Development and Validation of Stability Indicating HPLC Assay Method for Determination of Tapentadol in Tablet Formulation

Gaurang P. Pandya, Hitendra S. Joshi

Abstract - The purpose of the research described herein was to develop simple, precise and accurate isocratic stability indicating reversed phase HPLC assay method for determination of Tapentadol solid dosage forms. Isocratic RP-HPLC method was developed on Phenomenex Luna C8 150 × 4.6mm, 5μm column using mobile phase as methanol – 0.002 M potassium dihydrogen phosphate buffer pH 3.0 (60: 40 v/v) at a flow rate of 1.0 ml/min and the detection was carried out at 272 nm using photo-diode array detector. The drug was subjected to oxidation, hydrolysis, photolysis and heat to apply stress condition. The validation element investigated showed that the method has acceptable specificity, accuracy, linearity, solution stability, precision and robustness.

Index Terms - Tapentadol, Stability indicating assay, Method development, Method validation

1. Introduction

Tapentadol is chemically 3-((2R,3R)-1-(dimethylamino)-2-methylpentan-3-yl)phenol (Fig.1). Its molecular formula is C_{16}H_{23}NO having molecular weight 221.34 gm/mol. Tapentadol is centrally acting analgesic with broad analgesic efficacy that was approved by Food and Drug Administration in 2008. The drug has dual mode of action in single molecule as an agonist at μ-opioid receptor and as a norepinephrine reuptake inhibitor [1], [2]. With this mode of action Tapentadol provides analgesia at comparable levels of more potent narcotic analgesics such as morphine, oxycodone and hydrocodone [3],[4] but its μ-sparing effect reduces the frequency and/or severity of side effects. Its other action on noradrenaline re-uptake gives a high analgesic potency [5]. Major sites of action of this drug are the spinal μ-opioid receptors and that the block of the noradrenaline lead to potentially synergistic activation of the spinal α₂ adrenoceptors [6].

According to ICH guidelines stress testing is an integral part of developmental strategy and is carried out under more severe condition than that of accelerated conditions. These studies provide information of drug’s intrinsic stability. Stress testing is useful in developing and validating suitable analytical methods [7],[8],[9]. It is suggested in the ICH guidelines that stress testing should be done including the effect of temperature, light, oxidizing agent and susceptibility to hydrolysis across a wide range of pH values. It is also needed that analysis of stability sample should be carried out with the validated stability testing methods.

Few bioanalytical methods have been reported for determination of Tapentadol in blood plasma by using LC-MS-MS [10],[11],[12]. Besides, some methods have been reported for determination of Tapentadol in urine including LC-MS-MS and UPLC-MS.

To the best of our literature survey, so far there is no published report describing validated stability indicating HPLC method for determination of Tapentadol available in literature. This paper deals with forced degradation of Tapentadol under acidic hydrolysis, alkali hydrolysis and oxidation, thermal and photolytic stress condition and the validation of developed method for assay of Tapentadol from its dosage form (tablets).

2. Experimental

2.1 Instrumentation

The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10ATvp binary pump, a SPD-M10Avp photo-diode array detector and a rheodyne manual injector model 7725i with 20μl loop (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system (Class-VP 6.13 SP2, Shimadzu).

2.2 Reagents and Reference substance

Tapentadol standard was provided by Ami Life sciences Laboratories Ltd., Baroda (India). Tapentadol tablets containing 50 mg Tapentadol and the inactive ingredient used in drug matrix were obtained from market. HPLC grade methanol was purchased from Spectrochem Pvt. Ltd., Mumbai (India). HPLC grade water was produced in Milli-Q (Millipore, Millford, USA) system. Membrane filters of 0.45μm (Millipore) were used. Analytical grade ortho-phosphoric acid, hydrochloric acid, sodium hydroxide pellets and 30% v/v hydrogen peroxide...
solution were obtained from Ranbaxy Fine Chemicals, New Delhi (India).

2.3 Chromatographic conditions

Chromatographic analysis was performed on Phenomenex Luna C8 (150mm × 4.6mm i.d., 5µm particle size) column applying an isocratic elution using methanol – 0.002 M potassium dihydrogen phosphate buffer pH 3.0 (60: 40 v/v) as a mobile phase. The mobile phase was filtered through 0.45µm membrane filter and degassed for 30 minute in an ultrasonic bath prior to its use. Flow rate of mobile phase was adjusted to 1.00 ml/min and injection volume was 20 µL. Detection was performed at 272 nm.

2.4. Standard preparation

Tapentadol standard stock solution containing 500µg/ml was prepared in a 100 ml volumetric flask by dissolving 50.00 mg of Tapentadol and then diluted to volume with water as a diluent. Further take 10 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of 100µg/ml).

