Short Communication

Development and Validation of a Complexometric Titration Method for the Determination of Rosuvastatin Calcium in Raw Material.

ABSTRACT

Aims: a simple, sensitive and economical volumetric method for the determination of rosuvastatin calcium in raw material has been developed.

Methodology: The titrimetric method is based on the reaction of calcium with a solution of EDTA-Mg 0.01M. Hydroxynaphtol blue was used as indicator to detect the end point of the titration which changes from pink to blue at pH = 10. The method was validated by following the analytical performance parameters suggested by the International Conference on Harmonization (ICH).

Results: The calibration curve was linear from 80 to 120 % with r = 0.9998. Accuracy (mean recovery 99.0%) and precision were found to be satisfactory.

Conclusion: The proposed method can be used for quality control assay of rosuvastatin calcium in bulk drug.

Keywords: *Rosuvastatin calcium , Titrimetric, Assay, Complexometric titration, EDTA.Mg.*

1. INTRODUCTION

Rosuvastatin calcium (Fig. 1) is bis[(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2- [methyl(methylsulfonyl)amino] pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt. It is a competitive inhibitor of the enzyme HMG-CoA reductase, the enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, precursor for cholesterol. It is a cholesterol lower agent [1]. The usual doses are 5, 10, 20 and 40 mg.

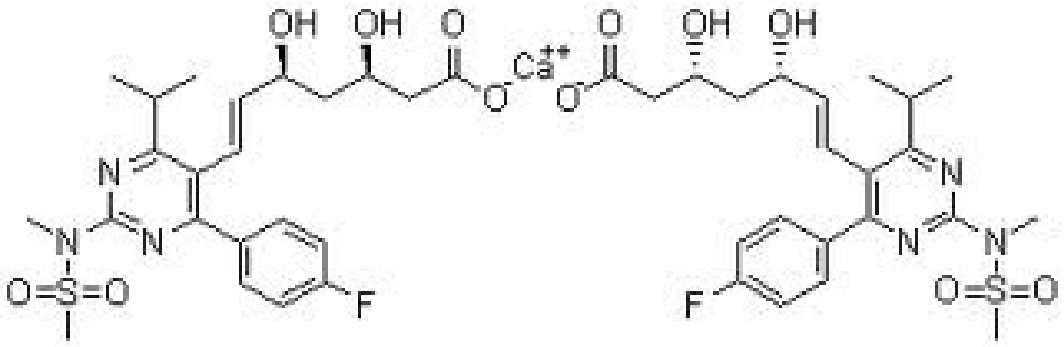

Figure 1 Rosuvastatin calcium

Rosuvastatin calcium alone has been determined by Spectrophotometric methods [2-4], TLC methods [5-7] and a Stability indicating HPTLC method [8].

A literature survey revealed some high-performance liquid chromatographic methods for the quantitation of rosuvastatin calcium [4, 9-13]. Most of the analytical techniques for rosuvastatin calcium described in the literature are based on the liquid chromatographic determination of this drug with another active drug substance [1, 14-22].

Due to the need for an absolute analytical technique for rosuvastatin calcium, we have developed this volumetric technique. The present technique is concerned using a complexometric titration for the quantification of Calcium (Ca). The formation of complex from many metal ions can serve as a basis of convenient titrations. These titrations are accurate and they offer the possibility of determinations of metal ions at concentrations at the millimole level. Many cations will form complexes in solution with a variety of

substances that have a pair of unshared electrons (e.g. on N, O, S atoms in the molecule) capable of satisfying the coordination number of the metal. The complexing agent is a Lewis base (electron pair donor) and the metal ions is a Lewis acid (electron pair acceptor). The ligand (complexing agent) use a number of molecules which depends on the coordination number of the metal and the complexing groups of it.

