# A LC-MS/MS METHOD FOR THE DETERMINATION OF COMMON SYNTHETIC CATHINONES IN MECONIUM

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**'Declarations of interest: none'**

**Highlights**

An LC-MS/MS method for common synthetic cathinones was developed in meconium.

Method validation was performed, fulfilling acceptance criteria for all parameters.

Application to suspicious clinical cases will improve neonatal care.

It will also help to estimate synthetic cathinones prevalence during pregnancy.

**Abstract**

New psychoactive substances have been introduced into the market in the last years due to their unregulated status. Synthetic cathinones are one of their main representatives, and they have shown to produce neonatal complications. It is important to have objective tools to identify in utero exposure to drugs that have shown to produce neonatal complications. An analytical method was developed and fully validated for the determination of common synthetic cathinones, including methylone, methedrone, mephedrone, 3,4-methylenedioxypyrovalerone (MDPV), (±)-4-fluoromethamphetamine and 4-fluoromethcathinone in meconium. Meconium (0.25 ± 0.02g) was homogenized with methanol by sonication for 30 minutes. After centrifugation, the sample was extracted with Oasis MCX columns. The analysis was performed by LC-MS/MS using an Atlantis T3 column (3 μm, 2.1x50 mm) and a gradient with acetonitrile and 0.1% formic acid in water. Method validation included the following parameters: selectivity (no endogenous or exogenous interferences), limits of detection (n=3, 0.5-1 ng/g) and quantification (n=3, 1-2 ng/g), linearity (n=5, LOQ-200 ng/g), imprecision (n=15, 0% to 10%), accuracy (n=15, 87.3% to 97.8%), matrix effect (n=10, -76% to -28.1%), extraction efficiency (n=6, 63.7% to 91.3%), total process efficiency (n=6, 16% to 60.2%) and stability for 72 h in the autosampler (n=3, %loss= -6.7% to 5.1%). The method was applied to 28 meconium specimens.

**Keywords**

Synthetic cathinones, new psychoactive substances, meconium, in utero drug exposure, LC-MS/MS

## 1. Introduction

New psychoactive substances (NPS) include a wide number of compounds that are not controlled by the international law and, therefore, they are used to replicate the effects of outlawed substances avoiding the legal prohibition for sale and consumption. Due to their unregulated status, they are sold in gas stations, stores or even on the Internet with the indications of "not for human use" or “not tested for hazards or toxicity” to avoid prosecution. In the last years, many countries started to ban the production and distribution of some NPS [1-4], and recently the EU published a new legislation to make NPS regulation easier and faster [5]. However, new drugs are constantly being introduced into the market. From 2009 to 2016, 739 different NPS were reported worldwide to the United Nations Office on Drugs and Crime (UNODC) [6], whereas by the end of 2017 the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) had monitored more than 670 NPS in Europe [7]. Synthetic cathinones along with synthetic cannabinoids represent around 80% of the NPS in confiscated seizures [7], and have recently emerged and grown to be popular drugs of abuse [8,9].

There is limited information about the prevalence of NPS, and sometimes users are not even aware of their consumption. In the last years, surveys on drug use performed in different countries disaggregated the use of these drugs among the general population. The use of NPS in adults aged 14-34 years old was 0.3% in Austria, and 1.6% in the Czech Republic and Ireland [10]. Specifically for synthetic cathinones, the use of mephedrone in United Kingdom in 16- to 34-year-olds was estimated at 0.5% [10]. In Spain, the majority of the population does not know about the existence of NPS (73.8% of survey responders), and the highest percentage of consumption is found among young male population (15 to 24 years old; 99% males). Regarding women of childbearing age

(from 15 to 44 years), between 0.6% and 0.7% admitted consumption of NPS at some time in their lives, and 0.1% recognized specifically having consumed the synthetic cathinone mephedrone [11]. According to data obtained by Energy Control (a Spanish non-governmental organization that seeks to reduce the risks of drug use), between 2010 and 2012 the more prevalent synthetic cathinones in Spain were mephedrone, methylone, 4-methylethcathinone (4-MEC) and 3,4-methylenedioxypyrovalerone (MDPV) [12]. In addition, in 2016 mephedrone and methylone continued to be the most requested cathinones in Spain [13].

Exposure to drugs during pregnancy can entail obstetric and fetal complications, and even later on the childhood and adulthood. Synthetic cathinones show similar sympathomimetic effects to amphetamine derivatives, including tachycardia, hypertension, abdominal pain, nausea, and anxiety [8,14,15], as well as paranoia, hallucinations or panic attacks [16,17]. Prenatal exposure to amphetamine and derivatives negatively affects fetal growth and infant neurobehavior [18]. Methylone, mephedrone and MDVP can cross the placenta and reach the fetus, as it has been shown in a recent study carried out with pregnant mice [19]. In addition, animal studies suggests that MDPV exposure during pregnancy may damage the fetal brain and, in the early pregnancy, is associated with a lower survival rate of the offspring [20]. Although there is no literature on the specific effects of synthetic cathinones in human fetus, exposure to cathinone through “khat-chewing” during pregnancy can produce neonatal low birth weight, probably due to its anorexic effects and placental blood circulation reduction [21,22]. In addition, the repeated use of these substances can lead to dependence development [8], which could result in neonatal abstinence syndrome (NAS), as recently reported [23]. Therefore, it is important to identify exposure to these drugs during pregnancy for the management of possible obstetric, fetal or neonatal complications.

Several methods have been published for the identification of synthetic cathinones in different biological matrices, including urine [24-27], blood, plasma or serum [27-30], oral fluid [27,31-33] and hair [34-36].

Meconium is considered the reference matrix for the identification of drug use during pregnancy, as its analysis provides information on the direct fetal exposure and has a wide window of detection (mainly from the third trimester of pregnancy) [37,38]. To our knowledge, only two research teams reported analytical methods for identification of synthetic cathinones in meconium [23, 39].

The goal of our research was to develop and validate a method for the determination of the most common synthetic cathinones in meconium by LC-MS/MS. This will allow to broaden the substances investigated in clinical and forensic cases where there is a suspicion of maternal drug use during pregnancy, improving neonatal care in those cases. In addition, its routine application will help to evaluate the prevalence of use of common synthetic cathinones among pregnant women.

