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Analytical Method Development and Validation for Amitriptyline Hcl (Psychoactive Drug) Using Hplc Instrument.

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Abstract: *This research manuscript describes simple yet sensitive, speedy, accurate and precise HPLC method for the analysis of Amitriptyline HCl in tablet form. The sample was analyzed by HPLC instrument using inertsil ODS 3V (150 mm X 4.6 mm, 5 µm Make, GL science) column as stationary phase and Phosphate Buffer: Acetonitrile(55:45 % v/v) as a mobile phase (where PH of the buffer was adjusted to 2.5 by using diluted ortho phosphoric acid) at a flow rate of 1.0 ml/min. UV detector was used for the detection at 254 nm. The retention time for Amitryptiline HCl was found about 4 minute. The linearity for the drug was obtained for the concentration of 45, 80, 100, 120 & 150µg/ml. This method would be successfully applied to pharmaceutical formulations because no significant interferences from tablet excipient were found. The method retained its accuracy and precision when certain variations in method parameters were applied.*

Keywords*: Amitryptiline HCl; Method development; Method validation; HPLC method.*

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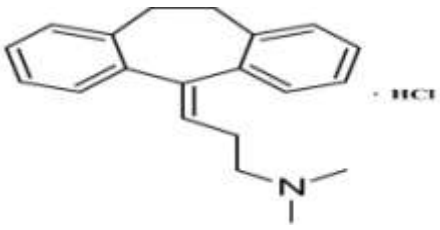
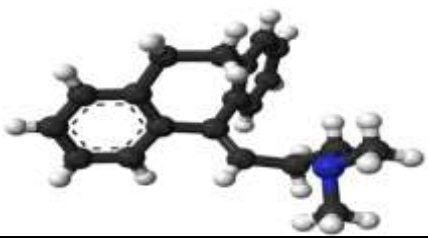
I. Introduction

[1,2]Amitriptyline hydrochloride is slightly different from Amitriptyline. The hydrochloride is just the salt form of Amitriptyline. The advantage of drug salt formation include development of controlled-release dosage forms, improved stability, targeted drug delivery in the gastrointestinal tract, improved taste, improved drug effectiveness, reduced pain on injection and ease of processing. Amitriptyline, sold under the brand name Elavil among others, is a medicine used to treat a number of mental illnesses. These include major depressive disorder and anxiety disorder, and less commonly hyperactivity disorder and bipolar disorder. Other uses include prevention of migraines, treatment of neuropathic pain such as fibromyalgia and post herpetic neuralgia, and less commonly insomnia. It is in the tricyclic antidepressant (TCA) class and its exact mechanism of action is unclear. Amitriptyline is taken by mouth. Amitriptyline HCl is 3-(10, 11-dihydro-5 *H*-dibenzo [*a,d*] cycloheptene-5-ylidene)- *N, N*-dimethyl-1-propanamine hydrochloride. Its molecular formula is $C_{20}H_{23}N$ • HCl. Amitriptyline HCl, a dibenzocycloheptadiene derivative, has a molecular weight of 313.87. It is a white, odorless, crystalline compound which is freely soluble in water

Figure 1: Molecular Structure of Amitriptyline Hydrochloride	Figure 2: Ball and stick model of Amitriptyline as found in amitriptylinium picrate
	

Amitryptiline HCl is official in Indian pharmacopeia [3], United States Pharmacopeia [4] and British pharmacopoeia[5]. Literature search reveals that various analytical methods like spectrophotometric development [6] ,HPLC [7] and HPTLC [8] methods are available for the estimation of Amitryptiline HCl but this work is more useful because it is more speedy, accurate and precise estimation of Amitryptiline HCl by HPLC. Aim of the present work is to develop a quick, accurate and precise HPLC method for estimation of Amitryptiline HCl in their marketed formulation which is more efficient method than available HPLC method. This method is developed and validated according to ICH [9,10] and USP [11] guideline.

II. Required Chemicals, Standard and Sample

Sr.	Chemical/ Standard	Acquired from
1	Amitryptiline HCl standard	Zydus-Cadila (Moraiya), Ahemedabad, India.
2	Sample : Amiline 10 mg (Torrent Pharmaceuticals, India)	Market
3	Mono basic sodium Phosphate AR grade	Finar Reagent, Ahemedabad India.
4	Acetonitrile HPLC grade	Finar Reagent., Ahmedabad, India
5	Ortho phosphoric acid	Finar Reagent., Ahmedabad, India
6	HCL HPLC grade	Finar Reagent, Ahemedabad India

Milli-Q Water was used throughout the study where ever is needed.

III. Instruments and Condition

The High Pressure liquid chromatography was performed using Agilent make HPLC system with UV detector. Chromatogram and data were recorded by using Chromeleon software. Separation was achieved by using inertsil ODS 3V (150 mm X 4.6 mm, 5 µm Make, GL science) as a stationary phase with Phosphate Buffer: Acetonitrile(55:45 v/v) as a mobile phase at a flow rate of 1.0 ml/min, Injection volume is 10µl and detection wavelength was 254 nm in UV detector. Column temperature is 35°C and Sample temperature is taken 25°C. Weigh machine of Mettler Toledo Company , pH meter of Mettler Toledo company and sonicator of Toshcon company- model SW1 were used in the study.

