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Development and Validation of Stability Indicating HPTLC method for Simultaneous Estimation of Paracetamol, Aceclofenac and Rabeprazole in Combined Tablet Dosage Formulation

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Abstract: A simple, sensitive, reliable and rapid HPTLC method has been developed for the determination of paracetamol, aceclofenac and rabeprazole in combined tablet dosage form. Determination was performed on aluminium backed silica gel $60F_{254}$ washed with methanol. The mobile phase used is ethyl acetate- methanol- glacial acetic acid (9: 1: 0.1). The spots were scanned at 275nm. The linearity of paracetamol, aceclofenac and rabeprazole was found to be 100-500µg/ml, 20-100µg/ml, 2-10µg/ml respectively. The method was validated for accuracy, precision, repeatability. The method was used for the determination of the compound in commercial pharmaceutical dosage forms.
Keywords: HPTLC, Pharmaceutical dosage form, Paracetamol, Aceclofenac and Rabeprazole, Method Development and Validation.

Introduction

Paracetamol {N- (4-hydroxyphenyl) acetamide} (Fig.1) and aceclofenac {2-[(2, 6-dichlorophenyl) amino] phenyl acetoxy acetic acid} (Fig.2) are NSAIDs which acts by inhibiting the synthesis of prostaglandins (Ryuta Yamazaki et al. 1999; Momin M Y et al. 2006). High performance liquid chromatography and pharmacokinetics of aceclofenac in rats have been reported by (Prashant Musmade et al.2007). Rabeprazole {2-[(4-(3-methoxypropoxy)-3-methyl-pyridine–2-yl) Methylsulfinyl- 1H benzoimidazole} (Fig.3) is an anti ulcer drug which is a proton pump inhibitor. Determination of rabeprazole enantiomers and their metabolites by High performance liquid chromatography with solid phase extraction was reported by (Masatomo Miura et al, 2006).[1-3] Spectrophotometer and chromatographic determination of rabeprazole in presence of its degradation products were done in both HPLC and HPTLC (Gindy A et al, 2003). No analytical method has been reported for the Simultaneous Estimation of Paracetamol, Aceclofenac and Rabeprazole in Combined Tablet Dosage Formulation. Hence the present study aims in developing simple, rapid, accurate, precise and validated methods for quantification of Paracetamol, Aceclofenac and Rabeprazole in Combined Tablet Dosage Formulation [4-7].

Figure 1: Chemical structure of Paracetamol

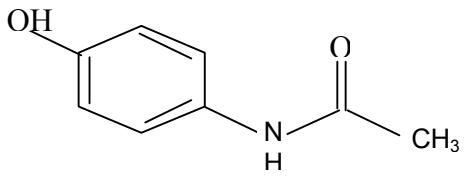


Figure 2: Chemical structure of Aceclofenac

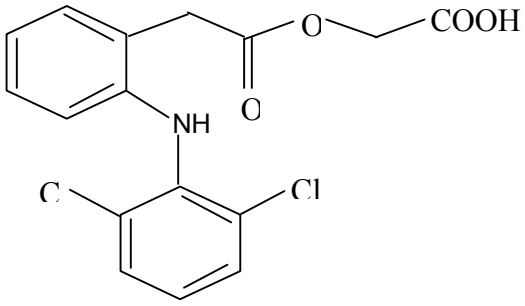
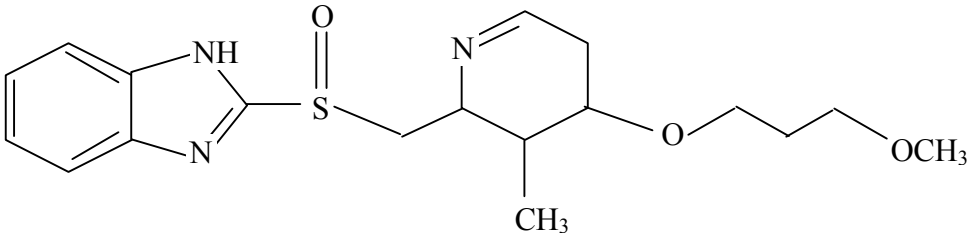


Figure 3: Chemical structure of Rabeprazole



Experimental

Materials and Reagents

An analytical pure sample of paracetamol and aceclofenac was a gift of Micro Laboratories. Rabeprazole was a gift of Glenmark Pharmaceuticals Ltd., Mumbai. All chemicals and solvents were supplied by S. D. Fine chemicals Ltd., India, Qaligens Fine Chemicals. Tablet formulation Ace proxyvon were obtained commercially.

