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Determination of lovastatin, mevastatin, rosuvastatin and simvastatin with HPLC by means of gradient elution

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A novel HPLC method with UV detection was developed and validated in skin penetration (in vitro) studies to identify and quantify lovastatin, mevastatin, rosuvastatin and simvastatin. A Venusil XBP C_{18} (2), 150 x 4.6 mm, 5 µm column (Agela Technologies, Newark, DE) was used with gradient elution (start at 45 % acetonitrile and increase linearly to 90 % after 1 min; hold at 90 % until 6 min and then re-equilibrate at start conditions) and the mobile phase consisted of (A) Milli-Q® water and 0.1% orthophosphoric acid, and (B) HPLC grade acetonitrile. The flow rate was set at 1 ml/min, 240 nm UV detection and an injection volume of 10 µl. Linearity was obtained over a range of 0.50-200.00 µg/ml and correlation coefficients ranging from 0.998-1.000 were obtained. Average recovery ranged from 95.9-100.6 %. The LOD and LOQ values obtained from the slope of a calibration curve and the standard deviation of the response ranged from 0.0138-0.0860 µg/ml and 0.0419-0.2615 µg/ml, respectively, where lovastatin and simvastatin could be detected at a concentration similar to the other statins, but could only be quantified at a higher concentration than the remaining statins. The specificity of the method was proved as accurate and quantification of statins was found, even within the incorporation of other compounds.

1. Introduction

Cholesterol is an essential requirement in a healthy human heart (Ma and Shieh 2006) and adequate amounts are produced by cells (endogenous cholesterol) to perform its function in the body. Consequently, elevation in plasma cholesterol can occur with additional dietary intake of cholesterol, along with other factors, i.e. age, gender and physical inactivity (Iversen et al. 2009) and pre-existing conditions (i.e. insulin resistance). Familial hypercholesterolemia (FH) is an inherited autosomal dominant disorder with several gene mutations implicated in the pathogenesis. These mutations vary in different populations, hence resulting in a diversity of symptoms and severity between countries (Nemati and Astaneh 2010). This widespread genetic disorder results in a lifelong increase in low-density lipoprotein (LDL) and a significant increase in the risk of coronary heart disease (CHD). Research suggests that worldwide as many as 34 million individuals are affected by FH and that every minute a new individual is born with this condition (Goldberg and Gidding 2016). Early diagnosis and treatment of FH is essential to mitigate the excess risk of premature atherosclerotic cardiovascular disease (Bouhairie and Goldberg 2015), which is primarily done with 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors or statins. These agents perform their pharmacological action primarily by decreasing cholesterol synthesis and further reduction of LDL (20-55 %) and triglycerides (8-30 %) occur due to up regulating of the LDL receptor gene. Lastly, inhibition of the synthesis of apolipoprotein B100 and triglyceride-rich lipoproteins (as well as secretion of last mentioned) causes a reduction in atherogenic lipoproteins (Amly and Karaman 2015).

Although these lipid lowering agents are generally well tolerated (Black 2002), side-effects such as hepatotoxicity (Law and Rudnicka 2006) and gastrointestinal effects (e.g. nausea, vomiting and abdominal cramps) (Mancini et al. 2013) have been reported with the use of statins. Yu et al. (2018) found that lower adherence can be associated with side-effects of statins, which results in limited pharmacological efficacy and benefits of statins (Al-Foraih and Somerset 2017). As metabolism of the statins (with the exception of rosuvastatin (Etemad, 2004)) is largely carried out by the cytochrome P450 (CYP450) family of enzymes, an increased probability for drug interactions can occur when co-administered with other drugs metabolized in the same enzymatic pathway (Muscari et al. 2002; Sica and Gehr 2002). These interactions can result in an increased risk of side-effects (Causevic-Ramosevac and Semiz 2013).

