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RESEARCH ARTICLE

Simultaneous Determination of Simvastatin with Caffeine in Bulk Drug, Formulation and their Monitoring in Mice Plasma Through HPLC-PDA Technique

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ARTICLE HISTORY

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Abstract: ***Objective:*** A simple, economical and rapid RP-HPLC method with PDA detector was developed and validated for the simultaneous determination of simvastatin and caffeine in pure form, in formulation and the method is applied to monitor these drugs in mice plasma. Caffeine containing food and drugs commonly co-administered with statins, there is also a possibility of the caffeine-statins combination as anti-influenza formulation implies a need for a reliable HPLC method for the simultaneous determination of these drugs.

Method: In the present work, the separation was carried out using methanol: water (90:10 v/v) as mobile phase at pH of 3.5 adjusted using phosphoric acid. At room temperature, isocratic separation carried out on C18 XBrigde^{TM} column (5 µm, 25x0.46 cm) at a flow rate of 1 mLmin^{-1} and drugs were detected at 230 nm by using a PDA detector. The established method was validated with respect to linearity, specificity, precision, accuracy and limits of detection and quantification.

Results: The method was linear at a concentration range of 5-50 µgmL^{-1} for both drugs with calculated limit of detection of 0.025 and 0.059 µgmL^{-1} for simvastatin and caffeine, respectively. This method was also successfully applied for the monitoring of these drugs in mice plasma.

Conclusion: The present study suggests that the simultaneous determination of simvastatin and caffeine will be helpful to pharmaceutical manufacturing companies and clinicians checking for drug-drug interactions and could save expenses in case of future combination determination.

Keywords: Simvastatin, caffeine, RP-HPLC, method development, anti-influenza.

1. INTRODUCTION

Statins, a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitors, are considered drugs of choice for the treatment of hypercholesterolemia [1]. Studies show that statins can interfere with cell structure and the environment necessary for viral replication [2, 3]. Literature also suggests that statins might have beneficial effects on influenza outcomes [4, 5]. Simvastatin is a widely used statin (Fig. **1**) [6] and is known to interact with different foods and drugs such as grapefruit juice which may lead to an increase in its concentration and its active form in the blood [7].

Some drugs such as amlodipine and colchicines have shown to increase the risk of myopathy in the case of co-administration with simvastatin [8]. The study performed on rats also indicated that simvastatin with fluoxetine and ascorbic acid is a radiant delayed stroke treatment [9].

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Caffeine (Fig. **2**), a known immune-stimulant, is a natural alkaloid and found in the leaves, seeds of coffee, cocoa beans, tea leaves and chocolate especially in the dark ones [10]. In America, 90% of adults consume caffeine on daily bases [11] and the major sources of caffeine in the adult diet in North America are coffee (60–75%) and tea (15–30%) [12]. It is known the other things to possess some antiviral activity [13].

It has been reported, in a patency application, that a mixture of selected statins and caffeine was designed to combat viral influenza through their administration in combination. The lovastatin/caffeine combination in certain dosage forms was more effective than TAMIFLU in treating both the H3N2 and H1N1 influenza viruses [14]. A different work performed by Liu *et al*., [15] suggested that statin/caffeine combination might be a viable treatment against influenza virus infections. The research results support the combination of these two agents for the treatment and prophylaxis of the aforementioned influenza virus strains and there was reported the development of a novel statin (PTPM7) and caffeine formulation by Canopus BioPharm [15].

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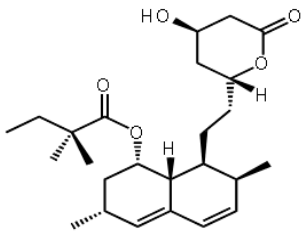

Fig. (1). Simvastatin.

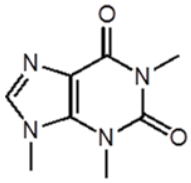

Fig. (2). Caffeine.

As seen before, there are multiple intersections between simvastatin and caffeine, starting from potential drug-food interaction to the possibility of drug-drug combination to treat viral influenza. For this purpose a highly sensitive analytical method is required to study the qualitative and quantitative behaviors of the two drugs and later to be used for the study of kinetics and the interaction profile along with an animal model to draw a conclusion.

