between these isomers ($<1 \text{ \AA}^2$). Isomeric opioids that have previously separated include: hydromorphone, morphine and norhydrocodone, via field asymmetric ion mobility spectrometry (FAIMS) MRM-MS [28] and codeine and hydrocodone using a modified differential mobility spectrometry (DMS) cell [47].

The influence of matrix effects on the “dilute and shoot” LC-TIMS-MS workflow was studied by comparing the separation of opioid standards in water and in human urine. Inspection of the 2D-IMS-MS plots show a single trendline, containing the opioids as well as other potential interferences from the urine sample. Closer inspection of the opioid region reveals the separation of the opioid signals; however, potential molecular interferences from the urine may lead to higher limits of detection when compared to

other IMS-MS-based DTM methods where the compounds of interest fall in a different trendline (data not shown) [37]. Moreover, the added advantage of liquid chromatography as a third dimension of separation allows for a clear separation of the potential matrix interferants as well as the separation of isomeric analytes that were not possible by TIMS-MS alone (Figure 4). The chromatographic program in this research had a final separation time of 12 min which is comparable to the reported LC-MRM times (e.g., 6-35 min) for opioid analysis [14]. Notice that the IS can be easily identified since they share the same retention time and CCS as their corresponding analyte. For example, naloxone and 6-AM can be separated by TIMS and by LC (retention times of 6.85 and 7.00 min, respectively). For quantification purposes, while the potential targets for naloxone and 6-AM isomers will have the same mass value, the IS of choice have different levels of deuteration so that they can be easily separated in the MS domain. That is, naloxone shows peaks at m/z 328.1542 and 333.1857 corresponding to the $[M+H]^+$ of the analyte and the IS $[M(D_5)+H]^+$ containing five deuterium atoms. The mass spectrum for 6-AM contains two main peaks at m/z 328.1542 and 331.1730, corresponding to the analyte $[M+H]^+$ and the IS $[M(D_3)+H]^+$ with three deuterium atoms (Figure 4a). Codeine and hydrocodone are not separated in the mobility domain, yet there is near-baseline separation in the LC (6.8 and 7.0 minutes, respectively) (Figure 4b). Analogous to the naloxone and 6-AM quantification, the IS for codeine and hydrocodone are chosen with different amounts of deuterium so that they can be easily separated in the MS domain. Norcodeine and norhydrocodone are not separated in the mobility domain, yet there is near-baseline separation in the LC (6.9 and 7.0 min, respectively) (Figure 4c).

Limits of detection (LODs) were compared between traditional two-dimensional separation (e.g., LC-TOF MS) and the currently proposed three-dimensional separation (e.g., LC-TIMS-TOF MS) for rapid and robust analysis of drugs of abuse and their metabolites. The LC-TOF MS and LC-TIMS-TOF MS results are summarized in Table 2; noteworthy are the LC-TIMS-TOF MS LODs for the common opioids in human urine: 1.4-31.2 ng/mL using a DTM method. These results compare to reported LODs of 0.6-2.5 ng/mL with 4-160 ng/mL linearity range using various extraction methods with MRM [14, 48-

50]. An increase in the LODs was generally observed in the presence of human urine which is consistent with increased background levels and/or decreased ionization yields associated with matrix effects. The limits of quantitation (LOQs) range from 30.2-156 ng/mL which are in agreement with reported LOQs of 0.1-126 ng/mL from single reaction monitoring (SRM) and MRM approaches [14, 48-50].

