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

Sojitra Rajanit[1*], Virani Paras[1], HashumatiRaj

[1*] Department of quality assurance, shreedhanvantry Pharmacy College, Kim, Surat.

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. 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.

1. 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 NIFprololSuccinate (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.

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

AdoublebeamUV/Visiblespectrophotometer(Shimadzumodel2450,Japan) with spectralwidth of 2nm, 1 cm quartzcells was used to measureabsorbance of allthe solutions. Spectrawere automaticallyobtained byUV-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 solutionofnifedipine (NIF)

Accuratelyweighedquantity of NIF10mg was transferred to 100mlvolumetricflask,dissolvedanddiluteduptomark with Methanol togivea stock solution havingstrength 100µg/ml.

2.4.2. Standard solutionofmetoprolol succinate (MET)

Accuratelyweighedquantity ofMET100mgwastransferredinto100mlvolumetric flask, dissolved anddiluted up to mark withMethanol togiveastock 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 mixtureformulation 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 processedto obtain first-derivative spectra. Data were recorded at aninterval of 1 nm. The two spectra were overlain and itappeared that NIF showed zero crossing at 283.80 nm, whileMET showed zero crossing at 242.60 nm. At the zero crossingpoint (ZCP) of NIF (283.80 nm), MET showed a firstderivativeabsorbance, 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 fordetermination of NIF and MET, respectively. These twowavelengths 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 ofwavelengthand method development fordetermination of Nifedipine and Metoprolol Succinate

ThestandardsolutionofNIFandMETwerescannedseparatelybetween200-400nm, and zero-order spectra were not showed overlapping peaks.(figure4.1.1)

Thus obtained spectrawere then processed to obtain first-derivativespectra.

Firstorderderivativespectrum for NIF showed four zero crossing points: 283.80 nm.Thewavelengthselected for estimation of NIF was283.80 nmbecauseitshowed r^2>0.998at this wavelength in mixture. (Figure4.1.2)

First or derderivative spectrum for MET showed two zero crossing points: 242.60 nm.Thewavelengthselected forestimationofMET was242.60 nmbecauseitshowedr^2>0.998 at this wavelength in mixture (Figure 4.1.2)

5. VALIDATION PARAMETERS

5.1. Linearity andRange

TheFirst-derivative spectra(fig.5.1.1)show edlinearabsorbanceat 283.80 nm (ZCPofMET)forNIF(5-25 µg/ml) and 242.60 nm(ZCPofNIF)forMET(25-125 µg/ml)withcorrelationcoefficient(r^2)of0.9980and0.9989forNIF and MET, respectively.

Thismethodobeyedbeer'slawintheconcentrationrange5-25 µg/mland25-125 µg/ml forNIF and MET, respectively. (Table 5.1.1)

Correlationcoefficient(r^2)for mcalibrationcurve of NIF and MET was found to be 0.9980 and 0.9989,respectively(figure5.1.2and 5.1.3)

Theregression line equation forNIF and MET are as following,

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

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

FromthecombinationsolutionofNIFandMETthedilutionweremadeinratioof 1:5 andabsorbancewererecorded(Table5.1.1)andcorrelationcoefficient(r^2)of 0.9980 (figure5.1.2) and 0.9989(figure5.1.3) for NIF and MET, respectively.

5.2. Precision

I. Intraday precision

ThedataforintradayprecisionforcombinedstandardsolutionofNIFand METis presented in Table 5.2.1

The%R.S.D wasfoundto be 0.457-0.687% forNIFand 0.630-0.863% for MET.

These%RSDvaluewasfoundtobelessthan±1.0indicatedthatthemethod is precise.

II. Interdayprecision

ThedataforinterdayprecisionforcombinedstandardsolutionofNIFand METis presented in Table 5.2.2

The% R.S.D was foundto be0.653-0.896% forNIF and0.712-0.890%for MET.

