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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR THE SIMULTANEOUS ESTIMATION OF NAPROXEN SODIUM AND CODEINE PHOSPHATE IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Two UV Spectrophotometric methods were developed and validated for the simultaneous estimation of Naproxen Sodium (NAP) and Codeine Phosphate (COD) in bulk and in its tablet dosage forms as per ICH guidelines. Sodium hydroxide is used as a solvent. The stock solutions were prepared by using 0.1 N sodium hydroxide as a solvent. In the Zero order derivative method, NAP showed maximum absorbance at 262 nm and COD showed maximum absorbance at 284 nm. In the Third order derivative method, NAP showed maximum absorbance at 264 nm and COD at 281 nm. Calibration graph was obtained in the concentration range of 50-250 µg/ ml for NAP and 5-25 µg/ ml for COD in both methods. The LOD and LOQ value of the drugs in zero order derivative method was found to be 3.59 µg/ ml and 10.89 µg/ ml for NAP and 0.29 µg/ ml and 0.90 µg/ ml for COD. In third order derivative method LOD and LOQ value of NAP is 5.21 µg/ ml and 15.81 µg/ ml and for COD is 0.41 µg/ ml and 1.25 µg/ ml. The developed methods were validated according to ICH guidelines. The developed methods were found to be simple, accurate and precise for the routine analysis of the NAP and COD in bulk and tablet dosage forms.

Keywords: Naproxen Sodium, Codeine Phosphate, UV-Spectrophotometric, Validation.

INTRODUCTION

NAP is known as non-steroidal anti-inflammatory drug and it is widely considered as strong pain killer as strong pain killer. It is designated chemically (+)-(S)-2-(6-Methoxynaphthalen-2-yl) propanoic acid with molecular formula $C_{14}H_{13}NaO_3$. **NAP** is an NSAID used to treat rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, polyarticular juvenile idiopathic arthritis, tendinitis, bursitis, acute gout, primary dysmenorrhea, and mild to moderate pain. NAP blocks arachidonate binding to competitively inhibit both cyclo-oxygenase (COX) isoenzymes, COX-1 and COX-2, resulting in analgesic and anti-inflammatory effects.

COD used to treat pain, cough, and diarrhea. It is made from opium or morphine and binds to opioid receptors in the central nervous system. Codeine phosphate is a type of opiate, a type of analgesic agent, a type of antitussive agent, and a type of antidiarrheal agent. is chemically known as 5α,6α)-7,8-didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol. With molecular formula $C_{18}H_{21}NO_3.H_3PO_4$. ½ H_2O. COD is an opioid and an agonist of the mu opioid receptor (MOR). It acts on the central nervous system to have an analgesic effect. It is metabolised in the liver to produce morphine which is ten times more potent against the mu receptor.

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The combination of NAP and COD is more effective in increasing threshold and tolerance to electrically induced pain.

Literature survey reveals that, till date no UV-Spectrophotometric method has been reported for the determination Nap and COD. In this study attempts has been made to develop and validate simple, accurate and economical UV methods for simultaneous estimation of NAP and COD.

DRUG PROFILE:

Naproxen sodium:

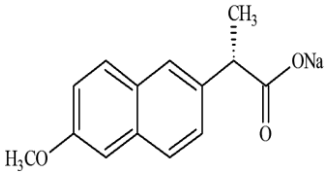


Figure 1: Structure of Naproxen sodium.

IUPAC Name : (+)-(S)-2-(6-Methoxynaphthalen-2-yl) propanoic acid.
Molecular formula : $C_{14}H_{13}NaO_3$.
Molecular weight : 230.26 g/mol.
Category : NSAIDs drug.
Physical state : Odourless, white to off-white crystalline substance.
Solubility : Soluble in water, chloroform, acetone, alcohol, organic solvents.

Codeine phosphate:

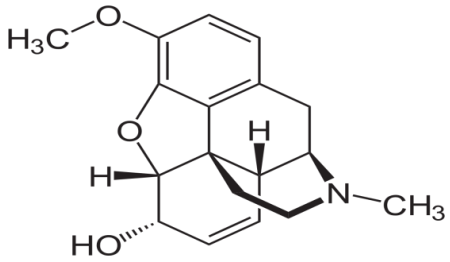


Figure 2: Structure of Codeine phoshphate.

IUPAC Name : 5α, 6α)-7, 8-didehydro-4, 5-epoxy- 3-methoxy-17- methylmorphinan-6- ol.
Molecular formula : $C_{18}H_{21}NO_3$, H_3PO_4. ½H_2O.
Molecular weight : 299.364 g/mol.
Category :Opioid analgesic (pain reliever) drug.
Physical state : Fine, white, needle shaped crystals or white crystalline in a powder.
Solubility : Soluble in four parts of water, slightly soluble in ethanol, Practically Insoluble in chloroform and ether.

