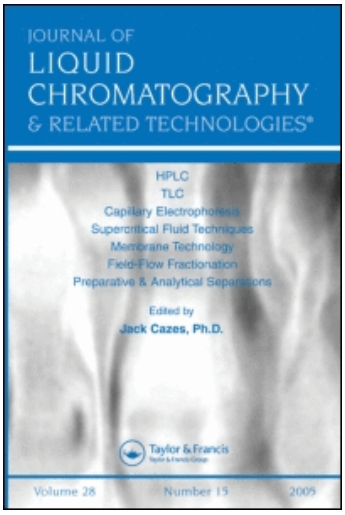

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DETERMINATION OF COTININE IN PLASMA BY LIQUID CHROMATOGRAPHY AFTER SOLID-PHASE EXTRACTION

Edward J. Dunn[a]
[a] Department of Laboratory Medicine, St. Joseph's Hospital, Hamilton, Canada

Online publication date: 13 January 2005

To cite this Article Dunn, Edward J.(1999) 'DETERMINATION OF COTININE IN PLASMA BY LIQUID CHROMATOGRAPHY AFTER SOLID-PHASE EXTRACTION', Journal of Liquid Chromatography & Related Technologies, 22: 13, 2043 — 2050
To link to this Article: DOI: 10.1081/JLC-100101784
URL: http://dx.doi.org/10.1081/JLC-100101784

J. LIQ. CHROM. & REL. TECHNOL., 22(13), 2043–2050 (1999)

DETERMINATION OF COTININE IN PLASMA BY LIQUID CHROMATOGRAPHY AFTER SOLID-PHASE EXTRACTION

Edward J. Dunn

Department of Laboratory Medicine
St. Joseph's Hospital
Hamilton, Ontario, Canada, L8N 4A6

ABSTRACT

A rapid method for the determination of cotinine concentration in plasma is described. Cotinine is extracted from plasma along with 2-phenylimidazole as an internal standard. Extraction is accomplished by solid phase extraction using BondElut C_{18} silica extraction columns. The extract is evaporated, reconstituted with mobile phase, and injected onto a reversed-phase C_{18} ion-pair column using an isocratic high performance liquid chromatograph. The mobile phase consists of acetonitrile and methanol in phosphate buffer (0.01M, pH 3.2) 7:13:80 (v/v/v) containing octanesulfonic acid (0.001 M). The flow rate was 1.5 mL/min. Absorbance was monitored at 260 nm. The detection limit is 5 μg/L for cotinine. The standard curve is linear from 0 to 1000 μg/L. The CVs at 40 μg/L and 100 μg/L are 7.4% and 4.8% respectively.

INTRODUCTION

Cotinine is a major metabolite of nicotine. The measurement of cotinine in urine and plasma is useful for determining individuals smoking status and for

monitoring nicotine patch replacement, nicotine chewing gum, and nasal spray therapy. Cotinine has been determined in plasma and saliva by liquid chromatography,[1,2] and in serum, urine, and oral samples, by gas chromatography-mass spectrometry.[3] Cotinine samples are generally prepared by liquid-liquid extraction using organic solvents such as methylene chloride[1,2] or toluene:butanol (9:1).[3]

Very recently a solid-phase extraction method for nicotine, but not cotinine, in serum and urine followed by isotope dilution gas chromatography-mass spectrometry was described.[4] This study describes a simple and efficient solid-phase extraction procedure for the determination of cotinine in plasma by liquid chromatography.

EXPERIMENTAL

Reagents and Chemicals

All reagents were of analytical grade. Racemic (±)-nicotine, (-)-cotinine, 2-phenylimidazole, and 1-octanesulfonic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Caffeine was obtained from BDH Inc. (Toronto, ON). HPLC grade acetonitrile and methanol were obtained from EM Science (Gibbstown, NJ). Water was freshly distilled prior to preparing the mobile phase. BondElut C_{18} SPE (1mL/100mg) columns were obtained from Varian Sample Preparation Products (Harbor City, CA).

Standards

Stock caffeine and cotinine solutions were prepared by dissolving 10.0-mg caffeine and 10.0 mg of (-)-cotinine in 10 mL of methanol respectively (1 g/L). Stock nicotine solution was prepared by dissolving 100 mg of (±)-nicotine in 10 mL of methanol (10 g/L). These stock solutions were stored at -20°C in tightly capped glass tubes. Plasma standards of cotinine (0 to 1000 μg/L) were prepared by diluting the appropriate volume of stock solution with pooled plasma.

