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Direct assignment of positional isomers by mass spectrometry: *ortho, meta* and *para* acyl and amidyl anilines and phenols and derivatives

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A direct MS/MS method for the *ortho, meta* or *para* configuration assignment of any single molecule that forms reference ions upon ionization and dissociation is demonstrated. Gas-phase structure diagnostic ion–molecule reactions with acetonitrile are shown to distinguish the isomeric 2-, 3- and 4-hydroxybenzoyl cations and the 2- from the 3- and 4-aminobenzoyl cations. These reference ions, which display indistinguishable 15 eV collision-induced dissociation product ion mass spectra, react with acetonitrile to yield characteristic ratios of product ions, most particularly for the 2-isomers. The reactivity of the 2-benzoyl cations is the most characteristic since the *ortho* configuration allows for [4⁺ + 2] polar cycloaddition that yields relatively stable heterocycles in *N*-protonated forms. Distinction of the reference isomeric 2-, 3- and 4-hydroxy- and aminobenzoyl cations permits, therefore, partially or completely, direct 'MS-only' positional assignment of either *ortho, meta* or *para* configuration for any single molecule that forms such reference ions upon ionization and dissociation. This ''class-universal'' method for direct MS assignment of a single positional isomer should therefore be applicable to many members of the homologous series of isomeric *ortho, meta* and *para* acyl and amidyl anilines and phenols and derivatives. Such molecules dissociate, or are likely to dissociate, after or during ionization processes to form the reference and structurally diagnostic *ortho, meta* or *para* hydroxy- or aminobenzoyl cations. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: pentaquadrupole mass spectrometry; acylium ions; cyclization reactions; isomer distinction; *ortho*, *meta* and *para* structural assignment

INTRODUCTION

Differentiation of isomers has always been a challenging task in mass spectrometry (MS). Ideally, for unequivocal MS structural elucidation, the ionized molecule (its radical cation or anion, the protonated, deprotonated or cationized molecule) of each isomer should dissociate to form a unique, structure diagnostic fragment ion. Otherwise, either the neutral or the ionized molecule of each isomer should react to form a unique, structure diagnostic product ion. These are, however, indirect structural assignments, since we must know in advance the configuration of each positional isomer (as determined by other techniques), and the whole set of isomers must be analyzed and their spectra compared under the same MS conditions to ensure the reliability of and to establish a comparative MS method for the distinction of a specific set of isomers.

For a new single isomeric molecule of unknown configuration, it is unlikely, therefore, that one would succeed in performing its direct structural assignment using only MS data and the methods just described.

A method for direct configuration assignment of positional isomers could be based, however, on the use of either collision-induced dissociation (CID)[1] or structure diagnostic ion–molecule reactions[2] not for a specific target molecule but for reference fragment ions in which the positional information of any substituent is preserved. A typical example is that of pyridyl and pyrimidyl cations. For these isomeric heteroaromatic cations, positional information of ring substituents is preserved upon $R^{\bullet}$ loss because the positive charge is placed after dissociation on a localized sp^2 orbital, and barriers for 'H-ring walk' are normally high enough to prevent isomerization (Scheme 1).[3] Unique dissociation behavior or structure diagnostic ion–molecule reactivity, or even both, could then reveal the location of the charge site in these 'reference ions', and thus determine the original position of literally any type of ring substituent (R) in the parent ion. This ''class-universal'' method could therefore be solely applied regardless the ring substituent to every single molecule within the whole series of analogue isomers. It would only be required that the single target molecule forms the respective reference ion upon ionization and dissociation.

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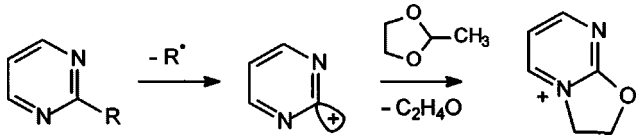
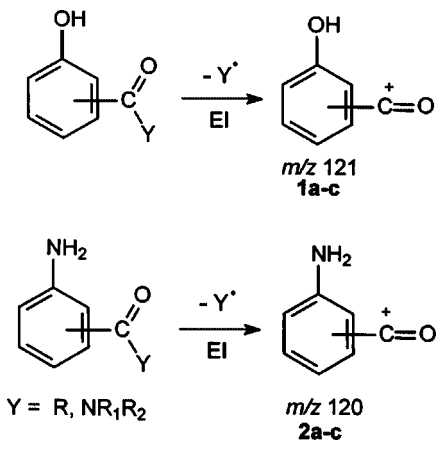


