Direct analysis of potentially genotoxic impurities by thermal desorption-field asymmetric waveform ion mobility spectrometry-mass spectrometry

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Thermal desorption has been combined with field asymmetric waveform ion mobility spectrometry and mass spectrometry for the rapid, direct analysis of isobaric potentially genotoxic impurities (PGIs) in a surrogate active pharmaceutical ingredient. FAIMS-selected PGIs were detected with limits of quantification <0.2 ppm, below the threshold of toxicological concern, with %RSD <8.4%, at the 1 ppm level.

Fig. 1 The structures of (a) 2,4,6-trimethylaniline, and (b) *N*,*N*-dimethyl-*m*-toluidine.

1 Introduction

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

Thermal desorption is an extraction technique, where an analyte is transferred into the gas phase from a solid or liquid. The technique requires minimal sample preparation and is often interfaced with gas chromatography-mass spectrometry.[7] Desorbed PGIs have been ionised *via* extractive electrospray in an electrospray ionisation source (ESI) and detected by mass spectrometry.[8,9] Thermal desorption has also been interfaced with drift tube ion mobility-mass spectrometry (IM-MS) for the
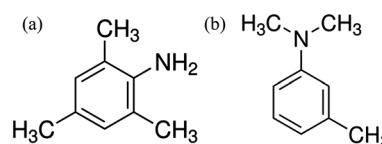
analysis of breath samples collected on solid adsorbents, with the ion mobility separation adding selectivity to the analyses.[10]

Field asymmetric waveform ion mobility spectrometry separates gas phase ions using a waveform with alternating low and high electric fields known as the dispersion field (DF). A compensation field (CF), is superimposed on the DF and may be scanned or fixed to transmit selected analytes based on their differential ion mobility under low and high electric fields conditions. FAIMS separation using a miniaturised, chip-based device is fast as a result of the short ion residence times (50–250 μs) and has been shown to enhance the selectivity of mass spectrometry analyses.[11,12] FAIMS separation is orthogonal to mass spectrometry and can distinguish between isobaric ions, making hyphenation of these techniques highly desirable.[11,13]

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

2 Materials and methods

2.1 Chemicals

2,4,6-Trimethylaniline (2,4,6-TMA), *N*,*N*-dimethyl-*m*-toluidine (*N*,*N*-DMT), starch, and HPLC grade acetonitrile, methanol, water and formic acid were obtained from Sigma Aldrich (Gillingham, UK).

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2.2 Sample preparation

Standard solutions of 2,4,6-TMA (50 ng mL^{-1}) and N,N-DMT (50 ng mL^{-1}) 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, 2.5 μg mL^{-1}) 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.), as shown in Fig. 2. The concentration of each of the PGIs in the starch was 1 ppm (w/w).

2.3 Instrumentation

TD-ESI-FAIMS-MS analyses were carried out using a Markes UNITY1 thermal desorption unit (Markes International, Swansea, UK) combined with an Agilent 6230 TOFMS (Agilent technologies, U.K.) fitted with a prototype chip-based FAIMS device (Fig. 2). 2,4,6-TMA and N,N-DMT were desorbed from the surrogate API (starch) at 230 °C for 2 min onto a cold trap (Tenax, −10 °C) and then rapidly desorbed from the trap (250 °C, >40 °C s^{-1}, 2 min). The thermal desorption cycle time was ~10 minutes. The heated transfer line from the thermal desorber, containing a fused silica capillary (0.25 mm i.d.) was maintained at 200 °C and introduced into the JetStream ESI of the TOFMS by removing the glass from a viewing window in the source housing. The tip of the fused silica capillary was positioned in the source at an angle of approximately 55° to the nebuliser, but set back ~10 mm to give the maximum response. Extractive electrospray and direct infusion electrospray were carried out in positive ion mode using a 10 μL min^{-1} infusion of methanol–water (50 : 50) with 0.1% formic acid. The ESI spray nebuliser pressure was set to 30 psig with an 8 L min^{-1} sheath gas flow at 250 °C. The nozzle voltage was set to 2000 V; the transfer capillary, spray shield and counter electrode were set to 3000 V and the drying gas flow to 6 L min^{-1} at 150 °C.

