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Original Research Paper

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CHLORTHALIDONE AND CILNIDIPINE IN BULK AND COMBINED TABLET DOSAGE FORM

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ABSTRACT

A simple, sensitive, linear, precise and accurate method by Gradient RP-HPLC for the simultaneous estimation of Chlorthalidone and Cilnidipine in bulk and in their combined Tablet Dosage form was developed and validated. The separation of the two drugs was based on Inertsil ODS 3V (250 × 4.6 mm, i.d., 5 µm) column in a Gradient mode. The mobile phase consisting of 0.025 M Potassium dihydrogen orthophosphate buffer whose pH was adjusted to 2.5 using dilute orthophosphoric acid (solvent A) and Acetonitrile (solvent B), set with gradient programming for 15 min at a flow rate of 1ml/min and the detection of the drugs at 240 nm using a variable PDA detector. The retention times of Chlorthalidone and Cilnidipine were found to be 3.872 minutes and 7.668 minutes respectively. The assay of CHL and CIL in bulk drug and in combined tablet dosage form was found to be 99.72% and 99.90% respectively. Calibration curves were linear for CHL and CIL at concentration ranges of range of 200-600 µg/ml and 160-480 µg/ml with the regression coefficient of 0.999 for both the drugs and precise with (% RSD < 2). The LOD was found to be 0.50 µg/ml and 0.40 µg/ml for CHL and CIL and LOQ was 1.50 µg/ml 1.20 µg/ml for CHL and CIL respectively. The developed method was validated by determining its linearity, accuracy, precision, system suitability and can be employed for routine quality control analysis as per ICH guidelines.

Keywords: Chlorthalidone, Cilnidipine, RP-HPLC, Gradient mode, Validation.

INTRODUCTION

Chlorthalidone (CHL) is a sulphamyl benzophenone derivative, chemically known as [2-chloro-5-(1-hydroxyl-3-oxo-2,3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide], structure shown in figure 1. It is a thiazide-like diuretic, as it acts in a similar manner to the thiazide drugs but does not include the benzothiadiazine structure. It is used in the treatment of edema coupled with congestive heart failure, cirrhosis of the liver, fluid retention caused due to kidney disease and hypertension by reducing the electrolyte salts and water in the body. It also used in the treatment of diabetes insipidus and prevent the formation of calcium kidney stones in people with increased levels of calcium in their urine (hypercalciuria).[1,2] Cilnidipine (CIL), is a dihydropyridine calcium channel antagonist, chemically known as 1,4- Dihydro- 2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxyl-lic acid 2-methoxyethyl(2E)-3-phenyl-propenyl ester, structure shown in figure 2. It obstructs L-type voltage-gated calcium channels in the

vascular smooth muscles and in the N-type calcium channels in sympathetic nerve endings that supply blood vessels. As a result, arterioles and venules are dilated, causing in the decrease of pressure in the capillary bed. Thus the fluid that is accumulated in the tissues flows back to the veins and thereby reducing the incidence of pedal edema.[3,4] A survey of literature demonstrates various methods such as UV-Visible Spectroscopy[5], RP-HPLC[6-12], HPTLC[13], Capillary Zone Electrophoreis[14] methods for the assessment of Chlorthalidone in bulk and combined dosage formulations and HPLC method in Biological Fluids.[15] Detailed study of the Literature shows that various methods such as, UV-Visible Spectroscopy[16-19], RP-HPLC[20], HPTLC[21] methods for the assessment of Cilnidipine in bulk and combined dosage formulations. However, no suitable method exists for the simultaneous estimation of CHL and CIL. Hence, the present research aims to develop a simple, rapid, accurate and sensitive method for simultaneous estimation of these two drugs for routine analysis with short run time and retention period. The developed method was validated in accordance with International Conference on Hormonization (ICH) guidelines.[22]

MATERIALS AND METHODS

Instrumentation

Liquid Chromatography, Waters separation 2996, with variable wavelength PDA detector module which is equipped with an automatic injector with the injection volume of 20 µl, and 2693 pump was employed for the quantitative determination. Chromatographic separation was achieved on Eclipse XDB C18 column (150 x 4.6 mm i.d; particle size - 5 micron). The data obtained was processed using Empower Software.

