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Review

Analytical Strategies for the Determination of Dapagliflozin in Pharmaceutical and Biological Matrices: A Comprehensive Review

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Abstract

Dapagliflozin (DAPA), a selective sodium–glucose cotransporter 2 inhibitor, is widely used in the management of type 2 diabetes mellitus, with additional indications in heart failure and chronic kidney disease. The growing analytical demand for DAPA determination in pharmaceutical formulations, fixed-dose combinations, and biological matrices has stimulated the development of diverse analytical methods. This review provides a comprehensive evaluation of reported techniques for DAPA quantification in different matrices. Approaches discussed include chromatographic methods (TLC, RP-HPLC, UHPLC, LC-MS/MS), electromigration techniques (CE), and spectroscopic methods (UV–Vis, spectrofluorimetry). Emphasis is placed on key performance characteristics such as selectivity, sensitivity, linearity, robustness, and applicability to stability studies and bioanalysis. Recent trends, including the application of Quality by Design, green analytical chemistry principles, and advanced hyphenated techniques, are also addressed. While RP-HPLC remains widely used for routine quality control due to its robustness and accessibility, LC-MS/MS is generally regarded as the method of choice for trace-level bioanalysis owing to its superior sensitivity and selectivity. CE and spectroscopic techniques offer cost-effective and environmentally friendly alternatives, though with certain limitations. This review highlights current methodological gaps and outlines future directions for developing more sensitive and sustainable analytical strategies.

Keywords: dapagliflozin; oral antidiabetic drugs; SGLT-2 inhibitors; pharmaceutical analysis; bioanalysis; stability-indicating analysis; analytical method validation

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1. Introduction

Diabetes mellitus (DM) is one of the most prevalent chronic metabolic disorders worldwide and represents a major public health challenge due to its increasing incidence and associated complications, including cardiovascular disease, nephropathy, neuropathy, and retinopathy. Effective glycemic control remains essential for reducing the risk of long-term complications and improving patient outcomes [1,2].

Oral antidiabetic agents exert their therapeutic effects through multiple mechanisms, including enhancement of insulin secretion, improvement of insulin sensitivity, and

modulation of glucose absorption and excretion [3]. Among oral antidiabetic agents, sodium-glucose co-transporter subtype 2 (SGLT-2) inhibitors have emerged as a novel class that reduce blood glucose levels via insulin-independent promotion of urinary glucose excretion [4].

SGLT-2 inhibitors lower blood glucose by inhibiting renal glucose reabsorption in the proximal tubule, thereby promoting urinary glucose excretion through an insulin-independent mechanism. SGLT-2 accounts for approximately 90% of renal glucose reabsorption, while SGLT-1 is responsible for the remainder [5]. Beyond glycemic control, SGLT-2 inhibitors have demonstrated cardiovascular and renal benefits, together with favorable effects on body weight and blood pressure [6].

To date, several SGLT-2 inhibitors have received regulatory approval for the treatment of type 2 diabetes, including canagliflozin (CANA), dapagliflozin (DAPA), and empagliflozin (EMPA) [7].

Chemically, DAPA is structurally described as (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol, with a molecular formula of $C_{21}H_{25}ClO_6$ and a molecular mass of 408.87 g/mol. The molecule contains multiple hydroxyl groups on the glucose ring, contributing to its polarity, while the substituted aromatic system determines lipophilic character, resulting in an amphiphilic structure. Unlike O-glucosides, DAPA contains a C-C glycosidic bond, conferring enhanced metabolic stability [8]. The chemical structure of DAPA is presented in Figure 1.

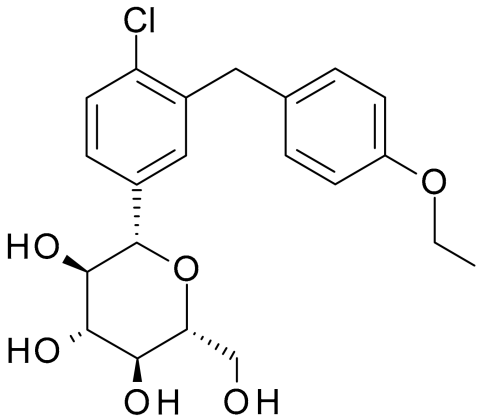

Figure 1. Chemical structure of DAPA.

From a stereochemical perspective, DAPA contains five stereogenic centers located within the glucose moiety, which are essential for selective binding to the SGLT-2 transporter. The presence of the para-substituted phenyl ring with a chloro group and an ethoxybenzyl substituent enhances hydrophobic interactions within the binding site, contributing to its potency and selectivity [8].

DAPA is characterized by favorable pharmacokinetic properties, including rapid oral absorption, high bioavailability, and metabolism predominantly via UGT1A9-mediated glucuronidation. Its elimination half-life of approximately 12–13 h allows convenient once-daily dosing [9].

DAPA is approved for the treatment of type 2 DM as monotherapy or in combination with other antidiabetic agents. In addition, its therapeutic indications have been expanded to include heart failure and chronic kidney disease [10].

Common combinations include fixed-dose combinations of DAPA with metformin (MET), as well as dual therapy with dipeptidyl peptidase-4 (DPP-4) inhibitors such as linagliptin (LINA), sitagliptin (SITA), saxagliptin (SAXA), teneligliptin (TENE), and vildagliptin (VILD). These combinations integrate insulin-independent glucose lowering

via SGLT-2 inhibition with enhanced incretin activity (via DPP-4 inhibition) or reduced hepatic glucose production (MET), resulting in additive or synergistic effects on glycemic regulation [11,12]. From a therapeutic perspective, fixed-dose combinations offer several advantages, including improved patient adherence, reduced pill burden, and simplified dosing regimens. In addition, they enable better targeting of multiple pathophysiological pathways of type 2 DM, potentially leading to improved efficacy with a lower risk of adverse effects compared to monotherapy [13].

The increasing use of DAPA in mono- and combination therapies, as well as its presence in complex matrices, necessitates the development of reliable, sensitive, and selective analytical methods. Analytical determination of DAPA is challenging due to its moderate polarity, structural complexity, and the need for simultaneous quantification in multi-component formulations and biological matrices.

DAPA may exist in different solid-state forms, including crystalline hydrates, solvates, and amorphous modifications [14]. The characterization of solid-state forms is important because polymorphism may influence physicochemical properties such as solubility, dissolution rate, stability, and bioavailability. Techniques commonly employed for polymorph identification include powder X-ray diffraction, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), infrared spectroscopy (IR), and Raman spectroscopy [15]. However, these approaches are primarily used during pharmaceutical development and solid-state characterization rather than for routine quantitative determination in pharmaceutical and biological matrices.

Despite the availability of several review articles, a comprehensive and critically integrated overview encompassing the full range of analytical methodologies reported for DAPA remains limited. Furthermore, recent developments in bioanalysis, stability-indicating methods, Quality by Design (QbD), and green analytical chemistry have not been comprehensively discussed within a single review. The novelty of the present work lies in the critical evaluation of analytical and bioanalytical methods for DAPA determination. Unlike previous reviews, which were mainly descriptive or focused on specific aspects, this review compares analytical performance, highlights recent methodological advances, and identifies current limitations and future research needs.

The review by Pathak & Mishra provides a broad overview of the analytical methods available for DAPA determination, covering mainly RP-HPLC and UV spectrophotometric approaches. While it compiles a considerable number of published articles, the manuscript remains largely descriptive, with limited critical comparison between techniques. Additionally, aspects related to method performance, validation, and green analytical chemistry are only marginally addressed, despite being mentioned as important considerations [16].

The review by Ganorkar et al. provides a comprehensive compilation of analytical methods reported for DAPA, including chromatographic, spectrophotometric, and hyphenated techniques. However, like the previous report, the manuscript remains largely descriptive, with limited critical comparison of method performance, selectivity, and practical applicability. Although the authors highlight trends in analytical approaches, deeper discussion on method validation, stability-indicating capability, and green analytical chemistry is insufficient. Furthermore, important gaps, such as the limited number of bioanalytical studies, incomplete impurity profiling, and lack of advanced degradation investigations, are acknowledged but not thoroughly analyzed [17].

The review by Suleman Basha et al. critically evaluates the current analytical landscape for DAPA determination, focusing primarily on environmental sustainability rather than analytical performance and characterization. By integrating greenness assessment tools and comparing traditional and emerging methodologies, it identifies key limitations,

methodological gaps, and opportunities for the development of more sustainable and efficient analytical strategies [18].

The present work aims to provide a critical overview of the currently available analytical strategies, emphasizing their analytical performance, practical applicability, advantages, limitations, and future perspectives.

2. Materials and Methods

A structured literature review was performed on the analytical determination of DAPA in pharmaceutical formulations and biological matrices, based on searches of major scientific databases and predefined inclusion criteria. Relevant scientific articles were identified through comprehensive searches of major bibliographic databases, including PubMed, Web of Science, Scopus, and Google Scholar. The search strategy employed combinations of keywords such as dapagliflozin, analytical methods, pharmaceutical analysis, HPLC, LC-MS/MS, capillary electrophoresis (CE), spectrophotometry, method validation, and green analytical chemistry. Only peer-reviewed articles published in English were considered.

The literature covered studies published between 2010 and 2025, corresponding to the period following the introduction and increasing clinical use of DAPA. The selected publications were evaluated with emphasis on analytical performance, including selectivity, sensitivity, linearity, and applicability.

The inclusion criteria were focused on analytical methods relevant to DAPA quantification in pharmaceutical or biological matrices, assessed through the reporting and adequacy of validation parameters (e.g., selectivity, sensitivity, linearity, accuracy, precision, robustness, and stability-indicating properties).

Exclusion criteria included conference abstracts, non-English publications, duplicate records, articles not focused on DAPA analysis, and studies lacking sufficient methodological information.

The identified methods were categorized according to their analytical approach, including chromatographic techniques (e.g., TLC/HPTLC, HPLC-UV/DAD, LC–MS/MS), electrophoretic methods (CE), spectroscopic techniques (UV–Vis and spectrofluorimetry), and hyphenated and advanced techniques used for bioanalysis and structural characterization. For each selected study, information regarding the analytical technique, chromatographic or electrophoretic conditions, validation characteristics (e.g., linearity, sensitivity, precision, accuracy, robustness), matrix type, and practical applicability was extracted and critically evaluated. Attention was given to methods intended for pharmaceutical quality control, bioanalysis, stability-indicating analysis, and approaches developed according to QbD or green analytical chemistry principles.

3. Analytical Approaches for the Determination of Dapagliflozin

The review is structured to cover chromatographic, electrophoretic, spectroscopic, and hyphenated techniques, followed by a critical comparison of their analytical performance and applicability.

The articles discussed below are presented in chronological order. Collectively, these studies illustrate the progressive evolution from conventional analytical methods to more advanced, sensitive, and sustainable approaches, including hyphenated techniques, green analytical chemistry, and QbD-based strategies.

3.1. Thin-Layer Chromatographic Methods (TLC/HPTLC)

The study by Abbas et al. describes a TLC-densitometric method for the simultaneous determination of DAPA and rosuvastatin (ROSV), a lipid-lowering agent belonging to the class of HMG-CoA reductase inhibitors, in pharmaceutical formulations and rabbit plasma. Chromatographic separation was achieved on silica gel 60 F254 plates using ethyl acetate:methanol (5:0.1, v/v) as mobile phase, with densitometric detection at 243 nm. The method provided efficient separation with Rf values of 0.23 (DAPA) and 0.44 (ROSV), with good selectivity in complex matrices such as plasma. Validation demonstrated a wide linearity range (10–2500 ng/band), good sensitivity (LOD 6.60 ng/band), and acceptable precision, in accordance with ICH guidelines. The method was successfully applied to rabbit plasma samples, with recoveries between 98 and 100%, indicating minimal matrix interference. Additionally, the method proved to be stability-indicating, enabling the monitoring of photodegradation kinetics under UV irradiation at 365 nm, with distinct degradation pathways observed for the two analytes [19].

