Research Article

Rapid Identification of Environmental Transformation Products of Dronedarone Using High Resolution Mass Spectrometry and Density Functional Theory

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ABSTRACT

Pharmaceuticals often prescribed in relatively high doses and a fraction of it has the potential to make its way into the environment where it can have a major impact on ecosystem. Although the human metabolites of these pharmaceuticals are well characterized, the identification and structural elucidation of metabolites formed in the environment presents a significant analytical challenge. In this present work the environmental transformation of a multichannel-blocking antiarrhythmic drug, dronedarone and its major transformation products have been studied. One of the predominant transformation products has been identified as *N*-Butyl-*N*-(3-(4-(2-butyl-5-(methylsulfonamido) benzofuran-3-carbonyl)phenoxy) propyl) butan-1-amine Oxide, using Liquid Chromatography High Resolution Mass Spectrometry and density functional theory calculations. Tandem mass spectrometry was exploited to confirm the proposed structure through the fragmentation patterns and neutral losses observed. Density functional theory with B3LYP functional and 6-31G* basis sets in Gaussian has been used to determine the most preferred oxidation site for this molecule.

Keywords: Dronedarone, Transformation products, HRMS, DFT

INTRODUCTION

Pharmaceuticals in the environment are now being discussed separately as unique environmental contaminants. Pharmaceuticals find their way to environment from production, from patient use and excretion or from improper disposal. Research into their fate and environmental toxic effects are currently gaining momentum as they may develop synergistic, antagonistic, or additive interactions causing potential health hazards.

The observed presence of pharmaceuticals in the environment can have potential adverse ecological effects and contamination of drinking water in particular need special attention.

With advancement of instrumentation and ever increasing sensitivity of analytical methods active pharmaceutical ingredients and their metabolites have been detected in aquatic environment in parts per billion to parts per trillion concentrations by various researchers in many countries, nevertheless the potential long-term effects of low concentrations of these substances and the potential combination effects need to be investigated further[1,2].

Pharmaceuticals, including analgesics, antibiotics, antiepilieptics, b-blockers, blood-lipid regulators and contraceptives and their transformation products resulting from structural change by fungi and bacteria in the environment and non-biotic agents such as oxidation, hydrolysis and photolysis in different environmental compartments including surface water, soil or sewage treatment also have the potential to cause adverse physiological changes in aquatic organisms in addition to being detrimental to human health.

The occurrence of numerous pharmaceuticals and personal care products has been reported in both ground and surface waters around the world[3–10].

The current study combines high resolution mass spectral analysis and density functional theory calculations to identify the key transformation products of antiarrhythmic agent dronedarone in environmental water.

Antiarrhythmic agents[11-13] are the drugs which are used to treat abnormal rhythms of the heart, such as atrial fibrillation (AF), atrial flutter, ventricular tachycardia, and ventricular fibrillation. AF the predominant dysrhythmia, affecting about 2-4 million people in the United States (US) and over 6 million Europeans[14]. Dronedarone, a new drug approved in both the USA and Europe in 2009, is a benzofuran derivative of amiodarone developed as an anti-dysrhythmic agent. It demonstrates anti-adrenergic and calcium antagonist properties, blocks sodium channels and potassium currents prolonging cardiac action potential and refractory periods.[15,16]. Chemically Dronedarone is *N*-(2-Butyl-3-(*p*-(3-(dibutylamino)propoxy)benzoyl)-5-benzofuranyl)methanesulfonamide and marketed as Multaq.

Liquid chromatography–tandem mass spectrometry has been evidently used to identify and quantify pharmaceutical compounds in environment[17,18].

Identification of transformation products can be best accomplished by acquiring high resolution mass spectral data and comparing the fragmentation pathway as structurally similar compounds may dissociate to give common fragmentation patterns, common product ions and/or common neutral losses. Therefore, the

identification of the product ions would necessarily help in identifying the unknown transformation products. Density functional theory (DFT) has been quite successful in predicting the product ions formed during collision active dissociation[19,20]. In this study authors have utilized DFT not only for the rationalization of product ions but also to predict the most favourable site of oxidation of DDR by comparing their relative energies.

