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Original Article

Application of Net Analyte Signal and dispersive liquid-liquid microextraction method based on solidification of floating organic drop for determination of rosuvastatin by spectrophotometry

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ABSTRACT

Hyperlipidemia is a major risk factor for the development of atherosclerosis and kidney disease progression. Rosuvastatin drug is used in the treatment of hypercholesterolemia. In this study, dispersive liquid-liquid microextraction based on solidification of floating organic drop (DLLME-SFO) combined with UV-Vis spectrophotometry Net Analyte Signal (NAS) was developed for the analysis of rosuvastatin in real samples. In this technique, 1- undecanol and ethanol were used as the extraction solvent and dispersive solvent, respectively and various parameters affecting extraction efficiency were optimized such as the type and volume of the extraction solvent disperser solvent, pH and extraction time. In the optimal condition, the linear calibration range was 0.034-0.517 μg ml^{-1} and the correlation coefficient was 0.9998. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.0069 μg ml^{-1} and 0.0231 μgml^{-1}, respectively. Moreover, the relative standard deviation of rosuvastatin was obtained 1.474 % for six replicates. Regarding spiking experiments on real samples, which was found by this method, was between 98.5 % and 110%. As a result, this method was successfully applied to the determination of the rosuvastatin in the pharmaceutical samples.

Keywords: DLLME-SFO, Spectrophotometry, Net Analyte Signal, Rosuvastatin.

Introduction

Hypercholesterolemia is an important risk factor for cardiovascular and renal diseases, which is associated with metabolic disorders of lipid profiles [1, 2]. Rosuvastatin is a solid substance with the molecular formula of $C_{22}H_{27}FN_3O_6S$ and PK_a =4.6, which has three enantiomers. This drug is hydrophilic due to its hydroxyl groups and methane sulphonamide group [3]. In addition, it has a sulfur agent that binds to numerous active sites of 3-hydroxymethyl glutaryl CO-A reductase [4]. This drug inhibits the HMG-COA reductase in the liver and prevents its conversion to the mevalonate, which is a limiting step in cholesterol biosynthesis [3-5]. Rosuvastatin also extends the number of LDL receptors on the surface of the liver cells and increases the absorption and catabolism of LDL [3, 6]. It also reduces the lipid profiles and has pleotropic properties including improved endothelial vascular function, decreased oxidative stress reduction and inflammation, inhibited thrombogenic response and increased nitric oxide production in the endothelial vascular system [4, 7]. Therefore, rosuvastatin prevents cardiovascular and renal diseases by treating hypercholesterolemia [4]. Liquid–liquid extraction (LLE) [8] and solid-phase extraction (SPE) [9] are important techniques for the extraction of analytes from liquid samples. Recently, several different types of liquid-phase microextraction (LPME) methods have been developed including dispersive liquid–liquid microextraction (DLLME)

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[10] and dispersive liquid–liquid microextraction based on the solidification of floating organic drop (DLLME-SFO) [11]. DLLME involves the use of a mixture of two solvents (extraction and dispersive), which are injected into the aqueous sample forming a cloudy solution. The high dispersion of extraction solvent accelerates the analyte extraction and after a centrifugation step, it becomes possible to collect the organic phase extract [10, 12]. The main characteristics of DLLME are simplicity, low cost, suitable analyte recovery, high preconcentration factors, low solvent consumption and short time for extraction [13]. Sample preparation plays an important role in the field of determination of pharmaceutical chemicals in various samples as a pre-concentration step. Recently, Huang et al. described a new liquid-liquid microextraction method based on solidification of the floating organic drop, which was successfully used for separation and pre-concentration of drugs and metals [11]. In this technique, the extraction solvent with a lower density than water, low toxicity along with a small volume of an organic solvent with the melting point near room temperature (in the range of 10-30^{oc}) was used. When an appropriate mixture of extractant and disperser solvent is injected into an aqueous sample containing the analyte of interest, a cloudy solution is formed [14]. Initially, the surface area between the extraction solvent and the sample equilibrium can be achieved quickly and enriched analyte in the floated phase and then organic solvent droplet can be floated on the surface of aqueous solution transferred into an ice bath. When the organic solvent is solidified, it is transferred into a small conical vial and the melted organic solvent is used for analyte determination by spectrophotometry method [15]. The advantages of DLLME-SFO method are the simplicity of operation rapidity, low cost, high enrichment factor and compatibility of the extraction solvent with instruments` analyses [16]. In addition, several experimental parameters influencing the extraction have been optimized such as type and volume of disperser solvent, extraction solvent, pH and extraction time. Chemometrics is a branch of science that is used for extraction of the data related to chemical and physical phenomena involved in the manufacturing process by the application of the statistical and mathematical methods. It can be applied in predictive issues of solving, like predicting the target properties and desired features. Chemometrics shows its application in the multivariate data collection and analysis [17]. The basic concept of Net Analyte Signal (NAS), as a chemometrics technique, is the part of the signal, which is directly related to the concentration predicted by the calibration model. In mathematical terms, it is the part of a spectrum, which is orthogonal to the space spanned by the spectra of all analytes except one. The objective of this study was utilizing DLLME-SFO in combination with UV-Vis spectrophotometry-Net Analyte Signal (NAS) to preconcentrate and measure rosuvastatin in the pharmaceutical samples, according to which several factors affecting the extraction were optimized. In order to Evaluate the method efficiency, the binary synthetic mixtures of rosuvastatin and atorvastatin were used and the proposed method was applied to the simultaneous determination of these components in synthetic samples.

