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Investigation and Optimisation of the Use of Spectrophotometry for the Assay of Simvastatin with *in situ* Bromine and Three Dyes as Reagents

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Abstract. Three simple and sensitive spectrophotometric methods are described for the determination of simvastatin (SMT) in bulk drug and in tablets using bromate-bromide as the bromination reagent in acid medium, and three dyes as subsidiary reagents. All the three methods are based on the bromination of SMT by a known excess of *in situ* generated bromine followed by determination of unreacted bromine by reacting with a fixed amount of methyl orange (method A), indigo carmine (method B) or meta-cresol purple (method C) and measuring the absorbance at 510, 610 or 540 nm. In all the methods, the amount of bromine reacted corresponds to the amount of SMT. The experimental conditions for the assay have been optimized. In all the methods, the absorbance is found to increase linearly with the concentration of SMT at the respective wavelengths. Beer's law is obeyed over the ranges 0.5-3.0, 2.5-15.0 and 2.5-15.0 μg mL^{-1} for method A, method B and method C, respectively and the respective molar absorptivity values are 1.0×10^5, 2.3×10^4 and 2.1×10^4 l mol^{-1} cm^{-1}. The limits of detection and quantification are reported for all the methods. The performance of the methods was validated according to the present ICH guidelines. The methods gave similar results in terms of precision and accuracy. The repeatability and intermediate precision, expressed by the RSD was better than 2%. The accuracy of the methods expressed as relative error was satisfactory. The proposed methods were applied to the analysis of tablet form of SMT and the results tallied well with the label claim. No interference was observed from the concomitant substances normally added to tablets. The results were statistically compared with those of a literature method by applying the Student's t-test and F-test. The accuracy and validity of the methods were further ascertained by performing recovery studies *via* spike method and standard-addition method.
Keywords. Simvastatin, spectrophotometry, bromate-bromide, pharmaceuticals, dyes.

Resumen. Se describen tres métodos espectrofotométricos simples y sensibles para la determinación de simvastatina (STM) en el medicamento a granel y en comprimidos usando bromato-bromuro como el reactivo de bromación en medio ácido, y tres colorantes como reactivos subsidiarios. Los tres métodos se basan en la bromación de STM por un exceso conocido de bromo generado *in situ,* seguida por la determinación del bromo que no reacciona, mediante una reacción con una cantidad fija de naranja de metilo (método A), carmín índigo (método B) o púrpura de metacresol (método C), y midiendo la absorbancia a 510, 610 o 540 nm. En todos los métodos, la cantidad de bromo que reacciona corresponde a la cantidad de STM. Las condiciones experimentales para la determinación fueron optimizadas. En todos los métodos, se encontró que la absorbancia se incrementa linealmente con la concentración de SMT a las respectivas longitudes de onda. La ley de Beer se cumple en los intervalos de 0.5-3.0, 2.5-15.0 y 2.5-15.0 μg mL^{-1} para el método A, el método B y el método C, respectivamente, y los valores de los respectivos coeficientes de absortividad molar son 1.0×10^5, 2.3×10^4 y 2.1×10^4 l mol^{-1} cm^{-1}. Los límites de detección y cuantificación se reportan para cada método. El desempeño de los métodos se validó de acuerdo con los procedimientos actuales ICH. Los métodos dieron resultados semejantes en términos de precisión y exactitud. La repetibilidad y la precisión intermedia, expresadas por el RSD fueron mejor que el 2%. La exactitud de los métodos, expresada como error relativo, fue satisfactoria. Los métodos propuestos fueron aplicados satisfactoriamente al análisis de STM en comprimidos y los resultados correspondieron con lo reportado en el marbete. No se observaron interferencias de las substancias concomitantes normalmente añadidas a los comprimidos. Los resultados fueron estadísticamente comparados con otros métodos publicados en la literatura aplicando la prueba t y F de Student. La exactitud y la validez de los métodos fueron adicionalmente comprobadas a través de la realización de estudios de recuperación de muestras fortificadas y el método de adiciones patrón.
Palabras clave. Simvastatina, espectrofotometria, bromato-bromuro, medicamentos, colorantes.

