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A SENSITIVE DUAL-RUN HPTLC TECHNIQUE FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE, GLIMEPIRIDE AND VOGLIBOSE IN COMBINED DOSAGE FORM

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ABSTRACT

A sensitive, selective, and precise high performance thin layer chromatography (HPTLC) based on dual-run technique has been developed for the simultaneous estimation of metformin (MET), glimepiride (GLI), and voglibose (VOG) in combined dosage form. Chromatographic separation was performed on aluminium plates precoated with silica gel 60 F_{254} as the stationary phase. A dual run technique was adopted for better resolution amongst all three drugs with solvent system initially toluene-methanol-ethyl acetate-formic acid (3:4:3:0.5, v/v/v/v) for separation of metformin. The plates were dried and developed again using another mobile phase system consisting of toluene-ethyl acetate-formic acid (5:5:1, v/v/v) for glimepiride and voglibose. Densitometric scanning was carried out in the absorbance mode at 254 nm. The linear regression data for calibration plots showed good linear relationship in the concentration range of 200-1200 ng/band for MET, 20-120 ng/band for GLI and VOG. The method was validated for precision, accuracy, robustness and recovery as per ICH guidelines and applied for quantification in their available combined pharmaceutical dosage form. Consider the merits of dual-run mode and sensitivity, the proposed method would be novel for the determination of MET, GLI and VOG.

Key words: Metformin, glimepiride, voglibose, tablet dosage form, densitometry, validation

INTRODUCTION

Diabetes mellitus is a major global health problem and an increasing cause of morbidity and mortality. The term diabetes mellitus describes a metabolic disorder of multiple etiologies, characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [1]. Monotherapy with an oral antidiabetic agent is not sufficient to reach target glycemic goals and multiple drugs may be necessary to achieve adequate control. Two or more antidiabetics agents from different pharmacological classes are often needed to achieve adequate blood glucose control. Combination therapy is an important option that combine efficacy of blood glucose reduction and a low side effect profile with convenient once daily dosing to enhance compliance.

Metformin (MET) chemically N, N-Dimethyl biguanide, acts by decreasing intestinal absorption of glucose reducing hepatic glucose production and increasing sensitivity [2]. Glimepiride (GLI) chemically 3-Ethyl-4-methyl-N-(4-[N-((1r, 4r)-4-methylcyclohexyl carbomyl) sulfamoyl] phenethyl)-2-oxo-2, 5-dihydro-1H pyrrole-1-carboxamide, is an oral blood-glucose-lowering drug of sulphonyl urea class. It lowers blood glucose by stimulating the release of insulin from pancreatic beta cells. Voglibose (VOG) chemically (1S, 2S, 3R, 4S, 5S)-5-(1, 3-Dihydroxypropane-2-ylamino)-1-(hydroxyl methyl) cyclohexane-1, 2, 3, 4-tetrol, is a potent α-glucosidase inhibitor and used for the treatment of diabetes mellitus. It acts as glucosidase inhibitor, remaining active within the gastrointestinal tract of humans by delaying the glucose absorption thereby preventing the sudden surge of glucose in the human

body after meals. The structures of MET, GLI, VOG are shown in Fig. 1.

Literature review revealed few methods for the determination of MET, GLI and VOG individually or in combination with other drugs. The methods include spectrophotometry, [3, 4] HPLC [5-8], LC-MS [9, 10] and HPTLC [11-15]. Till date there have been no published methods for simultaneous determination of MET, GLI and VOG in bulk or in combined dosage forms. So the present study reports for the first time the simultaneous quantification by HPTLC for these components in dosage form. The proposed method is validated as per ICH guidelines.

EXPERIMENTAL

Materials: Metformin, glimepiride and voglibose were obtained as gift sample from Micro Labs Ltd., Bangalore, India. Analytical grade methanol, toluene, ethyl acetate and formic acid were purchased from Merck Chemicals, Mumbai, India. Tablet formulation TRIPOSMEAL*2 (Unichem Laboratories Ltd., Mumbai, India) labeled to contain 500 mg metformin, 2 mg glimepiride and 0.2 mg voglibose were procured from local pharmacy.

