Gas Chromatography–Mass Spectrometry Designation and Prediction of Metabolic Dealkylation and Hydroxylation Reactions in Xenobiotics Exemplified by Tramadol

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Abstract

Metabolic dealkylation and hydroxylation reactions in xenobiotics are common and may take place at different sites in the molecules. Sometimes confusion may arise as to the nature and site of the resulting metabolic change when there is more than one potential site. The use of GC–MS in resolving the problem has been demonstrated by using tramadol as example. Human urine samples containing tramadol and its metabolites were extracted under basic pH conditions and analyzed by GC–MS, in the electron impact and chemical ionization modes, before and after trimethylsilyl (TMS) derivatization. By recognizing the mass-to-charge ratios of molecular and base-peak ions in the mass spectra, it was possible to predict and designate sites of demethylation and hydroxylation in tramadol metabolites. In addition to the designation of the known tramadol metabolites, the practice has led to the tentative characterization of hydroxytramadol and norhydroxytramadol as new metabolites of tramadol in humans. Possible extension of the modus operandi to other xenobiotics was discussed.

Introduction

N-Dealkylation from secondary amino (RNHR') and tertiary amino (RNR'_2) groups, *O*-demethylation from methoxy (OCH_3) groups bonded to rings or side chains and oxidation by addition of an oxygen atom to the molecule are common metabolic pathways of xenobiotics in humans (1,2). Xenobiotics that are metabolized by demethylation may contain both dimethylamino and methoxy groups in their molecules. Metabolic addition of oxygen atoms may take place at different sites in the molecule: at a basic nitrogen atom to give the *N*-oxide or at a ring or side chain to form a hydroxy compound. For pharmacokinetic, pharmacodynamic and analytical considerations, the sites of demethylation or oxidation in a xenobiotic molecule may need to be specified. Furthermore, the ability to predict the occurrence of such metabolic pathways in new molecules is a welcomed endeavor. Although methods based on liquid chromatographic separations and mass spectrometric (MS) detection are now widely used in metabolic characterizations (3–7), under favorable conditions of volatility and thermal stability, GC–MS can still be used, particularly in uncomplicated cases. In this study, tramadol, a narcotic/analgesic (8), has been chosen as a candidate for demonstrating the use of gas chromatography (GC)–MS in the prediction and designation of metabolic demethylation and oxidation reactions in xenobiotics for two reasons: it contains the necessary structural features and a large supply of urine samples containing it and its metabolites was available. Tramadol is reported to be metabolized through *N*- and/or *O*-demethylations to the five compounds shown in Figure 1 (9). It is of interest to note that hydroxytramadol, a known metabolite of tramadol in animals (10), has not been reported in humans. Therefore, a further objective of this study was to scrutinize the GC–MS data for urinary hydroxytramadol and norhydroxytramadol as possible tramadol metabolites in humans.

Materials and Methods

Materials

Urine samples from tramadol users were obtained from the Sharjah Police Rehabilitation Centre where tramadol is used in drug treatment programs for heroin addiction. Tramadol hydrochloride capsules were obtained from the pharmacy of the Sharjah Police Rehabilitation Centre. Bis(trimethylsilyl)trifluoroacetamide/1% trimethylchlorosilane (BSTFA/1% TMCS) and β-glucuronidase (from *Helix pomatia*) were purchased from Sigma-Aldrich (Deisenhofen, Germany). Toxi-Tubes A for basic drug extraction were obtained from Varian (Lake

Forest, CA). Acetic anhydride and pyridine were purchased from BDH Chemicals (Poole, England).

