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Development and Validation of HPTLC Method for Simultaneous Estimation of Anti-Diabetic Drugs from Their Combined Dosage Form

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ABSTRACT

A simple, accurate and precise HPTLC method for simultaneous determination of Metformin hydrochloride, Pioglitazone hydrochloride and Glibenclamide in tablet dosage form has been developed using Camag TLC system (Muttens, Switzerland) comprising of CAMAG Linomat 5 applicator. The chromatographic separation were carried out using precoated silica gel on aluminum plate 60 F254, (20×10 cm2 and 10×10 cm2) as stationary phase and butanol : 1,4-dioxane : glacial acetic acid in the proportion of 5:3:2 v/v/v and 2 drop of formic acid as mobile phase for Metformin hydrochloride, Pioglitazone hydrochloride and Glibenclamide. By using the developed method, Rf value was found to be 0.15 for Metformin hydrochloride, 0.72 for Pioglitazone hydrochloride and 0.85 for Glibenclamide. The method has been validated for linearity, accuracy and precision. Linearity of Metformin hydrochloride, Pioglitazone hydrochloride, and Glibenclamide was in the range of 2000 - 18000 ng/band, 60 - 540 ng/band and 10-100 ng/band respectively.

INTRODUCTION

Diabetes mellitus type 2 – generally – known as noninsulin dependent diabetes mellitus (NIDDM) or also as adult-onset diabetes. It is a metabolic issue involving high blood glucose (hyperglycemia) in the setting of insulin resistance and relative insulin lack.[1] Antidiabetic agents are useful in the treatment of patients who have Type 2 diabetes but who cannot be managed by diet alone. Metformin is an oral anti-diabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, particularly in overweight and obese people. The evidence is also mounting for its efficacy in gestational diabetes, although safety concerns still preclude its widespread use in this setting. It activates the AMP-activated protein kinase (AMPK). It is also used in the treatment of polycystic ovary syndrome and has been investigated for other diseases where insulin resistance may be an important factor.[2,3]

Pioglitazone hydrochloride [$C_{19}H_{20}N_2O_3$] is an agonist at peroxisome proliferator-activated receptors (PPAR) in target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. It is used in diabetes mellitus. Activation of PPAR-gamma receptors increases the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization. In this way, Pioglitazone enhances tissue sensitivity to insulin and also reduces hepatic gluconeogenesis. Thus, insulin resistance associated with type 2 diabetes mellitus is improved without an increase in insulin secretion by pancreatic β cells.[4]

Glibenclamide is a popular anti-diabetic drug, belonging to the class of sulfonylureas. The drug is widely used for treating type II diabetes. Glibenclamide binds to ATP-sensitive potassium channels on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Depolarization stimulates calcium ion influx through voltage-sensitive calcium channels, raising intracellular concentrations of calcium ions, which induces the secretion, or exocytosis of insulin.[5-7]

HPTLC has enhanced the form of thin layer chromatography. It is a powerful separation tool for quantitative analysis. Different changes can be made in the methods of thin layer chromatography to robotize the diverse steps, to expand the determination achieved and to permit more exact quantitative estimations. HPTLC is an effective instrument for the chromatographic data of complex blend of the organic, inorganic and natural compound. Widely used instrument software for the HPTLC is from CAMAG, Switzerland. It provides automated sample application, plate development, detection, and documentation.[8]

MATERIALS AND METHODS

Camag HPTLC system was used during the study. The chromatographic separation was carried out using precoated silica gel on the aluminum plate 60 F_{254}, (20×10 cm^2 and 10×10 cm^2) as the stationary phase and mobile phase consisting of butanol:1,4-dioxane: glacial acetic acid in the ratio of 5:3:2 v/v/v and 2 drops of formic acid.[9] The wavelength selected for detection was 226 nm. This method was used for determination of Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glibenclamide from pure powder mixture and tablet dosage form.

Preparation of Standard Stock Solution

For Metformin Hydrochloride[10-24]

Accurately weighted 250 mg of Metformin hydrochloride standard drug was transferred into a 25 mL volumetric flask. The drug was dissolved in few mL of methanol, the volume was made up to the mark with methanol. Then after 2 mL of this prepared solution was withdrawn and transferred into 10 mL volumetric flask. The volume was made up to mark with methanol to give a final standard stock solution of 2000 ng/µL of Metformin hydrochloride.

