DUAL RUN-DUAL WAVELENGTH HPTLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF FIVE ANTIDIABETIC DRUGS IN BULK AND THEIR PHARMACEUTICAL DOSAGE FORMS

ARPIT PATWARI[a]*, URVISH DESAI[a], BHANUBHAI SUHAGIA[b]

[a] Department of Quality Assurance, L.M. College of Pharmacy, Ahmedabad, Gujarat, India. [b] Faculty of Pharmacy, Dharamsinh Desai University, Nadiad, Gujarat, India. Email: patwari_arpit@yahoo.com

ABSTRACT

Objective: A sensitive, selective, precise and validated high-performance thin layer chromatographic method for analysis of five Antidiabetic drugs in bulk and their available combined dosage forms has been developed.

Methodology: The method employed TLC aluminum plates precoated with silica gel 60 F_{254} as the stationary phase. A dual run technique was adopted for better resolution amongst all five drugs with solvent system initially chloroform: methanol: Ammonia (9:1:0.2 v/v/v) upto half the height of the plate. The plates were dried and developed again in system chloroform: methanol: Ammonia (9:1.5:0.2 v/v/v) of higher polarity till 80 mm height. This was found to give compact spots for all five drugs. (Rf value of Metformin Hydrochloride, Repaglinide, Glimepiride, Pioglitazone and Sitagliptin Phosphate = 0.10 ±/0.02, 0.23 ±/0.02, 0.45 ±/0.02, 0.54 ±/0.02 and 0.66 ±/0.02 respectively). Densitometric analysis was carried out in the absorbance mode at 238 nm for Metformin Hydrochloride (MFH), Repaglinide (REP) and Glimepiride (GLM) while at 268nm for Pioglitazone (PIO) and Sitagliptin Phosphate (SGP).

Results: The linear regression data for the calibration plots showed good linear relationship with r^2 close to 0.9999 in the concentration range of 200-800 ng/spot for drugs other than SGP which showed linearity between 2000-8000ng/spot. The method was validated for precision, accuracy, ruggedness and recovery as per ICH guidelines and was applied for quantification in various formulations.

Conclusion: The developed method could be used for quantification of any of the selected five drugs in bulk or in their available combined pharmaceutical dosage forms.

Keywords: HPTLC Method, Antidiabetic Drugs, Dual Run Technique, Simultaneous Determination

INTRODUCTION

With advancement and modernization in field of drug development, newer and newer drugs have been invented every year. In past two decades several new categories of oral antidiabetic drugs have been invented and formulated in various combinations. A market survey carried out clearly indicated the use of oral antidiabetics i.e. Metformin Hydrochloride, Repaglinide, Glimepiride, Pioglitazone and newer DPP4 inhibitor drug Sitagliptin Phosphate, either alone or in combination with one another, the most by physicians and specialists. Metformin Hydrochloride (MFH) and Glimepiride (GLM) are sulphonylureas; Repaglinide (REP) a metaglinide; Pioglitazone

