

## Development And Validation of Analytical Method of Posaconazole and Aloin Drugs and Its Combination by UV-Spectrophotometer Method

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### Abstract

**Background:** Aloin and Posaconazole are therapeutically important compounds that require accurate and reliable analytical methods for their quantitative estimation in pharmaceutical formulations. The present study focuses on the development and validation of a simple, precise, and cost-effective UV spectrophotometric method.

**Materials and Methods:** Preformulation studies including organoleptic evaluation, melting point determination, solubility study, pH determination, and FTIR spectroscopy were performed for both drugs. Methanol was selected as the solvent for UV spectrophotometric analysis based on solubility studies. The wavelength of maximum absorption ( $\lambda_{max}$ ) was determined by scanning solutions between 200–800 nm. Calibration curves were prepared for Aloin (10–50  $\mu\text{g/ml}$ ) and Posaconazole (5–25  $\mu\text{g/ml}$ ). The developed method was validated according to ICH guidelines for specificity, linearity, precision, ruggedness, robustness, limit of detection (LOD), and limit of quantification (LOQ).

**Results:** Aloin appeared as a yellow-brown crystalline powder, while Posaconazole was white to off-white crystalline powder. The melting points were found to be 148°C and 170°C, respectively. Both drugs showed good solubility in methanol and acetone. The  $\lambda_{max}$  values were observed at 290 nm for Aloin and 276 nm for Posaconazole. The calibration curves exhibited excellent linearity with regression equations of  $y = 0.0057x + 0.054$  ( $R^2 = 0.9993$ ) for Aloin and  $y = 0.0151x + 0.098$  ( $R^2 = 0.9973$ ) for Posaconazole. Precision studies showed low %RSD values below 1%, confirming reproducibility of the method. LOD and LOQ values indicated satisfactory sensitivity.

**Conclusion:** The developed UV spectrophotometric method was found to be simple, precise, accurate, robust, and cost-effective for the routine quantitative estimation of Aloin and Posaconazole in

pharmaceutical formulations and quality control analysis.

**Keywords:** Aloin, Posaconazole, UV Spectrophotometry, Method Validation, FTIR Spectroscopy and Calibration Curve.

### I. Introduction

Method validation is important in analytical chemistry to obtain the reliability of an analytical method. Guidelines provided by the regulatory bodies can be used as a general framework to assess the validity of a method (Ismail *et al.*, 2019). Since these guidelines do not focus on the reliability of analytical results exclusively, this study was aimed to combine a few recently evolved strategies that may render analytical method validation more reliable and trustworthy (Logoyda, 2019). Aloin, also known as barbaloin, is a naturally occurring anthraquinone glycoside present in various Aloe species. It is a yellow-brown bitter compound traditionally used as a stimulant laxative for the treatment of constipation (Froldiet *et al.*, 2019). Aloin possesses several pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and laxative activities (Patel & Patel, 2013). Chemically, it is a C-glycoside anthracene derivative containing multiple hydroxyl functional groups that contribute to its biological activity (Zimbone *et al.*, 2024). Aloin, a naturally occurring anthraquinone glycoside from Aloe species, serves as a stimulant laxative with pharmacological properties including antioxidant and antimicrobial effects. Posaconazole is a broad-spectrum triazole antifungal drug effective for preventing and treating invasive infections from *Candida* and *Aspergillus*, primarily in immunocompromised patients (Gupta *et al.*, 2022). It is used frequently in those undergoing hematopoietic stem cell transplantation and chemotherapy. The drug works by inhibiting the enzyme sterol 14 $\alpha$ -demethylase, disrupting

ergosterol synthesis, which is vital for fungal cell membranes, thus leading to fungal growth inhibition and cell death (Frampton *et al.*, 2008). Posaconazole demonstrates greater potency and a wider antifungal spectrum compared to other azole derivatives. Due to the therapeutic importance of Posaconazole and Aloin, the development of simple, accurate, and reliable analytical methods for their identification and quantitative estimation is essential. UV spectrophotometric analysis offers a rapid, economical, and reproducible approach for routine pharmaceutical evaluation and quality control of these compounds.

