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Research Article

PURITY OF FIVE GENERIC BULK MONTELUKAST SODIUM USING LIQUID CHROMATOGRAPHY /MASS SPERCTROMETRY

SAMER HAIDAR *Ω STEFAN BOETTCHER+ M. AMER AL MARDINI* ROLF W. HARTMANN+

***Faculty of pharmacy, Damascus University, Damascus Syria, +Pharmazeutische und Medizinische Chemie, Saarland University, Saarbrücken, Germany. Email haidar@scs-net.org**

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ABSTRACT

Montelukast is frequently used drug for the treatment of asthma and allergic conditions. Montelukast was first marketed by Merck under the tread name SINGULAIR®, nowadays several companies around the globe are manufacturing this compound. In this work five Montelukast sodium samples from five different Asian companies were tested by LC-UV-MS to determine the purity of those compounds.

The purity of the tested samples was between 98.75 and 99.35%. An attempt was made to determine the structure of the impurities using LC-UV-MS/MS as well.

Key words: Montelukast, impurity, LC-MS, LC-MS-MS.

INTRODUCTION

Montelukast (MO), {1-[({(1*R*)-1-{3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl}-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl}thio-methyl]cyclopropyl}acetic acid monosodium salt, has a molecular formula of $C_{35}H_{35}ClNO_3S\bullet Na$ and a molecular weight of 608.17 g/mol.

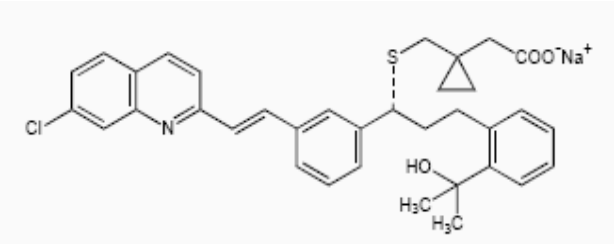

Fig. 1: Chemical structure of Montelukast sodium salt

Montelukast is a leukotriene receptor antagonist (LTRA) used orally for the treatment of asthma and to relieve symptoms of allergies. MO is a $CysLT_1$ antagonist; it blocks the action of leukotriene D4 on the cysteinyl leukotriene receptor $CysLT_1$ in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene, and results in less inflammation [1], MO is usually administered orally.

Several methods were developed for the determination of MO such as voltametric, capillary electrophoresis, spectroflurometry, spectrophotometery, and liquid chromatography [9-17]. Some methods were developed in pharmaceutical products [11, 14, 16, 17], and biological fluids [2, 10, 15]. Only few studies were involved in stability and isolation and identification of its impurities [15, 16].

The quality and safety of a drug can be significantly affected by the presence of impurities and its related compounds. According to the ICH guidelines the acceptable level of known and unknown impurities should be less than 0.15 and 0.10 %, respectively.

Ideally it is important to identify, synthesize and characterize the impurities in order to meet the regulatory requirement, but the aim of this study was only to verify the percentage of impurity of the tested drug from different sources in order to determine the quality of some Asian copies.

To rapidly obtain detailed structural information about the trace level impurities and degradation products observed in the bulk drug substance, liquid chromatography/mass spectrometry (LC-UV-MS) was used; furthermore LC-UV-MS/MS was also employed.

MATERIALS AND METHODS

Samples: Five samples of Montelukast sodium from five different Asian firms were purchased directly from these companies each provided with analytical sheets.

LC-UV-MS conditions

The SpectraSystems® LC system consisted of a pump, an autosampler, and a UV detector. Mass spectrometry was performed on a MSQ® electro spray mass spectrometer (Thermo Fisher, Dreieich, Germany). The system was operated by the standard software Xcalibur. A RP-C18 NUCLEODUR® 100-5(125x 3 mm) column (Macherey-Nagel GmbH, Dueren, Germany) was used as stationary phase. All solvents were HPLC grade.

