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Differentiating the aromatic positional isomers of methylbuphedrones and methoxybuphedrones via chemical ionization-mass spectrometry

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Abstract

Discrimination of aromatic positional isomers of methylbuphedrones and methoxybuphedrones was successfully achieved. Meta isomers were discriminated by chemical ionization-tandem mass spectrometry (CI-MS/MS) using acetonitrile as a reagent gas. Furthermore, all the aromatic positional isomers were discriminated by CI-MS/MS using vinyltrimethylsilane as a reagent gas.

KEYWORDS
buphedrones, chemical ionization, discrimination, tandem mass spectrometry, vinyltrimethylsilane

1 | INTRODUCTION

The social problems associated with numerous drugs have reached epidemic proportions.[1] This has increased the importance of the forensic identification of structural isomers because not all positional isomers commonly found in controlled drugs are regulated. Gas chromatography/mass spectrometry (GC/MS) plays an important role in drug analysis, but the analysis of positional isomers often results in identical mass spectra. In order to differentiate between drug isomers, an analytical method that combines GC with electron ionization (EI)-tandem MS generally is used.[2,3]

Chemical ionization (CI) is a soft ionization method for GC/MS. Methane, isobutane, ammonia, etc., are generally used as reagent gases for CI. In rare cases, small organic molecules such as acetonitrile, methanol, acetone, etc., are also used as a reagent gas.[4–11] Differentiating between positional aromatic isomers also is difficult when using CI-MS, and, therefore, tandem MS (MS/MS) using protonated molecules is used to differentiate aromatic positional isomers.[12,13]

On the other hand, CI using a silicon compound as a reagent gas has been reported, but it is used only for the analysis of linear alcohols and trinitrotoluene, and has never been used to discriminate structural isomers.[14–16] Kadentsev et al. reported the reactivity of functional groups toward H^+ and Me_3Si^+ ions where Me_3Si^+ ions react differently than protons toward a variety of functional groups.[17] We considered using silyl cations, which react differently than protons, for drug isomer analysis.

In the present study, we focused on GC/MS analysis to discriminate between the aromatic positional isomers of methylbuphedron (MeBP) and methoxybuphedron (MeOBP), and CI-MS/MS using vinyltrimethylsilane (VTMS) as the reagent gas allowed us to accomplish this goal. (Figure 1).

2 | EXPERIMENTAL

2.1 | Chemicals

Buphedrones (BPs) (MeBPs and MeOBPs) were synthesized using the method described in Figure 2.[18,19] Derivatization of BPs was performed via treatment with anhydrous heptafluorobutyric acid at room

Abbreviations: MeBP, methylbuphedron; MeBPs-HFB, heptafluorobutyryl derivatives of methylbuphedrons; MeOBP, methoxybuphedron; MeOBPs-HFB, heptafluorobutyryl derivatives of methoxylbuphedrons; VTMS, vinyltrimethylsilane.

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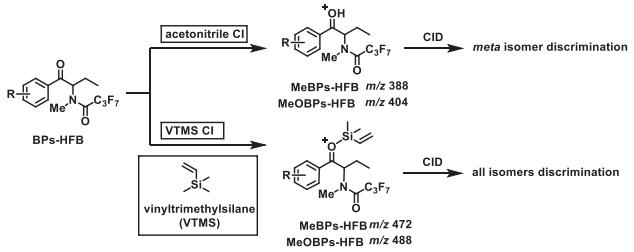

FIGURE 1 Differentiating the aromatic positional isomers of Buphedrones (BPs) after derivatization with heptafluorobutyric anhydride.

temperature for 30 min to give BPs-HFB (Figure 2).[20] All chemicals were reagent grade and used as received.

