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Novel spectrofluorimetric assessment of ondansetron hydrochloride based on excited state quenching of pararosaniline fluorophore

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ABSTRACT

In this work, pararosaniline hydrochloride (PA) was developed as the first fluorescent probe used for the spectrofluorimetric determination of ondansetron (OND). The analytical method is simple, rapid, sensitive and validated for studying the excited state interactions and measuring the quenching effect of OND on the fluorescence intensity of PA in dioxane. Under the optimized conditions, fluorescence quenching value ($\Delta F = F_{PA} - F_{PA\text{-}OND}$) showed a linear relation with OND concentration in the two ranges of 2.0×10^{-7} to 1.0×10^{-6} mol L^{-1} and 4.0×10^{-6} to 1.0×10^{-5} mol L^{-1}. The detection limit (DL) in the lower range is 2.0×10^{-8} mol L^{-1} and quantification limit (QL) is 6.05×10^{-8} mol L^{-1}. The % recovery for intra-day and inter-day assay ranged from 96.66 – 102.1 with percent relative standard deviation (%RSD) ranged from 0.0272 to 1.474 %, thus the developed method is accurate and precise in determining OND. Moreover, the developed method was successfully applied for determining OND in human urine samples and in pharmaceutical formulation with % recovery and %RSD values of 99.74 and 1.401 %, respectively.

1. Introduction

Ondansetron (OND) is one of an antiemetic drug. Chemically it is a 1,2,3,9-Tetrahydro-9-methyl-3-(2-methylimidazol-1-ylmethyl)-carbazol-4(9H)-onemonohydrochloride (Scheme 1). Nausea and vomiting that resulted from radiation therapy, cancer chemotherapy, surgery and during pregnancy may be eliminated by using OND [1]. It also used as an effective treatment against the irritable bowel syndrome, ameliorate pruritus, diarrhea associated with cryptosporidiosis or diabetes, anxiety and sleep disorders, chronic refractory diarrhea, vertigo, alcohol dependency, opiate withdrawal syndrome, Tourette's syndrome, psychosis in advanced Parkinson's disease and in gastroenteritis [2–4].

Maximum concentration of OND in plasma is reached after about 1.5 h from an oral dose of 8 mg and about 6 hours after a rectal dose. The absolute bioavailability of OND from dosage form is about 60%, mainly because of hepatic first-pass metabolism. The elderly subjects had remarkably higher bioavailability (65%) and clearance lower, may be due to reduced hepatic first-pass metabolism. The terminal elimination half-life is about 3 hours after oral or parenteral doses, and about 6 h after rectal use. The most frequently reported side effects of OND are itchiness, headache, diarrhea, sleepiness, constipation, hiccups and a sensation of flushing or warmth. Moreover, OND causes serious side effects include QT prolongation and severe allergic reaction [1].

Generally, there is a necessity to follow the drug level in different formulations to study the drug active metabolites, pharmacokinetics, toxicity, side effects and mechanistic pathway [5,6]. Therefore, during the continuous clinical development of OND for the prevention of nausea and vomiting, it is important to found fast, facile, accurate, precise and reliable analytical method of high selectivity and sensitivity to evaluate the quality of its dosage forms or to measure drug concentration in biological fluids.

Pararosaniline hydrochloride (PA) is a cationic triphenylmethane dye with molecular formula of $C_{19}H_{17}N_3HCl$ (Scheme 1). This dye is inflammable in nature and possesses anesthetic, bactericidal (gram positive), and fungicidal properties. It is used as a biological stain in order to detect acid-fast bacilli and useful in understanding microfissures. It is widely used as a coloring agent for textiles, paper, plastics, glass, leather, staining of collagen, muscle, mitochondria, and tubercle bacillus [7].

Various techniques have been utilized for the quantitative determination of OND in biological samples or pharmaceutical preparations such as capillary electrophoresis [8], spectrophotometry [9], voltammetry [10], HPLC [11–15], hyphenated LC–MS/MS [16] and HPTLC [17]. Spectrofluorimetric technique gains its importance not only because its novelty for determination of OND but also due to its sensitivity, short response time, convenience and

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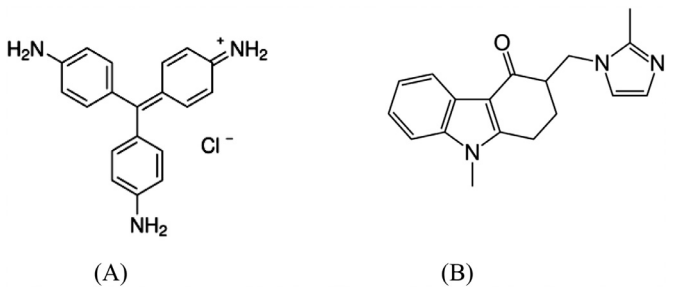

Scheme 1. Molecular structure of (A) pararosaniline hydrochloride (PA) and (B) ondansetron (OND)

simplicity of performance. Furthermore, fluorescence-based analysis could be employed in probing the structure and distribution of biomolecules. Organic fluorophores such as dye stuff are widely used as optical sensors in quantization of many organic and inorganic species [18–21].

