Research Article

Simultaneous Estimation of Paracetamol, Ambroxol Hydrochloride, Levocetirizine Dihydrochloride, and Phenylephrine Hydrochloride in Combined Tablet Formulation by First-Order Derivative Spectrophotometry

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Paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and phenylephrine hydrochloride are used in combination for the treatment of chronic sinusitis, rhinitis, fever, nasal discharge, sore throat, and wheezing. The present work deals with method development for simultaneous estimation of paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and phenylephrine hydrochloride in tablet formulation by first-order derivative spectrophotometry. For determination of sampling wavelength, 10 $\mu\text{g/mL}$ of each of paracetamol, ambroxol hydrochloride, levocetirizine dihydrochloride, and phenylephrine hydrochloride was scanned in 200–400 nm ranges and sampling wavelengths were found to be 305.5 nm for paracetamol, 321 nm for ambroxol hydrochloride, 244 nm for levocetirizine dihydrochloride, and 280 nm for phenylephrine hydrochloride in first-order derivative spectrophotometry. In this method, linearity was observed in the ranges of 20–140 $\mu\text{g/mL}$ for paracetamol and 10–70 $\mu\text{g/mL}$ for ambroxol hydrochloride, levocetirizine dihydrochloride, and phenylephrine hydrochloride. The % recovery was within the range between 98 and 102%, and % relative standard deviation for precision and accuracy of the method was found to be less than 2%. The method is validated as per International Conference on Harmonization Guidelines. The method can be successfully applied for the simultaneous analysis of these drugs in pharmaceutical dosage forms.

1. Introduction

Paracetamol (PARA), chemically known as *N*-(4-hydroxyphenyl)ethanamide (Figure 1(a)), inhibits the cyclooxygenase (COX) used as an analgesic, antipyretic, and nonnarcotic agent. Ambroxol hydrochloride (AMB), chemically known as *trans*-S-4-(2-Amino-3, 5-dibrombenzylamino)-cyclohexanol (Figure 1(b)), is an active *N*-desmethyl metabolite of the mucolytic bromhexine used as an oral mucolytic expectorant. Levocetirizine dihydrochloride (LEVO), chemically known as [2-[4-[(*R*)-(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-acetic acid dihydrochloride (Figure 1(c)), is *L*-enantiomer of cetirizine racemate. It works by blocking H_1 histamine receptors; it is used for allergic rhinitis and conjunctivitis, hay fever, and pollinosis; it controls

sneezing, runny but not blocked nose, and red, watering, and itchy eyes. Phenylephrine hydrochloride (PHEN), chemically known as (*R*)-3-hydroxy- α [(methylamino)methyl]benzenemethanol hydrochloride (Figure 1(d)), is a direct acting sympathomimetic agent. It is a selective α_1 adrenoceptor agonist and has negligible β action and is used as a nasal decongestant and for producing mydriasis when cycloplegia is not required. PARA, AMB, and PHEN are official in IP [1] and BP [2]. But LEVO is not official in any of the Pharmacopoeia.

Literature survey revealed that there are several methods that have been reported for the estimation of these drugs in combination with other drugs by using UV spectrophotometry [3–12], RP-HPLC [13–36], HPTLC [36–39], and LC-MS [40, 41]. PARA, AMB, LEVO, and PHEN in combination

FIGURE 1: Chemical structure of (a) PARA, (b) AMB, (c) LEVO, and (d) PHEN.

are not official in any pharmacopoeia. As per literature, no analytical method could be traced for the analysis of PARA, AMB, LEVO, and PHEN in combined tablet dosage form. Therefore, simple, rapid, and reliable method for simultaneous estimation of these drugs in combination seemed to be necessary.

Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. Under computer-controlled instrumentation, derivative spectrophotometry is playing a very important role in the multicomponent analysis of mixtures by UV molecular absorption spectrophotometry. Quaternary mixture can be easily resolved by means of a spectrophotometric method, which is based on the simultaneous use of “zero crossing” method. The aim of this work was to investigate the utility of derivative spectrophotometry and to develop reliable spectrophotometric procedure for the simultaneous determination of PARA, AMB, LEVO, and PHEN in combined tablet dosage form without any prior separation of individual drugs. The presently developed method was validated as per International Conference on Harmonization guidelines (ICH) [42, 43].

