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RP-HPLC Method Development and Validation of Paracetamol, Ambroxol, Hydrochloride Levocetirizine Dihydrochloride Pseudoephedrine Hydrochloride in Bulk and in Formulation

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Abstract:- A simple, exact, and reliable reverse phase liquid chromatographic technique was developed for the simultaneous detection of paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride in tablet dosage forms. The analysis was carried out using C_{18} (150 mm x 4.6 mm i.d.) and a mobile phase with an acetonitrile : water (pH6 adjusted with orthophosphoric acid) ratio of (60:40). The detection employed a flow rate of 1.0 ml/min and a wavelength of 257 nm. Levocetirizine dihydrochloride, pseudoephedrine hydrochloride, ambroxol hydrochloride, and paracetamol all had retention periods that were, respectively, 2.396 minutes, 4.829 minutes, 2.807 minutes, and 3.620 minutes. According to ICH recommendations, the method was authorized. The approach's specificity, precision, linearity, accuracy, and robustness were all confirmed. For paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride, the linearity ranged from 4 to 24 g/ml. The reported recoveries for levocetirizine dihydrochloride, pseudoephedrine hydrochloride, ambroxol hydrochloride, and paracetamol were 100.18% and 100.88%, respectively. The proposed method was successfully used to measure paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride in combination tablet formulation.

Keywords:- *Paracetamol, Ambroxol Hydrochloride, Levocetirizine Dihydrochloride, Pseudoephedrine Hydrochloride, RP-HPLC Method.*

I. INTRODUCTION

Cyclooxygenase (COX) is inhibited by the analgesic, antipyretic, and non-narcotic medication paracetamol (PARA), also known chemically as N-(4-hydroxyphenyl)ethanamide (Figure 1(a)). Ambroxol hydrochloride (AMB), sometimes referred to by its chemical name trans-S-4-(2-Amino-3, 5-dibrombenzylamino)-cyclohexanol (Figure 1(b)), is an active N-desmethyl metabolite of the mucolytic medication bromhexine and is used as an oral mucolytic expectorant. Levocetirizine is known by the chemical name [2-[4-[(R)-(4-chlorophenyl)phenylmethyl] (LEVO).The L-enantiomer of cetirizine is known as 1-piperazinyl]ethoxy]-acetic acid dihydrochloride (Figure 1(c)). Hay fever, allergic rhinitis, conjunctivitis, and sneezing are all treated with it. It also lessens red, watery, and itchy eyes. By preventing H1 histamine receptors, it works. PSE, also known chemically as (1s,2s)-2-methylamine-1-phenylpropan-1-ol hydrochloride (Figure 1(d)), is a nasal decongestant with a direct action.

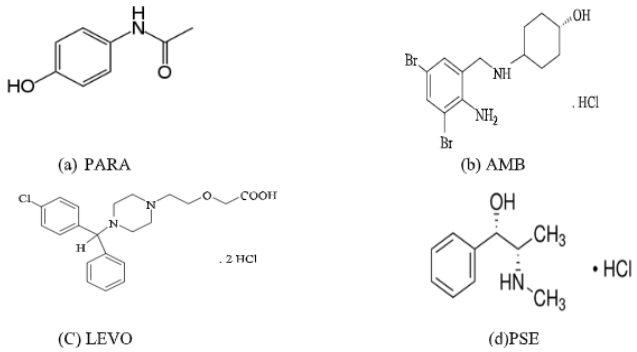

Fig 1 Chemical Structure of (a) PARA, (b) AMB, (c) LEVO, (d) PSE

HPTLC (Raja et al., 2012; Rathore et al., 2012; Dhaneshwar et al., 2011; Chitlange et al.) and RP-HPLC (Karunakaran et al., 2012; Arayne et al., 2010; Rathore et al., 2010; Singhvi, Neela Bhatia et al., 2006) have all been There is no established pharmacopoeia that combines PARA, AMB, LEVO, and PSE. There is no recognized analytical technique for the analysis of PARA, AMB, LEVO, and PSE in combination tablet dose form, according to the literature. Therefore, the following.Compared to