Quantification of Paracetamol, Aceclofenac and Rabeprazole

Selection of Wavelength

The sensitivity of HPTLC method that uses UV detector depends upon the proper selection of wavelength. UV spectra of Paracetamol, aceclofenac and rabebrazole on precoated plate were recorded. The λ_{max} of Paracetamol, aceclofenac and rabebrazole were found to be 250 nm, 277nm and 293 nm respectively. All the drugs shows significant absorbance at wavelength 275 nm. Hence 275 nm was selected for the study. (Fig.4)

Selection of Optimum Mobile Phase

A solvent system that would give dense compact spots, good separation from each other and separation from solvent front and application position was to be selected. Initially different solvent systems were tried. Ethyl acetate: Methanol: glacial acetic acid was selected because in this system good, compact, dense spots were obtained with good resolution between analytes, good separation from solvent front and sample application positions.

Seperation using Ethyl Acetate: Methanol: Glacial acetic acid

Different ratios of ethyl acetate : methanol : glacial acetic acid like 7:3:0.5, 3:6:1, 9:1:0.1% v/v/v were tried and the ratio 9: 1: 0.1% v/v/v was selected because it gave compact spots and good resolution between analytes good separation from solvent front and sample application positions. The standard chromatogram was shown in Fig. 5.

Figure 4: UV Spectrum of standard Paracetamol, Aceclofenac and Rabebrazole on TLC plate.

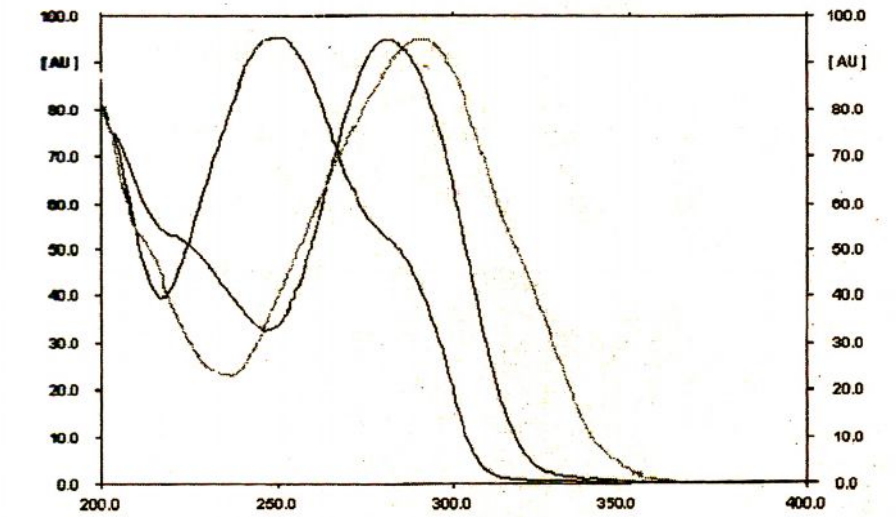
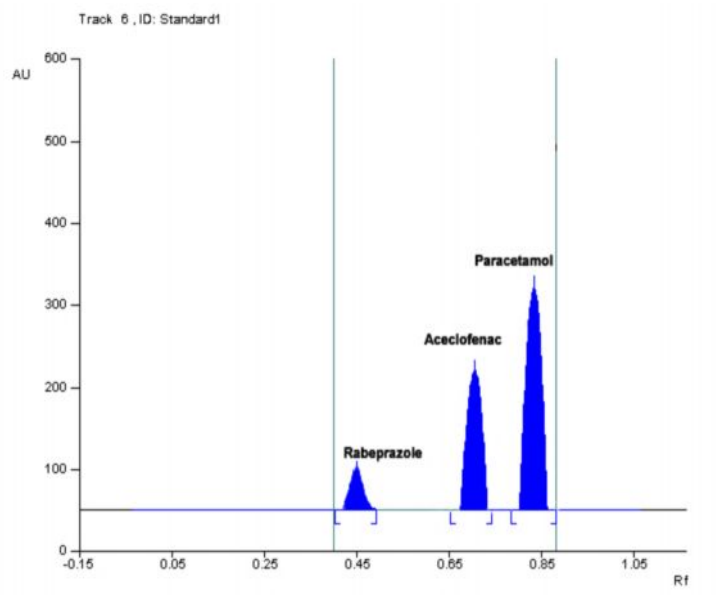


