SIMULTANEOUS ESTIMATION AND VALIDATION OF PARACETAMOL, CHLORPHENIRAMINE MALEATE AND PHENYLEPHRINE HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM BY USING DIFFERENT SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple, precise, accurate and economic simultaneous UV spectrophotometric method has been developed for the estimation of Paracetamol, Chlorpheniramine Maleate and Phenylephrine Hydrochloride in combination in bulk mixture and tablet. The estimation was based upon measurement of absorbance at absorbance maxima of 258 nm, 262 nm and 239 nm for Paracetamol, Chlorpheniramine Maleate and Phenylephrine Hydrochloride in methanol, respectively in bulk mixture and tablet. The Beer Lambert's law obeyed in the concentration range 4-24 µg/ml, for Paracetamol, Chlorpheniramine Maleate and Phenylephrine Hydrochloride respectively. The estimation of bulk mixture and tablet was carried out by simultaneous equation, Q-analysis and area under curve method for estimation of Paracetamol, Chlorpheniramine Maleate and Phenylephrine Hydrochloride. Recovery study was performed to confirm the accuracy of the methods. The methods were validated as per ICH guidelines.

Keywords: Paracetamol, Chlorpheniramine Maleate, Phenylephrine Hydrochloride, Ultraviolet spectroscopy, Simultaneous equation method, Absorption Ratio Analysis Method, Area Under Curve Method.

INTRODUCTION

Paracetamol (PARA) is chemically N-(4-hydroxyphenyl) acetamide, It has analgesic and antipyretic activity¹. Various analytical methods, such as, spectrophotometry^{2,3}, HPLC^{4,5}, HPTLC⁶ have been reported for the estimation of paracetamol from its formulations. Phenylephrine Hydrochloride is [(R)-2-methylamino-1-(3-hydroxyphenyl) ethanol hydrochloride], and used as alpha-adrenergic, sympathomimetic agent as well as vasoconstrictor with little effect on the myocardium or the central nervous system¹. From literature survey Phenylephrine Hydrochloride has been determined alone or in combination by using UV spectrophotometry^{2,3}, HPLC⁵, RP-HPLC⁷ methods. Chlorpheniramine maleate (CPM), (±) 2-[p-chloro-[2-dimethylamino) ethyl] benzyl] pyridine bimalate (Chlor-Trimeton). Chlorpheniramine (Maleate) is the maleate salt of Chlorpheniramine. Chlorpheniramine maleate used as an antihistaminic, it is also effective in nausea, motion sickness¹. UV spectrophotometry^{2,3}, HPLC⁵, RP-HPLC⁷ methods have been reported for the estimation of chlorpheniramine maleate. A mixture of this combination is widely used as an analgesic, antipyretic, decongestant and antihistamine. A combination of paracetamol, phenylephrine hydrochloride, chlorpheniramine maleate is commercially available in tablet dosage form. Literature reveals that no analytical method is available for simultaneous determination of these three drugs in combination. So we communicate here rapid and cost-effective quality-control tool for their routine quantitative analysis in pure and combined dosage forms by spectrophotometry.

MATERIAL AND METHOD

Materials

UV-visible double beam spectrophotometer, Jasco model 680 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used. The commercially available tablet, Febrex plus (Label claim: Paracetamol I.P.-500 mg, Chlorpheniramine Maleate 2 mg

and Phenylephrine hydrochloride) was procured from local market, Methanol, API of Paracetamol, Chlorpheniramine Maleate and Phenylephrine Hydrochloride.

Selection of common solvent

After assessing the solubility of drugs in different solvents Methanol was used as common solvent for developing spectral characteristics.

Preparation of standard stock and calibration curves

The standard stock solutions (250 µg/ml) of each of Paracetamol, Chlorpheniramine Maleate and Phenylephrine HCl were prepared separately by dissolving accurately about 25 mg of API in 20 ml of Methanol and volume was made up to 100 ml with methanol. Working standard solutions of 20 µg/ml were scanned in the entire UV range of 400-200 nm to obtain the absorbance. Solutions of 20 µg/ml of Paracetamol, Chlorpheniramine Maleate and Phenylephrine HCl were prepared separately. All these solutions were scanned in the spectrum mode from 200 - 400 nm. The maximum absorbance of Paracetamol, Chlorpheniramine maleate and Phenylephrine HCl were at 258 nm, 262 nm, 239 nm respectively. Paracetamol, Chlorpheniramine maleate and Phenylephrine HCl showed linearity in the concentration range of 4-24 µg/ml at their respective maxima. Accurately measured standard stock solution of Paracetamol, Chlorpheniramine Maleate and Phenylephrine HCl (1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml) were transferred to a separate series of 10 ml of volumetric flasks and diluted to the mark with Methanol. The absorbance of resulting solutions were measured at their respective max and plotted a calibration curve against concentration to get the linearity and regression equation.

