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Cerimetric determination of simvastatin in pharmaceuticals based on redox and complex formation reactions

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Abstract: Two sensitive spectrophotometric methods are described for the determination of simvastatin (SMT) in bulk drug and in tablets. The methods are based on the oxidation of SMT by a measured excess of cerium (IV) in acid medium followed by determination of unreacted oxidant by two different reaction schemes. In one procedure (method A), the residual cerium (IV) is reacted with a fixed concentration of ferroin and the increase in absorbance is measured at 510 nm. The second approach (method B) involves the reduction of the unreacted cerium (IV) with a fixed quantity of iron (II), and the resulting iron (III) is complexed with thiocyanate and the absorbance measured at 470 nm. In both methods, the amount of cerium (IV) reacted corresponds to SMT concentration. The experimental conditions for both methods were optimized. In method A, the absorbance is found to increase linearly with SMT concentration (r = 0.9995) whereas in method B, the same decreased (r = -0.9943). The systems obey Beer's law for 0.6-7.5 and 0.5-5.0 μg mL^{-1} for method A and method B, respectively. The calculated molar absorptivity values are 2.7 X 10^4 and 1.06 X 10^5 $Lmol^{-1}$ cm^{-1}, respectively; and the corresponding sandel sensitivity values are 0.0153 and 0.0039μg cm^{-2}, respectively. The limit of detection (LOD) and quantification (LOQ) are reported for both methods. Intra-day and inter-day precision, and accuracy of the methods were established as per the current ICH guidelines. The methods were successfully applied to the determination of SMT in tablets and the results were statistically compared with those of the reference method by applying the Student's t-test and F-test. No interference was observed from the common excipients added to tablets. The accuracy and validity of the methods were further ascertained by performing recovery experiments *via* standard addition procedure.

Keywords: Simvastatin; cerimetry; spectrophotometry; pharmaceuticals.

Introduction

Simvastatin (SMT), chemically known as (1S, 2S, 8S, 8aR)-1,2,6,8,8a-hexahydro-1-(2-((2R, 4R)-terahyro-4-hydroxy-6-oxo-2H-pyran-2-yl)-2,6-dimethylnapthalen-8-yl 2,2-dimethylbutanoate (Fig.1) is a cholesterol lowering lactone,and is identified as being among the most widely prescribed drugs in the world in 2007. It is found to lower cholesterol by inhibiting the synthesis of movalinic acid, which is a key precursor in cholesterol synthesis. SMT is administered as a prodrug, and in liver, it is hydrolysed to the β-hydroxy acid form [1].

The therapeutic importance of SMT justifies research to develop analytical methods for its determination in body fluids and pharmaceuticals. The most widely used technique for the assay of SMT has been the high performance liquid chromatography (HPLC) with UV, ms-ms or fluorescence detection. The drug in plasma has been assayed by LC

with UV-detection [2-6], mass spectrometric detection [7-13] and with fluorescence detection [14]; and in serum, the assay of drug has been accomplished by derivative UV-spectrophotometry [15] and voltammetry [16]. Not many methods have been reported for the determination of SMT in pharmaceutical substances. Here also, HPLC with UV detection [17-22] is the widely used technique although High-Performance Thin Layer Chromatography (HPTLC) [23], UV-spectrophotometry [15,24-28], voltammetry [16] and Micellar Electro kinetic Chromatography (MEKC) [29] have sparsely been used. The US pharmacopoeia [30] describes a HPLC-UV detection procedure for the assay of SMT in pharmaceuticals.