Instrumentation: The high performance thin layer chromatography (HPTLC) was performed on silica gel 60 F_{254} (10×10 cm, 250 µm thickness) (Merck, Germany). The plate is prewashed with methanol and activated at 110 °C for 5 minutes prior to application. Sample application was done by means of a 100 µL Hamilton (Reno, Nevada, USA) micro syringe, mounted on a Linomat V applicator (Camag, Muttenz, Switzerland). A constant application rate of 1 µL/band was used and the space between bands was 5 mm. Plates were left to equilibrate for 10 minutes in a 10×10 cm horizontal twin-trough chamber (Camag) using dual run technique. Initially toluene-methanol-ethyl acetate-formic acid in the ratio 3:4:3:0.5, v/v/v/v was used for MET. The plates were dried and developed again by mobile phase consisting of toluene-ethyl acetate-formic acid (5:5:1, v/v/v) for GLI and VOG. In order to reduce neck effect, the TLC chamber was saturated for 30 minutes with the help of saturation pads. Following the development, the TLC plate was dried in a stream of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner III at 254 nm which was selected experimentally on the basis of distinctive absorption spectra of compounds between 200 and 400 nm. winCATS software (VI, 4.2, Camag, Switzerland) was used for scanner control and data processing. The source of radiation utilized was deuterium lamp.

General Procedure

Preparation of standard stock solution and calibration curve: Standard stock solutions (1 mg/mL) were prepared separately by dissolving 25 mg each of GLI, MET and VOG in 25 mL methanol. From the stock solutions, suitable dilutions were made using methanol to obtain a combination solution containing 10 µg/mL of GLI, 100 µg/mL of MET and 10 µg/mL of VOG respectively. From this solution 2-12 µL solutions was spotted in TLC plates to furnish a concentration range of 20-120 ng/band of GLI, 200-1200 ng/band of MET and 20-120 ng/band of VOG respectively and their chromatograms were recorded. Calibration curve obtained from the data of peak area versus drug concentration was treated by linear least square regression analysis and the range was selected as working range for recovery and assay. Each standard was analyzed in triplicate and peak areas were recorded.

Assay of the pharmaceutical dosage form: The proposed HPTLC method was applied to the simultaneous estimation of MET, GLI, VOG in formulation (Trade name: TRIPOSMEAL*2, Label Claim: MET 500 mg, GLI 2 mg, VOG 0.2 mg). Ten tablets were weighed and the average weight was calculated. A quantity of tablet powder equivalent to 100 mg MET was weighed and transferred to 100 mL volumetric flask. The powder was dissolved in methanol and diluted to volume with same solvent. The solution was filtered through Whatman filter paper No. 41, then 1 mL of solution was transferred to a 100 mL volumetric flask and make up to volume with methanol to obtain a concentration of 1000 ng/band MET, 100 ng/band each of GLI and VOG. The plate was activated and 10 µL of sample solution was spotted. The procedure was repeated five times for analysis of homogenous samples and the possibility of excipient interference in the analysis was studied.

RESULTS AND DISCUSSION

Optimization of HPTLC method: Optimization of HPTLC method was very challenging for simultaneous estimation of MET, GLI and VOG respectively. In initial trial ammonium sulphate-methanol-ethyl acetate (1.4:0.1:0.5, v/v/v) was used. In this system GLI and VOG move along with the solvent front. In a system of ammonia-ethanol-ethyl acetate (0.1:0.8:0.1, v/v/v), MET was not separated. In a system of ammonia: methanol: toluene (0.1:1.4:0.6 v/v/v), GLI was not separated. After many trials, we concluded that a dual run technique can be adopted for better resolution amongst all three drugs. Therefore toluene-methanol-ethyl acetate-

formic acid (3:4:3:0.5, v/v/v/v) was used for development of MET. The plates were dried and developed again by toluene-ethyl acetate-formic acid (5:5:1, v/v/v) for development of GLI and VOG. The densitometric scanning was performed at 254 nm where all three drugs shows distinctive absorption.

Analytical Method Validation: After the successful optimization of the method, it was validated in accordance to ICH guidelines [16]. Parameters such as system suitability, specificity, sensitivity (LOD and LOQ), linearity, range, accuracy (recovery), precision (repeatability and intermediate precision) and robustness were all studied and validated.

Selectivity and specificity: The identity of GLI, MET and VOG in the samples were confirmed by overlaying their UV-absorption spectra with those of reference standards as well as by the coincidence of their respective R_f values using a TLC densitometric analysis. Fig. 2 and 3 shows typical chromatogram of both standard and sample of GLI, MET and VOG respectively.

Linearity and sensitivity: The calibration plots were linear in the concentration range of 20-120 ng/band (n = 6, r = 0.9999) for GLI, 200-1200 ng/band (n = 6, r = 0.9994) for MET and 20-200 ng/band (n=6, r=0.9996) for VOG respectively. The low values of standard deviation, standard error of slope and intercept of ordinate showed that the calibration plots did not deviate from linearity. The LOD and LOQ obtained by this method were 4.61 and 18.9 ng/band for GLI, 7.32 and 21.98 ng/band for MET and 2.89 and 8.7 ng/band for VOG respectively. Table1 shows the linearity parameters of calibration curve.