(PIO) a thiazolidinedione and Sitagliptin Phosphate (SGP) a DPP_4 inhibitor[1] (Figure 1) regulates the control of insulin in body via several mechanisms and maintains body's glucose levels. Today Indian markets are flooded with more than 800 generic brands of these drugs and same is the scenario globally [2]. Several methods like UV, HPLC, LC-MS, LC-MS MS and capillary electrophoresis [3-21] have been developed for quantification of these drugs alone or in combination. Due to its better resolution, cost effectiveness and higher sensitivity, High pressure thin layer chromatography is a widely adopted method of analysis. Thus it was thought of interest to develop a universal industrially applicable method for quantification of these drugs using HPTLC method.
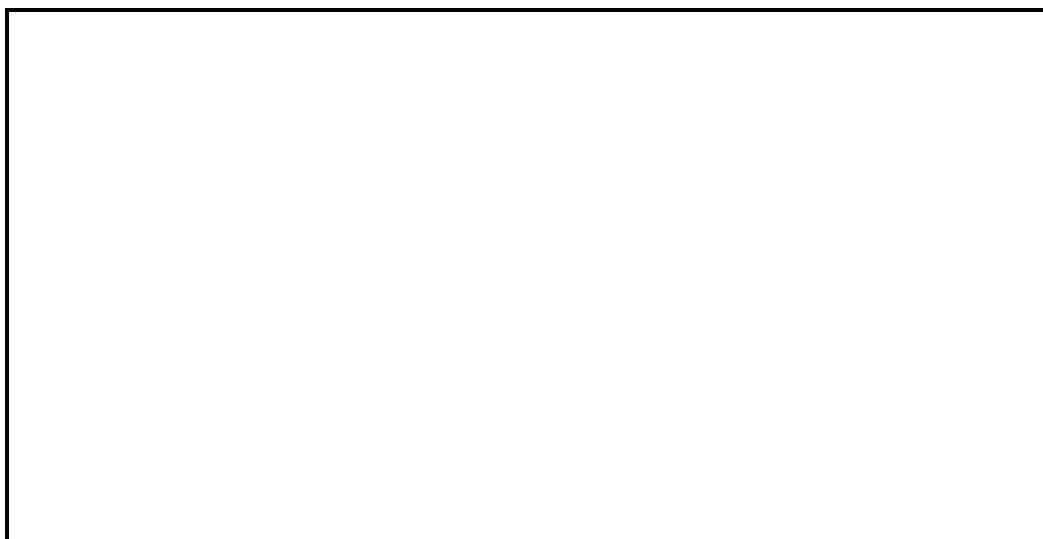

Fig. 1: Structures of Antidiabetic drugs

MATERIALS AND METHODS

Chemicals and Reagents

Pure drug samples of Metformin hydrochloride, Pioglitazone, Glimepiride and Repaglinide were gifted by Torrent Research Centre, Ahmedabad, Gujarat. Pure drug sample of Sitagliptin Phosphate was gifted from Sun Pharmaceuticals, Vadodara, Gujarat. Chloroform, methanol, ammonia and all other chemicals utilized during experiments were of analytical grade and purchased from SD Fine Chemicals, New Delhi, India. All the market formulations were purchased from local pharmacy.

Instrumentation and Chromatographic conditions

The HPTLC system (Camag, Switzerland) consisted of Linomat IV auto-spotter connected to a nitrogen cylinder, CAMAG TLC scanner-3 and CATS-4 software. A twin trough chamber (10×10 cm^2) was utilized for development of the plates. Pre-coated silica gel 60 F$_{254}$ TLC plates (10×10 cm^2, layer thickness 0.2 mm, Merck, Darmstadt, Germany) were used as a stationary phase. TLC plates were pre-washed with methanol and activated at 60 ^{0}C for 5 min prior to sample application. The standard and formulation samples in mixture of all drugs were spotted on precoated TLC plates in the form of narrow bands of lengths 5 mm. Linear ascending development was carried out in twin trough chamber (10 x 10 cm^2). Dual development technique was adopted for resolution amongst all drugs, where in initially plate was developed upto 4 cm height in mobile phase chloroform: methanol: ammonia (9: 1: 0.1 v/v/v), plate was dried and re-developed in more polar mobile phase system chloroform: methanol: ammonia (9: 1.5: 0.1 v/v/v) upto 8 cm height. Densitometric scanning was performed on CAMAG TLC scanner 3 in Absorbance/Reflectance mode, operated by CATS-4 software. The spots were analyzed at a dual wavelength mode i.e. at 238 nm for MFH, REP and GLM while 268 nm for PIO and SGP. The slit dimensions used in the analysis were length and width of 5 × 0.45 mm with a scanning rate of 20 mm/s.

