

*Review Article*

**A Review on Analysis of Dapagliflozin.**

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**ABSTRACT**

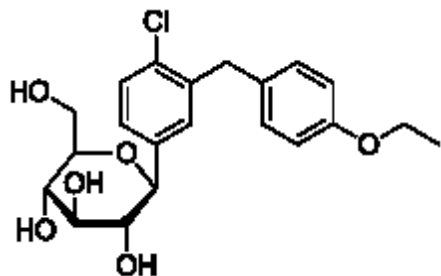
Dapagliflozin is a sodium-glucose co transporter 2 inhibitor (SGLT2) as a new class of oral anti-diabetic drugs. It is indicated for treatment of Diabetes mellitus type 2, either alone or in combination with other oral hypoglycaemic agents. The aim of this review is to focus on update of determination of Dapagliflozin in bulk and in pharmaceutical preparations using chromatographic and spectrophotometric methods. Dapagliflozin is estimated by RP-HPLC, UV, RP-UPLC, LC-MS methods. This review provides detailed information on separation conditions for Dapagliflozin alone, in the presence combination with other drugs and in presence of its degradation products.

**KEYWORDS**

Dapagliflozin, RP-HPLC, UV, RP-UPLC, LC-MS/MS.

## 1. INTRODUCTION

Dapagliflozin is a drug of gliflozin class. [1] Dapagliflozin is indicated for the management of diabetes mellitus type 2, and functions to improve glycemic control in adults when combined with diet and exercise. It is chemically known as (2S,3R,4R,5S,6R)-2-(4-Chloro-3-(4-ethoxybenzyl)phenyl)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol, molecular formula C<sub>21</sub>H<sub>25</sub>ClO<sub>6</sub> with molecular weight 408.875 g/mol.[34]



**Fig. 1:** Structure of Dapagliflozin

### 1.1. Mechanism of Action

Dapagliflozin is a selective sodium-glucose co-transporter subtype 2 (SGLT2) inhibitor with antihyperglycemic activity.

Dapagliflozin selectively and potently inhibits SGLT2 compared to SGLT1, which is the co-transporter of glucose in the gut. [2] Blocking this transporter mechanism causes blood glucose to be eliminated through the urine.[1] Using Dapagliflozin leads to heavy glycosuria (glucose excretion in the urine), which can lead to weight loss and tiredness. Dapagliflozin was approved by the FDA on Jan 08, 2014. Dapagliflozin is not recommended for patients with type 1 diabetes mellitus or for the treatment of diabetic ketoacidosis. [3,4]

### 1.3. HPLC Methods for Determination of Dapagliflozin

*Debata et al.* [3] described a new, simple, selective, accurate, rapid and precise reversed-phase high-performance liquid chromatographic technique for estimation of Dapagliflozin as per ICH Guidelines. Chromatographic separation was achieved using a Waters C18, 5  $\mu$ m particle size, 25 cm  $\times$  4.6 mm i.d. using phosphate buffer and acetonitrile in the ratio of 60:40 v/v as a mobile phase and a flow rate of 1.0 ml min<sup>-1</sup>. The eluent was monitored using UV detection at 237 nm. Total run time was 6.0 min. The retention time of Dapagliflozin was found to be 3.461 minutes. Validation of the developed method was done as per USP and ICH guidelines. Linear range of 10-60  $\mu$ g/ml with a correlation coefficient 0.9957 shows good relationship between area and concentration in calibration curve. Limit of detection were 0.02  $\mu$ g/ml and limit of quantification were 0.06  $\mu$ g/ml for Dapagliflozin. The results suggested that developed method is suitable method for assay, purity which can help in the analysis of Dapagliflozin in different formulations.

Another study by Verma et al. [4] who has develop precise, accurate and reproducible stability assay method by RP-HPLC for estimation of Dapagliflozin in API and pharmaceutical dosage form. The chromatographic condition consisted of column Agilent C18, combination of acetonitrile: di-potassium hydrogen phosphate with pH-6.5 adjusted with OPA (40:60 %v/v) as a

mobile phase with the flow rate of 1 ml/min (milliliter/minute). The effluent was detected at 222 nm (nanometer) using photo diode array detector. The retention time of Dapagliflozin API and Dapagliflozin tablet were 3.160 min (minute) and 3.067 min (minute) respectively. Linearity for Dapagliflozin was established in the range of 50-150µg/ml (microgram/milliliter) ( $R^2 = 0.99$ ) respectively. The % recoveries of Dapagliflozin API and tablet were found to be in the range of 99.00–99.99 % and 98.50–99.99 % respectively. Precision studies were carried out and the relative standard deviation values were less than two. LOD and LOQ were noted to be 5.14 µg/ml and 15.6 µg/ml for API. The method was found to be robust. The proposed method was found to be specific, accurate, precise and robust can be used for estimation of Dapagliflozin in API and Pharmaceutical dosage form.

