HPLC UV Impurity Identification using Prep-LC, SPE, NMR and and GC-MS

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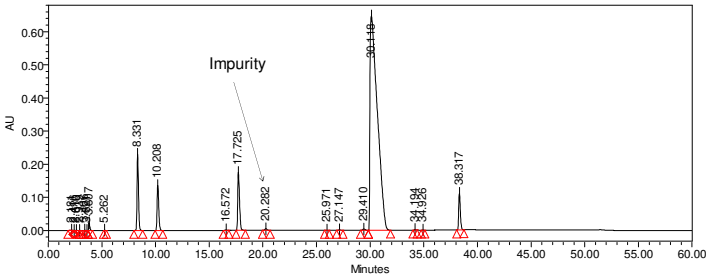
Background

FDA and International Conference on Harmonization (ICH) set strict requirements regarding impurities in formulated drug products. They define thresholds at which impurities must be identified. Liquid chromatography mass spectrometry (LC-MS) is ideally suited to aid in the structural elucidation of impurities although, as presented in this case study, it is not suitable for the identification of non polar, volatile substances. A flow diagram approach has been developed and used for the isolation and characterisation of an impurity in a formulated drug product. Each step of the process also gathers further information on the characteristics of the impurity.

HPLC Development

A previously unknown impurity was observed during HPLC analysis (J'Sphere, C18, 250 X 4.6mm, 4µm column) of an intravenous drug product. Since the phosphate buffer in the original mobile phase (H_2PO_4 /acetonitrile /methanol) was non-volatile, it was necessary to replace it with a volatile buffer, in this case ammonium formate. Since elution orders can change when mobile phases are substituted the UV spectrum of the impurity was recorded so it could be tracked. The UV spectrum also gave an initial insight into how closely related the impurity was to the API.

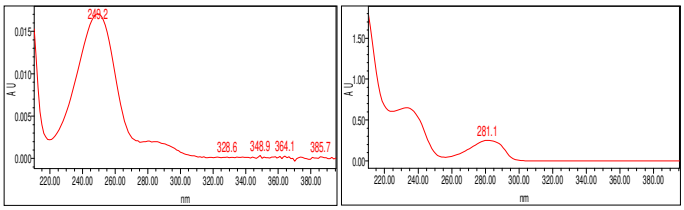
Figure 1: Original HPLC Chromatography Showing Impurity at 20.2 mins



UV Spectrum of Impurity

The use of ammonium formate allowed the original elution order to be retained. The UV spectrum of the impurity and the API were found to be very different indicating that the structures were dissimilar (Figure 2). When the sample was analysed by LC-MS the impurity peak failed to ionise whilst the API ionised easily. This provided further evidence that the API and impurity were not related structurally. Isolation of the impurity by Prep-LC was required to allow for further investigation.

Figure 2: UV Spectrum of Unknown Impurity (Left) and API (Right) in Phosphate Buffer Mobile Phase.



Prep-LC and SPE

All buffers were removed, thus employing only water, acetonitrile and methanol. The removal of buffers dramatically changed the elution profile, however the retention time of the impurity remained the same implying that the impurity was neither acidic nor basic in nature, and correlated with the fact that the compound failed to ionise during MS analysis. A buffer free Prep-LC method was developed using a Jupiter C18, 250 X 21.5 mm, 10µm column and the impurity was isolated in mobile phase (Figure 3). However when freeze dried, the impurity was found to disappear indicating that it was volatile. In order to concentrate the impurity in the eluent was passed through a C18 SPE cartridge and eluted with methanol. The isolation of the correct impurity was confirmed by analysis of the methanol extract using the original method (Figure 4)

Figure 3: UV Chromatogram (247nm) for Product using Buffer Free Mobile Phase and Jupiter C18 Column

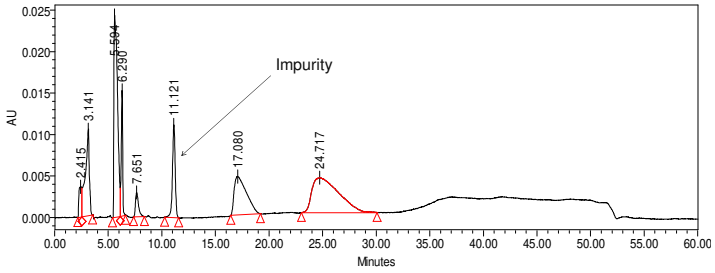
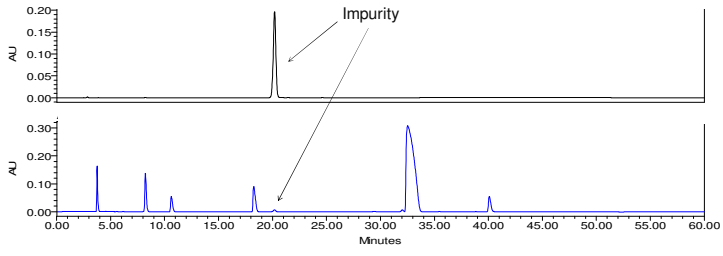


