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Research

Development and validation of RP-HPLC method for the simultaneous estimation of quinapril and hydrochlorothiazide in api form and pharmaceutical dosage form

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Abstract

A precise, simple, accurate and selective method was developed and validate for simultaneous estimation of Quinapril and Hydrochlorothiazide in API form and Pharmaceutical Dosage Form. Reversed phase high performance liquid chromatographic (RP-HPLC) method was developed for routine quantification of Quinapril and Hydrochlorothiazide in the API form as well as in combined pharmaceutical dosage form. Chromatographic separation was achieved on a Phenomenex Gemini C18 (4.6mm×250mm) 5µm particle size utilizing mobile phase of filtered and degassed mixture of Methanol and Phosphate buffer (pH-3.8) (40:60% v/v) at a flow rate of 1.0mL/min with UV detection at 225nm. The method has been validated for linearity, accuracy and precision. In RP-HPLC method, the calibration graphs were linear in the concentration range of 10-30µg/ml for Quinapril and 30-90µg/ml for Hydrochlorothiazide with percentage recoveries are within the limits. The results obtained by RP-HPLC methods are rapid, accurate and precise. Therefore proposed method can be used for routine analysis of Quinapril and Hydrochlorothiazide in the API form as well as in combined pharmaceutical dosage form.

Keywords: Quinapril and Hydrochlorothiazide, RP-HPLC, Validation, ICH Guidelines.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins,

carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.

The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Introduction to HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance. [6]

Types of HPLC Techniques

Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

Reverse phase chromatography: In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

Normal phase chromatography: In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted.[4]

Based on Principle of Separation

Liquid/solid chromatography (Adsorption): LSC, also called adsorption chromatography, the principle involved in this technique is adsorption of the components onto stationary phase when the sample solution is dissolved in mobile phase and passed through a column of stationary phase. The basis for separation is the selective adsorption of polar compounds; analytes that are more polar will be attracted more strongly to the active silica gel sites. The solvent strength of the mobile phase determines the rate at which adsorbed analytes are desorbed and elute. It is widely used for separation of isomers and classes of compounds differing in polarity and number of functional groups. It works best with compounds that have relatively low or intermediate polarity.[3]

Liquid/Liquid chromatography (Partition Chromatography): LLC, also called partition chromatography, involves a solid support, usually silica gel or kieselguhr, mechanically coated with a film of an organic liquid. A typical system for NP LLC column is coated with ß, ß'-oxy dipropionitrile and a non-polar solvent like hexane as the mobile phase. Analytes are separated by partitioning between the two phases as in solvent extraction. Components more soluble in the stationary liquid move more slowly and elute later.[1,2]

Ion exchange: In this the components are separated by exchange of ions between an ion exchange resin stationary phase and a mobile electrolyte phase. A cation exchange resin is used for the separation of cations and anion exchange resin is used to separate a mixture of anions. [3.16,17]

Size exclusion: In this type, the components of sample are separated according to their molecular sizes by using different gels (polyvinyl acetate gel, agarose gel). ex: separation of proteins, polysaccharides, enzymes and synthetic polymers. [3,15]

Chiral chromatography: In this type of chromatography optical isomers are separated by using chiral stationary phase.

Affinity chromatography: In this type, the components are separated by an equilibrium between a macromolecular and a small molecule for which it has a high biological specificity and hence affinity. [3]

MATERIALS AND METHODS

Quinapril (Pure)-provided by Sura Pharma Labs, Hydrochlorothiazide (Pure)-provided by Sura Pharma Labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck.

HPLC METHOD DEVELOPMENT
TRAILS
Preparation of standard solution
Accurately weigh and transfer 10 mg of Quinapril and Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 0.2ml of Quinapril and 0.6ml of Hydrochlorothiazide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization
Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Phosphate buffer in proportion 40:60 v/v respectively.