## 2. Materials and methods

### *2.1. Chemicals*

Methylone, methedrone, mephedrone, 3,4-methylenedioxypyrovalerone (MDPV), (±)-4-fluoromethamphetamine and 4-fluoromethcathinone standards at 1 mg/mL in methanol, and methylone-$d_3$, mephedrone-$d_3$, methamphetamine-$d_5$, and TFMPP-$d_4$ internal standards (IStd) at 100 µg/mL in methanol were supplied by Cerilliant (Round Rock, TX, USA). Dichloromethane, HCl 37%, formic acid and acetonitrile (ACN) were supplied by Scharlau (Sentmenat, Catalonia, Spain), purified water and ammonium hydroxide by VWR (Radnor, Pennsylvania, USA), and 2-propanol and methanol by Fisher Chemicals

(Loughborough, Leicestershire, UK). Oasis MCX cartridges (3 cc, 60 mg) were from Waters Corp. (Milford, MA, USA).

*2.2. Blank meconium samples*

Anonymized blank meconium specimens were supplied by the University Hospital of Vigo (Spain). These blank meconium specimens were used for the preparation of the calibrators and quality control (QC) samples.

*2.3. Preparation of calibration and QC solutions*

For the preparation of the calibration curves, mixed working solutions containing all the analytes were prepared in methanol at 10, 1, 0.5, 0.1, 0.05 and 0.01 μg/mL. An eight or seven-point calibration curve from 1 or 2 to 200 ng/g was generated by addition of 25 or 50 μL of the appropriate working solution to blank meconium samples. Different working solutions at 0.5, 0.1 and 0.01 μg/mL were used to elaborate low, medium and high quality control (QC) samples (3 ng/g, 30 ng/g and 150 ng/g, respectively). A working solution containing the internal standards (IStd) was prepared in methanol at a concentration of 1 μg/mL. All these solutions were stored at -20°C until use.

*2.4. Meconium sample preparation*

Meconium (0.25±0.02 g) was weighed into Pyrex® glass tubes. Then, 25 μL of the IStd solution at 1 μg/mL and 2 mL of methanol were added for sample homogenization. The sample was sonicated for 30 min and then centrifuged for 10 min at 4000 rpm. After the addition of 50 μL of 1% HCl in methanol, the supernatant was evaporated in a water bath at 35°C with a stream of nitrogen, and the extract was reconstituted in 2 mL of 2% formic acid in water for solid phase extraction (SPE).

*2.5. SPE procedure*

SPE was performed with Oasis MCX cartridges (3 cc, 60 mg). After cartridges conditioning with 2 mL of methanol and 2 mL of water, the sample was loaded. Cartridges were washed with 2 mL of 2% formic acid in water and 2 mL of methanol:water:formic acid (47.5:47.5:5, v/v/v), and then dried for 10 min under vacuum. Finally, analytes were eluted with 2 mL of dichloromethane:2-propanol:ammonium hydroxide (47.5:47.5:5, v/v/v), and subsequently evaporated to dryness with nitrogen at 35°C after the addition of 50 μL of 1% HCl in methanol to prevent analytes evaporation. Extracts were reconstituted with 100 μL of 0.1% formic acid:ACN (90:10, v/v), and 20 μL were injected into the LC-MS/MS.

*2.6. LC-MS/MS Instrumentation*

An Alliance 2795 Separation Module with an Alliance series column heater/cooler (Waters Corp.) was employed for the chromatographic separation using an Atlantis® T3 (2.1 mm × 50 mm, 3 μm) column (Waters Corp., Milford, MA, USA), maintained at 30°C. Formic acid (0.1%) in water (A) and acetonitrile (B) were used as mobile phase using the following chromatographic gradient: 0-0.5 min 10% B, 0.5-6 min from 10% to 60% B, 6.0-6.1 min from 60% to 100% B, 6.1-7.1 min 100% B, 7.1-7.5 min return to initial conditions and equilibrate until minute 12. Chromatographic separation of all the analytes was achieved in 6 minutes, with a total run time of 12 minutes.

The mass spectrometer employed was a Quattro Micro™ API ESCI triple quadrupole (Waters Corp.). The instrument was operated in electrospray in positive mode (ESI+). Capillary voltage was 1 kV; source block and desolvation gas temperature were 150°C and 500°C, respectively; and desolvation and cone gas flow rate were 800 L/h and 60 L/h, respectively. Data were recorded on multiple reaction monitoring (MRM) mode. MRM

transitions, cone voltages (CV) and collision energies (CE) for each analyte and the IStds (Table 1) were optimized by post-column infusion of each individual analyte (10 μg/mL) at 20 μL/min connected with a "T" valve to the chromatographic effluent (0.1% formic acid in water:ACN, 50:50, v/v). MassLynx 4.0 software was employed to control data acquisition and QuanLynx 4.1 for data-processing (Waters Corp.).

*2.7. Method validation*

Validation was performed according to the Scientific Working Group for Forensic Toxicology (SWGTOX) recommendations [40]. The following validation parameters were determined for a quantitative analysis: linearity; limit of detection (LOD); limit of quantification (LOQ); selectivity; accuracy; intra-assay, inter-assay and total imprecision; matrix effect; extraction and process efficiency; and autosampler stability. For those compounds just validated for qualitative purposes only LOD, selectivity, matrix effect, extraction and process efficiency, and autosampler stability were evaluated.

Linearity was evaluated by the analysis of calibration curves on five different days. Acceptable linearity was achieved when the coefficient of determination ($r^2$) was ≥0.99 and residuals were <20%.

Blank meconium samples from 3 different sources fortified at the lowest concentration of the calibration curve were analyzed in duplicate over three runs to evaluate the LOQ. The same process was performed for the LOD, but fortifying the sample at decreasing concentrations.

Selectivity of the method was evaluated for exogenous and endogenous interferences. Endogenous interferences were evaluated by the analysis of 10 different blank meconium samples fortified with the IStd. To evaluate the presence of exogenous interferences,

blank meconium samples were fortified with other common drugs of abuse and medicines (morphine, codeine, 6-acetylmorphine, methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, amphetamine, methamphetamine, 3,4-methylendioxyamphetamine, 3,4-methylendioxymethamphetamine, 3,4-methylendioxyethylamphetamine, cocaine, benzoylecgonine, ecgoninemethylester, cocaethylene, lysergic acid diethylamide, ketamine, norketamine, gammahydroxybutyric acid, nicotine, cotinine, fentanyl, amitriptyline, paroxetine, zolpidem, zopiclone, ibuprofen, omeprazole, paracetamol, diclofenac, naproxen, alprazolam, temazepam, lormetazepam, lorazepam, clonazepam, diazepam, nordiazepam, flunitrazepam, 7-aminoflunitrazepam, oxazepam, triazolam, nitrazepam and bromazepam) at 1000 ng/g.

