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Assessment of the quality of simvastatin capsules from compounding pharmacies

ABSTRACT

OBJECTIVE: To validate a method for determining the simvastatin content of compounded capsules, using high performance liquid chromatography.

METHODS: Eighteen samples of simvastatin 40 mg capsules from compounding pharmacies in the cities of São Paulo, Guarulhos, São Bernardo do Campo and Campinas, Southeastern Brazil, prescribed for fictitious patients were assessed. The analyses were based on the Brazilian Pharmacopoeia and on the high performance liquid chromatography method, optimized and validated in accordance with national and international standards for identification and quantification tests on compounded capsules.

RESULTS: The mean weight of the capsules ranged from 70 mg to 316 mg; four samples presented weight variation outside of the specification. The simvastatin content in the capsules was within the specification in 11 samples. In six, the content ranged from 4% to 87% of the declared quantity, thereby not complying with the content requirements for the active agent. For one sample, no content or uniformity determinations were performed. In the content uniformity test, 15 samples presented indices of less than 85%, with relative standard deviations greater than 6%. Three pharmacies had met the specification in this test. In the dissolution test, eight samples presented unsatisfactory results in the first stage of the test, while the remainder presented inconclusive results.

CONCLUSIONS: The method used was shown to be suitable for application to quality control, and it revealed the poor quality of the simvastatin capsules produced by some compounding pharmacies.

DESCRIPTORS: Simvastatin, standards. Capsules, chemistry. Drug Compounding. Chemistry, Pharmaceutical. Drug Quality.

INTRODUCTION

Atherosclerotic disease is the main underlying cause of mortality in Brazil.[11] It is a multifactorial disease and its prevention consists of identifying and controlling occurrences of hyperlipidemia, using antilipemics (including statins), along with risk factors resulting from inappropriate diet and lifestyle and sedentarism.[11]

Hydroxymethyl-glutaryl-coenzyme A (HMG-CoA) reductase is an enzyme that regulates the speed of cholesterol synthesis in the liver. Statins are known to inhibit this enzyme and are effective in reducing triglyceride and low-density lipoprotein (LDL) levels and increasing high-density lipoprotein (HDL) levels, with different lipid-lowering potentials.[22] Statins such as simvastatin present other types of pharmacological activity: anti-inflammatory action,[10] through improvement of endothelial function;[9] and antioxidant action,[18]

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through reduction of platelet adhesion and thrombus formation,[19] among other activity. Among the most serious adverse effects is the development of myopathy and rhabdomyolysis or muscle necrosis.[14]

Simvastatin is considered to be a prodrug, because after absorption it undergoes enzymatic hydrolysis of the lactonic ring to form the active metabolite simvastatin β-hydroxy acid, which acts as a potent competitive and reversible inhibitor of HMG-CoA reductase.[21]

Simvastatin was synthesized from the lovastatin molecule, which is produced through fermentation in yeast cultures of *Aspergillus terreus*.[4,6] It undergoes an oxidation process when exposed to air and/or high temperatures, and it should be conserved in well-closed receptacles at temperatures between 15°C and 30°C.[20]

The expiry of the patent on simvastatin has reduced the cost of this drug, thus giving rise to greater prescribed use for treating atherosclerosis, especially in the form of capsules produced by compounding pharmacies. Between 1998 and 2002, the number of compounding pharmacies in Brazil increased from 2,100 to 5,200.[a] The number of formulations prescribed cannot be calculated, given that they do not require registration with the health surveillance authority.[b]

The possibility that compounding pharmacies might offer medications at prices that are lower than those of industrially manufactured products has certainly contributed towards this expansion, in situations in which they had a competitive advantage over the industry in certain segments of the market.[b]

