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Research Article

SIMULTANEOUS ESTIMATION & VALIDATION OF PARACETAMOL, PHENYLEPHRINE HYDROCHLORIDE AND CHLORPHENIRAMINE MALEATE IN TABLETS BY SPECTROPHOTOMETRIC METHOD

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ABSTRACT

The present work describes two methods for simultaneous estimation of phenylephrine hydrochloride and chlorpheniramine maleate in pure and solid dosage forms. First method employs the application of simultaneous equation and second, is a multi-wavelength spectrophotometric analysis method. Both methods utilize 0.1N NaOH as solvent. Simultaneous equation develops using 256.8 nm, 236.8 nm and 222.4 nm as the λmax of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate respectively. Calibration curves were linear over the concentration ranges of 0-35 µg/mL for all drugs. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 1 %), while being simple, cheap and less time consuming, and hence can be suitably applied for simultaneous determination of three drugs in laboratory prepared mixtures and in commercial tablet preparation.

KEY WORDS: Paracetamol, Phenylephrine Hydrochloride, Chlorpheniramine Maleate, Multi-Wavelength Method, Simultaneous Equation Method.

INTRODUTION

Paracetamol is 4'-Hydroxyacetanilide. It is antipyretic and analgesic[1]. Paracetamol alone or in combination with other drugs is reported to be estimated by spectrophotometric method[2,3], HPLC[4], TLC[5], HPTLC[6], LC-MS[7], FT-IR[8], amperometric determination[9], Fluorimetry[10], and Micellar electrokinetic chromatographic method[11]. 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 system. Literature survey revealed that spectrophotometry[12], RP-HPLC[13], electrophoresis[14], liquid chromatography[15], methods have been reported for the estimation of phenylephrine hydrochloride in pharmaceutical formulations. Chlorpheniramine maleate is (RS)-3-(4-chlorophenyl)-3-(2-pyridyl) propyldimethylamine hydrogen maleate, used as an antihistaminic. For estimation of chlorpheniramine maleate, HPLC[16] chemometric-assisted spectrophotometric[17] methods have been reported. 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.

EXPERIMENTAL PROCEDURE

2.1 Materials

UV-visible double beam spectrophotometer, Shimadzu model 1700 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells were used. The commercially available tablet, Febrex plus (Label claim: paracetamol 500 mg, phenylephrine hydrochloride 10 mg and chlorpheniramine maleate 2 mg was procured from local market.

2.2 Selection of common solvent

After assessing the solubility of drugs in different solvents 0.1N NaOH has been selected as common solvent for developing spectral characteristics.

2.3 Preparation of standard stock and calibration curve

The standard stock solutions of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate were prepared by dissolving 25 mg of each drug in 10 mL of 0.1N NaOH and final volume was adjusted to 100 mL to get 250 µg/mL. Working standard solutions of 20 µg/mL were scanned in the entire UV range of 400-200 nm to obtain the absorbance spectra and overlain spectra. Seven working standard solutions for four drugs having concentration 5, 10, 15, 20, 25, 30, 35, µg/mL was prepared in 0.1N NaOH from stock solution. The absorbance of resulting solutions were measured at their respective λmax and plotted a calibration curve against concentration to get the linearity and regression equation.

2.4 Method I: Simultaneous equation method

Simultaneous equation method of analysis is based on the absorption of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate at the wavelength maximum (λmax) of each other. λmax for paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate are 256.8 nm, 236.8 nm and 222.4 nm respectively. The absorptivity values determined at 256.8 nm, 236.8 nm and 222.4 nm for paracetamol 0.0738(ax_1), 0.0465(ax_2), 0.0228(ax_3), for phenylephrine hydrochloride 0.0572(ay_1), 0.0442(ay_2), 0.0232(ay_3) and for chlorpheniramine Maleate 0.0152(az_1), 0.0169(az_2), 0.0444(az_3). These values are means of six estimations. The absorptivity coefficients were substituted in equation 1, 2 and 3 to obtain the concentration of drugs.

$$A_1 = 0.0738xC_{PA} + 0.0572xC_{PH} + 0.0152xC_{CM}$$

…….Eqn.1

$$A_2 = 0.0465xC_{PA} + 0.0442xC_{PH} + 0.0169xC_{CM}$$

…….Eqn.2

$$A_3 = 0.0228xC_{PA} + 0.0232xC_{PH} + 0.0444xC_{CM}$$

…….Eqn.3

Where $C_{PA,}$ C_{PH}, and C_{CM} are concentrations of paracetamol , phenylephrine hydrochloride and chlorpheniramine maleate respectively in µg/mL. A_1, A_2, and A_3 are the absorbance of the sample at 256.8 nm, 236.8 nm and 222.4 nm respectively.

