Ultra-sensitive method for the determination of nicotine in PK studies with next generation products

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Introduction

The pharmacokinetics (PK) of nicotine uptake with next (new) generation products (NGPs) such as electronic cigarettes (e-cigs), tobacco heating products and others are an essential criterion for evaluating the performance of the product. When using NGPs, the nicotine uptake is frequently found to be lower as compared to conventional cigarettes (CCs), sometimes even hardly exceeding the common background levels in human blood samples of non-users. Therefore, apart from having a highly sensitive analytical method for quantification of nicotine in serum or plasma, it is of paramount importance to reduce the ubiquitously occurring nicotine background levels as far as possible. This is required to be performed both at the side of the blood sample collection (the clinic) as well as at the side of the analysis (the laboratory).
Moreover, the method should be characterized by a very high throughput in order to be able to determine nicotine in large cohort PK studies.

UPLC-MS/MS analysis of nicotine

Sample preparation

- Plasma sample spiked with d_3-nicotine internal standard
- Liquid-liquid extraction with 1 mL of ethyl acetate
- Evaporation of the organic phase
- Reconstitution in acetonitrile for injection into the UPLC-MS/MS-system

Analytical method

- UPLC-MS/MS: Waters Acquity I-Class (UPLC); Xevo-TQS (MS/MS)
- Chromatographic separation:
  Waters Acquity UPLC BEH HILIC column (150 x 2.1 mm; 1.7 µ)
  Injection volume: 5 µL
  Eluents: acetonitrile-ammonium formate buffer (100 mM, pH = 3.2)
  Isocratic elution 80/20 (v/v); Flow rate: 0.4 mL/min

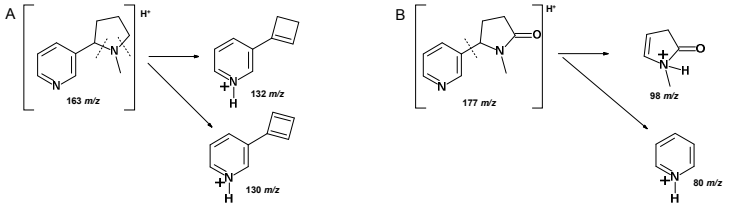

Fig. 1: Proposed fragmentation pattern for nicotine (A) and cotinine (B) according to [1]. The upper reaction scheme illustrates the quantifier mass transitions; qualifier mass transitions are given in the lower schemes.

Table 1: MS parameters (Quan: quantifier, Qual: qualifier, IS: internal standard, RT: retention time) for nicotine and cotinine.

Analyte	MRM [m/z]	IS (MRM) [m/z]	Collision [V]	RT [min]
Nicotine	163 -> 132 (Quan) 163 -> 130 (Qual)	Nic-d_3 (166 -> 132)	14 18	2.65
Cotinine	177 -> 98 (Quan) 177 -> 80 (Qual)	Cot-d_3 (180 -> 101)	18 18	1.44

Method is also suitable for the quantification of cotinine (Fig. 3) – the major primary metabolite of nicotine mainly formed by CYP450-induced oxidation in the liver (Fig. 2).

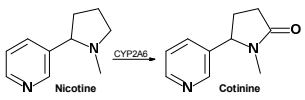

Fig. 2: CYP-induced formation of cotinine.

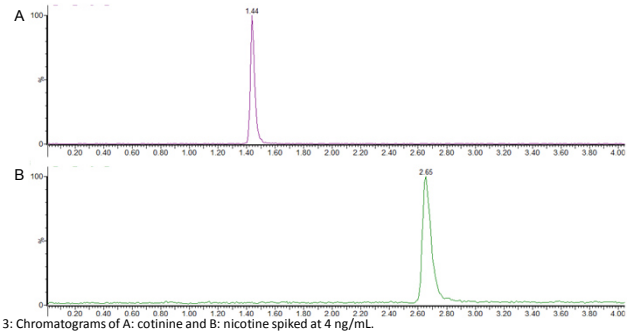

Fig. 3: Chromatograms of A: cotinine and B: nicotine spiked at 4 ng/mL.

