ORIGINAL ARTICLE

Validated isocratic liquid chromatographic method for the quantification of teneligliptin in the presence of internal standard

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Abstract

Introduction: Teneligliptin (TGP) is an oral dipeptidyl peptidase inhibitor used for the treatment of type 2 diabetes mellitus. A simple liquid chromatographic method has been established on isocratic mode for the quantification of TGP in pharmaceutical formulations in the presence of internal standard (IS) eplerenone, and the method was validated. **Materials and Methods:** Methanol and formic acid mixture was used with a flow rate of 0.4 ml/min (detection at 244 nm) using Agilent column on Shimadzu high-performance liquid chromatography system. **Results and Discussion:** TGP was eluted at 5.099 min and that of IS at 8.535 min. TGP has shown linearity 1–100 µg/mL with regression equation, y = 95722x–6775.4 correlation coefficient 0.9999. The limit of detection and limit of quantification are found to be 0.2598 µg/mL and 0.8134 µg/mL, respectively. **Conclusion:** It is observed that this reverse-phase ultra-fast liquid chromatography method is accurate and precise and can be used for the estimation of TGP tablets.

Key words: Eplerenone, isocratic mode, reversed-phase high-performance liquid chromatography, teneligliptin, validation

INTRODUCTION

Teneligliptin (TGP) is a dipeptidyl peptidase inhibitor,[1] and it is used for treating type 2 diabetes mellitus. Diabetes requires need lifelong medical treatment, and more attention has to be paid for the glycemic control.[2-4] Different techniques were developed for the estimation of TGP [Figure 1] in literature, but especially high-performance liquid chromatography (HPLC) methods[5-11] were very few. In the present paper, a simple liquid chromatographic method has been developed in the presence of internal standard (IS), eplerenone (EPL), which is an anti-hypertensive drug, and the method was validated.[12]

MATERIALS AND METHODS

Chemicals and Reagents

TGP and EPL were obtained as gift samples from Cadila Pharmaceuticals Ltd. and Zydus Cadila (India). TGP is available as tablet formulation with brand names - Zita Plus (Glenmark), Ziten (Glenmark Pharmaceuticals), Eternex T (Alembic Pharma), and Tenglyn (Zydus Cadila), with label claim 20 mg. All other chemicals are of AR grade, and all solvents are of HPLC grade. A stock solution of EPL and TGP was prepared by dissolving 25 mg of EPL and TGP in two separate 25 mL volumetric flasks with HPLC grade methanol and diluted with mobile phase mixture and used after filtration through membrane filter.

Method Validation

Chromatographic separation was performed on isocratic mode with methanol and formic acid mixture (75:25 v/v) at

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a flow rate of 0.4 mL/min and with detector signal at 244 nm. 0.1–100 µg/mL of TGP solutions were prepared along with the internal standard (IS) and EPL (10 µg/mL) from their stock solutions and injected into the SHIMADZU chromatographic system. The peak area (n = 3) of both TGP and EPL chromatograms was taken, and the mean peak area ratio (TGP/EPL) was calculated. A calibration curve was drawn by taking the concentration of TGP on the X-axis and the corresponding mean peak area ratio (TGP/EPL) on the Y-axis. Precision and accuracy (spiked 50%, 100%, and 150%) studies were conducted, and the percentage relative standard deviation (RSD) as well as the recovery values was calculated.

Assay of Tgp Tablets

Tablets of three different available marketed brands were collected and powdered. Powder holding 25 mg TGP was extracted with methanol and filtered after sonication. The resulting solutions were mixed with IS solution and then injected into the chromatographic system after mixing with the IS, and the calculations were done as explained for the pure API.

