International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 11, Issue 7, 2019

Original Article

A RAPID VALIDATED UNI-DIMENSIONAL DOUBLE DEVELOPMENT HPTLC-DENSITOMETRY METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE, GLICLAZIDE AND PIOGLITAZONE HYDROCHLORIDE

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Received: 20 Dec 2018 Revised and Accepted: 17 May 2019

ABSTRACT

Objective: To develop and validate a uni-dimensional double development high-performance thin layer chromatography (UDDD-HPTLC) for estimation of anti-diabetic medicine compromising of metformin (MET) gliclazide (GLZ) and pioglitazone hydrochloride (PIO).

Methods: The chromatographic separation of these drugs was carried out on precoated TLC plates silica gel 60F254by two mobile phases consisting of Ammonium Sulphate: Methanol: Acetonitrile: Water (4:3:2:1) for MET and PIO and Toluene: Ethyl Acetate: Formic Acid (6:4:0.5) for GLZ respectively for ideal separation and good resolution. The densitometric detection and quantification were carried out at 237 nm for MET and 200 nm for GLZ and PIO. The validation parameters were strictly followed as per the ICH guidelines.

Results: The linearity range was obtained at 3000-8000ng/spot, 360-960 ng/spot, 90-240 ng/spot for MET, GLZ and PIO with r^2value>0.999. The other parameters such as precision, reproducibility, robustness were efficiently obtained within the limits. The proposed method was successfully applied for simultaneous determination of MET, GLZ and PIO in the commercial formulation.

Conclusion: In simultaneous estimation, the different polarity of drugs makes it more cumbersome to develop and validate any chromatographic method. In the present study, a uni-dimensional double development high-performance thin layer chromatography (UDDD-HPTLC) for estimation of these drugs have been developed and validated to resolve the estimation problem. It is an effortless and speedy method which was developed and validated using ICH guidelines. The developed and validated method using ICH guidelines is effortless and speedy technique.

Keywords: HPTLC, Double-development, Metformin, Gliclazide, Pioglitazone, Validation, Simultaneous, ICH

DOI: http://dx.doi.org/10.22159/ijpps.2019v11i7.31586

INTRODUCTION

Metformin (MET) is chemically, 1-carbamimidamido-N-N-dimethyl-methanimidamide (fig. 1) [1]. It is an oral anti-diabetic drug from the biguanide class. It is the first-line drug for the treatment of type-2 diabetes, particularly in overweight and obese people and those with normal kidney function and evidences suggest it may be the best choice for the people with heart failure. The major action of MET is increasing glucose transport across the cell membrane in skeletal muscle [2].

Gliclazide (GLZ) is chemically N-(hexahydrocyclopenta(c) pyrrol-2-(1H)-ylcarbamoyl)-4-methyl) benzene sulphonamide (fig. 2). Gliclazide is an oral hypoglycemic (anti-diabetic) and is classified as a sulphonylurea. It is used in type 2 diabetes mellitus that is a non-insulin dependent diabetes mellitus. GLZ was proven to protect human pancreatic beta cells from hyperglycemia-induced apoptosis. It was also shown to have an anti-atherogenic effect (preventing the accumulation of fat in arteries) in type 2 diabetes. GLZ selectively binds to sulphonylurea receptors (SUR-1) on the surface of the pancreatic beta-cells [3].

Pioglitazone hydrochloride (PIO) is (±)-5-(p-[2-(5-Ethyl-2-pyridyl) ethoxy]benzyl)-2,4-thiazolidinedione hydrochloride (fig. 3). Pioglitazone belongs thiazolidinedione group which is a class of oral anti-diabetic drugs that enhance target tissue insulin sensitivity. Pioglitazone has been shown to affect abnormal glucose and lipid metabolism associated with insulin resistance by enhancing insulin action on peripheral tissues [4].

