ISSN: 2348-8948
Vol: 3; Issue: 6

RESEARCH ARTICLE

SYNTHESIS, CHARECTERIZATION, DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR PROCESS RELATED IMPURITY IN NIMODIPINE BULK AND FORMULATION

LUBNA SHAIKH[1], VISHAL PANDE[2], DEEPAK MUSMADE[3]

1.Research Scholar, Department of Quality Assurance Techniques
2.Associate Professor and Head, Department of Pharmaceutics and Quality Assurance Techniques
3.Assistant Professor, Department of Pharmaceutical Chemistry, Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Ahmednagar, Maharashtra, India.
E-mail: shaikh.lubna@ymail.com

Date Received:	Date of Accepted:	Date Published:
28-May-2015	18-Jun-2015	30-Jun-2015

Abstract:
The synthesis, characterization and quantitation of process related impurity in Nimodipine i.e.Diethyl 2, 6-dimethyl-4-(2-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate bulk and tablet formulation was performed by using Hantzch pyridine synthesis. This synthesis includes o-nitrobenzaldehyde, ethylacetoacetate in presence of ammonia and methanol as catalyst. The percentage yield was found to be 79%. The impurity was recrystallized and preliminary evaluation was done on lab scale viz. Melting point, TLC and elemental analysis. The melting point of impurity was found to be 156-158^{0}C. The TLC of impurity was carried out by using benzene and methanol (6:1) and the Rf was found to be 0.78 the conformation of synthesized Nimodipine impurity was carried out by using sophisticated instrument such as, FT-IR, NMR, GC-MS, and RP-HPLC method was developed to identify and quantify the Nimodipine impurity in bulk and formulation as per ICH Q2B guidelines. The method was found to be linear, precise, accurate, robust and rugged. Finally 1, 4-Dihydro-2, 6- Dimethyl-4-(o-nitrophenyl) pyridine-3, 5 dicarboxylate(Nimodipine Impurity) was quantified from bulk Nimodipine and its marketed tablet formulation. It was concluded that the amount of Nimodipine impurity, present in tablet was found to be 0.0876%and in bulk 0.0219% respectively.

Keywords: Impurity, IR, NMR, GC-MS, RP-HPLC, Validation.

Introduction

Chemically, Nimodipine is known as Isopropyl 2-methoxy ethyl 1, 4- Dihydro2, 6-dimethyl-4-(3-nitrophenyl)pyridine-3, 5-dicarboxylate.[1]It is a dihydropyridine calcium channel blocker and used for the treatment of high blood pressure. During the manufacturing process of an active pharmaceutical substance or product, some intermediates are formed. These intermediates may affect the safety and efficacy issues of the pharmaceutical products.[2]Pharmaceutical impurities are the unwanted chemicals that remain with the active pharmaceutical ingredients (API'S).[3]Various regulatory authorities like ICH, USFDA, Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in Active pharmaceutical ingredients (API) [4, 5]According to ICH guidelines on impurities in new drug products, identification of impurities less than 0.1% level is not considered to be necessary, unless potential impurities are expected to be unusually toxic or potent.

According to ICH, the maximum daily dose qualification threshold is considered as follows; ≤2g/day 0.1% or 1mg per day intake (whichever is lower) ≥ 2g/day 0.05 %[6]

Materials and Methods

Chemicals: O-nitrobenzaldehyde (AR), Ethylacetoacetate (AR), Ammonia (AR), Methanol(AR), Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (HPLC grade) were purchased from Merck chemicals, India.

Method

UV- Visible Spectrophotometer

The UV detection at wavelength 234 nm was selected by using UV- Vis Spectrophotometer (UV- 1650 PC) SHIMADZU INC.

FT-IR

The IR spectra were recorded by using Fourier Transform Infrared spectrophotometer by KBr press pellet technique.

