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A novel use of oxidative coupling reactions for determination of some statins (cholesterol-lowering drugs) in pharmaceutical formulations

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ABSTRACT

New, accurate and reliable spectrophotometric methods for the assay of three statin drugs, atorvastatin calcium (AVS), fluvastatin sodium (FVS) and pravastatin sodium (PVS) in pure form and pharmaceutical formulations have been described. All methods involve the oxidative coupling reaction of AVS, FVS and PVS with 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH) in the presence of Ce(IV) in an acidic medium to form colored products with λ_{max} at 566, 615 and 664 nm, respectively. Beer's law was obeyed in the ranges of 2.0–20.0, 4.9–35.4 and 7.0–30.0 μg mL^{-1} for AVS–MBTH, FVS–MBTH and PVS–MBTH, respectively. Molar absorptivities for the above three methods were found to be 3.24×10^4, 1.05×10^4 and 0.68×10^4 L mol^{-1} cm^{-1}, respectively. Statistical treatment of the experimental results indicates that the methods are precise and accurate. The proposed methods have been applied to the determination of the components in commercial forms with no interference from the excipients. A comparative study between the suggested procedures and the official methods for these compounds in the commercial forms showed no significant difference between the two methods.

1. Introduction

Statins are commonly used to treat several forms of hypercholesterolemia. They have potent cholesterol-lowering effects and they could reduce morbidity and mortality associated with coronary heart disease significantly, as proved by many clinical trials. However, some statins exhibit a number of adverse effects, such as myopathy or rhabdomyolysis, so it is useful to monitor the levels of statins in biological materials in order to establish an appropriate dosage scheme, which would minimize adverse effects and keep the cholesterol-lowering effect. Atorvastatin calcium (AVS) {[R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy-5(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate}, Fluvastatin sodium (FVS) {[R*,S*-(E)]-(±)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid, monosodium salt} and Pravastatin sodium (PVS) [hexahydro-6-hydroxy-2-methyl-8-(2-methylbutyryloxy)-1-naphthyl-3,5-dihydroxyheptanoate, monosodium salt] are the most commonly occurring drugs in commercially available pharmaceutical formulations used for the clinical treatment of hypercholesterolemia [1].

Many analytical procedures have been described for the individual determination of atorvastatin, fluvastatin or pravastatin, jointly with other pharmaceutical substances, including high performance liquid chromatography (HPLC) [2–22], spectrophotometry [23–27], capillary electrophoresis [28,29], and polarography [30–38] procedures. The simultaneous determination of atorvastatin and pravastatin has been carried out in pharmaceutical formulations using a computational program by HPLC [39].

The objective of the present study was to develop simple, precise, accurate and validated spectrophotometric methods by the application of oxidative coupling reaction for the estimation of AVS, FVS, and PVS in bulk and pharmaceutical formulations.

2. Experimental

2.1. Apparatus

A Jasco V-530 UV–VIS spectrophotometer (Japan) with 1 cm quartz cells was used for all absorbance measurements under the following operating conditions: scan speed medium (400 nm/min), scan range 400–800 nm and slit width 2 nm. Spectra were automatically obtained by Jasco system software. pH measurements were made with Consort C 830 (Belgium) with combined glass pH electrode.

2.2. Material and reagents

Atorvastatin calcium, $(C_{33}H_{34}FN_2O_5)_2Ca{\cdot}3H_2O$, 1209.42 g mol^{-1}, was supplied by CADILA Healthcare (Gujarat, India). Its

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purity was found to be 98.3% according to the compendial method. Fluvastatin sodium ($C_{24}H_{25}FNO_4Na$, 433.46 g mol^{-1}) was supplied by ALPHARM Chemical Co. (China). Its purity was found to be 99.2% according to the compendial method. Pravastatin sodium ($C_{23}H_{35}O_7Na$, 446.52 g mol^{-1}) was supplied by CHEMLINE Healthcare (Lugano, Switzerland), and had a purity of 99.3% according to the compendial method.

All other chemicals and reagents used were of analytical grade and all solutions were prepared with double distilled water.

2.3. Formulations

The following commercial formulations were subjected to the analytical procedures:

- Atoraz tablets (Razi Labs, Syria) labeled to contain 40 mg AVS/tablet.
- Lowlip tablets (Al Fares-Industries Co., Syria) labeled to contain 20 mg AVS/tablet.
- Almastatin capsules (Alma, Syria) labeled to contain 20 mg FVS/capsule.
- Fluvastatin capsules (Kimi, Syria) labeled to contain 40 mg FVS/capsule.
- Pravastatin tablets (Elsaad Pharma, Syria) labeled to contain 20 mg PVS/tablet.
- Pravastin tablets (Rasha, Syria) labeled to contain 40 mg PVS/tablet.

2.4. Solutions

Stock standard solutions of 0.5 mg mL^{-1} were prepared by dissolving the appropriate weight of AVS, FVS and PVS in 100 mL volumetric flask, 5 mL of methanol was added to AVS, the volume was then diluted to the mark with distilled water. 1×10^{-2} M MBTH (Fluka) solution was prepared with double distilled water and 1% $Ce(SO_4)_2$ (Merck) solution was prepared with sulfuric acid (0.368 M) medium. Freshly prepared solutions were always used.

