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REVIEW ARTICLE

A summary of Analytical methods of Empagliflozin: An SGL_2 inhibitor

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ABSTRACT
Now a day's very common metabolic disease called diabetes is a major problem of public health. So one of new category called phlozins are introduced as anti-diabetic agent, that given as orally. Out of all pholzins, one of highly selective drug is Empagliflozin which is approved by food drug and administration. Drug Empagliflozin also introduced in combination with other drugs like Metformin and Linagliptin. So this article states that the summary of different analytical methods for individual drug as well as in combination in pharmaceutical dosage form, biological fluids and active pharmaceutical ingredient. Through this study we can minimize the use of toxic and hazardous chemicals because they harm the environment as well as individual or community.
Keywords: Chromatographic analysis, Empagliflozin, Spectrophotometer, Antidiabetic, UPLC, HPTLC, LC/MS

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INTRODUCTION

Empagliflozin being highly selective in nature was the first to elucidate the decrease in cardiovascular death in patients with high CV risks and type 2 Diabetes. Being sodium-glucose cotransporter 2(SGLT2) inhibitor in nature, is probably the latest class of medicine in the treatment of T2DM. SGLT2 being glucose lowering agents shows an insulin independent mechanism which also proves their use in other treatments along with combination of other anti diabetec agents for the treatment of T2DM. After its first successful use in Japan in 2014, it was globally accepted. Empagliflozin or SGLT2 inhibitor clearly showed good results in reducing Blood Pressure (BP) and body weight. Apart from these, it also showed results for cardioprotective and renoprotective benefits, reduction in CV deaths, etc.

Other SGLT2 inhibitors such as Canagliflozin and Dapagliflozon have also showed their results in the treatment of cardiovascular and renal abnormalities but still Empalliflozin has passed all hurdles being the only SGLT2 inhibitor with proven cardiovascular and T2DM patients. Side effects such as UTI, GTI and ketoacidosis are being evaluated which is totally based on their mechanism of action, which is necessary for monitoring for such events. It has also been seen that they tend to decrease the Hyperglycemia which is achieved by increasing urinary glucose excretion. It blocks the reabsorption of glucose in the kidneys.

Empagliflozin is orally administrated drug and being SGLT2 in nature is located in the proximal convulated tubule (PCT) of the nephron and facilitates 90% of reabsorption. The chemical name of Empagliflozin is (Empagliflozin; 1-chloro-4-[b-D-glucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran – 3 – yl - oxy) benzyl]-benzene. The structure shown in figure 1. The SGLT2 inhibitors are derived from the phlorizin groups of compounds found in plants [1]. The physico-chemical parameters was shown in table 1. As per literaturethere are few analytical method has been reported as individual drug Empagliflozin but it has many analytical methods in combination of other drugs so in this review we segregate all the methods for helping the researcher for their studies.

PHARMACOLOGY

Kidney being am important organ plays a major role in the maintenance of glucose level in the body by the process of production, utilization and reabsorption of glucose via glomerular filtration. The renal

glucose reabsorption is facilitated by SGLT2 (an estimated of 90%). Empagliflozin being potent in nature is highly selective and competitive inhibitor of SGLT2 in the treatment of Type-2 DM patients with normal kidney functions. Patients of T2DM showed decreased glucose level both fasting and postprandial by-

- Increasing total glucose excretion
- Improving beta cell function
- Shifting substrate utilization from glucose to lipid.

Empagliflozin showed a 36% to 45% inhibition of glucose reabsorption after the administration of a single dose and subsequently maintained 36% to 48% inhibition after 27 days of administration. On comparison with placebo in a single rising dose study, Empagliflozin showed higher amount of glucose excretion.

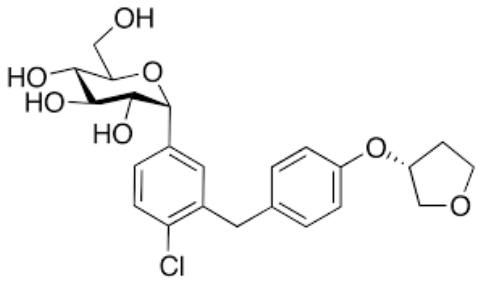

Figure 1: Structure of Empagliflozin

Parameter	Description
CAS Number	864077-44-0
Molecular formula	$C_{23}H_{27}ClO_7$
Molecular weight	450.9
Appearance	Crystalline Solid
Melting point	151-153° C
Solubility	Methanol, Ethanol, Dimethyl sulfoxide
Drug type	Approved

Table 1 Critical physiochemical parameters

PHARMACOKINETICS

With a limited number of drug-drug interaction and with no active metabolites, Empagliflozin is orally active and SGLT2 inhibitor with bioavailability of 78%. The overall kinetics was shown in Table 2.

