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Pharmaceutical Analysis of Linagliptin and Empagliflozin using LC-MS/MS

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ABSTRACT

*A new LC-MS/MS method was developed for determination of empagliflozin and linagliptin in pharmaceutical pure forms and dosage forms. Regression parameters, LOD, LOQ, accuracy and precision were investigated. Linearity was found to be acceptable over the concentration ranges of 25 - 800 ng mL^{-1} and 50 - 1600 ng mL^{-1} for linagliptin (LG) and empagliflozin (EG), respectively. S_b and S_a values of (4.43 * 10^{-3} - 2.04) and (2.46 * 10^{-3} - 2.27) were acceptable for LG and EG, respectively. Furthermore, LOD and LOQ were found to be (4.45 ng mL^{-1} -13.50 ng mL^{-1}) and (11.08 ng mL^{-1} - 33.57 ng mL^{-1}) for LG and EG, respectively. The results of accuracy and precision calculations including the mean of the recovery and the standard deviation were (99.73 % ±1.38) and (100.15 % ± 1.15) for LG and EG, respectively. The optimized method was proved to be accurate for the quality control of the investigated drugs either in bulk or in pharmaceutical formulation.*

Keywords: Linagliptin; Empagliflozin; Pharmaceutical Analysis; LC-MS/MS; Validation.

INTRODUCTION

Linagliptin (LG) is an inhibitor to dipeptidyl peptidase-4 while empagliflozin (EG) is an inhibitor of sodium glucose co-transporter-2 [1]. There is no reported LC-MS/MS method for the pharmaceutical analysis of LG and EG combination. Only some LC-UV and spectrophotometric methods were reported [1-12] for each drug, either alone or in different anti-diabetic combinations. The aim of the proposed method is to present the first LC-MS/MS method for determination of the drugs in pharmaceutical pure forms and dosage forms.

MATERIALS AND METHODS

Instrumentation

Waters Acquity UPLCH Xevo TQD system (USA) interfaced with a Waters Quattro Premier XE triple quadrupole mass spectrometer equipped with electrospray ionization was used. C_{18} (50 mm × 2.1 mm, 1.7 µm) column was selected for the experiment.

Reference samples and reagents

EG (99.80 %), LG (99.90 %) and Glyxambi® tablets nominally containing 5 mg of LG and 10 mg of EG per tablet were supplied from Boehringer Ingelheim pharmaceutical company (Germany). HPLC grade acetonitrile was purchased from Fisher Scientific (UK). Formic acid was purchased from Sigma Aldrich (Germany).

Stock and working solutions

Stock solutions of LG and EG (1 mg mL^{-1}) were prepared separately in methanol. Working solutions of LG (1 µg mL^{-1}) and EG (2 µg mL^{-1}) were prepared using the mobile phase.

Chromatographic conditions and detection parameters

A mixture of 0.1 % aqueous formic acid and acetonitrile in the ratio of (50:50, *v/v*) was used as a mobile phase at a flow rate of 0.2 mL min^{-1}. The optimum values of cone voltage and collision energy were set at 20 V and 25 eV, respectively. Detection was performed using multiple-reaction monitoring (MRM) in the positive mode, by monitoring the transition pairs of *m/z* 473.01 to 420.10 and *m/z* 451.24 to 71.29 for LG and EG, respectively.

Procedure and validation

Linearity was achieved using six calibrators over the concentration ranges of 25 - 800 ng mL^{-1} and 50 - 1600 ng mL^{-1} for LG and EG, respectively. Calibration curves were obtained by plotting peak area against concentration and the regression equations were calculated. Accuracy was checked using concentrations equivalent to (75, 150, 225, 300, 375 ng mL^{-1} of LG) and (150, 300, 450, 600, 750 ng mL^{-1} of EG). Precision was checked using concentrations of (400, 500, 600 ng mL^{-1} of LG) and (800, 1000, 1200 ng mL^{-1} of EG) three times within the same day and on three successive days. In addition, nine different ratios of the drugs (1:5, 2:5, 3:5…5:1) were prepared. Their concentrations were calculated using regression equations. Limit of detection (LOD) and limit of quantitation (LOQ) were determined. Furthermore, twenty tablets of Glyxambi® were weighed, powdered and mixed in a mortar. Accurately weighed amount equivalent to 20 mg of LG and 40 mg of EG was made up to 100 mL with methanol and sonicated to dissolve then the solutions were filtered and 50 µL was transferred to 100-mL volumetric flask and completed to volume with the mobile phase to prepare tablet solution of LG (100 ng mL^{-1}) and EG (200 ng mL^{-1}). The concentrations of the drugs were calculated using their regression equations. Then to check the validity of the proposed method, standard addition technique was applied.

