

Simultaneous quantification of sumatriptan succinate and prochlorperazine maleate in orodispersible films using two validated UV-spectroscopic methods

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Abstract: The study aimed at simultaneous quantification of sumatriptan succinate (SUM) and prochlorperazine maleate (PCP) in an orodispersible film using two validated spectroscopic methods *viz.* simultaneous equation (Method I) and the Q-absorption ratio (Method II). The Method I involved measurement of absorbances at λ_{\max} of both drugs while in Method II, absorbances were measured at isosbestic wavelength and λ_{\max} of one of the two components. Method validation were accomplished as per the ICH guidelines. A 1:1 mixture of the drugs and an orodispersible film (ODF) containing these drugs were assayed by both methods. The absorbance data of SUM and PCP in both methods were linear at respective wavelengths with correlation coefficient values >0.995. Both methods were precise as % RSD in repeatability, interday and intraday precision was less than 2. The estimation of SUM and PCP from the film dosage form by method I was 104.74% and 98.34% and by method II was 103.45% and 98.85%, respectively, with a standard deviation <2. The study concluded that both the methods were simple, reliable and robust and can be applied successfully for the simultaneous quantification of SUM and PCP in mixture and orodispersible film dosage form.

Keywords: Sumatriptan succinate, prochlorperazine maleate, UV-visible spectroscopy, simultaneous analysis, method validation.

INTRODUCTION

Fixed dose drug combinations (FDCs) are designed to contain more than one active ingredients in a single dosage form, which not only improves the patient compliance but also reduce treatment cost (Gautam *et al.*, 2008). Examples of FDCs include; tablets of metformin and pioglitazone, enalapril and hydrochlorothiazide, sumatriptan and naproxen sodium, amlodipine and simvastatin and metronidazole and diloxanide furoate (El-Ghobashy *et al.*, 2010; Hammouda *et al.*, 2015; Kanwal *et al.*, 2021; Karim *et al.*, 2007). As a result, multi-component analysis has emerged as among the most intriguing subjects in domains such as clinical chemistry, and medication analysis (Kamal *et al.*, 2016). There are several methods for the drugs' analysis in FDCs dosage forms, *i.e.*, electrophoresis, spectrophotometry and chromatography (Prashanth, 2014). However, simultaneous determination of drug through UV spectrophotometric methods is a rapid and cost-effective way of analyzing drug combinations in complex matrices by measuring the absorbance of ultraviolet (200-400 nm) or visible radiation (400-800 nm) of a dissolved substance (Behera *et al.*, 2012). The absorbance of radiations is associated with the excitation of electrons from the lower to higher energy levels in molecule. Only light possessing specific quantity of energy causes transitions from low energy to higher energy level. Thus,

different materials show absorbances at different wavelength known as λ_{\max} (Atole *et al.*, 2018).

In a mixture of drugs one drug may interfere with absorbance of radiations with other drug resulting in change in the λ_{\max} of each other. In order to overcome the problem, different UV spectrophotometric methods include the following: simultaneous equation method (Fernandes *et al.*, 2008), Q-absorbance ratio method (Patel *et al.*, 2014a), difference spectrophotometry (Sheth *et al.*, 2012), derivative spectrophotometry (Benamor *et al.*, 2008), absorbance ratio spectra (Kamal *et al.*, 2016), derivative ratio spectra (Issa *et al.*, 2011), successive ratio-derivative spectra (Afkhami *et al.*, 2005), absorptivity factor method (Samir *et al.*, 2012), dual wavelength method (Gangola *et al.*, 2011), absorption factor method (Prajapati *et al.*, 2011), multivariate chemometric methods (Ayoub, 2016), and isosbestic point method (Lotfy *et al.*, 2014).

Literature review revealed that UV and HPLC methods for sumatriptan succinate (SUM) and prochlorperazine maleate (PCP) have been reported either separately or combined with other active moieties in different dosage forms (Bhagwat, 2013; Pandey *et al.*, 2012; Prashanth, 2014; Solanki, 2011). However, no spectrophotometric method has been cited for the simultaneous determination of SUM and PCP in FDC. Though a SUM-PCP FDC has not been reported to the best of our knowledge, yet the SUM and PCP are the logical clinically indicated free

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combination, mostly prescribed (Loder, 2010; Silberstein, 2018) to prevent migraine and associated nausea. Among all triptans, SUM is more effective in migraine treatment (Jhee *et al.*, 2001; Zaman *et al.*, 2021) while the antiemetic PCP is better among antiemetics because it also acts as antimigraine (Ghelardini *et al.*, 2004) and prevents migraine-linked nausea (Coppola *et al.*, 1995; Jones *et al.*, 1996). Thus, an ODF containing 35mg of SUM and 5mg PCP was developed as fixed dose combination and drugs content in single film were determined. Therefore, the aim of present study was simultaneous quantification of SUM and PCP by employing simultaneous equation and Q absorption ratio methods and to use them for assay of both drugs in binary mixer and orodispersible film dosage form.

MATERIALS AND METHODS

Materials

Sumatriptan succinate (SUM) (Dr. Reddy's Laboratories India) was kindly gifted by Wilshire (Pvt.) Ltd., Lahore Pakistan and prochlorperazine maleate (PCP) was a gift sample from Polyfine ChemPharma (Pvt.) Ltd., Peshawar, Pakistan. Sodium hydroxide, hydrochloric acid and potassium dihydrogen phosphate were purchased from local vendor of Sigma Aldrich Germany. All the materials used were of analytical grade. An orodispersible film (ODF) containing SUM and PCP was prepared in research laboratory of Punjab University College of Pharmacy.