2. Materials and Methods

2.1. Apparatus and Instrument. A double beam UV-Visible spectrophotometer (Shimadzu, model pharm spec 1700) having two matched quartz cells with 1 cm light path and electronic analytical balance (Shimadzu AUX-220) and ultrasonication (Soltec 2200) were used. Volumetric flasks and pipettes of borosilicate glasses were used in the study.

2.2. Chemicals and Reagents. Pure drug samples of PARA and LEVO were obtained as gift sample from Medopharm Pvt. Ltd., Chennai, Tamil Nadu, India; AMB was gifted by Madras Pharmaceuticals Pvt. Ltd., Chennai, Tamil Nadu,

India; PHEN was gifted by APEX Pharmaceuticals, Chennai, Tamil Nadu, India. Methanol (HPLC grade) was purchased from Qualigens Pvt. Limited, Mumbai, India. Distilled water was obtained from the Double Distillation Unit in our laboratory.

2.3. Marketed Formulation. The marketed formulation studied was 1-AL total tablets manufactured by FDC Limited., Aurangabad, India. Each tablet contains 500 mg of PARA, 60 mg of AMB, 2.5 mg of LEVO, and 5 mg of PHEN.

2.4. Selection of Common Solvent. Methanol was selected as a common solvent to prepare the stock solution and further dilutions were made with distilled water for developing spectral characteristics of these drugs. The selection was made after assessing the solubility of these drugs in different solvents.

2.5. Preparation of Standard Solutions. 50 mg of PARA and 25 mg of AMB, LEVO, and PHEN were weighed accurately, transferred into 25 mL volumetric flask individually, and dissolved with methanol and the volume was made up to 25 mL with methanol. The stock solution contains 2 mg/mL of PARA and 1 mg/mL of AMB, LEVO, and PHEN.

2.6. Spectrophotometric Conditions

- (i) Mode: spectrum
- (ii) Scan speed: medium
- (iii) Bandwidth: 1 nm
- (iv) Wavelength range: 400–200 nm
- (v) Absorbance scale: 0.00 A–2.15 A
- (vi) Initial baseline correction: distilled water.

FIGURE 2: Overlay UV spectra of PARA, AMB, LEVO, and PHEN (10 $\mu\text{g/mL}$ concentration of each drug solution).

3. First-Order Derivative Spectrophotometric Method

The standard stock solution of PARA, AMB, LEVO, and PHEN was appropriately diluted with distilled water to get the concentration 10 $\mu\text{g/mL}$ of all the four drugs. Spectra of these diluted solutions were scanned in the spectrum mode between 200 nm and 400 nm using distilled water as a blank. The zero-order spectra of PAR, AMB, LEVO, and PHEN were transformed to corresponding first derivative spectra in the range of 200–400 nm. The overlay spectra (zero- and first-order) of PARA, AMB, LEVO, and PHEN are shown in Figures 2 and 3.

3.1. Selection of Wavelengths. A signal at 305.5 nm of first-order derivative spectrum was selected for the quantification of PARA where no interference due to AMB, LEVO, and PHEN was observed. Similarly, 321 nm was selected for quantification of AMB where PARA, LEVO, and PHEN did not interfere with the estimation of AMB. At 244 nm, LEVO and PARA showed marked absorbance where as AMB and PHEN have zero crossing point. The absorbance of PARA was interfered in the analysis of LEVO. Hence, the absorbance of PARA was corrected for interference from the total absorbance value. With the help of corrected absorbance, the amount of LEVO was calculated at 244 nm. A signal at 280 nm PHEN, PARA, and LEVO showed marked absorbance and AMB has zero crossing point. The absorbance of PARA and LEVO was interfered in the analysis of PHEN. Hence, the absorbance of PARA and LEVO was corrected for interference from the total absorbance value. With the help of the corrected absorbance, the amount of PARA was calculated at 280 nm.