2.2 | Analytical conditions for EI-MS

All GC/MS analyses were performed using an Agilent Technologies 240 Ion-trap mass spectrometer equipped with a 7890B GC system. The column was an ID-BPX5 (30 m × 0.25 mm i.d., 0.25 µm film thickness; SGE). The carrier gas was high-purity helium (99.9999%) with a constant flow of 1.2 mL/min. The GC oven temperature was set at 60°C for 1 min, which then was increased to 280°C at 10°C/min and held for 5 min. The mass spectrum was measured in an internal ionization mode. The injection, transfer line, ion trap, and manifold temperatures were set at 280, 300, 200, and 45°C, respectively. All injections were performed in the splitless mode with the split vent closed for 1 min. Full-scan EI data were acquired under the following conditions: mass range, *m/z* 50–600; scan time, 1 s/scan; and, emission current, 20 µA. CI was performed using a 240 ion-trap mass spectrometer equipped with a liquid CI apparatus.

2.3 | Analytical conditions for CI-MS using acetonitrile

Full-scan CI data with acetonitrile as a reagent gas were acquired under the following conditions: mass range, *m/z* 60–500; scan time, 1 s/scan; emission current, 10 µA; reagent low mass, *m/z* 35; reagent high mass, *m/z* 60; reaction storage level, *m/z* 35; ejection amplitude, 15 V; and, max reaction time, 100 ms. MS/MS was performed in resonant mode,

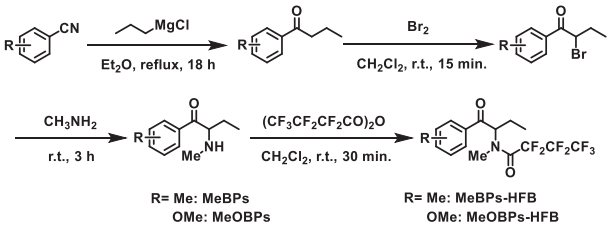

FIGURE 2 Preparation and derivatization of Buphedrones (BPs).

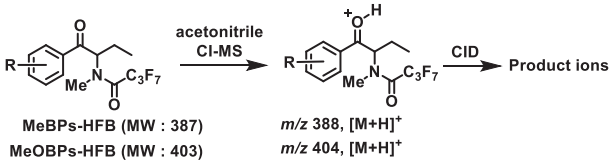

FIGURE 3 Acetonitrile chemical ionization (CI)-mass spectrometry of Buphedrones (BPs)-HFB.

and the precursor ions and excitation voltage were described in the mass spectra.

2.4 | Analytical conditions for CI-MS using VTMS

Full-scan CI data with VTMS as a reagent gas were acquired under the following conditions: mass range, *m/z* 150–500; scan time, 1 s/scan; emission current, 40 µA; reagent low mass, *m/z* 50; reagent high mass, *m/z* 100; reaction storage level, *m/z* 50; ejection amplitude, 15 V; and, max reaction time, 2000 ms. MS/MS was performed in resonant mode, and the precursor ions and excitation voltage are described in the figure.

3 | RESULTS AND DISCUSSION

BPs were identified by Maheux et al. in 2011 via ^{1}H NMR, ^{13}C NMR, and GC/MS.[21] The BPs, however, have never been differentiated. In this work, derivatization of BPs with heptafluorobutyric anhydride made analytes more volatile, which increased the ion intensity in GC/MS analyses.[20]

We initially used acetonitrile as a reagent gas in the CI-mass spectrometric study of BPs-HFB (Figure 3). Base peaks were detected at *m/z* 388 and *m/z* 404 for MeBPs and MeOBPs, respectively. These peaks correspond to $[M+H]^+$.

