

HPLC-UV Method for Nicotine, Strychnine, and Aconitine in Dairy Products

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The toxic nitrogen alkaloids nicotine, strychnine, and aconitine were quantitated in whole milk, skim milk, and cream using solid-phase extraction cleanup and HPLC-UV with dual wavelength detection. Samples were extracted in McIlvaine's buffer with EDTA and then partitioned with aqueous acetonitrile and hexane. The aqueous phase was concentrated and passed through an OASIS HLB column. The column was eluted with methylene chloride/ammonium hydroxide, 1 mL/1 μ L, v/v. The eluent was acidified with hydrochloric acid and evaporated. The sample was diluted for HPLC with acetonitrile/phosphate buffer pH 7.4. Chromatography was performed on an Xterra RP-18 column using a gradient of acetonitrile and ammonium bicarbonate buffer at pH 9.8. Nicotine and strychnine were monitored at 260 nm; aconitine was monitored at 232 nm. Calibration curves were generated from external standards in the range 0.2–10 μ g/mL using $1/x$ weighting. Mean recoveries in whole milk spiked between 0.1 and 10 ppm were the following: nicotine 89.2%, strychnine 75.7%, and aconitine 85.1%. Mean recoveries in skim milk spiked between 0.1 and 10 ppm were the following: nicotine 72.1%, strychnine 78.2%, and aconitine 82.9%. Mean recoveries in cream spiked between 0.2 and 20 ppm were the following: nicotine 87.9%, strychnine 76.9%, and aconitine 82.0%. Relative standard deviations of recovery were less than 20% in each case.

KEYWORDS: Nicotine; strychnine; aconitine; milk; skim milk; cream; HPLC; solid-phase extraction; partitioning.

INTRODUCTION

The U.S. Food and Drug Administration is responsible for protecting the public health by assuring the safety of our nation's food supply. Congress passed the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, which directs the FDA to provide for research to detect adulteration of food products (1). Nicotine, strychnine, and aconitine are highly toxic nitrogen alkaloids; the toxicology of these compounds has been thoroughly reviewed (2–4). These compounds are readily available from commercial sources. Nitrogen alkaloids such as these could contaminate food products either intentionally or unintentionally. Therefore, it is useful to know the fate of such compounds when added to food, particularly in the case where a raw agricultural commodity such as milk is contaminated and then processed into other food products.

Cow's milk is an extremely important food source throughout the world. Year 2003 per capita consumption figures in the United States are the following: whole milk 7.6 gal, low-fat

skim milk 13.9 gal, and cream 11.1 pt (5). Total consumption of milk in the United States for 2003 was 27 250 tons (6). Therefore, analytical methods for toxic nitrogen alkaloids in milk products are important tools for the detection of intentionally adulterated dairy products.

Milk and dairy products are difficult matrices to analyze since they contain protein, fat, and carbohydrate in varying amounts (7, 8). Analytical methods exist for nicotine in milk using HPLC-UV (9), GC-NPD (10), and GC-MS (11, 12). Numerous methods exist for analysis of nicotine in plasma (13–15) and urine (16–18). Some of the HPLC methods for nicotine employ ion-pair chromatography (19, 20), which can be less rugged and reproducible than reversed-phase HPLC methods. Methods are not readily available for the analysis of strychnine and aconitine in milk. Methods for analysis of strychnine in biological matrices using HPLC-UV (21), GC-MS (22), and LC-MS (23) have been reported. Methods for analysis of aconitine in biological matrices using HPLC-UV (24) and LC-MS (25, 26) have been reported. It is important to have a simple analytical method based on reversed-phase HPLC-UV to screen dairy products for toxic nitrogen alkaloids in complex matrices such as milk. Nicotine, strychnine, and aconitine are useful model compounds for such a method because they present different analytical problems. Nicotine in the free base form is

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volatile (bp_{745} 247, (27)), while aconitine is subject to base hydrolysis (25). Strychnine is the most stable of the three analytes. The relative polarities and subsequent reversed-phase HPLC retention of these compounds vary greatly. Nicotine is water-soluble and poorly retained under reversed-phase conditions. Aconitine has low solubility in water and is highly retained under reversed-phase conditions. Therefore, an effective gradient mobile phase is needed to efficiently separate these analytes in a reasonably short run time.

