Review Article

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Canagliflozin: A review with specific focus on analytical methods in biological matrices and pharmaceuticals

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Abstract: Sodium-glucose transporter 2 inhibitor emerges as the latest group of oral hypoglycemic agents, which shows insulin-independent pathology and provides an upper hand to enhance renal glucose elimination. Canagliflozin (CGN) was the number one drug, approved by FDA on 29th March 2013 for the treatment of type 2 diabetes mellitus. By totting up to its glucose-lowering effects, it exhibits beneficial effects on the heart and potentially on the kidneys. The study aims to summarize various analytical techniques, such as chromatography, spectrophotometry, and hyphenated techniques, such as Liquid chromatography with tandem mass spectrometry (LC-MS/MS) and Ultra performance liquid chromatography with tandem mass spectrometer (UPLC-MS) for the analysis of CGN. In the proposed work, we have reviewed various analytical methods reported for the estimation of CGN in biological matrices and Pharmaceuticals from various databases like ScienceDirect, Springer, PubMed, Scopus, Taylor & Francis, and Web of Science for the estimation of CGN. Various analytical methods adapted were high-performance liquid chromatography, UPLC, LC-MS/MS, high-performance thin-layer liquid chromatography, Fourier-transform infrared spectroscopy, spectrofluorimetry, and UV spectrophotometry. This current review presented the determination of CGN using various analytical techniques and biological matrices either in single or in combination with other hypoglycemic agents, as per International Conference on Harmonization guidelines. Further, some future trends that can be integrated were also suggested.

Keywords: canagliflozin, type 2 diabetes, analytical methods, validation, ICH guidelines, LC-MS/MS

Abbreviations

CGN	canagliflozin
DAD	diode array detector
DGN	dapagliflozin
EGN	empagliflozin
HPLC	high performance liquid chromatography
ICH	International Conference on Harmonization
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LNG	linagliptin
LLE	liquid–liquid extraction
LOD	limit of detection
LOQ	limit of quantitation
MFN	metformin
PDA	photo diode array
PPE	protein precipitation extraction
SGLT2	sodium glucose transporter 2
SPE	solid-phase extraction
T2DM	type 2 diabetes mellitus
TLC	thin layer chromatography
UPLC	ultra performance liquid chromatography
UV-Vis	ultra violet-visible spectroscopy
VWD	variable wavelength detector

1 Introduction

Type 2 diabetes mellitus (T2DM) resumes being a major non-communicable ailment with a universal burden of 366 million. India is considered the epicenter of diabetes

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with 77 million and is supposed to be 134 million by the year 2045. Sodium-glucose transporter 2 (SGLT2) inhibitor are the latest group of anti-diabetic medicines available in the market for the treatment of T2DM, preventing re-absorption of glucose from the blood, which is filtered through the kidneys and hence facilitating the excretion of glucose in urine. Canagliflozin (CGN) is the first approved member of the SGLT2 inhibitor class by the USFDA in March 2013 applicable to patients with T2DM as an adjunct to exercise and diet to enhance glycemic control [1]. SGLT2 inhibitors get inhibited in the proximal renal tubules by the action of CGN, lowering the urinary glucose threshold, which further enhances urinary glucose excretion. Cardiopathy is considered as a frequently developed etiology along with T2DM to cause morbidity and mortality among people with T2DM [2,3]. CGN was approved in the year 2018 to treat cardiovascular diseases, which includes blood pressure, body weight, and urinary function. CGN has superiority of SGLT2 inhibitors and cardiovascular treatment compared with other anti-diabetic drugs used to treat T2DM. CGN is chemically1-(beta-*d*-glucopyranosyl)-4-methyl-3-[5-(4-fluorophenyl)-2-thienylmethyl] benzene hemihydrate (Figure 1)

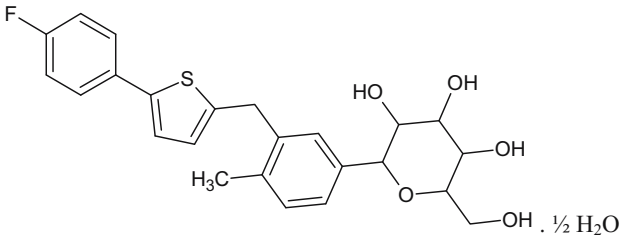

Figure 1: Structure of canagliflozin hemihydrate.

with chemical formula $C_{24}H_{25}FO_5S{\cdot}1/2H_2O$ and molecular weight of 453.52 g·mol^{-1}. It is freely soluble in methanol. It is yet to be monographed in any pharmacopoeias [4].

The threat of disease, access to medicine for the poor, and rising costs of medicine are the big challenges for the people of the world. For making affordable drugs available for people of the world that are extremely poor and prone to large epidemics, it is important to bring down the costs of research and development and thereby the production process and quality control. Simple, rapid (to reduce analytical down time in turn revenue), and cost-effective analytical method development is a thirst area of importance in the pharmaceutical industry. In view of the significance of CGN to the universal population, sensitive quality control methods are required for their analysis. Analytical methods have a major task in ensuring and assuring the quality of pharmaceuticals. Hence, currently, there is a need for the collection of the reported analytical methods of CGN. The main objective of the review is to summarize the multiple approaches available to estimate CGN in API, dosage form and biological matrix separately and drug combinations. The analytical methods were classified into four main methods: (1) UV-visible (UV-Vis) spectroscopy, (2) chromatography (Ultra performance liquid chromatography (UPLC), high-performance liquid chromatography (HPLC), high-performance thin-layer liquid chromatography (HPTLC)), and (3) bio-analytical. Figure 2 represents an overview of various analytical methods for the determination of CGN from various databases like ScienceDirect, Springer, PubMed, Scopus, Taylor & Francis, and Web of Science for the estimation of CGN.

