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**ORIENTAL JOURNAL OF CHEMISTRY**
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2013, Vol. 29, No. (4):
Pg. 1605-1610

# A Validated Spectral Discriminating Derivative Spectrophotometric Method for Simultaneous Quantification of Atorvastatin Calcium and Fenofibrate Combination in Tablets

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http://dx.doi.org/10.13005/ojc/290444

(Received: September 28, 2013; Accepted: November 07, 2013)

**ABSTRACT**

A simple and specific second-order-derivative spectorophotometric method has been developed and validated for simultaneous quantification of atorvastatin calcium (ATV) and fenofibrate (FEN) in tablet dosage forms. ATV was determined at a wavelength of 281 nm (zero-crossing wavelength point of FEN). Similarly, FEN was measured at 296 nm (zero-crossing wavelength point of ATV) in phosphate buffer, pH 2.8 as solvent. The second derivative amplitude-concentration plots were rectilinear over the range of 2-12 µg/mL for ATV and 1-30 µg/mL for FEN. The % assay in commercial formulation was found to be in the range 98.8 – 102.5 for ATV and 99.6 – 100.25 for FEN by the proposed method. The method was validated as per ICH guidelines. The proposed method can be effectively applied for routine analysis of ATV and FEN in tablets.

**Key words:** Atorvastatin calcium, Fenofibrate, Second-derivative, Simultaneous, Validation.

## INTRODUCTION

Atorvastatin Calcium (ATV) chemically [*R*-(*R*',*R*'))]-2-(4-Fluorophenyl)-β,δ-dihydroxy–5-(1–methylethyl)-3–phenyl–4-[(phenylamino)carbonyl]-1*H*-pyrrole–1–heptanoic acid, is a HMG-Co A reductase inhibitor with hypolipidemic properties [1-4]. Fenofibrate (FEN) chemically, Isopropyl 2-[4-(4-chlorobenzoyl)2-phenoxy] methyl propanoate, is a fibric acid derivative with lipid regulating properties exerting its therapeutic effects through activation of peroxisome proliferator activated receptor α (PPARα). The combined dosage form of ATV and FEN is therapeutically used for hyper lipidemic patients [5]. A detailed literature survey revealed that few simultaneous analytical methods reported for quantification of ATV and FEN by liquid chromatography, spectrophotometry (simultaneous

equation method) [6-10] but chromatographic methods are require complex mobile phase composition, expensive instrument set up, skilled operators and normal spectroscopy method was unsuitable for evaluation of drugs in multi-component analysis because of lack of specificity. Derivative spectroscopy provides a superior selectivity and spectral discrimination than common absorption spectroscopy. It is the dominant approach for resolution of one analyte whose peak is concealed by a large overlapping peak of another analyte in multi-component analysis [11-13]. To the best of our knowledge, only one second derivative spectrophotometric method [12-14] reported for simultaneous quantification of ATV and FEN in methanol as solvent, but methanol is environmental toxic and expensive than aqueous buffers. Literature data signify the need of simple, economic, eco friendly and specific analytical method for simultaneous quantification of ATV and FEN combination in tablets. Hence an attempt has been made to develop a simple, economic, eco-friendly and specific second derivative spectroscopic method for simultaneous quantification of ATV and FEN bulk drug and combination in tablet dosage form using acetate buffer, pH 2.8 as solvent[15].

## EXPERIMENTAL

Double beam 1800 UV-Visible spectrophotometer (Shimadzu, Japan), analytical balance (Shimadzu AUX 220, Japan), pH meter (Elico, Hyderabad) and ultrasonic cleaner (Sonica) were used for the study. ATV and FEN were obtained as a gift samples from Dr.Reddy's, Laboratories limited, Hyderabad, India. Methanol and sodium acetate were purchased from Sd Fine-Chem limited, Mumbai; Double distilled water was used throughout the study. ATV and FEN combination tablet formulations – Atocor (Dr.Reddy's, Laboratories Pvt. Ltd) and Fibator (Sun Pharma, Sikkim) were purchased from local market.