Methods

Solvent selection and preparation of standard stock and working solutions

UV/Vis absorbance of both the drugs was assessed in different solvents i.e., 0.1 N HCl, 0.1 N NaOH, distilled water, methanol, ethanol, and phosphate buffer (pH 6.8) to find out a common solvent for simultaneous determination of SUM and PCP. The spectra of all solutions were superimposed by software (Graph Pad Prism) to check deviation in λ_{\max} of two drugs in different solvents. From the results, the solvent giving maximum absorbance with minimum deviation in λ_{\max} was selected for further studies.

The standard stock solutions (10% w/v) of both SUM and PCP were prepared, separately in phosphate buffer (pH 6.8). For this purpose, 10 mg of both drugs were dissolved in the solvent to make up volume to 100mL, and the resulting solutions were sonicated using Greatsonic Ultrasonic Cleaner (GS-DS 230 China) for 10 min for complete dissolution and filtered using Whatman filter paper. A 5mL aliquot from the standard stock solution was diluted to 50mL using same solvent for obtaining working standard solution (10 μ g/mL).

Determination of λ_{\max} of both drugs and their 1:1 mixtures

The UV spectrum of working standard solutions (10 μ g/mL) and binary mixture of both drugs were measured using UV spectrophotometer from wavelength range of 200-400 nm (2550 Shimadzu, Kyoto, Japan equipped with a pair of 1cm matched quartz cells) against blank (phosphate buffer of pH 6.8). To observe the effect of concentration on λ_{\max} of drugs, different concentrations of SUM, PCP and their binary mixture was also subjected to UV scan.

Determination of isosbestic point

Isosbestic point was determined from overlay spectra of SUM and PCP and was confirmed by measuring UV-spectra of various concentrations of individual drugs and taking their overlay spectra.

Absorptivity values

The absorbance and absorptivity of SUM and PCP were determined using serial dilutions of standard solutions at their respective wavelength maximum using Equation 1 (Giriraj *et al.*, 2014; Sawant *et al.*, 2011).

$$\text{Absorptivity} = \frac{\text{Absorbance}}{\text{Conc. (g/100 mL)}} \quad \text{Equation 1}$$

Method I

In the simultaneous equation method, serial dilutions of SUM and PCP were prepared having concentrations between 1-10 μ g/mL (Series 1 for SUM) and 2-22 μ g/mL (Series 2 for PCP) in phosphate buffer pH 6.8 and absorbance of both Series was measured at two λ_{\max} , λ_1 and λ_2 for SUM and PCP, respectively and plotted calibration curves in Microsoft Excel (Anandakumar *et al.*, 2011). Thus, separate calibration curves were plotted for SUM and PCP relating the absorbances of serial dilutions at two wavelengths. In all the cases, phosphate buffer (pH 6.8) was used as blank.

The concentration of drugs SUM (C_x) and PCP (C_y) in sample solutions/mixture were determined by simultaneous equations (Equation 2 and 3 respectively); based on the absorbances of drugs at λ_1 and λ_2 and absorptivity values at these two wavelengths.

$$C_x = \frac{A_2, ay_1 - A_1, ay_2}{ax_2 ay_1 - ax_1 ay_2} \quad \text{Equation 2}$$

$$C_y = \frac{A_1, ax_2 - A_2, ax_1}{ax_2 ay_1 - ax_1 ay_2} \quad \text{Equation 3}$$

Where, C_x is the concentration (μ g/mL) of SUM and C_y is the concentration (μ g/mL) of PCP. A_1 is the absorbances of sample/mixture solution at λ_1 and A_2 at λ_2 , while ax_1 and ax_2 are absorptivities of SUM at λ_1 and λ_2 respectively, while ay_1 and ay_2 are absorptivities of PCP, respectively at λ_1 and λ_2 .

Method II

Absorption ratio method employs the ratio of absorptions at the two selected wavelengths, one of them is λ_{\max} of one of the two component (λ_1 i.e., λ_{\max} of SUM) and other being the isosbestic point (λ_2) (Singh *et al.*, 2012). Here λ_1 is same as in the simultaneous equation method so calibration curve of both drugs at λ_1 will remain same as for method I.

Dilutions of SUM and PCP were prepared in range of concentrations 1-9 $\mu\text{g/mL}$ for SUM (Series 1) and 2 to 32 $\mu\text{g/mL}$ for PCP (Series 3) in phosphate buffer (pH 6.8) and absorbances of both Series were measured at two λ_{\max} i.e., λ_1 (λ_{\max} of SUM) and λ_2 (isosbestic point) and plotted calibration curves. The concentration of SUM and PCP in the mixture was calculated by using the Equations 4 and 5 (Sawant *et al.*, 2011).

$$C_x = \frac{(Q_m - Q_y)}{(Q_x - Q_y)} \cdot A_1 a x_1 \quad \text{Equation 4}$$

$$C_y = \frac{(Q_m - Q_x)}{(Q_y - Q_x)} \cdot A_1 a y_1 \quad \text{Equation 5}$$

Where, C_x is concentration ($\mu\text{g/mL}$) of SUM and C_y is concentration ($\mu\text{g/mL}$) of PCP. $Q_m = A_2/A_1$, A_1 and A_2 are absorbances of mixture at λ_1 and λ_2 (Isosbestic point) respectively. $Q_x = a x_2/a x_1$, $a x_1$ and $a x_2$ represent absorptivities of SUM at λ_1 and λ_2 respectively. $Q_y = a y_2/a y_1$, $a y_1$ and $a y_2$ represent absorptivities of PCP at λ_1 and λ_2 .

