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HPTLC method development and validation for simultaneous estimation of Olmesartan medoximil, Amlodipine besylate and Hydrochlorothiazide in bulk drug and formulation

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ABSTRACT

In this study a new simple, precise and accurate HPTLC method has been developed for simultaneous estimation of Olmesartan medoxomil, Amlodipine besylate and Hydrochlorothiazide in pharmaceutical dosage forms. Chromatographic separation of the drugs was performed on precoated silica gel 60 F_{254} plates using Chloroform: Methanol: Formic acid (8.5:1.5:0.25, v/v/v). A TLC scanner set at 254 nm was used for the direct evaluation of the chromatogram in reflectance-absorbance mode. The drugs were satisfactorily resolved with R_f values of 0.57 ± 0.02, 0.36 ± 0.04 and 0.21 ± 0.02 for Olmesartan medoxomil, Amlodipine besylate and Hydrochlorothiazide. The accuracy and reliability of the method was assessed by evaluation of linearity (200-2000 ng/spot for OLME, 50-500 ng/spot for AMLO and 125-1250 ng/spot for HCTZ), precision (intra-day RSD 0.4510%, inter-day RSD, 0.2773 % and analyst to analyst RSD 0.1959 for OLME, intra-day RSD 1.0216 %, inter-day RSD 0.3137 %, analyst to analyst RSD 0.8557 % for AMLO) (intra-day RSD 0.4117 %, inter-day RSD 0.2974 % and analyst to analyst RSD 0.2038 % for HCTZ, accuracy for OLME, AMLO and HCTZ afford 98-102% and specificity in accordance with ICH guidelines. This HPTLC Method had the potential to determine these drugs simultaneously from dosage forms without any interference.

KEYWORDS

Olmesartan medoxomil;
Amlodipine besylate;
Hydrochlorothiazide;
Densitometric detection;
HPTLC;
ICH;
Method validation.

INTRODUCTION

Olmesartan medoxomil[1] (OLM), chemically it is 4-(1-Hydroxy-1-methylethyl)-2-propyl-1-[[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl]-1H-imidazole-5-carboxylic acid (5-Methyl-2-oxo-1, 3-dioxol-4-yl) methyl ester. Olmesartan medoxomil is hydrolyzed to olmesartan during absorption from the gastrointestinal tract. Amlodipine besylate[2] (AML) is 3 – Ethyl – 5 – methyl (±) 2 - [(2 – amino ethoxy) methyl] – 4 - (2 – chloro phenyl) – 1, 4 – dihydro – 6 – methyl - 3, 5 – Pyridine dicarboxylate, mono benzene Sulphonate. Hydrochlorothiazide[3] (HCTZ) is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide. The molecular weight of Olmesartan medoxomil, Amlodipine besylate, and Hydrochlorothiazide (Figure 1) are 558.6, 567.1 and 297.7 respectively. It is approved to treat high blood pressure. It works by relaxing the blood vessels, improving blood flow, and reducing blood volume. All those drugs are

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included in official books[1-5] which suggest different methods for their analysis. Literature survey reveals that there are some reported methods for the estimation of drugs using spectrophotometry[7-9], RP-HPLC[10-13], densitometry[13-18]. It is a fixed-dose triple combination has shown to be more effective at lowering blood pressure than using only two of the components of this medication alone. No HPTLC[6] method has been reported for simultaneous estimation of OLME, AMLO, and HCTZ in the combined dosage form. The objective of this work was to develop an accurate, precise, specific, reproducible and robust method for analysis of drugs in their formulations

