Quantitative Determination of Nicotine and Cotinine in Human Plasma by LC/MS/MS for Nicotine Replacement Studies
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Purpose
To develop and validate a LC/MS/MS method for nicotine and cotinine in human plasma that can be used to support nicotine replacement therapy in smoking cessation studies.

Methods
The range of quantitation is 0.500 to 100ng/mL for nicotine and 2.50 to 500ng/mL for cotinine. The method utilized a liquid-liquid extraction from 0.200mL of plasma. After the addition of combined internal standards to the plasma, 100µL of 1M sodium carbonate was added. Samples were extracted with 2mL of MTBE/methylene chloride (1:1) mixture. The organic layer was evaporated and reconstituted in 150µL of acetonitrile/water (4:1). HPLC separation was carried out on an Ascentis Express HILIC 2.7µm, 7.5cm x 3.0mm column at a flow rate of 0.700mL/min with an analysis time of 4 minutes. Mobile phase consisted of acetonitrile, water, formic acid and ammonium hydroxide (800:200:2.5:0.5) Nicotine-D4 and cotinine-D3 were used as the internal standards. Mass spectrometry detection was carried out with an ABSciex API5000 triple quadrupole mass spectrometer equipped with a Turbo IonSpray source. ESI mass spectra (m/z 163->117 for nicotine and m/z 177->80 for cotinine) were acquired in positive ion mode with multiple reaction monitoring.

Results
No matrix interference in six individual lots of plasma was observed indicating the specificity of the method. Acceptable intraday and interday assay precision and accuracy ranged from -1.2 to 4.6% over a linear range for both analytes. The mean extraction recovery was 86% for nicotine and 74% for cotinine. Mean %bias for Benchtop, freeze/thaw, and extract stabilities ranged from -4.0% to 9.5%. Selectivity at LLOQ ranged from 3.8% to 16.0% for nicotine and 2.8 to 14.0% for cotinine across six different lots of plasma. Hemolytic and lipemic plasma and whole blood stability were successfully evaluated.

Conclusion
A validated method which was reproducible, sensitive, specific, accurate, and reliable was developed. The method can be used to quantify human plasma for pharmacokinetic and drug safety studies for nicotine replacement therapy.