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ORIGINAL ARTICLE

Determination of Ondansetron by Spectrofluorimetry: Application to Forced Degradation Study, Pharmaceuticals and Human Plasma

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Abstract

The current manuscript describes a validated, responsive and rapid spectrofluorimetric method for quantifying ondansetron (OND) in authentic form, spiked human plasma and dosage forms. This is the first reported fluorescence study of Ondansetron in Triton X 100 system. Various variables affecting fluorescence response were studied precisely and optimised. The described method involved the fluorescence measurement in Triton X 100 system at $\lambda_{em}/\lambda_{ex}$ 354/317 nm. The calibration plot attained linearity over concentration range of 0.2 – 2 μg/mL. The developed method has been extensively applied to degradation studies of OND as per International Conference on Harmonisation (ICH) guidelines by exposing to oxidative, thermal, photo, acidic and alkaline conditions and also the degradation pathway has been proposed.

Keywords Ondansetron · Spectrofluorimetry · Pharmaceutical dosage forms · Spiked human plasma · Stability studies

Introduction

Ondansetron (OND) Fig. 1 is an anti-emetic drug that is employed in chemotherapy and radio therapy [1]. It is selectively a $5HT_3$ receptor antagonist. Ondansetron shows its onset of action by reducing the vagus nerve activity in turn affecting the both peripheral and central nerves thereby deactivating the serotonin receptors in chemoreceptor trigger zone. It is also employed for treating vomiting in post-surgery [2]. It is officially included in Indian Pharmacopeia, British Pharmacopeia and United States pharmacopeia [3–5]. It is one among the essential medicines proposed by World Health Organization needed in basic health system. Due to its importance therapeutically many analytical and bio analytical methods have been reported for quantification of OND. Various methods include UV Spectrophotometric methods [6–8], High performance Thin Layer chromatography (HPTLC) [9, 10], Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method for tablets and injection, in combination with rabeprazole, for human plasma [11–16]. For rat plasma a Liquid Chromatography and Liquid Chromatography – Mass Spectrometry (LC-MS/MS) method [17–19]. Spectrofluorimetric techniques are extensively applied in analysis of pharmaceuticals due its rapidity, selectivity and responsiveness [20–24]. Stability study is one of the significant aspects in early drug development process. Stability indicating assay method has to be developed as per International Conference on Harmonisation (ICH) Q1A R2 guidelines in order to explain the essential stability of the drug substances by application of accelerated degradation conditions [25]. Till now in literature no spectrofluorimetric method demonstrating stability studies for determination of ondansetron in a micelle enhanced medium, pharmaceutical preparations and human plasma has not yet reported. The proposed method is simpler and less time taking without any derivatisation reactions and validated according to ICH Q2 (R1) guidelines [26]. Spectrofluorimetric analysis stands top among other analytical techniques in terms of selectivity and ease. Hence the proposed method could be taken into account as an alternative and economical to already existing sophisticated and expensive set-up stability indicating analytical techniques.

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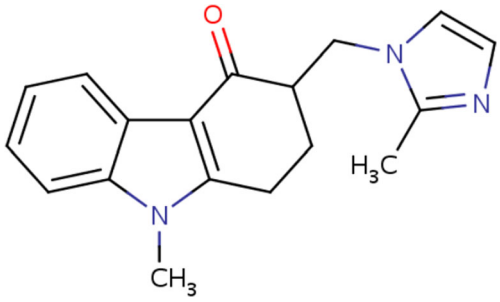

Fig. 1 Structure of ondansetron

Apparatus and Reagents Used

Instrumentation

For fluorescence measurements FLUOROMAX -4, HORIBA spectrofluorimeter with Xenon arc lamp and 1 cm quartz cell was utilised. Human plasma samples were sonicated using Remi Cyclo mixer CM 101. Digitalised Ultra sonic bath was adapted for sonicating the sample solutions. For weighing of standard and samples a Shimadzu AUW – 220D digital balance was employed. For centrifugation of spiked human plasma samples a REMI Labtech make ultracentrifuge was used.

