

**GC-MS QUANTIFICATION METHOD FOR MEPHEDRONE IN  
PLASMA AND URINE: APPLICATION TO HUMAN  
PHARMACOKINETICS**

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## **Abstract**

Increasing consumption has been observed among young people of new psychoactive substances (NPS), including synthetic cathinone derivatives. The most well-known of these is mephedrone whose use has been related to acute intoxication and fatality. Several methods able to detect mephedrone have been reported, although to date, none have been applied to human pharmacokinetic studies in a controlled setting. We developed a gas chromatography-mass spectrometry (GC-MS) technique for mephedrone quantification in human plasma and urine. Plasma after deproteinization and urine were submitted to a liquid-liquid extraction and derivatization of the extract with MSTFA prior to analysis. Calibration curves covered concentration ranges in plasma between 5–300 ng/mL and in urine between 20-1500 ng/mL. The method has been successfully applied to biological samples obtained from a pilot clinical trial intended to evaluate the human pharmacology of mephedrone and its relative bioavailability and pharmacokinetics. Six healthy males were administered 150 mg of mephedrone by the oral route in a randomized, double-blind, crossover controlled trial. Peak plasma concentration ( $C_{max}=122.6 \pm 32.9$  ng/mL) was reached at 1 hour (0.5-2 h) post-drug administration. Mephedrone showed a rapid elimination half-life ( $t_{1/2}=2.2$  h) compared to other psychostimulants. Less than 15% of the dose was excreted in urine as a free-form. Mephedrone concentrations displayed a relevant inter-subject variability.

## **1. Introduction**

There has recently been an increase among young people in the consumption of new psychoactive substances (NPS) including synthetic cathinone derivatives (1, 2). Mephedrone (4-methylmethcathinone, 4-MMC also known as “meow-meow” and “m-cat”), a beta-keto-amphetamine psychostimulant drug with structural and mechanistic similarities to methamphetamine and methylenedioxymethamphetamine (MDMA, ecstasy), is the best known of the synthetic cathinones (1, 3). It is available on the market as a white powder (hydrochloride salt) or a yellowish liquid (base) at ambient temperature (4). It is usually ingested nasally and/or orally (5–7) and, less frequently, intravenously. Acute intoxication and death associated with mephedrone misuse have been reported (8–11) although, with the exception of a recent publication, there is limited information about its pharmacology and pharmacokinetics in humans (12).

Mephedrone has been identified and quantified in humans by several methods and matrices. With gas chromatography-mass spectrometry (GC-MS) a technique based on a solid-phase extraction (SPE) has been reported (13). With liquid chromatography tandem mass spectrometry (LC-MS/MS) mephedrone was detected in several matrices such as dried blood spots, dried plasma, urine, oral fluid, and hair (14–17). Most of the available literature is limited to case reports of intoxications, screenings, and post-mortem analyses (11–20).

The evaluation of mephedrone pharmacology and its pharmacokinetics in humans requires the development of an analytical method for its detection and quantification in both plasma and urine.

The aim of the present study is to develop and validate a GC-MS analytical method for further application in controlled clinical studies. The technique has been employed in a clinical trial with plasma and urine samples from healthy subjects after the oral administration of a single 150 mg dose of mephedrone.

## **2. Material and Methods**

### **2.1. Chemical and reagents**

Mephedrone was purchased from LGC Standards Proficiency Testing (Lancashire, UK) and mephedrone-d<sub>3</sub> was obtained from TRC Toronto Research Chemicals (Ontario, Canada). Perchloric acid (70 %), methyl tert-butyl ether, sodium bicarbonate, sodium carbonate, and ultra-pure water Milli-Q were purchased from Merck (Darmstadt, Germany). N-Methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) was supplied by Macherey-Nagel (Düren, Germany). Drug-free plasma was obtained from the Hospital del Mar blood bank (Barcelona, Spain), and drug-free urine was collected from healthy volunteers.

### 2.2. Preparation of standards

A separate stock solution (1 mg/mL) of mephedrone and its internal standard (ISTD) mephedrone-d<sub>3</sub> (100 µg/mL) was prepared in HPLC-grade methanol and stored at -20 °C in amber vials. From the stock solution, working solutions of 100, 10, and 1 µg/mL mephedrone were obtained and used for the preparation of calibration curves. Mephedrone-d<sub>3</sub> was diluted in methanol in a working solution of 10 µg/mL and stored at the same previously described conditions until use.