RESULTS AND DISCUSSION

Method Development and Optimization

A new reverse-phase liquid chromatographic method was developed and validated for the determination of TGP in the presence of IS (EPL:10 µg/mL) using methanol and formic acid mixture. Initially, the method was optimized for the column suitability with the selected mobile phase, and in this study, Agilent column was proved to be suitable in terms of system suitability criteria. Tailing was reported when the mobile ratio was 30:70, 35:65, and 40:60 with a flow rate of 0.4 ml/min. Later, the mobile phase mixture was continued with 75:25 (methanol:formic acid) by which TGP was eluted at 5.099 ± 0.003 min and EPL at 8.535 ± 0.006 min with theoretical plates above 2000 (>6500 for TGP and >12,300 for EPL) and resolution >2.0 (Resolution 2.575). The optimized chromatographic conditions are shown in Table 1. The characteristic chromatograms of TGP and its IS were shown in Figures 2 and 3.

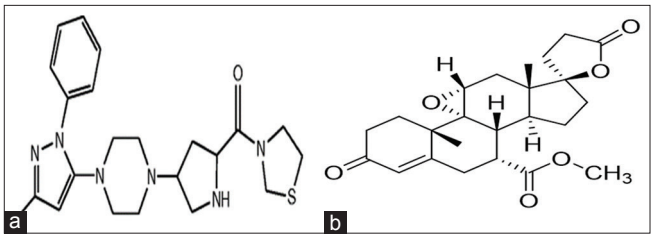

Figure 1: Chemical structure of (a) Teneligliptin and (b) Eplerenone

Table 1: Optimized conditions for the determination of TGP

Parameter	Optimized chromatographic conditions
Mobile phase	Methanol:formic acid (75:25 v/v)
Flow rate	0.4 mL/min
Detection range	244 nm
Retention time	5.099 min; 8.235 min (EPL)

TGP: Teneligliptin, EPL: Eplerenone

Table 2: Linearity of TGP

Concentration (µg/mL)	*Mean peak area		*Mean peak area ratio
	TGP	EPL	TGP/EPL
0	0		0
0.1	8493	1718123	0.0049
0.5	42451	1718325	0.0247
1	83789	1718068	0.0488
2	165295	1718026	0.0962
5	421584	1718247	0.2454
10	835774	1718374	0.4864
20	1651293	1718298	0.961
50	4316986	1718418	2.5122
100	8381694	1718321	4.8778

*Mean of three replicates. TGP: Teneligliptin, EPL: Eplerenone

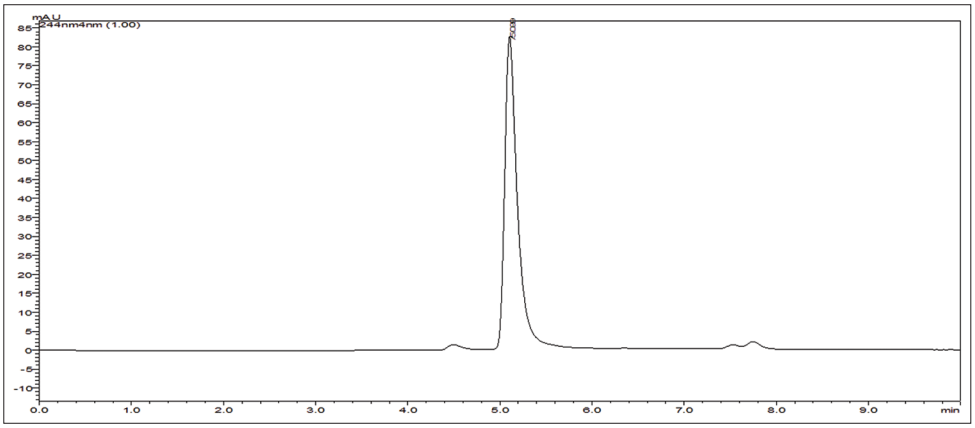

Figure 2: Characteristic chromatogram of teneligliptin (10 µg/mL) (Rt = 5.099 min; theoretical plates: 6550; tailing factor: 1.748)

Method Validation

TGP has shown linearity 0.1–100 µg/mL [Table 2] with linear regression equation, y = 0.049x + 0.0013 (R^2 = 0.9998) [Figure 4]. The LOD and LOQ were found to be 0.00273 and 0.08394 µg/mL. The precision studies have shown RSD as 0.0851–0.275 (intraday) [Table 3] and 0.087–0.276 (interday) [Table 4], and accuracy studies have shown 0.29–1.02 with percentage recovery 97.93–99.7 [Table 5].

