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Development and Validation of Simultaneous Derivative Spectrophotometric Methods for Determination of Nimesulide and Pantoprazole in Synthetic Mixture

Het Patel[1], Prof. Priyanka Malani[2], Dr. Umesh Upadhyay[3]
Student[1], Professor [2], Principal[3]
Sigma Institute of Pharmacy (Sigma University), Bakrol, Vadodara, Gujarat,390019, India.

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ABSTRACT
Simple, accurate, precise, economical and reproducible analytical method has been developed for the simultaneous estimation of Nimesulide and Pantoprazole in synthetic mixture form by UV spectrophotometric first order derivative method. The stock solutions were prepared in methanol. In the first order derivative method, the wavelengths at which PAN and NIM were analyzed were 249.6 nm and 297.2 nm respectively. At 249.6 nm PAN has absorbance while NIM shows zero absorbance. Similarly, at 297.2 nm NIM shows absorbance while PAN has zero absorbance. Thus, both the drugs do not interfere in the quantitation of one another. rom appropriate dilutions of the working standard stock solution, 6 µg/mL and 30 µg/mL concentration of Pantoprazole (PAN) and Nimesulide (NIM) were separately prepared and scanned in the UV range 200–400 nm. The linearity was obtained in the concentration range for NIM 10-50 µg/mL and for PAN 2-10 µg/mL.The percentage RSD for precision and accuracy of method was found to be less than 2 %. Therefore, proposed methods can be successfully used for routine analysis of Nimesulide and Pantoprazole in bulk as well as synthetic mixture.

[KEYWORD: UV (Ultravioletspectrophotometric), PAN(Pantoprazole), NIM(Nimesulide), First order derivative method, Development, Validation)**]**

I. INTRODUCTION:

Nimesulide is N-(4-Nitro-2-phenoxyphenyl)-meth-anesul fonamide. Nimesulide is a relativelyCOX-2 selective, non-steroidal anti-inflammatorydrug(NSAID) with analgesic and antipyretic properties.Its approved indications are the treatment of acute pain, the symptomatic treatment ofosteoarthritis andprimarydysmenorrhea in adolescents and adults above 12 years old. It is a potent selective Cyclooxygenase-2 inhibitor and is highlyeffective,withminimumdrug-related side effects, in the treatment of variousforms of pain and inflammatoryconditions.The drug is bestwith the disadvantage of poorwatersolubility.It is notofficialinUSP 23 (1995), BP (1998), and IP (1996). Nimesulide is yellow is h crystalline powder.

Pantoprazole was first studied in 1985, and in 1994 it was approved for medicinal usage in Germany. It is available as a generic drug. It's sold under the brand name Protonix, among others, is a proton pump inhibitor used for the treatment of stomach ulcer short-term treatment of erosive esophagitis due to gastroesophageal reflux diseases (GERD), maintenance of healing of erosive esophagitis, and pathological hypersecretory conditions including Zollinger Ellison syndrome.The benzimidazole group and the imidazopyridine group are the two subgroups of PPIs. Pantoprazole belongs to the class of PPIs known as benzimidazoles. Because benzimidazoles have a slower rate of metabolism than the other two families, their plasma presence is shorter. Pantoprazole permanently inhibited the H+/K+ ATP pumps according to its mode of action. With a drop in the pH of the environment, pantoprazole breaks down more quickly. The H+/K+ ATP pumps are found in the stomach; this medicine would function best there (specifically within the parietal cells of the stomach lining). Producing stomach acid ends at this stage. Pantoprazole binds to these pumps as a result, stopping acid output for up to 24 hours. The investigation medicinal drug was once designed to deal with OA pain alongside with dyspepsia, frequent adverse effect of stand-alone osteoarticular pain (musculoskeletal) pain drugs. The study outcomes suggest that pantoprazole may

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also have really useful effects on gastrointestinal function.

