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Der Pharmacia Lettre, 2016, 8 (8):302-307
(http://scholarsresearchlibrary.com/archive.html)

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ISSN 0975-5071
USA CODEN: DPLEB4

Estimation of Empaglifozin using Derivative spectrophotometry and Area under Curve in Bulk material and in *In-house* Tablet formulation

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ABSTRACT

Simple, Specific, rapid and accurate UV- spectrophotometric methods have been developed using methanol as solvent to determine Empaglifozin in bulk and in-house tablet formulation using derivative and Area Under Curve(AUC) techniques. "Method A" is Zero Order Derivative UV- spectrophotometry using absorbance, "Method B" is Zero Order Derivative UV- spectrophotometry using Area Under Curve (AUC) technique, 'Method C' is First Order derivative UV- spectrophotometry using amplitude, Method D is First Order Derivative UV- spectrophotometry using AUC, technique. The established methods have shown best findings in terms of linearity, accuracy, precision and LOD and LOQ for bulk drug and in-house tablets. In all Methods, Empaglifozin followed linearity in the concentration range of 4 - 21 µg/mL with (r^2 > 0.999).

Keywords: Derivative spectrophotometry; Area under curve; Empaglifozin

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INTRODUCTION

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed, scattered or emitted by atoms, molecules, or other chemical species. This absorption or emission is associated with changes in the energy states of the interacting chemical species and, since each species has characteristic energy states, spectroscopy can be used to identify the interacting species [1].

Empaglifozin, (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)- oxolan - 3 yl] oxyphenyl] methyl]phenyl]-6hydroxymethyl) oxane-3,4,5-triol. **(Figure 1)** is an inhibitor of sodium glucose co-transporter-2, which accounts for higher glucose reabsorption into the blood [2]. The literature survey disclosed that a UPLC method for estimation of empaglifozin and linagliptin and metformin various Pharmaceutical formulations was reported [3]. Also, RP-HPLC method for estimation of empaglifozin only in active pharmaceutical ingredient (API) was reported [4].

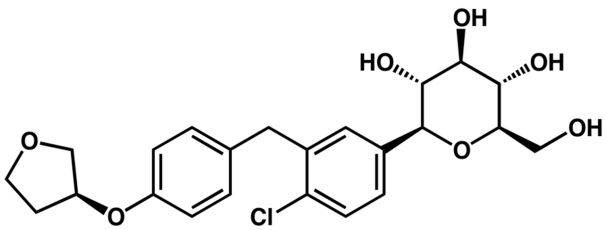

Fig. 1 Chemical structure of Empaglifozin

To our wisdom, no methods were reported in literature for determination of Empaglifozin in bulk and pharmaceutical formulation using derivative spectroscopic techniques. Therefore, our endeavor is to study zero order and first order derivative spectroscopy using amplitude and also area under curve (AUC) techniques. The AUC method is applicable where there is no sharp peak or when broad spectra are obtained. It engages the calculation of integrated value of area with respect to the wavelength between the two selected wavelengths $\lambda 1$ and $\lambda 2$. Choice of wavelength range is on the basis of repeated observations so as to get the linearity between AUC and concentration

[5, 6]. Further, methods were validated as per ICH guidelines [7].

2. Experimental Work

2.1 Materials

Empaglifozin working standard was obtained as gift sample from Macleod Pharmaceutical Ltd. *In- house* tablets containing 5 mg Empaglifozin were prepared using commonly used excipients.

All chemicals and reagents were of analytical grade and purchased from Merck chemicals, Mumbai, India.

2.2 Instrument

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe 2.21 with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 400- 200 nm; scan speed: medium; sampling interval: 10 nm; band width ($\Delta\lambda$): 1.0 nm; spectral slit width: 1 nm. An electronic balance (Model Shimadzu AUX 120) was used for weighing purpose.

2.3 Preparation of Stock Standard Solution

The stock standard solution was prepared by dissolving accurately weighed 10 mg of Empaglifozin in 100 mL of methanol to obtain a concentration i.e. 100 µg/mL.

