Nirma Univ J Pharm Sci; 2020, 7(1) 49-62

ISSN 2348 –4012

RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF ASPIRIN AND 5-FLUOROURACIL IN BULK AND DOSAGE FORM

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ABSTRACT

A simple, sensitive, selective, economical and reproducible Simultaneous estimation method by UV-Visible spectrophotometry method has been developed for the determination of Aspirin (ASP) and 5-Fluorouracil (5-FU) in bulk form. The methods are based on measurement of absorbance of ASP and 5-FU in 0.1N HCl at 234 nm and 266 nm respectively. Beer's law is obeyed over the linear range 12 – 32 µg/mL and 3.33 – 8.89 µg/mL for ASP and 5-FU respectively. The r^2 was 0.9971 and 0.9944 for ASP at 234 nm and 266 nm respectively. While 0.9988 and 0.9975 was found for 5-FU at 266 nm and 234 nm. Limits of Detection (LOD) were 0.16 µg/mL and 0.04 µg/mL for ASP and 5-FU respectively. Limit of Quantification (LOQ) were 0.48 µg/mL for ASP and 0.13 µg/mL for 5-FU. The method was validated in accordance with the current ICH guidelines. The precision results, expressed by intra-day and inter-day for ASP and 5-FU at 234 nm and 266 nm were within the limits (% RSD < 2 %). The accuracy was also satisfactory and % recovery was 98.00 – 102.38 % for ASP and 98.41 – 100.81 % for 5-FU. The assay of dosage form was 99.57 % and 98.97 % for ASP and 5-FU respectively.

Keywords: UV-Visible spectrophotometry, Aspirin, 5-Fluorouracil, Simultaneous Estimation, validation

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1. Introduction

Aspirin [2-(acetyloxy) benzoic acid] (Fig 1), acts as an inhibitor of cyclooxygenase which results in the inhibition of the biosynthesis of prostaglandins. It also inhibits platelet aggregation and is used in the prevention of arterial and venous thrombosis [1]. 5-Fluorouracil (Fig 2) is a pyrimidine analogue that irreversibly inhibits thymidylate syntheses. Blocking the synthesis of thymidylate which is required for DNA synthesis. Intracellular metabolites of 5-Fluorouracil exert cytotoxic effects by either inhibiting thymidylate synthesis or through incorporation into RNA & DNA, ultimately initiating apoptosis [2]. 5-Fluorouracil has been widely used to treat many gastrointestinal tract adenocarcinomas. Tegafur [4-Fluoro-1-(2-tetrahydrofuryl)-2,4(1H,3H)-pyrimidinedione] is a prodrug of 5-Fluorouracil (5-FU) and is converted into 5-FU by cytochrome P450 enzymes [3]. The use of tegafur in cancer treatment is due to its lower toxicity than 5-FU [4].

Various biochemicals, clinical and epidemiological studies have shown that aspirin (ASA) and other nonsteroidal anti-inflammatory drugs (NSAIDs) demonstrate antineoplastic properties, particularly in the gastrointestinal tract, inhibiting the proliferation of colorectal cancer cells. The mechanism of activity might be prostaglandin interceded through restraint of the COX enzymatic framework. This incorporates two iso-proteins, COX-I and COX-II, working together with the enactment of apoptosis, actuation of resistant observation, hindrance of expansion, and restraint of cancer-causing agent initiation. 5-Fluorouracil (5-FU) has demonstrated activity against colorectal cancer, leading to apoptosis of neoplastic cells, and ASA in combination with 5-FU, in colorectal cancer as evidenced by its effect on the HT-29 cell line [5].

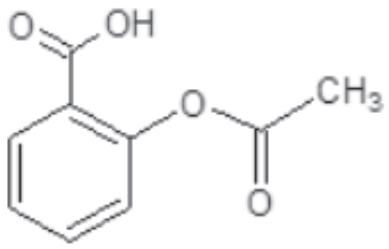

Fig 1: Aspirin [2-(acetyloxy) benzoic acid]

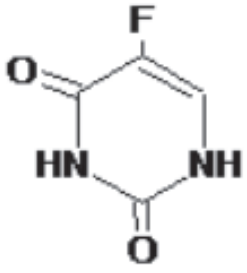

Fig 2: 5-Fluorouracil

Pharmaceutical analytical procedures are used for identification and quantitative analysis of the active moiety in the sample of drug substance or products. The development of assay procedures for multicomponent dosage forms poses considerable challenge.