Ultraviolet and Visible Spectrophotometry is one of the most frequently employed analytical tool in the pharmaceutical industry. Ultraviolet and visible absorption Spectrophotometry involves the measurement of absorption of monochromatic radiation by solutions of chemical substances, in the range of 200 nm to 400 nm, and 400 nm to 800 nmof the spectrum, respectively. The amount of absorption depends on the wavelength ofradiation and the structureof compound. The absorption ofradiation is due to the subtraction of energy from the radiation beam when electronsin orbital of lower energy are excited into orbital of higher energy. The various spectrophotometric methods which are used for estimation of drug in combine dos age forminclude;

- Simultaneous equation method
- Absorption ratiomethod (Q-ratio method)
- Geometric correctionmethod
- Orthogonal polynomialmethod
- Difference spectrophotometry
- Derivative spectrophotometry
- Chemical derivatisation method
- Absorption correction method
- Multi component method of analysis.

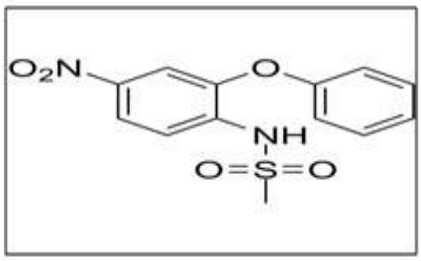

Figure 1. Structure formula of NIM

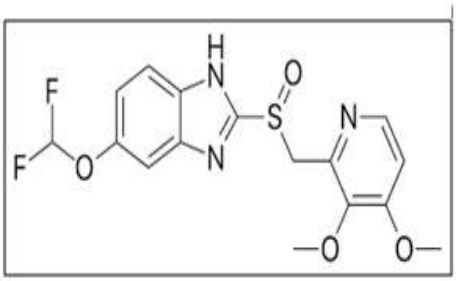

Figure 2. Structure formula of PAN

DERIVATIVE SPECTROPHOTOMETRY:

Derivative spectroscopy, as per name indicates, involves derivative of absorbance of zero order or simple absorption spectrum with respect to wavelength. Derivative spectroscopy follows principal additivity, and absorbance is also dependence on concentration. Using derivative spectroscopy, a wide range of complicated sources may be analyzed, including pharmaceutical dosage forms, inorganic materials including metals, biological samples, and samples containing food. The benefits of derivative spectroscopy are as follows.

1. Distinguish overlapped peaks in complicated data such ternary mixtures.
2. Spectral quality can be improved by removing baseline shift and scattering.
3. Direct UV analysis of complex-originated samples without chemical pretreatment of biologically-derived materials.
4. Enables impurity profile analysis at lower sample content levels.

MATERIALSAND METHODS:

Sample of Nimesulide and Pantoprazole procured from Zota Healthcare Pvt Ltd, Surat, Gujarat.

INSTRUMENT:

Adouble beam Shimadzu UV-1700 series spectrophotometer was used.Absorption and overlain spectra of both test and standard solutions were recorded over the wavelength range of 200 400nm using 1cm quartz cell atfastscanned speed and fixed slit width of 1.0nm.

SELECTION OF DILUENT:

Based on solubility, Nimesulide (NIM) and Pantoprazole (PAN) was soluble in methanol.Hence, methanol was selected as diluent.

PREPARATION OF STOCK SOLUTION:

Accurately weighed and transferred about 50 mg of Nimesulide (NIM) and 10mg of Pantoprazole (PAN) in to 100 ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with diluent. Concentration of Nimesulide (NIM) is 500 µg/ml and Pantoprazole (PAN) 100 µg/ml. Further diluted 5 ml of above solution to 50 ml volumetric flask and volume was make up to the mark with diluent. Concentration of Nimesulide (NIM) is 50 µg/ml and Pantoprazole (PAN) 10

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µg/ml. The optimumwavelength wasselected for the estimationwas 270 nmwhere gives good absorbance.