Figure 3 provides the graphical representation of the number of articles published for the quantification of CGN from 2014 to 2022.

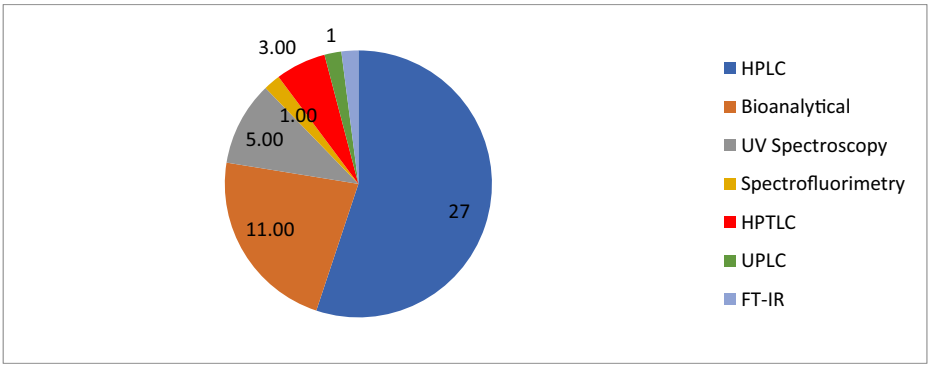

Figure 2: Analytical methods for the estimation of CGN.

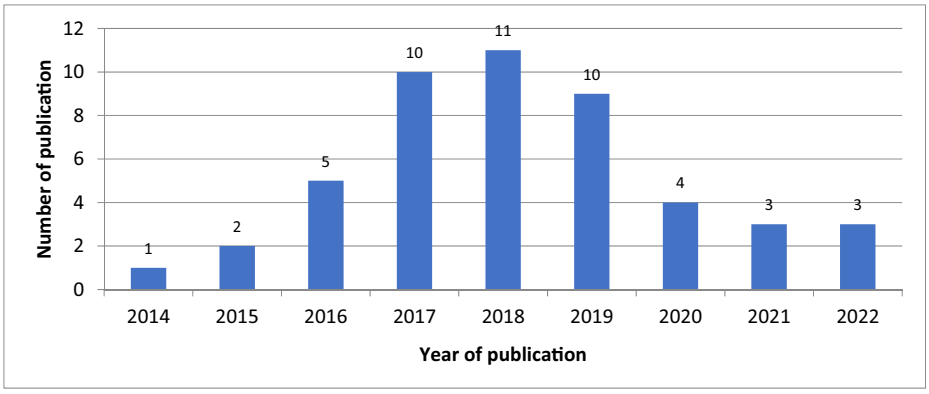

Figure 3: Number of analytical methods reported during 2014–2021. Database sources: ScienceDirect, Springer, PubMed, Scopus, Taylor & Francis, and Web of Science.

2 Spectroscopic methods

2.1 UV-Vis spectroscopic methods

UV-Vis spectroscopy methods are employed in pharmaceutical product estimation for their simplicity, versatility, accuracy, speed, and cost-effectiveness. Over a 40 years period, it has become the most important analytical instrument where expensive instruments like HPLC, Gas chromatography, Liquid chromatography with tandem mass spectrometry (LC-MS/MS) are not available. Singh et al. [5] developed a UV spectroscopic method using methanol as a diluent and measured the absorbance at 280 nm with a linearity of 5–50 $\mu g \cdot mL^{-1}$. Chinta et al. [6] reported a UV spectroscopic method using phosphate buffer as diluent at an absorbance wavelength of 289 nm and attained 99% purity with a linearity of 1–6 $\mu g \cdot mL^{-1}$. Ishpreet et al. [7] developed a UV spectroscopic method with a linearity of 5–10 $\mu g \cdot mL^{-1}$ at an absorbance of 290 nm and recovery within 80.00–120.00%. Vichare et al. [8] have reported two simultaneous methods, one based on absorbance correction UV spectroscopy (absorbance measurement at wavelengths 233 nm (λ_{max} of metformin (MFN)) and 291 nm (λ_{max} of CGN) and another based on first order derivative spectroscopy overlain spectra wavelengths 243 nm (zero absorbance of CGN) and 318 nm (zero absorbance of MFN) with a linearity of 0.75–4.5 $\mu g \cdot mL^{-1}$ for CGN and 2.5–15 $\mu g \cdot mL^{-1}$ for MFN, respectively. The percentage drug contents were found to be 98.48% ± 0.83% and 100.76% ± 1.29% for method A and 97.94 ± 0.96 and 97.22 ± 1.15 for CGN and MFN, respectively. The spectroscopic method existing for the determination of CGN as a single entity or in combination with other drugs [5–8] and the results (solvent, wavelength measured, linearity, accuracy, limit of detection (LOD), and limit of quantitation (LOQ)) obtained are represented in Table 1.

2.2 Spectrofluorimetry method

Spectrofluorimetry is a highly sensitive analytical method for the detection and determination of fluorescent compounds at ng or lower level. Nirav et al. [9] reported a specific, accurate, precise and robust spectrofluorometric method using methanol as the solvent, which emit excitation λ_{max} at 293 nm and emission λ_{max} at 349 nm. The method was linear over the range of 100–500 $ng \cdot mL^{-1}$ with a percentage recovery of 99.42–99.81%. The LOD and LOQ for the developed method were found to be

Table 1: Overview of published UV-Vis spectroscopic methods

Drug	Dosage form	Accuracy	LOD ($\mu g \cdot mL^{-1}$)	LOQ ($\mu g \cdot mL^{-1}$)	Reference
CGN	Bulk, tablets	99.46–100.31%	0.00945	2.8638	Singh et al. [5]
CGN	Bulk, tablets	80–120%	NA	NA	Chinta et al. [6]
CGN	Bulk, tablets	80–120%	0.084	0.255	Ishpreet et al. [7]
CGN and MFN	Bulk, tablets	CGN: 98.48% and MFN: 100.76%	NA	NA	Vichare et al. [8]
		CGN: 97.94% and MFN: 97.22%	NA	NA	

13.58 and 41.15 ng·mL^{-1}, respectively. The method was novel, sensitive, and can be applied for the routine analysis of CGN by spectrofluorimetry [9].

