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A Validated HPTLC Method for the Estimation of Amitriptyline HCL in Bulk and Its Tablet Dosage Form

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KEYWORDS

Amitriptyline HCL, HPTLC, Validation, Chromatography and Recovery

ABSTRACT: A simple, rapid, reliable and accurate HPTLC method has been developed for the quantitative determination of Amitriptyline HCL in bulk and tablets. Various aliquots of the sample solution were spotted automatically by means of camag ATS 4 applicator on precoated silica gel 60 F_{254} on aluminium sheet as stationary phase pre washed with methanol using Toluene: Methanol: Acetone: Ammonia (5:3:2:0.2)v/v/v/v as mobile phase. The spots were scanned at 254 nm. The R_f value of AMITRIPTYLINE HCL was 0.66 ± 0.02. Calibration curves were linear in the range of 67.5 - 472.5 ng/band. The limit of detection and limit of quantification were found to be 9.37 ng/band and 2.80 ng/band respectively. The suitability of this method for the quantitative determination of compound was proved by validation in accordance with requirements of pharmaceutical regulatory standards.

Objectives: This study aimed to enhance the stability indicating chromatographic method for determining Amitriptyline hydrochloride in the presence of degradation products and impurities, ensuring purity and stability of the bulk drug using HPTLC, in compliance with ICH recommendations.

Methods: The study used a pure gift sample of Amitriptyline HCL from Unichem Laboratories Ltd, GOA, India, and prepared a mobile phase with methanol, toluene, acetone, and ammonia. The substance's melting point was determined, and solubility was tested in various solvents. The ideal solvent was methanol, as it was completely soluble, stable, and economical. A standard stock solution of 0.1 mg/ml of Amitriptyline HCL was prepared by dissolving 10 mg in methanol. A sample was prepared by powdering 20 tablets, adding 50mL of methanol, sonicating, and filtering the solution.Chromatography was conducted on aluminium packed silica gel 60 F254 HPTLC plates, which were washed and dried before use. Samples were applied as 6 mm bands, and ascending development was performed at 25°C with a mobile phase of Toluene, Methanol, Acetone, and Ammonia. Densitometric scanning was performed using a Camag TLC scanner 4 with Wincats software. The following validation parameters are typically monitored for HPTLC method : Linearity, Sensitivity, Specificity, Precision, Data of repeatability, Interday Precision Data and Recovery. The mean weight of 20 tablets was determined, and a finely powdered powder equivalent to 1 tablet of Amitriptyline HCL was prepared, with a drug content of 99.53.

Results: The HPTLC method was validated according to ICH guidelines and was found to be linear, accurate, and precise for Amitriptyline HCL. Its sensitivity was found to be low, with a sensitivity coefficient of 0.999 and a coefficient of variation of 0.72%.

Conclusions: The developed HPTLC technique is precise, specific and accurate. The advantages lie in the simplicity of sample preparation and the low cost of reagents used. Statistical analysis proves that the method is suitable for the analysis of Amitriptyline HCL as bulk drug and in Pharmaceutical

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formulation without any interference from the excipient. Hence this HPTLC method can be used for routine drug analysis.

1. Introduction

Amitriptyline hydrochloride [10,11-dihydro-N,N-dimethyl-5H-dibenzo[a,d]cycloheptene-Δ^5 propylamine hydrochloride] is in the tricyclic antidepressant(TCA) drug. Amitriptyline is FDA approved medication to treat major depressive disorder (MDD) in adults. Amitriptyline Hydrochloride is an odorless, off-white crystalline powder. The empirical formula is $C_{20}H_{23}N.HCl$ having molecular weight of 313.87gm with melting point 195-198°C. It is freely soluble in water, alcohols like methanol and chloroform and slightly soluble in Benzene and diethyl ether[1,2,3]. Amitriptyline acts by blocking the reuptake of both serotonin and nor epinephrine neurotransmitters. Amitriptyline increases noradrenergic or serotonergic Neurotransmission by blocking the norepinephrine or serotonin transporter (NET or SERT) at presynaptic terminals. Chronic treatment with amitriptyline desensitizes presynaptic autoreceptors and heteroreceptors, producing long-lasting changes in monoaminergic neurotransmission. It is more sedating and has increased anti-cholinergic properties compared to other TCAs.[4,5]

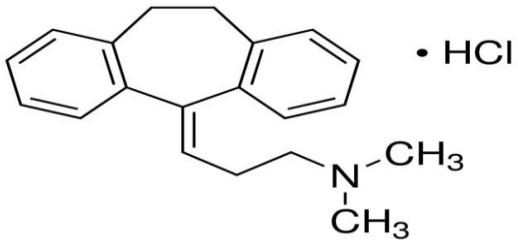

Figure 1: Structure of Amitriptyline Hydrochloride [15]

In pharmaceutical preparations, multiple analytical procedures have been reported for the analysis of Amitriptyline HCL when it is used as a single active principle or in combined dosage forms, UV and Spectrofluorimetric method [6-8], High-performance liquid chromatography [9-11] and High performance thin layer chromatography[12-14]. The aim of present study is to develop a HPTLC method for the estimation of Amitriptyline HCL in bulk and in tablets.

