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AN OVERVIEW ON ANALYTICAL METHODS FOR DAPAGLIFLOZIN - AN ANTIDIABETIC DRUG

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ABSTRACT: Dapagliflozin (DAPA) is a drug of gliflozin class. It is a white crystalline solid. Dapagliflozin is a selective sodium-glucose co-transporter subtype 2 (SGLT2) inhibitor with antihyperglycemic activity. Dapagliflozin blocks glucose reabsorption into the kidney, resulting in the elimination of blood glucose through the urine. DAPA is not official in any pharmacopeia. It comes under EU Pharmaceutical Product Classes, as per literature survey. Till date officially no analytical method has been developed or reported for this drug. This review focuses on collective literature on the analytical methods developed for DAPA.

INTRODUCTION: Dapagliflozin (DAPA) is a drug of gliflozin class. Chemically it is (2S, 3R, 4R, 5S, 6R)- 2- (4- chloro- 3- (4-methoxybenzyl) phenyl)-6- (hydroxymethyl)tetrahydro-2H-pyran-3, 4,5-triol with molecular formula of $C_{21}H_{25}ClO_6$ and 408.875g/ mol as its molecular weight. It is a white crystalline solid, having solubility in organic solvents like DMSO dimethylformamide and ethanol. Its melting point is 55-60 ºC [1]. Dapagliflozin is a selective sodium-glucose co-transporter subtype 2 (SGLT2) inhibitor with antihyperglycaemic activity. Dapagliflozin selectively and potently inhibits SGLT2 compared to SGLT1, which is the co-transporter of glucose in the gut.

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Dapagliflozin blocks glucose reabsorption into the kidney, resulting in the elimination of blood glucose through the urine [1]. DAPA is not official in any Pharmacopoeia as of now. Very few analytical methods have been reported so far. The purpose of this review is to collate all those published literature on analytical methods developed for DAPA by various researchers.

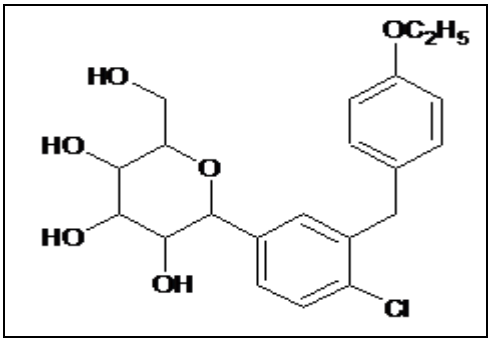

FIG. 1: CHEMICAL STRUCTURE OF DAPAGLIFLOZIN

Method of Pharmaceuticals:

Pharmacopoeial Methods: DAPA is not official in any pharmacopeia. It comes under EU

Pharmaceutical Product Classes, as per literature survey. So officially no analytical method has been developed for this drug as of now.

Titrimetric Methods: Titrimetric methods have not been reported for this drug.

UV- Spectrophotometric Methods: UV spectrophotometric methods were reported [4, 7]. Four different methods based on a spectrophotometric method for the determination of DAPA in tablet formulation are reported. The methods included are calibration curve, Area under the curve, First and second order derivative method. All the developed methods obeyed Beer-Lambert's Law in the concentration of 5-40 µg/mL, with correlation coefficient value less than 1, when measured at a selected wavelength using UV-Visible spectrophotometer with 1cm matched quartz cell using methanol-water mixture as solvent blank. Nearly 100% of the drug was estimated from these methods when applied to the dosage form.

In the second paper, a simple economic UV spectrophotometric method has been developed using ethanol and water as working solvent. The developed method describes that DAPA in API obeyed Beer's-Lambert's Law in the concentration of 0.5-0.9 µg/mL, with correlation coefficient value of 0.994, and LOD and LOQ were found to be 0.0925 µg/mL and 0.00129 µg/mL respectively. Intra-and inter-day precisions were approximately equivalent to 0.5% (relative standard deviation, RSD). The details of UV-spectrophotometric analysis are summarized in **Table 1**.

TABLE 1: PERFORMANCE CHARACTERISTIC OF UV SPECTROPHOTOMETRIC METHOD

Diluent	λ_{max} nm	Linearity Range µg/mL	LOD and LOQ µg/mL	Application	Reference
Methanol	Method I (Zero order): 224nm Method II (Area under Curve): 218-230 nm Method III (First order derivative UV-spectrophotometry using amplitude): 220 nm Method IV (Second order derivative UV-Spectrophotometry using amplitude): 224 nm and 235.5 nm	5-40	-	Tablet	Gajanan, 2017
Ethanol and working standard was prepared with distilled water	237 nm	0.5-0.9	0.0925 and 0.00129	API	Manasa, 2014

LOD: Limit of detection; LOQ: Limit of Quantification

Chromatographic Methods: In an effort to optimize a quantitative analysis of DAPA in formulation a liquid chromatographic method was developed [6], the separation was achieved on Waters C18 column, 5 µm particle size, 25 cm × 4.6 mm, with phosphate buffer and acetonitrile in the ratio of 60:40 v/v as a mobile phase and a flow rate of 1 mL/min with UV detection of 237 nm, showing a retention time of 3.461 min with linearity range concentration of 10-60 µg/mL with correlation coefficient value of 0.995 and LOD and LOQ values of 0.02 µg/mL and 0.06 µg/mL respectively. The method was applied to determine DAPA in tablet with the recovery of 99.4%-100.90%. The method was also shown to be accurate, precise, specific and robust.