In this report, a method is presented for the analysis of nicotine, strychnine, and aconitine added to uncontaminated whole milk, skim milk, and cream. A simple partitioning and solid-phase extraction procedure was developed to clean up samples prior to gradient elution HPLC-UV analysis. This method could be used to assess the partitioning of nicotine, strychnine, and aconitine into skim and cream after addition to milk prior to processing.

MATERIALS AND METHODS

Samples. Raw milk was obtained from a local source. Skim milk and cream were obtained by centrifugation of the raw whole milk with a Kamdhenu model KD-60E centrifugal separator (Kamdhenu LTD, Mumbai, India). Pasteurized whole milk, skim milk, and cream were obtained from local grocery stores. All samples were stored at 5–10 °C.

Chemicals and Reagents. Ammonium bicarbonate (Fluka LC-MS grade), anhydrous dibasic sodium phosphate (Sigma Ultra), and citric acid monohydrate (Sigma Ultra) were from Sigma-Aldrich (St. Louis, MO). Disodium EDTA (electrophoresis grade), ammonium hydroxide, hydrochloric acid, phosphoric acid (85% Certified ACS Plus), anhydrous sodium sulfate (Acros ACS Reagent), methanol, hexane, acetonitrile (ACN), water, and methylene chloride (DCM) were HPLC or Optima grade from Fisher (Fairlawn, NJ).

McIlvaine's buffer, pH 4, with EDTA (MB + EDTA) was prepared as described in a USDA procedure (28). SPE eluent was methylene chloride/ammonium hydroxide, 1 mL/1 μ L, v/v, and was used within a month of preparation. Ammonium hydroxide (1.5 N) and hydrochloric acid (6 N) were prepared by dilution of concentrated NH_4OH and HCl, respectively, with deionized water. Phosphate buffer, pH 7.4, 500 mM, was prepared from anhydrous sodium phosphate and phosphoric acid. HPLC diluent was 3/97 ACN/phosphate buffer, pH 7.4, 500 mM. HPLC diluent was stored at 5–10 °C and used within a month of preparation.

Standards. Nicotine hemisulfate 40% aqueous solution, strychnine, and aconitine were obtained from Sigma-Aldrich; listed purity was greater than 97% for the lots used.

Standard Solutions (Spiking). Individual stock standards were prepared in acetonitrile: aconitine 755 $\mu\text{g/mL}$, strychnine 822 $\mu\text{g/mL}$, and nicotine 1000 $\mu\text{g/mL}$. Aliquots of stock standards were blended and diluted with acetonitrile to produce a 100 $\mu\text{g/mL}$ mixed standard. A 10 $\mu\text{g/mL}$ mixed standard was prepared in acetonitrile from dilution of the 100 $\mu\text{g/mL}$ mixed standard. Standards were stored in amber vials and flasks at 5–10 °C.

Standard Solutions (HPLC Calibration). Calibration standards in the range 0.2–10 $\mu\text{g/mL}$ were prepared by serial dilution from the 100 $\mu\text{g/mL}$ mixed standard. Calibration standards were prepared weekly in 1/9 ACN/ H_2O or in HPLC diluent. Standards were stored in amber vials and flasks at 5–10 °C.

Apparatus and Instrumentation. Turbopap II and Turbopap LV evaporators were from Caliper Technologies (Hopkinton, MA). OASIS SPE columns, 6 mL, 0.2 g, were from Waters (Milford, MA). The HPLC system was an Alliance 2695 Solvent Module and 2996 Photodiode Array Detector from Waters. The column was an Xterra RP18, 15 cm \times 3.2 mm, 5- μm packing from Waters. The guard column was a Sentry Xterra RP18, 2 cm \times 3.2 mm, 3- μm packing from Waters.

Sample Preparation. Extraction. The 5-g sample for milk and skim milk was weighed into a 125-mL separatory funnel. Cream was heated to 50 or 60 °C in a water bath for 10–15 min, a 2.5-g aliquot was weighed into the separatory funnel, and 2.5 g of heated (50 or 60 °C)

water was added. The sample was shaken for 30 s with 10 mL of (MB + EDTA), then with 40 mL of 50/50 ACN/ H_2O . The sample was partitioned with 25 mL of hexane for 1 min. The aqueous phase was concentrated to a volume of about 28 mL in a Turbopap II 50-mL tube at temperature 40 °C with nitrogen pressure gradually increased from 4 to 12 in. during the course of evaporation.

