Development and Validation of HPTLC Method for Simultaneous Estimation of Amlodipine Besylate, Hydrochlorothiazide and Telmisartan In Their Combined Tablet Dosage Form

Bhavin Pankajbhai Marolia*, Kunjan Bharatbhai Bodiwala, Shailja Amritlal Shah, Pintu Bhagwanbhai Prajapati, Bhavik Himmatbhai Satani, Shailja Alkeshbhai Desai

Department of Quality Assurance, Maliba Pharmacy College, Bardoli-Mahuva road, Tarsadi, Dist.-Surat–394350, Gujarat, INDIA.

ABSTRACT

Background: The combination of Amlodipine Besylate, Hydrochlorothiazide and Telmisartan is prescribed for the treatment of hypertension. An Ultra Performance Liquid Chromatography (UPLC) method has been reported for simultaneous estimation of this combination. Objective: To develop and validate HPTLC Method for simultaneous estimation of Amlodipine Besylate, Hydrochlorothiazide and Telmisartan in their combined tablet dosage form. Materials and Method: The chromatographic separation was performed using aluminum plates pre-coated with silica gel 60F254 as stationary phase and chloroform: butan-1-ol: ammonia (6: 4: 0.1, v/v/v) as mobile phase. Spectro-densitometric scanning was performed at 254 nm. The developed method was validated according to ICH Q2R1 guideline. Results and Discussion: The linearity was established over a concentration range of 200-1000 ng/band, 500-2500 ng band and 1600-8000 ng band with correlation coefficient r² = 0.9992 and 0.9997 for Amlodipine besylate, Hydrochlorothiazide and Telmisartan, respectively. The Rf values of Amlodipine besylate, Hydrochlorothiazide and Telmisartan were found to be 0.27 ± 0.02, 0.43 ± 0.02 and 0.14 ± 0.02 respectively. Recovery of drug was achieved in the range of 99.43-101.57%, 100.22-101.54% and 100.12-100.44% for Amlodipine besylate, Hydrochlorothiazide and Telmisartan, respectively. The R² values of Amlodipine besylate, Hydrochlorothiazide and Telmisartan were found to be 0.9952, 0.9992 and 0.9979 for Amlodipine besylate, Hydrochlorothiazide and Telmisartan, respectively. Application: The developed HPTLC method was applied for simultaneous estimation of three drugs in their combined tablet dosage forms and results were found to be in good agreement with the labeled claim. Conclusion: The developed HPTLC method was found to be accurate, precise, specific and sensitive. It can be applied for routine analysis (assay) of tablets containing combination of Amlodipine besylate, Hydrochlorothiazide and Telmisartan.

Key words: Amlodipine besylate (AML), Hydrochlorothiazide (HCTZ), Telmisartan (TLM), High Performance Thin Layer Chromatography (HPTLC), Simultaneous estimation.

INTRODUCTION

Amlodipine besylate (AML): 3-ethyl-5-methyl(4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (Figure 1) is a widely prescribed anti-hypertensive drug. It is a calcium channel blocker.1,2,3,4 Hydrochlorothiazide (HCTZ): 6-chloro-3,4-dihydro-2H-1,2,4-benzothiazidine-7-sulfonamide 1,1-dioxane (Figure 2) is an anti-hypertensive diuretic drug belonging to thiazide class.1,2,3,4 Telmisartan (TLM): 4’-[[4-methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl][methyl]-2 biphénylcarboxylic acid (Figure 3) is an angiotensin II receptor antagonist widely used to treat hypertension.1,2,3,4 From the literature survey, it was found that HPLC, HPTLC, UVP-Spectrophotometry and Spectrodensitometric methods were reported for AML, HCTZ and TLM either individually or in combination with other drugs.5,6,7,8,9,10 Also an UPLC method has been reported for the simultaneous estimation of these three drugs in combination. However this method is complex and makes use of expensive instruments and chemicals which makes it unsuitable for the analysis at the small scale laboratory. Hence it was decided to develop and validate HPTLC method which is both cost effective and simple.

MATERIAL AND METHODS

Instrumentation

A HPTLC system (Camag Muttenz, Switzerland) comprising of semi-automatic sample applicator (Camag Linomat V), Hamilton syringe (100 μl), Camag TLC scanner IV, Camag WinCATS software, Camag twin trough chamber (10×10 cm), UV cabinet with dual wavelength UV lamps, Electronic analytical balance AUX-220 (Shimadzu) and Ultra-sonicator (Toshcon, model 4.5) were used during the study.

