DEVELOPMENT OF A NEW LC METHOD FOR THE IDENTIFICATION, SEPARATION AND ASSAY OF SIMVASTATIN AND ITS IMPURITIES

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Abstract

The new method described below, RP-HPLC (reversed phase - high performance liquid chromatography) on a cyanopropyl column, is a convenient and efficient but less explored alternative method for the separation and quantification of simvastatin and its impurities. The critical parameters for the newly developed method were studied, the final purpose being suitability assesment for the analysis of pharmaceutical dosage forms containing simvastastin.

The optimum experimental conditions (Nucleosil column 100-5CN 125 x 4.6 mm i.d, with 5 µm particles, gradient elution with a mixture of water and acetonitrile; room temperature; 2.5 mL/min flow rate; UV detection-238 nm) for the separation and assay of simvastatin were established.

Rezumat

Metoda nouă descrisă mai jos, cromatografie de lichide de înaltă perfomanță cu faze inversate pe o coloană ciano-propil, este un procedeu alternativ convenabil şi eficient, mai puţin utilizat pentru separarea şi cuantificarea simvastatinei şi impurităților sale. Au fost studiaţi parametrii critici pentru noua metodă dezvoltată cu scopul final de a verifica adecvarea metodei la analiza formelor farmaceutice dozate care conțin simvastatină.

Au fost stabilite condiţiile experimentale optime pentru separarea şi dozarea simvastatinei (coloana Nucleosil 100-5CN 125 x 4,6 mm d.i., cu particule de 5 µm, eluție în gradient cu amestec de apă și acetonitril, la temperatura camerei, debitul fazei mobile 2,5 mL/min și detecție în UV la 238 nm).

Keywords: simvastatin, lovastatin, RP-HPLC, cyanopropyl stationary phase

Introduction

Cholesterol lowering statins like simvastatin (the main statin used in clinical treatment) are frequently prescribed for their effect in reducing morbidity and mortality related to coronary heart disease. Beside changes in the lipid profile, there were revealed a lot of other beneficial effects associated with the statin therapy regarding the state of the endothelial function or decreasing the inflammation, oxidative stress and thrombogenic response inhibition *etc.* [1].

Due to the special synthesis route for simvastatin [2,3], the European Pharmacopoeia monograph of simvastatin describes seven synthesis impurities (Fig. 1) and indicates a specific RP-HPLC method for assay and purity testing of simvastatin, using a C18 stationary phase a mobile phase consisting in different ratios of acetonitrile and 0.1% aqueous phosphoric acid solution, according to a complex gradient program [4]. Also, reversed-phase liquid chromatographic methods using C8 or C18 stationary phases and various mixtures of aqueous acetate and formate buffer solutions and acetonitrile or methanol as mobile phases are frequently cited in the literature, both for the assay, identification of simvastatin or for the purity assessing tests [1, 5-9].

