Original Paper

A Selective Method for the Qualitative Determination of Paroxetine in Forensic Biological Samples by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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ABSTRACT

Paroxetine is a schedule H antidepressant drug. It has occasionally been implicated in lethal overdoses. To identify and estimate the level of this drug in toxicological samples is a forensic challenge. Attempts have been made in the past to extract and detect paroxetine in blood samples by using a variety of techniques such as gas chromatography, gas chromatography-mass spectrometry, high pressure liquid chromatography, liquid chromatography-mass spectrometry, etc. However, no studies have been reported in other biological samples. In this study, an attempt has been made to identify paroxetine in biological samples by liquid chromatography-tandem mass spectrometry in multiple reaction monitoring mode at 330.15192.11. The product ion spectra proved to be very helpful in identification of the drug. Furthermore, multiple reaction monitoring (MRM) enhances the reliability and specificity of the method. The use of modified mobile phase produces good quality of qualifier ions. This method appears to be simple, sensitive, specific, and reliable.

Key Words: Liquid chromatography-tandem mass spectrometry, LC-MS/MS, Multiple reaction monitoring, Qualifier ion, Paroxetine

Introduction

Paroxetine (5-(4-p-fluorophenyl-3-piperidylmethoxy)-1,3-benzodioxole, CAS-61869-08-7) is a phenylpiperidine compound, and is commercially available under various brand names as an antidepressant. It is also misused to counter stress, particularly among the working classes and students. The pharmacology of the drug suggests that the maximum blood level of the drug is reached in 2-8 hours after oral administration, and 95% of drug is bound to proteins. It is eliminated after transformation in the liver into pharmacologically inactive metabolites. There are some reports of overdose in humans resulting in death.[1] In order to prove that death has occurred due to overdose of paroxetine, a systematic forensic analysis of blood and tissues is required. A considerable amount of work has already been carried out to identify the drug in plasma by various methodologies including gas chromatography-mass spectrometry,[2,3] high pressure liquid chromatography,[4,5] and liquid chromatography-mass spectrometry.[6,7]

However, there does not appear to be any studies that have been done so far to identify paroxetine in biological tissues. In the present study, an attempt has been made to identify paroxetine in forensic biological tissues by using tandem mass spectrometric (MS/MS) analysis. The method has been modified to change the mobile phase, so as to avoid using formic acid which can give rise to an array of adduct ions that could cause confusion in the interpretation of results.

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Materials and Methods

Chemicals

Standard paroxetine was obtained from Zydus Pharmaceuticals (B.No.MF2306). Acetonitrile, methanol, diethylether, chloroform and water were of HPLC grade, while acetic acid, ammonium sulphate, and ammonium chloride were of analytical grade, all procured from Merck (India).

Equipment

The liquid chromatography (LC) system used was a Perkin Elmer 200 liquid chromatograph equipped with a gradient pump (200 series), an auto-sampler, and a degausser. Mass spectrometric analysis was performed by using LC-MS/MS 3200 Q-Trap from Applied Biosystems, USA. The data acquisition and system controller software was Analyst 1.4.1. Nitrogen was produced by an on-site nitrogen generator.

Packed C-18 column of dimensions 150 x 2.1 mm and 5 µm particle size from Restek was used. All analytical runs were preceded by a guard column packed with C18 from Restek.

Standard Solutions

Standard solution of paroxetine was prepared in methanol having a concentration of 1 mg/mL. Subsequently, serial dilution was carried out to produce a final concentration of 500 ng/ml by using acetonitrile: water (80:20 % v/v). This solution was injected into the pre-set LC-MS/MS, and the conditions were optimized.

LC-MS/MS Conditions

The chromatographic separation was carried out in isocratic mode at 21^{0}C, using mobile phase acetonitrile: water (80:20 % v/v), pumped at a flow rate of 200µL/min. The use of formic acid was avoided intentionally, since it produces a substantial amount of unstable adduct ions during ionization with parent molecule, which interferes with the accurate identification of the parent molecule. The injection volume was 20 µL, and the total run time was set for 5 minutes.

Mass spectrometric analysis was performed using triple quadrupole (QQQ) with ion trap capability, with ESI source in the positive ionization mode, where the protonation of parent molecules is identified under the m/z having +1 charge of proton or a hydrogen ion. The lower limit of detection was also estimated by using standards, and found to be 2 ng/ml. The optimised conditions are presented in Table 1.

