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STUDY TO RP-HPLC METHOD FOR DEVELOPMENT AND VALIDATION OF NAPUMETONE DRUG PRECISE PROCESS DETERMINATION

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Abstract: Since there are a growing number of drugs and drug combinations available on the market, it is necessary to create analytical instruments for quality control, which makes NSAIDs a very popular class of therapy for pain and inflammation. It is necessary to design the procedures in a way that minimizes design time while yielding the most reliable and accurate results. The USFDA-approved solid oral dosage forms of naproxenone are used in the current study's combination dose form. Creating a new analytical technique that will improve analysis's accuracy, cost, and time consumption. The measurement of nabumetone (NAB) in prescription medications using a precise and accurate FIA technique is explained. Based on the most effective carrier solvent solution, which was methanol:water (35:65% v/v), the developed method was implemented. This solvent was used to produce the sample solution (4.7X10-6 M NAB), which was then injected into the instrumental system at a flow rate of 1 ml/min. The drug was quantified on an Agilent Zorbax Bonus RP (250 x 4.6 mm, 5µ) column using a mobile phase consisting of methanol: water (35:65, % v/v) at a flow rate of 1 ml/min. The signals were detected using a UV detector at 270 nm RP-HPLC leave-taking. Using a DAD detector at 200–400 nm, quantification was achieved based on peak area and linear calibration curves for drug concentrations between 60 and 140 µg/ml. Nabumetone had a retention period of 2.88 minutes. The process had been useful for creating medicinal formulations one after the other. There was no introduction of chromatographic incursion from the tablet components. The linearity, recovery, and specificity of the devised approach were confirmed. In accordance with ICH rules, the intra- and inter-day precision and accuracy results fell within the recognition range.

Keywords: Nabumetone, NSAID, RP-HPLC, Method validation.

1. INTRODUCTION

Nabumetone [1] is non acidic prodrug, chemically, 4-(6-methoxy-2-naphthyl)-2-butanone [1], NSAID of 6-Phenylhexanoic acid group accustomed to contend ache and inflammation induced by rheumatism.[1] The literature survey discloses that colorimetric
[3] and some chromatographic systems had been reported for the analytical assay of nabumetone in various fluids like biological fluids and in medicinal preparations besides alone or other drugs. [5-6]

According to preceding have a look at Non-Steroidal Anti-Inflammatory Drugs (NSAIDs),[2] Nabumetone's is an energetic metabolite which constrains the cyclo-oxygenase enzyme favorably blocks the interest of COX-2 (Cyclo-Oxygenase - 2).During arthritis, cyclo-oxygenase-2 complements the charge of manufacturing of endoperoxides and prostaglandins E-2 and I2 (prostacyclin) that is not directly accountable for extreme ache withinside the patients. Nabumetone (NAB) comes beneath neath abrand-new elegance of non-steroidal anti-inflammatory drug which display much less capacity for GI sheath irritancy and inhibitsthe feature of platelet. It suggests much less effect now no longer simplest on nephric bradykinin emission however additionally on congestive coronary heart failure (CHF) compared to different antique pills of this elegance.[4]

There are a few articles to be had describing investigative tactics for NAB. This evaluation paper describes the easy, specific and sensitive HPLC approach that is recuperated and attested(documented) technique for estimation of Nabumetone as in step with ICH(International Conference on Harmonization) guidelines. Here, we evaluation provided novel approach for the decisiveness of Nabumetone which makes use of a totally reasonably-priced solvent device on a Waters ODS C18 analytical column. This form ofdevice primes to stepped forward preservation, very serrated and proportioned height figures and shows excellent selectivity for Nabumetone.[7]

