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Research Article

GC-MS TENTATIVE DETECTION AND CHARACTERIZATION OF HYDROXYTRAMADOL AS A METABOLITE OF TRAMADOL IN MAN

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ABSTRACT
The high concentrations of metabolites in the urine of tramadol abusers have made possible further investigation of the metabolism of this analgesic drug. In addition to the reported metabolites resulting from O- and N- demethylations, ring-hydroxylation has been detected in this work as a further route of tramadol metabolism. GC-MS and trimethylsilyl derivatization have provided the necessary data. Certain assumptions were necessary for corroborating the ring-hydroxylation route.
Keywords: Tramadol; Tramadol metabolism; hydroxytramadol; GC-MS; ring hydroxylation; trimethylsilyl derivatization

INTRODUCTION

Due to dose restrictions, controlled metabolic studies in humans may not result in sufficient metabolite concentration in urine to allow detection and unequivocal characterization by hyphenated chromatographic-spectroscopic techniques such as GC-MS. Tramadol is a widely used analgesic drug and due to its weak opioid mu-receptor agonistic effects with consequent euphoric effects; it is now widely abused[1]. The high concentrations of tramadol and its metabolites in the urine of drug abusers have provided the opportunity for testing the above statement.

Objective

The objective of this work was to characterize a tramadol-related compound detected in the GC-MS chromatograms obtained from urine samples of tramadol abusers in routine immunoassay confirmation analysis.

MATERIALS AND METHODS

Urine samples of tramadol abusers were obtained from Sharjah Police Forensic Science Laboratory, Sharjah, UAE. Sample preparation for GC-MS analysis involved basic extraction before and after β- glucuronidase hydrolysis. GC-MS analysis, both in electron impact (EI) and chemical ionization (CI) modes, was carried out before and after trimethylsilyl (TMS) derivatization.

RESULTS

The GC-MS chromatograms of the known metabolites of tramadol and the unknown compound X, before and after TMS derivatization, are shown in Figures 2 and 3, respectively. The EIMS and CIMS data of the compound labeled X in the chromatograms in Figures 2 and 3 are given in Table 1.

DISCUSSION

Hydroxylation of aliphatic side chains and alicyclic and aromatic rings is a common metabolic reaction of increasing polarity in xenobiotic molecules[2]. The common practice In GC-MS characterization of unknown compounds is MS library matching[3,4]. If this is not feasible, however, strategies based on assumptions followed by confirmation provide an alternative as has been developed in this laboratory. The increase of the molecular mass of a xenobiotic molecule by 16 Da is almost certainly due to metabolic addition of oxygen in the form of hydroxy group to an alkyl chain or ring, or oxidation of an amino group to the N-oxide. Both structural features, i.e. ring and amino group, are present in tramadol. Assigning of either metabolic change can be tentatively made by TMS derivatization and the observation of the increase of molecular mass by 72 Da or lack of it relative to both assumed metabolites, or by 88 Da relative to the hydroxy metabolite. Upon following such practice, hydroxytramadol has been detected as a metabolite of tramadol in human urine as indicated in the GC-MS chromatograms and spectra shown in Figures 1 and 2 and Table 1. It is worth mentioning that metabolic cyclohexane-ring hydroxylation of tramadol has been reported for rats and dogs[5]. Although the same site could be assumed to have occurred in man, as has been shown in this study, aromatic ring hydroxylation cannot be ruled out. Metabolic hydroxylation of aromatic rings is usually favored at electron-rich sites particularly those with electron-donating groups[6], such as the methoxy and cyclohexyl groups in tramadol. Due to resonance and steric effects, the position ortho to the methoxy group and para to the cyclohexyl ring is favored for metabolic hydroxylation of tramadol.

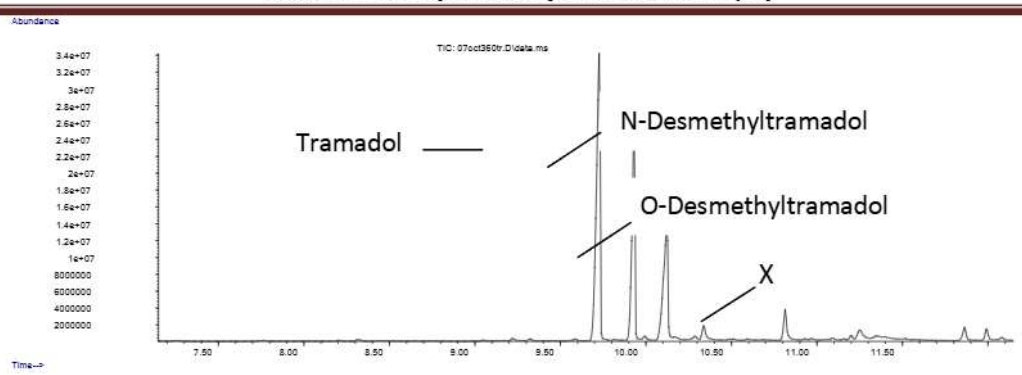

Figure 1: Total ion chromatogram of tramadol and its known metabolites as charcterized from MS spectra and of the compound X pending charaterization

