RESEARCH PAPER

A Simple and Cost-Effective UV Spectrophotometric Method for the Simultaneous Determination of MET, TNG, and PGL in Multicomponent Formulations

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ABSTRACT

Within the context of pharmaceutical formulations, the purpose of the current investigation was to evaluate the repeatability of analytical techniques that were established for the simultaneous measurement of metformin (MET), teneligliptin (TNG), and pioglitazone (PGL). For the purpose of determining whether or not the results were reproducible, five duplicates of each medication were examined over many runs at varied concentration levels. Over the course of the study, the mean recovery for MET varied from 98.94% to 99.78%, for TNG it was between 95.20% and 98.70%, and for PGL it was between 97.12% and 99.26%. There was a constant level of precision and repeatability in the procedure, as evidenced by the fact that the relative standard deviation (%RSD) values for all medicines across all concentrations were consistently above 0.15%. In the context of normal quality control applications, these data provide evidence that the analytical technique that was established is reliable...

Keywords: Metformin (MET), Teneligliptin (TNG), and Pioglitazone (PGL), method development, validation.

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INTRODUCTION

Diabetes mellitus is a metabolic condition that is defined by increased blood glucose levels. This elevated blood glucose level is caused by abnormalities in insulin production, insulin action, or both components of insulin[1]. In order to maintain optimal glycemic control, the therapy of type 2 diabetes frequently necessitates the utilization of a mix of therapeutic drugs that target several physiological pathways[2]. Because of its capacity to lower the amount of glucose that is produced by the liver, the biguanide known as metformin (MET) is frequently used as a first-line treatment. Teneligliptin (TNG), which is an inhibitor of dipeptidyl peptidase-4 (DPP-4), boosts the activity of incretin, which subsequently leads to an increase in insulin production and a decrease in glucagon consumption[3]. The thiazolidinedione known as pioglitazone (PGL) has the ability to enhance insulin sensitivity by exerting its influence on the peroxisome proliferator-activated receptor-gamma (PPAR-γ)[4].

Fixed-dose combos (FDCs) are becoming increasingly popular in the pharmaceutical industry, which calls for the development of analytical methods that are straightforward, economical, and precise in order to carry out simultaneous estimations of these combinations[5,6]. As a result of its accessibility, simplicity, and sensitivity, UV-Vis spectrophotometry is a very useful instrument for doing regular quality control examination. The overlapping spectra of multi-component formulations, on the other hand, provide a considerable difficulty that may be overcome by the application of mathematical methods such as the simultaneous equation technique. A UV-Vis spectrophotometric approach for the simultaneous assessment of MET, TNG, and PGL in a combination pharmaceutical dosage form (for example, Trijardy XR) is the subject of the present research, which intends to develop and validate the accuracy of the method. The purpose of this approach is to provide a dependable and cost-effective alternative for regular analysis in laboratories which are responsible for quality control.

MATERIAL AND METHODS

The proposed method estimates the marketed combination of three drugs (Metformin, Teneligliptin, and Pioglitazone) using UV Vis spectroscopy. The Trijardy XR Tablets, a recently launched diabetes treatment, contains Metformin 1000mg, Teneligliptin 15mg, and Pioglitazone 20mg. The method uses steps for estimating the marketed formulation.

Reagents and Standards

Reference standard of MET, TNG and PGL was a generous gift from pharmaceutical Company. Marketed formulation of MET, TNG and PGL were prepared in the ratio of (1000:15:20mg). Label claim of MET, TNG and PGL in tablets is 1000, 15 and 20mg respectively. Reverse osmosis water was used throughout the study.

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 10 mg of each drug in 5mL Methanol in

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10mlvolumetric flask. The flask was sonicated for about 10min to solubilize thedrugandthevolumewasmadeuptothemark10mlwithMethanoltogeta concentration of 1000 µg/ml (Stock-A) for both drugs.

Preparation of Working Standard Solution

0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with Methanol. This gave the solutions of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml respectively for MET.