IV. Preparation of Mobile Phase and Diluent

11.04 gm Mono basic Sodium Phosphate was dissolved in 900 mL of purified water. Then pH of buffer was adjusted 2.50 using ortho-phosporic acid and dilute it to 1000 ml with purified water. Filter it through 0.45 micron filter. From the prepared buffer solution 550 ml is mixed with 450 ml of Acetonitrile in 1000 ml volumetric flask to make a mobile phase ratio buffer : Acetonirile 55:45% v/v respectively. Diluent is prepared through diluting 85 ml Hydrochloric acid in 10000 ml water.

V. Preparation of Standard Solution

An accurately weighed 50 mg Amitryptiline HCl was transferred into 50 ml volumetric flask, 30 ml of diluent added in volumetric flask and sonicated for 5 minutes. Then the volume was made up to the mark with diluents & mixed well. After this, 5.0 ml of stock solution was transferred to 50.0 ml volumetric flask with the help of 5.0 ml pipette & made up to the mark using diluent & mixed properly. This solution contained concentration of 100 µg/ml of Amitryptiline HCl which is used as a standard solution throughout the study.

VI. Preparation of Sample Solution (10 mg strength)

Accurately weighed 20 tablets of Amiline 10 mg were taken and average weight was calculated. Then, 10 intact tablets were transferred to 1000 ml volumetric flask and 600 ml of diluent added to volumetric flask. Sonicated for 60 minutes and mixed well. Then, solutions was kept till it attains normal temperature and made up to the mark with diluent and mixed. Then the solution was filtered with 0.45 µm Millipore PVDF filter.

VII. Assay Test

The Assay test determine the content of Amitriptyline HCL in sample. Study design as follows.

Sr. No.	Sample	No. Of Injection
1	Diluent	1
2	Standard Solution (System Suitability)	5
3	Sample Solution	2

Calculation:

$$\text{Amitriptiline HCl} = \frac{ATi \times Ws \times 5 \times Ds \times P \times Avg.\ Wt.}{As \times 50 \times 50 \times WTi \times 100}$$

Where,

ATi = average peak area of sample
As = average peak area of standard
WS = Weight of standard
DS = Dilution of sample
WTi= Average weight of sample

Acceptance criteria decided according to the ICH guideline:
Assay value should be between 90.0% to 110.0%.
Result complies with acceptance criteria.

VIII. System Precision (System Suitability)

The precision of the method was checked by repeatedly injecting (n=5) injections of Amitryptiline HCl Standard solution (100 µg/ml) without changing the parameters.

Study design

Sr. No.	Sample	No. Of Injection
1	Diluent	1
2	Standard Solution (System Suitability)	5

Acceptance criteria

% RSD of 5 injections should not be more than 2.0%.

Result complies with acceptance criteria.

IX. Filter Saturation

The Filter Saturation study demonstrates its reliability for even changes in filter volume.

Study design

Sr. No.	Sample	No. Of Injection
1	Diluent	1
2	Standard Solution	5
3	Sample Solution (Unfiltered)	1
4	Sample Solution (filtered, 1 ml)	1
5	Sample Solution (filtered, 5 ml)	1
6	Sample Solution (filtered, 9 ml)	1

Acceptance criteria

Difference between unfiltered and filtered solution is NMT 2.0% .

Result complies with acceptance criteria.

X. Robustness

This test demonstrates its reliability even for minor changes in analysis conditions.

- Flow rate changed by 10%(i.e. 0.9 ml/min and 1.1 ml/min)
- Mobile phase pH changed by $\pm$0.2 units (i.e. pH 2.3 to 2.7)
- Column temperature changed by $\pm$5°C (i.e. 30°C to 40 °C)

Study design

Sr. No.	Sample	No. Of Injection
1	Diluent	1
2	Standard Solution	5

Assessment

This test will evaluate the minor changes in analysis condition do not vary any significant change in result.

XI. Linearity

This study establishes linearity of analyte with specified range. The Linearity of standard solution over the range of 45% to 150% of concentration was prepared.

Study design

Linearity level	Solution taken (ml)	Dilute volume with Diluent in ml	Final concentration in µg/ml
45%	4.5	100	45
80%	4.0	50	80
100%	5.0	50	100
120%	6.0	50	120
150%	7.5	50	150

Acceptance criteria

By Injecting solutions in HPLC system and record area of analyte peak plot a graph of concentration (X-axis) vs. analyte peak area (Y-axis).Correlation coefficient is NLT 0.995. Y-intercept is $\pm$2% of 100% linearity level response.

Result complies with acceptance criteria.

XII. Specificity

The specificity of the developed method was determined by injecting sample solutions which were prepared by forcibly degrading the sample in presence of stress conditions such as acid, base & oxidative medium and

application of light and heat. The stability signifying ability of the method was established from the acquired chromatographic data for Amitryptiline HCl .The results of force degradation study are explained in following table.

Specificity Study

Stress condition	Time duration	% Degradation
Acid degradation	1 Hour	21.54
Base degradation	1 Hour	26.21
Oxidative degradation	1 Hour	18.12
Thermal degradation	1 Hour	14.36
Photo degradation	48 Hour	17.80

Result complies with acceptance criteria.

XIII. Conclusion

A new and improved HPLC method has been developed and validated for the determination of Amitryptiline HCl in pharmaceutical dosage forms. The developed method was validated as per ICH guidelines and was found to be accurate, precise, robust, specific and less time consuming as compared to available methods. No interference from any other components of pharmaceutical dosage form or degradation products was observed, and the method can be successfully used to perform rapid and accurate analysis of Amitryptiline HCl in pharmaceutical dosage form.

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