Method 1: Simultaneous Equation Method

Simultaneous equation method of analysis is based on the absorption of Paracetamol, Phenylephrine Hydrochloride and

Chlorpheniramine Maleate at the wavelength maximum (λ -max) of each other. λ -max for Paracetamol, Phenylephrine hydrochloride and Chlorpheniramine maleate are 258 nm, 239 nm and 262 nm respectively. The absorptive values determined at 258 nm, 239 nm and 262 nm for Paracetamol 0.0724 (ax1), 0.0456 (ax2), 0.0220 (ax3), for Phenylephrine hydrochloride 0.0560 (ay1), 0.0430 (ay2), 0.0222 (ay3) and for Chlorpheniramine Maleate 0.0152 (az1), 0.0160 (az2), 0.0424 (az3). These values are means of six estimations. The absorptive coefficients were substituted in equation 1, 2 and 3 to obtain the concentration of drugs^{2,3,10-12}.

$$A1 = 0.0724 \times C_{Pa} + 0.0560 \times C_{Ph} + 0.0152 \times C_{Cm} \dots (1)$$

$$A2 = 0.0456 \times C_{Pa} + 0.0430 \times C_{Ph} + 0.0160 \times C_{Cm} \dots (2)$$

$$A3 = 0.0220 \times C_{Pa} + 0.0222 \times C_{Ph} + 0.0424 \times C_{Cm} \dots (3)$$

Where, CPA, CPH, and CCM are concentrations of Paracetamol, Phenylephrine Hydrochloride and Chlorpheniramine Maleate respectively in $\mu\text{g/mL}$, A1, A2, and A3 are the absorbance of the sample at 258 nm, 239 nm and 262 nm respectively.

Method 2: Absorption Ratio Method

For Q method, 242 nm (isobestic point) and 258 nm (λ -max of PARA) were selected for PARA and PHEN as wavelengths of measurements 250 nm (isobestic point) and 239 nm (λ -max of PHEN) were selected for PHEN and CHLOR as wavelengths of measurements. 234 nm (isobestic point) and 262 nm (λ -max of CHLOR) were selected for CHLOR and PARA as wavelengths of measurements. Concentrations of PARA, PHEN and CHLOR were determined using following equations^{2,3,10-12}.

$$C_{PARA} = \frac{(Q_{m1} - Q_{PHEN})}{(Q_{PARA} - Q_{PHEN})} \times \frac{A1}{a_{PARA1}} \dots (4)$$

$$C_{PHEN} = \frac{(Q_{m2} - Q_{CHLOR})}{(Q_{PHEN} - Q_{CHLOR})} \times \frac{A1}{a_{PHEN1}} \dots (5)$$

$$C_{PARA} = \frac{(X_{PHEN} \text{ at } 254 - 265 \times AUC_{PHEN} \text{ at } 232 - 244 - X_{PHEN} \text{ at } 232 - 244 \times AUC_{PARA} \text{ at } 254 - 265)}{(X_{PHEN} \text{ at } 232 - 244 \times X_{PARA} \text{ at } 232 - 244 - X_{PHEN} \text{ at } 232 - 244 \times X_{PARA} \text{ at } 254 - 265)} \dots (7)$$

$$C_{PHEN} = \frac{(X_{PARA} \text{ at } 232 - 244 \times AUC_{PARA} \text{ at } 254 - 265 - X_{PARA} \text{ at } 254 - 265 \times AUC_{PHEN} \text{ at } 232 - 244)}{(X_{PHEN} \text{ at } 254 - 265 \times X_{PARA} \text{ at } 232 - 244 - X_{PHEN} \text{ at } 232 - 244 \times X_{PARA} \text{ at } 254 - 265)} \dots (8)$$

Where; C_{PARA} and C_{PHEN} -Concentration of PARA and PHEN, respectively, AUC_{PARA} and AUC_{PHEN} - Area under curve of AMLB and HCT in bulk mixture. Similar procedure was applied for determination PARA and PHEN in tablet solution.

