World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
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Available online at: http://www.wjpsonline.org/
Original Article

Development and validation of analytical method for simultaneous estimation of diclofenac sodium and benzocaine in gel dosage form

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Received: 29-04-2015 / Revised: 18-05-2015 / Accepted: 20-05-2015

ABSTRACT

The present work involves the development and validation of RP-HPLC method and –UV Visible Spectroscopic method for the estimation of Diclofenac Sodium and Benzocaine in gel dosage form. A specific, precise and selective RP-HPLC method has been developed and validated using Zodiac C18 (150mm x 4.6mm i.d., 5µm). 0.1 % Glacial acetic acid in water: Acetonitrile (35:65) was selected as mobile phase, Flow rate 1.0 ml/min and detector wavelength was 243 nm. The retention time of Diclofenac Sodium and Benzocaine were observed at 6.58 min and 3.68 min respectively. The linearity range was found to be 0.6-4.2 µg/ml and 4-28 µg/ml for Diclofenac Sodium and Benzocaine respectively. The mean recovery was found to be 100.78% and 99.98% for Diclofenac Sodium and Benzocaine respectively. The developed methods were economic, precise and specific. The developed Ratio derivative spectroscopic method using pH 6.8 Phosphate buffer as solvent. Benzocaine and Diclofenac Sodium has absorbance at 321.5 nm and 233.5nm respectively The linearity was obtained in concentration range 0.6-3.0 µg/ml and 4-20 µg/ml for Diclofenac Sodium and Benzocaine respectively for all UV-Visible method. The mean recovery was 101.25% and 100.52% was obtained for Diclofenac Sodium and Benzocaine respectively.

Key words: Diclofenac Sodium, Benzocaine, RP-HPLC, Ratio Derivative Spectroscopic methods, Validation, LOD and LOQ

INTRODUCTION

Diclofenac sodium (DCS), chemically, sodium 2-[(2,6-dichlorophenyl)-amino] Phenylacetate is a non-steroidal anti -inflammatory agent (NSAID) with antipyretic and analgesic actions. Diclofenac sodium is official in I.P., B.P. and U.S.P. Diclofenac sodium inhibit both leukocyte migration and the enzyme cylooxygenase (COX-1 and COX-2),. As prostaglandins sensitize pain receptors, inhibition of their synthesis is responsible for the analgesic effects of Diclofenac. Antipyretic effects may be due to action on the hypothalamus, resulting in peripheral dilation, increased cutaneous blood flow, and subsequent heat dissipation. **Benzocaine** (BENZ), chemically, ethyl 4-aminobenzoat is a local anesthetic. Benzocaine is official in I.P., B.P., and U.S.P. Benzocaine used as local anesthetic in topical preparation. HPLC is a separation technique in which sample is separated into its analyte by distributing between stationary phase and mobile phase and forced degradation study carried out to detect primary degradent of products. Literature review reveals that methods reported for estimation of Diclofenac Sodium and Benzocaine as single component. [5-14] there was no reported HPLC method for Diclofenac Sodium and Benzocaine in combination. It is worthwhile thought to develop and validate HPLC methods for Diclofenac Sodium and Benzocaine in gel Dosage form

EXPERIMENTAL WORK:

HPLC Method development

Material: Diclofenac Sodium API was procured as gift sample from Centurion ltd., Vadodara. Benzocaine API was procured as gift sample from ICPA Health care, Ankleshwar. Gel dosage form was available from local market. Acetonitrile was available from. Acetonitrile and Glacial acetic acid obtained from Merk. All the chemicals were HPLC grade. Milli –Q-water was used throughout the experiment.

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Equipment: The waters HPLC system (shimadzu) with UV detector was used for method.

Chromatographic condition: The selected and optimized mobile phase was0.1% Glacial acetic acid: Acetonitrile (35: 65 v/v). The mobile phase was filtered 0.45 µm filter paper, mixed properly and degassed by sonication. The column used was Zodiac C18 (150mm x 4.6mm i.d., 5µm). The detector was set at 243 nm. The injection volume was 20 µl. The elution was carried out in isocratic mode with flow rate 1 ml/min. is given in table 1.

Preparation of standard solution: Standard Stock solution of 1000µg/ml of Benzocaine and 150 µg/ml of Diclofenac Sodium was prepared. From this 1 ml was transfer into 10 ml volumetric flask and make up the volume upto mark with diluent to make standard working solution of 10 µg/ml and 400 µg/ml of Benzocaine and Diclofenac Sodium.