For Pioglitazone Hydrochloride[10-24]

Accurately weighted 7.5 mg of Pioglitazone hydrochloride standard drug was transferred into a 25 mL volumetric flask. Drug was dissolved in few mL of methanol, the volume was made up to the mark with methanol. Then after 2 mL of this prepared solution was withdrawn and transferred into 10 mL volumetric flask. The volume was made up to mark with methanol to give a final standard stock solution of 60 ng/µL of Pioglitazone hydrochloride.

For Glibenclamide

Accurately weighted 5 mg of Glibenclamide standard drug was transferred to a 50mL volumetric flask. Drug was dissolved in few mL of methanol, then the volume was made up to the mark with methanol. Then after 1 mL of this prepared solution was withdrawn and transferred into 10 mL volumetric flask. The volume was made up to mark with methanol to give a final standard stock solution of 10 ng/µL of Glibenclamide.

Preparation of standard stock solution for mixtures of Metformin hydrochloride, Pioglitazone hydrochloride & Glibenclamide

A 2 mL stock solution each of Metformin hydrochloride (2000 ng/µL) and Pioglitazone hydrochloride (60 ng/µL) and 4 mL stock solution of Glibenclamide (10 ng/µL) were transferred in to the 10 mL volumetric flask and volume was made up to mark with methanol, so the final concentration of mixture becomes 400 ng/µL of MET, 12 ng/µL of PIO and 4 ng/µL of GLI.

Selection of Analytical wavelength

A standard stock solution of Pioglitazone hydrochloride, Metformin Hydrochloride and Glibenclamide was prepared by the appropriate dilution with methanol. All solutions were scanned to get suitable absorbance for selection of wavelength for the HPTLC. These three solutions were scanned between 200-400 nm in UV-Visible spectrophotometer. The appropriate wavelength was selected from the overlay spectra of Pioglitazone hydrochloride, Metformin hydrochloride, and Glibenclamide solutions.

Linearity[10-24]

Preparation of calibration curve of Metformin hydrochloride:

1 µL (i.e., 2000 ng) sample of Metformin hydrochloride solution of 2000 ng/µL was spotted on pre-coated silica gel 60 F_{254} TLC plates (E. Merck) using Camag Linomat V (Switzerland) automatic sample spotter. Similarly 3.0 µL (i.e., 6000 ng), 5.0 µL (i.e., 10000 ng) 7.0 µL (i.e., 14000 ng) and 9.0 µL (i.e., 18000 ng) samples of Metformin hydrochloride solution of 2000 ng/µL was spotted on pre-coated silica gel 60 F_{254} TLC plates (E. Merck) using Camag Linomat V (Switzerland) automatic sample spotter. The plate was developed in the solvent system consisting of butanol, 1, 4-dioxane, glacial acetic acid in a ratio of (5:3:2 v/v/v) and two drops of formic acid. The plate was dried at room temperature and scanned using CAMAG TLC scanner 3 at UV 226 nm and R_f values, absorption spectra of bands and their area were recorded. Calibration curve was plotted using area obtained at 226 nm.

Preparation of calibration curve of Pioglitazone hydrochloride:

1 µL (i.e., 60 ng) sample of Pioglitazone hydrochloride solution of 60 ng/µL was spotted on pre-coated silica gel 60 F_{254} TLC plates (E. Merck) using Camag Linomat V (Switzerland)

automatic sample spotter. Similarly 3.0 µL (i.e., 180 ng), 5.0 µL (i.e., 300 ng), 7.0 µL (i.e., 420 ng) and 9.0 µL (i.e., 540 ng) samples of Pioglitazone hydrochloride solution of 60 ng/µL was spotted on pre-coated silica gel 60 F_{254} TLC plates (E. Merck) using Camag Linomat V (Switzerland) automatic sample spotter. The plate was developed in the solvent system consisting of butanol, 1, 4-dioxane, glacial acetic acid in a ratio of (5:3:2 v/v/v) and two drops of formic acid. The plate was dried at room temperature and scanned using CAMAG TLC scanner 3 at UV 226 nm and R_f values, absorption spectra of bands and their area were recorded. Calibration curve was plotted using area obtained at 226 nm.