2.5. Test preparation

Twenty tablets were weighed and the average weight of tablet was determined. From these, five tablets were weighed and transfer into a 500 ml volumetric flask. About 50 ml of diluent was added and sonicated for a minimum 30 min. with intermittent shaking. Then content was brought back to room temperature and diluted to volume with diluent. The sample was filtered through 0.45µm nylon syringe filter. Further take 10 ml of this stock solution in 50 ml of volumetric flask and make up to mark with diluent. The concentration obtained was 100 µg/ml of Tapentadol.

The degradation samples were prepared by transferring powdered tablets, equivalent to 50 mg of Tapentadol into a 250 ml round bottom flask. Then prepared samples were employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After completing the degradation treatments, the stress content solutions were allowed to equilibrate to room temperature and diluted with mobile phase to attain 100 µg/ml concentrations of Tapentadol. Specific conditions were described as follows.

2.6.1. Acidic degradation condition

Acidic degradation study was performed by taking the drug content in 0.1 N HCl at room temperature for 2.0 hours and mixture was neutralized.

Fig. 3 Chromatogram of Acid degradation

2.6.2. Alkali degradation condition

Alkaline degradation study was performed by taking the drug content in 0.05 N NaOH at room temperature for 2.0 hours and mixture was neutralized.

Fig. 4 Chromatogram of Alkali degradation

2.6.3. Oxidative degradation condition

Oxidative degradation study was performed by taking the drug content in 30% v/v H2O2 at room temperature for 2 hours.

Fig. 5 Chromatogram of Oxidative degradation

2.6.4. Thermal degradation condition

Thermal degradation was performed by exposing solid drug at 80°C for 72 hours.
2.6.5. Photolytic degradation condition

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

2.7. Method validation

2.7.1 Specificity study

The evaluation of the specificity of the method was determined against placebo. The interference of the excipients of the claimed placebo present in pharmaceutical dosage form was derived from placebo solution. Further the specificity of the method toward the drug was established by means of checking the interference of the degradation products in the drug quantification for assay during the forced degradation study.

2.7.2 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.

2.7.3 Precision

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of Tapentadol 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 (interday) by another person under the same experimental condition.

Table I: Evaluation data of precision study

<table>
<thead>
<tr>
<th>Level</th>
<th>Theoretical Concentration (mg/ml)</th>
<th>Observed Concentration (mg/ml)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.04993</td>
<td>0.05001</td>
<td>100.15</td>
<td>0.429</td>
</tr>
<tr>
<td>100</td>
<td>0.10053</td>
<td>0.10059</td>
<td>100.06</td>
<td>0.614</td>
</tr>
<tr>
<td>150</td>
<td>0.15053</td>
<td>0.15004</td>
<td>99.67</td>
<td>0.662</td>
</tr>
</tbody>
</table>

2.7.4 Accuracy

An accuracy study was performed by adding known amounts of Tapentadol 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 triplicate.

Table II: Evaluation data of Accuracy Study

2.7.5. 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 flow rate (±0.1 ml/min), mobile phase composition methanol – 0.002 M potassium dihydrogen phosphate buffer pH 3.0 (62: 38 and 58: 42 v/v) and using different lot of LC column.
2.7.6. Solution stability

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

<table>
<thead>
<tr>
<th>Robust conditions</th>
<th>% Assay</th>
<th>System suitability parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Theoretical plates</td>
</tr>
<tr>
<td>Flow 0.9 ml/min</td>
<td>100.56</td>
<td>4235</td>
</tr>
<tr>
<td>Flow 1.1 ml/min</td>
<td>100.84</td>
<td>4430</td>
</tr>
<tr>
<td>Acetonitrile:water:0.1% acetic acid(62:38:0.1)</td>
<td>100.22</td>
<td>4316</td>
</tr>
<tr>
<td>Acetonitrile:water:0.1% acetic acid(58:42:0.1)</td>
<td>100.20</td>
<td>4615</td>
</tr>
<tr>
<td>Column change</td>
<td>99.82</td>
<td>4416</td>
</tr>
</tbody>
</table>

3. Result and discussion

To develop a rugged and suitable HPLC method for the quantitative determination of Tapentadol, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition and other chromatographic conditions. Our preliminary trials using different composition of mobile phases consisting of water with methanol or acetonitrile, did not give good peak shape. By keeping mobile phase composition as methanol – 0.002 M potassium dihydrogen phosphate buffer pH 3.0 (60: 40 v/v), best peak shape was obtained. For the selection of organic constituent of mobile phase, methanol was chosen and to attain good peak shape. Chromatogram of standard preparation is represented in (Fig. 2). A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same. For all system suitability injections, asymmetry was less than 2.0, theoretical plate was greater than 4000 and % RSD of peak area was found less than 2.0. 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 Tapentadol during the force degradation study. There was no interference of any peak of degradation product with drug peak. Major degradation was found in alkaline condition in which product was degraded up to 22%. The major impurity peak was found at 4.263 min. (Fig. 4). In oxidative degradation, it was found that around 12 % of the drug degraded and impurity peak was found at 5.374 min. (fig. 5) and in acidic condition around 7 % of the drug degraded and impurity peak was found at 5.602 min. (fig. 3). Tapentadol was found to be slightly degraded in thermal degradation while it was stable under the photolytic condition. Seven points calibration curve were obtained in a concentration range from 40-160 µg.ml for Tapentadol. The response of the drug was found to be linear in the investigation concentration range and the linear with correlation coefficient 0.999. The result of repeatability and intermediate precision study is shown in Table 1. The developed method was found to be precise as the % RSD values for the repeatability and intermediate precision studies were <0.51 % and <0.94 %, respectively, which confirm that method was precise. The HPLC area responses for accuracy determination are depicted in Table 2. The results show that best recoveries (99.67-100.15%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate. The result of robustness study of the developed assay method was established in Table 3. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. Table 4 shows the results obtained in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 hours at 2 - 5° C and ambient temperature as during this time the result was not decreased below the minimum percentage. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