Assay of pharmaceutical dosage form (sample preparation): Accurately weighed 50mg of gel transferred to a 10mL volumetric flask and dissolved in methanol with aid of ultra-sonication. Make up the volume to 50 ml with methanol. The solution was ultrasonicated for 20 minutes and filtered through 0.2µ membrane filter. The solution was further diluted to obtain the conc. 20 µg/ml of BENZ and 3.0 µg/ml of DIC

Method development: Ratio Derivative Spectrophotometric

Instrument: Instrument use was an UV Visible double beam spectrophotometer, make: SHIMADZU (model UV-1800)

Preparation of standard stock solutions:
Accurately Weighed 50 mg of Benzocaine and transferred into 50 mL volumetric flask and dissolved in pH 6.8 Phosphate buffer using ultra sonication and diluted up to mark to give a stock solution having concentration of 1000 µg/mL. Accurately Weighed 50 mg of Diclofenac Sodium and transferred into 50 mL volumetric flask and dissolved in pH 6.8 Phosphate buffer using ultra sonication and diluted up to mark to give a stock solution having concentration of 1000 µg/mL

Preparation of working standard solutions: 5ml of stock solution was taken from 50 mL volumetric flask and diluted to 50ml with pH 6.8 Phosphate buffer this resulting solution of 100 µg/mL. 5ml of stock solution was taken from 50 mL volumetric flask and diluted to 50ml with pH 6.8 Phosphate buffer this resulting solution of 100 µg/mL

Preparation of calibration curve: From the working standard solution, appropriate dilutions of BENZ in the range of 4-20 µg/mL and DIC 0.6-3.0 µg/mL were prepared for ratio derivative spectrophotometry method.

RESULT AND DISCUSSION:

Method development: Chromatographic parameters were preliminary optimized to develop HPLC method for estimation of Benzocaine and Diclofenac Sodium .243 nm was selected as detection wavelength as both drugs shows good absorbance at this wavelength**.** Column was ZODIAC C_{18} column (250 mm x 4.6 mm, 5 µm). Mobile phase was mixture of phosphate buffer (pH 3 with 0.05% OPA) and Acetonitrile in isocretic elution at flow rate of 1.0 ml/min. chromatogram is shown in figure no.1

System suitability: System suitability parameters were calculated for standard solution. Chromatogram is shown in figure no 1 and data shown in table 1.

Ratio Derivative Spectroscopic method
Instrument Conditions
Mode: Spectrum
Scan speed: Fast
Wavelength range: 200-400 nm
Wavelength accuracy: ± 0.3 nm
Spectral bandwidth: 0.5 nm
Absorbance scale: 0.00A – 4.00A
Initial base line correction: 6.8 pH phosphate buffer
Method validation for HPLC
Specificity: Specificity was performed by injecting blank, standard and sample solutions. Chromatograms are shown in figure no 1 and 3

Linearity and Range: The linearity of analytical procedure is its ability to obtained test results which are directly proportional to the concentration of analyte in the sample. Seven Concentration ranges of 4-28µg/ml of Benzocaine and 0.6-4.2 µg/ml of Diclofenac Sodium were prepared and analyzed. Results have been shown in table 2 and Overlay spectra have been shown in fig. no 2 and calibration curve has been shown in figure no 4 and 5 for Diclofenac Sodium and Benzocaine respectively.

Precision: For repeatability six replicates of concentration 20µg/ml of standard solution of Benzocaine and 3.0 µg/ml of Diclofenac Sodium was analyzed. For intraday precision three replicates of three concentrations 16 µg/ml, 20 µg/ml and 24 µg/ml of standard solution of Benzocaine and 2.4 µg/ml, 3.0 µg/ml, and 3.6µg/ml of Diclofenac Sodium was analyzed at

three different time intervals. For Interday precision three replicates of three concentrations of 16 µg/ml, 20 µg/ml and 24 µg/ml of standard solution of Benzocaine and 2.4 µg/ml, 3.0 µg/ml, and 3.6 µg/ml of Diclofenac Sodium was analyzed at three consecutive days. The %RSD was calculated (Table 2).

Accuracy: The accuracy of method was determined by standard addition method. Known amount of working standard was added to the fixed concentration of pre-analyzed tablet sample. For both the drugs recovery of was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 80%, 100% and 120% level and percentage recovery was calculated by subtracting the total area from pre-analyzed sample area. Results have been shown in table 2.

Robustness : Robustness of method was done by making slight deliberate change in chromatographic condition like change in flow rate (± 0.1 ml/min) and change in wavelength ((± 0.1).Results have been shown in table 2.

Assay of pharmaceutical dosage form: The proposed validated method was successfully applied to determine Benzocaine and Diclofenac Sodium in gel dosage form. The result obtained for Benzocaine and Diclofenac Sodium was comparable with corresponding labeled amounts. Results has been shown in table 3 and Spectra have been shown in Fig. no 3.