Preparation of standard stock solutions and calibration curves

For preparation of stock solutions, accurately weighed MFH, REP, GLM and PIO (10 mg) were transferred into a series of 10 mL volumetric flask, dissolved and diluted with methanol. Accurately weighed SGP (100 mg) was transferred into a 10 mL volumetric flask, dissolved and diluted with double distill water. For preparation of working standard solution, aliquot (1 mL) from each flask of stock solutions were transferred into a 10 mL volumetric flask and diluted with methanol upto the mark to obtain a final concentration of 100 µg/mL of MFH, REP, GLM and PIO while 1000 µg/mL of SGP. Calibration was obtained by applying mixture of working standard solutions ranging from 2.0 to 8.0 µL by Hamilton syringe with the help of Linomat IV auto-spotter on TLC plate that gave concentration 200-800 ng/spot for MFH, REP, PIO and GLM and 2000-8000 ng/spot for SGP. This was replicated for five times. From the developed plates calibration curve was plotted as mean peak areas versus concentration.

Analysis of marketed tablet formulations

1) OBIMAT-SR: (Metformin Hydrochloride Tablets - 500 mg)

Accurately weigh and powder 10 tablets. Powder equivalent to 500 mg of Metformin Hydrochloride was transferred to 50 mL volumetric flask. Methanol (30 mL) was added to it and was sonicated for 15 min and filtered using whatmann filter paper 41. The solution was diluted with methanol up to the mark. Aliquot quantity (1 mL) was transferred to 50 mL volumetric flask and diluted with methanol up to the mark. Take 2.5ml of above solution into 10ml volumetric flask and diluted to the mark with methanol. The solution (10 µL) was applied on TLC plate along with standard calibration curve.

2) JANUVIA: (Sitagliptin phosphate-100mg)

Accurately weigh and powdered 10 tablets. Powder equivalent to 100 mg of Sitagliptin phosphate was transferred to 50 mL volumetric flask. Triple distilled water (30 mL) was added to it and was sonicated for 15 min and filtered using whatmann filter paper

41. and diluted with triple distilled water up to the mark. The solution (2 µL) was applied on TLC plate along with standard calibration curve.

3) ACTOS: (Piglitazone-15mg)
Accurately weigh and powdered 10 tablets. Powder equivalent to 15 mg of Pioglitazone was transferred to 50 mL volumetric flask. methanol (30 mL) was added to it and was sonicated for 15 min and filtered using whatmann filter paper 41. and diluted with methanol up to the mark. The solution (2 µL) was applied on TLC plate along with standard calibration curve.

4) GLP: (Glimepiride-2mg)
Accurately weigh and powdered 10 tablets. Powder equivalent to 2 mg of Glimepiride was transferred to 50 mL volumetric flask. methanol (30 mL) was added to it and was sonicated for 15 min and filtered using whatmann filter paper 41. and diluted with methanol up to the mark. The solution (5 µL) was applied on TLC plate along with standard calibration curve.

5) ACTOPLUS MET: (Metformin hydrochloride-500mg + Pioglitazone-15mg)

Accurately weigh and powder 10 tablets. Powder equivalent to 500 mg of Metformin Hydrochloride was transferred to 50 mL volumetric flask. Methanol (30 mL) was added to it and was sonicated for 15 min and filtered using whatmann filter paper 41. The solution was diluted with methanol up to the mark. The solution (2 µL) was applied on TLC plate along with standard calibration curve for detection of pioglitazone. Aliquot quantity (1 mL) was transferred to 50 mL volumetric flask and diluted with methanol up to the mark. Take 2.5ml of above solution into 10ml volumetric flask and diluted to the mark with methanol. The solution (10 µL) was applied on TLC plate along with standard calibration curve for detection of Metformin hydrocloride.

6) EUREPA- TOR-MF: (Metformin hydrochloride-500mg + Repaglinide-2mg)

Accurately weigh and powder 10 tablets. Powder equivalent to 500 mg of Metformin Hydrochloride was transferred to 50 mL volumetric flask. Methanol (30 mL) was added to it and was sonicated for 15 min and filtered using whatmann filter paper 41. The solution was diluted with methanol up to the mark. The solution (5 µL) was applied on TLC plate along with standard calibration curve for detection of Repaglinide. Aliquot quantity (1 mL) was transferred to 50 mL volumetric flask and diluted with methanol up to the mark. Take 2.5ml of above solution into 10ml volumetric flask and diluted to the mark with methanol. The solution (10 µL) was applied on TLC plate along with standard calibration curve for detection of Metformin hydrocloride.

