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Research Article

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Development and validation of HPTLC method for simultaneous estimation of telmisartan and indapamide in pharmaceutical solid dosage form

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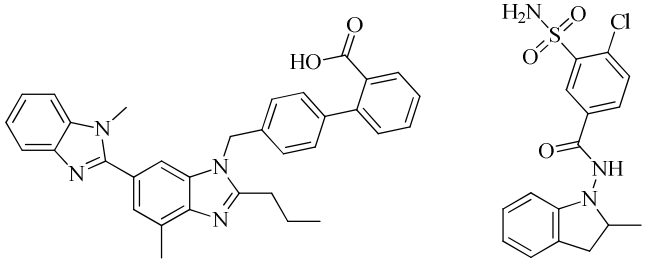
ABSTRACT

A simple, precise, accurate and specific high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of Telmisartan and Indapamide in pharmaceutical solid dosage form without separation of components. The method is based on high performance thin layer chromatographic separation of both drugs followed by the densitometric measurements at 249 nm. The separation was carried out on precoated silica gel 60 GF_{254} using mobile phase hexane: ethyl acetate: methanol: glacial acetic acid (14:6:2:1 v/v/v/v) with R_f values 0.21 and 0.36 for Telmisartan and Indapamide respectively. The calibration curve was found to be linear between 2000-7000 ng/spot for Telmisartan and 75-262.5 ng/spot for Indapamide with correlation co-efficient 0.9970 and 0.9959 respectively. It was observed that the proposed HPTLC method could be used for efficient analysis and monitoring of the Telmisartan and Indapamide in combined pharmaceutical solid dosage forms.

Keywords: Telmisartan, Indapamide, Simultaneous estimation, HPTLC.

INTRODUCTION

In patients with mild to moderate hypertension, a combination of Telmisartan and Indapamide shows synergistic effect to lower blood pressure with a high response rate. Telmisartan (TELM) is chemically 2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2 propylbenzimidazol-1-yl]methyl]phenyl]benzoic acid (Figure 1A). It is an angiotensin II type 1 receptor antagonist which interferes with the binding of angiotensin II to the angiotensin II AT_1-receptor by binding reversibly and selectively to receptors in the vascular smooth muscle and the adrenal gland effectively and reduce hypertension by blocking the renin-angiotensin system [1]. Various UV spectrophotometry [2,3], HPTLC [2], HPLC [4,5] and spectrofluorimetry method [2] have been reported for the estimation of TELM individually or in the combination with other drugs.



(A) **(B)**

Figure 1 Structures of (A) Telmisartan and (B) Indapamide

Indapamide (IND) is chemically 3-(aminosulfonyl)-4-chloro-N-(2,3-dihydro-2-methyl-1*H*-indol-1-yl)-benzamide (Figure 1B). It is thiazide like diuretic which inhibits reabsorption of sodium and calcium at the beginning of distal

convoluted tubules [6]. IND is also thought to stimulate the synthesis of the vasodilatory hypotensive prostaglandin PGE_2. Various UV spectrophotometric [7,8], colorimetric [9], HPTLC [10], HPLC [11,12] and LC-MS [13] methods have been employed for the quantitative estimation of IND in bulk and pharmaceutical formulations individually or in the combination with other drugs.

Literature survey revealed that only RP-HPLC [14] method has been described for the simultaneous estimation of this combined dosage form. Since the reported RP-HPLC method is expensive and involves complicated sample preparation, there is a need for an assay method that permits simultaneous quantification of TELM and IND. The aim of this work was to develop and validate a simple, rapid, selective and quite sensitive HPTLC assay method for simultaneous determination of TELM and IND in combined solid dosage form. In addition, the method will be cheap and does not require certain types of stationary phases. Therefore, the developed method becomes good alternative for the already existing RP-HPLC method.

EXPERIMENTAL SECTION

Chemicals and reagents

TELM and IND standards were procured as a gift sample from Cadila Healthcare Pvt. Ltd., Ahmedabad, India and Ami life sciences Pvt. Ltd., Baroda, India respectively. Marketed formulation (Inditel-D, Zydus Cadila) containing TELM (40 mg) and IND (1.5 mg) was procured from local market. All the chemicals and reagents were used of AR grade (Merck, Mumbai, India).