3 Chromatographic methods

3.1 HPLC

HPLC is the most versatile and dominant separation technique in modern pharmaceutical and biomedical analysis due to its highly efficient separation and enhanced detection sensitivity. Most of the drugs are analyzed by HPLC method due to its accuracy, ease of automation, rapidity, specificity, and accuracy. Vymyslicky et al. [10] reported a new stability indicating HPLC method using electrochemical detection which significantly saves time and reduces the consumption of active ingredients and solvents, thereby minimizing the costs of forced degradation. Using ammonium phosphate buffer with methanol in a 50/50 volume ratio as a working electrolyte, the authors electrochemically oxidized samples and analyzed them by HPLC. Oxidation with hydrogen peroxide required 7 days, whereas electrochemical oxidation was completed in 3 h. Reddy et al. [11] reported a HPLC method which eluted CGN at a longer retention of 7.3 min using acetonitrile:water (50:50) as the mobile phase. The method was found to be sensitive over the other reported methods. Sadasivuni and Gundoju [12], using C18 column and acetonitrile:pH 2.5 with orthophosphoric acid (50:50) as the mobile phase, had developed an accurate method but did not mentioned the retention time (Rt). Mounika et al. [13] had developed a cost-effective, accurate, and sensitive method compared to other methods using a cost-effective mobile phase and eluted CGN at a Rt of 3.3 min. Another author had used methanol:water (90:10) and eluted CGN at 4.4 min (Sushil et al. [14]). Singh et al. [5] reported an accurate, precise, and linear method using acetonitrile:orthophosphoric acid (0.01 M) (50:50) and C18 column and eluted CGN at 4.7 min. Bhatt et al. [15] used acetonitrile:ammonium acetate buffer (pH 4.5) (70%:30% v/v) to elute CGN at 4.5 min. Parida et al. [16] reported a method using methanol:phosphate buffer with pH 4 (65:35) as mobile phase and C18 column using variable wavelength detector (VWD) at 293 nm and eluted CGN at 2.9 min. The Rt was comparatively less than the other methods. Sreenivasulu et al. [17] had reported a novel method using ion pair reagent, acetonitrile:1-octane sulphonic acid buffer (70:30) as the mobile phase and Rt of CGN was found at 3.4 min. The authors had achieved a good retention by using an ion pair reagent. Another author reported a method using orthophosphoric acid (0.1%):acetonitrile (60:40) which had a longer Rt at 8.7 min (Rahul et al. [18]) compared to the other reported methods. Vijaya et al. [19] developed a sensitive, selective method using 0.02% formic acid:acetonitrile (40:60) as the mobile phase and C18 column and achieved separation at 4.4 min. Goutam et al. [20] separated CGN from its process and degradation related substances using Ascentis Express RP-amide (150 mm × 4.6 mm, amide groups chemically bonded to porous silica particles of 2.7 µm) column, and ammonium acetate buffer:acetonitrile as the mobile phase at 290 nm. This method can be used to estimate the related substances of CGN. Triveni et al. [21], Ishpreet et al. [22], and Ladva et al. [23] have reported rapid, sensitive, accurate, and linear method using C18 as the stationary phase and various ratios of buffers (formic acid in water (0.1% v/v), ortho phosphoric acid, and 0.1% ammonium acetate:organic solvent (acetonitrile, methanol) as the mobile phase. The detectors used for the estimation were UV and PDA detectors. Suma et al. [24] had developed a cost effective, accurate method which eluted CGN at 2.8 min using HPLC grade water:acetonitrile (55:45 v/v) as the mobile phase. Al-Shdefat et al. [25] developed a simple, easy, and precise stability indicating HPLC method with diode array detector (DAD) for the simultaneous determination of CGN and MFN in a combined dosage formulation using a triple combination of mobile phase 0.05 M H_3PO_4, acetonitrile, and methanol in the 45:45:10 (v/v). The Rt for MFN and CGN were obtained at 8 and 9.45 min, respectively, which was a longer Rt compared to the other reported methods and found to be linear over the range of 5–100 g·mL^{-1}. Authors Sunitha et al. [26], Bangaruthalli et al. [27], and Gandla et al. [28] performed simultaneous estimation of CGN and MFN using C18 as the stationary phase and wide choice of mobile phases. The methods were validated as per ICH guidelines. Moussa et al. [29] reported a simple and precise HPLC method for the quantitative estimation of CGN, empagliflozin (EGN), linagliptin (LNG), and MFN in their pharmaceutical formulation using a systematic and practical experimental design approach with minimum experimental trials. Authors selected three factors affecting the peaks significantly and determined using Plackett–Burman design. This method was found to be in good agreement with the observed value approving that design of experiment was simple methodology which was easy to instrument and chromatographically dependable in analyzing the single and multi-component drugs. Khalil et al., [30] developed

