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QUALITY BY DESIGN (QBD) BASED APPROCH TO ANALYTICAL VALIDATION RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF AMISULPRIDE IN BULK AND TABLETS

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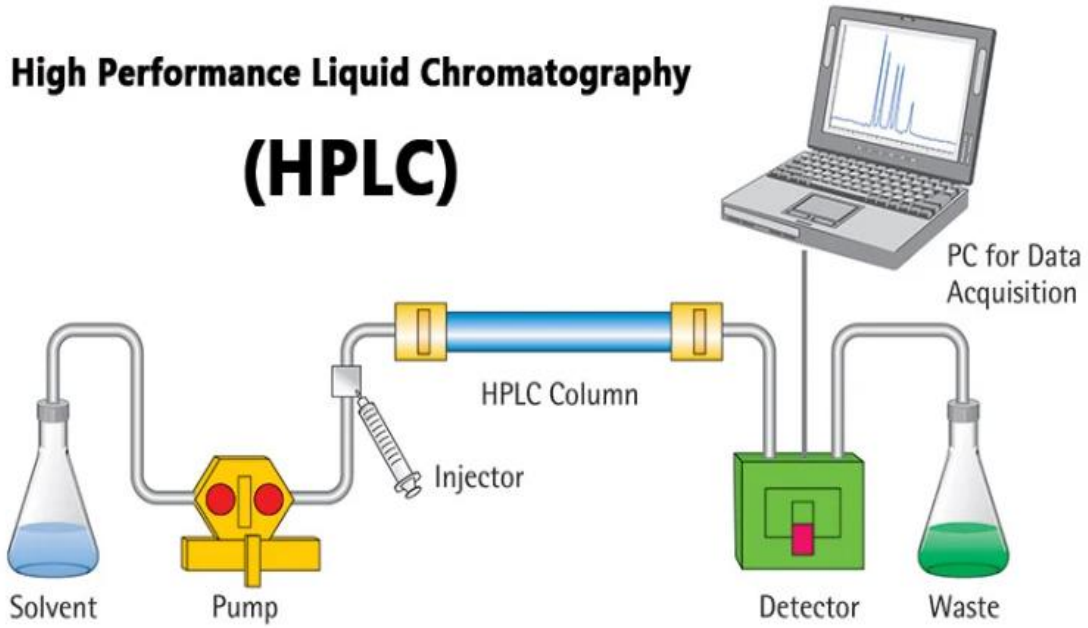
ABSTRACT

Amisulpride is a second-generation (atypical) antipsychotic belonging to the substituted benzamide class. It exhibits selective dopamine D2 and D3 receptor antagonism, distinguishing it from many other atypical antipsychotics that also target serotonin receptors. **Pharmacological profile:** At low doses (50–300 mg/day): Blocks presynaptic D2/D3 autoreceptors, enhancing dopamine transmission—useful for negative symptoms (e.g., apathy, social withdrawal). At high doses (400–800+ mg/day): Blocks postsynaptic D2/D3 receptors, reducing positive symptoms (e.g., hallucinations, delusions). **Therapeutic Uses:** (1) Schizophrenia: Approved for the treatment of both positive and negative symptoms, Especially beneficial in patients who show primary negative symptoms or are sensitive to sedation and weight gain caused by other antipsychotics. (2) Dysthymia and Bipolar Depression (off-label in some countries): Low-dose amisulpride has shown antidepressant effects. (3) Nausea and Vomiting: Low-dose IV amisulpride (Barhemsys®) is FDA-approved for postoperative nausea and vomiting (PONV). (4) Functional Gastrointestinal Disorders (investigational): Being explored for functional dyspepsia and gastroparesis due to its gastroprokinetic properties. **Advantages:** Lower risk of metabolic side effects (e.g., weight gain, diabetes), Minimal sedation, Good efficacy for both acute and maintenance treatment. **Limitations:** Risk of hyperprolactinemia, especially at higher doses, QT prolongation potential—caution in cardiac-compromised patients, Not approved in the U.S. for psychiatric use (available in Europe, Asia, etc.). **Aim of the study:** The primary aim of this study is to develop and validate a robust, precise, and accurate Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the quantitative analysis of Amisulpride in bulk and tablet dosage forms, employing the Quality by Design (QbD) approach. This methodology integrates systematic experimental design and risk assessment tools to identify and control critical method variables, thereby ensuring method robustness, reliability, and regulatory compliance in accordance with ICH Q2(R1) guidelines.

KEYWORD:- Amisulpride, Validation, RP- HPLC, Optimization, QBD.

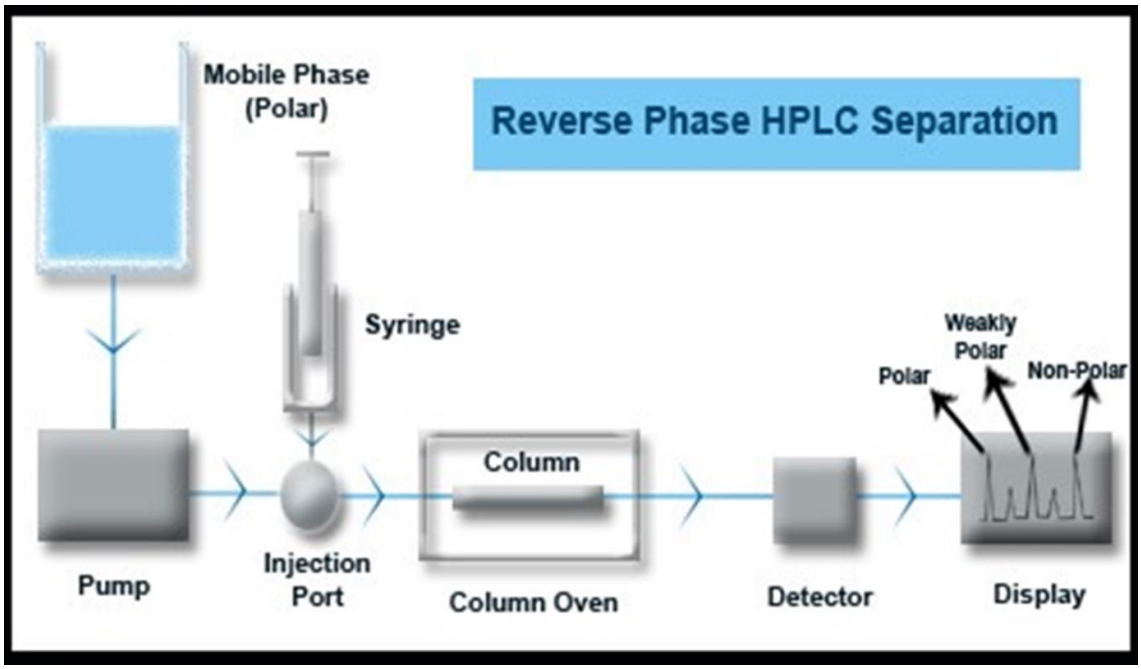
INTRODUCTION

- High-performance liquid chromatography or commonly known as HPLC, is an analytical technique used to separate, identify or quantify each component in a mixture. The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy. In the 1960s, the column chromatography LC with its low-pressure suitable glass columns was further developed to the HPLC with its high-pressure adapted metal columns. HPLC is thus basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres.



Reverse phase

- The column packing is non-polar (e.g C18), the mobile phase is water+ miscible solvent (e.g methanol). It can be used for polar, non-polar, ionizable, and ionic samples.



Validation of analytical method

"Validation of analytical method is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required sensitivity, accuracy, precision, ruggedness, etc."

