Chemistry & Biology Interface, 2016, 6, 1, 38-46

RESEARCH PAPER ISSN: 2249 –4820

CHEMISTRY & BIOLOGY INTERFACE

An official Journal of ISCB, Journal homepage; www.cbijournal.com

An Isocratic Assay Method Validation for the Determination of Five Most Potent Antihypertensive Drugs by a New Generation Liquid Chromatographic Technique

Hetal Jebaliya, Madhavi Patel, Batuk Dabhi, Yashwantsinh Jadeja, Anamik Shah*

Center of Excellence, NFDD Complex, Department of Chemistry, Saurashtra University, Rajkot – 360 005, India
**E-mail: anamik_shah@yahoo.com*
Received 4 November 2015; Accepted 27 January 2016

Abstract: The present investigation describes the development and validation of RP-UPLC method for the assay of Guanfacine hydrochloride, Sildenafil, Irbesartan, Losartan potassium and Indapamide in tablets by use of isocratic mobile phase. The chromatographic analysis was performed using Acquity UPLC @ HSS C18 (50mm X 2.1mm id, 1.8µm particle size) column with 40°C column oven temperature. The isocratic mobile phase was consisted 0.1% OPA and Acetonitrile (68:32, v/v). The detection was monitored at wavelength of 211nm. The flow rate was adjusted at 0.3ml/min with 0.5µl injection volume. Total analysis takes 3.5 minutes.

Keywords: An Isocratic, Assay Method Validation, Determination, Most Potent Antihypertensive Drugs, New Generation, Liquid Chromatographic

INTRODUCTION:

Antihypertensive are a class of drugs that are used to treat hypertension or high blood pressure.

Guanfacine hydrochloride is a centrally acting antihypertensive drug with alpha2a-adrenoceptor agonist properties. It is used to control symptoms of ADHD and treat to high blood pressure [1]. Guanfacine is also known as Intuniv, Estulic, Guanfacinum, Guanfacina, Guanfacinum [INN-Latin], Guanfacina [INN-Spanish]. The chemical designation of Guanfacine is N-Amidino-2-(2, 6-dichlorophenyl) acetamidemonohydrochloride. It is approved in 2009 by FDA.

Sildenafil is used to treat erectile dysfunction and pulmonary arterial hypertension. It acts by inhibiting cGMP-specific phosphordiesterase type 5 (PDE5) enzymes. Sildenafil is chemically known as 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl) phenylsulfonyl]-4-methylpiperazine. It was

originally discovered by Pfizer scientists Andrew Bell, David Brown, and Nicholas Terrett [2] [3]. Pfizer filled a patent covering the sildenafil and its use to treat cardiovascular diseases in 1992 [2]. The FDA has approved sildenafil for treatment of pulmonary hypertension, a rare, life-shortening lung disorder that causes high blood pressure in the lungs in June 2005 [4].

Irbesartan is also used to treat high blood pressure. It is an N-substituted heterocyclic derivative. Irbesartan is chemically known as 2-butyl-3-[[4-[2- (2*H*-tetrazol-5-yl) phenyl] phenyl] methyl]-1, 3-diazaspiro [4.4] non- 1-en-4-one [5]. It is an orally active lipophilic drug and possesses rapid oral absorption. It helps to protect kidney from damage due to diabetes. It is also used to decrease high blood pressure and helps to prevent strokes, heart attacks, and kidney problems. Irbesartan belongs to a class of drugs called angiotensin receptor blockers (ARBs) [6].

Losartan potassium is mainly used to treat high blood pressure (hypertension). It was the first angiotensin II antagonist to be marketed. It is the first member of a new chemical class of a non-peptide angiotensin II receptor antagonist, chemically known as (2-butyl-4-chloro-1-{[2'-(1*H*-tetrazol-5-yl) biphenyl-4-yl] methyl}-1*H*-imidazol-5-yl) methanol. Losartan potassium is an orally active, highly selective AT1 angiotensin II receptor inhibitor, effectively reduces blood pressure by direct receptor blockade [7]. Its side effects include dry cough diarrhea, muscle cramps, dizziness, insomnia, and nasal congestion [8].