Solid-Phase Cleanup. The SPE column was conditioned with 1 mL of ACN followed by 1 mL of H_2O , and a 60-mL reservoir was affixed to the column. Immediately prior to SPE cleanup, the sample was treated with 0.5 mL of NH_4OH . The basified (pH 10) solution was repeatedly withdrawn and dispensed on the solid precipitate formed from concentration of the aqueous phase. After dissolution of the precipitate, the sample was applied to the 60-mL reservoir. The 50-mL tube was rinsed with 5–10 mL of water, and the rinse water was added to the reservoir. The aqueous solution was passed through the SPE columns at a rate of about 1–2 drops/s. Vacuum was increased to about 12 in. for 1 min after the last drop eluted. The SPE column was washed with 3 mL of H_2O and then with 1 mL of 5/95 ACN/ H_2O . Vacuum was increased to about 12 in. for 1 min after the last drop eluted after each rinse. The SPE column was transferred to a clean SPE manifold and eluted with 2.2 mL of SPE eluent into a 12 mm \times 75 mm glass culture tube. The culture tube was removed, and a vacuum of 10–12 in. was applied to the SPE column for 20 min. The culture tube containing the sample was placed back into the SPE manifold. The column was eluted with 1.1 mL of SPE eluent. Vacuum was increased to about 12 in. for a few seconds after both elution steps.

Aqueous-Phase/Protein Removal. An aqueous surface layer containing protein was removed from the SPE eluent as follows. A volume of 10 μL of 10% NH_4OH was added to the sample and followed by 10 s of vortexing. A disposable pipet was fitted with a small plug of cotton and a 2–3-cm layer of anhydrous sodium sulfate. The pipet was rinsed with 1 mL of DCM. The aqueous layer and some of the SPE eluent were withdrawn off the top of the SPE eluent with a disposable pipet. The withdrawn solution was filtered through anhydrous sodium sulfate back into the culture tube containing the sample. The sodium sulfate/pipet was rinsed with 0.5 mL of SPE eluent into the sample.

Evaporation and Dilution for HPLC. The sample was vortexed with 50 μL of ACN and then with 25 μL of 6 N HCl for several seconds. The acidified SPE eluent was evaporated to near dryness (25 μL or less) in a Turbopap LV unit, temperature 40 °C, nitrogen pressure about 7 in. The sample was diluted with 1 mL of HPLC diluent and filtered through a 0.2- μm nylon syringe filter into an autosampler vial.

HPLC Analysis. A gradient mobile phase at flow rate 0.5 mL/min and temperature 35 °C was used with the Xterra RP-18 column. Mobile phase A was 14/86, v/v, ACN/ammonium bicarbonate buffer, pH 9.8, 10 mM. Mobile phase B was 59/41, v/v, ACN/ammonium bicarbonate buffer, pH 9.8, 10 mM. Mobile phase A was pumped for 2 min, followed by a linear 15-min gradient to mobile phase B. Mobile phase B was pumped for 5 min, followed by a step gradient to mobile phase A and 6 min of column conditioning. Injection volume was 60 μL . The PDA detector monitored 260 nm for nicotine and strychnine, and 232 nm for aconitine. At the end of each sample set, the column was washed with 10/90 methanol/water and 80/20 methanol/water, and an injection of 60 μL of water was made to flush the autosampler needle. Blanks and calibration standards in the range 0.2–10.0 $\mu\text{g/mL}$ were injected to generate calibration curves, followed by injection of samples. Standards were also injected periodically between samples and at the end of the sample set. Empower data system (Waters) was used to calibrate and quantitate nicotine, strychnine, and aconitine levels in ppm. Excel spreadsheet software was used to calculate recoveries, means, standard deviations, and relative standard deviations (RSD).