## II. Materials And Methods

### 2.2 Pre-formulation Studies

In this study, the organoleptic properties of Posaconazole and Aloin including their color, odor, appearance, and physical state were assessed to support their identification and suitability these attributes not only confirm the authenticity and quality of the substances but also offer insights into their potential behavior during processing and formulation (Kala & Juyal, 2016).

#### 2.2.1 Determination of melting point

The melting point device was used to determine the melting point. A capillary tube containing a tiny quantity of the pure medication Posaconazole and Aloin both drug placed in the melting point device and measurements were obtained.

#### 2.2.2 Solubility study

The solubility study of Posaconazole and Aloin was performed using the visual observation method to determine their solubility in various solvents. Posaconazole and Aloin showed highly soluble in acetone, and methanol (Zhenget *al.*, 2017).

#### 2.2.3 pH determination

In order to assess the physicochemical properties of posaconazole and aloin solutions and to guarantee their compatibility with biological environments and formulation components, their pH values were set at 5.0. A pH meter with a glass electrode as the indication electrode and a mercury electrode as the reference electrode was used to conduct the measurements. To ensure accuracy, each medication solution was tested in triplicate.

### 2.3 Fourier Transform Infrared Spectroscopy (FTIR)

The presence of distinctive functional groups in the pure samples of posaconazole and aloin was verified using FTIR spectroscopy, which helps to establish the medications' identity and purity. A Perkin

Spectrum BX spectrophotometer was used to perform the analysis (Sharma *et al.*, 2025).

### 2.4. UV-Vis Spectroscopy Method (Li *et al.*, 2025):

#### 2.4.1. Selection of solvent

Methanol was selected as the solvent for the development of the UV-Vis spectroscopic technique for both Posaconazole and Aloin based on the solubility investigation. The selection of methanol was based on its capacity to efficiently dissolve the medications, guaranteeing a transparent and stable solution for precise absorbance measurements. Because it directly affects the drug's spectrum characteristics and technique sensitivity, using the right solvent in UV-Vis analysis is essential to getting accurate and repeatable findings.

#### 2.4.2 Preparation of Standard Stock Solution for Calibration curve

The preparation of a standard stock solution of Posaconazole and Aloin is essential for developing a reliable calibration curve in UV-Vis spectroscopy. This calibration curve allows for accurate quantification of the drug concentration in unknown samples by establishing a relationship between absorbance and known concentrations.

#### 2.4.3. Determination of wavelength of maximum absorption ( $\lambda_{max}$ ) (Chaudhari *et al.*, 2024)

To find the wavelength of maximum absorption ( $\lambda_{max}$ ), the posaconazole and aloin solution is scanned across the ultraviolet-visible spectrum from 200 to 800 nm. An absorption spectrum, which shows how posaconazole and aloin interact with light throughout the UV and visible regions, is obtained by measuring the absorbance at each wavelength within this range.

### 2.5 Validation of the method

The International Council for Harmonization (ICH) requirements for the validation of analytical methods were followed in the development and validation of the analytical method. For the analytes posaconazole and aloin, the validation parameters assessed were linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD), and limit of quantitation (LOQ).

#### 1. Specificity

The UV spectra of standard posaconazole and aloin were examined separately in order to assess the method's specificity. The method's specificity for both analytes was verified by clear and distinct absorption peaks.

#### 2. Linearity

By examining various concentrations of the posaconazole and aloin standard solution, the linearity of measurement was assessed. The Beer

Lambert's concentration range for both methods was determined to be 5-25 and 10-50 µg/ml.

### 3. Precision

Precision was taken into account at three levels: repeatability, intermediate (intraday) precision and reproducibility (inter day precision).