The protonated ions at *m/z* 388, $[M+1]^+$ for MeBPs-HFB, were further investigated via CI-MS/MS using various levels of collision energy. The results appear in Figure 4A. The spectra represent the *ortho*-, *meta*-, and *para* isomers from top to bottom. The same investigation was performed for the protonated ions at *m/z* 404 $[M+1]^+$ for MeOBPs-HFB, which included the spectra for the CI-tandem mass, as displayed in Figure 4B. Common-product ions at *m/z* 268 were observed for both MeBPs-HFB and MeOBPs-HFB. These ions were detected in all compounds, which indicated independence from the position and type of substituents on the aromatic rings. The ion intensity at *m/z* 268 of the meta isomer was clearly lower than that of the other isomers. As shown in Figure 5, the product ions at *m/z* 268 are presumed to be iminium ions derived from the cleavage of the amide moieties of MeBPs-HFB or MeOBPs-HFB. Their formation could be promoted by the inductive effect of the aromatic rings of MeBPs and by the resonance effect of the aromatic rings of MeOBPs. Both effects are why the *ortho* and *para* isomers have intensities of *m/z* 268, which are stronger than those of the *meta* isomers. Figure 5 also shows the effects of the *para* isomers of

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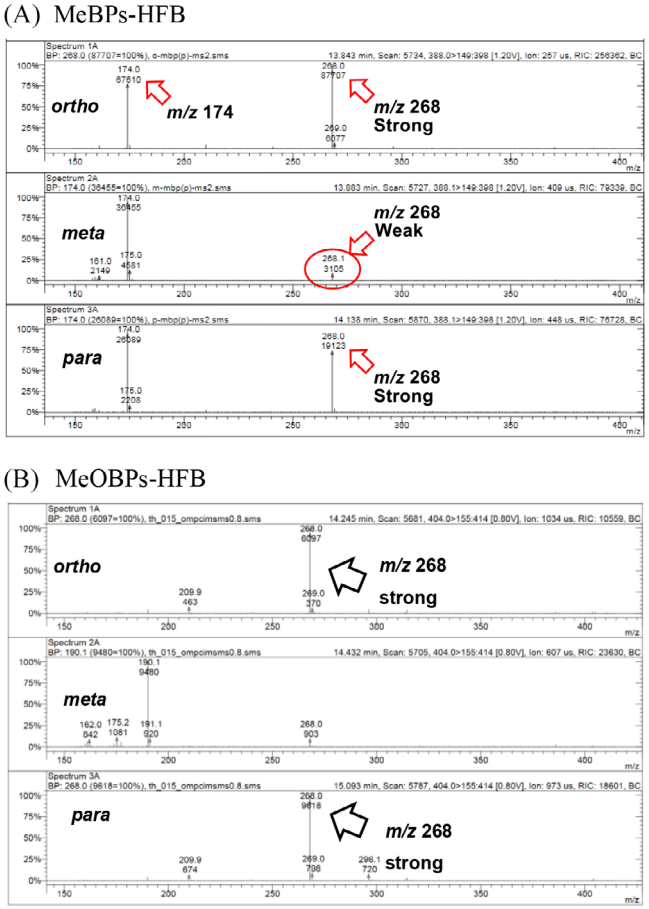

FIGURE 4 Acetonitrile chemical ionization (CI)-tandem mass spectra for (A) methylbuphedrons (MeBPs)-HFB (precursor ions: *m/z* 388, collision-induced dissociation [CID] voltage: 1.2 V) and (B) methoxybuphedrons (MeOBPs)-HFB (precursor ion: *m/z* 404, CID voltage: 0.8 V).

MeBP-HFB and MeOBP-HFB. The same stabilization can be assumed for *ortho* isomers but not for *meta* isomers. These results indicate that *meta* isomers can be differentiated via CI-MS/MS, but it is currently not possible to differentiate the *ortho* and *para* isomers.

