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Measurement of nicotine and cotinine in human milk by high-performance liquid chromatography with ultraviolet absorbance detection

Madhu Page-Sharp [a,*], Thomas W. Hale [b], L. Peter Hackett [c], Judith H. Kristensen [d], Kenneth F. Ilett [a,c]

[a] *Pharmacology Unit, School of Medicine and Pharmacology, The University of Western Australia, M510, 35 Stirling Highway, Crawley 6009, Australia*
[b] *Division of Clinical Pharmacology, Department of Pediatrics, Texas Tech University School of Medicine, Amarillo, TX, USA*
[c] *Clinical Pharmacology & Toxicology Laboratory, The Western Australian Centre for Pathology & Medical Research, Nedlands, Australia*
[d] *Pharmacy Department, King Edward Memorial Hospital, Womens and Childrens Health Services, Subiaco, Australia*

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Abstract

A high-performance liquid chromatographic (HPLC) assay for the determination of nicotine and cotinine in human milk was developed using an extraction by liquid–liquid partition combined with back extraction into acid, and followed by reverse-phase chromatography with UV detection of analytes. The assay was linear up to 500 μg/l for both nicotine and cotinine. Intra- and inter-day relative standard deviations (R.S.D.) were <10% (25–500 μg/l) for both nicotine and cotinine. Limits of quantitation (LOQ) were 10 and 12 μg/l for nicotine and cotinine, respectively, while the limits of detection (LOD) were 8 and 10 μg/l for nicotine and cotinine, respectively. The mean recoveries were 79–93% (range 25–500 μg/l) for nicotine and 78–89% (range 25–500 μg/l) for cotinine. The amount of fat in the milk did not affect the recovery. We found that this method was sensitive and reliable in measuring nicotine and cotinine concentrations in milk from a nursing mother who participated in a trial of the nicotine patch for smoking cessation.

Keywords: Nicotine; Cotinine

1. Introduction

The determination of nicotine and its major metabolite cotinine has been a subject of growing interest due to the adverse effect of smoking on human health. There are several published methods for analysis of nicotine and cotinine in plasma, urine, hair and saliva samples [1–7]. The most frequently used analytical techniques are gas chromatography (GC) or gas chromatography coupled to mass spectrometry (GC–MS) [1–4,7]. Other methods such as enzyme-linked immunoassay and radio-immunoassay have also been described [8,9] but have sensitivity and cross-sensitivity

* Corresponding author. Tel.: +61-8-9346-2985; fax: +61-8-9346-3469.
E-mail address: msharp@receptor.pharm.uwa.edu.au (M. Page-Sharp).

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limitations. High-performance liquid chromatography (HPLC) has been a preferred technique for many routine analyses [10–14].

There are only two published analytical methods for determination of nicotine and cotinine in human milk [15,16]. Both used GC as the separation/detection system. Unlike most other biological fluids, determination of drug concentrations in human milk is sometimes difficult [17] because of variable matrix composition. The aim of our study was to establish a reliable and robust HPLC method for measurement of nicotine and cotinine in human milk.

2. Experimental

2.1. Specimens

Milk samples were collected by hand expression or manual breast pump from a smoking nursing mother who was a participant in a study of the use of the nicotine transdermal patch for smoking cessation. The study protocol was approved by the Ethics Committee of King Edward Memorial and Princess Margaret Hospitals and by the Human Research Ethics Committee of the University of Western Australia and written informed consent was obtained from the mother. Immediately after expression, milk was stored at 4 °C and then transported on ice to the laboratory. Samples were aliquoted (1.5 ml) in Eppendorf® tubes and stored at −20 °C until assayed.

Drug-free breast milk samples for assay development were collected from non-smoking nursing mothers (excess samples from the Hospital Milk Bank) and used for standard curves and validation studies. These samples were aliquoted (10 ml) into polypropylene tubes and stored at −20 °C.

