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The Development and Validation of a Thin Layer Chromatography Densitometry Method for the Analysis of Diclofenac Sodium Tablets

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Abstract

A Thin Layer Chromatography (TLC) method for the qualitative and quantitative analysis of diclofenac sodium tablets was developed and validated according to ICH and USP guidelines. The method was developed using a mobile phase prepared with environment-friendly solvents: toluene, acetone and glacial acetic acid (10:15:0.2 v/v/v), on pre-coated TLC silica gel 60 F_{254} glass plates with a saturation time of 25 min and a densitometer detection wavelength of 284 nm in the reflectance absorbance mode. The R_f of diclofenac sodium was at 0.60 and the method was repeatable, robust with good selectivity and specificity. Regression functions were established over the range of 250-600 ng/spot with r^2 of 0.993 and 0.999 for the linear and polynomial regressions respectively. The accuracy at nominal concentration was found to be 100.2%, and the results of the assay of three brands for diclofenac sodium tablets were found to meet the USP 95 to 105% assay limits thus demonstrating the usefulness of the method for the assay of these products.

The developed assay method for diclofenac sodium in tablets is simple, accurate, and inexpensive, with good precision and should be especially useful in resource constrained countries.

Keywords: Thin Layer Chromatography; Diclofenac sodium; Validation

Introduction

Diclofenac sodium is 2-(2-(2, 6-dichlorophenylamino) phenyl) acetic acid (Figure 1). It is a Nonsteroidal Anti-Inflammatory Drug (NSAID) with anti-inflammatory, antipyretic, and analgesic action as a result of the blockade of prostaglandin synthesis by inhibition of the cyclooxygenase (COX) enzyme. It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis [1-4].

Analysis of the Active Pharmaceutical Ingredient (API) as well as Finished Pharmaceutical Product (FPP) is of vital importance to help ensure that good quality products are manufactured and supplied to the patient end users.

Literature review revealed that various methods have been reported for analysis of diclofenac salts in various formulations by High Performance Liquid Chromatography (HPLC) [4-7], High Performance TLC (HPTLC) [8-13], TLC [14], ultra violet-visible spectroscopy (UV-Vis) [15,16], spectrofluorometry [17] and colorimetry by derivatization of the diclofenac ring [18], and Gas Chromatography-Mass Spectrometry (GC–MS) for determination of

diclofenac in urine and plasma samples [19,20]. The developed TLC method has several advantages over other available methods such as ability to analyze several samples simultaneously in parallel, as well as using small quantities of solvents as a mobile phase which reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing environmental pollution [21-23].

Experimental

Solvents

Analytical grade reagents obtained from various manufacturers were used for the method development and validation. These consisted of methanol and glacial acetic acid (Scharlau Chemie, Gato Perez, Spain), ethyl acetate, toluene, acetone and ammonia solution (Fisher Scientific, Leicestershire, UK). Purified water was prepared by reverse osmosis in-house with a RO- Purification System Millipore® (France). Excipients used for simulation to determine specificity included microcrystalline cellulose (FMC BioPolymer, Philadelphia), sodium carboxymethyl cellulose and polyvinylpyrrolidone cross-linked (Associate Co. Ltd, Shenzhen, China), as well as magnesium stearate (Shandong Liaocheng Ehua Medicine Co. Ltd, China).
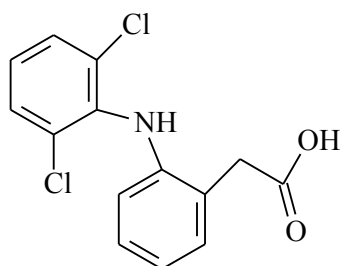

Figure 1: The Chemical structure of diclofenac.

Reference standards and sample tablets

Diclofenac sodium reference material was obtained from Sigma-Aldrich, Belgium. Diclofenac sodium tablets (50 mg) were obtained from Fourrts Company, India; Remedica, Cyprus; and Zenufa Laboratories, Tanzania.

Equipment

Instrumentation used during method development consisted of a densitometer with a TLC Scanner 3 operated with Wincats (version 1.4.3) planar chromatograph software that was used as data manager and integrator, a Linomat 5 automatic sample applicator with a Hamilton syringe of 100 µL capacity for sample application and a rectangular flat bottomed developing tank (CAMAG, Muntenz, Switzerland). The TLC plates used for chromatographic sample separations (5×10 cm and 20×10 cm)were pre-coated with silica gel 60 F_{254} (Merck, Darmstadt, Germany) [24].

Method development

Preparation of sample stock and working solutions: Samples were prepared by accurately weighing powdered tablets equivalent to 20 mg of diclofenac sodium into a 10 mL volumetric flask that was dissolved in methanol to obtain stock concentration of 2 mg/mL. Further dilution was made using methanol to obtain a working concentration of 0.2 mg/mL by diluting 1 mL of the stock solution into a 10 mL volumetric flask.

