Poster Number 13R1000

Quantification of Nicotine and its Metabolite Cotinine in Rat Tooth, Rat Alveolar Bone and Brain Using Triple Quad 5500 System

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2016 AAPS ANNUAL MEETING AND EXPOSITION
NOVEMBER 13–17, 2016
COLORADO CONVENTION CENTER, DENVER
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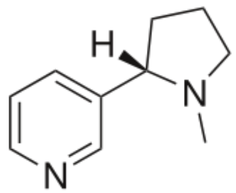
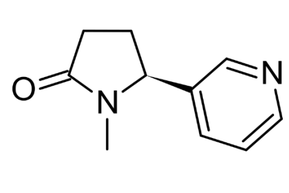
PURPOSE

Recently most people are aware of the impact of tobacco use on their oral health. Nicotine and its metabolite cotinine have been considered to be the main contributors. The purpose of this study was to develop a sensitive and specific LC-MS/MS assay for the simultaneous measurement of nicotine and its metabolite cotinine in rat tooth and brain samples and then applied to sample analysis to support the research of the effects of nicotine and cotinine on dental health. The lower limit of quantitation was 5 ng/g tissue.



Nicotine



Cotinine

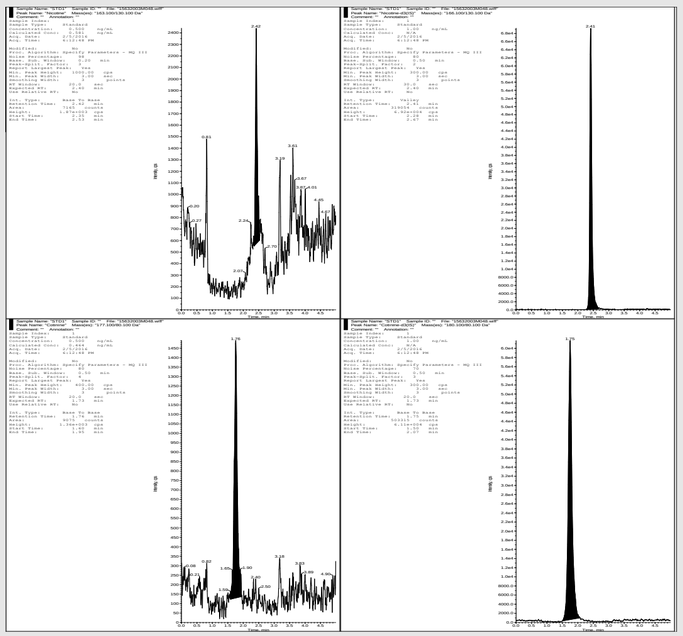


Figure 1. Representative chromatogram of Nicotine and Cotinine LLOQ in rat tooth. The upper left chromatogram is for Nicotine and upper right for Nicotine-d_3 (IS) ; the lower left for Cotinine and lower right for Cotinine-d_3 (IS).

METHOD

Sprague Dawley rats (280-330 g) were used in this study. Three dose groups (saline solution only, 0.8mg/kg nicotine, 3.2mg/kg nicotine) of total 18 rats were given intraperitoneal injection once a day and then fed with standardized diet and water. The rats were sacrificed after three months and the samples were collected for determinations of nicotine and cotinine in rat tooth, rat alveolar bone and rat brain.

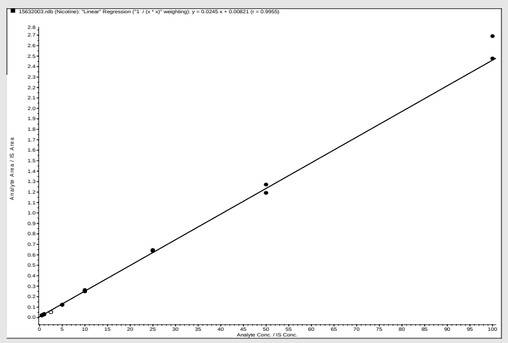
Nicotine, cotinine and the added internal standards were extracted from rat tooth and rat alveolar bone using solid phase extraction procedure. The ground rat teeth was dissolved in HCl overnight. The working solution and internal standard were spiked to the liquidized rat tooth matrix for preparing the calibration curve and QC samples. Then the neutralized sample was loaded for solid phase extraction and the eluent was ready for LC-MS/MS analysis. The rat brain samples were homogenized and then the rat brain homogenate samples were extracted using protein precipitation extraction procedure.

Liquid Chromatography:

Pump:	Shimadzu LC-30AD
Autosampler:	Shimadzu SIL-30AC
System Controller:	Shimadzu CBM-20A
Analytical Column:	Thermo Hypersil GOLD column, 2.1 x 100 mm, 5 µm
Gradient Flow:	The analyte was eluted using a gradient of mobile phase A (0.1% Heptafluorobutyric Acid in water and mobile phase B (0.1% Heptafluorobutyric Acid in methanol) from 5% to 30% mobile phase B in 2 min.
Injection Volume:	2 µL

Mass Spectrometry :

MS System:	Sciex Triple Quad 5500
Condition:	LC/(+)ESI-MS/MS
MRM transition:	
Nicotine:	163.1 → 130.1
Nicotine-d_3:	166.1 → 130.1
Cotinine:	177.1 → 80.1
Cotinine-d_3:	180.1 → 80.1



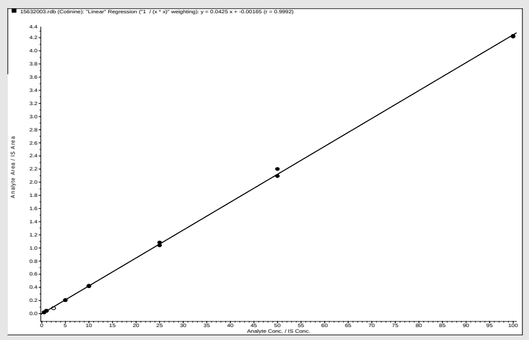


Figure 2. Typical Calibration Curve of Nicotine (left) and Cotinine (right) in Rat Tooth

RESULTS

Excellent linearity was obtained with a correlation coefficient ≥0.9965 for nicotine and ≥0.9971 for cotinine in rat tooth, ≥0.9973 for nicotine and ≥0.9978 for cotinine in rat brain. Both CV and biases (RE) met acceptance criteria at all QC levels. The methods have been applied to samples analysis from rat tooth, the alveolar bone and rat brain samples with different nicotine dosing levels. Results indicated: 1) In all three types of samples (tooth, alveolar bone and brain), cotinine, the metabolite of nicotine, is the major measurable compound which indicated the nicotine metabolism is fast; 2) The order of concentration for cotinine: tooth < alveolar bone < brain; 3) With dosing level increases, concentrations of cotinine in alveolar bone and tooth increase accordingly, but in brain, it was kept in a constant high concentration which is very noticeable and important.

CONCLUSION

Very specific and reproducible LC-MS/MS methods for the determination of nicotine and cotinine in both rat tooth, alveolar bone and rat brain have been established using Triple Quad 5500 System with a lower limit of quantitation of 5 ng/g. The methods have been used in sample analysis and provide very informative results for dental health.