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# VALIDATION OF STABILITY INDICATING RP-HPLC METHOD ANALYSIS FOR ASSAY OF AMISULPRIDE IN INJECTION

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## ABSTRACT

A simple and precise method was developed for the assay of Amisulpride from injection formulation. The solvent system and wavelength were optimized in order to maximize the sensitivity of the proposed method; Amisulpride shows the maximum absorbance at 280 nm. The separation was achieved on RP-HPLC Isocratic system equipped with HPLC Agilent 1200 series. The mobile phase was prepared with buffer pH 4.5: Acetonitrile: Methanol in the ratio of 75:20:5 O-phosphoric acid used for the pH adjustment (pH-4.5). The method was validated for accuracy, precision, linearity, specificity, stressed studies, robustness, etc. Linearity was observed in the concentration range of 70-130 % and gave mean correlation coefficient 0.999. The developed RP-HPLC method was found to be accurate, precise and was successful applied to a pharmaceutical injection formulation for qualitative estimation of Amisulpride.

**KEYWORDS:** Amisulpride, RP-HPLC, Force degradation, method validation.

## 1. INTRODUCTION

RP-HPLC involves the separation of compound. Good resolution that can be achieved under a wide range of chromatographic conditions. Chromatographic selectivity can be manipulated through changes in mobile phase characteristics and other important factors.

Amisulpride is an antipsychotic medication used to treat schizophrenia. It is also used to treat dysthymia. It is usually classed with the atypical antipsychotics Chemically it is a benzamide and like totally different benzamide antipsychotics, such as sulpiride, it is associated with a high risk of elevating blood levels of the lactation internal secretion, gonadotropic hormone (thereby doubtless inflicting the absence of the oscillation, breast

enlargement, even in males, breast milk secretion not associated with breastfeeding, impaired fertility, impotence, breast pain, etc.), and a low risk, relative to the typical antipsychotics, of causing movement disorders. It has conjointly been found to be with modesty more practical in treating psychosis than the standard antipsychotics. Amisulpride is believed to figure by reducing signalling via the monoamine neurotransmitter D2 receptor.

**Chemical Structure of Amisulpride**

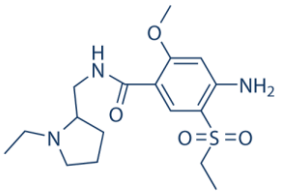


**Figure 1: Amisulpride.**

## 2. MATERIALS AND METHODS

### 2.1. Materials

Amisulpride, Active Pharmaceutical Ingredient (API) and working standard was supplied by Alkem Laboratories (Taloja, Navi Mumbai). Injection of Amisulpride as a sample for respected study was provided by the same firm.

### 2.2. Chemicals and Reagents

1) Potassium Dihydrogen Phosphate : AR grade or equivalent**.**
2) Water : HPLC grade or equivalent
3) Methanol : HPLC grade or equivalent
4) Acetonitrile : HPLC grade or equivalent
5) Orthophosphoric acid : AR grade or equivalent
6) Sodium Hydroxide : AR grade or equivalent

### 2.3. Apparatus and Equipment

1) HPLC equipped with pump, injector and UV Detector, Make: Agilent 1200 Series
2) Analytical Balance: Make: Mettler Toledo
3) Hot air oven: Make: Thermolab
4) Photostability Chamber: Make: Newtronic
5) pH meter: LAB INDIA, model PICO$^{+}$

6) Sonicator: Spectra lab, UCB 300D

### 2.4. Chromatographic conditions

Chromatographic separation was performed on a reverse phase Peerless basic $C_{18}$ shield. The mobile phase was a mixture of buffer pH 4.5: Acetonitrile: Methanol (75:20:5 % (v/v)).

| | |
|---|---|
| Column : | Peerless basic, 150mm x 4.6mm, 5µm (C18 or equivalent) |
| Flow rate | 1.5 ml /minute |
| Wavelength | 280 nm |
| Injection volume | 20µl |
| Column Temperature | 30°C |
| Sample Compartment Temperature | 25°C |
| Run Time | 5 minutes |
| Retention Time | 2.7 minutes of Ivabradine HCl |

#### a. Preparation of standard solution

Accurately about 25 mg of Amisulpride standard was weighed and transferred into 100 ml volumetric flask. About 70 ml of mobile phase was added, and then solution was sonicated to dissolve, then cooled at room temp and diluted up to the mark with mobile phase.

Further 5 ml of this solution was diluted to 100 ml with mobile phase.

#### b. Analysis of Marketed Formulation

Transfer 5ml of sample containing the equivalent of about 12.5mg of Amisulpride into a 100 ml volumetric flask. Add 70 ml of mobile phase sonicate for 5 minutes with intermittent shaking and make up the volume to 100 ml with mobile phase. Further dilute 5ml of this solution to 50 ml with mobile phase. Filter through 0.45µ PVDF filter/0.45µ nylon filter[#].

