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Trapped ion mobility mass spectrometry of new psychoactive substances: Isomer-specific identification of ring-substituted cathinones

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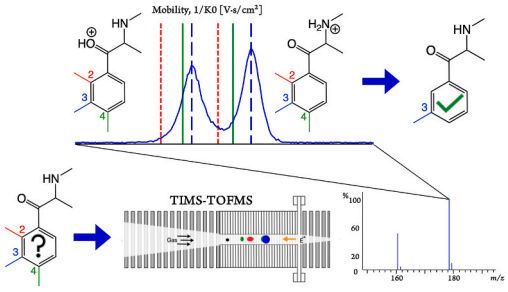
HIGHLIGHTS

- Novel TIMS-TOFMS approach developed for the separation of ring-positional isomers.
- Identification of ring-positional isomers based on distinct protomer mobilities.
- Unambiguous identification of ring-positional isomers of cathinones within 5 min.
- TIMS-TOFMS approach successfully identified cathinone isomers in case samples.

GRAPHICAL ABSTRACT



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ABSTRACT

New psychoactive substances (NPS) are synthetic derivatives of illicit drugs designed to mimic their psychoactive effects. NPS are typically not controlled under drug acts or their legal status depends on their molecular structure. Discriminating isomeric forms of NPS is therefore crucial for forensic laboratories. In this study, a trapped ion mobility spectrometry time-of-flight mass spectrometry (TIMS-TOFMS) approach was developed for the identification of ring-positional isomers of synthetic cathinones, a class of compounds representing two-third of all NPS seized in Europe in 2020. The optimized workflow features narrow ion-trapping regions, mobility calibration by internal reference, and a dedicated data-analysis tool, allowing for accurate relative ion-mobility assessment and high-confidence isomer identification. Ortho-, meta- and para-isomers of methylmethcathinone (MMC) and bicyclic ring isomers of methylone were assigned based on their specific ion mobilities within 5 min, including sample preparation and data analysis. The resolution of two distinct protomers per cathinone isomer added to the confidence in identification. The developed approach was successfully applied to the unambiguous assignment of MMC isomers in confiscated street samples. These findings demonstrate the potential of TIMS-

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TOFMS for forensic case work requiring fast and highly-confident assignment cathinone-drug isomers in confiscated samples.

1. Introduction

Ion mobility spectrometry (IMS) is a gas-phase separation technique capable of distinguishing ions based on their size, charge, and shape. This is achieved by traversing ions through a buffer gas using an electric field, where separation may occur based on differential ion mobility. Hyphenation of IMS with mass spectrometry (IM-HRMS) potentially allows for the separation of isomeric ions that cannot be discriminated by MS. IM-HRMS is particularly beneficial when tandem MS (MS/MS) does not enable isomer discrimination, e.g., due to identical fragmentation patterns. IM-HRMS techniques based on drift-tube (DTIMS), field-asymmetry (FAIMS), and trapped (TIMS) IM approaches have been successfully applied for isomer discrimination of compounds [1], such as glycans [2], lipids [3], peptides and proteins [4], metabolites [5], and even enantiomers [6]. In TIMS, ions are pushed forward through a tunnel by a buffer gas in the presence of an opposing electric field gradient (EFG). The EFG counteracts the drag force exerted by the buffer gas to trap and spatially separate ions based on their mobility [7–10]. The combined role of buffer gas velocity and EFG leads to higher mobility resolution compared to conventional DTIMS. Nevertheless, even with such increased resolving power, separating isomers of small organic molecules, particularly positional isomers, often remains challenging [11].

