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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF DULOXETINE AND CURCUMIN IN DOSAGE FORMS

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ABSTRACT:
The main objective of the present work is to develop a simple, precise, specific and stability method indicating reverse phase high performance liquid chromatography method for the estimation of Metformin hydrochloride and Pioglitazone in tablet dosage form. The HPLC system was utilized for estimation and separation of drugs, CRM and DXH. While selecting mobile phase different compositions such as Methanol-water, acetonitrile-water, Methanol-PO4 buffer, Acetonitrile-PO4 buffer, Methanol-Ammonium Acetate buffer, Acetonitrile-Ammonium Acetate buffer, Acetonitrile-5% v/v glacial acetic acid in varying ratios (Alanazi et al., 2015), pH and flow rate were used. The developed method was validated as per ICH Q2 (R1) guideline (Ich, 2005). The performance of system was verified using system suitability parameters. Six replicates injections of standard solutions (6µg/mL) of both the drugs were injected to HPLC. The number of theoretical plates, peak asymmetry, height equivalent to theoretical plate, and resolution and peak purity index were also measured. The % drug loading for CRM was found to be 92% and for DXH it was found to be 93% in SNEDDS. Similarly, the assay of tablet was found to be 98.96%.
Key words: Duloxetine, Curcumin, RP-HPLC, validation, Method Development

INTRODUCTION

Duloxetine ((+)-(*s*)-*N*-methyl-3-(1-naphthyloxy)-3-(thiophen-2-yl)-propan-1-amine) is a selective serotonin and norepinephrine reuptake inhibitor used primarily in the treatment of major depressive disorders and stress urinary incontinence . Duloxetine is also used to treat pain and tingling in diabetic neuropathy. Duloxetine, also known as LY248686 , is a potent dual inhibitor of reuptake of serotonin (5-hydroxytryptamine) and norepinephrine; its effect depends on binding to human serum albumin. It is approved by the United States Food and Drug Administration for the treatment of diabetic neuropathic pain.

From the literature survey, it was found that there are some analytical methods reported for Curcumin either individually (or) in combination with other drugs by HPLC, HPTLC in raw drug, herbal extract and turmeric powder. Still now, no RPHPLC method is available for the estimation of Curcumin in pharmaceutical Dosage form. So an attempt is made to develop RP-HPLC method for the estimation of Curcumin in Poly Herbal formulation. A literature survey reveals a good number of analytical methods for the Duloxetine and Curcumin individually or in combination with other drugs using ultraviolet (UV) spectrophotometry,[6] high performance liquid chromatography (HPLC),[7] HPTLC,[8] and LC-MS/MS[9] Hence, we tried to develop a simple stability indicating HPLC method for the estimation of the selected drugs. The developed method has been validated as per the guidelines of the ICH.[10] To establish the stability indicating nature of the method forced degradation studies were planned for the proposed method under acidic, alkaline, oxidative, thermal, photostability, and neutral conditions.

METHODS AND MATERIAL

Chemicals and Reagents: Pure Duloxetine was gifted by Sun Pharma, Mumbai, India. and Curcumin were were purchased from Central Drug House (CDH), New Delhi, India. HPLC grade Acetonitrile and Methanol from Merck, AR grade ortho phosphoric acid from Qualigens, and AR grade Potassium Dihydrogen Orthophosphate from Emplura, UV-1100 Shimadzu, HPLC, Sonicator from PCI.

Instruments and equipment used

HPLC system consisted of a mobile phase delivery pump (LC-20 AD; Shimadzu, Japan), a photodiode array detector (SPDM20A; Shimadzu, Japan) was used for quantitative estimation of the drug. Magnetic Stirrer, REMI, India was used. Spray dryer, SprayMate, Jay Instruments and systems, Navi Mumbai, India was used in the study. Magnetic Stirrer, vortex mixer, REMI, India was used for mixing of solutions. Dissolution apparatus DS8000, Lab India, Mumbai, India was used to carry out release studies.