This study was designed primarily for the development of a high resolution mass spectroscopy based method in combination with DFT calculations to be used in a larger and more systematic investigation of the transformation product of pharmaceuticals.

Experimental

MATERIALS AND REAGENTS

HPLC grade acetonitrile and methanol were purchased from Merck India limited (Mumbai, India). Ultrapure water (18.2 MΩ) was prepared using a Milli-Q plus water purification system from Millipore (Bedford, MA, USA). Oasis cartridges was obtained from waters (Bangalore, India).

Formic acid was obtained from Sigma-Aldrich Corporation (Bangalore, India). Generic tablets of 400 mg Dronedarone were purchased from local chemist shops in Bhubaneswar.

Analytical reagent grade ammonium acetate, were obtained from Qualigens India Limited (Mumbai, India).

Liquid Chromatography

All compound solutions were introduced into the ESI MS (electrospray ionisation mass spectrometry) source by high performance liquid chromatography (HPLC), Dionex ultimate 3000 (Thermo scientific, USA), using a hypersil BDS C18 column (150×4.6 mm, 5 µm, Thermo scientific, USA).

A mobile phase consisting of A, 10 Mm ammonium acetate adjusted to pH 3.2 ± 0.05 with formic acid and B, acetonitrile in gradient mode; T(min)/%B: 0/20, 10/35, 15/60, 20/60, 25/25, 30/70, 40/75, 45/20, 50/20. Column temperature was maintained at 35 °C and the flow rate was 1.0 mL/min. The samples were injected (10 µL) into the HPLC system in acetonitrile.

High-Resolution Mass Spectrometry

The MS and MS/MS studies were performed on Thermofisher Q-exactive mass spectrometer (Thermo Electron, Bremen, Germany) using electrospray ionization source and orbitrap mass analyzer.

Heated electrospray ionization source was used for ionization.

The temperature of the heater was kept at 450 °C and capillary of the ESI interface at 250 °C. Nitrogen was used both as sheath gas and auxiliary gas.

The electro spray and tube lens were set at 4.5 kv and 90 V respectively. The mass spectrometer was operated in full scan MS with data dependent MS2 mode in positive polarity.

The selected range was from 100 to 1000 m/z and the resolution was 70,000 full width half maximum (FWHM) with an isolation window applied, followed by a data dependent scan at a resolution of 17,500 FWHM with the fragmentation energy applied. The target capacity of the C-trap was defined at 1×10^6 charges and the maximum injection time was limited to 50 ms.

All the spectra were recorded under identical experimental conditions and average of 20–25 scans was performed. The data acquisition was under the control of xcalibur software.

Density Functional Theory

Gas-phase basicity, 3D structure and bond length calculations were performed using DFT, calculations at the B3LYP level using the 6-31G* basis set, with Gaussian09. The optimised geometry for the neutral molecule was calculated, basic sites were then protonated and the relating minimum energy geometry calculated for each possible structure. The energy differences between the most favourable cation (highest negative energy value in Hartrees) and all others were converted from Hartrees into kcal/mol using the conversion factor of 627.503.

Sample Preparation and Transformation Experiment

A 400 mg Dronedarone tablet was dissolved in 100 mL of water obtained from a farm pond near the city of Bhubaneswar, India. The sample was kept in a clear glass vial and capped.

The solution was exposed to natural sun light at ambient conditions over 120 days. The solution was decanted and passed through oasis cartridge with acetonitrile as eluent to remove sample matrix components before being subjected to mass spectral analysis.

RESULTS AND DISCUSSION

The chromatographic method was designed for the separation of DDR from its transformation products and fulvlic and humic materials present in natural water. The unknown peak observed at retention time of 23.43 min. was designated as TP and well separated from the principal peak of DDR and the orbitrap mass spectrometer yielded an exact mass of 573.3027 Th for its protonated molecular ion.

However, prior to identification of transformation product the product ion spectra of DDR were rationalized for understanding of its fragmentation pathway.

The exact mass of DDR was observed at m/z 557.3069 Da and produced major product ions at m/z 501.2447 Da, 435.2673 Da, 294.0806 Da, 170.1915 Da, 142.1600 Da, 114.1287 Da and 100.1131 Da as shown in Figure 1a.

