APPLICATION OF A SELECTIVE BONDED PHASE IN THE LIQUID CHROMATOGRAPHIC ASSAY OF ONDANSETRON HYDROCHLORIDE AND ITS IMPURITIES

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Abstract

The present paper reports a new HPLC method for the assay of ondansetron hydrochloride and its identified impurities in a chromatographic system using a *YMC basic* column.

YMC basic is a selective bonded, reverse-phase column developed by YMC Co. Ltd., Japan, designed for the analysis of basic compounds. It is obtained with an alternative bonding approach to reduce peak tailing, by bringing together a support containing neutral silanol groups, which have minimal interactions with ionisable compounds, and a novel bonded phase consisting of monomerically bonded C8 and smaller alkyl chains. This stationary phase has complementary selectivity to C8 and C18 materials and shows high resolution and good peak symmetry for basic compounds, without the need of an ion-pair reagent addition in the mobile phase.

Rezumat

Lucrarea prezină o nouă metodă lichid cromatografică de înaltă performanţă pentru identificarea şi separarea clorhidratului de ondansetron şi a potenţialelor sale impurităţi cunoscute, folosind coloana *YMC basic*.

Faza staţionară *YMC basic*, produsă de YMC Co. Ltd., Japonia, folosită în cromatografia lichidă în fază inversată prezintă, prin însăşi modul de obţinere, avantaje la separarea substanţelor cu funcţie bazică. A fost realizată prin grefarea pe structura silanolică, caracterizată prin interacţie minimă cu compuşii ionizabili, a grupărilor monomerice alchilice cu până la 8 atomi de carbon. Aceasta fază staţionară are selectivitate superioară fazelor staţionare clasice tip C8 sau C18 şi permite separarea cu o bună rezoluţie şi simetrie a picurilor compuşilor bazici, fară a fi necesară adăugarea în faza mobilă a reactivului formator de perechi de ioni.

Keywords: ondansetron hydrochloride and its impurities; liquid chromatographic assay

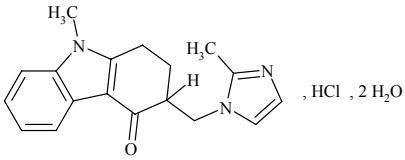
Introduction

In our previous research [1-3] we have already presented an isocratic HPLC method for the selective determination of ondansetron hydrochloride, a selective 5-HT_3 receptor antagonist for the prevention of chemotherapy-induced nausea and vomiting. The separation was achieved

by ion-pair reversed-phase chromatography on an Ultrasphere C8 column. A mixture of 0.3% sodium heptanesulphonate in phosphate buffer pH 3.0 solution and methanol (40/60, V/V) was used as mobile phase. The HPLC system was applied to the quantitative estimation of ondansetron hydrochloride in bulk and pharmaceutical dosage forms.

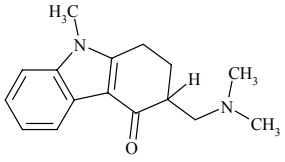
As an alternative to conventional C8 or C18, for the separation with high resolution and good symmetry of basic compounds without the addition of an ion-pair reagent in the mobile phase, we have used *YMC basic* column with a stationary phase containing monomeric bonding of several different alkyl silanes with chain length of C8 and smaller. The recommended pH range for *YMC basic* is 2.0 – 7.5 [4].

At acidic pH value, ondansetron, a weak basic compound, and its impurities act as an anion and could be retained on the stationary phase. On this basis we have developed and established the chromatographic conditions for the separation and assay of ondansetron hydrochloride and three of its identified impurities (figure 1).



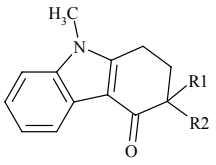
ondansetron hydrochloride dihydrate

(3RS)-9-Methyl-3[(2-methyl-1H-imidazol-1-yl)methyl]-
1,2,3,9-tetrahydro-4H-carbazol-4-one hydrochloride dihydrate



impurity A

(3RS)-3-[(Dimethylamino)methyl]-9-methyl-1,2,3,9-
tetrahydro-4H-carbazol-4-one hydrochloride dihydrate



impurity C; impurity D

impurity C: R1=R2=H
9-Methyl-1,2,3,9-tetrahydro-4H-carbazol-4-one

impurity D: R1+R2=CH_2
9-Methyl-3-methylene-1,2,3,9-tetrahydro-
4H-carbazol-4-one

Figure 1
Chemical structures of ondansetron hydrochloride dihydrate and its identified impurities [5]

Materials and methods

Liquid chromatography system and chromatographic conditions

A Thermo Electron Finnigan Surveyor LC System with diode array detection and a *YMC basic* column, 250 x 4.6 mm, 5 μm (Cat no. BA99S05-

2546WT, B-03-5, Serial No. 042528332 (W), Gel lot 1641) were used for the separation. The pH of the buffer solutions was adjusted using a Mettler Toledo pH Meter type 1120.