Depending upon the application, if delayed to an analytical procedure, it means that a method works reproducibly, when carried out by same or different persons, in same or different laboratories, using different reagents, different equipments, etc.[43]

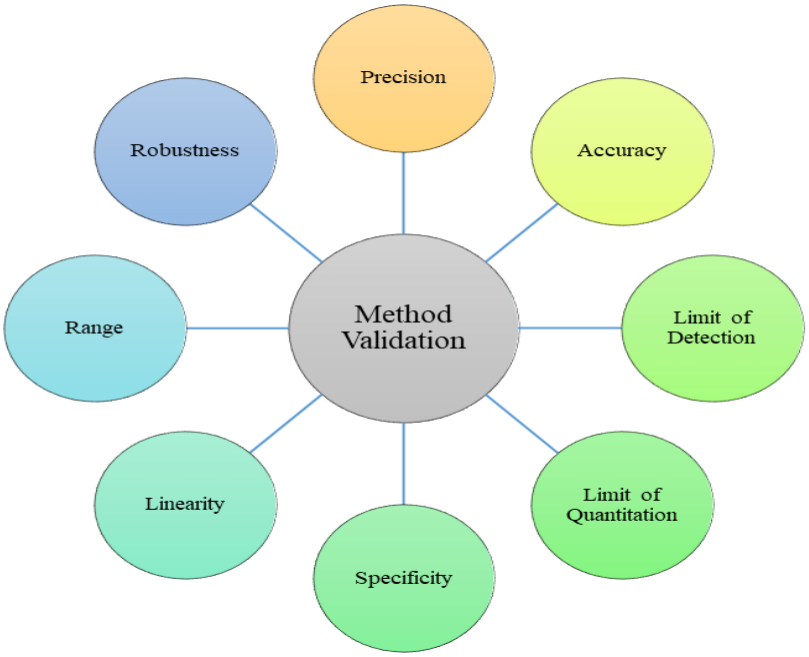
The different validation parameters are

a) Accuracy,
b) Precision,
c) Linearity and Range,
d) Limit of detection (LOD),
e) Limit of quantitation (LOQ),
f) Selectivity/ Specificity,
g) Robustness/ Ruggedness and
h) Stability and System suitability studies.

Reasons for validation

There are two important reasons for validation of assays in the pharmaceutical industry.

1. Assay validation is an integral part of the quality-control system and this is most important reason.



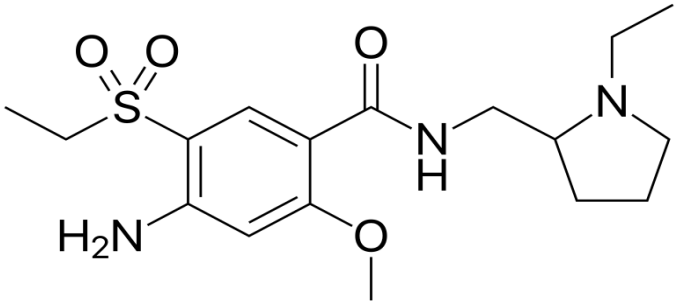
Introduction to quality by design

Quality has been given an importance by all regulatory bodies for pharmaceutical products. Quality indicates customer satisfaction in terms of service, product, and process. Many of these quality related activities reflect requirement for companies to excel in global competition. Customer demands the perfection in quality, reliability, low cost and timely performance. Customer satisfaction can be attained by two ways, first features and second free from deficiencies in goods. The features like performance, trustworthiness, robustness, ease of use, and serviceability have to build in the product and such product should be free from deficiencies. Quality, productivity, cost, cycle time and value are interconnected terms. Quality activities must try to find quality problems early enough to allows actions without demanding compromise in cost, schedule or quality. The emphasis must be on precaution rather than on just correction of quality problems. Quality can be the driving force to empower results in other parameters. Hence the quality has to be built in the product along with services through proper planning, thus the forth coming failure can be avoided. Mere analysis of final product will not work but the quality should be designed in the product. The concept of quality by design was summarized by a well-known quality expert Joseph Moses Juran; he believed that quality could be planned and that most quality related problems have their origin in the way which quality was planned in the first place. The principles of QbD have been used to advance the product and process quality in every industry. Because of need of potent drug with safety profile, pharmaceutical industries are investing billions of monies in the drug discovery and development process with endeavour to design quality product and.

Drug profile

Amisulpride

Amisulpride, sold under the brand names Solian and Barhemsys, is a medication used in the treatment of schizophrenia, acute psychotic episodes, depression, and nausea and vomiting. It is specifically used at lower doses intravenously to prevent and treat postoperative nausea and vomiting; at low doses by mouth to treat depression; and at higher doses by mouth to treat psychosis. It is usually classed with the atypical antipsychotics. Chemically it is a benzamide and like other benzamide antipsychotics, such as sulpiride, it is associated with a high risk of elevating blood levels of the lactation hormone, prolactin (thereby potentially causing the absence of the menstrual cycle, breast enlargement, even in males, breast milk secretion not related to breastfeeding, impaired fertility, impotence, breast pain, etc.), and a low risk, relative to the typical antipsychotics, of causing movement disorders. Amisulpride is believed to work by blocking, or antagonizing, the dopamine D2 receptor, reducing its signalling. The effectiveness of amisulpride in treating dysthymia and the negative symptoms of schizophrenia is believed to stem from its blockade of the presynaptic dopamine D2 and D3 autoreceptors.



Structure of amisulpride
IUPAC Name: (RS)-4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-ethylsulfonyl-2-methoxybenzamide
Molecular formula: $C_{17}H_{27}N_3O_4S$
Molecular weight: Average: 369.48 g/mol
Monoisotopic: 369.17
Pka: 19.10 (Strongest Acidic), 9.37 (Strongest Basic)
Categories: Antidepressive Agents, Second-Generation

Antiemetics
Antipsychotic agents
Solubility: Amisulpride is a white to off-white powder with the following solubility.

Characteristics
Water: Practically insoluble
Ethanol: Sparingly soluble, with a solubility of approximately 1 mg/ml
Methanol: Soluble
Dichloromethane: Freely soluble
DMSO: Soluble, with a solubility of approximately 15 mg/ml
Dimethyl formamide (DMF): Soluble, with a solubility of approximately 15 mg/ml
Aqueous buffers: Sparingly soluble, but can be dissolved in DMF and then diluted with the buffer

Mechanism of action
Dopamine is an essential and critical neurotransmitter produced in the substantia nigra and ventral tegmental regions of the brain. Dopaminergic projection function in the nigrostriatal, mesolimbic, and mesocortical systems. Hyperactive dopamine transmission in the mesolimbic areas, or dopamine dysregulation in various major brain regions, is understood as the key driver of positive and negative symptoms of schizophrenia. Many antipsychotic agents act as D2 receptor antagonists, as with amisulpride. Amisulpride is a selective dopamine D2 and D3 receptor antagonist. It has high preferential activity towards dopamine receptors in the limbic system rather than the striatum, leading to a lower risk of extrapyramidal side effects than other atypical antipsychotic agents. At low doses, amisulpride reduces negative symptoms of schizophrenia by blocking pre-synaptic dopamine D2 and D3 receptors, increasing the levels of dopamine in the synaptic cleft and facilitating dopaminergic transmission. At higher doses, amisulpride blocks postsynaptic receptors, inhibiting dopaminergic hyperactivity: this explains the drug improving positive symptoms. Amisulpride also works as an antagonist at 5-HT7A receptors 8 and 5-HT2A receptors, which may be related to its antidepressant effects.

The chemoreceptor trigger zone (CTZ), also commonly known as the area postrema (AP), is an important brain region located within the dorsal surface of the medulla oblongata. CTZ is involved in emesis: it contains receptors, such as dopamine receptors, that are activated in response to emetic agents in the blood and relay information to the vomiting center, which is responsible for inducing the vomiting reflex. Amisulpride is an antiemetic agent that works to limit signals that promote nausea and vomiting. Amisulpride binds to D2 and D3 receptors in the CTZ, leading to reduced dopaminergic signalling into the vomiting center.

Pharmacokinetics and Pharmacodynamics
Amisulpride is a selective dopamine D2 and D3 receptor antagonist with no affinity towards other dopamine receptor subtypes. Amisulpride is an atypical antipsychotic agent that works as an antagonist at dopamine receptors in the limbic system. Since it works preferentially in the limbic system, amisulpride is less likely to be associated with extrapyramidal adverse effects than other atypical antipsychotic agents. Amisulpride has no affinity for serotonin, alpha-adrenergic, H1-histamine, cholinergic, and sigma receptors. In clinical trials, amisulpride improved reduced secondary negative symptoms, affective symptoms, and psychomotor retardation in patients with acute exacerbation of schizophrenia. Notably, amisulpride has a differential target binding profile at different doses: at low doses, amisulpride selectively binds to presynaptic dopamine autoreceptors. At high doses, it preferentially binds to post-synaptic dopamine receptors. This explains how amisulpride reduces negative symptoms at low doses and mediates antipsychotic effects at high doses. One study alluded that the antinociceptive effects of amisulpride are mediated through opioid receptor acvitation and D2 receptor antagonism. The actions of amisulpride at opioid receptors may explain its pro-convulsant properties.

