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Development and Validation of First Order Derivative Spectrophotometric method for simultaneous estimation of Nifedipine and Metoprolol Succinate in Synthetic Mixture

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical first derivative spectrophotometric method for the simultaneous determination of Nifedipine (NIF)and Metoprolol Succinate (MET)in synthetic mixture. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra was obtained in methanol and the determinations were made at 283.80 nm (ZCP of nifedipine) for metoprolol succinate and 242.60 nm (ZCP of metoprolol succinate) for nifedipine. The linearity was obtained in the concentration range of succinate 5-25 µg/ml for nifedipine and 25-125µg/ml for Metoprolol Succinate. The mean recovery was 99.64 and 99.41 for Nifedipine and Metoprolol succinate, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of Nifedipine and Metoprolol succinate in synthetic mixture. The results of analysis have been validated statistically and by recovery studies.

Keywords: Spectroscopic method, First Order Derivativemethod, Nifedipine and MetoprololSuccinate.

INTRODUCTION

The aim of the present work was to develop a new simple, rapid, selective method for the simultaneous determination of components having overlapping spectra in binary mixtures, having the advantages of minimal data processing and a wider range of applications over the previously mentioned methods. To prove the ability of the newly described method in resolving the overlapping spectral data and simultaneous determination of each component, it was applied for the analysis of a mixture of Nifedipine (NIF) and Metoprolol SuccinateSuccinate (MET) formulated together in the form of synthetic mixture widely used for the treatment of heart related problems accompanying several hypertension.

Nifedipine is dimethyl 1, 4-dihydro-2, 6- dimethyl-4-(2-nitrophenyl)pyridine-3,5- dicarboxylate.[1][2] It is a calcium channel blocker, one of the most widely used coronary vasodilators.[3][4] Nifedipine acts by blocking the inward movement of calcium by binding to L-type calcium channels in the heart and smooth muscle of the coronary and peripheral arteriolar vasculature. This causes vascular smooth muscle to relax, dilating mainly arterioles.[5][6]Metoprolol succinate is chemically (*RS*)-1-(Isopropylamino)-3-[4-(2 methoxyethyl)phenoxy]propan-2-ol succinate [1], is a cardio selective β-blocker, used in the treatment of hypertension, angina pectoris, arrhythmia, myocardial infraction and heart failure[2]. It is official in IP[3],

BP[4]and USP[5]. Describe potentiometry method for its estimation. Literature survey reveals UV spectrophotometric method[6], RP-HPLC method[7], validated HPLC method for estimation of metoprolol in human plasma[8], simultaneous spectrophotometric method with other drug[9] and RP-HPLC method with other drug[10]in pharmaceutical dosage forms as well as in biological fluids.

(A) (B)

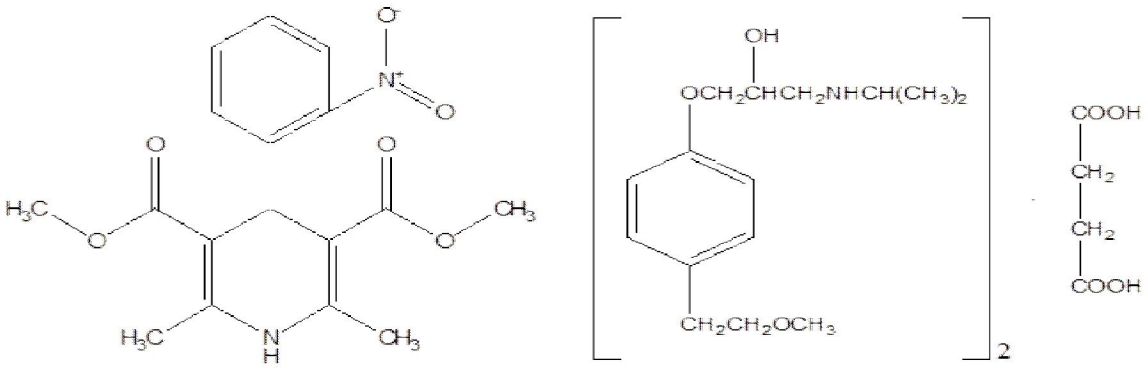

Fig.1(A) is Structure of Nifedipine and **(B)** is structure of Metoprolol Succinate.

