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Development And Validation Of UV Spectrophotometric Method For Estimation Of Paracetamol And Flupirtine Maleate In Bulk And Pharmaceutical Dosage Form

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Abstract: Two methods for simultaneous estimation of Paracetamol and Flupirtine maleate in combined dosage form have been developed. The first method is the application of Q-analysis method (absorbance ratio), which involves the formation of Q-absorbance equation at 302 nm (isobestic point) and at 248 nm, the maximum absorbance of Paracetamol. The linearity ranges for PCM and FLU were 2-35 µg/ml and 0.5-70µg/ml, respectively. The second method was based on the use of first derivative spectroscopy, in which derivative amplitudes were measured at selected wavelengths (234 nm ZCP of FLU for PCM and 334 nm ZCP of PCM for FLU), without mutual interference. The linearity ranges for PCM and FLU were 2-34 µg/ml and 2-65 µg/ml, respectively. The accuracy of the methods were assessed by recovery studies and was found to be 99.93% ±0.549 and 100.05% ±0.665 for Q absorbance ratio method and 99.54% ±0.591 and 99.41% ±0.792 for first derivative method, for PCM and FLU, respectively. These methods are simple, accurate and rapid; those require no preliminary separation and thereforecan be used for routine analysis of both drugs in quality control laboratories.
Keywords: Derivative and Q-analysis Spectrophotometric methods, Paracetamol, Flupirtine maleate.

INTRODUCTION

Paracetamol (PCM), chemically, [N-(4-hydroxyphenyl) acetamide] is an analgesic-antipyretic agent (Figure 1(a)). It is soluble in methanol and sparingly soluble in Water. It is effective in treating mild to moderate pain. PCM is official in Indian pharmacopoeia 2010[1], British pharmacopoeia [2] and unitedstate pharmacopoeia[3]. Flupirtine maleate (FLU), chemically, [Ethyl {2-amino-6-[(4-fluorobenzyl) amino] pyridin-3-yl}carbamate] (Figure 1 (b)). It is soluble in Methanol, Ethanol and DMSO [4]. It is indicated for the treatment of chronic and acute pain, for painful increased muscle tone of the posture and motor muscle, primary headache, tumour pain dysmenorrhoea and pain after orthopaedic operation and injuries. [5]

Literature review reveals that various analytical methods like UVSpectrophotometry[6,7,8], HPLC[9,10,11], Human Plasma by HPLC[12], LC-MS[13,14] and other analytical method have been developed for individually and combination with other drug. However no method has been reported for the estimation of PCM and FLU in their combine dosage form. Hence, it was proposed to develop simple, accurate and precise UV-visible Spectrophotometric methods for estimation of PCM and FLU in their marketed formulation.

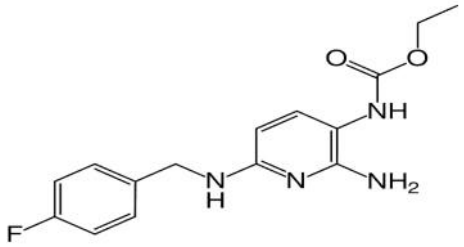

Figure 1 (a) Chemical Structure of Flupirtine maleate

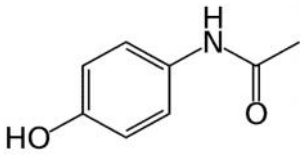

Figure 1(b) Chemical Structure of Paracetamol

MATERIALS AND METHODS

APPARATUS

A Shimadzu model 1800 double beam UV-visible Spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 nm matched quartz cells was used to measure absorbance. The instrument was equipped with Shimadzu UV- Probe 2.33 version software. Shimadzu AUX 220 analytical balance was used for weighing.

REAGENTS AND CHEMICALS

The PCM reference standard was gifted by Alembic Pharma Ltd (Baroda, Gujarat, India) and FLU reference standard was gifted by Lupin Pharma. Ltd.(Baroda, Gujarat, India). The commercial fixed dose formulation containing PCM 325 mg and FLU 100 mg, Lupirtin-P (LupinPharma Ltd) was procured from the local market. Methanol was used as a Solvent for the preparation of Stock and working standard solution.

PREPARATION OF STOCK SOLUTION AND WORKING STANDARD SOLUTION

Accurately weighed 10mg of PCM and FLU were transferred to two different 100 ml volumetric flask. The volume was made up to the mark with Methanol to obtain Stock solution of PCM and FLU having concentration 100µg/ml each. From this solution prepared working range concentration.

