Performance of the LTQ-Orbitrap mass analyzer for qualitative and quantitative analysis of drugs of abuse and relevant metabolites in sewage water

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ABSTRACT

This work illustrates the potential of liquid chromatography coupled to a hybrid linear ion trap Fourier Transform Orbitrap mass spectrometer for the simultaneous identification and quantification of 24 drugs of abuse and relevant metabolites in sewage water. The developed methodology consisted of automatic solid-phase extraction using Oasis HLB cartridges, chromatographic separation of the targeted drugs, full-scan accurate mass data acquisition under positive electrospray ionization mode over an *m/z* range of 50 - 600 Da at a resolution of 30,000 FWHM and simultaneous MS^n measurements to obtain information of fragment ions generated in the linear ion trap. Accurate mass of the protonated molecule, together with at least one nominal mass product ion and retention time allowed the confident identification of the compounds detected in these complex matrices. In addition to the highly reliable qualitative analysis, Orbitrap analyzer also proved to have satisfactory potential for quantification at sub-ppb analyte levels, a possibility that has been very little explored in the literature until now. The limits of quantification ranged from 4 to 68 ng L^{-1} in influent sewage water, and from 2 to 35 ng L^{-1} in effluent, with the exception of MDA, morphine and THC that presented higher values as a consequence of the high ionization suppression in this type of samples. Satisfactory recoveries (70 – 120%) and precision (< 30%) for the overall procedure were obtained for all compounds with the exception of *meta*-chlorophenylpiperazine, methylphenidate and ketamine. Isotope-labelled internal standards were added to sewage samples as surrogates in order to correct for matrix effects and also for possible losses during sample treatment. The methodology developed was applied to sewage water samples from the Netherlands (influent and effluent), and the results compared with those obtained by LC-MS/MS with triple quadrupole. Several drugs of abuse could be identified and quantified, mainly MDMA, benzoylecgonine, codeine, oxazepam and temazepam. Orbitrap also showed potential for retrospective investigation of ketamine metabolites in the samples without the need of additional analysis.

Keywords

Drugs of abuse, accurate mass, Orbitrap analyzer, high resolution mass spectrometry, quantitative analysis, sewage water.

1. INTRODUCTION

The presence of drugs of abuse, unaltered or as metabolites, in the water cycle has spurred researchers on to investigate their occurrence in sewage water, surface water and drinking water [1-3]. Although concentrations found are generally low (sub µg L^{-1} level), data obtained from analysis of urban wastewater can be used to study consumption and usage trends in communities [4]. Furthermore, environmental loads can be calculated, as their potential impact on aquatic organisms, human health and the environment may not be ruled out [1].

Most of the existing methods for determination of drugs of abuse in water are based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), using triple quadrupole (QqQ) analyzers. Despite its excellent sensitivity and selectivity , this approach also has some limitations [5, 6], the main being that other drugs, different from those included in the scope of the method, may be ignored in analyses, as analyte specific information is acquired and only the target analytes are normally detected and quantified. The increasing interest of using accurate mass High Resolution Mass Spectrometers (HRMS), e.g. Orbitrap and time-of-flight (TOF) instruments, in environmental sciences relies on its capability to perform both targeted as well as non-targeted analysis, based on full-spectrum accurate-mass acquisition at good sensitivity [7]. Efficient screening strategies using HRMS have allowed the detection and identification of various drugs of abuse in environmental and wastewater samples, in some cases even without the need of reference standards, but with high confidence due to the high mass accuracy measurements [8, 9].

Advantages of HRMS are widely recognized in qualitative analysis; however HRMS-based quantitative analyses have hardly been explored in the scientific literature until now. One of the classical criticisms concerns the relative low sensitivity and low linear dynamic range. This limitation was more evident in the first-generation instruments, e.g. first LC-TOF MS. However, the improved technology of the latest TOF instruments, i.e. higher sensitivity and resolving power, and wider linear dynamic range, provides better quantitative capabilities. This has allowed quantification of pesticides, pharmaceuticals and illicit drugs in wastewater by using LC-TOF MS [10, 11]. As for Orbitrap instruments, good quantitative performances, i.e. high sensitivity and selectivity, have been demonstrated in some applied fields [5, 12-15].

Nevertheless, to the best of our knowledge, the quantitative potential of Orbitrap has not been previously demonstrated for drugs of abuse in sewage water samples. The

determination of these compounds is complicated due to the complexity of the samples and low analyte concentrations. Sample pre-concentration is normally required, mostly based on solid phase extraction (SPE), but the key point is the quantification of analytes, which is problematic in LC-MS based methods due to the strong matrix effects commonly observed for this type of sample matrices. The use of isotope-labeled internal standards (ILIS) is the approach most frequently applied to solve this problem, although its application is difficult in multi-residue multiclass methods where a large number of ILIS would be required. Typically the own analyte ILIS is used, as the use of analogue compounds as internal standards does not always ensure an appropriate correction [10, 16].

In the present work, analytical methodology based on the use of SPE followed by LC coupled to a hybrid linear ion trap (LTQ) Fourier Transform (FT) Orbitrap MS, has been developed for the determination of 24 drugs of abuse and metabolites in urban wastewater. The acquisition of full-scan accurate-mass data by Orbitrap together with the simultaneous MS/MS measurements permitted by LTQ is a powerful combination for confident identification and confirmation. As the excellent qualitative potential of Orbitrap analyzer is widely accepted in the recent literature, an additional and relevant objective was to demonstrate the quantitative capabilities of this HRMS, a feature that has been little explored until now. To the best of our knowledge, the quantitative potential of Orbitrap has not been previously demonstrated for drugs of abuse in complex sewage water samples.

2. MATERIALS AND METHODS

2.1. Reagents

Drugs of abuse and metabolites reference standards: amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylene-dioxymethamphetamine (MDMA, or ecstasy), 3,4-methylenedioxyethylamphetamine (MDEA), cocaine, benzoylecgonine, heroin, morphine, 6-monoacetyl morphine (6-MAM), methadone, codeine, Δ-9-tetrahydrocannabinol (THC), 11-nor-9-carboxy-Δ-9-tetrahydrocannabinol (THC-COOH), 11-hydroxy-Δ-9-tetrahydrocannabinol (OH-THC), ketamine, methylphenidate, oxazepam, diazepam, temazepam, nordazepam, desalkyl-flurazepam, *meta*-chlorophenylpiperazine (mCPP), and fentanyl were obtained from Lipomed AG (Arlesheim, Switzerland) as solutions in methanol (MeOH), ethanol (EtOH) or acetonitrile (ACN) at a concentration of 1 g L^{-1}. Standard solutions of each compound were prepared at 36 mg L^{-1} in MeOH. A final mix solution was made by diluting aliquots from every compound individually to a concentration of 3.6 mg L^{-1}. Working mix solutions for calibration curves were made in MeOH. Before each analytical run, the calibration standards were diluted 10 times with ultrapure water resulting in a mix of water: MeOH (90:10 v/v) and were injected into the Orbitrap system. Final concentrations of standards ranged from: 0.7 to 288 µg L^{-1}.