These%RSDvaluewasfoundtobelessthan±1.0indicatedthatthemethod is precise.

5.3. Accuracy

Accuracyofthemethodwasdeterminedbyrecoverystudyfromsynthetic mixture at threelevels (80%, 100%, and 120%) of standard addition.

The% recoveryvalues aretabulated in Table 5.3.1and 5.3.2

PercentagerecoveryforNIFandMETbythismethodwasfoundintherange of98 to 102 % and99 to 101 %, respectively,

Thevalueof%RSDwithinthelimitindicatedthatthemethodisaccurateand percentagerecoveryshows that thereis no interferencefrom the excipients.

5.4. Limit of detection and quantitation

TheLODforNIFandMETwasconformedtobe0.032µg/mland0.831µg/ml, respectively.

TheLOQforNIFandMETwasconformedtobe0.098µg/mland2.520µg/m, respectively.

TheobtainedLODandLOQresults arepresentedin Table 5.4.1

5.5. Robustness andRuggedness

Theobtained Ruggedness andRobustness results arepresented in table5.5.1

The% R.S.D was foundto be 0.280-0.857 % forNIF and0.291-0.890 % for MET.

These%RSDvaluewasfoundtobelessthan±1.0indicatedthatthemethod is precise.

No significant changes in the spectrums were observed, proving that the developed method is ruggedand robust.

5.6. Application oftheproposed method for analysisof NIFand METin synthetic mixture

A first or derderivative spectrum of the sample solution containing4 µg/ml of NIF and 20 µg/mlofMET was recordedandtheabsorbanceat 283.80 nmand 242.60 nm werenotedforestimation of NIF and MET, respectively.

Theconcentration ofNIF and METinmixture was determinedusing the corresponding calibration graph.

Theresultsfromtheanalysisof synthetic mixturecontaining Nifedipine (4 mg)and Metoprolol Succinate (20 mg) in combination arepresented in Tablein 5.6.1

The percent as say shows that the reisnointerference from excipientsand the proposedmethodcansuccessfully appliedtoanalysisofcommercial formulationcontainingNIF andMET.The%assayvaluesare tabulatedin Table 5.6.1

6. CONCLUSION

Based on the results, obtained from the analysis of describedmethod, 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)=0.9980 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.

7. ACKNOWLEDGEMENTS

Declared none.

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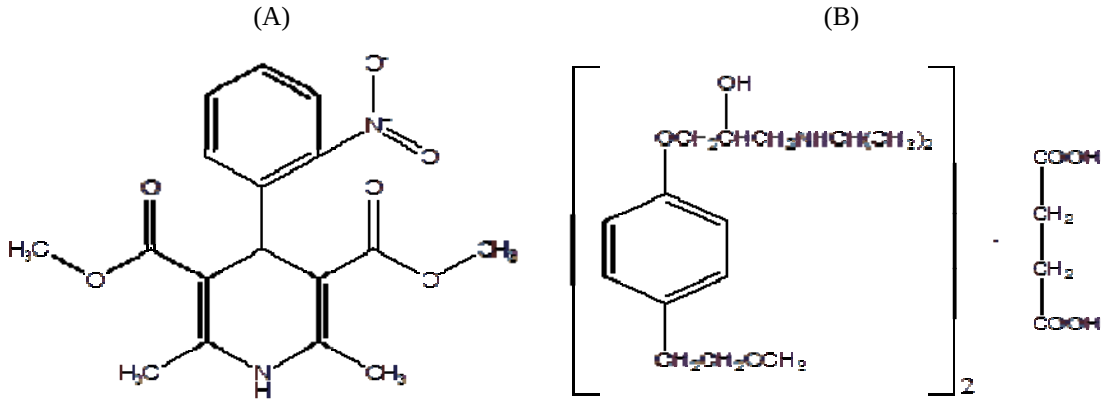

Fig.1(A) is Structure of Nifedipine and (B) is structure of Metoprolol Succinate.