METHODOLOGY

METHOD I: Q ABSORBANCE RATIO METHOD

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one, which is an iso absorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that PCM and FLU show an iso absorptive point at 302 nm. The second wavelength used is 248 nm, which is the λ-max of PCM (Figure 2(a). Working standard solutions having concentration 6.5, 13, 19.5, 26 and 32.5 µg/ml for PCM and 2, 4, 6, 8 and 10 µg/ml was prepared in methanol and the absorbances at 302 nm (iso absorptive point) and 248 nm (λ-max of PCM) were measured and absorptivitieswere calculated using Equation. The graph of absorbance v/s concentration was plotted at each wavelength and regression coefficients were calculated (Figure 3 (a) and (b)).

The concentration of two drugs in the mixture can be calculated using following equations.

$CX = [(QM – QY) / (QX - QY)] \times A1/ax1$..... (1)

$CY = [(QM – QX) / (QY - QX)] \times A1/ay1$ …… (2)

Where, A1 and A2 are absorbances of mixture at 302 nm and248 nm; ax1 and ay1 are absorptivities of PCM and FLU at 302 nm; ax2 and ay2 are absorptivities of PCM and FLU

Respectively at 248 nm; QM = A2 / A1, QX = ax2 / ax1 and QY =ay2 / ay1.

MEHOD II: FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD

SELECTION OF ANALYTICAL WAVELENGTHS

From appropriate dilutions of the working standard stock solution, 19.5µg/ml of PCM and 6 µg/ml of FLU were separately prepared and scanned in the UV range 200-400 nm. The overlain zero-order absorption spectra of PCM and FLU were obtained (Figure 2 (b)). These absorption spectra were converted to first-order derivative spectra by using the instrument mode. After observing the overlain first-order derivative spectra, zero crossing points of drugs were selected for the analysis of other drugs. The first wavelength selected was 234 nm (zero crossing point of FLU), where PCM showed considerable absorbance. The second wavelength selected was 334 nm (zero crossing pointof PCM), where FLU showed considerable absorbance.

Standard solutions having concentration 6.5, 13, 19.5, 26 and 32.5 µg/ml for PCM and 2, 4, 6, 8 and 10 µg/ml for FLU was prepared by appropriate dilutions from their respective standard stock solutions. The absorbances of resulting solutions were measured at 234 nm (ZCP of FLU) and 334 nm (ZCP of PCM). The graph of absorbance v/s concentration was plotted at each wavelength and regression coefficients were calculated (Figure 4 (a) and (b)).

VALIDATION OF DEVELOPED METHODS

The proposed methods have been statistically validated in terms of linearity, accuracy, precision, repeatability and reproducibility, limit of detection (LOD) and limit of quantification (LOQ) as per ICH Q2A guidelines (ICH, 1996)[15,16].

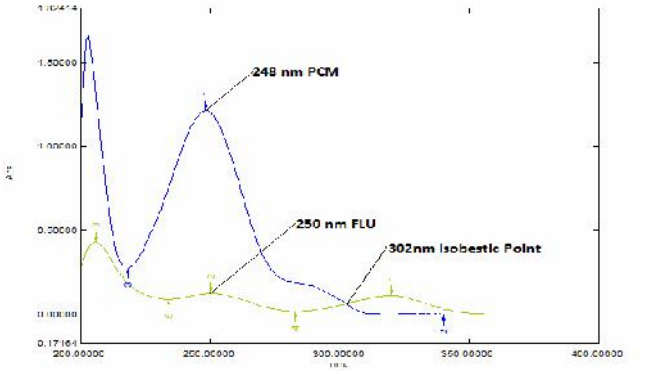

Figure 2 (a) Overlay Spectra of PCM (13 µg/ml) and FLU (4 µg/ml)

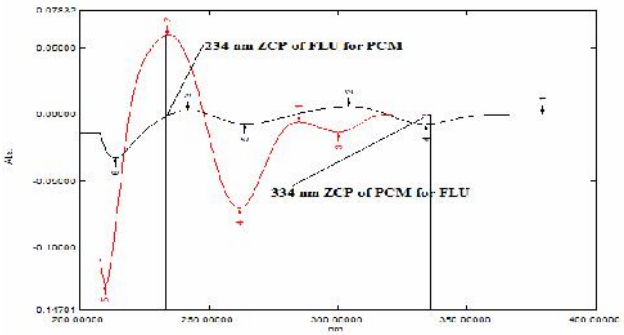

Figure 2 (b) Overlay Spectra of PCM (19.5 µg/ml) and FLU (6 µg/ml)

