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Original Article

# A Novel Sustainable UV Spectrophotometric Approach for the Quantification of Amisulpride in Bulk and Nanoformulations

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## ABSTRACT

**Background:** Schizophrenia is a persistent neuropsychiatric condition that requires precise drug delivery to the brain for effective treatment. Amisulpride, a second-generation antipsychotic, has low oral bioavailability and struggles to cross the blood–brain barrier, thus requiring innovative formulation techniques and dependable analytical methods. The aim of the proposed research work was to develop and validate an eco-friendly UV spectroscopy for measuring amisulpride in bulk drugs, niosomal formulations, and commercial products, promoting sustainable pharmaceutical quality control. **Materials and Methods:** A UV spectrophotometric approach was developed using a methanol: water (77:23) solvent mixture, with detection at 280 nm. Validation followed ICH Q2 (R1) guidelines, evaluating linearity, precision, accuracy, robustness, ruggedness, LOD, and LOQ. Amisulpride-loaded niosomes were formulated by the ethanol injection method using Span-60 and cholesterol. Particle size and Zeta potential were analyzed by Malvern Zetasizer. **Results:** The method showed excellent linearity ($r^2$ = 0.998), precision (%RSD < 2%), and recovery rates (97–101.3%). LOD and LOQ were found to be 0.58 µg/mL and 1.2 µg/mL, respectively. Niosomes had a particle size of 200.89 nm and a zeta potential of –23.6 mV. This method effectively detected amisulpride in the various formulations. **Conclusion:** The validated UV method provides a precise, robust, and sustainable alternative to chromatographic techniques, facilitating the routine quality evaluation of amisulpride in advanced nanoformulations.

**Keywords:** Amisulpride, UV-visible Spectrophotometry, Niosomes.

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## INTRODUCTION

Schizophrenia is a long-term and serious mental disorder that affects an individual's thoughts, emotions, and behaviours. Affected individuals often appear detached from reality and exhibit reduced involvement in everyday tasks. It presents with positive symptoms, such as hallucinations and delusions, negative symptoms, such as emotional disturbances, and cognitive challenges in mental functioning (Aubel, 2021). Globally, approximately 21 million people suffer from schizophrenia, with 12 million males and 9 million females affected (Chaudhari *et al.*, 2024).

Amisulpride (Figure 1) belongs to the second generation of antipsychotics with the molecular name 4-amino-n-((1-ethyl-2-pyrrolidinyl)methyl)-5-(ethylsulfonyl)-2-methoxy benzam, and a new atypical antipsychotic drug has demonstrated efficacy in addressing both the positive and negative symptoms of schizophrenia. Owing to its interaction with two central nervous system receptors, dopamine (D2) and serotonin (5-HT2A), it can be used to treat schizophrenia.

Compared with typical antipsychotic drugs, it exhibits a lower occurrence of extrapyramidal side effects and is better tolerated. At present, Amisulpride is widely utilized for the management of various forms of schizophrenia (Abdelbary & AbouGhaly, 2015; Purushottam Agrawal *et al.*, 2020; Telange *et al.*, 2016) .

Amisulpride is formulated into niosomes to enhance its delivery to the brain for schizophrenia treatment. Oral administration is associated with poor solubility and limited blood–brain barrier permeability, resulting in low cerebral bioavailability. Niosomes

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composed of nonionic surfactants improve drug encapsulation and stability. Intranasal delivery enables direct nose-to-brain transport via the olfactory and trigeminal pathways, thereby bypassing the blood–brain barrier. This targeted approach enhances brain uptake, reduces systemic exposure, and supports sustained release, ultimately improving the therapeutic efficacy (Patil *et al.*, 2023).

Pharmaceuticals hold a crucial and progressively important position throughout the entire progression of drug development (Mortimer, 2004). As the importance of amisulpride continues to expand in the management of psychiatric disorders, there is an increasing demand for convenient and rapid approaches to continuously evaluate and guarantee the quality of available formulations in the market (Behera, 2012).