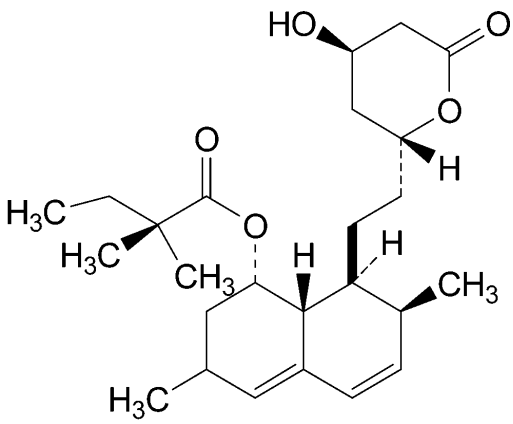

Figure 1. Structure of simvastatin

Although visible spectrophotometry is a rapid, fairly sensitive, accurate and precise, and cost-effective technique, it has not been applied yet to the assay of SMT in pharmaceuticals. The present study includes two new and indirect spectrophotometric methods for the sensitive determination of SMT. The study represents the use of cerium (IV) sulphate as the oxidimetric reagent, and ferroin, iron (II) and thiocyanate as subsidiary reagents.

Experimental details

Apparatus

A Systronics model 106 digital spectrophotometer provided with 1-cm matched quartz cells was used for all absorbance measurements.

Reagents and materials

All chemicals used were of analytical reagent grade and distilled water was used to prepare solutions.

Cerium (IV) sulphate (300 and 400 µg mL^{-1}). A stock standard solution equivalent to 0.01M cerium (IV) sulphate was prepared by dissolving 1.011 g of the chemical (LOBA Chemie, Mumbai,India, assay 99.9%) in 0.5 mol L^{-1} H_2SO_4 and diluting to 250 ml with the same acid in a 250 ml standard flask and standardised [31]. The stock solution was then diluted appropriately with 0.5mol L^{-1} H_2SO_4 to obtain a working concentrations of 300 µg mL^{-1} and 400 µg mL^{-1} in cerium (IV) sulphate for method A and method B, respectively.

Ferroin [36 µg ml^{-1} with respect to iron (II)]. Prepared by dissolving 44.8 mg of $FeSO_4.7H_2O$ (assay 99%) and 96 mg of 1,10-phenanthroline monohydrate (assay 100%) in water, and diluted to volume in a 250 ml calibrated flask.

Ferrous ammonium sulphate, FAS (400 µg ml^{-1}). Accurately weighed 100 mg of the chemical (LOBA Chemie, Mumbai, India, assay 99-101%) and dissolved in water containing 5 ml of dilute H_2SO_4 and diluted to volume in a 250 ml calibrated flask.

Potassium thiocyanate (3 mol L^{-1}). Prepared by dissolving 29 g of the chemical (S.d Fine Chem, Mumbai, India, assay 98%) in water and diluting to 100 ml.

Hydrochloric acid (5 mol L^{-1}). Concentrated hydrochloric acid (S.d. Fine Chem, Mumbai, India;sp.gr.1.18) was diluted appropriately with water.

Acetic acid (3:2). Prepared by diluting appropriately glacial acetic acid (S.d. Fine Chem, Mumbai, India; sp.gr. 1.05) with water.

Standard solution of simvastatin. Pharmaceutical grade simvastatin certified to be 99.9% pure was received as gift from Jubiliant Organosys, Nanjangud, India and used as received. A stock standard solution equivalent to 200 µg ml^{-1} SMT was prepared by dissolving accurately weighed 20 mg of pure drug in 3:2 acetic acid and diluting to the mark in a 100 ml calibrated flask with the same acid. The stock solution was diluted to obtain 25 µg ml^{-1} and 50 µg ml^{-1} SMT for use in method A and method B, respectively, with 3:2 acetic acid.

Dosage forms. The following dosage forms were purchased from local commercial sources and subjected to analysis: Simlip (10 and 20 mg), Simvas (5,10 and 20 mg) and Zosta (5,10 and 20 mg).

Procedures

Method A. Different aliquots (0.0,0.5,1.0…3.0 ml) of standard 25 µg ml^{-1} SMT solution were measured accurately and transferred into a series of 10 ml calibrated flasks .To each flask 1.5 ml of 5 mol L^{-1} HCl was added and the total volume was adjusted to 5 ml by adding water. One ml of cerium (IV) sulphate (300 µg ml^{-1}) was added to each flask, content mixed, and after 10 minutes, 1ml of ferroin was added, diluted to the mark with water and mixed well. The absorbance of the solution was measured at 510 nm against the reagent blank.