In the forensic field, the identification of positional isomers of so-called new psychoactive substances (NPS) represents an important task. NPS are defined by the United Nations Office on Drugs and Crime (UNODC) as "Substances of abuse, either in a pure form or in the form of a preparation, that are not controlled under the Single Convention on Narcotic Drugs of 1961 or the Convention on Psychotropic Substances of 1971, but that may pose a threat to public health". These substances can be analogues of existing controlled drugs or newly synthesized chemicals designed to mimic the psychoactive effects of controlled drugs" [12]. Different NPS with closely-related structures (including isomers) may have a different legal status and/or different psychoactive effects, also compared to the more traditional illegal recreative drugs [13]. Short- and long-term health risks of NPS are largely unknown, and consumers of such drugs, that are easily available online, are mostly unaware of potential health risks, which may even result in death [14]. Public health concerns have been triggered by the alarming number of new NPS reported each year (e.g., 52 in 2021 [15]) and the total number of 880 NPS being monitored Europe in 2021 [12,16]. Cathinones are an important category of NPS, second in terms of numbers of substances reported and accounting for 65% of NPS materials (i.e., 3.3 tons) seized in Europe in 2020 [15]. Many popular cathinones appear in isomeric forms, which only differ in the position of the same substituent on the aromatic ring. In the Netherlands, 4-methylmethcathinone (4-MMC) (Fig. S1) is a controlled substance (List I of the Opium Act, "hard drug") since 2012, while its positional isomer 3-methylmethcathinone (3-MMC) has been placed on List II of the Opium Act ("soft drug") in 2022, and 2-MMC currently remains unscheduled [17,18]. Legal amendments typically also trigger the emergence of alternative NPS, as observed with the recent introduction of the uncontrolled 3-chloromethcathinone (3-CMC) [15].

Reported methods for the differentiation of drug isomers involve nuclear magnetic resonance (NMR), mass spectrometry (MS) employing multivariate data analysis, liquid chromatography - mass spectrometry (LC-MS), and gas chromatography (GC) with MS or Fourier transform infrared spectroscopic (FTIR) detection [19–27]. More recently, GC with vacuum-ultraviolet detection (VUV) and infrared ion spectroscopy (IRIS) have been used for NPS isomer identification [19–22]. However, most of these methods are relatively complex, time consuming, require derivatization before analysis, and/or do not lead to sufficient resolution between isomers. LC-MS methods have been reported for the analysis of cathinone isomers, with sufficient resolution but at the expense of throughput, with total analysis times, including re-equilibration, of 25 min or longer [23–25]. IRIS is an interesting new development that provides adequate speed, sensitivity, and selectivity, but the necessary instrumentation is not routinely available and entails very dedicated expertise and infrastructure. NMR typically lacks sufficient sensitivity and is less suited for mixtures, such as street samples, which may contain cutting agents, excipients, and/or additional drugs [26,27]. Hence, new straightforward and fast approaches enabling the identification of NPS isomers with high confidence are needed in the forensic laboratory.

In the present work, we propose a new TIMS-TOFMS workflow for the fast analysis and isomer-specific identification of ring-substituted cathinones in confiscated street samples. Method development included the optimization of cathinone protomer separation, the use of an internal single-point mobility calibration, and the implementation of an automated data analysis and handling procedure. The applicability of the optimized TIMS-TOFMS method was demonstrated by the analysis of drug-mixture and confiscated samples, yielding the accurate assignment of ring-isomers cathinones.

2. Experimental section

Materials Acetonitrile (ACN) and methanol (MeOH), both LC-MS grade, were obtained from Biosolve (Valkenswaard, The Netherlands). Water was of Milli-Q grade (18.2 MΩ cm; Merck Millipore, MA, USA). Ketamine-D_4 hydrochloride solution (1.0 mg mL^{-1}) was acquired from Merck KGaA (Darmstadt, Germany). The substances 2-methylmethcathinone (2-MMC), 3-methylmethcathinone (3-MMC), 4-methylmethcathinone (4-MMC), 2-methylethcathinone (2-MEC), 3-methylethcathinone (3-MEC), 4-methylethcathinone (4-MEC), 2,3-methylone, 3,4-methylone, 2,3-ethylone and 3,4-ethylone, as well as the confiscated street samples, were provided by the Amsterdam Police Forensic Laboratory (The Netherlands). The Amsterdam Police seized the street samples based on the suspected presence of illicit drugs. Fig. S1 provides the molecular structures of the cathinones and information on their legal status in the Netherlands (January 2023). Stock solutions of the individual cathinones and confiscated street samples were prepared in MeOH at a concentration of 1 mg mL^{-1}. Working solutions were prepared by diluting the stock solutions in ACN/water (1:1, v/v) and comprised ketamine-D_4 hydrochloride and cathinone at a concentration of 200 ng mL^{-1}.

