A REVIEW ON ANALYTICAL METHODS FOR ESTIMATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN IN BULK AND IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
Dapagliflozin and Saxagliptin are very effectively used treatment for type II diabetes. They are very potent inhibitors of renal glucose reabsorption and dipeptidyl peptidase protein 4(DPP-4) and sodium glucose transport protein 2 and also they are called as DPP4 & SGLT2 inhibitors. They are generally administered as tablets. Determination of Dapagliflozin and Saxagliptin in pharmaceutical dosage form and bulk form, several analytical methods including UV, HPLC, LC-MS and HPTLC techniques has been developed. Methods indicating human plasma stability and impurity profiling are also described for both drugs. For qualitative and quantitative estimation of Dapagliflozin and Saxagliptin, these analytical methods can be used and it can also be used for its related degradants in bulk formulations and biological fluids. The following study depicts the review on analytical methods which includes estimating the antidiabetic drugs.

Keywords: Dapagliflozin, Saxagliptin, UV-Spectroscopy, RP-HPLC, LC-MS and Gas Chromatography,
1. Methods for determination of Dapagliflozin Single and combination with other drugs by UV-Spectroscopy and Chromatography and other techniques.

2. Methods for determination of Saxagliptin Single and combination with other drugs by UV-Spectroscopy and Chromatography and other techniques.

Table 1: Methods for determination of Dapagliflozin Single and combination with other drugs by UV Spectroscopy, Chromatography and other techniques

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<thead>
<tr>
<th>S.No</th>
<th>DRUGS</th>
<th>METHOD</th>
<th>DESCRIPTION</th>
<th>REF. NO</th>
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<tr>
<td>1</td>
<td>Dapagliflozin in tablet formulation</td>
<td>UV Spectrophotometric Method</td>
<td>Detection wavelength: 224 nm Mobile Phase: Methanol - Water Linearity range: 5-40 μg/ml Correlation coefficient: &lt; 1</td>
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<td>2</td>
<td>Dapagliflozin in bulk and pharmaceutical dosage forms</td>
<td>UV Spectrophotometric Method</td>
<td>Detection wavelength: 233.65 nm Mobile Phase: Ethanol: Phosphate buffer (1:1) Linearity range: 10-35 μg/ml Correlation coefficient: 0.9998 % Recovery: 99.7 LOD: 1.24 μg/ml LOQ: 3.62 μg/ml</td>
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<td>3</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 Metformin HCl-272 nm Solvent: Methanol Linearity range: Dapagliflozin-0.5-2.5 μg/ml Metformin-25-125 μg/ml Correlation co-efficient: Dapagliflozin-0.980 Metformin HCl-0.982 LOD: Dapagliflozin-0.009 μg/ml Metformin HCl-0.013 μg/ml LOQ: Dapagliflozin-0.039 μg/ml Metformin HCl-0.041 μg/ml</td>
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<td>4</td>
<td>Dapagliflozin API</td>
<td>UV Spectrophotometric Method</td>
<td>Wavelength: 237 nm Solvent: Ethanol Linearity range: 0.5-0.9 μg/ml Correlation coefficient: 0.994</td>
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<td>5</td>
<td>Dapagliflozin in API. RP-HPLC and UV- Spectroscopy.</td>
<td>RP-HPLC and UV-Spectroscopy.</td>
<td>Wavelength: 203 nm Mobile phase: Acetonitrile: Ortho phosphoric acid (55:45%) Linearity range: In HPLC- 25-150 μg/ml In UV-1-5 μg/ml Correlation co-efficient: 0.999 LOD: 0.01 μg/ml LOQ: 0.05 μg/ml</td>
