# Oxidation-assisted structural elucidation of compounds containing a tertiary amine side chain using liquid chromatography-mass spectrometry

**Short title:**

**Structure elucidation via oxidation and LC-MS**

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**Abstract**

A novel analytical technique for the structural elucidation of compounds bearing a tertiary amine side chain via "in vial" instantaneous oxidation and liquid chromatography-mass spectrometry (LC-MS) was developed. A series of lidocaine homologues and benzimidazole derivatives with a major/single amine-representative base peak in both their EI-MS and ESI-MS/MS spectra were subjected to oxidation by a 0.1% solution of hydrogen peroxide (including several $^{16}O/^{18}O$ exchange experiments), followed by LC-ESI-MS/MS analysis. The N-oxide counterparts promoted extensive fragmentation patterns with complete coverage of all parts of the molecule, enabling detailed structural elucidation and unambiguous identification of the un-oxidized analytes at low ng/ml levels.

**Keywords:** Structural elucidation, Tertiary amine side chain, Oxidation, LC-MS/MS, Water analysis

## 1. Introduction

Mass spectrometry is commonly applied to qualitatively and quantitatively profile small molecules. The increased availability of high-resolution mass spectrometry (HR-MS) for chemical analysis has dramatically improved the detection and identification of compounds in the fields of metabolomics, drug discovery, forensics, and environmental monitoring.[1] Modern mass spectrometers provide accurate measurements of the mass-to-charge ratios of ions with errors as low as sub-ppm depending on the instruments and calibration.[2] In addition to the mass accuracy, the ability of a mass spectrometer to faithfully measure the isotopic distribution of an ion is also important to determine the elemental composition of a molecule. Even a high mass accuracy and isotope distribution are not sufficient to determine the unique chemical structure of a molecule based on the mass value alone.[3] Evaluation of tandem MS (MS/MS) fragmentation is useful, however not always sufficient to propose structures.[4] For structure elucidation, which is the main bottleneck in the identification of unknown compounds,[5,6] MS/MS spectra with diagnostic and informative product ions representing all parts of the molecule are needed. The most common approach to determining the structure of unknown small organic molecules is searching the molecular formula against databases, such as Chemindex, NIST library or even Scifinder, for possible structures. Structure-fragmentation relations have been widely studied by Niessen,[7] Levsen et al.,[8] Holcapek et al.,[9] and our group.[10] Various algorithms that evaluate the structures of organic compounds and predict mass spectral fragments based on these rules have been developed (Mass Frontier, Mass Fragmenter, Advanced Chemistry Development, EPIC).[11-14] However, difficulties arise in cases where the ESI-MS/MS spectrum is uninformative or exhibits poor quality, leading to insufficient evidence to match the ESI-MS/MS spectra to the proposed structure. Several compounds containing an amine side chain, particularly tertiary

amines, present information-poor mass spectra with product ions mostly/only representing the amine-containing residue.[15-18] This lack of information is attributed to the strong tendency of the amine side chain to stabilize the positive charge and leads to unavoidable ambiguity in the identification process. In order to address this challenge, we have recently demonstrated a method for the structural determination of benzimidazoles containing a tertiary amine side chain employing multi-stage tandem MS, in combination with fragmentation pattern matching to an analogue utilizing LC-MS analysis.[16]

The objective of the current study was to develop a novel analytical technique based on oxidation for the structural elucidation of compounds containing tertiary amine side chains in aqueous solutions based on LC-MS analysis. The outcome of this study is discussed as a helpful tool when encountering such challenges. To the best of our knowledge, oxidation-assisted structure elucidation is demonstrated here for the first time.

## 2. Experimental

### *2.1. Materials and reagents*

Lidocaine hydrochloride monohydrate, L5647 (**A1**); 6-chloro-2-diethylamino-ortho-acetotoluidide hydrochloride, S423599 (**A2**); N-(4-tert-bu-2,6-dimethyl-ph)-2-diethylaminoacetamide, hydrochloride, S243205 (**A3**); ropivacaine hydrochloride monohydrate, R0283 (**A4**); bupivacaine hydrochloride, SML1092 (**A5**); amino-1-(2-(MeO-ph)-2-oxo-et)-3-(2-diethylamino-et)-benzimidazol-1-ium bromide, S20401 (**A7**); 2-amino-1-(2-(4-Cl-ph)-2-oxo-et)-3-(2-diethylamino-et)-benzimidazole-1-ium bromide, S20398 (**A8**); amino-1-(2-(di-MeO-ph)-2-oxo-et)-3-(2-morpholin-4-yl-et)-benzimidazol-1-ium bromide, S20320 (**A9**); 2-amino-1-(2-(4-Br-ph)-2-oxo-et)-3-(2-morpholin-4-yl-et)-benzimidazol-1-ium bromide, S20312 (**A10**), ammonium formate, 70221; 50 wt. % hydrogen peroxide solution in