Because of the problems and limitations associated with the oral administration of these compounds, the purpose of this study was to attempt the transdermal delivery of statins to overcome limitations experienced with oral administration, such as low plasma concentration, hepatic first-pass metabolism and in addition, gastrointestinal factors and poor patient compliance (Kaestli et al. 2008). Although literature suggests the use of HPLC methods for the analysis of statins, *in vitro* skin permeation studies will require the method to be sensitive enough to detect extremely low drug concentrations. Consequently a new method was developed, as no methods for the purpose of transdermal delivery of lovastatin, mevastatin, rosuvastatin and simvastatin was available in literature.

2. Investigations, results and discussion

The validation of this method was done by means of linearity, accuracy, precision (both inter-day and intra-day), ruggedness (sample stability and system repeatability), specificity, LOD (lowest limit of detection) and LLOQ (lowest limit of quantification). The Table represents the validation parameters obtained during the validation of this method.

The linearity of the selected statins was done by preparing a stock solution (±20 mg in 100 ml methanol (200 µg/ml)) for each of the statins. This stock solution was then utilised to prepare dilution 1 (±20 µg/ml) and dilution 2 (±2 µg/ml). The stock solution along with the dilutions were then transferred into HPLC vials and analysed (in duplicate) at injection volumes of 2.5 µl, 5.0 µl, 7.5 µl and 10.0 µl. Different concentration ranges were obtained (Table). The regression equations obtained from plotting peak area versus statin concentration are displayed in the Table.

Table: Validation parameters obtained for the four statins

Linearity				
	Lovastatin	Mevastatin	Rosuvastatin	Simvastatin
Concentration range (µg/ml)	0.480-191.900	0.624-249.500	0.488-195.300	0.547-218.900
Regression equation	y = 0.4916x + 0.0927	y = 0.3772x + 0.1555	y = 0.4159x - 0.0234	y = 0.4304x + 0.1386
Correlation coefficient (R^2)	1	1	1	0.9998
LOD and LLOQ				
LOD (µg/ml) DL (detection limit) =3.3 X σ/S	0.0806	0.0148	0.0863	0.0138
LLOQ (µg/ml) QL (quantification limit) =10 X σ/S	0.2441	0.0447	0.2615	0.0419
Accuracy				
Mean recovery (%)	99.7	98.6	95.9	100.6
%RSD	0.3	0.2	0.3	0.4
Precision				
Intra-day precision (%RSD)	0.73	0.70	0.79	0.58
Inter-day precision (%RSD)	2.72	2.48	2.65	1.12
Sample stability over 24 h				
Percentage recovery (%RSD)	0.99	1.23	0.61	0.74
Peak area (%RSD)	0.99	1.24	0.61	0.74
System repeatability				
Retention time (%RSD)	0.047	0.035	0.000	0.000
Peak area (%RSD)	0.92	0.10	0.12	0.08

(σ = Standard deviation of y-intercepts, S = Slope, RSD = relative standard deviation)

When observing the correlation coefficient (R^2), it was concluded that an excellent correlation between the peak area and the statins concentration was obtained within the tested concentration range. The recovery of the experiments was used to determine the accuracy of the proposed method. Lovastatin reached an average recovery of 99.7% (%RSD = 0.3), mevastatin 98.6% (%RSD = 0.2), rosuvastatin 95.9% (%RSD = 0.3) and simvastatin 100.6% (%RSD = 0.4). The percentage recovery is required to be within 98 to 102%, as this percentage is seen as a ratio between the estimated results and the obtained results, thus serving as an indicator of the accuracy of the methods (APVMA, 2004). Shabir (2005) also stated that 90-100% could serve as acceptable recovery range for active pharmaceutical ingredients (APIs) over a target concentration range of 80-100%. In addition, according to UNDOC (2009) recovery needs not necessarily be 100%, but rather to be consistent as displayed by the %RSD values. Therefore, it can be concluded that the method was accurate as recovery of the selected statins fell within the required limits. Calculations of LOD and LOQ were based on the slope of a calibration curve and the standard deviation of the response by utilising the formulas displayed in the Table for the LOD and LOQ, respectively (Swartz and Krull 2012). The sample was prepared by weighing ±5 mg of each selected statins into a 100 ml volumetric flask, made up to volume with methanol, where after 1 ml of the stock solution was transferred to another 100 ml volumetric flask and made up to volume with methanol. A volume of the dilution was then placed in a HPLC vial and injected six times at injection volumes of 2.5 µl, 5.0 µl, 7.5 µl and 10.0 µl.

