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Sensitive Extractive Spectrophotometric Method for the Determination of Some Statin Drugs in Pharmaceutical Preparations

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Abstract: Background: The statins (or HMG-CoA reductase inhibitors) formed a class of hypolipidemic drugs used to lower cholesterol levels in people with or at risk of cardiovascular disease. They lower cholesterol by inhibiting the enzyme HMG-CoA reductase, which is the rate-limiting enzyme of the mevalonate pathway of chloestrol synethesis. **The context and purpose:** Simple, sensitive and rapid extractive spectrophotometric method has been developed for the assay of statin drugs, simvastatin, pravastatin sodium and atorvastatin calcium, in pure form and in tablets. The method involves the formation of coloured ion-pairs between the drugs and the Mo (V)-thiocyanate binary complex followed by their extraction with 1, 2-dichloroethane and quantitative determination at 470 nm. The experimental conditions were optimized to obtain the maximum colour intensity. The method permits the determination of simvastatin, pravastatin and atorvastatin over a concentration range of 10-280, 10-150 and 10-180 μg mL^{-1}, respectively, with the detection limit of 1.2, 0.26 and 0.642 μg mL^{-1}, respectively. **Results:** The proposed methods are applicable for the assay of the investigated drug in different dosage forms and the results are in good agreement with those obtained by the official method that reported in the European pharmacopoeia and HPLC methods. The percentage recovery of 30-100 μg mL^{-1} of the drugs under investigation are found to be 97.00-100.3 with a relative standard deviations (%) less than 1%. No interference was observed from common excipients present in pharmaceutical formulations. **Conclusion:** Simvastatin, pravastatin sodium and atorvastatin calcium drugs have been determined in pure form and in tablets using spectrophotometric method.

Key words: Spectrophotometric determination, statin drugs, ion-pair extraction, Mo (V)

INTRODUCTION

Technological and scientific progress has led to the development of numerous synthetic drugs. It is therefore imperative to dispose of analytical methods to determine these drugs both in the quality control manufacturing phase of the pharmaceutical formulations, pure form and their determination in the human body. The statins (or HMG-CoA reductase inhibitors) formed a class of hypolipidemic drugs used to lower cholesterol levels in people with or at risk of cardiovascular disease. They lower cholesterol by inhibiting the enzyme HMG-CoA reductase, which is the rate-limiting enzyme of the mevalonate pathway of chloestrol synethesis.

Simvastatin (SVT) [1S [1α, 3α.7β, 8β (2S*, 4S*), 8αβ] 1, 2, 3, 7, 8, 8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran2yl) ethyl]-1-naphthalenyl-2,2-dimethylbutanoate, atorvastatin (AVT) [R (R*,R*)] (fluorophenyl) β, δ-dihydroxy-5-(1-methyl)-3-phenyl-4 [(phenylamino)carbonyl]1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate and pravastatin (PST)1-naphthalene-heptanoicacid, 1, 2, 6, 7, 8, 8a-hexahydro-β, δ, 6-trihydroxy-2-methyl-8-(2-methyl-1-oxobutoxy [1S [1α (βS*, δSβ (R*), 8aα] monosodium salt, are selective and competitive inhibitors of HMG-CoA reductase. Literature survey revealed that HPLC methods (Kim *et al*., 2004; Malenovic *et al*., 2004; Jemal *et al*., 2000; Zhang *et al*., 2004; Srinivasu *et al*., 2002; Cermola *et al*., 2006; Altuntas *et al*., 2004; Miao and Metcalfe, 2003; Van-Pelt *et al*., 2001; Dohalsky *et al*., 2006; Ma *et al*., 2007; Seshachalam and Kothapally, 2008; Shah *et al*., 2007; Erturk *et al*., 2003; Jamshidi and Nateghi, 2007; Zhu and Neirinck, 2003; Mulvana *et al*., 2000; Deng *et al*., 2008; Chaudhari *et al*., 2007), electrophoresis (Kocijan *et al*., 2005), UV spectroscopic (Erk, 2002, 2004, 2003; Siavash *et al*., 2007; Nagaraj and Rajshree, 2007) and electrochemical methods (Lovric and Nigovic, 2006; Ozkan *et al*., 2003; Nigovic, 2006) have been reported for the analysis of these drugs and their metabolites in biological fluids. So far no visible spectrophotometric method was reported for the quantitative determination of these drugs in pharmaceutical dosage forms. The present study describes development of simple, accurate, precise and reproducible method for the determination of statin drugs in tablet dosage forms. Extractive spectrophotometric