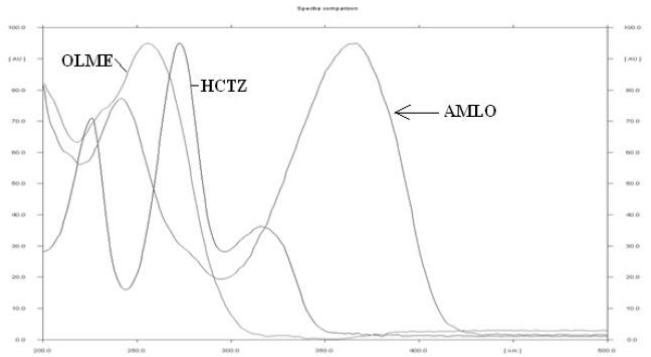

Figure 2 : In-situ overlain spectra of OLME, AMLO and HCTZ from 200 - 500 nm

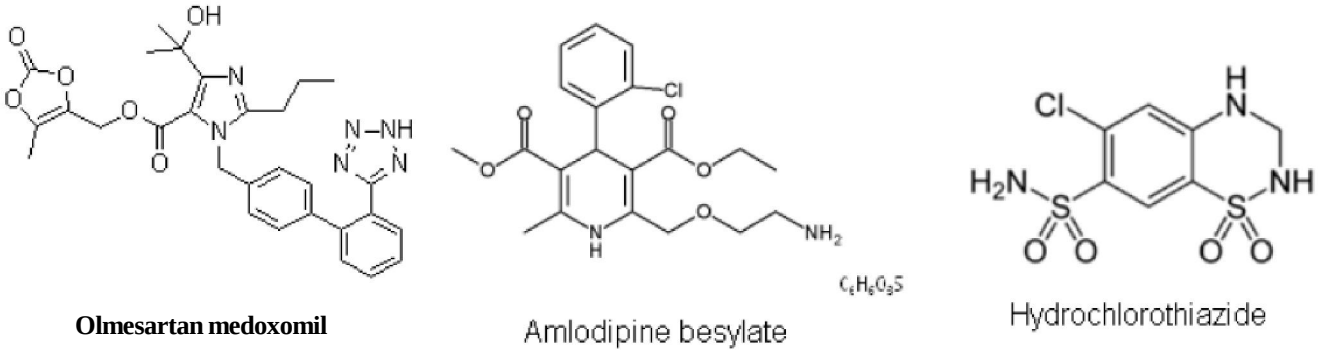

Figure 1 : Structure of Olmesartan, Amlodipine and Hydrochlorothiazide

EXPERIMENTAL

Materials

An analytical pure sample OLM, AML and HCTZ drugs (Accent Pharma. Pondy, INDIA), Analytical grade Methanol (LOBA, India Ltd) Chloroform (Thermo fisher scientific) and double distilled water was used in the present study. The commercially available tablets Olmat-AMH® tablets containing a combination of Olmesartan medoxomil 20mg, Amlodipine besylate 5mg and Hydrochlorothiazide 12.5mg were procured from Micro Labs, Bangalore from local pharmacy.

Instrumentation and chromatographic conditions

The method development was performed by using Camag HPTLC containing Camag Linomat IV applicator, Hamilton 100 microlitre sample syringe on E.MERCK KGaA silica gel *(Art. No.1.05554.0007)* precoated plate 60 F 254, [(20 × 10 cm) with 250 µm thickness; supplied by Anchrom Techno, Mumbai]. The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. A constant application rate of 0.1 µLs^{-1} was used and the space between two bands was 6 mm. The slit dimension was kept at 5mm × 0.45 mm and the scanning speed was 10 mm s^{-1}. The mobile phase was consists of Chloroform: Methanol: Formic acid (8.5:1.5:0.25, v/v/v) and 10 mL of the mobile phase was used for chromatography. It was observed that all the drugs showed considerable absorbance at 254 nm. So, 254 nm was selected as the wavelength for detection as shown in Figure 2. Linear ascending development was carried out

