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The Development of RP-HPLC Method For Simultaneous Quantitation of Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride and Metformin hydrochloride in Bulk and Its Tablet formulation

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ABSTRACT:

Recently, a newly anti-diabetic tablet formulation of Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride and Metformin hydrochloride was approved by CDSCO. Following ICH guidelines, the RP-HPLC method has been designed and validated for simultaneous estimation of Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride and Metformin hydrochloride in bulk and recently approved triple FDC. These 3 drugs were easily separated by 0.025 M KH2PO4 Buffer: Methanol: Acetonitrile (50:25:25 % v/v/v) at pH 3 at 225 nm wavelength and 1mL/min flow rate. The linear graph was obtained for 2-6 µg/mL- Teneligliptin hydrobromide hydrate, 1.5- 4.5 µg/mL for Pioglitazone hydrochloride and 50-150 µg/mL for Metformin hydrochloride. % Relative standard deviation was less than 2% for precision. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.021 and 1.159 µg/mL for Teneligliptin hydrobromide hydrate, 4.718 and 14.298 µg/mL for metformin hydrochloride and 0.417 and 1.189 µg/mL for Pioglitazone hydrochloride.

KEYWORDS: RP-HPLC, Degradation study, Teneligliptin hydrobromide hydrate, Metformin hydrochloride and Pioglitazone hydrochloride.

INTRODUCTION [1-4]

Diabetes is a condition in which a patient has a high blood sugar level. It is produced when the body is not reacting to the effects of insulin appropriately or when the pancreas produces very little or no insulin at all. Any age people can be affected by diabetes. This disease can be treated by medicine and /or lifestyle modifications, but mostly it is a chronic disease. Anti-diabetic drugs can be categorized into two classes:

A. Oral anti-diabetic drugs: This includes the following classes: sulphonylureas and non-sulphonylureas (Glinides/Meglitinide), Biguanides, Thiazolidinediones, α-glucosidase inhibitors, Di-peptidyl Peptidase-4 (DPP-4) inhibitors/gliptins, Sodium-glucose co-transporter 2 (SGLT2) inhibitors

B. Injectable anti-diabetic drugs: Insulin preparations Glucagon-like peptide 1 (GLP1) agonists.

Metformin hydrochloride is the medication of choice for all type-2 diabetes mellitus unless it is contraindicated or intolerable.

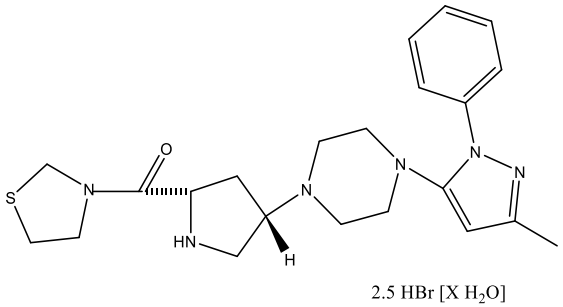

Figure 1 Structure of Teneligliptin hydrobromide hydrate.

The chemical name of Teneligliptin hydrobromide hydrate (TEN) is [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3-yl)piperazin-1-yl]pyrrolidin-2-yl]-(1,3-thiazolidin-3-yl) methanone; hydrate; pentahydrobromide. A chemical formula is C22H30N6OS, 2.5HBr, XH2O. Pioglitazone hydrochloride (PIO) has the chemical formula C19H21ClN2O3S. and its chemical name is 5-[[4-[2-(5-ethyl pyridine-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione;hydrochloride. Metformin hydrochloride is chemically 3-(diaminomethylidene)-1,1-dimethylguanidine; hydrochloride. Its chemical formula is C4H12ClN5. Fig. 1, 2 and 3 show the chemical structures of TEN, PIO and MET respectively. In Indian pharmacopoeia, all three drugs are official. [5-7]

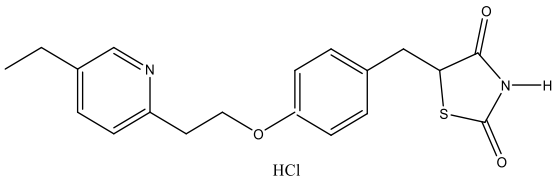

Figure 2 Pioglitazone hydrochloride structure

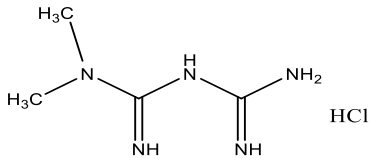

Figure 3 Metformin hydrochloride structure

A combination of TEN, PIO and MET was recently approved by the FDA. The literature review leads to the conclusion that different methods like HPLC, HPTLC, UV, LC/MS/MS were reported for determining TEN, PIO and MET in either a single drug or in combination with other drugs. There was only one method available to quantitatively determine them simultaneously in its combination. [8-20]

