RESEARCH ARTICLE

A solid-liquid extraction and high performance thin layer chromatographic determination of diacerein and aceclofenac in pharmaceutical tablet dosage form

Santosh Bhalerao, Santosh Tambe*, Vikas Pareek, Rupali Shinde, Lalit Kumar Gupta

Department of Pharmaceutical Chemistry, Mahatma Gandhi Vidya Mandir's Pharmacy College, Panchavati, Mumbai - Agra Road, Nashik - 422003, Maharashtra, India.

Address for correspondence: Mr. Santosh Tambe, Department of Pharmaceutical Chemistry, Mahatma Gandhi Vidya Mandir's Pharmacy College, Panchavati, Mumbai - Agra Road, Nashik - 422003, Maharashtra, India. E-mail: santoshtambe@indiatimes.com

A novel, simple and robust High Performance Thin Layer Chromatographic method was developed and validated for the determination of diacerein and aceclofenac in the combined pharmaceutical tablet dosage form. The developed method demonstrates extraction of diacerein and aceclofenac by solid – liquid extraction and densitometric determination of them. Paracetamol was used as an internal standard (IS). The precoated silica gel $60F_{254}$ aluminum plate was selected as the stationary phase and the mixture of ethyl acetate: methanol: glacial acetic acid in the ratio of (12: 0.5: 0.2 v/v/v) was used as developing solvents. The detection of diacerein and aceclofenac was carried out at 268 nm by TLC scanner-3(Camag). The developed method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation and robustness parameters. The correlation coefficient of diacerein and aceclofenac were 0.9997 and 0.9996 observed respectively. The average percentage recovery of diacerein and aceclofenac were found to be 100.14 ± 1.15 and 100.71 ± 0.33 respectively. Intra and inter day precision measured as coefficient of variation were less than 2% for both analytes. The proposed HPTLC method has potential applications for determination of diacerein and aceclofenac in combined tablet dosage form.

Keywords: *Diacerein, Aceclofenac, HPTLC, Solid-liquid extraction.*

INTRODUCTION

Diacerein is chemically as 4, 5-diacetoxy-9-10-dioxo-9-10-dihydroanthracene-2-carboxylic acid. It is a novel anti-inflammatory drug with pharmacological properties different from those of classical non steroidal anti-inflammatory agents. It is also known to inhibit interleukin-1. Diacerein is a readily obtained in few synthetic steps from naturally occurring glucopyranoside aloin. It is used in treatment of osteoarthritis and to prevent vascular diseases. Aceclofenac ([2-(2′, 6′-dichlorophenylamino) phenyl] acetoxyacetic acid) is a phenyl acetic acid derivative that shows analgesic properties and good tolerability profile in a variety of painful conditions. It is largely used in the symptomatic treatment of pain and of inflammatory or degenerative arthropathies like osteoarthritis, rheumatoid arthritis and ankylosing spondylities. Diacerein and aceclofenac is a recent combination available in the market for its synergetic effect in the treat - ment of different joint disorders. Literature survey revealed that various methods reported for diacerein and aceclofenac includes, Spectrophotometric and spectrofluorimetric [1], in human plasma by narrowbore HPLC using column- switching [2], HPLC [3], HPLC stability-indicating [4, 5], capillary electrophoresis [6], reverse phase HPLC [7], flow injection chemiluminescence analysis [8], high performance liquid chromatography and pharmacokinetics [9], visible spectrophotometric [10], LC-MS/MS [11], physico-chemical and structural characterization [12], isolation and structural elucidation [13]. These above methods demonstrated analysis of diacerein and aceclofenac alone or in combination of these with other drugs. However, we propose a solid-liquid extraction method followed by HPTLC determination of diacerein and aceclofenac in combined tablet dosage form.

MATERIALS AND METHODS

Materials

Methanol, ethyl acetate, glacial acetic acid (HPLC grade), were purchased from Fischer Scientific (India). Dimethyl sulfoxide (AR grade), was purchased from Merck Co. Diacerein, aceclofenac and paracetamol (IS) were kindly supplied by Blue Cross Lab. Pvt. Ltd., Nashik, India. The mobile phase includes ethyl acetate: methanol: glacial acetic acid (12: 0.5: 0.2 v/v/v).

