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High Performance Thin Layer Chromatographic Estimation of Tolterodine Tartarate

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ABSTRACT

A simple, sensitive and validated high performance thin layer chromatographic method has been developed for the estimation of Tolterodine tartarate in pure drug and its formulation. Aluminium plates precoated with Silica gel G 60 F_{254} was used as stationary phase and Acetonitrile:Water:Formic acid in the ratio of 50:50:3 was used as mobile phase. Quantification was carried out by the use of Densitometric absorbance mode at 281 nm. The content of Tolterodine tartarate in the formulation was calculated and found to be 99.1%. The proposed HPTLC method was quantitatively evaluated in terms of precision, repeatability, accuracy and calibration correlation proving its utility in routine analysis of its dosage form.

Keywords: HPTLC, Densitometric Absorbance mode.

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INTRODUCTION

Tolterodine tartarate, (+) – N, N-di-isopropyl-3-(2-hydroxy-5-methyl phenyl)-3-phenyl propyl amine L – hydrogen tartarate [1,2], belongs to the category of Anticholinergic [3] and antispasmodic [4] drug. It is a competitive muscarinic [5] receptor antagonist [6] with actions similar to atropine. It is used in the management of urinary incontinence [7].

Literature survey reveals that several methods like HPLC [8,9], (chiral HPLC for separation of enantiomers), LC-MS [10-12] and GC-MS [13,14] have been reported for the estimation of Tolterodine tartarate in its pure form and in the formulation. There were no reported methods for its estimation in tablet dosage form by HPTLC [15]. The present study described the development and validation of a simple, specific, accurate and precise HPTLC method for the determination of Tolterodine tartarate in pharmaceutical dosage form.

EXPERIMENTAL

A CAMAG HPTLC system comprising of CAMAG Linomat IV semiautomatic sample applicator, CAMAG TLC scanner 3, CAMAG twin trough chamber(10 x 10 cm), CAMAG CATS 4 software, Hamilton syringe (100µL) were used during the study. Tablets were purchased from local market. Acetonitrile and Water of HPLC grade (E.Merck India Ltd.) were used for preparing the mobile phase.

Chromatographic conditions

Following chromatographic conditions were uniformly followed in the experiment.

Stationary Phase: HPTLC precoated Silica gel G60 F_{254} (Merck)
Size: 10 x 10 cm
Mode of application: Band
Band size: 4.0 mm
Separation technique: Ascending
Temperature: Ambient
Saturation time: 15 min.
Migration time: 70 mm
Detection: UV
Scanning wavelength: 281 nm
Scanning mode: Absorbance/Reflectance
Slit dimension: 3 x 0.45 nm.

Linearity of detector response

A liquots of working standard solution (2,3,4,5,6 µl) of Tolterodine tartarate were spotted as sharp bands on the precoated TLC plate, using Camag linomat IV semiautomatic applicator under nitrogen stream. The plate was developed under chromatographic conditions

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mentioned above. The plate was removed from the chamber and dried in hot air dryer. Densitometric measurements were performed at 281 nm in absorbance mode. Data peak height and peak area of each band were recorded. The calibration curve was prepared by plotting peak area vs. concentration corresponding to each spot. The densitogram of Tolterodine Tartarate was presented in figure – 1.

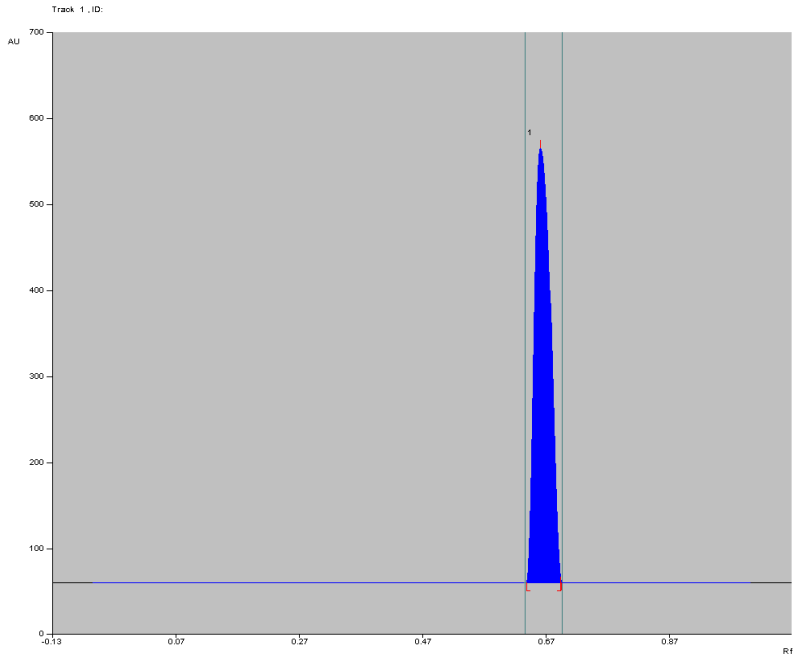

Figure 1 - DENSITOGRAM OF TOLTERODINE TARTARATE

Assay:

Stock solution A

An accurately weighed quantity of Tolterodine tartarate (50mg) was transferred into a 10ml volumetric flask. It was dissolved and diluted up to the mark with methanol to give a standard stock solution of 5 mg/ml. This 5mg/ml solution can be used as a working standard solution.

Preparation of sample solution

25 tablets were accurately weighed and average weight was calculated. Accurately weighed quantity of tablet powder equivalent to 50mg of the drug was transferred to 10ml volumetric flask. To it 8ml of methanol was added and shaken for 10 min and the volume was adjusted upto the mark with methanol and then filtered through Whatmann filter paper no.40. This solution is used as the sample solution.

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On the HPTLC plates spots of the standard and sample were applied. The plates were developed and after development the bands of the drugs were scanned at 281 nm. The peak height and area of the standard and sample bands were compared to obtain the concentration.

Method Validation:

Accuracy

Table 3 - Recovery studies

S.No	Amount Present (µg/µl)	Amount Added (µg/µl)	Amount Recovered (µg/µl)	%Recovery
1	10	5	15.1	100.1
2	10	10	19.9	99.1
3	10	15	25.1	99.4
Mean				100.03
Standard deviation				0.11547
% RSD				0.11505

Accuracy of the method was ascertained by performing the recovery studies using standard addition method. To a fixed amount of preanalysed drug were added at different levels. The total amount of the drug was determined by the above proposed method and the amount of pure drug was calculated. The average percent recovery was found to be nearly 100%(Table-3).

Precision

Table-1: Validation parameters of Tolterodine tartarate

Parameters	Tolterodine Tartarate
λ_{max} (nm)	281
Beer's law limit(µg/mL)	10 – 30
Limit of Detection (LOD)	21 ng
Limit of Quantification (LOQ)	53 ng
Regression equation(Y*)	
Slope(b)	683.1
Intercept(a)	328.4
Correlation coefficient(r)	0.997
Intraday %RSD **	0.05471
Inter day % RSD	0.07624
Analyst to analyst % RSD	0.04912

*Y = a + bx, where 'Y' is the absorbance and x is the concentration of the drug in µg/mL
**For six replicates

Precision of the analytical method was expressed as SD or %RSD of series of measurements by replicate estimation of the drugs by the proposed method (Table -1).

Ruggedness:

It was ascertained by analyst to analyst variation. The result was presented in Table -1

RESULTS AND DISCUSSION

Tolterodine tartarate was completely extracted from the tablet matrix with methanol. Combination of Acetonitrile: Water: Formic acid (50:50:3) offered optimum migration and resolution of Tolterodine tartarate from other components of the formulation matrix.

Table 2 - Estimation of Tolterodine Tartarate in formulation

S.No	Label Claim (mg)	Amount Estimated (mg)	%Label Claim
1	2	1.98	99
2	2	1.99	99.5
3	2	1.98	99
Mean*			99.1
Standard deviation*			0.2888
% RSD*			0.2912
Standard error*			0.1666

* Average of three determinations
*Y = a + bx, where 'Y' is the absorbance and x is the concentration of the drug in µg/mL
**For six replicates

The amount of Tolterodine tartarate in the formulation was calculated on applying suitable dilution factor and comparing peak height and peak area of the standard and sample solutions. The content of the drug in the formulation was found to be within the limits. (Table - 2)

The linearity of response was found to be in the range of 10 – 30 µg/ml. Also the percent recovery values were found to be within the limits. Lower values of intra-day and inter-day variation on the analysis indicate that the method is precise. Different validation parameters for the proposed HPTLC method have been summarized in Table-1.

CONCLUSION

The proposed HPTLC method was found to be simple, specific, precise and accurate. The sample recoveries in the formulations were in good agreement with their

respective label claims. Hence this method can be conveniently adopted for the routine analysis of Tolterodine tartarate in pure form and in its dosage form.

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