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Micellar liquid chromatography as a sustainable tool to quantify three statins in oral solid dosage forms

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ABSTRACT

A method based on micellar liquid chromatography has been developed to determine rosuvastatin, lovastatin and simvastatin in oral solid dosage forms. Samples were solved in mobile phase up to the target concentration, filtered and directly injected. The three statins were resolved in 30 min, using an aqueous solution of 0.10 M sodium dodecyl sulfate – 7.0% 1-butanol, buffered at pH 3 with 0.01 M phosphate salt as mobile phase, running under isocratic mode at 1 mL/min through a C_{18} column. Detection was at 240 nm. The effect of sodium dodecyl sulfate on elution strength was more important than that of the organic solvent. The procedure was successfully validated by the guidelines of the International Council for Harmonization in terms of: specificity, linearity ($r^2 > 0.990$), calibration range (1.5 - 15 mg/L for rosuvastatin, 0.5–10 mg/L for lovastatin and simvastatin), limit of detection (0.4, 0.2 and 0.15 mg/L for rosuvastatin, lovastatin and simvastatin, respectively), trueness (98.8–101.7%), precision (<2.7%), carry-over effect, robustness, and stability. Values were inside the acceptance criteria of the Methods, Method Verification and Validation, Food and Drug Administration-Office of Regulatory Affairs, thus ensuring the reliability of the results. The main feature was the low proportion of organic solvent used, thus making the procedure sustainable and green. Besides, it was easy-to-conduct and with high sample-throughput, and then useful for routine analysis in pharmaceutical quality control. Finally, it was applied to commercial pharmaceutical preparations.

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1. Introduction

Rosuvastatin (main brand name Crestor™), lovastatin (Mevacor™) and simvastatin (Zocor™) are lipid-lowering agents belonging to the group of statins, which are used to control the plasmatic concentration of lipids. These are first-line drugs for the treatment of hypercholesterolemia and in the prevention of cardiovascular events for patients at moderate to substantial risk. Compared to other lipid-lowering medication, they are more powerful in the reduction of low-density lipoprotein cholesterol (LDLc) (sometimes referred to as "bad cholesterol"), and very low-density lipoprotein cholesterol (VLDLc). They are generally well tolerated and exhibit minimal side and long-term effects. They are usually prescribed as a lifetime treatment, being non-adherence the main reason of failure. Their high benefit-to-risk, cost-effective profile and the considerable prevalence of dyslipidemia has resulted in this class being one of the most widely administered medications [1–4].

Lovastatin and semisynthetic simvastatin arise from fermentation products of *Aspergillus terreus*, have a remarkably close structure and physico-chemical properties, rather different from rosuvastatin, purely synthetic, as we can see in [1] and Table 1 [1,5], respectively. Lovastatin and simvastatin are highly hydrophobic and prodrugs which are metabolized to the active form, the β-hydroxyacid, while rosuvastatin is hydrophilic and the stem drug is the main responsible of the clinical activity. These three statins are considered equally effective from a clinical standpoint [1].

Rosuvastatin, lovastatin and simvastatin are usually administered by oral route, via tablets or capsules. They are marketed as a brand name and generic, mainly as immediate or extended-release tablets or capsules containing 5, 10, 20 or 40 mg for rosuvastatin (usually in calcium salt format), 20, 40 or 60 mg for lovastatin and

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Table 1
Physico-chemical characteristics of the three studied statins [1,5] and parameters of the calibration curve.

Parameter	Rosuvastatin	Lovastatin	Simvastatin
Molecular weight	481.5	404.5	418.6
Log Po/w	0.13	4.1	4.7
pKa (in the 2.5–7.5 interval)	4.0	––	––
Solubility in water	18 mg/L	0.0004 mg/L	0.03 mg/L
Charge at pH = 7	−1	0	0
Charge at pH = 3	−0.09	0	0
Structure			
Linear interval	1.5–15 mg/L	0.5–10 mg/L	0.5–10 mg/L
Slope	26.8 ± 0.4	37.3 ± 0.3	38.7 ± 0.3
Y-intercept	2 ± 3	5.0 ± 1.8	0.3 ± 1.6
Determination coefficient	0.9994	0.9997	0.9998
Limit of detection	0.4	0.2	0.15
Limit of quantification	1.3	0.5	0.45

5, 10, 20, 40 or 80 mg for simvastatin. Usual daily doses per day are 10–40 for rosuvastatin, 10–80 mg for lovastatin and 10–40 mg for simvastatin [3,4,6-8]. They are prescribed in monotherapy or combined with other lipid-lowering agents and/or cardiovascular drugs, although not with other statins [1,9].

