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Research Article

VALIDATED RP-HPLC METHOD FOR ESTIMATION OF RABEPRAZOLE AND ACECLOFENAC FROM HUMAN PLASMA

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ABSTRACT

Objective: Aim of present study was to develop a simple, fast and precise RP-HPLC method for estimation of Rabeprazole and Aceclofenac from human plasma.

Methods: Chromatographic separation of the said drugs was achieved on a HiQ SiL C_8 column (250 mm x 4.6 mm i.d, 5 µ) with a mobile phase comprising of 0.05 M Potassium diphosphate: Acetonitrile: Methanol in the ratio 4:4:3 (v/v/v) at a flow rate of 1 ml/min. UV detection was carried at 215 nm.

Result: The described method was found to be linear over a concentration range of 0.5-7.5 µg/ml. Separation was achieved within 8 mins. The recovery was calculated by standard addition method. Average recovery and precision was found to be in the limit given by USFDA guidelines.

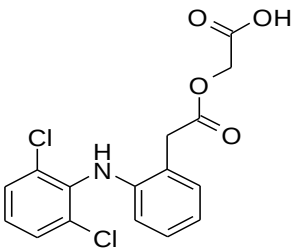
Conclusion: Method was validated as per US-FDA guidelines for selectivity, precision, recovery, accuracy and stability and robustness. Thus proposed method was found to be simple, accurate, precise and rapid for the estimation of Rabeprazole and Acelofenac from human plasma. It can be used for estimation of said drugs in human plasma, pharmacokinetic and interaction studies.

Keywords: RP-HPLC, Aceclofenac, Rabeprazole, Human plasma.

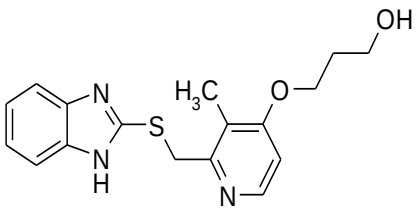
INTRODUCTION

Aceclofenac (Molecular formula: $C_{16}H_{13}Cl_2NO_4$; Molecular weight: 354.19), 2-[2-[2-(2,6- dichlorophenyl)aminophenyl]acetyl] oxyacetic acid is an effective anti-inflammatory drug. It has been widely used for the treatment of arthritis. It works by blocking the action of cyclooxygenases. Cyclooxygnases are involved in the production of various prostaglandins. It is official in Indian Pharmacopoeia [1].

Rabeprazole (Molecular formula: $C_{18}H_{20}N_3O_3S$; Molecular weight: 381.4 g/mol) is a substituted benzimidazole that inhibits gastric acid secreation and primarily used in the treatment of Ulcerative gastroesophageal reflux disease. It is chemically 2[[4-(3-methoxy propoxy)-3-methyl-2-pyridinyl]sulfinyl]-H-benzimidazole.
Rabeprazole belongs to a class of anti secreatory compounds that supress gastric acid secretion by inhibiting the gastric H+, K+ ATPase at the secretory surface of the gastric parietal cell. Because this enzyme is regarded as the acid pump within the parietal cell, rabeprazole has been characterised as a gastric proton pump inhibitor. It is official in Indian Pharmacopoeia [2].



Aceclofenac



Rabeprazole

A thorough literature survey has indicated several HPLC[3-9], HPTLC[10] and UV[11-13] methods for estimation of Aceclofenac and Rabeprazole as a single drug or in combinations with other drugs in pharmaceutical dosage forms and biological fluids. But there is no single HPLC method for simultaneous estimation of said drugs in human plasma. The present work describes a simple and rapid isocratic RP-HPLC method for simultaneous estimation of Aceclofenac and Rabeprazole in bulk drug and in human plasma. The developed method was validated as per US FDA guidelines [14].

