



Development and validation of HPLC dissolution assay of simvastatin tablets under normal and accelerated conditions

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ABSTRACT

The UV-Vis method required by the USP pharmacopeia for simvastatin tablet dissolution assays suffers from a lack of specificity and reproducibility which limit its use to perform kinetic dissolution profile between the innovator and its generics. This variability caused by the manganese dioxide treatment step or by the hydrolysed compound of simvastatin. In order to obtain an acceptable precision of the percent release of simvastatin under normal and accelerated conditions, a HPLC method was developed and validated according to ICH Q2R1 guidelines. The separation was achieved using a GL Science Inertsil ODS - 3 V (5 μ m, 150mm \times 4.6mm i.d.) column. The gradient elution mobile phase was composed of buffer (pH=4.2, 12 mM sodium acetate and adjust pH by glacial acetic acid and acetonitrile at a flow rate of 1.7ml/min. The detection was performed at 238 nm. It was demonstrated that the USP UV-Vis method is not suitable to assess the dissolution kinetic profiles and to perform a comparative study between the innovator and different references of simvastatin. This UV-visible method cannot distinguish between the simvastatin as principal active ingredient and its impurity A obtained by hydrolysis in the dissolution medium test. While it was demonstrated that the HPLC method is more suitable to quantify accurately and precisely the percent release of the innovator and the generics for simvastatin under normal and accelerated conditions

Keywords: Simvastatin, HPLC, Validation, Dissolution profile, Normal and Accelerated Conditions.

INTRODUCTION

Simvastatin (SIM) is a cholesterol-lowering agent belonging to the class of statins and it has been widely used in the treatment of hypercholesterolemia, dyslipidemia and coronary heart disease [1, 2]. SIM is a weak acid [3] classified as a BCS Class II substance [4,5], practically insoluble in water thus showing dissolution rate-limited absorption and low oral bioavailability [6]. Moreover, at higher pH values, the lactone form of simvastatin is unstable and the equilibrium favours hydrolysis opening the lactone and yielding the hydroxyl acid form (IMP-A) (Fig. 1) [7]

Drug dissolution testing is a critical step of the process development. Although dissolution cannot be used as a predictor of therapeutic efficiency; it can be used as a qualitative and a quantitative tool, which can provide important information about bioavailability of a drug as well as batch-to-batch consistency [8]. Moreover, it was reported that for the same active pharmaceutical ingredient, differences in solid-state properties, formulation excipients, or manufacturing can lead to differences in bioavailability from one product to another [9].

2.4 Analytical method validation

The in vitro dissolution method developed was validated according to current guidelines ICH Q2R1 [12]. Specificity, linearity, accuracy and precision were evaluated.

2.5 Assay content

Before starting the in vitro dissolution, the content of different products used for dissolution was determined under normal and stressed conditions (40 °C and 75% humidity for six months). An accurately weighed quantity of SIM standard was diluted by a suitable degassed mixture of buffer and acetonitrile (35:65, V/V) to obtain a solution having a known concentration of about 0.01 mg/ml. An accurately weighed portion of finely powder tablets, equivalent to about 10 mg of SIM was transferred to 50 ml volumetric flask, diluted to volume with the mixture solution, shaken mechanically for 10 minutes, and sonicated for about 10 minutes to obtain a clear solution. 5ml of this solution was transferred to a 100 ml volumetric flask, diluted to volume with the same diluent and mixed to obtain a solution having a concentration of about 0.01 mg per ml.

2.6 Dissolution test

The dissolution rate studies on conventional SIM tablets were carried out according to the USP paddle method (Apparatus 2), at a stirring rate of 50 rpm at 37 ± 0.5 °C. The dissolution medium was 900 ml of pH 7.0 buffer solution containing 0.5% sodium dodecyl sulfate in 0.01 M sodium phosphate prepared by dissolving 30 g of sodium dodecyl sulfate and 8.28 g of monobasic sodium phosphate in 6000 mL of water, and adjusting with 50% (w/v) sodium hydroxide solution to a pH of 7.0.

