

METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND NATEGLINIDE IN COMBINED DOSAGE FORM BY UV SPECTROSCOPY

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Abstract

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In the present work, the quantitative estimation of both the drugs in combined dosage forms was carried out. A new, simple, reliable, sensitive, rapid, and economical procedure for simultaneous estimation of atorvastatin calcium and nateglinide in a combined dosage form by UV spectroscopy using the simultaneous equation method was developed. Native ultraviolet absorbance maxima of the two chemotherapeutic agents were used. As both compounds do not interact chemically in methanol, the two wavelengths 246.15 nm for atorvastatin calcium and 206.6 nm for nateglinide were used. Both the drugs obeyed Beer's law in the concentration range (1-10 µg/ml) that was employed in this method. The method developed was validated to determine its linearity ($r^2=0.996$ for atorvastatin and $r^2=0.997$ for nateglinide), precision, reproducibility, and sensitivity.

Introduction

Multicomponent formulations have gained a lot of importance; the main reason being better patient acceptability, increased potency, and decreased side effects. Absorption and emission of radiant energy by molecules and atoms is the basis for optical spectroscopy. By interpretation, both qualitative and quantitative information can be obtained. The quantitative analysis of such multicomponent formulations is very important^{1,2}. One of the quantitative procedure for multicomponent formulations is the simultaneous spectrophotometric method, which utilizes the measurement of the intensity of electromagnetic radiation emitted or absorbed by the analyte. The quantitative estimation method has determined the amount of each constituent is present in the dosage form. Estimation of a drug in the dosage forms needs the quantitative analysis of that drug in it. The developed method is based on the native ultraviolet absorbance maxima of the two chemotherapeutic agents. As both compounds do not interact chemically in methanol³, the two wavelengths 246.15 nm for atorvastatin calcium and 206.6 nm for nateglinide were used⁴.

In multicomponent formulations the concentration of the absorbing substance is calculated from the three reported techniques: **(a) Single-component Mode of Analysis:** In such technique the concentration of a component in a sample which contains other absorbing substances is determined by a simple spectrophotometric measurement of absorbance, provided that the other components have a sufficient small absorbance at the wavelength of measurement. **(b) Absorbance correction Method:** If the identity, concentration and absorptivity values of the absorbing interferents are known, it is possible to calculate their contribution to the total absorbance of a mixture.⁵ **(c) Simultaneous equation method:** The method involves the quantitative estimation of two drugs in combined dosage form (X and Y) each of which absorbs at the λ_{max} of the other, it is possible to determine both drugs quantitatively by the technique of simultaneous equations (Vierodt's method). In the present work, the two drugs Atorvastatin and Nateglinide were estimated quantitatively in combined dosage form by using simultaneous equation method^{6,7}.
Atorvastatin is $[(\beta R, \delta S)-2-(4\text{-fluorophenyl})-\beta, \delta\text{-dihydroxy-5-(1-methylethyl)-3-phenyl-}$

4[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt is a lipid-lowering agent, acting through the inhibition of HMG Co-A reductase. It is used in hypercholesterolemia.^{1,2}

Nateglinide is 3-phenyl-2-[(4-propan-2-ylcyclohexanecarbonyl) amino] propanoic acid, which is a hypoglycaemic agent, It belongs to the meglitinide class of short-acting insulin secretagogues, which act by binding to β cells of the pancreas to stimulate insulin release. Nateglinide is an amino acid derivative that induces an early insulin response to meals decreasing postprandial blood glucose levels⁸. Although there are different methods developed for estimation of nateglinide and atorvastatin like HPTLC, HPLC, gravimetric analysis⁹. The quantitative evaluation of two drugs in bilayered tablets by UV spectroscopy is not reported yet, and UV spectroscopy technique is most routinely used in industries that lead to the development of such a method.

Materials and methods

Atorvastatin standard drug was obtained as a gift sample from Ranbaxy Pvt.Ltd. Dewas, Madhya Pradesh, India. Nateglinide standard drug was obtained as a gift sample from Dr. Reddy's Laboratories Hyderabad, India.