MATERIALS AND METHODS

Reagents and Chemicals

Pure drug samples of Acelofenac and Rabeprazole were obtained as gift samples from Aristo Pharmaceuticals, Mumbai and Wockhardt Pvt. Ltd, Aurangabad respectively with certificate of analysis. Methanol and Acetonitrile (HPLC grade) were procured from Thomas baker (Chemicals) Pvt. Ltd. Mumbai. Potassium Dihydrogen Phosphate (AR grade) was purchased from SISCO Research Lab. Pvt Ltd, Mumbai. HPLC grade water was used throughout the study. Pooled human plasma was purchased from Sahydri Hospital blood bank, Pune. Calibrated glassware's were used for the study.

Instrumentation

Chromatographic separation was performed with JASCO HPLC 2000 Series having PU-2080 HPLC isocratic pump, a JASCO UV- 2075 variable wavelength detector and Rheodyne injector (20µl). Borwin software version 1.5 was used for data analysis.

Preparation of stock solution

10 mg of each drug was taken in a separate 10 ml of volumetric flask and was dissolved in sufficient quantity of mobile phase. Solutions were diluted up to the mark with mobile phase to get stock solutions of strength 1000 µg/ml of each drug. For each validation parameter, appropriate dilutions were prepared from these stock solutions using mobile phase to get desired concentrations.

Preparation for spiked plasma samples

Human Plasma was distributed in small eppendrof tubes and stored at -20°C until analysis. For each study, plasma was thawed at room temperature and spiked with appropriate pure drug solutions (Drug-Plasma Stock). It was then treated with acetonitrile (4 times

the actual volume of solution) for protein precipitation and centrifuged at 4°C, at 5000 RPM for 5min. The clear supernatant liquid was evaporated; residue was reconstituted with mobile phase and injected in the stabilized HPLC system. The blank plasma sample was prepared by repeating whole procedure without drug sample.

Validation parameters

System suitability

Solution of standard and spiked plasma sample was analyzed for System suitability parameters namely resolution, tailing factor, number of theoretical plates and capacity factor.

Linearity

Solutions in the range of 0.5-7.5 µg/ml for both the drugs (bulk form and spiked in human plasma) were prepared separately. 20 µL of each solution was injected in six replicates. The chromatograms were recorded and the peak areas were computed. Peak areas were plotted versus concentration to get the correlation. First concentration of linearity range was taken as LLOQ (Lowest limit of quantification).

Recovery and Precision

The absolute recovery of Aceclofenac and Rabeprazole was determined at 3 concentrations (Low, medium and High level) by comparison of the peak areas from standard and that of extracted (spiked plasma) samples in triplicate. The intra-day and inter day precision was determined at three different concentrations (six replicates). The % Coeeficient of variation (CV) at each QC level was calculated for both the parameters.

Stability

According to USFDA guidelines stability study of stock solutions, spiked samples and processed samples was performed. All the analysis was performed at low and High concentration levels.

Freeze and thaw stability

Freeze and thaw stability of the spiked samples was determined after performing three freeze thaw cycles of samples stored at -20 °C and comparing them against the freshly spiked samples.

Short term room temperature stability

Short-term room temperature stability of the spiked samples was determined for a period of 4 hours. Samples were stored at room temperature and comparison was made against the freshly spiked samples.

Long term stability

Long-term stability of the spiked plasma solutions was determined for a period of 5 days by storing at 4^0C and comparing them against the freshly prepared spiked solutions.

Stock solution stability

Stock solution stability of the drug was determined for a period of 6 hrs stored at room temperature and by comparing them against the freshly prepared stock solution.

Post- preparative stability

It is the stability of the processed sample. Stability of the extracted samples for LQC and HQC levels was determined for a period of 5 hours at room temperature.

Robustness

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase, wavelength and flow rate of the mobile phase were altered and their effect on chromatographic characteristics was evaluated in terms of % RSD.

