STABILITY-INDICATING HPTLC METHOD FOR QUANTITATIVE ESTIMATION OF AGOMELATINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

Joshi Hitendra S. *, Rupareliya Reema H.

Department of Chemistry, Saurashtra University, Rajkot-360 005, Gujarat, India.

ABSTRACT
A simple, precise and stability-indicating high performance thin chromatographic method for analysis of Agomelatine in bulk drug and in a tablet formulation has been developed and validated. Aluminum foil TLC plates pre-coated with silica gel 60F were used as stationary phase Dichloro methane and methanol in the ratio of (95:5v/v) were used as mobile phase. A compact band (Rf 0.52±0.002) was obtained for Agomelatine. Densitometric analysis was performed in absorbance mode at 230 nm. Linear regression analysis revealed a good linear relationship (r² = 0.9982) between peak area and concentration in the range of 0.2-0.8 µg/spot. The precision (relative standard deviation: RSD) among a six sample preparation was 0.94% and 0.93%. The accuracy (recovery) was validated for specificity, linearity, precision, recovery and robustness. The limits of detection and quantization were also determined. Statistical analysis proved the method enables repeatable, selective and accurate for analysis of the drug. It can be used for identification and quantitative analysis of Agomelatine in bulk drug and in tablet formulation.

Keywords: Agomelatine, Stability-Indicating, Method Validation, HPTLC Method.

INTRODUCTION
Agomelatine is chemically N-[2-(7-methoxy napthalen-1-yl) ethyl] acetamide (Fig.1). Its molecular formula is C₁₅H₁₇NO₂ and its molecular weight is 243.301gm/mol. Agomelatine, a sleep modulating antidepressant was approved by the European Medicines Agency for the treatment of major depressive disorder (MDD) in 2009 [1]. The novel antidepressant...
agomelatine acts as a melatonergic receptor (MT₁/MT₂) agonist and serotonergic receptor (5-HT₂c) antagonist. Binding studies indicate that it has no effect on monoamine uptake and no affinity for α, β adrenergic, histaminergic, cholinergic, dopaminergic, and benzodiazepine receptors [2-3]. Agomelatine showed significant benefits over paroxetine due to the complete absence of side effects including the associated sexual effect that are troublesome with some antidepressant. Because of its action upon the melatonine receptors, agomelatine shows a marked improvement on sleep. Agomelatine has also proven to have anxiolytic properties and thus may prove to be very useful in the treatment of anxiety disorders [4-5].

![Fig. 1 Structure of Agomelatine](image)

The parent drug stability test guideline Q1A (R2) issued by International Conference on Harmonization (ICH) suggests that stress testing is an essential part of development strategy and is carried out under more severe conditions than accelerated conditions. These studies provide information to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical methods. [6-9]. According to ICH guidelines stress testing should include the effect of temperature, light, oxidizing agents as well as susceptibility across a wide range of pH values and separation of drugs from degradation products[10]. It is also suggested that analysis of stability sample should be done by using validated stability testing methods.

Methods reported in literature for analysis of Agomelatine include determination of agomelatine by RP-HPLC [11] and HPLC method for separating and analyzing agomelatine intermediate and final product thereof [12] and also Validated LC-MS/MS method for quantification of agomelatine in human plasma and its application in a pharmacokinetic study [5]. To our knowledge there has been no stability indicating HPTLC method reported for agomelatine in which ICH recommended stress conditions were applied. Therefore the stability indicating method was developed by applying different stress conditions like acidic, alkali, H₂O₂, thermal and photo degradation.
EXPERIMENTAL

Instrumentation
The chromatographic system used to perform development and validation of this assay method was Camag Linomat V Sample applicator, Camag Twin trough glass chamber and Camag TLC scanner III equipped with Cats 3 Version software.

Reagents and reference substance
Agomelatine standard was provided by Electron Inc.-Ahmadabad (India). Agomelatine tablets containing 25mg Agomelatine and the inactive ingredient used in drug matrix were obtained from market. HPLC grade methanol and Dichloro methane were obtained from Spectrochem Pvt. Ltd., Mumbai (India). All chemicals and reagents used were of AR grade.

