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Application of High performance thin layer chromatography-densitometry for the simultaneous determination of Metformin hydrochloride and Glipizide in bulk drug and tablet formulation

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ABSTRACT

Method describes an HPTLC method for the simultaneous determination of glipizide and metformin HCl from tablet dosage form. This employes a precoated silica gel 60 F_{254} (0.2 mm thickness) on aluminium sheets and a mobile phase Ammonium sulfate (0.5%): water: methanol: ethyl acetate in the ratio of 5.0: 5.0: 2.5: 2.5 (v/v/v/v), having chamber saturation for 30 min at room temperature. The mobile phase was run upto 8cm. The Rf values were found to be 0.31 and 0.70 for glipizide and metformin HCl respectively. The plate was scanned and quantified at 245 nm. The linear detector response was observed between 100 ng.spot^{-1} to 500 ng.spot^{-1} and 1000 ng.spot^{-1} to 5000 ng.spot^{-1} for glipizide and metformin HCl respectively. The method so developed was validated for its accuracy and precision. The LOD and LOQ were found to be 24.5 ng.spot^{-1} and 80 ng.spot^{-1} for glipizide and 60 ng.spot^{-1} and 200 ng.spot^{-1} for metformin HCl respectively. The recovery was carried out by standard addition method. The Average recovery was found to be 99.96 % and 99.77% for glipizide and metformin HCl respectively.

Keywords: High performance thin layer chromatography, Metformin HCl, Glipizide, Antidiabetic, Tablet formulation.

INTRODUCTION

For many patients with Type 2 diabetes, monotherapy with an oral antidiabetic agent is not sufficient to reach target glycaemic goals and multiple drugs may be necessary to achieve adequate control [1]. In such cases a combination of metformin and one of the sulfonylureas (SU) is used [2]. The most commonly prescribed medications for Type 2 diabetes are

metformin HCl chemically [1,1-dimethyl biguanide hydrochloride] (Fig 1) and the second generation sulfonylureas like glipizide [(3-azabicyclo[3.3.0]oct-3-yl)-3-(*p*-tolylsulphonyl)-urea] (Fig 2) .A combination of 500 mg of metformin and 5 mg of glipizide (T-1) and 250 mg of metformin and 2.5 mg of glipizide (T-2), are available commercially as tablets [3], which is used in the treatment of non insulin dependent diabetes mellitus (NIDDM).

Several methods are available for the estimation of metformin [4-9] and glipizide individually [11], in combination with other antidiabetic drugs [12-19] and in combination with each other [20-23].

Over the past decade HPTLC has been successfully used in the analysis of pharmaceuticals, plant constituents, and biomacromolecules. Several samples can be run simultaneously using a small quantity of mobile phase, thus lowering analysis time and cost per analysis. It also facilitates automatic application and scanning in situ.

To our knowledge, no article related to HPTLC determination of glipizide and metformin HCl in fixed dose combination has been reported in literature. The objective of the present work was to develop an accurate, specific and reproducible method for the simultaneous determination of glipizide and metformin in pharmaceutical formulations by HPTLC.
The proposed method was validated as per ICH guidelines [24] and its updated international convention [25-26].

MATERIALS AND METHODS

Pharmaceutical grade glipizide [GLIPI] (batch no. F144-07020) and metformin HCl [MET] (batch no. 1997418) working standards were obtained as generous gifts from Ranbaxy Pvt. Ltd. Indore, India respectively. Fixed-dose combination tablets Dibizide-M (batch no. DBMY0231) containing 5 mg of GLIPI and 500 mg MET were purchased from Microlabs limited (Tablet-A), Glimet (batch no. 13003802) containing 2.5 mg of GLIPI and 250 mg MET were purchased from Franco Indian Pharma (Tablet-B). All chemicals and reagents were of analytical-grade and were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

The HPTLC system consisted of a Camag Linomat 5 semi-automatic spotting device (Camag, Muttenz, Switzerland), a Camag twin-trough chamber (10 cm × 10 cm), Camag winCATS software 1.4.4.6337 and a 100 µl Hamilton syringe. Sample application was done on precoated silica gel 60 F_{254} TLC plates (10 cm × 10 cm). TLC plates were pre-washed with methanol and activated at 80°C for 5 min prior to the sample application. Densitometric analysis was carried out utilizing Camag TLC scanner 3.