Experimental:

Chemical and Reagents:

Rosuvastatin calcium was obtained from (Darmstadt, Germany) with the highest purity. Dispersive solvents including methanol, acetone, ethanol, and acetonitrile were purchased from Merck Ltd (Germany). 1-undecanol as extraction solvent was purchased from Merck (Darmstadt, Germany) and double distilled deionized water was used for the preparation of aqueous solutions.

Instrument and software

Spectrophotometry analysis of the samples was performed by Shimadzu (model UV- 1800) double-beam instrument. The pH of the solution was determined with an 827 Metrohm digital pH meter. Analytes were weighed with AND digital balance (EJ303-Japeanse) and a centrifuge (Behdad - Iran) was used to accelerate the phase separation process. The samples were sonicatly irradiated in a water bath (50 Hz frequency) for 15 min at 25^{o}C. For conducting NAS/CLS calibration as well as obtaining NAS signals in real samples, the MULTIVAR program available at www.iquir-conicet.gov.ar/descargas/mvc1.rar was used.

Standard solutions:

Accurately, a known amount of rosuvastatin calcium drug (25.0 mg) was transferred to a 50 ml volumetric flask dissolved in the sufficient methanol and then was sonicated for 15 min at 25 ^{o}C and was diluted to 50 ml with the same solvent , which obtained the concentration of drug solution of 500 µg /ml. This stock standard solution of rosuvastatin was stored at 4^{o}C for one month. Working solutions of rosuvastatin were prepared daily by diluting the stock solution with double distilled water before being used.

Real sample solution

Three tablets (Rosuvastatin 5mg) were accurately weighed and powdered. The powder equivalent to 5mg of rosuvastatin was weighed and transferred into a 50 ml volumetric flask and 30 ml methanol was added. The solution was sonicated for 20 min at 25^{0C} and then the volume was completed with MeoH. This solution filtered Whatman filter paper no 40. 5 ml , which was taken in a 50 ml volumetric flask , whose volume was made up to the mark with deionized water. Thus rosuvastatin of strength 10µg/ml was obtained.

Determination of maximum wavelength of rosuvastatin

The 1 ml of stock standard solution was diluted to 50 ml with the methanol, to get the concentration of 10 µg /ml, which was recorded in the range of 200-400 nm against methanol as a blank. The spectrum was recorded and λ_{max} was found to be 242 nm (Fig.1).

Theory and Methods

The principle of net analyte signal based methods was briefly described in this section. The matrices and vectors are used is as follows: an I×J calibration data matrix M containing responses of I samples at J wavelengths, an J×1 vector s_x composed of pure spectrum of analyte x at unit concentration and I×1 calibration concentration c_x vector of interested analyte. The variability of calibration data matrix M is composed of analyte x (Mx) responses and other components responses except analyte (M_{-x})

$$M = M_x + M_{-x} \quad (1)$$

On condition that one analyte of interest exists and provided that linearity is fulfilled, the M_x can be written as follows:

$$M_x = c_x s_x^T \text{ then } M = c_x s_x^T + M_{-x} \quad (2)$$

In calibration context, the net analyte calibration matrix (M_x^*) are vectors which are orthogonal to spaces spanned by the other vectors (M-x) except x [18]. In other words, they are the part of spectra that are orthogonal to the spaces which were created by the spectra of all other analytes except the interested one and is given by the following equation:

$$M_x^* = M R_{NAS} = (M_x + M_{-x}) R_{NAS} = M_x R_{NAS} = c_x s_x^T R_{NAS} \quad (3)$$

Where the R_{NAS} is an J×J preprocessing matrix which is orthogonal to M_{-x} and removes from calibration matrix all sources of variability except analyte x [19-21] hence:

$$M_x^* = c_x s_x^T R_{NAS} \quad (4)$$

The equation simply written as:

$$M_x^* = c_x (s_x^*)^T \quad (5)$$

Where M_x^* and $s_x^*$are net analyte calibration spectra matrix and net sensitivity of analyte x, respectively [22]. Thus, simply the net analyte calibration matrix can be fitted to the calibration concentrations (c_x) using classical least squares (CLS) and the calibration coefficient will be obtained as a result of usual least square fit [23]. The aforementioned equation leads to:

$$s_x^* = (M_x^*)^T c_x (c_x^T c_x)^{-1} \quad (6)$$

and this vector will subsequently predict the analyte x concentration in an unknown sample.

The R_{NAS} matrix projects the rows of calibration spectra (M) onto the NAS space [19-21] and is giving by the following equation:

$$R_{NAS} = I - (M_{-x})^+ M_{-x} \text{ and } M_x^* = M R_{NAS} \text{ then } M_x^* = M [I - (M_{-x})^+ M_{-x}] \quad (7)$$

Where I is an J×J unitary matrix and $(M_{-x})^+$ is pseudoinverse of M_{-x}. $(M_{-x})^+$ is calculated by applying singular value decomposition using K PCA factors [22] in the following equation:

$$R_{NAS} = I - U U^T \text{ and } M_x^* = M R_{NAS} \text{ then } M_x^* = M [I - U U^T] \quad (8)$$

Where U is an J× K matrix that contains the K first (with the largest eigenvalues) u_k eigenvectors of squares matrix $[(M_{-x})^+ M_{-x}]$. The optimum number of K can be estimated using cross validation methods [24, 25] or by minimizing the error values in a validation set [20]. In ideal systems, the number of K is equal to the number of components which create the space of M_{-x}.

