INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN: 2320-2831

IJPAR |Vol.10 | Issue 2 | Apr - Jun -2021

Journal Home page: www.ijpar.com

Research Study Open Access

Development and validation of a rapid and specific rp- hplc method for simultaneous estimation of benazepril and hydrochlorothiazide in pure form and in their marketed pharmaceutical dosage form

K.V. Subhasree*, Shankar Cheruku, Ramya Sri. S

Department of Pharmaceutical Analysis, Vijaya College of Pharmacy, Telangana, India.
Sura Pharma Labs, Dilsukhnagar, Hyderabad, Telangana-500060, India

ABSTRACT

A new, simple, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Benazepril and Hydrochlorothiazide in its pure form as well as in combined marketed formulation. Chromatography was carried out on a Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size column using a mixture of Methanol: Phosphate Buffer (pH-4.2) (37:63% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 275nm. The retention time of the Benazepril and Hydrochlorothiazide was found to be was 2.133, 3.692±0.02min respectively. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The method produce linear responses in the concentration range of 20-60mg/ml of Benazepril and 10-30mg/ml of Hydrochlorothiazide. The inter-day and intra-day precisions were found to be within limits. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Benazepril and Hydrochlorothiazide, RP-HPLC, Validation, Accuracy, Precision.

INTRODUCTION

The present study was designed to develop a simple, precise, and rapid analytical RP-HPLC procedure, which can be used for the analysis of assay method for simultaneous estimation of Benzepril and Hydrochlorothiazide as there was only individual methods reported for both drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of these two drugs in their combined dosage forms. Literature survey of clarithromycin and paracetamol revealed several methods for detecting these drugs individually but there is no method for their simultaneous estimation using RP-HPLC.

The developed method was validated as per ICH guidelines and its updated international convention. The linearity of response, precision, ruggedness and robustness of the described method has been checked.

Theory of Reversed Phase Chromatography

Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character can be separated by reversed phase chromatography with excellent recovery and resolution.

The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilised hydrophobic ligand, i.e. the stationary phase. The actual nature of the hydrophobic binding interaction itself is a matter of heated debate but the conventional wisdom assumes the binding interaction to be the result of a favourable entropy effect. The initial mobile phase binding conditions used in reversed phase

chromatography are primarily aqueous which indicates a high degree of organised water structure surrounding both the solute molecule and the immobilised ligand. As solute binds to the immobilised hydrophobic ligand, the hydrophobic area exposed to the solvent is minimised. Therefore, the degree of organised water structure is diminished with a corresponding favourable increase in system entropy. In this way, it is advantageous from an energy point of view for the hydrophobic moieties, i.e. solute and ligand, to associate **(Figure 1).**

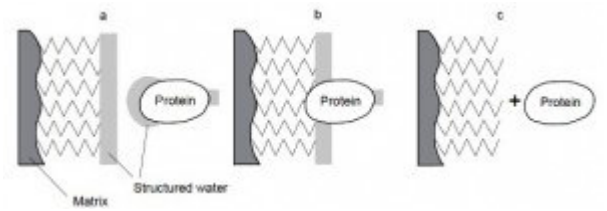

Figure 1: interaction of a solute with a typical reversed phase medium

Water adjacent to hydrophobic regions is postulated to be more highly ordered than the bulk water. Part of this 'structured' water is displaced when the hydrophobic regions interact leading to an increase in the overall entropy of the system.

Separations in reversed phase chromatography depend on the reversible adsorption/desorption of solute molecules with varying degrees of hydrophobicity to a hydrophobic stationary phase. The majority of reversed phase separation experiments are performed in several fundamental steps as illustrated in **Figure 2**.

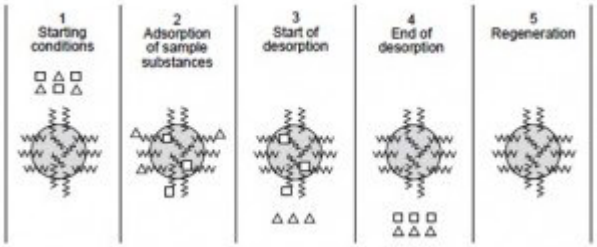

Figure 2: principle of reversed phase chromatography with gradient elution

Aim and objective

- In view of the need for a suitable RP-HPLC method for routine analysis of Benazepril and Hydrochlorothiazide in formulations, attempts were made to develop simple, precise and accurate analytical method for simultaneous estimation of Benazepril and Hydrochlorothiazide and extend it for their determination in formulation.
- Validation is a necessary and important step in both framing and documenting the capabilities of the developed method.
- The utility of the developed method to determine the content of Benazepril and Hydrochlorothiazide in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient.
- The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.