Urine sample preparation for GC–MS analysis

Without enzyme hydrolysis. Urine (4 mL) was extracted with a Toxi-Tube A by mechanical shaking for 10 min. The tube was then centrifuged at 3000 rpm for 5 min. After separating the layers, the organic layer was transferred to a 5-mL glass test tube and the solvent was evaporated under nitrogen at 40°C. The residue was dissolved in methanol (100 µL) and transferred to a 200-µL glass insert, and a 2-µL aliquot was injected into the GC–MS. After evaporation of the solvent under nitrogen at 40°C, the residue was heated with BSTFA/1% TMCS (100 µL) at 60°C for 10 min in a screw-capped 5-mL glass test tube. The reaction mixture was then transferred to a 200-µL glass insert, and a 2-µL aliquot was injected into the GC–MS. Another residue, obtained in the same way as described, was heated at 60°C for 30 min with of a 2:1 mixture of acetic anhydride/pyridine (500 µL). After evaporation of the excess reagent under nitrogen at 50°C, the residue was dissolved in methanol (100 µL) and transferred to a 200-µL glass insert. A 2-µL aliquot was injected into the GC–MS.

With enzyme hydrolysis. To a urine sample (4 mL) in a screw-capped glass vial were added 500 µL acetate buffer (2.0 M, pH 4.8) and 60 µL β-glucuronidase (6642 units). The vial was capped and heated at 56°C for 60 min. After cooling, a 1-mL aliquot of the reaction mixture was withdrawn for the immunoassay testing, and the remaining mixture was extracted with Toxi-A tube for basic drug extraction. After separating the two layers, the organic layer was transferred to a 5-mL glass test tube, and the solvent was evaporated under nitrogen at 40°C. The residue was heated with BSTFA/1% TMCS (100 µL) at 60°C for 10 min in a screw-capped 5-mL glass test tube. The reaction mixture was then transferred to a 200-µL glass insert, and a 2-µL aliquot was injected into the GC–MS instrument. Another residue obtained as described was heated with a 2:1 mixture of acetic anhydride/pyridine (500 µL) at 60°C for 30 min. After evaporation of the excess reagent, the residue was dissolved in methanol (100 µL), and a 2-µL aliquot was injected into the GC–MS.

GC–electron impact (EI) MS conditions

GC–EIMS analyses were conducted on an Agilent (HP) series 6980 GC interfaced with a 5973 Agilent mass selective detector (Palo Alto, CA) using an Agilent-5MS capillary column (cross-linked 5% phenyl-methyl polysiloxane, 30 m × 0.25-mm i.d., 0.25-µm film thickness, Agilent). The injector port and transfer line were maintained at 250°C and 280°C, respectively. Helium was used as the carrier gas at a flow-rate of 1 mL/min. Splitless injection of a 2-µL extract was performed with the following oven temperature program. The initial temperature of 100°C was held for 2 min and then raised at

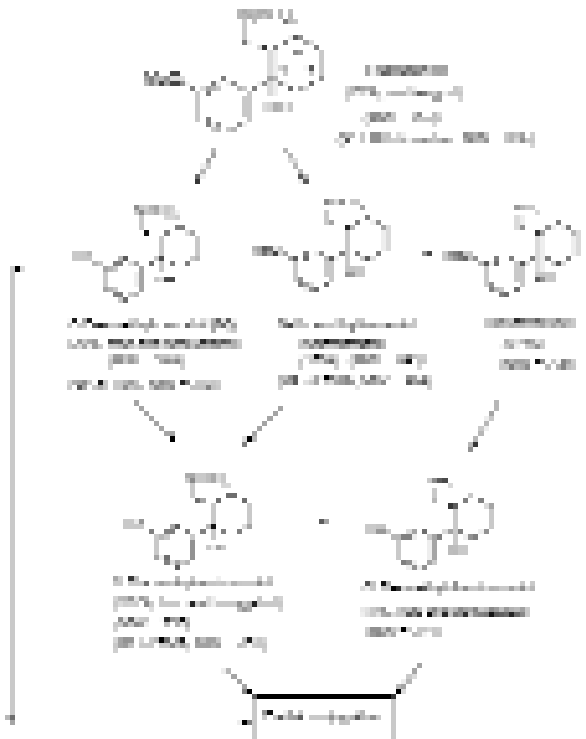

Figure 1. Chemical structures of tramadol and reported metabolites (9). The percent concentrations of the metabolites are for 72-h urine (9). Note: tramadol, nortramadol, and dinortramadol did not form glucuronide conjugates, which may be explained by steric hindrance occurring at the hydroxy group in position 1.