Preparation of standard and sample solutions

Standard stock solutions at a concentration of 100 $mg.mL^{-1}$ of MET and 10 $mg.mL^{-1}$ of GLIPI were prepared separately using methanol. From the standard stock solution, the mixed standard solution was prepared by methanol to obtain 10 $mg.mL^{-1}$ of MET and 0.1 $mg.mL^{-1}$ of GLIPI.

For the analysis of tablets, 20 tablets of each T-1 and T-2 were weighed and finely ground in a mortar. For T-1 the portion equivalent to 5 mg of GLIPI and 500 mg of MET was transferred in a 25 mL volumetric flask, 20 mL of diluent was then added, and sonication was done for 15 min with swirling. After sonication, the volume was made up to the mark with the diluent, and mixed well. The solution was filtered through Whatman filter paper 41.

For T-2, the portion equivalent to 2.5 mg of GLIPI and 250 mg of MET was taken and transferred in 25 mL volumetric flask, 20 mL of diluent was then added, and sonication was done for 15 min with swirling. After sonication, it was made up to volume with the diluent, and mixed well. The solution was filtered through Whatman filter paper 41.

For both T-1 and T-2, six determinations were performed.

HPTLC method and chromatographic Conditions

In the proposed HPTLC method, the samples were streaked on the precoated TLC plates in the form of a narrow band 6 mm in length, 10 mm from the bottom and margin and 10 mm apart at a constant application rate of 0.1 µ.L^{-s} by using a nitrogen aspirator. A Camag Twin Trough Chamber was saturated for 30 min at room temperature (25 ± 2 °C) with the mobile phase containing a mixture of ammonium sulfate (0.5%): water: methanol: ethyl acetate in the ratio of 5.0: 5.0: 2.5: 2.5 (v/v/v/v). After chamber saturation, the plates were developed to a distance of 80 mm and then dried in hot air. Densitometric analysis was carried out using a Camag TLC Scanner 3 (Camag) in the absorbance mode at 245 nm for all measurements. The slit dimension was kept at 5.0 mm × 0.45 mm and a scanning speed of 20 mm/s was employed. The chromatograms were integrated using winCATS evaluation software (Version 1.1.3.0).

Method validation

Validation of the optimized HPTLC method was carried out with respect to the following parameters.

Linearity and range

From the mixed standard stock solution 0.1 mg.mL^{-1} of GLIPI and 10 mg.mL^{-1} of MET, 1 to 5 µL solution were spotted on the TLC plate to obtain a final concentration of 100–500 ng.spot^{-1} for GLIPI and 1000–5000 ng.spot^{-1} for MET. Each concentration was applied six times on the TLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Limit of Detection and Quantification

The limits of detection (LOD) and quantification (LOQ) were calculated from the slope (s) of the calibration plot and the standard deviation of the response (SD).

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (100, 300, 500 ng.spot^{-1} and 1000, 3000, 5000 ng.spot^{-1} for GLIPI and MET respectively of the drug six

times on the same day. The intermediate precision of the method was checked by repeating studies on two different days.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for GLIPI and MET in sample was confirmed by comparing the R_f and spectra of the spots with that of standards. The peak purity of GLIPI and MET were assessed by comparing the spectra at three different levels, i.e. peak start, peak apex and peak end positions of the spot.

Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition like, ammonium sulfate (0.5%): water: methanol: ethyl acetate in the ratio of (5.0: 5.0: 2.4: 2.4 v/v/v/v), (4.0: 5.0: 2.5: 2.5 v/v/v/v), (5.0: 4.0: 2.5: 2.5 v/v/v/v), (5.0: 4.0: 2.5: 2.5 v/v/v/v) were tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ±5 %. The plates were prewashed by methanol and activated at 60 ^oC ± 5 for 2, 5, 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 minutes. Robustness of the method was done at three different concentration levels 100, 300,500 ng.spot^{-1} and 1000, 3000. 5000 ng.spot^{-1} for GLIPI and MET respectively.

Analysis of marketed formulation

The marketed formulation was assayed as described above. Fixed volumes solutions (1 µL) were spotted on plates and analyzed for MET and GLIPI in the same way as described earlier.

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (Nt and Mt combination tablets) to which known amounts of Nt and Mt standard powder corresponding to 80, 100 and 120% of label claim had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatograms in optimized mobile phase.

RESULTS AND DISCUSSION

The results of validation studies on the simultaneous estimation method developed for GLIPI and MET in the current study involving ammonium sulfate (0.5%): water: methanol: ethyl acetate in the ratio of 5.0: 5.0: 2.5: 2.5 (v/v/v/v) as the mobile phase which gives highest resolution, minimum tailing and R_f values of 0.31 and 0.70 for MET and GLIPI respectively (Fig 3).

UV scanning at 200-400 nm for both GLIPI and MET show that 245 nm is the suitable wavelength for detection of drugs (Fig 4).

Linearity

The GLIPI and MET showed a good correlation coefficient (r^2 = 0.9991 for GLIPI and 0.9993 for MET) in the given concentration range 100–500 ng.spot^{-1} for GLIPI and 1000–5000 ng.spot^{-1} for MET Table 1.

Precision

The repeatability and intermediate precision RSD (%) values for GLIPI was found to be 1.49. and 1.19, respectively, and the RSD (%) values for MET were found to be 1.30 and 1.84, respectively. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were <2%, respectively, as recommended by ICH guidelines Table 2.

LOD and LOQ

Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ, respectively. The LOD and LOQ were found to be 24.5 ng.spot^{-1} and 80 ng.spot^{-1} for GLIPI and 60 ng.spot^{-1} and 200 ng.spot^{-1} for metformin respectively.

Robustness of the method

The standard deviation peak of the areas was calculated for each parameter and the % RSD was found to be less than 2%. The low values of the % RSD indicated robustness of the method Table 3.

Specificity

The peak purity of GLIPI and MET was assessed by comparing their respective spectra at the peak start, apex, and peak end positions of the spot, i.e., r (S,M)=0.9983 and r (M, E)=0.9986. A good correlation (r^2=0.9991) was also obtained between the standard and sample spectra of GLIPI and MET, respectively. Also, excipients from formulation were not interfering with the assay.

Recovery studies

Good recoveries of the GLIPI and MET were obtained at various added concentrations for both T-1 and T-2 Table 4.

Table 1. Linear regression data for the calibration curves[a]

Compound	Linearity (ng.spot^{-1})	y = A + Bx		r^2
		A	B	
GLIPI	100-500	7.354	490.7	0.999
MET	10000-50000	0.723	52788	0.999

[a] *n = 6; r^2, coefficient of correlation*

Table 2. Intra and inter day precision of HPTLC method[a]

Compound	Interday precision		Intraday precision	
	S.D of areas.	% R.S.D.	S.D of areas.	% R.S.D.
GLIPI	15.89	1.49	20.34	1.30
MET	60.39	1.19	64.78	1.84

[a] *n = 6*

Table 3 Robustness testing[a]

Parameter	GLIPI		MET	
	SD of peak area	% RSD	SD of peak area	% RSD
Mobile phase composition	18.90	0.92	800.23	0.93
Amount of mobile phase	15.78	1.12	916.45	1.02
Temperature	15.93	0.82	856.83	1.84
Relative humidity	19.35	0.92	890.36	1.28
Plate pretreatment	20.38	0.02	780.45	0.90
Time from spotting to chromatography	19.33	0.91	901.36	0.88
Time from chromatography to scanning	19.73	0.85	950.01	0.60

