EVALUATION OF ION MOBILITY-MASS SPECTROMETRY COUPLED WITH HPLC FOR THE DETECTION OF TRACE LEVEL IMPURITIES IN FORMULATED DRUGS

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INTRODUCTION

The detection and identification of trace level impurities in formulated drugs, whether manufacturing impurities or degradation products, is often made difficult by the presence of excipients. The excipients are those components present in the final formulated product other than the active pharmaceutical ingredient (API) and are often very complex, including polymers, surfactants, sugars etc. Polyethylene glycols (PEGs), which are common excipients often present at high concentrations, are readily ionized making the detection of trace level impurities a challenge even when using hyphenated techniques like LC-MS.

Present methodology, involving sample preparation to remove excipients without removing any of the impurities, can be very time consuming and can result in recovery issues where components of interest are lost in the clean up. A potential alternative is ion mobility spectrometry (IMS), a gas phase separation technique which occurs on the millisecond timescale such that many separations can be acquired within the LC time frame.

This work investigates the use of a Waters Synapt high definition mass spectrometry (HDMS) IM-MS system to improve the separation of drug related materials from excipients [1].

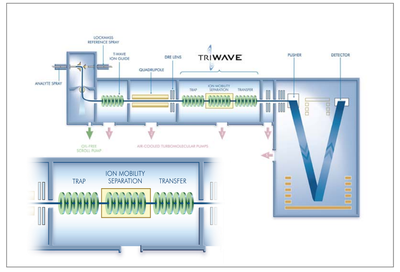

Figure 1. Schematic of Waters® SYNAPT™ HDMS™ system incorporating the enabling Triwave technology (see inset).

METHODS

Mass Spectrometry

A SYNAPT HDMS System, (Waters Corporation) was used in these studies, as shown in figure 1. Ions produced during electrospray ionisation are sampled by a Z-spray source and pass through a quadrupole, which may be set to transmit a wide mass range, or to isolate ions of a specific m/z. High efficiency ion mobility separation is then performed in the Triwave device. The Triwave consists of three travelling wave (T-wave) ion guides [2]. The first, the TRAP T-Wave, traps and accumulates ions which are then gated into the IMS T-Wave in which the ion mobility separation occurs. The TRANSFER T-Wave is then used to transport the separated ions into the oa-ToF for subsequent mass analysis. Acquisition of the mobility-MS data was through the MassLynx instrument control software and the data processing performed using the inbuilt Driftscope software package.

Samples

The samples employed in this study were the anti– viral medication Combivir and Avodart which is used to treat enlarged prostate. These are both GSK products.

A Combivir tablet sample (containing 150 mg of active ingredient in 750 mg of tablet) was prepared by crushing, shaking in 75/25 water/acetonitrile, centrifuging at 13,000 rpm for one minute and the supernatant removed and injected onto the HPLC. Sufficient tablet was taken to give 5mg/mL of the active ingredient, Zidovudine, in the final solution.

An Avodart soft capsule, containing 0.5mg of active ingredient dutasteride, was shaken in 5mL 70/30 water/acetonitrile, centrifuged at 13,000rpm for one minute and the supernatant removed for analysis by LC-MS.

Both the samples were analysed using generic LC methods developed by GSK.

RESULTS & DISCUSSION

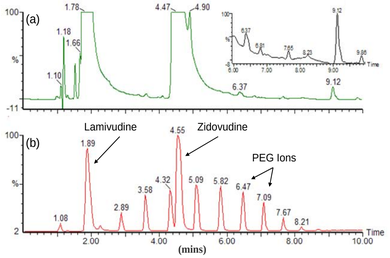

Figure 2. HPLC-MS analysis of Combivir tablet showing a) extracted UV chromatogram (245-285nm), inset is expanded region from 6-10minutes b) LC/MS TIC chromatogram

The major chromatographic peaks, which are saturated in the UV trace shown in figure 2, are from the active components lamivudine and zidovudine. The TIC shows the PEG which is present as an excipient in the formulation and is not apparent by UV. The PEG elutes in the same region as many of the low level potential impurities shown in the inset UV chromatogram.

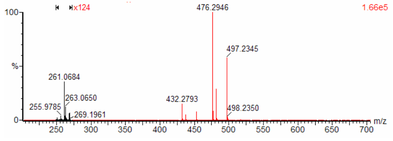

Figure 3. Background subtracted mass spectrum of known impurity A (magnified x 124) in presence of PEG interferences

Impurity A, 3'-chloro-3'deoxythymidine, is a known impurity of zidovudine, present at <1% of the API. Figure 3 shows the background subtracted mass spectrum of this impurity at m/z 261 in the presence of the PEG interfering ions. The signal from the protonated molecular ion of the known impurity has been magnified by a factor 124. It is only possible to identify the presence of this impurity because the retention time and expected molecular weight are known. This highlights the magnitude of the problem when looking for unknowns at levels of <1% in the presence of excipients such as PEG which are commonly used in formulations.

We therefore need to remove the PEG in order to look for trace level impurities. This can be done physically by using solid phase extraction however this can also result in the removal of impurities of interest. Ion mobility mass spectrometry was investigated as a potential additional orthogonal separation technique which could differentiate the PEG-related ions from the impurity ions.

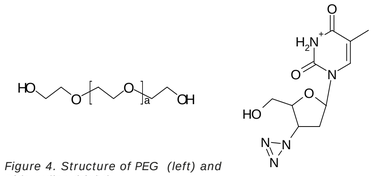

Figure 4. Structure of PEG (left) and Zidovudine (right)

IM separation relies on mobility differences of ion species as they drift through a gas under the influence of an electric field. The mobility of an ion species is related to it's structure. If we consider the structure of a PEG molecule (figure 4) it would be expected to have a different mobility compared to the active pharmaceutical ingredient Zidovudine and it's related impurities.