NMR

Characterization of impurities was achieved by NMR using $CDCL_3$ as a solvent. The ^1H and ^{13}C NMR chemical shift values were reported on the delta scale in ppm.

GC-MS

The Q- TOF Micro mass (YA-105) spectrometer capable of recording High Resolution Mass Spectrum (HRMS) both in atomic pressure chemical ionization (APCI) and Electron spray Ionization (ESR) were used for characterization of Nimodipine impurity.

RP-HPLC

The HPLC method was developed by using LC20AD Prominence Liquid Chromatography SPD 20-A Shimadzu, Japan. The UV- Vis detector and C18 column with dimension on 250x 4.6 mm was used for the HPLC method Development having flow rate of 0.8 ml/min at wavelength 234 nm. The Methanol:Acetonitrile: Water (35:40:25v/v/v) as a mobile phase was selected for development of validated method of Nimodipine impurity and various parameters according to ICH guidelines (Q2B) were studied.

Chromatographic Conditions-

Preparation of Mobile Phase

The selection of mobile phase was according to polarity & non polarity of solvents. The methanol: acetonitrile: water was selected as mobile phase in ratio of 35:40:25(v: v: v) and was filtered on membrane filter (0.45µ) to remove degassing.

Preparation of Stock Solution

The stock solution of 100µg/ml was prepared by dissolving 10mg Nimodipine impurity in 100 ml mobile phase. The dilution was prepared in various concentrations using stock solution and dissolved in mobile phase.

Preparation of Sample Solution (Formulation)

The sample solution of Nimodipine formulation was prepared as 100µg/ml stock solution for concentration using sample stock and dissolved in mobile phase for quantification of Nimodipine impurity in Nimodipine formulation.

Procedure

1.52 gm of o- nitrobenzaldehyde and 2.60 ml of ethylacetoacetate were added in round bottom flask. Then 5 ml of ammonia and 15 ml of methanol was added and stir vigorously. Refluxed for 5-6 hrs and the solution was poured in ice cold water and kept for overnight in freezer. Filtered at vaccum filter and recrystallized from Methanol.

Results and Discussion

Physicochemical properties:

UV Data[7, 8]

The λmax for Nimodipne impurity was found to be 234nm. Calibration curve data was constructed in the range of 2-18 µg/ml. Beer's law was obeyed over this concentration range. The correlation coefficient (R_2) was found to be 0.994. The regression equation Y= 0.053x + 0.035 was found to be linear.

IR Data

The major functional groups are primary amine, nitro and carbonyl groups. Obtained peaks in IR spectrum are as follows.

IR (KBr) cm^{-1}: 3327(NH-Stretch), 2937, 2978, 3078(C-H Stretch for aromatic), 2802, 2874(C-H Stretch for aliphatic), 1683(C=O Stretch), 1610(C=C Stretch), 1487, 1529(N-O Stretch), 1452(CH_3 Bend), 1357(NO_2 Stretch), 900-700(Oop), 833(Substitution at ortho position of benzene ring).[9, 10]

NMR Data

^{1}HNMR ($CDCL_3$)

δ = 5.830(1H,NH of 1,4-dihydropyridine), 1.159(6H,CH_3 of 1,4-dihydropyridine), 4.066 (4H,CH_2 proton of ester), 2.303(6H,CH_3 proton of ester), 6.498(1H, CH of 1,4-dihydropyridine ring), 7.283(2H, CH of nitrobenzene ring), 7.689(2H, CH of nitrobenzene ring). [10]

^{13}CNMR ($CDCL_3$)

δ= 13.99(2C, CH_3 Carbon attached to CH_2), 50.86(2C, CH_2 Carbon attached to CH_3), 167.34 (2C, Carbonyl carbon attached to 1,4- dihydropyridine ring), 19.15(2C, CH_3 Carbon attached to 1,4- dihydropyridine ring),