the first reverse phase HPLC (RP-HPLC) method for simultaneous determination of CGN, dapagliflozin (DGN), EGN, and MFN on C18 column (250 mm × 4.6 mm, 5 µm p.s) Inertsil ODS through isocratic elution using acetonitrile and 0.05 M potassium dihydrogen phosphate buffer pH 4 in a ratio of 65:35, v/v as a mobile phase at flow rate of 1 mL·min^{-1}. The method showed good linearity, accuracy, precision, and was successfully applied for determination of the four drugs in laboratory prepared mixtures and in the seven pharmaceutical dosage forms. Jyothi and Umadevi [31], Wafaa Zaghary et al. [32], Vinutha et al. [33], Kommineni et al. [34], Sonia et al. [35], and Panigrahy and Reddy [36] performed simultaneous estimation of CGN and MFN using C18 as the stationary phase and wide choice of mobile phases. The authors had developed RP-HPLC method for the estimation of CGN in single and in combined dosage forms by varying the chromatographic conditions and achieved better retention. The various HPLC methods (method, matrix, sample preparation, internal standard, column/mobile phase) available for quantification of CGN in pharmaceutical dosage forms as single and in combination [5,10–36] and the results (Rt, detection, and linearity) obtained are presented in Table 2.

3.2 UPLC technique

UPLC is a recent separation technique which reduces the cost and increases the rapidity and efficiency of analysis required for developing and validating the method. Wafaa et al. [32] validated an UPLC method for the determination of MFN and CGN in tablets using Hypersil Gold (50 mm × 3 mm, 1.9 µm) as the stationary phase and methanol:0.03 M phosphate buffer (80:20) considered as the mobile phase at 0.4 mL·min^{-1} flow rate. The Rt of CGN and MFN was 1 and 0.5 min using a detection wavelength of 240 nm. Accuracy of CGN and MFN was 99.47% ± 1.03% and 99.73% ± 0.89% with a linearity of 0.1–50 and 0.25–100 µg·mL^{-1} for CGN and MFN, respectively. LOD and LOQ were 0.463 and 1.406 µg·mL^{-1} for CGN and 0.614 and 1.862 µg·mL^{-1} for MFN, respectively [32].

3.3 HPTLC technique

HPTLC is a powerful technique for qualitative and quantitative analysis. It contains chromatographic layers with the highest separation efficiency, the possibility to use multiple detection methods on the same sample and plate. Accurate quantitative measurements and high resolution makes HPTLC to meet all quality requirements for today's analytical labs.

Vichare et al. [37] reported a novel HPTLC method for the simultaneous estimation of CGN and MFN using toluene:methanol:triethyl amine:glacial acetic acid (7:2.6:0.2:0.2, v/v/v/v) as a mobile phase. The detection was done at 254 nm with an excellent resolution of retardation factor (R_f) values 0.21 ± 0.02 and 0.50 ± 0.03 for MFN and CGN, respectively. Detection and quantitation limits were found to be 8.01 and 24.28 ng/band for MFN and 8.27 and 25.07 ng per band for CGN, respectively. The proposed method was applied to marketed formulation, the % drug contents were found to be 98.20% ± 0.24% and 99.33% ± 1.80% w/w for MFN and CGN, respectively. This HPTLC method was novel and found to be more linear, accurate, precise, and sensitive compared to the other reported methods [37].

Ishpreet et al. [38] validated a HPTLC method using toluene:ethyl acetate:methanol (2:2:1) as the mobile phase and silica gel 60 F_{254} as the stationary phase for the estimation of CGN in tablet dosage form. The densitometric detection was at 290 nm. The recovery was 99.04–99.82% with a linearity of 10–500 ng per spot. LOD and LOQ were 0.39 ng/spot and 119 ng/spot, respectively. The % assay of CGN tablets was found to be 99.8%. Forced degradation studies of CGN showed stability in acidic, alkaline, photolytic, and oxidation [38].

Bhole et al. [39] developed a combination of CGN and MFN using silica gel 60 F 254 (10 × 10 cm, 250 µm) as the stationary phase and toluene:ethyl acetate:methanol:ammonia (4:4:2:0.1) as mobile phase at a wavelength of 254 nm. The R_f of MFN and CGN was found to be 0.15 and 0.50 ng/band, respectively. The linearity of MFN and CGN was found to be 0.5–3 and 50–300 ng/band, respectively. The proposed HPTLC method selectively quantitated MFN and CGN in the presence of the degradation products [39].

4 Bioanalytical methods

Bio-analytical techniques are used for the quantitative determination of drugs and their metabolites in biological fluids, which plays a major role in the evaluation and interpretation of bioequivalence, pharmacokinetic, and toxicokinetic studies. Hence, the development of selective, sensitive, and reliable bio-analytical methods for the quantitative evaluation of drugs and their

Table 2: Summary of the reported HPLC methods for the determination of CGN in bulk and its dosage forms