Method Validation Experiments. Milk and skim milk samples were spiked at 0.1, 1.0, and 10 ppm by adding aliquots of the 10 $\mu\text{g/mL}$ or 100 $\mu\text{g/mL}$ mixed standards. Cream was spiked at 0.2, 0.4, 1.0, 2.0, 10.0, and 20.0 ppm. Method validation was usually performed on sets of 6–8 samples. Control samples were run concurrently with spiked samples.

RESULTS AND DISCUSSION

Figure 1a shows chromatograms of (a) diluent blank, (b) 0.2 $\mu\text{g/mL}$, and (c) 1.0 $\mu\text{g/mL}$ calibration standard acquired at

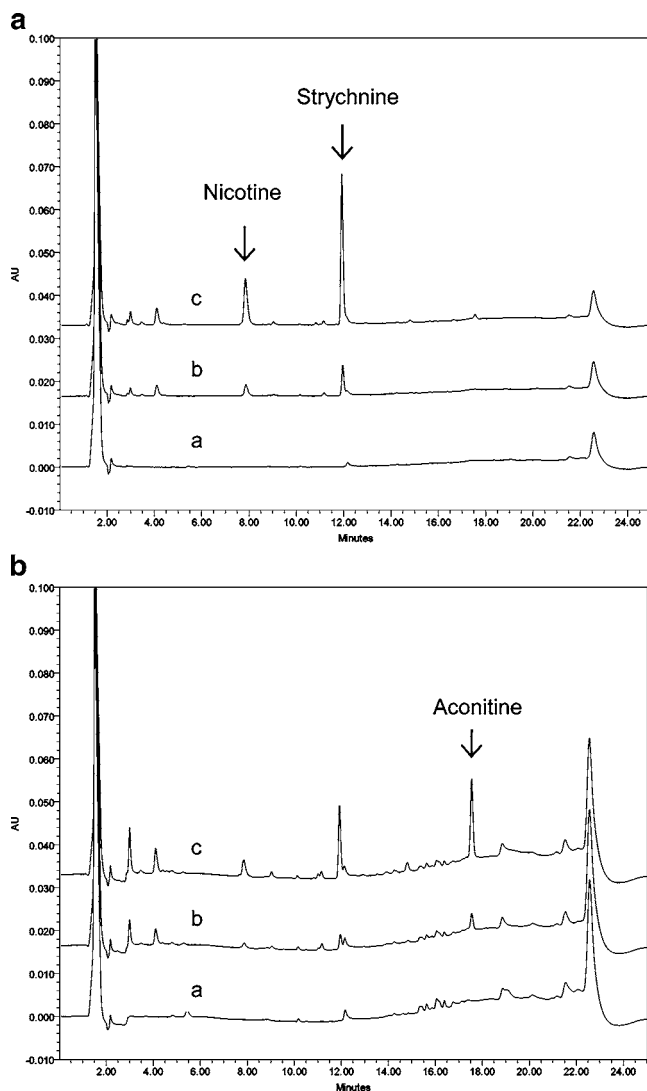


Figure 1. (a) Nicotine and strychnine calibration standards at 260 nm: chromatograms of (a) diluent blank, (b) 0.2 $\mu\text{g/mL}$, (c) 1.0 $\mu\text{g/mL}$. (b) Aconitine calibration standards at 232 nm: chromatograms of (a) diluent blank, (b) 0.2 $\mu\text{g/mL}$, (c) 1.0 $\mu\text{g/mL}$.

260 nm. Nicotine and strychnine were quantitated on the 260-nm data channel. The small peak which elutes after strychnine originated in the mobile-phase gradient; the magnitude of this peak was reduced when higher-grade ammonium bicarbonate was used for the mobile phase. The nicotine peak was somewhat broad when injected in HPLC diluent. The nicotine peak was sharper when injected in 1/9 ACN/H₂O. Most of the validation samples were calibrated with standards prepared in HPLC diluent in order to give similar peak shapes for nicotine. **Figure 1b** shows chromatograms of (a) diluent blank, (b) 0.2 $\mu\text{g/mL}$, and (c) 1.0 $\mu\text{g/mL}$ calibration standard acquired at 232 nm for aconitine.

Calibration curves with $1/x$ weighting were linear in the range 0.2–10 $\mu\text{g/mL}$, ($r^2 > 0.999$).