132.75(2C, C=C of 1,4- dihydropyridine ring), 34.41(1C, Carbon of 1,4- dihydropyridine), 147.49(6C, Carbon of phenyl ring).[10]

GC-MS Data

Gas Chromatography of Nimodipine Impurity shows a single peak at 26.62 min which indicates purity of synthesized Nimodipine Impurity. Mass spectrum at 26.62 minwas recorded. Peak appear at 374 indicates presence of molecular ion peak. Major base peak at 357 shows 100% abundance.[11, 12]

HPLC Method Development

Validation experiment was performed to demonstrate system suitability, linearity, precision, accuracy study, ruggedness and robustness as per ICH Q2B guidelines.

System Suitability Parameters

The area of respective concentrations, theoretical plates, number of theoretical plates per cm, Tailing factor and the peak symmetry was recorded.

Linearity

Dilution of standard impurity in the range of 2-12µg/ml were prepared by taking suitable aliquots of working standard solution in different 10ml volumetric flasks and diluting up to the mark with mobile phase. 20µl was injected from it each time on column at flow rate of 0.8ml/min. The standard from elute was monitored at 234 nm and corresponding chromatogram were obtained from these chromatograms peak area were calculated. A plot of peak area over concentration was constructed. Regression of the plot was computed by least square regression method.

Precision

Precision of analytical method was studied by multiple injections of homogeneous samples. 6 replicate of 4 ppm solution were prepared and injected for precision at the same flow rate of 0.8ml/min. The inter-day and intermediate precisions were used to study the variability of the method S.D. and %R.S.D. was calculated for both.

Accuracy

Accuracy of the method was studied using the method of standard addition. Standard impurity solutions were added to the unknown bulk and tablet formulation of Nimodipine. The percent recovery was determined at three different levels (50%, 100%, and 150%). Impurity content was determined and the percent recovery was calculated.

Robustness

Robustness was studied by changing parameters like change in flow rate. The S.D. and %R.S.D. between the change parameter were calculated.

Ruggedness

Ruggedness studied was carried out by using different analysts. The S.D. and %R.S.D. were calculated.

LOD and LOQ

Limit of detection and limit of Quantitation of the method was calculated by formula given below,

LOD= 3.3×S.D/Slope

LOQ=10×S.D./Slope

Quantitation of Impurity

The total amount of impurity present in Nimodipine bulk and formulations was calculated for synthesized compound and the result was compared to ICH limit for impurities in new drug substance is 0.1%.[13-18]

