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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ZALTOPROFEN AND PARACETAMOL BY HPTLC

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ABSTRACT

Objective: *A simple, precise, accurate and specific high performance thin layer chromatographic method has been developed for the simultaneous estimation of zaltoprofen and paracetamol and Quantitation in pharmaceutical dosage form.*

Methods*: The method employed silica gel 60F_{254} Precoated plates as stationary phase and a mixture of chloroform: acetone: toluene: acetic acid (6.5 :2.5:1:0.1v/v/v/v) as mobile phase.*

Results*: The developed method was validated for accuracy, linearity, precision, specificity, limit of detection and limit of quantitation as per ICH guideline. The Rf value for ZLT and PCM were found to be 0.63±0.2 and 0.36±0.2 respectively. The calibration curve was found to be linear between 100-500 ng/spot for ZLT and 406.25-2031.25 ng/spot for PCM. The limit of detection and limit of quantitation for ZLT were found to be 13.45 and 40.78 ng/spot and for PCM 16.33 and 49.55 ng/spot respectively.*

Conclusion: *The proposed method has been successfully applied for the estimation of Zaltoprofen and Paracetamol in their pharmaceutical formulation.*

KEYWORDS: *Zaltoprofen (ZLT), Paracetamol(PCM), HPTLC, Method development, Validation.*

INTRODUCTION

ZLT (Fig.1) and PCM(Fig.2) is a nonsteroidal anti-inflammatory drug. Zaltoprofen is a prefentially COX-2 inhibitor and selectively inhibits prostaglandin E2 production at inflammatory sites and induce apoptosis in variety of cell lines[3]. While paracetamol is generally considered to be weak inhibitor of the synthesis of prostaglandins. It may act through inhibition of central nervous system cyclo-oxygenase (COX-3) isoform. Zaltoprofen and Paracetamol combination is used as a potent analgesic and anti-inflammatory drug in the pain management and increase the effect and decrease the dose dependent side effect. A survey of literature revealed that RPHPLC and UV-visible spectrophotometric methods have been reported for estimation of Zaltoprofen and Paracetamol[10-13]. To the best of our knowledge there is no HPTLC methods reported for zaltoprofen and paracetamol in combined dosage form. So, the aim of work was to develop HPTLC method for simultaneous estimation Zaltoprofen and Paracetamol and to validate the method as per ICH guidelines.

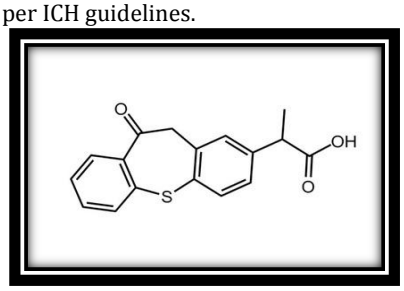

Fig. 1: Structure of Zaltoprofen

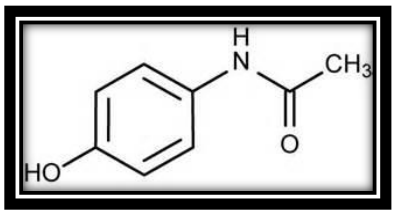

Fig. 2: Structure of Paracetamol

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MATERIALS AND METHODS

Reagents and chemicals:

Zaltoprofen and Paracetamol were kindly supplied as a gift sample by Anlon healthcare Pvt. Ltd. Rajkot, India and Sun pharmaceuticals Pvt. Ltd. Silvassa, India Respectively. All other chemicals and reagents used were of AR grade and were purchased from CDH Chemicals, New Delhi, India.

Instrumentation and chromatographic conditions:

The sample were spotted in the form of bands of width 8mm with a camag 100µL sample (Hamilton)syringe on Precoated silica gel aluminium pate 60 F_{254}(10cm × 10cm) with 250 µm thickness, E. Merk, Germany using a camag Linomat IV. The plates were prewashed by methanol and activated at 60^0C for 5min prior to chromatography. A constant application rate of 10 µL/spot was employed and space between two bands was 6mm.The slit dimension was kept at 6 mm × 0.45 mm and 10mm/s scanning speed was employed. The mobile phase consisted of chloroform: acetone: toluene: acetic acid (6.5:2.5:1:0.10v/v/v/v). The optimized chamber saturation time for mobile phase was 20 min at 27^0C±2^0C temperature. The TLC plate run distance was 80mm and TLC plates were dried and the densitometric scanning was performed on camag TLC scanner 3 in the reflection/absorbance mode at 269nm for all measurements and operated by CATS 4 software. the source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm.