Drug	Column (dimension)	Mobile phase	Detector, detection wavelength (nm)	Flow rate ($mL \cdot min^{-1}$)	Rt (min)	Linearity ($\mu g \cdot mL^{-1}$)	Accuracy (%)	LOD ($\mu g \cdot mL^{-1}$)	LOQ ($\mu g \cdot mL^{-1}$)	Reference
CGN	NA	Ammonium phosphate buffer:methanol (50:50)	Electrochemical detection	0.1 $mL \cdot h^{-1}$	NA	NA	NA	NA	NA	Vymyslicky et al. [10]
CGN	C18 (4.6 mm × 250 mm, 5 µm)	Acetonitrile:water (50:50)	UV: 290	1	7.3	From 98 $ng \cdot mL^{-1}$ to 50 $\mu g \cdot mL^{-1}$	98.9–99.8	0.048	0.098	Reddy et al. [11]
CGN	C18 (100 mm × 4.6 mm, 5 µm)	Acetonitrile: orthophosphoric acid with pH 2.5 (50:50)	UV: 235	1	NA	10–200	NA	NA	NA	Sadasivuni and Gundoju [12]
CGN	C18 (4.6 mm × 250 mm, 5 µm)	Acetonitrile:0.1% sodium acetate (pH 4.6) (20:80)	291	1	3.3	2–14	99.98	NA	NA	Mounika et al. [13]
CGN	Grace C18 (4.6 mm × 250 mm, 5 µm)	Methanol:water (90:10)	UV: 290	0.9	4.4	1–5	99.95–106.18	0.7212	2.1854	Sushil Patil et al. [14]
CGN	NA	Acetonitrile:orthophosphoric acid (0.01 M) (50:50)	280	0.9	4.732	2–40	91.70–102.03	NA	NA	Singh et al. [5]
CGN	C18 (4.6 mm × 250 mm, 5 µm)	Acetonitrile:ammonium acetate buffer (pH 4.5) (70:30%, v/v)	UV: 252	1	4.5	5–30	98–101	0.01	0.04	Bhatt et al. [15]
CGN	C18 (4.6 mm × 250 mm, 5 µm)	Methanol: phosphate buffer (pH 4) (65:35)	VWD: 293	1	2.9	10–125	99.33–99.92	0.9	2.7	Parida et al. [16]
CGN	C18 (4.6 mm × 250 mm, 5 µm)	Acetonitrile:1-octane sulphonic acid (70:30)	PDA: 245	1	3.4	10–100	98–102	0.017	0.1705	Sreenivasulu et al. [17]
CGN	X-bridge C18 (4.6 mm × 150 mm, 5 µm)	Orthophosphoric acid (0.1%):acetonitrile (60:40)	230	1	8.7	25–75	99.6–99.8	3.58	10.85	Rahul et al. [18]
CGN	C18 (4.6 mm × 250 mm, 5 µm)	0.02% formic acid:acetonitrile (40:60)	UV: 230	1.2	4.4	10–50	98.44–100.31	0.00136	0.00414	Vijayalakshmi et al. [19]
CGN, RS-1,2,3,4	RP-amide (4.6 mm × 150 mm, 2.7 µm)	Ammonium acetate:acetonitrile (50:50)	UV: 290	0.7	23	0.055–1.177	NA	0.0005	0.015	Goutam et al. [20]
CGN	C18 (4.6 mm × 150 mm, 5 µm)	Methanol:formic acid in water (0.1% v/v) (90:10)	UV: 220	1	2.4	100–300	99.9–100.8	0.0009	0.029	Triveni et al. [21]
CGN	C18 (4.6 mm × 250 mm, 5 µm)	Acetonitrile:ortho phosphoric acid (55:45)	PDA: 290	1	6.29	1–6	99.6–99.8	0.41	1.24	Ishpreet et al. [22]
CGN	C18 (4.6 mm × 250 mm, 5 µm)	Methanol:acetonitrile:0.1% ammonium acetate (40:40:20)	UV: 290	1.1	4.1	100–300	98.04–100.27	3.538	10.72	Ladva et al. [23]
CGN	ODS (150 × 4.6 mm, 5 µm)	Water:acetonitrile (55:45, v/v)	PDA: 214	1	2.8	25–150	NA	0.037	0.112	Suma et al. [24]

(*continued*)

Table 2: (*continued*)

Drug	Column (dimension)	Mobile phase	Detector, detection wavelength (nm)	Flow rate ($mL \cdot min^{-1}$)	Rt (min)	Linearity ($\mu g \cdot mL^{-1}$)	Accuracy (%)	LOD ($\mu g \cdot mL^{-1}$)	LOQ ($\mu g \cdot mL^{-1}$)	Reference
CGN	C18 (4.6 mm × 250 mm, 5 µm)	0.05 M H_3PO_4:acetonitrile:methanol (45:45:10 (v/v))	DAD: 238	1	CGN: 8.00	5–100	98–102	CGN: 0.653	CGN: 2.167	Al-Shdefat et al. [25]
MFN					MFN: 9.45			MFN: 0.261	MFN: 0.874	
CGN	Spolar C18 (4.6 mm ×	pH 6 phosphate buffer:acetonitrile (55:45)	UV: 254	0.8	CGN: 10.77	CGN: 5–30	NA	NA	NA	Sunitha et al. [26]
MFN	250 mm, 5 µm)				MFN: 3.24	MFN: 50–300				
CGN	Kromasil C18 (4.6 mm ×	Acetonitrile:phosphate buffer (pH 4.2):methanol (52:38:10)	254	1	CGN: 3.223	CGN: 5–30	NA	NA	NA	Bangaruthalli et al. [27]
MFN	250 mm, 5 µm)				MFN: 2.216	MFN: 50–300				
CGN	Primesil C18 (4.6 mm ×	Methanol:phosphate buffer (70:30)	PDA: 220	1	CGN: 3.47	CGN: 50–250	CGN: 99.38–99.53	CGN: 4.13	CGN: 0.112	Gandla et al. [28]
MFN	250 mm, 5 µm)				MFN: 2.413	MFN: 5–25	MFN: 99.18–99.91	MFN: 2.1	MFN: 0.0372	
CGN	C8 (250 mm × 4.6 mm, 5 µm)	pH 6 phosphate buffer (0.05 M):acetonitrile:methanol	UV	1.5	CGN: 8.3	50–350	98–102	12.96	39.29	Moussa et al. [29]
EGN		(50:25:25, v/v/v)			MFN: 2.2	500–3,500		57.51	174.29	
LNG					CGN/ EMG: 3.6	10–70		1.1	3.33	
MFN					EMG: 6.0	20–140		1.73	5.24	
					LNG/ MFN: 3.6	1.25–8.75		0.11	0.32	
CGN	Inertsil, ODS C18 (250 mm ×	Acetonitrile:0.05 M potassium dihydrogen phosphate (pH	UV: 212	1	CGN: 4.414	CGN: 7.5–225		CGN: 2.1	CGN: 6.5	Khalil et al. [30]
DGN	4.6 mm, 5 µm)	4) (65:35)			DGN: 3.560	DGN: 5–150		DGN: 0.7	DGN: 2.2	
EGN					EGN: 3.004	EGN: 6.5–187.5		EGN: 1.4	EGN: 2.3	
MFN					MFN: 1.898	MFN: 10–1,000		MFN: 3.2	MFN: 9.6	