7) JANUMET: (Metformin hydrochloride-500mg+Sitagliptin phosphate-100mg)

Accurately weigh and powder 10 tablets. Powder equivalent to 500 mg of Metformin Hydrochloride was transferred to 50 mL volumetric flask. Methanol (30 mL) was added to it and was sonicated for 15 min and filtered using whatmann filter paper 41. The solution was diluted with methanol up to the mark. The solution (2µL) was applied on TLC plate along with standard calibration curve for detection of sitagliptin phosphate. Aliquot quantity (1mL) was transferred to 50 mL volumetric flask and diluted with methanol up to the mark. Take 2.5ml of above solution into 10ml volumetric flask and diluted to the mark with methanol. The solution (10 µL) was applied on TLC plate along with standard calibration curve for detection of Metformin hydrocloride.

8) AMARYL-MZ: (Metformin hydrochloride-500mg+Glimepiride-2mg)

Accurately weigh and powder 10 tablets. Powder equivalent to 500 mg of Metformin Hydrochloride was transferred to 50 mL volumetric flask. Methanol (30 mL) was added to it and was sonicated for 15 min and filtered using whatmann filter paper 41. The solution was diluted with methanol up to the mark. The solution (5µL) was applied on TLC plate along with standard calibration curve for detection of Glimepiride and The solution (10 µL) was applied on TLC plate along with standard calibration curve for detection of Metformin hydrocloride.

9) TRIGLYNASE-2

(Metformin hydrochloride-500mg+Piogitazone-15mg+Glimepiride 2mg)

Accurately weigh and powder 10 tablets. Powder equivalent to 500 mg of Metformin Hydrochloride was transferred to 50 mL volumetric flask. Methanol (30 mL) was added to it and was sonicated for 15 min and filtered using whatmann filter paper 41. The solution was diluted with methanol up to the mark. The solution (5µL) was applied on TLC plate along with standard calibration curve for detection of Glimepiride and the solution (2 µL) was applied on TLC plate for analysis of pioglitazone. Aliquot quantity (1 mL) was transferred to 50 mL volumetric flask and diluted with methanol up to the mark. Take 2.5ml of above solution into 10ml volumetric flask and diluted to the mark with methanol. The solution (10 µL) was applied on TLC plate along with standard calibration curve for detection of Metformin hydrocloride.

Method Validation

Linearity

For linearity study 2.0 to 8.0 µL of working standard solution was spotted on precoated TLC plate. So the linearity responses were accessed in range of 200-800 ng/spot for MFH, REP GLM and PIO while 2000-8000 ng/spot for SGP.

Precision

Precision of the method was determined in the terms of intra-day and inter-day variation (%RSD). Intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

Accuracy

To the pre-analyzed sample a known amount of standard solution of pure drug was spiked at three different levels (80%, 100% and 120%). These solutions were subjected to re-analysis by the proposed method.

Sensitivity

The sensitivity of measurement of MFH, REP, GLM, PIO and SGP by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation. Based on the standard deviation of the response and the slope, LOD and LOQ were estimated using the formulae:

LOD= 3.3 σ/S

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

LOQ = 10 σ/S

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

LOD and LOQ were determined from the standard deviations of the responses for five replicate determinations.