EXPERIMENTAL

Chemicals and Reagents

NAP pure drug was procured as a free sample from Medo Pharma, Malur, Kolar District. COD pure drug was procured as a free sample from Med Rich pvt Ltd, Bangalore. All chemicals and reagents used were of analytical grade.

Instrumentation

The instrument used was Shimadzu model 1800 double beam UV-Visible Spectrophotometer with a spectral band width of 1 ± 0.2 nm, wavelength accuracy of ± 0.3 nm and a pair of quartz cuvettes having 1 cm path length was used.

MATERIAL AND METHOD

Method-1: Zero order derivative spectroscopy

Preparation of standard stock solution

Stock solution was prepared by accurately weighing 100 mg each of COD and NAP were weighed separately and transferred into two different 100 ml volumetric flasks. Both the drugs were dissolved in 100 ml of 0.1 N NaOH to obtain a final concentration of 1000 µg/ml of each component. (stock A and stock A^1 solution).

From the above stock A solution, 10 ml was pipetted out into a 100 ml volumetric flask and the volume was made up to the mark with 0.1 N NaOH to obtain the final concentration of 100 µg/ml of COD (stock B solution). The above stock A^1 solution is itself used as the standard stock solution for NAP.

Preparation of sample solution

Twenty tablets were weighed and their average weight was determined. The tablets were crushed to fine powder, tablet powder equivalent to 100 mg of NAP was weighed which also contains 5.42 mg of COD and transferred to 100 ml volumetric flask. Dissolved in 80 ml of the solvent. The solution was filtered through whatmann filter paper No.41, finally the volume was made up to 100 ml with the solvent to get a concentration of 1000 µg/ml of NAP and 54.2 µg/ml of COD and this solution was used as stock A solution.

From the above stock A solution, a required volume of the solution was pipetted. and the volume was made up to the mark with the solvent to obtain a solution in a desired concentration range of NAP and COD.

Method-2: Third order derivative spectroscopy.

Sample and standard stock solutions were prepared similarly as stated in the zero order method. Then the zero order peaks obtained were converted to third order by taking delta lambda of 8 with a scaling factor of 100. All the methodology and validation parameters were carried out similar to zero order method.

METHOD OF VALIDATION

A. Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

In both the developed methods NAP showed good linearity in the concentration range of 50-250 µg/ ml and COD at 5-25µg/ ml.

B. ACCURACY.

Procedure for determination of Accuracy

Recovery studies were carried out by adding 80%, 100% and 120% of the standard drug solution of NAP and COD to the known amount of sample solution by standard addition method.

Procedure for addition of 80 % standard solution of NAP to the known amount of sample solution

1 ml of sample stock solution (containing 1000 µg/ml of NAP) is transferred to the 10 ml volumetric flask and added 0.8 ml of standard stock solutions of NAP (800 µg/ml). The volume is made up to the 10 ml mark with 0.1 N NaOH. Absorbance was measured at 262 nm respectively.

Procedure for addition of 80 % standard solution of COD to the known amount sample solution

1 ml of sample solution (containing 54.2 µg/ml of COD) is transferred to the 10 ml volumetric flask and added 0.8 ml of standard stock solutions of COD (80 µg/ml). The volume is made up to the 10 ml mark with 0.1 N NaOH. Absorbance measured at 284 nm respectively.

Procedure for addition of 100 % standard solution of NAP to the known amount of sample solution

1 ml of sample stock solution (containing 1000 µg/ml of NAP) is transferred to the 10 ml volumetric flask and added 1 ml of standard stock solutions of NAP (1000 µg/ml). The volume is made up to the 10 ml mark with 0.1 N NaOH. Absorbance was measured at 262 nm respectively.

Procedure for addition of 100 % standard solution of COD to the known amount of sample solution

1 ml of sample stock solution (containing 54.2 µg/ml of COD) is transferred to the 10 ml volumetric flask and added 1ml of standard stock solutions of COD (100 µg/ml). The volume is made up to the 10 ml mark with 0.1 N NaOH. Absorbance was measured at 284 nm respectively.

Procedure for addition of 120 % standard solution of NAP to the knows amount of sample solution

1 ml of sample stock solution (containing 1000 µg/ml of NAP) is transferred to the 10 volumetric flask and added 1.2 ml of standard stock solutions of NAP (1200 µg/ml). The volume is made up to the 10 ml mark with 0.1 N NaOH. Absorbance was measured at 262 nm respectively.

Procedure for addition of 120 % standard solution of COD to the known amount of sample solution

1 ml of sample stock solution (containing 54.2 µg/ml of COD) is transferred to the 10 ml volumetric flask and added 1.2 ml of standard stock solutions of COD (120 µg/ml).The volume is made up to the 10 ml mark with 0.1 N NaoH Absorbance was detected at 284 nm respectively.