Indapamide is a non-thiazide sulphonamide diuretic drug, generally used in the treatment of hypertension, as well as decompensate heart failure, chemically it is known as 4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)-3-sulfamoyl-benzamide [9].

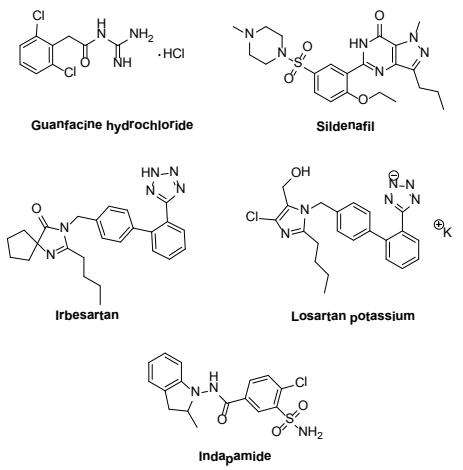


Figure 1: Structural formula of Guanfacine hydrochloride, Sildenafil, Irbesartan, Losartan potassium, Indapamide

The literature review regarding Guanfacine hydrochloride, Sildenafil, Irbesartan, Losartan potassium and Indapamide indicated that a variety of analytical methods such as Spectrophotometry, HPLC, LC-MS/MS and HPTLC have been reported for the determination of these five drugs in bulk, pharmaceutical dosage forms and in various biological fluids [10] to [22]. So far as per our present knowledge, there is no single method available for the simultaneous determination of these five most popular antihypertensive molecules. Nowadays pharmaceutical industries uses time consuming method and different mobile phase for different dosage form of drugs. But the current work requires only one mobile phase with 3.5 minutes runtime for simultaneous determination of these five drugs. So, the time and cost required for changing different mobile phase could be saved. The aim of proposed method is to estimate five drugs within sorter analysis time of 3.5 minute with simplest LC method.

EXPERIMENTAL CONDITION:

Materials and reagents

Reference standards of all active pharmaceutical

ingredients were provided from Devadhin Enterprises Ahmedabad, India and Anlon Research Organization, Rajkot, India. Tablet dosage form of all drugs was purchased from local market. HPLC grade Acetonitrile and Methanol were purchased from Merck India Limited, Mumbai and HPLC grade Orthophosphoric acid was provided from Spectrochem Mumbai, India. High purity deionised water was obtained from Milli-Q (Millipore, Miliford, MA, USA) purification system, 0.45µm membrane filters were purchased from Pall Life Sciences Mumbai; India and nylon syringe filters 0.45µm were purchased from Millex-Hn, Mumbai, India.

Instrumentation

Chromatographic separation was carried out using UPLC Acquity system (Waters, Milford, MA, USA), consisted a binary solvent manager, a sample manager, column oven and a PDA detector. The output signal was monitored and processed by Empower 2.0 version software. A microbalance obtained by LCGC Radwag weighing solution Pvt. Ltd. Mumbai, India. An ultrasonic water bath SONICA from Spincotech Pvt. Ltd. Mumbai, India and pH meter LI 610 purchased from ELICO Mumbai, India.

Chromatographic condition

The chromatographic analysis was performed using Acquity UPLC @HSS C18 (50 mm X 2.1mm id, 1.8µm particle size) column with 40°C column oven temperature. The isocratic mobile phase was consisted 0.1% OPA and Acetonitrile (68:32, v/v), detection was monitored at wavelength of 211nm with 0.3ml/min flow rate and 0.5µl injection volume. Total analysis takes 3.5 minutes.

Mobile phase preparation

An isocratic mobile phase was consisted 0.1% Orthophosphoric acid and Acetonitrile (68:32, v/v) degassed the mobile phase by ultrasonic bath before use.

Diluent preparation

Methanol and 0.1% Orthophosphoric acid used as diluent.

Stock solution preparation

The stock solution of 500µg/ml concentration was prepared by dissolving accurately weighted 50mg of each working standard into individual 100ml volumetric flask. Add 50ml of Methanol to each flask for dissolution purpose, sonicate the solution around 10 to 15 minute and then dilute to volume up to mark with methanol. For standard solution preparation pipette out 2.5ml of above stock solution from each flack into 50ml volumetric flask and dilute up to the mark with 0.1% Orthophosphoric acid. This solution contains 25µg/ml concentration of each drug.