Method validation

The selected UV spectrophotometric methods were validated based on the ICH guidelines, using parameters such as linearity and range, specificity, precision, robustness and accuracy (recovery) and the limit of detection (LOD) and limit of quantification (LOQ) (Engla *et al.*, 2016).

Determination of linearity and range

Linearity was determined for individual drugs (Series 1, 2 and 3) and binary mixer (Series 4 and 5). In the Series 4, 10 $\mu\text{g/mL}$ concentration of PCP was constant while that of the SUM was raised from 1 to 10 $\mu\text{g/mL}$ and dilutions were analyzed at λ_{\max} of SUM. In Series 5, the SUM concentration was constant (10 $\mu\text{g/mL}$) while PCP concentration was raised from 1 to 40 $\mu\text{g/mL}$ and the dilutions were measured at the λ_{\max} of PCP and isosbestic point. In all the cases, used the phosphate buffer solution as blank. The linearity was assessed by regression coefficient (R^2) value.

Determination of limit of detection (LOD) and limit of quantification (LOQ)

The signal to noise ratio for LOD is 3:1 while for LOQ it is 10:1 (Ramakrishna *et al.*, 2016; Shin *et al.*, 2017; Vaidya *et al.*, 2010). The above parameters were computed using the Equations 6 and 7.

$$\text{LOD} = 3 \text{ O/S} \quad \text{Equation 6}$$

$$\text{LOQ} = 10 \text{ O/S} \quad \text{Equation 7}$$

Where O is SD (standard deviation) of y-intercept and S is slope of calibration (Sharma *et al.*, 2014). The LOD and LOQ of SUM and PCP by the proposed methods were determined using calibration standards.

Precision

The methods' precision was determined as repeatability, intra- and inter- day precisions (Majithia *et al.*, 2020). Repeatability of the methods was carried out by analyzing mixture (containing 10 $\mu\text{g/mL}$ of both drugs) for six times. For intermediate precision interday and intraday precision studies were carried at three concentrations (4, 6 and 8 $\mu\text{g/mL}$) containing both SUM and PCP. For intra-day precision, study was performed three times analyzing drug mixture in a single day. For inter-day precision the samples of same concentrations were analyzed for three consecutive days (Gondalia, 2010; Sawale *et al.*, 2016).

Accuracy/Recovery

Standard addition method was used to determine accuracy of the system. Transferred the drug powder, equivalent to 10mg of SUM and PCP to three 100mL volumetric flasks, added 8mg, 10mg and 12 mg of SUM and PCP pure drugs into these Series of 100mL volumetric flasks for 80%, 100% and 120% level of recovery (Shinde *et al.*, 2016). Filtration and dilutions were performed with phosphate buffer (pH 6.8). Solutions were prepared in triplicate and analyzed. The formula for calculation of % recovery is given in Equation 8 (Shetty *et al.*, 2018).

$$\text{Recovery} = \left(\frac{A}{B+C} \right) * 100 \quad \text{Equation 8}$$

Where, A is the amount (total) of drug estimated, B is the quantity of drug found on pre analyzed basis and C is the amount of pure drug added.

Robustness

Robustness was determined by observing changes in UV spectra of mixed SUM and PCP solutions (10 $\mu\text{g/mL}$) in phosphate buffer after addition of small quantities (1mL) of 0.1 N HCL and 0.1 N NaOH (Abbas *et al.*, 2017).

Specificity

To evaluate the specificity and selectivity of method, the λ_{\max} of standard solutions of SUM and PCP were compared to their manufactured formulations. If there is no interference with excipients it indicate the specificity and selectivity of method (Murtaza *et al.*, 2011).

Testing validated method on orodispersible film dosage form

The validated method was employed for testing on two drugs in ODF, the second stage of study. ODF having 35mg of SUM and 5mg of PCP, prepared by solvent casting method was used to for assessing the developed

method. Drug content per film was determined by dissolving a 4cm² film in 50mL phosphate buffer, pH 6.8 and sonicated for 2 min. The filtered solution was diluted by using same solvent to get concentration of 7µg/mL of SUM and 2µg/mL of PCP. Absorbance of the solution was measured at 227 nm, 255nm and at 235.

STATISTICAL ANALYSIS

The mean and standard deviation (SD) was computed using MS Excel Version, 2019.

RESULTS

Selection of suitable solvent

UV/Vis absorbance of 10µg/mL solution of SUM and PCP in different solvents have been given in fig. 1. The highest concentration of SUM was found in methanol followed by phosphate buffer (pH 6.8) and PCP showed maximum absorbance at ethanol followed by methanol and phosphate buffer (table 1). As our final product i.e., ODF was to be determined in phosphate buffer which therefore, was chosen as a common solvent for the determination of both the drugs.

λ_{max} of SUM, PCP and their 1:1mixture

The individual spectra of SUM and PCP (10µg/mL) are presented in fig. 2. SUM showed peaks at 227 nm and at 282nm (fig. 2 A). In the present study the wavelength 227 was selected for the measurement of SUM in all the samples in both methods since it showed maximum absorbance at 227 nm. This is almost similar to the reported λ_{max} of SUM i.e., 227 (Fatima *et al.*, 2015). Spectrophotometric screening of the PCP showed absorbances at wavelengths 255nm and 306nm. In the present study, PCP was measured at wavelength of 255 nm in all the sample in method I. This is similar to the reported λ_{max} of PCP i.e., 255 nm (Shah, 2015) (fig. 2B).

