STUDIA UBB CHEMIA, LXVII, 4, 2022 (p. 19-26)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2022.4.02

DERIVATIZATION OF ROSUVASTATIN AS METHYL ESTER FOR ITS ANALYSIS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY IN PLASMA

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ABSTRACT. A new analytical procedure for rapid and selective derivatization of rosuvastatin directly in plasma into their corresponding methyl esters was developed for gas chromatography-mass spectrometry analysis. This work is the first method of selective derivatization of the carboxyl group from rosuvastatin to methyl ester. The lipids from plasma were extracted with heptane before derivatization. The methyl esters were obtained by reaction with methyl iodide in solution of dimethyl sulfoxide and in the presence of anhydrous potassium carbonate. The optimal conditions for selective methylation have been established. The derivatization was carried out in 2 min. The method was validated for the analysis of rosuvastatin in plasma. The methyl ester of rosuvastatin was identified by electron ionization mass spectrometry. The electron ionization mass spectrometer detector response was linear up to 300 ng/mL. The limit of detection and limit of quantification values in total ion chromatogram method were 0.4 ng/mL and 1.2 ng/mL, respectively.

Keywords: *Rosuvastatin, Plasma, Methyl esterification, Gas chromatography-mass spectrometry analysis.*

INTRODUCTION

Rosuvastatin is among the most effective anti-atherosclerotic drugs with a strong impact on cardiovascular diseases [1].

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Usually, the analysis of rosuvastatin in biological fluids was performed by liquid chromatography with a detector in the ultraviolet range [2], or in fluorescence [3] or by mass spectrometry (MS) [4]. Some liquid chromatographic (LC) analysis has many drawbacks such as low sensitivity, narrow linearity ranges, high sample volume, and expensive automated extraction procedure. Gas chromatography (GC) has been less used in the separation of statins, due to their high boiling points, which leads to a derivatization reaction to increase their volatility. However, the advantages of GC–MS analysis are a high sensitivity and selectivity to determine rosuvastatin in plasma. GC analysis of statins was performed without derivatization [5] or as silylated derivatives [6,7]. Rosuvastatin has not yet been derivatized as methyl ester for GC analysis.

Quantitative analysis of rosuvastatin from plasma is strongly influence by the presence of proteins and plasma lipids. Proteins can retain uncontrolled amount of drug and a high content of plasma lipids give unwanted peaks and a high background noise. The matrix effects are very evident in plasma samples from patients with high lipid levels [8]. Consequently, in both LC and GC analysis, sample preparation prior to analysis for protein solubilization and lipid elimination are important steps to avoid these disadvantages. Liquid-liquid extraction with different organic solvents could be a solution, because organic solvents generate precipitation and they extracted also plasma lipids. Chloroform, ethyl acetate, n-hexane, dichloromethane, n-hexane-dichloromethane- isopropanol (20:10:1, v/v/v) was investigated and ethyl acetate was found with no-concentration-dependent extraction recovery and acceptable matrix effect [9]. A mixture of chloroform–methanol has been widely used to extract lipids from different tissues and biological fluids, dichloromethane–methanol and methyl-tert-butyl ether–methanol solvent mixtures have also been reported as good solvents for lipid extraction that provide equivalent lipid recoveries [10]. However, rosuvastatin is slightly soluble in these solvents.

Solid phase extraction may have a higher selectivity for plasma lipids. Both cation and anion exchange resins have been used to retain lipids [8], but they will also retain rosuvastatin because it has a carboxylic group. Solid phase extraction on hydrophobic-lipophilic balanced copolymer sorbent [11] retains non-polar lipids.

The aim of this work is a selective derivatization of rosuvastatin as methyl ester in plasma for GC analysis. The work has a novelty value because it is the first method of derivatization of rosuvastatin in its methyl ester for GC-MS analysis. The method has the advantage that it requires less expensive equipment compared to liquid chromatography coupled with mass spectrometry.