### 2.3. Instrumentation

GC-MS analyses were carried out with an Agilent 6890 Series Plus gas chromatograph equipped with an Agilent 7683 autosampler and coupled to a 5973 N mass selective detector (Agilent Technologies, Palo Alto, CA, USA). Data acquisition and analysis were performed using standard software supplied by the manufacturer (Agilent Chemstation). Separation was carried out with a cross-linked 5 % phenylmethylsiloxane capillary column (ZB-5MS, Zebron Capillary GC Column, Phenomenex; California, USA). Helium was used as carrier gas at an initial flow rate of 1.2 mL/min under a constant pressure of 12 psi.

For plasma analysis, initial oven temperature was programmed at 100 °C (hold for 1 min), followed by a 15 °C/min ramp to 250 °C (hold for 2 min), and finally increased by a 40 °C/min ramp to reach 300 °C (hold 1 min). Total run time was 15.25 min and samples were injected in splitless mode. Injector and detector temperatures were set at 280 °C. The injection volume was 2 µL per sample.

In order to avoid a matrix interference at the mephedrone retention time observed in urine, the ramp temperature was slightly modified: initial oven temperature was 100°C (hold for 1 min), followed by a 5°C/min ramp to 120°C (hold for 2 min), then 15°C/min ramp to 250°C (hold for 1 min), and finally increased 40°C/min ramp to 320°C (hold for 1 min). Total run time was 19.42 min. Samples were injected in split (1:15) mode and the injection volume was 1 µL per sample.

The mass spectrometer was operated in electron ionization (EI) mode at 70 eV. Mass spectra of the analyte and ISTD were recorded in scan mode (scan range 40–550 m/z) to determine retention times and characteristic mass fragments. Three characteristic ions were monitored in the selected-ion-monitoring (SIM) mode. Ions at m/z 234 and m/z 219, and at m/z 237 and m/z 222, were chosen for the qualitative analysis of the *N*-TMS derivatives of mephedrone and mephedrone-d<sub>3</sub>, respectively. Ions at m/z 130 (mephedrone-*N*-TMS) and m/z 133 (mephedrone-d<sub>3</sub>-*N*-TMS) were selected for the quantitative analysis (see Figure 1).

## 2.4 Validation method

### 2.4.1 Interference studies

The absence of any inference is necessary to guarantee the proper detection and quantification of the targeted analyte. To check endogenous interferences, 10 blank plasma and urine samples were analyzed. Another set of 10 blanks for each matrix was prepared with a known concentration of the internal standard (200 ng/mL in plasma and 500 ng/mL in urine) to assess if there was an interaction between the analyte and the internal standard.

### 2.4.2 Linearity

The linearity of the method was checked by preparing four calibration curves in duplicate at six different concentrations: 5, 10, 25, 50, 100, and 300 ng/mL for plasma, and 20, 50, 200, 500, 1000, and 1500 ng/mL for urine, on three consecutive days. Calibration curves were prepared daily for each analytical batch by adding appropriate amounts of methanol working solutions of mephedrone and a constant concentration of the internal standard (200 ng/mL plasma; 500 ng/mL urine) to the blank matrix (1 mL of blank plasma; 0.5 mL of blank urine). A weighted (1/concentration) least-square regression analysis was used (Statistical Package for the Social

Sciences SPSS, version 18.0 for Microsoft Windows, Microsoft Corp., Seattle, USA). Calculations were performed with peak area ratios between mephedrone and mephedrone-d<sub>3</sub>. Plasma control samples containing mephedrone at four different concentrations: low limit of quantification (LLOQ) [5 ng/mL], low control (QC-L) [40 ng/mL], medium control (QC-M) [80ng/mL], and high control (QC-H) [200 ng/mL], were prepared in drug-free plasma and kept frozen at -20°C in 1 mL aliquots. Urine control samples were prepared with mephedrone at the following concentrations: LLOQ [20 ng/mL], QC-L [40 ng/mL], QC-M [800 ng/mL], and QC-H [1200 ng/mL]. All samples were randomly analyzed in order to assess carry-over. Blank samples used to evaluate carry-over effect were extracts of blank matrix samples. These blank samples were placed after samples with a high analyte concentration or calibration standard at the upper limit of quantification.