A Reverse Phase-HPLC method for estimation of DAPA from its tablet dosage form was developed. Aristocratic mode chromatographic separation was achieved with a mixture of Acetonitrile: 0.1% Triethylamine (pH-5.0) in the ratio of 50:50 v/v as mobile phase using Princeton C18 column at flow rate of 1mL/min and a detection wavelength of 224 nm, the retention time of drug was found to be 5.163 min. The proposed method obeyed Beer's-Lambert's law in the concentration range of 10-70 µg/mL, with correlation coefficient value 0.999 and LOD and LOQ values of 2.1 µg/mL and 6.39 µg/mL respectively. The method was applied to the marketed formulation and shown 100.57% mean value concerning label claim. Stress testing under various conditions such as pH (acid/base), oxidation, temperature, light, humidity, *etc*. was also carried out [8].

DAPA in API was chromatographed using Waters HPLC system, BDS column, a mixture of acetonitrile and orthophosphoric acid (55:45 v/v) was used as mobile phase at a detection wavelength of 245 nm [10]. The proposed method obeyed Beer's-Lambert's law in the concentration range of 25-150

µg/mL, with correlation coefficient value 0.999 and LOD and LOQ values of 0.60 µg/mL and 1.81 µg/mL respectively. The authors have also developed a UV spectroscopic method using methanol as solvent at a detection wavelength of 203 nm. The developed UV spectroscopic method showed a linearity range of 1-5 µg/mL with correlation coefficient value 0.999; LOD and LOQ values of 0.01 µg/mL and 0.05 µg/mL respectively. Both the methods were validated as per ICH guidelines.

A reverse phase high performance liquid chromatographic method was developed for the estimation of Dapagliflozin in tablet formulation and bulk drug using Zorbax Eclipse Plus C8 column, (150 × 4.6 mm) using buffer (pH 7.6) Tris: Methanol (60:40 v/v) as a mobile phase at a flow rate of 1 ml/min by employing UV detection at 224 nm, retention time was found to be 1.467 min, with linearity concentration range of 1-32 µg/mL. LOD and LOQ were found to be 0.207 and 0.693 µg/ml respectively. As per ICH guidelines, the method was found to be simple, precise and accurate when validated [2].

An RP-HPLC method was developed and validated for the analysis of DAPA in bulk and tablet formulation. The separation has been achieved using C18 column reversed-phase using mobile phase composition of methanol: water (75:25 v/v), utilizing Shimadzu SPD-20A Prominence UV-Visible detector at a flow rate of 1 mL/min and the absorption maxima 230 nm, retention time was found to be 3.107 min for DAPA. The proposed method obeyed Beer's-Lambert's law in the concentration range of 5-25 µg/mL, with correlation coefficient value 0.9252 and LOD and LOQ values of 2.5 µg/mL and 10 µg/mL respectively. The method was validated as per the ICH guidelines [5].

Few stability indicating analytical method development studies using HPLC were also reported. In a method, separation was effected on a Symmetry C18 column, 25 cm × 4.6 mm i.d. 5µm, particle size column using a mobile phase mixture of Methanol: Acetonitrile: Orthophosphoric acid in a ratio of 75:25:05 v/v/v at a flow rate of 1.0 mL/min. The detection was maintained at 246 nm. DAPA showed the linearity concentration range of 0-70 µg/mL. The LOD and LOQ were found to be 0.04 and 0.12 µg/mL respectively. The retention time of DAPA was found to be 2.797 min. The developed method was found to be simple, precise, accurate, specific, robust and rapid for the estimation of DAPA in bulk and tablet dosage form. This paper also reported the effect of stress conditions on DAPA. The various degradation pathway studied were acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation [12].

An RP-HPLC method has been developed for the DAPA in bulk and pharmaceutical dosage form. Chromatographic separation of DAPA was achieved on Symmetry C18, 250 mm × 4.6 mm i.d.5µm particle size and the mobile phase containing phosphate buffer: methanol in the ratio of 35:65 v/v with the flow rate was 1.0 ml/min, detection was carried out by absorption at 215nm using a UV detector at ambient temperature. The linearity of the proposed method was excellent over the range 0-70 µg/mL with the correlation coefficient of 0.999 with LOD of 0.09 µg/mL and LOQ of 0.36 µg/mL respectively [11].