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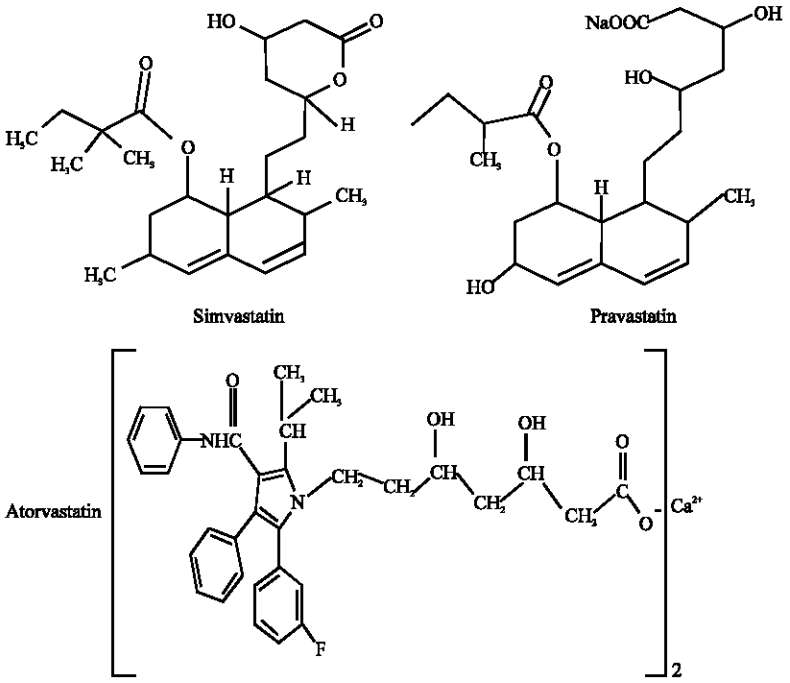

Fig. 1: The structures of statin drugs under investigation

procedures are popular for their sensitivity in the assay of drugs and therefore, ion-pair extractive spectrophotometry has received considerable attention or the quantitative determination of many pharmaceutical compounds. Quantitative analysis of the drugs applying extractive spectrophotometric methods is mainly founded on such factors as type of solvent extraction, nature of coordination agents, acidity of the solution, concentration of reagents, temperature, time of reaction and extraction. The structures of statin drugs under investigation are given in Fig. 1.

MATERIALS AND METHODS

Reagents: All chemicals and reagents used throughout this study were of analytical grade. Reagents used included ammonium molybdate (Mallinchrodt Chemical works, New York), ammonium-thiocyanate (Winlap,U.K.) and ascorbic acid (EL-Nasr.Co., Egypt). Solvents were always HPLC or spectroscopic grade. Doubly distilled water was used to prepare all solutions. Freshly prepared solutions were always employed.

Materials: Pharmaceutical grade atorvastatin calcium (AVT), simvastatin (SVT) and pravastatin sodium (PST) were used without further purification and were supplied by Egyptian Co. for chemical and pharmaceuticals, S.A.E, Egypt.

Apparatus: All the spectral analyses were made using Perkin-Elmer 601 spectrophotometer and quarts cell of 1 cm optical length was used. Automatic pipettes (Socorex Swis 200 μL and 200-1000 μL) were used.

Preparation of standard solutions: Stock solutions of AVT, SVT and PST containing 1 mg mL^{-1}, were prepared by dissolving 100 mg of drugs in 100 mL methanol in a calibrated measuring flask.

Stock solutions of pharmaceutical preparations as a pure base form of drugs: An accurately weighed amount, equivalent to 100 mg of each drug from composite of 20 powdered tablets, was transferred into a 100 mL calibrated flask and diluted to the mark with the appropriate solvent, sonicated for 20 min and filtered off through a Whidtman No.1 filter paper to obtain solutions of 1000 μg mL^{-1}. Further dilutions were made to obtain sample solution. All measurements were made at room temperature (25±1°C).