Stock internal standard (IS) solution (1 g/L) was prepared by dissolving 10 mg of 2-phenylimidazole in 10 mL of methanol. This solution was stored at -20°C. Working internal standard solution (1 μg/mL) was prepared prior to use by dilution of the stock in water.

Extraction

The SPE columns were placed on a VacElut device and washed once (one column volume) with of 1 N HCl, twice with methanol, and once with water. To 1.0 mL of sample were added 50 μL of 3M potassium hydroxide and 50 μL of working internal standard. After mixing, the sample was added to the washed SPE columns and passed through at a rate of approximately 1 mL/min. The columns were then washed once with 0.1 M HCl, once with water, and once with 10% aqueous methanol.

The columns were then transferred to correspondingly labeled 12 x 75 mm glass tubes previously washed with methanol. The columns were eluted with 0.5 mL of acetonitrile and the eluate collected and evaporated under a stream of nitrogen. The dried extract was reconstituted with 200 μL of mobile phase. A 50 μL aliquot was injected into the liquid chromatograph.

Instrumentation and Chromatography

The HPLC system consists of a model LC-7A bio-liquid chromatography pump, a model SPD-6AV UV-Vis detector (Shimadzu Scientific Instrument, Columbia, MD). The chromatogram was recorded on a Shimadzu integrator (Model CR501, Chromatopac). The 25 cm X 4.6 mm (i.d.) Ultrasphere octadecylsilane reversed phase column (5-μm particles, Beckman Instruments, Berkley, CA) was protected with a RP-18 1.5 cm guard column (5-μm particles, Applied Biosystems, Foster City, CA). The chromatography was performed at ambient temperature. The mobile phase consisted of acetonitrile and methanol in phosphate buffer (0.01M, pH 3.2) 7:13:80 v/v/v containing octanesulfonic acid (0.001 M). The flow rate was 1.5 mL/min and the detector wavelength was set at 260 nm.

RESULTS AND DISCUSSION

Cotinine is extracted from plasma by solid-phase extraction using C_{18} silica columns. Samples are made basic by the addition of potassium hydroxide and loaded onto the C_{18} SPE cartridges. The cartridges are then washed to remove acidic, polar, and neutral compounds. Cotinine and the internal standard are eluted with acetonitrile. 2-Phenylimidazole was chosen as the internal standard since its suitability has been described previously.[1,2] It absorbs well at 260 nm and its extraction efficiency from plasma is similar to cotinine. The recovery of 2-phenylimidazole and cotinine from plasma is typically greater than 85%.

