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Simultaneous estimation of irbesartan, telmisartan, hydrochlorothiazide and ramipril in combined dosage forms by validated HPTLC method

Abstract

A sensitive, specific and precise high performance thin layer chromatographic method for estimation of Irbesartan (IRB), Telmisartan (TEL), Hydrochlorothiazide (HCZ) and Ramipril (RAM) has been developed and validated. The method employed TLC aluminium plates pre-coated with silicagel 60 F254 as the stationary phase. The solvent system consisted of acetonitrile: toluene: methanol: formic acid (8:10:2:0.6 v/v/v/v). Densitometric analysis of drugs was carried out in the absorbance mode at 210nm. The method was validated with respect to linearity, specificity, precision, limit of detection, limit of quantification and recovery. The linear regression analysis data for the calibration plots showed a good linear relationship with R^2 of 0.999 for all the drugs in the concentration range of 250-1250ng/spot for TEL and RAM and 500-2500ng/spot for IRB and HCZ respectively. Recovery of all drugs was achieved in the range of 98.85%-101.31% by developed method. Statistical analysis proves that the method is repeatable and specific for the estimation of all four drugs. Developed method was successfully applied for estimation of all four drugs in their combined dosage form.

Keywords: irbesartan; telmisartan; hydrochlorothiazide; ramipril; hptlc; simultaneous estimation

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Abbreviations: HPTLC, high performance thin layer chromatography; ng, nanogram; R^2, correlation coefficient; TEL, telmisartan; HCZ, hydrochlorothiazide; RAM, ramipril; IRB, irbesartan; mg, milligram; SD, standard deviation; RSD, relative standard deviation; SE, standard error of mean; %, percentage

Introduction

For the determination of Irbesartan (IRB), Telmisartan (TEL), Hydrochlorothiazide (HCZ) and Ramipril (RAM), different analytical methods such as spectrophotometry,[1-6] spectrofluorimetry7,8 chemometrics,[9-14] high Performance Liquid Chromatography (HPLC),[15-35] LC-MS,[36-41] capillary zone electrophoresis[42] and High performance thin layer chromatography (HPTLC)[43-47] were reported individually or in combination with other drugs. But no method was reported so far for the simultaneous analysis of these drugs in ternary mixture. No HPTLC method was reported for simultaneous estimation of these drugs in combined dosage form. Therefore it was thought of interest to develop and validate HPTLC method for simultaneous estimation of IRB, TEL, HCZ and RAM in their combined dosage form. The present work deals with estimation of these two drugs in combined dosage form by HPTLC method.

Materials and methods

Instrumentation

The HPTLC system (CAMAG, Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (Camag, Muttenz, Switzerland), twin trough developing chamber (20×10cm), UV cabinet with dual wavelength lamps, win CATS software, syringe (100µl capacity, Hamilton) were used for chromatographic study.

Chemicals and reagents

IRB, TEL, HCZ and RAM were kindly supplied as gift samples by Madras Pharmaceuticals, Chennai. All chemicals and reagents used were of LR grade and purchased from S.D. Fine Chem Limited, Mumbai, India. Formulations containing IRB and HCZ, TEL and RAM were procured from local pharmacy.

Chromatographic conditions

Analysis was performed on 10×10cm aluminium plates pre-coated with 0.2mm layers of silica gel 60 F254 (Merck, Mumbai). Before use plates were washed with methanol, activated in an oven at 1050C for 20 min, then left to cool at room temperature. Standard solutions were applied to pre-washed activated plates, as 6mm bands, 6 mm apart, under a stream of nitrogen, by means of a Camag (Muttenz, Switzerland) Linomat V automated spray-on band applicator equipped with a Hamilton 100µl syringe (Reno, Nevada, USA). The plates were developed with 10ml of mobile phase, in a Camag twin trough chamber previously saturated with mobile phase vapour for 20 min.

After development the plates were removed from the chamber and dried in air. Densitometry was performed in reflectance mode with Camag TLC scanner 3 using WinCATS (version 1.4.4) software incorporating track position optimization. The slit dimensions were 4.00×0.30mm with scanning speed 20mm s-1.

Preparation of stock solutions of IRB, HCZ, TEL and RAM

A standard stock solution was prepared by adding 10mg of IRB, TEL and RAM and 20mg of HCZ to a 10 ml volumetric flask. The volume was made up to the mark with methanol. The stock solution containing 1mg/ml of each of IRB, TEL and RAM and 2mg of HCZ drug was then stored in refrigerator. The working solutions were freshly prepared by diluting 2.5ml of the stock solution to 10ml with methanol.

