VALIDATION OF A DISSOLUTION METHOD WITH HPLC-UV ANALYSIS FOR ESTIMATION OF OLMESARTAN MEDOXOMIL, AMLODIPINE BESYLATE AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORMULATION

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ABSTRACT
A simple, precise, sensitive and specific liquid chromatographic method was developed for the simultaneous estimation of olmesartan medoxomil (OLM), amlodipine besylate (AMLO) and hydrochlorothiazide (HCTZ) in tablet dosage form. Separation was achieved with Purospher ®-STAR, RP-C₁₈, 5μ, 250 mm x 4.6 mm column, using simple isocratic mode with mobile phase containing acetonitrile: potassium dihydrogen orthophosphate buffer adjusted to pH 3.0 with orthophosphoric acid (48: 52 v/v). The flow rate for analysis was 1.0 mL/min and UV detection at 238nm for hydrochlorothiazide, amlodipine besylate and olmesartan medoxomil. The selected chromatographic conditions effectively separated hydrochlorothiazide, amlodipine besylate and olmesartan medoxomil with retention time of 3.3, 3.9 and 7.7 minutes, respectively. Percent release for all three drugs achieved in dissolution study was more than 92% of labeled amount upto 60 min under optimized dissolution conditions. The HPLC method and dissolution method were validated with respect to system suitability, specificity, linearity, precision, accuracy and robustness. The results of the method were acceptable confirming that these methods can be applicable, without any interference from the excipients. In conclusion, a novel, simple, accurate and reproducible high performance liquid chromatographic method was developed and can be useful in routine quality control, dissolution study and pharmaceutical dosage form analysis.

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INTRODUCTION
Hypertension is the most common cardiovascular disease; its prevalence increasing with advancing age. Hypertension due to complex hemodynamics, is the principal cause of stroke, is a major risk factor for coronary artery disease and its complications, and is a major contributor to cardiac failure, renal insufficiency, and dissecting aortic aneurysm. The hemodynamic effects of antihypertensive agents provide potential complementary effects of concurrent therapy with two or more drugs. Concurrent use of drugs from different classes is an effective strategy for effective control of hypertension while minimizing dose-related adverse effects [1-2]. One of the triple combinations amongst various available and widely used is olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide.

Olmesartan medoxomil (OLM) (5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-[(4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl)methyl]-1H-imidazole-5-carboxylate, an angiotensin II receptor blocker, blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT_1 receptor in vascular smooth muscle [1-3]. Amlodipine besylate (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate is the benzene sulfonate (besylate) salt of amlodipine (AMLO), and is a dihydropyridine calcium channel blocker. AMLO as a calcium antagonist inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscles that in turn affects their contractile process and results in reduced blood pressure [1-4]. Hydrochlorothiazide, [6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide] is a thiazide diuretic that inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter in the distal convoluted tubule and is responsible for 5% of total sodium reabsorption [1-4].

![Chemical structure of Olmesartan medoxomil](image1.png)

(a) Olmesartan medoxomil

![Chemical structure of Amlodipine besylate](image2.png)

(b) Amlodipine besylate
Figure 1: Chemical structure of (a) Olmesartan medoxomil, (b) Amlodipine besylate and (c) Hydrochlorthiazide

Literature reviewed reports several analytical methods including HPLC [5-7], a stability-indicating HPLC [8-12], HPTLC [13-14] and UV-visible spectrophotometry [15-17] for the determination of OLM, AMLO and HCTZ either single or in combination with other drugs. Many analytical methods have been reported individually for AMLO [18-19] and HCTZ [19-20]. However, the literature is silent on the development and validation of High Performance Liquid Chromatographic method along with its application to in-vitro dissolution of these drugs for combined tablet dosage form.

Validation of the optimized dissolution methods, as well as other analytical methods, is an industrial and regulatory requirement to ensure that they are suitable for their intended use and give accurate and reliable data. Dissolution testing is generally performed using official or nonofficial methodology for studying the release kinetics from dosage formulation and these in vitro dissolution profiles also provide an insight to characterize the in vivo behavior of drugs with little success [21]. The main purpose of a dosage form after oral administration is absorption of the drug from dosage formulation and is dependent on release of the drug, followed by its dissolution and/or solubilization under physiological conditions and its permeability across the gastrointestinal tract. Moreover, in pharmaceutical industry, dissolution study is also used to guide development of new drug products and to assess lot to lot variability of drug products [22].

