Analytical method development for a pharmacokinetic-pharmacogenetic platform aimed to optimize smoking cessation pharmacotherapies

S. Fogli[1], A. Saba[2], M. Del Re[3], L. Carrozzi[4], F. Pistelli[4], A. Palla[4], R. Zucchi[2], M.C. Breschi[1], R. Danesi[3]

[1] Dept. of Pharmacy, University of Pisa, Italy

[2] Dept. of Surgery, Medical, Molecular, and Critical Area Pathology, University of Pisa, Italy

[3] Dept. of Clinical and Experimental Medicine, University of Pisa, Italy

[4] Cardio-Thoracic and Vascular Dept., University Hospital of Pisa, Italy

Several lines of evidence indicate that a major cause of the high percentage of failures in smoking cessation therapy relies on the inter-individual variability in nicotine metabolism that, in turn, may cause inadequate nicotine replacement after transdermal nicotine replacement therapy and variable response to bupropion and varenicline (Benowitz, 2006). Noteworthy, genetic variants in *CYP2A6* gene (that codes for the liver enzyme primarily responsible for the metabolism of nicotine) have been associated with altered nicotine metabolism and with effects on smoking behavior (Gold and Lerman, 2012). A pharmacokinetic (PK)-pharmacogenetic (PGx) platform able to predict drug response could be therefore a promising tool to select the most effective cessation treatment for an individual smoker. In this study, we developed a highly selective and sensitive liquid chromatography-tandem mass spectrometry method for the simultaneous assessment of nicotine, cotinine, 3-hydroxycotinine and anabasine in human plasma and PCR experimental conditions to be used for genotype analysis. LC-MS-MS analyses were carried out by an AB/Sciex API4000 triple quadrupole mass spectrometer equipped with an ESI source and coupled to a PerkinElmer 200 Series HPLC system. The HPLC separation was carried out by using a 100 x 2.0 mm, 4 µm Phenomenex Synergi Polar-RP column. A binary, linear gradient elution with 8 mM ammonium acetate in water (solvent A) and acetonitrile containing 0.1% formic acid (solvent B) was used at a flow rate of 0.4 ml/min. The mass spectrometer was operated in SRM mode, monitoring three transitions each compound, including the following quantifiers: 163→132 (nicotine), 163→118 (anabasine), 177→80 (cotinine), 193→80 (3-hydroxycotinine). Sample preparation was performed as follows: 500 µl of plasma were diluted with 0.1% HF (1:1) and loaded to aSPE cartridge (Waters Oasis® MCX 1 ml, 30 mg) previously conditioned with methanol and ammonium acetate buffer. Elution was carried out with an ammonium hydroxide-methanol solution and the so obtained eluate was dried by a gentle stream of nitrogen, reconstituted with 100 µl of a methanol/water (30/70, v/v) mixture, and injected into the HPLC-MS/MS system. PCR analysis was performed on the functionally impaired variants CYP2A6*2, *4, *9, and *12 following standard methods described in the literature (Schoedel et al., 2004). At present, we are running analyses in plasma samples derived from smokers who apply to the Smoking Cessation Centre of the University Hospital of Pisa, a structure operating within the Italy's National Health Service, where pulmonologists routinely conduct a standard smoking cessation program based on individual counseling and prescription of pharmacotherapy.

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