4. Conclusion

A new analytical method has been developed to be routinely applied to determine Tapentadol in pharmaceutical dosage form. In this study, stability of Tapentadol in present 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 and stability indicating. Hence, the method is recommended for routine quality control analysis and also for stability sample analysis.

Acknowledgements

The authors are thankful for 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


Development and validation of stability indicating HPLC assay method for simultaneous determination of amlodipine besylate, olmesartan medoxomil and hydrochlorothiazide in tablet formulation

Gaurang P. Pandya and Hitendra S. Joshi*

Department of Chemistry, Saurashtra University, Rajkot, Gujarat, India

ABSTRACT

The purpose of the research described herein was to develop simple, precise and accurate isocratic stability indicating reversed phase HPLC assay method for determination of simultaneous determination of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide solid dosage forms. Isocratic RP-HPLC method was developed on Phenomenex Gemini C18 250×4.6mm, 5µm column using mobile phase as 0.02M ammonium acetate buffer pH 4.5 - Acetonitrile (60:40, v/v) at a flow rate of 1.0 ml/min and the detection was carried out at 241 nm using photo-diode array detector. The drug was subjected to oxidation, hydrolysis, photolysis and heat to apply stress condition. The validation element investigated showed that the method has acceptable specificity, accuracy, linearity, solution stability, precision and robustness.

Keywords: Amlodipine besylate, Olmesartan medoxomil, Hydrochlorothiazide Stability indicating assay, Method development, Method validation

INTRODUCTION

Stress testing is a part of developmental strategy under International Conference on Harmonization (ICH) requirements and is carried out under more severe conditions than accelerated conditions. These studies serve to give information on a drug’s inherent stability and assist in the validation of analytical methods to be used in stability studies (1–3). It is suggested that stress testing should include the effects of temperature, light, oxidizing agents and susceptibility across a wide range of pH values. It is also recommended that analysis of stability samples should be accomplished through the use of a validated stability-testing method.

Amlodipine besylate is chemically described as 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate. Its empirical formula is C_{20}H_{25}ClIN_{2}O_{6}•C_{6}H_{4}O_{3}S, and its structural formula is shown in figure 1. Amlodipine belongs to the dihydropyridine (DHP) class of calcium channel blockers (CCBs), the most widely used class of CCBs. Similar to other DHP CCBs, amlodipine binds directly to inactive L-type calcium channels stabilizing their inactive conformation. Since arterial smooth muscle depolarizations are longer in duration than cardiac muscle depolarizations, inactive channels are more prevalent in smooth muscle cells. Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels. The vasodilatory effects of amlodipine result in an overall decrease in blood pressure. Amlodipine is a long-acting CCB that may be used to treat mild to moderate essential hypertension and exertion-related angina (chronic stable angina).
Olmesartan medoxomil is chemically \((5\text{-methyl-2-oxo-2H-1,3-dioxol-4-yl})\text{methyl} 4\text{-}(2\text{-hydroxypropan-2-yl})\text{2-propyl-1-(4\text{-}[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl)methyl})\text{-1H-imidazole-5-carboxylate}\) [figure 2]. Its molecular formula is \(C_{39}H_{30}N_{6}O_{6}\) having molecular mass 558.58 gm/mole. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the \(AT_{1}\) receptor in vascular smooth muscle. Its action is, therefore, independent of the pathways for angiotensin II synthesis.

Hydrochlorothiazide is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide. Its molecular formula is \(C_{7}H_{8}N_{3}O_{4}S_{2}\)Cl having molecular mass 297.74 gm/mole. Hydrochlorothiazide belongs to the thiazide class of diuretics. It reduces blood volume by acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electroneutral \(Na^{+}\text{-Cl}^{-}\) co-transporter by competing for the chloride site on the transporter. By impairing Na transport in the distal convoluted tubule, hydrochlorothiazide induces a natriuresis and concomitant water loss. Thiazides increase the reabsorption of calcium in this segment in a manner unrelated to sodium transport.

