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Research Article

Determination of Agomelatine in Tablets by Densitometric HPTLC

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A B S T R A C T

A simple and sensitive, HPTLC method has been developed for the quantitative estimation of agomelatine in its single component tablet formulation. Agomelatine was chromatographed on silica gel 60 F_{254} TLC plate using ethyl acetate: ammonia (33%): methanol in the ratio of 8.0:1.0:1.0 (v/v/v) as mobile phase. Agomelatine showed R_f value of 0.44±0.08 and was scanned at 229 nm using Camag TLC Scanner 3. The linear regression data for the calibration plot showed a good relationship with r =0.9987. The method was validated for precision and recovery. The limits of detection and quantification were 3 and 10 ng/spot respectively. The developed method was successfully used for the assay of Agomelatine tablet formulations. The method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

Keywords: High Performance Thin Layer Chromatography (HPTLC), Pharmaceutical Analysis, Agomelatine Tablet

Introduction

Agomelatine (AGM) is chemically N- [2-(7-methoxy napthalen-1-yl) ethyl] acetamide (Figure 1). Its molecular formula is $C_{15}H_{17}NO_2$ and its molecular weight is 243.301gm/mol. AGM is an acetamide naphthalene analogue of melatonin. AGM is a novel melatonergic antidepressant agent. It is a potential and well-tolerated medication for the treatment of major depressive disorder. AGM acts as a melatonergic receptor (MT1/MT2) agonist and serotonergic receptor (5-HT2C) antagonist. AGM works by restoring the balance of the circadian rhythm. AGM has also proven to have anxiolytic properties and thus may prove to be very useful in the treatment of anxiety disorders. Because of its action upon the melatonin receptors, AGM shows a marked improvement on sleep. Bioavailability is less than 5%. AGM is absorbed quickly in humans after oral administration. The mean half-life of AGM is 2.3 hours. AGM was bound to plasma proteins at 95% mainly to serum albumin (about 35%) and alpha1-acid AGMcoprotein (about 36%). The metabolism of AGM is almost completely hepatic. An extensive first pass hepatic effect is observed. It is practically insoluble in water and very soluble in organic solvents such as ethanol, methanol and dichloromethane.[1,2,3]

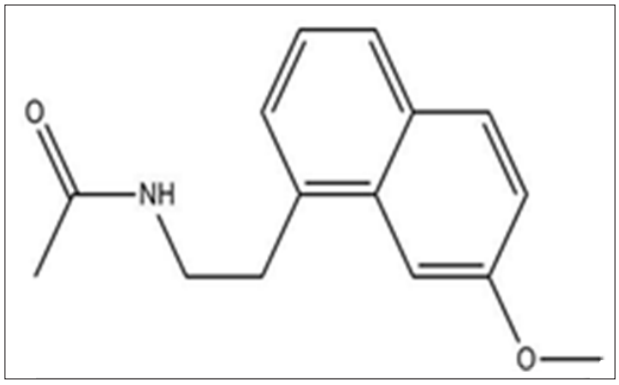

Figure 1.Chemical structure of Agomelatine (AGM)

The literature reports some analytical assays applied to AGM in different matrices. Among them, we highlighted UV,[4] HPLC chromatographic methods for quantitation in pharmaceuticals,[5-12] UHPLC-UV,[13] LC-MS-MS method.[14-20] In the present study we aimed to develop a HPTLC method for quantitative analysis of agomelatine in commercial sample, applying validation protocols.

There is a need for a simple, rapid, cost effective and reproducible method for assay of AGM in its dosage forms. Therefore, it was thought of interest to develop simple, rapid, accurate, specific and precise HPTLC method for the analysis of Agomelatine (AGM) in its tablet formulation. The objective of the current work is, therefore, to develop a simple HPTLC method for analysis of AGM in tablet formulations.

Materials and Methods

Experimental Materials

A generous gift sample of standard AGM was from Sun Pharma Laboratories Ltd, Sikkim (India). Silica gel 60 F_{254} TLC plates (10×10 cm, layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) were used as a stationary phase. All chemicals and reagents used were of analytical reagent grade and were purchased from Merck Chemicals, India. Agoprex containing 25 mg of AGM were purchased from Sun Pharma Laboratories Ltd (T1), Lupibliss containing 25 mg AGM were purchased from Lupin Ltd, Aurangabad, India (T2).

Instrumentation

The HPTLC system consisted of a Camag Linomat 5 semi-automatic spotting device (Camag, Muttenz, Switzerland), a Camag twin-trough chamber (10 cm×10 cm), Camag winCATS software 1.4.4.6337 and a 100 µl Hamilton syringe. Sample application was done on precoated silica gel 60 F_{254} TLC plates (10 cm×10 cm). TLC plates were pre-washed with methanol and activated at 80°C for 5 min prior to the sample application. Densitometric analysis was carried out utilizing Camag TLC scanner 3.