Chromatographic conditions
The mobile phase consisted of Dichloromethane-methanol (9.5:0.5 v/v). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm) previously saturated with mobile phase vapour for 30min. at room temperature and relative humidity 60 ± 5%. The development distance was approximately 80 mm. After development the plates were dried in current of air by use of an air dryer. Densitometric scanning, at 230nm was performed with a Camage TLC scanner III, operated by Wincats Software (V 1.4.2, Camage) in absorbance mode. The selection of wavelength was based on maximum absorbance for optimum sensitivity. The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the concentration range 190-400 nm. The slit dimensions were 5 mm x 0.45 mm.

Standard preparation
The standard Agomelatine 25 mg was weighted accurately and transferred to volumetric flask 50 ml. It was dissolved and sonicated for few minutes and diluted up to the mark with methanol to obtain stock solution. Then 10ml of stock solution was taken in 50 ml volumetric flask and diluted up to mark to obtain final concentration 100 µg/ml and the resulting solution was used as working standard stock solution.

Test preparation
To determine the content of Agomelatine in tablet formulations, 20 branded tables were weighed; their means weight was calculated and crushed to fine powder. Tablet power equivalent to 1 tablet of Agomelatine was weighed and transferred to 50 ml volumetric flask then dissolved with methanol and further diluted with methanol. It was kept for ultra
sonication for few min; the solution was then filtered through Whatmann filter paper No.41 and further dilution was with methanol to get final sample solution of 100µg/ml. The resultant solution was used as such for analysis.

**Degradation study**

The degradation samples were prepared by transferring powdered tablets, equivalent to 25.0 mg agomelatine into a 250 ml round bottomed flask. Then drug content were employed for acidic, alkaline and oxidant media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature and diluted with diluent to attain 100 µg/ ml agomelatine concentration. Specific degradation conditions were described as follows.

**Acidic degradation condition**

Acidic degradation study was performed by heating the drug content in 1 N HCl at 60°C for 30 min and mixture was neutralized.

**Alkali degradation condition**

Alkaline degradation study was performed by ambient temperature in 0.005N NaOH for 45 min and mixture was neutralized.

**Oxidative degradation condition**

Oxidation degradation study was performed by heating the drug content in 30% v/v H₂O₂ at 80°C for 45 min.

**Thermal degradation condition**

Thermal degradation was performed by exposing solid drug to dry heat of 80°C in a conventional oven for 72 hr.

**Photolytic degradation condition**

Photolytic degradation study was performed by exposing the drug content in UV-light for 72 hr.

**METHOD VALIDATION**

**Specificity study**

The specificity of the method was determined by checking the interference of placebo with analyte and the proposed method was eluted by checking the peak purity of agomelatine
during the force degradation study. The peak purity of the agomelatine was found satisfactory (0.99) under different stress condition. There was no interference of any peak of degradation product with drug peak.

**Linearity**

Linearity test solutions for the assay method were prepared at seven concentration levels from 40 % to 160 % of assay analyte concentration (40, 60, 80, 100, 120, 140 and 160µg/ml). The peak areas versus concentration data were evaluated by linear regression analysis.

**Precision**

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of Agomelatine test sample preparation and calculated the % RSD of assay (intraday). Intermediate precision of the method was checked by performing same procedure on the different day (inter day) by another person under experimental condition.

**Accuracy**

An accuracy study was performed by adding known amounts of agomelatine to the placebo preparation. The actual and measured concentrations were compared. Recovery of the method was evaluated at three different concentration levels (corresponding to 50, 100 and 150 % of test preparation concentration). For each concentration level, three sets were prepared and injected in duplicate.

**Robustness**

The robustness of study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions. The factors chosen for this study were the mobile phase composition [Dichloromethane-methanol (97:03 and 93:07, v/v)] and analyst change.