2.6.1 Assay based on the USP method with and without manganese treatment

The UV-Visible method proposed by USP monograph [10] is time-consuming due to the manganese dioxide treatment step. This step consisted of the preparation of the prewashed manganese dioxide by transferring about 10 g of manganese dioxide to 50 ml of dissolution medium in a suitable container, and was shaken vigorously for 5 minutes. The mixture was centrifuged, and the supernatant layer was isolated. This step was repeated twice, first with dissolution medium and then with water. Finally the solid was dried at 100 °C for 1 hour before use. The collected sample was transferred to a centrifuge tube containing about 10 mg of prewashed manganese dioxide per ml of the added solution and was mixed. The mixture solution was stand for 30 minutes with occasional shaking, centrifuged, and a portion of the clear supernatant was used. The dissolution medium is used as blank for all UV Visible measurements.

The amount the sample test and that of the standard solution with a known concentration of SIM which was prepared in the same medium and treated as the same way as the sample test was calculated from the UV absorbance difference corresponding to the wavelengths of 247 nm and 257 nm respectively.

The percent release of the originator (Zocor) and the five generic brands (A, B, C, D and E) with and without treatment with the manganese dioxide was assayed at 30 min by the USP method.

2.6.2 Assay based on the validated RP-HPLC method

The developed and validated method was used to assess the comparative dissolution profiles of the originator and the five generic compounds under normal and accelerated conditions.

Twelve tablets of each product were sampled. During the dissolution process, 5 ml aliquots were withdrawn at 10, 20, 30 and 45 minutes and filtered through 0.45 µm filters before their injection. The withdrawn aliquots were replaced by dissolution medium. The percent drug release was also quantified by the HPLC method using regression linear equation of the calibration curve.

2.7 Hardness Test

Hardness test was performed using a hardness tester on 10 tablets from each brand at normal and accelerated conditions.

RESULTS AND DISCUSSION

3.1. Validation of HPLC analytical methods

3.1.1. Specificity

The specificity of the developed RP - HPLC method for SIM was investigated in order to obtain indication of the possible interferences with active ingredient (SIM). The interference comes from the impurity A obtained from SIM under the accelerated condition. As shown in figure 2, neither BHA nor ascorbic acid interfere with the active ingredient (SIM)

