RESEARCH ARTICLE

Stability indicating ultrafast liquid chromatographic method for the estimation of Teneligliptin (An Anti-diabetic agent)

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Abstract

Introduction: Teneligliptin is used for the treatment of diabetes. It is generally used to reduce hyperglycemia. A new stability-indicating reversed-phase high-performance liquid chromatography method has been developed for the determination of teneligliptin in tablets and the method was validated. **Materials and Methods:** Teneligliptin was analyzed using formic acid:methanol:acetic acid mixture (25:75:0.1, v/v) as mobile phase with a flow rate of 0.4 mL/min (UV detection at 245 nm). Teneligliptin was exposed to different stress conditions and the chromatographic study was continued for its stability. **Results and Discussion:** Teneligliptin has shown linearity 1–100 µg/mL with regression equation, y = ×95722–6775.4 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. **Conclusions:** It is observed that this reverse-phase ultrafast liquid chromatographic method is accurate and precise and can be used for the estimation of teneligliptin tablets.

Key words: Isocratic mode, reverse-phase ultrafast liquid chromatography, stability indicating, teneligliptin, validation

INTRODUCTION

Teneligliptin [Figure 1] belongs to dipeptidyl peptidase-4 inhibitors.[1] Significant decrease in blood glucose levels was observed in patients taking teneligliptin for 12 weeks.[2,3] Teneligliptin is approved for the treatment of type 2 diabetes mellitus in India, Japan, and Korea in 2012.[4] Halabi *et al*. studied the metabolism and pharmacokinetic studies[5] of teneligliptin in patients with renal impairment. Analytical techniques such as spectrophotometry,[6-10] reversed-phase high-performance liquid chromatography (RP-HPLC),[11-13] ultra-performance liquid-chromatography–mass spectrometry (MS)[14] LC–MS/MS,[15] LC–MS,[16] and high-performance thin-layer chromatographic[17,18] methods were reported till now and only one stability-indicating RP-HPLC[19] method has been reported so far. The authors have developed a new HPLC method for the determination of teneligliptin in tablets and validated as per ICH guidelines.[20]

MATERIALS AND METHODS

Chemicals and reagents

Teneligliptin was procured from Zydus Cadila (India). Teneligliptin tablets are available with brand names - Ziten (Glenmark Pharmaceuticals), Zita Plus (Glenmark), Tenglyn (Zydus Cadila), and Eternex T (Alembic Pharma) with label claim 20 mg. All other chemicals are of AR grade and all solvents are of HPLC grade. Stock solution of teneligliptin was prepared by dissolving 25 mg of teneligliptin in a 25 mL volumetric flask with HPLC-grade methanol (1000 µg/mL),

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diluted with mobile phase and filtered through membrane filter.

Optimized chromatographic conditions

Chromatography study was performed on isocratic mode using a mixture of 0.1% formic acid and methanol:0.1% acetic acid (25:75:0.1, v/v) as mobile phase with flow rate of 0.4 mL/min (UV detection at 245 nm).

Method validation and assay of formulations

Different diluted solutions (1–100 µg/mL) were prepared from the stock and injected into the ultrafast liquid chromatographic (UFLC) system, and the peak area of the chromatogram was noted and calibration curve was plotted. Precision and accuracy experiments were performed and the recovery values were determined. The proposed method was checked for the robustness by changing the optimized chromatographic conditions.

20 tablets of available marketed formulations of different brands were procured, powdered, powder containing 25 mg teneligliptin was extracted with methanol, sonicated, and filtered, and solution from each brand was injected into the UFLC system and peak areas were noted from the respective chromatograms.

Forced degradation studies

Forced degradation studies[21] were performed by exposing teneligliptin to different stress conditions such as acidic hydrolysis, basic hydrolysis, and oxidation. Acidic degradation was performed by treating the drug solution with 0.1 N HCl for 30 min at 60°C in a thermostat, and later, the solution was cooled, neutralized using sodium hydroxide solution, and the solution was made up to volume to the required concentration with the mobile phase. Similarly, alkaline degradation was performed by treating the drug solution with 0.1 N NaOH for 30 min at 60°C in a thermostat, and later, the solution was cooled, neutralized using hydrochloric acid solution, and the solution was made up to volume to the required concentration with the mobile phase. Oxidative degradation was performed by treating the drug solution with 30% v/v H_2O_2 at 60 in the thermostat for 30 min.