MATERIALS AND METHODS

Table 1: Instruments used

S.No	Instruments And Glass wares	Model
1	HPLC	WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detectors.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table-2: Chemicals used

S.No.	Chemical	Brand names
1	Benazepril	Sura labs
2	Hydrochlorothiazide	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck
5	Potassium Dihydrogen Phosphate	Merck

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol: Phosphate Buffer (pH-4.2) (37:63 V/v)
Column : Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size
Column temperature : 35ºC
Wavelength : 275nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6minutes

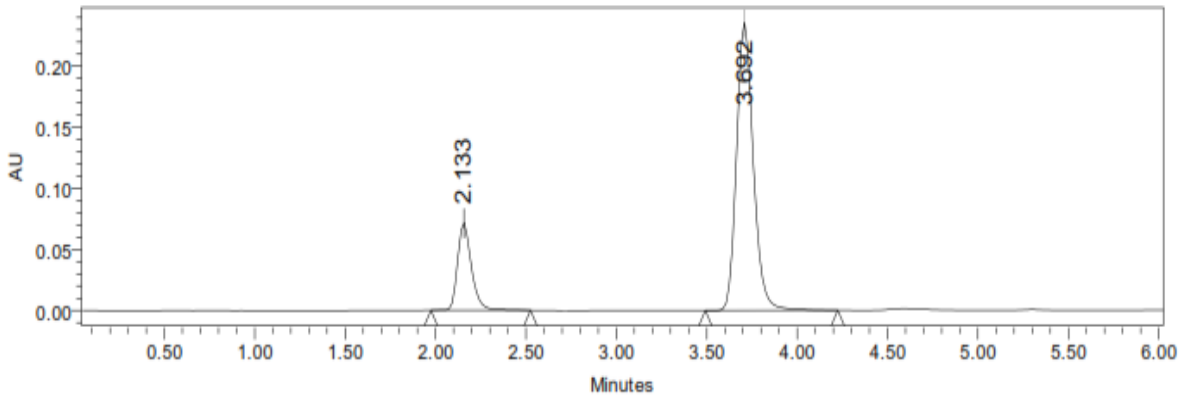

Figure-3: Optimized Chromatogram (Standard)

Table-3: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	US Tailing	US Plate Count	Resolution
1	Benazepril	2.133	526389	86756	1.56	5679	
2	Hydrochlorothiazide	3.692	1687285	367532	1.79	8685	9.8

Observation

From the above chromatogram it was observed that the Benazepril and Hydrochlorothiazide peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram

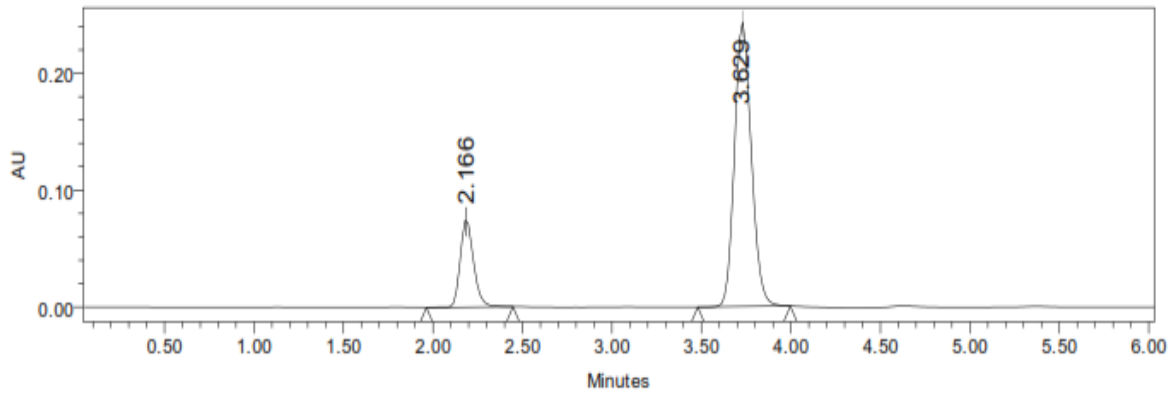

Figure-4: Optimized Chromatogram (Sample)