#### 2.4.3 Precision and accuracy

The within-day and between-day precision and accuracy of the method in both matrices were evaluated by the quadruplicate analysis of the LLOQ, QC-L, QC-M, and ULOQ over a three-day validation protocol.

Precision of the assay was expressed as the relative standard deviation (RSD) of the concentration values obtained from the control sample replicates. Accuracy was calculated as the percentage of difference between the observed concentrations and the nominal concentration (ERR). Values of RSD and ERR lower than 15% for the QC-L, QC-M, and ULOQ, and below 20% for the LLOQ were considered acceptable.

#### 2.4.4 Limit of detection and lower and upper limits of quantification

The limit of quantification (LLOQ) was the lowest calibration concentration experimentally tested with acceptable accuracy and precision. Thus the upper limit of quantification (ULOQ) was the highest calibration concentration point to enable an appropriate pharmacokinetic description of mephedrone (as suggested by the European Medicines Agency (21)). The limit of detection was estimated as the standard deviation (SD) of the LLOQ divided by the slope (of the calibration curve) and multiplied by 3.3.

Mephedrone detection was achieved by measuring the areas ratios between each ion. A quantitative ion was assigned as 100% and a percentage for each qualitative ion was obtained dividing its area by the quantitative one. For mephedrone identification to be considered acceptable, area ratios between each ion had to be constant in all samples (with a range of  $\pm 5\%$ , relative percentage).

#### 2.4.5 Recovery

Recovery (in both matrices) was calculated as the quotient of the response of standard solutions spiked into matrices before extraction, and the response of standard solutions spiked after extraction. The spiked concentrations for plasma mephedrone and mephedrone-d<sub>3</sub> concentrations were: 5 ng/mL and 200 ng/mL, respectively. Analyte and internal standard concentrations in urine were 20 and 500 ng/mL, respectively. Subsequently, the ratio between the areas before and after mephedrone and mephedrone-d<sub>3</sub> extraction peak was calculated and expressed as a percentage.

#### 2.4.6 Stability

Mephedrone intra-assay stability was evaluated throughout the time period of one analytical batch. Variance analysis was conducted taking into account the mephedrone area as a dependent variable, the concentration level as a factor, and injection time as a covariable.

The QC samples described in Section 2.4.2 were used to verify the storage stability of mephedrone in plasma and urine at -20°C for periods of 1 week, 2 weeks, 1 month, 2 months, 3 months, and 6 months in duplicate. The stability of the analyte after undergoing two freeze–thaw cycles at -20°C was also tested using a paired sample t-test (SPSS, version 18.0).

### 2.5. Pharmacokinetics study

#### 2.5.1 Study design

Our study forms part of a pilot dose-finding one. In order to select a definitive mephedrone dose so as to compare its effects with MDMA, mephedrone was administered at progressive quantities of 50 mg, 100 mg, 150 mg, and 200 mg. The study has been recently published and included 200 mg of mephedrone, 100 mg of MDMA, and placebo (12). We present here the data from the analytical method validation after one of the doses administered (150 mg). All the

trials were randomized double-blind and cross-over. Subjects were healthy, recreational users of MDMA and other stimulants including mephedrone. They signed an informed consent and were financially compensated for their participation. The protocol was authorized by the local ethical committee (CEIC-PSMAR reference 2013/5045) and registered at clinicaltrials.gov (NCT02232789). Six healthy male volunteers with medium age of  $36 \pm 4$  years, an average weight of  $68 \pm 4$  kg, and a BMI of  $21 \pm 2$  kg/m<sup>2</sup> participated. All the participants were CYP2D6 extensive metabolizers (tested with dextromethorphan/dextorphan ratio in urine following a 30 mg dose of dextromethorphan). CYP2D6 is described as the main responsible enzyme for the *in vitro* phase I metabolism of mephedrone (22). Treatment condition was 150 mg of mephedrone, administered orally with 250 mL of water to swallow. Mephedrone powder, obtained from the Spanish Ministry of Justice and the Ministry of Health, was administered as hard gelatine capsules. Other details of the pilot study have been previously published (12).