Precision for this analytical method was examined firstly, in terms of intra-day precision (repeatability) and secondly, according to inter-day precision (reproducibility). Triplicate samples were prepared of ±160 µg/ml, 200 µg/ml and 240 µg/ml for each of the statins. From intra-day precision (repeatability), these samples were analysed in duplicate. The acceptance criteria for intra-day precision are considered to be a %RSD equal or less than 2.00% (APVMA 2004; Snyder et al. 1997b), and therefore, it could be concluded that each of the selected statins displayed satisfactory results (%RSD ≤ 2%) as displayed in Table.

The samples used to determine inter-day precision (reproducibility) was prepared in similar to those used for intra-day precision, while for this assay only the intermediate concentration (200 µg/ml) was utilised. These three intermediate concentration samples were analysed in duplicate. By observing Table, it can be seen that the statins present with acceptable %RSD values within the acceptance criteria of equal or less than 3.00% (Rafael et al. 2007). The sample stability (over 24 h) and system repeatability was also determined. Sample stability was performed by injecting a standard (±20 mg in 100 ml methanol) hourly for 24 h. This analysis is performed so that sample solution will not be used for a period longer than it takes to degrade by 2%. System repeatability was done by injecting the standard sample (as prepared for 24 h) seven consecutive times to evaluate the repeatability of peak areas, as well as retention times of samples under same day conditions.

As the acceptance criteria for sample stability is considered a % RSD of equal or less than 2% (Shabir 2003; Suresh et al. 2010) and that of system repeatability less than 1% (Shabir 2004), it can be concluded from the Table that the statins met the criteria from both the percentage recovery and peak area as seen for sample stability, and from both the retention time and peak area as displayed by system repeatability (Table). After completion of sample stability and system repeatability, specificity of the analytical method was determined.

Specificity of an analytical method can be defined as the ability of the method to accurately detect an analyte in the presence of degradants, the matrix or impurities (Chandran and Singh 2007). Acceptable specificity can be seen as the pure identification of the target analyte in the presence of similarly structured compounds (ICH 2005); therefore, there should be no peak interference with the retention time of the API (Snyders et al. 1997).

Figures 1 A-D indicates that additional peaks formed as a result of degradation caused by stressing the sample; however, the respective statins peak retention times were not affected:

- Lovastatin: ±5.17 min
- Mevastatin: ±4.75 min
- Simvastatin: ±4.87 min
- Rosuvastatin: ±3.09 min

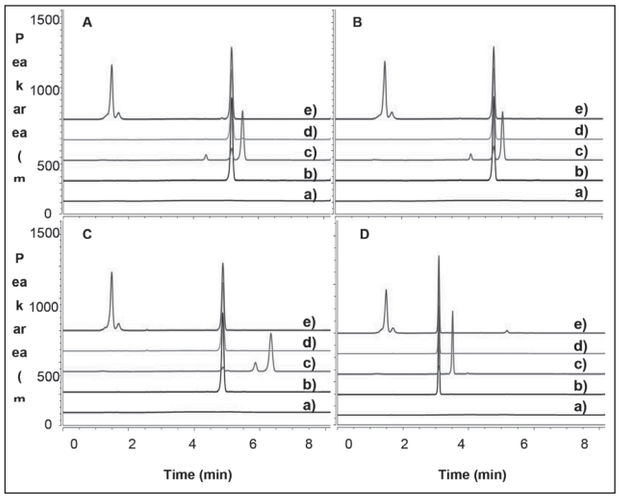

Fig. 1: HPLC chromatogram showing specificity data obtained: A) lovastatin, B) mevastatin, C) simvastatin and D) rosuvastatin. In addition for a) placebo solution, b) statin standard solution, following the sample solution of respective statin stressed with 200 µl of c) HCl, d) H_2O and e) H_2O_2