λ_{max} was confirmed by measuring UV-spectra of various concentrations of individual drugs and their mixture. The UV spectra of SUM and PCP at various concentrations have been given in fig. 3 A and B. The absorbances were directly proportional to concentrations. Both drugs showed same λ_{max} at all concentrations. There was no change in position of λ_{max} but intensity of absorbances was increased.

Spectra of binary mixture of different concentrations of SUM and PCP was also recorded (fig. 3C) and showed same peaks as individual drugs but the intensity of peaks was increased. Spectra of binary mixture did not show any kind of interaction or additional peak when the two drugs were combined.

Overlay spectra and determination of isosbestic point

The overlay spectra of drugs (fig. 4 A) showed that λ_{max} of SUM and PCP were separate from each other. SUM

showed negligible absorbance (0.063) at λ_{max} of PCP but PCP showed significant absorbance (0.352) at λ_{max} of SUM. The overlay spectra of individual drugs and their binary mixture (fig. 4B) indicated increased intensity of peak at λ_{max} of SUM in binary mixture (1.140 to 1.428). It was due to presence of PCP so they cannot be quantified exactly in a mixture at their respective λ_{max} and there is a need to develop method for their simultaneous estimation.

The points where spectra of individual drugs intersect each other at overlay spectra are called isosbestic points. Q absorption method uses isosbestic point for the determination of drugs concentration in a mixture of drugs. The overlay spectra of drugs (fig. 4A) showed four isosbestic points as the spectra of SUM and PCP intersect each other at four points i.e., 214, 235, 269 and 298 nm. 235 nm was selected as isosbestic point in our study as it was easy to estimate concentrations of both drugs at this point. Isosbestic points were found to be same as shown in overlay spectra of various different concentrations of drugs (fig. 4C).

Calibration curves of individual drugs

For simultaneous equation method

Minor interference in quantification of individual drugs at their respective λ_{max} was noted on the overlay spectra due to absorption of the other drug at that specific wavelength. Therefore, the concentration of drugs SUM (Cx) and PCP (Cy) in sample solutions/mixture were determined by simultaneous equations (Equation 2 and 3 respectively); based on the absorbances of both drugs at λ₁ and λ₂ and absorptivity values at these two wavelengths. Calibration curve of SUM (Series 1) and PCP (Series 2) at λ₁ (227 nm) and λ₂ (255 nm) (fig. 5) showed an increasing trend in absorbance with rising concentrations. The absorbance data of SUM at both wavelengths was linear over a concentration range of 1 to 9µg/mL with correlation coefficient, respectively of 0.999 and 0.992 (fig. 5 A and B). The absorbance of PCP was also linear over a concentration range, 1 to 20µg/mL with correlation coefficient 0.998 at λ₁ and 0.999 at λ₂ (fig. 5 C and D). Calibration curves of both drugs showed same behavior as manifested by overlay spectra. SUM showed negligible absorbance at λ₂ (fig. 5 B) but PCP showed significant absorbance at λ₁ (fig. 5 D).

For Q-absorption ratio method

In Q-Absorption Ratio Method, Series 1 and 2 were measured at λ₁ and isosbestic point i.e., 235nm. SUM and PCP showed linearity (R² =0.999) over a concentration range, 2-28µg/mL and 2-32µg/mL, respectively at λ₂ (fig. 6).

Absorptivity coefficients

The absorptivity coefficients of SUM and PCP at two wavelengths were calculated.

Table 1: λ_{\max} and Absorbance at 10 μ g/mL solution of SUM and PCP and their 1:1 mixture in different solvents

Solvents	λ_{\max} (nm)(Absorbance)					
	0.1 N HCl	0.1 N NaOH	Ethanol	Methanol	Distilled Water	Phosphate Buffer
SUM	226(1.078)	229(1.006)	228(1.237)	227(1.150)	226(1.115)	227(1.142)
PCP	254(0.516)	253(0.088)	257(0.623)	257(0.595)	255(0.393)	255(0.556)
Mixture	226(1.534)	228(1.137)	226(1.342)	226(1.552)	226 (1.597)	227(1.428)
	254(0.638)	253(0.144)	258(0.614)	257(0.750)	255(0.543)	255(0.472)

Table 2: Absorptivity values of SUM and PCP at 227 nm, 225 nm and 235 nm

Drug	Parameter	Average Absorptivity	
		Method I	Method II
SUM	ax ₁	0.100	0.101
	ax ₂	0.008	0.036
PCP	ay ₁	0.031	0.031
	ay ₂	0.047	0.030

Table 3: Optical characteristics

Parameters	Method I		Method II	
	SUM	PCP	SUM	PCP
λ_{\max} (nm)	227	255	227	235
Beer's law limits (μ g mL ⁻¹)	1-9	2-20	1-9	2-32
Intercept (b)	-0.003	0.010	0.007	-0.015
Slop (m)	0.101	0.048	0.099	0.031
Correlation Coefficient (R ²)	0.999	0.998	0.999	0.999
Standard error of intercept (SE)	0.006	0.009	0.003	0.004
Standard deviation of intercept (SD)	0.018	0.028	0.009	0.017
Limit of detection (LOD) (μ g mL ⁻¹)	0.20	0.62	0.100	0.440
Limit of quantification (LOQ) (μ g mL ⁻¹)	0.60	1.87	0.304	1.332
Sandell's sensitivity (μ g cm ⁻²)	0.0200	0.062	0.010	0.044
Molar Absorptivity (L mol ⁻¹ cm ⁻¹)	30219	17463	29924	10769