Method B. Varying aliquots (0.0,0.2,0.4…1.0 ml) of 50 µg ml^{-1} SMT solution were transferred into a series of 10 ml calibrated flasks by means of a micro burette and the total volume was adjusted to 3 ml by adding requisite volume of water. To each flask, it was added 3 ml of 5 mol L^{-1} HCl followed by 1 ml of 400 µg ml^{-1} cerium (IV) sulphate the last being added from a micro burette. The content was mixed and the flasks were let stand for 10 min before adding 1 ml of accurately measured 400 µg ml^{-1} FAS. After 5 min, 1ml of 3 mol L^{-1} thiocyanate solution was added to each flask, diluted to the mark with water and the absorbance measured at 470 nm against water blank.

In either method, analytical curve was prepared by plotting the increasing absorbance values in method A or decreasing absorbance in method B as a function of SMT concentration. The concentration of the unknown was read from the analytical curve or computed from the respective regression equation derived using Beer's law data.

Procedure for tablets. Twenty tablets were weighed accurately and finely powdered. A quantity of the powdered tablet containing 20 mg of SMT was weighed accurately and transferred into a 100 ml calibrated flask, 60 ml of 3:2 acetic acid and the content shaken thoroughly for 15-20 min to extract the drug into the liquid phase; the volume was finally diluted to the mark with the same acetic acid, mixed well and filtered using a Whatman No 42 filterpaper. First 10 ml of the filtrate was discarded, and suitable aliquots of the subsequent portion were diluted with 3:2 acetic acid to get tablet extracts containing 25 and 50 µg ml^{-1} SMT. A convenient aliquot was then subjected to analysis by either method described above.

Results and discussion

A number of oxidisable pharmaceutical substances have earlier been assayed using cerium (IV) sulphate by several workers [32-45] based on several reaction schemes. The present methods entail adding a known excess of cerium (IV) sulphate to SMT in acid medium followed by the determination of unreacted oxidant by two reaction schemes involving the use of ferroin, and iron (II) and thiocyanate as reagents. The possible reaction scheme is given in Fig.2.

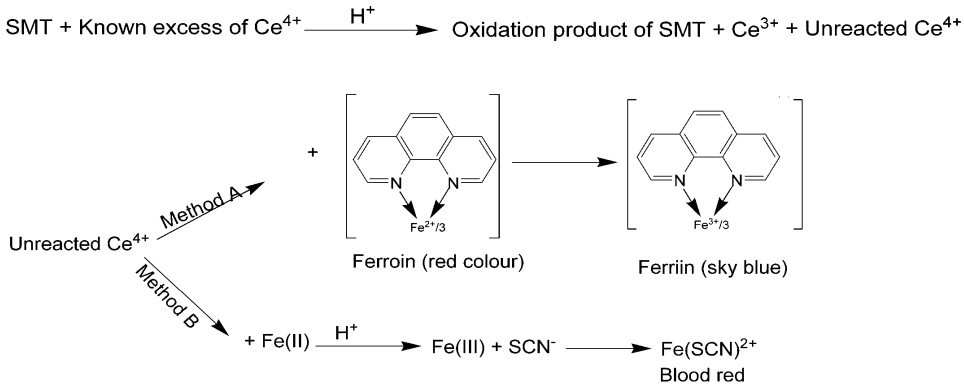

Figure 2. Possible reaction scheme of the methods.

Method A

One of the popular methods for cerium (IV) uses its oxidizing property and is based on the bleaching of the ferroin [46,47] by cerium (IV). In the present method, a known excess of cerium (IV) sulphate was reacted with SMT and after the oxidation of the drug was ensured to be complete, the unreacted oxidant was reacted with a fixed quantity of ferroin, and the resulting change in absorbance was measured at 510 nm, and related to the drug concentration. When a fixed concentration of cerium (IV) sulphate was made to react with increasing concentration of SMT, there occurred a concomitant fall in the oxidant concentration. The decreasing concentrations of cerium (IV) sulphate upon reacting with a fixed concentration of ferroin resulted in increasing absorbance at 510 nm due to the bleaching of ferroin colour by the oxidant, the bleaching being caused by the oxidation of ferroin to ferriin by cerium (IV) sulphate. This is observed as a proportional increase in the absorbance ferroin with increase in the SMT concentration which formed the basis for the assay.