In a gradient run the percentage of acetonitrile (containing 0.1 % triflouro-acetic acid) in 0.1 % triflorouro-acetic acid was increased from an initial concentration of 0% at 0 min to 100% at 15 min and kept at 100% for 5 min. The injection volume was 15 µl and flow rate was set to 800 µl/min. MS analysis was carried out in ESI mode at a spray voltage of 3800 V, a capillary temperature of 350 °C and a source CID of 10 V.

Mass spectra were acquired in positive mode from 100 to 1000 m/z. The UV trace was recorded at 254 nm and purities were calculated as percentage of the total UV peak area, using automated Avalon peak detection.

LC-UV-MS/MS conditions

A Surveyor® LC system was used, consisting of a pump, an autosampler, and a PDA detector. Mass spectrometry was carried out on a TSQ® Quantum electro spray mass spectrometer (Thermo Fisher, Dreieich, Germany), also using the Xcalibur software.

A RP-C18 NUCLEODUR® 100-3(125x 3 mm) column (Macherey-Nagel GmbH, Dueren, Germany) was used as stationary phase. All solvents were HPLC grade, using the above mentioned gradient and injection volume. The flow rate was set to 500 µl/min. MS analysis was carried out in ESI mode at a spray voltage of 3800 V, a capillary temperature of 350 °C and a source CID of 10 V. Spectra were acquired in positive mode from 100 to 1500 m/z and full scan UV trace (200-600 nm). MS/MS spectra were recorded from 100 to 600 m/z using a collision voltage of 35 V and a collision gas (Ar) pressure of 1.5 mTorr.

RESULTS

For the first sample nine peaks were detected with retention time (RT) corresponding to 10.8, 11.0, 12.1, 12.4 (M), 14.0, 15.0, 15.4, 16.4 and 16.6 min and the purity of this sample was 99.27 % (Figure 2).

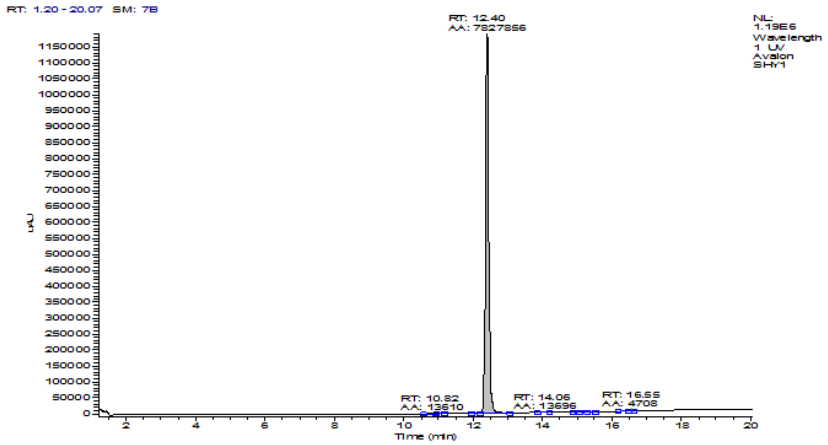

Fig. 2: Result of sample 1 using UV chromatogram of Montelukast sodium.

The highest number of peaks was detected in the second sample, with RT corresponding to 10.8, 11.8, 12.1, 12.4 (M), 13.0, 13.6, 13.8, 14.0, 14.9, 15.4 and 16.3 The purity of this sample was 98.75 % (Figure 3).

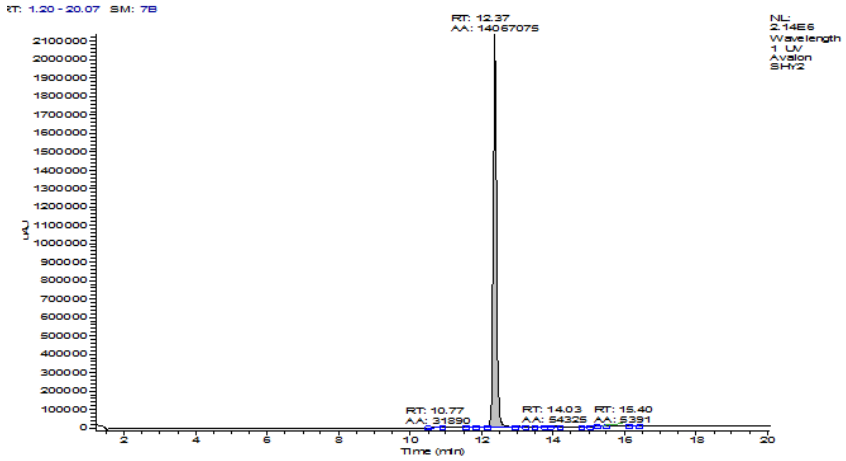

Fig. 3: Result of sample 2 using UV chromatogram of Montelukast sodium.

