RESEARCH PAPER

A Simple and Sensitive HPTLC Method for Simultaneous Determination of Metformin Hydrochloride, Teneligliptin Hydrobromide Hydrate and Dapagliflozin Propanediol Monohydrate in Tablet Dosage Form

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Abstract

A simple, sensitive, precise, accurate, and cost-effective high-performance thin-layer chromatographic (HPTLC) method was developed and validated for the simultaneous determination of metformin (MET), teneligliptin (TEN) and dapagliflozin (DAPA) in combined tablet dosage form. Chromatographic separation was performed on precoated silica gel 60 F254 TLC plates using acetonitrile: methanol: toluene: ammonium acetate: triethylamine (3:2.5:3:1:0.5, v/v/v/v/v) as the mobile phase. The chromatograms were evaluated by densitometric scanning in absorbance mode at 230 nm. The developed method was validated according to ICH Q2R2 guidelines with respect to linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and selectivity. The method demonstrated good linearity over the concentration ranges of 5000–25000 ng/band for MET, 200–1000 ng/band for TEN and 100–500 ng/band for DAPA, with correlation coefficients of 0.9991, 0.9990 and 0.9994, respectively. The recovery studies showed satisfactory recoveries for all three drugs, while precision studies produced %RSD values within the acceptable limit of 2%. The LOD values were 630.84, 22.95, and 45.45 ng/band, while LOQ values were 1911.65, 69.56, and 137.75 ng/band for MET, TEN, and DAPA, respectively. The proposed method was successfully applied to the analysis of marketed tablets, giving assay results of 99.46%, 100.77%, and 99.87% for MET, TEN and DAPA, respectively. Thus, the developed HPTLC method can be conveniently used for routine quality-control analysis of these drugs in combined pharmaceutical formulations.

Keywords: Metformin; Teneligliptin; Dapagliflozin; HPTLC; Validation; Tablet.

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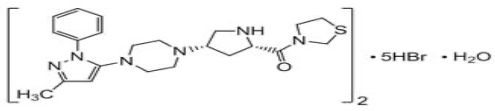
Introduction

Metformin hydrochloride (MET) 3-(diaminomethylidene)-1,1dimethylguanidine; hydrochloride (figure1(A)), Metformin stimulates tissue uptake of glucose, particularly in muscles, and is through to reduce gastro intestinal absorption of carbohydrates. The action of metformin does not involve stimulation of pancreatic insulin and there for it is still beneficial agent for when B cell function is declined [1]. Teneligliptin{(2S,4S)-4-[4-(3-Methyl-1-phenyl-1H-pyrazol-5-yl)-1-piperazineyl]-2-pyrrolidinyl} (1,3-thiazolidine-3-yl) methanone (figure2(B)) Teneligliptin inhibits human plasma DPP-4 activity and recombinant human DPP-4 activity in a concentration-dependent manner with

half-maximal inhibitory concentrations. Dapagliflozin(2S)-propane-1,2-diol(2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl}-6-(hydroxymethyl) oxane-3,4,5-triol hydrate(figure3(c)) [2]. Dapagliflozin hinders the sodium-glucose cotransporter 2(SGLT2) which is basically situated in the proximal tubule of the nephron. SGLT2 works with 90% of glucose resorption in the kidneys as its restraint considers glucose to be discharged in the pee [3].

A literature survey revealed that MET is official in IP [4], BP [5], and USP [6], while TEN is not yet official in any of the pharmacopoeia. DAPA monograph is present in IP [7]. A detailed literature survey found that HPLC method, RP-HPLC method [8], stability indicating HPLC method was done on MET, TEN, DAPA [9]. Metformin [10,11,12,13,15,16]. Metformin and sita [14,18]. Metformin and glipizide [17]. Vildagliptin and Metformin [19]. Teneligliptin [20,23,25]. Teneligliptin and metformin [22,24]. To our knowledge, this is the first report of HPTLC method for simultaneous estimation of MET, TEN and DAPA in a tablet dosage form. HPTLC method is useful for simultaneous processing of sample and standard, reduced need for internal standard, lower analysis time and less cost per analysis, simple sample preparation, no requirements of prior treatment for solvents like filtration and degassing, no interference from previous analysis, fresh stationary and mobile phases for each analysis with no contamination, the ability for visual detection with an open system, and to determine non UV absorbing compounds detected by post chromatographic derivatization. It reveals that proposed method require less time and less solvent for the analysis. So proposed method is cost effective as HPLC grade solvents are too costly.