Estimation of CHLOR in presence of PARA and PHEN by standard curve method

The absorbance of standard CHLOR solutions at different concentration ranging from 4-24 $\mu\text{g}/\text{ml}$ at 262 nm was measured. The regression equation was established by plotting the calibration curve of absorbance Vs concentration. The absorbance of bulk mixture and tablet solution was measured at 262 nm for CHLOR. The concentration of CHLOR was estimated by regression equation,

$$y = 0.021x - 0.008$$

Where, y-absorbance of CHLOR in bulk mixture,
x- concentration of CHLOR in tablet solution

$$C_{CHLOR} = \frac{Q_{m3} - Q_{PARA}}{(Q_{CHLOR} - Q_{PARA})} \times \frac{A1}{a_{CHLOR1}} \dots (6)$$

Where; $Q_{m1} = A2 / A1$, $Q_{m2} = A3 / A2$, $Q_{m3} = A4 / A3$, $Q_{PARA} = a_{PARA2} / a_{PARA1}$, $Q_{PHEN} = a_{PHEN2} / a_{PHEN1}$, $Q_{CHLOR} = a_{CHLOR2} / a_{CHLOR1}$, $A2$ = Absorbance of Mixture at 258nm, $A1$ = Absorbance of Mixture at 233 nm, $A3$ = Absorbance of Mixture at 250 nm, $A4$ = Absorbance of Mixture at 234 nm

a_{PARA1} = absorptivity of PARA at 233 nm, a_{PHEN1} = absorptivity of PHEN at 233 nm, a_{PARA2} = absorptivity of PARA at 258 nm, a_{PHEN2} = absorptivity of PHEN at 258 nm, a_{PHEN1} = absorptivity of PHEN at 250 nm, a_{CHLOR1} = absorptivity of CHLOR at 250 nm, a_{PHEN2} = absorptivity of PHEN at 239 nm, a_{CHLOR2} = absorptivity of CHLOR at 239 nm, a_{CHLOR1} = absorptivity of CHLOR at 234 nm, a_{PARA1} = absorptivity of PHEN at 234 nm, a_{CHLOR2} = absorptivity of CHLOR at 262 nm, a_{PARA2} = absorptivity of PARA at 262 nm

Method 3: Area under Curve Method

Area under curve method for PARA and PHEN in presence of CHLOR

For the simultaneous determination using the area under curve (AUC) method, suitable dilutions of the standard stock solutions (250 $\mu\text{g}/\text{ml}$) of PARA, PHEN and CHLOR were prepared separately in Methanol. The solutions of drugs were scanned in the range of 200-400 nm. For Area Under Curve method, calibration curve was plotted and the sampling wavelength ranges selected for estimation of PARA, PHEN and CHLOR are 254 nm - 265 nm (λ_1 - λ_2) and 232 nm - 244 nm (λ_3 - λ_4) and 257 nm - 267 nm (λ_5 - λ_6) respectively and area were integrated between these selected wavelength ranges for three drugs, which showed linear response with increasing concentration hence the same wavelength range were used for estimation of tablet formulations. By using integrated areas three simultaneous equations were constructed and solved to determine concentrations of analytes^{13,14}.

Analysis of the tablet formulations

Twenty tablets of marketed formulation were accurately weighed and powdered. Standard addition method was used for analysis of drugs. A quantity of powder equivalent to 50 mg of Paracetamol was weighed and dissolved in 100 ml of Methanol. Then the solution was filtered through Whatman filter paper no 41. From the above 10 ml of solution was diluted to 50 ml with Methanol to get 100 $\mu\text{g}/\text{ml}$ of Paracetamol and corresponding Phenylephrine hydrochloride and Chlorpheniramine maleate. From above 2.5 ml of solution was transferred in 10 ml volumetric flask. To this add 0.2 ml of stock solution (250 $\mu\text{g}/\text{ml}$) of pure Phenylephrine Hydrochloride and Chlorpheniramine Maleate and make-up volume up to the mark with Methanol. The purpose of this addition is to bring the concentration of Phenylephrine hydrochloride and Chlorpheniramine Maleate

in linearity range. With this addition, concentration of Paracetamol, Phenylephrine Hydrochloride and Chlorpheniramine Maleate in the samples was brought to 2.5, 5.5 and 5.1 µg/ml respectively. Analysis procedure was repeated six times with tablet formulation and result reported in Table 1.

Validation

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer- Lambert's concentration range is 4-24 µg/mL for all drugs. The linearity data for all methods are presented in Table 3^{1,15}.

Accuracy

Accuracy of the developed method was confirmed by recovery study as per ICH norms at three different concentration levels of 80 %, 100 %, 120 % by replicate analysis (n = 3). Here to a pre analyzed sample solution, standard drug solutions were added and then percentage drug content was calculated. The result of accuracy study was reported in Table 2. The recovery study indicates that the method is accurate for quantitative estimation of Paracetamol, Phenylephrine Hydrochloride and Chlorpheniramine Maleate in tablet dosage form as the statistical results are within the acceptance range (S.D. < 2.0).

