Highly Sensitive Nicotine Plasma and Metabolite Assays for Clinical Trials by LC-MS/MS

Yansheng Liu, Marsha Luna, X. Steven Yan, Julie Showalter, Kimberly Jackson and Gene Ray
KCAS Bioanalytical & Biomarker Services, Shawnee, KS 66216

ABSTRACT

A highly sensitive and selective liquid chromatographic tandem mass spectrometric method for determination of nicotine and its metabolites, cotinine and trans-3'-hydroxycotinine, in human plasma (K2EDTA) have been validated. The interference and contamination was controlled by the assays to achieve 0.20 ng/mL lower level of quantitation (LLOQ) for nicotine in the range of 0.20 to 40 ng/mL and 0.5 to 200 ng/mL. The range for its metabolites is from 1.00 to 300 ng/mL.

The precisions (%CV) and accuracies (% bias) for nicotine above the LLOQ were ≤ 7.3% and ≤ 3.9%, respectively. The precision and accuracy for nicotine at the LLOQ were 12.3% and 3.9%, respectively. Stability was documented for each analyte in plasma for 18.5 hr at ambient temperature, following 4 freeze/thaw cycles and for up to 48 days at -20°C. Stability was also documented in whole blood for 1.5 hr at room temperature.

Assay specificity was confirmed in the presence of menthol (as glucuronide metabolite) and in presence of lipemic and hemolytic plasma. These methods meets or exceeds FDA regulatory requirements and acceptable for clinical trial support.

INTRODUCTION

The new FDA regulatory requirements announced in July 2017 are aimed to reduce nicotine in both tobacco and electronic nicotine delivery systems (ENDS) to make them less addictive and lower health risks. With the reduction of nicotine levels in these and other products (i.e., gums, lozenges) lower assay ranges are essential for pharmacokinetic assessments.

In this presentation, we report the validated bioanalytical assays through simple protein precipitation extraction to achieve lower level of quantitation at 0.20 ng/mL for nicotine at a low curve assay and 0.5 ng/mL at a high curve assay, as well as the assay for its metabolites.

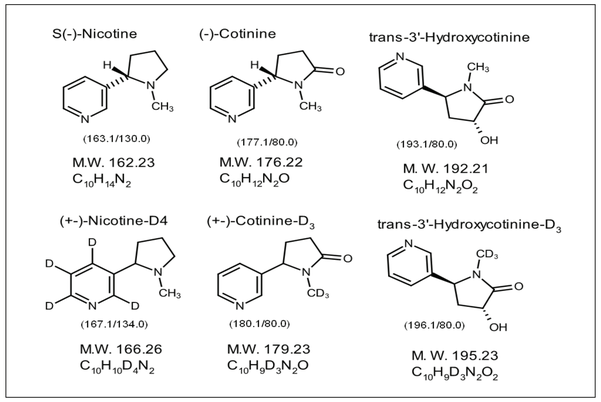

Figure 1. Chemical structures of nicotine, its metabolites and their internal standards as well as mass transitions used in analysis

METHOD

Nicotine and its metabolites were extracted in our nicotine-controlled laboratory environment from human plasma (100 uL) with simple protein precipitation method by adding 300 uL of methanol. After vortex and centrifuge, the supernatant was transferred to 96-well plate. It was then refrigerated until LC-MS/MS analysis.
The LC-MS/MS analysis was performed on a API 5000 mass spectrometer in MRM positive mode, coupled with Shimadzu UHPLC system. The mass transitions were listed in Fig. 1.

RESULTS

The assays were validated in terms of selectivity, sensitivity, linearity, accuracy, precision, matrix effect, recovery, dilution integrity, hemolytic and lipemic effect, stability in plasma and in whole blood, and column ruggedness in human plasma (K2EDTA) following currently regulatory guidelines. The intra- and inter-batch results for QC samples are shown in Table 1.