The preliminary tests were performed in order to identify the optimal chromatographic conditions for the separation of ondansetron and its impurities. A mixture of phosphate buffer pH 3.0 and methanol was selected as mobile phase. Different ratios of solvents were tested. The UV spectrum of each substance was recorded and presented in figure 2.

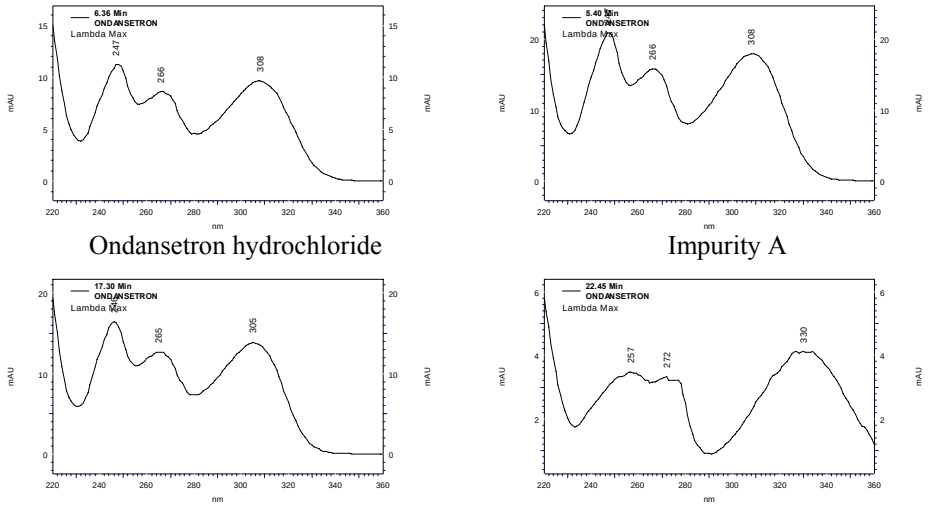


Figure 2

The UV spectra of ondansetron hydrochloride, impurity A, impurity C and impurity D

The 3D-UV overlaid spectrum of the active substance and each impurity is shown in figure 3.

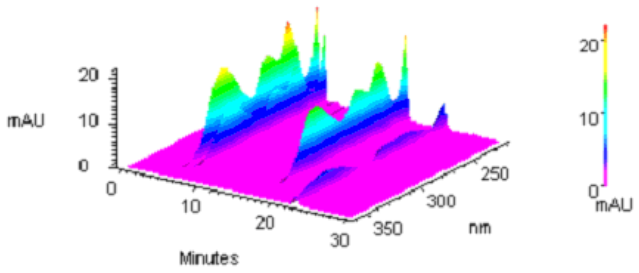


Figure 3

The UV-3D spectra of ondansetron hydrochloride, impurity A, impurity C and impurity D

As seen in the UV spectra, both ondansetron hydrochloride, impurity A and impurity C have absorption maxima at 305-308 nm. The absorption at 308 nm is also favorable for impurity D (with a maximum at 330 nm). So, the wavelength of 308 nm was selected for determination.

Chromatographic conditions:

- mobile phase: solvent A / solvent B 60 / 40 (V/V);
  - solvent A: 10 mM sodium dihydrogen phosphate buffer (potentiometrically adjusted to pH 3.0 with phosphoric acid)
  - solvent B: methanol
- UV detection: 308 nm (with full spectrum recording in the range 220-360 nm);
- flow rate: 1 mL/minute;
- sample solvent: mobile phase.

Reagents

Drug standards

Ondansetron hydrochloride dihydrate working standards was provided by Dr. Reddy's (India). The substance had 9.8% water content.

Impurity A, impurity C and impurity D were provided by Dr. Reddy's (India).