The study by Sen et al. presents the development and validation of an HPTLC-densitometric method for the simultaneous determination of DAPA and VILD in fixed-dose combinations. Chromatographic separation was performed on silica gel 60 F254 plates, using a mobile phase consisting of acetonitrile:benzene:glacial acetic acid (9:1:2, $v/v/v$), with densitometric detection at 210 nm. The optimized system provided well-resolved bands with Rf values of 0.21 (VILD) and 0.84 (DAPA). The method exhibited linearity in the ranges of 20–2500 ng/band (DAPA) and 2000–25,000 ng/band (VILD). Sensitivity was acceptable, with LOD values of 21.07 and 154.97 ng/band and corresponding LOQ values of 63.84 and 469.60 ng/band for DAPA and VILD, respectively. The method was successfully applied to commercial fixed-dose tablet formulations, with assay values close to the labeled claim, 99.6% (DAPA) and 98.9% (VILD), supporting its applicability for routine analysis [20].

The study by Shukla et al. describes the development and validation of a stability-indicating HPTLC-densitometric method for the simultaneous determination of DAPA and LINA in bulk and pharmaceutical dosage forms. Chromatographic separation was performed on silica gel 60 F254 HPTLC plates using a mobile phase consisting of toluene:chloroform:methanol:triethylamine (7:2:1:0.2, $v/v/v/v$), with densitometric detection at 224 nm. The optimized system provided well-resolved peaks with Rf values of 0.23 (DAPA) and 0.40 (LINA). The method exhibited linearity in the range of 200–1200 ng/band for both analytes. Sensitivity was adequate, with LOD values of 25.80 and 13.09 ng/band and corresponding LOQ values of 72.22 and 42.14 ng/band for DAPA and LINA, respectively. Forced degradation studies under acidic, basic, oxidative, photolytic, and thermal conditions demonstrated that both drugs are susceptible to degradation, while maintaining clear separation of degradation products from parent compounds, confirming the stability-indicating capability of the method. The method was successfully applied to tablet formulations, with assay values of approximately 100.7% (DAPA) and 99.8% (LINA), indicating suitability for routine analysis [21].

The study by Saiyed et al. reports the development and validation of a stability-indicating HPTLC-densitometric method for the simultaneous determination of DAPA and metoprolol succinate (METO), a β-blocker cardiovascular drug, in combined pharmaceutical formulations. Chromatographic separation was achieved on silica gel 60 F254 plates using a mobile phase composed of n-butanol:ethyl acetate:triethylamine (6:4:0.1, $v/v/v$), with densitometric detection at 223 nm. The optimized system produced well-resolved peaks with Rf values of 0.35 (METO) and 0.67 (DAPA). The method showed linearity over the concentration ranges of 200–1200 ng/band (DAPA) and 1000–6000 ng/band (METO). Sensitivity was acceptable, with LOD values of 21.97 and 223.58 ng/band and correspond-

ing LOQ values of 66.60 and 677.53 ng/band for DAPA and METO, respectively. Forced degradation studies revealed that both compounds are susceptible to acidic and basic hydrolysis, while remaining stable under oxidative, photolytic, and thermal conditions. The method was successfully applied to synthetic mixtures, yielding assay values of 96.17% (DAPA) and 99.76% (METO), with no interference from excipients [22].

The study by Prajapati et al. presents a HPTLC-densitometric method for the simultaneous determination of DAPA, MET, and VILD, optimized using analytical quality by design (AQbD) and a Box–Behnken design (BBD) response surface methodology. Chromatographic separation was carried out on silica gel 60 F254 plates using a mobile phase consisting of ethyl acetate:3% ammonium acetate in ethanol:triethylamine (7:3:0.3, $v/v/v$), with densitometric detection at 220 nm in absorbance-reflectance mode. The method enabled separation of the three analytes with Rf values of 0.19 (MET), 0.40 (VILD), and 0.60 (DAPA), demonstrating good selectivity and peak purity. Validation confirmed very good analytical performance, with linearity ranges of 300–900 ng/band (DAPA), 15,000–45,000 ng/band (MET), and 3000–9000 ng/band (VILD). The method showed good sensitivity, with LOD values of 50 ng/band (MET) and 100 ng/band (DAPA and VILD) and corresponding LOQ values of 150–300 ng/band. The method was successfully applied to multiple pharmaceutical formulations, with assay values within 98–102% of the labeled claim for all drugs. Importantly, the method integrates green and white analytical chemistry principles, using low-toxicity solvents (ethyl acetate and ethanol), minimal solvent consumption, and unified analytical conditions, achieving a high AGREE score (0.8) [23].

The study by Shubhangee et al. describes a QbD- and HSPiP (Hansen Solubility Parameters in Practice)-assisted HPTLC-densitometric method for the simultaneous determination of DAPA and LINA in fixed-dose combinations. Chromatographic separation was performed on silica gel 60 F254 plates, using an HSPiP-predicted mobile phase consisting of n-hexane:toluene:ethyl acetate:methanol:0.1% formic acid (40:10:5:40:5, $v/v/v/v/v$), enabling optimal solubility and separation conditions, detection was carried out densitometrically at 230 nm ensuring equal absorbance of both analytes. Optimization via BBD resulted in well-resolved peaks with Rf values of 0.41 (LINA) and 0.66 (DAPA). Method optimization identified saturation time, band length, and solvent front as critical parameters, with optimal conditions of 15 min saturation, 6 mm band length, and 80 mm solvent front, ensuring robust performance. Validation confirmed strong analytical performance, with linearity ranges of 1000–5000 ng/band (DAPA) and 500–2500 ng/band (LINA). Sensitivity was adequate, with LOD values of 29.7 and 4.38 ng/band and corresponding LOQ values of 90.2 and 13.28 ng/band for DAPA and LINA, respectively. Robustness testing showed minimal variation under slight changes in mobile phase composition, saturation time, and wavelength. The method integrates green analytical chemistry principles, achieving high AGREE greenness scores (0.87), due to low solvent consumption, minimal toxicity, and predictive solvent selection via HSPiP [24].

A summary of HPTLC- and TLC-based analytical methods reported for the determination of DAPA in single and combined pharmaceutical formulations is presented in Table 1.

TLC/HPTLC methods offer simplicity and low cost, but their sensitivity is generally inferior to chromatographic and mass spectrometric techniques, limiting their application mainly to pharmaceutical quality control.

Table 1. Summary of TLC- and HPTLC-densitometric methods for the determination of DAPA in pharmaceutical formulations and biological matrices.

Analytical Technique	Analytical Conditions	Matrix	Analytical Performance (DAPA)	Substances	Reference
TLC-densitometry	Silica gel 60 F254; ethyl acetate:methanol (5:0.1); detection 243 nm; Rf: 0.23 (DAPA), 0.44 (ROSV)	Tablets, rabbit plasma	linearity: 10–2500 ng/band LOD: 6.60 ng/band LOQ: 20.0 ng/band Precision (%RSD): 1.35% Stability-indicating (photodegradation kinetics)	DAPA, ROSV	[19]
HPTLC-densitometry	Silica gel 60 F254; acetonitrile:benzene:glacial acetic acid (9:1:2); detection 210 nm; Rf: 0.84 (DAPA), 0.21 (VILD)	Tablets	linearity: 20–2500 ng/band LOD: 21.07 ng/band LOQ: 63.84 ng/band Precision (%RSD): <2%	DAPA, VILD	[20]
HPTLC-densitometry (stability-indicating)	Silica gel 60 F254; toluene:chloroform:methanol: triethylamine (7:2:1:0.2); detection 224 nm; Rf: 0.23 (DAPA), 0.40 (LINA)	Tablets	linearity: 200–1200 ng/band LOD: 25.80 ng/band LOQ: 72.22 ng/band Precision (%RSD): <2% Stability-indicating	DAPA, LINA	[21]
HPTLC-densitometry (stability-indicating)	Silica gel 60 F254; n-butanol:ethyl acetate:triethylamine (6:4:0.1); detection 223 nm; Rf: 0.35 (METO), 0.67 (DAPA)	Synthetic mixture/tablets	linearity: 200–1200 ng/band LOD: 21.97 ng/band LOQ: 66.60 ng/band Precision (%RSD): <2% Stability-indicating	DAPA, METO	[22]
HPTLC-densitometry (AQbD optimized)	Silica gel 60 F254; ethyl acetate:ammonium acetate (3% in ethanol):triethylamine (7:3:0.3); detection 220 nm; Rf: 0.19 (MET), 0.40 (VILD), 0.60 (DAPA)	Tablets	linearity: 300–900 ng/band LOD: 100 ng/band LOQ: 300 ng/band Precision (%RSD): <2% AGREE score = 0.80	DAPA, MET, VILD	[23]
HPTLC-densitometry (HSPiP + QbD optimized)	Silica gel 60 F254; n-hexane:toluene:ethyl acetate:methanol:formic acid (40:10:5:40:5); detection 230 nm; Rf: 0.41 (LINA), 0.66 (DAPA)	Tablets	linearity: 1000–5000 ng/band LOD: 29.7 ng/band LOQ: 90.2 ng/band Precision (%RSD): <2% AGREE score = 0.87	DAPA, LINA	[24]

3.2. Liquid Chromatography Methods with UV/DAD Detection

Deepan et al. described the development of a stability-indicating RP-HPLC method for the simultaneous determination of DAPA and MET in pharmaceutical dosage forms. The method is based on isocratic separation on a C18 column, using a mobile phase consisting of acetonitrile:orthophosphoric acid buffer (pH 3.0) (70:30, *v*/*v*), with UV detection at 260 nm. The chromatographic system provided good separation, with retention times of 2.1 min (MET) and 3.7 min (DAPA). An important feature of this method is the comprehensive forced degradation study (acidic, alkaline, oxidative, photolytic, and thermal), demonstrating that the method is stability-indicating, with clear separation of degradation products. Validation results showed acceptable linearity, 5–25 µg/mL (DAPA) and 500–2500 µg/mL (MET), along with satisfactory precision and accuracy. The method is suitable for routine quality control and stability studies of fixed-dose combinations; however, it is less appropriate for trace-level or bioanalytical applications [25].

Deepan & Dhanaraju reported a stability-indicating RP-HPLC method for the simultaneous determination of DAPA and SAXA in bulk and tablet dosage forms. Chromatographic separation was achieved using an Xterra C18 column with an isocratic mobile phase of acetonitrile:water (60:40, *v*/*v*), at a flow rate of 1 mL/min, with UV detection at 248 nm. The method provides rapid analysis, with retention times of 2.09 min (DAPA) and 3.25 min (SAXA). The method was validated according to ICH guidelines, demonstrating good linearity (100–500 µg/mL for DAPA and 50–250 µg/mL for SAXA), along with satisfactory precision and accuracy. Forced degradation studies under acidic, basic, oxidative, photolytic, and thermal conditions confirmed the stability-indicating capability, with clear separation of degradation products. The main advantages of this method include its simplicity, short analysis time, and applicability to fixed-dose combinations, making it suitable for routine analysis in modern antidiabetic therapies [26].