(*continued*)

Table 2: (*continued*)

Drug	Column (dimension)	Mobile phase	Detector, detection wavelength (nm)	Flow rate ($mL \cdot min^{-1}$)	Rt (min)	Linearity ($\mu g \cdot mL^{-1}$)	Accuracy (%)	LOD ($\mu g \cdot mL^{-1}$)	LOQ ($\mu g \cdot mL^{-1}$)	Reference
CGN MFN	Inertsil ODS 3 VC18 (250 mm × 4.6 mm, 5 µm)	TFA in water (0.1% v/v):acetonitrile (20:80)	UV: 254	1	CGN: 4.20 MFN: 2.32	CGN: 20–40 MFN: 200–600	NA	NA	NA	Jyothi and Umadevi [31]
CGN MFN	C18 (100 mm × 4.6 mm, 5 µm)	Methanol:0.03 M phosphate buffer (75:25)	240	1.3	CGN: 0.9 MFN: 0.5	CGN: 1–50 MFN: 0.5–90	CGN: 99.81 ± 0.73 MFN: 99.37 ± 0.54	CGN: 0.7154 MFN: 0.9327	CGN: 2.1075 MFN: 2.8263	Wafaa et al. [32]
CGN MFN	Kromasil C18 (4.6 mm × 250 mm, 5 µm)	0.1% OPA (pH 2.8):acetonitrile (45:55)	PDA: 254	1	CGN: 2.671 MFN: 2.112	CGN: 2.5–15 MFN: 25–15	98.2–101.4	CGN: 0.01 MFN: 0.17	CGN: 0.50 MFN: 2.20	Vinutha et al. [33]
CGN MFN	Kromasil C18 (4.6 mm × 250 mm, 5 µm)	0.1% orthophosphoric acid (pH 2.8):acetonitrile (45:55)	UV: 254	1	CGN: 2.671 MFN: 2.112	CGN: 2.5–15 MFN: 25–150	98.22–101.54	CGN: 0.01 MFN: 0.17	CGN: 0.50 MFN: 2.20	Kommineni et al. [34]
CGN MFN	Gracesmart C18 (4.6 mm × 250 mm, 5 µm)	Acetonitrile:ammonium acetate (pH 4.5) (45:55)	PDA: 252	1	CGN: 5.76 MFN: 4.00	1–80	CGN: 99.19–100.57 MFN: 98.65–100.87	CGN: 0.124 MFN: 0.134	CGN: 0.376 MFN: 0.406	Sonia et al. [35]
CGN MFN	Kromasil C18 (4.6 mm × 250 mm, 5 µm)	0.01 M (pH 3.5) ammonium acetate:acetonitrile (65:55)	PDA: 254	1	CGN: 3.713 MFN: 2.440	CGN: 5–30 MFN: 50–300	CGN: 99.45–100.65 MFN: 99.95–100.74	NA	NA	Panigrahy and Reddy [36]

metabolites in biological matrices is important. An analytical method for the quantification of CGN in human plasma has been developed, validated, and enforced for the analysis of samples. The analytical method consists of extraction of the drug by protein precipitation method, the internal standard DGN was added to the plasma sample and extracted using protein denaturant acetonitrile followed by centrifugation. The supernatant was dried and reconstituted using the mobile phase and injected into the HPLC (Ajitha et al. [40]). Another method employed extraction of sample by liquid extraction using tertiary butyl methyl ether in human Plasma. In liquid–liquid extraction (LLE) the plasma was spiked with internal standard and tert-butyl methyl ether followed by centrifugation. The supernatant was dried and reconstituted with a solvent and then injected into the HPLC/LC-MS/MS. Determination was done using Zorbax XDB phenyl (7.5 × 4.6 mm, 3.5 mm) column and methanol:acetate (80:20) as the mobile phase by LC-MS/MS. The method used a deuterium labeled CGN as internal standard and eluted at 1.15 min with a linearity of 10–7,505 ng·mL^{-1} (Deepan et al. [41]). Udhayavani et al. [42] employed a LC-MS method to quantify CGN in human plasma using solid phase extraction. In solid-phase extraction (SPE), the plasma sample was spiked with ammonium acetate and internal standard into the SPE cartridge followed by conditioning and washing to extract CGN and injected into the HPLC/LC-MS/MS. Another author also followed a similar method but showed variation by using C18 (100 × 4.6 mm, 5 µm) as the stationary phase and 2 mM ammonium acetate:methanol (15:85) as the mobile phase (Somarouthu et al. [43]). Darshan et al. [44] estimated CGN by LC-MS/MS from rabbit plasma using EGN as internal standard. The author extracted CGN using LLE with tertiary butyl methyl ether using C18 (50 × 4.6 mm, 5 µm) column and 0.01 M ammonium acetate:methanol (30:70) as the mobile phase. CGN was extracted from rat plasma by LLE using LC-MS/MS (Kobuchi et al. [45]). Nalawade et al. [46] developed a simultaneous method and successfully applied for the determination of pharmacokinetics of MFN and CGN by using 100 µL of rat plasma. Mohamed et al. [47] performed the simultaneous estimation of CGN and MFN in biological samples (rat plasma) by protein precipitation extraction (PPE; acetonitrile) and LLE (ethyl acetate) using valsartan, CGN d4, MFN d6, tadalafil, and propranolol as the internal standard using LC-MS/MS. The authors estimated the plasma concentrations of the studied drugs in a pharmacokinetic study involving Egyptian health volunteers. Ramisetti et al. [48] reported a simple, rapid, and sensitive LC-MS/MS method for the simultaneous quantification of CGN and MFN. The authors used a one-step PPE for samples preparation and obtained highest recovery for the analytes. This method was successfully used for the *in vivo* plasma concentrations obtained from a pharmacokinetic study. Syeda et al. [49] used HPLC to estimate CGN and MFN in human plasma using PPE (2% v/v acetic acid in acetonitrile) with pioglitazone as the internal standard. The method was simple, accurate, precise, and can be applied in bioequivalence, pharmacokinetic, and toxicokinetic studies with desired precision and accuracy along with high-throughput. Another author also reported a sensitive, novel HPLC method using SPE as the extraction method (Deepan et al. [50]).The bio-analytical methods published for the quantitative analysis of CGN separately and in drug combination (40–50) and the results (method, matrix, sample preparation, internal standard, column/mobile phase, Rt, detection technique, and linearity) are represented in Table 3.

