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Effective quantitation of Acetaminophen, Phenylephrine hydrochloride, Cetirizine hydrochloride and Caffeine in pharmaceutical dosage form using UV spectroscopy

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ABSTRACT

Attempting to minimize the drawbacks of various organic solvents commonly used for solublization, two new spectrophotometric methods have been developed for simultaneous determination of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine in pure and tablet dosage form by using 0.1N NaOH. Jasco V-630 sepctrophotometer is used for quantitation. Without resolving mixtures of acetaminophen, phenylephrine hydrochloride, cetrizine hydrochloride and caffeine, simultaneous estimation has been successfully achieved by spectrophotometry. Method **I** employ formation and solving of mathematical simultaneous equation using 259 nm, 233 nm, 231 nm and 273 nm as the λmax of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine respectively. Method **II** is a multiwavelength spectrophotometric analysis in which the instrument is preprogrammed to collect and compile the spectral data from the scan of standards and matrix calculations. These methods were validated for accuracy, precision, linearity, specificity and sensitivity as per ICH norms. Calibration curves were linear over the concentration ranges of 0-40 µg/mL for all drugs. The validation study is statistically significant as all the statistical parameters are within the acceptance range (% COV< 2.0 and S.D. < 2.0) for both accuracy and precision. Both the methods are successfully applied to pharmaceutical formulation, with no interference from excipients as indicated by the recovery study. The proposed methods are simple, rapid, economic and accurate for routine simultaneous estimation of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine.

Keywords: Acetaminophen, Phenylephrine Hydrochloride, Cetirizine Hydrochloride, Caffeine, Simultaneous Equation Method, Multi-Wavelength Method.

INTRODUCTION

Plenty of dosage forms of drug combinations are available in the market. Due to their greater patient acceptability, increased potency, multiple action, fewer side effects and quicker relief, they have acquired a lot of importance nowadays. The instrumental techniques such as HPTLC, GLC, spectrophotometry, HPLC etc. are used for estimation of multi-component formulation. Simultaneous analysis procedures avoid time consuming extraction and separation; these are economical because of minimal use of expensive regents and equally accurate and precise. These methods are validated as per ICH guidelines [1]. Chemically, acetaminophen is 4-hydroxy acetanilide, used as an analgesic and antipyretic. Various analytical methods, such as, spectrophotometry [2] , HPLC [3] have been reported for the estimation of acetaminophen 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 system. Literature survey revealed that spectrophotometry [4-6], chromatography [7-9], micellar liquid chromatography [10], methods have been reported for the estimation of phenylephrine hydrochloride in pharmaceutical formulations. Cetirizine is a carboxylated metabolite of hydroxyzine and it has high specific affinity for histamine H1 receptor. Cetirizine is chemically known as 2-[4-(4-chlorobenzhydryl) piperazine-1-yl] ethoxy acetic acid. Chromatographic methods [11,12] have been reported for determination of cetirizine in pharmaceutical formulation. Caffeine is 3, 7- dihydro-1, 3, 7-trimethyl-1H-purine-2, 6-dione, used as central nervous system stimulant. For caffeine FTIR spectrophotometric [13] and UV/vis spectrophotometric [14] methods are available.

A combination of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine is commercially available in tablet dosage form. Literature reveals that no analytical method is available for simultaneous determination of these four 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.

MATERIALS AND METHODS

Materials

UV-visible double beam Jasco-V-630 spectrophotometer and a pair of 10 mm matched quartz cells were used. The commercially available tablet, Sumo cold (Label claim: acetaminophen 500 mg, phenylephrine hydrochloride 5 mg, cetirizine hydrochloride 2 mg, and caffeine 25 mg) was procured from local market.

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.

Preparation of standard stock and calibration curve

The standard stock solutions of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine 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 25 mg/mL were scanned in the entire UV range of 400-200 nm to obtain the absorbance spectra and overlain spectra. Eight working standard solutions for four drugs having concentration 5, 10, 15, 20, 25, 30, 35, 40 µg/mL was prepared in 0.1N

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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.

Method I: Simultaneous equation method

Simultaneous equation method of analysis is based on the absorption of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine at the wavelength maximum (λmax) of each other. λmax for acetami nophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine are 259 nm, 233 nm, 231 nm and 273 nm respectively. The absorptivity values determined at 231 nm, 233 nm, 259 nm and 273 nm for acetaminophen 0.0414(ax1), 0.0437(ax2), 0.0849(ax3), 0.0661(ax4), for phenylephrine hy drochloride 0.042(ay1), 0.0436(ay2), 0.0069(ay3), 0.0113(ay4), for cetirizine hydrochloride 0.0324(az1), 0.0318(az2), 0.0020(az3) 0.0013(az4) and for caffeine 0.0310(aw1), 0.0290(aw2), 0.0356(aw3) and 0.0606(aw4). These values are means of six estimations. The absorptivity coefficients were substituted in equation 1, 2, 3 and 4 to obtain the concentration of drugs.