Subrata Sarkar et al. [5] developed and validated a new simple, rapid, efficient and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of Dapagliflozin in Tablet dosage form. The separation was carried on a Symmetry C18, 25cmx 4.6mm i.d. 5µm, Particle size column. Mobile phase was mixture of Methanol: ACN: % OPA in a ratio of 75:25:05 v/v/v at a flow rate of 1.0ml/min. Dapagliflozin shows maximum absorbance at 246nm. Dapagliflozin was resolved at 2.797minutes. The Dapagliflozin drug was linear in the concentration range of 0 - 70µg/ml and correlation coefficient was 0.99. LOD & (LOQ) were found to be 0.04 & 0.12 µg/ml respectively. The developed method is also found to be simple, precise, accurate, specific, robust and rapid for the estimation of Dapagliflozin in bulk and tablet dosage form.

Manasa Sangapati et al. [6] reported an accurate, precise, specific and rapid RP-HPLC method for the determination of Dapagliflozin in API. The method was developed and validated as described in ICH guidelines. Better separation of the drug was performed by BDS column (250×4.5mm, 5µ). Mixture of ortho phosphoric acid and acetonitrile (45:55 v/v) as a mobile phase at a flow rate of 1ml/min. Eluent was monitored using PDA detector at 245nm. The developed method was validated for different parameters such as linearity, accuracy, precision, limit of detection (LOD), limit of Quantitation (LOQ), robustness. The retention time was found to be 2.963 min. Quantitative linearity was obeyed in concentration range of 25-150µg/ml with a correlation coefficient ( $r^2$ ) of 0.999. The LOD and LOQ of the method were calculated to be 0.6 and 1.8µg/ml respectively. The Precision was estimated by employing repeatability; intra-day and inter-day studies and the results were calculated as %RSD values were less than 2 % indicate method is precise. The average recovery of the analyte was found to be 99.8% which confirms the accuracy of the method. By studying all these validation parameters author concluded that the method was linear, accurate, precise, robust and rapid for the determination of Dapagliflozin in API.

Shaikh Shakirbasha et al. [7] develop and validate a simple, selective, precise, and accurate method for the estimation of Dapagliflozin using reversed-phase high-performance liquid chromatography (RP-HPLC) technique in bulk and tablet formulation. The chromatographic conditions consisted hypersil BDS (250 mm × 4.6 mm, 5 µ), column, mobile phase was mixture of buffer: acetonitrile (60:40) ratio. Instrument was set at flow rate of 1 ml/minute, column temperature was set at 30°C, and Detection wavelength was 245 nm. By injecting 5 times of the standard solution system suitability parameters were studied, and results were found well under

the acceptance criteria. The linearity study was performed by taking 25-150% levels, and the correlation coefficient value was found to be 0.999. Precision was noted to be 0.5 for repeatability and 0.31 for intermediate precision. The % recovery was found to be 99.89%. Limit of detection and limit of quantitation were found to be 0.60 µg/ml and 1.81 µg/ml, respectively. The % purity was found to be 99.71%. Degradation study on Dapagliflozin was performed and concluded that the purity threshold was more than purity angle and within the acceptable range. The author concluded that developed RP-HPLC method for Dapagliflozin was found to be simple, precise, accurate, reproducible, and cost effective. Statistical analysis of the developed method conforms that the proposed method is an appropriate and it can be useful for the routine analysis.

Bonagani Naresh et al. [8] has developed a simple, rapid, precise, accuracy, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method for estimation of the Dapagliflozin in bulk and pharmaceutical dosage form. Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size column was used for chromatographic separation of Dapagliflozin and the mobile phase containing Phosphate Buffer: Methanol in the ratio of 35:65 v/v. Eluent was eluted at flow rate of 1.0 ml/min, detection was carried out by absorption at 215 nm using a UV detector at ambient temperature. The proposed method was validated as per ICH guidelines for linearity, precision, accuracy, LOD and LOQ. A good linear response was obtained in concentration range of 0-70µg/ml form Dapagliflozin. The correlation coefficient was 0.999. Relative standard deviations of peak areas of all measurements were always less than 2.0%. Result of validation parameters indicates that the proposed method was also found to be accurate, precise, robust sensitive. The method can applied for routine quality-control analysis of these drugs in commercial tablets.

Jeyabaskaran. M, et.al. [9] Present simple, precise, accurate, reproducible and specific RP HPLC method for estimation of Dapagliflozin in bulk and pharmaceutical dosage forms. Chromatographic conditions consisted of Hypersil BDS 250mm x 4.6 mm, 5µ column. Analytes are eluted in isocratic mode with 0.1% ortho phosphoric acid buffer and acetonitrile 50:50 % v/v as mobile phase at a flow rate of 1ml/min. The injection volume was 10 µl and the total run time was set as 5min. PDA detector set at 245 nm was used for detection. The retention time for DGF was found to be 2.226 min. The proposed method shows good linearity in the range of 25 – 150 µg/ml and correlation coefficient 0.9998. Percentage recovery was found to be 100.12 %. The % RSD of intraday and inter day precision were found 0.6% and 0.29%. LOD and LOQ were 0.040, 0.121 respectively. The proposed method was found to be simple, rapid, economic and accurate and the method was applicable to routine laboratory analysis.