Sample Preparation

Ten mg of atorvastatin standard drug was dissolved in ten ml of methanol to form stock solution of 1000 μ g/ml solution, using the prepared stock solution, different dilutions up to 1-10 μ g/ml were prepared, and the standard curve was prepared using methanol as reference solvent by measuring the absorbance in UV spectrophotometer. (Shimadzu, Japan)

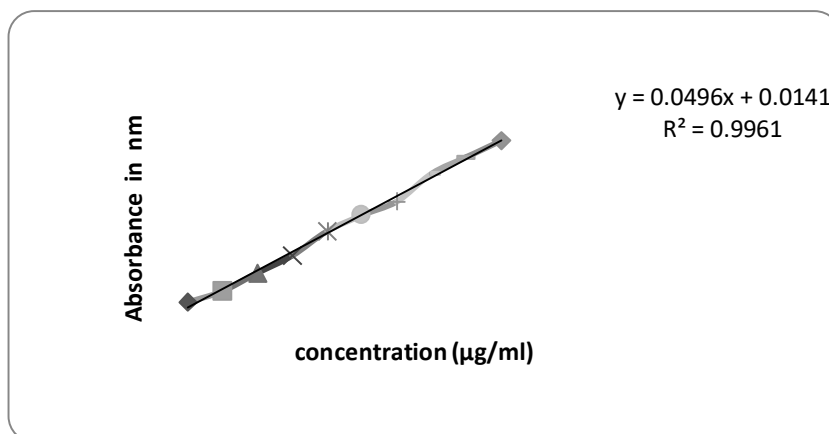


Fig 1. Standard curve of atorvastatin in methanol at λ_{max} 246.15nm

Similarly, 10 mg of nateglinide standard drug was dissolved in 10 ml of methanol to form stock solution of 1000 μ g/ml solution; from the prepared stock solution, different dilutions of 1-10 μ g/ml solutions were prepared, and the standard curve was prepared using methanol as blank using UV spectrophotometer.

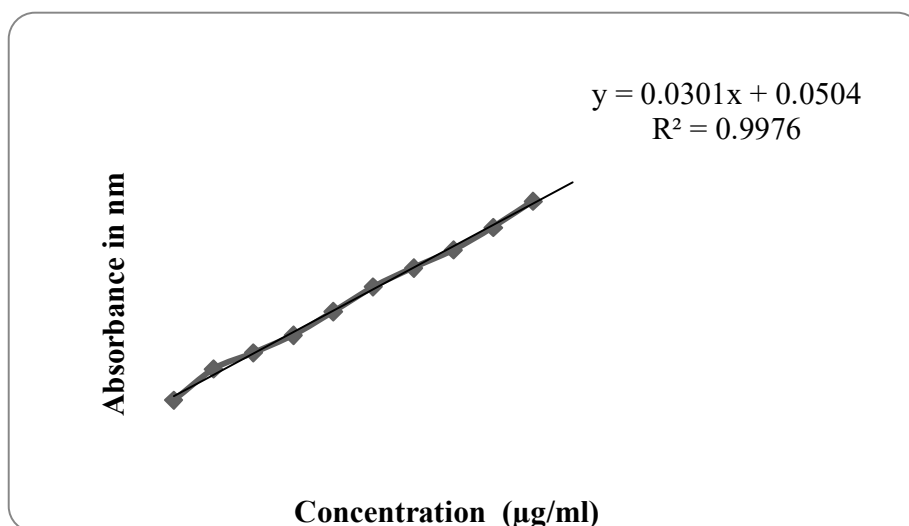


Fig 2. Standard curve of Nateglinide in methanol at λ_{max} of 206.5nm.

Determination of λ_{max} of both drugs

Ten mg of Atorvastatin drug was dissolved in 10 ml methanol to form 1000 µg/ml stock solution. From this, 10 µg/ml dilution was prepared and the sample was scanned between 400-200nm. The λ_{max} was found to be 246.15nm, which was compared with the reported literature and was found to be nearer to standard drug which was 247nm.

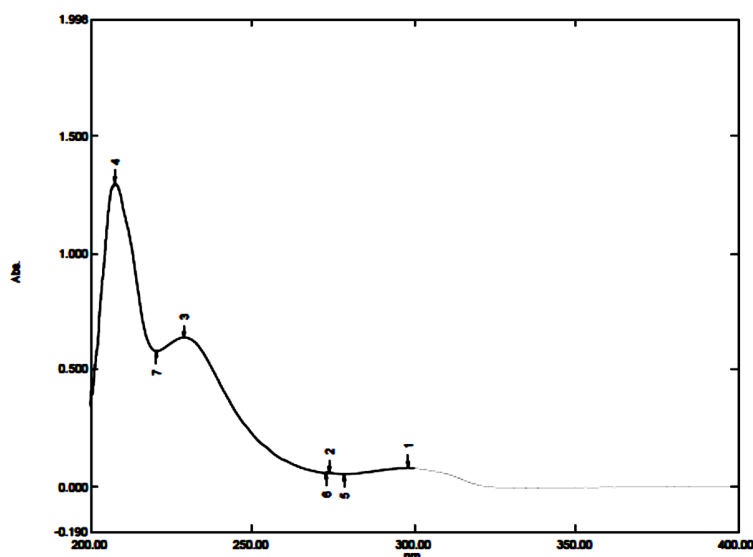


Fig.3. Graph showing λ_{max} of Atorvastatin

Similarly standard drug Nateglinide was also scanned by preparing 1000 µg/ml stock solution from which 10µg/ml dilution was prepared and was scanned between 400-200nm using methanol as reference solution. The λ_{max} was found to be 106.5 which were compared to reported literature.