The study by Singh et al. reports on the development of another stability-indicating RP-HPLC method for the simultaneous determination of DAPA and SAXA in fixed-dose combination tablets. Chromatographic separation was achieved on an Xterra C18 column using a mobile phase consisting of 20 mM phosphate buffer (pH 5.5):acetonitrile (53:47, v/v), at a flow rate of 1.2 mL/min, with UV detection at 230 nm. The optimized method provided good peak symmetry and resolution, with retention times of 6.1 min (DAPA) and 8.0 min (SAXA). The method was validated in accordance with ICH guidelines, demonstrating good linearity (2–14 µg/mL), as well as acceptable precision and accuracy. Importantly, the method showed good sensitivity, with a LOD of 0.32 µg/mL for DAPA. The study includes an extensive forced degradation evaluation (acidic, basic, oxidative, photolytic, and thermal conditions), confirming the stability-indicating nature of the method [27].

The study by Nasser et al. presents a comparative analytical approach using both RP-HPLC and HPTLC for the simultaneous determination of DAPA and MET in bulk and pharmaceutical formulations. For HPLC analysis, separation was achieved on a C18 column using a mobile phase consisting of 10 mM phosphate buffer (pH 3.5):acetonitrile (65:35, v/v) containing 0.1% triethylamine, with UV detection at 228 nm. The HPTLC method was developed using silica gel 60 F254 plates and a mobile phase consisting of acetonitrile:ammonium acetate:acetic acid (9:0.9:0.1, $v/v/v$), offering a rapid and cost-effective alternative. Both methods were validated according to ICH guidelines, demonstrating good linearity (2–20 µg/mL for DAPA by HPLC and 1–10 µg/spot by HPTLC), as well as satisfactory precision and accuracy. The study highlights that HPTLC exhibits higher sensitivity and throughput, while HPLC provides superior precision and robustness. This work demonstrates complementary chromatographic strategies (HPLC vs. HPTLC) for DAPA analysis and provides practical options for routine quality control laboratories [28].

The study by Manoharan et al. describes the development of a simple RP-HPLC method for the determination of DAPA in bulk and tablet formulations. Chromatographic separation was achieved using a C18 column with a mobile phase consisting of methanol:water (75:25, v/v), at a flow rate of 1.0 mL/min, with UV detection at 230 nm. The method provides rapid analysis, with a retention time of 3.1 min. The method was validated according to ICH guidelines, demonstrating linearity in the range of 5–25 µg/mL, along with good precision and satisfactory recovery. However, several limitations are evident. The reported correlation coefficient ($R \approx 0.925$) is unusually low for a validated HPLC method, raising concerns regarding linearity. Furthermore, the relatively high LOD (2.5 µg/mL) and LOQ (10 µg/mL) values indicate limited sensitivity, while the absence of a comprehensive forced degradation study provides insufficient evidence to support the claimed stability-indicating nature of the method. Such a correlation coefficient would not satisfy contemporary validation requirements and raises serious concerns regarding the reliability of the calibration model [29].

The study by Ameeduzzafar et al. presents a QbD-based bioanalytical RP-HPLC method for the quantification of DAPA in rat plasma, combined with forced degradation and pharmacokinetic evaluation. An important feature of this method is the application of a BBD for the optimization of critical analytical parameters, including mobile phase composition, flow rate, and detection wavelength. This approach enabled the identification of a robust design space and improved method performance in terms of peak area, retention time, and tailing factor. The optimized chromatographic conditions consisted of a C18 column with a mobile phase of acetonitrile:water (70:30, v/v), a flow rate of 0.5 mL/min, and detection at 235 nm, providing a well-defined peak with a retention time of 4.1 min. The method demonstrated good analytical performance, with linearity in the range of 10–1200 ng/mL, high sensitivity (LOD 2.15 ng/mL; LOQ 6.52 ng/mL), and acceptable precision and accuracy. Additionally, the method was shown to be stability-indicating,

as degradation products formed under acidic, basic, oxidative, and photolytic conditions were well resolved from the parent compound. The applicability of the method was further demonstrated through a pharmacokinetic study in rats, highlighting improved bioavailability of DAPA from solid self-nanoemulsifying drug delivery system formulations [30].

The study by Donepudi & Achanta reports an isocratic RP-HPLC method with UV detection for the simultaneous determination of DAPA and SAXA in human plasma, using LINA as an internal standard (IS). Chromatographic separation was achieved on an Eclipse XDB C18 column using a mobile phase consisting of 0.1% orthophosphoric acid (pH 4.5–5.0):acetonitrile (50:50, *v*/*v*), at a flow rate of 1 mL/min, with UV detection at 254 nm. The method enabled adequate separation, with retention times of approximately 2.7 min (LINA), 5.2 min (SAXA), and 7.2 min (DAPA), showing good resolution and absence of matrix interference. Validation demonstrated good linearity over the concentration ranges of 0.05–2 µg/mL for DAPA and 0.01–0.5 µg/mL for SAXA, along with acceptable accuracy and precision. The method showed adequate recovery (78–82%), good robustness across analysts and instruments, and satisfactory stability under various conditions, including freeze–thaw cycles and long-term storage (−28 °C and −80 °C). A notable strength is the simple sample preparation by protein precipitation with acetonitrile, making the method suitable for routine bioanalysis [31].

The study by Kazi et al. describes an RP-UHPLC method with UV/PDA detection for the simultaneous determination of DAPA and SITA in lipid-based self-nanoemulsifying formulations and biological matrices. Chromatographic separation was achieved on an Acquity BEH C18 column using an isocratic mobile phase consisting of methanol:acetonitrile:water (24:18:58, *v*/*v*/*v*), at a flow rate of 0.4 mL/min, with detection at 210 nm. The method provided good chromatographic performance, with well-resolved peaks at retention times of 0.79 min (SITA) and 5.17 min (DAPA). Validation demonstrated good linearity over a wide concentration range (10–10,000 ng/mL), along with satisfactory precision and high accuracy. Importantly, the method showed high sensitivity (LOD of 19 ng/mL for DAPA) and was successfully applied to both pharmaceutical formulations and in vivo pharmacokinetic studies in rat plasma [32].

The study by Gundala et al. describes the development of a QbD-assisted RP-HPLC method for the simultaneous determination of DAPA and SAXA in tablet dosage forms. Chromatographic separation was performed on Discovery C18 column using a mobile phase consisting of acetonitrile:0.1% orthophosphoric acid (50:50, *v*/*v*), at a flow rate of 0.98 mL/min, with detection at 210 nm. The optimized method enabled rapid separation, with retention times of approximately 2.8 min (SAXA) and 3.5 min (DAPA), providing good resolution and peak symmetry. A key feature of this study is the application of DoE using central composite design (CCD) for multivariate optimization of critical parameters, including mobile phase composition, flow rate, and column temperature, allowing establishment of a design space and improved method robustness. Validation demonstrated excellent linearity (25–150 µg/mL for DAPA and 12.5–75 µg/mL for SAXA), high accuracy, and good precision. Sensitivity was acceptable, with LOD values of 0.09 µg/mL and 0.13 µg/mL and LOQ values of 0.27 µg/mL and 0.39 µg/mL for DAPA and SAXA, respectively. The method is simple, rapid, and statistically optimized, with the QbD approach providing enhanced understanding of method performance and robustness, making it suitable for routine quality control analysis [33].

The study by Hassib et al. presents an LC method for the simultaneous determination of SGLT-2 inhibitors, CANA, DAPA, and EMPA, in combination with MET in the presence of its degradation impurity, cyanoguanidine. Chromatographic separation was achieved using an amino column Prontosil NH_2 with an isocratic mobile phase of 10 mM phosphate buffer (pH 2.8):acetonitrile (18.5:81.5, *v*/*v*) at a flow rate of 2 mL/min, and UV detection at 225 nm.

A key methodological aspect is the use of the NH_2 stationary phase, which enables efficient separation of highly polar compounds such as MET and cyanoguanidine, overcoming co-elution issues observed with conventional C18 columns. The method allowed rapid separation, with retention times between 2.45 and 3.25 min for all analytes, demonstrating high efficiency and suitability for routine analysis. Validation results confirmed excellent linearity, high accuracy, and precision, with low LOD values (0.068 µg/mL (MET) and 0.069–0.135 µg/mL for gliflozins). The method is stability-indicating, allowing selective quantification of the active substances in the presence of degradation products, making it suitable for quality control and stability studies [34].

The study by Sharif et al. describes a stability-indicating RP-HPLC-DAD method for the simultaneous determination of CANA, DAPA, and EMPA in pharmaceutical formulations. Chromatographic separation was achieved using a Hypercil™ C18 column with an isocratic mobile phase consisting of acetonitrile:0.1% formic acid buffer (pH 3.7) (60:40, *v*/*v*), at a flow rate of 1 mL/min. Detection was performed using a DAD at 230 nm (DAPA, EMPA) and 290 nm (CANA), allowing both quantification and peak purity assessment. The method achieved complete separation of all analytes and their degradation products within 7 min, confirming its suitability as a stability-indicating method. Validation demonstrated good linearity (4–160 µg/mL), high accuracy, and satisfactory precision, in accordance with ICH guidelines. Forced degradation studies (acidic, basic, oxidative, photolytic, and thermal conditions) confirmed the specificity of the method, with peak purity values <1.5, indicating the absence of co-elution [35].

The study by Gurrala et al. describes an AQbD-assisted RP-HPLC method for the simultaneous determination of DAPA and SAXA in pharmaceutical dosage forms, with application to dissolution and stability studies. Chromatographic separation was achieved on a Spolar C18 column using an isocratic mobile phase consisting of acetonitrile:phosphate buffer (pH 5.8) (26:74, *v*/*v*) at a flow rate of 0.96 mL/min, with UV detection at 236 nm. This method enabled rapid separation of both analytes, with retention times of 3.5 min (DAPA) and 5.0 min (SAXA), and good resolution. Method optimization was performed using CCD and response surface methodology, evaluating the influence of organic phase composition, pH, and flow rate on chromatographic performance. The generated design space ensured robust method performance within defined parameter ranges. Validation demonstrated excellent linearity over wide concentration ranges (0.2–300 µg/mL for DAPA and 0.1–150 µg/mL for SAXA), high precision and accuracy. The method showed good sensitivity (LOD 0.061 µg/mL for DAPA and 0.014 µg/mL for SAXA) and robustness within the design space. Forced degradation studies confirmed the stability-indicating capability of the method, with efficient separation of degradation products under acidic, alkaline, oxidative, photolytic and thermal conditions. Additionally, the method was successfully applied to in vitro dissolution studies, showing approximately 97% release of DAPA and 94% of SAXA within 45 min [36].