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<td>6</td>
<td>Metformin and Dapagliflozin in bulk and synthetic mixture</td>
<td>RP-HPLC method</td>
<td>Wavelength: 285 nm Mobile phase: Acetonitrile: Water (75:25% v/v) Flow rate: 1ml/min Injection volume: 10μl Retention time: Metformin-3.2 min Dapagliflozin-5.4 min Linearity range: Metformin-20-100 μg/ml Dapagliflozin-10-50 μg/ml % Recovery: 99.3-99.6% LOD: Metformin-5.0 μg/ml Dapagliflozin-3.7 μg/ml LOQ: Metformin-15.2 μg/ml Dapagliflozin-11.42 μg/ml</td>
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</tbody>
</table>
| 7    | Dapagliflozin in bulk and tablet dosage form.                               | RP-HPLC | **Wavelength**: 237 nm  
**Mobile phase**: Phosphate buffer : acetonitrile (75:25% v/v)  
**Flow rate**: 1.0 ml min⁻¹  
**Retention time**: 3.461 min  
**Linearity range**: 10-60 µg/ml  
**LOD**: 0.02 µg/ml  
**LOQ**: 0.06 µg/ml                                                                 | 15   |
| 8    | Metformin Hydrochloride and Dapagliflozin in tablet dosage form.            | RP-HPLC | **Wavelength**: 240 nm  
**Mobile phase**: Phosphate Buffer (pH 6.5): Methanol : Acetonitrile in the ratio of 50:30:20 v/v/v  
**Flow rate**: 1 ml/min  
**Retention time**: Metformin HCL-2.475 min  
**Linearity range**: Metformin HCL 85-510 µg/ml  
Dapagliflozin 0.5–3µg/ml  
**LOD**: Metformin HCL-2.469 ppm  
Dapagliflozin-3.649 ppm  
**LOQ**: Metformin HCl-0.997  
Dapagliflozin-0.9973  
**% Recovery**: Metformin HCl-100.67%  
Dapagliflozin-99.54%                                                                 | 16   |
| 9    | Metformin and Dapagliflozin in pharmaceutical dosage forms                  | RP-HPLC | **Wavelength**: 240 nm  
**Mobile phase**: Acetonitrile: phosphate buffer (70:30 %v/v)  
**Flow rate**: 1 ml/min  
**Retention time**: Metformin-2.463 min  
Dapagliflozin-3.760 min  
**Linearity range**: Metformin-50-250 µg/ml  
Dapagliflozin-5-25 µg/ml  
**% Recovery**: Metformin -97.0-98.0 %  
Dapagliflozin-100-103 %                                                                 | 17   |
| 10   | Dapagliflozin in API                                                        | RP-HPLC | **Wavelength**: 240 nm  
**Mobile phase**: Ortho phosphoric acid : Acetonitrile (45:55 v/v)  
**Flow rate**: 1 ml/min  
**Retention time**: 2.963 min  
**Linearity range**: 25-150 µg/ml  
**Correlation co-efficient**: 0.999  
**LOD**: 0.6 µg/ml  
**LOQ**: 1.8 µg/ml  
**% Recovery**: 99.8%                                                                 | 18   |
| 11   | Dapagliflozin in Bulk and Table Formulation                                | RP-HPLC | **Wavelength**: 210 nm  
**Mobile phase**: 0.1% Ortho phosphoric acid buffer : Acetonitrile (60:40 % v/v)  
**Flow rate**: 1 ml/min  
**Injection volume**: 10 µL  
**Runtime**: 5 min  
**Retention time**: 2.226 min  
**Linearity range**: 25 – 150 µg/ml  
**% Recovery**: 98.95 – 101.72 %  
**%RSD intraday precision**: 0.6%  
**%RSD inter day precision**: 0.4%                                                                 | 19   |
| 12   | Dapagliflozin and Saxagliptin in fixed-dose combination.                    | RP-HPLC | **Wavelength**: 230 nm  
**Mobile phase**: Sodium dihydrogen phosphate: Acetonitrile (53:47 v/v)  
**Flow rate**: 1.2 mL / min  
**Linearity range**: 2–14 µg/mL                                                                 | 20   |
| 13 | Saxagliptin Hydrochloride and Dapagliflozin in bulk and in tablet form | Stability indicating RP-HPLC method | Wavelength: 220 nm  
