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FLUOROMETRIC DETERMINATION OF LOVASTATIN AND SEMVASTATIN IN SPIKED HUMAN PLASMA

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ABSTRACT :

A spectrofluorometric method for the determination of the two antilipaemic drugs, lovastatin and semvastatin in plasma is described. The method was based on heating the drug with 0.1% orthophenelenediamine aqueous solution at 90°C(for 25 minutes for lovastatin and 15 minutes in case of semvastatin). The resulting reaction product, after dilution with methanol showed strong fluorogenic activity exhibiting wavelengths of maximum excitation and emission at 430 and 530 nm, respectively. The assay parameters were optimized to achieve maximum sensitivity and accuracy for the determination of the two drugs in spiked human plasma. The mean percentage recoveries of lovastatin and semvastatin from spiked human plasma samples in concentrations ranging from 0.3 to 0 .9 µg ml^{-1} were 99 . 76 ± S D 0 . 958 and 99 . 903 % ± SD 2 . 45, respectively.

INTRODUCTION

Lovastatin 1 and its methyl analogue semvastatin 2 (also known as mevolin and methl mevolin , respectively) are potent in - vivo inhibitors of hydroxy methylgluteryl coenzyme A (HM G - COA) reductase, the rate controlling enzyme in cholesterol biosynthesis. Both compounds 1 & 2 are considered cholesterol synthesis inhibitors and have been introduced in the last few years for the treatment of hypercholestremia[1].

Analytical reports for the determination of the two compounds are limited including GC - MS method for the determination of lovastatin[2] and simvastatin [3] serum ,flow injection systems with inseries ultraviolet and electrochemical detection method for lovastatin in tablets[4] and HPLC method for determination of lovastatin in plasma and bile [5].

In the present work, a fluorometric method for the determination of the two compounds 1 & 2 has been developed. The method is based on reaction with orthophenelenediamine (OPDA). The products of reaction

are highly fluorogenic at wavelengths of maximum excitation and emission at 430 and 530 nm , respectively. The method has been applied for the determination of the two drugs in human plasma.

EXPERIMENTAL

Apparatus :

A Shimadzu RF 500 spectrofluordmeter was used for recording spectra and fluorescence measurments .Sensitivity gains were adjusted at 2 and 5 during quantitative measurments of semvastatin and lovastatin, respectively .

Materials :

- Authentic samples of lovastatin and semvastatin " Merck Sharp & Dohm", kindly donated by the Drug Control Laboratory of U. A.E .
- Orthophenelenediamine , Riedel - de Haen, reagent grade
- Plasma samples were supplied by the local hospital blood bank
- Methanol and acetonitrile were of analytical grades.

Reagents and Solutions :

- Orthophenelenediamine (OPDA) solution was prepared in distilled water to contain 1.0 $\mu g\ ml^{-1}$.
- Standard drug solutions : stock solutions were prepared in methanol to contain 1.0 mg ml^{-1} of each of lovastatin and semvastatin Aliquots of these stock solutions were diluted with acetonitrile to give concentrations ranging from 1 to 10 $\mu g\ ml^{-1}$.
- Spiked drug plasma solutions : were prepared by diluting aliquots of the stock drug solutions (lovastatin or semvastatin) with human blank plasma to contain 30 - 100 $\mu g\ ml^{-1}$.

Preparation of Plasma Samples :

100 µl aliquot of the spiked plasma sample (containing 3 to 10 µg of the drug) was diluted to 1.0 ml with acetonitrile in a 10 ml centrifuge tube.The precipitated plasma protein was separated by centrifugation for 5 minutes at 5000 r.p.m. The clear supernatant layer was filtered through millipore filter (0.45 µm) . 100 µl aliquot from the filtrate was transferred into a 15 ml screw capped test tube followed by 900 µl of acetonitrile and used as the test solution for the general fluorometric procedure.