To guarantee the success of the treatment, the accurate dose must be taken by the patient. For this reason, the quantity of active component in the pharmaceutical formulation has to be verified by the pharmaceutical industry, as stressed by renowned drug regulatory agencies [10,11]. Otherwise, a black market existed around the world, mostly on the internet, where in pharmaceutical formulations are sold at minor cost by noncertified producers and retailers, because of the high price of legal formulations. Most of them are counterfeit or with questionable quality, and are sent under dubious shipping conditions, and would require an exhaustive control by national health regulatory agencies [12].

Methods based on HPLC-UV are the most adequate for pharmaceutical analyses, where there are neither serious selectivity nor sensitivity issues, due to their resolution power and easy automation [10]. Rosuvastatin [13], lovastatin [14], simvastatin [15] and lovastatin/simvastatin [16] have been individually determined in pharmaceutical formulations with the aid of this technique. Although these three statins are not usually found in the same pharmaceutical preparation, a method able to simultaneously determine them together could be useful for quality control laboratories to verify oral solid dosage forms containing either rosuvastatin or lovastatin or simvastatin in the same workday. Some HPLC-based methods have been proposed to simultaneously quantify rosuvastatin, lovastatin and simvastatin in pure and pharmaceutical forms [17,18,19,20] and for skin penetration studies [21,22]. Mobile phases contain high proportions of toxic, flammable, and volatile organic solvents (46 to 95%), like methanol and acetonitrile. Some of them are programmed as a gradient, which hinders the successive analysis of many samples [18,20-22]. Regarding the sample preparation, oral pharmaceutical forms, regardless of the statin analysed, are usually treated with organic solvent-rich solutions (50 to 100%), usually the mobile phase, to achieve the solubilisation of the active component [13–20]. The handling and waste of high amounts of these hazardous solutions represent a threat for the laboratory operator and the environment. Therefore, there is a need to develop a safe and sustainable alternative for the quantification of these statins, while keeping appropriate analytical and practical performances, following the trend of White Analytical Chemistry (WAC) [23]. Several authors have proposed the use of the less hazardous ethanol as organic modifier in both dosage form solubilisation solution and mobile phase for rosuvastatin [24] and simvastatin/rosuvastatin determination in pharmaceutical formulations [25].

Hybrid micellar liquid chromatography (HMLC), using the anionic surfactant sodium dodecyl sulfate (SDS), has been proposed as an alternative for the development of green and sustainable procedures to quantify the active component in pharmaceutical formulations [26]. Indeed, the hybrid micellar solutions are made up of innocuous and biodegradable salts and only a low proportion of organic solvent (<15%) [27,28]. Nevertheless, they can solubilize even poorly water-soluble drugs at a reasonable time because of the presence of the hydrophobic micellar pseudo-phase [10]. Regarding chromatographic separation, the introduction of this pseudo-phase increases the versatility of HMLC, which can resolve drug mixtures with a large difference of hydrophobicity using an isocratic mode. Besides, the retention is highly reproducible and can be modelled from the concentration of surfactant and organic modifier of the mobile phase [29]. Because of its excellent performances, this technique has been successfully employed to the development of sustainable procedure to determine natural dyes in plant root and water [30], synthetic colorants in sweat [31], pesticides in food products [32,33], drugs in plasma [34–37] and urine [36,37], as well as illicit additives in food [38].

The aim of the work was to develop a reliable, easy-to-handle, green and practical procedure to determine the statins rosuvastatin, lovastatin and simvastatin in pharmaceutical formulations for dosage quality control by micellar liquid chromatography. The effect of the composition of the mobile phase on retention was studied for pure theoretical purposes and as a part of the optimization. The method was validated as indicated by the guide Validation of Analytical Procedures: Text and Methodology Q2(R1) from the International Conference of Harmonization (ICH), to assess its suit-

ability [39]. The procedure was applied to commercial oral solid dosage forms.

2. Experimental

2.1. Standard and reagents

Powdered standards of rosuvastatin calcium (>98.0), lovastatin (pharmaceutical secondary standard) and simvastatin (>97.0%) were purchased from Sigma-Aldrich (Saint-Louis, MO, USA). Sodium dihydrogen phosphate monohydrate (>98.0%) and hydrochloric acid (37%) came from Panreac (Barcelona, Spain). SDS (>85.0%) was bought from Tokyo Chemical Industry (Tokyo, Japan), and methanol and 1-pentanol (HPLC grade) were from Scharlab (Barcelona, Spain). Ultrapure water was generated in the laboratory by purification of deionized water (supplied by the University as tap water) using a water purification system Adrona HPLC Connect (Adrona, Riga, Latvia). All aqueous solutions were prepared using this water.

2.2. Preparation of solutions

To prepare the micellar solutions, the proper amount of SDS and 0.01 M of $NaH_2PO_4.H_2O$ were solved in ultrapure water, and then the pH was adjusted to 3 dropwise using HCl solutions. Afterwards, the adequate volume of organic solvent was added, and the volumetric flask was filled up to the mark with ultrapure water. Finally, the solution was ultrasonicated to achieve solubilization, filtered through a 0.45-μm Nylon membrane filters (Micron Separations, Westboro, MA, USA), with the assistance of a vacuum pump, and then stored in amber bottles.

