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DEVELOPMENT AND VALIDATION OF SECOND ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF ATENOLOL AND NIFEDIPINE IN COMBINED DOSAGE FORM

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Atenolol (ATN), Nifedipine (NIF), Spectroscopy, Second order Derivative, Validation

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ABSTRACT: A simple, precise, accurate and reproducible spectrophotometric method has been developed for Simultaneous estimation of Atenolol and Nifedipine by employing second order UV derivative Spectrophotometric method in Water: ACN (60:40). The Second order derivative absorption at 245.63 nm (zero cross point of Nifedipine) was used for quantification of Atenolol and 218.7 nm (zero cross point of Atenolol) for quantification of Nifedipine. The linearity was established over the concentration range of 50-150 µg/ml and 20-60 µg/ml for Atenolol and Nifedipine with correlation coefficient (r^2) of 0.999 and 0.998 respectively. Interday and intraday studies showed repeatability of the method. The mean % recoveries were found to be in the range of 99.25% – 101.15% and 98.56% – 101.85% for Atenolol and Nifedipine, respectively. The method is successfully applied to pharmaceutical formulation, with no interference from excipients as indicated by the recovery study. The proposed method has been validated as per ICH guideline and successfully applied to the simultaneous estimation of Atenolol (ATN) and Nifedipine (NIF) in their combined Tablet dosage form. So it was recommended for routine analysis. The use of 60% Distilled water as a solvent makes method Cost effective and economical.

INTRODUCTION: Atenolol is chemically 2-(4-{2-hydroxy-3-[(1-methylethyl) amino] propoxy} phenyl)acetamide shown in **Figure 1A.** It acts by competing with sympathomimetic neuro-transmitters such as catecholamines for binding at β_1-adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation.

This results in a reduction in resting heart rate, cardiac output, systolic and diastolic blood pressure, and reflex orthostatic hypotension.

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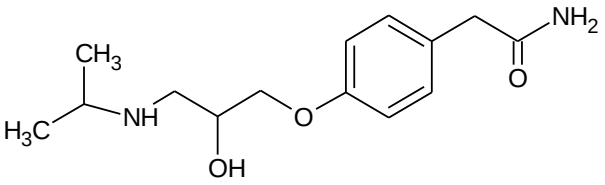
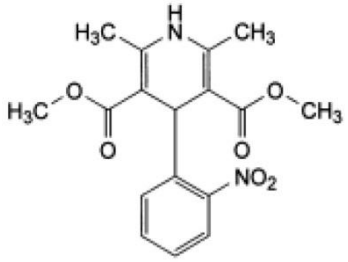
Nifedipine (3, 5-dimethyl 2, 6-dimethyl-4-(2-nitrophenyl)-1, 4-dihydropyridine-3, 5 dicarbo xylate) shown in **Figure 1B**. It acts by blocking the Ca^{++} channel. It is used in the treatment of Variant (Prinzmetal) Angina, Hypertension and Left ventricular dysfunction. The mixture of Atenolol and Nifedipine is commonly used for hypertension.

A Tablet Dosage form containing (ATN-50 mg & NIF-20 mg) is commercially available. Literature survey revealed the most recent methods for determination of Atenolol and Nifedipine like liquid chromatographic, electrochemical and spectrophotometric techniques. Different Spectro-photometric methods were available in literature survey. However, no 2^{nd} order derivative method in this solvent ratio had been reported till the date which makes its cost effective for the determination of this combination.



(A) ATENOLOL



(B) NIFEDIPINE

FIGURE 1: CHEMICAL STRUCTURE OF (A) ATN AND (B) NIF

MATERIALS AND METHODS [5-7]:

Reagents and chemicals: Analytically pure Atenolol and Nifedipine were used. Tablet of Atenolol and Nifedipine in combine dosage form, with a 50 mg ATN and 20 mg NIF label claim (NILOL), manufactured by Intas Pharmaceuticals, Ahmedabad-India, were procured from a local pharmacy.

Instruments: The Spectrophotometer used for study was Shimadzu UV/Vis 1800 double beam spectrophotometer with wavelength accuracy (± 0.3 nm), 1 cm matched quartz cells and UV probe 2.35 software was used for all the spectral measurements. Calibrated analytical balance Denver SI234, Germany was used for weighing purpose. All statistical calculations were carried out using Microsoft excel 2010 analytical tool.

Preparation of ATN standard stock solution: Accurately weighed 25 mg of ATN was transferred into 25 ml volumetric flask and dissolved in 10.0 ml of ACN and diluted up to the mark with water to get a stock solution containing ATN (1000 µg/ml ATN).

Preparation of NIF standard stock solution: Accurately weighed 6.25 mg of NIF was transferred into 25 ml volumetric flask and dissolved in 10.0 ml of ACN and diluted up to the mark with water to get a stock solution containing NIF (400 µg/ml NIF).