(a)



(b)



(c)

Figure1: Structure formula for (a) MET, (b) TEN and (c) DAPA

1. Experimental section

Materials

Ethanol (AR grade) were supplied by SD Fine Chemicals (Mumbai,India)

Distilled water was used throughout the study. Reference standard of MET was produced from Ami Life Science (Vadodara, gujarat). TEN was produced from Ami Life Science (Vadodara, gujarat). DAPA was produced from Ami Life Science (Vadodara, gujarat).

HPTLC Instrument

A CAMAG HPTLC system equipped with Linomat 5 autosampler, TLC scanner 4, and win CATS 1.4.8 software (CAMAG, Muttens, Switzerland) was used. The slit dimension was kept at 5.00×0.45mm, and 20mm/sec scanning speed was employed. Chromatography was performed on precoated silica gel 60 F254 TLC plates (10 × 10cm, catalogue number1.05554.007) (Merck, Darmstadt, Germany) using Acetonitrile: Methanol: Toluene: Ammonium Acetate: Triethylamine (3:2.5:3:1:0.5, v/v/v/v/v) as mobile phase. The band length 6mm and distance between bands 13mm were kept constant throughout the study. Numbers of applications on the plates were five for standards and three for test samples. The application speed was 150nL/sec. Ascending development to a distance of 85 mm was performed on 20×10 cm twin through chamber (CAMAG). Chromatograms were evaluated via peak area after scanning in absorbance mode at 230nm.

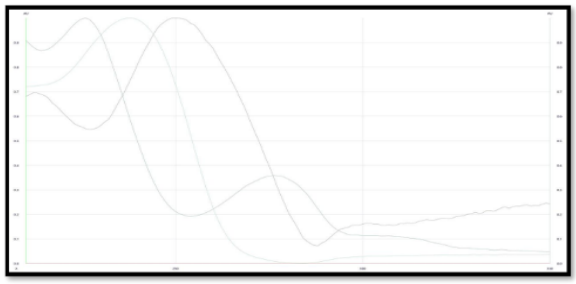


Figure2: Typical drug spectra for MET, TEN and DAPA Wavelength optimization by using drug solution.

Preparation of standard solution: MET (500mg), TEN (20mg) and DAPA (10mg) reference substance were accurately weighed and transferred to a 100ml volumetric flask. The powder was dissolved in and diluted to volume with methanol to furnish concentration of 5000 µg/ml MET, 200 µg/ml TEN and 100 µg/ml DAPA.

Preparation of sample solution Twenty tablets were weighed and ground to a fine powder. A quantity of powder equivalent to 500mg MET, 20mg TEN and 10mg DAPA Was weighed and transferd to 25ml

volumetric flask the powder was dissolved in methanol, sonicated for 30 min, diluted to volume upto mark to furnish a solution containing 5000 µg/ml MET,200 µg/ml TEN,100 µg/ml DAPA.

2. Method validation

Validation was done with respect to various parameters required under ICH guideline Q2R2.

