Characterization of API impurities and degradation products by ion mobility LC-timsTOF Pro with parallel accumulation serial fragmentation (PASEF)

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Introduction

Impurities and degradation products in active pharmaceutical ingredients (APIs) possess potentially toxic effects on the efficacy and safety of pharmaceutical products. It is important in drug development process to perform impurity profiling, identification, structure elucidation and quantitation which are strictly regulated by ICH, EMA, USFDA, CADTH and PMDA. API impurities could be derived from the processes of synthesis, formulation, packing, storage and transportation in the forms of reaction materials, synthesis intermediates, by products, stereochemistry (dimer, trimer) and crystallization (aggregates). LC based techniques in combination with mass spectrometry detection have had a large impact on pharmaceutical analysis [1]. In this work, Lumefantrine (API) was used as an example to evaluate the high throughput and sensitive metabolomics workflow for the characterization of API impurities and degradation products by direct infusion or LC-PASEF timsTOF Pro with ion mobility activated.

Methods

The stock solutions of 10 mg/mL Lumefantrine and its impurities (synthesized by SYN Pharmatech) were prepared in dichloromethane (Sigma-Aldrich). Its working solutions (1 mg/mL,

Figure 1. LC-PASEF timsTOF Pro system

50 μg/mL) were prepared by diluting respective stock solutions with methanol, bubbled with Argon and stored at 2-8^{0}C. Forced degradations of Lumefantrine (API) were conducted at 45^{0}C for overnight (~10 hrs) under oxidative (1% H_2O_2), acidic (1 M HCl) and alkaline (1 M NaOH) conditions. Samples were analyzed by direct infusion at 3 μL/min for 2 minutes or by Elute UHPLC-PASEF timsTOF Pro (Bruker, Figure 1) with ion mobility activated in ESI positive mode using a C18, 100 x 2.1mm (1.8μ) column, 8 minutes LC gradient elution and 5 μL sample injection. Data analysis was performed in DataAnalysis 5.2 and MetaboScape 5.0® (Bruker).

Results and Discussions

Impurity profiling

Lumefantrine was hardly dissolved in water, methanol and acetonitrile, and dichloromethane was found to be a good solvent to prepare its stock solution at 10 mg/mL. The chemical structures of major Lumefantrine impurities were listed in Figure 2 and their EIC profiles under different stability stress conditions were displayed in Figure 3. It was noticed (a) Desbenzylketo N-oxide (V, m/z 436) and Desbenzyl derivative (VII, m/z 406) only appear under oxidative stress; (b) Lumefantrine (mono-)desbutyl derivative (II, m/z 474) increases approximately at the same level under oxidative, acidic and basic stress conditions; (c) more DBK (III, m/z 420), Lumefantrine N-oxide (VI, m/z 544) or Lumefantrine oxide (VIII, m/z 544) were
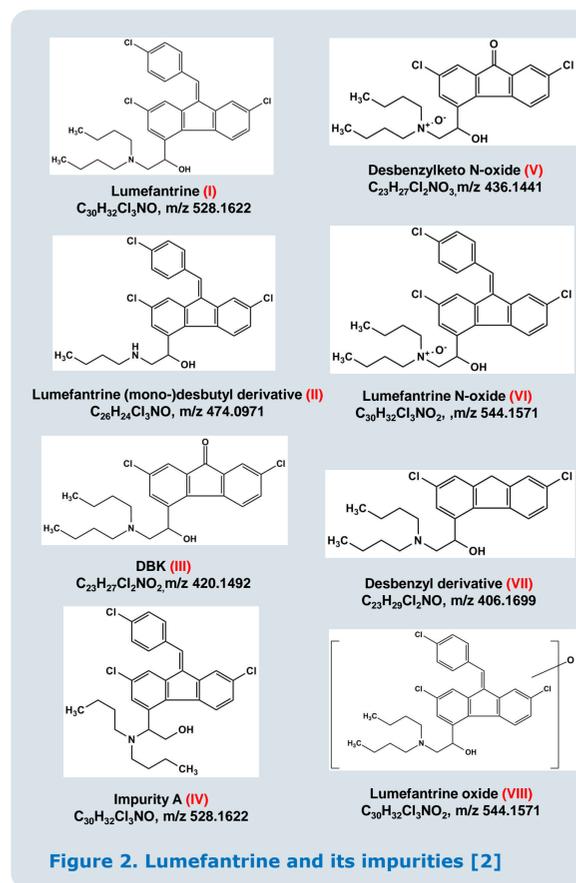

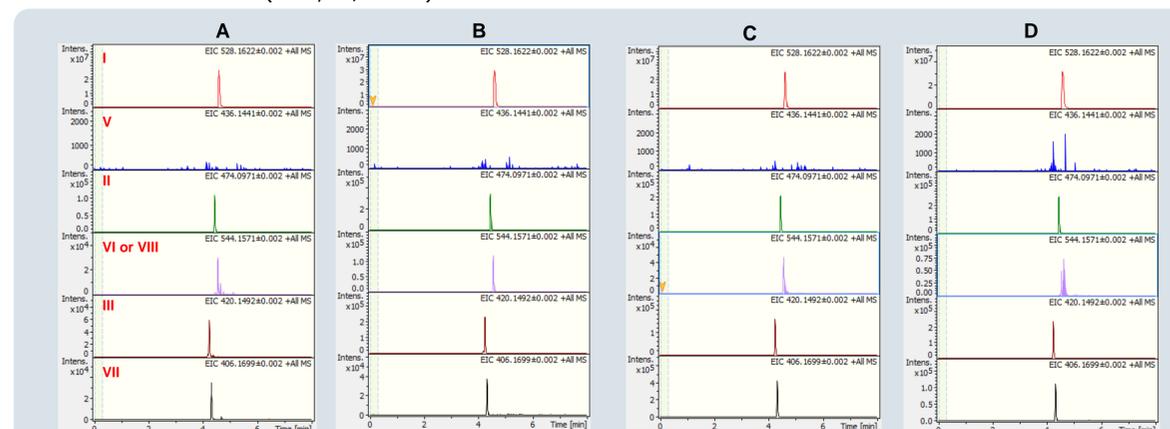**Figure 2. Lumefantrine and its impurities [2]**