$H_2O$, stabilized, 516813; and hydrogen peroxide $^{18}O_2$ solution, 2.2% in $H_2O$, 90% $^{18}O$ atoms, 609978 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Etonitazene (**A6**) was purchased from Tzamal D-chem Company (Israel). Water (LC-MS grade) and methanol (LC-MS grade) were obtained from Biolab Company (Israel).

### *2.2. Sample preparation*

Stock solutions were prepared in water (1 mg/ml) and diluted with water to concentrations in the range of 1 ng/ml- 1 µg/ml.

Oxidation of **A1**-**A10**

Hydrogen peroxide (2 µl, 50% in water) was added directly into an injection vial containing 998 µl of water with 0.001-1 µg of **A1**-**A10** to obtain a 0.1% solution of hydrogen peroxide. The analysis vial was stirred for 1 min prior to the LC-MS analysis.

$^{16}O/^{18}O$ oxygen isotope exchange

The $^{16}O/^{18}O$ exchange experiments were performed by exchanging the $H_2O_2$ for $H_2{}^{18}O_2$. The experiment was then run via the same method described earlier.

### *2.3. Instrumentation*

An HPLC-QTRAP-MS system and an HPLC-Q-Exactive Plus Orbitrap MS system were used in this study and data from both instruments is presented.

The analytes were separated using an Agilent 1290 high-performance LC system (Palo Alto, CA, USA) containing a 1290 infinity binary pump with a jet weaver V35 mixer, a 1290 infinity autosampler and a 1290 infinity thermostatted column compartment (TCC). HPLC conditions: gradient elution was performed on a reverse phase separation column (Gemini C18, 3.0 µm, 150 mm, 2.1 mm ID by Phenomenex, Switzerland) with a flow of 0.3 ml/min. The column was maintained at 40 ℃ in all the experiments. The gradient (solvent A,

water with 5% MeOH containing 1 mM ammonium formate; solvent B, MeOH containing 1 mM ammonium formate) was as follows: 0-10 min, linear increase from 0% B to 95% B, hold of 6 min at 95% B, followed by a 4 min equilibration period at 100% A.

MS and MS/MS experiments were carried out using two mass spectrometers in positive-ion mode: an Applied Biosystems 5500 QTRAP LIT quadrupole MS (AB SCIEX, Foster City, CA, USA) with "Analyst" software (version 1.6) equipped with a Turbo V ESI source and a Thermo Scientific Q-Exactive Plus Orbitrap MS (Thermo Fisher Scientific, Bremen, Germany) operated with a heated-ESI (HESI) source.

The QTRAP ESI operating conditions were as follows: gas 1, air (40 psi); gas 2, air (70 psi); ion spray voltage, 4500 V; ion source temperature, 600 ℃; curtain gas, nitrogen (35 psi). MS/MS experiments: The settings for the enhanced product ion (EPI) scans were as follows: collision gas 'high', and the collision energy was between 20 and 60 V. The fixed LIT fill time was set to 50 ms.

The orbitrap HESI operating parameters were as follows: electrospray voltage, 1.25 kV; sheath gas flow rate, 45 (arbitrary units); auxiliary gas flow rate, 10 (arbitrary units); sweep gas flow rate, 2 (arbitrary units); aux gas heater temperature, 400 ℃; capillary temperature, 275 ℃. The instrument was calibrated using a positive ESI calibration solution prepared according to the operating manual. All samples were analyzed using two alternating experiment types: full scan mode from *m/z* 60-900 at a resolving power of 70,000 and $1x10^6$ automatic gain control (AGC) target, and a data independent acquisition (DIA) experiment with an inclusion list at a resolving power of 35,000 and $5x10^5$ AGC target. The normalized collision energy (NCE) was set between 20 and 100 V.