Optimization of Column
The method was performed with various C18 columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µm was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used	: Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature	: 35ºC
Column	: Phenomenex Gemini C18 (4.6×250mm) 5µm particle size
Mobile phase	: Methanol and Phosphate buffer (pH-3.8) (40:60% v/v)
Flow rate	: 1ml/min
Wavelength	: 225nm
Injection volume	: 20µl
Run time	: 6minutes

Validation
Preparation of mobile phase
Preparation of mobile phase: Accurately measured 400ml of Methanol (40%) of and 600ml of HPLC Water (60%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.
Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio	: Methanol and Phosphate buffer (pH-3.8) (40:60% v/v)
Column	: Phenomenex Gemini C18 (4.6×250mm) 5µm particle size
Column temperature	: 35ºC

Wavelength : 225nm
Flow rate : 1ml/min
Injection volume : 20µl
Run time : 6minutes

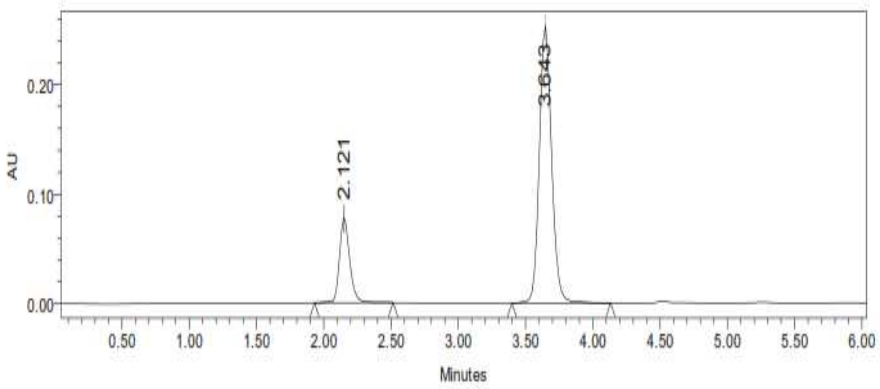

Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Quinapril	2.121	513567	78659	1.2	4536	
2	Hydrochlorothiazide	3.643	1625892	265321	1.1	7985	9.8

From the above chromatogram it was observed that the Quinapril 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

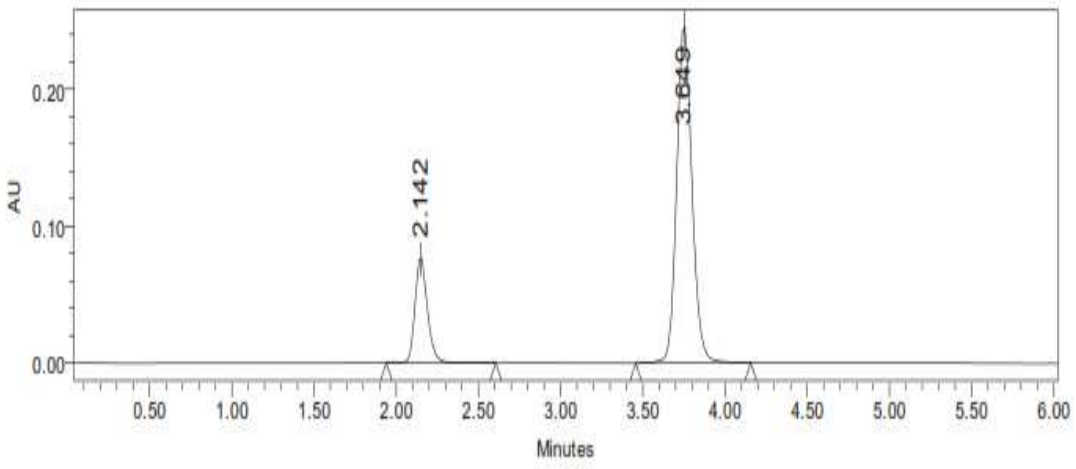

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.no	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Quinapril	2.142	512659	78956	1.2	4652	
2	Hydrochlorot	3.649	1615985	263587	1.1	7982	10.3