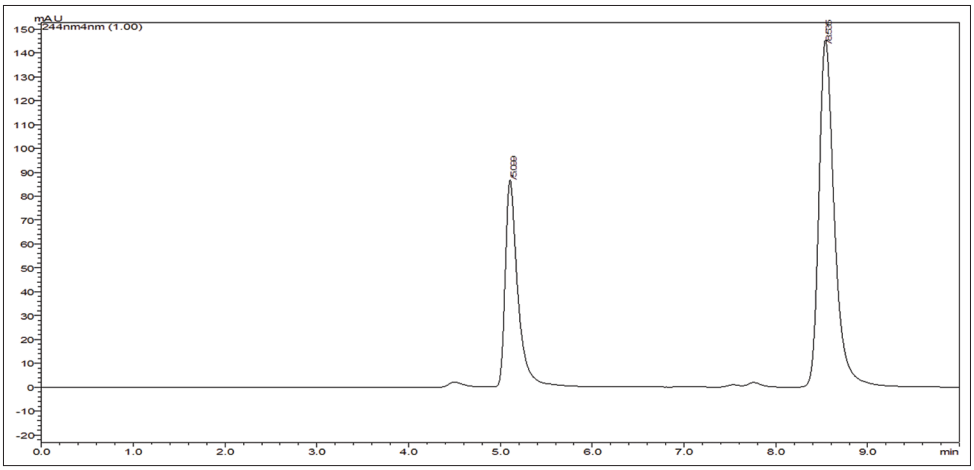

Figure 3: Characteristic chromatogram of teneligliptin (TGP) in the presence of eplerenone (EPL) (internal standard) (10 µg/mL) TGP (Rt: 5.099 min; theoretical plates: 6816; tailing factor: 1.743) EPL (Rt: 8.535 min; theoretical plates: 12357; tailing factor: 1.428)

Table 3: Intraday precision study

Concentration (µg/mL)	*Mean peak area		*Mean peak area ratio	% RSD
	TGP	EPL	TGP/EPL	
2	165562	1717985	0.0964	0.275
2	164956	1719756	0.0959	
2	164982	1718561	0.0960	
5	422156	1718247	0.2457	0.085
5	421965	1719552	0.2454	
5	421012	1716563	0.2453	
10	835692	1719355	0.4861	0.197
10	836259	1717965	0.4868	
10	838651	1718538	0.4880	

*Mean of three replicates. TGP: Teneligliptin, EPL: Eplerenone, RSD: Relative standard deviation

Table 4: Interday precision study

Concentration (µg/mL)	*Mean peak area		*Mean peak area ratio	% RSD
	TGP	EPL	TGP/EPL	
2 (day 1)	165482	1718945	0.0963	0.276
2	164895	1719821	00959	
2	164785	1713659	0.0962	
5 (day 1)	422264	1717986	0.2456	0.087
5	421786	1718452	0.2455	
5	421326	1716103	0.2455	
10 (day 1)	835584	1718321	0.4863	0.195
10	836351	1716235	0.4873	
10	838459	1718324	0.4879	

*Mean of three replicates. TGP: Teneligliptin, EPL: Eplerenone, RSD: Relative standard deviation