Precision

Precision was determined by studying the repeatability and intermediate precision.

Repeatability

Repeatability result indicates the precision under the same operating conditions over a short interval of time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated. Repeatability was performed for six times with tablets formulation. The results of statistical evaluation are given in Table 1.

Intermediate Precision (Inter-day and Intra-day precision)

An intermediate precision was carried out by intra and inter day precision study. In intra-day study concentration of drugs were calculated on the same day at an interval of one hour. In inter day study the drug contents were calculated on three different days. Study expresses within laboratory variation in different days. In both intra and inter-day precision study for the methods % COV were not more than 1.0 indicates good intermediate precision (Table 3).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of Paracetamol, Phenylephrine Hydrochloride and Chlorpheniramine Maleate by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3 /S and 10 /S respectively, where S is the slope of the calibration curve and is the standard deviation of response. The results of the same are shown in Table 3.

Table 1: Analysis data of tablet Formulation

Method	Drug	Label claim mg/tab	Amount found mg/tab	Label claim (%)	S.D.	% C.O.V.	S.E.
I	PARA	500	500.25	100.11	0.7608	0.7563	0.3106
	PHEN	10	9.936	99.34	0.1625	0.1611	0.0665
	CHLOR	2	1.9794	99.57	0.5137	0.4987	0.2105
II	PARA	500	495.0	99.10	0.5674	0.7811	0.3086
	PHEN	10	10.11	100.05	1.0077	1.2015	0.3537
	CHLOR	2	1.88	98.02	0.5480	0.4557	0.12227
III	PARA	500	486.98	94.55	0.2640	0.2640	0.1080
	PHEN	10	9.8732	97.32	1.0775	1.0865	0.4424
	CHLOR	2	1.87	98.01	0.9230	0.9289	0.3562

Table 2: Result of recovery studies

Method	Recovery level (added amount)	Drugs		
		PARA	PHEN	CHLOR
I	80 %	99.99 ± 0.1456	99.20 ± 0.1445	100.20 ± 0.3538
	100 %	98.90 ± 0.2052	99.70 ± 0.4567	98.89 ± 0.1406
	120 %	98.50 ± 0.6384	98.30 ± 0.1423	100.30 ± 0.3578
II	80 %	99.40 ± 0.2395	98.95 ± 0.5569	99.5 ± 0.0856
	100 %	100.30 ± 0.6568	99.90 ± 0.7560	99.32 ± 0.0856
	120 %	99.80 ± 0.3401	100.1 ± 0.3382	99.90 ± 0.0704
III	80 %	99.60 ± 0.1423	99.47 ± 0.3985	98.95 ± 0.9345
	100 %	99.75 ± 0.2376	98.92 ± 0.6258	99.47 ± 0.5641
	120 %	98.89 ± 0.2576	99.87 ± 0.5832	98.83 ± 0.3281

Table 3: Optical Characteristics data and validation parameters

Parameter	Values		
Drugs	PARA	PHEN	CHLOR
Working (nm)	258	239	262
Beer's law limit µg/ml	4-24	4-24	4-24
Absorptive	0.0724	0.0430	0.0424
Correlation coefficient	0.997	0.993	0.996
Intercept	-0.053	0.062	-0.008
Slop	0.098	0.052	0.021
LOD	0.4612	0.0793	0.3512
LOQ	1.6081	0.6858	0.5360
Intra-day (precision) (% C.O.V.)	0.7131	0.3216	0.3814
Inter-day (precision) (% C.O.V.)	0.9720	0.9582	0.5706

Figure 1: Overlay spectrum of Paracetamol

Figure 2: Overlay spectrum of Phenylephrine

Figure 3: Overlay spectrum of Chlorpheniramine

Figure 4: Overlay spectra of PARA, PHEN and CHLOR

RESULT

The proposed methods for simultaneous estimation of PARA, PHEN and CHLOR in combined dosage form were found to be accurate, simple and rapid which can be well understood from validation data as given in Table 3 and 4. The % R.S.D. Linearity was observed by linear regression equation method for PARA, PHEN and CHLOR in different concentration range. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. The assay results obtained by proposed methods as shown in Table 2, hence it can be used for routine analysis of two drugs in combined dosage forms. There was no interference from tablet excipients was observed in these methods. It can be easily and conveniently adopted for routine quality control analysis. These methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic and are validated as per ICH guidelines.

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