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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF OLMESARTAN MEDOXOMIL AND CHLORTHALIDONE IN COMBINED TABLET DOSAGE FORMS

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ABSTRACT

The present work describes development and validation of a new simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of Olmesartan medoxomil and Chlorthalidone in combined tablet dosage form. The chromatographic separation was carried out using aluminium plates precoated with silica gel $60F_{254}$ (10 × 10 cm) as stationary phase and Toluene: Ethyl acetate: Methanol (5: 3: 2, v/v/v) as mobile phase. Retention factors for Olmesartan medoxomil and Chlorthalidone were found to be 0.48±0.02 and 0.72±0.02, respectively. The wavelength selected for detection was 229 nm. Drug samples were subjected to different stress conditions like hydrolysis, oxidation, photolysis and thermal degradation. The developed method has been validated for linearity, accuracy, precision, limit of detection and limit of quantification and robustness, as per ICH guidelines. Results were found to be linear in the concentration range of 200-1200 ng $band^{-1}$ for Olmesartan medoxomil and 125-750 ng $band^{-1}$ for Chlorthalidone, respectively. The percentage drug contents obtained for Olmesartan medoxomil and Chlorthalidone were 99.71 and 100.45, respectively. The developed method can be used for the quantification of drugs in combined tablet dosage form as well as for routine analysis in quality control laboratories.

KEYWORDS: Olmesartan medoxomil, Chlorthalidone, HPTLC, Stability Studies, Validation.

INTRODUCTION

Olmesartan medoxomil (OLME), chemically, (5-methyl-2-oxo-1,3-dioxol-4-yl) methyl 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl] methyl] imidazole 4-carboxylate) is a specific angiotensin II type 1 (AT1) receptor antagonist used for the treatment of high blood pressure.[1] Chlorthalidone (CHLOR), (2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1yl)benzene-1-sulfonamide) is thiazide diuretic is often used in the management of hypertension and oedema.[2]

Extensive literature review reveals that several analytical methods like spectrophotometry[3-9], High Performance Liquid Chromatography (HPLC)[10-16], High Performance Thin Layer Chromatographic (HPTLC)[17] have been reported for determination of OLME in pharmaceutical formulations either as single or in combination with other drugs. Analytical methods reported for determination of CHLOR includes UV[18-20], HPLC[21-24] and HPTLC[25,26] in pharmaceutical formulations either as single or in combination with other drugs. Stability indicating RP-HPLC methods for simultaneous estimation of OLME and CHLOR in tablet dosage form are also found in the literature.[27, 28]

To best of our knowledge, no reports were available in the literature for simultaneous determination of OLME and CHLOR in the combined tablet dosage form by stability indicating HPTLC method. Based on this observation, we have developed a selective, accurate, precise and sensitive high performance thin layer chromatography method for the simultaneous estimation of OLME and CHLOR in combined tablet dosage form in accordance with International Conference on Harmonisation Guidelines.[29, 30]

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade working standard OLME and CHLOR was kindly supplied by Zuventus Healthcare Ltd. (Mumbai, India). The pharmaceutical dosage form used in this study was Olmin 20-CH tablets labeled to contain 20 mg of OLME and 12.5 mg of CHLOR were procured from the local market. Toluene, methanol and ethyl acetate (all AR grade) was purchased from Merck specialties Pvt. Ltd. Mumbai, India.

Instrumentation and chromatographic conditions
Chromatographic separation of drugs was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm ×10 cm with 250 µm layer thickness) from E. MERCK, (Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 5 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland). Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using toluene: ethyl acetate: methanol (5: 3: 2, v/v/v) as mobile phase. The mobile phase was saturated in chamber for 15 min.

After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner III at 229 nm for all developments operated by winCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of standard stock solutions
Accurately weighed 20 mg of OLME was dissolved in 10 mL of methanol to get the solution having concentration 2000 µg mL^{-1}. One mililitre of the above solution was diluted further with methanol to acquire final working concentration 200 ng μL^{-1}. Standard solution for CHLOR was prepared by dissolving accurately weighed 12.5 mg in 10 mL of methanol to get the solution having concentration 1250 µg mL^{-1} from which 1 mL of solution was diluted with same solvent to get solution having final concentration 125 ng μL^{-1}.

Selection of Detection Wavelength
After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 229 nm. So, 229 nm was selected as the wavelength for detection.

