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# Structural characterization of *in vitro* rat liver microsomal metabolites of antihistamine desloratadine using LTQ-Orbitrap hybrid mass spectrometer in combination with online hydrogen/deuterium exchange HR-LC/MS

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***In vitro* drug metabolism study is an integral part of drug discovery process. In this report, we have described the application of LTQ-Orbitrap hybrid mass spectrometer in conjunction with online hydrogen (H)/deuterium (D) exchange high resolution (HR)-LC/MS for structural characterization of *in vitro* rat liver microsomal metabolites of antihistamine desloratadine. Five metabolites M1 – M5 have been identified, including three hydroxylated metabolites M1 – M3, one N-oxide M4 and one uncommon aromatized N-oxide M5. Accurate mass data have been obtained in both full scan and MS$^n$ mode support assignments of metabolite structures with reported mass errors less than 3 ppm. Online H/D exchange HR-LC/MS experiments provide additional evidence in differentiating hydroxylated metabolites from N-oxides. This study demonstrates the effectiveness of this approach in structural characterization of drug metabolites. Copyright © 2008 John Wiley & Sons, Ltd.**

**Keywords:** *in vitro* metabolite identification; high resolution LC/MS; LC/MS$^n$; H/D exchange; Orbitrap MS

## Introduction

*In vitro* drug metabolism study is an important aspect of early drug discovery process in support of lead compound optimization in medicinal chemistry programs.[1] It employs simple and non-radiolabeled systems, such as microsomes, S9, recombinant P450 and hepatocytes. The experiments can be quickly carried out for cross species comparisons (rat, monkey, dog, human, etc.) and provide valuable information to medicinal chemists for lead compounds optimization. These studies are primarily designed for identification of metabolic soft-spots to address pharmacokinetics related issues in avoiding metabolic instability and subsequent poor oral bioactivity.[2] Additional benefits from these studies include assessment of metabolic hot-spots for early evaluation of reactivity of lead compounds so that potential drug safety concerns due to bioactivation to reactive metabolites can be minimized.[3,4] Because of its sensitivity, selectivity and speed of analysis, liquid chromatography/mass spectrometry (LC/MS) has emerged as the method of choice in metabolite identification.[5–7] Complex metabolite samples from biological matrix can be separated on a high-performance liquid chromatography (HPLC) column and analyzed by tandem mass spectrometry (MS/MS) online. The structures of metabolites are obtained by comparing fragmentation patterns of the parent compound and its metabolites using collision-induced dissociation (CID).

In spite of progress made over the last decade, there are still significant challenges in metabolite identification using LC/MS, including effect of complex biological matrix, ion suppression, and uncommon metabolites that are difficult to predict and analyze. Obtaining high quality MS/MS data with unambiguous assignments of ion structures often dictates the outcome of structural elucidation for metabolites. As one of the latest LC/MS instrumentation designs, LTQ-Orbitrap has the unique capability of performing high resolution (HR) LC/MS and LC/MS$^n$ experiments with resolving power (up to 100 000), excellent mass accuracy for both molecular ions and fragment ions (<3 ppm with external calibration) and large dynamic range (over $10^3$).[8–12] Unlike conventional Fourier transform (FT)-based HR LC/MS instruments (LTQ-FT, FT-ion cyclotron resonance (FT-ICR)), LTQ-Orbitrap is operated as an electrostatic ion trapping FT device without the use of superconducting magnet and offers cost-effective solutions to structural identification problems with ease of operations. Initial studies from several groups have focused on mixture analysis involving proteins/peptides using Orbitrap's low ppm mass accuracy.[13–20] There were also some literature reports on structural identification of impurities in drug substances[6,21] and drug metabolites using LTQ-Orbitrap,[22–26] demonstrating the power of this technology for providing robust accurate mass measurements in structural assignments regardless of relative ion abundance.

Another approach employed in structural characterization of small molecules is hydrogen/deuterium (H/D) exchange method. It has been used in MS for structural studies in both solution phase and gas phase. This method measures the difference in molecular

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weight of a compound before and after the deuterium exchange to determine the number of exchangeable hydrogen atoms in a molecule to assist structural elucidation.[7] Exchangeable hydrogen atoms are usually bound to N-, O-, or S atoms in functional groups such as OH, NH, $NH_2$, and COOH. Online H/D exchange LC/MS experiment can be readily set up using deuterated mobile phase for LC/MS analysis of mixtures.[27–34] The resultant change of retention time in chromatography owing to the use of deuterated mobile phase would not be an issue because of the use of mass identifications. In a previous study, we identified a trace level enol tautomer impurity in a novel Hepatitis C virus (HCV) protease inhibitor by online H/D exchange HR-LC/MS using LTQ-Orbitrap.[21] The HR-LC/MS experiments in a LTQ-Orbitrap mass spectrometer provided robust accurate mass data for unambiguous determinations of elemental compositions of unknowns. Multiple stage MS experiments further supported structural assignments. The enol and keto tautomers were readily differentiated by this method without prior isolation. In this report, we explore the use of LTQ-Orbitrap mass spectrometer in conjunction with online H/D exchange HR-LC/MS for rapid identification of drug metabolites from *in vitro* incubation of antihistamine desloratadine (SCH 34 117) in rat liver microsomes. As a long-acting tricyclic histamine antagonist with selective $H_1$-receptor histamine antagonistic activity, desloratadine is indicated for the treatment of seasonal and perennial allergic rhinitis. The drug's absorption, distribution, metabolism, and excretion (ADME) properties in human have been previously described in the literature.[35,36] The current studies illustrate structural characterization of *in vitro* metabolites of desloratadine including an uncommon aromatized N-oxide metabolite using accurate mass measurements of product ions in combination with online H/D exchange HR-LC/MS.

## Experimental

Desloratadine sample was obtained from Schering-Plough Research Institute, Kenilworth, NJ. *In vitro* microsomal incubation was carried out at 37 °C for 1 h in a shaking water bath. The incubation volume was 1 ml with the following components: 100 mM Trizma preset crystals buffer pH 7.4 (Sigma-Aldrich, St. Louis, MO), 1 mg/ml male Sprague-Dawley rat liver microsomes (In Vitro Technologies, Baltimore, MD), 1 mM NADPH (Sigma-Aldrich, St Louis, MO), and 50 μM desloratadine. The mixture of buffer, substrate solution and rat liver microsomes was preincubated for 5 min at 37 °C. The reaction was initiated by addition of the NADPH. The incubation was terminated by addition of trifluoroacetic acid (TFA) (Sigma-Aldrich, St Louis, MO). After precipitation of protein with TFA, the solutions were centrifuged and the supernatant was transferred to clean vials for LC/electrospray ionization (ESI)-MS analysis.

All LC/MS experiments were carried out on a Thermo Electron LTQ-Orbitrap (San Jose, CA) hybrid mass spectrometer equipped with a Thermo Electron Surveyor MS pump plus, Autosampler plus and PDA UV detector. The reversed-phase gradient LC/ESI-MS experiments were performed using a C18 column (Analytical Sales & Services, Pompton Plains, NJ) with a mobile phase A of water (Sigma-Aldrich, St Louis, MO) and a mobile phase B of acetonitrile (Sigma-Aldrich, St Louis, MO) with 0.1% formic acid at a flow rate of 1 ml/min. For online H/D exchange LC/MS experiments, deuterated solvents were used as mobile phases, including deuterium oxide ($D_2O$) (100% atom D, Fisher Scientific, Pittsburgh, PA) as mobile phase A and acetonitrile as mobile phase B with 0.1% D-formic acid (98% atom D, Sigma-Aldrich, St Louis, MO). Although the retention time was slightly shifted with deuterated solvents, individual metabolites detected were traced by mass measurements. The ESI needle was held at 5 kV with a capillary voltage at 49 V, and a nitrogen sheath gas (80 units) and a nitrogen auxiliary gas (20 units) were used to stabilize the spray. The heated capillary was set at 250 °C. The HR-LC/MS experiments were operated in the FTMS mode at a resolution of 15 000 with external calibration. The $MS^n$ data were acquired using data-dependent scanning with a parent ion list. The activation parameters used were a normalized collision energy of 30% with an isolation width of 2 Da and an activation time of 30 ms.