Validation according to FDA guidelines [2] currently running:

- Calibration range: ≈ 0.1 - 100 ng/mL nicotine
- LLOQ: ≈ 0.1 – 0.2 ng/mL for nicotine
- Estimated plasma volume needed: 0.2 mL

- Isocratic elution allows fast sample analysis (total run time of 4 minutes)
- Simple sample preparation with turn around of about 750 samples per week

➢ **High-throughput determination of nicotine for PK analysis of NGPs**

Challenge: Ubiquitous presence of nicotine

- NGPs like e-cigs often show lower nicotine uptake compared to CCs -> nicotine levels of 0.5 – 5 ng/mL need to be quantified
- Measurement of nicotine at trace levels < 2 ng/mL challenging due to its ubiquitous appearance
- Common nicotine background levels 0.5 – 2 ng/mL

➢ Elimination of nicotine contamination from all materials used in the study:

Precautions at the clinic:
- Use dedicated materials, lab coates, gloves, working benches…
- Use pre-cleaned tubes/vials for blood collection
- Samples should be handled by non-smoking / non-vaping staff

Precautions at the lab:
- Use dedicated materials, lab coates, gloves, working benches…
- Purification of solvents
- Wash all tubes, vials, pipette tips, etc. with purified solvents
- Samples should be handled by non-smoking / non-vaping staff

Development of a cleaning protocol: Elimination of nicotine traces in solvents & materials

Purification of solvents/eluents:
All solvents used during sample preparation and analysis (H_2O, CH_3CN, ethyl acetate) rinsed through cation exchange resin
-> Retention of nicotine due to ionic interactions with the resin

Fig. 4: Glass column packed with the cation exchange resin.

Elimination of nicotine traces is monitored by means of LC-MS/MS.

Treatment of polypropylene tubes with nicotine-free water: 2 wash cycles

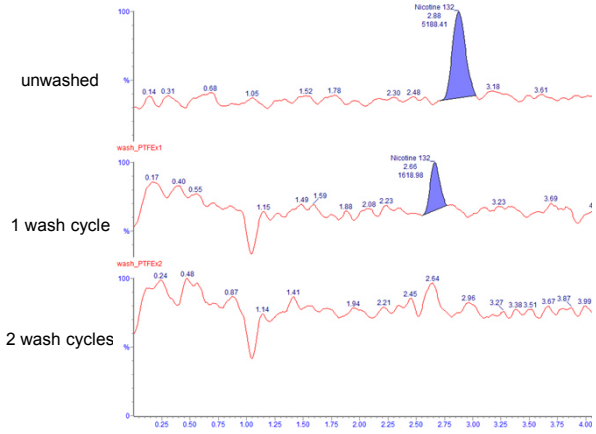

Fig. 5: Chromatograms of trace amounts of nicotine extracted from a polypropylene tube.
Unwashed: Direct extraction with acetonitrile;
1 wash cycle: Nicotine traces after the first wash cycle using nicotine-free water (LC-MS grade);
2 wash cycles: No nicotine traces detectable after the second wash cycle using nicotine-free water (LC-MS grade).

Summary and conclusion

- Highly sensitive nicotine analysis needed for PK analysis in NGPs
- LLOQs below 0.5 ng/mL shall be achieved for sufficient sensitivity
- Major challenge: Elimination of ubiquitous nicotine which contributes to background levels up to 2 ng/mL in solvents, eluents, tubes, pipette tips…
- Establishment of a cleaning protocol comprising the purification procedure for all solvents and eluents (Fig. 4) as well as a washing step for lab materials (Fig. 5)
- Validation and evaluation of sensitivity of the assay after removing background nicotine using pre-cleaned materials currently running at ABF

➢ Improvement of the sensitivity using nicotine-free materials should allow for analysis below 0.5 ng/mL

➢ Final aim of method development and validation will be to quantify nicotine with an LLOQ below 0.2 ng/mL

References:

[1] Medana, C., *et al.* (2016) Analysis of nicotine alkaloids and impurities in liquids for e-cigarettes by LC-MS, GC-MS, and ICP-MS. *Current Trends in Mass Spectrometry*, 5 (29), 20-28.

[2] Food and Drug Administration (FDA) (2001): Guidance for Industry - Bioanalytical Method Validation, 1-22.