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## **A review on analytical methods for determination of oral anti-diabetic drugs like biguanides, gliptins and gliflozins in bulk and in pharmaceutical dosage forms**

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*Received: 22-11-2017 / Revised Accepted: 13-12-2017 / Published: 02-01-2018*

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### **ABSTRACT**

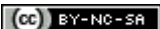
The aim of this review to focus on a comprehensive update of different analytical methods for determination of oral anti-diabetic drugs for the treatment of type 2 diabetes mellitus (T2DM), such as biguanides, dipeptidyl peptidase 4 inhibitors (gliptins), and sodium/glucose co-transporter 2 inhibitors (gliflozins) in their bulk materials and in pharmaceutical dosage forms. The review entails about analytical procedures like RP-HPLC, HPLC-UV, TLC/HPTLC and spectrophotometric (UV/VIS) methods taken from the literature over the past ten years (2007-2017). This review provides detailed information of estimation and separation conditions for biguanides, gliptins and gliflozins in alone or in combination with or without presence of their impurities and their degradation products.

**Keywords:** Biguanides; Gliptins; Gliflozins; HPLC; TLC/HPTLC and Spectrophotometric (UV/VS) method

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**How to Cite this Article:** Priyanka D. Patel, Saurabh S. Pandya. A review on analytical methods for determination of oral anti-diabetic drugs like biguanides, gliptins and gliflozins in bulk and in pharmaceutical dosage forms. World J Pharm Sci 2018; 6(1): 29-39.

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## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a worldwide problem affecting approximately 8% of the adult population, with predictions of more than 400 million cases by 2030 [1]. The prevalence of T2DM implies an urgent need for new treatments and preventative strategies. The disease results from progressive  $\beta$  cell dysfunction in the presence of chronic insulin resistance, leading to a progressive decline in plasma glucose homeostasis, increased glucagon secretion, gluconeogenesis, and renal glucose reabsorption and reduced incretin response. Treatments recommended by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) include drugs affecting all of the above processes [2]. In most patients, lifestyle changes and metformin (MET) from biguanides are recommended after diagnosis unless contraindications are present. If the therapeutic goal is not achieved after approximately three months, one of four oral treatment options can be considered in combination with MET: sulfonylureas (SUs), peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) agonists (glitazones), dipeptidyl peptidase 4 (DPP4) inhibitors (gliptins) or sodium/glucose co-transporter 2 (SGLT2) inhibitors (gliflozins). In patients with contraindications for MET, the initial drug one of these four types of drugs will be the initial treatment option. The choice of the treatment is always based on a particular patient and drug properties, with the goal of improving glycemic control and minimizing side effects [2].

The present review examines analytical methods used for the determination of biguanides, gliptins and gliflozins, the second choice drug options for oral treatment, excluding Sulfonylurea and glitazones.

**Biguanides:** Biguanides (mainly Metformin) are widely prescribed antihyperglycemic agents that suppress hepatic glucose production, increase peripheral glucose uptake, and moderately reduce LDL cholesterol and triglyceride levels. Glucose control with the aid of biguanides appears to decrease the risk of diabetes-related complications, and is not associated with weight gain. The most common adverse effect of biguanides is gastrointestinal distress, including diarrhoea, cramps, nausea, vomiting, and increased flatulence. Long-term use of biguanides has been associated with decreased absorption of vitamin B12 [3]. Metformin is usually prescribed as a single treatment (monotherapy), but it can also be combined with other medication in a single tablet - for example, metformin + pioglitazone

(Competact), metformin + vildagliptin (Eucreas), metformin + sitagliptin (Janumet), metformin + dapagliflozin (Xigduo XR). It's also sometimes prescribed in combination with insulin for people with type 1 diabetes [4].

**Gliptins:** Gliptins represent a novel class of agents that improve beta cell health and suppress glucagon, resulting in improved post-prandial and fasting hyperglycemia. They function by augmenting the incretin system (GLP-1 and GIP) preventing their metabolism by dipeptidyl peptidase-4 (DPP-4). Not only are they efficacious but also safe (weight neutral) and do not cause significant hypoglycemia, making it a unique class of drugs. The main drugs from this group are sitagliptin, vildagliptin, saxagliptin, linagliptin, and alogliptin [5, 6]

**Gliflozins:** Gliflozins are the newest class of approved oral antidiabetic agents that specifically inhibit sodium-glucose co-transporter 2 function in the kidney, thus preventing renal glucose reabsorption and increasing glycosuria in diabetic individuals while reducing hyperglycemia with a minimal risk of hypoglycemia. They reduce glycated hemoglobin and exert favorable effects beyond glucose control with consistent body weight, blood pressure, and serum uric acid reductions. The main drugs from this group are dapagliflozin, canagliflozin, empagliflozin and ipragliflozin [7-10].