3.2. Calibration Curves for PARA, AMB, LEVO, and PHEN. The standard solutions of PARA (2 mg/mL), AMB (1 mg/mL), LEVO (1 mg/mL), and PHEN (1 mg/mL) were used to prepare the working standard solution of PARA (20–140 $\mu\text{g/mL}$), AMB (10–70 $\mu\text{g/mL}$), LEVO (10–70 $\mu\text{g/mL}$), and PHEN (10–70 $\mu\text{g/mL}$), respectively. For, these aliquots of 1–7 mL of

FIGURE 3: Overlay first-order derivative UV spectra of PARA, AMB, LEVO, and PHEN (10 $\mu\text{g/mL}$ concentration of each drug solution).

standard stock solutions of PARA, AMB, LEVO, and PHEN were transferred separately to a series of 100 mL volumetric flasks and diluted up to the mark with distilled water. The absorbance was measured at 305.5 nm for PARA, 321 nm for AMB, 244 nm for LEVO, and 280 nm for PHEN, respectively. The values of first derivative $dA/d\lambda$ were plotted against corresponding concentrations to construct the calibration curves. The calibration curves are shown in Figure 4.

3.3. Analysis of Tablet Formulation. Marketed tablet formulation containing PARA (500 mg), AMB (60 mg), LEVO (2.5 mg), and PHEN (5 mg) was analyzed using this method. From the triturate of 10 tablets, the amount of tablet powder equivalent to 50 mg of PARA was accurately weighed and transferred into a series of 25 mL volumetric flasks. To that 4 mg/mL solution of AMB, 9.75 mg/mL solution of LEVO and 9.5 mg/mL solution of PHEN were added and dissolved with methanol and the solution was sonicated for 15 minutes. Then the final volume was made up to 25 mL with methanol. The solution was filtered through Whatmann filter paper Number 41. 3 mL of the stock solution was further diluted to 100 mL with distilled water to get the theoretical concentrations of 60 $\mu\text{g/mL}$, 12 $\mu\text{g/mL}$, 12 $\mu\text{g/mL}$, and 12 $\mu\text{g/mL}$ of PARA, AMB, LEVO, and PHEN, respectively. The absorbance values were measured at 305.5 nm, 321 nm, 244 nm, and 280 nm for PARA, AMB, LEVO, and PHEN, respectively. The concentration of each analyte was determined with the equations generated from calibration curve of respective drugs. The procedure was repeated for six times.

4. Results and Discussion

4.1. Selectivity. The UV spectra of standard mixture (PARA—60 $\mu\text{g/mL}$, AMB—12 $\mu\text{g/mL}$, LEVO—12 $\mu\text{g/mL}$, and PHEN—12 $\mu\text{g/mL}$) and sample solutions (tablet) were recorded between 200 and 400 nm and their $dA/d\lambda$ value was measured. The selectivity of the method was assessed by comparing spectra obtained from formulation

FIGURE 4: Calibration curve for (a) PARA, (b) AMB, (c) LEVO, and (d) PHEN.

solution with that obtained from standard drug solution. The UV absorption spectra obtained from standard and sample solutions were found to be identical, confirming the selectivity of the method.

4.2. Linearity. Linear correlation was obtained between $dA/d\lambda$ values versus concentrations of 20–140 $\mu\text{g/mL}$ for PARA, 10–70 $\mu\text{g/mL}$ for AMB, LEVO, and PHEN, respectively. Regression parameters are mentioned in Table 1.

4.3. Accuracy. Accuracy of the method was confirmed by recovery studies. Recovery studies were performed by standard addition method at three levels, namely, 80%, 100%, and 120%. Known amounts of pure PARA, AMB, LEVO, and PHEN were added to preanalyzed sample of marketed formulation, and they were subjected to analysis by the proposed method. The recovery was verified by estimation of drug in triplicate preparations at each specified concentration level and calculated %RSD. The recoveries were in the range of 99.77%–99.96%, 99.78%–101.07%, 99.80%–100.95%, and 100.27%–100.31% for PARA, AMB, LEVO, and PHEN, respectively. The low percentage RSD values indicated that there is no interference due to the excipients used in formulation. Hence the accuracy of the method was confirmed. The results of the recovery analysis are shown in Table 2.