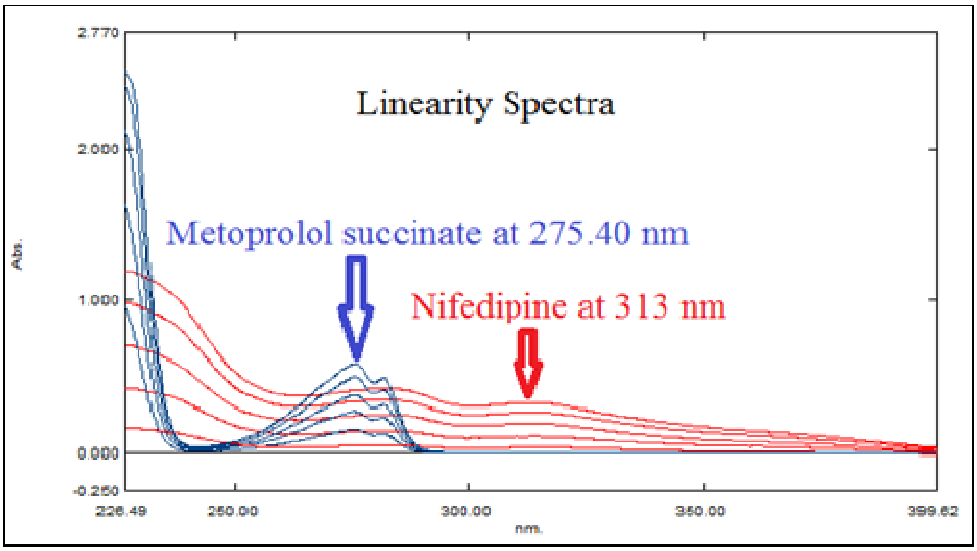

Figure4.1.1 Overlainzero orderspectra ofNIF andMET(1:5) ratios, respectively

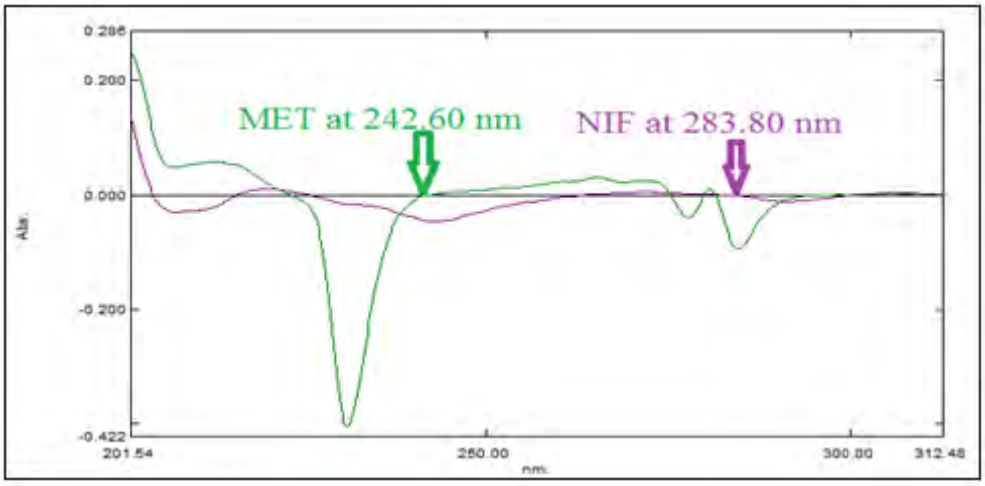

Fig.4.1.2 Overlain firstorderspectra ofNIF andMET in1:5 ratios

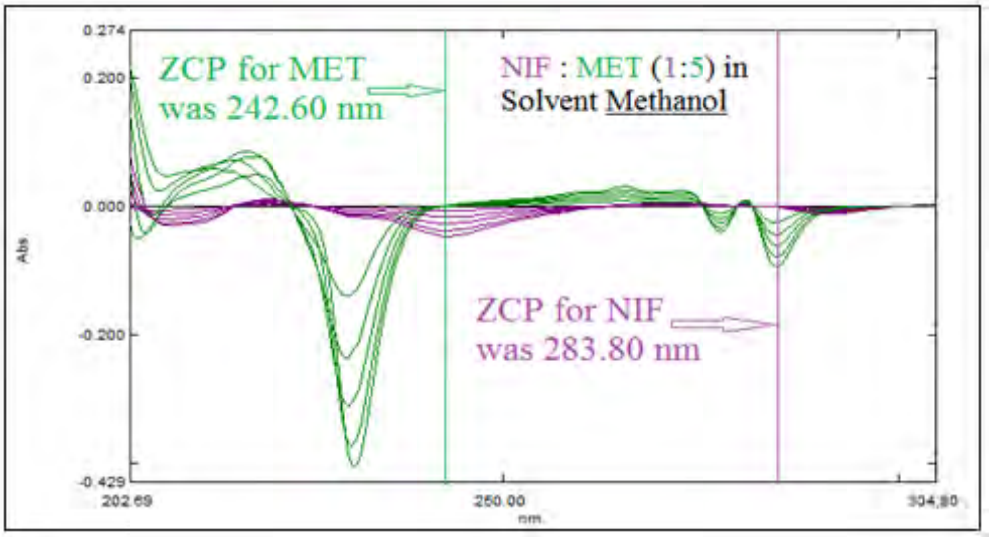

Fig.5.1.1 Overlainlinearfirstorderspectra ofNIF (Purple) andMET(Green) in1:5 ratios

Table 1Calibrationdata forNIF andMETat283.80 nmand242.60 nm, respectively. *(n=6)

Sr. No	Concentration (µg/ml)		Absorbance* (283.800 nm)±SD NIF	Absorbance* (245.60nm)±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