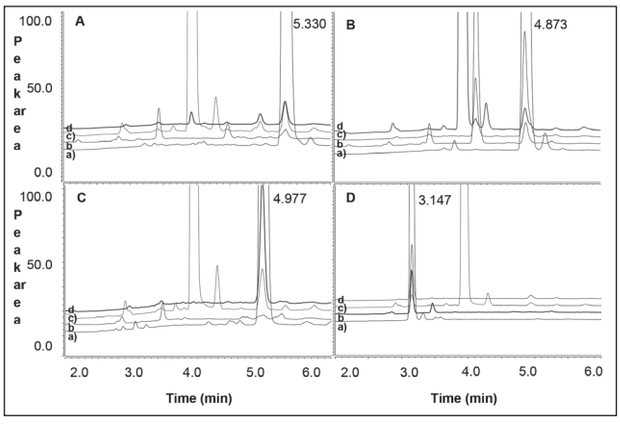

Fig. 2: Chromatographic representation of A) lovastatin, B) mevastatin, C) simvastatin and D) rosuvastatin. Chromatograms represents a) standard solution sample of respective statins, b) buffer (receptor phase) extraction sample, c) tape stripping sample of the statin and d) skin sample of the statin.

After completing the validation of the analytical method, the method was utilised during skin diffusion studies for analysis of diffusion (buffer phase), tape stripping and skin samples.

The chromatograms were obtained during skin diffusion studies performed on the respective statins. From Figs. 2 A-D it is clear that the peak of the tape stripping, skin and buffer (receptor phase) samples, displayed the same retention time as the standard solutions. The peak visible to the right in (c) of Figs. 2 A-C and to the left of Fig. 2 D is due to the 3M Scotch® Magic™ Tape used during tape stripping.

3. Experimental

A Dionex UltiMate 3000 dual system was used during this study consisting of ternary gradient pumps, column ovens, autosampler and diode array detectors operated on Chromeleon 7.2 data acquisition and analysis software (Thermo Fisher Scientific Inc., Waltham, MA). A Venusil XBP C_{18} (2), (150 x 4.6 mm, 5 µm) (Agela Technologies, Newark, DE) column was used for this method. Gradient elution was used with mobile phase A (A) consisting of Milli-Q® water and 0.1% orthophosphoric acid and mobile phase B (B) consisting of HPLC grade acetonitrile. The flow rate was set to 1 ml/min at 45% acetonitrile and to increase linearly to 90% after 1 min, where it was held at 90% for 6 min and then re-equilibrate at start conditions (45%). The default injection volume was set at 10 µl and the UV detector was set at 240 nm. The retention times were ±3.090, 4.673, 4.867 and 5.140 min for rosuvastatin, mevastatin, simvastatin and lovastatin, respectively, with a run time of 10.000 min. Methanol was used as a solvent during the course of the method validation.

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References

Al-Foraih M, Somerset S (2017) Factors affecting adherence to statins in hypercholesterolemic Kuwaiti patients: a cross-sectional study. Med Princ Pract 26: 35 – 40.

Amly W, Karaman R (2015) Lipid lowering medications – uses, side effects, pharmacokinetic properties and approaches to improve bioavailability. In: Karaman R (ed.) Commonly used drugs -uses, side effects, bioavailability and approaches to improve. New York: Nova Science Publishers. p. 131 – 172.

Australian Pesticides and Veterinary Medicines Authority (2004) Guidelines for the validation of analytical methods for active constituents, agricultural and veterinary chemical products. http://apvma.gov.au/sites/default/files/docs/guideline-69-analytical-methods.pdf Date of access: 21 Nov. 2017.

Black DM (2002) A general assessment of the safety of HMG CoA reductase inhibitors (statins). Curr Atheroscler Rep 4: 34 – 41.

Bouhairie VE, Goldberg AC (2015) Familial hypercholesterolemia. Cardiol Clin 33: 169 – 179.

