A Review On Chromatographic and Spectrophotometric Methods for Estimation of Dapagliflozin and Glimepiride In Bulk and In Different Dosage Forms

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ABSTRACT
Dapagliflozin and Glimepiride are very effectively used as type II diabetes. They very potent inhibit renal glucose reabsorption and inhibiting sodium glucose transport protein 2 and its called SGLT2 inhibitors. They used to enhance glycemic control as well as reduce body weight and systolic & diastolic blood pressure. They are generally administered as tablets. This review entails different methods developed for determination of the Dapagliflozin and Glimepiride like UV-spectroscopy and liquid chromatography.

Key-words: Dapagliflozin, Glimepiride, UV Spectroscopy, Liquid Chromatography, SGLT2 Inhibitors.

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INTRODUCTION:
Dapagliflozin and Glimepiride drugs are a class of pharmaceutical that inhibit renal glucose reabsorption and therefore lower blood glucose. They act by inhibiting sodium-glucose transport protein 2 (SGLT2), and are therefore also called SGLT2 inhibitors. Dapagliflozin and Glimepiride used in the treatment of type2 diabetes. As studied on Dapagliflozin and Glimepiride enhance glycemic control as well as reduce body weight and systolic and diastolic blood pressure [1].
SGLTs are responsible for mediating glucose reabsorption in the kidneys, as well as in the gut and the heart. SGLT-2 is primarily expressed in the kidney on the epithelial cells lining the S1 segment of the proximal convoluted tubule. It is the major transport protein that promotes reabsorption from the glomerular filtration glucose back into circulation and is responsible for approximately 90% of renal glucose reabsorption. By inhibiting SGLT-2 it prevents renal re-uptake from the glomerular filtrate and subsequently lowers the glucose level in the blood and promotes glycosuria [2, 3]. Selective and potent inhibition of SGLT-2 and its activity is based on each patient's underlying glycemic control and renal function. The results are decreased renal reabsorption of glucose, glycosuria effect increases with higher level of glucose in the blood circulation. Thereby Dapagliflozin and Glimepiride reduces the blood glucose concentration with a mechanism that is independent of insulin secretion and sensitivity, unlike many other anti-diabetic drugs. Functional β-cells are not necessary for the activity of the drug so it is convenient for patients with diminished β-cell function [2, 3]. Sodium and glucose are co-transported by the SGLT-2 protein into the tubular epithelial cells across the brush-border membrane of the proximal renal tubule. This happens because of the sodium gradient between the tubule and the cell, thereby it provides a secondary active transport of glucose. Glucose is later reabsorbed by passive transfer of endothelial cells into the interstitial glucose transporter protein. Different methods have been developed for determination of like UV-spectroscopy, liquid chromatography (HPTLC and HPLC) [2, 3].
Reported methods are categorized depending on the following considerations:
1. Single component analysed by UV-spectroscopy methods and chromatographic method.
2. Analysis of Dapagliflozin and Glimepiride from combination formulation by UV-spectroscopy methods and chromatographic method.

Table: 1 Analysis of dapagliflozin from combination formulation by liquid chromatography

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>DRUGS</th>
<th>METHOD</th>
<th>DESCRIPTION</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dapagliflozin API</td>
<td>UV Spectrophotometric Method</td>
<td>Wavelength-237 nm&lt;br&gt;Solvent-Water&lt;br&gt;Linearity range-0.5-0.9 µg/ml&lt;br&gt;Correlation co-efficient-0.994&lt;br&gt;LOD-0.0925 µg/ml&lt;br&gt;LOQ-0.00129 µg/ml</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Dapagliflozin in Bulk and Pharmaceutical dosage form</td>
<td>UV Spectrophotometric Method</td>
<td>Wavelength-233 nm&lt;br&gt;Linearity range-10-35 µg/ml&lt;br&gt;Correlation co-efficient-0.999&lt;br&gt;LOD-1.24&lt;br&gt;LOQ-3.62</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Simultaneous estimation of Dapagliflozin and Metformin HCL in synthetic mixture</td>
<td>UV Spectrophotometric Method</td>
<td>Wavelength-225-237 nm&lt;br&gt;Solvent-Methanol&lt;br&gt;Correlation co-efficient-0.993 for Metformin and 0.991 for Dapagliflozin&lt;br&gt;% RSD-1.102 of Metformin and 1.353 of Dapagliflozin</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>First derivative for simultaneous estimation of Dapagliflozin and Metformin HCL in synthetic mixture</td>
<td>UV Spectrophotometric Method</td>
<td>Wavelength-Dapagliflozin-235 nm&lt;br&gt;-Metformin HCL-272 nm&lt;br&gt;Solvent-Methanol&lt;br&gt;Linearity range-Dapagliflozin-0.5-2.5 µg/ml</td>
<td>7</td>
</tr>
</tbody>
</table>
5  Dapagliflozin API  RP-HPLC  **Mobile Phase**  
- Ortho phosphoric acid: Acetonitrile (45:55 v/v)  
**Stationary Phase**  
- BDS Column (250×4.5 mm,5µ)  
**Solvent**- Methanol  
**Flow rate**= 1 ml/min  
**Wavelength**-245 nm  
**Linearity range**  
- 25-150 µg/ml  
**Retention time**-2.963 min  
**Correlation co-efficient**-0.982  
**LOD**-0.009 µg/ml  
**LOQ**-0.013 µg/ml  
**% Recovery**-99.8%  