Analysis of marketed formulation
Twenty tablets were weighed accurately and finely powdered. A tablet powder equivalent to 20 mg OLME (12.5 mg of CHLOR) was weighed and transferred to a 10 mL volumetric flask having about 6 mL of methanol. The contents were sonicated for 15 min, filtered and volume was made up to the mark with methanol. From the above solution, 1 mL of solution was diluted using same solvent to achieve final concentration of 200 ng μL^{-1} for OLME and 125 ng μL^{-1} for CHLOR. Two micrilitre volume of this solution was applied on TLC plate to obtain final sample concentration of 400 ng $band^{-1}$ for OLME and 250 ng $band^{-1}$ for CHLOR. After chromatographic development peak areas of the bands were measured at 229 nm and the amount of each drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies of bulk drugs
The stability studies were performed by subjecting the bulk drugs individually to the physical stress (acid, base, peroxide, heat and light) and stability was accessed. The stress degradation studies were carried out at initial drug concentration of 2000 µg mL^{-1} of OLME and 1250 µg mL^{-1} of CHLOR in methanol. The hydrolytic studies were carried out by mixing the drug solutions of OLME and CHLOR with 0.1 N HCl and 0.1 N NaOH and were kept separately at room temperature for 2 h and 1 h to achieve degradation within the acceptable limit. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 200 ng $band^{-1}$and 125 ng $band^{-1}$ of OLME and CHLOR, respectively. Neutral hydrolysis study was performed by treating the drugs separately with water and the resulting solutions were kept at room temperature for 2 h. The oxidative degradation was carried out in 6 % H_2O_2 and the sample was diluted with methanol to obtain solution having concentration 200 ng $band^{-1}$ and 125 ng $band^{-1}$ of OLME and CHLOR, respectively. Thermal stress degradation was performed by keeping the solid drugs individually in oven at 60ºC for a period of 4 h. Photolytic degradation studies were carried out by exposing both drugs individually to UV light up to 200-watt h square $meter^{-1}$ for 1 d. Thermal and photolytic samples were diluted with methanol to get the concentration of 200 ng $band^{-1}$and 125 ng $band^{-1}$ of OLME and CHLOR, respectively.

RESULTS AND DISCUSSION

Method optimization
The prime aim in developing this stability indicating HPTLC method is to achieve the satisfactory resolution of drugs and their degradation products. Initially, many method trials were performed using different mobile phases in order to obtain better separation. Finally the mobile phase comprising toluene: ethyl acetate: methanol (5: 3: 2, v/v/v) was selected as optimal for obtaining well defined and resolved peaks for the drugs. Densitometric evaluation was carried out at 229 nm. The retention factors were found to be 0.48 ± 0.02 and 0.72± 0.02 for OLME and CHLOR, respectively. Representative densitogram of mixed standard solution of both drugs is shown in Figure 1.