SELECTION OF WAVELENGTH:

An ideal wavelength is the one that gives Maximum response for the drugs that was to be detected. From appropriate dilutions of the working standard stock solution, 6 µg/ml of PAN and 30 µg/ml of NIM were separately prepared and scanned in the UV range 200–400 nm. The overlain zero-order absorption spectra of PAN and NIM were obtained [Figure 3]. These absorption spectra were converted to first-order derivative spectra by using the instrument mode. After observing the overlain first-order derivative spectra with scaling factor = 2 and $\Delta\lambda = 2$ for PAN and NIM [Figure 4], zero crossing points of drugs were selected for the analysis of other drugs. The first wavelength selected was 249.80 nm (zero crossing of PAN), where NIM showed considerable absorbance. The second wavelength selected was 297.20 nm (zero crossing of NIM), where PAN showed considerable absorbance.

PREPARATION OF STOCK SOLUTION:

Accurately weighed and transferred about 50 mg of Nimesulide (NIM) and 10mg of Pantoprazole (PAN) in to 100 ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with diluent. Concentration of Nimesulide (NIM) is 500 µg/ml and Pantoprazole (PAN) 100 µg/ml. Further diluted 5 ml of above solution to 50 ml volumetric flask and volume was make up to the mark with diluent. Concentration of Nimesulide (NIM) is 50 µg/ml and Pantoprazole (PAN) 10 µg/ml. The optimumwavelength wasselected for the estimationwas 270 nmwhere gives good absorbance.

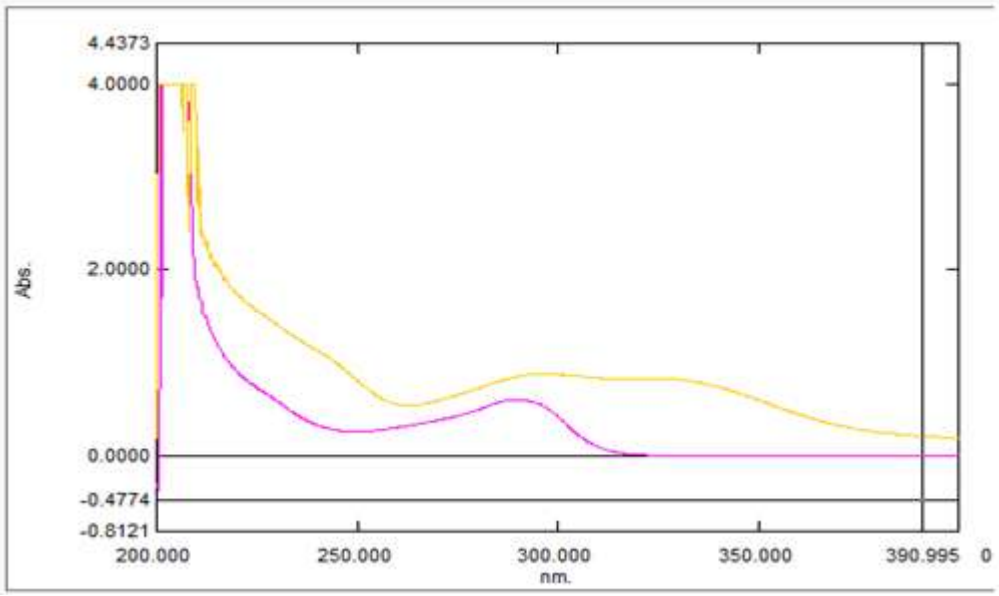

Figure 3. The Overlain Zero-Order Absorption Spectra ofPantoprazole (PAN) And Nimesulide (NIM) Were Obtained