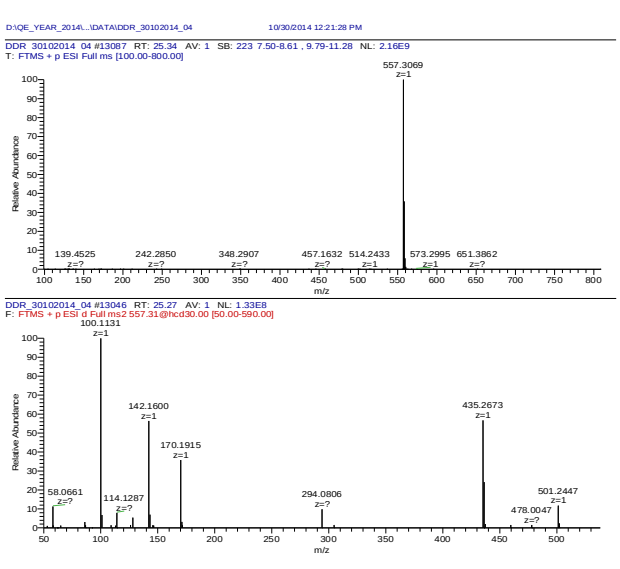

Figure 1a: Full scan and CID spectra of DDR

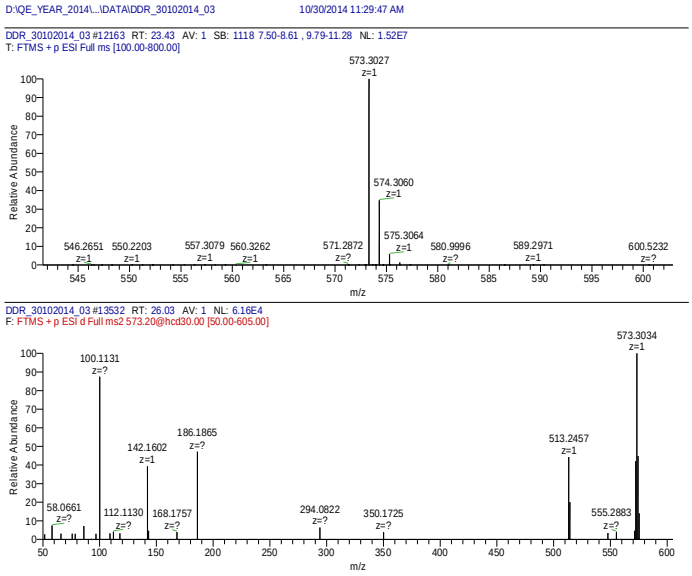

Figure 1b: Full scan and CID spectra of TP

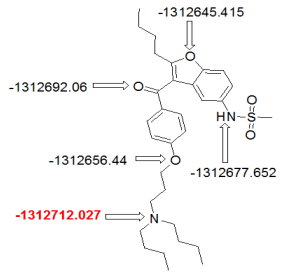

Figure 2: The energies of dronedarone molecule at different potential protonation sites in kcal mol^{-1} units.

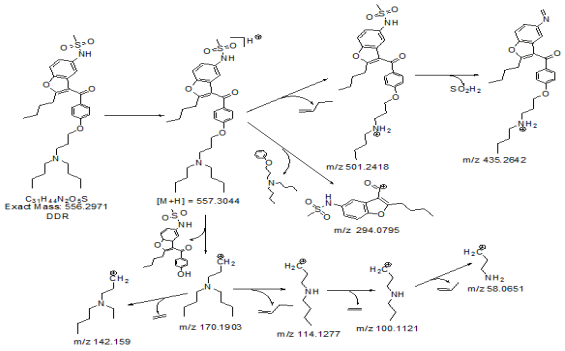

Figure 3a: Plausible fragmentation pathway of Dronedarone

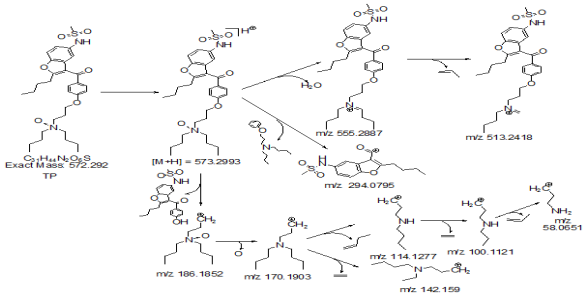

Figure 3b: Plausible fragmentation pathway of the transformation product of Dronedarone