Evaluation of reproducibility and effect of chemical environment for three identification parameters (CCS, RT and m/z) is illustrated across the calibration levels analyzed (Figure 5). In the CCS domain, marginal deviations were observed between samples with and without urine (relative percent deviation, RPD, <0.5%). Additionally, CCS values did not change across calibration levels, suggesting that CCS is a valid parameter for analyte identification in the tested range and that this parameter could be a valuable addition to the traditionally used for qualitative analysis such as retention time (RT) and, when possible, accurate mass. In this case, RTs were minimally affected in the presence of urine (RPD of samples analyzed in urine compared to water were below 0.5%) and a high mass accuracy (<1 ppm) was observed for all analytes across calibration levels in the presence of urine. In addition, intra-day reproducibility is shown by small (<0.25%) percent relative standard (%RSD) for individual analytes in water and human urine across the seven calibration points (Table 3). These results demonstrate the reliability of this methodology for identifications in multiple dimensions using LC-TIMS-MS for quantitative analyses at the low ng/mL levels. During the performance of the matrix effect experiments, no significant differences in the matrix factor (MF) of ten individual urine samples were observed for morphine, norhydromorphone, norcodeine, norhydrocodone, codeine and hydrocodone spiked at high (400 ng/mL) and low (75 ng/mL) concentrations (coefficient of variance, CV>15%) (See Figure S2).

Conclusions

For the first time, liquid chromatography, trapped ion mobility spectrometry and mass spectrometry were combined for fast separation, identification and quantitation of opioids and their metabolites in human

urine using a “dilute and shoot” approach. The proposed workflow provides analytical separation in the mobility and chromatographic domains within a 12 min analysis time, with LODs of 1.4 - 35.2 ng/mL with 0.5-500 ng/mL linearity range using DTM of opioids in urine. A good agreement was observed between the previously reported $^{DTIMS}CCS$, measured ^{TIMS}CCS , and the theoretical CCS of the candidate structures for the familiar opioids optimized at the DFT/B3LYP level. Beside the higher confidence during LC-TIMS-TOF MS analyses, similar LODs and LOQs are reported to those obtained using traditional LC-MRM measurements, with small relative percent deviations in retention times (<0.3%), and collision cross sections (<0.6%) and high mass accuracy (<1ppm). The need for complementary liquid and mobility separations prior to mass analysis is shown for the analysis of complex mixtures, with a two-fold increase in mobility resolving power ($R \sim 80-130$) compared to previous reports using DT-IMS ($R \sim 50-70$).

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Fig 1: Typical mobility profiles of analytes and their corresponding internal standards

