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DEVELOPMENT OF ANALYTICAL METHOD AND VALIDATION OF NADOLOLIN PURE AND PHARMACEUTICAL FORMULATIONS USING UV-SPECTROPHOTOMETRY AND SPECTROFLUORIMETRY

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Nadolol, Spectrophotometry, Spectrofluorimetry, Determination

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ABSTRACT: The development of analytical methods is in need for the estimation of drugs in pure and different pharmaceutical formulations. A simple, sensitive, rapid, accurate, precise and economic spectrophotometric and spectro-fluorimetric method was developed and validated for Nadolol in pure and pharmaceutical formulations. The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines. The wave length (λ_{max}) used for the estimation of Nadolol is 267 nm by spectrophotometry, excitation (λ_{Ex})-267 nm and emission (λ_{Em})-300 nm by spectrofluorimetry. The linearity of the calibration curve was validated by the high values of the correlation coefficient of regression. The percentage of drugs recovered 100.37 ± 0.94% and 99.9 ± 0.59% for spectrophotometric and spectrofluorimetric methods respectively. LOD and LOQ values for Nadolol were found to be 3.531 µg/ml and 10.70 µg/ml by spectrophotometry and 0.45 µg/ml and 1.37 µg/ml by spectrofluorimetry. The developed methods are simple and suitable for the determination of Nadolol in pure and pharmaceutical preparations.

INTRODUCTION: Nadolol, chemically is (2R, 3S)-5{[(2R)-3-tert-butylaminol-2-hydroxy-propyl] oxy} - 1, 2, 3, 4-tetrahydro naphthalene-2,3-diol **Fig. 1** is a non-selective β-blocker which is official in BP [1] and USP [2] used in the treatment of high blood pressure and chest pain. It has a preference for beta-1 receptors, which are predominantly located in the heart, thereby inhibiting the effects of catecholamines and causing a decrease in heart rate and blood pressure, inhibition of beta-2 receptors, which are mainly located in the bronchial smooth muscles of the airways leads to airway constriction similar to that seen in Asthma.

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Review of literature reveals that only a few methods like UV [3-5], Colorimetry [6-7], Fluorimetry [8], HPLC [5, 9-11], Biological fluids using HPLC [12], UHPLC–MS [13], and LC-MS [14], were developed for the determination of Nadolol in pure and Pharmaceutical preparation.

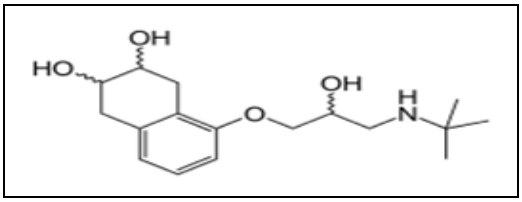

FIG. 1: STRUCTURE OF NADOLOL

MATERIALS:

Instrument: Absorption spectral measurements were carried out with a Systronics 2202 UV-Visible spectrophotometry, fluorescence spectra measurements were carried out with a Perkin Elmer LS 55 spectrofluorimetry, for sonication Branson 2510 sonicator was used.

Chemicals: Nadolol tablets (40 mg) were procured from Walmart Pharmacy 2051 Strachan Road S., Medicine Hat T1B OG4 from Canada. Hydrochloric acid was of AR grade from Nice Pharmaceuticals Pvt. Ltd., and in house produced distilled water was used. Nadolol working standard was obtained as a gift sample from a Pharma Industry, Industrial estate, Ahmedabad.

METHODS:

Preparation of Stock Solution: 25 mg of Nadolol was accurately weighed and transferred to a 25 ml volumetric flask. About 10 ml of water was added, vortexed for about 5 min. The volume was made up to 25 ml and mixed well with water to obtain a final concentration of 1 mg/ml.

Determination of Absorbance Maxima and Fluorescence Maxima: An appropriate aliquot portion of 2 ml of Nadolol from a standard stock solution of Nadolol was transferred to 100ml volumetric flask, mixed with water and the volume was made up to 100 ml with water to obtain the concentration 20 µg/ml of Nadolol. Drug solutions were further diluted necessarily and scanned in spectrophotometry and spectrofluorimetry to determine the absorbance maxima and based on absorbance maxima, emission maxima were determined.

Validation of the Proposed Method: The proposed method was validated according to the International Conference on Harmonization [15].

Linearity and Range:

Spectrophotometry: An appropriate aliquot portion of 2, 2.5, 3, 3.5, 4 and 4.5 ml of Nadolol from standard stock solution of Nadolol were transferred to 100 ml volumetric flask, mixed with water and the volumes were made up to 100 ml with water to obtain concentrations 20, 25, 30, 35, 40 and 45 µg/ml of Nadolol. The absorbance of all the resulting solutions was measured at 267 nm. The calibration curve was constructed by plotting drug concentration versus absorbance obtained.