An organic agent that has two or more groups capable of complexing with a metal ion is called a chelating agent. The complex is a chelate. Titration is called a chelometric titration, which is a particular type of complexometric titration. A pair of unshared electrons is located on each of the two nitrogen atoms and each of the four-carboxyl groups. There are six complexing groups in EDTA. The EDTA is represented by the symbol H4Y. It is a tetraprotic acid. The four hydrogens in the formula refer to the four acidic hydrogens on the four-carboxyl groups [23].

There are not satisfactory indicators for calcium by direct titration. Eriochrome Black T cannot be used as an indicator for the titration of calcium with EDTA, since it forms too weak a complex with calcium to give a sharp end point. However, calcium can be determined by direct titration with a small addition of magnesium chloride to the EDTA solution.

A solution containing the magnesium complex of EDTA, MgY^{2-}, is introduced into the titration mixture. Since Ca^{2+} forms a more stable complex with EDTA than magnesium, the following reaction occurs:

$$MgY^{2-} + Ca^{2+} <===> CaY^{2-} + Mg^{2+}$$

The equivalence point is detected with hydroxynaphthol blue which is itself a chelating agent. It changes from pink to blue in the presence of calcium at pH 10.

The present manuscript describes a simple, rapid, precise, accurate and economic volumetric method for the quantitation of rosuvastatin calcium in raw material.

The method was validated by following the analytical performance parameters suggested by the International Conference on Harmonization (ICH) [24].

2. MATERIALS AND METHODS

2.1 Apparatus

A standard borosil burrets, pipetts, standard flasks, measuring cilindres and conical flasks are calibrated as per International Conference on Harmonization (ICH) guidelines [25]

2.2 Materials

Rosuvastatin calcium (100.0%) was obtained from Optimus drugs (Hyderabad, India). Disodium EDTA was AR Grade (Merck Química Argentina, Argentina); Magnesium chloride hexahydrate AR Grade (Alcor, Argentina) Ammonia AR Grade (Conc 20%, Reactivo Raudo Analítico, Argentina), Ammonium chloride AR Grade (J.T.Baker, Mexico), Calcium carbonate ACS grade (Mallinckrodt, USA), Hydrochloric acid ACS grade (J.T.Baker, Mexico), Hydroxy naphthol blue AR Grade (Anedra, Argentina). Distilled water was passed through a 0.45 µm membrane filter.

2.2.1 Magnesium chloride 1% (w/v)

1% (w/v) $MgCl_2$ solution was prepared by dissolving 1.07 g of magnesium chloride hexahydrate in a 50 ml volumetric flask, dissolved with 30 ml of distilled water and made to volume with the same solvent.

2.2.2 Ammonia 6M

The 51 mL ammonia (NH_3) 20% was taken in 100 mL volumetric flask and made to volume with distilled water.

2.2.3 Disodium ethylene diaminotetraacetate (EDTA) - Magnesium Volumetric solution

The 0.01M EDTA - magnesium solution was prepared by dissolving 4.0 g of disodium EDTA dehydrate salt in 500 mL of distilled water in a 1 L volumetric flask. Add 10 mL of magnesium chloride 1% and 2 mL of ammonia 6M and made to 1 L with distilled water.

2.2.4 Ammonia – ammonium chloride buffer

Dissolve 6.0 g ammonium chloride in 100 mL of ammonia 6M. Stir until total dissolution.

2.2.5 Diluted hydrochloric acid (0.1N)

Prepare by diluting 100 ml of 1N hydrochloric acid solution with sufficient water to make 1000 mL.

2.3 Standardization

The 0.01M EDTA - magnesium solution was standardized with calcium carbonate. Place 30 mg of calcium carbonate previously dried at 110 ℃ to constant mass in a 250 mL conical flask, dissolve in 10 mL of distilled water and stir until dissolution. Add 10 mL of 0.1N hydrochloric acid. Add 10 mL of ammonia – ammonium chloride buffer and 30 mL of distilled water. Add 25 mg of hydroxy naphthol blue and shake during 1 min. The solution was titrated with 0.01M EDTA - magnesium solution until the pink colour changes to blue at the end of titration. . Each mL of 0.01 M EDTA - magnesium solution is equivalent to 1.0008 mg of calcium carbonate.