The exact nature of M_{-x} is not well defined in unknown samples. Hence, the numerous and different methods were used to find M_{-x}. However, they all present matrixes whose rows are linear combination of original (M_{-x}) matrix rows and generally these matrixes are also named M_{-x} and they all explain a common aspect [19-21]. This matrix is calculated by the following equation:

$$M_{-x} = M - c_x s^T \quad (9)$$

Where s is a spectrum or an appropriate linear combination of spectra which is containing the pure spectral profile of analyte x. one of these calculation and even attractive one is the projection of M matrix orthogonal to c_x [26]:

$$M_{-x} = [I - c_x (c_x^T c_x)^{-1} c_x^T] M \quad (10)$$

comparing equation 9 and 10 leads to:

$$s^T = (c_x^T c_x)^{-1} c_x^T M \quad (11)$$

Hence, s^T is a special linear combination of spectra which is containing in matrix M. This unique property makes M_{-x} orthogonal to c_x. This algorithm is named NAP (net analyte preprocessing) [22].

In summary, NAP/CLS is consist of two steps. The first, projection of M orthogonal to c_x in order to find M_{-x}, the space is spanned by all components except analyte x, and the second, projection of M orthogonal to U to get M_x^*, the analyte calibration data, and correlating this matrix to c_x through a CLS procedure (NAP/CLS) [27].

The NAP/CLS in details is as follows:

1 – pre-processing calibration data

1a - projection of M orthogonal to c_x in order to find M_{-x}

$M_{-x} = [I - c_x (c_x^T c_x)^{-1} c_x^T] M$

1b - applying SVD on M_{-x} and obtaining U with K factors

1c - projection of M orthogonal to U to get M_x^*

$M_x^* = M [I - U U^T]$

2 – pre-processing unknown sample

2a - projection of m orthogonal to U in order to find m_x^*

$m_x^* = [I - U U^T]m$

where m is an J×1 vector composed of unknown sample spectrum

3 - classical least square calibration and prediction

3a- CLS calibration to calculate the calibration coefficient s_x^*

$M_x^* = c_x (s_x^*)^T \rightarrow s_x^* = (M_x^*)^T c_x (c_x^T c_x)^{-1}$

3b- concentration prediction of x in unknown sample (c_{xun})

$m_x^* = c_{xun} (s_x^*)^T \rightarrow c_{xun} = (s_x^{*T} s_x^*)^{-1} s_x^{*T} m_x^*$

DLLME-SFO procedure:

A 10 ml of a standard solution was placed into 10 ml test tube and then the pH was adjusted to 4. A mixture of the disperser solvent (ethanol, 300 µl) and extraction solvent (1-undecanol, 300µl) was injected rapidly into the sample using microsyringe (Hamilton). Then, a cloudy solution was formed. At this step, rosuvastatin molecules were extracted into 1-undecanol. After centrifugation at 3800 rpm for 10 min, the tiny organic droplets were floated at the top of the glass tube. Then, the glass tube was cooled in an ice bath for 8 min to solidified droplets and organic phase were transferred to a conical vial. In this step, it was melted rapidly at room temperature. Subsequently, the extractant was transported to UV-Vis spectrophotometry using quartz microcell to measure its absorbance at (200-400 nm) for determination of rosuvastatin.

Results and Discussion:

In order to optimize the dispersive liquid-liquid microextraction based on solidification of floating organic drop (DLLME-SFO) in the determination of rosuvastatin the analytical factors that potentially affect sample extraction were studied.

Selection of extraction solvent:

The selection of an appropriate extraction solvent is of utmost importance for the optimization of the DLLME-SFO. The extraction solvent for DLLME-SFO should be able to form a cloudy solution in the aqueous phase. In addition, it must have a lower density than water, low solubility in water, low volatility, extract the desired analytes and have a melting point near room temperature (in the range of 10-30^{oc}). According to these considerations, several extraction solvents were examined and then 1-undecanol was selected because of higher extraction efficiency, sensitivity, stability, low toxicity, low vapor pressure , hence became ready to extract the desired analyte well [28].

Selection of extraction solvent volume:

In general, the volume of extraction solvent influences the surface area of the drop thus, the mass transfers the process of the analyte from the sample to the extractant. An increase in the extraction solvent volume also affects the final organic phase volume, leading to the dilution of the analytes. In addition, the very low amount of extraction solvent can lead to instability of the droplet or problems in its collection. In order to study the effect of different volumes of the solvent extraction on the extraction efficiency, various amounts of 1-undecanol (50-400µl) and constant volume of dispersive solvent (ethanol 0.5 ml) were tested. The obtained results showed that when the volume of 1-undecanol was 300 µl, the optimum level of the rosuvastatin was extracted (Fig. 2). Therefore, 300µl of 1-undecanol was selected as the extraction solvent volume in subsequent experiments.

Selection of disperser solvent:

The disperser solvent in DLLME-SFO must be miscible with both water and extraction solvents. The disperser solvent assists the dispersion of droplets of the extraction solvent in the aqueous phase, enhancing the surface between the phases and improving the extraction efficiency [29]. Thus, four types of disperser solvents including, ethanol, acetone, acetonitrile and methanol were investigated as the most suitable dispersing solvent. The experiments` results illustrated in (Fig. 3), showed that the ethanol was found to give the best efficiency. Therefore, ethanol was chosen as the dispersive solvent due to its low toxicity, low cost and compatibility with the spectrophotometry.