Table-4: Optimized Chromatogram (Sample)

S.No.	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Benazepril	2.166	536587	77464	1.57	5789	
2	Hydrochlorothiazide	3.629	1695846	378564	1.80	8795	10.01

Acceptance criteria

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Validation

Table-5: Results of system suitability for Benazepril

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Benazepril	2.152	526358	86598	5695	1.56
2	Benazepril	2.157	526548	86254	5652	1.57
3	Benazepril	2.141	526854	86598	5627	1.56
4	Benazepril	2.133	526598	86245	5692	1.57
5	Benazepril	2.166	524874	86521	5641	1.56
Mean			526246.4			
Std. Dev.			787.353			
%RSD			0.149617			

Acceptance Criteria

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable. (Table 6)

Table-6: Results of system suitability for Hydrochlorothiazide

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Hydrochlorothiazide	3.674	1682821	1686958	8659	1.56	9.8
2	Hydrochlorothiazide	3.631	1682726	1685745	8675	1.57	9.9
3	Hydrochlorothiazide	3.625	1687361	1685421	8692	1.56	9.8
4	Hydrochlorothiazide	3.692	1682811	1685242	8642	1.57	9.8
5	Hydrochlorothiazide	3.629	1683816	1685364	8635	1.58	9.8
Mean			1683907				
Std. Dev.			1982.03				
%RSD			0.117704				

Acceptance Criteria

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Benazepril and Hydrochlorothiazide in drug product. (Table-7, 8, 9 & 10)

Table-7: Peak results for assay standard of Benazepril

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Benazepril	2.152	526358	86598	1.56	5698	1
2	Benazepril	2.198	526584	86784	1.57	5687	2
3	Benazepril	2.179	529658	86253	1.56	5639	3

Table-8: Peak results for assay standard of Hydrochlorothiazide

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Hydrochlorothiazide	3.646	1687589	365879	1.80	8659	1
2	Hydrochlorothiazide	3.604	1685987	365854	1.79	8697	2
3	Hydrochlorothiazide	3.610	1685974	369854	1.80	8675	3

Table-9: Peak results for Assay sample of Benazepril

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Benazepril	2.152	536859	87584	1.58	5789	1
2	Benazepril	2.150	532654	87965	1.59	5784	2
3	Benazepril	2.187	532685	87465	1.58	5769	3

Table-10: Peak results for Assay sample of Hydrochlorothiazide

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Hydrochlorothiazide	3.646	1698568378562		1.81	8759	1
2	Hydrochlorothiazide	3.651	1698574375847		1.80	8795	2
3	Hydrochlorothiazide	3.601	1698547376584		1.81	8745	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 99.89%

The % purity of Benazepril and Hydrochlorothiazide in pharmaceutical dosage form was found to be 99.89%

Chromatographic data for linearity study of benazepril

Table-11: Chromatographic Data for Linearity Study of Benazepril

Concentration µg/ml	Average Peak Area
20	272897
30	402986
40	526389
50	649785
60	769287