Eight peaks were detected for the third sample with RT corresponding to 7.5, 10.4, 12.4 (M), 13.0, 14.0, 15.4. 16.4, and the purity of this sample was 98.89 % (Figure 4).

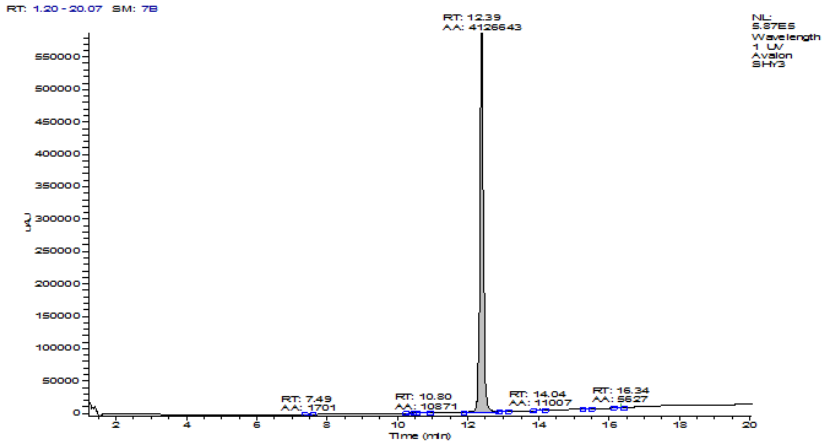

Fig. 4: Result of sample 3 using UV chromatogram of Montelukast sodium.

The purest sample turned out to be number 4 with only 6 peaks with RT corresponding 10.8, 12.4 (M), 10.0, 15.0, 15.4 and 16.4, the purity of this sample was 99.35% (figure5).

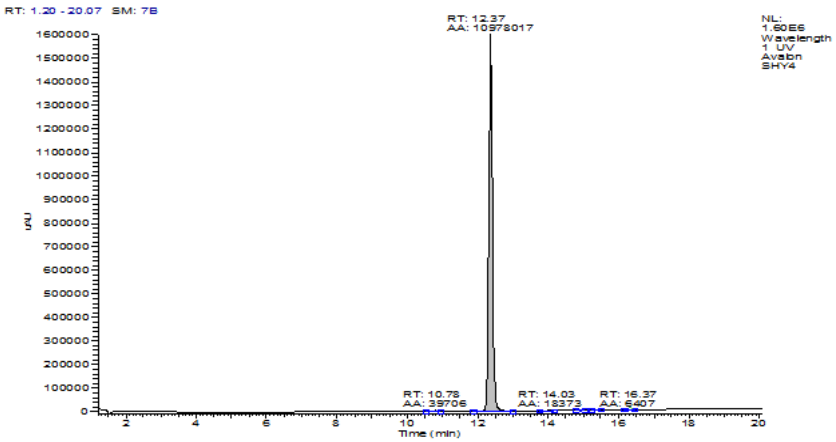

Fig. 5: Result of sample 4 using UV chromatogram of Montelukast sodium.

In the fifth sample, ten peaks were detected with corresponding RT of 10.8, 11.1, 11.9, 12.2, 12.4 (M), 14.1, 14.8, 15.6, 16.5 and 16.6. Here the purity was only 98.75% (Figure 6).