$$A_1 = 0.0414\,xC_{AC} + 0.042\,xC_{PH} + 0.0324\,xC_{CH} + 0.031\,xC_{CA}$$

$$A_2 = 0.0437\,xC_{AC} + 0.0436\,xC_{PH} + 0.0318\,xC_{CH} + 0.029\,xC_{CA}$$

$$A_3 = 0.0849xC_{AC} + 0.0069xC_{PH} + 0.0020xC_{CH} + 0.0356xC_{CA}$$

$$A_4 = 0.0661xC_{AC} + 0.0113xC_{PH} + 0.0013xC_{CH} + 0.0606xC_{CA}$$

Where C_{AC}, C_{PH}, C_{CH} and C_{CA} are concentrations of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine respectively in µg/mL. A1, A2, A3 and A4 are the absorbance of the sample at 231 nm, 233 nm, 259 nm and 273 respectively.

Analysis of the tablet formulations

Twenty tablets of marketed formulation were accurately weighed individually and their average weight was determined after that they were crushed to fine powder. Standard addition method was used for analysis. A quantity of powder equivalent to 50 mg of acetaminophen was weighed. An accurately weighed 9.85 mg of pure phenylephrine hydrochloride, cetirizine hydrochloride and 14.25 mg of caffeine was added to finely powdered samples to get the concentration of phenylephrine hydrochloride, cetirizine hydrochloride and caffeine in linearity range. With this addition, the ratio of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine in the samples was brought to 3:2:2:3 respectively. This sample powered dissolved in 100 mL of 0.1N NaOH. Then the solution was filtered through Whatman filter paper no 41. From the above 0.3 mL of solution was diluted to 10 mL with 0.1N NaOH to get 15µg/mL of acetaminophen and corresponding phenylephrine hydrochloride, cetirizine hydrochloride and caffeine. The absorbance of sample solution was recorded at selected wavelength. Analysis procedure was repeated six times with tablet formulation and result reported in Table 1.

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 acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine in the ratio of 10:5:5:10, 11:6:6:11, 12:7:7:12, 14:9:9:14, 15:10:10:15 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 (231 nm, 233 nm, 259 nm and 273nm.) were selected. 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.

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 0-40 µg/mL for all drugs. The linearity data for both methods are presented in Table 3.

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 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 acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine 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)

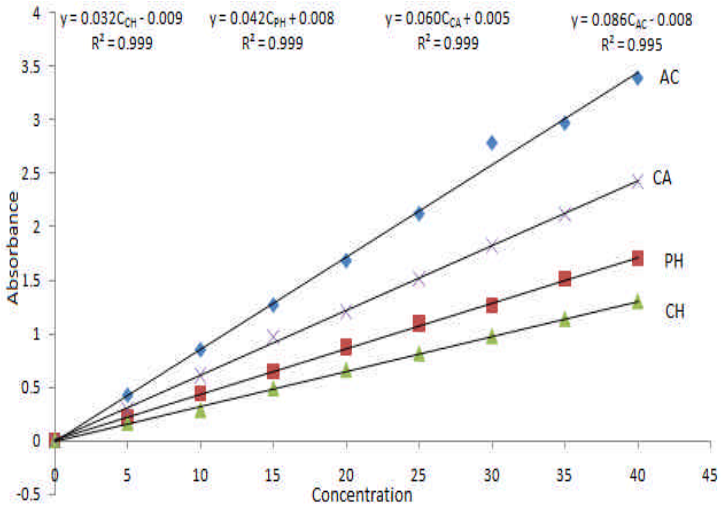
An intermediate precision was carried out by intra and inter day precision study. In intraday study concentration of drugs were calculated on the same day at an interval of one hour. In inter day study the drug contents were calculated on three different days. Study expresses within laboratory variation 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 acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine by proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\,\sigma/S$ and $10\,\sigma/S$ 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 0-40 µg/mL (Fig.1) for acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine at 259 nm, 233 nm, 231 nm and 273 nm (Fig.2) wavelengths with coefficient of correlation 0.9950, 0.9990, 0.9990 and 0.9990 respectively. All the drugs show good regression values at their respective wavelengths and the results



Where- C_{AC}, C_{PH}, C_{CH} and C_{CA} . Concentration of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine respectively.

Figure 1. Linearity graph of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine

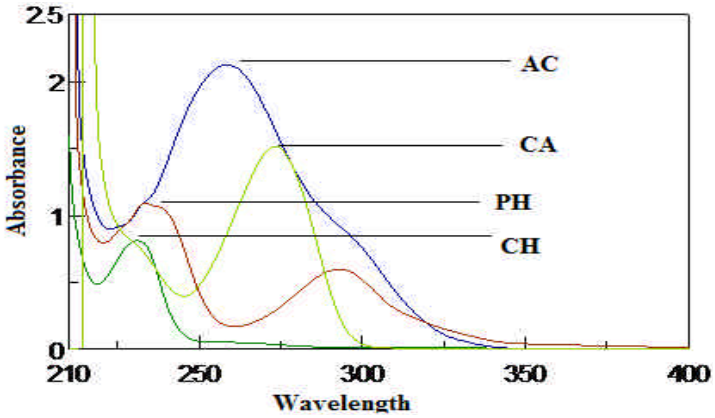


Figure 2. Overlain spectra of AC-Acetaminophen, PH-Phenylephrine hydrochloride, CH- cetirizine hydrochloride and CA-Caffeine showing maximum absorbance at 259 nm, 233 nm, 231 nm and 273 nm respectively

