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Review Article

Analytical Techniques for the Determination of Metformin and its Combinations with Oral Antidiabetic Agents in Pharmaceutical Dosage Forms: Combinations with Sulphonylurea Antidiabetic Drugs

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Abstract

The sulfonylurea class of antidiabetic agents includes a range of closely related compounds, allowing for similar analytical approaches when combined with metformin. This article provides a comprehensive review of published methods for determining sulfonylureas and metformin combinations in bulk and pharmaceutical preparations. Various techniques such as high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), capillary zone electrophoresis (CZE), and spectrophotometric methods have been widely used for these compounds, yielding reliable results. Additionally, the article discusses the number of citations for each method and its specific purpose, offering critical commentary.

Keywords: metformin; sulphonylureas; combination; determination; analytical techniques

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder marked by insulin resistance and a progressive decline in β-cell function [1]. Effective glycemic control is vital for managing diabetes, as elevated blood sugar levels are a major contributor to disease progression and vascular complications [2,3].

The growing global burden of diabetes significantly impacts individuals, families, and countries. According to the IDF Diabetes Atlas, 10.5% of adults aged 20-79 have diabetes, with nearly half of them unaware of their condition [4]. By 2045, The International Diabetes Federation (IDF) projections indicate that 1 in 8 adults, or approximately 783 million people, will be living with diabetes, representing a 46% increase [5].

Current guidelines from the American Association of Clinical Endocrinologists and the American College of Endocrinology suggest starting dual therapy—typically with metformin unless contraindicated or not tolerated, along with a second agent—for patients with an initial HbA1c level around 7.5%., for patients with an entry HbA1c level above 9.0% but without symptoms of hyperglycemia, dual or triple therapy with oral glucose-lowering agents is recommended, while symptomatic hyperglycemia necessitates insulin therapy alongside oral agents [6,7]. In contrast, the American Diabetes Association and the European Association for the Study of Diabetes recommend adding a second drug if the target HbA1c is not reached within 3 months of monotherapy. They also advise starting dual combination therapy when HbA1c is around 9.0% [8].

The American Diabetes Association guidelines recommend that if the target HbA1c is not achieved after approximately three months, and the patient does not have atherosclerotic cardiovascular disease (CVD) or chronic kidney disease (CKD), a combination therapy of metformin and one of the following six preferred treatment options should be considered:

- Sulfonylureas (SUs)
- Thiazolidinedione
- Dipeptidyl peptidase-4 inhibitor (DPP-4)
- Sodium–glucose linked transporter-2 inhibitor (SGLT2)
- Glucagon-like peptide-1 receptor agonist
- Basal insulin

For patients with T2DM and established CVD, the antihyperglycemic regimen should include SGLT-2 inhibitors or GLP-1 receptor agonists with proven cardiovascular benefits, taking into account both drug-specific and patient-related factors [9].

Metformin-based combination therapies

Metformin is the preferred first-line monotherapy for most patients with T2DM and is typically the primary component in combination therapy, as recommended by current treatment guidelines [10,11]. As an insulin sensitizer, metformin primarily enhances glycemic control by inhibiting hepatic gluconeogenesis, with additional effects in the muscle and adipose tissue [12,13]. It also reduces intestinal glucose absorption [14] and may increase endogenous glucagon-like peptide 1 (GLP-1) levels [15,16]. While the exact molecular mechanism of metformin is not fully understood, it is believed to involve changes in cell membranes, effects on respiratory chain oxidation, and activation of adenosine monophosphate-dependent protein kinase [12].

The International Diabetes Federation (IDF) provided specific recommendations in 2017 for dual therapy in managing T2DM [7]:

A second glucose-lowering drug (GLD) should be added if monotherapy with metformin (or its alternative) is not sufficiently effective in reaching or maintaining the target HbA1c level. The preferred add-on options include a sulfonylurea (excluding glibenclamide/glyburide), a DPP-4 inhibitor, or an SGLT-2 inhibitor. An alpha-glucosidase inhibitor is also an option. If weight loss is a priority and the drug is affordable, a GLP-1 receptor agonist can be considered (see Table 1).

The primary care physician should take into account the patient's profile, including age, body weight, complications, and duration of the disease, when selecting the most appropriate GLD.

For triple therapy in diabetes, the IDF recommendations (2017) are as follows [17]: A third GLD should be added if a combination of a GLD with metformin is not sufficiently effective in achieving or maintaining the HbA1c target. The most common option to add to two oral GLDs is basal insulin. Alternatively, if weight loss has been inadequate, a GLP-1 receptor agonist may be added. Triple therapy with three oral GLDs may be effective before introducing an injectable antidiabetic.