5 Multiple spectroscopic methods

Elnadi et al. [51] reported three novel, simple, accurate, and sensitive methods for the determination of CGN using FTIR, spectrofluorimetry, a stability-indicating UV-Vis spectroscopic method for the simultaneous estimation of CGN and MET. Method A is a green FTIR method using KBr disc for CGN determination measuring alkyl halide C–F peak area centered on 1,230 cm^{-1}. Method B is a spectrofluorimetry method using $\Delta\lambda$ = 50 nm synchronous mode at a peak maximum of 291.8 nm for CGN determination using methanol as diluent. Method C is a stability-indicating MCRS method measuring the peak amplitude of CGN and MET at 306.2 and 246.6 nm, respectively, in their mixture with complete CGN oxidation degradation. All the three spectroscopic methods can be used efficiently for routine analysis in QC laboratories [51].

6 Discussion

CGN is considered as a primary member in the gliflozins family, which has a considerable contribution since 2013. Authors Singh et al. [5], Ishpreet et al. [7], and Vichare et al. [8] used methanol as the diluent to develop an accurate and linear method for the estimation of CGN separately and in combined dosage forms by UV-Vis spectroscopic technique. Vichare et al. [8] reported two analytical methods for the simultaneous estimation of

Table 3: Summary of bio-analytical methods for the quantification of CGN alone and in combination with other drugs

Drug	Matrix	Sample preparation	Internal standard	Column/mobile phase	Rt (min)	Detection	Linearity (ng·mL^{-1})	Ref.
CGN	Human plasma	PPE	DGN	Phenomenex Luna C18 (150 × 4.6 mm, 5 µm)/0.01 M phosphate buffer (pH 3.5):acetonirile (45:55)	8.7	UV: 222 nm	60–2,400	Ajitha et al. [40]
CGN	Human plasma	Tertiary butyl methyl ether	CGN d4	Zorbax XDB Phenyl (7.5 mm × 4.6 mm, 3.5 mm) Methanol: acetate (80:20)	1.15	*m/z*: 462.2–267.10 for CGN *m/z*: 466–267.2 for IS	10–7,505	Deepan et al. [41]
CGN	Human plasma	SPE with ammonium acetate	CGN d4	Zodiac C18 (100 mm × 4.6 mm, 5 µm) Methanol:2 mM ammonium acetate (90:10)	1.5	MS/MS	10.253–6019.311	Udhayavani et al. [42]
CGN	Human plasma	SPE with ammonium acetate	CGN d4	C18 (100 mm × 4.6 mm, 5 µm) 2 mM ammonium acetate:methanol (15:85)	1.5	*m/z*: 462.2–267.10 for CGN *m/z*: 466–267.1 for IS	10.3–6,019	Somarouthu et al. [43]
CGN	Rabbit plasma	Tertiary butyl methyl ether	EGN	C18 (50 mm × 4.6 mm, 5 µm) 0.01 M ammonium acetate:methanol (30:70)	2	*m/z*: 462.1–267.10 for CGN *m/z*: 451.2–71.10 for IS	5×10^5 to 600	Darshan et al. [44]
CGN	Rat plasma	LLE with tertiary butyl methyl ether	EGN	Quicksorb ODS (150 mm × 2.1 mm, 5 µm) Acetonitrile:0.1% formic acid (90:10)	NA	*m/z*: 462.1–191 for CGN *m/z*: 451.2–71.0 for IS	NA	Kobuchi et al. [45]
CGN MFN	Rat plasma	NA	Valsartan	Methanol:0.1% formic acid (65:35)	CGN: 8.2 MFN: 1.83 IS: 6.2	MS/MS	200–8,000 for CGN 100–4,000 for MFN	Vivek et al. [46]
CGN	NA	Protein precipitation	Internal standard 1-CGN d4	Hypersil BDS C18 (100 mm × 4.6 mm, 5 µm) 5 mM ammonium acetate 0.01% formic acid:methanol (15:85)	CGN: 1.5	MS/MS	10–6,028 for CGN	Muralikrishna et al. [47]
MFN			Internal standard 2-MFN d6		MFN: 1.8		10–3,027 for MFN	
CGN	Human plasma	PPE with acetonitrile	CGN-Tadalafil	C18 (50 mm × 4.6 mm, 5 µm)	CGN: 4.5	*m/z*: 130.2–60.1 for MFN	50–5,000 for MFN	Ramisetti et al. [48]
MFN		LLE with ethyl acetate	MFN-propranolol	0.1% formic acid:acetonitrile	MFN: 0.9	462.3–191.0 for CGN 260.2–183.0 for propranolol 390.2–268.8 for tadalafil	10–1,000 for CGN	