Table 1 Source Parameters

S.No.	Parameter	Set Conditions
1.	Curtain gas	25 psi
2.	CAD gas	Medium
3.	IS	4500 V
4.	TEM	450^0 C
5.	GS1	30 psi
6.	GS2	40 psi
7.	Heater	On
8.	Declustering Potential	54 V
9.	Entrance potential	7 V
10.	Cell Ent. Potential	18 V
11.	Collision energy	29 V
12.	Cell Ext. Potential	3 V

Extraction

The biological samples were first deproteinated by adding ammonium sulphate with 5% acetic acid solution. The sample was then boiled for 3 hours, and filtered through Whatman filter paper no.42 to remove solids. The filtrates were basified by addition of ammonium hydroxide and extracted with chloroform to obtain the basic extract. The left-over filtrate was discarded. The basic extract was again passed through sodium sulphate to remove moisture and remaining solids.

The basic extract was dried in nitrogen drier at room temperature. After drying, the final extract was reconstituted in acetonitrile: water (8:2 v/v), and final volume was made to 2.0 ml.

Data Processing

Data acquisition and processing were done by Analyst 1.4.1 software, the MRM (multiple reaction monitoring) data were obtained from standard paroxetine, and the processed forensic samples were compared to establish the presence of paroxetine in the sample.

Results and Discussion

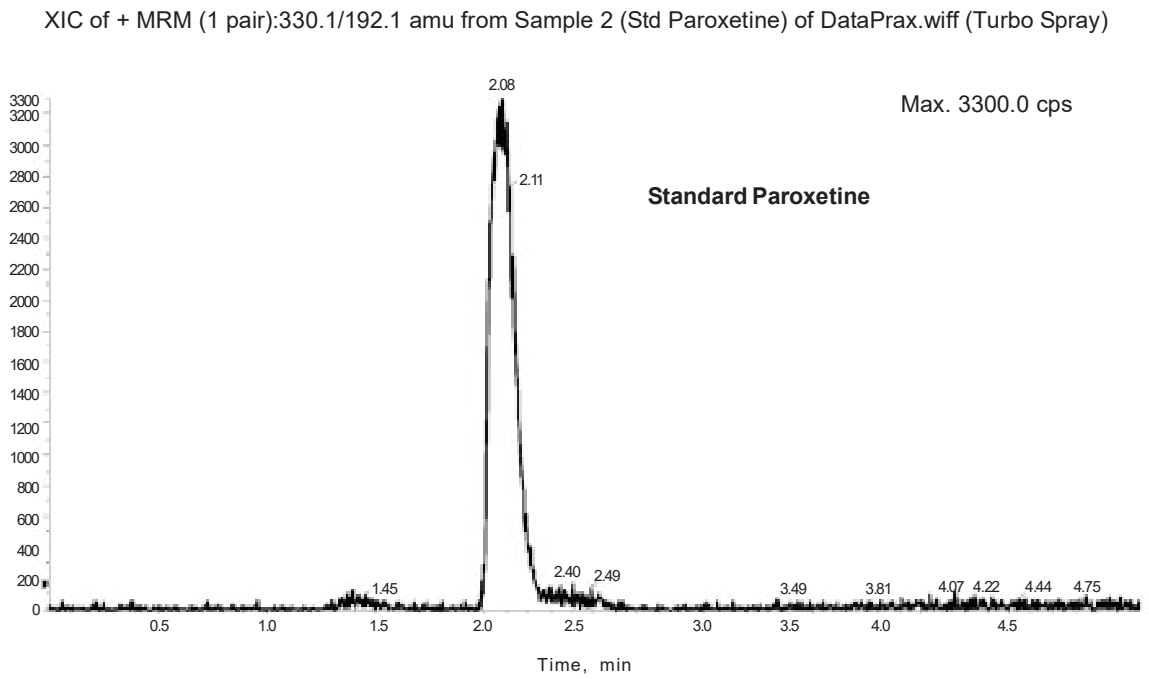

Fig. 1 Standard Paroxetine Multiple Reaction Monitoring (Conc.500ng/mL)