Amisulpride is also an antiemetic agent that prevents and alleviates postoperative nausea and vomiting. It primarily

works by blocking dopamine signalling in the chemoreceptor trigger zone, which is a brain area that relays stimuli to the vomiting center.

In clinical trials comprising Caucasian and Japanese subjects, amisulpride caused dose- and concentration-dependent prolongation of the QT interval; thus, intravenous infusion under a strict dosing regimen and close monitoring of patients with pre-existing cardiovascular conditions are recommended. Amisulpride increases plasma prolactin levels, leading to an association with benign pituitary tumours such as prolactinoma.

Absorption
Following oral administration, amisulpride is rapidly absorbed with absolute bioavailability of 48%.1 Amisulpride has two absorption peaks, with one rapidly achieved within one hour post-dose and a second peak occurring between three to four hours post-dose. Following oral administration of a 50 mg dose, two peak plasma concentrations were 39 ± 3 and 54 ± 4 ng/mL.

Following intravenous administration, the peak plasma concentration of amisulpride is achieved at the end of the infusion period and the plasma concentration decreases by 50% within approximately 15 minutes. The AUC(0-∞) increases dose-proportionally in the dose range from 5 mg to 40 mg, which is about four times the maximum recommended dose. In healthy patients receiving intravenous amisulpride, the mean (SD) Cmax was 200 (139) ng/mL at the dose of 5 mg and 451 (230) ng/mL at the dose of 10 mg. The AUC ranged from 136 to 154 ng x h/mL in the dose range of 5 mg to 10 mg. In patients undergoing surgery, the mean (SD) Cmax ranged from 127 (62) to 161 (58) ng/mL at the dose of 5 mg. At the dose of 10 mg, it was 285 (446) ng/mL. The AUC ranged from 204 to 401 ng x h/mL.

Volume of distribution
Following oral administration, the volume of distribution is 5.8 L/kg.11 Following intravenous infusion, the mean volume of distribution of amisulpride is estimated to be 127 to 144 L in surgical patients and 171 L in healthy subjects.

Protein binding
Plasma protein binding ranges from 25% to 30% in the concentration range from 37 to 1850 ng/mL. Amisulpride distributes into erythrocytes.

Interactions
The therapeutic efficacy of Teneligliptin can be decreased when used in combination with Bendroflumethiazide. The therapeutic efficacy of Teneligliptin can be decreased when used in combination with Benzthiazide. The risk or severity of hypoglycemia can be increased when Bepridil is combined with Teneligliptin.

Route of elimination
Following intravenous administration, about 74% of amisulpride is excreted in urine, where 58% of the recovered dose was excreted as unchanged amisulpride. About 23% of the dose is excreted in feces, with 20% of the excreted dose as unchanged parent drug. Following intravenous administration, about four metabolites were identified in urine and feces, accounting for less than 7% of the total dose administered. About 22 to 25% of orally administered amisulpride is excreted in urine, mostly as the unchanged parent drug.

Half Life
Elimination is biphasic. The elimination half-life of amisulpride is approximately 12 hours after an oral dose.

Clearance
The plasma clearance of amisulpride is 20.6 L/h in surgical patients and 24.1 L/h in healthy subjects following intravenous administration. Renal clearance was estimated to be 20.5 L/hr (342 mL/min) in healthy subjects.

Side effects
Hypoglycemia and constipation are the main adverse events.

Toxicity
In mice, oral LD50 is 1024 mg/kg, intraperitoneal LD50 is 175 mg/kg, and subcutaneous LD50 is 224 mg/kg. The Lowest published toxic dose (TDLo) following subcutaneous administration is 0.24 mg/kg in rats. The oral TDLo in men is 4.3 mg/kg.

Oral doses of amisulpride above 1200 mg/day are associated with adverse effects related to dopamine-2 (D2) antagonism. Cardiovascular adverse reactions include prolongation of the QT interval, torsades de pointes, bradycardia, and hypotension. Neuropsychiatric adverse reactions include sedation, coma, seizures, and dystonic and extrapyramidal reactions. As there is no specific antidote for amisulpride overdosage, management includes cardiac monitoring and treatment of severe extrapyramidal symptoms. Drug elimination with the use of hemodialysis is effective. Severe extrapyramidal effects may be managed with anticholinergic drugs.

MATERIAL AND METHOD

Table No. 6.1: Active pharmaceutical drug.

Sr. No.	Name	Description
1.	Amisulpride (API)	White to off-white crystalline powder; used to prevent and treat nausea and vomiting that may occur after surgery.
2.	Amisulpride Tablets 50mg IP.	50.0 mg drug contain each tablet, Manufactured by Mylan Laboratories Limited, Marketed by Mylan Pharmaceuticals Pvt. Ltd.

Table No. 6.2: List of chemicals use in research work.

Sr No.	Name of Chemical	Molecular Formula	Properties	Manufacturer
1.	Acetonitrile	C_2H_3N	Organic mobile phase component	Merck (Millipore)
2.	Methanol	CH_3OH	Organic mobile phase component	Merck (Millipore)
3.	Orthophosphoric Acid	H_3PO_4	pH adjustment of mobile phase (buffer)	Loba Chemie
4.	Potassium Dihydrogen Phosphate	KH_2PO_4	Buffer component for maintaining pH of mobile phase (buffer)	Merck (Millipore)
5.	Sodium Hydroxide	NaOH	pH adjustment	SD Fine-Chem
6.	Hydrochloric Acid	HCl	pH adjustment	SD Fine-Chem
7.	Distilled Water	H_2O	Universal Solvent, BP 100°C	Inhouse

Table No. 6.3: List of instruments.

Sr. No.	Name of Equipment's/ Instruments	Model /Specification	Manufacturer
1	HPLC	Utlimate3000	Thermo
	Pump	PU2080	
	Sample Injection Port	Rheodyne Injector	
	UV/Vis Detector	UV 2075 plus	
	Software	LabSoltuion	
	Kinetex C18	HPLC Column	
2	UV-Visible Spectrophotometer	UV-1800	Shimadzu
3	pH Meter	101	Mettler Toledo
4	Balance	AY-120	Shimadzu
5	Sonicator	UCB-40	Rolex
6	Oven (Hot Air or Vacuum)	AYT2022	Thermo Scientific
7	Deep Freezer	-	Blue Star
8	Refrigerator	-	Godrej

Validation

The proposed HPLC method was validated in terms of system suitability, specificity, precision, accuracy and robustness as per the International Conference on Harmonization (ICH) guidelines.[7]

1. Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 10-60 µg/ml for Amisulpride. Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves. The correlation coefficient was 0.9998. Linearity graph of concentration (as x-value) versus area (as y-value) were plotted and correlation coefficient, y-intercept and slope of the regression were calculated.

Weigh accurately and transferred about 10mg of Amisulpride standard into 100 ml volumetric flask. Added about 50 ml of Mobile Phase, sonicate with intermediate shaking to dissolved and dilute up to the volume with Mobile Phase. Mixed well and injected as 10 µg/ml to 60 µg/ml from stock solution (Concentration is 100 µg / ml and 1000 µg / ml). Preparation of linearity level solution: Prepare the linearity level as per the table below table 2.1.

Then, a graph was plotted between concentrations and peak area. Linearity plot was shown in Fig. 8.1.

Table 8.1: Linearity result of amisulpride.