Chromatographic condition

For HPTLC analysis the experiment was performed on aluminium plates pre-coated with silica gel 60F254 stationary phase (10×10 cm), prewashed with methanol and activated in an oven at 50°C for 10 min prior to chromatography), using mobile phase comprising of chloroform: butan-1-ol: ammonia (6: 4: 0.1 v/v/v). The solutions were applied on TLC plate in the form of bands of 6 mm width under a stream of nitrogen gas using a Camag Linomat V semi-automatic sample applicator. A constant application rate of 0.1 μl/sec was employed and space between two bands was auto-selected as 10 mm. Ascending development to 80 mm was performed in Camag (10×10 cm) twin trough glass chamber (Muttenz, Switzerland) saturated with the mobile phase for 30 min at room tem-
perature. The developed TLC plate was air dried and scanned between 200 to 400 nm using Camag TLC scanner IV using Win-CATS software. All three components show reasonably good response at 254 nm keeping the slit dimension of 4.00×0.30 mm and scanning speed of 20 mm/sec.

Preparation of solutions

Preparation of standard stock solution
AML (10 mg), HCTZ (25 mg) and TLM (80 mg) were weighed accurately and transferred in 10 ml volumetric flask for AML and HCTZ and 50 ml for TLM. The powder was dissolved and the solutions were diluted up to 1 ml with methanol to get the final concentration of 1000 μg/ml AML, 2500 μg/ml HCTZ and 1600 μg/ml TLM.

Preparation of working standard solution
From the standard stock solutions 1 ml aliquots were taken from AML and HCTZ while 5 ml aliquot was taken from TLM and diluted up to 10 ml with methanol to get the mixture solution containing the concentration of 100 μg/ml AML, 250 μg/ml HCTZ and 800 μg/ml. Further 4 ml of this mixture solution was taken and diluted to 10 ml with methanol to make the final concentration of 40 μg/ml AML, 100 μg/ml HCTZ and 320 μg/ml TLM.

Preparation of calibration curve
The series consisted of different concentration of AML, HCTZ and TLM ranging from 200-1000 ng/band, 500-2500 ng/band and 1600-8000 ng/band, respectively. The working standard solution (5, 10, 15, 20, 25 μl) was spotted and analysed by the proposed method. The calibration curves of peak area versus respective concentration were plotted and correlation coefficient and regression line equation were computed.

Validation of the Proposed Method

Linearity and range
The linearity response was determined by analyzing five independent levels of calibration curves in the range of 200-1000 ng/band, 500-2500 ng/band and 1600-8000 ng/band for AML, HCTZ and TLM, respectively. The calibration curves of peak area versus respective concentration were plotted and correlation coefficient and regression line equation were computed.

Specificity
The specificity of the method was ascertained by analysing standard drug and sample. The bands for AML, HCTZ and TLM in sample were found to be 237.5 nm, 270 nm and 254 nm respectively (n=5). The final solution contained 50 μg/ml, 125 μg/ml and 400 μg/ml of AML, HCTZ and TLM, respectively.

Repeatability of measurement and sample application
For repeatability of measurement working standard solution (15 μl) was spotted on pre coated TLC plate. The plate was developed, dried and the peak area was analysed as described under chromatographic conditions for seven times. For repeatability of sample application working standard solution (15 μl) was spotted on pre coated TLC plate seven times. The plate was developed, dried and analysed as described under chromatographic conditions.

Intermediate precision
Intra-day precision of the proposed method was evaluated by applying 5, 10, 15, 20 and 25 μl of working standard solution on the TLC plate three times on same day and analyzing over the entire concentration range as described under chromatographic conditions.

Inter-day precision of the proposed method was evaluated by applying 5, 10, 15 and 25 μl of working standard solution on the TLC plates three times on different days and analyzing over the entire concentration range as described under chromatographic conditions.

Accuracy
Accuracy was determined in terms of percent recovery. The proposed method was applied to determine AML, HCTZ and TLM in pharmaceutical dosage form. The recovery experiment was carried out in triplicate by spiking previously analyzed samples with three different concentrations of standards at 80%, 100% and 120%. The details of solution preparation are shown in Table 1. All the solutions were spotted (15 μl) and analysed as described under chromatographic conditions.