Introduction

Simvastatin (SMT), chemically known as (1*S*, 2*S*, 8*S*, 8a*R*)-1,2,6,7,8,8a-hexahydro-1-(2-((2*R*, 4*R*)-terahyro-4-hydroxy-6-oxo-2H-pyran-2-yl)-2,6-dimethylnapthalen-8-yl 2,2-dimethylbutanoate (Fig 1), belongs to the group of cholesterol lowering lactones known as statins which in 2007, have been identified as being the most widely prescribed drugs in the world. Statins lower cholesterol by inhibiting the synthesis of mevalonic acid, which is a key precusor in cholesterol synthesis. SMT, a lipid lowering agent that is derived synthetically from a fermentation product of *Aspergillus terreus* has been found to lessen both normal and elevated LDL-C concentrations. The drug is officially listed in the 2004 United States Pharmacopocia and the offical method of its determination is high-performance liquid chromatography [1]. Various other methods such as UV-spectrophotometry [2-6], HPLC [7-11], HPTLC [12], micellar electrokinetic chromatography [13] and voltammetry [14] have been reported for the assay of SMT in pharmaceuticals. There is only one report on the use of visible spectrophotometry which describes three procedures [15] for SMT in pharmaceuticals. One procedure is based on the reduction of iron (III) by SMT to iron (II) and subsequent formation of prussian blue with ferricyanide measurable at 730 nm. In the other two procedures, the iron (II) formed is chelated with 1,10-phenanthroline or 2,2'-bipyridine followed by measurement of absorbance at 480 or 490 nm. The present paper describes the development and optimisation of three visible spectrophotometric

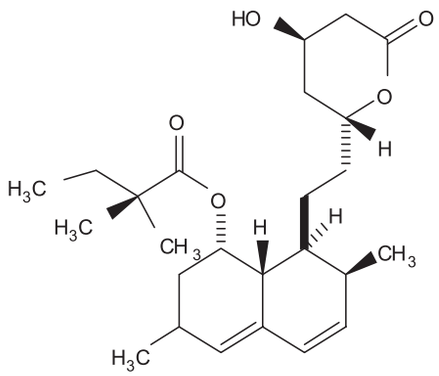

Fig. 1. Structure of Simvastatin.

methods using bromate-bromide mixture in acid medium as the brominating agent and methyl orange, indigo carmine and meta-cresol purple, as spectrophotometric agents. The proposed methods are characterized by simplicity, sensitivity, wide linear dynamic ranges, mild experimental conditions and above all cost-effectiveness. The performance characteristics of these methods are compiled in Table 1. Further, the methods were found to possess adequate accuracy and precision.

Experimental

Apparatus

All absorbance measurements were made on a Systronics model 106 digital spectrophotometer (Ahmedabad, India) provided with 1-cm matched quartz cells.

Materials

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

Bromate-bromide mixture (10, 50 and 40 µg/mL in $KBrO_3$). A stock standard bromate-bromide solution equivalent to 1000 µg/mL $KBrO_3$ was prepared by dissolving accurately weighed 100 mg of pure chemical (Sarabai M.Chemicals, Baroda, India) and 1 g of KBr (S.d fine chem., Munbai, India) in water and diluted to the mark in a 100 mL volumetric flask. The stock solution was diluted appropriately with water to get bromate-bromide solutions containing 10, 50 and 40 µg/mL $KBrO_3$ for use in method A, method B and method C, respectively.

Methyl orange (50 µg/mL). A stock solution equivalent to 500 µg/mL methyl orange was prepared by dissolving 58.8 mg of dye (S.d fine chem., Mumbai, India, 85% dye content) in water and diluting to 100 mL in a volumetric flask; and filtered using glass wool. It was diluted 10-fold to get 50 µg/mL dye solution.

Indigo carmine (200 µg/mL). A stock solution containing 1000 µg/mL indigo carmine was prepared by dissolving 108 mg of dye (Loba chemie, Mumbai, India, 93% dye content) in water and diluting to the mark in a 100 mL volumetric flask, and filtered. The filtrate was diluted with water to get a working concentration of 200 µg/mL dye.

Meta cresol purple (80 µg/mL). About 25 mg of the dye (S.d fine chem., Mumbai, India) was accurately weighed and dissolved in 2 mL of 0.1 N NaOH and diluted to volume with water in a 100 mL calibrated flask, and was diluted to get a working concentration of 80 µg/mL dye solution.