The miniaturised chip-based FAIMS (Owlstone Ltd., Cambridge), consisting of multiple parallel electrode gaps (100 μm) with a short path length (700 μm),[13–15] was located behind the spray shield of the TOFMS inlet. Scanning FAIMS-MS experiments were carried out by direct infusion of 2,4,6-TMA and N,N-DMT individually to evaluate the potential for separation of these isobaric compounds. The DF was stepped (10 Td) from 200 to 300 Td and the CF was scanned from −2 to +5 Td to determine optimum separation conditions. The mass spectrometer fragmentor voltage was set to 175 V for FAIMS-selected transmission experiments. The MS scan rates were 10 scans per s and 1 scan per s in the mass range m/z 70–1000 for direct infusion and thermal desorption experiments respectively.

3 Results and discussion

The thermal desorption of volatile compounds from a less volatile solid sample, such as an API, has the potential to speed
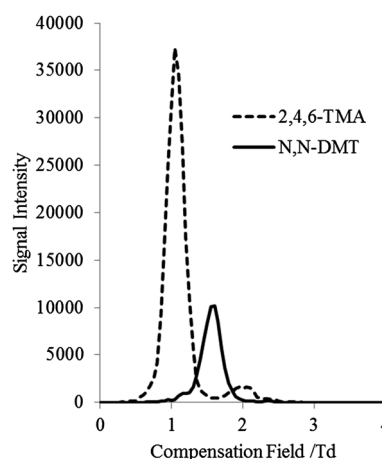
Fig. 3 FAIMS CF scan of 2,4,6-TMA (dashed) and N,N-DMT (solid) with DF set to 230 Td.
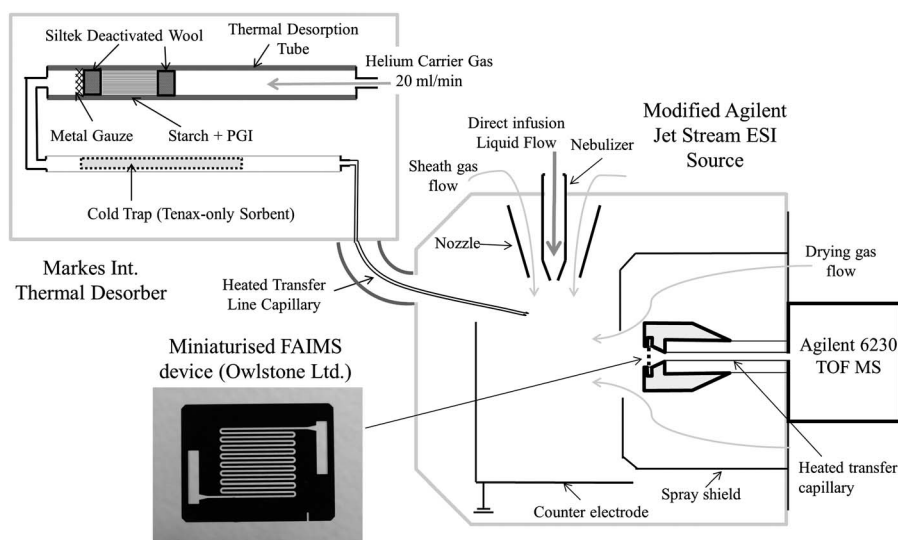
Fig. 2 A schematic diagram of the thermal desorber interfaced to the ESI-FAIMS-MS ion source.

