Quantitative Estimation and Validation with Using Hydrotropic Solubilization Phenomenon in Pharmaceutical Combined Tablet Dosage forms Atorvastatin calcium and Fenofibrate

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ABSTRACT: Attempting to minimize these drawbacks, three new, simple, accurate, environmental friendly, cost effective, safe, sensitive spectrophotometric methods have been developed for simultaneous estimation of Atorvastatin calcium and Fenofibrate in tablet dosage form using 7.0 M sodium benzoate aqueous solution, as a hydrotropic agent. Various organic solvents like methanol, chloroform, alcohol, dimethyl formamide; acetonitrile, hexane, acetone and carbon tetrachloride have been employed for solubilization of poorly water-soluble drugs for spectrophotometric estimations. Aqueous solubility of these model drugs were enhanced to a great extent 102 and 141 fold for Atorvastatin calcium and Fenofibrate in 7.0 M sodium benzoate solution respectively. Sodium benzoate solution and additives of tablet did not interfere in analysis, as sodium benzoate did not show any absorbance above 316 nm. In 7.0M sodium benzoate solution, Atorvastatin calcium and Fenofibrate shows maximum absorbance at wavelength 327 nm and 315 nm respectively and isobestic point was observed at 286 nm. Beer's law was obeyed in the concentration range 5-35 µg/ml for Atorvastatin calcium and 5-40µg/ml for Fenofibrate. Method-I is based on simultaneous equation method and method II is based on determination of Q-value. Results of analysis for methods were tested and validated for various parameters according to ICH guidelines, hence can be adopted for the routine analysis of Atorvastatin calcium and Fenofibrate in tablet dosage form. The proposed method is new, accurate, simple and economic.

Keywords: Hydrotropic Solubilization, Atorvastatin calcium Fenofibrate, Simultaneous equation method, Absorbance ratio method.

INTRODUCTION

Various techniques have been employed to enhance the aqueous solubility of poorly water soluble drugs such as alteration in pH of solvent, co-solvents, complexation etc. Various poorly water soluble drugs were analyzed using hydrotropic solubilization phenomenon. Viz. frusemide [1], cefixime [2], hydrochlorothiazide [3], ketoprofen [4], bulk sample of ketoprofen and salicylic acid [5], norfoxacin [6] in combination with tinidazole [7]. Hydrotropic Solubilization is one of them. Sodium salicylate sodium acetate, sodium citrate and urea sodium benzoate niacinamide have been employed as a hydrotropic agent which enhances the aqueous solubility of many poorly water soluble drugs. The pH of 7.0 M Sodium benzoate solution was 8.5. Various organic solvents like methanol, chloroform, alcohol, dimethyl formamide, and benzene have been employed for the solubilization of poorly water soluble drugs for spectrophotometric estimations. Increasing the aqueous solubility of insoluble and slightly soluble drugs is of major importance. Various techniques have been employed to enhance the aqueous solubility of poorly water-soluble drugs. Hydrotropic solubilization is one of them. The term hydrotropy has been used to designate the increase in solubility of various substances in water, due to the presence of large amounts of additives. Hydrotropy is a solubilization process where by addition of large amount of second solute results in an increase in aqueous solubility of another solute. Literature survey revealed that some methods are available for the determination Atorvastatin calcium [8], [9] (**AC**) is (β R, δ R)-2-(4-fluorophenyl)-β, δ-

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dihydroxy-5-(1-methyl ethyl)-3-phenyl-4-((phenyl amino)carbonyl)-1H-pyrrole-1-hepatonoic acid, a HMG CoA reductase inhibitors and fenofibrate [10],[11],[12],[13],[14],[15],[16] (**FB**) is 2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester. It is indicated for the treatment of hypercholesterolemia and mixed dyslipidemia [17].

MATERIALS AND METHOD

UV-visible double beam spectrophotometer, Shimadzu model 1700 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used. Pure sample of Atorvastatin calcium was obtained as gift samples from Sun Pharmaceutical Industries Dadra and Nagar Hawli India, and fenofibrate was obtained as gift samples from Lupin LTD, Mumbai. The commercially available tablets, Tablet formulation containing 10 mg of AC and 200 mg of FB is available (Lorilip Micro Labs. Ltd., Pondicherry, Tornet-TG Lupin LTD, Mumbai) was procured from local market.