Ratio Derivative Spectroscopic method

Validation

Linearity and Range: The linearity of analytical procedure is its ability to obtained test results which are directly proportional to the concentration of analyte in the sample. Five Concentration ranges of 4-20µg/ml of Benzocaine and 0.6-3.0 µg/ml of Diclofenac Sodium were prepared and analyzed. Results have been shown in table 4 and overlay spectra have been shown in Figure no 7 and 9and calibration curve has been shown in figure no 8 and 10 for Diclofenac Sodium and Benzocaine respectively.

Precision: For intraday precision three replicates of three concentrations 8.0 µg/ml, 12 µg/ml and 16 µg/ml of standard solution of Benzocaine and 1.2 µg/ml, 1.8µg/ml, and 2.4µg/ml of Diclofenac Sodium was analyzed at three different time intervals. For Inter-day precision three replicates of three concentrations of 8.0 µg/ml, 12 µg/ml and 16 µg/ml of standard solution of Benzocaine and 1.2 µg/ml, 1.8 µg/ml, and 2.4 µg/ml of Diclofenac Sodium was analyzed at three consecutive days. The %RSD was calculated (Table 4).

Accuracy: The accuracy of method was determined by standard addition method. Known amount of working standard was added to the fixed concentration of pre-analyzed tablet sample. For both the drugs recovery of was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 80%, 100% and 120% level and percentage recovery was calculated by subtracting the total area from pre-analyzed sample area. Results have been shown in (Table 4).

Robustness: Robustness of method was done by making slight deliberate change in chromatographic condition like change in wavelength (± 2). Results have been shown in table 4.

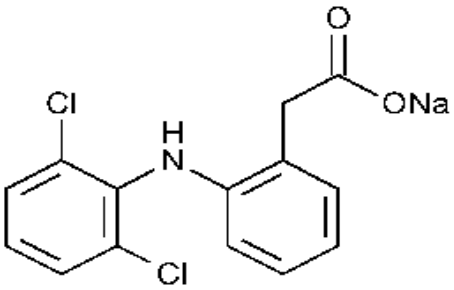
Assay of pharmaceutical dosage form: The proposed validated method was successfully applied to determine Benzocaine and Diclofenac Sodium in gel dosage form. The result obtained for Benzocaine and Diclofenac Sodium was comparable with corresponding labeled amounts. Results has been shown in table 5. And Spectra shown in Figure no 11 and 12 of Benzocaine and Diclofenac Sodium respectively

Conclusion: HPLC and UV method for Benzocaine and Diclofenac Sodium in pharmaceutical dosage form was developed. The developed method is accurate, precise and specific and has ability to separate the drugs Benzocaine and Diclofenac Sodium in gel dosage form. Developed HPLC and UV method was validated as per ICH guidelines and thus indicating general applicability of method for analysis of marketed formulation. The simplicity of method is allows its application in the laboratory for routine quality check. Overall method provides high throughput solution for determination of Benzocaine and Diclofenac Sodium with excellent sensitivity, precision and accuracy.

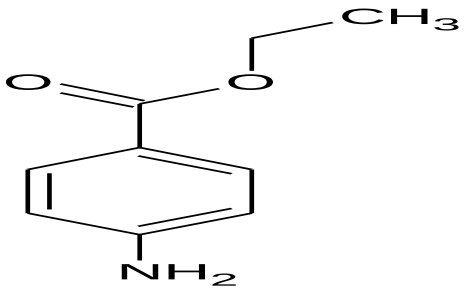
ACKNOWLEDGEMENT:

I am very grateful to **Dr. A. L. Prashad**, group leader of Omgene Life Science Baroda. I would also like to gratefully acknowledge the support of whole staff of the ADL, R&D and F&D Department of Omgene Life Science. For that I am very gratefulto, **Mr. Gaurav Kulkarni and Mr. Parag Patel.**

Structure of Diclofenac Sodium and Benzocaine



Diclofenac Sodium



Benzocaine

Table 1: System Suitability Report

System suitability parameters (20 + 3.0 µg/ml)	Benzocaine Mean ± S.D ,(n=6)	Diclofenac Sodium Mean ± S.D ,(n=6)
Area	264259±838.57	71858±402.86
Retention Time	3.68±0.005177	6.58±0.00687
Theoretical Plates	6873.95±240.2742	8268±279.8604
Tailing factor	0.96±0.0482	1.108±0.011023
Resolution	-	12.336±0.079

