A NEW VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PARACETAMOL AND FLUPIRITINE MALEATE IN TABLET DOSAGE FORM BY FIRST DERIVATIVE UV SPECTROPHOTOMETRIC METHOD

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ABSTRACT

This communication describe simple, sensitive, rapid, accurate, precise and cost effective First derivative spectrophotometric zero crossing method for the simultaneous determination of Paracetamol and Flupiritine Maleate in combined dosage form. The utility of first derivative data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The first order derivative absorption at 248 nm (zero cross point for Paracetamol) was used for estimation of Flupiritine Maleate and 254 nm (zero cross point for Flupiritine Maleate) was used for estimation of Paracetamol. Linear correlation was obtained between absorbance and concentrations of PARA and FLU in the concentration ranges of 2-14 μg/ml and 6.5-45.5 μg/ml, with $R^2$ value 0.999 at both the wavelength respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The RSD values of PARA and FLU were found to be less than 2%. LOD and LOQ values for PARA were found to be 0.122 and 0.402 μg/ml at 254 nm respectively. LOD and LOQ values for FLU were found to be 0.908 and 3.253 μg/ml at 248 nm, respectively.

KEY WORDS

Paracetamol and Flupiritine Maleate, Derivative spectrophotometric method, Zero crossing point.

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INTRODUCTION

Paracetamol (PARA) is chemically N-(4-Hydroxy Phenyl) Acetamide (amide derivative) (figure 1.). It functions as a weak inhibitor of the synthesis of prostaglandins (PGs).

![Structure of Paracetamol](image1.png)

**Fig. 1: Structure of Paracetamol.**

However, the in vivo effects of paracetamol are similar to those of the selective cyclooxygenase-2 (COX-2) inhibitors. Paracetamol also decreases PG concentrations in vivo. Flupiritine Maleate (FLU) is chemically ethyl [2-amino-6-[(4-fluorobenzyl)amino]pyridin-3-yl] carbamate (figure 2.) acts as selective neuronal potassium channel opener that also has NMDA receptor antagonist properties. Literature survey has revealed few methods for their quantification alone (or) in combination by UV spectrophotometry\(^3\)-\(^{12}\), HPLC\(^{13}\), but no UV derivative method was found for simultaneous estimation of both the drugs in dosage forms. Hence, the present work was designed and made attempt to develop first order derivative UV spectrophotometric method for PARA and FLU.
MATERIALS AND METHODS

Materials

A Lab India 3000+ double beam UV/VIS spectrophotometer with spectral width of 2nm, wavelength accuracy of 0.5nm & a pair of 10mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV Win 5.0 software. PARA bulk powder was kindly gifted by Dr.Reeddy’s Labs. Hyderabad, Andhra pradesh, India and FLU bulk powder was kindly gifted by Lupin pharmaceuticals, Mumbai, India. The commercial combination product was procured from the local market. Methanol was used in the study.

Preparation of standard solutions

10 mg of standard PARA and FLU were weighed and transferred to 10 ml volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 1000 µg/ml each of PARA and FLU. From the above solution, transfer 0.1 ml into 10 ml Volumetric flask and made up to mark with methanol to give a solution containing 10 µg/ml each of PARA and FLU.

Methodology

The working standard solutions of PARA and FLU were prepared separately in methanol having a concentration of 10µg/ml. They were scanned in wavelength range of 200-400 nm against solvent methanol as blank. The absorption spectra thus obtained were derivatised from first order to fourth order. First order overline spectra (figure 3.) were selected for analysis of both the drugs (figure 1&2).

![Figure 3. UV first order overline absorption spectra of PARA and FLU](image-url)
VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

Linearity (Calibration Curve)

Appropriate aliquots from the standard stock solutions of PARA and FLU were used to prepare two different sets of dilutions: Series A and B as follows. Series A consisted of different concentration of PARA (2-14 μg/ml). Aliquot from the stock solution of PARA (2-14 μg/ml) was pipetted out into a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range of 2-14 μg/ml (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 ml). Series B consisted of varying concentrations of FLU (6.5-45.5 μg/ml). Aliquot from the stock solution of FLU (6.5-45.5 μg/ml) was pipetted out into a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range of 6.5-45.5 μg/ml (0.65, 1.3, 1.62, 2.6, 3.25, 3.9, and 4.55 ml). The calibration curve were constructed by plotting drug concentration versus the absorbance values of first derivative spectrum 254 nm for PARA and 248 nm for FLU. The concentration of individual drugs present in the mixture was determined from the calibration curves in quantitation mode.

Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n= 6) for PARA and FLU (4, 8, 14 μg/ml for both drugs) without changing the parameter of the proposed spectrophotometric method.

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of PARA and FLU (4, 8, 14 μg/ml for PARA and 13, 26, 45.5 μg/ml for FLU). The result was reported in terms of % relative standard deviation (Table - 1).

