Indirect Oxygen Flask-Atomic Absorption Spectrometric Determination of Rosuvastatin Calcium

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Introduction

Rosuvastatin calcium, is a cholesterol lowering drug commonly referred to as “statins”, was approved for the treatment of dyslipidemia [1-3]. Rosuvastatin calcium (RSC) is chemically bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2- [methyl- (methyl sulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3, 5-dihydroxy hept-6-enoicacid] calcium salt (Figure 1). It is a synthetic lipid lowering agent, selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the key rate-limiting enzyme of cholesterol biosynthesis in liver.

ROS is used to reduce the amounts of LDL cholesterol, total cholesterol, triglycerides and a lipoprotein B in the blood. ROS also increases the level of HDL cholesterol in the blood. These actions are important in reducing the risk of atherosclerosis, which in turn can lead to several cardiovascular complications such as heart attack, stroke and peripheral vascular disease. The empirical formula for rosuvastatin calcium is (C22H27FN3O6S)2Ca and the molecular weight is 1001.14. Due its proved importance, the available literature reveals that Rosuvastatin Calcium (RSC) has been determined by different analytical techniques. Spectrophotometric [4-15], thin layer chromatography (TLC) [16], capillary electrophoresis [17], mass spectrometry [18-24], liquid chromatography (HPLC, UPLC) [25-51], electrochemical methods [52,53] and complex metric titration [54]. All of the previously published methods are direct ones.

The Schöniger flask or famously known as oxygen flask method (OF) [55] is a well proven technique for the combustion and then subsequent analysis of a range of elements including Chlorine, Bromine, Iodine, Fluorine and a number of metals. The combustion of the sample is a simple procedure and involves placing a few milliliters of absorbent solution in a flask. The sample is weighed...
Experimental

Pure drug by using Muffle Furnace Method

0.075 gm of the pure drug claimed to contain 3 mg Calcium (Ca) is transferred to a crucible and heated for 3 hours at 800°C. The sample is expected to turn to Ca oxide. The resulted oxides are dissolved in 0.1 M nitric acid and quantitatively transferred to 25 mL measuring flask with rinsing of the crucible and completion to the mark with 0.1 N nitric acid. The concentration of Ca in the flask is calculated to be 120 µg per mL. An aliquot is diluted to obtain a solution contains 12 µg per mL of Ca. This solution is measured using an atomic absorption spectrophotometer.

By using oxygen flask method

The combustion step: In a weighing stick, weigh accurately 76-128 mg of Ros-Ca which is equivalent to 3-5 mg of Ca element content. Transfer onto the conventional L-shaped ashless filter paper. Fold the latter and fix it to the platinum gauze sample holder. Charge the 1 L flask with 25 mL 0.1 M HNO₃ soln. Flush with oxygen, at a suitable rate, for 3 min., then combust as usual. The resulted solutions, after combustion, are quantitatively transferred to 50 mL measuring flasks and completed to the mark with 0.1 N nitric acid soln. These solutions are expected to contain 60.8 µg - 100.8 µg per mL Ca respectively. Dilution is carried out to obtain solutions of ≈12 µg per mL of Ca. These solutions are directly aspirated and measured using the atomic absorption spectrophotometer.

Tablet formulations

Weighed and transferred 10 tablets into a mortar and made a fine powder. Aliquots of the powder are weighed in a similar range for both decomposition by the oxygen flask or the muffle furnace.

Flame Atomic Absorption (FAAS) procedure

The concentration of the free Ca in the solution is directly measured at λᵣₙ equals 422.7 nm with detection limit of 50 µg/L using a mixture of Acetylene – Nitrous Oxide flame.

Results and Discussion

To carry out the idea of indirect determination of RSC, we firstly tried the mineralization process of the drug through simple evaporation of its concentrated nitric acid solution in a beaker over a hot plate till near dryness, but this procedure failed to achieve decomposition. Reflux of the drug’s nitric acid soln. for 3 hrs. failed, also to realize decomposition. Hence, the thought was directed towards the use of more efficient method for the oxidative destruction of RSC. We recalled the oxygen flask method of Schöniger [55] that has proved its potentiality as an elegant mineralization method. Our last application of it was about 20 years ago [56] where we used it for the successful decomposition of organo calcium compounds. Parallelly, we combusted samples of RSC contained in porcelain crucibles in the muffle furnace at 800°C for three hrs. As a conformational procedure both last gave quantitative calcium results, where each determined calcium atom is equivalent to two molecules of rosuvastatin. Good results have been obtained with the pure active ingredient (Table 1), but,
unfortunately and surprisingly, erroneous inconsistent results are obtained with all the tested tablet formulations.

This surprise has been dissipated on examining the group of active ingredients and excipients accompanying RSC in each of the tested tablet formulations. (Table 2) shows the presence of a number of metal salts and oxides, e.g., Ca phosphate, Mg stearate, titanium dioxide(E171), ferric oxide yellow(E172) and colloidal silicon dioxide are included side by side with RSC. On combustion by either method these elements interfere seriously with Ca.