Causevic-Ramosevac A, Semiz S (2013) Drug interactions with statins. Acta Pharm 63: 277 – 293

Chandran S, Singh RSP (2007) Comparison of various international guidelines for analytical method validation. Pharmazie 62: 4 – 14.

Etemad L (2004) Statins and potentially interacting medications: a managed care perspective. Prevent Med Managed Care 4: S27 – S29.

Goldberg AC, Gidding SS (2016) Knowing the prevalence of familial hypercholesterolemia matters. Circulation 133: 1054 – 1057.

International Conference of Harmonisation (2005) Validation of analytical procedures: text and methodology Q2 (R1). http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products /Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf Date of access: 21 Nov. 2017.

Iversen A, Jensen JS, Scharling H, Schmohr P (2009) Hypercholesterolaemia and risk of coronary heart disease in the elderly: Impact of age. The Copenhagen city heart study. Eur J Intern Med 20: 139 – 144.

Kaestli L, Wasilewski-Rasca A, Bonnabry, P, Vogt-Ferrier N (2008) Use of transdermal drug formulations in the elderly. Drugs Aging 25: 269 – 280.

Law M, Rudnicka AR (2006) Statin safety: a systematic review. Am J Cardiol 97: 53c – 60c.

Ma H, Shieh K (2006) Cholesterol and human health. J Am Sci 2(1): 46 – 50.

Mancini GBJ, RA, Tashakkor AY, Baker S, Bergeron J, Fitchett D, Frohlich J, Genest J, Gupta M, Hegele RA, Ng DS, Pearson GJ, Pope J (2013) Diagnosis, prevention and management of statin adverse effects and intolerance: Canadian working group consensus update. Can J Cardiol 29: 1553 – 1568.

Muscari A, Puddu GM, Puddu P (2002) Lipid-lowering drugs: are adverse effects predictable and reversible? Cardiology 97: 155 – 121.

Nemati MH, Astaneh B (2010) Optimal management of familial hypercholesterolemia: treatment and management strategies. Vasc Health Risk Manag 6: 1079 – 1088.

Rafael JA, Jabor JR, Casagrande R, Georgetti, SR, De Fátima Borin M, Fonseca MJV (2007) Validation of HPLC, DPPH and nitrosation methods for mesalamine determination in pharmaceutical dosage forms. Braz J Pharm Sci 43: 97 – 103.

Shabir GA (2003) Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. J Chromatogr A 987: 57 – 66.

Shabir GA (2004) A practical approach to validation of HPLC methods under current good manufacturing practices. J Valid Technol 10: 210 – 218.

Shabir GA (2005) Institute of Validation Technology: step-by-step analytical methods validation and protocol in the quality system compliance industry. http://www.ivtnetwork.com/sites/default/files/Step-byStep%20Analytical%20Methods%20Validation%20and%20Protocol%20in%20the%20Quality%20System%20Compliance%20Industry.pdf Date of access: 23 Aug 2018.

Sica DA, Gehr TW (2002) Rhabdomyolysis and statin therapy: relevance to the elderly. Am J Geriatr Cardiol 11: 48 – 55.

Snyders LR, Kirkland JJ, Glajch JL (1997). Completing the method: validation and transfer. In: Snyder LR, Kirkland JJ, Glajch JL (eds.) Practical HPLC method development. 2nd ed. New York: John Wiley & Sons. p. 685 – 713.

Suresh R, Anarthanan SVJ, Manavalan R, Valliappan K (2010) Aspects of validation in hplc method development for pharmaceutical analysis – comparison of validation requirements by FDA, USP AND ICH. Int J Pharm Sci Res, 1(12):123 – 132.

Swartz ME, Krull IS (2012) Handbook of analytical validation. Boca Raton: CRC Press.

UNODC (United Nations Office on Drugs and Crime) (2009) Guidance for the validation of analytical methodology and calibration of equipment used for testing of illicit drugs in seized materials and biological specimens. New York: United Nations. https://www.unodc.org/documents/scientific/validation_E.pdf Date of access: 21 Nov. 2017.