[a] *n = 6*

Table 4 Recovery studies [a]

T-1					T-2				
Label claim	Amount of drug added (%)	Total amount of drug present	Amount found	% Recovery	Label claim	Amount of drug added (%)	Total amount of drug present	Amount found	% Recovery
GLIPI 5 mg	80	360	360.54	100.15	GLIPI 2.5 mg	80	180	178.88	99.38
	100	400	400.92	100.23		100	200	199.86	99.23
	120	440	436.56	99.22		120	220	218.39	99.27
MET 500 mg	80	36000	36334.80	100.93	MET 250 mg	80	18000	17971.2	99.84
	100	40000	39812	99.53		100	20000	19958	99.79
	120	44000	43670	99.25		120	22000	21848.2	99.31

[a] *n = 6*

Table 5 Applicability of the HPTLC method for the analysis of the pharmaceutical formulations

Sample	Label claim (mg)	Drug Content (%)	% R.S.D.
T-1			
GLIPI	5	99.54	1.84
MET	500	99.48	0.72
T-2			
GLIPI	2.5	99.92	0.39
MET	250	100.08	0.87

Table 6 Summary of validation parameters

Parameter	GLIPI	MET
Linearity range ($ng.mL^{-1}$)	100- 500	10000- 50000
Correlation coefficient	0.999 ± 0.09	0.999 ± 0.01
Limit of detection ($ng.mL^{-1}$)	24.5	60
Limit of quantitation ($ng.mL^{-1}$)	80	200
Recovery (n = 6)		
T-1	99.86	99.90
T-2	99.52	99.64
Precision (% R.S.D.)		
repeatability	1.49	1.30
Inter day	1.19	1.84
Robustness	Robust	Robust
Specificity	0.9991	0.9996

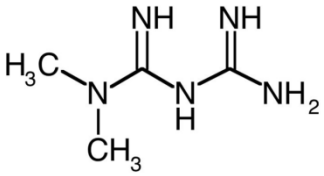

Fig 1. Chemical structure of Metformin

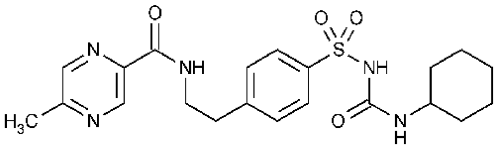

Fig 2. Chemical structure of Glipizide

Analysis of a formulation

Experimental results of the amount of GLIPI and MET in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets. Two different brands of fixed dose combination tablets were analyzed using the proposed procedures Table 5. The data of summary of validation parameters are listed in Table 6.