Linearity		
Sr. No	**Concentration (µg/mL)**	**Peak Area**
1	10	793208
2	20	1586416
3	30	2329624

4	40	3172832
5	50	3966040
6	60	4759248
Slope		79463.66
Standard Error		22625.31

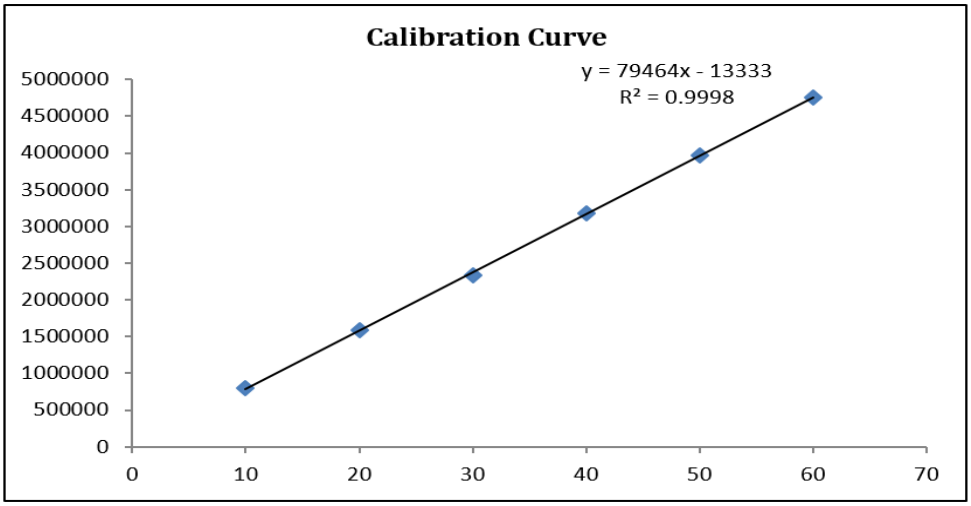


Figure 8.1: Calibration curve of amisulpride.

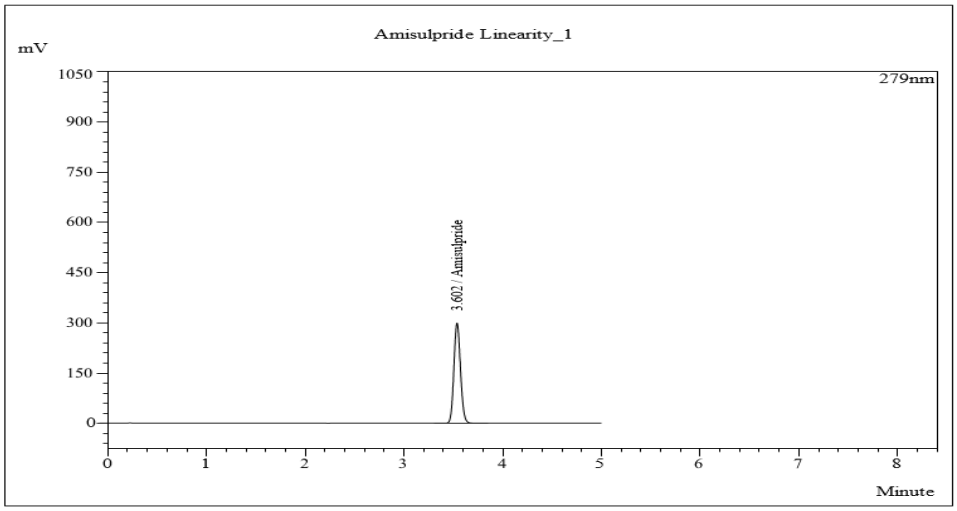


Figure 8.2: A typical chromatogram of amisulpride standard linearity 1.

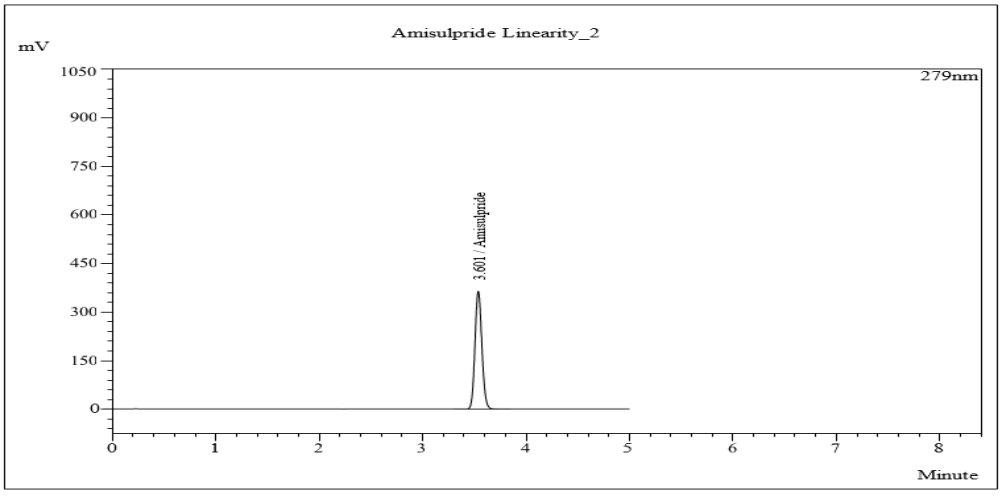


Figure 8.3: A typical chromatogram of amisulpride standard linearity 2.

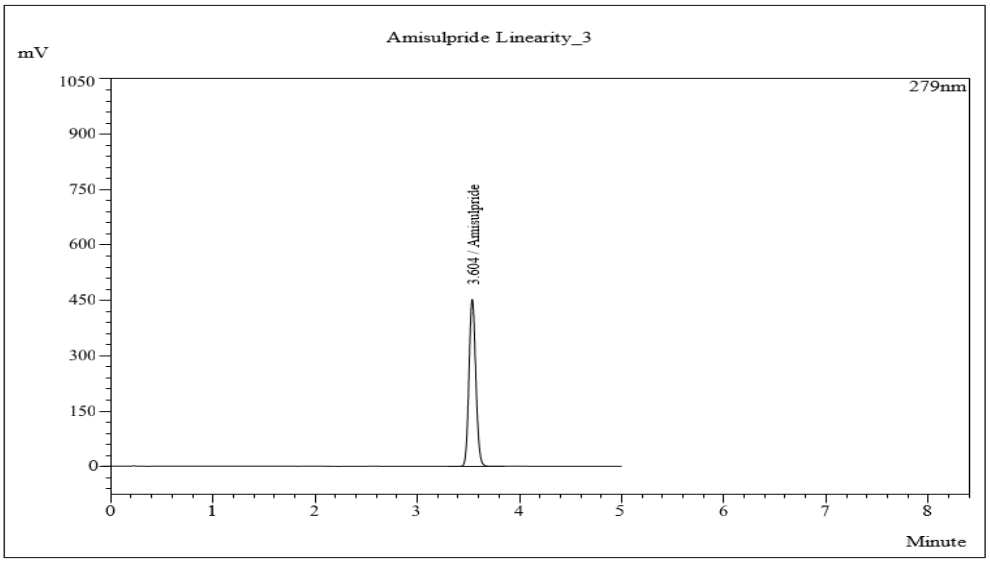


Figure 8.4: A typical chromatogram of amisulpride standard linearity 3.

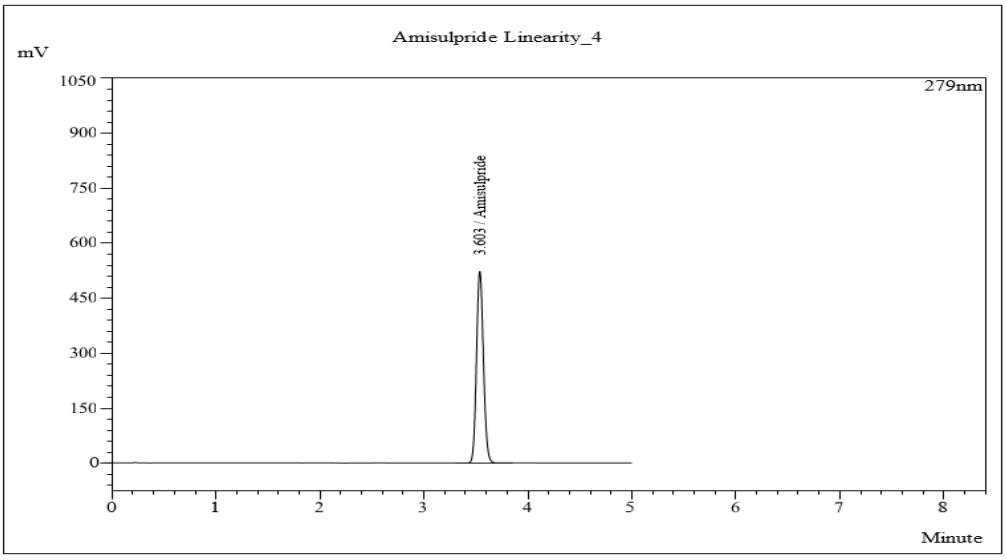


Figure 8.5: A typical chromatogram of amisulpride standard linearity 4.

