HPLC Method Development and Validation for Simultaneous Analysis of Diclofenac Sodium and Rabeprazole Sodium

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Abstract: A stable, simple, rapid, precise, accurate RP-HPLC method for simultaneous analysis of diclofenac sodium and rabeprazole sodium was developed and validated as per ICH guidelines without need of any internal standard. Separation was carried out using C8 column with triethyl amine buffer (pH 5): acetonitrile (50:50 v/v) as mobile phase with flow rate 2 mL min⁻¹. The detection was carried out at 284 nm. The parameters studied were retention time, linearity and range, accuracy, precision, detection limit, quantitation limit and bench top stability. The proposed method can be used for simultaneous estimation of diclofenac sodium and rabeprazole sodium in bulk drugs and pharmaceutical dosage forms.

Keywords: Diclofenac sodium, Rabeprazole sodium, RP-HPLC, Validation.

Introduction

Diclofenac sodium {2-[(2, 6-Dichlorophenyl) amino] benzene acetic acid sodium salt} (DICLO) is a phenyl acetic derivative used as non-steroidal anti-inflammatory agent. Rabeprazole sodium {2-[[[4-(3-methoxypropoxy)-3- methyl-2 pyridinyl] methyl] sulfinyl]-1H-benzimidazole sodium salt}(RAB), a benzimidazole derivative a proton pump inhibitor (PPI) used as acid suppressing agent. Several UV spectrophotometric, voltametric or HPLC methods are reported for DICLO and RAB as individual component or with other drugs¹⁻⁸. Reported simultaneous determination method for DICLO and RAB requires indapamide as an internal standard and using methanol as a solvent⁹. In present study the simple, rapid, precise, accurate, sensitive and stable RP-HPLC method has been developed without use of any internal standard as per ICH guidelines¹⁰. Alkaline borate buffer of pH 8 was utilized as

a solvent which can be used as dissolution medium for dissolution study of the DICLO and RAB in combined formulation. However 0.5 M sodium hydroxide was used for the stabilization of RAB. The method was developed for simultaneous estimation of DICLO and RAB in the pharmaceutical formulations.

Experimental

The chromatographic separation and simultaneous analysis of DICLO and RAB was carried out using Dionex chromatographic system (Germering, Germany) equipped with Automated sample injector (P680) plus HPLC pump (ASI 1000) plus UV detector (UVD170U), Rheodyne injector and Chromeleon® acquisition software Ver.6.70. BDS Hypersil C8 column (250 mm x 4.6 mm, 5 μ) was used for the separation. The 0.22 μ m nylon filters (Millipore) and Vacuum pump (Rocker pump 400 Today's) were used for filtration while ultrasonication was carried out with ultrasonicator (USR 100 Spectralab).

Diclofenac sodium (Glenmark Ltd., Nasik), rabeprazole sodium (Wockhardts Ltd., Aurangabad), triethyl amine (Merck Ltd., Mumbai), acetonitrile (Qualigens), boric acid (Qualigens), potassium chloride (Loba chem), orthophosphoric acid (Qualigens) and sodium hydroxide (Merck Ltd., Mumbai), *etc.* were used in this work.

General procedure

The chromatographic parameters were as given in Table 1.

Table 1. Chromatographic conditions

Mobile Phase	Acetonitrile (ACN): aqueous triethyl amine (TEA) buffer (50:50 v/v)
pH	5.00 (adjusted with phosphoric acid)
Flow rate	2 mL min ⁻¹
Injection volume	10 μ L
Elution type	Isocratic elution
Detection wavelength	284 nm
Column	BDS Hypersil C8 (250 mm x 4.5mm, 5 μ m)
Temperature	25 $\pm$ 2 $^{\circ}$ C

Preparation of standard stock solutions

Standard stock solutions of DICLO and RAB, 10 mg/dL were prepared in pH 8 alkaline borate buffer I.P. The standard solutions of DICLO and RAB in mixture were prepared from standard stock solution using pH 8 alkaline borate buffer I.P with one ml addition of 0.5 M sodium hydroxide to each solution in calibrated volumetric flask of capacity 5 mL. The prepared standard solutions of DICLO and RAB were ranging from 25-125 μ g mL⁻¹ and 5-25 μ g mL⁻¹ respectively. The representative chromatogram is shown in Figure 1. Use of non-actinic glass vials and incorporation of sodium hydroxide in standard solutions found to decrease degradation of RAB.

Preparation of calibration curve

An aliquot of 10 μ L of each standard solution was injected and chromatogram recorded for each solution. The calibration curves were obtained by plotting peak area *versus* concentration. All peaks were integrated by using software Chromeleon®. The equations of regression lines obtained are as follows:

For RAB: $y=0.1594x+0.2458$ ($r^2=0.9987$), For DICLO: $y=0.1925x-0.0546$ ($r^2=0.9985$).

