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# Development and Validation of RP-HPLC Method for Estimation of Amisulpride

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## ABSTRACT

A novel, simple, specific, accurate, precise method development and validated for the estimation of Amisulpride by RP-HPLC in bulk and marketed formulation. A High Performance Liquid Chromatography WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector withInertsil ODS C18 (4.6mm x 250mm, 5µm) column, with mobile phase composition of Methanol: Acetate Buffer (pH-4.2) (40:60% v/v) was used. The flow rate of 1.0 ml min-1 and effluent was detected at 280 nm. The retention time of Amisulpride was 3.388 minutes. Linearity was observed over concentration range of 60-140ng ml-1. The Limit of detection and limit of quantification was found to be 1.5ng ml-1 and 4.5ngml-1 respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98% to 102%. Then method was validated in terms of linearity, accuracy, precision, (repeatability, intermediate precision) specificity (by assay), robustness and system suitability.Thus, the validated method is can be successfully applied to routine analysis for regulate the quality. It also should be used for analytical research purpose.

**Keywords:** Amisulpride, RP-HPLC, Method Development, Validation, ICH Guidelines.

## INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called “Pharmacopoeia” (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental

analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs.

## MATERIALS AND METHODS

**Table 1: Instruments used**

| S.No. | Instruments and Glass wares | Model |
|---|---|---|
| 1 | HPLC | WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector. |
| 2 | pH meter | LabIndia |
| 3 | Weighing machine | Sartorius |
| 4 | Volumetric flasks | Borosil |
| 5 | Pipettes and Burettes | Borosil |
| 6 | Beakers | Borosil |
| 7 | Digital ultra sonicator | Enertech |

**Table 2: Chemicals used**

| S.No. | Chemical | Brand Names |
|---|---|---|
| 1 | Amisulpride | Sura labs |
| 2 | Water and Methanol for HPLC | LICHROSOLV (MERCK) |
| 3 | Acetonitrile for HPLC | Merck |

### Hplc method development

**TRAILS**

**Preparation of standard solution:** Accurately weigh and transfer 10 mg of Amisulpride working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol then sonicate to dissolve and removal of air completely and make volume up to the mark with Methanol.

Further pipette 1ml of the above Amisulpride stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

**Procedure:** Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

**Mobile Phase Optimization**: Initially the mobile phase tried was methanol: Water and Acetonitrile and water with varying proportions. Finally, the mobile phase was optimized to Methanol and Acetate Buffer in proportion 40:60 v/v respectively.

**Optimization of Column:**The method was performed with various C18 columns like ODS column, Xterra and AltimaC18 column. Inertsil ODS C18 (4.6mm x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0ml/min flow.

### Optimized chromatographic conditions

Instrument used :Waters HPLC with auto sampler and PDA detector 996 model.
Temperature : 35°C
Column :Inertsil ODS C18 (4.6mm x 250mm, 5µm)
Mobile phase:Methanol: Acetate Buffer (pH-4.2)(40:60 v/v)
Flow rate : 1.0ml/minute
Wavelength : 280nm
Injection volume : 10µl
Run time : 10minutes