On the other hand, there are reports of complaints forwarded to the Antibiotics Section of the Instituto Adolfo Lutz, by the health surveillance services, consumer protection bodies or private users. These complaints include suspicions of quality deviations in compounded medications and reports of adverse reactions, intoxication and/or therapeutic inefficacy, confirmed through the results from analyses. Such situations consist of overdoses or underdoses, heterogeneous distribution of the active agent and/or failure of the drug to release in various therapeutic classes that are compounded.[16]

There are several methods based on different analytical techniques for identifying and quantifying simvastatin in industrially manufactured pharmaceutical formulations and in biological fluids. Among these is spectrophotometry in the ultraviolet region with determination of first-derivative signals,[7] high-performance liquid chromatography (HPLC) with detection using mass spectrometry (HPLC/MS)[23] and HPLC coupled with an ultraviolet-visible detector (UV-VIS).[2,3,17]

Evaluation of compounded simvastatin capsules is not envisaged using the analytical methods in official compendiums. Thus, the present study aimed to validate a new method for evaluating the quality of simvastatin capsules produced in compounding pharmacies.

METHODS

To test the hypothesis that there was a correlation between the quality of simvastatin capsules and the socioeconomic conditions of the region, 60 capsules of 40 mg were assessed from 18 pharmacies, dispensed for fictitious patients, in 2007. These pharmacies were coded as letters according to the region: Campinas (A, B, C); São Paulo (northern zone: D; eastern zone: H, I, J; western zone: K, L; southern zone: M, N); Guarulhos (E, F, G); São Bernardo do Campo (O, P, Q, R).

The simvastatin and lovastatin standards used came from the United States Pharmacopeia[20] and the placebo consisted of magnesium stearate 0.5%, colloidal silicon dioxide 1%, sodium lauryl sulfate 1%, pharmaceutical talc 30% and sufficient maize starch to make up to 100% (Baldacci Laboratory, São Paulo, Brazil).

The evaluation on the appearance of the capsules (color and content) was done visually.

The mean content and weight variation of the capsules were measured using an analytical balance (Precisa, model 205 A SCS), and this was done on 20 capsules by pharmacy, in accordance with the procedures in the Brazilian Pharmacopoeia.[8]

To optimize the method, a high-performance liquid chromatograph (model CLASS-VP 10) was used, with detection at 238 nm (SPD-10AV ultraviolet-visible detector) at a temperature of 25°C, using an oven (model CTO-10 AC-VP), flow of 1.5 ml/min (LC-10AV-VP pump) in an isocratic system, and a Rheodyne 7725 injector with manual injection and loops of 20 µl. The chromatograms were processed using the SCL-10 AVP control system. All the modules were made by Shimadzu. A Chromolith RP-18 monolithic chromatographic column measuring 100 mm x 4.6 mm (Merck®) was used. The mobile phase consisted of 27 mM dibasic sodium phosphate buffer with pH adjusted to 3.0 by means of phosphoric acid, using a potentiometer (Denver, model 15) and acetonitrile (for HPLC) in the proportions 35:65 v/v. This was prepared on the day of use, and vacuum-filtered

[a] Agência Nacional de Vigilância Sanitária. Resolução n° 899, de 29 de maio de 2003. Guia para validação de métodos qualitativos e bioanalíticos. *Diario Oficial Uniao*. 02 Jun 2003[cited 2003, Sep 23];Seção 1:18221. Available from: http://www.anvisa.gov.br/legis/resol/2003/re/899_03re.htm

[b] Paumgartten FJ. Papel das farmácias magistrais deve ser complementar. *Bol Inf ANVISA*. 2005;56:4-5.

through a regenerated cellulose membrane of 0.45 µm (Sartorius®). The diluent used in preparing the samples and standard was the mobile phase.

Validation was performed in accordance with the guide for validation of qualitative and bioanalytical methods[20,a] and the pharmaceutical industry's guide from the International Conference on Harmonization.[12] The parameters determined were the selectivity, detection and quantification limits, accuracy, intermediate precision and analytical curves for simvastatin and lovastatin.