Preparation of Standard Solutions

A stock solution of AGM was prepared by dissolving 100 mg in 100 ml methanol (1000 µg/ml). Further standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 10 µg/ml.

Sample Preparation

Two brands of tablets T1 and T2 were selected. Twenty tablets were weighed and the average weight was calculated. The tablets were then powdered and an amount equivalent to one tablet was dissolved in 25 ml methanol. To ensure complete extraction of the drug it was sonicated for 45 min. This solution was filtered through a Whatman no. 41 paper.

HPTLC Method and Chromatographic Condition

In the proposed HPTLC method, the samples were streaked on the precoated TLC plates in the form of a narrow band 6 mm in length, 10 mm from the bottom and margin and 10 mm apart at a constant flow rate of 150 nl/s by using a nitrogen aspirator. A Camag Twin Trough Chamber was saturated for 20 min at room temperature (25±2 °C) with the mobile phase containing a mixture of ethyl acetate: ammonia (33%): methanol in the ratio of 8.0:1.0:1.0 (v/v/v). After chamber saturation, the plates were developed to a distance of 80 mm and then dried in hot air. Densitometric analysis was carried out using a Camag TLC Scanner 3 (Camag) in the absorbance mode at 229 nm for all measurements. The slit dimension was kept at 5.0 mm×0.45 mm and a scanning speed of 20 mm/s was employed. AGM was detected at R_f of 0.44±0.08. The chromatograms were integrated using winCATS evaluation software (Version 1.1.3.0).

Method Validation

Validation of the optimized HPTLC method was carried out with respect to the following parameters.

Linearity and Range

From the standard solution 10 µg/ml of AGM, 2 to 10 µl solutions were spotted on HPTLC plate to obtain final concentration of 20- 100 ng/spot for AGM. Each concentration was applied six times on the HPTLC plate. Peak area was recorded for each concentration and a calibration plot was obtained by plotting peak area against concentration.

Limit of Detection and Quantification

The Limits of Detection (LOD) and Quantification (LOQ) were calculated from the slope (s) of the calibration plot and the standard deviation of the response (SD).

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (20, 60, 100 ng/ spot) of the drug six times on the same day. The intermediate precision of the method was checked by repeating studies on two different days.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for AGM in sample was confirmed by comparing the R_f and spectra of the spot with that of standard. The peak purity of AGM was assessed by comparing the spectra at three different levels, i.e. peak start, peak apex and peak end positions of the spot.

Robustness of the Method

By introducing small changes in the mobile phase composition, the effects on the results were examined.

Mobile phases having different composition like, ethyl acetate: ammonia (33%): methanol (7.5:0.9:1.0, v/v/v), ethyl acetate: ammonia (33%): methanol (7.4:1.0:1.0, v/v/v), ethyl acetate: ammonia (33%): methanol (7.5:0.9: 0.9, v/v/v) were tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ±5 %. The plates were prewashed by methanol and activated at 60 °C ± 5 for 2, 5, 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 minutes. Robustness of the method was done at three different concentration levels 20, 60, 100 ng/spot for AGM.

Analysis of Marketed Formulation

Twenty tablets of each brands were weighed their average weight calculated, tablets finely powdered and the powder equivalent to containing 25 mg and 25 mg of AGM from T-1,T-2 respectively and dissolved in 25 ml of methanol. The solution was sonicated for 45 min and then filtered through Whatman filter paper No. 41. The residue was washed thoroughly with methanol. The filtrate and washings were combined. Each of these solutions (1 µl) were spotted on plates and analyzed for AGM in the same way as described earlier.

Recovery Studies

Recovery studies were carried out to check the accuracy of the method. Recovery experiments were performed by adding three different amounts of AGM i.e., 80, 100 and 120% of the labeled amount of AGM analyzed from the AGM formulations and the resultant were reanalyzed (n = 6).

Results and Discussion

Development of the Optimum Mobile Phase

Initially chloroform: methanol (8.0:2.0 v/v) in varying ratio tried. Then ethyl acetate: ammonia (33%): methanol was tried to above mobile phase in different ratios in order to achieve better R_f value. The mobile phase was ethyl acetate: ammonia (33%): methanol (8.0:1.0:1.0, v/v/v) gave good resolution with R_f value 0.44 for AGM gave a sharp and symmetrical peak. Well defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature (Figure 2). The analytical wavelength, 229 nm, was chosen on the basis of the absorption spectrum recorded in the range 400–200 nm.

Validation of the Method

Linearity

Linearity for AGM was observed in the range of 20–100 ng/spot with a correlation coefficient of 0. 9987 and the linear regression equation was y=14.627x + 27.238 (Table 1).

