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SPECTROPHOTOMETRIC METHODS FOR THE SIMULTANEOUS ESTIMATION OF ANTIHYPERLIPIDEMIC DRUGS (ATORVASTATINE CALCIUM AND EZETIMIBE)

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ABSTRACT
Spectrophotometric methods are used for the development and validation of simultaneous estimation of Atorvastatin calcium and ezetimibe. Method (I) is based on dual wavelength analysis whilemethod (II) is the mean centering of ratio spectra spectrophotometric (MCR) method. In method (I), two wavelengths were selected for each drug in such a way that the difference in absorbance was zero for the second drug. At wavelengths 226.6 and 244 nm EZ had equal absorbance values; therefore, these two wavelengths have been used to determine Atorvastatin calcium; on a similar basis 228.6 and 262.8 nm were selected to determine ezetimibe in their binary mixtures. In method II, the absorption spectra of both Atorvastatin calcium and ezetimibe with different concentrations were recorded over the range 200–350, divided by the spectrum of suitable divisor of both Atorvastatin calcium and ezetimibe and then the obtained ratio spectra were mean cantered. The concentrations of active components were then determined from the calibration graphs obtained by measuring the amplitudes at 215–260 nm for both Atorvastatin calcium and ezetimibe. Accuracy and precision of the developed methods have been tested; in addition, recovery studies have been carried out in order to confirm their accuracy. On the other hand, selectivity of the methods was tested by application for determination of different synthetic mixtures containing different ratios of the studied drugs. The developed methods have been successfully used for determination of Atorvastatin calcium and ezetimibe in their combined dosage form and statistical comparison of the developed methods with the reported spectrophotometric one using F and Student's t-tests showed no significant difference regarding both accuracy and precision.
KEYWORDS: *Atorvastatin; Ezetimibe; Dual wavelength method; Mean centring of ratio spectra;Spectrophotometry*

1. INTRODUCTION

Analytical chemistry studies and uses instruments and methods used to separate, identify, and quantify matter. In practice, separation, identification or quantification may constitute the entire analysis or be combined with another method. Separation isolates analytes. Qualitative analysis identifies analytes, while quantitative analysis determines the numerical amount or concentration[1,2].

Selection of a suitable analytical method for estimation of individual drug in any dosage form is a challenge for an analytical chemist. The method so selected should provide analytical data as accurate as required, technically sound, defensible with low limit of uncertainty and above all amenable to routine laboratory use and capable of being performed by personnel with minimal technical experience.[3,4]

Analytical method validation is the next important step in justification and acceptability of an analytical method, after method development. It enables scientists to communicate scientifically and effectively on technical matters. Set standards of evaluation procedures for checking compliance and taking remedial measures.

However, validation of equipment and analytical methods is necessary, not only due to regulations and accreditation standards, but also as prerequisite in terms of any good analytical practice and should be on going in the form of re-validation with method changes.[5,6]

1.1 DIFFERENT TECHNIQUES OF ANALYSIS

By using this process, the components of interest are separated and analysed by using the following techniques.

1.1.1 Classical Method

1.1.1.1 Qualitative analysis

- Chemical tests
- Flame test

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1.1.1.2 Quantitative analysis

- Gravimetric analysis
- Volumetric analysis

1.1.1.3 Instrumental methods

- Spectroscopy
- Mass spectrometry
- Electrochemical analysis
- Thermal analysis
- Separation
- Hybrid techniques
- Microscopy
- Lab-on-a-chip

1.1.2 Qualitative analysis - A qualitative analysis determines the presence or absence of a particular compound, but not the mass or concentration. That is, it is not related to quantity.

- **Chemical tests -** There are numerous qualitative chemical tests, for example, the acid test for gold and the Kastle-Meyer test for the presence of blood.
- **Flame test -** A flame test is an analytical procedure used in chemistry to detect the presence of certain elements, primarily metal ions, based on each element's characteristic emission spectrum.[7,8]

1.1.3 Quantitative analysis - Quantitative analysis is the measurement of the quantities of particular chemical constituents present in a substance[5].

- **Gravimetric analysis -** A known volume of sample solution is treated with an excess of suitable reagent which quantitatively precipitate the desired constituent present in the sample solution.[6] The precipitate which is of known composition is filtered, washed, dried and weighed. Knowing the weight of the precipitate, the amount of the desired constituent in the test solution is calculated.
- **Volumetric analysis -** In this type of analysis, to the sample solution of unknown concentration, a reagent solution of known concentration is gradually added till the reaction between them is just complete as shown by some indicator. The volume of the sample and reagent solutions are known, the concentration of the reagent solution is also known so the concentration of the given sample solution can be calculated.[9,10]

2. MATERIAL AND METHODS

A double beam UV/Visible model 1800, Electronics India, India, with software UV Probe 2.10 and 1 cm quartz cell, was used for analysis. Standard atorvastatin (ATR) and ezetimibe (EZ) was kindly supplied by Sun Pharma Industries, Baroda City, India. All other chemicals used were of analytical grade. Atoreza**s** tablets (10/10) (B.N. 1031061) labelled to contain Atorvastatin calcium equivalent to 10 mg ATR and 10 mg EZ.