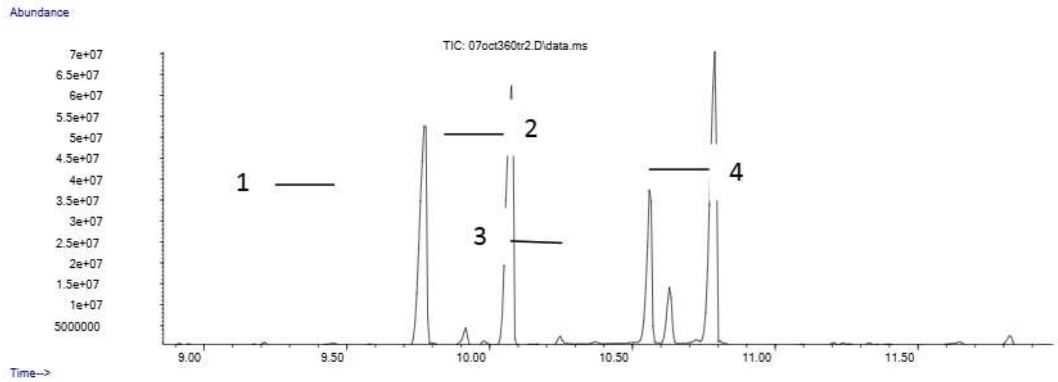

Figure 2: Total ion chromatogram of the TMS Derivatives of Tramadol (1); O-Desmethyltramadol (2); N-Desmethyltramadol (3); Compound X (4)

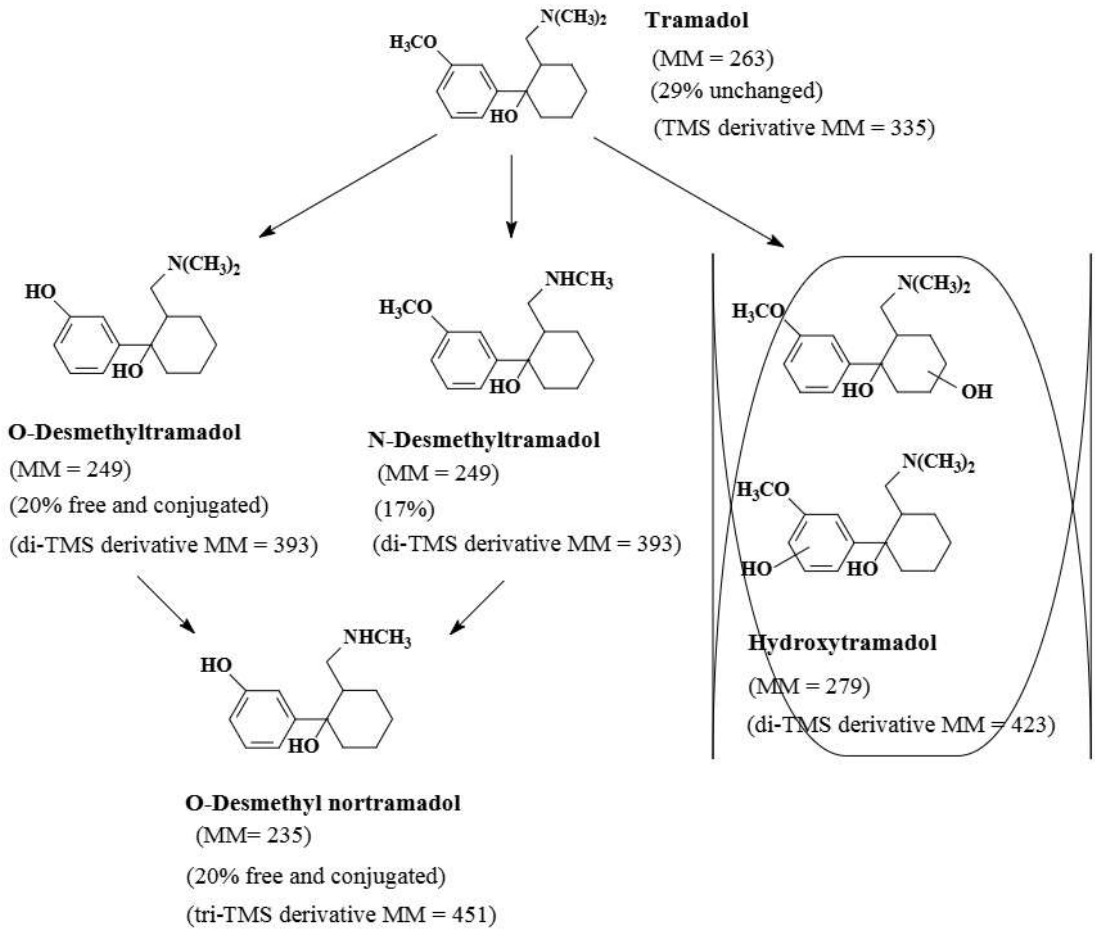

Figure 3: Structures of tramadol and its metabolites

Table 1: EIMS and CIMS data of compound X in Figure 1 and its corresponding TMS derivative, compound 4 in Figure 2

Compound	RT (min)	EIMS *m/z* (% relative abundance)	CIMS *m/z* (% relative abundance)
X (Figure 1) Underivatized	10.81	58 (100), 279 (3, MI), 135 (3)	280 (100, MH^+), 262 (5)
4 (Figure 2) TMS-derivatized	10.62	58 (100), 73 (30), 84 (11), 333 (34), 408 (14), 423 (8, MI)	424(100, MH+), 146(24), 409(21), 321(15)

CONCLUSION

Although the site of hydroxylation has not been unequivocally determined, hydroxytramadol has been detected and tentatively characterized as a metabolite of tramadol in man. GC-MS, with and without derivatization, can provide useful information regarding the detection and characterization of xenobiotic metabolites providing the appropriate strategy of making logical assumptions of chemical metabolic changes is followed.

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