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techniques like UV Spectroscopy and electrophorosis, RP-HPLC methods of analysis are more practical and less complicated. Simultaneous estimation RP-HPLC is a key component of the multicomponent analysis of mixtures by chromatography under computer-controlled apparatus. This study's objectives were to determine the effectiveness of the RP-HPLC method for simultaneous estimation and to provide a trustworthy Chromatographic approach for the simultaneous determination of PARA, AMB, LEVO, and PSE in combination tablet dosage form without the need for individual drug separation beforehand. According to guidelines set forth by the International Conference on Harmonization (ICH, 1994), the currently created technique has been validated.

II. EXPERIMENTAL

A. Chemicals and Reagents

Pure drug samples of PARA and LEVO were received as gifts from Medopharm Pvt. Ltd. in Chennai, Tamil Nadu, India; AMB was given as a gift by Tristar Pvt. Ltd. in Puducherry, India; and PSE was given as a gift by Sterile-Gene life science Pvt.Ltd. in Puducherry, India. Orthophosphoric acid, water, and acetonitrile were acquired from Qualigens Pvt. Limited in Mumbai, India. In our lab, we used a double distillation unit to produce distilled water.

B. Instruments

Shimadzu HPLC system with chromatographic system linked to UV-Detector and injector (20 l) was used for analysis. With the aid of a C_{18} (150 mm x 4.6 mm i.d.) and Shimadzu HPLC running Windows XP software, the chromatographic separation was completed.

III. METHOD

A. Chromatographic Conditions

The chromatographic separation process was finished at room temperature, and detection was carried out at 257 nm with a flow rate of 1 mL/min. Ten minutes was the maximum runtime. Prior to injecting the drug solution, the column was equilibrated for 30 min while the mobile phase was passing through the apparatus. 20 g/ml of injection volume was employed for the test level. The mobile phase for the analysis was C_{18}(150 mm x 4.6 mm i.d),and the acetonitrile : water (pH6 adjusted with orthophosphoric acid) ratio was 60:40%v/v.

B. Standard Preparation

The standard stock solutions of paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride were independently made by dissolving the working standard in the mobile phase and diluting with the same solvent. Standard calibration solutions of paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride with concentrations ranging from 4 to 24 g/ml were made by diluting stock solutions with mobile phase. A representative chromatogram of the standard preparation is shown in (Fig. 2).

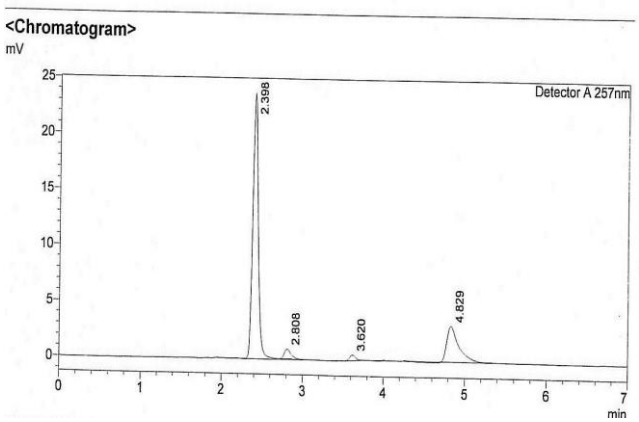

Fig 2 Chromatogram of Standard Preparation of PARA, LEVO, PSE, AMB.

C. Sample Solution

After accurately weighing twenty LV-PLUS tablets and calculating their average weight, the tablets were ground into a fine powder. From the triturate of 20 pills, an amount weighing 40 mg of paracetamol was carefully measured and added to a 10 ml volumetric flask. Pseudoephedrine hydrochloride (35.2 mg), levocetirizine dihydrochloride (39.6 mg), and ambroxol hydrochloride (37.6 mg) were carefully weighed as raw chemicals and combined. The remaining volume was then created using mobile phase after it had been dissolved in methanol. The solution was sonicated for around 15 minutes before being filtered with whatmann filter paper no.41.