One rapid, precise and accurate RP-HPLC method has been reported by G.V. Mante et al. [10] for estimation of Dapagliflozin from its tablet dosage form. The method was developed and validated as per ICH guidelines. The analysis of eluents were carried on Princeton C18 column by isocratic mode. Mixture of Acetonitrile: 0.1% Triethylamine (pH-5.0) in the ratio of 50:50v/v as mobile phase pumped at flow rate of 1mL/min and detection wavelength of 224nm. Using optimized chromatographic conditions, retention time of drug was found to be 5.163min. The proposed method obeyed Beer's-lambert's law in the concentration range of 10-70µg/mL, with correlation coefficient value 0.999. The mean percent amount of drug estimated was 100.57%.

The validation parameters like accuracy, precision, ruggedness, robustness, linearity and range were studied for proposed method and were found to be within limits. Stress testing under various conditions such as pH (acid/base), oxidation, temperature, light, humidity, etc. was also carried out. From the obtained results the proposed validated HPLC methods can be used as stability indicating assay method for selected drug in their bulk and tablet formulation.

Santosh Illendula et al. [11] focused in their study on developing and validating a new, simple, fast, selective, precise and accurate RP HPLC method for the estimation of Dapagliflozin from bulk and marketed formulations. The chromatographic separation was performed on HPLC Waters ODS C 18 column, 5m, 25 cm x 4.6mm i.d.

Observation done with UV detector with an injection volume of 20 µl was injected. Mobile phase consist of Buffer (Potassium hydrogen orthophosphate & pH adjusted to 4.2 with ortho phosphoric acid) and Methanol in a ratio of 65:35. Mobile phase is pumped at a flow rate of 1.0 ml/min and detected by UV detector at 225nm. Ambient column temperature has maintained. Dapagliflozin was resolved at 2.93 min. Calibration curve display linearity in the concentration range of 20-100 µg/ml for Dapagliflozin with correlation coefficient 0.999. The proposed method was found to be precise and reproducible with %RSD of 0.42 for Dapagliflozin. Amount of the drug obtained in the range of 99.25 to 101.16% w/w for Dapagliflozin. The LOD & LOQ were found to be 0.003 & 0.009 µg/ml respectively. The method was validated according to the ICH guidelines. The results of the stress studies indicated the specificity of the method that has been developed. Dapagliflozin was stable in both oxidation & acidic stress conditions. The result shows the developed method is suitable method for assay & stability which can help in the analysis of Dapagliflozin in different formulations.

#### UV/VIS Methods for Determination of Dapagliflozin

Manasa Sangapati et al. [12] reported a simple, novel approach to develop a safe, sensitive and economic UV- Spectrophotometric method for the estimation of a Type II anti diabetic drug, Dapagliflozin. The developed method was validated as per ICH guidelines. The drug showed two different wavelengths of maximum absorption, at 203nm and 237nm. This method can be successfully applied for the estimation of Dapagliflozin in bulk for routine analysis with UV detection at 237nm. A Labindia UV-Visible spectrophotometer with 1cm matched quartz cells and ethanol solvent were employed in this method. The Developed method obeyed Beer's-Lambert's law in the concentration range of 0.5-0.9µg/ml, having correlation coefficient of 0.994. Different validation parameters like, precision (intra-day and inter-day studies), limit of detection, limit of quantitation, ruggedness and robustness were studied and were found to be within the limits.

K. Priya et al. [13] reported a unique specific, sensitive and economical UV spectroscopic method has been for the estimation of Dapagliflozin in Bulk and its pharmaceutical dosage forms. Dapagliflozin shows maximum absorption at 233.65 nm. Dapagliflozin obeyed Beer's law in the concentration range from 10-35 µg / ml. Proposed method was developed and validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values with correlation coefficient of 0.9998. The percentage recovery of Dapagliflozin was found to be 99.7 in

pharmaceutical dosage form. Results of the analysis for accuracy, precision, LOD, LOQ and were found to be satisfactory. The proposed method is simple, rapid and suitable for the routine quality control analysis.

Another study depending on UV spectroscopy has been developed by Atul Hemke et al. [14] for the estimation of Dapagliflozin and validated as per ICH guidelines. These methods includes Calibration curve, Area under curve (AUC), First and second order derivative method. Absorbance are measured at a selected wavelengths using UV-visible spectrophotometer with 1 cm matched quartz cell and methanol with water as a solvent. All developed methods obeyed Beer's-lambert's law in the concentration range of 5-40 µg/mL, with correlation coefficient value less than 1. The percent amount of drug estimated by these methods was nearly 100%, found to be in good agreement with label claim of marketed tablet formulation. The recovery study was carried out at five different levels and results were found to be satisfactory. The results of estimation and validation parameters like accuracy, precision, ruggedness, linearity and range were studied for all the developed methods and were found to be within limits. The proposed method can be adopted for routine quality control for estimation of drug in formulation.