Next, we attempted to use VTMS as a CI reagent gas instead of acetonitrile. VTMS was converted into various silyl cations via ion-molecule reactions. Formation of dimethylhydrosilyl cations (*m/z* 59), trimethylsilyl cations (*m/z* 73), vinyldimethylsilyl cations (*m/z* 85), and divinylmethylsilyl cations (*m/z* 97) was confirmed (Figure 6). These silicon reagent ions are expected to attack the target aromatic positional

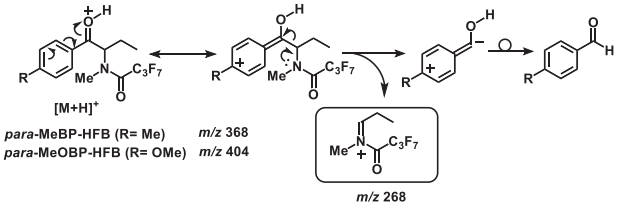

FIGURE 5 A plausible mechanism of the specific product ions at *m/z* 268 for *para*-MeBP-HFB and *para*-MeOBP-HFB.

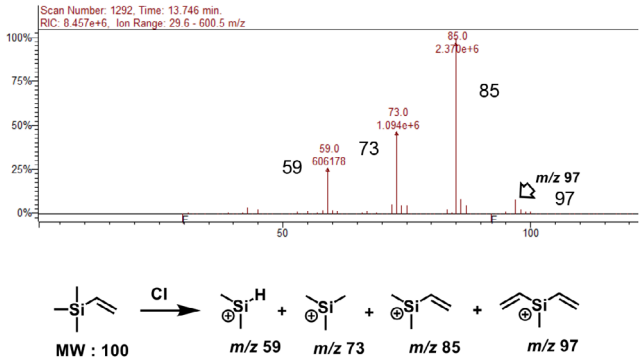

FIGURE 6 Reagent ions generated from vinyltrimethylsilane (VTMS) under chemical ionization (CI) conditions.

isomers and induce specific fragmentations that are not produced by acetonitrile reagent ions.

Figure 7A shows the CI-mass spectra of MPBs-HFB (MW 387) via VTMS. Protonated ions $[M+1]^+$ at *m/z* 388 were not observed. Instead, ions at *m/z* 174, *m/z* 460, and *m/z* 472, were detected for all isomers. The plausible structures of these produced ions are depicted under the spectra in Figure 7. The fragment ions at *m/z* 174 are assumed to be of the cyclic iminium variety. Two fragment ions that are larger than the molecular ions at *m/z* 460 and *m/z* 472 are assumed to be the adducts of trimethylsilyl cations $[M+73]^+$ and vinyldimethylsilyl cations $[M+85]^+$, respectively. Figure 7B shows the CI-mass spectra when VTMS was loaded to MeOBPs. A similar feature was observed with MeBPs-HFB. The fragment ion peaks at *m/z* 190, *m/z* 476, and *m/z* 488 were observed for all the isomers. The fragment ions at *m/z* 190 are of the cyclic iminium variety. Two fragment ions that are larger than the molecular ions at *m/z* 476 and *m/z* 488 are assumed to be the adducts of trimethylsilyl cations $[M+73]^+$ and vinyldimethylsilyl cations $[M+85]^+$, respectively.

For both MeBPs-HFB and MeOBPs-HFB, the CI-mass spectra showed the same ions among all aromatic positional isomers, so a clear differentiation of isomers was not successful. We then performed CI-MS/MS of the fragment ions for each aromatic positional isomer and focused on the fragment ions at *m/z* 472 for MeBPs-HFB and *m/z* 488 for MeOBPs-HFB, which are adducts with vinyldimethylsilyl cation $[M+85]^+$ (Figure 8).

Figure 9A shows the CI-MS/MS for MeBPs-HFB that came from the precursor ions at *m/z* 472 corresponding to $[M+85]^+$. It is noteworthy that the *ortho* and *meta* isomers afforded the fragment ion peaks at *m/z* 444, whereas the *para* isomer afforded ions with small peaks. Plausible structures of the fragment ions at *m/z* 444 are depicted under the spectra. These results show that three positional isomers of MeBPs-HFB were discriminated by acetonitrile CI and VTMS CI-MS/MS. Figure 9B shows the CI-MS/MS for MeOBPs-HFB that originated from the precursor ions at *m/z* 488 corresponding to $[M+85]^+$. Similar features were obtained in the case of MeBPs-HFB. The characteristic fragment ions at *m/z* 460 of the *para* isomer had a smaller intensity than that of the *ortho* and *meta* versions. The fragment ions at *m/z* 386 of the *meta* isomer had an intensity that was smaller than that of either the *ortho* or

