Research Article

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DEVELOPMENT AND VALIDATION OF REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF DIASEREIN AND PARACETAMOL IN TABLET DOSAGE FORM.

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Abstract:

This research manuscript describes simple, sensitive, accurate, precise and repeatable reverse phase high performance liquid chromatography method for the simultaneous determination of Diaserein and Paracetamol in tablet dosage form. The sample was analyzed by reverse phase C_{18} column (Phenomenex C_{18}, 250 mm × 4.6 mm, 5µ) as stationary phase; acetonitrile : methanol : water (20 : 50 : 30 , v/v/v) as a mobile phase at a flow rate of 1.0 ml/min. Quantification was achieved with Photo Diode Array detector at 254 nm. The retention time for Diaserein was found to be 3.6 min and for Paracetamol was found to be 6.3 min. The linearity was obtained in the concentration range of 2-24 µg/ml for both Diaserein and Paracetamol. The method was successfully applied to tablet dosage form because no chromatographic interferences from formulation excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.

Keywords: Diaserein, Paracetamol, RP-HPLC, Photo Diode Array, Method Validation.

Introduction

Diacerein (DIA) [1], 4, 5-diacetoxy-9, 10-dihydro-9, 10-di oxo-anthracene-2-carboxylic acid, inhibits the stimulation of interleukin-1 beta production and production of nitrous oxide [2]. It also significantly reduces severity of pathological changes of osteoarthritis compared to placebo and increases the expression of transforming growth factor (TGF) - beta 1and TGF-beta 2, with, potential cartilage repairing properties. DIA does not alter renal or platelet cyclo-oxygenase activity and may therefore be tolerated by patients with prostaglandin-dependent renal function. DIA is involved liquid chromatographic-tandem mass spectrometry (LC MS/MS), flow Injection chemiluminescence method and spectrophotometric method [3-5]. Paracetamol (PARA) is an analgesic-antipyretic agent. It is effective in treating mild-to-moderate pain such as headache, neuralgia, and pain of musculo-skeletal origin [6]. Owing to widespread use of PARA in different kinds of pharmaceutical preparations, rapid and sensitive methods for the determination of PARA individual and in combination are being investigated. The most recent methods for determination of PARA include chromatographic [7–10], electrochemical [11–14], spectrophotometric [15–18] and fluorescence spectroscopic [19] techniques. There has been no report in literature on the simultaneous determination of DIA and PARA in tablets. The present work describes the development of validated RP-HPLC method, which can quantify these components simultaneously from a tablet dosage form. The proposed RP HPLC method was validated in accordance with ICH guidelines [20], by assessing its selectivity, linearity, accuracy, precision, and limits of detection and quantification

MATERIALS & METHODS

Apparatus

The chromatography was performed on a Shimadzu (Japan) RP-HPLC instrument (LC-2010C_{HT}) equipped with Photo Diode Array (PDA) detector and LC-solution software, Phenomenex (Torrance, CA) C_{18} column (250 mm × 4.6 mm id, 5µm particle size) was used as stationary phase. Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic cleaner (Frontline FS 4, Mumbai, India), Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad) were used in the study.

Reagents and materials

DIA and PARA standards were kindly supplied as a gift samples from Astron research limited, Ahmedabad, Gujarat, India. The tablet formulation (Lecerein P) containing 50 mg DIA and 500 mg PARA was obtained from local market. Acetonitrile, Methanol, triple distilled water (S. D. Fine Chemicals Ltd., Mumbai, India) used were of HPLC grade. Nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India) and Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

Chromatographic Condition

Stationary phase: C_{18} column (150 mm x 4.6 mm id., 5 µm).
Mobile phase: Acetonitrile: Methanol: Water (20: 50: 30, v/v/v)
Flow rate: 1.0 ml/min
Injection volume: 20 µL
Temperature: 40 °C
Detection: At 254 nm using PDA detector.