#### 2.5.2 Biological sample collection

Blood samples were obtained through a catheter inserted in a peripheral vein both before ( $t = 0$ , baseline) and after drug administration at 15, 30, 45 minutes and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours. Blood was collected in heparin tubes (6 mL) and centrifuged at 3000 rpm for 10 min at 4 °C. Plasma was stored in polypropylene tubes at -20 °C until analysis.

Urine was collected before ( $t = 0$ ) and at several time interval periods after mephedrone administration (0-4 h, 4-8 h, 8-12 h, 12-24 h, and 24-48 h). Excreted volume was measured and aliquots of 10 mL were stored at -20 °C until analysis.

#### 2.5.3 Biological sample preparation

An aliquot of 1 mL of human plasma was transferred into 1.5 mL Protein Low Binding Micro tube (Sarstedt AG & Co, Germany) and spiked with 20 µl of mephedrone-d3 (from a 10 µg/mL solution). The sample was precipitated with 100 µl perchloric acid (70 %), mixed in a vortex, and centrifuged for 10 minutes at 4 °C/13000 r.p.m. The supernatant was transferred into 15 mL screw-capped amber glass tubes. Samples were neutralized with 400 mg of NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub> (1:2 p/p) and the pH adjusted to 10.5-11 with 2 ml of 0.05 M Na<sub>3</sub>PO<sub>4</sub> buffer (pH=11). Methyl tert-butyl ether (5 mL) was added, the samples shaken for 30 min in a rocking mixer and then

centrifuged at 3500 rpm for 5 min. The organic layer from each sample was transferred into a clean amber tube and evaporated to dryness under nitrogen stream at <30 °C (10-15 psi pressure). Trimethylsilyl derivatives were formed with 100 µL of MSTFA as a derivatizing agent in a dry bath at 60 °C for 30 min.

In the case of urine, sample preparation was practically the same as that of plasma but without protein precipitation. As the range of concentrations was notably higher than that of plasma the amount of urine added to each sample was diluted 1:1 with water (0.5 mL of urine/0.5 mL of MilliQ grade pure water).

#### 2.5.4 Experimental Pharmacokinetics Analysis

The maximum plasma concentration ( $C_{max}$ ) and the time to reach peak concentration ( $T_{max}$ ) were determined experimentally from time-course plasma concentrations. Terminal slope ( $K_e$ ) and elimination half-life ( $t_{1/2}$ ) were calculated using the concentrations of the elimination phase. The area under the curve (AUC) was calculated by the trapezoidal method at different time periods (0-12 h and 0-24 h). Plasmatic clearance ( $Cl_{plasmatic}$ ) and the absolute distribution volume ( $V_d$ ) were calculated considering a hypothetical bioavailability of 10% of mephedrone (estimated in rats by Martínez-Clemente J. et al (23)). Renal clearance ( $Cl_{renal}$ ) was obtained as the result of the total amount of excreted free mephedrone in urine divided by the  $AUC_{0-24h}$ . The percentage of mephedrone eliminated in urine was estimated as:  $Cl_{renal} / Cl_{plasmatic} * 100$ . All values were calculated using pharmacokinetic functions for Microsoft Excel<sup>1</sup>. All samples (for each subject) were analyzed in the same batch of analysis.

### **3. Results**

#### 3.1. Analytical method

Following the previously described chromatographic conditions, mephedrone was successfully separated from background noise. In the case of urine a different temperature program was applied to avoid interfering peaks. Figure 2 shows the representative extract ion chromatograms in plasma and urine.

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Calibration curves in plasma and urine were linear between the 5-300 ng/mL and 20-1500 ng/mL concentration ranges, respectively. In both matrices coefficients of determination ( $r^2$ ) were greater than 0.99. The values of precision and accuracy for between run (inter-assay of the three validation days) and for within run (intra-assay of one of the validation days) are reported in Table 1. All values correspond to the criteria of the *Guideline on bioanalytical method validation* (European Medicines Agency; 2012 (21)). Moreover, no significant carry-over was detected when blank samples were injected after the highest point of the calibration curve.

Regarding mephedrone stability, no significant correlation ( $p > 0.05$ ) between the area response of mephedrone and time were found. There were no significant changes in mephedrone concentration in either plasma or urine samples when stored for up to 6 months at  $-20\text{ }^\circ\text{C}$ , even when both matrices had undergone two freeze-thaw cycles.