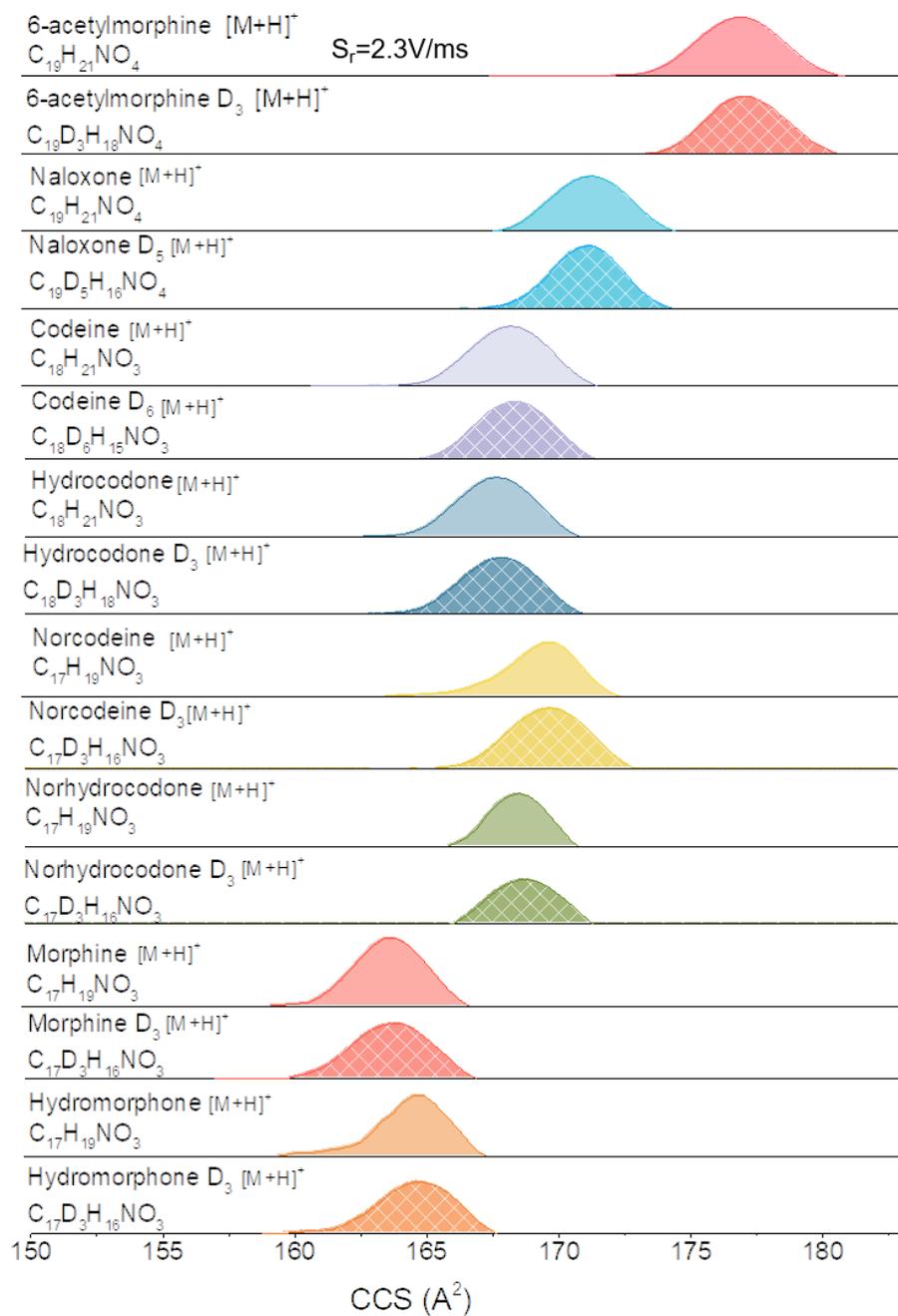
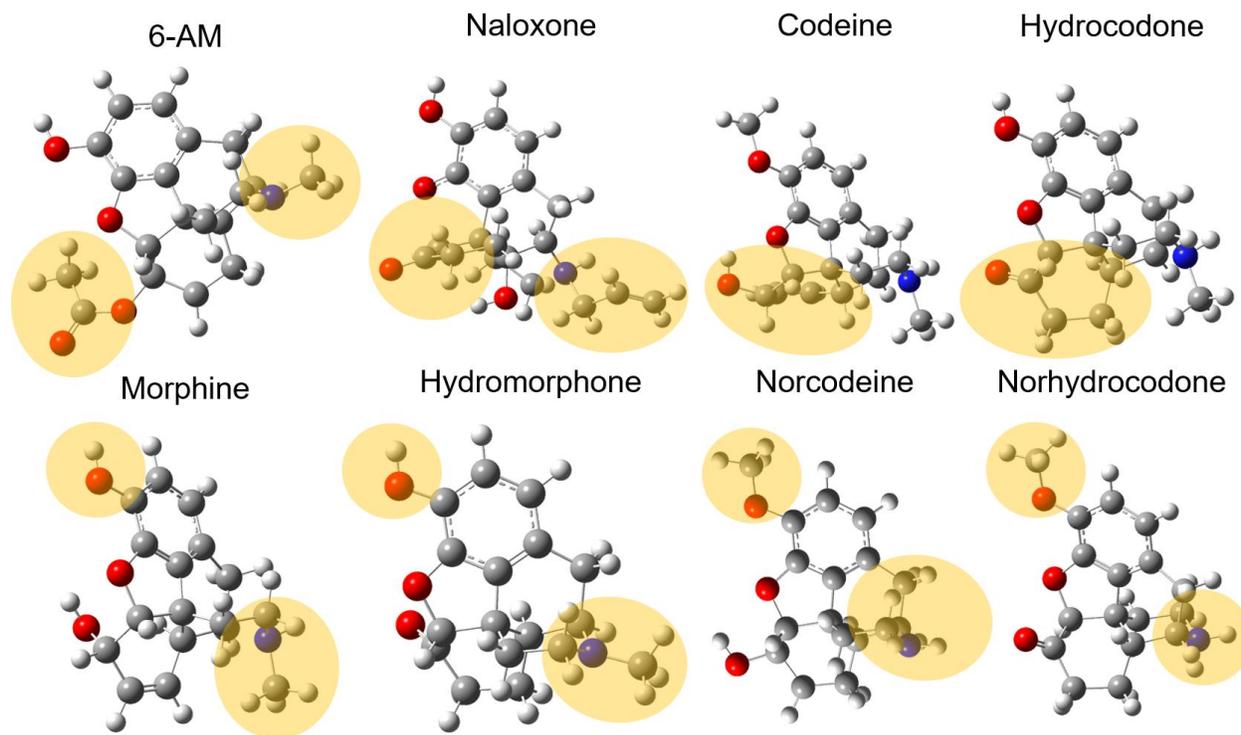