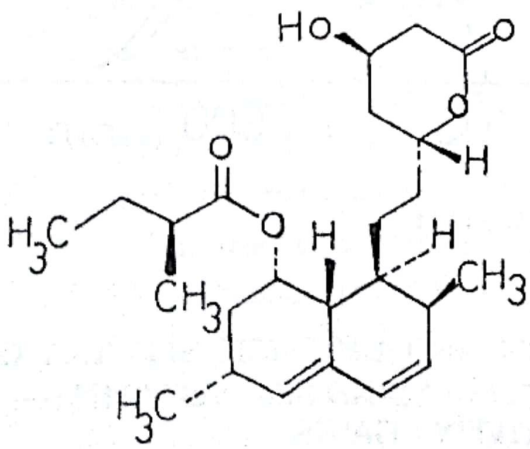
An equal volume of blank human plasma was treated similarly and used in the preparation of the blank reagent in the general fluorometric procedure.

General Fluorometric Procedure :

Each 1.0 ml of drug solution (0.1-1µg ml^{-1}) or the test solution prepared from plasma samples was transferred into a 10 ml screw capped test tube . 700 µl of OPDA solution was then added, the tube was capped and heated in water bath for 25 minutes at 90° C. The tube was cooled under tap - water and diluted with Me OH to 5 ml. The fluorescence of the resulting solution was measured at the wavelengths of maximum excitation and emission at 430 and 530 nm, respectively against a reagent blank experiment. Concentrations of lovastatin or semvastatin were calculated from the corresponding calibration graph prepared simultaneously.

RESULTS AND DISCUSSION

Lovastatin 1 and semvastatin 2 are the most recent antilipemic drugs introduced in medicine during the last decade. Both drugs are mivinic acid lactone derivatives and semvastatin is the methyl analouge of lovastatin. Their importance have aroused from their ability to inhibit cholesterol synthesis in the liver through inhibition of HMC - COA reductase , the rate controlling enzme in cholesterol biosynthesis [1]. Lovastatin and semvastatin are available in tablet form under the brand names Movicor and Zocor,* respectively .



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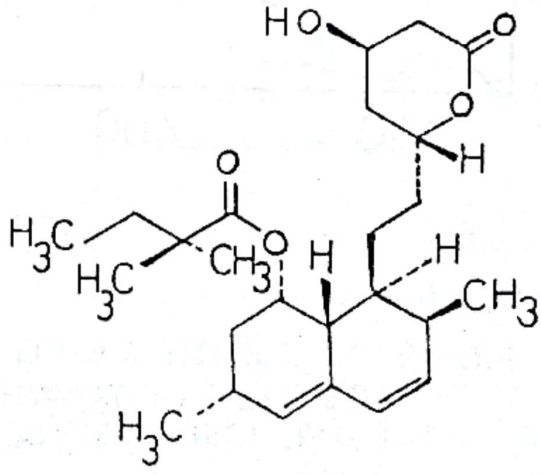

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* - Merck Sharp and Dohm , U. S . A