Preparation of calibration curve of Glibenclamide:

1 µL (i.e., 10 ng) sample of Glibenclamide solution of 10 ng/µL was spotted on pre-coated silica gel 60 F_{254} TLC plates (E. Merck) using Camag Linomat V (Switzerland) automatic sample spotter. Similarly 3.0 µL (i.e., 30 ng), 5.0 µL (i.e., 50 ng) 7.0 µL (i.e., 70 ng) and 9.0 µL (i.e., 90 ng) samples of Glibenclamide solution of 10 ng/µL was spotted on pre-coated silica gel 60 F_{254} TLC plates (E. Merck) using Camag Linomat V (Switzerland) automatic sample spotter. The plate was developed in the solvent system consisting of butanol, 1, 4-dioxane, glacial acetic acid in a ratio of (5:3:2 v/v/v) and two drops of formic acid. The plate was dried at room temperature and scanned using CAMAG TLC scanner 3 at UV 226 nm and R_f values, absorption spectra of bands and their area were recorded. Calibration curve was plotted using area obtained at 226 nm.

Chromatographic separation using standard stock solution for mixtures of Metformin hydrochloride, Pioglitazone hydrochloride & Glibenclamide:

A 10 µL sample of mixed standard of Metformin hydrochloride, Pioglitazone hydrochloride and Glibenclamide containing 400 ng/µL of MET, 12 ng/µL of PIO and 4 ng/µL of GLI (4000 ng/spot MET, 120 ng/spot PIO and 40 ng/spot GLI) was applied on pre-coated silica gel 60 F_{254} TLC plates (E. Merck) using Camag Linomat V (Switzerland) automatic sample spotter. The plate was developed in the solvent system consisting of butanol, 1, 4-dioxane, glacial acetic acid in a ratio of (5:3:2 v/v/v) and two drops of formic acid. After complete separation of three drugs, the plate was dried at room temperature and scanned using CAMAG TLC scanner 3 at UV 226 nm and R_f values, absorption spectra of bands and their area were recorded and concentrations of each drug from standard drug mixture were calculated using standard calibration curve and data was tabulated.

This study was performed six times.

Precision[10-24]

Intra-day precision

Standard solution containing Metformin hydrochloride (2000 ng/µL), Pioglitazone hydrochloride (60 ng/µL) and Glibenclamide (10 ng/µL) were analyzed six times on the same day and area of band were measured as per the analysis of standard drug mixture analysis and % RSD was calculated.

Inter-day precision

Standard solution containing Metformin hydrochloride (2000 ng/µL), Pioglitazone hydrochloride (60 ng/µL) and Glibenclamide (10 ng/µL) were analyzed six times on the different day and area of band were measured as per the analysis of standard drug mixture analysis and % RSD was calculated.

Robustness

The solution containing 2000 ng/µL of Metformin hydrochloride, 60 ng/µL of Pioglitazone hydrochloride and 20 ng/µL of Glibenclamide was applied to the TLC plate by the Linomate-5 applicator and area of the band were measured as per standard drug mixture analysis under different parameters of mobile phase and % RSD was calculated. Robustness study was carried out using three different volume of butanol in mobile phase used for robustness study i.e., 4 mL, 5 mL and 6 mL.

Analysis of marketed formulation[10-24]

Accurately a 20 tablet containing Metformin hydrochloride, Pioglitazone hydrochloride and Glibenclamide were weighed. Their average weight was determined. A tablet powder equivalent to 500 mg MET, 15 mg of PIO and 5 mg of GLI was weighed and transferred to a 50 mL volumetric flask. Few mL (around 20 mL) of methanol was added to the flask. It was shaken well and sonicated for 30 min. The volume was made up to the mark with methanol. It was filtered with Whatman filter paper. The filtrate was collected in a beaker. Then 2 mL of filtrate was transferred into 10 mL volumetric flask. The volume was made up to the mark

with methanol to obtain a concentration of 2000 ng/µL of Metformin hydrochloride, 60 ng/µL of Pioglitazone hydrochloride and 20 ng/µL of Glibenclamide.

A 2 µL sample of tablet containing 2000 ng/µL of Metformin hydrochloride, 60 ng/µL of Pioglitazone hydrochloride and 20 ng/µL of Glibenclamide (4000 ng/spot MET, 120 ng/spot PIO and 40 ng/spot GLI) was applied on pre-coated silica gel 60 F_{254} TLC plates (E. Merck) using Camag Linomat V (Switzerland) automatic sample spotter. The plate was developed in the solvent system consisting of butanol, 1, 4-dioxane, glacial acetic acid in a ratio of (5:3:2 v/v/v) and two drops of formic acid. After complete separation of three drugs, the plate was dried at room temperature and scanned using CAMAG TLC scanner 3 at UV 226 nm and R_f values, absorption spectra of bands and their area were recorded and concentrations of each drug from tablet solution were calculated using standard calibration curve and data was tabulated.