Table 4: Repeatability, intraday and interday precision and robustness of methods

Parameters	Method I				Method II			
	SUM		PCP		SUM		PCP	
	% Average Drug recovered \pm SD	%RSD	% Average Drug recovered \pm SD	%RSD	% Average Drug recovered \pm SD	%RSD	% Average Drug recovered \pm SD	%RSD
Repeatability	99.88 \pm 0.13	0.13	99.17 \pm 0.99	1.00	101.03 \pm 0.29	0.29	99.64 \pm 0.96	0.97
Interday	95.77 \pm 1.54	1.61	91.46 \pm 1.84	1.99	102.95 \pm 0.80	0.78	99.26 \pm 1.64	1.65
Intraday	98.38 \pm 1.13	1.15	91.44 \pm 1.56	1.73	103.62 \pm 1.55	1.49	98.40 \pm 1.86	1.90
Robustness	106.93 \pm 0.44	0.47	95.86 \pm 1.65	1.81	105.10 \pm 0.54	0.51	87.18 \pm 0.83	0.97

Table 5: % Recovery of SUM and PCP in both methods

Level of recovery	Method I				Method II			
	SUM		PCP		SUM		PCP	
	% Average Drug recovered \pm SD	%RSD	% Average Drug recovered \pm SD	%RSD	% Average Drug recovered \pm SD	%RSD	% Average Drug recovered \pm SD	%RSD
80%	92.17 \pm 0.19	0.21	90.38 \pm 0.26	0.29	100.05 \pm 0.88	0.88	100.22 \pm 0.83	0.83
100%	99.98 \pm 0.14	0.14	97.72 \pm 0.45	0.46	101.15 \pm 0.73	0.72	99.26 \pm 1.09	1.10
120%	98.36 \pm 0.31	0.32	99.79 \pm 0.33	0.33	99.02 \pm 1.00	1.01	104.93 \pm 1.55	1.48

*Average \pm standard deviation from three determinations

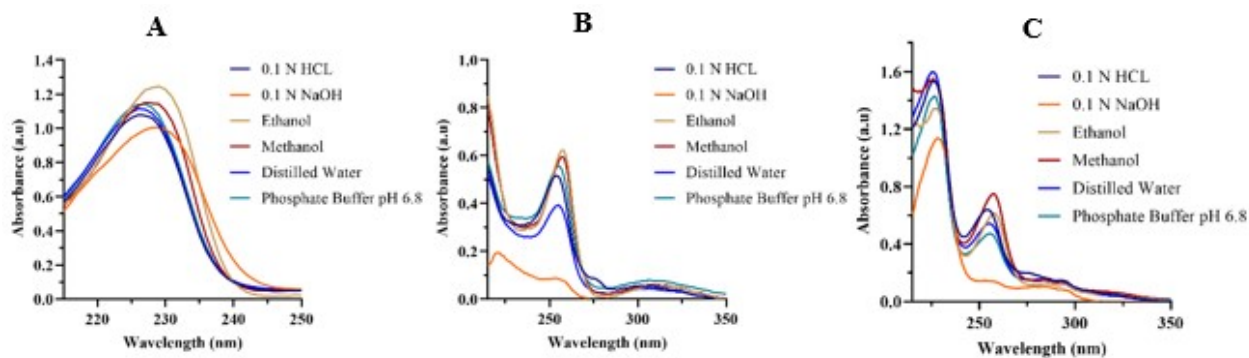


Fig. 1: λ_{max} of drugs SUM (A), PCP (B) and their 1:1 mixture (C) in different solvents

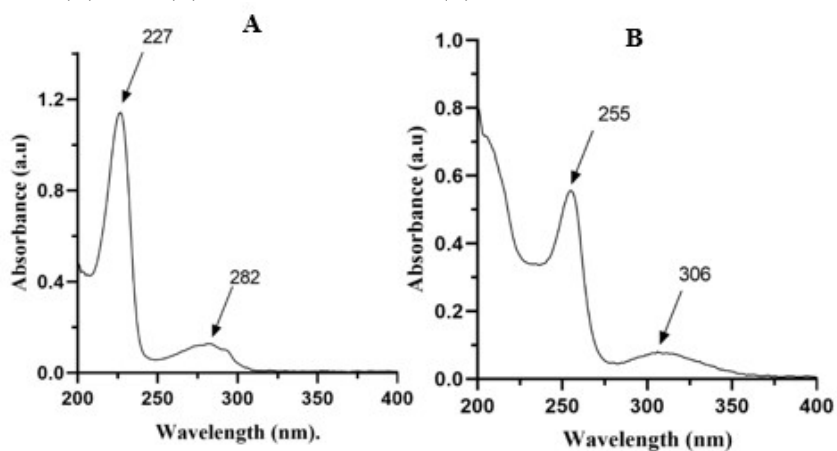


Fig. 2: UV Spectra of (10µg/mL) SUM (A) and PCP (B) showing their respective λ_{max}

