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Research Article

HPLC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF HYDROCHLOROTHIAZIDE AND PERINDROPRIL IN TABLET DOSAGE FORM

SURAJ SAHOO*, PRASANNA KUMAR PANDA[1], SAGAR KUMAR MISHRA[1], SABUJ SAHOO[1]

*School of Pharmaceutical Education & Research, Berhampur University, Berhampur 760007, [1]University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar751004.**Email:** surajuniversity@rediffmail.com

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ABSTRACT

A precise, accurate, sensitive and economic HPLC method was proposed for simultaneous estimation of Hydrochlorothiazide and perindopril from tablet dosage forms using a mobile phase A that is mixture of equal volumes of water (pH adjusted to 2.5 with O- phosphoric acid) and perchloric acid and mobile phase B that is 0.03 % V/V solution of perchloric acid in acetonitrile at a flow rate of 1.0 ml/min with an injection volume of 20µl. A C8 RP-Column (Zorbax XDB 150mm x 4.6 mm 5 µm) column was used as stationary phase. Wavelength at which assay method studied was 215nm. The retention time of Hydrochlorothiazide and perindopril were found to be 3.26 min. and 7.31 min. respectively. Linearity was obtained in the concentration range of 5-45µg/ml for Hydrochlorothiazide, 20-100 µg/ml for perindopril. The method was validated statistically according to International Conference on Harmonization (ICH) guidelines and observed results were found in the acceptance range.

Keywords: Hydrochlorothiazide, Perindopril, RP-HPLC, Validation

INTRODUCTION

Hydrochlorothiazide belongs to the thiazide class of diuretics and is used for the treatment of hypertension, congestive heart failure whereas Perindopril is a long-acting ACE inhibitor and is used to treat high blood pressure, heart failure or stable coronary artery disease. Various UV- Spectrophtometric[1], HPLC[2-7], HPTLC[8] and Volta metric assay[9] methods are reported in the literature for the estimation of Hydrochlorothiazide and perindopril individually and in combination with other drugs. According to literature survey there is no method reported for the simultaneous estimation of Hydrochlorothiazide and perindopril in combined solid dosage forms.

Procedures and acceptances criteria were adapted according to ICH[10] guidelines for validating the proposed method. The developed method designed in such a way that it avoids use of buffer which proves economy of the method with respect to time and cost, as column washing with solvents is not required. Thus the objective of the present work was the development and validation of a precise, accurate, economic RP-HPLC method for simultaneous estimation of Hydrochlorothiazide and perindopril in tablet dosage form.

MATERIALS AND METHODS

Active Pharmaceutical Ingredients were procured from Cellogen Pharma, Navi Mumbai. HPLC grade solvents used were of E-Merck India. Other chemicals and reagents used were of analytical grade. HPLC grade water used was obtained using Millipore purification system.

Liquid chromatography instrument equipped with a dual pump (LC-10ATVP) in gradient mode. Column used as stationary phase was (Zorbax XDB, C8- 150mm x 4.6 mm 5 µm) and the column was maintained at 30° c. UV-detector (SPD-10A VP) was implemented for detection and execution of chromatogram.

Chromatographic conditions

Column:	Zorbax XDB, C8- 150mm x 4.6 mm, 5 µm
Wavelength:	215nm
Injection volume:	20 µl
Flow rate:	1.0 ml/min, Mobile phase A: Mobile phase B in the ratio 95:5
Temperature:	30°c
Run time:	10min
Mobile phase A:	mixture of equal volumes of water (pH adjusted to 2.5 with O-phosphoric acid) and perchloric acid
Mobile phase B:	0.03 % V/V solution of perchloric acid in acetonitrile
Diluent:	Mobile phase A

Assay Preparations

Hydrochlorothiazide standard stock solution

An accurately weighed quantity 50 mg of Hydrochlorothiazide was transferred in to a 100 ml volumetric flask, dissolved in a sufficient quantity of diluent. The volume was made up to the mark with diluent.

Perindopril standard stock solution

An accurately weighed quantity 40 mg of Perindopril was transferred in to a 100 mL volumetric flask, dissolved in a sufficient quantity of diluent. The volume was made up to the mark with diluent.

Standard and sample solution

5.0 ml from Hydrochlorothiazide standard stock solution and 10.0ml from Perindopril standard stock solution were diluted to 100ml with diluent to get final concentration of 25 µg/ml and 40 µg/ml respectively. Sample solution using diluent was prepared keeping same concentration as that of standard concentration. The solution was filtered with 0.45 µm filter.

Standard solution and sample filtrate was injected and chromatographed. System suitability parameters and retention time obtained are shown in Table-1. The amount of the drugs present were calculated, assay for Hydrochlorothiazide and Perindopril found to be 100.0 % and 99.8% respectively. The standard chromatogram obtained, shown in Figure -1.

