Direct Analysis of Potentially Genotoxic Impurities in Active Pharmaceutical Ingredients using Miniaturized Field Asymmetric Waveform Ion Mobility Spectrometry

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Introduction

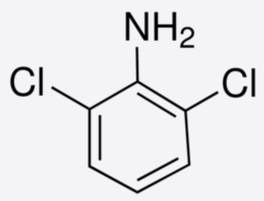
Potentially genotoxic impurities (PGIs) are a group of compounds which may be present in active pharmaceutical ingredients (APIs). PGIs can be formed as synthetic by-products from unwanted side reactions during drug synthesis, from residues of starting materials or may result from degradation of the API itself. PGIs have the potential to cause dangerous side effects make them a subject of concern for the pharmaceutical industry and the monitoring of PGIs in drugs has in recent years become increasingly important

The threshold for toxicological concern (TTC) is defined the maximum dose at which the risk from the genotoxic effects of a PGI are determined as being low. The current TTC value gives a maximum daily dose of 1.5 µg/day per PGI. Assuming a daily dose of 1g of a drug substance per day this gives a rough control level of 1.5 ppm in the drug. Drug substances where the concentration of a PGI is above this level are therefore deemed not suitable for human consumption. Currently active pharmaceutical ingredients are screened for PGIs using either LC with mass spectrometric and/or UV detection, or by GC-MS.[1] These approaches both require the development of a chromatographic method and, in the case of GC-MS, prior extraction and cleanup of the sample prior to analysis.

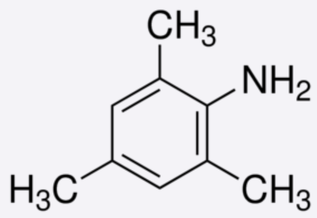
Here we demonstrate rapid and direct analysis of PGIs using thermal desorption in conjunction with a prototype chip-based FAIMS device hyphenated with a time-of-flight mass spectrometer. Separation of isobaric PGIs is achieved and mobility preseparation can be used to select precursor ions for in-source fragmentation.

Experimental

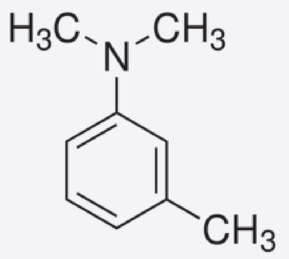
▪Three PGI compounds: 2,6 Dichloroaniline (DCA), 2,4,6 Trimethylaniline (TMA) and N,N Dimethyltoluidine (NNDMT) were analyzed in these experiments (Fig 1).



2,6-dichloroaniline (DCA) $[M+H]^+$ = 161.9877



2,4,6-Trimethyaniline (TMA) $[M+H]^+$ = 136.1126



N,N-dimethyltoluidine (NNDMT)) $[M+H]^+$ = 136.1126

Figure 1. Structures of the three of the PGI compounds analyzed.

•All analyses were carried out using a prototype Owlstone miniaturized FAIMS chip based differential mobility spectrometer hyphenated to an Agilent 6230 time-of-flight mass spectrometer.

•The FAIMS chip consists of multiple planar electrode channels with a 100 µm gap and 700 µm depth. High dispersion fields of up to 300 Td with short residence times (50-250 µs) enable fast scan rates.

•The FAIMS chip is placed inside the spray shield of the mass spectrometer in front of the capillary (Fig. 2).

•Samples for thermal desorption were placed inside a hollow thermal desorption tube. PGIs were thermally desorbed using a Markes UNITY thermal desorber and the heated transfer line was introduced into the electrospray source

•PGIs were spiked into starch to simulate a solid API.

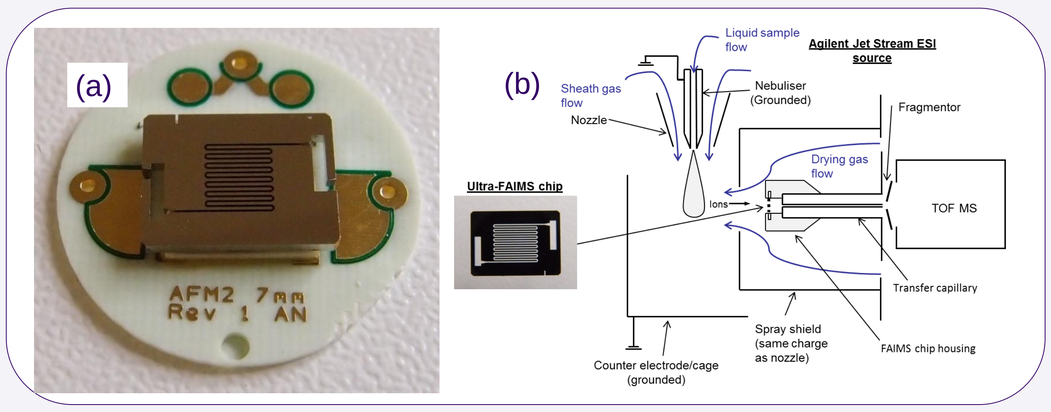


Figure 2. (a) The miniaturised chip-based FAIMS and **(b)** interfaced with the TOF-MS.

Results – Solution studies of PGIs

•PGI compounds were analyzed both individually and as a mixture.

•Optimum performance for the PGIs tested was achieved using chip with a trench length of 78.1mm at a dispersion field of 228 Td (Fig. 3).