A literature survey reveals that there is a limited UV spectroscopy method for quantifying amisulpride in bulk, nanoformulation, and marketed formulations.

The present study aimed to develop and validate a UV spectrophotometric method, which is simple, accurate, highly efficient, and repeatable for analyzing amisulpride in bulk, nano formulation, and marketed tablets.

## MATERIALS AND METHODS

### Materials

Amisulpride was provided as a gift sample from Symed Laboratories, Hyderabad. The reagents and chemicals used in the analysis were obtained from KLE College of Pharmacy, KAHER, Belagavi, and they were of pure and high analytical quality. For the analysis, a Shimadzu UV-Spectrophotometer, model UV-1900, equipped with UV probe software was employed. The drug sample was weighed using a calibrated weighing balance during the investigation.

### Method Development

Amisulpride was quantified using a Shimadzu UV-1900 spectrophotometer equipped with UV-Probe software, utilizing a solvent mixture of methanol and water in a 77:23 ratio, with a detection wavelength at 280 nm. The method was validated in accordance with ICH Q2(R1) guidelines, evaluating parameters such as linearity (ranging from 2 to 10 µg/mL), precision (across six replicates), accuracy (at recovery levels of 50%, 100%, and 150%), ruggedness, robustness, and the Limits of Detection (LOD) and Quantification (LOQ). Niosomes were prepared by ethanol injection using Span-60 and cholesterol, and their particle size, zeta potential, and morphology were assessed using dynamic light scattering and Transmission Electron Microscopy (TEM). Statistical analysis involved calculations of %RSD, regression coefficients, and standard deviation-based determinations of LOD and LOQ.

### Method Validation

To authenticate the newly formulated method parameters in accordance with the ICH guidelines (specifically, ICH guidance Q2A and Q2B), a validation process was pursued (El Assasy *et al.*, 2019). Validation refers to the process outlined by (ICH). It involves obtaining documented evidence to ensure a high level of confidence in the consistent production of a desired outcome of readings that meet predetermined specifications and quality standards. The method validation procedure involved the assessment of the following parameters (Humaira & Dey, 2008).

### Preparation of the standard solution

A precise quantity of 10 mg of amisulpride was weighed and placed into a 10 mL volumetric flask. The volumetric flask was filled with a methanol-water mixture (77:23 v/v). The clear solution was created by sonicating it using a bath sonicator (Gschwend *et al.*, 2006).

### Selection of wavelength

The analyte's identity was assessed by examining a 10 µg/mL concentration of the respective stock solution within the spectral range of 400 to 200 nm for Amisulpride using a methanol-water mixture (77:23 v/v).

### Specificity and selectivity

Accurately measuring Amisulpride in bulk, niosome, and commercial formulations is essential. These factors were assessed to ensure that there was no interference from excipients or other external substances. The absence of absorbance at 280 nm in blank samples confirmed the method's specificity and selectivity.

### Linearity and range

Linearity was assessed by analysing amisulpride dilutions (2-10 µg/mL) at 280 nm. This process was repeated three times to ensure its consistency.

### Limit of Detection and Limit of Quantification

The Detection Limit (LOD) is the smallest quantity of analyte in a sample that can be detected. The Quantification Limit (LOQ) is the minimum concentration of the analyte within a sample that can be properly and precisely quantified (Manoja *et al.*, 2012).

The LOD and LOQ were calculated using the following set of equations: LOD = 3.3 SD of the y-intercept divided by the slope of the calibration curve and LOQ = 10 times SD of the y-intercept divided by the slope of the calibration curve(Papoutsis *et al.*, 2014) .

LOD = 3.3 regression standard deviation/ Slope.

LOQ = 10 regression standard deviation/ Slope.

### Precision

Precision tests were performed to determine the reliability of the proposed analytical approach. Six duplicates at a concentration of 4 µg/mL were analysed for repeatability. Absorbance was measured on the same day to ensure intraday accuracy. Precision analysis involved preparing a 4 µg/mL drug solution and testing it at three-time intervals during the day to assess inter-day precision. This technique was performed for three separate days to compute the percentage Relative Standard Deviation (RSD) as a measure of accuracy (Sharma *et al.*, 2010; Naik *et al.*, 2025).