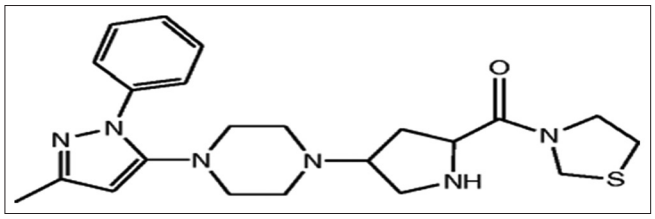

Figure 1: Chemical structure of teneligliptin

RESULTS AND DISCUSSION

Method development and optimization

A simple stability-indicating reverse-phase ultrafast liquid chromatography method has been chosen for the determination of teneligliptin. Mobile phase containing formic acid:methanol (25:75, v/v) with flow rate of 0.5 mL/min was used initially, but theoretical plates were very low, i.e., 1400 (<2000). Small amount of acetic acid was incorporated into the mobile phase and thereby peak tailing was completely avoided [Table 1]. Mixture of formic acid:methanol:acetic acid (25:75:0.1, v/v) with flow rate of 0.4 mL/min was found to be more appropriate to satisfy the system suitability parameters, and the optimized chromatographic conditions were shown in Table 2. Teneligliptin was eluted at 4.982 min [Figure 2].

Method validation

The proposed method was validated by linearity, precision, accuracy, and robustness as per the ICH guidelines. The calibration curve was drawn by taking concentration of teneligliptin on x-axis and the corresponding mean peak area values on the y-axis. Teneligliptin obeys Beer–Lamberts law over the

Table 1: Method optimization

Trails	Column	Mobile phase (v/v)	Flowrate (mL/min)	Rt (min)	Comments	Figure
1	C8 Phenomenex	25:75	0.5	4.060	Theoretical plates <2000 tailing	2A
2	C8 Phenomenex	25:75	0.4	5.056	Theoretical plates <2000 tailing	2B
3	C8 Phenomenex	30:70	0.4	5.564	Theoretical plates <2000 tailing factor >2	2C
4	C8 Phenomenex	35:65	0.4	5.857	Theoretical plates <2000 tailing factor >2	2D
5	C8 Phenomenex	35:65	0.4	5.786	Theoretical plates <2000 tailing factor >2	2E
6	C8 Phenomenex	35:65:Acetic acid	0.4	5.584	Theoretical plates <2000 tailing factor >2	2F
7	C8 Phenomenex	30:70:Acetic acid	0.4	5.415	Theoretical plates <2000	2G
8	C8 Phenomenex	25:75:Acetic acid	0.4	4.982	Theoretical plates 6337 method optimized	2H

Table 2: Optimized conditions for determination of teneligliptin

Parameter	Optimized chromatographic conditions
Mobile phase	Formic acid: methanol:acetic acid (25:75:0.1,v/v)
Stationary phase	C_8 (phenomenex) column (250 mm×4.6 mm i.d., 5 µm particle size)
Flow rate	0.4 mL/min
Detection range	245 nm
Column temp.	(25° ± 2°C)
Injection volume	20 µL
Detector	SPD M20A prominence photodiode array detector
Elution	Isocratic mode
Retention time	4.982±0.02 min
Total run time	10 min

concentration range of 1–100 µg/mL [Table 3] with regression equation, y = 95722x – 6775.4 [Figure 3] 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. The percentage RSD in intraday and interday was found to be 0.03–0.15 and 0.02–0.12, respectively, indicating that the method is precise [Tables 4 and 5]. The percentage RSD in accuracy study was found to be 0.89–1.06 with a recovery of 98.67–99.88% [Table 6]. The system suitability parameters are within the acceptable criteria.

Assay of teneligliptin commercial formulations (tablets)

Teneligliptin has shown 99.56–99.78 [Table 7] recovery in the marketed formulations and the chromatogram obtained in one of the marketed formulations was shown in Figure 4.