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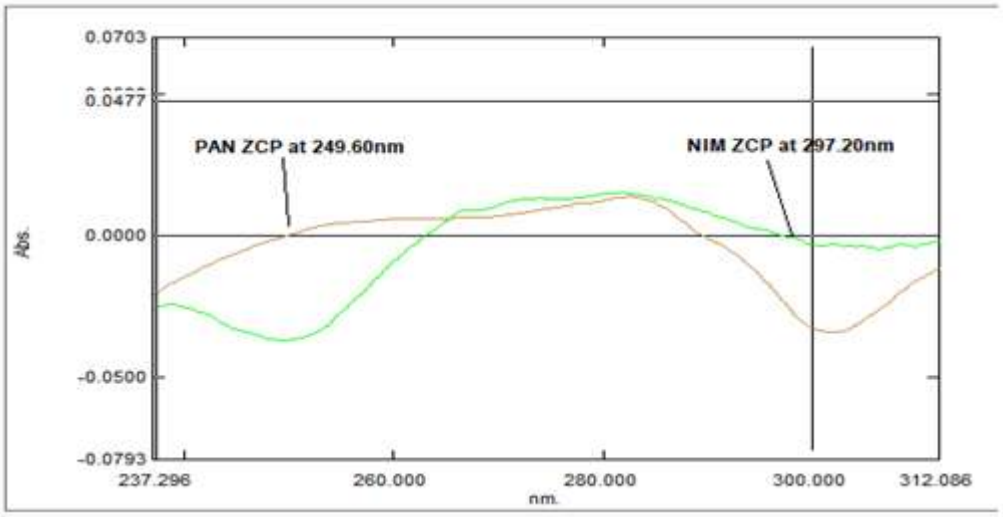

Figure 4 First-Order Derivative Spectra with Scaling Factor = 2 And Δλ = 2 For Pantoprazole (PAN) And Nimesulide (NIM)

II. RESULT AND DISCUSSION:

VALIDATION OF PROPOSED METHOD:

LINEARITY AND RANGE: The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range. The linearity and range of the method was determined by plotting a calibration curve over the concentration range of 2 - 10 µg/ ml for PAN and 10- 50 µg / ml for NIM, respectively. The calibration curve was constructed by plotting areas versus concentrations of 2 - 10 µg/ ml for PAN and 10- 50 µg / ml for NIM, respectively shown in figure 8 and figure 9. Linearity Data shown in table 1. The regression equation was found to be y = 0.0021x + 0.0062 and correlation coefficient was found to be 0.9999 for PAN. The regression equation was found to be y = 0.0013x - 0.0006 and correlation coefficient was found to be 0.9988 for NIM. Each response was the average of three determinations. The Statistical analysis data of calibration curve intercept, slope, and regression equation are shown in Table1.

