LC – MS/MS Determination Of Nicotine And Its Metabolite Cotinine In Human Plasma

Inas Abdallah[1], Priyanka Ghosh[2], Bryan Newman[2], Sam Raney[2], Audra L. Stinchcomb[1], Hazem E. Hassan[1]

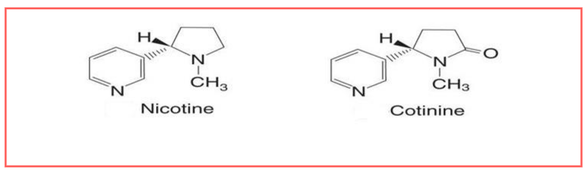
[1]Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland Baltimore

[2] U.S Food and Drug Administration

Abstract

A sensitive and specific liquid chromatographic tandem mass spectrometric (LC – MS/MS) method capable of quantifying nicotine and its metabolite cotinine in human plasma was developed and validated. In this method, the drug was extracted from a 0.1 mL plasma sample using liquid – liquid extraction method. Chromatographic separation was performed on HILIC column and detection was achieved using Waters® Acquity Triple quadrupole tandem mass spectrometer employing electro – spray ionization in the positive ion mode along with multiple reaction monitoring (MRM). The quantification range is 0.75 to 15 ng/mL for nicotine and 7 to 150 ng/mL for cotinine. The method can be applied to analyze human plasma samples following pharmacokinetic studies.

Background



Nicotine, is an alkaloid with a tertiary amine group. It is excreted partially unchanged by the kidney in the form of 20 or more different metabolites. The major primary metabolite is cotinine. Analyzing a basic polar drug as nicotine by reversed phase columns (e.g. C18 columns) has some drawbacks (i.e., peak tailing and limited retention which is unfavorable for subsequent ESI – MS detection as it is eluted in the ion suppression zone). On the other hand, the use of Hydrophilic interaction liquid chromatography (HILIC) columns with polar compounds (e.g., nicotine) enhances retention of these compounds, elutes them well outside of the ion suppression zone and results in high resolution chromatography. In this method we report a simple, sensitive, specific and validated method of nicotine and cotinine quantification according to the FDA guidance for bioanalytical method validation (2013).

Methods

• Liquid Chromatography

- HPLC system: An Alliance ® HPLC system (Waters®, Milford, MA).
- Analytical column: Phenomenex Luna®HILIC column (150 mm x 3.0 mm)
- Mobile phase: A: 100 mM ammonium formate buffer (pH =3.2)
  B: Acetonitrile.
- Isocratic elution (A: 10%, B: 90 %)
- Flow rate: 0.4 ml/min.
- Injection volume: 15 µL

• Mass Spectrometry

- MS System: Acquity TQD(Waters®, Milford, MA).
- Condition: LC – ESI (+) – MS/MS (MRM).

Table 1: MRM Transitions for nicotine, cotinine and their internal standards

Compound	MRM	Cone voltage	Collision energy
Nicotine	163.05→131.97	28	20
Nicotine – d4 (IS)	167.08→134.00	28	20
Cotinine	177.01→145.97	35	20
Cotinine – d3 (IS)	180.06→100.90	35	20

• Sample Preparation

100 µL plasma
12.5 µL appropriate nicotine working solution
12.5 µL appropriate cotinine working solution
5 µL nicotine – d4 working solution (Final concentration 6 ng/mL) (IS)
5 µL cotinine – d3 working solution (Final concentration 50 ng/mL) (IS)

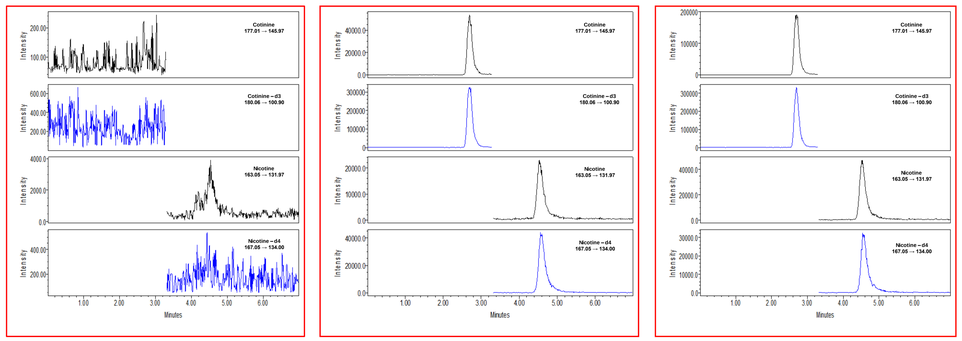
↓

Vortex and add 1 mL of ethyl acetate, vortex again and shake for 20 min at high speed shaker. Centrifuge at 14,000 rpm and 4° C for 15 min.

↓

Transfer the upper organic layer in a clean Eppendorf and evaporate under light stream of liquid nitrogen at 37 ° C till dryness
Reconstitute the residue in 50 µL acetonitrile.

Results



A B C

Figure 1: MRM Chromatograms of blank human plasma (A); LLOQ (0.75 ng/mL for nicotine and 7 ng/mL for cotinine) (B) and ULOQ (10 ng/mL for nicotine and 100 ng/mL for cotinine) .

Table 2: Summary of the method validation for both nicotine and cotinine

	Nicotine	Cotinine
Limit of detection (LOD)	0.25 ng/mL	5 ng/mL
Lower Limit of quantitation (LLOQ)	0.75 ng/mL	7 ng/mL
Calibration curve	0.75 – 15 ng/mL	7 – 150 ng/mL
Inter – run accuracy (for each QC concentration) (%RSD)	Low QC (2.5 ng/mL) = 4.28 % Medium QC (4 ng/mL) = 1.67 % High QC (10 ng/mL) = 1.43 %	Low QC (20 ng/mL) = 1.68 % Medium QC (40 ng/mL) = 4.51% High QC (100 ng/mL) = 1.19 %
Inter – run precision (for each QC concentration) (%RSD)	Low QC (2.5 ng/mL) = 7.58 % Medium QC (4 ng/mL) = 5.62 % High QC (10 ng/mL) = 3.62 %	Low QC (20 ng/mL) = 3.80 % Medium QC (40 ng/mL) = 4.11% High QC (100 ng/mL) = 1.73 %
Selectivity (%CV)	7.57 %	6.57 %
Recovery (extraction efficiency)	93.62 %	94.35%
Matrix effect	107 %	103%
Short – term stability (%CV)	1.39 %	1.05 %
Freeze – thaw stability (%CV)	4.55 %	3.56 %
Processed sample stability (%CV)	1.34 %	2.43 %

Conclusions

A sensitive, specific, accurate, reproducible method was developed and validated for quantification of nicotine and its major metabolite cotinine according to the FDA guidance for bioanalytical method validation. The implementation of HILIC separation with a simple single step extraction significantly increased the sensitivity of the method compared to reversed phase separation. The method can be used to quantify nicotine and cotinine in human plasma.

Acknowledgement

Funding for this project was made possible, in part, by the Food and Drug Administration through grant U01FD004955-01. The views expressed in this poster do not necessarily reflect the official policies of the Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.