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應用手冊

The Characterization of Different Manufacturing Routes using the MarkerLynx Application Manager

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Abstract

The MarkerLynx Application Manager for MassLynx Software has been used to highlight characteristic manufacturing impurities between different manufacturing routes of a GSK drug substance.

Introduction

The use of MarkerLynx has been well documented for Metabonomic/Metabolomic applications. However, its use in the area of impurity analysis has not been widely explored. Impurities in chemical compounds can arise in samples from any number of potential sources such as degradation, contamination, or side reactions in the synthetic route. The levels of impurities are very strictly controlled, and are usually below 0.1% peak area ratio relative to the main component (PAR). However, these trace level impurities can provide interesting information about the history of the samples. Possible applications in pharmaceutical and other industries include: monitoring of batch-to-batch variation during manufacturing and other quality control applications, comparison of samples made by different manufacturing routes, and monitoring of changes in impurity profiles on degradation.

This application note shows how LC-MS combined with Principal Components Analysis (PCA) can be used to highlight minor characteristic differences in trace level impurities as a result of different manufacturing routes.

Experimental

Study Details

Three different manufacturing routes were investigated: Routes A, B, and C. Three batches from Routes A and B, and two batches from Route C were tested in this study. These were experimental batches, and all contained very low levels of impurities (well below 0.1% PAR).

Each batch was chromatographically analyzed using a standard analytical HPLC-MS method on a Waters Micromass Q-Tof Ultima Mass Spectrometer. Duplicate injections from each batch were made. LC-MS data were collected in centroid mode and PCA was performed on the low level impurities present in each batch using MarkerLynx.

Results and Discussion

A representative TIC chromatogram from one of the samples after enrichment of the impurities is shown in Figure 1.

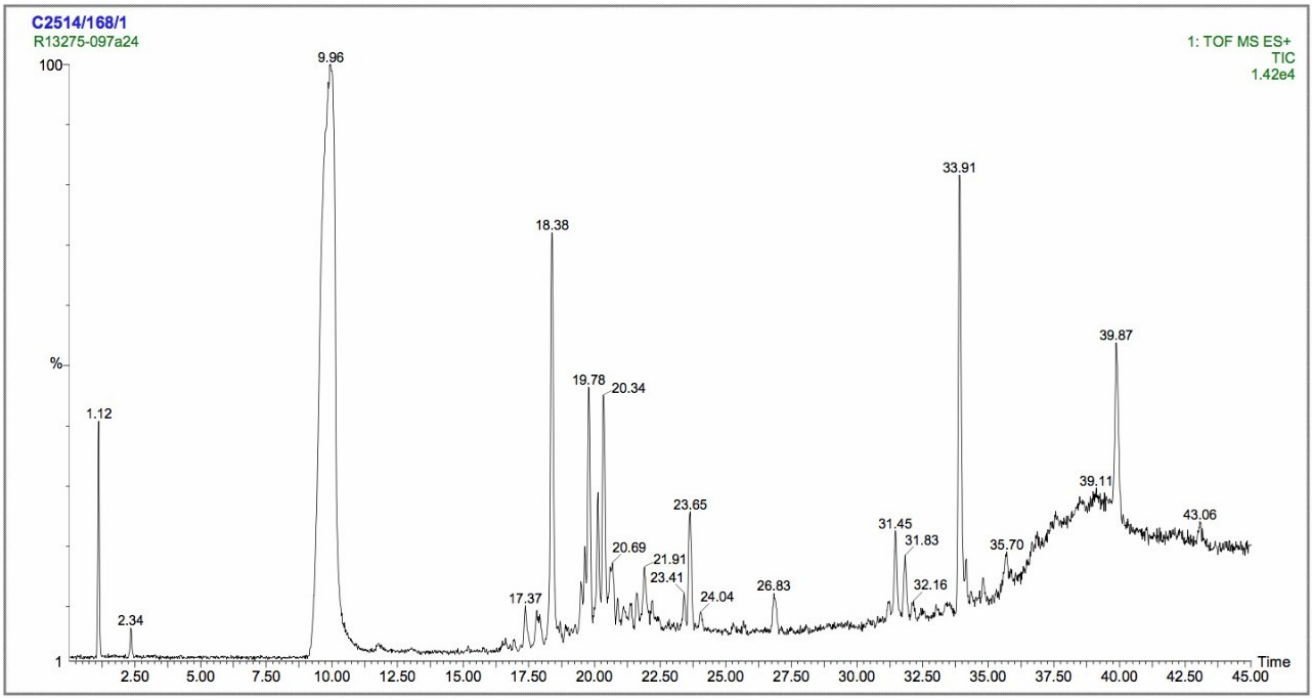

Figure 1. Representative TIC chromatogram after enrichment of the impurities.