The study by Chan-Jiang et al. describes a stability-indicating LC-DAD method for the determination of DAPA in bulk substance and tablet formulations in the presence of its degradation products. Chromatographic separation was achieved on a core-shell Ascentis® Express C18 column using an isocratic mobile phase consisting of acetonitrile:water (35:65, *v*/*v*), with DAD detection at 225 nm. The method enabled the separation of DAPA, propyphenazone (IS), and two degradation products within 6 min, with retention times of 5.2 min (DAPA), 4.7 min (IS), and 2.1–3.1 min (degradation products), demonstrating adequate selectivity. Method validation showed good linearity over the concentration range of 50–150 µg/mL, along with high precision and accuracy, in accordance with ICH guidelines. The method exhibited moderate sensitivity (LOD 0.28 µg/mL, LOQ 0.86 µg/mL) and acceptable robustness, although slight variations in mobile phase composition were found

to affect peak resolution, indicating a certain sensitivity of the method to compositional changes. Forced degradation studies revealed that DAPA is stable under acidic, basic, oxidative, and photolytic conditions, but undergoes degradation under neutral hydrolysis, thermal stress, and combined humidity/thermal conditions, yielding two degradation products identified by LC-MS/MS. The degradation process was consistent with apparent first-order kinetics, with an increased degradation rate under humidity/thermal stress, highlighting the importance of controlled storage conditions. The method was successfully applied to commercial tablet formulations, with assay results close to the labeled content, confirming its suitability for routine pharmaceutical analysis [37].

The study by Bodiwala et al. describes the development of a stability-indicating RP-HPLC method combined with Design of Experiments (DoE) for the investigation of oxidative degradation kinetics of DAPA. Chromatographic separation was performed on C18 column using an isocratic mobile phase of acetonitrile and 0.01% triethylamine (pH 5 adjusted with orthophosphoric acid) in a ratio of 70:30 (v/v), at a flow rate of 1.0 mL/min, with UV detection at 220 and 270 nm. The method enabled efficient separation of DAPA and its degradation products within a short runtime (10 min), ensuring good selectivity under various stress conditions (acidic, alkaline, oxidative, and photolytic). Validation demonstrated good linearity over the range of 1–60 µg/mL, high precision, and good accuracy. The method showed adequate sensitivity (LOD 0.07 µg/mL, LOQ 0.22 µg/mL) and robustness against small variations in chromatographic conditions. Forced degradation studies confirmed that DAPA undergoes degradation under acidic, alkaline, oxidative, and photolytic conditions, with the highest degradation observed in oxidative media. Kinetic analysis revealed that oxidative degradation follows first-order kinetics, with degradation rate strongly influenced by temperature and hydrogen peroxide concentration, as demonstrated by full factorial DoE modeling and response surface analysis [38].

The study by Vankalapati et al. describes a stability-indicating RP-HPLC method for the simultaneous determination of DAPA, MET, and SAXA in bulk and tablet dosage forms. Chromatographic separation was performed on Kromasil C18 column using a mobile phase consisting of phosphate buffer (pH 3.0):acetonitrile (60:40, v/v), at a flow rate of 1.0 mL/min, with detection at 230 nm. The method enabled rapid separation, with retention times of 2.17 min (MET), 2.68 min (DAPA), and 3.45 min (SAXA). A representative RP-HPLC chromatogram of DAPA, MET, and SAXA separation under optimized conditions is shown in Figure 2. Validation demonstrated good linearity over wide concentration ranges (1.25–7.5 µg/mL for DAPA, 125–750 µg/mL for MET, and 0.625–3.75 µg/mL for SAXA), along with high precision and accuracy. Forced degradation studies under acidic, basic, hydrolytic, oxidative, photolytic, and thermal conditions demonstrated significant degradation, particularly under acid, base, and oxidative stress, while maintaining adequate separation from degradation products, confirming the stability-indicating nature of the method. Robustness testing indicated that small variations in flow rate, mobile phase composition, and temperature did not significantly affect assay results. The method was successfully applied to commercial tablets, confirming its suitability for routine quality control analysis of the fixed-dose triple combination [39].

The study by Sunkara & Tummalapalli describes the development of a stability-indicating gradient RP-HPLC method for the determination of DAPA in the presence of its process-related impurities (5-BC and 4-BC) and degradation products, supported by LC-MS identification. Chromatographic separation was performed on XBridge Phenyl C18 column using a gradient elution with 0.05% trifluoroacetic acid (aqueous phase) and acetonitrile, at a flow rate of 1.0 mL/min, with UV detection at 210 and 240 nm. The optimized method enabled efficient separation of DAPA and its impurities, with retention times of approximately 7.3 min (DAPA), 7.9 min (5-BC), and 17.1 min (4-BC), ensuring good

selectivity and peak resolution. The method exhibited very high sensitivity for impurity detection, with LOD values in the low ppm range (0.000053 ppm and 0.0000165 ppm) and corresponding LOQ values (0.00016 ppm and 0.00005 ppm) for 4-BC impurity and 5-BC impurity, respectively, making it suitable for trace-level impurity profiling. Forced degradation studies revealed that DAPA undergoes significant degradation under acidic and basic hydrolysis (~96%), forming a common degradation product (m/z 320.42), while remaining stable under oxidative, photolytic and thermal conditions. LC-MS analysis enabled characterization of degradation products and proposed fragmentation pathways, providing insight into the degradation mechanism [40].

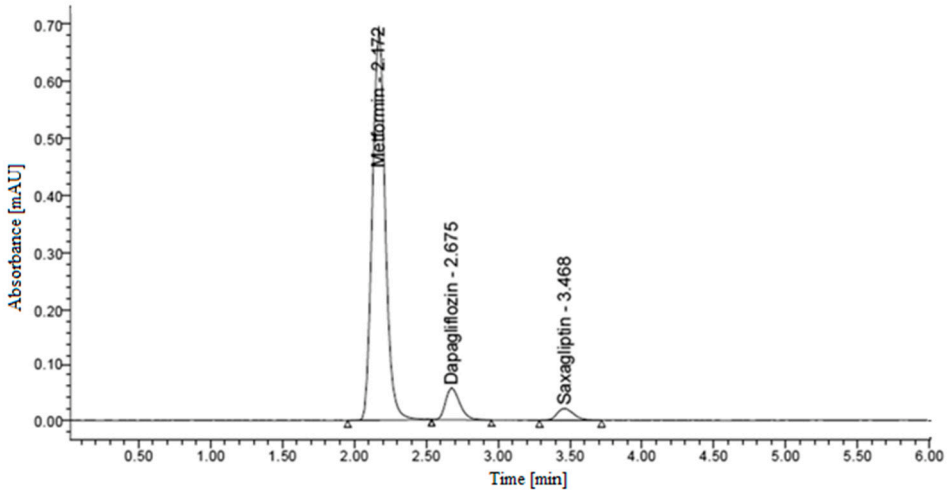

Figure 2. Typical chromatogram of a standard mixture containing DAPA, MET, and SAXA obtained under optimized RP-HPLC conditions. Chromatographic conditions: Kromasil C18 column (150 × 4.6 mm, 5 µm), mobile phase: phosphate buffer (pH 3.0):acetonitrile (60:40, v/v), flow rate 1.0 mL/min, column temperature 30 °C, UV detection at 230 nm. Reprinted from Vankalapati et al., 2022 [39] with permission from Wiley.

The study by Kashyap et al. reports the development of a stability-indicating RP-HPLC method for the simultaneous determination of DAPA and TENE in tablet formulations. Chromatographic separation was achieved using a Zorbax Eclipse Plus C18 column. The mobile phase consisted of 10 mM ammonium acetate buffer (pH 6.3):methanol:acetonitrile (40:50:10, $v/v/v$), delivered at a flow rate of 0.6 mL/min, with detection at 224 nm. The method provided good chromatographic resolution, with retention times of approximately 6.6 min (TENE) and 12.6 min (DAPA). Linearity was demonstrated over concentration ranges of 12.5–50 µg/mL for DAPA and 50–150 µg/mL for TENE. Sensitivity was adequate, with LOD values of 0.5 and 2 µg/mL and corresponding LOQ values of 1.56 and 6.25 µg/mL for DAPA and TENE, respectively. Robustness testing indicated that small variations in chromatographic conditions did not significantly affect analytical performance. Forced degradation studies demonstrated that DAPA is stable under all tested conditions, whereas TENE undergoes degradation under acidic, alkaline, and oxidative stress. Degradation products of TENE were structurally characterized using LC–MS/MS, with major degradation products identified at m/z 356 (DP1) and 443 (DP2), enabling the proposal of degradation pathways [41].

The study by Prajapati et al. reports the development of a green RP-HPLC method based on White Analytical Chemistry (WAC) and AQbD for the simultaneous determination of DAPA and VILD in fixed-dose combinations. Chromatographic separation was performed using a Shim-Pack C18 column. The mobile phase consisted of ethanol:water (60:40, v/v), adjusted to pH 3.0 with orthophosphoric acid, representing a greener alter-

native to conventional acetonitrile- or methanol-based systems. The flow rate was set at 0.8 mL/min, with detection at 210 nm using a UV/PDA detector. The method achieved rapid separation, with retention times of approximately 2.34 min (VILD) and 3.85 min (DAPA). Linearity was demonstrated over concentration ranges of 10–50 µg/mL for DAPA and 100–500 µg/mL for VILD. Sensitivity was high, with LOD values of 0.0223 µg/mL and 0.0332 µg/mL and corresponding LOQ values of 0.0669 µg/mL and 0.0996 µg/mL for DAPA and VILD, respectively. Robustness testing indicated no significant influence of small variations in mobile phase composition, flow rate, temperature, or wavelength. The method was successfully applied to tablet formulations, with assay results within 95–105% of the labeled claim. A key innovation is the integration of AQbD (DoE, risk assessment, design space) and WAC evaluation (RGB model, AGREE, GAPI), demonstrating improved greenness and robustness compared to conventional RP-HPLC methods [42].

Another study by Prajapati et al. describes a stability-indicating RP-HPLC method for the simultaneous determination of DAPA and LINA in pharmaceutical formulations, coupled with LC-MS/MS identification of degradation products. Chromatographic separation was performed on Eclipse Plus C18 column using gradient elution with 0.1% formic acid (aqueous phase) and acetonitrile, at a flow rate of 0.7 mL/min, with detection at 225 nm. The method provided efficient separation of analytes and degradation products, with retention times of 9.44 min (LINA) and 13.29 min (DAPA), ensuring good selectivity and peak purity. Validation demonstrated good linearity over the ranges of 50–150 µg/mL for DAPA and 25–75 µg/mL for LINA, along with acceptable precision and accuracy, in accordance with ICH guidelines. Sensitivity was moderate, with LOD values of 4 and 5 µg/mL and corresponding LOQ values of 12 and 15 µg/mL for LINA and DAPA, respectively. The method proved to be stability-indicating, as forced degradation studies under acidic, alkaline, oxidative, photolytic, and thermal conditions showed significant degradation, particularly for LINA, while maintaining clear separation from degradation products. LC-MS/MS analysis enabled the structural elucidation of multiple degradation products, revealing complex degradation pathways for LINA, whereas DAPA showed higher stability under the tested conditions. Interestingly, the presence of DAPA influenced the degradation behavior of LINA, suggesting possible drug–drug interactions affecting degradation pathways [43].

The study by Shah & Kotadiya describes a stability-indicating RP-HPLC method for the simultaneous determination of DAPA, MET, and LINA in pharmaceutical formulations. Chromatographic separation was performed on Phenomenex Luna C18 column using a mobile phase consisting of acetonitrile:phosphate buffer (pH 6.8) (40:60, *v*/*v*), modified with triethylamine and orthophosphoric acid, at a flow rate of 0.8 mL/min, with detection at 230 nm. The method provided well-resolved peaks with retention times of approximately 3.09 min (MET), 5.97 min (LINA), and 11.71 min (DAPA), ensuring adequate resolution and peak symmetry. Validation demonstrated good linearity over the ranges of 0.6–2.8 µg/mL (DAPA), 20–140 µg/mL (MET), and 0.2–1.4 µg/mL (LINA), along with high precision and accuracy, in accordance with ICH guidelines. Sensitivity was acceptable, with LOD values of 0.16 µg/mL, 6.20 µg/mL, and 0.03 µg/mL and corresponding LOQ values of 0.47 µg/mL, 18.8 µg/mL, and 0.09 µg/mL for DAPA, MET, and LINA, respectively. Forced degradation studies under acidic, basic, oxidative, photolytic, and thermal conditions demonstrated significant degradation, particularly under acidic conditions, while maintaining clear separation between analytes and degradation products, confirming the stability-indicating capability of the method. The method was successfully applied to commercial tablets, with assay values of approximately 100%, confirming its suitability for routine quality control analysis [44].

