Journal of Pharmaceutical Analysis 2012;2(6):443–449

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

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ORIGINAL ARTICLE

Cathodic adsorptive stripping voltammetry of an anti-emetic agent Granisetron in pharmaceutical formulation and biological matrix

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Received 27 November 2011; accepted 10 March 2012
Available online 18 March 2012

KEYWORDS

Granisetron;
Human plasma;
Solid-phase extraction;
Pharmaceutical formulation;
Voltammetry;
Hanging mercury drop electrode

Abstract Granisetron showed one well-defined reduction peak at Hanging Mercury Drop Electrode (HMDE) in the potential range from −1.3 to −1.5 V due to reduction of C=N bond. Solid-phase extraction technique was employed for extraction of Granisetron from spiked human plasma. Granisetron showed peak current enhancement of 4.45% at square-wave voltammetry and 5.33% at cyclic voltammetry as compared with the non stripping techniques. The proposed voltammetric method allowed quantification of Granisetron in pharmaceutical formulation over the target concentration range of 50–200 ng/mL with detection limit 13.63 ng/mL, whereas in human plasma 50–225 ng/mL with detection limit 11.75 ng/mL.

1. Introduction

Nausea and vomiting are two of the most severe side effects of cytotoxic chemotherapy or radiotherapy and prolonged vomiting may also induce severe complications [1]. Granisetron (endo-1-methyl-*N*-[9-methyl-9-azabicyclo (3.3.1) non-3-yl]-1H-indazole-3-carboxamide) (Fig. 1) is a highly selective 5-hydroxytryptamine 3 (5-HT3) receptor antagonist, and is considered to be a potent anti-emetic agent in the control of chemotherapy-induced nausea and vomiting. Its main effect is to reduce the activity of the vagus nerve, which is a nerve that activates the vomiting center in medulla oblongata [2]. Granisetron is a well-tolerated drug, and breaks down slowly, staying in the body for a long time. It is broken down by the liver's cytochrome P450 system and removed from the body by the liver and kidneys. Its metabolism involves N-demethylation and aromatic ring oxidation followed by conjugation.

However, higher dose and prolonged exposure of drug may cause some mild and long-term side effects in a subset of patients. Therefore, it is necessary to establish fast and sensitive analytical

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

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10.1016/j.jpha.2012.03.005

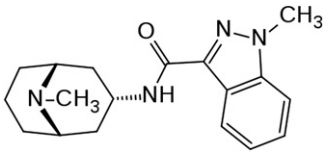

Figure 1 Chemical structure of Granisetron.

method for detection and quantification of Granisetron in biological matrix for clinical, toxicological and pharmacological studies. The widespread use of this compound also requires analytical method to assay the drug in pharmaceutical formulations and doses form. Several techniques have been explored for determination of Granisetron in pharmaceutical formulation and biological fluids such as high-performance liquid chromatography [3–6], potentiometric liquid membrane sensor [7], high performance thin layer chromatography [8] and liquid chromatography tandem mass spectrometry [9–11]. Although spectrophotometry and chromatography are the most commonly employed techniques, but the demand of expensive and sophisticated instrumentation, highly skilled personnel and time consuming extraction purification approaches prior to final analysis restrict their use in routine analysis. Since the last decade, electroanalytical techniques have been widely used in the field of pharmaceutical analysis [12–18]. These techniques have been found more selective, inexpensive, time saving and do not require time consuming purification steps after extraction, when compared with other analytical techniques [3–11]. However, no electroanalytical methods have yet been reported for the quantification of Granisetron in pharmaceutical formulation and human plasma. This work describes an analytical method with acceptable analytical characteristic of suitability and reliability for detection and quantification of Granisetron in pharmaceutical formulation and human plasma.