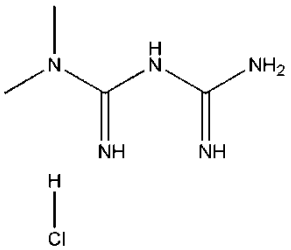
It is observed through a literature review that many spectrophotometric and chromatographic methods have been reported for the estimation of single as well as combined formulations containing Metformin, Gliclazide and Pioglitazone hydrochloride [5-15].

MET has been simultaneously determined with PIO by spectrophotometric methods, [16, 17], by HPLC in the binary mixture [18-21], and with other components by HPLC [22-25]. GLZ has been simultaneously determined with MET by spectrophotometric methods, [26-28], by LC in dosage forms [29-33], and in human plasma [34, 35]. Spectrophotometric determinations have been reported for mixtures composed of more than two drugs [36-38]. Literature also reveals the use of the ion-pairing technique [39-42], and micellar liquid chromatography [43], to develop a successful HPLC method for the determination of gliclazide and/or metformin.

Among different analytical tools TLC is one of the multi-applicative, quickest, accurate and robust method. However, the literature survey does not reveal any HPTLC method for simultaneous estimation of MET, GLZ and PIO in combined tablet dosage form. The reason for this can be the polarity difference among the three drugs. But, TLC application is so diverse that it offers various special development modes such, as multidimensional development, uni-dimensional multiple development (UMD), incremental multiple development (IMD), gradient multiple development (GMD), etc. [44]. All these methods, improve the resolution of the components because of each consecutive development results in band re-concentration and thus, it enhances the efficiency of the separation [44, 45]. In this current research work, we have applied the same principle and have successfully developed and validate UDDD-HPTLC method for the simultaneous estimation of MET, GLZ and PIO.

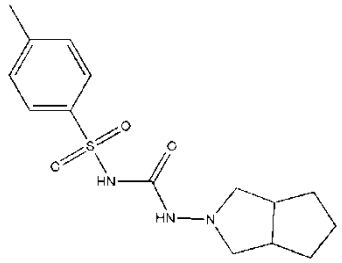
Therefore, a simple and new UDDD-HPTLC method has been developed and validated for their estimation of MET, GLZ and PIO in bulk and combined tablet dosage form. UDDD-HPTLC techniques allow separation of compounds with a large difference in polarity.

When the combined formulation have drugs with different polarity the chromatographic study like HPLC becomes costly, tedious and more time-consuming affair. Thus, in such case, HPTLC method with robust, easy detection and time-saving characteristics can be effectively employed.



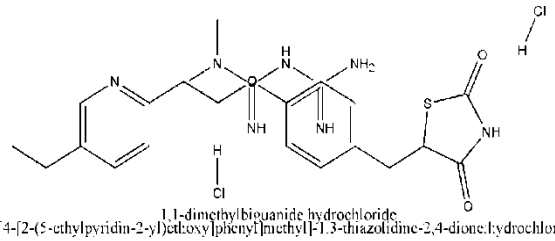
1,1-dimethylbiguanide hydrochloride

Fig. 1: Structure of metformin (MET)



1-(3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrol-2-yl)-3-(4-methylphenyl)sulfonylurea

Fig. 2: Structure of gliclazide (GLZ)



1,1-dimethylbiguanide hydrochloride
5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione;hydrochloride

Fig. 3: Structure of pioglitazone hydrochloride (PIO)

MATERIALS AND METHODS

Chemical and reagents

MET, GLZ and PIO API procured as a gift sample from Ipca Laboratories Limited Mumbai. The marketed formulation (Glycinorm Total) with fixed dose combination tablets of the three compounds, (MET, GLZ, PIO) was purchased from retail pharmacy in Mumbai (Maharashtra, India). Other chemicals and reagents of analytical grade were purchased from Merck (India).

