ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Enantioseparation and purity determination of Ondansetron by amylose based Chiral HPLC method: A Chemometric approach

Ganna Anitha* and Pandey VP

Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalainagar - 608 002, Tamil Nadu, India

Article History:

Received on: 03.02.2018
Revised on: 12.05.2018
Accepted on: 16.05.2018

Keywords:

Chiral separation, Central composite, Derringer's desirability, Multicriteria decision, Ondansetron

ABSTRACT

Amylose based chiral HPLC method for enantioselective separation of ondansetron was developed, optimized and validated on Chiral Pak AS-3R analytical column (150 mm × 4.6 mm i.d., 3 µm). Mobile phase comprising of Acetonitrile: 0.2% Triethylamine: Methanol (15.49:30:54.51) and at a flow rate of 1.33mL/min. The separation was detected at a wavelength of 254 nm and run time was achieved within 6.0 min. Applied Central composite design (CCD) to validate the impact of three independent variables: 0.2% triethylamine (TEA), Acetonitrile (ACN) and flow rate (FL) keeping the methanol concentration constant and the responses selected were capacity factor (k_1), resolution ($Rs_{2,1}$), and retention time (tR_2). Using this approach, a mathematical model was elucidated and response surface plots were acquired for the separation. CCD results were integrated into a multi-criteria decision making approach in order to prevail a set of optimal experimental conditions directing to the most desirable compromise between resolution and analysis time. This method can be applied for the routine analysis.

* Corresponding Author

Name: Ganna Anitha
Phone: +91-9362788046
Email: anitha.royal@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v9i3.1551

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

In compliance with the global drug regulatory agencies issued guidelines stating that ideally, only the zestful enantiomer of a chiral drug should be introduced to the market (Gal, 2006) the enantiomeric separation became vital in the pharmaceutical industry considering the difference in the pharmacokinetic and pharmacological properties, even toxicity. The Enantioseparation advanced since the early 1980s from an academic inquisitiveness to an extremely proficient collection of allied techniques. Enantioselective separation emerged as one of the major workhorses to cope with the stereoselectivity issues in the pharmaceutical industry especially after the USFDA issued guidelines for the marketing of racemic compound in 1992 (USFDA, 1992). Ondansetron was first medically used in 1990. It is one of the WHO list of essential medicines, the most effective safe medicines required in the health system.

Ondansetron, {1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1Himidazol-1-yl)methyl]-4H-carbazol -4-one} is a selective 5-HT_3 receptor antagonist used in the treatment of post-operative as well as chemotherapy- and radiotherapy-induced nausea and emesis (Currow, 1997; Butcher, 1993). Acts by minimising the activity of the vagus nerve, which triggers the vomiting centre in the medulla oblongata, and furthermore by blocking serotonin receptors at the chemoreceptor trigger zone. It shows little effect on vomiting due to motion sickness, and no effect on dopamine receptors or muscarinic receptors.

The main objective of the current work was the development of a direct chiral HPLC method, optimisation by applying Response Surface Methodology – a Chemometric approach and

quantification of ondansetron enantiomers in formulations, also suitable for plasma which provides the scope for assessing the pattern of bioavailability and pharmacokinetics of the enantiomers individually. Since the R-Ondansetron (Eutomer) is approximately eight times potent than the S-ondansetron (Distomer) (Butler and Hill 1988) instead the eutomer administration avoided the racemic ondansetron associated adverse effects viz headache, constipation and increased transaminase levels (James 1993).

Literature review reveals that no method has been reported for the optimisation of ondansetron racemic mixture employing Central composite design. Only few chiral methods have been reported for determination of enantiomers in human plasma (Kelly, 1993; Siluveru and Stewart 1997; Liu, 2008; Liuand and Stewart, 1997) and a number of achiral procedures (Chandrasekar, and Ramakrishna, 2004; Colthupa and Palmer, 1989; Xu, 2000; Depot, 1997; Bauer, 2002). Recently Valliappan developed a direct chiral method applying D-Optimal design with a mobile phase comprising 85% MeOH and at a flow rate of 1.5mL/min and no criteria was optimised for biologicals (Valliappan and Selvakumar 2017) which motivated to develop a method suitable for biological matrices.

EXPERIMENTAL

Apparatus

Chromatography was done using a Shimadzu (Tokyo, Japan) model with a LC10AD and LC10 ADvp solvent delivery module, SPD 10A UV-Visible detector, a Rheodyne injector (model 7125, USA) valve fitted with a 20µl loop, and UV detector (SPD-10A). The system was controlled through a system controller (SCL-10A) and a personal computer using a Shimadzu chromatographic software (LC Solution, Release 1.11SP1) installed on it. The Branson sonicator (Branson Ultrasonics Corporation, USA) was used for degassing the mobile phase. Absorbance spectra were recorded using a double beam UV-Visible spectrophotometer: 2202 Systronics, employing quartz cell of 1.00 cm of path length.

Software

Design-Expert® trial version 7.0.0. (Stat-Ease Inc., Minneapolis) was used for Experimental design, data analysis and desirability function calculations and the remaining calculations for the analysis were accomplished using Micro soft Excel 2007 software (Microsoft, USA).

Chemicals and reagents

Working standards of ondansetron racemic mixture were gifted by M/S. Pharma analytical Lab., Puducherry, India. The pure enantiomer of R-OND was obtained from Emcure Pharmaceuticals (Pune, India). S-OND was procured from Sigma-Aldrich, India. ACN of HPLC grade, purchased from SD Fine Chemicals, Mumbai, India. The HPLC grade water was prepared by using Milli-Q Academic, Millipore, Bangalore, India. The pharmaceuticals: Emeset-8 tablets containing (RS)OND 8 mg (Cipla Ltd Mumbai, India) and Zordil-4 tablets containing pure enantiomer of R-OND 4 mg (Emcure Pharmaceuticals Ltd, India) were procured from pharmacy retail shop.

