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Application of ion-trap mass spectrometry for identification and structural determination of an unknown impurity in simvastatin

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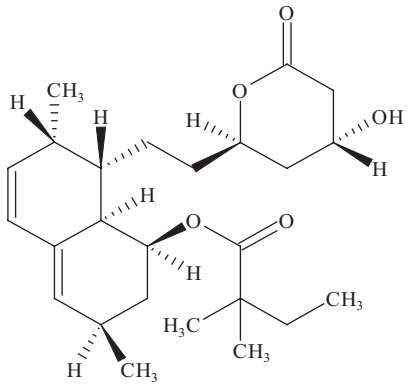
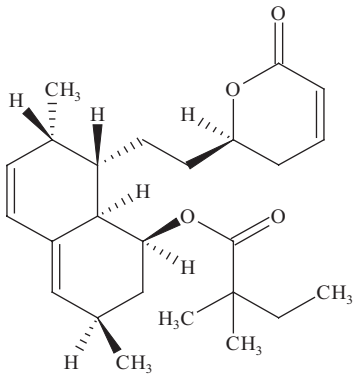
Anhydro-simvastatin and simvastatin dimer are the two main impurities in the fermentation broth as well as in the final product of simvastatin, which is a hypolipidemic drug. An unknown impurity with m/z 451 for $[(M+H)^+]$ was detected in the analysis of final simvastatin drug sample. By using reverse phase high performance liquid chromatography (HPLC)-mass spectrometry (MS) and MS/MS spectra, the unknown impurity was detected and identified. Separation was achieved on ACE-5 C18 (150 × 4.6 mm, 3 μm column) at the flow rate of 1.2 ml·min^{-1} applying gradient elution of mobile phase A consisting of Milli-Q water of pH 3.0 with formic acid and B consisting of acetonitrile. MS/MS spectrum of the unknown impurity was obtained using HPLC-MS equipped with positive electrosoray ionization (ESI). The unknown impurity is named as 7-[7-(2,2-dimethyl-butyryloxy)-2,6-dimethyl-1,2,6,7,8,8a-hexahydro-naphthalen-1-yl]-3-hydroxy-5-hydroxymethyl-heptanoic acid.

1. Introduction

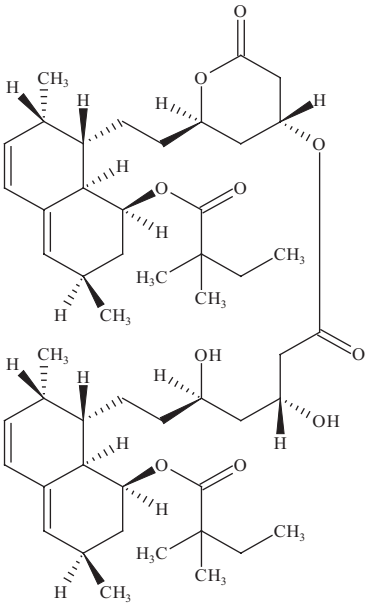
Simvastatin is a hypolipidemic drug with the chemical name (1*S*,3*R*,7*S*,8*S*,8*aR*)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]-ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2, 2-dimethylbutanoate. It is a lipid-lowering agent, synthetically derived from a fermentation product of *Aspergillus terreus* and belongs to the class of pharmaceuticals named statins. Statins include natural, lovastatin; semi-synthetic, simvastatin, and pravastatin; and synthetic compounds, fluvastatin, atorvastatin, cerivastatin, rosuvastatin and pitavastatin and are potent, specific and competitive inhibitors of 3-hydroxy-3-methlyglutaryl coenzyme A (HMG-CoA) reductase. The treatment with these statins is continued for a long time; therefore a high purity of drug substance and the knowledge of the impurity profile are most important criteria in the manufacturing process.