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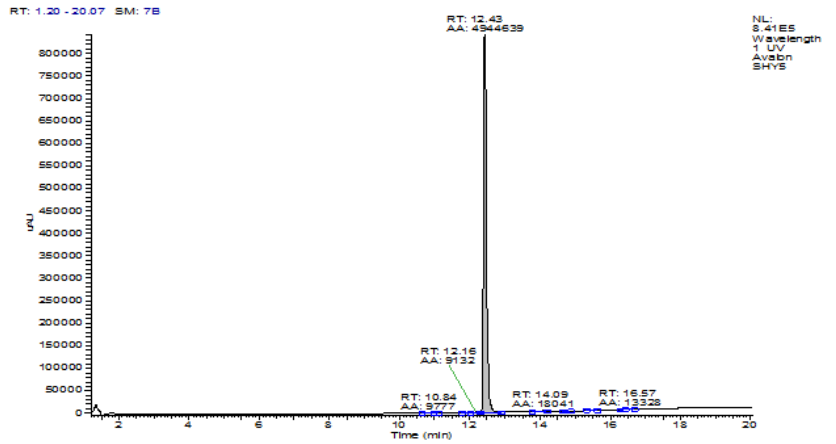


Fig. 6: Result of sample 5 using UV chromatogram of Montelukast sodium.

For the characterization of the main byproduct, which is present in all the samples at 10.8 min in the LC-MS standard method, for sample 2 a UV full scan was performed on a LC-UV-MS/MS device and the two highest peaks were identified with corresponding retention times 9.77 and 11.48 min. Those peaks were further analyzed using MS/MS. The difference in the relative amounts, in comparison to the analytical measurement of sample 2, are due to the wavelength full scan and the very high amount of sample, which overloaded the Montelukast sodium peak, but which was necessary to have a suitable amount of the byproduct, to be analyzed in MS/MS.

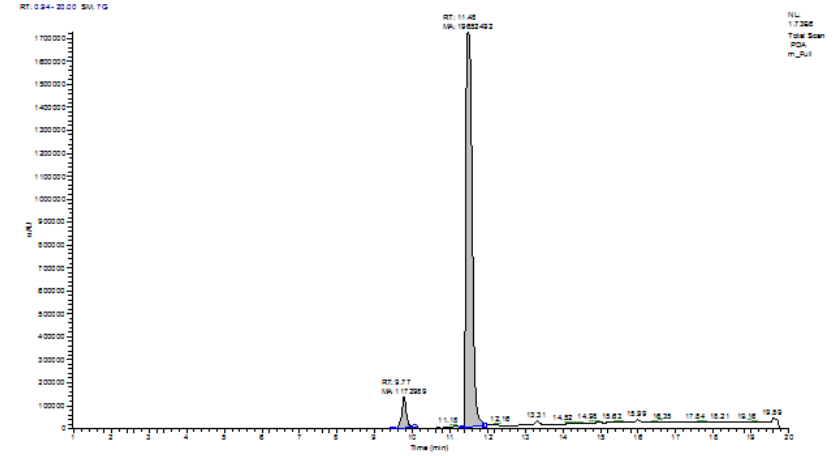


Fig. 7: Chromatogram of full scan PDA of sample 2.

The analysis of the first peak with RT 9.77 using MS and MS/MS is shown in figure 8.