Scheme 1

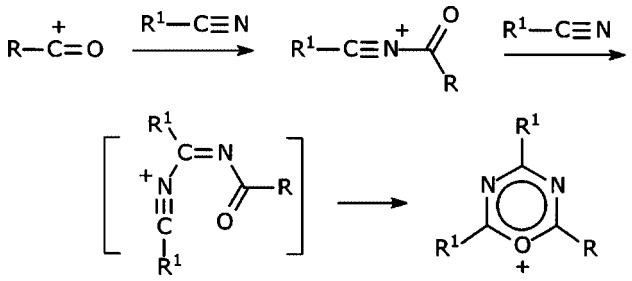
With the application of such direct MS-only method in mind, we have recently demonstrated[4] that both CID and a structure diagnostic polar transacetalization reaction[5] occurring most pronouncedly for the 2-isomer (Scheme 1) can be used to distinguish (locate the charge site) the 2-, 4- and 5-pyrimidyl cations, a set of reference ions that can be used for the direct *ortho*, *meta* or *para* configuration assignment of any single molecule of a monosubstituted pyrimidine that forms such ions upon ionization and dissociation.

We wish, therefore, to demonstrate further the generality of this method. *Ortho, meta* and *para* series of isomeric monosubstituted acyl and amidyl anilines and phenols such as the 2-, 3- and 4-isomeric sets of acetylanilines and -phenols, and aminobenzamides,[6] often display nearly identical, undistinguishable 70 eV electron ionization (EI) mass spectra, so even the comparative MS methods of configuration assignments based on EI-MS would certainly fail. Other approaches based on the intact ionized molecules such as chemical ionization (CI)-MS/MS would also tend to fail. Fortunately however, these isomeric molecules readily form upon 70 eV EI the 2-, 3- or 4-isomeric sets of hydroxy (**1a**–**c**) and amino (**2a**–**c**) benzoyl cations, respectively (Scheme 2). These acylium ions are stable gaseous ions with a diverse gas-phase chemistry[7] that makes them good candidates to reference ions for configuration assignment.

The isomeric precursors (and if so then any single known or new analogue as long as it forms the reference benzoyl cation) could then be otherwise distinguished if **1a**–**c** and **2a**–**c** are found to display unique CID behavior or ion–molecule reactivity, or both. Several mass spectrometric studies with mass-selected ions have investigated extensively the gas-phase ion–molecule chemistry of acylium ions,[7] and a variety of novel reactions have been found. For instance, nitriles add to acylium ions in a tandem cyclization process to yield 1,3,5-oxadiazinium ions (Scheme 3).[8]