Materials and methods

Materials, reagents and pharmaceutical formulation

TEN, PIO and MET standards were acquired from reputed pharmaceutical companies in Gujarat as gift samples. HPLC-grade acetonitrile, methanol, and water were purchased from E. Merck chem. Limited, Mumbai. All other required chemicals were of analytical grade (CYNOR Pharma Private Limited).

Instrumentation

As a chromatographic system, Agilent 1200 infinity II LC was used as a High-Performance liquid chromatograph equipped with a 1260 Quat Pump VL and PDA detector. Sapphires Column C18 (250mm ×4.6mm, 5 µm particle size) and EZ Chrome software was used for data acquisition and integration.

Mobile phase selection and optimization

A combination of 0.025 M KH2PO4 Buffer, Methanol and Acetonitrile in the ratio of 50:25:25 % v/v/v pH 3 adjusted by 1% Orthophosphoric acid at 1 mL/min flow rate gave better separation with quantification and having nice peak shape, high no. of theoretical plates and asymmetry for simultaneous estimation of TEN, PIO and MET in its triple FDC. Figure 4 displays the HPLC chromatogram of TEN, PIO and MET at this optimized condition.

Selection of detection wavelength

At 225 nm, the combination of three drugs produced a satisfactory response.

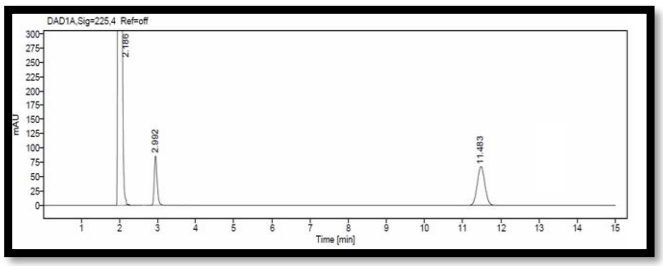

Figure 4 HPLC Chromatogram of Metformin hydrochloride- 1000 µg/mL, Teneligliptin- 40 µg/mL and Pioglitazone hydrochloride- 30 µg/mL in Acetonitrile: methanol: 0.025 M KH2PO4 Buffer (25:25:50 %v/v/v) pH 3 adjusted by OPA (Optimized condition)

Procedure

Preparation of mobile phase

0.025 M KH2PO4 Buffer was prepared by dissolving 1.7 g KH2PO4 in HPLC grade water in 500 mL. 1% orthophosphoric acid was added to adjust pH 3.0 of the solution. A mixture of 500 mL of prepared buffer, 250 mL of Acetonitrile and 250 mL of Methanol and mixed for 5 minutes under sonication produces the final mobile phase. This solution was filtered from 0.45µ Whatman filter paper and was loaded into the system.

Preparation of solutions

15 mg PIO, 20 mg TEN, and 500 mg MET standards were accurately weighed and transferred in different 100 mL volumetric flasks to prepare 150 µg/mL PIO, 200 µg/mL TEN, and 5000 µg/mL MET standard stock solutions respectively. 2mL from all three standard stock solutions was pipette out respectively and transferred to the same 10 mL volumetric flask and volume was made up to the mark with methanol to prepare 30 µg/mL PIO, 40 µg/mL TEN and 1000 µg/mL MET stock solution respectively.

Preparation of sample solution

Twenty tablets were accurately weighed and then the average weight was taken. After that, the finely crushed

tablet powder equivalent to 1.5 mg PIO, 2 mg TEN and 50 mg MET was weighed and transferred to a 100 mL volumetric flask. Then sonicated for 15 minutes and the volume was made up with methanol. The solution was filtered through Whatman filter paper no.41. 2 mL from this sample stock solution was taken and transferred to a 10 mL volumetric flask and made up the volume to the mark with the methanol to get 3 µg/mL PIO, 4 µg/mL TEN and 100 µg/mL MET concentration. 20 µL of this solution was injected for assay analysis.