Literature review reveals that till date individual methods for analysis of ASP[6-8]and 5-FU[9-11]were reported but not analytical methods have been reported for simultaneous estimation of ASP and 5-FU from bulk form. Therefore, it was endeavoured to develop accurate, precise and sensitive UV-Visible spectroscopic method for estimation of ASP and 5-FU from their bulk form.If a sample contains two absorbing drugs each of which absorbs at the $\lambda_{max}$of the other, it may be possible to determine both drugs by the technique of simultaneous equations provided that certain criteria apply.

2. Materials and Methods

2.1 Instrumentation

UV-Visible spectrophotometer with model: UV-1800 equipped with UV probe 3.24 software; manufactured by Shimadzu Inc, Japan and Analytical balance with model ML204 which is manufactured by METLER TOLEDO were used.

2.2 Apparatus

Volumetric flasks (10mL and 100mL); pipettes (1mL and 10mL) and beakers (100mL, 250mL and 500mL) of borosilicate class A were used.

2.3Methodology

2.3.1 Selection of solvent for ASP and 5-FU

As per the solubility test, 0.1N HCl was ideal solvent for spectrophotometric analysis of aspirin and 5-FU. 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32 µg/mL solutions of aspirin and 3.33, 3.89, 4.44, 5.00, 5.55, 6.11, 6.67, 7.22, 7.78, 8.33. 8.89 µg/mL solutions of 5-FU were prepared in 0.1N HCl and spectrums were recorded between 200-400 nm.

2.3.2 Preparation of standard stock solution

2.3.2.1 ASP standard stock solution (100 µg/mL)

10 mg of aspirin standard was weighed and transferred to 100mL volumetric flask. The flask was shaken and volume was made up to the mark with 0.1N HCl to give a solution containing 100 µg/mL aspirin.

2.3.2.2 5-FU standard stock solution (100 µg/mL)

10 mg of 5-FU was weighed and transferred to 100 mL volumetric flask. The flask was shaken and volume was made up to the mark with 0.1N HCl to give a solution containing 100 µg/ml 5-FU.

2.3.3 Selection of analytical wavelength for aspirin and 5-FU

For aspirin 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32 µg/mL solutions were prepared in diluents and spectrum were recorded

between 200-400 nm. Similarly, 3.33, 3.89, 4.44, 5.00, 5.55, 6.11, 6.67, 7.22, 7.78, 8.33. 8.89 µg/mL solutions of 5-FU were prepared in diluents and spectrum were recorded between 200-400 nm. The wavelength that at which maximum absorbance was obtained was considered as λ_{max} of the drug.

2.3.4 Establishment of calibration curve

A calibration curve was plotted over a concentration range of 12-32 µg/mL for aspirin and 3.33-8.89 µg/mL for 5-FU, individually. Accurately measured working stock solution of aspirin and 5-FU were transferred to 10mL volumetric flasks and diluted to the mark with 0.1N HCl. Absorbance of each solution was measured at selected wavelength 234 and 266nm. Calibration curves were constructed for aspirin and 5-FU by plotting absorbance versus concentration and regression coefficient was reported.

2.3.5 Preparation of test sample solution

For the estimation of drugs in the developed formulations, 20 capsules containing 180mg aspirin and 50 mg 5-FU pellets were weighed and average weight was calculated. The pallets were crushed and powdered in glass mortar. For the analysis of drugs, a standard addition method was used. Drugs were diluted with 0.1N HCl and final concentration was 14µg/mL for ASP and 3.89 µg/mL for 5-FU.

2.4 Simultaneous Equation Method

For estimation of aspirin and 5-FU using spectrophotometry, simultaneous equation methods were used. In this method two wavelength are required. One wavelength is selected at which aspirin shows maximum absorbance, while second wavelength is selected at which 5-FU shows maximum absorbance. The absorptivity of both the drugs determined, at selected wavelengths, i.e. 234nm and 266nm.