RESULTS AND DISCUSSION

Linearity was found to be acceptable over the concentration ranges of 25 - 800 ng mL^{-1} and 50 - 1600 ng mL^{-1} for LG and EG, respectively. The analytical data of the calibration curves are summarized in (Table 1). S_b and S_a values of ($4.43 * 10^{-3}$ - 2.04) and ($2.46 * 10^{-3}$ - 2.27) were acceptable for LG and EG, respectively. Furthermore, LOD and LOQ were found to be (4.45 ng mL^{-1} -13.50 ng mL^{-1}) and (11.08 ng mL^{-1} - 33.57 ng mL^{-1}) for LG and EG, respectively. The results obtained for accuracy and precision calculations including the mean of the recovery and the standard deviation were (99.73 % ± 1.38) and (100.15 % ± 1.15) for LG and EG, respectively. The accuracy and precision results are shown in (Table 2), the laboratory prepared mixture results are shown in (Table 3). Furthermore, Standard addition technique and dosage form results are illustrated in (Table 4).

A linear relationship between peak area and component concentration was obtained for each drug and the regression equations were computed over the concentration ranges of 25 - 800 ng mL^{-1} and 50 - 1600 ng mL^{-1} for LG and EG, respectively. Accuracy was calculated by % recovery of concentrations equivalent to (75, 150, 225, 300, 375 ng mL^{-1} of LG) and (150, 300, 450, 600, 750 ng mL^{-1} of EG). In addition, accuracy was confirmed by % recovery of different laboratory prepared mixtures. The results including the mean of the recovery and standard deviation were calculated. Furthermore, Standard addition technique was applied to confirm and ensure the accuracy of the method. To check the precision, the % R.S.D were calculated for the three concentrations of each drug using concentrations of (400, 500, 600 ng mL^{-1} of LG) and (800, 1000, 1200 ng mL^{-1} of EG), within the same day and on three successive days, and found to be less than 2 % as shown in (Table 1). Specificity was confirmed by % recovery of different concentrations of each drug in the presence of the other drug in their laboratory prepared mixtures. The proposed method was successfully applied to the pharmaceutical dosage form (Figure 1). Standard addition technique was applied and the concentrations were calculated using the corresponding regression equations as in (Table 4). Statistical comparison of the results obtained by the proposed methods and the reference method [1] was carried out by "SPSS statistical package version 11" at P=0.05 as shown in (Table 5).

Table 1: Results of assay validation of the proposed UPLC-MS/MS method for determination of of LG and EG in bulk powder

Item	LG	EG
Range of linearity (ng mL^{-1})	25-800	50-1600
Regression equation	Area = 2.1753 $C_{ng/mL}$+0.3483 (Equation 1)	Area = 0.9712 $C_{ng/mL}$+5.6418 (Equation 2)
Regression coefficient (r)	0.9998	0.9999
LOD (ng mL^{-1})	4.45	11.08
LOQ (ng mL^{-1})	13.50	33.57
S_b (standard error of slope)	4.43×10^{-3}	2.46×10^{-3}
S_a (standard error of intercept)	2.04	2.27
Confidence limit of the slope	2.1753±4.45	0.9712±2.21
Confidence limit of the intercept	$0.3483 \pm 1.54 \times 10^{-3}$	5.6418±0.014
Standard error of the estimation	2.94	3.26
Intraday %R.S.D	0.12 – 0.19 – 0.24	0.21 – 0.26 – 0.37
Interday %R.S.D	0.10 – 0.25 – 0.29	0.13 – 0.28 – 0.32

Table 2: Results for determination of LG and EG in bulk powder by the proposed UPLC-MS/MS method.

LG			EG		
Pure (ng mL^{-1})	Found (ng mL^{-1})	*Recovery %	Pure (ng mL^{-1})	Found (ng mL^{-1})	*Recovery %
75	76.47	101.96	150	147.61	98.41
150	148.19	98.79	300	304.12	101.37
225	224.96	99.98	450	442.09	98.24
300	299.43	99.81	600	599.63	99.94
375	375.74	100.20	750	755.11	100.68
Mean±S.D.		99.73±1.38	Mean±S.D.		100.15±1.15

**Mean of three determinations.*

Table 3: Simultaneous determination of LG and EG by UPLC-MS/MS method in laboratory prepared mixtures.

Ratio LG:EG	LG			EG		
	Pure (ng mL^{-1})	Found (ng mL^{-1})	*Recovery %	Pure (ng mL^{-1})	Found (ng mL^{-1})	*Recovery %
5:1	500	504.90	100.98	100	100.84	100.84
4:1	400	399.87	99.97	100	99.46	99.46
3:1	300	294.85	98.28	100	98.08	98.08
2:1	200	197.03	98.52	100	101.76	101.76
1:1	100	101.27	101.27	100	101.76	101.76
1:2	100	100.25	100.25	200	196.45	98.23
1:3	100	101.27	101.27	300	298.05	99.35
1:4	100	98.19	98.19	400	404.24	101.06
1:5	100	99.22	99.22	500	501.7	100.34
	Mean ± S.D.		99.77 1.27			100.10 1.40

**Mean of three determinations.*

Table 4: Simultaneous determination of LG and EG in pharmaceutical dosage form and standard addition technique by UPLC-MS/MS method.