**Analytical methods for determination of oral antidiabetics:** Pharmaceutical analysis has become one of the most important stages in the therapeutic process. Drug analysis includes analytical investigations of bulk drug materials, the intermediate products, drug formulations, impurities and degradation products. Analytical techniques play a significant role in understanding the chemical stability of the drug, in evaluating the toxicity of some impurities and in assessing the content of drug in formulations. Also, they are fundamental tools in pharmacokinetic studies where the analysis of a drug and its metabolites in biological fluids must be performed. Polypharmacy, which has become an integral part of therapy for many diabetic patients, further supports the importance of drug analysis. To support polypharmacy, methods suitable for two or more components are needed for quality control of such combined formulations as well as for assays in biological samples. This review presents analytical procedures such as HPLC, TLC/HPTLC and spectrophotometric (UV/VIS) methods elaborated for the listed drugs. It is based on a review of the literature from the past ten years (2007-2017).

**Table 1: HPLC methods for the analysis of biguanides, gliptins and gliflozins in bulk materials and formulations**

Sr. No.	Drug	Sample	Description	Detection mode	Ref. No.
1	Metformin	Bulk substance	<b>Column :</b> Inertsil-Extend C18 (250 × 4.6mm, packed with 5µm) <b>Mobile phase :</b> 1- Octane sulfonic acid : Acetonitrile (80 : 20) <b>Flow rate:</b> 1.0 ml/min <b>Linearity :</b> 1 – 250 µg/ml with $r^2 = 0.999$	PDA 232 nm	11
2	Metformin	Tablet dosage form	<b>Column :</b> Kromasil -ODS C18 <b>Mobile phase:</b> Methanol : orthophosphoric acid (50:50) <b>Flow rate:</b> 1.0 ml/min	UV 270 nm	12
3	Metformin	Microspheres and Tablet dosage form	<b>Column :</b> Phenomenex C <sub>18</sub> ODS (5 µ) 250 × 4.60 mm <b>Mobile phase :</b> Acetonitrile : phosphate buffer (65:35) pH adjusted to 5.75 with o-phosphoric acid <b>Flow rate:</b> 1.0 ml/min <b>Linearity :</b> 0 – 25 µg/ml with $r^2 = 0.999$	UV 233 nm	13
4	Sitagliptin	Bulk substance and Tablet dosage form	<b>Column :</b> Eclipse XDB C18 column (150.00 mm × 4.60 mm, 5µ) <b>Mobile phase :</b> 0.01M Phosphate buffer : methanol (50:50) pH adjusted to 2.5 with orthophosphoric acid <b>Flow rate :</b> 0.7 ml/min <b>Linearity :</b> 5 – 30 µg/ml with $r^2 = 0.999$ <b>LOD :</b> 0.60 µg/ml <b>LOQ :</b> 1.9 µg/ml	PDA at 267 nm	14
5	Vildagliptin	Bulk substance	<b>Column :</b> Shield C18 column (3.5 µm, 4.6×150mm) <b>Mobile phase :</b> 50mM ammonium bicarbonate (pH 7.8) : acetonitrile <b>Flow rate :</b> 1.0 ml/min <b>Linearity :</b> 10 – 120 µg/ml with $r^2 = 0.997$	UV at 210 nm	15
6	Saxagliptin	Tablet dosage form	<b>Column :</b> Kromasil C18 column (150 mm × 4.6 mm i.d., 5 µm) <b>Mobile phase :</b> phosphate buffer (pH 4.5) : methanol (65:35) <b>Flow rate :</b> 1.0 ml/min <b>Linearity :</b> 1 – 10 µg/ml with $r^2 = 0.9899$ <b>LOD :</b> 0.10 µg/ml <b>LOQ :</b> 0.28 µg/ml	UV at 230 nm	16

7	Linagliptin	Tablet dosage form	<p><b>Column :</b> Symmetry C18</p> <p><b>Mobile phase</b> Methanol : water (83:17)</p> <p><b>Flow rate :</b> 1.0 ml/min</p> <p><b>Linearity :</b> 5 – 30 µg/ml with <math>r^2 = 0.999</math></p> <p><b>LOD :</b> 0.025 µg/ml</p> <p><b>LOQ :</b> 0.08 µg/ml</p>	UV at 241 nm	17
8	Alogliptin	Bulk substance and Tablet dosage form	<p><b>Column :</b> Angilent Zobax SB-CN column (250 × 4.6 mm; 5 µm)</p> <p><b>Mobile phase:</b> Water : acetonitrile : trifluoroacetic acid (1900:100:1)</p> <p><b>Flow rate :</b> 1 ml/min</p> <p><b>Linearity :</b> 5 – 1000 ng/ml with <math>r^2 = 0.9991</math></p> <p><b>LOD :</b> 8.32 ng/ml</p> <p><b>LOQ :</b> 24.96 ng/ml</p>	UV at 278 nm	18
9	Canagliflozin	Bulk substance and Tablet dosage form	<p><b>Column :</b> Hypersil BDS, C18 (100 x 4.6 mm, 5 µm) column</p> <p><b>Mobile phase:</b> 0.1% ortho phosphoric buffer : acetonitrile (53:47)</p> <p><b>Flow rate :</b> 1.1 ml/min</p> <p><b>Linearity :</b> 75 – 450 µg/ml with <math>r^2 = 0.9999</math></p> <p><b>LOD :</b> 0.23 µg/ml</p> <p><b>LOQ :</b> 24.96 µg/ml</p>	UV at 240 nm	19
10	Dapagliflozin	Bulk substance	<p><b>Column :</b> BDS Column (250×4.5mm, 5µ)</p> <p><b>Mobile phase:</b> Acetonitrile : orthophosphoric acid (55:45)</p> <p><b>Flow rate :</b> 1.0 ml/min</p> <p><b>Linearity :</b> 25 – 150 µg/ml with <math>r^2 = 0.999</math></p> <p><b>LOD :</b> 0.6 µg/ml</p> <p><b>LOQ :</b> 1.8 µg/ml</p>	PDA at 245 nm	20, 21
11	Empagliflozin	Bulk substance	<p><b>Column :</b> Intersil column (150x40mm, 5 µm)</p> <p><b>Mobile phase:</b> 0.01 M acetate buffer : methanol (30:70)</p> <p><b>Flow rate :</b> 2.0 ml/min</p> <p><b>Linearity :</b> 2 – 150 µg/ml with <math>r^2 = 0.999</math></p> <p><b>LOD :</b> 0.7 µg/ml</p> <p><b>LOQ :</b> 1.91 µg/ml</p>	PDA at 260 nm	22