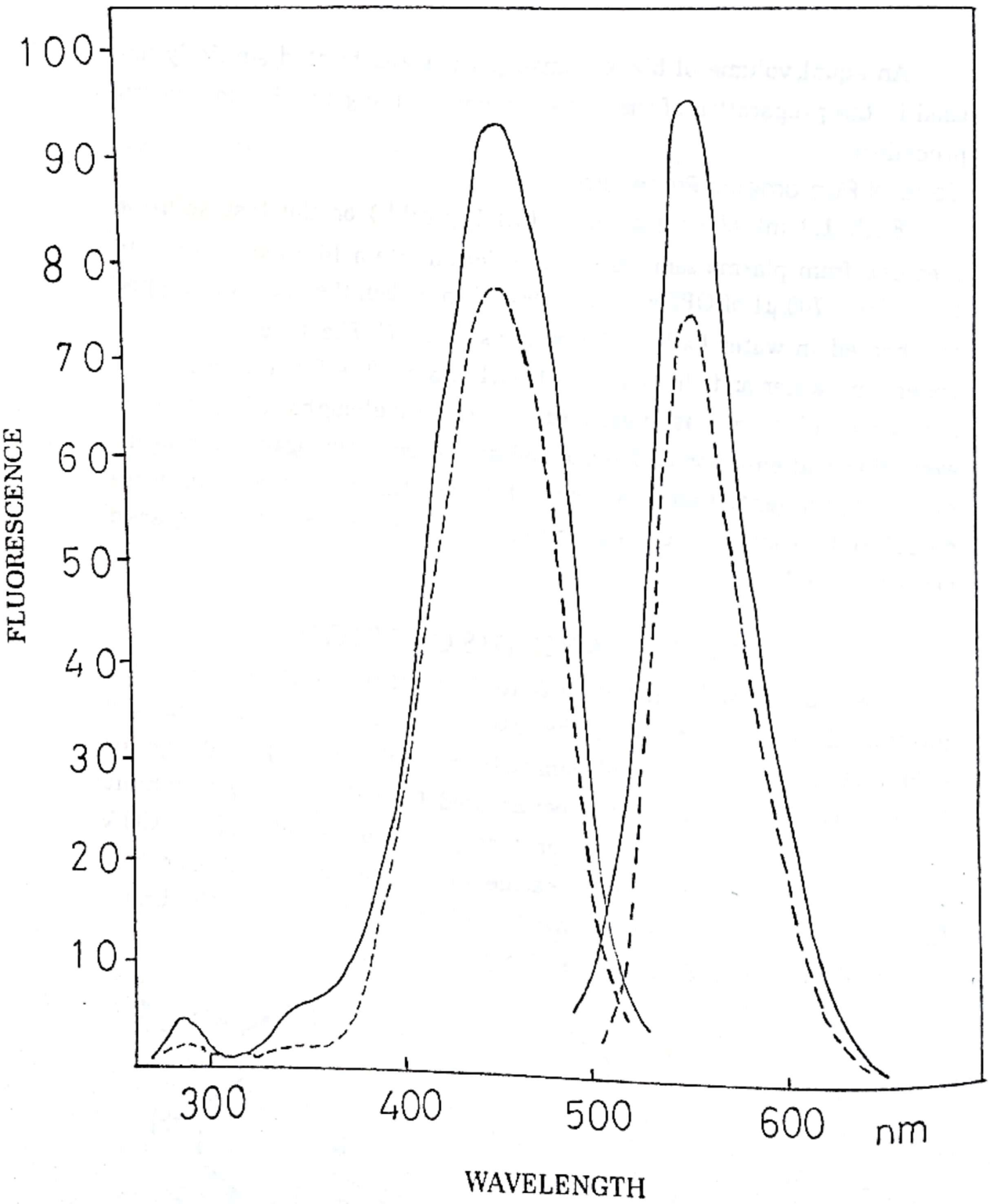

FIG 1 : UNCORRECTED EXCITATION AND EMISSION SPECTRA OF 0.5 μ G ML^{-1} OF LOVASTATIN (——)AND SEMVASTATIN (-------) USING DIFFERENT SENSITIVITY GAINS.

Interest in developing analytical method for determination of the two drugs has aroused from their therapeutic importance and limited reported procedures for their determination.

When lovastatin or semvastatin was heated with OPDA solution, strong fluorogenic products were obtained. The resulting products exhibit wavelengths of maximum excitation and emission at 430 and 530 nm, respectively (Figure 1).

Although the two drugs 1 & 2 are showing the same wavelengths of maximum excitation and emission , the intensity of semvastatin reaction product was found to be relatively higher than that of lovastatin . For this reason quantitive measurments of semvastatin were done at lower sensitivity gain (gain 2) to avoid "out - of scale" fluorescence measurments while lovastatin measurments were done at higher sensitity gain (gain 5) of the spectrofluorometer.

The optimum reaction conditions were studied for each drug to obtain a maximum sensitivity .The rate of formation of the fluorogenic product of lovastatin and semvastatin with OPDA were found to be 25 minutes and 15 minutes heating at 90° C, respectively (Figure 2 & 3).

The maximum fluorescence for 1 & 2 were obtained by using 0.7ml of 1.0 mg ml^{-1} OPDA aqueous solution (Figure 4).