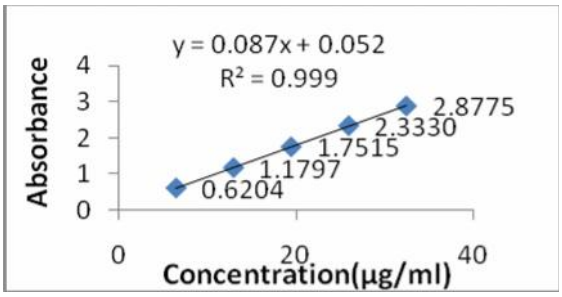

Figure 3 (a) Calibration Curve of PCM at 248 nm (Method I)

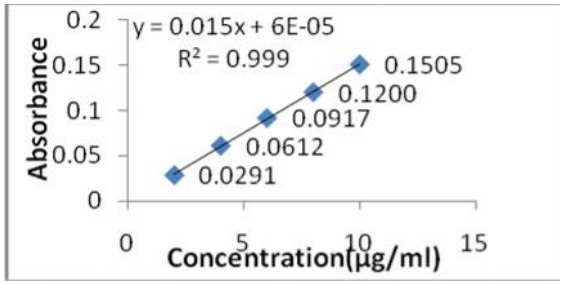

Figure 3 (b) Calibration Curve of FLU at 302 nm (Method I)

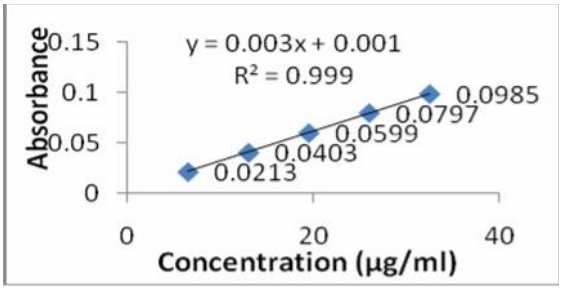

Figure 4 (a) Calibration Curve of PCM at 234 nm (Method II)

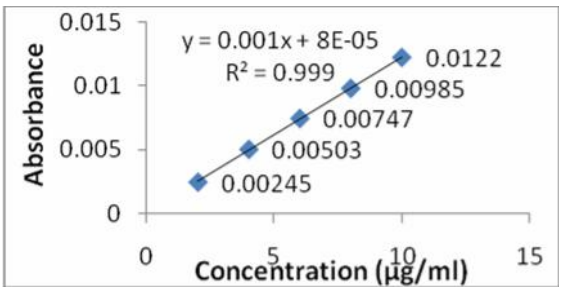

Figure 4 (b) Calibration Curve of FLU at 334 nm (Method II)

LINEARITY

Linearity was observed in the range of 6.5-32.5 µg/ml for PCM and 2-10 µg/ml for FLU respectively for Method I (Table 1) and Method II (Table 2). The r^2value was observed for both the methods was >0.995.

PRECISION

Variations of results within the same day (Intraday) and between days (interday) were analyzed. The Intraday and Interday precision was determined by analyzing three different concentrations of PCM (6.5, 19.5 and 32.5 µg/ml) and FLU (2, 6, and 10 µg/ml), obtained by dilution from stock solutions for three times in a day (Intraday) and for three consecutive days (Interday). The %RSD value of <2% suggests that the developed methods are precise. The results are reported in Table 1 and 2 for method I and method II respectively.

Table No 1: Summary of validation Parameters for Method I

Parameters	PCM	FLU
Wavelength (nm)	248 nm	302 nm
Linearity range (µg/ml)	2-35	1-70
r^2 Value	0.9999	0.9996
Linear regression equation	y=0.0872x+0.0522	y=0.151x+6E-05
Slope	0.0872	0.151
Intercept	0.0522	8E-05
Average Absorptivity	(ax1) = 49.543 (ax2) = 908.59	(ay1) = 150.491 (ay2) = 309.349
Interday Precision(%RSD) (n=3)	1.113	1.397
Intraday Precision (%RSD) (n=3)	0.531	0.475
LOD (µg/ml)	0.551	0.0864
LOQ (µg/ml)	1.672	0.261

Table No 2: Summary of validation Parameters for Method II

Parameters	PCM	FLU
Wavelength (nm)	234 nm	334 nm
Linearity range (µg/ml)	2-35	1-70
r^2 Value	0.9999	0.9998
Linear regression equation	y=0.003x+0.0019	y=0.0012x+8E-05
Slope	0.003	0.0012
Intercept	0.0019	8E-05
Interday Precision(%RSD) (n=3)	0.911	0.804
Intraday Precision (%RSD) (n=3)	0.492	0.541
LOD (µg/ml)	0.558	0.343
LOQ (µg/ml)	1.692	1.039