## 3. Results and discussion

### *3.1. Chemical derivatization*

Because N-oxides undergo thermal deoxygenation during a GC-MS analysis,[19,20] we focused on an LC-MS-based method. To address the problem of information-poor MS/MS spectra, we sought a derivatizing agent that would react with tertiary amines, reduce their stability as protonated product ions in preference to the main core, and as a result, improve the mass spectral quality in LC-MS/MS analysis. The absence of an active hydrogen on tertiary amines renders traditional derivatization reactions, such as acylation, ineffective. Moreover, most derivatization reactions, such as acylation or silylation, usually require anhydrous reaction conditions because these derivatization reagents are very sensitive to moisture and may hydrolyze.[21] Because our investigated compounds may be found in aqueous solutions, it is desirable that these amine transformations be carried out in water. Our goal was to develop a new derivatization method that allows structural elucidation of compounds containing a tertiary amine side chain. The reaction between hydrogen peroxide and tertiary amines has been documented to lead to the formation of N-oxides.[22] Mass spectral analysis of various N-oxide compounds, including nitrogen mustards, N,N-dialkylaminoethyl-2-chlorides and N,N-dialkylaminoethanols etc., demonstrated a distinctive fragmentation pattern compared with the intact parent amine.[23,24,25] Therefore, we decided to explore the oxidation reactions of various tertiary amines and their effect on the MS/MS fragmentation behavior.

### *3.2. Analyte selection*

A series of lidocaine-related structures bearing a tertiary amine side chain with varying aromatic cores (**A1**, **A2**, **A3**) or tertiary amine side chains (ropivacaine (**A4**),

bupivacaine (**A5**)), and a series of benzimidazole derivatives containing a tertiary amine side chain (**A6**-**A10**), which all present information-poor $MS^2$ spectra with insufficient information about the core, were chosen to be analyzed and evaluated (Fig. 1).

### *3.3. Reaction conditions (reaction time, oxidant concentration, base addition)*

Several reaction parameters were investigated, including reaction time (1 min- 24 h), $H_2O_2$ concentration (0.01%- 1%) and effect of base addition (potassium carbonate). We noticed that the oxidation rate of **A1**-**A10** increased with $H_2O_2$ concentration up to 0.1%, while no substantial increase was further observed in the concentration range from 0.1% to 1%. Base addition only slightly improved the signal intensity. Extending the reaction time improved the signal intensity to some extent; after 1 min stirring, between 10-20% of the analytes were converted to the oxide form. The analysis after 3 h revealed up to a 40% conversion with a higher signal intensity, and a 40-60% conversion was obtained after 24 h. Even though the N-oxide counterparts were less sensitive to detect than the non-oxidized compounds, their MS/MS spectra provided the information needed to enable the identification of the analytes. Hence, utilizing 0.1% $H_2O_2$ with only 1 min of stirring enables the formation of adequate amounts of N-oxide counterparts, allowing unambiguous identification of the analytes at low ng/ml levels. In cases where the analyte concentration is at the detection limit, prolonged reaction time of up to 24 h may improve signal intensity by a factor of 3-5.

### *3.4. MS/MS spectra of aromatic amides containing a tertiary amine side chain [$R_1$-NH-CO-$R_2$] (Lidocaine homologues, A1-A5)*

This interesting class of aromatic amides contains some local anesthetic agents. Their general structures are presented in Fig. 1, which shows that these molecules can be

characterized by two sub-structures: the "core" substituted ring ($R_1$) and the tertiary amine side chain ($R_2$). In general, the tertiary amine side chain of these compounds determines the fragmentation pattern for both EI and ESI, regardless of the substituent present on the core ring.

**A1**-**A3** have the structure of $R_1$-NH-CO-$CH_2$-N($C_2H_5$)$_2$ with different aromatic cores, where $R_1$=2,6-dimethyl phenyl (**A1**), $R_1$=6-chloro-2-methyl phenyl (**A2**) and $R_1$=4-tert-butyl-2,6-dimethyl phenyl (**A3**). The only product ions observed in their MS/MS spectra were at *m/z* 86 (corresponding to $CH_2=N^+(C_2H_5)_2$) and *m/z* 58, corresponding to $CH_2=N^+H(C_2H_5)$ (sequential loss of ethylene). Lidocaine (**A1**), ropivacaine (**A4**) and bupivacaine (**A5**) have an identical core with different tertiary amine side chains, and their EI-MS and ESI-MS/MS spectra exhibit fragments/product ions representing only the tertiary amine side chain (*m/z* 86, 126, and 140, respectively) without core-representative product ions. For example, *m/z* 126 and 140 in **A4** and **A5** correspond to the cleavage between the amide carbon and piperidyl ring. The MS/MS spectra of **A1**-**A3** and **A5** are shown in Fig. 2 (left) (**A4**, not shown).