The study by Marie et al. reported a stability-indicating green micellar liquid chromatography (MLC) method for the simultaneous determination of DAPA and MET in pharmaceutical tablets. Chromatographic separation was achieved on a BDS Thermo-Hypersil C8 column using a hybrid micellar mobile phase composed of sodium dodecyl sulfate (SDS), 2-propanol, triethylamine, and water adjusted to pH 3.3, providing complete separation of both analytes within 10 min. The optimized method showed satisfactory analytical performance, with validated linearity, accuracy, precision, selectivity, and robustness according to ICH recommendations. Stability-indicating capability was demonstrated through forced degradation studies performed under acidic, alkaline, oxidative, photolytic, and thermal stress conditions, with successful resolution of the analytes from their degradation products. Furthermore, the environmental impact of the procedure was evaluated using the Analytical Eco-Scale, AGREE, and ComplexMoGAPI tools, confirming the greener character of the method compared with conventional RP-HPLC approaches relying on higher organic solvent consumption [45].

A comparative summary of LC-based analytical methods reported for the determination of DAPA in single and combined pharmaceutical formulations, as well as biological matrices, is presented in Table 2.

Table 2. Summary of RP-HPLC and other LC-based methods for the determination of DAPA in pharmaceutical formulations and biological matrices.

Analytical Technique	Analytical Conditions	Matrix	Analytical Performance (DAPA)	Substances	Reference
RP-HPLC (UV detection)	C18 column (150 × 4.6 mm, 5 µm); mobile phase: acetonitrile:0.1% orthophosphoric acid (70:30, pH 3.0); isocratic; flow 1.0 mL/min; detection at 260 nm; RT: MET 2.1 min, DAPA 3.7 min	Tablets (fixed-dose combination)	linearity: 5–25 µg/mL LOD: 2.98 µg/mL LOQ: 9.98 µg/mL Precision (%RSD): 1.2% Stability-indicating	DAPA, MET	[25]
RP-HPLC (UV detection)	Xterra C18 column (150 × 4.6 mm, 5 µm); mobile phase: acetonitrile:water (60:40); isocratic; flow 1.0 mL/min; detection at 248 nm; RT: DAPA 2.09 min, SAXA 3.25 min	Bulk and tablets (fixed-dose combination)	linearity: 100–500 µg/mL LOD: 3.00 µg/mL LOQ: 9.98 µg/mL Precision (%RSD): 0.4–0.8% Stability-indicating	DAPA, SAXA	[26]
RP-HPLC (UV detection)	Xterra C18 column (150 × 4.6 mm, 3.5 µm); mobile phase: 20 mM phosphate buffer (pH 5.5):acetonitrile (53:47, *v*/*v*); flow 1.2 mL/min; detection at 230 nm; RT: DAPA 6.1 min, SAXA 8.0 min	Tablets (fixed-dose combination)	linearity: 2–14 µg/mL LOD: 0.32 µg/mL LOQ: 0.97 µg/mL Precision (%RSD): <2% Stability-indicating	DAPA, SAXA	[27]
RP-HPLC (UV detection)	C18 column (250 × 4.6 mm, 5 µm); mobile phase: 10 mM phosphate (pH 3.5):acetonitrile (65:35, *v*/*v*) + 0.1% triethylamine; flow 1.2 mL/min; detection at 228 nm	Bulk and tablets (fixed-dose combination)	linearity: 2–20 µg/mL LOD: 0.58 µg/mL LOQ: 1.78 µg/mL Precision (%RSD): 0.23–0.93% Stability-indicating	DAPA, MET	[28]
RP-HPLC (UV detection)	C18 column (150 × 4.6 mm, 5 µm); mobile phase: methanol:water (75:25, *v*/*v*); flow 1.0 mL/min; detection at 230 nm; RT: DAPA 3.1 min	Bulk and tablets	linearity: 5–25 µg/mL LOD: 2.5 µg/mL LOQ: 10.0 µg/mL Precision (%RSD): 0.42%	DAPA	[29]
RP-HPLC (UV detection, QbD-optimized)	C18 column (250 × 4.6 mm, 5 µm); mobile phase: acetonitrile:water (70:30, *v*/*v*); flow 0.5 mL/min; detection at 235 nm; RT: DAPA 4.1 min; BBD optimization (acetonitrile %, flow, wavelength)	Rat plasma (bioanalysis; pharmacokinetic study)	linearity: 10–1200 ng/mL LOD: 2.15 µg/mL LOQ: 6.52 µg/mL Precision (%RSD): 0.24–3.98% Stability-indicating	DAPA	[30]
RP-HPLC (UV detection, with IS)	Eclipse XDB C18 column (150 × 4.6 mm, 5 µm); mobile phase: 0.1% phosphoric acid (pH 4.5–5.0):acetonitrile (50:50, *v*/*v*); flow 1 mL/min; detection at 254 nm; RT: SAXA 5.2 min, DAPA 7.2 min; I.S: LINA	Human plasma	linearity: 0.05–2.00 µg/mL LLOQ: 0.05 µg/mL LOQ: 0.05 µg/mL Precision (%RSD): 4.35–7.90%	DAPA, SAXA	[31]
RP-UHPLC (UV/PDA detection)	BEH C18 column (2.1 × 50 mm, 1.7 µm); mobile phase methanol:acetonitrile:water (24:18:58); flow 0.4 mL/min; detection 210 nm; RT: SITA 0.79 min, DAPA 5.17 min	Lipid-based nanoemulsions, tablets (fixed-dose combination), plasma	linearity: 10–10,000 ng/mL LOD: 19 ng/mL LOQ: 61 ng/mL Precision (%RSD): ≤4.25%	DAPA, SITA	[32]

Table 2. *Cont.*

Analytical Technique	Analytical Conditions	Matrix	Analytical Performance (DAPA)	Substances	Reference
RP-HPLC (QbD-optimized)	Discovery C18 column (250 × 4.6 mm, 5 µm); mobile phase: acetonitrile:0.1% orthophosphoric acid (50:50, *v*/*v*); flow 0.98 mL/min; detection at 210 nm; RT: SAXA 2.8 min, DAPA 3.5 min; CCD (DoE) optimization (mobile phase %, flow, temperature)	Tablets (fixed-dose combination)	linearity: 25–150 µg/mL LOD: 0.09 µg/mL LOQ: 0.27 µg/mL Precision (%RSD): 0.40–0.60	DAPA, SAXA	[33]
HPLC (UV detection)	Prontosil NH_2 column (250 × 4.6 mm, 5 µm); mobile phase 10 mM phosphate (pH 2.8):acetonitrile (18.5:81.5); flow 2 mL/min; detection 225 nm	Tablets (fixed-dose combination)	linearity: 0.3075–2.46 µg/mL LOD: 0.077 µg/mL LOQ: 0.233 µg/mL Precision (%RSD): 0.28–1.31 Stability-indicating	CANA, DAPA, EMPA, MET, + cyanoguanidine	[34]
RP-HPLC (DAD detection)	Hypercil™ C18 column (250 × 4.6 mm, 5 µm); acetonitrile:0.1% formic acid (pH 3.7) (60:40); flow 1 mL/min; detection 230/290 nm	Tablets	linearity: 4–160 µg/mL LOD: 0.12 µg/mL LOQ: 0.34 µg/mL Precision (%RSD): 0.5–1.2 Stability-indicating	CANA, DAPA, EMPA	[35]
RP-HPLC (stability-indicating, AQbD/DoE optimized)	SPOLAR C18 column (250 × 4.6 mm, 5 µm); acetonitrile:phosphate buffer (pH 5.8) (26:74); flow 0.96 mL/min; detection 236 nm	Tablets (fixed-dose combination), dissolution + stability	linearity: 0.2–300 µg/mL LOD: 0.061 µg/mL LOQ: 0.18 µg/mL Precision (%RSD): 1.05–1.15 Stability-indicating	DAPA, SAXA	[36]
LC–DAD (stability-indicating)	Core–shell C18 column (150 × 4.6 mm, 5 µm); acetonitrile:water (35:65); detection 225 nm; RT: DAPA 5.1 min	Tablets	linearity: 50–150 µg/mL LOD: 0.28 µg/mL LOQ: 0.86 µg/mL Precision (%RSD): 0.85–1.65 Stability-indicating	DAPA	[37]
RP-HPLC (stability-indicating, kinetic/DoE study)	C18 column (150 × 4.6 mm, 5 µm); mobile phase: acetonitrile:0.01% triethylamine (pH 5.0) (70:30); flow 1.0 mL/min; detection 220/270 nm	Bulk/degradation studies	linearity: 1–60 µg/mL LOD: 0.07 µg/mL LOQ: 0.22 µg/mL Precision (%RSD): 0.59–0.95% Stability-indicating	DAPA (degradation kinetics)	[38]
RP-HPLC–DAD (stability-indicating)	Kromasil C18 column (150 × 4.6 mm, 5 µm); phosphate buffer pH 3.0:acetonitrile (60:40); detection 230 nm; RT: MET 2.33 min, DAPA 2.89 min, SAXA 3.78 min	Tablets (fixed-dose combination)	linearity: 1.25–7.5 µg/mL LOD: NR LOQ: NR Precision (%RSD): 0.4 Stability-indicating	DAPA, MET, SAXA	[39]
RP-HPLC (gradient, stability-indicating, LC–MS supported)	XBridge Phenyl C18 column (250 × 4.6 mm, 5 µm); mobile phase: 0.05% trifluoroacetic acid (aqueous):acetonitrile (gradient); flow 1.0 mL/min; detection 210–240 nm; RT: DAPA 7.3 min, 7.9 min (impurity 5-BC), 17.1 min (impurity 4-BC)	Bulk (impurity profiling)	Impurity profiling LOD: 0.0000627 ppm (impurities) LOQ: 0.00019 ppm (impurities) Precision (%RSD): 0.4 Stability-indicating	DAPA + process impurities (5-BC, 4-BC)	[40]
RP-HPLC–PDA + LC-MS/MS (stability-indicating)	Zorbax Eclipse Plus C18 column (150 × 4.6 mm, 5 µm); ammonium acetate buffer:methanol:acetonitrile (40:50:10); flow 0.6 mL/min; detection 224 nm; RT: TENE 6.6 min, DAPA 12.6 min	Tablets (fixed-dose combination)	linearity: 12.5–50 µg/mL LOD: 0.50 µg/mL LOQ: 1.56 µg/mL Precision (%RSD): 1.67 Stability-indicating	DAPA, TENE	[41]
RP-HPLC–UV/PDA (WAC + AQbD optimized)	Shim-Pack C18 column (250 × 4.6 mm, 5 µm); ethanol:water (60:40, pH 3.0); flow 0.8 mL/min; detection 210 nm; RT: VILD 2.34 min, DAPA 3.85 min	Tablets (fixed-dose combination)	linearity: 10–50 µg/mL LOD: 0.0223 µg/mL LOQ: 0.0669 µg/mL Precision (%RSD): 0.56–0.79 greenness evaluated by AGREE, GAPI and RGB models	DAPA, VILD	[42]
RP-HPLC–PDA (LC-MS/MS supported)	Eclipse Plus C18 column (150 × 4.6 mm, 5 µm); gradient 0.1% formic acid:acetonitrile; flow 0.7 mL/min; detection 225 nm; RT: LINA 9.44, DAPA 13.29	Tablets	linearity: 50–150 µg/mL LOD: 5 µg/mL LOQ: 15 µg/mL Precision (%RSD): 0.75–1.74 Stability-indicating	DAPA, LINA	[43]
RP-HPLC–UV (stability-indicating)	Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm); acetonitrile:phosphate buffer (pH 6.8) (40:60); flow 0.8 mL/min; detection 230 nm; RT: MET 3.09, LINA 5.97, DAPA 11.71	Tablets	linearity: 0.6–2.8 µg/mL LOD: 0.16 µg/mL LOQ: 0.47 µg/mL Precision (%RSD): 1.31–1.62 Stability-indicating	DAPA, MET, LINA	[44]
MLC-PDA (stability-indicating)	BDS Thermo-Hypersil C8 column (150 × 4.6 mm, 5 µm); hybrid micellar mobile phase SDS:2-propanol: triethylamine (pH 3.3); flow 1.0 mL/min; detection 223 nm; RT: DAPA 5.37, MET 7.64	Tablets	linearity: 1–100 µg/mL LOD: 0.16 µg/mL LOQ: 0.49 µg/mL Precision (%RSD): ≤1.86% Stability-indicating greenness assessed using AGREE, GAPI and RGB/WAC tool	DAPA, MET	[45]