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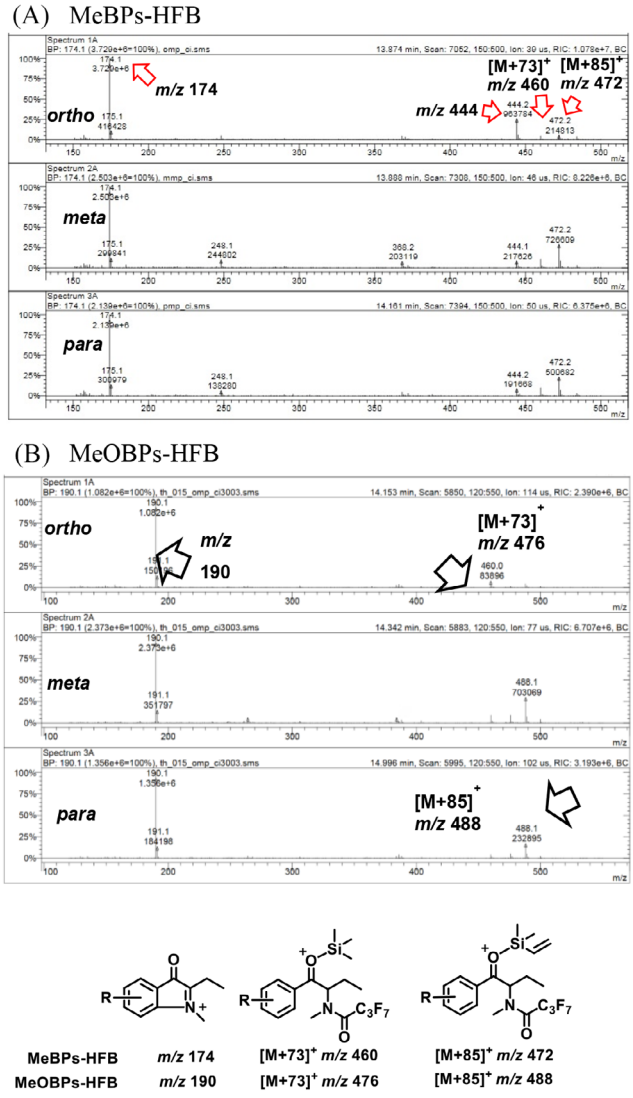

FIGURE 7 Vinyltrimethylsilane (VTMS) chemical ionization (CI)-mass spectra for (A) methylbuphedrons (MeBPs)-HFB and (B) methoxybuphedrons (MeOBPs)-HFB.