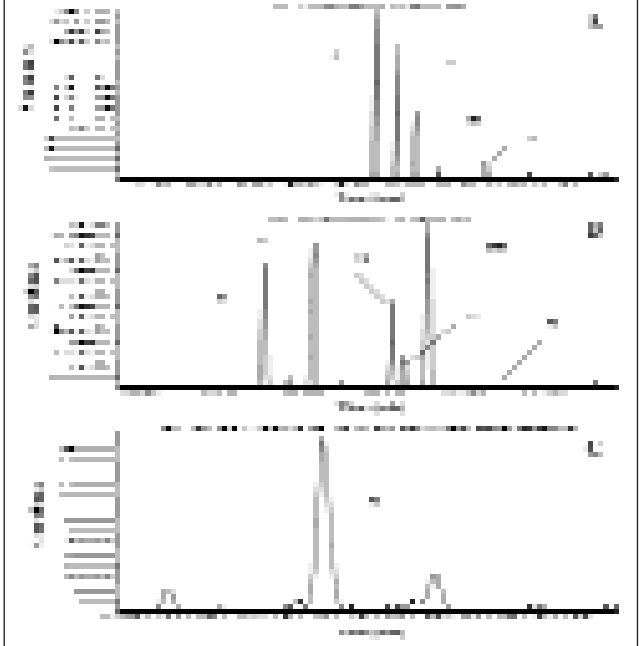

Figure 2. Total-ion chromatograms (A and B) and extracted-ion chromatogram (C) obtained from a urine sample of a tramadol user. Sample preparation: The urine sample (3 mL) was extracted with a Toxi-tube for basic drug extraction. After evaporation of the solvent, the residue was dissolved in methanol (100 µL), and a 2-µL aliquot was injected into the GC–MS (A); heated with BSTFA at 60°C for 10 min, and a 2-µL aliquot was injected into the GC–MS (B); and extracted-ion chromatogram (at *m/z* 481) showing compound X in chromatogram (B) on a larger scale (C).

20°C/min to 280°C with a final hold time of 5 min. Electron impact ionization (EI) analysis was performed with ionization energy at 70 eV. Data were acquired in the scan mode in a range of *m/z* 50–550 at a rate of 2 scans/s. The ion source temperature was 230°C.

GC–chemical ionization (CI) MS

A Saturn 2200 GC–MS system from Varian (Walnut Creek, CA) was used in both EI and CI modes of analysis. The system consisted of a Varian 3800 GC interfaced with Saturn MS 2200 ion trap, a capillary 1177 split/splitless injector and a Varian 8200 autosampler. Analysis was performed on a 5% phenyl 95% dimethylpolysiloxane (30 m × 0.25 mm, 0.25-µm film thickness) column (CP-Sil 8 CB Low Bleed MS, Varian). A 2-µL splitless injection was performed with the following oven program: an initial temperature of 100°C held for 2 min, then ramped to 190°C at 10°C/min, and then to 280°C at 20°C/min with a final hold time of 14 min. The carrier gas was helium with a flow rate of 1 mL/min. The injector and interface temperatures were set at 250°C and 260°C, respectively. The split ratio was 1:20. A scan range of 47–650 was used at a scan rate of 2 scans/s. The ion trap temperature was set at autotune value, and the target total ion chromatogram was set at the threshold value of 20,000 counts for EI and 5000 counts for CI.

For electron impact (EI) ionization, the filament current was set at 10 µA, and the maximum ionization time was 15,000 µs with a background mass of *m/z* 45.

For chemical ionization (CI), the filament current was set at 20 µA, and the storage level was *m/z* 19. The background mass was *m/z* 65 with the maximum ionization time of 2000 µs and a maximum reaction time of 60 ms. The reagent gas was isobutane at a pressure of 5 psi.