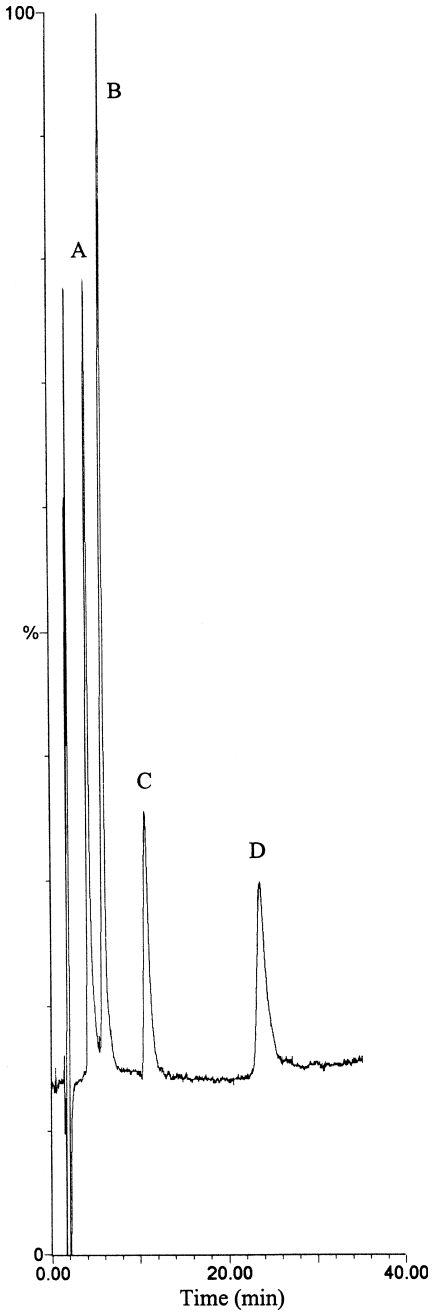

Table 1

Effect of pH on Retention Times

	Retention Times (Min.)			
pH	**Cotinine**	**Caffeine**	**Nicotine**	**I.S.**
4.05	4.55	5.59	9.08	27.70
3.71	4.42	5.59	9.58	27.40
3.41	4.34	5.57	10.04	27.10
3.20	4.30	5.57	10.44	26.93
3.11	4.25	5.55	10.74	26.68
3.07	4.19	5.54	11.40	26.37
2.94	4.13	5.55	11.78	25.91

Table 2

Precision Data for the Cotinine Method

	Mean (SD)	**CV%**	**N**
Within-Batch	104 (5)	4.8	5
	41 (3)	7.4	5
Between-Batch	114 (13)	11.4	5
	47 (5)	10.2	5

The mobile phase was chosen to optimize the separation of cotinine from caffeine. Figure 1 illustrates that cotinine is well separated from caffeine. Good resolution of these two peaks is important for accurate quantitation of cotinine since caffeine is often present in plasma in very large concentrations. Nicotine is also well separated from caffeine and cotinine, however, nicotine is not well extracted by this method. The pH of the mobile phase has a dramatic effect on the retention time of nicotine. The effect of pH on retention time of each analyte is shown in Table 1. Decreasing the pH increases the retention time of nicotine but decreases the retention times of cotinine and 2-phenylimidazole.

Figure 1 (left). Chromatogram of standards: A, cotinine, 125 ng; B, caffeine, 125 ng; C, nicotine,125 ng; D internal standard, 12.5 ng (on-column amounts). Retention time for the peaks (A) 4.1, (B) 5.8, (C) 10.6, and (D) 23.6 min.

