Development and validation of stability indicating UPLC method for the simultaneous determination of anti-diabetic drugs in pharmaceutical dosage forms

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ABSTRACT

In this work, a rapid, precise and specific stability indicating ultra performance liquid chromatography (UPLC) method was developed and validated for the simultaneous determination of anti-diabetic drugs in pharmaceutical dosage forms. Comparison of system performance with conventional HPLC was made with respect to analysis time, efficiency and sensitivity. The chromatographic separation of all the drug components were achieved on Waters Acquity BEH C18, 50×2.1 mm, 1.7 µm UPLC column within a short runtime of 1.5 min. The newly developed UPLC method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, forced degradation, solution stability and selectivity. Three unknown degradants were not detected in the alkaline degradation of pioglitazone and glimepiride, having not been reported previously. The structure of the degradants was not determined using a combination of UPLC/MS and mechanistic chemistry. These impurities were characterized as methyl 3-(4-(2-(5-ethylpyridin-2-yl) ethoxy) phenyl)-2-mercaptopropanoate (impurity A) arising from the alkaline degradation of pioglitazone, methyl 4-(N-(4-methylcyclohexyl) carbamoyl) sulfamoyl) phenethylcarbamate (impurity B) and (Z)-methyl 2-ethyl-3-methyl-4-(3-(4-(N-(4-methylcyclohexyl) carbamoyl) sulfamoyl) phenethyl)ureido) but-2-enoate (impurity C), both arising from the alkaline degradation of glimepiride, respectively. The degradation pathway of the drug's API is proposed, which is not yet reported.

Keywords: UPLC; Anti-diabetic drugs; Simultaneous determination; Stability; Validation.

INTRODUCTION

Drugs belonging to class such as sulfonyl ureas (e.g. chlorpropamide, tolbutamide, glipizide, glibenclamide, gliclazide, glimepiride) and thiazolidinedione (TZD) derivatives (pioglitazone) are commonly prescribed hypoglycemic drugs for the treatment of non-insulin dependent type II diabetes mellitus. The use of combination of sulfonyl ureas and TZDs is commonly observed in clinical practice. The seven anti-diabetic drugs chosen for this study were chlorpropamide (CHL), glipizide (GLZ), tolbutamide (TOL), pioglitazone (PGL), gliclazide (GLC), glibenclamide (GLB) and glimepiride (GLM). CHL, GLZ, TOL, GLC, GLB and GLM are sulfonylurea oral hypoglycemic drugs that are individually used to lower blood glucose levels in patients with type II non-insulin-dependent diabetes mellitus. PGL belongs to the thiazolidinedione class, which exert their glucose-lowering effect by binding to peroxisome proliferator-activated receptors gamma, thus increasing the receptor sensitivity to insulin [1, 2].

A few analytical methods including gas chromatography [3,4], capillary electrophoresis [5] and fluorescence detection [6] are available in the literature. Several analytical methods have been reported for the detection of anti-diabetic drugs in plasma or urine, including liquid chromatography–tandem mass spectrometry (LC–MS/MS) [7-12]. Ho et al. [9] developed an LC–MS/MS method for simultaneous detection of ten anti-diabetics in plasma and urine, Lin et al. [10] reported a LC–MS/MS method for simultaneous detection of GLZ and rosiglitazone. These two methods, however, were not suitable for routine assay determination in the pharmaceutical formulations because of LC–MS/MS.

Aburuz et al. [13] described a HPLC-UV method for the simultaneous determination of MET, GLB, GLM (method 1) and MET, GLB, GLC (method 2) in plasma, respectively. Venkatesh et al. [14] described a ternary gradient elution for the simultaneous estimation of GLB, GLC, GLZ, PIO, repaglinide and rosiglitazone of pharmaceutical formulations. All these methods were based on the conventional HPLC columns ranging from 50 mm – 250 mm with a run time of 10 - 45 minutes. Most of the analytical methods reported, so far, are based on high-performance liquid chromatography (HPLC) with UV detection [15-21] for the determination of inactive metabolite of individual drugs in the biological fluids. Moreover, some of these methods were not sufficiently specific and sensitive, some were not validated and some were time-consuming and tedious.