Developing dissolution methods for poorly soluble compounds has been a consistent challenge for the pharmaceutical scientist. Published literature reports none of the official validated method for dissolution study for the available antihypertensive combination. The objective of the present article is the application of validated, accurate and reliable RP-HPLC method for the estimation of OLM, AMLO and HCTZ release from solid dosage formulation for in-vitro dissolution study.

MATERIALS AND METHODS

Chemicals and reagents
Olmesartan medoxomil, amlodipine besylate and hydrochlorthiazide were kindly supplied by Glenmark Generics Limited, Pune, Prudence PharmaChem, Ankleshwar and Ipcal Laboratories Limited, Ratlam respectively. Phosphoric acid (85%), potassium dihydrogen orthophosphate, sodium hydroxide pellets were obtained from Merck Specialities Private Limited. Acetonitrile was procured from Rankem Pvt. Ltd. Double distilled water was used. Marketed formulation Triolmezest tablet, Sun Pharmaceutical Ind. Ltd. was procured from local market.

Instrumentation
HPLC system, Shimadzu LC-2010 CHT series with an automated injector, UV-visible detector and a column oven was used. In-vitro dissolution study was performed on dissolution tester (USP) TDT-08L (Electrolab). An analytical balance from Denver instrument was used. A pH meter CL54+ from Toshcon Industries Pvt. Ltd., Haridwar was used to adjust the pH. Spectrophotometric measurements were carried out using a double beam
UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) with 1 cm quartz cell and a fixed slit width (2 nm) connected to an IBM-PC computer loaded with Shimadzu UV-probe personal software (version 2.34).

**Chromatographic conditions**
The chromatographic analysis was performed on a reversed-phase Purospher ®-STAR, C18 (250 mm x 4.6 mm, 5μm) column, kept at 25 □ C. The mobile phase consisted of a mixture of acetonitrile: phosphate buffer 0.025mM adjusted to pH 3.0 (48:52v/v). The phosphate buffer was prepared from potassium dihydrogen orthophosphate buffer adjusted to pH 3 by orthophosphoric acid. The mobile phase was prepared daily, filtered, sonicated before use, and delivered at a flow rate of 1.0ml/min and the injection volume was 10μl. Mobile phase was used as a diluent and eluent was monitored at 238 nm.

**Preparation of standard solutions**
**Preparation of standard stock solution**
40.00mg of OLM, 10.00mg of AMLO and 25.00mg of HCTZ were accurately weighed and dissolved separately in mobile phase in 100 ml volumetric flask to obtain a stock solution having concentration of 400μg/ml OLM, 100μg/ml AMLO and 250μg/ml HCTZ.

**Preparation of calibration curve standard solution**
Appropriate aliquots from stock solution were diluted with mobile phase to prepare final concentration in the range of 12-28μg/ml, 3-7μg/ml and 7.5-17.5μg/ml for OLM, AMLO and HCTZ respectively. Calibration plots were constructed by plotting the area of the main peak versus the concentration of the drug and linear regression analysis performed.

**Optimization of HPLC method**
For method development in HPLC, the most important parameters that modify the selectivity in the chromatographic separation such as the pH of the mobile phase, the concentration of the buffer and the organic modifier percentage were studied in order to explore a novel, rapid and precise method to quantify these three drugs in tablets. The HPLC procedure was optimized with a view to develop a simultaneous estimation method for OLM, AMLO and HCTZ respectively. The mixed standard stock solution (20μg/ml for OLM and 5μg/ml for AMLO and 12.5μg/ml for HCTZ) was injected; and for HPLC method optimization, different ratios of methanol, acetonitrile, 0.1% orthophosphoric acid and 0.025M potassium dihydrogen orthophosphate as mobile phase were tried. The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives optimum response at a single wavelength for all drugs that are to be detected. In the present study, drug solutions of 10μg/ml of OLM, 10μg/ml of AMLO and 10μg/ml of HCTZ were prepared separately in acetonitrile and scanned in the range of 200-400nm to determine the optimum wavelength of detection.