2.2. Chemicals and reagents

Stock solutions of nicotine and cotinine were prepared separately at a concentration of 1 mg/ml in methanol. These methanolic solutions are stable for at least 3 months at 4 °C [12]. *N*-acetylprocainamide was used as an internal standard (IS) and a 1 mg/ml stock solution was prepared as above. All stock solutions were stored in the dark at 4 °C. Working standards were prepared in 10% methanol as required and stored as above. Nicotine, cotinine and *N*-acetylprocainamide were purchased from Sigma, St. Louis, MO, USA. Citric acid, triethylamine and dichloromethane were obtained from BDH Laboratory Supplies, Dorset, England. Acetonitrile and methanol were purchased from Merck, Damstadt, Germany. All other chemicals were of analytical or HPLC grade.

2.3. Sample preparation

Milk samples (1 ml) were aliquoted and spiked with IS (50 ng). The samples were alkalinised in 10 ml polypropylene tubes with 100 μl of 10 M NaOH and extracted with 8 ml of dichloromethane by vigorous manual shaking for 10 min. After centrifugation at 1500 × *g* for 10 min, the supernatant was aspirated to waste and the remaining dichloromethane (6.5 ml) was transferred to a clean polypropylene tube. Samples were then back extracted into 3 ml of 0.1 M HCl by shaking vigorously for 5 min followed by centrifugation as above. The upper acidic aqueous extract (2.9 ml) was transferred to a clean polypropylene tube to which 8 ml of dichloromethane and 200 μl of 10 M NaOH was added. Samples were then extracted by shaking vigorously for 10 min. After centrifugation as above the upper aqueous layer was aspirated to waste and 7.5 ml of dichloromethane was transferred into a round-bottomed borosilicate glass tube. Concentrated HCl (20 μl) was added to each sample tube to prevent the volatilisation of nicotine [14], the samples were vortexed gently and evaporated to dryness at 45 °C under a gentle stream of dry nitrogen. Residues were reconstituted in 100 μl of the HPLC mobile phase and 50 μl aliquotes were injected onto the HPLC column.

2.4. HPLC instrumentation and chromatographic conditions

The HPLC system comprised of a Waters Millipore Solvent Select Valve coupled to an Hewlett Packard isocratic pump, autosampler and a variable wavelength UV detector (Series 1100, Agilent Technology, Waldbronn, Germany). Separation was performed on a Zorbax SB-C8 column, 2.1 mm × 100 mm, 3.5 μm (Agilent Technology) in series with an Eclipse XBD-C8 2.1 mm × 12.5 mm, 5 μm (Agilent Technology) guard column. The mobile phase contained 2.5% (v/v) acetonitrile in phosphate-citrate buffer

(30 mM K_2HPO_4, 30 mM citric acid, 0.5% (v/v) triethylamine-adjusted to pH 6.7 with 10 M NaOH). The mobile phase was pumped at 0.25 ml/min and analytes were detected by their UV absorbance at 260 nm. Analysis and quantification of chromatograms (peak area) were undertaken using Chemstation Software (Version 9, Agilent Technology, Waldbronn, Germany).

2.5. Measurement of milk fat content

The percentage of milk fat content was determined using the creamatocrit technique [18] as modified by Lucas et al. [19].

2.6. Validation tests

The calibration curve and validation studies for nicotine and cotinine were performed on blank milk samples collected from non-smoking nursing mothers.

2.6.1. Calibration curve

For each unknown analytical batch a five-point calibration curve ranging from 12.5 to 500 μg/l was prepared by spiking blank milk (previously shown to be free from nicotine or cotinine) with appropriate volumes of working standards.

2.6.2. Quality control and validation range

Quality control (QC) standards were run with each batch of samples; QC concentrations of 25 and 50 ng were used. Validation measures used spiked milk samples at low, mid and high concentrations (25, 100 and 500 μg/l) of the calibration curve.

