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Review Article

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Review on Analytical Methods of Dapagliflozin combinations

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Abstract Dapagliflozin is an oral hypoglycemic agent. It is a medication used to treat type 2 diabetes. It is a selective sodium-glucose co-transporter subtype 2 (SGLT2) inhibitor that inhibits glucose reabsorption and induces glucosuria. Dapagliflozin is used with pioglitazone, saxagliptin, metformin, sitagliptin, and glimepiride.
Considering the therapeutic significance of dapagliflozin, the reliability of its application is dependent on bioanalytical data; therefore, in this review, we focus on critical compilation and the evaluation of analytical methods for the analysis of dapagliflozin and its available combinations.
This review focuses on previously reported analytical techniques for determining dapagliflozin in biological samples and pharmaceutical formulations using a combination of sitagliptin, saxagliptin, metformin, pioglitazone, and glimepiride. Therefore, this attempt of critical compilation of data will help to design proper analytical methods for the analysis of dapagliflozin in combination with its dosage form and biological fluid.
The current review article may be successfully used to perform future analytical research for dapagliflozin's estimate.

Keywords Dapagliflozin, Analytical methods , Diabetes mellitus type 2

1. Introduction

Diabetes currently affects 463 million people worldwide and is expected to reach 700 million people by 2045 [1]. Diabetes mellitus is complex and chronic disease that necessarily involves ongoing medical attention along with multifactorial risk reduction strategies in addition to glycemic control [2].
DAPA is approved for used in treatment of diabetes type 2 as an addition to diet and exercise [3]. The FDA authorized dapagliflozin to be used in the treatment of diabetes type 2 in January 2014. Since 2012, this chemical has been authorized and is in use across Europe. It is available as 10 mg tablets that are administered orally once a day with or without meals [4].
DAPA is a competitive, reversible, and SGLT2 inhibitor with excellent selectivity; inhibition of SGLT2 decreases glucose reabsorption and induces glucosuria [5].
Sodium glucose cotransporter 2 inhibitor are insulin-independent oral antihyperglycemic drug used by physicians to treat type-2 diabetes [3].

The cotransporter SGLT2 is responsible for renal glucose reuptake; inhibiting the cotransporter enables higher renal glucose excretion, which results in lower plasma glucose level [6].
DAPA, as an SGLT2 inhibitor, causes prolonged dose-dependent glucosuria in healthy persons without lowering blood glucose [7].

2. Structure

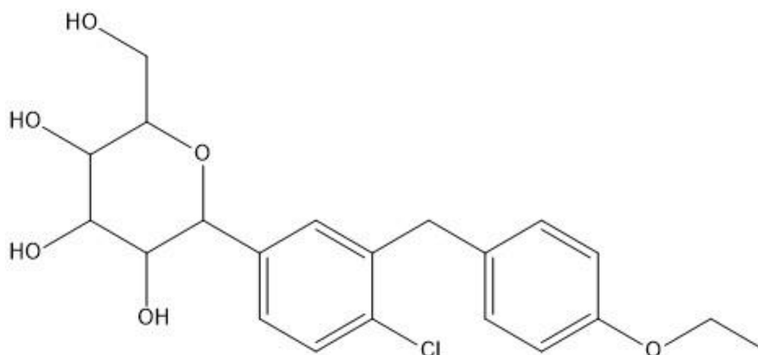

Figure 1: Structure of Dapagliflozin

3. Physiochemical properties
chemically (1 s)-1, 5-anhydro-1-C-[4-chloro-3-[(4- ethoxyphenyl) methyl]-D-glucite [8].
The molecular structure that was rationally constructed with a C-linked glucoside, such that the aglycone component is bound to glucose by a carbon-carbon bond, conferring metabolic stability against glucosidase enzymes. The molecular weight is 408.87 g/mol and the molecular formula $C_{21}H_{25}ClO_6$ [9].
DAPA is white crystalline powder that is soluble in ethanol, methanol, dimethyl-sulfoxide, and dimethyl-formamide.
European Medicines Agency reports (EMA), dapagliflozin is classified as category III in the Biopharmaceutical Classification System (BCS), which indicates it is high solubility and low permeability [8].
DAPA melting point is 65°C and should be stored in hermetically sealed containers at temperatures below 20 ◦C [10].
Dapagliflozin in combination with saxagliptin, metformin, pioglitazone glimepiride or sitagliptin [11].

4. Mechanism of Action
The sodium-glucose co-transporter-2 is produced by epithelial cells lining in proximal convoluted tubule's first portion, which reabsorbed 90% of glucose which is filtered and just 1% of filter glucose reached the urine. The remaining 10% reabsorbed in the nephron through sodium-glucose co-transporter-1. The luminal surface of the proximal tubular epithelium is the beginning point of the glucose reabsorption cycle, and sodium-glucose co-transporter-2 plays a critical role in transferring glucose from the glomerular filtrate to the epithelial cells [12].
DAPA reduces the reabsorption of glucose into the blood by inhibiting SGLT2 [6].
It suppresses the transporter protein in the kidneys, reducing renal reabsorption and increasing urine glucose excretion, lowering blood glucose [10].

5. Pharmacokinetics
DAPA is easily and thoroughly absorbed after oral treatment. The plasma concentration reaches their peak within two hours of treatment. Bioavailability of the 10 mg once-daily dosage is 78%. It can be taken with or without food. It is 91% protein-bound and unaffected by hepatic or renal impairment [12].
Uridine diphosphate glucuronosyltransferase-1A9 metabolites it in the kidney [13] and liver for major metabolism and cytochrome 450-mediated for minor metabolism [6].

UGT1A9 predominantly metabolites dapagliflozin to dapagliflozin 3-O-glucuronide, which is an inactive form of a metabolite [5].

By suppressing SGLT2 in the PCT, dapagliflozin causes urine glucose excretion, which decreases blood glucose [12].

The pharmacokinetics were studied in the presence of two possible UGT1A9 pathway modulators: rifampicin (an inducer) and mefenamic acid (an inhibitor). Rifampin coadministration causes a substantial dose reduction of dapagliflozin (an AUC reduction of 22%). Coadministration of mefenamic acid causes a substantial dose increase for dapagliflozin (an AUC increase of 51%) [14].

DAPA causes consistent rates of glucosuria in healthy volunteers and diabetes type 2 patients, equivalent to 70 g of glucose excreted daily [14].

DAPA is strongly protein-bound, with just about 2% of the parent molecule eliminated in urine. Likewise, fewer than 70% of metabolites are discharged by kidney. The half-life of approximately 12–13 hours at a dosage of 10 mg, making it suitable for once-daily administration [5].

About 9% of the dosage was oxidatively metabolized, with approximately 29% of these metabolites also being glucuronidated [9]. DAPA treatment results in increased renal glucose excretion [15].

6. Pharmacodynamics

DAPA is an SGLT2 transporter inhibitor that is both reversible and selective. SGLT2 Proteins are found in proximal convoluted tubule (PCT) of the kidneys, where they are Consider sodium and glucose reabsorption from the glomerular filtrate [16].

The major pharmacodynamic parameter impacted by dapagliflozin is glucosuria [5]. It works by reabsorbing glucose from liver, resulting in increased glucose excretion in urine and so improving glycemic control in people with type II diabetes [8].

Inhibiting renal SGLT2 by reducing filtered glucose reabsorption reduces the physiological renal glucose threshold, increasing glucose excretion [10].

7. Physiological activity

Patients using dapagliflozin had a lower total body weight [14]. Weight loss has been consistently reported in all existing SGLT2 inhibitor Phase III studies [17].

DAPA, alone or in conjunction with other anti-diabetic drugs, effectively reduced blood pressure [18]. The reduction in blood pressure due to a diuretic effect caused by increased sodium excretion during SGLT2 blockage [19].

Patients taking SGLT2 inhibitors have significantly lower systolic blood pressure. Dapagliflozin reduces systolic blood pressure by 2-9 mmHg without increasing heart rate or syncopal episodes [17].

DAPA Lower systolic blood pressure by acting as an osmotic diuretic in people taking antihypertensive medications [6].