One ml of ferroin (36 μgml^{-1} in Fe^{2+}) in a total volume of 10 ml was found to give a convenient maximum absorbance at 510 nm and this was bleached to a minimum and constant absorbance by 1 ml of 300 $\mu g\ ml^{-1}$ cerium (IV) sulphate in acid medium. Hence, different concentrations of SMT were reacted with 1ml of 300 μgml^{-1} cerium (IV) sulphate in acid medium and the unreacted oxidant was determined by reacting with 1 ml of ferroin equivalent to 36 $\mu g\ ml^{-1}$ iron (II). One and a-half ml of 5 mol L^{-1} HCl in a total volume of 6 ml was found optimum for the oxidation of SMT by cerium (IV) sulphate which was complete in 10 min. Hydrochloric acid volume upto 2.5 ml and reaction time upto 60 min had no adverse effect on the absorbance. The same acid concentration was maintained for the reaction between the cerium (IV) and ferroin, which was instantaneous. The colour was found to be stable for several hours in the presence of the reaction product/s.

Method B

Cerium (IV) in the past has been determined by reducing it by known excess of iron (II) and complexing the iron (III) with thiocyanate [48]. This method is based on the oxidation of SMT by a measured excess of cerium (IV) sulphate in HCl medium, reduction of the residual oxidant by a fixed amount of iron (II) and subsequent formation of iron (III)-thiocyanate complex, which is measured at 470 nm. When a fixed concentration of cerium (IV) sulphate is reacted with increasing concentrations of SMT, there will be a proportional decrease in the concentration of the oxidant. The unreacted oxidant, when treated with a fixed concentration of iron (II) accounts for a proportional decrease in the iron (III) concentration. This is observed as a proportional decrease in the absorbance of iron (III)-thiocyanate complex with the drug concentration, which formed the basis for the assay of drug.

The conditions for the determination of iron (III) by thiocyanate are well established [49]. Hence, experimental variables for the oxidation of SMT by cerium (IV) sulphate and its reduction by iron (II) were optimized. Three ml of 5 $molL^{-1}$ HCl in a total volume of 7 was determined to be optimum for the oxidation step which was complete in 10 min and the same acidic condition was used for the reduction of residual cerium (IV) by iron (II) and subsequent formation of iron (III)-thiocyanate complex. The last two reaction steps were instantaneous. The colour was stable for at least 60 min in the presence of reaction product/s.

Taking 5.5 $\mu g\ ml^{-1}$ iron (III) as the upper limit that could be determined by thiocyanate method, stoichiometrically this could be generated from 386.2 $\mu g\ ml^{-1}$ FAS by 400 $\mu g\ ml^{-1}$ cerium (IV) sulphate in a total volume of 10 ml. However, a slightly higher concentration (400 $\mu g\ ml^{-1}$ FAS) was used to ensure the complete reduction of residual oxidant. Hence, different concentrations of SMT were reacted with 1 ml of 400 $\mu g\ ml^{-1}$ cerium (IV) sulphate followed by the reduction of residual oxidant by 1 ml of 400 $\mu g\ ml^{-1}$ FAS to determine the concentration range within which the drug could be determined by the present method.

Method validation

Linearity. A linear relation is found between absorbance and concentration in the ranges given in Table 1.In method B, Beer's law is obeyed in the inverse manner. The calibration graphs were described by the equation:

$$Y=a + bX$$

where Y=absorbance, a=intercept, b=slope and X=concentration in µg ml^{-1} obtained by the method of least squares. Correlation coefficients, intercepts and slopes for the calibration data are also presented in Table 1.