LODs of the presented method were 1.1 ng/mL in plasma and 4.5 ng/mL in urine. LLOQs were 5 ng/mL in plasma and 20 ng/mL in urine. Recoveries were  $54.7\% \pm 2.4$  in plasma and  $92.3\% \pm 12.9$  in urine for mephedrone.

### 3.2. Human pharmacokinetics

The average plasma concentration-time curve and mean recovery of unaltered mephedrone in urine are provided in Figure 3. Pharmacokinetic parameters are summarized in Table 2. The average peak plasma concentration of mephedrone was 122.6 ng/mL (range between 79.5 and 162.3 ng/mL).

All pre-dose plasma samples were negative for mephedrone (LOD, 1.1ng/mL). After mephedrone administration, only 33 % of the volunteers at 15 minutes had concentrations above LLOQ. At 12 h post-drug administration, only 66% of the subjects had concentrations over LLOQ.. In urine all values fitted within the range described by the calibration curve (between 20-1500 ng/mL).

## **4. Discussion**

Mephedrone has psychoactive effects and physiological actions similar to MDMA in humans (12) and appears to have been responsible for several acute intoxications and fatalities (20). The

development of an analytical method for its quantification in the main biological fluids can lead to a better understanding of its pharmacology.

In the present report, we have developed and validated a simple GC/MS method for the quantification of mephedrone in conventional human matrices: urine and plasma. This technique successfully evaluated mephedrone pharmacokinetics in a controlled study with healthy subjects within the framework of the evaluation of its clinical pharmacology.

One of the strengths of the method presented here is that the same procedure can be applied to plasma and urine, the two main human matrices. The use of mephedrone-d<sub>3</sub> as an internal standard is recommended.

The technique presented in this work had an appropriate LLOQ in urine, as all experimental values were within the calibration curve. Nevertheless, in plasma some extreme values were below the LLOQ and were excluded, in part due to high variability in mephedrone bioavailability. A further improvement in the sensitivity of the method is recommended for the plasma matrix. A change in the instrumental technique from GC/MS to a triple quadrupole one (GC/MS/MS) should suffice without any further modification in sample preparation procedure.

The pharmacokinetic parameters demonstrated that mephedrone at 150 mg has a rapid absorption, as the maximum plasma concentration was reached 1 hour after administration. The results are similar to those obtained after 200 mg administration (12). It is substantially faster than other related psychostimulants such as MDMA (T<sub>max</sub> = 2.3 h) (24), cathinone, cathine, and norephedrine (T<sub>max</sub> = 2.3 h, 2.6 h, and 2.8 h, respectively (25)). The elimination of mephedrone is also relatively rapid as the plasma half-life is 2.2 hours.

No data are available on mephedrone oral bioavailability in humans, an estimation of 10 % oral bioavailability performed in rats was used as a reference (23). With this value, around 15 % of mephedrone (for a 150 mg dose) was eliminated unmodified in urine. Mephedrone is essentially cleared by an extensive and complex metabolism as supported by the literature (26).

An interesting outcome of our work is the large inter-subject variability observed in the subjects throughout the study (see Table 2, Figure 3). This could have been due to their being extensive metabolizers for CYP2D6, an isoenzyme of cytochrome P450 displaying a polymorphism (22).

Nevertheless, taking into account the complexity of mephedrone metabolism (26), other sources of variability not as yet defined cannot be discarded. The addition of mephedrone metabolites in the presented method was not possible due to the lack of reference substances which are currently being synthesized.

Another factor is the low bioavailability of the substance when ingested orally as previously described in rats [20]; we hypothesize that this also takes place in humans. As a result of fluctuating bioavailability and the large quantity of metabolites, mephedrone plasma concentrations and AUC differ more than expected among individuals. Drugs with greater oral bioavailability, such as MDMA which presents approximately 80 %, display less interindividual-variability (24).

## **5. Conclusions**

A simple GC-MS method for the quantification of mephedrone in human urine and plasma samples has been developed and validated. The technique has been applied to a controlled bioavailability study in humans. The pharmacokinetics variability observed when mephedrone is ingested orally may have clinical relevance, as consumers could experience differing pharmacological and adverse effects for the same dose.

