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Isocratic RP-HPLC Method for Simultaneous Estimation of Paracetamol and Lornoxicam in combined tablet Dosage Form and its Dissolution Assessment

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Abstract: A simple, rapid and precise Reverse Phase High Performance Liquid Chromatographic method with *invitro* Dissolution assessment was developed for simultaneous estimation of Paracetamol and Lornoxicam in Tablet. 25 cm × 4.6 mm i.d, 5-µm particle; Phenomenex Luna C18 reversed-phase column, with mobile phase, ethyl acetate: Methanol: Water (2.5: 70:28.5 v/v),pH was adjusted to 4.0 with acetic acid. The flow rate was 1.0 mL/min and individual component were measured at 234 nm. The retention time of Paracetamol and Lornoxicam was 4.350 and 7.23 respectively. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH and USP guidelines. The *invitro* release of various test units was compared for their similarity using the f_2 test which limits were found within the acceptance criteria. The assay and recovery studies show Paracetamol and Lornoxicam in the range from 99 to 101 % were obtained at various added concentrations. Ambroxol was used as an internal standard. The procedures were successfully applied for simultaneous determination of both drugs in laboratory prepared mixtures as well as commercial tablet dosage form.

Keywords: RP-HPLC; Paracetamol, Lornoxicam, Ambroxol.

Introduction

Paracetamol (PARA), chemically 4-hydroxy acetanilide, is a centrally and peripherally acting nonopioid analgesic and antipyretic[1-3]. Literature survey revealed the most recent methods for determination of paracetamol like chromatographic[2-4], electrochemical[5-6]and spectrophotometric[7-9]techniques. Lornoxicam (LOX) is 6-chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno-[2,3-e]-1,2- thiazine-3-carboxamide 1,1-dioxide; is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties. LOX belongs to the chemical class oxicams, which includes lornoxicam, tenoxicam and meloxicam. LOX, which is commercially available in tablet, is used to treat inflammatory diseases of the joints, osteoarthritis, pain after surgery, and sciatica [10], Paracetamol and Lornoxicam combination by HPLC method[11]. It works by blocking the action of cyclooxygenase, an enzyme involved in the production of chemicals, including some prostaglandins in the body[12-15]. Author of the article and his research team has developed a HPLC method development in different pharmaceutical dosage form [16-24].This paper describes a simple, accurate, sensitive and validated RP-HPLC, *in-vitro* Dissolution method for simultaneous quantification of these compounds as the bulk drug and in tablet dosage forms.The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines[25].

Materials and method

Chromatographic Condition

The HPLC system consisted of a solvent delivery module Agilent 1100 Series Isocratic pump equipped with 20 µl loop and G1365B Multi Wavelength Detector. Integration was achieved by using the software Chemstation. Separation was carried out on a columns containing different stationary phases, the final choice giving satisfactory resolution and run time was the 25 cm × 4.6 mm i.d, 5-µm particle; Phenomenex Luna C18 reversed-phase column. The mobile phase was prepared by mixing solvents, ethyl acetate: Methanol: Water (2.5: 70:28.5 v/v), pH was adjusted to 4.0 with acetic acid. The prepared mobile phase was filtered through a Millipore 0.45 µm membrane filter and ultrasonically degassed prior to use. , ethyl acetate: Methanol: Water (2.5: 70:28.5 v/v) was used as diluents throughout the experiment. The detection wavelength was set at 234 nm. The elution was done at a flow rate of 1.0 ml/min under ambient condition.

Preparation of Standard Stock Solutions

Standard stock solution of Paracetamol was prepared separately by dissolving 10 mg of drug in 10 mL mobile phase to get concentration of 1000 µg mL^{-1}. One mL of this stock solution was further diluted to 10 mL with mobile phase to get a working standard solution having concentration 100 µg mL^{-1}. Standard stock solution of Lornoxicam was prepared by dissolving 5 mg of drug in10 mL mobile phase to get concentration of 500 µg mL^{-1}. One mL of this stock solution was further diluted to 10 mL with mobile phase to get a working standard solution having concentration 50 µg mL^{-1}.

Procedure for Analysis of Tablet Formulation

Twenty tablets were weighed accurately and powdered and Ambroxol. A quantity of tablet powder equivalent to 10 mg of Paracetamol (500 mg of Paracetamol) was weighed and transferred to 10 mL volumetric flask containing about 7 mL of mobile phase and ultrasonicated for 15 min and volume was made up to the mark with the mobile phase The solution was filtered through Whatmann paper No. 41. One mL of this solution was transferred to 10 mL calibrated volumetric flask and volume was made up to the mark with the mobile phase to get solution of concentration 100 µg $mL^{-1}$for Paracetamol and 20 µg mL^{-1} for Lornoxicam. Further one mL of above solution was transferred to 10 mL calibrated volumetric flask and volume was made up to the mark with the mobile phase to get solution of concentration 10 µg $mL^{-1}$for Paracetamol. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

System Suitability

The system suitability was assessed by six replicate injections of the mixture containing 10 µg mL^{-1} of both the drugs. The resolution, peak asymmetry, number of theoretical plates and HETP were calculated as represented in *Table 1*. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination. Mean retention time and standard deviation was found to be 3.432 ± 0. 21 for Paracetamol and 6.143 ± 0.13 min for Lornoxicam respectively.

