



Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Drotaverine Hydrochloride and Aceclofenac in Bulk and Tablet Dosage Form

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Abstract

Objective: To develop a simple, reproducible and economical reverse phase high performance liquid chromatographic (RP-HPLC) method for the simultaneous estimation of drotaverine hydrochloride and aceclofenac in bulk and tablet dosage form and to validate it as per international conference on harmonization (ICH) guidelines. **Methods:** The quantification was carried out on a Hypersil ODS 250 mm×4.6 mm, 5μ column in isocratic elution mode with mobile phase ortho-phosphoric acid buffer (pH 2.8) and acetonitrile in the ratio 20:80 v/v. The mobile phase was pumped at a flow rate of 1.0 ml/min. The detection was carried out at 210 nm using photo diode array detector. **Results:** The retention times of drotaverine hydrochloride and aceclofenac were found to be 3.152 and 2.921 minutes respectively. The developed method was linear for drotaverine hydrochloride and aceclofenac over the concentration range of 25-150 % and 20-120 % with correlation coefficient (r^2) values 0.9998 and 0.9996 respectively. The LOD and LOQ for drotaverine hydrochloride and aceclofenac were 0.11, 0.06 and 0.34, 0.18 μg/ml correspondingly. The mean percent recoveries of drotaverine hydrochloride and aceclofenac were 100.04% and 100.3% and were within the limits (98%-102%). **Conclusion:** The validation of the method revealed that the proposed method was found to be selective, linear, accurate, precise and robust and hence it can be used for routine analysis.

Keywords

RP-HPLC, drotaverine hydrochloride, aceclofenac, validation and ICH.

INTRODUCTION

Drotaverine hydrochloride and aceclofenac are used to treat abdominal pain and cramps. Drotaverine

hydrochloride is an anti-spasmodic medicine which relieves contractions (spasms) associated with smooth muscles in the abdomen. Drotaverine

hydrochloride ($C_{24}H_{31}NO_4 \cdot HCl$) is structurally related to papaverine. It is a selective inhibitor of phosphodiesterase 4 and has no anticholinergic effects. It inhibits phosphodiesterase by hydrolyzing cAMP, thereby increasing cAMP concentration, decreasing calcium uptake of the cells and changing

the distribution of calcium among the cells. It may also have minor allosteric calcium channel blocking properties. Drotaverine hydrochloride has been shown to possess dose-dependent analgesic effects in animal models.

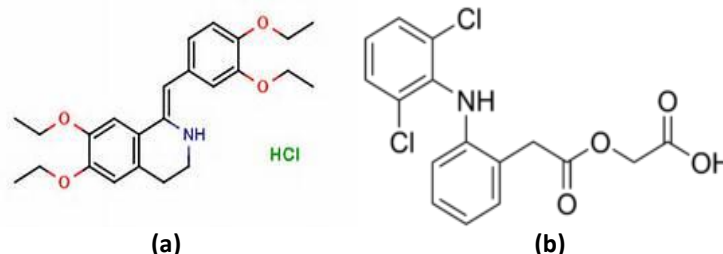


Fig.1: Chemical structure of (a) Drotaverine hydrochloride (b) Aceclofenac

Aceclofenac ($C_{16}H_{13}Cl_2NO_4$) the glycolic acid ester of diclofenac, is a non-steroidal anti-inflammatory drug (NSAID). It is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. The mechanism of action of aceclofenac involves the inhibition of cyclo-oxygenase (COX), which is a key enzyme in the inflammatory cascade. Cyclo-oxygenase is involved in the production of prostaglandins, swelling and inflammation. The inhibition of COX leads to the suppression of pro-inflammatory prostaglandins and cytokines. It is metabolized in human hepatocytes and human microsomes to form [2-(2', 6'-dichloro-4'-hydroxy- phenylamino) phenyl] acetoxyacetic acid as the major metabolite, which is then further conjugated.