hiazide

- *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.*

System suitability

Table 3: Results of system suitability for Quinapril

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Quinapril	2.152	513652	78542	4698	1.2
2	Quinapril	2.157	513524	78654	4785	1.2
3	Quinapril	2.141	513425	78541	4682	1.2
4	Quinapril	2.133	513647	78454	4854	1.2
5	Quinapril	2.166	514824	78655	4872	1.2
Mean			513814.4			
Std. Dev.			572.2004			
% RSD			0.111363			

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

Table 4: 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	1635285	265421	7985	1.1	10.1
2	Hydrochlorothiazide	3.631	1635241	265484	7898	1.1	10.1
3	Hydrochlorothiazide	3.625	1652547	253498	7954	1.1	10.1
4	Hydrochlorothiazide	3.692	1658458	265241	7965	1.1	10.1
5	Hydrochlorothiazide	3.629	1652894	265348	7985	1.1	10.1
Mean			1646885				
Std. Dev.			10865.58				
% RSD			0.659766				

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

Assay (Standard)

Table 5: Peak results for assay standard of Quinapril

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Quinapril	2.152	513538	78074	1.2	4562	1
2	Quinapril	2.198	513975	79001	1.2	4620	2
3	Quinapril	2.179	513283	78048	1.2	4652	3

Table 6: Peak results for assay standard of Hydrochlorothiazide

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Hydrochlorothiazide	3.646	1625632	265325	1.1	7949	1
2	Hydrochlorothiazide	3.604	1635458	265423	1.1	7919	2
3	Hydrochlorothiazide	3.610	1635241	265874	1.1	7926	3

Assay sample

Table 7: Peak results for Assay sample of Quinapril

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Quinapril	3.651	513265	78548	1.2	4582	1
2	Quinapril	2.150	513254	78547	1.2	4658	2
3	Quinapril	2.187	513876	78498	1.2	4597	3

Table 8: Peak results for Assay sample of Hydrochlorothiazide

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Hydrochlorothiazide	3.646	1625284	78569	1.1	7985	1
2	Hydrochlorothiazide	3.651	1624613	78547	1.1	7898	2
3	Hydrochlorothiazide	3.601	1625874	78462	1.1	7854	3

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

The % purity of Quinapril and Hydrochlorothiazide in pharmaceutical dosage form was found to be 99.7%

Linearity

Chromatographic Data For Linearity Study Of Hydrochlorothiazide

Concentration µg/ml	Average Peak Area
10	245899
15	365687
20	481526
25	589854
30	705882