Accuracy/Recovery[10-24]

Recovery studies were carried out by applying the method to drug sample present in tablet dosage form to which known amount of Metformin hydrochloride, Pioglitazone hydrochloride and Glibenclamide corresponding to 80, 100, 120% of label claim was added (standard addition method). In 80% recovery study, amount of standard added is 400 mg of Metformin hydrochloride, 12 mg of Pioglitazone hydrochloride and 4 mg of Glibenclamide per tablet. In 100% recovery study, the amount of standard added is 500 mg of Metformin hydrochloride, 15 mg of Pioglitazone hydrochloride and 5 mg of Glibenclamide per tablet. In 120% recovery study, the amount of standard added is 600 mg of Metformin hydrochloride, 18 mg of Pioglitazone hydrochloride and 6 mg of Glibenclamide per tablet.

In each case, after the addition of pure standards into tablet triturate, the required amount of mixed powder (powder equivalent to 500 mg MET, 15 mg of PIO and 5 mg of GLI) was weighed and transferred to a 50 mL volumetric flask. Few mL (around 20 mL) of methanol was added to the flask. It was shaken well and sonicated for 30 min. The volume was made up to the mark with methanol. It was filtered with Whatman filter paper. The filtrate was collected in a beaker. Then 2 mL of filtrate was transferred into 10 mL volumetric flask. The volume was made up to the mark with methanol to obtain a concentration of 2000 ng/µL of Metformin hydrochloride, 60 ng/µL of Pioglitazone hydrochloride and 20 ng/µL of Glibenclamide.

A 2 µL sample of recovery studies (containing 4000 ng/spot MET, 120 ng/spot PIO and 40 ng/spot GLI) was applied on pre-coated silica gel 60 F_{254} TLC plates (E. Merck) using Camag Linomat V (Switzerland) automatic sample spotter. The plate was developed in the solvent system consisting of butanol, 1, 4-dioxane, glacial acetic acid in a ratio of (5:3:2 v/v/v) and two drops of formic acid. After complete separation of three drugs, the plate was dried at room temperature and scanned using CAMAG TLC scanner 3 at UV 226 nm and R_f values, absorption spectra of bands and their area were recorded and concentrations of each drug from samples of recovery studies were calculated using standard calibration curve and data was tabulated.

At each level, three different determinations were performed for recoveries study.

RESULTS

Linearity

Linearity of Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glibenclamide

Table 1. Calibration Table of Metformin Hydrochloride, Pioglitazone hydrochloride, and Glibenclamide at 226 nm

Concentration (ng/band)			AUC		
MET	**PIO**	**GLB**	**MET**	**PIO**	**GLB**
2000	60	10	14204.7	2540.18	267.9
6000	180	30	30126.9	6965.94	624.01
10000	300	50	39471.1	9750.45	845.86
14000	420	70	51317.6	12147.3	1136.43
18000	540	90	62371.8	15406.7	1510.26
-	-	100	-	-	1761.66

