International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

R E S E A R C H A R T I C L E

Novel stability indicating RP-HPLC method for the determination of Ondansetron impurities in Ondansetron Injection

Dr. Srinivas Jagarlapudi*[1], A. Ravi Kumar[2], Panchumarthi Srinivas[2], M. Raveendra Babu[2], P Ramyasree[2]

[1]*Sri Krishnadevaraya University, Anatapuramu. Andhra Pradesh, India-515003.*
[2]*Sree Vidyanikethan College of Pharmacy, Sree Sainath Nagar, Tirupathi, A. Ranganpet, Andhra Pradesh-517102.*

A B S T R A C T

The author developed a novel and unique RP-HPLC method for the determination of all the impurities of Ondansetron with adequate resolution in Ondansetron Injection.The current official USP monograph uses two methods for the determination of Ondansetron impurities in Ondansetron Injection and the resolution between impurity E (imidazole) and impurity F (2-methyl imidazole) is not appropriate. The analytical method uses a Zodiac C18 (250 x 4.6 mm, 3 µm) column and the mobile phase consists of 1-octane sulfonic acid (ion pair), water and adjusted the pH to 2.5 with diluted ortho phosphoric acid with a column temperature of 30°C and injection volume of 25 µL. To finalize the above chromatographic conditions several C18 stationary phases were used. pH condition of the mobile phase was optimized with different pH. To improve the retention time and resolution for impurity E and Impurity-F ion pairing reagent was introduced in the mobile phase. Gradient elution mode was selected for the separation all known and degradants impurities from the analyte peak. The analytical validation covered all the parameters including robustness.

Keywords: Ondansetron injection, Development, Validation, Gradient, Stationary phase, HPLC, ion-pair.

A R T I C L E I N F O

Corresponding Author
Dr Srinivas Jagarlapudi
Sri Krishnadevaraya University,
Anatapuramu. Andhra Pradesh, India-515003.
Email:vasujagarla@gmail.com
MS-ID: IJCTPR3680

PAPER QR-CODE

Article History: Received 19 April 2018, **Accepted** 25 May 2018, **Available Online**15 July 2018

Citation: Dr Srinivas Jagarlapudi, *et al.Novel stability indicating RP-HPLC method for the determination of Ondansetron impurities in Ondansetron Injection*. *.Int. J. Curnt. Tren. Pharm, Res., Res*., 2018, 6(4): 103-109.

C O N T E N T S

1. Introduction

Ondansetron (ODS) is used to prevent nausea and vomiting caused by cancer chemotherapy, radiation therapy, and surgery. ODS blocks the actions of chemicals in the body that can trigger nausea and vomiting. ODS is in a class of medications called serotonin 5-HT3 receptor antagonists. It works by blocking the action of serotonin, a natural substance that may cause nausea and vomiting. While its mechanism of action has not been fully characterized, ODS is not a dopamine-receptor antagonist. Serotonin receptors of the 5-HT3 type are present both peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the area postrema. It is not certain whether ODS's antiemetic action is mediated centrally, peripherally, or in both sites. However, cytotoxic chemotherapy appears to be associated with release of serotonin from the enter chromaffin cells of the small intestine. ODS is a chemically (RS)-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-2,3-dihydro-1H-carbazol-4(9H)-one [1].