Results and Discussion

The GC–EIMS data of the tramadol-related compounds detected in urine are shown in Figure 2 and Table I. The molecular weights of the compounds (corresponding to the molecular ions in Table I) were confirmed by GC–CIMS (Table I). Although GC–MS is considered an ancillary technique in metabolic studies of xenobiotics, it can yield very useful standalone information for some simple metabolic reactions, particularly when the volatility and thermal stability of the separated compounds allow and derivatization is used. This has been well demonstrated in the present study for tramadol metabolic demethylation and hydroxylation reactions. Usually, the first step in the prediction of metabolic chemical changes in a xenobiotic is the calculation of the difference between the molecular weights of the derivatized and underivatized (expected or hypothesized) metabolites from that of the corresponding derivatized and underivatized parent compound. The necessary information can be obtained from EIMS and/or CIMS data. The data in Table II can then be used to give a tentative indication as to the nature of the metabolic chemical change that had occurred. The second step is to designate the site of the chemical change from the mass spectral data. Because phase I metabolic chemical changes almost invariably result in the generation of polar groups, their sites in the molecule can be determined by derivatization when GC–MS is used in their prediction. This will be demonstrated for tramadol metabolites in the following discussion.

The key mass fragmentations used in the designation of tramadol and its metabolites from GC–MS data are the base-

Table I. Mass Spectral Data of Underivatized and TMS-Derivatized Tramadol and its Metabolites*

Compound	Figure	RT† (min)	*m/z* (% Relative Abundance) CIMS	*m/z* (% Relative Abundance) EIMS
Underivatized				
I	2A	9.73	58(100), 263(17, MI), 135(9), 218(2))	264(100, MH+), 246(4.3
II	2A	9.93	189(100), 73(72), 121(68), 202(39), 135(35), 261(26, MI), 159(23)	262(100, MH+), 202(5)
III	2A	10.12	58(100), 249(6, MI), 121(4)	250(100, MH+), 232(4), 175(4)
IV	2A	10.81	58(100), 279(3, MI), 135(3)	280(100, MH+), 262(5)
TMS-Derivatized				
V	2B	9.78	58(100), 335(43, MI), 245(21), 73(20), 220(10), 216(6), 84(6),	336(100, MH+), 290(5)146(48), 245(14), 321(13)
VI	2B	10.09	58(100), 393(54, MI), 73(38), 378(25), 303(21), 274(10), 84(6)	394(100, MH+), 146(42), 217(16), 304(13), 177(12), 58(10)
VII	2B	10.57	116(100), 73(49), 393(20, MI), 303(5), 278(5), 289(4), 246(4), 190(4), 147(6)	146(100), 394(70, MH+), 303(32), 379(30)
VIII	2B	10.62	58(100), 73(30), 84(11), 333(34), 408(14), 423(8, MI)	424(100, MH+), 146(24), 409(21), 321(15)
IX	2B	10.79	116(100), 73(100), 142(17), 451(13, MI), 190(13), 361(6), 436(5)	452(100, MH+), 116(18), 73(13)
X	2B, 2C	11.27	116(100), 73(40), 147(11), 481(8, MI), 466(6)	482(100, MH+), 116(19)

* The chemical structures of the compounds are shown in Figure 1, and the experimental conditions for sample preparation are described in Figure 2.
† Abbreviations: RT, retention time; TMS, trimethylsilyl; EIMS, electron impact mass spectrum; CIMS, chemical-ionization mass spectrum; and MH+, protonated molecular ion.

peak ions of *m/z* 58 and 116, which result from the intact dimethylaminomethyl group [$((CH_3)_2N=CH_2)^+$] and the TMS-derivatized monodesmethylaminomethyl group [$(CH_3N(TMS)CH_2)^+$], respectively. The ion of *m/z* 58 is found in compounds I, III, IV, V, VI, and VIII (Table I), whereas that of *m/z* 116 is found in compounds VII, IX, and X (Table I).