Test solution preparation

Twenty tablets of Sildenafil, Irbesartan, Losartan potassium, forty tablets of Indapamide and thirty tablets of Guanfacine hydrochloride were accurately crushed; weighted and average weight has been calculated individually. The equivalent weigh of each powder drug has been taken into individual 100ml volumetric flask and add 50ml of methanol to each flask, dissolve the substance by sonication then dilute to volume up to mark with methanol. Filter this solution with 0.45µm membrane filter. These all solution contains 500µg/ml concentration of each drug. For test solution preparation pipette out 2.5ml from each stock solution and dilute up to 50ml with diluent. This solution contains 50µg/ml concentration of each drug.

RESULT AND DISCUSSION:

Development of UPLC method

The objective of this study was to separate five antihypertensive molecules. Several exploratory runs have been made on the basis of literature survey, but initially proper selectivity and resolution between all these drug substances were not properly achieved. After endowing more importance to the literature, 0.1% Orthophosphoric and Acetonitrile (68:32, v/v) gave the maximum resolution. Another most important part of method development is column selection. Most appropriate column chemistry was achieved by Acquity UPLC @HSS C18 (50mm X 2.1mm id, 1.8µm particle size) column with 40°C column oven temperature. For UV detection screened the standard solution over 190nm to 400nm using the advantage of photo diode array detector. On the basis of peak absorption maxima and peak purity index the 211nm was decided as the detection wavelength which also gives the maximum chromatographic compatibility to the method.

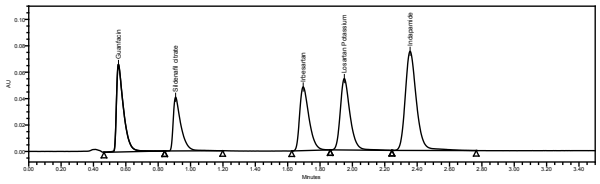


Figure 2: Chromatogram of standard preparation

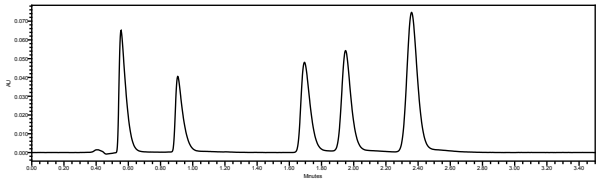


Figure 3: Chromatogram of test preparation

Method validation parameters

The developed LC method for bulk as well as pharmaceutical dosage form is validated as per ICH guidelines. The method was validated by several validation parameters such as Accuracy, Precision, Linearity, Limit of Detection, Limit of Quantitation, Robustness, and Specificity. Whole validation was performed as per ICH guidelines [23][24] to ensuring that the present method was suitable for its intended purpose.

System suitability study

A system suitability test for the chromatographic system was performed before each validation experiment. Five replicate injections of standard preparation were injected and Asymmetry, Theoretical Plates and %RSD of peak area were determined for same. The theoretical plates should be more than 5000, Asymmetry should be less than 2.0 and %RSD also should be less than 2.0. As the data suggested the system suitability was within the criteria in each validation experiment. Hence the system was found suitable to perform the validation experiment which confirms the reliability of the data generated during the method validation.

Parameters	GFH	SDC	IBS	LSP	IDP
Accuracy (%Recovery)	99-100	99-101	99-101	99-101	99-101
Linearity (Concentration range µg/ml)	10-40	10-40	10-40	10-40	10-40
Regression equation	y = 7906.4x -1966.5	y = 5180.1x + 3489.5	y = 7065.3x + 5247.2	y = 8779.1x - 2748.2	y = 13080x + 2624.3
Co-relation coefficient (R^2)	0.9996	0.9994	0.9987	0.9995	0.9999
Intermediate precision (%RSD)	0.86	0.84	1.07	0.36	0.67
Method precision (%RSD)	0.83	1.26	1.00	0.78	1.06
Robustness (%RSD)	0.44	1.00	0.82	0.99	1.12
Specificity (%RSD)	0.84	0.97	1.24	1.45	1.24
Limit of Detection (µg/ml)	0.09	0.06	0.04	0.08	0.11