## Results and Discussion

Incubated rat liver microsome sample from desloratadine was analyzed by HR-LC/MS, as illustrated in selected ion chromatograms of desloratadine and its metabolites M1–M5 (Fig. 1). Desloratadine was eluted at 5.43 min with detected molecular ion at *m/z* 311. It has a characteristic one chlorine isotopic pattern. The accurate mass measurement gave the elemental composition of $C_{19}H_{20}ClN_2$ ([M + H$^+$], 0.68 ppm), consistent with the structure of desloratadine (Scheme 1). Figure 2 displays both experimental and theoretical isotopic distribution patterns for desloratadine molecular ion. They are remarkably similar to each other, demonstrating the effectiveness of isotopic distribution fit for accurate mass assignments. The mass spectra of desloratadine metabolites all exhibit the same characteristic one chlorine isotopic pattern. Accurate mass measurements suggest that M1–M4 are oxidative metabolites (addition of one oxygen atom) and M5 (*m/z* 323) has an elemental composition of $C_{19}H_{16}OClN_2$ ([M + H$^+$], 1.56 ppm). Their measured isotopic distribution patterns are matched very well with their theoretical isotopic distribution patterns, as shown in the case of M1 (Fig. 3). To further elucidate structures of these metabolites,

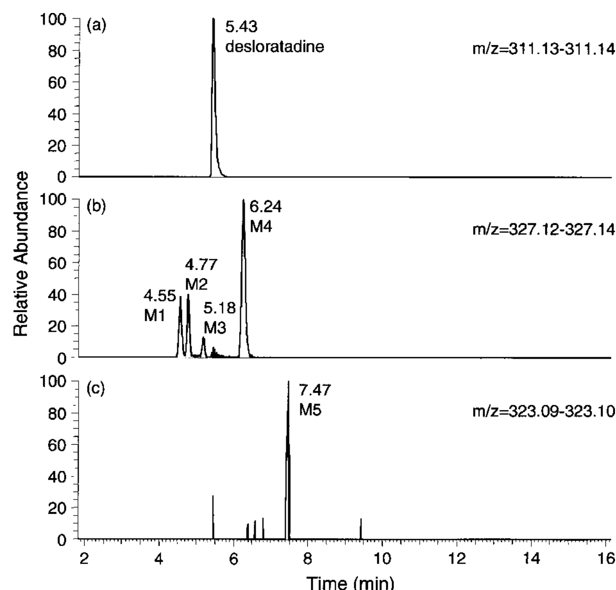


**Figure 1.** Selected ion chromatograms of desloratadine from *in vitro* rat liver microsome incubations. Its metabolites M1–M4 have the same elemental compositions of $C_{19}H_{20}OClN_2$ ([M + H$^+$], <2 ppm). M5 has a measured elemental composition of $C_{19}H_{16}OClN_2$ ([M + H$^+$], 1.56 ppm).



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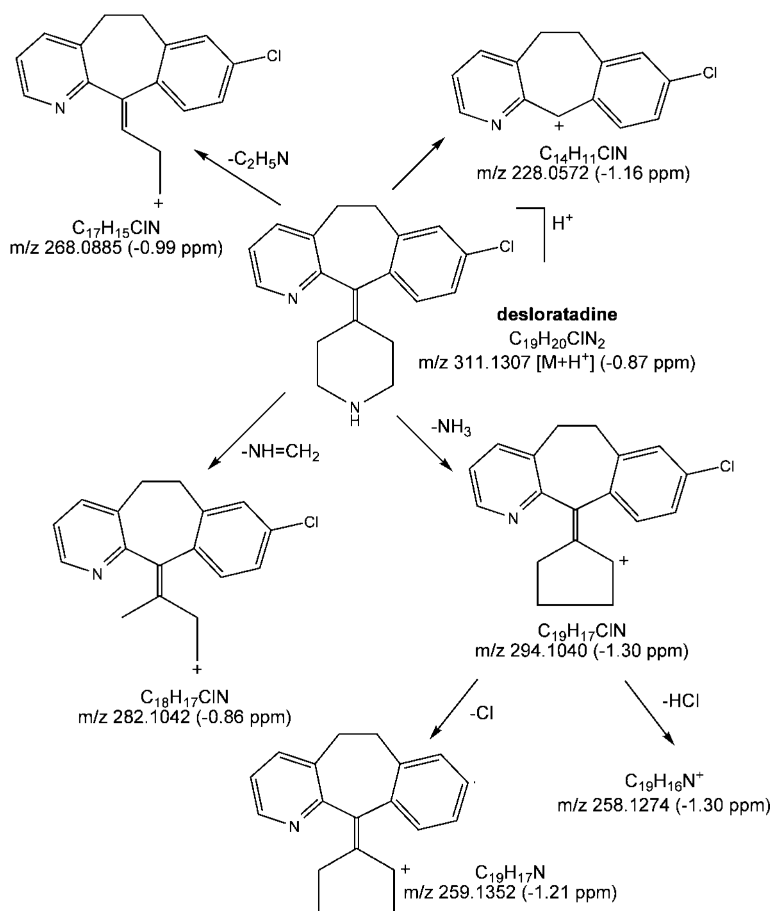


**Scheme 1.** Proposed fragmentation patterns for desloratadine molecule.

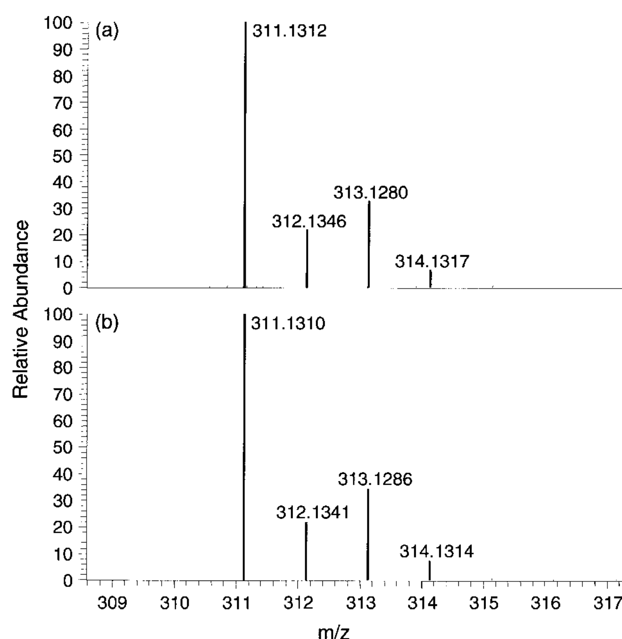


**Figure 2.** (a) Experimental isotopic distribution patterns for desloratadine molecular ions. (b) Theoretical isotopic distribution patterns based on the elemental composition of $C_{19}H_{20}ClN_2$ for desloratadine molecular ions.