The concentration of COD and NAP were calculated. At each level of recovery dies, three determinations were performed. The results obtained were compared and statistically validated

Table 1: Result of calibration curve for NAP at 262 nm by zero order derivative method.

Concentration (µg/mL)	Absorbance Mean ± Std. Deviation(n=6)	%CV
5	0.222 ± 0.002258	1.017117
10	0.402± 0.001966	0.489055
15	0.614± 0.004761	0.775407
20	0.812±0.003189	0.392773
25	1.037± 0.005947	0.574589

Table 2: Result of calibration curve for COD at 284 nm by zero order derivative method.

Concentration (µg/mL)	Absorbance Mean ± Std. Deviation(n=6)	%CV
50	0.25 ±0.002317	0.9268
100	0.45 ±0.436888	0.436888
150	0.656±0.827439	0.827439
200	0.85±0.398352	0.398352
250	1.07± 0.810159	0.810159

Table 3: Statistical validation data for tablet formulation by zero order method..

Components	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
NAP	99.51	0.902783	0.907228	0.36999
COD	99.115	0.817698	0.824999	0.33512

Table 4: Result of calibration curve for NAP at 264 nm by Third order derivative method.

Concentration (µg/mL)	Absorbance Mean ± Std. Deviation(n=6)	%CV
50	0.083±0.000753	0.907229
100	0.165±0.001366	0.828036
150	0.249±0.001862	0.74775
200	0.324±0.001211	0.373784
250	0.413±0.002714	0.657182

Table 5: Result of calibration curve for COD at 281 nm by Third -order derivative method.

Concentration (µg/mL)	Absorbance Mean ± Std. Deviation(n=6)	%CV
5	-0.051±0.000408	-0.80049
10	-0.104±0.001033	-0.99307
15	-0.164±0.001366	-0.83309
20	-0.210±0.001602	-0.7629
25	-0.271±0.001871	-0.69034

Table 6: Statistical validation data for tablet formulation by third order method.

Components	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
NAP	99.54167	0.82921	0.833028	0.33984
COD	99.10494	0.751592	0.75838	0.30802

n*=6

Table 7: Determination of accuracy of by zero order method

Level of %recovery	Amount taken from formulation (µg/mL)		Amount of standard drug added (µg/ml)		Total amount recovered (µg/ml)		% Recovery	
	NAP	COD	NAP	COD	NAP	COD	NAP	COD
80%	100	5.42	80	8	179.99	13.48	99.99	100.44
	100	5.42	80	8	178.92	13.59	99.4	101.26
	100	5.42	80	8	181.96	13.42	101.08	100
100%	100	5.42	100	10	199.96	15.37	99.98	99.675
	100	5.42	100	10	200.99	15.5	100.49	100.51
	100	5.42	100	10	199.96	15.4	99.98	99.87
120%	100	5.42	120	12	219.18	17.35	99.62	99.59
	100	5.42	120	12	219.98	17.43	99.99	100.05
	100	5.42	120	12	218.95	17.38	99.52	99.77

Table 8: Determination of accuracy by third order method

Level of % recovery	Amount taken from formulation (µg/mL)		Amount of standard drug added (µg/ml)		Total amount recovered (µg/ml)		% Recovery	
	NAP	COD	NAP	COD	NAP	COD	NAP	COD
80%	100	5.42	80	8	179.92	13.49	99.95	100.52
	100	5.42	80	8	178.95	13.58	99.4	101.19
	100	5.42	80	8	181.98	13.44	101.1	100.1
100%	100	5.42	100	10	199.95	15.36	99.97	99.61
	100	5.42	100	10	200.97	15.49	100.48	100.45
	100	5.42	100	10	199.95	15.39	99.97	99.80
	100	5.42	120	12	219.17	17.38	99.62	99.77

	100	5.42	120	12	219.99	17.41	99.99	99.94
120%	100	5.42	120	12	218.97	17.37	99.53	99.71

Table 9: Statistical validation Data for accuracy determination by zero order and third order method

Level of Recovery	Zero order method						Third order method					
	Mean*		Standard Deviation*		Co-efficient Variation*		Mean*		Standard Deviation*		Coefficient Variation*	
	NAP	COD	NAP	COD	NAP	COD	NAP	COD	NAP	COD	NAP	COD
80%	100.161	100.571	0.85669	0.64245	0.85669	0.6388	100.15	100.62	0.85963	0.52866	0.85828	0.5254
100%	100.152	100.022	0.29734	0.44143	0.29734	0.44133	100.23	99.95	0.36062	0.44143	0.3598	0.44162
120%	99.7136	99.8086	0.24575	0.232	0.24575	0.23245	99.71	99.80	0.24568	0.1195	0.24638	0.11973