Individual stock solutions of rosuvastatin, lovastatin and simvastatin (100 mg/L) were prepared using methanol as solvent. Working solutions were made by successive dilutions of these ones in mobile phase.

2.3. Chromatographic equipment and operational conditions

Chromatographic analyses were conducted using an HP1100 system (Agilent Technologies, Palo Alto, CA, USA) with a degasser, an isocratic pump, an autosampler (loop), and an UV-Absorbance diode array detector (DAD). The control of the instrumentation, as well as the visualization and registration of the signal was carried out using the software Agilent ChemStation (Rev. A.10.01). The working specifications in HMLC to avoid damaging the chromatograph can be revised in [37].

The column was Kromasil C_{18} with the following characteristics: 150 × 4.6 mm; particle size, 5 μm; pore size 10 nm (AkzoNobel, Amsterdam, The Netherlands). The mobile phase was an aqueous solution of 0.05 M SDS – 7% butanol, buffered at pH 3 with 0.01 phosphate salt, running under isocratic conditions at 1 mL/min. Injection volume and absorbance detection wavelength were 20-μL and 240 nm, respectively. The temperature was not controlled. The solutions to-be-injected were filtered by pushing through a 0.45-μm Nylon membrane filter (Micron Separations) using a 3-mL syringe and then introduced into the chromatographic vial, which were placed in the autosampler tray. The experimental dead time was 0.9 min. Retention factor (k), efficiency (number of theoretical plates, N) and tailing factor (T) were calculated as in [10].

2.4. Sample preparation

Pharmaceutical formulations containing one of the studied statins as active principle ingredient (API) were randomly bought from local suppliers.

Table 2
Effect of the composition of the mobile phase on retention.

Mobile phase		Retention factor		
SDS	1-butanol	Rosuvastatin	Lovastatin	Simvastatin
0.05 M	1%	8.9	46.7	58.8
0.05 M	7%	5.9	22.3	28.9
0.10 M	4%	4.1	15.2	18.6
0.15 M	1%	3.8	19.1	24.2
0.15 M	7%	2.8	8.6	10.0
SDS	1-pentanol		Retention factor	
0.05 M	2%	5.8	30.2	38.3
0.05 M	6%	3.9	15.8	19.4
0.10 M	4%	2.7	12.6	15.6
0.15 M	2%	2.5	14.5	18.5
0.15 M	6%	1.8	6.8	8.1

Five tablets or capsules from the same package were crushed into fine powder using an Agatha mortar and homogenized. The proper mass was introduced in a beaker and few milliliters of methanol were added, and the mixture was hand shaken. Afterwards, it was diluted in mobile phase up to 40 mL, shaken using a magnetic stirrer for 15 min and then ultrasonicated 15 min to achieve solubilization. This solution was transferred to a volumetric flask, and then filled up to 50 mL. Finally, an aliquot was diluted in mobile phase to get a target concentration of 5 mg/L of the API.

Analyzed solutions were discarded, while tablets, capsules and the obtained powder were kept at −4 °C.

3. Results and discussion

3.1. Optimization of the chromatographic conditions

The use of a C_{18} column with a hybrid micellar mobile phase of SDS, running under isocratic mode at 1 mL/min, has been proven a valuable alternative to determine drugs in a wide variety of matrices, including pharmaceutical preparations [26,29]. In the frame of the current work, we optimize the composition of the mobile phase and the detection conditions.

3.1.1. Effect of the mobile phase composition on retention

The pH selection was considered in the working range of the column (2.5 – 7.5). Lovastatin and simvastatin would be barely affected by this parameter, as they are neutral in the entire interval. However, rosuvastatin is an acidic compound with a low pKa (4.0), and then exhibits a global negative charge, which decreases at higher pHs (Table 1), thus being repelled by the stationary phase and the micelles. Besides, it is moderately polar, which would result in poor retention. Consequently, the pH was set to 3 as the charge of this statin is nearly neutral, in order to maximize the retention of this compound.

The addition of an organic solvent was envisaged to increase elution and improve peak shape. 1-propanol was discarded, considering the expected retention of simvastatin and lovastatin, due to their high hydrophobicity. Therefore, 1-pentanol and 1-butanol were tested. Standard solutions of the three statins were analyzed using mobile phases containing SDS/1-butanol and SDS/1-pentanol selected using a full factorial design plus the central point ($n = 5$), taking as levels the minimum and the maximum concentration recommended in HMLC for these solvents [31,34]. Results can be seen in Table 2.