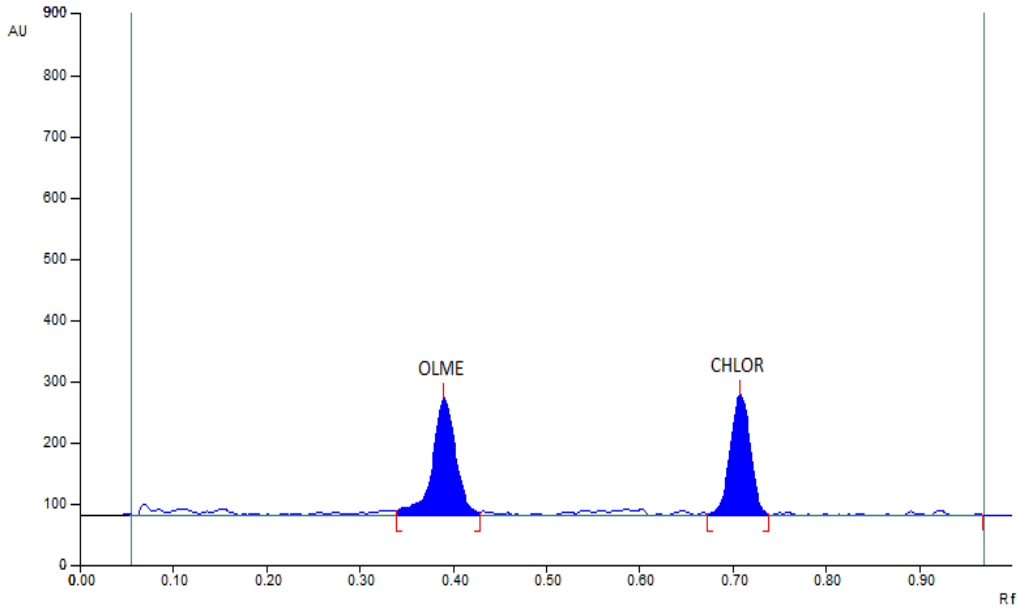
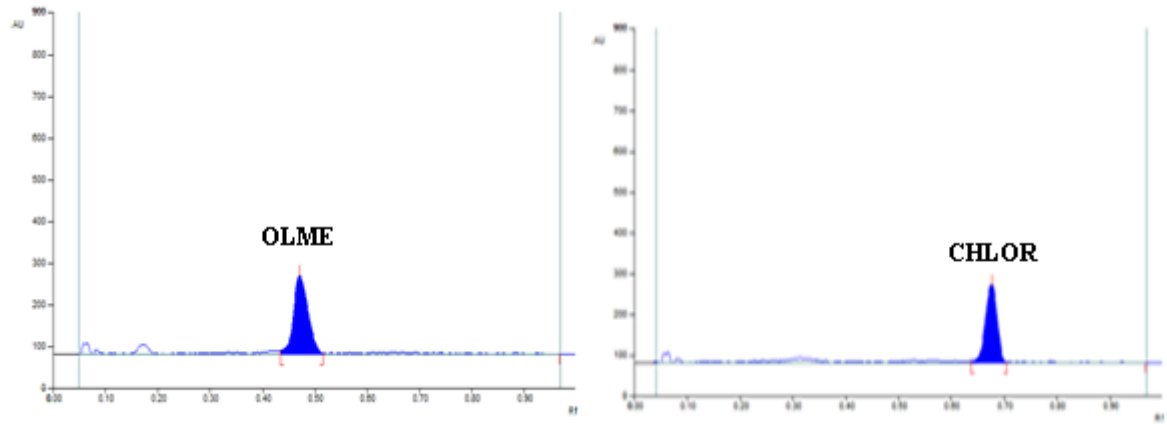


Fig. 1: Representative densitogram of standard solution of OLME (600 ng band^{-1}, Rf= 0.48 ± 0.02) and CHLOR (375 ng band^{-1}, Rf= 0.72 ± 0.02)

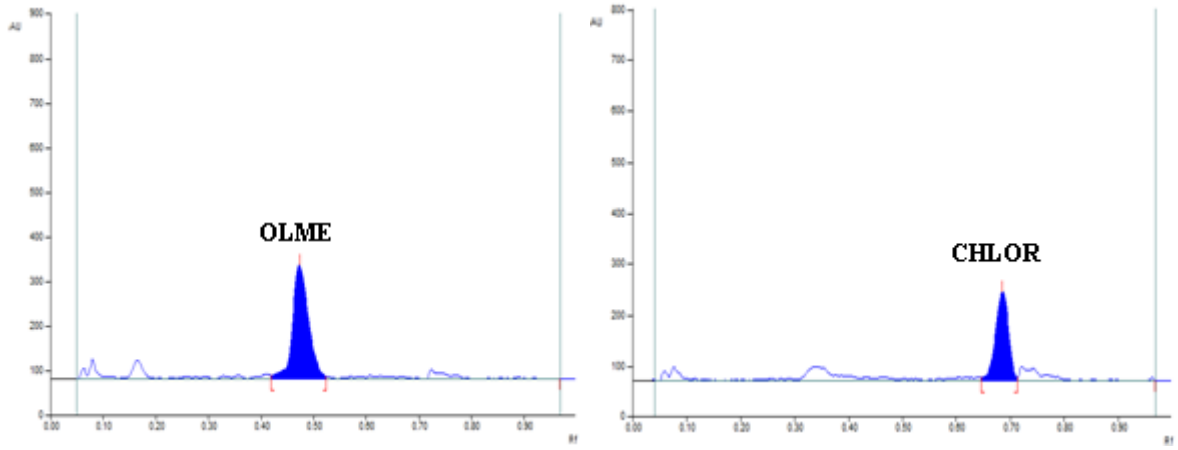
Forced degradation studies

The stress degradation results revealed the susceptibility of both the drugs to hydrolytic, oxidative, thermal and photolytic stress conditions. Marked degradation in the densitograms was observed without appearance of degradation peaks but there was reduction in the peak areas of both drugs after degradation. Figures 2 and 3 shows the densitograms of acid and alkali hydrolytic degradation, while Figures 4 and 5 shows the densitograms of neutral and oxidative degradation, respectively.