Table 10: Statistical validation data for intra-day precision

Components	Zero order method			Third order method		
	Mean*	Standard Deviation*	Co-efficient of Variation*	Mean*	Standard Deviation*	Co-efficient of Variation*
NAP	99.44	0.833181	0.837873	99.31	0.908075	0.914384
COD	99	0.77974	0.787616	99.3	0.689928	0.694791

***n=6.**

Table 11: Statistical validation data for inter-day precision by zero order and third order method

Components	Zero order method			Third order method		
	Mean*	Standard Deviation*	Co-efficient of Variation*	Mean*	Standard Deviation*	Co-efficient of Variation*
NAP	99.64	0.913841	0.917143	99.69778	0.4445693	0.445917
COD	99.63	0.6509	0.653317	99.53433	0.3320654	0.333619

***n = 3**

Figure 3: Calibration curve of NAP at 262 nm by zero order derivative method

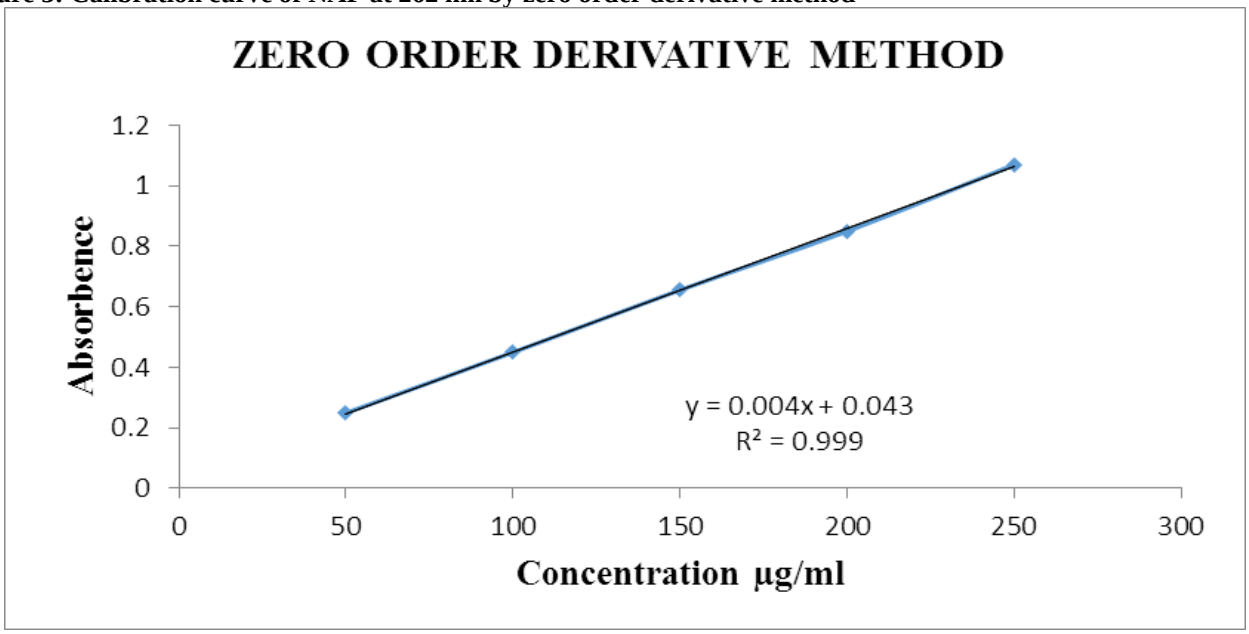


Figure 4: Calibration curve of COD at 284 nm by zero order derivative method.

