Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2014

Research Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PARACETAMOL AND ACELOFENAC IN COMBINED DOSAGE FORM

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Received: 07 Nov 2013, Revised and Accepted: 02 Dec 2013

ABSTRACT

Objective: The simple, accurate and precise method for simultaneous determination of Paracetamol and Acelofenac in tablets by HPTLC methods is developed.

Methods: In this method, the chromatograms were developed using a mobile phase of Toluene: Methanol (3.5:1 v/v).The method uses aluminum plates coated with silica gel 60 F_{254} as stationary phase. Densitometric evaluation of the separated bands was performed at 266 nm.

Results: The R_F values for Paracetamol and Acelofenac were 0.48 and 0.62 respectively. The linearity of paracetamol and Acelofenac is 300 to 1800 ng/spot and 100 to 600 ng/spot respectively. Recovery of paracetamol and Acelofenac is in between 99.98 to 101.98. Precision, Robustness and Ruggedness are shown in validation section.

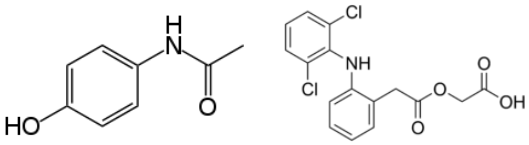
Conclusion: The proposed method HPTLC method is simple, economic, accurate & reproducible & can be used in routine analysis for simultaneous determination of Paracetamol and Acelofenac in combined dosage form.

Keywords: Paracetamol, Acelofenac, HPTLC and Validation.

INTRODUCTION

Paracetamol and Acelofenac are very commonly used as pain killer drug. First time maximum parameters in validation are mentioned in this method. Few milliliter of mobile phase is required for this HPTLC method that's why it is less expensive method. This method is developed and validated according to ICH guidelines. Cost per analysis is very low as compared to hplc. In case of hptlc, stationary phase is HPTLC plates which are less expensive than hplc column, that's why hptlc method is selected for method development and validation. Due to auto sampler less solvent is required.

Paracetamol is chemically *N*-(4-hydroxyphenyl) ethanamide *N*-(4-hydroxyphenyl) acetamide. It is having molecular formula $C_8H_9NO_2$ & its molecular weight is 151.17 g/mol. Paracetamol is used for the relief of pains associated with many parts of the body. It is analgesic in nature and it has relatively little anti-inflammatory activity. Literature survey reveals that several analytical methods have been reported for estimation of Paracetamol and Acelofenac by colorimetric and HPLC method. Acelofenac is 2-[2-[2-[(2, 6-dichlorophenyl) amino] phenyl] acetyl] oxyacetic acid. It is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis.It is having molecular formula $C_{16}H_{13}Cl_2NO_4$ and its molecular weight is 354.18472 g/mol.



1. Structure of Paracetamol 2. Structure of Acelofenac

MATERIALS AND METHODS

Instrumentation

HPTLC system equipped with CAMAG LINOMAT V automatic sampler applicator; CAMAG TLC SCANNER; Integrator controlled by CATS4 software; CAMAG twin through glass chamber with stainless steel lid. Precoated silica gel F_{254} on aluminum sheets (20 × 10 cm).

Chemicals and reagents

Standard sample of Paracetamol and Acelofenac were provided by central drug Testing Lab. Mumbai. Tablets of combined form were procured from the market. All other reagents were analytical grade. Toluene and methanol were obtained from science house (Mumbai, India). Stationary phase used is silica gel F_{254} precoated aluminum plates.

Preparation of stock solution

Accurately 10mg of Paracetamol and 10mg of Acelofenac reference std is weighed and transfer in 10 ml volumetric flask 5ml methanol is added, sonicated for 15 minutes and diluted to prepare stock solution with methanol.

Sample preparation

To determine the content of Paracetamol and Acelofenac in tablet (label claim: 500mg of Paracetamol and 100mg of Acelofenac). 5 tablets were weighed; average weight is taken and crushed to fine powder. 10 mg from it is transferred to 10 ml volumetric flask 5ml methanol is added and sonicated and diluted further with methanol.

Chromatography

Linear ascending development was carried out in a 20cm × 10cm twin though glass chamber (CAMAG) using the mobile phase Toluene: Methanol (3.5:1) (v/v). The chamber is saturated for 10 minutes. Plates were dried in a current of air with the help of hair dryer. The source of radiation utilized in deuterium lamp emitting a continuous UV spectrum between 200nm to 400nm. Slit dimension were 5nm × 0.45nm and the scanning speed of 20mms^{-1}. For preparation of the calibration curve 1mg/ml of working standard of Paracetamol and Acelofenac is prepared in methanol. Sample was spotted on precoated TLC plates by using Linomat 5 automatic sampler. TLC plates were developed up to 8cm contents of Paracetamol and Acelofenac were determined by comparing area of the chromatogram of sample with calibration curve of working standard both.

RESULT AND DISCUSSION

Validation of method

Development method is validated in terms of linearity, accuracy, precision, LOD, LOQ, robustness.