2.6.3. Precision and accuracy

Five replicates of blank milk spiked at 25, 100 and 500 μg/l ($n = 15$ in total) were used to construct a standard curve. Precision was determined as the percentage relative standard deviation (R.S.D.) of the replicate measurements, where

$$\text{R.S.D. (\%)} = \frac{\text{standard deviation}}{\text{mean[nicotine or cotinine]}} \times 100.$$

Both intra- and inter-day R.S.D. were calculated using peak area ratios. Intra-day accuracy of the determined values to the true concentrations was calculated from the average of the five replicates at each concentration using the formula:

$$\text{accuracy (\%)} = \frac{\text{calculated[nicotine or cotinine]}}{\text{known[nicotine or cotinine]}} \times 100.$$

2.6.4. Limits of quantification and detection

The limit of quantification (LOQ) was determined as the lowest concentration measured with R.S.D. of ≤20%, while the limit of detection (LOD) was determined as the concentration with a signal to noise ratio of 3.

2.6.5. Specificity

Interference by the presence of any endogenous constituents in milk was assessed by the analysis of blank milk samples. The retention times of a range of drugs that were likely to be extracted and chromatograph under our assay conditions were measured by injecting solutions of these compounds onto the HPLC column.

2.6.6. Storage stability

The stability of nicotine and cotinine in milk was evaluated at 25, 100 and 500 μg/l. At each concentration, replicates ($n = 5$) were assayed when freshly prepared and again after storage at −20 and −80 °C for 1, 2 or 3 months. Storage stability was assessed by repeated measures one-way analysis of variance (ANOVA; SigmaStat Version 2.0, SPSS Inc., Chicago, IL, USA). Stability of nicotine and cotinine with final reconstituted samples that was injected onto the HPLC was at 50 and 500 μg/l. The samples prepared in mobile phase were kept at room temperature (22 °C) in the autosampler, and aliquots were injected onto the HPLC immediately after the preparation and again hourly for 18 h. Stability of nicotine and cotinine in these samples was assessed by linear regression analysis (SigmaStat Version 2.0, SPSS Inc., Chicago, IL, USA).

2.6.7. Absolute recovery

Absolute recovery was measured as the area response of a processed spiked standard expressed as a percentage of the response of pure standards prepared in mobile phase at concentrations indicative of 100% extraction. The recovery was compared to the extracted samples at 25, 100 and 500 μg/l for

both nicotine and cotinine, using milk samples with varying lipid content (range 6.5–12.7%). Mean recoveries between the different fat contents and analyte concentrations were compared by two-way analysis of variance (SigmaStat Version 2.0, SPSS Inc., Chicago, IL, USA).

2.6.8. Application

This HPLC method was used to study the concentration–time profiles of nicotine and cotinine in the milk from a 33-year-old smoking nursing mother, while she was smoking an average of 20 cigarettes per day, and later when she had ceased smoking with assistance of a transdermal nicotine patch (Nicabate® CQ, GlaxoSmithKline Healthcare, Ermington, Australia; 21 or 14 mg per day strength). Average concentrations of nicotine and cotinine in milk from this subject were calculated as area under the milk concentration–time curve divided by the time over which the collection was made. Area under the curve was calculated using linear trapezoidal rule as implemented in SigmaPlot Version 8 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Validation tests

3.1.1. Specificity

Retention times for nicotine, *N*-acetylprocainamide and cotinine were 11, 20 and 24.5 min, respectively (Fig. 1b and c). There were slight variations in these retention times over different days. No other interfering peaks were observed in drug-free milk ($n = 14$) studied during the study period. A typical chromatogram from blank milk is shown in Fig. 1a. Retention times of other drugs likely to be co-extracted with nicotine and cotinine are shown in Table 1. None of these interfered with the analytes of interest. However, caffeine, which was found in most of the milk samples had a retention time of around 35 min and this necessitated a long run time.