Table 2: Result of validation parameter

Sr.No.	Parameter	BENZ	DIC
1	Linearity range (µg/ml)	4-28	0.6-4.2
2	Regression line equation	14103x-19046	24879x-4214.7
3	Correlation co-efficient (r2)	0.9988	0.9984
4	Precision		
	Repeatability Intraday Interday	0.31 0.56-0.82 0.91-1.15	0.56 0.56-0.76 0.88-1.20
5	Robustness		
	Change in flow rate Change in wavelength Change in mobile phase ratio	0.51 0.78 0.82	0.91 0.72 0.83
5	Accuracy (%)	99.94-100.73	99.94-101.60
6	LOD(µg/ml)	0.21	0.19
7	LOQ(µg/ml)	0.61	0.57

Table 3: Data of assay of pharmaceutical dosage form

Parameter	Value	
	BENZ	DIC
% Estimation	100.73	100.66
% RSD	0.5041	0.88

Table: 4Validation parameter of UV Spectrophotometer

Sr.No.	Parameter	BENZ	DIC
1	Linearity range (µg/ml)	4-20	0.6-3.0
2	Regression line equation	-5.806x-0.4597	0.0103x-0.0014
3	Correlation co-efficient (r2)	0.9999	0.9990
4	Precision		
	Intraday Interday Different analyst Different instrument	0.56-0.59 0.85-1.14 0.28-0.68 0.61-0.76	0.28-0.98 0.92-1.15 0.60-0.73 0.47-0.97
5	Accuracy	99.30-101.25	99.16-102
6	Robustness		
	Change in wave length	0.86-1.05	0.90-1.02
6	LOD(µg/ml)	0.15	0.12
7	LOQ(µg/ml)	0.31	0.28

Table: 5Assay data for BENZ and DIC in gel formulation

Parameter	Value	
	DIC	BENZ
% Estimation	99.24±0.28	99.037±0.36
% RSD	0.36	0.28

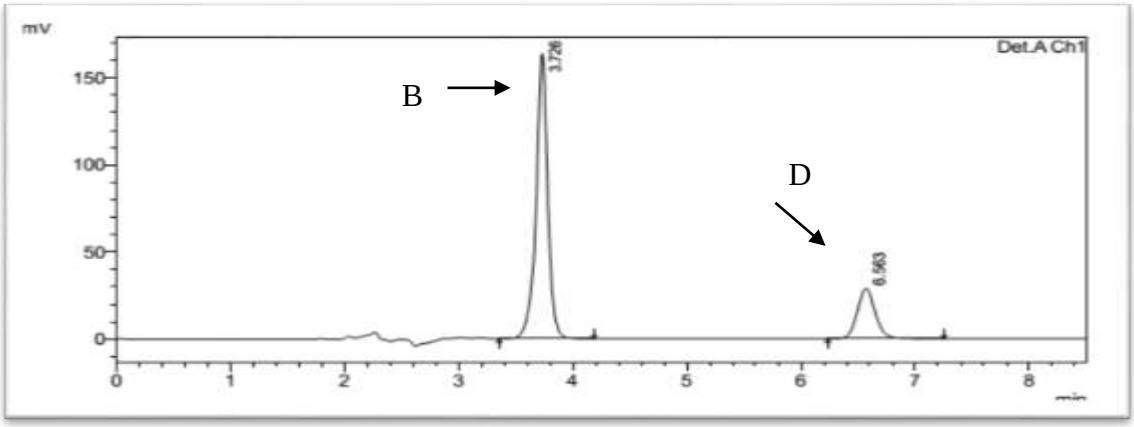


Fig No: 1 RP-HPLC chromatogram of Standard solution

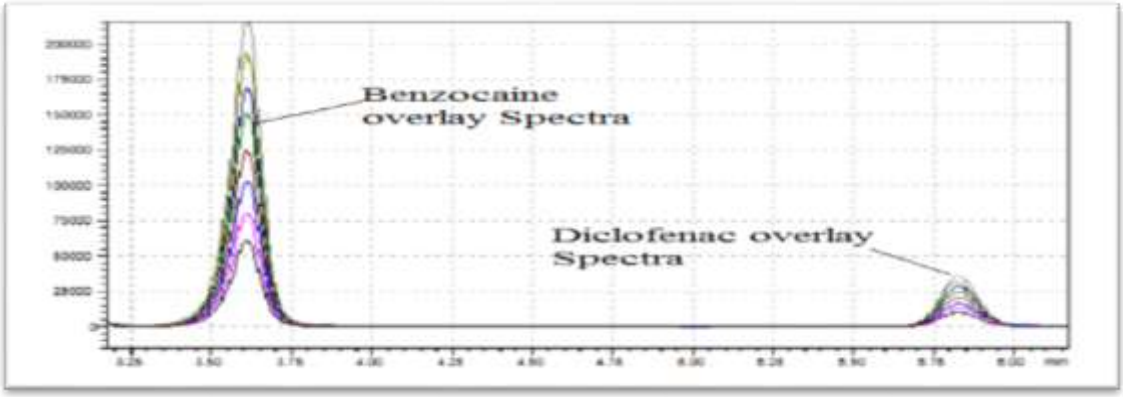


Fig No: 2 Chromatogram of mixture BENZ and DIC (4-28:0.6-4.2)