Under the described conditions, a linear relationships between fluorescence responses and concentrations of 1 & 2 were obtained over the range of 0.1— 1 μg ml^{-1} (Table 1).To determine the recoveries of 1 & 2 , known concentrations were analyzed by the proposed procedure and calculated from the regression equation of calibration curves prepared simultaneously .

The mean percentage recoveries were 100.159% ± S.D,0.753 and 99.744% ± S.D.1. 63 for lovastatin and semvastatin , respectively in concentrations ranging from 0. 1 to 1.0 μg ml^{-1}.

The use of this method for the determination of 1 & 2 in biological fluids was established by spiking blank human plasma with each drug and subscequent determination by the proposed method. The determinations have been done in plasma after deprotienization with acetonitrile prior to application of the fluorogenic procedure. Blank

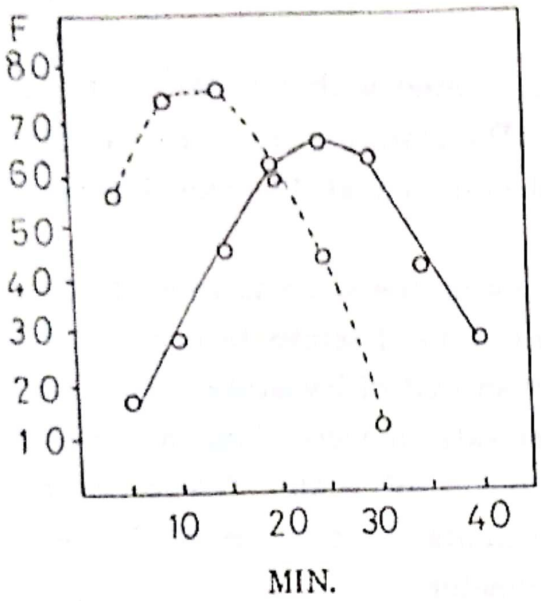

FIG 2: EFFECT OF HEATING TIME
1 (————) & 2 (----------)

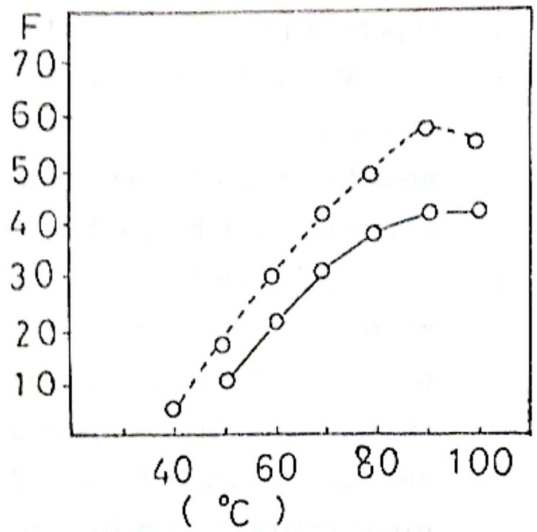

FIG 3 : EFFECT OF HEATING TEMP.
1 (————) & 2 (----------

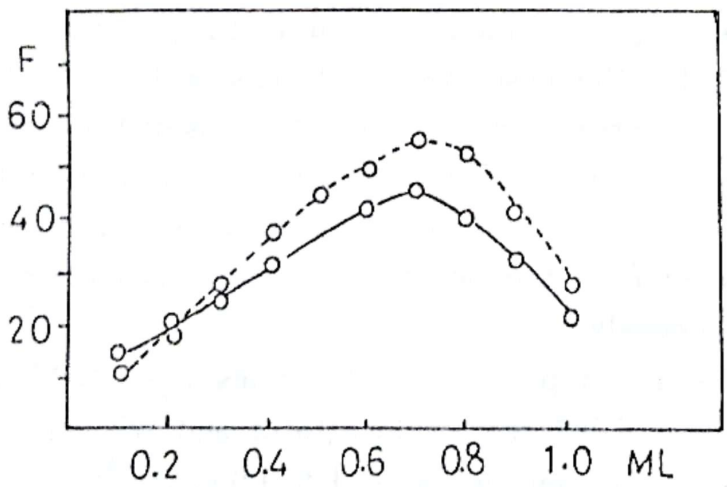

FIG 4 : EFFECT OF OPDA REAGENT VOLUM E
1 (————) & 2 (----------)

human plasma was used for the preparation of the blank reagent experiment.