2.4 Methods A (Zero Order UV- Spectrophotometry) and Method B (Zero order UV- Spectrophotometry–AUC)

From the stock standard solution, an appropriate volumes 0.4 – 2.1 mL was transferred into series of 10 mL volumetric flask and volume was made up to the mark to obtain concentration in the range of 4 - 21 µg/mL. In Method A, absorbance was measured at 225 nm is shown in **Figure 2**; whereas in Method B, area under curve was selected in the wavelength range of 220.50 - 231.00 nm is shown in **Figure 3**. The calibration curves were constructed by plotting concentration *versus* absorbance or AUC in Method A and B, respectively.

2.5 Methods C (First order derivative – UV Spectrophotometry and D (First order derivative UV-Spectrophotometry-AUC)

For Method C and D, spectra of above prepared solutions in the range of 4 - 21 µg/mL were derivatives into first order using software UV-Probe 2.21 with delta lambda 10 and scaling factor 10. In Method C, the amplitude was recorded at 234 nm is shown in **Figure 4**, while in Method D, AUC of the derivative spectrum was studied at 228 - 244 nm is shown in **Figure 5.** The calibration curves were constructed by plotting concentration *versus* amplitude in Method C, while in Method D; it was studied as concentration versus AUC- of First order Derivative spectra between selected wavelengths.

