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Research Article

Absorbance Correction Method for Simultaneous Estimation of Nifedipine and Metoprolol Succinate in Their Synthetic Mixture Using From Spectrophotometry

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Abstract

A new simple, economical, precise and accurate method are described for the simultaneous determination of Nifedipine (NIF) and Metoprolol Succinate (MET) in combined tablet dosage form. The proposed method was applied for the determination of Nifedipine and Metoprolol Succinate in synthetic mixture, for determination of sampling wavelength, 10µg/ml of each of NIF and MET were scanned in 200-400 nm range and sampling wavelengths were 313nm for NIF and 275.40nm for MET are selected for development and validation of absorption correction method. For this method linearity observed in the range of 5-25µg/ml for NIF and 25-125µg/ml for MET, and in their pharmaceutical formulation with mean percentage recoveries 100.68 and 100.33, respectively. The method was validated according to ICH guidelines and can be applied for routine quality control testing.

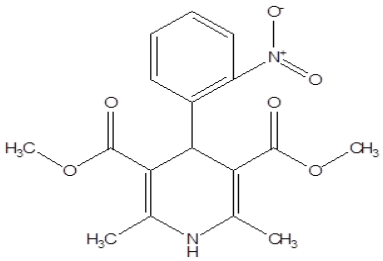
Keywords: Spectroscopic method, absorption correctedmethod, Nifedipine and Metoprolol Succinate.

1. Introduction

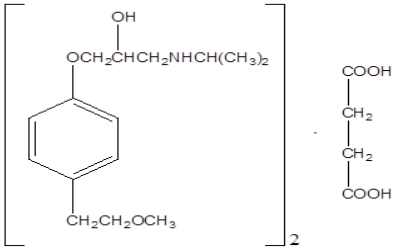
The aim of the present work was to develop a new simple, rapid, selective method for the simultaneous determination of components having overlapping spectra in binary mixtures, having the advantages of minimal data processing and a wider range of applications over the previously mentioned methods. To 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 Metoprolol Succinate (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 a selective β-adrenergic antagonist, which is used in the treatment of cardiovascular disorders such as hypertension, angina pectoris, cardiac arrhythmias, congestive heart failure and myocardial infarction. Metoprolol is administered orally as tablet. Chemically Metoprolol succinate is (RS)-1-(Isopropylamino)-3-[p-(2-methoxyethyl) phenoxy] propan-2- ol succinate with molecular formula $C_{34}H_{54}N_2O_{10}$.[7,8]

Fig.1 [A] is Structure of Nifedipine and [B] is structure of Metoprolol Succinate



[A]



[B]

1.1 Theory

This method is modification of simultaneous equation method. This method uses the absorbances at two selected wavelengths, one at λmax of one drug where other drug also shows considerable absorbance (λ2) and other being the wavelength at which the first drug has practically nil absorbance (λ1).

The concentration of two drugs (X and Y) in sample solution was calculated by using following equations:

$Cy = A_2 / ay2$ ..... (1)

$Cx = A_1 - ay1 * y/ax1$ .....(2)

Where,

A_1 and A_2 are the absorbances of mixture at λ1 and λ2 respectively,

ay1 and ay2 are absorptivities of y at λ1 and λ2 respectively,

ax1 is absorptivity of X at λ2,

CX is concentration of X,

CY is concentration of Y.

2. Material and Method

2.1. Apparatus

A double beam UV/Visible spectrophotometer (Shimadzu model 2450, Japan) with spectral width of 2nm, 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 NIF 10 mg was transferred to 100ml volumetric flask, dissolved and diluted upto 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 MET 50mg was transferred into 100mlvolumetric flask, dissolved and diluted up to mark with Methanol to give a stock solution having strength 500µg/ml.

2.4.3. Preparation of standard mixture

Pipette out accurately 0.5ml of NIF stock solution (100µg/ml), 0.5ml of MET stock solution (500µg/ml) in 10 ml volumetric flask and make up the volume up to the mark with Methanol. It gives solution containing NIF 5µg/ml, MET 25µg/ml.