Selective SGLT2 inhibitor and orally active with 78% bioavailability.
Empagliflozin exhibits rapid absorption after oral administration and reaches peak level after single dose.
Empagliflozin is distributed to body fluids and tissues with Volume of Distribution (Volume of distribution) of 73.8L at a steady rate.
No major metabolite of the drug is present in plasma yet three conjugates are present with each metabolite.
Empagliflozin exerts renal excretion with a total body clearance of 10.6L per hour.

Table 2 Pharmacokinetics of Empagliflozin

Absorption

After being orally administered, it shows rapid absorption by reaching peak levels 1.5 hours after a single administration.

Distribution

Being distributed to tissues and fluids, it shows around 86.2% protein bound in healthy individuals. It has a population based Volume of distribution of somewhat around 73.8L at a steady rate.

Metabolism

Empagliflozin shows no major metabolites in plasma, however it showed three conjugates with each metabolite, having less than 10% of the total drug in circulation.

Excretion

The estimated half-life of Empagliflozin is 12.4hours having a total body clearance of 10.6L/hour. It exhibits renal excretion and also showed that the radio –labeled dose of oral Empagliflozin was recovered in feces, most of which were unchanged drug.

Dosage and Administration

Initial dose is 10mg orally in the morning with a subsequent increase to a maximum dose of 25mg once daily, if necessary[2-5].

Chromatographic method (high performance liquid chromatography) of Empagliflozin

Jaiswal [6] et al gave exhaustive separation method on high performance liquid chromatography 0.1% orthophosphoric acid and acetonitrile in ratio of 50:50 at 230 nm. They used 1.2 mL/min flow for the analysis and obtain the retention time 11.51 min. Due to the high retention time, there were need to some more new and novel methods for separation analysis. Padmaja [7] et al produce a specific and new method using methanol and acetonitrile (50:50) at 265 nm. Peak observed at 2.80 minutes at 1mL/min flow rate. Padmaja [8] gave one more method using different mobile phase 0.01M acetate buffer and methanol (30:70) at 260 nm. Retention time observed early at 1.23 minute in comparison to previous one. Shyamala [9] et al developed and validated a different method for separation using 0.1% orthophosphoric acid and acetonitrile (70:30) at 233 nm. Mounika [10] et al produce a simple method of chromatographic separation using mobile phase methanol and water (70:30). Retention time observed at 6.20 minutes using 1 mL/min flow. Shirisha [11] et al gave a novel method using phosphate buffer and acetonitrile (45:55) at 228 nm. Peak observed at 3.8 minutes using buffer in 1 mL/min flow. Wem-jimg HAO [12] et al developed a precise, robust and rugged method using a three solvents as mobile phase that is phosphoric acid water, methanol and acetonitrile (1:1) at 224 nm. Madhurima [13] et al produce a new method for single drug using acetonitrile and water (60:40). The peak obtained at 5.41 minutes. Joanna [14] et al use acetonitrile and water (72:28) in a different ratio of same mobile phase as previous one at 230 nm but the retention time is early at 3.84 minutes in this method. After the many single drug methods, there are some methods in combination with different days. Patil [15] et al developed a selection method for combination of Empagliflozin and metformin using a methanol and water (80:20). The pH of water maintained at 3.0. The wavelength used for separation in combination is 227 at 0.8mL/min flow. Gopal [16] et al gave a new method using buffer in same as previous combination. Method using phosphate buffer and methanol (30:70) at 240 nm with 1 mL/min flow in instrument.Godasu [17] et al gave a method for separation analysis for Empagliflozin and metformin using same mobile phase as previous method of gopal. Geetha [18] et al produce a new method using phosphate buffer, acetonitrile and methanol (15:80:5) at 227 nm for Empagliflozin and metformin combination. For the above same combination Patel [19] et al gave specific and linear method using 0.05 M potassium dihydrogen phosphate buffer and acetonitrile (50:50)at 227 nm. Vinay [20] et al developed a method for combination Empagliflozin and metformin using a different mobile phase 0.1 % orthophosphoric acid and acetonitrile (50:50) at 260nm. Irfan Ali [21] et al produce specific method for the same combination and using a buffer and acetonitrile (45:55) for analysis. Alaas amin [22] et al produce a method using the same phosphate buffer and acetonitrile (60:40). The wavelength used was 255nm. Dighe [23] et al use the new mobile phase for the same combination that is methanol and dihydrogen phosphate buffer for separation at 227nm. Gampa vijay [24] et al gave a robust and rugged method using methanol and phosphate buffer (70:30) at 260nm.the flow rate was 0.8 mL/min. Jaffer [25] et al produce a method for same combination and using same mobile phase as above method but only ratio was changed that is (40:60) at 255nm. After this combination several researcher developed method for different combination of Empagliflozin and Linagliptin. For this combination Sharmila [26] et al uses 0.1% orthophosphoric acid and acetonitrile (68:32) at 218 nm. Naazneen [27] et al used 0.1% perchloric acid and acetonitrile (60:40) at 230 nm. Rao [28] et al gave a little bit different method from sharmila's analysis. Used a same mobile phase but different ratio that is (60:40) and different wavelength at 230 nm with 0.8 mL/min flow. Jyothirmai [29] et al gave selected method using methanol, acetonitrile and 0.1%