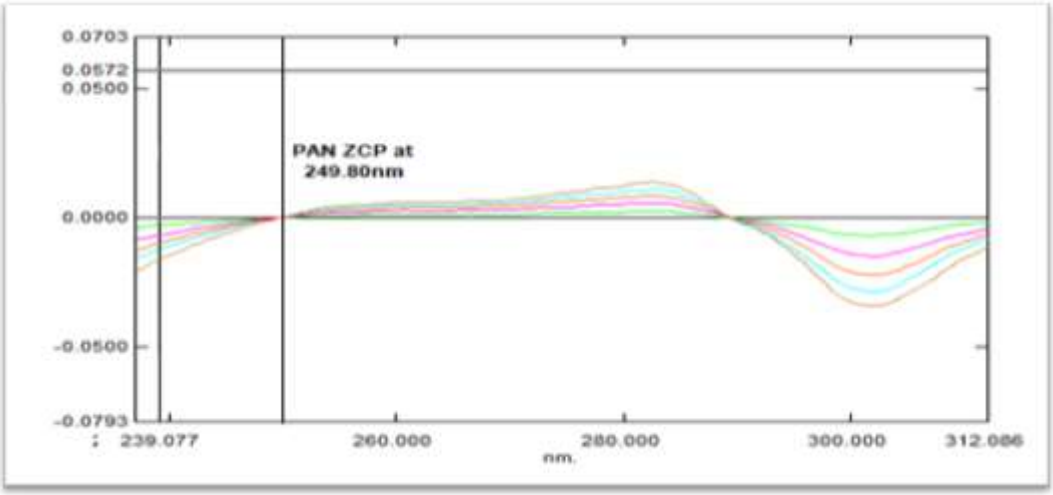

Figure 5 calibration curve of individual drug PAN at 249.80 nm

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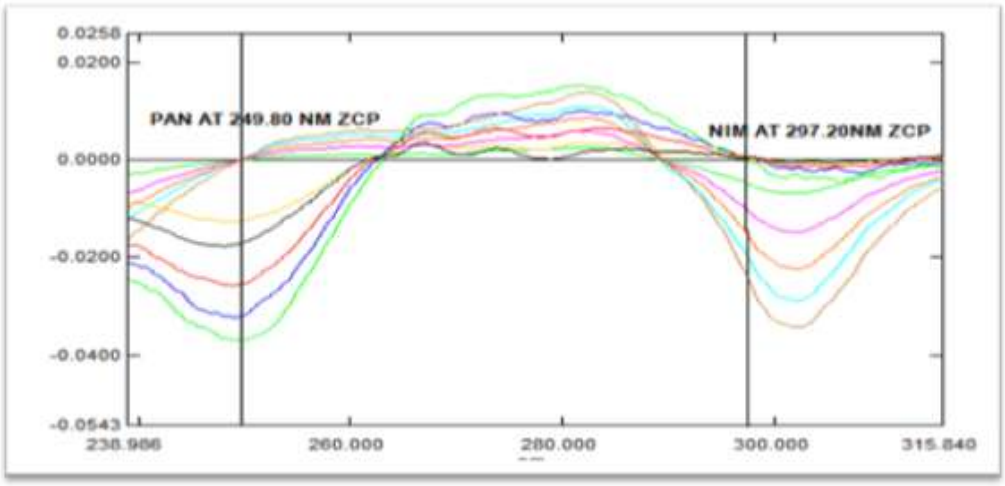

Figure 6. Calibration curve of combine drug PAN and NIM

Table 1. Linearity Data

Drug	Conc.	Absorbance	% RSD	Drug	Conc.	Absorbance	% RSD
PAN	2	0.0105 ± 0.00019	1.81	NIM	10	0.0126 ± 0.00019	1.49
	4	0.0147 ± 0.00019	1.29		20	0.0256 ± 0.00041	1.61
	6	0.0190 ± 0.00032	1.68		30	0.0372 ± 0.00062	1.65
	8	0.0231 ± 0.00039	1.68		40	0.0521 ±0.00066	1.26
	10	0.0275 ± 0.00026	0.94		50	0.0642 ± 0.00093	1.45