The PCA scores plot obtained in MarkerLynx after deconvolution and alignment of the complete data set is shown in Figure 2.

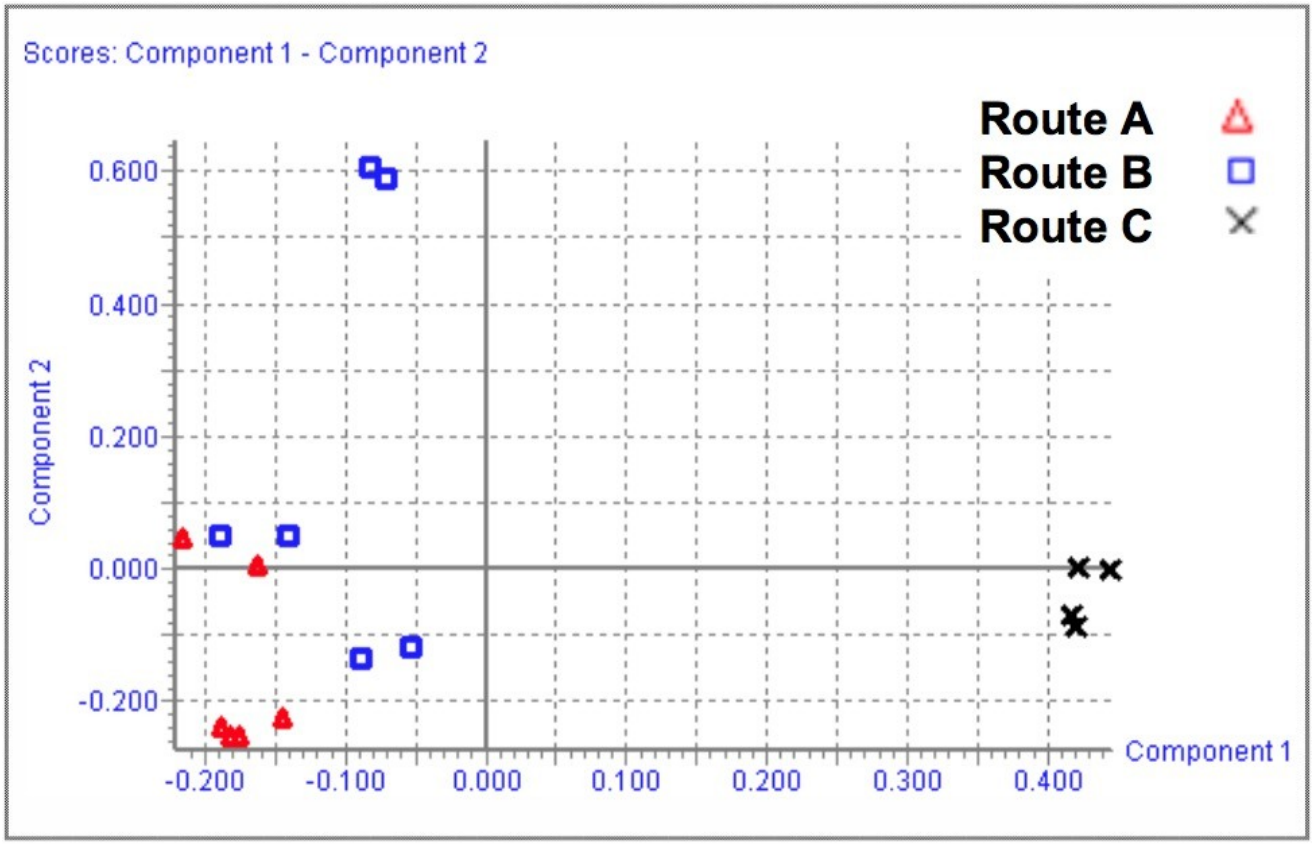

Figure 2. PCA scores plot.