Trapped ion mobility mass spectrometry IM-HRMS experiments were carried out using a TIMS-quadrupole time-of-flight mass spectrometer (timsTOF instrument, Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionization (ESI) source. Working solutions were infused with an Agilent G1329A autosampler at a flow rate of 6 μL min^{-1} using the syringe pump of the timsTOF instrument. Sequence analysis was performed using Compass Hystar 3.2 software from Bruker. MS acquisition was carried out in ESI positive mode. The source parameters were: end plate offset, 500 V; capillary voltage, 3500 V; nebulizer pressure, 0.6 bar; dry gas flow rate, 3 L min^{-1}; drying temperature, 180 °C. The MS parameters were: RF funnel 1, 100 Vpp; RF multipole and funnel 2, 200 Vpp; deflection delta, 40 V; isCID, 5 eV. For the quadrupole settings, ion energy was set at 4.0 eV and the low mass at 150 *m/z*. The collision cell parameters were: collision energy, 2.0 eV; collision cell RF, 600 Vpp; transfer time and pre-pulse storage, 40.0 and 8.0 μs, respectively. The ion-mobility spectra were obtained within 0.78–0.93 $1/K_0$ [V·s cm^{-2}] (0.61–0.73 V·s cm^{-2} post calibration) using the following parameters: scan rate (β), 950 ms; accumulation time, 2

ms; IM entrance funnel RF, 230 Vpp. DC potentials (D1-6) were set to 5, 3, 5, −16, 0, and 5 V, respectively. The tunnel-in pressure was set at 3.00 mbar. The TOFMS analyzer was calibrated according to a standard procedure using a tuning mix of Agilent Technologies (Waldbronn, Germany) [28]. For optimized cathinone measurements, the $1/K_0$ (IMS) scale was single-point calibrated using ketamine-D_4 as internal calibrant, as described in the section Data Analysis.

Data Analysis An MS data handling algorithm (workflow scheme in Fig. S2) was written in MATLAB 2022a (MathWorks, Natick, MA, USA). The raw data was transformed to mzML type format [29] with MSConvert [30]. The mobility, *m/z*, and signal intensity of analyte ions were extracted from the mzML files. The *m/z* and mobility values were binned to 4 decimals to improve sensitivity and speed up the data processing. Extracted-ion mobilograms (EIMs) for *m/z* values of interest were constructed using a ±0.01 *m/z* window. Cumulative EIMs were obtained over the total duration (4 min) of measurement during direct sample infusion. Gaussian distributions were fitted to the recorded EIMs to accurately determine mean ion-mobilities of analytes. This method is less impacted by noise than conventional peak-top determination [31]. A single Gaussian distribution was fitted to the EIM of the internal standard ketamine-D_4. Small shifts in absolute ion mobility observed for the analytes over time were corrected using ketamine-D_4 as internal calibrant. The reference mobility of ketamine-D_4 was determined using a wide mobility-trapping region allowing for calibration according to standard procedure using the tuning-mix standards [28]. The cathinone IM distributions were fitted based on the mobility profiles obtained for the protomers using a genetic algorithm with a population size of 10,000 and limited to 3000 generations. Unknown cathinone isomers were identified by comparing both the measured *m/z* (i.e., cathinone specific) and mean ion-mobilities (i.e., isomer specific) from the respective EIMs with in-house established data obtained from reference cathinones (Table 1). Cathinone isomer identity was assigned when measured ion-mobility values for the protomers were within their 99.9% prediction intervals (stated in Table S1) determined for each isomer from repeated experiments and registered in a database.