2.4.4. Test sample preparation

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

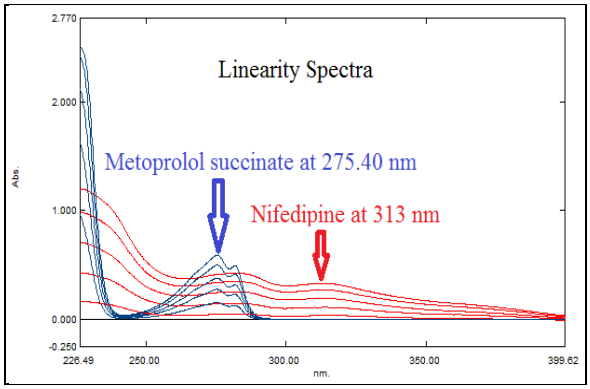
2.5. Procedures

2.5.1. Construction of calibration curves (linearity)

This series consisted of five concentrations of standard NIF solution ranging from 5-25µg/ml. The solutions were prepared by pipetting out standard NIF stock solution (0.5ml, 1ml, 1.5ml, 2.0ml, 2.5ml) was transferred into a series of 10ml volumetric flasks and volume was adjusted upto mark with Methanol. A zero order spectra of the resulting solutions were recorded, measured the absorbance at 313nm against are agent blank solution (Methanol). Calibration curve was prepared by plotting absorbance versus respective concentration of NIF.

This series consisted of five concentrations of standard MET solution ranging from 25-125µg/ml. The solutions were prepared by pipetting out Standard MET stock solution (0.5ml, 1ml, 1.5ml, 2.0ml, 2.5ml) was transferred into a series of 10ml volumetric flasks and volume was adjusted upto mark with Methanol. A zero order spectra of the resulting solutions were recorded and measured the absorbance at 275.40nm against a reagent blank solution (Methanol). Calibration curve was prepared by plotting absorbance versus respective concentration of MET.

Fig. 2: Overlain linear zero order spectra of NIF (Red) and MET (Blue) in1:5 ratio



2.5.2. Analysis of laboratory-prepared mixtures.

Laboratory-prepared mixtures containing different ratios of NIF and MET were prepared. By applying the procedure under linearity, absorbances at 313nm were recorded for NIF and 275.40nm were recorded for MET. The concentration of each drug in each mixture was calculated from its corresponding Cx and Cy equation. Validity of the method was assessed by spiking the pharmaceutical formulation by known amounts of standard drug powders (standard addition technique). The recovery of the added standards was then calculated after applying the proposed method.

2.5.3. Application of the proposed method for the simultaneous determination of NIF and MET in synthetic mixture.

In that mixture the excipient were like HPMC, silicon dioxide and guar gum were taken as per the required weight. With the Nifedipine and Metoprolol Succinate with the ratio dissolved in methanol with all excipient.

Finally the in the Synthetic mixture had the concentration 100µg/ml and 500µg/ml respectively for NIF and MET. After that from this solution 1ml was pipette out and diluted upto 10ml with methanol. So the concentration was 10 µg/ml and 50 µg/ml for NIF and MET respectively.

3. Results and Discussion

The absorbance wavelength for NIF and MET found to be 313nm and 275.40nm, respectively, which are different and hence non-overlapping. Thus simultaneous determination of NIF and MET in bulk mixture-I and synthetic mixture solution-I was found to be successful by absorption corrected.

3.1. Specificity

The specificity of the method was investigated by observing any interference of one drug with other two drugs in bulk mixture and formulation solution. Similarly the interference of excipients of synthetic mixturewith drugs was investigated.

3.2. Linearity and Range

The linearity of method is its ability within a given range to obtain test results which are directly or through a mathematical transformation, proportional to the concentration of analyte. Linearity of the method was determined at five concentration levels for NIF and MET independently.

3.3. Accuracy

The accuracy of an analytical method is the closeness of the test results to the true value. It was tested by spiking standard NIF solution in different concentration 80, 100 and 120% to a tablet solution. The synthetic mixture solution was analyzed at 313nm for estimation of NIF. Similarly, the accuracy for MET was determined at 275.40nm, respectively.

3.4. Precision

The intra-day precision (repeatability) of method was determined by measuring the absorbance of synthetic mixture solution-I at 313nm and 275.40nm for NIF and MET, respectively. Within a laboratory over a short period of time. The inter-day precision (intermediate precision) was determined by measuring the absorbance of synthetic mixturesolution-I at 313nm and 275.40nm for NIF and MET, respectively. Within a laboratory on three consecutive days, by different analysts. The %RSD was calculated for intra and inter-day precision.

3.5. LOD and LOQ

The LOD of an analytical method is the lowest amount of analyte in a sample which can be detected but not necessarily quantified. The detection limit (DL) of method was determined by equation, DL = (3.3 σ)/S, where, σ– standard deviation of blank response, S– slope of the calibration curve. The quantitation limit (QL) of analyte was determined by equation DL = (10 σ)/S, where, σ– standard deviation of blank response, S– slope of the calibration curve

3.6. Robustness and Ruggedness

Robustness and ruggedness of the method has been evaluated at two different levels i.e. change in stock solution and changing the instrument.