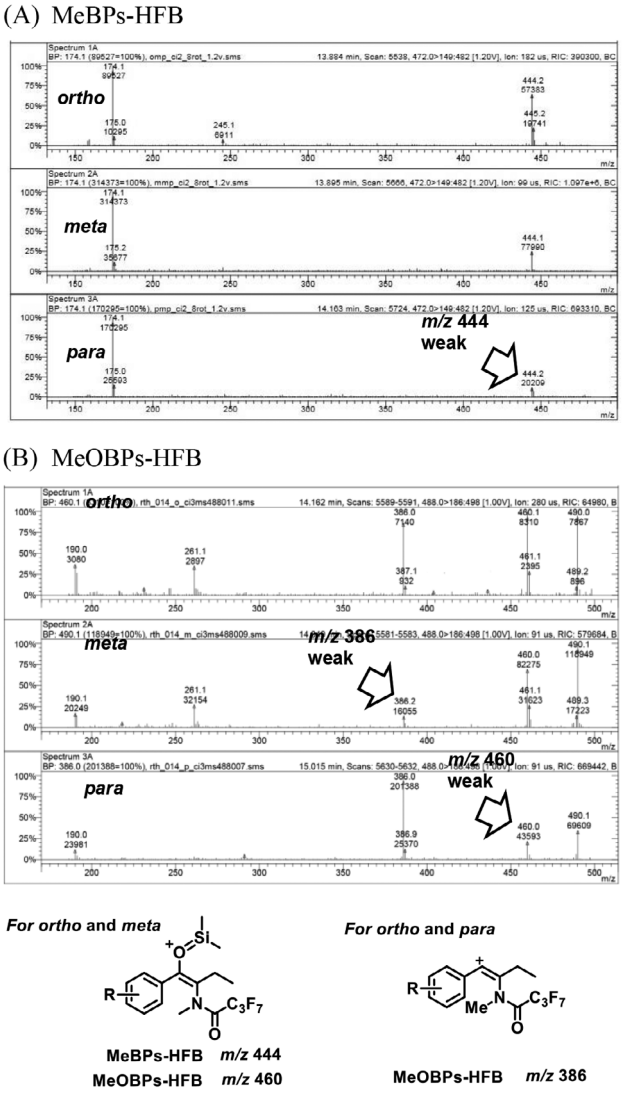

FIGURE 9 Vinyltrimethylsilane (VTMS) chemical ionization (CI)-tandem mass spectra for (A) methylbuphedrons (MeBPs)-HFB (precursor ions: *m/z* 472, collision-induced dissociation [CID] voltage: 1.2 V) and (B) MeOBPs-HFB (precursor ions: *m/z* 488, CID voltage: 1.0 V).

para versions. Plausible structures of the ions at *m/z* 460 and 386 are depicted under the spectra 9B. These results indicate that the three positional isomers of MeOBPs-HFB were easily differentiated via the large differences in the intensity ratios for the specific peaks at *m/z* 386 and 460 in the VTMS CI-MS/MS.

Figure 10 shows how the specific product ions are generated from the precursor ions at *m/z* 472 for MeBPs-HFB and at *m/z* 488 for

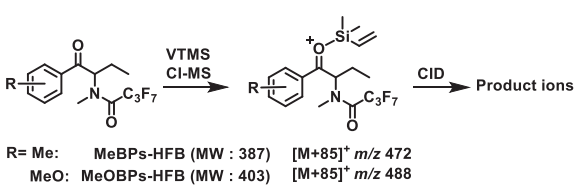

FIGURE 8 Structures of the precursor ions for chemical ionization (CI)-tandem mass spectrometry for methylbuphedrons (MeBPs)-HFB and methoxybuphedrons (MeOBPs)-HFB.

MeOBPs-HFB ([M+85]$^+$). As shown in Figure 10A, the product ions at *m/z* 444 for MeBPs-HFB and at *m/z* 460 for MeOBPs-HFB are assumed to have been formed by the rearrangement of the acidic α-proton of the carbonyl group to the vinyl group and by the subsequent elimination of ethylene from the 2-silylethyl cations **I**. The preferable formation of 2-silylethyl cations **I** was likely caused by the stabilization of the β-cation by silicon ($\sigma-\pi$ interaction).[22] In addition, for *ortho* and *meta* isomers, cations **I** am likely to form because the vinylsilane moiety and the α-protons are favorably in close proximity due to the steric hindrance of aromatic substituents such as the ***o*-[M+85]$^+$**, as depicted in Figure 10A. The vinylsilyl moiety of the *para* isomers ***p*-[M+85]$^+$**, however, is not near the α-protons. As shown in Figure 10B, the specific product ions at *m/z* 386 for MeOBPs-HFB are presumed to have been the result of a neutral loss of silanol. *Ortho* and *para* isomers are able to generate the stable intermediates **II-*o*** and **II-*p*** due to the resonance

