RESEARCH ARTICLE

Simultaneous Determination of Metformin and Teneligliptin by Liquid Chromatography in tablets

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Abstract

Introduction: Metformin and Teneligliptin are used for the treatment of diabetes. A new liquid chromatographic method has been developed for the simultaneous determination of Metformin and Teneligliptin and the method was validated. **Materials and Methods:** Shimadzu Model CBM-20A/20 Alite, Ultrafast liquid chromatographic system with C8 Phenomenex column (250 mm × 4.6 mm i.d., 5 µm particle size) and PDA detector was used for the simultaneous determination of Metformin and Teneligliptin. A mixture of methanol and formic acid was used as mobile phase with a flow rate of 0.5 ml/min (UV detection at 210 nm). **Results and Discussion:** Metformin and Teneligliptin have shown linear regression equations, y = 159069.66x - 139280.39 (R^2 = 0.9998) and y = 4863932.73x - 12592.06 (R^2 = 0.9999). The LOD and LOQ are found to be 0.7631 µg/mL and 2.3147 µg/mL for Metformin and 0.0029 µg/mL and 0.0084 µg/mL for Teneligliptin respectively. **Conclusions:** The present liquid chromatographic method is precise, accurate and can be used for the simultaneous estimation of Meformin and Teneligliptin tablets

Key words: Isocratic mode, metformin, reverse phase ultra-fast liquid chromatography, teneligliptin, validation.

INTRODUCTION

Metformin (MTF) is a biguanide derivative acting as an antidiabetic.[1] Teneligliptin (TGL) also acts as an antidiabetic by increasing the insulin secretion from the pancreas. The combination of these two drugs provides good control over diabetic patients.[2] A review was given by Manish *et al*., regarding the chemistry and pharmacological activity of MTF and TGL in combined dosage forms.[3] Only one spectrophotometric method[4] and some liquid chromatographic (LC) methods have been developed so far for the determination of MTF and TGL[5-7] and in the present study the authors have proposed a new validated[8] reverse phase ultra-fast LC (RP-UFLC) method for the simultaneous determination of MTF and TGL in tablets [Figure 1].

Chemicals and reagents

The combination of MTF and TGL is available with brand names Tenglyn M 500 (Label claim: TGL 20 mg and MTF 500) (Zydus Cadila (India), Tenglyn M 1000 (Label claim: TGL 20 mg and MTF 1000), and Afoglip M (Torrent Pharma) as tablets. Stock solutions of MTF and TGL were prepared by dissolving 25 mg of MTF and TGL in a 25 mL volumetric flasks separately with high-performance liquid chromatography (HPLC) grade methanol (1000 µg/mL) and diluted with mobile phase as per requirement.

Method validation

MTF (2.5–200 µg/mL) and TGL (0.1–2.0 µg/mL) solutions were prepared from their stock solutions, and 20 µL of each solution were injected into the LC system, and the

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peak area of the chromatograms was observed. Calibration graph was plotted using a concentration of MTF and TGL on the X-axis and the mean peak area on the Y-axis separately. Intraday and interday precision was studied at different levels for MTF and TGL on the same day and on 3 consecutive days, respectively. The accuracy of the method was proved from their recovery values robustness was studied by incorporating small changes in their method optimized parameters.

Assay of commercial formulations

A total of 20 tablets of combined dosage forms of MTF and TGL of different brands were procured, powdered and extracted with methanol, sonicated for 30 min and filtered, and the filtrate was diluted with mobile phase 20 µL of this combined dosage form solution of each brand was injected into the UFLC system, and the peak area of the two drugs was noted from the chromatogram.

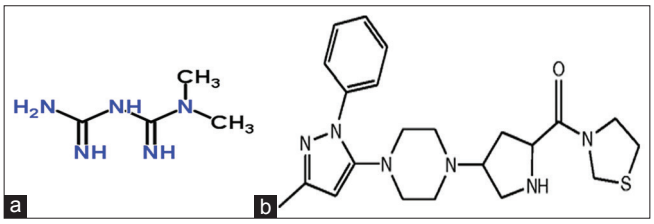

Figure 1: Chemical structure of (a) metformin (b) teneligliptin

RESULTS AND DISCUSSION

Method development and optimization

A new RP-UFLC method has been developed and validated for the determination of MTF and TGL in tablets. A comparative study of the previously established methods in the literature with the present proposed method has been shown in Table 1. Initially, C8 Phenomenex column was used with mobile phase composition Formic acid: methanol (25:75, v/v) with flow rate 0.6 mL/min with ultraviolet (UV) detection at 244 nm and only MTF was eluted at 3.362 min but TGL was not eluted, and the system suitability parameters were not within acceptable criteria. Therefore, the flow rate was changed to 0.5 mL/min, and both MTF and TGL were eluted at 4.024 min and 6.234 min, respectively, with UV detection at 210 nm [Figure 2]. The method optimized conditions were shown in Table 2.