Instrumentation, chromatographic conditions and optimization of procedure

Initially various compositions of mobile phases were tried for chromatographic development of diacerein and aceclofenac simultaneously, but no proper resolution of diacerein and aceclofenac bands were obtained. So the solid liquid extraction method was selected for separation of diacerein and aceclofenac and both analytes were quantited separately by densitometer. Samples were applied in the form of bands of 8 mm width with a Camag 100 µL syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminum plate 60 F254 (20x10 cm) with 25 mm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologist, (Mumbai) using a Camag Linomat 5 (Switzerland). Plates were prewashed by methanol (HPLC grade) and activated at 120°C for 20 min prior to chromatographic developments. A constant application rate of 0.2 µl/s was employed. Slit dimension 6.00x0.45 mm and scanning speed 20 mm/s was employed. Mobile phase consisted of ethyl acetate: methanol: glacial acetic acid (12: 0.5: 0.2, v/v/v) and about 13 mL of mobile phase was used for each chromatographic development. Linear ascending developments were carried out in twin trough glass chamber (Camag, Muttenz, Switzerland). Optimized chamber saturation time with developing solvents was 15 minutes at 23±2°C and relative humidity was 60 ± 5%. The length of migration front was 70 mm and run time was around 7 minutes. Subsequent to the development, TLC plate was dried by hot air in fuming cupboard. Densitometric scanning was performed on Camag TLC scanner 3 in absorbance mode at 268 nm. The source of radiation used was deuterium lamp emitting continues UV spectrum between 400 - 200 nm. R_f value of paracetamol, diacerein and aceclofenac were found 0.41±0.03, 0.56±0.03 and 0.61±0.03 respectively. Figure 1 indicates integrated peak and R_f value of 200 ng paracetamol (IS) and 150 ng diacerein and Figure 2 indicates integrated peak and R_f value of 200 ng paracetamol (IS) and 300 ng aceclofenac. Evaluation was done via peak areas. Peak response (ratio of peak areas of diacerein and aceclofenac with peak areas of paracetamol) was considered for all the statistical determination.

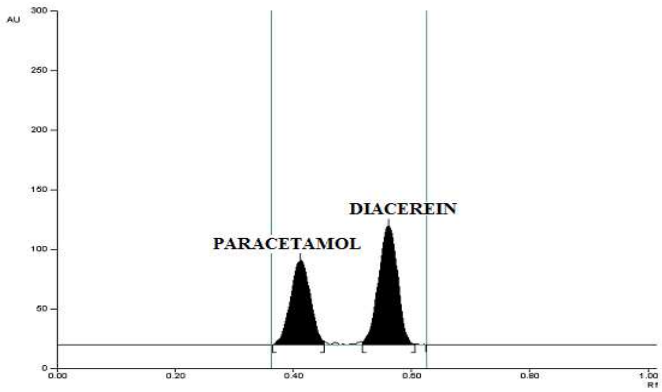

Figure 1. It shows HPTLC chromatogram of paracetamol 200 ng (0.41±0.03) and diacerein 150 ng (0.56±0.03)

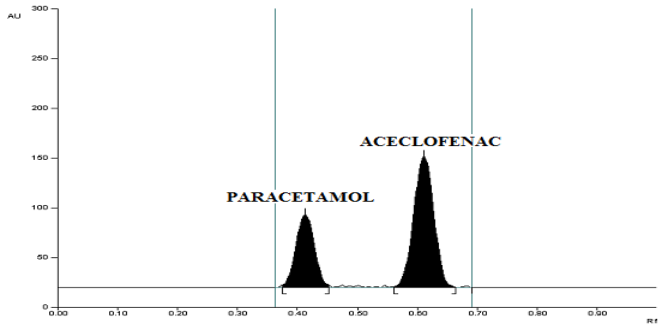

Figure 2. It shows HPTLC chromatogram of paracetamol 200 ng (0.41±0.03) and aceclofenac 300 ng (0.61±0.03)

Selection of scanning wavelength

After chromatographic development, bands were scanned over the range of 200-400 nm. Both drugs showed considerable absorption at 268 nm and hence the densitometric analysis was performed at 268 nm for all the measurements. Figure 3 shows overlay spectra of 200 ng diacerein and 400 ng aceclofenac.

Standard stock solutions

Aceclofenac 20 mg and diacerein 10 mg were weighed accurately on shimadzu balance (AUW-120 D) and transferred to a single centrifuge tube. To this powder mixture 7 mL of methanol (HPLC grade) was added and sonicated for 2 minutes to extract aceclofenac. The resulting mixture of solid and liquid was centrifuged for 5 min at 5000 rpm (Remi Centrifuge). The supernatant clear solution of aceclofenac was separated from residue of diacerein by filtration using whatman filter paper and made up the volume up to 10 mL with methanol. The residue of diacerein was extracted in sufficient dimethyl sulfoxide and made up the volume up to 10 mL with methanol. Finally the stock solution of diacerein 1mg/mL in dimethyl sulfoxide and methanol, aceclofenac 2 mg/mL in methanol were obtained. The paracetamol 1 mg/mL as internal standard in methanol was prepared.