2.4 General Procedure

250 mg of rosuvastatin calcium was accurately weighed, transferred to a 500 ml conical flask, and dissolved in 200 mL of distilled water and 25 mL of ammonia – ammonium chloride buffer. Stir during 10 min. Add 25 mg of hydroxy naphthol blue and shake during 1 min (pH= 10.0) Titrate with 0.01M EDTA - magnesium solution until the pink colour changes to blue at the end of titration.

Each mL of 0.01 M EDTA - magnesium solution is equivalent to 10.0114 mg of rosuvastatin calcium.

2.5 Method Validation

2.5.1 Linearity

The linearity of the volumetric method was determined by analysis of six replicates at 80%, 100% and 120% and three replicates at 90% and 110% of analyte concentration.

2.5.2 Precision

Precision of the assay method was performed by analyzing six individual samples of rosuvastatin calcium raw materia. Intermediate precision was checked by analyzing six individual samples on different days by two different analysts.

2.5.3 Accuracy

Recovery studies were done to evaluate the accuracy of the method, 6 samples each at three different levels (80%, 100% and 120%). The amount of rosuvastatin calcium recovered in relation to the added amount was calculated.

3. RESULTS AND DISCUSSION

The proposed procedure has been satisfactorily applied to the quantitation of rosuvastatin calcium in raw material.

The linearity of the volumetric method was determined by analysis of six replicates at 80%, 100% and 120% and three replicates at 90% and 110% of analyte concentration. The calibration curve showed good linearity over the concentration range. The correlation coefficient (“r”) value was 0.9998. Typically, the regression equation for the calibration curve was found to be y = 0.1035x + 0.2495. The linearity of the calibration graphs was validated by the high value of the correlation coefficient and the intercept value that was not statistically (P = .05) different from zero (Table 1 and Figure 2).

TABLE 1 Linearity

% of nominal value	Weighed (mg) (RSD)	Vol cons (RSD)

80	200.6 (0.6)	21.0 (0.7)
90	226.4 (0.5)	23.7 (0.4)
100	251.8 (0.6)	26.3 (0.7)
110	276.2 (0.1)	28.7 (0.3)
120	301.7 (0.5)	31.5 (0.4)