This data shows that Route C is significantly different than the two other manufacturing routes and the Route C samples also form a tighter group. This suggests that the impurities present from the different Route C batches are very similar. The loadings plot shown in Figure 3 indicates the retention times and masses of the impurities responsible for any clustering shown in the scores plot.

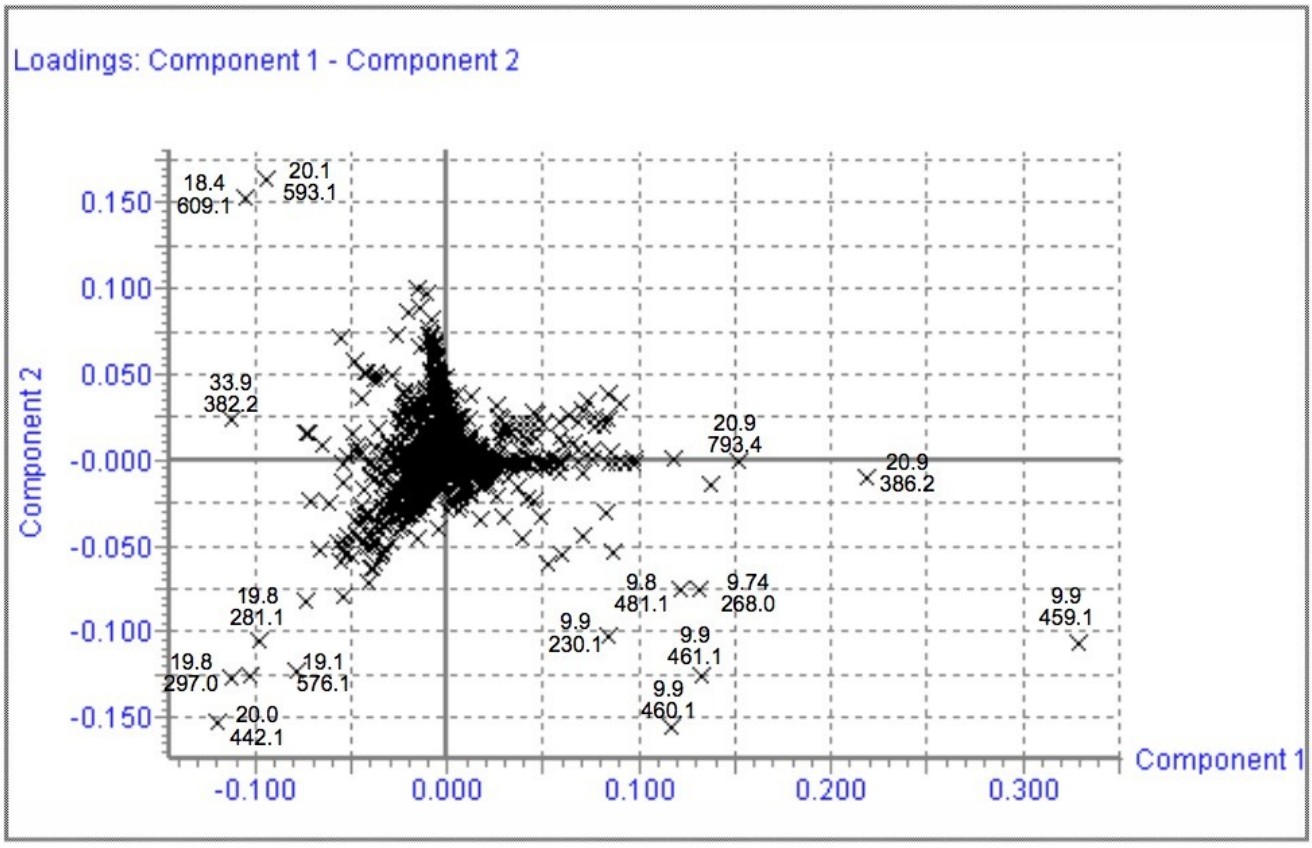

Figure 3. PCA loadings plot.