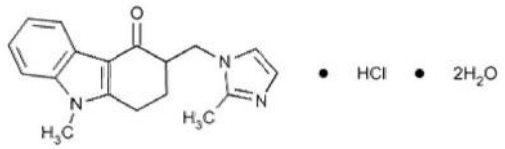

Figure 1: Structure of Ondansetron Hydrochloride

There are several process impurities/related substances associated with the synthesis of ODS. Different process related impurities are observed with various synthetic routes. Five of the known ODS-related substances have been mentioned here; chemical structures for ODS and its related substances, vizimp-A, imp-C, imp-D, imp-E and imp-F. The analytical procedures published in the literature were done on UV [2-3], visible and derivative techniques [4-6] which are not stability indicating methods. High performance thin layer chromatography [7] and LCMS methods [8-9] were used to determine of assay of ODS in biological samples. Capillary Electrophoresis [10] with solid phase extraction method was used to determine enatiomeric selection of ODS. HPLC methods [11-17] were reported in the literature for the determination of ODS. The author in the present work developed common method for determination of all the impurities with adequate separation from ODS in ODS injection. The method was validated as per ICH guidelines [18] and is simple, rapid and reliable gradient RP-HPLC method with UV detection for the determination of ODSimpurities in ODS injection.

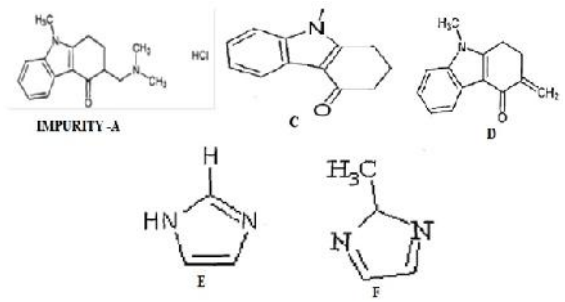

Figure 2: Ondansetron impurities A, C, D,E, F

2. Materials and Methods

Chemicals and Reagents:

1-Octane sulfonic acid (Analytical grade), Acetonitrile (HPLC grade) and Methanol (HPLC grade) were purchased from Merck (Mumbai, India). Milli-Q water produced from Milli- Q system, Merck Millipore (Mumbai, India). ODS working standardODS injection sample & ODS placebo were manufactured at Market Sample Limited (Hyderabad, India). ODS Impurity Aand ODS Impurity D were procured from Clearsynth (Hyderabad, India). ODS Impurity C was procured from TLC Pharma (Hyderabad, India). ODS Impurity E and ODS Impurity Fwere procured from Simson Pharma (Mumbai, India). 0.9% Sodium Chloride Injection and 5%Dextrose Injection were procured from Claris Otsuka Limited (Ahmedabad, India).

Instrumentation and Chromatographic conditions:

The chromatographic system consisted of Agilent 1260 Infinity series model equipped with quaternary pump (DEABJ02269), vacuum degasser (DEABJ02269), auto injector with variable injection valve (DEABE04741), Diode Array Detector (DEAAX01521) and column oven (DEAAK05395). A Zodiac C18 column, 3µm particle size was used for separation. Flow rate and injector volume were 0.9 mL/min. and 25µL respectively. Column oven temperature and wavelength were set at 30°C and 216 nm respectively.

Preparation of Mobile phase:

Mobile phase-A consisted of an ion-pair 0.05M (pH 2.5) and Mobile phase-B consisted of Acetonitrile, Methanol and Waterin the ratio 800:150:50%v/v. Before preceding the analysis mobile phase was degassed and filtrations with 0.45µ membrane filter.

Method Development:

The method development has been started based on the USP official method by using cyano (L10) column 250 x 4.6 mm, 5 µm as a stationary phase, 216 nm detection and the mobile phase containing pH 5.4 phosphate buffer and acetonitrile in the ratio of 50:50 v/v and with the flow rate of 1.5 mL/min. in which the impurities E & F are co-eluted and other impurities are well seperated from the main analyte peak. For separation of these peaks (impurity E & F) gradient elution method was initiated and found that there is no separation observed between these impurities. If the percentage of organic ratio is more than 5% of the actual value, both impurities E & F will be co-eluted with less than 1.0 resolution. Phosphate buffer, perchlorate buffer with different combinations of 1-pentane sulfonic acid sodium salt, 1-heptane sulfonic acid sodium salt and 1-octane sulfonic acid sodium salts were used at different pH (2.5, 3.0 & 5.4) to improve the resolution between impurity E & F. 1-octane sulfonic acid sodium salt with pH 2.5 showed better resolution between impurity E & F. The reverse phase stationary columns C18, C8, C2, phenyl, pentafluorophenyl and amino columns were used for the separation of impurity E & F. However, better resolution for impurities E & F were achieved in Zodiac C18 stationary phase column.