Hydrochloric acid. Required volume of concentrated acid (Merck, Mumbai, India, sp.gr. 1.18) was diluted with water to get 5M acid for all the three methods.

Acetic acid. Glacial acetic acid (Merck, mumbai, India, sp.gr.1.05) was diluted appropriately with water to get 3:2 acid.

Standard drug solution. Pure SMT was gifted by Jubiliant Organosis, Nanjangud, India, and was used as received. A standard stock solution equivalent to 100 µg/mL SMT was prepared by dissolving accurately weighed 25 mg of pure drug in 150 mL of concentrated acetic acid, followed by dilution to 250 mL with water in a volumetric flask. This stock solution was diluted with 3:2 acetic acid to get working concentration of 10 µg/mL for method A and, 50 µg/mL for method B and method C.

Procedures

Method A

Different aliquots (0.5-3.0) of 10 µg/mL SMT solution were accurately transferred into a series of 10 mL calibrated flasks using micro burette and the total volume was adjusted to 3.0 mL by adding 3:2 acetic acid. To each flask were added 1 mL each of 5 M HCl and bromate-bromide (10 µg/mL w.r.t $KBrO_3$). The flasks were stoppered, the content was mixed and the flasks were let stand for 15 min with occasional shaking. Then, 1 mL of 50 µg/mL methyl orange was added to each flask by means of micro burette, diluted to the mark with water, mixed and absorbance of each solution was measured at 510 nm against a reagent blank after 10 min.

Method B

Varying aliquots (0.5-3.0 mL) of SMT solution (50 µg/mL) were accurately measured into a series of 10 mL calibrated flasks by means of micro burette and the total volume was brought to 3 mL by adding 3:2 acetic acid. To each flask were added 1 mL of 5M HCl followed by 1 mL of $KBrO_3$-KBr solution (50 µg/mL w.r.t. $KBrO_3$). The flasks were stoppered immediately, content mixed, and kept aside for 15 min with occasional shaking. Lastly, 1 mL of 200 µg/mL indigo carmine solution was added to each flask and let stand for 5 min before diluting up to the mark with water. The absorbance of each solution was measured at 610 nm against reagent blank.

Table 1. Comparison of the performance characteristics of the existing methods with the proposed methods.

Sl. No.	Method	Conditions	Linear range, $\mu g\ mL^{-1}$	LOQ μgmL^{-1}	Remarks	Ref.
1.	UV-Spectrophotometry	Measurement of first derivative signals at 241.6, 245.9 and 249.1 nm	12.0-28.0	NA	Narrow linear range	2
2.	UV-Spectrophotometry	Absorbance measured at 238 nm in ethanol	2.0-16.0	NA	RSD = 0.50% Av. Recovery = 100.2%	3
3.	UV-Spectrophotometry	First derivative signal measured at 224 & 250 nm.	4.0-12.0	NA	Narrow linear range	4
4.	RP-HPLC	SB-C 18 column, CH_3CN-0.025 M NaH_2PO_4 (65:35) (flow rate: 1.0 mL min^{-1}) was the mobile phase, UV detection at 238 nm.	2.0-200	NA	RSD ~ 3%	7
5.	HPLC	ODS 5-µm column, MeCN-0.1 % H_3PO_4 was the mobile phase.	0.5-5.0 µg	NA		10
6.	HPTLC	Precoated silica gel $60F_{254}$, chloroform: methanol:toluene (6:2:2, v/v/v) as mobile phase.	NA	200 ng/spot		12
7.	Micellar electrokinetic chromatography (MEKC)	Electrolyte system: 12% acetronitrile (v/v) in 25 mM sodium borate buffer pH 9.3 containing 25 mM sodium dodecyl sulphate with an extended light path capillary (48.5 cm × 50µ i.d., 40 cm to detector)	NA	10.6	Expensive experimental setup, critically dependent on pH.	13
8.	a. Cyclic voltammetry	SMT in aq. alcohol medium at a stationary glassy carbon electrode, oxidation peak between pH 2 & 8.	NA	NA	Expensive experimental setup.	14
	b. Differential pulse	SMT in 0.1 M H_2SO_4 & a constant amount of methanol (20%)-supporting electrolyte.	2×10^{-6} 1.0×10^{-4} M	2.71×10^{-7} M		
	c. Square wave voltammetry			5.50×10^{-7}		
9.	Visible Spectrophotometry	**a.** Method A: Reaction of SMT with Fe (III) and potassium ferricyanide. λ_{max} = 730 nm.	NA	NA	-	15
		b. Method B & Method C: Oxidation of SMT by Fe (III) & chelation of Fe (II) produced with 1,10 phenanthroline or 2,2'-bipyridine. (480 or 490nm)	NA			
10.	Visible Spectrophotometry	Method A: Bromate-bromide-methyl orange as reagent in acid medium. λ_{max} = 510 nm	0.5-3.0 ($\varepsilon = 1.0 \times 10^5$)	0.08	Mild acidic conditions, wide linear dynamic ranges, highly sensitive (Method A), good accuracy & precision.	This work.
		Method B: Bromate-bromide-Indigocarmine as reagent in acid medium. λ_{max} = 610 nm.	2.5-15.0 ($\varepsilon = 2.3 \times 10^4$)	0.40		
		Method C: Bromate-bromide-Meta cresol purple as reagent in acid medium λ_{max} = 540 nm.	2.5-15.0 ($\varepsilon = 2.1 \times 10^4$)	0.77		