The selectivity of the method for determining the simvastatin content was evaluated from the diluent and mobile phase injections, placebo solution, placebo with simvastatin standard added, simvastatin standard and simvastatin capsule sample, in order to observe possible interference with the analyte retention time.

The signal/noise was determined using a placebo solution. The detection and quantification limits for simvastatin and lovastatin were established from the concentrations for which the area was three and ten times the signal/noise area, respectively.

The accuracy was calculated as the percentage recovery of the known quantity of analyte that had been added to the placebo, or as the percentage difference between the means and the accepted true value. Anvisa recommends verification from at least nine determinations, taking into consideration the linear interval of the procedure, i.e. three concentrations (low, medium and high), each determined three times. The recovery accuracy is expressed as the ratio between the mean concentration determined experimentally and the corresponding theoretical concentration. In the present study, the accuracy was determined as the recovery performed in triplicate on independent samples (n = 9) of placebo added to the simvastatin standard in solution at the concentrations of 49.85, 99.70 and 149.55 µg/ml. Each sample of the triplicate was injected three times.

The analytical curve for simvastatin was established through preparing a stock solution: 100.3 mg of simvastatin standard of potency 99.4% dissolved in diluent in a volumetric flask of 200 ml. Aliquots were measured and diluted in appropriate volumetric flasks in order to obtain concentrations of 49.85, 74.77, 99.7, 124.62 and 149.55 µg/ml. The solutions were filtered and injected in quadruplicate.

The analytical curved for lovastatin was established through preparing a stock solution starting with 25 mg of lovastatin standard of potency 100%, dissolved in diluent in a volumetric flask of 100 ml. Aliquots were measured and diluted in appropriate volumetric flasks in order to obtain concentrations of 0.50, 0.75, 1.00, 1.25 and 1.50 µg/ml. The solutions were filtered and injected in quadruplicate.

The precision was determined as the repeatability, using a standard solution of simvastatin prepared at a concentration of 100.0 µg/ml. This was filtered through a membrane of 0.45 µm and injected nine times. The relative standard deviation (RSD%) of these results was calculated.

The intermediate precision was determined using the same solution, which was conserved in a refrigerator, filtered and injected nine times on two consecutive days (n = 18).

The system suitability test was evaluated using a standard solution of simvastatin at a concentration of 99.7 µg/ml, injected six times. The parameters determined were the resolution, asymmetry, number of theoretical plates, capacity factor and retention time.

To determine the content uniformity, standard solutions of simvastatin and lovastatin were prepared. To prepare the standard solution of lovastatin, 25 mg was dissolved in the diluent in a volumetric flask of 500 ml. For the standard solution of simvastatin, 25.2 mg of simvastatin was dissolved in a volumetric flask of 250 ml. Before making up the volume, an aliquot of 5 ml of the lovastatin standard solution was added. The mixture of standard solutions of simvastatin and lovastatin resulted in concentrations of 100.19 µg/ml and 1.0 µg/ml, respectively.

The samples were prepared from ten capsules, as described in the Brazilian Pharmacopoeia.[8] They were placed individually in volumetric flasks of 200 ml and 100 ml of diluent was added. The solutions were stirred mechanically for 15 minutes, subjected to an ultrasound bath (made by Unique) for 20 minutes, cooled and made up to the volumes indicated. Then 5.0 ml was taken from each solution and diluted in a volumetric flask of 10 ml.

The solutions of the samples and the mixture of simvastatin and lovastatin standards were filtered and 20 µl was injected twice into the chromatographic system.

The simvastatin concentration was determined by calculating the mean from ten content uniformity results on each sample.