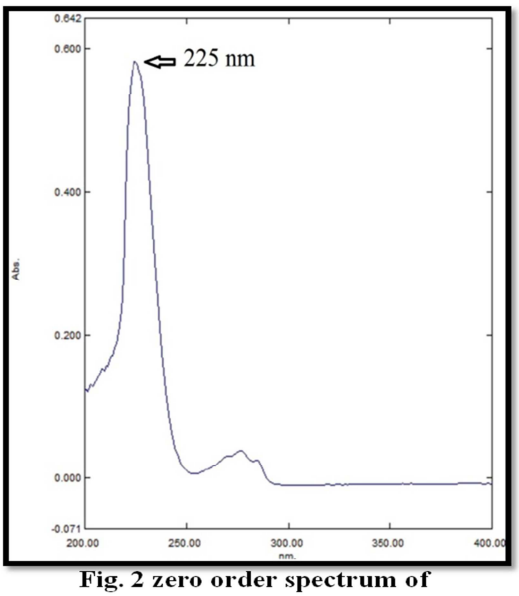

Fig. 2 zero order spectrum of Empaglifozin in methanol

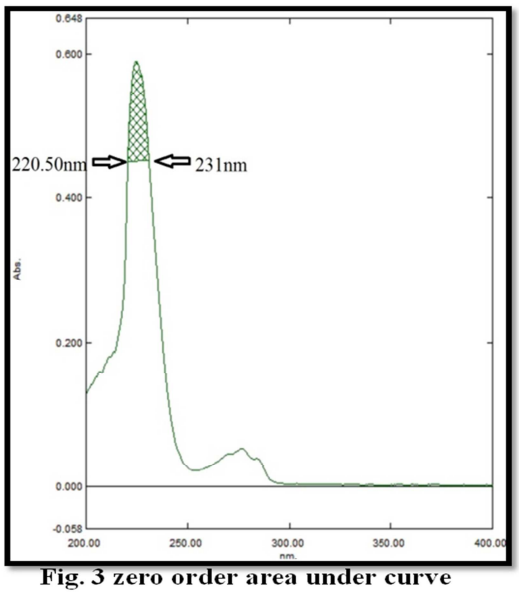

Fig. 3 zero order area under curve spectrum of Empaglifozin in methanol

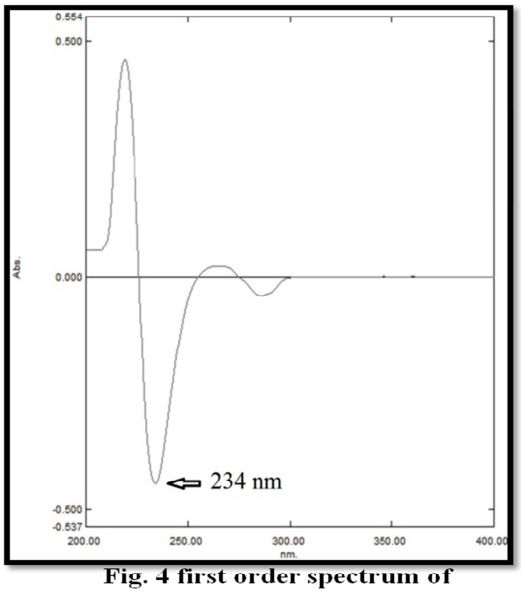

Fig. 4 first order spectrum of Empaglifozin in methanol

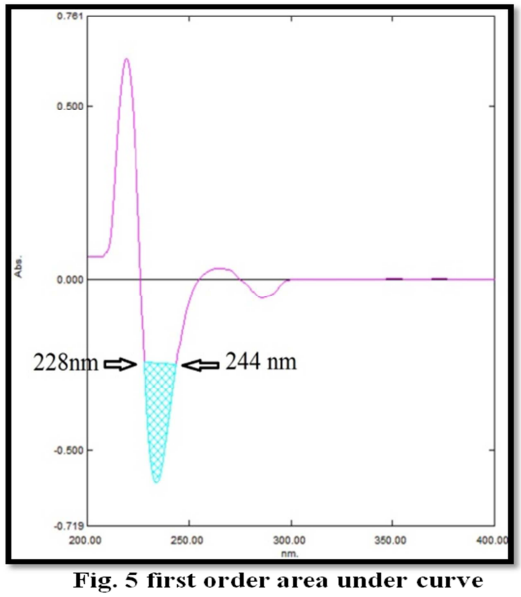

Fig. 5 first order area under curve spectrum of Empaglifozin in methanol

2.6 Preparation of *in-house* tablet formulation

In-house tablets, containing 5 mg of Empaglifozin per tablet, were prepared using simple direct compression technique.

3. Validation of methods

The developed Method A, B, C and D were validated as per ICH guidelines.

3.1 Linearity

In Method A, B, C and D Empaglifozin followed linearity in the concentration range of 4- 21 µg/mL. The details of optical characteristics and linearity data are furnished in **Table 1.**

Table 1: Optical Characteristics of Empaglifozin

Parameters	Method A	Method B	Method C	Method D
Beer-Lambert's range (µg/mL)	4-21	4-21	4-21	4-21
λ max (nm)/Wavelength range (nm)	225	220.50- 231	234	228 - 244
Slope	0.046	0.0966	0.0278	0.1641
Intercept	0.024	0.0355	0.0062	0.0638
Correlation coefficient	0.9991	0.9992	0.9995	0.9996

3.2 Accuracy/ Recovery study

The accuracy of all methods was assessed by measurement of recovery. To the pre-analyzed sample solutions (8 µg/mL), known amounts of stock standard solutions were added at different levels, i.e. 80 %, 100%, and 120 %. The solutions were re-analyzed by the proposed methods. The experiments were repeated for three times at each level for each method. Results are shown in **Table 2.**

Table 2: Accuracy Studies

Method	Initial Amount (µg/mL)	Amount Added (µg/mL)	Amount Recovered (µg/mL, n=3)	% Recovered	% RSD
A	8	6.4	6.43	100.51	0.51
	8	8	8.01	100.80	0.27
	8	9.6	9.56	99.65	0.59
B	8	6.4	6.21	97.16	0.16
	8	8	7.82	97.65	0.35
	8	9.6	9.35	97.43	0.11
C	8	6.4	6.29	98.37	0.56
	8	8	8.03	100.46	0.46
	8	9.6	9.54	99.40	0.59
D	8	6.4	6.19	96.79	0.09
	8	8	7.77	97.15	0.11
	8	9.6	9.58	99.84	0.06

3.3 Precision

Precision of the methods was studied as intra-day and inter-day variations. In all the methods precision was determined by analyzing the 8, 12 and 16 µg/mL of Empaglifozin solutions as intra-day and inter-day variations; results are shown in **Table 3.**

Table 3: Precision Studies

Method	Concentration (µg/mL)	Intra-day (n=3)	% RSD	Inter-day (n=3)	% RSD
A	8	8.03	0.97	8.04	0.61
	12	12.08	0.27	12.06	0.21
	16	16.06	0.25	16.00	0.16
B	8	7.517	0.18	7.57	0.18
	12	11.16	0.14	11.16	0.14
	16	15.17	0.10	15.17	0.10
C	8	8.06	0.33	8.06	0.33
	12	12.09	0.17	12.08	0.17
	16	16.13	0.13	16.13	0.13
D	8	8.01	0.11	8.01	0.11
	12	12.12	0.03	12.09	0.03
	16	15.76	0.04	15.76	0.04