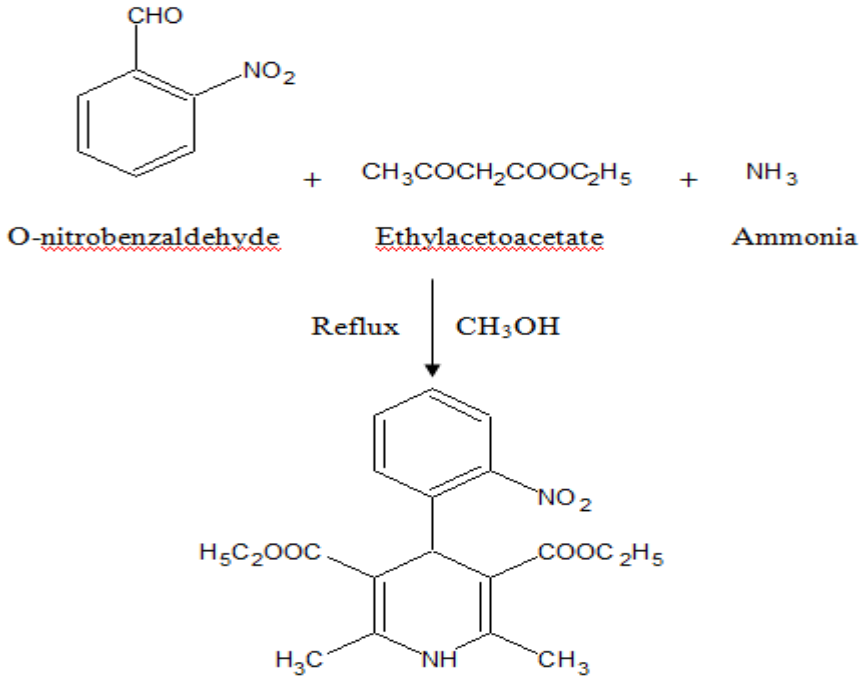
Conclusion

The synthesis of process related impurity of Nimodipine was successfully carried out by suitable synthetic procedure. The preliminary evaluation was done on laboratory scale such as melting point, TLC, and elemental analysis. The characterization of synthesized impurity was performed by IR, ^{1}HNMR, ^{13}CNMR and GC-MS. Based on the spectral data, the structure of impurity was characterized as diethyl 1, 4- dihydro-2, 6-dimethyl-4(o-nitro phenyl) pyridine-3, 5 dicarboxylate. An efficient isocratic RP-HPLC method was developed and validated to identify and quantify the process related impurity in Nimodipine bulk and formulation. The method was found to be linear, precise, specific, sensitive and accurate. The amount of impurity present in tablet and bulk was found to be 0.0876% and 0.0219% respectively. Thus, it was found that the impurity was found to be within the limit laid down as per ICH guidelines (not more than 0.1%). Thus from the present study we can conclude that impurity profiling may act as a prominent quality control tool in pharmaceutical analysis of API.

Acknowledgement

Authors wish to express their sincere thanks to Principal, SRES's Sanjivani College of Pharmaceutical Education and Research Kopargaon, for his constant encouragement and support.

Synthesis of Nimodipine Impurity



Diethyl 2, 6-dimethyl-4-(2-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate.

Figure 1 Scheme for Synthesis of Nimodipine Impurity

Table 1 Physicochemical Properties of Nimodipine Impurity

Molecular Formula	Molecular Weight	M.P.°C	Rf Values	% Yield
$C_{19}H_{22}N_2O_6$	374	156-158	0.78	79%

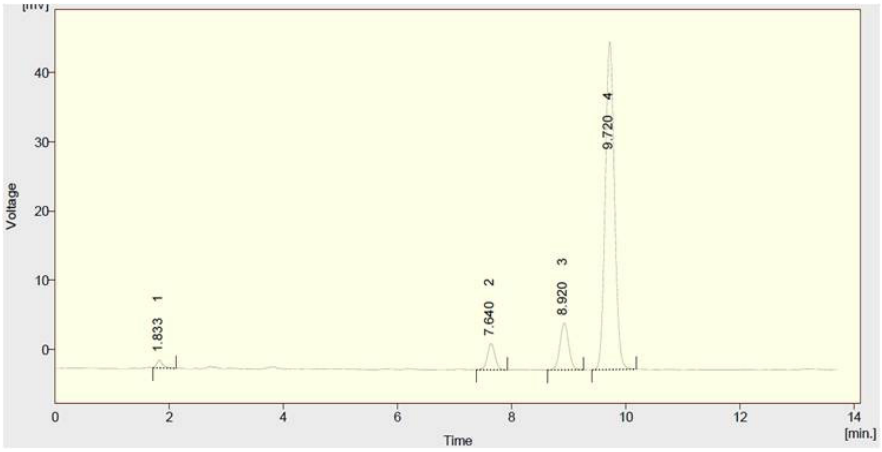


Figure 2: HPLC Chromatogram of Nimodipine.