1.1.THEORY

We can find out concentration of both the drug from combination mixture using the linearity equation. In this method using the absorbance of both the drug and mixture at their wavelength and put this value in following equation and we can find out the concentration of drugs present in combination.

$$Y = mx + c \quad \text{---------- (1)}$$

Where,

Y = Absorbance

m = Slop

x = Concentration

c = Intercept

2. MATERIAL AND METHOD

2.1.Apparatus

A double beam UV/Visible spectrophotometer(Shimadzumodel2450,Japan) with spectral width of 2 nm, 1 cm quartz cells was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software.

2.2. Reference samples

NIF and MET reference standard are kindly supply by J.B. Chemicals, Ankleshwar and CTX Life Science, Surat as a gift sample respectively.

2.3. MATERIALS AND REAGENTS

Methanol AR grade (RANKEM)

2.4.STANDARD SOLUTIONS

2.4.1. Standard solution of nifedipine (NIF)

Accurately weighed quantity of NIF10mg was transferred to 100ml volumetric flask, dissolved and diluted up to mark with Methanol to give a stock solution having strength 100µg/ml.

2.4.2. Standard solution of metoprolol succinate (MET)

Accurately weighed quantity of MET100mg was transferred in to100mlvolumetric flask, dissolved and diluted up to mark with Methanol to give a stock solution having strength 1000µg/ml.

2.4.3. Preparation of standard mixture

Pipette out accurately 0.5 ml of NIF stock solution (100µg/ml), 0.25 ml of MET stock solution (1000µg/ml) in 10 ml volumetric flask and make up the volume up to the mark with Methanol. It gives solution containing NIF 5 µg/ml, MET 25µg/ml.

2.4.4. Test sample preparation

Dissolve synthetic mixture formulation in 100 ml volumetric flask containing 100 ml methanol. Take 1 ml tablet sample solution in 10 ml volumetric flask and make up volume up to mark with methanol.

3. METHODOLOGY

The standard solutions of NIF (10 µg/ml) and MET (50µg/ml) were scanned separately in the UV range of 200-400nm. The zero-order spectra thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 1 nm. The two spectra were overlain and it appeared that NIF showed zero crossing at 283.80 nm, while MET showed zero crossing at 242.60 nm. At the zero crossing point (ZCP) of NIF (283.80 nm), MET showed a first derivative absorbance, whereas at the ZCP of MET (242.60nm), NIF showed a first-derivative absorbance. Hence 242.60and 283.80 nm was selected as analytical wavelengths for determination of NIF and MET, respectively. These two wavelengths can be employed for the determination of

NIFand MET without any interference from the other drug intheir synthetic mixture formulation.

4. RESULTAND DISCUSSION

4.1.Selection of wavelength and method development for determination of Nifedipine and Metoprolol Succinate

The standard solution of NIF and MET were scanned separately between200-400nm, and zero-order spectra were not showed overlapping peaks.(figure4.1.1)

