Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(10):63-66
(http://derpharmachemica.com/archive.html)

Review of HPLC/UPLC-UV Methods for Sensitive Determination of Nicotine in Plasma

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ABSTRACT

A concise review of HPLC/UPLC-UV methods for determination of nicotine in human plasma is presented in this manuscript in the form of tables with full description of the used stationary phases, mobile phases, detection wavelength, flow rate and linearity ranges. Also full description of plasma extraction techniques is described for all the reported LC-UV methods. This review permits the application of the reported methods for further pharmacological and clinical studies while design of new nicotine formulations. The reported methods may also be used in comparative studies using different dosage forms and cigarettes to investigate the pharmacokinetic parameters of new nicotine formulations in vivo.

Keywords: Nicotine; Human plasma; HPLC/UPLC-UV; Chromatographic conditions; Sample preparation; Pharmacological applications.

INTRODUCTION

Nicotine, (S) -3- [1-methylpyrrolidin-2-yl] pyridine (Figure 1) is a potent alkaloid found in cigarettes. To the best of the author' knowledge, one UPLC-UV method for sensitive determination of nicotine in human plasma was developed [1]. On the other hand, many HPLC-UV methods [2-11] have been reported for nicotine determination lacking the UPLC major advantages of consuming less solvent and less time.

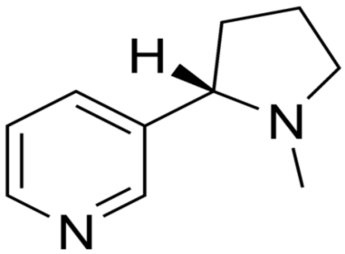

Figure 1: Chemical structure of nicotine

Literature review of plasma extraction techniques and chromatographic conditions of the reported HPLC/UPLC-UV methods

Many extraction techniques [1-11] were described for nicotine extraction from human plasma prior to injection into LC-UV system as shown in (table 1). In addition, all the reported chromatographic conditions were summarized in (table 2).