Chromatography: Since the TLC plates were activated, they were freshly removed from their packing only when in use and it was not necessary to pre-wash them. Before spotting the plate was labeled and solvent front marked at 70 mm from the bottom. 5 µL were applied at 8 mm from the bottom as 8 mm band using a Linomat 5 applicator. After application the plate was dried with hot air before developing. Filter paper was placed to aid saturation at one side of the developing tank and the mobile phase poured by wetting it and the lid closed for 25 minutes to allow the tank to saturate before the plate was developed. After the plate was developed and dried it was scanned using a TLC Scanner 3 in the reflectance absorbance mode with Wincats (version 1.4.3) planar chromatograph software for data acquisition and some calculations.

Selection of suitable mobile phase: From literature survey the following solvents were identified as potential candidates for the method development: toluene, methanol, ethyl acetate, acetone, glacial acetic acid, formic acid and chloroform. Chloroform was omitted due to its carcinogenic potential. Toluene was substituted for chloroform and glacial acetic acid was used in place of formic acid [2,3,8-13]. The solvents were combined in various ratios taking into consideration their polarities and the lipophilicity of the analyte to optimize the mobile phase for separation of diclofenac sodium.

Method validation: After method optimization, a validation protocol was prepared based on the ICH Q2R1 guidelines and USP guidance and the validation was performed [2,24].

Results and Discussion

Selection of suitable mobile phase

Various combinations of solvents were used during method development, such as toluene:methanol (17:3 v/v); toluene: methanol (20:10v/v) (Figure 2); toluene; methanol (15:15 v/v); toluene:methanol:ammonia solution (17:3:0.2 v/v/v); toluene:methanol:ammonia solution (20:5:0.1 v/v/v); toluene:methanol:glacial acetic acid (20:5:1 v/v/v); toluene:ethyl acetate (17:4v/v);toluene:ethylacetate(20:10v/v);toluene:ethylacetate:ammonia solution (20:10:0.1 v/v/v); toluene:ethylacetate:glacial acetic acid (20:10:0.2 v/v/v) and toluene:acetone (10:20 v/v). The mobile phase containing toluene:acetone (10:20 v/v) was selected for optimization. The mobile phase toluene:acetone:glacial acetic acid was varied as [(10:20:0.05), (10:20:0.1), (10:18:0.2), (10:18:0.5), (10:15:0.2) v/v/v]. Diclofenac sodium with a working concentration at 100% at 0.2 mg/mL showed good peak resolution with mobile phase composition of toluene: acetone:glacial acetic acid (10:15:0.2 v/v/v). The optimum saturation time was determined to be 25 minutes and the development time of 6 minutes had a migration distance of 70 mm at room temperature (23 to 28°C) with a relative humidity between 36 and 54%. The optimum wave length was determined to be 284 nm after scanning the developed plate from 200 nm to 800 nm using the deuterium and tungsten lamps.

Method validation

The assay method was validated for specificity, linearity, precision, accuracy and robustness [24].

Method robustness: The robustness of the assay method was tested by varying the acetone: glacial acetic acid mobile phase solvent composition ratio ± 5% and the saturation times that were established during method development against critical parameter: R_f. The changes in the R_f values were found to be 0.60 ± 0.02 which showed that the method was robust.

