ISSN 2063-5346

SIMULTANEOUS ESTIMATION OF CLONIDINE AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORM BY VALIDATED STABILITY INDICATING RP-HPLC METHOD

Purushottam Prajapati[1], M.S. Ranawat[2]

[1]Research scholar, Faculty of Bhupal Nobles' University, Udaipur (Rajasthan)

[2]Dean, Faculty of Pharmacy, Bhupal Nobles' University, Udaipur (Rajasthan)

Email: peeru.om@gmail.com

Article History: Received: 01.02.2023 **Revised:** 07.03.2023 **Accepted:** 10.04.2023

Abstract

Objective: To develop and validate a simple, efficient and cost-effective stability indicating RP-HPLC method for simultaneous estimation of Clonidine and Hydrochlorothiazide in Tablet dosage form.

Methods: Phosphate buffer (ph6.0)-Methanol and Triethylamine (70:30:0.1) used as mobile phase and stationary phase (250*4.6mm C18, Hypersil BDS) column, wavelength was selected 226 nm, Flow rate 1.0 ml/ min, injection volume 20 µl and column oven temperature 25°C. Prepared Standard solution and sample solution at working concentration, used Mobile phase as diluent.

Results: Elution order of both peaks, Clonidine (Retention time 3.197 min.) eluted first and Hydrochlorothiazide (Retention time 5.083 min.) second with good resolution and fulfil System suitability parameters. Precision results shows % Relative standard deviation of Clonidine and Hydrochlorothiazide 1.6 and 1.4 respectively. Linearity results of Clonidine and Hydrochlorothiazide found acceptable in range 0.5 µg/ml to 3.0 µg/ml and 100.0 µg/ml to 600.0 µg/ml, respectively. Calibration curve shows good linearity and Correlation coefficient was 0.9999 and 0.9997 of Clonidine and Hydrochlorothiazide, respectively. Recovery results of Clonidine and Hydrochlorothiazide from matrix of tablet formulation were 100.2% and 99.9%, respectively. Robustness and ruggedness results were found well within the acceptance limit.

Conclusion: The results shows that the proposed simple, precise and accurate method can be successfully applied for simultaneous estimation of Clonidine and Hydrochlorothiazide in Tablet dosage form.

Keywords: Clonidine and Hydrochlorothiazide, RP-HPLC, Stability indicating, Simultaneous estimation.

INTRODUCTION

Clonidine and Hydrochlorothiazide is a combination of two medicines: Clonidine and hydrochlorothiazide which lower blood pressure effectively. Clonidine is an alpha-2 agonist. It works by relaxing blood vessels which makes the heart more efficient at pumping blood throughout the body. Hydrochlorothiazide is a diuretic which lowers blood pressure by removing extra water and certain electrolytes from the body. Clonidine is crosses the blood-brain barrier Mechanism which has the chemical name N-(2,6-dichlorophenyl)-4,5-dihydro-1H-imidazol-2-amine. It is an imidazoline derivative and a centrally acting α_2-adrenergic agonist and thereby inhibits sympathetic outflow from CNS. It crosses blood brain barrier and acts in hypothalamus to induce a fall in blood pressure.[1] It is a White to off-white crystalline powder which is freely soluble in water and methanol.

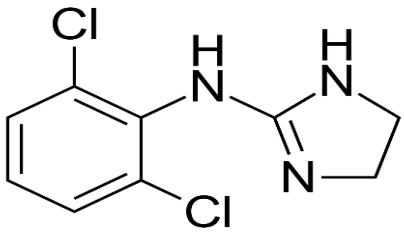


Fig. 1: Structure of Clonidine

Hydrochlorothiazide is thiazide class of diuretics. used in the treatment of hypertension, it is inhibits Na+ and Cl- ions reabsorption in the distal convoluted tubule by blocking the Na+ /Cl-Symporter. IUPAC name was 6- chloro-3,4-dihydro-7-sulfamoyl-2H-1,2,4-benzothiadiazine - 1,1-dioxide with molecular formula $C_7H_8ClN_3O_4S_2$.[2] It acts by inhibiting the kidney's ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and by other mechanism, is believed to lower peripheral vascular resistance.[3] Hydrochlorothiazide was Sparingly soluble in methanol.