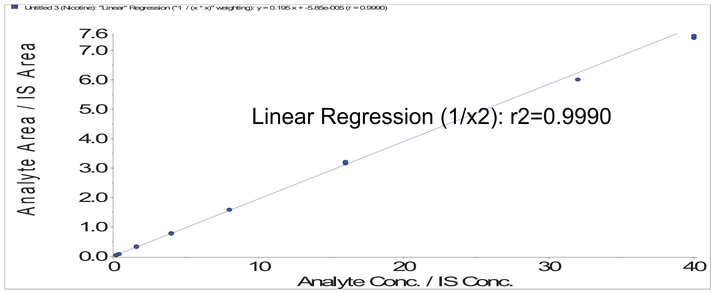

Figure 1. Chemical structures of nicotine, its metabolites and their internal standards as well as mass transitions used in analysis

Batch	QCs	0.200 LLOQ	0.600 Low	12.0 Mid	30.0 High
1	Mean	0.217	0.636	12.6	30.0
	% CV	12.6	8.3	2.8	2.5
	% Bias	8.4	5.9	4.7	0.1
2	Mean	0.194	0.609	12.2	30.6
	% CV	7.0	9.3	4.3	3.1
	% Bias	-3.2	1.5	1.9	1.9
3	Mean	0.213	0.625	12.4	29.9
	% CV	14.2	3.8	2.7	3.4
	% Bias	6.6	4.2	3.4	-0.3
Overall	Mean	0.208	0.623	12.4	30.2
	% CV	12.3	7.3	3.3	3
	% Bias	3.9	3.9	3.4	0.6

Table 1. Precision and accuracy results for nicotine control samples (n=6 for each QC per batch)

RESULTS (continued)

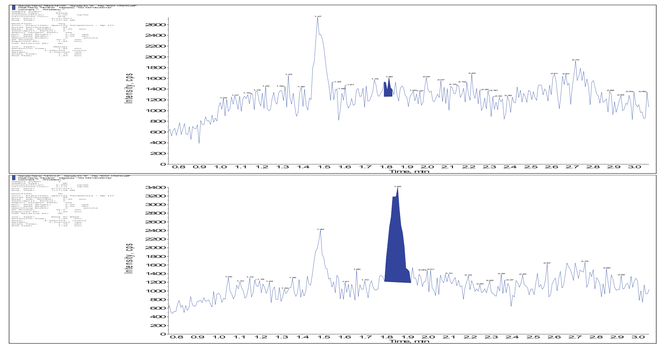

Figure 3. A typical ion chromatograms for blank human plasma (upper panel) and LLOQ of nicotine (0.200 ng/mL, lower panel)

The overall recovery (using low, mid and high QCs, n=6, three batches) for nicotine and its I.S. is 96.2% and 95.8%, respectively, within 2.7% of precisions (%CV). The precision and accuracy for nicotine high curve assay (0.50 to 200 ng/mL) are comparable to that of low curve assay.

Cotinine and trans-3'-hydroxycotinine linearity range are the same, i.e. from 1.00 to 300 ng/mL. Their intra- and inter-batch results for QC samples are shown in Table 2.

Cotinine / Trans-3'-hydroxycotinine

Batch	QCs	1.00 LLOQ	3.00 Low	120 Mid	300 High
1	Mean	1.02 / 0.967	3.23 / 3.10	125 / 122	300 / 293
	% CV	4.8 / 9.5	5.4 / 5.3	3.0 / 2.2	5.4 / 2.9
	% Bias	2.1 / -3.3	7.7 / 3.4	4.5 / 1.6	0.0 / -2.5
2	Mean	1.05 / 1.06	3.25 / 3.12	123 / 120	300 / 291
	% CV	6.0 / 10.4	2.0 / 6.0	1.9 / 1.0	2.1 / 4.7
	% Bias	4.7 / 5.5	8.2 / 4.0	2.6 / 0.0	0.1 / -3.1
3	Mean	1.10 / 1.09	3.28 / 3.28	125 / 122	299 / 297
	% CV	6.6 / 4.7	2.6 / 3.8	3.3 / 4.6	3.3 / 2.9
	% Bias	10.4 / 8.7	9.4 / 9.4	3.9 / 1.4	-0.3 / -1.1
Overall	Mean	1.06 / 1.04	3.25 / 3.17	124 / 121	300 / 293
	% CV	6.5 / 9.4	3.5 / 5.4	2.8 / 2.9	3.6 / 3.5
	% Bias	5.7 / 3.7	8.4 / 5.6	3.7 / 1.0	-0.1 / -2.2

Table 2. Precision and accuracy results for cotinine and trans-3'-hydroxycotinine control samples (n=6 for each QC per batch)

CONCLUSION

Human plasma assays for nicotine and its key metabolites (cotinine and trans-3'-hydroxycotinine) have been validated in terms of sensitivity, selectivity, carryover, linearity, accuracy, precision, dilution integrity, recovery, matrix effect and stability. The low limit of quantitation value for nicotine at 0.2 ng/mL and analytical range allows adequate pharmacokinetics or bioequivalence studies in clinical trials.