RESULTS AND DISCUSSION

Pure drug's chromatogram were run in different mobile phases containing Methanol, Acetonitrile, Water and different Buffers in different ratios, different stationary phases

(e.g. C8, C18) of different dimensions. Detection was performed at 215 nm where both the drugs show significant absorption. The retention time and tailing factor was evaluated for each drug. Finally 0.05 M Potassium dihydrogen Phosphate, Acetonitrile and Methanol as a mobile phase in the volume of ratio 4:4:3 v/v and HiQ SiL C8 analytical column was selected which gave a sharp and symmetrical peaks with minimum tailing and appropriate retention time. **Fig. 1** indicates a chromatogram of blank plasma while **Fig. 2** shows a typical chromatogram of Rabeprazole and Aceclofenac in human plasma.

The chromatogram developed has well resolved peaks of said drugs without any interference of each other as well as plasma components. Results of system suitability parameters are shown in **Table 1**.

Calibration graph was found to be linear at range 0.5 to 7.5 µg/ml for both the drugs. Correlation coefficient (r^2) was found to be 0.990 and 0.997 for aceclofenac and rabeprazole respectively. Details are shown in **Table 2.** It indicates that excellent correlation exists between peak area and concentration.

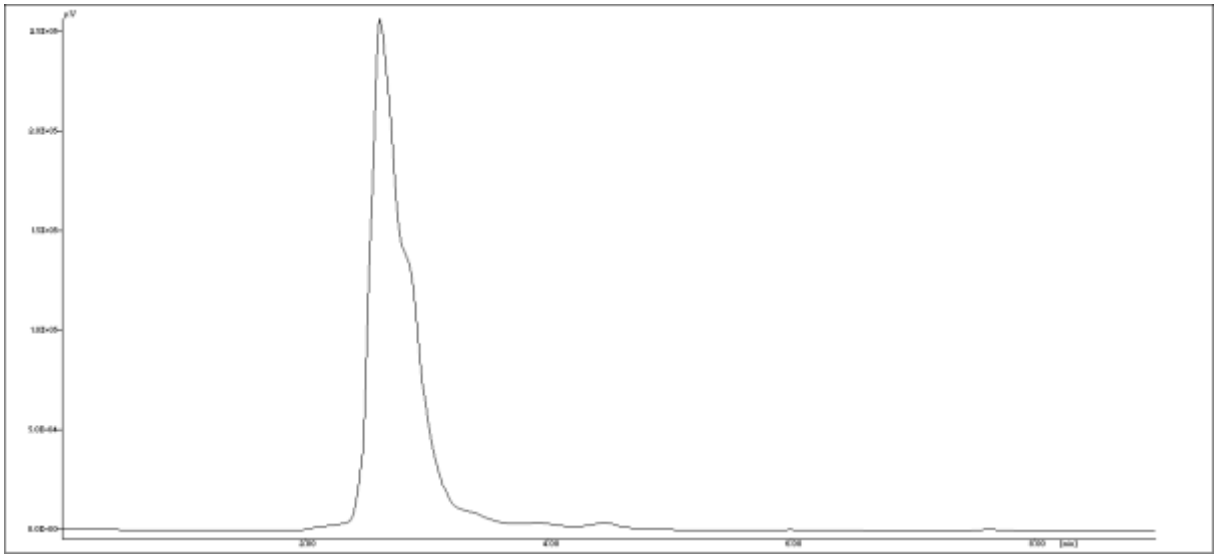


Fig. 1: Chromatogram of blank plasma

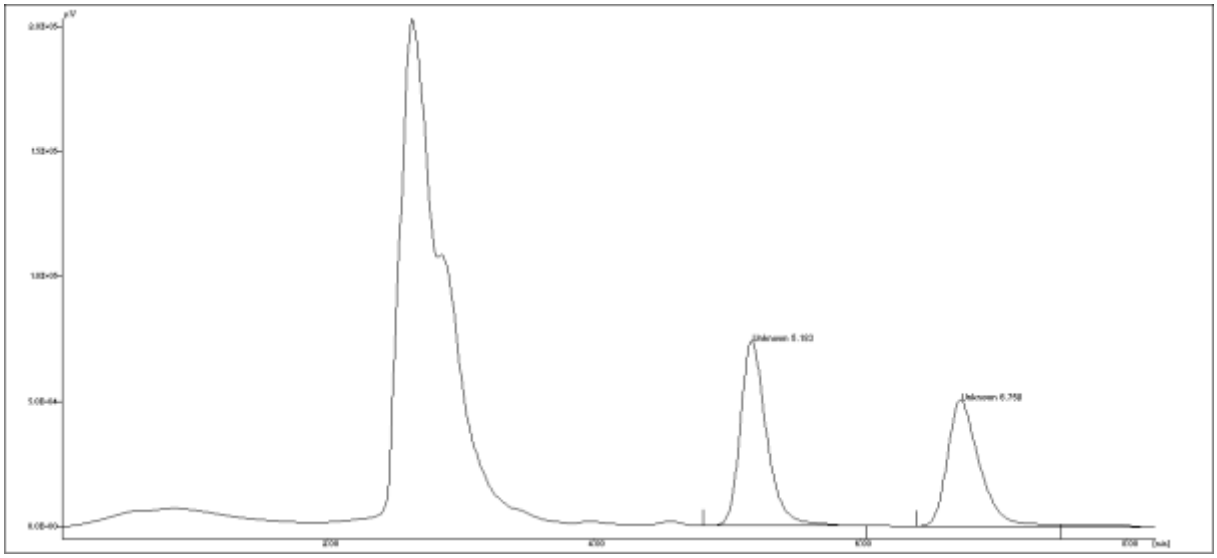


Fig. 2: A typical chromatogram of Rabeprazole and Aceclofenac in human plasma

Table 1: System suitability parameters

Parameters	Rabeprazole	Aceclofenac
Retention time(min)	5.2	6.7
Resolution	6.56	4.60
Theoretical plates	5351.44	6104.35
Asymmetry	1.03	1.00

Table 2: Results of Linearity

Name of drugs	R^2	Equation
Aceclofenac	0.990	y = 10222x + 77049
Rabeprazole	0.997	y = 12509x + 14664