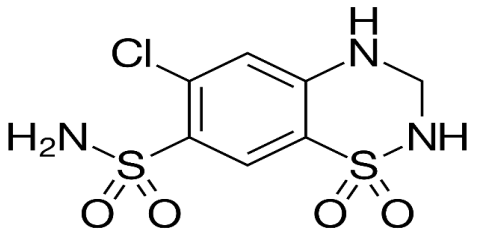


Fig. 2: Structure of Hydrochlorothiazide

The literature survey revealed many methods for Hydrochlorothiazide individual and with combination of other drug including LC-MS,[4] spectrophotometry [5] and HPLC.[6] Clonidine was determined by fluorimetry,[7] HPLC,[8] LCMS.[9]

MATERIAL AND METHODS

Table 1: Instruments details

HPLC	**Make**	Shimadzu
	Pump	LC-20 AT
	Software	Spinchrom CFR Software
	Column	Hypersil BDS C18
UV-Visible Spectrophotometer	**Make**	Systronic
	Model	119
Analytical Balance	**Make**	Shimadzu
	Model	ATX-224
pH Meter	**Make**	Ana lab Scientific Pvt Ltd

Table 2: Chemical and Regents details

Sr. No.	Chemical Name	Make	Grade
1	Water	Milli-Q	HPLC
3	Ortho phosphoric acid	Merck	HPLC
4	Methanol	Rankem	HPLC
4	Triethylamine	Rankem	HPLC

Table 3: Drug substances and Drug product details:

Name of Drug and Drug Product	Supplier and Manufacturer
Clonidine	Molecule Laboratory
Hydrochlorothiazide	Molecule Laboratory
Clonidine and Hydrochlorothiazide Tablets	Torrent Pharmaceuticals Ltd.

Method development

Wavelength selection

Clonidine Standard Solution

Weigh equivalent 10 mg of Clonidine Standard and transferred in to 100 ml volumetric flask. Added 70 ml of methanol and sonicated to dissolve it. Dilute to volume with methanol and mixed well. Further pipette 1.0 ml and dilute up to 10 ml with methanol and mixed well. (10 µg/ml)

Hydrochlorothiazide Standard Solution

Weigh equivalent 20 mg of Hydrochlorothiazide Standard and transferred in to 100 ml volumetric flask. Added 70 ml of methanol and sonicated to dissolve it. Dilute to volume with methanol and mixed well. Further pipette 1.0 ml and dilute up to 10 ml with methanol and mixed well. (20 µg/ml)

Procedure:

Taken UV spectra of above two solutions individually between the range of 200nm-400nm using methanol as a blank. Overlay both the spectra and find iso-absorptive point.

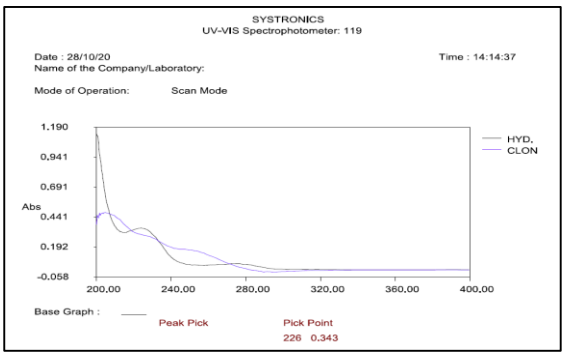


Fig. 3: UV spectra of Clonidine and Hydrochlorothiazide

Mobile phase Selection

By the literature survey it is found that Buffer pH 5.00 to 6.00 suitable for the elution of both components. Taken trials by using different pH buffers, methanol and Triethylamine mixture to find better resolution between both components. Finally optimized below chromatographic condition.