Imprecision and accuracy were determined at the three QC levels. These parameters were assessed by the analysis of 3 replicates for each QC on 5 different days (n=15). Intra-assay, inter-assay and total imprecision were determined by calculating the coefficient of variation (%CV) using SPSS software (version 24.0, SPSS Inc., Chicago, IL) [41]. Imprecision and accuracy should be less than 20%.

Matrix effect, extraction and process efficiency were determined at two concentration levels (low and high QC). Evaluation of matrix effect was performed by comparing average analyte peak area in blank meconium samples (n=10, from different neonates) fortified after extraction with average peak area when the analyte was directly added to a clean tube (neat, n=6). Extraction efficiency was calculated comparing average analyte peak area in blank meconium samples fortified before extraction (n=6) with average peak area in blank meconium samples fortified after extraction (n=10). Finally, process efficiency was calculated by comparing average analyte peak area in blank meconium samples fortified before extraction (n=6) with average peak area of neats (n=6) [42].

Autosampler analyte stability was evaluated at three QC levels (low, medium and high) by comparing mean concentration after storage in the autosampler at 6˚C for 72h (n=3) with mean concentration of freshly prepared QCs (n=3). %Loss <15% was considered acceptable.

*2.7. Application to real specimens*

The present method was applied to 28 meconium specimens that have previously tested positive for one or more drugs of abuse (cannabis, opiates, cocaine and/or amphetamine). These specimens were collected at the University Hospitals of Santiago de Compostela and Vigo, Spain, from January 2012 to December 2015 as part of a broad study to evaluate drug use during pregnancy. Real samples collection was approved by the Ethics Committee of the University of Santiago de Compostela (Spain) and by the Galician Clinical Research Ethics Committee (Xunta de Galicia, Spain).

In addition, to assess method performance, staff from the National Institute of Toxicology and Forensic Sciences (INTCF, Madrid, Spain) prepared quality controls by fortification of three blank meconium samples with some of the analytes included in the present method using a single blind method. The samples were sent to our laboratory for their analysis.

## 3. Results

*3.1 Method validation*

The above-mentioned assays for a full validation of the method were performed. Methedrone and fluoromethcathinone did not satisfy acceptance criteria for those parameters to guarantee an appropriate quantification of the analytes (linearity, precision

and accuracy). Therefore, for these 2 analytes, the method was only validated for qualitative purposes.

Linearity for methylone, fluoromethamphetamine, mephedrone and MDPV was verified from 1 or 2 to 200 ng/mL, applying a 1/x-weighting factor. The curves were fitted to a linear regression model, obtaining a $r^2 \geq 0.99$ in all cases. LOD and LOQ were 0.5 and 1 ng/g, respectively, for methylone, fluoromethamphetamine and mephedrone, and 0.5 ng/g and 2 ng/g for MDPV. LOD for methedrone and fluoromethcathinone was 1 ng/g. Calibration parameters for each analyte are summarized in Table 2. Fig. 1 shows the chromatograms of the main MRM transitions for each analyte in a blank meconium sample (1A) and in a blank meconium sample fortified at the LOD (1B).

No interferences were detected in blank meconium samples from 10 different neonates or in blank meconium samples fortified with other common drugs; therefore, endogenous and exogenous selectivity was verified for all analytes.

For methylone, fluoromethamphetamine, mephedrone and MDPV intra-assay, inter-assay and total imprecision were satisfactory, with %CV <7.9%, 8.9% and 10%, respectively. Acceptable results were also achieved for accuracy, with calculated concentrations within 87.3%-97.8% of the nominal concentration (Table 3).

Matrix effect, extraction efficiency and process efficiency results are shown in Table 4. All the analytes showed signal suppression at low and high QC concentrations, ranging from -28.1 to -76% (%CV= 8.4-18.8%). However, behavior of the IStd was similar (-15.6% to -66%, %CV= 7.1-18.1%), even for those compounds for which their deuterated analogue was not employed as IStd (Table 4), compensating matrix effect on quantitative parameters. Extraction efficiency ranged from 63.7% to 91.3% (%CV= 1.9-19.2%), and overall process efficiency from 16% to 60.2%.

All analytes were stable for 72 h in the autosampler at 6°C, with a %loss ranging from -0.1% to -6.7% (Table 5).

*3.2. Application to real samples*

Twenty-eight specimens of meconium that had already tested positive for other drugs of abuse were analyzed to evaluate the possible presence of synthetic cathinones. None of the synthetic cathinones were detected in any of these selected cases.

In relation with the analysis of the blind meconium samples fortified at the INTCF, all the analytes added to the samples were correctly identified, and quantified within ±10% of the nominal concentration, proving the correct performance of the method. Specifically, Sample A showed a concentration of 5.3 ng/g of methylone (5 ng/g theoretical concentration) and 22.1 ng/g of mephedrone (20 ng/g theoretical concentration), Sample B 107.9 ng/g of MDPV (100 ng/g theoretical concentration), and Sample C methedrone (qualitatively validated) and 50.9 ng/g of fluoromethamphetamine (50 ng/g theoretical concentration).

## 4. Discussion

The manuscript describes the first validated analytical method for the simultaneous determination of the most common synthetic cathinones (methylone, methedrone, mephedrone, MDPV, fluoromethamphetamine, fluoromethcathinone) in meconium by LC-MS/MS.

In the present method, all the analytes were chromatographically separated in 6 min, with a total run time of 12 min. The method was satisfactorily validated, with LODs and LOQs ranging from 0.5 to 1 ng/g and 1 to 2 ng/g, respectively, using only 0.25 g of meconium. As meconium is a matrix frequently scarce, and the determination of the common illegal

drugs of abuse is usually the main objective, it is a priority to develop analytical methods using a small amount of sample.