Table 1.Linear regression data for the calibration curves[a]

Linearity (ng/ spot)	r±SD	Slope ±SD	Intercept ±SD
20-100	0. 998±0.05	27.23±0.07	14.62±1.02

[a]n=6

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and found to be 0.94. The results shown in Table 2, revealed intra- and inter-day variation of AGM at three different concentration levels 20, 60, 100 ng/spot. The % RSD for within and day-to-day analysis was found to be <2%.

Table 2.Intra- and inter-day precision of HPTLC method[a]

Intra-day precision		Inter-day precision	
SD of areas	% R.S.D.	S.D of areas	% R.S.D.
0.91	1.07	1.26	0.81

[a]n = 6, Average of three concentrations 20, 60, 100 ng/spot.

Robustness of the Method

The standard deviation of peak area was calculated for each parameter and % R.S.D. was found to be less than 2%. The low values of % R.S.D as shown in Table 3, indicated robustness of the method.

Table 3.Robustness testing[a]

Parameter	SD of peak area	% RSD
Mobile phase composition	0.37	1.12
Amount of mobile phase	0.17	1.85
Temperature	0.60	0.07
Relative humidity	1.26	1.78
Plate pretreatment	0.07	0.05
Time from spotting to chromatography	0.12	0.08
Time from chromatography to scanning	0.09	0.03

[a]n=6, Average of three concentrations 20, 60, 100 ng/spot.

LOD and LOQ

The signal to noise ratios 3:1 and 10:1 were considered as LOD and LLOQ respectively. The LOD and LOQ were found to be 3 and 10 ng/spot respectively.

Specificity

The peak purity of AGM was assessed by comparing the

spectra of standard at peak start, peak apex and peak end positions of the spot i.e., r (start, middle)=0.9987 and r (middle, end)=0.9993. Good correlation (r=0.9990) was also obtained between standard and sample spectra of AGM.

Analysis of Marketed Formulations

A single spot at R_f 0.44 was observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The results, given in Table 5, indicate that the amount of drug in the tablets is within the requirement of 99.33-100.92% of the label claim.

Table 4.Recovery studies[a]

Label claim (mg)	Amount of drug added (%)	Total amount of drug present (mg)	Amount found (mg)	% Recovery
T1	80	45	45.31	99.32
	100	50	50.44	99.13
	120	55	54.82	100.32
T2	80	45	45.39	99.14
	100	50	50.46	99.09
	120	55	55.33	99.40

[a] n=6

Table 5.Applicability of the HPTLC method for the analysis of the pharmaceutical formulations

Formulation	Label Claim (mg)	Drug Content (%)	% R.S.D.
T1	25	99.33	0.05
T2	25	100.92	0.33

[a] n=6

Recovery Studies

The proposed method when used for extraction and subsequent estimation of AGM from pharmaceutical dosage form after spiking the preanalysed sample with 80, 100 and 120 % of label claim of AGM afforded recovery of 99.09-100.32 % as listed in Table 4.

The data of summary of validation parameters are listed in Table 6.

Table 6.Summary of validation parameters

Parameter	Data
Linearity range	20-100 ng/ml
Correlation coefficient	0. 9987± 0.09
Limit of detection	3 ng/ml
Limit of quantitation	10 ng/ml
Recovery (n=6)	
T-1	
T-2	
Precision (% RSD)	
Repeatability of application	0.94
Inter day (n=6)	1.07
Intra day (n=6)	0.81
Robustness	Robust
Specificity	0.9986

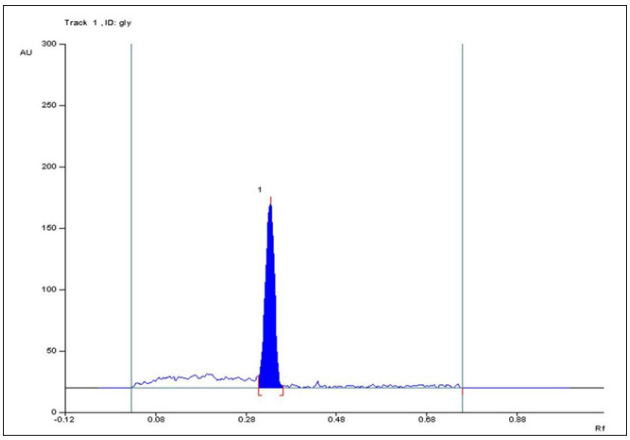

Figure 2. Densitogram of standard AGM (1000 ng/spot); peak 1 (R_F: 0.44±0.08). ethyl acetate: ammonia (33%): methanol (8.0:1.0:1.0, v/v/v).

Conclusion

A new HPTLC method has been developed for the identification and quantification of AGM in formulations. The method was found to be simple, sensitive, precise, accurate and specific for estimation and can be conveniently employed for the routine quality control analysis of AGM from tablets.

Conflicts of Interest: None

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