There is additional evidence that SGLT2 inhibitors increase beta cell activity, resulting in long-term HbA1c decreases when compared to other oral diabetes medications [16]. Lowering plasma glucose concentrations using dapagliflozin enhances β cell performance significantly, giving substantial evidence in humans for the glucotoxic effect of hyperglycemia on β cell function [13].

The Serum uric acid levels are significantly reduced while using SGLT2 inhibitors [8].

SGLT2 inhibitors significantly reduce risk of mortality, adverse cardiac events, non-fatal myocardial infarction, and heart failure in patient with type 2 diabetes [20].

8. Analytical Method

A thorough literature review revealed that various analytical approaches for identifying and quantifying pharmaceuticals and other compounds of interest in Pharmaceutical Matrices have been discovered. More analytical approaches for determining Dapagliflozin in biological fluids and pharmaceutical formulations were developed.

Methods such as high-performance liquid chromatography, liquid chromatography-electrosprayionization-tandem mass spectrometry, and UV-Visible spectrophotometric methods of dapagliflozin estimation in combination with others such as pioglitazone, metformin, glimepiride, saxagliptin, and sitagliptin have been reported [21].

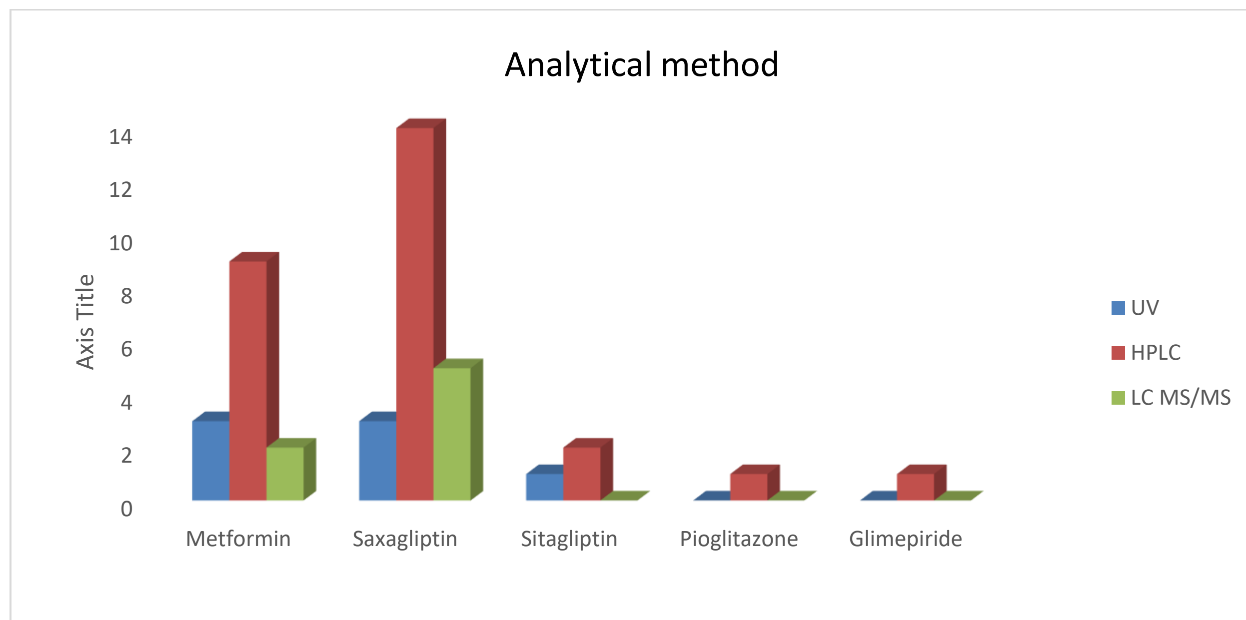

Figure 2: Dapagliflozin with combination for different analytical methods

8.1 HPCL Method

In the late 1960s and early 1970s, high-performance liquid chromatography (HPLC) was invented. (22)

A HPLC system is comprised of five components: pump, sample injection, column, detector and data processor. The sample and solvents delivered by the pump, after passing through column sample transported to the detector. An autosampler (automatic) and an injector (manual) are commonly used for sample injection.

The column is the heart of an HPLC system. Column having great effect on resolution. (C3) Propyl, (C4) Butyl, and (C5) Pentyl column are useful for ion pairing chromatography and peptides containing hydrophobic residues, and other large molecules. Non-polar solute retains less in C3-C5 columns than C8 and C18 column. Longer alkyl chain column has more hydrolysis resistance than this column. Octyl (C8, MOS) column have a wide range of application but less effective than C18 column, this column is useful for pharmaceuticals, steroids and nucleosides. Small diameter porous particles size use for column packing, the most common sizes are 5 µm, 3.5 µm and 1.8 µm

The stationary phase can be supported by a variety of matrices, including silica, alumina, polymer and zirconium. The most common matrix for HPLC columns is silica.

The detector used to detect the separated components, and depending on the target sample, a variety of detectors can be used. A highly sensitive fluorescence detector or a UV/Vis detector can be used to detect only a few specific components. A PDA detector, a differential refractive-index and an evaporative light-scattering detector is more suitable for detecting components. On a computer, the data processor displays and analyses the detected signal.

Most compounds are separated using one of four major separation modes: Reversed phase chromatography, Normal phase chromatography, Size exclusion chromatograph and Ion exchange chromatography.

The goal of the method is to quantify and separate drug as well as degradant, synthetic intermediates, and any impurities. HPLC primary and essential analytical tool used in preformulation, drug discovery, development, and production in the modern pharmaceutical industry. Because of its advantages such as rapidity, accuracy, specificity, precision, robustness, the HPLC method can be used for analysis of the majority of drugs in dosage

forms. Hplc used for trace analysis in component for toxic chemicals, high purity product, impurities, and research purposes.

Raju et al. It was created a simple, fast, and exact approach for estimating saxagliptin and dapagliflozin in tablet dosage form. BDS C8 column (50 x 4.6 mm, 5u) was used to conduct the chromatogram. The mobile phase included a 55:45 ratio of potassium dihydrogen phosphate to acetonitrile, and the pH was adjusted to 3.8 using diluted orthophosphoric acid. flow rate was 1 ml/min. The column temperature was kept constant at 30 °C. The selected wavelength was 210 nm. Dapagliflozin and saxagliptin had retention times of 2.266 and 2.805 minutes, respectively. The LOD and LOQ value for dapagliflozin was found 0.20 ,0.26 and for saxagliptin was found 0.60, 0.79, respectively. Dapagliflozin 25-150 ug/ml and Saxagliptin 12.5-75 ug/ml linearity ranges were chosen.(23)

M. Patel et al. the RP-HPLC technique was created using an HPLC machine and a PDA detector. The separation was using C18 Inertsil ODS (150 x 4.6 mm, 5 u). The mobile phase was phosphate buffer: acetonitrile (55:45), pH 4.0 adjusted with glacial acetic acid. column with a flow rate of 0.8 mL/min and a detection wavelength of 220 nm. The column temperature was held at 25 °C. DAPA and SAXA retention times were determined to be 3.156 and 2.144 minutes, respectively. The approach has been verified for SAXA and DAPA at concentrations ranging from 8 to 22 ug/ml and 16 to 44 ug/ml, respectively [24]

Gurrala et al. This approach uses an acetonitrile: phosphate buffer having pH of 5.8 (26:74% v/v) and flow rate of 0.96 mL/min on a SPOLAR C18 (250 mm x 4.6 mm, 5 u) column, a run period of 6 minutes, and wavelength selected was 236 nm. DAPA and SAXA had retention times of 3.5 and 5.0 min, respectively. Linearity concentration range for dapagliflozin and saxagliptin 0.2–300 ug/mL and 0.1–150 ug/mL, respectively. The LOD value was found to be 0.061 and 0.014 ug/ml and LOQ value was found to be 0.18 and 0.043 ug/ml, respectively [25].

Rsch et al. The technique was developed using ammonium dihydrogen phosphate buffer pH 6.8 and methanol in a 65:35 v/v ratio as mobile phase on an Intersil ODS C18 (250 mm x 4.6 mm) column. Detection wavelength of UV was 280 nm. The technique demonstrated linearity in the 2.5–40.0 ug/ml range for DGFZ and the 1.2–20.0 ug/ml range for SGPT. DAPA and SAXA LOQ values of 0.312 ug/ml and 0.156 ug/ml, respectively. The LOD values for DAPA and SAXA are 0.156 g/ml and 0.078 g/ml, respectively [26].