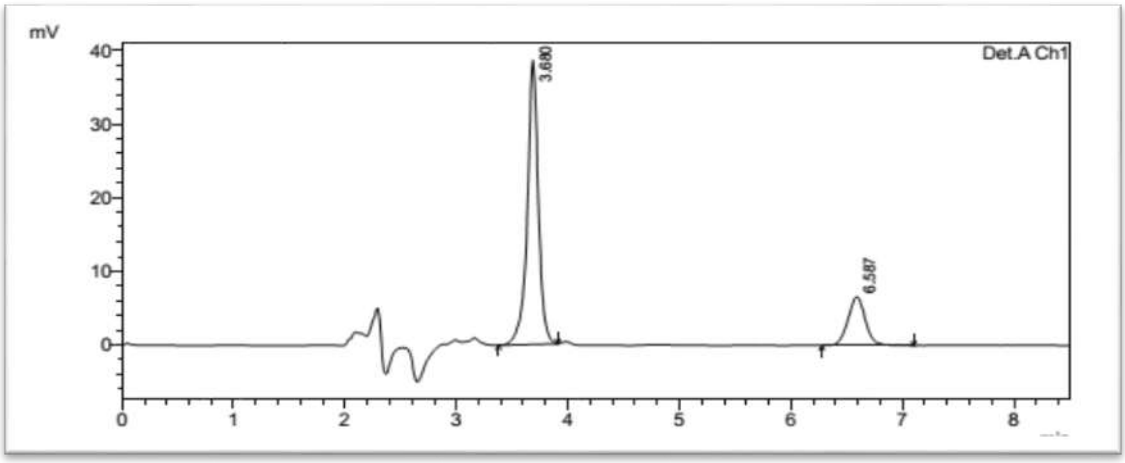


Fig No: 3 RP-HPLC chromatogram of Sample solution (Formulation)