Stock and Working Standard Solutions

Stock standard solutions of racemic OND and R-OND, at 1000 µg mL^{-1}, were prepared individually using a mixture of MeOH, ACN and water in 80:20 v/v and stored at 4°C protected from light. The stock solutions of (RS)-OND further diluted with the mobile phase to give a series of standard mixtures having a final concentration in the range of 2–10 µg mL^{-1}. The solution prepared for the optimisation procedure comprised of (RS)-OND, at 10 µg mL^{-1}.

Preparation of the Sample Solution

Twenty tablets of Emeset-8 (RS)-OND and Zordil-4 (R-OND) were weighed and analysed separately. An amount of powder equivalent to 10 mg was weighed and transferred in a 10 mL volumetric flask, and 5 mL of mobile phase was added. This mixture was subjected to sonication for approximately 15 min to ensure complete solubility of the drugs, and the solution was made up to the mark with mobile phase and further dilutions were made to obtain a concentration of (RS)-OND 10 µg mL^{-1} and R-OND 5.0 µg mL^{-1}. The resulted solution was centrifuged at 4000 rpm for 10 min, and the clear supernatant was collected and filtered through a 0.2 µm membrane filter (Gelman Science, India) and 20 µl of this solution was injected for HPLC analysis.

Chromatographic procedure

The chromatographic separation was carried out on a Daicel Chiral Pak AS-3R column (150 mm × 4.6 mm i.d., 3 µm) connected with Daicel Chiral Pak AS-3R guard cartridge. The mobile phase consisted of ACN/MeOH/0.2 % triethylamine. Before use, the mobile phase was degassed for 15 min in an ultrasonic bath and vacuum filtered through 0.45 µm membrane filter (Gelman Science, India). The wavelength of 254 nm was selected for detection. An injection volume of the sample was 20 µL. The

HPLC system was used in an air-conditioned laboratory atmosphere (20 ± 2°C).

RESULTS AND DISCUSSION

The main objective of the current study was to develop, optimise and validate a direct chiral HPLC method for the stereoselective separation of OND by using Chiral Pak AS-3R where the amylose tris[(S)-αmethylbenzylcarbomate] coated on 3 µm silica gel acts as stereoselective CSP in reversed-phase (RP) mode. The principle involved in separating the enantiomers of Ondansetron using this CSP might be because of hydrogen bonding interactions, dipole–dipole interactions, pi–pi interactions and aromatic functionalities provide an additional stabilizing effect on the solute-CSP complex by inclusion of the aromatic group into chiral cavity (Scriba, 2016; Hariram, 2014; Scriba, 2013; Ahuja Satinder 2011).

Preliminary Screening

Preliminary Experiments were performed with water, ACN, DEA/TEA enantiomer peaks were not separated and hence included the methanol component too. Mobile-phase additives have a vital role in the separation of analytes consisting of basic or acidic functional groups (Mosiashvili, 2013; Gogaladze, 2015). The structure of OND (Fig. 1) contains the basic functionalities (tertiary amine group in carbazol and imidazole ring), hence basic additives viz, DEA and triethylamine were selected. The first series of trials were performed for comparing TEA and DEA. The mobile phase comprising ACN, MeOH and water with TEA showed better baseline resolution of OND enantiomers along with good K value than DEA and also the concentration of DEA (0.1–0.3% v/v) has no significant effect on enantiomeric resolution and peak shape while TEA concentration effects the K value, and from the next series of trials fixed the TEA as 0.2%. Furthermore, another series of trials were performed for obtaining the best responses comparing the existing methods by altering the ratios of the components of mobile phase and selected the lower and upper levels of ACN, 0.2%TEA keeping methanol concentration fixed and optimised using CCD.

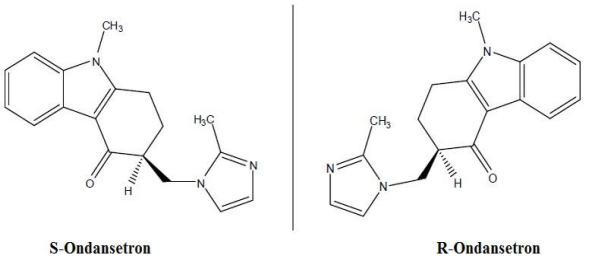

Figure 1: The chemical structures of Ondansetron enantiomers (S & R)

Optimization by Central Composite Design Analysis

Initially, the 2^k Factorial design was applied and investigated and found that curvature is significant for the $Rs_{2,1}$ response since p-value is less than 0.05. This implies that a quadratic model should be considered to model the separation process (Ting, 2009). In order to obtain second order predictive model, central composite design (CCD) is employed, which is a design type under RSM. CCD is chosen due to its flexibility and can be applied to optimise HPLC separation by gaining a better understanding of factor's main and interaction effects (Wang 2006a, 2006b). The selection of factors for optimisation was based on preliminary experiments and prior knowledge from literature, as well as certain instrumental limitations. All experiments were conducted in randomised order to minimise the effects of uncontrolled variables that may introduce a bias on the measurements. Replicates (n=6) of the central points were performed to estimate the experimental error. For an experimental design with three factors, the model including linear, quadratic, and cross terms can be expressed as:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (1)$$

Where, Y is the response to be modelled, β is the regression coefficient and X_1, X_2 and X_3 represent factors A, B and C respectively. Statistical parameters obtained from ANOVA for the reduced models are given in (*Table 3*). The insignificant terms (P > 0.05) were eliminated from the model through a backward elimination process to obtain a simple and realistic model. Since R^2 always decreases when a regressor variable is eliminated from a regression model, in statistical modelling the adjusted R^2 which takes the number of regression variables into account, is usually selected (Parajo, 1992).

Based on the preliminary experiments, the low level and high level of selected factors were fixed at 0.2% TEA (A: 30 -35mL), ACN (B: 15 -20mL) and flow rate (C: 1.0 – 1.5mL/min) whereas MeOH was not included as a factor and the proportion is taken is the remaining volume to make 100mL. To judge the quality of the method under different experimental conditions, the following responses of interest were defined: (1) retention factor of the first eluted peak S-OND (k1); (2) resolution between OND enantiomers ($Rs_{2,1}$); and (3) retention time of second peak (tR_2).