Limit of detection and limit of quantification
Limit of detection was calculated using following equation as per ICH guidelines.

\[
LOD=3.3\times N/S
\]

\[
LOQ=10\times N/S
\]

Where N is the standard deviation of the Y-intercepts of the five calibration curves and S is mean slope of the five calibration curves.

Analysis of drug formulation
Twenty tablets were accurately weighed, finely powdered and mixed thoroughly. The powder equivalent to 40 mg of TLM (5 mg AML and 12.5 mg HCTZ) was accurately weighed and transferred into 100 ml volumetric flask, 70 ml of methanol was added and the solution was sonicated for 20 min and the volume was made up to the mark with methanol. The solution was filtered through whatman filter paper No. 41. The final solution contained 50 μg/ml, 125 μg/ml and 400 μg/ml of AML, HCTZ and TLM, respectively.

Sample solution (15 μl) was applied on TLC plate and analysed by the developed method. Analysis of marketed formulations was done for three times and percentage of AML, HCTZ and TLM were calculated from the calibration curve.

RESULTS AND DISCUSSION

Selection of wavelength
Zero order spectra of AML, HCTZ and TLM were taken in methanol. The λ<sub>max</sub> for AML, HCTZ and TLM were found to be 237.5 nm, 270 nm and 297 nm, respectively. Overlaid derivative spectra of AML (10 μg/ml), HCTZ (10 μg/ml) and TLM (10 μg/ml) are depicted in Figure 4. The wavelength selected for determination of AML, HCTZ and TLM was 254 nm.

Calibration curve
Calibration curves of AML, HCTZ and TLM were prepared in the range of 200-1000 ng/band, 500-2500 ng/band and 1600-8000 ng/band respectively (n=5). The calibration curves of peak area versus respective concentration were plotted. Calibration curves for AML, HCTZ and TLM were found to be linear in selected rage.

Method validation

Linearity and range
The calibration curves were found linear in the given range with a correlation coefficient of 0.9952, 0.9992 and 0.9979 for AML, HCTZ and TLM respectively. A 3D chromatogram showing linearity of AML, HCTZ and TLM.
Table 1: Preparation of solution for accuracy study

<table>
<thead>
<tr>
<th>Pre-analysed Mixture Drug Solution (ml) [AML-100 μg/ml; HCTZ-250 μg/ml; TLM-800 μg/ml]</th>
<th>Standard Mixture Solution Spiked (ml) [AML-100 μg/ml; HCTZ-250 μg/ml; TLM-800 μg/ml]</th>
<th>Volume Made up (ml)</th>
<th>Volume Spotted (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>2.4</td>
<td>10</td>
<td>15</td>
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</table>

Table 2: Summary of validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AML</th>
<th>HCTZ</th>
<th>TLM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (ng/spot)</td>
<td>200-1000</td>
<td>500-2500</td>
<td>1600-8000</td>
</tr>
<tr>
<td>Correlation Coefficient ($r^2$)</td>
<td>0.9952</td>
<td>0.9992</td>
<td>0.9979</td>
</tr>
<tr>
<td>Precision (RSD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability of measurement (n=7)</td>
<td>0.24</td>
<td>0.97</td>
<td>0.40</td>
</tr>
<tr>
<td>Repeatability of sample application (n=7)</td>
<td>0.27</td>
<td>0.95</td>
<td>0.40</td>
</tr>
<tr>
<td>Intra-Day (n=3)</td>
<td>0.20-1.22</td>
<td>0.38-1.89</td>
<td>0.40-1.89</td>
</tr>
<tr>
<td>Inter-Day (n=3)</td>
<td>0.21-1.87</td>
<td>0.46-0.90</td>
<td>0.44-1.58</td>
</tr>
<tr>
<td>Accuracy (% Recovery)</td>
<td>99.43-101.57</td>
<td>100.22-101.54</td>
<td>100.12-100.44</td>
</tr>
<tr>
<td>LOD (ng/spot)</td>
<td>8.6</td>
<td>58.0</td>
<td>186.9</td>
</tr>
<tr>
<td>LOQ (ng/spot)</td>
<td>26.1</td>
<td>175.8</td>
<td>566.4</td>
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</tbody>
</table>