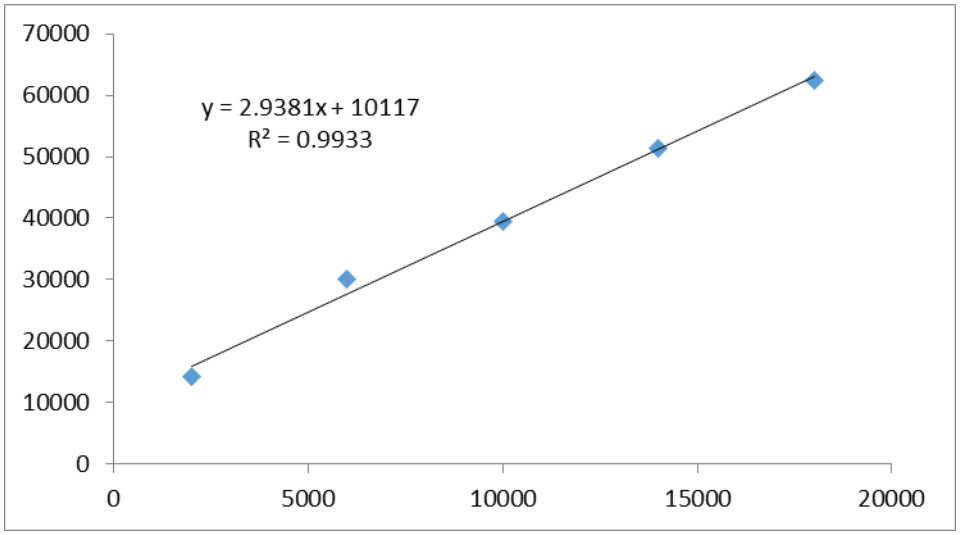

Figure 1. Standard Calibration Curve of Metformin Hydrochloride

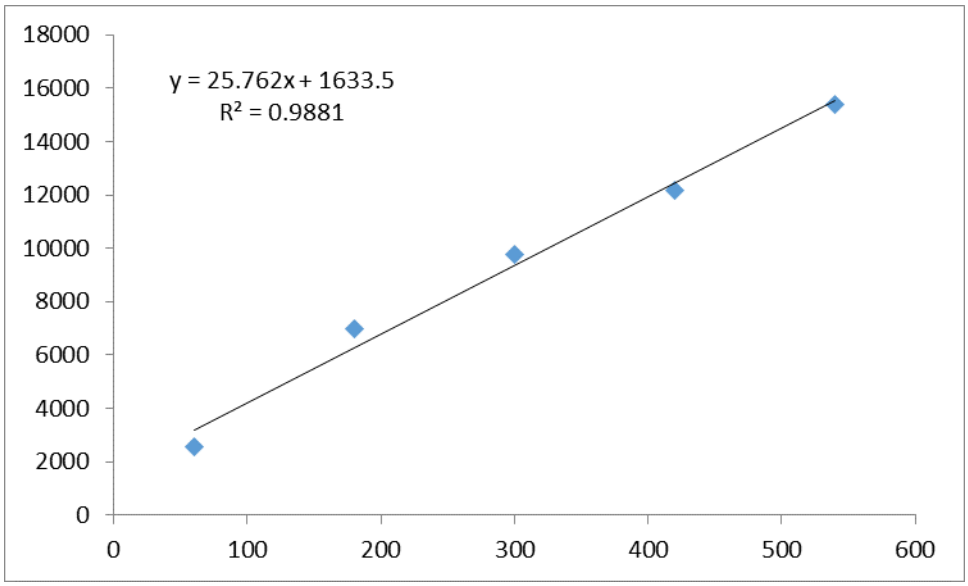

Figure 2. Standard Calibration Curve of Pioglitazone hydrochloride

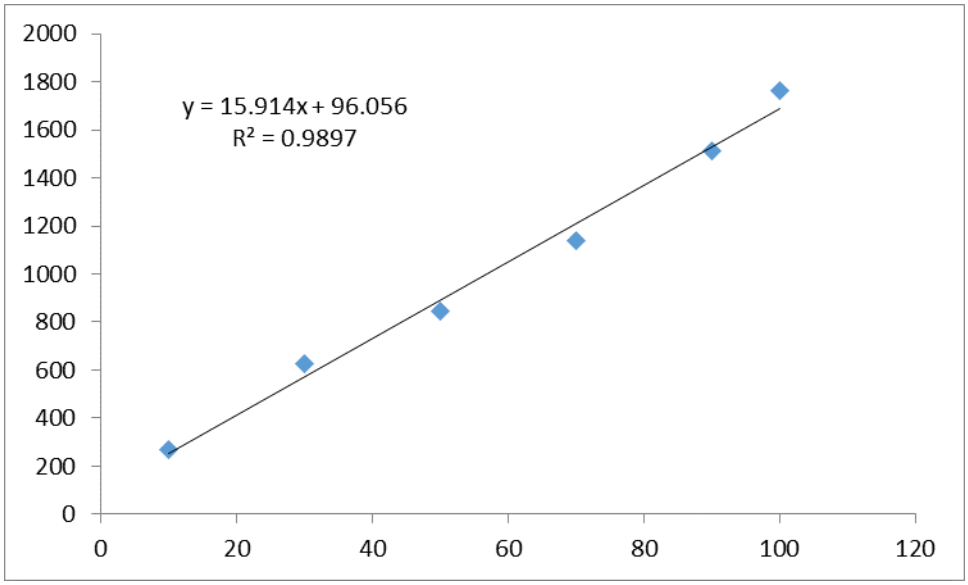

Figure 3. Standard Calibration Curve of Glibenclamide

Regression data for linearity of Metformin Hydrochloride

Table 2. Regression data for linearity of Metformin Hydrochloride

Parameter	Mean Drug found*	Standard Deviation*	% Relative Standard Deviation*	Standard Error*
Slope	2.94	2.37	80.61	9.69
Intercept	10102.1	4.167	4.167	1.701
Regression coefficient (r^2)	0.993	0.000	0.000	0.000