In Precision Repeatability was performed and % RSD (Relative Standard Deviation) was found to be 1.51 and 1.03 for aceclofenac and rabeprazole respectively. The results obtained for intra-day precision (%RSD) were 3.34 and 1.01. For inter day precision % CV was 1.76 and 1.97 for aceclofenac and rabeprazole respectively. The low values of standard deviation and coefficient of variation at each level indicate high reproducibility of the method. Percent recovery was determined by comparing the standard solution and the spiked drug. The percentage recovery of the aceclofenac and rabeprazole was found to be 100.66% and 100.25% respectively. Stability of the drugs aceclofenac and rabeprazole was checked as short term room temperature, long term, freeze and thaw, stock solution and post preparative stability. % Mean stability for short term room temperature was found to be 91.82 and 88.38 for rabeprazole and aceclofenac respectively. % Mean stability for long term stability, freeze and thaw and stock solution stability was found to be 93.47 and 94.65, 92.65 and 89.23, 96.13 and 88.35 for rabeprazole and aceclofenac respectively. % Mean stability for post preparative stability studies was found to be 101.41 and 89.63 for rabeprazole and aceclofenac respectively. These all results indicate that plasma samples obtained for testing can be stored up to stated stability periods before analysis. **Table 3** shows summary of results obtained for all validation parameters.

Table 3: Summary of all validation parameters

Parameters	Rabeprazole	Aceclofenac
Linearity range (µg/ml)	0.5-7.5	0.5-7.5
Retention time (min)	6.7	5.4
R^2	0.990	0.997
Repeatability	1.51	1.03
Precision (% CV)		
Intra day Precision (% RSD)	3.34	1.01
Inter day Precision (% RSD)	1.76	1.97
Mean Recovery (%)	100.66	100.25
Short-term room temperature stability	91.82	88.38
Long-term stability	93.47	94.65
Stock solution stability	96.13	88.35
Post-Preparative stability	101.41	89.63
Freeze-Thaw stability	92.65	89.23

As all results are within limits of USFDA guidelines, the proposed HPLC method can be successfully applied for measurement of said drugs in human plasma and as bulk drugs.