Method C

Varying aliquots (0.5-3.0 mL) of 50 µg/mL SMT were accurately measured into a series of 10 mL calibrated flasks and the total volume was brought to 3 mL by adding 3:2 acetic acid. To each flask, 1 mL of 5 M HCl followed by 1 mL of bromate-bromide reagent (40 µg/mL w.r.t. $KBrO_3$) were added. The flasks were stoppered, content mixed and shaken occasionally for 15 min before adding 1 mL of 80 µg/mL meta cresol purple. The flasks were again stoppered and let stand for 5 min, followed by diluting to the mark with water. The absorbance was measured at 540 nm against reagent blank.

Procedure for Tablets

Twenty tablets were weighed accurately and ground into a fine powder. Powder equivalent to 50 mg of SMT was weighed accurately and transferred into a 100 ml volumetric flask, 60 mL of 3:2 acetic acid added and content shaken for 15-20 min and diluted to volume with the same acid and mixed well; and filtered using a Whatman No. 42 filter paper. First 10 mL portion of filtrate was discarded and a suitable aliquot of the subsequent portion was subjected to analysis by method A, method B, or by method C, after appropriate dilution with 3:2 acetic acid.

Method Development

The methods are based on the bromination of SMT (either at 1,3 or 1,2 postion) by known excess of *in situ* generated bromine in hydrochloric acid medium and subsequent determination of unreacted bromine by reacting with methyl orange, indigo carmine or meta-cresol purple and measuring the resulting absorbance at 510, 610 or 540 nm. The possible reaction scheme is given in Fig.2. In all the methods, the absorbance increased linearly with a increasing concentration of SMT, the observation which served as the basis of quantization of the drug.

SMT, when added in increasing concentration to a fixed concentration of bromine, consumed the latter and there occurred a concomitant fall in its concentration. When a fixed concentration of either dye was added to decreasing concentrations of bromine, a concomitant increase in the concentration of dye resulted. This was observed as a proportional increase in the absorbance at the respective λ_{max} with increasing concentration of SMT as shown in Fig 3. The change in absorbance as a function of concentration of SMT at 510 nm is depicted in the form of absorption spectra for the method using methyl orange in Fig. 4. Similar absorption spectra can be drawn for methods using indigocarmine and metacresol purple.

Preliminary experiments were performed to determine the maximum concentration of each dye spectrophotometrically; and these were found to be 5, 20 and 8 µg/mL for methyl orange, indigo carmine and meta-cresol purple, respectively. The bromate concentration (in the presence of excess KBr) required to bleach the dyes completely in acid medium was also determined and they were found to be 1 µg/mL for methyl orange, 5 µg/mL for indigo carmine and 8 µg/mL for meta-cresol purple. Hence, different concentrations of SMT were reacted with 1.0 mL each of 10 µg/mL $KBrO_3$ in method A, 50 µg/mL $KBrO_3$ in method B and 40 µg/mL $KBrO_3$ in method C, before determining the residual bromine as described under the respective procedures.