A total of twenty experimental runs obtained from the design were subjected to experiment in order to generate the response variables. (Table 2) summarises the conducted experiments and the

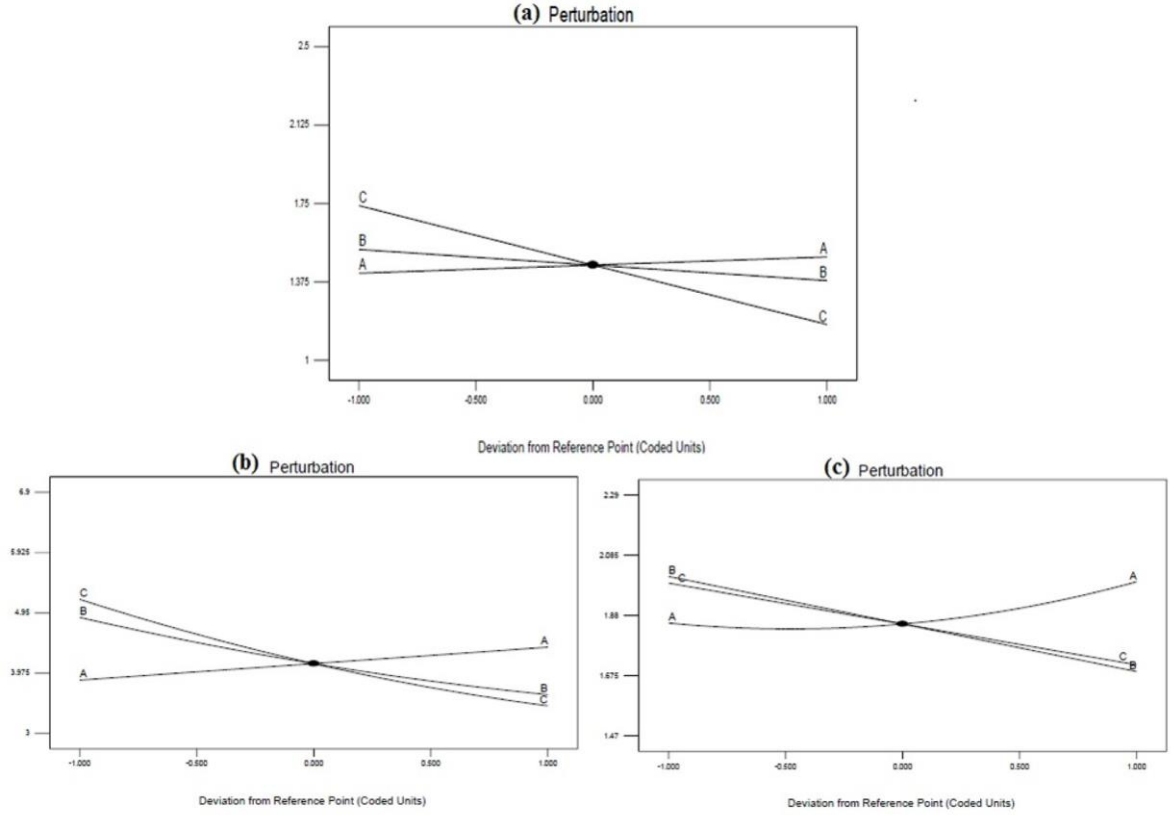

Figure 2: Perturbation plots showing the effect of each of the independent variables on k_1, tR_2 and $Rs_{2,1}$. Where A is the concentration of 0.2% TEA, B the concentration of ACN and C the mobile phase flow rate.

Table 1: Experimental responses and central Factorial design

Design Points	Coded factors levels			Responses		
	A (%v/v)	B (%v/v)	C(mL/min)	K_1	$Rs_{2,1}$	tR_2
1	-1	-1	-1	1.388	5.492	2.124
2	-1	-1	1	1.148	3.535	1.756
3	1	-1	-1	2.174	6.829	2.177
4	0	0	0	1.577	4.207	2.108
5	1	-1	1	1.148	4.374	1.997
6	0	0	0	1.575	4.205	2.109
7	0	0	0	1.578	4.206	2.107
8	1	1	1	1.28	3.396	1.616
9	0	0	0	1.57	4.21	2.108
10	0	0	0	1.574	4.207	2.106
11	-1	1	-1	2.489	4.527	1.805
12	-1	1	1	1.072	3	1.475
13	1	1	-1	1.25	5.142	1.791
14	0	0	0	1.576	4.207	2.106

responses. All experiments were conducted in randomised order to minimise the effects of uncontrolled variables that may introduce a bias on the measurements. Two replicates were performed for each experiment in order to know the experimental error variance and to test the predictive validity of the model. The obtained results were entered and analysed and found that the model is significant for all the three factors since the P value < 0.05, therefore, proceeded for optimization and selected the optimal condition having Derringers desirability value of 0.754, mobile phase comprises of 0.2% TEA : ACN: MeOH (30.00:15:49:54.51) at 1.33 mL/min flow rate, simultaneously the condition suitable for plasma was also selected having Derringers desirability value 0.986, mobile phase comprises of 0.2% TEA : ACN: MeOH (35:15:55) at 1.09 mL/min flow rate and results were tabulated in Table 4 and Table 5.