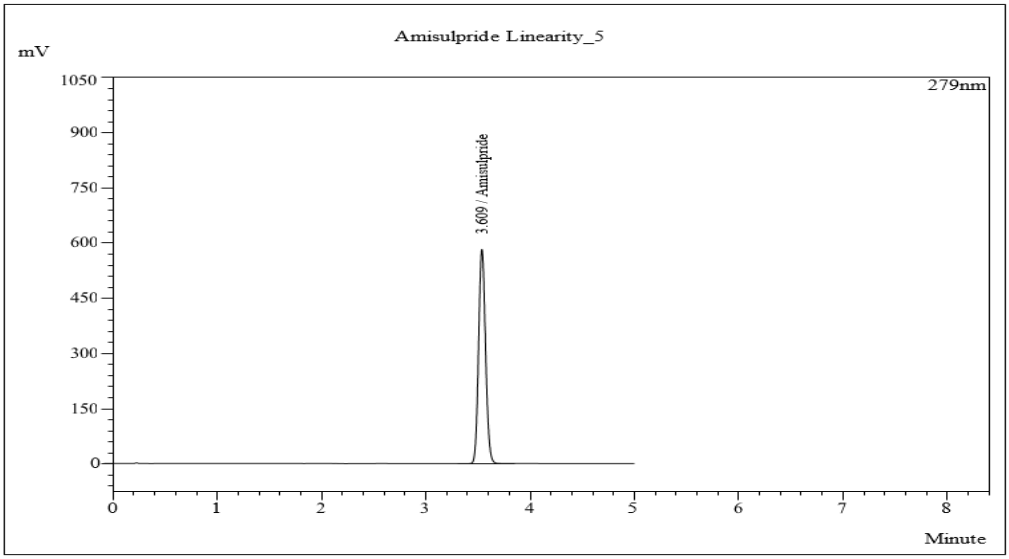


Figure 8.6: A typical chromatogram of amisulpride standard linearity 5.

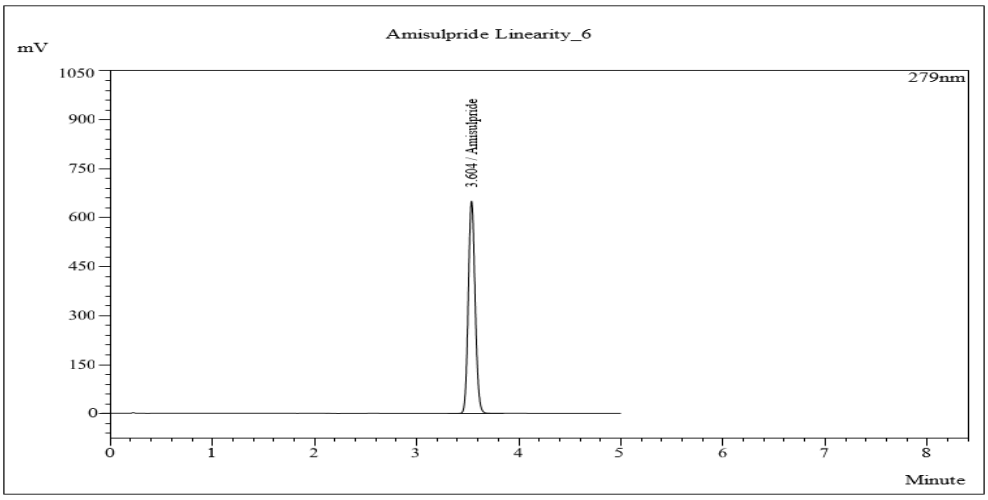


Figure 8.7: A typical chromatogram of amisulpride standard linearity 6.

Table 8.2: Characteristic parameters of Amisulpride for the proposed HPLC method.

Parameter	Result
	Amisulpride
Calibration range (µg/ml)	10-60
Detection wavelength (nm)	279
Mobile phase	Methanol: Buffer
Regression equation (y*)	y = 79464x - 13333
Slope (b)	79464.10
Intercept (a)	13333
Correlation coefficient(r2)	R^2 = 0.9999

2. System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 50 µg/ml. The results which are given in Table 8.3 were within acceptable limits.

Table 8.3: System suitability studies of Amisulpride by HPLC method.

System suitability parameter		
Retention time (min)	**Concentration (µg/mL)**	3.602
Peak area	10	793208
Theoretical plates		12709
Asymmetric Factor		1.121

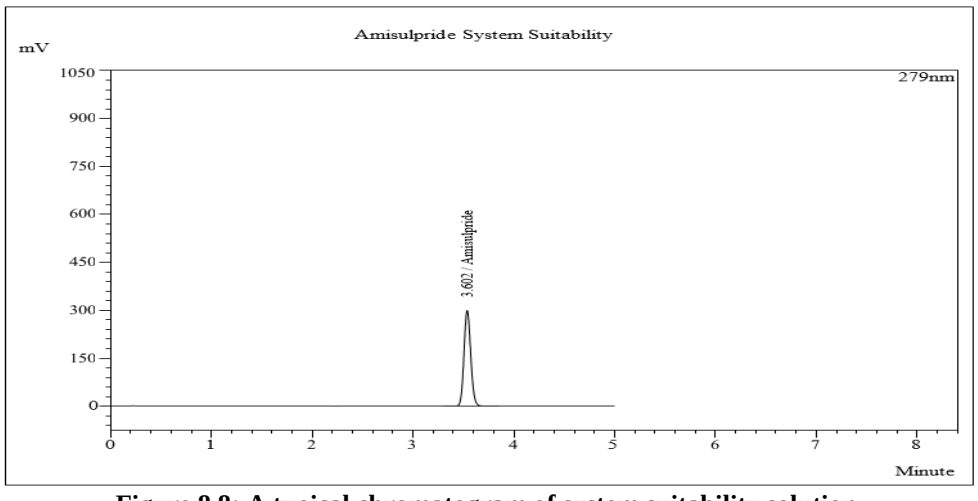


Figure 8.8: A typical chromatogram of system suitability solution.

3. Specificity

Chromatogram of blank was taken as shown in Fig No.8.10. Chromatogram of Amisulpride showed peak at a retention time of 3.604 min. The mobile phase designed for the method resolved the drug very efficiently. The Retention time of Amisulpride was 3.601 ± 0.0051min. The wavelength 279 nm was selected for detection because; it resulted in better detection sensitivity for the drug. The peak for Amisulpride from the tablet formulation was Amisulpride.

Table 8.4: Specificity of Amisulpride by HPLC method.

Specificity				
Sample	**Label Claim (mg)**	**Amount Found**	**Recovery**	**Retention Time**
Tablet	50	49.91	99.82	3.602

Table 8.5: Formulation used in specificity of amisulpride.

Formulation	
Name of Formulation	Amisulpride Tablets 50mg IP.
Type of Formulation	Tablet
Concentration (mg)	50

Figure 8.9: A typical chromatogram of blank.

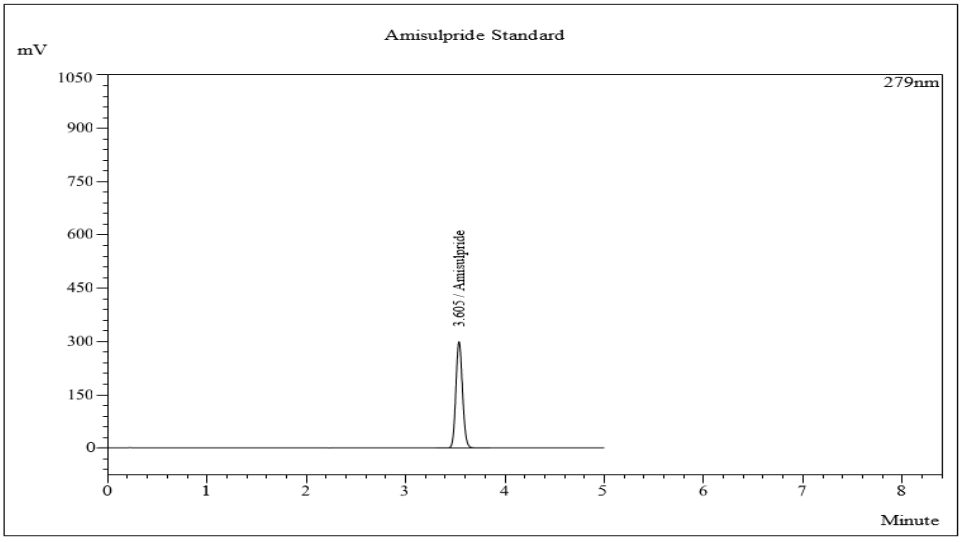


Figure 8.10: A typical chromatogram of standard.