Fig 2: Candidate structures optimized at the DFT/B3LYP/6-311G(d,p) of the opioids considered



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Fig 3: Typical IMS separations of binary mixtures: top) 6-acetylmorphine and naloxone; middle) hydromorphone and norhydrocodone; bottom) morphine and norcodeine

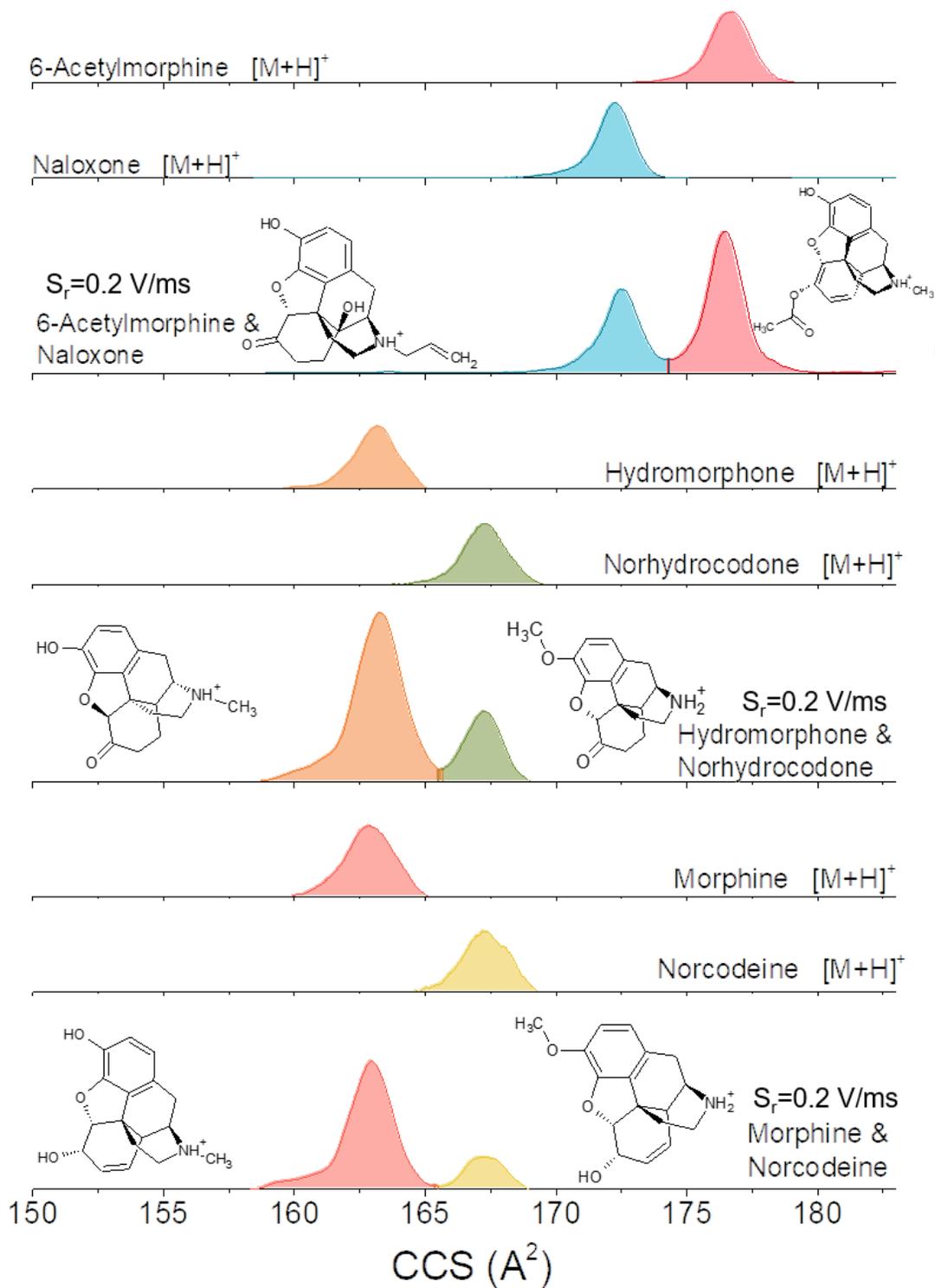


Fig 4: Typical LC-TIMS-TOF MS analysis of isomeric opioids. 2D-IMS-MS contour plots are shown for the highlighted LC bands