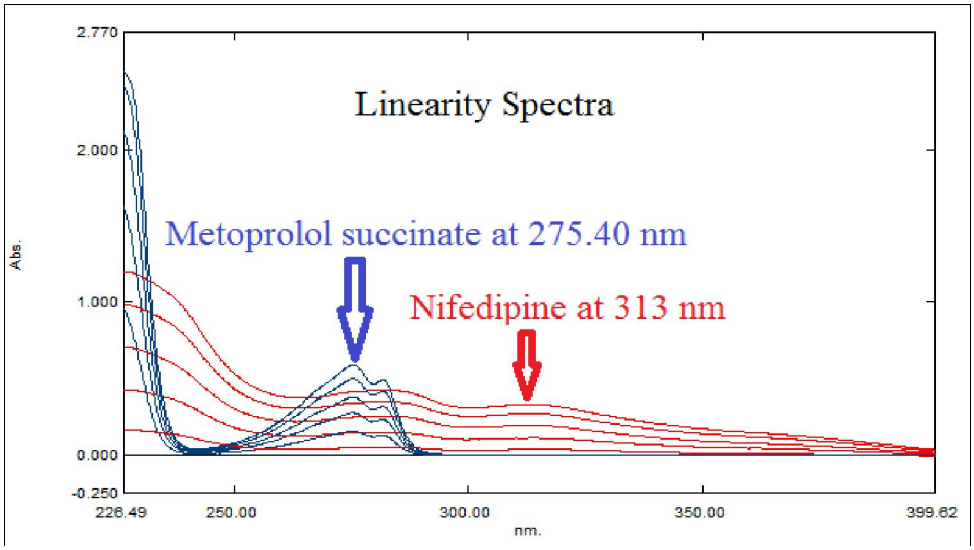

Figure4.1.1 Overlain zero order spectra of NIF and MET(1:5) ratios, respectively

Thus obtained spectra were then processed to obtain first-derivative spectra.

First order derivative spectrum for NIF showed four zero crossing points: 283.80 nm. The wavelength selected for estimation of NIFwas242.60 nm because it showed r^2>0.9984at this wavelength in mixture. (Figure4.1.2)

First order derivative spectrum for MET showed two zero crossing points: 242.60 nm. The wavelength selected for estimation of MET was283.80 nmbecauseitshowedr^2>0.998 at this wavelength in mixture (Figure 4.1.2)

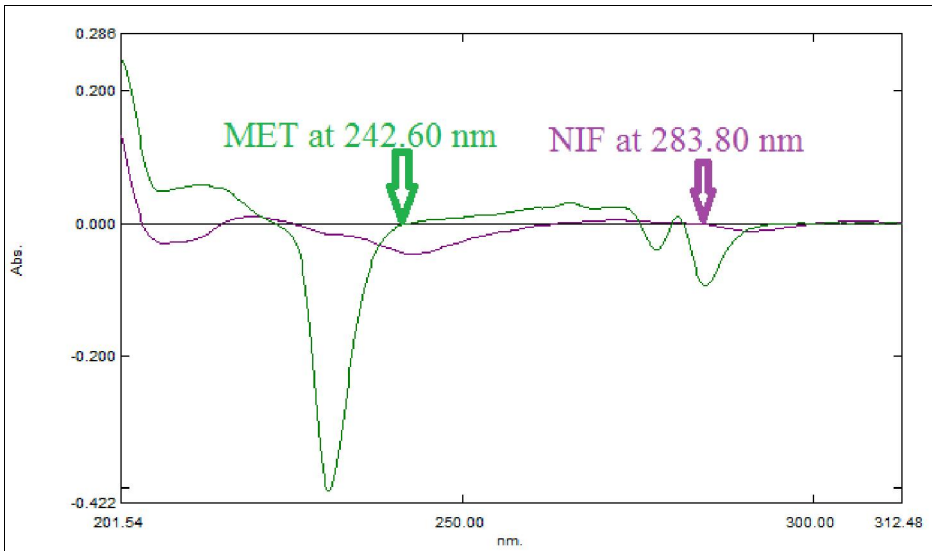

Fig.4.1.2 Overlain first order spectra of NIF and MET in1:5 ratios

5. VALIDATION PARAMETERS

5.1. Linearity and Range

The First-derivative spectra (fig.5.1.1) showed linear absorbance at 242.60 nm (ZCP of MET) for NIF (5-25 µg/ml) and 283.80nm (ZCP of NIF) for MET (25-125 µg/ml) with correlation coefficient(r^2) of 0.9984 and 0.9989 for NIF and MET, respectively.