Table 1
List of *m/z* values and ion mobilities determined for the two protomers of each cathinone isomer studied. The ion mobilities are calibrated on the ion mobility of the ketamine-D_4 reference. Mobility P1 and P2 refer to the mobilities of the *O*-protonated and *N*-protonated species, respectively, and are stated with the associated standard deviation.

Cathinone isomer	*m/z* of the $[M+H]^+$ ion	Mobility P1 $1/K_0$ [V·s cm^{-2}]	Mobility P2 $1/K_0$ [V·s cm^{-2}]
2-MMC	178.1226	0.64154 ± 0.00008	0.65766 ± 0.00008
3-MMC	178.1226	0.65011 ± 0.00013	0.66687 ± 0.00008
4-MMC	178.1226	0.64737 ± 0.00011	0.66176 ± 0.00007
2-MEC	192.1383	0.66517 ± 0.00006	0.67651 ± 0.00006
3-MEC	192.1383	0.67334 ± 0.00003	0.68569 ± 0.00003
4-MEC	192.1383	0.67112 ± 0.00016	0.68080 ± 0.00009
2,3-Methylone	208.0968	0.66948 ± 0.00032	0.68833 ± 0.00011
3,4-Methylone	208.0968	0.68026 ± 0.00090	0.69126 ± 0.00009
2,3-Ethylone	222.1125	0.69103 ± 0.00034	0.70539 ± 0.00005
3,4-Ethylone	222.1125	0.70197 ± 0.00026	0.71060 ± 0.00009
Ketamine-D_4	242.1244	0.70306 ± 0.00094	Not present

3. Results and discussion

Method optimization Recent studies have reported the separation of ring-substituted positional isomers by IM-HRMS [32], albeit only for isomers with relatively large substituents [33] or employing complexation with molecular shape sorters, such as cyclodextrins, to create sufficient IM differences [34]. Moreover, these studies focused on positional isomers for which the specific substituents are protonation sites, which potentially enhances mobility differences due to charge relocation [35]. The substituents of cathinone drugs are mostly small and not ionizable, which make the respective isomers difficult to resolve by IMS.

The TIMS separation performance is usually expressed by the resolving power (*R*) according to Eq. (1):

$$R = v_g * \sqrt[4]{\frac{2L_p}{\beta}} * \frac{1}{\sqrt[4]{K_0^3}} * \sqrt{\frac{q}{16\ ln\ 2 k_b T}} \quad (1)$$

where v_g is the drift gas velocity, L_p the electric field gradient (EFG) plateau length, β the scan rate, K_0 the reduced analyte ion mobility (i.e., normalized for pressure and temperature), q the charge of the analyte ion, k_b the Boltzmann constant, and T the temperature. The most significant TIMS parameters for optimizing analyte separation are v_g and β, controlled by the pressure at the tunnel entrance (P_{ent}) and the gradient ramp time, respectively [36]. Applying standard values for these parameters did not yield sufficient resolution to adequately resolve the ion mobility distributions of the studied cathinones (Fig. S3). In order to maximize *R*, v_g was increased by setting P_{ent} to 3.00 mbar and β was minimized by increasing the ramp time to 950 ms, respectively. Additionally, β was further minimized by reducing the EFG magnitude, so that only analytes within a narrow mobility range were trapped. However, the latter hindered proper calibration of the mobility ($1/K_0$) scale when using a standard tuning mixture [28] containing a series of hexasubstituted oxyphosphazenes. These calibrants cover a relatively wide *m/z* and mobility range (*m/z* 118.09–2721.89 and 0.542–2.168 $1/K_0$ [V·s cm^{-2}], respectively). Therefore, a single point internal calibration method was implemented using ketamine-D_4 as calibrant, as this compound falls within the narrow mobility window (i.e., 0.612–0.730 $1/K_0$ [V·s cm^{-2}]). The developed method provides adequate selectivity to resolve mobility peaks for isomer identification (Fig. S3).