## RESULTS AND DISCUSSION

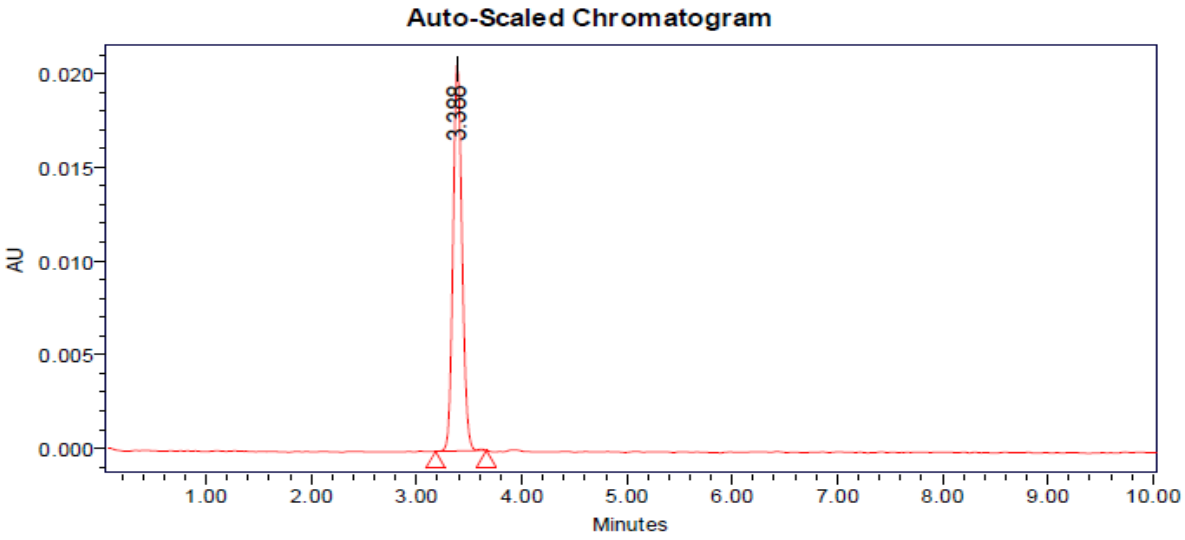


**Fig 1: Optimized Chromatogram (Standard)**

**Table 3: Results of Optimized Chromatogram (Standard)**

| S.No. | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Amisulpride | 3.388 | 145867 | 32546 | 1.76 | 8457 |

In this trial it shows proper plate count, tailing and baseline in the chromatogram. So it's an optimized chromatogram.

### Optimized Chromatogram (Sample)

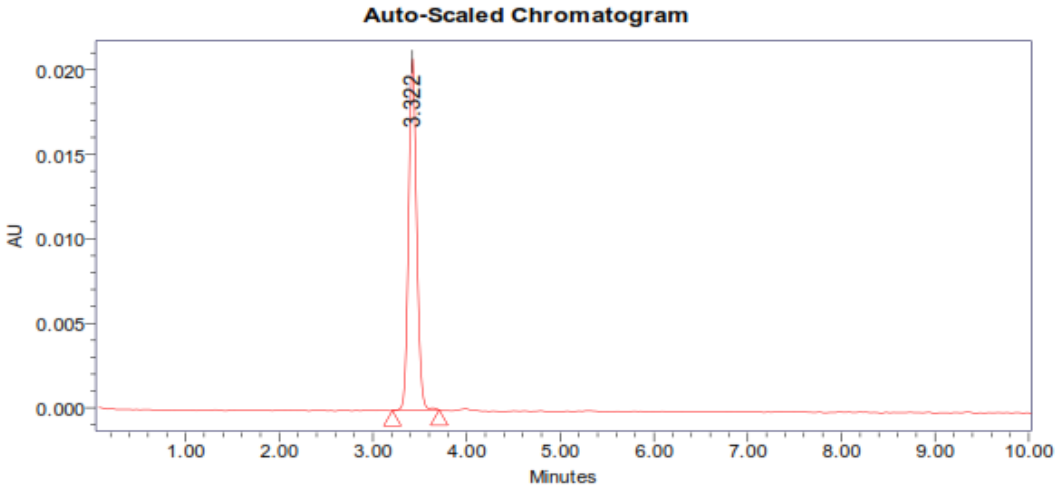


**Fig 2: Optimized Chromatogram (Sample)**

**Table 4: Results of Optimized Chromatogram (Sample)**

| S.No. | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Amisulpride | 3.322 | 145658 | 32654 | 1.78 | 8547 |

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.

## Method validation

### System suitability

**Table 5: Results of System Suitability for Amisulpride**

| S. No | | | Area | Height | | |
|---|---|---|---|---|---|---|
| 1 | Amisulpride | 3.398 | 145965 | 32653 | 8475 | 1.78 |
| 2 | Amisulpride | 3.324 | 146857 | 32785 | 8495 | 1.79 |
| 3 | Amisulpride | 3.349 | 145985 | 32598 | 8492 | 1.80 |
| 4 | Amisulpride | 3.388 | 146697 | 32695 | 8463 | 1.76 |
| 5 | Amisulpride | 3.364 | 145982 | 32675 | 8458 | 1.77 |
| **Mean** | | | 146380.25 | | | |
| **Std. Dev.** | | | 462.762 | | | |
| **% RSD** | | | 0.316137 | | | |

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

### Specificity

**Table 6: Peak results for assay standard**

| S.No | | | Area | Height | | |
|---|---|---|---|---|---|---|
| 1 | Amisulpride | 3.379 | 145857 | 32654 | 8546 | 1.76 |
| 2 | Amisulpride | 3.303 | 145874 | 32587 | 8574 | 1.77 |
| 3 | Amisulpride | 3.322 | 145685 | 32564 | 8759 | 1.76 |
| 4 | Amisulpride | 3.327 | 145876 | 32854 | 8598 | 1.76 |
| 5 | Amisulpride | 3.310 | 145682 | 32415 | 8564 | 1.77 |
| **Mean** | | | 145794.8 | | | |
| **Std. Dev.** | | | 101.8759 | | | |
| **% RSD** | | | 0.069876 | | | |