2. Experimental

2.1. Reagents and chemicals

Granisetron hydrochloride standard (99.20%) was kindly provided by Veeda Clinical Research Pvt. Ltd., Ahmedabad (India). Tablets containing Granisetron hydrochloride (*Graniforce*®, 1 mg/Tab) manufactured by Discovery Mankind were obtained from commercial source. Ultra pure water was obtained from Milli-Q purification system (Millipore Corp., Milford, MA, USA) and used throughout the studies. Phosphate buffer in the pH range 2.0–12.0 was prepared in ultra pure water and used without filtration. All chemicals used were of analytical reagent grade and employed without further purification. Drug free human blood plasma lots anti-coagulated with sodium heparin were procured from Radha Swami blood bank, Gwalior, India. These plasma lots were preserved at −20 °C in freezer and used after gentle thawing at room temperature.

2.2. Instrumentation and electroanalytical methods

Electrochemical measurements were performed using a μ Autolab Type III potentiostat-galvanostat (Eco-Chemie B.V., Utrecht, The Netherlands) with 757 VA computrace software. The electrodes utilized in the study were Hanging Mercury Drop Electrode (HMDE) as a working electrode, platinum wire as an auxiliary electrode and Ag/AgCl (3 M KCl) as a reference electrode. All pH measurements were performed on Decible DB-1011 digital pH meter fitted with a glass electrode as a working and saturated calomel electrode as a reference, which was previously standardized with buffer solutions of known pH. The electrochemical behavior of Granisetron hydrochloride was studied using square-wave cathodic adsorptive stripping voltammetry (SWCAdSV) and cyclic voltammetry. The voltammetric experiments were carried out at room temperature using 0.2 M phosphate buffer as supporting electrolyte. For electrochemical measurement a volume of phosphate buffer 9.9 mL and 0.1 mL analyte solution was added to the electrochemical cell and purged with pure nitrogen for 25 s. The required accumulation potential (E_{acc}) was applied to the working electrode for a selected accumulation time (t_{acc}), while the solution was stirred continuously at 2200 rpm. The stirring was stopped and after equilibrium time of 10 s, a negative-going potential scan was initiated using the parameters those are reported in Section 3.4.

2.3. Preparation of standard and test solutions

Standard stock solution of Granisetron hydrochloride (500 μg/mL) was prepared in methanol and further diluted with phosphate buffer to achieve a final concentration (100 ng/mL) in the working range. Ten tablets of Granisetron (*Graniforce*®, 1 mg/Tab) were ground to fine powder and mixed well. Sufficient amount of powder equivalent to 5 mg of Granisetron was taken for preparation of a stock solution (500 μg/mL) into 10 mL volumetric flask and the volume was made up to mark with methanol. This solution was vortexed and sonicated for 5 min to dissolve all contents of tablets properly. Clear supernatant liquid was withdrawn and diluted with phosphate buffer for preparation of test solutions in the calibration range. Stock and test solutions were stored at 2–8 °C in refrigerator until analysis.

2.4. Preparation of plasma calibration and quality control samples

Standard stock solution of Granisetron (1 mg/mL) was prepared by dissolving pure compound in methanol. Intermediate solutions were prepared from stock solution using a mixture of methanol/water (50:50, v/v) as a diluent. Working solutions were prepared from intermediate solutions using the same diluent, at proposed concentration levels of 0.50–2.25 μg/mL. Plasma calibration and quality control (QC) samples were prepared by 10% spiking of respective working solutions in blank plasma. The final methanol content of all calibrators and quality control samples was less than 5%.

2.5. Extraction techniques

Solid-phase extraction (SPE) technique was used to extract Granisetron from spiked human plasma samples. A volume of 0.5 mL each of calibrator and quality control samples was transferred to 1.5 mL pre-labeled rivals. Then 0.2 mL phosphate buffer (pH 2.5) was added to each sample, vortexed for 1 min and centrifuged at 4800 rpm for 5 min. SPE was performed using oasis mixed mode cation-exchanger (*MCX*) cartridges (30 mg/cc), a vacuum manifold device and a vacuum source. SPE cartridges were conditioned with 1 mL of methanol and equilibrated with 1 mL of phosphate buffer (pH 7.0).