Spectrofluorimetry: An appropriate aliquot portion of 1, 2, 3, 4 and 5 ml of Nadolol from standard stock solution of Nadolol were transferred to 100 ml volumetric flask, mixed with water and the volumes were made up to 100 ml with water to obtain the concentrations 1, 2, 3, 4 and 5 µg/ml of Nadolol. The fluorescence of the resulting solutions with water was measured at excitation (λ_{Ex})-267 nm, emission (λ_{Em})-300 nm.

Precision: 10 tablets were weighed accurately and crushed into a fine powder using glass mortar and pestle. An accurately weighed quantity of tablet powder equivalent to 25 mg of Nadolol transferred into to 25 ml volumetric flask. 10ml of water was added and sonicated for 5 min, the volume was made up to 25 ml with water, mixed well and filtered it. For spectrophotometric method, 1 ml of the solution of the filtrate was pipetted out and transferred into 25 ml of volumetric flask and the volume was made up to 50 ml with water so that the final solution concentration will be 20 µg/ml. The absorbance of the resulting solution was measured at 267 nm.

For the spectrofluorimetric method, 1 ml of a solution of the filtrate was pipetted out and transferred into 100 ml of volumetric flask and the volume was made up to 100 ml with water. From the above solution, 2 ml was pipetted out and transferred into a 10 ml volumetric flask and made up the volume up to 10 ml with water so that the final solution concentration will be 2 µg/ml. The fluorescence intensity of the resulting solution was measured at excitation (λ_{Ex})-267 nm, emission (λ_{Em})-300 nm using water as a solvent blank.

Accuracy:

Preparation of Stock Solution: The first step is the preparation of the stock solution (500 mg of pure drug of Nadolol was dissolved in 25 ml of water).

Spectrophotometry & Spectrofluorimetry:

Accuracy for 50%: 206 mg of tablet powder weighed accurately (equivalent to 40 mg of Nadolol) and transferred into three different 25 ml volumetric flask. 10ml of water was added and 1ml of the stock solution which contains 20 mg/ml of Nadolol. The solution was sonicated for 3 min, made up the volume up to 25 ml with water. The resulting solutions were filtered separately, and from the filtrate, 1 ml of the solution was pipette out and transferred to a 100 ml volumetric flask and made up the volume up to 100 ml with water. It was repeated for three times of three different weighings.

Accuracy for 100%: 206 mg of tablet powder weighed accurately (equivalent to 40 mg of Nadolol) and transferred into a 25 ml volumetric flask. 10ml of water and 2 ml of the stock solution, which contain 40 mg/ml of Nadolol was added. The solution was sonicated for 3 min and made up the volume up to 25 ml with water. The resulting solutions were filtered and from the filtrate 1 ml was pipetted out transferred into 100 ml volumetric flask and made up the volume up to 100 ml with water. It was repeated for three times of different weighing.

Accuracy for 150%: 206 mg of tablet powder weighed accurately (equivalent to 40 mg of Nadolol) and transferred into a 25 ml volumetric flask. 10 ml of water and 3 ml of the stock solution, which contain 20 mg/ml of Nadolol was added. The above solutions were sonicated for 3 min and made up the volume up to 25 ml with water. The resulting solutions were filtered and from the filtrate 1 ml was pipetted out transferred to 100 ml volumetric flask and make up the volume up to 100 ml with water. It is repeated for three times of different weighing.

For Spectrophotometry the absorbance of all the resulting solutions was measured at 267 nm and for Spectrofluorimetry the intensity of fluorescence of all the resulting solutions was measured at excitation (λ_{Ex})-267 nm and emission (λ_{Em})-300 nm using water as solvent blank.

Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Detection limit (DL) may be expressed as:

$$DL = 3.3\ \sigma / S$$

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

The slope 'S' is estimated from the calibration curve of the analyte. The estimation of σ was carried out using a calibration curve.

Limit of Quantitation: The quantitation limit of an individual analytical procedure is the lowest analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities or degraded products.

$$QL = 10\ \sigma / S$$

Ruggedness: Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. Ruggedness is determined by the analysis of aliquots by different analysts. 20 µg/ml and 2 µg/ml solutions were prepared and analyzed using spectrophotometer and spectrofluorometer respectively.

Robustness: The robustness of an analytical procedure is a measure of is capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was carried out by changing the wavelength by one nm at 266 nm and 269 nm for spectrophotometry and emission wavelength at 299 nm and 301 nm respectively.