Calibration curve preparation

Amount of standard solution equivalent to 0.25 to 1.25µg of TEL, IRB and RAM and 0.5 to 2.5µg of HCZ were applied to the TLC plate,

developed, dried and scanned as mentioned above. This was done by spotting 1,2,3,4 and 5µl of working solution (0.5µg/ml of HCZ and 0.25µg/ml of other drugs). Calibration plots were constructed by plotting peak areas against the corresponding amount of the drugs.

Method validation

Specificity: The specificity of the method was ascertained by analyzing drugs from standard and sample. The bands for all four drugs from the sample were confirmed by comparing the Rf and UV spectra of the respective band with those that obtained from standard. The peak purity was assessed by comparing UV spectra acquired at three different positions on the band, i.e. peak start (s), peak apex (m) and peak end (e).

Linearity: For TEL, IRB and RAM response was a linear function of amount in the range 250-1250ng per spot, for HCZ the linear range was 500-2500ng per spot. The correlation coefficients, r were 0.9991 for TEL, 0.9991 for HCZ, 0.9992 for RAM and 0.9990 for IRB. The average linear regression equations were calculated and shown in (Table 1 & Figure 1).

Table 1 Linearity Data for all four drugs

Drug	Linearity range (ng/spot)	R2	Slope	intercept
Telmisartan	250-1250	0.9991	394.11	25.279
Hydrochlorothiazide	500-2500	0.9991	1200.7	93.564
Ramipril	250-1250	0.9992	510.85	51.568
Irbesartan	250-1250	0.999	800.14	96.021

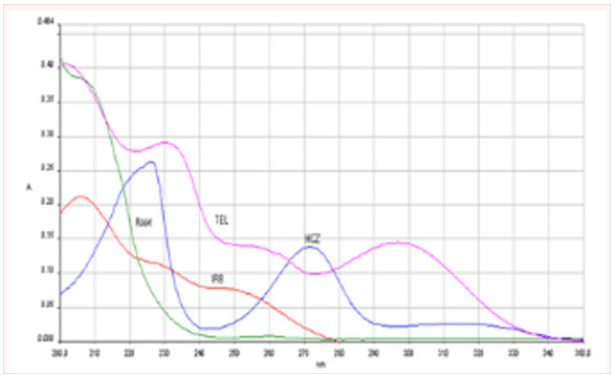

Figure 1 Typical absorption spectra of Irbesartan, Telmisartan, Hydrochlorothiazide and Ramipril.

Precision: Precision was determined by analysis of standard solutions containing concentrations of TEL, HCZ, RAM and IRB covering the entire calibration range. The precision of the method, as intra-day variation (RSD, %) was determined by analysis of these solutions three times on the same day. Inter-day precision (RSD, %) was assessed by analysis of these solutions on three different days over a period of one week. The results of precision studies are shown in (Table 2 & Figure 2).

Accuracy: The accuracy of the method was determined by analysis of standard additions at three different levels, i.e. multiple-level recovery studies. Sample stock solution obtained from the tablet formulations was spiked with amounts equivalent to 80, 100 and 120% of amounts of drugs in original solution. When these solutions were analyzed the recoveries were found to be within the acceptable limits.

Sensitivity: The sensitivity of measurement of TEL, HCZ, IRB and RAM was estimated in terms of limit of quantitation (LOQ). The smallest amounts detected under the chromatographic conditions used were estimated in terms of the limit of detection (LOD). LOQ and LOD were calculated by use of the equations LOD= 3.3×N/B and LOQ= 10×N/B, where N is the standard deviation of the peak areas of the drugs, taken as the measure of noise and B is the slope of the corresponding calibration plot.

Table 2 Recovery Data of all four drugs in tablet formulation

Drug	recovery level (%)	Initial amount (ng)	Amount added (ng)	Mean recovery (%) (n=3)	RSD (%)
	80	500	400	100.33	0.521
TEL	100	500	500	99.98	0.432
	120	500	600	100.25	0.332
	80	1000	800	101.31	0.411
HCZ	100	1000	1000	98.85	0.572
	120	1000	1200	100.34	0.38
	80	500	400	99.85	0.109
RAM	100	500	500	100.08	0.403
	120	500	600	100.21	0.233
	80	500	400	99.35	0.117
IRB	100	500	500	99.28	0.342
	120	500	600	100.17	0.61

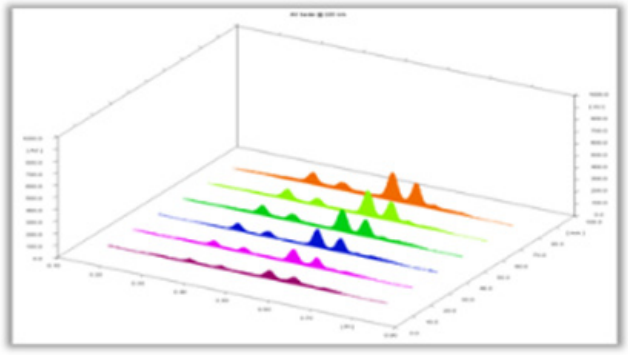

Figure 2 Three dimensional densitogram showing linearity and resolution of drugs.