Deuterated compounds were purchased from Lipomed AG as solutions in MeOH, EtOH or ACN at a concentration of 1 g L^{-1} and were used as surrogate isotope labelled internal standards (ILIS) for quantification: amphetamine-d_{11}, methamphetamine-d_5, 3,4-methylenedioxyamphetamine-d_2 (MDA-d_2), 3,4-methylenedioxymethamphetamine-d_5 (MDMA-d_5), 3,4-methylenedioxyethyl-amphetamine-d_5 (MDEA-d_5), cocaine-d_3, benzoylecgonine-d_3, morphine-d_3, 6-monoacetyl morphine-d_3 (6-MAM-d_3), Δ^9-tetrahydrocannabinol-d_3 (THC-d_3), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol-d_3 (THC-COOH-d_3), 11-hydroxy-Δ^9-tetrahydrocannabinol-d_3 (OH-THC-d_3), oxazepam-d_5, diazepam-d_5, nordazepam-d_5. A mixed ILIS working solution was prepared in MeOH and added to all calibration standards to get a final ILIS concentration of 72 µg L^{-1}, as well as to the influent and effluent sewage water samples prior to sample treatment (final ILIS concentration in sample of 360 ng L^{-1} and 180 ng L^{-1}, respectively). All standard and working solutions were stored in amber glass bottles at -18 °C.

The ultrapure water was obtained by purifying demineralized water in a Milli-Q system from Millipore (Bedford, MA, USA). Formic acid (98 – 100%), HPLC-grade MeOH, EtOH and ACN were acquired from Mallinckrodt Baker (Deventer, The Netherlands).

Glass fibre filters (1 µm, type A/E) were purchased from Pall Corporation (Port Washington, NY, USA). Polyethersulfone filters (0.45 µm) with disposable setup were acquired from Nalgene (Rochester, NY, USA).

SPE cartridges, built of a hydrophilic and a lipophilic monomer (Oasis-HLB; 6 mL, 150 mg) were purchased from Waters (Milford, MA, USA).

Polytyrosine-1,3,6 standard used for mass axis calibration was purchased from Cs Bio Co. (Menlo Park, CA, USA).

2.2. Water samples

24-hours flow dependent influent and effluent composite-samples from different sewage treatment plants (STPs) located in the Netherlands were taken on the same weekend day, without accounting for lag-time. Samples were collected in amber glass bottles, and stored in the dark at 4 °C. Upon reception in the laboratory, the samples were immediately analysed.

2.3. Extraction procedure

Prior to SPE, samples were vacuum filtered through 1 µm type A/E glass fibre filters, followed by 0.45 µm polyethersulfone (PES) filters with disposable setup. Subsequently, 200 mL of effluent sewage water, or 100 mL of influent sewage water sample, were spiked with a mixed internal standard solution to give a concentration for each compound in sample of 180 ng L^{-1} and 360 ng L^{-1}, respectively. SPE was performed automatically using a GX-274 ASPEC (Gilson). Oasis HLB cartridges were conditioned by washing and rinsing with 8 mL of ACN, 8 mL of MeOH and 8 mL of Milli-Q water. Samples were loaded onto the cartridges at 5 mL min^{-1}, and then cartridges were washed with 8 mL of Milli-Q water and dried with nitrogen for 15 min at a pressure of 1 bar. Analytes were eluted using 8 mL of MeOH at a flow of 0.5 mL min^{-1}.

The SPE eluates (MeOH) were evaporated to 200 µL at 35°C under a gentle stream of nitrogen. Then, 250 µL of Milli-Q water was added and the remaining MeOH (200 µL) evaporated. Evaporation of the extracts was performed automatically using Barkey optocontrol (Germany). The final extract was then made up, by weight, to exactly 250 µL with Milli-Q water. As a final step, the volume was adjusted to 500 µL, by weight, with

water:MeOH (80:20 v/v) to achieve a final percentage of 10% MeOH. An aliquot of the sample extract (20 µL) was injected directly into the LC-LTQ FT Orbitrap system.

2.4. Liquid Chromatography

A hybrid linear ion trap Fourier Transform (LTQ FT) Orbitrap mass spectrometer was interfaced to a Surveyor HPLC system, consisting of a Surveyor auto sampler model Plus and a Surveyor quaternary gradient HPLC-pump (Thermo Fisher Scientific, Breda, The Netherlands). Chromatographic separation of the compounds was made using an XBridge C_{18} column (150 mm x 2.1 mm I.D., particle size 3.5 µm) (Waters). The pre-column used was a 4.0 x 2.0 mm I.D. Phenomenex Security Guard column (Bester, Amsterdam, the Netherlands). The analytical column and the guard column were maintained at a temperature of 21 °C in a column thermostat. An optimized gradient was used at a constant flow rate of 0.3 mL min^{-1} using Milli-Q water (Solvent A) and MeOH (Solvent B) both with 0.05% formic acid. The percentage of organic modifier (B) was changed linearly as follows: 0 min, 5%; 20 min, 100%; 30 min, 100%; 32 min, 5%. Between consecutive runs, the analytical column was re-equilibrated for 10 min.

2.5. LTQ FT Orbitrap mass spectrometry

An LTQ FT Orbitrap mass spectrometer (Thermo Electron, Bremen, Germany) was used. The LTQ part of this system was equipped with an Ion Max Electrospray Ionization (ESI) probe and operated in the positive ion mode. The conditions in ESI positive mode were: source voltage 4.0 kV, heated capillary temperature 300 °C, capillary voltage 30 V and tube lens 45 V. In the LTQ component of the instrument, the temperature was set to 26 °C and helium was used as damping gas. All measurements were done using the automatic gain control (AGC) of the LTQ to adjust the number of ions entering the trap. Products ions were generated in the LTQ trap at a normalized collision energy setting of 40% and using an isolation width of 2 Da.

Full-scan accurate mass spectra (mass range from 50 to 600 Da) were obtained at a mass resolution of 30,000 FWHM (*m/z* 400). The total cycle time depends upon the resolution; at the selected resolution the total cycle time is about 0.55 s. The mass spectrometer operated under data-dependent-acquisition (DDA) mode during the complete chromatographic run, in which both MS and MS^n spectra were acquired simultaneously. The instrument was initially set to operate in full-scan ('survey') mode with accurate mass measurements. When an ion exceeded a preset threshold and corresponded to the target mass

list specified by the user, the instrument switched to product-ion scan mode (MS^n) in the ion-trap part with nominal mass measurements. In this way, relevant information for identification and confirmation, e.g. retention time, molecular weight and fragmentation, was obtained in a single analysis. All data were acquired and processed using Xcalibur version 2.1 software.

Mass calibration was performed with every batch run just prior to starting the batch by using flow injection of a Polytyrosine-1,3,6 solution ($[M+H]^+$ 182.01170 / 508.20783 and 997.39781) at a flow rate of 10 µL min^{-1}.

Identification and quantification of target compounds was performed using the accurate mass of the protonated molecule within a mass window of 5 ppm. For confirmation of the identity of the compounds, in addition to the accurate mass of the precursor ion, at least one nominal mass product ion was used together with retention time, which was compared with that of the reference standards (within 2.5%) [17, 18].

2.6. Method validation

The performance of the method was evaluated in terms of linearity, limits of quantification, trueness and precision. The overall recovery (including sample treatment and potential matrix effects) was studied and evaluated.

Instrumental linearity was estimated by analyzing standard solutions in triplicate. Satisfactory linearity was assumed when the coefficient of determination (r^2) was > 0.99, based on analyte/internal standard peak areas, except for those compounds that were quantified without ILIS (absolute response).