Mobile Phase	Water pH 6: Methanol: TEA (70:30:0.1)
Column	Hypersil BDS 250*4.6mm* 5µm C18,
Flow rate	1.0 ml/ min
Column oven temperature	25°C
Injection volume	20 µl
Wavelength	226 nm
Run Time	10 min
Diluent	Used Mobile Phase

Buffer preparation (0.05M potassium dihydrogen phosphate, ph-4.0)

Taken 1000ml water and adjust pH 6.0 of this solution with Orthophosphoric acid or Sodium hydroxide solution.

The pH of the solution remains Constant throughout Experiment.

Mobile Phase

Prepare a mixture of Water pH-6.0, Methanol and Triethylamine in the ratio of 70:30: 0.1 ml (%v/v) and mix well. Degas it by sonication.

Diluent: Used Mobile phase as diluent.

Standard Preparation

Clonidine Standard Stock Solution

Weigh equivalent 10 mg of Clonidine Standard and transferred in to 200 ml volumetric flask. Added 140 ml of methanol and sonicated to dissolve it. Dilute to volume with methanol and mixed well. (50 µg/ml)

Hydrochlorothiazide Standard Stock Solution

Weigh equivalent 200 mg of Hydrochlorothiazide Standard and transferred in to 100 ml volumetric flask. Added 70 ml of methanol and sonicated to dissolve it. Dilute to volume with methanol and mixed well. (2000 µg/ml)

Standard Solution

Pipette 1.0 ml of Clonidine Standard Stock Solution and 5.0 ml of Hydrochlorothiazide Standard Stock Solution and transfer in to 50 ml volumetric flask. Dilute up to volume with mobile phase and mixed well.