Standard solutions and calibration graphs:

Stock solution was prepared by dissolving 0.08gm ZLT (800µg/ml) and 0.325gm of PCM (325µg/ml) in 100ml methanol. Working standard were prepared by diluting the stock solution in methanol and obtain concentrations of 10-50 µg/ml of ZLT and 40.625-203.125 µg/ml of PCM. Each concentration was spotted five times on the TLC plate. The plate was developed on the chromatographic condition. The peak area was plotted against the concentration to obtain the calibration graphs.

Validation of Developed Method:

The developed method was validated as per the ICH guidelines Q2(R1) for linearity, accuracy, precision, limit of detection, limit of quantitation, specificity, and robustness.

Linearity and Range:

linearity of response for ZLT and PCM was assessed by analysis of five levels of calibration curve in the range of 100-500 ng/spot for ZLT and 406.25-2035.25 ng/spot for PCM (n = 5). 10µL of each solution were spotted on the TLC plate, plates were developed and analysed.

Precision:

Repeatability of sample application and Scanning:

Repeatability of sample application and scanning was performed by application of 10µL of working standard solution of ZLT 30 µg/mL and PCM 121.875 µg/mL for six times on a same TLC plate and for scanning, scan six times without changing position of band. The plate was developed, dried and analysed as described in chromatographic condition. The area was measured and % RSD was calculated.

Intermediate precision:

Precision was carried out by spotting 10µL for three different concentrations (ZLT 10,30 and 50µg/mL and PCM 40.625,121.875 and 203.126µg/mL) three times on same day for intraday precision and three times on different days for interday precision. Plate was developed and scanned. % RSD was calculated on the basis of peak area.

Limit of detection and Limit of quantitation:

The limits of detection and quantitation of the developed method were calculated from the standard deviation of the intercepts and mean slope of the calibration curves of ZLT and PCM using the formula as given below.

$$LOD=3.3\times\sigma/S \text{ and } LOQ=10\times\sigma/S$$

Where, σ = Standard deviation of the Y intercept regress lines and S = Slope of the calibration curve equation.

Specificity:

The peak purity of Zaltoprofen and Paracetamol was determined by comparing the UV spectra of standard and sample (marketed formulation) scanned at peak start (S), peak apex(M) and peak end (E) position of band.

Accuracy:

Accuracy was determined by calculating recovery of both drug by standard addition method at three different level (80%,100% and 120%). From the test stock solution (ZLT:80µg/mL and PCM:325µg/mL) 2.5 mL was pippet out in four different 10mL volumetric flask and from the standard solution 2 ml for 80%, 2.5 mL for 100% and 3ml for 120% was added and in forth flask standard was not added and considered as 0% and make upto the mark with methanol.

10µLof the above solution was spotted on TLC plate; plate was developed as mention in chromatographic conditions. The plate was dried and scanned. % recovery was calculated on the basis of peak area.

Robustness:

Robustness was determined by change in various parameter like change in mobile phase (±0.2mL acetone), saturation time(±2min), Run distance(±5mm) and detection wavelength (±2nm) were done by spotting 10µL solution of three concentrations (ZLT:10,30 and 50µg/mL and PCM:40.625,121.875 and 203.125µg/mL) on TLC plate three times and %RSD were calculated based on peak area.

Assay of marketed formulation:

Twenty tablets were weighed accurately and Powder equivalent to 325mg of PCM and 80mg of ZLT was transferred to 100 mL volumetric flask and dissolved in methanol and the volume was made upto the mark with methanol (3250 µg/ mL PCM and 80 µg/mL ZLT).

From the above test stock solution 5mL was transferred in 50mL volumetric flask and diluted with methanol. Aliquots of 3.75mL from the above solution were pipetted out and transferred to 10 mL volumetric flasks in triplicate and made upto the mark with methanol. 10µL was spotted along with the standard solutions of different concentrations. The content of PCM and ZLT was calculated from the linear regression equation on the basis of peak area, SD and % assay was calculated.

RESULTS

Optimization of chromatographic condition:

The method employed silica gel $60F_{254}$ Precoated plates as stationary phase and a mixture of chloroform: acetone: toluene: acetic acid (6.5 :2.5:1:0.1v/v/v/v) as mobile phase provide optimum resolution of ZLT and PCM.The Rf value for ZLT and PCM were found to be 0.63±0.2 and 0.36±0.2 respectively (figure 3).