Construction of Calibration Plots

From the standard stock solutions of all the two drugs, different dilutions were prepared and chromatographic and the peak areas were measured. Calibration plot of concentration against peak area were then constructed for Paracetamol and Lornoxicam. From the calibration plots it was found that response to Paracetamol and Lornoxicam was a linear function of concentration in the range 05-100 µg mL^{-1} and 02-20 µg mL^{-1} respectively. Unknown assay samples were quantified by reference to these calibration plots.

Buffer preparation:

Dissolve 2.0 g of sodium dihydrogen orthophosphate in to 1000 mL of Milli Q water and adjust pH 3.0 with orthophosphoric acid. Filtered it through 0.45 µ HVLP nylon filter.

Applied method to compare dissolution profiles:

The description of the in vitro dissolution profiles was calculated by using model-independent method [26-28]. In this study, as model-independent approaches, two fit factors were applied to the dissolution data that compare the dissolution profiles of a pair of drug product. These fit factors directly compare the difference between the percent drug dissolved per unit time for a test and reference product. The fit factors are f_1 (difference factor) and f_2 (similarity factor).

Validation

The method was validated for linearity, accuracy, precision, repeatability, selectivity and specificity. Accuracy was studied by measurement of recovery at three different levels 80, 100, and 120% of the amount expected in the formulation, in accordance with ICH guidelines. Precision was measured both intra-day and inter-day. In the intra-day study the concentrations of all two drugs were calculated three times on the same day at intervals of an hour. In the inter-day study the

concentrations of all the two drugs were measured on three different days. The limits of detection and quantitation of the method were studied to detect the lowest amount of analyte and quantitative determination of analyte in a sample respectively.

Method Validation

Linearity

The linearity of the calibration curves was determined for intra- and interday precision on 3 different days. Aliquots of 0.3, 0.4, 0.5, 0.6, and 0.7 mL of a 50.0 µg/mL standard solution of Paracetamol and 1000 µg/mL standard solution of Lornoxicam were transferred to 10 mL volumetric flasks and diluted to volume with mobile phase. The calibration curves were constructed by plotting the absolute peak area (y) versus the concentration (x), by using linear regression analysis. The LOQ (defined as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy) and the LOD (defined as the lowest absolute concentration of analyte in a sample that can be detected but not necessarily quantified) were calculated according to the ICH specifications.

Accuracy

The accuracy of the method was confirmed by studying recovery at two different concentrations 80, 100, and 120% of those expected, in accordance with ICH guidelines, by replicate analysis (n = 6). Standard drug solutions were added to a pre analyzed sample solution and percentage drug content was measured. The results from study of accuracy are reported in (table-2).From these results it was clear that the method enables very accurate quantitative estimation of Paracetamol and Lornoxicam in tablet dosage form, because all the results were within acceptable limits, i.e. COV < 2.0% and S.D. < 1.0.

Selectivity and Specificity

The selectivity of the method was checked by injecting solutions of all the three drugs. It was observed that three sharp peaks for Paracetamol and Lornoxicam were obtained at retention times 4.35, min and 7.23 min respectively (table-2). The retention times of the standards drug and the drugs from sample solutions were same, so the method was specific. Specificity of the method was evaluated by preparing a placebo tablet containing the same excipients as in the commercial product. The solution was prepared by using the procedure described in Preparation of Sample Solutions and injected three times. Moreover, it was used as the chromatographic peak purity tool, which is another way to verify the specificity of the method.

Table 1: Results from assay of the Tablet formulation

Drug	Label claim (mg)n=6	Amount Found in mg	Drug Concentration (%)	SD	COV (%)	SE
Paracetamol	500	500.11	100.11	0.26	0.483	0.11
Lornoxicam	8	7.99	99.99	0.72	0.215	0.36

S.D- standard deviation; COV- coefficient of variance; S.E- standard error;
n- No, of replicates

Table 2: System suitability parameters

Property	Paracetamol	Lornoxicam
R'	4.35	7.23
T_f	1.24	1.21
K'	0.32	0.46
N	6438	8548
R_s	1.98	2.57

Rt, retention time; *Tf*, tailing factor; *k*′, capacity factor; *N*, number of theoretical plates *R*s, resolution