Aliquots of 0.01ml, 0.02ml, 0.03ml, 0.04ml and 0.05ml withdrawn with help of pipette fromstandardstocksolution(Stock-B)separatelyin10mlvolumetricflaskandvolumewas made up to 10ml with Methanol. This gave the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml respectively for TNG.

Aliquots of 0.02ml, 0.04ml, 0.06ml, 0.08ml and 0.10ml withdrawn with help of pipette fromstandardstocksolution(Stock-B)separatelyin10mlvolumetricflaskandvolume was made up to 10ml with Methanol. This gave the solutions of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml respectively for PGL[7].

Selectionofwavelengthforlinearity

Solutionsof100µg/mlofMET,1.5µg/mlTNGand2.0µg/mlPGLwereprepared separately.The solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximumabsorbanceofMET,TNGandPGLwasobservedat226.0nm,256.0nmand230.0nm,respectively.METshowedlinearityintheconcentrationrangeof20-100µg/ml andTNGshowedthelinearityintheconcentrationof1-5µg/mlandPGLshowedlinearity 2-10µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration[8].

Studyofoverlayspectra

Working standard solution from the standard stock solution prepared in concentration 100µg/mlofMET,1.5µg/mlofTNGand2µg/mlofPGLwerescannedinthespectrum modeovertherangeof200-400nmagainstMethanolasblankandtheoverlainspectraof thetwowererecorded.METshowedanabsorbancepeakat226.0nm,whereasTNGat256.0nmandPGLat224.0nm.Theoverlainspectraalsoshowedisoabsorptivepointsat230.00nm.Dueto differenceinabsorbancemaximaandhavingnointerferencewitheach other so both drug can be simultaneously estimated by simultaneous equation method.

Validation of simultaneous equation method

Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated bydividing theabsorbancewithrespectiveconcentration.Thena graphwas plotted between concentration and response ratio.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levelsi.e.80%,100%,120%.Therecoverystudieswerecarried outbyaddingknownamount of standard solution of MET, TNG and PGL to preanalysed Capsule solutions. The resulting solutions were then re-analysed by proposed methods. Wholeanalysisprocedure wasrepeatedtofindouttherecoveryoftheaddeddrugsample.Thisrecoveryanalysis was repeated at 3 replicate of 5 concentrations levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzingsameconcentrationofdrugsforfivetimes.Daytoday wasperformedby analyzing 5 different concentration of the drug for three days in a week.

Analysisoftabletsformulation

Twentytabletsweretakenanddeterminetheaverageweight,tabletsgroundtoafine powder; amount equal to 100mg of MET (1.5mg TNG and 2mg PGL) was taken in 10 ml volumetric flask. Then 5ml of Methanol was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with methanol. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with Methanol to get the final concentrations of all threedrugsintheworkingrange.Theabsorbancesoffinaldilutionswereobservedat selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times[9].