up analysis by removing time consuming sample preparation and chromatographic separation steps. Furthermore, there is an even greater potential advantage in that such approaches are matrix independent and can be applied to the analysis of a wide range of APIs without the need to develop individual specific methods for each PGI/API combination. Increasing sample throughput is important for efficient quality control, but selectivity may be compromised to reduce analysis times, particularly for isobaric analytes that cannot be distinguished by mass spectrometry. Miniaturised, chip-based field asymmetric waveform ion mobility spectrometry incorporated into the ion source of a time-of-flight mass spectrometer (FAIMS-MS), was therefore investigated for the separation of the isobaric PGIs 2,4,6-trimethylaniline (2,4,6-TMA) and *N,N*-dimethyl-*m*-toluidine (*N,N*-DMT) (Fig. 1).[16,17]

3.1 Direct infusion FAIMS-MS studies

The two substituted anilines, 2,4,6-TMA and *N,N*-DMT, were introduced by direct infusion into the ESI-FAIMS-MS to explore the potential for a rapid separation step which would be compatible with the thermal desorption of these analytes. A DF stepping experiment, with the CF scanned at each DF, was used to determine the optimum DF for maximum separation without compromising sensitivity.[12] The CF spectrum obtained at the optimum DF, which was determined to be 230 Td, is shown in Fig. 3. The spectrum contains two peaks corresponding to 2,4,6-TMA and *N,N*-DMT centered at a CF at 1.0 and 1.5 Td

respectively. There is also a small peak at CF 2 Td in Fig. 3, which is assigned to the protonated dimer of 2,4,6-TMA. The *N,N*-DMT peak lies between the 2,4,6-TMA monomer and dimer peaks where there is no significant interference from either. 2,4,6-TMA and *N,N*-DMT were sufficiently resolved at a DF of 230 Td to enable the selective transmission of each without significant interference from the other.

3.2 TD-FAIMS-MS analysis of 2,4,6-TMA and *N,N*-DMT

2,4,6-TMA and *N,N*-DMT are sufficiently volatile to be thermally desorbed from a surrogate API (starch) and transferred to the ESI source *via* a heated transfer line. The desorbed molecules were ionised using extractive electrospray (EESI) and detected using FAIMS-time-of-flight mass spectrometry (TOF MS). The FAIMS CF was set to transmit either 2,4,6-TMA (CF 1.0 Td) or *N,N*-DMT (CF 1.5 Td) selectively at a DF of 230 Td following ionization. Fig. 4 shows typical thermal desorption profiles (*m/z* 136) for the FAIMS pre-selected 2,4,6-TMA and *N,N*-DMT, spiked as a mixture into a surrogate API (starch) at 1 ppm (w/w). A good peak shape for the thermal desorption profile of each PGI was achieved by using a single sorbent (Tenax) in the desorber cold trap. The time axis corresponds to the mass spectrometry acquisition time, which was started 1 minute before the heating of the cold trap. Fig. 4a and b inserts show mass spectra corresponding to the maximum point of the thermal desorption profiles of FAIMS-selected 2,4,6-TMA and *N,N*-DMT respectively, showing the protonated ions at *m/z* 136. The use of a FAIMS
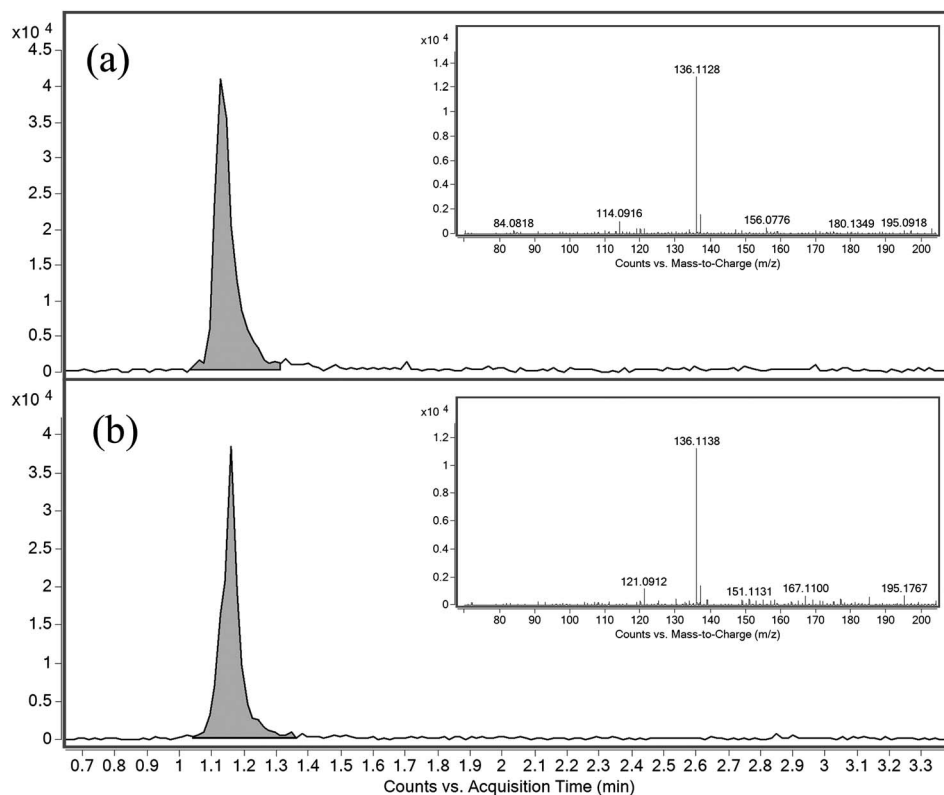