Figure 5: Standard Chromatogram



Fixed Chromatographic Conditions

Stationary phase	: Pre coated silica gel $60F_{254}$ on aluminium sheets
Mobile phase	: Ethyl acetate: methanol: glacial acetic acid (9: 1: 0.1% v/v/v)
Chamber saturation	: 20 minutes
Migration distance	: 80 mm
Band width	: 6 mm
Slit dimensions	: 5 × 0.45 mm
Source of radiation	: Deuterium lamp
Scanning wavelength	: 275 nm
R_f value	
Paracetamol	: 0.79 ± 0.03
Aceclofenac	: 0.63 ± 0.03
Rabebrazole	: 0.39 ± 0.03

Standard Stock Solution

500mg of paracetamol and 100 mg of aceclofenac and 10 mg of rabeprazole were accurately weighed. A standard stock solution of paracetamol (50 mg/ml), aceclofenac (10mg/ml) and rabeprazole (1 mg/ml) were prepared in methanol. These solutions were further diluted to obtain a series of concentrations ranging from 100 - 500 µg/ml of paracetamol, 20 – 100 µg/ml of aceclofenac and 2 – 10 µg/ml of rabeprazole.

Sample Preparation

Twenty tablets each containing quantity equivalent to 500 mg of paracetamol, 100 mg of aceclofenac and 10 mg of rabeprazole were weighed, powdered and average weight was calculated. Quantity equivalent to 100 mg of aceclofenac was weighed and transferred to a 100 ml volumetric flask. The drug was extracted by addition of methanol with shaking and finally volume was made up to the mark. The solution was filtered through Whatman filter paper (No: 14). The solution was further diluted with methanol. The formulation was assayed by spotting 1 µl of the solution on to the plate followed by development and scanning. The concentrations of the drugs were calculated from peak area obtained using standard calibration graph. (Table 1)

Chromatography

From the above stock solution different volumes from 0.2 to 1µl were spotted on 20 × 10 aluminium backed silica gel $60F_{254}$ HPTLC plates with help of Linomat 5 applicator equipped with 100µl syringe (Hamilton). Ascending development of plates, migration distance of 85mm, was performed at 25 ± 2^0 C with ethyl acetate: methanol: glacial acetic acid (9:1:0.1% v/v/v) as mobile phase in Camag twin-tough TLC chamber previously saturated in mobile phase for 10 min. The average development time is 15 minutes. After development the plates were dried in air for 10 minutes. Densitometric scanning at 275nm was then performed with a Camag TLC Scanner equipped with win cats software, using deuterium light source; the slit dimension is 6.00 × 0.45mm.The peak areas of paracetamol, aceclofenac and rabeprazole were recorded and calibration graph was plotted against concentration of standard Vs peak area for paracetamol, aceclofenac and rabeprazole respectively (Fig. 6-8), (Table 2).

Tabe 1: Assay of formulation

Drug	Amount (mg/tablet)		% label claim	% RSD*
	Labelled	Estimated		
Paracetamol	500	498.76	99.75	0.31
Aceclofenac	100	99.25	99.52	0.23
Rabebrazole	10	9.82	99.26	0.15

*Mean RSD of six observations

Table 2: Calibration Data

Paracetamol			Aceclofenac			Rabeprazole		
Volume (µl)	Conc (µg/ml)	Peak area	Volume (µl)	Conc (µg/ml)	Peak area	Volume (µl)	Conc (µg/ml)	Peak area
0.2	100	15678	0.2	20	11801	0.2	2	1569
0.4	200	26331	0.4	40	17996	0.4	4	2558
0.6	300	36988	0.6	60	24191	0.6	6	3544
0.8	400	47640	0.8	80	30384	0.8	8	4531
1.0	500	58292	1.0	100	36578	1.0	10	5520