Table 1: Indirect determination of RSC after combustion in oxygen flask and muffle furnace.

| Parameters | Sample conc. (μg per mL) | Diluted from conc. (μg per mL) | Weight of drug (gm) | Found conc. (μg per mL) | Mean | Standard deviation | % Recovery | Method of digestion | Conditions of digestion |
|------------|--------------------------|-------------------------------|---------------------|--------------------------|------|-------------------|------------|----------------------|------------------------|
| 1          | 12                       | 121.6                         | 0.076               | 12 12 11.8               | 11.9 | 0.08              | 99.2       | Oxygen flask          | Absorbing and washing solution 0.1 M nitric acid |
| 2          | 12                       | 201.6                         | 0.126               | 12 11 13                 | 12   | 0.8               | 100        | Oxygen flask          | Absorbing and washing solution 0.1 M nitric acid |
| 3          | 12                       | 120                           | 0.075               | 13 12 11.5               | 12.2 | 0.62              | 101.6      | Muffle furnace        | Temperature 800 Celsius degree for 3hr, Absorbing and washing solution 0.1 M nitric acid |

Table 2: List of assayed pharmaceutical preparations and their inactive ingredients content.

| Commercial name | Manufacturing company | Pharmaceutical form | No. of tablets/ stripe | Active ingredient’s concentration | Inactive ingredients |
|-----------------|-----------------------|---------------------|------------------------|----------------------------------|----------------------|
| 1 Cholerose 5mg | Marcyrlpharmaceutical industries | Round film coated tablet | 7 | RSC 5.2 mg | Lactose monohydrate, microcrystalline cellulose, Ca phosphate, crospovidon, Mg stearate, hypomellose 29105m, glycerol tri acetate, titanium dioxide, ferric oxide yellow. |
| 2 Crestor 5mg | Astrazeneca-Egypt | Round, yellow film-coated tablet | 7 | RSC 5.2 mg | Tablet core :- lactose monohydrate, microcrystalline cellulose, Ca-phosphate, crospovidone, Mg stearate, Tablet coat: lactose monohydrate, hypomellose, glycerol tri acetate, titanium dioxide (E171), ferric oxide yellow (E172). |
| 3 Estero-map 10mg | Multi-apex for pharmaceutical industries | Round, yellow film-coated tablet | 10 | RSC 10.4 mg | Lactose monohydrate, microcrystalline cellulose povidoneK30, crospovidone, Mg stearate, colloidal silicon dioxide , methocelE5, talc, titanium dioxide, yellow iron oxide, PEG6000 . |
| 4 Justechol 10 mg | AUG PHARMA | Oval film-coated tablet | 7 | RSC 10.4 mg | Tablet core:- micro crystalline cellulose spray dried lactose, dibasic Ca phosphate, cross camellose sodium, hydroxypropyl methyl cellulose, Mg stearate Tablet coat: hydroxypropyl methyl cellulose, poly ethylene Glycol, titanium dioxide,177891, talc powder; yellow iron oxide ec: 77492. |
| 5 Crestolip 10 mg | Global Napi Pharmaceutical | Round, white film coated tablet | 10 | RSC 10.4 mg | Micro crystalline cellulose (avicel PH102), lactose spray dried, Mg stearate, PVPK30. |
| 6 Suvikan 20mg | HIKMA PHARMA | Round film coated tablet | 7 | RSC 20.8 mg | Tablet core:- lactose monohydrate ,micro crystalline cellulose, Ca-phosphate, crospovidone, Mg stearate Coat-opadry. |
| 7 Advochol 10 mg | Advocure pharmaceutical | Round film coated tablet | 7 | RSC 10.4 mg | NA |
The classical technique deals with weights and the encountered difficulty in its decomposition is verified by the forced degradation studies as acidity, alkalinity, oxidation, heat and photo degradation which are performed in our recent HPLC study [51] according to ICH guidelines [57]. Also, the DSC data of pure RSC [58] show the endothermic peak at 156.47°C of the pure RSC where there was no sharp change in melting point of the drug and indicate the melting point value (122 °C) which was reported in literature.

Although the present method has succeeded only for the determination of RSC in its pure form and failed to be applied for the determination of its concentration in tablet formulations due to the causes illustrated in (Table 2), however the method is considered the first indirect one for its determination through its chelated Ca atom besides it comprises essential modifications introduced on the classical Schöniger method concerning the following:

a) **Weight taken:** The classical technique deals with weights in the range 3-5 mg, here we combusted, safely, more than twenty times large weights in the range 76-126 mg, to compensate the low Ca ratio relative to the large MW (1001.14) of RSC.

b) **Volume of the combustion flask:** The former modification necessitated the use of 1L Erlenmeyer flask instead of the classical 250 mL or 500 mL flasks.

c) **Volume of the absorption soln:** The classical 5 mL absorbent soln. has been increased to 25 mL to absorb and dissolve the combustion products of such larger weights.

d) **Oxygen flushing time:** Has been increased from 1 min. to 3 min. to supply the sufficient quantity of oxygen for achieving complete combustion.

To extend the application of the proposed method to the determination of tablet formulations, further work is planned, in the near future, to be applied on the soln. after combustion, based on the use of highly selective colorimetric reagent for Ca in the presence of efficient masking agents for the other interfering elements that are present in the inactive ingredients illustrated previously.

**Conclusion**

An indirect method has been developed for the determination of RSC through its chelated Ca atom after decomposition by the oxygen flask and /or the muffle furnace. Essential modifications have been introduced on the classical Schöniger method concerning the weight taken, volume of the combustion flask, volume of the absorption soln. and the flushing time.

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