Table 1. Analytical and regression parameters.

Parameter	Method A	Method B
$\lambda_{max,}$ nm	510	470
Beer's law limits, µg mL^{-1}	0.6-7.5	0.5-5.0
Molar absorptivity, L mol^{-1} cm^{-1}	2.7 x 10^4	1.06 x 10^5
Sandel sensitivity, µg cm^{-2}	0.0153	0.0039
Limit of detection (LOD), µg mL^{-1}	0.10	0.08
Limit of quantification (LOQ), mg mL^{-1}	0.29	0.26
Regression equation, y*		
Intercept (a)	0.0027	0.743
Slope (b)	0.064	-0.085
Correlation coefficient, (r)	0.9995	-0.9943
S_a	±0.0168	±0.1905
S_b	±0.00254	± 0.0455

*Y=a+bX, where Y is the absorbance and X concentration in µg mL^{-1}
S_a.standard deviation of intercept.
S_b.standard deviation of slope.

Sensitivity. Sensitivity parameters such as molar absorptivity and Sandel sensitivity, and limits of detection and quantification calculated as per the current ICH guidelines [50] are compiled in Table1. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae:

$$LOD=3.3\sigma/s \text{ and } LOQ=10\sigma/s$$

where σ is the standard deviation of seven reagent blank determinations and s is the slope of the calibration curve.

Precision and accuracy. Intra-day precision and accuracy of the methods were evaluated from the results of seven replicate determinations on pure drug solution at three concentration levels. The mean values and relative standard deviation values were calculated. To determine the inter-day precision, analyses were performed over a period of *five days by preparing all solutions freshly* each day. The accuracy of methods were determined by calculating the percentage deviation observed in the analysis of pure drug solution as expressed as the relative error (%). Table 2 summarizes the intra-day precision and accuracy data as well as the inter-day precision data obtained for the determination of SMT by the proposed methods. From the data, it is implied that the intra-day precision and accuracy were less than 3.5% whereas inter-day precision was less than 4%.

Table 2. Evaluation of accuracy and precision Method

SMT	SMT taken	Range found*	RE	SD %	SEM	RSD[a]	RSD[b] %	%
A	2.0	1.98	0.20	1.05	0.061	0.023	3.1	1.6
	4.0	3.95	0.29	1.23	0.1	0.038	2.5	2.8
	6.0	5.95	0.12	0.87	0.044	0.017	0.74	3.5
	1.5	1.52	0.145	1.07	0.035	0.013	2.3	2.2
B	3.0	2.94	0.21	2.13	0.07	0.027	2.4	3.6
	4.5	4.51	0.13	0.11	0.046	0.017	1.02	2.1

*SMT taken/found, range, SD and SEM are in µg mL^{-1}.
RE. Relative error; SD. Standard deviation; SEM. Standard error of mean; RSD. Relative standard deviation; a. Intra-day precision, b. Inter-day precision.

Robustness and ruggedness. To evaluate the method robustness, two experimental variables *such as* HCl volume and reaction time were slightly altered, and the same were found to have no significant effect on the accuracy and precision of the methods when the studies were made on a single concentration of SMT. The ruggedness of the methods was assessed by calculating the RSD for results obtained by performing the analysis using three different instruments and by three different persons. The inter-instrumental RSD values ranged from 2.5 to 4.5% whereas the inter-personal RSD values varied from 2.5-3.5% for three concentrations of SMT employed for accuracy and precision studies.

Effect of interferences. To assess the usefulness of the methods, synthetic mixtures containing SMT and inactive ingredients with the following composition were prepared and analysed for SMT content: SMT (20 mg), talc (40 mg), starch (50 mg), sodium alginate (50 mg), magnesium stearate (20 mg), lactose (20 mg), calcium gluconate (30 mg), calcium dihydrogen orthophosphate (50mg) and titanium dioxide (10mg). The percent recovery of SMT by method A was 96.58±1.6 (n=5) and 97.62±2.1 (n=5) by method B indicating that there is no interference from the common excipients added to tablet preparations.