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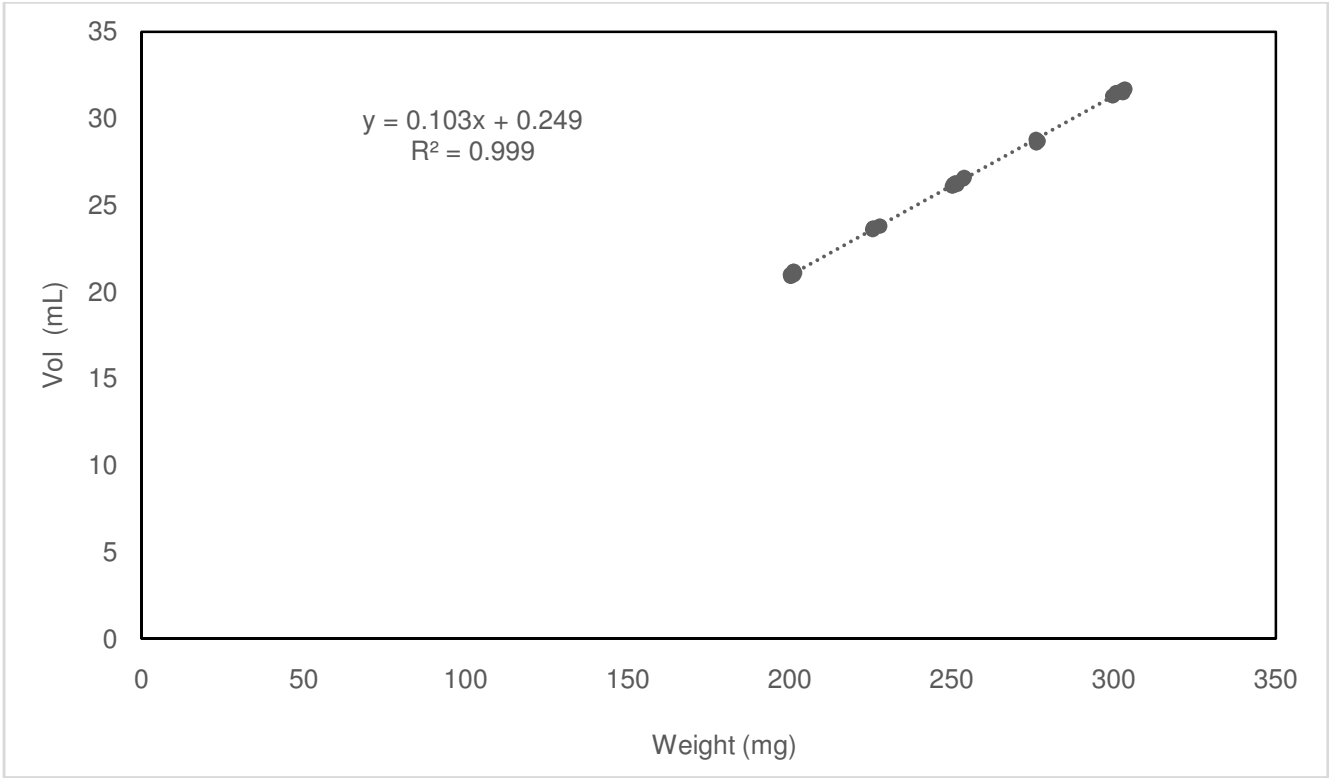
Slope[(a)]	0.1035. ± 0.0011
Intercept[(b)]	0.2495 ± 0.3706

[a]Confidence limits of the slope (p= 0.05)
[b]Confidence limits of the intercept (p= 0.05)



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135 **Figure 2 Linearity**
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The precision of an analytical procedure expresses the closeness of the agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

The intra-day precision of the volumetric method was performed by assaying the samples on two different days by two different analysts. The results were given both individually and as the average. For each precision assay the results were as follows: mean values 99.5 and 100.9 %, RSD 0.4% and 0.5%. "*t*" Test comparing two samples with 95% confidence for 10 degrees of freedom disclosed that both results were not significantly different *inter se* ($t_{n-2,\ \alpha:0.05}$) = 2.23 (Table 2).

TABLE 2. Intermediate precision

Analyst 1 Sample N°	Weighed (mg)	Percentage	Analyst 2 Sample N°	Weighed (mg)	Percentage
1	249.8	99.3	1	250.6	100.9
2	250.2	99.1	2	252.3	101.3
3	250.4	99.4	3	250.1	101.1
4	249.7	100.1	4	252.0	101.4
5	250.8	99.6	5	250.0	100.3
6	251.8	99.3	6	250.9	100.4
Mean	250.4	99.5		251.0	100.9
RSD	0.3	0.4		0.4	0.5

The percentage recovery range and RSD values were found to be 98.5-99.7% and within 0.3% respectively. Method accuracy of the volumetric method was also demonstrated by plotting the amount of rosuvastatin calcium measured against the amount present in the samples, both expressed in mg. Linear regression analysis rendered slopes not significantly different from 1 (t test *P*=.05), intercepts not significantly different from zero (t test *P*=.05) and r = 0.9999, the RSD was 0.3. (Table 3 and Figure 2)