RESULTS AND DISCUSSION

Method development

The TLC procedure was optimized for determination of all the chosen antidiabetic drugs. Dual run development resulted in good resolution with sharp and symmetrical peaks at Rf of MPH, REP, GLM, PIO and SGP = 0.10 ±/0.02, 0.23 ±/0.02, 0.45 ±/0.02, 0.54 ±/0.02 and 0.66 ±/0.02 respectively (Figure 2). Scanning of the plates was done at dual wavelength (238 and 268 nm) as MFH, REP and GLM showed wavelength maximum at 237-238 nm while PIO and SGP showed at 268 respectively. It was observed that pre-washing of TLC plates with methanol (followed by drying and activation) ensured good reproducibility and peak shape of all the drugs.
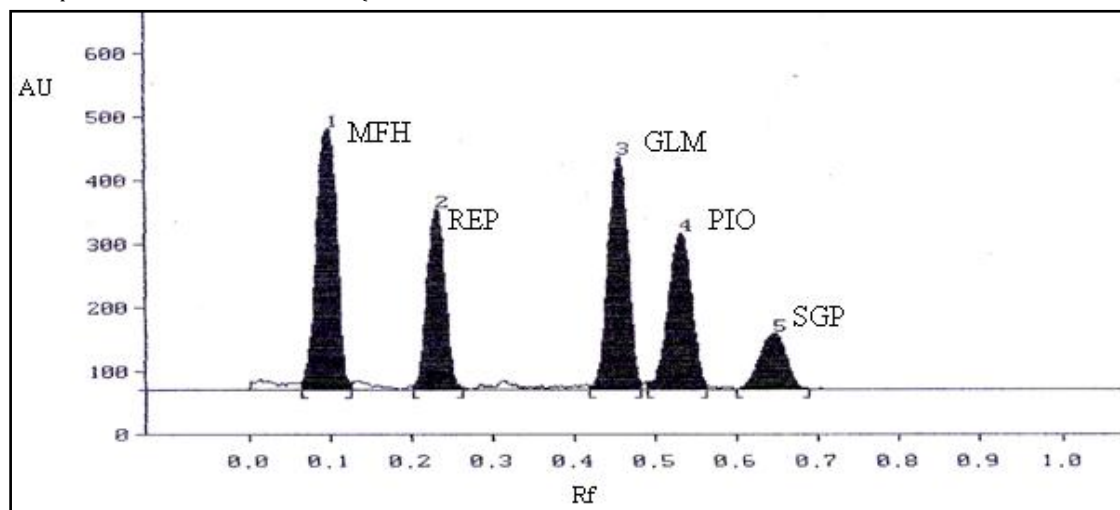

Fig. 2: Chromatogram of standard drugs in mixture

Validation

Linearity

Linear regression data for the calibration plots revealed good linear relationships between area and concentration over the ranges 200-800 ng/spot for MFH, REP, GLM and PIO while 2000-8000 ng/spot for SGP. The linear equations and regression for the calibration plots are given in Table 1.

Precision

The precision of the method was expressed as relative standard deviation (RSD %). The %RSD values for intra-day precision study and inter-day study listed in Table 2 were ≤2.0%, confirming that the method was sufficiently precise.

Accuracy

When the method was used for accuracy and subsequent analysis of all the drugs from the pharmaceutical dosage form, and spiked with 80, 100, and 120% of additional pure drug, the recovery was found close to 100 % confirming the accurateness of the method. (Table 3)

Sensitivity

The LOD and LOQ were calculated by equation. The LOD and LOQ values for all the drugs were as listed in Table 4.

Analysis of marketed tablet formulations

Several available brands in varying combinations of all selected anti diabetic drugs when analyzed using developed method gave sharp and well defined peaks at their respective Rf. The result in table also indicates that there was no interference from the excipients which are commonly present in tablet dosage form.

Table 1: Calibration details of antidiabetic drugs

Sr. No	Drug	Linearity Equation	Regression (r^2)
1.	MFH	y = 10.63x + 1376	r^2 = 0.999
2.	REP	y = 5.78x + 572.9	r^2 = 0.997
3.	GLM	y = 10.33x + 2414	r^2 = 0.998
4.	PIO	y = 6.60x + 1867	r^2 = 0.998
5.	SGP	y = 0.74x + 1164	r^2 = 0.999