Limit of Quantitation (µg/ml)	0.40	0.30	0.0.25	0.37	0.42

Table 1: Validation study evaluation data

Specificity study

The specificity study of the method was determined against diluent application and each of the molecules to another drug substance, which were taken in to the consideration. The peak purity of the Guanfacine hydrochloride, Sildenafil, Irbesartan, Losartan potassium and Indapamide has been found satisfactory. Excipients of all tablets are practically insoluble in diluent whereas the active pharmaceutical ingredients are freely soluble. The interference of the diluent and each molecule was derived by injecting each individual drug substance solution and diluent. The retention time of each drug is separated from diluent and other excipients. Hence it is prove that any interference was not observed from blank or excipients to the peak of interest. The specificity of proposed method is satisfactory with respect to the diluent and excipients in the commercial sample.

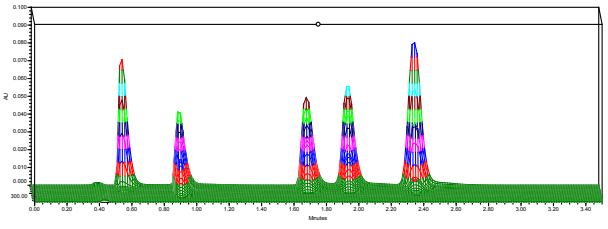
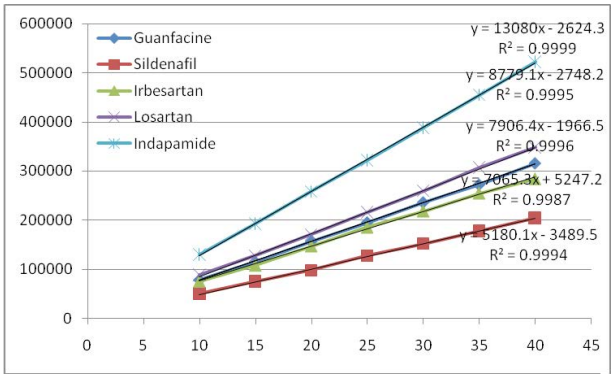


Figure 4: 3D Chromatogram obtained by specificity study

Linearity study

The linearity study was determined by analyzing seven solutions in the concentration range between 10-40µg/ml of Guanfacine hydrochloride, Sildenafil, Irbesartan, Losartan potassium and Indapamide. These concentration levels were respectively corresponding to 40, 60, 80, 100, 120, 140 and 160% of standard solution concentration. The plot of peak area against concentration data were evaluated by linear regression analysis. The response of the drug was found to be linear in the investigation. The correlation coefficient and linear regression equation of each drug is describe below. Where y is the peak area in absorbance units; x is the concentration in µg/ml. which proves the method is highly linear over the working range between 10-40µg/ml.



X axis = Concentration (µg/ml), Y axis = Peak Area in absorbance units

Chart 1: Linearity curve for Guanfacine, Sildenafil, Irbesartan, Losartan potassium and Indapamide

Linearity & Range		
Drug name	**R²**	**y = mx + c**
GUANFACINE HYDROCHLORIDE	0.9996	y = 7906.4x -1966.5
SILDENAFIL	0.9994	y = 5180.1x + 3489.5
IRBESARTAN	0.9987	y = 7065.3x + 5247.2
LOSARTAN POTASSIUM	0.9995	y = 8779.1x - 2748.2
INDAPAMIDE	0.9999	y = 13080x + 2624.3

Where R^2 = Co-relation co efficient and Y = mX + c is regression analysis equation