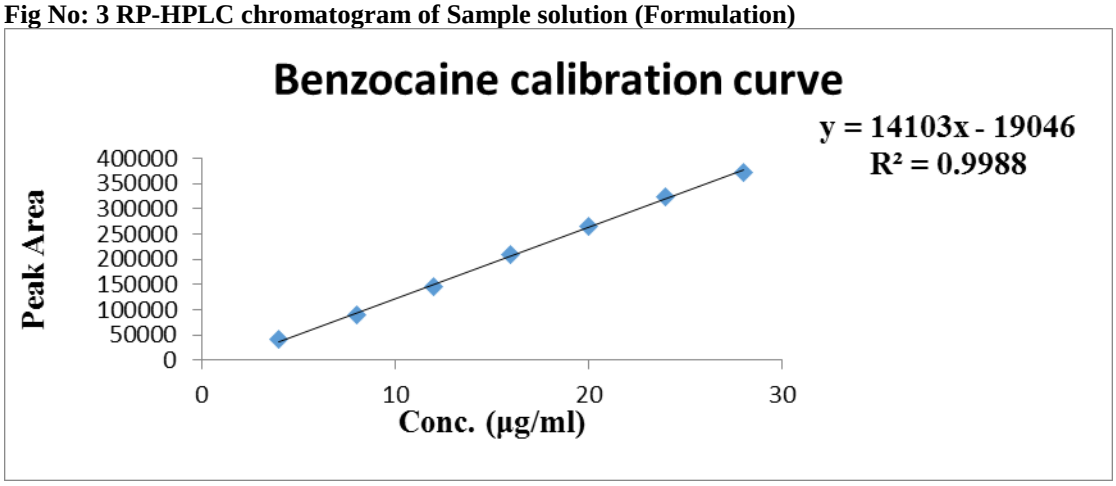


Fig No 4: Benzocaine calibration curve

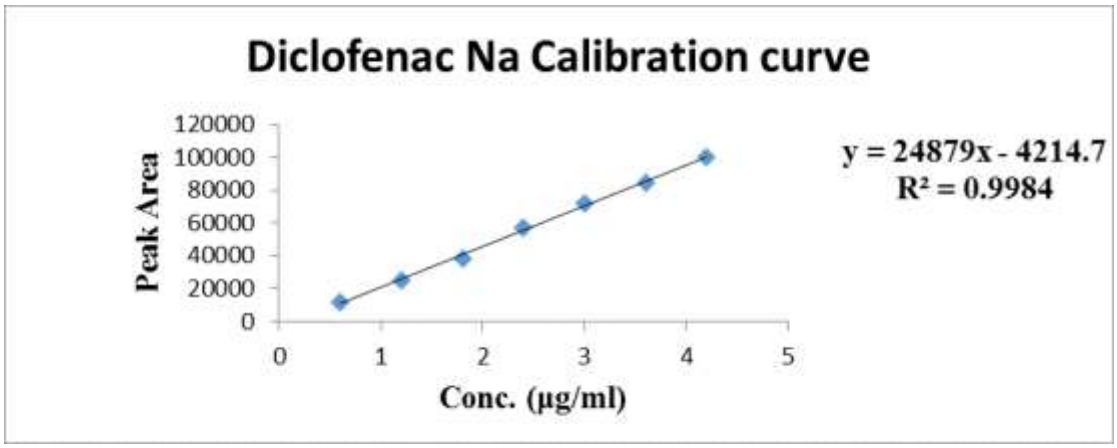


Fig No 5:Dicfenac Sodium calibration curve

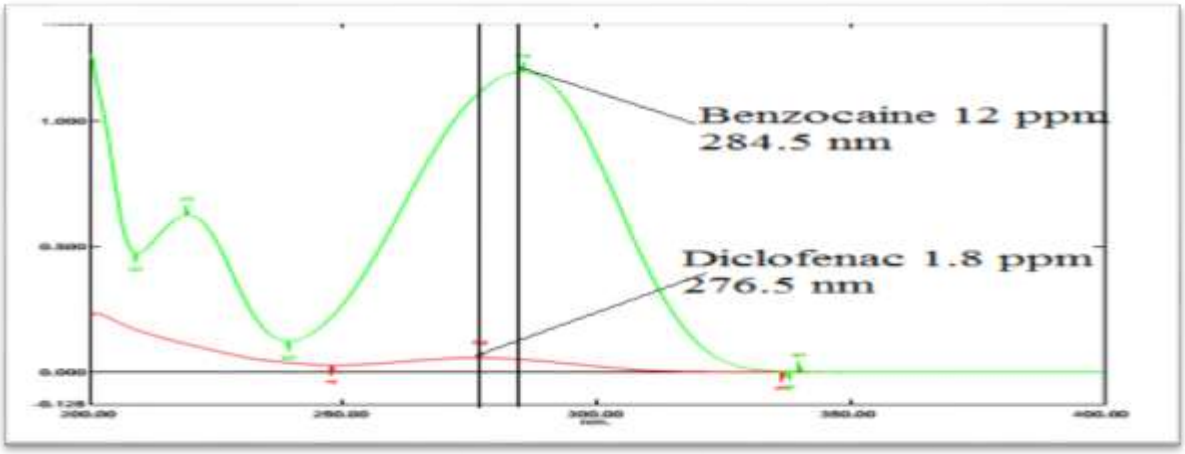


Fig No 6: selection of wavelength

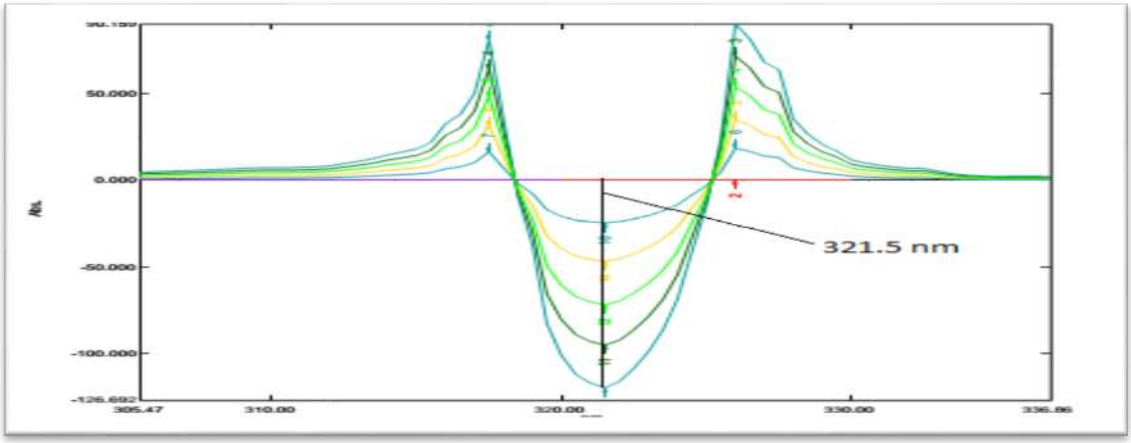


Fig No 7: Overlay first derivative ratio spectra of Benzocaine at divisor of 1.8 ppm Diclofenac sodium