From the above data of absorptivity, the generated equations for both the drugs are as under simultaneous equation

$$A1 = ax1\ cx + ay1\ cy \quad (1)$$

$$A2 = ax2\ cx + ay2\ cy \quad (2)$$

Where,

ax1 and ax2 are absorptivity of aspirin at 234 and 266 nm respectively

ay1 and ay2 are absorptivity of 5-FU at 234 and 266 nm respectively

A1 and A2 are absorbance of diluted test sample at 234 and 266 nm respectively

Using cramer's rule and matrices, the equation (1) and (2) can be written as,

$$Cx = (A2\ ay1 - A1\ ay2)/ (ax2\ ay1 - ax1\ ay2)$$

$$Cy = (A1\ ax2 - A2\ ax1)/ (ax2\ ay1 - ax1\ ay2)$$

By putting the values of absorptivity

Where, 1) A1 and A2 are absorbance of sample at 234nm and 266nm respectively

2) Cx is concentration of aspirin in µg/mL

3) Cy is concentration of 5-FU in µg/Ml

2.5 Optimized Spectrophotometric Conditions

Solvent – 0.1N HCl

λ_{max}= 234 nm for ASP and 266 nm for 5-FU

Scanning speed - Fast

2.6 Preparation of Solutions for Method Validation

2.6.1 Linearity

Calibration curve for ASP and 5-FU: Appropriate aliquots from ASP (1.2 – 3.2 mL) and 5-FU (0.33 – 0.88 mL) standard stock solution were transferred to different volumetric flasks of 10 mL capacity separately. The volume was made up to the mark with 0.1N HCl to obtain concentration of 12-32µg/mL of ASP and 3.33-8.89 µg/mL of 5-FU. The solutions were analysed at 234nm and 266nm and calibration curve was constructed by plotting average absorbance Vs. Conc. and regression equation was computed. The mean absorptivity values were also calculated for ASP and 5-FU.

2.6.2 Precision

2.6.2.1 Intraday Precision

Lower, middle and higher concentrations of calibration curve were selected for intraday precision for ASP and 5-FU. Appropriate aliquots of ASP (1.4, 2.2 and 3.2 mL) and 5-FU (0.38, 0.61 and 0.88 mL) were transferred to different volumetric flasks of 10 mL individually. The volume was made up to the mark with 0.1N HCl to obtain concentration of 14, 22 and 32 µg/mL of ASP and 3.89, 6.11 and 8.89 µg/mL of 5-FU respectively. The solutions were analysed at 234 nm and 266 nm for three times in a same day and absorbances were recorded.

2.6.2.2 Interday Precision

Lower, middle and higher concentrations of calibration curve were selected for inter day precision for ASP and 5-FU. Appropriate aliquots of ASP (1.4, 2.2 and 3.2 mL) and 5-FU (0.38, 0.61 and 0.88 mL) were transferred to different volumetric flasks of 10 mL individually. The volume was made up to the mark with 0.1N HCl to obtain concentration of 14, 22 and 32 µg/mL of ASP and 3.89, 6.11 and 8.89 µg/mL of 5-FU respectively. The solutions were analysed at 234 nm and 266 nm for three times on three consecutive day and absorbances were recorded.

2.6.3 Repeatability

Aliquots of 2.2 mL of ASP and 0.61 mL of 5-FU were pipette out and transferred to volumetric flasks of 10 mL individually. The volume was made up to the mark with 0.1N HCl to obtain concentration of 22 µg/mL of ASP and 6.11 µg/mL of 5-FU respectively. Repeatability was established by performing the experiment for six time

consecutively and absorbance were recorded.

2.6.4 Accuracy

The validity and reliability of proposed method was assessed by recovery study using standard addition method. To check the accuracy of proposed method recovery study were carried out from pre analysed samples at 3 different levels of standard addition (80%, 100% and 120% of label claim).

Solution A: tablet equivalent to 14 mg was taken and dissolved in 10 mL of 0.1N HCl. The solution was filtered through whatman filter paper no. 41. The final solution having concentration 14µg/mL of QTF was analysed.