A literature survey on the analytical methods of drotaverine hydrochloride and aceclofenac revealed that a few UV Spectrophotometric [1, 2 & 3], RP-HPLC [4, 5, 6 & 7] and HPLC [8 & 9] methods were available for their estimation in dosage form in addition to other techniques. Some of these methods have certain drawbacks like complexity in composition of mobile phase, higher amounts of buffer that can affect the column performance, elution technique, long run time, less resolution, lack of sufficient sensitivity, precision and accuracy and some methods lacked proper documentation. Hence, attempts have been made to develop simple, fast, accurate, economical and precise RP-HPLC method for determination of drotaverine hydrochloride and aceclofenac.

MATERIALS AND METHODS

Chemicals and reagents

Drotaverine hydrochloride and aceclofenac standard drugs were procured from Spectrum pharma

solutions, Kukatpally, Hyderabad and tablet dosage forms of drotaverine hydrochloride and aceclofenac were procured from local market. All the solvents water, methanol, acetonitrile, ortho-phosphoric acid of HPLC grade and hydrochloric acid, hydrogen peroxide and sodium hydroxide of analytical grade were purchased from the Merck Company.

Instrumentation

HPLC instrument used was WATERS HPLC 2965 SYSTEM with auto Injector and PDA detector. Software used was EMPOWER 2. UV/VIS spectrophotometer PG Instrument T60 with special band width of 2 mm and 10 mm and matched quartz was used for measuring absorbance of drotaverine hydrochloride and aceclofenac solutions, SARTORIUS Micro-balance was used for weighing and digital pH meter used was METSAR (DP^H 504).

Selection of wavelength (λ_{max})

In order to ascertain the optimum wavelength, the solutions of drotaverine hydrochloride and aceclofenac were prepared with diluent and the solutions were scanned in UV region. Both the drugs showed significant absorbance at 210 nm. Hence, 210 nm was selected for analysis.

Preparation of ortho-phosphoric acid buffer (pH 2.8)

0.1% Ortho-Phosphoric Acid buffer was prepared by adding 1ml of ortho phosphoric acid to 1000 ml with HPLC grade water and dissolved. It was sonicated for 5min to degas and finally pH was adjusted to 2.8.

Preparation of Mobile Phase (Buffer: Acetonitrile) (20:80)

200 ml of Ortho-phosphoric acid buffer (pH 2.8) was taken in a clean and calibrated 1000 ml measuring cylinder and 800 ml of acetonitrile was added. The solution was filtered through 0.45 μ nylon filter paper and then sonicated for 10 min.

Preparation of Diluent

Diluent was prepared by taking HPLC grade water and HPLC grade acetonitrile in the ratio 30:70. This solution was used as blank.

Preparation of standard stock solution

An accurately weighed quantity of drotaverine hydrochloride 10 mg and aceclofenac 8 mg were taken separately in a clean and dry 10 ml volumetric flask each and dissolved by using small amount of Diluent. It was sonicated for 5 min. The final volume was made with diluent. From the above two solutions 1ml was taken into 10 ml volumetric flasks and diluted to 10 ml with the diluent.

Preparation of sample stock solution

20 tablets were weighed, and average weight was calculated. 10 mg equivalent weight of powdered tablets was weighed and then transferred into a 100 ml volumetric flask. The powder was dissolved in

small amount of Diluent. It was sonicated for 25 min and finally make up to the volume with diluent. From the filtered solution 1ml was pipetted out into 10ml volumetric flask and made up to 10ml with diluent.

Procedure for assay [10]

20 tablets were taken, accurately weighed and powdered. A quantity equivalent to 100 mg was taken into a 100 ml volumetric flask and made up to the mark with diluent. The resultant solution was sonicated and filtered. Then it was diluted to get a concentration in the linearity range of bulk drug to get a sample solution. Standard solutions were made from active pharmaceutical ingredients. 10 µl of blank, standard and sample solutions were injected into the chromatographic System. Peak area of drotaverine hydrochloride and aceclofenac and sample solution were used for calculating the percentage assay by using the formula.

$$\% \text{ Assay} = \frac{\text{Sample area} \times \text{standard dilution} \times \text{potency} \times \text{average weight} \times 100}{\text{Standard area} \times \text{sample dilution} \times 100 \times \text{label claim}}$$

Method Validation

System suitability

The system suitability was checked by giving six replicate injections of standard solution (10 µg/ml) in to HPLC system. The acceptance criteria % RSD for the peak area of principle peak from 6 replicate injections of each standard solution should be not more than 2%. The no. of theoretical plates (N) for the drug peak should not less than 2500. The tailing factor (T) for the drug peak should not more than 2.0.