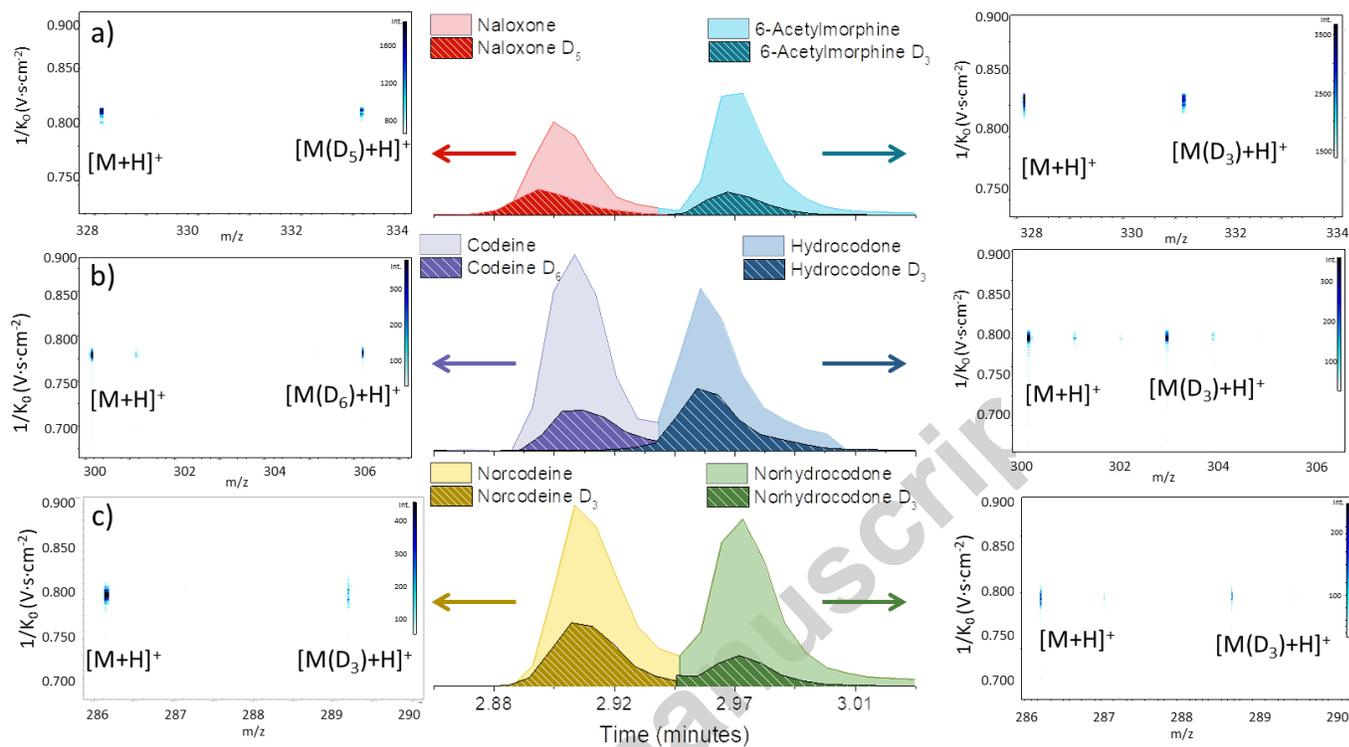