Precision

a) System precision: The repeatability of sample application and measurement of peak area were verified by proposed method for system precision study. The repeatability of sample application was carried out by making six measurements on three different concentrations. Repeatability of measurement of peak area was determined by scanning the developed bands six times without changing the plate position. In both the cases, %RSD measurement of peak area was taken to evaluate the system precision and result obtained was less than 2, which meets the accepted requirements as shown in Table 2.

b) Method precision: Precision of proposed method was determined in relation to repeatability (intraday) and intermediate precision (inter day). In order to evaluate the repeatability of the method, six samples were determined during same day for three concentrations (low, medium and high levels) of GLI, MET and VOG respectively. Intermediate precision was studied by comparing the assays performed on three consecutive days (fresh samples were prepared everyday) using above concentrations. This method meets the accepted requirements as shown in Table 3.

Accuracy (Recovery): The accuracy of the method determined by use of standard addition method (Fig. 4), performed at three different concentrations in triplicate showed good recoveries; 96.7 - 100.8% for GLI, 99.75 - 99.99% for MET and 98.7 -100.13% for VOG respectively (Table. 4.), the %SEM in all cases was within the acceptable limit (< 2%).

Robustness: Predetermined variations were performed under the experimental conditions of HPTLC method to assess its robustness. The variations imposed on the chromatographic method are summarized in Table 5. The parameters selected for the robustness study were development distance (cm), sample saturation time (min) and time of spotting to chromatogram (min). By introducing small changes in these parameters, the effects on the results were examined. The %RSD values showed no significant change in final assay results.

Applicability of the method to marketed formulations: It is evident from the results obtained that the validated method gave satisfactory results with respect to the analysis of both drugs. The validated method is applied to a commercially available formulation (Triposmeal*2) as shown in Table 6.

CONCLUSION

The developed HPTLC method for the simultaneous determination of metformin, glimepiride and voglibose is rapid, simple, precise, specific, accurate, selective, sensitive and reproducible. The amount found in assay was well agreed with label claim. The proposed method was successfully applied for determination of both drugs in tablet dosage form.

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Table 1: Regression and statistical parameters for the determinations of GLI, MET and VOG using the proposed HPTLC method

Validation Parameter	GLI	MET	VOG
Specificity	Specific	Specific	Specific
Linearity (ng/band)	20-120	200-1200	20-120
Repeatability of sample measurement (%RSD)	0.4670	0.3177	0.3016
Repeatability of sample application (%RSD)	0.5013	0.3446	0.2986
LOD (ng/band)	4.61	7.32	2.89
LOQ (ng/band)	18.91	21.98	8.7
Correlation coefficient (r)	0.9999	0.9994	0.9996
Retention factor (R_f)	0.22	0.43	0.56

Table 2: System precision of proposed method

Drug Concentration (ng/band)		System precision Calculated area * ± SD	%RSD
GLI	2.0	9028.21 ± 163.23	1.81
	4.0	10132.70 ± 131.55	1.29
	6.0	16937.33 ± 219.05	1.30
MET	200	10899.21 ± 157.33	1.47
	400	80732.66 ± 581.05	0.72
	600	12936.51 ± 221.66	1.71
VOG	0.2	8978.56 ± 120.43	1.34
	0.4	10156.12 ± 85.96	0.85
	0.6	12985.06 ±101.07	0.79

*Mean of six replicates

Table 3: Results of precision studies of proposed method

Drugs	Nominal concentration (ng/band)	Intra-day		Inter-day	
		SD*	%RSD	SD*	%RSD
MET	200	0.6345	0.3177	0.9834	0.4933
	400	0.9459	0.2367	1.0342	0.2586
	600	0.9432	0.1573	1.0334	0.1723
GLI	20	0.1789	0.890	0.2041	1.0242
	40	0.2137	0.5354	0.2191	0.5488
	60	0.1643	0.2741	0.1897	0.3162
VOG	20	0.1329	0.6639	0.1549	0.7745
	40	0.0753	0.1880	0.0515	0.1292
	60	0.2066	0.3450	0.2811	0.4696