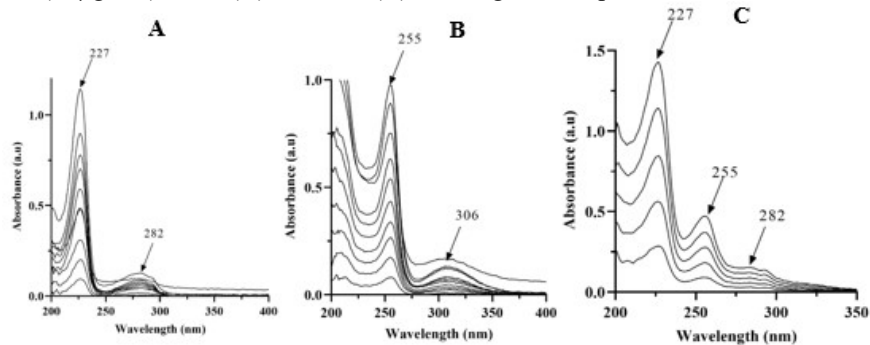


Fig. 3: UV spectra of different concentrations of SUM (1-10µg/ml) (A), PCP (2-22µg/ml) (B) and 1:1 binary mixture of (2-10µg/ml) of SUM and PCP(C)

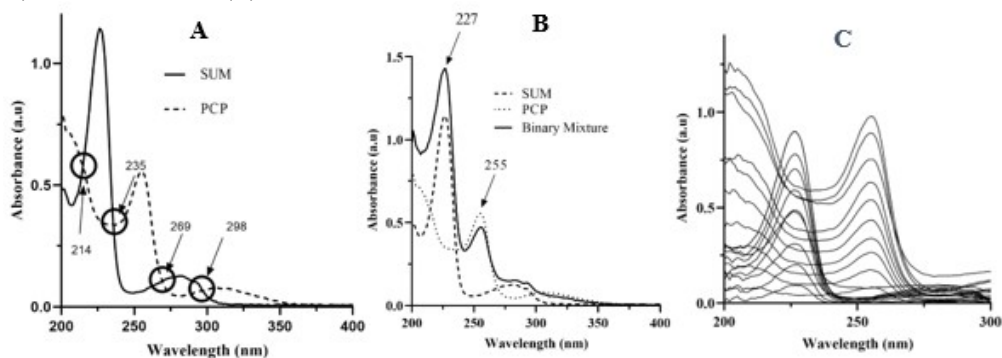


Fig. 4: Overlay spectra of SUM and PCP (A), SUM, PCP, their binary mixture (B) and binary mixture at different concentrations (C)

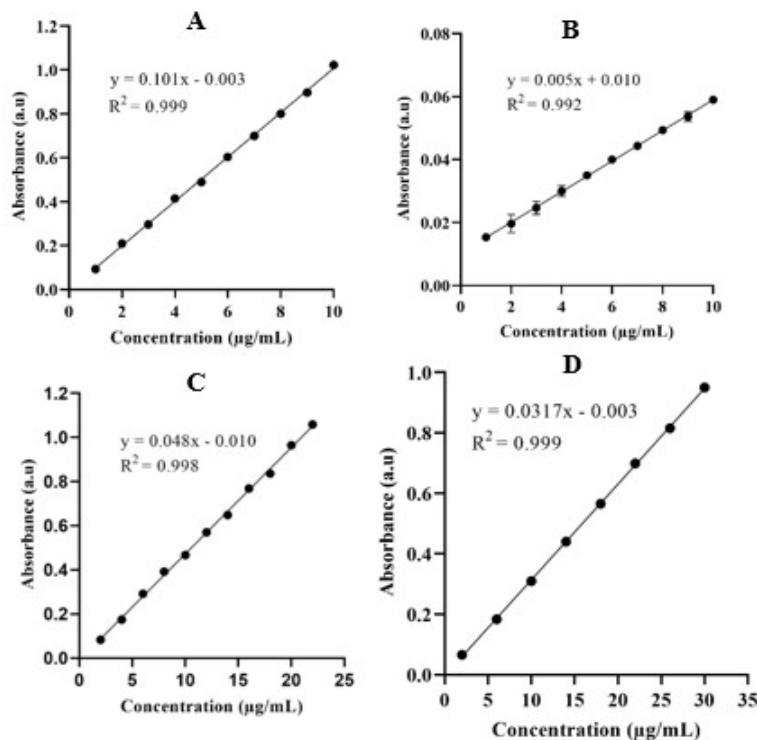


Fig. 5: Calibration curves of SUM at 227 (A), 255(B) and PCP at 255 (C) and 227(D)

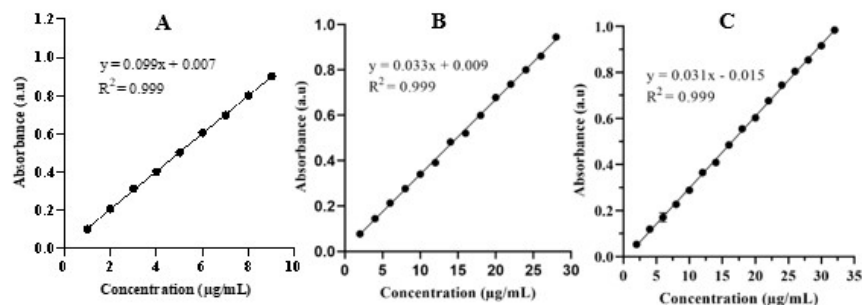


Fig. 6: Calibration curve of SUM at 227 nm (A), 235 nm (B) and PCP at 235 nm (C).