Two analytical methods have been published to date for the detection of NPS in meconium. Pichini et al. [23] developed a method in relation to a case report of a Spanish newborn with neonatal abstinence syndrome (NAS) after maternal consumption of 4-MEC and methadone during pregnancy. The sample was extracted by SPE after homogenization with methanol using 1 g of sample. This high amount of meconium may not be available in some cases, especially if other analytical methods should be applied, as in this reported case (meconium was also analyzed for methadone, cocaine, amphetamines, opiates and cannabis). Unfortunately, method validation for 4-MEC was not described or mentioned, and a medicine (pentazocine) was used as IStd, which is unadvisable. Meconium specimen was positive for 4-MEC (0.7 ng/g), although the LOQ for this analyte was not clearly indicated in the manuscript. Recently, Nemeskalová et al. [39] published a method for the detection of amphetamine-like stimulants, including common synthetic cathinones such as methedrone, methylone, MDPV, α-pyrrolidinopentiophenone (α-PVP), butylone, flephedrone and naphyrone, using salting-out liquid-liquid extraction. Although our method does not include some of these cathinones (α-PVP, butylone, flephedrone and naphyrone), it allows the identification of others (mephedrone, fluoromethamphetamine and fluoromethcathinone). In addition, in spite of using a similar amount of meconium (0.2 g vs 0.25 g), our sensitivity was from 5 to 10-fold higher for all the analytes. This could have a great impact on cathinones detection, as in the only real sample in which the authors found a synthetic cathinone (methylone) its concentration was below their LOQ.

The proposed method was applied to the analysis of 28 meconium specimens that had previously tested positive for other common illicit drugs. In these real samples no synthetic cathinones were detected. Although prevalence of consumption for these novel drugs is lower than the prevalence of the traditional ones, other authors reported their use among pregnant women in different European countries. Jones et al. described the use of mephedrone in pregnant women (according to maternal interview) and the presence of NAS in neonates in-utero exposed to this drug at the 2011 International Congress of the European Association of Poisons Centers and Clinical Toxicologists [43]. Two other authors identified synthetic cathinones in meconium specimens [23,39], and one of them [23] also concluded that fetal exposure to these NPS may have poor neonatal outcomes, including NAS. However, the concomitant maternal use of methadone in that case make it difficult to discriminate between the effects of both drugs. Nevertheless, recent animal studies showed that synthetic cathinones can reach the fetal brain in high concentrations, being fetal risk, definitely, a concern [19, 20]. Therefore, analysis of meconium for NPS, and specifically for synthetic cathinones, is advisable.

The main limitation of our method is the restricted number of NPS included, taking into account the large number of new drugs reported each year, and the evolution in the use of these substances over time. However, the present method includes some of the still more common synthetic cathinones available on the drug market. Moreover, the method could be easily expanded for the identification of other new synthetic cathinones if a request for a specific analyte was made. The method described in this manuscript has been included in our routine protocol for drugs of abuse testing in meconium, which can help in the rapid detection of cases were these compounds may be involved, improving neonatal care. In addition, its application to all the routine meconium cases received in

our laboratory for drug analysis will allow to have objective data on synthetic cathinones prevalence among pregnant women.

## 5. Conclusions

A method for the determination of the most common synthetic cathinones in meconium was developed and validated. This method is suitable for the application to the analysis of real meconium samples and can contribute to provide real data on the prevalence of these synthetic cathinones among pregnant women.

## Acknowledgements

We would like to thank the participants, and E. Lendoiro would like to thank the Consellería de Cultura, Educación e Ordenación Universitaria, Xunta de Galicia, for her postdoctoral contract (ED481B2016/103-0). We would also like to thank Dr. Óscar Quintela from the INTCF (Madrid, Spain) for providing fortified meconium samples to proof method performance.

## Funding

This work was supported by the Plan Nacional Sobre Drogas, Ministerio de Sanidad, Servicios Sociales e Igualdad, Gobierno de España (grant 2016I005).

## References

[1] Orden SPI/201/2011, Boletín Oficial del Estado (BOE) num. 34, Spain, 9 February 2011. https://www.boe.es/boe/dias/2011/02/09/pdfs/BOE-S-2011-34.pdf (accessed 28 March 2019).

[2] Orden SSI/1091/2015, Boletín Oficial del Estado (BOE) num. 140, Spain, 12 June 2015. https://www.boe.es/boe/dias/2015/06/12/pdfs/BOE-A-2015-6496.pdf (accessed 28 March 2019).

[3] Council Implementing Decision 2015/1875, Official Journal of the European Union, L275, European Union, 8 October 2015. http://www.emcdda.europa.eu/system/files/attachments/10001/Council-decision-2015-1875-25i-nbome_ah-7921_mdpv_methoxetamine.pdf (accessed 28 March 2019).

[4] Drug Enforcement Administration. Part 1308 - Schedules of Controlled Substances. https://www.deadiversion.usdoj.gov/21cfr/cfr/1308/1308_11.htm. (accessed 28 March 2019).

[5] Regulation 2017/2101, Official Journal of the European Union, L305 , European Union, 21 November 2017. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2017:305:FULL&from=EN (accessed 28 March 2019).

[6] United Nations Office on Drugs and Crime, World Drug Report 2017 (ISBN: 978-92-1-148291-1, eISBN: 978-92-1-060623-3, United Nations publication, Sales No. E.17.XI.6) https://www.unodc.org/wdr2017/field/Booklet_1_EXSUM.pdf (accessed 28 March 2019).

[7] European Monitoring Centre for Drugs and Drug Addiction (2018), European Drug Report 2018: Trends and Developments, Publications Office of the European Union, Luxembourg. http://www.emcdda.europa.eu/system/files/publications/8585/20181816_TDAT18001ENN_PDF.pdf (accessed 28 March 2019).

[8] M.T. Zanda, L. Fattore, Novel Psychoactive Substances: A New Behavioral and Mental Health Threat, in: R.R. Watson, S. Zibad (Eds.), Addictive Substances and Neurological Disease, Academic Press, 2017, pp. 341-353. https://doi.org/10.1016/B978-0-12-805373-7.00029-3

[9] J. Jerry, G. Collins, D. Streem, Synthetic legal intoxicating drugs: the emerging 'incense'and 'bath salt'phenomenon, Cleve. Clin. J. Med. 79 (2012) 258-264 https://www.doi.org/10.3949/ccjm.79a.11147

[10] European Monitoring Centre for Drugs and Drug Addiction (2017), European Drug Report 2017: Trends and Developments, Publications Office of the European Union, Luxembourg. https://www.emcdda.europa.eu/system/files/publications/4541/TDAT17001ENN.pdf (accessed 28 March 2019).