(a) simvastatin



(b) anhydro impurity



(c) dimer impurity of simvastatin

The impurities of drugs can originate from raw materials and reagents, intermediates, synthetic or bio-synthetic by-products, or degradation products. It is important to know that some impurities can have safety and efficacy effects. According to the guidelines of the International Conference on Harmonization (ICH), identification and quantification of trace impurities is an increasing requirement in drug development and manufacturing. An impurity is any component of an active pharmaceutical ingredient (API) which is not the chemical entity of active substance or excipient, present at levels higher than 0.1% or in some cases higher than 0.2%, depending on daily recommended dosage, need to be identified and qualified with appropriate toxicological studies. If the impurities are expected to be very toxic, then the identification and qualification would be required even at lower concentrations (Krstulovic et al. 2002). The minor impurities present in the sample are diagnostic of the route of synthesis employed in the manufacture of the API.

Few methods to identify simvastatin-related impurities have been reported (Wang et al. 2001; Vuletic et al. 2005; Plumb et al. 2007) by LC–MS. Wang et al. (2001) developed a LC-MS method in which the impurities are commercially available or already described in the literature and other methods (Vuletic et al. 2005; Plumb et al. 2007) were applied to determine the impurities in the simvastatin tablets. The present work describes a simple method for detection of an unknown impurity in final simvastatin drug at trace level by reversed phase HPLC and its structural determination using CAD (MS/MS).

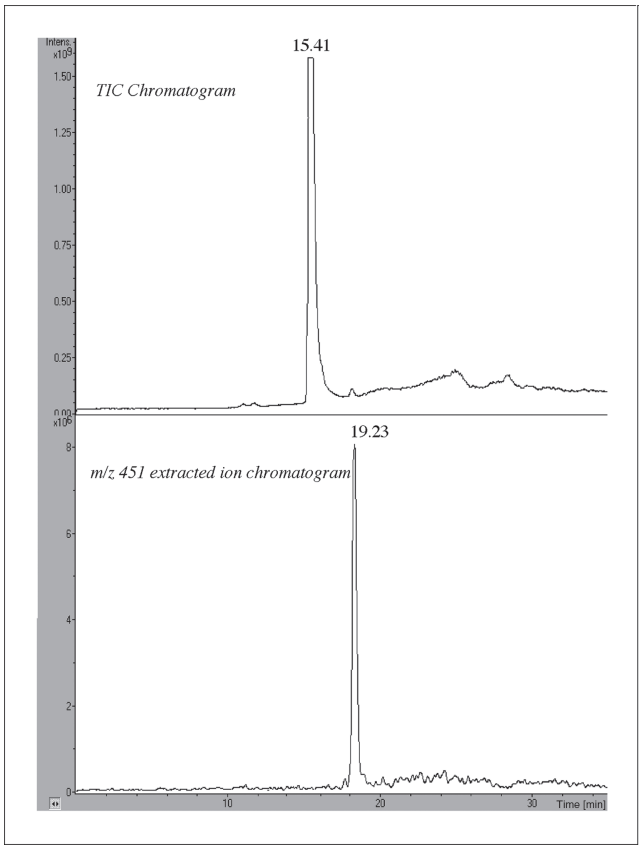


Fig. 1: HPLC-MS chromatograms of simvastatin sample (a) TIC and (b) extracted ion chromatogram m/z 451

2. Investigations, results and discussion

It was necessary to ensure that the quality of the pharma product was consistent with the prescribed acceptance criteria during