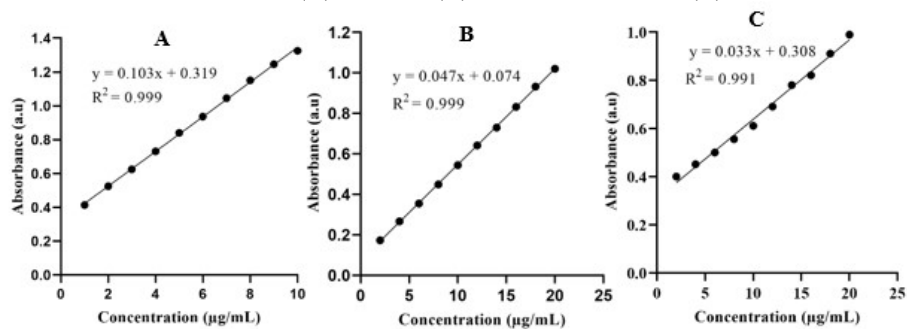


Fig. 7: Calibration curve of SUM at 227 nm in presence of PCP (A), PCP in presence of SUM at 255 (B) and 235 (C)

Table 6: Results from assay of SUM and PCP in the formulation

Drug	%Amount Found (Mean) ±SD	
	SUM	PCP
Label Claim	35 mg/film	5 mg/film
Method I	104.74 ± 0.07	98.34 ± 1.62
Method II	103.45 ± 0.50	98.85 ± 0.54

For simultaneous equation method

The average absorptivity of SUM at λ_1 (ax_1) and λ_2 (ax_2) was 0.100 and 0.008, respectively, whereas absorptivity of PCP at λ_1 (ay_1) and λ_2 (ay_2) was 0.031 and 0.047, respectively (table 2). It showed that SUM didn't show any significant absorbance at the λ_{max} of PCP but PCP exhibited absorbance at λ_{max} of SUM so estimation of SUM at its λ_{max} (227) in the presence of PCP may show more values of absorbance and give false values of SUM concentration. So, this problem can be rectified by determining the concentration of individual drugs in mixture by using simultaneous equation.

For Q-absorption ratio method

The average absorptivity of SUM at λ_1 (ax_1) and λ_2 (ax_2) was 0.101 and 0.0363 respectively, whereas absorptivity of PCP at λ_1 (ay_1) and λ_2 (ay_2) was 0.031 and 0.030 respectively (table 2). It showed that SUM and PCP both exhibited absorbance at λ_{max} of each other so estimation of SUM at its λ_{max} (227) in the presence of PCP and estimation of PCP at isosbestic point (235) in the presence of SUM may give false values of concentration. So, this problem can be rectified by determining the concentration of individual drugs in mixture by using Q absorption ratio method.

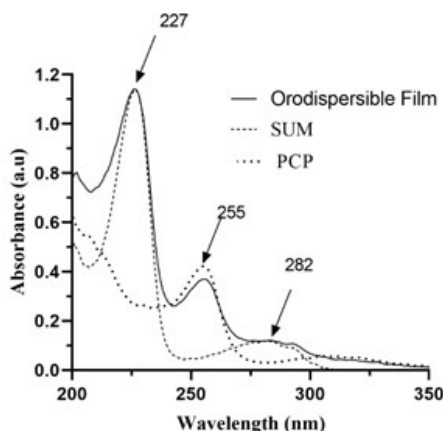


Fig. 8: The λ_{max} of standard solutions of SUM and PCP compared with ODF showing no interaction with excipients.

Validation of spectroscopic methods**Linearity**

Linearity of drugs was determined for individual drugs as well as binary mixture. As both the drugs were used in combination for the formulation of fast dissolving films so there was a need to construct calibration curves of drug's mixture at both wavelengths for each method to check whether there is linearity in absorbance versus concentration graphs of binary solutions. In the mixture the absorbance of SUM was observed for each concentration at its λ_{max} in presence of PCP and absorbance of PCP was observed at its λ_{max} and isosbestic point in presence of SUM.

SUM exhibited a linear relationship in the concentration range between 1 to 9 $\mu\text{g/ml}$ in the both methods while PCP showed linearity in concentration range between 2 to 20 $\mu\text{g/ml}$ by simultaneous equation method and 2-32 $\mu\text{g/ml}$ in method of Q-absorption ratio. The calibration curves of individual drugs are shown in figs. 5 and 6. The UV absorption of SUM measured at λ_1 with rising concentrations, up to 10 $\mu\text{g/ml}$ in combination with PCP (10 $\mu\text{g/ml}$) demonstrated a linear relation (fig. 7 A). The UV absorbance of PCP, at 225 and 235 with elevating concentrations, up to 20 $\mu\text{g/ml}$ in combination with SUM (10 $\mu\text{g/ml}$) were linearly correlated also (fig. 7 B and C). The above observations confirmed that the absorbance profiles for SUM or PCP (either alone or in combination) in both methods followed the Beer Lambert law.

The optical attributes such as intercept, slope, LOD, LOQ, R^2 , molar absorptivity and Sandell's sensitivity for both the drugs for both methods were calculated and are shown in table 3.

Limits of detection and quantification

LOD refers as lowest possible detectable amount of the drug while LOQ is the least possible quantifiable amount of the drugs which were determined by signal to noise ratio methods. For method I the values of LOD and LOQ for SUM were computed, respectively as 0.20 $\mu\text{g/ml}$ and 0.60 $\mu\text{g/ml}$. PCP's LOD and LOQ were 0.62 and 1.87 $\mu\text{g/ml}$. For method II LOD and LOQ for SUM, respectively were 0.100 $\mu\text{g/ml}$ and 0.304 $\mu\text{g/ml}$ and the LOD and LOQ for PCP were respectively 0.440 and 1.3 $\mu\text{g/ml}$ (table 3).