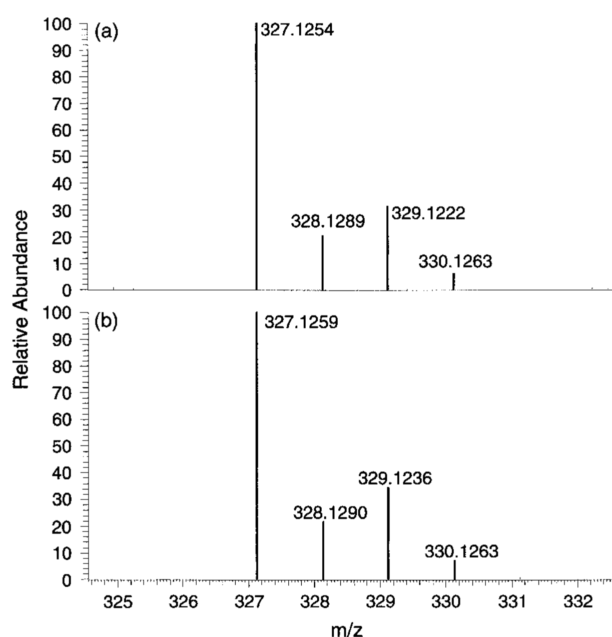


**Figure 3.** (a) Experimental isotopic distribution patterns for M1 molecular ions. (b) Theoretical isotopic distribution patterns based on the elemental composition of $C_{19}H_{20}OClN_2$ for M1 molecular ions.


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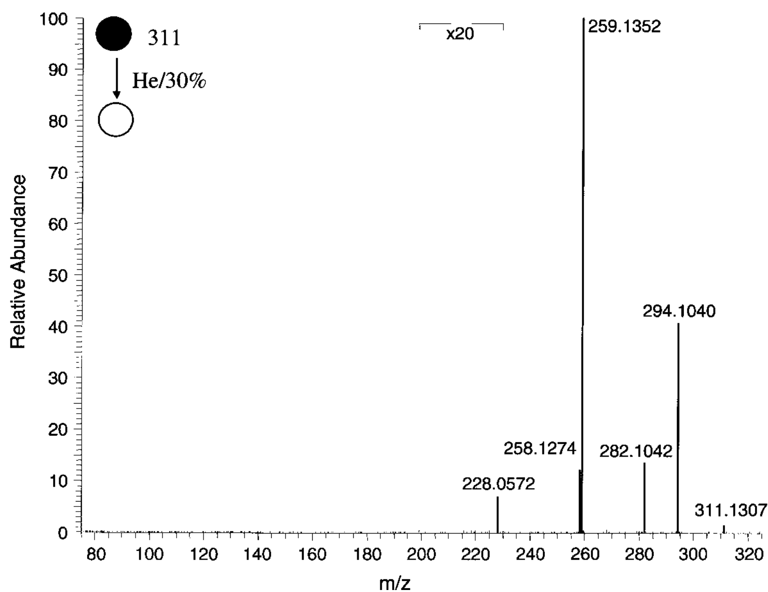


**Figure 4.** Product ion mass spectrum of *m/z* 311 for desloratadine.

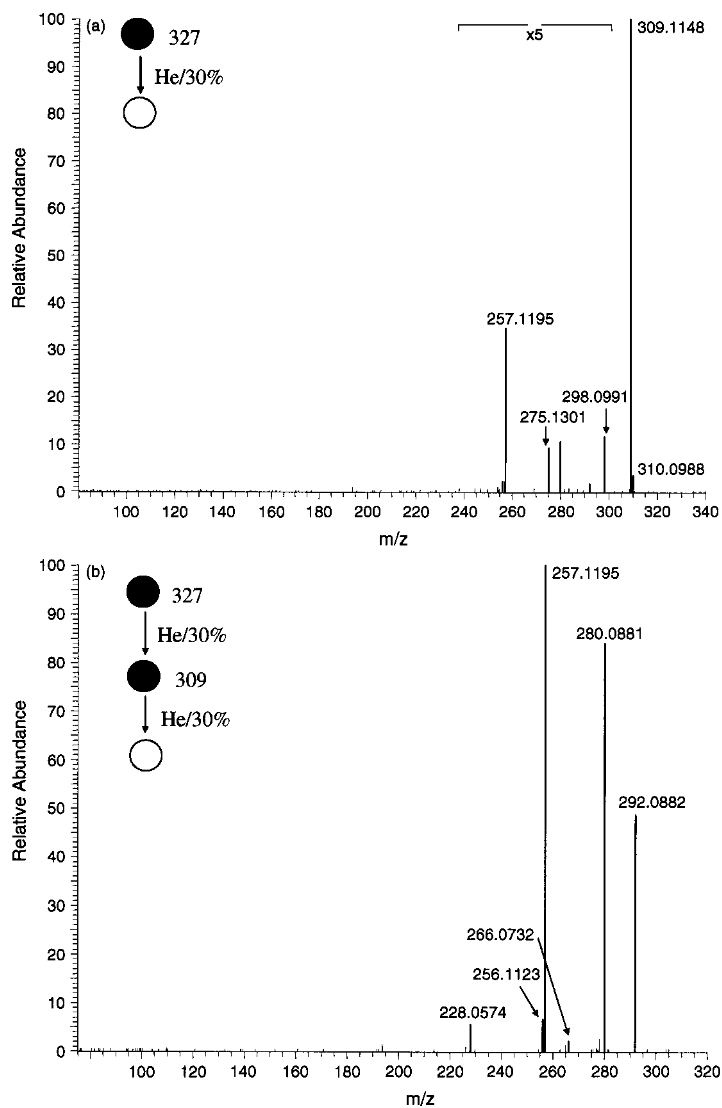


**Figure 5.** Product ion mass spectra of *m/z* 327 for M1.




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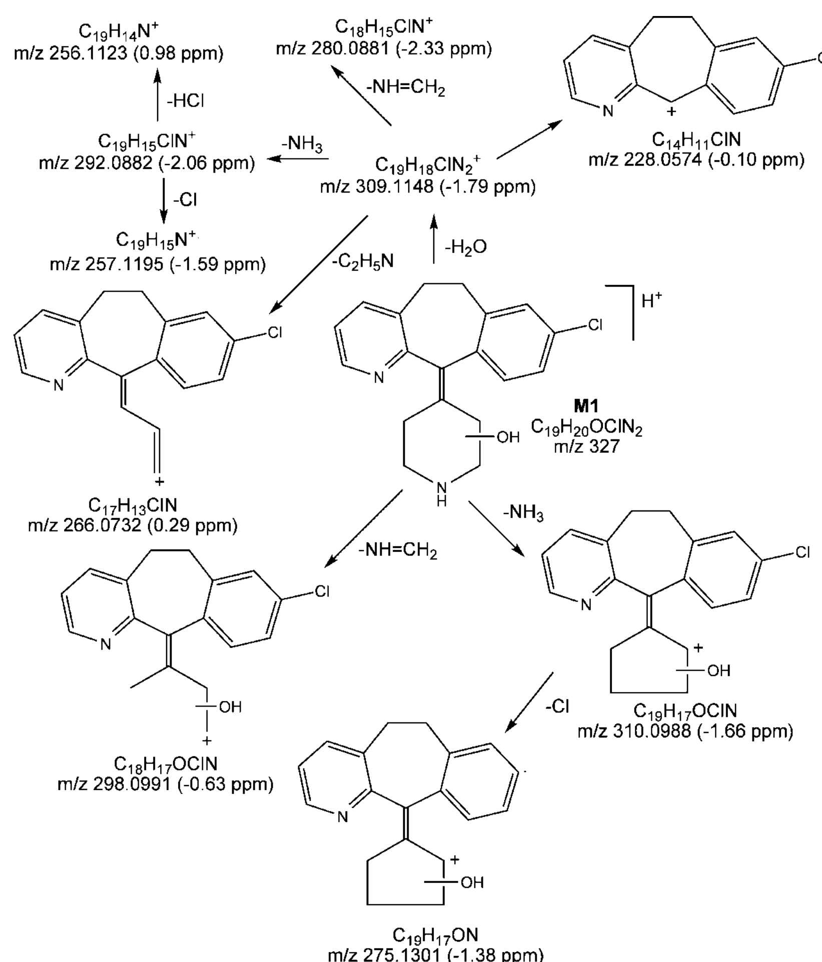


**Scheme 2.** Proposed fragmentation patterns for metabolite M1.

MS/MS experiments were performed on desloratadine and its metabolites. Online H/D exchange HR-LC/MS experiments were also carried out to facilitate structural identification. Note that a narrow isolation width (2 Da) was used to select the most abundant isotopic peak in all tandem MS experiments. This approach was in contrast to a broad band mass selection method where the parent ions were selected to include all isotopic forms by using low resolution mass selection in the first quadrupole mass analyzer to facilitate recognition of product ions through their characteristic isotopic patterns.[37–39] The current procedure reduces complexity of product ion spectra because of the possible distortion of isotopic patterns for product ions during isolation steps in ion traps.