Materials and Reagents

Tokyo Chemical Industry Co. Tamil Nadu, India provided the authentic sample of Ondansetron Hydrochloride (Batch No. 4EIYI-KQ). Solvents used were: Water used was bidistilled. Merck, Mumbai, India supplied Acetone, Dimethyl Sulfoxide (DMSO) and Acetonitrile. Finar chemicals, Ahmedabad, Gujarat, India supplied Ethanol and methanol. From Merck, Mumbai Sodium hydroxide and Hydrochloric acid were purchased. Thermo Fischer Scientific Mumbai, India supplied 30% Hydrogen peroxide. Alfa Aesar, England supplied Triton X 100, From Loba chemie, Mumbai, India Methyl β – Cyclodextrin (β –CD), Sodium Dodecyl sulphate (SDS), and Cetrimide (CTAB) were obtained. Human plasma was procured from Merck, Germany and kept frozen at −20 °C till analysis. Emeset tablets (Batch No.GG61637, Cipla Ltd., Goa, India) labelled to contain 4 mg OND per tablet, Emeset syrup (Batch No. A270237 Cipla Ltd., Ahmedabad, India) labelled to contain 2 mg/5 mL OND, and Emeset Injection (Batch No. EMS0084, Cipla Ltd.; Goa, India) labelled to contain 1 mg/2 mL OND were used.

Experimental

Standard Stock Solutions

A 10 mg of authentic drug is dissolved in 60 mL bidistilled water; sonicated and made upto mark in 100 mL standard flask to attain 100 μg/mL concentration solution. Further dilutions was done in 10 mL volumetric flasks to get working standard solutions of concentration range of 0.2 - 2 μg/mL. The solutions were refrigerated at 6 °C for a minimum period of ten days and were found to be stable.

Calibration Curves

Determination of OND in Aqueous Medium

The native fluorescence of OND was measured at emission wavelength of 353 nm upon exciting at wavelength of 316 nm for the working standard solutions ranging in the concentration of 0.2 - 2 μg/mL.

Determination of OND in Micelle – Enhanced Medium

The enhanced fluorescence of OND was measured at an emission wavelength of 354 nm upon exciting at wavelength of 317 nm by addition of 0.5 mL of Triton X - 100 to each calibrated flask of working standard solutions in final concentration range of 0.2 - 2 μg/mL.

Pharmaceutical Preparations

Analysis of OND in Tablets, Syrup and Injection

Aliquots of weight or volume nearly equal to 10 mg of OND were measured and taken to 100 mL standard flasks and the contents were filtered after sonication for 15 min. Then the working concentration range aliquots (0.2 - 2 μg/mL) were prepared in 10 mL calibrated flasks and the methodology under 'Calibration Curves' was followed and the nominal contents were obtained from respective regression equation.

OND Determination in Spiked Human Plasma

0.5 mL plasma was transferred to centrifugation tubes, spiked with different portions of OND; vortexed for 5 min, 5 mL methanol was added for full precipitation of proteins and subjected to centrifugation at 2500 rpm for 20 min. The upper layers of liquid was removed and filtered. Then, 1 mL of filtrate was taken into 10 mL calibrated flasks and made-up with DDW to obtain final concentrations of 0.2 - 2 μg/mL followed by blank plasma experiment. The methodology under 'calibration curves' section was followed'.

Forced Degradation Study Procedure

Alkaline and Acidic Degradation

To a set of conical flasks, small portions of OND stock solutions (500 μg/mL) was transferred and 2 mL 1 N

sodium Hydroxide or 2 N Hydrochloric acid was added, heated over a time period of 15, 30, 45 and 60 min at 80 °C. Solutions were cooled to attain room temperature and neutralized with 2 N HCl or 1 N NaOH and completed to mark with water. In 10 mL volumetric flask a 0.5 mL solution was taken and the methodology under 'calibration curves' section was applied.

Oxidative Degradation

Small portions of OND stock solution (500 μg/mL) was taken into 10 mL standard flasks followed by 2.5 mL addition of 30% H_2O_2 and maintained at room temperature for one day. The 0.5 mL of solutions was transferred to each flask after heating on water bath for 5 min to remove additional hydrogen peroxide and the methodology under 'calibration curves' section was applied.

Photo Degradation

In 10 mL volumetric flask, 1 mL of OND stock solution (500 μg/mL) was taken made upto volume with water and exposed to direct sunlight for two days and the methodology under 'calibration curves' section was applied.

Thermal Degradation

Pure drug of 10 mg in solid state was exposed as a thinlayer in a petriplate and placed in hot air oven at 80 °C for three days. After three days the drug was taken in 100 mL volumetric flask and made up using bidistilled water. From that solution an aliquot of 1 mL has been transferred to 10 mL calibrated flask and the methodology under 'calibration curves' section was applied.