Table 1
Retention times of drugs tested for non-interference ($\lambda = 260$ nm)

Drug	Retention time (min)
Procainamide	7.1
Paracetamol	8.4
Chloroquine	8.3
Pseudoephedrine	13.3
D-Amphetamine	16
Caffeine	35.3

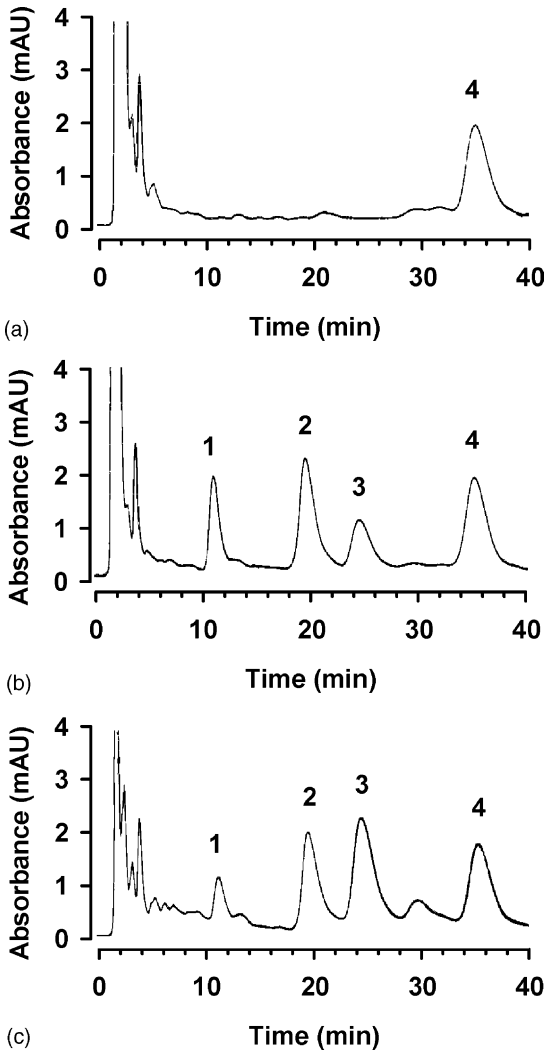

Fig. 1. Chromatograms showing (a) an extract of blank milk, (b) an extract of milk spiked with nicotine (100 μg/l), cotinine (100 μg/l) and internal standard, and (c) an extract of a milk sample from a patient treated with the nicotine patch showing nicotine (57 μg/l), cotinine (247 μg/l) and internal standard. Labelling of peaks: (1) nicotine; (2) *N*-acetylprocainamide (internal standard 50 μg/l); (3) cotinine; (4) caffeine. The additional peak at around 30 min in the patient sample (c) is an endogenous compound that chromatographs in some milk samples.

Table 2
Estimated intra- and inter-day relative standard deviations (R.S.D.) and accuracy of the assay method

Compound	Concentration (μg/l)	R.S.D. (%)	Accuracy (%)
Intra-day ($n = 5$)			
Nicotine	25	6.4	105
	100	4.5	110
	500	2.2	101
Cotinine	25	8.1	104
	100	2.3	108
	500	2.9	98
Inter-day ($n = 5$)			
Nicotine	25	8.2	97
	100	5.7	95
	500	5.6	101
Cotinine	25	8.0	102
	100	5.5	104
	500	5.2	99

3.1.2. Linearity

A five-point milk standard curve that was constructed for each assay batch was linear over the range of 12.5–500 μg/l ($r^2 \geq 0.998$) for both nicotine and cotinine.

3.1.3. Precision and accuracy

These data are summarised in Table 2. Both the intra- and inter-day R.S.D. were less than 10% over the range 25–500 μg/l for both nicotine and cotinine. At the same concentrations, accuracy ranged from 98 to 110% intra-day and from 95 to 104% inter-day.