Table 2: Intraday and Interday precision data

	Conc. (ng/spot)	Intraday Area Mean(n=3) ± SD	% RSD	Interday Area Mean(n=3) ± SD	% RSD
MFH	200	3454.03 ± 32.39	0.93	3498.06 ± 65.42	1.87
	500	6658.70 ± 35.28	0.52	6684.66 ± 50.31	0.75
	800	9624.40 ± 26.15	0.27	9807.00 ± 73.47	0.74
REP	200	1657.20 ± 32.92	1.98	1656.46 ± 29.42	1.77
	500	3534.56 ± 34.40	0.97	3509.06 ± 29.25	0.83
	800	5052.50 ± 27.74	0.54	5097.13 ± 45.30	0.88
GLM	200	3162.30 ± 44.15	1.39	3126.96 ± 56.57	1.81
	500	5257.63 ± 35.17	0.66	5169.23 ± 57.11	1.10
	800	7039.40 ± 27.10	0.38	7052.27 ± 52.00	0.73
PIO	200	4353.27 ± 43.41	0.99	4396.67 ± 77.86	1.77
	500	7533.40 ± 49.12	0.65	7575.80 ± 39.09	0.51
	800	10655.50 ± 43.46	0.40	10581.13 ± 73.43	0.67
SGP	2000	2654.36 ± 33.76	1.27	2652.67 ± 49.58	1.89
	5000	4926.33 ± 52.61	1.07	4853.53 ± 55.12	1.13
	8000	7190.90 ± 39.02	0.54	7206.43 ± 56.90	0.79

Table 3: Results of % Recovery studies (n=3) by standard addition technique

% Recovery	MFH	PIO	GLM	SGP
0%	98.83	98.10	98.50	99.51
50%	100.17	97.47	97.83	98.84
100%	97.29	99.07	100.04	97.93
150%	98.35	98.76	96.39	99.98
Mean ± SD	98.60 ± 1.46	98.43 ± 0.85	98.08 ± 1.84	98.92 ± 1.03

Table 4: LOD and LOQ data for drugs

	MFH	REP	GLM	PIO	SGP
LOD (µg/mL)	23.12	20.26	20.16	28.44	225.92
LOQ (µg/mL)	70.07	61.41	61.08	86.18	684.62

Table 5: Assay results for marketed tablet formulations

Sr. No.	Brand	Company	Drug	% Assay ± SD
1.	OBIMET-SR	Abbott	Metformin Hydrochloride-500mg	98.47 ± 1.18
2.	JANUVIA	Merck	Sitagliptin Phosphate-100mg	99.86 ± 1.31
3.	ACTOS	Mylan	Pioglitazone-15mg	98.45 ± 1.03
4.	GLP	IPCA	Glimepiride-2mg	97.27 ± 1.16
5.	ACTOPLUS MET	Mylan	Metformin Hydrochloride-500mg	99.63 ± 0.28
			+ Pioglitazone-15mg	97.48 ± 0.90
6.	EUREPA MF	Torrent	Metformin Hydrochloride-500mg	98.73 ± 0.52
			+ Repaglinide-2mg	98.50 ± 1.24
7.	JANUMET	Merck	Metformin Hydrochloride-500mg	98.02 ± 0.45
			+ Sitagliptin Phosphate-100mg	97.67 ± 0.97
8.	AMARYL-M2	Sanofi Aventis	Metformin Hydrochloride-500mg	99.73 ± 0.26
			+ Glimepiride-2mg	95.28 ± 0.73
9.	TRIGLYNASE-2	USV	Metformin Hydrochloride-500mg	98.14 ± 0.34
			+ Pioglitazone-15mg	97.85 ± 1.16
			+ Glimepiride-2mg	97.26 ± 0.91

CONCLUSION

The developed HPTLC method is simple, precise, accurate and reproducible and can be used for determination of MFH, REP, GLM, PIO and SGP in bulk and their available combined pharmaceutical dosage forms. The method was validated as per International Conference on Harmonization (ICH) guidelines.