However, none of these methods describes the UPLC assay for simultaneous determination of anti-diabetic drugs. Therefore, the objective of the present study was to develop and validate a stability indicating UPLC method for the simultaneous determination of seven anti-diabetic drugs viz. CHL, GLZ, TOL, PGL, GLC, GLB and GLM (Fig. 1) in a single run for application in pharmaceutical formulations with UV detection. During stability studies, an unknown degradants of PGL and GLM were observed during alkaline degradation when exposed to sodium hydroxide in presence of methanol. This paper deals with the investigation of a novel degradation mechanism for these drugs.

2.0. MATERIALS AND METHODS

2.1. Reagents

An active pharmaceutical ingredient (API) working standards of TOL, CHL, PGL and GLM were obtained from IPCA Laboratories Limited, Mumbai; MET, GLP, and GLZ were obtained as gift samples from Bal Pharma Limited, Bangalore. GLB was obtained from Sigma-Aldrich. The test samples obtained from commercial drug store had the following combination of drugs: PGL – GLZ (15 mg, 2 mg); MET – GLB (400 mg, 2.5 mg); MET – GLZ (500, 5 mg); MET – GLC (500 mg, 80 mg); TOL (500 mg); CHL (250 mg). HPLC grade acetonitrile was obtained from Merck, Mumbai, India. Formic acid (85%) was from Thomas Baker, Mumbai, India. High purity de-ionized water was obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system.

2.2. Apparatus

2.2.1 High-performance liquid chromatography

A Surveyor HPLC system (Thermo Fisher, USA) equipped with quaternary gradient pump, auto sampler, column oven and photodiode array detector (PDA) was employed for analysis. Chromatographic data was acquired using ChromQuest 4.2 software.

2.2.2 Ultra performance liquid chromatography

A Waters Acquity UPLC system (Waters, USA) equipped with binary gradient pump, auto sampler, column oven and photodiode array detector (PDA) was employed for analysis. Chromatographic data was acquired using Empower 2 software.

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2.2.3 Ultra performance liquid chromatography – mass spectrometry (UHPLC-MS)

A Thermo-Finnigan Accela UHPLC system consisting of auto sampler, Accela pump, photo diode array detector was interfaced with a Thermo Finnnigan LCQ Advantage Max ion trap mass spectrometer via an ESI probe.

2.3. Chromatographic conditions

2.3.1 Method 1

An X-Terra C18, 250×4.6 mm, 5µm column was used for separation. Chromatographic separation was achieved using timed gradient. The mobile phase consisting of A: buffer (5 mM ammonium acetate pH 4.0 adjusted with formic acid) and B: a mixture of 90% acetonitrile and 10% buffer with a timed gradient programme was used. The gradient condition of the mobile phase was: 0 min 45% solvent B, 0.6 min 45% solvent B, 8.0 min 65% solvent B, 15 min 80% solvent B, 18 min 80% solvent B, 20 min 100% solvent B and 21 min 45% solvent B with further 4 min for system equilibration. The flow rate of the mobile phase was 1.0 mL/min with detection at 245 nm. The column temperature was kept ambient and the injection volume was 10 µL.

This method is applied for the simultaneous determination of all the selected drugs by HPLC.

2.3.2 Method 2

An Acquity UPLC BEH C18 (50 nm × 2.1 mm, 1.7 µm) (Waters, Ireland) column was used as a stationary phase. The mobile phase consisting of A: buffer (5 mM ammonium acetate pH 4.0 adjusted with formic acid) and B: a mixture of 90% acetonitrile and 10% buffer with a timed gradient programme was used. The gradient condition of the mobile phase was: 0 min 40% solvent B, 1.2 min 80% solvent B, 1.3 min 60% solvent B and 1.5 min 40% solvent B. The flow rate of the mobile phase was 613 µL/min with detection at 245 nm. The column temperature was kept ambient and the injection volume was 0.2 µL. Forced degradation studies were carried out with a 2996 photo diode array detector.

This method is applied for the simultaneous determination of all the selected drugs by UPLC.