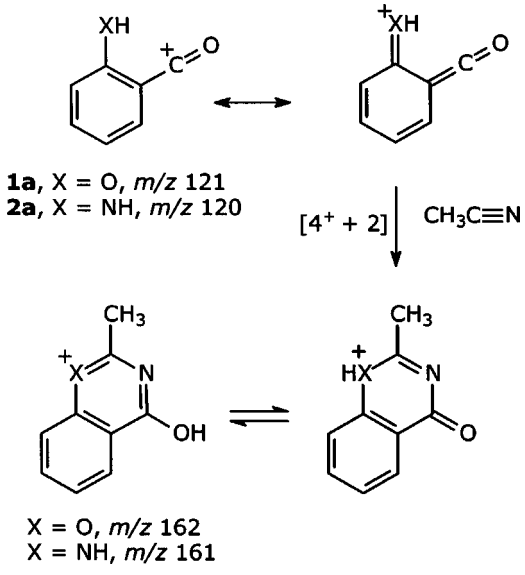
Scheme 2



Scheme 3

For the isomeric benzoyl cations **1a**–**c** and **2a**–**c**, reactions with nitriles therefore appear promising. Particularly for the 2-isomers, single nitrile addition to both the 2-hydroxybenzoyl (**1a**) and 2-aminobenzoyl cation (**2a**) could proceed via polar $[4^+ + 2]$ cycloaddition[9] to form relatively stable heteroaromatic species, that is, the protonated forms of the heterocycles 2-methyl-1*H*-quinazolin-4-one and 2-methylbenzo[*e*][1,3]oxazin-4-one (Scheme 4). Note that **1a** corresponds the gaseous protonated form of a cyclic α-oxoketene, that is, of the *o*-quinonoid ketene, and **2a** to its *N*-analogue. α-Oxoketenes are highly reactive, usually transient and important building blocks in organic synthesis,[10] and $[2 + 2]$ cycloadditions so common for other ketenes are often overridden by $[4 + 2]$ cycloadditions with dienophiles. We found recently that ionized *o*-quinonoid ketene of *m/z* 120 reacts readily via polar $[3^+ + 2]$ cycloaddition with several enol ethers and ketones.[11]

Here we report a multiple-stage pentaquadrupole (QqQqQ) mass spectrometric study[12] in which an 'MS-only' method was tested for the direct structural assignment of single molecules of *ortho, meta* or *para* acyl and amidyl anilines and phenols and derivatives. The isomeric acylium ions **1a**–**c** and **2a**–**c** were selected as the reference ions, and both CID and ion–molecule reactions with acetonitrile were evaluated for their distinction.



Scheme 4

EXPERIMENTAL

MS^2 and MS^3 experiments were performed with an Extrel pentaquadrupole mass spectrometer.[13] The $Q_1q_2Q_3q_4Q_5$ consists of three mass-analyzing quadrupoles (Q_1, Q_3, Q_5) in which ion-mass selection and analysis are performed, and two reaction quadrupoles (q_2, q_4) which are used to perform either low-energy (near zero eV) ion-molecule reactions or 15 eV CID with argon. For the two-stage MS^2 experiments, the ion of interest was generated by the dissociative 70 eV EI of the following precursors: **1a** (2-hydroxybenzaldehyde), **1b** (3-hydroxybenzaldehyde), **1c** (4-hydroxybenzaldehyde), **2a** (2-aminoacetophenone), **2b** (3-aminoacetophenone), and **2c** (4-aminoacetophenone). After ion-molecule reactions or CID in q_2, Q_5 was scanned to record the product ion spectra, while operating Q_3 in the non-mass analyzing rf-only mode. For the MS^3 experiments, a q2-product ion of interest was mass-selected by Q3 for further 15 eV collision-induced dissociation (CID) with argon in q4, while scanning Q5 to record the mass spectrum. The collision energies, calculated as the voltage difference between the ion source and the collision quadrupole, were typically near 1 eV for ion-molecule reactions and 15 eV for CID both in MS^2 and MS^3 experiments.

Molecular orbital calculations were performed with GAUSSIAN98.[14] Optimized structures and the total energies for the species of interest (available from the authors upon request) were obtained with DFT calculations at the B3LYP/6-311G(d,p) level of theory.

RESULTS AND DISCUSSION

CID behavior

Unfortunately, all three isomeric hydroxy- (**1a–c**) and aminobenzoyl cations (**2a–c**) display nearly identical, indistinguishable CID behavior upon 15 eV collisions with argon (spectra not shown). Ions **1a–c** of m/z 121 dissociate readily and similarly by sequential loss of two CO molecules to form the fragment ions of m/z 93 and 65. The ions **2a–c** dissociate readily and similarly upon 15 eV CID by CO loss to form fragment ions of m/z 92, and then by the loss of a neutral HNC molecule to form fragment ions of m/z 65.

Reactions with acetonitrile

Fortunately, each isomeric hydroxy benzoyl cation **1a–c** is found to react with acetonitrile in a distinctive, structure-diagnostic fashion (Fig. 1). The 2-isomer **1a** forms nearly exclusively the adduct of m/z 162 (Fig. 1(a)). The great impetus of **1a** towards addition to acetonitrile may therefore result, as expected, from $[4^+ + 2]$ cycloaddition and formation of a relatively stable aromatic species, that is, protonated 2-methyl-1*H*-quinazolin-4-one of m/z 162 (Scheme 4).