Preparation of Solutions

Preparation of standard stock solutions of DIA

Accurately weighed DIA (10 mg) was transferred to a 100 ml volumetric flask, dissolved in 10 ml DMSO and diluted to the mark with methanol to obtain a standard stock solution (100 µg/ml).

Preparation of standard stock solutions of PARA

Accurately weighed PARA (10 mg) was transferred to a 100 ml volumetric flask, dissolved in 10 ml DMSO and diluted to the mark with methanol to obtain a standard stock solution (100 µg/ml).

Preparation of Calibration Curve

Aliquots equivalent to 0.2, 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 ml working standard solution of DIA and 0.2, 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 ml working standard solution of PARA were transferred into a series of seven 10 ml volumetric flasks separately and volume was adjusted to the mark with solvent (1:9 DMSO: Methanol) to get concentrations 2, 4, 8, 12, 16, 20 and 24 µg/ml of DIA and 2, 4, 8, 12, 16, 20 and 24 µg/ml of PARA. 20 µl of each of the solution were injected into HPLC system and analyzed. Calibration curve was obtained by plotting respective peak area against concentration in µg/ml and the regression equation was computed.

Preparation of sample solution

The average weight of 10 tablets was determined and was ground in a mortar. An accurately weighed amount of powder equivalent to 50 mg of DIA or 500 mg of PARA was transferred to 100 ml volumetric flask, dissolved in DMSO (10 ml) and further diluted with Methanol (HPLC grade) and sonicate for 10 min. The content was filtered through Wattmann filter paper and made up to mark with methanol. This solution contains 500 µg/ml of DIA and 5000 µg/ml of PARA. From the above solution, 1 ml of solution was transferred to 10 ml volumetric flask and dilute up to mark with methanol (HPLC grade) to get the concentration of 50 µg/ml of DIA and 500 µg/ml of PARA. From the above solution, 0.4 ml of solution was transferred to 10 ml volumetric flask and dilute up to mark with methanol to get the concentration of 2 µg/ml of DIA and 20 µg/ml of PARA. An aliquot (20 µl) of sample solution was injected under the operating chromatographic condition as described above and responses were recorded.

Method Validation

The method was validated in compliance with ICH guidelines [20].

Accuracy (recovery study)

To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (50%, 100% and 150%). A known amount of drug was added to preanalyzed sample powder and percentage recoveries were calculated.

Method precision (Repeatibility)

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of DIA (12 µg/ml) and PARA (12 µg/ml) without changing the parameters. The results were reported in terms of relative standard deviation (% RSD).

Intermediate Precision (Reproducibility)

Precision of the method was determined by performing interday variation and intraday variation (%RSD). Intraday precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days. The results were reported in terms of relative standard deviation (% RSD).

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations

designated by International Conference on Harmonization (ICH) guidelines [20].

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and
S = slope of the calibration curve.

Robustness

The robustness was studied by analyzing the same samples of DIA and PARA by deliberate variations in the method parameters. The change in the responses of DIA and PARA were noted. Robustness of the method was studied by changing the extraction time of DIA and PARA from sample by ± 2 min, composition of mobile phase by ± 2 % of organic solvent, flow rate by ± 2 ml/min and column oven temperature by 40 ± 2°C. The parameters used in system suitability test were asymmetry of the chromatographic peak, tailing factor and theoretical plates, as %RSD of peak area for replicate injections.