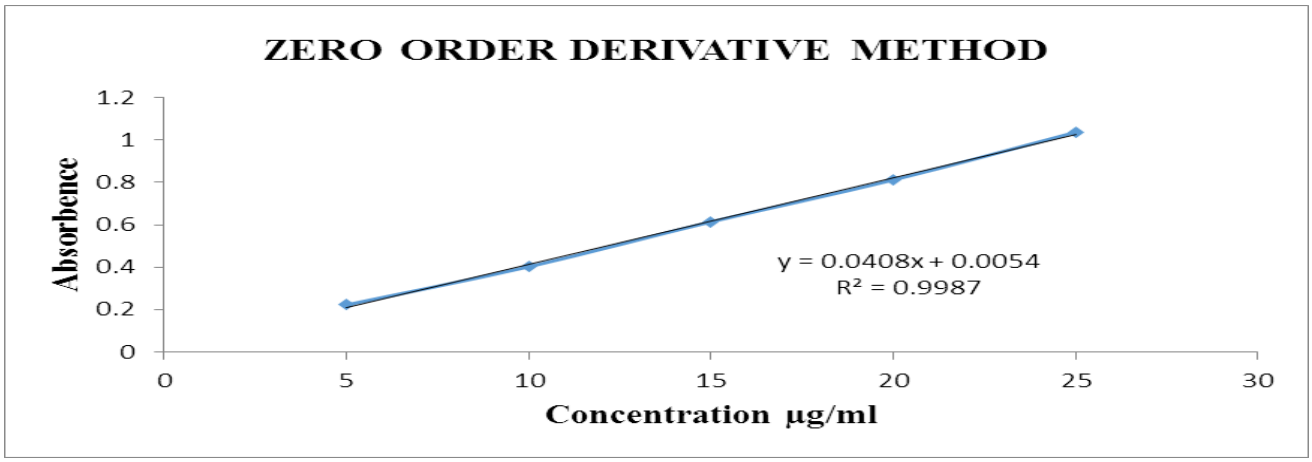


Figure 5: Zero order derivative spectrum of NAP at 262 nm.

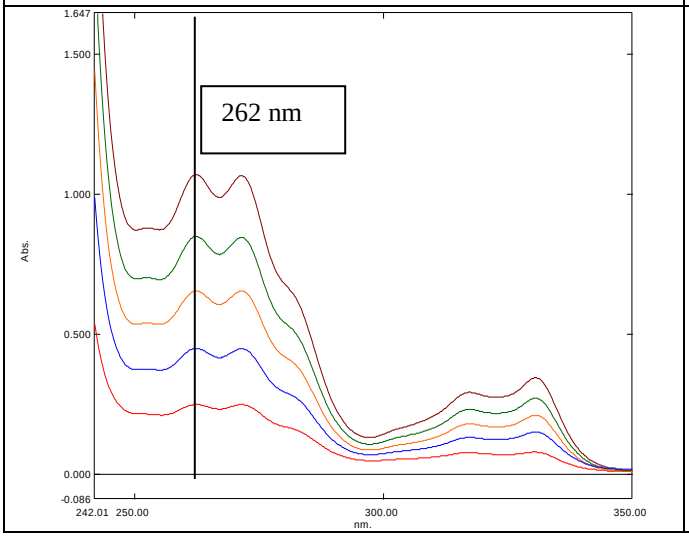


Figure 6: Zero order derivative spectrum of COD at 284 nm.

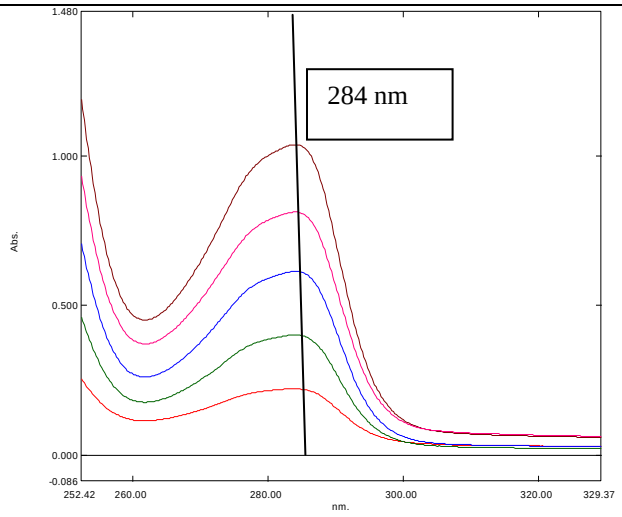


Figure7: Zero order derivative spectrum of mixture.