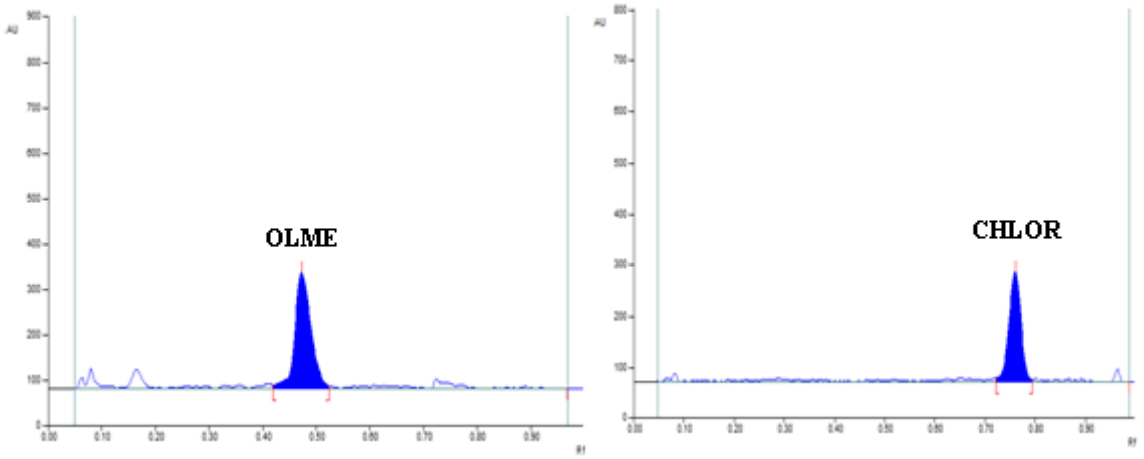
(a) **(b)**

Fig. 2: Densitogram of (a) OLME and (b) CHLOR obtained after acid degradation



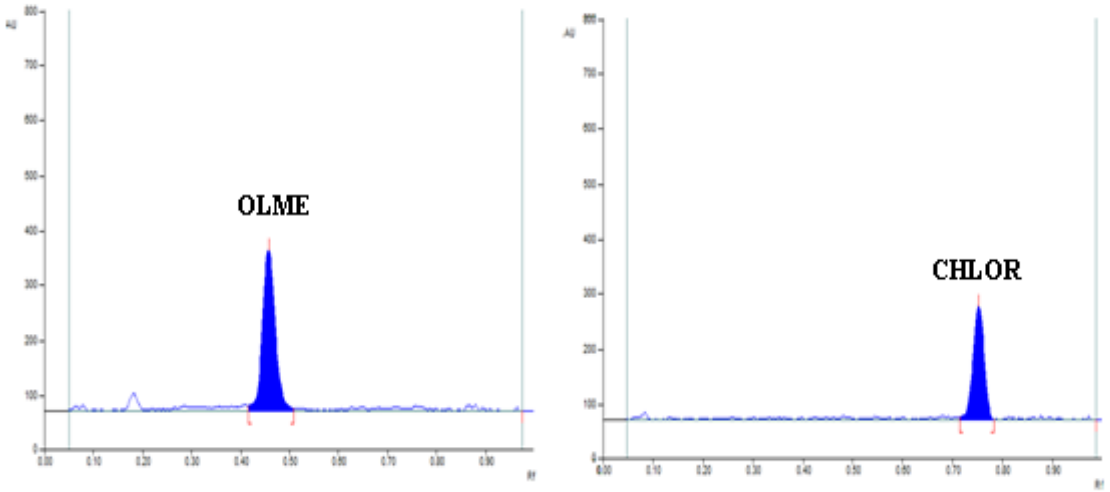
(a) **(b)**

Fig. 3: Densitogram of (a) OLME and (b) CHLOR obtained after alkali hydrolysis



(a) **(b)**

Fig. 4: Densitogram of (a) OLME and (b) CHLOR obtained after neutral hydrolysis



(a) **(b)**

Fig. 5: Densitogram obtained for (a) OLME and (b) CHLOR after peroxide treatment

Peak purity results greater than 991 indicate that peaks for both drugs are homogeneous in all stress conditions tested. The unaffected assay of tablet formulation confirmed the stability indicating power of the method. The findings of degradation studies are represented in Table 1.

