A Comprehensive Review on HPLC Based Analytical Methods for Quantification of Linezolid and Canagliflozin

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***Abstract*: In this review we have studied about common analytical method used for quantification of drugs which is High Pressure Liquid Chromatography (HPLC). We used Linezolid and canagliflozin for HPLC study. These drugs are of different categories, first one is Linezolid is from and class has an antibacterial activity, it is synthetic antibiotic which disrupts bacterial growth by inhibiting the initiation process of protein synthesis. Second one Canaglifozin, is a antidiabetic drug, usually used in type 2 diabetes. Which has recently approved by USFDA, used in Diabetes Mellitus either alone or combined with other hypoglycaemic drugs. The focus of the review is on chromatographic study of various pharmaceutical and biological preparations of Linezolid and Canagliflozin.**

***Keywords*: RP-HPLC, LC-MS, HPLC Based Estimation, Linezolid, Canaglifozin, Diabetic Mellitus, quantification of analyte.**

INTRODUCTION:

HPLC: High Pressure Liquid Chromatography or High Performance Liquid Chromatography is an important quantitative and qualitative technique used for estimation of pharmaceutical and biological products. It is versatile, safest, least time consuming technique. It is the special technique used for analysis, identification and quantification of an active compound[1].

Principles - HPLC is separation based technique in which analyte is distributed between mobile phase and stationary phase, separation of mixture into individual component takes place in analytical column[2].

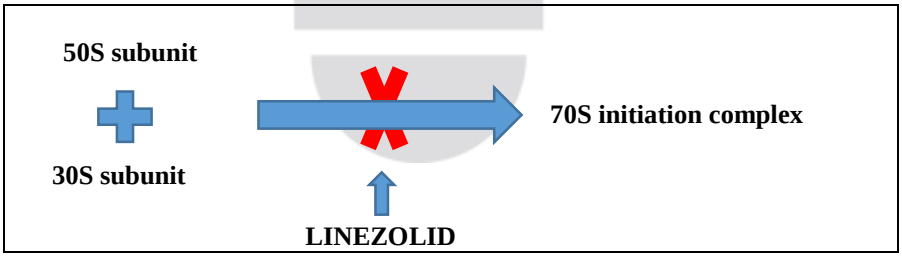
HPLC Analytical Studies of Drugs:

A) LINEZOLID:

Linezolid is the first member of the class of oxazolidinone antibiotics. Linezolid was approved by the US-FDA in 2000. It is used for the treatment of infectious disease caused by gram –positive pathogen e.g. Methicillin-resistant *Staphylococcus aureus* and Tuberculosis[3]. Linezolid has no antibacterial activity against the gram negative bacteria. Linezolid is possible to administered orally as well as parenterally. Linezolid is completely absorbed by oral administration with bioavailability 100 and drug is eliminated by renal and non-renal route with plasma elimination half-life between 4.5 and 5.5 h%[4]. Linezolid is prepared from 3,4 difluoronitrobenzene and morpholine, in presence of suitable base as N,N di- iso propyl ethylamine or triethylamine with suitable solvent such as acetonitrile, tetrahydrofuran(THF) or ethyl acetate[5].If patient have briefly drug resistant TB and having limited drug treatment then we have to used pretonamid in combination with bedaquilin and linezolid with TB bactericidal activity[6].

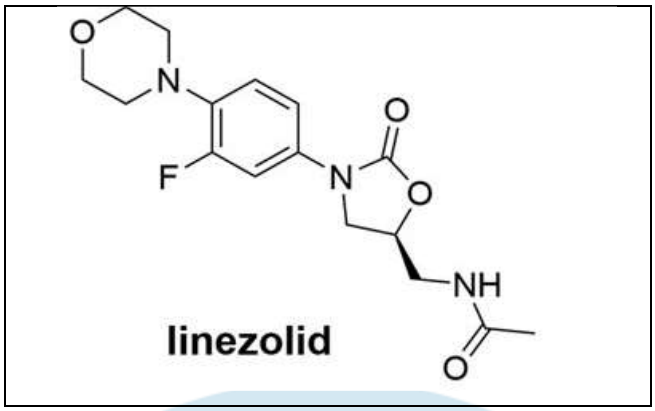
Mechanism of action: Linezolid has bacteriostatic activity, which inhibits protein synthesis by binding Ribosomal RNA on both site of 30S and 50S subunit and prevents the formation of functional 70S initiation complex, which is essential for bacterial reproduction[7].Brand name of Linezolid is **Zyvox**

Linezolid also used in serotonin toxicity, when it is used in combination with Serotonin Reuptake Inhibitor, since Linezolid is non-specific inhibitor of Monoamine Oxidase[8,9].