Hydrochloric acid was the ideal medium for bromination reaction as well as the determination of residual bromine by using any of the studied dyes. The reaction between SMT and bromine (*in situ*) was unaffected when 0.5-3.0 mL of 5 M hydrochloric acid was used in about 5 mL. Hence, 1 mL of 5M HCl was used for both steps of the reaction. Further at this acid concentration, the time required to bleach the dyes was 5 min for meta cresol purple and indigo carmine and 10 min for methyl orange. At lower acid concentrations, the bleaching took a longer time. For quantitative reaction between SMT and bromine (*in situ*), a contact time of 15 min was found sufficient in all three methods and constant absorbance readings were obtained when contact times were extended up to 30 min. The measured color in all the three methods was stable for several hours even in the presence of reaction product.

Results and Discussion

An acidified solution of bromate-bromide behaves as an equivalent solution of bromine and has been widely used for the determination of many organic substances of pharmaceutical importance [16-21]. The present methods make use of brominating ability and bleaching action of *in situ* generated bromine.

Method validation

Analytical parameters

A linear relation is found between absorbance and concentration within the Beer's law range given in Table 2. The calibration graphs are described by the equation:

$$Y = a + b\,X$$

(where Y = absorbance, a = intercept, b = slope and X = concentration in µg/mL) obtained by the method of least squares. Correlation coefficients, intercepts and slopes for the calibration data are summarized in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values, the limits of detection and quantification are also presented in Table 2 and speak of the excellent sensitivity of the proposed methods.