Reagents and Chemicals

Pure drug samples of CHL and CIL were obtained as gift samples from Aurobindo Drugs, Hyderabad. Acetonitrile (HPLC grade) was procured from Standard Reagents, Hyderabad, India, Water (HPLC grade) was procured from Merck (India) Ltd. Potassium dihydrogen orthophosphate and Orthophosphoric acid were purchased from SD Fine chemicals, Mumbai. Commercial Nexovas-CH Tablets (CHL-12.5 mg and CIL-10.0 mg) were purchased from the local market.

Preparation of 0.025 M Potassium Dihydrogen Orthophosphate Buffer (pH 2.5)

3.5 gm of Potassium dihydrogen orthophosphate was weighed and dissolved in 1000 ml of water. The pH of the solution was adjusted to 2.5 by using dilute orthophosphoric acid and was filtered through 0.45 µ membrane filtered.

Preparation of Mobile Phase

The contents of the mobile phase were 0.025 M Potassium dihydrogen orthophosphate buffer whose pH was adjusted to 2.5 using dilute orthophosphoric acid (solvent A) and Acetonitrile (solvent B). They were filtered through a 0.45 µm membrane filter and sonication was done to degases before use.

Preparation of Standard Solution

Preparation of the standard stock solution of the drugs was done by dissolving 250 mg of CHL and 200 mg CIL into 50 ml volumetric flask containing about 25 ml of the diluent (Acetonitrile: Water 50:50), sonicated to ensure complete solubility of the drug and finally the volume was done up to 50 ml with the diluent. Further, 5 ml of the prepared solution was taken into a 50 ml volumetric flask and the volume was made up to the mark with diluent to get working standard solution having concentration of 500 µg/ml for CHL and 400 µg/ml for CIL.

Preparation of Sample Solution

Twenty tablets of Nexovas-CH of combined dosage form were weighed and powdered. About 2000 mg of powdered tablets with weight equivalent to 250 mg of CHL and 200 mg of CIL was accurately weighed and mixed with 25 ml of diluent (Acetonitrile: Water 50:50) in a 50 ml of volumetric flask. The mixture was sonicated to ensure complete solubility. Further, 5 ml of the above prepared solution was taken into a 50 ml volumetric flask and filled up to the mark with diluent to get the working sample solution containing 500 µg/ml for CHL and 400 µg/ml for CIL respectively.

Optimization of the Method

Systematic study of the effects of various parameters like column, different buffers, pH of the aqueous phase, composition of organic phase in mobile phase, organic modifiers and flow rate were undertaken by varying one parameter at a time and controlling all other parameters. The mobile phase consisting of 0.025 M Potassium dihydrogen orthophosphate buffer whose pH was adjusted to 2.5 using dilute orthophosphoric acid (solvent A) and Acetonitrile (solvent B), set with gradient programming for 15 min at a flow rate of 1ml/min and detection of the drugs at 240 nm using a variable PDA detector, was optimized for efficient separation of drugs with good peak shapes and retention times. Table1 shows Time programming of gradient elution. The retention time was found to be 3.90 minutes for CHL and 7.67 minutes for CIL. The % assay was calculated for CHL and CIL and was found to be 99.72% and 99.90% respectively. Chromatogram of blank solution (placebo), standard solution and sample solution are shown in figure 3, 4, and 5 respectively.

METHOD VALIDATION

System Suitability

To determine the adequate resolution and repeatability of the proposed method, system suitability tests were carried out. The parameters like retention time, no. of theoretical plates, asymmetry factor were studied by injecting standard solutions of the drugs six times. The values given in table 2, were obtained within the limits.

Linearity and Range

The linearity is the ability of the method to give the test results which are directly related to the concentration of the analyte in sample. Aliquots of standard solution of the drugs were taken to prepare sample solutions in the concentration range of 200-600 µg/ml for CHL and 160-480 µg/ml for CIL. Each of the solution was analyzed in triplicates. Linear fit was illustrated graphically by plotting peak area versus concentration of CHL and CIL and was demonstrated using Linear Regression analysis. Calibration curves of CHL and CIL are given in figure 6 and 7 respectively and the linearity regression data is given in table 3.

Precision

The precision at 100 % concentration of the assay method was estimated by carrying out six independent assays of CHL and CIL. System Precision and Method Precision was carried out and the results are given in table 4 and 5.