This method obeyed beer's law in the concentration range 5-25 µg/ml and 25-125 µg/ml for NIF and MET, respectively. (Table 5.1.1)

Correlation coefficient (r^2) form calibration curve of NIF and MET was found to be 0.9980 and 0.9989, respectively (figure5.1.2and 5.1.3)

The regression line equation for NIF and MET are as following,

y = -0.0006x - 0.0101 for NIF _____ (1)

y = -0.002x + 0.002 for MET _____ (2)

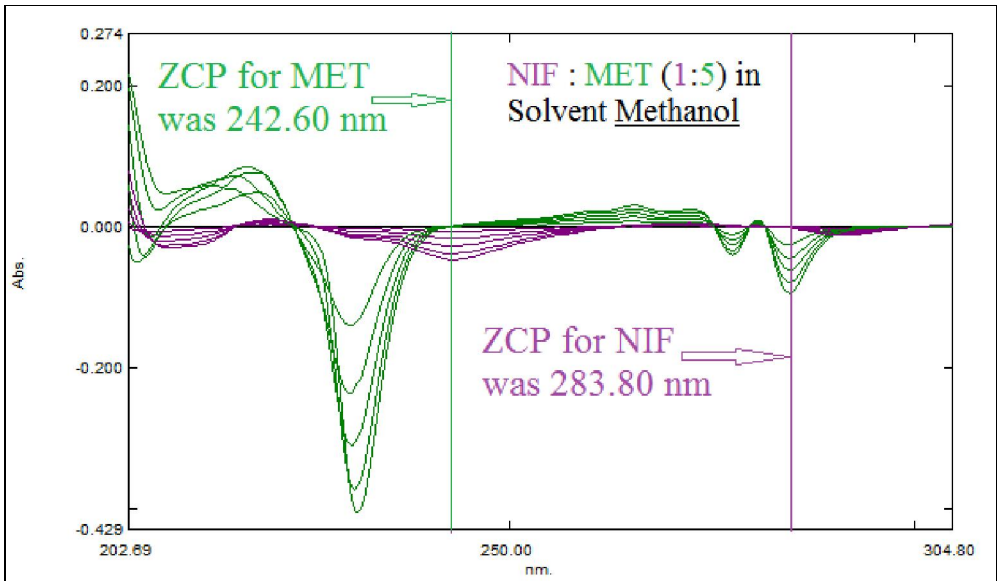

Fig.5.1.1 Overlain linear first order spectra of NIF (Purple) and MET (Green) in1:5 ratios

From the combination solution of NIF and MET the dilution were made in ratio of 1:5 and absorbance were recorded (Table 5.1.1) and correlation coefficient (r^2)of 0.9980 (figure5.1.2) and 0.9989 (figure5.1.3) for NIF and MET, respectively.

Table 1 Calibration data for NIF and MET at 283.80 nm and 242.60 nm, respectively. *(n=6)

Sr. No	Concentration (µg/ml)		Absorbance* (242.60nm)±SD NIF	Absorbance* (283.80nm)±SD MET
	NIF	MET		
1	5	25	-0.025±0.00011	-0.008±0.00011
2	10	50	-0.042±0.00016	-0.017±0.00010
3	15	75	-0.055±0.00024	-0.028±0.00012
4	20	100	-0.072±0.00015	-0.038±0.00014
5	25	125	-0.086±0.00023	-0.047±0.00015