**Table-7: Peak results for Assay sample**

| S.No. | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | Amisulpride | 3.310 | 146425 | 32658 | 1.78 | 8457 | 1 |
| 2 | Amisulpride | 3.398 | 146874 | 32547 | 1.77 | 8495 | 2 |
| 3 | Amisulpride | 3.388 | 146524 | 32658 | 1.78 | 8475 | 3 |

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Amisulpride in pharmaceutical dosage form was found to be 99.57%

### Linearity

**Table 8: Chromatographic Data for Linearity Study**

| Concentration µg/ml | Average Peak Area |
|---|---|
| 60 | 85784 |
| 80 | 112564 |
| 100 | 139867 |
| 120 | 165248 |
| 140 | 189586 |

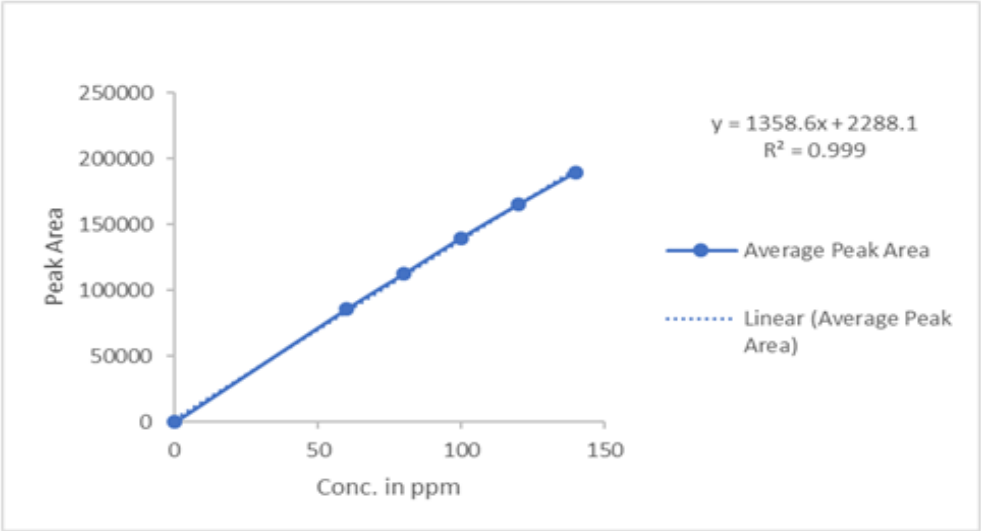


**Fig 3: Calibration Curve of Amisulpride**

Correlation Coefficient (r) is 0.99, and the intercept is 2288. These values meet the validation criteria.

## Accuracy

**Table 9: The accuracy results for Amisulpride**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 70031.67 | 50 | 49.884 | 99.768% | 99.86% |
| 100% | 138413.33 | 100 | 100.239 | 100.239% | |
| 150% | 205138 | 150 | 149.374 | 99.582% | |

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

## Limit of detection for amisulpride

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

**LOD= 3.3 × σ / s**

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

**Result:**

LOD=

=1.5µg/ml

**Quantitation limit**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

**LOQ=10×σ/S**

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

**Result:**

LOQ =

=4.5µg/ml

### Robustness

**Table 10: Results for Robustness**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 145867 | 3.388 | 8457 | 1.76 |
| Less Flow rate of 0.9 mL/min | 146854 | 3.595 | 8152 | 1.74 |
| More Flow rate of 1.1 mL/min | 135262 | 3.122 | 7985 | 1.73 |
| More organic phase (about 5 % Increase in Methanol) | 143652 | 3.119 | 8142 | 1.72 |
| Less organic phase (about 5 % decrease in Methanol) | 142546 | 3.545 | 7985 | 1.75 |

## CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Amisulpride in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Amisulpride was found to be soluble in organic solvents such as DMSO and dimethyl formamide (DMF). It is very slightly soluble in ethanol and sparingly soluble in acetone and tetrahydrofuran (THF), very poorly soluble in water. Methanol: Acetate Buffer (pH-4.2) (40:60 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed inTables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Amisulpride in bulk drug and in Pharmaceutical dosage forms.

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