Amit et al. It was performed on an ODS C18 (250 x 4.6 mm, 5 m) column with isocratic elution. The ideal mobile phase consists of an aqueous phase (sodium acetate buffer, pH 4) and an organic phase (acetonitrile: methanol, 40:40) at a ratio of 20:80. Flow rate was 1.0 ml/min and detected at 228 nm. This approach demonstrated high linearity across a range 2.0–12 ug/mL for DAPA and 1-6 ug/mL for SAXA. Dapagliflozin and Saxagliptin samples had retention periods of 2.31 and 2.90 minutes, respectively. The calculated LOD and LOQ for DAPA were 0.44 ug/ml and 1.33 ug/ml, respectively, whereas for SAXA they were 0.26 ug/ml and 0.78 ug/ml, respectively [27].

Sharmila et al. An Eclipse XDB C18 (150 x 4.6 mm, 5 um) column with mobile phase of 0.1% OPA and ACN (50:50) with pH set to 5.0 at a 1 mL/min flow rate was used to separate the analytes. At 254 nm, analyte was detected. The retention times was found to be for saxagliptin, dapagliflozin, and internal standard were 5.173, 7.218, and 2.746 minutes, respectively. The linearity range of dapagliflozin was 0.05–2 ug/mL, and that of saxagliptin was 0.01–0.5 ug/mL [28].

*K.P.R. Chowdary et al.*An XTerra C-18 (4.6 x 150 mm, 5 um) column at 30 °C ambient temperature was used for chromatographic separation. The Phosphate buffer pH 4 adjusted by OPA: Acetonitrile 50:50 v/v selected as mobile phase to elute the samples at a 1 ml/min of flow rate. The experiments were carried out with a 10-ul injection volume and wavelength selected was 225 nm. The retention time was 5.3 minutes for Dapa, 3.2 minutes for Saxa, and 9.56 minutes for Dapagliflozin. Linearity was found in concentration range of 20–300 ng/mL for saxagliptine and 100–1500 ng/mL for dapagliflozin [29].

Nima Suthar et al. The separation was accomplished using an LC-20 AT C18 (250 mm X 4.6 mm, 2.6 um) column with a buffer (pH 6.0) and acetonitrile (70:30) having flow rate of 1 ml/min and an injection volume of 20 uL. The detection wavelength was 275 nm. The retention time was 5.263 and 3.593 minutes, for SAXA and DAPA, respectively. Linearity shown in concentration range 10–30 ug/mL and 5–15 ug/mL for dapagliflozin and

saxagliptin, respectively. The limit of detection and quantification value was found to be 1.817 ug/ml and 5.508 ug/ml for dapagliflozin, while 0.352 ug/ml and 1.067 ug/ml for saxagliptin, respectively [30].

Deepan et al. For the separation, an Xterra RP18 (4.6 x 150 mm, 5 um) column with acetonitrile: water (60:40) was used. The wavelength of detection was 248 nm using the isocratic mode at 1 mL/min of flow rate. DAPA and SAXA retention times were around 2.09 and 3.24 minutes, respectively. The calibration curves for DAPA were found to be linear in the 100–500 ug/ml range and in the 50–250 ug/ml range for SAXA. DAPA detection and quantification values were 3.0 and 9.98 ug/ml, respectively, while SAXA detection and quantification values were 3.0 and 10 ug/ml [31].

R. Usman et al. For the quantitative measurement of saxagliptin (SAXA) and dapagliflozin (DAPA), a high-performance liquid chromatographic technique that is accurate, precise, and reproducible was established. Agilent gradient system with a UV detector and RP C18 Thermo column (250mm x 4.6mm, 5.5 um). The mobile phase was methanol and 0.1% o-phosphoric acid (60:40). Flow rate was 1 ml/min, and detection wavelength was 220 nm. SAXA and DAPA have a retention time of 5.263 and 3.593 minutes, respectively. concentration range for Saxagliptin 10–50 ug/mL, and dapagliflozin 20–100 ug/mL. Dapagliflozin's LOD and LOQ are 0.1230 and 0.5460, respectively, whereas saxagliptin's are 0.040 and 0.01230 [32].

K.P.R.Chowdary et al. Separation takes place on an XTerra C-18 column (150mm x 4.6mm, 5 um). Mobile phase selected phosphate buffer pH 4 and ACN (50:50 v/v) at 1 ml/min flow rate. The UV detection at 225 nm. Saxagliptine's LOD and LOQ are 1.63 ug/ml and 5.39 ug/ml, respectively, whereas Dapagliflozin's are 1.94 ug/ml and 6.50 ug/ml. Saxagliptine and dapagliflozin had retention times of 2.10 and 2.81 minutes, respectively, with a resolution of 3.26. Linearity was established for both SAXA and DAPA in the range 20–60 ug/ml and the 40–120 ug/ml , respectively [33].

R. Aswini et.al The Dapagliflozin and Saxagliptin were separated chromatographically using an Inertsil-ODS C18 (250 mm x 4.6 mm, 5 um) column with a mobile phase of a 45:55 v/v mixture of methanol and potassium dihydrogen phosphate buffer supplied at a flow rate of 1.0 ml/min. The wavelength selected was 210 nm. The retention times of dapagliflozin was 4.707 and saxagliptin was 6.684 minutes, respectively. linearity studies revealed concentration ranges of 20–70 ug/ml for both dapagliflozin and saxagliptin. LODs of 0.109 ug/ml and 0.58 ug/ml and LOQs of 0.332 ug/ml and 1.77 ug/ml, for dapagliflozin and saxagliptin, respectively [34].

*Singh et al.*The drugs were separated chromatographically using a buffer and acetonitrile 53:47 v/v in the mobile phase on an Xterra C 18 column (150 x 4.6 mm, 3.5 u). The buffer in the mobile phase was 20 mM sodium dihydrogen phosphate that had been pH adjusted to 5.5 ± 0.02 using OPA. The wavelength of the UV-visible detector was 230 nm, and the flow rate was 1.2 ml/min. The approach shows acceptable linearity throughout a 2–14 ug/mL range. The LOD for DAPA and SAXA were 0.32 and 0.25 ug/mL, respectively, whereas the LOQ were 0.97 and 0.71 ug/mL [35].

Swamy et al. The samples were eluted isocratically at 247 nm on a C18 (250 cm x 4.6 cm, 5 u) Primesil ODS column with mobile phase potassium dihydrogen phosphate buffer (pH 6.0) and acetonitrile (45:55 v/v). DAPA and SAXA had retention times of 2.3 and 3.2 minutes, respectively. At 5–30 ug/mL, saxagliptin hydrochloride showed a linear response, as does dapagliflozin at 10–60 ug/mL. DAPA and SAXA had LODs of 0.76 ug/ml and 1.57 ug/ml and LOQs of 1.3 ug/ml and 3.96 ug/ml, respectively [36].

Nachiket S Dighe et al. A Cosmosil C-18 (250 mm x 4.6 mm, 5 m) column was used to standardize the chromatographic conditions. The wavelength selected was 228 nm. Mobile phase was Methanol: Potassium Dihydrogen Phosphate Buffer with pH 3.0 (80:20 v/v), and the retention times for Metformin and Dapagliflozin were 3.6 and 5.2 minutes, respectively. Metformin has a linearity range of 100–500 ug/mL, while dapagliflozin has linearity range of 1–5 ug/mL. LOD and LOQ for dapagliflozin are 0.052 and 0.158, respectively, whereas those for sitagliptin are 0.837 and 2.538 [37].

Bhavyasri et al. Phenomenex C18 (250 x 4.6 mm) stationary phase was used for chromatographic conditions. A 50:50 combination of water and methanol was used in the mobile phase. The flow rate was set to 1.0 ml/min. The detecting wavelength was set to 230 nm. Linearity was found to be for Metformin (2–7 ppm) and for Dapagliflozin (60–210 ppm). LOD values were found to be 345000 ppb and 263000 ppb, and LOQ values were found to be

415000 ppb and 324000 ppb, for dapagliflozin and saxagliptin, respectively. The retention times for DAPA and MET were 3.338 and 2.178 minutes, respectively [38].