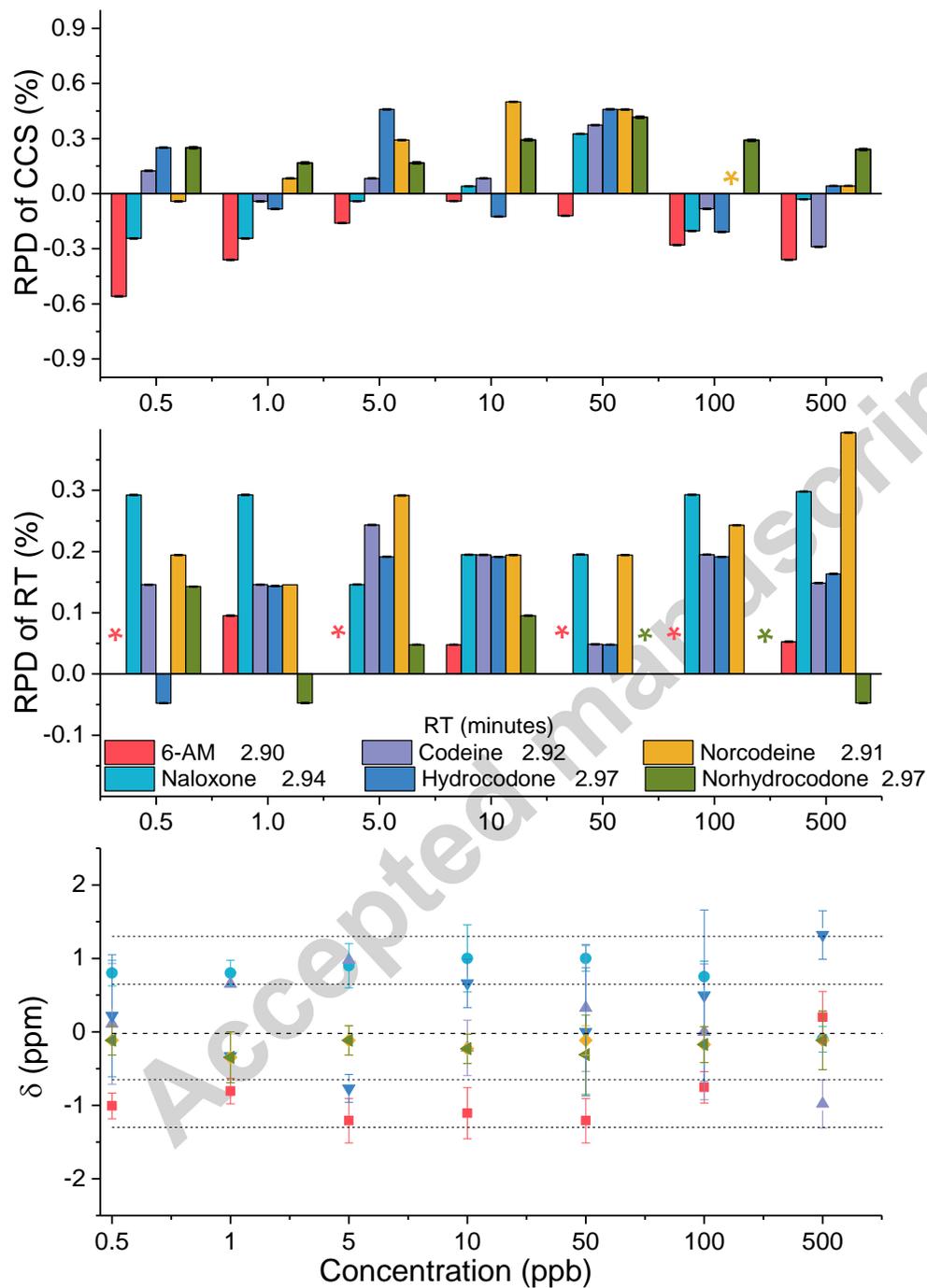