The effect of dispersive solvent volume:

The influence of the volume of ethanol in the range of 50-350µl on the extraction efficiency of rosuvastatin was tested. According to the obtained results (Fig. 4), the absorbance of analyte was maximized when the volume of ethanol was 300 µl. When the volume of ethanol was lower than 300 µl, the 1-undecanol was not completely dispersed in the aqueous phase and the extraction efficiency was low, whereas the volume higher than 300 µl of ethanol had low efficiency due to the increase of solubility of the analyte in the aqueous solution. Therefore, 300 µl of ethanol was used as the optimum volume of dispersive solvent.

The effect of sample pH:

The pH of the sample is an important factor that may affect the extraction efficiency of analytes in the aqueous samples. In this study, the effect of pH was investigated by varying the pH in the range of 2-6. The results illustrated in (Fig. 5) showed that the absorbance was maximum and nearly constant at the pH 4. This may be explained by considering that analytes in neutral forms may be extracted into the organic phase. The decrease in the absorbance at pH less than 4 may be related to the protonation of rosuvastatin and at pH greater than 4, it could be the anion form, which prevents its extraction into the organic phase. Therefore, in the future experiments, a pH of 4 can be selected as an optimum value for the extraction of rosuvastatin.

The effect of extraction time:

In DLLME-SFO techniques, extraction time is defined as the interval time between the injection of the solution of disperser and extraction solvents before starting to centrifuge. The extraction time is dependent on the mass transfer rate of the analyte from the aqueous sample into the extractant. In addition, in dispersive techniques equilibrium is very rapidly attained due to the infinitely large surface area between both phases after the formation cloudy solution. The effect of extraction time has been studied in the range of (5-20) min, while the other experimental condition remained constant. The results in (Fig. 6) showed that the absorbance of rosuvastatin increased in 10 min, but the difference was not significant. Therefore, 10 min was selected as the optimum extraction time.

Analytical characteristics of the method

Validation parameters of the proposed method such as linearity, the limit of detection (LOD), the limit of quantitation (LOQ), precision were evaluated under the optimized conditions. The results are shown in Table (1). The linearity of the method was determined by extracting standard solutions of rosuvastatin at different concentrations. In the optimal condition, the linear calibration range was 0.034-0.517 μg ml^{-1} for rosuvastatin after extraction. The r-square value of the calibration curve was 0.9998, which confirmed the linearity of the method. The limit of detection (LOD) calculated based on 3b/m and LOQ, were 0.0069 μg ml^{-1} and 0.0231 μg ml^{-1} , respectively. The precision based on the relative standard deviation (RSD) for 10 μg ml^{-1} solution of rosuvastatin was calculated to be 1.474 % for six replicates.

Real sample analysis:

In order to evaluate the method efficiency in real sample analysis, a known amount of standard rosuvastatin solutions in the linear range were added to real samples (rosuvastatin tablet solution) and were analyzed by the proposed method (Fig.7). The results showed that the percentage of recovery of the real sample was between 98.5 and 110%, which indicated the accuracy of the measurement of the drug. For the measurement of rosuvastatin in the presence of unknown interferences, atorvastatin was added to the analyte solution as a model component and binary mixture of these components was prepared according to (Table 2). Calibration and prediction sample sets of the binary mixture was investigated using a randomized design method in the linear range of the two compounds. To evaluate the feasibility of the proposed method in real samples analysis, the samples were extracted in optimal conditions and the absorbance spectra were recorded by spectrophotometry in the range of 200-400 nm. Finally, the concentrations of components were examined by the proposed method using MULTIVAR toolbox in MATLAB software. According to the obtained results in (Table 2), the percentage of recovery of rosuvastatin in the presence of atorvastatin were between 93% and 108%, which indicated the accuracy of the measurement for rosuvastatin.

Comparison with other methods:

Determination of rosuvastatin in the aqueous samples by the developed DLLME-SFO was compared with the other preconcentration methods used for their determination , whose results are summarized in Table (3). As shown, the extraction time was shorter than the other reported method. The detection limit was lower than the other spectrophotometric methods and was comparable to the method that used more sensitive detectors. The RSD of the method was better than some of the reports. Furthermore, the solvent used in this study was less toxic than other liquid extraction methods. DLLME-SFO UV-Vis, on the other hand, was relatively simple and inexpensive than other methods.

Conclusion:

In this research by combining DLLME-SFOD with spectrophotometer instrument equipped with micro liter cells and by recording the absorbance of extracted pharmaceutical, the rosuvastatin was successfully determined at trace level. The spectrum of extracted analyte has sever overlap with other pharmaceuticals such as atorvastatin in whole studied spectra regions. Hence, the multivariate calibrations were used for determination of analyte. In DLLME-SFO measurements, the nature and matrix of environmental samples are unknown. Hence, the calibration samples will be dissimilar to prediction samples. In order to resolve this problem, the net analyte signal (NAS) based calibration method was used so as to preprocessing the spectrophotometric data. NAS is a part of signal which is directly proportional to the model predicted concentration. In the current work, the combination of NAS and CLS method, which called NAS/CLS, was used to minimize the un-calibrated interferences in calibration set. In summary, this new method offered many features such as sensitivity, cost effectiveness, rapid, reproducible, friendly to the environment and low toxic. The experimental results revealed that NAS/CLS combined with DLLME-SFO UV-Visible is provided good accuracy and very low LOD and precision for the determination of low concentration level of rosuvastatin in pharmaceuticals within a short analysis time.