**VALIDATION OF HPLC METHOD**
The developed method was validated by determination of various analytical method validation parameters like accuracy, precision, linearity, range, limit of detection, limit of quantitation, robustness, specificity, and system suitability according to ICH guideline Q2 (R1) [23].
System suitability
System suitability standard solution at a concentration of 20μg/ml of OLM, 5μg/ml of AMLO and 12.5μg/ml of HCTZ was prepared daily using the stock solution. System suitability was determined from six replicate injections of the standard solution before sample analysis. **Linearity and range**
The linearity of the method was evaluated from 60%-140% of the nominal assay concentration for OLM, AMLO and HCTZ by evaluation of regression coefficient. Moreover, Lack of Fit (LOF) and Bartlett’s test was also applied for checking deviation from linearity and homogeneity of variance.

**LOD and LOQ**
As per ICH guideline, limit of detection and quantitation of the developed method were calculated from the standard deviation of the response and slope of the calibration curve of each drug using the formula,

Limit of detection=3.3*σ/S  
Limit of quantitation=10*σ/S  
Where, “σ” is Standard deviation of response  
“S” is Slope of calibration curve

**Accuracy**
The accuracy of the proposed method was evaluated by recovery study. The placebo was spiked with standard at three different concentration levels of 80, 100 and 120 % and the mixtures were analyzed in triplicate by the proposed method.

**Precision**
Precision was evaluated by performing repeatability and intermediate precision for concentration of OLM (20.00 μg/ml), AMLO (5.00 μg/ml) and HCTZ (12.50 μg/ml). Six replicates at 100% test concentration for each drug were analyzed on the same day for repeatability, and on different days to ascertain intermediate precision.

**Specificity**
The standard and sample at nominal concentration were injected into chromatographic system. The specificity of method was evaluated by comparing retention time of all three drugs in marketed formulation with that of standard. Moreover, a placebo solution prepared from a synthetic blend of the tablet excipients and diluent as blank was also injected.

**Robustness**
Method robustness was evaluated by deliberate changes of flow rate (0.9 and 1.1 ml/ min), mobile phase composition (49:51, 47:53), wavelength scan (236 and 240 nm) to evaluate the impact on the performance of the method and the results were expressed in terms of %RSD.

**Solution stability**
The combined standard solution of OLM, AMLO and HCTZ was stored, unprotected from light, at ambient condition and analyzed after 0, 2, 4, 6, 8, 12 and 24hr against a freshly prepared standard solution.

**Analysis of marketed formulation**
The proposed method was used for simultaneous estimation of OLM, AMLO and HCTZ in tablet dosage forms. Twenty tablets were ground in fine powder form and powder equivalent to 20.00mg of OLM, 12.50mg of HCTZ and 5.00 mg of AMLO accurately weighed was transferred into 100ml of volumetric flask. About 15ml
of mobile phase was added and the mixture after sonication for 15 minutes diluted to the mark with mobile phase, mixed well and filtered to obtain the sample stock solution. For the determination, 5 ml of stock solution was diluted to 50 ml in volumetric flask with mobile phase followed by analysis as per standard procedure.

APPLICATION OF HPLC METHOD TO IN-VITRO DISSOLUTION STUDY
Preparation of standard solution
Standard stock solution of OLM, AMLO and HCTZ were prepared separately by transferring accurately weighed 22.22 mg OLM, 7.69 mg AMLO and 13.88 mg HCTZ into 100 ml volumetric flask, followed by dissolution in acetonitrile (10 ml). The volume was made up to the mark with dissolution medium to obtain a final concentration of OLM, AMLO and HCTZ of 22.22 μg/ml, 5.55 μg/ml and 13.88 μg/ml respectively.

Dissolution method
Dissolution testing was performed in compliance with USP-NF 25 by means of paddle apparatus after optimization of various conditions, rotational speed in rpm, selection of dissolution media. The temperature was maintained at 37±0.5°C and the 1-liter glass dissolution vessels were covered to minimize evaporation. Nine hundred milliliters of freshly prepared and degassed pH 7.5 phosphate buffer was used as dissolution medium. The final optimized conditions were phosphate buffer of pH 7.5 (900 ml) with a rotational speed of 100 rpm. Six tablets were evaluated and dissolution sample aliquots (10 ml) were withdrawn at 10, 20, 30, 40, 50 and 60 min by manual sampling using a glass hypodermic syringe equipped with a stainless steel needle. The solutions were immediately filtered using nylon filter 0.45 μ and analyzed by HPLC.

Validation of dissolution method
Precision
Precision was evaluated by performing repeatability and intermediate precision, for dissolution of six tablets. Six replicates at 100% test concentration for each drug were analyzed on the same day for repeatability, and on different days to ascertain intermediate precision. Precision was expressed as %RSD of the analyte peaks.