Figure 6: Linearity graph of Paracetamol

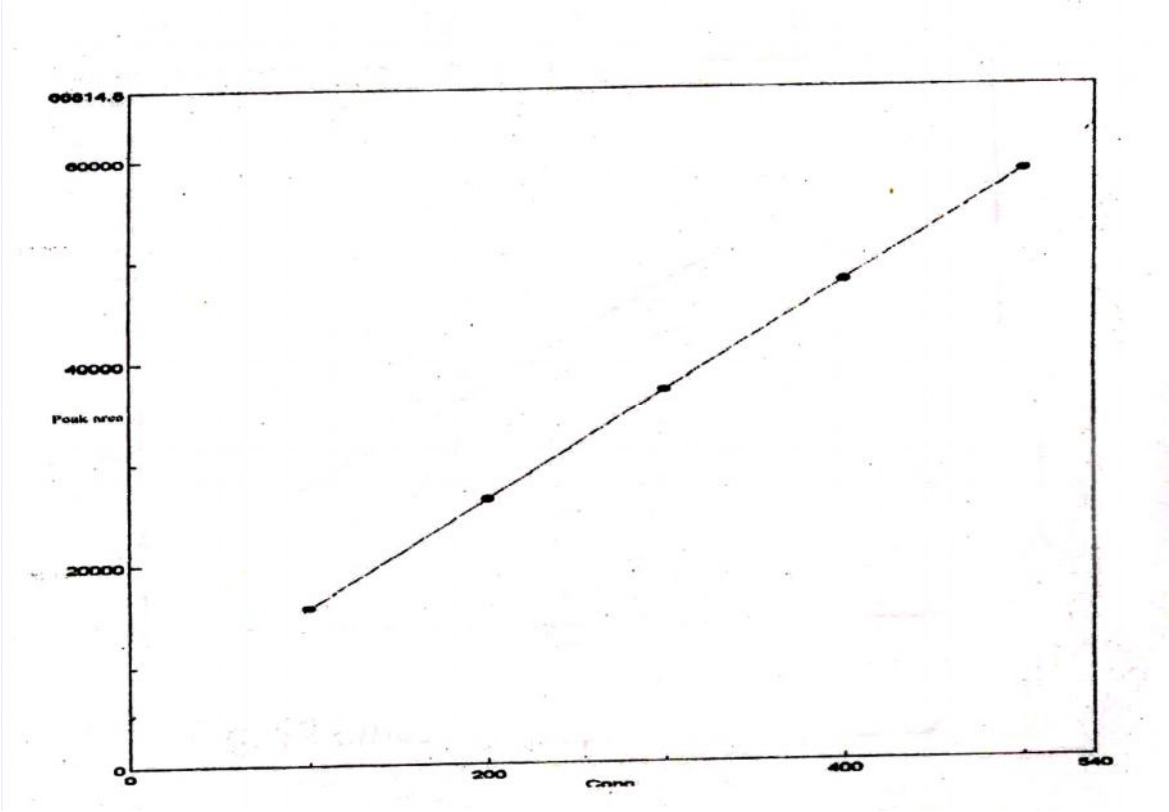


Figure 7: Linearity graph of Aceclofenac

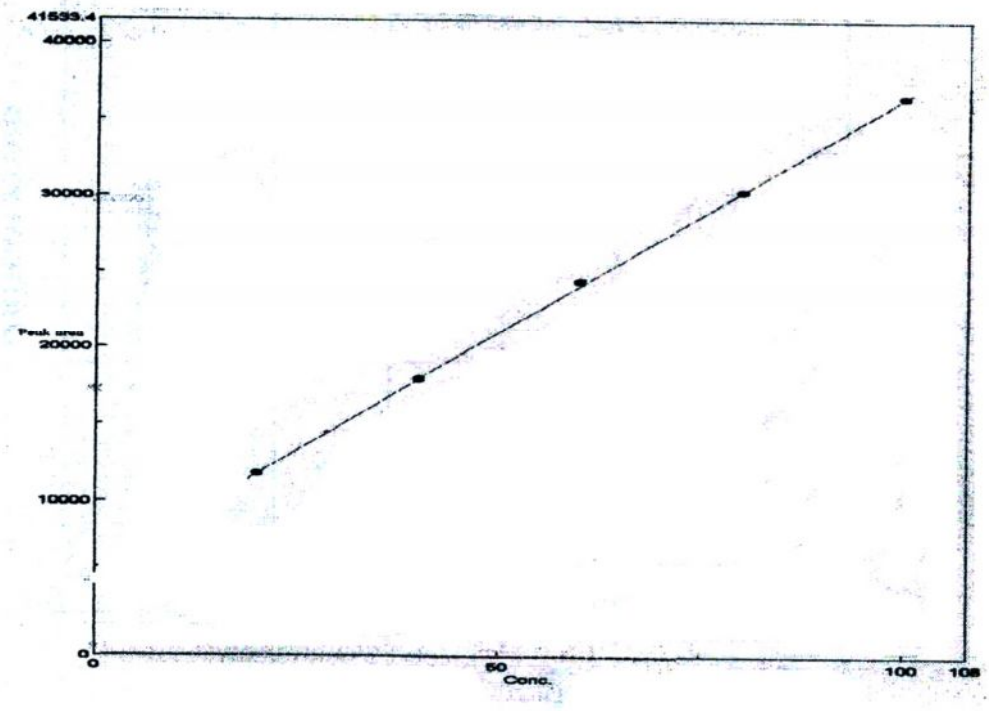
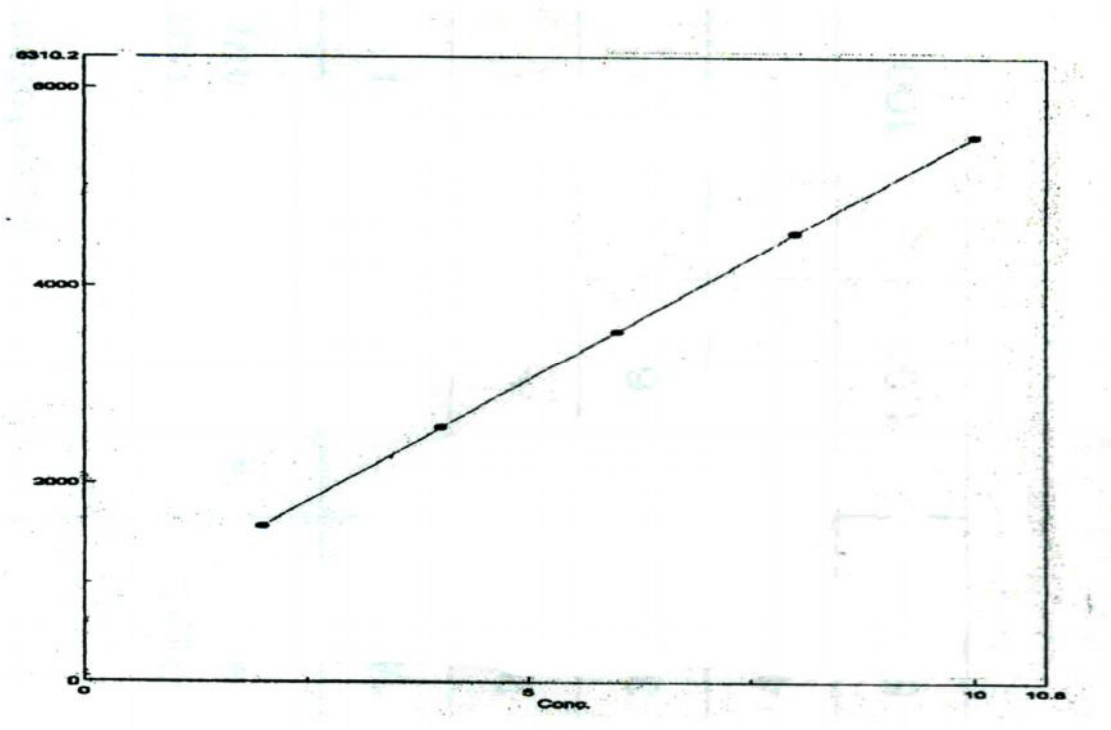


Figure 8: Linearity graph of Rabebrazole



Method Validation

The method validation was based on the international conference on harmonization guidelines (ICH/CPMP 1994) for the validation of analytical procedures. The parameters used were required for the assay of a dosage form linearity, quantification limit, accuracy, specificity and precision [8-10].