Dhale et.al chromatographic separation was performed on a Thermo Fisher ODS C18 (4.6 mm x 250 mm,5 um) column containing a mobile phase of water and acetonitrile 65:35% v/v of pH 6.8 adjusted with 0.1% OPA using flow rate 1 ml/min. The measured wavelength was 240 nm. The retention times for MET and DAPA were determined to be 2.13 minutes and 5.41 minutes, respectively. The suggested technique was shown to be linear for MET in the range of 100–600 ug/ml (R^2 = 0.9999) and DAP in the ranges of 1–6 ug/ml (R^2 = 0.9996). LOD and LOQ were determined to be 0.1230 ug/ml and 0.546 ug/ml for dapagliflozin and 0.04 µg/ml and 0.01230 µg/ml for metformin, respectively [39].

Pottabathula et al. The Phenomenex Luna C18 (4.6 mm x 250 mm, 5 µm) column and mobile phase was acetonitrile: water (75:25% v/v), with a flow rate of 1 ml/min was used for separation. The detecting wavelength was 285 nm, and the injection volume was 10 ul. MET and DAPA retention times were reported to be 3.2 minutes and 5.4 minutes, respectively. Linearity concentration range of metformin and dapagliflozin was 20–100 ug/ml and 10–50 ug/ml, respectively. The LOD and LOQ values were 5.0 ug/ml, 15.2 ug/ml, and 3.7 ug/ml, 11.4 ug/ml for MET and DAPA , respectively [40].

Hadi et al. The separation was done on the Kromasil C18 column (250 mm x 4.6 mm, 5 um), using a mobile phase of buffer: acetonitrile (60:40 v/v), at a flow rate of 1.0 ml/min. The wavelength for detection was 266 nm. Metformin and dapagliflozin had retention times of 2.330 and 3.098 minutes, respectively. The linearity was tested at concentrations ranging from 125 to 750 ppm for metformin and 1.25 to 7.5 ppm for dapagliflozin, respectively. LOD and LOQ for metformin were 0.90 and 2.73, respectively, and 0.02 and 0.07 for dapagliflozin [41].

T. Deepan et al. The chromatographic separation was accomplished using isocratic flow using an inspire (4.6 mm x 150 mm, 5 um) column. Acetonitrile and 0.1 M orthophosphoric acid buffer (70:30, v/v) were used as the mobile phase, and the flow rate was 1.0 mL/min. UV detection was performed at 260 nm. MET and DAPA retention times were 2.097 and 3.691 minutes, respectively. A linear response was seen across the concentration range of 5–25 ug/mL for DAPA and 500–2500 µg/mL for MET; corresponding LOD and LOQ values for DAPA were 2.98 and 9.95 ug/mL, and for MET, they were 3.05 µg/mL and 10.07ug/mL, respectively [42].

*K. J. Patel et al.*The separation was performed using an Inertsil ODS C18 column (250 mm x 4.6 mm, 5 u), a UV detector set at 227 nm, and a mobile phase of 0.05 M Potassium Dihydrogen Ortho Phosphate Buffer (pH 3.5, adjusted with 0.1% orthophosphoric acid) and Acetonitrile in a 50:50% v/v ratio at a flow rate of 1.0 ml/min. Dapagliflozin and metformin HCl, the technique is linear throughout concentration ranges of 5–15 and 25–75 ug/mL, respectively. DAPA and MET had retention times of 2.633 and 5.620 minutes, respectively. LOD and LOQ for DAPA were 0.28 and 0.85 ug/mL, respectively, and 0.78 and 2.37 ug/mL for MET [43].

Urooj et al. The Phenomenex Luna C18 (4.6 mm x 250 mm, 5 um) column was utilized for chromatographic separation. The mobile phase was Acetonitrile: Water (75:25% v/v), and the flow rate adjusted was 1 ml/min. The wavelength used for detection was 285 nm, and the injection volume was 10 µl. Metformin and Dapagliflozin retention times were reported to be 3.2 minutes and 5.4 minutes, respectively. MET and DAPA have linearities in the ranges of 20–100 ug/mL and 10–50 ug/mL, respectively. The LOD and LOQ values for MET and DAPA were found to be 5.0 ug/ml, 15.2 ug/ml, and 3.7 ug/ml and 11.4 ug/ml, respectively [44].

yunoos et al. Chromatography was performed on a hypersil BDS C18 (250 mm x 4.6 mm, 5 um) column with a mobile phase of buffer (0.1% orthophosphoric acid) set to pH 6.8 with triethylamine: acetonitrile in a 50:50 % v/v ratio with a flow rate of 1 ml/minute. The wavelength was set to 240 nm. The retention times for MET hydrochloride were 2.791 minutes and 3.789 minutes for DAPA, respectively. The proposed method demonstrated linearity in the concentration ranges of 0.5–3.0 ug/ml for DAPA ($r2$ = 0.99978) and 85–510 ug/ml for MET ($r2$=0.99995).The value for LOD and LOQ for MET and DAPA was 1.32 ,.3.95 ug/ml and , 0.43 ,1.43 ug/ml, respectively [45].

Y. D. Patel et al. An Inertsil ODS C-18 (250 mm x 4.6 mm, 5 m) column using Methyl Nitrile (25 parts) and 0.02 M KH2PO4 buffer containing 0.02 M triethylamine with a neutral pH adjusted by orthophosphoric acid (75 parts) in isocratic mode at a flow rate of 1 ml/minute, while the chromatogram was monitored at 210 nm. DAPA and SITA

retention times were 2.89 and 16.41 minutes, respectively. Dapagliflozin's linearity range is 5–15 ug/ml, while sitagliptin's linearity range is 50–150 ug/ml [46].

Gupta et al. Mobile phase potassium phosphate monobasic buffer pH 3.0 (mobile phase A) and methanol and ACN in the ratio of 60:40 v/v (mobile phase B) was used in gradient elution to accomplish chromatographic separation on a Hypersil BDS C18 (250mm x 4.6mm, 5 um) column. Time/Mobile phase A%/Mobile phase B% is 0 minutes/55/45, 3 minutes/55/45, 9 minutes/20/80, 13 minutes/20/80, 15 minutes/55/45, and 20 minutes/55/45. Dapagliflozin's linearity varies from 25 to 76 ug/mL, and Sitagliptin's linearity ranges from 247 to 743 ug/ml. The wavelength selected was 215 nm, while column temperature was controlled at 35 °C and the injection volume was 10 ul. Dapagliflozin retention time is 9.416 minutes, while sitagliptin retention duration is 2.76 minutes [47].

Patel et al. The drug separation on a Kromstar Vertex C18 analytical column (250 x 4.6 mm, 5 um) with ACN: Dihydrogen Phosphate Buffer pH 4 was adjusted by adding OPA and UV detection at 228 nm with a 20-uL injection volume. The flow rate was 1 ml / min. The retention times of DAPA and PIO was 3 and 6.5 minutes, respectively. The linearity range for DAPA was determined to be 2–10 ug/mL and 3–15 ug/mL for PIO. The LOD and LOQ were 0.041 ug/mL and 0.13 ug/mL for dapagliflozin, and 0.105 ug/mL and 0.34 ug/mL for pioglitazone HCl, respectively [48].

Patel et al. A Pearless C-18 (4.6 x 250 mm, 5 u) column used for separation and mobile phase of ACN and 10% orthophosphoric acid in water pH 6.0 (70:30% v/v) at a 1.0 ml/min flow rate. chromatogram was recorded at 228 nm. The RP-HPLC process's linearity was assessed for DAPA and GLM over concentration ranges of 1–5 g/ml for both, respectively. DAPA has a retention time of 3.100 minutes, while GLM has a retention time of 6.760 minutes. LOD values for DAPA and GLM were found to be 0.0024 µg/ml and 0.013 µg/ml respectively. The LOQ values for DAPA and GLM were found to be 0.0072 µg/ml and 0.039 µg/ml respectively [49].

In Table 1-5, there is information on the stationary phase, mobile phase, flow rate, wavelength, retention time, linearity, limit of detection and limit of quantification of reported literature for dapagliflozin combination.

