Direct analysis of potentially genotoxic impurities

Rapid FAIMS separation provides selectivity on direct analysis timescales

UltraFAIMS offers a high-speed method of separating isobaric analytes that would be indistinguishable by mass spectrometry alone. In this application, the addition of an ultraFAIMS device to a thermal desorber-mass spectrometer enables selective transmission, as well as rapid detection of isobaric potentially genotoxic impurities (PGIs) at a level below the threshold of toxicological concern.

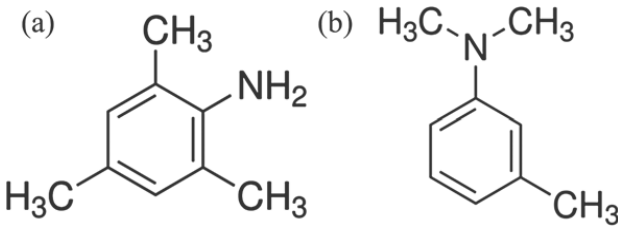

Figure 1: The structures of (a) 2,4,6-trimethylaniline, and (b) N,N-dimethyl-m-toluidine

Introduction

Potentially genotoxic impurities (PGIs) have characteristic structures that may be carcinogenic.[1,2] PGIs are monitored during the production of active pharmaceutical ingredients (APIs) to ensure that their concentrations remain at safe levels and below the threshold value of toxicological concern (TTC), required by the European Medicines Agency.[3,4] The TTC is typically 1.5 µg per day which is equivalent to 1.5 ppm assuming a 1 g per day dose. Gas chromatography (GC) and high performance liquid chromatography (LC) are often combined with mass spectrometry and are common approaches for monitoring the levels of PGI compounds in APIs. However, these conventional methods require lengthy sample preparation and chromatographic separation, prolonging the analysis time. There is a need for new analytical strategies to meet the needs of the fast-paced pharmaceutical research and discovery environment.[5,6]

This application note shows a rapid method for the determination of the isobaric PGIs 2,4,6-trimethylaniline and N,N-dimethyl-*m*-toluidine (Figure 1) by thermal desorption from a surrogate API with a FAIMS separation prior to mass spectrometry detection. Good precision was observed at the 1 ppm level for the FAIMS pre-selected PGIs, with a limit of quantification well below the required TTC.

Experiments

The experiments were carried out using a Markes Unity Series 1 Thermal Desorber interfaced with an Agilent 6230 time-of-flight mass spectrometer with an Agilent JetStream ESI source (AJS). The ultraFAIMS device was located in front of the transfer inlet capillary, behind a modified spray shield within the ESI source (see Figure 2).

Compounds	LOQ /ppm	RSD /%
2,4,6-Trimetylaniline	0.19	8.4
N,N-Dimethyl-*m*-toluidine	0.13	7.5

Table 1: Summary of quantitative performance for PGI analysis by TD-FAIMS-MS

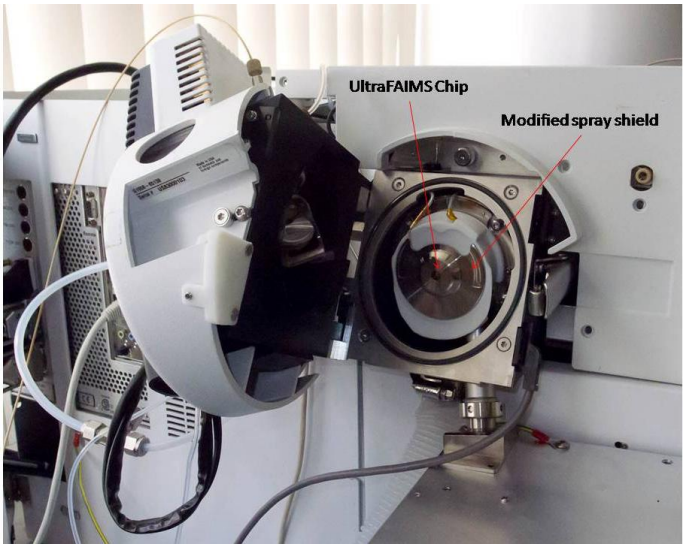

Figure 2: UltraFAIMS interface installed on Agilent 6230 TOF

Standard solutions of 2,4,6-TMA (50 ng/ml) and N,N-DMT (50 ng/ml) were prepared in methanol-water (50:50) with 0.1% formic acid for infusion studies and in acetonitrile for thermal desorption studies. The PGI mixture (4 µl, each at 2.5 µg/ml) was added to a surrogate API, starch (10 mg), which was immediately inserted into a thermal desorption tube between two pieces of Siltek Deactivated Wool (Borosilicate, Markes Int.). The concentration of each of the PGIs in starch was 1 ppm (w/w).