Figure 4: HPLC Analysis of Isolated Impurity Overlayed with Original Chromatography containing the Impurity



GC-MS Analysis

The concentrated isolate sample of the impurity in methanol was analysed by GC-MS with EI ionisation. This identified the impurity as benzaldehyde using the NIST08 library with a high level of confidence (Figure 5). To provide further confirmation, a sample was again loaded on an SPE cartridge and eluted using deuterated methanol. This sample was analysed by ^{1}HNMR against a standard of benzaldehyde and provided additional confidence in the positive identification (Figure 6). Lastly bezaldehyde was spiked into the original drug product sample to confirmation the retention of the impurity.

Figure 5: GC-MS Mass Spectum of Unknown Impurity in the SPE Extract Containing Impurity and Benzaldehyde from the NIST08 library

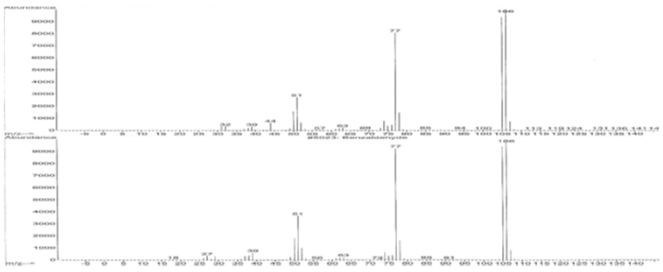
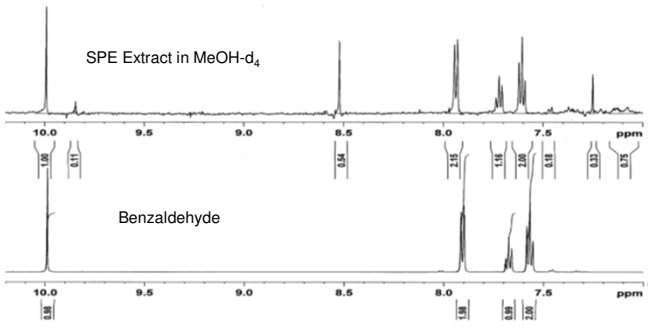
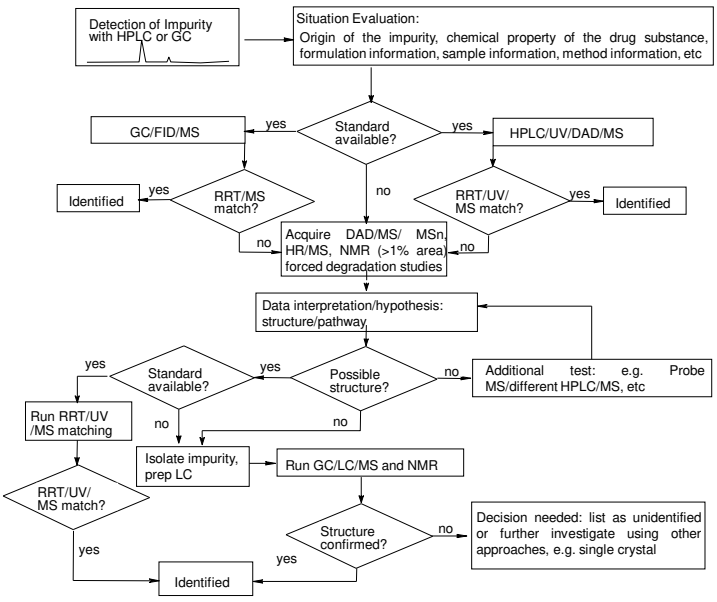


Figure 6: ^{1}HNMR of Isolated Impurity and Benzaldehyde



Flow Diagram Illustration for Impurity Identification



Conclusion

The structure of the trace impurity was successfully determined using a combination of Prep-LC, GC-MS and NMR. Final identity was confirmed by the overlaying of the proposed molecule chromatography against the original chromatography containing the impurity.