Mobility distribution of cathinone isomers Extracted ion mobilograms (EIMs) for the $[M+H]^+$ ions of 2-, 3-, and 4-MMC (all detected at *m/z* 178.122) are shown in Fig. 1A. All isomers exhibited a bimodal mobility distribution, with distinct mean mobilities for each isomer (Table 1). The bimodal distribution is caused by the presence of two protomers per isomer (i.e., *O*- and *N*-protonated species), which are expected to be formed during ESI of cathinones under the conditions used in these experiments [5,35,37]. Indeed, NPS with similar structures but without the β-keto group (i.e., phenylethylamines), exhibited a single mobility distribution, as the *O*-protonated species cannot be formed (Fig. S4). Based on earlier reported observations, in the bimodal pattern obtained for the cathinones, the peak of higher mobility (i.e., lower $1/K_0$) was attributed to the *O*-protonated species and the peak of lower mobility to the *N*-protonated species, respectively [38,39]. A bimodal mobility distribution was also observed for the 2-, 3-, and 4-positional isomers of MEC (Fig. S5).

The positional isomers of the cathinones methylone (Fig. 1B) and ethylone (Fig. S6) also showed a bimodal mobility distribution, indicating the formation of protomers. However, the *O*-protonated species were substantially less abundant for the 3,4-isomers of methylone and ethylone. Although the protomers of 3,4-methylone and 3,4-ethylone are not baseline separated with the developed workflow, the mobility differences between the two protomers are sufficient to enable the deconvolution of their signals, as highlighted in Table 1. The 3,4-isomers are listed as controlled substances in the Netherlands, whereas the 2,3-substituted cathinone isomers are not. Conventional methods, such as GC-MS, are often not capable of reliably distinguishing 2,3- from 3,4-

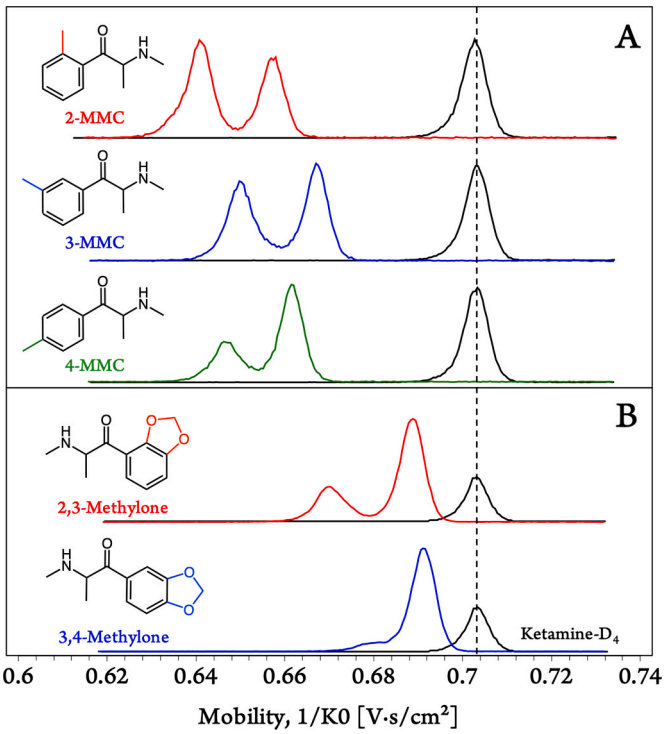

Fig. 1. Extracted ion mobilograms of the calibrant ketamine-D_4 (black) (*m/z* 242.1244), (**A**) 2-methylmethcathinone (MMC) (red), 3-MMC (blue), 4-MMC (green) (*m/z* 178.1226); and (**B**) 2,3-methylone (red) and 3,4-methylone (blue) (*m/z* 208.0968). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

isomers during methylone and ethylone analysis [20].

