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Research Paper

Targeted mrm to identify molecular species of dimetridazole according to 2002/657/ec guidelines

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The current study was conducted to establish and validate a confirmatory method using ESI-LC-MS/MS for determination of dimetridazole in aqueous according to the European Commission Decision 2002/657/EC after optimization. the following parameters linearity, limit of quantification (LOQ) and range of linearity were studied and results obtained for each of them i.e. r2 > 0.99, LOQ 0.20ng ml_1 and range of linearity 0.20to 200 ng/ml were within acceptable values. This validated method was found to be satisfactorily linear and selective that can be used for confirmatory analysis of dimetridazole in any matrices.

Key words: dimetridazole; Liquid chromatography–tandem mass spectrometric detection; identification points.

INTRODUCTION

Nitroimidazoles are imidazole heterocycles with a nitrogen group incorporated in the structure. Examples of these compounds are dimetridazole (1,2-dimethyl-5-nitroimidazole, DMZ), metronidazole (1-(2-hydroxyethyl)-2-methyl-5- nitroimidazole, MNZ), ronidazole (1-methyl-2-[(carbamoyloxy) methyl]- 5-nitroimidazole, RNZ), ipronidazole (2-isopropyl-1-methyl-5-nitroimidazole, IPZ), carnidazole (1-(2-ethylcarbamothioic acid O-methyl ester)-2-methyl-5-nitroimidazole, CNZ), ornidazole (1- (3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole, ONZ) and ternidazole (2-Methyl-5-nitroimidazole-1-propanol; 3-(2-methyl- 5-nitroimidazol-1-yl)propan-1-ol, TRZ). These examples are known as 5-nitroimidazoles as they contain a NO_2 group on the 5th position on its ring which is seen in Fig. 1.

5-Nitroimidazoles are primarily used for the prophylactic and therapeutic treatments of diseases such as histominiasis and coocidiosis in poultry, hemorrhagic enteritis in pigs and genital tricchoniasis in cattle. It has been reported that RNZ, DMZ and MNZ show mutagenic, carcinogenic and toxic properties (2-4)

In order to ensure consumer's safety and to facilitate food trade, Community Reference Laboratory (5) has already set a minium required performance limit

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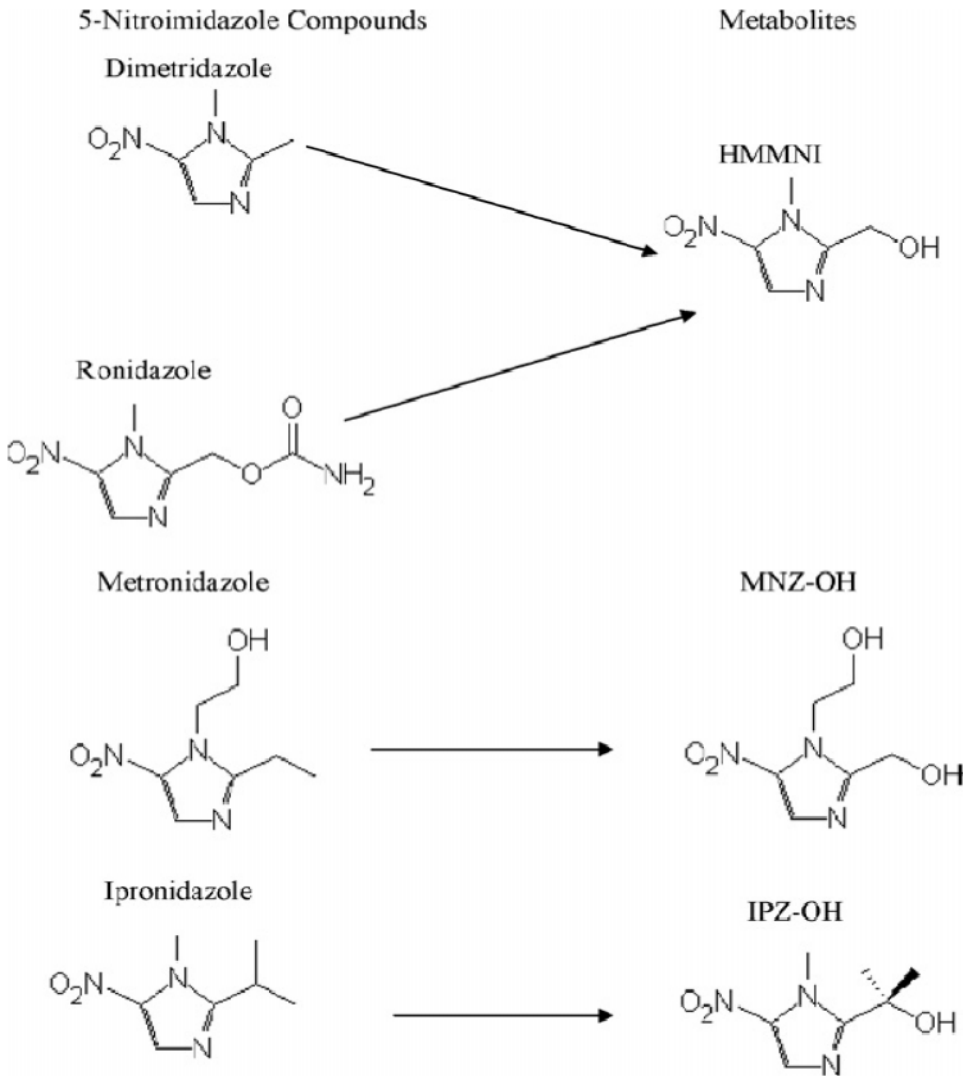