Fig. 4 TD-ESI-FAIMS-MS selected ion profiles (*m/z* 136.1) of (a) 2,4,6-trimethylaniline (CF = 1 Td, DF 230 Td); and (b) *N,N*-dimethyl-*m*-toluidine (CF = 1.5 Td, DF 230 Td), desorbed directly from starch at 1 ppm (w/w). Inset are the corresponding mass spectra at the desorption peak maxima.

Table 1 Quantitative determination of 2,4,6-TMA and *N,N*-DMT by TD-FAIMS-MS

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

separation at the appropriate DF and CF determined from the direct infusion studies, adds selectivity to the analysis by discriminating between *N,N*-DMT and 2,4,6-TMA.

The quantification limits and precision were evaluated to test performance of the prototype TD-FAIMS-MS analysis (Table 1). The limit of quantification (LOQ) was determined to be 0.19 ppm and 0.13 ppm for the FAIMS pre-selected 2,4,6-TMA and *N,N*-DMT respectively (10 : 1 signal : noise), spiked as a mixture into 10 mg of the surrogate API. These LOQs are approximately an order of magnitude below 1.5 ppm, the TTC limit assuming a 1 g per day dose (European Medicines Agency). However, detection of PGIs at 'as low as reasonably possible' (the so-called ALARP concept) levels[1,2] is encouraged, but not essential, for unusually toxic impurities where detection below the TTC may be required. This requirement is easily met using TD-FAIMS-MS, as well as reducing sample preparation and analysis times.

4   Conclusion

Chip-based FAIMS combined with TOF MS has been used for the rapid determination of the isobaric PGIs 2,4,6-TMA and *N,N*-DMT, without the need for a chromatographic separation. The ability to filter ions based on differential mobility, an orthogonal technique to mass spectrometry, provides an added dimension of separation to the TD-MS analysis. Direct thermal desorption of 2,4,6-TMA and *N,N*-DMT from a solid matrix was demonstrated at 1 ppm, below the TTC, with good precision (<8.4%). The combination of good reproducibility and limits of quantification almost an order of magnitude below the TTC, shows that TD-FAIMS-MS has the potential as a rapid, quantitative screening method for monitoring volatile PGIs in active pharmaceutical ingredients. Other API matrices would need to be investigated to demonstrate the ubiquity of this technique. The approach may also be used for the analysis of volatile organic compounds in other solid matrices.