Linearity

A series of standard drug solution were applied to a pre- washed TLC plate. The plate was developed, dried and scanned as described above. A calibration plot was constructed by plotting peak area against concentration. The linear regression data showed good linear relationship over a concentration range of 100 to 500 µg/spot for paracetamol, 20 to 100 µg/spot for aceclofenac and 2 to 10 µg/spot for rabeprazole. The slope, intercept and correlation co-efficient values for paracetamol were found to be 112.60, 131.0and 0.99987 respectively. The slope, intercept and correlation co-efficient values for aceclofenac were found to be 5607.40, 309.71 and 0.99976 respectively. The slope, intercept and correlation co-efficient values for rabeprazole were found to be 581.90, 493.75 and 0.99985 respectively.(Table 3)

Table 3: Linearity and Range

Drug	Linearity and range	Correlation coefficient (r)	Slope	Intercept
Paracetamol	100-500	0.99987	112.60	131.0
Aceclofenac	20-100	0.99976	5607.40	309.71
Rabebrazole	2-10	0.99985	581.90	493.72

Sensitivity

The sensitivity of the method was estimated in terms of the Limit of Quantification and Limit of Detection. LOD and LOQ were determined by applying decreasing amount of the drug in triplicate on the plate. The lowest concentration at which the peak is detected is called 'Limit of Detection' and the lowest concentration at which the peak is quantified is called 'Limit of Quantification'. The LOD and LOQ were calculated by the use of equation LOD= 3 ×N/B and LOQ= 10×N/B where N is the standard deviation of the peak area of the drug taken as a measure of noise and B is the slope of the corresponding calibration plot. The Limit of Quantification (LOQ) was found to be 120, 20 and 50ng/spot respectively for paracetamol, aceclofenac and rabeprazole.(Fig. 9,10,11) The Limit of Detection (LOD) was found to be 40, 10 and 20ng/spot respectively for paracetamol, aceclofenac and rabeprazole. (Fig. 12, 13, 14).