CONCLUSION

The high performance liquid chromatographic method for the determination of Aceclofenac and Rabeprazole from human plasma was found to be rapid, accurate and precise. Thus, the proposed RPHPLC method can be successfully applied for the estimation of drug levels in human plasma, Pharmacokinetic evaluation and drug drug interaction studies.

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REFERENCES

1. Indian Pharmacopoeia, Volume II. Govt. of India, New Delhi: Ministry of Health and Family Welfare; 681-683, 2007.
2. Indian Pharmacopoeia. Volume III. Govt. of India, New Delhi: Ministry of Health and Family Welfare; 1647-1648,2007.
3. Choudhary B, Goyal A, Khokra, SL, Kaushik D Simultaneous Estimation of Diclofenac sodium and Rabeprazole by HPLC method in dosage form. Int. J. of Pharmaceutical Sciences and Drug Research 2009; 1(1): 43-45.
4. Obula Reddy MK, Bodepudi C Method Development and Validation of Rabeorazole in Bulk and Tablet dosage form by RP HPLC. Int J of Chem Tech Research 2011; 3: 1580-1588.
5. Battu PR, Reddy MS Development and Validation of RP HPLC for Rabeprazole sodium in Pharmaceutical Formulation and Human Plasma. Asian J Research Chem 2009; 2(1): 49-51.
6. Gopinath R, Rajan S, Krishnaveni N, Siresh B A RP HPLC Method for simultaneous estimation of Paracetamol and Aceclofenac in Tablets. Indian J pharm sci 2007; 69(1): 137-140.
7. Godse VP, Deodhar MN, Bhosale AV, Sonawane RA, Sakpal PS, Borkar DD Reverse Phase HPLC Method for Determination of Aceclofenac and Paracetamol in Tablet Dosage Form. Asian J Research Chem 2009; 2(1): 37-40.
8. Vaidya VV, Singh GR, Choukekar MP, Kekare MB Simultaneous RP HPLC determination of Aceclofenac, Paracetamol and Tizanide in Pharmaceutical Preparations. E-Journal of Chemistry 2010;7(1): 260-264.
9. Nema RK, Kartikeyan V, Yuvaraj G Simultaneous Estimation of Paracetamol, Chlorzoxane and Aceclofenac in Pharmaceutical Formulation by HPLC Method. Int J of Chem Tech Research 2009; 1(3): 457-460.
10. Bharekar VV, Mulla TS, Rajput MP, Yadav SS, Rao JR Validated HPTLC method for simultaneous estimation of Rabeprazole Sodium, Paraetamol and Aceclofenac in bulk drug and formulation. Der Pharma Chemica 2011; 3 (4): 171-179.
11. Jain J, Patadia R, Chauhan R, Vanparia D, Shaha S Dual Wavelength Spectophotometric Method For Simultaneous estimation of Drotaverine HCl and Aceclofenac in their combined tablet dosage form. Int J. Pharm Pharm Sci 2010; 2(4): 76-79.
12. Mandhanya M, Dubey N, ChaturvediSC, Jain DK Simultaneous Estimation of Paracetamol, Aceclofenac and Rabeprazole in Tablet Dosage form using UV Spectroscopy. Asian Journal of Pharm and Life Science 2011; 1(2):113-117.
13. Patel AH, Patel JK, Patel KN, Rajput GC, Rajgor NB Development and Validation of Derivative Spectrophotometric Method for Simultaneous Estimation of Domperidone and Rabeprazole Sodium in Bulk and Dosage Forms. International Journal on Pharmaceutical and Biological Research 2010; 1(1): 1-5.
14. U.S. Department of Health and Human Services. Food and Drug Administration. Guidance for Industry: Bioanalytical Method Validation. 2001.