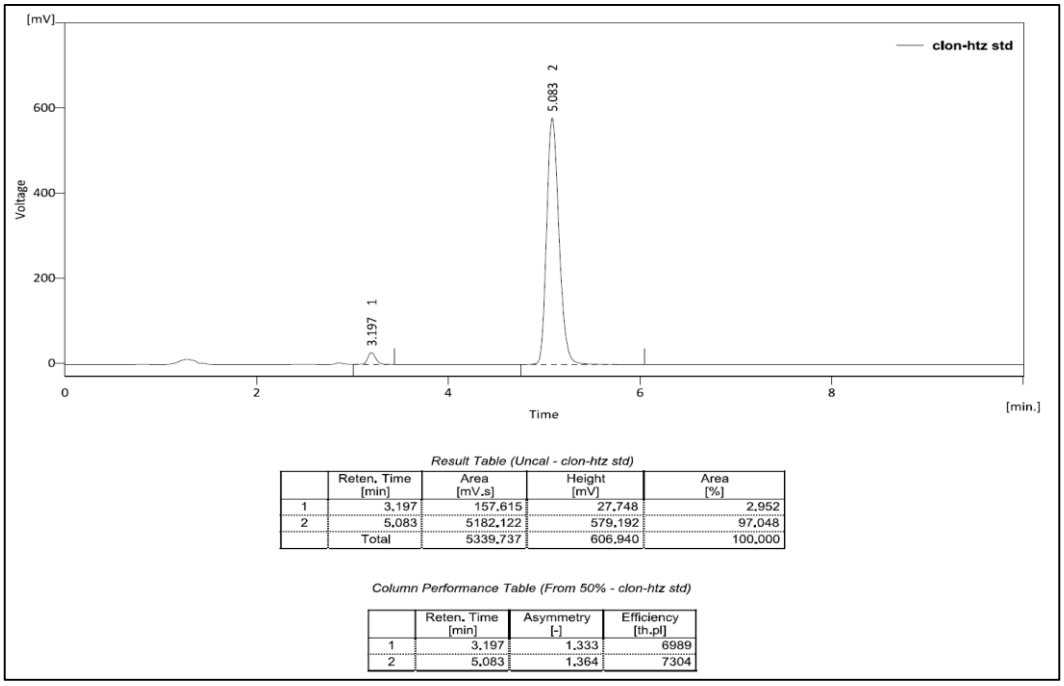
Sample preparation

Sample stock solution: Weigh and powdered 20 tablets. Take tablet powder equivalent to 1 mg Clonidine /200 mg Hydrochlorothiazide in to a 100ml volumetric flask. Add 60 ml methanol. Shake the solution for 15 minutes and sonicate it for 10 minutes. Make up volume with methanol. Filter it through Whatman filter paper no 1. (Clonidine 10 µg/ml, Hydrochlorothiazide 2000 µg/ml)

Working sample preparation

Taken 1.0 ml from sample stock solution into a 10 ml volumetric flask and make up with diluent. (Clonidine 1 µg/ml, Hydrochlorothiazide 200 µg/ml)

Retention time of Clonidine and Hydrochlorothiazide found 3.197 min and 5.083 min, respectively and % Relative standard deviation found 1.56 and 1.41%, respectively.



Result Table (Uncal - clon-htz std)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.197	157.615	27.748	2.952
2	5.083	5182.122	579.192	97.048
	Total	5339.737	606.940	100.000

Column Performance Table (From 50% - clon-htz std)

	Reten. Time [min]	Asymmetry [-]	Efficiency [th.pl]
1	3.197	1.333	6989
2	5.083	1.364	7304

Fig. 4: Chromatogram of Standard Solution of Clonidine and Hydrochlorothiazide

Table 4: System Precision Results

Injection	**Clonidine 1 µg/ml Area**	**Hydrochlorothiazide 200 µg/ml Area**
1	157.434	5172.715
2	159.808	5254.547
3	161.182	5295.818
4	162.944	5336.132
5	164.661	5393.552
6	161.893	5295.574
Mean	161.320	5291.390
Std Dev.	2.5113	74.7224
%RSD	1.56	1.41

METHOD VALIDATION

Method validation was carried out by as per ICH guidelines. Parameters included Specificity, Linearity, Method Precision, Intermediate Precision, Accuracy and Robustness.

Forced degradation Study

Forced degradation study performed by Chemical and Physical degradation like Acid stress, Alkali stress, Oxidation stress, Heat stress and Light stress condition.

RESULTS AND DISCUSSION

Specificity

Specificity has been evaluated by assuring no interference observed at the retention time of Clonidine and Hydrochlorothiazide peak in the chromatogram obtained from the Blank solution, Standard solution and Sample solution.

Linearity

Linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in Sample. The linearity of Clonidine and Hydrochlorothiazide are established by analysing linearity solutions of different concentration from 50% to 300% of working concentration of assay method. The linearity curve is plotted of peak area versus concentration. The results are summarized in Table 5. The linearity graph of Clonidine and Hydrochlorothiazide are shown in Fig. 5 and Fig.6, respectively.

Table 5: Linearity results

Linearity level	Azilsartan Medoxomil		Chlorthalidone	
	Concentration (µg/ml	Area	Concentration (µg/ml	Area
50 %	0.5	79.73	100	2618.471
80 %	0.75	116.049	150	3812.16
100 %	1	158.867	200	5143.224
120 %	1.25	197.8	250	6503.569
150 %	1.5	233.658	300	7563.68
250 %	2.5	394.421	500	12974.854
300 %	3	473.124	600	15404.286
Correlation Coefficient	0.9999		0.9997	
Slope (B)	157.75		25.762	
Y-Intercept	-0.386		-11.463	