Instrumentation and conditions

Precoated Silica gel 60 GF_{254} TLC plates (10*10 cm) on aluminium sheet (layer thickness 0.2 mm) procured from Merck, Mumbai, India, was used as stationary phase. A Camag HPTLC system containing Camag Linnomate V semiautomatic applicator (band application by spray on technique), Hamilton, Bonaduz, Schweiz applicator syringe - 100 µL, Camag twin trough glass chamber (20*10 cm), Camag TLC scanner III (spectral range 190-800 nm), Camag UV cabinet with dual wavelength UV lamp (dual wavelength 254/366 nm) and Sonistar ultrasonicator were used during the study.

Preparation of standard solution

Accurately weighed 100 mg each of TELM and IND were dissolved in 100 mL methanol to get final concentration of 1000 µg/mL (stock solution) of each drug. From stock solutions suitable aliquots was transferred to prepare standard mixture solution having concentration of 1000 µg/mL of TELM and 37.5 µg/mL of IND for simultaneous quantitative studies of both drugs. The stock solutions were further diluted with methanol to obtain a working standard solution with final concentrations of 2000, 3000, 4000, 5000, 6000 and 7000 ng/mL for TELM and 75, 112.5, 150, 187.5, 225 and 262.5 ng/mL for IND respectively, which were used for calibration purposes.

Preparation of sample solution

For analysis of solid dosage form, twenty capsules (each containing 40 mg TELM and 1.5 mg IND) were weighed and their average weight was calculated. The capsule powder equivalent to 100 mg of TELM and 3.75 mg of IND was transferred to a 100 mL volumetric flask, dissolved and diluted up to mark with methanol. The solution was sonicated for 15 min, filtered through the Whattman No. 41 filter paper and the residue was washed with methanol. This solution was further diluted with methanol to get the same concentration as that of the final standard solution.

Chromatographic conditions

The chromatographic conditions comprises use of precoated silica gel 60 GF_{254} as stationary phase, hexane: ethyl acetate: methanol: glacial acetic acid (14:6:2:1 v/v/v/v) as mobile phase, chamber and plate saturation time of 20 minutes, migration distance allowed was 90 mm, ambient temperature was 25-26° C, wavelength scanning was done at 249 nm keeping the slit dimensions at 4*0.45 mm. TLC plates were prewashed with methanol. Activation of plates was done in an oven at 50° C for 5 minutes. Standard solution of TELM and IND was spotted on activated TLC plate. The plate was scanned at 249 nm for the both drugs and peak area was measured with Camag TLC scanner 3 using WinCATS software. The concentration of each drugs were determined using straight line equations.The standard calibration curve was generated with the help of Microsoft excel using regression analysis. Sample solutions of the marketed formulation were spotted on to the same plate and scanned after development under same chromatographic conditions. The analysis was carried out in triplicate. The drug content was calculated from the peak areas of the chromatogram recorded.

RESULTS AND DISCUSSION

Selection of Mobile Phase

A solvent system that would give good separation of both drugs along with sharp and compact spots was desired for quantification. Different mobile phases were tried using different solvent systems containing non-polar and relatively polar solvents along with acetic acid. Acetic acid is useful to prevent tailing of IND which is weakly acidic and highly interacting with the stationary phase. Among the different mobile phase combinations tested, hexane: ethyl acetate: methanol: glacial acetic acid (14:6:2:1 v/v/v/v) was found to be the most suitable which gave the better resolution and sharper peaks with the R_f values 0.21 and 0.36 for TELM and IND, respectively. Figure 2 represents a typical HPTLC chromatogram of TELM and IND using the optimal conditions.