Globally, we observe the elution order remains the same, whatever the mobile phase. In all cases, retention factor diminishes at increasing values of SDS, pointing to a bending behavior between the statins and the micelles, probably by hydrophobic interaction. Globally, the use of 1-pentanol leads to a lower retention time and peak bandwidth than 1-butanol, as expected due to

Table 3
Equation adjustment of retention factor modelization for the three analytes.

SDS/1-butanol	Rosuvastatin	Lovastatin	Simvastatin
c_0±SD (p)	0.229±0.008 (0.02)	0.060±0.003 (0.03)	0.0498±0.0019 (0.02)
c_1±SD (p)	0.083±0.009 (0.07)	0.026±0.003 (0.08)	0.022±0.002 (0.06)
c_2±SD (p)	0.038±0.009 (0.14)	0.022±0.003 (0.08)	0.020±0.002 (0.07)
c_{12}±SD (p)	0.009±0.009 (0.48)	0.010±0.003 (0.19)	0.009±0.002 (0.14)
c_1/c_0	0.36	0.43	0.44
c_2/c_0	0.17	0.37	0.40
c_{12}/c_0	0.04	0.17	0.18
R^2	0.991	0.992	0.995
| error |	<6.5%	<9.3%	<7.9%
SDS/1-pentanol			
c_0±SD (p)	0.351±0.010 (0.02)	0.0784±0.0005 (0.004)	0.06385±0.00012 (0.001)
c_1±SD (p)	0.132±0.011 (0.05)	0.0299±0.0006 (0.01)	0.02496± 0.00014 (0.004)
c_2±SD (p)	0.060±0.011 (0.11)	0.0271±0.0006 (0.01)	0.02371±0.00014 (0.004)
c_{12}±SD (p)	0.018±0.011 (0.35)	0.0120±0.0006 (0.03)	0.01099±0.00014 (0.008)
c_1/c_0	0.38	0.38	0.39
c_2/c_0	0.17	0.35	0.37
c_{12}/c_0	0.05	0.15	0.17
R^2	0.994	0.9998	0.99998
| error |	<5.6%	<1.3%	<0.4%

SD: standard deviation; R^2: multiple determination coefficient:
error = $(k_{calculated} - k_{experimental})/k_{experimental}$ *100.

its higher hydrophobicity. Otherwise, the elution power of the mobile phases was higher at increasing proportions of organic solvent. Rosuvastatin was eluted far before the other two statins because it is significantly more polar and has a slight negative charge. Simvastatin and lovastatin exhibited a similar retention time, as their structures are rather similar (only an additional methyl- group for simvastatin) and have nearly the same hydrophobicity, and then would partially overlap in case mobile phases with a strong elution power were used or their concentration was too high. Therefore, the main challenge was to maximize the separation between these two statins.

3.1.2. Modeling of the retention factor

The retention factor, as a response, was modelled depending on SDS and alcohol proportion (φ), as independent variables for each statin, using Eq. (1), which has been proven accurate for moderately hydrophobic drugs, when using a hybrid mobile phase running under isocratic mode through a C_{18} column saturated with SDS [29].

$$1/k = c_0 + c_1[\text{SDS}] + c_2\,[\text{organic modifier}] + c_{12}\,[\text{SDS}]\,[\text{organic modifier}] \quad (1)$$

The constant c_0 indicates the elution strength (inverse of retention) at intermediate values while c_1, c_2 and c_{12} represents the effect of each factor and their interaction, respectively. The higher these constants, the stronger the power displayed by the mobile phase. The relative effect of SDS, alcohol and the interaction compared to the retention at central experimental conditions (c_x/c_0) can be used to compare their influence on retention factor between different solutes.

The equation was adjusted, one per statin and organic solvent, using the experimental data from Table 2 with concentration values normalized from −1 to +1, by curve-fitting non-linear least-square regression (1 freedom degree) [40]. Results can be seen in Table 3. What is more, fitted equations allow the determination of the retention factor at intermediate concentrations of SDS and alcohol without performing the chromatographic run. The six constructed models were enough accurate, as the multiple determination coefficient (R^2) were > 0.991 and the regression residuals were < 9.5%.

In all cases, the importance of each factor on the elution strength was SDS > alcohol > interaction, and the most significant constant was c_0, indicating that the concentration of surfactant and alcohol exhibited a limited influence on the elution strength of the mobile phase in the studied interval. For lovastatin and simvastatin, the relative influence of the two factors and that of the interaction were quite comparable, and rather different from that of rosuvastatin. Effect of SDS was similar for the three compounds, while the influence of the alcohol and the interaction was more significant for simvastatin and lovastatin. Indeed, as these ones are more hydrophobic and water-insoluble, their solubility in the bulk mobile phase benefits more from the addition of alcohol.