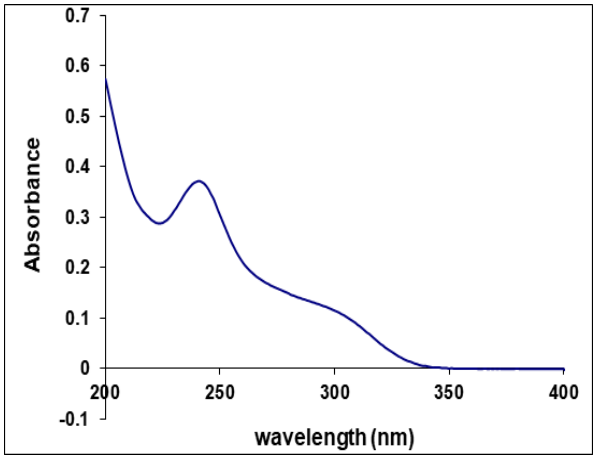


Fig. 1. Plot of the measured spectrophotometric spectrum of rosuvastatin. Conditions: aqueous volume: 10 ml, pH: 3, 1-Undecanol : 300 µL, Ethanol: 300 µL, Contact time: 10 min, Stirring rate: 3800 rpm, Centrifuging time: 10 min (4500 rpm), Solidification time: 8 min, Freezing temperature: 4 °C. All experiments were performed in triplicate (n=3)

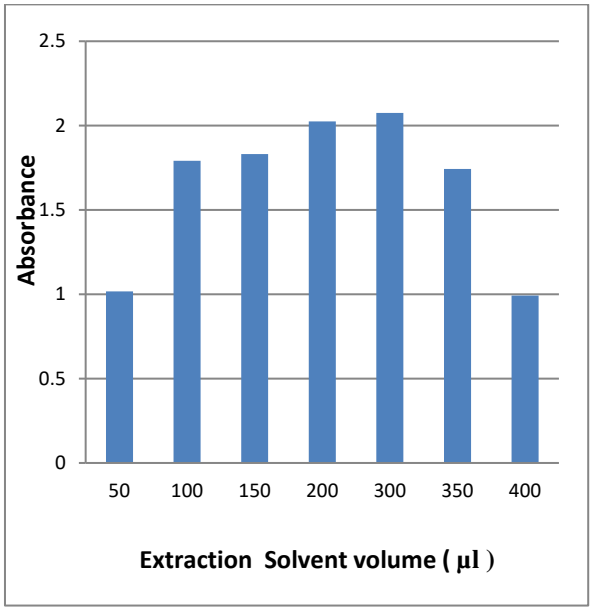


Fig. 2. Effect of volume of extraction solvent, 1-undecanol, on DLLME-SFO efficiency, Conditions: aqueous volume: 10 ml, pH: 3, Ethanol: 300 µL, Contact time: 10 min, Stirring rate: 3800 rpm, Centrifuging time: 10 min (4500 rpm), Solidification time: 8 min, Freezing temperature: 4 °C. All experiments were performed in triplicate (n=3)

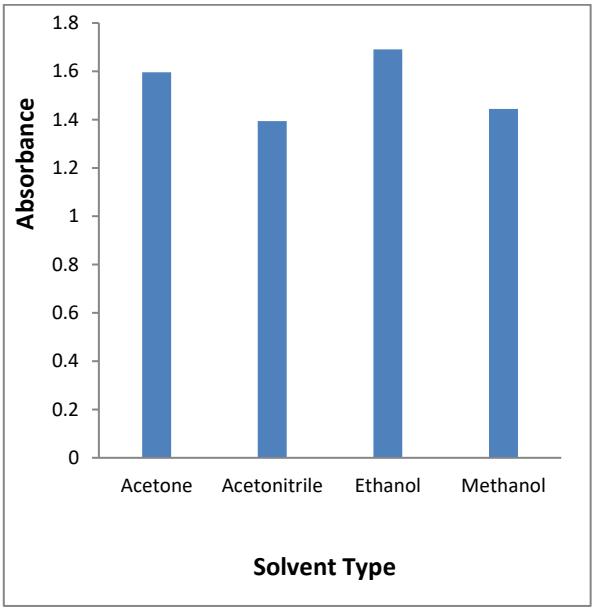


Fig. 3. Effect of disperser solvent type on DLLME-SFO efficiency, Conditions: aqueous volume: 10 ml, pH: 4, 1-Undecanol : 300 µL , disperser: 300 µL, Contact time: 10 min, Stirring rate: 3800 rpm, Centrifuging time: 10 min (4500 rpm), Solidification time: 8 min, Freezing temperature: 4 °C. All experiments were performed in triplicate (n=3)

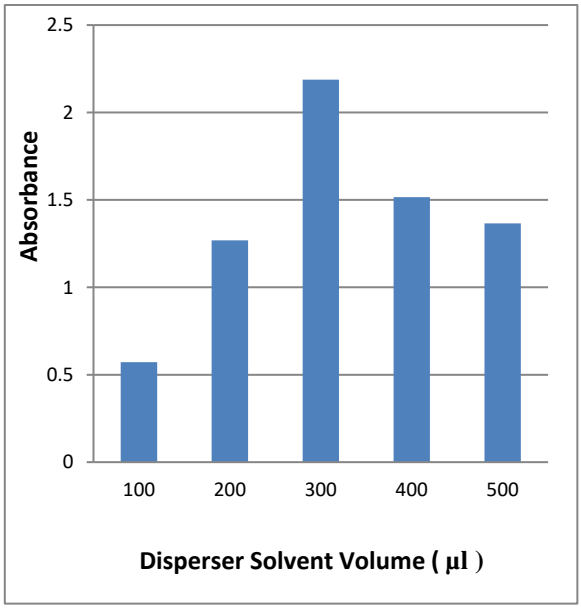


Fig. 4. Effect of volume of disperser solvent, ethanol, on DLLME-SFO efficiency, Conditions: aqueous volume: 10 ml, pH: 4, 1-Undecanol : 300 µL, Contact time: 10 min, Stirring rate: 3800 rpm, Centrifuging time: 10 min (4500 rpm), Solidification time: 8 min, Freezing temperature: 4 °C. All experiments were performed in triplicate (n=3)