Table 1: Metformin containing dual fixed-dose combinations for type 2 diabetes management. [18]

Metformin	+	SUs (glibenclamide, glipizide, gliclazide, glimepiride)
		DPP-4 (sitagliptin, linagliptin, saxagliptin, gemigliptin, teneligliptin)
		SGLT2 inhibitors (empagliflozin, canagliflozin, dapagliflozin)
		Glitazones (pioglitazone, rosiglitazone)
		AGIs (voglibose, acarbose)

Commercially available dual therapy fixed-dose combinations containing metformin are listed in Table 2.[19]. Fixed-dose combinations (FDCs) offer several advantages and disadvantages. They reduce pill burden by combining multiple medications into a single pill, which decreases the number of pills a patient needs to take and reduces medication errors by simplifying the regimen. FDCs can enhance therapeutic effects through synergistic drug combinations and lower manufacturing and logistics costs. They also simplify dosing by requiring patients to track only one medication expiry date, leading to improved therapy adherence and treatment outcomes. However, FDCs have limitations such as no dosing flexibility, which can be necessary for optimal treatment, and a risk of drug interactions that may alter therapeutic effects and cause harm. Incompatible pharmacokinetics of individual components in irrational FDCs can lead to ineffective treatment. Sometimes, FDCs can be more expensive than the sum of single-dose tablets. Additionally, if a patient is allergic to even one component of the FDC, they must discontinue the entire combination, complicating treatment [20-22].

Table 2: Commercially available dual therapy fixed-dose combinations containing metformin

Drug class	Generic component	Form	Tablet size, mg/mg
Metformin + sulfonylurea	Glyburide/metformin	IR	1.5/500, 2.5/500, 5/500
	Glipizide/metformin	IR	1.5/500, 2.5/500, 5/500
Metformin + meglitinide	Repaginate/metformin	IR	1/500, 2/500
Metformin + DPP-4 inhibitor	Sitagliptin/metformin	IR	50/500, 50/1000
	Saxagliptin/metformin	XR	5/500, 5/1000, 2.5/1000
	alogliptin/metformin	IR	12.5/500, 12.5/1000
Metformin + TZD	Pioglitazone/metformin	IR	15/00, 15/850
	Pioglitazone/metformin	XR	15/1000, 30/1000
	Rosiglitazone/metformin	IR	2/500, 4/500, 2/1000, 4/1000
Metformin + SGLT2 inhibitor	dapagliflozin/ metformin	XR	5/500,10/500 5/1000, 10/1000
	canagliflozin/ metformin	IR	50/500, 50/1000 150/500, 150/1000

The sulfonylurea class consists of a variety of closely related compounds. These drugs all share the phenyl-sulfonyl-urea structure (Figure 1), which is responsible for their hypoglycemic effects, while the R and R1 radicals (see Table 1) influence their pharmacokinetic and pharmacotoxicological properties [23].

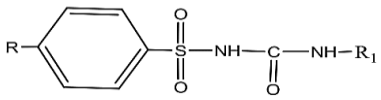


Figure 1: General chemical structure of hypoglycemic sulfonylureas

The key clinical differences between individual sulfonylurea drugs are attributed to variations in their half-lives, metabolic and excretion pathways, and receptor binding affinities. Second-generation sulfonylureas, in particular, bind more strongly to the specific sulfonylurea receptors on pancreatic B cells, allowing them to be effective at lower doses.

The primary action of sulfonylureas is to stimulate insulin secretion, a response that is sustained over the long term. The glucose-lowering effectiveness of sulfonylureas is directly related to the initial blood glucose level—the higher the fasting blood glucose, the more significant the reduction achieved with sulfonylurea therapy.

Many methods have been reported in literature for the simultaneous estimation of the combination of metformin with one sulphonylureas.

Methodology

The literature for analysis was selected from accessible publications spanning the years 2010 to 2024. Journal articles were sourced from specialized databases, including Science Direct, Taylor & Francis, Springer Link, PubMed, Scopus, Google Scholar, and Wiley. A combination of keywords was used to retrieve relevant studies, such as "analytical method for determination of metformin and sulfonylureas combination" or "determination of metformin and individual sulfonylurea group members."

Once identified, the articles were manually screened based on their titles and abstracts. Duplicates and irrelevant studies were excluded, while those meeting the inclusion criteria were incorporated into the analysis. The inclusion criteria were as follows: (1) original research published in peer-reviewed journals; (2) studies presenting various methods for the determination of metformin and sulfonylureas combinations; (3) quantification performed in dosage forms; and (4) articles published in English. Exclusion criteria applied to articles written in languages other than English and those that did not meet the aforementioned inclusion criteria.

Summary tables were created to interpret key findings from the included studies. These tables provided details such as the matrix in which the analysis was conducted, the analytical method used, the detector and wavelength employed for detection, stationary and mobile phases, flow rate, linearity range, and limits of detection (LOD).

Analytical Techniques

Official methods

The United States Pharmacopoeia monographs for metformin in combination with either glibenclamide or glipizide outline two distinct methods for determining the concentrations of metformin alongside glibenclamide or glipizide [24].

The determination of glibenclamide in combination with metformin is performed using an octylsilane column (4.6 mm × 15 cm; 5 µm) maintained at 40°C. The mobile phase consists of a 0.25M monobasic ammonium phosphate buffer and acetonitrile in a 40:60 ratio, adjusted to a pH of

5.3, with a flow rate of 1.2 mL/min. The analyte is detected at 230 nm. In contrast, metformin is analyzed using a C18 column (3.9 mm × 30 cm; 10 µm) maintained at 30°C. The mobile phase is composed of a buffer (0.5 g/L each of sodium heptanesulfonate and sodium chloride, adjusted to a pH of 3.85) and acetonitrile in a 90:10 ratio, with a flow rate of 1.0 mL/min. Detection is carried out at 218 nm.