2.1 Identification and Characterization of Atorvastatine and Ezetimibe

2.1.1 Identification:

The gift samples of Atorvastatin and Ezetimibe procured were evaluated for the spectral analysis to check their purity and authenticity.

2.2 FTIR Spectrum of Atorvastatin and Ezetimibe

The drug sample (Atorvastatin and Ezetimibe) were evaluated by the FT-IR spectroscopy (Bruker Alpha-II courtesy by, AIPS Sagar, M.P.).

2.3 Physicochemical Characteristics of Atorvastatin and Ezetimibe

The following parameters were selected i.e., melting point and solubility.

2.3.1 Melting point

Melting point of the drug sample was analyzed by Digital Melting point apparatus. Melting point of Atorvastatin and Ezetimibe was found to be 164-180˚C and 163˚C respectively. that defines the purity of the drug sample.

2.3.2 Solubility

The solubility of drug samples (Atorvastatin and Ezetimibe) was carried out by various solvent systems and data of solubility used in selection of solvent for the UV analysis. Different solvents were used for the solubility analysis of drugs.

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2.3 UV spectrophotometric analysis by Dual wavelength analysis method

Application of dual wavelength method is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the component of interest, independent of the interfering components. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration.

Different aliquots equivalent to 60–260 and 80–400 μg/mL of ATR and EZ, were separately transferred from their respective standard working solutions (0.1 mg/mL) into two separate series of 10-mL volumetric flasks and then the volume was completed using methanol. The prepared solutions were scanned in the range of 200–350 nm and the absorbance values at 226.6 and 244 nm (for ATR) and at 228.6 and 262.8 nm (for EZ) were measured. ATR was determined by plotting the difference in absorbance values at 226.6 and 244 nm (difference is zero for EZ) against its corresponding concentration. Similarly for determination of EZ, the difference in absorbance values at 228.6 and 262.8 nm (difference is zero for ATR) was plotted against the corresponding concentrations.

2.4 Standard drug solution and scanning in UV:

Before the selection of working conc. range, we tried various conc. ranges to find out appropriate conc. range for the work ahead.

2.5 Stock solution of Ezetimibe

Stock solution of Ezetimibe was prepared by dissolving accurately weighed 1000 µg of standard ezetimibe in 10 ml of solvent system to get concentration 100 µg/ml of stock solution. Then taken 10 ml of solution from 100 µg/ml stock solution and diluted with solvent system to get 1000 µg/ml concentration and after appropriate dilutions with solvent system to get concentration of 80-400 µg/ml and this was scanned in UV range.

2.6 Stock solution of Atorvastatin

Stock solution of atorvastatin was prepared by dissolving 1000 µg (accurately weighed) of standard atorvastatin in 100 ml of solvent system and give concentration 100 µg/ml stock solution. Then taken 10 ml of solution from 100 µg/ml stock solution and after dilution with solvent system to obtained 60-260 µg/ml. The prepared solutions were scanned in the range of 200–350 nm and the absorbance values at 226.6 and 244 nm (for ATR) were measured. ATR was determined by plotting the difference in absorbance values at 226.6 and 244 nm (difference is zero for EZ) against its corresponding concentration.

2.7 Analysis of marketed formulation (Atorvastatin and Ezetimibe)

This Ezetimibe + Atorvastatin is a Tablet combination available in market. Fourteen tablets of Atorezas tablets were powdered and mixed well. Accurately weighed amount of the powdered tablets equivalent to 100 mg of ATR and EZ was transferred to a 100-mL volumetric flask and 75 mL of methanol was added. The prepared solution was sonicated for 15 min, cooled and the volume was completed to obtain 1 mg/mL stock solution and then the solution was filtered. Appropriate dilutions of the prepared solution were made to prepare its working solution (0.1 mg/mL) and the procedures under linearity were followed.

2.8 Recovery studies

To study the accuracy of the proposed methods, recovery studies were carried out by application of the standard addition technique. Known amounts of the studied drugs were separately added to a definite amount of the powdered tablet; the prepared samples were then analyzed as under linearity and the percentage recoveries were then calculated.

2.9 Validation of Method

Validation of the method has been carried out according to ICH recommendations.