ICH method validation

As per ICH guidelines, different validation parameters like Accuracy, Precision, Range, Linearity, Limit of detection, System suitability, Robustness, Limit of Quantitation, Specificity and System suitability were checked using 3 µg/mL PIO, 4 µg/mL TEN, and 100 µg/mL MET concentration.

RESULT

Results of method validation

Linearity and Range- Table 1 displays a linear correlation between peak area and concentration. Figures 5-7 show calibration curves of TEN, PIO and MET respectively.

Table 1 Linearity data of TEN, PIO and MET (n=3)

	TEN		PIO		MET	
	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Area of peak	Conc. (µg/mL)	Peak area
1	2	2241.67	1.50	4782.00	50	224795.31
2	3	3132.33	2.25	7149.33	75	340684.23
3	4	4137.66	3.00	9469.33	100	459596.00
4	5	4970.67	3.75	11357.33	125	552682.75
5	6	6244.00	4.50	14269.00	150	676485.00

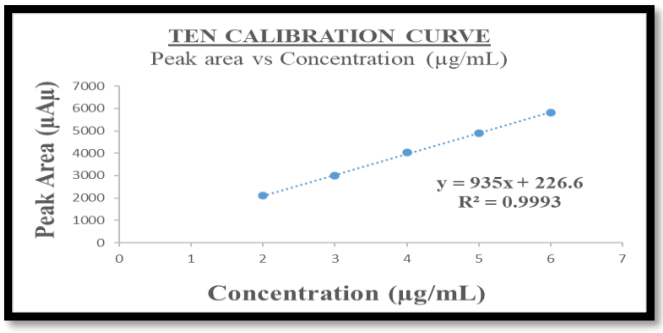

Figure 5 Calibration curve of Teneligliptin hydrobromide hydrate

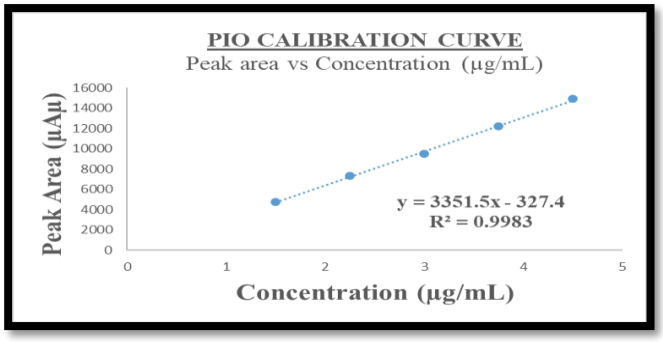

Figure 6 Calibration curve of Pioglitazone hydrochloride

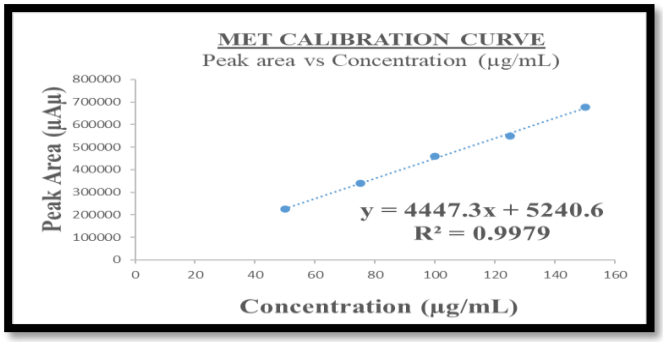

Figure 7 Calibration curve of Metformin hydrochloride.

Accuracy study- % Recoveries were found in a range of 99.35 % -100.06 %, 99.18 % -100.31 % and 99.35 % -100.12% for Metformin hydrochloride, Teneligliptin hydrobromide hydrate and Pioglitazone hydrochloride respectively. Results are given in Table 2.

Table 2 % Recovery data

	TEN		PIO		MET	
No.	**Amount added (µg/mL)**	**% Mean Recovery ± S.D.**	**Amount added (µg/mL)**	**% Mean recovery ± S.D.)**	**Added amount (µg/mL)**	**% Mean Recovery ± S.D.**
1	2	99.64 ± 1.05	1.5	99.22 ± 1.65	50	100.12 ± 0.16
2	4	99.35 ± 0.83	3	99.18 ± 1.44	100	99.91 ± 0.39
3	6	100.06 ± 1.17	4.5	100.04 ± 1.74	150	99.35 ± 1.42

Precision – Tables 3 and 4 represent results of intraday, Interday precision and repeatability.