Simultaneous estimation of CRM and DXH on RP-HPLC

The HPLC system was utilized for estimation and separation of drugs, CRM and DXH. While selecting mobile phase

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different compositions such as Methanol-water, acetonitrile-water, Methanol-PO4 buffer, Acetonitrile-PO4 buffer, Methanol-Ammonium Acetate buffer, Acetonitrile-Ammonium Acetate buffer, Acetonitrile-5% v/v glacial acetic acid in varying ratios (Alanazi et al., 2015), pH and flow rate were used. Finally, based on the observations, acetonitrile - 5% v/v glacial acetic acid pH 2.35 in the ratio of 60:40 v/v, were used as mobile phase. The wavelength for detection was 289 nm and flow rate was 1.0 mL/min for simultaneous estimation of both the drugs (Kumar et al., 2017)

Preparation of standard stock solution

The stock solutions of CRM and DXH containing 1 mg/mL of each of the drugs, prepared separately by dissolving 100mg of their reference standards in 10mL of mobile phase [acetonitrile - 5% v/v glacial acetic acid pH 2.35 in the ratio of 60:40 (v/v)] in 100mL standard volumetric flask. Further the volume was adjusted to 100mL using mobile phase and diluting the same in suitable diluents (Kumar et al., 2017).

Preparation of sample solutions

SNEDDS (1mL) containing 30 mg of both the drugs, CRM and DXH and diluted to 100mL. In order to achieve concentration of 3 mg/mL further it is diluted to 100 mL to get 30µg/mL. From this, aliquot (2mL) withdrawn and diluted to 10 mL in order to get the concentration of 6µg/mL (Kumar et al., 2017).

Development of calibration curve

In order to prepare the calibration curve for CRM and DXH, aliquotes (10mL) was withdrawn from the standard stock solution and diluted to 100mL using mobile phase to achieve 100 µg/mL. Further, 10 mL of solution was withdrawn and diluted to 100 mL to get 10 µg/mL. From this, 2, 4, 6 and 8 mL of solutions were withdrawn to 10ml standard volumetric flasks and the volume was adjusted to 10ml in order to achieve concentrations of 2, 4, 6, 8, 10 µg/mL, respectively. The linearity of the method was assessed by analyzing various samples of CRM and DXH. About 25µL of each of the five working standard solutions were injected into the RP-HPLC. The elution was done as depicted above, and calibration curves were acquired by plotting the concentration of standard solutions versus peak area. The study was carried out five times and mean data was recorded (Kumar et al., 2017).

Validation of Method

The developed method was validated as per ICH Q2 (R1) guideline (Ich, 2005). The performance of system was verified using system suitability parameters. Six replicates injections of standard solutions (6µg/mL) of both the drugs were injected to HPLC. The number of theoretical plates, peak asymmetry, height equivalent to theoretical plate, and resolution and peak purity index were also measured (Kumar et al., 2018; Kumar et al., 2017).

Specificity studies

Specificity was evaluated by analyzing the chromatograms of placebo solutions of SNEDDS, working standard solutions containing mixture of CRM and DXH as well as SNEDDS sample solution containing mixture of CRM and DXH at concentration of 6 µg/mL. The placebo solution was prepared with the same composition as the SNEDDS formulation without the addition of drugs and treated in the same manner asthe sample solution and the reference solution (Kumar et al., 2018; Kumar et al., 2017) To this placebo solution, SFP solution (5 µg/mL) was also added.

Linearity and range

The calibration curve was developed by graph between mean peak area of five replicates versus corresponding concentrations of CRM and DXH, and the regression equation was recorded (Kumar et al., 2017).