It is apparent from the loadings plot that Route C samples may contain a route specific impurity, e.g. *m/z* 386.2 at retention time (RT) 20.9 minutes. This impurity had been previously observed in samples produced by route C by manual interrogation of the data. The trend plot for this impurity, which indicates the level present across all of the samples, is shown in Figure 4.

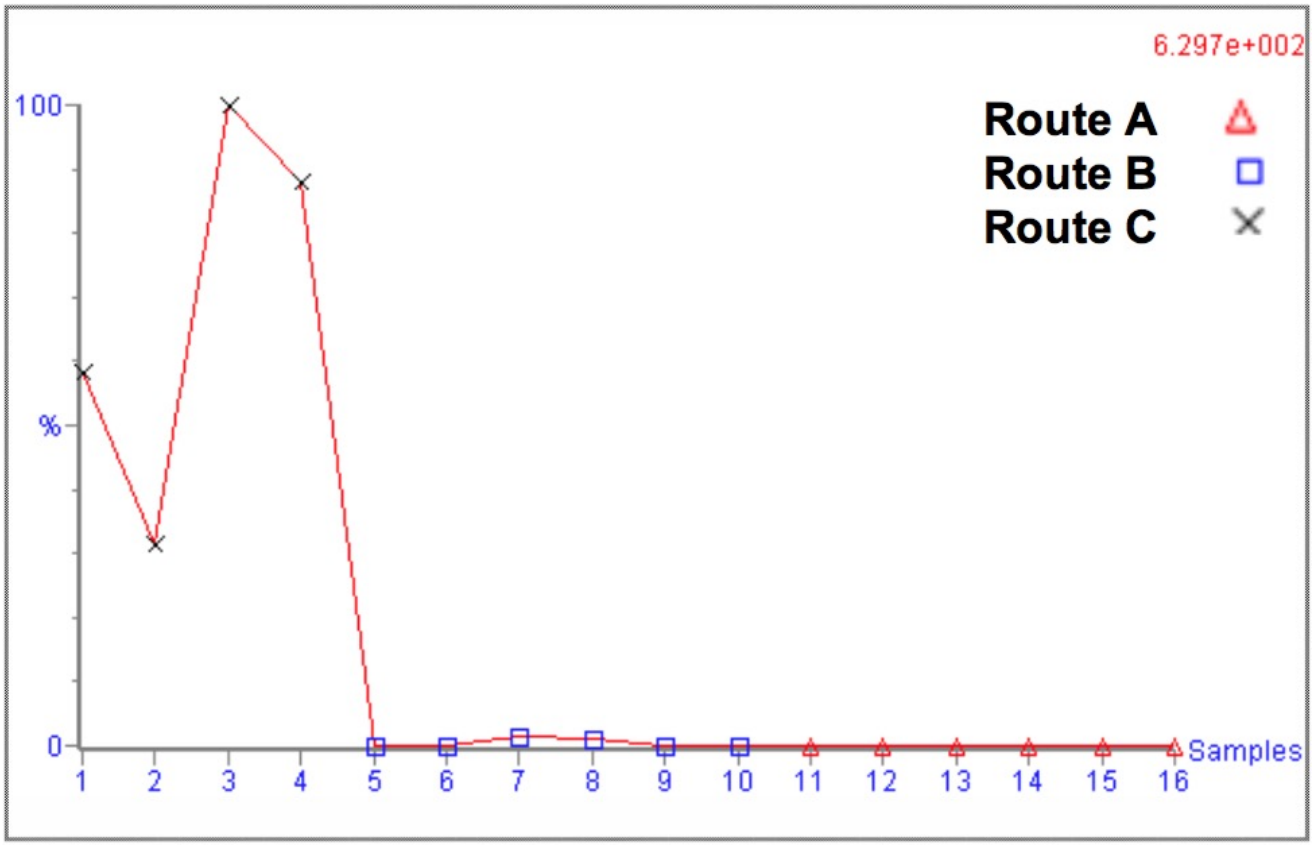

Figure.4. Trend plot for m/z 386.2 at 20.9 minutes.

The results in Figure 4 indicate that this impurity is also present at low levels in the Route B samples. Thus, its presence or absence cannot be used alone as a direct indication of Route C and other factors must be considered. Further examination of the data revealed Route C-specific impurities, one of which is shown in Figure 5.