The difference in the molecular weights (MWs) of tramadol and compound III is 14 (263–249), which indicates metabolic monodemethylation of the former compound at either the dimethylamino- or the methoxy group (designation a, Table II). However, from the *m/z* 58 of the base-peak ion, compound III is designated as *O*-desmethyltramadol (Figure 1). In fact, a good match has been obtained for compound III as *O*-desmethyltramadol by the NIST database mass spectral library.

The molecular weight of 393 of both compounds VI and VII (Table I) corresponds to di-TMS derivative of monodemethylated tramadol [i.e., (263 – 14) + 144 (designations a/h, Table II)]. From the base-peak ion of *m/z* 58, compound VI is designated as the di-TMS derivative of *O*-desmethyltramadol (Figure 1), whereas from the base-peak ion *m/z* 116, compound VII is designated as di-TMS derivative of *N*-desmethyltramadol (nortramadol, Figure 1). Further differentiation between the two metabolites can be made from the ion of *m/z* 348, which is present in the mass spectrum of compound VI but not in that of

Table II. Common Numerical Changes of Molecular Weights (MW) of Xenobiotics Resulting from Metabolic Processes or Chemical Manipulation During Sample Preparation or GC Artifact Formation

Designation	Change in MW of Parent Compound*	Chemical Change	Cause of Change
a	14 (–)	Loss of a methyl group from amino or methoxy group (*N*- or *O*-demethylation, respectively)	Metabolic
b	28 (–)	Loss of two methyl groups from amino and/or methoxy groups	Metabolic
c	42 (–)	Loss of three methyl groups from amino and/or methoxy groups	Metabolic
d	18 (–)	Dehydration (loss of a molecule of water); usually from underivatized compounds in which the OH group is part of alicyclic ring or aliphatic side chain; dehydration does not take place when the OH is bonded to an aromatic ring	Thermal effect (high temperature in GC)
e	44 (–)	Decarboxylation: loss of CO_2 from a carboxylic acid (COOH) group	Thermal effect (high temperature in GC)
f	16 (+)	Oxidation: addition of oxygen (O) as *N*-oxide (in compounds containing amino groups or heterocyclic nitrogen) or OH group substitution in rings or aliphatic side chains	Metabolic
g	72 (+)	Formation of TMS derivatives at intrinsic OH, NH (in amines and amides) and COOH groups	Analytical manipulation
h	144 or 216 (+)	TMS derivatization at two or three intrinsic sites, respectively	Analytical manipulation
i	28 (–), 56 (–)	Loss of one or two ethyl groups, respectively	Metabolic
j	44 (+)	Loss of ethyl group (–28) followed by TMS derivatization (+72)	Metabolic/analytical manipulation
k	58 (+)	Demethylation (–14) followed by TMS derivatization (+72)	Metabolic/analytical manipulation
l	116 (+)	Loss of two methyl groups (–28) addition of two TMS groups (+144)	Metabolic/analytical
m	174 (+)	Loss of three methyl groups (–42) followed by TMS derivatization at the three generated active sites (+216)	Metabolic/analytical manipulation
n	88 (+)	Hydroxylation (+16) followed by TMS derivatization of the newly generated OH group	Metabolic/analytical manipulation
o	176	Hydroxylation at 2 sites (+32) followed by TMS derivatization (+144)	Metabolic/analytical manipulation
p	146	Hydroxylation (+16), *N*-demethylation (–14) followed by TMS derivatization (+ 144)	Metabolic/analytical manipulation
q	102	Oxidation of alkyl group attached to ring to carboxyl group (COOH) followed by TMS derivatization	Metabolic/analytical manipulation

* (–) indicates loss, and (+) indicates gain.

compound VII (Table I). This ion results from the loss of the neutral dimethylamine [$(CH_3)_2NH$] of mass 45 Da, which is only feasible from compound VI. The ion of *m/z* 303 in the mass spectra of both compounds VI and VII results from the loss of mass 90 Da corresponding to trimethylsilanol [$HOSi(CH_3)_3$]. Because such neutral loss occurs at aliphatic side chains or alicyclic rings (7), in which the adjacent carbon atom is saturated and carries a hydrogen, it can be assigned to the trimethylsilyloxy group ($OSi(CH_3)_3$ at position 1 in both compounds VI and VII (Figure 1). It follows that in xenobiotics that do not contain intrinsic hydroxy groups, the detection of the ion corresponding to the loss of the neutral molecule [$HOSi(CH_3)_3$] in the mass spectrum of the parent compound may be taken to indicate metabolic hydroxylation at an aliphatic side chain or alicyclic ring.