6  Dapagliflozin and Metformin HCL in bulk drug and tablet  RP-HPLC  **Mobile Phase**  
- Triethylamine : Acetonitrile (50:50 % v/v)  
**Stationary Phase**  
- Hypersil BDS C₁₈ (250×4.6 mm,5µ Particle size)  
**Solvent**- Methanol  
**Flow rate**= 1 ml/min  
**Wavelength**-240 nm  
**Linearity range**  
- 85-510 µg/ml for Metformin and 0.5-3.0 µg/ml for Dapagliflozin  
**Correlation co-efficient**  
- 0.99995 for Metformin and 0.99978 for Dapagliflozin  

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**Table: 2 Analysis of Glimepiride from combination formulation by liquid chromatography**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Drug</th>
<th>Method</th>
<th>Description</th>
<th>Ref No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glimepiride in pharmaceutical dosage form</td>
<td>UV Spectrophotometric Method</td>
<td><strong>Detection wavelength</strong> : 249 nm</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Linearity range</strong>: 5-30 µg/ml</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Correlation coefficient</strong>: 0.999732</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Precision</strong>: 0.159437</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Limit of Detection</strong>: 0.4 µg/ml</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Limit of Quantification</strong>: 1.2 µg/ml</td>
<td></td>
</tr>
</tbody>
</table>
| 2  | Glimepiride in tablet dosage form | RP-HPLC Method | **Detection wavelength:** 210 nm  
**Mobile Phase:** Acetonitrile: 0.05M monophasic potassium phosphate (pH 6.0) (40:60) (v/v).  
**Stationary Phase:** Hypersil C18 column (15x3.9mm)  
**Retention time:** 7.8 min  
**Flow rate:** 1.5 ml/min  
**Recoveries:** 99-101% | 11 |
| 3  | Glimepiride in tablet formulation | Stability indicating RP-HPLC Method | **Detection wavelength:** 228 nm  
**Mobile Phase:** potassium phosphate buffer (pH 6.5; 27.5 mmol/L)-methanol (34 + 66, v/v)  
**Stationary Phase:** C18 column (250 x 4.6 mm, 5.0 pm)  
**Flow rate:** 1 ml/min  
**Retention time:** 9 min  
**linearity** 2 to 40 mg/L  
**LOD:** 0.315 mg/L  
**LOQ:** 1.050 mg/L | 12 |
| 4  | Glimepiride in supersaturatable Self Nano-emulsifying (SNE) formulation | RP-HPLC Method | **Detection wavelength:** 228 nm  
**Mobile Phase:** potassium di-hydrogen phosphate buffer (pH-4): Acetonitrile (50:50 v/v)  
**Stationary Phase:** Kromasil C18 column (150 x 4.6 mm; 5μ)  
**Retention time:** 0.9152 min  
**Flow rate:** 1.0ml/min | 13 |
| 5  | Pioglitazone and Glimepiride in bulk and combine dosage form | UV Derivative(1st order) Spectrophotometric Method | **Detection wavelength:**  
Pioglitazone: 225 nm  
Glimepiride: 248 nm  
**Solvent:** 0.1 N HCL  
**Linearity range:**  
Pioglitazone: 5-30µg/ml  
Glimepiride: 4-20 µg/ml  
**Correlation coefficient:**  
Pioglitazone: 0.9912  
Glimepiride: 0.9964  
**Limit of Detection:**  
Pioglitazone: 0.0187 µg/ml  
Glimepiride: 0.132 µg/ml  
**Limit of Quantification:**  
Pioglitazone: 0.056µg/ml  
Glimepiride: 0.40µg/ml | 14 |
| 6  | Pioglitazone and Glimepiride in tablets | RP-HPLC Method | **Detection wavelength:** 225 nm  
**Mobile Phase:** Phosphate buffer (pH-4.5): Acetonitrile (45:55 v/v)  
**Stationary Phase:** Inertsil ODS (250x4.6mm, 5µm in particle size) | 15 |
<table>
<thead>
<tr>
<th>Table</th>
<th>Method</th>
<th>Detection wavelength</th>
<th>Mobile Phase</th>
<th>Stationary Phase</th>
<th>Retention time</th>
<th>Flow rate</th>
<th>Correlation coefficient</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Simultaneous UV Spectrophotometric Method</td>