**Table 2: HPLC methods for the analysis of biguanides, gliptins and gliflozins in combined formulations and mixtures**

Sr. No.	Drug	Sample	Description	Detection mode	Ref. No.
1	Metformin + Pioglitazone	Tablet dosage form	<b>Column :</b> Eclipse XDB plus C18 Column (4.6 × 150 mm, 5µm) <b>Mobile phase :</b> Methanol : Tetrahydrofuran (70:30) <b>Flow rate :</b> 1.0 ml/min <b>Retention time :</b> 1.02 and 2.51 <b>Linearity :</b> 10 – 50 µg/ml with $r^2 = 0.998$ and 10 – 50 µg/ml with $r^2 = 0.999$ <b>LOD :</b> 0.019 µg/ml and 0.010 µg/ml <b>LOQ :</b> 0.059 µg/ml and 0.030 µg/ml	UV at 230 nm	23
2	Sitagliptin + Metformin	Bulk substance and Tablet dosage form	<b>Column :</b> C18 column (Phenomenex, 250 x 4.6 mm, 5 µm) <b>Mobile phase :</b> Phosphate buffer : pH : 4.3 acetonitrile (55:45) <b>Flow rate :</b> 1.0 ml/min <b>Retention time :</b> 2.34 and 3.20 <b>Linearity :</b> 4 – 20 µg/ml with $r^2 = 0.997$ and 10 – 50 µg/ml with $r^2 = 0.998$ <b>LOD :</b> 1.24 µg/ml and 2.64 µg/ml <b>LOQ :</b> 3.76 µg/ml and 8.0 µg/ml	UV at 252 nm	24
3	Vildagliptin + Metformin	Bulk substance and Tablet dosage form	<b>Column :</b> Zodiac C18 Column (250 mm X 4.6 mm; 5µm) <b>Mobile phase :</b> Phosphate buffer : methanol (73.5 :26.5) <b>Flow rate :</b> 1.0 ml/min <b>Retention time :</b> 4.243 and 2.490 <b>Linearity :</b> 7.5 – 17.5 µg/ml with $r^2 = 0.998$ and 75 – 175 µg/ml with $r^2 = 0.9988$ <b>LOD :</b> 0.38 µg/ml and 0.09 µg/ml <b>LOQ :</b> 1.15 µg/ml and 0.28 µg/ml	UV at 200 nm	25
4	Saxagliptin + Metformin	Bulk substance and Tablet dosage form	<b>Column :</b> Enable C18 G (250 × 4.6 mm; 5 µm particle size) <b>Mobile phase :</b> 0.05 M KH <sub>2</sub> PO <sub>4</sub> buffer (pH 4.5) : Methanol : Acetonitrile (60 : 20 : 20) <b>Flow rate :</b> 0.6 ml/min <b>Retention time :</b> 6.92 and 4.38 <b>Linearity :</b> 7.5 – 17.5 µg/ml with $r^2 = 0.998$ and 75 – 175 µg/ml with $r^2 = 0.9988$ <b>LOD :</b> 0.029 µg/ml and 0.112 µg/ml <b>LOQ :</b> 0.096 µg/ml and 0.373 µg/ml	UV at 220 nm	26
5	Alogliptin + Metformin	Bulk substance and Tablet dosage form	<b>Column :</b> X Bridge C18 column (4.6×150mm), 5µ <b>Mobile phase :</b> Buffer : Methanol : ACN (20:60:20) <b>Flow rate :</b> 1.0 ml/min <b>Retention time :</b> 3.907 and 2.365 <b>Linearity :</b> 1.5 – 2.5 µg/ml with $r^2 = 0.999$ and 60 – 100 µg/ml with $r^2 = 0.999$	UV at 290 nm	27