Method validation

The method was validated as per the ICH guidelines.

MTF and TGL obey Beer-Lamberts law 0.01–2.0 and 2.5–200 µg/mL [Table 3], respectively. MTF and TGL have shown linear regression equations, y = 159069.66x -139280.39 (R^2 = 0.9998) and y = 4863932.73x -12592.06 (R^2 = 0.9999), respectively. The limit of detection and limit of quantification are found to be 0.7631 µg/mL and 2.3147 µg/mL for MTF and 0.0029 µg/mL and 0.0084 µg/mL for TGL, respectively.

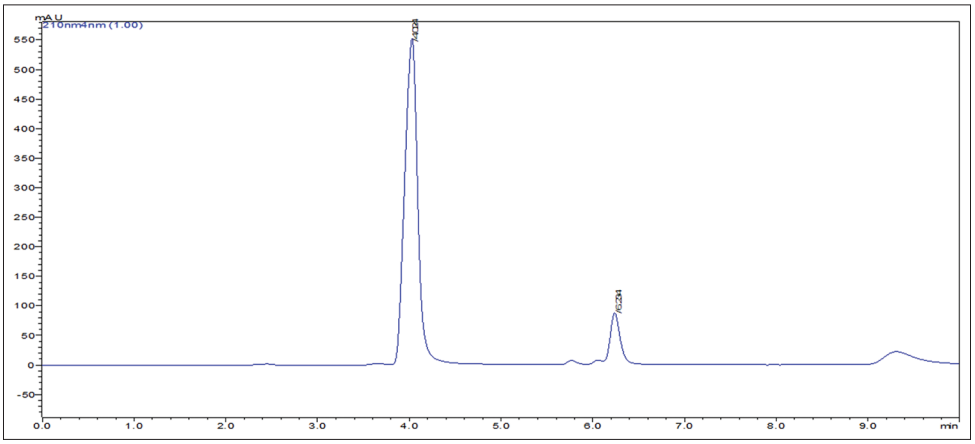

Figure 2: Typical chromatograms of metformin (Rt 4.024 min) and teneligliptin (Rt 6.234 min)

Table 1: Comparison of the previously reported methods with the present method

Mobile phase/reagent	Method	Reference
Methanol	Spectrophotometry	[4]
Methanol and water (pH 3.5) (50:50 v/v)	RP-HPLC	[5]
Methanol: water (pH 3) (70:30 v/v)	RP-HPLC	[6]
Methanol: water 0.05% OPA (50:50 v/v)	RP-HPLC	[7]
Methanol: formic acid: acetic acid (75: 25: 0.1 v/v)	RP-HPLC	Present work

RP-HPLC: Reversed-phase high-performance liquid chromatography

The percentage relative standard deviation in intraday [Table 4], interday [Table 5] precision and accuracy [Table 6] (% recovery 97.50–99.44 for TGL and 99.64–99.70 for MTF) was found to be <2.0% indicating that the method is precise and accurate. The theoretical plates were found to be 3466 and 13534 for MTF and TGL (>2000), respectively, whereas the tailing factor is found to be <1.5 in all the chromatographic observations [Figure 3].

Table 2: Optimized conditions for determination of MTF and TGL

Parameter	Optimized chromatographic conditions
Mobile phase	Methanol: formic acid: acetic acid (75:25:0.1, v/v)
Flow rate	0.5 mL/min
Detection range	210 nm
Retention time	MTF (4.024±0.02 min) and TGL (6.234±0.03 min)

MTF: Metformin, TGL: Teneligliptin

Table 3: Linearity of MTF and TGL

Conc (µg/mL)		*Mean peak area		% RSD	
TGL	MTF	TGL	MTF	TGL	MTF
0.01	2.5	46559	353162	0.12	0.65
0.02	5	96678	779121	0.15	0.59
0.05	12.5	255835	1565347	0.26	0.57
0.1	25	482990	3830671	0.19	0.53
0.2	50	979793	7882297	0.28	0.61
0.4	100	1946151	15622751	0.36	0.69
0.6	150	2830159	23483193	0.24	0.56
0.8	200	3825735	31922898	0.18	0.52
1	-	4841256	-	0.31	-
1.5	-	7256241	-	0.26	-
2	-	9778569	-	0.24	-