•The three PGIs are partially resolved under these conditions with clear peaks visible for each different PGI. Comparison with individual PGI standards was used to identify the PGI peaks. Identification is possible using accurate mass assignment (Fig. 4)

Figure 3. ESI-FAIMS-MS spectrum of a mixture of the three PGI's overlaid with individual components

Figure 4. FAIMS selected mass spectra from the three component mixture **(a)** DCA **(b)** TMA and **(c)** NNDMT

Results – FISCID-MS of PGIs

• Mobility pre-selection of precursor ions can be used prior to in-source collision induced dissociation (FISCID-MS) This can be used to give additional selectivity and to identify PGIs from their fragmentation pattern as well as *m/z*.

•Tandem mass spectrometry of three PGIs was used to determine unique fragment ions for each PGI. FISCID-MS spectra show fragmentation patterns which is comparable to those observed in conventional MS/MS.

•Scanning FISCID-MS shows that characteristic fragment ions (Fig. 6) can be correlated to distinct CV peaks for each PGI enabling separation and identification of isobaric PGIs.

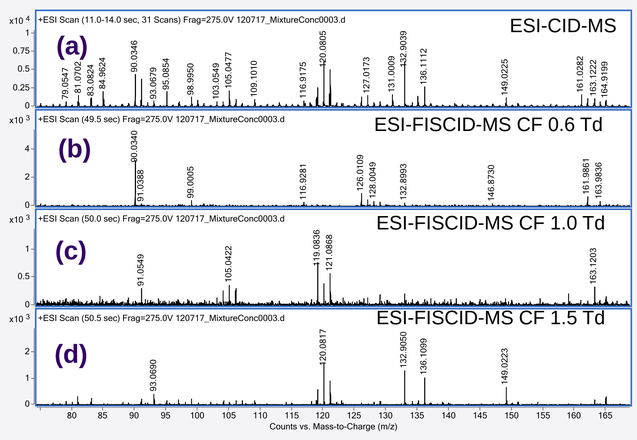


Figure 5. ESI-FISCID-MS spectra of **(a)** fragment ion spectrum (no FAIMS) **(b)** DCA **(c)** TMA **(d)** NNDMT

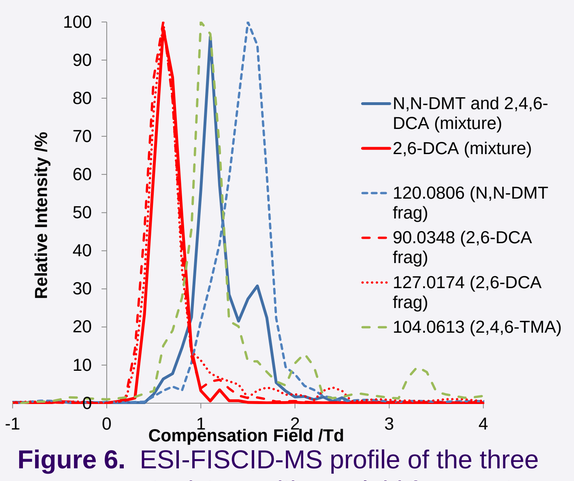


Figure 6. ESI-FISCID-MS profile of the three component mixture with overlaid fragments

Results – Thermal desorption of PGIs

•Thermal desorption experiments were performed on both liquid and solid samples.

•In the example given (Fig. 7) 50 ng of NNDMT was spiked into 50 mg of starch to simulate a PGI concentration of 1 ppm. This was placed into a TD tube and thermally desorbed.

•The chip was set to transmit ions at a compensation field of 1.4 Td to preselect NNDMT.

•A thermal desorption profile for the molecular ion of NNDMT (m/z 136.1121) was obtained (Figure 7)

•This profile shows a peak-to-peak signal to noise ratio of 11.8:1 indicating that this response is in the quantifiable range.

•The calculated limit of detection for NNDMT is 0.25 ppm demonstrating the suitability of TD-FAIMS-MS for fast toxicological profiling of APIs.

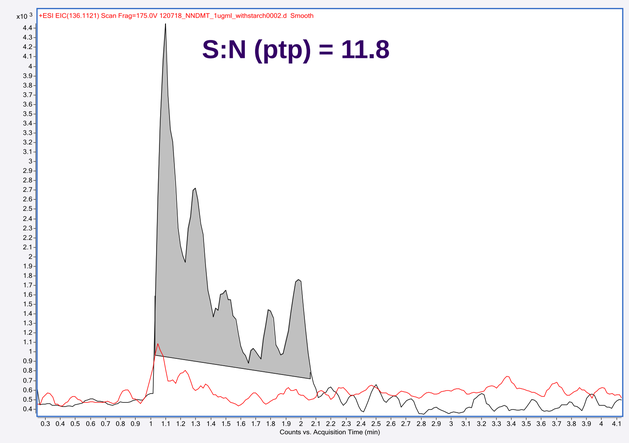


Figure 7. TD-ESI-FAIMS-MS selected ion profile of 1ppm NNDMT desorbed directly from starch compared to starch only blank

Conclusions

• UltraFAIMS in conjunction with TOF-MS is capable of rapidly separating and analyzing isobaric PGIs without a chromatographic preseparation.

•FISCID-MS can be used in a pseudo MRM experiment giving additional selectivity

•Direct thermal desorption from solid samples is demonstrated to be capable of detecting PGIs at the TTC level and is therefore suitable for rapid toxicological screening.

Acknowledgements

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References

1. Taylor, G.E; Gosling, M; Pearce, A, *J. Chrom. A* (2005) **1119**, 231-237
2. Brown, L.J; Smith, R.W, Toutoungi, D.E, Reynolds J.C *et. al, Anal. Chem* (2012) **84**, 4095-4103.