Selection of Analytical Wavelength: Solutions of ATN and NIF were prepared in Water: ACN (60:40) by appropriate dilution and spectrum was recorded between 200-400 nm and all zero order spectrums (D0) were converted to second derivative spectrum (D2). The overlain second derivative spectrums of ATN and NIF at different concentration were recorded. The zero crossing point (ZCP) of ATN was found to be 218.7 nm and ZCP of NIF was found to be 245.63 nm.

Method validation [8]: The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility.

Linearity: Appropriate volume of aliquot from ATN and NIF standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted upto the mark with Water : ACN (60:40) to give a solution containing 50-150 µg/ml of ATN and 20-60 µg/ml NIF. All D2 Spectrum were recorded using above spectrophotometric condition. D2 absorbance at 245.63 nm and 218.7 nm were recorded for ATN and NIF respectively. Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs.

Accuracy: Accuracy was assessed by determination of the recovery study. Addition of standard drug to the pre quantified sample preparation at three different concentration levels 80, 100 and 120%. Each concentration was analyzed three times and average recoveries were measured.

Precision: The intraday and interday precision study of ATN and NIF was carried out by estimating different concentrations of ATN (50-150 µg/ml) and NIF (20-60 µg/ml), three times on the same day and on three different days and the results were reported in terms of % RSD.

Detection limit and Quantitation limit: ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve.

In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3σ/S and 10σ/S criterions, respectively;

Where, σ is the standard deviation of y-intercepts of regression lines and, **S** is the slope of the calibration curve.

Determination of ATN and NIF from combined Tablet dosage form: Accurately weighed 118.00 mg (equivalent to 50 mg of ATN and 20 mg of NIF) of 20 tablet powder was transferred into 25 ml of volumetric flask, diluted up to mark with solvent and sonicated for 30 minutes. The resulting solution (1.0 ml) was transferred to 10 ml volumetric flask and diluted with mobile phase to get a solution containing 100 µg/ml of ATN and 40 µg/ml of NIF.

RESULTS & DISCUSSION: For the solvent ratio Water: ACN(60:40) the second order derivative spectra shows 245.63 nm (Zero crossing point of NIF) and 218.7 nm (zero crossing point of ATN) for the simultaneous estimation of ATN and NIF which are shown in **Figure 2**.

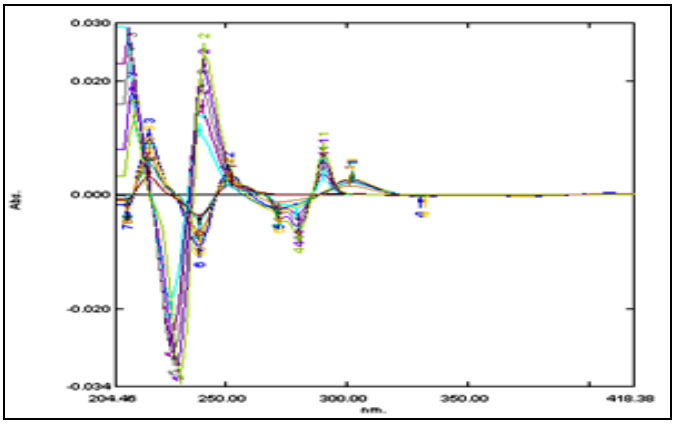


FIGURE 2: OVERLAY OF SECOND ORDER DERIVATIVE OF ATN (50-150 µg/ml) and NIF (20-60 µg/ml)

The linearity range of 50-150 µg/ml & 20-60 µg/ml for ATN and NIF was taken respectively. Straight line equations were obtained from these calibration curves which were shown in **Figure 3 & 4**. The Correlation Coefficients (r^2) for ATN & NIF was found to be 0.999 & 0.998 respectively.