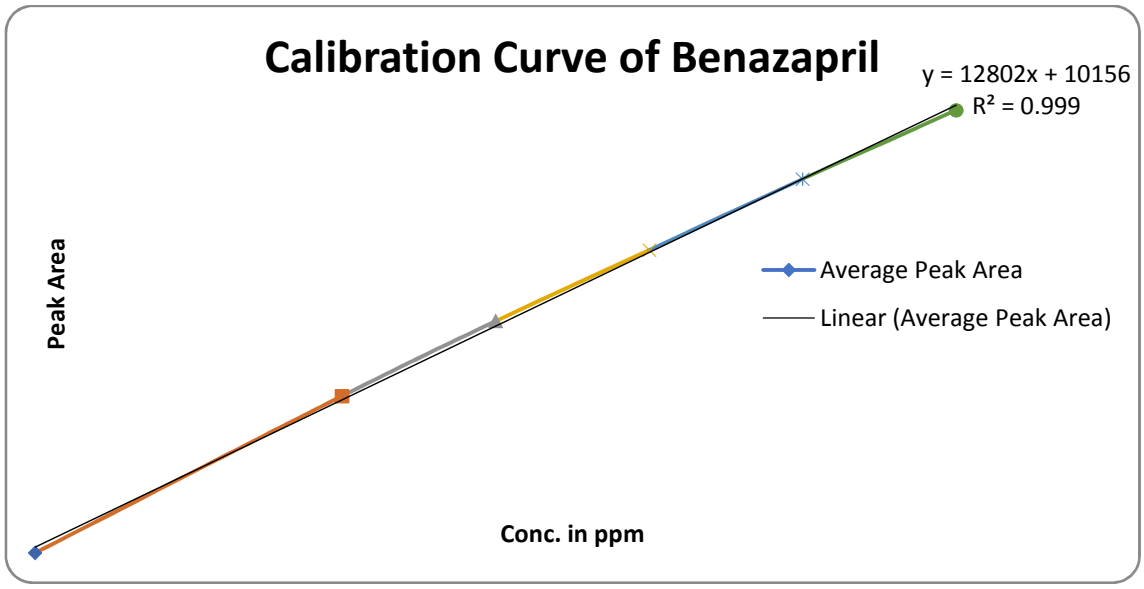

Fig-5: Calibration Curve of Benazepril

Linearity plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Benazepril is a straight line.

Y = mx + c

Slope (m) =12802

Intercept (c) = 10156

Correlation Coefficient (r) = 0.99

Validation criteria

The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Chromatographic data for linearity study of hydrochlorothiazide

Table-12: Chromatographic Data for Linearity Study of Hydrochlorothiazide

Concentration µg/ml	Average Peak Area
10	1000237
15	1448768
20	1887285
25	2365897
30	2826845

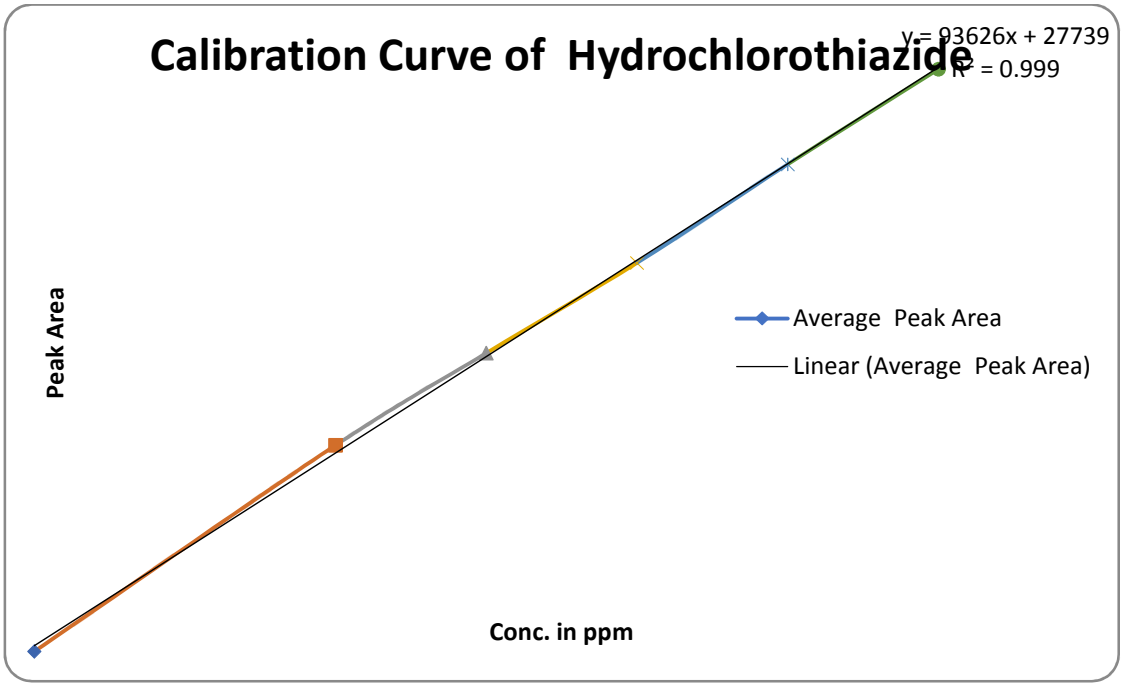

Fig-6: Calibration Curve of Hydrochlorothiazide

Linearity plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Hydrochlorothiazide is a straight line.
Y = mx + c