2.10 Linearity and range

The calibration range for ATR and EZ was established through considerations of the practical range necessary according to adherence to Beer–Lambert's law and the concentrations of ATR and EZ present in the pharmaceutical dosage form to give accurate, precise and linear results.

2.11 Accuracy

The degree of agreement between a value recognised as a conventional true value or an accepted reference value and the value produced is defined as the accuracy of an analytical procedure. The recovery tests were carried out in triplicate by spiking previously analysed samples with three different doses of standards. The accuracy of the results was checked by applying the proposed methods for determination of different blind samples of ATR and EZ and the concentrations were obtained from the corresponding regression equations.

Accuracy of the methods was further assured by applying the standard addition technique where good percentage recoveries

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were obtained, confirming the accuracy of the proposed methods.

2.12 Precision

2.12.1 Repeatability.

It is the concordance of a series of measurements of the same quantity when the experiments are conducted under same conditions (analyst, apparatus, instrument, and day) in a rapid succession. Three concentrations of ATR and EZ (12, 16, 20 mg/mL) were analyzed three times intra-daily using the proposed methods.

2.12.2 Intermediate precision.

The intermediate precision expresses with in laboratories variation: different day, different analyst, different equipment etc. The previous procedures were repeated inter- daily on three different days for the analysis of the three chosen concentrations.

2.12.3 Specificity

Specificity of the methods was achieved by analysis of different laboratory prepared mixtures of ATR and EZ within their linearity ranges.

3. RESULT AND DISCUSSION

3.1 Characterization of drugs

3.1.1 FTIR spectra of ezetimibe

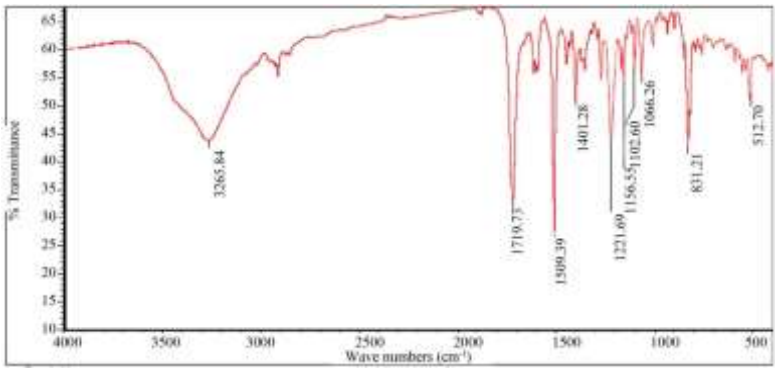

Figure 1. FTIR spectra of Ezetimibe

3.1.2 FTIR spectra of atorvastatin

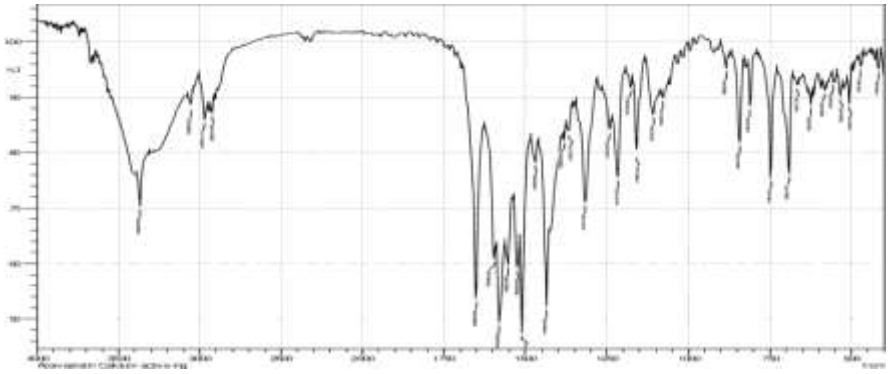

Figure 2. FTIR spectra of Atorvastatin

3.1.3 Melting point of atorvastatin and ezetimibe

Both the drug was also identified by determining their melting. Both the drugs showed the melting point in the standard range so the analysed samples of drugs were pure.

Drug	Melting point (°C)
Atorvastatin	164-180 ˚C
Ezetimibe	162-163 ˚C

Table 1. Melting point of atorvastatin and ezetimibe

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3.1.4 Solubility study of drugs

Different solvents were used for the solubility analysis of drugs.