[11] Observatorio Español de la Droga y las Adicciones (OEDA), 2017. Encuesta sobre Alcohol y Drogas en España (EDADES). Centro de publicaciones del Ministerio de Sanidad, Servicios Sociales e Igualdad. http://www.pnsd.mscbs.gob.es/profesionales/sistemasInformacion/informesEstadisticas/pdf/2017OEDA-INFORME.pdf (accessed 28 March 2019).

[12] F. Caudevilla-Gálligo, M. Ventura, B.I. Indave Ruiz, I. Fornís, Presence and composition of cathinone derivatives in drug samples taken from a drug test service in Spain (2010–2012), Hum. Psychopharmacol. Clin. Exp. 28 (2013) 341-344. https://www.doi.org/10.1002/hup.2296.

[13] Informe sobre las sustancias analizadas en España en 2016 por Energy Control. 2016; Available at: https://energycontrol.org/files/analisis/informeas2016_full2.pdf. (accessed 28 March 2019).

[14] J.M. Prosser, L.S. Nelson, The toxicology of bath salts: a review of synthetic cathinones, J. Med. Toxicol. 8 (2012) 33-42. https://www.doi.org/10.1007/s13181-011-0193-z.

[15] P.I. Dargan, R. Sedefov, A. Gallegos, D.M. Wood, The pharmacology and toxicology of the synthetic cathinone mephedrone (4-methylmethcathinone), Drug. Test. Anal. 3 (2011) 454-463. https://www.doi.org/10.1002/dta.312

[16] National Institute on Drug Abuse; National Institutes of Health; U.S. Department of Health and Human Services. Synthetic Cathinones ("Bath Salts"). 2018; Available at: https://www.drugabuse.gov/publications/drugfacts/synthetic-cathinones-bath-salts (accessed 28 March 2019).

[17] L. Karila, B. Megarbane, O. Cottencin, M. Lejoyeux, Synthetic cathinones: a new public health problem, Curr. Neuropharmacol. 13 (2015) 12-20. https://www.doi.org/10.2174/1570159X13666141210224137

[18] M. Behnke, V.C. Smith, and Committee on Substance Abuse, Prenatal Substance Abuse: Short- and Long-term Effects on the Exposed Fetus, Pediatrics. 131 (2013) e1009-e1024. https://www.doi.org/10.1542/peds.2012-3931

[19] L.G. Strange, K. Kochelek, R. Keasling, S.D. Brown, B.B. Pond, The pharmacokinetic profile of synthetic cathinones in a pregnancy model, Neurotoxicol. Teratol. 63 (2017) 9-13. https://www.doi.org/10.1016/j.ntt.2017.08.001

[20] S. Chomchai, J. Phuditshinnapatra, P. Mekavuthikul, C. Chomchai, Effects of unconventional recreational drug use in pregnancy, Semin. Fetal Neonatal Med. 24 (2019) 142-148 https://www.doi.org/10.1016/j.siny.2019.01.010

[21] N.A. Ghani, M. Eriksson, B. Kristiansson, A. Qirbi, The influence of khat-chewing on birth-weight in full-term infants, Social Science & Medicine. 24 (1987) 625-627. https://doi.org/10.1016/0277-9536(87)90068-2

[22] M. Eriksson, N.A. Ghani, B. Kristiansson, Khat-chewing during pregnancy-effect upon the off-spring and some characteristics of the chewers, East Afr. Med. J. 68 (1991) 106-111.

[23] S. Pichini, M.C. Rotolo, J. García, N. Girona, L. Leal, O. García-Algar, R. Pacifici, Neonatal withdrawal syndrome after chronic maternal consumption of 4-methylethcathinone, Forensic Sci. Int. 245 (2014) e33-e35 . https://www.doi.org/10.1016/j.forsciint.2014.10.027

[24] M. Concheiro, S. Anizan, K. Ellefsen, M.A. Huestis, Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resolution mass spectrometry, Anal. Bioanal. Chem. 405 (2013) 9437-9448. https://www.doi.org/10.1007/s00216-013-7386-z

[25] P.M. O'Byrne, P.V. Kavanagh, S.M. McNamara, S.M. Stokes, Screening of stimulants including designer drugs in urine using a liquid chromatography tandem mass spectrometry system, J. Anal. Toxicol. 37 (2013) 64-73. https://www.doi.org/10.1093/jat/bks091

[26] Y. Al-Saffar, N.N. Stephanson, O. Beck, Multicomponent LC-MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine-experience from the Swedish population, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 930 (2013) 112-120. https://www.doi.org/10.1016/j.jchromb.2013.04.043

[27] L. Mercolini, M. Protti, M.C. Catapano, J. Rudge, A.E. Sberna, LC–MS/MS and volumetric absorptive microsampling for quantitative bioanalysis of cathinone analogues in dried urine, plasma and oral fluid samples, J. Pharm. Biomed. Anal. 123 (2016) 186-194. https://www.doi.org/10.1016/j.jpba.2016.02.015

[28] L.K. Sørensen, Determination of cathinones and related ephedrines in forensic whole-blood samples by liquid-chromatography–electrospray tandem mass spectrometry, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 879 (2011) 727-736. https://www.doi.org/10.1016/j.jchromb.2011.02.010

[29] A. Wohlfarth, W. Weinmann, S. Dresen, LC-MS/MS screening method for designer amphetamines, tryptamines, and piperazines in serum, Anal. Bioanal. Chem. 396 (2010) 2403-2414. https://www.doi.org/10.1007/s00216-009-3394-4

[30] M.J. Swortwood, D.M. Boland, A.P. DeCaprio, Determination of 32 cathinone derivatives and other designer drugs in serum by comprehensive LC-QQQ-MS/MS analysis, Anal. Bioanal. Chem. 405 (2013) 1383-1397. https://www.doi.org/10.1007/s00216-012-6548-8