**1] Intraday Precision:** Solutions containing 15 µg/ml of posaconazole and 30 µg/ml aloin was analyzed three times on the same day and %R.S.D was calculated.

**2] Interday Precision:** Solutions containing 15 µg/ml posaconazole and 30 µg/ml aloin was analyzed on three different successive days and %R.S.D was calculated.

**3] Repeatability:** Method precision of the experiment was performed by preparing the standard solution of posaconazole and aloin (15 and 30 µg/ml) for three times.

### 4. Ruggedness study

To determine the robustness, the same procedure was carried out by changing the temperature and the result is compared with the same previous procedure.

**Limit of Detection (LOD):** The detection limit was calculated using the following equation in accordance with ICH rules.

**Limit of Quantification (LOQ):** The quantification limit was calculated using the following equation in accordance with ICH rules (Kumar *et al.*, 2012).

## III. Results and discussion

### 3.1 Organoleptic properties of Aloin and Posaconazole

Table 1: Organoleptic Properties of Aloin and Posaconazole

S. No.	Organoleptic Properties	Observations	Observations
1	Colour	Yellow, brownish-yellow	White to off-white
2	Odor	Odorless, characteristic odor	Odorless
3	Physical appearance	Crystalline powder	Crystalline powder
4	State	Solid	Solid

### 3.2 Melting point of Aloin and Posaconazole

Table 2: Melting point of Aloin and Posaconazole

S. No.	Drug	Reference range	Observation
1	Aloin	148-149 °C	148 °C
2	Posaconazole	170-172 °C	170 °C

### 3.3 pH of Aloin and Posaconazole

Table 3: pH of Aloin and Posaconazole

S. No.	Drug	Reference range	Observation
1	Aloin	3.0 to 6.5 pH	6.25 pH
2	Posaconazole	3.6 to 7.0 pH	5.03 pH

### 3.4 Solubility Study (A) Aloin and Posaconazole

The drug solubility profile shows a significant reliance on the kind of solvent. The material's high affinity for highly polar aprotic solvents is suggested by its free solubility in DMSO. It is only weakly soluble in acetone, although it is soluble in methanol, suggesting a moderate compatibility with polar solvents. Due to its restricted aqueous solubility and very low

interaction with less polar protic solvents, the chemical is very weakly soluble in ethanol and only marginally soluble in water. In general, this pattern indicates that the molecule has restricted hydrophilicity and dissolves more readily in particular organic solvents. This information is crucial for choosing the right media for formulation and analytical purposes.

### 7.3 Fourier Transform Infrared Spectroscopy FTIR of Aloin and Posaconazole

Table 4: FTIR study

S.NO	Aloin				Pasconazole			
	Frequency Range	Group Absorption (cm-1)	Group	Compound Class	Frequency Range	Group Absorption (cm-1)	Group	Compound Class

1	3400-3300 (cm-1)	3384.28	N-H stretching	aliphatic primary amine	3700-3584 (cm-1)	3660.06	O-H stretching	alcohol
2	3000-2840 (cm-1)	2929.36	C-H stretching	alkane	3200-2700 (cm-1)	3114.73	O-H stretching	alcohol
3	1420-1330 (cm-1)	1375.34	O-H bending	alcohol	3000-2840 (cm-1)	2902.11	C-H stretching	alkane
4	1342-1266 (cm-1)	1289.71	C-N stretching	aromatic amine	1440-1395 (cm-1)	1425.65	O-H bending	carboxylic acid
5	1210-1163 (cm-1)	1187.45	C-O stretching	ester	1275-1200 (cm-1)	1274.86	C-O stretching	alkyl aryl ether
6	1085-1050 (cm-1)	1080.45	C-O stretching	primary alcohol	1150-1085 (cm-1)	1139.60	C-O stretching	aliphatic ether
					1085-1050 (cm-1)	1075.76	C-O stretching	primary alcohol