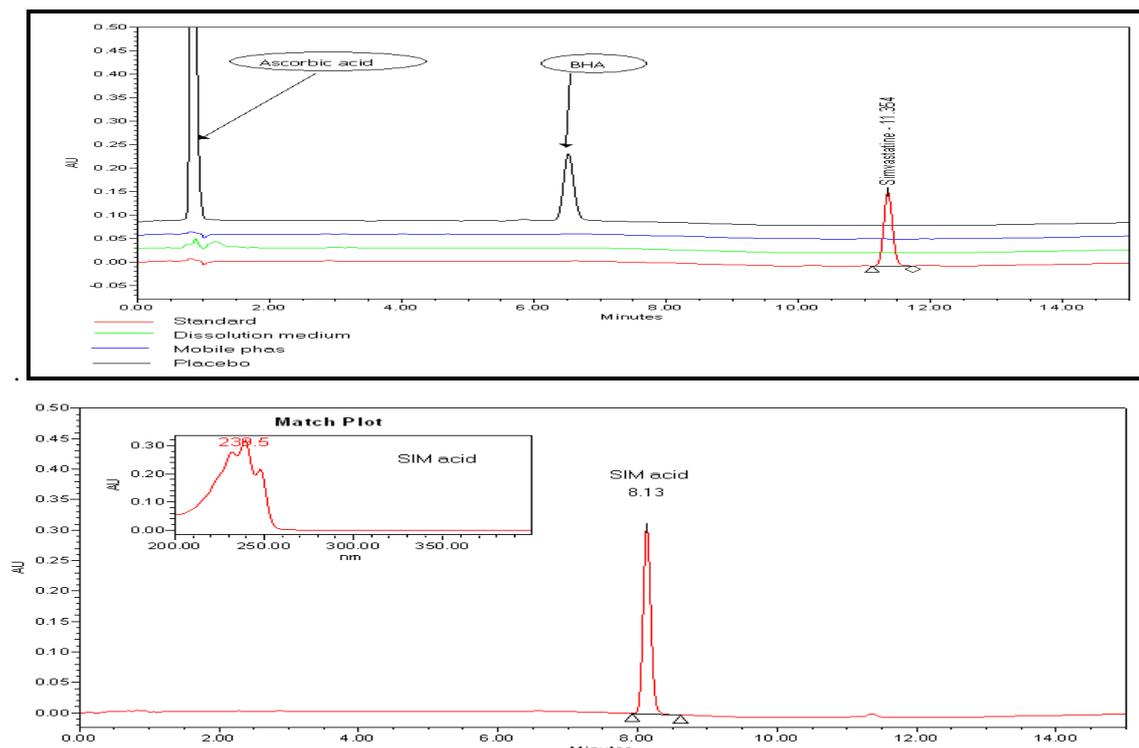


Fig.2: Specificity of the HPLC method at the accelerated conditions

3.1.2 Calibration curve

In order to bracket the target concentration of SIM after the dissolution experiments, linearity in the range of 2.5 $\mu\text{g/ml}$ to 16.66 $\mu\text{g/ml}$ was evaluated using five concentration levels with two replicate for each level. A linear regression equation was obtained ($y = 17328x + 10235$) with a regression coefficient (r) of 0.99996.

3.1.3 Precision (repeatability and intermediate precision)

The repeatability (within-day precision) was validated as described on the ICH Q2R1 guidelines, by performing six replicate samples of 11.11 $\mu\text{g/ml}$ of SIM in the same conditions. The calculated mean relative standard deviation (R.S.D) was 1.5% .

The intermediate precision (day-to-day precision) was assessed by the CV% calculated from data obtained by performing six measurements for the nominal concentration (11.11 $\mu\text{g/ml}$) for three independent series. The CV % was 1.8 which confirmed the reproducibility of the HPLC assay.

3.1.4. Accuracy

The accuracy of the method was determined by spiking the placebo with standard SIM at five concentration levels, covering the range of 50 - 140 % of the target concentration. The mean percent recovery 99.99 % falls inside the 95 % confidence interval of 98.01%-101.98%.

3.2. Assay content (HPLC)

The results of SIM content in tablets under normal and accelerated conditions were reported in Table 2. The results show that the percent recoveries for all the SIM products met the acceptance criteria (100 ± 5 %).

Table 3: Assay results of tablets at normal and accelerated conditions

Product	Normal Conditions	Accelerated Conditions
	Mean \pm RSD (%)	Mean \pm RSD (%)
Originator	100.8 \pm 0.2	98.5 \pm 0.6
Generic A	102.4 \pm 1.1	97.4 \pm 0.5
Generic B	98.4 \pm 0.8	95.9 \pm 1.3
Generic C	99.4 \pm 0.9	97.1 \pm 1.0
Generic D	98.7 \pm 1.2	96.4 \pm 0.9
Generic E	99.5 \pm 1.1	99.4 \pm 1.0

3.3. Hardness Test

The mean hardness with respect to the standard deviation, and coefficient of variation of the test at normal and accelerated conditions for originator and generics tablets were reported in Table 4,. The hardness varied from brand

to brand and without significant change caused by accelerating conditions, except of the brand B which have shown a clear decrease in hardness around 50%.