6	Canagliflozin + Metformin	Bulk substance and Tablet dosage form	<b>Column</b> : Kromosil C18 (250 × 4.6 mm, 5 μm) <b>Mobile phase</b> : phosphate buffer : Acetonitrile (65 : 35) <b>Flow rate</b> : 1.0 ml/min <b>Retention time</b> : 3.57 and 2.43 <b>Linearity</b> : 5 – 30 μg/ml with $r^2 = 0.9999$ and 50 – 300 μg/ml with $r^2 = 0.999$ <b>LOD</b> : 0.361 μg/ml and 0.300 μg/ml <b>LOQ</b> : 1.094 μg/ml and 0.910 μg/ml	UV at 248 nm	28
7	Dapagliflozin + Metformin	Tablet dosage form	<b>Column</b> : BDS column (250× 4.6mm, 5μ) <b>Mobile phase</b> : Phosphate buffer : methanol: acetonitrile (50:30:20) <b>Flow rate</b> : 1.0 ml/min <b>Retention time</b> : 3.647 and 2.475 <b>Linearity</b> : 0.5 – 3 μg/ml with $r^2 = 0.997$ and 85 – 510 μg/ml with $r^2 = 0.997$ <b>LOD</b> : 0.0365 μg/ml and 0.0247 μg/ml <b>LOQ</b> : 0.0365 μg/ml and 0.0247 μg/ml	PDA at 240 nm	29
8	Empagliflozin + Metformin	Bulk substance and Tablet dosage form	<b>Column</b> : C18 column (150× 4.6mm, 5μ) <b>Mobile phase</b> : Phosphate buffer : methanol (60:40) <b>Flow rate</b> : 1.0 ml/min <b>Retention time</b> : 4.210 and 2.463 <b>Linearity</b> : 3 – 7 μg/ml with $r^2 = 0.995$ and 60 – 140 μg/ml with $r^2 = 0.994$	UV at 255 nm	30

**Table 3: TLC/HPTLC methods for the analysis of biguanides, gliptins and gliflozins in bulk materials and formulations**

Sr. No.	Drug	Sample	Description	Detection wave length (nm)	Ref. No.
1	Metformin	Bulk substance and Tablet dosage form	<b>Stationary phase</b> : Silica gel 60 F <sub>254</sub> <b>Mobile phase</b> : Ammonium sulphate (0.5%) :2-propanol: methanol (8.0:1.6:1.6) <b>Linearity</b> : 200 – 1000 ng/ml with $r^2 = 0.9991$ <b>LOD</b> : 95 ng/ml <b>LOQ</b> : 200 ng/ml	238	31
2	Sitagliptin	Bulk substance and Tablet dosage form	<b>Stationary phase</b> : Silica gel aluminium plate 60 F <sub>254</sub> <b>Mobile phase</b> : Toluene : methanol (8:2) <b>Linearity</b> : 2000 – 12000 ng/band <b>LOD</b> : 193.13 ng/band <b>LOQ</b> : 579.18 ng/band	265	32
3	Vildagliptin	Bulk substance and Tablet dosage form	<b>Stationary phase</b> : Silica gel aluminium plate 60 F <sub>254</sub> <b>Mobile phase</b> : Ethyl acetate : methanol (8.5:1.5) <b>Linearity</b> : 200 –1000 ng/band <b>LOD</b> : 61 ng/band <b>LOQ</b> : 102 ng/band	217	33

4	Saxagliptin	Bulk substance and Tablet dosage form	<b>Stationary phase</b> : Silica gel aluminium plate 60 F <sub>254</sub> <b>Mobile phase</b> : Methanol : Chloroform (6:4) <b>Linearity</b> : 400 –1200 ng/band <b>LOD</b> : 7.96 ng/band <b>LOQ</b> : 26.54 ng/band	222	34
5	Linagliptin	Tablet dosage form and in biological sample	<b>Stationary phase</b> : Silica gel aluminium plate 60 F <sub>254</sub> <b>Mobile phase</b> : Methanol : toluene (7:3) <b>Linearity</b> : 50 –300 ng/band	232	35
6	Alogliptin	Bulk substance and Tablet dosage form	<b>Stationary phase</b> : Silica gel aluminium plate 60 F <sub>254</sub> <b>Mobile phase</b> : Acetonitrile : 1% ammonium acetate in methanol (4.5:5.5) <b>Linearity</b> : 100 – 5000 ng/band with r <sup>2</sup> = 0.999 <b>LOD</b> : 2.356 ng/band <b>LOQ</b> : 7.14 ng/band	277	36
7	Canagliflozin	Bulk substance and Tablet dosage form	<b>Stationary phase</b> : Silica gel aluminium plate 60 F <sub>254</sub> <b>Mobile phase</b> : Toluene : ethyl acetate : methanol (2:2:1) <b>Linearity</b> : 10 – 500 ng/spot with r <sup>2</sup> = 0.9988 <b>LOD</b> : 0.39 ng/spot <b>LOQ</b> : 1.19 ng/spot	290	37

**Table 4: TLC/HPTLC methods for the analysis of biguanides, gliptins and gliflozins in combined formulations and mixtures**