<td>Metformin : 236 nm Glimepiride: 228 nm</td>
<td>Ortho-phosphoric acid (pH -9.2) Methanol(60:40 v/v)</td>
<td>Water symmetry shielde Rp 18 column(250x4.6mm, 5µm in particle size)</td>
<td>Metformin : 2.344 min Glimepiride: 3.725 min</td>
<td>1.0ml/min</td>
<td>0.9999</td>
<td>99.98%</td>
</tr>
<tr>
<td>8</td>
<td>RP-HPLC Method</td>
<td>285 nm</td>
<td>20 mM phosphate buffer, adjusted to pH 3.0 and an organic phase (methanol:acetonitrile;62.5:37.5) in the ratio of 80:20.</td>
<td>JASCO Finepak SIL (250 mm × 4.6 mm i.d. 5µm)</td>
<td>Metformin HCL:2.75 min Glimepiride: 5.87 min</td>
<td>1.0ml/min</td>
<td>0.9999</td>
<td>99.98%</td>
</tr>
<tr>
<td>9</td>
<td>Stability-Indicating RP-HPLC Method</td>
<td>230nm</td>
<td>an aqueous phase (20 mM phosphate buffer, adjusted to pH 3.0) and an organic phase (methanol:acetonitrile;62.5:37.5) in the ratio of 80:20</td>
<td></td>
<td>Metformin HCL:2.75 min Glimepiride: 5.87 min</td>
<td>1.0ml/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>HPLC Method</td>
<td>231nm</td>
<td>Methanol: Water (90:10%v/v)</td>
<td>C18 column20 (250 x 4.6 mm; 5µ)</td>
<td>Glimepiride: 4.286 min Metformin HCL :2.262 min</td>
<td>1 ml/min</td>
<td>0.9998</td>
<td>99.98%</td>
</tr>
</tbody>
</table>
| 11 | Pioglitazone and Glimepiride in tablet-Dosage form | UV By multi wavelength Spectroscopy | Metformin HCL: 99.9%  
Assay: % Purity  
Glimepiride: 98.05  
Metformin HCL: 99.69 | 20 |
| 12 | Rosuvastatin Calcium and Glimepiride in Tablet Dosage Form | UV Spectrophotometric Method | Detection wavelength:  
280nm and 238nm  
Solvent: 0.1 N NaOH  
Linearity range:  
Pioglitazone: 10-50 µg/ml  
Glimepiride: 1-5 µg/ml  
% RSD:  
Pioglitazone: 0.74  
Glimepiride: 0.96  
% Recovery:  
Pioglitazone: 101.0  
Glimepiride: 100.9 | 21 |
| 13 | Glimepiride in self-Nano emulsifying powder (SNEP) formulation | RP-HPLC method and its dissolution study | Detection wavelength:  
228 nm using PDA detector.  
Mobile Phase:  
Acetonitrile:0.2 M phosphate buffer (pH = 7.4)  
40:60 v/v  
Stationary Phase:  
Octadecylsilane (ODS) column (250x4.6mm, 5µm in particle size)  
Flow rate: 1.0ml/min  
Linearity range:  
Glimepiride: 0.2-2 µg/ml  
Correlation coefficient:  
Glimepiride: 0.999  
Limit of Detection:  
Glimepiride: 0.38 µg/ml  
Limit of Quantification:  
Glimepiride: 1.17 µg/ml | 22 |
| 14 | Rosiglitazone and Glimepiride in combined dosage forms and human plasma | RP-HPLC method | Detection wavelength:  
235 nm using nicardipine as an internal standard.  
Mobile Phase:  
Acetonitrile : 0.02M Phosphate buffer(pH5) (60:40 v/v)  
Stationary Phase:  
C18 column (150 x 4.6 mm; 5µ)  
Retention time:  
Rosiglitazone: 3.7 min  
Flow rate: 1.0ml/min  
Linearity range: | 23 |
<table>
<thead>
<tr>
<th>Sequence</th>
<th>Study Details</th>
<th>Methodology</th>
<th>Detection Parameters</th>
</tr>
</thead>
</table>
| 15       | Pioglitazone and Glimepiride in pharmaceutical dosage form | RP-HPLC Method | **Detection wavelength:** 230nm  
**Mobile Phase:** Acetonitrile: KH₂PO₄ buffer (pH6) (60:40 v/v)  
**Stationary Phase:** Phenomenex Luna (150x4.6mm, 5μm in particle size)  
**Retention time:** Pioglitazone: 4.4min  
Glimepiride: 2.7 min  
**Flow rate:** 1.5ml/min  
**Linearity range:** Pioglitazone: 2.40-360μg/ml  
Glimepiride: 32-48 μg/ml |
| 16       | Glimepiride, Pioglitazone, and Metformin in Pharmaceutical Dosage Forms | RP-HPLC Method | **Detection wavelength:** 228nm  
**Mobile Phase:** Buffer (pH5) : Acetonitrile : Tetrahydrofuran: (40 : 50 : 10)  
**Stationary Phase:** Inertsil ODS-3V (250 mm × 4.6 mm, 5μm)  
**Resolution Run time:** Glimepiride: 5 min  
Pioglitazone: 3.9min  