The observed bimodal ion-mobility distribution added confidence in the isomer identification, as at least one specific protomer mobility was observed for each cathinone isomer (Table 1). For instance, the mobility difference between the *O*-protonated species of 3- and 4-MEC is marginal (0.002 V·s cm^{-2}), whereas the *N*-protonated isomers show a higher difference in their mobilities. In this case, using only the *O*-protomers would provide a less reliable identification. A procedure based on two isomer-dependent protomer mobilities is considered advantageous due to the variety of cathinones currently on the market and those expected to be introduced in the market.

Interestingly, different protomer-intensity ratios were observed for each positional isomer. The proximity of the substituent to the protonation site had a distinct effect on the protonation efficiency. For MMC and MEC, the relative abundance of the *N*-protomer with respect to the *O*-protomer increased in the order of ortho > meta > para for the cathinone ring substitution (Fig. S7), while for methylone and ethylone the relative *N/O*-protomer abundance of the 2,3-iomers was higher than of the 3,4-isomers (Fig. S8). The relative abundance of each protomer may in theory be used to further increase the confidence of isomer identification. However, protomer ratios may be affected by the ionization conditions, including solvent and sample constituents [37–39]. As the composition of street samples is variable, potentially comprising different solvents, excipients, additives and/or impurities, the information on protomer-intensity ratios was not used in the developed cathinone-isomer identification procedure.

Cathinone isomer assignment The mobilities recorded for both protomers of each tested cathinone are listed in Table 1, highlighting that each isomer has a unique set of protomer mobilities. Therefore, the assignment of cathinone isomers in a sample involved both the *m/z* (specific for cathinone type) and the mean mobilities determined for both protomers (specific for positional isomer). The mean mobility (i.e., the first moment of the fitted mobility distribution) determined for each protomer was selected as discriminative parameter, because mobility peak shape (e.g., width and asymmetry) may be affected by the experimental conditions, such as solvent and analyte concentration, due to space charge effects [40,41]. The standard deviation (Table 1) and prediction interval (i.e., estimate of the interval within which a future observation of the mobility would fall with a certain probability, Table S1) of the mean mobilities obtained from the mobility distributions were very small, allowing for mobility determination and discrimination with high precision. The combination of accurate *m/z* and precise ion mobilities strongly diminishes the chances of making wrong assignments or obtaining false positive results. The differences between the mobilities of the isomers of a specific cathinone were much larger than the standard deviations obtained for the protomer mobilities (Table 1). For example, for the most critical protomer pair (i.e., P1 of 3- and 4-MEC), the difference in ion mobility was 0.00222 V·s cm^{-2}, whereas the standard deviations of the separate mobility values were 0.00003 and 0.00016 V·s cm^{-2}, respectively. The assignment was considered valid when the measured *m/z* was within a 0.01 *m/z* tolerance with respect to the reference *m/z* in the database (Table 1), and when the two measured protomer mobilities fell within 99.9% prediction interval (See Table S1 for the tolerances) of the reference database mobilities (Table 1). A false positive would only be possible if the *m/z* value of the compound is within 0.01 difference and shows two protomers with the same mobility within the prediction interval, which is unlikely.

Isomer identification in cathinone mixtures NPS products sold on the (black) market or (dark) web typically contain a single psychoactive compound, but may (intentionally or unintentionally) also consist of mixtures of two or more substances, including cathinones. In order to mimic the composition of drug products seized on the market, the developed TIMS-TOFMS workflow was applied to the analysis of different mixtures of cathinone standards. Fig. 2 shows the EIMs of MMC, MEC, methylone, and ethylone isomers measured in the different mixtures. All cathinones were present at a concentration of 200 ng mL^{-1} except for the MEC, which had a concentration of 20 ng mL^{-1}. Fig. 2 displays the protomer reference mobilities of the relevant cathinone isomers (straight vertical colored lines). All cathinone isomers in the mixture were correctly assigned by matching the measured *m/z* and protomer mobilities to the reference database. Notably, the MEC isomer was also unambiguously identified, despite its ten times lower concentration. A successful identification was also achieved in other cathinone isomer mixtures (Fig. S9). Remarkably, the presence of multiple cathinones did not influence the mobility behavior and, thus, the identification process.

