Enhanced nicotine metabolism in HIV-1-positive smokers compared to HIV-negative smokers: Simultaneous determination of nicotine and its four metabolites in their plasma using a simple and sensitive ESI-LC-MS/MS technique

Ravinder Earla, Anusha Ande, Carole McArthur, Anil Kumar, Santosh Kumar

Division of Pharmacology and Toxicology, School of Pharmacy, University of Missouri-Kansas City, Kansas City, MO, 64108 (R.E., A.A., A.K., S.K.)

Department of Oral and Craniofacial Science, School of Dentistry, University of Missouri-Kansas City, Kansas City, MO, 64108 (C.M.)

Running title: Nicotine metabolism in HIV-1 positive and negative smokers

Corresponding author

Santosh Kumar, School of Pharmacy, University of Missouri-Kansas City, 2464 Charlotte Street, Kansas City, MO 64108, Tel: 816-235-5494, Fax: 816-235-1776, Email: Kumarsa@umkc.edu

Table 1. Electrospray Ionization Mass Spectrometry (ESI-MS) with a proton adducts $[M+H]^+$ based on *mass* to *charge* ratio (*m/z)* parameters for nicotine, nornicotine, cotinine, norcotinine, and *trans* 3'-hydroxycotinine.

Analyte	**Precursor ion Q1(MS1)**	**Product ions Q2(MS2)**	**DP(V) (MS1)**	**EP(V) (MS1)**	**CEP(V) (MS1)**	**MRM *trans*ition [Q1→Q3]**	**CE (V)**
Nicotine	163.3	84.1, 106.0, **117.1**, 130.3, 132.4	36.0	5.5	10.0	163.3→117.1	33.0
Nornicotine	149.5	80.1,117.1, 130.1, **132.3**	36.0	5.0	11.0	149.5→132.3	17.1
Nicotine-d4(IS)	167.3	84.3, 110.5, **121.4**, 134.2, 136.4	36.0	7.0	9.5	167.3→121.4	35.0
Cotinine	177.5	**80.3**, 98.1, 146.2	46.0	7.0	12.0	177.5→80.3	23.0
Norcotinine	163.4	**80.3**, 84.1, 135.2, 146.3	51.0	6.5	10.0	163.4→80.3	25.0
Trans 3'-hydroxy cotinine	193.2	**80.1**, 134.3, 149.2	51.0	5.5	11.5	193.2→80.1	25.0
Cotinine-d3(IS)	180.3	80.3, **101.2**, 118.1, 146.0	46.0	7.5	12.0	180.3→101.2	29.0

MS- mass spectrometry; **m/z**-mass-to- charge ratio; **DP**- declustering potential; **CE**- collision energy; **CEP**: Cell exit potential; **CAD**-collisionally activated dissociation gas : 3.0 psi; **MRM**- multiple monitoring reactions; **Dwell time**: 500 ms; **Source gas (GS1)** : 30.0 psi; **Source gas (GS2)**: 30.0 psi; **Source temperature (TEM)** : 400^0C ; **Ion spray voltage (IS)** : 5500 Volts; **Interface heater**: on and **Analytical total run time** : 4.0 min;

Table-2. LC-MS/MS system suitability test for nicotine, nornicotine, cotinine, norcotinine and *trans* 3'-hydroxycotinine with internal standards, nicotine-d4, and cotinine-d3 were used.

	MRM peak area counts(1.0 µg/mL)			***MRM peak area counts(1.0 µg/mL)***	
Nicotine	**Nicotine-d4 (IS)**	**Area ratio (Analyte/IS)**	**Nornicotine**	**Nicotine-d4 (IS)**	**Area ratio (Analyte/IS)**
1141682	1897707	0.5911	2185983	1897707	1.1519
1149019	1931564	0.5949	2142384	1931564	1.1091
1171239	1943119	0.6028	2162761	1943119	1.1130
1148919	1939139	0.5925	2173457	1939139	1.0980
1161239	1943519	0.5975	2152861	1943519	1.1077
1184409	1979449	0.5984	2153761	1979449	1.0881
Mean		**0.596198**			**1.11130**
SD		**0.0042781**			**0.021844**
%CV		**0.72**			**1.97**

MRM peak area counts (1.0 µg/mL)	***MRM peak area counts (1.0 µg/mL)***	***MRM peak area counts (1.0 µg/mL)***