Figure 9: LOQ of Paracetamol

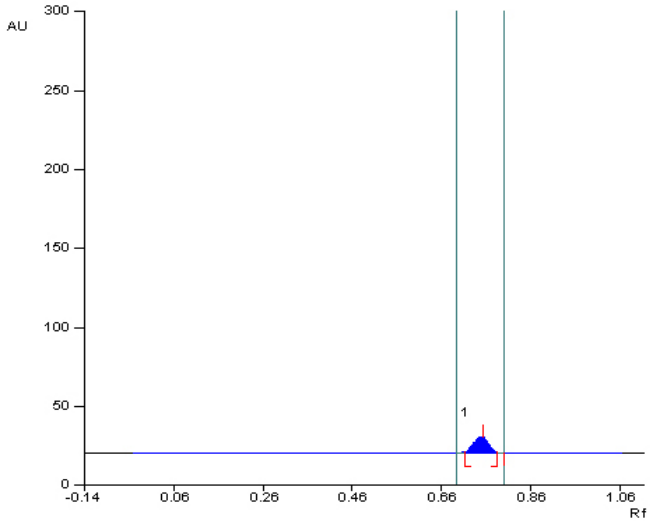


Figure 10: LOQ of Aceclofenac

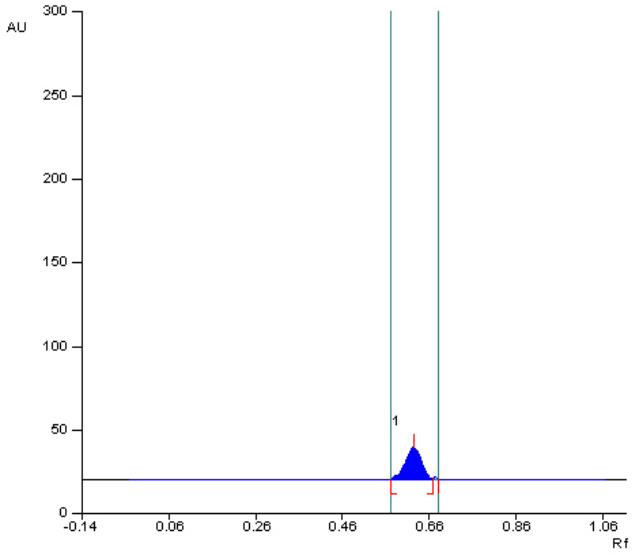


Figure 11: LOQ of Rabeprazole

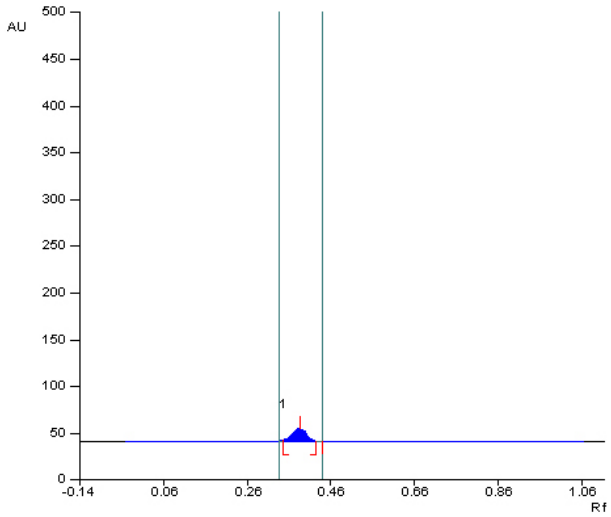


Figure 12: LOD of Paracetamol

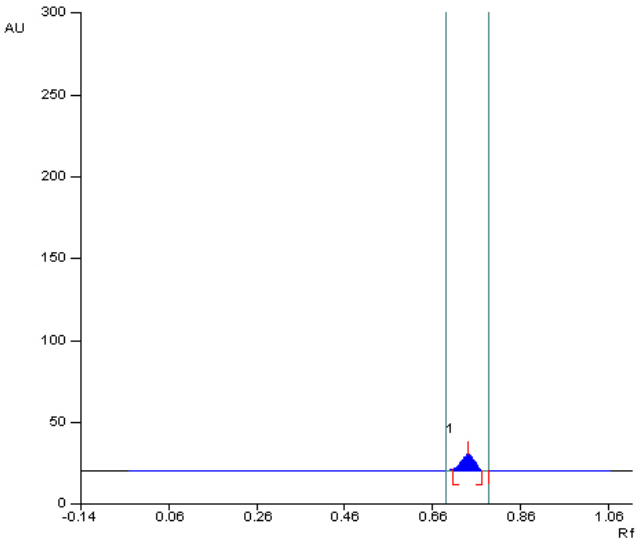


Figure 13: LOD of Aceclofenac

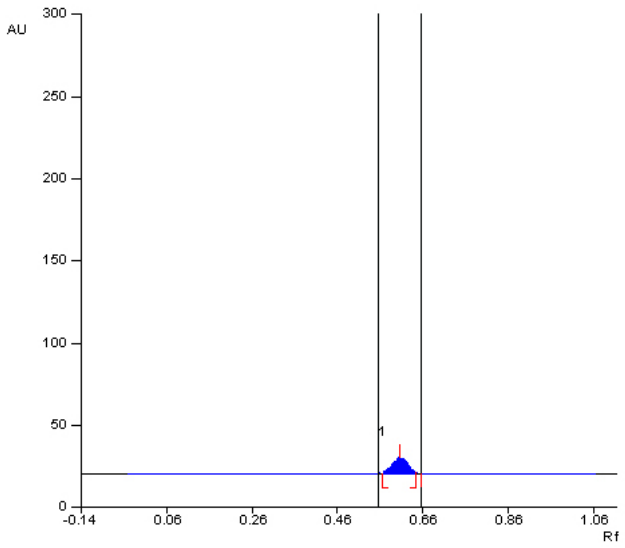
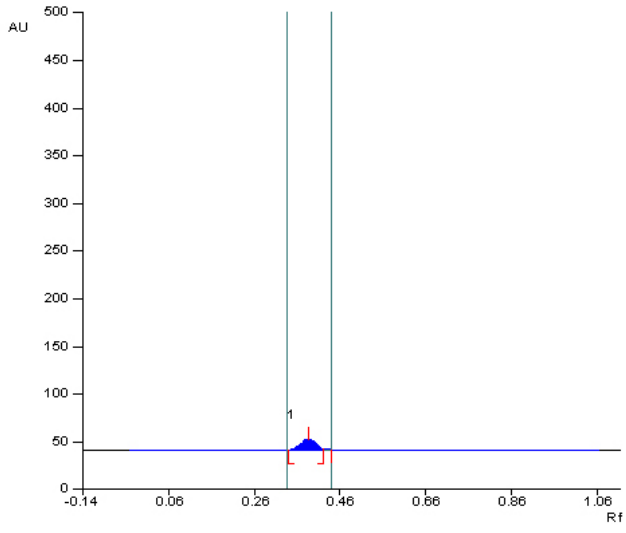


Figure 14: LOD of Rabeprazole



Precision:

Intraday Precision

Intraday precision was found out by carrying out the analysis of the standard drugs at two different concentrations in the linearity range of drugs for three times on the same day. Each concentration was applied in duplicate and percentage RSD was calculated (Table 4).