Determination of lovastatin as a contaminant was performed by means of a lovastatin limit test, based on the areas of the lovastatin peaks obtained from the injections in the content uniformity tests on the capsules. The percentage lovastatin was determined in relation to the quantity of simvastatin in each sample, and this should not exceed 1%, according to the reference in the United States Pharmacopoeia.[20]

The dissolution test determined the percentage of the active agent released into the dissolution medium, in relation to the value declared on the product label,

within the period specified on the monograph. In the first stage, each capsule was expected to release not less than 80% simvastatin (Q = 75% + 5%).[20]

The chemical adequacy test on the dissolver (Erweka, model DT 800) was performed before carrying out the dissolution test, using calibrating tablets of prednisone and acetylsalicylic acid from the same pharmacopoeia.[20] The dissolution test on simvastatin tablets used apparatus 2 (paddles), rotation of 50 rpm, temperature of 37°C, duration of 30 minutes and a sinker to avoid capsule floatation. The dissolution medium was prepared using 30 g of sodium dodecyl sulfate (Merck®) and 8.28 g of monobasic sodium phosphate (Merck®), dissolved in 6,000 ml of water, with adjustment to pH 7 using 50% sodium hydroxide solution.

Six capsules of each sample and one empty capsule (to determine any interference and taken to be a blank) were place individually in each vessel containing 900 ml of the dissolution medium.

The standard solution for reading the absorbance in the spectrophotometer (Pharmaspec UV-Vis model UV-1700, Shimadzu) was prepared from a stock solution of the simvastatin standard at a concentration of 450 µg/ml, diluted to obtain 9 µg/ml, and a sample solution adjusted to the same concentration, using the dissolution medium as the diluent. The quantity of simvastatin released in the dissolution test was determined as the difference between the absorbance readings at 247 and 257 nm, respectively, for the sample and standard. The absorbance of the blank solution was used to correct the readings on the standard and sample.

For the method to be considered valid, the values found for the parameters of selectivity, linearity, detection limits, quantification, accuracy and intermediate precision in the validation process needed to comply with the values established in the standards.[12,20,a]

RESULTS

The gelatin capsules were hard, either with two or one color, and contained homogeneous white powder.

The mean content of the capsules ranged from 70 mg to 316.73 mg, for the same prescription of 40 mg of simvastatin (Table 1). Table 1 also presents the values for the weight variation and the numbers of capsules that were outside of the reference values. The four samples from the pharmacies A, B, D and G presented more than two capsules at the upper or lower limits of variation, thus indicating lack of homogeneity among these capsules. In addition, insufficient numbers of capsules in these samples made it impossible to proceed with the second or third-stage determinations that the reference required.

Table 1. Mean content of the capsules of simvastatin 40 mg, weight variation and number of capsules outside of the specifications, produced in compounding pharmacies. São Paulo, Guarulhos, São Bernardo do Campo and Campinas, Southeastern Brazil, 2007.

Pharmacy	Medan weight (mg)	Weight variation (%)		Number of capsules outside of the specifications
		Maximum	Minimum	
A	114	11	12	3
B	11	12	18	3
C	116	9	10	2
D	70	17	13	4
E	142	8	8	0
F	265	5	8	0
G	129	5	10	3
H	113	4	6	0
I	115	4	6	0
J	130	7	11	1
K	317	8	7	0
L	153	4	6	0
M	242	3	5	0
N	88	5	9	0
O	172	9	10	0
P	133	4	7	0
Q	165	3	3	0
R	195	3	3	0

The chromatographic conditions resulting from the optimization were a monolithic column of Cromolith RP 18 measuring 100 mm x 4.6 mm, a mobile phase consisting of 27 mM dibasic sodium phosphate buffer with pH 3 and acetonitrile 35:65 v/v, detection in UV at 238 nm, temperature of 25°C and flow of 1.5 ml/min.

The method optimized by means of HPLC-UV was validated and the results for simvastatin and lovastatin were respectively: detection limit 0.07 µg/ml and 0.03 µg/ml; quantification limit 0.41 µg/ml and 0.4 µg/ml; linearity 0.9997 and 0.9998. For simvastatin, the accuracy was between 101% and 103%, and the precision (n = 9) was calculated via RSD% as 0.37%.