Many methods, either individual or in a combination of Amlodipine, Olmesartan and Hydrochlorothiazide have been reported, including simultaneous determination of Amlodipine with Olmesartan, Olmesartan with Hydrochlorothiazide and Amlodipine with Olmesartan. Few methods are also reported for simultaneous determination of Amlodipine with Olmesartan and Hydrochlorothiazide in combination formulations by ultraviolet (UV) absorption and UV derivative spectrophotometry, spectrophotometric determination with artificial neural network, high performance liquid chromatography (10–12), ultra high performance liquid chromatography and thin-
layer chromatographic determination (13). Furthermore these methods are not impressionable to achieve the high throughput study which can be possible by optimizing the method in such a way which includes shortest runtime with maximum selectivity. Hence, it can be maximum utilize for the analysis of formulation development and stability testing as well as at quality control laboratory for routine use.

This paper deals with forced degradation of Amlodipine, Olmesartan and Hydrochlorothiazide under acidic hydrolysis, alkali hydrolysis and oxidation, thermal and photolytic stress condition and the validation of developed method for assay of Amlodipine with Olmesartan and Hydrochlorothiazide from its dosage form (tablets).

MATERIALS AND METHODS

2.1 Instrumentation
The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10ATvp binary pump, a SPD-M10Avp photo-diode array detector and a rheodyne manual injector model 7725i with 20µl loop (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system (Class-VP 6.13 SP2, Shimadzu).

2.2 Reagents and Reference substance
Olmesartan medoxomil and Amlodipine besylate standards were provided by Cadila pharmaceuticals Ltd., Ahmadabad (India) and Hydrochlorothiazide standard was provided by Alembic pharmaceuticals Ltd., Baroda (India). Olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide tablets containing 40mg Olmesartan medoxomil, 10mg amlodipine besylate and 25mg hydrochlorothiazide and the inactive ingredient used in drug matrix were obtained from market. HPLC grade acetonitrile was obtained from Spectrochem Pvt. Ltd., Mumbai (India). Analytical grade ammonium acetate, hydrochloric acid, glacial acetic acid, sodium hydroxide pellets and 30% v/v hydrogen peroxide solution were obtained from Ranbaxy Fine Chemicals, New Delhi (India).

2.3 Chromatographic conditions
Chromatographic analysis was performed on Phenomenex Gemini C18 (250mm × 4.6mm i.d., 5µm particle size) column applying an isocratic elution using a mixture of 0.02M ammonium acetate buffer pH 4.5 - Acetonitrile (60:40, v/v) as a mobile phase. The mobile phase was filtered through 0.45µm membrane filter and degassed for 30 minute in an ultrasonic bath prior to its use. Flow rate of mobile phase was adjusted to 1.00 ml/min and injection volume was 20 µL. Detection was performed at 241 nm. Normal run time was chosen as 20 minutes but for degradation study chromatographic run was carried out up to 60 minutes to confirm that any degradation peak is eluted after 20 minutes or not.

2.4. Standard preparation
Olmesartan medoxomil standard stock solution containing 400µg/ml was prepared in a 100 ml volumetric flask by dissolving 40.00 mg of Olmesartan medoxomil and then diluted to volume with diluent. Further take 25 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of 200µg/ml). Amlodipine besylate standard stock solution containing 100µg/ml was prepared in a 100 ml volumetric flask by dissolving 10.00 mg of Amlodipine besylate and then diluted to volume with diluent. Further take 25 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of 50µg/ml). Hydrochlorothiazide standard stock solution containing 250µg/ml was prepared in a 100 ml volumetric flask by dissolving 25.00 mg of Hydrochlorothiazide and then diluted to volume with diluent. Further take 25 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of 125µg/ml).

2.5. Test preparation
Twenty tablets were weighed and the average weight of tablet was determined. From these, five tablets were weighed and transfer into a 500 ml volumetric flask. About 50 ml of diluent was added and sonicated for a minimum 30 minute with intermittent shaking. Then content was brought back to room temperature and diluted to volume with diluent. The sample was filtered through 0.45µm nylon syringe filter. Further take 25 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent. The concentration obtained was 200 µg/ml of Olmesartanmedoxomil, 50µg/ml of Amlodipine besylate and 125 µg/ml of Hydrochlorothiazide.
2.6 Degradation Study
2.6.1. Acidic degradation condition
Acidic degradation study was performed by taking the drug content in 0.1 N HCl at room temperature for 2.0 hours and mixture was neutralized.

2.6.2. Alkaline degradation condition
Alkaline degradation study was performed by taking the drug content in 0.05 N NaOH at room temperature for 2.0 hours and mixture was neutralized.

2.6.3. Oxidative degradation condition
Oxidative degradation study was performed by taking the drug content in 30% v/v H₂O₂ at room temperature for 2 hours.