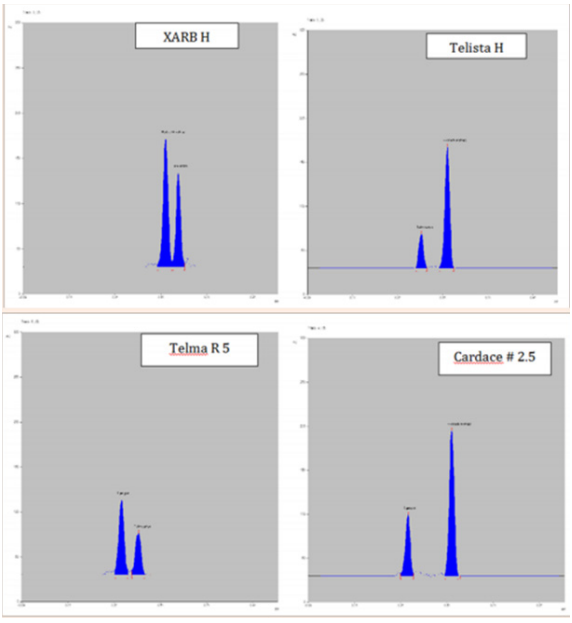

Figure 3 Densitogram of Marketed Formulations. XARB H: Irbesartan (150mg) and Hydrochlorothiazide (12.5mg). Telista H: Telmisartan (40mg) and Hydrochlorothiazide (12.5mg). Telma R: Telmisartan (40mg) and Ramipril (2.5mg). Cardace #2.5: Ramipril (2.5mg) and Hydrochlorothiazide (12.5mg).

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Table 3 Results of evaluation of precision

Drug	concentration (ng per Spot)	Intra-day precision (RSD, %, n=3)	Inter-day precision (RSD, %, n=3)
	250	0.823	1.231
TEL	500	0.674	0.984
	750	0.543	1.134
	500	0.456	0.873
HCZ	1000	0.762	0.985
	1500	0.342	0.885
	250	0.566	1.203
RAM	500	0.672	1.073
	750	0.487	0.983
	250	0.771	1.332
IRB	500	0.652	1.07
	750	0.472	0.768

Table 4 Analysis of Marketed Formulation

Drug	label Claim amount (mg/Tablet)	found[a] (mg)	Drug Content[a] (%)	Standard Deviation (SD)	RSD (%)	SE
XARB H (Formulation 1)						
IRB	150	150.43	100.28	0.402	0.4	0.131
HCZ	12.5	12.58	100.64	0.543	0.539	0.21
Telista H (Formulation 2)						
TEL	40	40.56	101.4	0.344	0.339	0.231
HCZ	12.5	12.57	100.56	0.441	0.438	0.208
Telma R5 (Formulation 3)						
TEL	40	40.14	100.35	0.624	0.621	0.312
RAM	5	4.96	99.2	0.562	0.566	0.22
Cardace # 2.5 (Formulation 4)						
RAM	2.5	2.53	101.2	0.076	0.075	0.125
HCZ	12.5	12.46	99.68	0.354	0.355	0.233

*Average of six estimations: T1: XARB H-Nicholas Piramal, Mumbai. Irbesartan: 150mg and Hydrochlorothiazide-12.5mg. T2: Telista H-Lupin Pharma, Mumbai. Telmisartan: 40mg and Hydrochlorothiazide-12.5mg. T3: Telma R5-Glenmark, Mumbai. Telmisartan: 40mg and Ramipril-5mg. T3: Cardace # 2.5-Aventis Pharma, Goa. Ramipril: 2.5mg and Hydrochlorothiazide-12.5mg

Analysis of marketed formulation

Twenty tablets of each preparation were weighed and powdered. Suitable amount of powder was transferred in to a 50ml volumetric flask. After addition of 3ml of methanol, the solution was centrifuged at 600rpm for 15min to ensure complete extraction of drugs from matrix. The volume was made up with methanol and filtered through a 0.45µ filter (Millipore, Milford, MA, USA). Standard addition technique was followed for drugs of lesser amount in formulations to meet the calibration limits.

Results and discussion

Selection of wavelength

To investigate the appropriate wavelength for simultaneous determination of HCZ, IRB, TEL and RAM, solutions of these compounds in methanol were scanned by UV-visible spectrophotometry (Perkin Elmer Lamda 25 model) in the range of 200-350nm. Suitable wavelength choices were selected for monitoring the drugs in HPTLC system from the overlapped UV spectra. Suitable wavelength choices obtained from the overlapped UV spectra were 260, 230 and 210nm. Solutions of each substance were spotted on the TLC plate and the responses (peak area) were recorded at 260, 230 and 210nm after development. It was observed that 210nm as the most appropriate wavelength for analysis of all the substances with suitable sensitivity.