Specificity

Specificity is the ability of the analytical method to assess the analyte in the presence of components which may be expected to be present in the sample. Specificity is to find out the peaks of interest. Here four samples were prepared i.e. blank (only diluents), standard (active Pharmaceutical Ingredient), sample (marketed formulation), placebo (only additives) and injected into the HPLC system to check the interference. Chromatograms of standard and sample should be identical with near retention time.

Linearity

Six concentrations ranging from 20% to 150 % for both the drugs were prepared and injected twice into the HPLC system and peak area was measured. A graph was plotted by taking peak area on y- axis and concentration on x- axis. The slope, intercept and correlation coefficient of regression line was determined.

Accuracy

The accuracy of the method can be known by percentage recovery using standard addition

method. To the sample solution (10 µg/ml), a known amount of standard drug was added at 50%, 100% and 150 % level. Triplicate injections for each level accuracy sample was injected in to HPLC system, the average percentage recovery for each level was calculated.

System precision

Six replicate injections of standard solution were injected in to HPLC system and system precision of the proposed method was analyzed over a short period of time by same analyst on same equipment and on same day. The percentage RSD of the results were calculated.

Method precision

Six different standard solutions were prepared and injected in to HPLC system and method precision of the proposed method was analyzed with 24 hours' time lag. The percentage RSD of the results were calculated.

LOD and LOQ

The limit of detection and limit of quantification can be determined based on standard deviation of the response (SD) and slope of calibration curve (S). LOD and LOQ can be calculated using following equations.

$$\text{LOQ} = 3.3 \times \text{SD}/S$$

$$\text{LOQ} = 10 \times \text{SD}/S$$

Robustness

The robustness is the ability of a method to remain unaffected by small changes in parameters like changes in flow rate, change mobile phase composition, change in temperature etc. To

demonstrate robustness two sets of analysis were carried out by using the same homogenous sample by making individual small deliberate changes in the sample like change in flow rate (± 0.1 ml), change in mobile phase ($\pm 5\%$ organic solvent) and change in temperature ($\pm 5^\circ\text{C}$).

Forced degradation studies

From prepared stock solutions, 1 ml of drotaverine hydrochloride and aceclofenac were taken separately and subjected to different forced degradation conditions. Then the resultant solutions were diluted to obtain $100\mu\text{g/ml}$ & $80\mu\text{g/ml}$ solutions respectively. $10\mu\text{l}$ of each sample was injected into HPLC system and the chromatograms were recorded to assess the stability of the sample.

Oxidation

1 ml of 20% hydrogen peroxide (H_2O_2) was added separately to 1 ml of stock solutions of drotaverine hydrochloride and aceclofenac, the solutions were kept for 30 min at 60°C . The solutions were brought to ambient temperatures and the volume was made up to 10 ml with diluent.

Acid degradation studies:

1 ml of 2N Hydrochloric acid was added separately to 1 ml of stock solutions of drotaverine hydrochloride and aceclofenac and refluxed for 30 mins at 60°C . The solutions were brought to ambient temperatures and the volume was made up to 10 ml with diluent.

Alkali degradation studies

To 1 ml of stock solutions of drotaverine hydrochloride and aceclofenac, 1 ml of 2N sodium hydroxide was added and refluxed for 30 mins at

60°C . The solutions were brought to ambient temperatures and the volume was made up to 10 ml with diluent.

Neutral degradation studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hours at a temperature of 60°C . The solutions were brought to ambient temperatures and the volume was made up to 10 ml with diluent.

Dry heat degradation studies:

The standard drug solution was placed in oven at 105°C for 6 hours to study dry heat degradation. The solutions were brought to ambient temperatures and the volume was made up to 10 ml with diluent.

Photo degradation studies:

The photo stability of the drug was studied by exposing the $1000\mu\text{g/ml}$ & $800\mu\text{g/ml}$ solutions of drotaverine hydrochloride and aceclofenac to UV Light by keeping the beaker in UV Chamber for 7 days or 200-Watt hours/ m^2 in photo stability chamber. The solutions were diluted to get $100\mu\text{g/ml}$ & $80\mu\text{g/ml}$ with diluent.