Table1: system suitability parameters for the proposed method

Parameters	Hydrochlorothiazide	Perindopril
Retention Time (min)	3.26	7.31
Theoretical plates	2461	2098
Tailing Factor	0.6	0.8

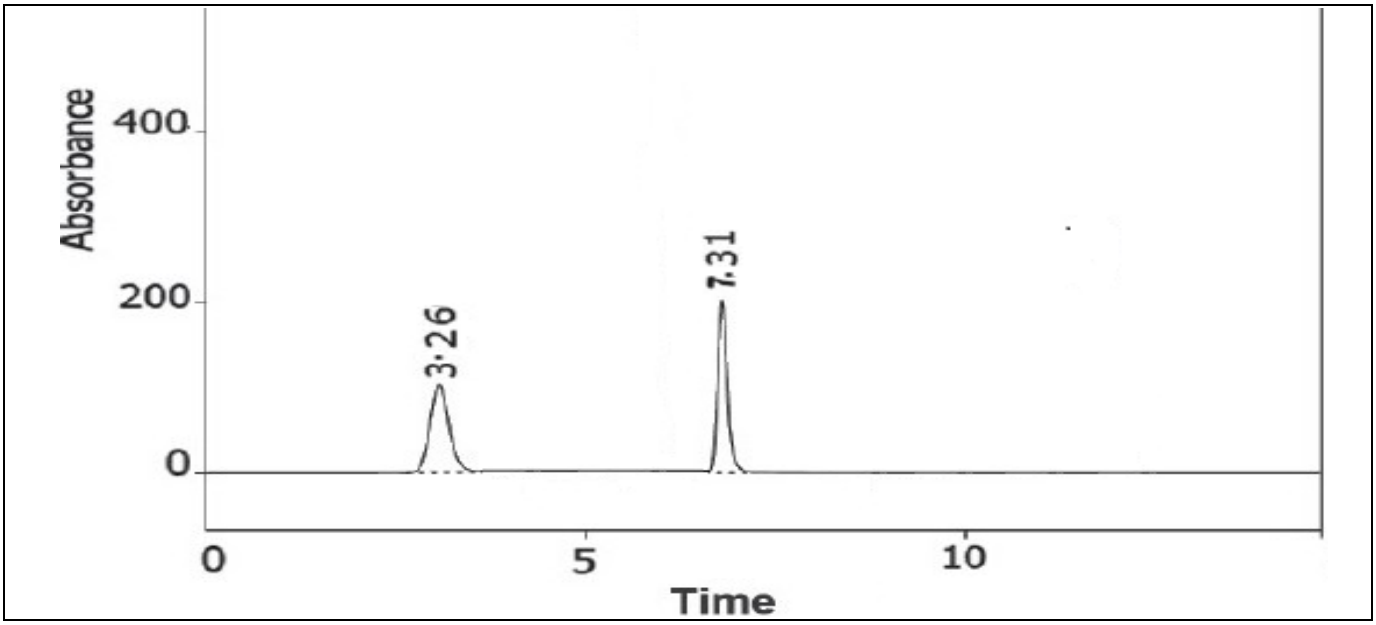

Figure 1: standard chromatogram showing well separated peaks for hydrochlorothiazide and perindopril.

Method validation

The proposed analytical method was validated with respect to parameters which are specificity, linearity, precision, accuracy, robustness, and ruggedness and are executed as per the ICH guidelines. The results obtained are narrated in tables ahead (Table 1-6).Results obtained with respect to individual parameter are within the acceptance criteria and as stated earlier are validated as per ICH guidelines.

RESULTS AND DISCUSSION

Linearity

The linearity of the method was studied at five different concentrations including the test concentration. *i.e*., 50, 75, 100, 125, and 150%. The response obtained was linear which apparently indicates the capability of method to reproduce the results within the linear range. The statistical results obtained were shown in Table 2.

table 2: regression analysis of the calibration curve

Parameters	Hydrochlorothiazide	Perindopril
Linearity range (µg/ml)	10-40	20-60
Slope	415	523.3
Intercept	1.20	121
Correlation coefficient	0.9999	0.9999

Precision

The system precision was studied by preparing the standard solution at test concentration and injected repeatedly for six times. The method precision results obtained for five different preparations are tabulated showing % relative standard deviation. The results obtained are shown in Table- 3.

table 3: results for precision showing % rsd for system and method

Drug	System Precision	Method Precision
	% RSD	% RSD
Hydrochlorothiazide	0.81	0.28
Perindopril	0.17	0.18

Accuracy

The % recovery has been studied by spiking method from 50 to 150% of test concentration. The results obtained are tabulated in Table- 4.

table 4: results for accuracy

Levels	Hydrochlorothiazide		Perindopril	
	% Recovered	%RSD	% Recovered	%RSD
50%	100.0	0.8	100.7	1.5
100%	98.9	1.4	99.8	1.7
150%	100.4	1.0	100.4	0.7

Ruggedness

Ruggedness was studied by 2nd analyst on another day; the results obtained are within the acceptance criteria and are shown in Table-5.

table 5: results for ruggedness

Drug	Intraday assay(Analyst 1)		Inter day assay(Analyst 2)	
	% Obtained	% RSD	% Obtained	% RSD
Hydrochlorothiazide	100.0	0.28	100.2	0.64
Perindopril	99.8	0.18	100.0	0.71

Robustness

The robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed by small variation in the chromatographic conditions and found to be unaffected by small variations like

- variation in volume of mobile phase composition,
- flow rate of mobile phase,
- Variation in pH.

table 6: results for robustness

Factor	Change	Retention time		%RSD	
		Hydrochlorothiazide	Perindopril	Hydrochlorothiazide	Perindopril
Flow rate(ml/min)	-0.2	3.27	7.5	0.2	1.0
	+0.2	3.26	7.3	0.4	0.9
% of Acetonitrile	-0.5	3.27	7.4	0.5	0.8
	+0.5	3.25	7.3	0.7	0.9
pH	-0.2	3.25	7.3	0.5	0.6
	+0.2	3.26	7.3	0.5	0.8

CONCLUSION

The proposed reverse phase high performance liquid chromatographic method has been evaluated over the linearity, precision, accuracy, ruggedness and robustness and proved to be effective for the determination of Hydrochlorothiazide and Perindopril in given application. Thus the proposed method is Precise, accurate, sensitive and economic.

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