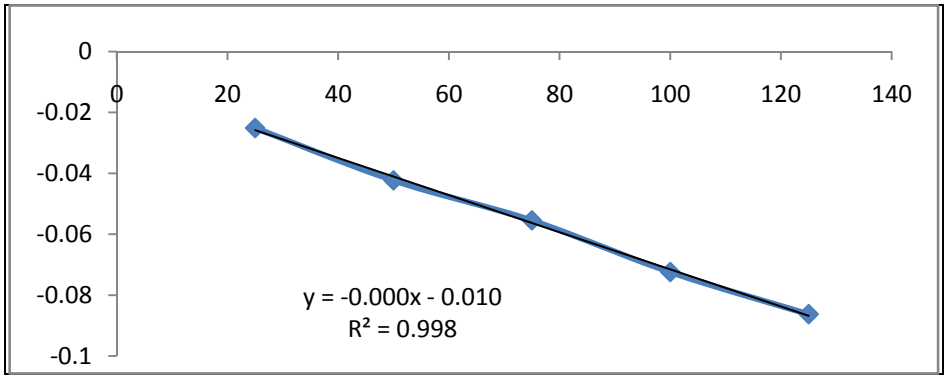

Figure5.1.2CalibrationcurveforNIF at 283.80 nm

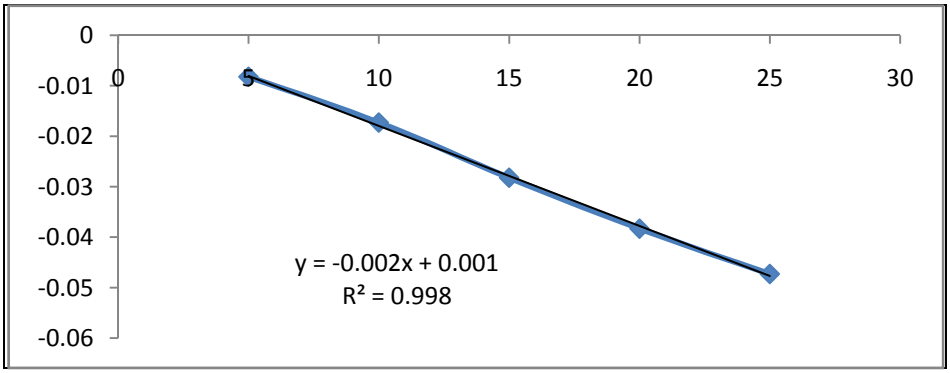

Figure5.1.3CalibrationcurveforMET at 242.60 nm

Table 2 Intraday precision data for estimation ofNIF andMET*(n=3)

Conc. (µg/ml)		Abs.* (NIF) Avg. ± SD(271.20nm)	% RSD	Abs. (MET)* Avg.± SD(245.60nm)	% RSD
NIF	MET				
2	50	-0.0151 ± 0.00025	0.612	-0.0096 ± 0.00060	0.863
3	75	-0.0303 ± 0.00040	0.687	-0.0201 ± 0.00077	0.738
4	100	-0.0455 ± 0.00036	0.457	-0.0304 ± 0.0085	0.630

Table 3Interdayprecision data for estimation ofNIF andMET*(n=3)

Conc. (µg/ml)		Abs. (NIF)* Avg. ± SD(271.20nm)	% RSD	Abs. (MET)* Avg.± SD(245.60nm)	% RSD
NIF	MET				
2	50	-0.0272 ± 0.00020	0.764	-0.0685 ± 0.00060	0.875
3	75	-0.0402 ± 0.00036	0.896	-0.1048 ± 0.00096	0.910
4	100	-0.0548 ± 0.00036	0.653	-0.1371 ± 0.00111	0.812

Table 4Recovery data ofNIF*(n=3)

Conc. ofNIF from formulation (µg/ml)	Amount of Std. NIF added (µg/ml)	Total amount of NIF (µg/ml)	Total amount ofNIF found (µg/ml) Mean*± SD	% Recovery* (n=3)	% RSD NIF
4	3.2	7.2	7.18 ± 0.00015	99.76	0.213
4	4.0	8.0	7.95 ± 0.00026	99.37	0.315
4	4.8	8.8	8.58 ± 0.00035	99.80	0.402

Table 5 Recovery data ofMET*(n=3)