REFERENCES

1. Ramchandran A: Current Status of Diabetes in India and Need for Novel Therapeutic Agents. Journal of the Association of Physicians of India 2010, 58, 7-9.
2. Chaung L, Chyan Y: Dipeptidyl Peptidase-IV Inhibitors: An evolving treatment for type 2
3. diabetes from the incretin concept. Recent Patents on Endocrine, Metabolic & Immune Drug Discovery 2007; 1: 15-24.
4. Kong A, Vanessa N: Dipeptidyl Peptidase (DPP)-IV Inhibitor: A novel class of oral anti-hyperglycemic agents. Drug Review 2007; 12: 5, 1-2.
5. Elkady E, Ayuob B, Bagary R: Spectrophotometric methods for the determination of Sitagliptin and Vidagliptin in Bulk and Dosage Forms. International Journal of Biomedical science 2011; 7 (1): 55-61.
6. Sahoo P, Sharma R, Chaturvedi S: Simultaneous estimation of Metformin Hydrochloride and Pioglitazone Hydrochloride by RP-HPLC method from combined tablet dosage form. Indian Journal of Pharmaceutical Science 2008; 70 (3): 383-386.
7. ICH Draft Guideline on Validation of Analytical procedures: Definitions and Terminology, Federal register, Switzerland: IFPMA. 1995.
8. ICH, Stability Testing of New Drug Substance and Products (Q1A).International Conference on harmonization. Geneva: IFPMA. 2000.
9. Kar A, Chaudhury J: HPLC method of metformin hydrochloride. Indian Journal of Pharmaceutical Sciences 2009; 6: 317-319.
10. Dayade GK et al: Estimation of metformin hydrochloride and glimepiride in tablet formulations by UV-Visible spectrophotometry. Indo-American journal of pharma research 2013; 3(1): 220-225.
11. Prasanth VG et al: Development and validation Of UV Spectroscopic Methods for the estimation of repaglinide and metformin hydrochloride in synthetic mixture. International Journal Of Pharmaceutical Science And Health Care 2012; 2(2): 2249-2255.
12. Riad SM, Rezk MR, Mahmoud GY: Spectrophotometric determination of Sitagliptin and Metformin in their pharmaceutical formulation. Pharmacie Globale 2012; 3 (5): 1-4.
13. Yuen HH, Peh KK: Simple high performance liquid chromatographic method for the determination of metformin in human plasma. J.Chromatography B 1988; 710: 243–246.
14. Betteridge J: Chromatographic method of analysis of metformin hydrochloride. Clin Pharmacol 1998; 25: 243-58.
15. Lotfy S: Determination of Pioglitazone hydrochloride in tablets by high performance liquid chromatography. Pak. J. Anal. Environ. Chem 2008; 9(2): 118- 121.
16. Wanjari DB and Gaikwad NJ: Stability Indicating RP-HPLC method for determination of pioglitazone from tablets. Indian Journal of Pharmaceutical Sciences 2005; 67 (2): 256-258.
17. Jedlicka A, Klimes J and T Grafnetterová: Reversed-phase HPLC methods for purity test and assay of pioglitazone hydrochloride in tablets. Die Pharmazie 2004; 59(3): 178-182.
18. Alexandar S, Diwedi R, and Chandrasekar M J: A RP-HPLC method for simultaneous estimation of metformin and pioglitazone in pharmaceutical formulation. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2007; 1(4): 858.
19. Singh D, Dwivedi S, Kushnoor A: Development and validation of a hptlc method for simultaneous estimation of pioglitazone and glimepiride in bulk and tablet dosage form. International Journal of Biomedical and Advanced Research 2011; 2 (9): 368-377.
20. Kakde R and Kale D: Simultaneous determination of pioglitazone, metformine and glimepiride in pharmaceutical preparations. Journal of Planner Chromatography 2011; 24: 331–336.
21. Sumitra M: Devolopment of RP-HPLC method and it's validation for simultaneous estimation of sitagliptin and Metformin. International Journal of Pharmaceutical and Chemical Sciences 2012; 1(1): 360-364.
22. Maleshwararao C, Suryanarayana M, Mukkanti K: Simultaneous determination of sitagliptin phosphate monohydrate and metformin hydrochloride in tablets by a validated UPLC method. Scientica Pharmaceuticam 2011; 8 (1): 1-9.
23. Kadamb A, Havele S, Dhaneshwar S: Validated HPTLC method for simultaneous estimation of lafutidine and domperidone in bulk drug and formulation. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(4): 221-225.
24. Mohite V, Potawale S, Gabhe S: Development and validation of HPTLC method for simultaneous estimation of Paracetamol, diclofenac potassium and chlorzoxazone in bulk drug and tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(2): 432-435.