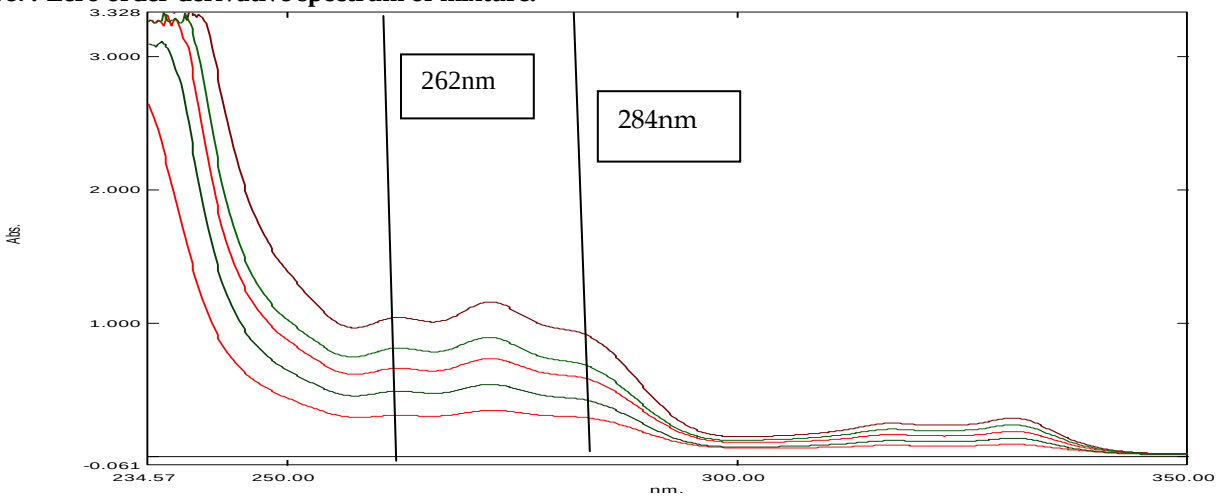


Figure 8: Calibration curve of NAP at 264 nm by third order derivative method.

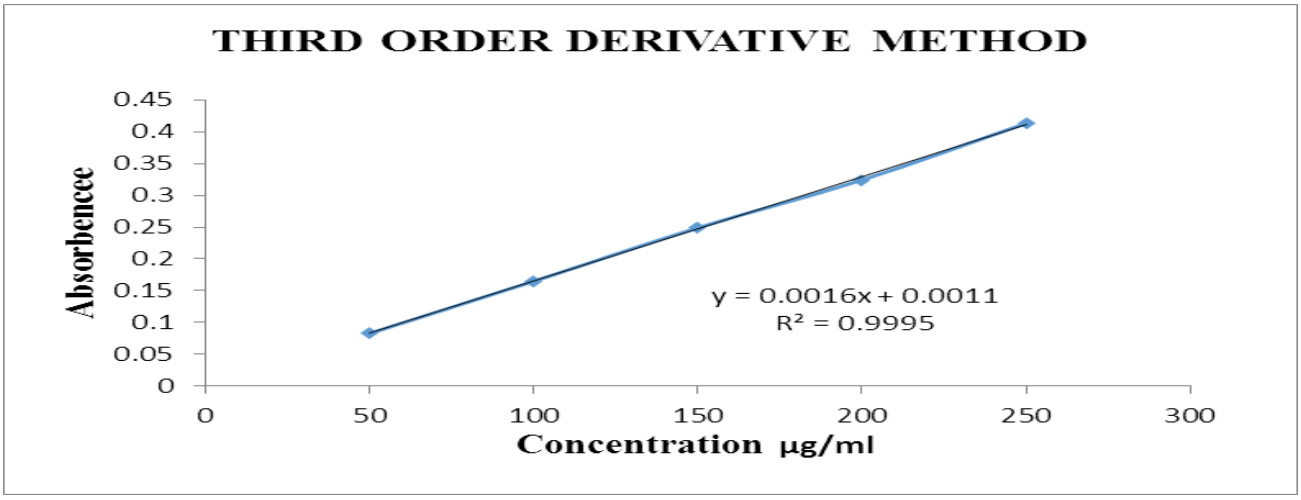


Figure 9: Calibration curve of COD at 281 nm by Third order derivative method.

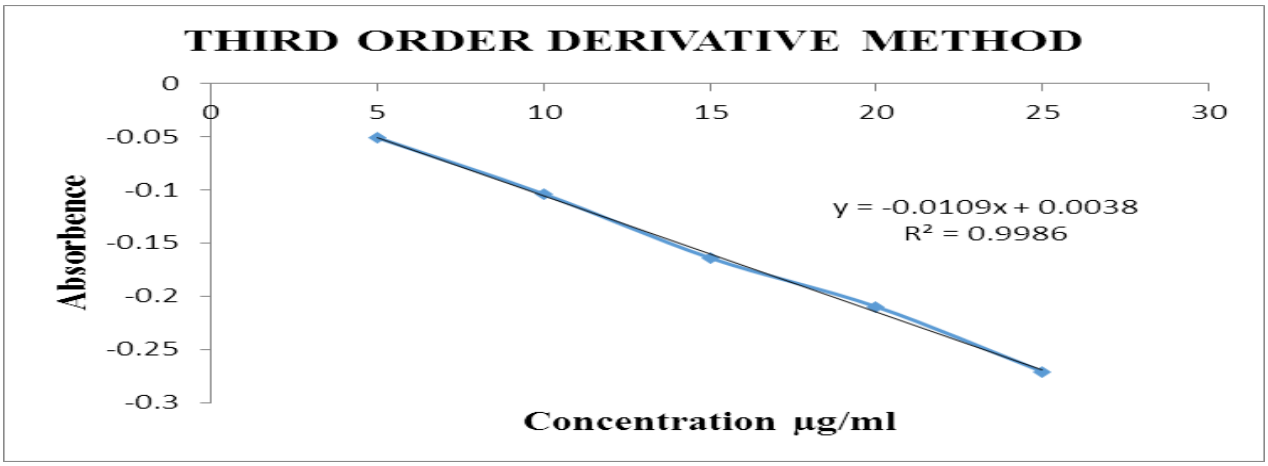


Figure 10: Third order derivative spectrum of NAP at 264 nm and zero crossing at 262 nm