RESULTS AND DISCUSSION:

Determination of Absorption Maximum: Absorbance maxima were determined in the spectro-photometer by taking 20 µg/ml Nadolol drug which is dissolved in water and scanned from 200-400 nm using UV-Visible Spectrophotometer. The absorption spectra presented in **Fig. 2.** It was found that the absorption maximum of 267 nm was identified in the spectra.

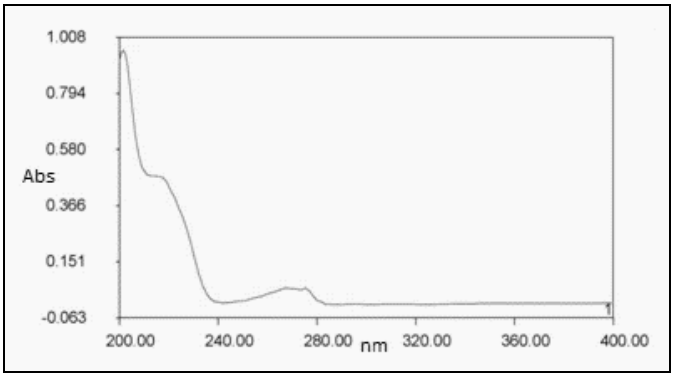

FIG. 2: UV-SPECTRUM OF NADOLOL WITH WATER

Determination of Emission Maxima: Absorbance maxima were determined in spectrofluorimetry by taking 3 µg/ml Nadolol drug which is dissolved in water and excitation maxima were fixed at 267 nm. Since the wavelength shows the highest absorbance

the emission spectra were scanned from 270-500 nm. The emission spectrum is presented in **Fig. 3**. It was found that an emission maximum 300nm was identified in the spectra.

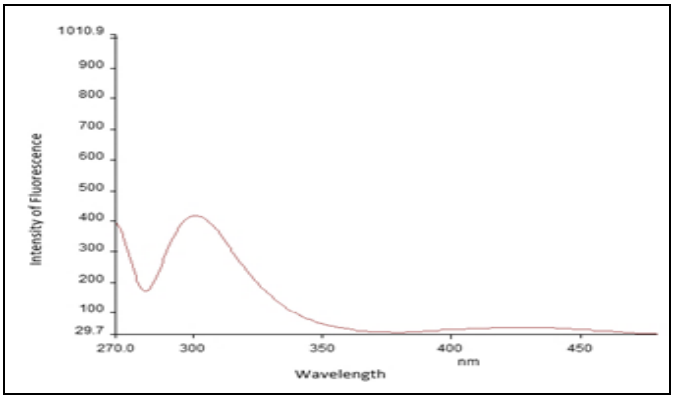

FIG. 3: FLUORIMETRY SPECTRUM OF NADOLOL USING WATER

Linearity and Range: In spectrophotometry Calibration standards for Nadolol covering a range of 20-45 µg/ml were prepared in serial dilutions that were made with water. The absorbance of all resulting concentrations was measured at 267 nm. The graph between the concentration and absorbance was plotted. The regression equation was found to be y = 0.004x - 0.005. The correlation coefficient (R^2) of the standard curve was found to be 0.985. The obtained data are presented in **Table 1** and the calibration graph is presented in **Fig. 4** respectively.

TABLE 1: LINEARITY AND RANGE OF NADOLOL USING SPECTROPHOTOMETER

S. no.	Concentration (µg/ml)	Absorbance
1	20	0.089
2	25	0.112
3	30	0.135
4	35	0.157
5	40	0.181
6	45	0.207

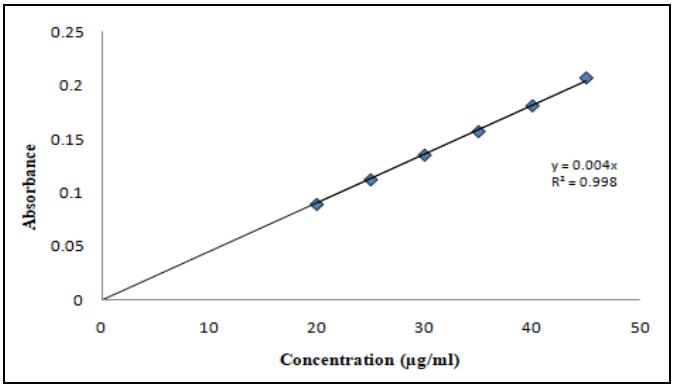

FIG. 4: CALIBRATION GRAPH OF NADOLOL USING SPECTROPHOTOMETER

In spectrofluorimetry calibration standards for Nadolol covering a range of 1 to 5 µg/ml were prepared in serial dilutions that were made with water. The intensity of fluorescence of all the resulting concentrations was measured at excitation (λ_{Ex})-267 nm and emission (λ_{Em})-300 nm.