Table 3: Results from determination of intra-day and inter-day precision, and LOD and LOQ

Drugs	Intra-day precision (COV, %)	Inter-day precision (COV, %)			LOD ng mL^{-1}	LOQ ng mL^{-1}
		Day 1	Day 2	Day 3		
Paracetamol	4.765	3.165	2.987	1.432	0.021	0.060
Lornoxicam	2.432	1.987	1.143	0.876	0.058	0.043

A Mean from six determinations COV, coefficient of variance; LOD, limit of detection; LOQ, limit of quantitation

Table 4.System Suitability and System Precision

Compound	RT (Mean ± SEM)	n	k'	R	T	α
Paracetamol	5.11	6549	0.362	3.26	1.29	0.332
Lornoxicam	7.32	8743	0.174	2.43	1.23	1.327
RT- Retention time n: Theoretical plates, k': Capacity Factor, R: Resolution,T: Asymmetry = Selectivity						

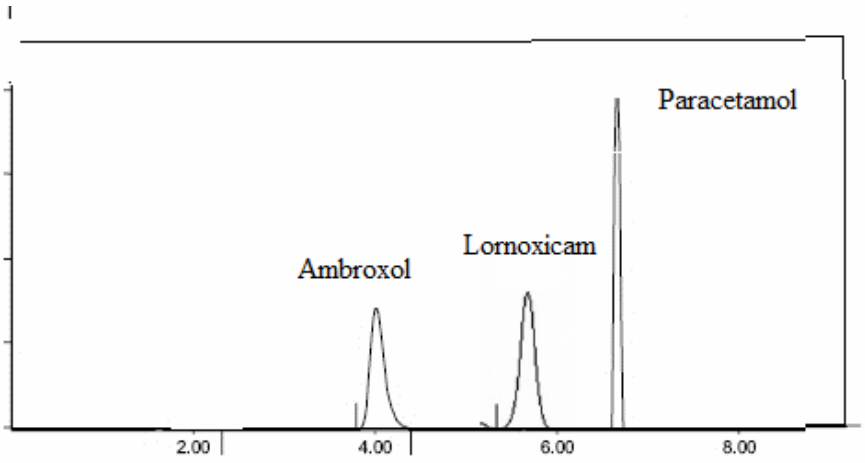

Fig. RP-HPLC chromatograms Paracetamol, Lornoxicam and Ambroxol (internal standard)

Result and discussion

The mobile phase conditions were optimized so that the tablets components were free from interference from the solvent and from excipients. Other criteria, for example time required for analysis, appropriate *k* range for eluted peaks, assay sensitivity, solvent noise, and use of the same solvent system for extraction of the drug from formulation matrices during drug analysis were also considered. After trying columns containing different stationary phases, the final choice giving satisfactory resolution and run time was the 25 cm × 4.6 mm i.d, 5-µm particle; Phenomenex Luna C18 reversed-phase column. Column chemistry, solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the buffer solution), detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. To determine the appropriate wavelength for simultaneous determination Paracetamol and Lornoxicam solutions of these compounds in mobile phase were scanned by UV–visible Spectrophotometry (Shimadzu 1700) in the range 200–400 nm. From the overlain UV spectra, suitable wavelengths considered for monitoring the drugs were 234 nm. Solutions of each substance in mobile phase were also injected directly for HPLC analysis and the responses (peak area) were recorded at 234 nm. Under the optimum chromatographic conditions, the retention times obtained for Paracetamol and Lornoxicam were 4.350 and 7.23 respectively. The values obtained for *k* and *R*S ($1 < k < 10$, $RS > 2$) show these chromatographic conditions are appropriate for separation and quantification of both the compounds. The number of plates (*N*) is a measure

of column efficiency; which shows the high separation efficiency of the column used.Stability of the standard solutions were studied by injecting the prepared solutions at periodic intervals into the chromatographic system ≤24 h, when stored at room temperature and when refrigerated. The solutions maintained at least 99.5% of their initial concentration under the test conditions. Before an analytical method is applied to quality control, it is necessary to validate the method. The validation ensures that the procedure is suitable for its intended purpose. The guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human describe the analytical parameters that should be evaluated in a method validation. The type of method and its respective use determine which parameters should be evaluated. It is the responsibility of the analyst to select the parameters considered relevant for each method. A model-independent method was used for the comparison of in vitro dissolution profiles. In this study f_1 (difference factor) and f_2 (similarity factor) was calculated. The use of these factors was also recommended for dissolution profile comparisons in the FDA's guides for industry. This method dissimilarity factor (f1) was found to be 5.11 and 7.32 (table-4) and similarity factors (f2) were found to be 66.54 and 55.43 for Paracetamol and Lornoxicam respectively.

Conclusion

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of Paracetamol and Lornoxicam in combined tablet dosage form.

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