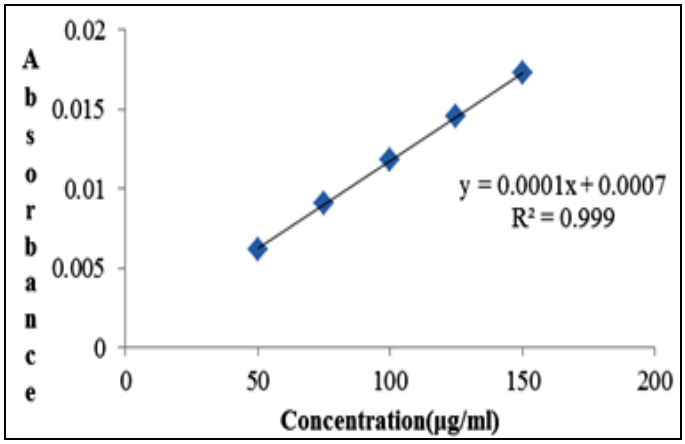


FIGURE 3: CALIBRATION CURVE OF ATN

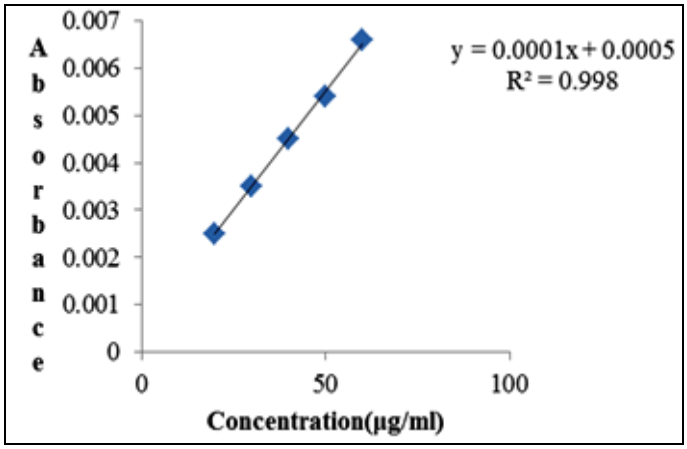


FIGURE 4: CALIBRATION CURVE OF NIF

For the accuracy of the method, recovery studies were done and the percentage recovery found was 99.25% – 101.15% and 98.56% – 101.85% respectively for ATN and NIF Shown in **Table 1**.

TABLE 1: RESULTS OF RECOVERY STUDIES (TABLET)

Level	Amt. of sample (µg/ml)		Amt. of std drug added (µg/ml)		Amt. recovered (µg/ml)		%Mean recovery ± SD	
	ATN	NIF	ATN	NIF	ATN	NIF	ATN	NIF
80%	50	20	40	16	89.56 89.42 89.00	35.20 35.25 36.00	99.25±0.32	98.56±1.24
100%	50	20	50	20	100.95 99.87 101.45	41.17 40.95 40.10	100.75±0.80	101.85±1.41
120%	50	20	60	24	109.10 111.50 110.17	45.00 44.12 44.40	101.15±1.2	101.15±1.02

The intraday and interday precision was expressed in terms of relative standard deviation (RSD). For intraday & interday precision % RSD for ATN & NIF was found to be satisfactory as shown in **Table 2 and 3**. The summary of the validation parameter was shown in **Table 4**.

TABLE 2: INTRADAY PRECISION DATA FOR ATN AT 245 nm AND NIF AT 218 nm

Concentration (µg/ml)		Mean absorbance± SD		%RSD	
ATN	NIF	ATN	NIF	ATN	NIF
50	20	0.0061±0.0001	0.0026±0.0001	1.16	1.89
75	30	0.0090±0.0004	0.0036±0.0002	1.20	1.50
100	40	0.0117±0.0002	0.0046±0.0001	1.70	1.98
125	50	0.0145±0.0002	0.0055±0.0004	1.37	1.70
150	60	0.0172±0.0003	0.0067±0.0006	1.74	1.85

TABLE 3: INTERDAY PRECISION DATA FOR ATN AT 245 nm AND NIF AT 218 nm

Concentration(µg/ml)		Mean absorbance± SD		%RSD	
ATN	NIF	ATN	NIF	ATN	NIF
50	20	0.0062±0.0001	0.0025±0.0001	1.61	0.48
75	30	0.0091±0.0002	0.0035±0.0003	1.98	1.80
100	40	0.0118±0.0001	0.0045±0.0001	0.84	1.99
125	50	0.0146±0.0004	0.0054±0.0004	1.85	1.08
150	60	0.0173±0.0002	0.0066±0.0006	1.73	1.61

TABLE 4: SUMMARY OF VALIDATION PARAMETERS

Parameter	Atenolol	Nifedipine
Linearity (µg/ml)	50 –150 µg/ml	20 –60 µg/ml
Co-relation coefficient(r^2)	0.999	0.998
Slope	0.0001	0.0001
Intercept	0.0007	0.0005
LOD (µg/ml)	5.214	0.52
LOQ (µg/ml)	15.81	1.58
Precision		
Intraday (n=3) %RSD	1.434	1.784
Interday (n=3) % RSD	1.602	1.392

The assay of marketed formulation was found to be 98.26% for ATN & 97.25% for NIF respectively shown in **Table 5**.

Hence, the proposed method was evaluated statistically and was validated in terms of linearity, accuracy, precision and ruggedness. The present work provides an accurate and sensitive method for the analysis of ATN and NIF in bulk and tablet formulation.

TABLE 5: RESULTS OF ANALYSIS OF TABLET FORMULATION

Tablet	Label claim (mg/tablet)		Assay ± SD (% of label claim)	
NILOL	ATN	NIF	ATN	NIF
	50	20	98.26±1.58	97.25±0.59

CONCLUSIONS: Based on the results obtained, it was concluded that the proposed method is accurate, reproducible & economical and can be employed for routine quality control of Atenolol and Nifedipine in bulk and pharmaceutical dosage form.

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