Figure 4 shows a product ion mass spectrum of desloratadine. The base peak in the spectrum is the fragment ion at *m/z* 259 proposed as a distonic ion. It is likely to have resulted from the loss of $NH_3$ and Cl moieties. This is confirmed by accurate mass measurement of this ion with an elemental composition of $C_{19}H_{17}N$ (−1.21 ppm). Other low intensity fragment ions are generated, mostly from the breakdown of the piperidine ring, as illustrated in Scheme 1. Accurate mass measurement data support the proposed structures. Note that a low abundant fragment ion at *m/z* 228 representing the tricyclic portion of the molecule is also observed in the spectrum with consistent accurate mass measurement data for its elemental composition of $C_{14}H_{11}ClN$ (−1.16 ppm). The low relative abundance of this ion indicates that its formation is likely to have resulted from the rearrangement of the double bond from the piperidine ring. Additional online H/D exchange HR-LC/MS experiments gave molecular ion of *m/z* 313 with an elemental composition of $C_{19}H_{18}D_2ClN_2$ ([M + D$^+$], 0.15 ppm), indicating one exchangeable hydrogen atom in the molecule (NH of the piperidine ring).

The oxidative metabolite M1 got eluted at 4.55 min with a molecular ion at *m/z* 327. It fragments readily to give a dominant ion at *m/z* 309, a loss of $H_2O$ from the parent ion (Fig. 5(a)). The highly abundant water-loss fragment ion suggests that the oxygen addition as a form of hydroxylation does not take place at the aromatic rings. Online H/D exchange HR-LC/MS experiment produced molecular ion at *m/z* 330 with an elemental composition of $C_{19}H_{17}D_3OClN_2$ ([M+D$^+$], −0.78 ppm), suggesting two exchangeable hydrogen atoms. This data eliminates the possibilities of N-oxidation at the aromatic ring and hydroxylation at NH-group in the piperidine ring. Both cases would only yield one exchangeable hydrogen atom. Studies on M2 and M3 metabolites indicate that they are metabolites hydroxylated at cyclohepta ring (see the following section). Thus, M1 is characterized to be a metabolite hydroxylated at the piperidine ring.

In addition to the base peak at *m/z* 309, there are other low intensity fragment ions in Fig. 5(a). For example, the loss of $NH_3$ and NH=$CH_2$ from M1 leads to fragment ions at *m/z* 310 and 298, respectively. The ion at *m/z* 310 likely generates the fragment ion at *m/z* 275 when it loses one chlorine atom, similar to the case in desloratadine (*m/z* 294 → *m/z* 259, Scheme 1). Further activation

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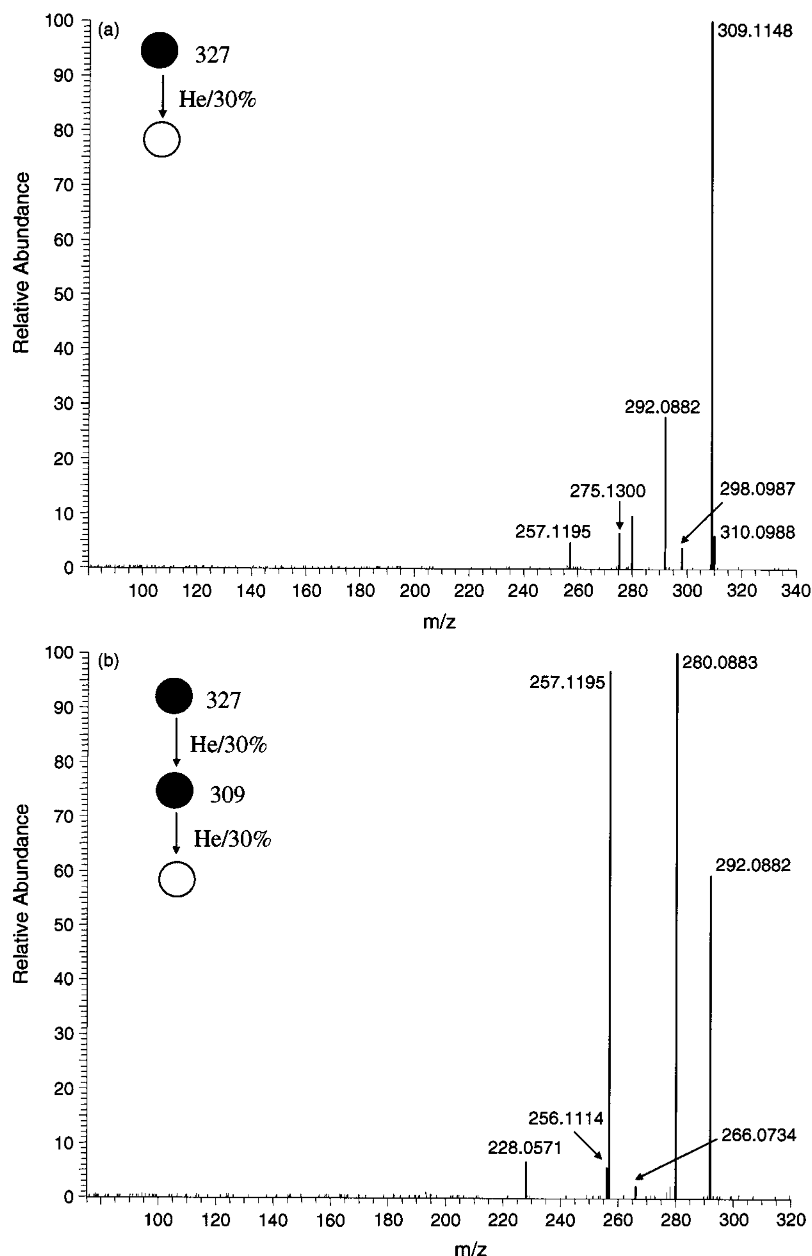


**Figure 6.** Product ion mass spectra of *m/z* 327 for M2.

of water-loss fragment ion at *m/z* 309 produces abundant product ions at *m/z* 292 (loss of $NH_3$), 280 (loss of $NH{=}CH_2$), and 257 (loss of $NH_3$ and Cl) along with some low intensity ions at *m/z* 266 (loss of $C_2H_5N$) and 228 (tricyclic portion of the molecule) (Fig. 5(b)). Accurate mass measurements on fragment ions provide supporting evidence on proposed structures (Scheme 2).