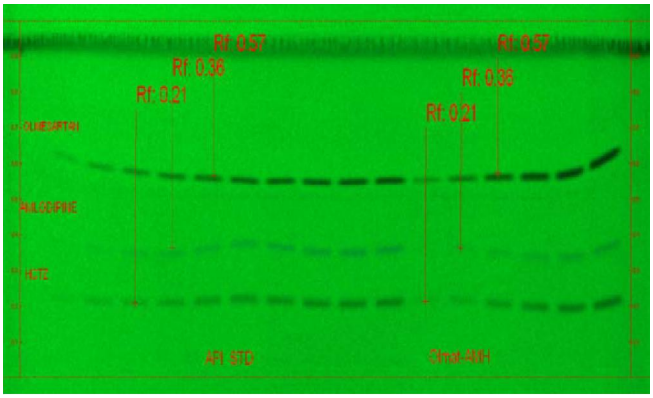

Figure 3 : Densitogram spots obtained in standard and formulation @ 254 nm

in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25°C ± 2). Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode and operated by winCATS software (V1.4.3). The source of radiation used was a deuterium lamp emitting a continuous UV spectrum between 200 and 500 nm. Dissolution of the compounds was enhanced by sonication on a Shimadzu sonicator and REMI centrifuge.

Preparation of standard stock solution

Standard stock solution was prepared separately by dissolving of OLM, AMLO and HCTZ diluted with methanol with ultrasonication for 5 min to get a final concentration of 600 µg/ ml, 400 µg/ ml and 200 µg/ ml of Olmesartan medoxomil, Hydrochlorothiazide and Amlodipine besylate respectively.

Method validation

The method was validated in compliance with ICH guidelines[19, 20].

Linearity

Stock solutions were further diluted to obtain a series of concentrations ranging from 200 - 2000 ng/spot of olme, 50 – 500 ng/spot of amlo and 125 – 1250 ng/ spot of HCTZ were applied on the TLC Plate. The TLC Plate was dried, developed and analyzed photometrically. Linearity of the method was studied by injecting separately of each concentration with six times. Calibration curves of OLME, AMLO and HCTZ were plotted separately of peak area with concentrations.

Specificity

The specificity of the method was confirmed by comparing the R_f values and spectra of the spots with that standards and test samples of olmesartan, amlodipine and hydrochlorothiazide. The peak purity of samples was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot. The peak purity was determined on WinCATS software V 1.4.3.

Analysis of marketed formulation

Powdered tablet equivalent to 20 mg Olmesartan medoxomil, 5 mg equivalent of Amlodipine and 12.5 mg Hydrochlorothiazide was transferred to a 100 ml volumetric flask containing 30 ml methanol and sonicated for 20min. The volume was then made up to the mark with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the supernatant solution was filtered through Whatmann paper No. 41. From the filtrate (200 µg/ml), sample solution of 5µl of this solution was applied six times on the TLC plate to give spot concentrations of 1000 ng/band of OLM, 250 ng/ band of AML and 625 ng/band of HCTZ respectively. The plate was developed in the previously described chromatographic conditions. The peak areas of the spots were measured at 254 nm and concentrations in the samples were determined using multilevel calibration.

Precision

To study the precision, The ICH Guideline recommended that repeatability should be assessed by using minimum of nine determinations in the specified range (i.e. 3 concentrations and 3 replicates of each concentration or using a minimum of 6 determinations of the test concentration). Repeatability, intra-day inter-day precision and Analyst to analyst were applied. Intraday precision was studied by taking three different concentrations 400, 800 and 1200 ng/ band of Olmesartan, 200, 400 and 600 ng/band of Amlodipine and 250, 500 and 750 ng/band of Hydrochlorothiazide. The given concentrations were minimum of 6 determinations of the test concentration for repeatability, For intraday and inter-day precision, applied 3 concentrations and 3 replicates of each concentration to see the variation of their peak area within a day and for three different days.

Accuracy

To study the recovery of formulation, standard drugs of Olmesartan, Amlodipine and Hydrochlorothiazide at 80%, 100%, 120% were added to the labeled claim of olmesartan 20 mg (i.e. the spiked amounts were 800, 1000, 1200 ng/band). To study the recovery of amlodipine, standard were added to the labeled claim of amlodipine 5 mg (i.e. the spiked amounts were 400, 500 and 600 ng/band). Similarly, to study recovery of hydrochlorothiazide, standards were added to the labeled claim of hydrochlorothiazide 12.5 mg (i.e. the spiked amounts were 1000, 1250, 1500 ng/band). The % recovery and % RSD were calculated and found to

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be within the limits.