RESULTS ANDDISCUSSION

Determination of λ_{max} Wavelength

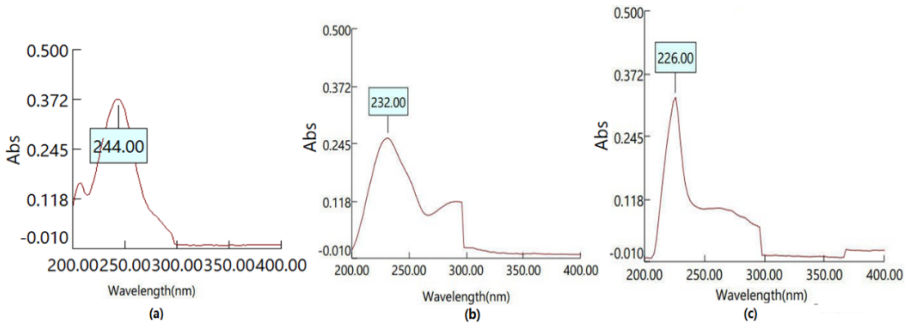

Fig.1 λ_{max} of Teneligliptin, Metformin and Pioglitazone

Table 1: Linearity of TNG, PGL and MET

Linearity of TNG At λ_{max} = 250.0 nm			**LinearityofPGLAtλ_{max}=230.0nm**		**Linearity of MET At λ_{max}=232.0nm**		
Standard Conc. (µg/ml)	**Mean**	**%RSD**	**Mean**	**% RSD**	**Standard Conc. (µg/ml)**	**Mean**	**%RSD**
0	0	0	0	0	0	0	0
10	0.2219±0.0007	0.2999	0.2016±0.0002	0.1215	20	0.316±0.002	0.500
20	0.4407±0.0003	0.0574	0.4018±0.0004	0.1037	40	0.562±0.001	0.178
30	0.6607±0.0002	0.0328	0.6009±0.0007	0.1116	60	0.793±0.001	0.154
40	0.8753±0.0008	0.0878	0.8059±0.0014	0.1715	80	1.024±0.002	0.154
50	1.1074±0.0005	0.0477	1.0190±0.0005	0.0474	100	1.304±0.001	0.064

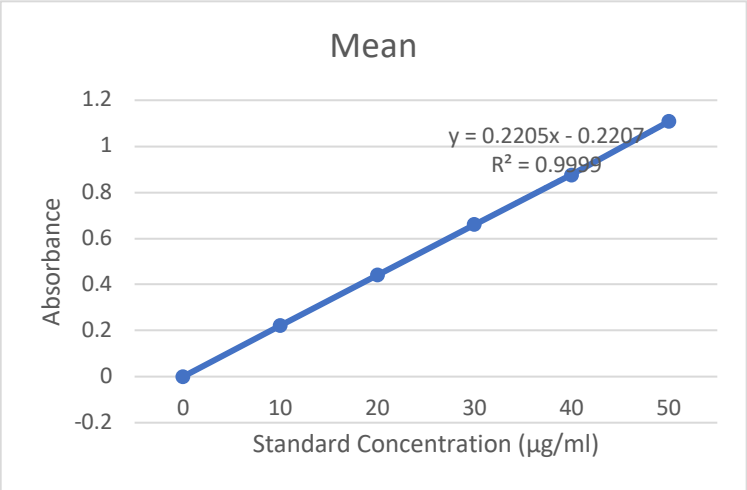

Fig.2:CalibrationCurveofTNG

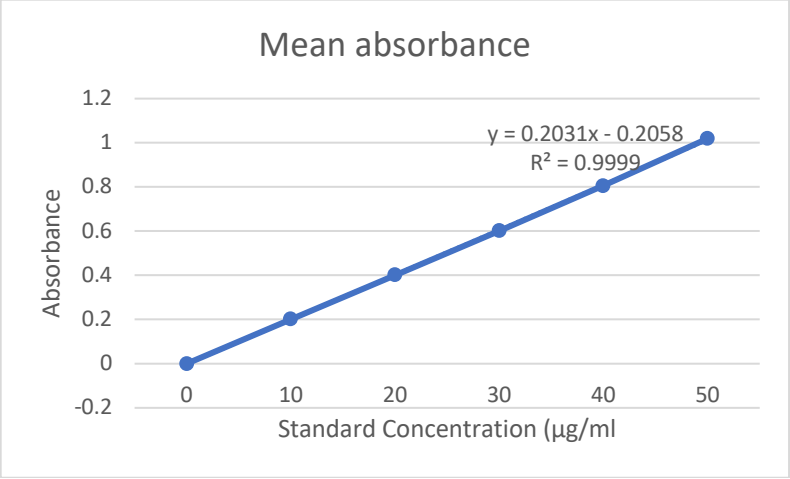

Fig.3:CalibrationCurveofPGL

ResultsofAccuracy

Table 2. ResultsofAccuracy

Level (%)	Drug	Mean Recovery (%)	SD	% RSD
80	TNG	96.460	1.453	1.486
100	TNG	98.267	0.729	0.742
120	TNG	98.900	0.577	0.583
80	PGL	98.862	0.352	0.356
100	PGL	98.463	0.524	0.532
120	PGL	99.181	0.594	0.599