Upon application of the method to spiked human plasma it was noticed that the significant part of the standard calibration curves of 1&2 has been started from 0. 3 $\mu g\ ml^{-1}$.

The mean percentage recovery was 99.76% SD ± 0.976 for lovastatin at concentration ranging from 0.3 to 0.9 $\mu g\ ml^{-1}$ and 99. 903% , SD± 2.45 for semvastatin at concentration range of 0.3 to 0.8 $\mu g\ ml^{-1}$ (table 2).

The mechanism of the reaction has not yet been investigated, but there is a possibility of a nucleophillic attack of OPDA with subsequent cleavage of the mivinic acid lactone ring with the formation of the corresponding hydroxy amide. This amide may suffer dehydration and subsequent formation of the benzimidazole ring capable of forming an internal hydrogen bond with the hydroxyl group at position 4 as shown in scheme I. A similar reaction was observed with secondary alkyl amines and morpholin-2- ones which resulted in ring opening and the formation of the corresponding hydroxy amides [6].

In conclusion, the reaction of lovastatin and semvastatin with OPDA as a fluorogenic reagent , is simple, accurate, sensitive and precise. Thus, the proposed method can be recommended for the determination of compounds 1 & 2 in biological fluids.

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Table 1 : Assay Parameters for Fluorometric Determination of Lovastatin and Semvastatin Using Orthophenylenediamine (OPDA).

Drug	Conc. range µg ml	Optimum reaction time (min) at 90° C	Regression Equation * intercept (a)	slope (b)	corrl. coeff. (r)	Recovery Data** % ± SD.
Lovastatin	0.1 to 1	25	- 0 .200	80.272	0.9996	100.159 ±0.753
Semvastatin	0.1 to1	15	0.733	63.393	0.9995	99.74±1.63

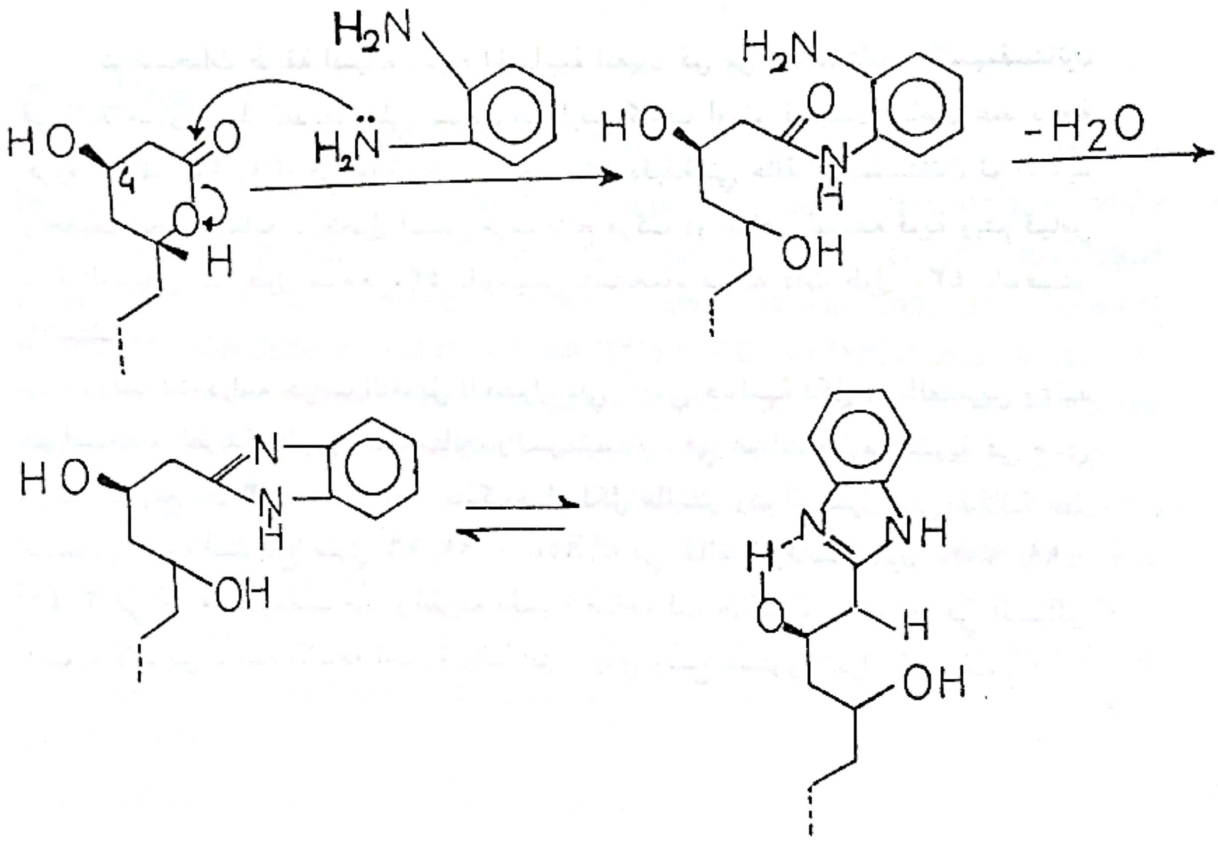
* Y= a + b c where : **y** is the fluorescence and C is the Conc . µg ml^{-1}