In the present study, the adjusted R^2 was well within the acceptable limits of $R^2 \geq 0.80$ (Lundstedt, 1998) which revealed that the experimental data

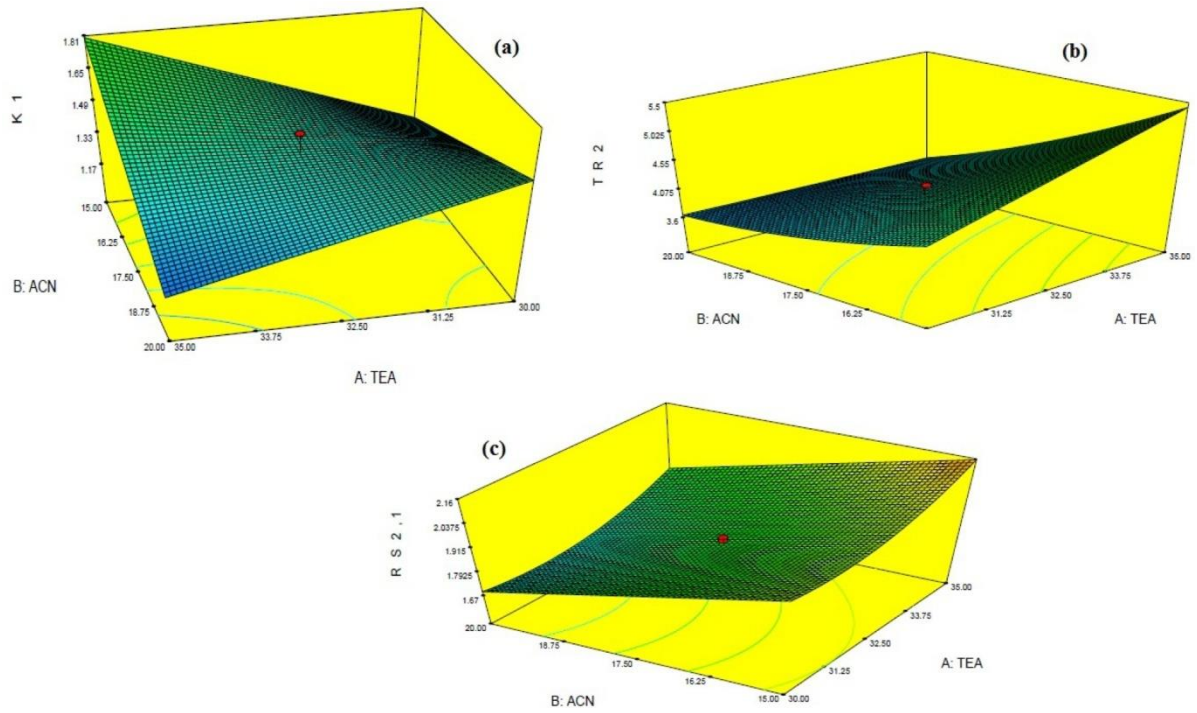

Figure 3: Response surfaces related to percentage 0.2% TEA concentration (A) and Acetonitrile concentration (B) Mobile phase flow rate (C) was kept constant : (a) capacity factor of the first peak (k_1), (b) retention time of the second peak (tR_2), and (c) resolution between two enantiomer peaks ($Rs_{2,1}$).

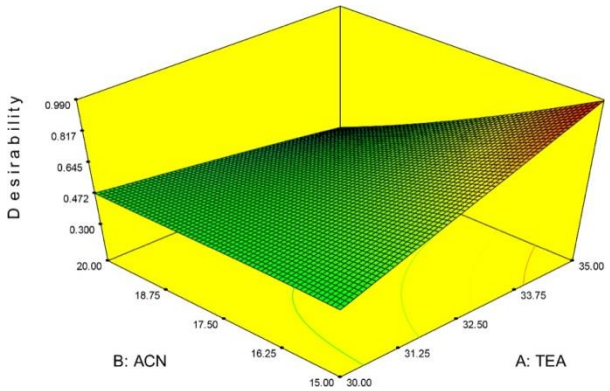

Figure 4: Response surface representation of the overall desirability function for criteria – I *D.* (*D* = 0.754) were 0.2% TEA Conc. (*A*) of 30.00%, Acetonitrile concentration (*B*) of 15.49%, and flow rate (*C*) of 1.33 mL/min.

shows a good fit with the second-order polynomial equations. For all the reduced models, *P* value of < 0.05 is obtained, implying these models are significant. The adequate precision value is a measure of the signal (response) to noise (deviation) ratio". A ratio greater than 4 is desirable (Beg, 2003). In this study, the ratio was found to be in the range of 8.11–14.63, which indicates an adequate signal and therefore the model is significant for the separation process.

As can be seen in (Table 3), the interaction term with the largest absolute coefficients among the fitted models is *BC* (+ 0.28) of tR_2 model. The positive interaction between *B* and *C* is statistically significant (< 0.0001) for tR_2. The study reveals that changing the fraction of ACN from low to high results in a rapid decline in the retention time of R-Ondansetron peak both at the low and high level of flow rate. Further at a low level of factor *B*, an increase in the flow rate results in a marginal decrease in the retention time. This may be due to reduced amylose effects as a result of higher flow rate used. Therefore, when the ACN concentration is set at its lowest level, the flow rate has to be at its highest level to shorten the runtime. Especially this interaction is synergistic, as it led to a decrease in runtime.

In (Fig.2) perturbation plots are presented for predicted models in order to gain a better understanding of the investigated procedure. This type of plots shows the effect of an independent factor on a specific response, with all other factors

Table 2: Experimental responses and Central composite rotatable design

Design points	Factor levels			Responses		
	A (%v/v)	B (%v/v)	C(mL/min)	K_1	$Rs_{2,1}$	tR_2
1	30	15	1	1.388	5.492	2.124
2	35	15	1	2.174	6.829	2.177
3	30	20	1	2.489	4.527	1.805
4	35	20	1	1.25	4.044	1.894
5	30	15	1.5	1.148	3.535	1.756
6	35	15	1.5	1.18	4.374	1.997
7	30	20	1.5	1.072	3	1.475
8	35	20	1.5	1.28	3.396	1.616
9	28.3	17.5	1.25	1.278	3.621	2.026
10	36.7	17.5	1.25	1.722	4.54	2.285
11	32.5	13.3	1.25	1.751	5.489	2.269
12	32.5	21.7	1.25	1.026	3.547	1.706
13	32.5	17.5	0.83	2.063	6.214	2.203
14	32.5	17.5	1.67	1.312	3.135	1.76
15	32.5	17.5	1.25	1.557	4.154	1.828
16	32.5	17.5	1.25	1.286	4.158	1.746
17	32.5	17.5	1.25	1.286	4.149	1.853
18	32.5	17.5	1.25	1.286	4.158	1.81
19	32.5	17.5	1.25	1.286	4.151	1.871
20	32.5	17.5	1.25	1.286	4.156	1.817

arrangements[a]