Solvents	Ezetimibe	Atorvastatin
Water	Sparingly soluble	Very slightly Soluble
Acetone	Soluble	Insoluble
DMSO	Soluble	Sparingly Soluble
Ethanol	Soluble	Slightly Soluble
Methanol	Freely Soluble	Freely Soluble

Table 2. Solubility of drugs

3.2 Dual wavelength analysis method

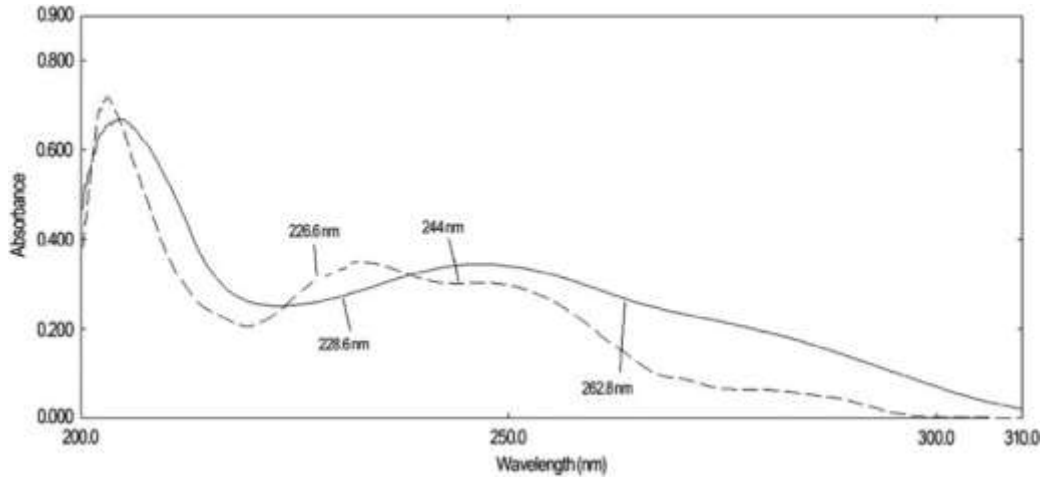

Figure 3. Zero-order absorption spectra of 8 mg/mL each of ATR (—) and EZ (- - -) using methanol as a solvent.

Parameters	Dual wavelength method	
	ATR	EZ
Linearity range (mg/mL)	6-26	8-40
Slope	0.0092	0.0202
Intercept	0.0097	0.009
Correlation coefficient	0.9997	0.9999
Precision		
Repeatability	0.924	1.071
Intermediate precision	1.273	1.145
Accuracy	100	101.66
Specificity (%)	99.897 ± 1.594	100.877 ± 1.292

Table 3. Linear regression and analytical parameters of the proposed method for determination of ATR and EZ

3.3 Analysis of marketed formulation (Atorvastatin and Ezetimibe)

Parameters	Dual wavelength method	
	ATR (%)	EZ (%)
Tablet formulation	99.97±1.80	101.54±1.06
Standard addition[a]	100.6±1.02	101.17±1.85
F-test (6.388) [b]	2.491	1.124
Student's t-test (2.306) [b]	0.006	1.147

Table 4. Determination of the studied drugs in the laboratory prepared mixtures (L.P.), pharmaceutical preparations by the proposed method and statistical analysis.

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3.4 Linearity and range

Parameters	Dual wavelength method	
	ATR	EZ
Linearity range (mg/mL)	6-26	8-40
Slope	0.0092	0.0202
Intercept	0.0097	0.009
Correlation coefficient	0.9997	0.9999

Table 5. Determination of the linearity ranges of both ATR and EZ

3.5 Accuracy

Precision Parameters	Dual wavelength method	
	ATR	EZ
Accuracy	100	101.66

Table 6. Accuracy data of both ATR and EZ by the proposed method

3.6 Repeatability

Precision Parameters	Dual wavelength method	
	ATR	EZ
Repeatability	0.924	1.071

Table 7. Repeatability data of both ATR and EZ

3.7 Intermediate precision

Precision Parameters	Dual wavelength method	
	ATR	EZ
Intermediate precision	1.273	1.145

Table 8. Intermediate precision data of both ATR and EZ

3.8 Specificity

Precision Parameters	Dual wavelength method	
	ATR	EZ
Specificity (%)	99.897 ± 1.594	100.877 ± 1.292

Table 9. Specificity (%) data of both ATR and EZ

5. CONCLUSION

The developed dual wavelength and mean centering of ratio spectra (MCR) spectrophotometric methods have been successfully applied for simultaneous determination of ATR and EZ in their combined marketed sample; they are found to be rapid, simple, accurate and easy to be understood and applied. On the developed dual wavelength method once the equations were constructed, analysis required only measuring the absorbance values of the sample solution at the elected wavelengths followed by a few simple calculations. On the other hand, the MCR method does not need derivatization steps or complex algorithms. When the suggested methods were completely validated, they showed satisfactory data for all the method validation parameters tested. Recovery studies indicated that practically there was no interference from the tablets additives, so these methods can be easily and conveniently adopted for routine quality control analysis of ATR and EZ.

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