Table 1: HPLC Method for Dapagliflozin with Saxagliptin

Stationary Phase	Mobile Phase	λ max	Flow rate (ml / min)	Retention time (min)	Linearity	LOD	LOQ	Ref
BDS C8 column (50 × 4.6 mm, 5µ)	Potassium dihydrogen phosphate: Acetonitrile (55:45) pH was adjusted to 3.8 with dilute orthophosphoric acid	210	1	DAPA 2.266 min SAXA 2.805 min	DAPA 25-150µg/ml SAXA 12.5-75µg/ml	DAPA 0.20 SAXA 0.26	DAPA 0.60 SAXA 0.79	23
C18 Inertsil ODS (150×4.6) mm, 5µ)	Phosphate buffer: Acetonitrile (55:45), pH 4.0 adjusted by glacial acetic acid	220	0.8	DAPA 2.144 min	DAPA 8–22 µg/mL	-	-	24
SPOLAR C18 column (250 × 4.6 mm, 5 µ)	acetonitrile: phosphate buffer, pH 5.8 (26:74% v/v)	236	0.96	DAPA 3.5 min SAXA 5.0 min	DAPA 0.2-300 µg/mL SAXA 0.1-150 µg/mL	DAPA 0.061 SAXA 0.014	DAPA 0.18 SAXA 0.043	25
Intersil ODS C18 column (250 mm × 4.6 mm × 5 µ).	ammonium dihydrogen phosphate buffer (pH 6.8) and methanol in a ratio of 65:35 v/v	280	1.5	DAPA 4.67 min SAXA 6.74 min	DAPA 2.5-40.0 µg/ml SAXA 1.25-20.0 µg/ml	DAPA 0.156 SAXA 0.078	DAPA 0.312 SAXA 0.156	26
Eclipse XDB C18 column	0.1% orthophosphoric acid and acetonitrile	254	1	DAPA 7.218	DAPA 0.05-2	-	-	27

(150 x 4.6 mm, 5 um)	(50:50) with pH set to 5.0			SAXA 5.173	ug/ml SAXA 0.01-0.5 ug/ml	-	-	
ODS C18 column (250 mm x 4.6 mm, 5 µ)	aqueous phase (sodium acetate buffer, pH 4) and an (organic phase acetonitrile: methanol 40:40) at a ratio of 20:80	228	1	DAPA 2.31 min SAXA 2.90 min	DAPA 2.0-12 µg/ ml SAXA 1-6 µg /ml	DAPA 0.44 SAXA 0.26	DAPA 1.33 SAXA 0.78	28
XTerra C 18 column (4.6 x 150mm, 5 µm)	Phosphate buffer: ACN (50:50v/v) PH 4 adjusted by OPA	225	1	DAPA 5.37 min SAXA 3.26 min	DAPA 100-1500ng/ml SAXA 20-300ng/ml	- -	- -	29
LC- 20 AT C18 column (250mm x 4.6 mm x 2.6 µm)	Buffer (pH 6.0): Acetonitrile (70:30)	275	1	DAPA 3.593 min SAXA 5.263 min	DAPA 10-30 µg/ml SAXA 5-15 µg/ml	DAPA 1.817 SAXA 0.352	DAPA 5.508 SAXA 1.067	30
Xterra RP C-18 column (4.6×150 mm, 5 µm)	Acetonitrile: water (60:40)	248	1	DAPA 2.091 min SAXA 3.249 min	DAPA 100-500 µg/ml SAXA 50-250 µg/ml	DAPA 3.0 SAXA 3.02	DAPA 9.98 SAXA 10	31
Thermo C18 column (250mm x 4.6 mm x and 5 µm)	Methanol 0.1 % o-phosphoric acid (60:40)	220	1	DAPA 5.41 min SAXA 7.30 min	DAPA 10-50 µg/mL SAXA 20-100 µg/ml	DAPA 0.1230 SAXA 0.040	DAPA 0.5460 SAXA 0.01230	32
XTerra C 18 column (150mm x 4.6mm x5µm)	phosphate buffer (pH 4) and Acetonitrile (50:50v/v	225	1	DAPA 2.1 min SAXA 2.8 min	DAPA 20-60ug/ml SAXA 40-120ug/ml	DAPA 1.94 SAXA 1.63	DAPA 6.50 SAXA 5.39	33
Inertsil-ODS, C18 column (250 × 4.6 mm; 5 µm	Methanol and Potassium dihydrogen phosphate buffer in the ratio of 45:55 v/v	210	1	DAPA 4.707 min SAXA 6.684 min	DAPA 20-70 µg/m SAXA 20-70 µg/m	DAPA 0.109 SAXA 0.332	DAPA 0.58 SAXA 1.77	34
Xterra C-18 analytical column (150 mm × 4.6 mm x 3.5 µ)	buffer and acetonitrile (53:47 v/v)	230	1.2	DAPA 6.11±0.03 min SAXA 8.01±0.02 min	DAPA 2-14 µg /mL SAXA 2-14 µg / mL	DAPA 0.32 SAXA 0.25	DAPA 0.97 SAXA 0.71	35
Primesil ODS C18 column C18 (250 cm x 4.6cm x 5µ)	Potassium dihydrogen phosphate Buffer (pH 6.0): Acetonitrile (45:55 v/v)	247	1	DAPA 3.2 min SAXA 2.3 min	DAPA 10-60 µg/mL SAXA 5-30 µg/mL	DAPA 0.76 SAXA 1.3	DAPA 1.57 SAXA 3.96	36

Table 2: HPLC Method for Dapagliflozin with Metformin

Stationary Phase	Mobile Phase	λ max	Flow rate (ml / min)	Retention time (min)	Linearity	LOD	LOQ	Ref
Cosmosil C18 column (250mm x 4.6mm x 5µm)	Methanol: Potassium dihydrogen phosphate buffer with pH 3.0 (80:20%v/v)	228	0.9	DAPA 3.6 min MET 5.2 min	DAPA 100-500µg/ml MET 1-5µg/ml	DAPA 0.052 MET 0.837	DAPA 0.158 MET 2.538	37
Phenomenex C18 (250mm x 4.6 mm x 5µm)	Water: Methanol in the ratio of 50:50	230	1	DAPA 3.338 min MET 2.178 min	DAPA 60-210 ppm MET 2-7 ppm	DAPA 345000 ppb MET 263000 ppb	DAPA 415000 ppb MET 324000 ppb	38
THERMO fisher ODS C18 column (4.6mm x 250mm; 5µm)	water and acetonitrile 65:35 % v/v of pH 6.8 adjusted with 0.1 % ortho phosphoric acid	240	1	DAPA 5.41 min MET 2.13 min	DAPA 1-6 ug/ml MET 100-600 ug/ml	DAPA 0.1230 MET 0.04	DAPA 0.546 MET 0.01230	39
Phenomenex Luna C18 (4.6mm × 250mm x 5µm)	Acetonitrile: Water (75:25% v/v)	258	1	DAPA 5.4 min MET 3.2 min	DAPA 10–50µg/ml MET 20–100µg/ml	DAPA 3.7 MET 5.0	DAPA 11.4 MET 15.2	40
Kromasil C18 column (250mm × 4.6mm X 5µm)	Buffer: Acetonitrile (60:40 v/v)	266	1	DAPA 3.098 min MET 2.330 min	DAPA 1.25-7.5 ppm MET 125-750 ppm	DAPA 0.02 MET 0.90	DAPA 0.07 MET 2.73	41
Inspire column (4.6 x 150mm, 5µm)	Acetonitrile and 0.1M orthophosphoric acid buffer (70:30, v/v)	260	1	DAPA 3.691 min MET 2.097 min	DAPA 5-25µg/ mL MET 500-2500µg/ mL	DAPA 2.98 MET 3.05	DAPA 9.95 MET 10.07	42
Inertsil ODS C18 column (250mm x 4.6 mm, 5µ)	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	227	1	DAPA 2.633 min MET 5.620 min	DAPA 5-15 MET 25-75µg/ml	DAPA 0.28 MET 0.78	DAPA 0.85 MET 2.37	43
Themosil C18HPLC column (150mm x 4.6mm x 5µm)	0.1M orthophosphoric acid: acetonitrile: methanol (35:40:25v/v/v)	234	1.2	DAPA 4.105 min MET 2.102 min	DAPA .5-2.5µg/ml MET 0.5-25µg/ml	DAPA 2.84 MET 3.02	DAPA 9.93 MET 10.6	44