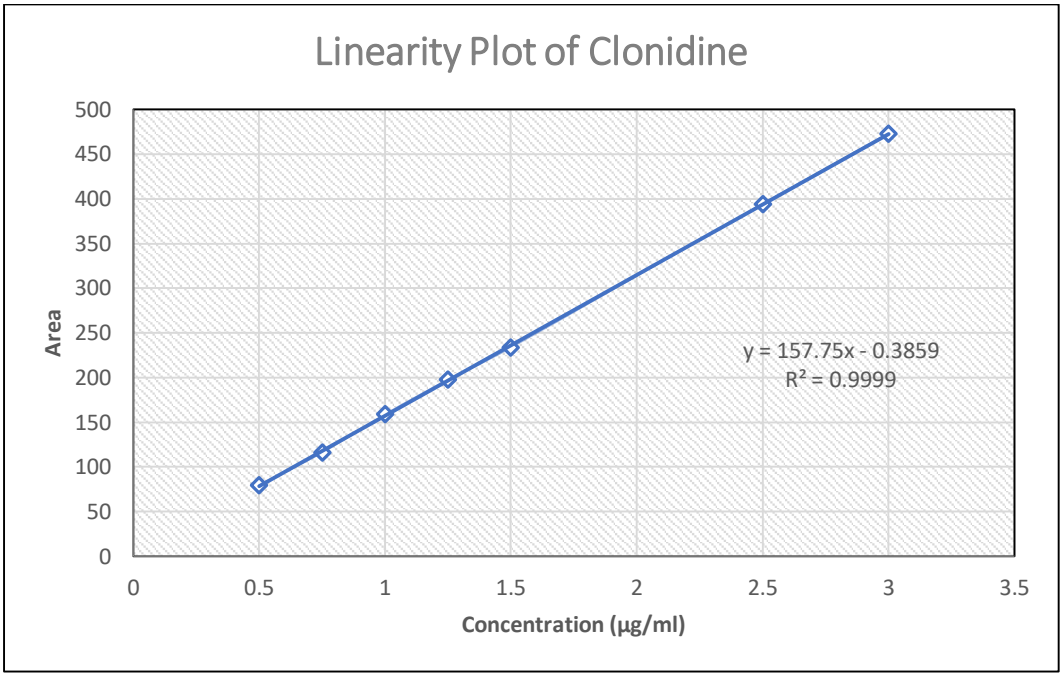


Fig. 5: Linearity Curve of Clonidine

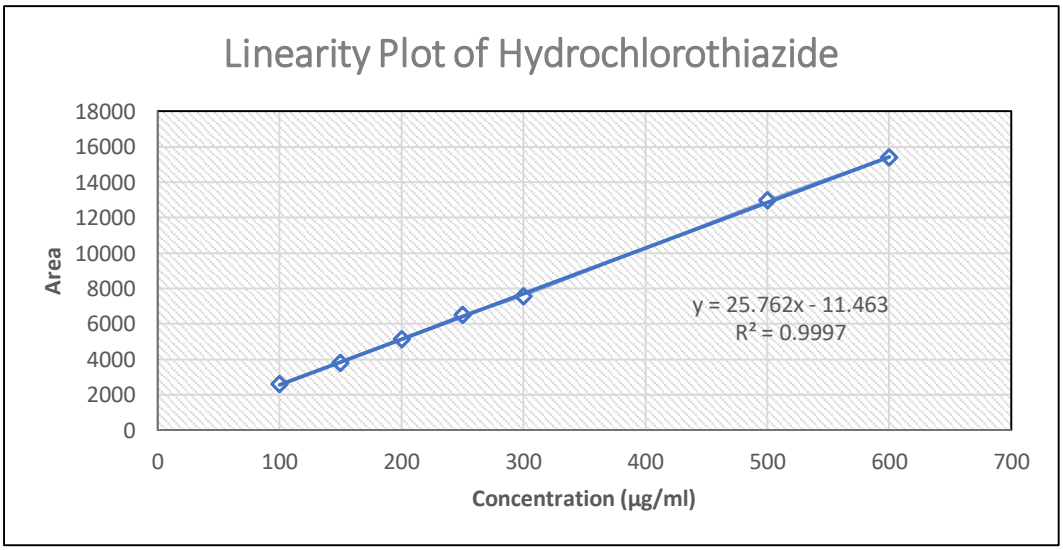


Fig. 6: Linearity curve of Hydrochlorothiazide

Method Precision

The precision of analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same sample under the prescribed condition.