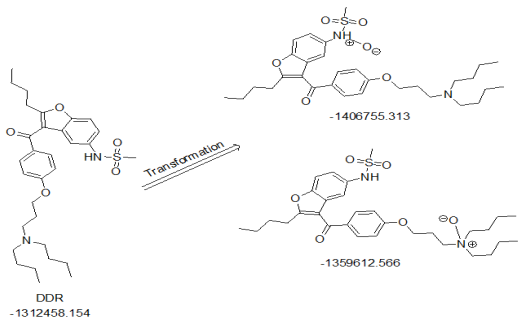

Figure 4: The relative of energies of DDR and two possible transformation products obtained through DFT calculation in kcal mol^{-1} units.

Loss of 1-butene (C4H8, 56.0626 Da) led to the formation of fragment ion at m/z 501.2447 Da which subsequently lost a molecule of Hydrosulphurous acid(H_2SO_2, 69.9775 Da) and generated a daughter ion at m/z 435.2673 Da. Cleavage of C-N bond is also supported by DFT calculation which indicates the tertiary nitrogen atom to be the preferred site of protonation (Figure 2) and elongation of bond as a result of protonation often induces fragmentation[20].

The product ions formed at m/z 294.0806 Da due to the neutral loss of N-butyl-N-(3-phenoxypropyl)butan-1-amine.

The details of the fragmentation pathway depicting the formation of product ions of DDR are shown in Figure 3a.

The TP with protonated exact mass of 573.3027 Th is also analyzed in the same fashion as that of DDR. The mass difference of 15.9958 Da between the protonated molecular ions of DDR and TP indicated it to be an oxidized transformation product of DDR and the most plausible structure was proposed as the *N*-oxide of DDR.

Product ions obtained through a data dependent mass spectral analysis were investigated and CID spectra as shown in Figure 1b showed key fragment ions at m/z 555.2883 Da, 513.2457 Da, 350.1725 Da, 294.0822 Da, 186.1865 Da, 142.1602 Da and 100.1131 Da. The neutral loss of water (H_2O, 18.0106 Da) followed by loss of propene (C_3H_6, 42.047 Da) produced the base peak at m/z 513.2457 Da.

The product ion at m/z 186.1865 Da formed due to cleavage of C-O bond with a neutral loss of *N*-(2-butyl-3-(4-hydroxybenzoyl)-3a, 7a-dihydrobenzofuran-5-yl) methanesulfonamide ($C_{20}H_{23}NO_5S$, 389.1297 Da) was consistent with the product ion of DDR at 170.1915 Da differing by the mass of one oxygen atom (15.995 Da) and served as diagnostic fragment to the proposed *N*-oxide structure.

The comparative fragmentation profile of TP is depicted in Figure 3b.

DFT calculations also supported the predicted *N*-oxide structure of TP and indicated it to be more favourable energetically by -34.375 kcal mol^{-1}.

The result of DFT calculation is graphically illustrated in Figure 4.

CONCLUSION

The high resolution mass spectral data, CID spectra and DFT calculations have been successfully carried out on DDR and its major transformation product in natural pond water eco system has been identified as *N*-Butyl-*N*-(3-(4-(2-butyl-5-(methylsulfonamido) benzofuran-3-carbonyl)phenoxy) propyl) butan-1-amine Oxide.

The incorporation of DFT generated complimentary structural information for the unequivocal identification of TP along with high resolution CID spectra.