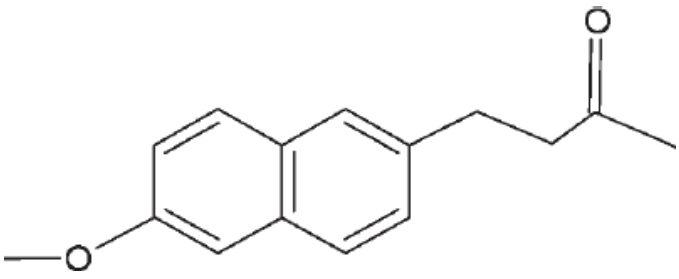

Figure 1. Structure of Nabumetone

Analytical methods are classified as non-instrumental and instrumental. Different instrumental methods involve spectroscopy, chromatography, mass spectroscopy, Calorimetry, microscopy, electrochemistry, environmental analysis, forensic, crystallography,etc. The process of chromatography is applied for the

application of separation, purification of the test substances. The mixture which is to be applied for separations is placed on a stationary phase (solid or liquid), and a pure solvent which includes water or any kind of gas is permitted slowly to travel over the stationary phase, transporting the components separately according to their solubility in the pure solvent. Chromatography differs from most other physical and chemical separation processes in that two mutually incompatible phases are brought into contact; one is stationary and the other is mobile. A sample is conveyed through a column (manifold) containing a dispersed stationary phase after being put into a mobile phase. The ever-increasing number of medications and drug combinations on the market necessitates the development of analytical tools for monitoring their quality. Theprocedures must be developed in such a way that they take less time to design and produce the most accurate and robust results possible. The current study dosage form comprises nabumetone in solid oral dosage forms, which was recently approved by the USFDA. The goal of this project is to develop and validate a simple, precise, accurate, and cost-effective RP-HPLC technique for the estimation of nabumetone in bulk and pharmaceutical dosage forms that follows ICH recommendations.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

The Nabumetone changed into procured from Aadhar Life Sciences Pvt. Ltd. Other analytical reagents encompass orthophosphoric acid (OPA) of HPLC grade changed into bought from the Avantor Performance cloth India Ltd. Thane, Maharashtra. MEOH and water of HPLC grade have been bought from the Merck Specialities Pvt. Ltd., Shiv Sager Estate 'A' Worli, Mumbai. Analytical grade reagents and chemical substances have been used on this study. Fixed dose aggregate used in this test grow to be Niltis P tablets (Ipca laboratories Ltd., India) categorized to comprise 500 mg of Nabumetone.

2.2. Selection of wavelength:

The pattern changed into scanned from 200-400 nm with DAD detector. The Wavelength decided on for evaluation selected changedinto 270 nm on foundation of suitable depth of Nabumetone.

2.3. Instrumentation and Chromatographic Conditions:

Agilent 1260 Infinity II system consisting of Compatible with conventional and ultrahigh performance LC: 1260 Infinity II Quaternary Pump and 1260 Infinity Binary Pump operate up to 600 bars, a perfect match for our Infinity Lab Poroshell 120 columns.The peaks were quantified by means of PC based Lab Advisor software. A reverse phase Agilent Zorbax Bonus-RP (250 x 4.6 mm,5 µ) column equilibrated with mobile phase Methanol: Water (35:65 % v/v) was used. Mobile phase flow rate was maintained at 1ml/min and effluents were monitored at 270 nm. The temperature of the system was maintained at 30^0 C. The

sample was injected using a 10 µl fixed loop, and the total run time was 5 min.

2.4. Preparation of Standard Stock Solution

Prepare a Standard Stock Solution (SSS-I) of by adding 10 mg of Nabumetone in 10 ml volumetric flask & add 5 ml diluent (methanol: water, 50: 50 %v/v), mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Nabumetone = 1000 µg/ml). Then add 1.0 ml of ASSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent.(Conc. of Nabumetone = 100 µg/ml).

2.5. Drug Product Sample Preparation for Assay: [Tablet Sample Solution (TSS)]

10 Tablets were weighed and average weight was calculated and Tablets was crushed & mixed in mortar and pestle. Powder weightequivalent to 10 mg Nabumetone was weighed into 10 ml volumetric flask & add 5 ml diluent, sonicate for 10 minutes and make the volume to 10 ml with diluent. (Conc. of Nabumetone = 1000 µg/ml).

2.6. Calibration of standards:

The mobile phase and stationary phase were allowed to equilibrate until OPA by baseline was achieved. Pipette 10 mg nabumetoneinto a 10 ml volumetric flask from the freshly made standard stock solution. It was then diluted with the mobile phase. To reach the final concentration, 0.6, 0.8, 1.0, 1.2 and 1.4 of the solution were pipette out into a 10 ml volumetric flask, and volume was brought up to 10 ml with the mobile phase. Nabumetone (60, 80, 100, 120 and 140 g/ml). Samples were injected and peaks were recorded at 275 nm, as shown in the graph plotting drug concentration versus peak area.