Active substance and dosage forms

Ondansetron hydrochloride dihydrate active substance was also provided by Dr. Reddy's (India). A value of 9.9% water content was determined by Karl Fischer titration.

As pharmaceutical dosage form was selected Osetron® 4 mg, injectable solution (i.v.), manufacturer: Dr. Reddy's, India (composition: 4 mg ondansetron as ondansetron hydrochloride dihydrate and excipients: citric acid monohydrate, sodium citrate, sodium chloride, water for injections to 2 mL).

Solvents and chemicals

All solvents were LiChrosolv® HPLC grade, obtained from Merck (Germany). Ultrapure water obtained with a Milli-Q UF Plus water purification system was used throughout the study.

Standard and sample solutions

Standard ondansetron hydrochloride dihydrate solution (a): an accurately weighted amount of 10 mg ondansetron hydrochloride dihydrate working standard, corresponding to 8 mg ondansetron, was dissolved in a 5 mL volumetric flask with mobile phase. After a brief sonication, it was brought to volume with the same solvent. A dilution of 1 mL in a 10 mL

volumetric flask with mobile phase was prepared (0.2 mg/mL ondansetron hydrochloride dihydrate, corresponding to 0.16 mg/mL ondansetron).

Standard ondansetron hydrochloride dihydrate solution (b): a dilution of 1 mL standard solution (a) in a 50 mL volumetric flask with mobile phase was prepared (4 μg/mL ondansetron hydrochloride dihydrate).

Standard impurity A solution: an accurately weighted amount of 1 mg impurity A was dissolved in a 10 mL volumetric flask with mobile phase. After a brief sonication, it was brought to volume with the same solvent. A dilution of 1 mL in a 25 mL volumetric flask with methanol was prepared (4 μg/mL impurity A).

Standard impurity C solution: an accurately weighted amount of 1 mg impurity C was dissolved in a 10 mL volumetric flask with mobile phase. After a brief sonication, it was brought to volume with the same solvent. A dilution of 1 mL in a 25 mL volumetric flask with methanol was prepared (4 μg/mL impurity C).

Standard impurity D solution: an accurately weighted amount of 1 mg impurity D was dissolved in a 10 mL volumetric flask with mobile phase. After a brief sonication, it was brought to volume with the same solvent. A dilution of 0.5 mL in a 25 mL volumetric flask with methanol was prepared (2 μg/mL impurity D).

Standard impurity A, impurity C and impurity D mixture solution: 1 mL of *Standard impurity A solution, 1* mL of *Standard impurity C solution and 1* mL of *Standard impurity D solution* were diluted in a 10 mL volumetric flask with mobile phase (0.4 μg/mL ondansetron impurity A, 0.4 μg/mL ondansetron impurity C and 0.2 μg/mL ondansetron impurity D).

System suitability solution: 1 mL of *Standard ondansetron hydrochloride dihydrate solution (b)*, 1 mL of *Standard impurity A solution,* 1 mL of *Standard impurity C solution* and 1 mL of *Standard impurity D solution* were diluted in a 10 mL volumetric flask with mobile phase (0.4 μg/mL ondansetron hydrochloride dihydrate, 0.4 μg/mL impurity A, 0.4 μg/mL impurity C and 0.2 μg/mL ondansetron impurity D).

S*ample solutions of active substance:* were prepared in the same way as the *Standard ondansetron hydrochloride dihydrate solution (a)*.

S*ample solutions of pharmaceutical dosage forms:* 0.8 mL of injectable solution was diluted in a 10 mL volumetric flask with mobile phase.

Results and discussion

A volume of 20 μL *System suitability solution* was injected. The peak tailing of each compound should not be more than 1.5. The resolution between the peaks of impurity A and ondansetron should not be less than 2.0.

The chromatogram obtained for *System suitability solution* injection is presented in figure 4.