Table 3: Data of peak purity

<table>
<thead>
<tr>
<th>Drug</th>
<th>Standard Drug</th>
<th>Tablet Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_{(s,m)}$</td>
<td>$r_{(m,e)}$</td>
</tr>
<tr>
<td>AML</td>
<td>0.9999</td>
<td>0.9990</td>
</tr>
<tr>
<td>HCTZ</td>
<td>0.9999</td>
<td>0.9993</td>
</tr>
<tr>
<td>TLM</td>
<td>0.9998</td>
<td>0.9990</td>
</tr>
</tbody>
</table>

Table 4: Data of recovery study for AML, HCTZ and TLM

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken (ng)</th>
<th>Amount Spiked (ng)</th>
<th>Total area (Mean ± SD; n=3)</th>
<th>Recovered amount in ng (Mean ± SD; n=3)</th>
<th>Mean % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>300</td>
<td>-</td>
<td>6119.49 ± 101.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>240</td>
<td>8743.33 ± 132.75</td>
<td>243.38 ± 0.90</td>
<td>101.41</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>300</td>
<td>9335.41 ± 147.41</td>
<td>298.30 ± 0.74</td>
<td>99.43</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>360</td>
<td>10061.45 ± 192.50</td>
<td>365.64 ± 0.71</td>
<td>101.57</td>
</tr>
<tr>
<td>HCTZ</td>
<td>750</td>
<td>-</td>
<td>879.54 ± 15.27</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>600</td>
<td>1568.07 ± 28.04</td>
<td>609.27 ± 0.76</td>
<td>101.54</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>750</td>
<td>1729.01 ± 31.87</td>
<td>751.67 ± 0.80</td>
<td>100.22</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>900</td>
<td>1907.38 ± 35.90</td>
<td>909.51 ± 1.11</td>
<td>101.06</td>
</tr>
<tr>
<td>TLM</td>
<td>2400</td>
<td>-</td>
<td>2545.04 ± 41.53</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2400</td>
<td>1920</td>
<td>3523.03 ± 69.79</td>
<td>1926.44 ± 0.44</td>
<td>100.34</td>
</tr>
<tr>
<td></td>
<td>2400</td>
<td>2400</td>
<td>3772.99 ± 74.73</td>
<td>2410.59 ± 1.32</td>
<td>100.44</td>
</tr>
<tr>
<td></td>
<td>2400</td>
<td>2880</td>
<td>4013.84 ± 72.43</td>
<td>2883.41 ± 0.95</td>
<td>100.12</td>
</tr>
</tbody>
</table>
Table 5: Assay data of formulation

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label Claim (mg)</th>
<th>Assay (% Label Claim) (Mean ± SD; n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AML</td>
<td>HCTZ</td>
</tr>
<tr>
<td>Brand A</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>Brand B</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>Brand C</td>
<td>5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Figure 1: Chemical structure of Amlodipine besylate (AML) (at column width).

Figure 2: Chemical structure of Hydrochlorothiazide (HCTZ) (at column width).

Figure 3: Chemical structure of Telmisartan (TLM) (at column width).

Figure 4: Overlay Spectra for selection of wavelength; Solution of AML, HCTZ and TLM (10 µg/mL, each) was used. (at column width).

Figure 5: A 3D chromatogram showing linearity of AML, HCTZ and TLM; Rf=0.14: Telmisartan, Rf=0.27: Amlodipine besylate and Rf=0.43: Hydrochlorothiazide (at column width).

Figure 6: Overlay spectra showing the peak purity of AML standard and sample (at column width).
TLM good correlation was obtained which indicate that the peaks are pure and any excipient do not interfere in separation of the drugs (Figure 6, 7 and 8).

**Precision**

**Repeatability of measurement and sample application**

The data for repeatability of measurement of peak area is summarized in Table 2. RSD for peak area was found to be 0.24, 0.97 and 0.40 for AML, HCTZ and TLM, respectively. The data for repeatability of sample application is summarized in Table 2. RSD for peak area was found to be 0.27, 0.95 and 0.40 for AML, HCTZ and TLM, respectively.

**Intermediate precision**

The data for intra-day precision is summarized in Table 2. RSD for peak area was found to be in the range of 0.20–1.22, 0.38–1.89 and 0.40–1.89 for AML, HCTZ and TLM, respectively. The data for inter-day precision is summarized in Table 2. RSD for peak area was found to be in the range of 0.21–1.87, 0.46–0.90 and 0.44–1.58 for AML, HCTZ and TLM, respectively.