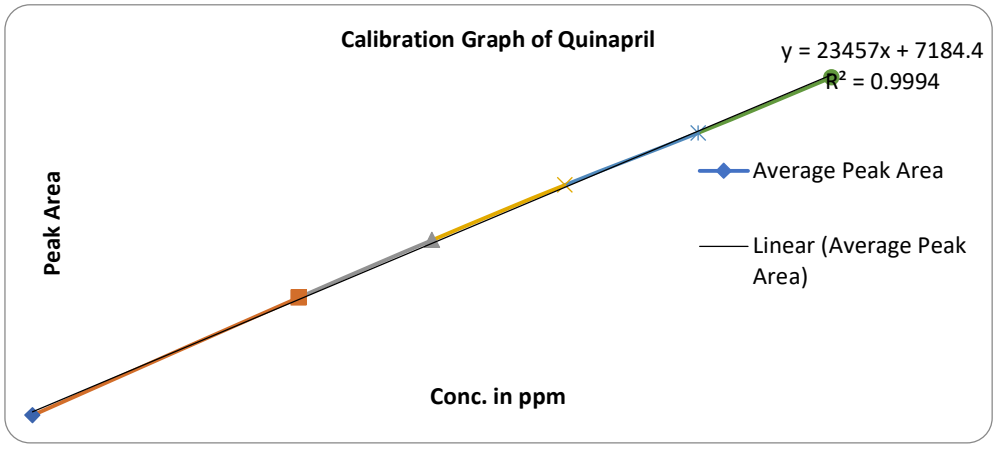

Fig 3: Calibration Graph of Quinapril

Chromatographic Data For Linearity Study Of Hydrochlorothiazide

Concentration µg/ml	Average Peak Area
30	863094
45	1249397

60	1678592
75	2050412
90	2468444

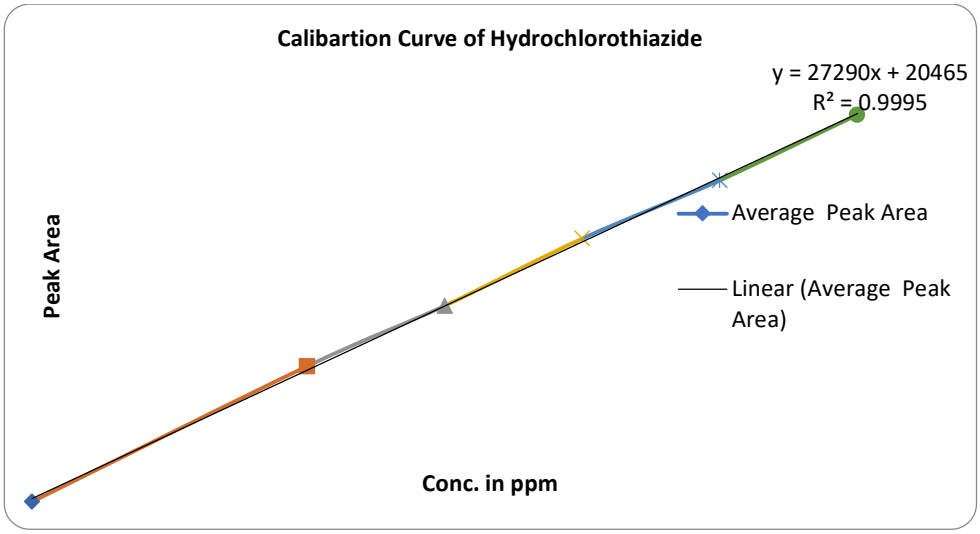

Fig 4: Calibration Curve of Hydrochlorothiazide

Repeatability

Table 9: Results of repeatability for Quinapril:

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Quinapril	2.157	513568	78546	1.2	4528
2	Quinapril	2.159	513685	78541	1.2	4572
3	Quinapril	2.186	513659	79852	1.2	4598
4	Quinapril	2.160	513254	78498	1.3	4529
5	Quinapril	2.170	513647	77898	1.2	4572
Mean			513562.6			
Std.dev			177.9475			
%RSD			0.03465			

- *%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 10: 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	1635625	265325	1.1	7985
2	Hydrochlorothiazide	3.608	1658744	264588	1.1	7859
3	Hydrochlorothiazide	3.600	1652985	265985	1.2	7845
4	Hydrochlorothiazide	3.696	1645898	264898	1.1	7969
5	Hydrochlorothiazide	3.629	1652364	268489	1.1	7846
Mean			1649123			
Std.dev			8811.631			
%RSD			0.534322			

Intermediate precision

Table 11: Results of Intermediate precision for Quinapril

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Quinapril	2.198	514658	78698	4658	1.2
2	Quinapril	2.196	514354	78599	4598	1.2
3	Quinapril	2.160	513985	79854	4652	1.2
4	Quinapril	2.160	514875	79879	4561	1.2
5	Quinapril	2.160	514658	79865	4659	1.2
6	Quinapril	2.186	516452	79854	4589	1.2
Mean			514830.3			
Std. Dev.			852.3705			
% RSD			0.165563			