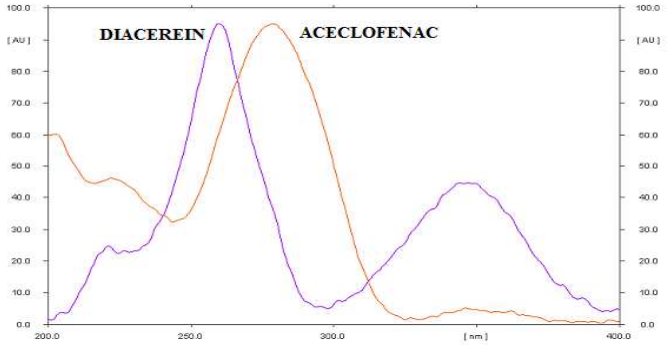

Figure 3. It shows overlay spectrum of diacerein 150 ng and aceclofenac 300 ng at 268 nm

Tablet sample preparation

Pharmaceutical preparation of diacerein and aceclofenac in combined tablet dosage form was purchased from local market. The contents of twenty tablets were weighed accurately on balance (Shimadzu AUW-120D) and finely powdered. A portion of the powder equivalent to 10 mg of diacerein and 20 mg of aceclofenac was accurately weighed and transferred to a single centrifuge tube. To this formulation powder added 7 mL of methanol (HPLC grade) was added and ultrasonicated for 10 minutes to extract aceclofenac and same procedure described in section of standard stock solution was followed. From this extracted contents 100 µg/mL and 200 µg/mL of diacerein and aceclofenac respectively were prepared using methanol as a solvent.

Method Validation

Linearity

From the separate stock solution of diacerein 100 µg/mL and aceclofenac 200 µg/mL, 0.50, 1.0, 1.5, 2.0. 2.5 µL were applied separately on TLC plates to obtain concentration range 0.05 to 0.25 µg/mL of diacerein and 0.1 to 0.5 µg/mL of aceclofenac. The concentration of paracetamol (IS) 0.2 µg/mL was constant for all bands. The calibration curve was developed by peak area ratio of respective drug and paracetamol versus the corresponding drug concentration. Regression coefficient and % RE was determined.

Accuracy

To study the accuracy of the proposed method, recovery experiments were carried out by addition of known amount of standard drug to the pre analysed tablet formulation, in 50%, 100% and 150% of label claim. At each level of amount three determinations were performed.

Precision

Precision of the method was determined with the formulation. An amount of the formulation powder equivalent to 100% of the label claim of diacerein and aceclofenac was accurately weighed and assayed. Method repeatability was assessed by repeating assay three times in a same day. Intermediate precision was assessed by the assay of three concentrations of three replicates on three consecutive days (Inter day Precision).

Limit of detection and limit of quantitation

The detection limit and quantitation limit was computed to assess lower limit of detection and minimum quantity of analyte measurable by proposed HPTLC method.

Robustness

Robustness of method was evaluated by small and deliberate changes in the method parameters like; time from chromatograph development to scanning (0 min, 15 min and 30 min) and in the time of plate activation (120^0C for 10 min, 20 min and 30 min). The effect of working area temperature (21^oC±1^oC, 23^oC±1^oC, 25°C±1^oC), mobile phase volume (12.5

mL, 12.8 mL, 13 mL) and its composition was also evaluated as the parameter of robustness studies.

RESULTS AND DISCUSSION

The proposed HPTLC method is based on the isolation of diacerein and aceclofenac from its combined tablet dosage form by solid liquid extraction procedure followed by densitometric determination without any interference of other analyte and excipients. Mobile phase solvent(s) selection is a critical area of separation technique (HPTLC). Often incomplete resolution, low or high retention and tailing can be blamed on the mobile phase composition. Many efforts made to simplify the components of mobile phase. The solid liquid extraction method is best alternative for isolation of those analytes having poor resolution or same retention factor on chromatographic developments. Mobile phase selection is a time consuming and tedious part of separation technique. The proper selection of mobile phase for good separation of targeted analyte and avoid interference of other analyte need to perform several trials with different solvents which increase the cost of analysis, but we have used extraction of targeted analyte from other components and not needed several trials for mobile phase selection, ultimately reduced the cost of analysis. Extraction method also reduced the time of mobile phase selection because isolated analytes were chromatograph separately. Correlation coefficient 0.9997 for diacerein and 0.9996 for aceclofenac were obtained. Table 1 and 2, shows the regression parameters of the calibration curve generated from each weighting factor (Wi*)* and the respective percentage relative errors (%RE).