orthophosphoric acid (30:60:10) at 246nm. One more method given by Jayalaxmi [30] et al using methanol and phosphate buffer (70:30) at 254nm. Anjali [31] et al using the same mobile phase as above but ratio is changed that is phosphate buffer and methanol (70:30) at 240 nm. Sirigiri [32] used mobile phase phosphate buffer, water and acetonitrile at 225nm. The flow rate was 1 mL/min. Sruthi [33] et al gave same method as Jayalaxmi's method. After these two combination a new combination study reported by Khalil [34] et al that is novel method for combination of gliflozins like Canagliflozin, Dapagliflozin, Empagliflozin and metformin using a acetonitrile and phosphate buffer (65:35) at 212 nm. The summary of all methods shown in Table 3.

Spectrophotometric method for Empagliflozin

Some spectrophotometric methods also available for the Empagliflozin and its combination. Patil [35] et al produce an exhaustive method using methanol and water as solvent in a ratio of (90:10) at 224 nm. Jyothirmai [36] et al also gave a spectrophotometric method using methanol solvent at different wavelengths. Patil [37] and padamaja [38] et al gave a method for Empagliflozin and metformin using a methanol solvent. The summary of all methods shown in Table 3.

High performance thin layer liquid chromatography methods for Empagliflozin

There are some thin layer chromatography methods for Empagliflozin and its combination so bhole [39] et al gave a method at 230 nm using toluene and methanol (70:30) for individual drug. Dedhiya [40] et al gave a method for combination of Empagliflozin and metformin at 230 nm using toluene, ammonium acetate in methanol, ethyl acetate and ammonia. The summary of all methods shown in Table 3.

Ultra performance liquid chromatography methods for Empagliflozin

Mabrok [41] et al. gave a method for Empagliflozin at 210 nm using aqueous tetra-flouro acetic acid and acetonitrile (60:40) and flow rate was 0.5 mL/min. Padmaja [42] et al gave a method for Empagliflozin and metformin at 254 nm using 0.1% and methanol (40:60). The flow rate was 0.25 mL/min. A new method was given by Ayoub [43] for the combination of Empagliflozin linagliptin and metformin at 225 nm. The flow rate was 0.4 mL/min using phosphate buffer and methanol (50:50).LC/MS method for a combination of Empagliflozin and Linagliptin was given by Abdul ghany et al. method using 0.1 % aqueous formic acid and acetonitrile in the ratio of (50:50) at 0.2mL/min flow rate. The summary of all methods shown in Table 3.

Liquid Chromatography-Mass Spectroscopy analysis of Empagliflozin

Ayoub [44] et al also gave one method for combination of Empagliflozin and metformin at 215 nm using 0.1% aqueous formic acid and acetonitrile (75:25). Tejas [45] et al. gave one method for same combination using methanol and ammonium tri-fluoroacetic (90:10) in human plasma at 224 nm. Ghany [46] et al. gave method for one more combination of Empagliflozin and linagliptin. The summary of all methods shown in Table 3.