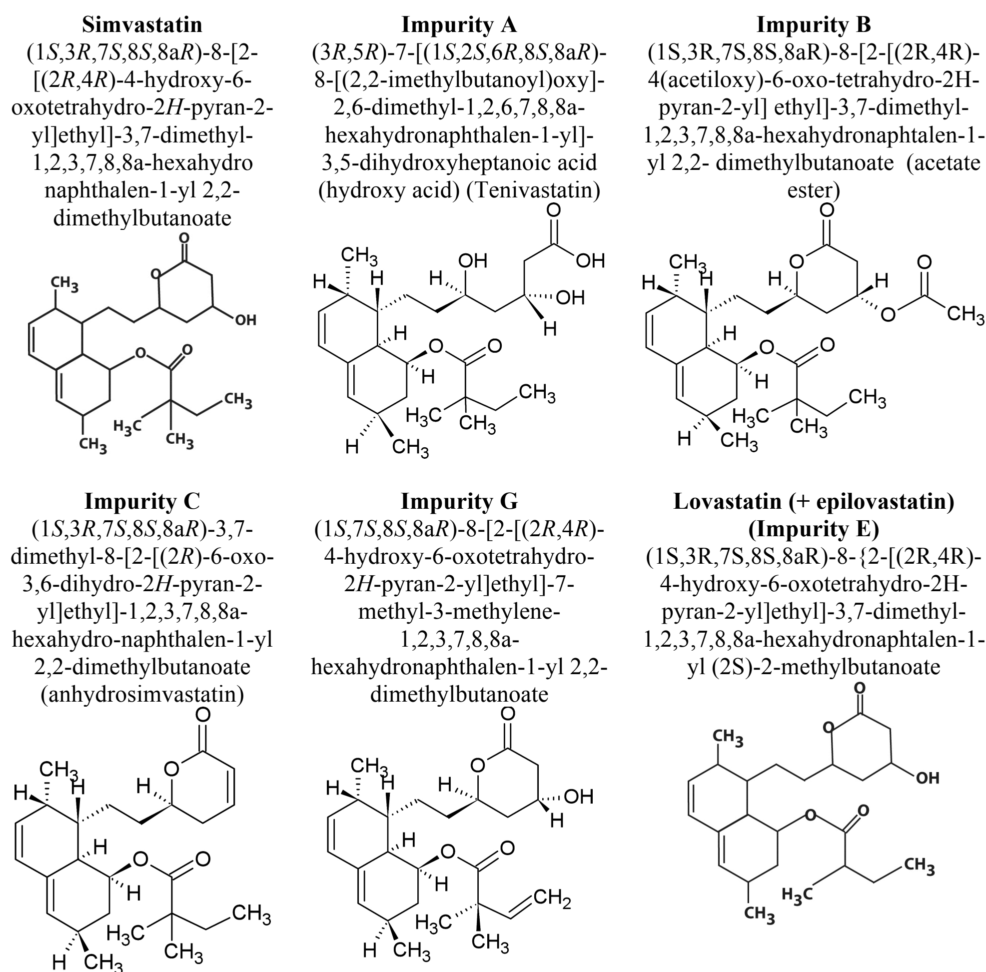

Figure 1

Chemical structures of simvastatin and its synthesis impurities

As simvastatin and most of its impurities are polar highly lipophilic species, it was interesting to investigate the suitability of a stationary phase with a wide selectivity such as cyano-propyl (CN) bonded stationary phase. The CN columns are often used for the analysis of polar substances in various, even very complex matrices, like biological fluids and the separation power is mostly due to the possibility to use it both in normal-phase and in reversed-phase mode [10, 11]. We have tested the capacity of the CN column to solve the mixture of simvastatin and its impurities cited in the Ph. Eur. (A, B, C, G and E) in the RP-HPLC mode. The results allowed us to validate the method as an efficient alternative method for the assay and purity testing of simvastatin in bulk and pharmaceutical dosage forms.

Materials and Methods

Liquid chromatography system and chromatographic condition

We used in our studies a Varian HPLC equipment, consisting of a quaternary pump Varian Prostar 240, equipped with a Rheodyne 7725i injector (20 µL calibrated sample loop), on-line degasser and PDA detector Varian Prostar 330; a STAR Chromatography Workstation 6.6 software was used for data acquisition and integration. The column was a stainless-steel column Nucleosil 100-5CN (125 x 4.6 mm i.d, with 5 µm particles). Data acquisition channels were 238 and 253.6 nm, with full spectra recorded between 200-600 nm.

For the analysis, a mixture of water and acetonitrile (MeCN) was used as mobile phase with a flow rate of 2.5 mL/min, at room temperature (24-26ºC), according to the following gradient program:

Time (min)	% Water	% MeCN
0	90	10
18	80	20
30	80	20

Reagents

All the solvents used (acetonitrile, methanol, tetrahydrofuran) were HPLC-grade (Merck KGaA, Germany), filtered and degassed before use. Ultrapure water for chromatography was prepared with a Barnstead EasyPure RoDi equipment.

Simvastatin and its impurities were reference standards EDQM.

The standard stock solutions were prepared by dissolving 5.00 mg simvastatin reference standard and 0.50 mg, respectively, for individual impurities B, C, G and E in 5.0 mL MeCN; standard solution of impurity A

was prepared by dissolving 0.50 mg in 25.0 mL of a 1:1 mixture of water and MeCN. Standard solutions were suitable diluted further, according to the validation procedure.

A mixture of simvastatin and its impurities in the ratio 15 / 1, respectively, was prepared for method development.

Sample solution of simvastatin was prepared by dissolving about 15.00 mg simvastatin in 5.0 mL MeCN (solution A). 2.0 mL solution A were diluted to 10.0 mL with MeCN.