Levocetirizine dihydrochloride, pseudoephedrine hydrochloride, ambroxol hydrochloride, and the filtrate were all diluted to achieve a theoretical concentration of 8 g/ml, then 5 ml to 25 ml, then 80 g/ml, and finally 1 ml to 10 ml volumetric flask. This answer is applied for more analysis. A stable baseline was acquired when the chromatographic parameters were improved. After the baseline had stabilized for 30 minutes, six test solutions of the formulation were injected, and the chromatograms were recorded. A typical chromatogram for sample preparation is shown in Figure 3.

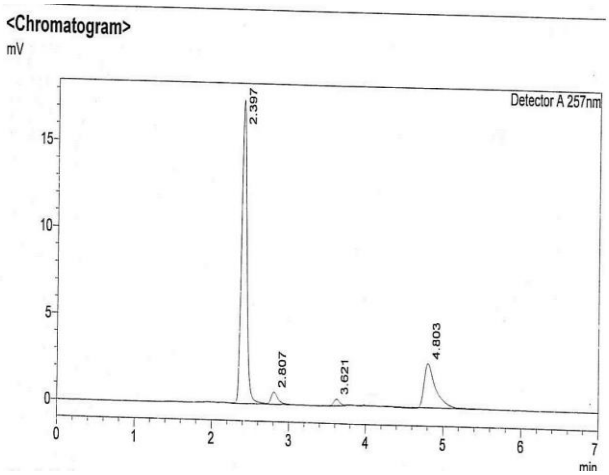

Fig 3 Chromatogram of Sample Preparation of PARA, LEVO, PSE, AMB

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IV. ANALYTICAL METHOD VALIDATION

A. Specificity

To evaluate the method's specificity, tablet powder without paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride was made using the same excipients as those used in commercial formulation. The solution for RP-HPLC was prepared using the same technique as was used to prepare the analytical sample. A placebo solution was added to the HPLC instrument after the test conditions. Any reactions to the peaks on the chromatogram were quantified. The chromatogram shows no interference from Levocetirizine dihydrochloride, pseudoephedrine hydrochloride, ambroxol hydrochloride, or the placebo.

B. Precision

The standard for precision was the applicability and measurement's reproducibility. The accuracy of the method was evaluated using the% RSD of the peak regions of three duplicate injections for three different standard doses. The average RSD values for paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride were found to be 0.2417%, 0.2198%, 0.7513%, and 0.3852%, respectively. The RSD is less than 2%, which indicates that the method's accuracy is acceptable.

C. Calibration and Linearity

To confirm the method's linearity, the calibration curve is constructed. For paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride, the linearity of calibration curves (analyte peak area ratio vs concentration) was studied over concentration ranges of around 4-24 g/ml. The entire eluting process lasted 10 minutes. A regression line relating typical medication concentrations was made using regression analysis. In the studied range, the calibration curves were linear, and regression analysis equations were generated.

$Y=10634.04446x+2203.6785$; $r^2=0.9998$ for PARA
$Y=3020.9821x+179.9285$; $r^2=0.9996$ for AMB
$Y=518.9642x+109.0000$; $r^2=0.9998$ for LEVO
$Y=211.7053x+56.9642$; $r^2=0.9993$ for PSE

The mean ± standard deviation (SD) for the slope; intercept and correlation coefficient of standard curves were calculated.

D. Accuracy

A known volume of a pure standard drug was added to the tablet powder solution in order to conduct the recovery investigations. After that, the standard was added to the sample in amounts equal to 80%, 100%, and 120% of the test. The resulting spiked sample solutions underwent three independent tests, and the percentage findings were computed by contrasting the obtained results with the expected ones. In accordance with Table 1, the recovery rates for Levocetirizine Dihydrochloride, Pseudoephedrine Hydrochloride, Ambroxol Hydrochloride, and Paracetamol were 100.13 to 100.50%, 99.80 to 100.57%, 99.96 to 100.40%, and 100.20 to 101.70%, respectively.