The shift from 1-butanol to 1-pentanol as organic modifier gave rise to an increase of the elution strength of the mobile phase (c_0), slightly more significant for rosuvastatin (x1.5) than for lovastatin and simvastatin (x1.3). Besides, we noticed a decrease on the relative influence of SDS concentration for these two statins.

As previously discussed, lovastatin and simvastatin exhibited a similar retention behavior, as there are equally affected the change of experimental conditions, due to the similarity between the structures and the physico-chemical properties. The most significant difference was in the elution strength at intermediate values (c_0).

3.1.3. Optimization of the composition of the mobile phase

The optimal conditions were selected by studying the chromatographic responses obtained from the assays described in Section 3.1.1.

The organic solvent 1-pentanol was discarded as organic solvent because it provokes low retention times for rosuvastatin; thus, increasing the risk of its overlapping with excipients and/or impurities, which usually are eluted near the dead time, when analyzing a pharmaceutical formulation.. Therefore, 1-butanol was selected, which provides useful retention times for rosuvastatin. Low proportions of 1-butanol (1 - 4%) provided excessively broad peaks for simvastatin and lovastatin, indicating an excessively slow transfer of these solutes from the mobile to the stationary phase. Indeed, these are nearly water solutions, and their polarity and viscosity hinder the mobility of such water-insoluble compounds. This behavior was not observed for rosuvastatin, due its moderate polarity. Therefore, the proportion of 1-butanol was set to 7%, where their peak width was adequate.

In mobile phases containing SDS 0.1 M and 0.15 M, a noticeable overlapping between simvastatin and lovastatin was observed. Therefore, the SDS concentration of SDS was set to 0.05 M in or-

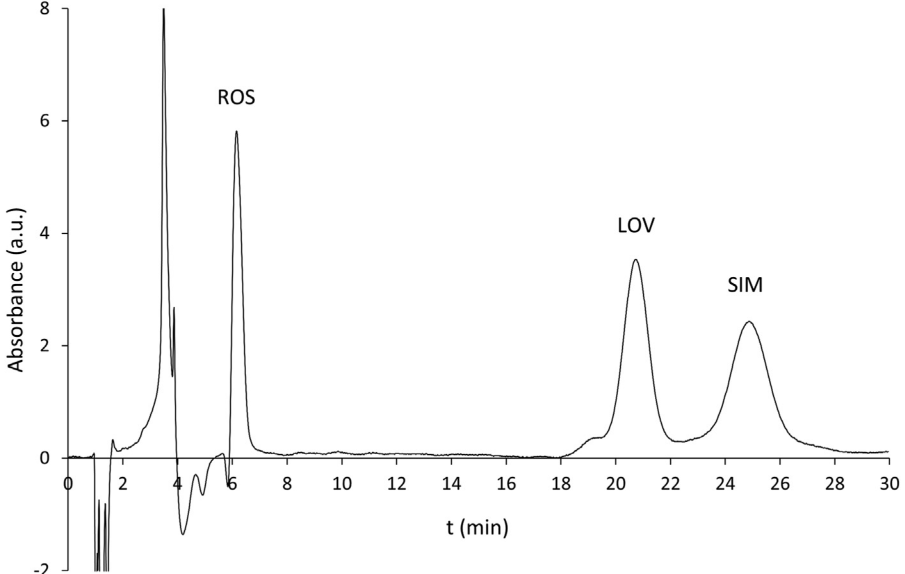

Fig. 1. Chromatogram obtained by the analysis of a mixture of rosuvastatin, lovastatin and simvastatin 5 mg/L under the optimal conditions.

der to minimize the elution strength of the mobile phase and increase the resolution between the compounds. Under these conditions, both peaks were fully resolved, although the end tail of lovastatin is very close to the beginning of the front of simvastatin.

3.1.4. Optimization of the detection conditions

The spectroscopic properties of the solutes change when solved in a micellar environment. Therefore, the UV absorption spectra of the three compounds were in-time registered at their respective retention time during chromatographic run using the optimized experimental conditions. The spectra at 50%- and 5%- fronting and tailing edge were also recorded for peak purity studies [10]. The three statins exhibited an absorption band between 200 – 260 nm, with the maximum near 240 nm, and the detection wavelength was set to this value. Spectra of simvastatin and lovastatin were nearly indistinguishable, due to their structural similarity, and rather different to that of rosuvastatin.

3.1.5. System suitability testing

A system suitability testing was performed by the analysis of a mixture containing 5 mg/L of each statin solved in mobile phase (six replicate injections), under the previously optimized conditions (0.05 M SDS – 7% 1-butanol buffered at pH 3, and detection wavelength 240 nm) to appraise the consistency of the results and ensure the proper working of the instrumentation. The obtained chromatogram is shown in Fig. 1.