Accuracy

It was developed through calculation of absolute recovery of the drugs from the mobile phase. The dilutions were made at three different levels i.e. lower quantified concentration [LQC (80%)], medium quantified concentration [MQC (100%)] and high quantified concentration [HQC (120%)] of method concentration (i.e. 6µg/mL). In order to carry out this, suitable aliquots of 4.8, 6 and 7.2 mL were withdrawn from 10 µg/mL of standard stock solution and transferred individually into 10 mL standard volumetric flask. Further 2 mL of aliquot was withdrawn from 30 µg/mL of sample stock solution and transferred to each of the volumetric flask that were containing LQC, MQC and HQC of standard stock solution. Finally, volume of each of the flasks (containing quantified concentration plus sample solution) was adjusted to 10 mL in order to achieve concentration of 10.8, 12 and 13.2 µg/mL. Percentage absolute recovery was calculated by dividing the actual recovery of drug to their theoretical concentration and multiplying them by hundred (eq.1). The study was carried out five times and mean data was recorded (Kumar et al., 2017).

$$\text{Absolute \% recovery} = \frac{\text{Actual concentration recovered} \times 100}{\text{Theoretical concentration}} \quad \text{(eq.1)}$$

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Precision

It was evaluated in terms of repeatability as well as intermediate precision. Repeatability was tested by injecting five times the samples of LQC, MQC and HQC on the same day and under same experimental conditions. The intermediate precision was evaluated by determining LQC, MQC and HQC samples five times on each of three different days (inter-day) as well as by the three different analysts (inter-analyst) under the same experimental conditions. The mean data was recorded and percentage relative standard deviation was (Kumar et al., 2017).

Robustness

This study was carried out by varying pH of the mobile phase (4.3, 4.5 and 4.7), flow rate (0.8, 1.0 and 1.2 mL/min) and ratio of mobile phase [58 (A):42 (B); 60 (A): 40 (B), and 62 (A):38 (B)], respectively. Five replicates of method concentration (6µg/mL) plus sample solution (i.e. 6 µg/mL) were injected and their effect on area of the peak, recovery and retention time was observed. The mean and percentage relative standard deviation was calculated (Kumar et al., 2018; Kumar et al., 2017).

RESULT AND DISCUSSION:

Method development on HPLC for simultaneous estimation of CRM and DXH

Different compositions of mobile phase were used for method development using HPLC. These used mobile phases were methanol-water, acetonitrile-water, methanol-phosphate buffer, acetonitrile-phosphate buffer, methanol-ammonium acetate buffer, acetonitrile-ammonium acetate buffer (Kumar et al., 2017), acetonitrile-5% v/v acetic acid in varying ratios, pH and flow rate. Among them the compositions having varying ratios of acetonitrile and 5% v/v acetic acid (pH 2.35) in various ratios at a flow rate of 1mL/min were able to provide acceptable chromatograms (Kumar et al., 2017).