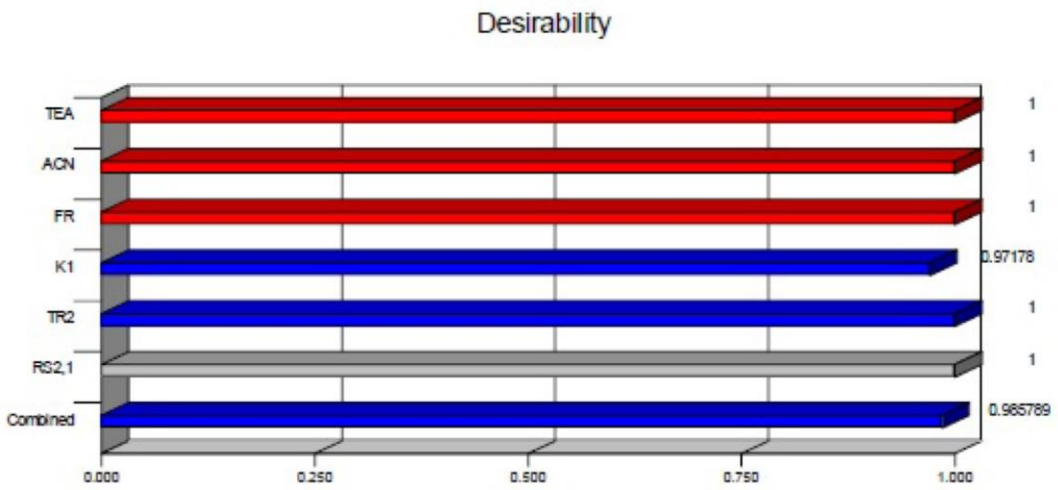

Figure 5: Graphical representation of the overall desirability function for criteria II *D.* (*D* = 0.986) were 0.2% TEA Conc. (*A*) of 35.00%, Acetonitrile concentration (*B*) of 15.00%, and flow rate (*C*) of 1.09 mL/min

held constant at a reference point (Derringer and Suich 1980). A steepest slope or curvature indicates sensitiveness of the response to a specific factor. Fig. 2a and 2b reveal that Flow rate (factor C) had the most important effect on k_1 and $Rs_{2,1}$ followed by the Factor B. Fig. 2c shows factor B shown a more significant effect on tR_2 than the effect of Factor C. The factor A had shown a significant effect on $Rs_{2,1}$ whereas the effect is less significant on k_1 and tR_2 than effect shown by the factors B and C.

Response surfaces plots for k_1, $Rs_{2,1}$ and tR_2 are illustrated in Fig. 3. Fig.3a, 3b and 3c plotted for acetonitrile concentration against the buffer concentration with flow rate held at constant at the centre value). Analysis of the perturbation plots and response plots of optimisation models revealed that factor B and *C* had the significant effect on separation of the analytes, whereas the factor A, i.e. the buffer concentration, is of little significance.

Global Optimization.

In the present study, the identified criteria for the optimisation were: resolution between the critical peaks, capacity factor and elution time. Derringer's desirability function was used to optimise three responses with different targets (Sree Janardhanan, 2012). The Derringer's desirability

Table 3: Response models and statistical parameters obtained from ANOVA for CCD

Responses	Regression model	Adjusted R^2	Model *P* value.	%C.V	Adequate precision
K_1	+1.46+0.039*A-0.075*B-0.28*C-0.23*A*B	0.9120	0.0106	19.97	8.116
$Rs_{2,1}$	+1.85+0.070*A-0.16*B-0.14*C+0.073*A^2	0.9443	<0.0001	5.34	14.63
tR_2	+4.13+0.27*A-0.62*B-0.86*C-0.28*A*B+0.28*B*C+0.12*B^2+0.18*C^2	0.9929	<0.0001	3.82	34.044

Table 4: Criteria for the optimisation of the individual responses

Responses	Lower limit	Upper limit	Criteria I		Criteria II	
			Goal	Importance	Goal	Importance
K_1	1.026	2.489	Target = 1.2	5	Target = 2	5
$Rs_{2,1}$	1.475	2.285	Maximize	5	In Range	5
tR_2	3	6.829	Target = 3.7	5	Target = 6.2	5

function, *D*, is defined as the geometric mean, weighted, or otherwise, of the individual desirability functions. The expression that defines the Derringer's desirability function is:

$$D = [d_1^{p_1} \times d_2^{p_2} \times d_3^{p_3} \times \ldots \times d_n^{p_n}]^{\frac{1}{n}} \quad (2)$$

Where *pi* is the weight of the response, *n* the number of responses and *di* is the individual desirability function of each response. Desirability function (*D*) can take values from

0 to 1. Weights can range from 0.1 to 10. Weights lower than 1 give less importance to the goal, whereas weights greater than 1 give more importance to the goal. In the present study, *pi* values were set at 1 for all the four responses. A value of *D* close to 1, indicates that the

combination of the different criteria is matched in a global optimum (Sree Janardhanan, 2011; Valliappan, 2013; ICH, Q2A 1995). The criteria for the optimisation of each response are shown in (Table 4). Criteria I had been proposed for selecting an optimum experimental condition for analysing routine quality control samples. As can be seen under criteria I, the responses tR_2 was targeted at 3.7, in order to shorten the analysis time and to compete the existed methods. On the other hand, $Rs_{2,1}$ was maximised to allow baseline separation of enantiomers. In order to separate the first eluting peak (S-OND) from the solvent front, *k*1 was targeted at 1.2. Importance can range from 1 to 5, which emphasises a target value. Following the conditions and restrictions above, the optimisation procedure was carried out. The response surface obtained for the global desirability function is presented in (Fig. 4). From the figure it can be concluded that there was a set of coordinates producing high desirability value (D = 0.754) were 0.2% TEA concentration of 30.00mL, ACN 15.49mL and flow rate of 1.33 mL/min. The predicted response values corresponding to the latter value of *D* were: k_1 = 1.2, $Rs_{2,1}$ = 1.94 and tR_2 = 3.88 min. The prediction efficiency of the model was confirmed by experimenting with the optimal condition and the corresponding chromatogram is shown in (Fig. 6).