Different HPLC methods have been reported for the determination of each simvastatin and caffeine in dosage forms and in biological fluids. Barrett *et al*., determined simvastatin and its metabolite simvastatin hydroxyacid on an electron spray ionization triple quadrupole mass spectrometer equipped with an ESI interface [16]. In one method, simvastatin was determined by HPLC technique using an acetonitrile-phosphate buffer-methanol (5: 3: 1, v/v/v) as a mobile phase [17]. One method based on RP-HPLC for the simultaneous determination, was reported for tocotrienol isoforms and simvastatin in SIM–TRF nanoparticles using UV-vis detector [18]. A simultaneous method for the determination of simvastatin with ezetimibe in pharmaceutical dosage forms using isocratic RP-HPLC method was also reported [19]. Guzik compared and validated two methods of analysis of simvastatin using HPLC [20]. A method was reported for the quantification of simvastatin in the dissolution medium by HPLC for simvastatin capsules [21]. An HPLC method also reported for the determination of lovastatin, pravastatin sodium and simvastatin in bulk drug or dosage forms [22]. Munir et. al., reported an HPLC method to check the simvastatin solubility in different oils and surfactants [23]. Simultaneous determination of simvastatin and other statins with cetirizine [24] and with antidiabetic drugs [25] are also reported. Recently, a sensitive LC-MS/MS method for the simultaneous estimation of simvastatin and simvastatin acid in human plasma, utilizing isocratic elution on a C18 column has been reported. Using a mobile phase of methanol: ammonium acetate (10 mM) and formic acid in a ratio of (90:10:0.02, v/v/v), at a flow rate of 1.0 mL/min, the method was applied for the pharmacokinetic of a simvastatin and extended release niacin combination tablet while freeze-thaw stability of simvastatin and simvastatin acid were also studied [26].

Similarly, there are several methods reported for the determination of caffeine by RP-HPLC [10,27,28]. Wanyika developed a method for the determination of caffeine content of tea and instant coffee using water, acetic acid and methanol as mobile phase **[**10**]**. Syed and Muhammad reported HPLC method for the determination of caffeine level in human and in synthetic plasma and studied their freeze and thaw stability **[**28**]**.

To the best of our knowledge, still there is no method reported for the simultaneous determination of simvastatin and caffeine in literature. The purpose of present work was to look for the possibility of developing a practical, valid and efficient method for the simultaneous determination of simvastatin and caffeine through RP-HPLC technique in their pure forms, formulation and its application in mice plasma. The established method was validated as per parameters like linearity, precision, accuracy, limit of detection and quantification (LOD and LOQ) according to International Conference on Harmonization (ICH) [29] and FDA bioanalytical method validation guidelines [30].

2. EXPERIMENTAL

2.1. Materials and Instrumentations

Simvastatin (purity ≥97%) reference standard was purchased from Sigma Aldrich Chemical Co. USA while tablets having label claim of 20 mg active (Gulf Pharmaceutical Industries U.A.E) were purchased from local market. Methanol HPLC grade, caffeine and phosphoric acid were of analytical grade (Merck Germany) and freshly prepared deionized water was used throughout the experiment. In formulation tablets of caffeine, an equivalent amount of 20 mg per tablet was prepared by using caffeine and common excipients like steric acid, avicel, magnesium strearate etc. Waters HPLC system with Waters 2998 PDA (Photodiode Array Detector) and Water e2695 separation modules pump with auto injector were used along with Waters XBrigdeTM C18 column (5 µm, 25x0.46 cm) and connected with guard column. Empower 2 software; Waters Corporation was utilized for data acquisition.

2.2. Animals

Mice (weighing 20–22 g) of either sex housed under the controlled conditions of temperature and humidity (25±2°, 55±2%) and received a standard pelleted diet. The Institutional Animal Ethics Committee (IAEC) approved (UQU-COP-EA#143616) the use of animals.