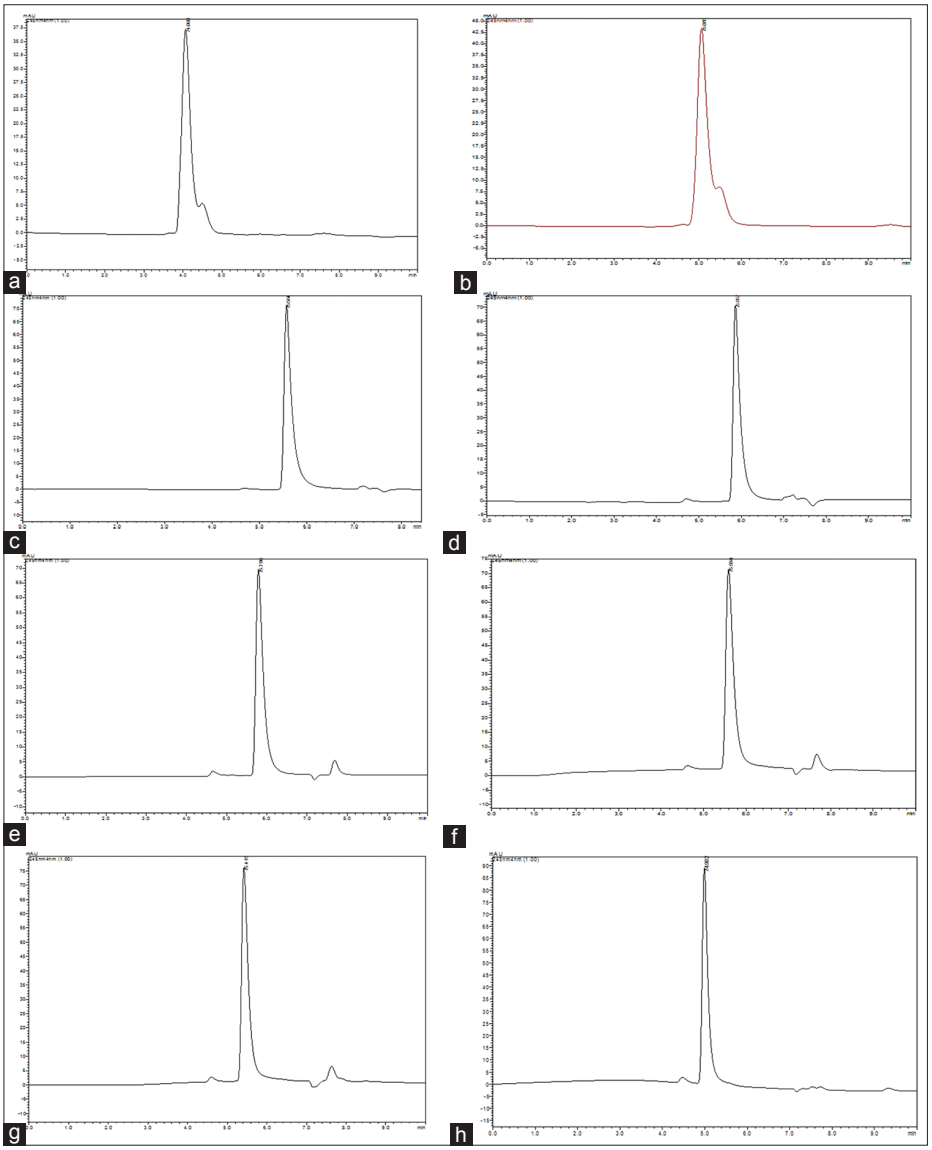

Figure 2: (a-h) Chromatograms of teneligliptin observed during method optimization

Table 3: Linearity of teneligliptin

Concentration (µg/mL)	*Mean peak area	% RSD
1	93890	0.16
5	476525	0.10
10	934022	0.23
20	1913065	0.12
50	4800673	0.61
80	7569461	0.87
100	9620341	0.22