2.6.4. Thermal degradation condition
Thermal degradation was performed by exposing solid drug at 80˚C for 72 hours.

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

2.7. Method validation

2.7.1 Specificity study
The evaluation of the specificity of the method was determined against placebo. The interference of the excipients of the claimed placebo present in pharmaceutical dosage form was derived from placebo solution. Further the specificity of the method toward the drug was established by means of checking the interference of the degradation products in the drug quantification for assay during the forced degradation study.

2.7.2 Linearity
Linearity test solutions for the assay method were prepared at seven concentration levels from 40 to 160 % of assay analyte concentration. The peak areas versus concentration data were evaluated by linear regression analysis.

2.7.3 Precision
The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of 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 (interday) by another person under the same experimental condition.

2.7.4 Accuracy
An accuracy study was performed by adding known amounts of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide 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 triplicate.

2.7.5. 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 flow rate (±0.1 ml/min), mobile phase composition 0.02M ammonium acetate buffer pH 4.5 - Acetonitrile (62: 38 and 58: 42 v/v) and using different lot of LC column.

2.7.6. Solution stability
The stability of solution for test preparation was evaluated. The solution was stored at ambient temperature and 2-5°C and tested at interval of 12, 24, 36 and 48 hours. The responses for the aged solution were evaluated against a freshly prepared standard solution.

![Chromatogram of Acidic forced degradation study](image)

![Chromatogram of Alkali forced degradation study](image)
RESULTS AND DISCUSSION

To develop a rugged and suitable HPLC method for the quantitative determination of amlodipine, olmesartan and hydrochlorothiazide, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition and other
chromatographic conditions. Our preliminary trials using different composition of mobile phases consisting of water with methanol or acetonitrile, did not give good peak shape. By keeping mobile phase composition as 0.02M ammonium acetate buffer pH 4.5 - Acetonitrile (60:40, v/v), best peak shape was obtained. For the selection of organic constituent of mobile phase, Acetonitrile was chosen to reduce run time and to attain good peak shape. Chromatogram of standard preparation is represented in (Fig. 2). A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same. For all system suitability injections, asymmetry was less than 2.0 and % RSD of peak area was found less than 2.0. 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 all drug peaks during the force degradation study. There was no interference of any peak of degradation product with drug peak. Major degradation was found in oxidative condition in which drug products were degraded up to 24%. (Fig. 3). In oxidative degradation, it was found that around 15 % of the drugs contents were degraded (fig. 5) and in alkaline condition around 8 % of the drugs contents were degraded and impurity peak was found at 5.602 min (Fig. 5). Drug contents were found to be degraded around 2% in thermal degradation while slightly degradation was observed under the photolytic condition. For linearity seven points calibration curve were obtained in a concentration range from 50.0-200.0 µg/ml for Amlodipine, from 80-320 µg/ml for Olmesartan and from 50-200 µg/ml for Hydrochlorothiazide. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was $y = 2E+07x + 3802$ with correlation coefficient 0.999 for Amlodipine, $y = 7E+07x + 15600$ with correlation coefficient 0.999 for Olmesartan and was $y = 1E+08x + 53918$ with correlation coefficient 0.999 for Hydrochlorothiazide. The result of repeatability and intermediate precision study is shown in Table 1. The % RSD values for intraday precision study and interday precision study was < 2.0 % for all drugs which confirm that the method is precise. The HPLC area responses for accuracy determination are depicted in Table 2. The results show that best recoveries (99.72-98.70% for Amlodipine, 99.09-99.47% for olmesartan and 99.23-99.67% for Hydrochlorothiazide) of the spiked drug were obtained at each added concentration, indicating that the method was accurate. Table 3 shows the results obtained in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 hours at 2 - 5˚ C and ambient temperature as during this time the result was not decreased below the minimum percentage. The result of robustness study showed that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

CONCLUSION

A new analytical method has been developed to be routinely applied to simultaneous determination of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide in pharmaceutical dosage form. In this study, stability of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide in present 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 and stability indicating. Hence, the method is recommended for routine quality control analysis and also for stability sample analysis.

Acknowledgements

The authors are thankful for 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


Stability Indicating HPTLC Method for Estimation of Modafinil in the Bulk and Tablet Formulation

Gaurang P. Pandya, Dr. Hitendra S. Joshi*
Department of Chemistry, Saurashtra University, Rajkot-360 005, Gujarat, India.

Abstract: A simple, selective, precise and stability-indicating high-performance thin layer chromatographic method for analysis of Modafinil, both as the bulk drug and in a tablet formulation has been developed and validated. Aluminum foil TLC plates precoated with silica gel 60F 254 were used as stationary phase ethyl acetate, acetone and methanol in the volume ratio of (7:2:1 v/v) respectively as mobile phase. A compact band (Rf 0.42±0.02) was obtained for modafinil. Densitometric analysis was performed in absorbance mode at 232 nm. Linear regression analysis revealed a good linear relationship (r²=0.9995) between peak area and concentration in the range of 80-320 ng /spot. The method was validated for precision, recovery, and robustness. The limits of detection and quantitation were 15 and 50 ng/spot, respectively. Modafinil was subjected to acid and alkaline hydrolysis, oxidation, photochemical and thermal degradation and underwent degradation under all these conditions. Statistical analysis proved the method enables repeatable, selective, and accurate analysis of the drug. It can be used for identification and quantitative analysis of Modafinil in the bulk drug and in tablet formulations.