3.4 Sensitivity

The sensitivity of measurement of Empaglifozin by the use of the proposed methods was estimated in terms of the limit of quantification (LOQ) and the limit of detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3*N/B and LOQ = 10*N/B; Where, 'N' is the standard deviation of the absorbance, amplitude and peak areas of the Empaglifozin (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. The results are shown for LOD Method **A- 0.08**, **B- 0.03**, **C- 0.08** and **D- 0.32**. and for LOQ Method **A- 0.27**, **B- 0.09**, **C- 0.25** and **D- 0.99**.

3.5 Repeatability

Repeatability was determined by analyzing 16 µg/mL concentration of Empaglifozin solutions for six times for all methods and results are shown in **Table 4.**

Table 4: Repeatability Studies

Method	Amount taken (µg/mL)	Amount found (µg/mL)	% Amount found (n=6)	Mean ± SD	% RSD
A	16	15.98	99.93	99.93 ± 0.14	0.14
B	16	15.18	94.92	94.92 ± 0.09	0.10
C	16	16.15	100.96	100.96 ± 0.05	0.34
D	16	15.76	98.52	98.52 ± 0.06	0.06

3.6 Ruggedness

Ruggedness of the proposed methods was determined for 16 µg/mL of Empaglifozin by analysis of aliquots from a homogenous slot by two analysts using the same operational and environmental conditions for all methods. The results are in acceptable range that is % RSD values < 2 for all the methods as shown in **Table 5.**

Table 5: Ruggedness Studies

Method	Analyst I		Analyst II	
	% Amount found (µg/mL)	%RSD	% Amount found (µg/mL)	%RSD
A	99.97 ± 0.15	0.15	100.43 ± 0.03	0.03
B	94.82 ± 0.08	0.08	94.92 ± 0.09	0.09
C	100.84± 0.27	0.27	100.43± 0.03	0.03
D	98.50 ± 0.06	0.06	98.52 ± 0.06	0.06

3.7 Analysis of *in-house* tablet Formulation

Twenty *in-house* tablets were weighed accurately and ground into fine powder. An amount of powdered drug equivalent to 10 mg was accurately weighed and transferred into a 100 mL volumetric flask containing methanol, sonicated for 15 min and volume was made up to the mark followed by filtration through Whatmann filter paper number 41. From this solution; an appropriate volumes of 0.6 mL were diluted to 50 ml using same solution. The resulting solutions were scanned using UV-Spectrophotometer in the range of 400 - 200 nm. The amounts of drug estimated using various proposed methods were determined from respective linearity equations and results are reported in **Table 6.**

Table 6: Analysis of *in-house* tablet formulation

	Tablet Assay		
Method	Amount found (µg/mL)	% Amount found	% RSD
A	16.00	100.02 ± 0.20	0.19
B	15.18	94.92 ± 0.09	0.09
C	15.81	98.84 ± 0.49	0.49
D	15.63	97.71 ± 0.04	0.04

RESULTS AND DISCUSSION

In methanol, Empaglifozin obeyed linearity in the concentration range of 04 – 21 µg/mL. The maximum absorbance/wavelength range and correlation coefficient for all method given in **table 1.** *In-house* tablet of Empaglifozin were analyzed. The amounts of Empaglifozin determined by all methods are given in **Table 5.** In all methods precision was studied (% RSD < 2) and inter-day and intra-day variations (% RSD < 2) for Empaglifozin. The accuracy of all methods was determined by calculating mean percentage recovery. It was determined at 80, 100 and 120 % level. The ruggedness of the methods was studied by two different analysts using the same operational and environmental conditions. The % recovery, repeatability data, ruggedness data are represented in **Table 2, 4,** and **5,** respectively.

CONCLUSION

All four methods were developed for the determination of Empaglifozin based on different analytical techniques UV spectrophotometric derivative and AUC methods. The methods were validated and found to be simple, sensitive, accurate and precise. Hence, the methods can be used successfully for routine analysis of pharmaceutical formulation and bulk of Empaglifozin.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgement
The authors are thankful to R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur for providing necessary laboratory facilities.

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