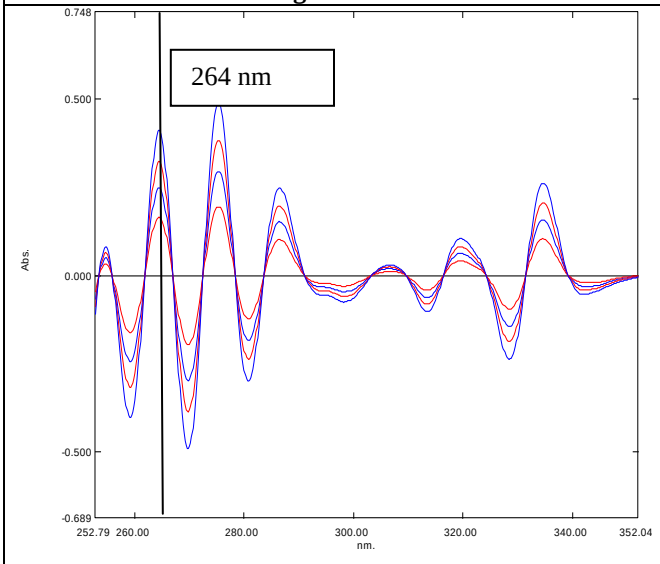


Figure 11: Third order derivative spectrum of COD at 281 nm and zero crossing at 284 nm

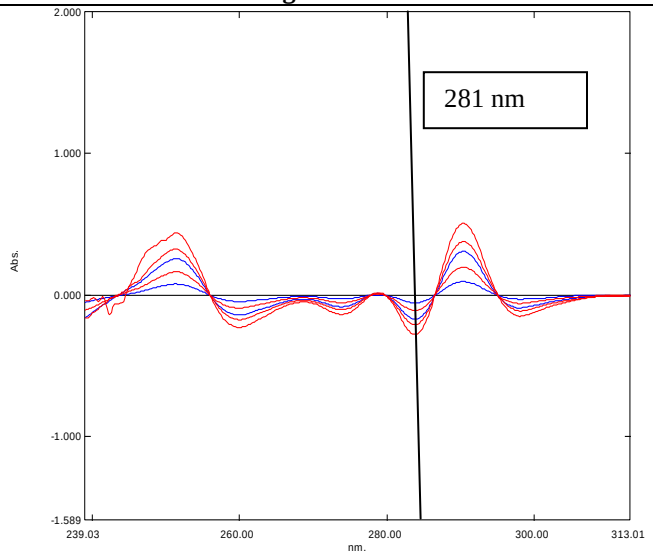
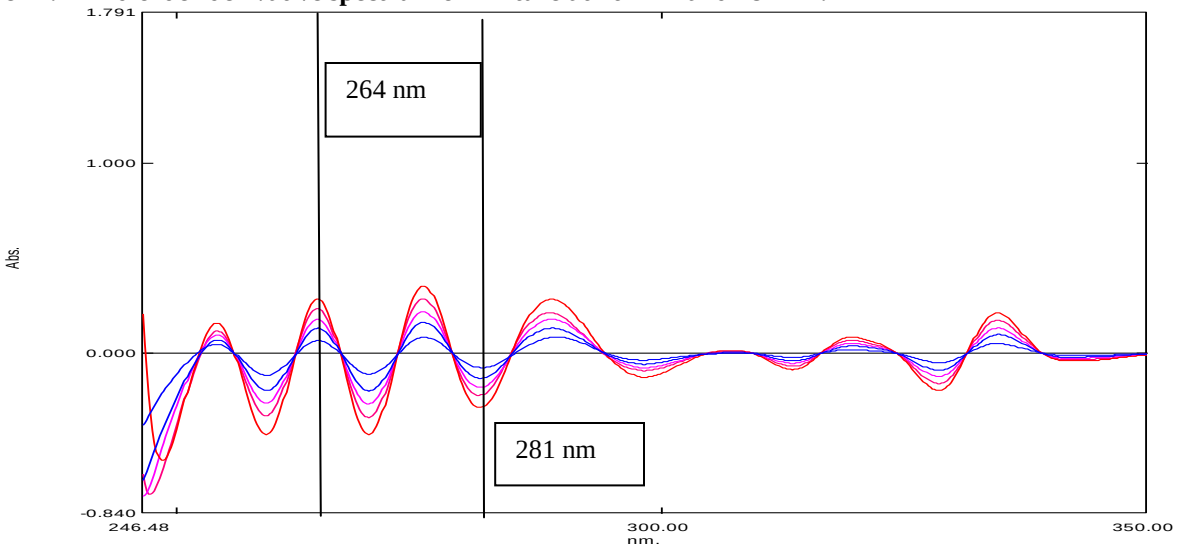


Figure 12: Third order derivative spectrum of mixture at 264 nm and 281 nm.