Results and Discussion

In general the fluorescence intensity greatly enhances by employment of micellar medium. This paved the way for quantification of OND in Triton X – 100 micellar system, spiked human plasma and pharmaceutical dosage forms by Spectrofluorimetry. The enhanced fluorescence property of analyte molecules aids in decreasing the limit of detection which is highly beneficial. The strong microenvironment provided by organised micellar systems restricts the power of fluorophore units by decreasing the non radiative process, creating consistent environment and obstruct quenching which plays a major role in increasing fluorescence intensity of guest molecules [27, 28]. The enhanced fluorescence is also a cause to creation of strong microenvironment ahead of OND and restricting the free spinning motions which are typical for luminescent emission [29]. Hence for the proposed spectrofluorimetric method has been developed by employing Triton X − 100 micellar medium.

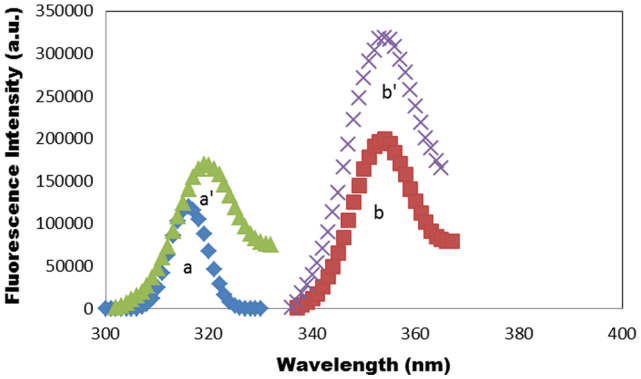

Fig. 2 Fluorescence Spectra of OND (1 μg/mL) in: (a′, b′) 0.5% Triton X-100 system and (a, b) OND (1 μg/mL) in aqueous system, where: (a, a′) excitation spectra and (b, b′) are the emission spectra

Optimizations

Fluorescence Spectra of OND in Aqueous Solution

The increment in fluorescence intensity was found to be 1.60 fold in triton X 100 micellar medium to that of native fluorescence of OND in its aqueous solution (Fig. 2).

Different Organised Media Effect

Different surfactants like Triton x-100, SDS, CTAB and a macromolecule β-CD were prepared as 0.5% solutions and their fluorescence action was studied carefully. Except Triton x − 100, remaining all caused sharp decrease in fluorescence intensity. The reason for enhancement may be perhaps the hydrogen bond formation between terminal hydroxyl groups of triton X - 100 and carbonyl oxygen, imidazole nitrogen of OND which promotes lowest molecular electronic state protection of analyte molecule in small environment of aggregated medium (Fig. 3).

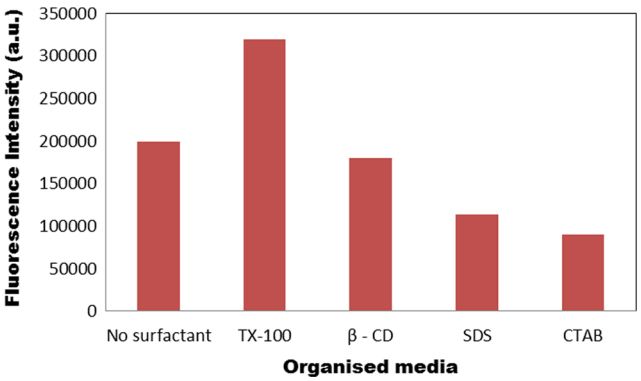

Fig. 3 Effect of different Surfactants and Macromolecule

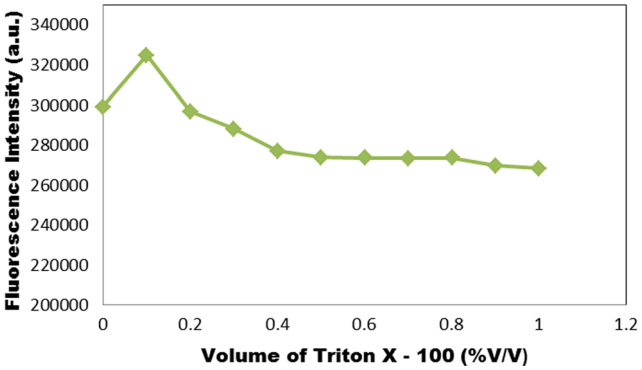

Fig. 4 Concentration (%*v*/v) Effect of Triton X – 100 on FI of OND (1 μg/mL)

Triton X– 100 Concentration Effect

By increasing the concentration of triton X - 100 fluorescence intensity also steadily increased; finally set to a constant level at 0.4% *v*/v of Triton X − 100; after which no further rise in fluorescence response was observed. Hence as suitable concentration triton X − 100 of 0.5% v/v was adapted (Fig. 4).