2. Objectives

The goal of this work was to increase stability indicating chromatographic approach for the determination of Amitriptyline hydrochloride in presence of degradation products and associated impurity for the evaluation of purity of bulk drug and stability of its bulk dosage form the usage of HPTLC. The technique was validated in compliance with ICH recommendations and its up-to-date international convention.

The approach needs to be simple, correct, precise repeatable and stability indicating; also, it ought to decrease the length of evaluation and have to be appropriate for routine determination of Amitriptyline hydrochloride in the Tablet dosage form.

3. Methods

Materials

An analytically pure gift sample of Amitriptyline HCL from Unichem Laboratories Ltd, GOA, India was used as working standard. Methanol, Toluene, Acetone and Ammonia of AR grade were used to prepare the mobile phase.

Melting point (M.P)

Sample obtained was characterized for melting point of the substance. The melting point was determined by introducing small amount of substance in capillary and constant heat was supplied. The drug substance was tested in the temperature range of 197°C and the melting point was noted.

Solubility

The solubility of drug sample was tested in various solvents like acetonitrile, methanol and water. The observed results were then compared with drug profile.

Selection of solvent

The ideal property of a solvent should be that the drug should be completely soluble in the solvent used. The drug should be stable in the solvent used and should be economical. After suitable literature survey, practical

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experience and taking above factors into consideration the suitable solvents selected was Methanol.

FT-IR spectra of Amitriptyline

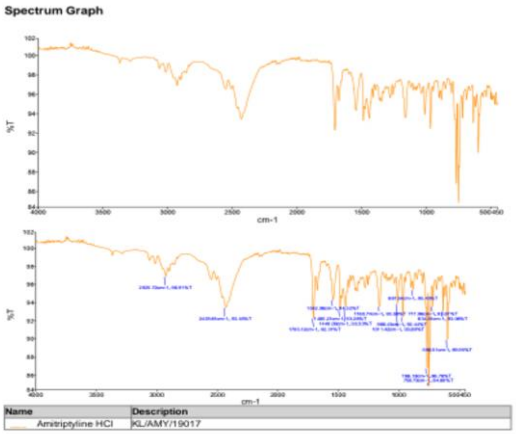

Figure 2: FT-IR spectra of Amitriptyline HCL

Camag (Switzerland) ATS 4 applicator, a Camag Twin trough TLC Chamber. Camag TLC scanner 3, Camag Wincats Software. Hamilton (Reno, Nevada, USA) syringe (100 µL). HPTLC conditions are given in Table 1.

Table 1. HPTLC Conditions

Stationary Phase	TLC aluminium sheets Silica gel 60 F_{254}
Sample Solvent type	Methanol
Mobile Phase	Toluene: Methanol: Acetone: Ammonia (5:3:2:0.2)v/v/v/v
Migration distance	70 mm
Slit Dimensions	4.00 x 0.45 mm, micro
Wavelength scanning	254 nm
R_f value of Amitriptyline HCL	0.66 ± 0.02

Standard preparation

Standard stock solution containing 0.1 mg/ml of Amitriptyline HCL was prepared by dissolving 10 mg standard drug in 100 ml methanol and used as working standard solution.[18]

Sample preparation

Twenty tablets were weighed and powdered. An amount of powder equivalent to 10mg of Amitriptyline HCL transferred to 100 ml calibrated volumetric flask. 50mL of Methanol is added and sonicated for 10 minutes; the solution was made up of volume with the same solvent and filtered. A sample solution was spotted for the assay of Amitriptyline HCL.[15]

Chromatography

Chromatography was performed on 10 cm x 20 cm aluminium packed silica gel 60 F_{254} HPTLC plates. Before use, the plates were washed with methanol and dried in an oven at 50°C for 5 min. Samples were applied as 6 mm bands by spraying at a rate of 15 µL S^{-1} by means of a Camag Linomat ATS 4 applicator equipped with a 100 µL syringe, the distance between the bands was 10.5 mm. Ascending development of the plate, migration distance 70 mm, was performed at 25 ± 2°C with Toluene: Methanol: Acetone: Ammonia (5:3:2:0.2) v/v/v/v as mobile phase in a Camag twin-trough chamber previously saturated for 20 min. The average development time was 20 min. Densitometric scanning was performed with Camag TLC scanner 4 equipped with Wincats software at λ_{max} 254 nm using Deutrium light source, the slit dimensions were 4.00 x 0.45 mm, micro.