The 3-isomer **1b** also reacts with acetonitrile forming an adduct of m/z 162, but a second product ion of m/z 134 is

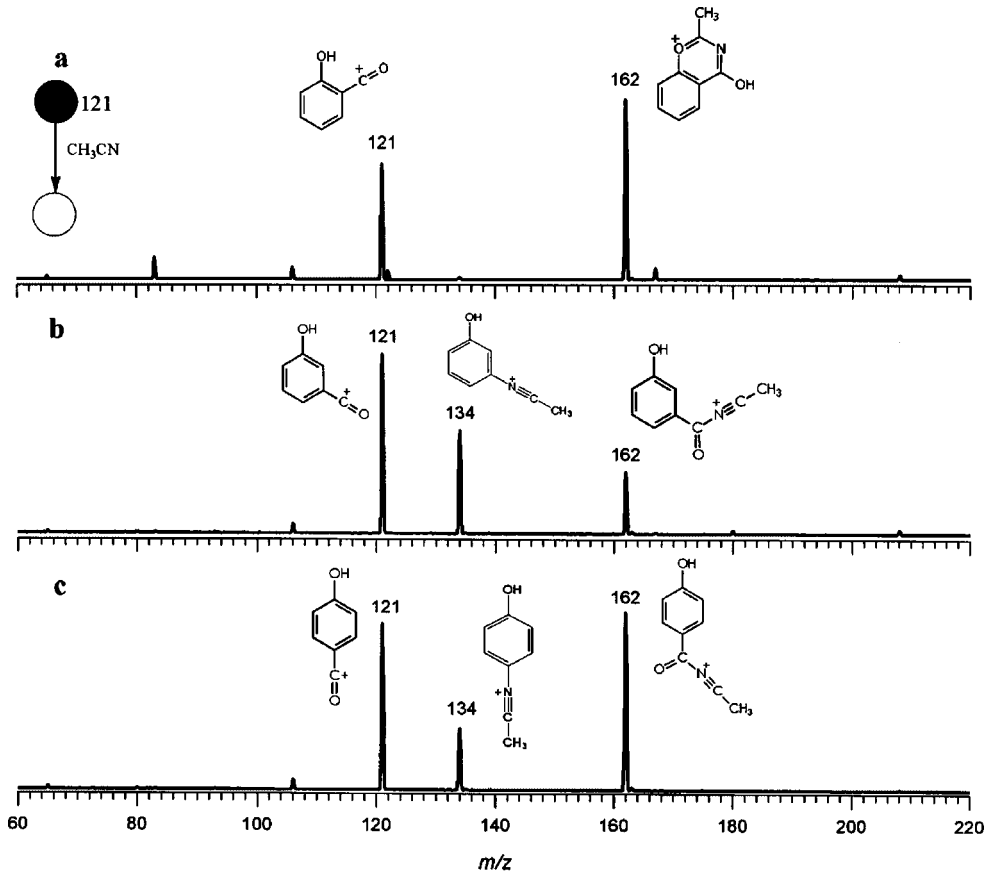
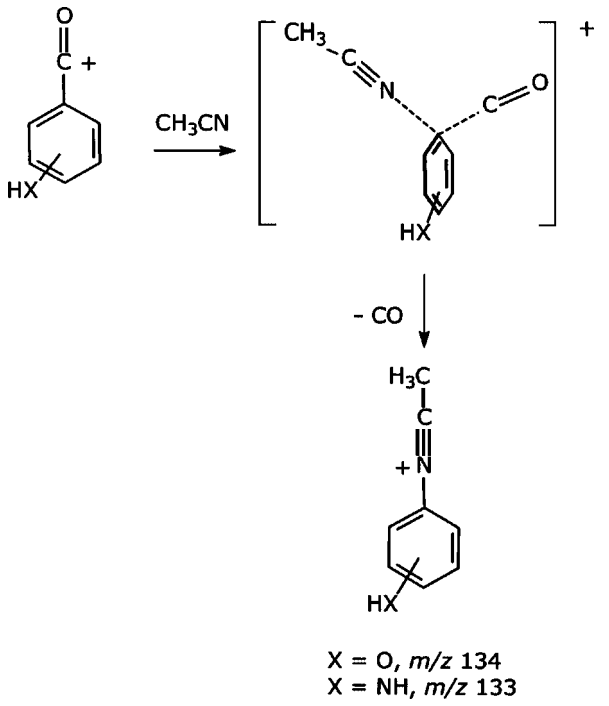


Figure 1. Product ion mass spectra for the reactions of the isomeric *ortho*, *meta* and *para* (2-, 3- and 4-) hydroxybenzoyl cations **1a–c** of *m/z* 121 with acetonitrile. Note for each isomer the characteristic distribution of the two major product ions: the intact adduct of *m/z* 162 and the CO-by-CH_3CN '*ipso* substitution' product ion of *m/z* 134.