Interday Precision

Inter day precision was found out by carrying out the analysis of the standard drugs at two different concentrations in the linearity range of drugs for two days for three times and the percentage RSD was calculated (Table 5).

Accuracy

Recovery studies of the drugs were carried out for the accuracy parameters. It was done by mixing a known quantity of standard drug with the pre analysed sample formulation and the contents were reanalysed by the proposed method. This was carried out at 50% and 100% levels. (Table 6).

Table 4: Intraday Assay

Volume applied(µl)	Peak area		
	Paracetamol	Aceclofenac	Rabeprazole
0.8	47640 47600 47653	30384 30401 30378	4531 4545 4521
% RSD	1.08	0.69	0.45
1	58292 58301 58284	36578 36590 36564	5520 5499 5432
% RSD	0.91	0.74	0.77

Table 5: Interday Assay

Volume applied(µl)	Day	Peak area		
		Paracetamol	Aceclofenac	Rabeprazole
0.8	Ist	47640 47630 47653	30384 30396 30366	4531 4545 4529
	% RSD	1.60	0.64	0.81
	2nd	47652 47622 47635	30388 30390 30378	4530 4546 4555
	% RSD	0.67	0.14	0.107
1	Ist	58293 58278 58275	36580 36578 36566	5525 5534 5519
	% RSD	1.79	0.86	0.10
	2nd	58258 58268 58270	36588 36590 36572	5529 5519 5522
	% RSD	1.13	0.53	0.82

Table 6: Recovery Studies

Drug	% Recovery		% RSD*	
	50%	100%	50%	100%
Paracetamol	101.04	98.89	0.52	0.69
Aceclofenac	99.88	100.08	0.73	0.56
Rabeprazole	99.22	98.98	0.47	0.89

***Mean of six observations**

Repeatability of Sample Application

Repeatability of sample application was assessed by spotting 1.0 µl of drug solution six times on pre – coated TLC plate followed by development of plate and % RSD was calculated. **(**Table 7). Repeatability of measurement of peak area was determined by spotting 1.2 µl of standard drug solutions on pre – coated TLC plate. After development of plate, the separated spots were scanned six times without changing position of the plate and % RSD was calculated (Table 8).

Stability of the Plate

To test the stability of the drugs on the TLC plates, the freshly prepared solutions of the analyte were applied to the plates and developed and scanned at different intervals. No decomposition of the drug was observed during chromatogram development. No significant decrease in peak area was found for a stock solution after storage at room temperature for 4 hours. These observations suggest that the drug is stable under the typical processing and storage conditions of the analytical procedure (Table 9).

Table 7: Repeatability of Sample Application

Volume applied(µl)	Peak Area		
	Paracetamol	Aceclofenac	Rabeprazole
1	58292 58300 58288 58308 58290 58279	36585 36575 36579 36589 36581 36574	5520 5519 5508 5525 5524 5531
% RSD	1.09	0.60	0.55

Table 8: Repeatability of Measurement

Volume applied(µl)	Peak area		
	Paracetamol	Aceclofenac	Rabeprazole
1	58295	36570	5528
	58288	36578	5519
	58305	36600	5516
	58312	36592	5530
	58281	36588	5520
	58294	36575	5510
% RSD	0.81	0.59	0.63

Table 9: Stability of Plate

Volume applied(µl)	Time in hours	Peak area		
		Paracetamol	Aceclofenac	Rabeprazole
1µ1	0	58295	36578	5528
	½	58288	36571	5522
	1	58291	36565	5519
	2	58078	36256	5501
	3	57957	35932	5437
	4	57221	35265	5358

Conclusion

The HPTLC method was developed for the simultaneous estimation of paracetamol, aceclofenac and rabeprazole in combined tablet dosage form using ethyl acetate: methanol: glacial acetic acid (9:1:0.1% v/v) as mobile phase. The peak area of the densitogram was quantified by densitometer at 275nm. The proposed method is simple, sensitive and accurate with good precision and is suitable for routine analysis of this drug in formulations.

Acknowledgement

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