RP-HPLC methods with UV/DAD detection remain the most widely applied techniques for DAPA analysis due to their robustness, accessibility, and suitability for stability studies, although their sensitivity and selectivity are inferior to LC-MS-based approaches.

3.3. LC-MS/MS-Based Bioanalytical Methods

The study by Aubry et al. presents one of the first validated LC-MS/MS bioanalytical methods for the quantification of DAPA in rat plasma, developed to support preclinical pharmacokinetic studies. The method involves solid-phase extraction (SPE) followed by LC-MS/MS detection using electrospray ionization (ESI) in negative mode, which provided improved sensitivity compared to positive ionization. A key analytical challenge, adduct formation in the presence of formic or acetic acid, was addressed by employing a simple acetonitrile:water mobile phase, enhancing signal stability and reproducibility. Method validation demonstrated good linearity (5–2000 ng/mL), precision, and accuracy, with acceptable recovery. Matrix effects were effectively compensated using a stable isotope-labeled IS. An additional strength of the study is the comparison between gradient and isocratic elution modes, providing flexibility for different analytical setups. However, several limitations should be considered: the method is restricted to rat plasma, limiting direct clinical applicability; furthermore, matrix effects are mitigated rather than eliminated, and the method focuses exclusively on the parent compound, with no comprehensive evaluation of metabolites [46].

The study by Shah et al. describes a validated LC-MS/MS method for the simultaneous quantification of DAPA and MET in human plasma. The method employs ion-pair SPE followed by RP-LC separation on an ACE 5CN column using an acetonitrile:15 mM ammonium acetate buffer (pH 4.5) (70:30, v/v) mobile phase. Detection was performed using a triple quadrupole (QTRAP) mass spectrometer operating in multiple reaction monitoring (MRM) mode, with polarity switching to accommodate the different ionization behaviors of DAPA (negative ESI via acetate adduct formation) and MET (positive ESI). The method demonstrated excellent analytical performance, with wide linearity ranges (0.1–200 ng/mL for DAPA and 1–2000 ng/mL for MET), high sensitivity (LOQ of 0.1 ng/mL for DAPA), and good precision and accuracy. The use of stable isotope-labeled IS effectively compensated for matrix effects, while the optimized ion-pair SPE procedure ensured consistent recoveries (78% for DAPA and 86% for MET). As shown in Figure 3, DAPA and MET exhibit distinct fragmentation patterns under electrospray ionization, enabling the selection of specific MRM transitions, m/z 467.1 $\rightarrow$ 329.1 (DAPA) and m/z 130.1 $\rightarrow$ 60.1 (MET), which are critical for achieving high selectivity in LC–MS/MS-based bioanalysis. A key strength of this method is the simultaneous extraction and quantification of two compounds with markedly different polarity, which represents a significant analytical challenge. The use of sodium dodecyl sulfate (SDS) as an ion-pairing agent significantly improved MET retention during extraction, highlighting an important methodological innovation. The method was successfully applied to a pharmacokinetic study in human subjects, demonstrating its robustness and practical applicability. Moreover, the short analysis time (4 min) and the low plasma volume required (50 µL) enhance its suitability for high-throughput bioanalysis. However, matrix effects are not fully eliminated but rather compensated through internal standardization, and the method remains inherently targeted, limiting its applicability for impurity profiling or non-target screening [47].

The study by El-Zaher et al. presents a validated LC-MS/MS bioanalytical method for the simultaneous determination of DAPA, MET, and SAXA in human plasma, designed for pharmacokinetic and bioequivalence studies. Chromatographic separation was achieved using a Zorbax C18 column with an acetonitrile:0.1% formic acid (45:55, v/v) mobile phase, coupled with tandem MS detection in positive ESI mode using MRM transitions (DAPA

m/*z* 407 → 329). The method shows high efficiency, with short retention times (2.45 min for DAPA) and rapid overall analysis. A notable strength of this method is the optimized dual extraction strategy, combining liquid–liquid extraction (LLE) (DAPA and SAXA) with protein precipitation (MET), addressing the significant differences in polarity between analytes. Validation demonstrated excellent analytical performance, with high sensitivity (LOQ—5 ng/mL for DAPA), wide linearity (5–500 ng/mL); precision, accuracy, recovery, and stability. The major advantage of this method is its ability to simultaneously quantify three antidiabetic drugs in human plasma, including newly introduced ternary combinations, which represent a clear advancement over earlier methods analyzing single compounds or binary mixtures. Limitations include the relatively complex sample preparation (two-step extraction) and the requirement for specialized LC–MS/MS instrumentation, which may limit routine applicability in quality control laboratories [48].

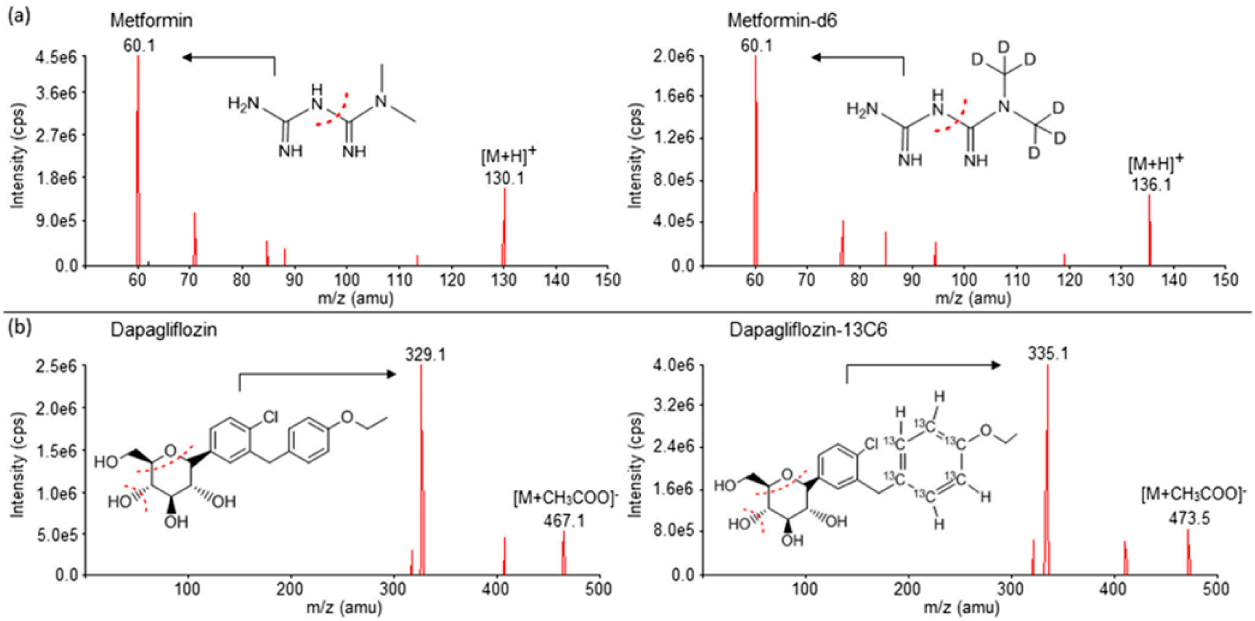

Figure 3. Representative product ion mass spectra (MRM transitions) of (**a**) MET (*m*/*z* 130.1 → 60.1) and MET-d6 (*m*/*z* 136.1 → 60.1) obtained in ESI+ mode, and (**b**) DAPA (*m*/*z* 467.1 → 329.1) and DAPA-$^{13}C_6$ (*m*/*z* 473.5 → 335.1) obtained in ESI− mode. Spectra were acquired using a triple quadrupole LC-MS/MS system operated in polarity-switching mode, with chromatographic separation performed on an ACE 5CN column (50 × 4.6 mm, 5 µm) using an acetonitrile:15 mM ammonium acetate mobile phase (70:30, *v*/*v*). Reprinted from Shah et al., 2019 [47] with permission from Wiley.

The study by Surendran et al. describes the development and validation of a stability-indicating LC-MS/MS method for the simultaneous determination of DAPA and SAXA in rat plasma, using a polarity-switching approach to accommodate their different ionization behaviors. Chromatographic separation was achieved on an Eclipse Plus C18 column with a gradient mobile phase consisting of 0.01% ammonia solution:acetonitrile, at a flow rate of 1.0 mL/min. Detection was performed on QTRAP mass spectrometer operating in MRM mode, with negative ESI for DAPA and positive ESI for SAXA. The optimized method provided good chromatographic performance, with retention times of 2.04 min (SAXA) and 4.81 min (DAPA). The method was validated according to US FDA bioanalytical guidelines, demonstrating good linearity over the ranges of 5–2000 ng/mL (DAPA) and 0.2–80 ng/mL (SAXA), along with acceptable precision and accuracy. Sensitivity was high, with LLOQ values of 0.2 ng/mL (SAXA) and 5 ng/mL (DAPA). SPE provided high and consistent recoveries (84% for SAXA and 92% for DAPA) with negligible matrix effects. A key strength of this method is the implementation of polarity switching within a single analytical run,

enabling simultaneous quantification of analytes with opposite ionization preferences without compromising sensitivity or peak shape. The method was successfully applied to a pharmacokinetic study in rats, confirming its robustness and applicability for preclinical bioanalysis [49].

The study by van der Aart-van der Beek et al. reports a simple, fast, and robust LC-MS/MS method for the simultaneous quantification of SGLT-2 inhibitors, CANA, DAPA, and EMPA, in human plasma, serum, and urine. Chromatographic separation was achieved using a Waters ACQUITY UPLC HSS T3 column with gradient elution of 20 mM ammonium acetate (pH 5.0):acetonitrile, at a flow rate of 0.8 mL/min, coupled with negative ESI and MRM detection. A major advantage of this method is the extremely short runtime of only 1 min, with retention times of approximately 0.74 min for DAPA, enabling very high-throughput analysis. Sample preparation is simplified through protein precipitation using methanol, requiring only 200 µL plasma, which further enhances method efficiency. The method demonstrated excellent analytical performance, with wide linearity (1–500 µg/L for DAPA), good correlation, high accuracy, and precision within regulatory limits. The method was validated across multiple biological matrices (plasma, serum, urine) and successfully applied in pharmacokinetic studies, confirming its clinical applicability [50].