RESULTS AND DISCUSSION

Rosuvastatin is a synthetic drug administered as calcium salt. Rosuvastatin molecules are taken up by albumin, up to the saturation of the sites available for this purpose. Rosuvastatin molecules are released more easily from albumin when the albumin is solubilized in the reaction solvent. Among the dipolar aprotic solvents, dimethyl sulfoxide has the highest solubilizing power of albumin [12].

The esterification of rosuvastatin with methyl iodide is a nucleophilic substitution [13]. The main reactions that occur during the esterification of rosuvastatin with methyl iodide (CH_3I) and solid K_2CO_3 are shown in the Scheme 1.

The direct reaction between the calcium salt of rosuvastatin and methyl iodide does not lead to methyl esters. For this reason, calcium salt is transformed into potassium salt by adding solid potassium carbonate.

The first step is the dissolution of the solid K_2CO_3 in dimethyl sulfoxide. The solubility of K_2CO_3 in dimethyl sulfoxide is very low (47 g/L). However, the dissolved K_2CO_3 is consumed in this reactions with rosuvastatin (Eq.1), but is continuously replaced by the dissolution of the solid base. Finally, the rosuvastatin anion will react with methyl iodide and will generate methyl esters (Eq. 2). Anhydrous powder of K_2CO_3 is hygroscopic was also used in this method for the purpose of retaining water from the system. For this reason, potassium carbonate was used in molar excess up 40 compared to rosuvastatin. The volumetric excess of methyl iodate was up to 10.

$$(\text{Rosuv-COO})_2\text{Ca} + \text{K}_2\text{CO}_3 \longrightarrow 2\text{Rosuv-COOK} + \text{CaCO}_3 \quad (1)$$

$$\text{Rosuv-COOK} + \text{CH}_3\text{I} \longrightarrow \text{Rosuv-COOCH}_3 + \text{KI} \quad (2)$$

Scheme 1: The main reactions of the esterification process

Studies under certain stress conditions have shown that rosuvastatin is stable in the presence of bases and degrades in an acidic environment as well as in heat [14]. Therefore, performing the reaction in a weak basic medium of K_2CO_3 and at room temperature avoids possible degradation reactions.

The injection of methylated products may introduce small amounts of plasma lipids into the injector port. Proteins and other polar compounds were partially retained on solid K_2CO_3. In order to avoid this contamination, the capillary column must be protected with a guard capillary column.

Figure 1 shows the GC–MS total ion chromatogram. The chromatographic peaks have a symmetrical shape.

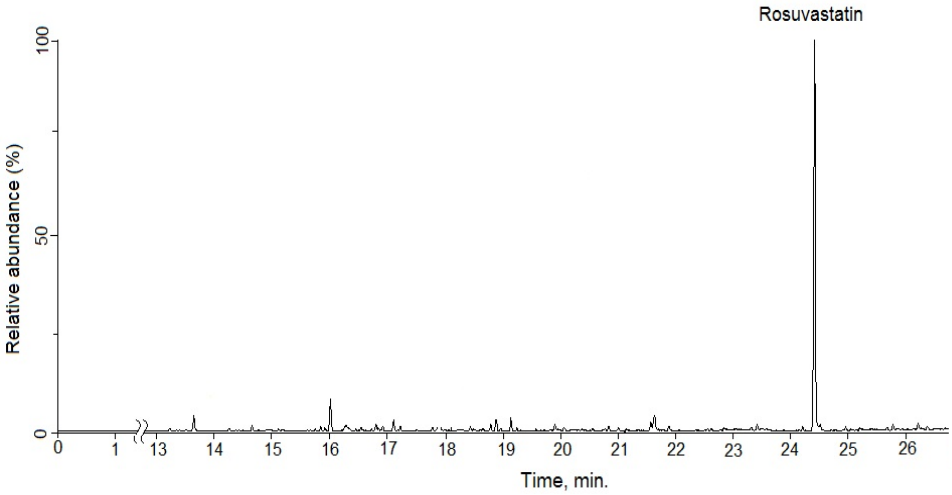

Figure 1. GC–MS total ion chromatogram of rosuvastatin as methyl ester into a plasma sample. Chromatographic and mass spectrometric conditions are given in the Experimental section.