- *%RSD of five different sample solutions should not more than 2.*

Table 12: Results of Intermediate precision for Hydrochlorothiazide

S.No	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Hydrochlorothiazide	3.623	1645875	266589	7985	1.1	10.1
2	Hydrochlorothiazide	3.611	1658554	265898	8001	1.1	10.1
3	Hydrochlorothiazide	3.696	1649854	265415	7985	1.1	10.1
4	Hydrochlorothiazide	3.696	1659842	265154	7956	1.1	10.1
5	Hydrochlorothiazide	3.696	1645985	266598	7985	1.1	10.1
6	Hydrochlorothiazide	3.642	1659852	265341	8002	1.1	10.1
Mean			1653327				
Std. Dev.			6838.733				
% RSD			0.413635				

- *%RSD of five different sample solutions should not more than 2.*

Table 13: Results of Intermediate precision Day 2 for Quinapril

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Quinapril	2.198	514658	78572	4672	1.2
2	Quinapril	2.196	514895	78516	4639	1.2
3	Quinapril	2.178	514658	78572	4783	1.2
4	Quinapril	2.142	514784	78372	4623	1.2
5	Quinapril	2.177	515268	78592	4639	1.2
6	Quinapril	2.177	514598	78526	4737	1.2
Mean			514810.2			
Std. Dev.			248.5224			
% RSD			0.048275			

- *%RSD of five different sample solutions should not more than 2.*

Table 14: Results of Intermediate precision Day 2 for Hydrochlorothiazide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Hydrochlorothiazide	3.611	1638732	264384	7985	1.1	10.1
2	Hydrochlorothiazide	3.623	1637438	265827	7946	1.1	10.1
3	Hydrochlorothiazide	3.684	1638474	266382	7943	1.1	10.1
4	Hydrochlorothiazide	3.697	1634273	269183	7964	1.1	10.1
5	Hydrochlorothiazide	3.684	1636372	261931	7968	1.1	10.1
6	Hydrochlorothiazide	3.684	1639283	264356	7982	1.1	10.1
Mean			1637429				
Std. Dev.			1860.366				

% RSD	0.113615

- *%RSD of five different sample solutions should not more than 2.*

Accuracy

Table 15: The accuracy results for Quinapril

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	245954	10	10.179	101.79%	101.36%
100%	483747	20	20.316	101.58%	
150%	715961	30	30.	100.72%	

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

Table 16: The accuracy results for Hydrochlorothiazide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	842287	30	30.114	100.38%	100.26%
100%	1659744	60	60.068	100.113%	
150%	2483885	90	90.268	100.297%	

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

Robustness
Quinapril

Parameter used for sample	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.0 mL/min	513567	2.121	4536	1.2
Less Flow rate of 0.9 mL/min	523652	2.210	4462.3	0.9
More Flow rate of 1.1 mL/min	502146	2.184	4325.1	1.0
Less organic phase	521574	2.200	4632.4	0.9
More Organic phase	502416	2.172	4190.8	0.8

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

Hydrochlorothiazide

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.0 mL/min	1625892	3.643	4536	1.1
Less Flow rate of 0.9 mL/min	1758455	4.498	4426.4	0.9
More Flow rate of 1.1 mL/min	1742514	3.505	4421.5	0.8
Less organic phase	1726451	4.504	4355.1	0.9
More organic phase	1725466	3.512	4426.6	0.9

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

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Quinapril and Hydrochlorothiazide in bulk drug and pharmaceutical dosage forms. Quinapril was found to be freely soluble in water, Soluble in Acetone, Dimethyl sulfoxide, Ethanol, 0,1N HCl, very soluble in methanol and Hydrochlorothiazide was found to be Soluble in dilute ammonia, or sodium hydroxide; also soluble in methanol, 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. Methanol and Phosphate buffer (pH-3.8) (40:60% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Quinapril and Hydrochlorothiazide in bulk drug and in Pharmaceutical dosage forms.

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