For 80% level of recovery, 1 mL of standard solution having concentration of 11.2 µg/mL and 1 mL of solution A were pipette in 10 mL volumetric flask. The volume was made up to the mark with methanol to obtain final concentration of 25.2 µg/mL.

For 100% level of recovery, 1 mL of standard solution having concentration of 14 µg/mL and 1 mL of solution A were pipette in 10 mL volumetric flask. The volume was made up to the mark with methanol to obtain final concentration of 28 µg/mL.

For 120% level of recovery, 1 mL of standard solution having concentration of 16.8 µg/mL and 1 mL of solution A were pipette in 10 mL volumetric flask. The volume was made up to the mark with methanol to obtain final concentration of 30.8 µg/mL. Solutions were analysed for three times and absorbances were recorded.

2.6.5 Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. Calibration curve was repeated six times and the standard deviation of the intercepts and mean of slopes were calculated. Then LOD was measured by using mathematical expression.

$LOD = 3.3\ \sigma/S$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

2.6.6 Limit of Quantification (LOQ)

The quantification limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Calibration curve was repeated six times and the standard deviations of the intercepts and means of slopes were calculated. Then LOD was measured by using mathematical expression.

$LOQ = 10\ \sigma/S$

Where, σ = the standard deviation of the

response

S = the slope of the calibration curve

2.6.7 Robustness

The robustness of an analytical procedure is a measurement of its capacity to remain unaffected by small, but deliberate variations in method parameters. Robustness of the developed method was tested by varying detection wavelength (±2 nm) of optimized conditions.

2.7 Assay of Developed Formulation Containing ASP and 5-FU

A quantity of equivalent to 14 mg of was taken and dissolved in 100 mL of 0.1N HCl. The solution was filtered through whatman filter paper no. 41. From this mL was pipette out in 10 mL volumetric flask and volume was made up to mark to obtain 14 µg/mL of ASP and 3.89 µg/mL of 5-FU. The final solution having concentration 14 µg/mL of ASP and 3.89 µg/mL of 5-FU was analysed.

3. Results and Discussions

3.1 Method Validation

Linearity

Linearity was found between 12 to 32 µg/mL for ASP as shown in fig 3 and 3.33 to 8.89 µg/mL 5-FU as mentioned in fig 4 respectively. Linear regression data for the calibration curves (n=6) shows a good linear relationship over the concentration range of 12 to 32 µg/mL (Fig 5 and 6) and 3.33 to 8.89 µg/mL 5-FU (Fig 7 and 8)