Key Words: Modafinil HPTLC Validation Stability-indicating Degradation

I. Introduction

Modafinil is chemically 2-[(diphenylmethyl)sulfinyl]acetamide having molecular formula C_{18}H_{15}NO_{2}S and molecular mass 273.35 gm/mol (Figure 1). Modafinil is an analeptic drug manufactured by Cephalon, and is approved by the U.S. Food and Drug Administration (FDA) for the treatment of narcolepsy, shift work sleep disorder and excessive daytime sleepiness associated with obstructive sleep apnea. Narcolepsy is caused by dysfunction of a family of wakefulness-promoting and sleep-suppressing peptides, the orexins. Orexin neurons are activated by modafinil. Modafinil also affects tuberomammillary nucleus [1-2].

![Figure-1 Structure of Modafinil](Image)

However extensive research into the interaction of modafinil with a large number of neurotransmitter systems has been done, an exact mechanism or set of mechanisms of action remains uncertain. It seems that modafinil, like other stimulants, increases the release of monoamines [3-4], specifically the catecholamine, norepinephrine [5-6] and dopamine from the synaptic terminals. Though, modafinil also elevates hypothalamic histamine levels, leading some researchers to consider Modafinil a "wakefulness promoting agent" rather than a classic amphetamine-like stimulant [7-8]. Even though modafinil's histaminergic action, it still partially shares the actions of amphetamine-class stimulants due to its effects on norepinephrine and dopamine [9-11].

Modafinil has been prohibited by World Anti Doping Agency since 2004 so that there are so many bioanalytical methods are reported to detect Modafinil in urine and blood plasma by GC-MS[12], LC-MS/MS[12-14],UPLC-MS[15-17] and HPLC[22]. Some methods are also reported for chiral resolution of modafinil enantiomers by HPLC [18-21]. Besides bioanalytical methods few methods are reported to determine Modafinil in tablet dosage form by HPLC [23-25]. Furthermore these methods are not impressionable to achieve the high throughput study which can be possible by optimizing the method in such a way which includes cost effectiveness with optimum selectivity. Hence, it can be maximum utilize for the analysis of formulation development and stability testing as well as at quality control laboratory for routine use.

In recent times, there is an increase tendency towards the development of stability-indicating assay, using the approach of stress testing as mentioned in the ICH guidelines (Q1A). It is also recommended to carry

www.iosrjournals.org
out stress testing on the drug substance to establish its inherent stability characteristics and to hold up the suitability of the proposed analytical method. In this study our approach is to study degradation behavior of Modafinil and development of a validated stability indicating HPTLC assay method.

II. Experimental

2.1 Chemicals and reagents

The modafinil working standard was provided as a gift sample by Alembic pharmaceutical Ltd, Baroda. All chemicals and reagents used were of AR grade. The formulation, 100 mg and 200 mg is available in market by brand name PROVIGIL.

2.2 HPTLC Instrumentation and conditions

Chromatography was performed on Merck TLC plates pre-coated with silica gel 60 F254 (10 cm × 10 cm with 250 μm layer thicknesses) from E. Merck, Germany. Before use the plates were prewashed with methanol then dried in the current of dry air and activated at 110 °C for 5 min. The samples were applied onto the plates as a band with 4 mm width using Camag 100 μl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The mobile phase consisted of ethyl acetate-acetone-methanol (7:2:1 v/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 30 min 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 232 nm, was performed with a Camag TLC scanner III, operated by Wincats Software (V 1.4.2, Camag) 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 range 190–400 nm. The slit dimensions were 5 mm × 0.45 mm.

2.3 Preparation of standard solution

The standard modafinil 10 mg was weighed accurately and transferred to volumetric flask 100 ml. It was dissolved and sonicated for few minutes and diluted up to the mark with methanol to obtain final concentration of 100 μg/ml and the resulting solution was used as working standard stock solution.

2.4 Preparation of sample solution

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

2.5 Method validation

The method was validated in compliance with ICH guidelines. The following parameters were performed and evaluated for the validation of developed method.