Figure 2a shows chromatograms of whole milk (a) control, (b) 0.1 ppm spike, and (c) 1.0 ppm spike acquired at 260 nm. **Figure 2b** shows chromatograms of whole milk (a) control, (b) 0.1 ppm spike, and (c) 1.0 ppm spike acquired at 232 nm. The rising baseline near the strychnine retention window is likely to be protein and is more prominent in the 232-nm channel. When strychnine is quantitated on the 260-nm channel, the protein interference is minimized.

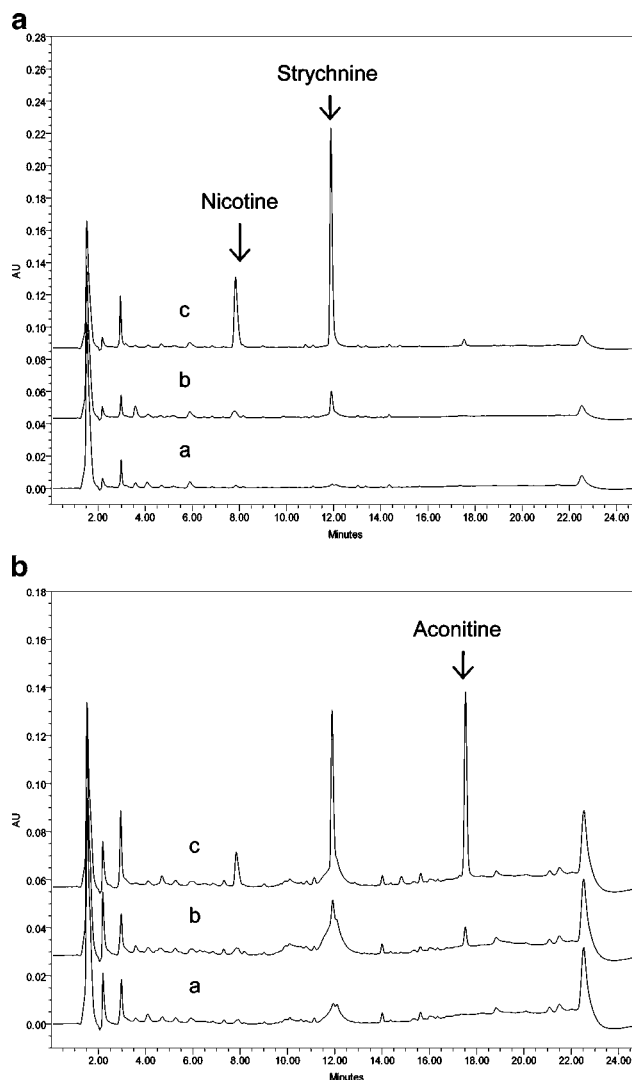


Figure 2. (a) Whole milk samples, nicotine and strychnine at 260 nm: chromatograms of (a) control, (b) 0.1 ppm spike, (c) 1.0 ppm spike. (b) Whole milk samples, aconitine at 232 nm: chromatograms of (a) control, (b) 0.1 ppm spike, (c) 1.0 ppm spike.

Figure 3a shows chromatograms of skim milk (a) control, (b) 0.1 ppm spike, and (c) 1.0 ppm spike acquired at 260 nm. **Figure 3b** shows chromatograms of skim milk (a) control, (b) 0.1 ppm spike, and (c) 1.0 ppm spike acquired at 232 nm. The analyte retention windows are very clean at both wavelengths. A very small peak elutes immediately after strychnine in the 260-nm chromatogram, but it is baseline-resolved from strychnine.

Figure 4a shows chromatograms of cream (a) control, (b) 0.2 ppm spike, and (c) 2.0 ppm spike acquired at 260 nm. **Figure 4b** shows chromatograms of cream (a) control, (b) 0.2 ppm spike, and (c) 2.0 ppm spike acquired at 232 nm. Analyte retention windows again are clean. A small peak elutes after strychnine in the 260-nm chromatogram. The magnitude of this peak is much smaller than the strychnine peak in the 0.2 ppm spike chromatogram (**Figure 4a**, chromatogram b), and it does not adversely affect integration of the strychnine peak.