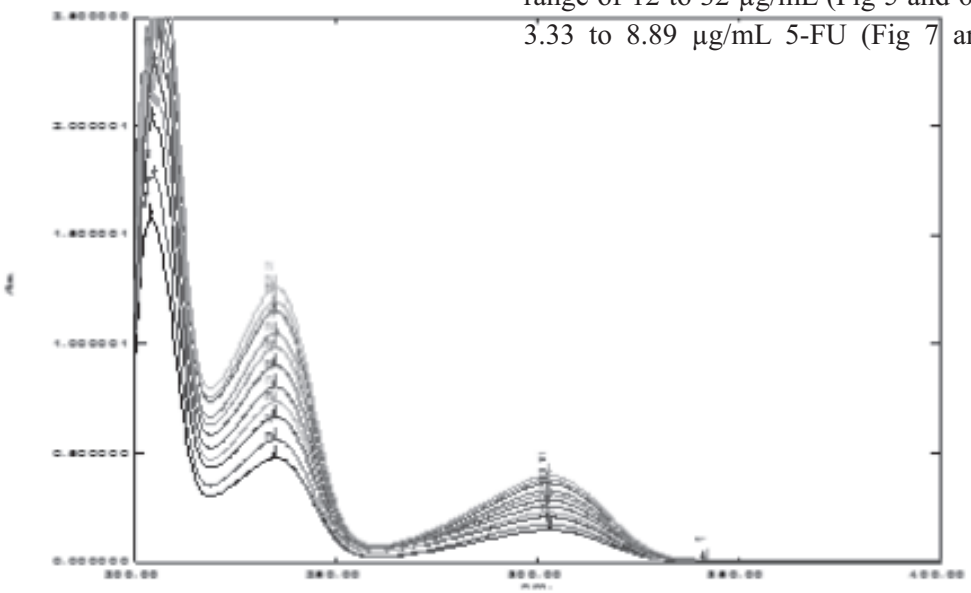

Figure 3: Overlay spectra of ASP (12 to 32µg/mL) in 0.1N HCl at 234 nm

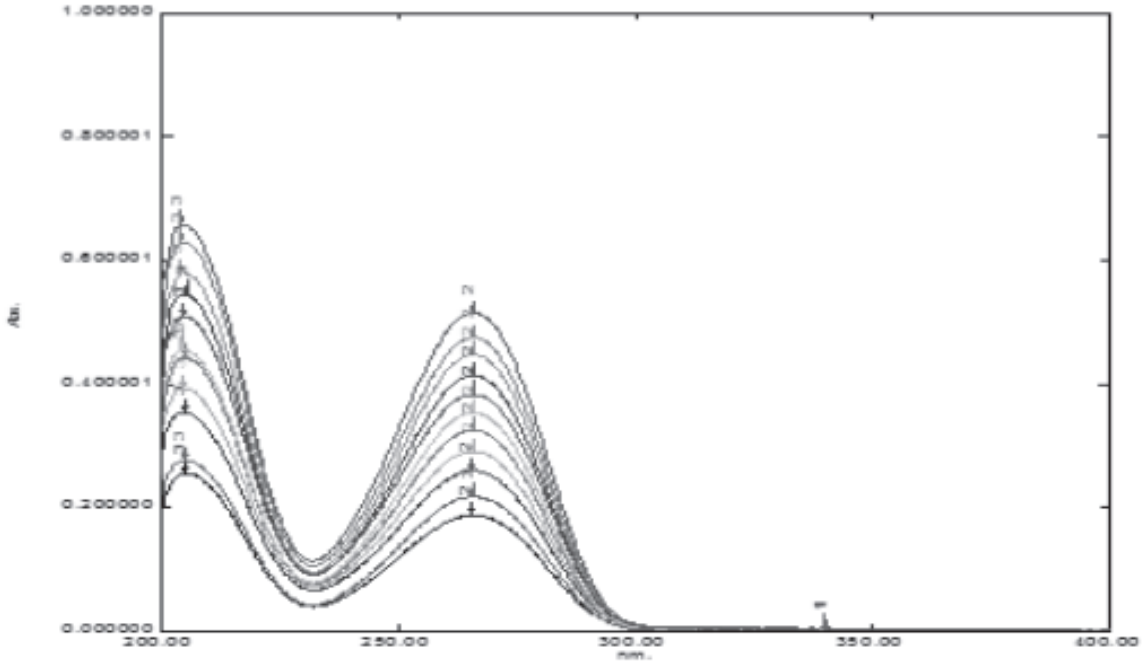

Figure 4: Overlay spectra of 5-FU (3.33 to 8.89µg/mL) in 0.1N HCl at 266 nm

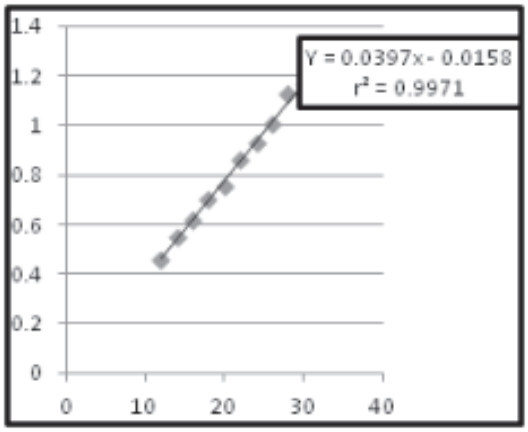

Fig 5: Calibration curve for ASP at 234nm

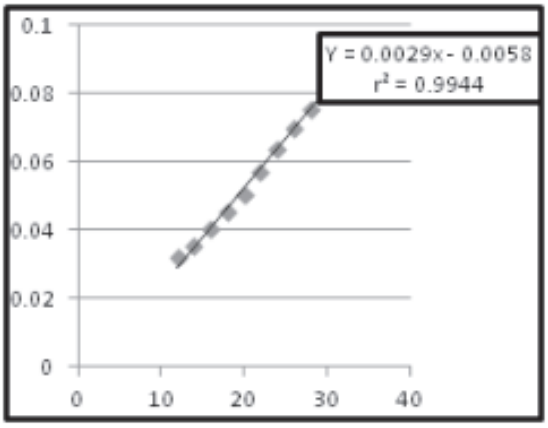

Fig 6: Calibration curve for ASP at 266nm

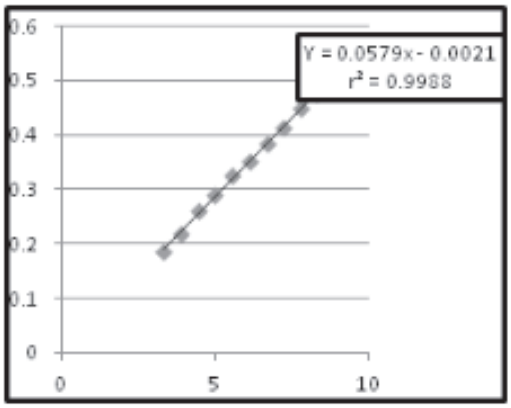

Fig 7: Calibration curve for 5-FU at 266nm

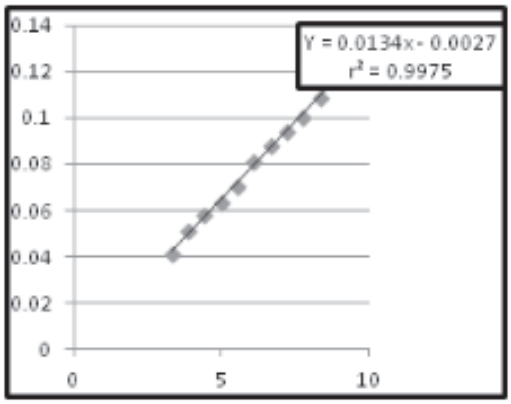

Fig 8: Calibration curve for 5-FU at 234 nm

respectively.

Precision

Intraday Precision

Intraday precision was performed and %RSD values were within the acceptance criteria.

Interday Precision

Interday precision was performed and %RSD values were well within the acceptance criteria.

Repeatability

It was performed and %RSD values were found to be less than 2%.

Accuracy

Accuracy was performed and % Recovery was found to be within 98-102% at all

Table 1: Accuracy (% Recovery) data for ASP

Level of recovery	Sample Conc. (µg/mL)	Conc. Of Std added (µg/mL)	Total Conc. (µg/mL)	Abs	Amt Recovered (µg/mL)	% Recovery	Mean % Recovery ± SD	%RSD