The determination of glipizide in combination with metformin is carried out using an octylsilane column (4.6 mm × 15 cm; 5 µm). The mobile phase consists of three solutions: Solution A (0.02M monobasic ammonium phosphate buffer, pH 8), Solution B (acetonitrile, water, and Solution A in a 1:14:5 ratio), and Solution C (acetonitrile, water, and Solution A in a 2:1:1 ratio). A gradient elution is employed, starting with 100% Solution B for the first 3 minutes, followed by a gradual transition to 100% Solution C over 15 minutes, which is then maintained for an additional 2 minutes. The flow rate is set at 1.0 mL/min, and detection occurs at 223 nm. For metformin, analysis is performed using a phenyl column (4.6 mm × 15 cm; 3.5 µm). The mobile phase is composed of Solution A (50 mM hexanesulfonic acid buffer, adjusted to a pH of 2.0), Solution B (acetonitrile and water in a 40:60 ratio), and water, mixed in a 3:2:5 ratio. This is pumped at a flow rate of 1.0 mL/min, with detection at 218 nm.

Separation techniques

High-Performance Liquid Chromatography (HPLC)

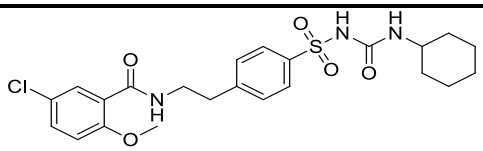
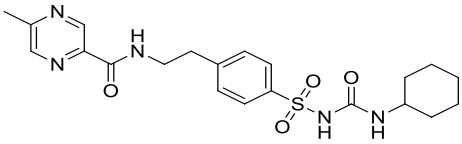
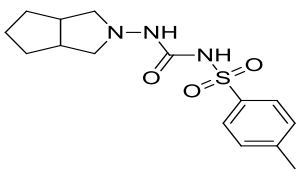
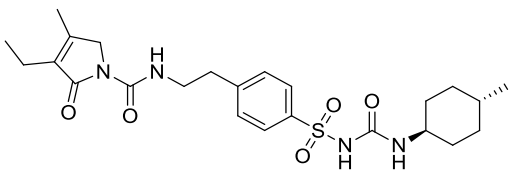
Reversed-phase high-performance liquid chromatography (RP-HPLC) is the most commonly employed separation technique in the pharmaceutical industry. Over the past 20 years, the development of a wide variety of robust reverse-phase columns has made RP-HPLC the favored "first-line" method for content analysis. Most of the reported methods employed isocratic elution using a reversed-phase column (C8 or C18) and mobile phases composed of organic solvent and buffers mixtures adjusted to specific pH levels.

Although RP-HPLC remains the most frequently used approach for analyzing combination drug products, alternative separation techniques may provide more effective solutions for these complex analyses.

Analyzing combination drug products, which contain two or more active pharmaceutical ingredients (APIs), presents several challenges for RP-HPLC, including difficulties with analyte retention, separating impurities, distinguishing excipients, maintaining peak shape, and ensuring column longevity.

The polarity and ionization potential of molecules play a crucial role in determining their retention on organic reverse-phase high-performance liquid chromatography (RP-HPLC) columns. When active pharmaceutical ingredients (APIs) in a combination drug exhibit significant differences in polarity, it often becomes necessary to develop multiple chromatographic methods. This method development and validation process can be both resource- and time-intensive. A common example of this challenge is seen in drug combinations containing metformin and sulfonylurea compounds. Metformin, with its high polarity, contrasts sharply with sulfonylureas, which have medium-to-low polarity, as indicated by their respective log P values in Table 3.

Table 3: Structure and Log P (octanol/water) of sulphonylureas

Compound	Chemical Structure	Log P	Reference
Glibenclamide (GLB)		4.8	61
Glipizide (GLZ)		1.9	61
Gliclazide (GLC)		2.1	61
Glimepiride (GLM)		3.8	62

To assess whether the chromatographic methods provided satisfactory separation, i.e., adequate interaction between the stationary and mobile phases, the retention factor (k) was calculated. This calculation was based on the retention times and column characteristics provided by the authors, following the guidelines set by the United States Pharmacopeia (USP) [24]. According to the USP, an acceptable retention factor falls within the range of $2 < k < 10$ [24]. However, in many of the methods reviewed, metformin exhibited a retention factor (k) of less than 1. In some cases, the retention time of metformin was very close to the dead time, resulting in a k value approaching zero [25-28, 30-42, 44-47, 49-50, 52-57, 59,60]. These findings indicate that metformin had minimal or no interaction with the stationary phase, leading to poor chromatographic separation. As a result, metformin often eluted near the void volume and was prone to co-elution with polar interferents and matrix components from the formulation.

The challenge of metformin's low affinity for reversed-phase packing material was further compounded by the presence of sulfonylurea compounds. At a retention time where metformin could achieve adequate retention (i.e., $k > 2$), the sulfonylureas would elute at unacceptably long times. To address this issue, ion-pairing reagents such as sodium dodecyl sulfate, octane sulfonic acid, hexane sulfonic acid, and pentane sulfonic acid [29,35,48,51] have been employed to enhance metformin's retention. Gedawy et al. [43,58] successfully overcame the retention challenges posed by metformin and sulfonylureas by using a cyanopropyl column. This approach allowed for the effective separation of metformin from gliclazide or glipizide and also moved the metformin peak away from the solvent front. To address this challenge, the USP established two distinct methods for metformin and its associated sulfonylurea.