To substantiate the flexibility of the optimisation strategy and to search for an optimum experimental condition for analysing plasma samples, criteria II was established by varying the response goals and their importance values (Table 4). For instance, the high value of k_1 has to be selected for the separation of S-OND from the initial disturbances of plasma components. Therefore, k_1 was targeted at 2 and a high importance value of 5 was assigned. Following the response goals above, the optimisation procedure was carried out for which optimal condition II with the maximum desirability value of D = 0.986 was obtained. In order to investigate the predictability of the proposed model, the agreement between experimental and predicted responses for both the predicted optimums I and II are shown in (Table 5). The Percentage of prediction error was calculated by Eq. (4). The average percentage predicted errors for K_1, tR_2 and $Rs_{2,1}$ were < 6 %, indicating a good correlation between the experimental and the predicted responses (Sree Janardhanan, 2012).

Predicted Error = Experimental – Predicted / Predicted x 100 (4)

Method Validation

Validation studies were conducted using the optimised assay conditions based on the principles of validation described in the ICH guidelines ''Text on Validation of Analytical Procedures'' and ''Q2B, Validation of Analytical Procedures: Methodology'' (Danzer and Currie 1998). Key analytical parameters, including, specificity, accuracy, precision, linearity, detection limit, and the quantitation limit were evaluated.

Table 5: The comparison of observed and predictive values of different objective functions under optimal conditions

Optimum conditions	TEA (mL)	ACN (mL)	Flow(ml/min)	K_1	$Rs_{2,1}$	tR_2
I	Desirability Value (D) = 0.754					
	30	15.49	1.33			
		Experimental value		1.13	2.04	3.78
		Predicted value		1.2	1.94	3.88
		Percentage Error		5.83	5.2	2.6
II	Desirability Value (D) = 0.986					
	35	15	1.1			
		Experimental value		2.01	2.31	6.29
		Predicted value		1.98	2.24	6.2
		Percentage error		1.85	3	1.48

Table 6: Validation summary for the determination S-OND and ROND

Validation Parameters	S-OND		R-OND	
	Concentration	Results	Concentration	Results
Linearity (n=6)	2-10 μgmL^{-1}	y = 48752x +16.66	2-10 μgmL^{-1}	y = 37331x+ 45.44
	R^2 = 0.9988		R^2 = 0.9986	
LOD	1.219 $ngmL^{-1}$		1.11 $ngmL^{-1}$	
LOQ	3.696 $ngmL^{-1}$		3.365 $ngmL^{-1}$	
Specificity	No excipient peaks were co-eluted with the enantiomer peaks which reveals the developed method is selective and specific.			
Accuracy (mean % recovery) (n=3)				
	80%W/W	99.35	80%W/W	100.1
	100%W/W	100.03	100%W/W	100.95
	120% W/W	100.03	120% W/W	101.2
(mean % recovery, %CV((n=9)		99.80,0.37		99.83, 0.54
Precision (%CV)(n=6)				
(a)Intraday precision	1	1.91	1	1.53
	3	1.7	3	1.26
	5	1.2	5	0.08
(b)Interday precision	1	2.04	1	1.98
	3	1.88	3	2.3
	5	0.91	5	0.94
Robustness (% Assay, %CV)				
% TEA conc. (30.00 ± 0.5%)	99.99, 0.33		99.73, 0.61	

Linearity

Linearity was established for the concentration range of 2.0-10µg/mL for Ondansetron (approximately 20% to 200% of the nominal range of the analyte). Calibration curves were plotted for both S-OND and R-OND peak area (Y) versus their respective concentrations (x). Performed linear regression analysis for the resultant calibration curves and Correlation coefficients (R^2) were determined as more than 0.999 for both the enantiomers. The representative linear regression equations were: y = 56203x + 940.86 and y = 54871x + 433.52 for S-OND and R-OND, respectively. Since the correlation coefficients alone are not good indicators of linearity studies for an analytical procedure (ICH Q2B, 1997) also performed one-way ANOVA for both the enantiomers, and the calculated F – value (Fcalc) was found to be less than the theoretical F – value (Fcrit) at 5% significance level, indicating that there was no significant difference between replicate determinations for each concentration level.

Specificity

Specificity defined the method's ability to distinguish the analyte from interfering substances and was assessed by checking the separation and resolution of the enantiomer peaks from tablet placebo (prepared in compliance with the qualitative and quantitative composition of the

tested pharmaceutical formulation). Fig. 6 shows no excipient peaks were co-eluted with the enantiomer peaks which reveals the developed method is selective and specific.

Limit of Detection and Quantitation

The limit of detection (LOD) and quantitation (LOQ) for S-OND and R-OND were determined according to ICH guideline Q2B (Crowther, 2001). LOD was defined as 3.3r/S and LOQ was 10r/S based on 'standard deviation of the response and slope' of the calibration curve specially constructed in a low region of 0.05 to 1.0% of the target analyte concentration (Kleinschmidt, 2005). The standard deviation of y-intercepts of the regression lines was used as r (the standard deviation of the response) and S is the slope of the calibration curve. The LOD and LOQ were estimated at 1.219 and 3.696 ng/mL for S-OND, 1.110 and 3.365 ng/mL for R-OND respectively.