Diluting Solvents Effect

Various diluting solvents like bidistilled water, dimethyl sulfoxide, acetonitrile, ethanol, methanol and acetone were used. Bidistilled water is selected as the good diluent for analysis due to its highest fluorescence response and lower blank value. DMSO initiates inter system crossing and cause sharp decrease in fluorescence response [30]. The noticeable fall in fluorescence response with ethanol and methanol is due to greater solubilisation of aqueous phase small chain alcohols creating disturbance to microenvironment of micellar medium there by causing size reduction, micellar breakdown at high concentration and change in solvent properties [31]. The denaturing effect of acetonitrile and acetone is the reason for decrease fluorescence response (Fig. 5).

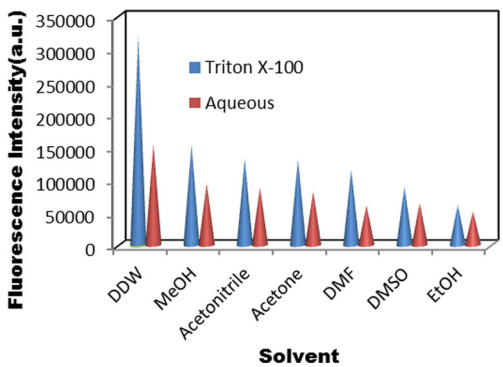

Fig. 5 Different diluting solvents effect on FI of OND (1 μg/mL) in presence of Triton X – 100 (0.5% v/v) and in aqueous media

Table 1 A brief regression data of the developed method in Aqueous and Triton X-100

Parameter	In Aqueous System	In Triton X-100 System
λ_{ex} (nm)	316	317
λ_{em} (nm)	353	354
Linearity range (μg/mL)	0.2–2	0.2–2
Limit of detection LOD (μg/mL)	0.04	0.02
Limit of quantitation LOQ (μg/mL)	0.12	0.07
SD of slope (S_b)	2.1	64
SD of residuals ($S_{y/x}$)	3.25	116
SD of intercept (S_a)	2.36	79
Correlation coefficient (r)	0.9992	0.9998
% RSD	1.28	1.33
Intercept (a)	8760	192,776
Slope (b)	192	11,472

Time and Temperature Effect

It was noticed that development of the fluorescence was immediate and remained for about 4 h. The entire procedure was carried out under room temperature since the standard solutions placed in thermostatically controlled water bath over a certain temperature ranging from i.e.45–85 °C, caused rise in the temperature there by causing vast decrease in fluorescence response, since at elevated temperature there is high chance of internal conversion which causes nonradiative deactivation of excited singlet state [32].

Method Validation

Linearity

Statistical analysis [33] of regression data was performed by taking concentration on x-axis and fluorescence intensity on y-axis for determination of OND from calibration graph shows a good linear relationship for entire range of concentration 0.2–2 μg/mL. Results are depicted in Table 1.

Table 2 The assay results of OND in pure form by developed and reference methods

Amount taken (μg/mL)	Amount found (μg/mL)	% Found*	Reference method [13]
0.2	0.20	100.58	103.1
0.4	0.40	101.19	103.83
0.6	0.61	101.15	103.41
0.8	0.81	101.14	
1.0	0.99	98.95	
Mean		100.60	103.45
±SD		0.96	0.37
t		4.80	
F		6.85	

*Each result is average of three separate determinations

The values of the tabulated t and F are 2.45 and 19.25 respectively at $P = 0.05$ [33]

Table 3 Precision Study for the OND by developed method

	Amount taken (μg/mL)	% Found*	% RSD	% Error
Triton X-100	0.4	98.9 ± 1.2	1.21	0.69
Inter-day	0.8	107.98 ± 0.73	0.68	0.42
	1.2	108.08 ± 1.57	1.45	0.91
Triton X-100	0.4	101.9 ± 0.8	0.74	0.43
Intra-day	0.8	110.37 ± 0.44	0.40	0.26
	1.2	107.1 ± 1.7	1.61	1.0

*Each result is average of three separate determinations

Accuracy

The developed method accuracy was evaluated by application of F- test and T-test which exhibited insignificant difference between developed method and reference method [13]. The outputs of test are depicted in Table 2.