Table 4: Hardness of tablets at normal and accelerated conditions

Product	Normal Conditions	Accelerated Conditions
	Mean (N) ± RSD (%)	Mean (N) ± RSD (%)
Originator	116 ± 2.0	113 ± 4.3
Generic A	64 ± 5.6	62 ± 6.1
Generic B	107 ± 2.2	64 ± 9.7
Generic C	124 ± 2.9	120 ± 2.6
Generic D	80 ± 5.1	73 ± 5.5
Generic E	99 ± 3.5	91 ± 4.4

3.4. Dissolution test

3.4.1 UV-VIS method

The results of the dissolution test of tablets treated with and without a manganese dioxide were reported in (table-5). These results indicated that the originator, the generics products (C and E) present a significant difference ($p < 0.05$) in the percent release obtained with or without manganese dioxide treatment at normal conditions. While for the generics (A, B and D) the results are not significant ($p > 0.05$). It is noticed that at the earlier time measurement points e.g at $t = 10$ minutes, the relative standard deviation is higher (28 %) than the other times points (less than 10 %) when the USP method was applied to quantify the percent release. Accordingly, the USP method seems to be not adequate for assessing the kinetic dissolution profiles between the innovator and the others references.

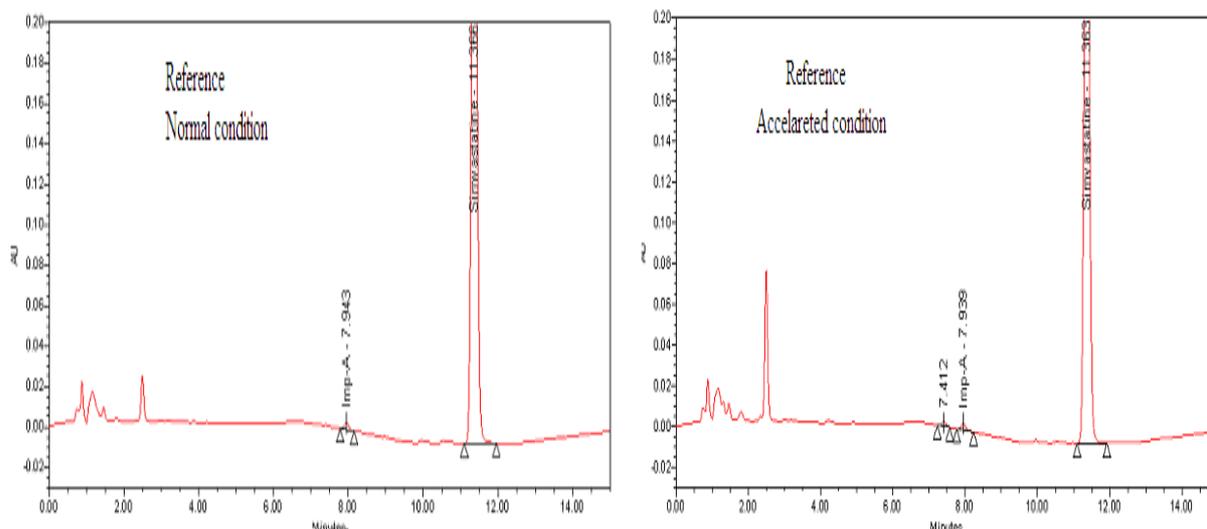
Table 5: Percent release with (T) and without (NT) the manganese dioxide treatment at normal conditions, t (min) = 45

Products	T	NT
	Mean (%) ± RSD (%)	Mean (%) ± RSD (%)
Originator	90 ± 7.2*	98 ± 8.1*
Generic A	94 ± 12.5	92 ± 9.3
Generic B	91 ± 13.1	94 ± 13.3
Generic C	124 ± 2.9*	120 ± 2.6*
Generic D	89 ± 17.0	91 ± 10.5
Generic E	92 ± 19.1*	107 ± 14.7*

* $p < 0.05$

3.4.1 HPLC method

The kinetic dissolution profiles for Simvastatin innovator and references at different conditions obtained by HPLC method are given in figure 3. The RSD showed at different times points don't exceed 10 % compared to the dispersion obtained by the USP method. As shown in Fig. 3 all drug products, except the generic B at accelerated conditions, complied with the dissolution specification, ($Q=80\%$ with 30 min), stated in the USP 34 [10]. The decrease in the percent release of SIM ($Q=55\%$) of B product at accelerated condition can be explained by the increase of the impurity A (retention time = 7.9 min). This difference cannot be highlighted by the USP monograph based on the UV-Visible method.