Table 1: Extraction techniques of nicotine

Method	Sample preparation details
Method [1]	Series of 1 ml plasma samples was spiked with 10 µL of different nicotine working solutions (2.5, 5, 10, 20, 30, 40 and 50 ng/10 µL) separately followed by spiking with 5 µl of Quinine working solution (100 ng/5 µL) then alkalinized with 0.1 ml of 10 M NaOH before adding the organic extracting phase (2 ml Diethyl ether) and vortex for 3 minutes at 3000 RPM, The samples were centrifuged for 12 minutes at 6000 RPM then the upper layer was separated (diethyl ether). 5 µl concentrated HCL was added to the separated diethyl ether layer then vortex was used for 3 min at 3000 RPM followed by vacuum evaporated at 40 C˚ - 1400 RPM till complete dryness of the sample, Reconstituted with 0.5 ml mobile phase, vortex for 3 minutes at 3000 RPM followed by filtration using syringe filter and finally transferred to the vials in the auto sampler and 2 micro liters were injected into the C_{18} column.
Method [2]	A 0.5 mL aliquot of plasma was placed into a screw capped glass test tube 15 × 100 mm with 100 µL of 11 ppm acetanilide in 50% methanol (internal standard). Each sample was alkalinized with 100 µL of 2.5 M NaOH for plasma samples, then vortex mixed at 2800 RPM for 30 s. A 3 mL aliquot of dichloromethane-diethylether (1:1, *v/v*) was used for one-step single extraction, and then vortex mixed at 2800 RPM for 2 min. The organic layer, after being centrifuged at 3500 RPM for 3 min, was transferred to a new glass tube containing 20 µL of 0.25 M HCl. The organic phase was then evaporated by a stream of nitrogen at 35ºC until dryness and reconstituted to 250 µL with mobile phase.
Method [3]	Passive sampler consisted of sodium bisulfate impregnated filter and filter holder was used. The size of the filter was 25 or 47 mm. Three types of collection filter were tested; (glass fiber filter GB-100R, GA-55, and quartz fiber filter QR-100). These collection filters was dipped in sodium bisulfate aqueous solution, dried and set in the filter holder. The filter holder was for asbestos. Each sampler was put into the aluminum/polyethylene bag and sealed. Nicotine was collected as nicotine sulfate. After sampling, the filter was put into 10 mL of the test tube. Purified water treated with Direct-Q was added to it. The sample was ultrasonicated for 10 minutes, and centrifuged at 3000 RPM for 10 minutes. The supernatant was taken to vial for autosampler of HPLC.
Method [4]	Solid Phase Extraction by silica columns that were conditioned with methanol and washed with water prior to the addition of plasma. Columns were washed with water and dried under vacuum.
Method [5]	A 1.5 ml aliquot of serum, with 100 µL of NENC (N-ethylnorcotinine) added, was mixed with 1.4 ml of 0.5 M sodium hydroxide and transferred to an Extrelut-3 glass column, which was preconditioned with 12 ml of dichloromethane; for one day before the experiment. After 15 min, the analytes were eluted under gravity with 10 ml of dichloromethane - isopropyl alcohol (9:1, *v/v*). The organic phase, with 300/A of methanolic HC1 (25 mM) added, was evaporated to dryness under nitrogen and redissolved in 100 µL of water.
Method [6] and Method [9]	Spiked and treated samples were acidified with 1N acetic acid (pH 5.0). Disposable C18 Sep-Pak Vac 3 cm3 (500 mg) cartridges (Waters Corporation) were conditioned with 3 mL of acetonitrile then equilibrated using 3 mL of water prior to use. The spiked urine and plasma samples were vortexed for 30 s and centrifuged for 5 min at 1000 g. The supernatant was then loaded into the disposable cartridges, washed with 3 mL of water, and then eluted twice by 1 mL of methanol, twice using 2 mL of acetonitrile, and reduced to 500 µL using a gentle stream of nitrogen at room temperature.
Method [7]	The mixture was extracted with 4 ml of dichloromethane by shaking for 10 min. After centrifugation at 1000 *g* for 10 min, 25 ml of conc. HCl was added to the organic fraction for determination of nicotine concentration. The organic fraction was evaporated with a vacuum evaporator. The residue was reconstituted in 100 ml of the mobile phase and then an 80-ml portion of the sample was subjected to HPLC.
Method [8]	Extraction in 10-mL screw-capped Teflon tubes with methylene chloride after deproteinization with trichloroacetic acid. Reconstitution of the extract in 30 ml of mobile phase.
Method [10]	A 0.5 ml aliquot of serum with 100 ml of NENC (N-ethylnorcotinine) (3 µg/ml) added was mixed with 0.4 ml of 0.5 M NaOH and transferred to an Extrelut 1 glass column, which was preconditioned with 8 ml of dichloromethane the day before the experiment. After 10 min, the analytes were eluted under gravity with 5 ml of dichloromethane. The organic phase, with 25 mM methanolic HCl added, was evaporated to dryness under nitrogen and redissolved in 100 µl of HPLC mobile phase.
Method [11]	Using Lichrolut RP-select B SPE cartridges with tetrahydrofuran as eluent, requiring small volumes, 200 µL of blood serum.