2.7. Validation of A Method for Analysis of Nabumetone:

Specificity & Assay:

Individual samples of Nabumetone were prepared of 100 µg/ml and peaks were for identified from Retention Time. Blank wasinjected to ensure there is no blank peak interfering with the main analyte peaks. (Table No. 1)

Linearity:

The mathematical treatment of test findings acquired by analysis of samples with analyte concentrations across the specified rangedetermines the analytical method's linearity. The area as a function of analyte concentration is visually shown. Fittings of percentage curves are calculated.

Accuracy (recovery):

A known amount of analyte is added in the system is used for the analysis of the accuracy. The percentage of analyte recovered by the assay is used to compute the accuracy from the test findings.

Precision:

Intra-day precision:

Sample solutions containing 10 mg of nabumetone three different concentration (80 µg/ml, 100 µg/ml, 120 µg/ml) nabumetone. Nabumetone were determined three times on the same day and %R.S.D. was calculated.

Inter-day precision:

Sample solutions containing 10 mg of Nabumetone three different concentration (80 µg/ml, 100 µg/ml, 120 µg/ml) nabumetone. Nabumetone were determined three times on the same day and %R.S.D. was calculated.

Detection Limit:

By using the below formula, it can be calculated

$$DL = \frac{3.3\sigma}{S}$$

Where, σ = the S.D. of the y-intercepts of regression lines. S = the slope of the calibration curve.

The calibration curve can be used to estimate the slope S and S.D. was used should be calculated from the y-intercepts of theregression line in the calibration curve.

Quantitation Limit:

It was calculated by the below formula.

$$QL = \frac{10\sigma}{S}$$

Where, σ = the S.D. of the y-intercepts of regression lines. S = the slope of the calibration curve.

The calibration curve can be used to estimate the slope S and S.D. was used should be calculated from the y-intercepts of the regression line in the calibration curve.

3. RESULTS AND DISCUSSION:

3.1. Specificity:

Specificity for the nabumetone as comparing to marketed formulation (Niltis 500mg Tablet, Ipca Laboratories Pvt. Ltd.) was foundas mentioned in the following table no. 01 and mentioned figures 02 & 03. The % Assay of nabumetone for this developed techniquewas found to be 97.50.

Table No. 1: Specificity and Assay:

Sample (API Name)	**Nabumetone**
Standard Solution	1525414
Marketed Formulation	1487272
% Assay	97.50