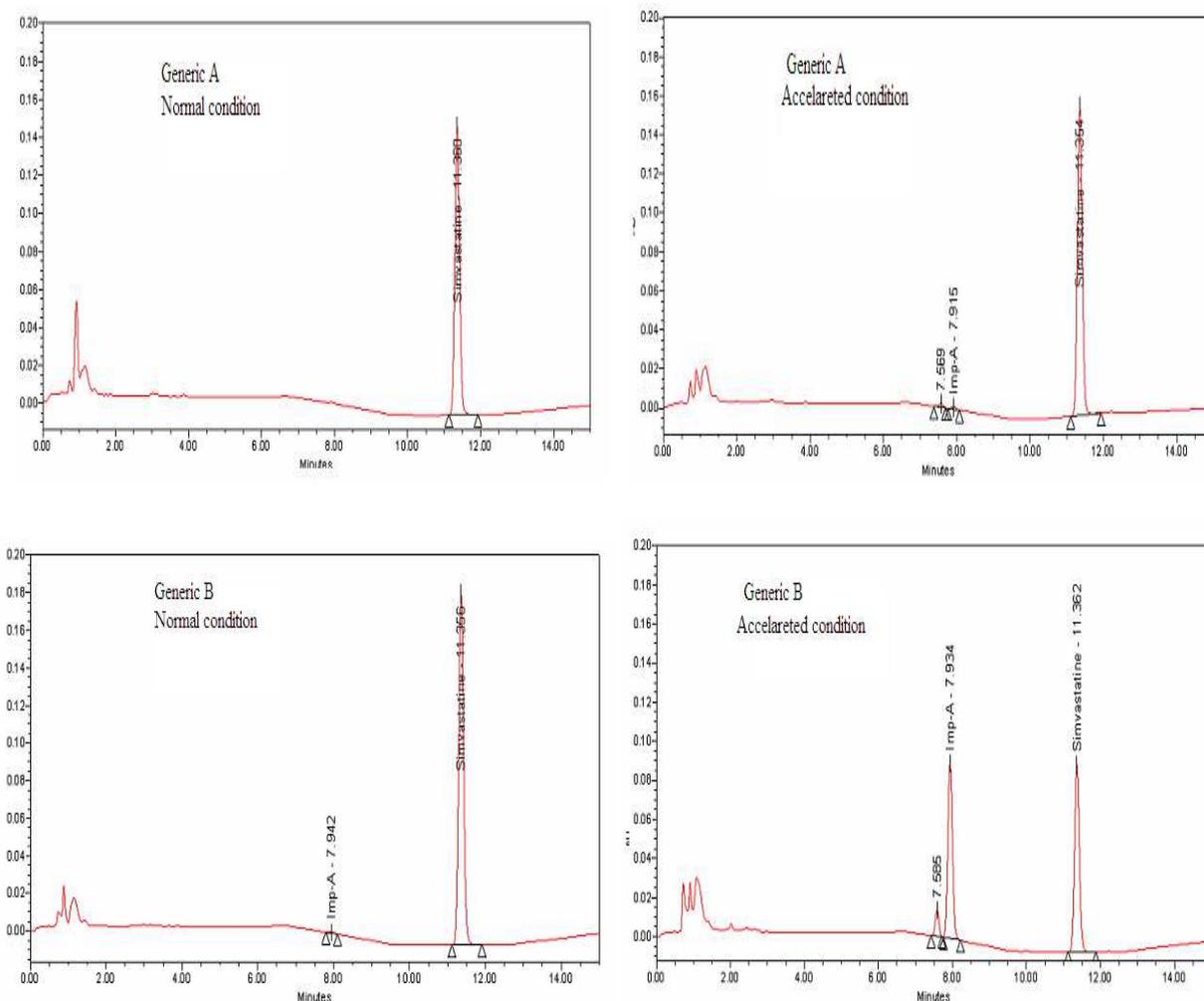
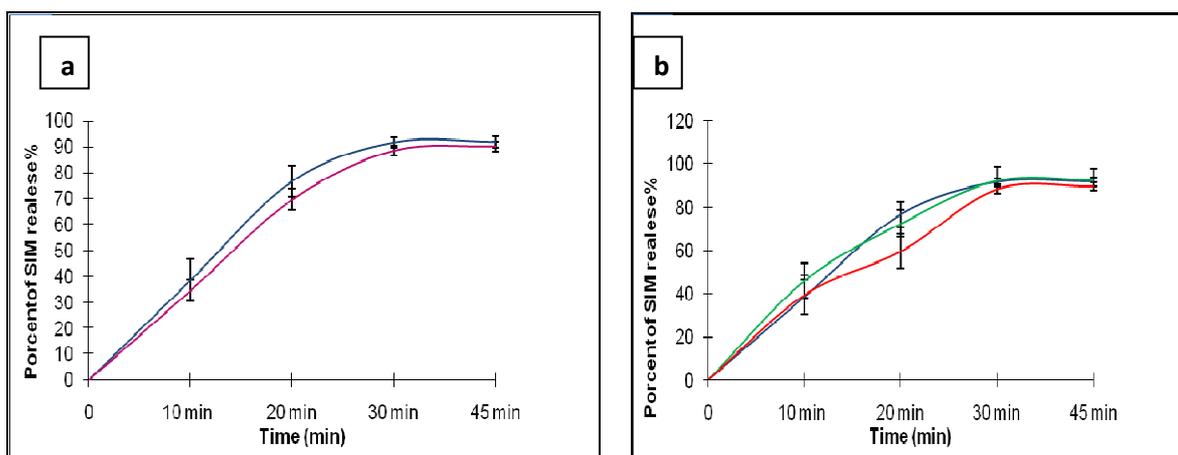