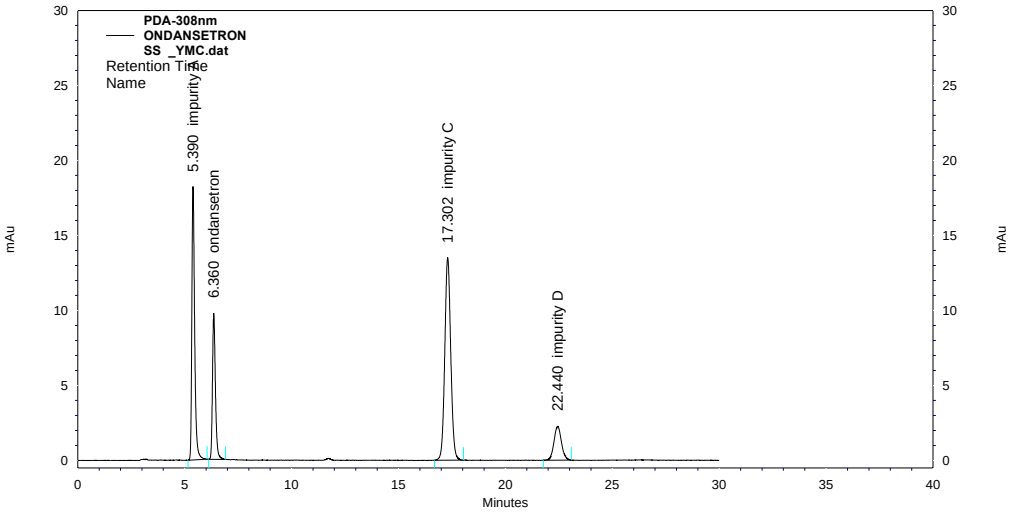


Figure 4
The chromatogram of System suitability solution

Six replicates of *Standard ondansetron hydrochloride dihydrate solution (a)* and two replicates of six independent sample solutions (of active substance and dosage pharmaceutical form) were injected. The overlaid chromatograms are presented in figure 5.

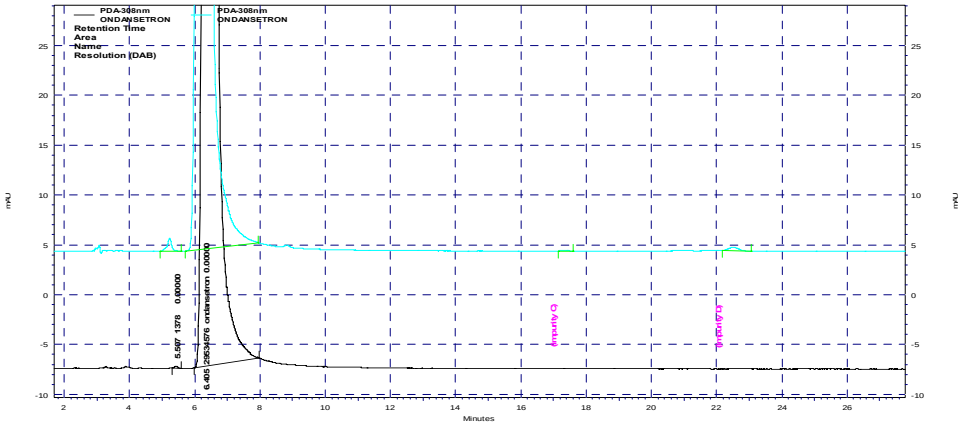


Figure 5
The chromatograms of active substance and pharmaceutical dosage forms sample solutions

The results obtained for both samples (active substance and pharmaceutical dosage forms) are reported in table I.

Table I
Results obtained in the assay of ondansetron hydrochloride active substance and dosage forms

Preparation type	Assay results (%)						Average (%)	RSD (%)	Confidence interval (n=6, P=95%)
Active substance	98.35	99.48	101.13	99.01	98.67	99.70	99.39	0.99	98.35 – 100.43 % (as ondansetron hydrochloride)
Osetron®	99.57	98.42	98.88	99.27	99.95	99.85	99.32	0.60	98.70 – 99.94 % (1.97– 2.00 mg/mL) (as ondansetron)

Assay of the related compounds: Two replicates of *Standard impurity A, impurity C and impurity D mixture solution* and two replicates of six independent sample solutions were injected. The relative retention time with reference to the retention time of ondansetron (~ 6 minutes), according to figure 4, are presented in table II.

Table II
Retention time and relative retention time for the active substance and its impurities

Substance name	Retention time (minutes)	Relative retention time
Impurity A	5.390	0.844
Ondansetron hydrochloride	6.360	1.000
Impurity C	17.302	2.762
Impurity D	22.440	3.589

No impurity was quantified in the active substance sample. Impurity A was below the limit of quantification.