Sr. No.	Drug	Sample	Description	Detection wave length (nm)	Ref. No.
1	Metformin + Repaglinide	Bulk substance and Tablet dosage form	<b>Stationary phase</b> : Silica gel 60 F <sub>254</sub> <b>Mobile phase</b> : Methanol : ammonium sulphate (0.25%) (2.5:7.5) <b>Linearity</b> : 500 – 2500 ng/band with r <sup>2</sup> = 0.9999 and 100 – 500 ng/band with r <sup>2</sup> = 0.995 <b>LOD</b> : 98 ng/band and 17 ng/band <b>LOQ</b> : 296 ng/band and 51 ng/band	236	38
2	Sitagliptin + Simvastatin	Bulk substance and Tablet dosage form	<b>Stationary phase</b> : Silica gel 60 F <sub>254</sub> <b>Mobile phase</b> : Chloroform : methanol (8:2) <b>Linearity</b> : 2000 – 7000 ng/spot with r <sup>2</sup> = 0.9983 and 250 – 750 ng/spot with r <sup>2</sup> = 0.9974 <b>LOD</b> : 150 ng/spot and 50 ng/spot <b>LOQ</b> : 2000 ng/spot and 660 ng/spot	217	39
3	Vildagliptin + Metformin	Bulk substance and Tablet dosage form	<b>Stationary phase</b> : Silica gel 60 F <sub>254</sub> <b>Mobile phase</b> : Ammonium acetate in methanol (1%) : toluene (10:0.5) <b>Linearity</b> : 500 – 2000 ng/spot with r <sup>2</sup> = 0.991 and 1000 – 5000 ng/spot with r <sup>2</sup> = 0.999 <b>LOD</b> : 34.60 ng/spot and 17.22	214	40

			ng/spot <b>LOQ</b> : 104.85 ng/spot and 52.20 ng/spot		
4	Linagliptin + Metformin	Tablet dosage form	<b>Stationary phase</b> : Silica gel 60 F <sub>254</sub> <b>Mobile phase</b> : Acetone : methanol : toluene : formic acid (4:3:2:1) <b>Linearity</b> : 20 – 100 ng/spot with $r^2 = 0.9993$ and 400 – 2000 ng/spot with $r^2 = 0.9991$ <b>LOD</b> : 10 ng/spot and 20 ng/spot <b>LOQ</b> : 20 ng/spot and 100 ng/spot	259	41
5	Alogliptin + Metformin	Bulk substance and Tablet dosage form	<b>Stationary phase</b> : Silica gel 60 F <sub>254</sub> <b>Mobile phase</b> : Acetonitrile : Ammonium acetate in methanol (1%) (4.5:5.5) <b>Linearity</b> : 100 – 2500 ng/spot and 100 – 2500 ng/spot	253	42

**Table 5: Spectrophotometric (UV/VIS) methods for the analysis of biguanides, gliptins and gliflozins in bulk materials and formulations**

Sr. No.	Drug	Sample	Description	Detection wave length (nm)	Ref. No.
1	Metformin	Tablet dosage form	<b>Solvent</b> : 0.01N sodium hydroxide <b>Linearity</b> : 8 – 13 µg/ml with $r^2 = 0.9999$ <b>LOD</b> : 1.0 µg/ml <b>LOQ</b> : 3.0 µg/ml	233	43
2	Sitagliptin	Bulk substance and Tablet dosage form	<b>Solvent</b> : Methanol <b>Linearity</b> : 20 – 60 µg/ml with $r^2 = 0.991$ <b>LOD</b> : 6.03 µg/ml <b>LOQ</b> : 18.28 µg/ml	267	44
3	Vildagliptin	Tablet dosage form	<b>Solvent</b> : Water <b>Linearity</b> : 12 – 200 ppm with $r^2 = 0.999$	266	45
4	Saxagliptin	Bulk substance and Tablet dosage form	<b>Solvent</b> : Methanol <b>Linearity</b> : 5 – 40 µg/ml with $r^2 = 0.999$ <b>LOD</b> : 0.0607 µg/ml <b>LOQ</b> : 0.1821 µg/ml	208	46
5	Linagliptin	Tablet dosage form	<b>Solvent</b> : Methanol <b>Linearity</b> : 5 – 40 µg/ml with $r^2 = 0.9985$ <b>LOD</b> : 0.3649 µg/ml <b>LOQ</b> : 1.1059 µg/ml	228	47
6	Alogliptin	Bulk substance and Tablet dosage form	<b>Solvent</b> : Water + Bromate – bromide solution <b>Linearity</b> : 1 – 10 µg/ml with $r^2 = 0.9994$ <b>LOD</b> : 0.115 µg/ml <b>LOQ</b> : 0.348 µg/ml	505	48
7	Canagliflozin	Bulk substance and Tablet dosage form	<b>Solvent</b> : Methanol <b>Linearity</b> : 5 – 10 µg/ml with $r^2 = 0.9989$ <b>LOD</b> : 0.084 µg/ml <b>LOQ</b> : 0.255 µg/ml	290	49
8	Dapagliflozin	Bulk and substance	<b>Solvent</b> : Phosphate buffer solution (pH 7.2)	233.65	50



		Tablet dosage form	<b>Linearity</b> : 10 – 35 µg/ml with $r^2 = 0.9998$ <b>LOD</b> : 1.24 µg/ml <b>LOQ</b> : 3.62 µg/ml		
9	Empagliflozin	Bulk substance and Tablet dosage form	<b>Solvent</b> : Ethanol <b>Linearity</b> : 2 – 12 µg/ml with $r^2 = 0.999$ <b>LOD</b> : 0.02 µg/ml <b>LOQ</b> : 0.07 µg/ml	247	51