General procedure

Batch measurements: The ion-pair distribution ratio was determined at room temperature by shaking equal volumes (5 mL) of the organic and aqueous phases of a given composition in a 50 mL separating funnel for 15 min. After the phases were separated by gravity, an aliquot of the organic phase was used. A calibration curve for spectrophotometric determination of the drugs was

prepared by taking known amounts of standard solution of the drugs forming the complex and measuring the absorbance of the organic phase vs. reference solution at 470 nm for all the drugs under investigation.

RESULTS AND DISCUSSION

Anionic thiocyanate complexes of Mo (V) formed ion associates with the positively charged drugs, the ion association complex, with two oppositely charged ions, behaved as a single unit held together by an electrostatic force of attraction.

Spectral characteristics: In an attempt to increase the sensitivity of the assay, the absorption spectra of the extracted Mo (V)-thiocyanateBSVT, Mo (V)-thiocyanate BPST and Mo (V)-thiocyanateBAVT ion-pairs in 1, 2-dichloroethane are scanned against blank reagent at different wavelengths ranged from 400 to 550 nm as shown in Fig. 2. Maximum absorbance with highest

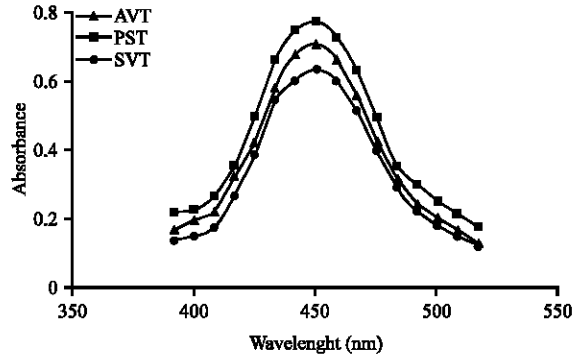

Fig. 2: Absorption spectra Mo (V)-statins ion-pairs

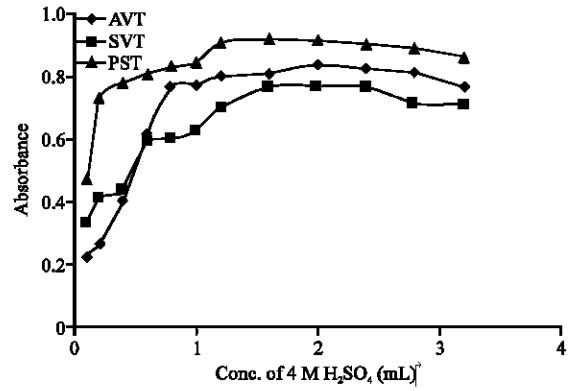

Fig. 3: Effect of sulphuric acid concentration on the spectra of the ion-pairs of simvastatin, atorvastatin and pravastatin drugs at λ_{max} = 470 nm

sensitivity is accomplished at 470 nm for all the drugs under study.

Optimization of variables: Optimum conditions necessary for rapid and quantitative formation of coloured ion-pair complexes with maximum stability and sensitivity were established by a number of preliminary experiments. Extraction of Mo (V)-thiocyanate complex with drugs from sulphuric, hydrochloric and nitric acid solutions in 1, 2-dichloroethane was investigated It is found that, maximum absorbance and molar absorpitivity (ε) of the 1, 2-dichloroethane extract are obtained by using sulphuric acid. from Fig. 3. It is obvious that, 1.6-2 mL of 4M H_2SO_4 is suitable for the ionBpairs formations. The effect of ascorbic acid concentration on the formation and extraction of the ion-pairs is examined by varying the ascorbic acid concentration in the aqueous phase. The absorbance of the extracted ion-pairs is increased by increasing the concentration of ascorbic acid first and then decreased later which means that 1×10^4 and 3×10^4 μg mL^{-1} of ascorbic acid for SVT, PST and AVT drugs, respectively, are sufficient for complete conversion of Mo (IV) to Mo (V) (Fig. 4a).