Conc. ofMET from formulation (µg/ml)	Amount of Std. MET added (µg/ml)	Total amount of MET (µg/ml)	Total amount ofMET found (µg/ml) Mean*± SD	% Recovery* (n=3)	% RSD MET
40	16	36	35.75 ± 0.00025	99.30	0.351
40	20	40	39.90 ± 0.00057	99.75	0.436
40	24	44	43.65 ± 0.00042	99.20	0.514

Table 6LOD andLOQ dataofNIF andMET *(n=10)

Conc. (µg/ml)		Abs.* (NIF) Avg. ± SD(283.80 nm)	% RSD	Abs.* (MET) Avg. ±SD(242.60 nm)	% RSD
NIF	MET				
5	25	-0.02217 ± 0.000048	0.805	-0.01128 ± 0.00012	0.614
LOD (µg/ml)		0.032		0.831	
LOQ (µg/ml)		0.098		2.520	

Table 7RobustnessandRuggedness dataof NIF andMET*(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

Table 8 Analysisdata ofcommercial formulation*(n=3)

Sr. No.	Formulation (synthetic mixture)		Absorbance* (283.80 nm) NIF	%Assay NIF±SD	Absorbance* (242.60 nm) 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 9Summary ofvalidation parameters

PARAMETERS	First-derivativeUV 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)	0.9980	0.9989
Accuracy(%Recovery) (n=3)	99.64	99.41
Intra-dayPrecision (%RSD) (n=3)	0.657-0.987	0.630-0.863
Inter-dayprecision (%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