#### Determination of Dapagliflozin in Combination with Other Drugs

Useni Reddy Mallu et al. [15] has developed and validated a simple, precise and stability-indicating Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method for simultaneous quantification of Dapagliflozin and Saxagliptin in combined dosage form. The chromatographic separation was carried out on Intersil ODS C18 column (250 mm × 4.6 mm × 5 µ) with ammonium dihydrogen phosphate buffer (pH 6.8) and methanol in a ratio of 65:35 v/v as mobile phase at a flow rate of 1.5 ml/min. It was detected by UV detector at with correlation coefficient values of 0.9992 and 0.999 for Dapagliflozin and Saxagliptin. The percent recoveries of two drugs found within the limits of (98.00-102.0%). The Limit of Quantification (LOQ) concentrations of Dapagliflozin and Saxagliptin are 0.312 µg/ml and 0.156 µg/ml respectively. The Limit of Detection (LOD) concentrations of Dapagliflozin and Saxagliptin are 0.156 µg/ml and 0.078 µg/ml respectively. According to International Conference on Harmonization (ICH) guidelines forced degradation study has been conducted at standard concentration. Author concluded that this method can be used for the estimation of Dapagliflozin and Saxagliptin in bulk drug and in a formulation.

N. Singh et al. [16] focused in their study on developing simple and precise stability indicating method for the simultaneous estimation of Dapagliflozin and Saxagliptin in combined tablet dosage using RP-HPLC. The chromatographic separation of the drugs was performed with an Xterra C-18 analytical column (150 mm × 4.6 mm i.d., particle size 3.5 µ) using buffer and acetonitrile (53:47 v/v) as the mobile phase. The buffer used in mobile phase contained 20 mm sodium dihydrogen phosphate and its pH was adjusted to 5.5 ± 0.02 with ortho phosphoric acid. The instrument was set at a flow rate of 1.2 mL min<sup>-1</sup> at ambient temperature and the wavelength of the UV-visible detector was 230 nm. The excellent linearity was achieved over a range of 2–14 µg mL<sup>-1</sup> for all the drugs. The correlation coefficients for Dapagliflozin and Saxagliptin were found to be 0.997 and 0.996, respectively. The mean recovery values were found to be 99.16% and 100.58%. The proposed method can be used for quantitative determination of these drugs in pharmaceutical preparations and also for quality control in bulk

manufacturing. Stress testing, which covered acid, base, peroxide, photolytic and thermal degradation, was performed on each test to prove the specificity of the method and that the degradation was achieved. No interferences were observed from the stress degradation products. The F-test and t-test at 95% confidence level were applied to the data for statistical analysis.

One study for development and validation of a new stability indicating RP HPLC method for simultaneous estimation of Saxagliptin and Dapagliflozin in bulk and dosage forms has been reported by K.P.R.Chowdary et al. [17] The separation was performed by XTerra C 18 column (150mm x 4.6mm x5 $\mu$ m particle size) with UV detector and mobile phase consisted of phosphate buffer (pH 4) and Acetonitrile (50:50v/v). The flow rate was 1ml/min and detection achieved at 225nm. Retention time was 2.1min (Saxagliptin), 2.8min (Dapagliflozin). The proposed method was validated according to the ICH guidelines. Linearity was established in the range of 20-60ug/ml (Saxagliptin), 40-120ug/ml (Dapagliflozin) with correlation coefficient 0.999 and 0.998 respectively. Percentage recovery was found to be 99.99-100.50% for both drugs. Precision was 0.78% and 0.44% for Saxagliptin and Dapagliflozin. LOD and LOQ are 1.63ug/ml and 5.39ug/ml for Saxagliptin, 1.94ug/ml and 6.50ug/ml for Dapagliflozin. From the results it is concluded that developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust.

A simple and precise stability indicating reversed-phase high-performance liquid chromatography method was developed and validated by Y. Mohammad et al. [18] for the simultaneous determination of Metformin hydrochloride and Dapagliflozin in bulk and pharmaceutical dosage form. Chromatography was carried out on hypersil BDS C18 (250 mm x 4.6 mm, 5  $\mu$  particle size) column. Mobile phase composed of buffer (0.1% ortho phosphoric acid) adjusted to pH 6.8 with triethylamine: acetonitrile in the ratio of 50:50%/v/v at a flow rate of 1 ml/minutes. The analyte was monitored using photodiode array detector at 240 nm. The retention time was found to be 2.791 minutes and 3.789 minutes for Metformin hydrochloride and Dapagliflozin respectively. Linearity was established in the concentration range of 85-510  $\mu$ g/ml for Metformin ( $r^2=0.99995$ ) and 0.5-3.0  $\mu$ g/ml for Dapagliflozin ( $r^2=0.99978$ ), respectively. The mean % recoveries obtained were found to be 99.66-100.23% for Metformin and 99.61-100.38% for Dapagliflozin respectively. Stress testing which covered acid, base, peroxide, photolytic and thermal degradation was performed on under test to prove the specificity of the method and the degradation was achieved. The developed method has been statistically validated according to ICH guidelines. Thus, the proposed method can be successfully applied for the stability indicating the simultaneous determination of Metformin hydrochloride and Dapagliflozin in bulk and combined tablet dosage form and in the routine quality control analysis.