also formed, and to a greater extent. Such a product formally arises from CO loss from the intact adduct, and Scheme 5 (X = O) provides a rationalization for its formation via *ipso* attack of acetonitrile with CO substitution. Note that the *ipso* carbon carries by far the most of the positive charge for benzoyl cations.[15] Finally, the 4-isomer **1c** also reacts with acetonitrile to form both product ions of *m/z* 134 and 162, but for **1c** the ion of *m/z* 162 is more abundant than that of *m/z* 134.

The isomeric 2-, 3- and 4-aminobenzoyl cations (**2a**–**c**) also react with acetonitrile in rather distinct fashions, but again most particularly so for the 2-isomer **2a** (Fig. 2). The *ortho* isomer **2a** forms both the intact adduct of *m/z* 161 and the CO-by-CH_3CN '*ipso*-substitution' product ion of *m/z* 133 (Scheme 5, X = NH). In contrast, both **2b** and **2c** nearly fail to form the intact adduct of *m/z* 161, and the '*ipso*-substitution' product ion of *m/z* 133 dominates. The following *m/z* 161: 133 ion abundance ratios were calculated for the three isomers: **2a** (1.50), **2b** (0.04) and **2c** (0.09). Hence this ratio for **2a** is nearly 30 times greater than those for **2b** and **2c**. Therefore, **2a**, owing to its *ortho* configuration, reacts selectively with acetonitrile probably via polar [$4^+ + 2$] cycloaddition to yield a stable, aromatic heterocycle in its protonated form: 2-methylbenzo[*e*][1,3]oxazin-4-one of *m/z* 161 (Scheme 4, X = NH). Likewise, the '*ipso*-substitution' product ion of *m/z* 133 for **2a** (Scheme 5) is likely to undergo cyclization via intramolecular attack



Scheme 5

of the amino group to form, after intramolecular proton transfer, a much more thermodynamically stable (see DFT calculations below) species, that is, the *N*-protonated form

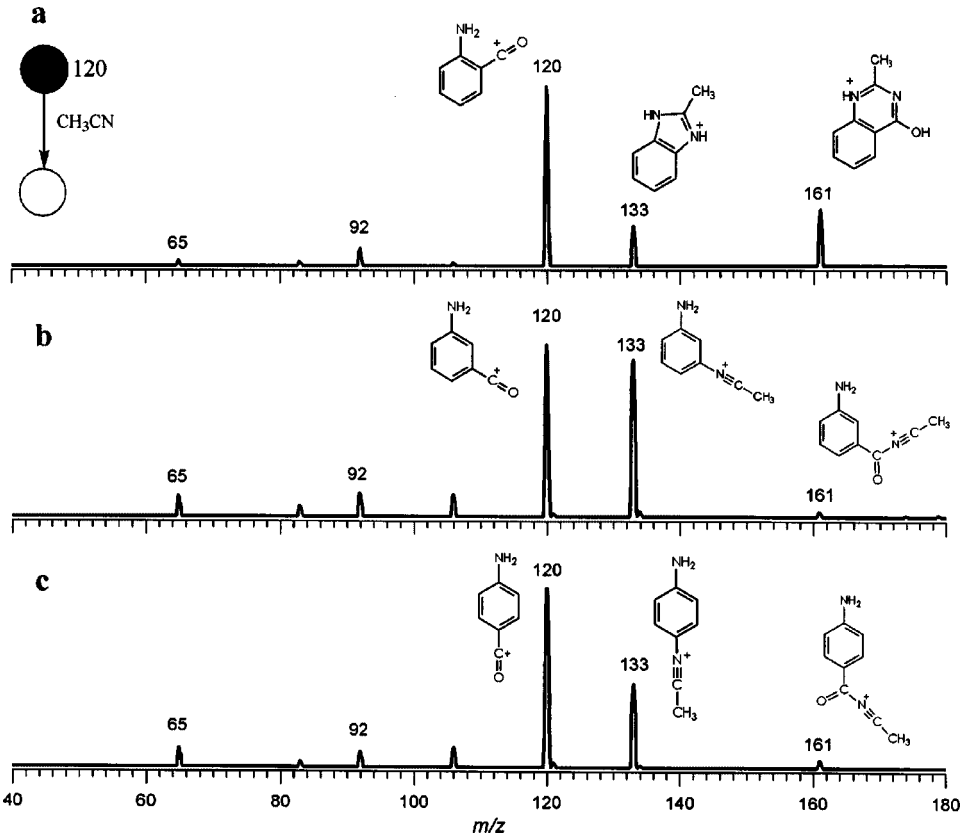
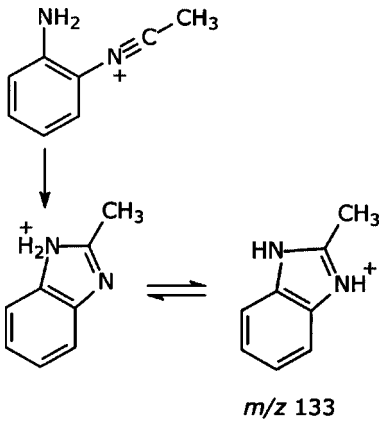