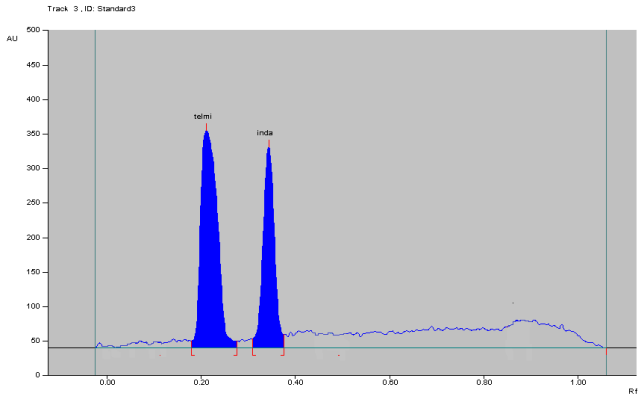


Figure 2 Chromatogram of Telmisartan and Indapamide

Selection of Wavelength

The wavelength for the quantitation of TELM and IND is selected by scanning the plate in the UV light from 400-200 nm. TELM show maximum absorbance at 299 nm and IND at 237 nm. The photometric measurement was carried at 249 nm, the iso-absorptive point for both drugs showing good peak areas,with the help of Camag TLC scanner 3 (reflectance mode). The overlay spectrum of TELM and IND is shown in the Figure 3.

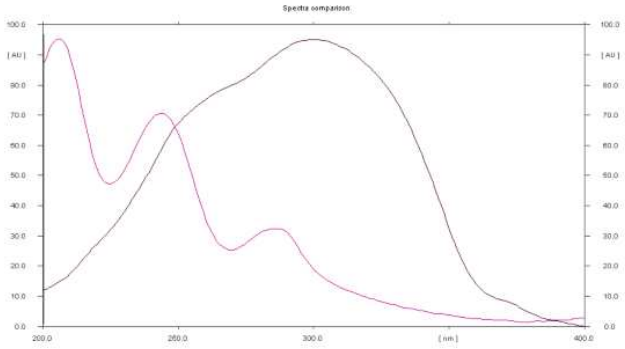


Figure 3 Overlay spectrum of Telmisartan and Indapamide

Method validation

The developed method was validated according to International Conference on Harmonization (ICH) [15,16] guidelines in terms of linearity and range, accuracy, intra-day and inter-day precision, specificity, limit of detection and limit of quantification.

Linearity and range

Linear relationship between peak area and concentration of the drugs were evaluated over the range of concentrations expressed in ng/band by analyzing six independent concentration levels for both drugs. Peak areas were found to have good linear relationship with the concentration than the peak heights. Figure 4 represents 3D chromatogram of linearity for TELM and IND.

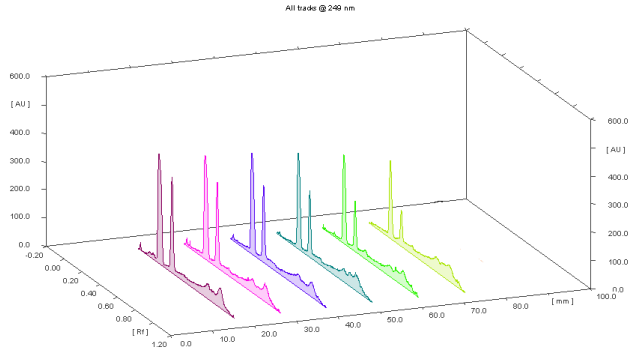


Figure 4 3D chromatogram of linearity for Telmisartan and Indapamide

The correlation coefficients, y-intercepts and slopes of the regression lines of the two compounds were calculated and presented in Table 1.

Table 1 Linearity results

Drugs	Concentration range (ng/spot)	Regression Equation	Correlation coefficient r^2
TEL	2000-7000	y = 1.3369x + 4028.5686	0.9970
IND	75-262.5	y = 26.8767x + 257.1029	0.9959

Accuracy

Accuracy of the method was confirmed by recovery study at three level of standard addition i.e. multiple level recovery studies. The recovery studies were carried out at 80%, 100% and 120% of the test concentration as per ICH guidelines. The results of the recovery studies and its statistical validation are given in Table 2.