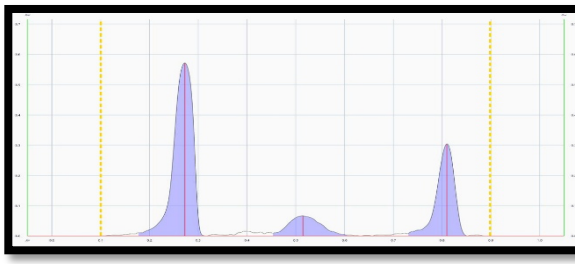


Figure 3: Chromatogram for standard of MET, TEN and DAPA

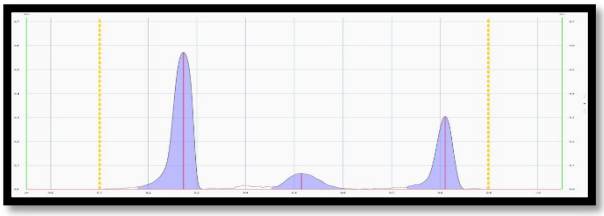


Figure 4: Chromatogram for sample of MET (5000ng/band), TEN (200ng/band) and DAPA 100(ng/band)

Table 1: Statistical evaluation of linear part of calibration dependence of MET, TEN, DAPA

PARAMETER	MET	TEN	DAPA
Range(ng/band)	5000-25000	200-1000	100-500
Slope	0.1019	0.513	0.244
Intercept	3339.4	344.9	105.4
R	0.9991	0.999	0.9994

Linearity: calibration curve was plotted over a concentration range from 5000 to 25000ng/band for MET, 200 to 1000ng/band for TEN and 100 to 500ng/band for DAPA were applied to the plate. The plate was developed in a developing chamber previously saturated with the mobile phase for 15 minutes and each reading was the average of three determinations.

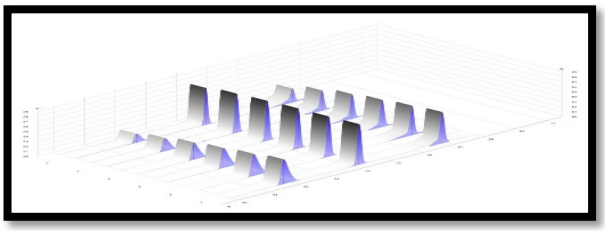


Figure 5: 3D Image of Linearity for MET (5000-25000ng/band), TEN (200-1000ng/band) and DAPA (100-500 ng/band) under optimized conditions.

Accuracy (percent of Recovery): The accuracy of the method was determined by calculating recoveries of MET, TEN, DAPA by the standard addition method. The analysed samples (5000 µg/ml, 200 µg/ml and 100 µg/ml) were spiked with extra concentration levels of 5000,10000 and 15000 µg/ml for met, ten and dapa mixtures were reanalysed by the proposed method. Recovery analyses were repeated three times for each level of all samples.

Precision: The precision of developed method was checked by repeatedly (n=6) injecting15000 ng/band for MET, 600 ng/band for TEN and 300 ng/band for DAPA without changing the position of plate for HPTLC method. Repeatability of a sample application and measurement of the peak area were determined on the same day by the repeated application (n=6) of sample solutions, while intermediate precision was evaluated by comparing the assays for three different days.

Limit of Detection and Limit of Quantification: Limit of detection (LOD) and Limit of quantification (LOQ) were calculated using the equations LOD =3.3 × N/B and LOQ=10 × N/B, where N is standard deviation of the peak area (n=3), taken as measure of the noise, and B is the slope of the corresponding calibration curve.

Selectivity: Selectivity of the method was tested by comparison of peaks of test compounds with those of standards prepared as mentioned before.

Procedure for Assay: Twenty tablets were weighed and powdered, and an amount of tablet powder equivalent to 500mg MET, 20mg TEN and 10 mg DAPA in to 100 ml volumetric flask and add 10-20 ml of methanol and diluted up to 100ml with methanol. Sonicate the solution then filtered with filtered with filtered with Whatman filter paper (No.42) to obtain 5000µg/ml MET, 200µg/ml TEN and 100µg/ml DAPA. 2µl of solution was applied to precoated TLC plates by Hamilton syringe under continuous steam of nitrogen gas to obtain a band containing 5000 ng/band for MET, 200 ng/band for TEN, 100ng/band for DAPA and analyzes it by the proposed method.