Fig 5: Relative percent deviation of RT, CCS compared to non-matrix sample and δ m/z across calibration levels (*= no change)



Graphical Abstract

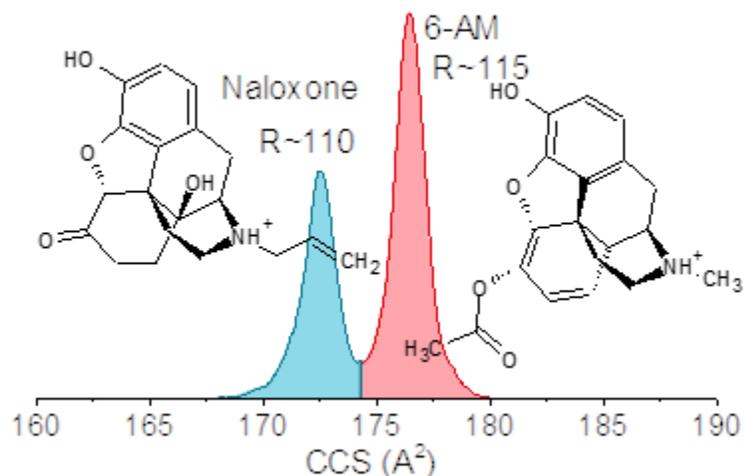


Table 1: Experimental and theoretical m/z and CCS values for the opioid analytes considered. Note: values in parentheses refer to previously reported data from DT-IMS_{Air} [18, 24, 26, 44-46]

