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

Hapse Sandip Appasaheb*, Kapare Parmeshwar Subhash, Dhurnal Virashri Atmaram, Damale Pallavi Shankar
PDVVP’S College of Pharmacy, Post- MIDC, Vilad Ghat, Ahmednagar, (MS), India

*Corresponding Author Email: sandiphapse@gmail.com

Article Received on: 19/09/13 Revised on: 21/10/13 Approved for publication: 10/10/13

DOI: 10.7897/2230-8407.041010

IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com
© All rights reserved.

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 Paracetol, 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 activity. Various analytical methods, such as, spectrophotometry, HPLC, HPTLC 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 system. From literature survey Phenylephrine Hydrochloride has been determined alone or in combination using UV spectrophotometry, HPLC, RP-HPLC methods. Chlorpheniramine maleate (CPM), (±) 2-[p-chloro-[2-dimethylamino] ethyl] benzyl] pyridine bimaleate (Chlor-Trimeton). Chlorpheniramine (Maleate) is the maleate salt of Chlorpheniramine. Chlorpheniramine maleate used as an antihistaminic, it is also effective in nausea, motion sickness. UV spectrophotometry, HPLC, RP-HPLC 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 Phenolinephine hydrochloride) was procured from local market, Methanol, API of Paracetamol, Chlorpheniramine Maleate and Phenolinephine 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 to 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 absorbative 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

\[ A1 = 0.0724\text{xCPA} + 0.0560\text{xCPH} + 0.0152\text{xCCM} \]  
\[ A2 = 0.0456\text{xCPA} + 0.0430\text{xCPH} + 0.0160\text{xCCM} \]  
\[ A3 = 0.0220\text{xCPA} + 0.0222\text{xCPH} + 0.0424\text{xCCM} \]

Where, CPA, CPH, and CCM are concentrations of Paracetamol, Phenylephrine Hydrochloride and Chlorpheniramine Maleate respectively in µg/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

\[ \text{CPARA} = \frac{Qm1\text{-QPHEN}}{Q\text{PARA-QPHEN}} \times \frac{A1}{a\text{PARA1}} \]  
\[ \text{CPHEN} = \frac{Qm2\text{-QCHLOR}}{Q\text{PHEN-QCHLOR}} \times \frac{A1}{a\text{PHEN1}} \]

Where, Qm1, Qm2, Qm3, QPARA, QPHEN, QCHLOR are absorbance of PARA at 233 nm, PHEN at 239 nm, CHLOR at 262 nm and PARA, PHEN, CHLOR respectively.

**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 µg/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 determination of PARA, PHEN and CHLOR are 254 nm - 265 nm (λ1-λ2) and 232 nm - 244 nm (λ3-λ4) and 257 nm - 267 nm (λ5-λ6) 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 analytes13,14.

\[ \text{C}_{\text{PARA}} = \frac{(\text{XPHEN at 254 - 265} \times \text{AUCPHEN at 232 - 244} - \text{XPHEN at 252 - 246}) \times \text{AUCPARA at 254 - 265}}{(\text{XPHEN at 252 - 246} \times \text{XPARA at 232 - 244} - \text{XPHEN at 252 - 246} \times \text{XPARA at 254 - 265})} \]  
\[ \text{C}_{\text{PHEN}} = \frac{(\text{XPARA at 254 - 265} \times \text{AUCPARA at 254 - 265} - \text{XPARA at 252 - 244}) \times \text{AUCPHEN at 254 - 246}}{(\text{XPHEN at 254 - 246} \times \text{XPARA at 232 - 244} - \text{XPHEN at 252 - 244} \times \text{XPARA at 254 - 265})} \]

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 µg/3/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

**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 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 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.

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.