Robustness
The small deliberate changes studied were rpm, dissolution medium volume and pH of the dissolution medium. These variables were chosen as they were deemed the most significant factors that can potentially affect dissolution results. The effect of deliberate variations in parameters; rotation speed (94 rpm and 104 rpm), dissolution volume (890 ml and 910 ml), dissolution medium as phosphate buffer (pH 7.3 and 7.5) were evaluated. The effect of these changes on both Rt and peak areas was evaluated by calculating the % relative standard deviation (%RSD) for each parameter.

Specificity
The specificity of method was ascertained by comparing retention time of all three drugs in marketed formulation with that of standard in dissolution media. Moreover, dissolution medium as blank was also injected, to check for the presence of any interferents.

Solution stability
The combined standard solution of Olmesartan medoxomil, Amlodipine besylate and Hydrochlorothiazide was stored, unprotected from light, at ambient condition and assayed after 0, 2, 4, 6, 8, 12 and 24 hr against a freshly prepared standard solution in dissolution medium.
RESULTS AND DISCUSSION

Optimization of HPLC method

The mobile phase conditions were optimized for resolution of the three drugs. Different solvents in different ratios like acetonitrile, methanol with orthophosphoric acid and buffer were tried. But appropriate retention time, resolution, tailing factor, theoretical plates were obtained by using acetonitrile instead of methanol and best results were obtained by using acetonitrile : 0.025M potassium dihydrogen orthophosphate adjusted to pH 3.0 with orthophosphoric acid (OPA) (48:52 v/v). Under optimum chromatographic conditions, the retention time of HCTZ, AMLO and OLM were 3.3, 4.3 and 7.7 min, respectively (Figure 2). The wavelength selected for detection was 238nm, the intersection point of AMLO and OLM, where HCTZ was also showing optimum response.

![HPLC chromatogram](image)

Figure 2: HPLC chromatogram of tablet sample containing 20μg/ml for OLM, 5 μg/ml for AMLO and 12.5 μg/ml for HCTZ standard

VALIDATION OF HPLC METHOD

System suitability

The limit of number of theoretical plates and tailing factor was fixed as not less than 6000 and not more than 2 respectively. All the chromatograms under optimized chromatographic conditions showed number of theoretical plates above 6000 and tailing factor was less than 1.3. %RSD of the peak area, tailing factor, theoretical plates, capacity factor, and retention time showed adherence to the limits indicating that the developed method was valid and is suitable for routine laboratory analysis (Table 1).

Table 1: Results of system suitability parameters for the proposed HPLC method for the determination of OLM, AMLO and HCTZ

<table>
<thead>
<tr>
<th>System suitability parameters</th>
<th>OLM</th>
<th>AMLO</th>
<th>HCTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>7.78 ± 0.023</td>
<td>3.95 ± 0.0147</td>
<td>3.32 ± 0.004</td>
</tr>
<tr>
<td>Repeatability of peak area (%RSD)</td>
<td>0.33</td>
<td>0.81</td>
<td>0.05</td>
</tr>
<tr>
<td>Resolution (Rs)</td>
<td>16.21</td>
<td>3.46</td>
<td>-</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.06</td>
<td>1.214</td>
<td>1.24</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>12155.87</td>
<td>6744.09</td>
<td>6089.02</td>
</tr>
</tbody>
</table>

*RSD= relative standard deviation

Linearity and Range

The linear concentration range of OLM, AMLO and HCTZ was in the range of 12-28μg/ml, 3-7μg/ml and 7.5-17.5μg/ml respectively. The data presented indicate acceptable linearity for all three drugs (Table 2). Linearity was verified by Bartlett’s test and the response of peak area for all three drugs showed homogenous variance;
exemplified by the $\chi^2$ value less than the tabulated value. Moreover, linearity was also confirmed by Lack of Fit (LOF) test, where the deviation of the regression line when computed in terms of $F$ ratio for all three drugs was less than the tabulated one (Table 2).