Table 2: Linearity study-Regression analysis data

Limit of Detection & Limit of Quantitation study

LOD is the lowest amount of the drug content which can be detected by the proposed method while LOQ is the lowest amount which can be quantified by the method. The guideline suggest minimum signal to noise ratio (S/N) more than 3.3 for LOD and more than 10 for LOQ. The LOD concentrations were found at 0.09, 0.06, 0.04, 0.08, 0.11 and LOQ concentration were found at 0.40, 0.30, 0.25, 0.37, 0.42 simultaneously for Guanfacine, Sildenafil, Irbesartan, Losartan Potassium, Indapamide. It have been established by evaluating the minimum level at which the analyte could be readily detected and quantified accurately. On the basis of linearity data theoretically it can be also calculated by the given formula. The data of linearity extension up to LOQ level also suggest that the analytes can be quantified up to 0.30µg/ml accurately. All the results of LOD and LOQ data were within the acceptance criteria,

$$LOD = 3.3(\sigma / S)$$

$$LOQ = 10(\sigma / S)$$

Where, σ = Standard deviation of regression line

S = Slope of the calibration curve

Precision study

In the precision study, six different preparations of all five drugs were analysed by performing multiple preparations of a single sample on the same and different day. Precision study was established by evaluating method precision and intermediate precision study.

Method precision

Method precision of the analytical method was determined by analyzing six sets of sample solution preparation. Assay of all six replicate sample preparations was determined and mean %Assay value, Standard deviation and %Relative standard deviation for the same was calculated.

Intermediate precision

Intermediate precision of the analytical method was determined by performing same experiment as method precision on another day by another analyst using different make of raw materials under same experimental condition. Assay of all six replicate sample preparations was determined and mean %Assay value, Standard deviation and % Relative standard deviation also calculated.

Overall assay value of method precision and intermediate precision was compared and % Difference and overall % Relative standard deviation was calculated.

Accuracy study

Accuracy study was assessed by determination of the recovery of the method at three different concentrations (corresponding to 50, 100 and 150% of test solution concentration). Known amounts of Guanfacine hydrochloride, Sildenafil, Irbesartan, Losartan potassium and Indapamide (12.5, 25 and 37.5µg/ml) were added to sample preparation. For each concentration, three sets were prepared and injected in duplicate. % Recovery was calculated at each level. The mean recovery of each drug was between 99 to 101% and % RSD is less than 2% for all levels which are indicate accuracy of the method.

	Set no.	GFH	SDC	IBS	LSP	IDP
		% Assay				
Method Precision study	1	99.99	100.80	100.81	100.68	100.04
	2	100.94	99.51	100.42	100.36	99.97
	3	99.70	99.80	100.83	100.72	99.28
	4	99.82	100.16	100.11	100.17	99.60
	5	100.06	100.63	100.61	100.36	99.88
	6	100.32	100.34	100.78	100.64	99.41
	Mean	100.14	100.21	100.59	100.49	99.70
	Stdev	0.45	0.49	0.28	0.22	0.31
	% RSD	0.45	0.49	0.28	0.22	0.31
	95% confidence level	0.36	0.39	0.23	0.18	0.25
Intermediate Precision study	1	99.03	99.68	100.16	100.12	100.60
	2	99.97	100.62	100.12	100.88	100.86
	3	99.07	99.47	100.14	100.04	100.63
	4	100.72	100.84	100.75	100.94	100.58
	5	99.15	100.08	99.52	100.61	100.77
	6	100.10	100.39	99.76	100.44	100.16
	Mean	99.67	100.18	100.08	100.51	100.60
	Stdev	0.70	0.54	0.42	0.38	0.24
	% RSD	0.70	0.54	0.42	0.37	0.24
	95% confidence level	0.56	0.43	0.33	0.30	0.19

Table 3: Precision study evaluation data

Parameters for 50 %	**GFH**	**SDC**	**IBS**	**LSP**	**IDP**
Amount added (µg/ml)	12.53	12.58	12.54	12.55	12.54
Amount found (µg/ml)	12.43	12.55	12.56	12.57	12.43
Mean Recovery %	99.21	99.84	100.54	100.03	99.76
Stdev	0.10	0.74	0.40	0.98	0.68
% RSD	0.10	0.74	0.40	0.98	0.68
Parameters for 100 %					
Amount added (µg/ml)	25.01	25.14	25.0	25.26	24.98
Amount found (µg/ml)	24.93	24.99	25.0	25.27	25.07
Mean Recovery %	99.93	100.76	99.87	100.01	100.36
Stdev	0.60	1.24	0.57	0.26	0.54
% RSD	0.60	1.24	0.57	0.26	0.54
Parameters for 150 %					
Amount added (µg/ml)	37.59	37.55	37.66	37.62	37.59
Amount found (µg/ml)	37.61	37.5	38.0	37.81	37.92
Mean Recovery %	99.55	100.32	100.54	99.87	100.75
Stdev	0.53	0.44	0.43	0.54	0.2
% RSD	0.53	0.44	0.43	0.54	0.2