Precision

The results (Table 3) of Interday and Intraday precision was assessed by measuring three replicates and three concentrations over a time period of three recurring occasions and three recurring days. The low % RSD values show good reproducibility of the developed method.

Robustness

The robustness was demonstrated by minor and careful variation in volume of Triton X - 100 0.4 ± 0.2 mL. The small changes that had taken place during analysis did not cause

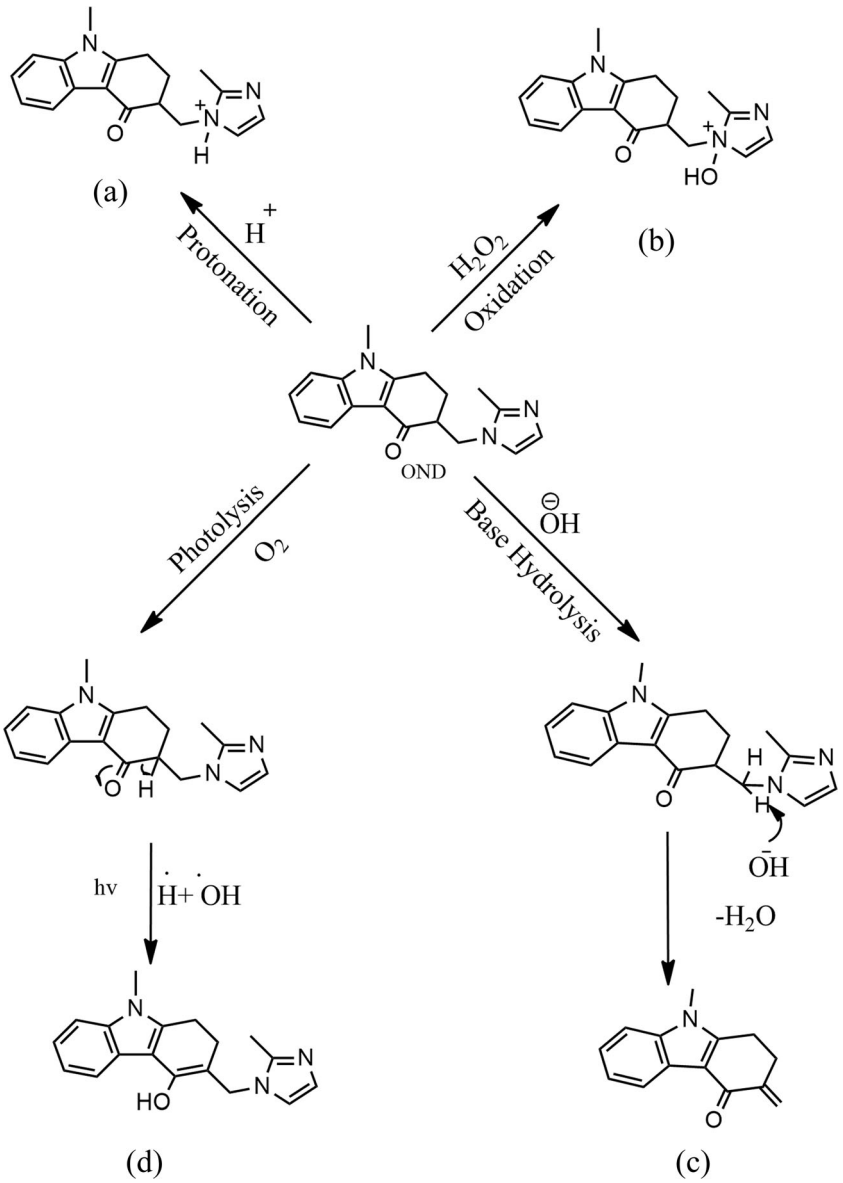

Fig. 6 Forced Degradation study Pathway Proposal of OND

Table 4 Assay results for the OND in (Emeset) Tablets, Syrup and Injection by the developed method