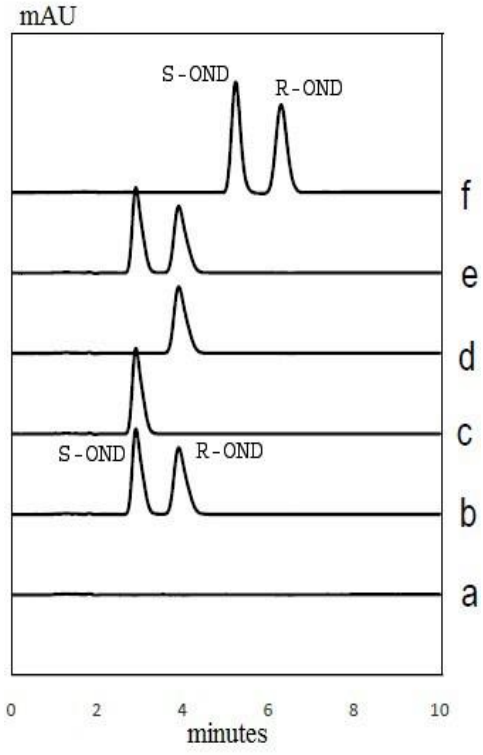

Figure 6: Chromatograms corresponding to (a) a placebo solution; (b) a synthetic mixture of S-OND and R-OND (c) Pure S-OND enantiomer (d) Zordil-4 (R-Ondansetron-4mg) (e) Emeset-8-tablets (±ondansetron-8mg) (f) synthetic mixture of OND enantiomers, under optimum assay conditions II for plasma.

Accuracy/Recovery

The accuracy of the method was determined by performing the recovery experiment at 80, 100 and 120% levels of the labelled amount of the analytes in the commercial formulation. Three replicate samples of each concentration level were prepared by spiking the standard drugs with the placebo or tablet excipients and the %recovery at each level (n = 3), and mean %recovery (n = 9) were determined (Table 6). The recoveries for S-OND and R-OND were found to be 99.80 and 99.83 %, respectively, which were within acceptable ranges of 100 ± 2%

Precision

Six injections, of three different concentrations, were given on the same day and the per cent relative standard deviations (%RSD) were calculated to determine intra-day precision. These studies were also repeated on six consecutive days to determine inter-day precision. The data obtained from precision experiments are given in Table 6. The %RSD values for the intra-day precision study were ≤ 2 and for the inter-day study ≤ 3, confirming that the method was sufficiently precise (Danzer and Currie 1998).

Application of the method.

As a final step, two commercial tablet products Emeset (±ondansetron-8mg) and Zordil (R-Ondansetron-4mg) were assayed by the proposed HPLC method. Representative chromatograms are presented in (Fig. 6). The results achieved when analysing Emeset tablets were 3.97 (0.34) mg of S-OND and 3.96 (0.29) mg of R-OND with the values within parentheses being the % CV of the six replicates. Good agreement was found between the assay results and the label claim of the product. The %C.V. for both the tablets were < 2, indicating the precision of the analytical methodology.

SUMMARY

An enantioselective direct chiral reversed-phase high-performance liquid chromatography method was developed, optimised and validated for the separation and estimation of the ondansetron enantiomers in pharmaceutical formulations. The developed chiral HPLC method could be of immense relevance and value since in India ondansetron is chiefly prescribed during chemotherapy and radiotherapy induced emesis. The developed method can be stated as cost effective since it reduces overall assay development time and consumption of organic solvents. The method furnishes crucial data relating to the sensitivity of diverse chromatographic factors and their interaction effects on the attributes of separation. Time of analysis, resolution and quality of the peaks were simultaneously optimised by applying useful tools of chemometrics: central composite design and Derringer's desirability function. The validation study assisted in selecting the assay conditions by assuring that the selected assay was highly sensitive, specific, precise, robust and linear.

Hence, the developed HPLC method can be used for usual quality control analysis in a pharmaceutical environment. The outcome of the study manifests the comfort of applying this approach in choosing optimum conditions for the determination of drugs in pharmaceutical formulation and plasma samples.

CONCLUSION

The newly developed and chemometrics assisted optimised enantioselective method is highly sensitive than the other existing methods. It is cost minimising simple and fast (<4 min) method with good linearity, precision. The obtained results confirmed that the proposed method could be successfully adopted in enantiopurity control, a chiral switch of Ondansetron and further studies such as bioavailability and pharmacokinetics will be continued based on the developed plasma condition (criteria II).

ACKNOWLEDGEMENT

Authors are thankful to University Grants Commission, New Delhi for UGC –BSR Fellowship during the research tenure. Authors are also thankful to Prof. and HOD Dr. P.K. Manna for providing all research facilities to carry out this entire work.

REFERENCES

A.Butler & J.M. Hill, 1988. Pharmacological properties of GR38032F, a novel antagonist at 5HT3 receptors. Br. J. Pharmacol. 94, 397–412.

Ahuja Satinder, 2011. Chiral separation methods for pharmaceutical and biotechnological products. Wiley, Hoboken.

B.Hariram, R. Suresh Kumar, Anireddy Jaya Shree, Dama Venugopala Rao, L. Kalyanaraman and Katkam Srinivas 2014. Development of a stereoselective method for the quantification and geometrical isomer of pitavastatin calcium by the enhanced approach. Chromatographia. 77, 901–912.

Bauer S, Störmer E, Kaiser R, Tremblay PB, Brockmöller J, Roots I. 2002. Simultaneous determination of ondansetron and tropisetron in human plasma using HPLC with UV detection. Biomed. Chromatogr. 16, 187–190.

Butcher ME, 1993. Global experience with ondansetron and future potential. Oncology. 50, 191–197.

Crowther JB, 2001. Validation of pharmaceutical test methods. In: Ahuja S, Scypinski S (eds) Handbook of modern pharmaceutical analysis. Academic Press, New York, pp. 415–443.

Currow DC, Coughlan M, Fardell B, Cooney NJ. 1997. Use of ondansetron in palliative medicine. The Journal of Pain and Symptom Management. 13, 302–307.

D. Chandrasekar, and S. Ramakrishna, 2004. A rapid, sensitive and validated method for the determination of ondansetron in human plasma by reversed-phase high-pressure liquid chromatography. Arzneimittel for schung. 54, 655–659.

Danzer, K. and Currie, L. A., 1998. Pure Appl. Chem. 70, 993-1014.

G. Derringer and R. Suich, 1980. Simultaneous optimisation of several response variables. J Qual Technol. 12, 214–219.

Gal J, 2006. Chiral drugs from a historical point of view. In: Eric Francotte and Wolfgang Lindner, Chirality in Drug Research. Wiley-VCH Verlag GmbH and Co. KGaA, pp. 33:1–26.