Although all the reported methods have been validated according to the International Conference on Harmonization (ICH) requirements [63], however only few have been optimized [28,41,60], while HPLC methods, robustness, and ruggedness should be tested earlier in the development stage of the method to ensure the efficiency of the method over the lifetime of the product. Otherwise, it can take considerable time and energy to redevelop, revalidate, and retransfer analytical methods if a non-robust or non-rugged system is adapted. Few methods [30, 34, 44, 45, 51-53] were further demonstrated to be stability-indicating through the analysis of forced degradation samples. Description of the reported HPLC methods is given in Table 4.

Table 4. High performance liquid chromatographic methods used for the analysis of metformin and sulphonylureas combination

No.	Metformin +	Column	Mobile Phase	Detection λ (nm)	Working range (µg/mL)	LOD (µg/mL)	Ref
1	glibenclamide tablets	C_{18}, 4.6 x 100 mm, 5 µm	acetonitrile:water (60 : 40, v/v) mobile phase at 0.9 ml/min flow rate	254	0.06-0.24 GLB 1.5-6.0 MET	0.003 GLB 0.16 MET	25
2	Glibenclamide or gliclazide tablets	C_{18}, 3.0 x 100 mm, 2.2 µm, 30˚C	acetonitrile: water: trifluoroacetic acid: trimethylamine (54:46:0.1:0.1v/v/v/v) at a flow rate of 0.38 mL/min	230	5-50 for the three	0.010 GLB and GLC 0.025 MET	26
3	glibenclamide tablets	C_{18}, 15 cm x 4.6 mm, 5µ	acetonitrile: 0.1% w/w mono basic sodium phosphate Buffer pH to 2.5 (50:50) at a flow rate of 0.38 mL/min	228	125-450 MET 0.25-2.0 GLB	0.019 MET 0.033 GLB	27
4	glibenclamide tablets	C_{18}, 25 cm x 4.6 mm, 5µ, 30˚C	acetonitrile-water (50:50, v/v) pH 5.0, at a flow rate of 0.8 mL/min	225	5–100 MET 2.5–80 GLB	NA	28
5	glibenclamide tablets	C_{18}, 25 cm x 4.6 mm, 5µ,	methanol: phosphate buffer (pH 6.5) containing 0.01 M sodium dodecyl sulphate (50:50, v/v) at a flow rate of 1.5 mL/min	225	2.5-7.5 GLB 250-750 MET	0.01 GLB 0.002 MET	29
6	glibenclamide tablets	C_{18}, 25 cm x 4.6 mm, 5µ,	acetonitrile: 0.05 M KH_2PO_4 (60:40v/v) adjusted to pH 3, at flow rate of 1 mL/min	210	5–75 MET 2-45 GLB	0.64 MET 0.02 GLB	30
7	glibenclamide tablets	C_{18}, 25 cm x 4.6 mm, 5µ,	methanol: acetonitrile: water in 30:60:10 (v/v/v), at a flow rate of 1.0 mL/min	228	2-4.5 GLB 200-450 MET	0.01 GLB 0.30 MET	31
8	glibenclamide tablets	C_8, 25 cm x 4.6 mm, 5µ,	0.1 M ammonium acetate (pH 5.0) and methanol (23:77, v/v), delivered at a flow rate of 0.7 mL/ min	230	0.1-300 MET 0.89-311.0 GLB	0.026 MET 0.089 GLB	32
9	glibenclamide tablets	C_{18}, 25 cm x 4.6 mm, 5µ, 30°C	0.1% w/w NaH_2PO_4, pH 2.5: acetonitrile (50:50 v/v) delivered at a flow rate of 1 mL/ min	228	125-450 MET 0.25-2.0 GLB	0.019 MET 0.033 GLB	33
10	glibenclamide tablets	C_{18}, 25 cm x 4.6 mm, 5µ	methanol: water solution in the ratio (70:30) delivered at a flow rate of 1 mL/ min	226	10-50 GLB 500-2500 MET	0.012 GLB 0.24 MET	34