3.1.4. Limits of quantification and detection

The LOQ for the assay was 10 μg/l for nicotine and 12 μg/l for cotinine with intra-day R.S.D. of 12.1 and 15.8%, respectively, and inter-day R.S.D. of 14.1 and 17.6%, respectively. The LOD was 8 μg/l for nicotine and 10 μg/l for cotinine.

3.1.5. Storage stability

Nicotine and cotinine reconstituted in mobile phase were stable for at least 18 h. Linear regression of the peak area ratio (nicotine/IS) versus time gave a slope of 0.0002 with ($F = 3.3$, $P = 0.102$) at 25 μg/l and slope of 0.002 with ($F = 2.7$, $P = 0.13$) at 500 μg/l, respectively. Similarly the linear regression of the peak area ratios (cotinine/IS) versus time up to 18 h yielded slope of 0.00016 with ($F = 0.308$, $P = 0.59$) at 25 μg/l and slope of 0.002 with ($F = 2.05$, $P = 0.18$) at 500 μg/l, respectively.

One-way analysis of variance showed that nicotine and cotinine at (25, 100 and 500 μg/l) were stable for up to 3 months when stored at either −20 or −80 °C. For nicotine, compared to the concentration measured on day 0 (spiked at 25, 100 or 500 μg/l), the measured concentrations after 1, 2 and 3 months of storage were not significantly different ($F = 0.47$, $P = 0.71$; $F = 0.82$, $P = 0.5$; $F = 0.41$, $P = 0.75$, respectively). Similarly for cotinine, compared to the concentration measured on day 0 (spiked at 25, 100 or 500 μg/l), the measured concentrations after 1, 2 and 3 months of storage also were not significantly different ($F = 3.1$, $P = 0.05$; $F = 0.23$, $P = 0.87$; $F = 2.4$, $P = 0.1$, respectively).

3.1.6. Absolute recovery from milk with different fat content

The mean recoveries of both nicotine and cotinine at 25, 100 and 500 μg/l in milk samples containing varying fat concentrations (6.5, 9.3 and 12.7%) are presented in Table 3. Two-way ANOVA showed that

Table 3
Absolute recoveries of nicotine and cotinine from milk samples with different lipid content ($n = 5$ at each of three concentrations)

Lipid (%)	Compound	Concentration (μg/l)	Absolute recovery (mean ± S.D.) (%)
6.5	Nicotine	25	83.4 ± 7.2
		100	82.0 ± 2.0
		500	87.9 ± 4.2
6.5	Cotinine	25	84.0 ± 5.3
		100	78.6 ± 3.7
		500	78.4 ± 5.1
9.3	Nicotine	25	81.3 ± 3.9
		100	87.4 ± 2.3
		500	92.5 ± 7.4
9.3	Cotinine	25	86.9 ± 6.6
		100	81.4 ± 2.0
		500	83 ± 2.1
12.7	Nicotine	25	82.0 ± 4.3
		100	84.3 ± 9.4
		500	79.0 ± 2.1
12.7	Cotinine	25	88.6 ± 1.4
		100	81.6 ± 4.3
		500	80.2 ± 1.6

recoveries for nicotine were similar for all three different fat concentrations ($F = 2.77$, $P = 0.08$) and across the three drug concentrations tested ($F = 1.81$, $P = 0.18$). Similarly, cotinine recovery was not influenced by either fat content ($F = 1.91$, $P = 0.17$) or by drug concentration ($F = 2.86$, $P = 0.08$).

3.1.7. Application of method during smoking and use of the nicotine patch

The concentrations of nicotine and cotinine in milk from a lactating woman (subject 19; 31 years, 65 kg) during smoking, and sequentially later whilst on the 21 or 14 mg nicotine patch are shown in Fig. 2. Average concentrations in milk while smoking were 32 μg/l for nicotine and 115 μg/l for cotinine. By comparison, during the period when the 21 mg patch was in use, average milk concentrations were 28 μg/l for nicotine and 114 μg/l for cotinine. When the patch strength was decreased to 14 mg, average milk concentrations were 25 μg/l for nicotine and 84 μg/l for cotinine.