M2 and M3 metabolites were eluted at 4.77 min and 5.18 min, respectively. Accurate mass measurements on molecular ion at *m/z* 327 yield the same elemental composition of $C_{19}H_{20}OClN_2$ ($[M + H^+]$). Both metabolites show the same product ion spectra, suggesting isomeric structures. The ready loss of $H_2O$ from M2 and M3 during CID excludes the possibility of hydroxylation at aromatic rings (Fig. 6(a)). Onine H/D exchange HR-LC/MS data gave molecular ions at *m/z* 330 with elemental composition of $C_{19}H_{17}D_3OClN_2$ ($[M + D^+]$) for both M2 and M3, indicating two exchangeable hydrogen atoms in the molecule. Similar to the case of M1, M2 and M3 could not be N-oxides at the aromatic ring or hydroxylation metabolites at NH-group in the piperidine ring. The relatively high abundant fragment ions at *m/z* 292 and 280 reflect their highly conjugated tricyclic structures and the relative ease of losing $NH_3$ and $NH{=}CH_2$ moieties in the piperidine ring from water-loss fragment ion at *m/z* 309 (Fig. 6(a), Scheme 3), when compared with M1 (Fig. 5(a)). This observation may suggest less likelihood of the piperidine ring being modified (hydroxylated). To verify this proposal, synthetic standards of desloratadine hydroxylated at cyclohepta ring (5-OH and 6-OH desloratadines) were synthesized and they exhibited the same fragmentation patterns as M2 and M3 along with the same retention time as those of M2 and M3. The identity of metabolites M2 and M3 is confirmed to be 5-OH and 6-OH desloratadines.

The base peak (*m/z* 309) in product ion mass spectrum of M2 corresponds to the loss of water from M2 (Fig. 6(a)). Other low


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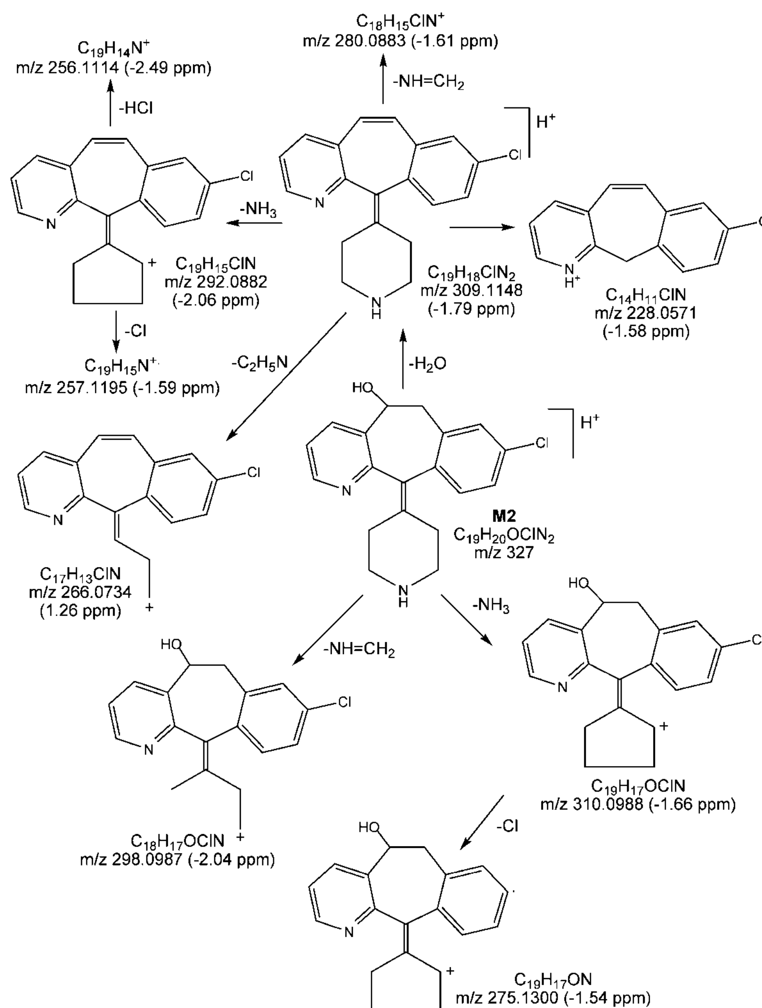


**Scheme 3.** Proposed fragmentation patterns for metabolite M2.

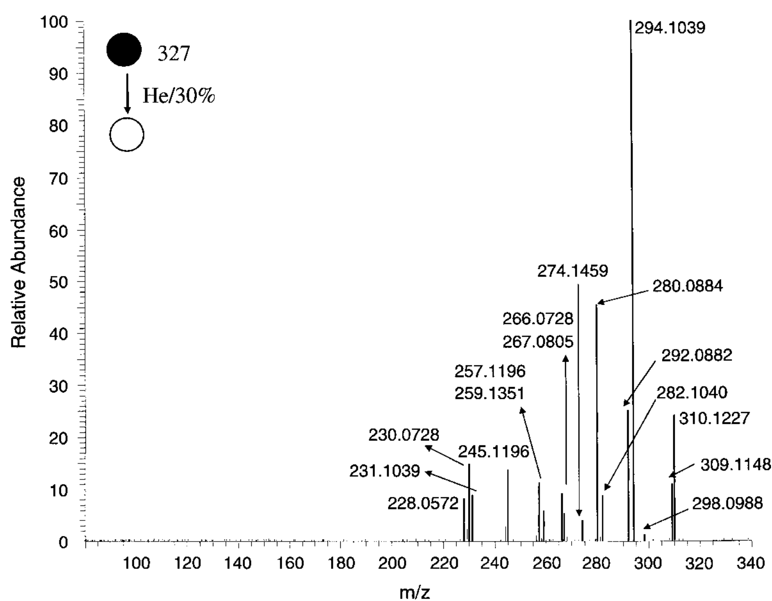


**Figure 7.** Product ion mass spectrum of *m/z* 327 for M4.



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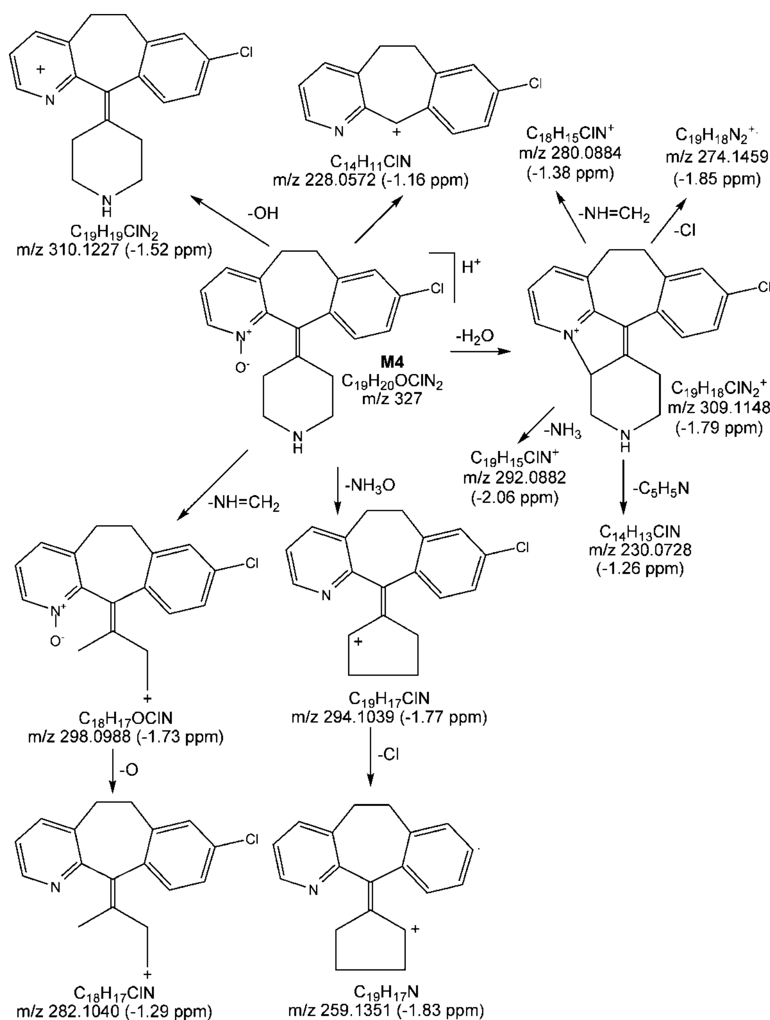