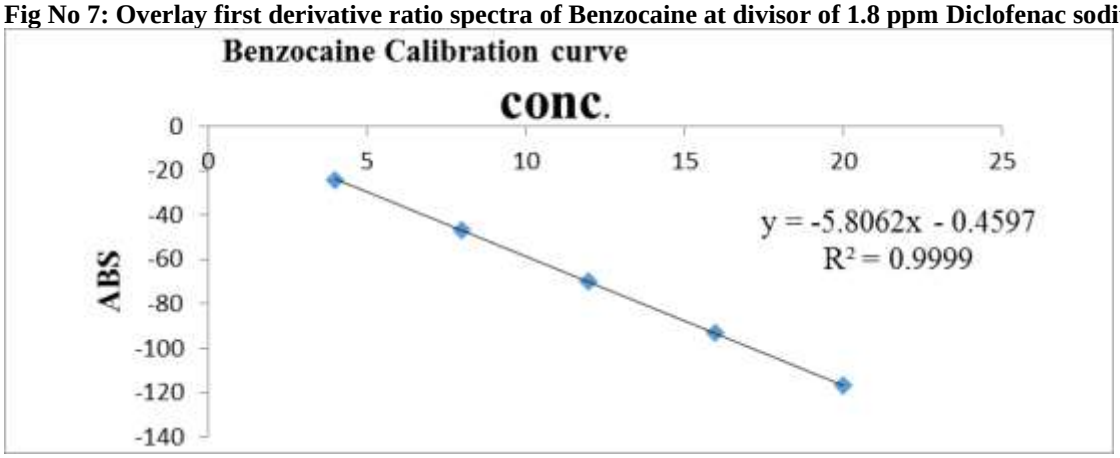


Fig No 8: Benzocaine calibration curv

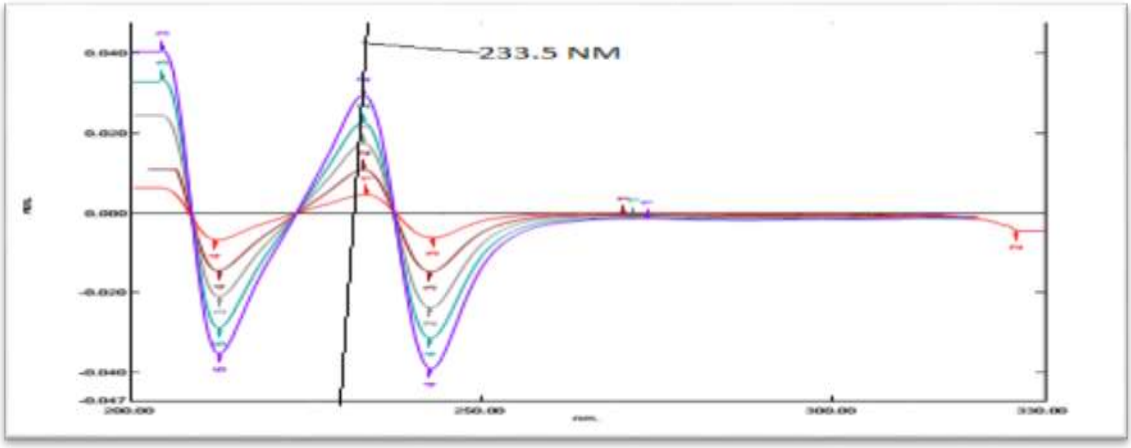


Fig No 9: Overlay first derivative ratio spectra of Diclofenac sodium at divisor of 12 ppm Benzocaine