**Table 5: Spectrophotometric (UV/VIS) methods for the analysis of biguanides, gliptins and gliflozins in combined formulations and mixtures**

Sr. No.	Drug	Sample	Description	Detection wave length (nm)	Ref. No.
1	Metformin + Pioglitazone	Tablet dosage form	<b>Solvent</b> : Methanol <b>Linearity</b> : 2 – 10 µg/ml with $r^2 = 0.9993$ and 2 – 20 µg/ml with $r^2 = 0.9996$ <b>LOD</b> : 0.4 µg/ml and 0.9 µg/ml	225 and 237	52
2	Sitagliptin + Gliclazide	Bulk substances and Tablet dosage form	<b>Solvent</b> : Methanol + water <b>Linearity</b> : 20 – 100 µg/ml with $r^2 = 0.998$ and 7 – 27 µg/ml with $r^2 = 0.996$ <b>LOD</b> : 0.23 µg/ml and 0.31 µg/ml <b>LOQ</b> : 0.69 µg/ml and 0.93 µg/ml	267 and 226	53
3	Vildagliptin + Metformin	Tablet dosage form	<b>Solvent</b> : Water <b>Linearity</b> : 60 – 100 µg/ml with $r^2 = 0.998$ and 10 – 50 µg/ml with $r^2 = 0.996$ <b>LOD</b> : 0.23 µg/ml and 0.31 µg/ml <b>LOQ</b> : 0.69 µg/ml and 0.93 µg/ml	218.25 and 225.50	54
4	Linagliptin + Metformin	Tablet dosage form	<b>Solvent</b> : Distilled water <b>Linearity</b> : 10 – 40 µg/ml with $r^2 = 0.999$ and 2 – 14 µg/ml with $r^2 = 0.999$ <b>LOD</b> : 1.03 µg/ml and 0.34 µg/ml <b>LOQ</b> : 5.18 µg/ml and 1.71 µg/ml	294.4 and 230.4	55
5	Alogliptin + Metformin	Tablet dosage form	<b>Solvent</b> : Methanol <b>Linearity</b> : 0.05 – 0.25 µg/ml with $r^2 = 0.9996$ and 2 – 10 µg/ml with $r^2 = 0.9993$	225 and 237	56
6	Dapagliflozin + Metformin	Synthetic mixture	<b>Solvent</b> : Methanol <b>Linearity</b> : 0.5 – 2.5 µg/ml with $r^2 = 0.983$ and 25 – 125 µg/ml with $r^2 = 0.985$	225 and 237	57
7	Empagliflozin + Metformin	Bulk substance and Tablet dosage form	<b>Solvent</b> : Methanol <b>Linearity</b> : 5 – 25 µg/ml with $r^2 = 0.999$ and 2 – 12 µg/ml with $r^2 = 0.999$	272 and 234	58

## CONCLUSION

In this literature review, a broad range of analytical methods have been presented for the analysis of oral anti-diabetics in bulk materials and pharmaceutical dosage forms. HPLC with UV detection and UV spectrophotometry were mainly used, due to their accuracy, precision, reliability,

repeatability, analysis time and sensitivity. These methods are adequate to analyse the drugs in single component formulation as well as combination preparation. Also, TLC/HPTLC with densitometry detection and VIS spectrophotometry were widely used for typical analysis in pharmaceutical formulation. UV/VIS spectrophotometric methods and TLC/HPTLC methods were frequently

proposed as an alternative to HPLC due to its simplicity and cost effectiveness. Groups of oral anti-diabetics are still extensively developed, and almost every year a new drug similar to these described drugs appears on the market. The

analytical methods in this article were gathered together and compared to facilitate steps in designing, in examining, in manufacturing already discovered drugs as well as upcoming new substances.