Compound IX has a molecular weight of 451 and a base peak ion of *m/z* 116, both of which are consistent with tri-TMS derivative of *O*-desmethylnortramadol (Figure 1, 263 – 28 + 216, designations b/h, Table II).

The difference of 16 (atomic weight of oxygen) between the MWs of compound IV and tramadol (279 – 263) indicates metabolic oxidation of tramadol to give either hydroxytramadol (Figure 3) or tramadol-*N*-oxide (designation f, Table II). Hydroxytramadol has been reported as a major metabolite of tramadol in animals (rats and dogs) (11) but not in humans (9). Tramadol-*N*-oxide was found to have been formed in vitro upon using rat hepatic S9 fraction in the presence of NADPH generating system (11). In the present study, the lack of detection of tramadol-*N*-oxide in human urine is not sufficient evidence to exclude its metabolic formation because, due to thermal instability, GC–MS is not the appropriate technique for its detection; LC–MS is recommended for the detection of such type of compounds (12). Evidence that compound IV is hydroxytramadol was obtained from the mass spectrum of compound VIII (Table I) where the MW of 423 represents di-TMS-derivatized hydroxytramadol (263 + 16 + 144, designations f/h, Table II) and the *m/z* 58, which results from the intact dimethylaminomethyl group. In fact, a 90% match quality for hydroxytramadol was obtained on the Wiley mass spectral database library for compound IV. The site of metabolic hydroxylation of tramadol is most probably the aromatic ring because hydroxylation at the cyclohexane ring would have resulted in thermal dehydration under the GC–MS experimental conditions used in this study (designation d, Table II). However, cyclohexane-ring hydroxylation has been reported for rats and dogs as well as in vitro metabolic studies of tramadol using rat hepatic S9 fraction in the presence of NADPH-generating system (8).

Compound X (Figure 2) has a molecular weight of 481 and a base-peak ion of 116, both of which are consistent with tri-TMS derivative of norhydroxytramadol (263 + 16 – 14 + 216) (Figure 3; designations a/f/h, Table II). This is the first time that this compound has been detected and characterized as a metabolite of tramadol in any species.

Despite being major metabolites of tramadol, nortramadol and *O*-desmethylnortramadol (Figure 1) have only been detected after TMS derivatization in most of the urine samples tested, an observation that emphasizes the need for derivatization in metabolic studies by GC–MS to increase the sensitivity of detection and/or to overcome artifact formation under the analytical conditions used.

It should be noted that the two reported tramadol metabolites, dinortramadol and *O*-desmethyldinortramadol (Figure 1), have not been detected under the experimental conditions used in this study, possibly because of their low metabolic yields (Figure 1).

Compound II (Figure 2 and Table I) has been detected when analyzing both urine samples of tramadol users and methanolic extracts of tramadol capsules by GC–MS. It is, therefore, a possible impurity or analytical artifact of tramadol. However, because its characterization is not within the domain of this work, it will not be further discussed; its GC–MS data are presented for future consideration of its possible source and identity.

Acetyl derivatization of the urine sample extracts containing tramadol and its metabolites resulted in very low abundance mass spectra that were deficient in molecular ions and therefore structurally uninformative (data not shown).

