INTRODUCTION

It is a regulatory submission requirement to fully characterize the impurities of the active pharmaceutical to ensure that sufficient toxicological information is obtained during the safety evaluation process [1,2]. The ROA requires the following:

- Pharmacokinetics at levels greater than 1 g/day (3): Impurities ≥ 0.2% must be quantified.
- For dosing levels up to 0.5 g/day, impurities ≥ 0.1% must be quantified.
- For dosing levels up to 0.1 g/day, impurities ≥ 0.02% must be quantified.

Liquid chromatography coupled to mass spectrometry is the primary technique used for the identification of impurities [3]. This normally requires more than one analytical run to produce the high-resolution mass spectrometry (HRMS) information required to identify the chemical structure of the impurities. The combined with the long analysis times make this a time consuming process.

Bateman et al. described a new approach to this problem of acquiring MS and MS/MS data using alternating high-low collision energy acquisition using a hybrid quadrupole TOF instrument to provide both precursor and product ion information on one analytical run [4]. The approach was shown to provide accurate mass measurement in-vitro samples providing rapid accurate identification of drug metabolites by generating accurate mass precursor and product ion data in one analytical run [2-5].

Here we describe the use of sub-2 µm porous particle LC combined with high-speed high resolution chromatography and MS detection on the Q-TOF II mass spectrometer. The sub-2 µm particle LC operated at elevated flow rates allowed for the collection of high quality accurate mass data under exact mass conditions in one run. The high collision energy data was used to identify all drug impurities (Figure 2). The fragment ion information and accurate mass data was used to confirm the identities of the impurities (Figure 4).

METHODS

UPLC/MS/MS Analysis Techniques

United States Pharmacopeia Simvastatin-RS (Boehringer, MD).
Reagents: Acetone, Optimas, Fisher Scientific (Fairlawn, N.J.), Gradipore, Merck, Acetic acid and Dimethyl formamide (DMF), suitable solvents.

UPLC Conditions

Column: Acquity UPLC C18 SB 1.7 µm 2.1 x 100 mm, Waters (Milford, MA).
Mobile Phase: A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile.
Gradient: 25% A for 0.5 minutes, then linear gradient to 50% A over 6 minutes.
Flow Rate: 0.6 mL/min.
Injection Volume: 1.0 µL.
Extraction Cone: 4.5 V
Sample Cone: 35V for reference.
Detection: ACQUITY UPLC PDA @ 238 nm.
Acquisition Range: 100 - 800 Da.
Scan Time: 0.095 s.

RESULTS

The rapid identification of the impurities of simvastatin using UPLC® / Q-TOF™ technology

The data derived from the identification of the known impurities and the information provided in the fragmentation pathway allowed for a preliminary structure of the unknown impurities to be proposed (Figure 5). The UPLC/MS/MS data showed a ≥2 mass unit shift in the mass fragments of the impurity. The data below illustrates that it is most likely simvastatin related (Figure 5).

DISCUSSION

The rapid identification of the impurities of simvastatin using UPLC® / Q-TOF™ technology and an intelligent data mining approach

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