Statistical Analysis:

All the data were analyzed using Graph Pad Prism 6 software (Graph Pad software Inc., La Jolla, CA, USA).

The results were expressed as Mean +SD and % RSD. A value of $p<0.5$ was considered as significant, $p<0.05$ as highly significant while $p<0.005$ was considered as extremely significant. A value of $p>0.5$ was considered to be statistically insignificant.

3.Results and Discussion

Impurities Validation

The proposed HPLC method is validated as per ICH Q2 (R1). System suitability, method precision, intermediate precision, linearity, accuracy, specificity and forced degradation, solution stability, LOD and LOQ, pH variation, organic variation, temperature and flow variation were performed as a part of validation. The results for the validated parameters were discussed in the following sections.

Validation Parameters

Specificity:

A study was conducted to demonstrate the interference of placebo and blank at the retention time of known impuritiesand ODS peak. Placebo was prepared in duplicate and injected into HPLC system. The impurities were prepared as per stability specification level and the results are presented in Table 1.Specificity of the Ondansetron peak in the presence of placebo (n=2 preparations) and known impurities.

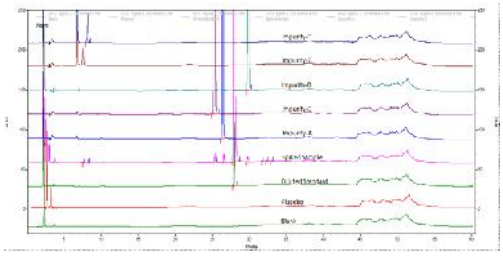

Figure 3: Overlay Spectra of Specificity

Linearity:

To demonstrate the linearity, impurity standard solutions (Impurity A, C, D, E & F) of ODS were prepared with concentration ranging from LOQ to 300% of the sampletarget concentration. The peak area ratio of the drug was considered for plotting the graph between concentration and response.Regression analysis shows linear relationship between concentration and the response of Pemetrexed for injection with in specified range. The linearity was evaluated by linear regression analysis and was calculated by the least square regression method and presented in Fig. 4, 5, 6, 7, 6 and 8) and Table 2,3,4

.