The graph obtained is shown below:

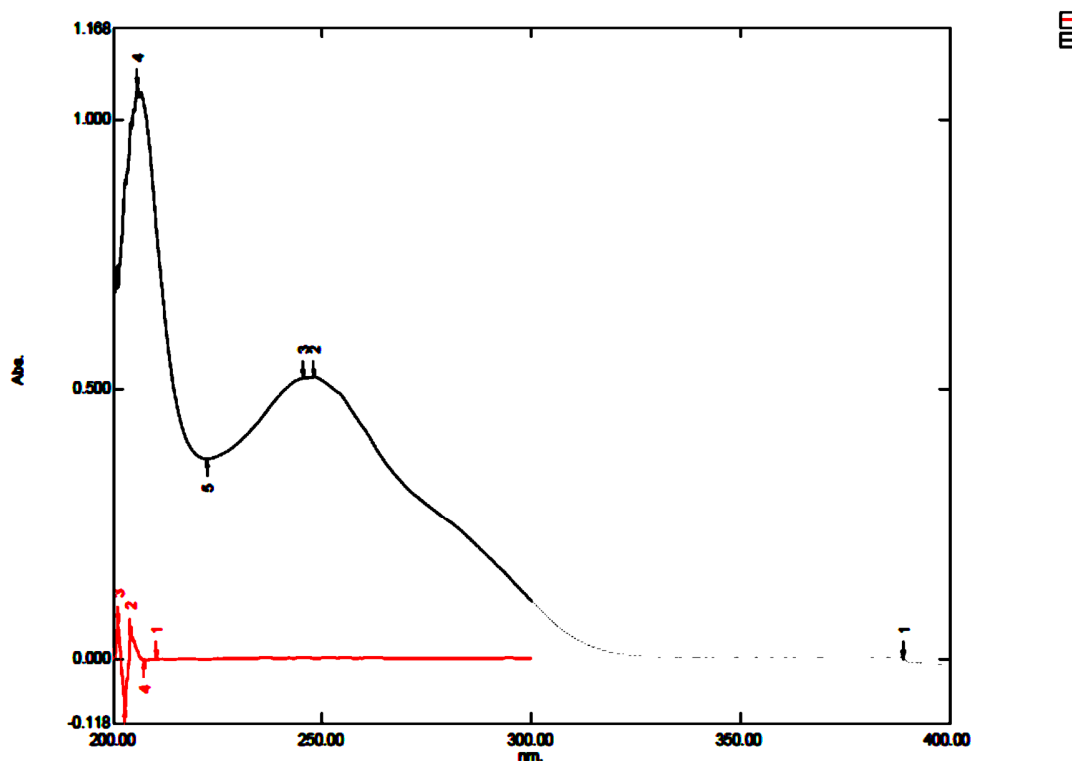


Fig.4. Graph showing λ_{max} of Nateglinide

Formulation of bilayered Tablets containing Atorvastatin and Nateglinide:

The bilayered tablets were prepared by the combination of immediate released layer and sustained released layer using multi station punching machine. During the preparation of bilayered tablets the optimized formulation of immediate release layer was introduced firstly in the die cavity followed by slight precompression to allow uniform distribution of this layer after that the granules of sustained release layer was added and the final compression was made to form bilayered tablet. After that the quantitative estimation of both the drugs was done using simultaneous equation method.

Estimation of both drugs using simultaneous equation method

Ten mg of drug 1 (nateglinide) was dissolved in 10 ml methanol to form 1000 $\mu\text{g/ml}$ of stock solution. From this 10 $\mu\text{g/ml}$ dilution was prepared and the sample was scanned at the range of 400-200nm. After scanning of nateglinide, using the same reference the scanning was followed by using the standard 10 $\mu\text{g/ml}$ of the atorvastatin solution. It was found that atorvastatin also showed the absorbance at the λ_{max} of nateglinide.

Thus at $\lambda 1$ (λ_{max} of Nateglinide)

Absorbance of nateglinide: 0.789

Absorbance of atorvastatin: 0.776

As above the stock solution of atorvastatin 10mg/ml was prepared and from the stock 10 $\mu\text{g/ml}$ was prepared using methanol as solvent the prepared sample solution of atorvastatin was scanned at the range of 400-200nm using methanol as reference solvent. When the sample solution of atorvastatin was scanned the absorbance was noted and the sample of 10 $\mu\text{g/ml}$ of nateglinide was also scanned at continuous manner, thus the drug nateglinide also showed some absorbance in the presence of atorvastatin.