Validation of the HPTLC method

Linearity

Amount of standard solutions equivalent to 67.5 - 472.5 ng/band was spotted on the prewashed HPTLC plates. The plates were developed, dried and scanned as described above. The calibration curve was constructed by plotting the peak areas against the corresponding concentrations in ng. The linearity response for Amitriptyline HCL assessed in the concentration range is 67.5 - 472.5 ng/band. The statistical analysis of data correlation coefficient and coefficient of variation are found to be 0.999 and 0.72% respectively, over the concentration range studied with six replicate readings of each concentration. The chromatogram of Amitriptyline HCL Standard, Sample and Calibration plot are showed in Fig. 2, 3 and 4 respectively [18]

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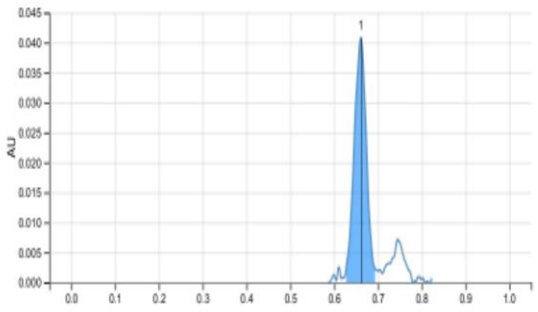

Figure 3: Chromatogram of Standard Amitriptyline HCL

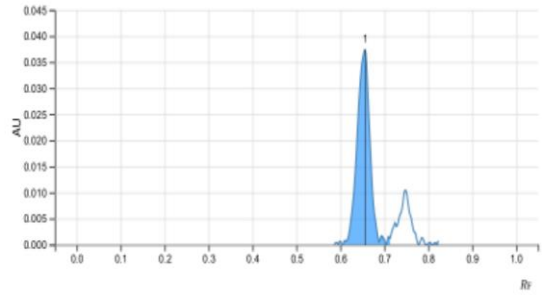

Figure 4 : Chromatogram of Sample Amitriptyline HCL

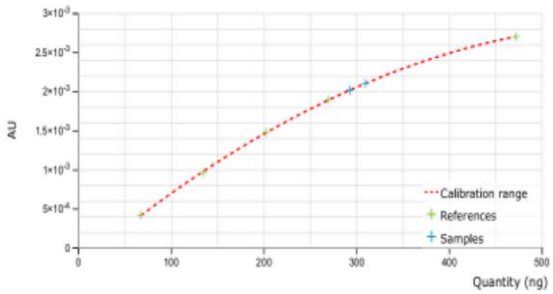

Figure 5: Calibration curve of Amitriptyline HCL

SENSITIVITY

The sensitivity of proposed method is estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated by the use of equation LOD = 3 X N/B and LOQ = 10 X N/B, where N is the standard deviation of peak areas of the drug taken as a measure of noise, and B is the slope of the corresponding calibration curve. The limit of detection and limit of quantification for Amitriptyline HCL were found to be 9.37 ng/band and 2.80 ng /band respectively.[17]

SPECIFICITY

Specificity of the method was determined by analyzing standard drug and sample. The specificity of the method was ascertained by analyzing Amitriptyline HCL. The ability of the method to separate the drug from tablet excipients indicates the specificity of the method. There was no interference or co elution from excipients at Rf value of drug, The band of Amitriptyline HCL was confirmed by comparing the Rf of the sample with that of standard, indicating absence of interference of mobile phase, diluent and excipients.

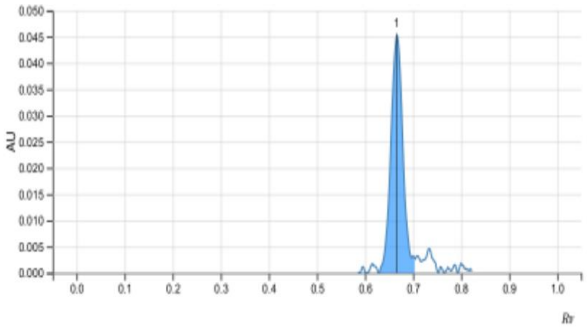

Figure 6 : Chromatogram of Standard Amitriptyline HCL

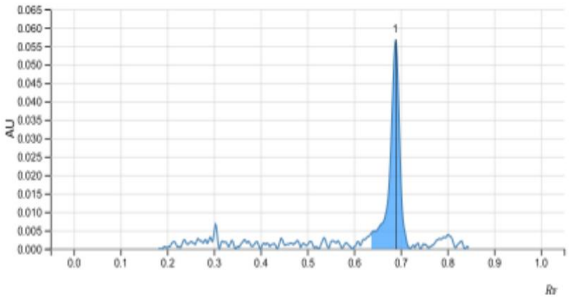

Figure 7: Chromatogram of Sample Amitriptyline HCL

Percent Amount spiked	Area x 106	Average Area	Area Obtained	Excepted Area	Percentage	Mean Percentage
Sample (100%)	0.00079 08					
1µl						