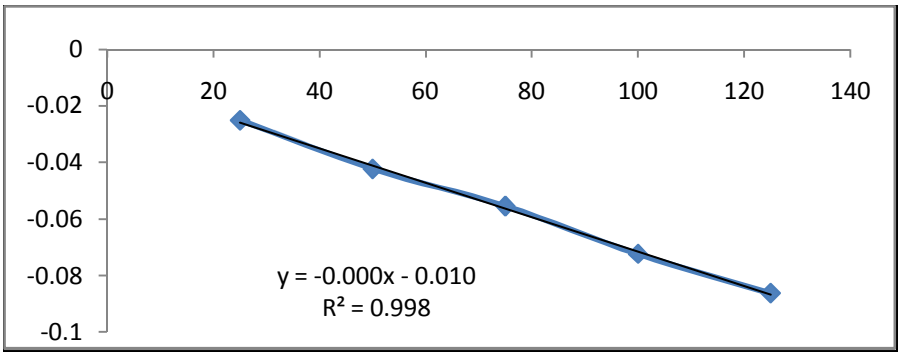

Figure5.1.2 Calibration curve for NIF at 242.60 nm

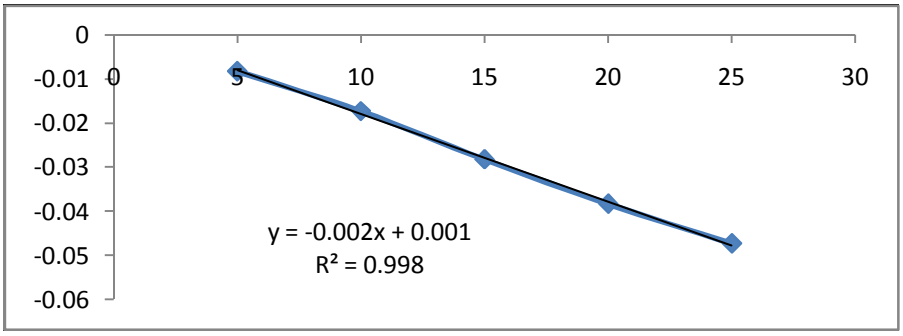

Figure5.1.3 Calibration curve for MET at 283.80 nm

5.2.Precision

I. Intraday precision

The data for intraday precision or combined standard solution of NIF and MET is presented in Table 5.2.1

The % R.S.D was found to be 0.457-0.687% for NIF and 0.630-0.863% for MET.

These % RSD value was found to be less than±1.0 indicated that the method is precise.

Table 2 Intraday precision data for estimation of NIF and MET*(n=3)

Conc. (µg/ml)		Abs.* (NIF)	%	Abs. (MET)*	%
NIF	MET	Avg. ± SD(242.60nm)	RSD	Avg.± SD(283.80nm)	RSD
5	50	-	0.612	-	0.863
10	75	-	0.687	-	0.738
25	100	-	0.457	-	0.630

II. Inter day precision

The data for inter day precision for combined standard solution of NIF and MET is presented in Table 5.2.2

The % R.S.D was found to be0.653-0.896% for NIF and 0.712-0.890%for MET.

These % RSD value was found to be less than±1.0 indicated that the method is precise.

Table 3 Inter day precision data for estimation of NIF and MET*(n=3)

Conc. (µg/ml)		Abs. (NIF)* Avg. ± SD(242.60nm)	% RSD	Abs. (MET)* Avg.± SD(283.80nm)	% RSD
NIF	MET				
2	50	-	0.764	-	0.875
3	75	-	0.896	-	0.910
4	100	-	0.653	-	0.812

5.3.Accuracy

Accuracy of the method was determined by recovery study from synthetic mixture at three levels (80%, 100%, and 120%) of standard addition.

The % recovery values are tabulated in Table 5.3.1and 5.3.2

Percentage recovery for NIF and MET by this method was found in the range of 98 to 102 % and 99 to 101 %, respectively,

The value of % RSD within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients.

Table 4 Recovery data of NIF*(n=3)

Conc. Of NIF from formulation (µg/ml)	Amount of Std. NIF added (µg/ml)	Total amount of NIF (µg/ml)	Total amount of NIF found (µg/ml) Mean*± SD	% Recovery* (n=3)	% RSD NIF
4	3.2	7.2			0.213
4	4.0	8.0			0.315
4	4.8	8.8			0.402

Table 5 Recovery data of MET*(n=3)

Conc. Of MET from formulation (µg/ml)	Amount of Std. MET added (µg/ml)	Total amount of MET (µg/ml)	Total amount of MET found (µg/ml) Mean*± SD	% Recovery* (n=3)	% RSD MET
40	16	36			0.351
40	20	40			0.436

40	24	44			0.514

5.4.Limit of detection and quantitation

The LOD for NIF and MET was conformed to be 0.032µg/ml and 0.831µg/ml, respectively.

The LOQ for NIF and MET was conformed to be 0.098µg/ml and 2.520µg/m, respectively.