RESULTS AND DISCUSSION

Optimized chromatographic conditions

Hypersil ODS 250 mm $\times$ 4.6 mm, 5μ column with mobile phase phosphate buffer (pH 2.8) and acetonitrile in the ratio 20:80 v/v. The elution was achieved isocratically at a flow rate of 1 ml /min. Column temperature was maintained at 30°C and chromatograph was recorded at wavelength 210 nm and the run time was 10 minutes. The representative chromatogram was shown in Fig.2.

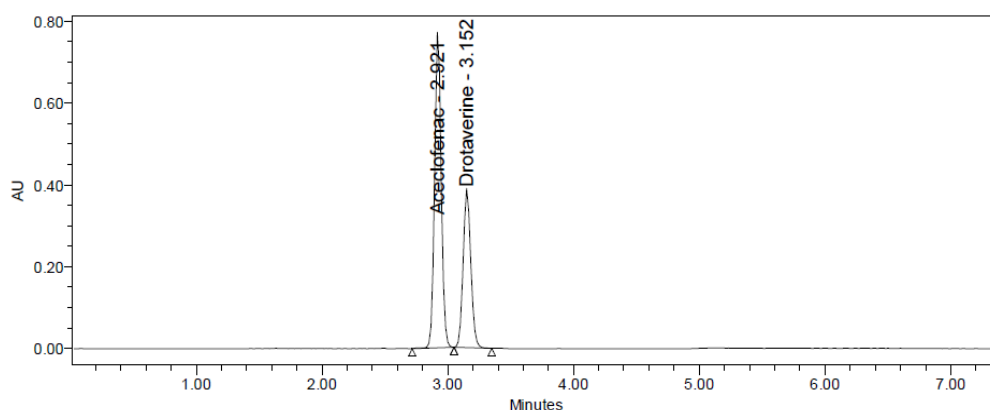


Fig 2: Optimized method chromatogram

Assay

Drugs in the dosage form were estimated by taking the standard as the reference. The percentage purity of drotaverine hydrochloride and aceclofenac in pharmaceutical dosage form was found to be 100.15% and 100.49% respectively.

System suitability

System suitability parameters like resolution and peak asymmetry were performed and the obtained results indicate that the overall system performed well, and the results were within the acceptance limits.

Specificity

The specificity tests were performed for drotaverine hydrochloride and aceclofenac. The absence of interfering peaks due to excipients in the tablet dosage form at retention times of drotaverine hydrochloride and aceclofenac proved the specificity of the method. Also, there is good correlation between the retention times of standard and sample.

Linearity

Linearity was evaluated by analyzing a series of various concentrations of both the drugs. The method was linear in the concentration range of 25µg/ml-150µg/ml for drotaverine hydrochloride and 20µg/ml-120µg/ml for aceclofenac and the correlation co-efficients were found to be 0.9998 and 0.9996 respectively. Linearity plots were shown in Fig.3 and Fig 4.