A summary of representative LC-MS/MS-based bioanalytical methods developed for the determination of DAPA in biological matrices is presented in Table 3.

Table 3. Summary of LC-MS/MS-based bioanalytical methods for the determination of DAPA in biological matrices.

Analytical Technique	Analytical Conditions	Matrix	Analytical Performance (DAPA)	Substances	Reference
LC-MS/MS (ESI−, MRM)	SPE; C18 column (50 × 2.1 mm, 5 µm); mobile phase: water:acetonitrile; gradient (20–80% acetonitrile) or isocratic (60:40 water:acetonitrile); flow 0.3 mL/min; negative ESI; transitions m/z 407 → 329; I.S: 13C6-DAPA	Rat plasma (preclinical samples)	linearity: 5–2000 ng/mL LLOQ: 5 ng/mL Precision (%RSD): 1.19–7.97	DAPA; IS (13C6-DAPA)	[46]
LC-MS/MS (ESI−/+, MRM, ion-pair SPE)	ACE 5CN column (50 × 4.6 mm, 5 µm); mobile phase: acetonitrile:15 mM ammonium acetate (pH 4.5) (70:30, v/v); flow 0.3 mL/min; polarity switching (ESI− for DAPA via acetate adduct, ESI+ for MET); MRM; ion-pair SPE (SDS); IS: stable isotope-labeled	Human plasma (pharmacokinetic study)	linearity: 0.10–200 ng/mL LOD: 0.03 ng/mL LOQ: 0.10 ng/mL Precision (%RSD): 1.4–4.0	DAPA, MET	[47]
LC-MS/MS (ESI+, MRM)	Zorbax C18 column (50 × 4.6 mm, 5 µm); mobile phase: acetonitrile:0.1% formic acid (45:55, v/v); flow 0.5 mL/min; positive ESI; MRM transitions: DAPA m/z 407 → 329; dual extraction (LLE + protein precipitation); RT: DAPA 2.45 min	Human plasma (bioanalysis; pharmacokinetic study)	linearity: 5–500 ng/mL LLOQ: 5 ng/mL Precision (%RSD): 8.88–10.65	DAPA, MET, SAXA	[48]
LC–MS/MS (ESI+/−, MRM, polarity switching, SPE)	Eclipse Plus C18 column (150 × 4.6 mm, 3.6 µm); mobile phase: 0.01% ammonia:acetonitrile; gradient elution; flow 1.0 mL/min; polarity switching (ESI+ for SAXA, ESI− for DAPA); MRM transitions: SAXA m/z 316 → 180, DAPA m/z 407 → 329; SPE; RT: 2.04 min (SAXA), 4.81 min (DAPA)	Rat plasma (preclinical pharmacokinetic study)	linearity: 5–2000 ng/mL LLOQ: 5 ng/mL Precision (%RSD): 1.71–8.23	DAPA, SAXA (IS: EMPA, VILD)	[49]
LC-MS/MS (ESI−, MRM, gradient)	UPLC HSS T3 column (2.1 × 50 mm, 1.8 µm); mobile phase: 20 mM ammonium acetate (pH 5.0):acetonitrile; flow 0.8 mL/min; negative ESI; MRM; protein precipitation (methanol)	Human plasma, serum, urine	linearity: 1–500 µg/L LLOQ: 1 µg/L Precision (%RSD): 13.7	CANA, DAPA, EMPA	[50]

LC–MS/MS methods represent the gold standard for DAPA quantification in biological matrices, offering superior sensitivity, selectivity, and suitability for pharmacokinetic

studies, despite higher cost, methodological complexity, and limited applicability for routine quality control.

Matrix effects remain one of the major challenges in LC-MS/MS bioanalysis, particularly when analyzing complex biological matrices. Although isotopically labeled internal standards represent the most effective strategy for compensating ion suppression or enhancement, other approaches such as optimized sample preparation, solid-phase extraction, chromatographic separation of interfering components, sample dilution, and optimization of ionization conditions may also contribute to minimizing matrix-related effects.

3.4. Electromigration Techniques

The study by Maher et al. presents a stability-indicating CE method with DAD detection for the simultaneous determination of DAPA, MET, and SAXA in pharmaceutical tablets. Separation was performed using a fused silica capillary (65 cm total length, 75 μm ID) with a 30 mM phosphate buffer (pH 6.0) as background electrolyte (BGE), with detection at 203 nm (DAPA, SAXA) and 250 nm (MET). The method enabled efficient separation within a short migration window (2.7–4.4 min), with good peak symmetry and high efficiency. Validation demonstrated good linearity over wide concentration ranges (1.25–50 μg/mL for DAPA, 7.5–1000 μg/mL for MET, and 10–200 μg/mL for SAXA), along with high precision and accuracy. A key strength of the method is its stability-indicating capability, as degradation products formed under acidic, basic, oxidative, photolytic, and thermal conditions were well separated from the parent compounds. The method is characterized by low solvent consumption and environmentally friendly conditions, reflecting the inherent advantages of CE techniques. However, the method exhibits moderate sensitivity and is primarily applicable to pharmaceutical formulations, with limited suitability for biological matrices [51].

The study by Bueno et al. presents a green CE method for the determination of DAPA in pharmaceutical tablets, developed using a DoE optimization approach. Separation was performed on fused silica capillary (48.5 cm total length, 50 μm ID) using a 40 mM sodium tetraborate buffer (pH 10.5) as BGE, selected to ensure improved peak shape and migration behavior. Ranitidine was employed as an IS to enhance precision and correct migration time variability. The optimized method provided migration times of approximately 5.0 min (IS) and 6.5 min (DAPA), with good resolution. As shown in Figure 4, the electropherograms demonstrate a well-resolved and symmetrical peak for DAPA, while no interfering signals are observed in the excipient matrix, highlighting the selectivity of the method. Linearity was demonstrated over the range of 50–175 μg/mL, while sensitivity was moderate, with LOD and LOQ values of 6.2 μg/mL and 18.8 μg/mL, respectively. Robustness testing confirmed that small variations in capillary temperature, separation voltage, and injection time did not significantly affect method performance. The method showed no interference from excipients or degradation products, demonstrating good selectivity under stress conditions. In addition, the use of aqueous buffers and minimal solvent consumption highlights the green analytical character of the method. However, the method exhibits limited sensitivity compared to LC-based techniques and is primarily suitable for pharmaceutical formulations rather than trace-level or bioanalytical applications [52].

A summary of representative CE-based methods reported for the determination of DAPA in pharmaceutical formulations is presented in Table 4.

Electromigration techniques offer a green and cost-effective alternative for DAPA analysis, although their applicability may be limited by lower sensitivity and challenges in separating neutral compounds.

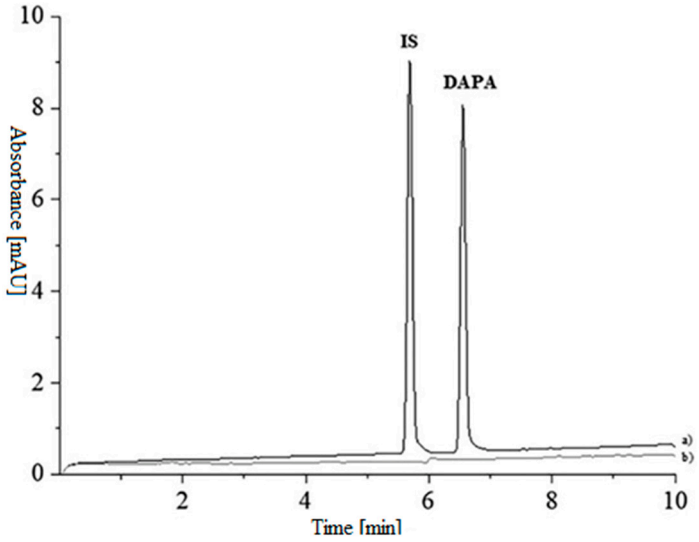

Figure 4. Representative electropherograms obtained for (a) a standard solution containing DAPA (75 µg/mL) and IS (75 µg/mL) and (b) excipient solution under optimized CE conditions. Electrophoretic conditions: fused silica capillary (48.5 cm total length, 40 cm effective length, 50 µm I.D.), 40 mM sodium tetraborate BGE, pH 10.5, separation voltage 14 kV, capillary temperature 25 °C, UV detection at 230 nm. Reproduced Bueno et al., 2025 [52] under the terms of the Creative Commons CC BY license.

Table 4. Summary of CE methods for the determination of DAPA in pharmaceutical formulations.

Analytical Technique	Analytical Conditions	Matrix	Analytical Performance (DAPA)	Substances	Reference
CE-DAD	Fused silica capillary (65 cm × 75 µm); BGE: 30 mM phosphate buffer pH 6.0; 30 kV; 25 °C, pressure 20 mbar; injection 40 s; detection: 203 nm (SAXA/DAPA), 250 nm (MET)	Tablets (fixed-dose combinations)	linearity: 1.25–50 µg/mL LOD: 0.4 µg/mL LOQ: 1.25 µg/mL Precision (%RSD): 1.14–1.97 Stability-indicating	DAPA, MET, SAXA	[51]
CE-DAD	Fused silica capillary (48.5 × 50 µm); 40 mM tetraborate buffer pH 10.5; 14 kV; 25 °C; 230 nm; I.S (ranitidine)	Tablets	linearity: 50–175 µg/mL LOD: 6.2 µg/mL LOQ: 18.8 µg/mL Precision (%RSD): 2.52–3.05%	DAPA	[52]

3.5. Spectroscopic Methods

The study by Meira et al. focuses on the development and validation of a dissolution method for DAPA tablets, coupled with UV-Vis spectrophotometric quantification. The dissolution test was performed using USP apparatus II (paddle) in 900 mL simulated gastric fluid (pH 1.2) at 37 °C and 50 rpm, conditions selected based on systematic optimization studies. Quantification was achieved by UV detection at 224 nm, offering a simple and cost-effective approach suitable for routine quality control. The method demonstrated good analytical performance, with linearity in the range 0.5–15 µg/mL, precision, and low LOD/LOQ (0.05 and 0.15 µg/mL). Robustness was confirmed using a factorial experimental design. An important contribution of this study is the evaluation of dissolution profiles for multiple commercial batches, showing high dissolution (>85% within 15 min) and similarity between batches. This supports the classification of DAPA as a BCS Class III drug and highlights its rapid dissolution behavior [53].

The study by Lotfy et al. introduces three univariate UV spectrophotometric methods based on the concept of factorized response spectra (FRS) for the simultaneous determination of DAPA and SAXA in binary mixtures and tablets. A key analytical challenge addressed is the severe spectral overlap between DAPA (λmax 224 nm) and SAXA (λmax 209 nm), which prevents direct simultaneous quantification using conventional UV methods. The proposed FRS approach enables mathematical reconstruction of individual spectra

from overlapped mixtures, eliminating the need for prior separation. The methods were successfully applied to fixed-dose formulations, with acceptable recovery and dosage unit uniformity. All methods exhibited satisfactory analytical performance, characterized by wide linearity ranges (2.5–50 μg/mL for DAPA and 2.5–60 μg/mL for SAXA), excellent correlation coefficients, low LOD (0.40–0.67 μg/mL), and high accuracy with good precision. The main advantage of this work is the high selectivity achieved using simple mathematical manipulation within standard spectrophotometer software, avoiding the need for chromatographic separation or advanced chemometric tools [54].