2.5 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 0.1N NaOH. Then the solution was filtered through Whatman filter paper no 41. From the above 10 mL of solution was diluted to 50 mL with 0.1N NaOH to get 100 µg/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 µg/mL) of pure phenylephrine hydrochloride and chlorpheniramine maleate and make-up volume upto the mark with 0.1N NaOH. 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 25, 5.5 and 5.1 µg/mL respectively. Analysis procedure was repeated six times with tablet formulation and result reported in Table 1.

2.6 Method II: Multiwavelength spectroscopy

In this method, the instrument is preprogrammed to collect and compile the spectral data from the scan of standards and produces the result by matrix calculations. Five mixed standards of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate in the ratio of 5:0.1:0.02, 10:0.2:0.04, 15:0.3:0.06, 20:0.4:0.08, 25:0.5:0.1, were prepared in 0.1N NaOH by diluting appropriate volumes of the standard stock solutions and scanned in the region of 400 nm to 200 nm. Sampling wavelengths (256.8 nm, 236.8 nm and 222.4 nm) were selected on the trial and error basis. The concentration of individual drug was feed to the multi-component mode of the instrument. The instrument collects and compiles the spectral data from mixed standards and concentration of each component were obtained by spectral data of sample solution with reference to that of five mixed standards. A tablet sample solution was prepared as described under method I. The spectrophotometric analysis of the resulting solution was carried out using the multicomponent mode of the instrument.

2.7Validation[18]

2.7.1 Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer- Lambert's concentration range is 0-35 µg/mL for all drugs. The linearity data for both methods are presented in Table 3.

2.7.2 Accuracy

Accuracy of the developed method was conformed by recovery study as per ICH norms at three different concentration levels of 80 %, 100 %, 120 % by replicate analysis (n = 3). Here to a preanalysed 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).

2.7.3 Precision

Precision was determined by studying the repeatability and intermediate precision.

2.7.4 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.

2.7.5 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).

2.7.5 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 σ/S and 10 σ/S respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 3.

RESULTS AND DISCUSSION

The Beer- Lambert's concentration range is 0-35 µg/mL for paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate at 256.8 nm, 236.8 nm and 222.4 nm wavelengths with coefficient of correlation 0.9990, 0.9990 and 0.9960 respectively. All the drugs show good regression values at their respective wavelengths and the results of recovery study reveals that any small change in the drug concentration in the solution could be accurately determined by the proposed methods. Percentage estimation of the drugs found in tablet dosage form are 100.03, 99.45, 99.47 using method I where as 99.20, 100.25, 99.55 using method II for paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate respectively with standard deviation < 2.

In method II five mixed standard and three sampling wavelengths were selected through rational experimentation keeping in view the amount of drugs in the formulation and molar absorptivity coefficients (Fig. 2). The method requires no manual calculations, produces comparable results to the first method and is more suitable as compared to method I.

The validity and reliability of proposed methods are assessed by recovery studies. Sample recoveries for both the methods are in good agreement with their respective label claims, which suggests non-interference of formulation additives in estimation (Table-2). Precision was determined by studying the repeatability and intermediate precision. 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 are calculated for paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate (Table 1). Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for both the methods % COV are not more than 1.0 indicates good intermediate precision (Table 3). The LOD values are 0.4612, 0.0793, 0.3512µg/mL and LOQ values are 1.6081, 0.6858, 0.5360 µg/mL for paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate respectively. Low values of LOD and LOQ indicated good sensitivity of proposed methods.