## **Conflict of interest**

The authors declare no potential conflicts of interest.

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Figure 1. Electron ionization mass spectra of (A) mephedrone-N-TMS and (B) mephedrone-d<sub>3</sub>-N-TMS.

Figure 2. Mephedrone-N-TMS extract ion chromatograms (m/z 130) from (A) human blank plasma (top), LLOQ (5 ng/mL, medium) and plasma from a subject (86,3 ng/mL, bottom) and (B) human blank urine (top), LLOQ (20 ng/mL, medium) and urine from the same subject (85,3 ng/mL, bottom).

Figure 3. (A) Time course of mephedrone plasma concentration (n=6 human subjects, dose=150 mg) (B) Quantity of free-mephedrone excreted in urine (n=6, dose=150 mg).

Table 1. Within run and between run accuracy and precision data mephedrone analysis in plasma and urine.

Table 2. Data of main pharmacokinetics parameters (n=6 and dose=150 mg mephedrone). Mean values ± standard deviation are calculated with the exception of T<sub>max</sub> whose median (range) is reported. §Values calculated with a hypothesized bioavailability of 10%.

Table 1. Within run and between run accuracy and precision data mephedrone analysis in plasma and urine.

| Matrices      | Controls | Concentration<br>(ng/mL) | Within Run                         |                      |                        | Between Run                        |                      |                        |
|---------------|----------|--------------------------|------------------------------------|----------------------|------------------------|------------------------------------|----------------------|------------------------|
|               |          |                          | Estimated<br>concentration<br>± SD | Precision<br>(CV, %) | Accuracy<br>(error, %) | Estimated<br>concentration ±<br>SD | Precision<br>(CV, %) | Accuracy<br>(error, %) |
| <b>Plasma</b> | LLOQ     | 5                        | 5.2 ± 0.5                          | 8.9                  | 6.9                    | 4.9 ± 0.5                          | 9.7                  | 8.5                    |
|               | QC-L     | 40                       | 36.4 ± 0.9                         | 2.6                  | 9.1                    | 37.3 ± 2.4                         | 6.5                  | 8.3                    |
|               | QC-M     | 80                       | 74.0 ± 9.8                         | 13.3                 | 11.8                   | 76.8 ± 7.4                         | 9.6                  | 8.4                    |
|               | QC-H     | 200                      | 201.1 ± 3.3                        | 1.6                  | 1.5                    | 201.0 ± 5.6                        | 2.8                  | 2.1                    |
| <b>Urine</b>  | LLOQ     | 20                       | 21.6 ± 0.8                         | 3.5                  | 8.0                    | 22.7 ± 1.5                         | 6.7                  | 13.2                   |
|               | QC-L     | 40                       | 42.1 ± 2.6                         | 6.2                  | 7.0                    | 44.3 ± 3.1                         | 7.1                  | 10.6                   |
|               | QC-M     | 800                      | 814.2 ± 40.1                       | 4.9                  | 4.5                    | 792.6 ± 43.6                       | 5.5                  | 4.3                    |
|               | QC-H     | 1200                     | 1270.1 ± 37.8                      | 3.0                  | 5.8                    | 1255.2 ± 31.2                      | 2.5                  | 4.9                    |

Table 2. Data of main pharmacokinetics parameters (n=6 and dose=150 mg mephedrone). Mean values  $\pm$  standard deviation are calculated with the exception of T<sub>max</sub> whose median (range) is reported. § Values calculated with a hypothesized bioavailability of 10%.

| Parameters                        | Units           | Mean $\pm$ SD                 |
|-----------------------------------|-----------------|-------------------------------|
| C <sub>max</sub>                  | ng/mL           | 122.6 $\pm$ 32.9              |
| AUC <sub>0-12h</sub>              | ng·h/mL         | 438.7 $\pm$ 171.3             |
| AUC <sub>0-∞</sub>                | ng·h/mL         | 460.9 $\pm$ 187.7             |
| K <sub>e</sub>                    | h <sup>-1</sup> | 0.32 $\pm$ 0.04               |
| T <sub>1/2</sub>                  | h               | 2.20 $\pm$ 0.28               |
| T <sub>max</sub>                  | h               | 1.0 (0.5-2)                   |
| Vd <sup>§</sup>                   | L               | 123.5 <sup>§</sup> $\pm$ 64.0 |
| Cl <sub>plasma</sub> <sup>§</sup> | L/h             | 41.1 <sup>§</sup> $\pm$ 26.1  |
| Cl <sub>renal</sub>               | L/h             | 5.6 $\pm$ 2.6                 |
| Urine excretion                   | %               | 15.4 $\pm$ 8.4                |

Figure 1. Electron ionization mass spectra of (A) mephedrone-N-TMS and (B) mephedrone-d<sub>3</sub>-N-TMS.