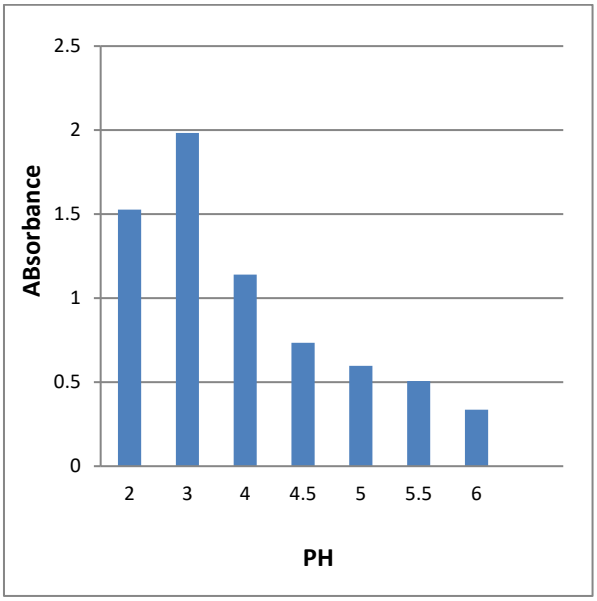


Fig. 5. Effect of pH on the extraction of rosuvastatin, Conditions: aqueous volume: 10 ml, 1-Undecanol : 300 µL, Ethanol: 300 µL, Contact time: 10 min, Stirring rate: 3800 rpm, Centrifuging time: 10 min (4500 rpm), Solidification time: 8 min, Freezing temperature: 4 °C. All experiments were performed in triplicate (n=3)

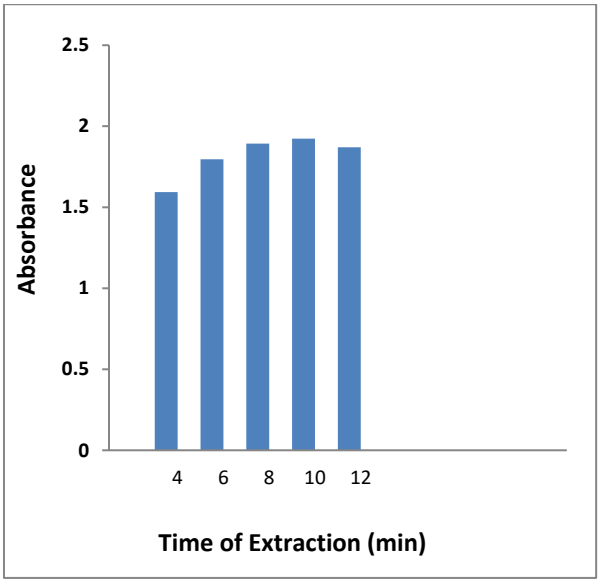


Fig. 6. Effect of the extraction time on DLLME-SFO efficiency, Conditions: aqueous volume: 10 ml, pH: 3, 1-Undecanol : 300 µL, Ethanol: 300 µL, Stirring rate: 3800 rpm, Centrifuging time: 10 min (4500 rpm), Solidification time: 8 min, Freezing temperature: 4 °C. All experiments were performed in triplicate (n=3)

Table 1. Analytical characteristics of DLLME –SFO for the determination of rosuvastatin

Parameter	Analytical feature
Maximum wavelength	242 nm
Dynamic range µgml^{-1}	0.034 – 0.517
Correlation coefficient (r^2)	0.9998
Limit of detection (LOD) µgml^{-1}	0.0069
Limit of quantitation(LOQ) µgml^{-1}	0 .0231
Relative standard deviation (RSD %)	1.474 %
Concentration factor	29
Percentage of recovery of real sample	110 %

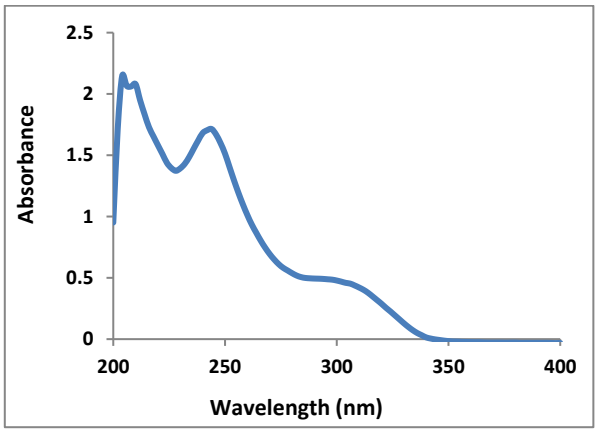


Fig. 7. Absorption spectra spiking experiments in real sample, Conditions: aqueous volume (10µgml^{-1} Real sample, 3 µgml^{-1} Standard RC): 10 ml, pH: 3, 1-Undecanol : 300 µL, Ethanol: 300 µL, Stirring rate: 3800 rpm, Centrifuging time: 10 min (4500 rpm), Solidification time: 8 min, Freezing temperature: 4 °C. All experiments were performed in triplicate (n=3)

Table 2. Determination and recovery of analyte in binary synthetic mixtures of rosuvastatin (ppm) and atorvastatin using NAP/CLS (Each value is the mean of three replicates).