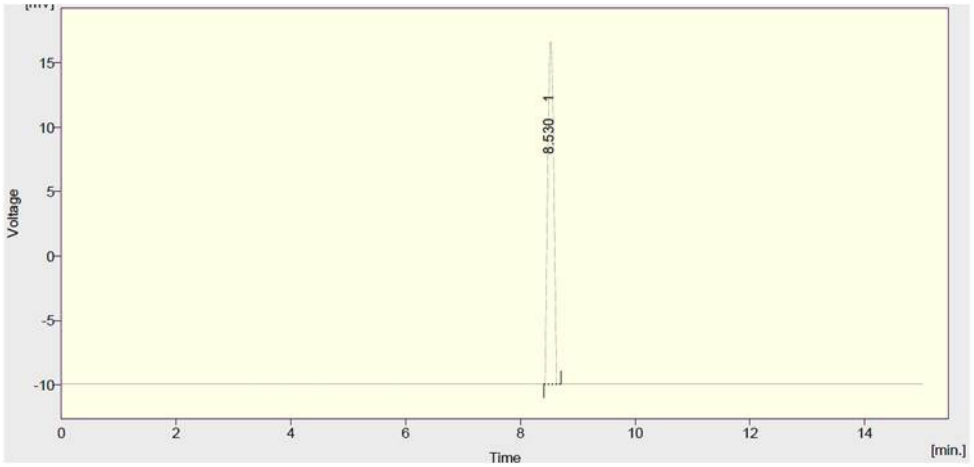


Figure 3: HPLC Chromatogram of Nimodipine Impurity

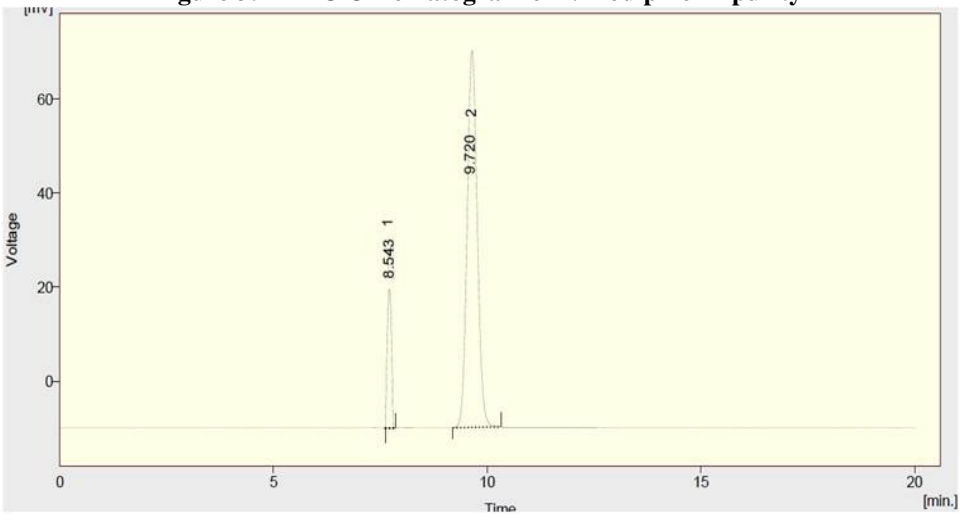


Figure 4: HPLC Chromatogram of Nimodipine Impurity and Nimodipine Mixture

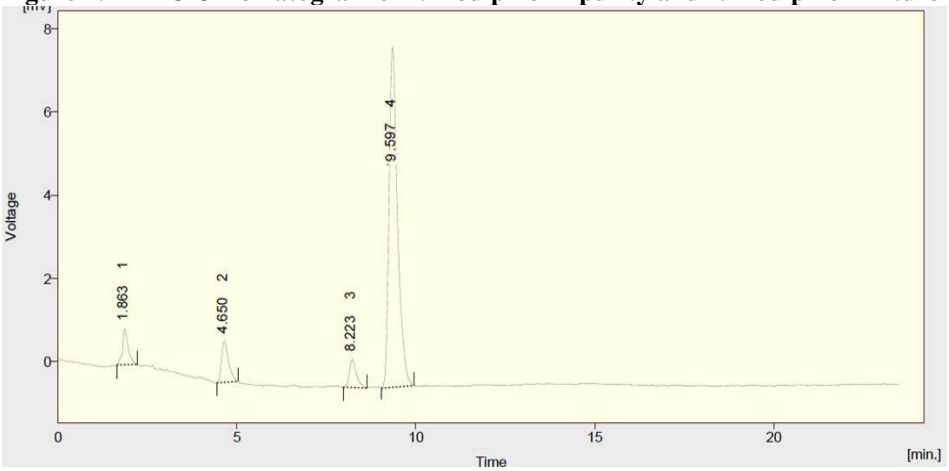


Figure 5: HPLC Chromatogram of Nimodipine Tablet

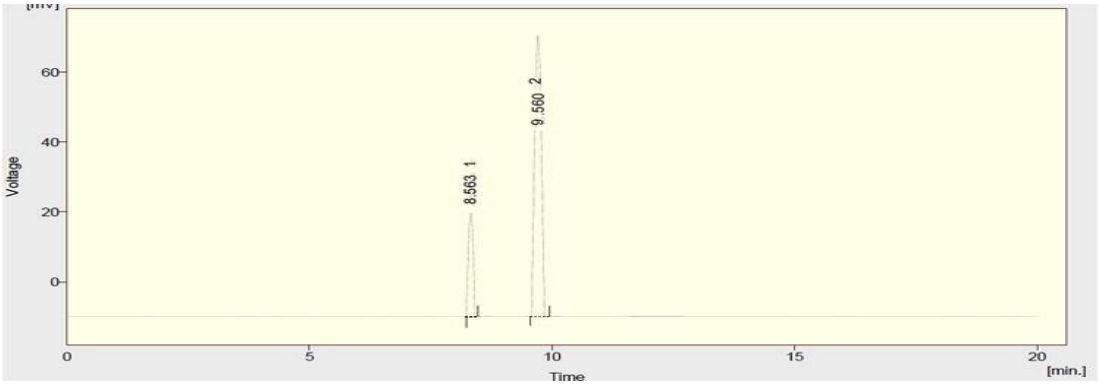


Figure 6: HPLC Chromatogram of Nimodipine Impurity and Tablet Mixture

Table 2 Linearity Data of Nimodipine Impurity.

Parameter	Observation
Linearity Range	2-12 µg/ml
Slope	14.97
Intercept	48.96
Correlation Coefficient	0.980
LOD	0.2177 µg/ml
LOQ	0.6597 µg/ml

Table 3 Result of Repeatability Studies.

Parameter	SD	%RSD
Precision	0.987	0.824
Intraday Precision	0.901	0.755
Interday Precision	1.279	1.046
Robustness	1.970	0.467
Ruggedness		

Table 4 Result Accuracy Study of Nimodipine impurity.

Drug/ Formulation	Amount of Drug (µg/ml)	Amount of Impurity Added (µg/ml)	Amount Recovered (µg/ml)	Percentage Recovery	Mean	SD	%RSD
	4	2(50%)	5.82	97			
Bulk	4	4(100%)	7.89	98.62	98.27	1.140	1.160
	4	6(150%)	9.92	99.2			
	4	2(50%)	5.87	97.83			
Tablet	4	4(100%)	7.91	98.87	98.73	0.843	0.854
	4	6(150%)	9.95	99.5			

Table 5: System Suitability Parameters.

Property	Values	Official limits
Retention time (t_R)	8.530	-
Theoretical plates (N)	4614	N≥ 2000
Resolution (R)	4.310	R≥ 2
Tailing factor (T)	0.96	T≤ 2

Table 6: Quantitation of Nimodipine Impurity in Bulk and Tablet.