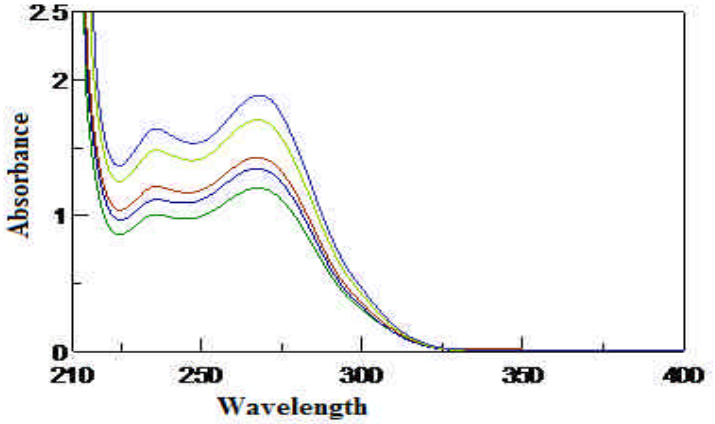


Figure 3. Overlain spectra of five mixed standards of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine in the ratio of 10:5:5:10, 11:6:6:11, 12:7:7:12, 14:9:9:14, 15:10:10:15 in 0.1N NaOH.

Table 1 Analysis data of tablet formulation

Method	API Component	Label Claim mg/tab	Amount Found mg/tab	Label Claim %	S.D.*	% COV	S.E*.
I	AC	500	499.00	99.80	0.9959	0.9978	0.9980
	PH	5	5.00	100.03	0.8358	0.8355	1.0003
	CH	5	4.99	99.83	0.7763	0.7776	0.9983
	CA	25	24.83	99.35	0.8215	0.8268	0.9935
II	AC	500	501.75	100.35	1.0559	1.0522	1.0035
	PH	5	4.96	99.25	0.5576	0.5618	0.9925
	CH	5	5.00	100.05	0.9690	0.9685	1.0005
	CA	25	25.07	100.28	0.7111	0.7091	1.0028

AC: acetaminophen, PH: phenylephrine hydrochloride, CH: cetirizine hydrochloride and CA: caffeine. * Average of six determination. 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# AC	PH	CH	CA
I	80%	99.30±0.3412	98.10±0.4561	101.20±0.3558	99.50±0.2341
	100%	100.10±0.8711	99.40±0.3419	99.10±0.7812	100.20±0.4561
	120%	98.60±0.6719	99.30±0.4512	101.20±0.451	99.50±0.2781
II	80%	98.70±0.3451	99.90±0.3471	99.80±0.4561	100.30±0.5410
	100%	99.50±0.4516	98.40±0.1234	99.40±0.5612	100.40±0.4510
	120%	98.70±0.3142	99.80±0.9819	100.60±0.7812	99.80±0.3412

AC: acetaminophen, PH: phenylephrine hydrochloride, CH: cetirizine hydrochloride and CA: caffeine S.D.: standard deviation, # Average of three estimation at each level of recovery.

Table 3. Optical characteristics data and validation parameters

Parameters	Values AC	PH	CH	CA
Working λmax	259 nm	233nm	231 nm	273 nm
Beer's law limit (µg/ml)	0-40	0-40	0-40	0-40
Absorptive*	0.0849	0.0436	0.0324	0.0606
Correlation coefficient*	0.9950	0.9990	0.9990	0.9990
Intercept*	-0.0080	0.008	-0.009	0.005
Slope*	0.086	0.042	0.032	0.060
LOD* (µg/ml)	0.7123	1.4563	1.3561	0.4687
LOQ*(µg/ml)	0.3412	0.9812	0.7812	0.2187
Intra-Day* (Precision)(% COV)	0.6754	0.2317	0.3410	0.4561
Inter-Day (Precision) (% COV) n=3	0.4532	0.9872	0.5671	0.0987

AC: acetaminophen, PH: phenylephrine hydrochloride, CH: cetirizine hydrochloride and CA: caffeine, COV: coefficient of variation, * Average of six determination.

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 98.80,100.03,99.83,99.35 using method I where as 100.35, 99.25, 100.05,100.28 using method II for acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine respectively with standard deviation < 2.0. 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.3). 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 noninterference 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 acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine (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 0.712, 0.0543, 1.456, 1.3561, 0.4687 µg/mL and LOQ values are 0.3412, 0.9812, 0.7812, 0.2187 µg/mL for acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine respectively. Low values of LOD and LOQ indicated good sensitivity of proposed methods.

CONCLUSION

The proposed methods are simple, rapid, economic and accurate for routine simultaneous estimation of acetaminophen, phenylephrine hydrochloride, cetirizine hydrochloride and caffeine.

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