2.3.3 LC/MS/MS analysis

All data were collected in the positive and negative ion mode. Instrument parameters were heated capillary 320°C; sheath gas (N2) 55; auxiliary gas (N2) 15; total two micro scans; maximum injection time 200 ms. For MS experiments, the mass range scanned was m/z 50–900. MS/MS parameters were IW 1.0; RCE 40%. Chromatography was performed using method 2.

2.4. Solution preparation

2.4.1. Standard stock solution

Individual standard stock solutions of CHL (50 mg/mL); TOL (100 mg/mL), PGL and GLB (each 2 mg/mL); GLM and GLZ (1mg/mL) and GLC (12.8 mg/mL) were prepared separately by dissolving appropriate amounts of the compounds in methanol and water mixture (1:1). The stock solutions were stored at approximately 5°C and found to be stable for a week except GLC, which was found to be stable only for 24 hrs.

2.4.2. System suitability solution

Composite system suitability standard solution was prepared from standard stock solutions with mobile phase in 2 mL, volumetric flask to get final concentration of CHL (5000 µg/mL); GLZ (100 µg/mL); TOL (10000 µg/mL); PGL (3000 µg/mL); GLC (1600 µg/mL); GLB (50 µg/mL) and GLM (40 µg/mL).

2.5. Sample solution

To determine the content of specific anti-diabetic drugs simultaneously in conventional tablets, 10 tablets each of the selected combined pharmaceutical dosage forms (section 2.2) were weighed individually, their mean weight determined and were ground to a fine powder using a glass mortar and pestle. An equivalent of 500 mg of CHL, 10 mg of GLZ, 1000 mg of TOL, 30 mg of PGL, 160 mg of GLC, 5 mg of GLB and 4 mg of GLM was accurately weighed and transferred to a individual 100 mL volumetric flask, respectively. The volume was adjusted with methanol and water (1:1 v/v) and the resultant solution was sonicated for 15 minutes, filtered through a 0.2µm nylon filter (Millipore, Milford, USA) and injected directly onto the UPLC column using the optimized conditions.

2.6 Validation procedure

System suitability test was performed by making six repeated injections of standard solution to check parameters such as % relative standard deviation, theoretical plates, capacity factor, asymmetry factor and peak purity. The specificity of the method was determined by injecting the sample solution containing excipients without drug having concentration same as that of the sample.

2.6.1 Linearity

Linearity solutions were carried out at six concentration levels from 10% to 150% of analyte concentration in triplicate. The curves were constructed by plotting drug concentration verses peak areas. Linear calibration curves were generated by linear regression analysis and obtained over the respective standard concentration ranges. The standard solution for linearity was prepared as per section 2.4.

2.6.2 Accuracy

The accuracy of the method was carried out by adding known amount of each drug corresponding to the concentration levels 50%, 80%, 100%, 120% and 150% of the label claim along with the excipients in triplicate. The samples were given the same treatment as described in Section 2.5.

2.6.3 Precision

Precision of the method was checked by carrying out six replicate assays of combination drugs against qualified working standard. Intermediate precision was performed analyzing the samples by two different analysts using different instruments on different days.

2.6.4 Solution stability

The stability of each drug in solution was determined for 48 hours at room temperature. The samples were checked at periodic intervals and the data were compared with freshly prepared samples.

2.6.5 Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 613 µL/min to 575 µL/min and 650 µL/min. Standard solution was injected five times in replicate for each change, % RSD for all the drug components and resolution between drug components and their impurities were monitored for all robustness parameters. Respective peak areas, dilution factors, sample and standard weights were taken into account to quantitate the amounts of drug substance in mg per tablet.

2.7 Computation

The UPLC method was obtained by converting the HPLC method using the Method Translator and Cost Saving Calculator version 2.0 (www.chem.agilent.com). The flow rate of the UPLC method was obtained using the equation 1.

\[ Q_{\text{column}} - Q_{\text{column}} = Q_{\text{column}} \times (\frac{d_p}{d_{p1}})^2 \times (\frac{d_{p2}}{d_{p2}}) \quad \text{Eq. 1} \]

Where, \( Q_{\text{column}} \) and \( Q_{\text{column}} \) are the flow rates, \( d_p \) and \( d_{p1} \) are the diameters and \( d_{p2} \) are the particle size of the HPLC and UPLC columns, respectively.