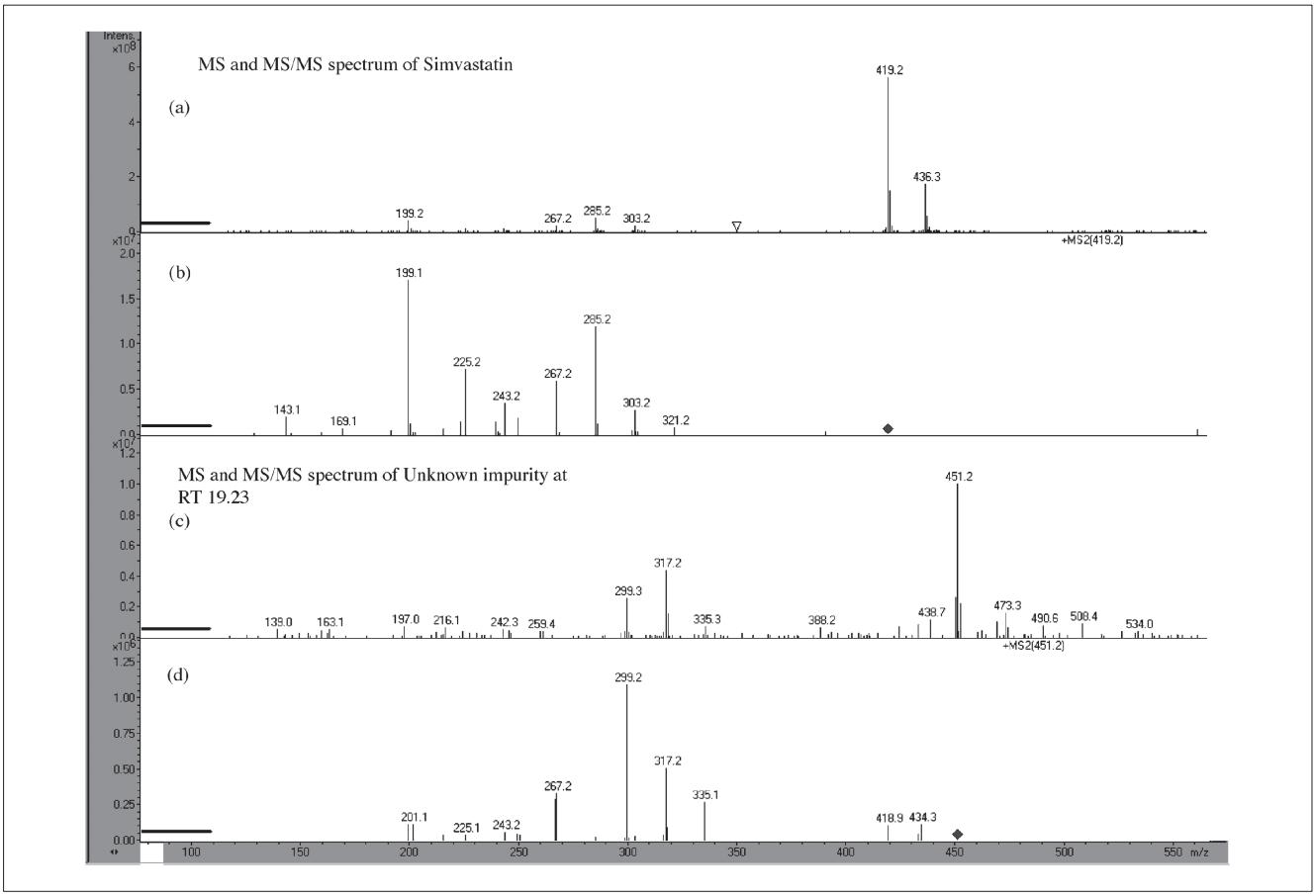
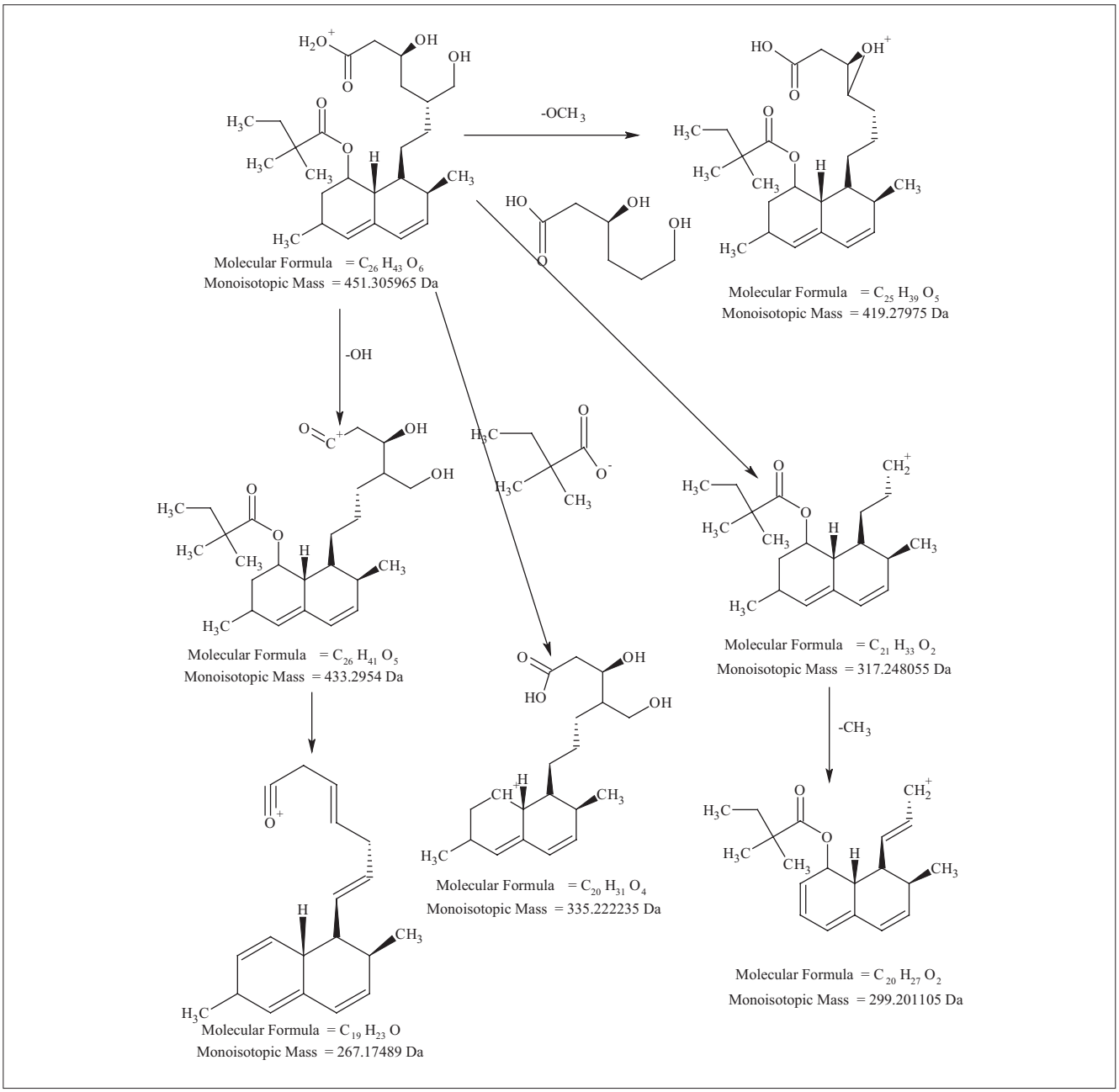