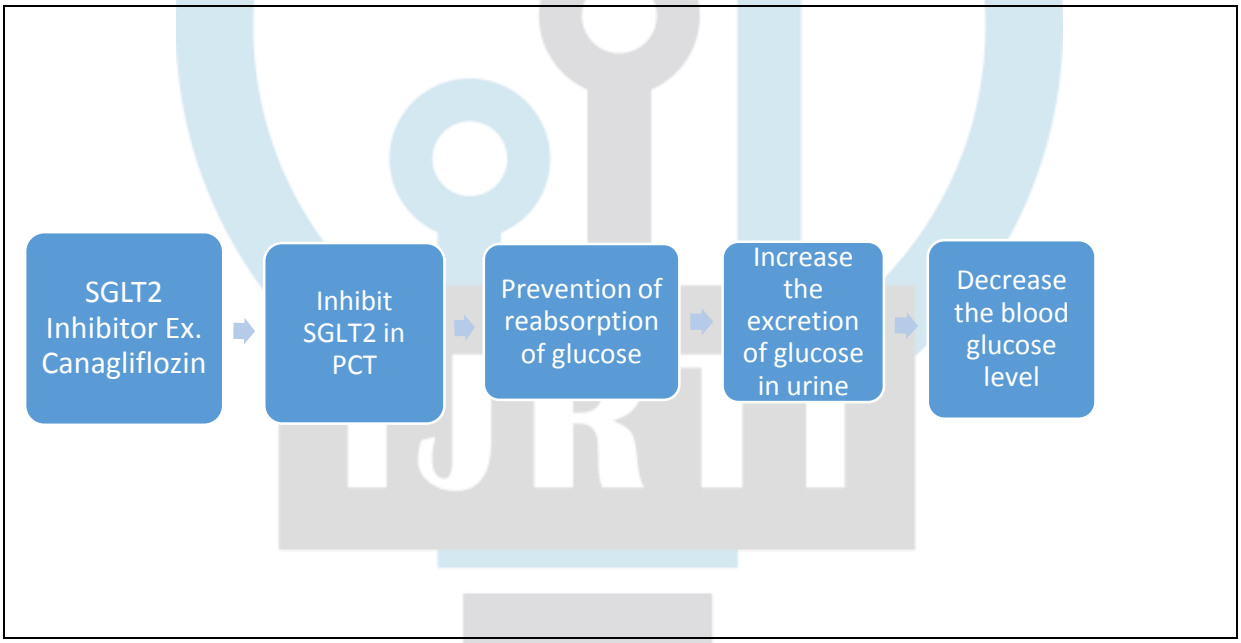
Drug Profile:

Chemical name: N-(3-(3-fluoro-4-morpholinylphenyl)-2-oxa-5-oxazolidinyl)methyl)acetamide
Molecular formula: C16H20FN3O4
Molecular weight: 337.351g/mol
Appearance: white crystalline powder,solubility of 3mg/ml in water and is slightly soluble in ethanol and ethyl acetate[10].