Thermal desorption-FAIMS-MS Conditions

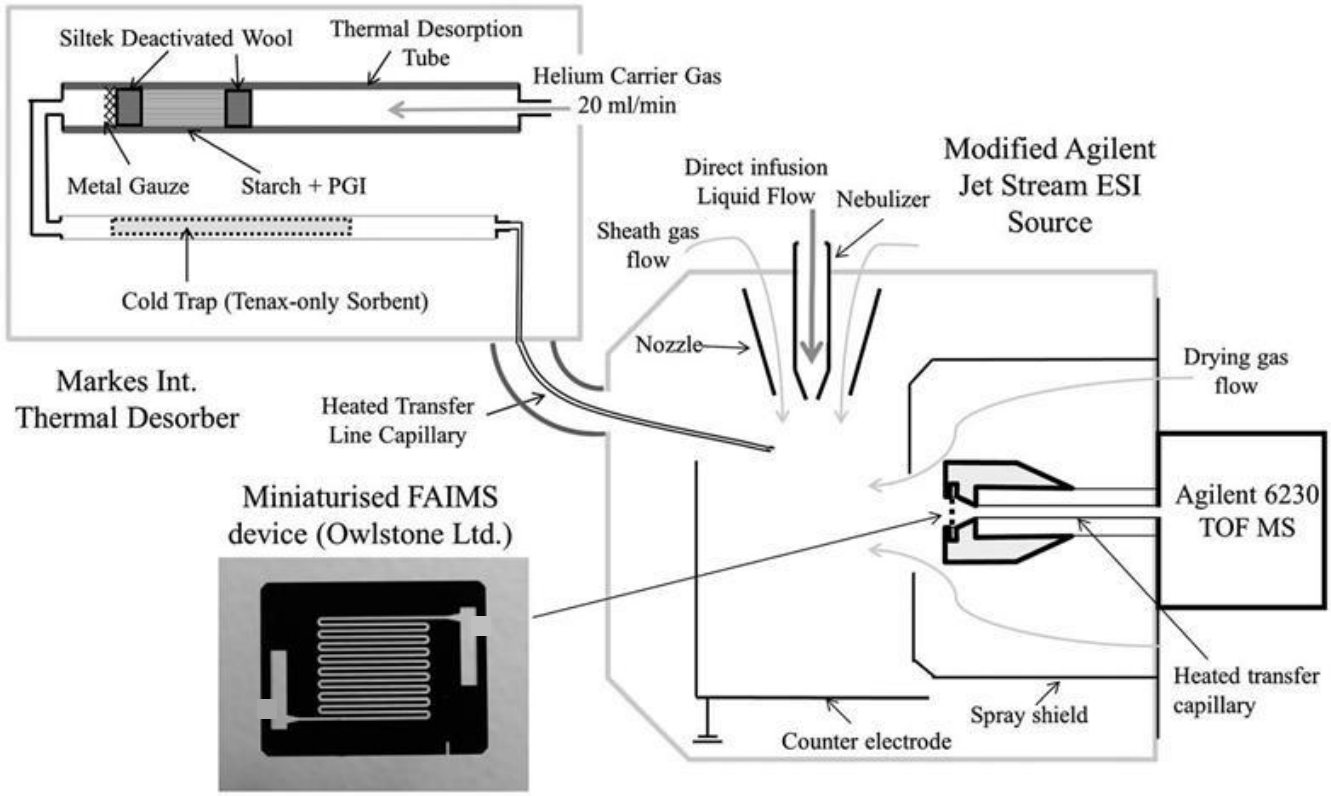

Figure 3: A schematic showing the thermal desorber interfaced with the ESI source and FAIMS-MS

The PGIs were desorbed from starch at 230˚C for 2 minutes onto a cold trap (Tenax, -10˚C) and then rapidly desorbed from the trap (250˚C, >40˚C/s, 2 minutes) with a cycle time of 10 minutes. Thermally desorbed compounds travelled down a heated transfer line (fused silica capillary, 0.25 mm i.d., 200˚C) to the heated JetStream ESI of the mass spectrometer. The tip of the fused silica capillary was positioned in the source at 55˚ to the nebulizer and set back ~10 mm. Ionization by extractive electrospray and direct infusion electrospray were carried out in positive ion mode using a 10 µl/min flow of methanol/water (50:50) with 0.1% formic acid. MS settings are shown in Table 2.