Optimum conditions are fixed by varying one parameter at a time while keeping other parameters constant and observing the effect on the absorbance at 470 nm. The effect of ammonium molybdate concentration is studied by extracting the coloured ion-pair species at different concentrations of ammonium molybdate varied from 5 to 160 μg mL^{-1}. The absorbance of the extracted ionBpairs is increased by increasing the molybdate concentration up to 50 μg mL^{-1} for AVT, SVT or PST drugs, respectively (Fig. 4b). The effect of adding different concentrations of ammonium thiocyanate ranged from (5×10^3 to 7×10^4 μg mL^{-1}) on the formation of ionBpairs is performed. The results obtained showed that, 25×10^3 to 40×10^3 μg mL^{-1} of ammonium thiocyanate are sufficient to give sensitive and reproducible results for microdetermination of SVT, PST and AVT drugs. In the presence of excess thiocyanate concentration over Mo (V), it is possible to obtain ion-pair species having various numbers of coordinate thiocyanate anions, so the colour of the aqueous solutions of Mo (V) thiocyanate is unstable which makes the quantitative measurements somewhat difficult (Fig. 4c).

The effect of time on the formation of the ionBpairs is studied carefully and illustrated in Fig. 5 which shows the increase of absorbance with time up to 15, 15 and 10 min for SVT, PST and AVT drugs, respectively. Then the extract gradually lost its orange red colour, so all measurements are made within a few minutes after phase separation because the variation of the absorbance is not

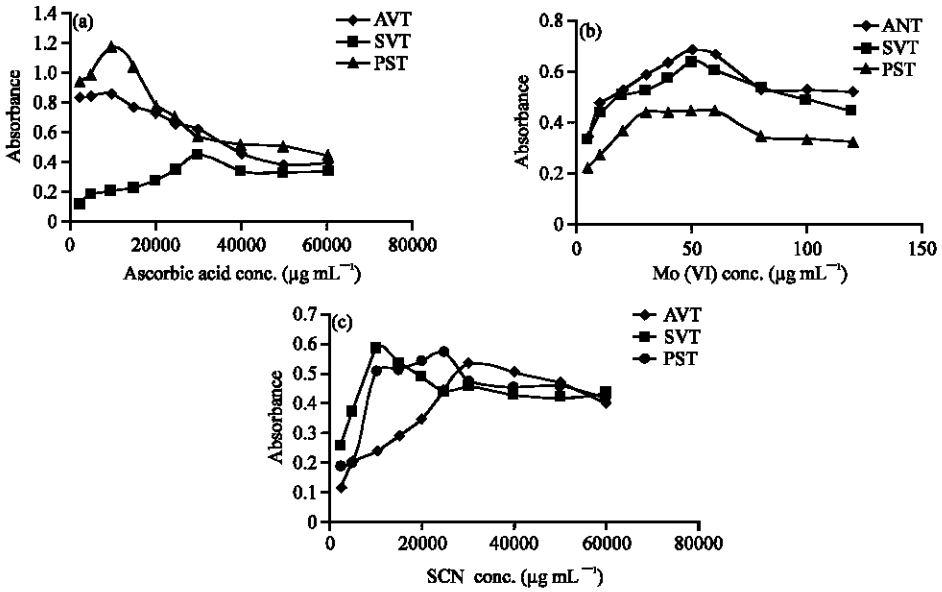

Fig. 4: The effect of variable concentrations of different reactants on the ion-pairs (a) effectof ascorbic acid concentration (b) effect of Mo (VI) concentration and (c) effect of SCN concentration

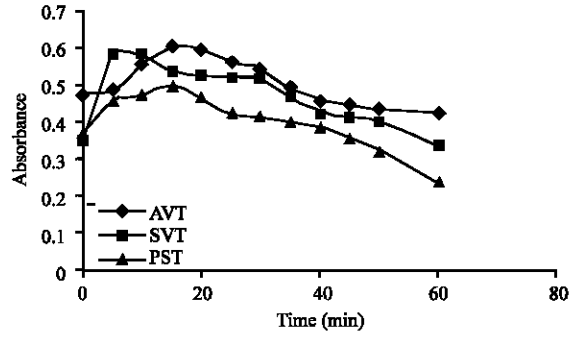

Fig. 5: Effect of time on the spectra of the ion-pairs at λ_{max} = 470 nm

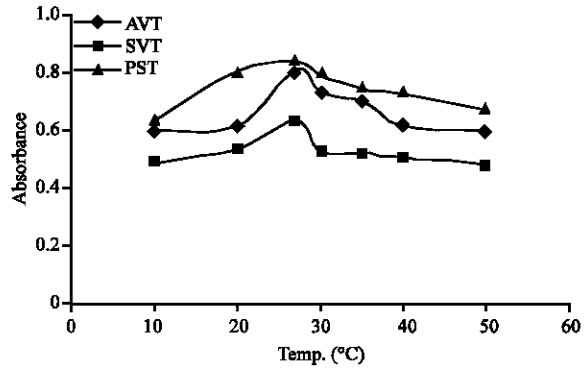

Fig. 6: Effect of temperature on the spectra of the ion-pairs at λ_{max} = 470 nm