Pharmaceutical dosage form	*% R ± S.D.		Standard addition technique							
	LG	EG	Claimed concentrtion (ng mL^{-1})		Pure added (ng mL^{-1})		Pure found		* % R pure added	
			LG	EG	LG	EG	LG	EG	LG	EG
Glyxambi® tablets (linagliptin and empagliflozin) 5 mg/ 10 mg B.N.:25061N	100.19 ± 1.25	99.63 ± 0.97	150	300	50	100	50.45	98.84	100.90	98.84
					100	200	101.93	199.97	101.93	99.99
					150	300	147.24	305.7	98.16	101.90
					200	400	203.87	401.32	101.94	100.33
					250	500	249.17	509.82	99.67	101.96
Mean ± S.D.									100.52 ±1.62	100.60 ± 1.33

Table 5: Statistical comparison between the proposed method and the reference method

Statistical term	LG		EG	
	Reference Method [b]	UPLC-MS/MS	Reference Method [b]	UPLC-MS/MS
Mean	100.21	99.73	99.71	100.15
S.D.±	1.29	1.38	0.97	1.15
%RSD	1.29	1.38	0.97	1.15
n	5	5	5	5
V	1.66	1.90	0.94	1.32
***t* ([a] 2.306)**		0.57		0.65
***F* ([a] 6.39)**		1.14		1.40

[a] *Figures in parentheses are the theoretical t value at (p=0.05).*

[b] *Reference method [6]: aliquots of standard solutions containing 10-50 µg mL^{-1} LG and 1-32 µg mL^{-1} EG were measured at 225 nm using LC-UV.*

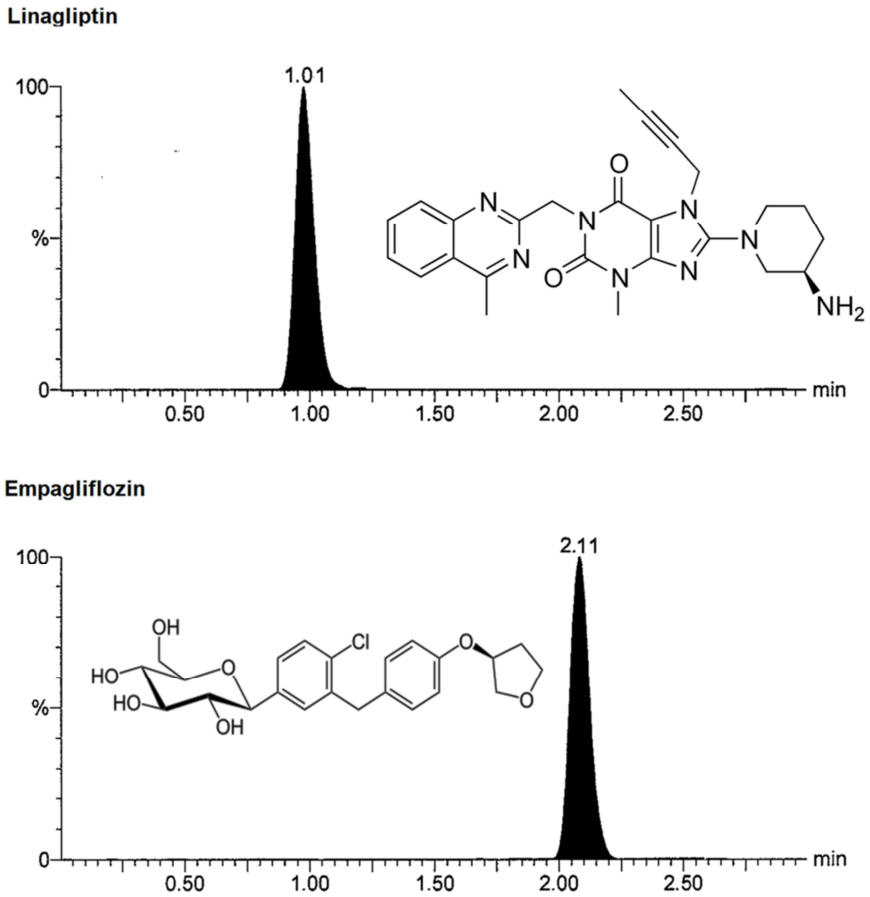

Figure 1: MS Chromatograms of linagliptin and empagliflozin.

CONCLUSION

The proposed LC-MS/MS method proved to be sensitive for determination of LG and EG and it was validated showing satisfactory data for all the parameters tested and can be used by quality control laboratories for the routine analysis of the drugs in their pure form and in their pharmaceutical formulations.

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