Chromatographic conditions

A CAMAG HPTLC system equipped with Linomat 5 autosampler, TLC scanner 3, and winCATS 1.2.2 software (CAMAG, Muttens, Switzerland) was employed. The slit dimension was kept at 5.00 × 0.45 mm, and 20 mm/sec scanning speed was employed. Chromatography was performed on precoated silica gel 60 F254 TLC plates (20 × 10 cm,) (Merck, Darmstadt, Germany) using Ammonium Sulphate: Methanol: Acetonitrile: Water (4:3:2:1) for MET and PIO and Toluene: Ethyl Acetate: Formic Acid (6:4:0.5) for GLZ respectively as mobile phase. The band length 6 mm and distance between bands 15 mm were kept constant throughout the study. The application speed was 150 nl/sec. Ascending development to a distance of 85 mm was performed on 20×10 cm twin through the chamber (CAMAG). Densitometric scanning was achieved over a camag TLC scanner III operated using winCATS software (V 1.4.4. CAMAG). The source of radiation used was a UV Spectrophotometer, using spectral data the λmax was determined for each compound.

Preparation of solutions

Standard stock solution

The standard stock solutions with a concentration of 1 mg/ml of the individual standard were prepared in methanol.

Sample stock solution

Twenty tablets were weighed and crushed to a fine powder. The quantity of powder equivalent to 500 mg MET, 60 mg GLZ and 15 mg PIO was weighed and transferred to a 100 ml volumetric flask. The solution containing 5 mg/ml MET and 0.6 mg/ml GLZ 0.15 mg/ml PIO. The solution was filtered through Whatmann filter paper number 41.

Validation of the method

Linearity

The standard stock solution with 1 mg/ml each MET and GLZ, PIO were prepared in methanol. Different volumes of each solution were applied to the HPTLC plate to deliver 3000-8000 ng of MET, 360-960 ng of GLZ, 90-240 ng of PIO per spot. Each concentration was analyzed in duplicate [46].

Precision

The precision of the developed method was evaluated by performing intra-day and inter-day precision. Intra-day precision was assessed based on triplicate of three different concentrations of MET (3000, 5000, 7000ng/spot), GLZ (360,600,960ng/spot) and PIO (90,150,240 ng/spot). The inter-day precision of the method was verified by performing a similar method on different days under the same set of experimental conditions. The repeatability study of the same application and calculation of the peak area for the analyte was articulated in terms of the % RSD [46].

Accuracy

The accuracy of the method was assessed by determination of recovery of the method at 3 different concentrations (80%, 100% and 120% levels) by addition of known amounts of MET, GLZ and PIO. At each level, six determinations were performed to evaluate the accuracy studies. The results were recorded in the form of percent recovery and percent RSD of all three drugs [46].

Limit of detection and limit of quantitation

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), the signal to noise ratio (S/N) of 3 and 10 was determined for six to replicate determinations for each drug [46].

Specificity

The specificity of the method was by means of complete separation of pure drugs in the presence of other excipients normally present in the formulation. Peak purity of MET, GLZ and PIO was assessed by comparing their respective spectra at peak start (S), peak apex (M) and peak end (E) position of the spots [46].

Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. Following the introduction of small changes in the mobile phase composition (±0.1 ml for each component), the amount of mobile phase was varied over the range of±5%. The time from spotting to chromatography and from chromatography to scanning was varied

by+10 min. The effect of these changes on both the Rf values and peak areas was examined by calculating the %RSD for each parameter [46].

Analysis of tablet sample

The validated method was used for the simultaneous quantitation of Metformin, Gliclazide and Pioglitazone hydrochloride in the tablet dosage form. Further, 5 ml of stock solution of the sample was diluted with 10 ml of methanol to get the concentration of 2.5 mg/ml MET, 0.3 mg/ml GLZ and 0.75 mg/ml PIO. The analysis was repeated in triplicate [46].

RESULTS AND DISCUSSION

Chromatographic separation of the standard solution of MET, GLZ and was performed. Briefly, the spot of the standard solution was applied on TLC plates. The TLC plates were developed by linear ascending development by using various solvents such as acetone, benzene, chloroform, ethyl acetate, methanol and toluene. The experimental condition for the HPTLC method such as mobile phase composition and the wavelength of detection was optimized to provide accurate, precise and reproducible, compact, flat bands for simultaneous determination of MET, GLZ and PIO.