### Preparation of standard stock solutions

Each of standard ATV (10 mg) and FEN (10 mg) were weighed and transferred into two separate 10 mL volumetric flasks and dissolved in methanol. The flasks were shaken and volume was made up to the mark with methanol. From this 1 mL solution was diluted to 10 mL with acetate buffer pH 2.8 to obtain standard solution of ATV and FEN having final concentration of 100 µg /mL of each.

### Selection of wavelengths

Standard solution of ATV and FEN were diluted appropriately with acetate buffer pH 2.8 to obtain a solution containing ATV (10 µg /mL) and FEN (10 µg/ mL). Spectra of these diluted solutions were scanned in the spectrum mode between 200 to 400 nm using acetate buffer pH 2.8 as a blank. These zero-order spectra were transformed to corresponding first and second-derivative spectra in the range of 200 to 400 nm.

### Preparation of sample solutions

Twenty tablets of two different brands (ATOCOR and FIBATOR), containing 10 mg of ATV and 160 mg/145mg (ATOCOR/FIBATOR) of FEN were taken and accurately weighed. Average weight was determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 10 mg ATV and 160 mg/145mg (ATOCOR/FIBATOR) of FEN was transferred to 100 mL volumetric flask. Methanol 20 mL was added to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with acetate buffer. The solution was filtered through whatmann filter paper (No- 41).The filtrate was further diluted with acetate buffer, pH 2.8 to obtaine sample solutions of concentrations within linearity range. The derivative absorbance of sample solutions were measured at selected wavelengths used for the quantification of drugs.

### Method Validation

The selected method was validated [13] for linearity, accuracy, precision, specificity, LOD and LOQ by the following procedures.

### Linearity

Appropriate aliquots of standard stock solutions of atorvastatin calcium (100 µg/mL) and fenofibrate (100 µg/mL) were taken in two different sets of 10 mL volumetric flasks and diluted upto the mark with acetate buffer, pH 2.8 to obtain final concentrations of 2 – 12 µg /mL ATV and 1-30 µg /mL FEN. The second-derivative spectra were recorded using the prepared solutions against acetate brffer, pH 2.8 as blank. The values of second-derivative absorbance were plotted against corresponding concentrations to construct the calibration curves.

**Accuracy**

The accuracy of the method was determined by calculating recoveries of ATV and FEN by the method of standard additions. Known amounts of ATV and FEN (80%, 100% and 120%) levels were added to pre quantified sample solutions. These solutions were further diluted with acetate buffer, pH 2.8 and analyzed by using acetate buffer, pH 2.8 as blank. The recovery was verified by the estimation of drug in triplicate at each specified concentration level and calculated % RSD.

**Precision**

The intra-day and inter-day precision of the proposed second-derivative spectrophotometric simultaneous method was determined by estimating the corresponding response three times on the same day (intra-day) and for three repeated days (inter-day) for three different concentrations of ATV (2, 6 and 12 µg/mL) and FEN ( 1,15 and 30 µg/mL). The results are reported in terms of relative standard deviation (% RSD).

**Limit of detection (LOD) and limit of quantification (LOQ)**

The limit of detection (LOD) and limit of quantification (LOQ) for the procedure were performed on sample containing very low concentrations of analyte as per ICH guidelines.

## RESULTS AND DISCUSSION

The technique of derivative spectroscopy may be used with minimum error for the quantification of one analyte, whose peak is hindered by a large overlapping peak of another analyte. Fig.1 shows overlaid zero-order spectra of standard solution of ATV and FEN at 10 µg/mL and spectra were found to be similar in nature and overlapping. It was observed that ATV and FEN mystify significantly

**Table 1: Optimized conditions for the proposed method**

| S.No. | Parameter | ATV | FEN |
|---|---|---|---|
| 1 | Absorption maxima (nm) | 281 | 296 |
| 2 | Beer's Law Limit (mcg/ml) | 2-12 | 1-30 |
| 3 | slope | -0.0004 | -0.002 |
| 4 | Intercept | - 0.0001 | -0.001 |
| 5 | Correlation coefficient | 0.9971 | 0.998 |
| 6 | Regression equation | y = - 0.0004x -0.0001 | y = -0.002x -0.001 |