Acknowledgements

References

1 L. Muller, R. J. Mauthe, C. M. Riley, M. M. Andino, D. D. Antonis, C. Beels, J. DeGeorge, A. G. M. De Knaep, D. Ellison, J. A. Fagerland, R. Frank, B. Fritschel, S. Galloway, E. Harpur, C. D. N. Humfrey, A. S. Jacks, N. Jagota, J. Mackinnon, G. Mohan, D. K. Ness, M. R. O'Donovan, M. D. Smith, G. Vudathala and L. Yotti, *Regul. Toxicol. Pharmacol.*, 2006, **44**, 198–211.

2 *Genotoxic Impurities: Strategies for Identification and Control*, ed. A. Teasdale, John Wiley and Sons Inc., New Jersey, 2010.

3 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002903.pdf, accessed 16 December 2012.

4 http://www.fda.gov/downloads/Drugs/.../Guidances/ucm079235.pdf, accessed 16 December 2012.

5 T. McGovern and D. Jacobson-Kram, *TrAC, Trends Anal. Chem.*, 2006, **25**, 790–795.

6 B. A. Olson, B. C. Castle and D. P. Myers, *TrAC, Trends Anal. Chem.*, 2006, **25**, 796–805.

7 J. F. Pankow, W. Luo, L. M. Isabelle, D. A. Bender and R. J. Baker, *Anal. Chem.*, 1998, **70**, 5213–5221.

8 J. Ding, S. Yang, D. Liang, H. Chen, Z. Wu, L. Zhang and Y. Ren, *Analyst*, 2009, **134**, 2040–2050.

9 J. C. Reynolds, G. J. Blackburn, C. Guallar-Hoyas, V. H. Moll, V. Bocos-Bintintan, G. Kaur-Atwal, M. D. Howdle, E. L. Harry, L. J. Brown, C. S. Creaser and C. L. P. Thomas, *Anal. Chem.*, 2010, **82**, 2139–2144.

10 N. A. Devenport, L. C. Sealey, F. H. Alruways, D. J. Weston, J. C. Reynolds and C. S. Creaser, *Anal. Chem.*, 2013, **85**, 6224–6227.

11 L. J. Brown, R. W. Smith, D. E. Toutoungi, J. C. Reynolds, A. W. T. Bristow, A. Ray, A. Sage, I. D. Wilson, D. J. Weston, B. Boyle and C. S. Creaser, *Anal. Chem.*, 2012, **84**, 4095–4103.

12 R. W. Smith, D. E. Toutoungi, J. C. Reynolds, A. W. T. Bristow, A. Ray, A. Sage, I. D. Wilson, D. J. Weston, B. Boyle and C. S. Creaser, *J. Chromatogr., A*, 2013, **1278**, 76–81.

13 L. J. Brown, D. E. Toutoungi, N. A. Devenport, J. C. Reynolds, G. Kaur-Atwal, B. Boyle and C. S. Creaser, *Anal. Chem.*, 2010, **82**, 9827–9834.

14 A. A. Shvartsburg, R. D. Smith, A. Wilks, A. Koehl, D. Ruiz-Alonso and P. Boyle, *Anal. Chem.*, 2009, **81**, 6489–6495.

15 A. A. Shvartsburg, K. Tang, R. D. Smith, M. Holden, M. Rush, A. Thompson and D. Toutoungi, *Anal. Chem.*, 2009, **81**, 8048–8053.

16 M. E. Kugler-Steigmeier, U. Friederich, U. Graf, P. Maier and C. Schlatter, *Arch. Toxicol., Suppl.*, 1998, **12**, 337–340.

17 G. Vanhoenacker, E. Dumont, F. David, A. Baker and P. Sandra, *J. Chromatogr., A*, 2009, **1216**, 3563–3570.