During the stage of method development different mobile phases were tried and the mobile phase comprising of Ammonium Sulphate: Methanol: Acetonitrile: Water (4:3:2:1) for MET and PIO and Toluene: Ethyl Acetate: Formic Acid (6:4:0.5) for GLZ respectively were confirmed. The spectral data revealed the λ max for MET at 237 nm and for GLZ and PIO at 200 nm. The chromatographic conditions confirmed for the analysis gave well-resolved peaks for each standard. (fig. 4 and fig. 5).

A good linear relationship was obtained over the concentration ranges 200-1000, 360-960 and 90-240 ng/spot for MET, GLZ and PIO respectively. The linear regression data showed a regression coefficient of 0.9993 for MET (fig. 6), 0.9987 for GlZ (fig. 7) and 0.9993 for PIO (fig. 8). The LOD with signal/noise ratio was found to be 357.7, 150.24 and 61.745 ng/spot for MET, GLZ, and PIO respectively. The LOQ with signal/noise ratio was found to be 1084.0, 455.27 and 187.105 ng/spot for MET, GLZ, and PIO respectively.

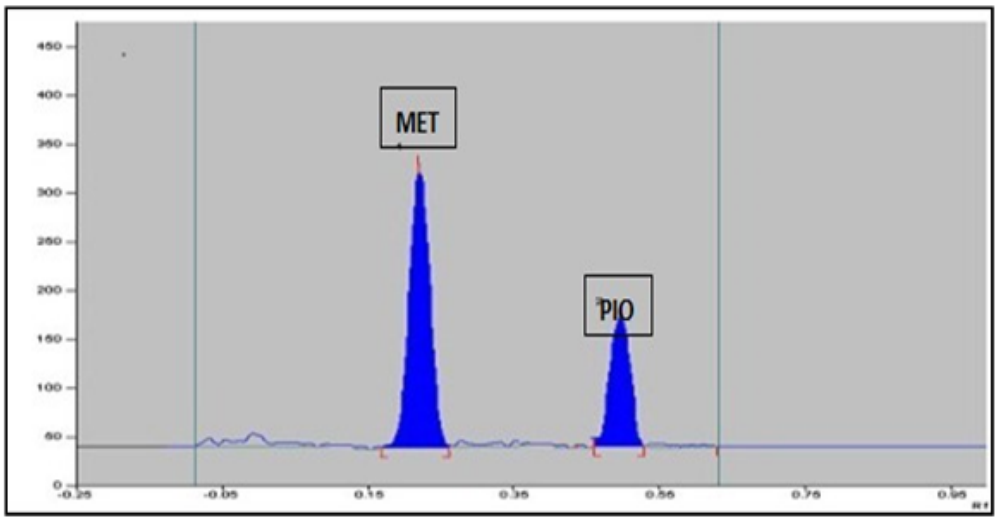


Fig. 4: HPTLC profile of MET and PIO

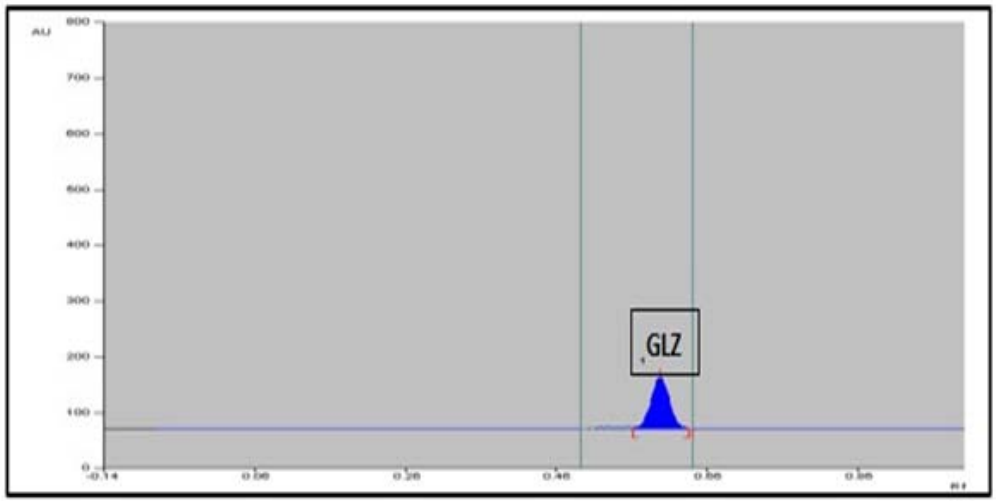


Fig. 5: HPTLC profile of GLZ

The intraday and inter-day precision showed excellent % RSD less than 2 % (table 1, 2 and 3). The recovery was 101.6, 101.4 and 100.6% for MET, 99.8, 100.4 and 100.6 % for GLZ and 100.6, 99.1, 101.7 % for PIO at 80%, 100% and 120% levels (table 4). The robustness parameter was also successfully carried and the results were satisfying and within the limits. (table 5.1, 5.2, 5.3)