Fig.1: Chemical Structures of NMZs and its metabolites

(MRPL) of 3µg/Kg for nitroimidazoles (DMZ, MNZ and RNZ) in aquaculture products, milk, honey, poultry and pigs.

Furthermore, several bioassay methods are in place to detect nitroimidazole residues in different matrices such as Enzyme Liquid Immune Sorbent Assay (ELISA) (6) and Surface plasma resonance (SPR) based biosensors (7-8).These assays are either qualitative or semi quantitative in nature that could only be used as a screening method. On the face of it, most of these methods are for a class of drugs rather than a single specific drug and are also known for their false positive results.

2. Materials and methods

2.1 Chemicals and Reagents

Dimetridazole (Analytical grade standard) was obtained from Sigma Aldrich, (Fluka Analytical, USA (Batch: SZB8129XV)), acetronitrile and methanol were of HPLC ultragradient solvent from J.T Baker,

USA. Formic acid was obtained from Suprapur, Merck, Germany. De-ionized water was prepared using milli-Q water purification system from Millipore, France. Mobile phases used in this study were filtered through 0.22µm Nylon membrane filters from Millipore, Ireland, using vacuum filtration assembly and was sonicated using a bath type ultrasonicator before LC-MS/MS analysis.

2.2 Preparation of Standard and working drug solutions:

Analytical grade standard of dimetridazole obtained from Sigma Aldrich (Fluka Analytical, USA) with 99.82% purity, were used for this study. Stock solution of 1mg/ml was prepared in absolute acetonitrile. From this stock solution, intermediate stock solutions of 100, 10, and 1ppm were prepared using a diluent consisting of water and acetonitrile in 50:50, v/v. Using these stocks, nine working calibration standards solution of the following concentrations viz. 0.20, 0.50, 1.60, 8.00, 40.00, 80.00, 120.00, 160.00 and 200 ng/ml were prepared using the same diluents mentioned above. All these stock and working standard solutions were stored at 4 ºC in the refrigerator until use.

2.3 Chromatography

An Agilent 1200 series HPLC with C_{18} Phenomenex column (250×4.6mm, 5µm) was used as an analytical column for the separation process. A mixture of acetonitrile with 0.1% acetic acid in water and 0.1% acetic acid in acetonitrile was used as a gradient mobile phase for elution of dimetridazole. The flow rate of the mobile phase was maintained at $0.5 ml min^{-1}$.

2.4 Mass spectrometric detection

The tandem mass spectrometric detection was used to monitor the elution of dimetridazole. Turbo Ion Spray was employed as the ionization source and operated in positive mode. The Ion Spray voltage was set at 5500 V. The source temperature was set at 400°C. The nebulizer gas and heater gas was maintained at 55 and 50psi respectively. The curtain gas was set at 20 $L min^{-1}$and the CAD gas to medium. Declustering potential (DP) and entrance potential (EP) was set at 46 and 10 V respectively. Unit resolution was used for both Q1 and Q3. The collision energies were 23 and 33 eV. The collision exit potentials were 4.00 and 4.00V. The dwell time was set at 150 ms. m/z 142.1→96.0 was monitored for quantification transition and m/z 142.1→81.0 for confirmation transition as shown in Fig. 2 & 3. A typical chromatogram of LLOQ sample spiked with dimetridazole and aqueous blank are shown in Fig. 4. Analyst version 1.4.2 was

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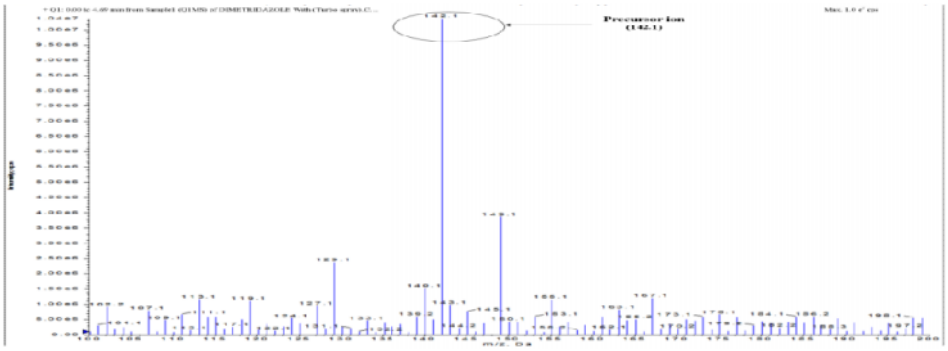