Table 1 Summary of Validation Parameters for the Proposed Method

PARAMETERS	PARA	AMB	LEVO	PSE
Retention time (min)	2.396	4.829	2.807	3.620
Theoretical plates	4674	6067	4262	8204
Tailing factor	1.330	1.465	1.504	1.147
Linearity range (µg $mL^{-1)}$	4-24	4-24	4-24	4-24
Correlation coefficient (r)	0.9998	0.9996	0.9998	0.9993
Accuracy (%)	100.13-100.50%	99.80-100.57%	99.96-100.40%	100.20-101.70%

E. Robustness

The method's robustness was assessed by assessing standard solutions under typical operating settings and under different flow rates, pH levels, and detection wavelengths.

F. 4.6 LOD & LOQ

The equations 3.3S,D/S, and 10S was used to determine LOD and LOQ.D/S, where S.D is the Y-intercept standard deviation and S is the calibration curve slope.

V. RESULTS AND DISCUSSION

For the purpose of optimizing the HPLC process, several solvents and ratios were investigated, but it was found that the peaks changed in shape. Different acetonitrile to water ratios and pH values were tested. It was found that producing good peaks required a ratio of 60:40%v/v acetonitrile:water (pH6 adjusted with orthophosphoric acid) at a flow rate of 1.0 mL/min. The following parameters were used C_{18} (150 mm x 4.6 mm i.d.) column, 1 mL/min flow rate, 20 L injection volume, Ambiebt temperature, and 257 nm wavelength as the mobile phase. Acetonitrile:water (pH6 adjusted with orthophosphoric acid) in the ratio 60:40%v/v.This mixture was discovered to be efficient. These chromatographic conditions were used to calculate the retention periods of paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride. There were no peaks at the paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, or pseudoephedrine hydrochloride retention times when the specificity of the method was checked by injecting a placebo solution. The specified process was followed to prepare a sample solution, which was afterwards examined initially and hourly for up to 24 hours at room temperature. Levocetirizine dihydrochloride, paracetamol, ambroxol hydrochloride, and pseudoephedrine hydrochloride were shown to have a good linear relationship over the range of 4-24 g/ml, as revealed by correlation coefficient values of 0.9998, 0.9996, 0.9998, and 0.9993. The results were within acceptable bounds even when the

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analyst, column, and chemical manufacturer were changed while the chromatographic parameters remained the same. The robustness of the approach was evaluated by deliberately altering the chromatographic parameters, such as flow rate, pH, and wavelength. The tailing factor and retention period parameters show that the limits were followed. The accuracy of the approach was evaluated using recovery studies, and the recovery % was computed. Levocetirizine dihydrochloride, pseudoephedrine hydrochloride, ambroxol hydrochloride, and paracetamol recovered between 100.13 and 100.5%, 99.96 to 100.4%, 100.20 to 101.70%, and 99.80 to 100.57%, respectively. The recommended liquid chromatographic method was used to determine the amounts of paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride in their combined dose forms (Tablet LV-PLUS). For paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride, the results were equivalent to the stated amounts (Table 2).

Table 2 Assay Results of Combined Dosage using Proposed Method

Drug	Labelled amount (mg)	Average (%)	% Recovery
PARA	500	100.11	100.32
AMB	60	99.98	100.19
LEVO	5	99.83	100.18
PSE	30	100.09	100.88

VI. CONCLUSION

The HPLC method is exact, accurate, and has a short run time. It was developed for the evaluation of a mixture of paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and pseudoephedrine hydrochloride in pharmaceutical preparations. The method had undergone thorough validation, and each of the tested method validation parameters yielded satisfactory findings. Using the developed method, the quality control division may quickly determine the assay of pharmaceutical items.

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