Limit of quantification (LOQ): To facilitate the fourier transformation of the acquired frequency data and conversion to *m/z* in the orbitrap, noise is filtered out. This is why the common approaches to evaluate the limits of quantification do not apply [19]. Therefore, a different approach was applied as previously reported by de Voogt *et al.* [20]. It is based on the matrix suppression of the deuterated analogue and the identification criteria [18] to reach enough identification points. The matrix effect (expressed as defined by Matuszewski *et al.* [21]) is calculated by using the area of the accurate mass signal of the deuterated standard, spiked before extraction (in matrix), divided by the average area of the deuterated standard in the calibration curve (in solvent). By using the lowest standard visible in the calibration curve which meets all the identification criteria (typically the absence/presence of the confirmation product ion is the critical parameter) and corrected for the matrix suppression and the concentration factor, the LOQ can be determined.

For those analytes for which deuterated analogues were unavailable, either the closest deuterated structure, the deuterated analyte with a similar polarity or the closest eluting compound was selected for correction. If none of the above was feasible, the LOQ was calculated based on the lowest point of the calibration line and translated to concentration in sample, taking into account the pre-concentration factors.

Trueness (estimated by means of recovery experiments) and *precision* (expressed as repeatability in terms of coefficients of variation) was evaluated by analyzing influent and effluent wastewater samples spiked at 360 ng L^{-1} and 180 ng L^{-1}, respectively.

3. RESULTS AND DISCUSSION

3.1. Sample treatment.

Optimization of the extraction process and evaluation of matrix effects were made using analyte ILIS, except for some compounds of which internal standards were not available. It is expected that ILIS are affected by potential losses associated to the sample treatment and by matrix effects in the same way as the analyte [22]. An advantage of using ILIS for this evaluation is that they are not present in sewage water samples, since getting a representative genuine blank sample is one of the main difficulties for researchers working in this area.

In search for an optimum matrix effect/pre-concentration factor ratio, different volumes of wastewater sample (900 mL, 600 mL, 300 mL and 100 mL) were evaluated **Figure 1** demonstrates the influence of intake volume on matrix suppression in a typical influent. To prevent the SPE material from clogging, samples were filtered prior to SPE through a 0.45 µm polyethersulfone filter. All experiments were performed in triplicate. A satisfactory compromise for ion suppression/enhancement versus pre-concentration factor was found by loading the cartridges with 100 mL of influent or 200 mL of effluent sewage water.

A comparison between SPE carried out manually and automatically was made. Although recoveries were slightly lower (around 5%), automated SPE using a GX-274 ASPEC (Gilson) was preferred over the more time-consuming manual SPE.

3.2. LC-MS conditions

The most relevant parameters selected for the measurement of each analyte are shown in **Table 1**. All analytes were measured in positive mode, and the precursor ion selected was in all cases $[M+H]^+$. The instrumental configuration used in this article allows accurate mass measurement of the precursor ions in full-scan mode in the Orbitrap and, based on response thresholds, simultaneously nominal mass product ions scan in the iontrap. The exact masses of precursor ions, together with the product ions selected, measured in nominal mass, are also shown in **Table 1**. It is worth mentioning the difference with QTOF instruments where parallel acquisition cannot be performed.

Acceptable chromatographic separation of the selected drugs of abuse was achieved, with the exception of methylphenidate/mCCP, but this couple did not pose any problem for identification because of their *m/z* difference. Under the chromatographic conditions selected,

the analytes retention times varied from 4.71 min (morphine) to 26.26 min (THC) (**Table 1**). A satisfactory compromise between mass resolving power and chromatographic peak shape was obtained when mass resolution was set at 30,000 FWHM with a total cycle time of about 0.55 s (including the simultaneous MS/MS acquisition in the iontrap). Accordingly, the number of data points (i.e. accurate mass scans) across each peak was at least ten and the concept of HRMS instruments (resolution ≥ 20,000 FWHM and mass accuracy ≤ 5 ppm) is maintained. The instrumental LOQs were in the 14-144 pg range (**Table 2**) except for THC and MDA (720 pg) they compare favorably to that of QTOF instruments (10-100pg) [11] but they are considerably higher than those reported on UPLC-QqQ-MS/MS instruments but on the same order of magnitude of those achieved with a standard LC-QqQ-MS/MS system (12-530 pg) [11, 22].

3.3. Method validation

The instrumental linearity was studied by analyzing standard solutions in triplicate at nine concentrations in the range from 0.7 to 288 µg L^{-1} (this would be equivalent to 3.6 – 1440 ng L^{-1} or to 1.8 – 720 ng L^{-1} in influent and effluent sewage water, respectively, taking into account the pre-concentration factor). The r^2 ranged satisfactorily between 0.991 and 0.999 (**Table 2**).

The matrix effects of influents and effluents were evaluated by analyzing samples from 5 different STPs. **Figure 2** shows that in general a suppression of the signal can be observed in both influents and effluents. The matrix effects range from moderate suppression (oxazepam) to almost complete suppression (THC). Influents exhibit in general stronger suppression than effluent, despite the fact that sample intakes were twice as low.

A graph showing the mass deviation of the protonated ions of selected deuterated analytes in relation to the theoretical mass over a period of 55h is presented in **Figure 3**. In contrary to QTOF systems [9, 11] a reference solution for continuous calibration of the mass axis was not necessary as mass accuracy remained within a deviation of 5 ppm over the whole period, illustrating the mass stability of the Orbitrap analyzer during validation and analysis.

In general, LOQs varied between 4 and 68 ng L^{-1} in influent sewage water and between 2 and 35 ng L^{-1} in effluent sewage water (**Table 2**). As previously described, theoretical LOQs can be calculated using the lowest calibration standard divided by the matrix suppression. For MDA, morphine and THC high suppression resulted in to high theoretical LOQs (influents: 439, 486 and 29221 ng L^{-1}, respectively; effluents THC: 5747 ng L^{-1}). However in practice, concentrations lower than these theoretical LOQs could be

satisfactorily quantified. For these compounds, the concentrations in spiked samples that were used for method validation were used as LOQs (360 ng L^{-1} for all three analytes in influent and 180 ng L^{-1} for THC in effluent). Analyte- or analogue-ILIS were used for all drugs of abuse as shown in **Table 1**, with the exception of heroine, methadone, ketamine, methylphenidate and mCCP, for which no appropriate ILIS was found.

Recoveries, calculated from relative responses analyte/ILIS, were tested for influent and effluent samples spiked at 360 ng L^{-1} and 180 ng L^{-1}, respectively, which led to a concentration of 72 µg L^{-1} in the final extract (**Table 2)**. Relative recoveries for most of the drugs in each matrix were between 70 and 120 %, with the exception of mCCP (influent, 52%; effluent, 62%), methylphenidate (influent, 45%; effluent, 65%) and ketamine (influent, 48%). Precision was < 30% for heroin, methadone, codeine, ketamine, methylphenidate, desalkyl-flurazepam, mCCP and fentanyl, for the remaining compounds it was ≤ 15% (**Table 2**). Best recoveries and precision were observed for those compounds that could be corrected by the use of ILIS, illustrating the importance of adequate matrix correction. For the 5 compounds of which no ILIS were available recovery data were obtained without ILIS correction, and were poorer as expected, ranging from 45% (methylphenidate in influent) to 81% (ketamine in effluent). In the analysis of sewage water samples (see next section), results for these 5 compounds were corrected using quality control (QC) recoveries, included in every sequence of analysis. The fortified sewage waters (QC) selected for correction were taken from the same STP during the same sampling period in order to minimize matrix composition differences owing to temporal variations or from different locations, obtaining a sample set with a more uniform matrix [23]. Although results always need to be critically evaluated, this approach was considered satisfactory in absence of appropriate ILIS, as the treatment procedure for samples and QCs (fortified sewage water) is the same and matrix effects are expected to be comparable for samples taken within the same period of sampling and within the same STP.