Table 4: Accuracy study evaluation data

Robustness study

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions such as flow rate (± 0.05ml/min), the proportion of 0.1% OPA: ACN (66:34, v/v and 70:30, v/v) and changing the column oven temperature (± 2°C). For each different analytical condition the standard solutions and test solutions of all six drugs were prepared separately. The results were observed in terms of assay value and chromatographic compatibility (system suitability test), the result obtained from assay of the test solution was not affected by varying the conditions and was in accordance with the true value. System suitability data were also found to be satisfactory during variation of the analytical conditions. The analytical method therefore remained unaffected by slight but deliberate changes in the analytical conditions.

Solution stability study

Solution stability study was evaluated for the standard solution and the test preparation. The solutions were prepared and stored at 5°C and at ambient temperature without protecting of light and tested after 12, 24, 36 and 48 h. the responses for the aged solution were evaluated by comparison with freshly prepared solutions. During study of the stability of stored solutions of standards and test preparations for assay determination the solutions were found to be stable for up to 48 h. Assay values obtained after 48 h were not statistically match with standard assay values. So, the solutions were found to be stable up to 48 h.

APPLICATION OF CURRENT WORK:

a) The developed method is recommended for reaction monitoring of preparation for all molecules and quality control analysis

Robust conditions	% Assay	System suitability parameter		% Assay	System suitability parameter	
		Theoretical plates	Asymmetry		Theoretical plates	Asymmetry
	Guanfacine			Sildenafil		
0.25 ml/min flow rate	99.30	15235	1.08	100.27	16985	1.11
0.35 ml/min flow rate	99.32	16859	1.09	100.34	17580	1.09
0.1% OPA: ACN (66:34, v/v)	100.68	15475	1.05	100.05	16368	1.07
0.1% OPA: ACN (70:30,v/v)	100.37	17859	1.10	99.28	17582	1.06
38˚C column temperature	100.71	17902	1.03	99.28	18530	1.07
42˚C column temperature	99.62	15968	1.08	99.92	17581	1.08
	Irbesartan 1.04			Losartan potassium		
0.25 ml/min flow rate	100.70	16898	1.06	99.09	18785	1.09
0.35 ml/min flow rate	99.47	18785	1.05	99.37	17885	1.05
0.1% OPA: ACN (66:34, v/v)	100.85	16475	1.09	99.17	16985	1.7
0.1% OPA: ACN (70:30, v/v)	99.41	15745	1.05	100.61	15785	1.08
38˚C column temperature	100.80	16980	1.8	99.95	17580	1.07
42˚C column temperature	99.34	17585	1.07	99.16	16897	1.06
	Indapamide					
0.25 ml/min flow rate	99.64	17582	1.06			
0.35 ml/min flow rate	99.59	16258	1.08			
0.1% OPA: ACN (66:34, v/v)	99.37	15238	1.07			
0.1% OPA: ACN (70:30, v/v)	99.21	16980	1.06			
38˚C column temperature	99.98	15802	1.05			
42˚C column temperature	99.76	18569	1.08			

Table 5: Robustness study evaluation data

b) This method can be used for the simultaneously quantification of all five most significant antihypertensive in bulk drug as well as in pharmaceutical dosage form in routine or as a special test.

c) This method can be used for the determination of all five analyte individually or in combination dosage form.

d) This method has also application over the chromatographic purity of all analytes.

CONCLUSION:

The observation and results obtained from each validation experiment including Specificity, Linearity, LOD and LOQ, Precision, Accuracy, Robustness, Solution stability and System suitability lies well inside the acceptance criteria of ICH guideline. Since, all the results are with-in the limit. So the developed analytical method is considered as validated and suitable for possible use.