Slope (m) = 93626
Intercept (c) = 27739
Correlation Coefficient (r) = 0.99

Validation criteria

The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD. (Table-13)

Table-13: Results of repeatability for Benazepril

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Benazepril	2.157	526358	86598	5689	1.56
2	Benazepril	2.159	524856	86542	5687	1.57
3	Benazepril	2.186	526985	86578	5684	1.56
4	Benazepril	2.160	528654	86354	5689	1.56
5	Benazepril	2.170	528457	86958	5639	1.56
Mean			527062			
Std.dev			1569.114			
%RSD			0.297709			

Acceptance Criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise. (Table-14, 15)

Table-14: Results of Repeatability for Hydrochlorothiazide

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Hydrochlorothiazide	3.603	1687589	367859	8659	1.79
2	Hydrochlorothiazide	3.608	1685987	368547	8679	1.80
3	Hydrochlorothiazide	3.600	1685987	367985	8645	1.80
4	Hydrochlorothiazide	3.696	1685754	365874	8695	1.79
5	Hydrochlorothiazide	3.629	1685985	364589	8625	1.79
Mean			1686260			
Std. Dev			749.493			
%RSD			0.044447			

Table-15: Results of Intermediate precision for Benazepril

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Benazepril	2.198	546585	87589	5898	1.58	100%
2	Benazepril	2.196	548758	87985	5879	1.59	100%
3	Benazepril	2.160	549854	87452	5868	1.58	100%
4	Benazepril	2.160	548798	87421	5847	1.59	100%
5	Benazepril	2.160	542659	87963	5896	1.58	100%
6	Benazepril	2.186	548754	87254	5874	1.59	100%
Mean			547568				
Std. Dev.			2631.576				
%RSD			0.480593				

Acceptance criteria

- %RSD of five different sample solutions should not more than 2. (Table 16)

Table 16: Results of Intermediate precision for Hydrochlorothiazide

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution	%Assay
1	Hydrochlorothiazide	3.623	1698587	385482	8789	1.81	9.8	98%
2	Hydrochlorothiazide	3.611	1698574	385698	8759	1.80	9.8	98.2%
3	Hydrochlorothiazide	3.696	1698532	385748	8754	1.81	9.9	98.7%
4	Hydrochlorothiazide	3.696	1698574	386958	8754	1.81	10.01	99.7%
5	Hydrochlorothiazide	3.696	1698532	385755	5798	1.80	9.98	98.5%
6	Hydrochlorothiazide	3.642	1698547	386558	8762	1.80	10.02	98.2%
Mean			1698558					
Std. Dev.			23.77113					
%RSD			0.001399					

Acceptance criteria

- %RSD of five different sample solutions should not more than 2. (Table-17, 18, 19, 20)

Table-17: Results of Accuracy for concentration-50%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Benazepril	2.165	266848	45878	1.06	2856	1
2	Hydrochlorothiazide	3.696	973682	178542	1.15	4586	1
3	Benazepril	2.155	266754	45967	1.07	2875	2
4	Hydrochlorothiazide	3.684	972534	178598	1.16	4587	2
5	Benazepril	2.173	267432	45265	1.06	2865	3
6	Hydrochlorothiazide	3.688	972413	178568	1.15	4527	3

Table-18: Results of Accuracy for concentration-100%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Benazepril	2.156	523289	86598	1.57	5789	1
2	Hydrochlorothiazide	3.618	1898547	365895	1.80	8795	1
3	Benazepril	2.226	523456	86254	1.58	5749	2
4	Hydrochlorothiazide	3.650	1903242	364875	1.81	8859	2
5	Benazepril	2.226	524512	86359	1.57	5784	3
6	Hydrochlorothiazide	3.650	1898578	368985	1.80	8798	3

Table-19: Results of Accuracy for concentration-150%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Benazepril	2.148	776587	98695	1.78	6859	1
2	Hydrochlorothiazide	3.668	2848985	469852	1.86	9945	1
3	Benazepril	2.195	778798	99862	1.77	6925	2
4	Hydrochlorothiazide	3.633	2855486	465874	1.87	9987	2
5	Benazepril	2.186	779987	98745	1.78	6935	3

6	Hydrochlorothiazide	3.6682848985465325	1.86	9969	3

Table-20: The accuracy results for Benazepril

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	267011.3	20	20.063	100.315%	100.28%
100%	523752.3	40	40.118	100.295%	
150%	778457.3	60	60.133	100.221%	