It is important to note that mixtures containing cathinones with the same molecular formula, i.e., isomeric mixtures, were not included in this study. Indeed, compounds with the same *m/z* will lead to combined EIMs, which complicates the identification process, depending on the number of isomers present and their relative concentration. Structural isomeric mixtures do not pose problems, as each structural isomer can be distinguished based on its specific fragmentation pattern. Indeed, post-IMS collision-induced dissociation (CID) can provide unique fragments of structural isomers that yield isolated EIMs containing precursor mobilities. This approach is not possible with ring isomeric cathinone mixtures, as they lead to identical fragments. Luckily, mixtures of two or more ring isomers are rarely encountered in real case samples. Hence, the mixtures explored in this study were limited to cathinones with unique molecular formulas. We are currently working on an alternative TIMS-TOFMS-based approach for NPS isomer separation, which will solve this limitation.

Identification of cathinone isomers in forensic case material The feasibility of the proposed TIMS-TOFMS workflow in forensic case work was assessed with the analysis of confiscated street samples. Street samples are mainly encountered as tablets, powders, and liquids, and may contain more than one psychoactive substance, excipients (e.g., tablet fillers, colorants), and potential impurities. A powder (case

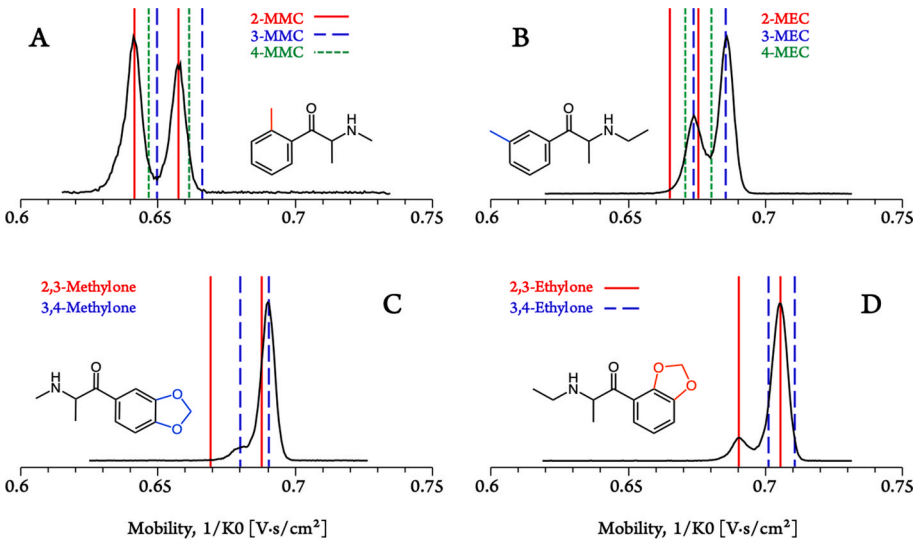

Fig. 2. EIMs obtained during TIMS-TOFMS analysis of a mixture of (**A**) a methylmethcathinone (MMC) (*m/z* 178.1226), (**B**) a methylethcathinone (MEC) (*m/z* 192.1383), (**C**) a methylone (*m/z* 208.10), and (**D**) an ethylone (*m/z* 222.1125) isomer. The vertical straight colored lines indicate the protomer reference mobilities of each isomer. For each cathinone the concentration was 200 ng mL^{-1}, except for MEC (20 ng mL^{-1}).