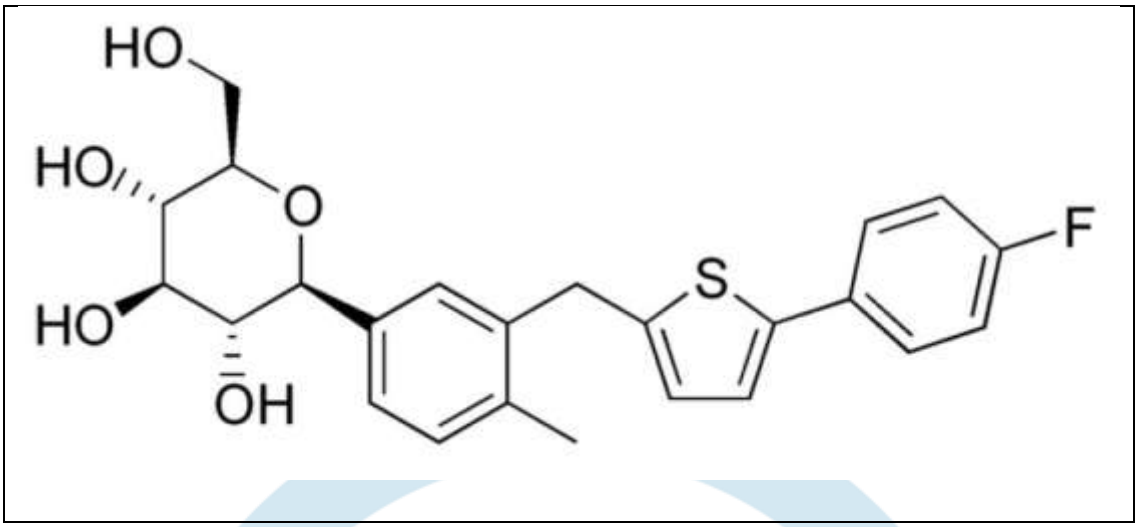


B) Canagliflozin: It is an oral antidiabetes drug; belong to gloflozin class .Especially used to treat type 2 Diabetes Mellitus. Indication of Canagliflozin is an adjunct to diet and exercise to improve glycaemic control in adult with type 2 DM[11].

Mechanism of action:
Canagliflozin is agliflozin class which inhibit the sodium glucose co-transporter 2(SGLT2). The main role of SGLT2 is to inhibit the reabsorption of glucose in proximal tubule of kidney.Gliflozin class of drug are more potent to treat T2DM.Canagliflozin decrease the reabsorption of glucose and increase the urinary glucose excretion[12].The potency of these drug depend on the amount of glucose is filtered throught the glomeruli and enter in tubular lumen therefore patient with have uncontrolled T2DM show maximum effect[13].
After taking these drug calories are lost from body in the form of glucose in urine ,it cause loss of weight which is good in patient having T2DM.These drug also help in reduction of BP due to mild weight loss anddiuretic action caused by it[14],[15].



Drug Profile:
Chemical name: (2S, 3R, 4R, 5S, 6R)-2-{3-[5-(4-fluoro-phenyl)-thiophen-2-ylmethyl]-4-methyl-phenyl}-6-hydroxymethyltetrahydro-pyran-345triol.
Molecular formula: C24H25FO55
Molecular weight: 444.52g/mol
Appearance: It is white solid, having melting point 95-1050c, soluble in many organic solvent and insoluble in aqueous solvent[16],[17],[18].



Structure of Canagliflozin[19]

In these review we are going to highlights the analytical methods especially HPLC based method used for quantification of drug. Table 1. We have to mention the overview of chromatographic method for estimation of Linezolid. Table 2. We compile the list of chromatographic estimation of Canagliflozin.

Table 1. Summary of HPLC based method for estimation of Linezolid

Sr N0	Matrix	Method	column	Mobile phase	Detection	1)LOQ 2)LOD	1)FR& 2)RT	Ref
1	Tablets	HPLC	Column C18 Hypersil ODS (250x4.6 mm; 5 µm)	Isocratic: methanol, water and (50:50 v/v)	UV at 254nm	1)0.01983 &2)0.0663µg/ ml	1)1.0ml/min, 2)5.11min	20
2	Tablets	HPLC	Column C18 Waters (250_4.6 mm; 5 lm)	Isocratic: aqueous 1% acetic acid, methanol and acetonitrile (50:25:25,v/v/v)	UV 254nm	1)0.21&2)0.63 µg/ml	1)1.0ml/min 2)4.6min	21
3	Human plasma	HPLC	Column C8 Zorbax RX-8 (150_4.6 mm; 5 lm)	Isocratic: water and acetonitrile (80:20, v/v)	UV 251nm	N.A.& 0.01µg/ml	1)1.0mi/min 2)10.0min	22
4	Human serum	HPLC	Column C18 Hypersil 5ODS (100_4.6 mm; 5 lm)	Isocratic: water with 2 g/L heptane sulfonic acid (pH 5), methanol, and ortho-phosphoric acid (69:30:1, v/v/v)	UV at 254 nm	0.1µg/ml N.A.	1)1.0ml/min 2) 6.5min	23
5	Human plasma	HPLC	Column C8 Zorbax RX-8 (250_4.6 mm; 5 lm)	Isocratic: acetic acid, tetrahydrofuran, methanol, and water (0.1:1.2:25:73.7, v/v/v/v)	UV at 251nm	N.A and 0.01µg/ml	1)1.0ml/min 2)7.0min	24
6	Human serum and urine	HPLC	Column C18 Nucleosil (250_4.6 mm; 5 lm)	Isocratic: sodium dihydrogen phosphate buffer (50 mM, pH 5.0) and acetonitrile (75:25,	UV at 260 nm	1)0.1and 2)0.3µg/ml	1)1.0ml/min 2) N.A.	25