significant after the first 20 min. Absorbance-temperature curve represents the reaction of AVT, SVT or PST drugs with Mo (V)-thiocyanate at λ_{max} = 470 nm, at the temperature range from 0 to 50°C (Fig. 6).

Figure 6 shows that the absorbance is generally increased by temperature increase and reached a maximum value at 27°C (room temperature) for SVT, PST and AVT drugs, respectively and slightly decreased above this temperature due to dissociation of the formed ion-pair complexes. Therefore, the temperature chosen is 25±2°C as the best temperature range for microdetermination of the drugs under study in pure and in pharmaceutical forms which confirmed the fact that ion-pairs are more stable at room temperature.

The effect of organic extracting solvents is investigated. A number of organic solvents such as 1, 2-dichloroethane, chloroform, petroleum ether, dichloromethane and ethyl acetate are studied for extraction of the complexes in order to provide an applicable extraction procedure. 1, 2-dichloroethane is preferred as it gives the highest absorbance of coloured extracts and have the highest molar absorptivity values.

Stoichiometry of the formed ion-pairs: The composition of ion-pairs is determined by the contineous variation and the molar ratio methods using equimolar solution to check the ratio between Mo (V) and SVT, PST and AVT drugs to select the optimum conditions for their microdetermination. The results indicate that a 1:1 Mo (V): AVT, Mo (V): SVT or Mo (V): PST ion-pairs are formed

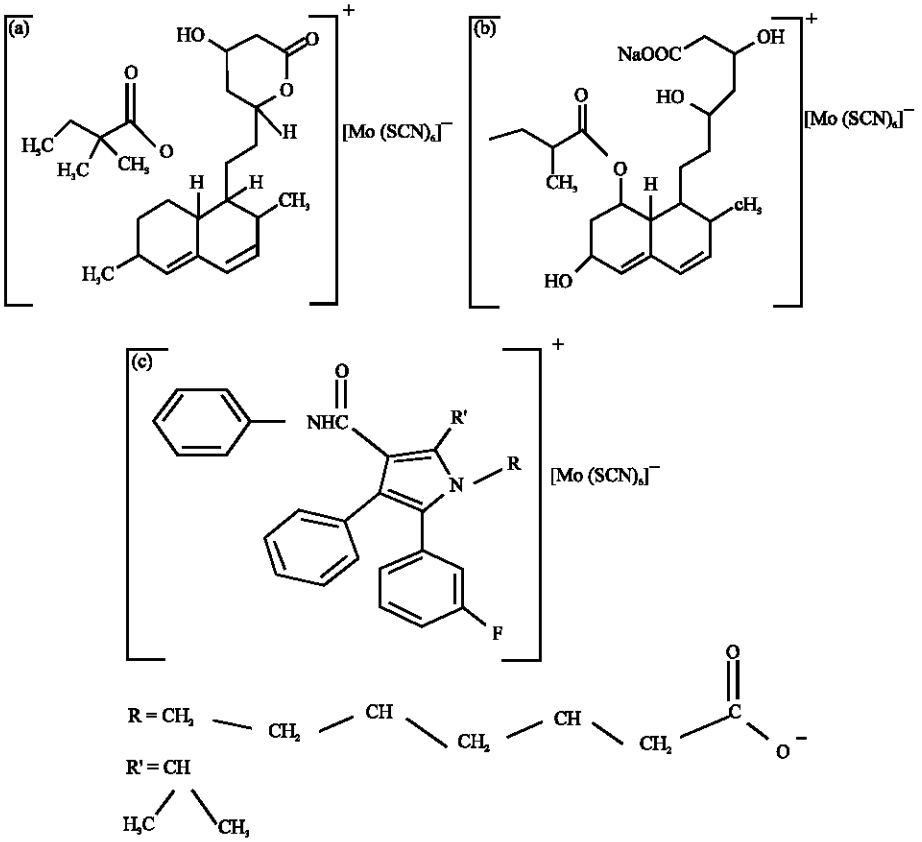

Fig. 7: The proposed structures of (a) SVT (b) PST and (c) AVT-Mo (V)-thiocyanate ion-pairs