Table 1. Analysis data of tablet formulation

Method	Drug	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.*	% COV	S.E*.
I	PA	500	500.15	100.03	0.2646	0.2645	0.1080
	PH	10	9.945	99.45	1.0795	1.0854	0.4432
	CM	2	1.9894	99.47	0.9232	0.9281	0.3789
II	PA	500	496.0	99.20	0.5674	0.7811	0.3068
	PH	10	10.025	100.25	1.0077	1.2015	0.3537
	CM	2	1.991	99.55	0.5480	0.4557	0.1272

PA: paracetamol, PH: phenylephrine hydrochloride and CM: chlorpheniramine maleate S.D.: standard deviation**,** COV: coefficient of variation, S.E.: standard error, *Average of six estimation of tablet formulation.

Table 2. Result of recovery studies

Method	Recovery level (Added amount)	Percent recovery ± SD#		
		PA	PH	CM
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

PA: paracetamol, PH: phenylephrine hydrochloride and CM: chlorpheniramine maleate S.D.: standard deviation, # Average of three estimation at each level of recover

Table 3. Optical characteristics data and validation parameters

Parameters	Values		
	PA	PH	CM
Working λmax	256.8 nm	236.8 nm	222.4 nm
Beer's law limit (µg/ml)	0-35	0-35	0-35
Absorptive*	0.0738	0.0442	0.0444
Correlation coefficient*	0.9990	0.9990	0.9960
Intercept*	0.0170	-0.0020	0.0250
Slope*	0.072	0.044	0.042
LOD* (µg/ml)	0.4612	0.0793	0.3512
LOQ*(µg/ml)	1.6081	0.6858	0.5360
Intra-Day* (Precision) (% COV)	0.7131	0.3216	0.3814
Inter-Day (Precision) (% COV) n=3	0.9720	0.9582	0.5706

PA: paracetamol, PH: phenylephrine hydrochloride and CM: chlorpheniramine maleate COV: coefficient of variation, * Average of six determination.

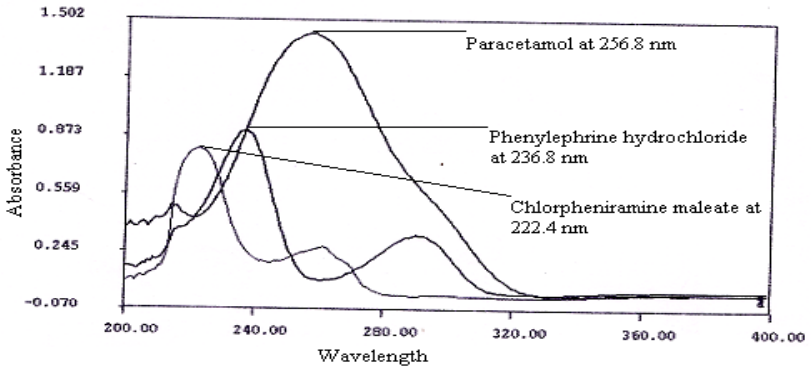

Figure 1. Overlain spectra of paracetamol, phenylephrine hydrochloride,and chlorpheniramine maleate

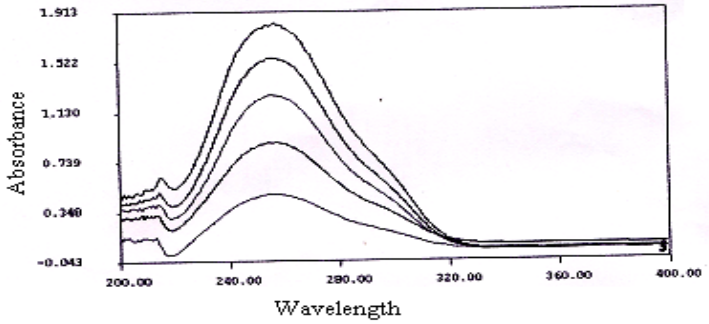

Figure 2. Overlain spectra of mixed standard of paracetamol, phenylephrine hydrochloride, and chlorpheniramine maleate.

CONCLUSION

This method represents a fast analytical procedure for the simultaneous estimation of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate. Standard deviation was satisfactorily low indicating accuracy and reproducibility of the methods. Recovery studies were satisfactory which shows that there is no interference of excipients. The developed methods were found to be simple, rapid, and accurate and can be used for routine estimation of three drugs from tablet formulations.

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