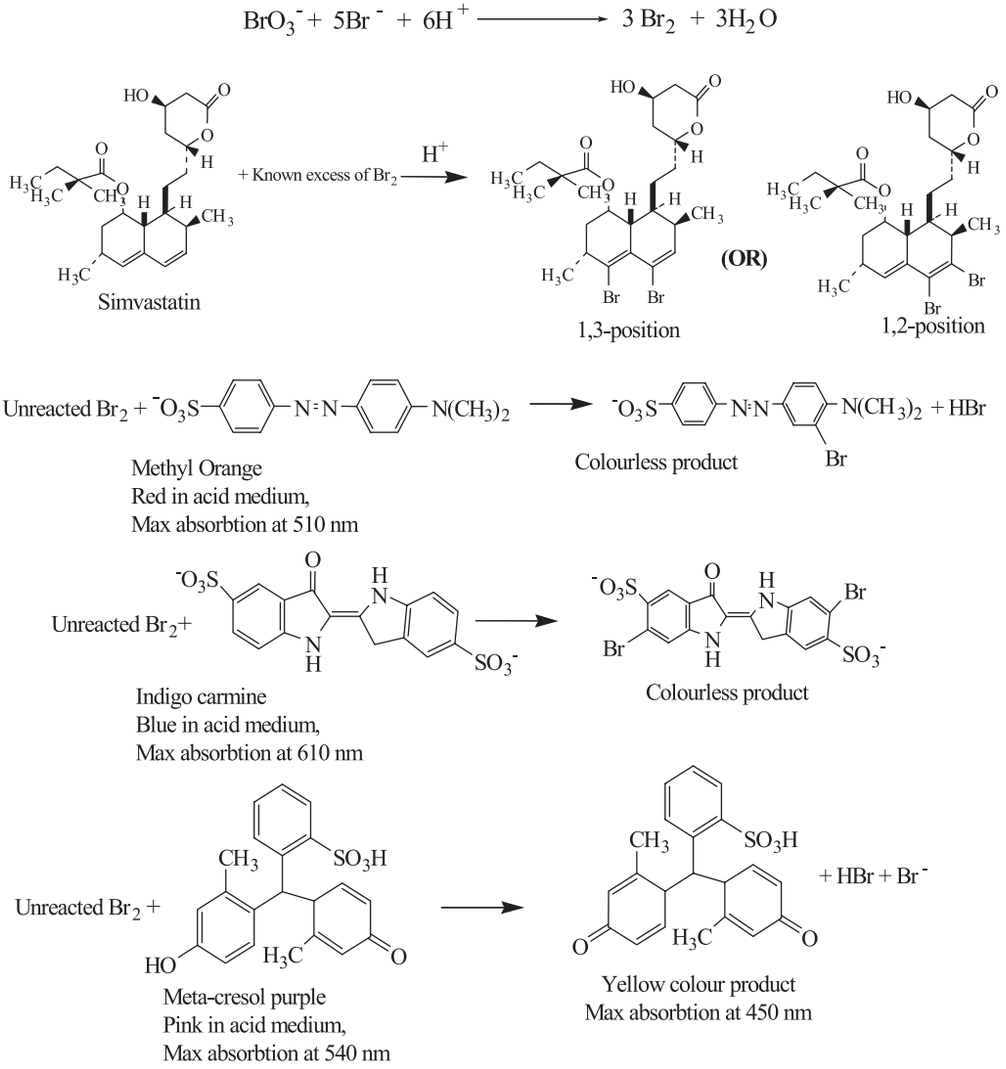

Fig. 2. Reaction scheme.

Assay precision and accuracy

The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day) [22]. Three different concentrations of SMT were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The RSD (%) values of intra-day and inter-day studies showed that the precision was good (Table 3). The accuracy of an analytical method expresses the closeness between the reference value and the found value [22,23]. Accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for SMT (Bias %). The results obtained are compiled in Table 3 and show that the accuracy is good. In order to check the interference from the excipients, a synthetic mixture consisting of 20 mg sodium alginate, 30 mg magnesium stearate, 20 mg lactose, 20 mg acacia, 50 mg talc and 30 mg starch besides 20 mg of SMT was prepared and analysed after extraction into acetic acid as described under analysis of tablets. The percent recoveries of SMT were 98.56 ± 0.68, 100.6 ± 1.16 and 99.75 ± 0.92 for method A, method B and method C, respectively. These results further confirm the good accuracy as well as precision of the proposed methods.

Application to analysis in tablets

The proposed methods were applied to determine SMT in two brands of tablets with three different doses. The results were compared with those of the literature method [6] which consisted of measurement of absorbance of the tablet extract at 240 nm in methanolic medium. Statistical analysis of the results using Student's t-test for accuracy and F-