*Mean of three replicates

Table 4: Intraday precision study of teneligliptin

Concentration (µg/mL)	*Mean peak area	Statistical analysis *Mean±SD (% RSD)
20	1913065	1916472±2781.39 (0.15)
20	1919878	
20	1913456	
50	4800673	4804979±3331.24 (0.07)
50	4808787	
50	4805478	
100	9620341	9623987±2578.70 (0.03)
100	9625842	
100	9625780	

*Mean of three replicates

Stress degradation studies

Teneligliptin peak was totally destroyed in alkaline and acidic hydrolysis, and it may be due to the carbonyl moiety and the heterocyclic moiety present in the chemical structure of teneligliptin, respectively. During oxidation and hydrolysis, <5% degradation was observed. In all the degradation studies, it was found that the drug peak was well separated in the presence of degradation conditions indicating that the method is selective and specific. The system suitability parameters were well in the acceptance criteria [Table 8]. The individual chromatograms, as well as the 3D chromatograms

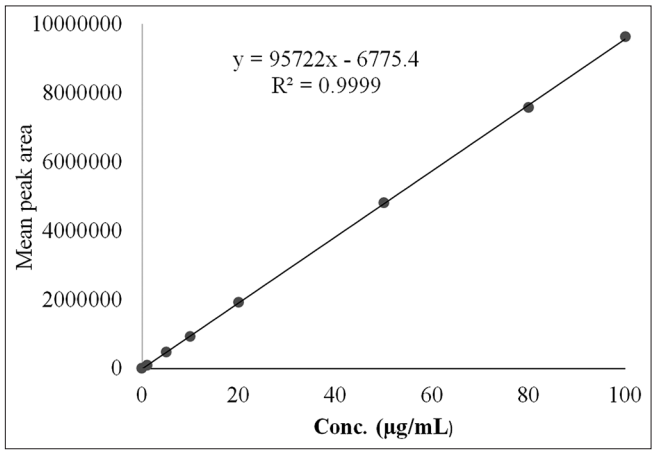

Figure 3: Calibration of teneligliptin

Table 5: Interday precision study of teneligliptin

Concentration (µg/mL)	*Mean peak area			*Mean±SD (% RSD)
	Day 1	Day 2	Day 3	
20	1913489	1918543	1917865	1916632±2239.84 (0.12)
50	4801258	4809087	4801253	4803866±3691.80 (0.08)
100	9629878	9625467	9629076	9628140±1918.47 (0.02)

*Mean of three replicates

Table 6: Accuracy study of teneligliptin

Concentration (µg/mL)			*Mean (%RSD)	% recovery
Formulation	Pure drug	Total		
20	16	36	35.5193 (0.89)	
20	16	36		98.67
20	16	36		
20	20	40	39.956 (0.91)	
20	20	40		99.88
20	20	40		
20	24	44	43.823 (1.05)	
20	24	44		99.59
20	24	44		

*Mean of three replicates

Table 7: Assay of teneligliptin tablets

Formulation	Label claim (mg)	*Amount found (mg)	*Recovery (%)
Brand I	20	19.91	99.56
Brand II	20	19.96	99.78
Brand III	20	19.93	99.65

*Mean of three replicates

observed during the stress degradation studies, were shown in Figures 4 and 5, respectively.

CONCLUSIONS

The proposed stability-indicating method for the determination of teneligliptin is more economical. Teneligliptin is known to

Table 8: Stress degradation studies of teneligliptin

Stress condition medium/temp.	Rt (min)	% recovery	% drug degradation	Theoretical plates	Tailing factor
Standard drug	4.982	100	-	6337	1.881
Acidic hydrolysis 0.1 N HCl/60°C	5.911,6.389	-	-	9841986	-
Alkaline hydrolysis 0.1 N NaOH/60°C	5.912, 6.389	-	-	12291849	-
Oxidation 30% H_2O_2	5.088	96.69	3.31	4595	1.940
Hydrolysis H_2O/60°C	5.113	99.89	0.11	4052	2.182