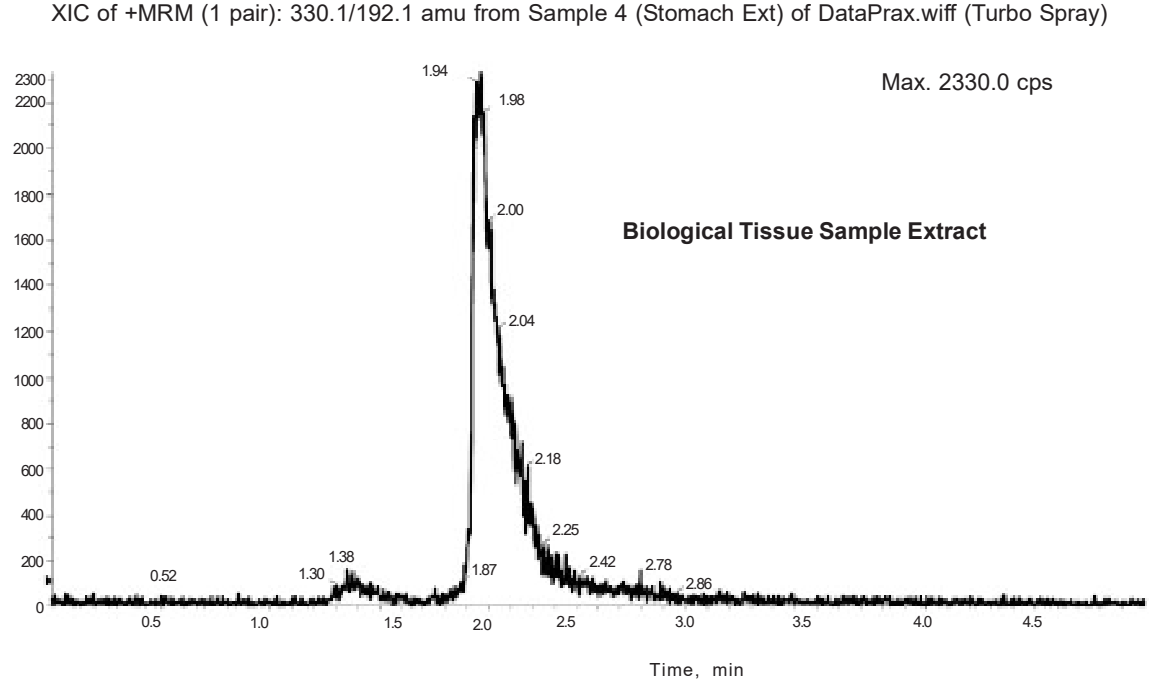

Fig. 2 Multiple Reaction Monitoring Chromatogram of Extracted Biological Sample

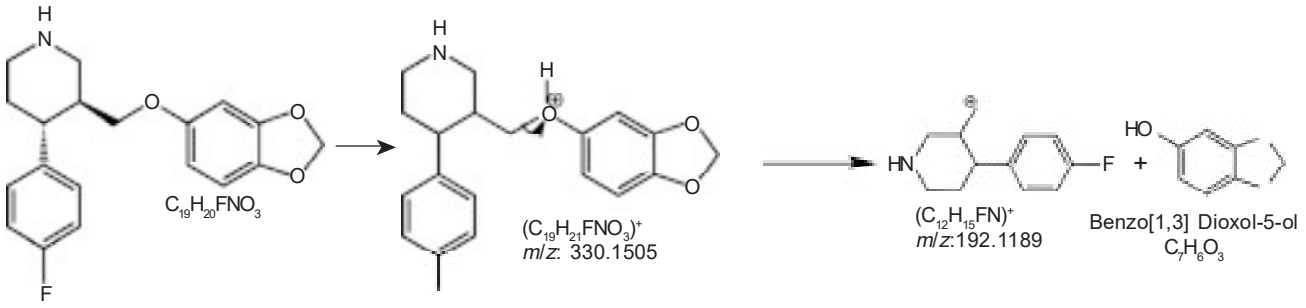

Fig. 3 Cleavage Pattern of Paroxetine Ion

Standard paroxetine shows the presence of protonated molecules (MH^+) of m/z 330.15. The cleavage of the paroxetine ion as shown in **Fig 3** produces an ion of $C_{12}H_{15}FN^+$ of m/z 192.11 and $C_7H_6O_3$ (Benzo [1,3,]dioxaol-5-ol). The intensity of $C_{12}H_{15}FN^+$ ion was found to be in the order of maximum stability, and thus considered to be best qualifier ion. By selecting 192.11 as a stable product ion, paroxetine could be identified in the biological samples through MRM.

Conclusion

The identification of paroxetine ion of m/z 330.15 in biological samples by Multiple Reaction Monitoring (MRM) with the qualifier product ion 192.11 provides a sensitive and specific method of identifying the drug in biological samples at relatively low concentrations (LOD-2.0 ng/ml). The modified mobile phase (without formic acid) proved to be effective, as it produced adequate number of qualifier ions without unwanted cluster ions, which otherwise could have caused ambiguity in the interpretation of results.

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