Name	Chemical Formula	Theoretical m/z $[M+H]^+$	Experimental m/z $[M+H]^+$	Error (ppm)	Theoretical CCS (\AA^2)	Experimental TIMS CCS _{N₂} (\AA^2)	Experimental K_0 ($\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$)
6-Acetylmorphine	C ₁₉ H ₂₁ NO ₄	328.1543	328.1545	0.609	166.2	176.7 (167-171.1)	1.182
6-Acetylmorphine-D3	C ₁₉ H ₁₈ D ₃ NO ₄	331.1732	331.1733	0.302	166.3	176.9	1.189
Naloxone	C ₁₉ H ₂₁ NO ₄	328.1543	328.1542	0.305	166.7	171.1	1.221
Naloxone-D5	C ₁₉ H ₁₆ D ₅ NO ₄	333.1857	333.1855	0.600	166.6	171.0	1.229
Codeine	C ₁₈ H ₂₁ NO ₃	300.1594	300.1596	0.600	171.6	168.2 (168.9-178.9)	1.268
Codeine-D6	C ₁₈ H ₁₈ D ₆ NO ₃	306.1971	306.1969	0.653	171.7	168.0	1.256
Hydrocodone	C ₁₈ H ₂₁ NO ₃	300.1594	300.1592	0.666	171.8	167.8	1.271
Hydrocodone-D3	C ₁₈ H ₁₈ D ₃ NO ₃	303.1782	303.1783	0.330	171.7	167.9	1.257
Morphine	C ₁₇ H ₁₉ NO ₃	286.1438	286.1437	0.349	162.6	162.9 (172.8-189.0)	1.290
Morphine-D3	C ₁₇ H ₁₆ D ₃ NO ₃	289.1626	289.1625	0.346	162.4	164.0	1.289
Hydromorphone	C ₁₇ H ₁₉ NO ₃	286.1438	286.1437	0.349	161.6	163.2 (160.3)	1.287
Hydromorphone-D3	C ₁₇ H ₁₆ D ₃ NO ₃	289.1626	289.1625	0.692	161.5	164.4	1.286
Norcodeine	C ₁₇ H ₁₉ NO ₃	286.1438	286.1440	0.699	168.8	167.9 (196.1)	1.252
Norcodeine-D3	C ₁₇ H ₁₆ D ₃ NO ₃	289.1626	289.1625	0.346	168.9	167.9	1.259
Norhydrocodone	C ₁₇ H ₁₉ NO ₃	286.1438	286.1438	0.000	168.9	167.4	1.256

Norhydrocodone-D3	$C_{17}H_{16}D_3NO_3$	289.1626	289.1625	0.692	168.9	168.0	1.259
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Table 2: Calibration results for analytes with (Matrix) and without urine (No Matrix) for LC-TIMS-qTOF MS and LC-qTOF MS

Analyte	LC-TIMS-qTOF MS						LC-qTOF MS					
	Water			Urine			Water			Urine		
	LOD (ng/mL)	LOQ (ng/mL)	R ²	LOD (ng/mL)	LOQ (ng/mL)	R ²	LOD (ng/mL)	LOQ (ng/mL)	R ²	LOD (ng/mL)	LOQ (ng/mL)	R ²
Codeine	2.0	10.4	0.994	9.9	49.6	0.996	1.4	6.9	0.997	3.0	15.0	0.994
Hydrocodone	3.0	15.1	0.994	6.0	30.2	0.996	1.8	9.1	0.997	7.6	38.2	0.995
Morphine	7.9	39.5	0.996	27.9	138.6	0.993	7.9	39.5	0.996	31.9	159.4	0.999
Norcodeine	8.3	41.6	0.997	31.2	156.0	0.999	7.4	37.3	0.997	35.2	176.0	0.999
Norhydrocodone	8.1	40.4	0.995	29.1	145.8	0.996	8.1	40.7	0.996	20.7	103.5	0.996

Table 3: Intraday Variability of CCS and RT with and without urine represented by percent relative standard deviation (%RSD)

Intraday Variability	RT (% RSD)		CCS (% RSD)	
	Water	Urine	Water	Urine
6-Acetylmorphine	0.07	0.04	0.18	0.22
Naloxone	0.12	0.12	0.19	0.23
Codeine	0.08	0.10	0.19	0.18
Hydrocodone	0.08	0.10	0.22	0.27
Norcodeine	0.09	0.07	0.22	0.21
Norhydrocodone	0.05	0.07	0.20	0.22

Highlights

- Complementary LC, TIMS and MS for DTM of isomeric opioids and metabolites
- LOD: 1.5-35 ng/mL with linearity up to 500 ng/mL
- High precision RT (<0.3%), CCS (<0.6%) and mass accuracy (<1 ppm)
- Mobility separation between isomeric opioids with high resolving power (R~80-130)
- Higher confidence in ID compared to traditional LC-MRM