[31] A. de Castro, E. Lendoiro, H. Fernández-Vega, S. Steinmeyer, M. López-Rivadulla, A. Cruz, Liquid chromatography tandem mass spectrometry determination of selected synthetic cathinones and two piperazines in oral fluid. Cross reactivity study with an on-

site immunoassay device, J. Chromatogr. A. 1374 (2014) 93-101. https://www.doi.org/10.1016/j.chroma.2014.11.024

[32] S. Strano-Rossi, L. Anzillotti, E. Castrignanò, F.S. Romolo, M. Chiarotti, Ultra high performance liquid chromatography–electrospray ionization–tandem mass spectrometry screening method for direct analysis of designer drugs,"spice" and stimulants in oral fluid, J. Chromatogr. A. 1258 (2012) 37-42. https://www.doi.org/10.1016/j.chroma.2012.07.098

[33] P. Amaratunga, B. Lorenz Lemberg, D. Lemberg, Quantitative measurement of synthetic cathinones in oral fluid, J. Anal. Toxicol. 37 (2013) 622-628. https://www.doi.org/10.1093/jat/bkt080

[34] E. Lendoiro, C. Jiménez-Morigosa, A. Cruz, M. Páramo, M. López-Rivadulla, A. de Castro, An LC-MS/MS methodological approach to the analysis of hair for amphetamine-type-stimulant (ATS) drugs, including selected synthetic cathinones and piperazines, Drug Test. Anal. 9 (2017) 96-105. https://www.doi.org/10.1002/dta.1948

[35] A. Salomone, G. Gazzilli, D. Di Corcia, E. Gerace, M. Vincenti, Determination of cathinones and other stimulant, psychedelic, and dissociative designer drugs in real hair samples, Anal. Bioanal. Chem. 408 (2016) 2035-2042. https://www.doi.org/10.1007/s00216-015-9247-4

[36] S. Strano-Rossi, S. Odoardi, M. Fisichella, L. Anzillotti, R. Gottardo, F. Tagliaro, Screening for new psychoactive substances in hair by ultrahigh performance liquid chromatography–electrospray ionization tandem mass spectrometry, J. Chromatogr. A. 1372 (2014) 145-156. https://www.doi.org/10.1016/j.chroma.2014.10.106

[37] J. Lozano, O. García-Algar, O. Vall, R. de la Torre, G. Scaravelli, S. Pichini, Biological matrices for the evaluation of in utero exposure to drugs of abuse, Ther. Drug Monit. 29 (2007) 711-734. https://doi.org/10.1097/FTD.0b013e31815c14ce

[38] M. Concheiro, M.A. Huestis, Drug exposure during pregnancy: analytical methods and toxicological findings, Bioanalysis. 10 (2018) 587-606. https://www.doi.org/10.4155/bio-2017-0260

[39] A. Nemeškalová, M. Bursová, D. Sýkora, M. Kuchař, R. Čabala, T.Hložek, Salting out assisted liquid-liquid extraction for liquid chomatography tandem-mass spectrometry determination of amphetamine-like stimulants in meconium, J Pharm Biomed Anal. 172 (2019) 42-49. https://www.doi.org/10.1016/j.jpba.2019.04.036.

[40] Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology, J. Anal. Toxicol. 37 (2013) 452-474. https://doi.org/10.1093/jat/bkt054

[41] J.S. Krouwer, R. Rabinowitz, How to improve estimates of imprecision, Clin. Chem. 30 (1984) 290-292.

[42] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC−MS/MS, Anal. Chem. 75 (2003) 3019-3030 https://www.doi.org/10.1021/ac020361s

[43] Abstracts of the 2011 International Congress of the European Association of Poisons Centres and Clinical Toxicologists, 24–27 May 2011, Dubrovnik, Croatia Y1 - 2011/03/01, Clin. Toxicol. 49 (2011) 197-269. https://www.doi.org/10.3109/15563650.2011.568269

**Tables**

Table 1. MRM transitions, cone voltage (CV), collision energy (CE), retention time (Rt) and internal standard (IStd) for each compound.

| Compound | MRM transition | CV (V) | CE (eV) | Rt (min) | IStd |
|---|---|---|---|---|---|
| Methylone | <u>208.3 > 160.2</u><br>208.3 > 190.2 | 20 | 18<br>14 | 2.2 | Methylone-$d_3$ |
| Methylone-$d_3$ | 211.4 > 163.3 | 25 | 20 | 2.2 | |
| FMCAT | <u>182.3 > 164.2</u><br>182.3 > 149.1 | 20 | 14<br>20 | 2.3 | Mephedrone-$d_3$ |
| Methedrone | <u>194.3 > 176.2</u><br>194.3 > 161.2 | 20 | 12<br>20 | 3 | Methylone-$d_3$ |
| FMAMP | <u>168.3 > 109.0</u><br>168.3 > 137.1 | 20 | 20<br>12 | 3.7 | MAMP-$d_5$ |
| MAMP-$d_5$ | 155.4 > 121.1 | 20 | 12 | 3. | |
| Mephedrone | <u>178.3 > 160.2</u><br>178.3 > 145.1 | 20 | 12<br>20 | 3.8 | Mephedrone-$d_3$ |
| Mephedrone-$d_3$ | 181.4 > 163.3 | 20 | 14 | 3.8 | |
| MDPV | <u>276.4 > 126.1</u><br>276.4 > 135.1 | 35 | 24<br>26 | 4.9 | TFMPP-$d_4$ |
| TFMPP-$d_4$ | 235.4 > 190.2 | 40 | 24 | 5.2 | |

MRM transition selected for quantification is underlined.