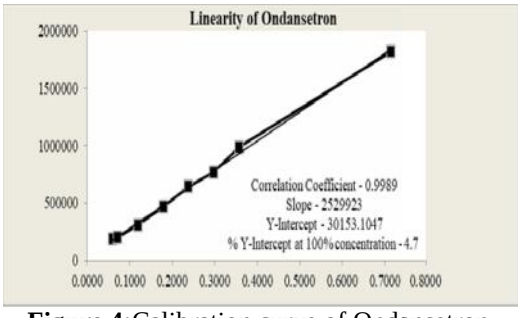

Figure 4: Calibration curve of Ondansetron

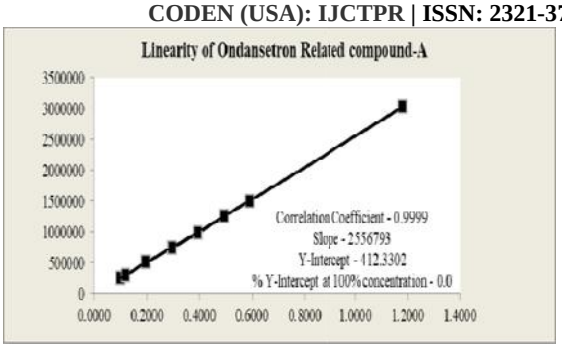

Figure 5: Calibration curve of Ondansetron impurity-A

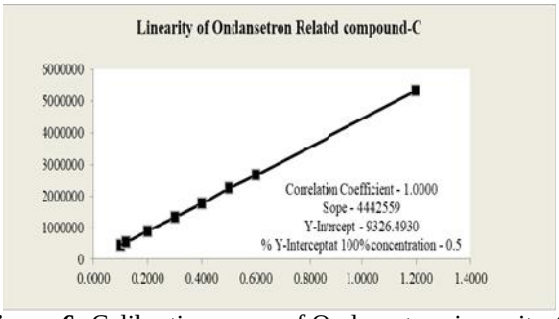

Figure 6: Calibration curve of Ondansetron impurity-C

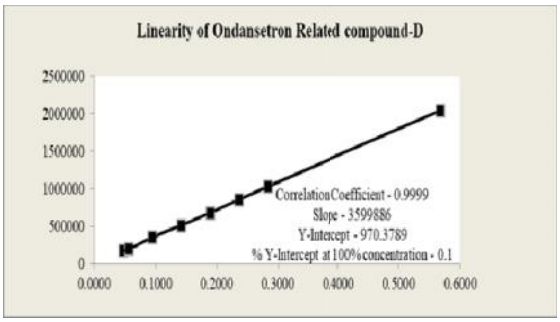

Figure 7: Calibration curve of Ondansetron impurity-D

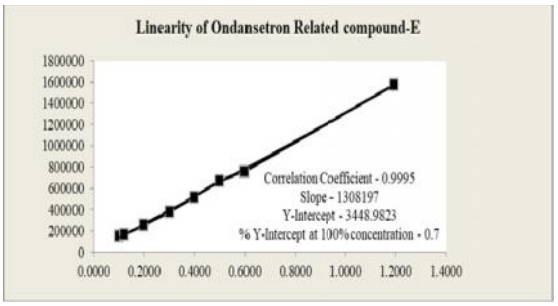

Figure 8: Calibration curve of Ondansetron impurity-E

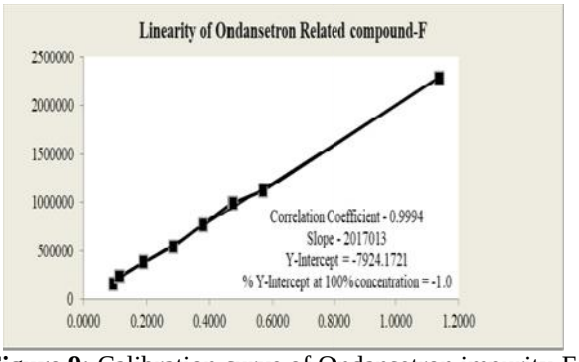

Figure 9: Calibration curve of Ondansetron impurity-F

Accuracy: Known amount of analyte, impurity standard solutions (Impurity A, C, D, E & F) of ODS were prepared with concentration ranging from LOQ to 300% of the stability specification of impurities and analyzed by the proposed method. The results are tabulated in the following Table 5 and 6.

Precision

To demonstrate the reproducibility of the method, six samples from the same batch of formulation werespiked with the known impurities as per stability specification level and analyzed individually. The % RSD for six determinations was calculated for each determination and presented in Table 7.Precision analysis of Ondansetron impurities in Ondansetron pharmaceutical dosage form of different analyst on different days (n=6) for each analyst with mean, variance and relative standard deviation was calculated.

LOQ and LOD

The LOD/LOQ of ODS & impurities were established by Slope method. The concentrations are presented inTable 8.

Forced Degradation studies:

Forced degradation studies were conducted to establish the stability indicating capability of the method. For each stress experiment, drug product was stressed under conditions shown in below Table 6and results are presented in Table 9.Peak purity establishment for Ondansetron impurities in acid stress, base stress, oxidation, UV light, water, Humidity and thermal stress (n=1 for each condition).

