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DEVELOPMENT AND VALIDATION OF A NORMAL-PHASE HPTLC METHOD FOR THE SIMULTANEOUS ANALYSIS OF PARACETAMOL AND ONDANSETRON IN FIXED-DOSE TABLETS

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ABSTRACT

A new precise method was developed for simultaneous quantification of PARACETAMOL and ONDANSETRON in tablets by HPTLC method. The chromatograms were developed using a mobile phase of Chloroform: Dichloro methane: Methanol (4:6:2) on pre-coated plate of TLC silica gel 60 F254 and quantified by densitometric absorbance mode at 254 nm. The *Rf* values were 0.69 and 0.61 for Ondansetron and Paracetamol respectively. The linearity of the method was found to be within the concentration range between 1-5 mcg/spot for both Paracetamol and Ondansetron. The lower limits of detection and quantification were 0.0634µg/spot and 0.1921µg/spot for Ondansetron and 0.0112µg/spot and 0.1921µg/spot for Paracetamol. The method was also validated for precision, accuracy and recovery. This developed method was used to analyze fixed dosage tablets (Lupisetron plus, Lupin Ltd) samples of Paracetamol and Ondansetron.

KEYWORDS: Paracetamol, Ondansetron and HPTLC.

INTRODUCTION

Paracetamol is chemically *N*-(4-hydroxyphenyl)ethanamid, *N*-(4-hydroxyphenyl)acetamide which is generally considered to be a weak inhibitor of the synthesis of prostaglandins (PGs). Paracetamol also decreases PG concentrations in vivo, but, unlike the selective COX-2 inhibitors, paracetamol does not suppress the inflammation of rheumatoid arthritis.[9] The main mechanism proposed is the inhibition of cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-2. Paracetamol reduces the oxidized form of the

COX enzyme, preventing it from forming pro-inflammatory chemicals. This leads to a reduced amount of Prostaglandin E2 in the CNS, thus lowering the hypothalamic set-point in the thermoregulatory centre.

Ondansetron is chemically (*RS*)-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-2,3-dihydro-1*H*-carbazol-4(9*H*)-one. Ondansetron is a potent, highly selective $5HT_3$ receptor-antagonist. Chemotherapeutic agents and radiotherapy may cause release of 5HT in the small intestine initiating a vomiting reflex by activating vagal afferents via $5HT_3$ receptors. Ondansetron blocks the initiation of this reflex.

The literature review supports that there was no HPTLC method had been reported for the determination of Paracetamol and Ondansetron in tablet dosage form.

So an experiment was taken to expand and corroborate a HPTLC method for the determination of Paracetamol and Ondansetron in tablet dosage forms.

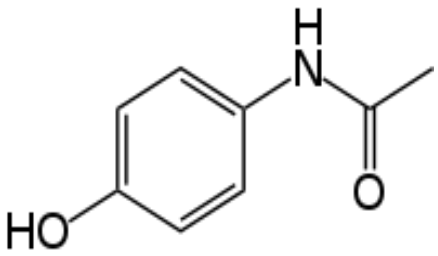

Fig. 1 PARACETAMOL

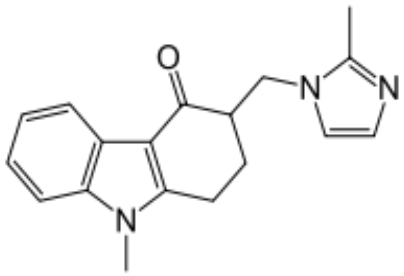

Fig. 2 ONDENSETRON

MATERIALS

Paracetamol and Ondansetron were earned from Ideal analytical and research institution Puducherry, India. All chemicals worn were analytical standard. The pharmaceutical tablet dosage form used in this study was Lupisetron with a label claim of Paracetamol 500 mg and Ondansetron 4mg were purchased from local pharmacy.

Preparation of solutions

Standard Solution

Weighed accurately 100 mg of Ondansetron and 100mg paracetamol into 100 ml standard flask, dissolved and diluted to volume with methanol.