FMCAT: 4-fluoromethcathinone; FMAMP: (±)-4-fluoromethamphetamine; MAMP-$d_5$: methamphetamine-$d_5$; MDPV: 3,4-methylenedioxypyrovalerone; TFMPP-$d_4$: 3-trifluoromethylphenylpiperazine-$d_4$

Table 2. Limits of detection (LOD), limits of quantification (LOQ) and calibration parameters.

| Compound | LOD (ng/g) | LOQ (ng/g) | Range (ng/g) | Intercept ± SD | Slope ± SD | $r^2$ ± SD |
|---|---|---|---|---|---|---|
| Methylone | 0.5 | 1 | 1-200 | 0.0452 ± 0.0543 | 0.2914 ± 0.0169 | 0.9979 ± 0.0017 |
| FMCAT | 1 | - | - | - | - | - |
| Methedrone | 1 | - | - | - | - | - |
| FMAMP | 0.5 | 1 | 1-200 | 0.0898 ± 0.0687 | 0.3458 ± 0.0544 | 0.9967 ± 0.0016 |
| Mephedrone | 0.5 | 1 | 1-200 | 0.0654 ± 0.0510 | 0.2752 ± 0.0252 | 0.9981 ± 0.0014 |
| MDPV | 0.5 | 2 | 2-200 | 0.3885 ± 0.1320 | 0.5548 ± 0.0923 | 0.9960 ± 0.0055 |

FMCAT: 4-fluoromethcathinone; FMAMP: (±)-4-fluoromethamphetamine; MDPV: 3,4-methylenedioxypyrovalerone

Table 3. Imprecision and accuracy in meconium at low (3 ng/g), medium (30 ng/g) and high (150 ng/g) QC concentrations for those analytes validated for quantitative purposes.

| Compound | Intra-assay imprecision (n=15; %CV) | | | Inter-assay imprecision (n=15; %CV) | | | Total imprecision (n=15; %CV) | | | Accuracy (n=15; % target concentration) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Low QC | Medium QC | High QC | Low QC | Medium QC | High QC | Low QC | Medium QC | High QC | Low QC | Medium QC | High QC |
| Methylone | 5.7 | 1.6 | 2.4 | 4 | 6.6 | 7.6 | 6.9 | 6.8 | 7.9 | 94.9 | 94.6 | 95.3 |
| FMAMP | 7.9 | 2.7 | 4.5 | 5.4 | 5.9 | 8.9 | 9.6 | 6.5 | 10 | 92.9 | 89.2 | 92.9 |
| Mephedrone | 4.5 | 1.9 | 1.7 | 5.6 | 5.7 | 6.6 | 7.2 | 6 | 6.8 | 95.8 | 97.5 | 96.4 |
| MDPV | 5.7 | 6 | 3.4 | 0 | 4 | 8.8 | 5.7 | 7.2 | 9.4 | 87.3 | 97.8 | 94.8 |
| FMAMP: (±)-4-fluoromethamphetamine; MDPV: 3,4-methylenedioxypyrovalerone | | | | | | | | | | | | |

Table 4. Matrix effect (ME), extraction efficiency (EE) and process efficiency (PE) at low (3 ng/g) and high (150 ng/g) QC concentrations.

| Compound | ME (%) (%CV) (n=10) | | EE (%) (%CV) (n=6) | | PE (%) (n=6) | |
|---|---|---|---|---|---|---|
| | Low QC | High QC | Low QC | High QC | Low QC | High QC |
| Methylone | -40.2 (18.8) | -41.4 (13.3) | 70 (4.8) | 78.5 (5.3) | 41.9 | 46 |
| Methylone-$d_3$ | -38.8 (18.1) | -34.2 (13.7) | 67.7 (4.9) | 77.6 (4.9) | 41.5 | 51 |
| FMCAT | -76 (17.7) | -72.1 (15) | 66.8 (6.8) | 65.7 (19.2) | 16 | 18.3 |
| Methedrone | -39.1 (11.8) | -41.3 (8.4) | 70.9 (4.5) | 77.7 (3.6) | 43.2 | 45.6 |
| FMAMP | -36.2 (13.2) | -28.1 (10) | 78.8 (2.8) | 83.7 (2.3) | 50.3 | 60.2 |
| MAMP-$d_5$ | -18.1 (10) | -15.6 (7.1) | 81.1 (3.5) | 81.9 (4.9) | 66.4 | 69.1 |
| Mephedrone | -67.7 (15.7) | -66.2 (11.8) | 63.7 (5) | 71.6 (9.1) | 20.6 | 24.2 |
| Mephedrone-$d_3$ | -66 (16) | -62.6 (11.6) | 60.5 (5.1) | 71.6 (7.6) | 20.6 | 26.8 |
| MDPV | -63.8 (10.9) | -51.8 (9.9) | 85.9 (5.4) | 91.3 (1.9) | 31.1 | 41.4 |
| TFMPP-$d_4$ | -62.1 (12.1) | -58.5 (11.5) | 74.1 (6.8) | 84.3 (8.4) | 28.1 | 35 |

FMCAT: 4-fluoromethcathinone; FMAMP: (±)-4-fluoromethamphetamine; MAMP-$d_5$: methamphetamine-$d_5$; MDPV: 3,4-methylenedioxypyrovalerone; TFMPP-$d_4$: 3-trifluoromethylphenylpiperazine-$d_4$; MAMP-$d_5$: methamphetamine-$d_5$

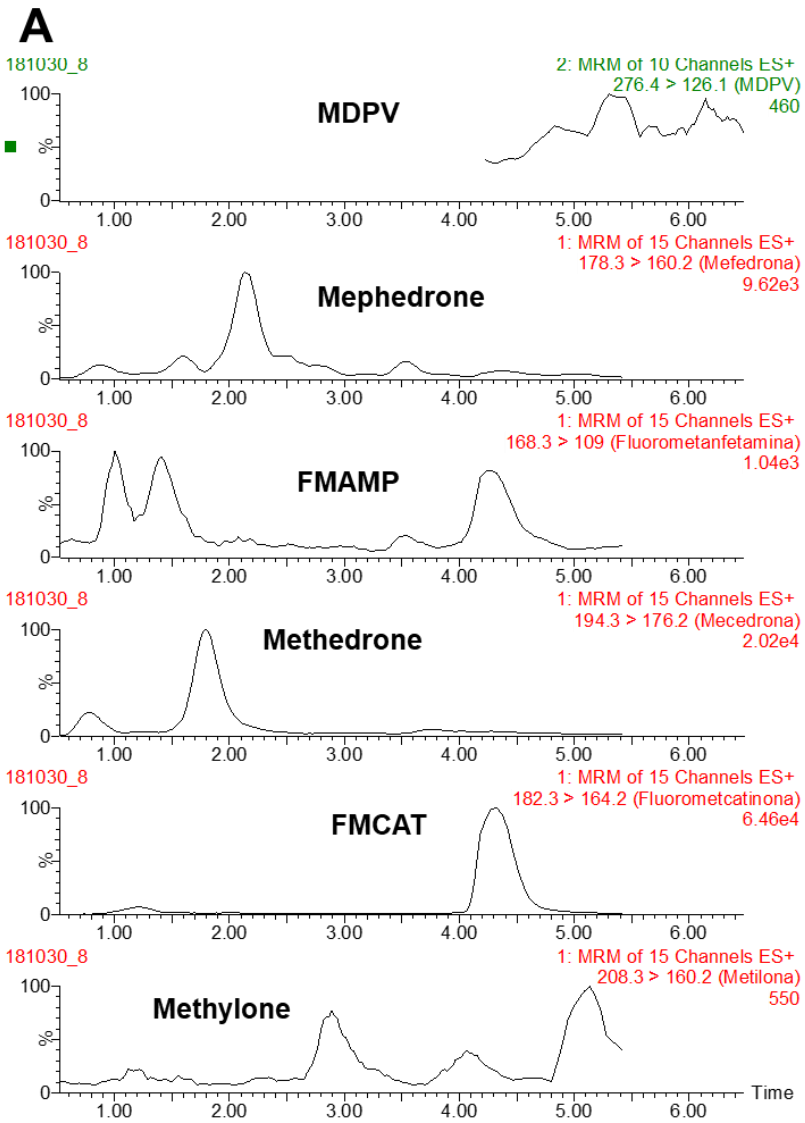
Table 5. Stability at low, medium and high QC concentrations after storage in the autosampler (6°C) for 72 h, expressed as %loss.