2.5.1 Linearity

Appropriate volume of aliquots from standard modafinil stock solutions were prepared and applied on the TLC plate covering the range from 200-800 ng/spot with the help of micro liter syringe using an automatic sample applicator. The plates were developed, dried and scanned densitometrically at 232 nm. Each concentration was applied seven times to the plate and the plate was developed as described above. Peak areas were plotted against corresponding concentrations to furnish the calibration plot.

| Table 1 | Regression analysis of calibration curve |
| Parameters | Result |
| Linearity Range(ng/Band) | 200-800 |
| correlation coefficient | 0.998 |
| Slope | 82.46 |
| Intercept | 120.2 |
2.5.6 Accuracy
To check the recovery of the drug at different levels in formulations, samples were analysed at 3 different level 50, 100, and 150% of modafinil standard by proposed method and the recovered amount of drugs were calculated. The experiment was conducted in triplicate.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Level of Recovery</th>
<th>Amount of drug taken(mg)</th>
<th>Amount of drug found(mg)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>50</td>
<td>50.11</td>
<td>100.22</td>
<td>1.859</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>100</td>
<td>100.31</td>
<td>100.31</td>
<td>1.797</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>150</td>
<td>149.97</td>
<td>99.38</td>
<td>1.491</td>
</tr>
</tbody>
</table>

a) Mean of three determinations

2.5.3 Precision
Precision of the developed method was measured by repeatability, intra-day and inter-day precision. In repeatability study analyses of six replicates of same concentration (500 ng) were done by developed method. For intra-day and inter-day precision, three sample sets of tablet sample and standard were analyzed in triplicate by developed method on the same day and on three different days, respectively.

2.5.3 Robustness
Small changes in the chromatographic conditions were introduced and the effects on the results were examined. Slight changes in the composition of mobile phase (±0.1ml) were made in the used optimized mobile phase. The time from spotting to chromatography and time from chromatography to scan was varied at ±5 minutes. The chromatographic development distance was varied in the range of ±5 mm. The effects of these changes on both $R_F$ value and peak area were assessed by calculating relative standard deviation (%RSD).

<table>
<thead>
<tr>
<th>Condition</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mobile phase composition</td>
<td>1.34</td>
</tr>
<tr>
<td>amount of mobile phase</td>
<td>0.95</td>
</tr>
<tr>
<td>chamber saturation time</td>
<td>1.21</td>
</tr>
<tr>
<td>time from application to chromatography</td>
<td>1.58</td>
</tr>
<tr>
<td>time from chromatography to scanning</td>
<td>0.66</td>
</tr>
<tr>
<td>Distance from application to solvent front</td>
<td>0.83</td>
</tr>
</tbody>
</table>

2.5.4 Limits of Detection and Quantification
To determine the limits of detection (LOD) and limit of quantification (LOQ), different dilution of standard solution of drug were applied along with methanol as the blank. The LOD and LOQ were determined on the basis of signal to noise ratio until the average responses of triplicate analysis were obtained approximately 3 and 10 times the responses of the blank respectively.

2.5.5 Specificity
The specificity of the method was determined by analysis of drug standards and samples. The band for modafinil in the sample was identified by comparing the $R_F$ value and spectrum of the band with those of the band from a standard. The peak purity of modafinil was assessed by comparing spectra acquired at three different positions on the peak, i.e. the peak start (S), peak apex (M), and peak end (E) positions of the peak.

2.6 Analysis of Marketed Formulation
Assay of marketed tablet was done through developed HPTLC method. The sample solution was made as depicted in section 2.4. Same volume (5 µL) of Standard solution and sample solution were applied on TLC. The experiment was carried out for six sample solutions. The chromatogram obtained is shown in Figure 2.
Table 4  
**Summary of validation parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda) (nm)</td>
<td>232</td>
<td></td>
</tr>
<tr>
<td>(R_F)</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Linear Range (ng/Band)</td>
<td>200-800</td>
<td></td>
</tr>
<tr>
<td>Accuracy (at 100% level)</td>
<td>100.31</td>
<td>1.797</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraday precision</td>
<td>99.98</td>
<td>1.579</td>
</tr>
<tr>
<td>Interday precision</td>
<td>99.57</td>
<td>0.983</td>
</tr>
<tr>
<td>Limit Of Detection (ng/Band)</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Limit Of Quantitation (ng/Band)</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Figure-2  Chromatogram of Modafinil drug sample

Table 5  
**Analysis of tablet formulation**

<table>
<thead>
<tr>
<th>Method</th>
<th>Drug</th>
<th>Label claim [mg/tablet]</th>
<th>Amount found [mg/tablet] (^1)</th>
<th>Drug Assay [%] (^2)</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC-Densitometry</td>
<td>Modafinil</td>
<td>100</td>
<td>99.84</td>
<td>99.84</td>
<td>0.786</td>
</tr>
</tbody>
</table>

\(^1\) Mean of six determinations

2.7 **Forced Degradation studies**  
A stock solution containing 10 mg modafinil in 10 ml methanol was prepared. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of the method. In all degradation studies the average peak area of modafinil after application (500 ng/spot) of seven replicates was obtained after development and scanning of the plate as described above.