For the sample of the pharmaceutical dosage form impurities A and D were quantified, as presented in table III. The results were below the admissibility limits from European Pharmacopoeia / United States Pharmacopoeia (max. 0.2% for the impurities A and C, max. 0.1% impurity D). Impurity C was not detected.

Table III
Results obtained in the assay of ondansetron hydrochloride impurities in pharmaceutical dosage forms

Recovery of impurity A (%)	Average = 0.0229% RSD (%) 1.96 Confidence interval (n=6, P=95%) μ = 0.0224 – 0.0233%	Recovery of impurity D (%)	Average = 0.0151% RSD (%) 2.81 Confidence interval (n=6, P=95%) μ = 0.0146 – 0.0155%
0.0229		0.0151	
0.0225		0.0143	
0.0221		0.0149	
0.0231		0.0154	
0.0233		0.0153	
0.0231		0.0153	

Validation of the chromatographic procedure

The analytical procedure was validated according to ICH requirements [6].

Assay of the active substance

Linearity study was carried out with the linearity solutions containing ondansetron in 5 different concentrations (i.e. 80, 90, 100, 110 and 120% of the target concentration). An accurately weighted amount of 20 mg of ondansetron hydrochloride dihydrate working standard, corresponding to 16 mg ondansetron, was dissolved in a 10 mL volumetric flask with mobile phase. With the exception of 100% target solution which was injected for 6 times, each other concentration of the linearity solutions of 0.16–0.24 mg/mL ondansetron hydrochloride dihydrate was injected in triplicate. The obtained overlaid chromatograms are presented in figure 5. A calibration curve was plotted in the figure 6 and the detector response factor is presented in figure 7. The results show a good correlation between response and concentration.

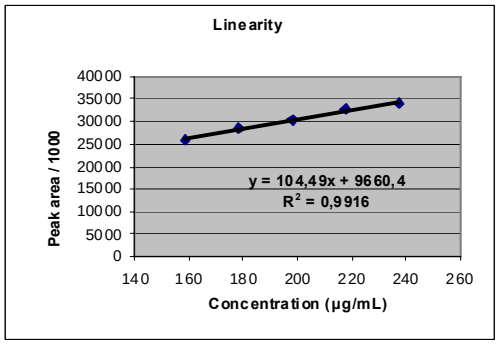


Figure 6
Assay linearity – regression line

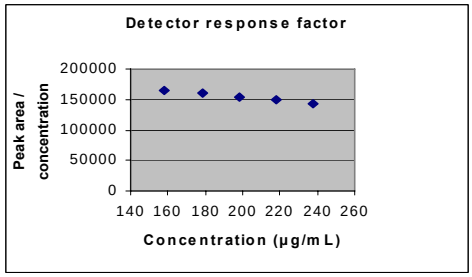


Figure 7
Assay linearity - detector response factor

Assay of ondansetron impurity A and ondansetron impurity C

Linearity study was carried out with the linearity solutions containing each impurity in 5 different concentrations (i.e. 80, 90, 100, 110 and 120% of the target concentration). Impurity A, impurity C and impurity D stock solutions were prepared according to previously described methodology. Successive dilutions of each impurity stock solution (0.8; 0.9; 1.0; 1.1 and 1.2 mL) in 10 mL volumetric flasks were prepared (80–120% from the target value of 0.4 µg/mL impurity A and impurity C, equivalent to 0.2% from the standard solution of 0.2 mg/mL ondansetron hydrochloride dihydrate and the target value of 0.2 µg/mL impurity D, equivalent to 0.1% from the standard solution of 0.2 mg/mL ondansetron hydrochloride

dihydrate). Additionally, in order to assure the completeness with the chromatographic system suitability requirements (especially the resolution between impurity A and ondansetron hydrochloride), in each volumetric flask was added the corespondent volume from the stock concentrated solution of 2 ml/mL active substance (0.8; 0.9; 1.0; 1.1 and 1.2 mL, coresponding to 80-120% of the target value of the active substance). The solution was diluted to volume with mobile phase.

With the exception of 100% target solution which was injected for 6 times, each other concentration of the linearity solutions was injected in triplicate. The obtained overlaid chromatograms are presented in figure 8.