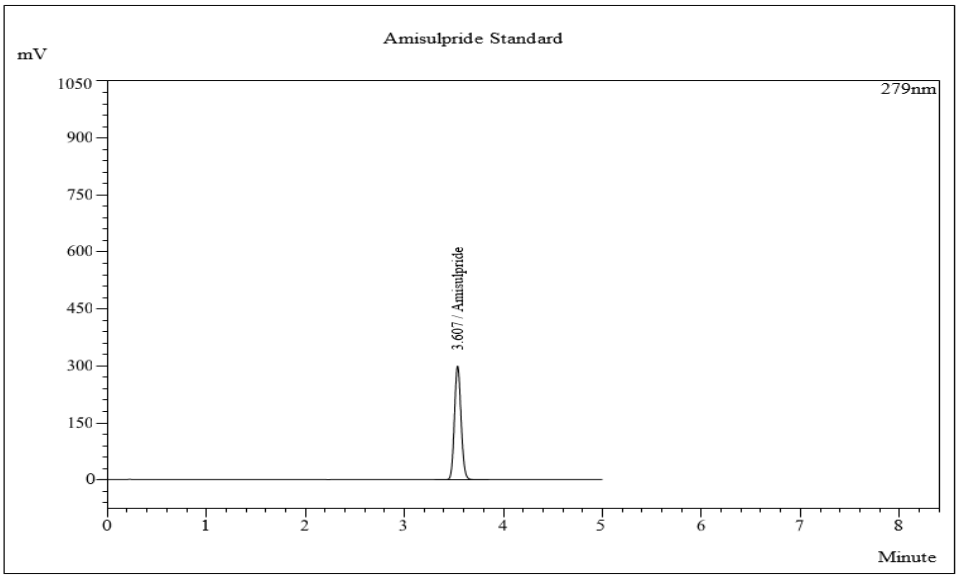


Figure 8.11: A typical chromatogram of sample.

4. Sensitivity

The sensitivity of measurement of Amisulpride by use of the proposed method was estimated in terms of the limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ were calculated by the use of signal to noise ratio. In order to estimate the LOD and LOQ values, the blank sample was injected six times and the peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level, while ten times the noise value gave the LOQ.

Table 8.6: Sensitivity.

LOD & LOQ		
1	LOD (µg/mL)	0.9396
2	LOQ (µg/mL)	2.8473

5. Precision

The precision of the analytical method was studied by multiple sampling of the homogenous sample. The precision was done at two levels (intraday and inter day). Intraday precision was done by analysing the intermediate concentration of each drug for six times. Interday precision was measured over six consecutive days for the same drug concentrations for six times. The %RSD values were found to be within limit (< 2.0 %) and the low RSD values indicate that the method is precise. The results are given in Table 1 and 2.

Table 8.7: Repeatability.

Precision		
Repeatability		
Sr. No	**Concentration (µg/mL)**	**Peak Area**
1	40	3178050
2	40	3158050
3	40	3178063
4	40	3171176
5	40	3228050
6	40	3175838
Average		3181537.8
Standard Deviation		21905.7
RSD%		0.689

6. Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analysed samples of Amisulpride (120 µg/ml) were spiked with 80, 100, and 120 % extra Amisulpride standard and the mixtures were analysed by the proposed method.

Standard deviation of the % recovery and % RSD were calculated and reported in Table No. 8.7.

Table 8.8: Accuracy of Amisulpride.

Accuracy				
Sr. No	**Concentration (µg/mL)**	**Peak area**	**Found Concentration (µg/mL)**	**% Recovery**
1	32	2526147.2	31.99	99.96
2	40	3157684	39.99	99.98
3	48	1894610.4	48.09	100.18

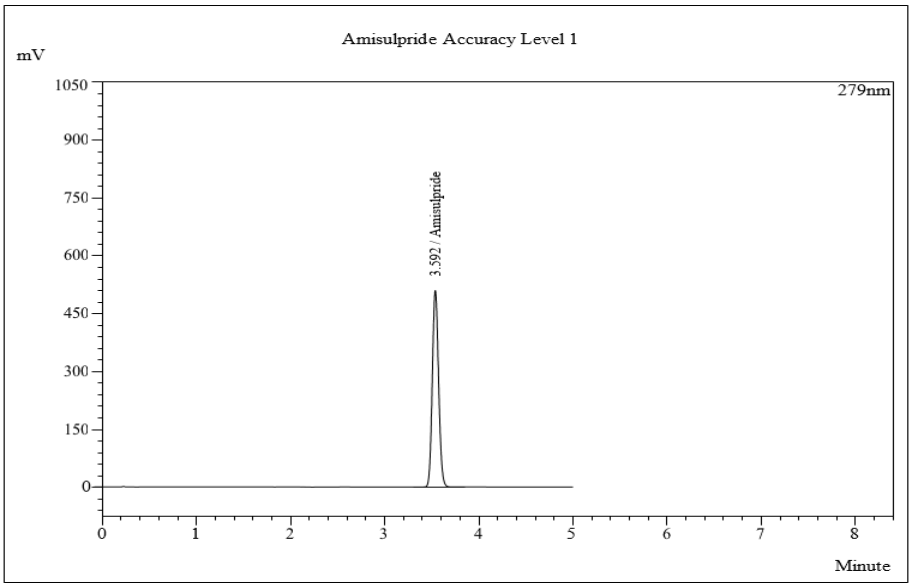


Figure 8.12: Amisulpride Accuracy level 1.

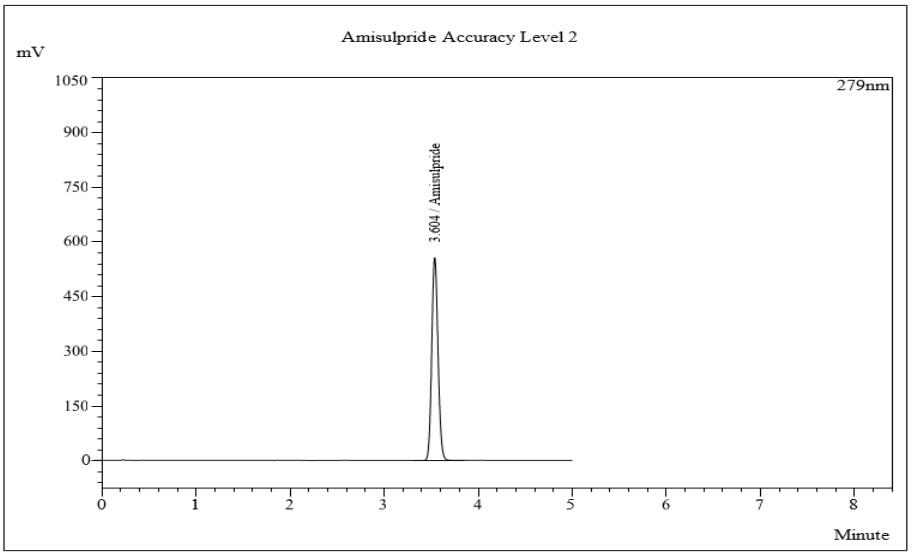


Figure 8.13: Amisulpride Accuracy level 2.