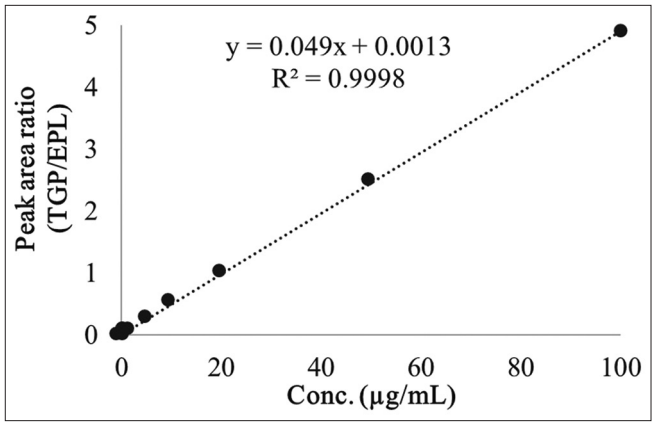

Figure 4: Calibration of teneligliptin in the presence of eplerenone

Assay of TGP Tablets

TGP tablets have shown 99.30–99.70 [Table 6] of recovery, and the characteristic chromatogram observed during the assay of TGP tablets is shown in Figure 5. Interference of excipients was not observed.

CONCLUSION

The present liquid chromatographic method for the quantification of TGP is simple and economical. The reversed-phase HPLC method was validated as per the ICH guidelines and can be used for the assay of tablets.

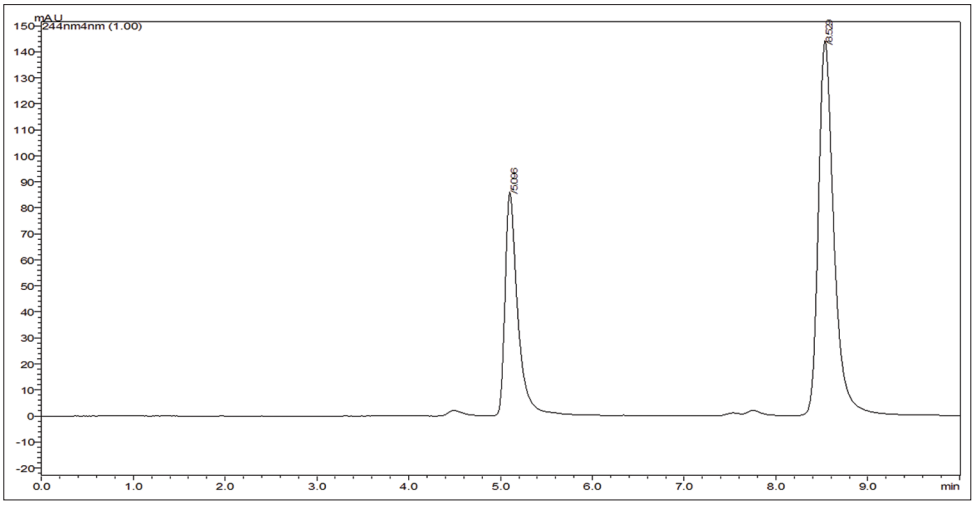

Figure 5: Characteristic chromatogram of teneligliptin tablet in the presence of eplerenone (internal standard) (10 µg/mL) TGP (Rt: 5.096 min; theoretical plates: 6669; tailing factor: 1.748) EPL (Rt: 8.529 min; theoretical plates: 12348; tailing factor: 1.427)

Table 5: Accuracy study

*Concentration (µg/mL)				% Recovery
Formulation	Pure drug	Total	Obtained (% RSD)	
10	5	15	14.69 (0.29)	97.93
10	5	15		
10	5	15		
10	10	20	19.94 (0.76)	99.70
10	10	20		
10	10	20		
10	15	25	24.79 (1.02)	99.16
10	15	25		
10	15	25		

*Mean of three replicates, RSD: Relative standard deviation

Table 6: Assay of TGP tablets

Tablet brands	Label claim (mg)	*Amount found (mg)	*Recovery(%)
I	20	19.91	99.55
II	20	19.86	99.30
III	20	19.94	99.70

*Mean of three replicates TGP: Teneligliptin

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