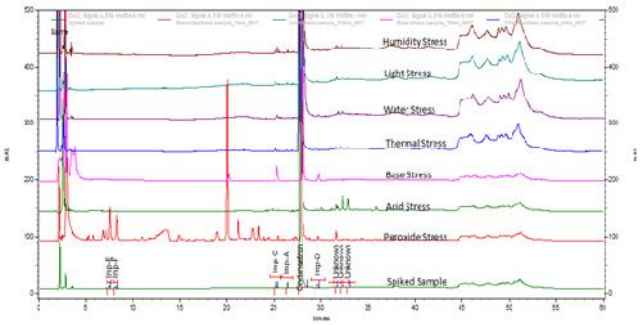

Figure 10: Overlay Chromatogram of Forced degradation Studies

Robustness

System suitabilityand impurity spiked samples were injected into HPLC after making deliberate changes in the mobile phase like flow rate (0.2 mL/min)and pH variation (±0.2) from developed HPLC conditions. Results are tabulated in Table 10.

Solution stability test

Spiked impurity sample solution and standard solutions (2 ppm) were prepared and stored at ambient temperature and refrigerated conditions. The analysis of each solution was repeated at periodic intervals covering a time period of 72 hrs. Standard solutions were stable for 72 hr when stored at room temperature and at refrigerated conditions. Sample solutions were stable for 48 hr when stored at room temperature and 72 hr when stored at refrigerated conditions.

Table 1: Specificity results

S.No	Standard/Impurity Name	Retention Time
1	Placebo	Not Detected
2	Ondansetron	27.687
3	Ondansetron Impurity-A	26.307
4	Ondansetron Impurity-C	25.120
5	Ondansetron Impurity-D	29.560
6	Ondansetron Impurity-E	7.413
7	Ondansetron Impurity-F	8.027

Table 2: Linearity data of Ondansetron

S.No	Level	PPM	Peak area
1	LOQ	0.06	190535
2	30%	0.07	198918
3	50%	0.12	310071
4	75%	0.18	468319
5	100%	0.24	646513
6	125%	0.3	770092
7	150%	0.36	987786
8	300%	0.71	1817035
Correlation Coefficient			**0.9989**
Slope			**2529923**
Y-Intercept			**30153.1047**
% Y-Intercept at 100% concentration			**4.7**

Table 3: Linearity results for Impurities A, C and D

S. No	Level	Impurity A		Impurity C		Impurity D	
		PPM	**Peak area**	**PPM**	**Peak area**	**PPM**	**Peak area**
1	LOQ	0.1	265753	0.1	461839	0.05	173394

2	30%	0.12	300420	0.12	540565	0.06	192985
3	50%	0.2	514266	0.2	898391	0.09	356145
4	75%	0.3	746531	0.3	1326670	0.14	511234
5	100%	0.39	989946	0.4	1766583	0.19	673042
6	125%	0.49	1262351	0.5	2251832	0.24	855362
7	150%	0.59	1507017	0.6	2674015	0.28	1027850
8	300%	1.18	3028334	1.2	5333075	0.57	2043533
Correlation Coefficient			**0.9999**		**1.0000**		**0.9999**
Slope			**2556793**		**4442559**		**3599886**
Y-Intercept			**412.3302**		**9326.4930**		**970.3789**
% Y-Intercept at 100% concentration			**0.0**		**0.5**		**0.1**

Table 4: Linearity results for Impurities E and F

S. No	Level	Impurity E		Impurity F	
		PPM	**Peak area**	**PPM**	**Peak area**
1	LOQ	0.1	151601	0.09	155825
2	30%	0.12	167042	0.11	234266
3	50%	0.2	253691	0.19	383461
4	75%	0.3	379148	0.28	545857
5	100%	0.4	520978	0.37	768605
6	125%	0.5	669687	0.46	992710
7	150%	0.6	761094	0.56	1118539
8	300%	1.19	1573330	1.11	2282100
Correlation Coefficient			**0.9995**		**0.9994**
Slope			**1308197**		**2017013**
Y-Intercept			**3448.9823**		**-7924.1721**
% Y-Intercept at 100% concentration			**0.7**		**-1.0**