Capacity factor ‘k’ gives an indication of how long each component is retained on the column. In the present study, the capacity factor of each peak in both HPLC and UPLC was obtained using the equation 2.

\[ k' = \frac{t_{R} - t_{n}}{t_{n}} \quad \text{Eq. 2} \]

Where ‘\( t_{n} \)’ is the unresolved peak’s retention time and ‘\( t_{R} \)’ is retention time of the
### Table 1: Comparison of system performance of HPLC and UPLC

<table>
<thead>
<tr>
<th>Drug Component</th>
<th>Retention time (min)</th>
<th>Capacity Factor</th>
<th>Resolution</th>
<th>USP Tailing</th>
<th>Peak capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpropamide</td>
<td>8.7</td>
<td>0.69</td>
<td>2.5</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Glipizide</td>
<td>9.4</td>
<td>0.76</td>
<td>2.8</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>10.2</td>
<td>0.82</td>
<td>3.1</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>11.9</td>
<td>0.99</td>
<td>3.8</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>12.8</td>
<td>1.04</td>
<td>4.1</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>15.4</td>
<td>1.27</td>
<td>5.2</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Glimeperide</td>
<td>16.4</td>
<td>1.36</td>
<td>5.5</td>
<td>1.0</td>
<td>1.3</td>
</tr>
</tbody>
</table>

### Table 2: Linear regression equations and correlation coefficient

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Equation</th>
<th>RSD (%)</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpropamide</td>
<td>y = 8.7x + 0.69</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Glipizide</td>
<td>y = 9.4x + 0.76</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>y = 10.2x + 0.82</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>y = 11.9x + 0.99</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>y = 12.8x + 1.04</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>y = 15.4x + 1.27</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Glimeperide</td>
<td>y = 16.4x + 1.36</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

### Table 3: Accuracy data (analyte recovery)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Theoretical Amount (µg/mL)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpropamide</td>
<td>50</td>
<td>2500</td>
<td>99.7</td>
<td>1.2</td>
<td>100.6</td>
</tr>
<tr>
<td>Glipizide</td>
<td>80</td>
<td>4020.0</td>
<td>99.5</td>
<td>1.1</td>
<td>98.3</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>100</td>
<td>5010.0</td>
<td>99.2</td>
<td>0.9</td>
<td>99.2</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>120</td>
<td>6002.0</td>
<td>99.4</td>
<td>0.7</td>
<td>101.3</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>150</td>
<td>7595.0</td>
<td>98.6</td>
<td>0.4</td>
<td>97.9</td>
</tr>
<tr>
<td>Glimeperide</td>
<td>180</td>
<td>9193.0</td>
<td>98.3</td>
<td>0.3</td>
<td>98.9</td>
</tr>
</tbody>
</table>

### Table 4: Intermediate precision and ruggedness

<table>
<thead>
<tr>
<th>Component</th>
<th>Analyst 1 Mean Assay (%)</th>
<th>RSD (%)</th>
<th>Analyst 2 Mean Assay (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpropamide</td>
<td>101.0</td>
<td>0.7</td>
<td>100</td>
<td>0.8</td>
</tr>
<tr>
<td>Glipizide</td>
<td>99.8</td>
<td>0.6</td>
<td>99.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>98.2</td>
<td>1.0</td>
<td>100.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>98.3</td>
<td>0.9</td>
<td>100.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>99.2</td>
<td>0.5</td>
<td>100.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>98.8</td>
<td>0.3</td>
<td>99.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Glimeperide</td>
<td>98.8</td>
<td>1.0</td>
<td>101.3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Fig. 1: Chemical structures of (a) Tolbutamide (TOL); (b) Chlorpropamide (CHL); (c) Glipizide (GLZ); (d) Glimeperide (GLM); (e) Glibenclamide (GLB); (f) Pioglitazone (PGL); and (g) Gliclazide (GLC).

Fig. 2: a. HPLC chromatograms of anti-diabetic drugs analyzed by means of the expression ‘π/2L’. Approximately 70% of the column volume then constitutes the void volume (is the volume that is not taken up by the stationary phase) of the column.

The dwell volume becomes increasingly important when employing a fast gradient with small columns typically used with UPLC system because it effects the peak of interest. Unretained peak’s retention time (t<sub>0</sub>) is determined by injecting uracil under same chromatographic conditions.