Accuracy (Recovery Study)

The accuracy of the method was determined by calculating the recoveries of PARA and FLU by the standard addition method. Known amounts of standard solutions of PARA and FLU were added at 80, 100 and 120% level to pre quantified sample solutions of PARA and FLU (6 μg/ml for PARA and 16.25 μg/ml for FLU). The amounts of PARA and FLU were estimated by applying obtained values to the respective regression line equations (Table – 2).
Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

$$\text{LOQ} = 10 \times \frac{\sigma}{S}$$

Where, $\sigma$ = the standard deviation of the response and $S$ = slope of the calibration curve

Analysis of tablet Sample

Twenty tablets of PRUF P were weighed and finely powdered and tablet powder equivalent to 100 mg of both paracetamol and flupiritine maleate is weighed and extracted with methanol in a 100 ml volumetric flask. The flask was sonicated for 15 min and volume was made up to the mark with methanol. 1 ml was transferred into a 10 ml volumetric flask and the volume was made up to the mark with water, and 0.6 & 1.625 ml of above solution is added to 10 ml volumetric flask and made up to the mark with water, finally the solution is filtered by using syringe filter to obtain 6 & 16.25 µg/ml of paracetamol and flupiritine maleate. The absorbance of the solution was measured under UV spectrophotometer. The assay procedure was made triplicate and weight of sample taken for assay was calculated. The percentage of drug found in formulation, mean and standard deviation in formulation were calculated.

RESULTS AND DISCUSSION

The standard solutions of PARA and FLU were scanned separately in the UV range and First-order spectra for PARA and FLIU were recorded. The first order derivative absorption at 254 nm (zero cross point for PARA) was used for Flupiritine Maleate and 248 nm (zero cross point for FLU) was used. These two wavelengths can be employed for the determination of PARA and FLU without any interference from the other drug in their combined formulations.

Linear correlation was obtained between absorbance and concentrations of PARA and FLU in the concentration ranges of 2-14 µg/ml and 6.5-45.5 µg/ml, with $R^2$ value 0.999 (Table-1) at both the wavelength respectively.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PARA</th>
<th>FLU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>248</td>
<td>252</td>
</tr>
<tr>
<td>Beer’s law limit (µg /ml)</td>
<td>2-14</td>
<td>6.5-45.5</td>
</tr>
</tbody>
</table>
Regression equation
(y = a + bc)
Slope (b)  
Intercept (a)  
Correlation coefficient ($r^2$)
LOD$^a$ (μg/ml)  
LOQ$^b$ (μg/ml)  
Precision (% RSD$^c$, n = 3)
Interday  
Intraday  
Accuracy (% Recovery, n = 5)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount of sample (μg/ml)</th>
<th>Recovery level</th>
<th>Amount of drug added (μg/ml)</th>
<th>Amount found (μg/ml)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>6</td>
<td>80%</td>
<td>4.8</td>
<td>4.72</td>
<td>99.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>6.0</td>
<td>5.96</td>
<td>99.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120%</td>
<td>7.2</td>
<td>7.20</td>
<td>100.45</td>
</tr>
</tbody>
</table>

$^a$LOD = Limit of detection, $^b$LOQ = Limit of quantification, $^c$RSD = Relative standard deviation.
$^d$S.D. = Standard deviation.

The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The RSD values of PARA and FLU were found to be less than 2%. LOD and LOQ values for PARA were found to be 0.122 and 0.402 μg/ml at 254 nm respectively (Table-1). LOD and LOQ values for FLU were found to be 0.986 and 3.253 μg/ml at 248 nm, respectively (Table 1). These data shows that method is sensitive for the determination of PARA and FLU.

The recovery experiment was performed by the standard addition method.

**Table 2: Recovery Data of PARA and FLU by Spectrophotometric method**
The recoveries of PARA and FLU were found to be in the range of 99.25-100.45% and 98.87-101.30% for PARA and FLU, respectively (Table-2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated spectroscopic method was successfully applied to combined dosage form (tablet). The results obtained for PARA and FLU were comparable with the corresponding label claim percentage (Table-3).

Table 3. Analysis of PARA and FLU by Spectrophotometric method

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug</th>
<th>Label claim</th>
<th>Amount found ± SD</th>
<th>Assay</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRUF P</td>
<td>Paracetamol</td>
<td>100mg</td>
<td>100.16±12.36mg</td>
<td>100.16%</td>
<td>0.123</td>
</tr>
<tr>
<td></td>
<td>Flupiritine Maleate</td>
<td>325mg</td>
<td>324.92±11.25mg</td>
<td>99.75%</td>
<td>0.034</td>
</tr>
</tbody>
</table>

No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of PARA and FLU in pharmaceutical dosage forms.

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of PARA and FLU in tablet dosage form. The method utilizes easily available and cheap solvent for analysis of PARA and FLU hence the method was also economic for estimation of PARA and FLU from tablet dosage form. The common excipients and other additives are usually present in the tablet dosage form which do not interfere in the analysis of PARA and FLU in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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REFERENCES