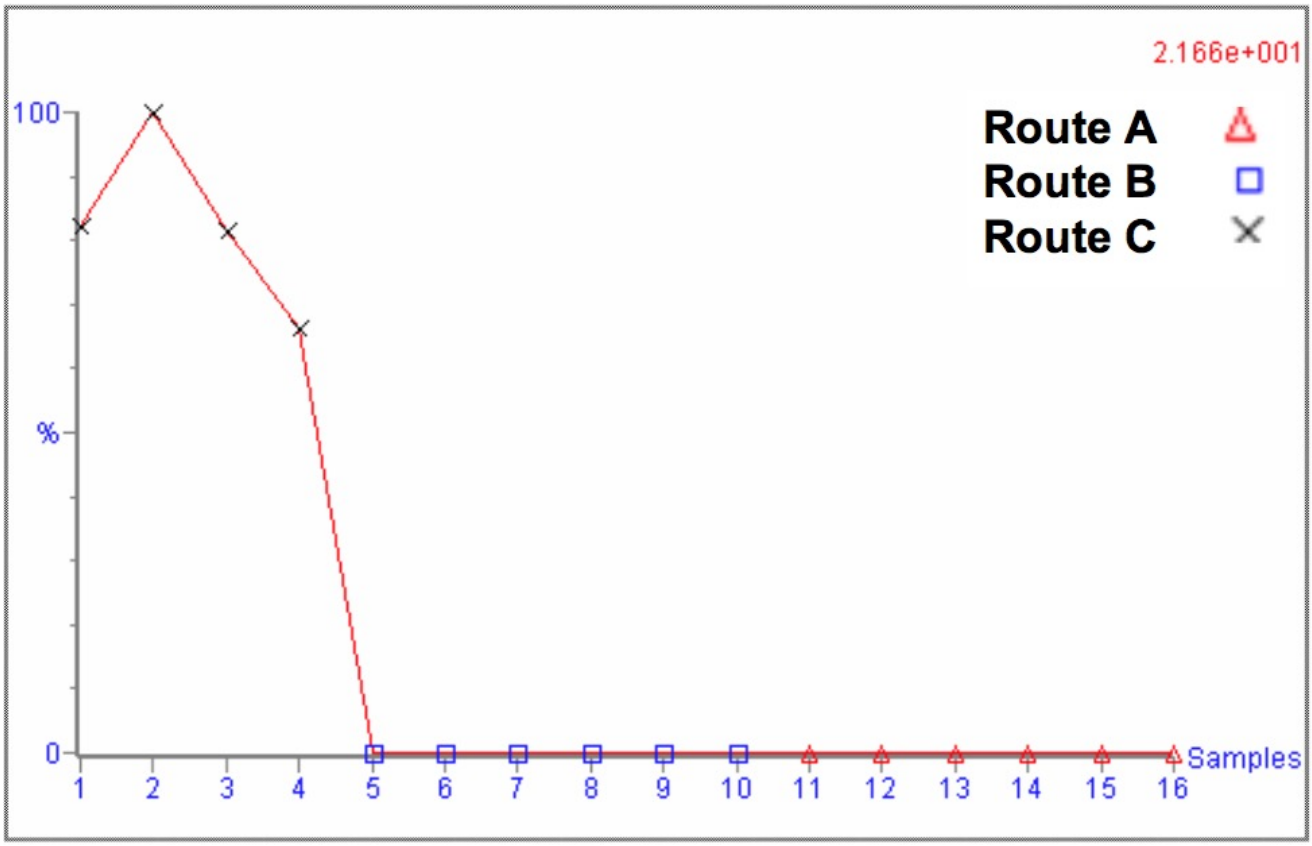

Figure 5. Trend plot for m/z 426.3 at 23.3 minutes.

From the PCA scores plot in Figure 2, it is unclear if there are significant differences between Routes A and B. However, from an in-depth analysis of the MarkerLynx results, two impurities have been highlighted as being potentially route-indicative for Routes A and B.

The trend plot for *m/z* 362.1 at RT 20.4 minutes is shown in Figure 6 and is indicative of the Route B samples. It was not observed in any of the other samples.