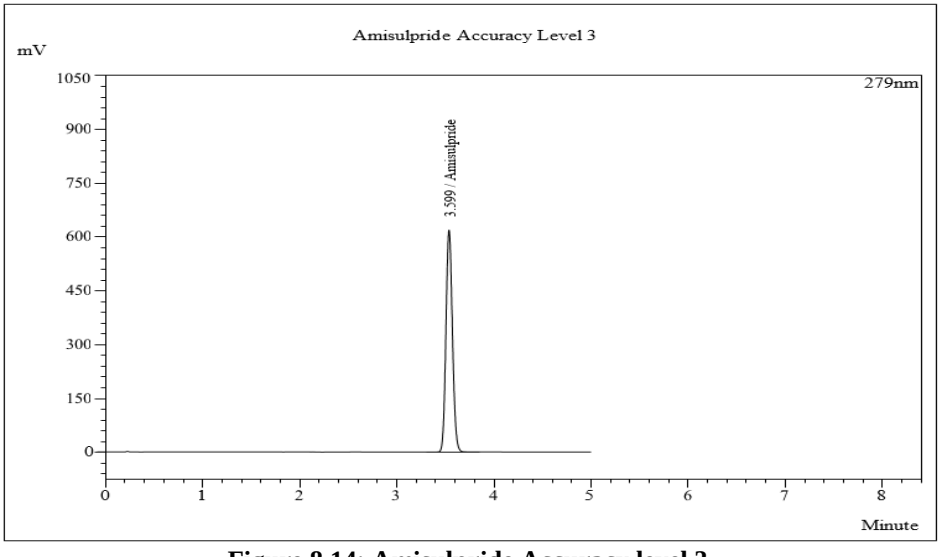


Figure 8.14: Amisulpride Accuracy level 3.

7. Robustness

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of mobile phase flow rate by 1.0 ml/min (1.0 and 1.1 ml/min), pH and wavelength had no significant effect on the retention time and chromatographic response of the 40 µg/ml solution, indicating that the method was robust. The results are shown in Table No. 8.9.

Table 8.9: Robustness of amisulpride.

Robustness					
Sr. No	**Parameter**		**Response**	**Parameter**	**Response**
Acetonitrile : Potassium Phosphate Buffer (V/V)			**Retention Time (min)**	**Detection Wavelength** (nm)	**Peak Area**
1	69	31	3.600	277	3114705
2	70	30	3.602	279	3173020
3	71	29	3.581	281	3206284
Average			3.595	Average	3164670
Standard Deviation			0.080	Standard Deviation	37850.36
RSD%			1.115	**RSD%**	1.196
Flow Rate (mL/min)			**Retention Time (min)**	**pH of Buffer** (mmol/L)	**Peak Area**
1	0.9		3.593	6.3	3201092
2	1		3.602	6.5	3173193
3	1.1		3.679	6.7	3097572
Average			3.624	Average	3157286
Standard Deviation			0.0627	Standard Deviation	43733.13
RSD%			1.641	**RSD%**	1.385

Optimization

Optimization result

Screening design for suitable chromatographic condition

Determination of solvent system based on peak parameters. Methanol: water/ ACN: water and Methanol: Ammonium Formate Buffer, these three mobile phases were selected for screening study on C18 columns at pH 5.5 and 6.5. These mobile phases were screened by varying the organic phase composition from 70 to 90 % v/v. Flow rate was varying form 1 ml/min.

Results of various trials, having organic phase composition 70 % v/v are shown in following tables.

Table 7.2: Trials performed on C18 column at mobile phase (70:30 v/v) with aqueous phase pH 6.5.

Sr. no.	Composition	Observation	Remarks
1	**Methanol: buffer**	**Less peak asymmetry with more theoretical plates and good retention time with greater peak height**	**Extremely Satisfactory**
2	Methanol: water	Less peak asymmetry but less theoretical plates	Satisfied
3	ACN: water	Greater peak Asymmetry and lower theoretical plates	Not satisfactory

Table 7.2: Trials performed on C18 column at mobile phase (70:30 v/v) with aqueous phase pH 5.5.

Sr. no.	Composition	Observation	Remarks
1	Methanol: buffer	Good peak properties	Satisfactory
2	Methanol: water	More retention time	Not satisfactory
3	ACN: water	Small Peak Observed	Dissatisfactory

Results of various trials, having organic phase composition 90 % v/v are shown in following tables.

Table 7.3: Trials performed on C18 column at mobile phase (90:10 v/v) with aqueous phase pH 6.5.

Sr. no.	Composition	Observation	Remarks
1	Methanol: buffer	Less Retention time but less theoretical plates	Partly Satisfactory
2	Methanol: water	Broad Peak Appeared	Dissatisfactory
3	ACN: water	Greater Peak asymmetry with less theoretical plates and more retention time	Partly Satisfactory

Table 7.4: Trials performed on C18 column at mobile phase (90:10 v/v) with aqueous phase pH 5.5.

Sr. no.	Composition	Observation	Remarks
1	Methanol: buffer	Good Peak Property but less theoretical plates	Partly Satisfactory
2	Methanol: water	No Peak Appearance	Dissatisfactory
3	ACN: water	Good Peak Properties but More Retention Time.	Partly Satisfactory

This methodology is initially based on constructing a desirability function for each individual response. The scale of individual desirability function ranges between i= 0, for completely undesirable response and i =1, for fully desired response. Selection of trial was based on maximum desirability value. Therefore, first trial which was having desirability one (i=1) selected for method optimization.

Table 7.5: Optimized trials suggested by software based on desirability value.

Sr. no.	Amount of Methanol	pH of buffer	Flow rate	Retention time	Tailing factor	Theoretical plates	Desirability
1	83	6.5	1.0	3.602	1.121	8030	0.702

Optimized chromatographic conditions

Mobile phase: Ammonium Formate buffer: Methanol (30: 70 v/v), pH of buffer: 6.5, Analytical column: C_{18} column Waters XBridge (4.6× 250mm id. particle size 5µm), UV detection: 279 nm, Injection volume: 10 µL, Flow rate: 1.00 mL min^{-1}, Temperature: Ambient, Run time: 10 min

Effect of independent variables on retention time (X)

After applying experimental design, suggested Factorial Model was found to be significant with model F value of 63660000, *p* value less than 0.005 and R^2 value of 1.000. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R^2 were 00 and 1.000 respectively. The model for response X (Retention time) is as follows:

The equation for Factorial model is as follows

Retention Time = +13.49500 -0.11695 * Mobile Phase -1.00000E-003 * pH of Aqueous Phase

Fig.3 (b) shows a graphical representation of pH of buffer (B) and amount of ACN (A), while flow rate (C) is maintained constant at its optimum of 1 mL min^{-1}.

If there is change in mobile phase concentration from 70 to 90 v/v, shown drastic change in retention time but if there is change in pH of mobile phase, shown negative effect.

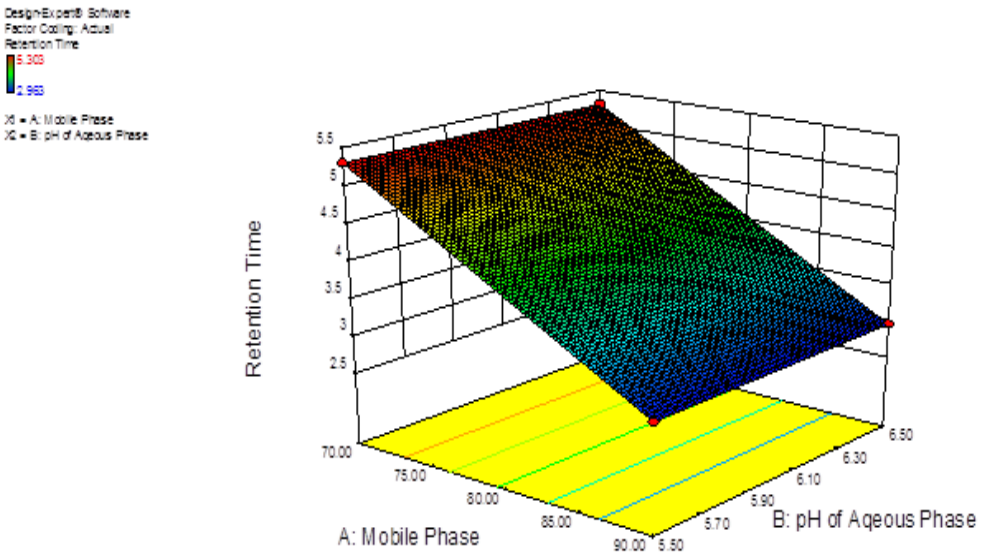


Figure 7.1: (b) Three-dimentional plot for retention time as a function of pH of buffer and amount of buffer. Constant factor (flow rate- 1mL min^{-1}).

Fit summary: 2FI model was suggested by the software.

ANOVA: ANOVA of developed 2 level factorial models for retention time (Y_1).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant.

In this case All factors are significant model terms.