C. PRECISION

It is the procedure which expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions.

Procedure for determination of Intra-day precision

In intra-day precision the sample mixture containing 50 µg/ml of NAP and 5 µg/ml of COD were prepared and analyzed six times at different time intervals in the same day at their selected analytical wavelengths by both the developed methods. The variation of the results within the same day was analyzed and statistically validated.

Procedure for determination of Inter-day precision

In inter-day precision the above sample mixtures containing 50 µg/ml of NAP and 5 µg/ml of COD were prepared and analyzed six times at same time on three different days of a week at their selected analytical wavelengths by both the developed methods. The variation of the results within the same day was analyzed and statistically validated.

D. LIMIT OF DETECTION

The limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Based on the Standard deviation of the response and the Slope, the limit of detection (LOD) may be expressed as:

$$LOD = 3.3\ \sigma / S$$

Where, σ is the standard deviation of the response & S is the slope of the calibration curve.

E. LIMIT OF QUANTITATION

The limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Based on the Standard deviation of the response and the Slope,

The limit of quantitation (LOQ) may be expressed as:

$$LOQ = 10\ \sigma / S$$

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

RESULTS AND DISCUSSION

In Zero order derivative method graph were recorded in the wavelength of 262 & 284 nm for NAP and COD and 264 & 281 nm for NAP and COD in third order method. The UV visible spectroscopic method for the NAP and COD was found to be simple, accurate, economical and reproducible.

The drug concentrations were found to belinear i n the range NAP 50-250 µg/ml for NAP and 5-25 µg/ml for COD. The regression equations of calibration curves of NAP is $y = 0.0408x + 0.0054$ with the correlation coefficient value 0.9987 simillarly regression equations of calibration curves of COD $y = 0.004x + 0.043$ with the correlation coefficient value of 0.999 in zero order derivative method and for Third order derivative method regression equations of calibration curves of NAP $y = 0.0016x + 0.0011$ with the correlation coefficient value of 0.9995 and $y = 0.0109x + 0.0038$ with the correlation coefficient value of 0.9986 for COD, which indicates that developed method was linear. The accuracy of the method was assessed by recovery studies at three different levels i.e. 80 %, 100 % and 120 %. The values of standard deviation were satisfactory and the recovery studies were close to 100 %. The percentage recovery was found in the range of 99.71 % – 100.23 % which

indicates that the developed methods are accurate. For Precision, intra-day and inter-day precision results in terms of percentage relative standard deviation values were found to be 0.833181and 0.77974 (intra day) 0.913841and 0.6509 (Inter day) for zero order derivative method respectively in same way Precision, intra-day and inter-day precision results in terms of percentage relative standard deviation values were found to be 0.91438 and 0.69479 (Intra day) and 0.4445693, 0.3320654 (Inter day) in third order derivative method.

For zero order derivative method LOD and LOQ values were found to be 0.906 µg/ml & 10.8925 µg/ml for NAP, 0.2989 µg/ml & 3.594525 µg/ml for COD respectively. In the same way in third order derivative method LOD and LOQ values were found to be 5.21806 µg/ml & 15.81 µg/ml for NAP, 0.414494 µg/ml & 1.250643 µg/ml for COD. The result of the analysis for pharmaceutical formulation by the developed methods were consistent with the label claim which is highly reproducible and reliable. The method can be used for routine quality analysis of NAP and COD in bulk and pharmaceutical formulations.

CONCLUSION

Two UV-method was developed for the determination of NAP and COD in bulk and pharmaceutical formulation. The proposed method is simple, accurate, precise and this method is suitable for routine analysis of NAP and COD in bulk and pharmaceutical formulations. Detection and Quantification limits achieved, describe that the method is sensitive. High recoveries and acceptable %RSD values confirms accuracy and precision of developed method. Assay results show that the method can be successfully applied for routine analysis of Naproxen and Codeine in bulk and pharmaceutical formulations.

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The Author's thank to Medo Pharma,Malur Kolar District for NAP pure drug was procured as a free sample and Med Rich pvtLtd, Bangalore for COD pure drug was procured as a free sample and also we are thankful to National College of Pharmacy for providing adequate facilities to carry out the work.

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