The data is presented in **Table 2** and the graph between the concentration and absorbance was plotted in **Fig. 5**. The regression equation was found to be y = 130.9x + 6.5. The correlation coefficient (R^2) of the standard curve was found to be 0.99.

TABLE 2: LINEARITY AND RANGE OF NADOLOL USING SPECTROPHOTOMETER

S. no.	Concentration (µg/ml)	Intensity of Fluorescence
1	1	151
2	2	237
3	3	418
4	4	532
5	5	658

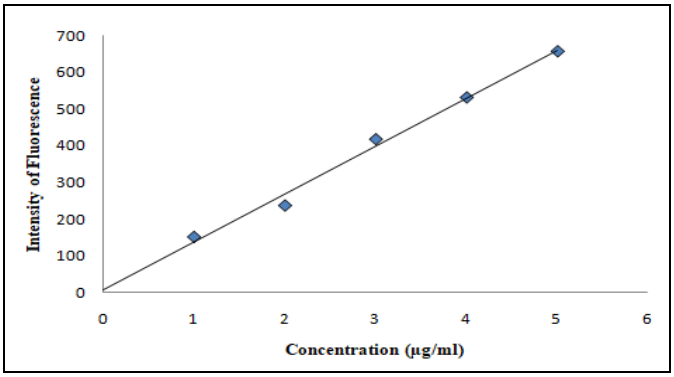

FIG. 5: CALIBRATION GRAPH OF NADOLOL USING SPECTROFLUOROMETER

Precision: The method was carried out as described. The results were presented in **Tables 3** and **4** for spectrophotometry and spectrofluorimetry respectively.

It was found that the amount of drugs found to be present in the sample was found to be 100.25 ± 0.20 and 100.65 ± 0.035 for spectrophotometry **Table 3** and spectrofluorimetry **Table 4,** respectively.

The values obtained in the repeatability (precision) shows that there is no significant difference in the precision value. Hence, that developed method can be used to analyte the Nadolol in tablet formulation. There is no evidence of interference of excitation with Nadolol.

TABLE 3: PRECISION STUDY OF NADOLOL USING SPECTROFLUOROMETER

S. no.	Weight of the tablet powder(mg)	Absorbance	Drug content present (mg)	Percentage found (%)
1	206.05	0.103	40.40	101
2	206.04	0.102	40.04	100.1
3	206.01	0.101	39.71	99.27
4	206.03	0.102	40.11	100.24
5	206.02	0.103	40.50	101.25
6	206.04	0.101	39.72	99.3
		Mean		100.25
		S.D		0.207
		RSD		0.002

The mean precision vale was found to be 100.19 ± 0.828%. The value was obtained from 99.3 to 101.25% by the spectrophotometric method.

TABLE 4: PRECISION STUDY OF NADOLOL USING SPECTROFLUOROMETER

S. no.	Weight of the tablet powder (mg)	Intensity of fluorescence	Drug content present (mg)	Percentage found (%)
1	206.03	231.02	40.33	100.82
2	206.01	230.19	40.15	100.37
3	206.02	230.91	40.31	100.77
4	206.04	229.97	40.15	100.37
5	206.03	232.04	40.50	101.25
6	206.05	230.14	40.15	100.37
		Mean		100.65
		S.D		0.0357
		RSD		0.003

The mean precision vale was found to be 100.65 ± 0.0357%. The value was obtained from 100.37 to 100.82% by the spectrofluorimetric method.

TABLE 5: ACCURACY STUDY OF NADOLOL USING SPECTROPHOTOMETER

S. no.	Percentage level	Sample weight (mg)	Drug in the tablet powder (mg)	Pure drug added (mg)	Total drug content (mg)	Absorbance	Amount found (mg)	Amount Recovered	Percentage recovery (%)
1	50	206.05	40	20	60	0.231	60.14	20.14	100.7
2	50	206.06	40	20	60	0.232	60.39	20.39	101.95
3	50	206.04	40	20	60	0.230	59.98	19.88	99.4
4	100	206.01	40	40	80	0.308	80.2	40.2	100.5
5	100	206.03	40	40	80	0.306	79.67	39.67	99.17
6	100	206.04	40	40	80	0.308	80.18	40.19	100.47
7	150	206.02	40	60	100	0.385	100.25	60.25	100.6
8	150	206.05	40	60	100	0.383	99.71	60.71	99.27
9	150	206.01	40	60	100	0.386	100.51	60.51	101.27
				Mean					100.37
				SD					0.940
				RSD					0.009

Accuracy: From the data drug-excipients interactions and drug-solvent interactions have not been found. Hence, there is no interference of any component with the drug has been proved. The percentage of recovery was found to be 100.37 ± 0.940%. The value was obtained from 99.27 to 101.95% by the spectrofluorimetric method. The resulting data is presented in **Table 5**.