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	0.00087 12	0.00081 25				
Standard (80%)	0.00164 9					
0.8µl	0.00151 6	0.00158 9	0.00158 9	0.00150 7	105.41	
Standard (100%)	0.00167 6					
1.0µl	0.00160 4	0.00164 4	0.00164 4	0.00168 8	97.41	100.28
Standard(120 %)	0.00177					
1.2µl	0.00169 8	0.00172 4	0.00172 4	0.00175 9	98.01	

DATA OF REPEATABILITY

Conc (2µl)		**Avg = 0.001**	**CV=1.36%**
Sr.No	**Rf**	**Peak area**	**Deviation**
1	0.0663	0.00128	-1.26%
2	0.0656	0.0013	0.12%
3	0.0652	0.00129	-.0.80 %
4	0.0656	0.00127	-1.62%
5	0.0661	0.00132	2.15%
6	0.0668	0.0013	0.14%

Table 2 : Data of Repeatability (n=6)

INTERDAY PRECISION DATA

Conc (2µl)		**Avg = 0.002**	**CV=1.88%**
1	0.653	0.00183	-0.17%
2	0.655	0.00179	-2.56%
3	0.65	0.00186	1.10%
4	0.648	0.00182	-0.82%
5	0.642	0.00183	-0.52%
6	0.642	0.00189	2.97%

Table 3 : Day 1

Conc (2µl)		**Avg = 0.002**	**CV=1.17%**
Sr.No	**Rf**	**Peak area**	**Deviation**
1	0.653	0.00177	0.87%
2	0.652	0.00175	-0.25%
3	0.65	0.00173	-1.88%
4	0.645	0.00176	0.12%
5	0.644	0.00175	-0.39%
6	0.647	0.00179	1.52%

Table 4 : Day 2

RECOVERY

Recovery was carried out to determine accuracy of the method. Recovery was determined at 3 concentration level at 80% (0.8µl), 100% 1µl) and 120% (1.2µl). Recovery percentage was found to be 100.28%. Sample (0.1mg/ml) and Standard (0.1mg/ml) were diluted in methanol.[17]

Conc (2µl)		**Avg = 0.002**	**CV=1.88%**
1	0.653	0.00183	-0.17%
2	0.655	0.00179	-2.56%
3	0.65	0.00186	1.10%
4	0.648	0.00182	-0.82%
5	0.642	0.00183	-0.52%
6	0.642	0.00189	2.97%

Table 5 : Data of Recovery

Analysis of Marketed formulation

Twenty tablets were weighed, their mean weight was determined and was finely powdered Powder equivalent to 1 tablet of Amitriptyline HCL was weighed. Sample preparation (10mg/100ml) was made and Drug content per tablet was determined by performing assay. The percent content was found to be 99.53. [21]

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4. Results

HPTLC method was validated as per ICH guidelines. The developed method was found to be linearwithin the range of 67.5 - 472.5 ng with correlation coefficient and coefficient of variation are found to be 0.999 and 0.72% respectively for Amitriptyline HCL. The accuracy of method was determined at 80%, 100%, 120% level. The % recovery was found to be 100.28%. The LOD for Amitriptyline HCL was found to be 9.37 ng/band and the LOQ Amitriptyline HCL was found to be 2.80 ng/band indicating the sensitivity of the method. The developed method was found to be precise as the % RSD values for intra-day and inter-day were found to be less than 2%. Summary of the results of validation parameters is shown in table 6

PARAMETERS	Amitriptyline HCL
Linearity [ng/band]	67.5 - 472.5 ng/band.
LOD [ng/band]	9.37
LOQ [ng/band]	2.8
Precision (%RSD) Intra-day (n=6)	1.354
Precision (%RSD) Inter-day (n=6)	1.5175
Specificity	Specific
% Recovery	100.28%.

TABLE 6 : Summary Of Validation Parameters

5. Discussion

The developed HPTLC technique is precise, specific and accurate. The advantages lie in the simplicity of sample preparation and the low cost of reagents used. Statistical analysis proves that the method is suitable for the analysis of Amitriptyline HCL as bulk drug and in Pharmaceutical formulation without any interference from the excipient. Hence this HPTLC method can be used for routine drug analysis.

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