Precision

Table 3.ResultsofRepeatability

Drug	Conc. (mg)	Mean Found (mg)	% Mean	SD	% RSD
TNG	10	9.752	97.52	0.105	0.108
TNG	20	19.744	98.72	0.086	0.087
TNG	30	27.730	92.43	4.518	4.888
TNG	40	39.772	99.43	0.093	0.094
TNG	50	49.786	99.57	0.093	0.094
TNG Avg	—	—	**97.535**	**0.979**	**1.054**
PGL	10	9.750	97.50	0.113	0.116
PGL	20	19.708	98.54	0.122	0.123
PGL	30	29.740	99.13	0.091	0.092
PGL	40	39.758	99.40	0.113	0.114
PGL	50	49.800	99.60	0.102	0.102
PGL Avg	—	—	**98.834**	**0.108**	**0.109**

ResultsofIntermediatePrecision

Table 4.ResultsofDay-to-DayVariation

Drug	Conc. (mg)	Mean Found (mg)	% Mean	SD	% RSD
TNG	10	9.653	96.533	0.087	0.091
TNG	20	19.677	98.383	0.136	0.138
TNG	30	29.777	99.256	0.168	0.169
TNG	40	39.647	99.117	0.095	0.096
TNG	50	49.453	98.907	0.442	0.447
TNG Avg	—	—	**98.439**	**0.186**	**0.188**
PGL	10	9.570	95.700	0.221	0.231
PGL	20	19.767	98.833	0.111	0.112
PGL	30	29.810	99.367	0.164	0.165
PGL	40	39.760	99.400	0.111	0.112
PGL	50	49.440	98.880	0.278	0.281
PGL Avg	—	—	**98.436**	**0.177**	**0.180**

ResultsofAnalysttoanalystvariation

Table 5. ResultsofAnalysttoanalystvariation

Drug	Conc. (mg)	Mean Found (mg)	% Mean	SD	% RSD
TNG	10	9.735	97.350	0.163	0.167
TNG	20	19.895	99.475	0.035	0.036
TNG	30	29.750	99.167	0.141	0.143
TNG	40	39.710	99.275	0.042	0.043
TNG	50	49.595	99.190	0.035	0.036
TNG Avg	—	—	**98.891**	**0.083**	**0.085**
PGL	10	9.715	97.150	0.092	0.095
PGL	20	19.645	98.225	0.134	0.137
PGL	30	29.755	99.183	0.177	0.178
PGL	40	39.815	99.538	0.049	0.050
PGL	50	49.730	99.460	0.071	0.071
PGL Avg	—	—	**98.711**	**0.105**	

ResultsofReproducibility

Table 6. ResultsofReproducibility

Drug	Conc. (mg)	Mean Found (mg)	% Mean	SD	% RSD
TNG	10	9.780	97.80	0.091	0.093
TNG	20	19.680	98.40	0.225	0.228
TNG	30	29.752	99.17	0.144	0.145
TNG	40	39.720	99.30	0.093	0.093
TNG	50	49.774	99.54	0.090	0.090
TNG Avg	—	—	**98.844**	**0.128**	**0.130**
PGL	10	9.756	97.56	0.084	0.086
PGL	20	19.712	98.56	0.108	0.110
PGL	30	29.754	99.18	0.119	0.120
PGL	40	39.722	99.305	0.105	0.106
PGL	50	49.676	99.352	0.081	0.082
PGL Avg	—	—	**98.791**	**0.100**	**0.101**

Resultsofanalysisoftabletsample

Table7:AnalysisoftabletformulationofTNGandPGL

Drug	Labelclaim (mg)	Amountfound (mg)	Labelclaim (%)	S.D.	%RSD
TNG	20	19.85	99.25	0.125	0.136
PGL	15	14.83	98.87	0.223	0.228