80%	14	11.2	25.2	0.9755	24.89	98.76	98.85 ± 0.0850	0.09
				0.9768	24.93	98.93		
				0.9758	24.91	98.85		
100%	14	14	28	1.1242	28.68	102.46	102.38 ± 0.0721	0.07
				1.1225	28.65	102.32		
				1.1230	28.66	102.36		
120%	14	16.8	30.8	1.1837	30.20	98.05	98.0067 ± 0.0513	0.05
				1.1822	30.17	97.95		
				1.1829	30.19	98.02		

Table 2: Accuracy (% Recovery) data for 5-FU

Level of recovery	Sample Conc. (µg/mL)	Conc. Of Std added (µg/mL)	Total Conc. (µg/mL)	Abs	Amt Recovered (µg/mL)	% Recovery	Mean % Recovery ± SD	%RSD
80%	3.89	3.11	7.00	0.4048	7.03	100.41	100.4667 ± 0.2205	0.22
				0.4055	7.05	100.71		
				0.4040	7.02	100.28		
100%	3.89	3.89	7.78	0.4320	7.73	99.36	98.4167 ± 0.8558	0.87
				0.4217	7.64	98.20		
				0.4210	7.60	97.69		
120%	3.89	4.67	8.56	0.4981	8.65	101.05	100.8167 ± 0.3062	0.30
				0.4979	8.64	100.93		
				0.4963	8.60	100.07		

three levels. This indicates that the ASP and 5-FU can be recovered successfully in presence of excepients. (As shown in table 1 and 2)

LOD and LOQ

LOD and LOQ values were calculated using the mathematical equation.