Acceptance Criteria

- The percentage recovery was found to be within the limit (98-102%). (Table-21)

Table-21: The accuracy results for Hydrochlorothiazide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972876.3	10	10.094	100.94%	100.48%
100%	1900122	20	19.998	99.99%	
150%	2851152	30	30.156	100.52%	

- The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Limit of detection

- The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

- σ = Standard deviation of the response
- S = Slope of the calibration curve

BENAZEPRIL

Result

- = 1.04µg/ml

Hydrochlorothiazide

- **Result:** = 3.12µg/ml

Quantitation limit

- The quantisation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.
- **LOQ=10×σ/S**

Where

- σ = Standard deviation of the response
- S = Slope of the calibration curve

Benazepril

- **Result:** =2.1µg/ml

Hydrochlorothiazide

- **Result**: =6.3µg/ml

Benazepril

Table-22: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	526389	2.133	5679	1.56
Less Flow rate of 0.9 mL/min	542685	2.210	5264	1.54
More Flow rate of 1.1 mL/min	526483	2.184	5426	1.52
Less organic phase	516854	2.200	5163	1.57
More Organic phase	506898	2.172	5098	1.51

Acceptance criteria

- The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Hydrochlorothiazide

Table 23

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1687285	3.692	8685	1.79
Less Flow rate of 0.9 mL/min	1725468	4.498	8265	1.68
More Flow rate of 1.1 mL/min	1652847	3.505	8415	1.59
Less organic phase	1687485	4.504	8326	1.62
More organic phase	1674524	3.512	8415	1.63

Acceptance criteria

- The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Summary

Summary of Validation data for Benazepril

Table 24

S.No.	Parameter	Observation	Acceptance Criteria
1	**System suitability**		
	Theoretical plates	5679	Not less than 2000
	Tailing	1.56	Not more than 2
	%RSD	0.14	Not more than 2.0%
2	**Specificity**		
	%Assay	99.89%	98-102%
3	**Method Precision** (%RSD)	0.29	Not more than 2.0%
4	**Linearity**	20-60 µg/ml	
	Slope	12802	
	Correlation coefficient(r^2)	0.999	≤0.99
5	**Accuracy**		
	Mean % recovery	100.28%	98 - 102%
6	**Robustness**	All the system suitability parameters are within the limits.	

	a)	Flow rate variation
	b)	Organic phase variation

Summary of validation data for Hydrochlorothiazide

Table 25

S.No	Parameter	Observation	Acceptance criteria
1	**System suitability**		
	Theoretical plates	8685	Not less than 2000
	Tailing	1.79	Not more than 2
	%RSD	0.11	Not more than 2.0%
2	**Specificity**		
	%Assay	99.89%	98-102%
3	**Method Precision** (%RSD)	0.044	Not more than 2.0%
4	**Linearity**	10-30 µg/ml	
	Slope	93626	
	Correlation coefficient(r^2)	0.999	≤0.99
5	**Accuracy**		
	Mean % recovery	100.48%	98 - 102%
6	**Robustness**		
	a) Flow rate variation	All the system suitability parameters are within the limits.	
	b) Organic phase variation		

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Benazepril and Hydrochlorothiazide in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Benazepril was found to be freely soluble in water; soluble in alcohol, in methanol, ethanol and in glacial acetic acid and also soluble in Acetonitrile. Hydrochlorothiazide was found to be is slightly soluble in water, freely soluble in sodium hydroxide solution, in n-butyl amine, and in dimethyl formamide; sparingly soluble in methanol; insoluble in ether, in chloroform, and in dilute mineral acids.

ACKNOWLEDGEMENT

The Authors are thankful to Sura Labs, Dilshukhnagar, and Hyderabad for providing the necessary facilities for the research work.