Validationofsimultaneousequationmethod

Resultsoflinearity

Table8:ResponseRatioofMET,TNGandPGL

MET			TNG			PGL		
Conc. (µg/ml)	**ABS**	**Response Ratio**	**Conc. (µg/ml)**	**ABS**	**Response Ratio**	**Conc. (µg/ml)**	**ABS**	**Response Ratio**
0	0	0	0	0	0	0	0	0
20	0.316	0.016	1	0.124	0.124	2	0.123	0.062
40	0.562	0.014	2	0.254	0.127	4	0.247	0.062
60	0.793	0.013	3	0.356	0.119	6	0.365	0.061
80	1.024	0.013	4	0.4752	0.119	8	0.477	0.060
100	1.304	0.013	5	0.6042	0.121	10	0.587	0.059

ResultsofAccuracy

Table 9. ResultsofAccuracy

Drug	Level	Mean Recovery (%)	Standard Deviation (SD)	% RSD
MET	80%	99.411	0.535	0.539
	100%	99.321	0.447	0.450
	120%	99.467	0.385	0.387
TNG	80%	98.483	0.563	0.571
	100%	97.254	1.750	1.799
	120%	98.894	0.602	0.609
PGL	80%	99.587	0.601	0.603
	100%	98.439	0.327	0.332
	120%	99.050	0.560	0.565

ResultsofRepeatability

Table10:RepeatabilityofMET,TEG and PGL

RepeatabilityofMET				
Conc. (µg/mL)	**Mean Found**	**% Mean**	**SD**	**% RSD**
20	19.842	99.21	0.0887	0.0894
40	39.800	99.50	0.1177	0.1183
60	59.600	99.33	0.3885	0.3911
80	79.444	99.31	0.3758	0.3784
100	98.752	98.75	0.6246	0.6325
Overall	—	**99.22**	**0.319**	**0.322**
RepeatabilityofTNG				
Conc. (µg/mL)	**Mean Found**	**% Mean**	**SD**	**% RSD**
1	0.970	97.00	0.0158	0.0163
2	1.750	87.50	0.4205	0.4806
3	2.878	95.93	0.0705	0.0735
4	3.878	96.95	0.0867	0.0894
5	4.880	97.60	0.0704	0.0721
Overall	—	**94.997**	**0.133**	**0.146**
RepeatabilityofPGL				
2	1.914	95.70	0.0623	0.0651
4	3.828	95.70	0.1279	0.1337
6	5.878	97.97	0.0760	0.0775
8	7.906	98.83	0.0532	0.0538
10	9.736	97.36	0.0838	0.0861
Overall	—	**97.11**	**0.081**	**0.083**

ResultsofIntermediatePrecision

Table 11. ResultsofDay-to-DayVariation

Drug	**Nominal Conc. (µg/mL)**	**Mean Found (µg/mL)**	**% Mean**	**SD**	**% RSD**
MET	20	19.87	99.35	0.1015	0.1022
	40	39.82	99.55	0.1572	0.1579
	60	59.76	99.60	0.1015	0.1019
	80	79.22	99.03	0.5484	0.5538
	100	99.43	99.43	0.5689	0.5722
TNG	1	0.953	95.33	0.006	0.006
	2	1.93	96.50	0.0700	0.0725
	3	2.863	95.44	0.0907	0.0951
	4	3.937	98.42	0.0586	0.0595
	5	4.897	97.93	0.0569	0.0581
PGL	2	1.950	97.50	0.0300	0.0310
	4	3.797	94.92	0.1159	0.1221
	6	5.907	98.44	0.1102	0.1119
	8	7.930	99.13	0.0700	0.0706
	10	9.793	97.93	0.1504	0.1536

ResultsofAnalyst-to-AnalystVariation

Table 12. Analyst-to-AnalystVariation

Drug	**Nominal Conc. (µg/mL)**	**Mean Found (µg/mL)**	**% Mean**	**SD**	**% RSD**
MET	20	19.820	99.10	0.157	0.157
	40	39.747	99.37	0.1002	0.1002
	60	59.490	99.15	0.4804	0.4804
	80	79.483	99.35	0.5686	0.5686
	100	98.917	98.92	0.8731	0.8731
TNG	1	0.965	96.50	0.021	0.022