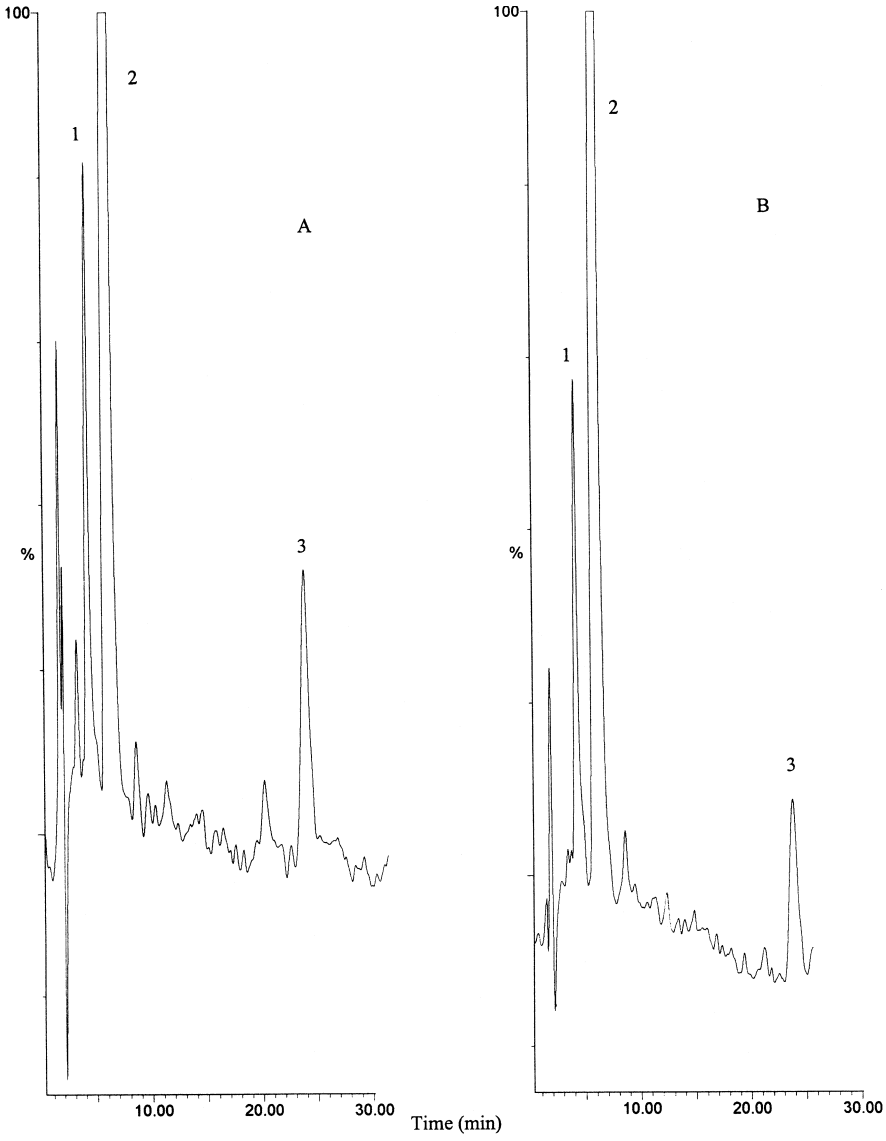

Figure 2. Liquid chromatograms of 1-mL plasma extracts. Plasma of non-smoker spiked with 200 μg/L cotinine (1) and 50 μg/L of internal standard (3). The sample also contains an undetermined amount of caffeine (2). Plasma of a smoker contains 256 μg/L cotinine, an unknown amount of caffeine (2) and 50 μg/L of internal standard (3).

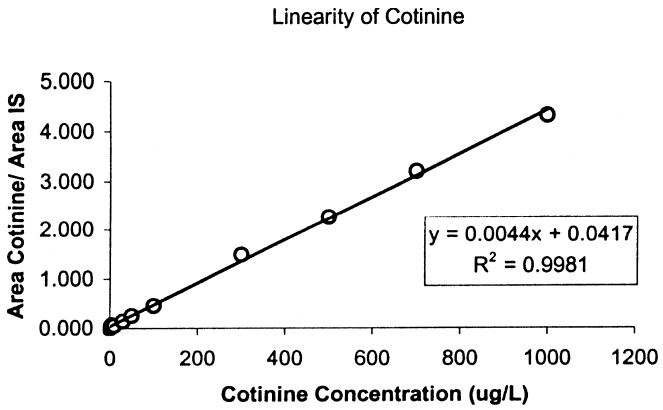

Figure 3. Standard curve of cotinine area/internal standard area versus cotinine concentration.

This is in contrast to the study by Harlharan,[2] which reported that the retention time for nicotine increased with increasing pH from 3.0 to 7.3, using their analytical procedure.

The retention time of caffeine is unaffected. The compromise for separation of cotinine from caffeine is that the retention time for the internal standard is quite long. Increasing the organic composition of the mobile phase will decrease the retention time of the internal standard as well as the separation of cotinine and caffeine.

Figure 2 shows representative chromatograms of plasma extracts. Figure 2A is blank plasma spiked with 200 μg/L of cotinine and Figure 2B is a plasma extract of a known smoker. Both chromatograms illustrate separation of the cotinine peak from the much larger caffeine peaks. It can also be seen that there are very few extraneous peaks in the chromatograms indicating that the extraction is quite specific. Plasma, which contained no cotinine, was spiked with cotinine at concentrations ranging from 0 to 1000 μg/L.

These standards were extracted and injected onto the liquid chromatograph. The resulting standard curve (Figure 3) demonstrates that the method is linear to 1000 μg/L and passes through the origin. The limit of detection is 5 μg/L. The precision data are given in Table 2 indicating acceptable precision of the method.

In summary, a liquid chromatographic method for determination of the concentration of cotinine in plasma was developed that uses solid-phase extraction. This extraction technique is simpler and as efficient as more laborious liquid-liquid extractions. The method is linear over a wide range of concentrations with a limit of detection of 5 μg/L and has acceptable precision at low concentrations.

REFERENCES

1. D. A. Machacek, N.-S. Jiang, Clin. Chem., **32**, 979-982 (1986).

2. S. A. McAdams, M. L. Cordeiro, J. Chromatog., **615**, 148-153 (1993).

3. M. Harlharan, T. VanNoord, J. F. Greden, Clin. Chem., **34**, 724-729 (1988).

4. L. B. Baskin, R. W. Anderson, J. R. Charlson, R. D. Hurt, G. M. Lawson, Ann. Clin. Biochem., **35**, 522-527 (1998).

Received September 5, 1998
Accepted October 18, 1998
Manuscript 4885

Request Permission or Order Reprints Instantly!