ACCURACY (RECOVERY)

The accuracy of the developed methods was determined by calculating % recovery at three different levels (50%, 100% and 150%) in pre analyzed samples using standard addition method. The results of recovery studies are reported in Table 3 for the method I and method II respectively. The % recovery for PCM and FLU for Method I and Method II are within 98%-102%, assuring that the both the developed methods can estimate the drugs successfully in presence of excipients.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

LOD and LOQ were determined using mathematical equations.
LOD = 3.3 X σ/S and LOQ = 10 X σ/S
Where, σ = Standard deviation of the response
S = Slope of the calibration curve.
The results are reported in Table 1 for Method I and Table 2 for Method II.

ANALYSIS OF MARKETED FORMULATION

For determination of the content of PCM and FLU in their marketed formulation, tablet powder equivalent to 32.5 mg of PCM and 10 mg FLU was taken in 100 ml volumetric flask. The volume was made up to the mark with Methanol and Sonicatedfor 20 min. The solution thus formed was filtered using Whatman filter paper to remove the suspended particles. Further dilution was made from this solution to get the final working concentration of PCM (13 µg/ml) and FLU (4 µg/ml). For Method, I, the absorbance of the sample solution, i.e. A1 and A2 were recorded at 302 nm and 248 nm respectively and concentration of FLU and PCM were determined using above equation (1) and (2). For method II, the absorbance of resulting solutions was measured at 234 nm (ZCP of FLU) and 334 nm (ZCP of PCM) and concentration of PCM and FLU were determined from the equation of the calibration curve. The result of the analysis of the tablet formulation is shown in Table 4.

Table No 3: Results of Recovery study for Method I and Method II

Level of Accuracy (%)	Sample Conc. (µg/m)	Amt. of Std spiked (µg/ml)	Total Conc. (µg/m)	Average Amount recovered (µg/ml) Method I	% Recovery ± SD* Method I	Average Amount recovered (µg/ml) Method II	% Recovery ± SD* Method II
PCM							
50	4	2	6	19.48	99.909±0.541	19.496	99.982±0.287
100	4	4	8	25.716	98.92±0.507	25.691	98.811±0.694
150	4	6	10	32.813	100.961±0.599	32.446	99.835±0.793
FLU							
50	13	6.5	19.5	5.986	98.735±0.834	5.922	98.703±0.429
100	13	13	26	7.903	100.775±0.629	7.888	98.611±1.321
150	13	19.5	32.5	10.08	100.77±0.533	10.088	100.88±0.625

(n = 3 Determination)

Table No 4: Analysis of Marketed Formulation

Label Claim (n=3)	Conc. of Drug taken	Content estimated (µg/ml) Method I	% Assay ± SD Method I	Content estimated (µg/ml) Method II	% Assay ± SD Method II
325 mg (PCM)	19.5	19.683	100.939 ± 1.025	19.415	99.566 ± 0.936
100 mg (FLU)	6	6.026	100.425 ± 0.913	5.963	99.398 ± 0.930

(n = 3 Determination)

RESULTS AND DISCUSSION

The developed methods, Q Absorbance and first-order derivative method, for estimation of PCM and FLU were found to be accurate, simple and precise. The simplicity and ease of the developed methods lie in using Methanolas solvent. The Q Absorbance method (Method I), generally, used to estimate two absorbing compounds (X and Y), show Isobestic point. Molar Absorptivity of PCM and FLU at 302nm was found 49.543 and 150.491,respectively and 908.598 and 309.349 when measured at 248 nm. The Q Absorbance ratio equations were constructed by placing these values in equation 1 and 2 and were used to determine the concentrations of PCM and FLU by the method I.

$CX = [(QM – QY) / (QX - QY)] \times A1/ax1$ ..... (1)
$CY = [(QM – QX) / (QY - QX)] \times A1/ay1$ …… (2)

Derivative Spectrophotometry method (Method II) is a useful method in the determination of mixtures with two or more component is having been overlapping spectra. The developed methods were validated as per ICH guidelines.

CONCLUSION

The developed Q Absorbance ratio method (Method I) and first derivative method (Method II) were found to be simple, rapid and cost effective. The results of validation confirmed the sensitivity, accuracy and precision of the developed methods. The developed methods could be successfully applied for simultaneous estimation of PCM and FLU from their marketed formulations and for routine quality control of these drugs.

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