The amide bond in all the derivatives **A1**-**A5** appears to not be cleaved, unlike that in peptides. This is probably due to amide bond stabilization by virtue of resonance with the aromatic group.[26] It is noteworthy that our attempt to obtain more abundant ions related to the main core utilizing EPI (MS/MS) experiments with a broad range of collision induced dissociation (CID) energies failed, even when we increased the analyte concentration up to µg/ml levels.

### *3.5. Oxidation effect on MS/MS spectra of the aromatic amides containing a tertiary amine side chain [$R_1$-NH-CO-$R_2$] (Lidocaine homologues, A1-A5)*

Oxidation of **A1-A5** employing a 0.1% hydrogen peroxide solution resulted in the addition of an oxygen atom. The N-oxide counterparts with a 16 Da increase in mass, as expected, were characterized by LC-ESI-MS/MS, revealing a different CID-MS/MS pattern with product ions indicative of both the core ring and tertiary amine side chain, though the main peak, that seems to exclude the formation of N-oxides, was still at *m/z* 86 for **A1-A3** and *m/z* 126 and 140 for **A4** and **A5**, respectively, for all collision energies. These peaks can be rationalized however by OH-group migration in the protonated N-oxide[27] or by loss of an OH radical, followed by a homolytic carbon-carbon cleavage via a concerted process, as reported in the fragmentation of N-oxide of N,N-dialkylaminoethyl-2-chlorides and N,N-dialkylaminoethanols.[23,24] The ESI-MS/MS spectra of **A1-A3** and **A5** after the oxidation are depicted in Fig. 2 (right). At a low collision energy (20 eV, not shown), cleavage at the C-N bond in the amide position with charge retention on the tertiary N-oxide side chain was observed in the ESI-MS/MS spectra (*m/z* 130 in **A1-A3**, *m/z* 170 in **A4** and *m/z* 184 in **A5**). At a higher collision energy (40 eV), an ion at *m/z* 88 (loss of $CH_2$=CO from *m/z* 130) was observed for **A1-A3**, and an ion at *m/z* 100 (loss of CO and propylene or alternatively butylene) was observed for **A4** and **A5**. The ions attributed to the N-oxide of the tertiary amine side chain strengthened our assumption that oxygen was incorporated in the tertiary amine rather than the aromatic or benzylic sites. The formation of the ions at *m/z* 130 and *m/z* 88 in lidocaine has been discussed in the literature,[27] demonstrating the incorporation of oxygen on the right-hand side of the amide bond and supporting the assumption of N-oxide formation. Furthermore, when the reaction was performed using a solution of 0.1% $^{18}$O-labeled hydrogen peroxide, the expected 2 Da mass increase was observed for those ions (*m/z* 90 and 132 in **A1-A3**, *m/z* 102 and 172 in **A4** and *m/z* 102 and 186 in **A5**). The MS/MS

spectra of **A1** after oxidation with $^{18}$O-labeled hydrogen peroxide are depicted in the supporting information, Fig. S1. At a collision energy of 40 eV, some cleavages with charge retention on the core were observed in the ESI-MS/MS spectra. For **A1** and **A2**, product ions were observed at *m/z* 164 and 184, respectively. These ions are attributed to cleavage at the C-N position (α to the tertiary N-oxide) with a proton transfer to the main core. The equivalent ion for **A3** at *m/z* 220 was not observed; however, an ion at *m/z* 164 was observed, which apparently is the secondary product of the *m/z* 220 ion after a sequential loss of the tert-butyl group. In addition, ions at *m/z* 148, 168, and 204 were observed for **A1**, **A2** and **A3**, respectively, and correspond to cleavage between the carbonyl and the carbon α to the tertiary N-oxide. These ions sequentially lose the carbonyl group, resulting in the formation of ions at *m/z* 120, 140 and 176, respectively. Another common dissociation process was observed at the C-N amide bond, which resulted in the formation of ions at *m/z* 122, 142 (for **A1** and **A2**) and *m/z* 122 (**A3**) (C-N cleavage with sequential loss of the tert-butyl group). A comparison between the ESI-MS/MS spectra of the N-oxides of lidocaine (**A1**), ropivacaine (**A4**) and bupivacaine (**A5**), all with an identical core, reveals a repetitive fragmentation pattern with ions at *m/z* 148, 120 and 105, which is indicative of the aromatic amide core. The ion at *m/z* 105 corresponds to cleavage at the aromatic C-N bond, leading to the formation of a 2,6-dimethyl phenyl cation, in addition to the aforementioned ions at *m/z* 148 and 120. However, the ion at *m/z* 164 was only observed in lidocaine and not in **A4** and **A5** due to their ring system, preventing cleavage at the C-N position (α to the tertiary N-oxide). All the formulae of the discussed ion structures were supported by orbitrap-$MS^2$ exact mass measurements (< 2 ppm error) [Table 1]. It is noteworthy that the formation of the N-oxide counterparts leads to an increase in polarity, and therefore, shorter LC retention times of all the oxide derivatives were observed utilizing reverse-phase C18 chromatography.