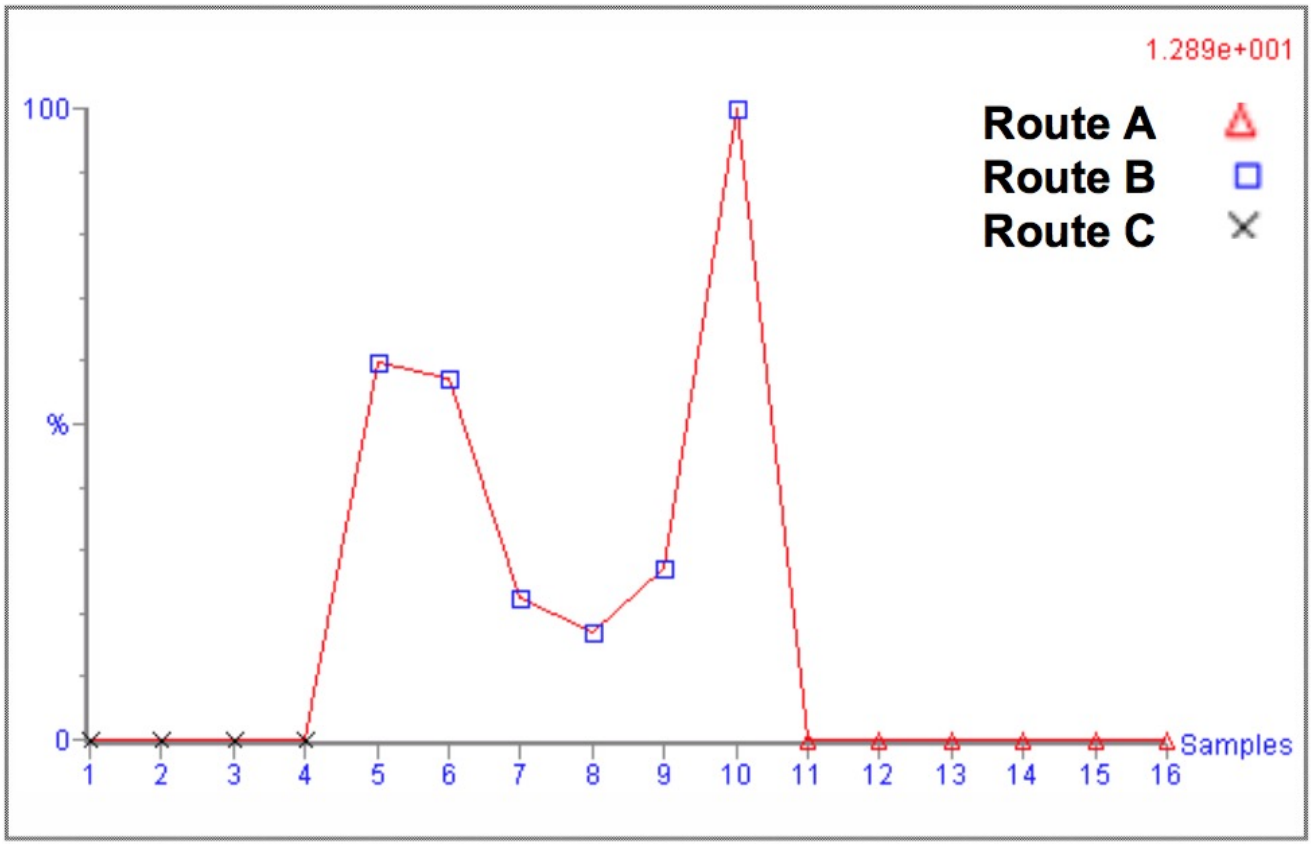

Figure 6. Trend plot for m/z 362.1 RT 20.4 minutes.

The trend plot for a potential Route A indicative impurity, *m/z* 362.1 at RT 25.3 minutes, is shown in Figure 7.

Once again, this impurity is not observed in the other two sets of samples.