Mobile phase: Potassium dihydrogen phosphate Buffer (pH 6.0) : Acetonitrile (45:55 v/v)  
Linearity range: Saxagliptin HCl 56-54 μg/ml  
Dapagliflozin 112-168 μg/ml |  
% Recovery:  
Dapagliflozin - 99.16%  
Saxagliptin - 100.58%  
Correlation coefficients:  
Dapagliflozin - 0.997  
Saxagliptin - 0.996 |
|---|---|---|---|
| 14 | Saxagliptin and Dapagliflozin in bulk and dosage forms | Stability indicating RP-HPLC method | Wavelength: 220 nm  
Mobile phase: Phosphate Buffer : Acetonitrile (50:50 v/v)  
Flow rate: 1.2 mL/ min  
Linearity range: Saxagliptin 20-60 μg/ml  
Dapagliflozin 40-120 μg/ml  
Retention time: Saxagliptin 2.1 min  
Dapagliflozin 2.8 min  
Accuracy range: 99.99-100.50 %  
Precision:  
Saxagliptin 0.78 %  
Dapagliflozin 0.44%  
LOD:  
Saxagliptin 1.63 μg/ml  
Dapagliflozin 1.94 μg/ml  
LOQ:  
Saxagliptin 5.39 μg/ml  
Dapagliflozin 6.50 μg/ml  
% Assay: 100.24-100.43 % |
| 15 | Dapagliflozin and Saxagliptin in combined tablet dosage forms. | Stability indicating RP-HPLC method | Wavelength: 220 nm  
Mobile phase: Acetonitrile:0.1% Orthophosphoric acid in water(50:50 v/v)  
Flow rate: 1.0 mL/ min |  
Linearity range:  
Metformin 85-510 μg/ml  
Dapagliflozin 0.5-3.0 μg/ml  
Retention time:  
Metformin 2.791 min  
Dapagliflozin 3.789 min |
Mobile phase: Buffer (0.1% Orthophosphoric acid) adjusted to pH 6.8 with Triethylamine: Acetonitrile in the ratio of 50:50% v/v  
Flow rate: 1.0 mL/ min.  
Linearity range:  
Metformin 85 - 510 μg/ml  
Dapagliflozin 0.5-3.0 μg/ml  
Retention time:  
Metformin 2.791 min  
Dapagliflozin 3.789 min |
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</table>
| 1 | Saxagliptin Hydrochloride and Metformin Hydrochloride in API | UV-VIS Spectroscopy method | **Wavelength**: Saxagliptin HCL 274 nm Metformin HCL 231 nm  
**Linearity range**: Saxagliptin HCL 50-90 µg/ml Metformin HCL 2-10 µg/ml  
**Correlation co-efficient**: 0.990  
**% Recovery**: Saxagliptin HCL 100.1% Metformin HCL 99.88% | 25 |
| 2 | Saxagliptin in Hydrochloride in Bulk and tablet Dosage Form | RP-HPLC method | **Wavelength**: 210 nm  
**Mobile phase**: Phosphate buffer: Acetonitrile (80:20 v/v)  
**Flow rate**: 1 ml/min  
**Retention time**: 5.43±0.03 min  
**Linearity range**: 0.10–0.30 mg/ml  
**Correlation co-efficient**: 0.999  
**LOD**: 9 µg/ml  
**LOQ**: 27 µg/ml  
**% Recovery**: 100.28%  
**%RSD for repeatability**: 0.630%  
**%RSD for intermediate precision**: 0.529%  
**Accuracy**: 99.96% | 26 |
| 3 | Metformin and Saxagliptin in API | RP-HPLC method | **Wavelength**: 220 nm  
**Mobile phase**: 0.05M KH₂PO₄ buffer (pH4.5): Methanol: Acetonitrile (60:20:20% v/v)  
**Flow rate**: 0.6 mL/min  
**Run time**: 10 min  
**Retention time**: Metformin-4.38 min Saxagliptin-6.92 min  
**Injection volume**: 10µl  
**LOD**: Metformin-0.112 µg/ml Saxagliptin-0.029 µg/ml  
**LOQ**: Metformin-0.373 µg/ml Saxagliptin-0.096 µg/ml | 27 |
| 4 | Metformin and Saxagliptin in Pharmaceutical Dosage Form | RP-HPLC method | **Wavelength**: 208 nm  
**Mobile phase**: Buffer: Methanol (55:45 v/v)  
**Flow rate**: 1 ml/min  
**Linearity range**: Metformin 60–100 µg/ml Saxagliptin 0.6–1.0 µg/ml  
**LOD**: Metformin-0.17 µg/ml Saxagliptin-0.064 µg/ml  
**LOQ**: Metformin-0.08 µg/ml Saxagliptin-0.02 µg/ml  
**Correlation co-efficient**: 0.999  
**Accuracy**: Metformin-101.07% Saxagliptin-101.25% | 28 |
| 5 | Saxagliptin and Methyldopa in a laboratory mixture | Analytical method | **Wavelength**: 211-280 nm  