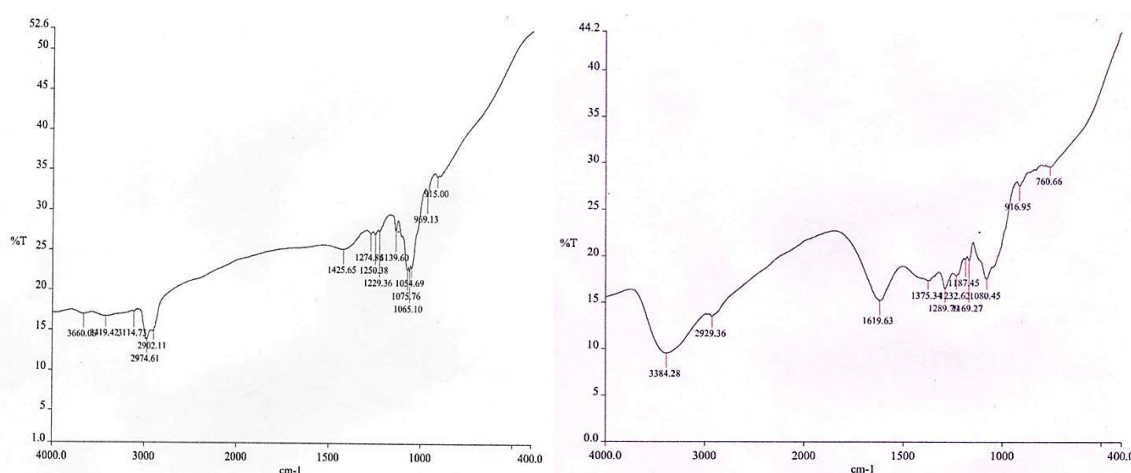


Figure 1: FTIR Spectra of Aloin and Posaconazole

### 3.4 Method Development by UV spectroscopy

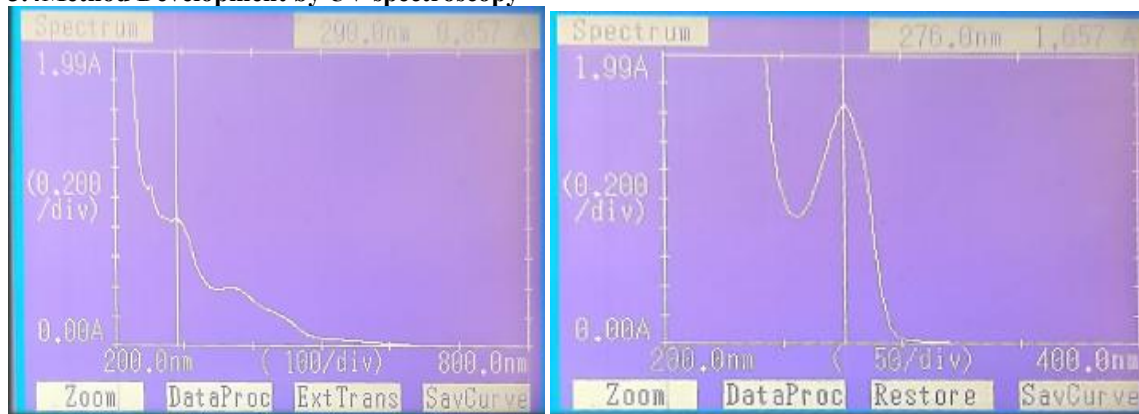


Figure 2: UV Spectrum of Aloin and Posaconazole

### 3.5 Calibration curve (Linearity):

#### Calibration Curve (Linearity) of Aloin

Calibration curves for Aloin and Posaconazole were established. The Aloin curve, involving concentrations from 10µg/ml to 50µg/ml, yielded a

regression equation of  $y = 0.0057x + 0.054$  with an  $R^2$  value of 0.9993, indicating strong linearity. For Posaconazole, standard solutions from 5µg/ml to 25µg/ml showed absorbance values increasing from 0.166 to 0.472, confirming a linear relationship per