**Accuracy**

The mean of %recovery of AML, HCTZ and TLM was found to be 99.43-101.57%, 100.22-101.54% and 100.12-100.44%, respectively. The data for accuracy is presented in Table 4.

**Limit of detection and limit of quantification**

The data for LOD and LOQ are presented in Table 2.

**Assay of marketed formulations**

Tablets were analyzed by the proposed HPTLC method. Results were in good agreement with the label claim. The assay results are shown in Table 5. Chromatogram for AML, HCTZ and TLM in their combined tablet dosage form is shown in Figure 9.

**CONCLUSION**

An HPTLC method was developed for simultaneous estimation of AML, HCTZ and TLM in bulk. The method was validated as per ICH (Q2 R1) guidelines. The proposed method was found to be specific, accurate, precise and sensitive. The developed method was applied for assay of combination tablets of three drugs and results were found to be in good agreement with the label claim. The proposed method can be applied for routine analysis of combination dosage forms of three drugs.

**ACKNOWLEDGEMENTS**

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**CONFLICT OF INTEREST**

The author have no conflict of interest.

**ABBREVIATION USED**

AML: Amlodipine besylate  
HCTZ: Hydrochlothiazide,  
TLM: Telmisartan,  
LOD: Limit of Detection,  
LOQ: Limit of Quantitation,  
HPTLC: High Performance Thin Layer Chromatography,  
HPLC: High Performance Liquid Chromatography,  
UPLC: Ultra Performance Liquid Chromatography,  
UV: Ultra violet,  
CV: Co-efficient of Variance,  
SD: Standard Deviation.
REFERENCES


PICTORIAL ABSTRACT

SUMMARY

• HPTLC method was developed and validated as per ICH (Q2R1) guidelines for simultaneous estimation of Amlodipine Besylate, Hydrochlorothiazide and Telmisartan.
• The analysis was done using aluminium plates pre-coated with silica gel 60F254 as stationary phase and chloroform: butan-1-ol: ammonia (6: 4: 0.1 v/v/v) as mobile phase. The estimation was done at 254 nm.
• The developed method was applied for simultaneous estimation of three drugs in their combined tablet dosage form.
• The assay results were found to be in good agreement with the labelled claim.

ABOUT AUTHORS

Dr. Bhavin P. Marolia: Obtained his Ph. D. Degree in 2013 from Veer Narmad South Gujarat University, Surat. He is working as Assistant Professor in Department of Quality Assurance and Pharmaceutical Analysis at Maliba Pharmacy College of Uka Tarsadia University, Bardoli. He has over ten years of teaching and research experience. He has guided 24 students for their M. Pharm. research projects and has 25 publications in national and international journals.

Dr. Pintu B. Prajapati: Obtained his Ph. D. Degree in 2014 from Veer Narmad South Gujarat University, Surat. He is working as Assistant Professor in Department of Quality Assurance and Pharmaceutical Analysis at Maliba Pharmacy College of Uka Tarsadia University, Bardoli. He has over eight years of teaching and research experience. He has guided 16 students for their M. Pharm. research projects and has 11 publications in national and international journals.

Dr. Kunjan B. Bodiwala: Obtained his Ph. D. Degree in 2016 from Veer Narmad South Gujarat University, Surat. He is working as Assistant Professor in Department of Quality Assurance and Pharmaceutical Analysis at Maliba Pharmacy College of Uka Tarsadia University, Bardoli. He has over eight years of teaching and research experience. He has guided 20 students for their M. Pharm. research projects and has 10 publications in national and international journals.

Dr. Shailesh A. Shah: Is Principal, Maliba Pharmacy College of Uka Tarsadia University, Bardoli. He is Professor in Department of Quality Assurance and Pharmaceutical Analysis. He obtained his Ph. D. Degree in 1986 from Gujarat University, Ahmedabad. He has over thirty seven years of teaching and research experience. He has guided 76 M. Pharm. students for their research projects. 10 students have been awarded Ph. D. degrees under his guidance and has 92 publications in national and international journals to his credit.

Mr. Bhavik H. Satani: Is working as Assistant Professor in Department of Pharmacognosy at Maliba Pharmacy College of Uka Tarsadia University, Bardoli. He has over four years of teaching and research experience. He has co-guided 02 students for their M. Pharm. research projects and has 7 publications in national and international journals.