sample 1) and a liquid (case sample 2) provided by the Amsterdam Police were analyzed using the developed workflow. A previous analysis by GC-MS indicated the presence of an MMC isomer in case sample 1 and the presence of an MMC and an ethylone isomer in case sample 2. However, the GC-MS method was not able to unequivocally assign the specific isomeric forms (Fig. S10) [21,42,43]. In contrast to the GC-MS experiments, which involved a derivatization step, the sample preparation for the TIMS-TOFMS workflow is very straightforward, namely, dissolution and/or dilution in ACN/water (1:1, v/v). Owing to the low limits of detection of the developed method (i.e., down to 10–50 nM for target analytes), the case samples could be substantially diluted to minimize potential interferences caused by additives, excipients, and/or impurities. The workflow applied for the analysis of the samples only focused on the *m/z* values present in the database; other peaks detected in the mass spectra corresponding to other substances, including additives, excipients, and/or impurities, were ignored. The mass spectra revealed the presence of an MMC isomer (*m/z* 178.123 for $[M+H]^+$, Fig. S11) in both samples. Fig. 3A and B shows the EIMs for *m/z* 178.123 of the two samples (black trace) with the protomer reference mobilities of each MMC isomer (vertical straight colored lines). Based on the specific ion mobilities measured for each protomer, the MMC cathinones in case samples 1 and 2 were unambiguously assigned to 4-MMC and 3-MMC, respectively. In case sample 2, the mass spectrum also indicated the presence of an ethylone (*m/z* 222.113), which via the constructed EIM was identified as the 3,4-isomer (Fig. 3C). These two examples demonstrate the potential of the developed TIMS-TOF MS workflow to reliably, selectively, and quickly (i.e., within 5 min) assign cathinone isomers in real case samples of unknown composition without the need for extensive sample preparation.

4. Conclusions

A novel TIMS-TOFMS workflow was developed and optimized for the direct isomer-specific assignment of cathinones, an important category of NPS, in forensic case samples. Narrow ion-trapping regions, mobility calibration by internal reference, and a dedicated data analysis tool allowed for adequate resolution, accurate relative ion mobility assessment, and unequivocal isomer identification. The presence of protomers with distinct ion mobilities for each cathinone isomer supported the isomer assignment. The analysis of confiscated street samples (powder and liquid) showed the capability of the developed TIMS-TOFMS workflow to identify cathinone isomers in forensic mixtures. Identification was achieved within 5 min only, with sample dissolution and/or dilution as sample pretreatment. The current workflow is applicable to the analysis of cathinone-containing street samples, which opens up great possibilities for high-volume, routine analysis of NPSs in forensic laboratories. Finally, the sensitivity achieved by this method also suggests a future potential for this workflow for analysis of cathinones in biological samples. Further work is needed for a successful application of TIMS-TOFMS to the discrimination of multiple isomers of a cathinone present in the same sample.

CRediT authorship contribution statement

Hany A. Majeed: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Tijmen S. Bos:**

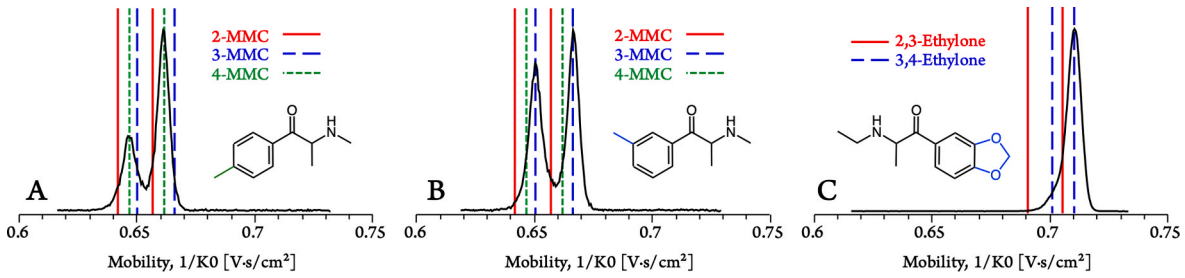

Fig. 3. Extracted ion mobilograms (EIM) (black trace) obtained during TIMS-TOFMS of case sample 1 (powder, **A**), *m/z* 178.1226 and case sample 2 (solution, **B** and **C**), *m/z* 178.1226 (B) and *m/z* 222.1125 (C). Protomer reference mobilities of MMC and ethylone isomers are depicted as colored lines.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2023.341276.

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