(*continued*)

Table 3: (*continued*)

Drug	Matrix	Sample preparation	Internal standard	Column/mobile phase	Rt (min)	Detection	Linearity ($ng \cdot mL^{-1}$)	Ref.
CGN	NA	Protein precipitation	Pioglitazone	Spursil (Dikma) ODS C18 (4.6 mm × 250 mm, 5 µm)	CGN: 8.352	NA	250–1,250 for MFN	Syeda et al. [49]
MFN		2% v/v acetic acid in acetonitrile		Acetonitrile:phosphate buffer (pH 3.0) (15:85% v/v)	MFN: 4.738		25–125 for CGN	
					Pioglitazone: 10.995			
CGN	Human plasma	SPE	Pioglitazone	Inertsil ODS C18 (4.6 mm × 250 mm, 5 µm) (pH 3 with sodium hydroxide) phosphate buffer:acetonitrile (85:15)	CGN: 8.10	NA	5,000–25,000 for MFN	Deepan et al. [50]
MFN					MFN: 4.6		500,000–1,250,000 for CGN	
					Pioglitazone: 10.64			

CGN and MFN using methanol as the diluent. Both the methods showed good recovery with a linearity of 0.75–4.5 and 2.5–15 $\mu g \cdot mL^{-1}$ for CGN and MFN, respectively. Whereas Chinta et al. [6] developed a cost effective, sensitive method using phosphate buffer as the diluent which is a better method. HPLC method is utilized for the estimation of CGN separately and in combination with other drugs in bulk and pharmaceutical formulations. Rahul et al. [18] reported a cost-effective method for the estimation of CGN using X-Bridge C18 (4.6 × 150 mm, 5 µm) as stationary phase and orthophosphoric acid (0.1%):acetonitrile (60:40) as the mobile phase. Rt of CGN is about 8.7 min with a linearity of 25–75 $\mu g \cdot mL^{-1}$ and recovery of 99.6–99.8% at detection wavelength of 230 nm. This method had a higher Rt of 8.7 min compared to all the other reported methods. The authors would have tried by varying the mobile phase composition for a better method. Another author Reddy et al. [11] reported a HPLC method in which CGN eluted at 7.3 min, but achieved a linearity in the range from 98 $ng \cdot mL^{-1}$ to 50 $\mu g \cdot mL^{-1}$ indicating the sensitiveness of the method. Mounika et al. [13] developed a method which was cost effective method using ecofriendly solvents compared to the other reported methods. Panigrahy et al. [36] reported a cost-effective method for the simultaneous estimation of CGN and MFN using kromasil C18 (4.6 × 250 mm, 5 µm) as the stationary phase and 0.01 M (pH 3.5) ammonium acetate:acetonitrile (65:55) as the mobile phase at a detection wavelength of 254 nm. CGN eluted at 3.713 min with % recovery of 99.45–100.65%. MFN eluted at 2.44 min with % recovery of 99.85–100.74%. This method was validated as per ICH guidelines and found to be sensitive, accurate, and precise. Many bio-analytical methods using LC-MS/MS and a few HPLC methods were reported for CGN separately and in combination with other drugs. Deepan et al. [41] reported a LC-MS/MS method for the estimation of CGN using Zorbax XDB phenyl (7.5 × 4.6 mm, 3.5 mm) as the stationary phase and methanol:acetate (80:20) as the mobile phase with CGN d4 as the internal standard. LLE was performed by using tert butyl methyl ether as the extraction solvent. CGN eluted at 1.15 min with an m/z: 462.2–267.10 for CGN and m/z: 466–267.2 for CGN d4 and a linearity of 10–7505 $ng \cdot mL^{-1}$. The method was selective and sensitive compared to all the reported methods. Deepan et al. [50] reported a simultaneous method for the estimation of CGN and MFN by HPLC in human plasma using Inertsil ODS C18 (4.6 × 250 mm, 5 µm) as the stationary phase and (pH 3 with sodium hydroxide) phosphate buffer:acetonitrile (85:15) as the mobile phase. CGN eluted at 8.10 min and MFN eluted at 4.6 min with a linearity of 5–25 $\mu g \cdot mL^{-1}$

for MFN and 500–1,250 $\mu g \cdot mL^{-1}$ for CGN, respectively. Bioanalytical method using HPLC is always cost effective compared to the sophisticated LC-MS/MS techniques. But LC-MS/MS methods are sensitive and selective compared to the HPLC methods. All reviewed methods were validated as per ICH guidelines.

7 Conclusion

This detailed review explains the existing methods for the determination of CGN in pharmaceutical formulations and in biological fluids using spectroscopy, UPLC, HPLC, HPTLC, and LC-MS/MS. This review has presented the complete review of the methods reported right from the drug approval time to up-to-date. In future, researchers can take efforts to develop more methods using eco-friendly solvents for the determination of CGN in pharmaceutical dosage forms and employ sample extraction methods using green analytical techniques (single-drop micro-extraction, liquid-phase micro-extraction, liquid–liquid–liquid micro-extraction, single short column, solid-phase micro-extraction, stir-bar-sorptive extraction, matrix solid-phase dispersion, supercritical-fluid extraction, pressurized-liquid extraction, subcritical-water extraction, microwave-assisted extraction, sonication-assisted solvent extraction). More methods based on LC-MS/MS can pave the way for the toxicokinetic studies and therapeutic monitoring of CGN.

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