Table 2 Recovery analysis for Telmisartan and Indapamide

Theoretical content (mg)	Level (%)	Amount of drug added	Total amount of drug (mg)	Amount recovered (mg) ± S.D.	% Recovery ± S.D.
Telmisartan 60	80	20	80	79.49 ± 0.17	101.37 ± 0.86
	100	40	100	99.18 ± 0.93	99.58 ± 0.86
	120	60	120	119.71 ± 0.84	100.83 ± 1.41
Indapamide 2.25	80	1.2	3.45	3.41 ± 0.01	98.26 ± 0.27
	100	1.5	3.75	3.72 ± 0.03	99.74 ± 1.51
	120	1.8	4.05	4.01 ± 0.02	99.14 ± 1.05

The mean percentage recovery for each compound was calculated at each concentration level and reported with its standard deviation. For TELM, the recoveries were found between 99.58% and 101.3% and for IND the recoveries were found between 98.26% and 99.74%. Recovery between 98-102% justifies the accuracy of the method. The low RSD value indicated the applicability of the method for routine analysis of TELM and IND in solid dosage forms.

Precision

The precision of the developed method was studied by measuring intra-day variation or repeatability and inter-day variation or intermediate precision.To study intra-day variation, six replicates of sample solutions containing TELM (40 ng/spot) and IND (1.5 ng/spot) were analyzed on the same day. To study inter-day variation, analysis of three replicates of sample solutions with the same concentration was performed on three different days. Intra-day variation as RSD was 1.34% for TELM and 1.37% for IND, and inter-day variation as RSD was1.14% for TELM and 1.63% for IND. The coefficients of variation for both the inter-day and intra-day precision of the method was found to be less than 2% for both drugs which indicate that the method is precise.

Limits of detection and quantification

The limits of detection and quantification of the developed method were calculated using 3.3*a/S and 10*a/S phenomena for the limits of detection and quantification, respectively [17] where a is the standard deviation of the y-intercepts and S is the slope of the calibration curve. The limit of detection was found to be 531.40 ng/spot and 21.42 ng/spot for TELM and IND, respectively. The limit of quantification was found to be 1610.32 ng/spot and 64.90 ng/spot TELM and IND, respectively.

Specificity

The specificity of the method was ascertained by purity of the chromatographic peaks. The spots of dosage forms were scanned at three different levels in spectral scanning mode of the WinCATs software. The peak purity for

TELM and IND was tested by correlation coefficients of spectra acquired at the peak start (s), peak maximum (m), and peak end (e) positions. Correlation coefficients of these spectra were calculated and summarized in Table 3.

Table 3 Specificity data of Telmisartan and Indapamide

Drugs	Co-relation r (s, m)	Co-relation r (m, e)	Peak purity
Telmisartan	0.999842	0.999398	> 0.999
Indapamide	0.999788	0.996963	> 0.996

The spectra of dosage form and reference standards were also compared for both studied drugs. The closeness of peak purity values to 1 indicates that the spots were only attributed to a single compound and the other the excipients present within the formulation did not interfere with the peaks of TELM and IND.

Analysis of marketed formulation:

Analysis of samples of marketed capsules containing TELM 40 mg and IND 1.5 mg was carried out and the amounts recovered were expressed as a percentage amount of the drug found. The mean drug content was found to be 39.66 mg and 1.49 mg of TELM and IND with a % R.S.D of 0.22 and 0.55, respectively. The drug content of the marketed formulation was found to be within the limits as recorded in Table 4

.

Table 4 Analysis of marketed formulation of TEL and IND

Brand Name	Label Claim (mg/capsule)	Amount found mg	% of drug found	% RSD
Inditel-D	TEL 40	39.66	99.15	0.22
	IND 1.5	1.49	99.33	0.55

CONCLUSION

The proposed HPTLC method provides simple, quick, accurate and reproducible quantitative analysis for simultaneous determination of simultaneous estimation of TELM and IND in bulk drug and in combined dosage form. The method was validated according to ICH guidelines. It has some advantages like less use of mobile phase per run, less time consuming etc. over HPLC methods in general.

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