4.4. Precision

4.4.1. Repeatability. The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions ($n = 6$) of PARA (10 $\mu\text{g/mL}$),

AMB (10 $\mu\text{g/mL}$), LEVO (10 $\mu\text{g/mL}$), and PHEN (10 $\mu\text{g/mL}$) without changing the parameters of the proposed method. The %RSD values for PARA, AMB, LEVO, and PHEN were found to be 0.6874, 1.8892, 0.6264, and 1.1750, respectively (Table 3). Low relative standard deviation (<2) indicates that the proposed method is repeatable.

4.4.2. Intermediate Precision. Precision of both methods was determined in terms of intraday and interday variations (%RSD). Intraday precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Interday precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days. The intraday and interday precisions were determined, results of which are given in Table 4.

4.5. LOD and LOQ. LOD and LOQ of the drug were calculated as per ICH guideline. LOD values for PARA, AMB, LEVO, and PHEN were found to be 0.0352 $\mu\text{g/mL}$, 0.0373 $\mu\text{g/mL}$, 0.0645 $\mu\text{g/mL}$, and 0.0557 $\mu\text{g/mL}$, respectively. LOQ values for PARA, AMB, LEVO, and PHEN were found to be 0.1070 $\mu\text{g/mL}$, 0.1132 $\mu\text{g/mL}$, 0.1954 $\mu\text{g/mL}$ and 0.1687 $\mu\text{g/mL}$, respectively (Table 1). These data show that the proposed method is sensitive for the determination of PARA, AMB, LEVO, and PHEN.

4.6. Analysis of PARA, AMB, LEVO, and PHEN in Marketed Formulation. Content of PARA, AMB, LEVO, and PHEN found in the marketed formulation from the proposed

TABLE 1: Regression analysis data and summary of validation parameters for the proposed method.

Parameters	First derivative spectrophotometric method			
	PARA*	AMB*	LEVO*	PHEN*
Wavelength	305.5 nm	321 nm	244 nm	280 nm
Linearity range ($\mu\text{g/mL}$)	20–140	10–70	10–70	10–70
Slope (m)	0.00012	0.00026	0.00119	0.00051
Intercept (c)	−0.0002	−0.000064	0.000085	0.00031
Correlation coefficient (r^2)	0.9992	0.9990	0.9990	0.9995
LOD ($\mu\text{g/mL}$)	0.0352	0.0373	0.0645	0.0557
LOQ ($\mu\text{g/mL}$)	0.1070	0.1132	0.1954	0.1687
% Recovery	99.77–99.96	99.28–101.07	99.80–100.95	100.27–100.31
Precision (RSD) %				
Repeatability ($n = 6$)	0.1661	1.9231	1.6141	1.1750
Interday ($n = 3$)	0.0726	1.6244	0.0750	0.0763
Intraday ($n = 3$)	0.4977	0.9453	0.0752	0.0765

* Mean of six observations.

TABLE 2: Recovery data for the proposed method.

Drug	Amount present ($\mu\text{g/mL}$)*	Amount added ($\mu\text{g/mL}$)*	Amount estimated ($\mu\text{g/mL}$)*	Amount recovered ($\mu\text{g/mL}$)*	Percentage recovered ($n = 6$)*	% RSD
PARA	59.6533	47.3221	107.8898	48.2365	101.93	0.2757
	59.6533	60.0016	119.3033	59.6500	99.41	0.2891
	59.6533	71.8727	131.4650	71.8117	99.92	0.1703
AMB	12.1440	9.6417	21.6966	9.5526	99.08	0.4154
	12.1440	12.0892	24.2021	12.0581	99.74	0.3285
	12.1440	14.3880	26.3979	14.2539	99.07	0.7261
LEVO	12.1176	9.5284	21.6460	9.5284	100.00	0.3518
	12.1176	12.0916	23.9843	11.8667	98.14	0.3848
	12.1176	14.3880	26.3683	14.2507	99.05	0.4868
PHEN	11.9592	9.5524	21.6182	9.6590	101.11	0.9930
	11.9592	12.1304	24.2703	12.3111	101.49	0.6889
	11.9592	14.3957	26.5306	14.5714	101.22	0.7734

* Mean of six observations. RSD: relative standard deviation.