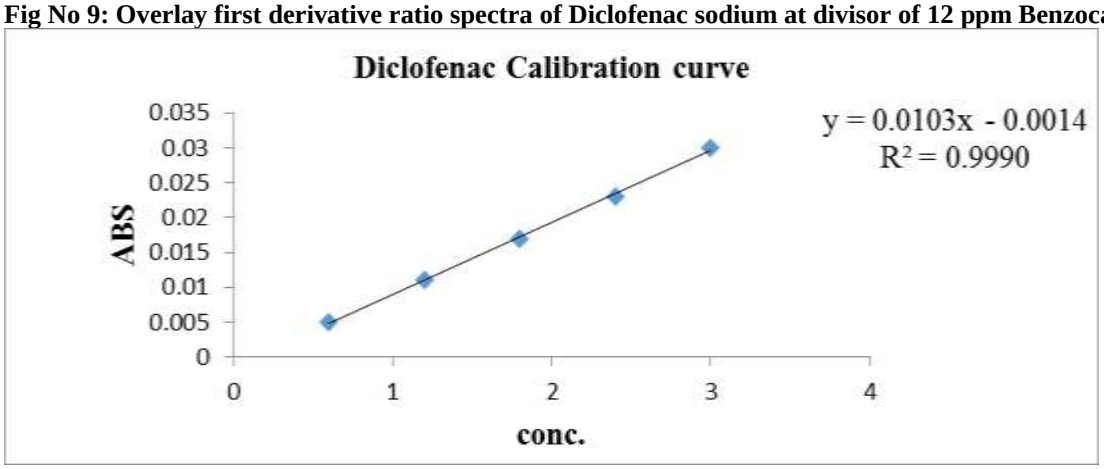


Fig No 10: Diclofenac Sodium calibration curve

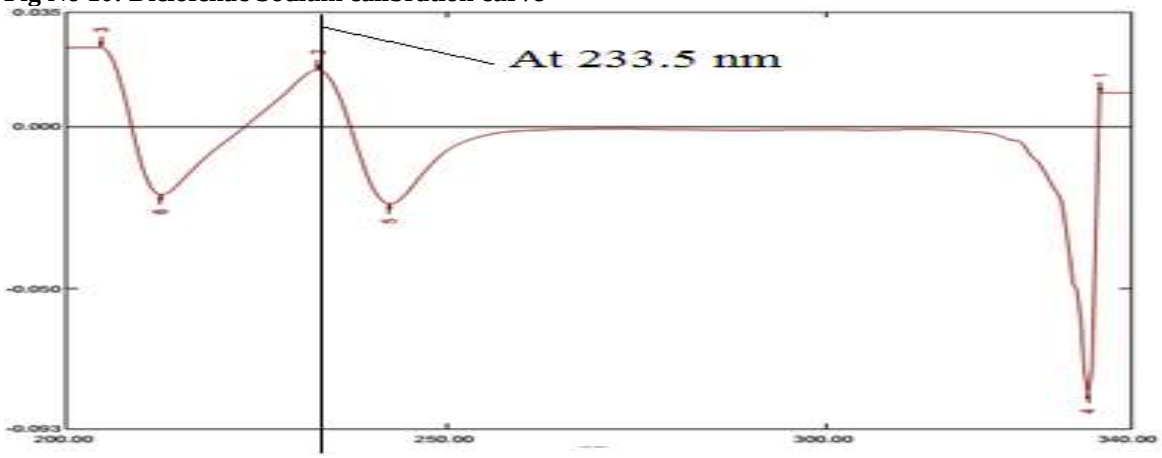


Fig No 11: Spectra of Formulation (1.8/12 ppm) Divisor used 12 ppm BENZ

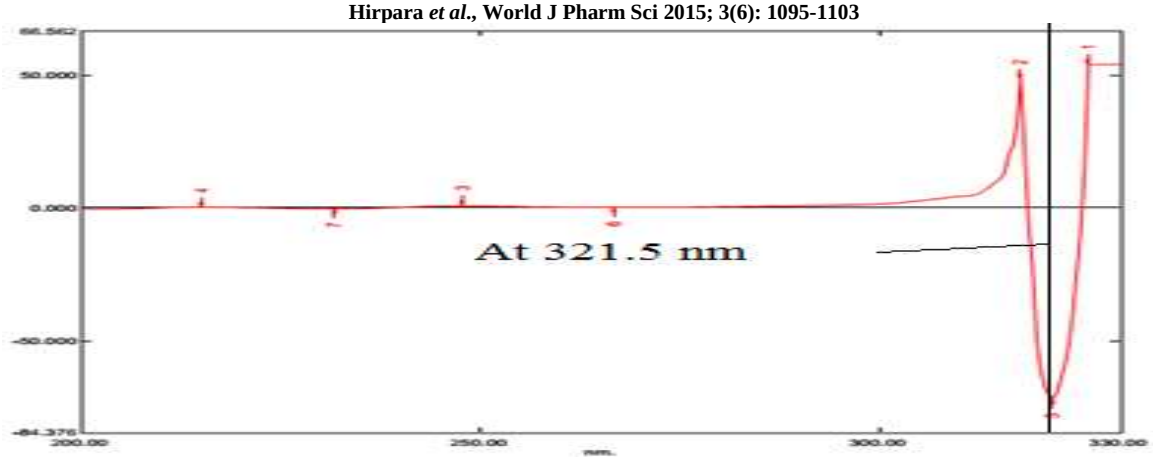


Fig No 12: Spectra of Formulation (1.8/12 ppm) Divisor 1.8 ppm DIC

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