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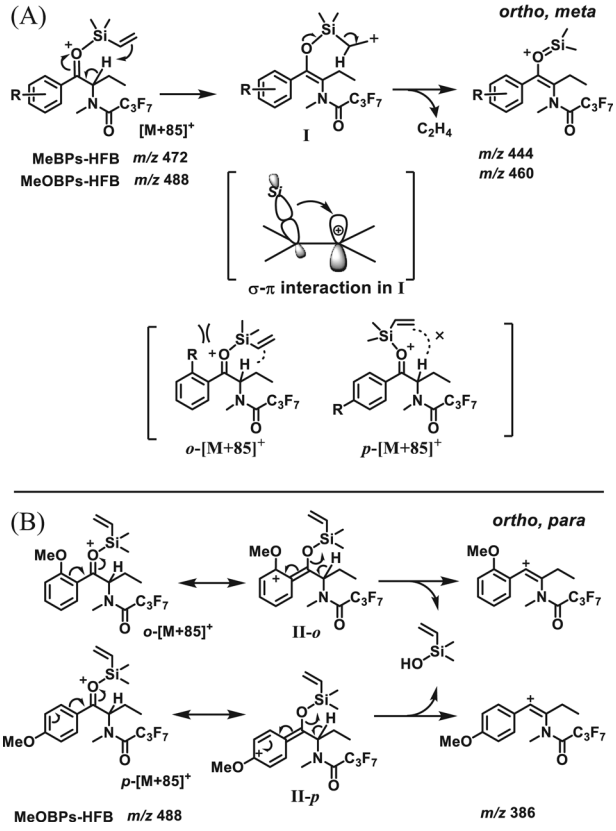

FIGURE 10 Plausible fragmentation pathways of specific fragment ions. (A) The ions at *m/z* 444 for methylbuphedrons (MeBPs)-HFB and 460 for MeOBPs-HFB (precursor ions at $[M+85]^+$). (B) The ions at *m/z* 386 for MeOBPs-HFB (precursor ions at $[M+85]^+$).

effect of the MeOBP methoxy groups. We assume that product ions at *m/z* 386 were not observed in the *meta* isomer of MeOBPs-HFB due to the absence of the resonance effect of the methoxy group. We also assume that the corresponding silanol elimination was not observed in all MeBPs-HFB isomers because there was no resonance effect on the methyl groups.

4 | CONCLUSIONS

We used CI-MS/MS to differentiate the aromatic positional isomers of MeBPs and MeOBPs. CI-MS/MS with acetonitrile as a reagent gas was used to differentiate the smaller peak intensities of Meta isomers, at *m/z* 268, which were generated by precursor ions at *m/z* 388. Furthermore, all the aromatic positional isomers were also discriminated via CI-MS/MS, but with the use of VTMS as a reagent gas wherein silylated precursor ions induced characteristic product ions. These results mark the first use of a silicon compound as a reagent gas for CI in order to differentiate drug isomers (Supporting Information).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available upon request.