Application to tablet assay. Of the seventeen brands of tablets available in the Indian market, three representative brands were subjected to analysis by the proposed methods. Results presented in Table 3 reveals that there is a close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically with those obtained by a reference method [30] by applying Student's t-test for accuracy and F-test for precision. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values suggesting that the proposed methods are as accurate and precise as the reference method.

Recovery study. Accuracy and validity of the methods were further ascertained by performing recovery studies *via* standard-addition procedure. Pre-analysed tablet powder was spiked with pure SMT at three different concentration levels (50,100 and 150% of the level in the tablet) and the total was found by the proposed methods. Each experiment was repeated three times. The recovery of pure SMT added to tablet powder ranged from 95.6 to 104.1% (Table 4) demonstrating that commonly added tablet excipients did not affect the results of assay.

Table 3. Results of assay of tablets and statistical comparison.

Tablet Brand name*	Nominal amount, mg	% found* ± SD		
		Reference method	Method A	Method B
Simvas	5	98.65 ± 1.5	99.12 ± 1.8 t=0.45 F=1.44	99.76 ± 2.1 t=0.97 F=1.96
	10	102.1 ± 1.2	101.6 ± 1.4 t=0.61 F= 1.36	102.8 ± 1.8 t= 1.05 F=2.25
	20	97.84 ± 2.1	97.26 ± 1.9 t= 0.46 F=1.22	98.52 ± 2.5 t= 0.46 F= 1.42
Zosta	5	103.6 ± 1.7	102.9 ± 2.2 t=0.76 F=1.67	103.5 ± 1.9 t= 0.09 F= 1.25
	10	99.74 ± 1.4	100.6 ± 1.8 t= 0.85 F= 1.65	101.1 ± 2.6 t= 1.43 F= 3.45
	20	98.44 ± 1.2	99.26 ± 1.7 t= 0.89 F= 2.00	99.04 ± 1.5 t= 1.15 F= 1.56
Simlip	10	100.6 ± 2.2	101.2 ± 1.9 t=0.46t=0.45 F=1.34	99.95 ± 2.3 F= 1.09
	20	101.3 ± 1.6	102.5 ± 2.4 t=0.95 F= 2.25	100.8 ± 2.5 t= 0.39 F=2.44

*Mean value of five determinations
#Marketed by; a.Cipla Ltd ; b. Morepen Labs. Ltd; c. Glenmark Pharm. Ltd.,
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39.

Table 4. Results of recovery study.

Method	Tablet studied	SMT in tablet, µg	Pure SMT added, µg	Total found, µg	Pure SMT recovered* (Percent ± SD)
A	Simlip	20.5	10.0	30.82	103.2 ± 2.8
	20 mg	20.5	20.0	41.06	102.8 ± 1.7
		20.5	30.0	51.73	104.1 ± 2.2
	Simvas	20.32	10.0	30.82	105.0 ± 3.0
	10mg	20.32	20.0	41.06	103.7 ± 3.2
		20.32	30.0	51.7	104.6 ± 2.8
B	Simlip	20.16	10.0	30.29	101.3 ± 1.8
	20mg	20.16	20.0	40.72	102.8 ± 2.5
		20.16	30.0	51.42	104.2 ± 3.2
	Simvas	20.56	10.0	31.07	105.1 ± 2.6
	10 mg	20.56	20.0	41.90	106.7 ± 3.0
		20.56	30.0	51.25	102.3 ± 2.1

*Mean value of three determinations.

Conclusions

This is a first report on the application of visible spectrophotometry for the assay of simvastatin. The methods are based on well-established and characterised chemical reactions and use cheaper and readily available chemicals. The procedures do not involve any critical experimental condition or tedious sample preparation. The methods are highly sensitive as shown by the molar absorptivity values besides being fairly accurate and precise, the last two parameters being unaffected by slight variations in experimental conditions such as acid concentration and reaction time. The methods have been demonstrated to be free from interference from common tablet excipients and additives.

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