**Solution stability**

The stability study of solution for test preparation was carried out. The solution was stored at ambient temperature and 2-5°C and tested at interval of 12, 24, 36 and 48 hr. The responses for the aged solution were evaluated using a freshly prepared standard solution.

**RESULT AND DISCUSSION**

TLC procedure was optimized with a view to develop a stability indicating assay method. The drug reference standards were spotted on the TLC plates and developed in different
solvent systems. Different mobile phases were tried to resolve Agomelatine and degradants. Best suited mobile phase found was Dichloro methane and methanol in the volume ratio of (9.5:0.5 v/v) respectively. Developed mobile phase enabled good resolution, and a sharp and symmetrical peak at Rf 0.52 for Agomelatine form a compact and non-diffuse band. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min (the optimum saturation time) ensured good reproducibility and peak shape. Chromatogram of standard preparation is represented in (Fig. 2).

Fig. 2: Chromatogram for standard preparation

The drug substance was easily extracted from the pharmaceutical dosage form using methanol. The tablet dispersed readily in water and the drug substance was freely soluble in methanol. Solutions of standard and test preparation were found to be stable in methanol which was used as a diluent. Chromatogram of sample preparation is represented in (Fig. 3).

Fig. 3: Chromatogram for test preparation
After development of the analytical method, it was validated in accordance with ICH and USP guidelines. This furnished evidence the method was suitable for its intended purpose. The intensive approach described in this manuscript was used to develop and validate a high performance thin layer liquid chromatographic analytical method that can be used for assay validation of agomelatine a pharmaceutical dosage form. Degradation product produced as a result of stress did not interfere with detection of agomelatine and the assay method can thus be regarded as stability indicating. Optimized Chromatographic conditions are shown below (Table 1).

**Table 1 Optimized chromatographic conditions for Agomelatine**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Chromatographic Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development chamber</td>
<td>CAMAGE Twin Trough Chamber</td>
</tr>
<tr>
<td>Stationary phase</td>
<td>Silica gel GF254 pre-coated on aluminum sheet</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Ethyl acetate: Hexane(80:20)</td>
</tr>
<tr>
<td>Chamber saturation</td>
<td>45 min.</td>
</tr>
<tr>
<td>Sample applicator</td>
<td>CAMAGE LINOMAT V</td>
</tr>
<tr>
<td>Band</td>
<td>6mm</td>
</tr>
<tr>
<td>Space</td>
<td>9 mm</td>
</tr>
<tr>
<td>Scanning speed</td>
<td>20mm/sec</td>
</tr>
<tr>
<td>Development distance</td>
<td>8 cm</td>
</tr>
<tr>
<td>Drying of plate</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Densitometric scanner</td>
<td>CAMAGE TLC SCANNER</td>
</tr>
<tr>
<td>Lamp</td>
<td>Deuterium</td>
</tr>
<tr>
<td>Wavelength</td>
<td>230 nm</td>
</tr>
<tr>
<td>Volume</td>
<td>5µl</td>
</tr>
</tbody>
</table>

The specificity of the method was evaluated by checking the interference of placebo with analyte and the proposed method was evaluated by checking the peak purity of Agomelatine during the force degradation study. There was no interference of any peak of degradation product with drug peak.

Major degradation was found in acidic condition that product was degraded up to 19.50% (Fig.4). The Drug was slightly degraded into alkali, H2O2 and photolytic degradation while it was found to be stable into thermal degradation.
To determine linearity a calibration graph was obtained by plotting Agomelatine concentration against peak area (Fig.5). Linearity was good in the concentration range 40 to 160µg/ml. The response of the drug was found to be linear regression equation for agomelatine was $y = 63,032.1429 \times + 1,287.7857$ with correlation coefficient 0.9982, where $x$ is the concentration in mg/ml and $y$ is the peak area in absorbance units (AU).

For assay ($n=6$) the system precision was 0.74% on the same day (intra-day) and 0.62% on different days (inter-day). The mean values of method precision (repeatability) were 99.61%, RSD 0.94%, on the same day (intra-day) and 100.05%, RSD 0.93% on different days (inter-day). Intermediate precision was established by determining the overall (intraday and inter day) method precision for assay.