A simple, specific, accurate, precise and reproducible and robust method have been developed and validated by Khyati J. Patel et al. [19] for the Simultaneous estimation of Dapagliflozin and Metformin HCl in RP-HPLC Method. The chromatographic separation of Dapagliflozin and Metformin HCl was carried out using Inertsil ODS C18 column (250mm x 4.6 mm, 5 $\mu$ ) as stationary phase. Combination of 0.05M Potassium Dihydrogen ortho Phosphate buffer (pH- 3.5, adjusted with 0.1% orthophosphoric acid) and Acetonitrile in the ratio of 50:50% v/v used as mobile phase. The detection was observed by UV detector set at 227 nm and flow rate was 1.0

ml/min. The method is linear over concentration range of 5-15 and 25-75 $\mu$ g/ml for Dapagliflozin and Metformin HCl respectively. The retention time observed for Dapagliflozin and Metformin HCl were 2.633 min and 5.620 respectively. The % recoveries of Dapagliflozin and Metformin HCl were found within range of 100.40%-101.27%, 100.18%-100.63% respectively. Method was statistically validated for accuracy, precision, specificity, LOD, LOQ and robustness according to ICH guidelines. LOD and LOQ for Dapagliflozin were noted to be 0.28 and 0.85 respectively. LOD and LOQ for Metformin HCl were noted to be 0.78 and 2.37 respectively. Author concluded that the method was successfully applied for the estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture.

Patel P. D. et al. [20] focused in their study on developing and validating a new, precise, rapid, accurate RP – HPLC method for simultaneous estimation of Dapagliflozin and Saxagliptin Hydrochloride in bulk and in tablet dosage form. The chromatography was carried out on a Hypersil BDS C18 (250 mm  $\times$  4.6 mm) 5 $\mu$ m column with an isocratic mobile phase composed of Phosphate buffer (pH 4.5): Methanol in the ratio of 85:15 v/v at a flow rate of 1 ml/min and detection wavelength of 222 nm. The retention time of Dapagliflozin and Saxagliptin HCl found to be 4.080 min and 5.343 min. Calibration curve displayed excellent linearity over the concentration range of 10 – 30  $\mu$ g/ml for Dapagliflozin and 5 – 15 for Saxagliptin HCl with correlation coefficient 0.998 for Dapagliflozin and 0.993 for Saxagliptin HCl. The limit of detection (LOD) and limit of quantification (LOQ) of Dapagliflozin were found to be 1.16  $\mu$ g/ml and 3.52  $\mu$ g/ml, while those of Saxagliptin HCl were found to be 0.53  $\mu$ g/ml and 1.62  $\mu$ g/ml. From the results we can conclude method was found to be rapid, sensitive, linear, specific, accurate, precise and economic for estimation of Dapagliflozin and Saxagliptin HCl in marketed tablet dosage form without interference of excipients and impurities.

One study for simultaneous estimation of Dapagliflozin and Saxagliptin in combined tablet formulations has been reported by Sarath Nalla et al. [21] who has developed and validated a rapid and sensitive stability indicating RP-HPLC method. Chromatography was performed a Kromasil C18 HPLC Column (250 x 4.6 mm; 5m; 30°C). Elution done with a mobile phase consisting of a 50:50 v/v mixture of acetonitrile and 0.1 % orthophosphoric acid in water at a flow rate of 1.0 ml/ min. The detection wavelength was set at 220 nm. Accuracy was assessed by using standard addition method. The developed HPLC method was validated with respect to precision, specificity, accuracy, linearity and robustness. Forced degradation studies on the formulation were conducted by adopting the proposed method to assess the stability of the analytes under acid, base, peroxide, thermal and photolytic conditions and suitability of the method to resolve the degradation products.

Afshan Urooj et al. [22] has developed a simple, accurate and precise reverse phase high performance liquid chromatography method for simultaneous estimation of Metformin and Dapagliflozin in bulk and synthetic mixture. The column used for separation was waters (Model: Alliance 2695) with Phenomenex Luna C18 (4.6mm I.D.  $\times$  n 5 $\mu$ m). It contains waters injector and PDA Detector (Deuterium). Mobile phase consists of Acetonitrile: Water (75:25% v/v) and flow rate adjusted was 1ml/min. Analytes were monitored using PDA detector at 285nm and injection volume was 10  $\mu$ l. The developed method was fully validated for the parameters as per ICH guidelines. By using the developed method, retention time of Metformin and Dapagliflozin



was found to be 3.2min and 5.4min respectively. The proposed method has permitted quantification of Metformin and Dapagliflozin over linearity in the range of 20–100µg/ml and 10–50µg/ml respectively. The percentage recoveries obtained for Metformin and Dapagliflozin were found to be in range of 99.3 – 99.6%. LOD and LOQ were found to be 5.0µg/ml and 15.2µg/ml for Metformin 3.7and 11.4µg/ml for Dapagliflozin. Rapidity, usage of simple mobile phase and easy sample preparation steps shows method is simple. Results of validation parameters demonstrated that the analytical procedures is suitable for its intended purpose and meets the criteria defined in ICH Q2R1