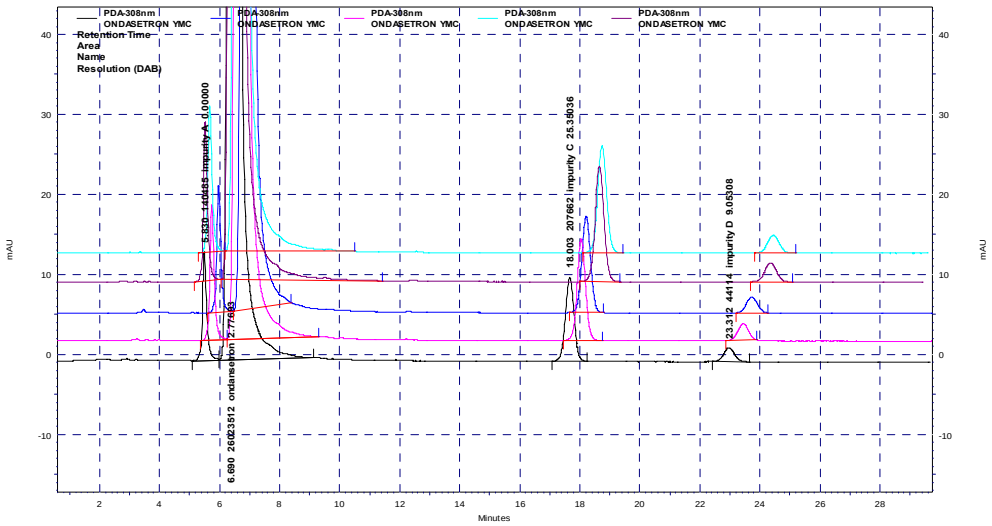


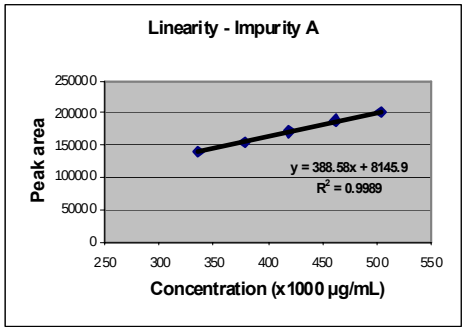
Figure 8

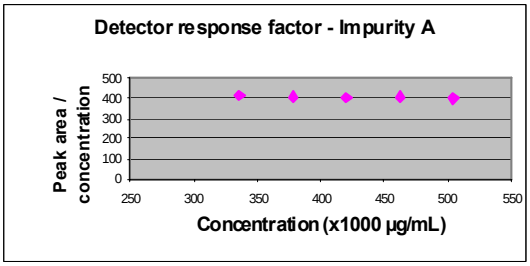
Overlaid chromatograms for the calibration curve of impurities A, C and D in the linearity range in the presence of ondansetron hydrochloride

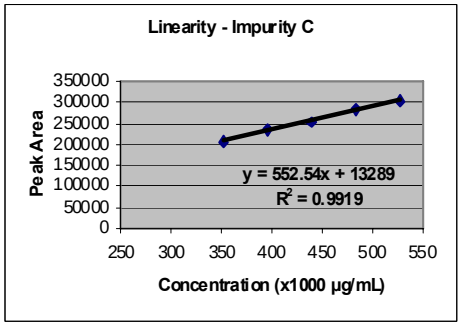
The calibration curve obtained for each impurity was plotted (figure 9) and the detector response factor is presented in figure 10. The results show a good correlation between response and concentration.

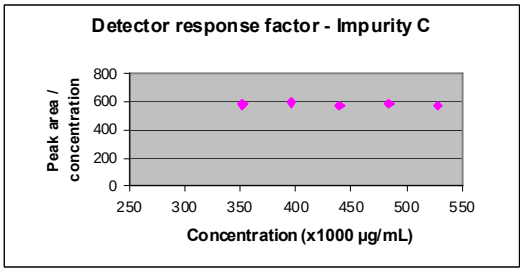
Repeatability: RSD% (6 injections) for ondansetron hydrochloride dihydrate was 1.67, for impurity A was 1.36, for impurity C was 1.34 and for impurity D 3.21.

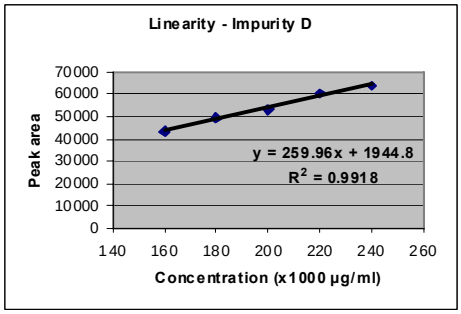
Specificity: no interference with solvent mixture (mobile phase) and placebo was detected.