The Driftscope mobility trace (figure 5) shows that the PEG peaks are mobility separated making it possible to remove the polymer related signal and improve the likelihood of detection and subsequent identification of the impurities.

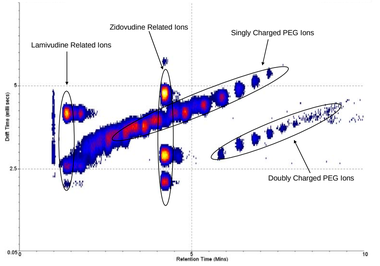

Figure 5. A retention time vs drift time plot for the LC-IMS-MS analysis of the Combivir tablet sample.

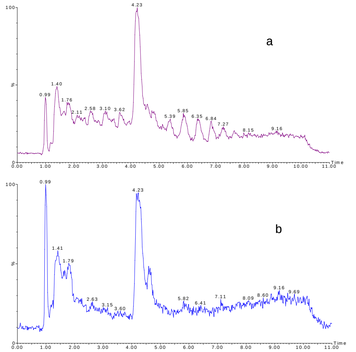

Figure 6. a) Reconstructed LC ion mobility separated chromatogram of the total Combivir sample and c) after removal of selected regions

Figure 6a shows the LC ion mobility separated chromatogram for the total Combivir sample while Figure 6b shows the TIC chromatogram after removal of selected regions associated with the PEG excipient. Whilst these data are still quite 'crude', the peaks for Lamivudine and Zidovudine can be seen and the PEG peaks have been reduced

The generic approach to the removal of the PEG interference shown in figure 6 dramatically cleans up the entire data set and closer examination of the reconstructed ion mobility separated trace after removal of the PEG peaks shows that IMS-MS allows detection of unknown, low-level, peaks in the TIC which were not previously observed. These could be manufacturing impurities or excipients that have not been removed and need to be investigated further but this clearly shows that as a "proof of principle" ion mobility is a viable additional separation technique.

The analysis of low level impurities in Avodart is a much more challenging case as the active ingredient, dutasteride is only present at 0.5mg in the soft capsule formulation which also contains many polymeric excipients with varying properties. As the excipients are of differing polarity this rules out extraction techniques such as solid phase extraction (SPE). The dutasteride is also highly potent so techniques with the minimum of handling possible are desirable.

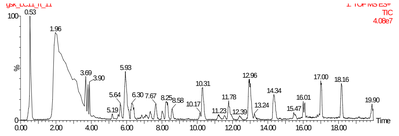

Figure 7. Reconstructed ion mobility chromatogram from analysis of an Avodart capsule.

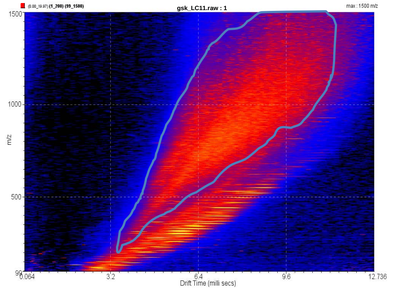

Figure 8. 3D Driftscope mobility plot of drift time versus m/z for Advodart showing selected region containing much of the interference associated with the excipients.

Figure 7 shows the reconstructed LC-IMS-MS from the analysis of Avodart. The Driftscope mobility plot (figure 8) of drift time against m/z lacks the obvious structure observed when PEG was a significant part of the formulation as demonstrated in figure 5. Figure 9 a shows the reconstructed LC-IMS-MS TIC from the region of the mobility plot containing much of the excipient interference as selected in figure 8. The ability to zoom in and exclude several regions of the mobility plot is demonstrated in figure 9b.

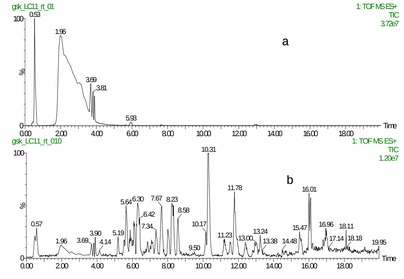

Figure 9. Reconstructed LC-IMS-MS TIC for a) region selected in figure 8 and b) after exclusion of several selected regions to remove excipients

The reconstructed LC-IMS-MS TIC after exclusion of most of the excipients (figure 9b) reveals the flexibility of this approach which provides a simple, unique route to removal of interferences, such as the dosing vehicle, and enhanced MS detection of low level impurities. Not all of the excipients have been excluded to ensure that the impurity peaks are not inadvertently removed. As the excipients have not been physically removed it is simple to go back to the raw data at any time to interrogate it further thus improving the robustness and precision of the overall method.

The active ingredient at 10.3min, which was only present at 0.5mg in the original capsule, is now the biggest peak in the chromatogram and several previously unidentified trace level impurities, associated with the synthetic route, have now been identified.

CONCLUSION

- **Ion mobility spectrometry (IMS), coupled with LC/MS offers an additional rapid, orthogonal separation capability and is therefore an ideal tool for finding trace level impurities**
- **Rapid removal of chemical noise and interferences and enhanced impurity detection was achieved without physically removing the excipients**
- **By removing the sample preparation step there are no recovery issues and the robustness and precision of the overall method is improved**
- **Although the use of HDMS does not negate the need for LC separation it affords significant advantages for the analysis of very complex mixtures.**

References

1. Eckers C., Laures A.M.-F, Giles K., Major H. and Pringle S., Rapid Comm. Mass Spec., 21 (2007) 1255-1263
2. The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).

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