Fig. 2: MS and MS/MS spectra of simvastatin (a, b) and unknown impurity (c, d) at RT 19.23



Scheme: Proposed fragmentation pattern of the unknown impurity

the analysis of the impurities in pharmaceutical products. For this purpose, it was necessary to have a sufficient resolution chromatography system and a high sensitivity detection system, such that any differences between products can be separated, detected, and identified. Reversed-phase LC with UV or photodiode array detection has become the technique of choice for this operation, due to its compatibility with the samples, resolution, specificity, and sensitivity. MS provides not only mass of analytes but also of fragment ions gained through MS/MS experiment, thus giving an additional dimension to MS/MS data for structural determination. So, initially a sample of simvastatin was analyzed using reversed phase HPLC-MS in positive electrospary ionization. The main impurities which appeared during the preparation of simvastatin were anhydrosimvastatin and simvastatin dimer. During the analysis of final simvastatin, at the retention time of 15.41 min, the simvastatin peak was confirmed. At retention time 19.23 min, an unknown peak with molecular mass 451 Da was detected. By comparing the retention times of the main impurities to unknown peak retention time, it was confirmed as unknown impurity, (The retention times of the main impurities, anhydrosimvastatin and dimer simvastatin were 27.52 and 41.98, respectively). The HPLC-MS chromatograms were shown in Fig. 1. (Fig. 1a. represents total ion chromatogram (TIC) and Fig. 1b. represents extracted ion chromatogram at m/z 451).