Sample Preparation

To determine the content of paracetamol, ondansetron in synthetic mixture(both the drugs 100 mg, lactose100 mg ,talc 100 mg ,magnesium stearate 100 mg). The amount equivalent to 100mg of the drug was transferred into a 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 mins and diluted to 100 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 5mins and supernatant was analyzed for drug content (1000 $\mu g.mL^{-1}$). One microlitre of the above solution (1000 $ng.spot^{-1}$) was applied on the TLC plate followed by development and scanning under described condition.

Chromatographic conditions

Paracetamol and Ondansetron reference standard solutions were prepared using methanol as solvent. Solutions of 2 µL were applied to the HPTLC plates as spot bands of 6 mm using Linomat IV with CAMAG 100µl syringe. Application positions were at least 15 mm from the sides and 10 mm from the bottom of the plates by using the chamber CAMAG twin trough chamber (20X10). Mobile phase components like Chloroform: Dichloro methane: Methanol(4:6:2) were mixed prior to use and the development chamber was left for saturation with mobile phase vapor for 10 min before each run. Development of the plate was carried out by the ascending technique to a migration distance of 7 cm. Then the plates were dried on a hot plate. All the analyses were carried out in a laboratory with temperature control (20–24 °C).

Densitometry scanning was done in absorbance mode at 2546 nm using a deuterium lamp. The slit dimensions were set at 6 mm×0.30 mm, the scanning speed of 10 mm/s, and the data resolution at 100µm/step. Development time was20mints and Single wavelength detection was performed since the main components were only analyzed.

METHOD VALIDATION

The developed method was validated as per the International Conference on Harmonization (ICH) guidelines with respect to linearity and range, precision, accuracy, recovery studies, limit of detection and limit of quantification.

RESULTS AND DISCUSSIONS

ASSAY

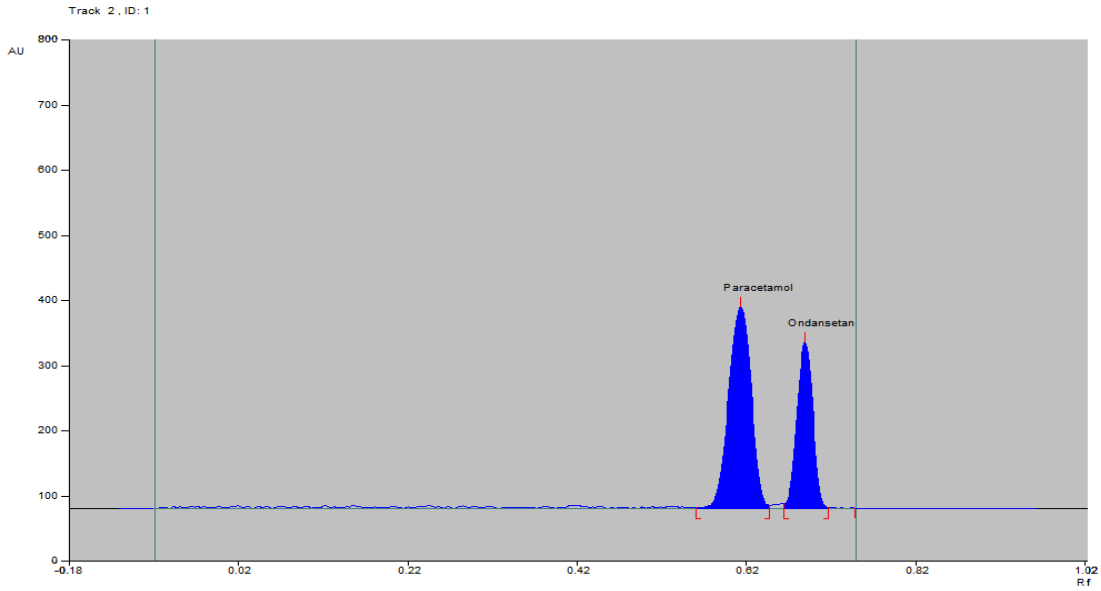

Fig. No. 3 Chromatogram (standard)