ACKNOWLEDGEMENT:

The authors are grateful to the Department of Chemistry, Saurashtra University (UGC-SAP Sponsored and DST-FIST Funded) Rajkot, Gujarat, India. Special thanks are to the Center of Excellence in Drug Discovery, Department of Chemistry, Saurashtra University, Rajkot, Gujarat, India for providing the instrumental facilities. The author is also thankful to UGC for providing financial assistance in terms of BSR-SRF fellowship.

REFERENCES:

1. M.P. Cruz, Pharmacy and Therapeutics, 2010, 35, 448-451
2. US Patent 5250534 A, Andrew SB, et al., Pyrazolopyrimidinone antianginal agents, 1993 Oct-5
3. M. Boolell, M. J.Allen, S.A. Ballard, A. S. Gepi, G. J. Muirhead, A. M. Naylor, I. H. Osterloh, C. Gingell, International Journal of Impotence Research, 1996, 8, 47-52
4. http://www.webmd.com/lung/news/20050608/viagra-ingredient-okd-for-lung problem
5. W. Lin, W. Jingkang, B. Ying, Li. Tonghe, Journal of Chemical and Engineering, 2007, 52, 2016-2017
6. http://www.medicinenet.com/irbesartan-oral/article.htm
7. J. M. Mallion, D. C. Bradstreet, L. Makris, A. I. Goldberg, S. Halasz, C. S. Sweet, N. Y. Lim, O. Madonna, Journal of Hypertension Supplement, 1995, 13, 35-41
8. http://www.rxlist.com/cozaar-side-effects-drug-center.htm
9. B. Sweta, S. Moha, American Journal of Pharmtech Research, 2013, 3, 704-711
10. K. A. Vinod, R.S. Dnyaneshwar, S. S. Vinayak, V. T. Vinod, P. M. Rajendra, B. N. Rajesh, P. P. Rajendra, International Journal of Industrial Chemistry, 2011, 2, 69-77
11. M. G. Suryanarayana, S. M. N. Yadagiri, B. K. Chandrasekher, B. R. Challa, Z. A. Bahlul, Journal of Pharmaceutical Analysis, 2013, 3, 472-480
12. N. Daraghmeh, M. Al-Omari, A. A. Badwan, A. M. Jaber, Journal of Pharmaceutical And Biomedical Analysis, 2001, 25, 483-492
13. K. Vijay, Y. Sanjeeva, U. R. Vinay, M. K. Uma, B. Radhika, K. Naresh, Journal of Scientific Research in Pharmacy, 2012, 1, 13-14
14. R. B. Prasanna, R. V. Sudhakar, R. V. Srinivasa, R. C. Surendranath, R. B. Ravindra, International Journal of Applied Biology and Pharmaceutical Technology, 2010, 1, 104-111
15. R. A. Ramprasad, G. S. Kumar, S. B. Puranik, G. Peerla, K. A. Sridhar, International Journal of Pharmaceutical, Chemical and Biological Sciences, 2012, 2, 696-703
16. R. R. Ramesh, N. Bujjibabu, Pharmacophore an International Research Journal, 2011, 2, 145-149
17. K. Sujana, D.Sankar, K. Abbulu, O. Souri, International Journal of Pharmaceutical Research and Development, 2011, 3, 67-73
18. K. R. Srinivasa, K. Srinivas, Indian Journal of Pharmaceutical Sciences, 2010, 72, 108-111
19. R. P. Priyanka, U. R. Sachin, P. N. Dhabale, K. B. Burade, International Journal of Chemtech Research, 2009, 1, 464-469
20. M. P. Vanessa, L. Carolina, M. Ana, ActaPharmaceuticaBonaerense, 2005, 24, 250-255
21. G. S. Kumar, Asian Journal of Biomedical and Pharmaceutical Sciences, 2012, 2
22. B. P. Jyoti, A. S. Sathishkumar, B. Gopinath, C. Gangaprasad, International Journal of Pharmtech Research, 2011, 3, 1482-1487
23. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Definitions and Terminology, 1996, Q2A, Geneva
24. International Conference on Harmonization, (ICH), Q2 (R1), Guideline on Validation of Analytical Procedure: Text and Methodology, 2005, Geneva, Switzerland