Limit of detection and quantification

Determinations of limit of detection and quantification were based on the standard deviation of the response and the slope as:

LOD = 3.3 σ/S, and LOQ = 10σ/S

Where σ is the standard deviation of y-intercepts of regression line and S is the slope of the corresponding standard curve. LOD and LOQ were determined by measuring the magnitude of analytical background by spotting a blank and calculating the signal-to-noise ratio for OLME, AMLO and HCTZ by spotting a series of solutions until the S/N ratio 3 for LOD and 10 for LOQ.

Robustness:

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. Small changes in the mobile phase composition (±0.1 mL), the effect on the results were examined. Mobile phases having different proportions of components, e.g. Chloroform: Methanol: Formic acid in the ratio of (8.6:1.5:0.25, v/v/v), (8.4:1.5:0.25, v/v/v), (8.5:1.6:0.25, v/v/v), (8.5:1.4:0.25, v/v/v) etc., were tried. The time from spotting to chromatography and from chromatography to scanning was varied by 10 min and analysed. The robustness of the method was determined at different proportions of mobile phase. The effect of changes on R_f values and peak area was evaluated by calculating the relative standard deviations (RSD) for each parameter.

RESULTS AND DISCUSSION

Method validation

Linearity and calibration curves

Calibration graphs for the three drugs were constructed by plotting peak areas against the corresponding concentrations (ng/band). According to ICH guidelines, validation of analytical methods[21], linear relationship was found to be less precise due to the minimal fitting of the residuals on the calibration line indicating lower precise correlations of these drugs. Plots of residuals against the concentrations of Olmesartan, Amlodipine and Hydrochlorothiazide (Figure 4, 5 and 6) showed against their concentrations were distributed both above and below the zero residual line for Olmesartan, Amlodipine and Hydrochlorothiazide