During in-process monitoring of impurities at the drug discovery or manufacturing phases, fragmentation patterns of unknown impurities were compared against the database of fragments formed from analogs and the API (Nicolas and Scholz 1998; Volk et al. 1996). So, by comparing the fragmentation pathway of $[(M+H)^+]$ of simvastatin with the fragmentation of the unknown impurity the structure could be predicted. The MS spectra of simvastatin and unknown impurity were obtained using low collision energy and were shown in Fig. 2a and Fig. 2c, respectively. By using an ion trap mass spectrometer, the main fragmentation pathway of simvastatin in positive and negative ionization modes were determined by analyzing

low energy CAD of protonated $(M+H)^+$ and deprotonated $(M-H)^-$ molecules (Wang et al. 2001; Qin 2003). The CAD spectra of simvastatin and unknown impurity were shown in Fig. 2b and Fig. 2d, respectively and were obtained using high collision energy. By the application of the additional voltages on the ring and end cap electrodes in the ion trap instrument, the product ion spectra of the unknown impurity of simvastatin eluting at retention time 19.23 min were taken. The main fragments of the unknown impurity observed were at m/z values of 434, 419, 335, 317, 299 and 267.

By comparing the fragment ion molecules of the unknown impurity with simvastatin, it was obvious that the unknown impurity is to some extent a structural analogue of simvastatin. This is due to some of the fragmented ions (m/z 419, 267 and 199) which were observed in the unknown impurity and are similar to simvastatin. The proposed fragmentation pathway of the unknown impurity is shown in the Scheme. From this fragmentation, it can be explored that the impurity should result from a break of the cyclic five membered ring. In the further study the impurity was isolated using preparative HPLC to obtain a more pure compound. From the MS fragmentation, it has been confirmed that the unknown impurity is a new impurity and named, 7-[7-(2,2-dimethyl-butyryloxy)-2,6-dimethyl-1,2,6,7,8,8a-hexahydro-naphthalen-1-yl]-3-hydroxy-5-hydroxymethyl-heptanoic acid and this was also supported by NMR and IR measurements.

3. Experimental

3.1. Materials

Simvastatin samples and its impurities, anhydro-simvastatin and simvastatin dimer were supplied by Sun pharmaceutical Ltd (Hyderabad, India). HPLC grade acetonitrile (ACN) and methanol (MeOH) were obtained from Merck Co (Darmstadt, Germany). Formic acid and ammonium acetate were obtained from Sigma-Aldrich (Steinheim, Germany). A Milli-Q purification system (Millipore, Bedford, USA) was used to purify the deionized water. Standard solution of simvastatin was prepared at a concentration of 0.1 mg ml^{-1} in 10 mM ammonium acetate and ACN (50:50).

3.2. HPLC-MS and MS/MS

Analyses were performed using a HPLC system consisted of an Agilent 1100 series Vacuum degasser, G1311A Quaternary pump, G1329A Auto sampler with column oven and G1316A PDA Detector. Separation was achieved on ACE-5 C18 (150 × 4.6 mm, 3 μm) column at the flow rate of 1.2 ml min^{-1}. Column temperature was 30 °C and gradient elution was applied for separation. Gradient elution was carried out with mobile phase A consists of Milli-Q water pH adjusted to 3.0 with formic acid and B consists of ACN. The total run time was set to 55 min. The mobile phase gradient started at 50% B up to 5 min, increased up to 95% in 25 min and continued up to 44 min and decreases in 5 min to initial gradient. The injection volume was 20 μl.

MS and MS/MS experiments were carried out using an Agilent Technologies (Waldbornn, Germany) 1100 series LC/MSD Trap (SL) (Concord, Canada), interfered with an ESI ion source. ESI source parameters were: Vaporizer temperature, 325 °C; Nebulizer gas (nitrogen), 40 psi; Dry gas, 9.0 l min^{-1}; and Capillary exit, 132 V. Isolation and fragmentation were obtained by applying 1.5 amplitude to ring and end cap electrodes and multiplier voltage was 2604 V. Mobile phase consists of 0.01 M ammonium acetate and 50% MeOH, the flow rate was 1.0 ml·min^{-1}. Helium gas was used as collision gas. Sample was introduced with loop injection of 10 μl. The data were acquired in used in positive mode and analyzed using chemstation software.

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