The results were (rosuvastatin; lovastatin, simvastatin; acceptance criteria [10]): retention time (6.19±0.03 min; 21.0 ± 0.2 min; 25.1 ± 0.3 min; —-), relative standard deviation of retention time (0.7%; 1.0%; 1.0%; ≤ 1.0%), relative standard deviation of peak area (0.4; 0.5; 1.0; <1.0), capacity factor (5.9; 22.3; 28.9; >2.0), efficiency (2013; 2055; 2031; >2000 number of theoretical plates) and tailing factor (1.2; 1.0; 1.0; 0.8–1.6). The resolution between lovastatin and simvastatin was 1.5, the minimum required for a complete separation.

The obtained parameters indicated the experimental conditions and instrumentation are useful to achieve the determination of the statins. Indeed, the provided responses were quite reproducible and the chromatographic parameters points to an adequate elution process. The chromatogram obtained by the analysis of a mixture containing 5 mg/L of each statin is shown in Fig. 1.

3.2. Method validation

A critical step in method development is called validation. It is carried out to verify the reliability of the qualitative and quantitative results and evaluate the range and conditions of applicability of the procedure. This was performed following the guidelines of the ICH Validation of Analytical Procedures: Text and Methodology Q2(R1) [39] (specially developed to determine active principle ingredients in pharmaceutical preparations) and Methods, Method Verification and Validation, Food and Drug Administration – Office of Regulatory Affairs (FDA-ORA) Laboratory Manual Volume II [41], assisted by other documents about validation [42]. Unless specified, the assays were performed in standard solutions.

3.2.1. Calibration range and linearity

Standard solutions containing up to 20 mg/L of each statin were analyzed by sextuplicate. Peaks corresponding to simvastatin and lovastatin overlapped at concentrations over 10 mg/L, then the linearity was limited to this value for these two solutes. The homoscedasticity was verified by comparing the minimum and maximum variance of peak area by a F-two-tailed-test (α = 5%). Average peak area was plotted *vs.* concentration by least square regession method (6 levels) to calculate the slope, y-intercept, determination coefficient and to set the linear interval of the calibration curve. Limit of detection and limit of quantification were the smallest concentration that could be distinguished from the baseline noise and quantified with enough reliability, respectively, and were calculated as 3.3 and 10 times, respectively, the standard deviation of the blank (taken as the standard deviation of the y-intercept) and the sensitivity (slope of the calibration curve). Results can be seen in Table 1.

An adequate linearity was obtained, as r^2 were > 0.995, the residuals were randomly distributed around 0. The y-intercept accounts for less than 5% of the signal at the target concentration, and its confidence interval covers 0, thus pointing out the absence of systematic error. Calibration range cover the 80–120% of the target concentration (5 mg/L), and the LOQ was enough under this value.

3.2.2. Trueness and precision

These parameters were determined at x0.8, x1 and x1.2 of the target concentration (5 mg/L). Six standard solutions containing

Table 4
Trueness and precision for the studied drugs.

Statin	Nominal concentration (mg/L)	Found concentration (mg/L)	Trueness[a] (%)	Repeatability[a] (RSD,%)	Intermediate precision[b] (RSD,%)
	4	4.01±0.03	100.2	0.7	1.3
Rosuvastatin	5	4.96±0.04	99.1	0.8	1.1
	6	6.08±0.08	101.3	1.3	1.8
	4	4.02±0.02	100.5	0.6	1.4
Lovastatin	5	4.940±0.020	98.8	0.4	1.2
	6	6.09±0.03	101.5	0.5	1.0
	4	4.07±0.05	101.7	1.2	2.3
Simvasatin	5	4.96±0.05	99.2	1.1	2.5
	6	5.93±0.08	98.9	1.4	2.7

[a] $n = 6$.
[b] $n = 5$.

the adequate concentration of the three statins, different from those used in the calibration study, were consecutively analyzed. Trueness, calculated as relative recovery, was the quotient between the average found concentration and the true value, while repeatability was the relative standard deviation of the six values. To evaluate the effect of time in the dispersion of the results, the same approach was repeated, five different days on a three-month period, by renewing the solutions. Intermediate precision was the relative standard deviation of these found concentrations. Results can be seen in Table 4.

Both relative recovery (98.8 – 101.7%) and dispersion (<2.7%) were inside the acceptance criteria (trueness, 97–103%; repeatability <2.0; intermediate precision, <3.0% [41], thus indicating the quantitative data provided by the method are reliable. This was achieved by the solubilization power of micellar solutions for small organic molecules, and the simplicity of sample treatment. Therefore, the concentration taken from one measurement can be considered as trustworthy, and then replicate analysis can be avoided. This represents a considerable saving of time and resources in routine practice.

3.2.3. Carry-over effect

A solution containing 20 mg/L of each drug (more than has been fixed as maximum limit of quantification) and a blank were analyzed in successive injections. This high concentration was selected to ensure that, if no cross contamination was detected at these values, we could consider that there would not be at the working concentrations in common practice. Neither peaks nor baseline oscillation were visualized at the window time of the analytes, thus pointing out the absence of cross contamination.