The obtained LOD and LOQ results are presented in Table 5.4.1

Table 6 LOD and LOQ data of NIF and MET *(n=10)

Conc. (µg/ml)		Abs.* (NIF)	%	Abs.* (MET)	%
NIF	MET	Avg. ± SD(242.60nm)	RSD	Avg. ±SD(283.80nm)	RSD
5	25	-0.02217 ± 0.000048	0.805	-0.01128 ± 0.00012	0.614
LOD (µg/ml)		0.26		0.20	
LOQ (µg/ml)		0.80		0.61	

5.5.Robustness and Ruggedness

The obtained Ruggedness and Robustness results are presented in table5.5.1 The % R.S.D was found to be 0.280-0.857 % for NIF and 0.291-0.890 % for MET.

These % RSD value was found to be less than±1.0 indicated that the method is precise. No significant changes in the spectrums were observed, proving that the developed method is rugged and robust.

Table 7RobustnessandRuggedness data of NIF and MET*(n=3)

Conc. (PPM)	Nifedipine (Mean Abs.* ±% RSD)			
	Instrument 1	Instrument 2	Stock – 1	Stock – 2
2	-0.0273 ± 0.857	-0.0231 ± 0.827	-0.0253 ± 0.605	-0.0222 ± 0.657
3	-0.0350 ± 0.390	-0.0324 ± 0.755	-0.0313 ± 0.487	-0.0312 ± 0.560
4	-0.0549 ± 0.471	-0.0531 ± 0.553	-0.0543 ± 0.280	-0.0523 ± 0.521
	Metoprolol Succinate (Mean Abs.* ±% RSD)			
50	-0.0157 ± 0.338	-0.0101 ± 0.731	-0.0151 ± 0.686	-0.0111 ± 0.513
75	-0.0268 ± 0.713	-0.0232 ± 0.438	-0.276 ± 0.489	-0.0.245 ± 0.629
100	-0.0282 ± 0.138	-0.0288 ± 0.669	-0.291 ± 0.291	-0.0281 ± 0.709

5.6.Application of the proposed method for analysis of NIF and MET in synthetic mixture

A first order derivative spectrum of the sample solution containing 4µg/ml of NIF and 20 µg/ml of MET was recorded and the absorbance at 242.60 nm and 283.80 nm were noted for estimation of NIF and MET, respectively.

The concentration of NIF and MET in mixture was determined using the corresponding calibration graph.

The results from the analysis of synthetic mixture containing Nifedipine (4 mg) and Metoprolol Succinate (20 mg) in combination are presented in Table in 5.6.1

The percent assay shows that there is no interference from excipients and the proposed method can successfully applied to analysis of commercial formulation containing NIF and MET. The % assay values are tabulated in Table 5.6.1

Table 8 Analysis data of commercial formulation*(n=3)

Sr. No.	Formulation (synthetic mixture)		Absorbance* (242.60nm) NIF	%Assay NIF±SD	Absorbance* (283.80nm) MET	%Assay MET±SD
	NIF	MET				
1	4	20	-0.0026	99.87 ± 0.776	-0.0070	99.52 ± 0.861
2			-0.0025		-0.0068	
3			-0.0023		-0.0068	

Table 9 Summary of validation parameters

PARAMETERS	First-derivative UV Spectrometry	
	Nifedipine	Metoprolol Succinate
Concentration range (µg/ml)	5-25	25-125
Regression equation	y = -0.0006x - 0.0101	y = -0.002x + 0.002
Correlation Coefficient(r^2)	0.9984	0.9989
Accuracy (%Recovery) (n=3)	99.64	99.41
Intra-day Precision (%RSD) (n=3)	0.657-0.987	0.630-0.863
Inter-day precision (%RSD) (n=3)	0.653-0.896	0.812-0.910
LOD(µg/ml)	0.032	0.831
LOQ(µg/ml)	0.098	2.520
Ruggedness and Robustness	0.280-0.857	0.291-0.890
%Assay	99.87	99.52

6. CONCLUSION

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 5-25 µg/ml and 25-125 µg/ml for NIF and MET, respectively with co-efficient of correlation, (r2)=0.9984 and (r2) = 0.9989 for NIF and MET, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of NIF and MET. The method can be used for the routine analysis of the NIF and MET in synthetic mixture form without any interference of excipients.

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