S.No	Matrix	Techniques	Method description	Wavelength (nm)	Flow rate (mL/min)	Retention time	Reference
1	Empagliflozin	HPLC	0.1%Orthophosphoric acid(50):Actonitrile(50)	230	1.2	11.51	[6]
2	Empagliflozin	HPLC	Methanol(50):Acetonitrile(50)	265	1	2.80	[7]
3	Empagliflozin	HPLC	0.01 M acetate buffer(30): methanol(70)	260	2	1.23	[8]
4	Empagliflozin	HPLC	0.1% OPA(70):Acetonitrile(30)	233	1	3.17	[9]
5	Empagliflozin	HPLC	Methanol(70):water(30)	233	1	6.20	[10]
6	Empagliflozin	HPLC	Phosphate buffer(45):Acetonitrile(55)	228	1	3.8	[11]
7	Empagliflozin	HPLC	Phosphoric acid water:methanol:acetonitrile(1:1)	224	1	-	[12]
8	Empagliflozin	HPLC	Acetonitrile(60):water(40)	223	1	5.41	[13]
9	Empagliflozin	HPLC	Acetonitrile(72):water(28)	230	1	3.84	[14]
10	Empagliflozin and Metformin	HPLC	Methanol (80): Water (20) pH 3.0	227	0.8	2.63 5.13	[15]
11	Empagliflozin and Metformin	HPLC	phosphate buffer(30) pH-3:methanol(70)	240	1	1.18 1.71	[16]
12	Empagliflozin and Metformin	HPLC	Methanol(70):phosphate buffer(30) pH-3	240	1	2.40 3.90	[17]
13	Empagliflozin and Metformin	HPLC	Phosphate buffer(15):acetonitrile(80):methanol(5)	227	1	2.52 1.41	[18]
14	Empagliflozin and Metformin	HPLC	0.05M Potassium Dihydrogen Phosphate buffer (50) pH-3.5: Acetonitrile(50)	227	1	2.63 4.38	[19]
15	Empagliflozin and Metformin	HPLC	0.1% ortholphosphoric acid(50):acetonitrile(50)	260	1	2.19 3.20	[20]

16	Empagliflozin and Metformin	HPLC	Buffer(45):Acetonitrile(55)	226	1.1	2.18 2.90	[21]
17	Empagliflozin and Metformin	HPLC	Phosphate buffer(60):Acetonitrile(40)	255	1	6.40 2.60	[22]
18	Empagliflozin and Metformin	HPLC	Methanol(60):dihydrogenphosphate buffer(30)	227	0.8	6.54 5.27	[23]
19	Empagliflozin and Metformin	HPLC	Methanol(70):phosphate buffer(30)	260	0.8	-	[24]
20	Empagliflozin and Metformin	HPLC	Methanol (40):phosphate buffer(60)	255	1	4.21 2.46	[25]
21	Empagliflozin and Linagliptin	HPLC	0.1% orthophosphoric acid (68): acetonitrile (32)	218	1	0.35 0.47	[26]
22	Empagliflozin and Linagliptin	HPLC	0.1%perchloric acid(60):acetonitrile(40)	230	1	2.05 4.10	[27]
23	Empagliflozin and Linagliptin	HPLC	0.1%o-phosphoric acid (60): acetonitrile (40)	230	0.8	2.13 2.75	[28]
24	Empagliflozin and Linagliptin	HPLC	Methanol(30):Acetonitrile(60):0.1%ortho phosphoric acid(10)	246	1	4.16 5.73	[29]
25	Empagliflozin and Linagliptin	HPLC	Methanol(70):phosphate buffer(30)	254	1	-	[30]
26	Empagliflozin and Linagliptin	HPLC	Potassium dihydrogen phosphate buffer(70): methanol (30)	240	1	3.02 3.96	[31]
27	Empagliflozin and Linagliptin	HPLC	Potassium dihydrogen phosphate mobile phase A water:acetonitrile (5:95) phase B	225	1	5.38 8.39	[32]
28	Empagliflozin and Linagliptin	HPLC	Buffer (70):Acetonitrile (30)	286	1	1.92 3.69	[33]
29	Empagliflozin, Canagliflozin, Dapagliflozin, Metformin	HPLC	Acetonitrile (65):0.05M Potassium dihydrogen phosphate (35)	212	1.1	3.00 4.41 3.56 1.89	[34]
30	Empagliflozin	UV	Water (90):Methanol(10)	224	-	-	[35]
31	Empagliflozin	UV	Methanol	247	-	-	[36]
32	Empagliflozin and Metformin	UV	Methanol	224 230	-	-	[37]
33	Empagliflozin and Metformin	UV	Methanol	272 234	-	-	[38]
34	Empagliflozin	HPTLC	Toluene: Methanol (7:3)	230	-	-	[39]
35	Empagliflozin and Metformin	HPTLC	Toluene: 3% Ammonium Acetate in Methanol: Ethyl acetate: Ammonia (3: 5: 2: 0.4)	230	-	-	[40]
36	Empagliflozin	UPLC	aqueous trifluoroacetic acid (0.1%, pH 2.5): acetonitrile (60:40)	210	0.5	0.60	[41]
37	Empagliflozin and Metformin	UPLC	0.1% ortho phosphoric acid buffer: methanol in the ratio (40:60)	254	0.25	3.4 0.8	[42]
38	Empagliflozin, Linagliptin and Metformin	UPLC	potassium dihydrogen phosphate buffer pH (4) methanol (50 : 50)	225	0.4	4.7 2.3 1.6	[43]
39	Empagliflozin and Metformin	LC/MS	0.1% aqueous formic acid:acetonitrile (75:25)	215	0.2	-	[44]
40	Empagliflozin and Metformin	LC/MS	methanol and 10 mM ammonium trifluoroacetate (90:10)	224	0.8	-	[45]
41	Empagliflozin and Linagliptin	LC/MS	0.1 % aqueous formic acid and acetonitrile (50:50)	220	0.2	-	[46]