Table 1: Forced degradation studies of OLME and CHLOR

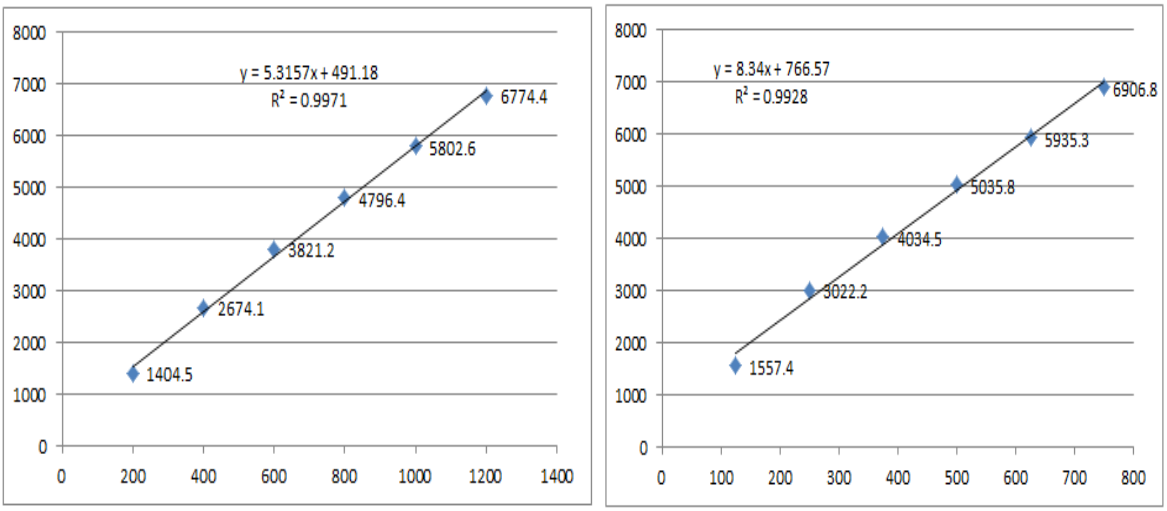
Stress conditions/ duration	OLME		CHLOR	
	% Assay	% degradation	% Assay	% degradation
Acidic / 0.1N HCl	71.52	28.47	76.83	23.16
Alkaline /0.1 N NaOH	88.59	11.40	72.78	27.21
Oxidative /6 % H_2O_2	73.20	26.79	82.86	17.13
Neutral/H_2O	85.64	14.35	91.52	8.47
Photolysis: UV light 200 watt h square meter^{-1} / 1 d	94.62	5.37	90.02	9.97
Dry heat/ 60ºC/ 4 h	92.26	7.73	78.68	21.31

Method Validation

The optimized method was validated in accordance with ICH guidelines with respect to linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness.[29, 30]

Linearity

Aliquots of 1, 2, 3, 4, 5 and 6 µL of the standard stock solutions of OLME and CHLOR (200 ng µL^{-1}and 125 ng µL^{-1}) were applied by overspotting on TLC plate to obtain the concentration in the range 200-1200 ng band^{-1} for OLME and 125-750 ng band^{-1} for CHLOR, respectively. Results were found to be linear in the concentration range indicated above. The linear regression equation and correlation coefficient were found to be y = 5.3157x + 491.18 and R^2 = 0.997 for OLME and 8.34x + 766.57 and R^2 = 0.992 for CHLOR, respectively. The calibration curves and 3 D spectra obtained for OLME and CHLOR are represented in Figure 6 and 7, respectively.