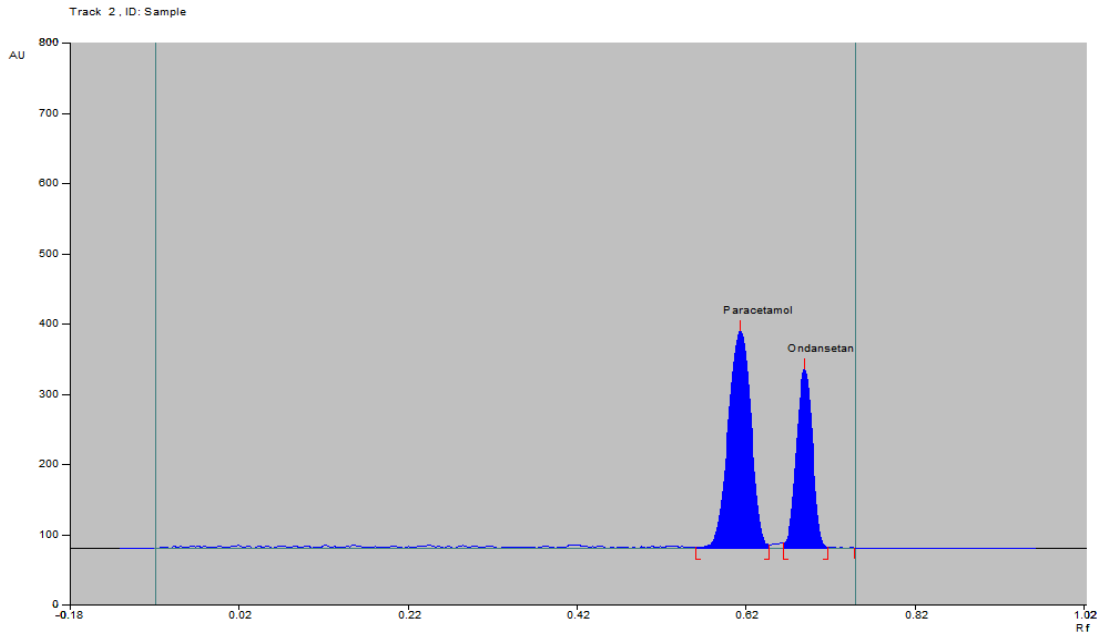

Fig. No. 4 Chromatogram (sample)

Table No.1: Assay of Paracetamol.

SI	Drug	Concentration (µg/spot)	Peak area	Amount found (mg)	% Purity (w/w)	Label claim
1.	Paracetamol	3	9364.8	100.0117475	100.0117	100mg
		3	9345	99.99464983	99.9946	
		3	9322.9	99.53344864	99.5334	

Table No. 2: Assay of Ondansetron.

SI	Drug	Concentration (µg/spot)	Peak area	Amount found (mg)	% Purity (w/w)	Label claim
1.	Ondansetron	3	6478.7	100.8154	100.8154	100mg
		3	6516.3	99.05751	99.0575	
		3	6478.2	99.8335	99.8335	

LINEARITY

The response for paracetamol and ondansetron was linear (R^2= 0.999 Paracetamol and R^2 =0.999Ondansetron) in the concentration range between 1-5 mcg/spot. The mean (±RSD) values of slope, intercept and correlation coefficient were 3160.66667, 73.6667, 0.999 for paractamol and 2240.33333, 89.6633, 0.999 for ondansetron respectively.

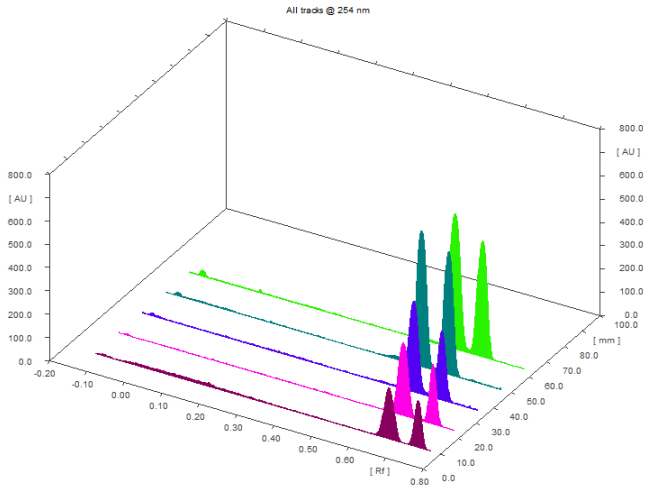

Fig. No. 5: HPTLC densitogram for linearity.