Pharmaceutical dosage form: We have tested *Vasilip*® tablets (10 mg simvastatin per tablet, *Krka Slovenia*). 0.100 g tablet powder (corresponding to 10 mg simvastatin) was weighed in a 10.0 mL volumetric flask and 7 mL of MeCN were added. The mixture was sonicated 10 minutes, then the volume was completed with MeCN. After filtering through 0.45 μm porous membrane, 1.0 mL solution was diluted with acetonitrile in a 25.0 mL volumetric flask.

Validation study

The analytical procedure was validated according to ICH requirements for the following parameters: linearity, precision (repeatability), specificity, accuracy, robustness, limit of detection (LOD) and quantification (LOQ), solution stability [3, 12-14].

Detection limit (DL) and quantification limit (QL) were estimated from the signal-to-noise ratio: DL as the lowest concentration resulting in a peak area of three times the baseline noise and QL as the lowest concentration that provides a signal-to-noise ratio higher than 10, with precision and accuracy within specific acceptable range.

The calibration curve was constructed with ten concentrations (simultaneously prepared) ranging from 4.9 – 78.4 μg/mL for simvastatin. The linearity was assessed by linear regression analysis, which was calculated by the least square method.

Precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) for 3 consecutive days [3, 12-14]. Three different concentrations of simvastatin were analyzed in three independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision). Every sample was injected in triplicate.

The specificity tests of the analytical method verified the ability to discriminate between simvastatin and its impurities or other inactive components, such as dosage form excipients [11, 12]. All experiments were performed in triplicates.

The absolute recovery was computed from the peak area of simvastatin standard solutions at three different concentrations.

Results and Discussion

Throughout method development we have studied the effect of the mobile phase composition, of the flow rate and temperature on separation efficacy.

Several elution programs, both isocratic and gradient have been tested. The first gradient run proved that cyanopropyl stationary phase is able to discriminate the components of the tested mixture, as all species in the mixture could be identified in the chromatogram (Figure 2).

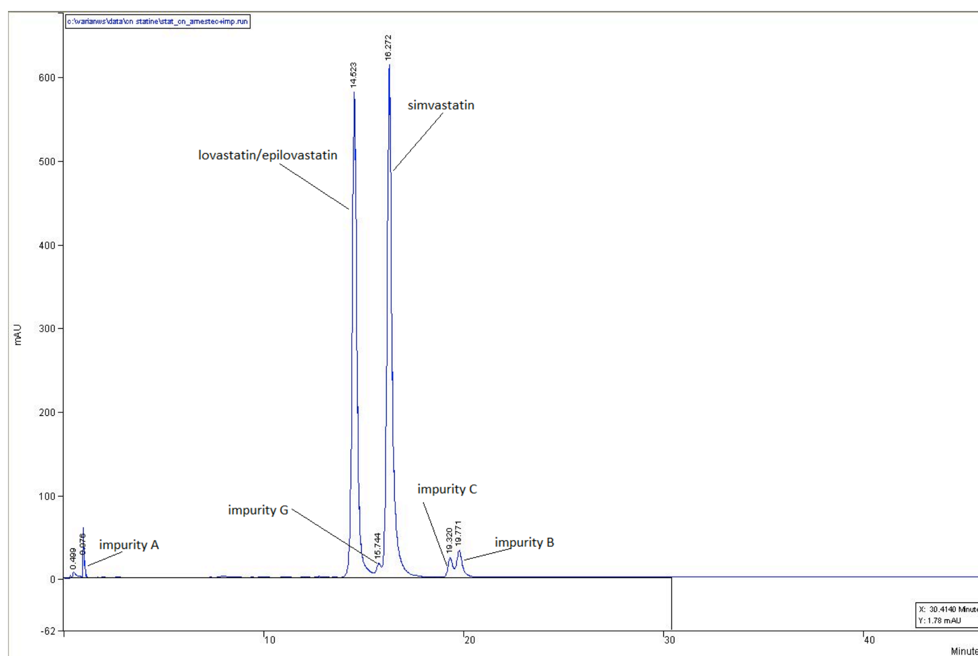

Figure 2

Chromatogram of a mixture containing simvastatin (660 µg/mL), lovastatin/epilovastatin (580 µg/mL), impurity A (70 µg/mL), impurity B (40 µg/mL), impurity C (60 µg/mL) and impurity G (50 µg/mL) using a Nucleosil 100-5CN column (150x4.6 mm, 5 µm); the mobile phase was water (solvent A) : MeCN (solvent B), 60 min. linear gradient from 10 to 45% solvent B