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for DIA and PARA were obtained with a mobile phase comprising of acetonitrile: methanol: water (20: 50: 30, v/v/v) at a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 254 nm based on peak area. The peak with clear baseline was obtained (Figure 1). The retention time for DIA and PARA was found to be 3.6 min and 6.3 min, respectively (Figure 1). Linear correlation was obtained between peak area versus concentrations of DIA and peak area versus concentration of PARA in the concentration ranges of 2-24 µg/ml for both the drugs (Figure 2 & 3). The method was found to be specific as no significant changes in the responses of DIA and PARA were observed after 24 hrs. The mean recoveries obtained were 99.89 ± 0.79 % for DIA and 98.79 ± 0.69 % PARA which indicates accuracy of the proposed method. The % RSD value for DIA and PARA was found to be <2 %, which indicates that the proposed method is repeatable. The low % RSD values of interday and intraday variations for both the drugs reveal that the proposed method is precise. LOD value for DIA and PARA were found to be 0.1122 µg/ml and 0.1250 µg/ml and LOQ value for DIA and PARA were found to be 0.3367 µg/ml and 0.3751 µg/ml. These data show that the proposed method is sensitive for the determination of DIA and PARA.

CONCLUSION

A simple, sensitive, repeatable and specific RP-HPLC method has been developed for the simultaneous estimation of Diacerein and Paracetamol using a PDA detector. The method was validated for accuracy, precision, linearity, specificity, LOD & LOQ and robustness. In this proposed method the linearity is observed in the concentration range of 2-24 µg/ml for both the drugs with co-efficient of correlation (r^2) = 0.9975 for DIA and (r^2) = 0.9967 for DIA and PARA respectively at 238 nm. The result of the analysis of tablet by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drugs. The method can be used for the routine analysis of the DIA and PARA in pharmaceutical dosage form without any interference of excipients.

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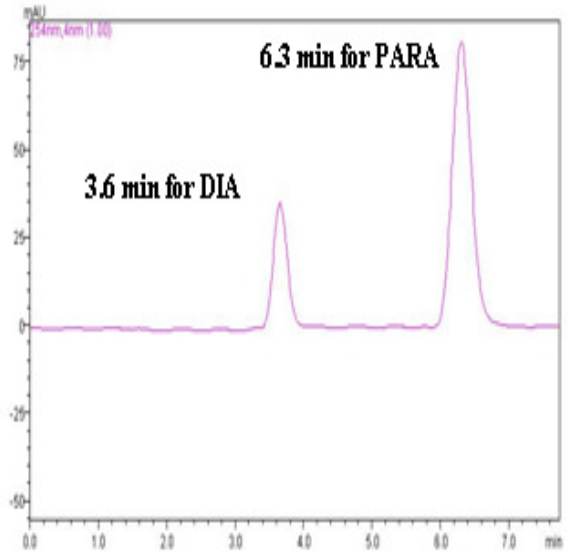