### *3.6. MS/MS spectra of benzimidazole derivatives containing a tertiary amine side chain (A6-A10)*

The dissociation pathways of five benzimidazoles containing a tertiary amine side chain, **A6-A10,** were previously studied.[16] The most abundant product ions in the ESI-MS/MS spectra of **A6-A8** are at *m/z* 100, which corresponds to the charge-favorable N,N-diethylaziridinium ion and *m/z* 72 (sequential loss of ethylene). For **A9-A10**, the dominant ion is at *m/z* 114, corresponds to the charge-favorable vinyl morpholinium ion, which further dissociates to *m/z* 84 (sequential loss of formaldehyde) and *m/z* 70 (sequential loss of acetaldehyde). The MS/MS spectrum of **A6** is depicted in Fig. 3 (left), and the MS/MS spectra of **A7-A10** are available in the supporting information, Figs. S2 and S3 (left).

All the above mentioned product ions are representative of only the amine-containing residue. As mentioned before, this is attributed to the strong tendency of the amine side chain to stabilize the positive charge rather than the benzimidazole core. A cleavage of the C-N bond (α to the tertiary amine) that leads to an ion resulting from dimethylamine loss, which is typical of alkyl amine groups, is not favorable here, as we previously reported.[10] This type of cleavage may have provided structural information about the benzimidazole core. However, only low-abundance, core-indicative product ions were observed. Therefore, an interpretation based on these low-abundance ions was impractical.

### *3.7. Oxidation effect on MS/MS spectra of benzimidazole derivatives containing a tertiary amine side chain (A6-A10)*

In general, benzimidazole derivatives are very stable towards heat and oxidizing agents.[28] The two lone pair electrons contribute to the aromatic sextet by an imino group, and the other nitrogen is a pyridine-like base with a pKa value of the conjugate acid ~5, versus ~11 for the tertiary amine side chain. Thus, we assumed oxidation would preferably occur on

the amine side chain moiety (similarly to **A1**-**A5**). Employing an oxidation reaction resulted in high-intensity, rich and informative ESI-MS/MS spectra of all the N-oxide derivatives with product ions representing all parts of the molecule. The ESI-MS/MS spectrum of the N-oxide of etonitazene (**A6**) is presented in Fig. 3 (right).

For etonitazene, ions representative of the amine side chain (*m/z* 100, 72) were mainly observed, in addition to trace-abundance ions indicative of the benzimidazole core and the ethoxybenzyl side residue (*m/z* 278, 135, 107). For the N-oxide counterpart, product ions indicative of the core were primarily observed. This can be attributed to the less favorable tendency of the N-oxide group to stabilize a positive charge, leading to the more favorable formation of core-structure indicative product ions. The ions at *m/z* 324 (loss of diethyl amine), *m/z* 278 (sequential loss of $NO_2$), *m/z* 250 (loss of ethylene from *m/z* 278), *m/z* 202 (loss of ethoxy benzene from *m/z* 324), and *m/z* 156 (loss of phenol from *m/z* 250) are all indicative of the benzimidazole core. Comparison of the MS/MS fragmentation of the oxidized etonitazene to the $MS^3$ fragmentation of the un-oxidized etonitazene, as we previously reported,[16] revealed identical product ions.