Table 2: Chromatographic conditions for the reported methods

Stationary phase	Mobile phase	λ_{max}	Flow rate	Linearity range
C 18 column (100 mm × 2.1 mm, 2.2 µm)	Methanol: Acetonitrile: Phosphate buffer (pH 2.7) with the ratio (20:30:50, *v/v/v*)	260 nm	0.2 ml/min	2.5-50 ng/mL [1]
C 18 column (125 cm x 4 mm , 5 µm)	0.272 g of KH_2PO_4, 0.184 g of sodium n-heptane sulfonate in 820 mL of water and 180 mL of methanol, (PH 3.2)	254 nm	1.0 ml/min	1-5000 ng/mL [2]
C 18 column (4.6 mm x 50 mm, 2.7 µm)	Methanol and 0.5% ammonium formate: 80% methanol for 2 minutes at the beginning, and 80% methanol to 70% methanol in 2.6 minutes.	254 nm	1.0 ml/min	10-70 ng/mL [3]
C 18 column (4.6 x 100 mm , 3 µm)	3.65 g/L triethylamine hydrochloride, 0.6 g/L heptanesulfonic acid, 4.08 g/L potassium phosphate monobasic, 8.82 g/L citric, and 90 mL/L acetonitrile dissolved in HPLC grade water (pH 6.2)	256 nm	0.7 ml/min	1.25-10 ng/ml [4]
C 8 column (25 cm x 4.6 mm ,5 µm)	Binary gradient: Solvent A was water-acetonitrile (96.4:3.6, *v/v*) containing 2 ml/l of triethylamine and 0.012 M each of sodium heptanesulphonate, K_2HPO_4 and citric acid. Solvent B was water-acetonitrile (80.3:19.7, v/v) containing 2 ml/1 of triethylamine and 0.012 M each of sodium heptanesulphonate, K_2HPO_4 and citric acid.	254 ng/ml	1.5-1.8 ml/min.	10-500 ng/ml [5]
C 18 column (3.9 x 300 mm, 10 µm)	10% methanol in water for 5 min. changed to a gradient of acetonitrile in water at 6 min, started at 20% acetonitrile, increased to 35% at 10 min, and then returned to 10% methanol in water at 12 min.	260 nm	0.8 ml/min	50-1000 ng/mL [6]
C 18 column (150 x 4.6 mm, 5 µm)	7% Methanol, 2 m*M* sodiumdihydrogen ortho phosphate, 0.2% phosphoric acid, and 1 m*M* heptane sulfonate sodium.	260 nm	1.0 ml/min	0.2 - 25.0 ng/ml [7]
C 18 column (15 mm x 0.2 cm, 3 µm)	Citrate phosphate (30 mmol/liter) buffer mixture containing 50 mL of acetonitrile and 1 mmol of sodium heptanesulfonate/liter.	256 nm	0.3 mL/min	10 to 700 µg/l [8]
C 18 column (3.9 x 300 mm, 10 µm)	Gradient elution of methanol, acetonitrile and water.	260 nm	0.8 ml/min	200-2000 ng/ ml [9]
C 8 column (25 cm x 4.6 mm, 5 µm)	Water-acetonitrile (80:9, *v/v*) containing 5 ml of triethylamine, 670 mg/l sodium heptanesulphonate, and 0.034 M each of k_2HPO_4 and citric acid.	254 nm	1.6 ml/min.	10-500 ng/ml [10]
C 8 column (250 x 4 mm, 5 µm)	Consisted of A: 0.05M ammonium acetate and phase B: methanol at a volume ratio 60:40.	262 nm	1.4 ml/min	0.2-20 ng/µl [11]

DISCUSSION

Many HPLC/UPLC-UV methods were reported for determination of nicotine in human plasma and they are suitable for further pharmacological studies while design of new nicotine formulations. The reported methods showed satisfactory data for all the parameters tested within the limits of bioanalytical assays. The lower limit of quantification permits application of the methods on human volunteers and suitable for further pharmacological studies. The authors are going through future work to prepare new dosage form containing nicotine. The *in vivo* studies and pharmacokinetic investigations of this new dosage form will be conducted, using this review as a guide for their work. C_{18} was the most common column in the literature and it was selected by the authors for their future investigation as Cyano column (and other columns) failed to give satisfactory validation parameters for analysis of nicotine and its internal standard in the preliminary investigations in spite of its successful use by the same authors with sharp peaks for the analysis of many pharmaceutical formulations [12-15].

UPLC methods are preferable than HPLC, with many associated advantages such as that UPLC operates at much higher pressure. This ultra-pressure ensures the advantages of improved resolution and fewer consumables. One of the key advantages is the resolution, as demonstrated by the peak shape. HPLC typically produces broad peaks that skilled operators can characterize very well, including peak heights and peak widths. Another important advantage is a faster run time. The significant reduction in solvent use is another important advantage of UPLC [16]. Some methods for nicotine analysis in the literature included spiking technique in which nicotine was spiked onto the sample so that the total nicotine content after spiking was twice the amount prior to spiking [17] similar to the common well established spiking technique that commonly used in spiking pharmaceutical formulations [18]. The use of spiking sample enrichment technique may be applicable to nicotine analysis in plasma to increase the sample concentration up to the level which can be measured using the ultraviolet detector instead of the high cost mass detector.

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