4. Discussion

Some 26% of women smoke cigarettes whilst breast feeding [20]. This practice continues despite the widespread recognition of the proven adverse consequences of environmental tobacco exposure for the neonate [21,22], and the possibility of unwanted pharmacological effects in neonates from direct exposure to nicotine and its metabolic products via breast milk [23]. Using nicotine replacement therapy to assist women smokers to quit is one way of avoiding adverse health effects in the breast-fed infant [23]. The method described in this manuscript was developed to support a planned study of the nicotine patch in reducing exposure of the breast-fed infant to nicotine and its metabolite cotinine.

Most previously described methods for quantifying nicotine and cotinine have concentrated on plasma or urine as the sample matrix [1–5]. Semi-quantitative bioassay techniques for nicotine in milk have been described in very early literature [24,25], and a GC method was reported in 1976 [26]. GC-based measurements of both nicotine and/or cotinine in milk also have been reported [27–30], but mostly with minimal methodological detail. Milk can sometimes be a difficult analytical matrix, mostly because of its variable

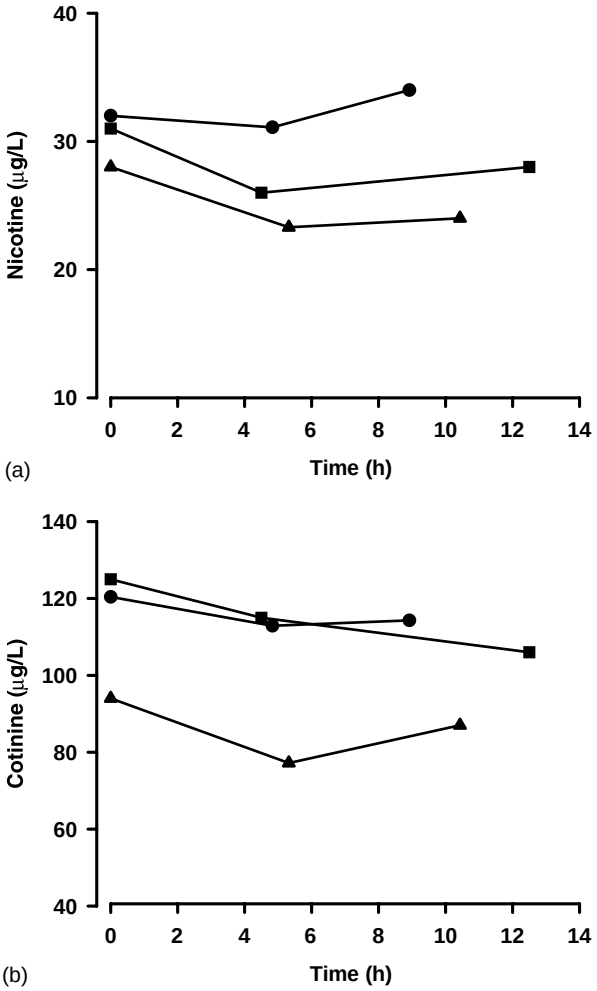

Fig. 2. Concentrations of nicotine (a) and cotinine (b) in milk from subject 19 whilst smoking 20 cigarettes per day (●) and later after being sequentially stabilised (>1 week) on the 21 mg (■) or 14 mg (▲) nicotine patch.

fat content [17]. To our knowledge, a method for the measurement of nicotine and cotinine in milk using HPLC has not been described.