The oxidation-assisted method was applied to 4 additional, different benzimidazole-containing tertiary amine side chain compounds (**A7**-**A10**) to reveal their core structure (Figs. S2 and S3 in the supporting information). In contrast to **A1**-**A5**, no product ions exhibiting the incorporation of oxygen were observed for **A6**-**A10**, hence $^{18}O$-labeled oxidation experiments have no added value and were not performed. The generated N-oxide counterparts of **A6**-A**10**, completely altered the charge distribution during MS/MS fragmentation. In the non-oxidized compounds (**A6**-**A10**), the C-N bond cleavage (α to the tertiary amine side chain), resulted in extremely low abundance ions indicative of the benzimidazole core, with MS/MS spectra dominated by product ions representing the amine side chain. However, in the N-oxide counterparts of **A6**-**A10**, the same C-N bond cleavage (α

to the tertiary amine-oxide side chain), resulted in high abundance ions indicative of the benzimidazole structure. From all investigated benzimidazole derivatives (**A6**-**A10**), only the MS/MS spectra of the N-oxide of **A6** show, in addition, ions at *m/z* 100 (representing the tertiary amine side chain N,N-diethylaziridinium ion) and *m/z* 72 (sequential loss of acetaldehyde), as supported by HRMS data, and not the expected 16 Da oxygen mass increase. As mentioned before for **A1**-**A5**, this can be rationalized by OH-group migration[27] or by loss of an OH radical, followed by C-N bond cleavage in a concerted process.[23,24] All the observed product ions were supported by ESI-orbitrap-$MS^2$ exact mass measurements (< 2 ppm error), enabling detailed structural elucidation and unambiguous identification of the analytes. For the exact mass measurements of the product ions of **A6**, see Table 1, and for **A7**-**A10,** see Figs. S2 and S3 in the supporting information.

## 4. Conclusions

We have demonstrated a new, convenient and very simple analytical technique for rapid structural elucidation of compounds containing a tertiary amine side chain (lidocaine homologues and benzimidazole derivatives). The method is based on instantaneous oxidation with a 0.1% solution of hydrogen peroxide in water that forms an N-oxide, altering the charge distribution during ESI-MS/MS fragmentation and revealing structural information that is otherwise not available and can easily be traced back to the original, un-oxidized compound. Although a sensitivity loss of approximately one order of magnitude was observed with the N-oxide derivatives, extensive fragmentation patterns with complete representation of both sides of the molecule were achieved, allowing unambiguous identification of such compounds at trace levels. It is noteworthy, that the mild oxidation carried out in this study, generally resulted in one significant peak of the N-oxide product, with no measurable amount of other oxidation products. However, in some derivatives, a few small LC peaks of other oxidized species were observed (3-10% of the main peak), with MS/MS spectra similar to the original un-oxidized compounds. We assume that oxidation in other positions, distant from the tertiary amine side chain site (for example, on the benzimidazole ring) would not significantly change the charge distribution during MS/MS fragmentation. Several substrates that can potentially be oxidized in a competitive reaction to the tertiary amine (for example, compounds containing a sulfur group), may result in the formation of positional isomers. LC separation prior to ESI-MS analysis is usually beneficial to separate such positional isomers and their distinction can be achieved based on differences in their MS/MS spectra. Most challenging are rare cases in which two isomers co-elute, leading to ambiguity in the identification process. Careful LC analysis with improved separation and MS/MS interpretation should be performed in such cases. The method

described here can be elaborated for different cores attached to various aliphatic and cyclic amines and may facilitate the identification of "unknowns".

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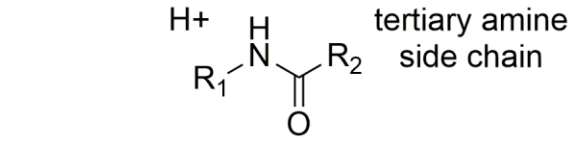
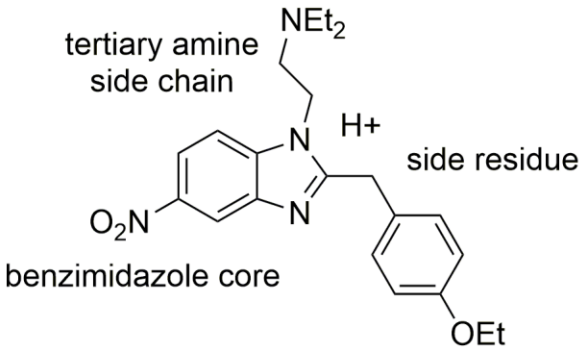
**Table 1.** Exact mass measurements of product ions of 10 ng/ml solutions of **A1-A6** and their N-oxide counterparts (mass errors < 2 ppm).