Calibration Prediction	Sample Number	Atorvastatin (ppm)	Rosuvastatin (ppm)	Rosuvastatin (ppm) Obtained-CX	recovery Percent
Calibration	1	2	2	-	-
Prediction	2	7	4	4.32	% 108
Calibration	3	2	6	-	-
Calibration	4	8	7	-	-
Calibration	5	3	8	-	-
Calibration	6	7	4	-	-
Prediction	7	2	5	5.25	%105
Calibration	8	6	4	-	-
Prediction	9	4	2	1.86	%93
Calibration	10	3	7	-	-

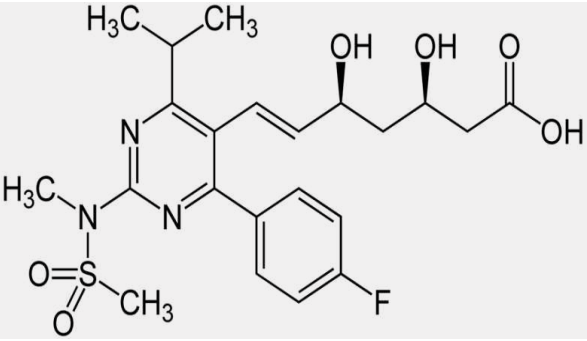
Table 3. Comparison of the proposed with other reported methods for the determination of. rosuvastatin in different samples

Technique	Matrix	Linear range	LOD	RSD%	LOQ	Ref
HPLC	Tablet	3.0-1602 µgml^{-1}	0.12 µgml^{-1}	2.40	0.39 µgml^{-1}	[30]
RP-HPLC	Tablet	0.5-80 µgml^{-1}	0.1 µgml^{-1}	-	0.5 µgml^{-1}	[31]

HPLC – UV	Plasma	20-200 $ngml^{-1}$	7.2 $ngml^{-1}$	-	8.5 $ngml^{-1}$	[32]
UV –Vis	Tablet	1-60 μgml^{-1}	0.33 μgml^{-1}	-	NR	[33]
RP-HPLC	Tablet	5-30 μgml^{-1}	0.14 μgml^{-1}	-	0.46 μgml^{-1}	[34]
DLLME-HPLC-Q-TOF-MS[a]	Waste water	NR[b]	5.30 ngl^{-1}	10.8	NR[b]	[35]
DLLME-SFO	Water	0.034-0.517 μgml^{-1}	0.0069 μgml^{-1}	1.474	0.0231 μgml^{-1}	This work

a) Dispersive liquid-liquid microextraction with HPLC and quadruple TOF-MS

b) Not reported.



Structure of rosuvastatin calcium.

References

1. Tsimihodimos V, Mitrogianni Z, Elisaf M. Dyslipidemia associated with chronic kidney disease. The open cardiovascular medicine journal. 2011;5:41.
2. Wu Y, Wang Y, An C, Dong Z, Liu H, Zhang Y, et al. Effects of rosuvastatin and atorvastatin on renal function. Circulation Journal. 2012;76(5):1259-66.
3. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. Fundamental & clinical pharmacology. 2005;19(1):117-25.
4. Xilifu D, Abudula A, Rehemu N, Zhao L, Zhou X, Zhang X. Effect of rosuvastatin on hyperuricemic rats and the protective effect on endothelial dysfunction. Experimental and therapeutic medicine. 2014;8(6):1683-8.
5. Al-Dujaili ANG, Al-Shemeri MK. Effect of Silver Nanoparticles and Rosuvastatin on Endothelin and Obestatin in Rats Induced By High Fat-Diet. research journl of pharmaceutical biological and chemical sciences. 2016;7(3):1022-30.
6. Gómez-García A, Torres GM, Ortega-Pierres LE, Rodríguez-Ayala E, Álvarez-Aguilar C. Rosuvastatin and metformin decrease inflammation and oxidative stress in patients with hypertension and dyslipidemia. Revista espanola de cardiologia. 2007;60(12):1242-9.
7. Herbet M, Gawrońska-Grzywacz M, Jagiełło-Wójtowicz E. evaluation of selected biochemical parameters of oxidative stress in rats pretreated with rosuvastatin and fluoxetine. Acta poloniae pharmaceutica. 2015;72(2):261-5.
8. Foster GD, Gates PM, Foreman WT, McKenzie SW, Rinella FA. Determination of dissolved-phase pesticides in surface water from the Yakima River Basin, Washington, using the Goulden large-sample extractor and gas chromatography/mass spectrometry. Environmental science & technology. 1993;27(9):1911-7.
9. Jönsson JÅ, Mathiasson L. Liquid membrane extraction in analytical sample preparation: II. Applications. TrAC Trends in Analytical Chemistry. 1999;18(5):325-34.
10. Rezaee M, Assadi Y, Hosseini M-RM, Aghaee E, Ahmadi F, Berijani S. Determination of organic compounds in water using dispersive liquid–liquid microextraction. Journal of Chromatography A. 2006;1116(1-2):1-9.
11. Leong M-I, Huang S-D. Dispersive liquid–liquid microextraction method based on solidification of floating organic drop combined with gas chromatography with electron-capture or mass spectrometry detection. Journal of Chromatography A. 2008;1211(1-2):8-12.
12. Pereira ER, Soares BM, Maciel JV, Caldas SS, Andrade CF, Primel EG, et al. Development of a dispersive liquid–liquid microextraction method for iron extraction and preconcentration in water samples with different salinities. Analytical Methods. 2013;5(9):2273-80.
13. Rezaee M, Yamini Y, Faraji M. Evolution of dispersive liquid–liquid microextraction method. Journal of Chromatography A. 2010;1217(16):2342-57.
14. Pebdani AA, Shabani AMH, Dadfarnia S, Talebianpoor MS, Khodadoust S. Preconcentration of valsartan by dispersive liquid–liquid microextraction based on solidification of floating organic drop and its determination in urine sample: Central composite design. Journal of separation science. 2016;39(10):1935-44.
15. Topaç N, Karadaş C, Kara D. Development of a preconcentration method for indomethacin in natural waters using dispersive liquid–liquid microextraction based on solidification of floating organic drop technique. Water Quality Research Journal. 2018;53(1):41-50.
16. Kocúrová L, Balogh IS, Šandrejová J, Andruch V. Recent advances in dispersive liquid–liquid microextraction using organic solvents lighter than water. A review. Microchemical Journal. 2012;102:11-7.