11	glibenclamide tablets	C_{18}, 25 cm x 4.6 mm, 5µ	70.00% organic (ethanol) to 30.00% aqueous 10.00 mM KH_2PO_4, pH 3.0 containing 50.00 mM octanesulphonic acid, delivered at a flow rate of 1 mL/ min	250	0.50-100 for both drugs	0.15 MET 0.15 GLB	35
12	glibenclamide tablets	C_{18}, 25 cm x 4.6 mm, 5µ	methanol and acetonitrile (70:30 % v/v) as mobile phase at pH of 3.5, delivered at a flow rate of 1 mL/ min	232	0.025-0.5 MET 0.025-0.2 GLB	0.025 MET 0.029 GLB	36
13	gliclazide tablets	C_{18}, 25 cm x 4.6 mm, 5µ	buffer (0.1 % each orthophosphoric acid and triethylamine) and methanol at the ratio of 60:40, at a flow rate of 1 mL/min	230	1-50 MET 0.16-8 GLC		37
14	gliclazide tablets	C_{18}, 25 cm × 4.6 mm, 5 µ	KH_2PO_4 buffer pH 6.6 and acetonitrile in the ratio 60:40 v/v , at a flow rate of 1 mL/min	261	125-750 MET 20-120 GLC	2.3 MET 0.43 GLC	38
15	gliclazide tablets	C_{18}, 25 cm × 4.6 mm, 5 µ	0.1% orthophosphoric acid and acetonitrile in the ratio of 35:65 v/v in isocratic mode at a flow rate of 0.8 ml/min	230	20-60 MET 3.2-9.6 GLC	0.018 MET 0.03 GLC	39
16	gliclazide tablets	C_{18}, 25 cm × 4.6 mm, 5 µ	10 mM phosphate buffer (pH 3) :acetonitrile (70:30 % v/v) at a flow rate of 1 mL/min	230	10-60 ??? 2-12 GLC	NA	40
17	gliclazide tablets	C_{18}, 25 cm × 4.6 mm, 5 µ	water: methanol: acetonitrile: triethylamine (60:20:20:0.5 % (v/v) respectively, adjusted to pH 7.0, at a flow rate of 1 mL/min	227	50–150 for both	0.035 GLC 0.08 MET	41
18	gliclazide, glimepiride tablets	C_8, 25 cm × 4.6 mm, 5 µ	methanol: 0.025M KH_2PO_4 pH 3.20 (70: 30, v/v), at a flow rate of 1 mL/min	235	5 - 100 for the three	0.05 MET 1.21 GLC 0.11 GLM	42
19	gliclazide tablets	CN, 25 cm × 4.6 mm, 5 µ	20 mM ammonium formate buffer (pH 3.5) and acetonitrile (45:55,v/v) in isocratic elution mode at 1 mL/min	227	1.25-150 GLC 2.5-150 MET	0.8 GLC 0.97 MET	43
20	gliclazide tablets	C_{18}, 25 cm × 4.6 mm, 5 µ	phosphate buffer: acetonitrile (40:60, v/v) at a flow rate of 0.8 ml/min	240	100-700 MET 20-140 GLC	43.59 MET 8.96 GLC	44
21	gliclazide tablets	C_{18}, 100 mm x 2.6 mm, 1.6µ	trifluoroacetic acid buffer: acetonitrile (70: 30, v/v) at a flow rate of 1 ml/min	227	25.0-375.0 MET 4.0-60.0 GLC	0.25 MET 0.04 GLC	45

22	gliclazide tablets	C_{18}, 25 cm × 4.6 mm, 5 µ	acetonitrile, methanol and water in the ratio of (300 : 250 : 450) and pH adjusted to pH 3.5 , at a flow rate of 1 ml/min	228	10-60 MET 1.2-7.2 GLC	NA	46
23	glimepiride tablets	C_{18}, 10 cm × 4.6 mm, 5 µ	acetonitrile: ammonium acetate buffer pH 3.0 (55: 45, v/v) at a flow rate of 1.5 ml/min	270	2000-8000 MET 4-16 GLM	NA	47
24	glimepiride tablets	C_8, 10 cm × 4.6 mm, 5 µ, 50°C	Gradient of: pentane sulfonic acid sodium salt buffer pH 3.5 and acetonitrile: 0-8 min: 90% buffer, 8-30 min: 100% acetonitrile, 30-35 min: 90% buffer	230	0.02-4.0 GLM 0.50-10 MET	0.03 GLM 0.5 MET	48
25	glimepiride tablets	C_{18}, 10 cm × 4.6 mm, 5 µ	20 mM KH_2PO_4 buffer, pH 3.0 and an organic phase (methanol: acetonitrile; 62.5:37.5) in the ratio of 80:20. The flow rate was1 mL/minute	230	5-30 MET 1-10 GLM	0.73 MET 0.24 GLM	49
26	glimepiride tablets	C_{18}, 15 cm × 4.6 mm, 5 µ	0.05 M KH_2PO_4(pH 3.0): ACN (40:60). The flow rate was 1.0ml/min	230	5-25 MET 10-50 GLM	0.011 MET 0.024 GLM	50
27	glimepiride tablets	C_{18}, 15 cm × 4.6 mm, 5 µ	25 mM hexane sulphonic acid buffer adjusted to pH 2.5 with ortho-phosphoric acid and acetonitrile (45:55 v/v), at a flow rate of 1.0 mL/min	229	150-750 MET 0.75-4.5 GLM	0.29 MET 0.08 GLM	51
28	glipizide tablets	C_{18}, 25 cm × 4.6 mm, 5 µ, 30°C	acetonitrile: water, 0.2% triethylamine (pH 3.0 adjusted with orthophosphoric acid) (60:40 v/v), at flow rate 0.8 ml/min	258	100-500 MET 1-5 GLZ	4.19 MET 0.113 GLZ	52
29	glipizide tablets	C_{18}, 25 cm × 4.6 mm, 5 µ	Acetate buffer (pH 4.0): Acetonitrile (60:40 v/v) and at a flow rate of 1 ml/min	257	60-140 MET 10-50 GLZ	0.287 MET 0.065 GLZ	53
30	glipizide tablets	C_{18}, 15 cm × 4.6 mm, 5 µ	methanol: 0.05 M KH_2PO_4 (65:35), pH 4.5 at flow rate 0.8 ml/min	225	100-500 MET 1-5 GLZ	2.96 MET 2.94 GLZ	54
31	glipizide tablets	C_{18}, 25 cm × 4.6 mm, 5 µ	methanol and water (60:40, pH 3 adjusted with orthophosphoric acid) in an isocratic mode and flow rate of 0.8 mL/min	226	100-500 MET 1-5 GLZ	1.68 MET 0.055 GLZ	55
32	glipizide tablets	C_{18}, 25 cm × 4.6 mm, 5 µ	acetate buffer (pH 4.0) and acetonitrile in the ratio of 60:40 v/v, at a flow rate of 1.0 mL/min.	257	60-140 MET 10-50 GLZ	0.287 MET 0.065 GLZ	56