	2	1.885	94.25	0.049	0.053
	3	2.865	95.50	0.120	0.126
	4	3.905	97.63	0.078	0.080
	5	4.915	98.30	0.064	0.065
PGL	2	4.815	96.30	0.049	0.051
	4	9.865	98.65	0.120	0.122
	6	14.865	99.10	0.120	0.121
	8	19.910	99.55	0.057	0.057
	10	24.735	98.94	0.064	0.064

Reproducibility

Table 13. Reproducibility

Drug	Nominal Conc. (µg/mL)	Mean Found (µg/mL)	% Mean	SD	% RSD
MET	20	9.894	98.94	0.090	0.091
	40	19.854	99.27	0.120	0.121
	60	29.800	99.33	0.109	0.110
	80	39.800	99.50	0.124	0.125
	100	49.888	99.78	0.113	0.113
TNG	1	0.952	95.20	0.024	0.025
	2	1.962	98.10	0.013	0.013
	3	2.948	98.27	0.016	0.017
	4	3.948	98.70	0.019	0.019
	5	4.898	97.96	0.080	0.081
PGL	2	4.856	97.12	0.084	0.086
	4	9.788	97.88	0.134	0.137
	6	14.790	98.60	0.132	0.134
	8	19.852	99.26	0.122	0.123
	10	24.724	98.90	0.080	0.081

Analysisoftabletsformulation

Table14:Assayoftabletsformulation

	%Conc.Found		
	MET	**TNG**	**PGL**
Replicate1	99.12	99.45	99
Replicate2	99.05	99.12	99.02
Average	99.085	99.285	99.01
S.D.	0.049	0.233	0.014
%RSD	0.050	0.235	0.014

DISCUSSION:

Metformin (MET), Teneligliptin (TNG), and pioglitazone (PGL) were all measured in a combination tablet dose form utilizing UV-Visible spectrophotometry in this investigation. Based on their respective absorbance maxima, the λmax values for MET (226 nm), TNG (256 nm), and PGL (230 nm) allowed for the construction of a simultaneous equation approach. An isoabsorptive point at 230 nm was also noted, which helped in the development of the approach, and overlay spectra verified that simultaneous estimation was possible without spectral interference.

From 20-100 µg/mL for MET, 1-5 µg/mL for TNG, and 2-10 µg/mL for PGL, the linearity of all three medicines was proven. The suggested approach was confirmed to be consistent and reliable as the association between concentration and absorbance shown good linearity with extremely low %RSD values.

We used recovery trials at three different concentration levels (80%, 100%, and 120%) to evaluate the accuracy. The technique is accurate and free from matrix interference from tablet excipients, as indicated by the percentage recovery values for TNG and PGL, respectively, which ranged from 96.46% to 98.90% and 98.46% to 99.18%.

Research on precision showed high levels of repeatability and moderate accuracy. The repeatability %RSD values for TNG and PGL were consistently below 5%, and in the majority of cases, they were considerably below 1%,

demonstrating outstanding consistency. Similarly, %RSD values consistently under 0.2% for both analyst-to-analyst and day-to-day variability, showing solid intermediate accuracy.

Tests on a commercially available tablet formulation (Trijardy XR) demonstrated that the proposed approach accurately measured MET, TNG, and PGL. Consistent and very variable findings validate this spectrophotometric method's usefulness for everyday quality control.

CONCLUSION

In the current investigation, a straightforward, precise, and reproducible UV-Vis spectrophotometric approach was successfully devised and validated for the simultaneous measurement of Metformin (MET), Teneligliptin (TNG), and Pioglitazone (PGL) in a formulation that is currently on the market. The technique exhibited high mean recovery rates and low %RSD values, which indicated that it had exceptional accuracy and reproducibility. Additionally, the approach revealed excellent linearity within the concentration ranges that were evaluated for each medication. The study of the overlay spectra demonstrated that there was only a limited amount of interference between the three medications, which made it possible to do an accurate simultaneous quantification using the simultaneous equation approach. Furthermore, the approach demonstrated its appropriateness for regular quality control of combination antidiabetic formulations such as Trijardy XR by demonstrating its robustness across repeatability and intermediate precision trials...

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