The nature and the concentration of the organic modifier in the mobile phase have a significant influence on the separation results, so we have tested different mixtures water - organic solvents (i.e. acetonitrile, tetrahydrofuran-THF and methanol-MeOH) having the same eluting strength, i.e. MeCN:H_2O 20:80, MeOH:H_2O 30:70, THF:H_20 15:85 and 30:70 and the most convenient organic solvent proved to be MeCN (Figure 3).

We have recorded the retention profile for each of the six species analyzed against the MeCN level in the mobile phase. As it can be easily seen in Fig. 4, mobile phases containing less than 30% MeCN provide higher selectivity and separation of the complex mixture studied.

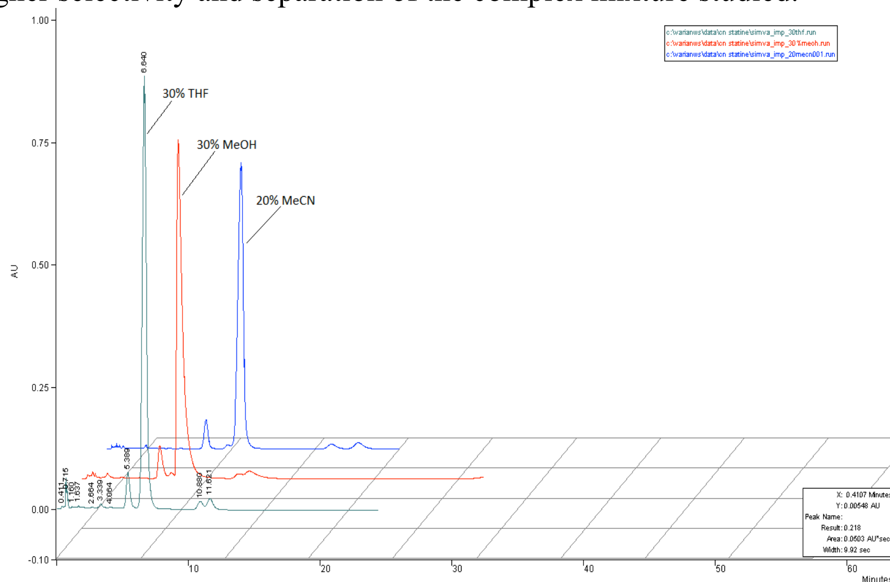

Figure 3

Chromatograms of a mixture containing simvastatin (660 µg/mL), lovastatin/epilovastatin (50 µg/mL), impurity A (70 µg/mL), impurity B (40 µg/mL), impurity C (60 µg/mL) and impurity G (50 µg/mL) using a Nucleosil 100-5CN column (150x4.6 mm, 5 mm) with mobile phases containing 20%MeCN (-), 30% MeOH (-) and 30%THF (-).

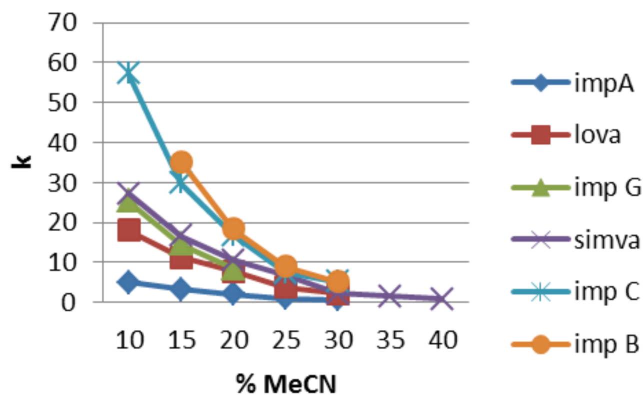

Figure 4

Retention profile of simvastatin and its impurities against MeCN level in the mobile phase

After several runs, we have established the gradient program able to provide us with the complete separation of simvastatin and its synthesis impurities (A, G, E, B, C).

The hypothesis that the pH value has a less significant effect on retention unless it is determining structural changes of the analyzed species was confirmed. Only impurity A retention is affected, as acidic solution are responsible for the presence of its molecular, less polar structure and results in 0.2 - 0.5 min longer retention times.