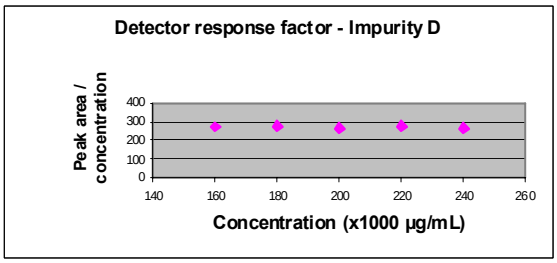


Figure 9
Linearity of impurity A, C and D – regression line

Figure 10
Linearity of impurity A, C and D – detector response factor

Accuracy: the recovery of ondansetron hydrochloride dihydrate working standard and its impurities in 3 diluted samples with known concentration of 95, 100 and 105% from the target value of 200 µg/mL ondansetron hydrochloride dihydrate spiked with known concentration of 95, 100 and 105% from the target value of each impurity (0.4 µg/mL impurity A and C, 0.2 µg/mL impurity D) was determined. "Osetron synthetic mixtures" containing known quantities of active substance (95,

100 and 105% from the target value of 2.5 mg ondansetron hydrochloride dihydrate corresponding to 2 mg/mL ondansteron) in placebo mixtures, spiked with known concentration of 95, 100 and 105% from the target value of each impurity (0.4 µg/mL impurity A and C, 0.2 µg/mL impurity D) were prepared and diluted as prescribed. The obtained statistical data are presented in table IV.

Table IV

Recovery of ondansetron hydrochloride and its impurities in active substance and synthetic mixtures

Sample type	Active substance Average (%) / Confidence interval (n=3, P=95%)	Impurity A Average (%) / Confidence interval (n=3, P=95%)	Impurity C Average (%) / Confidence interval (n=3, P=95%)	Impurity D Average (%) / Confidence interval (n=3, P=95%)
Active substance	99.97 µ = 97.85 – 102.08	98.6 µ = 97.01 – 100.19	99.63 µ = 97.81 – 101.45	97.92 µ = 95.89 – 99.95
Synthetic mixture	99.41 µ = 98.82 – 100.00	99.33 µ = 97.88 – 100.79	100.02 µ = 97.86 – 102.18	98.36 µ = 95.64 – 101.08

The limit of detection (LOD value) was determined for signal/noise (S/N)=3 and the limit of quantification (LOQ value) was determined for S/N=10. The obtained values are presented in table V.

Table V

LOD and LOQ for ondansetron hydrochloride and its impurities

Substance	LOD (µg/mL)	LOD (%)	LOQ (µg/mL)	LOQ (%)
Active substance	0.0015681	0.0007841	0.004752	0.002376
Impurity A	0.0016632	0.0008316	0.00504	0.00252
Impurity C	0.0017424	0.0008712	0.0088	0.0044
Impurity D	0.000792	0.000396	0.02	0.01

The stability of ondansetron and its impurities in solution was studied. The solutions used for assay of ondansetron hydrochloride are stable for 24 hours, at room temperature or in refrigerator (2-8ºC). The impurities solutions are not stable for 24 hours at room temperature or in refrigerator and should be injected immediately after preparation.

A comparative study between the new established method for the assay of ondansetron hydrochloride dihydrate and the USP method was performed [7].

The results are presented in table VI. The F value proved that there is no significant difference between the two sets of measurements.

Table VI
Comparative results for the assay of ondansetron hydrochloride (n=6, P=95%)

Sample type	YMC basic		Spherisorb CN		F value
	Average (%)	Confidence interval (%)	Average (%)	Confidence interval (%)	
Active substance	99.39	98.35 – 100.43	99.73	99.20 – 100.27	1.59
Osetron 2 mg/mL	99.32	98.70 – 99.94	100.60	100.46 – 100.75	3.01

Conclusions

The qualitative and quantitative estimation of ondansetron hydrochloride and its identified impurities, in bulk and pharmaceutical dosage forms, was developed in a new chromatographic system using a *YMC basic* column. The method was validated and the results were similar to those obtained using the current USP monographs.

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