**Table 2: Precision of the method**

| | ATV | | | |
|---|---|---|---|---|
| **Concentration (µg/mL)** | **Intra-day precision Concentration estimated (µg/mL) (AM ± SD) (n=3)** | **% RSD** | **Inter-day precision Concentration estimated (µg/mL) (AM ± SD) (n=3)** | **%RSD** |
| 2 | 2.25 ± 0.034 | 1.511 | 2.32± 0.025 | 1.077 |
| 6 | 5.75 ± 0.025 | 0.434 | 6.25 ± 0.045 | 0.720 |
| 12 | 12.08 ± 0.235 | 1.903 | 12.02± 0.246 | 2.046 |
| | | FEN | | |
| 1 | 0.9± 0.115 | 1.22 | 1.0± 0.011 | 1.1 |
| 15 | 16.9 ± 0.062 | 0.36 | 15.52 ± 0.054 | 0.347 |
| 30 | 32.25± 0.426 | 1.32 | 31.15± 0.525 | 1.685 |

Acceptance Criteria: % RSD should not be more than

**Table 3: Analysis of commercial tablets (assay)**

| Formulation | ATV | | | FEN | | |
|---|---|---|---|---|---|---|
| | Label claim (mg) | Amount found (mg) (AM ± SD) (n=3) | % RSD | Label claim (mg) | Amount found (mg) (AM ± SD) (n=3) | % RSD |
| Atocor | 10 | 10.25 ± 0.125 | 1.219 | 160 | 160.4±0.252 | 0.157 |
| Fibator | 10 | 9.88 ± 0.112 | 1.133 | 145 | 144.5 ±0.454 | 0.314 |

at their corresponding $\lambda_{max}$ value for absorbance. Hence, the derivative graphical method was used to estimate ATV and FEN in presence of each other. First-order-derivative overlaid spectra of ATV and FEN shown in fig.2 revealed that there was no zero crossing point for ATV to the determination of FEN. Hence, first derivative spectrum was not suitable for quantification of ATV and FEN combination in tablets. This problem was minimized by second-order-derivative method, which was used to choose the suitable wavelengths that make the quantification proportional to ATV and FEN concentrations with zero crossing (Fig.3) The second-derivative spectrum of FEN has zero absorbance at 281 nm, where ATV gives the significant derivative response, while the second-derivative spectrum of ATV has zero absorbance at 296 nm, where FEN gives the significant derivative response. Therefore, 281nm was selected for estimation of ATV and 296 nm selected for the estimation of FEN.