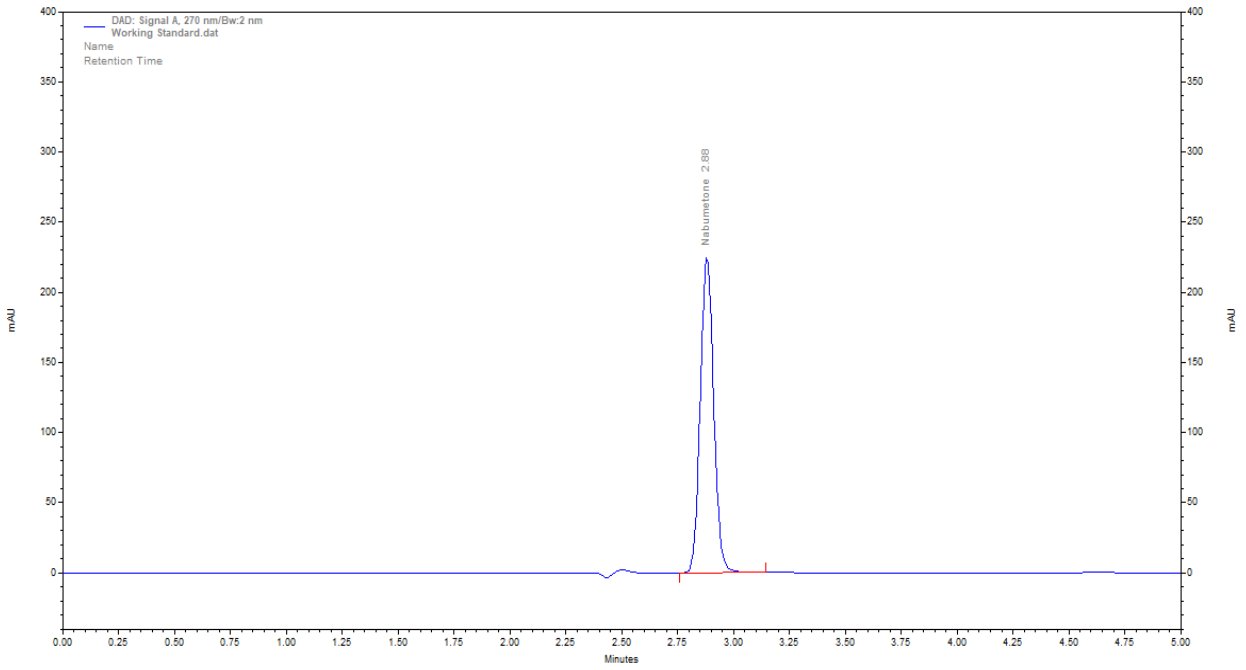

Fig. 02: Chromatogram of Working Standard

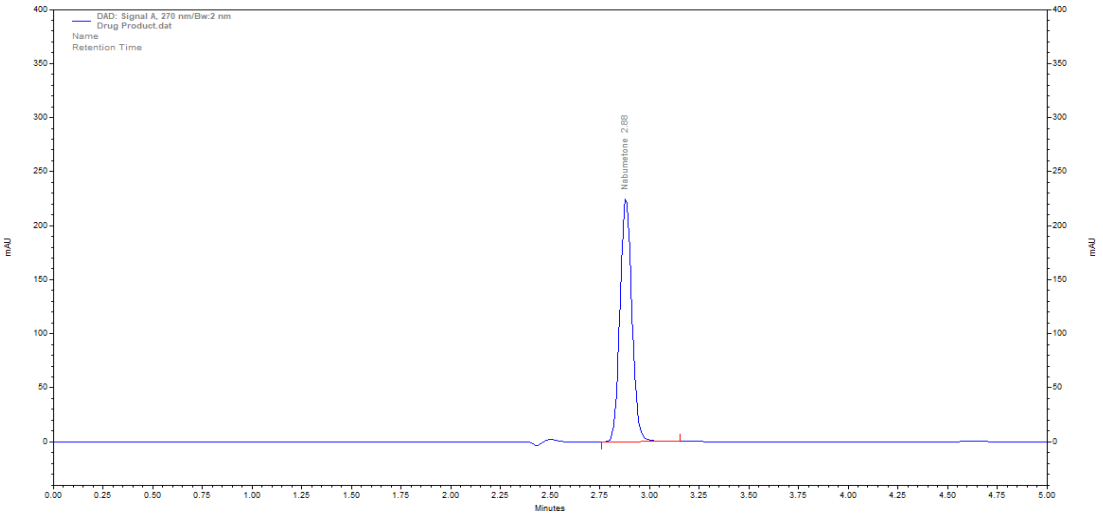

Fig. 03: Chromatogram of Drug Product

3.2. Linearity:

The data revealed a linear association between peak areas and concentrations in the ranges of 2-140 ug/mL for Nabumetone. The linear equation for Nabumetone was y = 15262x - 23.3, where x represents the concentration of the drug and y represents the peakarea. The correlation coefficient was 1.000 and the calibration curve of Nabumetone is depicted in figure 4. Linearity data for the Nabumetone is represented in table no. 02.

Table No. 02: Linearity and range:

% Conc	Conc (ug/ml)	Nab AREA
60%	60.00	914382
80%	80.00	1224309
100%	100.00	1525733
120%	120.00	1827682
140%	140.00	2138925