***n=6**

Table 3. Regression data for linearity of Pioglitazone Hydrochloride

Parameter	Mean Drug found*	Standard Deviation*	% Relative Standard Deviation*	Standard Error*
Slope	25.75	0.050	0.1941	0.020
Intercept	1633	20.455	1.2526	8.351
Regression coefficient (r^2)	0.987	0.00081	0.082	0.00033

Table 4. Regression data for linearity of Glibenclamide

Parameter	Mean Drug found*	Standard Deviation*	% Relative Standard Deviation*	Standard Error*
Slope	16.025	0.5133	3.203	0.2096
Intercept	97.54	23.684	0.526	9.669
Regression coefficient (r^2)	0.9898	0.0021	0.212	0.00087

Powder mixture analysis

Table 5. Table of powder Mixture Analysis

Sr. No.	Amount of drug present (ng/band)			Amount of drug found (ng/band)			% Drug found		
	MET	PIO	GLI	MET	PIO	GLI	MET	PIO	GLI
1	4000	120	20	3980	118.5	19.72	99.5	98.8	98.6
2	4000	120	20	3968	119.5	19.98	99.2	99.6	99.9
3	4000	120	20	3940	118.8	20.1	98.5	99.0	100.5
4	4000	120	20	4040	118.6	20.2	101	98.9	101
5	4000	120	20	3996	119.5	19.9	99.9	99.6	99.8
6	4000	120	20	4008	120	19.7	100.2	100.0	98.7

Statistical Validation of powder mixture analysis

Table 6. Data for Statistical Validation of powder mixture analysis

Drug	% Mean Drug found*	Standard Deviation*	% Relative Standard Deviation*	Standard Error*
MET	99.71	0.8612	0.8637	0.3516
PIO	99.31	0.4834	0.4867	0.1973
GLI	99.75	0.9566	0.9589	0.3905

*** n = 6**

Precision

Intra-day Precision

Table 7. Intra-day Precision for Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide

Sr. No.	Amount of drug present (ng/band)			Amount of drug found (ng/band)			% Drug found		
	MET	PIO	GLI	MET	PIO	GLI	MET	PIO	GLI
1	4000	120	20	4040	119.5	19.74	101	99.6	98.7
2	4000	120	20	3952	118.2	19.78	98.8	98.5	98.9
3	4000	120	20	3976	120.8	20.2	99.4	100.7	101
4	4000	120	20	4004	119.2	19.9	100.1	99.4	99.5
5	4000	120	20	3948	121.2	20.08	98.7	101	100.4
6	4000	120	20	3996	120.24	19.86	99.9	100.2	99.3

Statistical Validation of Intra-day precision

Table 8. Statistical Validation of Intra-day Precision

Drug	% Mean Drug found*	Standard Deviation*	% Relative Standard Deviation*	Standard Error*
MET	99.65	0.8689	0.8719	0.3547
PIO	99.9	0.9209	0.9218	0.3759
GLI	99.63	0.8937	0.8970	0.3648

*** n = 6**

Inter-day Precision

Table 9. Inter-day Precision for Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide

Sr. No.	Amount of drug present (ng/band)			Amount of drug found (ng/band)			% Drug found		
	MET	PIO	GLI	MET	PIO	GLI	MET	PIO	GLI
1	4000	120	20	3996	118.32	20.2	99.9	98.6	101
2	4000	120	20	3940	120.12	20.08	98.5	100.1	100.4
3	4000	120	20	3940	119.4	19.96	98.5	99.5	99.8
4	4000	120	20	4028	119.04	19.74	100.7	99.2	98.7
5	4000	120	20	4040	118.2	19.78	101	98.5	98.9
6	4000	120	20	3968	120	19.92	99.2	100	99.6

Statistical Validation of Inter-day precision

Table 10. Statistical Validation of Inter-day Precision

Drug	% Mean Drug found*	Standard Deviation*	% Relative Standard Deviation*	Standard Error*
MET	99.63	1.080	1.0840	0.4410
PIO	99.31	0.6795	0.6842	0.2774
GLI	99.73	0.8756	0.8779	0.3575

***n=6**

Robustness

Table 11. Robustness Data for Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide

Drug	mL of Butanol in Mobile Phase	Level	Rf Value	Amount Found (%)
MET	4	+1	0.14	98.8
	5	1	0.15	100.2
	6	-1	0.16	99.9
PIO	4	+1	0.70	101
	5	1	0.72	98.6
	6	-1	0.74	99.8
GLI	4	+1	0.86	99.1
	5	1	0.85	98.5
	6	-1	0.83	100.1

Analysis of Marketed Formulation

Chromatogram for Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glibenclamide

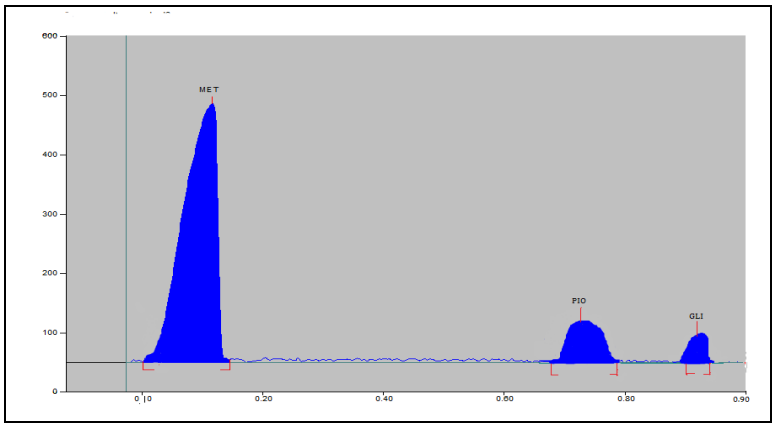

Figure. 4. Chromatogram for Marketed Formulation in butanol: 1, 4-dioxane: glacial acetic acid (5:3:2) + 2 drops of formic acid at 226 nm

Analysis of Marketed Formulation data for Metformin Hydrochloride

Table 12. Analysis of Marketed Formulation data for Metformin Hydrochloride

Sr. No.	Amount Present (mg/tab)	Amount of drug found (mg/tab)	% Drug found in formulation
1	500	502.5	100.5
2	500	494.5	98.9
3	500	499.5	99.9
4	500	500.5	100.1
5	500	498	99.6
6	500	493.5	98.7

Analysis of Marketed Formulation data for Pioglitazone Hydrochloride

Table 13. Analysis of Marketed Formulation Data for Pioglitazone Hydrochloride

Sr. No.	Amount Present (mg/tab)	Amount of drug found (mg/tab)	% Drug found in formulation
1	15	14.82	98.8
2	15	14.86	99.12
3	15	15.15	101.01
4	15	14.82	98.8
5	15	14.95	99.7
6	15	15.06	100.4

Analysis of Marketed Formulation data for Glibenclamide

Table 14. Analysis of Marketed Formulation Data for Glibenclamide

Sr. No.	Amount Present (mg/tab)	Amount of drug found (mg/tab)	% Drug found in formulation
1	5	4.94	98.9
2	5	4.98	99.7
3	5	5.05	101
4	5	4.98	99.6
5	5	4.94	98.8
6	5	5.02	100.4

Statistical Validation of Marketed Formulation

Table 15. Statistical Validation of Marketed Formulation

Drug	% Mean Drug found*	Standard Deviation*	% Relative Standard Deviation*	Standard Error*
MET	99.61	0.6998	0.7025	0.2857
PIO	99.63	0.9099	0.9132	0.3715
GLI	99.73	0.8524	0.8547	0.3480

Accuracy/Recovery

Recovery study for Metformin Hydrochloride

Table 16. Recovery Data for Metformin Hydrochloride from Formulation

Sr. No.	Concentration level (%) Recovery	Amount present in formulation (mg/tab)	Amount of standard added/Spiked (mg)	Concentration of prepared solution (ng/band)	Concentration of Solution Found (ng/band)	Total Amount Recovered (mg)	% Recovery
1	80	500	400	4000	4020	904.5	100.5
2	80	500	400	4000	3984	896.4	99.6
3	80	500	400	4000	4040	909	101
4	100	500	500	4000	3968	992	99.2
5	100	500	500	4000	3940	985	98.5
6	100	500	500	4000	3988	997	99.7
7	120	500	600	4000	3956	1087.9	98.9
8	120	500	600	4000	4000	1100	100
9	120	500	600	4000	4036	1109.9	100.9