2.5. General procedures

Aliquots of standard AVS (0.10–1.0 mL, 0.5 mg mL^{-1}), FVA (0.245–1.77 mL, 0.5 mg mL^{-1}) or PVS (0.35–1.50 mL, 0.5 mg mL^{-1}) solution were transferred into a series of 25 mL calibrated volumetric flasks. Then 1.0 mL of MBTH solution was added and kept aside for 3, 7 and 3 min for AVS, FVS and PVS, respectively. After that, 1.0 mL of $Ce(SO_4)_2$ solution was added. The volume was made up to the mark with distilled water and the absorbance was measured after 7 min at 566 nm for AVS–MBTH, 12 min at 615 nm for FVS–MBTH and 15 min at 664 nm for PVS–MBTH against a similar reagent blank. The amount of AVS, FVS and PVS was computed from its Beer's law plot prepared with standard drug solution under identical conditions.

2.6. Procedures for formulations

Twenty tablets (or the entire content of twenty capsules) containing AVS, FVS, or PVS were weighed and pulverized. Amount of the powder equivalent to 25 mg of the cited drug was dissolved in a 25 mL of methanol and mixed for about 5 min. and then filtered through Whatman filter paper number 40. The methanol was evaporated to about 5 mL in the case of AVS and to the dryness for FVS and PVS. The remaining portion of solution was diluted in a 50 mL volumetric flask to the volume with double distilled water to achieve a concentration of 0.5 mg mL^{-1}. The general procedure was then followed in the concentration ranges mentioned above.

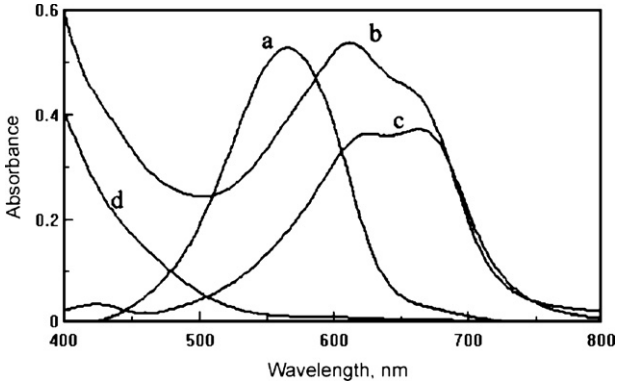

Fig. 1. Absorption spectra of (a) AVS–MBTH–$Ce(SO_4)_2$, (b) FVS–MBTH–$Ce(SO_4)_2$, (c) PVS–MBTH–$Ce(SO_4)_2$ systems against reagent blank (d) vs. distilled water. [AVS or PVS] = 20 μg mL^{-1} and [FVS] = 25 μg mL^{-1} + 1 mL of 10^{-2} M MBTH + 1 mL of 1% $Ce(SO_4)_2$.

3. Results and discussion

The optimum conditions for the development of methods were established by varying the parameters one at a time and keeping the others fixed and observing the effect produced on the absorbance of the colored oxidative coupling products.

In order to establish experimental conditions, the effect of various parameters such as volumes of MBTH, $Ce(SO_4)_2$, addition of buffer solutions, waiting time, order of addition of reagents and the stability of colored oxidative coupling products were studied at room temperature. The applicability of MBTH in combination with various oxidizing agents such as $FeCl_3$, KIO_4, $NaIO_4$, $Ce(SO_4)_2$, $K_2Cr_2O_7$ and $KMnO_4$ was examined. $Ce(SO_4)_2$ was found to be optimal to form colored oxidative coupling products (AVS–MBTH, FVS–MBTH and PVS–MBTH) and enhanced the final color. Addition of KCl–HCl or britton buffer solutions affected negativity on the formation of the three colored oxidative coupling products. Addition of drug, MBTH and $Ce(SO_4)_2$ in that order gave maximum absorbance. The laboratory temperature (25 ± 0.5 °C) was found to be optimal for all the experiments. Final colors were achieved with 5, 10 and 12 min and the color products were stable for at least 1200, 15 and 18 min up to 50, 40 and 40 °C and were measured at 566, 615 and 664 nm for AVS–MBTH, FVS–MBTH and PVS–MBTH, respectively. Under the experimental conditions each pure drug showed a negligible absorbance at the corresponding maximum (Fig. 1).

Apparent molar absorptivities of the drugs were found to be 3.24×10^4, 1.05×10^4 and 0.68×10^4 L mol^{-1} cm^{-1} for AVS–MBTH, FVS–MBTH and PVS–MBTH, respectively. Sandell's index represents the number of micrograms or nanograms of the determinant per millilitre of a solution having an absorbance of 0.001 for the cell path length of 1 cm and is a suitable parameter for expressing and comparing the sensitivity of developed spectrophotometric method. Sandell's sensitivity coefficients of AVS, FVS, and PVS were found to be 0.075, 0.087 and 0.133 μg cm^{-2}/0.001 A, respectively (Table 1).

A volume of 1.0 mL of 0.01 M MBTH and 1.0 mL of 1% $Ce(SO_4)_2$ was found to be optimal for maximum color development, since the absorbance was found to be maxima at the mentioned volumes (Figs. 2 and 3).

3.1. Stoichiometric relationship

The composition of colored oxidative coupling products was determined by Job's method of continuous variation and mole-ratio method [40], indicated a molar ratio of 1:1 and 1:2 drug to MBTH for AVS–MBTH and FVS–MBTH, and 1:1 for PVS–MBTH.