Bulk/ Formulation	Quantitation of Nimodipine Impurity
Bulk Nimodipine	0.0219%
Nimodipine Tablet	0.0876%

References

1. British Pharmacopoeia, Volume 2nd, The Department of Health, British Pharmacopoeia commission Office, 1st edition, (2011):1547-1548.
2. Ahuja, S, Impurities Evaluation of Pharmaceuticals, 1st edition, Marcel Dekker: 85-108, (2006)
3. Solanki, R., 2012. Impurity Profiling of Active Pharmaceutical Ingredients and Finished Drug Products. Int. J. Drug. Res. Tech. 2, 231-238.
4. Shah, R.S., Patel, M.A., Naik, M.V., Pradhan, P.K., 2012. Recent Approaches of Impurity Profiling in Pharmaceutical Analysis: A Review. Int. J. Pharm. Sci. Res. 3, 3603-3617.
5. Bari, S.B., Kadam, B.R., Jaiswal, Y.S., Shirkhedkar, A.A., 2007. Impurity Profile: Significance in Active Pharmaceutical Ingredient. Eurasian J. Anal. Chem. 2, 32-53.
6. Federal Register, International Conferences on Harmonization, Impurities in New Drug Products, European Medicines Agency, and Q3B (R2), 2006: 3-14.
7. Beckett, A.H., Stenlake, J.B, Practical Pharmaceutical Chemistry, 4th edition, Althone Press: 115-119 (1988)
8. Skoog, D.A., Holler, F.J., Crouch, S.R, Fundamentals of Analytical Chemistry, 8th edition, Cengage learning: 411-420 (2004)
9. Silverstein, R.M., Webster, F.X, Spectrometric Identification of Organic Compounds, 6th edition Wiley India: 81-109 (2010)
10. Pavia, D.L., Lampman, G.M., Kritz, G.S., Vyvyan, J.R, Spectroscopy, 8th edition, Cengage Learning: 38-39, 126-129, 177-178 (2007)
11. Tamer, A., Clark, C.R., DeReuiter, J, 2007. GC-MS Analysis of Acylated Derivatives of The Side-Chain Regioisomers of Studies on Side Chain Regioisomers of 4-Methoxy-3-Methyl-Phenylamines Related to Methylene dioxymethamphetamine. J. Chromatogr. Sci. 45, 477-485.
12. Hadir, M.M., Tamer, A., DeRuiter, J., Clark, C.R., 2012. GC-MS and GC-IRD Studies on Dimethoxyphenylamines (DMPEA): Regioisomes Related to 2, 5-DMPEA. J. Chromatogr. Sci. 50, 1-9.
13. Federal Register, International Conferences on Harmonization, Guidance for Industry, Validation of Analytical Procedures: Methodology U.S. Department of Health and Human Services Food and Drug Administration, (CDER), (CBER), Q2B, (1996):1-10
14. Patil, P., Vaidya, I., 2013. Overview on Impurity Profiling. IJPRS. 2, 54- 65.
15. Snyder, L.R., Kirkland, J.J., Glajch, J.L., 1997. Practical HPLC Method Development, 2. Wiley and Sons, New York, 31-42.
16. Chandorkar, J.G., Kotwal, V.B., Dhande, N.S., Gurav, S.G., Pande, V.V., Yadav, P.V., 2008.A Sensitive HPLC Method For Simultaneous Estimation of Tamsulosin Hydrochloride and Its Impurity. Pak. J. Pharm. Sci. 21, 307-310.
17. Chandorkar, J.G., Kotwal, V.B., Dhande, N.S., Pachpor, M.P., Pande, V.V., 2008. Development and Validation of High Performance Liquid Chromatography Method For Analysis of Sibutramine Hydrchliride and Its Impurity. Pak. J. Pharm. Sci. 21, 121-124.
18. Shaikh, L.B., Pande, V.V., Musmade, D.S., Patil, P.P., 2015.Development and Validation of RP-HPLC Method for Estimation of Process Related Impurity in Nimodipine Bulk and Formulation. Der Pharmacia Lettre. 7, 287-290.