## REFERENCES

1. Shaw JE et al. Global estimates of the prevalence of diabetes. *Diab Res Clin Pract* 2010; 87: 4-14.
2. Inzucchi SE et al. Management of hyperglycaemia in type 2 diabetes: a patient centred approach update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2015; 38: 140-149.
3. Sofer S et al. A diet with carbohydrates eaten primarily at dinner: an innovative, nutritional approach to end the vicious cycle of abdominal obesity. *Nutr Pre Treat Obe* 2014; 401-414.
4. <https://www.diabetes.co.uk/diabetes-medication/biguanides.html>
5. Gupta V, Kalra S. Choosing gliptin. *Ind J Endo Meta* 2011; 15(4): 298-308.
6. Kumar KV, Gupta AK. Clinical audit of patients using DPP4 inhibitors in longstanding type 2 diabetes. *Diabetes Metab Syndr Clin Res Rev* 2015; 9: 277-279.
7. Bonner C et al. Inhibition of the glucose transporter SGLT2 with dapagliflozin in pancreatic alpha cells triggers glucagon secretion. *Natr Med* 2015; 21: 512-517.
8. Scheen AJ. SGLT2 Inhibitors: Benefit/Risk Balance. *Current Diabetes Reports* 2016; 16: 92.
9. Kellani TM. Gliflozins: initial review of litera. *Integr Obesity Diabetes* 2016; 2(5): 276.
10. Basile JN. The potential of sodium glucose cotransporter 2 (SGLT2) inhibitors to reduce cardiovascular risk in patients with type 2 diabetes (T2DM). *J Diabetes Complicat* 2013; 27: 280-286.
11. Madhukar A et al. Simple and sensitive analytical method development and validation of metformin hydrochloride by RP-HPLC. *Int J Pharm Pharm Sci* 2011; 3(3): 117-120.
12. Gadipally S. Method development of metformin hydrochloride RP-HPLC. *World J Pharm Pharm Sci* 2014; 3(3): 1149-1159.
13. Kar M, Choudhury PK. HPLC method for estimation of metformin hydrochloride in formulated microspheres and tablet dosage form. *Ind J Pharm Sci* 2009; 71(3): 318-320.
14. Lavanya R, Yunoo MD. Development and validation of RP-HPLC method for the estimation of sitagliptin phosphate in bulk and its tablet dosage form. *J Adv Pharm Edu Res* 2013; 3(4): 475-479.
15. Pharne AB et al. Bioanalytical method development and validation of vildagliptin a novel dipeptidyl peptidase IV inhibitor by RP-HPLC method. *Int J Pharm Pharm Sci* 2014; 4(3): 119-123.
16. Abdalla SA. Validation of stability indicating RP-HPLC method for the estimation for saxagliptin in tablet formulations. *Indo Am J Pharm Res* 2014; 4(8): 3550-3558.
17. Badugu LR. A validated RP-HPLC method for the determination of linagliptin. *Am J Pharm Res* 2012; 2(4): 462 - 470.
18. Zhang K et al. A developed HPLC method for the determination of alogliptin benzoate and its potential impurities in bulk drug and tablets. *As J Pharm Sci* 2015; 10(2): 152-158.
19. Suneetha A, Sharmila D. A validated stability indicating RP-HPLC method for estimation of canagliflozin in dosage form. *Res J Pharm Bio Chem Sci* 2015; 6(5): 1186-1194.
20. Manasa S et al. Development and validation of stability- indicating RP-HPLC method for determination of dapagliflozin. *J Adv Pharm Edu Res* 2014; 4(3): 350-353.
21. Manasa S et al. Development and validation of a RP-HPLC method for the estimation of dapagliflozin in API. *Int J Phram Sci Res* 2014; 5(12): 5394-5397.
22. Padmaja N, Veerhadram G. Method development and validation of RP-HPLC method for the estimation of empagliflozin in API. *Int J Phram Sci Res* 2016; 7(2): 724-727.
23. Dr. Usharani N et al. Development and validation of RP-HPLC and UV derivative spectrophotometric methods for the determination of metformin HCL and pioglitazone in pharmaceutical formulations. *World J Pharm Res* 2015; 4(12): 1117-1125.
24. Jeyabalan G, Nyola N. Simultaneous estimation of sitagliptin phosphate monohydrate and metformin hydrochloride in bulk and pharmaceutical formulation by RP-HPLC. *J Pharm Edu Res* 2012; 3(2): 24-28.
25. Gattu MP et al. Development and validation of simultaneously estimation of vildagliptin & metformin hydrochloride by RP-HPLC in bulk and oral dosage form. *Int J Adv Pharm Gen Res* 2014; 2(1): 24-33.
26. Prasad PBN et al. Development and validation of a method for simultaneous determination of metformin and saxagliptin in a formulation by RP-HPLC. *Am J Anal Chem* 2015; 6: 841-850.
27. Koduru S et al. Method development for the simultaneous estimation of metformin and alogliptin by using RP-HPLC. *Int J Pharm Res Health Sci* 2015; 3(3): 747-753.
28. Gaware D et al. A validated stability indicating RP-HPLC method for simultaneous determination of metformin and canagliflozin in pharmaceutical formulation. *World J Pharm Pharm Sci* 2015; 4(12): 631-640.
29. Shyamala et al. Validated RP-HPLC method for simultaneous estimation of metformin hydrochloride and dapagliflozin in tablet dosage form. *Am J Bio Pharm Res* 2015; 2(2): 109-113.
30. Hussain BJ et al. Method development and validation of metformin and empagliflozin in pharmaceutical dosage forms in RP-HPLC. *As J Res Chem Pharm Sci* 2016; 4(3): 91-100.