*Mean of three replicates. % RSD: % Relative standard deviation, MTF: Metformin, TGL: Teneligliptin

Assay of metformin and teneligliptin commercial formulations (Tablets)

The combined dosage form containing MTF and TGL has shown 98.95–99.9 and 99.54–99.79 recovery, respectively [Table 7], in the tablet formulations.

CONCLUSIONS

The present study gives a new validated[8] RP-UFLC method for the simultaneous determination of Metformin and Teneligliptin in tablets, and this method can be applied for the kinetic studies also *in vitro* as well as *in vivo* studies.

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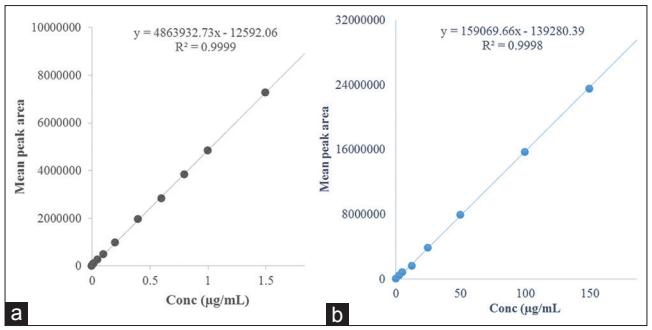

Figure 3: Calibration curve of (a) teneligliptin (b) metformin

Table 4: Intraday precision study of MTF and TGL

Conc. (µg/mL)		Statistical analysis	
		*Mean peak area±SD (% RSD)	
TGL	MTF	TGL	MTF
0.2	2.5	976886.33±3129.29 (0.32)	354512±2587.94 (0.73)
0.2	2.5		
0.2	2.5		
0.4	25	1947365±1716.86 (0.09)	3835647±31068.74 (0.81)
0.4	25		
0.4	25		
0.6	50		
0.6	50	2830239±18962.60 (0.67)	7884568±72538.03 (0.92)
0.6	50		

*Mean of three replicates. % RSD: % Relative standard deviation, MTF: Metformin, TGL: Teneligliptin

Table 5: Interday precision study of MTF and TGL

Conc (µg/mL)		Statistical analysis	
		*Mean peak area±SD (% RSD)	
TGL	MTF	TGL	MTF
0.2	2.5	977589.33±2052.94 (0.21)	354985±2946.38 (0.83)
0.4	25	1948456.29±12175.28 (0.63)	3835368±34134.78 (0.89)
0.6	50	2831502.43±20103.67 (0.71)	7889654±56016.54 (0.81)

*Mean of three replicates. % RSD: % Relative standard deviation, MTF: Metformin, TGL: Teneligliptin

Table 6: Accuracy study of MTF and TGL

Conc. (µg/mL)				*Total found		% RSD		% Recovery	
Formulation		Pure drug		Conc. (µg/mL)					
TGL	MTF	TGL	MTF	TGL	MTF	TGL	MTF	TGL	MTF
0.2	5	0.16	4	0.358	8.97	0.78	0.63	99.44	99.67
0.2	5	0.16	4						
0.2	5	0.16	4						
0.2	5	0.2	5	0.39	9.97	0.85	0.81	97.50	99.70
0.2	5	0.2	5						
0.2	5	0.2	5						
0.2	5	0.24	6	0.43	10.96	0.91	1.03	97.73	99.64
0.2	5	0.24	6						
0.2	5	0.24	6						

*Mean of three replicates. % RSD: % Relative standard deviation, MTF: Metformin, TGL: Teneligliptin

Table 7: Assay of MTF and TGL tablets

Formulation	Label claim (mg)		*Amount found (mg)		*Recovery (%)	
	TGL	MTF	TGL	MTF	TGL	MTF
Brand I	20	500	19.96	498.95	99.8	99.79
Brand II	20	500	19.98	497.68	99.9	99.54
Brand III	20	500	19.79	498.91	98.95	99.78

* Mean of three replicates. MTF: Metformin, TGL: Teneligliptin

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