Figure 2 shows the electron ionization (EI) mass spectrum of rosuvastatin methyl ester. The methyl ester of rosuvastatin was identified based on the molecular ion (M+•) at m/z 495 and the characteristic fragment ions. The fragment ions at m/z 463 and m/z 459 are generated by the loss of one methanol molecule and two water molecules respectively from the molecular ion. The fragment ion at m/z 445 may result from the m/z 463 ion due to the loss of a water molecule. The ion at m/z 421 may be the McLafferty rearrangement ion at the methyl ester group in the hydrocarbon chain. The fragment ion at m/z 403 may represent the expulsion of a water molecule from m/z 421. The fragment ions at m/z 392, m/z 378, m/z 348, and m/z 322 may result from the cleavage of the C-C bonds from the hydrocarbon chain with the carboxylic methyl ester group. Thus, for example, the ion at m/z 392 results from the cleavage between C3 and C4. One pathway for the formation of the base ion at m/z 258 may be the elimination of a sulphur dioxide molecule from the ion at m/z 322.

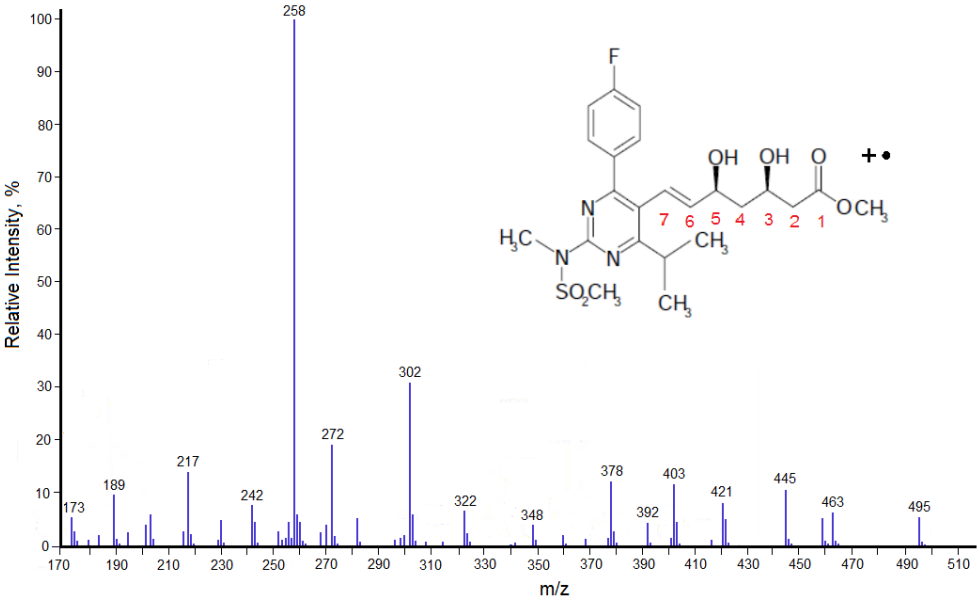

Figure 2. Electron ionization spectrum and chemical structure of rosuvastatin methyl ester.

Quantitative evaluation in this method was performed by the internal standard method [15], using anthracene a standard, which is not affected in the derivatization process. The MS detector response was linear up to 300 ng/mL. The limit of detection (LOD) and limit of quantification (LOQ) values in TIC method were 0.4 ng/mL and 1.2 ng/mL, respectively. The accuracy was tested by adding 6 ng/µL, 30 ng/mL and 60 ng/mL of rosuvastatin into the sample. The average recovery values (n = 5), expressed as mean ± standard deviation (SD), were 98.2±1.2; 98.8±0.9; and 99.3±0.5, respectively.

CONCLUSIONS

This paper provides a sensitive and selective method for the derivatization of rosuvastatin as a methyl ester for GC analysis. The methyl ester of rosuvastatin was identified by mass spectrometry based on molecular ion and characteristic fragment ions. The proposed method was optimized and validated. This work is the first derivatization method of the carboxylic group from rosuvastatin to methyl ester. The derivatization was

performed in 2 min. The lipids from plasma were extracted three times with heptane before derivatization. Dimethyl sulfoxide was used because it proved to be the best reaction medium, but also because it is very good for releasing rosuvastatin from plasma albumin. The method is simple and fast and can have applications in monitoring the concentration of rosuvastatin in plasma in patients with atherosclerosis [16] that can affect different organs.