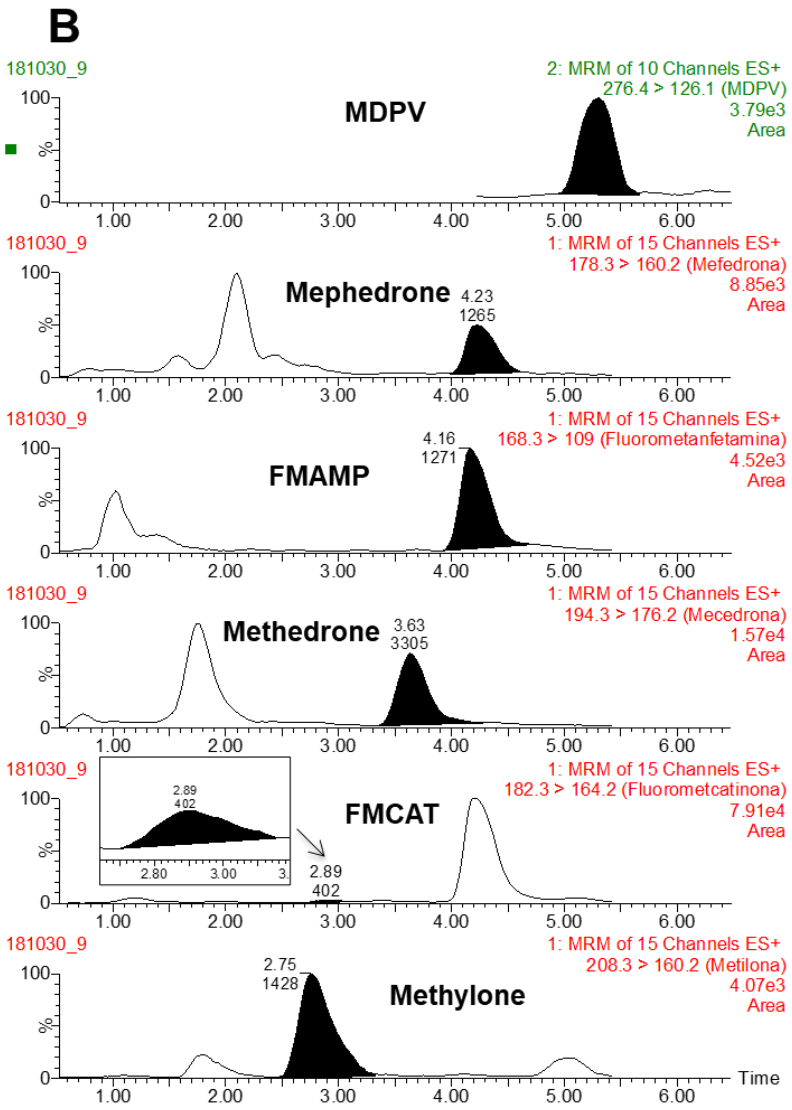
| Compounds | Autosampler stability (n=3) | | |
|---|---|---|---|
| | Low QC | Medium QC | High QC |
| Methylone | -4.3 | -4.2 | 0.9 |
| FMCAT | -3.9 | -3.6 | -0.1 |
| Methedrone | 1 | -2.9 | -4.1 |
| FMAMP | 2.3 | 1.2 | -3.9 |
| Mephedrone | -6.7 | -4.6 | -2.9 |
| MDPV | 5.1 | -6.4 | -6.3 |

FMCAT: 4-fluoromethcathinone; FMAMP: (±)-4-fluoromethamphetamine; MDPV: 3,4-methylenedioxypyrovalerone

**Figure**

Figure 1. MRM chromatograms of the quantifier transition for all the analytes in a blank meconium sample (A) and in a blank meconium sample fortified at the corresponding LOD for each analyte (B). FMCAT: 4-fluoromethcathinone; FMAMP: (±)-4-fluoromethamphetamine; MDPV: 3,4-methylenedioxypyrovalerone




B
181030_9
2: MRM of 10 Channels ES+
276.4 > 126.1 (MDPV)
3.79e3
Area
MDPV
100
%
0
1.00
2.00
3.00
4.00
5.00
6.00
181030_9
1: MRM of 15 Channels ES+
178.3 > 160.2 (Mefedrona)
8.85e3
Area
Mephedrone
4.23
1265
181030_9
1: MRM of 15 Channels ES+
168.3 > 109 (Fluorometanfetamina)
4.52e3
Area
FMAMP
4.16
1271
181030_9
1: MRM of 15 Channels ES+
194.3 > 176.2 (Mecedrona)
1.57e4
Area
Methedrone
3.63
3305
181030_9
1: MRM of 15 Channels ES+
182.3 > 164.2 (Fluorometcatinona)
7.91e4
Area
FMCAT
2.89
402
2.80
3.00
181030_9
1: MRM of 15 Channels ES+
208.3 > 160.2 (Metilona)
4.07e3
Area
Methylone
2.75
1428
Time