Preliminary Solubility Studies of Drugs

Solubility of both drugs was determined at 25 ±1°C. An excess amount of drug was added to three screw capped 70 ml glass vials containing different aqueous system viz. distilled water, buffer of pH 8.5, 7.0M Sodium benzoate solution. The vials were shaken for 15 hrs at 25±1°C in a mechanical shaker. These solutions were allowed to equilibrate for the next 33 hrs and then centrifuged for 5 minutes at 2100rpm. The supernatant of each vial was filtered through Whatman filter paper No. 41. The filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blank.

Preparation of Standard Stock, Calibration Curve and Binary Mixture Solutions

The standard stock solutions of Atorvastatin calcium and fenofibrate were prepared by dissolving 50mg of each drug in 50ml of 7.0M sodium benzoate solutions and final volume was adjusted with distilled water in 100µl of volumetric flask. From the above solution 10 ml of solution was taken and diluted to 50ml with distilled water to get a solution containing 100 µg/ml of each drug. Working standard solutions were scanned in the entire UV range of 400-200 nm to determine the λ max of both drugs. The λ max of Atorvastatin calcium and fenofibrate were found to be 327 nm and 315 nm respectively and from overlain spectra (Fig. 1) it is evident that isobestic point was obtained at 286 nm. Eight working standard solutions for both drugs having concentration 5, 10, 15, 20, 25, 30, 35, 40µg/ml were prepared in distilled water from stock solution. The absorbance's of resulting solutions for both drugs were measured at 315, 327 nm for method I, 286, 327 nm for method and plotted a calibration curve against concentration to get the linearity and regression equation of both drugs. Six mixed standards solutions with concentration of Atorvastatin calcium and fenofibrate in µg/ml of 30:5, 25:10, 20:15, 15:20, 10:25, 5:30 were prepared in distilled water by diluting appropriate volumes of the standard stock solutions.

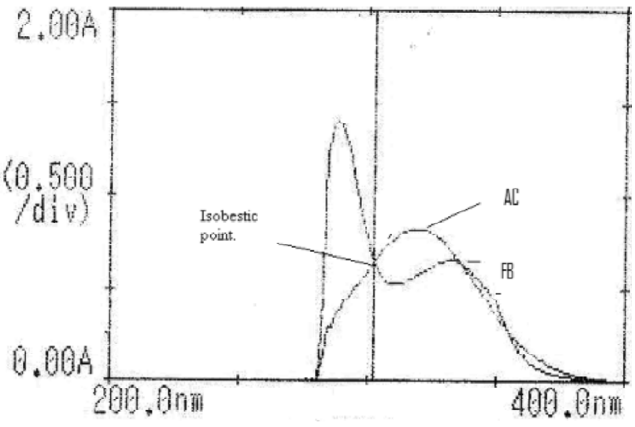

Figure 1: Overlain Spectra and Isobastic Point of AC-Atorvastatin Calcium, FB: Fenofibrate

Method-I (Simultaneous Equation Method)

Simultaneous equation method [18] of analysis was based on the absorption of drugs (Atorvastatin calcium and fenofibrate) at the wavelength maximum of the each other. Two wavelengths were selected for the development of the simultaneous equations was 327 nm and 315 nm, lmax of Atorvastatin calcium and fenofibrate respectively. The absorbances of both the drugs were measured at 327 nm and 315 nm. The absorptivity values E (1%, 1cm) determined for Atorvastatin calcium at 327 nm and 315 nm were 321 (ay_2) and 295 (ay_1) while respective values for fenofibrate were 342 (ax_2) and 386 (ax_1). These values were means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in following

equations to obtain the concentration of both drugs.

$$CAC = \frac{(A_1 x342) - (A_2 X386)}{-23152} \quad (1)$$

$$CFB = \frac{(A_2 x321) - (A_1 x295)}{-23152} \quad (2)$$

Where C_{AC} and C_{FB} are concentration of Atorvastatin calcium and fenofibrate respectively in g/ 100mL. A_1 and A_2 are the absorbance of the mixture at 315nm & 327 nm respectively.