The first reversed phase high performance liquid chromatographic method for simultaneous determination of Empagliflozin, Canagliflozin, Dapagliflozin and Metformin has been developed and validated by Ismail Salama et al. [23] to be a simple, sensitive, rapid, specific, precise, and accurate method. Chromatographic separation was performed with C18 column (250×4.6 mm-5µm p.s) Inertsil ODS with isocratic mobile phase composed of acetonitrile and 0.05 M potassium dihydrogen phosphate buffer Ph 4 in a ratio [65:35, v/v] at flow rate of 1ml/min. It was detected by UV detector at 212 nm and injection volume was 10 µl. Retention time for Canagliflozin, Dapagliflozin, Empagliflozin and Metformin was found to be 4.414, 3.560, 3.004, and 1.898. Linearity was obeyed for Canagliflozin, Dapagliflozin, Empagliflozin and Metformin in concentration range of 7.5-225, 5-150, 6.5-187.5 and 10-1000 µg/ml, respectively; with correlation coefficient 1 for all drugs. LOD was found to be 2.1, 0.7, 1.4, and 3.2 for Canagliflozin, Dapagliflozin, Empagliflozin and Metformin respectively. LOQ was found to be 6.5, 2.2 2.3, 9.6 for Canagliflozin, Dapagliflozin, Empagliflozin and Metformin respectively. The proposed method was successfully applied for determination of the four drugs in laboratory prepared mixtures and in the seven pharmaceutical dosage forms and so it is suitable for quality control of them.

Shyamala et al. [24] described a new, precise, rapid, accurate RP-HPLC method for the Simultaneous Estimation of Dapagliflozin and Metformin HCL in tablet dosage form. After optimization the good chromatographic separation was achieved by BDS (250 x 4.6 mm, packed with 5 micron) column. Analytes are eluted with Isocratic mode with a mixture of Phosphate Buffer (pH 6.5): methanol: acetonitrile in the ratio of 50:30:20 v/v/v as the mobile phase at flow rate of 1 mL/min and detection wavelength of 240 nm. The retention time of Metformin HCl and Dapagliflozin found to be 2.475min and 3.647min respectively. The linearity was obeyed in the concentration range of 85 µg/mL to 510 µg/mL for Metformin HCL and 0.5 µg/mL to 3µg/mL for Dapagliflozin. The correlation coefficient R<sup>2</sup> value is found to be 0.997 for Metformin HCL and 0.9973 for Dapagliflozin. The LOD and LOQ for metformin HCL were found to be 2.469 ppm and 2.468ppm respectively. The LOD and LOQ for Dapagliflozin were found to be 3.650ppm and 3.649ppm respectively. Percentage recovery metformin HCL and Dapagliflozin were found to be 100.67 and 99.54 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage form. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, specificity and Robustness. Method was found to be simple, sensitive, accurate, and precise.

M.D. Dhanaraju et al. [25] describe a simple, precise and stability indicating HPLC method for the simultaneous determination of Metformin hydrochloride and Dapagliflozin in pharmaceutical dosage forms. The separation was performed by an Inspire (4.6 x 150mm, 5 $\mu$ m) column with isocratic flow. The mobile phase at a flow rate of 1.0mL/min composed of Acetonitrile and 0.1M orthophosphoric acid buffer (70:30, v/v). The UV detection was carried out at 260nm. The retention times for Metformin and Dapagliflozin were found to be 2.097min and 3.691min, respectively. Parameters such as linearity, precision, accuracy, specificity and ruggedness are studied as reported in the International Conference on Harmonization guidelines. A linear response was obtained over the concentration range of 5-25 $\mu$ g/mL for Dapagliflozin and 500-2500 $\mu$ g/mL for Metformin respectively. Limit of detection and limit of quantification for Dapagliflozin were 2.98 and 9.95 $\mu$ g/mL and for Metformin were 3.05 $\mu$ g/mL and 10.07 $\mu$ g/mL respectively. Individual drugs (Metformin and Dapagliflozin) were exposed to thermal, photolytic, hydrolytic and oxidative stress conditions. The resultant stressed samples were analyzed by the proposed method. The method gave high resolution among the degradation products and the analytes. The analysis concluded that the method was selective for simultaneous estimation of Metformin and Dapagliflozin which will help to improve quality control and contribute to stability studies of pharmaceutical tablets containing these drugs.