Table 1: Analytical characteristics, precision and accuracy of the proposed method

Drug	Simvastatin	Atorvastatin	Pravastatin
λ_{max} (nm)	470	470	470
Beer`s law (μg mL^{-1})	10-280	10-180	10-150
Sandell Sensitivity (μg cm^{-2})	0.064	0.054	0.059
ϵ (L mol^{-1}cm^{-1})	32.27×103	99.02×103	53.81×103
SD	0.013 0.057	0.011-0.073	0.019-0.064
RSD (%)	0.122-0.835	0.114-0.832	0.11-0.688
Linear regression equation[a]			
Slope (a)	0.0061	0.0104	0.0127
Intercept (b)	0.1105	0.0649	0.0688
Limit of detection (LOD) (μg mL^{-1})	1.2	0.642	0.26
Limit of quantification (LOQ) (μg mL^{-1})	3.9	2.1	0.89
Correlation coefficient (r^2)	0.9983	0.9987	0.999

[a] Y = ax+b, where x is the concentration in μg mL^{-1}

through the electrostatic attraction between positive protonated drugs, AVT^+, SVT^+ or PST^+ and thiocyanate negative complex $[Mo(SCN)_6]^-$ as shown by the proposed strucures given in Fig. 7.

Linearity and range: Beer's law range, molar absorptivity, regression equation and correlation coefficient determined for each drug are given in Table 1. A linear relationship is found between the absorbance at λ_{max} and the concentration of the drug in the range of 10-280, 10-150 and 10-180 μg mL^{-1} for SVT, PST and AVT, respectively, Regression analysis of Beer's plots at λ_{max} reveals a good correlation. The graphs show negligible intercepts and are described by the regression equation obtained by the Least-squares method. The correlation coefficients are found between 0.9983-0.9987 indicating good linearity. The high molar absorptivity values of 32.27×10^3, 99.02×10^3 and 53.81×10^3 L mol^{-1} cm^{-1} of the ion-pairs of SVT, AVT and PST, respectively, indicate the high sensitivity of the method.

Validation of the methods: Examined samples were prepared and tested at four levels of drug using the proposed procedures. The complete set of validation assays is performed for the drug as determined by the proposed method. The results obtained for the pure drug are given in Table 2. The precision and accuracy of the methods were tested by analyzing five replicates of the drug. The standard deviation, relative standard deviation, recovery and 95% confidence limits of different amounts tested were determined from the calibration curve, as

Table 2: The Inter- and Intra-day precision and accuracy data for simvastatin, atorvastatin and pravastatin determination obtained by the proposed method, n = 5

Drug	Drug taken (μg mL^{-1})	Drug found (μg mL^{-1})	Percent recovery (%)	SD	RSD (%)
	30.00	30.00	100.00	0.075	0.40
	50.00	49.55	99.10	0.027	0.35
Simvastatin	100.00	100.00	100.00	0.082	0.24
	30.00	29.90	99.66	0.043	0.20
Atorvastatin	50.00	50.00	100.00	0.019	0.45
	100.00	100.10	100.10	0.025	0.32
	30.00	30.10	100.30	0.034	0.22
Pravastatin	50.00	49.50	97.00	0.054	0.32
	100.00	99.94	100.00	0.029	0.26

Table 3: Spectrophotometric microdetermination of simvastatin, atorvastatin and pravastatin drugs in their pharmaceutical preparations using the Mo(V)-thiocyanate method