Table 6: Results of Method Precision

Sr. No.	Clonidine		Hydrochlorothiazide	
	RT (Min.)	% Assay	RT (Min.)	% Assay
1	3.167	99.4	5.033	99.0
2	3.170	100.7	5.040	100.2
3	3.167	101.5	5.033	101.1
4	3.177	99.2	5.047	98.6
5	3.177	102.3	5.047	101.9
6	3.170	103.2	5.040	101.5
Mean	3.171	101.1	5.040	100.4
SD		1.5909		1.3556
% RSD		1.6		1.4

Intermediate Precision

Table 7: Results of Intermediate Precision

Sr. No.	Clonidine		Hydrochlorothiazide	
	Precision	Intermediate Precision	Precision	Intermediate Precision
1	99.4	101.3	99.0	102.0
2	100.7	99.7	100.2	100.1
3	101.5	102.8	101.1	102.5
4	99.2	103.4	98.6	103.1
5	102.3	101.7	101.9	101.4
6	103.2	103.2	101.5	102.8
Mean	101.1	102.0	100.4	102.0
SD	1.5909	1.4077	1.3556	1.1017
% RSD	1.6	1.4	1.4	1.1
Over all Mean	101.5		101.2	
Over all SD	1.5186		1.444	
Over all % RSD	1.5		1.4	

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value found. To demonstrate the accuracy of this method by spiking Clonidine and Hydrochlorothiazide standard solution into sample solution are added quantitatively from 50% to 150% for Clonidine and Hydrochlorothiazide of working concentration of this method at each level with triplicate preparation and analysed using the test method. The results are tabulated **Table no 8**.

Table 8: Accuracy results

Recovery level	Clonidine			Hydrochlorothiazide		
	% recovery	Mean % recovery	% RSD	% recovery	Mean % recovery	% RSD
50 % Set-1	101.8	100.2	1.4	100.3	99.2	1.1
50 % Set-2	99.2			99.2		
50 % Set-3	99.7			98.2		
100% Set-1	100.9	100.1	0.8	100.0	100.5	0.5
100% Set-2	100.0			101.0		
100% Set-3	99.4			100.5		
150% Set-1	100.1	100.1	0.8	99.3	100.0	0.6
150% Set-2	101.0			100.3		
150% Set-3	99.3			100.4		
Mean % recovery	100.2			100.2		
% RSD	0.9			1.2		

Robustness

Robustness study was performed by analysing the standard at different conditions the results obtained with altered conditions were compared against results obtained under normal chromatographic conditions.

Table 9: Robustness results of Clonidine

Change in Parameters	Value	Retention time (min.)	Asymmetry factor	Theoretical Plates	% RSD
Control	As Such	3.197	1.333	6989	1.6
Flow rate	+ 0.2 ml/ min	3.037	1.286	6801	1.2
	0.2 ml/min	3.323	1.318	7024	0.7
Mobile Phase Composition	+2 % Solvent	3.053	1.286	6876	1.5

	-2 % Solvent	3.323	1.318	7024	1.3
Buffer pH	+ 0.2 pH	3.177	1.333	7443	0.6
	-0.2 pH	3.167	1.333	6859	1.3

Table 10: Robustness results of Hydrochlorothiazide

Change in Parameters	**Value**	**Retention time (min.)**	**Asymmetry factor**	**Theoretical Plates**	**% RSD**
Control	As Such	5.083	1.364	7304	1.4
Flow rate	+ 0.2 ml/ min	4.823	1.387	7250	0.8
	-0.2 ml/min	5.283	1.353	7189	1.7
Mobile Phase Composition	+2 % Solvent	4.850	1.355	7330	0.9
	-2 % Solvent	5.283	1.353	7189	1.6
Buffer pH	+ 0.2 pH	5.047	1.406	7199	0.4
	-0.2 pH	5.033	1.333	7161	0.7

Forced degradation

To demonstrate stability indicating properties of the method force degradation was conducted by applying heat light, acid alkali and oxidation stress to the drug product.