*Mean of six replicates

Table 4: Result of recovery studies

Drugs	Initial amount (ng/band)	Fortified amount (ng/band)	Amount recovered ± SD (ng/band)	SEM
GLI	40	20	58 ± 0.013	0.5307
		40	79.8 ± 0.014	0.5715
		60	100.08 ± 0.025	1.0206
MET	400	200	598.5 ± 0.010	0.4082
		400	799.5 ± 0.017	0.6940
		600	999.98 ± 0.011	0.4490
VOG	40	20	59.6 ± 0.020	0.8164
		40	80.01 ± 0.010	0.4083
		60	99.7 ± 0.021	0.8573

*Mean of six replicate, SEM-Standard error mean

Table 5: Result of robustness studies

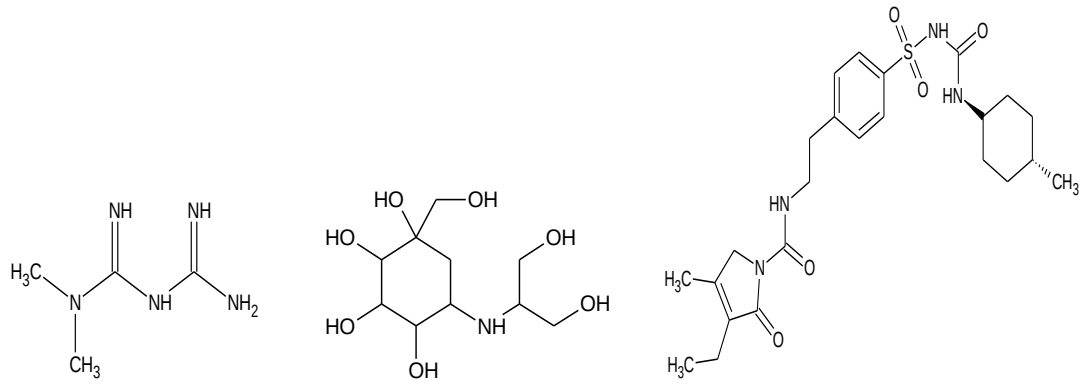
Condition		Retention factor (R_f)			Assay* (%)			%RSD		
		GLI	MET	VOG	GLI	MET	VOG	GLI	MET	VOG
Development distance (cm)	6	0.35	0.43	0.56	99.5	100.05	99.56	0.981	1.76	0.431
	8	0.34	0.42	0.55	98.98	100.01	99.87	0.569	0.997	0.590
Time of spotting to chromatogram (min)	9	0.35	0.43	0.55	99.08	99.98	98.92	0.590	1.21	0.397
	11	0.34	0.43	0.56	100.01	101.02	99.91	0.498	1.71	0.429
Saturation time (±5 min)		0.35	0.43	0.56	99.56	100.06	98.89	0.983	1.09	0.298

*Mean of three determinations

Table 6: Result from analysis of pharmaceutical formulation

*Mean of five replicates

Label claim (mg)			Amount present (mg)			SD*			%RSD		
GLI	MET	VOG	GLI	MET	VOG	GLI	MET	VOG	GLI	MET	VOG
2	500	0.2	1.96	499.62	0.198	0.021	1.58	0.001	1.07	0.316	0.505



a **b** **c**

Figure 1: Chemical structures of (a) Metformin, (b) Voglibose and (c) Glimepiride