Specificity and selectivity: Both the solvent and simulated excipients were used during each chromatographic plate run. The densitograms that were obtained during method development showed that the method was specific for the assay and selective for the API and that there were no interferences from the excipients or solvents showing that the method was acceptable for assay of diclofenac sodium (Figures 3-6).
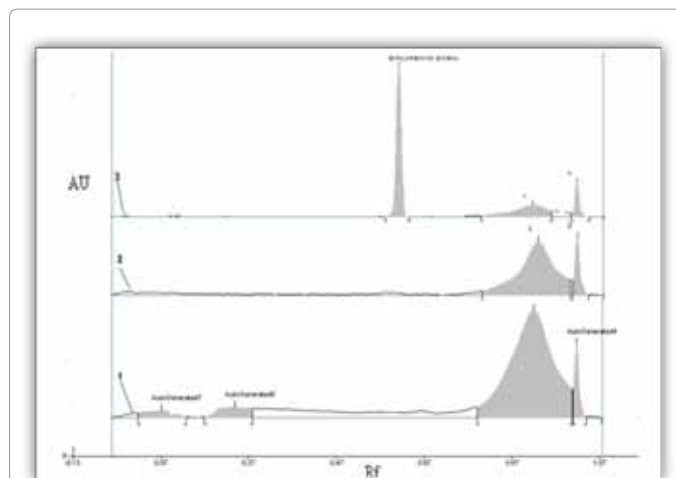
Linearity: Evaluation of linearity of the diclofenac sodium assay was demonstrated by preparing five standard concentrations ranging from 50% to 120% (500 to 1200) ng/spot using serial dilutions from a stock solution. Spots were applied at the plate for each concentration starting with lowest concentration to avoid carryover effect. The procedure
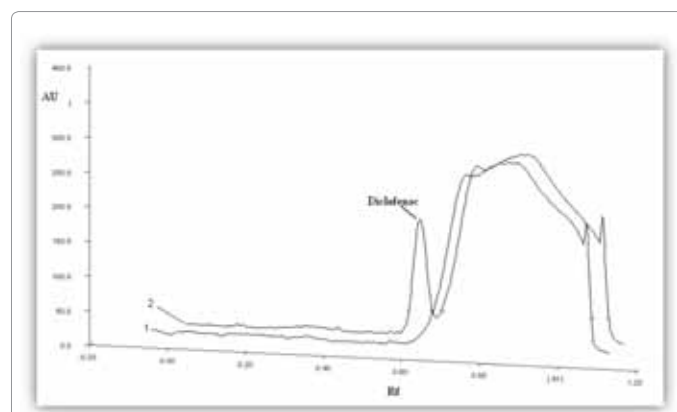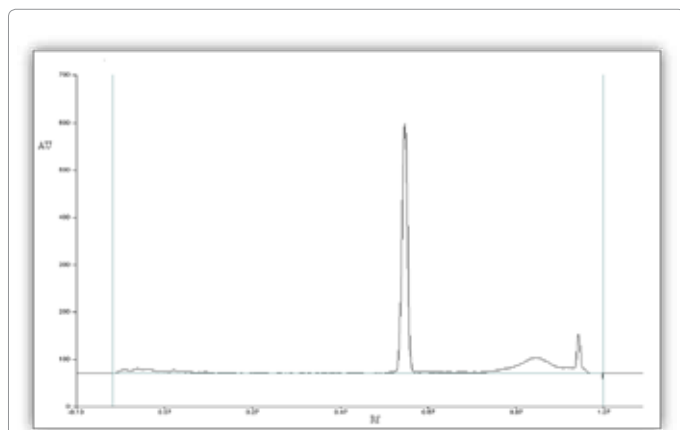

Figure 2: Typical densitograms of a solution containing diclofenac sodium using a mobile phase containing toluene:methanol (20:10 v/v), saturation time of 20 min using 5 cm×10 cm TLC silica gel 60 F_{254} plate and application volume was 5 µL at a detection wave length of 284 nm. Where track 1: Blank/methanol and track 2: diclofenac sodium (0.1 mg/mL), R_f 0.55.
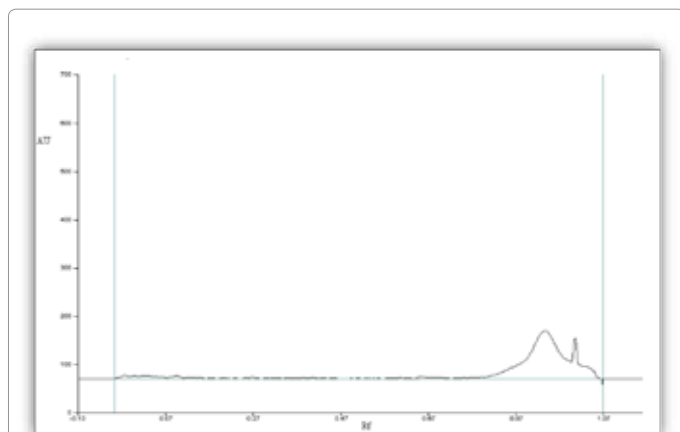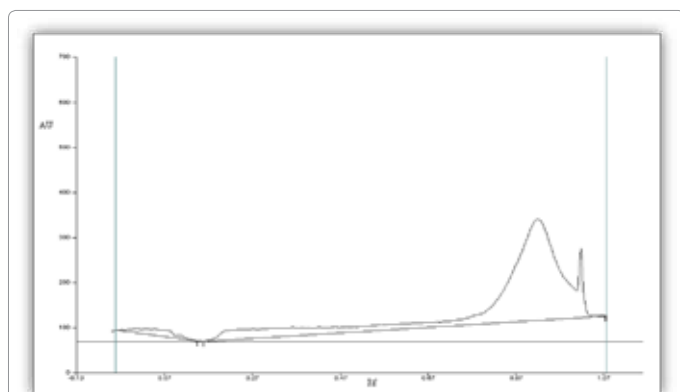
Figure 3: Typical densitogram of methanol used as a blank by using a mobile phase containing toluene: acetone: glacial acetic acid (10:15:0.2 v/v/v), saturation time of 20 min using 5 cm×10 cm TLC silica gel 60 F_{254} plate and application volume was 5 μL at a detection wave length of 284 nm.