Hypersil BDS C18 column (250 mm × 4.6 mm, 5 µ)	Buffer (0.1% orthophosphoric acid) adjusted to pH 6.8 with triethylamine: acetonitrile (50:50%/v/v)	240	1	DAPA 3.789 min MET 2.791 min	DAPA 0.5-3.0 µg/ml MET 85-510 µg/ml	DAPA 0.43 MET 1.32	DAPA 1.43 MET 3.95	45

Table 3: HPLC Method for Dapagliflozin with sitagliptin

Stationary phase	Mobile phase	λ max	Flow rate (ml/min)	Retention time (min)	Linearity	LOD	LOQ	Ref
inertsil ODS C18 column (250 mm x 4.6 mm x 5µm)	Methyl Nitrile (25 parts) and 0.02 M KH2 PO4 buffer having neutral PH adjusted by ortho-phosphoric acid and 1 ml triethylamine (75 parts)	210	1	DAPA 2.86 SITA 16.41	DAPA 5 to 50 µg/ml SITA 15 to 150 µg/ml	- -	- -	46
Hypersil BDS C18 column (250mm x 4.6mm, 5µm)	potassium phosphate monobasic buffer pH 3.0 (mobile phase A) while methanol: acetonitrile ratio of 60:40 v/v (mobile phase B)	215	-	DAPA 9.416 SITA 2.769	DAPA 25 to 76 µg/ml SITA 247 to 743 µg/ml	- -	- -	47

Table 4: HPLC Method for dapagliflozin with pioglitazone

Stationary phase	Mobile phase	λmax	Flow rate	Retention time	Linearity	LOD	LOQ	Ref
Kromstar Vertex C18 analytical column (250 ×4.6 mm, 5µm)	ACN: Potassium Dihydrogen Phosphate Buffer (pH 4) was adjusted by adding OPA (25:75% v/v)	228	1	DAPA 3 min PIO 6.5 min	DAPA 2-10 µg/ml PIO 3–15 µg/mL	DAPA 0.041 µg/mL PIO 0.105 µg/mL	DAPA 0.13 µg/mL, PIO 0.32 µg/mL	48

Table 5: HPLC Method for Dapagliflozin with Glimepiride

Stationary phase	Mobile phase	λ max	Flow rate (ml/min)	Retention time (min)	Linearity	LOD	LOQ	Ref
Pearless C 18 (4.6 mm x 250mm x 5µ) column	ACN: 10% OPA in water pH 6.0 (70:30% v/v)	228	1	DAPA 3.100 min GLM 6.76 min	DAPA 1 -5 µg/ml GLM 1 -5 µg/ml	DAPA 0.0024 GLM 0.013	DAPA 0.0072 GLM 0.039	49

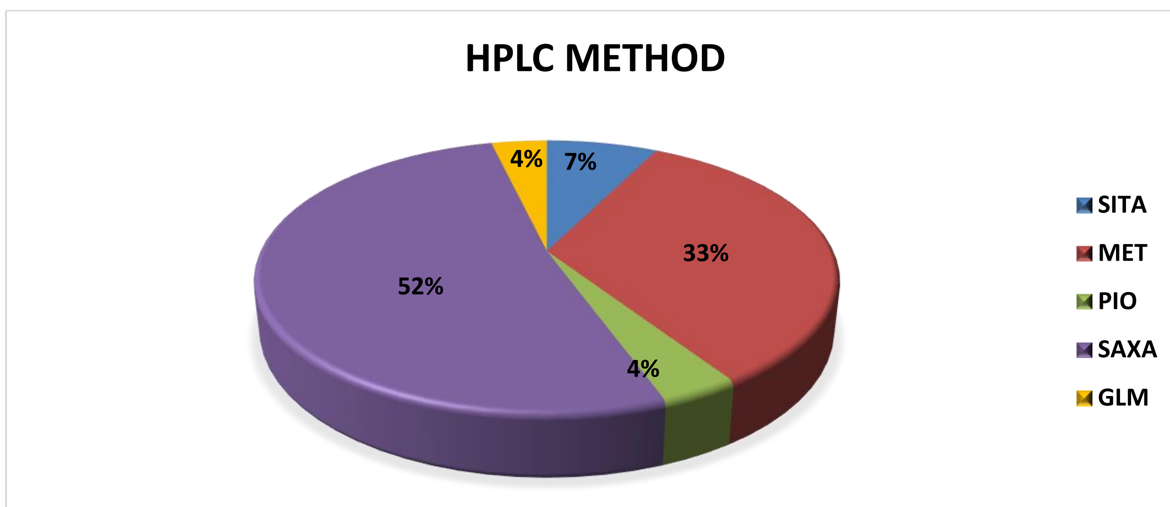

Figure 3: Percentage for various drugs in combination with dapagliflozin in HPLC Analytical Method

8.2 LC-MS Method

Liquid chromatography separation based on affinity for stationary and mobile phase. Mass spectrometry (MS) is ionizes the chemical compound to generate mass-to-charge ratios (m/z), and measures the intensity of each ion. The fruitful coupling of LC with MS results structural information and molecular mass of eluted components, supplementing qualitative information.

A LC unit's basic components are as follows: (1) Pump – for constant flow of solvent (2) Column - for sample separation, and (3) Autosampler - introduces the samples, (4) Detector - separated component are analyse.

The instrumentation for an LC-MS system comprised of: (1) LC unit, (2) LC-MS interface, (3) Ion source (4) Ion guide (5) Mass analyzer (6) Detector.

Types of interfaces are Thermos pray ionization, Electrospray ionization, Atmospheric pressure chemical ionization and Atmospheric pressure photoionization and different mass analyzer are quadrupole mass filter, Time to flight, ion trap analyzer. MS detectors are microchannel plate (MCP) and electron multiplier that function via secondary electron emission process.

LC-MS used in the food industry detect trace level of contaminant. The pharmaceutical industry is a common application for LC-MS, impurities in pharmaceutical products, assay of drug substances, intermediates, drug product and their related compounds.

LC-MS used to investigate drug absorption, metabolism, and excretion. Quantitative determination of drugs or metabolites is measured in biological matrix, pharmacodynamics, clinical trials, and in-vitro dissolution tests are all examples of comparative bioequivalence studies, also it is used to identify the various components of pesticides and fertilizers.

Goday et al. In isocratic mode, chromatographic separation on a reverse phase Hypersil Gold C-18 (50 mm x 3.0 mm, 5 um) column using a combination of 10 mM ammonium acetate and methanol (20:80, v/v) and flow rate was 0.5 ml/min. Using the liquid-liquid extraction approach, a 20 ul injection volume was employed for simultaneous assessment of SAXA and DAPA in K2EDTA human plasma (LLE). Internal standards were dapagliflozin d5 and saxagliptin d2. Under multiple reaction monitoring (MRM) circumstances, an electrospray ion interface working in positive mode for quantification of DG, DGd5, SG, and SGd2, the mass transitions were 410.2/250.6, 415.3/250.6, 316.1/272.4, and 318.2/272.3, respectively. For both analytes, the technique demonstrated good linearity across the concentration range of 50.00–10000.00 pg/mL [50].

Surendran et al. An Agilent Eclipse Plus C-18 (150 x 4.6 mm,3.6 um) column using gradient elution with 0.01 % ammonia solution and ACN as mobile phase. As a sample preparation method, a solid-phase extraction procedure was applied. Internal standards were vildagliptin (IS1) and empagliflozin (IS2). The flow rate was 1.0 mL/min.

Using a polarity switching technique, the ion transitions were measured in both positive and negative polarity. The approach demonstrated good linearity in the 0.2–80 ng/mL range for SAXA and the 5–2000 ng/mL range for DAPA. The analytes were detected at m/z 316.3–180 for SAXA and m/z 304.2–154.1 for IS1 in the positive mode, and at m/z 407.2–329.0 for DAPA and m/z 449.0–371.2 for IS2 in the negative mode [51].