Over a temperature range between 25°C and 35°C no marked variations of the retention times and retention factors are recorded (Fig. 5), so the recommended temperature is room temperature (25°C±2°C).

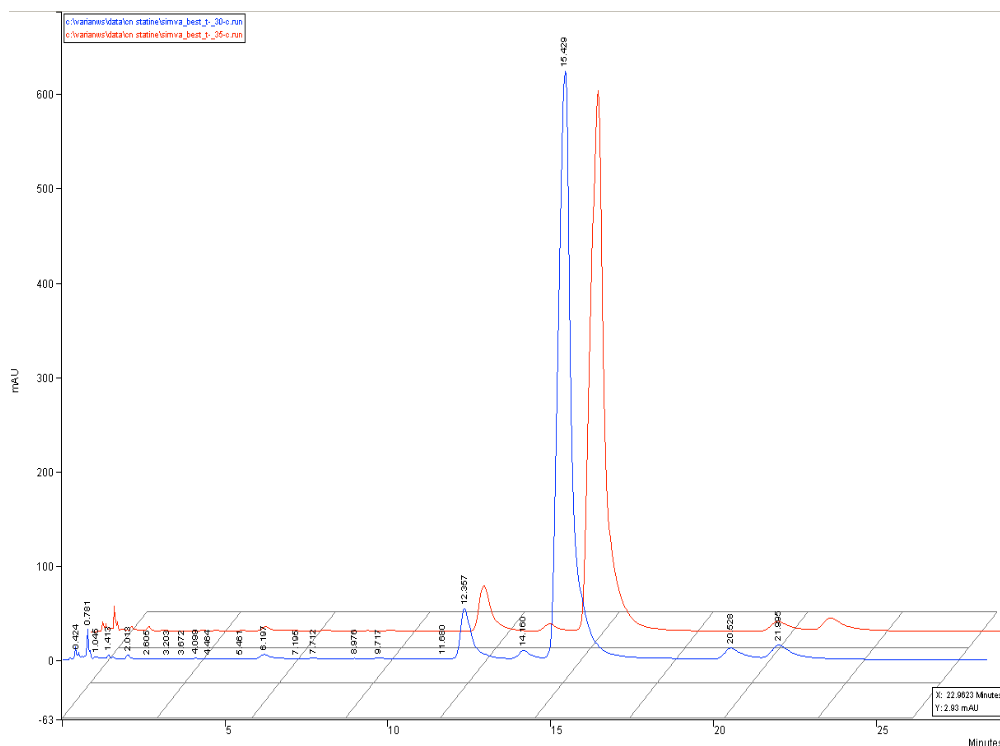

Figure 5
Chromatogram of simvastatin and its impurities mixture at 25°C and 35°C

Using flow rates between 1.5 and 3mL/min, the theoretical plate's height was estimated and the optimum flow rate ranges were found to be between 2-2.5 mL/min. Due to the complexity of the mixture, in order to

keep the assay time within reasonable limits the flow rate was set to 2.5 mL/min.

Using the selected experimental conditions within a runtime of 30 minutes, the complete separation of the species in the mixtures was achieved; the chromatographic resolution was 1.4 for impurity G - simvastatin and 1.7 for impurity C – impurity B, while the tailing was 1.15 and 0.91 for lovastatin and simvastatin, respectively (Fig. 6).