EXPERIMENTAL SECTION

Standards and reagents

Rosuvastatin calcium salt (≥98%) and anthracene (internal standard) were from Sigma-Aldrich (MO, USA). Methyl iodide, dimethyl sulfoxide (DMSO), chloroform, dichloromethane, potassium carbonate, heptane, and molecular sieves were purchased from Merck (Darmstadt, Germany). All reagents were analytical grade. The potassium carbonate powder was dehydrated by heating at 200°C and then stored in an airtight system to prevent the contact of the powder with moisture.

Collection and storage of blood sample

This study compiled the Declaration of Helsinki regarding the ethical principles for medical research and was approved by the Ethics Committee of the Scientific Research of the University of Medicine and Pharmacy Timisoara. All participants gave written informed consent. The blood sample was taken into commercially available anticoagulant-treated tubes by antecubital venipuncture after at least 12 h of fasting and was immediately centrifuged at 1500xg at room temperature for 10 min. The plasma was apportioned into single use aliquots, which were then stored at -70 °C until analysis.

Statistical analysis

Microsoft Excel 2016 (Microsoft Corp. USA) and Statistical Test Calculator online version 2018 from Social Science Statistics (https://www.socscistatistics.com/tests/) were used for statistical analyses.

Derivatization method of rosuvastatin as methyl ester

The methylation reactions were carried out in 2 mL glass vials with silicone septa lined screw caps. An aliquot of plasma sample (200 µL) was introduced with a chromatographic syringe into the glass vial with 0.5-1.0 mL of dimethyl sulfoxide. Rosuvastatin was dissolved in dimethyl sulfoxide and

was added to the sample. Anhydrous powder of potassium carbonate salt (40 mg) was added to the sample solution and was vortexed for 2 min at room temperature. After the addition of a volume of 0.5 mL of heptane, the mixture was vortexed a few seconds and the heptane layer was removed with a syringe. This lipid extraction step was repeated three times.

Then methyl iodide (10 µL) was added and the glass vial was vortexed at room temperature (20°C) for 2 min. Methylation was performed without inert gas atmosphere or special drying conditions. Rosuvastatin methyl ester was extracted with chloroform (3x0.5 mL). The extraction solvent can be evaporated under nitrogen flow and the dried residue containing rosuvastatin methyl ester was re-dissolved in dichloromethane for GC injection.

Instrumentation

All GC–MS analyses were carried out on a gas chromatograph that had installed both a FID detector and a mass spectrometer model Varian 450 GC 240 MS Ion Trap (Agilent, CA, USA). A Varian Workstation software was used for data acquisition and processing. The GC was equipped with HP-1 fused silica capillary column from Hewlett-Packard (SUA) with 30m length, 0.25mm internal diameter, and 0.25µm polydimethylsiloxane cross-bonded film. The capillary column was protected by contaminants with a guard capillary column (5 m length and 0.25 mm inner diameter). A split/splitless injector was used in split mode with a split ratio of 50:1. Sample injection was splitless for 1.0 min. The GC inlet linear had a plug of silylated glass wool for improving the vaporization and for the protection of the capillary column. The carrier gas was high-purity helium at a flow rate of 1 ml/min. The GC oven temperature was started from 120°C and then increased to 270°C with 30°C/min and kept for 20 min. After that, the temperature was increased at 50°C rate to 310°C and kept at the final temperature for 5 min. The temperature of the injector was set to 300°C. The transfer line temperature was 280°C. The temperature of the ion source was set to 220°C. The mass spectra were recorded in the positive-ion electron impact ionization mode in the scan range m/z 50–600. The energy of the emitted electrons was 70 eV and was turned off for the first 3 min of the chromatographic run to avoid filament damage during sample analysis. Chromatograms were assessed in total ion current mode for both qualitative and quantitative analyses.

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