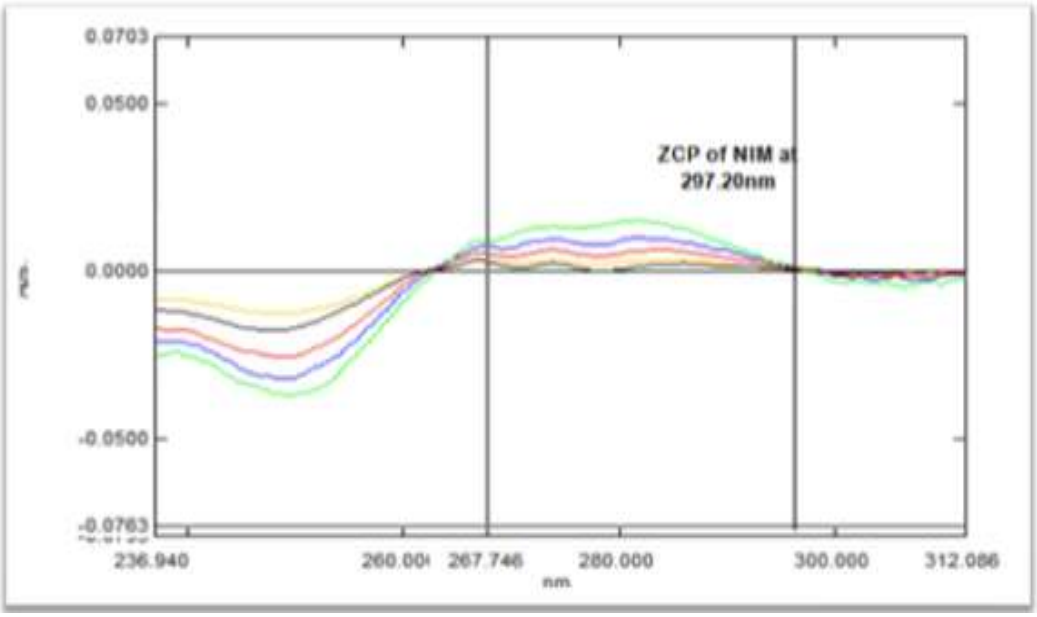

Figure 7. calibration curve of individual drug NIM at 297.20 nm

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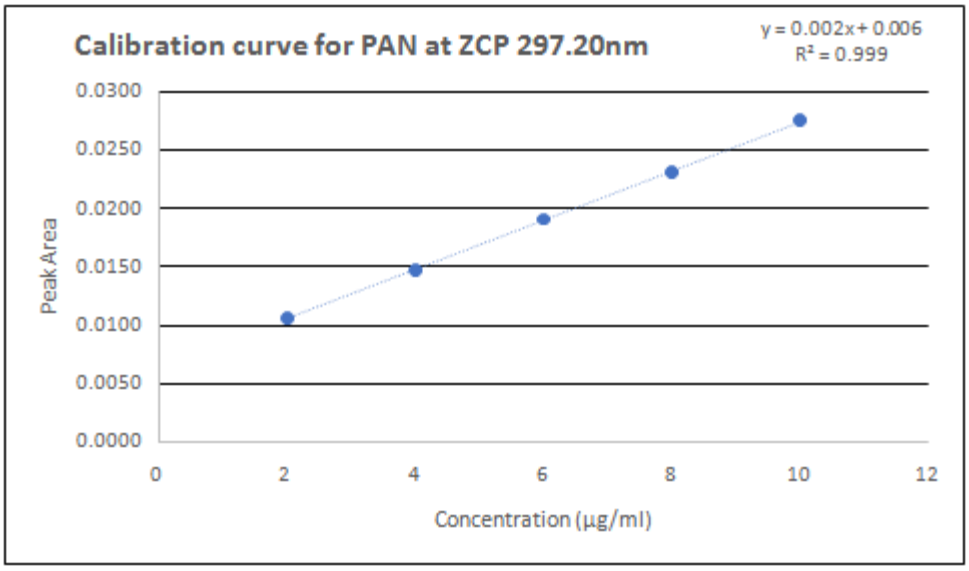

Figure 8. Calibration curve of PAN standard

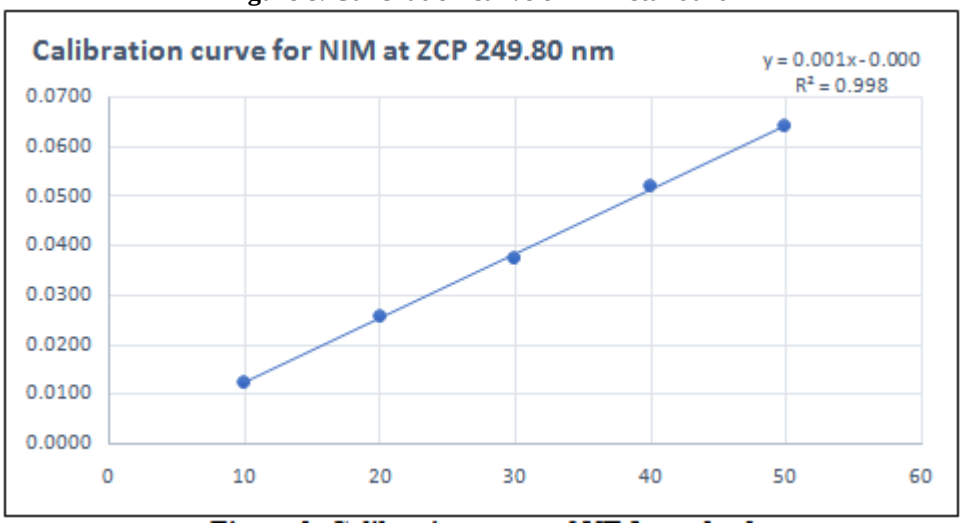

Figure 9. Calibration curve of NIM standard

Table 25. Statistical analysis data of calibration curve

Parameters	**PAN**	**NIM**
Linear Range	2 – 10 µg/ml	10 – 50 µg/ml
Regression Equation	y = 0.0021x + 0.0062	y = 0.0013x – 0.0006
Regression Coefficient (r^2)	0.9999	0.9988
Mean of slope	0.00215	0.00653
Standard deviation of intercept	0.00015	0.00041