3.4. Application to sewage water samples

The strong potential of Orbitrap for identification purposes comes from its high resolution, as recently illustrated for different organic pollutants [6, 8, 24]. However, Orbitrap has been much less used for quantification purposes. The main objective of the present work was to demonstrate that a confident quantification can also be made by Orbitrap for drugs of abuse in sewage water in addition to its, already expected, excellent capabilities for identification/elucidation purposes.

The methodology developed was applied to sewage water samples collected from the Netherlands. The samples formed part of a pilot study on drugs of abuse in which two other laboratories also participated analyzing the same samples but applying different methods, all based on the use of LC-MS/MS with triple quadrupole. The analytical methodologies were in-house validated using ILIS for matrix effects and sample handling errors correction. The most frequently detected drugs were MDMA, benzoylecgonine, morphine, codeine, oxazepam and temazepam, which were present in at least 75% of influent and effluent sewage water samples. The highest drug levels were found in influent sewage water and corresponded to oxazepam (average concentration 1167 ng L^{-1}) and benzoylecgonine (average concentration 1703 ng L^{-1}), the main metabolite of cocaine, highlighting the widespread consumption of benzodiazepines and cocaine. Data from this study and more detailed information can be found elsewhere [25, 26].

The fact that samples were analyzed by different techniques allowed us to perform an additional validation step of the analytical method applied, a relevant aspect taking into account that LC-MS/MS with QqQ is the most widely applied technique for the determination of drugs of abuse in sewage water. This could be done for six target drugs that were included by all participants and were detected in several of the samples analyzed. **Table 3** shows the concentrations found in four of these samples, which have been taken as illustrative examples because they were positive for several analytes included in this work. In this table, data reported for two laboratories using LC-MS/MS QqQ are compared with our data using Orbitrap.

Data for amphetamine, MDMA and benzoylecgonine were, in general, in good agreement, with the exception of MDMA in influent sample 1 for QqQ 1, where the concentrations reported were notably lower than by QqQ 2 and Orbitrap. The overall (inbetween laboratories) deviation for these three analytes was $\leq$ 30%, with the above mentioned exception of MDMA in influent sample 1 and benzoylecgonine found at trace level in effluent sample 3. Few data were available for THC-COOH and ketamine. However, it can be noticed that Orbitrap was able to detect THC-COOH in the only sample that was positive by this compound (influent sample 1, QqQ 1), although it could not be quantified despite the concentration reported by QqQ was higher than the LOQ estimated for Orbitrap in influent. In relation to ketamine, in the effluent sample 4 where this compound was quantified by QqQ 2 (16 ng L^{-1}), the concentration reported by Orbitrap was about the same order (6 ng L^{-1}). Data for cocaine were less consistent, although it could be detected by Orbitrap in the two influent samples, and the level reported in the effluent 4 (8 ng L^{-1}) was about the same

order than that obtained by QqQ 1 (14 ng L^{-1}). More data are required in future monitoring to have a more realistic overview of the Orbitrap applicability to THC-COOH and cocaine analysis. In the time of writing this report, an intercomparison study between 13 laboratories have been made for several illicit drugs (including those mentioned above), which will give more light to this issue. These inter-laboratory comparison data will be the subject of a specific publication in the very near future. In addition to the six common compounds monitored by all participants, other target drugs could be quantified by Orbitrap in these four samples. In both influents, codeine, oxazepam and temazepam were also found. In the effluents, codeine, oxazepam, temazepam, nordazepam, diazepam (sample 4) and methylphenidate (sample 3) were also detected.

As illustrative example, **Figure 4** shows $[M+H]^+$ extracted-ion chromatograms (exact mass ± 5 ppm) and MS/MS spectra for several drugs of abuse detected in influent and effluent samples.

An advantage of HRMS, derived from the full-acquisition accurate mass data, is the possibility to perform retrospective analyses [27]. Although it was not the objective of our work, we briefly explored this feature in order to tentatively confirm the presence of ketamine in several samples. Occasionally, ketamine was suspected to be present, based on the finding of the accurate mass of the $[M+H]^+$ ion at the expected retention time. However, due to the high matrix complexity, the product ions, normally used for confirmation of the identity of ketamine, could not be detected in the samples These samples were considered on a case-by-case basis, and were further investigated retrospectively thanks to the useful information acquired by LTQ-FT Orbitrap searching for the ketamine metabolites norketamine and dehydronorketamine. Although it is not clear if the latter is a true metabolite or an artifact, both compounds have been largely found in urine, even at concentrations higher than ketamine [28]. Hence one would expect to find these compounds in sewage water, assuming no further degradation in the sewer. **Figure 5** shows the retrospective search of norketamine and dehydronorketamine using their exact masses with a maximum error of 5 ppm. The presence of chromatographic peaks in the extracted ion chromatograms at the exact masses of the two metabolites is an indication of their presence in the sample analyzed. One may conclude from this observation that ketamine is likely to be present in the sample. Obviously, more research would be required to unequivocally confirm the presence of these metabolites, *e.g.* injecting reference standards, but these findings are illustrative of the potential of the Orbitrap analyzer.

4. CONCLUSIONS

Analytical methodology based on full-spectrum accurate-mass and MS/MS acquisition provided by LC-LTQ FT Orbitrap MS has been developed for the simultaneous quantification and confirmation of 24 target drugs of abuse at ng L^{-1} levels in sewage water. Although Orbitrap is recognized as an excellent analyzer for qualitative purposes, its suitability to perform quantitative analysis has not been much explored. In this work, Orbitrap has been applied for the first time to the quantitative analysis of drugs of abuse in sewage water. Our data showed that this analyzer can be used for the reliable quantification with almost the same sensitivity than the most commonly used methodologies based on LC-MS/MS with triple quadrupole. The quantitative applicability has been demonstrated by method validation and the analysis of quality control samples included in each sample sequence, and also via a comparison with data reported by triple quadrupole analysis. In addition, MS data provided by Orbitrap have allowed retrospective analysis leading to an indication of the presence of two ketamine metabolites. In conclusion, this unique feature of high-resolution accurate-mass spectrometry demonstrates that ketamine is likely to be present in several samples.

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Table 1: Exact masses of the protonated target drugs of abuse, nominal masses and relative abundance of product ions, together with their retention times and isotope labelled internal standards used for quantification.