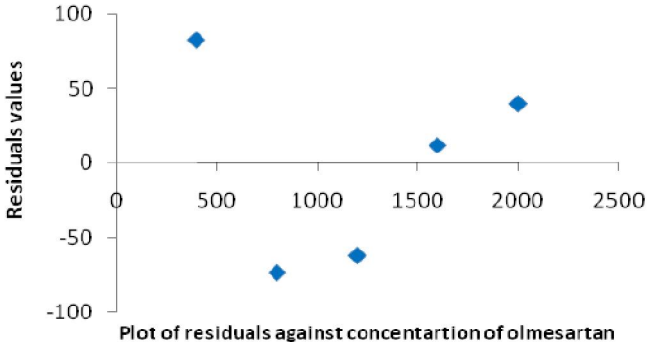

Figure 4 : Residual plots of Olmesartan medoxomil

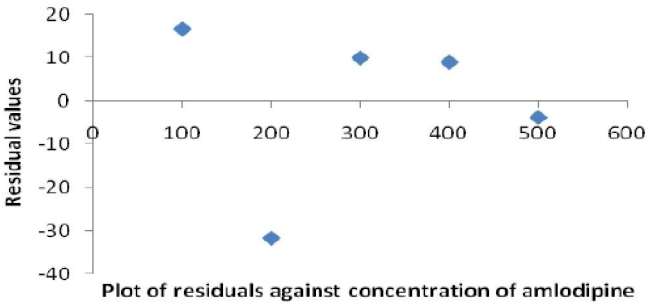

Figure 5 : Residual plots of amlodipine besylate

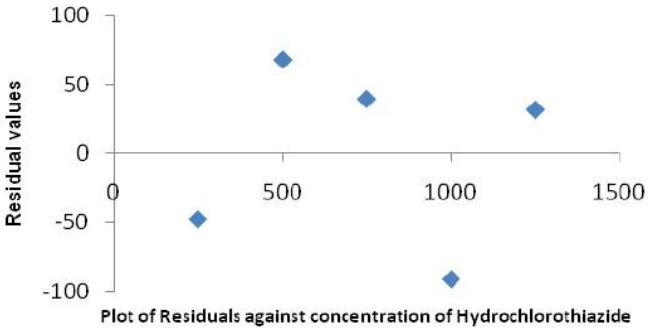

Figure 6 : hydrochlorothiazide

Specificity

Peak purity for the drugs was 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 Olmesartan r(S, M) = 0.9998 and r(M, E) = 0.9995, for Amlodipine r(S, M) = 0.9996 and r(M, E) = 0.9993 and for Hydrochlorothiazide r(S, M) = 0.9997 and r(M, E) = 0.9994. It can be concluded that no impurities or degradation products were eluting with the peaks obtained from the standard drug solution. The in-situ overlain spectral comparison of the spots of the standards and dosage forms were presented in

Figure 2 and Figure 3

Analysis of marketed formulation

The spots at R_f 0.57 ± 0.02, 0.36 ± 0.04 and 0.21 ± 0.02 for OLM, AML and HCTZ were observed respectively in the densitogram of the drug samples extracted from tablets. The OLM, AML and HCTZ content was found to be close to 99.96±0.58, 98.91±1.25 and 98.75±0.81 % and the results are summarized in TABLE 2. The low % RSD value indicated the suitability of this method for routine analysis.

Precision

The developed method was found to be precise % RSD values for intraday, interday precision and analyst to analyst precision studies were < 2 %, respectively as recommended by ICH guidelines[19, 20]. The results are shown in TABLE 3.

Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100%, and 120% Known amounts of standard OLM, AML and HCTZ were added to pre-analyzed samples and were subjected to

TABLE 1 : Summary of method validation parameters for calibration curves of Olmesartan, Amlodipine and Hydrochlorothiazide using peak areas

Parameters	Olmesartan	Amlodipine	Hydrochlorothiazide
Linearity range (ng/band)	200-2000	50-500	125-1250
Correlation coefficient (r)	0.9997	0.9993	0.9995
Regression equation(Y=mx+c)	Y =5.0491x +76.4929	Y = 2.8368x + 27.7161	Y = 5.1809x + 126.056
Slope (m)	5.0491	2.8368	5.1809
Intercept (c)	76.4929	27.7161	126.056
Limit of detection(ng/band)	2.9917	2.3811	4.3326
Limit of quantification(ng/band)	9.0658	7.2154	13.1292
Standard deviation, n=6	1.78	1.59	1.83

TABLE 2 : Assay results of the fixed dose combination tablets (n=5)

Parameters	Olmesartan	Amlodipine	Hydrochlorothiazide
Label claim(mg/tab)	20mg	5mg	12.5mg
Actual amount added(ng/band)	1000ng	250ng	625ng
drug content	99.96 ± 0.6254	98.91 ± 0.9008	98.75 ± 0.4792
% RSD	0.6256	0.9107	0.4852

TABLE 3 : Intra and Inter-day precision, analyst to analyst precision for Olmesartan, Amlodipine and Hydrochlorothiazide (n=6)

Drug	Amount labeled (mg/tab)	Percentage Obtained*			SD			%RSD		
		Intra day	Inter day	Analyst to analyst	Intra day	Inter day	Analyst to analyst	Intra day	Inter day	Analyst to analyst
	20	99.82	100.32	100.23						
OLM	20	100.46	99.94	100.21	0.4508	0.2773	0.1965	0.4510	0.2773	0.1959
	20	99.59	99.78	100.56						
Mean		99.95	100.01	100.33						
	5	98.45	98.89	98.56						
AML	5	100.08	99.25	99.76	1.0105	0.3113	0.8485	1.0216	0.3137	0.8557
	5	98.23	99.51	99.97						
Mean		98.92	99.21	99.16						
	12.5	99.24	99.52	98.96						
HCT	12.5	98.46	98.93	99.11	0.4067	0.2951	0.2020	0.4117	0.2974	0.2038
	12.5	98.65	99.24	99.36						
Mean		98.78	99.23	99.14						