Robustness

Robustness of development method was checked by varying the λ_{max}. The results show that % RSD values are less than 2% at varied maxima indicating that developed method is robust in terms of change in λ_{max} by ±2 nm.

Analysis of Developed Formulation

Table 3: Assay data for ASP and 5-FU

Drug	Label Claim (mg)	Sample conc. (µg/mL)	Abs at 234 nm	Abs at 266 nm	%Assay ± SD	%RSD
ASP	180	14	0.5485	0.0356	99.57 ± 0.6217	0.62
			0.5410	0.0349		
			0.5502	0.0361		
5-FU	50	3.89	0.0508	0.2175	98.97 ± 1.6028	1.62
			0.0524	0.2151		
			0.0513	0.2193		

The developed methods were validated according to ICH guidelines and summary of validation results mentioned in table 4.

Table 4: Summary of Validation Parameters of UV-Visible Spectrophotometric Method

Parameters	ASP		5-FU	
λ_{max}	234 nm	266 nm	266 nm	234 nm
Linearity	12-32 µg/mL		3.33-8.89 µg/mL	
r^2	0.9971	0.9944	0.9988	0.9975
Regression Equation	Y = 0.0397x-0.0158	Y = 0.0029x-0.0058	Y = 0.0579x-0.0021	Y = 0.0134x-0.0027
LOD (µg/mL)	0.16	-	0.04	-
LOQ (µg/mL)	0.48	-	0.13	-
Intraday Precision (%RSD) (n = 3)	0.59 - 0.82	1.38 - 1.79	0.21 – 1.54	1.82 – 2.12
Interday Precision (%RSD) (n = 3)	0.97 – 1.36	1.49 – 1.85	0.59 – 1.16	0.89 – 1.65
Repeatability (%RSD) (n = 6)	0.13	1.00	0.37	1.10
Accuracy(%Recovery) (n=3)	0.05 – 0.09		0.22 – 0.87	
% Assay	99.57		98.97	

The results of assay were found to be within of label claim for formulations containing ASP and 5-FU as shown in table 3

4. Discussion

The estimation of Aspirin and 5-fluorouracil in dosage form was performed using simple, accurate, precise and sensitive UV-Visible spectrophotometric method. In UV-Visible spectrophotometer, quantification was done by absorbance. The estimating wavelength at 234 nm and 266 nm for ASP and 5-FU respectively. The linear concentration range was found to be 12 to 32 µg/mL for ASP and 3.33 to 8.89 µg/mL for 5-FU. The regression equation was Y = 0.0397x- 0.0158 (r^2 = 0.9971) and Y = 0.0029x- 0.0058 (r^2 = 0.9944) for ASP at 234 nm and 266 nm respectively. The regression equation was Y = 0.0579x- 0.0021 (r^2 = 0.9988) and Y = 0.0134x- 0.0027 (r^2 = 0.9975) for 5-FU at 266 nm and 234 nm respectively. LOD was found to be 0.16 µg/mL for ASP and 0.04 µg/mL for 5-FU. While LOQ was found to be 0.48 µg/mL for ASP and 0.13 µg/mL for 5-FU. % Assay was found to be 99.57% for ASP and 98.97% for 5-FU.

5. Conclusion

The present study involves systematic Chemometric, based development of a simple, rapid, precise, and cost-effective UV Spectrophotometric method for simultaneous estimation of ASP and 5-FU. Furthermore, the validation study supported the selection of the best conditions by confirming that the method was selective, specific, accurate, linear, precise, and robust. The values of % assay for analysis of formulations were found to be within 98-102%. The developed methods were validated as per ICH Q2 (R1) guidelines. In conclusion, a simple, selective, sensitive and accurate UV Spectroscopic method was developed and validated as per ICH guidelines Q2 (R1) for the routine quality for analysis of dosage form containing ASP and 5-FU. This developed method satisfies the ICH Q2 (R1) guidelines and is suitable for regulatory submission under regulatory flexibility.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgements

We are thankful to Ramanbhai Patel College of Pharmacy and Charotar University of Science and Technology, Charusat for providing necessary infrastructure and research facilities to conduct research.

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