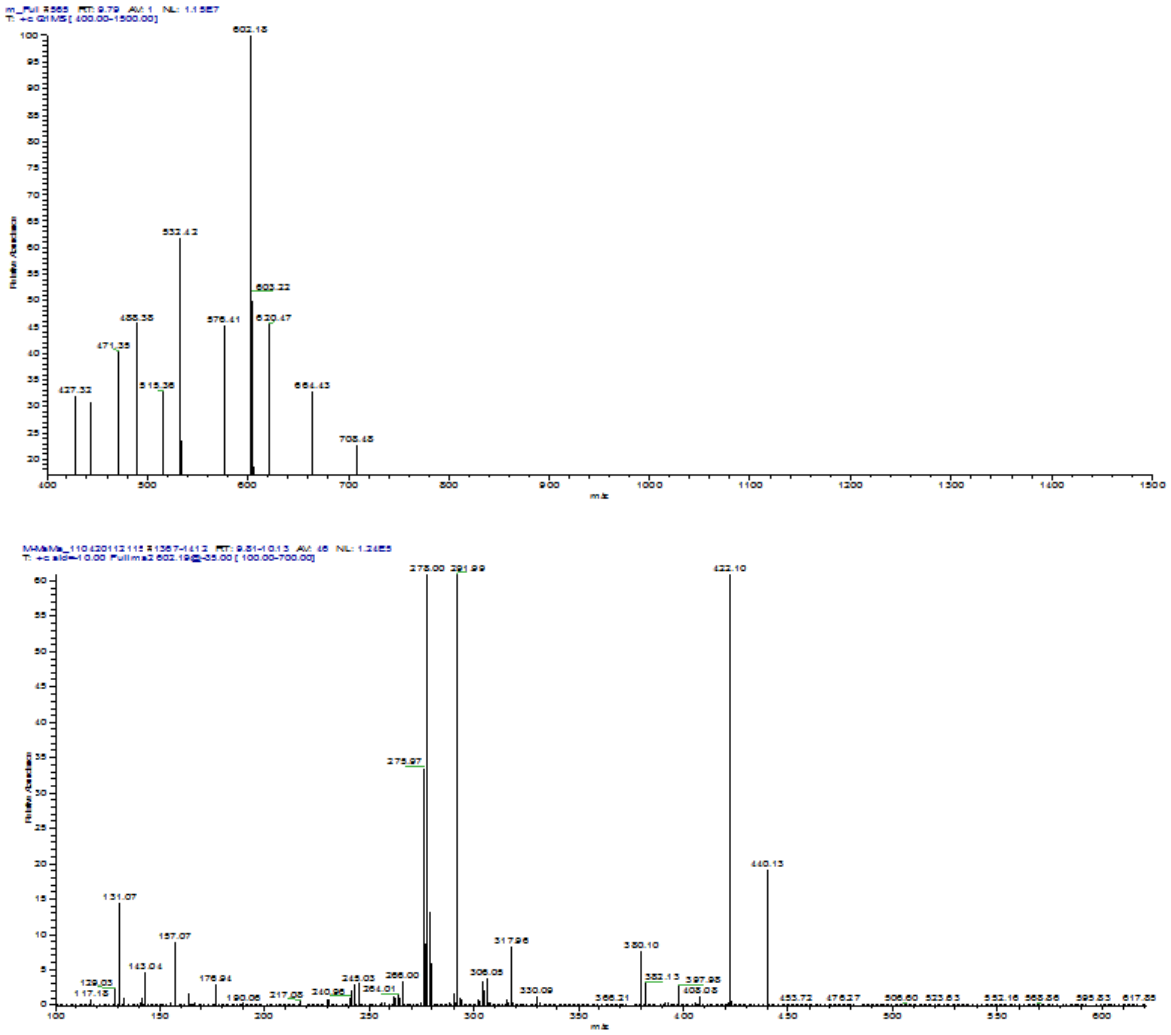
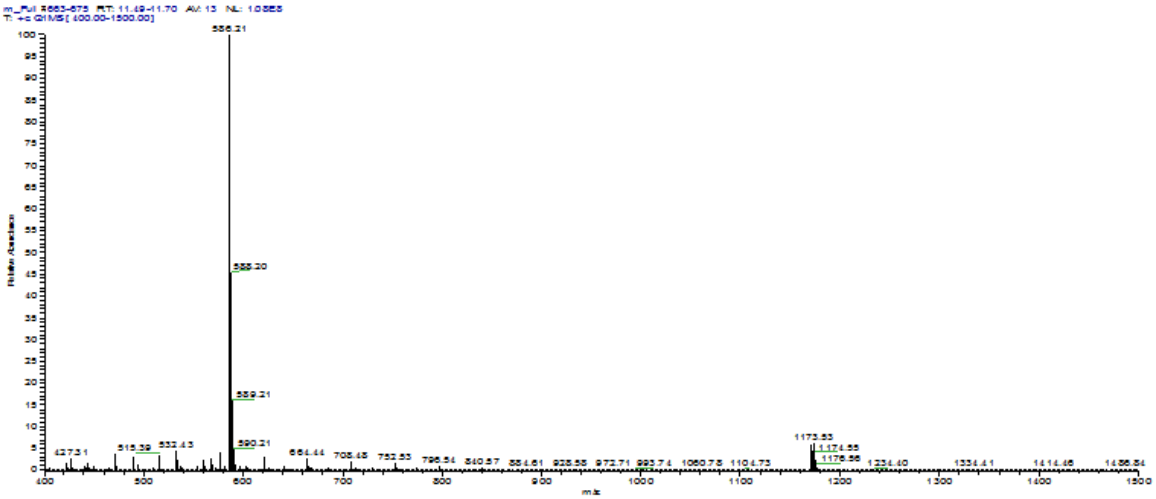