Specificity

The effect of wide range of excipients and the other additives usually present in the formulation of CHL and CIL under optimum conditions of the proposed method were investigated. The excipients commonly present in the formulation did not interfere with the elution or quantification of the method as observed in chromatograms presented in figure 3, 4 and 5.

Accuracy

The accuracy of the method was evaluated in triplicates by recovery studies at three different concentration levels of 80%, 100 % and 120%. Known amounts of standard drug concentrations were added to the sample. The accuracy data and the corresponding results are as shown in table 6.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD is defined as the lowest concentration of the analyte that gives a detectable response. LOD is based on S/N ratio (signal/noise) typically for HPLC methods. The LOQ is defined as the lowest concentration of the analyte that can be quantified within a specified level of accuracy and precision. LOQ is determined on the basis of S/N ratio (signal/noise) which is typically used for HPLC methods. A signal-to-noise ratio (S/N) of 3 was generally accepted for estimating LOD and signal-to-noise (S/N) ratio of 10 was used for estimating LOQ. The Concentration and peak areas obtained for determining the LOD and LOQ values are given in table 7.

Robustness

Robustness was evaluated by slight deliberate changes made in chromatographic conditions like proportion of flow rate and use of different

columns. The results obtained by Robustness study are presented in the table 8.

RESULTS AND DISCUSSION

Use of the mixture of 0.025 M Potassium dihydrogen orthophosphate buffer; pH was adjusted to 2.5 using dilute orthophosphoric acid (solvent A) and Acetonitrile (solvent B) as mobile phase in gradient mode was found to be the most suitable to obtain a good chromatographic separation with well defined peaks, free from tailing. The flow rate was found to be optimum at 1ml/ min resulting in short retention time, good baseline stability with low noise level. In the present developed RP-HPLC method, the standard and sample preparation required less time and no tedious extraction was involved. By the application of the proposed method the retention time of CHL and CIL was found to be 3.872 minutes and 7.668 minutes respectively. The assay of CHL and CIL in bulk drug and in combined tablet dosage form was found to be 99.72% and 99.90%. A good linear relationship, r^2=0.997 for CHL and r^2=0.997 for CIL was observed between the concentration range of 200-600 µg/ml and 160-480 µg/ml respectively. The number of theoretical plates was 18991.77 and 36057.29 respectively indicates efficient performance of the column. The LOD values were found to be 0.50 µg/ml and 0.40 µg/ml and LOQ values were found to be 1.50 µg/ml 1.20 µg/ml for both CHL and CIL respectively. Low values of standard deviation of retention time and peak areas indicate high precision of the method. From the recovery studies data, it was found that the mean % recovery was within the limits, indicated high accuracy of the proposed method. There are no additional peaks observed in the chromatogram, which indicates non-interference of the common excipients used in the formulation. No marked changes were observed in % assay of the optimized conditions with that of the altered conditions in the robustness study indicating that the method is robust.

CONCLUSION

A simple and efficient method was developed for the simultaneous estimation of Chlorthalidone and Cilnidipine within a short analysis time with no interference of the common additives present in the pharmaceutical formulation. The proposed method can be applied for routine quality control and analysis in bulk and in tablet dosage forms.