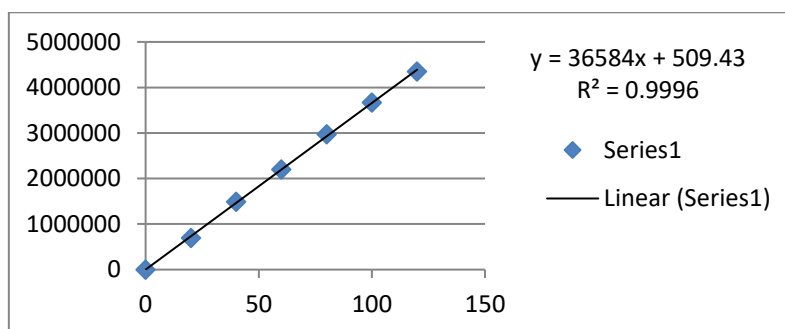


Fig 3: Linearity plot of Aceclofenac

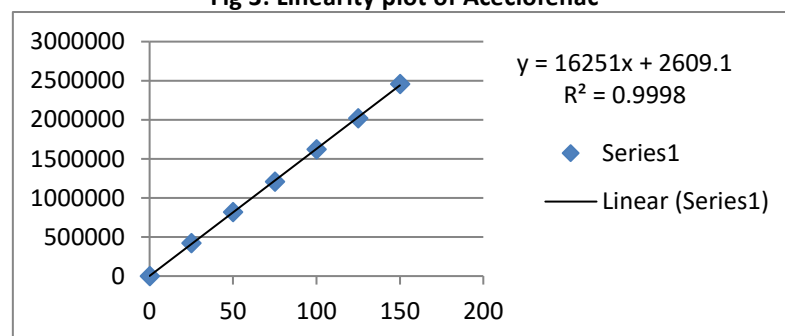


Fig 4: Linearity plot 1 of Drotaverine

Accuracy

The accuracy of the method was evaluated with the help of standard addition method. Recovery studies were performed at three levels of concentrations 50%, 100% and 150% for drotaverine hydrochloride and aceclofenac and analyzed in triplicate to

calculate accuracy. The mean % recoveries of drotaverine hydrochloride and aceclofenac were 100.2% and 101.12% respectively and were within the limits (NLT-98% & NMT-102%). Mean accuracy results were tabulated in Table. 1.

Table 1: Mean Accuracy results for Aceclofenac and Drotaverine Hcl

Drug name	Conc (%)	% Recovery	Mean % recovery
Aceclofenac	50% 3 injections	100.0067	100.04
	100% 3 injections	100.2567	
	150% 3 injections	99.86	
Drotaverine Hcl	50% 3 injections	100.28	100.3
	100% 3 injections	100.83	
	150% 3 injections	99.70667	

Precision

The system and method precisions were demonstrated by calculating percentage RSD, tailing factor, and number of theoretical plates. Both

system and method precisions were satisfactory, since the percentage RSD for the obtained results was less than 2.0%.

LOD and LOQ

The limit of detection and limit of quantification were quantitatively calculated with suitable precision. The LOD and LOQ for drotaverine hydrochloride were found to be 0.11 µg/ml and 0.33 µg/ml and for aceclofenac 0.06 µg/ml and 0.18 µg/ml.

Robustness

The results of robustness testing showed that the small changes like change in flow rate by ± 0.1 ml/min, change in concentration of organic phase in mobile phase by $\pm 5\%$ and temperature by $\pm 5^\circ\text{C}$ had no considerable influence on the method. In all conditions the method showed good recovery and the results were within limits with % RSD not more than 2.0%. Robustness data was tabulated in Table.2.

Table 2: Results of Robustness Studies

S.no	Robustness conditions	Aceclofenac %RSD	Drotaverine %RSD
1	Flow minus	0.1	0.1
2	Flow plus	0.1	0.4
3	MP minus	0.7	0.7
4	MP plus	0.2	1.8
5	Temperature minus	0.1	0.1
6	Temperature plus	0.1	0.1

Table 3: Forced Degradation studies data for drotaverine hydrochloride and aceclofenac

Type of degradation	Drotaverine hydrochloride			Aceclofenac		
	Area	% RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	1653898	97.45	2.55	2860060	98.09	1.91
Base	1645909	96.98	3.02	2829252	97.03	2.97
Peroxide	1670411	98.42	1.58	2858143	98.02	1.98
Thermal	1690295	99.60	0.4	2889559	99.10	0.9
Uv	1681510	99.08	0.92	2893248	99.23	0.77
Water	1681510	99.08	0.92	2898763	99.41	0.59

Forced degradation studies

In the stress degradation studies, it was observed that response of peak area and retention time of drotaverine hydrochloride and aceclofenac were nearly same. In all applied stress conditions additional peaks were observed only in acid and base degradation studies. The percentage degradation was found to be less than 5 % so the method was stable in all the stress conditions.

CONCLUSION

The developed RP-HPLC method was found to be simple, accurate, precise for simultaneous estimation of drotaverine hydrochloride and aceclofenac in tablet dosage form. Degradation studies revealed that the developed method was a stability indicating method. Retention times and run time were decreased than the reported methods. The developed method was simple and economical that can be adopted in regular quality control test for the simultaneous estimation of drotaverine hydrochloride and aceclofenac in tablet dosage form.

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