Fig. 8: MS and MS/MS result of the first peak.

The analysis of the second peak with RT 11.48 using MS and MS/MS is shown in figure 9.



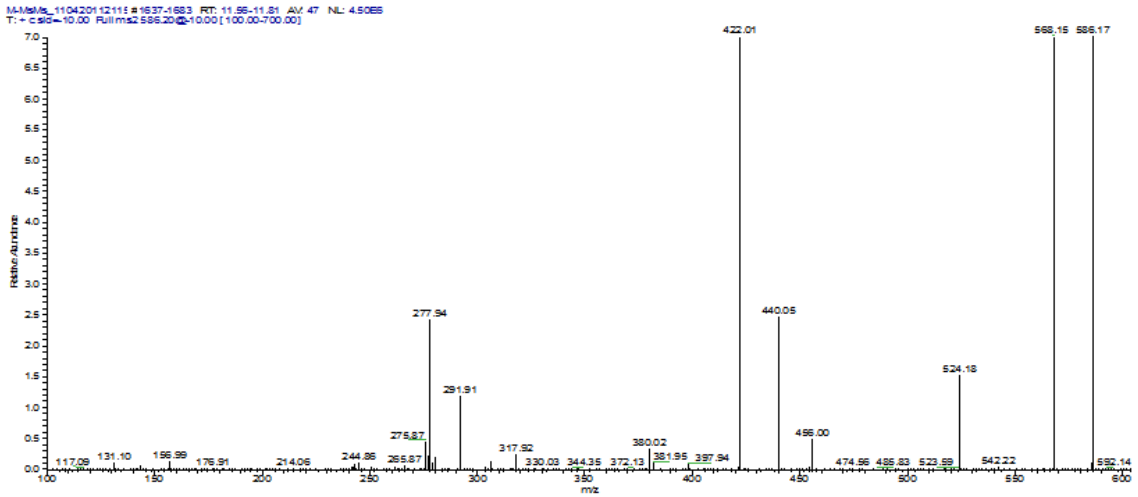


Fig. 9: MS and MS/MS result of the second peak

DISCUSSION

The mass (M) of Montelukast is 586 amu (for M+H+ instead of Na^+ in the MS). The mass of the byproduct (6.7 % in UV trace at 254 nm) is 602 amu ($M+H^+$) so the difference is 16, which is most probably an oxygen atom. An MS/MS experiment was performed and found that the fragment at 440 amu is present as well in the montelukast spectrum as in the byproduct. 440 would be the molecule of the tested compound minus the thio-ether side chain. So the oxidation took place at the side-chain. With respect to the structure of the side-chain it is most likely that the sulfur is oxidized.

According to Saravanan et.al. this impunity has an olefin double bond reduced to alkane group and the general formula of this impurity is $C_{35}H_{38}ClNO_3S$ [15]. In the last few decades the Asian companies especially the Chinese and Indian were producing medicinal raw materials with competitive price comparing to western companies which drive third world countries to purchase from these recourses; nevertheless the quality was sometimes a concern.

The currently employed method was able to detect the impunities percentage of the tested samples. None of the Montelukast samples were within the accepted level of purity recommended by the ICH, some were contaminated with around 1.25% impurities. Although the impurities were not characterized in order to verify whether they are toxic or nontoxic but they exceed the accepted level.

As a conclusion it is recommended to test the raw material carefully before manufacturing them in their formulations and to verify the toxic impurities.

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