B. Hari Babu et al. [26] reported developed and validated a stability indicating reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous quantitative analysis of Dapagliflozin and Metformin in bulk and tablets. Dapagliflozin, Metformin and stress degradation products were separated on the Supelco C8, 250 mm x 4.6 mm, 5  $\mu$ m analytical column by using 0.1 M dipotassium hydrogen phosphate, acetonitrile and methanol (60:30:10 v/v/v), as a mobile phase pumped at 1.2 mL/min flow rate. The column temperature was set 30°C and 10  $\mu$ L of the samples were injected. Detection was done by PDA detector at 285 nm. According to ICH guidelines, the developed RP-HPLC method was validated. The retention times of Dapagliflozin and metformin were 2.847 min and 3.804 min, respectively. The proposed method shows linearity in the concentration range of 2-6  $\mu$ g/mL ( $R^2 = 0.9999$ ) for Dapagliflozin and 200-600  $\mu$ g/mL ( $R^2 = 0.9998$ ) for metformin. The LOD for Dapagliflozin and metformin were found to be 0.004  $\mu$ g/mL and 0.272  $\mu$ g/mL, respectively. The percent recovery and percent relative standard deviation for the selected drugs were in the range of 99.00%-99.82% and 0.098%-0.291%, respectively. Non - interference of peaks from stress degradation products in acidic, alkaline, oxidative, thermal and photolytic conditions demonstrated the stability indicating power of the method. The proposed RP-HPLC method can be used as a stability indicating method and can be used for assay of Dapagliflozin and metformin simultaneously in bulk and tablet dosage form.

Ashish Patel et al. [27] has developed and validated an accurate, precise and reproducible UV-spectrophotometric methods and liquid chromatographic assay method for the determination of Dapagliflozin propanediol and Glimepiride in synthetic mixture. Spectrophotometric estimation was done by derivative spectroscopic method and methanol as solvent. In this method  $\lambda_{max}$  for Dapagliflozin propanediol and Glimepiride were selected at 288 nm and 224nm. RP-HPLC analysis of samples was carried out using Pearless C-18 column (4.6 x 250mm, 5 $\mu$  particle size) Mobile phase containing Acetonitrile : 10% Ortho-phosphoric acid in water pH 6.0 (70:30%

v/v) at a flow rate of 1.0 ml/min and chromatogram was recorded at 228 nm. Linearity was observed over the concentration range of 5 -30 µg/ml and 5-30 µg/ml for Dapagliflozin propanediol and Glimepiride respectively in UV spectrophotometric. In RP-HPLC method linearity was evaluated over the concentration range of 1 -5 µg/ml and 1-5 µg/ml for Dapagliflozin propanediol and Glimepiride respectively. Correlation coefficient was found to be 0.9978 and 0.995 found were by UV method for Dapagliflozin and Glimepiride and 0.997 and 0.996 found were by RP-HPLC method for Dapagliflozin and Glimepiride respectively. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Hence from the results it is concluded that the both methods can be used for routine monitoring of Dapagliflozin and Glimepiride in the assay of synthetic mixture of both drug.

Gandla Kumara Swamy et al. [28] has described a simple, rapid, precise, accurate and robust stability-indicating RP-HPLC method to estimate Saxagliptin hydrochloride and Dapagliflozin in bulk and in tablet form. The samples were isocratically eluted using a C18 (250 cm x 4.6cm\*5µ) Primesil ODS column. Other chromatographic conditions including mobile phase Potassium dihydrogen phosphate Buffer (pH 6.0): Acetonitrile (45:55 v/v) and spectrum was run at wavelength 247 nm. A proposed method permitted quantification of analytes over linearity range from 5-30 µg/mL for Saxagliptin hydrochloride and 10-60 µg/mL for Dapagliflozin. LOD and LOQ were noted to be 0.76 and 1.57 for Dapagliflozin and 1.3, 3.96 for Saxagliptin respectively. The method was quantitatively evaluated in terms of linearity, precision, accuracy (recovery), selectivity and robustness as per ICH guideline. Forced degradation conditions of hydrolysis (neutral, acidic and alkaline), oxidation, photolysis and thermal stress, as suggested in the ICH guideline Q1A (R2) were estimated. It can successfully applied for estimation of Saxagliptin and Dapagliflozin in its pharmaceutical dosage form.

Sherif A. Abdel- Gawad et al. [29] developed a reversed phase high-performance liquid chromatographic (RP-HPLC) method and validated as a stability indicating method for the simultaneous quantification of Dapagliflozin and Metformin hydrochloride in presence of their degradation products. The degradation process was carried out under acidic, basic, oxidative and thermal conditions, as recommended by the International Conference on Harmonization (ICH)-guidelines. Validation parameters such as linearity, accuracy, precision, specificity, limits of detection and quantification (LOD & LOQ) were determined. The best chromatographic separation pattern was achieved using Hypersil TM ODS C18 column (150 x 4.6 mm, 5 µm) as a stationary phase. Eluents are eluted by isocratic mode in 0.05 M potassium dihydrogen phosphate buffer (adjusted to pH 4.6 using orthophosphoric acid): acetonitrile: methanol (5:4:1, by volumes), as a mobile phase. It was pumped with flow rate of 0.5mL/min. and UV detection at 236 nm. The calibration graph was linear in the range of 0.5 -20 µg/mL, for Dapagliflozin and 50 – 550 µg/mL, for Metformin. The proposed method is accurate, sensitive and precise, so it can be successfully adopted for the simultaneous determination of Dapagliflozin and Metformin in either their bulk or tablet forms.