Column volume (CV) for both HPLC and UPLC columns were calculated by using the expression ‘π/2L’. Approximately 70% of the column volume then constitutes the void volume (is the volume that is not taken up by the stationary phase) of the column.

The dwell volume becomes increasingly important when employing a fast gradient with small columns typically used with UPLC system because it effects the...
time taken for the gradient to reach the head of the column. The dwell volumes for both HPLC and UPLC systems were estimated by employing respective columns as used in this study. The mobile phase consisting of A: methanol and B: 0.1% acetone in methanol was used. The flow rates for HPLC and UPLC were kept at 1.0 mL/min and 0.613 mL/min, respectively. Detector was set at 260 nm. Gradient programme was started from 100% A to 100% B in 20 minutes and then calculated the time taken to reach 50% absorbance ($T_{0.5}$). The difference in time interval between $T_{0.5}$ and 10 minutes (half the gradient time) was multiplied by the flow rate to determine the dwell volume.

Since the peak capacity [22] is a good tool to determine the quality of a gradient separation, performance of both the methods to separate all the selected drug compounds in the present study was compared with the peak capacity. The peak capacity ($P$) was calculated using the equation 3.

$$P = 1 + \frac{1}{n} \sum_{i=1}^{n} \frac{w_i}{t_g}$$  \hspace{1cm} \text{Eq. 3}

Where, ‘n’ is the number of peaks selected for the calculation, $t_g$ is the gradient run time and ‘$w$’ is peak width. Thus peak capacity is simply the gradient run time divided by the average peak width.

The mobile phase linear velocity (mm/sec) at a given flow rate was calculated by dividing the column length (mm) by the retention time of unretained peak (sec) i.e, void volume of peak time.
3.1. LC method development and transfer to UPLC

Initially, the isocratic and gradient HPLC conditions were optimized with an objective to separate all the six drug components selected in this study. The UV spectra of the analytes were independently determined. Each drug has exhibited different maximum UV absorbance. At a UV$_{max}$ range of 210–240 nm, baseline drift towards the negative absorbance was observed in the chromatograms, whereas at wavelength 245 nm, we could detect all the selected drugs simultaneously with good separation, sensitivity and consistent baseline.

The chromatographic separation was achieved on X-Terra C18, 250×4.6 mm, 5µm column maintained at ambient temperature. The feasibility of various mixture(s) of solvents (acetonitrile and methanol) using different buffers (ammonium acetate, acetic acid and formic acid) with variable pH range of 3–6, along with altered flow-rates (in the range 1.0 – 1.5 mL/min), was tested for complete chromatographic resolution of all the selected drugs. The use of acetic acid and formic acid (pH 3) led to a poor resolution between GLB and GLM, while PGL shown lesser capacity factor (0.8 and 0.3, respectively). Finally, the use of ammonium acetate (pH 4) based buffer with a timed gradient was optimized as mentioned in Section 2.3.1, which provided adequate separation with less tailing which resulted in best resolution between all the selected drugs amongst the buffers tested.

The basic chromatographic conditions like, stationary phase, solvents and UV detection, employed in HPLC were taken into account while developing the new UPLC method. The detection wavelength, column temperature, buffer and solvent used in HPLC were kept constant. The stationary phase C18 was chosen in order to have similar chemistry as that used in the HPLC. A BEH C18, 50×2.1 mm, 1.7µm column was employed for the separation. The injection volume was scaled down to 0.2 µl from 10µl as used in HPLC. As per van Deemter curves [23] for 2.1 mm i.d columns with 1.7 µm particle size, the maximum efficiency can be achieved between 3 and 7 mm/sec of linear velocity. Based on this theory, initial mobile phase linear velocity was kept at 4.2 mm/sec (0.5 mL/min). Unretained peak was found at 11 seconds. Under these conditions, though a satisfactory separation was achieved between all the selected drug components, tailing was observed for GLM in a total run time of 3-4 min. A backpressure of 6500 psi was observed. Taking into account the capability of high operating pressure of UPLC, the mobile phase linear velocity was increased to 5.6 mm/sec (0.613 mL/min) with a backpressure of 8,600 psi. This linear velocity, by using the ammonium acetate (pH 4) based buffer with a timed gradient as mentioned in Section 2.3.2, provided adequate resolution with less tailing which resulted in the best separation. The runtime was decreased to 1.5 min without affecting the separation of all the drug components. Hence, the gradient mode was preferred for UPLC analysis.