DAPA in the presence of its degraded products was identified by RP HPLC method. DAPA was subjected to acid, alkali hydrolysis, oxidation, thermal and photolytic degradation. The degradation studies indicated that DAPA was more susceptible to thermal degradation. The separation was achieved using Agilent C18 (4.6 × 150 mm, 5 µm), mixture of acetonitrile: di-potassium hydrogen phosphate with pH-6.5 adjusted with orthophosphoric Acid (40:60% v/v) as a mobile phase with the flow rate of 1 mL/min and the effluent was monitored at 222 nm using photodiode array detector.

The linearity of the proposed method was excellent over the range 50-150 µg/mL with the correlation coefficient of 0.99 with LOD of 5.14 µg/mL and LOQ of 15.6 µg/mL respectively. The retention time of DAPA was reported as 3.160 min for API and 3.067 for DAPA in tablets [9]. The details of HPLC analysis were summarized in **Table 2** and LC-MS analysis in **Table 3**. A fully automated high throughput liquid chromatography/ tandem mass spectrometric (LC-MS/MS) method was developed for DAPA using normal and

Zucker diabetic fatty rat plasma. The assay was carried out using solid phase extraction and LC-MS/MS analysis in negative ion electrospray ionization mode. The mobile phase used was water and acetonitrile [3].

TABLE 2: PERFORMANCE CHARACTERISTICS OF HPLC METHODS

Stationary and mobile phase	λ_{max} (nm)	Linearity Range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Application	Reference
Waters C18, 5 µm particle size, 25 cm × 4.6 mm i.d., with phosphate buffer and acetonitrile in the ratio of 60:40 v/v as a mobile phase and a flow rate of 1.0 ml/min	237	10-60	0.02	0.06	Tablet	Jitendra, 2017
Acetonitrile: 0.1% Triethylamine (pH-5.0) in the ratio of 50:50 v/v as mobile phase using Princeton C18column at flow rate of 1mL/min	224	10-70	2.1	6.39	Tablet	Mante, 2018
Waters HPLC system, BDS column, A mixture of Acetonitrile and Orthophosphoric acid was used as the mobile phase in the ratio of 55:45 v/v	245	25-150	0.60	1.81	API	Sanagapati, 2014
Zorbax Eclipse Plus C8 column, (150 × 4.6 mm) using a buffer (pH 7.6) Tris: Methanol (60:40 v/v) as a mobile phase at a flow rate of 1mL/min	224 nm	1-32	0.207	0.693	Bulk drug and pharmaceutical formulations	Asmita, 2018
C (18) column reversed-phase using mobile phase composition of Methanol: Water (75:25 v/v). The flow rate was adjusted to 1 mL/minute	230	5-25	2.5	10.0	SIAM, bulk drug, and Tablet Formulation	Gunasekar, 2018
Symmetry C18, 25cm × 4.6 mm i.d. 5µm, Particle size column using a mobile phase mixture of Methanol: Acetonitrile: Orthophosphoric acid in a ratio of 75:25:05 v/v/v at a flow rate of 1.0mL/min	246 nm	0-70	0.04	0.12	SIAM and bulk drug and tablets	Subrata, 2017
Symmetry C18, 250 mm × 4.6 mm i.d.5µm particle size and the mobile phase containing Phosphate Buffer: Methanol in the ratio of 35:65 v/v. The flow rate was 1.0 mL/min	215	0-70	0.09	0.36	SIAM and bulk drug and Pharmaceutical dosage form	Sanjeev, 2017
Agilent C18 (4.6 mL 150,5 µm, a mixture of acetonitrile: di-potassium hydrogen phosphate with pH-6.5 adjusted with OPA (40:60 %v/v) as a mobile phase with the flow rate of 1 mL/min	222	50-150	5.14	15.6	SIAM, API and pharmaceutical dosage form	Mitali, 2017

- LOD: Limit of Detection
- LOQ: Limit of Quantification
- SIAM: Stability Indicating Analytical Method
- API: Active Pharmaceutical Ingredient

TABLE 3: PERFORMANCE CHARACTERISTICS OF LIQUID CHROMATOGRAPHY-MASS SPECTROPHOTO-METRIC (LC-MS/MS) METHODS

Method	Detection	Linearity Range	LOD and LOQ	Application	Reference
LC-MS/MS	Negative ion electrospray ionization mode	5-40 µg/mL	-----	Normal and ZDF rat plasma	Aubry, 2010

CONCLUSION: The review of the analytical methods reported for dapaglifloxin showed that spectrophotometric, HPLC with UV- visible detector have been reported. So far only one paper

has been published based on its assay in rat plasma. In general, for the determination of the drugs in biological samples, HPLC-MS/MS is ideal for estimation.

So, it greater option to estimate the dapagliflozin in a biological sample by HPLC MS/MS can be carried out. This review represents an overview of the current analytical methods for the determination of dapagliflozin in an active pharmaceutical ingredient, tablet, and pharmaceutical dosage forms.

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