**Scheme 4.** Proposed fragmentation patterns for metabolite M4.

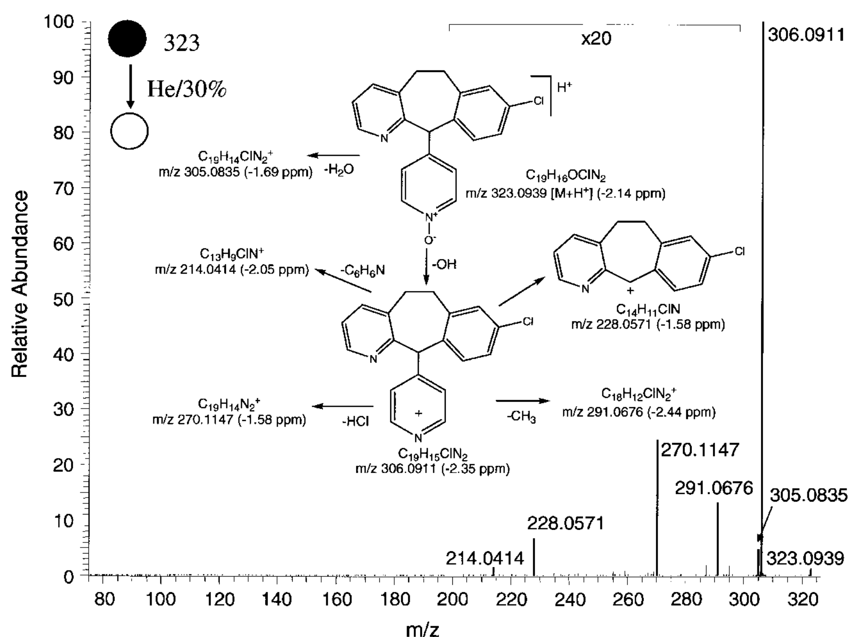


**Figure 8.** Product ion mass spectrum of *m/z* 323 for M5. The insert shows proposed fragmentation patterns for M5.




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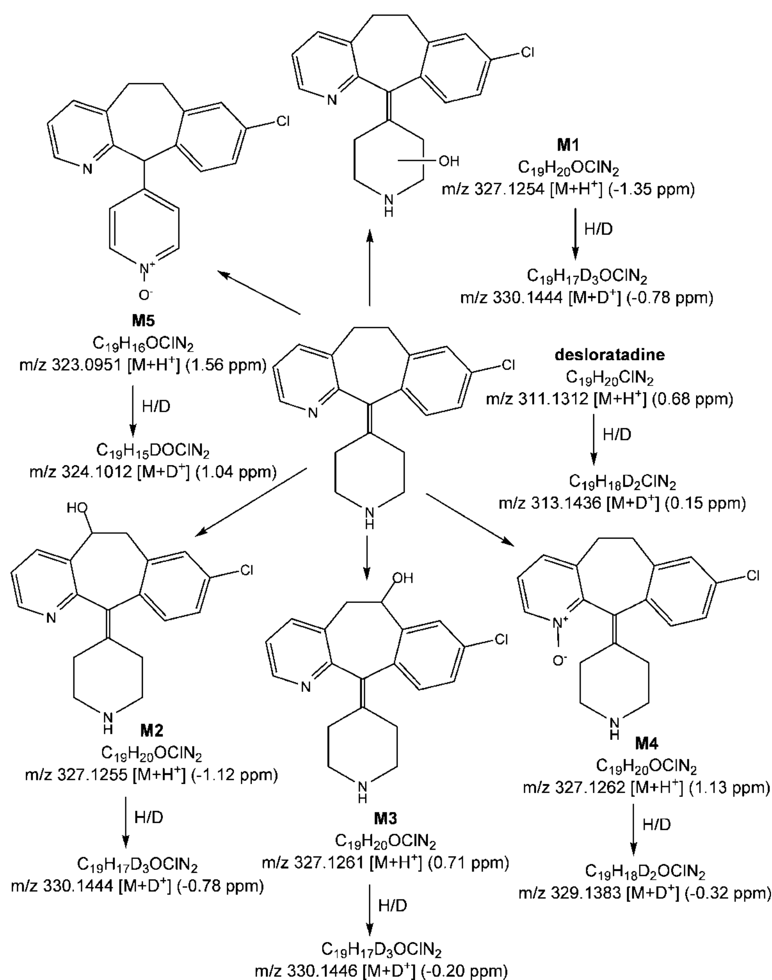


**Scheme 5.** Metabolic pathways of desloratadine from *in vitro* incubations in rat liver microsomes along with online H/D exchange HR-LC/MS data.

intensity fragment ions at *m/z* 310 and 298 are generated by the loss of $NH_3$ and $NH{=}CH_2$ from M2, respectively. The tandem mass spectrum of *m/z* 309 displays the same fragment ions as in the case of M1, although their relative abundances are different as a reflection of possible stabilities of the ionic structures (Fig. 6(b)). Scheme 3 summarizes M2 fragmentation patterns with accurate mass data in support of the proposed structures.

The most abundant oxidative metabolite M4 has a measured elemental composition of $C_{19}H_{20}OClN_2$ ($[M + H^+]$, *m/z* 327). It fragments to yield abundant product ions at *m/z* 309 and 310, corresponding to the loss of $H_2O$ and OH radical, respectively from M4 (Fig. 7). The assignments are confirmed by the measured elemental compositions for *m/z* 309 ($C_{19}H_{18}ClN_2$, −1.53 ppm) and *m/z* 310 ($C_{19}H_{19}ClN_2$, −1.52 ppm) (Scheme 4). The loss of $H_2O$ and OH moieties from M4 suggests the possibility of M4 being the N-oxide of desloratadine. Another evidence for the modification taking place at the tricyclic portion of desloratadine is the observed low abundant fragment ion at *m/z* 298. Its measured elemental composition of $C_{18}H_{17}OClN$ (−1.73 ppm) indicates a loss of $NH{=}CH_2$ from unmodified piperidine ring in the molecule (Scheme 4). Online H/D exchange HR-LC/MS experiments on M4 yield molecular ions at *m/z* 329 with the elemental composition of $C_{19}H_{18}D_2OClN_2$ ($[M + D^+]$), indicating one exchangeable hydrogen atom in M4. This is consistent with M4 being N-oxide of desloratadine. Structures of hydroxylated metabolites would have two exchangeable hydrogen atoms.

The product ion mass spectrum of M4 shows a number of fragment ions. The dominant peak at *m/z* 294 has an elemental composition of $C_{19}H_{17}ClN$ (−1.77 ppm), likely to have resulted from the loss of $NH_3O$ moiety from M4 on the basis of elemental composition measurement of *m/z* 294 (Scheme 4). Similarly, the fragment ion at *m/z* 282 ($C_{18}H_{17}ClN$, −1.29 ppm) could be derived from the ion at *m/z* 298 with a loss of oxygen atom. The observation of loss of oxygen atom in MS is not unprecedented. Earlier studies by Pramanik and coworkers indicated that nitro-sugars and some other nitro compounds can lose one oxygen atom under fast-atom bombardment MS conditions.[40,41] They also observed a loss of an oxygen atom in self-ionization of some nitro compounds under negative chemical ionization conditions.[42] In a more recent study, Chowdhury *et al*. found that N-oxides lose an oxygen atom under atmospheric pressure chemical ionization (APCI) conditions.[43] It is likely that deoxygenation of N-oxide in CID process depends on the nature of chemical bonds and ionization/activation process. Further investigation on the observation of loss of one oxygen atom during ESI/MS/MS may provide more insight into the process.