**Table 2:** Summary of validation parameters for OLM, AMLO and HCTZ of the proposed HPLC method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OLM</th>
<th>AMLO</th>
<th>HCTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linearity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibration range(μg/ml)</td>
<td>12-28</td>
<td>3-7</td>
<td>7.5-17.5</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.9998</td>
<td>0.9999</td>
<td>0.9994</td>
</tr>
<tr>
<td>Slope ± S.D.</td>
<td>22389.36±49.77</td>
<td>27243.9±88.44</td>
<td>7129.08±62.72</td>
</tr>
<tr>
<td>Confidence interval of slope</td>
<td>22451.32-22327.40</td>
<td>27353.10-27132.90</td>
<td>7207.157-7051.00</td>
</tr>
<tr>
<td>Intercept ± S.D.</td>
<td>8267.66±840.21</td>
<td>2856.74±395.52</td>
<td>3970.78±868.34</td>
</tr>
<tr>
<td>Confidence interval of intercept</td>
<td>9313.59-7221.74</td>
<td>3349.11-2364.37</td>
<td>5051.73-2889.83</td>
</tr>
<tr>
<td>Bartlett’s test ($\chi^2$)</td>
<td>0.000143</td>
<td>0.000314</td>
<td>0.001853</td>
</tr>
<tr>
<td>Lack of fit (LOF)</td>
<td>3.29</td>
<td>3.66</td>
<td>2.61</td>
</tr>
<tr>
<td><strong>Sensitivity (μg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limit of Detection</td>
<td>0.1238</td>
<td>0.0479</td>
<td>0.4215</td>
</tr>
<tr>
<td>Limit of quantitation</td>
<td>0.3753</td>
<td>0.1452</td>
<td>1.2772</td>
</tr>
<tr>
<td><strong>Precision (%RSD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability studies</td>
<td>0.367-1.125</td>
<td>0.736-0.801</td>
<td>0.651-1.377</td>
</tr>
<tr>
<td>Intermediate precision</td>
<td>0.314-0.929</td>
<td>0.671-1.397</td>
<td>0.566-1.150</td>
</tr>
<tr>
<td><strong>Accuracy (%RSD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.166-0.208</td>
<td>0.510-0.611</td>
<td>0.473-0.741</td>
</tr>
<tr>
<td>Mobile phase ratio</td>
<td>0.414-0.472</td>
<td>0.327-0.217</td>
<td>0.479-0.592</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.378-0.423</td>
<td>0.274-0.414</td>
<td>0.404-0.643</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OLM</th>
<th>AMLO</th>
<th>HCTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Robustness (%RSD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.166-0.208</td>
<td>0.510-0.611</td>
<td>0.473-0.741</td>
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<td>0.404-0.643</td>
</tr>
</tbody>
</table>

$^a$ n= five replicates, $^b$ $\chi^2$ critical value = 9.488 at $\alpha=0.05$, $^c$ n= six replicates, $^d$ n=three replicates at three concentration levels, $^e$ n= five replicates, S.D.= standard deviation, RSD=relative standard deviation

**Accuracy**
The accuracy of the method was evaluated at 80, 100 and 120% of the nominal assay concentration for the OLM, AMLO and HCTZ, and the average percent recoveries ranged from 100.23 - 102.23% for OLM, 99.32 - 101.94% for AMLO and 99.04 - 101.13% for HCTZ respectively indicating suitability of the method for analysis of tablet formulation (Table 2).

**Precision**
In both intra and inter-day precision studies for the methods, %RSD values were not more than 2% showing acceptablerelpeatability and reproducibility of the developed method (Table 2).

**Specificity**
Figure 3 (a) and (b) shows no system, filter or excipient-related peaks that interfered with the quantitation of either drug, demonstrating specificity of the method. Similarly, $R_f$ value for all three drugs appeared at same position in chromatogram of tablet dosage formulation when compared with standard without any additional peak of intereference (Figure 3 (c) and (d)).
**Figure 3:** Specificity showing $R_f$ value of tablet dosage formulation same as standard a) placebo, b) blank, c) standard and d) tablet formulation

**Robustness**

Deliberate change in different parameters like flow rate (0.9 and 1.1 ml/min), mobile phase composition (49:51, 47:53), wavelength scan (236 and 240 nm) showed % relative standard deviation of peak area less than 2%, indicating that none of these variables significantly affects the performance of proposed method, thereby confirming robustness of method (Table 2).