Statistical Validation of Recovery Studies for Metformin Hydrochloride

Table 17. Statistical Validation of Recovery Studies for Metformin Hydrochloride

Drug	Recovery Level	% Mean Drug found*	Standard Deviation*	% Relative Standard Deviation*	Standard Error*
MET	80 %	100.36	0.7095	0.7069	0.4096
	100 %	99.13	0.6028	0.6080	0.3480
	120 %	99.93	1.002	1.002	0.5783

3.7.2 Recovery study for Pioglitazone Hydrochloride

Table 18. Statistical Validation of Recovery Studies for Pioglitazone Hydrochloride

Sr. No.	Concentration level (%) Recovery	Amount present in formulation (mg/tab)	Amount of standard added/Spiked (mg)	Concentration of prepared solution (ng/band)	Concentration of Solution Found (ng/band)	Total Amount Recovered (mg)	% Recovery
1	80	15	12	120	119.4	26.86	99.5
2	80	15	12	120	121.2	27.27	101
3	80	15	12	120	120.8	27.18	100.7
4	100	15	15	120	119.9	29.97	99.92
5	100	15	15	120	118.2	29.55	98.5
6	100	15	15	120	118.4	29.61	98.7
7	120	15	18	120	120.9	33.26	100.8
8	120	15	18	120	118.6	32.80	98.9
9	120	15	18	120	119.1	32.76	99.3

Statistical Validation of Recovery Studies for Pioglitazone Hydrochloride

Table 19. Statistical Validation of Recovery Studies for Pioglitazone Hydrochloride

Drug	Recovery Level	% Mean Drug found*	Standard Deviation*	% Relative Standard Deviation*	Standard Error*
PIO	80 %	100.4	0.7937	0.790	0.4583
	100 %	99.04	0.7686	0776	0.4438
	120 %	99.66	1.002	1.005	0.5783

*** n = 3**

Table 20. Statistical Validation of Recovery Studies for Glibenclamide

Sr. No.	Concentration level (%) Recovery	Amount present in formulation (mg/tab)	Amount of standard added/Spiked (mg)	Concentration of prepared solution (ng/band)	Concentration of Solution Found (ng/band)	Total Amount Recovered (mg)	% Recovery
1	80	5	4	5	4.93	8.874	98.6
2	80	5	4	5	4.97	8.946	99.4
3	80	5	4	5	5.02	9.036	100.4
4	100	5	5	5	5.04	10.08	100.8
5	100	5	5	5	4.945	9.89	98.9
6	100	5	5	5	4.96	9.92	99.2
7	120	5	6	5	4.985	10.967	99.7
8	120	5	6	5	4.955	10.901	99.1
9	120	5	6	5	5.03	11.066	100.6

Table 21. Statistical Validation of Recovery Studies for Glibenclamide

Drug	Recovery Level	% Mean Drug found*	Standard Deviation*	% Relative Standard Deviation*	Standard Error*
GLI	80 %	99.46	0.9018	0.9066	0.5207
	100 %	99.63	1.021	1.0247	0.5897
	120 %	99.8	0.7550	0.7565	0.4359

DISCUSSION

Metformin hydrochloride is Biguanides, Pioglitazone hydrochloride is Thiazolidinediones and Glibenclamide are Sulfonylureas class of anti-diabetic drug which used for diabetes. Metformin hydrochloride, Pioglitazone hydrochloride, and Glibenclamide are available in different dosage form, they widely used for the diabetes patient to give orally.

The present work dealt with simultaneous estimation of Metformin hydrochloride, Pioglitazone hydrochloride, and Glibenclamide in bulk and multi-component formulation (pioglucored forte) by HPTLC method. The working wavelength selected for HPTLC method is 226 nm. This chromatographic method was developed, validated and successfully applied

to the multi-component tablet dosage form for determination of the amount of Metformin hydrochloride, Pioglitazone hydrochloride, and Glibenclamide. The results of tablet analysis and recovery studies revealed that developed method can be successfully applied to the routine determination of these three drugs from tablet dosage forms.

CONCLUSION

The developed HPTLC method offers several advantages such as rapidity, usage of simple mobile phase and easy sample preparation steps. Further, improved sensitivity makes it specific and reliable for its intended use. Hence, this method can be applied to the analysis of pure drug and pharmaceutical tablet dosage forms.

From the present study, it can be concluded that the proposed method is simple, sensitive, precise, specific, accurate and reproducible. Results of validation parameters demonstrated that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2A/B.

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