Table No. 3: Linearity parameters for Paracetamol.

Linearity	Correlation coefficient(y)	Slope(m)	Intercept(c)
Linearity 1	0.999	3161	77.4
Linearity 2	0.999	3169	82.07
Linearity 3	0.999	3152	61.53
Average	0.999	3160.66667	73.6667

Table No. 4 Linearity parameters for Ondansetron.

Linearity	Correlation coefficient(y)	Slope(m)	Intercept(c)
Linearity 1	0.999	2232	115.9
Linearity 2	0.999	2244	39.99
Linearity 3	0.999	2245	113.1
Average	0.999	2240.33333	89.6633
Std deviation		43.0411	

LIMIT OF DETECTION

The signal: noise ratios of 3:1 and 10:1 were considered as LOD and LOQ respectively. The limit of detection was found to be 0.0112µg/spot and 0.0634µg/spot for Paracetamol and Ondansetron respectively. The limit of quantification was found to be 0.0341µg/spot and0.1921µg/spot for Paracetamol and Ondansetron, respectively, which was lower than that reported earlier

PRECISION

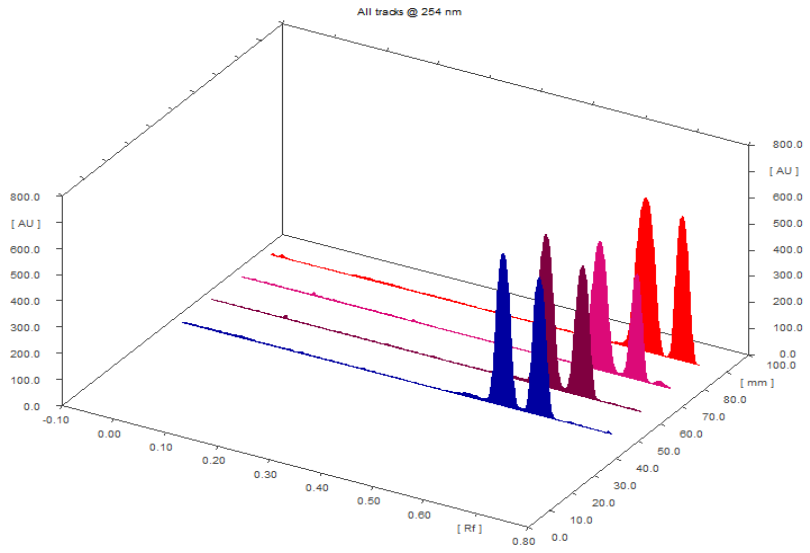

Fig. No. 6: HPTLC densitogram for precision.

Table No. 5 Precision for paracetamol and ondansetron.

concentration	Inter assay		Intra assay			
	Day 1		Day 1		Day 2	
	Precision 1		Precision 2		Precision 3	
	PARA	ONDAN	PARA	ONDAN	PARA	ONDAN
3µg/Spot	9367.9	6489.5	9345	6459.4	9445	6459.4
3µg/Spot	9467.9	6497.5	9367.7	6408.3	9567.7	6367.3
3µg/Spot	9369	6432.9	9356	6334.8	9455.5	6456.8
3µg/Spot	9364	6498.7	9345	6458.2	9645	6428.2
3µg/Spot	9547.8	6375	9546.7	6429.8	9456.7	6356.8
3µg/Spot	9458.5	6457	9457.4	6459.8	9457.4	6459.8
Mean	9429.1833	6458.4333	9402.9666	6425.05	9504.55	6421.38
SD	74.906486	48.431133	82.209650	48.856105	82.678866	47.57715
%RSD	0.7944111	0.7498898	0.8742948	0.7604003	0.8698872	0.740918