Parameters	Pharmaceutical dosage forms									Reference Method [13]
	Emeset Tablets (4 mg)			Emeset Syrup (2 mg/5 mL)			Emeset Injection (2 mg/mL)			
	Amount taken (μg/mL)	Amount found (μg/mL)	% Found*	Amount taken (μg/mL)	Amount found (μg/mL)	% Found*	Amount taken (μg/mL)	Amount found (μg/mL)	% Found*	
	0.2	0.19	97.12	0.2	0.20	98.26	0.2	0.20	97.57	98.2
	1.0	0.98	98.36	1.0	0.97	96.50	1.0	0.98	98.21	97.62
	2.0	1.99	99.47	2.0	1.94	97.15	2.0	1.98	98.94	98.62
Mean			98.31			97.30			98.24	98.15
± SD			1.18			0.89			0.69	0.5
t			0.22			1.43			0.18	
F			5.50			3.12			1.86	

*Each result is average of three separate determinations

The values of the tabulated t and F are 2.78 and 19 respectively at P = 0.05 [33]

greater effect on fluorescence intensity and it remained steady with experimental operation.

Results of Stability Studies

OND was stable only under thermal degradation. Noteworthy amount of drug was degraded by exposing the drug to photolytic, acidic, alkaline and oxidative degradation. The drug was degraded readily in alkaline medium. On subjecting the drug with 1 N sodium hydroxide at optimum temperature degradation was fast that 33.9% of degradation was observed. Initially 1 N HCl was used for acidic degradation study but no degradation was observed, since degradation rate in acidic medium is slow to that in basic medium. So upon subjecting the drug to 2 N HCl nearly 23.8% degradation was observed. Then on subjecting the drug to oxidative degradation 30% hydrogen peroxide at 25 °C for 24 h significant degradation was observed. The quantity of degraded drug was volume dependant, 9.18% drug was degraded upon addition of 1.5 mL of 30% hydrogen peroxide to the drug. The drug was very much liable to photo degradation. 43.4% drug was degraded on exposing the drug to direct sunlight for two days. A brief description of degradation study is listed in Table 5.

Table 5 Stress study of OND under various degradation conditions using developed method

Conditions	Time (h)	% Degradation
2 N HCl, 80 °C	1	23.8
1 N NaOH, 80 °C	1	33.9
30% (*w*/*v*) H_2O_2, RT	24	9.18
Photolytic degradation	48	43.4

Pathway of Degradation

Under acidic condition the drug was converted to protonated form resulting in compound (A). Under oxidative condition the drug was just converted to oxidised form of ondansetron by the loss of fluorescence resulting in compound (B). The alkaline hydrolysis involves a two-step process where the first step involves protonated form of drug and the second step involves elimination of methyl substituted imidazole group from the drug with the loss of fluorescence and resulting in formation keto derivative of OND (C). The photolytic cleavage involves loss of H_2O by addition of hydroxyl group which may be due to keto-enol tautomerization of the drug in solution and resulting in loss of fluorescence with formation of enol form (D). The proposed degradation pathway for basic and photolytic degradation was related to previous studies on OND [34]. The pathway for OND is presented in Fig. 6.

Pharmaceutical Applications

The developed method was employed for estimation of OND in injection, tablets and syrup. Statistical analysis [33] performed depicted in Table 4 showed satisfactory results with those obtained by reference method [13].

Table 6 OND determination in spiked human plasma by developed method

Amount taken (μg/mL)	% Found*	% RSD	% Error
0.4	94.7 ± 0.7	0.78	0.43
0.8	97.3 ± 1.1	1.13	0.64
1.2	93.69 ± 0.62	0.66	0.36

*Each result is average of three separate determinations

Spiked Human Plasma Samples Application

OND is given as oral or intra-muscular or intra-venous forms. It undergoes limited first-pass metabolism. It is bound to plasma protein around 70% - 76%. The half-life time lasts for 5.7 h. It is rapidly and completely absorbed when taken orally. The maximum dosage of OND per day is only 8 mg. The bioavailability of drug is 32% when administered orally and 75% when administered subcutaneously [35]. The calculated standard deviations and percentage recovery ranging from 93.69% to 97.25% for OND are within the acceptable limits. Results are shown in Tables 5 and 6.

Conclusion

A consistent and responsive spectrofluorimetric method for estimation of OND was proposed. The proposed methodology is rapid, cost effective, time saving without involvement of pretreatment procedures, derivatisation reactions and cumbersome extraction steps with instantaneous enhanced fluorescence intensity compared with chromatographic techniques. Hence the proposed methodology can be employed with ease for estimation of OND in various available pharmaceutical formulations and spiked human plasma. In addition it is suitable for stability studies of OND under different degradation conditions. Further it serves as an alternative approach to other analytical techniques.

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