$A_1 = 386xC_{AC} + 295xC_{FB}$ and $A_2 = 342xCA + 295xC_{FB}$

Method-II (Absorbance Ratio Method)

Absorbance ratio method [18] of analysis was based on the absorbance's at two selected wavelengths, one of which is an iso-bestic point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Fig. 1) 312 nm (Iso-bestic point) and 320.5 nm (l max of Atorvastatin calcium) were selected for the formation of Q absorbance equation (Eqn. 3 and 4). The absorbances of both drug measured at 312 and 320.5 nm. The absorptivity values E (1%, 1cm) determined for Atorvastatin calcium at 320.5 and 312 nm were 319.5 (ax_2) and 301.14 (ax_1) while respective values for fenofibrate were 365 (ay_2) and 348.90 (ay_1). These values were means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in following equations to obtain the concentration of both drugs.

$$CAC = \frac{Q_M - 3.8912}{-1.3417} x \frac{A_1}{301.14} \quad (3)$$

$$CFB = \frac{Q_M - 0.54631}{0.73241} x \frac{A_1}{319.5} \quad (4)$$

Q_M, Q_X, and Q_Y were obtained as bellow:

$$Q_M = \frac{A_2}{A_1},\ Q_X = \frac{ax_2}{ax_1} = 0.21301,\ Q_Y = \frac{ay_2}{ay_1} = 2.8471$$

Where C_{AC} and C_{FB} are concentration of Atorvastatin calcium and fenofibrate respectively in g/100mL. A_1 and A_2 were the absorbance of the sample at 303 nm and 332 nm respectively,

A_1 = 301.14$xCAC$ + 319.5$xCFB$ and A_2 = 365xC_{AC} + 301.14$xCFB$

Analysis of the Tablet Formulations

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to 50 mg of Atorvastatin calcium and fenofibrate was transferred to 100 ml volumetric flask and dissolved in 50 ml of 7.0 M sodium benzoate with frequent shaking for 15 minutes and final volume was made up with distilled water. The sample solution was then filtered through Whatman filter paper No.41 and first few ml were rejected. From the above solution 10ml of solution was taken and diluted to 50ml with distilled water to get a solution containing 100 µg/ml of Atorvastatin calcium and corresponding concentration of fenofibrate. This solution contains Atorvastatin calcium and fenofibrate in the proportions of 2:4:7.5.0 ml of solution was transferred in 10ml volumetric flask and diluted with distilled water to obtain final concentration of 5 µg/ml of Atorvastatin calcium and 16 µg/ml of fenofibrate. For method-I absorbance of the sample solution viz. A_1 and A_2 were recorded at 315 and 327 nm respectively and concentration of two drugs in the sample were determined using Eqn.1 and 2. For method-II, absorbances of the sample solution viz. A_1 and A_2 were recorded at 312 nm (iso-bestic point) and 327 nm, lmax of Atorvastatin calcium respectively and ratio of absorbance were calculated viz. A_2/A_1. Concentration of two drugs in the sample were calculated using Eqn. 3 and 4.

VALIDATION

Precision and Repeatability

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablets formulation. The standard deviation, coefficient of variation and standard error was calculated. The results of statistical evaluation are given in Table 2.

Intermediate Precision- (Inter-day and Intra-day precision)

The inter-day and intra-day precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The results of the same are presented in Table 3.

Table 1
Optical Characteristics Data of Atorvastatin Calcium and Fenofibrate

Parameters	*Method-A*		*Method-B*	
	AC	*FB*	*AC*	*FB*
Working λ (nm) in 7.0M sodium benzoate solution	327	315	320.5	312
Beer's law limit (µg/ml)	5-35	5-35	5-40	5-40
Absorptive E (1%,1cm)*	264	282	307	338
Correl Correlation Coefficient*	0.9999	0.9997	0.9999	0.9998
Intercept*	0.0031	0.0002	0.0021	0.0087
Slope*	0.0032	0.0417	0.0087	0.0190