Intermediate precision was established by determining the overall (intraday and inter day) method precision. For intermediate precision ($n=12$), overall % assay and % RSD value was 99.83% and 0.0.82% respectively for Agomelatine. The precise result for content uniformity was indicative of uniform distribution of the drug in the tablets without significant variation;
this is accordance with the USP, which stipulates acceptance limits for drug content uniformity and RSD as 85 - 115 % and < 6% respectively [13].

The accuracy of the method was assessed by determination of recovery for three concentrations covering the range of the method. Known amounts of agomelatine (12.5, 25, 37.5µg/ml) were added to a placebo preparation and the amount of agomelatine recovered, in the presence of placebo interface, was calculated. The mean recovery of agomelatine was 100.32%, 99.31% and 99.09% respectively (Table 2).

Table 2 Results of accuracy study of Agomelatine

<table>
<thead>
<tr>
<th>Level (%)</th>
<th>Theoretical concentration(^a) (µg/ml)</th>
<th>Observed concentration(^a) (µg/ml)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agomelatine</td>
<td>50</td>
<td>50.17</td>
<td>50.33</td>
<td>100.32</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100.1</td>
<td>99.41</td>
<td>99.31</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>150.03</td>
<td>148.67</td>
<td>99.09</td>
</tr>
</tbody>
</table>

\(^a\) Each value corresponds to the mean of three determinations.

The robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions. For each different analytical condition the standard solution and test solution were prepared separately. The result obtained from assay of the test solution was not affected by varying the conditions and was in accordance with the true value (Table 3). The analytical method therefore remains unaffected by slight but deliberate changes in the analytical conditions.

Table 3 Evaluation data of robustness study of Agomelatine

<table>
<thead>
<tr>
<th>Robust conditions</th>
<th>% Assay</th>
<th>System suitability parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Area</td>
</tr>
<tr>
<td>MDC: Methanol (97: 3)</td>
<td>98.90</td>
<td>6798</td>
</tr>
<tr>
<td>MDC: Methanol (93: 7)</td>
<td>99.69</td>
<td>8037</td>
</tr>
<tr>
<td>Analyst Change</td>
<td>98.79</td>
<td>7412</td>
</tr>
</tbody>
</table>

During study of the stability of stored solutions of standards and test preparations for assay determination the solutions were found to be stable for up to 48hr. Assay values obtained after 48hr were statistically identical with the initial value without measurable loss.

Before each measurement of validation data a system suitability test was performed by measurement of general characteristics such as peak asymmetry, number of theoretical plates.
and RSD (%) of peak area observed for a standard solution. The values obtained were satisfactory and in accordance with in-house limits (Table 4).

Table 4 Evaluation data of system suitability study of Agomelatine

<table>
<thead>
<tr>
<th>System suitability data</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-House limit</td>
<td>NMT&lt;sup&gt;b&lt;/sup&gt;2.0</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.99</td>
</tr>
<tr>
<td>Linearity</td>
<td>0.43</td>
</tr>
<tr>
<td>Precision For Assays</td>
<td>0.94</td>
</tr>
<tr>
<td>Intermediate Precision For Assays</td>
<td>0.93</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.36</td>
</tr>
</tbody>
</table>

CONCLUSION
A new analytical method has been developed to be routinely applied to determine Agomelatine in pharmaceutical dosage form was established through employment of ICH recommended stress condition. The developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear, precise, accurate and robust. Hence, the method is recommended for routine control analysis and stability sample analysis.

ACKNOWLEDGEMENT
The authors are thankful to quality Electron Inc.-Ahmadabad for providing Agomelatine Standard and also thankful to facilities & grants given under UGC- Special Assistance Programme Department Research Support (DRS) and Department of Science & Technology (DST) New Delhi, Fund For Improvement of Science & Technology (FIST), National Facility for Drug Discovery (NFDD) and Department of Chemistry, Saurashtra University, Rajkot – 360 005 (INDIA) for providing analytical facilities.

REFERENCES