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REFERENCES

1. Cunningham V L, Binks S P, Olson M. J, Human Health Risk Assessment from the Presence of Human Pharmaceuticals in the Aquatic Environment, *Regul. Toxicol. Pharmacol.*, 53, 2009, 39-45.
2. Lin A.Y.C, Yu T.H, Lin C.F, Pharmaceutical Contamination in Residential, Industrial and Agricultural Waste Streams: Risk to Aqueous Environments in Taiwan, *Chemosphere*, 74, 2008. 131-141.
3. Petrovic M, Eljarrat E, Lopez de Alda M.J, Barcelo D, Endocrine Disrupting Compounds and Other Emerging Contaminants in the Environment: A Survey on New Monitoring Strategies and Occurrence Data, *Anal. Bioanal. Chem*. 378, 2004, 549-562.
4. Bendz D, Paxeus N.A, Ginn T.R, Loge F.J, Occurrence and Fate of Pharmaceutically Active Compounds in the Environment, A Case Study: Hje River in Sweden, *J. Hazard*. Mater, 22, 2005, 195-204.
5. Bester K, Huffmeyer, Schaub E, Klasmeier J, Surface Water Concentrations of the Fragrance Compound OTNE in Germany-A Comparison between Data from Measurements and Models, *Chemosphere*, 73, 2008, 1366-1372.
6. Kasprzyk-Hordern B, Dinsdale R.M, Guwy A.J, The Removal of Pharmaceuticals, Personal Care Products, Endocrine Disruptors and Illicit Drugs during Wastewater Treatment and Its Impact on the Quality of Receiving Water, *Water Res*, 43, 2009, 363-380.
7. Kuster M, Analysis and Occurrence of Pharmaceuticals, Estrogens, Progestogens and Polar Pesticide in Sewage Treatment Plant Effluents, River Water and Drinking Water in the Llobregat River Basin, *J. Hydrol*, 358, 2008, 112-123.
8. Bartelt-Hunt S.L, The Occurrence of Illicit and Therapeutic Pharmaceuticals in Wastewater Effluent and Surface Waters In Nebraska, *Environ. Pollut*, 157, 2009, 786-791.
9. Zhang X, Synthetic Musks in the Aquatic Environment and Personal Care Products in Shanghai, China, *Chemosphere*, 72, 2008, 1553-1558.
10. Zhou J.L, Pharmaceuticals Residues in Wastewater Treatment Works Effluents and Their Impact on Receiving River Water, *J. Hazard. Mater*., In press, 166, 2009, 655-661.
11. Vaughan Williams, E. M. Symp. *Card. Arrhythmias,* 1970, 449–472.
12. Bigger J. T., Jr. *Am. J. Cardiol.* 5, 1984, 8B–16B.
13. Peter M. D.; Kowey R, *Arch. Intern. Med*. 158, 1998, 325–332.
14. European Heart Rhythm A, European Association for Cardio-Thoracic S, Camm A J, Guidelines for the management of atrial fibrillation: the Task Force for the

Management of Atrial Fibrillation of the European Society of Cardiology (ESC). *Eur Heart J*, 31, 2010, 2369–2429.

15. Hodeige D, Heyndrickx JP, Chatelain P, Manning A, a new amiodarone like antiarrhythmic agent: anti-adrenoceptor activity in anaesthetized and conscious dogs. *Eur J Pharmacol*, 279, 1995, 25–32.
16. Sun W, Sarma JS, Singh BN, Electrophysiological effects of dronedarone (SR33589), a noniodinated benzofuran derivative, in the rabbit heart: comparison with amiodarone. Circulation, 100, 1999, 2276–2281.
17. Kosjek T, Heath E, Petrovic M, Barceló D, Mass spectrometry for identifying pharmaceutical biotransformation products in the environment. *Trends Anal. Chem*. 26, 2007, 1076–1085.
18. Radjenovic J, Petrovic M, Barceló D, Advanced mass spectrometric methods applied to the study of fate and removal of pharmaceuticals in wastewater treatment. *Trends Anal. Chem*. 26, 2007, 1132–1144.
19. Bandu M.L, Watkins K R, Bretthauer M.L, Moore C.A, Desaire H, Prediction of MS/MS data. A focus on pharmaceuticals containing carboxylic acids. *Anal. Chem.*, 76, 2004, 1746-1753.
20. Wright P, Alex A, Pullen F. S., Predicting collision-induced dissociation spectra: Semi-empirical calculations as a rapid and effective tool in software-aided mass spectral interpretation. *Rapid Commun. Mass Spectrum*, 28, 2014, 1127–1143.

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