Table 1. It shows regression parameters of the calibration curve generated for each weighting factor (Wi) and the respective relative errors (%RE) of diacerein

Model	Wi	b	a	R	% RE
1	1	0.0100	0.099	0.9997	1.12
2	$1/y^{1/2}$	-0.0032	1.3574	0.9483	24.09
3	1/y	-0.0060	1.7216	0.9093	32.96
4	$1/y^2$	-0.0120	2.6916	0.8368	34.80
5	$1/x^{1/2}$	-24.108	3.8110	0.9438	10.29
6	1/x	-111.00	2.6203	0.9044	44.71
7	$1/x^2$	-4096.11	2.0872	0.8302	214.91

Where, Wi – Weighting factor, b - Slope, a - Y-intercept, R - Correlation coefficient, % RE - % Relative Error, y - Response, x – Concentration

The best weighting factor is chosen according to a percentage relative error (%RE), which compares the regressed concentration (C_{found}) computed from the regression equation obtained from each Wi with the nominal standard concentration ($C_{nominal}$). The best result was found from unweighted factor (model 1).

$$\%RE = \frac{C_{found} - C_{nominal}}{C_{nominal}} \times 100 \qquad (1)$$

Table 2. It shows regression parameters of the calibration curve generated for each weighting factor (Wi) and the respective relative errors (%RE) of aceclofenac

Model	Wi	b	A	R	% RE
1	1	0.0050	0.121	0.9996	1.20
2	$1/y^{1/2}$	-0.0015	1.2627	0.9437	24.65
3	1/y	-0.0026	1.4985	0.9083	32.57
4	$1/y^2$	-0.0045	2.0298	0.8370	35.24
5	$1/x^{1/2}$	-39.2067	4.3810	0.9452	10.32
6	1/x	-258.0906	3.0408	0.9088	29.52
7	$1/x^2$	-19063.733	2.4216	0.8349	258.49

Where, Wi – Weighting factor, b - Slope, a - Y-intercept, R - Correlation coefficient, % RE - % Relative Error, y - Response, x – Concentration

On the basis of different weighting factors regression equation was computed but not found satisfactory. On the basis unweighting factor regression equation was found more satisfactory as compared to weighting factors. Average percentage recovery 100.14±1.15 for diacerein and 100.71±0.33 for aceclofenac were found (Table 3).

Table 3. It shows results for accuracy study of diacerein and aceclofenac in tablet formulation

S. No.	% Recovery*	Diacerein	Aceclofenac
01	50%	99.32 ± 1.41	100.32 ± 0.92
02	100%	99.34 ± 0.81	101.13 ± 1.41
03	150%	101.78 ± 0.24	100.69 ± 0.37
	Mean	100.14 ± 1.15	100.71 ± 0.33

*n=3, Mean ± Standard deviation

Method precision (% RSD) were 1.45, 1.10 (Intra day) and 0.84, 1.48 (Inter day) for diacerein and aceclofenac respectively (Table 4). The limit of detection 0.0064 µg/mL and limit of quantitation 0.0194 µg/mL of diacerein was found. The limit of detection 0.017 µg/mL and limit of quantitation 0.054 µg/mL of aceclofenac was found (Table 4). Small but deliberate changes in the composition of developing solvents, volume of developing solvent

did not affect the chromatograph determination.

Table 4. It shows results of robustness study, precision and limit of detection, limit of quantitation

S. No.	Parameters checked	Diacerein	Aceclofenac
		% R.SD of area	% RSD of area
1	Time from Chromatographic development to Scanning	1.47	1.26
2	Mobile phase composition	1.28	1.36
3	Volume of developing solvent used	1.54	1.45
4	Time and Temperature of plate activation	0.99	1.38
5	Effect of working area temperature	1.67	1.08
6	Precision		
7	Intraday*	1.45	1.10
8	Interday*	0.84	1.48
9	LOD	0.0064 µg/mL	0.0194 µg/mL
	LOQ	0.017 µg/mL	0.054 µg/ML

*average of nine determinations, LOD - limit of detection, LOQ - limit of quantitation

This method also remains unaffected by small changes in the time gap between chromatographic development and scanning of plate and time of plate activation. Even small change in the working area temperature did not show any changes in Rf values and peak areas of both the drugs. Required statistical parameters were evaluated for the determination of robustness and found satisfactory. The results of robustness study are reported in (Table 4). The proposed HPTLC method was found to be simpler and robust.

A solid-liquid extraction method supported by HPTLC determination of diacerein and aceclofenac in pharmaceutical solid dosage form has proved to be cost effective and robust. Solid–liquid extraction method plays an important role in separation of analytes on the basis of solubility. The extraction method is a best alternative for the separation of those analytes were not properly resolved. Determination of %RE by applying various weighting factors indicates that the unweighted factor (model 1) gives best suitable regression equation for statistical evaluation. No chromatographic interference from formulation excepients was found during the study.

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