Mobile phase optimization

A variety of mobile phases, for example chloroform-methanol, chloroform-toluene, chloroform-toluene-methanol, chloroform-toluene-acetic acid, acetonitrile-toluene-acetic acid and methanol-toluene-acetic acid mixtures were investigated for separation of TEL, IRB, HCZ and RAM. Finally a mixture of acetonitrile, toluene and methanol gave good resolution, but peak shapes were missing. Formic acid was added to get good peak shape. Addition of small amount of formic acid reduced peak tailing and improved peak characteristics. Acetonitrile:toluene:methanol:formic acid 8:10:2:0.6 (*v/v/v/v*) was found to result in best peak shape. The drugs RAM, TEL, HCZ and IRB were satisfactorily resolved with R_f values 0.35, 0.42, 0.54 and 0.60 respectively. Pre-saturation of TLC chamber with mobile phase vapour for 20 min ensured more reproducible migration of the drugs and better resolution.

Method validation

Linearity and range: Calibration plots were constructed by plotting peak areas against the corresponding amount of the drugs. For TEL, IRB and RAM response was a linear function of amount in the range 250-1250ng per spot, for HCZ the linear range was 500-2500ng per spot. The correlation coefficients, r were 0.9991 for TEL, 0.9991 for HCZ, 0.9992 for RAM and 0.9990 for IRB. The average linear regression equations were calculated and shown in Table.

Specificity: Peak purity for the drugs were tested by acquiring spectra at the peak start (S), peak apex (A) and peak end (E) positions. Results from correlation of the spectra were: for RAM, r(S, A) = 0.9996 and r(A,E) = 0.9994, for TEL r(S, A) = 0.9998 and r(A,E) = 0.9995, for HCZ r(S, A) = 0.9998 and r(A,E) = 0.9997 and for IRB r(S, A) = 0.9999 and r(A,E) = 0.9998 respectively. It can thus be concluded that no impurities or degradation products were eluted along with the peaks obtained from the standard drug solution.

Precision: The repeatability of sample application was assessed by application of 1µl of standard drug solution seven times to an HPTLC plate, development of plate, and recording peak height and peak area of the zones. The RSD (%) of peak height and area were 0.65 and 0.54 respectively for RAM, 0.55 and 0.43 for HCZ, 0.33 and 0.43 for TEL and 0.21 and 0.42 for IRB.

Repeatability of measurement of peak height and area were determined by application of 1µl standard drug solution to an HPTLC plate, developing the plate and scanning the zone seven times without changing the position of the plate. RSD (%) of peak height and area were 0.21 and 0.13 respectively for RAM, 0.08 and 0.11 for HCZ, 0.04 and 0.12 for TEL and 0.09 and 0.05 for IRB.

Accuracy: The accuracy of the method was determined by analysis of standard additions at three different levels, i.e. multiple-level recovery studies. Sample stock solution obtained from the tablet formulations was spiked with amounts equivalent to 80, 100 and 120% of amounts of drugs in original solution. When these solutions were analyzed the recoveries were found to be within the acceptable limits ranging from 98.85% to 101.31% for all four drugs.

Sensitivity: LOQ and LOD were calculated by use of the equations LOD =3.3×N/B and LOQ = 10×N/B, where N is the standard deviation of the peak areas of the drugs, taken as the measure of noise and B is the slope of the corresponding calibration plot. LOQ and

LOD for TEL were 136.23 and 44.38ng, for RAM they were 168.33 and 56.78ng, for IRB they were 180.54 and 65.66ng, for HCZ they were 268.44 and 98.55ng respectively.

Assay of marketed formulation

The applicability of the method was verified by the determination of all the drugs in respective formulations. The content of all the drugs were found to be within the acceptance limit and are mentioned in (Table 3 & 4) (Figure 3). No interference from the excipients present in the marketed formulations was observed. The low % RSD value indicated the suitability of the method for routine analysis of all four drugs in various formulations.

Conclusion

HPTLC method was developed for estimation of IRB, HCZ, TEL and RAM in tablet formulation. The method was validated as per ICH (Q2 R1) guidelines. The proposed method was found to be specific, accurate, precise and sensitive. The developed method was successfully applied for quantitative analysis of all four drugs in their combined tablet formulation. Results were found to be in good agreement with label claim of their combined tablet formulation. The proposed method can be applied for routine analysis of IRB, HCZ, TEL and RAM in their combined dosage form.

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Conflicts of Interset

None.

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