4. Analysis of synthetic mixture

The proposed method was successfully applied to the analysis of both mixtures in their pharmaceutical preparations. Results obtained were precise and in good agreement with the labelled claim as concluded from the satisfactory values of % recovery and RSD (%) gathered in table 2 and 3. Proposed method is precise and accurate, and give same result in same day and between the day and this data is gathered in table 1. When we change in standard stock concentration or changed the instrument that time also this proposed method give good result this data also gathered in table 4 and 5.

Table 1: Interday and intraday precision data for Nifedipine and Metoprolol Succinate in three different concentration ranges

Precision	Conc. µg/ml	Nifedipine 313nm	Conc. µg/ml	Metoprolol Succinate 275.40nm
Intraday (n=3) Abs.±%RSD	5	0.038± 0.64	25	0.157± 0.65
	10	0.117± 0.54	50	0.272± 0.49
	15	0.191± 0.46	75	0.386± 0.40
Interday (n=3) Abs.±%RSD	5	0.040± 0.71	25	0.160± 0.75
	10	0.118± 0.60	50	0.172± 0.54
	15	0.192± 0.40	75	0.388± 0.22

Table 2: Accuracy data for Nifedipine and Metoprolol Succinate with % recovery and % RSD

Level of recovery	Initial conc. (µg/ml)		Quantity of Std. Added (µg/ml)		Total Amount (µg/ml)		Result of recovery study			
							Total Quantity Found* (µg/ml)± %RSD		%recovery± %RSD	
LEVEL	**NIF**	**MET**	**NIF**	**MET**	**NIF**	**MET**	**NIF**	**MET**	**NIF**	**MET**
Placebo	10	50	-	-	10	50	10.05± 0.26	50.15± 0.30	100.50± 0.30	100.30± 0.25
80%	10	50	8	40	18	90	18.11± 0.16	90.19± 0.11	100.61±0.19	100.46±0.17
100%	10	50	10	50	20	100	20.15± 0.19	100.29±0.13	100.68± 0.22	100.29± 0.15
120%	10	50	12	60	22	120	22.17± 0.13	120.47±0.39	100.77± 0.15	100.78± 0.13

Table 3: Robustness and ruggedness data into that change in instrument and change in (±0.2nm) wavelength of both drug

Condition	Conc.	Different Instrument		Λmax (± 0.2nm)	
		UV-2450	**UV-1800**	**312.80nm**	**313.20nm**
Nifedipine Mean(n=3) ±%RSD	5	0.039± 0.71	0.038± 0.33	0.038± 0.37	0.039±0.45
	10	0.117± 0.32	0.118± 0.43	0.116± 0.32	0.117± 0.51
	15	0.191± 0.41	0.193± 0.29	0.191± 0.42	0.192± 0.75
		UV-2450	**UV-1800**	**275.20nm**	**275.60nm**
Metoprolol Succinate Mean (n=3) ±%RSD	25	0.159± 0.41	0.158± 0.59	0.157± 0.22	0.160± 0.65
	50	0.272± 0.39	0.273± 0.38	0.271± 0.38	0.274± 0.22
	75	0.388± 0.52	0.389± 0.25	0.387± 0.47	0.389± 0.17

Table 4: LOD&LOQ data for Nifedipine and Metoprolol Succinate

Drugs	LOD(µg/ml)	LOQ(µg/ml)
Nifedipine	0.038	0.066
Metoprolol Succinate	0.115	0.200

Table 5: Result of all validation and development parameters for this proposed method for Nifedipine and Metoprolol Succinate

Sr. No.	Parameter	Nifedipine	Metoprolol succinate
1	Linearity(µg/ml)(n=6)	5-25	25-125
2	Regression equation	y=0.0742x-0.0361	y=0.1094x+0.0498
3	Correlation coefficient(r)	0.9998	0.9991
4	Accuracy(%Recovery)	100.68	100.33
5	LOD (µg/ml) (n=10)	0.066	0.20
6	LOQ(µg/ml) (n=10)	0.030	0.64
7	Precision: Intra-day (%RSD)(n=3) Inter-day(%RSD)(n=3)	0.46-0.64 0.40-0.71	0.40-0.65 0.22-0.75
8	Robustness(%RSD)	0.29-0.75	0.17-0.59

5. Conclusion

A novel, simple, rapid and sensitive method is proposed for the analysis of two binary mixtures with overlapping spectra. The method involves the generation of absorbance spectra followed by measurement of the absorbance. The proposed method does not require any sophisticated mathematical treatment for the absorption data, and it exhibits several advantages over other spectrophotometric methods for resolution of binary mixtures. The applicability of the developed method was evaluated through the determination of drug

combinations in several laboratory-prepared mixtures with good accuracy and precision. Therefore, the presented methodology is adequate for the routine quality control analysis of these fixed-dose combinations.

Conflict of Interest: The authors confirm that this article content has no conflict of interest.

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