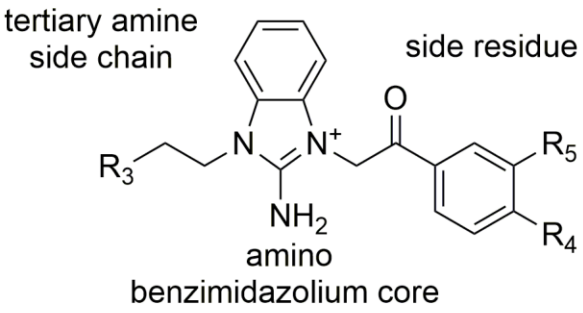
| Compound | RT (min) | Measured mass ($MH^+$) | Product ion masses |
|---|---|---|---|
| A1 | 7.6 | 235.1806 | 86.0964, 58.0654 |
| A1 oxide | 6.2 | 251.1754 | 164.1069, 148.0755, 130.0862, 122.0964, 120.0808, 105.0698, 88.0757, 86.0964 |
| A2 | 8.0 | 255.1260 | 86.0964, 58.0654 |
| A2 oxide | 5.9 | 271.1206 | 184.0524, 168.0211, 142.0416, 140.0261, 130.0863, 88.0757, 86.0965 |
| A3 | 9.6 | 291.2432 | 86.0964, 58.0654 |
| A3 oxide | 8.5 | 307.2382 | 204.1383, 176.1434, 164.1070, 130.0863, 122.0964, 88.0757, 86.0964 |
| A4 | 8.8 | 275.2120 | 126.1277, 98.0964, 84.0808 |
| A4 oxide | 7.5 | 291.2066 | 170.1176, 148.0756, 126.1277, 120.0808, 105.0699, 100.0757, 98.0964, 84.0808 |
| A5 | 8.6 | 289.2276 | 140.1434, 98.0965, 84.0808 |
| A5 oxide | 8.0 | 305.2223 | 184.1332, 148.0756, 140.1435, 120.0808, 105.0700, 100.0757, 98.0965, 84.0809, 70.0652 |
| A6 | 9.3 | 397.2234 | 278.1409, 135.0803, 107.0491, 100.1121, 72.0809 |
| A6 oxide | 7.8 | 413.2183 | 324.1342, 296.1028, 278.1417, 250.1096, 202.0610, 156.0683, 135.0805, 107.0492, 100.1121, 72.0809 |




**A1**: $R_1$= 2,6-dimethyl phenyl, $R_2$= $CH_2N(C_2H_5)_2$ (Lidocaine)
**A2**: $R_1$= 6-chloro-2-methyl phenyl, $R_2$= $CH_2N(C_2H_5)_2$
**A3**: $R_1$= 4-tert-butyl-2,6-dimethyl phenyl, $R_2$= $CH_2N(C_2H_5)_2$
**A4**: $R_1$= 2,6-dimethyl phenyl, $R_2$= N-propyl-2-piperidyl (Ropivacaine)
**A5**: $R_1$= 2,6-dimethyl phenyl, $R_2$= N-butyl-2-piperidyl (Bupivacaine)




**A6**: Etonitazene




**A7**: $R_3$= $N(C_2H_5)_2$, $R_4$= OMe, $R_5$=H
**A8**: $R_3$= $N(C_2H_5)_2$, $R_4$=Cl, $R_5$=H
**A9**: $R_3$= Morpholinyl, $R_4$= OMe, $R_5$=OMe
**A10**: $R_3$= Morpholinyl, $R_4$= Br, $R_5$=H

**Fig. 1.** Structures of the amino amide (**A1**-**A5**) and benzimidazole (**A6**-**A10**) compounds studied in this work.