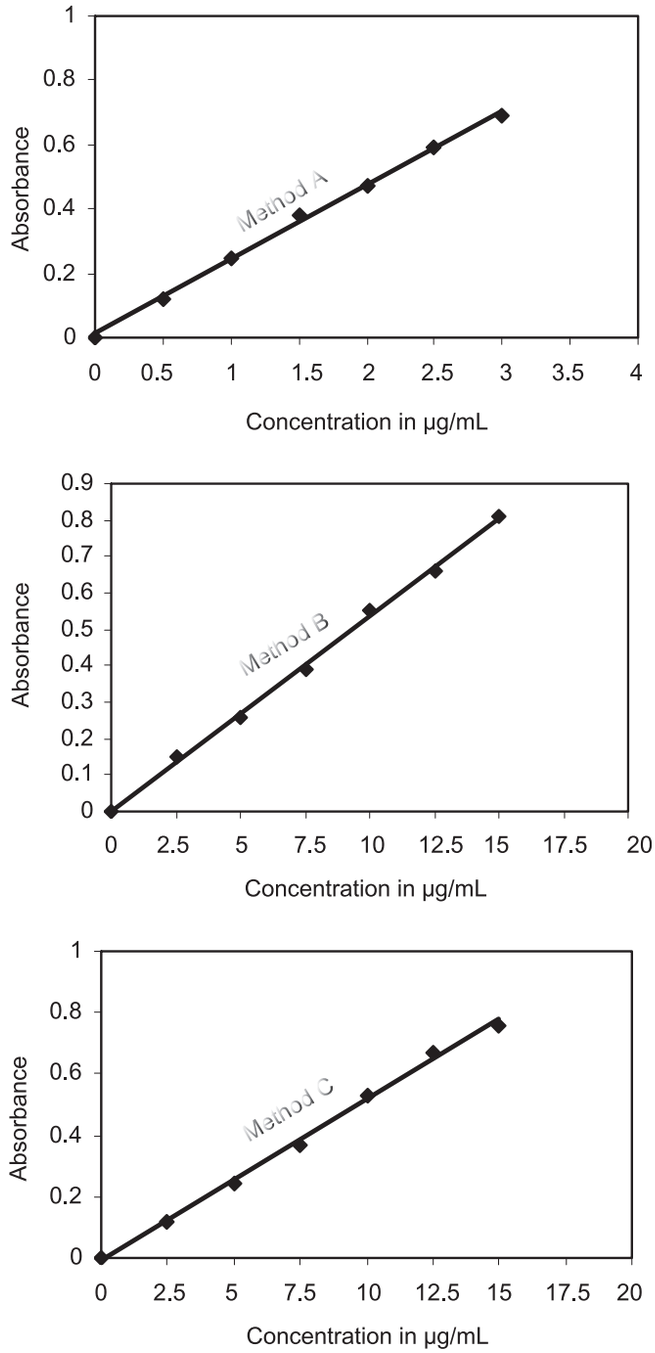

Fig. 3. Beer's law curves for method A, method B and method C

test for precision revealed no significant difference between the proposed methods and the literature method at the 95 % confidence level with respect to accuracy and precision (Table 4).

Recovery study

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analysed tablet powder was spiked with pure SMT at three concentration levels (50,100 and 150% of that in tablet powder) and the total was found by the proposed methods. In all cases, the added SMT recovery percentage values ranged between 98.42 and 103.7% with relative standard deviation < 2%. The results of this study given in Table 5 indicated that the recovery was good, and that the co-formulated substances did not interfere in the determination.

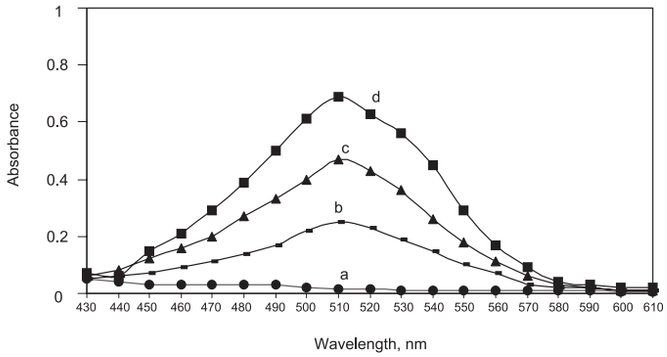

Fig. 4. Absorption spectra of the reaction mixture containing: **a.** 0.0 µg mL^{-1} SMT; **b.** 1.0 µg mL^{-1} SMT; **c.** 2.0 µg mL^{-1} SMT and **d.** 3.0 µg mL^{-1} SMT in method A (using methyl orange)

Conclusion

This is a second report on the determination of simvastatin by visible spectrophotometry. All the three methods developed are simple, rapid and cost-effective. The methods are free from stringent experimental conditions and are characterized by wide linear dynamic ranges of response and high sensitivity. The colored species are stable for long duration and are independent of any experimental variables which perhaps accounts for high precision of results. Another significant advantage is that the absorbance is measured at longer wavelengths where the interference from co-formulated substances is far less compared to shorter wavelengths. The methods could be put to use in industrial quality control laboratories for routine analysis.

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Table 2. Regression and analytical parameters

Parameter	Method A	Method B	Method C
λ_{max}, nm	510	610	540
Beer's law limits, µg / mL	0.5-3.0	2.5-15.0	2.5-15.0
Molar absorptivity, L/ mol /cm	1.0×10^5	2.3×10^4	2.1×10^4
Sandell sensitivity, µg/cm^2	0.0042	0.0184	0.0198
Limit of detection, µg/ mL	0.03	0.13	0.25
Limit of quantification, µg/ mL	0.08	0.40	0.77
Regression equation, Y			
*Intercept (a)	0.0207	0.0040	-0.0167
Slope (b)	0.2263	0.0533	0.0531
Correlation coefficient, (r)	0.9984	0.9987	0.9977
S_a	0.0133	0.0139	0.0186
S_b	0.00637	0.0013	0.0018