## 3. METHOD VALIDATION

The method was validated for linearity, accuracy, precision, Specificity, robustness, filter paper study, solution stability and forced degradation study.

### 3.1. Accuracy

Placebo was spiked with the known amount of Amisulpride at 70%, 100% and 130% of test concentration as Amisulpride injection (2.5mg Amisulpride per ml). The amount of Amisulpride was quantified as per the test method. The percentage recovery was calculated from the amount found and the actual amount added.

### 3.2. Precision

The instrument precision was evaluated by determining the absorbance of the standard solution six times repeatedly. The results are reported in terms of relative standard deviation for the same. The intra-and inter-day variation for the determination was carried out in triplicate for the standard solution.

### 3.3. Linearity

Linearity for Amisulpride performed in the range of 8.877mcg/ml to 16.486 mcg/ml (about 70% to 130%) test concentration. A graph was plotted with concentration (in mcg/ml) on x-axis and peak areas on y-axis. Slope, y-intercept, correlation coefficient (r-value) and residual sum of squares (RSS) were determined from the obtained results.

### 3.4. Specificity

Blank (mobile phase), Placebo, standard and sample solution were injected into the HPLC system. There was no interference from the blank and placebo at the retention time of Amisulpride peak. Peak purity data reveals that Amisulpride peak was homogeneous and there were no co-eluting peaks at the retention time of Amisulpride peak.

### 3.5. Stability in analytical solution

Stability of Amisulpride peak in analytical solution was verified by analyzing the standard solutions and sample solutions, initially and also at different time intervals as mentioned below by storing in sample compartment of HPLC instrument at 25°C. Calculated the % assay for standard solution and sample solutions.

### 3.6. Filter paper selection study

Selection of filter paper was evaluated by preparing the assay preparation in triplicate as per test method. Filtered the test solution through 0.45µm PVDF filter and 0.45µm Nylon filter analysed the samples against centrifuged a portion of sample solution. The % assay of Amisulpride was calculated and compared the results with method precision results.

### 3.7. Robustness

Robustness of the method was verified by deliberately varying the following instrumental conditions.

a. By changing the temperature by ± 5°C

b. By changing the flow rate by ± 10%

c. By changing the organic content in mobile phase by ± 2% absolute.

### 3.8. Forced Degradation

Forced degradation study was carried out by treating the sample under the following conditions along with control sample.

| Degradation Conditions | Condition Detail |
|---|---|
| Thermal | 60° C for 24 Hrs. |
| UV | 254 nm for 48 Hrs. |
| Acid | 5 mL 0.1 N HCl for 24 Hrs. |
| Alkali | 5 mL 0.1 N NaOH for 24 Hrs. |
| Oxidation | 5 mL 0.3% $H_2O_2$ for 24 Hrs. |

### 3.9. System Suitability

1. %Relative standard deviation for five replicate injections of Standard preparation should be not more than 2.

2. Tailing factor for Amisulpride peak should not be more than 2.0.

## 1. RESULT AND DISCUSSION

The method given here was found to be highly specific and linear according to the results. The peaks of the active ingredients and other additives as well as the degradation products were separated out by the HPLC method. Therefore, HPLC method is highly specific and stability indicating for degradation products of the dosage form and formulation excipients. The method can be applied for routine analysis of Amisulpride in liquid dosage form. Developed HPLC method was found to be stability indicating and very specific for estimation of Amisulpride in presence of other degradation products and various excipients used in solid dosage form. Complete validation studies for the method proved it to be specific, linear, robust and reproducible.

### 4.1. System Suitability

System Suitability was daily performed during the entire validation to ensure the suitability of method.

The system suitability and system precision results of developed HPLC method, are given in Table 3.