REFERENCES

1. Kelly JP. Cathinone derivatives: a review of their chemistry, pharmacology and toxicology. *Drug Test Anal*. 2011;3:439-453.
2. Inoue H, Negishi S, Nakazono Y, et al. Differentiation of ring-substituted bromoamphetamine analogs by gas chromatography–tandem mass spectrometry. *Forensic Toxicol*. 2016;34:125-132.
3. Kohyama E, Chikumoto T, Tada H, Kitaichi K, Horiuchi T, Ito T. Differentiation of ring-substituted bromoamphetamine analogs by gas chromatography–tandem mass spectrometry. *Anal Sci*. 2016;32:831-837.
4. Oldham NJ. Ion/molecule reactions provide new evidence for the structure and origin of $[C_3H_4N]^+$ from acetonitrile chemical ionization plasma. *Rapid Commun Mass Spectrom*. 1999;13:1694-1698.
5. Oldham NJ, Svatos A. Determination of the double bond position in functionalized monoenes by chemical ionization ion-trap mass spectrometry using acetonitrile as a reagent gas. *Rapid Commun Mass Spectrom*. 1999;13:331-336.
6. Tzing SH, Ghule A, Chang JY, Ling YC. Chemical ionization of substituted naphthalenes using tetrahydrofuran as a reagent in gas chromatography with ion trap mass spectrometry. *Rapid Commun Mass Spectrom*. 2003;17:811-815.
7. Brodbelt J, Liou CH, Donovan T. Selective adduct formation by dimethyl ether chemical ionization in a quadrupole ion trap mass spectrometer and a conventional ion source. *Anal Chem*. 1991;63:1205-1209.
8. Moore C, Guzaldo F, Hussain MJ, Lewis D. Determination of methadone in urine using ion trap GC/MS in positive ion chemical ionization mode. *Forensic Sci Int*. 2001;119:155-160.
9. Ding WH, Tzing SH. Analysis of nonylphenol polyethoxylates and their degradation products in river water and sewage effluent by gas chromatography–ion trap (tandem) mass spectrometry with electron impact and chemical ionization. *J Chromatogr A*. 1998;824:79-90.
10. Buchanan MV. Mass spectral characterization of oxygen-containing aromatics with methanol chemical ionization. *Anal Chem*. 1984;56:546.
11. Wu HF, Lin PY. Ethylenediamine as a liquid chemical reagent to probe hydrogen bonding and host-guest interactions with crown ethers in an ion trap tandem mass spectrometer. *Rapid Commun Mass Spectrom*. 2004;18:1365-1373.
12. Negishi S, Nakazono Y, Iwata YT, et al. Differentiation of regioisomeric chloroamphetamine analogs using gas chromatography–chemical ionization-tandem mass spectrometry. *Forensic Toxicol*. 2015;33:338-347.
13. Westphal F, Junge T. Ring positional differentiation of isomeric N-alkylated fluorocathinones by gas chromatography/tandem mass spectrometry. *Forensic Sci Int*. 2012;223:97-105.
14. Odiorne TJ, Harvey DJ, Vouros P. Chemical ionization mass spectrometry using tetramethylsilane. *J Phys Chem*. 1972;76:3217-3220.
15. Pitt CG, Bursey MM, Chatfield DA, Greenberg RS. Some gas phase reactions of silicenium ions derived from *sym*-Tetramethyldisiloxane. *J Organomet Chem*. 1975;90:269-277.
16. Crellin KC, Widmer M, Beauchamp JL. Chemical ionization of TNT and RDX with trimethylsilyl cation. *Anal Chem*. 1997;69:1092-1101.
17. Kadentsev VI, Chuvylkin ND, Stomakhin AA, Kolotyrkina NG, Chizhov OS. Reactivity of functional groups toward H+ and $SiMe_3^+$ ions. *Russ Chem Bull*. 2000;49:570-571.

18. Wessig P, Glombitza C, Müller G, Teubner J. Photochemical preparation of highly functionalized 1-Indanones. *J Org Chem*. 2004;69:7582-7591.
19. Meltzer PC, Butler D, Deschamps JR, Madras BK. 1-(4-Methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (Pyrovalerone) analogues: a promising class of monoamine uptake inhibitors. *J Med Chem*. 2006;49:1420-1432.
20. Weintraub ST, Lear CS, Pinckard RN. Analysis of platelet-activating factor by GC-MS after direct derivatization with pentafluorobenzoyl chloride and heptafluorobutyric anhydride. *J lipid res*. 1990;31:719-725.
21. Maheux CR, Copeland CR. Chemical analysis of two new designer drugs: buphedrone and pentedrone. *Drug Test Anal*. 2012;4:17-23.
22. Nguyen KA, Gordon MS, Wang GT, Lambert JB. Stabilization of ß positive charge by silicon, germanium, or tin. *Organometallics*. 1991;10:2798-2803.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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