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TABLE 4 : Recovery study for Olmesartan, Amlodipine and Hydrochlorothiazide (n=3)

Drug	Label claim (mg/tablet)	Amount Added		Amount Recovered (ng/band)	% Recovery
		%	(ng/band)		
Olmesartan	20	80%	800	796.43	99.55 ± 0.16
		100%	1000	998.21	99.82± 0.38
		120%	1200	1191.57	99.29± 0.23
Amlodipine	5	80%	400	393.28	98.32± 0.51
		100%	500	496.83	99.36± 0.29
		120%	600	598.61	99.76± 0.31
Hydrochlorothiazide	12.5	80%	1000	997.84	99.78± 0.16
		100%	1250	1249.58	99.96± 0.24
		120%	1500	1492.47	99.49± 0.19

[1]**Average value ± relative standard deviation from three analyses**

the proposed method. Results of recovery studies are shown in TABLE 4.

Lod loq

Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ shown in the TABLE 1.

Robustness

The robustness of the method was determined by variations in mobile phase composition, volume of mobile phase, development distance, time from application to development and time from development to scanning mobile phase composition and volume variation on R_f values shown in TABLE 5. The method was found to be unaffected by small changes with R_f values shows

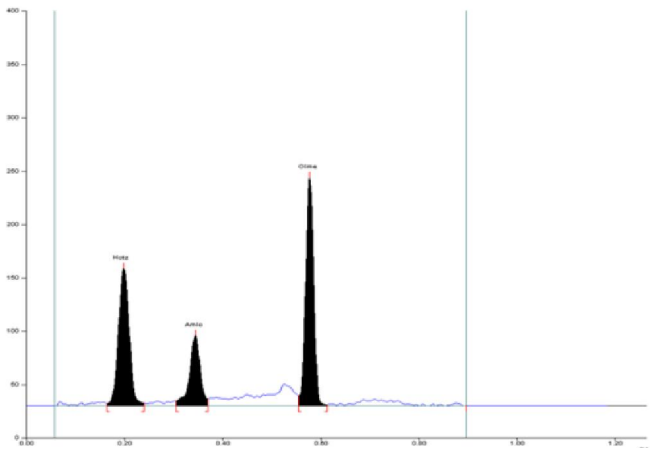

Figure 7 : HPTLC densitogram obtained solution of Olmesartan, Amlodipine and Hydrochlorothiazide

TABLE 5 : Effect of mobile phase composition and volume variation on R_f values

Mobile phase composition, v/v/v Chloroform: Methanol: Formic acid	R_f value		
	Olmesartan	Amlodipine	Hydrochlorothiazide
8.5:1.5:0.25 (optimized)	0.61	0.34	0.20
8.7:1.5:0.25	0.60	0.32	0.20
8.3:1.5:0.25	0.57	0.32	0.21
8.5:2.0:0.25	0.59	0.30	0.18
8.5:1.0:0.25	0.64	0.33	0.21
8.5:1.5:0.50	0.65	0.35	0.24
8.5:1.5:0.15	0.61	0.30	0.21

TABLE 6 : Peak area Robustness study for the developed method (n= 6)

Parameter studied	% RSD		
	Olmesartan	Amlodipine	Hydrochlorothiazide
Composition of mobile phase (±2%)	1.12	1.23	0.98
Volume of mobile phase (±5%)	0.84	0.78	0.67
Time from spotting to development (10 min)	0.41	0.37	0.32
Time from development to scanning (10min)	0.78	0.63	0.83

*** % RSD were calculated from the peak areas of densitograms**

the % RSD less than 2%, indicating that the method is robust shown in the TABLE 6.

CONCLUSION

The proposed HPTLC densitometric method was validated as per ICH guidelines. This validated HPTLC method[21] proved to be simple, fast while comparing with other methods and thus can be used for routine analysis of olmesartan, amlodipine and hydrochlorothiazide in combined tablet dosage forms.

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