Figure 4: Typical densitogram of a solution containing diclofenac sodium using a mobile phase containing toluene: acetone: glacial acetic acid (10:15:0.2 v/v/v), saturation time of 20 min using 5cm×10 cm TLC silica gel 60 F_{254} plate and application volume was 5 μL at a detection wave length of 284 nm, diclofenac sodium (0.2 mg/mL) had R_f 0.60.

Figure 5: Typical densitogram of simulated Placebo mixture of excipients used as a blank by using a mobile phase containing toluene: acetone: glacial acetic acid (10:15:0.2 v/v/v), saturation time of 20 min using 5 cm×10 cm TLC silica gel 60 F_{254} plate and application volume was 5 μL at a detection wave length of 284 nm.

Figure 6: Typical densitograms obtained during method specificity test (1) Placebo mixture of excipients in methanol (2) methanol and (3) diclofenac sodium. The chromatographic conditions consisted of mobile phase toluene: acetone: glacial acetic acid (10:15:0.2v/v/v) at a saturation time of 25 min, on a 20 cm×10 cm TLC silica gel 60 F_{254} plate with application volume of 5 μL, and detection at 284 nm. Diclofenac sodium (0.2 mg/mL) had a R_f of 0.60.

was repeated for three days. The results were analyzed via peak area of the developed spot. Both the linear (r^2=0.993) and polynomial (r^2=0.999) functions were evaluated and both cases had r^2>0.98 though polynomial regression which was superior to that shown in table 1 and was selected for use during accuracy testing.

Precision: Repeatability and intermediate precision were done for the assay method using sample tablets in which six replicate sample solutions were prepared independently, corresponding to 100% level of assay concentration. Intermediate precision was done by two analysts performing the procedure on different days. The calculated percentage relative deviations (% rsd) by using peak areas were found to be 1.1 and 3.8 for repeatability and intermediate precision as shown in tables 2 and 3.

Accuracy: Accuracy was evaluated by determination of percentage of standard diclofenac sodium spotted as controls by using a calibration curve at 80,100 and 120% of the assay concentrations. The controls were weighed in triplicate for each concentration and were spotted on a separate plate in triplicate. Assay of sample tablet was done where percentage assay at 100% was determined using the calibration curve. The accuracy was within limits at concentration of 100 and 120% according to USP [2] with value of 100 and 99% while at a concentration of 80% the accuracy was 103% (Table 4) of the various brands of diclofenac sodium tablets were tested using the method and all were found to be within the USP [2] limits of 95% to 105% (Table 5).

Conclusion

A facile and robust HPTLC assay technique for diclofenac sodium tablets was developed and validated according to ICH and USP guidelines [2,24,25]. Compared to some previously reported methods, [4-7,19,20] the method is simple, accurate, and inexpensive for the routine analysis of diclofenac sodium tablets using environmentally friendly solvents.

The method is repeatable and robust with good selectivity and specificity. Moreover, the assay results obtained showed that the sample tablets met the USP acceptance criteria of 95 to 105% of the label claim

Parameter	Polynomial regression	Linear regression
Concentration range(ng/spot)	500 -1200	500 -1200
x^2-coefficient	-0.0001	-
x-coefficient	4.509	2.890
y-intercept	1,819	2,464
r^2	0.999	0.993

Table1: Linear and polynomial regression equations obtained from the TLC densitometric method for diclofenac sodium.

Parameter	Sample repeatability
% Concentration	100
Mean	5146
Sd	56.6
% rsd	1.1
n	6

n=number of replicate samples

Table 2: Repeatability of diclofenac sodium assay.

Parameter	Analyst-1	Analyst -2	Average
% Concentration	100.00	100.00	100.00
Mean	4,806.02	5,081.47	4,943.74
Sd	76.14	156.63	185.68
% rsd	1.58	3.08	3.80
n	6	6	12

n= number of replicate samples

Table 3: Intermediate precision for diclofenac sodium using two analysts.

Concentration levels	80%	100%	120%
% Recovery	103.37	100.19	98.94
Sd	7.07	5.42	13.61
% rsd	1.60	1.10	2.60

Table 4: Accuracy values for diclofenac sodium.

Product	Manufacturer	Percentage per label claim
Ziclofen-50 Batch-1	Zenufa Laboratories Ltd Tanzania	103.71
Ziclofen-50 Batch-2	Zenufa Laboratories Ltd Tanzania	103.14
Remethan 50	Remedica Cyprus	101.23
Diclofen 50	Fourrts India	100.61

Table 5: Assay of diclofenac sodium tablet using the developed method.

which shows that the method is suitable for quantifying diclofenac sodium in tablets and thus is suitable for routine drug screening and analysis especially in resource constrained countries.

Acknowledgement

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