Beer–Lambert’s law. This curve had a regression equation of  $y = 0.0151x + 0.098$  and an  $R^2$  of

0.9973, also demonstrating good linearity

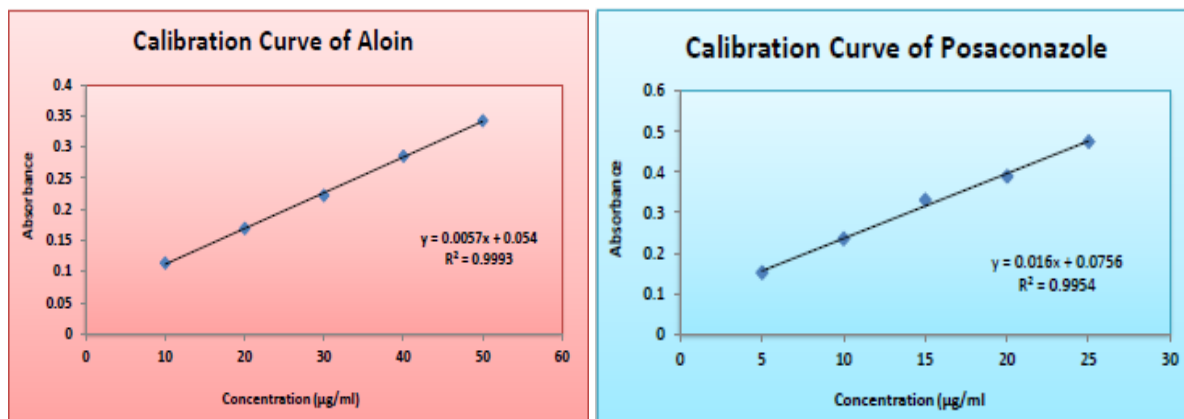


Figure 3: Calibration Curve of Aloin and Posaconazole

### 3.6 Method Validation via UV spectroscopy for Aloin and Posaconazole

#### 1. Precision study

##### a) Intraday Precision

Table 5: Result of Intraday Precision (three times on the same day) of Aloin and Posaconazole

Aloin				Posaconazole			
Concentration (µg/mL)	Day 1 Absorbance (Morning) at 290nm	Day 1 Absorbance (Afternoon) at 290nm	Day 1 Absorbance (Evening) at 290nm	Concentration (µg/mL)	Day 1 Absorbance (Morning) at 276nm	Day 1 Absorbance (Afternoon) at 276nm	Day 1 Absorbance (Evening) at 276nm
30	0.222	0.221	0.223	15	0.331	0.332	0.333
30	0.220	0.223	0.225	15	0.331	0.334	0.335
30	0.224	0.220	0.224	15	0.333	0.330	0.334
Mean	0.222	0.2213	0.224	Mean	0.331	0.332	0.334
SD	0.002	0.0015	0.001	SD	0.0011	0.002	0.001
%RSD	0.900	0.452	0.446	%RSD	0.302	0.602	0.299
AVG % R.S.D	0.465			AVG % R.S.D	0.401		

##### b) Interday Precision

Table 6: Result of Interday Precision (three times on the different day) of Aloin and Posaconazole

Aloin				Posaconazole			
Concentration (µg/mL)	Day 1 Absorbance (Morning) at 290nm	Day 1 Absorbance (Afternoon) at 290nm	Day 1 Absorbance (Evening) at 290nm	Concentration (µg/mL)	Day 1 Absorbance (Morning) at 276nm	Day 1 Absorbance (Afternoon) at 276nm	Day 1 Absorbance (Evening) at 276nm
30	0.222	0.223	0.222	15	0.331	0.331	0.331
30	0.224	0.221	0.220	15	0.332	0.334	0.333
30	0.222	0.220	0.223	15	0.333	0.331	0.334
Mean	0.222	0.221	0.221	Mean	0.332	0.330	0.332
SD	0.0011	0.0015	0.0015	SD	0.001	0.0017	0.0015
%RSD	0.450	0.452	0.452	%RSD	0.301	0.303	0.301
AVG % R.S.D	0.451			AVG % R.S.D	0.301		