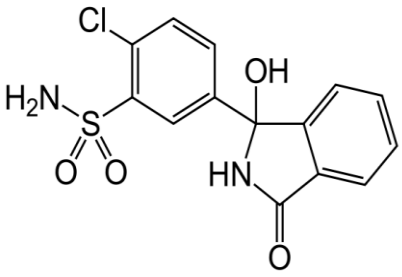

Figure 1: Structure of Chlorthalidone

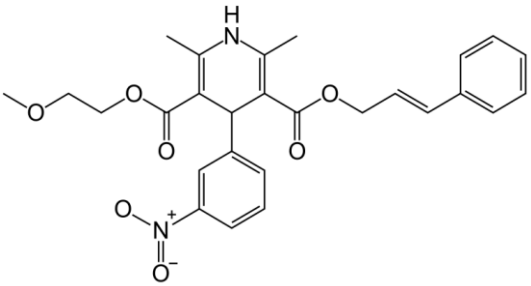

Figure 2: Structure of Cilnidipine

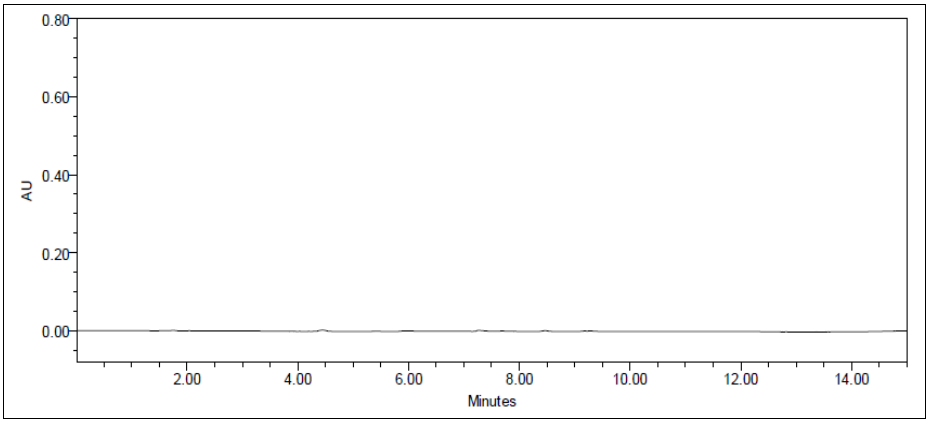

Figure 3: Chromatogram of blank solution

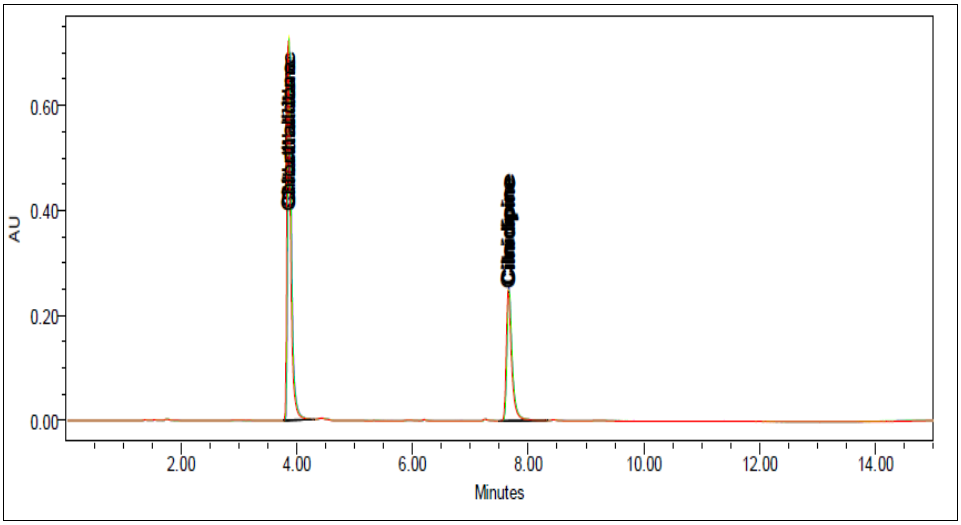

Figure 4: Chromatogram of working standard solution

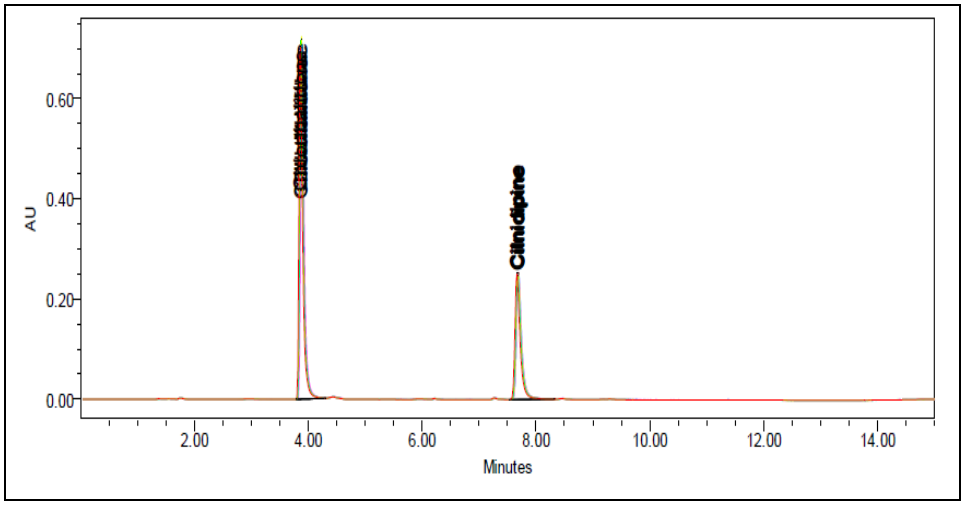

Figure 5: Chromatogram of working sample solution (Nexovas-CH)