TABLE 3. Accuracy

% of nominal value	Added amount (mg)	Found amount (mg)	Recovery (%)	Average recovery (n=3)	RSD (%)
80	194.0	191.6	98.8	99.1	0.4
	194.2	192.5	99.1		
	193.1	190.7	98.8		
	192.2	189.8	98.7		
	193.1	191.6	99.2		
	193.0	191.6	99.3		
	194.0	193.5	99.7		
100	241.2	238.2	98.8	98.9	0.2
	242.5	239.1	98.6		
	241.5	239.1	99.0		
	242.1	240.0	99.2		
	244.3	241.8	99.0		
	244.7	242.7	99.2		
120	288.8	285.6	98.9	98.9	0.2
	292.5	289.3	98.9		
	290.3	287.5	99.0		
	289.9	287.5	99.2		
	291.7	288.4	98.9		
	291.9	287.5	98.5		
Mean (n=18)				99.0	0.3

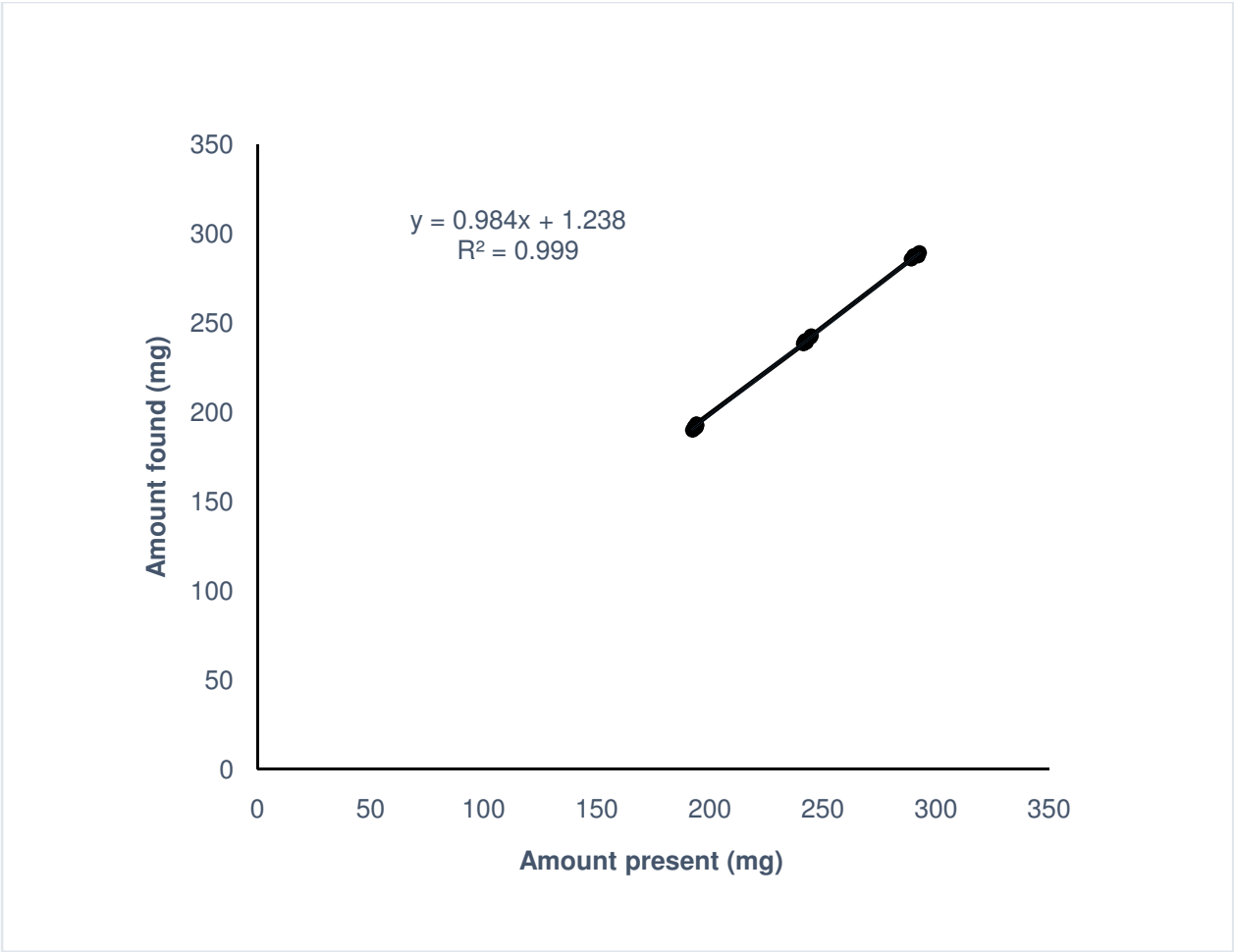

Figure 3. Accuracy

4. CONCLUSIONS

The volumetric method proposed is simple, rapid and inexpensive and can therefore be applied to the determination of rosuvastatin calcium in raw material. Method validation yielded good results and included linearity, precision and accuracy.

REFERENCES

[1] Beludari MI, Prakash KV, Mohan GK, RP-HPLC method for simultaneous estimation of Rosuvastatin and Ezetimibe from their combination tablet dosage form. Int J Chem Anal. Sci. 2013;4:205-209

[2] Uyar B, Celebier M, Altinoz S, Spectrophotometric determination of rosuvastatin calcium in tablets. Pharmazie 2007;62(6):411-413

[3] Dannana GS, Marothu VK, Extractive Spectrophotometric methods for the determination of Rosuvastatin calcium in pure form and in pharmaceutical formulations by using safranin O and methylene blue. E. J. Chem. 2007;4:46-49.

[4] Gupta A, Mishra P, Shah K, Simple UV Spectrophotometric Determination of Rosuvastatin Calcium in Pure Form and in Pharmaceutical Formulations. E. J. Chem. 2009;6(1):89-92.

[5] Astarita A, DellaGreca M, Iesce MR, Montanaro S, Previtera L, Temussi F, Polycyclic compounds by sunlight exposure of the drug rosuvastatin in water. J. Photoch. Photobio A. 2007;187: 263-268.

[6] Fabris J, Makuc D, Casar Z, Plavec J, Conformational analysis of E/Z-isomeric pairs of rosuvastatin and its lactonized analogues. Tetrahedron 2013;69: 6262-6268.