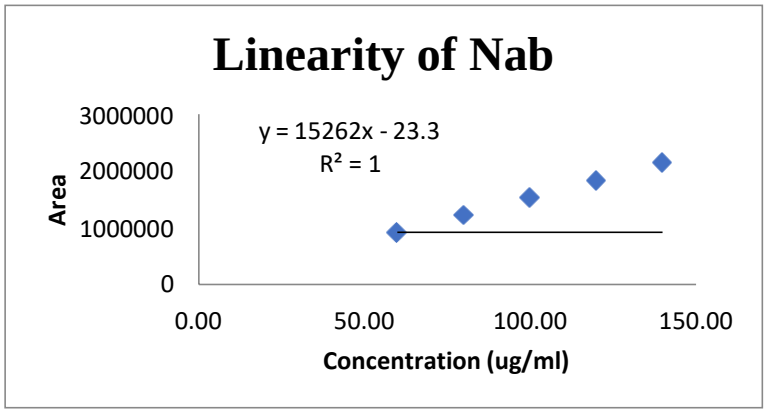

Fig. 04: Calibration curve (linearity) of nabumetone

3.3. System Suitability:

The system suitability was assessed by six replicate injections of the mixture containing as internal standard. The resolution, peak asymmetry, number of theoretical plates and HETP were calculated are represented in table no.03.

Table No. 03: System Suitability:

Sr. No.	Parameters	Nabumetone
1	Theoretical Plates	23509
2	Retention Time	2.88
3	Asymmetry factor	1.01

3.4. Accuracy:

Validation of Recovery Studies which is done statistically is represented in table no. 04 which shows the effects of Nabumetone. Recovery studies were carried out to ensure that the developed approach was accurate. The solution which is previously analyzed means the specific standard drug concentration 80, 100, and 120 percentages were mixed and then allowed for the recovery analysis.Recovery experiments at various concentration levels are used to verify the accuracy of the RP-HPLC and UV Spectrophotometric methods. The recovery rate was determined to be between 98 and 101%.

Table No. 04: Accuracy of Nabumetone:

Level of Recovery	Sample No. (ug/ml)	Spiked Amount	Spiked Amount wrt Sample	Area ATV	Amount Recovered (ug/ml)	%Recovery	% RSD
80%	Reps1(80)	79.76	79.76	1229830	80.38	100.78	0.45
	Reps2(80)	79.76	79.76	1224309	80.02	100.33	
	Reps3(80)	79.76	79.76	1218782	79.66	99.87	
100%	Reps1(100)	99.7	99.70	1528732	99.92	100.22	0.10
	Reps2(100)	99.7	99.70	1525733	99.72	100.02	
	Reps3(100)	99.7	99.70	1527638	99.85	100.15	
120%	Reps1(120)	119.64	119.64	1828782	119.53	99.91	0.05
	Reps2(120)	119.64	119.64	1827682	119.46	99.85	
	Reps3(120)	119.64	119.64	1826783	119.40	99.80	

3.5. Precision:

Intraday and inter-day precision investigations on the RP-HPLC method for nabumetone demonstrate high precision percent amounts ranging from 97 to 101 percent, indicating an analytical procedure that was concluded. Table no. 05 shows the results of intraday and inter-day precision experiments on the RP-HPLC technique for Nabumetone.

Table No. 05: Precision for Nabumetone

Instrument Precision	Peak Area
Parameter	**ATV**
Rep 1(50 ug/ml)	1529432
Rep 2(50 ug/ml)	1527204
Rep 3(50 ug/ml)	1527273
Rep 4(50 ug/ml)	1524395
Rep 5(50 ug/ml)	1520934
Rep 6(50 ug/ml)	1523246
Average	**1525414**

SD	**3118.117701**
%RSD	**0.20**

3.6. Limit Detection:

Depending on the standard deviation of response and slope, the limit of detection means LOD is detected. The LOD is the lowest limit that can be detected. The value of LOD of Nabumetone was observed as 0.613600684 (ug/mL), the analytical method that concluded.

Limit of detection = 3.3X6032.658809/32444.185 =0.613600684 (ug/mL)

3.7. Limit Quantification:

The LOQ is that the lowest concentration which will be quantitatively measured. The value of LOQ for Nabumetone was observedas 0.613600684 (ug/mL) for the concluded method.

Limit of Quantitation = 10X12.80/177.8=1.85939601 (µg/mL).