Fig.3: Chromatograms of SIM release under normal and accelerated conditions

The Comparative kinetic dissolution profiles of the innovator and the generics at normal and accelerated conditions were shown in Figure 4. This kinetic is similar for each for all products under the conditions studied except for the generic B which present a difference in the shape of the kinetic curve at different conditions.



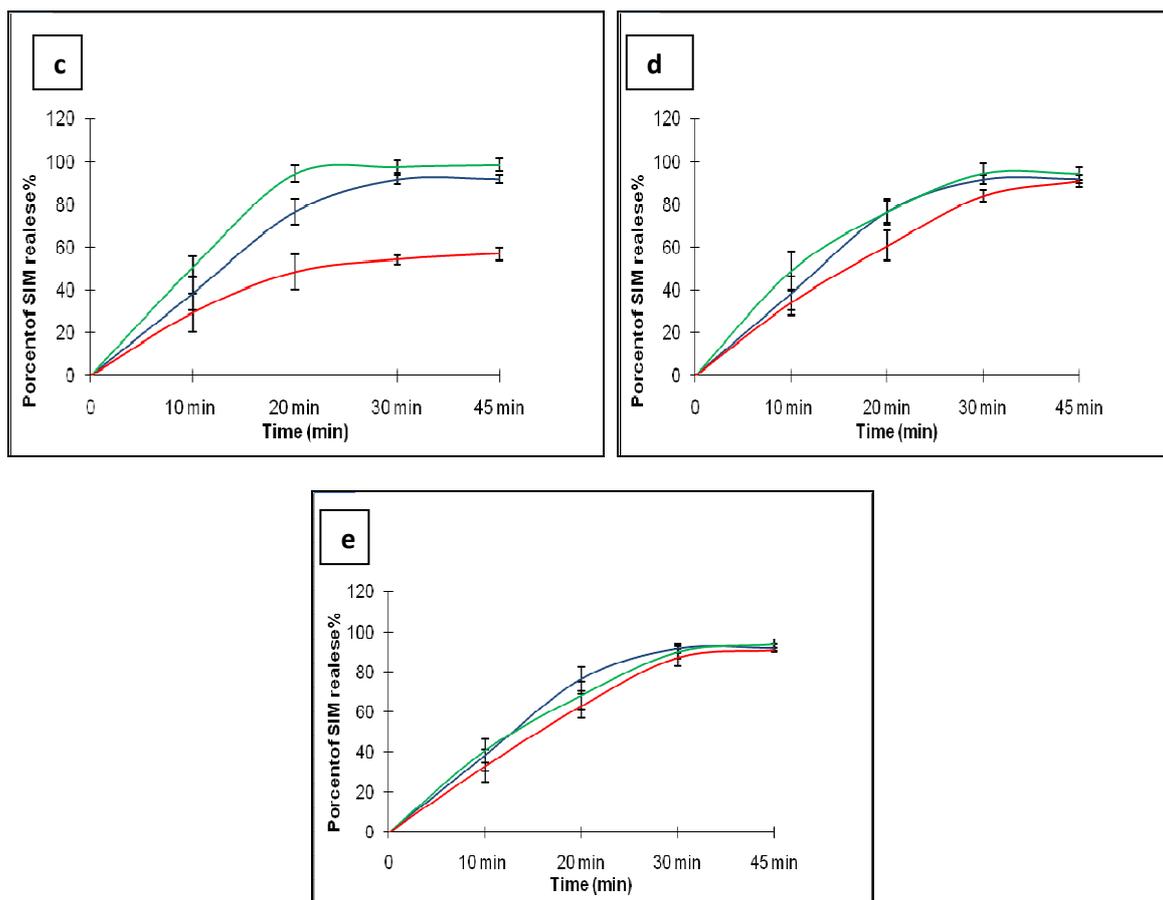


Fig.4: Comparative kinetic dissolution profile of the innovator and the references at normal and accelerated conditions obtained by HPLC method

Légende : — Originator normal conditions — Originator accelerated conditions
 — Generic normal conditions — Generic accelerated conditions

In order to check this similarity between the two curves related to the dissolution profile at normal and accelerated condition for each product, similarity factor (F_2) and dissimilarity factor (F_1) were calculated [14] table 8. According to FDA's guides for industry [13], F_1 factor is proportional to the average difference between the two profiles, whereas F_2 factor is inversely proportional to the average squared difference between the two profiles, with emphasis on the larger difference among all the time points. According to this guideline, generally, F_1 values up to 15 (0–15) and F_2 values greater than 50 (50–100) ensure sameness or equivalence of the two curves.