			Drug found (μg mL^{-1})		Recovery (%)					
Drug	Name of preperation	Drug taken (μg mL^{-1})	Proposed method	Official method	Proposed method	Official method	SD*	SD**	F-test#	t-test#
Simvastatin	Simvastate	20.00	20.10	20.03	100.5	100.00	0.020	0.040	4.10	2.10
		100.00	100.20	99.50	100.2	99.50	0.040	0.010	6.25	1.33
Atorvastatin	Atorstate	120.00	121.00	120.80	103.0	100.80	0.010	0.019	4.45	1.97
		140.00	140.00	141.00	100.0	101.00	0.039	0.089	5.20	2.10
		20.00	20.05	19.90	100.3	99.00	0.034	0.089	5.19	2.21
		30.00	30.50	30.00	100.5	100.00	0.056	0.120	4.49	1.56
		50.00	50.00	50.00	100.0	100.00	0.021	0.049	5.10	1.56
		80.00	80.10	80.12	100.1	100.10	0.087	0.110	5.79	2.50
Pravastatin	Lipostate	20.00	20.10	20.05	100.1	100.00	0.025	0.020	5.30	1.44
		100.00	100.50	100.00	100.5	100.00	0.021	0.010	3.20	1.37
		120.00	120.10	119.90	100.0	99.90	0.030	0.090	6.90	2.21
		140.00	140.10	140.20	100.1	100.20	0.050	0.098	2.60	2.31

No. of replicates (n) =5, #Standared F-values at 95 % confidence level = 6.39, #Standared t-values at 95% confidence level = 2.77, *standard deviation values using proposed method, ** Standard deviation values using official method

recorded in Table 2. The accuracy of the method is indicated by the excellent recovery (98.50-100.2), (98.80-102.0) and (98.50-100.1) for SVT, AVT and PST, respectively and the precision is supported by the low standard deviation, (SD = 0.013 to 0.057, 0.019 to 0.064 and 0.011 to 0.073 for SVT, PST and AVT drugs, respectively) and relative standard deviation (RSD% = 0.24 to 0.40, 0.22 to 0.32 and 0.20 to 0.45 % for SVT, PST and AVT drugs, respectively).

Precision, accuracy and specificity: Day-to-day precision and accuracy were evaluated by analyzing five samples of three different concentrations, which are prepared and analyzed on the same day (Table 2). Sample-to-sample variability is assessed using five samples o three different concentrations analyzed on four different days over a period of a week. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that there were no significant intra-day or inter-day differences.

Interferences in pharmaceutical analysis: It is important to test the selectivity towards the excipients and fillers added to the pharmaceutical formulations. The concentration of the drugs in the dosage forms is calculated from the appropriate calibration graphs. There is no shift in the absorption maximum due to the presence of other constituents of the dosage forms. No interferance from common excipents, which indicates the selectivity of the proposed method.

Application: The validity of the proposed method is tested by the determination of SVT, AVT and PST drugs in dosage forms manufactured in the local companies. Table 3 shows the results obtained during the determination of SVT, AVT and PST drugs in the dosage forms. The results are compared with those obtained applying the official method (reported in the European pharmacopoeia) for SVT and PST drugs and official method for AVT drug. The results obtained are compared statistically by t-test and F-test with those obtained by official method on the sample of the same batch. It is obvious from this table that the percentage recoveries, SD and RSD values obtained applying the proposed method are very close to those obtained by the official method. The t-test and F-test values obtained at the 95% confidence level and degree of freedom (n = 4) did not exceed the theoretical tabulated value indicating that there is no significant difference between accuracy and precision of the proposed and the official methods.

CONCLUSION

A rapid, simple, sensitive and accurate extractive spectrophotometric method has been developed which can be used for determination of SVT, AVT and PST drugs in pharmaceutical formulations. There are some methods available for this determination, which use UV-spectrophotometry; however, these have several disadvantages: the cost of equipment, manner of performance, time required, difficulty of reaction conditions and analytical procedures. We described the extractive spectrophotometric method for determination of SVT, AVT and PST drugs based on the formation of ion pair with thiocyanate of Mo (V). The method makes use of simple reagents, which an ordinary analytical laboratory can afford and validation showed them to be suitable for routine determination of SVT, AVT and PST drugs in its formulations. The commonly used additives such as starch, silicon dioxide, magnesium stearate, glucose, glycerin, talc, sodium lauryl sulphate and sodium saccharin do not interfere with the assay procedures. The main The main advantages of these procedures are low cost of reagents, apparatus used and short time of analysis .the method are Characterized by good precision, reproducibility of determination and high sensitivity.

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