

## **LC/MS Analysis of Nicotine and Its Metabolites in Urine, Oral Fluid, and Blood**

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Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of nicotine and its metabolites that include- nicotine, anabasine, cotinine, trans-hydroxy-cotinine, NNAL, NNN, NNK, NAB and norcotinine by QQQ. Simple sample preparation techniques such as dilute and shoot for urine and oral fluid, protein crash for Blood, and one dimensional (1D) chromatographic configurations achieved the required sensitivity and is capable of quantitating the analytes over their relevant dynamic range.

An Agilent 6460 QQQ with Jet Stream technology in positive Electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 100 µl of human urine, oral fluid and blood was used for the analysis of the compounds. Various columns were evaluated and an Agilent Poroshell 120 were used with a water:acetonitrile mixture containing 0.1% formic acid gradient achieved baseline chromatographic separation in an approximately 6 minute run time for all matrices. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using reference materials from UTAK and human samples.

Good linearity and reproducibility were obtained across the dynamic range of the drugs with a coefficient of determination  $R^2 > 0.995$  for all drugs in the various matrices. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined, and excellent reproducibility was observed for all compounds ( $CV < 15\%$ ) in all matrices.

A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of nicotine and its metabolites in urine, oral fluids and blood.