produced under acidic stress than alkaline and oxidative conditions; (d) Only one EIC peak of m/z 544 was observed which could be the isobaric impurities of Lumefantrine N-oxide (VI, m/z 544) or Lumefantrine oxides (VIII, m/z 544). It is impossible to verify the isomer based on all available accurate mass, isotope pattern and MS/MS fragmentation information. By analyzing its ion mobility data, multiple EIM peaks of m/z 544 are observed (Figure 4A) which confirm m/z 544 could be Lumefantrine N-oxide or Lumefantrine oxides; (e) Impurity A (IV, m/z 528) is an impurity of Lumefantrine and it is also an isobaric isomer of Lumefantrine, but no chromatography separation was achieved. Based on the EIM of m/z 528 (Figure 4B), two major peaks and some minor peaks were observed; and one of the minor peaks could be Impurity A since its level is significantly lower than Lumefantrine. By using direct infusion and further modifying the ion mobility experimental parameters, better EIM separation was achieved.
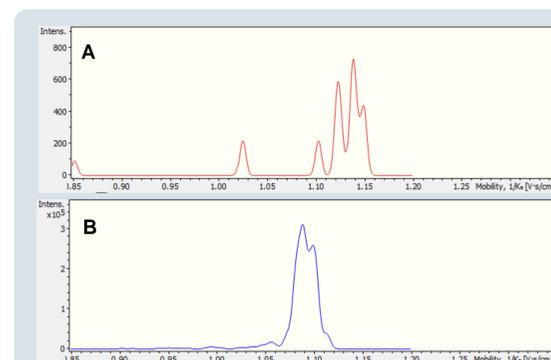

Figure 4. Extracted ion mobilogram (EIM)
A – m/z 544.1571; B – m/z 528.1622

Impurity identification and confirmation

Lumefantrine data of LLC-PASEF timsTOF Pro were processed in MetaboScape 5.0® using the powerful T-ReX ® 4D algorithm, and the extra fast PASEF MS/MS acquisition speed at full sensitivity enables to deeply perform low abundant impurities identification (Figure 5). The results of BAK (III, m/z 420) and Desbenzyl derivative (VII, m/z 406) were listed in Figure 6 and annotated with Analyte List and Bruker MetaboBASE® Personal Library. Desbenzyl derivative was confirmed based on
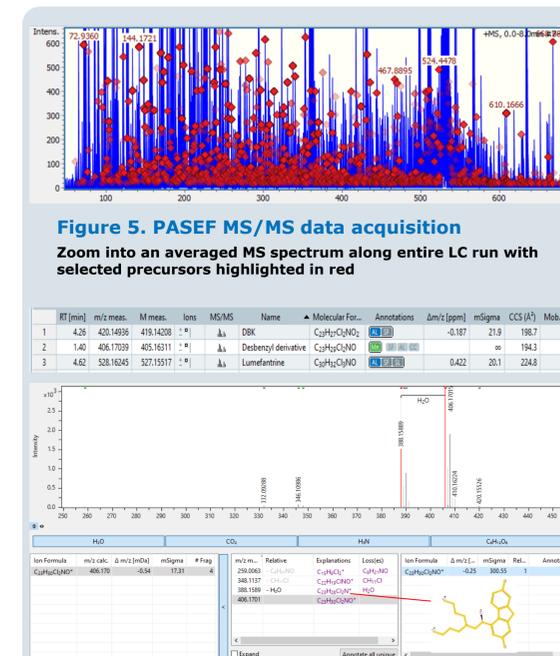

Figure 5. PASEF MS/MS data acquisition
Zoom into an averaged MS spectrum along entire LC run with selected precursors highlighted in red

Figure 6. Impurity identification and confirmation

SmartFormula (SF)/SF3D with four fragment ions were concluded and it was further verified by MetFrag in-silico fragmentation.

References

(1) Beccaria M et al. Analyst, 2020, 145 (4):1129
(2) Verbeken et al. Malaria Journal 2011, 10:51

Conclusions

- LC-timsTOF Pro with PASEF enables fast MS/MS acquisition for deeply profiling and identification of Lumefantrine (API) impurities
- Ion mobility of timsTOF Pro provides additional confidence for the characterization of Lumefantrine (API) impurities
- MetaboScape 5.0® is a powerful tool to automatically extract feature compounds and enable confident identification.

Figure 3. Extracted ion chromatogram (EIC) of Lumefantrine and its impurities
Lumefantrine stability testing at 45^{0}C overnight: A – control; B – 0.1 M HCl; C – 0.1 M NaOH; D – 1% H_2O_2

API impurity profiling