TABLE 6: ACCURACY STUDY OF NADOLOL USING SPECTROFLUORIMETER

S. no.	Percentage level	Sample weight (mg)	Drug in the tablet powder (mg)	Pure drug added (mg)	Total drug content (mg)	Intensity of fluorescence	Amount found (mg)	Amount Recovered	Percentage recovery (%)
1	50	206.01	40	20	60	301.04	59 .99	19.99	99.97
2	50	206.07	40	20	60	303.12	60.37	19.37	100.92
3	50	206.04	40	20	60	302.13	60.21	19.21	100.52
4	100	206.05	40	40	80	402.54	80.11	40.11	100.27

5	100	206.03	40	40	80	400.56	79.83	39.83	99.57
6	100	206.05	40	40	80	401.23	79.95	39.95	99.87
7	150	206.01	40	60	100	499.26	99.51	59.51	99.18
8	150	206.06	40	60	100	499.13	99.46	59.46	99.1
9	150	206.02	40	60	100	501.02	99.86	59.86	99.76
				Mean					99.90
				SD					0.596
				RSD					0.005

The percentage of recovery was found to be 99.90 ± 0.596%. The value was obtained from 99.1 to 100.92% by the spectrophotometric method. The resulting data is presented in **Table 6.**

LOD and LOQ: The LOD was found to be 3.53 µg/ml and the LOQ concentration was found to be 10.7 µg/ml with water by spectrophotometric method.

The LOD was found to be 0.45 µg/ml and the LOQ concentration was found to be 1.37µg/ml with Water by the spectrofluorimetric method.

Ruggedness: Ruggedness data is presented in **Table 7** for spectrophotometric and spectro-fluorometric methods.

TABLE 7: RUGGEDNESS OF NADOLOL

S. no.	Analyst -1	Analyst-2
Absorbance		
1	0.089	0.085
2	0.087	0.087
3	0.088	0.086
Intensity of fluorescence		
1	237	238
2	236	237
3	237	236

Robustness: There is no significant difference in absorbance and fluorescence observed when the minor changes like the one-nanometer difference in spectrophotometer and spectrofluorometer.

TABLE 8: VALIDATION PROFILE OF NADOLOL WITH WATER

Parameters	Spectrophotometry	Spectrofluorimetry
Linearity range (µg/ml)	20-45	1-5
Precision (%)	100.25 ± 0.20%.	100.65 ± 0.035%
Accuracy (%)	100.37 ± 0.94 %.	99.90 ± 0.59%
50%	100.68±1.27	100.47±0.46
100%	100.04±0.75	99.90±0.35
150%	100.38±1.01	99.34±0.50
LOD (µg/ml)	3.53	0.45
LOQ (µg/ml)	10.7	1.37

Validation Profile: Performing replicate analysis of the standard solutions was used to assess the accuracy and precision and reproducibility of the proposed methods. The selected concentration within the calibration range was prepared in water and analyzed with the relevant calibration curves to determine the intra-day and inter-day variability. The validation profile of Nadolol with water is presented in **Table 8** by spectrophotometric and spectrofluorimetric methods.

CONCLUSION: Spectrophotometric and spectrofluorimetric method for quantifying Nadolol in pure and formulation has been developed and validated. The developed method is precise, accurate and linear over the concentration range from 20-45 µg/ml and 1-5 µg/ml for spectrophotometric and spectrofluorimetric method respectively. The precision was found to be 100.25 ± 0.2% and 100.65 ± 0.03% for spectrophotometry and spectrofluorimetry respectively.

The percentage of drugs recovered 100.37 ± 0.94% and 99.9 ± 0.59% for spectrophotometric and spectrofluorimetric methods respectively. The LOD and LOQ were found to be 3.53 µg/ml and 10.70 µg/ml for spectrophotometry and LOD and LOQ were found to be 0.45 µg/ml and 1.371 µg/ml for spectrofluorimetry with water. Among the two developed methods, the spectrofluorimetric method is highly sensitive than the spectrophotometric method. These methods are simple and suitable for the determination of Nadolol in pure and pharmaceutical preparations.

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CONFLICT OF INTEREST: The authors have declared no conflicts of interest.

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