Our method uses solvent extraction, followed by reverse-phase HPLC with UV detection at 260 nm. Nicotine and cotinine have somewhat different oil:water partition coefficients ($\log_{10} P = 0.72$ and -0.228, respectively) and pK_a values (8.0 and 4.72, respectively) [31], and previous authors working with plasma have had to resort to the use of separate extraction steps involving two different solvents

(diethyether for nicotine and dichloromethane for cotinine) [1]. Our method uses a dual extraction process followed by evaporative concentration in the presence of HCl to minimise volatilisation of nicotine. Recoveries through the process were high for both nicotine (mean 79–92%) and cotinine (mean 78–89%) with R.S.D. of <10%. Moreover, recovery was not affected by the fat content of milk. The robustness of the overall method for both analytes was excellent with intra- and inter-day R.S.D. ranging from 2.2 to 8.2% and accuracy ranging from 95 to 100%. Linearity for both nicotine and cotinine was demonstrated up to 500 μg/l. The LOQs of 10 and 12 μg/l for nicotine and cotinine, respectively, were not as low as those that can be achieved with GC assays (approximately 0.2 μg/l [16,27]. Nevertheless, recent studies from our laboratory have shown that mean (95% CI) concentrations of nicotine in milk from patients on a 7 mg patch were 27 μg/l (21–35 μg/l), and our LOQ would therefore allow the detection of nicotine in milk at patch doses equivalent to theoretical dose of about 2 mg per day [32]. Caffeine, a drug that is commonly found in milk did not interfere in the assay, and the procedure was also free from interference from a range of other amine drugs and from endogenous constituents in the milk. The stability of nicotine and caffeine at relevant concentrations in milk stored both at −20 and −80 °C was demonstrated for up to 3 months, as was stability of the final extract (while reconstituted in mobile phase and awaiting injection onto the HPLC) for a period of 18 h.

Utility of the method was demonstrated using breast milk collected from a smoker, before and during use of nicotine patches. These data showed similar average concentrations of nicotine and cotinine for the smoking period and when the 21 mg patch was in use. This was expected since the manufacturer suggests that the 21 mg patch is approximately equivalent to smoking 20 cigarettes per day. Average concentrations of both nicotine and cotinine decreased with use of the 14 mg patch. The data suggest that the exposure of the breast-fed infant to nicotine and its metabolites will be proportionally decreased as the patient progresses through the process of quitting smoking by use of the nicotine transdermal patch. However, data from our laboratory for a group of 15 nursing smokers show that the decrease is significant only at the 7 and 14 mg patch levels, compared to smoking or the 21 mg patch [32]. The concentrations of nicotine and cotinine in milk from nursing smokers in our case report are similar to those reported previously. One study found nicotine in milk from nursing smokers (5–40 cigarettes per day) ranged from 2–62 μg/l, while cotinine ranged from 12–222 μg/l [27]. In the other, the mean nicotine concentration in milk from smokers was 55 μg/l (range 10–140 μg/l) and mean cotinine was 136 μg/l (31–467 μg/l) [16].

5. Conclusions

We have developed a HPLC/UV method for quantification of nicotine and cotinine in human milk. This method is simple, sensitive, reliable and applicable to routine analysis of these analytes in milk during smoking and use of the nicotine patch.