The chromatographic parameters determined by applying the system suitability test to chromatograms on the simvastatin standard were: capacity factors, 2.21 and 3.1; asymmetry, 1.45 and 1.0; and efficiency (number of theoretical plates), 6,229 and 2,648, for simvastatin and lovastatin, respectively, with a resolution of 3.3. These values indicated that the performance of the optimized chromatographic system was good and compatible with the recommended parameters for chromatographic methods.

The content uniformity results on the simvastatin capsules are presented in Table 2.

Pharmacies A, B, C, D, E, G, H, L, N and Q presented more than one capsule with values outside of the range from 85% to 115% and RSD% greater than 6%. These test results were unsatisfactory, since only one capsule can be outside of this range. For pharmacies E and N, all the capsules were outside of the reference range. Pharmacies F, J, M and O should have been retested with more than 20 capsules, which was not possible because of insufficient sample size. Only pharmacies I, P and R presented content uniformity with values that complied with the reference. For pharmacy K, technical problems with the chromatograph while carrying out the test invalidated the results from this sample.

The capsules from all the pharmacies presented simvastatin content less than 100% of the declared amount. Pharmacies B, D, E, G, M and P presented values between 4% and 87% of the declared amount, thus indicating great fluctuation in the quantities of simvastatin (Figure). These results did not meet the requirements for simvastatin content, and the value of 4% characterized therapeutic underdosing.

The limit test for lovastatin as a contaminant of simvastatin presented results between 0.4% and 1% in all the capsules, thus meeting the requirements of this test.

In the dissolution test, if the gelatin capsules float, they partially dissolve and this may lead to crosslinking and avoid the release of simvastatin into the dissolution medium. Therefore, the test was carried out using sinkers so that the capsules would not float. The dissolution test results showed that the simvastatin capsules from pharmacies A, C, E, G, H, I, N and P failed at the first stage, while the samples from pharmacies B, D, F, J, K, L, M, O, Q and R presented inconclusive results from the first stage. The test could not be concluded because of insufficient sample size (Table 3).

Table 2. Content uniformity of the capsules of simvastatin 40 mg produced in compounding pharmacies. São Paulo, Guarulhos, São Bernardo do Campo and Campinas, Southeastern Brazil, 2007.

Pharmacy	Content uniformity		
	Maximum	Minimum	RSD%
A	105	84	8
B	94	67	9
C	94	84	4
D	95	73	7
E	5	4	10
F	105	84	7
G	93	75	7
H	97	75	8
I	98	90	2
J	111	83	8
L	99	79	7
M	107	86	7
N	85	75	5
O	100	80	7
P	99	90	3
Q	90	79	7
R	97	92	2

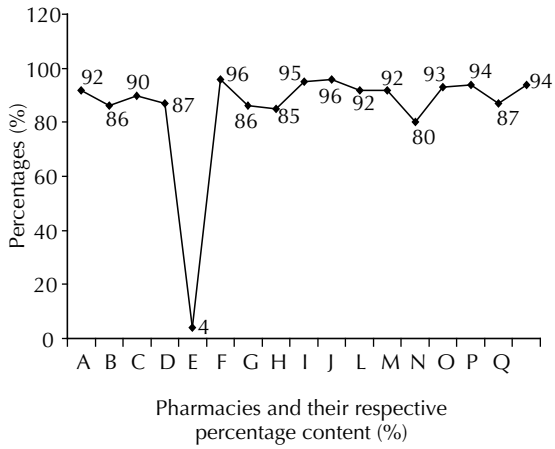

Figure. Percentage of simvastatin content found in relation to what was declared for the capsules produced in compounding pharmacies. São Paulo, Guarulhos, São Bernardo do Campo and Campinas, Southeastern Brazil, 2007.

Table 3. Dissolution test on the capsules of simvastatin 40 mg. São Paulo, Guarulhos, São Bernardo do Campo and Campinas, Southeastern Brazil, 2007.

