Impurity Analysis of Selective High-Affinity Ligands: Comparison of Bench-Scale vs. Production Syntheses by Label-Free Differential Analysis

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Overview

Purpose: Impurity analysis using LC-MS and differential analysis software to determine impurities between two synthetic pathways for a drug with potential in targeting non-Hodgkin's lymphoma.

Methods: A bench-scale synthesis of the drug was compared to the first batch of a pilot production synthesis. LC-MS (positive mode) using a Thermo Scientific™ Open Accela™ autosampler coupled to a Thermo Scientific LTQ Orbitrap™XL mass spectrometer was performed with Thermo Scientific® SIEVE™ software (version 2.0). Thermo Scientific Mass Frontier software version 7.0 was used for determination of fragments and fragmentation pathways.

Results:

Structural Elucidation from Differential Analysis

Label-free differential analysis (SIEVE 2.0 software) was used for determination of low-level impurities. Classic alignment and fragmenting was used since the samples studied are multiply charged by ES/MS. Analyzed groups were: a bench-scale synthesis of SH7144 (also called standard), the pilot production synthesis of SH7144, and solvent blanks (50:50 acetonitril:H2O 5% (v/v)). The standard bench-scale synthesis group was set as the control group and the pilot production synthesis and solvent blanks were selected as the trend points. This allowed for background removal. The retention-time window for the analysis was from 7 to 20 min. Other parameter settings include: ‘frame width’ set to 10 ppsm (typical for HR-MS data). ‘Frame Retention Time Width’ set to 0.75 min. and ‘Intensity Threshold’ to 10,000 (Figure 2).

Tabulated results after filtering (130 frames, 31 components) are shown in Figure 3. The column ‘Product MW’ provides the deconvoluted molecular weight, which is very useful for a first pass check at common LC-MS adducts (Mass Spectrometry Adduct Calculator). No common adducts found. Ratios of Prod/Std column filled yellow of < 1 represent mpk peaks found in higher abundance in the bench-scale synthesis than in the pilot production scale and ratios > 2 represent mpk values that occur in higher abundance in the production-scale sample.

The elucidation of differences between the two preparations, by comparing LC traces, would not have been possible without prior differential analysis by SIEVE software.

FIGURE 2. SIEVE software version 2.0 showing alignment of chromatographic peaks as a first step in the workflow. The results are also used in the analysis are also shown.

A principal component analysis (PCA) plot reveals that, as expected, the two synthetic products are very similar to each other and very different from the solvent blank (data not shown). However, differences between the two synthetic products are also evident (shown in Figure 4).

FIGURE 3. Principal component analysis results window. m/z 1272.468 appears in greater abundance in the standard bench-scale synthesis than in the pilot production one.

Two components, m/z values: 745.971 and 1036.475, with ratios (production/bench-scale) of 29.02 and 0.00 respectively, were picked from Figure 3 for MS/MS analysis. The fragmentation spectra from those were then compared to the MS/MS of the parent and abundant compounds to look for similarities.

Figure 5 shows the comparison for MS/MS of m/z 745.971 vs. m/z 1186.415 (M+H+) peak in the production-scale sample. SIEVE software results indicated this component was more abundant in the production than in the bench-scale. It displays one common fragment with the parent compound. Its fragmentation pathway was elucidated using the “fragments and mechanisms” feature in Mass Frontier software. SIEVE software provided the deconvoluted MW of 745.97 as 2235.8944 amu, which was 87.04 difference from the parent compound. A likely pathway is the loss of 1558.54 amu, structure shown in Figure. 5. ChemSpider was used to elucidate the difference between parent compound and impurity (ongoing work).

FIGURE 5. Ion trap CID for [M+H+]2+ m/z 1186.415 (M+H4744, top trace) and CID of derivative at m/z 745.97 found by SIEVE software to be more abundant in the production scale synthesis.

Conclusions

A tridentate selective high-affinity ligand (SHAL) was successfully studied with a hybrid high-resolution mass spectrometer. High resolution, accurate mass data was used in conjunction with label-free differential analysis to determine differences in the two synthetic products from two syntheses. Ion trap MS data was utilized for structure elucidation of those differences.

Two compounds identified by SIEVE software to be more abundant in the bench-scale synthesis than in the pilot production synthesis were further analyzed by ion trap MS. The elucidation of the differences by SIEVE software opens both fast and easy interpretation of results, therefore is ideally suited for impurity analysis of drugs. It handles multiply charged spectra especially well, spectra that are more complex to visualize and compare directly from LC traces.

References