Kommineni et al. Ace Phenyl column (150 x 4.6 mm, 5 um) at a fixed flow rate of 0.8 mL/min and mobile phase was ACN: 5 mM ammonium acetate buffer 70:30 v/v using isocratic mode. The analyte and Internal standard were separated from the human plasma using solid-phase extraction using the Cleanert PEP-H extraction cartridge. Internal standards were dapagliflozin-d5 HCl and saxagliptine-d2 HCl. The linearity range was 0.50–227 ng/mL, for DAPA while 0.103–76.402 ng/mL for SAXA. MS/MS detection using positive ions mode. The analytes were detected in various reaction transitions at m/z 409.14 to 135, m/z 412.43 to 135, m/z 316.2 to 180.13, and m/z 318.10 to 180.13 for dapagliflozin, dapagliflozin-d5, saxagliptine, and saxagliptine-d2, respectively [52].

El-zaher et al.On a Zorbax C18 column (50 x 4.6 mm, 5 um), dapagliflozin (DAPA), metformin (MET), and saxagliptin (SAXA) and their internal standards were separated and detected using mobile phase acetonitrile: 0.1% formic acid (45:55, v/v) and an electrospray ionization (ESI) source is in positive ion mode. Flow rate was 0.5 ml/minute. Due to the differences in the physicochemical characteristics of pharmaceuticals, sequential liquid extraction and precipitation procedures were utilized for extraction. Carbamazepine, vildagliptin, and tadalafil were employed as internal standards. The MS/MS response for DAPA, SAXA, and MET was linear over concentration ranges of 5-500 ng/ml, 2-50 ng/ml, and 10-4000 ng/ml, respectively. The mass transition ion pair for dapagliflozin was chosen to be m/z 407.3 to m/z 329.1, m/z 316.5 to 180.2 for saxagliptin, and m/z 130.2 to m/z 71.1 for metformin [53].

Surendran et al. An Agilent Eclipse Plus C-18 (150 x 4.6 mm,3.6 um) column using gradient elution with 0.01 % ammonia solution and ACN as mobile phase. As a sample preparation method, a solid-phase extraction procedure was applied. Internal standards were vildagliptin (IS1) and empagliflozin (IS2). The flow rate was 1.0 mL/min. Using a polarity switching technique, the ion transitions were measured in both positive and negative polarity. The approach demonstrated good linearity in the 0.2–80 ng/mL range for SAXA and the 5–2000 ng/mL range for DAPA. The analytes were detected at m/z 316.3–180 for SAXA and m/z 304.2–154.1 for IS1 in the positive mode, and at m/z 407.2–329.0 for DAPA and m/z 449.0–371.2 for IS2 in the negative mode [51].

Parle et al. The separation was performed an Agilent Infinity Lab Poroshell 120 EC C-18 (2.1 x 100 mm, 2.7 µm) column. Mobile phase of 5 mM ammonium acetate and ACN (20:80 v/v) in isocratic mode, a column temperature of 35 °C, and a flow rate of 0.2 mL/min. In the positive mode, the mass spectrometer was employed with electrospray ionization to monitor the transition pair (precursor to product ion) of m/z 426.20-107.20 and 130.10-60.10 for dapagliflozin and metformin, respectively. The approach demonstrated linearity in concentration ranges of 25–500 ng/mL and 100–2000 ng/mL for DAPA and MET, respectively. The LOD value was 6.83 ng/mL and 29.45 ng/mL, and LOQ value was 20.70 ng/mL and 89.24 ng/mL for dapagliflozin and metformin, respectively [54].

Shah et al. The analytes were separated on a reversed-phase ACE 5CN (150 x 4.6 mm, 5 um) column using acetonitrile and 15 mM ammonium acetate PH 4.5 (70:30, v/v). In mass spectrometric analyzation, the polarity switching strategy was used to improve analyte selectivity and sensitivity. The positive and negative ion transitions studied using multiple reaction monitoring mode were m/z 130.1 [M+H] +/60.1 for MET and m/z 467.1 [M+CH3COO]-/329.1 for DAPA, respectively. The assay's linear calibration range was set at 1.00–2000 ng/mL for MET and 0.1–200 ng/mL for DAPA. The LOD value was 0.39 ,0.03 ng/ml and LOQ value was 1.0, 0.1 ng/ml, for MET and DAPA, respectively [55].

Table 6: LC MS Method for Dapagliflozin with saxagliptin

Stationary Phase	Mobile Phase	Method	λ max	Quantification method	Linearity	m/z ion	Ref
Hypersil Gold C18 column (50mm x 3.0 mm ,5µm)	10mM ammonium acetate: Methanol	Liquid –liquid Extraction method (LLE)	0.5	electro spray ion interface operating in positive mode	DAPA (56)	DAPA 410.2/250.6 IS1 415.3/250.6	53

	(20:80 v/v)				SAXA 50.00-10000.0 pg/mL DAPA 0.2–80 Ng/mL	SAXA 316.1/272.4 IS2 318.2/272	
Agilent Eclipse Plus C-18 column (150 x 4.6 mm, 3.6 mm)	0.01% ammonia solution and acetonitrile	solid phase extraction process	1.0	positive and negative polarity using a polarity switching approach	SAXA 0.2–80 ng /mL	SAXA 316.3–180 IS 1 304.2–154.1 in positive mode DAPA 407.2–329.0 IS2 449.0–371.2 in negative	54
Ace Phenyl column (150 x 4.6 mm, 5 um)	Acetonitrile and 5 mM ammonium acetate buffer (70:30 v/v)	Solid-phase extraction	0.8	electrospray ionization (ESI) source in positive ion mode	DAPA 0.502-227 ng/mL SAXA 0.103-76.402 ng/mL	DAPA 135 DAPA d5 412.43 - 135 SAXA 316. 20 -180.13 SAXA d2 318.10	55
Zorbax C-18 column (50 X 4.6 mm, 5 µm)	Acetonitrile: 0.1% formic acid (45:55, v/v)	Liquid liquid extraction and precipitation techniques	0.5	electrospray ionization (ESI) source in positive ion mode	DAPA 5-500 ng/ml SAXA 2-50 ng/ml MET 10-4000 ng/ml	DAP 407.3 - 329.1 SAX 316.5 - 180.2 MET 130.2 - 71.1	56

Table 7: LC MS Method for Dapagliflozin with metformin

Stationary Phase	Mobile Phase	Method	λ max (ml/min)	Quantification Method	Linearity	m/z ion	Ref
Agilent Infinity Lab Poroshell 120 EC-C18column (2.1mm × 100 mm x 2.7 µm)	5mM ammonium acetate: acetonitrile (20:80, v/v)	-	0.2	electrospray ionization in the positive mode	DAPA 25-500 ng/mL MET 100-2000 ng/mL	DAPA 426.20 - 107.20 MET 130.10-60.10	57
ACE 5CN (150 x 4.6 mm, 5 µm) column	acetonitrile: 15 mM ammonium formate buffer, pH 4.5 (70:30, v/v)	solid phase extraction	1.0	positive and negative polarity switching approach	DAPA 0.10-200 ng/ mL MET 1.00-2000	DAPA 467.1/329.1 DAPA 13C6 473.5/335.1	58

	ng/mL	MET 130.1/60.1 MET-d6 136.1/60.1

8.3 UV Visible Spectrophotometry Method

The UV-Vis spectrophotometer's design incorporates both the spectrometer and the photometer. A light source, monochromator, sample cell and slits are all part of the spectrometer. The monochromator's grating is controlled by the microcontroller, while the photometer is composed of preamplifier, photodiode and data acquisition system.

Single beam spectrophotometers, double beam spectrophotometers, and split beam spectrophotometers are the three types of UV-Vis spectrophotometers.

There are different types of uv spectroscopic analytical technique which are simultaneous equation method, absorbance ratio spectra, Q-absorbance ratio method, derivative spectrophotometry, difference spectrophotometry, derivative ratio spectra, successive ratio - derivative spectra, dual wavelength method, absorptivity factor method, absorption factor method, multivariate chemometric methods, and isosbestic point method.

Mixture of two or more samples analyses using methods like simultaneous and derivative spectroscopy, with derivative spectroscopy being advantageous for closely absorbing peaks resolution and simultaneous spectroscopy being superior in terms of simplicity.

UV-vis spectrophotometers measure the absorption or light transmission that occurs through a medium as a function of wavelength. UV-Vis measurements cover wavelengths ranging from about 200 nm to 800 nm.