### Ruggedness & Robustness

The method's ruggedness and robustness were validated through systematic absorbance measurements of amisulpride at 280 nm. Ruggedness was confirmed by testing across different analysts and UV spectrophotometers, showing consistent results via mean, standard deviation, and %RSD. Robustness was demonstrated by slightly altering the detector wavelength (+2 nm) and analyzing the statistical consistency of the absorbance data, affirming the method's reliability under minor, intentional variations in analytical conditions (Kochling *et al.*, 2016).

### Accuracy

The accuracy of the proposed method was evaluated using recovery experiments by increasing the concentration of pure amisulpride (50, 100, and 150%) in the samples (Khan *et al.*, 2017).

### Formulation of Amisulpride-loaded niosomal formulation

Amisulpride-loaded Niosomes (AMSNs) were developed using the ethanol injection method. Span-60, cholesterol, and amisulpride (50 mg) were precisely weighed and dissolved in 10 mL of ethanol by bath sonication at approximately 60°C. At the same temperature, the clear organic solution was promptly transferred into a water solution, which was vigorously agitated with a Teflon-coated bead at 500 revolutions per minute (Remi magnetic stirrer). The aqueous solution immediately changed to a milky solution, indicating niosome production. To evaporate ethanol, the resultant solution was vacuumed for 15 min and agitated for an hour. Finally, the volume of the niosomal dispersion was adjusted to 30 ml by adding water (Patil *et al.*, 2023).

### Characterization of Niosomes

#### *Vesicle Size Analysis*

Amisulpride-loaded niosomes were physicochemically characterized to determine their average particle size, polydispersity index, and zeta potential. This assessment was performed using a Zetasizer (Malvern Instruments, Malvern, UK). The niosomal dispersion was appropriately diluted in distilled water (1:10) for the measurements. Folded capillary cells (DTS 1060) were used, and all measurements were performed at 25 °C. The measurements were repeated three times to ensure accuracy (Sita *et al.*, 2020).

### Transmission Electron Microscopy (TEM) Analysis

The morphology and surface characteristics of the niosomes were assessed using high-resolution Transmission Electron Microscopy (TEM) at DST-SAIF, Cochin. A small volume of the amisulpride-loaded niosomal formulation was placed on a carbon-coated copper grid, and the sample was analyzed using TEM (Swartz & Krull, 2018).

## RESULTS

The proposed UV Spectrophotometric technique offers an accurate, cost-effective, and convenient approach for amisulpride analysis. This approach underwent thorough validation following ICH Q2 (R1) guidelines, confirming its dependability, precision, and adherence to regulatory standards.

### Determination of maximum wavelength

The highest absorbance in a methanol/water mixture (77:23) was found at a wavelength of 280 nm (Figure 2).

### Standard calibration curve

The calibration plot for amisulpride was linear with a coefficient of correlation of 0.998 (Figure 3).

### Linearity

Linearity was assessed by analysing amisulpride dilutions (2-10 µg/mL) at 280 nm; the correlation coefficient ($r^2$) confirmed the linearity of the calibration curves. The correlation coefficient for amisulpride was 0.998.

### Detection Limit and Quantification Limit

LOD and LOQ were observed to be 0.58 µg/mL and 1.2 µg/mL, respectively.

### Precision

Intraday and interday experiments were conducted on separate days, and the results indicated minimal differences between them.