3.2. Comparison study of chromatographic performance

A comparative data on chromatographic performance of HPLC and UPLC has been obtained by injecting a solution of system suitability standard. Column volumes for HPLC and UPLC columns were found to be 4.15 mL and 0.19 mL, respectively. The dwell volumes for both HPLC and UPLC systems were found to be 1.6 mL and 0.11 mL, respectively.

The elution time of all the drug compounds in UPLC were observed to be reduced by more than 10-fold to that of HPLC employing 5-micron columns. The resolution obtained for all the drug compounds selected in this study by UPLC showed comparatively better separation efficiency than HPLC. The higher peak capacity (P = 76) in UPLC as against HPLC (P = 57) conforms to better gradient separation efficiency and resolving power of UPLC. The performance parameters of both the systems are shown in Table 1.
Under these optimized conditions, the analyte peaks were well resolved and free from tailing. The tailing factors were <$2.0$ for all the peaks. The nominal retention times of CHL, GLZ, TOL, PGL, GLC, GLB and GLM were found to be $8.7, 9.4, 10.2, 11.9, 12.8, 15.4$ and $16.4$ minutes respectively, at a flow rate of $1.0 \text{ mL/min}$ using UPLC while with UPLC, it was found to be $0.64, 0.70, 0.76, 0.88, 0.94, 1.11$ and $1.19$ minutes at a flow rate of $0.613 \text{ mL/min}$, respectively. The typical chromatograms obtained from final HPLC and UPLC conditions are depicted in Fig. 2a and 2b.

3.3. UPLC Method validation
The newly developed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, and robustness [24]. The system suitability parameters like capacity factor ($k'$), resolution (R) tailing factor (T) and peak capacity (P) were calculated and presented in Table 1.

3.3.1. Specificity
The different force degradation samples were analyzed and it was found that the drug peaks in acid, alkalai, peroxide, UV and photo degraded drug sample solutions have passed the purity test. The purity angles for the selected drug components in all stress conditions were found to be less than the threshold angle. This study confirms the specificity of the developed method.

The overall degradation in acidic and basic condition was found to be around $5\%$ for all the drug compounds except for GLC and PGL. In both acidic and basic stress condition, GLC showed $20\%$ degradation, while PGL showed $15\%$ degradation in alkaline stress condition. In GLC, the presence of sulfonylurea moiety makes it susceptible to hydrolysis and photolytic degradation [25, 26]. In our proposed method all the known and unknown impurities [27] were well separated from the drug substance.

The drug components in tablet sample were found to be stable when treated with $30\%$ hydrogen peroxide at $100^\circ \text{C}$. Overall degradation of $7\%$ including known and unknown impurities were achieved, when drug products were exposed to peroxide oxidation. The baseline resolution was achieved between all the impurities and drug components.

3.3.2. Structural identification of degradants

3.3.2.1 Pioglitazone
During stress degradation studies of pioglitazone hydrochloride, four major impurities were detected by LC-MS: 1, 2, 3 and 4. Since, Ramulu et al. [28] has already explained the formation of $m/z$ 331 (degradant 1) and dimer with $m/z$ 660 (degradant 2), only $m/z$ 330 (degradant 3) and $m/z$ 345 (degradant 4) were chosen for particular attention for further characterization in the present study. Since we could not isolate these degradation impurities by preparative chromatography, only LCMS analysis provided primary means for structure determination.

Fig. 3a and 3b represents the UPLC chromatogram and corresponding total ion chromatogram of pioglitazone obtained from alkaline degradation, respectively. Since, the chromatographic conditions employed for both LCMS and UPLC experiment were same, correlation of the peaks by both the methods were straightforward. Fig. 4 represents the positive ion mass spectra of PGL degradation products. In absence of other spectral data except LCMS, all our efforts in identifying the structure of degradant 3 was not successful.