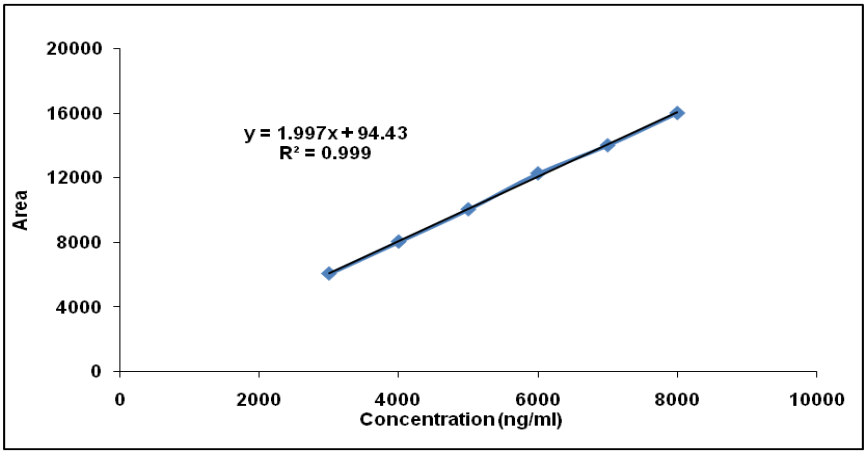


Fig. 6: Linearity graph of MET

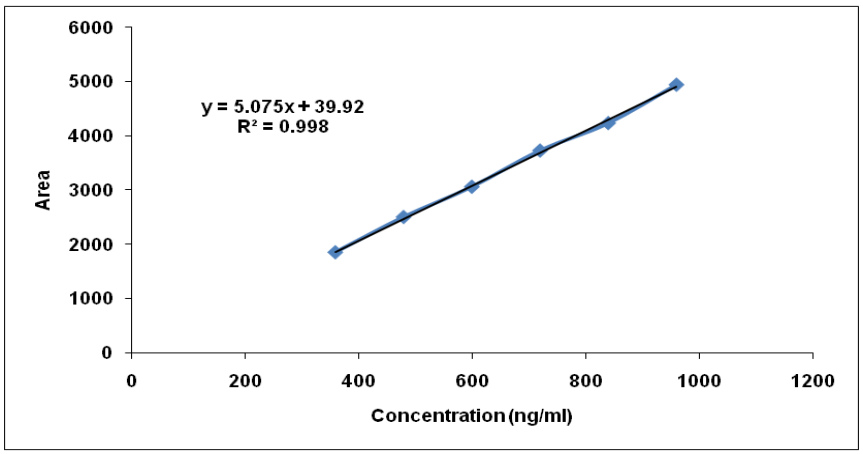


Fig. 6: Linearity graph of GLZ

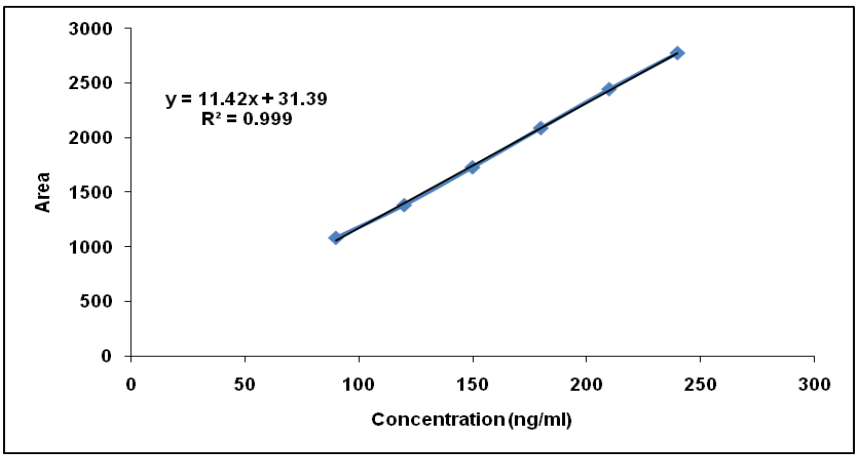


Fig. 6: Linearity graph of PIO

Table 1: Evaluation of intra-day and inter-day precision of MET

MET taken (ng/spot)	Intra-day precision		Inter-day precision	
	MET found(ng/spot) mean±SD	% RSD	MET found (ng/spot) mean±SD	% RSD
3000	3080.4±50.52	1.64	3037.8±26.13	0.86
5000	5134.8±91.91	1.79	5220.7±81.96	1.57
7000	7119.7±128.87	1.81	7356.3±100.78	1.37

SD=Standard deviation, RSD= relative standard deviation

Table 2: Evaluation of intra-day and inter-day precision of GLZ

GLZ taken (ng/spot)	Intra-day precision		Inter-day precision	
	GLZ found(ng/spot) mean±SD	% RSD	GLZ found (ng/spot) mean±SD	% RSD
360	357.3±4.93	1.38	355.6±3.13	0.88
600	598.6±6.46	1.08	597.1±5.37	0.90
960	969.4±13.77	1.42	965.6±15.16	1.57

SD=Standard deviation, RSD= Relative Standard deviation

Table 3: Evaluation of intra-day and inter-day precision of PIO

PIO taken (ng/spot)	Intra-day precision		Inter-day precision	