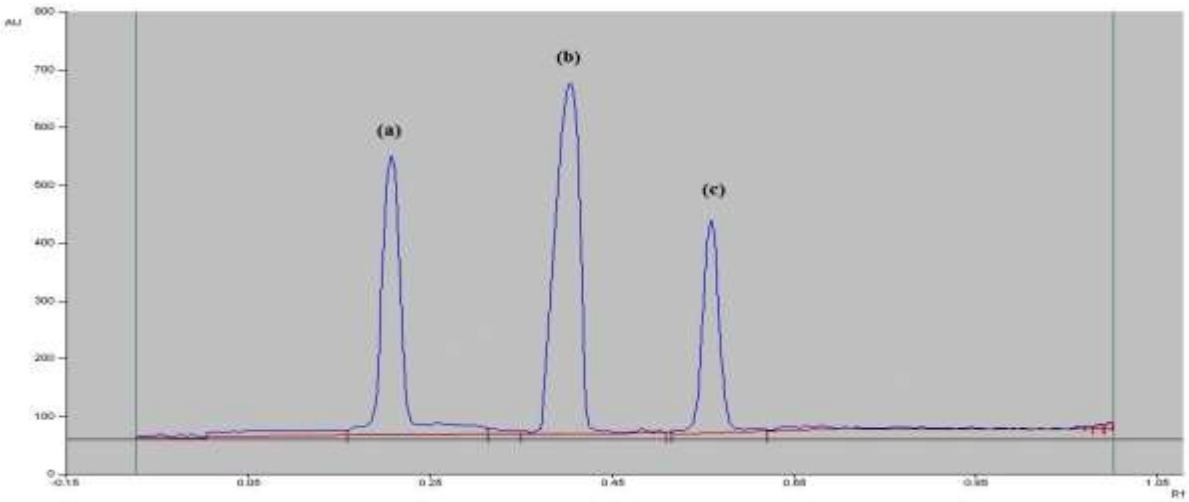


Figure 2: Typical chromatogram of standard (a) GLI, (b) MET and (c) VOG

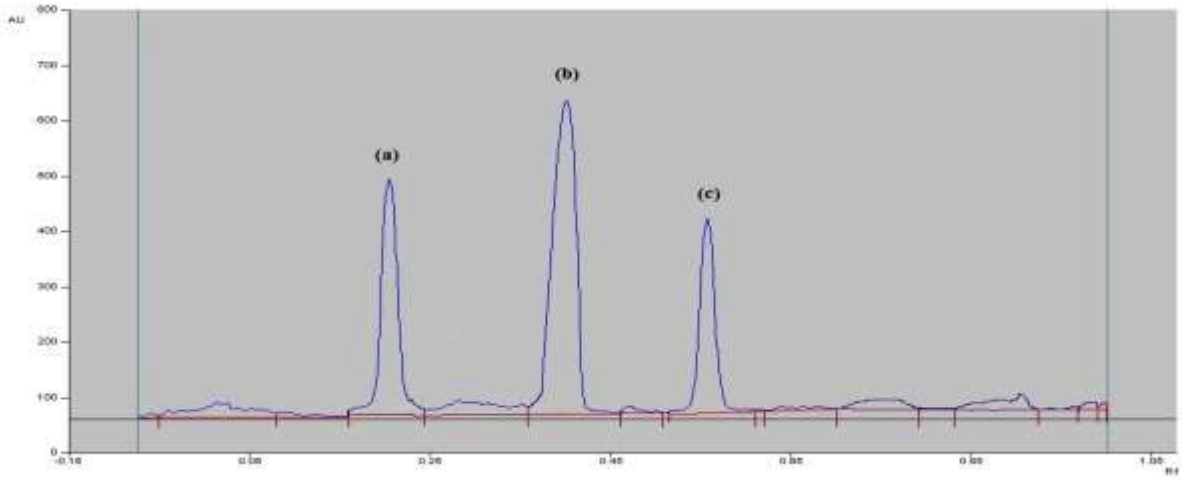


Figure 3: Typical chromatogram of sample (a) GLI, (b) MET and (c) VOG

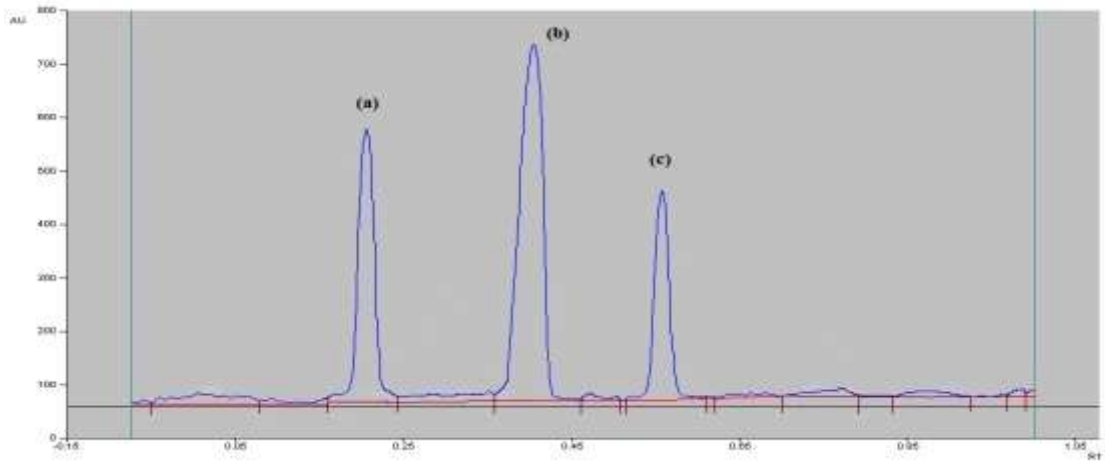


Figure 4: Typical chromatogram showing recovery (a) GLI, (b) MET and (c) VOG

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