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LOD (µg/ml)	0.224	0.207
LOQ (µg/ml)	0.678	0.629

PRECISION:

The repeatability of developed method was determined by analyzing 6µg/ml for PAN solution six times on the same day. The percentage RSD was found to be 1.81. The repeatability of developed method was determined by analyzing 30µg/ml for NIM solution six times on the same day. The percentage RSD was found to be 1.12. The results of repeatability data are shown in **Table 2**.

Table 2. Repeatability study

Concentration	**PAN 6 (µg/ml)**	**NIM 30 (µg/ml)**
Area	0.01910	0.03750
	0.01950	0.03680
	0.01880	0.03760
	0.01860	0.03680
	0.01940	0.03780
	0.01900	0.03730
Mean	**0.0191**	**0.0373**
SD	**0.00034**	**0.00041**
% RSD	**1.81**	**1.12**

SD: Standard Deviation
RSD: Relative Standard Deviation

The results of the intermediate precision (Intraday precision and Interday precision) experiments are shown in **Table 3**for PAN. Replicate analyses of three different concentrations PAN (2, 6, 10 µg/ml) solutions showed good reproducibility. The percentages RSD of intraday and interday studies werefound to be 0.97 – 1.39% and 0.91 - 1.61% respectively for PAN.The results of the intermediate precision (Intraday precision and Interday precision) experiments are shown in **Table 4** for NIM. Replicate analyses of three different concentrations NIM (10, 30, 60 µg/ml) solutions showed good reproducibility. The percentages RSD of intraday and interday studies was found to be 0.41 – 1.37 % and 0.46–1.91% respectively for NIM.The developed method was found to be precise and repeatable on the basis of the mean CV values for the repeatability and intermediate precision studies which were < 2 for PAN and NIM respectively. The separations of the drug and various degradation products in a mixture of stressed samples were found to be similar when the analyses were performed with an LC system on different days.

Table 3. Intraday and Interday Precision study for PAN

Intraday Precision		
Conc. (µg/ml)	**(Absorbance ± S.D) (n=3)**	**% RSD**
2	0.0103 ± 0.0001	0.97

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6	0.0191 ± 0.0003	1.39
10	0.0275 ± 0.0003	1.09
Inter day Precision		
2	0.0105 ± 0.0002	1.80
6	0.0189 ± 0.0003	0.90
10	0.0276 ± 0.0003	0.57

n=Three determination

Table 4. Intraday and Interday Precision study for NIM

Intraday Precision		
Conc. (µg/ml)	**(Absorbance ± S.D) (n=3)**	**%RSD**
10	0.0125 ± 0.0002	1.23
30	0.0372 ± 0.0005	1.21
50	0.0651 ± 0.0006	0.89
Interday Precision		
10	0.0126 ± 0.0002	1.66
30	0.0376 ± 0.0007	1.81
50	0.0640 ± 0.0008	1.25

n=Three determination

ACCURACY:

The recovery of the method was carried out by the standard addition to the preanalysed test sample at three different concentration levels 50%, 100% and 150%. Triplicate determinations were made at each concentration level. The accuracy of the method was determined by calculating recoveries of 2, 4, 6 µg/ml of PAN and 10, 20, 30µg/ml of NIM in the preanalysed concentration 4 µg/ml Pantoprazole (PAN) and 20 µg/ml Nimesulide (NIM) by method of standard addition. The recoveries of PAN and NIMwere calculated by putting the absorbance of the added concentration of PAN and NIMin the regression equation of calibration curve respectively. The recoveries found to be 98.02 % - 102.06 % for PAN and 98.55 %-102.05% for NIM, respectively. The result of the method is indicating good accuracy for chromatographic method. The accuracy result shown in **Table 5**.