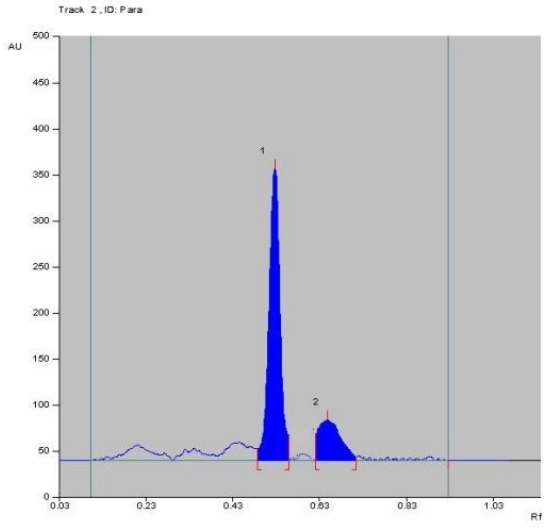


Fig. 1: Densitogram of Paracetamol and Acelofenac

Linearity

Appropriate volume of aliquots from standard Paracetamol and Acelofenac stock solutions were prepared and applied on the TLC plate in the range of 1.2-2.4 µL to give a series of spots covering the range from 300 to 1800 ng/spot and 100 to 600 ng/spot respectively. The linear regression coefficient for paracetamol is 0.999 and for Acelofenac is 0.999.

Precision

The intraday and inter day precision of the method were estimated by performing six determination of drugs solution at two different concentrations; the results obtained as shown in table 1&2.

Table 1: It shows precision for Paracetamol

Amount µg/band	Intra-day precision		Inter-day precision	
	Mean area (AU)	RSD (%)	Mean area (AU)	RSD (%)
600	6200	0.18	5800	0.90
900	13800	0.38	12800	1.62

n=6 (Paracetamol)

Table 2: It shows precision for Acelofenac

Amount µg/band	Intra-day precision		Inter-day precision	
	Mean area (AU)	RSD (%)	Mean area (AU)	RSD (%)
200	290	0.17	300	0.98
300	604	0.40	625	1.49

n=6 (Acelofenac)

Robustness

Robustness was measured by analysis of the sample solution by making small changes to mobile phase composition. Toluene: Methanol in the ratio 3.6:0.9 (v/v) and Toluene: Methanol in the ratio 3.4:0.6 (v/v) were selected with different distances 8 & 9cm for different amount of Paracetamol and Acelofenac. The low values of RSD obtained after introduction of small changes in mobile phase shown in table 3 were indicative of the robustness.

Table 3: It shows Robustness for Paracetamol

Condition	Mean	RSD in %
Toluene: Methanol in the ratio 3.6:0.9 (v/v)	100.5	0.78
Toluene: Methanol in the ratio 3.4:0.6 (v/v)	99.19	1.21
Development distance		
8cm	101.29	0.89
9cm	99.99	1.18

Table 4: It shows Robustness for Acelofenac

Condition	Mean	RSD in %
Toluene: Methanol in the ratio 3.6:0.9 (v/v)	100.80	1.08
Toluene: Methanol in the ratio 3.4:0.6 (v/v)	99.02	1.12
Development distance		
8cm	102.9	1.02
9cm	99.98	1.23

Limit of detection & Limit of quantitation

The limits of detection (LOD) and limit of quantitation (LOQ) were calculated from slopes of the calibration plots and the standard deviation (SD) of the response by the use of the equations LOD 3.3 XSD/S and LOQ 10 XSD/S. the limit of detection and limit of quantitation obtained by this method for Paracetamol 5mcg and 480mcg; Acelofenac 10mcg and 450mcg respectively.

Specificity

Specificity of the method is ascertained by analyzing reference standard and samples. The bands for Paracetamol and Acelofenac formulations were confirmed by comparing R_f values and U.V spectra of these separated bands with those from standard the peak purity of Paracetamol and Acelofenac accessed by comparing the spectra acquired at the peak start(S) peak apex(N) and peak end(E) of a band. It was found that r(S, M) = 0.999 and r (M, E) = 0.999, it was in good correlation(r=0.999) was also obtained between standard and spectra of samples containing Paracetamol and Acelofenac.

Recovery

The analyzed samples was spiked with an additional 500,600,700 mcg of Paracetamol and 150,200,250 mcg of Acelofenac standard 7 mixture were analyzed again, in triplicate by proposed method, to check different amounts if the drug from the formulation, Recovery was 99.98-101.09% which is shown in table 5 & 6.

Table 5: It shows Recovery for Paracetamol

Amount of Drug added (%)	Theoretical Content (mcg)	Recovery (%)	RSD
500	600	100.80	1.08
600	600	101.45	1.39
700	600	101.98	1.34

Table 6: It shows Recovery for Acelofenac

Amount of Drug added (%)	Theoretical Content (mcg)	Recovery (%)	RSD
150	200	99.989	1.02
200	200	100.34	1.32
250	200	101.01	1.08

Ruggedness

Ruggedness is measure of reproducibility of a test results under normal, expected operating Condition from instrument & from analyst to analyst, ruggedness was tested by analysis of 900,300mcg Paracetamol and Acelofenac for per band were listed in table 7 & table 8 respectively.

Table 7: It shows Ruggedness for Paracetamol

Variable	Mean	RSD
Analyst I	98.98	1.12
Analyst II	100.98	1.21

Table 8: It shows Ruggedness for Acelofenac

Variable	Mean	RSD
Analyst I	100.02	1.01
Analyst II	99.922	0.999

CONCLUSION

The proposed method HPTLC method is simple, economic, accurate, & reproducible & can be used in routine analysis for simultaneous determination of Paracetamol and Acelofenac in combined dosage form.

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