Table 3 Different Analytical methods of Empagliflozin

CONCLUSION

This review consist many analytical methods of Empagliflozin in different forms like formulations, active pharmaceutical ingredient and biological fluids. Analysis of these methods applicable to check the quality of products of drug. Mostly used chromatographic method is high performance liquid chromatography for combination with other drugs. Various chemicals and solvents have used in methods which are not applicable to green chemistry. Used chemicals must not harmed to environment as well as individual so according to this literature researcher can go for further studies used any of method mention here and one more conclusion was that it is desired to develop a new, simple, cheap and green method for Empagliflozin as single drug.

REFERENCES

1. Cavaiola TS, Pettus J. (2018). Cardiovascular effect of sodium glucose cotransport-2 inhibitors. Diabetes Metab Syndr Obes; 11:133-148.

2. Bakris GL, Fonseca VA, Sharma K, et al.[2009]. Renal sodium-glucose transport: role in diabetes mellitus and potential clinical implications. Kidney Int;75(12):1272–1277.
3. Grempler R, Thomas L, Eckhardt M, et al. [2012]. Empagliflozin a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterization and comparison with other SGLT-2 inhibitors. Diabetes Obes Metab;14(1):83–90.
4. Jardiance (empagliflozin). [2014]. prescribing information. Ridgefield, Connecticut: Boehringer Ingelheim Pharmaceuticals.
5. HeiseT, Seewaldt-Becker E, Macha, S,et al. [2013]. Safety, tolerability, pharmacokinetics and pharmacodynamics following 4 weeks' treatment with Empagliflozin once daily in patients with type-2 diabetes. Diabetes Obes Metab;15(7):613–621.
6. Jaiswal SH, Katariya1 MV, Katariya VR, Karva GS, koshe K. [2017]. Validated stability indicating HPLC method for determination of process related impurities in Empagliflozin drug substances. World Journal of Pharmaceutical Research; 6(7): 1025-1037.
7. Padmaja N, Veerabhadram G. [2016] Development and validation of a novel stability-indicating RP-HPLC method for the determination of Empagliflozin in bulk and pharmaceutical dosage form. Int J Pharm Sci Res; 7: 4523-4530.
8. Padmaja N, Veerabhadram G. [2016] Method development and validation of RP-HPLC for the estimation of Empagliflozin in API. Int J Pharm Sci Res; 7: 724-727.
9. Shyamala NK, Moanika J, Nandine B. [2016].Validated Stability indicating RPHPLC method for determination of Empagliflozin. Scholar. Res. Lib; 8(2):457-464.
10. Mounika PS, Hemant TK, Srinivasa YR, Rao VPK. [2016]. RP-HPLC Method for Quantification of Empagliflozin in Pharmaceutical Formulation. Scholar. Res. Lib; 10(2):547-554.
11. [11] Shirisha V, Krishnaveni B, Illendulal S, Rao KNV, Dutt HR. [2019]. A New Simple Method Development, Validation and Forced Degradation Studies of Empagliflozin by Using RP-HPLC. International Journal of Pharmacy and Biological Sciences; 9 (1): 25-35
12. Hao WJ, Tao Z,Hua H,Hao C, Xiao-dong Z. [2016]. Determination of related substances in empagliflozin tablets by HPLC. Chinese Journal of Pharmaceutical Analysis; 36(5): 902-910