Other low intensity fragment ions in the product ion spectrum of M4 are mostly from cleavages of the piperidine ring, such as *m/z* 292 (loss of $NH_3$ from *m/z* 309) and *m/z* 280 (loss of $NH{=}CH_2$ from *m/z* 309). One of the features in the fragmentation of M4


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appears to be the loss of chlorine atom during activation. For example, the fragment ion at *m/z* 294 can lose one chlorine atom to give a distonic ion at *m/z* 259, as supported by accurate mass measurements. Other cases include *m/z* 274 (loss of chlorine atom from *m/z* 309). All these fragmentation patterns described are supported by elemental composition measurements and are consistent with the proposed N-oxide structure, as shown in Scheme 4.

A very low level metabolite M5 was detected at 7.47 min. It has a molecular weight of 322 Da with a measured elemental composition of $C_{19}H_{15}OClN_2$. This corresponds to the addition of one oxygen atom and loss of four hydrogen atoms from desloratadine molecule. Compared with calculated double bond equivalency (DBE) or rings plus double bonds[44] of desloratadine ($C_{19}H_{19}ClN_2$, DBE = 11), the DBE of M5 is calculated to be 13. If the four-ring structure of desloratadine remains to be intact, two extra DBE of M5 may suggest formation of two additional double bonds in the structure. Product ion mass spectrum of M5 shows a major fragment ion at *m/z* 306, corresponding to the loss of OH radical (Fig. 8). There is also a less abundant water-loss product ion at *m/z* 305. Online H/D exchange HR-LC/MS experiments indicate no exchangeable hydrogen atom in M5 (observed *m/z* 324.1012, $C_{19}H_{15}DOClN_2$ $[M + D^+]$, 1.04 ppm), consistent with the N-oxide structure. The appearance of fragment ion at *m/z* 228 corresponding to the tricyclic portion of the molecule also illustrates the possibility of a modification occurring at the piperidine ring. Furthermore, no fragment ions due to cleavages of the piperidine ring are observed, suggesting that the nature of structural modifications possible is the aromatization of the piperidine ring (converting piperidine ring to pyridine N-oxide). The proposed M5 structure was confirmed by a synthetic standard.

On the basis of accurate mass data and online H/D exchange HR-LC/MS results, all five metabolites M1–M5 have been identified, including three hydroxylated metabolites M1–M3, one N-oxide M4 and one aromatized N-oxide M5. Scheme 5 summarizes identified metabolites of desloratadine from *in vitro* incubations of rat liver microsomes, including accurate mass data for H/D exchange experiments.

## Conclusions

The present studies demonstrate the practical utility of LTQ-Orbitrap in conjunction with online H/D exchange HR-LC/MS for structural elucidation of unknown metabolites from *in vitro* incubation of desloratadine in rat liver microsomes. The HR-LC/MS experiments in a LTQ-Orbitrap mass spectrometer provide robust accurate mass data for unambiguous determinations of elemental compositions of unknowns. Accurate mass data obtained in MS/MS mode strongly support assignments of ion structures and establishment of fragmentation patterns. The online H/D exchange HR-LC/MS data clearly facilitate the differentiation of hydroxylated metabolites from N-oxides and give additional information on structures of metabolites. The current approach represents a simplified and effective method for rapid structural identification of drug metabolites in drug discovery.

## References

[1] E. H. Kerns, L. Di. Utility of mass spectrometry for pharmaceutical profiling applications. *Current Drug Metabolism* **2006**, *7*, 457.
[2] A. P. Watt, R.J. Mortishire-Smith, U. Gerhard, S. R. Thomas. Metabolite identification in drug discovery. *Current Opinion in Drug Discovery and Development* **2003**, *6*, 57.
[3] A. S. Kalgutkar, J. R. Soglia. Minimizing the potential for metabolic activation in drug discovery. *Expert Opinion on Drug Metabolism and Toxicology* **2005**, *1*, 91.
[4] S. Ma, R. Subramanian. Detecting and characterizing reactive metabolites by liquid chromatography/tandem mass spectrometry. *Journal of Mass Spectrometry* **2006**, *41*, 1121.
[5] C.E.C.A. Hop . LC-MS in drug disposition and metabolism. In *The Encyclopedia of Mass Spectrometry*, *Volume 3, Biological Applications, Part B: Carbohydrate, Nucleic Acids and Other Biological Compounds*, R. M. Caprioli, M. L. Gross (eds). Elsevier: Oxford, **2006**, 233.
[6] G. Chen, B. N. Pramanik, Y. H. Liu, U. Mirza. Applications of LC/MS in structure identifications of small molecules and proteins in drug discovery. *Journal of Mass Spectrometry* **2007**, *42*, 279.
[7] G. Chen, L. K. Zhang, B. N. Pramanik. LC/MS: theory, instrumentation and applications to small molecules. In *HPLC for Pharmaceutical Scientists*, Chapter 7, Y. Kazakevich, LoBrutto R (eds). Wiley: New York, **2007**, 281.
[8] M. Hardman, A. A. Makarov. Interfacing the orbitrap mass analyzer to an electrospray ion source. *Analytical Chemistry* **2003**, *75*, 1699.
[9] Q. Hu, R. J. Noll, H. Li, A. Makarov, M. Hardman, R. G. Cooks. The orbitrap: a new mass spectrometer. *Journal of Mass Spectrometry* **2005**, *40*, 430.
[10] M. Scigelova, A. Makarov. Orbitrap mass analyzer – overview and applications in proteomics. *Proteomics* **2006**, *6*, 16.
[11] A. Makarov, E. Denisov, A. Kholomeev, W. Balschun, O. Lange, K. Strupat, S. Horning. Performance evaluation of a hybrid linear ion trap/orbitrap mass spectrometer. *Analytical Chemistry* **2006**, *78*, 2113.
[12] A. Makarov, E. Denisov, O. Lange, S. Horning. Dynamic range of mass accuracy in LTQ orbitrap hybrid mass spectrometer. *Journal of the American Society for Mass Spectrometry* **2006**, *17*, 977.
[13] J. V. Olsen, L.M. de Godoy, G. Li, B. Macek, P. Mortensen, R. Pesch, A. Makarov, O. Lange, S. Horning, M. Mann. Parts per million mass accuracy on an orbitrap mass spectrometer via lock mass injection into a c-trap. *Molecular and Cellular Proteomics* **2005**, *4*, 2010.
[14] J. R. Yates, D. Cociorva, L. Liao, V. Zabrouskov. Performance of a linear ion trap-orbitrap hybrid for peptide analysis. *Analytical Chemistry* **2006**, *78*, 493.
[15] J. Adachi, C. Kumar, Y. Zhang, J. V. Olsen, M. Mann. The human urinary proteome contains more than 1500 proteins, including a large proportion of membranes proteins. *Genome Biology* **2006**, *7*, R80.
[16] B. Macek, L. F. Waanders, J. V. Olsen, M. Mann. Top-down protein squencing and MS3 on a hybrid linear quadrupole ion trap-orbitrap mass spectrometer. *Molecular and Cellular Proteomics* **2006**, *5*, 949.
[17] J. R. Wisniewski, A. Zougman, S. Kruger, M. Mann. Mass spectrometric mapping of linker histone H1 variants reveals multiple acetylations, methylations, and phosphorylation as well as differences between cell culture and tissue. *Molecular and Cellular Proteomics* **2007**, *6*, 72.
[18] X. Li, S. A. Gerber, A. D. Rudner, S. A. Beausoleil, W. Haas, J. Villen, J. E. Elias, S. P. Gygi. Large-scale phosphorylation analysis of alpha-factor-arrested saccharomyces cerevisiae. *Journal of Proteome Research* **2007**, *6*, 1190.
[19] J. D. Venable, J. Wohlschlegel, D. B. McClatchy, S. K. Park, J. R. Yates. Relative quantification of stable isotope labeled peptides using a linear ion trap-orbitrap hybrid mass spectrometer. *Analytical Chemistry* **2007**, *79*, 3056.
[20] R. Shi, C. Kumar, A. Zougman, Y. Zhang, A. Podtelejnikov, J. Cox, J. R. Wisniewski, M. Mann. Analysis of the mouse liver proteome using advanced mass spectrometry. *Journal of Proteome Research* **2007**, *6*, 2963.
[21] G. Chen, A. Khusid, I. Daaro, P. Irish, B. N. Pramanik. Structural identification of trace level enol tautomer impurity by on-line hydrogen/deuterium exchange HR-LC/MS in a LTQ-orbitrap hybrid mass spectrometer. *Journal of Mass Spectrometry* **2007**, *42*, 967.
[22] M. Thevis, G. Sigmund, A. K. Schiffer, W. Schanzer. Determination of N-desmethyl- and N-bisdesmethyl metabolites of sibutramine in doping control analysis using liquid chromatography-tandem mass spectrometry. *European Journal of Mass Spectrometry* **2006**, *12*, 129.
[23] S. M. Peterman, N. Duczak, A. S. Kalgutkar, M. E. Lame, J. R. Soglia. Application of a linear ion trap/orbitrap mass spectrometer in