The dimethylaminomethyl [$(CH_3)_2NCH_2^-$, DMAM] group, which has given rise to the base-peak ion of *m/z* 58 in its intact form in the mass spectra of tramadol and some of its metabolites, is present in a number of other drugs (Table III). One of the metabolic pathways of these drugs is monodemethylation (13). The resulting methylaminomethyl group (MAM) is expected to give the base-peak ions of *m/z* 116 when the compounds are TMS-derivatized (same as has been found for tramadol mono-*N*-desmethyl metabolites). Therefore, screening urine for the presence of drugs containing DMAM group and their monodesmethyl metabolites by GC–MS can be done by extracting ion chromatograms at *m/z* 58 and 116 of the underivatized and TMS-derivatized samples, respectively, in addition to determining the molecular weights using CIMS. Furthermore, MAM group is present per se in some drugs (Table III) and gives rise to the base-peak ion of *m/z* 44 in the mass spectra of these drugs. Upon TMS derivatization, the base-peak ion resulting from MAM will have a mass-to-charge ratio of 116. Thus, in addition to determining the molecular weights, the values of *m/z* 44 and 116 can be used to search for MAM-containing drugs in urine from GC–MS data. This approach can be extended to drugs that contain the homologue diethylaminomethyl [$(CH_3CH_2)_2NCH_2$, DEAM] group (Table III) and are metabolized, to a minor or large extent, through monodesethylation, to give ethylaminomethyl (EAM)-group-

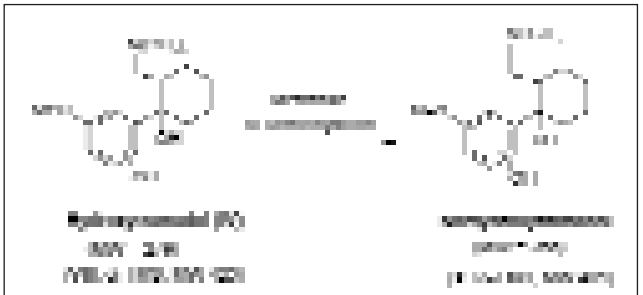

Figure 3. Chemical structures of hydroxytramadol and norhydroxytramadol.

containing compounds. In its intact form, DEAM gives rise to the base-peak ion *m/z* 86 in the mass spectra of the compounds containing it. On the other hand, EAM is expected to give rise to base-peak ion *m/z* 130 in the TMS-derivatized samples (86 – 28 + 72). Therefore, the DEAM-containing drugs in Table III and their deethylated metabolites can be screened for by extracting ion chromatograms at *m/z* 86 and 130 for the underivatized and TMS-derivatized sample in addition to determining the molecular weights of the corresponding compounds

Metabolic oxidation, involving the introduction of an oxygen atom, and its possible site in the molecule are other designations that can be derived from GC–MS data. An increase of the molecular weight of the metabolite by 16 relative to that of the parent drug indicates the addition of an oxygen atom as *N*-oxide or hydroxyl group (designation f, Table II). Differentiation between the two possibilities can be made by derivatization: TMS derivatization would result in increase of the molecular weight of the hydroxy metabolite by 88 relative to that of the parent drug (designation n, Table II); the *N*-oxide contains no active hydrogen and will not be TMS-derivatized.

Upon comparing the GC–MS results obtained from hydrolyzed and unhydrolyzed urine containing tramadol and its metabolites, before and after TMS derivatization, no qualitative differences were observed. This result has two implications. Firstly, it substantiates literature reports that tramadol and its metabolites are only partially glucuronide-conjugated (9). Secondly, it suggests that initial GC–MS metabolic screening of xenobiotics can be carried out on unhydrolyzed urine with the advantage of considerably reducing matrix interferences and therefore obtaining good quality mass spectra for use in unknown characterization in case glucuronide and/or sulfate conjugation of the parent drug and/or metabolites were only partial. The use of database mass spectral libraries in unknown characterization should always be scrutinized since faulty results may be obtained. This has been clearly demonstrated in the tramadol urine cases by a compound, which had given molecular and base-peak ions of *m/z* 249 and 44, respectively, and was characterized by the Wiley mass spectral library as *O*-desmethyltramadol. Certainly, the base-peak ion of *m/z* 44 rules out this labeling because it must have resulted from methylaminomethyl (CH_3NHCH_2) group which is only feasible with *N*-desmethyltramadol (i.e., nortramadol) and not *O*-desmethyltramadol.