Fig.2: Parent ion of dimetridazole

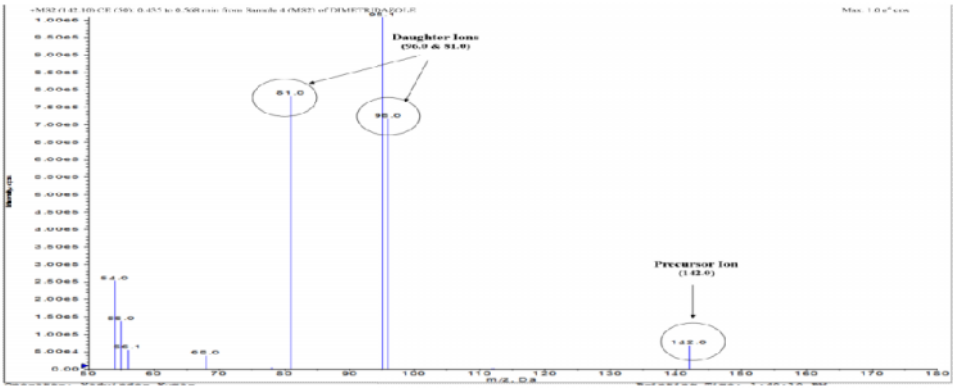

Fig.3: Daughter ions of dimetridazole

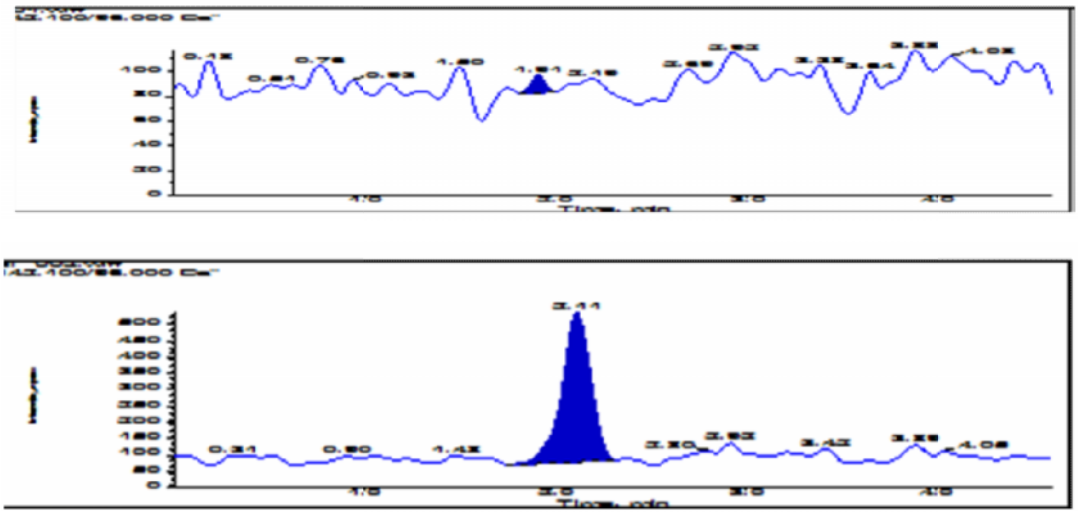

Fig. 4: Representing Blank and LLOQ in Aqueous

used for the integration of all samples in same batch. Calibration curves were established by the linear least squares fitting technique.

The proposed method was validated according to the directives from European Commission Decision 2002/657/EC (16).The following MS/MS parameters

Table 1: MRM parameters for MS/MS analysis

Compound	$[M+H]^+$	Quantification Transition (MRM1)	Confirmation Transition (MRM2)	DP (V)	EP (V)	Linearity range (ng/ml)
dimetridazole	142.1	142.1 → 96.0 (23)	142.1 → 81.0 (33)	46	10	0.2-200

Table 2: Identification point earned

LC-MS/MS technique	Identification point earned
1 Precursor	1
2 daughter ions (1.5×2)	3
Total	4

were assessed for confirmatory analysis of dimetridazole in aqueous viz. linearity, limit of quantification (LOQ) which are summarized in Tables 1 and 2 and are in compliance with EU recommendations.

4. Conclusions

An ESI-LC-MS/MS method to confirm the identity of dimetridazole in aqueous was developed according to the European Commission Decision 2002/657/EC (16). This method proved to be good with the chromatographic conditions, quantification transition, confirmation transition and relative ion intensities were in accordance with the Commission Decision 2002/657/EC. The use of two MS/MS transitions to identify the parent ion and two of its daughter ions makes this procedure an appropriate one in compliance with EU for determination and confirmation of the target dimetridazole residues in aqueous.

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