	PIO found (ng/spot) mean±SD	% RSD	PIO found (ng/spot) mean±SD	% RSD
90	97.6±1.82	1.86	97.3±170.28	1.75
150	149.7±2.77	1.85	150.0±1.38	0.92
240	235.4±4.26	1.81	234.8±3.38	1.44

SD=Standard deviation, RSD= Relative Standard deviation

Table 4: Recovery data of MET, GLZ and PIO

Level (%)	Amount added (ng)			Amount found (ng) mean±SD			% Recovery		
	MET	GLZ	PIO	MET	GLZ	PIO	MET	GLZ	PIO
80	4800	580	150	4868±16.99	582±10.23	149±1.29	101.4	100.4	99.1
100	6000	720	180	6094±41.63	718±5.06	181±2.39	101.6	99.8	100.6
120	7200	860	220	7245±84.02	865±12.65	224±1.15	100.4	100.6	101.7

SD=Standard deviation, RSD= Relative Standard deviation

Table 5.1: Robustness study for developed study of metformin

Parameter	MET			
Concentration in ng/spot	3000	5000	7000	%RSD
Mobile phase composition (±0.1 ml) mean±SD	6425.33±99.51	10425.33±100.50	14300.33±77.72	1.02
Amount of mobile phase (±5%) mean±SD	6315±95.69	10319.3±73.42	14342.7±167.73	1.13
Time from spotting to chromatography (±10 min) mean±SD	6328±72.08	10526.33±204.26	14461.67±204.26	1.63
Time from chromatography to scanning (±10 min) mean±SD	6863.67±115.94	10718.33±170.10	14692.67±159.10	1.45