2.3. Chromatographic Conditions

Methanol and water in a ratio of 90:10 v/v (pH of 3.5 by phosphoric acid buffer) were used as a mobile phase for the separation. Flow rate of 1.0 $mLmin^{-1}$ was chosen and wavelength of 230 nm was selected for the quantification. The retention times were found to be 5.48 for simvastatin and 2.99 minutes for caffeine. A representative chromatogram is shown in Fig. (**3**).

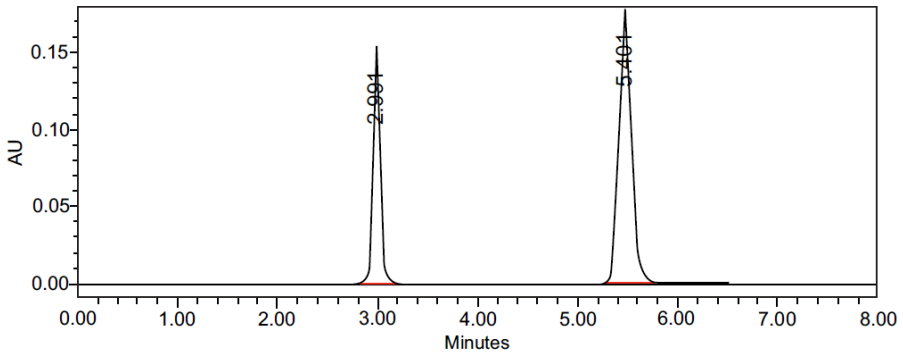

Fig. (3). Representative chromatogram of caffeine (at 2.99 min) and simvastatin (at 5.40 min).

2.4. Solution Preparations

All solutions were prepared using methanol as diluent. Initially stock solutions were prepared by weighing 10 mg of both compounds to 100 mL volumetric flasks. Different volumes of these solutions were diluted with methanol in order to obtain solutions with a final concentration of 5-50 μgmL^{-1} for simvastatin and caffeine. These sets of dilutions were injected into the system and then kept in a cool place. Twenty tablets of each drug, were crushed and equivalent to 10 mg of powder were weighed and transferred in 100 mL flasks. The powder was dissolved in methanol and this solution was shaken for ~60 min before performing filtration through Whatman filter paper in order to separate out the insoluble excipients. The required dilution and concentration were then prepared.

2.5. Validation Procedures

Validation process was followed according to the guidelines set by the ICH for technical requirements for the registration of pharmaceuticals for human use [29].

2.6. Linearity, Accuracy and Precision

For linearity, standard calibration curve and linear regression analysis were performed in the concentration range from 5-50 μgmL^{-1} (5, 10, 20, 30, 40, 50 μgmL^{-1}) for simvastatin and caffeine. The precision of the method was evaluated by 6 consecutive runs at a fixed amount of samples. For accuracy, recovery studies were performed at different amounts of samples within linearity range.

2.7. Limit of Detection and Quantitation

According to ICH, [29] limit of detection and quantitation were calculated using following equations;

$$LOD = \frac{3.3\ \sigma}{S}$$

$$LOQ = \frac{10\ \sigma}{S}$$

Where σ is the standard deviation of the response, S is the slope of the calibration curve.

2.8. Application in Mice Plasma

50 μgmL^{-1} of simvastatin and caffeine each was injected by subcutaneously. Under ether anesthesia, blood samples (0.2 mL) were collected after 1 hour by heart puncture of mice by micro-capillary in heparinized tubes and later on plasma was separated from it by centrifugation at 3000×g [31-33]. Plasma (0.5 mL) collected from mice was deproteinized by adding 1 mL acetonitrile so that the drugs could be extracted out. This mixture was then centrifuged at 5000 g for 10 min. The supernatant was filtered and 20µL was injected in to the system.

2.9. Stability Studies

Stability studies were carried out for QC samples of high and low concentration keeping at room temperature for 6 h [30, 33].

3. RESULTS

3.1. Method Optimization

To get optimum conditions for sensitivity, good separation and peak shape, different mobile phase combinations of methanol and water with phosphate buffer were investigated. After a number of trials, we found that methanol and water at a ratio of 90: 10, v/v having pH of 3.5 was the best choice in order to perform simultaneous determination of these drugs. Hence, this mobile phase was used for the analysis and validation studies of the simultaneous method.