Parameter	Setting
TOF acquisition rate	10 scans/sec for scanning FAIMS experiments; 1 scans/sec for TD-FAIMS-MS
Capillary voltage	3kV
Nozzle voltage	2kV
Skimmer voltage	65V
Fragmentor voltage	175V
Drying gas temperature	150^{O}C
Sheath gas temperature	250^{O}C
Nebulizer pressure	30psig
Drying gas flow	6L/min
Sheath gas flow	8L/min

Table 2: ESI-MS conditions for direct infusion and thermal desorption

Step 1: Optimise ultraFAIMS separation

A direct infusion of each PGI was used to explore the best conditions for separation of the isobaric compounds. Compensation field (CF) was scanned from -2 to +5 Td across a DF range 200 to 300 Td at each 10 Td step; requiring a total of 140 seconds to complete a DF vs CF scan. It was found that a DF of 230 Td provided sufficient resolution to transmit each PGI individually (Figure 4). There is a second smaller peak observed for 2,4,6-TMA which is contributed by a dimer which fragments to the monomer ion after FAIMS separation; the peak maximum for N,N-DMT is located in the space between the 2,4,6-TMA monomer and dimer.

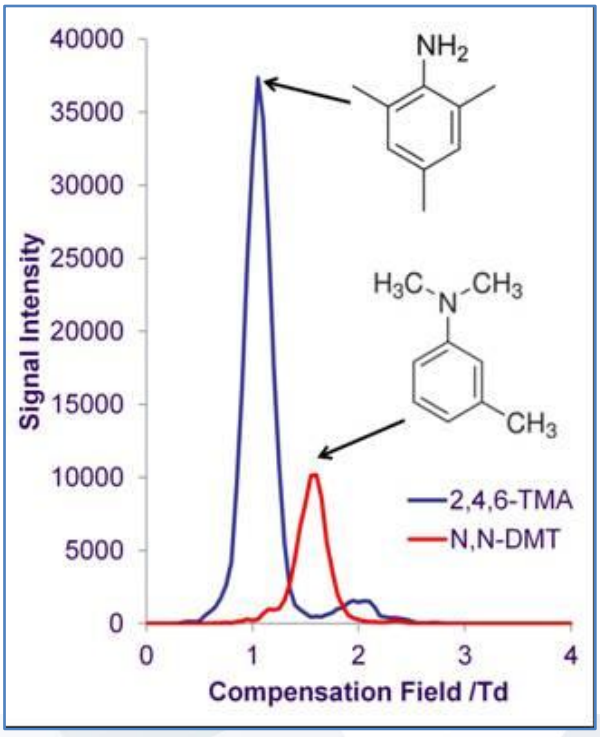

Figure 4: Selected ion response (m/z 136.1) during direct infusion of each PGI separately with FAIMS CF scan underway at DF=230 Td. 2,4,6 TMA monomer is optimally transmitted at CF=1.0 Td, whilst N,N-DMT is optimally transmitted at CF=1.5Td.

Step 2: TD-FAIMS-MS analysis

2,4,6-TMA and N,N-DMT are sufficiently volatile that they can be thermally desorbed from starch (surrogate API). Both of the desorbed compounds are simultaneously ionized and detected by EESI-MS, but as they have the same molecular formula, protonated 2,4,6-TMA and N,N-DMT ions cannot be distinguished by mass spectrometry.

The FAIMS device was set to transmit either: 2,4,6-TMA (CF 1.0 Td, DF 230 Td) or N,N-DMT (CF 1.5 Td, DF 230 Td), while filtering out isobaric interference from the other as they elute from the transfer line of the thermal desorber. Typical thermal desorption profiles for the PGI mixture (1 ppm) after FAIMS pre-selection are shown in Figure 5 (the time axis is from the MS acquistion, started 1 minute after heating the cold trap). The selected ion responses (*m/z* 136) for each FAIMS pre-selected PGI

show that the PGIs can be thermally extracted from starch and distinguished in-source to remove isobaric interference prior to MS detection.