Pharmacy	% released		Number of capsules outside of the specifications
	Maximum	Minimum	
A	59	40	6
B	84	72	6
C	70	28	6
D	74	60	6
E	8	2	4
F	95	74	6
G	86	49	5
H	69	43	6
I	77	43	6
J	87	65	3
K	81	65	5
L	81	55	4
M	95	75	1
N	63	49	6
O	78	60	6
P	75	47	6
Q	86	75	2
R	85	72	4

DISCUSSION

The results from method validation studies have shown sensitivity, specificity, accuracy, precision and linearity. The aim in optimizing the USP-31 chromatographic method[20] was to obtain a system in which the elution times were low, with good resolution, while maintaining the symmetry of the chromatographic peaks. To achieve this, the adjustments made included reducing the pH of the sodium phosphate buffer to 3.0, replacing the column of 30 cm in length packed with particles with a monolithic (en bloc) of 10 cm in length, and setting the temperature at 25°C. These modifications have resulted in a new chromatographic system with a reduction in elution time of around 60%. The time for lovastatin is 2.61 minutes and for simvastatin, 3.28 minutes, with excellent chromatographic efficiency according to the number of theoretical plates. This economizes on solvents and shortens analysis run times.

Using this validated optimized method, the 18 formulations evaluated presented quality deviations independent of the locations of the pharmacies where they were produced. This shows non-compliance with good compounding practices. There was no correlation with the socioeconomic conditions of the regions in which the pharmacies were located or the quality of the capsules produced.

The variation in the quantity of excipient used in the formulations analyzed may have interfered with the solubility of the drug and its pharmaceutical equivalence, thereby changing its performance.

The crystalline form of the simvastatin, the grain size of the ingredients and the type of excipient present in the formulation determine the solubility of the drug. The influence of these factors may lead to low quantities of drug dissolved and determined in the dissolution test, thereby altering the amount absorbed and therefore the pharmacological activity level.

Evaluation of the dosage uniformity determined by the content uniformity expresses the distribution of the drug in the unit dose.[8] This is one of the tests that best assesses the conditions of the compounding process.[1,c] The formulations studied presented heterogeneous distribution of the active agent in each unit dose in the same pack, thus confirming previous studies[5] on other formulations produced at compounding pharmacies.

The quality deviations encountered related to: lower quantities of active agent than what was declared on the label, therapeutic underdosing; encapsulation problems; and heterogeneous distribution of the drug in each unit dose in the same pack. These inadequacies reflected problems in the ingredient mixing process and excipient and drug grain size variation, which altered the pharmacotechnics of the formulations and affected the release of the active agent of the capsules in most of the dissolution test samples.[13,15]

The results suggest that the controls over the raw materials, compounding process and finished product quality were faulty or nonexistent in the pharmacies from which the formulations were dispensed. They also suggest that the pharmacies were not following the good practices for compounding medications for human use laid down in RDC Resolution No. 67 of October 8, 2007.[c] This Resolution also establishes that the drugs compounded should consist of individualized doses that are not provided by the pharmaceutical industry. Therefore, the pharmacies studies were infringing the current legislation through compounding simvastatin 40 mg formulations, given that the pharmaceutical industry provides these doses in the form of tablets.

Correct compounding of capsules of simvastatin 40 mg avoids health risks in treating dyslipidemia and supports regulatory health measures for establishing rational use of the medication. This makes it possible for the Pharmacovigilance Center of the State of São Paulo to monitor the quality, efficacy and safety of the use of medications while they are on the market, in order to protect users' health.

[c] Agência Nacional de Vigilância Sanitária. Resolução RDC n° 67, de 08 de outubro de 2008. Dispõe sobre boas práticas de manipulação de preparações magistrais e oficinas para uso humano em farmácias. Diario Oficial Uniao. 09 Oct 2008 [cited 2008, Jul 28];Seção1:29-58. Available from: http://www.e-legis.brs.br/leis

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