**Linearity range**: Saxagliptin-5-30 µg/ml Methyldopa-2-12 µg/ml  
**% Recovery**: 98-101% | 29 |
| 6 | Saxagliptin in Tablet Dosage Form | RP-HPLC method | **Wavelength**: 220 nm  
**Mobile phase**: Acetonitrile: Potassium Di-hydrogen Phosphate Buffer  
**Flow rate**: 1 ml/min | 30 |
<table>
<thead>
<tr>
<th>Table</th>
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<th>Method Details</th>
<th>Results/Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Saxagliptin and Metformin in Bulk and Pharmaceutical Dosage Form,</td>
<td>Stability indicating RP-HPLC method</td>
<td>Retention time: 3.487 min, Linearity range: 50-150 µg/ml, Correlation co-efficient: 0.9999</td>
</tr>
<tr>
<td>8</td>
<td>Saxagliptin in Pharmaceutical Dosage form</td>
<td>Stability indicating RP-HPLC method</td>
<td>Wavelength: 225 nm, Mobile phase: 0.1% phosphoric acid: methanol (70:30, v/v), Flow rate: 1 ml/min, Linearity range: 15.0-100.0 µg/ml, Correlation coefficients: &gt; 0.999, % Accuracy: 99.42-101.59%</td>
</tr>
<tr>
<td>9</td>
<td>Metformin hydrochloride and Saxagliptin in bulk and combined tablet dosage form</td>
<td>Stability indicating Quantitative RP-HPLC method</td>
<td>Wavelength: 211 nm, Mobile phase: Phosphate buffer: Acetonitrile: Methanol (25:50:25 % v/v/v), Flow rate: 1.0 ml/min, Linearity range: Metformin HCL 125-750 µg/ml, Saxagliptin 1.25-7.5 µg/ml, Retention time: Metformin 2.246 min, Saxagliptin 4.516 min, Correlation coefficients: 0.999, % Recovery: Metformin HCl 99.62-99.93%, Saxagliptin 99.66-99.80%</td>
</tr>
<tr>
<td>11</td>
<td>Saxagliptin in Tablet Formulations</td>
<td>Stability Indicating RP-HPLC method</td>
<td>Wavelength: 230 nm, Mobile phase: Phosphate buffer: methanol (65:35), Flow rate: 1 ml/L/min, Linearity range: 1 to 10 µg/ml, Retention time: 5.12 min, Correlation coefficients: 0.9899, LOD: 0.10 µg/ml, LOQ: 0.28 µg/ml, Intraday precision: 0.22 – 0.71%, Inter day precision: 0.28 – 0.76%, % Recovery: 99.90 – 101.03%</td>
</tr>
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<td>12</td>
<td>TEMPO in Saxagliptin monohydrate drug substance</td>
<td>GC method</td>
<td>Linearity range: 6µg/g-450 µg/g, Correlation coefficients: 0.995, LOD: 2 µg/g, LOQ: 6 µg/g, % Recovery: 89%</td>
</tr>
<tr>
<td>13</td>
<td>Saxagliptin levels and its pharmacokinetic application in presence of sucralse in animal’s serum</td>
<td>HPLC method</td>
<td>Wavelength: 230 nm, Mobile phase: Phosphate buffer: Methanol (70:30 v/v), Flow rate: 1 ml/min, Injection volume: 50 µl</td>
</tr>
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CONCLUSION
This review depicts the reported Spectroscopic and Chromatographic methods developed and validated for estimation of Dapagliflozin and Saxagliptin. According to this review it was concluded that for Dapagliflozin and Saxagliptin 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 Saxagliptin 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.

REFERENCES
14. Debata Jitendra, Sundeep Kumar, Sajal Kumar Jha and Amjad Khan. A new RP-HPLC Method Development and

Intra-day precision : CV % values range (0.14-4.03) Accuracy % range : 99.5-104
Inter-day precision:
CV % range (0.15-2.81) Accuracy % range: 99.9-116
Run time : 10 min
Correlation coefficients : 0.999