3.2.4. Robustness

The influence of the deviation of the experimental conditions from those optimal on the chromatographic responses (peak area and retention time) was evaluated. A standard solution containing 5 mg/L of each drug was analyzed at the optimal, and the minimal and maximal value of the expected interval of oscillation that can usually occur during the laboratory practice, for the evaluated experimental parameter, while keeping the other ones at their optimal value. The examined parameters (tested interval) were: SDS concentration (0.045–0.055 M), 1-butanol proportion (6.8–7.2%), pH (2.8–3.2), flow rate (0.98–1.02 mL/min), injection volume (18–22 μL) and detection wavelength (235–245 nm).

RSD% between the three values was <5% in all cases, and then the method can be considered enough robust to be unaffected by small variation of the main experimental conditions.

3.2.5. Specificity

The presence of interfering compounds was evaluated by the analysis of oral solid dosage forms containing one of the analytes as active principle ingredient, as no blank were available. Neither peaks close to the window time of the analytes nor overlapping peaks were noticed by visual observation of the analytes.

A peak-purity study was carried out, by taking the absorption spectra at the retention time and at 50% and 5% of the leading and tailing edge, and visually comparing each one with those obtained from the analysis of the standard solution containing the three statins at the same points (3.1.4) by overlaying the spectra [10,11,43]. No significant difference was noticed, thus indicating the absence of coeluting interfering compounds.

As a result, the three statins can be reliably identified in pharmaceutical samples from the peaks detected at their retention time, and the entire peak can be assigned to the corresponding analyte for quantification purposes.

3.2.6. Stability

The possible decay of the statins through time in a processed sample (short-term bench-top stability) and its effect on the reliability of the results were explored. For each statin, the corresponding oral solid dosage (containing 20 mg of active component) form was processed. The obtained solution was placed in the autosampler tray and analyzed each 2 h for 2 days. Neither significant diminishing of the peak area (<3%) nor peak from degradation product were noticed, and then the analytes remain unaltered during this period. This allows the processing of all the samples to-be-analyzed, followed by their chromatographic injection in the same sequence run, with a maximum duration of 2 days.

3.3. Analysis of pharmaceutical preparations

The method was applied to quantify the studied statins in several pharmaceutical preparations containing them as a unique active component, marketed as oral solid dosage, in order to verify its applicability in a routine pharmaceutical analysis: Rosuvastatina Cinfa 5, 10 and 20 mg (Laboratorios Cinfa, Pamplona, Spain) for rosuvastatin, Colesvir 20 and 40 mg for lovastatin (Industria Quimica Y Farmaceutica Vir, S.A., Madrid, Spain) and Simvastatina Normon 10, 20 and 40 mg for simvastatin (Laboratorios Normon, Madrid, Spain).

Firstly, all samples were processed and then the extracts were placed in the autosampler tray. Several blanks and QC samples of 5 mg/L were added between the samples to estimate drift measurement. All these solutions were injected in the same sequence, and the data treated the day after. The quantitative data were consistent (QC relative recovery were 97–103%) and the label claim values were inside the acceptance criteria (95 – 105%). The statins were detected without overlapping. Representative chromatograms can be seen in Fig. 2.

The method allows the analysis of a maximum of 40 samples in a single workday and requires generic and widely available reagents and instrumentation. Only one solution has to be