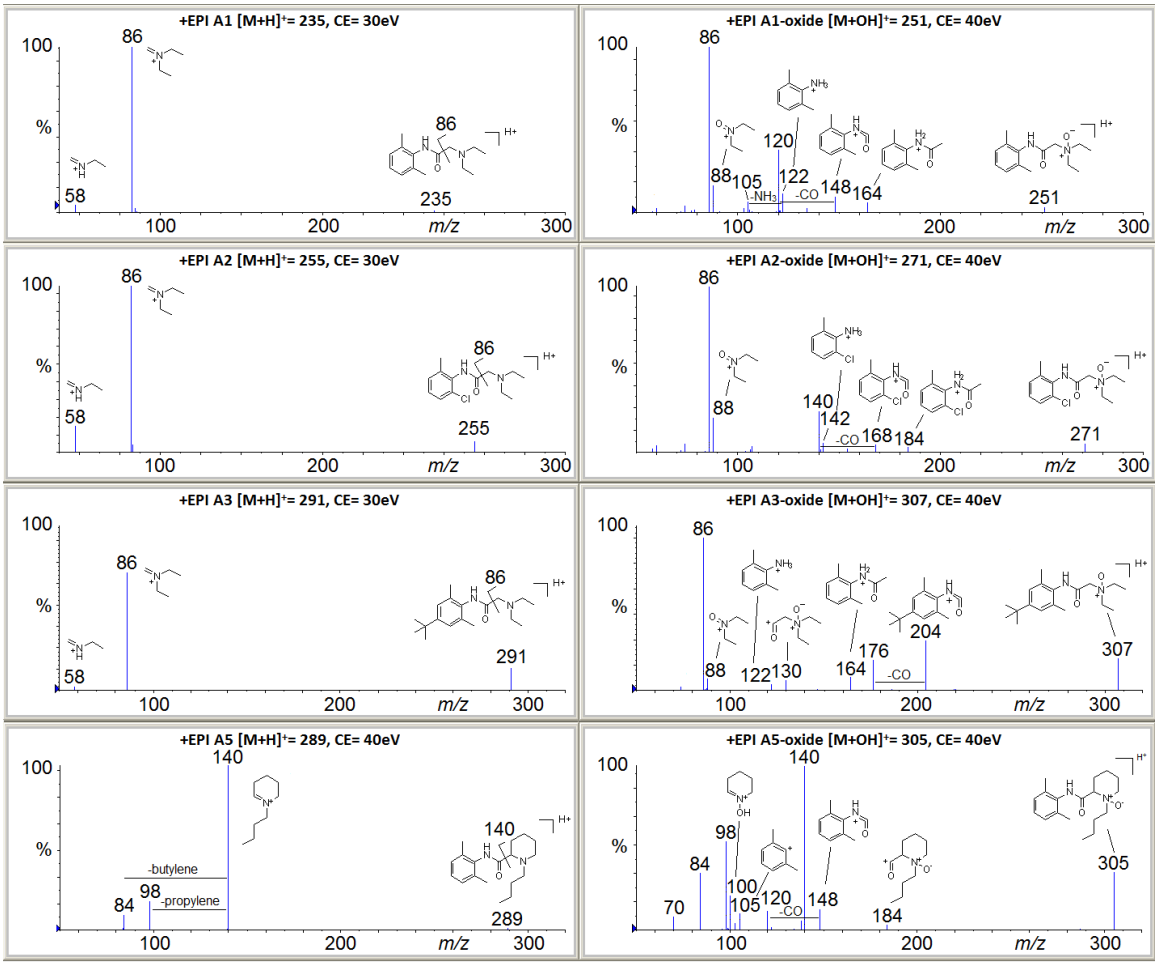



**Fig. 2.** Enhanced product ion (EPI) MS/MS spectra of 10 ng/ml solutions of **A1**-**A3**, **A5** (left) and their N-oxide counterparts (right).

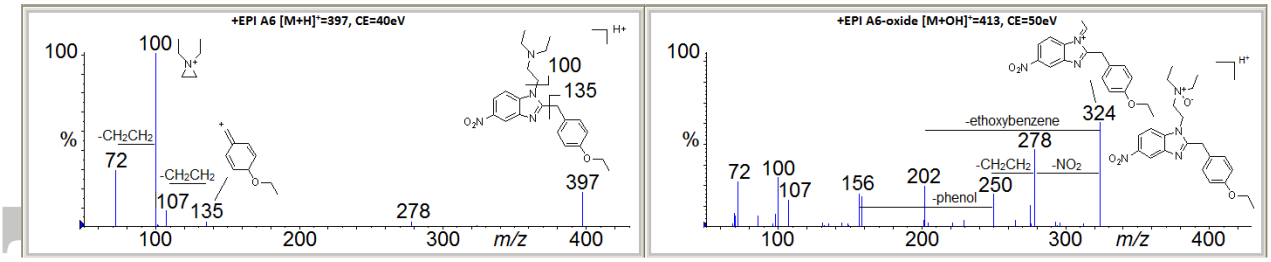



**Fig. 3.** Enhanced product ion (EPI) MS/MS spectra of a 10 ng/ml solution of **A6** (left) and its N-oxide counterpart (right).