Table 1: Analysis data of tablet Formulation

<table>
<thead>
<tr>
<th>Method</th>
<th>Drug</th>
<th>Label claim mg/tab</th>
<th>Amount found mg/tab</th>
<th>Label claim (%)</th>
<th>S.D.</th>
<th>% C.O.V.</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>PARA</td>
<td>500</td>
<td>500.25</td>
<td>100.11</td>
<td>0.7608</td>
<td>0.7563</td>
<td>0.3106</td>
</tr>
<tr>
<td></td>
<td>PHEN</td>
<td>10</td>
<td>9.936</td>
<td>99.34</td>
<td>0.1625</td>
<td>0.1611</td>
<td>0.0665</td>
</tr>
<tr>
<td></td>
<td>CHLOR</td>
<td>2</td>
<td>1.9794</td>
<td>99.57</td>
<td>0.5137</td>
<td>0.4987</td>
<td>0.2105</td>
</tr>
<tr>
<td>II</td>
<td>PARA</td>
<td>500</td>
<td>495.0</td>
<td>99.10</td>
<td>0.5674</td>
<td>0.7811</td>
<td>0.3086</td>
</tr>
<tr>
<td></td>
<td>PHEN</td>
<td>10</td>
<td>10.11</td>
<td>100.05</td>
<td>1.0077</td>
<td>1.2015</td>
<td>0.3537</td>
</tr>
<tr>
<td></td>
<td>CHLOR</td>
<td>2</td>
<td>1.88</td>
<td>98.02</td>
<td>0.5480</td>
<td>0.4557</td>
<td>0.1227</td>
</tr>
<tr>
<td>III</td>
<td>PARA</td>
<td>500</td>
<td>486.98</td>
<td>94.55</td>
<td>0.2640</td>
<td>0.2640</td>
<td>0.1080</td>
</tr>
<tr>
<td></td>
<td>PHEN</td>
<td>10</td>
<td>9.8732</td>
<td>97.32</td>
<td>1.0775</td>
<td>1.0865</td>
<td>0.4424</td>
</tr>
<tr>
<td></td>
<td>CHLOR</td>
<td>2</td>
<td>1.87</td>
<td>98.01</td>
<td>0.9230</td>
<td>0.9289</td>
<td>0.3562</td>
</tr>
</tbody>
</table>

Table 2: Result of recovery studies

<table>
<thead>
<tr>
<th>Method</th>
<th>Recovery level (added amount)</th>
<th>PARA</th>
<th>PHEN</th>
<th>CHLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>80 %</td>
<td>99.99 ± 0.1456</td>
<td>99.20 ± 0.1445</td>
<td>100.20 ± 0.3538</td>
</tr>
<tr>
<td></td>
<td>100 %</td>
<td>98.99 ± 0.2052</td>
<td>99.70 ± 0.4567</td>
<td>98.89 ± 0.1406</td>
</tr>
<tr>
<td></td>
<td>120 %</td>
<td>98.50 ± 0.6384</td>
<td>98.30 ± 0.1423</td>
<td>100.30 ± 0.3578</td>
</tr>
<tr>
<td>II</td>
<td>80 %</td>
<td>99.40 ± 0.2395</td>
<td>98.95 ± 0.5569</td>
<td>99.5 ± 0.0856</td>
</tr>
<tr>
<td></td>
<td>100 %</td>
<td>100.30 ± 0.6568</td>
<td>99.90 ± 0.7560</td>
<td>99.32 ± 0.0856</td>
</tr>
<tr>
<td></td>
<td>120 %</td>
<td>99.80 ± 0.3401</td>
<td>100.1 ± 0.3382</td>
<td>99.90 ± 0.0704</td>
</tr>
<tr>
<td>III</td>
<td>80 %</td>
<td>99.60 ± 0.1423</td>
<td>99.47 ± 0.3985</td>
<td>98.95 ± 0.9345</td>
</tr>
<tr>
<td></td>
<td>100 %</td>
<td>99.75 ± 0.2376</td>
<td>98.92 ± 0.6258</td>
<td>99.47 ± 0.5641</td>
</tr>
<tr>
<td></td>
<td>120 %</td>
<td>98.89 ± 0.2576</td>
<td>98.87 ± 0.5832</td>
<td>98.83 ± 0.3281</td>
</tr>
</tbody>
</table>

Parameter

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 |
| Slope | 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 |

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 precision 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.
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.

ACKNOWLEDGEMENT

The authors are thankful to P.D.V.V.P.F’s College Of Pharmacy, Vilad Ghat, Ahmednagar, India for providing facilities to carry out this work.

REFERENCES


Source of support: Nil, Conflict of interest: None Declared