(a) **(b)**

Fig 6: Calibration curve for (a) OLME (b) CHLOR

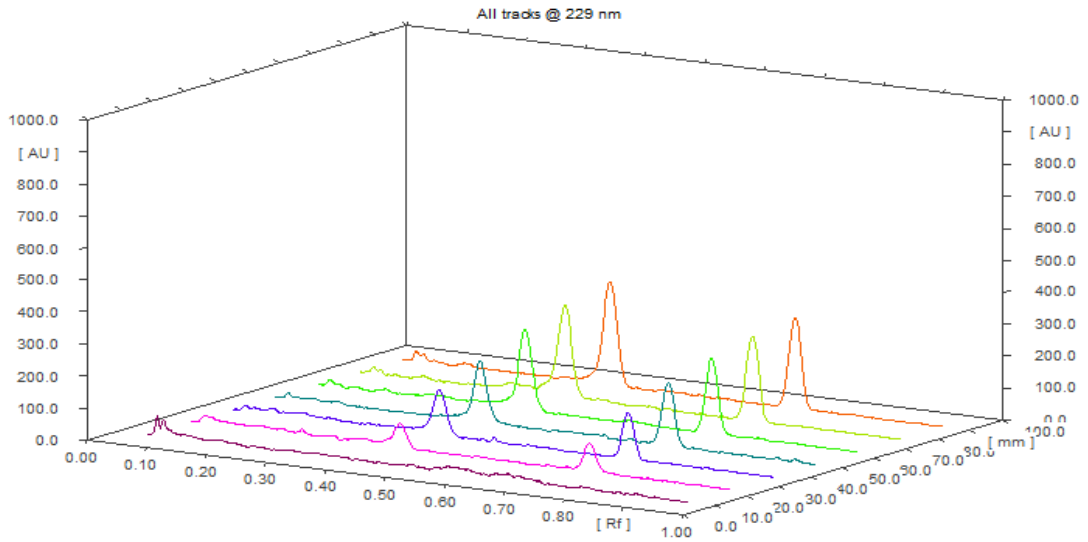


Fig. 7: 3 D spectra of linearity for OLME and CHLOR

Precision

Set of three different concentrations in three replicates of mixed standard solutions of OLME and CHLOR were prepared. All the solutions were analyzed thrice on the same day and on three consecutive days in order to record intra-day and inter-day variations in the results. The % R.S.D. values for intra-day and inter-day precision were found to be in the range of 1.08-1.30 and 0.49-1.23 for OLME and 0.59-1.18 and 0.89-1.33 for CHLOR, respectively. The smaller values of % R.S.D. obtained indicate that the developed method is precise.

Table 2: Intra-day precision

Drug	Spotted concentration (ng band^{-1})	Mean area	S.D.	% R.S.D.*
OLME	600	3694.1	6.10	1.08
	800	4762.9	10.50	1.30
	1000	5807.4	12.20	1.21
CHLOR	375	3667.4	07.00	1.18
	500	4709.9	04.70	0.59
	625	5869.9	08.90	0.88

*Average of three determinations.

Table 3: Inter-day precision

Drug	Spotted concentration (ng band^{-1})	Mean area	S.D.	% R.S.D.*
OLME	600	3881.4	4.6	1.23
	800	4890.7	2.4	0.49
	1000	5966.3	7.4	1.19
CHLOR	375	3877.5	3.8	1.03
	500	4947.0	6.6	1.33
	625	5915.9	5.5	0.89

*Average of three determinations

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. LOD and LOQ were found to be 53.73 ng band^{-1} and 162.83 ng band^{-1} for OLME and 7.43 ng band^{-1} and 22.52 ng band^{-1} for CHLOR, respectively.

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to pre-analysed sample at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 400 ng band^{-1} for OLME and 250 ng band^{-1} for CHLOR from tablet solution. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drugs in combined tablet dosage form.

Table 4: Recovery studies of OLME and CHLOR

Drug	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Total amount found (ng band^{-1})	% Recovery	% R.S.D.*
OLME	400	320	713.03	99.03	0.63
	400	400	791.67	98.95	0.76
	400	440	886.84	100.77	0.78
CHLOR	250	200	448.11	99.58	0.69
	250	250	494.70	98.94	0.44
	250	300	553.56	100.64	0.82

*Average of three determinations.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, detection time was altered and the effect on the area of drugs was noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

CONCLUSION

A simple, precise, accurate, reproducible and stability-indicating HPTLC method without interference from the excipients has been developed and validated for the determination of OLME and CHLOR as bulk drugs and in combined tablet dosage form. The developed method can be used for quantitative analysis of OLME and CHLOR in pharmaceutical dosage form. The method was developed by using easily available and economic solvents for analysis of drug hence can be considered as economic. As the method is stability indicating one it may be extended to study the degradation kinetics of drugs.

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