13. Basak M, Gouru SR, Bera A, Nagappan KV. [2019]. A Rapid and Sensitive RP-HPLC Method for the Quantitative Analysis of Empagliflozin in bulk and Pharmaceutical Dosage Form. International Journal of Applied Pharmaceutics; 11(5): 60-65.
14. Manoel JW, Primieri GV, Bueno LM, Wingert NR,Volpato NM,Garcia CV, Schapovala EES, Steppea.M. [2020].the application of quality by design in the development of the liquid chromatography method to determine Empagliflozin in the presence of its organic impurities. RSC Adv; 10: 7313-7320.
15. Patil SD, Amurutkar SV, Chatpalliwar VA, Upasani CD. [2017]. Development and validation of RP-HPLC method for Empagliflozin and Metformin HCL. JIPBS; 4 (4):185-189.
16. Gopal NM, Sridhar AC. [2017].Validated stability indicating an ultra-performance liquid chromatographic method for simultaneous determination of metformin hydrochloride and Empagliflozin in bulk drug and tablet dosage form. Int J Appl Pharm;9(3):45-50.
17. Godasu SK, Sreenivas SA. [2017]. A new validated RP-HPLC method for the determination of metformin HCL and Empagliflozin in its bulk and pharmaceutical dosage forms. Int J Pharm Sci Res; 8(5): 2223-32.
18. Geetha SP, Lakshmana RK, Prasad KRS, Suresh BK. [2016]. Development and validation of stability indicating reversed phase high-pressure liquid chromatography method for simultaneous estimation of metformin and Empagliflozin in bulk and tablet dosage form. Asian J Pharm Clin Rese; 9: 126-135.
19. Patel AM, Chaudhary AB. [2017]. Stability Indicating RP-HPLC Method Development and Validation for estimation of Empagliflozin and Metformin HCL. World Journal of Pharmacy and Pharmaceutical Sciences; 6(9): 872-885.
20. Kumar VD, Rao S. [2018]. A new validated stability indicating RP-HPLC method for simultaneous estimation of metformin hydrochloride and Empagliflozin in tablet dosage forms. IRJPMS; 1:16–22.
21. Irfan SA, Kumar BR. [2017]. Stability indicating simultaneous estimation of metformin Empagliflozin in pharmaceutical tablet dosage form by RP-HPLC. Asian J Res Chem; 10:783–788.
22. Amin AS. [2019] Simultaneous For the Estimation of Metformin and Empagliflozin in Pharmaceutical Dosage Form by HPLC Method. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS); 14(1): 75-80.
    Dighe NS, Shinde GS, Vikhe JJ. [2019].Simultaneous Estimation, Validation and Force Degradation Study of Metformin Hydrochloride and Empagliflozin by RP-HPLC Method. Research Journal of Science and Technology; 11(2):135-147.
23. Gampa VK, Sowjanya P,Shyam N. [2016]. RP-HPLC Method Development and Validation for Simultaneous Estimation of Metformin and Empagliflozin in Bulk and Pharmaceutical Dosage Forms. Pharma research library; 4(3):1-8.
24. Hussain JB et al. [2016]. Method development and validation of Metformin and
25. Empagliflozin in pharmaceutical dosage forms in RP-HPLC. Asian Journal of Research in Chemistry and Pharmaceutical Sciences; 4(3): 91-100.
26. Donepudi S, Achanta S. [2018]. Validated HPLC-UV method for simultaneous estimation of linagliptin and Empagliflozin in human plasma. Int J Appl Pharm; 10: 56–6.