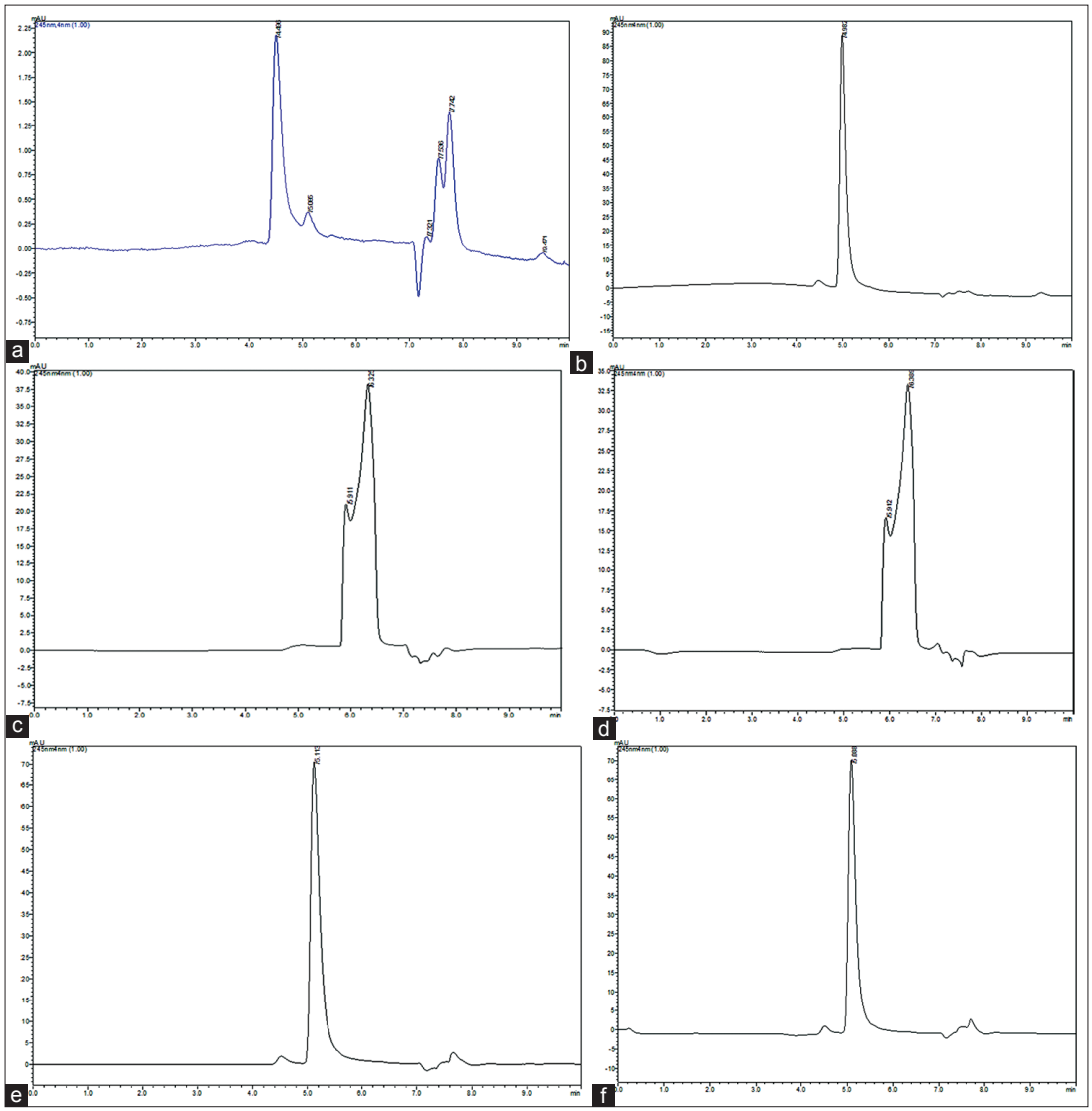

Figure 4: Typical chromatograms of teneligliptin (10 µg/mL) (a) blank (b) formulation (tablets) (c) acidic hydrolysis (d) alkaline hydrolysis (e) hydrolysis (f) oxidation