Compound	t_R	Precursor ion $[M+H]^+$	Product ion 1	Product ion 2		Internal standard
	(min)	*m/z*	*m/z*	*m/z*	RA (%)	
Amphetamine	10.28	136.11208	119.1	91.1	0.5	Amphetamine-d_{11}
Methamphetamine	10.64	150.12773	119.0	91.1	9.0	Methamphetamine-d_5
MDA	10.75	180.10191	163.2			MDA-d_2
MDMA	10.90	194.11755	163.1	58.0	1.0	MDMA-d_5
MDEA	11.66	208.13321	163.1	72.0	2.7	MDEA-d_5
Cocaine	13.42	304.15433	182.1	150.2	2.6	Cocaine-d_3
Benzoylecgonine	12.51	290.13868	168.2	272.2	4.8	Benzoylecgonine-d_3
Heroin	13.07	370.16490	328.2	268.2	99.1	n/a
Morphine	4.71	286.14334	201.1	229.1	51.9	Morphine-d_3
6-MAM	10.33	328.15433	211.2	268.2	73.7	6-MAM-d_3
Methadone	18.80	310.21654	265.1	247.2	0.1	n/a
Codeine	9.10	300.15942	215.2	243.1	47.7	6-MAM-d_3
THC	26.26	315.23186	259.2	193.2	76.7	THC-d_3
THC-COOH	24.84	345.20604	327.2	299.3	6.1	THC-COOH-d_3
OH-THC	24.48	331.22677	313.3			OH-THC-d_3
Ketamine	12.43	238.09932	220.1	207.1	23.9	n/a
Methylphenidate	13.61	234.14886	84.0	174.2	0.3	n/a
Oxazepam	19.53	287.05818	269.1	241.1	3.9	Oxazepam-d_5
Diazepam	20.62	285.07892	257.1	222.2	30.4	Diazepam-d_5
Temazepam	19.85	301.07383	283.0	255.2	9.2	Nordazepam-d_5
Nordazepam	20.13	271.06327	243.1	208.1	37.7	Nordazepam-d_5
Desalkyl-flurazepam	19.67	289.05385	261.1	140.0	44.5	Nordazepam-d_5
mCCP	13.62	197.08400	154.0	119.1	6.9	n/a
Fentanyl	15.66	337.22744	188.2	216.3	5.6	Nordazepam-d_5

RA: relative abundance of product ions.

n/a: adequate internal standard was not available.

Table 2: Method validation in influent (n = 4) and effluent (n = 4) sewage water

Compound	Influent			Effluent			Linearity	
	R[a] (%)	CV[b] (%)	LOQ (ng L^{-1})	R[a] (%)	CV[b] (%)	LOQ (ng L^{-1})	r^2	Instrumental LOQ (pg)
Amphetamine	104	8	40	105	6	4	0.9960	58
Methamphetamine	98	4	15	92	7	5	0.9994	28
MDA	113	13	360[c]	92	4	158	0.9996	720
MDMA	102	4	12	97	7	4	0.9999	14
MDEA	101	4	17	98	5	4	0.9999	14
Cocaine	70	6	40	93	6	6	0.9999	14
Benzoylecgonine	111	8	10	93	7	2	0.9999	14
Heroin	70	20	19	72	21	7	0.9943	28
Morphine	102	5	360[c]	98	12	125	0.9996	144
6-MAM	117	11	19	119	15	7	0.9995	14
Methadone	73	19	45	76	5	6	0.9917	14
Codeine	90	15	19	120	26	7	0.9988	28
THC	109	11	360[c]	94	12	180[c]	0.9995	720
THC-COOH	102	7	33	90	5	7	0.9995	28
OH-THC	82	4	68	85	10	35	0.9988	58
Ketamine	48	21	10	81	7	2	0.9956	14
Methylphenidate	45	20	20	65	27	2	0.9969	14
Oxazepam	97	6	14	91	6	4	0.9994	28
Diazepam	100	4	18	94	7	6	0.9998	20
Temazepam	105	6	4	90	7	2	0.9980	28
Nordazepam	96	5	4	91	8	2	0.9995	28
Desalkyl-flurazepam	88	25	4	109	15	2	0.9994	28
mCCP	52	28	20	62	18	6	0.9911	14
Fentanyl	73	16	4	74	14	2	0.9964	14

[a] Trueness, estimated by means of recovery experiments.

[b] Precision, expressed as repeatability in terms of coefficients of variation.

[c] These values were derived from validation experiments (for detailed explanation see text).

Table 3: Comparison of concentrations of drugs of abuse detected in influent and effluent sewage waters analyzed by LC-MS/MS with triple quadrupole (two different laboratories) and by the Orbitrap method presented in this work. Each number is the result of a single measurement of the pertaining sample using a specific MS detection technique

Compound	Influent sewage water (ng L^{-1})						Effluent sewage water (ng L^{-1})					
	Sample 1			Sample 2			Sample 3			Sample 4		
	QqQ 1[a]	QqQ 2[b]	Orbitrap	QqQ 1	QqQ 2	Orbitrap	QqQ 1	QqQ 2	Orbitrap	QqQ 1	QqQ 2	Orbitrap
Amphetamine	95	117	123	282	249	245	-	-	-	-	-	-
MDMA	21	96	144	-	56	86	84	88	137	50	54	76
Cocaine	439	296	d[c]	179	114	d[c]	2	-	-	14	-	8
Benzoylecgonine	1178	1136	1637	528	645	615	26	19	45	85	77	99
THC-COOH	378	n/a[d]	d[c]	-	n/a	-	-	n/a	-	-	n/a	-
Ketamine	n/a	-[e]	-	n/a	-	-	n/a	-	2	n/a	16	6

[a] Pre-treatment: centrifugation; Pre-concentration by SPE (Oasis MCX, 150 mg); pre-concentration factor: influent 10x, effluent, 50x; [29]

[b] Pre-treatment: none; Pre-concentration by SPE (Oasis HLB, 200 mg); pre-concentration factor: influent 50x, effluent 250x; [25]

[c] d: detected

[d] n/a: no data available

[e] - : not detected

FIGURE CAPTIONS

Figure 1: The influence of intake volume (100, 300, 600 and 900 mL) on matrix effect in a typical influent

Figure 2: Average matrix effects [21] observed in both influents (100 mL) and effluents (200 mL) for 5 different STPs.

Figure 3: The mass deviation (mass drift) of the protonated ions of selected deuterated analytes in relation to the theoretical mass over a period of 55 hours.

Figure 4: LC-MS (ESI + mode) extracted-ion chromatograms (left) and MS/MS spectra (right) of drugs of abuse detected in influent (A) and effluent (B) sewage water from the sewage treatment plant of Amsterdam. Concentrations found in these samples were the following (influent and effluent, respectively); MDMA: 136 and 190 ng L^{-1}; benzoylecgonine: 3701 and 155 ng L^{-1}; THC-COOH: 431 and 22 ng L^{-1}; Oxazepam: 430 and 422 ng L^{-1}. Arrows indicate chromatographic peak of MDMA.

Figure 5: LC-MS (ESI+ mode) extracted-ion chromatograms of ketamine, norketamine and dehydronorketamine in an influent sewage water sample from Eindhoven (retrospective search).