Figure 1: Chromatogram of DIA (10 µg/ml) and PARA (10 µg/ml) at 254 nm

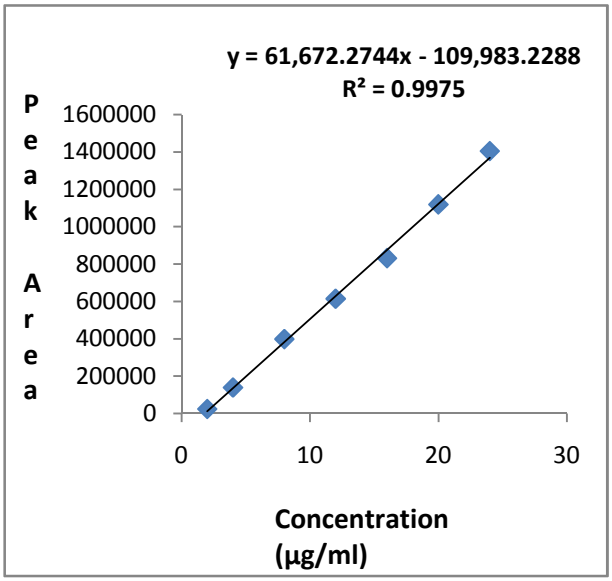

Figure 2: Calibration curve of DIA at 254 nm

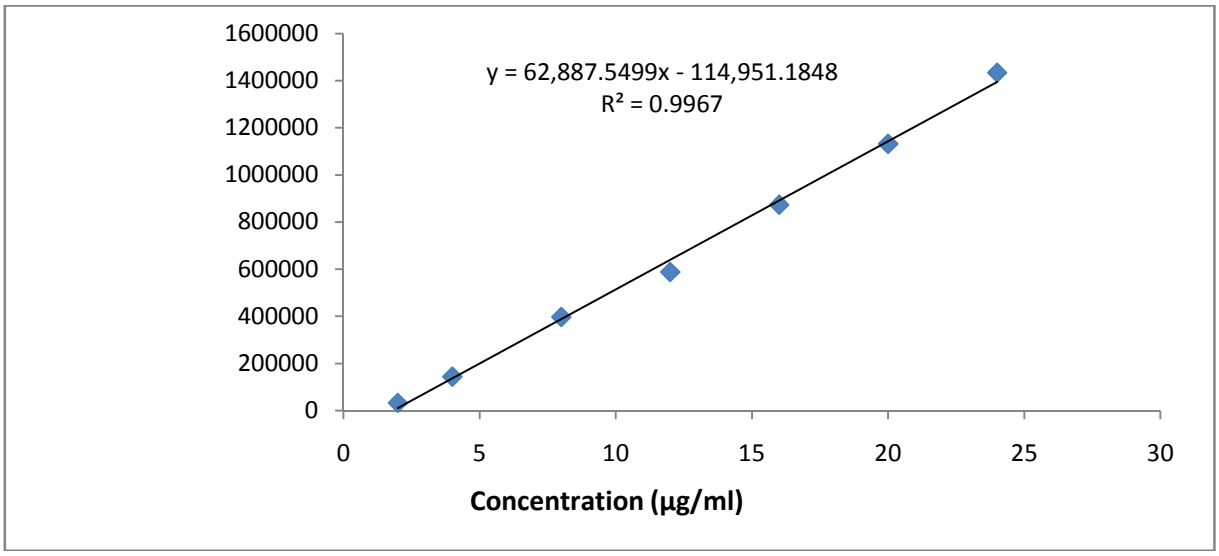

Figure 3: Calibration curve of PARA at 254 nm

Table 1 Regression analysis data and summary of validation parameter for the proposed RP-HPLC method

Parameters	DIA	PARA
Concentration Range (µg/ml)	2-24	2-24
Slope	61672	62887
Intercept	109983	114951
Correlation coefficient	0.9975	0.9967
LOD	0.1122	0.3367
LOQ	0.1250	0.3751
Accuracy	99.89 ± 0.79 %	98.79 ± 0.69 %
Repeatibility (% RSD, n=6)	0.82	0.75
Precision (% RSD) **Interday (n=3)** **Intraday (n=3)**	 0.58-1.08 0.10-0.46	 0.38-1.53 0.33-0.76

Table 2 Recovery data for the proposed method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± % RSD (n=3)
DIA	I	8	50 %	99.71 ± 0.76
	II	8	100 %	100.19 ± 0.75
	III	8	150 %	99.79 ± 0.87

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± % RSD (n=3)
DIA	I	8	50 %	99.71 ± 0.76
	II	8	100 %	100.19 ± 0.75
	III	8	150 %	99.79 ± 0.87

Table 3 System suitability test parameters for DIA and PARA for the proposed RP-HPLC method

Parameters	DIA ± % CV (n=6)	PARA ± % CV (n=6)
Retention Time (minutes)	3.655 ± 0.122	6.303 ± 0.085
Tailing Factor	1.223±1.195	1.13133± 1.2817
Theorical Plate	2280 ± 1.915	3623.5 ± 1.880
Ressolution	5.2943 ± 1.170	

Table 4 Analysis of tablet formulation of DIA and PARA by proposed RP-HPLC method (n = 3)

Formulation	Label Claim (mg)	Amount Found (mg)	% Label claim ± % RSD (n=3)
DIA	50	50.19	100.38 ± 0.67
PARA	500	498.91	99.78 ± 1.06