The calculate factors F_2 and F_1 (table 8) indicated that the A, C and E generic products presented a similar profile for different conditions as the originator and are considered meeting the quality requirement. Concerning the B product, under accelerated condition the F_1 value 36.8 (table 8) revealed that this product is not similar to the innovator and its quality don't be guaranteed.

Table 8: Similarity factor (F_2) and dissimilarity factor (F_1) of different brands

Products	Conditions	dissimilarity factor (F2)	Similarity Factor(F1)
	N	---	---
Originator	Acc	53.7	5.3
	N	54	4.5
Generic A	Acc	52.8	7.8
	N	37.4	14.2
Generic B	Acc	26.4	36.8
	N	50.1	5.3
Generic C	Acc	51.7	9.7
	N	53.3	4.7
Generic E	Acc	55.6	8.2

CONCLUSION

HPLC method was developed and validated using ICH Q2R1 guidelines for the determination of SIM percent release in different generic and innovator tablets. It was demonstrated that the USP UV-Vis method is not suitable to assess the dissolution kinetic profiles and to perform a comparative study between the innovator and different generics of simvastatin. This UV-visible method cannot distinguish between the SIM as principal active ingredient and its impurity A obtained by hydrolysis at the dissolution medium test. While it was demonstrated that the HPLC method is more suitable to quantify accurately and precisely the percent release of the innovator and the generics for simvastatin under normal and accelerated conditions

Acknowledgements

The authors thank the Moroccan National Drug Control Laboratory (Laboratoire Nationale de Contrôle des Médicaments LNCM) for supporting this study.

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