Figure 2. Product ion mass spectra for the reactions of isomeric *ortho*, *meta* and *para* (2-, 3- and 4-) aminobenzoyl cations **2a**–**c** of *m/z* 120 with acetonitrile. Note the distinct reactivity of the *ortho* isomer **2a**, which forms both the adduct of *m/z* 161 and the CO-by-CH_3CN '*ipso* substitution' of *m/z* 133 to considerable extents, whereas **2b** and **2c** reacts similarly to each other, yielding predominantly the respective CO-by-CH_3CN '*ipso* substitution' product ion of *m/z* 133.



m/z 133

Scheme 6

of the aromatic heterocycle 2-methyl-1*H*-benzoimidazole (Scheme 6).

DFT calculations

DFT calculations at the B3LYP/6–311G(d,p) level show that the acyclic adduct of the 2-hydroxybenzoyl cation **1a** with acetonitrile lies -12.1 kcal mol^{-1} (1 kcal = 4.184 kJ) below the energy of the reactants, whereas the aromatic cyclic adduct of *m/z* 162, that is, protonated 2-methyl-1*H*-quinazolin-4-one (Scheme 4, X = O), lies much lower in energy, that is, -39.1 kcal mol^{-1} below the reactants. Hence the formation of the cyclic adduct is far the most thermodynamically favored process. Similarly, the acyclic adduct for reactions of the 2-aminobenzoyl cation **2a** with acetonitrile is found to lie -12.9 kcal mol^{-1} below the reactants, but the aromatic cyclic adduct, *N*-protonated 2-methyl-benzo[*e*][1,3]oxazin-4-one (Scheme 4, X = NH) is also placed much lower in energy, that is, it lies -42.5 kcal mol^{-1} below the reactants.

DFT calculations also shows that cyclization of the CO by CH_3CN '*ipso* substitution' product ion of *m/z* 133 for **2a** (Scheme 5) is highly thermodynamically favored: the acyclic ion is placed -78.3 kcal mol^{-1} below the reactants, but the cyclic *N*-protonated 2-methyl-1*H*-benzoimidazole is found as far below as -119.7 kcal mol^{-1}!

CONCLUSION

A direct MS/MS method using gas-phase ion–molecule reactions with acetonitrile for the *ortho, meta* and(/or) *para* structural assignment of any single molecule that forms reference hydroxy- or aminobenzoyl cations upon ionization and dissociation has been demonstrated. This ''class-universal'' method is expected to be applicable to either known or new molecules of *ortho, meta* or *para* acyl or amidyl anilines or phenols or derivatives. Sets of isomers within this class are known to display nearly identical EI mass spectra. The product yield may vary as a function of the conditions used for ion–molecule reactions with the reference ions, but such ions could be easily formed from standards, as in this study, to calibrate product ion ratios before the analysis of amino or hydroxybenzoyl cations arising from molecules of unknown configuration. The method could be applied for molecules ionized by different techniques such as EI and CI or even electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Under atmospheric pressure ESI and APCI conditions, in-source CID could induce dissociation to form the reference benzoyl cations for their further gas-phase reactions. Even atmospheric pressure in-source ion–molecule reactions with acetonitrile (a common HPLC eluent and ESI solvent) occurring under APCI and ESI conditions[16] could eventually be performed.

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