Table 3 Intermediate precision results (n=3)

Drug	Conc. (µg/mL)	Interday		Intraday	
		Peak area ± S.D.	%R.S.D.	Area of peak ± S.D.	% R.S.D.
TEN	2.0	2215 ± 41.00	1.85	2236 ± 38.16	1.71
	4.0	4085 ± 64.82	1.59	4062 ± 60.86	1.50
	6.0	6255 ± 85.58	1.37	6248 ± 74.65	1.19
PIO	1.5	4704 ± 73.57	1.56	4710 ± 64.39	1.37
	3.0	9497 ±139.57	1.47	9475 ± 125.58	1.30
	4.5	14190 ± 195.11	1.37	14192 ± 178.07	1.25
MET	50	228024 ± 4109.43	1.08	225024 ± 3342.36	1.49
	100	466182 ± 7289.24	1.56	469182 ± 6127.15	1.31
	150	676185 ± 8283.20	1.22	675852 ± 6801.48	1.01

Table 4 Repeatability data (n=6)

	TEN (4µg/ mL)		PIO (3 µg/ mL)			MET (100µg/ mL)		
Area	**Mean ± S.D.**	**% R.S.D.**	**Area**	**Mean ± S.D.**	**% R.S.D.**	**Area**	**Mean ± S.D.**	**% R.S.D.**
4070	4085.50 ±	1.07	9470	9490.67 ±	1.46	463350	46301015 ±	1.40
3993	72.16		9319	138.91		456667	6497.49	
4198			9697			455112		
4124			9527			472621		
4031			9362			466994		
4097			9569			463350		

Limit of Detection (LOD) and Limit of Quantitation(LOQ)- Results are given in Table 5

Specificity- The difference in retention time of reference and sample was found to be ± 0.009 min for TEN, ± 0.008 min for PIO and ± 0.003 min for MET

Table 5 Table 5: Results of LOD and LOQ in µg/mL

	TEN	PIO	MET
LOD	0.473	0.417	4.718
LOQ	1.432	1.189	14.298

Robustness- pH, ratio of mobile phase and flow rate were changed to study robustness. % RSD was found to be less than 2. Results are given in Table 6.

System suitability- Parameters are given in Table 7.

Analysis of a marketed formulation- Table 8 provides a % assay of sample solution.

Table 6 : Robustness results

	%R.S.D. (n=6)					
	Flow rate (mL/min)				pH	Mobile phase (%v/v/v)
Drug	**0.8**	**1.2**	**2.8**	**3.2**	**(23:23:54)**	**(27:27:46)**
TEN	1.27	1.10	0.78	0.90	0.54	0.85
PIO	1.00	1.23	1.13	0.70	1.51	1.01
MET	0.82	1.09	0.36	1.12	0.72	1.40

Method conclusion- Zita-Pio-Met 500 tablet is recently approved triple FDC. So, no methods are available for simultaneous quantification of them. The current research presents an approach dealing with the development of RP-HPLC assay. A cost-effective, specific, simple, and

sensitive method was developed which is suitable for simultaneous determination in the bulk and its tablet formulation. This method has complied with ICH guidelines. This method is also less time-consuming, cost-effective and easy to perform. Thus, it can be applied for regular analysis of the simultaneous determination of TEN, PIO and MET.

Table 7 System suitability

Parameters, (n=3)	TEN	PIO	MET	Standard limits
Asymmetry ± RSD	1.01 ± 0.223	1.03 ± 0.121	1.09 ± 0.181	A_s of ≤ 2
Theoretical Plates ± RSD	4905 ± 0.86	12644 ± 0.27	3549 ± 0.12	>2000
Retention time (min) ± RSD	2.992 ± 0.030	11.483 ± 0.007	2.186 ± 0.002	-
Resolution ± RSD	2.68 ± 0.14	21.22 ± 0.11	-	>2

Table 8 % Assay of marketed sample

Tablet	Zita Pio Met 500		
Label claim	**TEN (20 mg)**	**PIO (15 mg)**	**MET (500 mg)**
Assay (% Mean ± S. D.)	99.23 ± 0.14	98.51 ± 0.19	99.15 ± 0.49

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