Table 5: LOQ recovery of Ondansetron, Impurity A, C, D, E and F

Sample	**% of Recovery**					
	Ondansetron	Imp-A	Imp-C	Imp-D	Imp-E	Imp-F
Preparation-1	127.1	93.6	94.1	98.5	94.8	82.2
Preparation-2	126.4	98.7	101.3	93.0	115.7	92.1
Preparation-3	116.0	102.9	98.5	90.1	108.7	76.5
Preparation-4	122.0	95.2	98.9	92.2	99.0	85.1
Preparation-5	94.8	102.1	93.5	108.7	123.3	79.9
Preparation-6	125.7	101.7	98.1	95.6	110.9	71.2
Average	118.7	99.0	97.4	96.4	108.7	81.2
SD	12.40	3.89	3.01	6.71	10.53	7.19
%RSD	10.45	3.93	3.09	6.97	9.68	8.86

Table 6: Recovery of Ondansetron, Impurity A, C, D, E and F

Sample	**% of Recovery**					
	Ondansetron	Imp-A	Imp-C	Imp-D	Imp-E	Imp-F
50% Preparation-1	94.8	96.0	95.6	94.7	98.3	102.1
50% Preparation-2	94.8	97.5	96.6	105.1	85.2	97.9
50% Preparation-3	94.8	93.9	92.0	94.9	89.3	99.6
100% Preparation-1	98.1	92.7	93.5	89.9	95.9	100.4
100% Preparation-2	103.3	92.8	92.5	95.00	93.3	98.1
100% Preparation-3	100.8	91.2	92.5	93.5	90.9	101.8
150% Preparation-1	96.0	93.8	94.3	95.2	96.3	100.2
150% Preparation-2	98.1	93.7	93.4	95.00	89.8	93.8
150% Preparation-3	113.7	93.3	94.2	93.4	86.8	97.4
300% Preparation-1	94.1	94.2	93.6	93.9	92.3	100.3
300% Preparation-2	93.5	93.9	93.7	93.8	96.3	99.3
300% Preparation-3	93.7	93	94	93.4	95.3	98.4
300% Preparation-4	94.2	93.6	93.5	92.6	88	97.8
300% Preparation-5	96.6	96.2	93.7	94.1	96.2	100.4
300% Preparation-6	93.9	93.5	93.8	96.1	95.8	98.2

Average	97.36	93.95	93.79	94.71	92.65	99.05
SD	5.33	1.56	1.15	3.21	4.08	2.05
%RSD	5.48	1.66	1.23	3.39	4.40	2.07

Table 7: Precision results for Ondansetron impurities

Parameter		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Avg	Std. dev	% RSD
Imp A	I.P	0.186	0.18	0.181	0.185	0.182	0.181	0.183	0.004	2.05
	M.P	0.184	0.185	0.182	0.184	0.173	0.186			
Imp C	I.P	0.189	0.187	0.185	0.192	0.186	0.188	0.190	0.003	1.59
	M.P	0.190	0.192	0.192	0.191	0.195	0.194			
Imp D	I.P	0.116	0.115	0.115	0.119	0.115	0.123	0.121	0.006	4.92
	M.P	0.117	0.122	0.119	0.120	0.136	0.124			
Imp E	I.P	0.193	0.191	0.198	0.197	0.192	0.204	0.198	0.004	2.03
	M.P	0.202	0.197	0.197	0.201	0.200	0.197			
Imp F	I.P	0.142	0.146	0.138	0.143	0.141	0.147	0.144	0.003	2.28
	M.P	0.140	0.148	0.145	0.149	0.143	0.142			