				v/v)				
7	Human serum and urine	HPLC	Column C18 Nucleosil-100 (125_4 mm; 5 lm)	Isocratic: acetonitrile, acetate buffer (50 mM), and 4 M sodium hydroxide solution (18:82:0.05, v/v/v)	UV at250nm	1)0.07 and 2)0.14µg/ml	1)1.3ml/min 2) 6.6min	[26]
8	Rabbit eye	HPLC	Column C18 Ultra sphere XL-ODS (150_4.6 mm; 5 lm)	Isocratic: ammonium acetate (25 mM, pH 5) and acetonitrile (76:24, v/v)	UV at 251nm	1)0.1µg/ml 2)N.A.	1)1.0ml/min 2) N,A.	[27]
9	Dried blood spotting	HPLC	Column C18 Kinetex EVO (100_4.6 mm; 2.6 lm)	Gradient: (A) ammonium acetate buffer (10 mM, pH 3.5) with acetic acid; (B) acetonitrile and methanol, (80:20, v/v)	UV at 251nm	1)0.015 µg/ml 2)0.05µg/ml	1)1.0 ml/min 2)8.9min	[28]
10.	Aqueous	HPLC	Column C18 Apollo (150_4.6 mm; 5 lm)	Isocratic: phosphate buffer (pH 7; 11 mM) and methanol (40:60, v/v)	UV at 253 nm	N.A.	1)1.0ml/min 2)7.0min	[29]
11	Human plasma and saliva	HPLC	Column C18 Capcell Pak(150×4.6mm;5µm)	Isocratic:acetonitrile,tetrahydrofuran,and0.5%ammonium acetate buffer(pH4.4)(17:5:78,v/v/v)	UV at 254nm	N.A.	1)0.8ml/min 2)N.A	[30]
12.	Standard	HPLC-MS	Column C18 Kromasil(250×4.6mm;5µm)	Ammonium acetate and methanol	MS using m/z 330>220	N.A	1)1ml/min 2)14.04min	[31]
13	Human plasma	HPLC-UV	Column C18 Ascentis Express(50×2.1mm; 2.7µm)	Hydrochloric acid; acetonitrile and methanol(80:20 v/v)	UV at 254nm	1)0.007 and 2)0.01µg/ml	1)0.6ml/min 2)2.82 min	[32]
14	Standard	HPLC-UV	Column Chiralcel OJ-RH(150×4.6mm;5µm)	Isocratic:150mMdi-sodium hydrogen phosphate buffer and acetonitrile(86:14,v/v)	UV at 220nm	1)94µg/ml 2)375µg/ml	1)0.5ml/min 2)19.5min	[33]
15	Human serum	HPLC-MS	Column C18 Fortis(100×2.1mm; 3µm)	10mMammonium formiate in water-formic acid; methanol	MS using m/z 338.3>235.0	1)N.A. 2)0.12µg/ml	1)0.5ml/min 2)2.31min	[34]