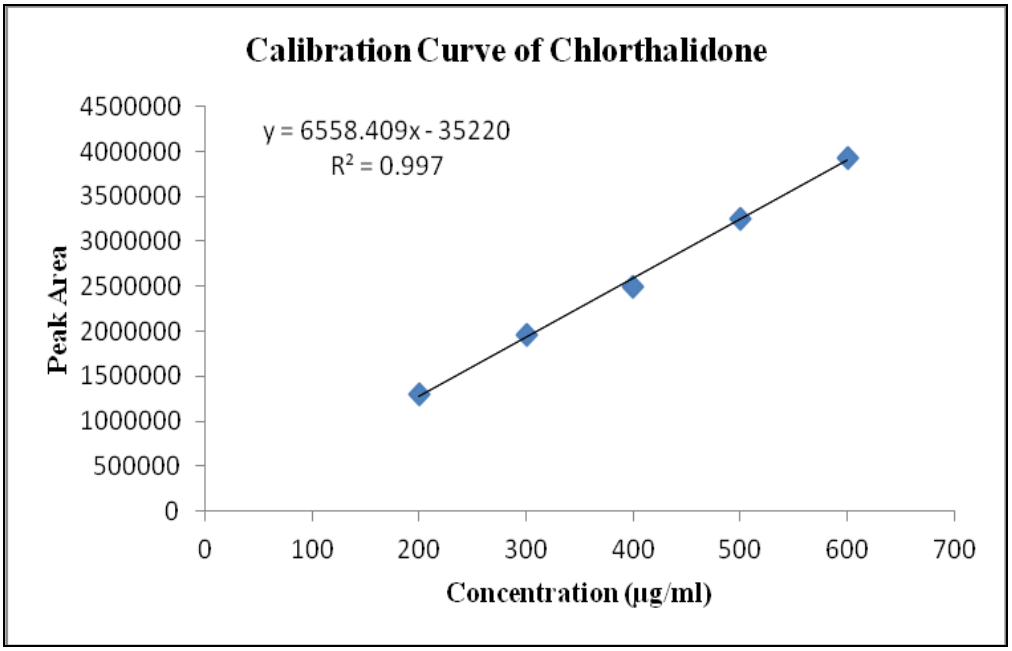

Figure 6: Calibration curve of Chlorthalidone

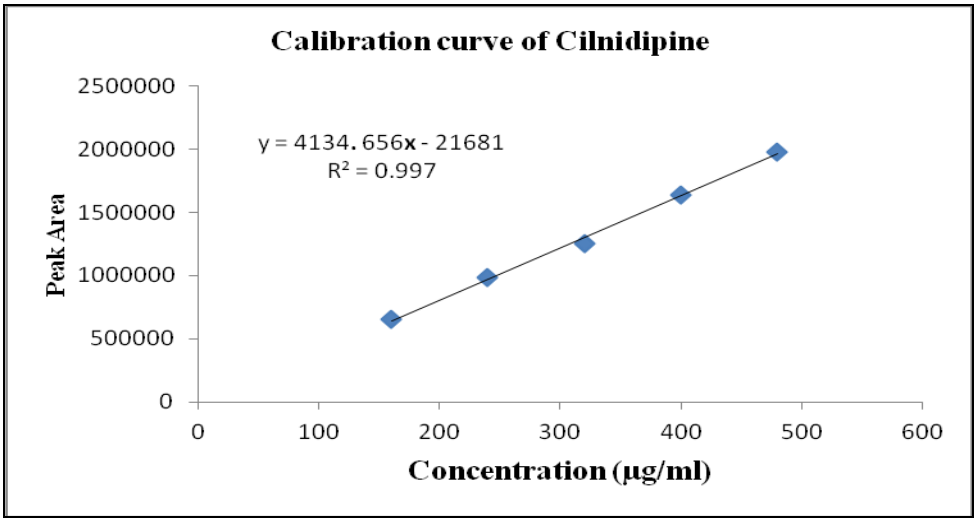

Figure 7: Calibration curve of Cilnidipine

Table 1: Time programming of gradient elution

Time in Minutes	% Mobile phase-A (0.025 M Potassium dihydrogen orthophosphate)	% Mobile phase-B (Acetonitrile)
0	80	20
4	20	80
10	20	80
12	80	20
15	80	20