The sharp peaks were achieved by using a single sorbent (Tenax) instead of the more commonly-used multiple sorbents cold traps. Trapping the PGIs on a cold trap (Figure 3) with a single sorbent results in a single desorption profile, aiding peak integration of the area for quantification. The inserts to Figure 5 show the corresponding mass spectra obtained at the peak maxima of the thermal desorption peaks.

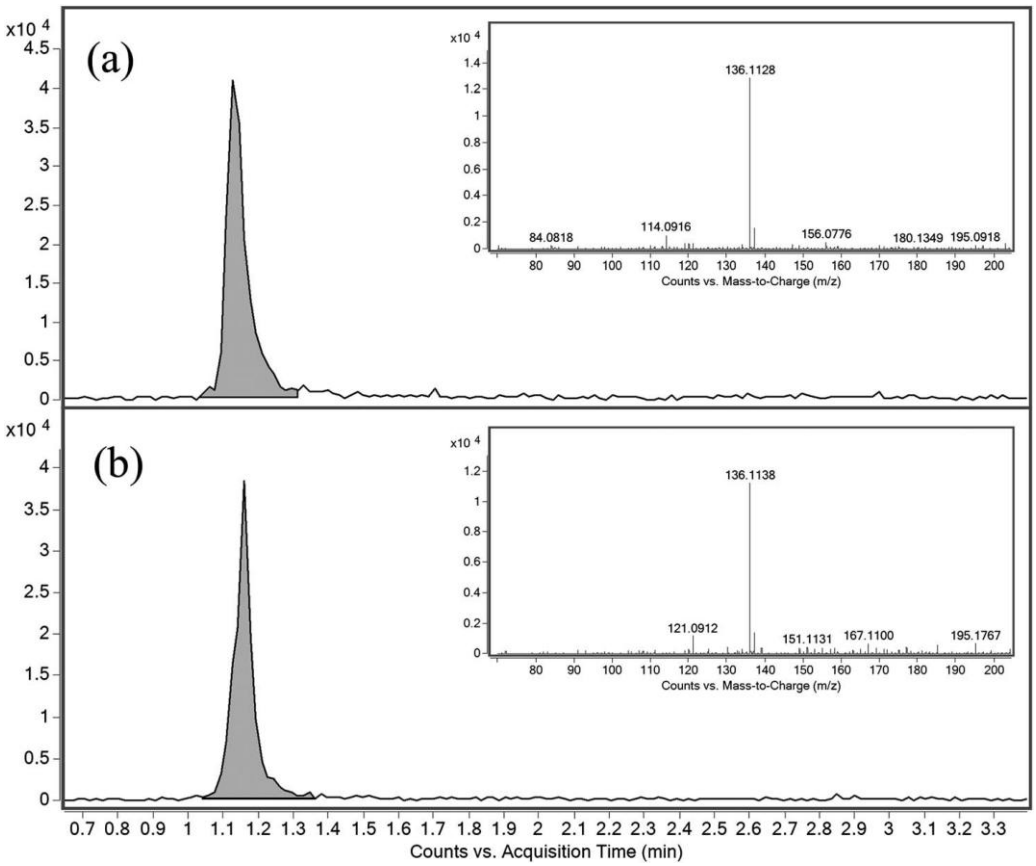

Figure 5: Selected ion response (m/z 136.1) of TD-FAIMS-MS analysis of a mixture of 2,4,6-TMA and N,N-DMT with FAIMS set to transmit: (a) 2,4,6-TMA (DF 230 Td, CF 1.0 Td); and (b) N,N-DMT (DF 230 Td, CF 1.5 Td); each with corresponding mass spectra from TD peak maximum

Quantitative Performance

As summarized earlier in Table 1:

- The limit of quantitation (LOQ; signal-to-noise 10:1) for 2,4,6-TMA and N,N-DMT was 0.19 and 0.13ppm, respectively – approximately an order of magnitude below the TTC limit, assuming a 1g/day dose.
- Precision was evaluated at 1 ppm and found to be acceptable, with RSD less than 8.4%

These data demonstrate that the addition of ultraFAIMS to TD-MS adds the ability to distinguish between isobaric compounds while easily meeting the requirements for screening PGIs with significantly less sample preparation and analysis times.

References

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