REFERENCES

1. Weston A, Phyllisr. Brown, HPLC principle and practice. 1st ed. Academic press; 1997. P. 24-37.
2. Kazakevich Y, Lobrutto R. HPLC for Pharmaceutical Scientists, 1stedition. Wiley Interscience a John Wiley & Sons, Inc Publishing Group; 2007. P. 15-23.
3. Chromatography [online]. Available from: http://en.wikipedia.org/wiki/Chromatography [cited 31/3/2021].
4. Meyer VR. Practical high-performance liquid chromatography. 4th ed. England: John Wiley & Sons Ltd; 2004. P. 7-8.
5. Sahajwalla CG a new drug development. Vol. 141. New York: Marcel Dekker, Inc; 2004. P. 421-6.
6. Snyder LR practical HPLC method development. 2nd ed. New York: John Wiley & sons; 1997. P. 180-2.
7. Skoog DA, West DM, Holler FJ. Introduction of analytical chemistry. Sounder college of publishing. Harcourt Brace college publishers; 1994. P. 1-5.
8. Sharma BK. Instrumental method of chemical analysis Meerut; 1999. P. 175-203.
9. Breaux J, Jones K. Understanding and implementing efficient analytical method development and validation. Pharm Technol. 2003; 5:110-4.
10. Dr. Kealey, Haines PJ. Analytical Chemistry, 1stedition. Bios Publisher; 2002. P. 1-7.
11. Braith Wait A, Smith FJ. Chromatographic methods, 5thedition. Kluwer Academic Publishers; 1996. P. 1-2.
12. Introduction to column [online]. Available from: http://amitpatel745.topcities.com/index_files/study/columncare.pdf [cited 31/3/2021].
13. Detectors used in HPLC (online). Available from: http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC [cited 31/3/2021].
14. Detectors [online]. Available from: http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html [cited 31/3/2021].
15. Detectors [online]. Available from: http://www.dionex.com/enus/webdocs/64842-31644-02_PDA-100.pdf [cited 31/3/2021].

16. Detectors [online]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8867705 [cited 31/3/2021].
17. Detectors [online]. Available from: http://www.chem.agilent.com/Library/applications/59643559.pdf [cited 31/3/2021].
18. Detectors [online]. Available from: http://hplc.chem.shu.edu/new/hplcbook/detector [cited 31/3/2021].
19. Draft ICH. Guidelines on Validation of Analytical Procedures Definitions and terminology. Fed Regist. 1995; 60:1126.
20. Code. Q2B, validation of analytical procedures; methodology. ICH harmonized tripartite guidelines. 1996:1-8.
21. Introduction to analytical method validation [online], available from. Available from: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf [cited 31/3/2021].
22. Data elements required for assay validation [online] available from. Available from: http://www.labcompliance.com/tutorial/methods/default.aspx [cited 31/3/2021].
23. Willard Hy, Merritt LL, Dean JA, Settle FA. Instrumental methods of analysis. 7th ed CBS publisher and distributors. New Delhi; 1991. P. 436-9.
24. ICH. 'validation of analytical methods, definitions and terminology', ICH Harmonized tripartite guideline. Vol. Q2A; 1999.
25. Available from: https://www.drugbank.ca/drugs/DB00542 [cited 31/3/2021].
26. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Benazepril [cited 31/3/2021].
27. Available from: https://en.wikipedia.org/wiki/Benazepril [cited 31/3/2021].
28. Available from: https://www.drugbank.ca/drugs/DB00999 [cited 31/3/2021].
29. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Hydrochlorothiazide [cited 31/3/2021].
30. Available from: https://en.wikipedia.org/wiki/Hydrochlorothiazide [cited 31/3/2021].
31. Parmar V, Chhalotiya U, Shah D, Bhatt K, Baldania S. Quantification of benazepril hydrochloride and hydrochlorothiazide in tablet dosage form by simultaneous equation spectro photometric method. J Appl Chem. 2013; 2013, 5 pages: Article ID 316137. doi: 10.1155/2013/316137.
32. Chhalotiya UK, Varsha LP, Dimal AS, Kashyap KB, Sunil LB. Stability indicating LC method for the estimation of benazepril HCl and hydrochlorothiazide in pharmaceutical dosage form, chromatography separation. Techniques; 2014. 5(2):1-7.
33. Ashour S, Alhaj A. Sakur2 and Manar Kudemati1, A validated stability-indicating liquid chromatographic method for the simultaneous determination of amlodipine and benazepril in capsules dosage form, Canadian chemical. Transactions. Year 2014 | Volume 2 | Issue 4 |. Page 418-33.
34. Safeer K, Anbarasi B, Senthil Kumar N. Analytical Method Development and Validation of amlodipine and hydrochlorothiazide in combined dosage form by RP-HPLC. Int J ChemTech Res. Jan–Mar 2010; 2(1):21-5.