In this study, we present a novel spectrofluorimetric method for the determination of OND in bulk, pharmaceutical formulations and in human urine samples. The technique is based on a fluorescence quenching of (PA) via a selective non-radiative charge transfer from PA fluorophore to (OND) as a result of static quenching. The method validation, precision and accuracy has been evaluated by intraday and interday recovery experiments and compared to other reported methodology.

2. Experimental

2.1. Materials and reagents

All chemicals were of analytical grade and were used as received. Ondansetron HCl dihydrate (OND) was provided from T3A Pharma Group (Egypt). Pararosaniline hydrochloride (PA) was purchased from Sigma. Methanol, Ethanol, Acetonitrile, 1,4-dioxane, Chloroform, dichloromethane, Dimethylformamide, Sodium hydroxide and hydrochloric acid were obtained from Scharlau, Spain. Zemitron® tablets (Advanced Pharmaceutical Industries Co. Ltd. Abdullah II Bin Al-Hussein Industrial Estate Street H, Sahab-Jordan), labeled to contain 4 mg of ondansetron per tablet, was kindly provided by Prince Mutaib Bin Abd Ulaziz Hospital, Sakaka, Aljouf, Saudi Arabia.

2.2. Instruments

UV-vis. absorption spectra were recorded using Agilent spectrophotometer. A Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, America) equipped with a 1 cm quartz cell was used for all measurements at 10 nm slit widths for excitation and emission wavelength**.** pH measurements were measured using HI 2211 pH/ORP meter, Hanna Instruments.

2.3. Methods

2.3.1. Preparation of stock solutions

A stock solution of OND was prepared by dissolving appropriate amount of OND in a mixture of methanol and acetonitrile, and then was completed to 25 mL with 1,4-dioxane. An accurate weighed quantity of PA was transferred into a 50 mL measuring flask, dissolved in 5 mL methanol and then diluted to the volume with 1,4-dioxane. To obtained a specific concentration of the working solutions, the standard solutions were diluted to a fixed volume with 1,4-dioxane.

2.3.2. Effect of OND on the fluorescence intensity of PA: Developing a calibration curve

A 0.1 mL of 1.0×10^{-3} mol L^{-1} PA was added to a 5 mL measuring flask, then being completed to the mark with 1,4-dioxane and the contents were shaking well before monitoring the fluorescence intensity at λ_{ex} 368 nm where an emission band assigned at $\lambda_{em} = 486$ nm. To develop a calibration curve, the influence of different concentrations of OND on the fluorescence intensity of a constant concentration of PA (2.0×10^{-5} mol L^{-1}) was investigated. The working concentrations of OND were in the range from zero to 2.0×10^{-5} mol L^{-1}.

2.3.3. Determination of quantum yield

Fluorescence quantum yield (Φ) illustrates the efficacy of getting emitted photons with respect to absorbed photons [22]. Estimating quantum yield could be helpful in addressing radiationless transitions, excited electronic states, sample purity and its chemical structure. Fluorophore quantum yield could be relatively determined via a comparison to a standard of known quantum yield under similar working conditions. To determine the relative quantum yield of our systems, we take quinine sulfate as standard and apply in the following equation [23]:

$$\emptyset = \frac{\emptyset_s F_u \ (OD_s) n_u^2}{F_s (OD_u) n_s^2} \quad (1)$$

Where Φ_s is the quantum yield of quinine sulfate reference of value 0.55 in 0.05 mol L^{-1} H_2SO_4, F_u and F_s are fluorescence integrals of unknown and standard, respectively. OD_s, OD_u, n_s and n_u are the optical densities and refractive index of standard and unknown, respectively.

2.3.4. Determination of OND in pharmaceutical formulation and in human urine samples

Five tablets of Zemitron® pharmaceutical product were ground to a fine powder. A quantity of the powder equivalent to 5 mg of OND was putted into 20 mL measuring flask, 5 mL of each methanol and acetonitrile was added to the flask, shake it vigorously for several minutes using vortex mixer, then the flask was placed in an ultrasonic bath for 10 min, and then made up to the volume with 1,4-dioxane. The insoluble matter in solution was removed by filtration. An appropriate concentration for measurements was obtained by diluted the prepared solution with 1,4-dioxane.

On the other hand, fresh human urine samples from healthy volunteers were spiked with convenient amounts of OND stock solutions. The spiked urine completed to 5 mL with 1,4-dioxane. where the tested final OND concentrations were 4.0 and 10 µM.

3. Results and discussion

3.1. UV-vis. spectroscopic characterization

Fig. 1 shows the absorption spectra of OND, PA and PA/OND in dioxane solutions. The absorption spectrum of OND shows absorption band at 298 nm due to π-π^* transition. For PA, a strong shoulder appeared on the blue side. This could be ascribed to desymmetrization that gives rise to two isomeric excited states being in equilibrium [24]. The reduced symmetry could be inferred to a dipole or a counter anion interacting with the amino group and thus split both the ground and the excited states of PA. If the isomer in the ground state reduces the symmetry, it will lead to a splitting of the degenerated excited state [25]. Addition of OND to PA has no much effect on the assigned shoulder, otherwise refining of the absorption profile around 500 nm and 560 nm as well as a dramatic increase in absorbance at 298 nm is noticed.

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