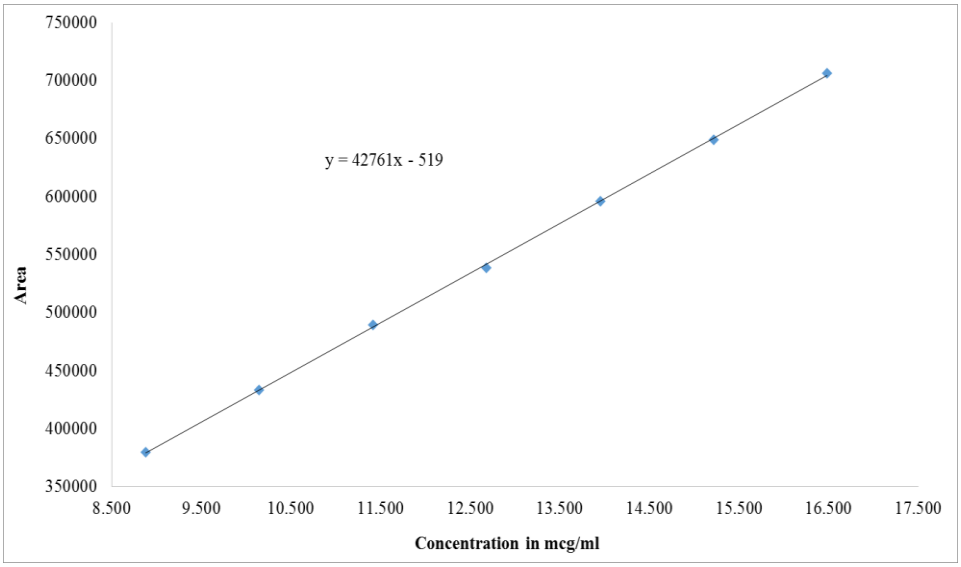
**Table 3: System suitability and System Precision.**

| Retention Time (Mean ± SEM) | Theoretical Plates (n) | Asymmetry/Tailing (T) |
|---|---|---|
| 2.462 | 5829 | 1.2 |

### 4.2. Linearity

Characteristics of the method from Standard calibration curve:

| | |
|---|---|
| Slope | 42761 |
| y-intercept | -519 |
| r-value | 0.99989 |
| RSS | 17894347 |




### 4.3. Specificity

The data demonstrate that there is no interference of blank, known impurity and placebo at retention time of Amisulpride.

Peak Purity in HPLC method.

| Sample type | Peak name | Retention Time | Purity match |
|---|---|---|---|
| Control Sample | Amisulpride | 2.464 | 999.993 |
| Standard | Amisulpride | 2.465 | - |

### 4.4. Stability indicating nature of the Developed Method-Forced degradation

Placebo preparation of Amisulpride, Amisulpride API, and samples i.e., injection of Amisulpride were exposed to various stress conditions showed the peak purity as that of the normal condition. The degradation study clearly indicated that Amisulpride degrades in peroxide and photolytic condition. The degradation results were shown in Table.

| Sr. No. | Condition | % Assay | % Degradation | Purity Match / Purity factor |
|---|---|---|---|---|
| 1 | Untreated Sample* | 102.36 | - | - |
| 2 | Acid Treated sample | 100.51 | 1.81 | 999.993 |
| 3 | Base Treated sample | 100.26 | 2.05 | 999.993 |
| 4 | Peroxide Treated sample | 99.92 | 2.38 | 999.992 |
| 5 | Heat Treated sample | 100.03 | 2.28 | 999.993 |
| 6 | UV-Visible Treated sample | 90.97 | 11.13 | 999.991 |

### 4.5. Method Precision

The method precision was obtained by determining the assay by preparing six-sample preparation. The low value of standard deviation i.e., >2 proved the method to be very precise and accurate.

| SR No | % Assay |
|---|---|
| 1 | 101.96 |
| 2 | 102.05 |
| 3 | 103.12 |
| 4 | 100.76 |
| 5 | 103.51 |
| 6 | 102.78 |
| Mean | 102.36 |
| SD | 0.989 |
| % RSD | 0.97 |

### 4.6. Intermediate Precision

Ruggedness of methodology(the tactic, the strategy) was verified by analysing the six samples of same batch that was used for methodology preciseness as per take a look at method by completely different analyst mistreatment different instrument and different column on different day. The % relative standard deviation (%RSD) for % assay of the six samples should not be more than 2 and the overall % RSD should not be more than 2. The results are tabulated in table.

| Sample No. | % Assay | |
|---|---|---|
| | Analyst-I | Analyst-II |
| 1 | 101.96 | 102.42 |
| 2 | 102.05 | 103.37 |
| 3 | 103.12 | 105.22 |
| 4 | 100.76 | 102.75 |
| 5 | 103.51 | 104.24 |
| 6 | 102.78 | 106.99 |
| **Mean** | 102.36 | 104.17 |
| **SD** | 0.989 | 1.719 |
| **%RSD** | 0.97 | 1.65 |

| | |
|---|---|
| **Over all Mean** | 103.26 |
| **Over all SD** | 1.635 |
| **Over all %RSD** | 1.58 |

### 4.7. Method Recovery

To determine the reliability and accuracy of the method recovery studies were carried out In triplicate at 70%, 100% and 130% of target concentration. Results of accuracy study are found to be within the range of 98% to 102% and RSD < 1%.

| **Level no. (Spike level in %)** | **Mean (%)** | **SD** | **% RSD** |
|---|---|---|---|
| **Level – 1 (70%)** | 98.47 | 0.155 | 0.16 |
| **Level – 2 (100%)** | 99.17 | 0.294 | 0.30 |
| **Level – 3 (130%)** | 99.53 | 0.246 | 0.25 |
| **Over all mean 99.06** | | | |
| **Over all SD 0.511** | | | |
| **Over all % RSD 0.52** | | | |