AC- Atorvastatin calcium, FB: fenofibrate, *Average of six estimation

Table 2
Analysis Data of Tablet Formulation, Statistical Validation and Recovery Studies

Method	*Drug*	*Label claim mg/tab*	*Amount found* mg/tab*	*Label claim (%)*	*S.D.**	*% COV*	*S.E.**	*Amount added at (%)*	*% Recovery #*
I	AC	10	9.98	99.87	0.3421	0.2812	0.0931	80	99.92
								100	99.77
								120	100.20
	FB	200	199.98	99.95	0.1092	0.1061	0.2516	80	99.66
								100	92.75
								120	100.0
II	AC	10	10.02	100.07	0.9861	0.6872	0.8712	80	100.05
								100	99.96
								120	100.01
	FB	200	200.04	100.02	1.0613	0.3241	0.6510	80	99.77
								100	100.07
								120	99.99

AC- Atorvastatin calcium, FB: fenofibrate, S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation, # Average of three estimation at each level of recovery

Table 3
Validation Parameters

Method	*Drug*	*LOD* µg/ml*	*LOQ* µg/ml*	*Intraday n=6*	*Precision (% COV) Interday**		
					First day	*Second day*	*Third day*
I	AC	0.1642	0.2209	0.8904	0.6509	0.6902	0.6771
	FB	0.0536	0.2182	0.9063	0.8946	0.7891	0.6812
II	AC	0.02517	0.5719	0.6781	0.8104	0.7610	0.7701
	FB	0.1829	0.0832	0.5690	0.4581	0.5615	0.8032

AC- Atorvastatin calcium, FB: fenofibrate, COV: Coefficient of variation, *Average of six determination

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method-I and II, the Beer-Lambert's concentration range was found to be 5-35 µg/ml for AC and 5-40 µg/ml for FB. The linearity data for both methods are presented in Table 1.

Accuracy

To check the accuracy of the proposed methods, recovery studies were carried out at 80,100, and

120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are given in Table 2.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD & LOQ of Atorvastatin calcium and fenofibrate by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 2.5 σ/S and 8.5 σ/S, respectively, where S is the slope of the calibration curve and s is the standard deviation of response. The results of the same are shown in Table 3.

RESULTS AND DISCUSSIONS

The validity and reliability of proposed methods were assessed by recovery studies. Sample recoveries for both the methods are in good agreement with their respective label claims, which suggested non interference of formulation additives and hydrotropic solubilizing agent sodium benzoate in estimation. (Table 2).

Percentage estimation of both drugs was found in tablet dosage form were 100.05 and 99.98 in method I, 100.05and 99.95 in method II for Atorvastatin calcium and fenofibrate respectively with standard deviation <2 (Table 2).Solubility studies indicated that aqueous solubility of Atorvastatin calcium and fenofibrate were enhanced more than 102 and 141 folds in 7.0 M sodium benzoate solution as compared to solubility in distilled water and buffer of pH 8.5 respectively.

Linearity range for Atorvastatin calcium and fenofibrate were found to be 5-35 µg/ml and 5-40 µg/ml at respective selected wavelengths and coefficient of correlation were found 0.9999, 0.9997,0.9993 for Atorvastatin calcium at 315,327, 286, nm and 0.9999, 0.9996,0.9989 for fenofibrate at 320,327,301 nm respectively (Table 1).

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated for Atorvastatin calcium and fenofibrate. The results were mentioned in Table 2. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for both the methods %COV were not more than 1.0% indicates good repeatability and intermediate precision (Table 3).The present paper describes application of hydrotropic solubilization phenomenon for the simultaneous estimation of Atorvastatin calcium and fenofibrate in tablet dosage form by simultaneous equation method, absorbance ratio method. Both drugs showed good regression values at their respective wavelengths and the results of recovery study reveled that any small change in the drug concentration in the solution could be accurately determined by the proposed methods and low values of LOD and LOQ indicated good sensitivity of proposed methods. Hence proposed methods are new, simple, cost effective, accurate, sensitive, and precise and can be adopted for routine analysis of Atorvastatin calcium and fenofibrate in tablet dosage form.

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