SD=Standard deviation, RSD= Relative Standard deviation

Table 5.2: Robustness study for developed study of Gliclazide

Parameter	GLZ			
Concentration in ng/spot	**360**	**600**	**960**	**%RSD**
Mobile phase composition (±0.1 ml) mean±SD	1878.67±16.50	3085.33±36.64	4759.33±85.01	1.28
Amount of mobile phase (±5%) mean±SD	1845.67±23.01	3083.00±35.79	4937.67±35.10	1.04
Time from spotting to chromatography (±10 min) mean±SD	1855.33±12.34	3079.67±41.05	4892.3±14.2	0.76
Time from chromatography to scanning (±10 min) mean±SD	1848.00±3.61	3045.67±45.65	4881.33±62.07	0.99

SD=Standard deviation, RSD= Relative Standard deviation

Table 5.3: Robustness study for developed study of Pioglitazone hydrochloride

Parameter	PIO			
Concentration in ng/spot	90	150	240	%RSD
Mobile phase composition (±0.1 ml) mean±SD	1126.67±14.503	1725.67±3.512	2741.33±25.813	0.81
Amount of mobile phase (±5%) mean±SD	1129±14.526	1747±28.919	2739±36.497	1.42
Time from spotting to chromatography (±10 min) mean±SD	1117.67±4.163	1729±11.14	2706.33±77.37	1.29
Time from chromatography to scanning (±10 min) mean±SD	1116.33±7.51	1735.33±15.31	2717±37.47	0.98

SD=Standard deviation, RSD= Relative Standard deviation

This developed and validated the method for the simultaneous analysis of MET, GLZ and PIO in pharmaceutical preparations was successfully employed. The assay results of tablet dosage form are shown in table 6.

Assay results show excellent label claim of 99.4% for Metformin, 100.1% for Gliclazide and 99.7 % for Pioglitazone hydrochloride. Final, optimum characteristics and validation parameters are summarized in table 7.

Table 6: Assay results of tablet dosage form

Formulation	Actual added (mg)			Amount found±(mg)			% drug found±SD		
Glycinorm Total	MET	GLZ	PIO	MET	GLZ	PIO	MET	GLZ	PIO
	500	60	15	498	58.8	14.2	99.4±0.15	100.1±0.09	99.7±0.08

SD=Standard deviation, RSD= Relative Standard deviation

Table 7: Optical characteristic and validation parameter of MET, GLZ and PIO

Parameter	MET	GLZ	PIO
Wavelength	237	200	200
Linearity range (ng/Spot)	3000-8000	360-960	90-240
Correlation co-efficient (r2) mean±SD	0.9993±0.0019	0.9987±0.0006	0.9993±0.0012
Retention factor	0.22	0.60	0.48
Intercept	94.43	39.92	31.39
Slope of regression	2.00	5.03	11.43
Limit of detection (LOD, ng/spot)	179.06	29.59	5.40
Limit of quantitation (LOQ, ng/spot)	542.61	89.65	13.60
Assay (%) mean±SD	99.4±0.15	100.1±0.09	99.7±0.08

SD=Standard deviation, RSD= Relative Standard deviation

CONCLUSION

The developed UDDD-HPTLC method is new, less time consuming, precise, accurate and robust. The double development feature in TLC method enables estimation of any kind of compounds simultaneously. It is concluded that the developed method offers several advantages such as rapid, cost-effective, simple mobile phase, easy sample preparation steps and improved sensitivity made it reliable and easily reproducible in quality control set-up providing all the parameters are followed accurately for its intended use. The proposed UDDD-HPTLC method is novel, less expensive, simpler, rapid, and more flexible than HPLC. This method allows simultaneous estimation of all API on one single TLC plate with a single application and thus, it can be employed for routine quality control analysis.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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