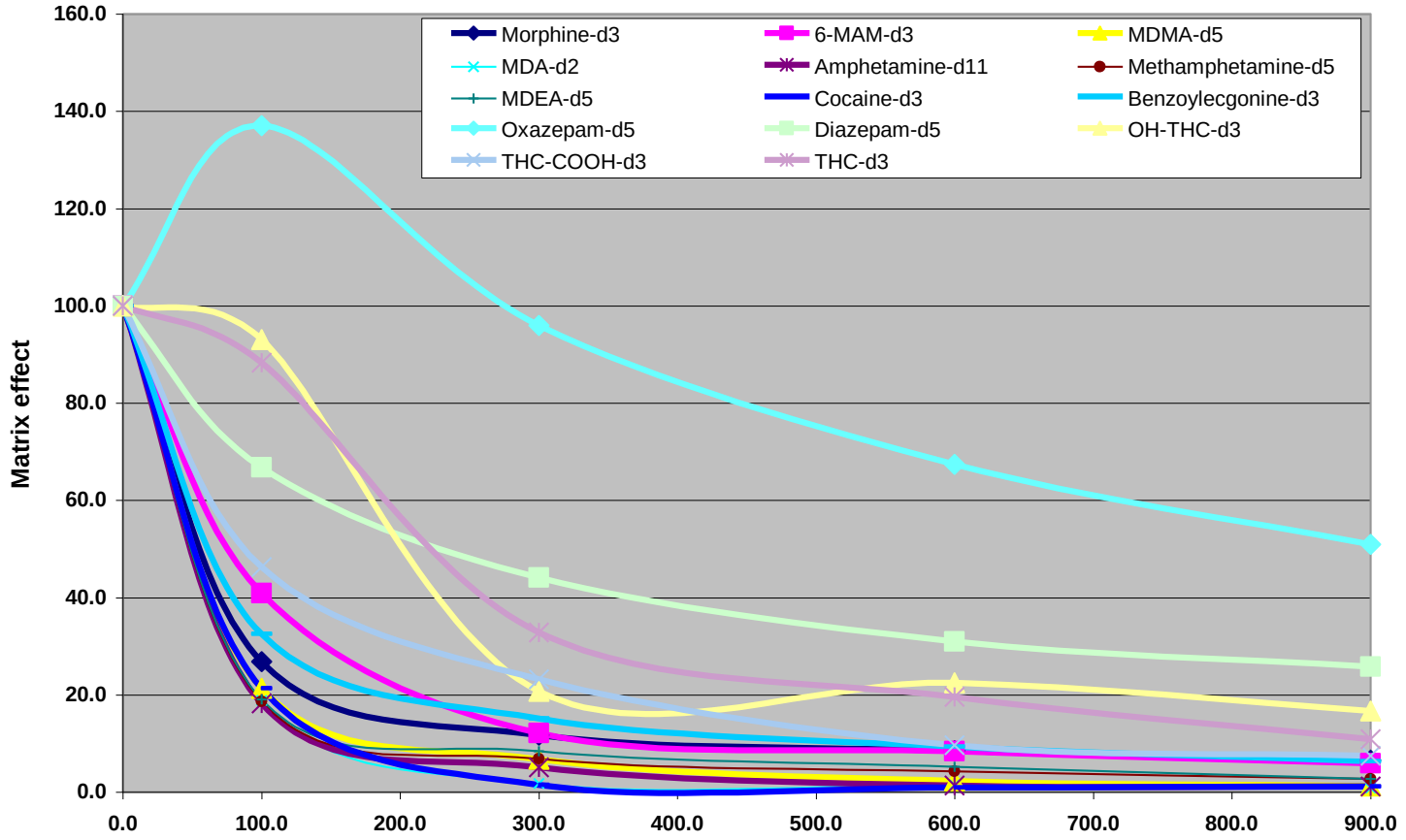

Morphine-d3
6-MAM-d3
MDMA-d5
MDA-d2
Amphetamine-d11
Methamphetamine-d5
MDEA-d5
Cocaine-d3
Benzoylecgonine-d3
Oxazepam-d5
Diazepam-d5
OH-THC-d3
THC-COOH-d3
THC-d3
Matrix effect
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
0.0
100.0
200.0
300.0
400.0
500.0
600.0
700.0
800.0
900.0

Figure 1

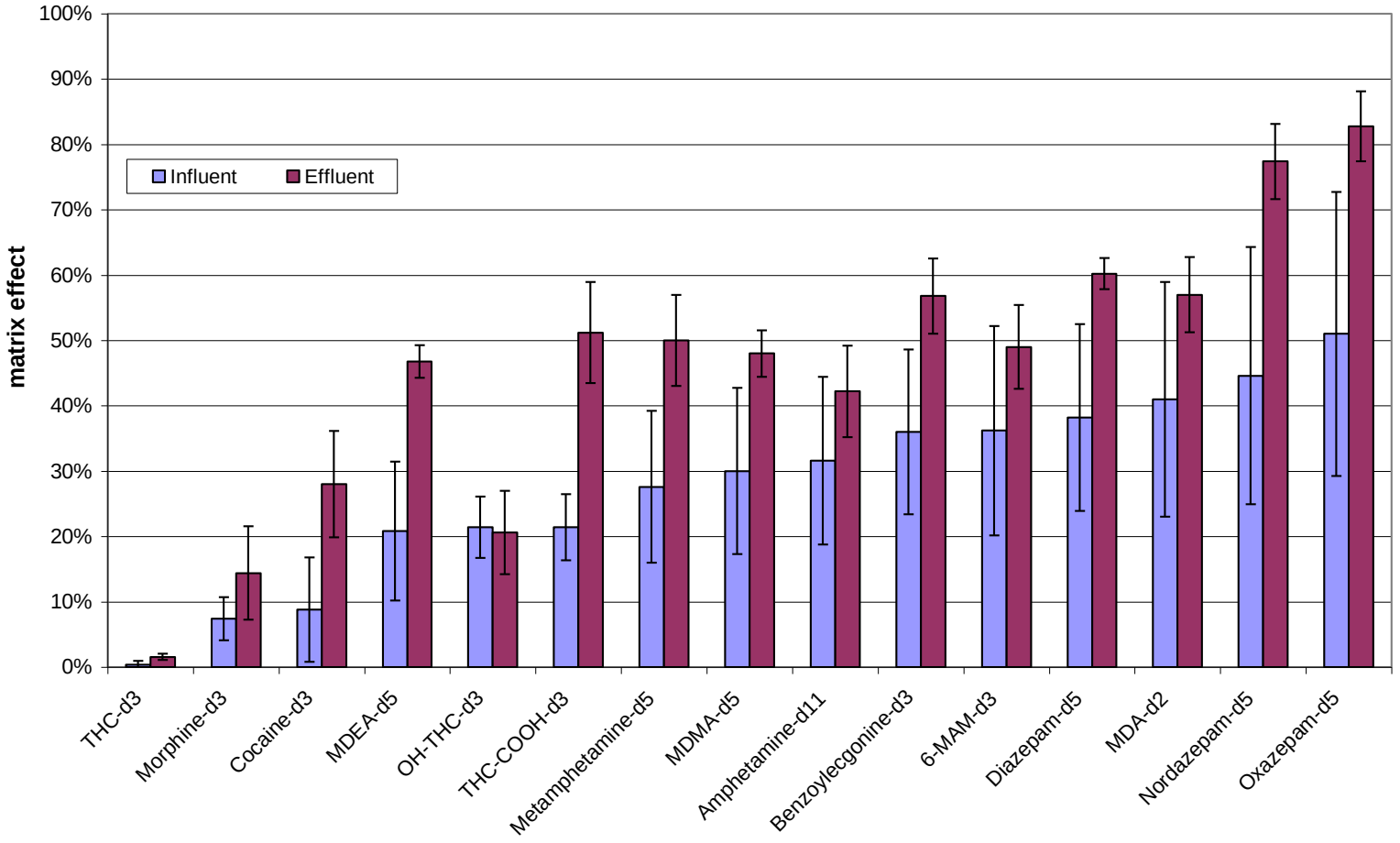

matrix effect
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Influent
Effluent
THC-d3
Morphine-d3
Cocaine-d3
MDEA-d5
OH-THC-d3
THC-COOH-d3
Metamphetamine-d5
MDMA-d5
Amphetamine-d11
Benzoylecgonine-d3
6-MAM-d3
Diazepam-d5
MDA-d2
Nordazepam-d5
Oxazepam-d5