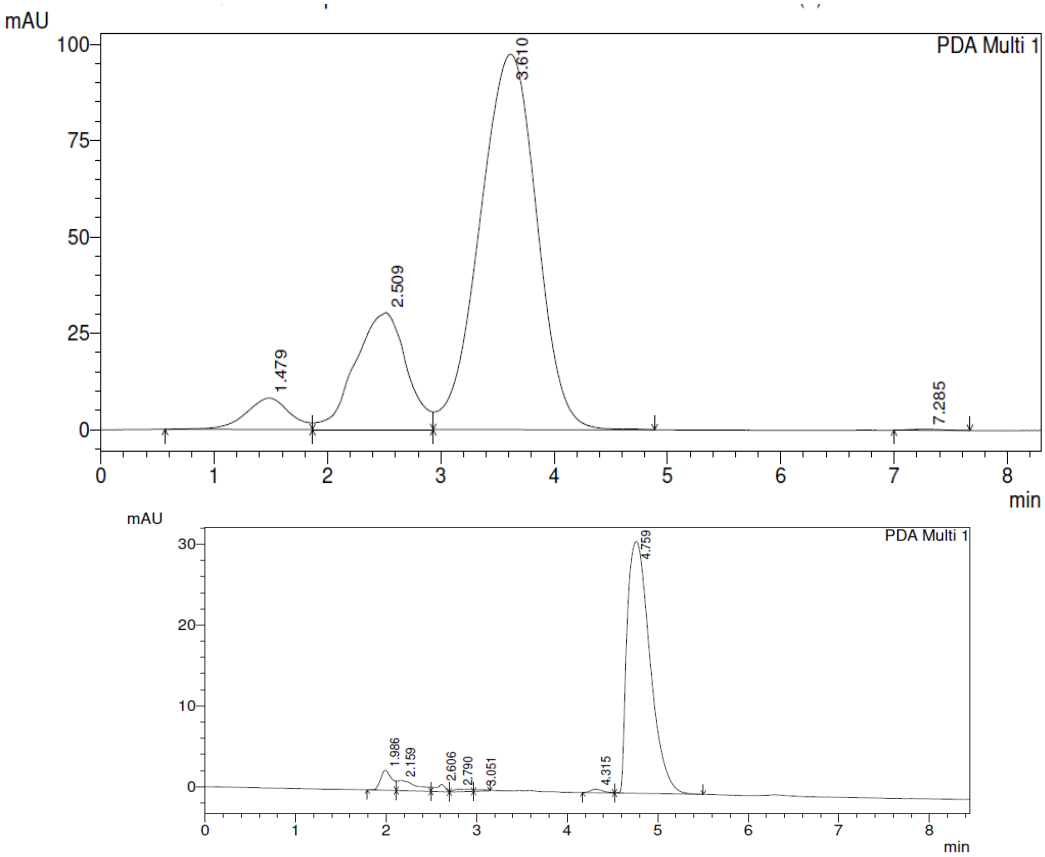

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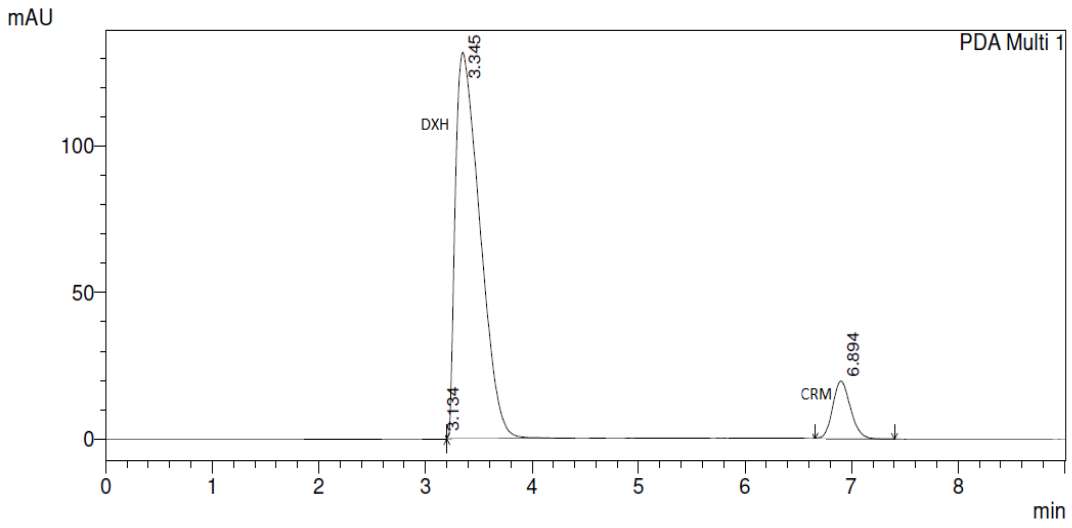
Fig.1. Chromatogram of a. CRM and b. DXH in acetonitrile (A) and 5% v/v acetic acid (pH 2.35) (B) at 50:50 v/v; c. CRM and d. DXH in A and B at 75:25 v/v; e. CRM and f. DXH in A and B at 85:15 v/v; g. CRM and h. DXH in A and B at 60:40 v/v; i. CRM and DXH in A and B at 60:40 v/v.