33	glipizide tablets	C_{18}, 25 cm × 4.6 mm, 5 µ	phosphate buffer pH(8.0):acetonitrile (50:50) in an isocratic mode, at a flow rate of 2 mL/min	257	60-140 MET 3.6-8.4 GLZ	NA	57
34	Glipizide tablets	CN, 25 cm × 4.6 mm, 5 µ	20 mM ammonium formate buffer (pH 3.5) and acetonitrile (45:55,v/v) in isocratic elution mode at 1 mL/min	227	1.25-150 GLC 2.5-150 MET	1.069 MET 0.796 GLZ	58
35	gliclazide, glipizide, glibenclamide and glimepiride	C_8, 2.1 × 50 mm, 1.7 µ, 30°C	Buffer (1 mL orthophosphoric acid + 1 ml triethylamine in 1000 ml of water): Acetonitrile (60: 40), at a flow rate of 0.3 mL/min	225	50-150 MET 0.5-1.5 GLZ 8-24 GLC 0.5-1.5 GLB 0.2-0.6 GLM	NA	59
36	gliclazide, glipizide, glibenclamide and glimepiride	C_{18}, 15 cm × 4.6 mm, 5 µ	0.1 % acetic acid in water: acetonitrile mixture was adopted as mobile phase (32.9:67.1, % v/v) at a flow rate: 0.469 mL/min	230	0.01-150 MET 0.1-200 GLZ 0.5-350 GLC 0.02-200 GLB 0.1-200 GLM	0.0168 MET 0.0357 GLZ 0.1620 GLC 0.0077 GLB 0.035 GLM	60

High-Performance Thin Layer Chromatography (HPTLC)

Densitometric thin-layer chromatography methods have been developed for the determination of metformin in combination with sulfonylurea antidiabetic agents in pharmaceutical formulations [65-71]. Detection was typically carried out using spectrophotometry [65-68, 70, 71] or by measuring fluorescence intensities after scraping off the analyte spots and reacting them with dansyl chloride [69].

While the method reported by Adlina et al. [69] for determining metformin and glimepiride appears highly sensitive, the extensive sample manipulation required may limit its practicality for routine use. On the other hand, the method developed by Mohamed et al. [70] is considered the most reliable of the reported approaches. It has been optimized for factors such as mobile phase composition, ammonium sulfate concentration, and chamber saturation time, and also demonstrates stability-indicating capabilities. Description of the reported HPLC methods is given in Table 5.

Table 5: Thin-layer chromatographic methods used for the analysis of metformin and sulphonylureas combination

Meformin+	Matrix	Plate	Mobile Phase	Detection λ (nm)	Working range (ng/spot)	LOD (ng/spot)	Ref.
Glibenclamide	tablets	silica gel 60 F_{254}	methanol: water: glacial acetic acid (6:4:0.25)	237 MET 300 GLB	4000-8000 MET 300-400 GLB	232.3 MET 12.5 GLB	65
Glibenclamide	tablets	silica gel 60 F_{254}	methanol: water: 0.4 % sodium sulphate in water (7: 5:11)	232 MET 238 GLB	250-1750 for both	1.2412 MET 0.994 GLB	66
Gliclazide	tablets	silica gel 60 F_{254}	toluene, acetonitrile, ethanol, Ammonium sulphate (0.25%) (4 : 4 : 4 : 3, v/v/v/v)	228	200-1000 for both	86.14 GLC 106.11 MET	67
Gliclazide	tablets	silica gel 60 F_{254}	ammonium sulfate (0.25%): methanol: ethyl acetate 10.0:2.5:2.5 (v/v/v)	236	100-500 GLC 1000-5000 MET	40 GLC 60.7 MET	68
Glimepiride	tablets	silica gel 60 F_{254}		238	300-500 GLM 150000-250000 MET	30 GLM 95 MET	
Glimepiride	tablets	silica gel 60 F_{254}	methanol: water: glacial acetic acid (6:4:0.25)	Fluorescence 483 MET 489 GLM	0.5 – 1.4 µg/mL 0.1 – 1.0 µg/mL	0.07 µg/mL 0.13 µg/mL	69
Glimepiride	tablets	silica gel 60 F_{254}	0.025 M aqueous ammonium sulfate and acetonitrile (7:3, v/v)	237	60-1400 for both	12.17 GLM 12.83 MET	70
Glipizide	tablets	silica gel 60 F254	water:methanol:0.5% w/v ammonium sulfate solution (6:3:1.5v/v/v)	236	5000-25000 MET 50-250 GLZ	91.30 MET 9.57 GLZ	71

Capillary zone electrophoresis (CZE)

Capillary zone electrophoresis (CZE) is a separation technique that relies on the differential migration of ionic or ionizable compounds under the influence of an electric field. This method is widely applicable for analyzing a variety of sample types. One of the key advantages of CE is its instrumental simplicity, as it consists primarily of a capillary column in which the separation process takes place. The most commonly employed electrophoretic techniques in pharmaceutical analysis are capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). While CZE is effective for separating charged analytes, it is not suitable in its standard form for separating neutral molecules.