17. Singh I, Juneja P, Kaur B, Kumar P. Pharmaceutical applications of chemometric techniques. ISRN Analytical Chemistry. 2013;2013.
18. Lorber A, Faber K, Kowalski BR. Net analyte signal calculation in multivariate calibration. Analytical Chemistry. 1997;69(8):1620-6.
19. Xu L, Schechter I. A calibration method free of optimum factor number selection for automated multivariate analysis. Experimental and theoretical study. Analytical Chemistry. 1997;69(18):3722-30.
20. Berger AJ, Koo T-W, Itzkan I, Feld MS. An enhanced algorithm for linear multivariate calibration. Analytical chemistry. 1998;70(3):623-7.
21. Goicoechea HC, Olivieri AC. Enhanced synchronous spectrofluorometric determination of tetracycline in blood serum by chemometric analysis. Comparison of partial least-squares and hybrid linear analysis calibrations. Analytical chemistry. 1999;71(19):4361-8.
22. Goicoechea HC, Olivieri AC. A comparison of orthogonal signal correction and net analyte preprocessing methods. Theoretical and experimental study. Chemometrics and intelligent laboratory systems. 2001;56(2):73-81.
23. Ghorbani-kalhor E, Maryam Sajjadi S, Naseri A, Abolhasani J, Taghi Vardini M. Modified model-based rank annihilation factor analysis to quantitative analysis of pH-modulated mixture samples. Current Analytical Chemistry. 2014;10(4):552-64.
24. Goicoechea HC, Olivieri AC. Wavelength selection by net analyte signals calculated with multivariate factor-based hybrid linear analysis (HLA). A theoretical and experimental comparison with partial least-squares (PLS). Analyst. 1999;124(5):725-31.
25. Collado MaS, Mantovani VcE, Goicoechea HC, Olivieri AC. Simultaneous spectrophotometric-multivariate calibration determination of several components of ophthalmic solutions: phenylephrine, chloramphenicol, antipyrine, methylparaben and thimerosal. Talanta. 2000;52(5):909-20.
26. Goicoechea HC, Olivieri AC. Determination of bromhexine in cough–cold syrups by absorption spectrophotometry and multivariate calibration using partial least-squares and hybrid linear analyses. Application of a novel method of wavelength selection. Talanta. 1999;49(4):793-800.
27. Goicoechea HC, Olivieri AC. MULTIVAR. A program for multivariate calibration incorporating net analyte signal calculations. TrAC-Trends in Analytical Chemistry. 2000;19(10):599-605.
28. Peng G, He Q, Al-Hamadani SM, Zhou G, Liu M, Zhu H, et al. Dispersive liquid–liquid microextraction method based on solidification of floating organic droplet for the determination of thiamphenicol and florfenicol in environmental water samples. Ecotoxicology and environmental safety. 2015;115:229-33.
29. Siti UM, Wan A, Aboul-Enein H. Application of dispersive liquid-liquid microextraction based on solidification of floating organic droplet to the analysis of antidepressant drugs in water samples. Sains Malaysiana. 2013;42(2):149-57.
30. Ashour S, Omar S. Validated high-performance liquid chromatographic method for the estimation of rosuvastatin calcium in bulk and pharmaceutical formulations. International journal of biomedical science: IJBS. 2011;7(4):283.
31. Kaila H, Ambasana M, Thakkar R, Saravaia H, Shah A. A new improved RP-HPLC method for assay of rosuvastatin calcium in tablets. Indian journal of pharmaceutical sciences. 2010;72(5):592.
32. Al-Akkam EJ, Rasool AA, Badwan A, Qinna N. Development and validation of a sensitive and accurate method for determination of atorvastatin and rosuvastatin in rat plasma by reversed-phase high performance liquid chromatography with UV detection. Int J Pharm Pharm Sci. 2013;5:211-9.
33. Uyar B, Celebier M, Altinoz S. Spectrophotometric determination of rosuvastatin calcium in tablets. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2007;62(6):411-3.
34. Pandya CB, Channabasavaraj K, Chudasama JD, Mani T. Development and validation of RP-HPLC method for determination of rosuvastatin calcium in bulk and pharmaceutical dosage form. Inter J Pharm Sci Rev Res. 2010;5:82-6.
35. Martín J, Buchberger W, Alonso E, Himmelsbach M, Aparicio I. Comparison of different extraction methods for the determination of statin drugs in wastewater and river water by HPLC/Q-TOF-MS. Talanta. 2011;85(1):607-15.