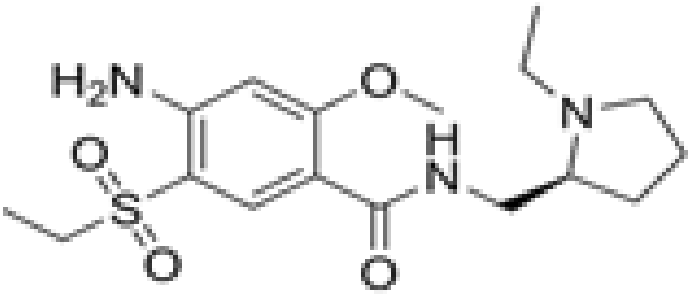


**Figure 1:** Chemical Structure of Amisulpride.

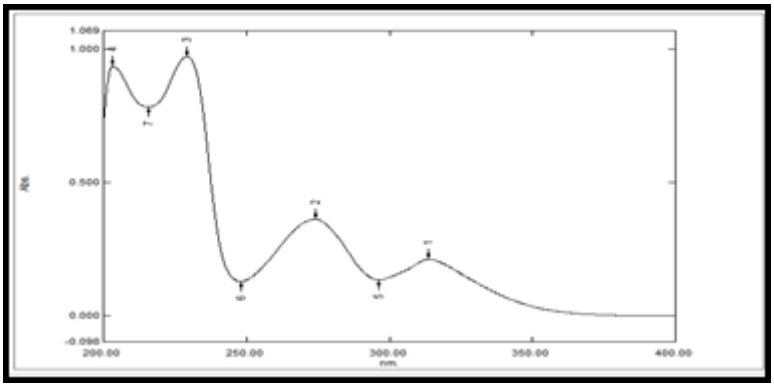

**Figure 2:** UV-spectrum of amisulpride in methanol and water (77:23).

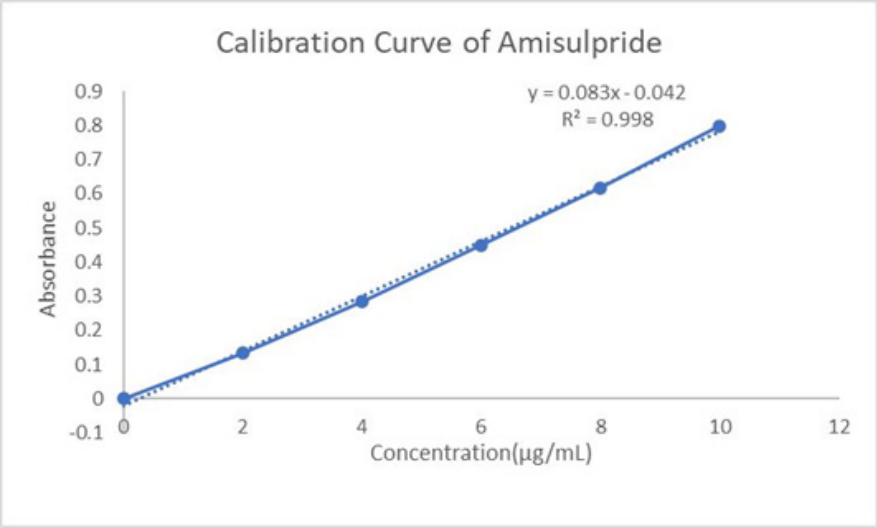


**Figure 3:** Standard calibration curve for Amisulpride.

This result suggests that the proposed methodology is consistent and reliable. The precision results, along with the relative variance calculations, are presented in Tables 1 and 2, respectively (Raman *et al.*, 2015).

## Ruggedness & Robustness

Ruggedness was assessed by performing the assay under the same conditions on different days using different analysers, with different tools, and at different times. The test results consistently fell within the range of 99-101%, indicating the robustness of the method. The robustness was further confirmed by performing the assay at different wavelengths. The Relative Standard Deviation (%RSD) was below 2%, which was well within the specified limit, as shown in Table 3 (Rathod & Desai, 2015).

## Accuracy

The accuracy of the proposed method was verified using the classical addition method, recovering in the range of 97-101.30% (Rapalli *et al.*, 2020). The results are depicted in Table 4.