Cotinine	Cotinine -d3 (IS)	Area ratio (Analyte/IS)	Norcotinine	Cotinine -d3 (IS)	Area ratio (Analyte/IS)	*Trans* 3'-hydroxy cotinine	Cotinine-d3 (IS)	Area ratio (Analyte/IS)
2999425	2273971	1.3190	3771038	2273971	1.6583	3479011	2273971	1.5299
3060224	2316229	1.3212	3804754	2316229	1.6427	3549332	2316229	1.5324
3134471	2313156	1.3551	3815535	2313156	1.6495	3589533	2313156	1.5518
3033583	2290737	1.3243	3781464	2290737	1.6508	3485113	2290737	1.5214
3100583	2390728	1.3243	3790161	2390728	1.6508	3525243	2390728	1.5214
3103501	2298747	1.3243	3879214	2298747	1.6508	3486313	2298747	1.5214
Mean		**1.32803**			**1.65048**			**1.52971**
SD		**0.013436**			**0.004958**			**0.011852**
%CV		**1.01**			**0.31**			**0.77**

CV- Coefficient of variation ((SD/mean) X100; **MRM-** Multiple reactions monitoring

Figure 1: Extracted blank plasma for nicotine, cotinine, trans-3-hydroxycotinine, nornicotine and norcotinine.

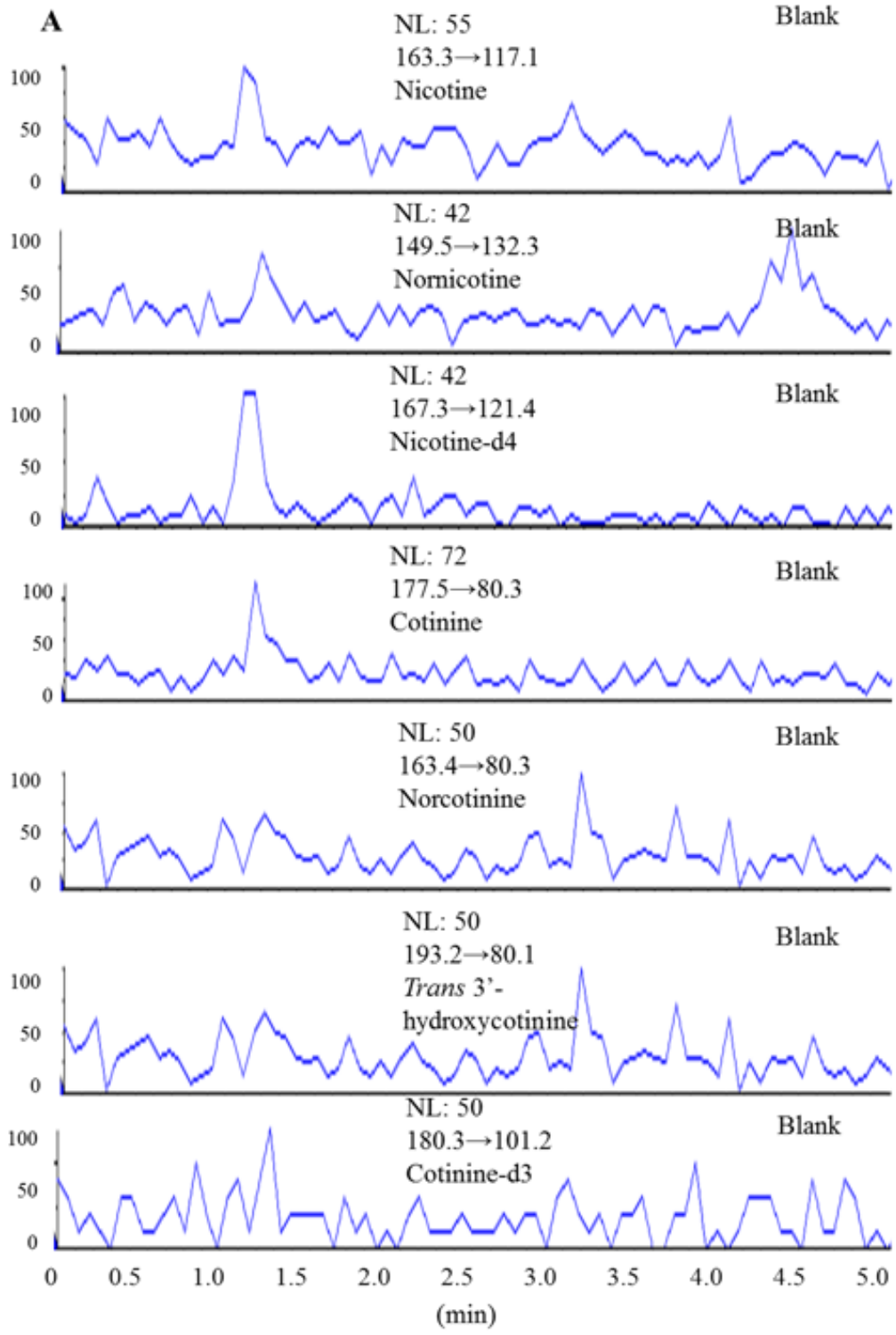

Figure 2: Calibration curve standards and linear regression lines for analysis of nicotine and its metabolites in non-smokers plasma that were used for calculating their concentrations in the plasma of HIV-1-positive, and HIV-negative smokers.