This method is low-cost, simple, flexible, non-destructive analytical technique also advantages in terms of robustness, less troubleshooting, and physicochemical interferences and analyses a wide range of organic compounds and some inorganic species. commonly used method in many fields of science, including bacterial culture, drug identification, nucleic acid purity checks, and quantification, as well as chemical research.

Jani et al. This approach used the first derivative method, which measured absorbance at wavelengths, 235 nm and 272 nm, using a UV-visible spectrophotometer with 1 cm matched quartz cells and methanol solvent. The developed approach followed Beer's Lambert's in concentration range of 0.5–2.5 ug/mL for DAPA and 25–125 ug/mL for MET [56].

Jani et al. A UV-visible spectroscopic method for estimating dapagliflozin (DAPA) and metformin hydrochloride (MET) concurrently, this approach involved solving simultaneous equations based on absorbance measurements at wavelengths, 225 nm and 237 nm. The Dapagliflozin has a linearity range of 0.5 to 2.5 ug/mL, and Metformin has a linearity range of 25 to 125 ug/mL. The method uses methanol as solvent [57].

Bhavyasri et al. Dapagliflozin and metformin UV spectrophotometry measurements were taken at 222 nm and 232 nm, respectively. The Q absorption ratio technique was used for measurement linearity ranges of 2–32 ug/ml and 1–20 ug/ml, for dapagliflozin and metformin respectively. Water was used as solvent [58].

P. Patel et al. The Vierdot approach is used, commonly known as the simultaneous equation method. As a solvent, methyl alcohol is used. Vierdot's method resulted in wavelengths of 224 nm for sitagliptin and 267 nm for dapagliflozin. The linearity range for sitagliptin was 50–150 ug/mL and 5–15 ug/mL for dapagliflozin [59].

Suthar et al. The method involved determining absorbance difference between 214.40 nm and 220.0 nm for saxagliptin hydrochloride and 208.0 nm and 209.0 nm for dapagliflozin propendiol monohydrate. Linearity was achieved in concentration ranges of 4–16 ug/ml for SAXA and 10–22 ug/ml for DAPA, respectively. Methanol was used as a solvent [60].

Bhadauria and AgarwaI In this approach, simultaneous equations are computed based on absorbance measurements at two wavelengths, 222 nm for SAXA and 276 nm for DAPA in phosphate buffer having pH 6.8. Both drugs have 5–25 ug/mL concentration ranges and follow Beer's rule [61].

M. Patel et al. A Shimadzu UV-1800 Spectrophotometer was used to quantify SAXA and DAPA absorption in methanol using a simultaneous equation UV approach at 210 nm and 224 nm, respectively. Methanol was used as a solvent. The concentration ranges used 6–22 ug/mL for SAXA and 12–44 ug/mL for DAPA, respectively [24].

Dapagliflozin with various combinations was in literature using spectrophotometry, Table, review of published techniques revealing fundamental principle, λ max, solvent, linearity.

Table 8: UV-Visible spectrophotometry for Dapagliflozin

Drug combination	Method	Solvent	λ max	Linearity	Ref
DAPA and MET	First derivative spectroscopic method	methanol	DAPA 235 nm MET 272 nm	DAPA 0.5-2.5 µg/ml MET 25-125 µg/ml	59
DAPA and MET	Simultaneous equation method at two wavelengths	methanol	DAPA 225 nm MET 237 nm	DAPA 0.5-2.5 µg/ml MET 25-125 µg/ml	60
DAPA and MET	Q absorption ratio method	water	DAPA 222 nm MET 232 nm	DAPA 2 – 32 µg/ml MET 1 – 20µg/ml	61
DAPA and SITA	Vierdot's method	methyl alcohol	DAPA 224 and SITA 267 nm	DAPA 5-15 µg/mL SITA 50-150 µg/mL	62
DAPA and SAXA	Simultaneous equation method for the absorbance difference	methanol	DAPA 208.0 nm - 209.0 nm SAXA 214.40 nm - 220.0 nm	DAPA 10-22 µg/ml SAXA 4-16 µg/ml	63
DAPA and SAXA	Simultaneous equation method	phosphate buffer pH 6.8	DAPA 276 nm SAXA 222 nm	DAPA 5-25µg/ml SAXA 5-25µg/ml	64
DAPA and SAXA	Simultaneous equation UV method	Methanol	DAPA 224 nm SAXA 210 nm	DAPA 12-44 µg / mL SAXA 6-22 µg / mL	65

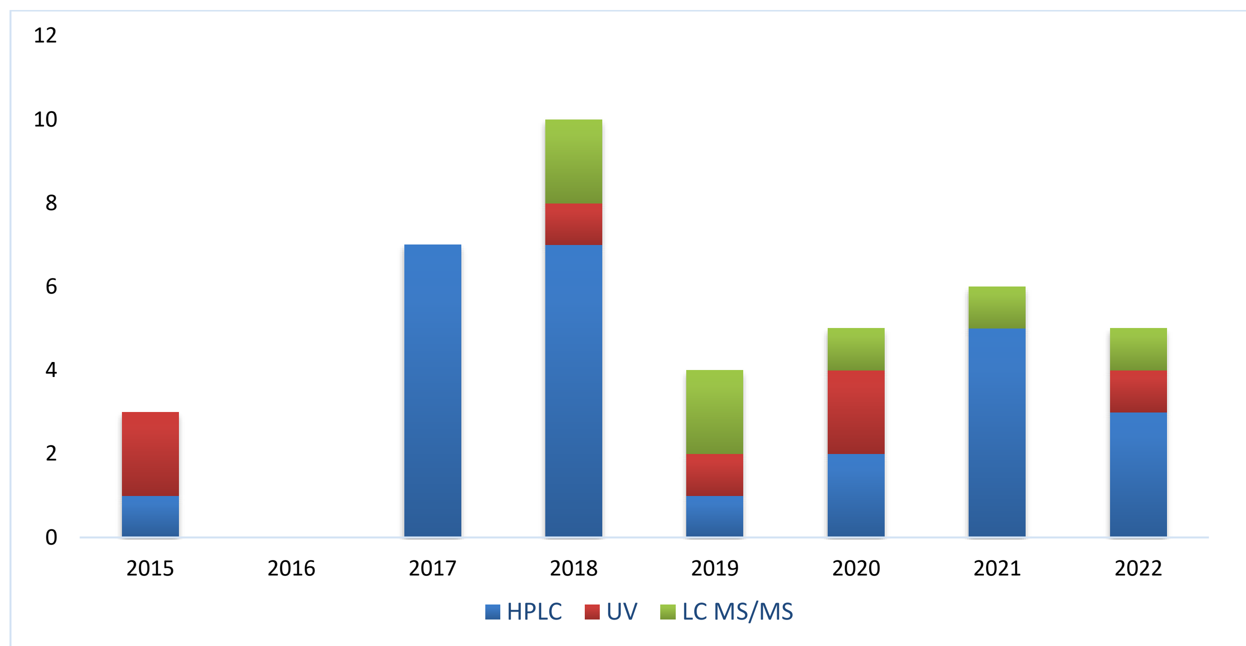

Figure 4. Analytical methods year wise data of dapagliflozin with combination

4. Conclusion

The current review summaries several analytical techniques published in the literature for determining Dapagliflozin with combination in bulk, pharmaceutical formulations, and biological matrices such as urine and blood plasma. The Analytical methods for analyzing dapagliflozin in combination with sitagliptin, sitagliptin, metformin, pioglitazone, and glimepiride was a prime focus.

This study focuses on the most current state-of-art analytical approaches for determining dapagliflozin. Dapagliflozin analysis in biological samples and pharmaceutical formulations may be accomplished using variety of methods. Data from in-depth investigation indicated that HPLC was widely employed for determining dapagliflozin in various combinations.

The most prevalent approach is HPLC hyphenated with a UV detector because it offers reliable results at a reasonable cost compared to more complex detection techniques. Furthermore, using MS methods in LC provided distinct selectivity and sensitivity, as well as a method of choice for analyzing dapagliflozin and its metabolites in biological materials. Among all combinations, dapagliflozin and saxagliptin have the highest ratio of analytical techniques published in the literature.

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