Figure 1. Representative chromatogram of DICLO and RAB

Procedure for assay of marketed formulation

Twenty capsules (Trade Name: Safediclo, Emcure, Ltd. Pune) procured from market were weighed by individually emptying the contents of capsules. The granules were powdered and powder equivalent to average weight of granules from 20 capsules were added to 100 mL calibrated volumetric flask wrapped with aluminum foil. The 20 mL of 0.5 M NaOH solution was immediately added to the flask with further addition of 30 mL ice cold pH 8 alkaline borate buffers. The contents of flask were sonicated by keeping flask in ice cold bath for 10 min. Then flask was again cooled and volume was made up to 100 mL with pH 8 alkaline borate buffer. The solution was transferred to centrifuge tubes and centrifuged at 3500 rpm for 5 min, supernatant was collected and filtered through 0.22 μm nylon filter (Millipore) using a syringe filter (Tarsons) mounted on the Luer Lok syringe (Becton Dickinson). The one mL of filtrate was diluted to ten mL in calibrated volumetric flask with pH 8 alkaline borate buffer and 10 μL was injected in triplicate to column. The concentration of DICLO and RAB in the formulation was calculated by comparing with standard calibration curve.

Validation of RP-HPLC method

The HPLC method was validated based on ICH guidelines with validation parameters *viz.* linearity and range, accuracy, precision, limit of detection and limit of quantitation. The bench top stability and system suitability studies were also performed.

Linearity and range

The linearity of the method was determined at five concentration levels ranging from 25-125 $\mu\text{g mL}^{-1}$ for DILCO and 5-25 $\mu\text{g mL}^{-1}$ for RAB.

Accuracy

The accuracy of method was determined using three concentrations in triplicate at ratios 75:15, 100:20 and 125:25 $\mu\text{g mL}^{-1}$ for DILCO: RAB. The percent recovery within 98-100% illustrates accuracy of the method.

Precision

The method is validated using six determinations at 100% of test concentrations for repeatability and three concentrations at 3 levels for intermediate precision. The RSD not more than 2 indicates precise method.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection and limit of quantitation were calculated from the standard deviation approach.

Bench top stability study

The standard solution containing 100 $\mu\text{g mL}^{-1}$ and 20 $\mu\text{g mL}^{-1}$, DICLO and RAB respectively were kept at room temperature for 24 h and then samples were analyzed.

System suitability

The asymmetry factor not more than 2, relative standard deviation of peak areas of six replicate injections not more than 2 and resolution of peaks of DICLO and RAB not less than 2 and theoretical plates not less than 2000 qualifies the system suitability.

Results and Discussion

The RP-HPLC method was developed for DICLO and RAB with sharp and well resolved peaks. The use of 0.5 M NaOH and non-actinic glassware found to improve stability of RAB in solution. The different mobile phases at various pH and different flow rates were examined and mobile phase with ACN: TEA (50:50) pH 5.00 was selected as optimal mobile phase based on system suitability parameters with well resolved peaks of DICLO and RAB.

The wavelength for the detection of both compounds was 284 nm with best detector response. The method was found to be linear at range of 25-125 $\mu\text{g mL}^{-1}$ for DICLO and 5-25 $\mu\text{g mL}^{-1}$ for RAB. The developed method was found to be accurate, precise and quite stable with acceptable values of LOD and LOQ. Validation and other parameters are reported in Table 2.

Table 2. Validation parameters

Parameters	RAB	DICLO
Retention time	2.06 min	3.30 min
Wavelength, nm	284	284
No. of data points	5	5
Range, mg L^{-1}	5-25	25-125
Regression coefficient	0.9987	0.9985
Slope	0.1594	0.1924
Intercept	0.2458	-0.05464
Accuracy, %	99.3073 $\pm$ 1.0323	99.7556 $\pm$ 0.2127
Intraday Precision	0.2401	0.0156
Interday Precision	0.4494	0.3649
Repeatability	0.5279	0.3649
Assay, %	99.5857 $\pm$ 0.4649	100.0776 $\pm$ 0.2510
LOD $\mu\text{g mL}^{-1}$	0.00824	0.07342
LOQ $\mu\text{g mL}^{-1}$	0.02508	0.22248
Bench top recovery, %	97.4253 $\pm$ 0.4267	99.7251 $\pm$ 0.1014
No. of Theoretical Plates	7483	7567

Conclusion

The RP-HPLC method developed for simultaneous analysis of DICLO and RAB can be used for routine quality control of their bulk drug mixture and their combined dosage form.

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