I.P –Intermediate precision; M.P –Method precision

Table 8: Establishment of LOD and LOQ by using slope method

Peak	Conc. of LOQ(PPM)	Conc. of LOD(PPM)
Ondansetron	0.05	0.02
Ondansetron Imp-A	0.10	0.04
Ondansetron Imp-C	0.10	0.04
Ondansetron Imp-D	0.05	0.02
Ondansetron Imp-E	0.10	0.04
Ondansetron Imp-F	0.10	0.04

Table 9: Results forced degradation Studies

Stress Type	% Assay	% Degradation	Peak Purity
Control sample	100.1	0.175	**Pass**
Acid Hydrolysis	99.3	0.797	**Pass**
Base Hydrolysis	99.7	0.375	**Pass**
Oxidation	85.4	14.72	**Pass**
Thermal	100.1	0.091	**Pass**
UV (photolytic)	100.1	0.093	**Pass**
Water	100.1	0.124	**Pass**
Humidity	**100.1**	**0.174**	**Pass**

Table 10: Robustness results

Parameter	Flow Rate variation			pH variation		
	Actual (0.9 mL/min)	Change-1 (0.7mL/min)	Change-2 (1.1mL/min)	Actual pH 2.5	Change-1 pH 2.3	Change-2 pH 2.7
Recovery of standard sensitivity solution	98.45	102.00	103.95	98.45	101.43	98.03
The % RSD for 3 replicate injections of diluted standard solution	0.14	0.36	1.19	0.14	0.53	0.09
The Theoretical plates of diluted standard	411204	421761	356107	411204	419166	419915

4. Conclusion

The developed analytical method to determine the impurities of ODS in ODS Injection is precise, accurate, linear and stability indicating. All the validation parameters are validated spastically according to ICH guidelines Q2(R1). The above method is novel, rapid and cost effective when compared with other analytical methods and can be employed in commercial scale for the impurities of ODS in ODS Injection rather than using two or more methods for analysis.

5. References

[1] USP (38) NF-Online (33) (2015) 4641.
[2] S. Pillai, I. Singhvi, Indian J.Pharm.Sci. 69(2007) 601.
[3] A. Raza, A. Subhan, J. Chinese Chem. Soc.54 (2007) 223.
[4] P. Ravi Kumar, M. Murali Krishna, P. Bhanu Prakash, E-J. Chem. 3(2006) 134.
[5] P. Shirish, L.J. Patel, Asian J. Pharm.Clin. Res.5 (2012) 2.
[6] L. Zamora, T. Francis 29(1996) 785.
[7] P. B. Raval, S. J. Wadher, P. G. Yeole, Indian J. Pharm.Sci. 70(2008) 386.
[8] L.Pang, Q. Wang, Y. Wang, Latin A. J. Pharm. 31 (2012) 305.
[9] Y. Dotsikas, C Kousoulos, G Tsatsou, J Chromatogr. 836(2006) 79.
[10] M. Siluveru, J Chromatogr. 691 (1997)217.
[11] S. Ravi, Y. Darwis, N. Khan, Chrom.graph.70 (2009)75.
[12] A. Varvara, C-M.Monciu, C. Arama, C. Popescu, Farmacia 56 (2008) 154.
[13] A. Varvara, C-M.Monciu, C. Arama, C.Popescu, Farmacia, 57 (2009) 570.
[14] J.W.Kelly, J. Chromatogr, 622 (1993) 291.
[15] S.R. Patel, J. Patel, P.Yogeshvar,Int. J. Chem.Tech. Res.2 (2010) 1531.
[16] Z. Dedania, R. Dedania, V. Karkhanis, Asian J. Res. Chem.2 (2009) 2.
[17] M. D. M.Basha, B. Praveena, M. Srinidhi, SK.A. Rahaman, Int. J.PharmTech Res.5 (2013) 86.
[18] ICH Q2 (R1), Geneva (2005) 1.