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Table 5. Accuracy study

Level	Drug added (µg/ml)	Drug Recovered (µg/ml) [a]	% Drug Recovered ± SD
PAN			
0	4	4.03	100.79 ± 1.81
50	6	6.06	101.06 ± 3.30
100	8	7.84	98.02 ± 1.23
150	10	10.21	102.06 ± 1.80
NIM			
0	20	20.31	101.54 ± 0.77
50	30	29.56	98..55 ± 0.65
100	40	40.82	102.05 ± 0.29
150	50	49.41	98.82 ± 0.58

a=Average of Three determination

LIMIT OF DETECTION AND LIMIT OF QUANTITATION:

According to ICH, the approach based on the standard deviation of the response and mean of slope was used for determining the Limit of detection (LOD) and limit of quantitation (LOQ). The detection limits for PAN and NIMwere found to be 0.22 µg/ml and 0.207 µg/ml respectively, while quantitation limits were found to be 0.68 µg/ml and 0.629 µg/ml respectively. Data Shown in table no.6The above data shows that a microgram quantity of PAN and NIMthe drugs can be accurately and precisely determined. The values of LOD and LOQ of PAN and NIM respectively indicate the sensitivity of proposed method.

Table 6. LOD and LOQ Data

	NIM	PAN
LOD	0.207	0.220
LOQ	0.629	0.680

ROBUSTNESS:

Robustness is the measure of the capacity of a method to remain unaffected by small variations in the method parameters. Robustness of the method was determined in triplicate at a concentration level of 6 µg/mlPantoprazole (PAN) and 30 µg/mlNimesulide (NIM). Robustness of proposed method was performed by changing UV analyst and keeping the remaining conditions (solvent, dilution, UV spectrophotometer) same and RSD of absorbance calculated. The method was found to be robust, as small but deliberate changes in method parameters have no detrimental effect on the method performance as shown in **table 7**. The low value of percentage relative standard deviation indicates that the method is robust.

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Table 7. Robustnessdata

Condition	PAN		NIM	
	Absorbance ± SD	%RSD	Absorbance ± SD	%RSD
Analyst 1	0.0191 ± 0.0004	1.84	0.0371 ± 0.0004	1.08
Analyst 2	0.0190 ± 0.0003	1.69	0.0368 ± 0.0007	1.93

ANALYSIS OF SYNTHETIC MIXTURE:

The developed first derivative UV spectrophotometry method was successfully applied for the estimation of Pantoprazole (PAN) and Nimesulide (NIM) in synthetic mixture. The absorbance of sample measured at ZCP of both drugs, indicating that there is no interference of the excipients present in synthetic mixture. The content of Pantoprazole (PAN) and Nimesulide (NIM) was calculated by measure the absorbances of sample and put this value in the regression equation.

Table 8. Results of synthetic mixture

Formulation	Drug	Amount Taken (µg/ml)	Amount Found^{n} (µg/ml)	%PAN ± SD	%NIM ± SD
Synthetic mixture	Pantoprazole (PAN)	4	3.95	98.80 ± 1.19	98.20 ± 0.58
	Nimesulide (NIM)	20	19.64		

n= Average of Three determination

III. CONCLUSION:

Simple and sensitive first order derivative UV spectrophotometry method were developed for simultaneous estimation of Pantoprazole (PAN) and Nimesulide (NIM) in their synthetic mixture. Based on the results and the statistical parameters obtained, it was concluded that the proposed method of analysis is simple, rapid, accurate, precise and economical. The method did not utilize any extraction step for recovering the drug from the formulation excipient matrixes and they're by decreased the degree of error, time in estimation of drugs and the overall cost of the analysis. The developed method can be employed for routine quality control analysis of Pantoprazole (PAN) and Nimesulide (NIM) in bulk and pharmaceutical formulations.

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