**Solution stability:** The assay results of standard and test solution stability at 0, 2, 4, 6, 8, 12 and 24hr were within 99-101% of the initial value and no peak for degradation product was observed in any of the chromatogram, indicating stability under normal laboratory conditions.
Analysis of marketed formulation

The percentage amount found for all the drugs in the marketed formulation were within the range of 99.99%-100.94% w/w, which proves applicability of developed method in routine analysis of pharmaceutical dosage form (Table 3).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim(mg)</th>
<th>%amount of drug found a</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCTZ</td>
<td>12.50</td>
<td>100.32</td>
<td>0.47</td>
</tr>
<tr>
<td>AMLO</td>
<td>5.00</td>
<td>100.94</td>
<td>0.46</td>
</tr>
<tr>
<td>OLM</td>
<td>20.00</td>
<td>99.99</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Table 3: Percent amount of HCTZ, AMLO and OLM found in marketed formulation

Dissolution method

In vitro dissolution was used to assess the release rate of tablet formulation expressed in terms of percentage and to assure the quality of solid dosage forms. On the basis of solubility and screening study, various solvents, purified water, 0.1 N hydrochloric acid and phosphate buffer pH 6.8 and 7.5 were used as dissolution medium. Phosphate buffer pH 7.5 provided highest drug release profile with greater stability and was selected as best dissolution medium from the screening study. Moreover, at 100 rpm, the release of drug was more compared to 50 and 75 rpm using phosphate buffer pH 7.5 as dissolution medium. The optimized dissolution study conditions selected by screening were phosphate buffer pH 7.5 as dissolution medium (900ml), rotation speed of 100 rpm and 60min duration for drug release kinetic study. Hence, % release of all three drugs vs. time(min) under optimized conditions for tablet formulation is as shown in Figure 4.

Validation of Dissolution method

%RSD values for both repeatability and intermediate precision were not more than 2% that indicates acceptable precision of the developed method. Figure 5 (a), shows that there was no system, filter or blank related additional peaks at the retention time of all three drugs that interfered with the quantitation of either drug, demonstrating specificity of the method. Additionally, Rf value for all three drugs appeared at same positions in the chromatogram of dissolution sample when compared with dissolution standard without any additional peak as shown in figure 5 (b) and (c). Typical variations in dissolution conditions were used to evaluate the

Figure 4: Percent release of HCTZ, AMLO and OLM within 60min duration, in pH 7.5 buffer at 100 rpm
robustness of the dissolution method. The results of robustness shows %RSD values less than 2, indicating that the method is robust (Table 4).

**Table 4**: Robustness study of the HPLC method for determination of OLM, AMLO and HCTZ for dissolution study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variation</th>
<th>HCTZ</th>
<th>AMLO</th>
<th>OLM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%Assay</td>
<td>%RSD</td>
<td>%Assay</td>
<td>%RSD</td>
</tr>
<tr>
<td>Rpm</td>
<td>96</td>
<td>94.17</td>
<td>0.28</td>
<td>96.49</td>
</tr>
<tr>
<td></td>
<td>104</td>
<td>105.53</td>
<td>0.95</td>
<td>100.06</td>
</tr>
<tr>
<td>Volume</td>
<td>890</td>
<td>101.54</td>
<td>0.22</td>
<td>101.77</td>
</tr>
<tr>
<td></td>
<td>910</td>
<td>94.49</td>
<td>1.04</td>
<td>95.05</td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>102.24</td>
<td>0.16</td>
<td>101.53</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>100.72</td>
<td>0.49</td>
<td>103.68</td>
</tr>
</tbody>
</table>

*mean of three replicates, % RSD=Relative Standard Deviation

a) Chromatogram of dissolution medium

b) Chromatogram of dissolution standard solution

c) Chromatogram of dissolution sample solution
**Figure 5 (a-c):** Specificity showing R<sub>f</sub> value of tablet formulation same as standard a) dissolution medium, b) dissolution standard solution and c) dissolution sample solution

**Solution stability**
The results for solution stability at 0, 2, 4, 6, 8, 12 and 24hr time period were within 99-101% of the initial value and no peak for degradation product was observed in any of the chromatogram. The standard solution is therefore stable for at least 24 hr under normal laboratory conditions.

**CONCLUSION**
An isocratic RP-HPLC method has been reported for simultaneous estimation and invitro dissolution studies to determine the percent release of olmesartan medoxomil, amlodipine and hydrochlorothiazide in marketed formulation. The validation of proposed HPLC method was carried out according to ICH guideline and proved that the method is simple, precise, reliable, sensitive and robust. The dissolution study revealed that the percent drug released was in accordance to the acceptance criteria. Hence these methods can be applicable, without any interference from the excipients, for the routine quality control and determination of the combination tablet formulation in pharmaceutical industry.

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**REFERENCES**