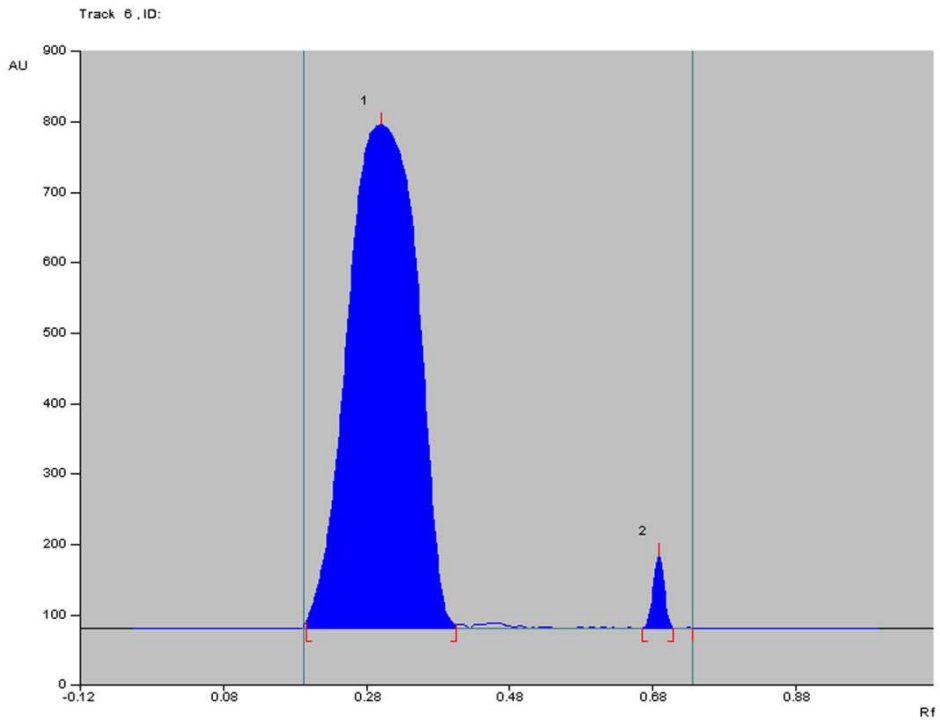

Fig 3. Densitogram of of metformin HCl (2000 ng.spot^{-1}); peak 1 (standard) (R_f : 0.31, glipizide (200 ng.spot^{-1}); peak 2 (standard) (R_f: 0.70). Ammonium sulfate (0.5%): water: methanol: ethyl acetate in the ratio of 5.0: 5.0: 2.5: 2.5 (v/v/v/v)

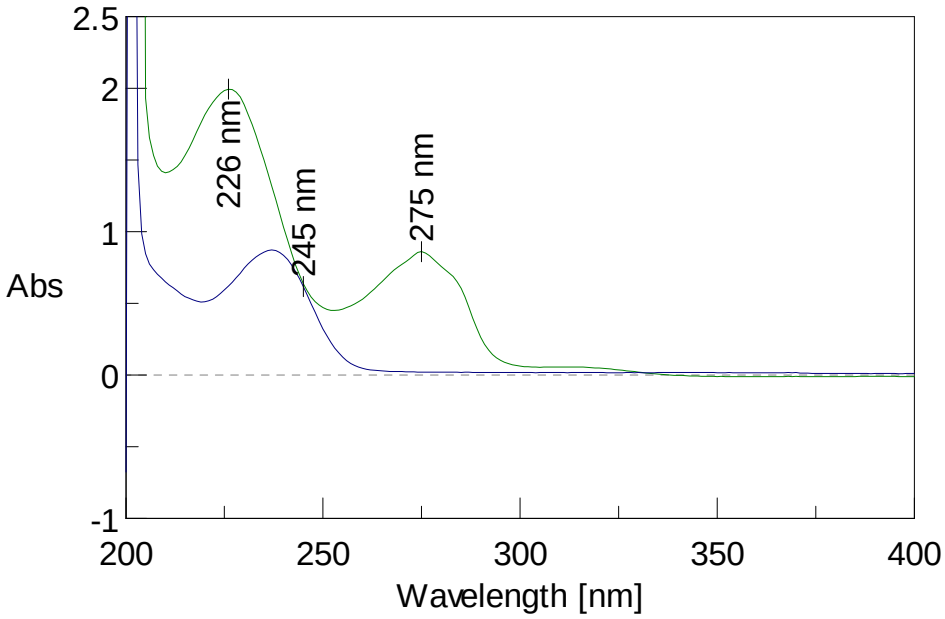

Fig.4 Overlain UV spectrum of metformin HCl and glipizide measured from 200 to 400 nm

CONCLUSION

Introducing HPTLC into pharmaceutical analysis represents a major step in terms of quality assurance. Today, HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput, and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC; thus reducing the analysis time and cost per analysis. The developed HPTLC technique is precise, specific, and accurate. Statistical analysis proves that the method is suitable for the analysis of GLIPI and MET as a bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of GLIPI and MET and also for its estimation in plasma and other biological fluids.

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