Table 11: Forced degradation study results

	Clonidine		**Hydrochlorothiazide**	
	% Assay	**% Degradation**	**% Assay**	**% Degradation**
As such	98.8	N/A	102.5	N/A
Acid Stress	72.7	26.1	88.7	13.8
Alkali Stress	78.6	20.2	86.3	16.2
Oxidation Stress	86.5	12.3	83.0	19.5
Heat Stress	82.9	15.9	79.0	23.5
Light Stress	75.6	23.2	74.6	27.9

Conclusion: The results shows that the proposed simple, precise and accurate stability indicating method can be successfully applied for simultaneous estimation of Clonidine and Hydrochlorothiazide in Tablet dosage form. During validation study method was found specific, precise, accurate, rugged and robust for the intended use.

REFERENCES:

1. Reitz, DJ, Piletz, JE. Theimidazoline receptor in control of blood pressure by clonidine and allied drugs. American Journal of Physiology, 1997, 5: 273.
2. Merck Index Encyclopaedia of Chemicals Drugs and Biologicals, 13th edition, Merck Research Laboratories Division of Merck & Co INC, 2001, 2414 and 4802.
3. Duarte JD, Cooper-DeHoff RM. Mechanism for blood pressure lowering and metabolic effects of thiazide and thiazide-like diuretics, 2010, 8 (6): 793-802.
4. Lia H, Wanga Y, Tanga Y. Wanga J, Zhaob L, Gau J, A Liquid Chromatography/tandem Mass Spectrometry Method for the Simultaneous Quantification of Valsartan and Hydrochlorothiazide in Human Plasms, Journal of Chromatography B, 2007, 852 (1-2): 436-442.
5. Sachan A, Jain DK and Trivedi P, Simultaneous Estimations of Captopril and Hydrochlorothiazide in two Component Tablets by Ultra Violet Absorption Spectrophotometry, Indian Journal of Pharmaceutical Sciences, 1997, 29-31.
6. Safeer K, Anbarasi B, Kumar NS, Analytical Method development and Validation of Amlodipine and Hydrochlorothiazide in Combine Dosage Form by RP-HPLC, International Journal of Chem Tech Research, 2010, 2(1): 21-25.
7. Fawzy A. El, Bedair M & Korany MA, Spectrophotometric and Fluorometric Determination of Clonidine hydrochloride, Analyst, 1986, 111 (4): 477-478.
8. Devanaboyina N, Kumar BC, Vijay B, Bhanu MA, Gayathri V, Liquid Chromatography Method Development, and Validation for Analysis of Clonidine in Pharmaceutical Dosage, Journal of Atoms and Molecules, 2012, 93-102.
9. Ghosh C, Singh RP, Inamdar S, Mote M and Chakraborty BS, Sensitive, Selective, Precise and Accurate LC-MS Method for Determination of Clonidine in Human Plasma, Springer Link Chromatographia, 2009, 69 (11-12): 1227-1232.
10. Choudhari VP, Bari NA, Shah A, Sharma SN, Katariya PM, Bhise SS. Simultaneous Estimation of Aliskiran And Hydrochlorothiazide In Pharmaceutical Formulation By Rp-Lc-Pda. Int. J. Pharm. Sci. Rev. Res. 2012; 14(1):10-14.
11. Khan HN, Asha C, Swarali M, Zameeruddin MD, Bharkad VB. Development and Validation of Area under curve Method for the Estimation of Clonidine HCI in Bulk and Pharmaceutical dosage form. Asian J. Research Chem. 2018; 11(1):40-42.
12. International Conference on Harmonization, "Q2A: Text on Validation of Analytical Procedures. Federal Register 1995; 60:11260–2.
13. International Conference on Harmonization, "Q2B: Validation of analytical procedures: Methodology. Federal Register 1996; 62:27463–7.