Thus at λ_2 (λ_{max} of Atorvastatin)

Absorbance of nateglinide: 0.461

Absorbance of atorvastatin: 0.536

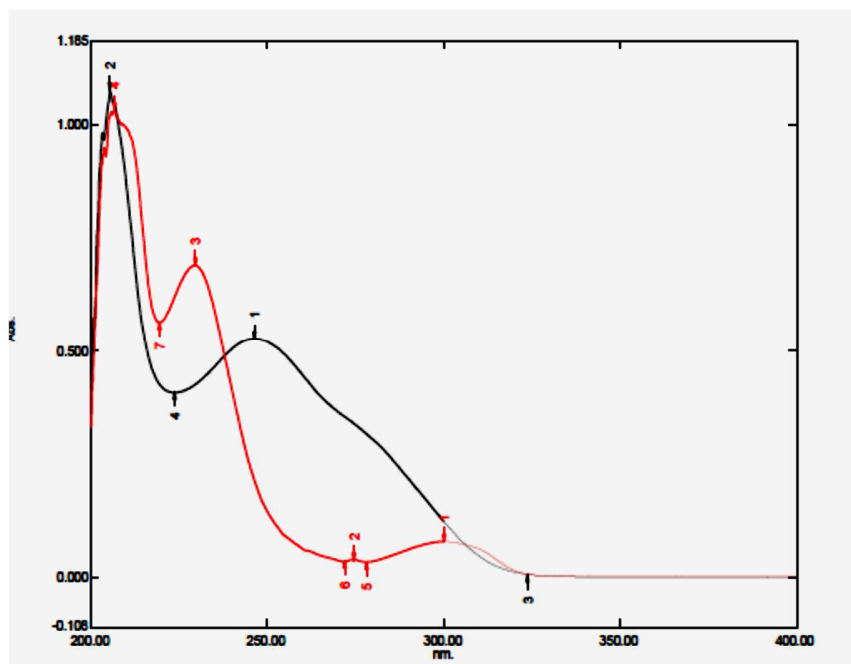


Fig.5. Spectra obtained formed as shown in graph:

The absorptivities ($a=ebc$) of drug 1 (nateglinide) at λ_1 and at λ_2 is ax_1 & ax_2 where b is 1cm c is concentration of the stock solutions prepared, so:

As per the formula:

$$ax_1 = 0.015$$

$$ax_2 = 0.007$$

The absorptivities of drug 2 (atorvastatin) at λ_1 and at λ_2 is ay_1 & ay_2 so:

$$ay_1 = 0.0155$$

$$ay_2 = 0.00672$$

So,

The absorbance of the mixture is the sum of individual absorbances of atorvastatin and nateglinide.

$$A_1 = ax_1bc_x + ay_1bc_y$$

$$= 0.015 \times 50 + 0.015 \times 50$$

$$A_1 = 1.50$$

$$A_2 = ax_2bc_x + ay_2bc_y$$

$$= 0.007 \times 50 + 0.006 \times 50$$

$$= 0.75$$

Concentration of Nateglinide can be estimated using the formula:

$$C_{nat} = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2}$$

$$= \frac{0.75 \times 0.015 - 1.5 \times 0.006}{0.007 \times 0.015 - 0.015 \times 0.006}$$

$$= 28.57 \mu g.$$

Concentration of Atorvastatin can be estimated using the formula:

$$C_{atorv} = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2}$$

$$=1.5 \times 0.007 - 0.75 \times 0.015 / 0.007 \times 0.015 - 0.015 \times 0.006$$
$$=57.14 \mu\text{g}.$$

Thus concentration of nateglinide was found to be 28.57 μg and of atorvastatin was found to be 57.14 μg .

Result and discussions

The simultaneous estimation of both the drugs prepared in the dosage form was successfully estimated. The prepared bilayered tablets consist of two drugs with different λ_{max} thus the concentration of both the drugs was determined using Vierhodt's Method. By using the formula, after that the absorbance of both the drugs was found in λ_{max} of both the drugs individually, which was found as per the values given. The absorptivities values of both the drugs was determined and using the formula and the absorbance value of both drugs was determined in the mixture, now the concentration of both the drugs in the dosage form was determined and was found to be 28.57 μg of nateglinide and that of atorvastatin was found to be 57.14 μg .

Conclusion

The proposed method for estimation of atorvastatin and nateglinide by utilizing simultaneous equation method in combined dosage form was found to be simple, accurate, precise, economical and rapid.

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