Validated HPLC/MS/MS method for determination of nicotine in human plasma and its application of Pharmacokinetics study of Nicotine transdermal system in Healthy Korean Male Smokers

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A sensitive, rapid liquid chromatographic electrospray ionization mass spectrometric method for determination of nicotine in human plasma was developed and validated. Nicotine in plasma (1 mL) was extracted with dichloromethane 5 mL, the organic layer 4 mL was transferred to another test tube, adding 35 % HCl 40 μL and evaporated to dryness at 40 °C and reconstituted in methanol. The extracted sample was separated using a C18 column (5.0 μm, 2.1 x 150 mm, Eclipse Zobax, Agilent), with a mobile phase containing of methanol : acetonitrile : water = 55 : 25 : 20 and was isocratically eluted at a flow rate of 0.32 mL/min. Nicotine and its internal standard, nicotine amide were measured by electrospray ion source in positive ion mode (SRM mode). This method was validated by examining the precision and accuracy for inter- and intra-day analysis. The standard curve was linear (R2 = 0.9991) over the concentration range of 2 ~ 75 ng/mL. The limit of quantification for nicotine in human plasma was 2 ng/mL. The intra-day and inter-day precision ranged from 3.3 % to 6.4 % and 6.8 % to 10.7 % (R.S.D.), respectively. And the intra-day and inter-day accuracy ranged from 92.0 % to 97.6 % and 96.1 % to 103.2 %, respectively. For analysis of pharmacokinetic properties, the blood samples were drawn at 0, 2, 4, 6, 8, 10, 12, 16, 24, 25, 26, 28, 32 and 36 hours after attaching the transdermal patch. The attached patch was removed at 24 hour. The established method has been successfully applied to pharmacokinetic study.