## Solvent and standard stock solution stability

The stability of amisulpride in the solution was evaluated by calculating the Relative Standard Deviation (% RSD) of the absorbance values obtained from both freshly prepared solutions and previously prepared solutions containing amisulpride. Upon evaluation, the analytical results were found to be within acceptable limits, confirming the stability of the standard stock solution. The investigation on solution stability spanned a period of four days, (Skibiński *et al.*, 2007).

## Characterization of Niosomal Dispersion

The zeta potential of apremilast niosomes was determined to be -23.6 mV, with a particle size of 200.89 nm. TEM confirms smooth, oval-shaped particles

## Quantification of amisulpride in different marketed formulations and prepared Niosomal Formulation

Amisulpride was quantified in bulk, in various marketed products, such as Solian tablet and Sulpitac tablet, along with the Niosomal formulations. (Sen *et al.*, 2016). The formulations showed a spectrum at a wavelength of approximately 277 nm, which was close to the observed absorption maxima of amisulpride. The

corresponding peaks in the spectrum can be attributed to the different excipients used in the formulations (Figure 4).

## DISCUSSION

The developed UV spectrophotometric method represents a distinct advancement in the quantitative analysis of amisulpride, combining simplicity, sensitivity, and environmental sustainability. Unlike earlier methods such as that of (Humaira & Dey, 2008), which utilized 0.1 N HCl at 226.5 nm and risked analyte instability, the present approach employs a methanol:water system at 280 nm, optimizing excipient transparency and reducing instrument corrosion. While visible spectrophotometric methods reported by (venumadhav, 2010) and (Rao, n.d.) achieved high linearity (0.9995–0.9999) through diazotization reactions, their reliance on toxic reagents and multistep procedures made them inconsistent with green analytical chemistry principles. The newly developed method addresses these limitations through a non-toxic and cost-efficient solvent system that achieves superior LOD (0.58 µg/mL) and LOQ (1.2 µg/mL) values, thereby enabling sensitive and reliable quantification. As the first validated UV spectrophotometric method for amisulpride-loaded niosomes, it supports advanced formulation studies while maintaining compliance with ICH Q2(R1) guidelines. Demonstrating excellent linearity ($r^2$ = 0.998), precision (%RSD < 2%), and recovery (97–101.3%), the method achieves HPLC-comparable accuracy with minimal infrastructure requirements, making it suitable for routine quality control in resource-limited environments. Robustness and ruggedness evaluations confirmed reproducibility across

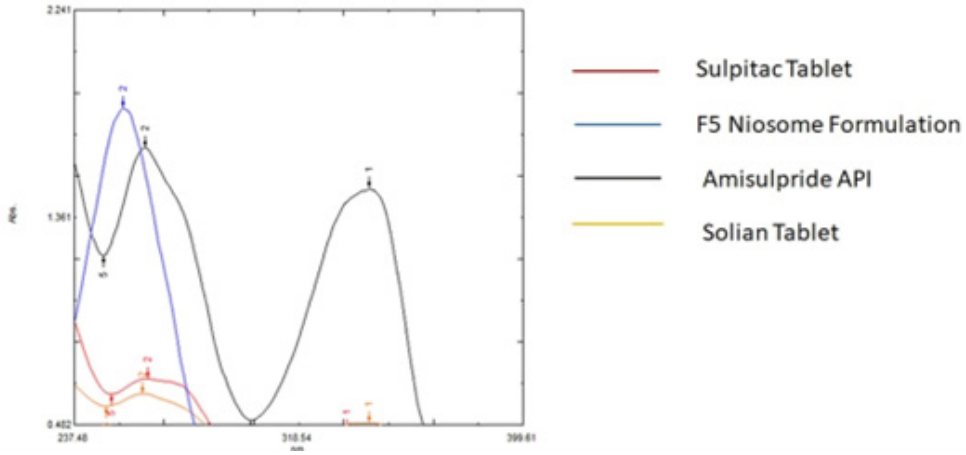


**Figure 4:** UV Spectrum of marketed products and Niosomal Formulation of Amisulpride.

**Table 1: Interday precision data of amisulpride.**

| Concentration (µg/mL) | Absorbance 1 (11:00 am) | Absorbance 2 (1:00 pm) | Absorbance 3 (3:00 pm) | Average %RSD |
|---|---|---|---|---|
| 4 | 0.280 | 0.283 | 0.284 | 1.398 |
| 4 | 0.282 | 0.285 | 0.284 | |
| 4 | 0.277 | 0.279 | 0.276 | |
| 4 | 0.280 | 0.282 | 0.283 | |
| 4 | 0.284 | 0.286 | 0.289 | |
| 4 | 0.277 | 0.275 | 0.277 | |