##### C) Repeatability

Table 7: Result of repeatability of Aloin and Posaconazole

Sr. No.	Concentration (µg/ml)	Absorbance		Statistical analysis	Concentration (µg/ml)	Absorbance	Statistical analysis
1	30	0.222	Mean	0.222	15	0.331	Mean 0.3321

2	30	0.220	SD	0.0017	15	0.333	SD	0.0011
3	30	0.222	% RSD	0.450	15	0.332	% RSD	0.301
4	30	0.221			15	0.331		
5	30	0.223			15	0.334		
6	30	0.225			15	0.332		

### 1. Ruggedness-

Table 8: Result of ruggedness of Aloin

Aloin				Posaconazole			
Analyst-1		Analyst-2		Analyst-1		Analyst-2	
Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Concentration (µg/ml)	Absorbance	Absorbance	Concentration (µg/ml)	Absorbance
30	0.222	15	15	0.332	0.331	15	0.332
30	0.224	15	15	0.334	0.333	15	0.334
30	0.222	15	15	0.335	0.330	15	0.335
Mean	0.2226	Mean	Mean	0.333	0.331	Mean	0.333
SD	0.0011	SD	SD	0.0015	0.0015	SD	0.0015
% RSD	0.450	% RSD	% RSD	0.300	0.302	% RSD	0.300

### 2. Robustness

Table 9: Results showing robustness of Aloin

Temperature 250C		Temperature 300C	
Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
30	0.222	30	0.220
30	0.220	30	0.224
30	0.223	30	0.224
Mean	0.22167	Mean	0.22267
SD	0.0015	SD	0.0023
% RSD	0.450	% RSD	0.900

Table 10: Results showing robustness of posaconazole

Temperature 250C		Temperature 300C	
Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
15	0.331	15	0.334
15	0.332	15	0.333
15	0.334	15	0.333
Mean	0.332	Mean	0.332
SD	0.0015	SD	0.0015
% RSD	0.301	% RSD	0.301

### 3.7 LOD and LOQ

Table 11 :Results showing LOD and LOQ of Aloin and Posaconazole

S. No.	Drug name	Wavelength	LOD (µg/ml)	LOQ (µg/ml)
1	Aloin	290.0nm	0.40110	1.2154
2	Posaconazole	276.0nm	1.9948	6.0449

### IV. Discussion

The analysis of Aloin and Posaconazole shows that both substances have acceptable physicochemical properties. Aloin is a yellow to brown crystalline solid, while Posaconazole is white

and odorless, indicating good purity. Their melting points confirmed identity: 148°C for Aloin and 170°C for Posaconazole. The pH values were within acceptable ranges at 6.25 and 5.03, respectively. Solubility studies indicated low water solubility but

good solubility in organic solvents. FTIR analysis supported their structural identity. UV spectrophotometric methods revealed  $\lambda_{\max}$  values of 290 nm for Aloin and 276 nm for Posaconazole, with high correlation coefficients demonstrating linearity. Precision studies showed low %RSD, confirming high reproducibility. Reliability under various conditions was evidenced by ruggedness and robustness results with %RSD below 1%. The methods demonstrate good sensitivity with LOD and LOQ values. Overall, this UV spectrophotometric method is accurate, precise, and suitable for routine estimation in pharmaceutical formulations.

## V. Conclusion

The study effectively conducted preformulation and analytical evaluation of Aloin and Posaconazole using UV spectrophotometric techniques. Characterization methods confirmed the identity, purity, and suitability of the drugs. The UV method exhibited excellent linearity and was validated following ICH guidelines, demonstrating accuracy, precision, and robustness with low %RSD values. It offers good sensitivity, is simple, reliable, cost-effective, and suitable for quantitative estimation in pharmaceutical applications.

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