Conclusions

Despite limitations imposed by thermal stability and volatility, GC–MS could be used in the prediction and designation of metabolic dealkylation and hydroxylation reactions in xenobiotics. Differentiation was possible between the following metabolic reactions: *N*- and *O*-demethylation, hydroxylation and *N*-oxide formation, and hydroxylation at aromatic ring on one hand and alicyclic ring or aliphatic side chain on the other.

Table III. Drugs Containing Alkylaminomethyl and Dialkylaminomethyl Groups that Give Rise to Base-Peak Ions of *m/z* 44, 58, 72, and 86 in the Corresponding Mass Spectra*

Drugs Containing Methylaminomethyl (CH_3NHCH_2, MAM) Group†	Drugs Containing Dimethylaminomethyl [$(CH_3)_2NCH_2$, DMAM] Group‡	Drugs Containing Diethylaminomethyl [$(C_2H_5)_2NCH_2$, DEAM] Group§
Maprotiline, Metaraminol, Methylthiamfetamine, Oxedrine, Phenylephrine, Phenylpropanolamine, Rimantadine	Alimemazine, Almotriptan, Amiodarone, Benactyzine, Bufotenine, Butetamate, Carbinoxamine, Carbocromen, Chloroprocaine, Chloropyramine, Chloroquine, Chlorphenamine, Chlorpromazine, Chlorprothixene, Cinchocaine, Clomifene, Clomipramine, Cloricromen, Cyclobenzapine, Cyclopentoate, Dexchlorphenamine, Dextropropxyphene, Dibenzeoin, Diltiazem, Dimethoxanate, Dimethyltryptamine, Dimetindene, Diphenhydramine, Dosulepin, Doxylamine, Ephedrine, Ethylnoradrenaline, Etilefrine, Hordenine, Hydroxyephedrine, Imipramine, Iprindole, Levomepromazine, Levopropoxyphene, Meclofenoxate, Mepyramine, Metamfetamine, Methapyriline, Methoxyphenemine, MDEA, MDMA, Methylephedrine, Moxisylyte, Normethadone, Noxiptline, Orphenadrine, Psilocin, Psilocybine, Rizatriptan Tolpropamine, Tolterodine	Benactyzine, Butetamate, Caramiphen, Carbetapentane, Carbocromen, Chlor Diamethazole, Dicycloverine, Diethazine, Doxepin, Etamiphylline, Flurazepam, Lidocaine, Metoclopramide, Nadolol, Nicametate, Oxeladin, Phenglutarimide, Prilocaine, Procainamide, Procaine, Propoxycaine, Proxymetacaine, Salbutamol

* The drugs have been compiled from Clarke's Analysis of Drugs and Poisons (12).
† Ion: $(CH_3NH{=}CH_2)^+$, *m/z* = 44 [Clarke's]. Ion from TMS derivative: $(CH_3N(Si(CH_3)_3){=}CH_2)^+$, *m/z* = 116.
‡ The drugs are metabolized by monodemethylation to give MAM-containing metabolites. Ion: $((CH_3)_2N{=}CH_2)^+$, *m/z* = 58 [Clarke's]. Ion from TMS derivative of MAM: $(CH_3N(Si(CH_3)_3){=}CH_2)^+$, *m/z* = 116.
§ The drugs are metabolized by monodeethylation to give ethylaminomethyl-group (EAM)-containing metabolites. Ion: $((C_2H_5)_2N{=}CH_2)^+$, *m/z* = 86 [Clarke's]. Ion of TMS-derivatized EAM: $(C_2H_5N(Si(CH_3)_3){=}CH_2)^+$, *m/z* = 130.

Derivatization, particularly by trimethylsilylation, is an essential step in the designation of the dealkylation site and confirmation of hydroxylation. There is always the possibility of identifying new metabolites of candidate xenobiotics providing the appropriate approach is adopted.

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