The positive ion mass spectrum of degradant 4 as shown in Fig. 4 possesses $M+1$ ion of 346. This molecular weight (MW) requires the presence of an odd number of nitrogens. The only site which can be susceptible to hydrolysis is thiazolidine moiety. Hence, the proposed structure of degradant 4 must account for the destruction of pyrrolone ring to form an ester carbonyl moiety. Since, the chromatographic conditions employed for both LCMS and UPLC experiment were same, correlation of the peaks by both the methods were straightforward. Fig. 5. Formation of fragmented ions of $m/z$ 286 by MS/MS experiment, as shown in Fig 6, indicates the loss of methyl formate (59 amu) from the precursor of impurity A.

3.3.2.2 Glimepiride
During stress degradation studies of glimepiride, four major impurities were detected by LC-MS: 5, 6, 7 and 8. Since, degradant 5 with $m/z$ 382 is already reported in the literature [29-31], only $m/z$ 397 (degradant 6), $m/z$ 522 (degradant 7) and $m/z$ 520 (degradant 8) were chosen for particular attention for further characterization in the present study. Only LCMS analysis provided primary means for structure determination. Fig. 7a and 7b represents the UPLC chromatogram and corresponding total ion chromatogram of glimepiride obtained from alkaline degradation, respectively.

3.3.2.3 Assay
The validated method was applied to the determination of CHL, GLZ, TOL, PGL, GLC, GLB and GLM in commercially available tablets. The result of the assays ($n = 6$) undertaken yielded 101.0% ($\text{RSD} = 0.7\%$), 99.8% ($\text{RSD} = 0.6\%$), 98.2% ($\text{RSD} = 0.30\%$), 98.3% ($\text{RSD} = 0.9\%$), 99.2% ($\text{RSD} = 0.5\%$), 98.4% ($\text{RSD} = 0.3\%$) and 98.8% ($\text{RSD} = 1.0\%$) of label claim for CHL, GLZ, TOL, PGL, GLC, GLB and GLM respectively. The results for all the tested compounds are listed in Table 4, which indicates that the method has a good reproducibility and intermediate precision.

3.3.3. Robustness
In all deliberately varied conditions, the RSD of peak areas of CHL, GLZ, TOL, PGL, GLC, GLB and GLM were found to be well within the acceptable limit of 2%. The tailing factor for all the peaks was found to be $<2.0$. The elution order and total retention time of CHL, GLZ, TOL, PGL, GLC, GLB and GLM respectively, which are well within the acceptable limit of 2.0. The RSDs for intermediate precision were found to be $0.8\%$, $0.9\%$, $1.1\%$, $0.6\%$, $0.8\%$, $0.6\%$ and $0.9\%$ for CHL, GLZ, TOL, PGL, GLC, GLB and GLM respectively. The results for all the tested compounds are listed in Table 4, which indicates that the method has a good reproducibility and intermediate precision.

3.3.4. Assay
The validated method was applied to the determination of CHL, GLZ, TOL, PGL, GLC, GLB and GLM in commercially available tablets. The result of the assays ($n = 6$) undertaken yielded 101.0% ($\text{RSD} = 0.7\%$), 99.8% ($\text{RSD} = 0.6\%$), 98.2% ($\text{RSD} = 0.30\%$), 98.3% ($\text{RSD} = 0.9\%$), 99.2% ($\text{RSD} = 0.5\%$), 98.4% ($\text{RSD} = 0.3\%$) and 98.8% ($\text{RSD} = 1.0\%$) of label claim for CHL, GLZ, TOL, PGL, GLC, GLB and GLM respectively. The results for all the tested compounds are listed in Table 4, which indicates that the method is selective for the analysis of all the selected drugs without interference from the excipients used to formulate and produce these tablets.
4.0 CONCLUSION
The newly developed UPLC method for the simultaneous determination of CHL, GLZ, TOL, PGL, GLC, GLB and GLM in pharmaceutical dosage forms was found to be capable of giving faster retention times, maintaining good resolution than that achieved with conventional HPLC. The method was completely validated showing satisfactory data for all the parameters tested. This method exhibited an excellent performance in terms of sensitivity and speed. It is a stability indicating method suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

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6.0 REFERENCES

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