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

Table- 3. intra-day and inter-day precission and accuracy results

Method	SMT taken µg/mL	Intra-day[d]			Inter-day[e]		
		SMT found[a] (µg/mL)	Precision[b]	Accuracy[c]	SMT found[a] (µg/mL)	Precision[b]	Accuracy[c]
	1.00	1.02	0.85	2.00	1.025	1.46	2.5
A	2.00	2.02	0.96	1.00	2.03	1.72	1.5
	3.00	2.96	0.54	1.33	2.94	1.27	2.00
	2.5	2.55	1.04	2.00	2.58	1.85	3.2
B	7.5	7.41	0.84	1.20	7.38	2.16	1.6
	12.5	12.68	0.63	1.44	12.72	1.74	1.76
	2.5	2.56	0.98	2.40	2.54	1.38	1.6
C	7.5	7.41	0.67	1.47	7.39	1.02	1.47
	12.5	12.78	1.34	2.24	12.72	0.82	1.76

a. Mean ± standard error, b. Relative standard deviation (%), c. Bias %: (found-taken/taken) x 100, d. n = 7, e. n = 5

Table. 4. Results of assay of tablets and statistical evaluation

Tablet brand name[**]	Nominal amount mg	Found (% of nominal amount ± SD)			
		Literature method	Method A	Method B	Method C
	10	99.42 ± 0.65	100.1 ± 0.85 **t** =1.43 **F** =1.7	98.74 ± 0.36 **t** =2.1 **F** =3.3	99.08 ± 0.74 **t** =0.77 **F** =1.3
Simvofix[a]	20	101.5 ± 1.01	100.4 ± 0.76 **t** =1.96 **F**=1.77	102.6 ± 0.92 **t** =1.8 **F** =1.21	100.8 ± 0.56 **t** =1.41 **F** =3.30
	40	98.66 ± 0.82	99.42 ± 1.2 **t** =1.19 **F** =2.14	97.85 ± 0.76 **t** =1.62 **F** =1.16	98.94 ± 0.3 **t** =0.42 **F** =2.51
	5	100.6 ± 1.11	101.5 ± 0.92 **t** =1.4 **F** =1.46	99.76 ± 1.36 **t** =1.07 **F** =1.50	100.1 ± 0.79 **t** =0.83 **F** =1.97
Zosta[b]	10	99.33 ± 0.96	100.5 ± 1.45 **t** =1.53 **F** =2.28	101.04 ± 0.83 **t** =3.02 **F** =1.34	98.50 ± 0.42 **t** =1.76 **F** =5.22
	20	97.44 ± 0.75	98.36 ± 1.06 **t** =1.61 **F** =1.9	97.12 ± 0.95 **t** =0.59 **F** =1.60	98.67 ± 0.86 **t** =2.41 **F** =1.31

[*]Mean value of five determinations
[**]Marketed by: a. Bal Pharma (Servetus); b. USV (Corvette).
Tabulated t-value at the 95% confidence level is 2.77; Tabulated F-value at the 95% confidence level is 6.39.

Table 5. Results of recovery experiments by standard addition method

Formulation studied	Method A				Method B				Method C			
	SMT in tablet, µg/mL	Pure SMT added, µg/mL	Total found, µg/mL	Pure SMT recovered*, Percent ± SD	SMT in tablet, µg/mL	Pure SMT added, µg/mL	Total found, µg/mL	Pure SMT recovered*, Percent ± SD	SMT in tablet, µg/mL	Pure SMT added, µg/mL	Total found, µg/mL	Pure SMT recovered*, Percent ± SD
Zosta,20 mg	0.98	0.50	1.48	99.66 ± 1.30	4.86	2.50	7.32	98.42 ± 1.96	4.43	2.50	7.46	101.1 ± 1.52
	0.98	1.00	1.99	101.3 ± 1.66	4.86	5.00	9.89	100.5 ± 1.26	4.93	5.10	10.06	100.6 ± 0.94
	0.98	1.50	2.52	102.5 ± 1.12	4.86	7.50	12.46	101.3 ± 1.72	4.93	12.50	17.72	102.3 ± 1.44
Simvofix,40 mg	0.99	0.50	1.49	100.6 ± 1.90	4.89	2.50	7.38	99.50 ± 1.85	4.95	2.50	7.56	101.3 ± 1.63
	0.99	1.00	2.03	103.5 ± 1.02	4.89	5.00	10.00	102.3 ± 1.98	4.95	5.00	10.14	103.7 ± 1.58
	0.99	1.50	2.51	101.3 ± 1.86	4.89	7.50	12.40	100.1 ± 1.2	4.95	12.50	17.65	101.6 ± 1.31

*Mean value of three determinations

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