Doomkaew et al. [72] developed a capillary zone electrophoretic method to determine metformin, glibenclamide, and gliclazide. This method utilized a capillary with a 56.0 cm effective length and a 50 µm inner diameter. The separation was conducted at a voltage of 20 kV and a temperature of 25°C, with detection occurring at a wavelength of 210 nm. A 50 mM borate buffer at pH 9.0 served as the background electrolyte solution. The method's working ranges were 800–1200 µg/mL for metformin, 8–12 µg/mL for glibenclamide, and 128–192 µg/mL for gliclazide. The limits of detection (LOD) were 2 µg/mL for both metformin and glibenclamide, and 4 µg/mL for gliclazide.

Amăriuţei et al.[73] described a microemulsion electrokinetic chromatography method (MEEKC) for determining metformin, glibenclamide, and gliclazide in bulk. The method employed a fused-silica capillary with a 50 µm internal diameter and a total length of 48 cm (40 cm effective length). The running electrolyte was a microemulsion composed of 3.3% SDS, 6.6% n-butanol, 0.8% n-heptane, and 89.3% 20 mM borate buffer (pH 9.0). The separation was achieved with an applied voltage of 23 kV at a temperature of 30°C, and direct UV detection was performed at 208 nm. The method exhibited a linear range of 30-300 µg/mL for all three analytes, with limits of detection (LOD) of 5.47 µg/mL for metformin, 7.34 µg/mL for gliclazide, and 9.51 µg/mL for glibenclamide.

Spectrophotometric methods

Analytical chemists encounter significant challenges when analyzing and controlling the quality of combination drugs using direct spectrophotometry due to overlapping spectral bands. To address this issue, various methods involving mathematical manipulation of spectral data have been developed. The effectiveness of these methods depends on the degree of overlap and the number of components involved.

Several spectrophotometric methods have been reported for analyzing combinations of metformin with sulphonylureas in pharmaceutical formulations. The majority of these analyses were performed using simultaneous equation spectrophotometry [74, 78, 79, 81, 85-87, 89, 92, 93,] first derivative spectrophotometry [25, 30, 77, 80, 84], and a few using second derivative spectrophotometry [25, 30]. Additionally, some methods employed area under the curve [83, 93], direct UV [76, 90], and multicomponent spectrophotometry[80, 82, 88]. The experimental conditions and technical details of these methods are summarized in Table 6.

Among the spectrophotometric methods reported for the combination of metformin and glibenclamide, Alhemiary et al[30] presented data indicating that their method is the most sensitive, achieving the lowest LOD values (0.21 and 0.29 µg/mL for MET and GLB, respectively) compared to other spectrophotometric and first derivative methods. Other spectrophotometric techniques, including simultaneous equation [74, 78, 79, 81, 85-87, 89, 92, 93], direct UV [76, 90], area under the curve [83, 93], multicomponent spectrophotometry [80, 82, 88], have also been proposed, all with quantitation limits sufficiently sensitive and appropriate for determining these drugs in bulk and pharmaceutical preparations. Chemometric-assisted spectrophotometric methods (principal component regression (PCR) partial least-squares regression (PLS)) also have been reported [75], their use in routine quality control analysis remains limited.

Table 6: Spectrophotometric methods used for the analysis of metformin and sulphonylureas combination

No.	Metformin +	Matrix	Technique	Wavelengths nm	Solvent	LOD (µg/mL)	Linear range (µg/mL)	Ref.
1	glibenclamide	tablets	first derivative	261 GLB	ethanol	0.6 GLB		25
			second derivative	235 MET		3.3 MET	10-55 GLB	
			first derivative of	241 GLB		3.6 MET	20-200 MET	
			the ratio spectra	227 MET		0.64 GLB		
2	glibenclamide	tablets	first derivative	236 MET 275.7 GLB	methanol	0.21 MET	5-120 MET	30
						0.29 GLB	1-20 GLB	
			second derivative	244.6 MET 229 GLB		0.46 MET	5-120 MET	
						0.30 GLB	1-20 GLB	
3	glibenclamide	tablets	simultaneous equation	237.0		0.21 MET		74
				229.2	methanol	0.72 GLB	2-10 MET	
							2-14 GLB	
			absorption ratio method	237 MET		0.18 MET		
				225 isosbestic		0.68 GLB		
4	glibenclamide	tablets	principal component regression (PCR) partial least-squares regression (PLS)	200-400	ethanol	NA	40-200 MET 1-10 GLB	75
5	glibenclamide	tablets	direct UV	233 MET	water	NA	8-12 MET	76
				301 GLB	Acetonitrile: Methanol (1:1)		80-120 GLB	
6	glibenclamide	tablets	first derivative	314.7 GLB 228.6 MET		2.100 GLB	10-125 GLB	77
			first derivative of	314.7 GLB	methanol	0.250 MET	2-18 MET	
			the ratio spectra	238.0 MET		1.800 GLB	10-125 GLB	
						0.150 MET	2-18 MET	