2.6.1 **Acid and Base Degradation**  
Modafinil (10 mg) was separately dissolved in 10 ml of methanolic solution of 0.2 N HCl and 0.5 N NaOH. These solutions were refluxed at 80°C for 2 h in the dark in order to exclude the possible degradative effect of light. 1 ml of treated solution were taken and neutralized and then diluted up to 10 ml with methanol. Then this diluted sample was used to apply on TLC plate in triplicate (500 ng/spot). The plates were chromatographed and analysed by proposed method.
2.6.2 Oxidative Degradation

Modafinil (10 mg) was dissolved in 10 ml of methanolic solution of hydrogen peroxide (30%, v/v) and the mixture was kept for 48 h at room temperature in the dark, to exclude the possible degradative effect of light. The solutions (1 ml) were diluted to 10 ml with methanol and further proceed as described for acid and base degradation.

2.6.3 Dry Heat Degradation

Dry heat studies were performed by keeping powdered drug in oven (60°C) for 6 hrs. Samples were withdrawn, dissolved in methanol, diluted suitably and appropriate volumes of resultant solution were applied on TLC plate and densitograms were developed.

2.6.4 Photo Degradation

Photo degradation studies were carried out by keeping modafinil drug sample in sunlight for 4 days. Sample solution was taken and diluted in methanol, and appropriate volume of the resultant solution were applied and chromatographed as described above.

III. Results And Discussion

3.1 Development of the optimum mobile phase

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 modafinil and degradation products. Best suited mobile phase was found to ethyl acetate, acetone and methanol in the volume ratio of (7:2:1 v/v) respectively. Developed mobile phase enabled good resolution, and a sharp and symmetrical peak of Rf 0.42 for modafinil (Fig. 2) form a compact and non-diffused 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.

3.2 Validation of the developed stability-indicating method

The response for the drug was found to be linear in the concentration range 200-800 ng/band for modafinil. The results of regression analysis are depicted in Table 1. The accuracy of the method was determined at 50, 100 and 150% level. Results of accuracy study are expressed in terms of % recovery (Table 2). According to results of robustness studies shown in Table 3, %RSD value does not exceed more than 2. So the developed method is robust. The %RSD value for precision study was found to be not more than 2 % for modafinil, thus confirming precision of the method. The Limit of Detection and Limit of Quantititation were found to be 15 ng/band and 50 ng/band respectively for analyte. The results are shown in Table 4. The specificity of the method was ascertained by peak purity profiling studies. In analysis of marketed formulation the mean assay result was found to be 99.84% (Table 5). From the result obtained we can conclude that there is no interference of the excipients present in the formulation. The low %RSD suggests the suitability of developed method for routine analysis.

3.3 Degradation observed

After applying different stress conditions upon modafinil, following results were observed by analyzing samples using the developed method. About 21.50% drug was degraded (Figure 3) under oxidative condition and major degradation peaks were observed at 0.18, 0.20 and 0.23 Rf values. In acid hydrolysis modafinil was degraded (Figure 4) about 17.35% and degraded product was observed at 0.14 and 0.19 Rf value, however in alkali hydrolysis some harsh condition was required compared to that in acid degradation. Initially, 0.1 N NaOH was used and refluxed at 80°C for 2 hours, yet no degradation was observed; then strength of alkali was increased to 0.5 N and refluxed at 80°C for 2 hours resulting in 8.20% degradation (Figure 5) and degradation peaks were observed at 0.23, 0.27 and 0.32 Rf values. Under dry heat (Oven, 80°C, 6 hr), drug was found to be degraded up to 9.25% with decrease in area only, whereas under Photolytic studies, no additional peaks were observed and drug peak area remained constant. This indicates stability of modafinil in UV light. In all forced degradation condition the drug peak was well resolved from degradation products.
Stability Indicating HPTLC Method for Estimation of Modafinil in the Bulk and Tablet Formulation

Figure 3. Chromatogram of Modafinil after Oxidative degradation

Figure 4. Chromatogram of Modafinil after Acid degradation

Figure 5. Chromatogram of Modafinil after Alkali degradation
IV. Conclusion

Based on the above study and result, it can be concluded that the developed HPTLC method is simple, precise, accurate, rapid, selective and sensitive for the determination of modafinil in tablet formulation as well as in bulk drugs. The products formed after forced decomposition studies were resolved from the bulk drug response. From the peak purity profile studies, it was confirmed that the peak of the degradation product was not interfering with the peak of drugs. It confirms that peak for degradation product of drug can be resolved from the drug peak by this method; hence it can be used as stability indicating method. The method can minimize the time and cost of analysis. It has also utilized the merit of applying several samples on TLC plates which can be advantageous for regulatory quality control laboratories. The method does not require expensive chemical and solvents, thus it can stand for a good alternative for already existing hplc methods. It is proposed for routine analysis of these drugs in presence of degradation products in stability study.

Acknowledgements

The authors are thankful for facilities & grants given under UGC- Special Assistance Programme (SAP), Department Research Support (DRS), 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