The study by Omar et al. describes a simple and highly sensitive spectrofluorimetric method for the determination of DAPA as pure substance and tablet formulations, with application to content uniformity testing. The method is based on the native fluorescence of DAPA, measured at an emission wavelength of 303 nm after excitation at 278 nm, exploiting the intrinsic fluorophore properties of the molecule. Optimization studies demonstrated that methanol provides the highest fluorescence intensity, while pH adjustment and surfactants had no significant effect, simplifying the analytical procedure. The method showed excellent sensitivity, with a linear range of 100–1000 ng/mL, a very low LOD of 26.49 ng/mL, and LOQ of 79.48 ng/mL, outperforming many conventional UV-based methods. Validation confirmed high accuracy and good precision with no interference from common excipients, demonstrating good selectivity. A notable advantage is the direct applicability to content uniformity testing, with results well within USP acceptance criteria [55].

The study by Elhassan et al. describes the development of four UV spectrophotometric methods (three univariate and one multivariate) for the simultaneous determination of DAPA and SAXA in bulk and pharmaceutical dosage forms. Spectral analysis was performed in methanol over the 200–400 nm range, where a strong overlap between DAPA and SAXA spectra was observed in the 200–250 nm region, preventing direct determination at their λmax values (210 nm for SAXA and 225 nm for DAPA). The ratio difference method was applied using amplitude differences at 214 and 235 nm (DAPA) and 213 and 235 nm (SAXA) after division by suitable standard spectra, enabling selective quantification of each component. The ratio subtraction method with constant multiplication was based on the extended spectrum of DAPA (250–292 nm), allowing reconstruction of the zero-order spectra and measurement at 210 nm (SAXA) and 225 nm (DAPA). The second derivative method was applied with $\Delta\lambda = 10$ and scaling factor 100, enabling zero-crossing determination at 235 nm (SAXA) and 303 nm (DAPA). The multivariate partial least-squares (PLS) method was developed using spectra in the 205–300 nm range, with a calibration set of 17 mixtures and validation set of 8 mixtures, and an optimal number of 8 latent variables based on RMSECV. Validation demonstrated good linearity over the ranges 2.5–50 μg/mL (DAPA) and 5–60 μg/mL (SAXA), with good precision, and accuracy close to 100% recovery. LOD values were in the range of 0.51–0.78 μg/mL and 1.53–1.63 μg/mL, with corresponding LOQ values of 1.54–2.36 μg/mL and 4.65–4.94 μg/mL, for DAPA and SAXA, respectively. The methods were successfully applied to laboratory-prepared mixtures and simulated combined tablets, with assay values close to 100% and no significant difference compared to a reported spectrophotometric method based on statistical analysis (*t*-test, F-test) [56].

A summary of representative spectroscopic methods developed for the determination of DAPA in single and combined pharmaceutical formulations is presented in Table 5.

Spectroscopic techniques offer rapid, low-cost, and straightforward analytical solutions for DAPA determination in pharmaceutical formulations; however, their limited selectivity and dependence on mathematical data processing restrict their applicability in complex matrices.

Table 5. Summary of spectroscopic methods for the determination of DAPA in pharmaceutical formulations.

Analytical Technique	Analytical Conditions	Matrix	Analytical Performance (DAPA)	Substances	Reference
UV-Vis spectrophotometry (dissolution method)	USP apparatus II (paddle); 900 mL simulated gastric fluid (pH 1.2); 37 ± 0.5 °C; 50 rpm; detection at 224 nm; linear range 0.5–15 µg/mL	Pharmaceutical tablets (dissolution testing)	linearity: 0.5–15 µg/mL LOD: 0.05 µg/mL LOQ: 0.15 µg/mL Precision (%RSD): 0.11–1.39	DAPA	[53]
UV-Vis spectrophotometry (FZM, FDM, FRM–FRS approach)	Methanol solvent; spectral range 200–400 nm; FZM: λ 224 nm (DAPA), 209 nm (SAXA); FDM: D1 (Δλ = 4, scaling 10); FRM: ratio spectra (235–214 nm); no separation required	Tablets (fixed dose combination)	linearity: 2.5–50.0 µg/mL LOD: 0.67 µg/mL (FZM), 0.64 µg/mL (FDM), 0.40 µg/mL (FRM) LOQ: 2.03 µg/mL (FZM), 1.94 µg/mL (FDM), 1.21 µg/mL (FRM) Precision (%RSD): 0.95–1.29 (FZM), 1.05–1.58 (FDM), 0.98–1.24 (FRM)	DAPA, SAXA	[54]
Spectrofluorimetry	λex = 278 nm; λem = 303 nm; solvent: methanol; no buffer required	Tablets	linearity: 100–1000 ng/mL LOD: 26.49 ng/mL LOQ: 79.48 ng/mL Precision (%RSD): ≤1.15	DAPA	[55]
UV–Vis spectrophotometry (univariate + PLS)	Methanol; 200–400 nm; ratio difference, ratio subtraction, second derivative, PLS (205–300 nm)	Simulated tablets	linearity: 2.5–50.0 µg/mL LOD: 0.51–0.78 µg/mL LOQ: 1.54–2.36 µg/mL Precision (%RSD): 0.34–1.06	DAPA, SAXA	[56]

4. Discussion

Overall, clear differentiation can be established among analytical techniques used for DAPA determination in different matrices based on their intended application, performance, and practical constraints.

RP-HPLC methods remain the most widely employed approaches for routine pharmaceutical analysis due to their robustness, operational simplicity, and broad availability in quality control laboratories. These methods are well suited for assay determination and stability studies; however, their inherent limitations in sensitivity and selectivity become evident when dealing with trace-level quantification or determination from complex biological matrices.

From a practical perspective, stability-indicating RP-HPLC methods developed for fixed-dose combinations containing DAPA and MET, SAXA, LINA, SITA, or VILD appear particularly suitable for routine quality control applications. These methods provide simultaneous quantification of all active components while ensuring adequate separation from degradation products generated under stress conditions.

LC-MS/MS techniques represent the gold standard for bioanalytical applications, offering higher sensitivity, selectivity, and structural confirmation capabilities. Their ability to operate in MRM mode enables highly specific quantification even in the presence of significant matrix interference, making them useful for pharmacokinetic and bioequivalence studies. Nevertheless, these methods are associated with higher operational costs, increased methodological complexity, and the requirement for specialized instrumentation and expertise, which may limit their routine implementation in standard quality control settings.

CE emerges as an attractive alternative, particularly from the perspective of green analytical chemistry, due to its minimal solvent consumption, high separation efficiency, and relatively short analysis times. However, despite these advantages, CE methods are still limited in the literature and are generally characterized by moderate sensitivity, restricting their applicability primarily to pharmaceutical formulations rather than bioanalysis.

Spectroscopic techniques, including UV-Vis and spectrofluorimetric methods, offer simple, rapid, and cost-effective analytical solutions, especially for routine quality control. However, their limited selectivity, particularly in the presence of co-formulated drugs or complex matrices, significantly constrains their applicability, often necessitating mathematical manipulation or chemometric approaches to resolve overlapping signals.

Despite the considerable number of analytical methods reported, several important limitations and gaps remain evident. A large proportion of studies focus predominantly on pharmaceutical dosage forms, whereas comprehensive bioanalytical investigations, particularly in diverse biological matrices, remain comparatively limited. In addition, impurity profiling and detailed elucidation of degradation pathways are insufficiently explored, especially for fixed-dose combinations, where potential drug–drug interactions may influence stability and degradation behavior. Furthermore, although stability-indicating methods are frequently reported, the depth of forced degradation studies and the extent of structural characterization of degradation products vary considerably, often limiting the mechanistic understanding of degradation processes.

Another critical aspect is the relatively limited integration of green analytical chemistry principles. While recent studies employing AQbD and WAC frameworks demonstrate promising advancements in method optimization and environmental sustainability, most reported methods still rely heavily on conventional organic solvents such as acetonitrile and methanol. Consequently, there is a clear need for the development of more sustainable analytical strategies that balance performance with environmental impact, without compromising analytical reliability. It should be noted that the term "green" is used in a relative sense and refers to methods exhibiting reduced environmental impact compared with conventional analytical procedures, rather than the complete elimination of solvents or reagents.

Although the primary focus of this review is the analytical determination of DAPA in pharmaceutical and biological matrices, it should be noted that the characterization of its solid-state forms, including crystalline, amorphous, and solvated forms, also represents an important area of pharmaceutical analysis requiring complementary analytical techniques.

These observations highlight the need for future analytical developments that integrate enhanced sensitivity, broader applicability across matrices, comprehensive impurity profiling, and sustainability considerations, thereby supporting the evolving analytical requirements associated with modern antidiabetic therapies and complex pharmaceutical formulations.

5. Conclusions

The current review provides a comprehensive and critical evaluation of the analytical methodologies reported for the determination of DAPA in pharmaceutical formulations and biological matrices. Compared to previously published reviews, which are often primarily descriptive or focused on specific analytical approaches, the present work emphasizes a comparative assessment of method performance, applicability, and emerging trends, including sustainability considerations.

The analysis highlights that RP-HPLC methods remain the most widely applied techniques for routine quality control due to their robustness and accessibility, whereas LC-MS/MS approaches offer superior sensitivity and selectivity, making them indispensable for bioanalytical applications. CE and spectroscopic methods represent valuable complementary approaches, particularly in the context of cost-efficiency and green analytical chemistry, although their applicability may be limited by lower sensitivity or selectivity.

Despite the large number of published methods, relatively few studies address impurity profiling, metabolite determination, and comprehensive bioanalytical applications.

Future developments are expected to focus on the integration of high-resolution mass spectrometry (HRMS) for non-target screening and impurity identification, the implementation of miniaturized and microfluidic systems, and the broader application of green and QbD-based strategies. Such advances are essential to meet the increasing analytical

demands associated with complex pharmaceutical formulations and evolving therapeutic applications of DAPA.

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Abbreviations

The following abbreviations are used in this manuscript:

AGREE	Analytical GREEnness metric
AQbD	Analytical Quality by Design
BBD	Box–Behnken Design
BGE	Background electrolyte
CANA	Canagliflozin
CCD	Central Composite Design
CE	Capillary electrophoresis
DAD	Diode array detection
DAPA	Dapagliflozin
DM	Diabetes mellitus
DoE	Design of experiments
DPP-4	Dipeptidyl peptidase-4
EMPA	Empagliflozin
ESI	Electrospray ionization
GAPI	Green analytical procedure index
HSPiP	Hansen solubility parameters in practice
IS	Internal standard
ICH	International Council for Harmonisation
LINA	Linagliptin
LLE	Liquid–liquid extraction
MET	Metformin
METO	Metoprolol
MLC	micellar liquid chromatography
MRM	Multiple reaction monitoring
PDA	Photodiode array detection
ROSV	Rosuvastatin
SAXA	Saxagliptin
SGLT-2	Sodium–glucose cotransporter 2
SITA	Sitagliptin
SPE	Solid-phase extraction
TENE	Teneligliptin
VILD	Vildagliptin
WAC	White analytical chemistry

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