7	glibenclamide	tablets	simultaneous equation	226.60 233	0.01N NaOH	NA	2-10 for both	78
8	glibenclamide	tablets	simultaneous equation	229.5 237	methanol	NA	3-15 GLB 2-10 MET	79
9	glimepiride	tablets	first derivative multicomponent	249 GLM 258 MET 222 and 228	0.1N NaOH	0.91 GLM 0.05 MET 0.52 GLM 0.02 MET	3-15 GLM 1.0-5.0 MET 2.0-10.0 GLM 0.5-2.5 MET	80
10	glimepiride	tablets	simultaneous equation	233 MET 228.4 GLM	0.1N NaOH	0.0841 MET 0.0429 GLM	5-10 MET 3-18 GLM	81
11	glimepiride	tablets	multicomponent	233 and 228	0.1N NaOH	0.0823 MET 0.04213 GLM	5-10 MET 3-18 GLM	82
12	glimepiride	tablets	wavelength maxima area under the curve	236 MET 228 GLM 217-247 213-239	methanol	0.7480 MET 0.7904 GLB NA	5-25 for both	83
13	glimepiride	tablets	first derivative	238.6 GLM 230.0 MET	methanol	2.0 MET 5.0 GLB	4 -30 5 -30	84
14	glimepiride	tablets	simultaneous equation	234 239	0.1N NaOH	26.91 MET 5.24 GLB	5-25	85
15	glipizide	tablets	simultaneous equation	224 236	methanol	0.02 GLZ 0.75 MET	0.125-0.75 GLZ 12.5-75 MET	86
16	glipizide	tablets	simultaneous equation	272 232	water	0.214 MET 0.608 GLZ	5-25 MET 20-50 GLZ	87
17	glipizide	tablets	multicomponent	276 237	methanol	NA	2-20 GLZ	88
18	glipizide	tablets	simultaneous equation	238				89

				275	water	NA	1.2-6.0 GLZ	
			absorbance ratio	259.5			2-10 MET	
				275.0				
19	glipizide	tablets	direct UV	233 MET	acetonitrile and methanol (1:1)	NA	8 -12	90
				275 GLZ				
20	gliclazide	tablets	absorbance ratio	229	water	NA	2-12	91
				233				
21	gliclazide	tablets	simultaneous equation	227.0	methanol	NA	5-25 GLC	92
				237.5			2.5-12.5 MET	
22	gliclazide	tablets	simultaneous equation	228	water methanol (60:40)		2-24 GLC	93
				234			2-14 MET	
			area under the curve	223-233 GLC		0.33 GLC	2-24 GLC	
				229-239 MET		0.2940 MET	2-14 MET	

Conclusion

In recent years, numerous analytical methods have been developed for the quantitative estimation of drugs in combined pharmaceutical dosage forms. This article introduces methods for determining metformin and sulfonylurea antidiabetic agents in both bulk and formulated products. This review highlights the widespread use of HPLC as the primary method for analyzing these combinations, accounting for 52.9% of reported methods. This prevalence is likely due to HPLC's versatility, sensitivity, and ability to separate complex mixtures. Spectrophotometric methods are also widely used, comprising 32.4% of the reported methods, probably because of their simplicity and cost-effectiveness. Although TLC methods are less common, making up 11.9%, they still play a significant role in analyzing these combinations. Figure 2 illustrates the distribution of the analytical techniques used for the analysis of the combinations. It has been observed that the application of experimental design approaches for method optimization is limited, suggesting potential for improvement in this area. Statistical tools and experimental designs can help identify optimal separation conditions and enhance the robustness of analytical methods. It is noteworthy that while most reported methods are validated according to international guidelines, some may not be suitable for routine quality control testing due to their lack of robustness. This underscores the need for more stringent standards in the publication of analytical methods.

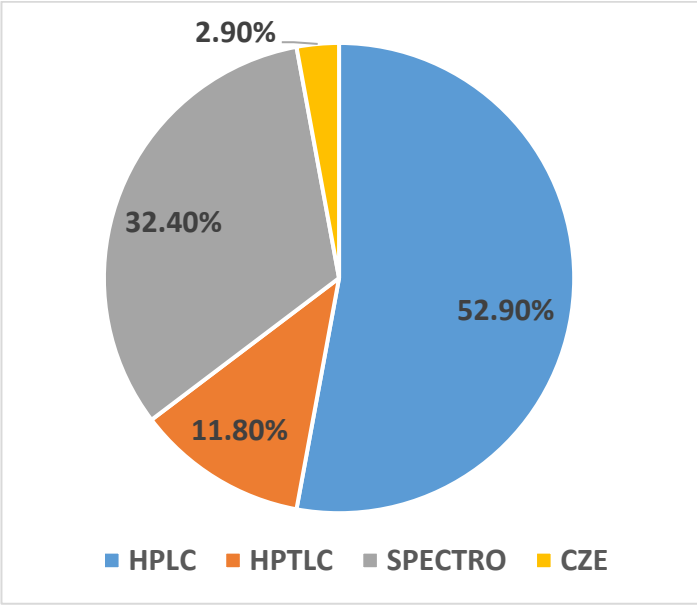


Figure 2: Percent distribution of analytical methods described in the literature for determination of metformin combinations with sulphonylureas antidiabetic agents in period between 2010 and 2024.

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