Hence, it was decided to develop chromatograms by fixing the flow rate and concentration of acetic acid 5% w/v as well as pH 2.35 and varying the volume ratio of acetonitrile and acetic acid. Results of some of the trials are given fig.20. In trial 1, CRM showed poor peak resolution and appearance of multiple peaks (Fig 20a.) in acetonitrile (A) and 5% v/v acetic acid (pH 2.35) (B) at 50:50 v/v, whereas, DXH showed tailing and broadened peaks (Fig 20b.). When the ratio of A and B was changed to 75:25 v/v, tailing was observed for CRM (Fig.20c), whereas, poor peak resolution was observed with DXH (Fig.20d). When the ratio of A and B was changed to 85:15 v/v, bifurcated peaks were observed (Fig 20e) for CRM whereas, peak broadening and peak tailing were observed with DXH (Fig 20f). Single peak with absence of fronting and tailing was observed for CRM (Fig 20g) and DXH (Fig 20h) at the ratio of 60:40 v/v for A and B (Kumar et al., 2017). Therefore, the optimum mobile phase consisting of A (60% v/v) and B (40 % v/v) was selected with an optimized flow rate of 1.0 mL/ min (Kumar et al., 2017).

Specificity studies

Specificity study was carried out by injecting placebo solutions, standard and sample solution of both the drugs (Fig.3). The chromatograms observed for the excipients (castor oil, tween-80, Transcutol®, SFP, CCS and talc) did not interfere with the chromatograms of CRM and DXH.



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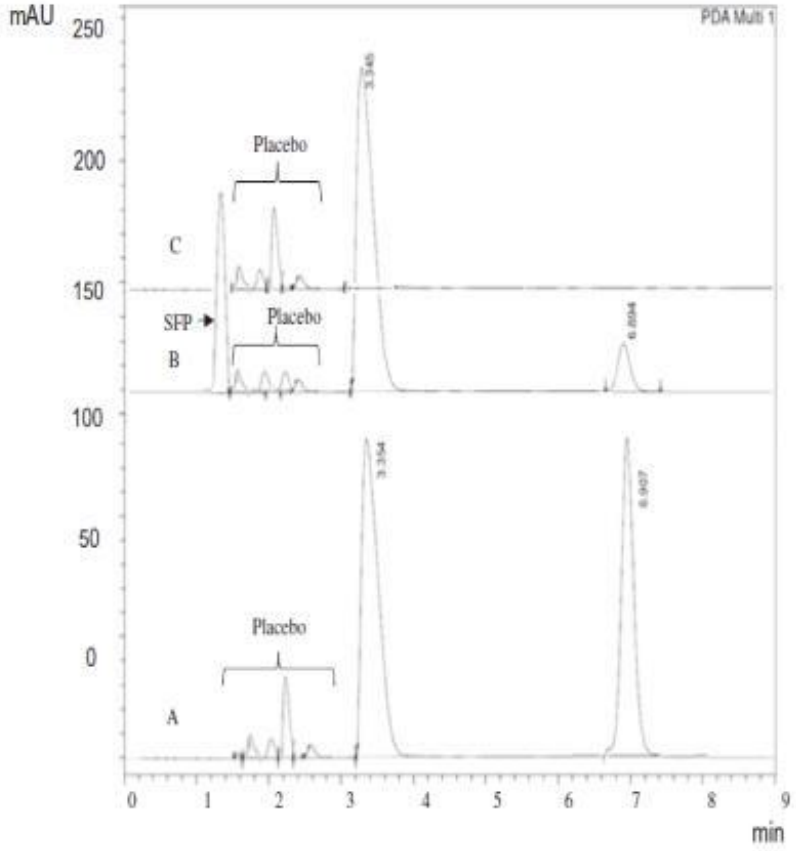

Fig.3. Chromatograms deciphering overlay of A. L-SNEDDS sample solution containing CRM and DXH; B. Spray drying of S-SNEDDS sample solution containing CRM and DXH using SFP as carrier; C. Placebo solution

Moreover, they were eluted out before the appearance of DX

H peak. Hence, the study deciphered that the developed method was specific for the determination of CRM and DXH (Kumar et al., 2017).

CONCLUSION

The present work involved the development of accurate, precise, simple and suitable RPHPLC method for estimation of the drugs in multicomponent formulations. Hence the present study was undertaken with an objective of developing suitable, sensitive and simple analytical method like RP-HPLC method for simultaneous estimation of both drugs in their combined dosage form. The proposed method is found to be accurate, precise, linear, specific and robust.

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