Table 2: System suitability test parameters of the proposed method

S. No	Parameters	Chlorthalidone	Cilnidipine	Limits
1	Relative retention time(minutes)*	3.872	7.668	--
2	% RSD of Retention Time	0.2	0.1	Not more than 2
3	Peak Area*	3233611.5	1611191.4	--
4	% RSD of Peak area	0.8	0.3	Not more than 2
5	Theoretical plates	18991.77	36057.29	More than 2000
6	Tailing factor	1.6	1.5	Less than 2
7	Resolution	---	26.61	More than 2

*Mean of six determinations

Table 3: Regression analysis data of the proposed method

Parameters	**Chlorthalidone**	**Cilnidipine**	**Limits**
Linearity range	200-600 µg/ml	160-480 µg/ml	__
Regression Equation (y= mx+c)	y=6558.409x-35220	y=4134.656x-21681	__
Correlation coefficient (r^2)	0.997	0.997	$r^2 \geq 0.99$

Where, y- is the peak area and x is the Concentration in µg/ml

Table 4: Results of System Precision

Injection No	Chlorthalidone		Cilnidipine	
	Retention Time in Minutes	% Assay	Retention Time in Minutes	% Assay
1	3.86	100.05	7.66	99.87
2	3.87	99.44	7.67	99.71
3	3.87	101.20	7.66	99.61
4	3.87	99.43	7.67	100.43
5	3.87	98.32	7.67	99.92
6	3.88	99.85	7.67	99.81
Mean	3.9	99.72	7.7	99.90
Std. Dev	0.0	0.94	0.0	0.29
% RSD	0.2	0.94	0.1	0.29

Table 5: Results of Method Precision

Injection No	Chlorthalidone		Cilnidipine	
	Retention Time in Minutes	% Assay	Retention Time in Minutes	% Assay
1	3.87	99.01	7.67	99.56
2	3.88	99.63	7.68	99.60
3	3.88	100.28	7.68	99.58
4	3.88	99.09	7.70	99.37
5	3.89	99.46	7.70	99.88
6	3.88	100.65	7.69	99.65
Mean	3.9	99.69	7.7	99.61
Std. Dev	0.0	0.65	0.0	0.17
% RSD	0.2	0.66	0.2	0.17

Table 6: Recovery studies of Chlorthalidone and Cilnidipine

Drug/ Parameters	Chlorthalidone			Cilnidipine		
Concentration	80 %	100 %	120 %	80 %	100 %	120 %
Amount present in µg/ml	400	500	600	320	400	480
Amount spiked in µg/ml	50	50	50	40	40	40
Amount after spiking in µg/ml	450	550	650	360	440	520
Total amount recovered	518.40	520.85	718.90	416.88	446.82	561.60
% recovery*	115.20	94.71	110.60	115.80	101.55	108.00
Mean % recovery	106.83			108.45		

* Mean of three determinations

Table 7: LOD and LOQ values

S. No.	Parameters	Chlorthalidone	Cilnidipine
1	LOD(µg/ml)	0.50 µg/ml (0.10 %)	0.40 µg/ml (0.10 %)
2	LOQ(µg/ml)	1.50 µg/ml (0.30 %)	1.20 µg/ml (0.30%)

Table 8: Results of Robustness by variations in Flow rate and column

Parameter	Modification	Chlorthalidone			Cilnidipine		
		RT*	% RSD of RT	% Assay	RT*	% RSD of RT	% Assay
Optimized conditions		3.872	0.2	99.71	7.668	0.1	99.89
Flow-rate	0.9 ml/min	4.24	0.4	99.35	8.30	0.3	99.76
	1.1 ml/ min	3.62	0.1	99.28	7.30	0.1	99.05
Different Column	---	3.90	0.2	99.80	7.77	0.0	99.89

* Mean of three determinations, *Retention time in Minutes

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