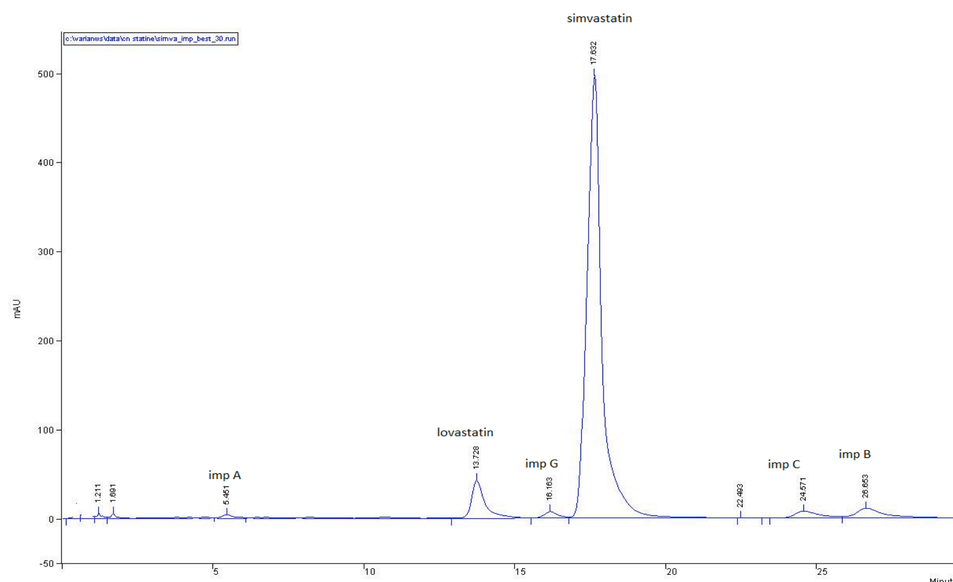

Figure 6

Chromatogram of a mixture containing simvastatin (660 μg/mL), lovastatin/epilovastatin (58 μg/mL), impurity A (70 μg/mL), impurity B (40 μg/mL), impurity C (60 μg/mL) and impurity G (50 μg/mL) using a Nucleosil 100-5CN column (150x4.6 mm, 5 μm); the mobile phase was water (solvemt A) : MeCN (solvent B), 30 min. gradient elution according to the stated program

The new method was validated in order to assess its suitability for the assay of simvastatin and also for related impurities detection and quantification. Validation data are summarized in table I.

Table I
Summary of the validation data obtained for the new HPLC method for the assay of simvastatin and its impurities

Validation criteria		Results
Linearity (simvastatin)	Regression equation	Y= 25.08X +1.82
	Correlation coefficient	0.9995
Concentration range		4.9 – 78.4 µg/mL
Precision (simvastatin)	Repeatability	RSD% = 0.55
	Intermediary precision	RSD% = 0.53
Accuracy		99.55 ± 0.35 %
Detection Limit (simvastatin)		2.6 µg/mL
Quantification Limit (simvastatin)		8.7 µg/mL
Detection Limit (simvastatin impurities): – lovastatin/epilovastatin (impurity E) - impurity A - impurity G - impurity C - impurity B		 2.5 µg/mL 6 µg/mL 1.25 µg/mL 4.5 µg/mL 4.5 µg/mL

For the estimated 3/1 signal/noise ratio the measured peak area was 24 mAU·s, corresponding to a simvastatin level of 2.8 µg/mL. Detection limits for simvastatin impurities are 2.5 µg/mL (lovastatin/epilovastatin - Impurity E); 6 µg/mL (Impurity A) and 4.5 µg/mL (Impurities G, C and B).

Peak area for a 10/1 signal/noise ratio, when using the newly established method, was 80 mAU·s, corresponding to 22.66 µg simvastatin/mL.

As discussed above, the specificity of an HPLC method is the ability to detect the analytes in the presence of other ingredients. Excipients (lactose monohydrate, pregelatinized starch, microcrystalline cellulose, magnesium stearate, hypromelose and talcum did not exhibit any peaks, and therefore no interferences were detected.

The method proved to be suitable for the assay of simvastatin in bulk and pharmaceutical dosage forms, tablets, as it is stated in table II.

Table II
Results reported for the assay of simvastatin in bulk and pharmaceutical dosage forms

Sample type							Average %	RSD %	Confidence interval (n=6; P=95%)
Active substance	100.52	100.24	99.98	100.04	99.87	100.08	100.12	0.21	100.12 ± 0.54
Vasilip®	99.58	100.03	99.86	99.94	99.82	99.96	99.87	0.14	99.87 ± 0.12

Conclusions

The RP-HPLC method using a cyanopropyl stationary phase proved to be an efficient alternative to RP-HPLC methods recommended in the literature, useful in resolving complex mixtures as the one described above.

The optimum conditions selected in this study provide separations with good resolutions for all critical pairs of analytes (higher than 1.4, with baseline separation for critical pairs in the chromatogram). The validation data and the results obtained in the assay of simvastatin assess method suitability for detection or/and quantification of simvastatin and its synthesis impurities in bulk and in pharmaceutical dosage forms (tablets).

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