27. Naazneen S, Sridevi A. [2016]. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of Empagliflozin and linagliptin in tablet formulation. Pharm Lett 2016; 8: 57-65.
28. Rao MS, Rambhau K. [2018]. Development and validation for the simultaneous Estimation in of metformin and empagliflozin in drug product by RP-HPLC. EuropeanJournal of biomedical and pharmaceutical sciences; 5(2): 404-410.
29. khatiza NJ, Begum MD, supriya P. [2016].Novel stability indicating RP-HPLC methodfor the simultaneous estimation of Empagliflozin and linagliptin in bulk and pharmaceutical formulation, journal of atom and molecule; 6(3): 977.
30. Jayalaxmi, Rajesh T, Gampa VK. [2017]. A Validated RP- HPLC Method for the Simultaneous Estimation of Empagliflozin and Linagliptin in its Bulk and Pharmaceutical Dosage Forms. Pharma research library; 5(3):58-64.
31. Bakshi A, Mounika A, Bhutada S, Bhagvan MR. [2018]. Simultaneous Estimation of Empagliflozin and linagliptin by RP-HPLC Method. World Journal of Pharmacy and Pharmaceutical Sciences; 7(8): 1062-1071.
32. Sirigiri N, Subramanian NS, Reddy GNK. [2018].Stability Indicating Method Development and Validation for Simultaneous Estimation of Linagliptin and Empagliflozin in Tablets by HPLC. Saudi J. Med. Pharm. Sci; 4(8): 884-896.
33. Sruthi V, Poornima B et al.[2019]. Analytical Method Development and Validation for Simultaneous Estimation of Linagliptin and Empagliflozin in Bulk and Pharmaceutical Formulation by RP-HPLC. Ijppr.Human; 16 (2): 555-568.
34. Khalil GA, Salama I, Gomma MS, Helal MA.[2018] Validated RP-HPLC method for simultaneous determination of canagliflozin, dapagliflozin, empagliflozin and metformin. Int J Pharm Chem Biol Sci ; 8:1–13.
35. Patil SD,Chaure SK, Rahman MAH, Varpe PU. [2017]. Development & Validation of simple UV-Spectrophotometric Method for the determination of Empagliflozin. Asian Journal of Pharmaceutical Analysis; 7 (1): 18-22.
36. Jyothirmai N, Nagaraja B, Anil KM. [2016]. Novel UV Visible Spectrophotometric Methods for the analysis of Empagliflozin a type 2 Diabetic drug in Bulk and Pharmaceutical Formulations. J. de Africana; 3(1): 177-187.
37. Patil SD, Chaure SK, Kshirsagar S. [2017]. Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs. Asian J. Pharm. Ana; 7(2): 117-123.
38. Padmaja N, Babu MS, Veereabhadram G. [2016]. Development and validation of UV spectrophotometric method for simultaneous estimation of Empagliflozin and metformin hydrochloride in bulk drug and combined dosage forms. Pharm Lett; 8: 207-213.
39. Bhole RP, Tamboli FR. [2018]. Development and Validation of Stability Indicating HPTLC-MS Method for Estimation of Empagliflozin in Pharmaceutical Dosage Form. Analytical Chemistry Letters; 8(2): 244-256
40. Praful, Singh RS, Shah PJ, Shah SA. [2019].Development and Validation of High Performance Thin Layer Chromatographic Method for Estimation of Metformin Hydrochloride and Empagliflozin in Combined Tablet Dosage Form Using Quality by Design Approach. Pharma Science Monitor; 10(2): 83-100.
41. Mabrouk MM, Soliman SM, El-Agizy HM,Mansour FR. [2019]. A UPLC/DAD method for simultaneous determination of Empagliflozin and three related substances in spiked human plasma. BMC Chemistry; 13(83): 1-9.
42. Padmaja N, Veerabhadram G .[2017]. A novel stability indicating RP-UPLC- DAD method for determination of metformin and Empagliflozin in bulk and tablet dosage form. Orient J Chem ; 33:1949–1958.
43. Ayoub BM. UPLC simultaneous determination of Empagliflozin, linagliptin and metformin. RSC Adv 2015; 5: 95703-95709.
44. Ayoub BM, Mowaka S. [2017]. LC–MS/MS determination of Empagliflozin and metformin. J Chromatograp Sci; 55: 742–747.
45. Wattamwar T , Mungantiwar A, Halde S, Pandita N. [2019]. Development of simultaneous determination of Empagliflozin and metformin in human plasma using liquid chromatography–mass spectrometry and application to pharmacokinetics. European Journal of Mass Spectrometry; DOI: 10.1177/1469066719879297
46. Abdel-Ghany MF, Abdel-Aziz O, Ayad MF, Tadros MM. [2016]. Pharmaceutical analysis of linagliptin and empagliflozin using LC-MS/MS. Pharma Chem; 8: 186-189.