3.8. Analysis of Tablet Formulation:

The quantity of Nabumetone in each tablet was estimated by the extrapolation method by taking the value of area from the calibration curve. The process of Analysis procedure was done repeatedly about five times by using tablet formulation. For calculation of % Label claim and %RSD, tablet assay was estimated. In Table no. 06 and figure 05, results are represented. Brand Name Niltis tablet 500 mg (IPCA laboratories) was used for the analysis. The average weight of the tablets was 1.5328 gms /Tab. Its equivalent weight for 10 mg nabumetone would be 100X 1.5328 / 500 =0.0304 grams. Hence, 167.6 mg in 10 ml water was taken and sonicated 10 min. Analysis of marketed formulation was analyzed and % Label Claim was observed as 100-101% whichwas concluded satisfactorily.

Table 06: Analysis of marketed formulation:

Assay	drug	mg	potency	% Label Claim	S.D.	%R.S.D.
RP-HPLC Method	Nabumetone	10	97.5	97.50	3118.117701	0.20

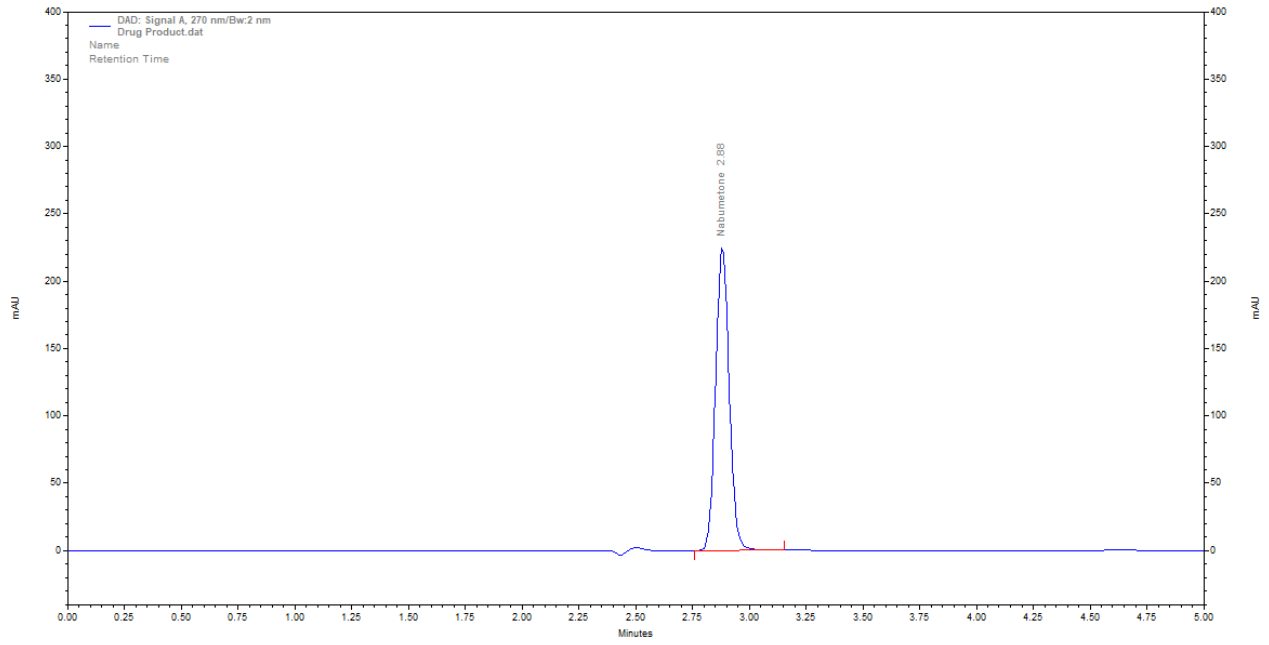

Fig. 05: Chromatogram for Marketed Formulation

4. CONCLUSION:

The developed new method proved to be simple in procedure and it produced more accurate results. Hence, the methods effective for the routine analysis of nabumetone in bulk and tablet dosage form. The simple, accurate and sensitive validated RP-HPLC method for simultaneous determination of Nabumetone has been developed. The method may be recommended for routine and quality control analysis of the investigated drugs in pharmaceutical formulations. The data demonstrate that the RP-HPLC methodwe have developed showed acceptable linearity, specificity, accuracy, precision and LOD & LOQ in the concentration range of 2-140 µg/ml for Nabumetone as per the requirement of ICH guidelines. In this study, stability of drug was established according to ICH-recommended stress conditions. In conclusion, the proposed method could be routinely used for the analysis of drug in pharmaceutical dosage form.

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