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References

[1] N. Hengen, M. Hengen, Clin. Chem. 24 (1978) 50.
[2] P. Jacob, M. Wilson, N.L. Benowitz, J. Chromatogr. 222 (1981) 61.
[3] P. Jacob, L. Yu, M. Wilson, N.L. Benowitz, Biol. Mass Spectrometr. 20 (1991) 247.
[4] H.S. Shin, J.G. Kim, Y.J. Shin, S.H. Jee, J. Chromatogr. Biomed. Sci. Appl. 769 (2002) 177.
[5] C. Eliopoulos, J. Klein, M.K. Phan, B. Knie, M. Greenwald, D. Chitayat, G. Koren, JAMA 271 (1994) 621.
[6] T.P. Moyer, J.R. Charlson, R.J. Enger, L.C. Dale, J.O. Ebbert, D.R. Schroeder, R.D. Hurt, Clin. Chem. 48 (2002) 1460.
[7] J.G. Kim, U.S. Shin, H.S. Shin, Bull. Korean Chem. Soc. 23 (2002) 1496.
[8] H. Van Vunakis, H.B. Gjika, J.J. Langone, IARC Sci. Publ. (1987) 317.
[9] G.J. Knight, P. Wylie, M.S. Holman, J.E. Haddow, Clin. Chem. 31 (1985) 118.
[10] A. Sioufi, C. Parisot, N. Sandrenan, J.P. Dubois, Methods Find. Exp. Clin. Pharmacol. 11 (1989) 179.
[11] L.J. Lewis, J.D. Lamb, D.J. Eatough, L.D. Hansen, E.A. Lewis, J. Chromatogr. Sci. 28 (1990) 200.
[12] C. Oddoze, A.M. Pauli, J. Pastor, J. Chromatogr. Biomed. Sci. Appl. 708 (1998) 95.
[13] J. Baranowski, G. Pochopien, I. Baranowska, J. Chromatogr. Biomed. Sci. Appl. 707 (1998) 317.

[14] M. Nakajima, T. Yamamoto, Y. Kuroiwa, T. Yokoi, J. Chromatogr. Biomed. Sci. Appl. 742 (2000) 211.
[15] W. Luck, H. Nau, Eur. J. Pediatr. 146 (1987) 21.
[16] A. Dahlstrom, B. Lundell, M. Curvall, L. Thapper, Acta Paediatr. Scand. 79 (1990) 142.
[17] E.J. Begg, S.B. Duffull, L.P. Hackett, K.F. Ilett, J. Hum. Lact. 18 (2002) 323.
[18] I.R. Fleet, J.L. Linzell, J. Physiol. 175 (1964) 15.
[19] A. Lucas, J.A. Gibbs, R.L. Lyster, J.D. Baum, Br. Med. J. (Clin. Res. Edn.) 1 (1978) 1018.
[20] H.C. Boshuizen, P.H. Verkerk, J.D. Reerink, W.P. Herngreen, B.M. Zaadstra, S.P. Verloove-Vanhorick, Am. J. Epidemiol. 147 (1998) 117.
[21] A.B. Becker, J. Manfreda, A.C. Ferguson, H. Dimich-Ward, W.T. Watson, M. Chan-Yeung, Arch. Pediatr. Adolesc. Med. 153 (1999) 689.
[22] M.A. Mascola, H. Van Vunakis, I.B. Tager, F.E. Speizer, J.P. Hanrahan, Am. J. Public Health 88 (1998) 893.
[23] B.S. Schatz, J. Hum. Lact. 14 (1998) 161.
[24] W.B. Thompson, Am. J. Obstet. Gynecol. 26 (1933) 662.
[25] H.H. Perlman, A.M. Dannenberg, N. Sokoloff, J. Am. Med. Assoc. 120 (1942) 1003.
[26] B.B. Ferguson, D.J. Wilson, W. Schaffner, Am. J. Dis. Child 130 (1976) 837.
[27] W. Luck, H. Nau, Br. J. Clin. Pharmacol. 18 (1984) 9.
[28] W. Luck, H. Nau, Eur. J. Pediatr. 146 (1987) 21.
[29] J.A. Mennella, G.K. Beauchamp, New Engl. J. Med. 339 (1998) 1559.
[30] A. Woodward, N. Grgurinovich, P. Ryan, J. Epidemiol. Community Health 40 (1986) 309.
[31] Anonymous, SciFinder Scholar, Chemical Abstracts Services, American Chemical Society, Washington, DC, USA, 2003.
[32] K.F. Ilett, T.W. Hale, M. Page-Sharp, J.H. Kristensen, R. Kohan, L.P. Hackett, Clin. Pharm. Ther. (2003) in press.