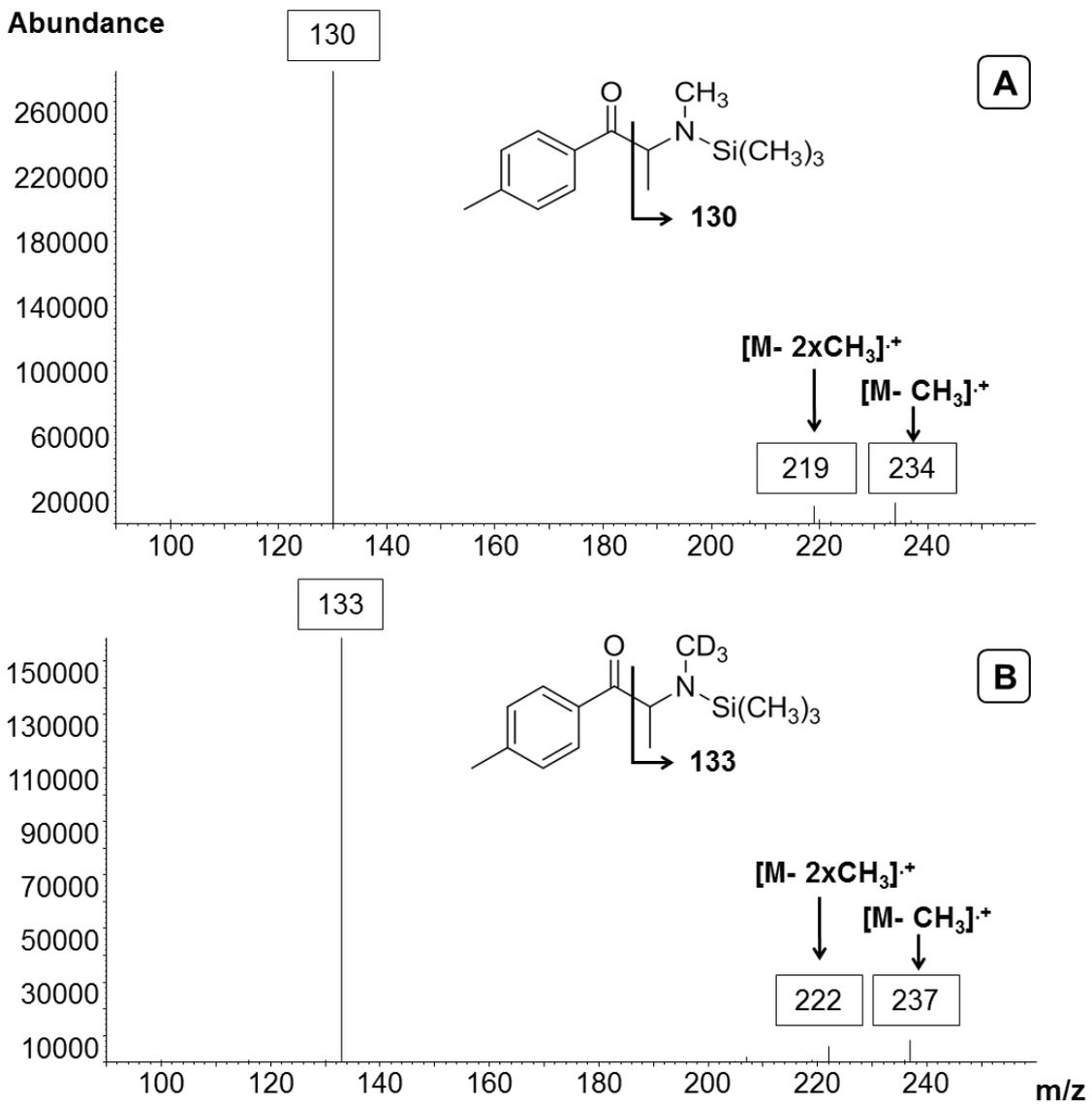


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