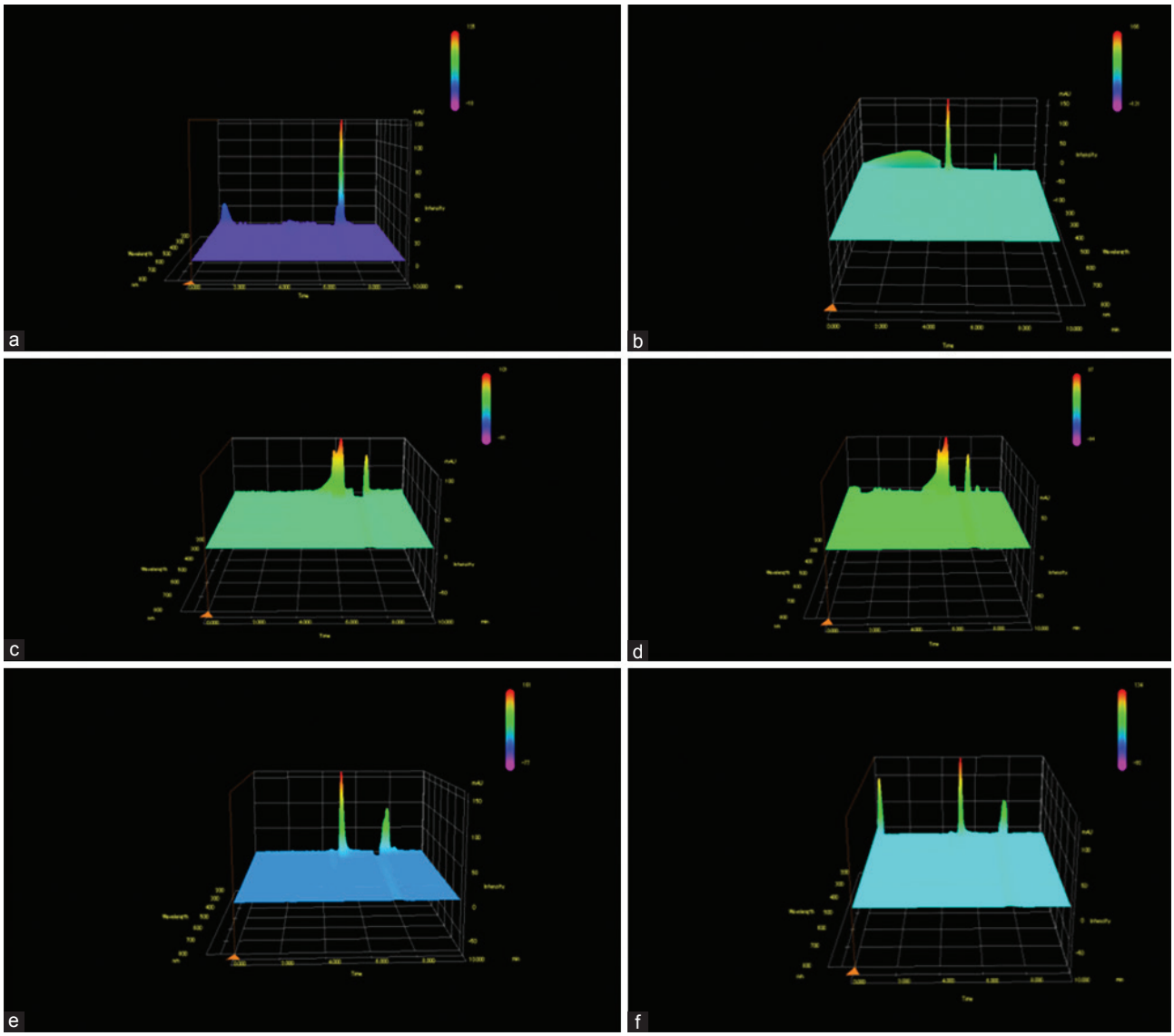

Figure 5: 3D chromatograms of teneligliptin (10 µg/mL) (a) blank (b) standard (c) acidic hydrolysis (d) alkaline hydrolysis (e) hydrolysis (f) oxidation

be more sensitive toward acidic as well as basic environment, and the method can be satisfactorily applied for the determination of teneligliptin tablets.

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