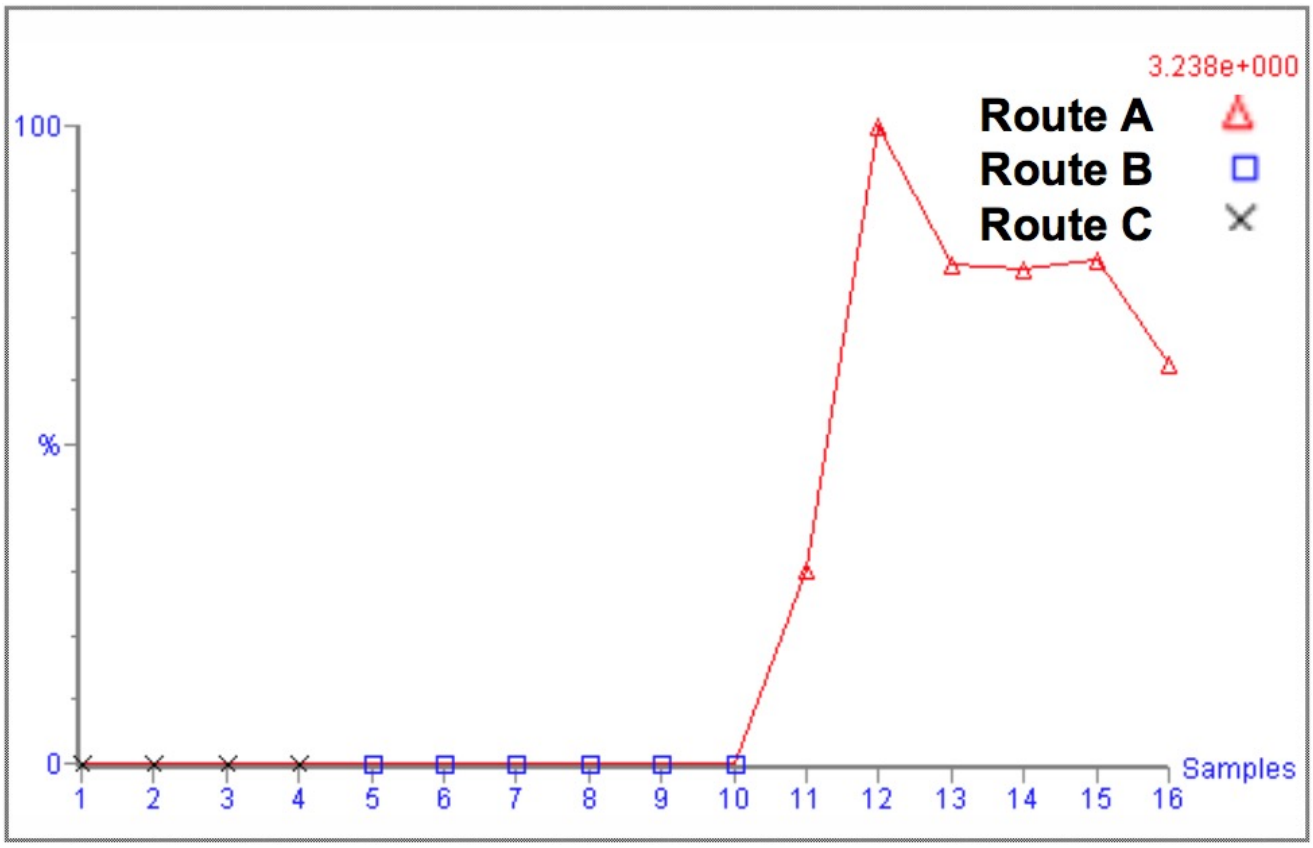

Figure 7. Trend plot for m/z 362.1 RT 25.3 minutes.

The results were confirmed by examination of the mass chromatograms for these impurities in the samples from the different manufacturing routes. Using MarkerLynx, it was possible to highlight impurities which had not been previously identified from Routes A and B. Work is currently under way to identify the structures of these impurities.

Conclusion

The combination of LC-MS (ToF) and the MarkerLynx Application Manager have been used to characterize different manufacturing routes for a pharmaceutical compound. The trend plots within MarkerLynx facilitated the detection of impurities which could be specific for the three routes examined. Further work will be necessary to identify these impurities and explain their association with the differences in the chemistry. One of these impurities had been observed in samples when previous manual interpretation of the data was carried out, thus confirming the results obtained by MarkerLynx. However, the other impurities MarkerLynx identified as potential sample differentiators had not been detected before. Thus, MarkerLynx has been shown to provide a rapid and

effective method for distinguishing between these different batches of compounds.

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