**Table 2: Intraday precision data of amisulpride.**

| Concentration (µg/mL) | Absorbance 1 (day 1) | Absorbance 2 (day 2) | Absorbance 3 (day 3) | Average %RSD |
|---|---|---|---|---|
| 4 | 0.291 | 0.278 | 0.279 | 0.9422 |
| 4 | 0.281 | 0.282 | 0.282 | |
| 4 | 0.279 | 0.279 | 0.280 | |
| 4 | 0.283 | 0.284 | 0.284 | |
| 4 | 0.278 | 0.280 | 0.282 | |
| 4 | 0.282 | 0.275 | 0.276 | |

**Table 3:** Robustness and Ruggedness data for amisulpride.

| Concentration (µg/mL) | Absorbance | RSD |
|---|---|---|
| Analyst-1& 280nm. | | Average=0.281 |
| 4 | 0.277 | SD=0.0026 |
| 4 | 0.281 | % RSD = 0.931 |
| 4 | 0.279 | |
| 4 | 0.284 | |
| 4 | 0.282 | |
| 4 | 0.283 | |
| Concentration (µg/mL) | Absorbance | RSD |
| Analyst-2 & 282nm. | | |
| 4 | 0.281 | Average=0.281 |
| 4 | 0.278 | SD=0.0023 |
| 4 | 0.282 | %RSD=0.82 |
| 4 | 0.279 | |
| 4 | 0.284 | |
| 4 | 0.281 | |
| %RSD | 0.82 | 0.931 |

**Table 4:** Accuracy data of amisulpride.

| Label claim | Theoretical Value | Level addition | Amount of drug added | Average % recovery |
|---|---|---|---|---|
| 1 | 1.5 | 50% | 1.523 | 101.5333 |
| 1 | 2 | 100% | 2.0189 | 100.945 |
| 1 | 2.5 | 150% | 2.439 | 97.56 |

analysts and wavelengths. The optimized niosomal formulation exhibited a mean particle size of 200.89 nm and a zeta potential of -23.6 mV, indicative of colloidal stability favorable for intranasal delivery in schizophrenia treatment. Overall, this eco-friendly and reproducible UV spectrophotometric approach provides a sustainable, practical, and analytically sound alternative for the quantification of amisulpride in bulk, niosomal, and commercial formulations.

## CONCLUSION

A validated UV spectrophotometric method was successfully developed for precise quantification of amisulpride in bulk, niosomal, and commercial formulations. Demonstrating excellent linearity, precision, and accuracy with recovery rates of 97–101.3%, this eco-sustainable approach offers a cost-effective alternative to chromatographic techniques, aligning with ICH Q2 (R1) standards. The formulated niosomes exhibited optimal colloidal stability (particle size: 200.89 nm, zeta potential: –23.6 mV), supporting effective intranasal brain delivery for schizophrenia therapeutics.

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## ABBREVIATIONS

**API:** Active Pharmaceutical Ingredient; **UV Spectroscopy:** Ultraviolet Spectroscopy; **ICH:** International Conference on Harmonisation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **HPLC:** High Performance Liquid Chromatography; **PDI:** Polydispersity Index; **TEM:** Transmission electron microscopy; **RSD:** Relative standard deviation.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ARTIFICIAL INTELLIGENCE (AI)

This manuscript benefited from the use of Microsoft Copilot, the Paperpal AI tool, to enhance grammar, clarity, and overall readability.

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