** Mean of ten determinations in the concentration range of 0.1 -1 µg ml^{-1}

Table 2 : Determination of Lovastatin and Semvastatin in Spiked Human Plasma .

Conc. * µg ml^{-1}	Lovastatin found µg ml^{-1}	Recovery %	Conc. µg ml^{-1}	Semvastatin found µg ml^{-1}	Recovery %
0.3	0.3022	100.72	0.3	0.3068	10228
0.4	04009	100.22	0.4	0.3979	99.48
0.5	0.4995	97.95	0.5	0.5054	101.09
0.6	0.5987	99.71	0.6	0.5726	95.43
0.7	0.6669	99.56	0.7	0.6972	99.60
0.8	0.7955	99.45	0.8	08123	101.54
0.9	0.9066	100.73	–	–	–
	Mean ±SD	99.76 ±0 .958		Mean ± SD	99.903 ± 2.45

* Conc. subjected to the general procedure , after treatment of spiked drug plasma solutions (30 - 100) µ g ml -1.



SCHEME 1

طريقة لصيغة لتقييم اللوفاستاتين والسمفستاتين في البلازما البشرية

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تم استحداث طريقة لصيغة شديدة الحساسية لتعيين كل من اللوفاستاتين والسيمفستاتين في البلازما وتعتمد الطريقة علي تسخين العقارمع كاشف أورثو فينيللين دياميين عند درجة حرارة ٩٠ لمدة ٢٥ دقيقة في حالة اللوفاستاتين و ١٥ دقيقة في حالة السيمفستاتين ثم التبريد وتخفيف المحلول الناتج بالكحول المثيلي حيث ينتج مركب ذو خواص لصيغه قوية ويتم قياس شدة الوميض عند طول موجه ٥٣٠ نانوميتر باستخدام موجه ذات طول ٤٣٠ نانوميتر للاستثارة.

ولقد تمت دراسة جوانب التفاعل للحصول علي أقصي حساسية لكل من العقارين وعليه تم استخدام الطريقة لتقييم اللوفاستاتين والسيمفستاتين في عينات بلازما بشرية في مدي تركيزات يقع بين ٠,٣ - ٠,٩- ميكروجرام لكل ملليتر وتم الحصول علي علاقة خط مستقيم بنسب استرجاع منوي ٩٩,٧٦ ± ٠,٩٥٨ في حالة اللوفاستساتين ، ٩٩,٩٠± ٢,٤٥ في حالة السيمفستاتين والطريقة الجديدة صالحة لتعيين كل من الدوائين في السوائل الحيوية لاغراض دراسة الاتاحة الحيوية والتكافؤ الحيوي وتتبع مستوي الدواء في الجسم.