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metabolite characterization studies: examination of the human liver microsomal metabolism of the non-tricyclic anti-depressant nefazodone using data-dependent accurate mass measurements. *Journal of the American Society for Mass Spectrometry* **2006**, *17*, 363.

[24] R. Breitling, A. R. Pitt, M. P. Barrett. Precision mapping of the metabolome. *Trends in Biotechnology* **2006**, *24*, 543.

[25] H. K. Lim, J. Chen, C. Sensenhauser, K. Cook, V. Subrahmanyam. Metabolite identification by data-dependent accurate mass spectrometric analysis at resolving power of 60,000 in external calibration mode using an LTQ/Orbitrap. *Rapid Communications in Mass Spectrometry* **2007**, *21*, 1821.

[26] J. Ding, C. M. Sorensen, Q. Zhang, H. Jiang, N. Jaitly, E. A. Livesay, Y. Shen, R. D. Smith, T. O. Metz. Capillary LC coupled with high-mass measurement accuracy mass spectrometry for metabolic profiling. *Analytical Chemistry* **2007**, *79*, 6081.

[27] N. Ohashi, S. Furuuchi, M. Yoshikawa. Usefulness of the hydrogen-deuterium exchange method in the study of drug metabolism using liquid chromatography-tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* **1998**, *18*, 325.

[28] M. A. Olsen, P. G. Cummings, S. Kennedy-Gabb, B. M. Wagner, G. R. Nicol, B. Munson. The use of deuterium oxide as a mobile phase for structural elucidation by HPLC/UV/ESI/MS. *Analytical Chemistry* **2000**, *72*, 5070.

[29] D. Q. Liu, C. E. Hop, M. G. Beconi, A. Mao, S. H. Chiu. Use of on-line hydrogen/deuterium exchange to facilitate metabolite identification. *Rapid Communications in Mass Spectrometry* **2001**, *15*, 1832.

[30] W. Lam, R. Ramanathan. In electrospray ionization source hydrogen/deuterium exchange LC-MS and LC-MS/MS for characterization of metabolites. *Journal of the American Society for Mass Spectrometry* **2002**, *13*, 345.

[31] A. Tolonen, M. Turpeinen, J. Uusitalo, O. Pelkonen. A simple method for differentiation of monoisotopic drug metabolites with hydrogen-deuterium exchange liquid chromatography/electrospray mass spectrometry. *European Journal of Pharmaceutical Sciences* **2005**, *25*, 155.

[32] J. C. Wolff, A. M. Laures. 'On-the-fly' hydrogen/deuterium exchange liquid chromatography/mass spectrometry using a dual-sprayer atmospheric pressure ionisation source. *Rapid Communications in Mass Spectrometry* **2006**, *20*, 3769.

[33] T. J. Novak, R. Helmy, I. Santos. Liquid chromatography-mass spectrometry using the hydrogen/deuterium exchange reactions as a tool for impurity identification in pharmaceutical process development. *Journal of Chromatography B* **2005**, *825*, 161.

[34] D. Q. Liu, C.E.C.A. Hop. Strategies for characterization of drug metabolites using liquid chromatography-tandem mass spectrometry in conjunction with chemical derivatization and on-Line H/D exchange approaches. *Journal of Pharmaceutical and Biomedical Analysis* **2005**, *37*, 1.

[35] A. Ghosal, Y. Yuan, N. Hapangama, A. D. Su, N. Alvarez, S. K. Chowdhury, K. B. Alton, J. E. Patrick, S. Zbaida. Identification of human UDP-glucuronosyltransferase enzyme(s) responsible for the glucuronidation of 3-hydroxydesloratadine. *Biopharmaceutics and Drug Disposition* **2004**, *25*, 143.

[36] R. Ramanathan, L. Reyderman, A. D. Su, N. Alvarez, S. K. Chowdhury, K. B. Alton, M. A. Wirth, R. P. Clement, P. Statkevich, J. E. Patrick. Disposition of desloratadine in healthy volunteers. *Xenobiotica* **2007**, *37*, 770.

[37] D. F. Hunt, W. M. Bone, J. Shabanowitz, J. Rhodes, J. M. Ballard. Sequence analysis of oligopeptides by secondary ion/collision activated dissociation mass spectrometry. *Analytical Chemistry* **1981**, *53*, 1704.

[38] D. F. Hunt, A. M. Buko, J. M. Ballard, J. Shababowitz, A. B. Giordani. Sequence analysis of polypeptides by collision activated dissociation on a triple quadrupole mass spectrometer. *Biomedical Mass Spectrometry* **1981**, *8*, 397.

[39] G. Chen, S. H. Hoke, R. G. Cooks. Ion/molecule reactions leading to $WF_n^+$ ($n = 1-5$):implications for the mechanisms of ion/surface reactions. *International Journal of Mass Spectrometry and Ion Processes* **1994**, *139*, 87.

[40] B. N. Pramanik, A. K. Mallams, P. L. Bartner, R. R. Rossman, J. B. Morton, J. H. McGlotten. Special techniques of fast atom bombardment mass spectrometry for the study of oligosaccharide containing macrotetronolide antibiotic, kijanimicin. *Journal of Antibiotics* **1984**, *37*, 818.

[41] B. N. Pramanik, A. K. Ganguly. Fast atom bombardment mass spectrometry: a powerful technique for study of oligosaccharide antibiotics. *Indian Journal of Chemistry* **1986**, *25B*, 1105.

[42] B. N. Pramanik, P. R. Das. Self-ionization of nitrocompounds under negative chemical ionization conditions. *Organic Mass Spectrometry* **1987**, *22*, 742.

[43] R. Ramanathan, A. D. Su, N. Alvarez, N. Blumenkrantz, S. K. Chowdhury, K. Alton, J. Patrick. Liquid chromatography/mass spectrometry methods for distinguishing N-oxides from hydroxylated compounds. *Analytical Chemistry* **2000**, *72*, 1352.

[44] F. W. McLafferty, F Tureček. *Interpretation of Mass Spectra*, 4th ed. University Science Books: Sausalito, **1993**, 27.

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