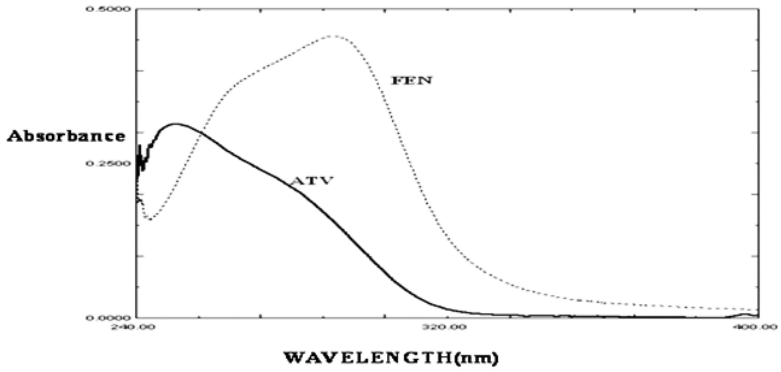


**Fig. 1: Zero-order UV overlaid spectrum**

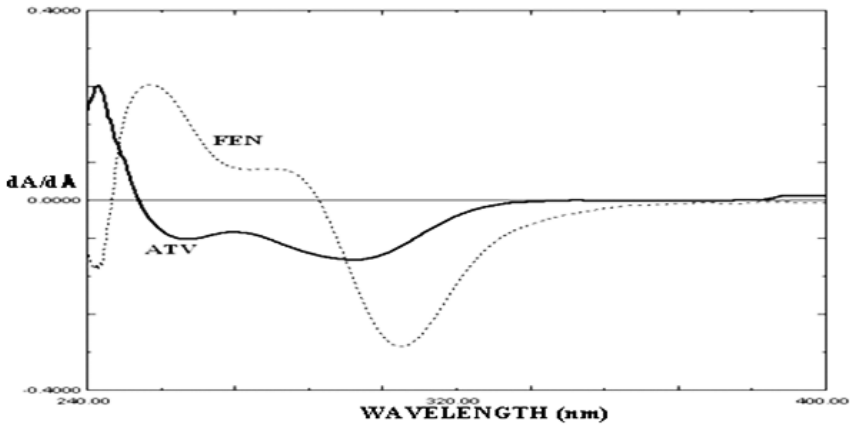


**Fig. 2: First-order UV overlaid spectrum**

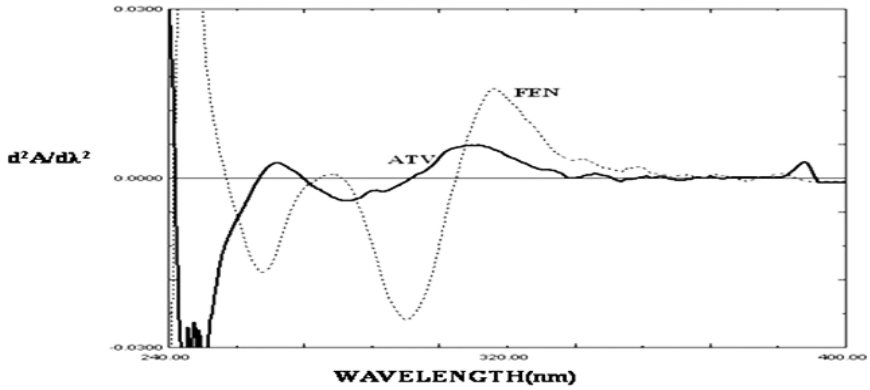


**Fig. 3: Second-order UV overlaid spectrum**

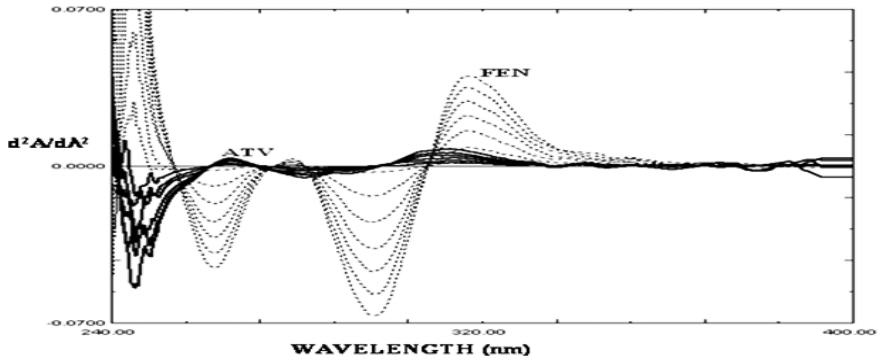


**Fig. 4: UV Second-derivative linearity spectra**

The calibration curves shows that, the developed method was linear in the concentration range of 2-12 µg/mL for ATV and 1-30 µg/mL FEN (Fig.4) .Limit of detection and limit of quantification values were indicated that the method shows high sensitivity. The optimized conditions for developed method were shown in Table 1. No significant difference between intra-day and inter-day precision, revealed that the method is reproducible (Table 2). The % recovery was within the range between 98.8-102.5 and 99.6-100.25 for ATV and FEN respectively and %RSD for commercial formulation was shown less than 2 (Table 4). This indicates that the method is accurate and reliable.

## CONCLUSION

In this present investigation a simple, eco-friendly, sensitive, specific and economic second derivative spectrophotometric method for simultaneous quantification ATV and FEN in pure form and in tablet dosage forms by using acetate buffer pH 2.8 as solvent. The assay values were in good concurrence with their respective labeled claim, which suggested no interference of formulation excipients in the estimation and obtained results from validation evidenced the proposed method was scientifically sound. Therefore, the developed method can be readily accepted by pharmaceutical quality control laboratory for routine analysis.

## ACKNOWLEDGMENTS

The authors are grateful to the management of Gokaraju Rangaraju College of pharmacy for providing needful facilities and supporting for this investigation.

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