A simple and more economic isocratic stability-indicating RP-HPLC method was developed and subsequently validated by G. Veerabhadram et al. [30] for the simultaneous determination of Metformin and Dapagliflozin in bulk and pharmaceutical dosage form. The chromatographic

conditions consisted of a Themosil C18 HPLC column with 150mm in length and internal diameter of 4.6mm with size 5 $\mu$ m. The analyte detection was carried out by using a PDA detector set at a wavelength of 234 nm. Combination of 0.1M orthophosphoric acid: acetonitrile: methanol (35:40:25v/v/v) used as mobile phase. The retention time was found to be 2.102 minutes and 4.105 minutes for Metformin and Dapagliflozin respectively. The calibration curves of two drugs shows good linearity between peak area and concentration with correlation coefficients of 0.999 and 0.999 over a concentration range of 0.5-2.5 $\mu$ g/ml for Metformin and 5-25 $\mu$ g/ml for Dapagliflozin. Metformin and Dapagliflozin were subjected to different degradation stress conditions. The degradation products were well resolved from that of pure standard drugs (Metformin and Dapagliflozin) with significant different retention time values. The current method has been statistically validated according to the ICH guidelines and this method has been subsequently developed and applied successfully to determine the levels of Metformin and Dapagliflozin in a combined formulation and in the routine quality control. The developed RP-HPLC method is stability indicating provides simple, economical, accurate, precise quantitative analysis for simultaneous determination of Metformin and Dapagliflozin in bulk and pharmaceutical dosage form.

#### *1.4. RP-UPLC Method for Determination of Dapagliflozin*

S. Madhavi et al. [31] has described a rapid, an accurate and precise Ultra Performance Liquid Chromatography (UPLC) method for simultaneous estimation of Saxagliptin and Dapagliflozin in its tablet dosage form (10mg Dapagliflozin and 5mg Saxagliptin). The UPLC method was developed using 2.1  $\times$  100 mm, reverse phase C18 column (Acquity UPLC ethylene bridge hybrid (BEH) C18 1.7  $\mu$ m). Analysis done in mobile phases containing 0.1% ortho phosphoric acid and acetonitrile (40:60) as mobile phase. Instrument was set at flow rate of 0.3 ml/min with PDA detection at ( $\lambda_{max}$ ) 254 nm and the injection volume was set at 1  $\mu$ l with run time 3 min. The method was validated by using various validation parameters like accuracy, precision, linearity and robustness. The calibration curves plotted for Saxagliptin and Dapagliflozin were linear over the concentration range of 12.5-75  $\mu$ g/ml for Saxagliptin, 25-150  $\mu$ g/ml for Dapagliflozin. Percent recovery of Saxagliptin ranged from 99.62% to 100.94% and for Dapagliflozin 98.71% to 101.28%. LOD for Saxagliptin and Dapagliflozin was found to be 0.13  $\mu$ g/ml & 0.53  $\mu$ g/ml respectively. LOQ for Saxagliptin and Dapagliflozin was found to be 0.38  $\mu$ g/ml & 1.59  $\mu$ g/ml respectively. Method can applied as a quality control tool for analysis of the drug in its tablet dosage forms in pharmaceutical industries.

#### *1.5. LC-MS/MS Methods or Determination of Dapagliflozin*

Qin C. et al. [32] reported a liquid chromatography–tandem mass spectrometry (LC–MS/MS) bioanalytical assay of Dapagliflozin in human plasma. A lower limit of quantitation (LLOQ) at 0.2 ng/mL with 50  $\mu$ L of plasma was obtained, which reflects a 5-fold improvement of the overall assay sensitivity in comparison to the previous most sensitive assay using the same mass spectrometry instrumentation. In this new assay, acetate adduct ions in negative electrospray ionization mode were used as the precursor ions for selective reaction monitoring (SRM) detection. Sample preparation procedures and LC conditions were further developed to enhance the column life span and achieve the separation of Dapagliflozin from potential interferences, especially its epimers. The assay also quantifies Dapagliflozin's major systemic circulating

glucuronide metabolite, BMS-801576, concentrations in human plasma. The assay was successfully transferred to contract research organizations (CROs), validated, and implemented for the sample analysis of pediatric and other critical clinical studies. This assay can be widely used for bioanalytical support of future clinical studies for the newly approved drug Farxiga or any combination therapy containing Dapagliflozin.

One of the LC–MS/MS method reported by Anne-Francoise et al. [33] in negative ion electrospray ionization mode. Because Dapagliflozin readily forms adducts in the presence of formic acid, the mobile phases were simple mixtures of water and acetonitrile. The assay was validated in the concentration range of 5–2000 ng/ml with good intra- and inter-day precisions and acceptable sample stability. From the observations it is concluded that validated assay was successfully applied to the quantitation of Dapagliflozin in plasma in support of preclinical studies in both normal and diabetic rats.

## **2. CONCLUSION**

Various methods for determination of Dapagliflozin have been reported. Some RP- HPLC assay methods were used to estimate Dapagliflozin. Some articles provides determination of Dapagliflozin alone or in combination with Metformin, Saxagliptin in pharmaceutical dosage forms. UV methods are also reported. Research papers on UPLC, LC-MS, and LC-MS/MS are also reported. Bioanalytical methods are also reported in which Dapagliflozin is determined in human and rat plasma.

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