TABLE 3: Repeatability data for proposed method.

Serial number	Concentration ($\mu\text{g/mL}$)	Absorbance of PARA at 305.5 nm	Absorbance of AMB at 321 nm	Absorbance of LEVO at 244 nm	Absorbance of PHEN at 280 nm
1	10 ppm	0.0199	0.0027	0.0121	0.0054
2	10 ppm	0.0200	0.0028	0.0120	0.0053
3	10 ppm	0.0203	0.0027	0.0119	0.0052
4	10 ppm	0.0200	0.0027	0.0120	0.0052
5	10 ppm	0.0201	0.0028	0.0120	0.0051
6	10 ppm	0.0200	0.0027	0.0121	0.0053
	Mean	0.0201	0.0027	0.0120	0.0052
	SD	0.00014	0.000052	0.000075	0.0001
	% RSD	0.6874	1.8892	0.6264	1.9977

SD: standard deviation; RSD: relative standard deviation.

TABLE 4: Intraday and interday precision data of PARA, AMB, LEVO and PHEN.

Drug	Amount labeled (mg/tab)	Percentage obtained ($n = 6$)		SD		% RSD	
		Intraday	Interday	Intraday	Interday	Intraday	Interday
PARA	500	99.47	99.60	0.4936	0.0723	0.4977	0.0726
	500	98.61	99.48				
	500	99.46	99.61				
	Mean	99.18	99.56				
AMB	60	98.56	97.85	0.9408	1.6197	0.9453	1.6244
	60	100.44	100.83				
	60	99.57	100.44				
	Mean	99.52	99.72				
LEVO	2.5	99.88	100.01	0.0750	0.0751	0.0752	0.0750
	2.5	100.01	99.88				
	2.5	99.88	100.01				
	Mean	99.92	99.96				
PHEN	5	98.21	98.34	0.0750	0.0752	0.0765	0.0763
	5	98.34	98.22				
	5	98.21	98.34				
	Mean	98.25	98.29				

SD: standard deviation; RSD: relative standard deviation; CI: confidence interval; SE: standard error.

TABLE 5: Assay results of marketed formulation.

Parameters	PARA	AMB	LEVO	PHEN
Actual concentration ($\mu\text{g/mL}$)	60	12	12	12
Percentage purity	99.62	101.02	100.98	99.66
% RSD	0.1661	1.9231	1.6141	1.1750

method is shown in Table 5. The percentage purity was $99.62\% \pm 0.1655$ for PARA, $101.02\% \pm 1.9427$ for AMB, $100.98\% \pm 1.6300$ for LEVO, and $99.66\% \pm 1.1711$ for PHEN.

5. Conclusion

In this proposed method, the linearity was observed in the concentration ranges of 20–140 $\mu\text{g/mL}$, 10–70 $\mu\text{g/mL}$, 10–70 $\mu\text{g/mL}$, and 10–70 $\mu\text{g/mL}$ while coefficients of correlation were $r^2 = 0.9992$, $r^2 = 0.9990$, $r^2 = 0.9990$, and $r^2 = 0.9995$ for PARA, AMB, LEVO, and PHEN at 305.5 nm, 321 nm, 244 nm, and 280 nm, respectively. The result of the analysis of combined mixture by the proposed method was found to be highly reproducible and reliable. The additive present in the combined mixture of the assayed samples did not interfere with determination of PARA, AMB, LEVO, and PHEN. So, the developed first derivative UV spectrophotometric method is simple, precise, accurate, and reproducible and can be effectively used for the simultaneous determination of PARA, AMB, LEVO, and PHEN in combined tablet dosage form.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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