### 4.8. Method Robustness

Robustness of the method was determined by small deliberate changes in some Important chromatographic conditions like flow rate, organic phase ratio, buffer pH and column oven temperature. Even when these changes content of the drug failed to have an effect on adversely. The low values of relative standard deviation indicating that the HPLC method is robust for this particular parameter.

| **Sr. No.** | **Method Precision** | **Minus Flow** | **Plus Flow** | **Minus Temp.** | **Plus Temp.** | **Minus Org. Content** | **Plus Org. Content** | **Minus pH** | **Plus pH** |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 101.96 | 102.37 | 102.43 | 102.57 | 102.55 | 100.82 | 100.74 | 100.98 | 100.92 |
| 2 | 102.05 | 103.50 | 103.77 | 103.24 | 103.29 | 101.22 | 101.14 | 100.96 | 100.97 |
| 3 | 103.12 | 103.90 | 104.03 | 104.14 | 104.10 | 102.80 | 102.77 | 102.68 | 102.72 |
| 4 | 100.76 | | | | | | | | |
| 5 | 103.51 | | | | | | | | |
| 6 | 102.78 | | | | | | | | |
| Over all Mean | | 102.66 | 102.71 | 102.68 | 102.68 | 102.11 | 102.09 | 102.09 | 102.09 |
| Over all SD | | 0.984 | 1.034 | 0.997 | 0.994 | 1.013 | 1.032 | 1.012 | 1.022 |
| Overall % RSD | | 0.96 | 1.01 | 0.97 | 0.97 | 0.99 | 1.01 | 0.99 | 1.00 |

**Where,**

| Sr. No | Experiment (Actual Value) |
|---|---|
| 1 | Method Precision data |
| 2 | Minus flow (1.35ml) |
| 3 | Plus flow (1.65ml) |
| 4 | Minus temperature (25°C) |
| 5 | Plus Temperature (35°C) |
| 6 | Minus organic content (– 2 % absolute) |
| 7 | Plus organic content (+ 2% absolute) |
| 8 | Minus pH (4.3) |
| 9 | Plus pH (4.7) |

### 4.9. Solution Stability

Standard and sample solutions in HPLC method were evaluated at room temperature for 16 Hours. The solutions were analysed after 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 Hours the relative standard deviation was found to be below 2.0% in both the methods. It proves the Solution stability of standard and sample solution is at room temperature. The results of solution stability HPLC method are shown in Table.

Solution stability for standard solution.

| Time in hours | % Assay | Difference |
|---|---|---|
| Initial | 100.55 | - |
| 1 | 100.58 | 0.03 |
| 4 | 100.61 | 0.06 |
| 6 | 100.66 | 0.11 |
| 8 | 100.81 | 0.26 |
| 10 | 100.90 | 0.35 |
| 12 | 100.93 | 0.38 |
| 14 | 100.86 | 0.31 |
| 16 | 100.88 | 0.33 |
| 18 | 100.87 | 0.32 |
| 20 | 101.10 | 0.55 |
| 22 | 100.99 | 0.44 |
| 24 | 101.16 | 0.61 |

Solution stability for sample solution.

| Time in hours | % Assay | Difference |
|---|---|---|
| Initial | 101.93 | - |
| 1 | 101.98 | 0.05 |
| 4 | 102.13 | 0.20 |
| 6 | 102.17 | 0.24 |
| 8 | 102.38 | 0.45 |
| 10 | 102.40 | 0.47 |
| 12 | 102.45 | 0.52 |

| | | |
|---|---|---|
| 14 | 102.50 | 0.57 |
| 16 | 102.51 | 0.58 |
| 18 | 102.44 | 0.51 |
| 20 | 102.63 | 0.70 |
| 22 | 102.53 | 0.60 |
| 24 | 102.81 | 0.88 |

### 4.10. Assay

The developed and validated method was successfully applied for the estimation of Amisulpride in injection dosage form. The assay results were about 98-100%. The mean retention time was about 2.46. The results of assay by using this method indicate that the method is specific for the analysis of Amisulpride without interference from the excipients used to prepare and formulate these injections.

## 2. CONCLUSION

The above developed method is suitable for determination of Amisulpride in injection. The method was found to be highly sensitive, precise, and accurate for determination of Amisulpride in pharmaceutical dosage form. These methods are quite useful and reasonably satisfy all the standards. Therefore, these methods can be used for routine analysis of Amisulpride in pharmaceutical dosage form i.e., injection form. More over the HPLC method has also proved to be stability indicating because it can separate degradation peaks from the main peaks and accurately quantifies it in the stability samples.

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