ACCURACY

The accuracy was assessed by the methodological recovery. The recovery of the method was calculated by comparing the determined concentration of spiked samples to the theoretical concentrations. The mean percentage recovery for each compound was calculated at each

concentration level and reported with its standard deviation. The intra-day and inter-day percentages of accuracy obtained for Paracetamol at the concentrations of 2, 3, 4 ng/spot, and for Ondansetron at the concentrations of 2, 3 and 4 ng/spot, are respectively. The percentage recovery of paracetamol and ondansetron was found to be in the range of 99.103-99.804% and 99.1412-101.2573% which is well within the acceptance limit of 97% - 103%w/w.

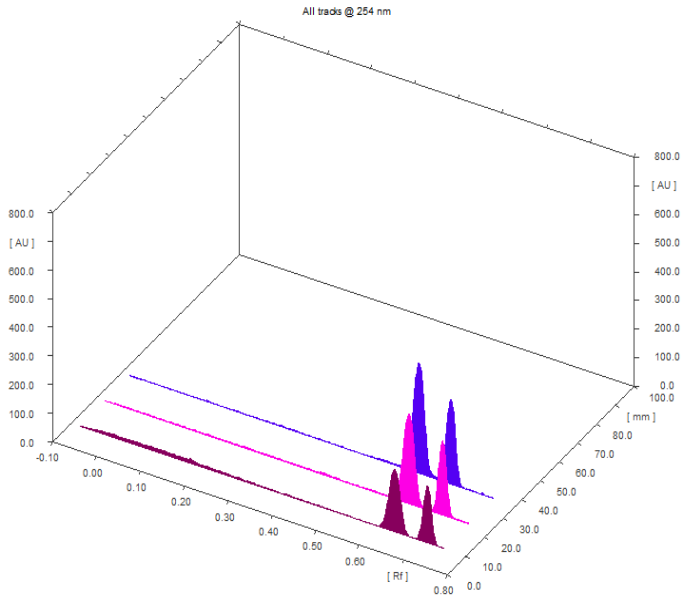

Figure-31: HPTLC densitogram for accuracy.

RECOVERY

The estimated percentage recovery was found to be close to 100% and hence prove the recovery of the method. The percentage recovery of paracetamol and ondansetron was found to be in the range of 99.103-99.804% and 99.1412-101.2573% which is well within the acceptance limit of 97% - 103%w/w.

Table No. 6: Summary of validation for simultaneous estimation of paracetamol and ondansetron by hptlc.

Parameters	**Results**		**Acceptance**
	Paracetamol	**Ondansetron**	**Criteria**
Linearity-Range (µg/spot)	1-5(µg/spot)	1-5(µg/spot)	---
Correlation coefficient	0.999	0.999	>0.9960
Slope	3160.66667	2240.33333	---
Intercept	73.6667	89.6633	---
Method Precision (% RSD)	0.846197742	0.751603892	< 2%
Assay (% purity)	99.533 – 100.01%	99.057-100.815%	97 – 103%w/w
Percentage recovery	99.103 – 99.804%	99.141 – 101.37%	97 – 103%w/w

CONCLUSION

A simple, and selective high performance thin layer chromatographic method was developed and validated for estimation of paracetamol and ondansetron in synthetic mixture. The method employed precoated TLC aluminum plates with silica gel 60F254 as the stationary phase. The solvent system comprised Chloroform: Dichloro methane: Methanol(4:6:2v/v/v). The retention factor is 0.61min and 0.69 min for paracetamol and ondansetron respectively. Spectrodensitometric scanning integration was performed 254 nm. The polynomial regression data for the calibration graph showed good linear relationship with R^2 = 0.999 in the concentration range of 1-5 µg/spot. The developed method was validated for precision and recovery. The proposed method is applied for determination for paracetamol and Ondansetron.

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