Figure 2

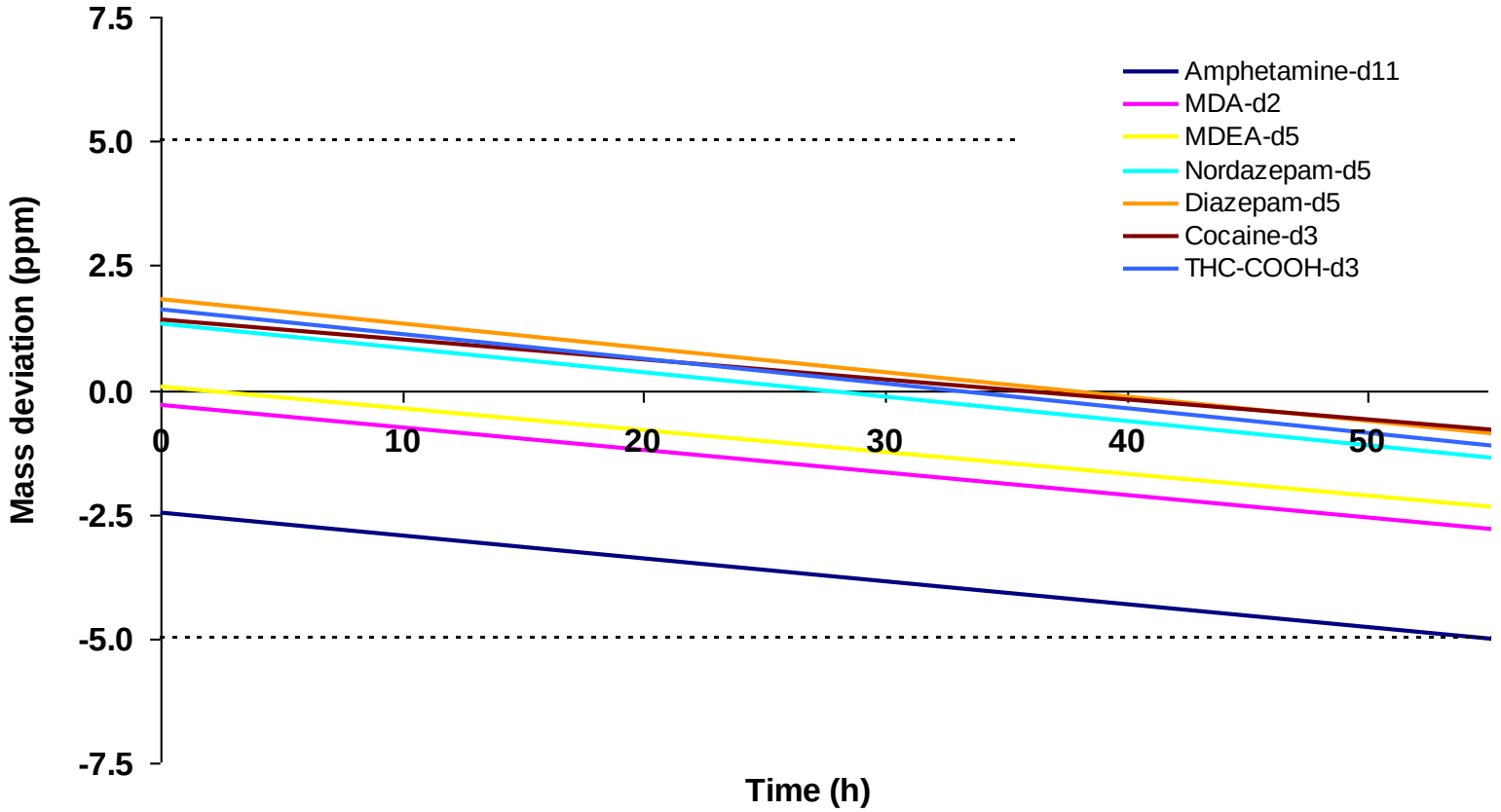
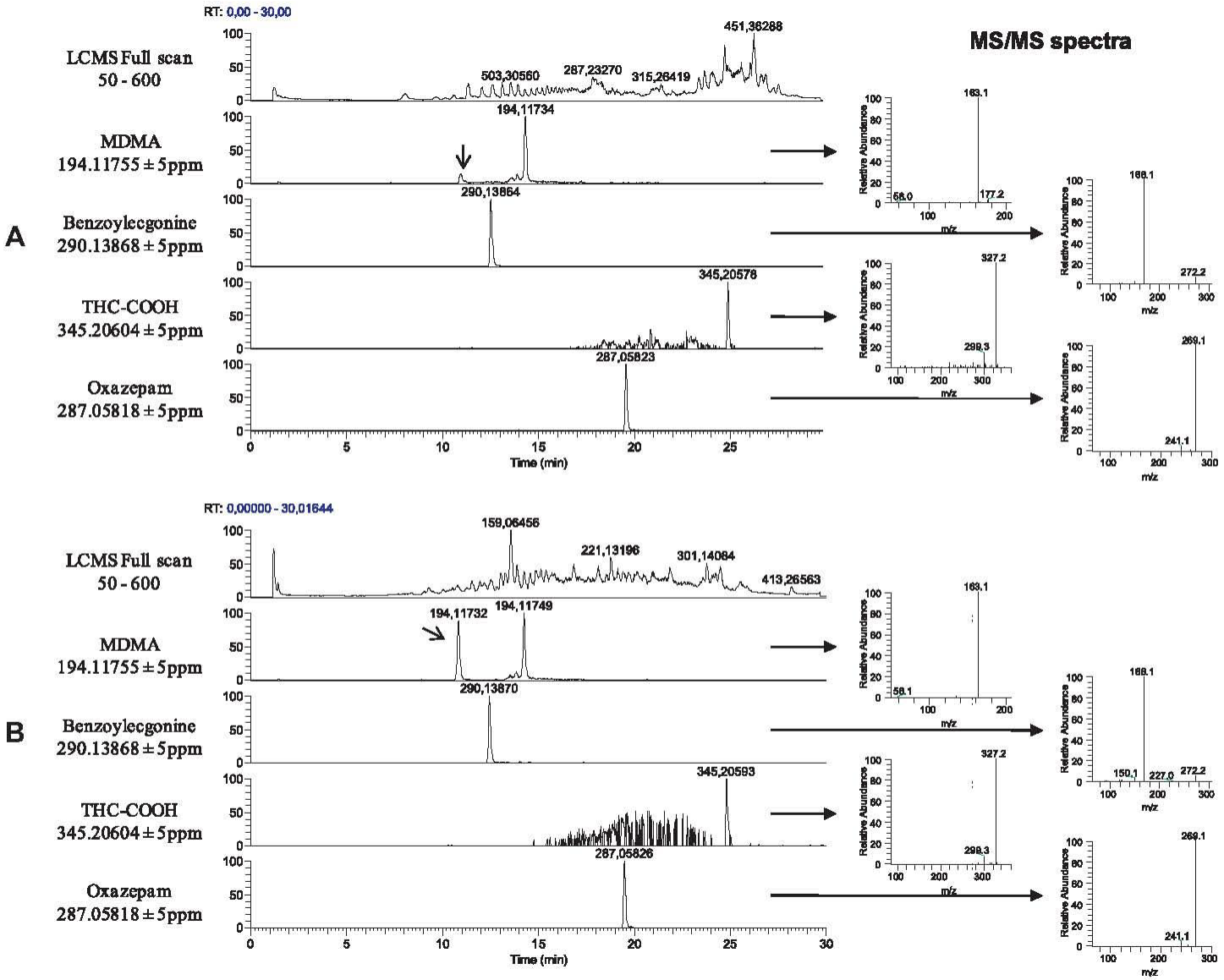