3.2. Method Validation

The method was validated in accordance with ICH guidelines [29]. Linearity, method accuracy (%recovery), method precision (%RSD), limit of detection and quantification were tested.

3.3. Linearity

In our method, calibration curves showed linearity over a concentration range from 5 to 50 μgmL^{-1} for both drugs. The determination coefficients simvastatin and caffeine obtained by linear regression of curve were 0.9975 and 0.9964, respectively. Table **1** summarizes the statistical parameters of the experimental data, such as slopes, intercepts and determination coefficients.

3.4. Accuracy, Precision and Recovery

Accuracy was studied, in term of percent recoveries, by using three different concentrations, containing 8, 10 and 12 μgmL^{-1} of simvastatin and caffeine. According to Table **2**

Table 1. Linear regression functions and their statistical parameters.

Drug	Simvastatin	Caffeine
Regression equation	y=29064x+ 31215	y=14288x+ 20584
r^2	0.9975	0.9964
LOD ($mgmL^{-1}$)	0.025	0.059
LOQ ($mgmL^{-1}$)	0.077	0.179
Retention time (min±%rsd)	5.48±0.2	2.99±0.2

Table 2. Accuracy of method.

Analyte	Measuring Concentration (μgmL^{-1})	Measured Concentration (μgmL^{-1})	Accuracy %	Bias %
Simvastatin	8	8.01	100.18	0.12
	10	9.94	99.39	-0.60
	12	12.01	100.10	0.08
Caffeine	8	8.29	103.64	3.62
	10	10.04	100.44	0.40
	12	11.68	97.32	-2.67

[34], recovery values were found within the range, from 99.39 to 100.18% for simvastatin and from 97.32 to 103.64% of caffeine, satisfying the acceptance criteria for the study. The precision of the method investigated in terms of %RSD. Precision was calculated from six replicate injections of each simvastatin and caffeine at the analytical concentration of about 50 μgmL^{-1} and the %R.S.D. was found 1.63% and 0.21%, respectively (Table **3**). Known amounts of stock solutions were added in to the samples having known content for calculating recovery tests and percentage of recovery was calculated (Table **4**).

3.5. Limit of Detection and Quantitation

The calculated LOD and LOQ values for simvastatin and caffeine are 0.025, 0.077 and 0.059 and 0.179 μgmL^{-1}, respectively (Table **1**).

3.6. Specificity and Selectivity

Chromatogram (Figs. **3**-**5**) shows that the components present in the sample matrix (in formulation and in mice plasma) are not interfering in the analysis. The specificity and selectivity of the method were evident as in the presence or absence of excipients and serum metabolites no extra peak or changes in retention times of the drugs was observed.

3.7. Application to Mice Plasma

Plasma samples obtained from mice blood were diluted and then injected in to the HPLC system. The represented chromatograms were generated for the two drugs in the mice plasma, shown in Fig. (**5**). No interfering peaks from serum metabolites were observed in the generated chromatograms of the two drugs. The method was found to be applicable in the mice plasma and can be used later on for the pharmacokinetics and interaction studies on the two drugs.

3.8. Stability Results

Stability studies were performed and it is observed that the analytes were stable at room temperature (25–30 °C). No significant decrease in the measured concentration or change in chromatographic pattern was observed (Table **5**).

4. DISCUSSION

It is well known that statins alone and in combination with other drugs is commonly used for the prevention of cardiovascular diseases [6, 35, 36]. Caffeine is used as a pain reliever, in cold medications and diet pills [12, 37, 38]. Cardiovascular disease needs long term therapies with the chance of simultaneous consumption of statins with caffeine, which may affect the total amount of drug absorbed [39] on the other hand, the synergistic effects of the two drugs have been established for antiviral therapy. In fact caffeine and statins combination was found to be effective against avian influenza and was found to ameliorate lung damage and inhibited viral replication [15]. In fact, there is also a possibility of combination of statins and caffeine in the treatment of influenza.