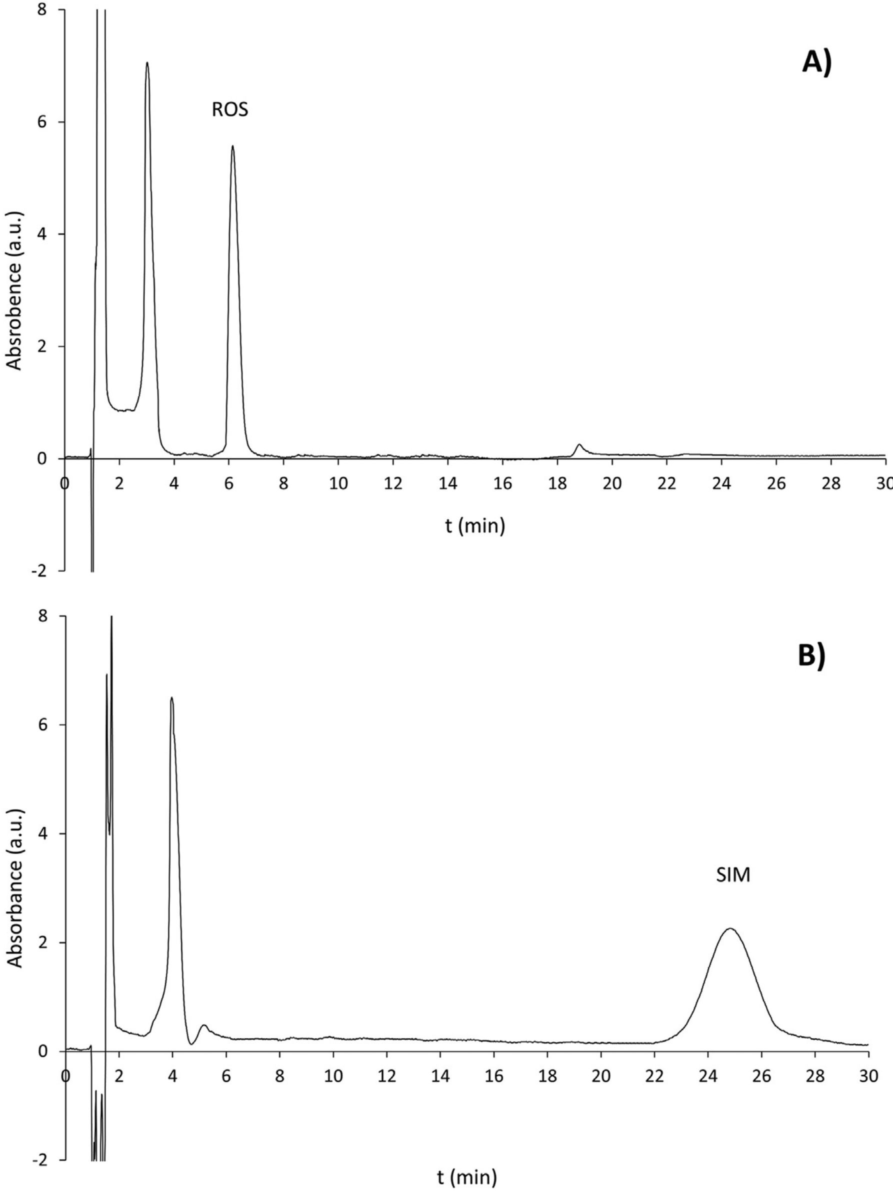

Fig. 2. Chromatograms obtained from the analysis of A) Rosuvastatina Cinfa 20 mg (label claim 99.5%) and Simvastatina Normon 20 mg (label claim 101.8%).

prepared, for the extracting solution and mobile phase exhibited the same composition, what expedites the experimental work. This one mainly contains innocuous and biodegradable reagents, and only a low amount of toxic, volatile and flammable solvent (<7.0%). In hydroorganic RP-HPLC, a pure organic solvent is usually employed in sample pretreatment, to solve the active component, and the organic modifier in the mobile phase are acetonitrile or methanol, more hazardous than 1-butanol, and at higher proportions (up to 100%), and thus the current proposal is more ecofriendly and safer. Otherwise, it requires generic and available chemicals, laboratory material and instrumentation.

Sample treatment was performed using an approach allowing the simultaneous processing of many samples, with a minimal participation of the operator. Regarding the chromatographic analysis, the use of the same mobile phase for the three studied drugs, even though they are rarely prescribed together, and the chromatographic run becomes excessively long, enables the analysis of oral solid dosages containing different statin as active component the same workday. In addition, working under an isocratic mode eliminates the need of equilibration time between successive injections, thus providing a more stable baseline and chromatographic response, and shortening and facilitating the analysis of a large set of samples.

4. Conclusions

Hybrid micellar liquid chromatography has been demonstrated as a valuable tool for the determination of rosuvastatin, lovastatin and simvastatin in oral solid dosage forms. The main feature is the use of barely hazardous solutions, in both sample preparation and chromatographic analysis. Indeed, the incorporation of the surfactant in the aqueous environment increases the solubility of the statins, without having to resort to high proportions of organic solvents. Besides, formulations separately containing these three statins can be analyzed using the same experimental conditions. The method was successfully validated by the guidelines of the International Council of Harmonization, thus proven its reliability. It displays interesting practical advantages, as an acceptable sample throughput and is cost-effective, easy-to-conduct and globally

sustainable, and then useful for routine analysis in pharmaceutical quality control.

Otherwise, the analytical challenge of resolving three compounds, two very similar and the third one rather different, has been overcome from the experimental data taken from only five assays per alcohol. These same results were used to construct a chemometric model to quantify and explain the effect of the main component of the mobile phase, SDS and alcohol, on statin retention on the basis of their physico-chemical properties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Lidia García-López: Validation, Formal analysis, Investigation, Data curation, Writing – original draft. **Juan Peris-Vicente:** Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Devasish Bose:** Software, Project administration, Funding acquisition. **Abhilasha Durgbanshi:** Conceptualization, Software, Resources, Writing – review & editing, Supervision. **Samuel Carda-Broch:** Methodology, Investigation, Resources, Visualization, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

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