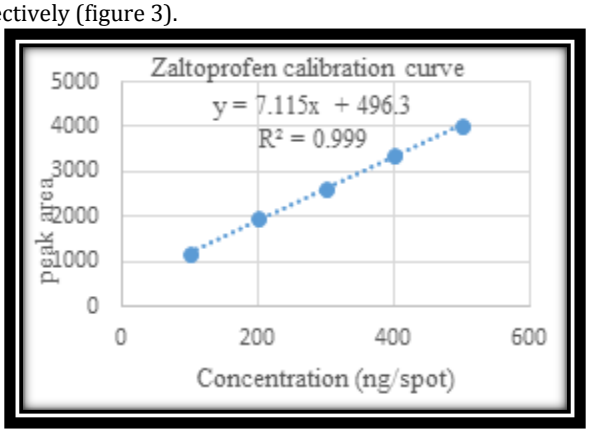

Fig. 3: Calibration curve of Zaltoprofen

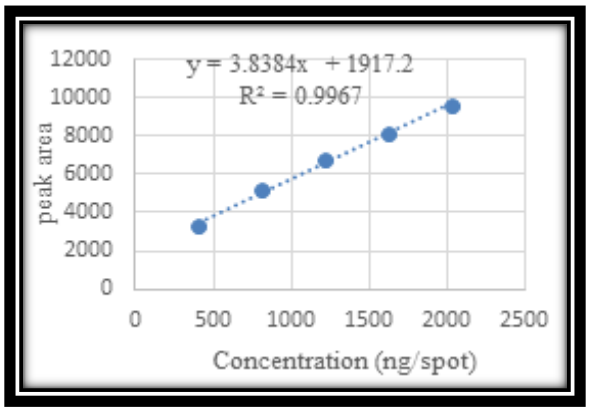

Fig. 4: Calibration curve of Paracetamol

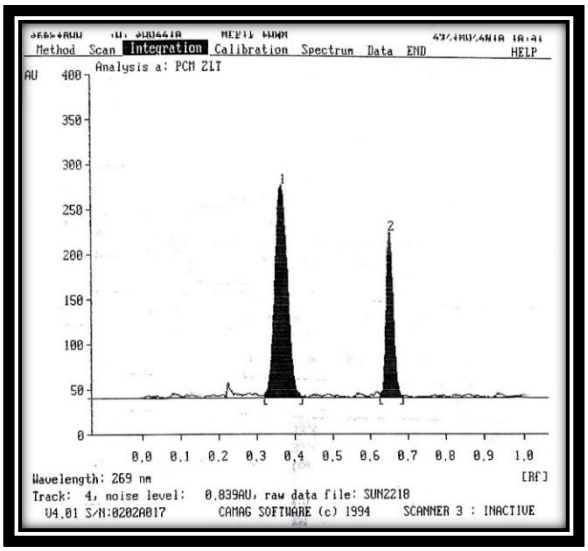

Fig. 5: Standard chromatogram of ZLT and PCM

Linearity:

Linear regression for calibration curve of Zaltoprofen was found to be 0.999 and for Paracetamol was found to be 0.996. the regression line equation is as follows: (figure 3&4)

y = 7.115x+496.3 for Zaltoprofen and y=3.838x+1917 for Paracetamol

Precision:

The %RSD for peak area was found to be 0.17 and 0.43 of Zaltoprofen and Paracetamol respectively by spot application six times. The %RSD for peak area was found to be 0.26 and 0.31 of ZLT and PCM respectively by spot scanning six times.

The %RSD for peak area was found to be 0.17 and 0.43 of Zaltoprofen and Paracetamol respectively by spot application six times. The %RSD for peak area was found to be 0.26 and 0.31 of ZLT and PCM respectively by spot scanning six times.

The %RSD for intraday precision was found to be 0.62-0.91for ZLT and 0.13-0.17 for PCM. Interday precision was performed by analyzing three different concentrations within linearity range, three times in different day. The %RSD for Interday precision was found to be 0.68-1.62 for ZLT and 0.55-1.04 for PCM (Table 1&2).

LOD and LOQ:

The LOD and LOQ and were found to be 13.45, 40.78 ng/spot and 16.33, 49.55 ng/spot respectively for Zaltoprofen and Paracetamol.

Specificity:

The peak purity of ZLT and PCM was assessed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot, i.e. r (M, E) =0.999596, 0.999801. good correlation (r=0.999780 and r=0.99857) was obtained between standard and sample spectra of ZLT and PCM respectively.

Accuracy:

To check the accuracy of the method, recovery studies were carried out by addition of standard solution to sample solutions at three different levels, 80,100,120%. Chromatogram

was developed and % recovery was found to be 100.36% and 100.05% for ZLT and PCM respectively (Table 3).

Assay of marketed dosage form:

The assay concentration was taken 300ng/spot for ZLT and 1218.75ng/spot for PCM.The drug content was found to be 101.60%±01.53 and 100.37%±00.54 for ZLT and PCM respectively.