3. Results and Discussion

Wavelength optimization

UV-vis spectra (Figure2) of MET, TEN and DAPA were measured from 223 to 274 nm and 230nm was used as the optimum wavelength throughout the experiment for substances.

Method validation

Linearity

The calibration curve was linear from 5000 to 25000ng/band for MET, 200 to 1000ng/band for TEN and 100 to 500ng/band for DAPA, respectively. Statistical evaluation of the linear part of calibration dependence of MET, TEN and DAPA are presented table1.

Accuracy (percent of recovery)

The analyzed samples were spiked with extra concentration levels of 5000,10000, and 15000ng/band for met, 200 ,400 and 600ng/band for TEN and 100,200,300ng/band for DAPA and the mixture were analyzed by the proposed method. Recovery analyses were repeated three times for each level of all samples. this data is present in table 2.

Table 2: The recovery studies of MET and SITA

Compound	Amount taken (ng)	Amount added (ng)	Recovery (%) ±RSD (%) (n=3)
MET	10000	5000	101.57±0.40
	10000	10000	100.59±0.19
	10000	15000	98.21±0.27
TEN	400	200	101.17±1.18
	400	400	101.67±0.73
	400	600	98.83±0.66
DAPA	200	100	103.28±1.26
	200	200	103.10±1.02
	200	300	101.37±1.37

Table 3: Data indicating various validation parameters of HPTLC method (n=6)

	MET	TEN	DAPA
Precision (n=6) Mean ±SD; RSD (%)			
Method precision	0.18	0.95	1.40
Intraday precision	0.94-1.68	0.90-1.60	1.06-1.70
Interday precision	0.99-1.62	0.60- 1.25	0.84-1.12
LOD (ng/band)	630.84	22.95	45.45
LOQ (ng/band)	1911.65	69.56	137.75

Precision

The precision of the developed method was expressed as a percentage of relative standard deviation (percent of RSD) for repeatability (intraday precision) and intermediate precision (interday precision). The data obtained were within 2% RSD(Table3).

Limit of Detection and Limit of Quantification

The LOD and LOQ values were 630.84 ng/band and 1911.65 ng/band of MET, 22.95 ng/band and 69.56 ng/band of TEN and 45.45 ng/band and 137.75 ng/band of DAPA, respectively (Table3). The data shows that the method is sensitive for the determination of MET, TEN and DAPA

Selectivity

Typical absorption spectra of MET, TEN and DAPA are shown in figure3. selectivity of the method was tested by comparison of peaks of test compound with those of standards prepared as mentioned before. Related chromatogram is presented in figure 3. Thus, it can be concluded that the excipients did not interfere with the peaks from standard drug solutions.

Application of the validation method to a pharmaceutical preparation

The proposed validated method was successfully applied to determine MET, TEN and DAPA in their combined dosage form. The label claim was 500mgMET, 20mgTEN, 10mgDAPA as per tablet. The percentage of assay of MET, TEN and DAPA found in tablets by the proposed method is 99.46%,100.77% and 99.87% with percentage of RSD 0.92%,1.52% and 1.78% respectively (figure3).

Conclusions

A new HPTLC method for simultaneous determination of MET, TEN and DAPA in pharmaceutical tablet formulation has been developed. The method was found to be simple, sensitive, precise, accurate, and specific for quantification of MET, TEN and DAPA in pharmaceutical formulation. It does not suffer from interference from common excipients present in the

pharmaceutical preparation and can be conveniently adopted for quality-control analysis

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Conflict of Interest

The author/editor has no conflicts of interest, financial or otherwise, to declare.

Statement of Contribution of Researchers

Concept – G.P, D.P., PKP.; Design – D.P., M.P.; Supervision – D.P.,G.P., D.B.M; Data Collection and/or Processing – K.P., I.P., D.S., Analysis and/or Interpretation – K.P., I.P., Literature Search – M.P; Writing – M.P., ; Critical Reviews – D.P., G.P., D.B.M.

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