Gogaladze K, Chakvetadze L, Tsintsadze M, Farkas T and Chakvetadze B, 2015. Effect of basic and acidic additives on the separation of some basic drug enantiomers on polysaccharide-based chiral columns with acetonitrile as mobile phase. Chirality. 27, 228–234.

H.T. Ting and K.A. Abou-El-Hossein, H.B. Chua, 2009. Predicting of etching rate of aluminosilicate glass by RSM AND ANN. J.Scientific & Industrial Research. 68, 920-924.

J. Liu and J.T. Stewart 1997. High-performance liquid chromatographic analysis of ondansetron enantiomers in human serum using a reversed-phase cellulose-based chiral stationary phase and solid phase extraction. J. Chromatogr. B Biomed. Sci. Appl. 694, 179– 184.

J.C. Parajo, J.L. Alonso, M.A. Lage and D. Vazquez, 1992. Empirical modelling of eucalyptus wood processing, Bioprocess Eng. 8, 129 –136.

J.W. Kelly, L. He and J. T. Stewart, 1993. High-performance liquid chromatographic separation of ondansetron enantiomers in serum using a cellulose-derivatized stationary phase and solid-phase extraction. J. Chromatogr. 622: 291–295.

K. Danzer and L.A. Currie, 1998. Guidelines for Calibration in Analytical Chemistry Part 1: Fundamentals and Single Component Calibration. Pure and Appl Chem.70, 993-1014.

K. Liu, X. Dai, D. Zhong, and X. Chen, 2008. Quantitative determination of ondansetron in human plasma by enantioselective liquid chromatography–tandem mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 864, 129–136.

K. Valliappan, V Sree Janardhanan and P.Venkatesan, 2013. Direct Chiral HPLC method for the simultaneous determination of warfarin enantiomers and its impurities in raw materials and pharmaceutical formulation: application of chemometric protocol, J. Chromatographia. 76, 287–292.

Kleinschmidt G, 2005. Case study: validation of an HPLC method for identity, assay, and related impurities. In: Ermer J, Miller JHM (eds), Method validation in pharmaceutical analysis: a guide to best practice. Wiley-VCH, Weinheim, 195–226.

M. Depot, S.Leroux and G.Caille, (1997). A high-resolution liquid chromatographic method using ultraviolet detection for determination of ondansetron in human plasma. J. Chromatogr. B Biomed. Sci. Appl. 693, 399–406.

M. Siluveru and J.T. Stewart, 1997. Enantioselective determination of S-(+)- and R-(−)-ondansetron in human serum using derivatised cyclodextrin-modified capillary electrophoresis and solid-phase extraction. J. Chromatogr. B Biomed.Sci. Appl. 691, 217–222.

Mosiashvili L, Chakvetadze L, Farkas T, Chakvetadze B 2013. On the effect of basic and acidic additives on the separation of the enantiomers of some basic drugs with polysaccharide based chiral selectors and polar organic mobile phases. J Chromatogr A 1317:167–174.

P.V. Colthupa and J.L. Palmer 1989. The determination in plasma and pharmacokinetics of ondansetron. Eur. J. Cancer Clin. Oncol. 25(1), S71–S74.

Proceedings of the International Conference on Harmonization (ICH), Q2B: Validation of Analytical Procedures: Methodology, US FDA Federal Register, 1997.

Q. Beg, V. Sahai and R. Gupta, 2003. "Statistical media optimization and alkaline protease production from Bacillus mojavensis in a bioreactor", Process Biochem.39, 203–209.

Scriba GK, 2013. Chiral recognition in separation science: an overview. Methods Mol. Biol. 970, 1–27.

Scriba GK, 2016. Chiral recognition in separation science—an update. J Chromatogr A. 1467, 56–78.

T. Lundstedt, E. Seifert, L. Abramo, B. Thelin, A. Nystrom, J. Pettersen and R. Bergman, 1998. *Design and optimisation.* Chemom. Intell. Lab. Syst, 42, 3–40.

US FDA Federal Register, 1995. Proceedings of the International Conference on Harmonization (ICH), Q2A: Text on Validation of Analytical Procedures: Definitions and Terminology.

US Food Drug Administration, 1992. Chirality. 4, 338-340.

V Sree Janardhanan, R. Manavalan and K. Valliappan, 2012. HPLC method for the simultaneous determination of proton-pump inhibitors with domperidone in human plasma employing response surface design, Int J Pharm Pharm Sci, 4(1), 309-317.

V. Sree Janardhanan, R. Manavalan and K. Valliappan, 2012. Chemometric technique for the optimisation of chromatographic system: Simultaneous HPLC determination of Rosuvastatin, Telmisartan, Ezetimibe and Atorvastatin used in combined cardiovascular therapy. Arabian Journal of Chemistry. http://dx.doi.org/10.1016/j.arabjc.2012.03.001 .

Valliappan Kannappan and Selvakumar Kanthiah, 2017. Enantiopurity Assessment of Chiral Switch of Ondansetron by Direct Chiral HPLC. Chromatographia 80, 229-236.

W.J. James. U.S. patent 5470868. Methods for treating emesis and nausea using optically pure R(+)ondansetron.

X. Xu, M.G. Bartlett, and J.T. Stewart, 2000. Determination of ondansetron and its hydroxy metabolites in human serum using solid-phase extraction and liquid chromatography/positive ion electrospray tandem mass spectrometry. J. Mass Spectrom. 35, 1329–1334.

Y. Wang, M. Harrison and B. J. Clark, 2006. Optimising reversed-phase liquid chromatographic separation of an acidic mixture on a monolithic stationary phase with the aid of response surface methodology and experimental design, J. Chromatogr. A.1105, 199-207.

Y. Wang, M. Harrison and B.J. Clark, 2006. Experimental design for a basic mixture on a fluorinated packing. The effect of composition of the mobile phase. J. Chromatogr.A, 1105, 77–86.