Quantification of these drugs simultaneously was determined in commercial products using this method which was found to be reliable. Structurally these drugs are different and the only common behavior of these drugs was found

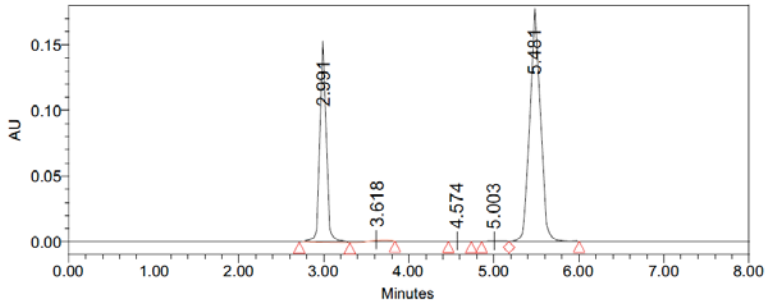

Fig. (4). Representative chromatogram of caffeine and simvastatin in formulations.

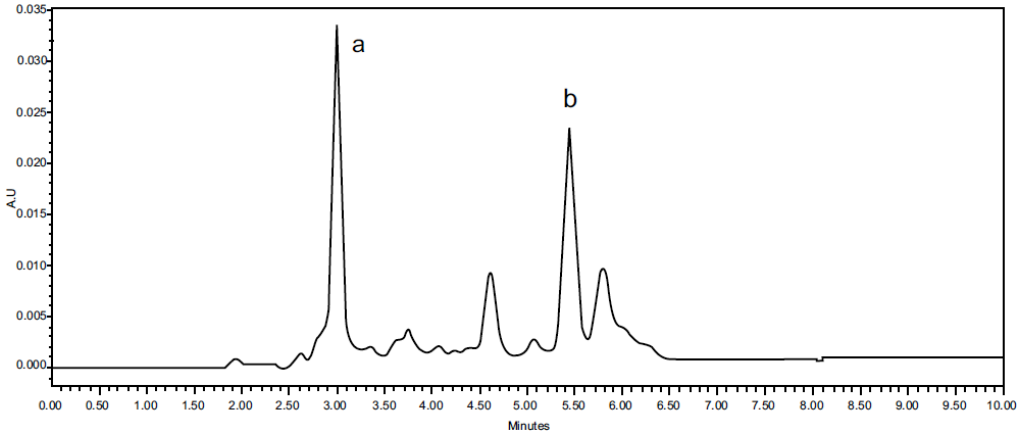

Fig. (5). Representative chromatogram of caffeine (**a**) at 2.99 min and simvastatin (**b**) at 5.40 min in mice serum.

Table 3. Precision of method.

Analyte	Measuring Concentration (μgmL^{-1})	No. of Runs (n)	RSD %
Simvastatin	50	6	1.63
Caffeine	50	6	0.21

Table 4. Recovery of simvastatin and caffeine.

Simvastatin			Caffeine	
Concentration (μgmL^{-1})	Found (μgmL^{-1})	Recovery %	Found (μgmL^{-1})	Recovery %
10	10.03	100.3	9.97	99.70
15	15.10	100.66	14.94	99.60
20	20.14	100.7	20.33	101.65
25	25.11	100.44	24.88	99.52

Table 5. Stability of the method.

Analyte	Measuring Concentration (μgmL^{-1})	Mean Recovery (μgmL^{-1})
Simvastatin	5	4.91
	25	24.88
Caffeine	5	4.85
	25	24.91

to be their solubility profile in the mobile phase. This method is based on simple sample preparation steps thereby avoiding laborious time-consuming sample preparations and extraction procedures. The peak areas measurement was accurate due to the sharp and symmetrical peaks obtained. Tablets interference was not observed as no interfering peaks were found (Fig. **3**), establishing the specificity of the method.

CONCLUSION

We have reported the development of a practical method for the simultaneous determination of caffeine and simvastatin by RP-HPLC in pure form, formulation and in its monitoring in mice plasma. The experimental results showed the validity and practicality of the developed method in addition to being the first time, according to the available literature. For future research, it is recommended the study of possible chemical or kinetic interactions between these two agents in different conditions similar to the physiological conditions of our organism through the application of RP-HPLC.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

We have provided the ethical committee approval letter to conduct experiments on animals.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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