Robustness:

Developed method was found to be robust by changing the parameter saturation time (±2min), detection wavelength (±2nm), mobile phase (±0.2ml of acetone) and run time (±5 min) in 3 levels and % RSD was found to be below 2% (Table 4&5)

Table No. 1: Intraday data of ZLT and PCM

Sr.no.	Concentration (ng/spot)	Average area(n=3)mean± SD	%RSD
Zaltoprofen			
1	100	1167.50±10.69	0.91
2	300	2638.23±16.38	0.62
3	500	4036.03±35.22	0.87
Paracetamol			
1	406.25	3309.33±5.20	0.17
2	1218.75	6755.830±9.11	0.13
3	2031.25	9584.7±16.75	0.17

Table No. 2: Interday data of ZLT and PCM

Sr.no.	Concentration (ng/spot)	Average area(n=3)mean± SD	%RSD
Zaltoprofen			
1	100	1190.73±08.11	1.62
2	300	2678.70±28.41	1.06
3	500	4119.12±66.75	0.68
Paracetamol			
1	406.25	3414.80±35.38	0.55
2	1218.75	6825.46±44.55	0.65
3	2031.25	9726.00±43.14	1.04

Table No. 3: Accuracy data of Zaltoprofen and Paracetamol

% Level	Amount of Test (ng)	Amount of Standard spike (ng)	Amount of standard Recovered (ng)±SD	% Recovered ±SD	% Avg. recovered
Zaltoprofen					
80	200	160	158.87±13.43	99.29±08.93	
100	200	200	203.67±10.98	101.83±05.49	100.36
120	200	240	239.98±03.18	99.99±01.32	
Paracetamol					
80	812.5	650.0	652.32±03.26	100.35±00.50	
100	812.5	812.5	807.85±14.35	99.42±01.77	100.05
120	812.5	975.0	978.97±14.30	100.40±01.46	

Table No. 4: Robustness data of Zaltoprofen

Parameter	Mean area(n = 3)			Mean ± SD	%RSD
	Optimized condition	Changed condition			
		-	+		
Mobile phase(±0.2mL)	1162.6	1152.63	1185.62	1166.95±16.91	1.44
	2652.9	2612.32	2715.02	2660.08±51.72	1.94
	3994.2	3889.96	4020.22	3968.12±68.93	1.73
Saturation time(±2min)	1162.6	1125.25	1160.36	1149.40±20.94	1.82
	2652.9	2612.02	2700.25	2655.05±44.15	1.66
	3994.2	3895.43	4045.66	3978.43±76.34	1.91
Run distance(±5mm)	1162.6	1135.25	1178.98	1158.94±22.09	1.90
	2652.9	2599.63	2702.36	2651.63±51.37	1.93

	3994.2	3889.96	4012.88	3965.68±66.23	1.67
Detection wavelength (±2nm)	1162.6	1185.98	1200.87	1183.15±19.29	1.63
	2652.9	2612.00	2712.23	2659.04±50.39	1.89
	3994.2	3991.25	4112.52	4032.65±69.17	1.71

Table No. 5: Robustness data of Paracetamol

Parameter	Mean area(n = 3)			Mean ± SD	%RSD
	Optimized condition	Changed condition			
		-	+		
Mobile phase(±0.2mL)	3310.20	3299.89	3365.25	3325.11±35.13	1.05
	6752.06	6689.36	6920.93	6787.45±119.77	1.76
	9598.51	9512.22	9659.78	9590.16±74.13	0.77
Saturation time(±2min)	3310.20	3256.32	3378.94	3315.15±61.45	1.85
	6752.06	6894.80	6909.11	6851.95±86.79	1.26
	9598.51	9449.23	9664.52	9570.65±110.25	1.15
Run distance(±5mm)	3310.20	3275.42	3400.88	3328.83±64.77	1.94
	6752.06	6675.12	6908.63	6778.65±118.93	1.75
	9598.51	9453.26	9746.25	9599.23±146.49	1.52
Detection wavelength (±2nm)	3310.20	3272.51	3386.77	3323.16±58.22	1.75
	6752.06	6701.28	6885.37	6779.57±95.07	1.40
	9598.51	9538.53	9848.81	9661.84±164.63	1.70

DISCUSSION

Developed chromatographic method was validated according to ICH guidelines. Linearity study indicated that area was directly proportional to concentration (r^2=0.999 for ZLT and r^2=0.996 for PCM) and that the method was linear. % RSD for precision study was less than 2% showing that the method was precise. The LOD and LOQ for ZLT were found to be 13.45 and 40.78 ng/spot and for PCM 16.33 and 49.55 ng/spot respectively. Recovery study was carried out at concentration level of 80%,100% and 120%. Mean % recovery was found to be 100.63 and 100.05 for ZLT and PCM respectively. In Robustness study, % RSD was found to be less than 2% indicating that small changes in parameter such as mobile phase, saturation time, run time and detection wavelength did not show any major changes in results. In quantitation of pharmaceutical dosage form % was found to be 101.60 and 100.37 for ZLT and PCM respectively.

CONCLUSION

The developed HPTLC method provides simple, accurate and reproducible quantitative analysis for simultaneous determination of ZLT and PCM in tablets dosage form. The method was validated as per ICH guideline.

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