31. Havele S, Dhaneshwar S. Estimation of metformin in bulk drug and in formulation by HPTLC. *J Nanomedic Nanotechnol* 2010; 1(1): 102-105.
32. Sarowar GS et al. Stability-indicating HPTLC method development and validation for estimation of sitagliptin phosphate in tablet dosage form. *Euro J Pharm Med Res* 2016; 3(1): 213-218.
33. Butle SR, Deshpande PB. Validated stability-indicating HPTLC method development for determination of vildagliptin as bulk drug and in tablet dosage form. *Euro J Pharm Med Res* 2015; 2(6): 234-237.
34. Salvala S et al. Development and validation of a high performance thin layer chromatographic method for quantitative analysis of saxagliptin. *Am J Anal Chem* 2015; 6: 797-806.
35. Bhole RP et al. High performance thin layer chromatographic determination of linagliptin in pharmaceutical formulations and in biological samples. *Int J Pharm Chem Anal* 2017; 4(1): 13-19.
36. Sharma K, Parle A. Development and validation of HPTLC method for the estimation of alogliptin benzoate in bulk drugs and tablet dosage form. *Int Bull Drug Res* 2015; 5(8): 81-89.
37. Kaur I et al. Development and validation of a stability-indicating high performance thin layer chromatography (HPTLC) method for estimation of canagliflozin in bulk and pharmaceutical dosage form. *J App Pharm Sci* 2016; 6(5): 051-057.
38. Ahir KB et al. Simultaneous estimation of metformin hydrochloride and repaglinide in pharmaceutical formulation by HPTLC-densitometry method. *J Chromat Sep Tech* 2013; 4(1): 1-5.
39. Rathod S et al. Development and validation of HPTLC method for the estimation of sitagliptin phosphate and simvastatin in bulk and marketed formulation. *Int J Drug Dev Res* 2012; 4(3): 292-297.
40. Shirode AR et al. RP-HPLC and HPTLC methods for simultaneous estimation of metformin hydrochloride and vildagliptin from bulk and marketed formulation: development and validation. *Br J Pharm Res* 2014; 4(20): 2370-2386.
41. Rajasekaran A et al. Development and validation of HPTLC method for simultaneous estimation and stability indicating study of metformin HCl and linagliptin in pharmaceutical formulation. *World J Pharm Sci* 2014; 2(4): 317-327.
42. Tejjpal et al. Development and validation of HPTLC method for simultaneous estimation of metformin hydrochloride and alogliptin benzoate in bulk drugs and combined dosage forms. *J Pharm Care Hea Sys* 2015; 2(5): 116.
43. Mishra K et al. Method development and validation of metformin hydrochloride in tablet dosage form. *Euro J Chem* 2011; 8(3): 1309-1313.
44. Jeyabalan G, Nyola N. Analytical method development and validation of sitagliptin phosphate monohydrate in pure and tablet dosage form by derivative spectroscopy. *J App Pharm Sci* 2013; 3(1): 95-98.
45. Safila N et al. Using UV spectrophotometric method to determine the linearity of vildagliptin brands. *Am J Inn Res App Sci* 2015; 1(1): 6-9.
46. Kalaichelvi R., Jayachandran E. Validated spectroscopic method for estimation of saxagliptin in pure and from tablet formulation. *Int J Pharm Pharm Sci* 2011; 3(3): 179-180.
47. Sangeetha RK, Subashri T. Analysis of linagliptin in tablet dosage form by UV spectroscopy method, its derivatives and difference spectra. *Euro J Pharm Med Res* 2016; 3(11): 536-540.
48. Sunil kumar et al. Spectrophotometric determination of alogliptin in bulk and tablet dosage form using bromate bromide mixture as brominating agent. *Kar Int J Mod Sci* 2017; 3: 8-17.
49. Kaur I et al. Development and validation of UV spectroscopic method for determination of canagliflozin in bulk and pharmaceutical dosage form. *Pharm Meth* 2015; 6(2): 82-86.
50. Chitra KP et al. Unique UV spectrophotometric method for reckoning of dapagliflozin in bulk and pharmaceutical dosage forms. *J Chem Pharm Res* 2015; 7(9): 45-49.
51. Jyothirmai N et al. Novel UV and visible spectrophotometric methods for the analysis of empagliflozin a type 2 diabetic drug in bulk and pharmaceutical formulations. *J de Afri* 2016; 3(1): 177-187.
52. Sujana K et al. Simultaneous estimation of pioglitazone hydrochloride and metformin hydrochloride using UV spectroscopic method. *J Biomed Sci Res* 2010; 2(2): 110-115.
53. Reichal RC, Rao GM. Development and validation of spectrophotometric method for simultaneous estimation of gliclazide and sitagliptin phosphate monohydrate in bulk and pharmaceutical dosage form. *Int J Pharm Pharm Sci* 2015; 7(6): 372-376.
54. Karajgi SR et al. First derivative spectrophotometric simultaneous determination of vildagliptin and metformin in tablet formulations. *Pharmacophore* 2016; 7(2): 109-117.
55. Konari SN et al. Stability indicating UV spectrophotometric method for linagliptin and metformin in pharmaceutical dosage form. *Pharm Meth* 2017; 8(2): 121-126.
56. Malathi S, Vijayalakshmi M. Development and validation for simultaneous estimation of alogliptin and metformin in combined dosage form by UV method. *Am J Pharm Hea Res* 2017; 5(8): 88-97.
57. Jani BR et al. Development and validation of UV spectroscopic method for simultaneous estimation of dapagliflozin and metformin hydrochloride in synthetic mixture. *Int J Res Dev Pharm L Sci* 2015; 4(3): 1569-1576.
58. Padmaja N et al. Development and validation of UV spectrophotometric method for simultaneous estimation of empagliflozin and metformin hydrochloride in bulk drugs and combined dosage forms. *Der Pharm Let* 2016; 8 (13): 207-213.