Table 2. Summary of HPLC based method for estimation of Canagliflozin

Sr. No.	Matrix	Method	Column	Mobile phase	Detection	1)LOQ 2)LOD	1)FR 2)RT	Ref
1	Human Plasma	RP-HPLC	ZORBX C18(4.6 mmx250 mm, 5µm)	:Acetate buffer Acetonitrile(60:40)	UV at 260nm	1)30µg/ml 2)10.2µg/ml	1)1ml/min 2)….	35
2	Raw material	HPLC	Hypersil BDS, c18,4.6	0.1% orthophosphoacetonitrile(53:47),water &acetonitrile(50:50)	UV at 240nm	1)0.23µg/ml 2)0.7µg/ml	1)1.1 ml/mi & 2)3.3± 0.2min	36
3	Pharmaceutical dosage form	RP-HPLC	ODS column (4.6x150 mm,5µ)	Water and acetonitrile(55:45v/v)	PAD at 214nm	1)….. 2)…..	1)1.ml/min 2)….	37
4	Bulk	HPLC	C18 column (250x4.6 mm,5µm)	Acetonitrile:Orthophophoric acid (55:45)	PDA at 290nm	1)0.41µg/ml and 2)1.24µg/ml	1)1ml/min and 2)6.29 min	38
5	Bulk	HPLC	Grace smart RP-C18 (250x4.6 mm,5µ)	Acetonitrile and ammonium acetate buffer (45:55v/v)	PDA at 252nm	1)….. 2)…..	1)1ml/min and 2) 4-5.76min	39
6	Canagliflozin and metformin HCl	HPLC	ODS250 x4.6mm, 5µm	Buffer Acetonitrile and methanol	PDA at 212nm	1)… 2)…	1)1ml/min&2)3.781 min	40
7	Tablet	HPLC	SPOLAR C18(250 x4.6mm, 5µ)	Potassium di hydrogen othro-phosphate buffer and acetonitrile	UV 254nm	1)0.5 2)1.67µg/ml	1)1.22 ml/min	41
8	API	HPLC	Agilent Zobrax-x SB-C18(250 x4.6mm, 5µm)	0.05Mortho-phosphoric acid buffer, acetonitrile and methanol(45:45:10)	DAD 210-340nm	1)0.6532)2.167µg/ml	1ml/min	42
9	API	HPLC	Kromasil C18 (100mm ×4.6mm, 5µm)	Acetonitrile :water:orthophosphoric acid(50:50v/v)	UV at 260nm	1)… 2)….	1ml/min	43
10	Tablet	RP-HPLC	BDS(250 x4.6x5µ)	Acetonitrile and Sodium dihydrogen ortho phosphate	UV246nm	1)0.0032)0.011µg/ml	1ml/min	44
11	Human plasma	RP-HPLC	EA874(250x4.6m m,5mm) column	36.46mMAcetate buffer:acetonitrile:methanol(30:50:20v/v)	290nm	1)…. 2)…	1)1ml/min and 2)5.1min	45

12	API	RP-HPLC	Inertsil ODS-3(250x4.6mm,5µm)	Ammonium acetate bufferand acetonitrile 30:70v/v	UV at 252nm	1)0.01 2)0.04µg/ml	1)1ml/min&2)4min	[46]
13.	Human plasma	RP-HPLC	IntersilC18 column (250mm x4.6mm, 5mm)	Phosphate buffer with orthophosphoric acid and acetonitrile(85:15v/v)	UV at 280nm	1)2.98µg/ml and 2)9.98µg/ml	1ml/min	[47]
14.	Human plasma	LC-MS	Zobrax XDB phenyl (75 x4.6mm, 3.5mm)	Methanol : acetate buffer(80:20 v/v)	Turbo spray ionization	……	1)1.15 min and 2)1min	[48]
15	Bulk	RP-HPLC	Kromosil C18 column(250mm×4.6mm,5mm)	Phosphate buffer and Acetonitrile (65:35v/v)	UV at 254nm	1)0.361 µg/ml and 2)1.094 µg/ml	1)1ml/min and 2)3.548min	[49]
16	Bulk and Pharmaceutical dosage form	RP-HPLC	Inertsil ODS-3(250×4.6mm,5µ) column	0.02%Formic acid: Acetonitrile(40:60)	UV at 230	1)0.00136µg/ml and 2)0.00414µg/ml	1.2ml/min	[50]

Conclusion –

The overall summary we concluded that RP- HPLC base method used for the estimation Linezolid and Canagliflozin. We have used different category of drugs, first is Linezolid which is antimicrobial drug and second one is Canagliflozin is antidiabetes drug. It was observed that in most of the chromatographic method Acetonitrile, water, methanol and buffer used as mobile phase to produce a greater resolution. We observed that flow rate of both drug is almost constant that is 1ml/min. In present review, most of the methods included have used HPLC system, along with UV detectors. One of methods include Mass spectrometer as a detector, emerged recently, which a is expensive method, preventing its use in most of the laboratories. The advantages of HPLC systems coupled with UV Detectors or Mass Spectrometer includes high specificity, accuracy, high sesnsitivity and speed of analysis.

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