Metformin: 1.3 min  
**Flow rate:** 1.7 ml/min  
**Linearity:** 150%, 125%, 100%, 75%, and 50% solutions |
| 17       | Metformin, Voglibose, Glimepiride in Bulk and Combined Tablet Dosage Form | Gradient RP-HPLC | **Detection wavelength:** 230nm using Photodiode array detector.  
**Mobile Phase:** 0.02M phosphate buffer (pH 2.5): Acetonitrile (v/v)  
**Stationary Phase:** Inertsil ODS 3V (150x4.6mm, 5μm in particle size) column in a gradient mode.  
**Retention time:** Metformin: 2.423min  
Voglibose: 8.191min  
Glimepiride: 11.708min  
**Flow rate:** 1.0ml/min  
**Linearity range:**  
Pioglitazone: 240-360μg/ml  
Glimepiride: 32-48 μg/ml  
**Gradient programming:** 18 min  
**%Assay:** Metformin: 99.92%  
Voglibose: 99.32%  
Glimepiride: 99.72%  
**Linearity range:**  
Metformin: 200-600 μg/ml  
Voglibose: 0.08-0.24 μg/ml |
| 18 | Glimepiride and sildenafil citrate in rat plasma | RP-HPLC method And application to pharmacokinetic studies | The drug samples were extracted by liquid-liquid extraction with 300 μl of acetonitrile and 5 ml of diethyl ether. **Detection wavelength:** 230nm **Mobile Phase:** Methanol: Water (85:15 v/v) **Stationary Phase:** C18 column **Retention time:** Glimepiride: 2.5min Sildenafil: 4min **Flow rate:** 1.0ml/min **Total run time:** 7 min |
| 19 | Metformin, pioglitazone, and glimepiride in pharmaceutical dosage forms | Liquid chromatography | Detection wavelength: 240nm using a UV-SPD-10AVP detector **Mobile Phase:** Methanol : Acetonitrile: 15 mM potassium dihydrogen phosphate (pH 4) 40:35:25 (v/v) **Stationary Phase:** Phenomenex-ODS-3 (C-18) column (250 × 4.60 mm, 5 µm) **Retention time:** Metformin : 2.85 ± 0.03 min Pioglitazone: 4.52 ± 0.03 min GLIMEPIRIDE: 7.08 ± 0.02min **Flow rate:** 1.0ml/min **Linearity Range:** Metformin : 0.2–50 μg/ ml Pioglitazone & Glimepiride : 0.2–30 μg/ml **Precision** - Metformin : Intra-day % RSD : 1.01–3.24 Inter-day % RSD : 1.54–4.09 Pioglitazone: Intra-day % RSD : 1.03–2.09 Inter-day % RSD : 2.26–3.10 Glimepiride: Intra-day % RSD : 1.00–3.15 and Inter-day % RSD : 1.58–3.07 **Accuracy** - Metformin : 99.66 ± 0.14 Pioglitazone: 98.46 ± 0.40 Glimepiride: 98.62 ± 0.39 |
| 20 | Sildenafil and Glimepiride in Rat Plasma | LC-Ms Method and their Applications in Pharmacokinetic | **Mobile phase:** A mixture of 70% methanol, 30% of 0.1% formic acid in water **Stationary phase:** ACE 5 C18 column |
CONCLUSION:
This review depicts the reported Spectroscopic and Chromatographic methods developed and validated for estimation of Dapagliflozin and Glimepiride. According to this review it was concluded that for Dapagliflozin and Glimepiride different Spectroscopic and Chromatographic methods are available for single and combination also it was found that the mobile phase containing Acetonitrile, water, and Phosphate buffer were common for most of the chromatographic method to provide more resolution. It was observed that most common combination of Dapagliflozin and Glimepiride were with Metformin. For chromatographic method flow rate is observed in the range 1.0-1.5 ml/min to get good resolution time. For most of the Spectroscopic methods common solvent is Methanol. Hence this all methods found to be simple, accurate, economic, precise and reproducible in nature. Most of Methods were of RP-HPLC and UV absorbance detection because these methods provided with best available reliability, repeatability, analysis time and sensitivity.
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