7.5
5.0
2.5
0.0
-2.5
-5.0
-7.5
0
10
20
30
40
50
Mass deviation (ppm)
Time (h)
Amphetamine-d11
MDA-d2
MDEA-d5
Nordazepam-d5
Diazepam-d5
Cocaine-d3
THC-COOH-d3

Figure 3


A
RT: 0,00 - 30,00
LCMS Full scan
50 - 600
MDMA
194.11755 ± 5ppm
Benzoylecgonine
290.13868 ± 5ppm
THC-COOH
345.20604 ± 5ppm
Oxazepam
287.05818 ± 5ppm
451,36288
503,30560
287,23270
315,26419
194,11734
290,13864
345,20578
287,05823
Time (min)
MS/MS spectra
163.1
177.2
327.2
298.3
168.1
272.2
269.1
241.1
B
RT: 0,00000 - 30,01644
LCMS Full scan
50 - 600
MDMA
194.11755 ± 5ppm
Benzoylecgonine
290.13868 ± 5ppm
THC-COOH
345.20604 ± 5ppm
Oxazepam
287.05818 ± 5ppm
159,06456
221,13196
301,14084
413,26563
194,11732
194,11749
290,13870
345,20593
287,05826
Time (min)
163.1
168.1
150.1
227.0
272.2
327.2
298.3
269.1
241.1

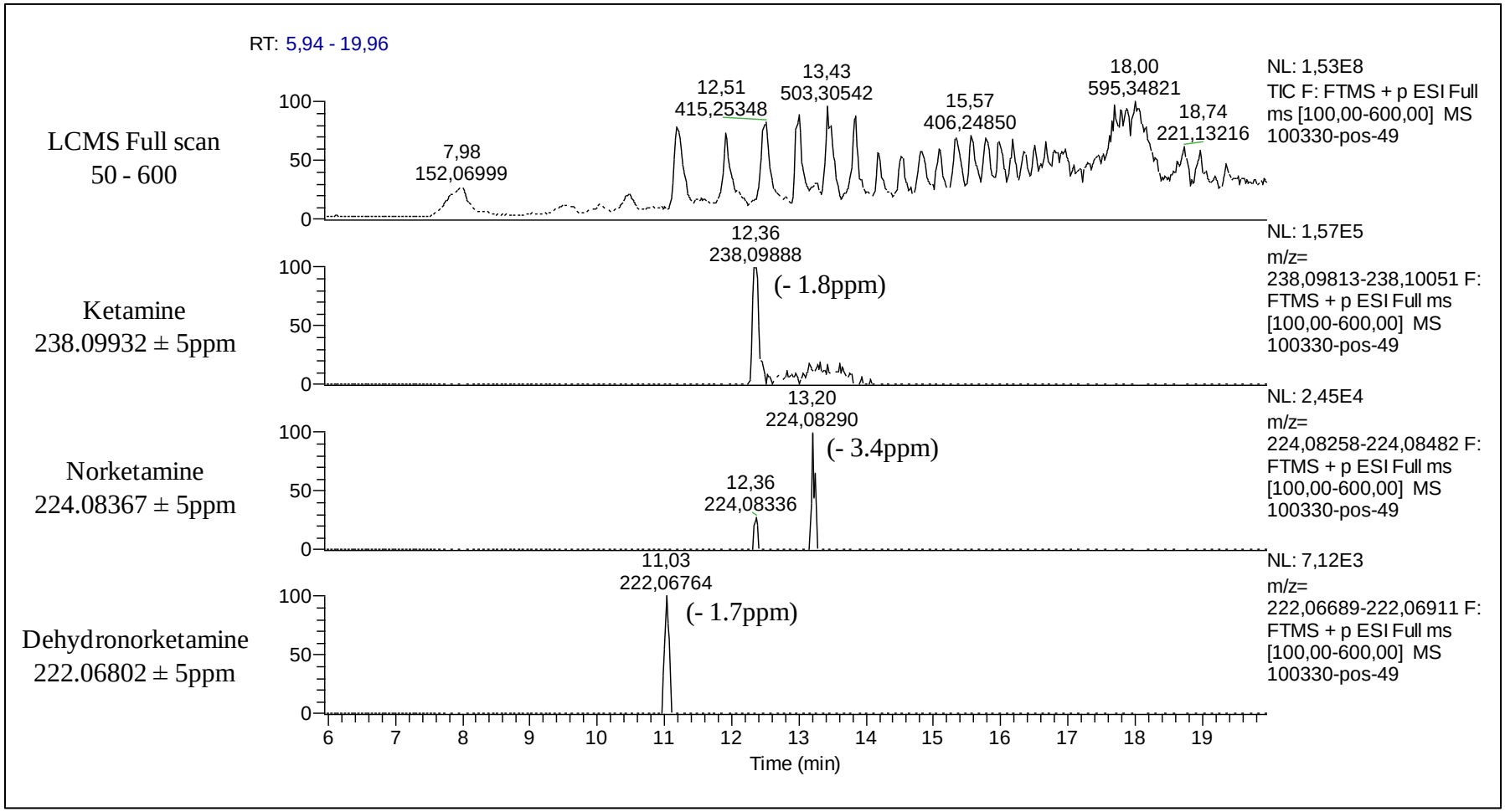

RT: 5,94 - 19,96
LCMS Full scan
50 - 600
7,98
152,06999
12,51
415,25348
13,43
503,30542
15,57
406,24850
18,00
595,34821
18,74
221,13216
NL: 1,53E8
TIC F: FTMS + p ESI Full ms [100,00-600,00] MS 100330-pos-49
Ketamine
238.09932 ± 5ppm
12,36
238,09888
(- 1.8ppm)
NL: 1,57E5
m/z= 238,09813-238,10051 F: FTMS + p ESI Full ms [100,00-600,00] MS 100330-pos-49
Norketamine
224.08367 ± 5ppm
13,20
224,08290
(- 3.4ppm)
12,36
224,08336
NL: 2,45E4
m/z= 224,08258-224,08482 F: FTMS + p ESI Full ms [100,00-600,00] MS 100330-pos-49
Dehydronorketamine
222.06802 ± 5ppm
11,03
222,06764
(- 1.7ppm)
NL: 7,12E3
m/z= 222,06689-222,06911 F: FTMS + p ESI Full ms [100,00-600,00] MS 100330-pos-49
100
50
0
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Time (min)

Figure 5