Table 7.6: Significance of *p* value on model terms of retention time.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	Significant
Model	5.47	2	2.74	6.366E+007	< 0.0001	Significant
A-Mobile Phase	*5.47*	1	*5.47*	*6.366E+007*	*< 0.0001*	
B-pH of Aqeous Phase	*1.000E-006*	1	*1.000E-006*	*6.366E+007*	*< 0.0001*	

Effect of independent variables on tailing factor (Y):
After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 83.65, *p* value less than 0.005 and R^2 value of 0.9940. There is only a 7.75% chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R^2 were 3.22 and 0.9820 respectively. The model for response
Y (Tailing factor) is as follows:
Asymmetric Factor = +4.59300 +3.20000E-003 * Mobile Phase -0.57500* pH of Aqueous Phase

Fig.6.(b) shows a graphical representation of pH of buffer (B) and amount of Methanol (A), while flow rate (C) is maintained constant at its optimum of 1.0 mL min^{-1}.

As increases in pH of buffer had antagonistic effect on response while increase in Methanol showed Increases the asymmetric factor.

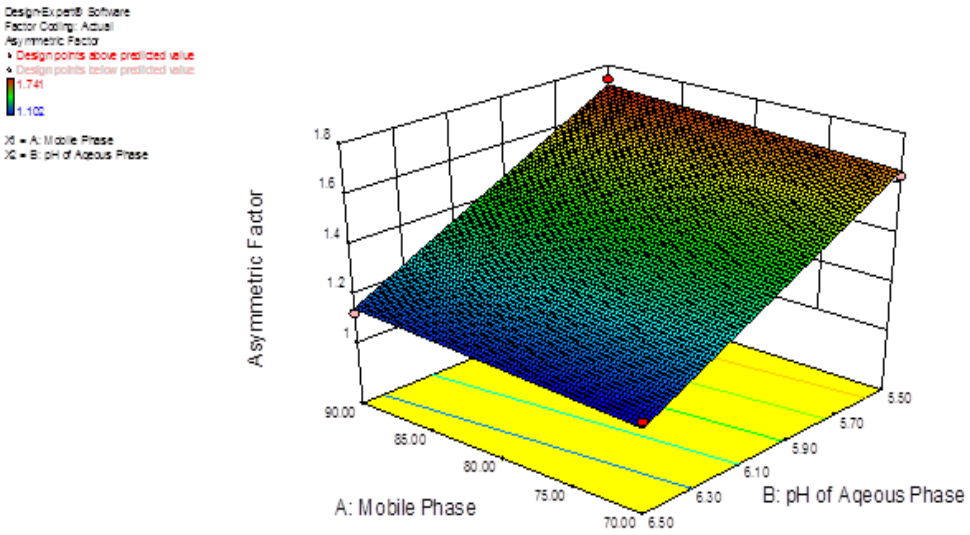


Figure 7.2: (b) Three-dimentional plot for tailing factor as a function of pH of buffer and % v/v of buffer. Constant factor (flow rate- 1mL min^{-1})
Fit summary: Response Surface Linear Model was suggested by the software.
ANOVA : ANOVA of developed factorial model for tailing factor (Y).
Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant.
In this case A, B are significant model terms.

Table 7.7: Significance of *p* value on model terms of tailing factor.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	Significant
Model	0.33	2	0.17	82.65	0.0775	Significant Insignificant
A-Mobile Phase	*4.096E-003*	1	*4.096E-003*	*2.02*	*0.3901*	
B-pH of Aqeous Phase	*0.33*	1	*0.33*	*163.27*	*0.0497*	

Effect of independent variables on theoretical plates (Z)
After applying experimental design, suggested Response Surface Quadratic Model was found to be significant with model F value of 22.94, *p* value less than 0.005 and R^2 value of 0.9787. There is only a 14.60 % chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R^2 were 69.42 and 0.9367 respectively. The model for response Z (theoretical plates) is as follows:
Theoretical Plates = +17605.50000 -199.80000 * Mobile Phase +1078.00000 * pH of Aqeous Phase
Fig.9.(b) shows a graphical representation of amount of methanol (A) and pH of buffer (B), while flow rate (C) is maintained constant at its optimum value 1mL min^{-1}.

A increases in pH of buffer showed increase in number of theoretical plates (Z), while increase in amount of Methanol showed decreases in response. Combination of amount of Methanol and pH of buffer showed synergistic effect on it.

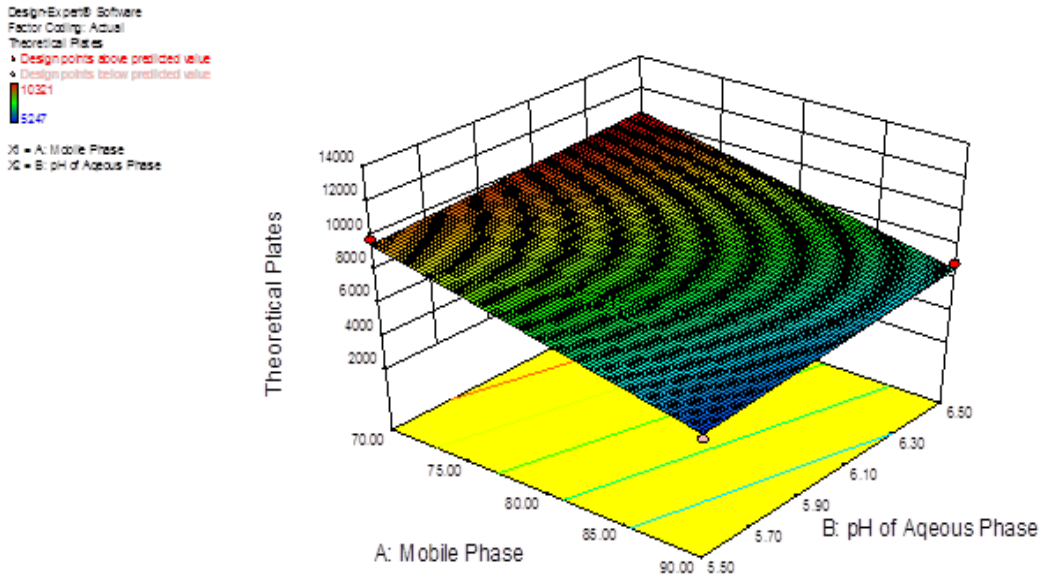


Figure 7.8: (b) Three-dimentional plot for theoretical plates as a function of pH of buffer and % v/v of buffer. Constant factor (flow rate- 1 mL min^{-1})

Fit summary: Factorial model was suggested by the software

ANOVA: ANOVA of developed CCD model for theoretical plates (Z).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant.

In this case model is significant.

Table 7.8: Significance of *p* value on model terms of theoretical plates.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	Significant
Model	1.713E+007	2	8.565E+006	22.94	0.1460	Significant Insignificant
A-Mobile Phase	*1.597E+007*	1	*1.597E+007*	42.77	0.0966	
B-pH of Aqeous Phase	*1.162E+006*	1	*1.162E+006*	3.11	0.3283	

CONCLUSION

Establishing techniques capable of quickly and accurately evaluating a large number of samples is always important for regular analytical purposes. In the Indian Pharmacopoeia, Amisulpride is recognised as a drug.

Only a few analytical techniques, such as HPLC, HPTLC, and UV-visible spectrophotometric techniques, have been reported in the literature for the measurement of Amisulpride. In light of the aforementioned fact, the development of certain straightforward analytical techniques that were sensitive, accurate, precise, and affordable was proposed. In the current study, an HPLC method (using Quality by Design) for the quantitative determination of Amisulpride in pharmaceutical formulations and bulk medication has been established in accordance with ICH requirements. The findings of linearity, precision, accuracy, specificity, system adaptability, and robustness of the HPLC techniques were validated, and they pass the threshold. In HPLC. There was no any interference of excipients in the recovery study. The low value of %RSD, molar extinction coefficient (L mol-1 cm-1) suggested that the developed methods are sensitive. The proposed high-performance liquid chromatographic method has also been evaluated over the accuracy, precision and robustness and proved to be convenient and effective for the quality control of Amisulpride. Developed method was found simple and cost effective for the quality control of Amisulpride.

Additionally, the reduced solvent use makes the spectroscopic process economical and environmentally benign. As a result, the suggested methodology is quick, selective, only needs a quick sample preparation step, and is a good method for Amisulpride.

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