198 [7 Litvic M, Smic K, Vinkovic V, Filipan-Litvic M, A study of photodegradation of drug rosuvastatin calcium
199 in solid state and solution under UV and visible light irradiation: The influence of certain dyes as efficient
200 stabilizers. J. Photoch. Photobio A. 2013;252:84-92.
201 [8] Trivedi HK, Patel MC, Development and Validation of a Stability-Indicating RP-UPLC Method for
202 Determination of Rosuvastatin and Related Substances in Pharmaceutical Dosage Form. Sci. Pharm.
203 2012; 80:393-406.
204 [9] Bergman E, Forsell P, Tevell A, Persson AM, Hedeland M, Bondesson U, Knutson L, Lennernäs H,
205 Biliary secretion of rosuvastatin and bile acids in humans during the absorption phase. Eur. J. Pharm. Sci.
206 2006;9:205-214.
207 [10] Lan K, Jiang X, Li Y, Wang L, Zhou J, Jiang Q, Ye L, Quantitative determination of rosuvastatin in
208 human plasma by ion pair liquid–liquid extraction using liquid chromatography with electrospray ionization
209 tandem mass spectrometry. J Pharm Biomed. Anal. 2007;44:540-546.
210 [11] Zhang R, Li Y, Jiang X, Wang L, Pharmacokinetics and Tolerability of Multiple-Dose
211 Rosuvastatin: An Open-Label, Randomized-Sequence, Three-Way Crossover Trial in Healthy Chinese
212 Volunteers. Curr. Ther. Res. 2009;70(5):392-404.
213 [12] Hauptstein S, Müller C, Dünnhaupt S, Laffleur F, Bernkop-Schnürch A, Preactivated thiomers:
214 Evaluation of gastroretentive minitablets. Int. J. Pharm. 2013;456:473-479.
215 [13] Balakumar K, Raghavan CV, Selvan NT, Prasad RH, Abdu S, Self-nanoemulsifying drug delivery
216 system (SNEDDS) of Rosuvastatin calcium: Design, formulation, bioavailability and pharmacokinetic
217 evaluation. Colloid Surface B 2013;112:337-343.
218 [14] Trivedi RK, Kallem RR, Mullangi R, Srinivas NR, Simultaneous determination of rosuvastatin and
219 fenofibric acid in human plasma by LC–MS/MS with electrospray ionization: Assay development,
220 validation and application to a clinical study. J Pharm Biomed. Anal. 2005;39:661-669.
221 [15] Lee HB, Peart TE, Svoboda ML, Backus S, Occurrence and fate of rosuvastatin, rosuvastatin
222 lactone, and atorvastatin in Canadian sewage and surface water samples. Chemosphere 2009; 77:1285-
223 1291.
224 [16] Shah Y, Iqbal Z, Ahmad L, Khan A, Khan MI, Nazir S, Nasir F, Simultaneous determination of
225 rosuvastatin and atorvastatin in human serum using RP-HPLC/UV detection: Method development,
226 validation and optimization of various experimental parameters. J. Chromatogr. B 2011;879:557-563.
227 [17] Nasir F, Iqbal Z, Khan A, Ahmad L, Shah Y, Khan AZ, Khan JA, Khan S, Simultaneous
228 determination of timolol maleate, rosuvastatin calcium and diclofenac sodium in pharmaceuticals and
229 physiological fluids using HPLC-UV. J. Chromatogr. B, 2011;879:3434-3443.
230 [18] Arayne MS, Sultana N, Tabassum A, RP-LC simultaneous quantitation of co-administered drugs for
231 (non-insulin dependent) diabetic mellitus induced dyslipidemia in active pharmaceutical ingredient,
232 pharmaceutical formulations and human serum with UV-detector Clin. Chim. Acta 2013;425:54-61.
233 [19] Lee D., Roh H., Son H., Jang S.B., Lee S., Nam S.Y. and Park K., Pharmacokinetic Interaction
234 Between Rosuvastatin and Metformin in Healthy Korean Male Volunteers: A Randomized, Open-label, 3-
235 period, Crossover, Multiple-dose Study. Clin. Ther. 2014;36(8):1171-1181
236 [20] Roh H, Son H, Lee D, Chan HC, Yu C, Park K, Pharmacokinetic Interaction Between Rosuvastatin
237 and Olmesartan: A Randomized, Open-label, 3-period, Multiple- dose Crossover Study in Healthy Korean
238 Male Subjects. Clin. Ther. 2014; 36(8):1159-1170.
239 [21] Assassi AL, Roy CE, Perovitch P, Auzerie J, Hamon T, Gaudin K, Green analytical method
240 development for statin analysis J. Chromatogr. A 2015; 1380:104-111.
241 [22] Narapusetti A, Bethanabhatla SS, Sockalingam A, Repaka N, Saritha V, Simultaneous determination
242 of rosuvastatin and amlodipine in human plasma using tandem mass spectrometry: Application to
243 disposition kinetics. J. Adv. Res. 2014, Available from: http://dx.doi.org./10.1016/j.jare.2014.08.010
244 [23] Skoog DA, West DM, Holler FJ, Química Analítica 6st Ed., Ed. Mc GrawHill, México, 1995
245 [24] International Conference on Harmonization. ICH Q2(R1) Guideline on Validation of Analytical
246 Procedures: Text and Methodology (2005).
247 [25] International Conference on Harmonization. ICH Q7 Guideline on Good Manufacturing Practice Guide
248 for Active Pharmaceutical Ingredients (2010).
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