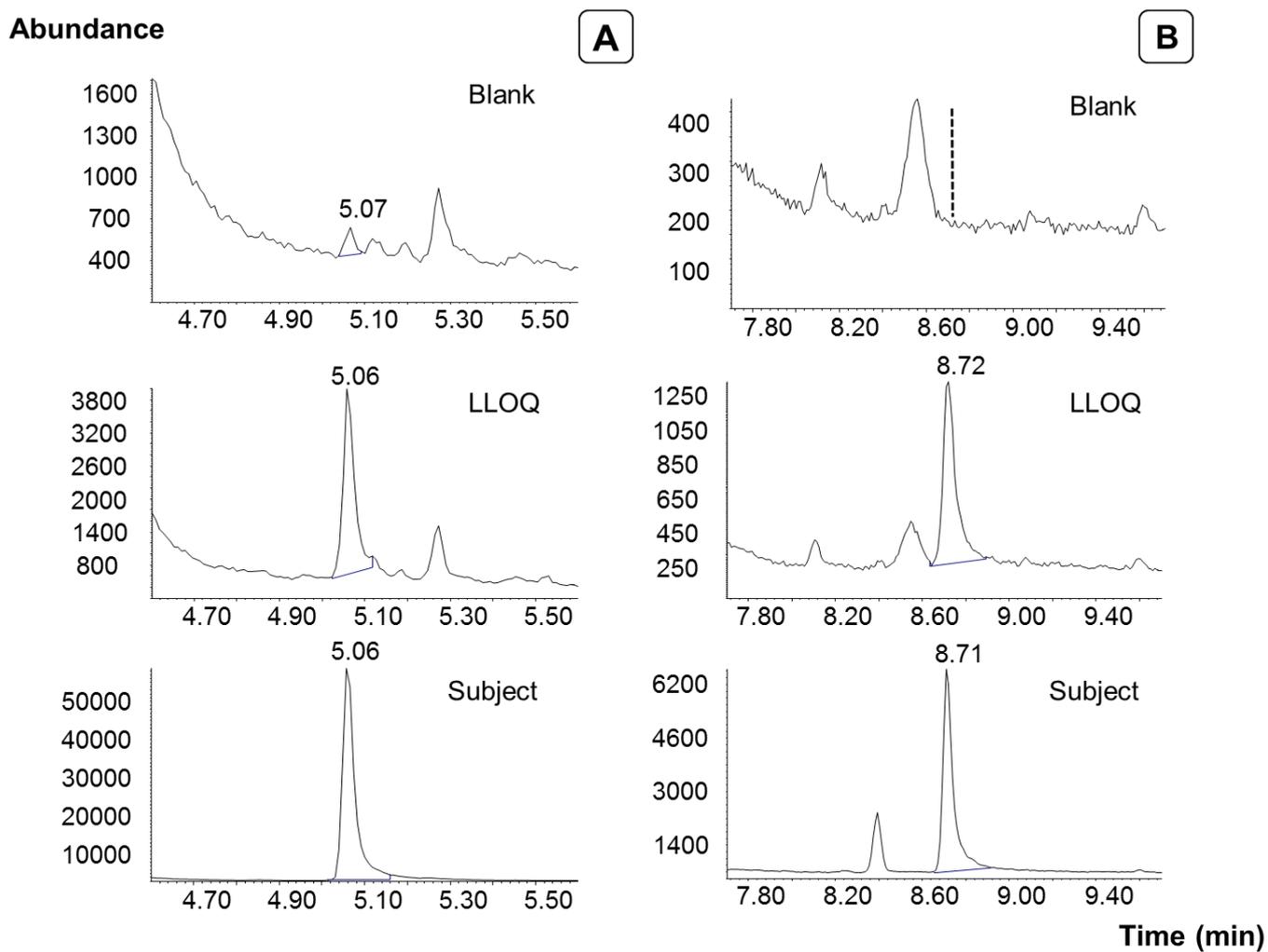


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