Precision

Precision is the close agreement of different values to each other. The RSD (%) for repeatability, inter day and intraday analysis were observed to be <2%. The results were presented in table 4. The low RSD values are suggested that the amounts found were in good agreement with the actual amounts.

Accuracy/Recovery

As per ICH guidelines accuracy, in terms of recovery studies is calculated by spiking the known concentration of the drugs at 80%, 100% and 120% in pre analyzed sample solutions (Nalluri *et al.*, 2012). Three values for absorption were noted for each concentration to easily calculate mean, SD and RSD. The recoveries (%) for all the solutions were in the range of 90-104%, as demonstrated in table 5.

Robustness

Robustness, a capability of the analytical method to remain unaffected by minor alterations in the method parameters, was investigated by observing modification in λ_{max} in response to small fluctuations in temperature and pH. The above findings demonstrated that this approach

was appropriately robust in terms of change in pH (table 4).

Specificity

Efficiency of the method is usually assessed by its specificity. The λ_{\max} of standard solutions of SUM and PCP were compared to their manufactured formulations. There was no interference with excipients it indicated the specificity and selectivity of method (Figure 8). UV scan of individual film showed there was no interaction with excipients.

Assay results for SUM-PCP Film

The developed methods were applied to ODF having 35 mg of SUM and 5 mg of PCP. The % assay of SUM and PCP was 104.74% and 98.34% respectively, in method I and 103.45% and 98.85% respectively, in method II.

DISCUSSION

UV methods are frequently chosen in testing the quality control of dosage forms when faster, more reliable, and easier methods are required for analysis (Atole *et al.*, 2018). Majority of researchers used simultaneous equation and Q absorption ratio methods for the simultaneous determination of two drugs in a pharmaceutical dosage form and declared them easy, precise and economic (Bhaskar *et al.*, 2020; Sawant *et al.*, 2011). In this study simultaneous equation (Method I) and Q absorption ratio (Method II) were used for the simultaneous determination of SUM and PCP in a physical mixture and formulated ODFs containing both SUM and PCP (Majithia *et al.*, 2020; Shetty *et al.*, 2018). Both methods were found to be precise, accurate, and robust. Phosphate buffer was employed as the solvent since this was a good solvent for SUM and PCP. The λ_{\max} of SUM and PCP were observed to be 227 nm and 255 nm respectively, and isosbestic wavelength was 235 nm. The λ_{\max} were selected on the basis of maximum absorbance of individual drugs. The calibration curves were linear for SUM in the range 1 to 9 $\mu\text{g/mL}$ for both methods while PCP showed linearity in the range, 2 to 20 $\mu\text{g/mL}$ in method I and 2-32 $\mu\text{g/mL}$ in method II. In case of binary solutions, slope and intercept increased from 0.101 to 0.103 and -0.003 to 0.319, respectively for SUM. The increments in the absorbance values are attributed to the presence of PCP. The UV absorbance of PCP in binary solution differs slightly from that of single solution only with respect to the intercept that was raised from 0.010 to 0.074 in method I and 0.015 to 0.308 in method II. The calibration curves of both drugs in the presence of other drug were found to be linear. For the purpose of methods validation, LOD and LOQ were determined (Pandey *et al.*, 2012). Both methods were found to be sensitive for SUM and PCP. Method I may determine a minimum concentration, 0.60 $\mu\text{g/mL}$ for SUM, and 1.87 $\mu\text{g/mL}$ for PCP. Method II can quantify

0.303 $\mu\text{g/mL}$ of SUM and 1.3 $\mu\text{g/mL}$ of PCP. The reported LOD and LOQ of SUM alone (non FDC formulation) by UV method are 0.31 $\mu\text{g/mL}$ and 0.94 $\mu\text{g/mL}$, respectively (Solanki, 2011) and 0.37 $\mu\text{g/mL}$ and 1.14 $\mu\text{g/mL}$ respectively for PCP alone (Patel *et al.*, 2014b). The reported LOD and LOQ values for SUM by HPLC are 0.065 $\mu\text{g/mL}$ and 0.22 $\mu\text{g/mL}$ respectively (Pandey *et al.*, 2012) and 0.025 $\mu\text{g/mL}$ and 0.412 $\mu\text{g/mL}$ respectively for PCP (Lew *et al.*, 2011). Though HPLC being sensitive method can determine low quantities yet in this study, the LOD and LOQ of both methods are in the range to estimate drugs concentrations in the dosage forms and final diluents during dosage form development. The repeatability and the intra- and inter-day precisions of the current analytical methods were examined. All of these studies yielded RSD values that were within the range, with a highest RSD of 1.99 %. Accuracy studies were performed at three levels (i.e., 4, 5, 6 $\mu\text{g/mL}$) for both drugs (Sawale *et al.*, 2016). The accuracy, as demonstrated by the percentage recovery for SUM and PCP were within 90 to 104%. The response of the developed methods to minor variations in temperature and pH was used to describe the method's robustness (Abbas *et al.*, 2017). Our findings suggested that minor changes in pH did not change the amounts of drugs recovered. The currently developed methods for simultaneous measurement of SUM and PCP were assessed for its validity by assaying the drugs' physical mixture and also ODFs and no interference due to excipients of the film formulation were observed. The results showed that both methods were successfully employed on these samples and gave almost similar results.

CONCLUSION

Simultaneous equation and Q-absorption ratio methods were observed to be simple, sensitive, accurate and precise for the quantification of SUM and PCP in film dosage form without any interference. The results demonstrated that both the methods could be employed conveniently for the routine quality control testing of SUM and PCP as the analysis is more economical than the RP-HPLC method.

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