Tables 1–3 summarize recovery data for whole milk, skim milk, and cream, respectively. The tables include validation data from raw and pasteurized samples. In general, there was little difference in the chromatographic behavior between raw and pasteurized samples. In most cases, recoveries were slightly lower for the low-level spikes (0.1 and 0.2 ppm) compared to

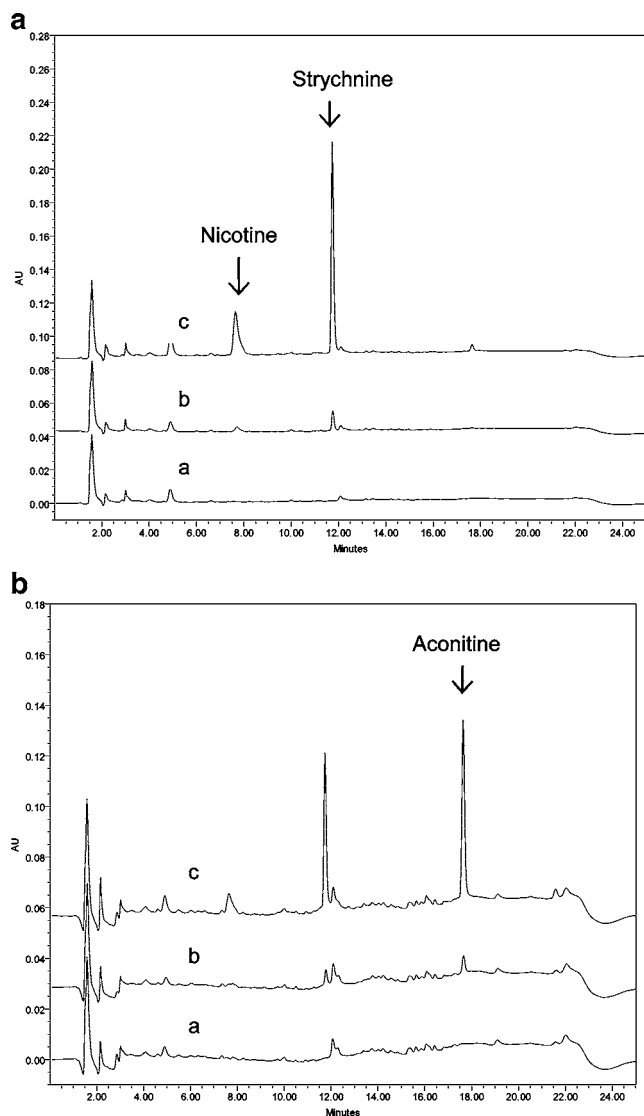


Figure 3. (a) Skim milk samples, nicotine and strychnine at 260 nm: chromatograms of (a) control, (b) 0.1 ppm spike, (c) 1.0 ppm spike. (b) Skim milk samples, aconitine at 232 nm: chromatograms of (a) control, (b) 0.1 ppm spike, (c) 1.0 ppm spike.

samples spiked at higher levels. **Table 4** shows individual recoveries grouped by fortification level for whole milk. Single-factor ANOVA of this data showed no significant difference in recovery between spike levels at $\alpha = 0.05$, $F_{crit} = 4.737$. Too few replicates of high-level spikes were run to enable ANOVA to be performed for skim and cream spike levels. The mean and RSD of three recoveries for aconitine, nicotine, and strychnine in skim milk at the lower limit of method validation (LLOMV) were 84.7 ± 8.9 , 67.0 ± 18.1 , and 77.0 ± 6.0 , respectively. The mean and RSD of five recoveries for aconitine, nicotine, and strychnine at the LLOMV in cream were 79.5 ± 12.5 , 94.1 ± 23.5 , and 70.8 ± 19.5 , respectively.

Cream is a difficult matrix to analyze due to high fat content; heavy cream contains at minimum 36% fat by weight (29). We did not find references on analysis of cream for contaminants such as those studied here. The hexane partition employed in the method removed lipid matrix components. The defatted cream samples were readily passed through the SPE columns. Recoveries of aconitine, nicotine, and strychnine from cream were generally as good as recoveries from whole and skim milk. Single-factor ANOVA of mean overall recovery for each analyte between matrices showed no significant difference for aconitine

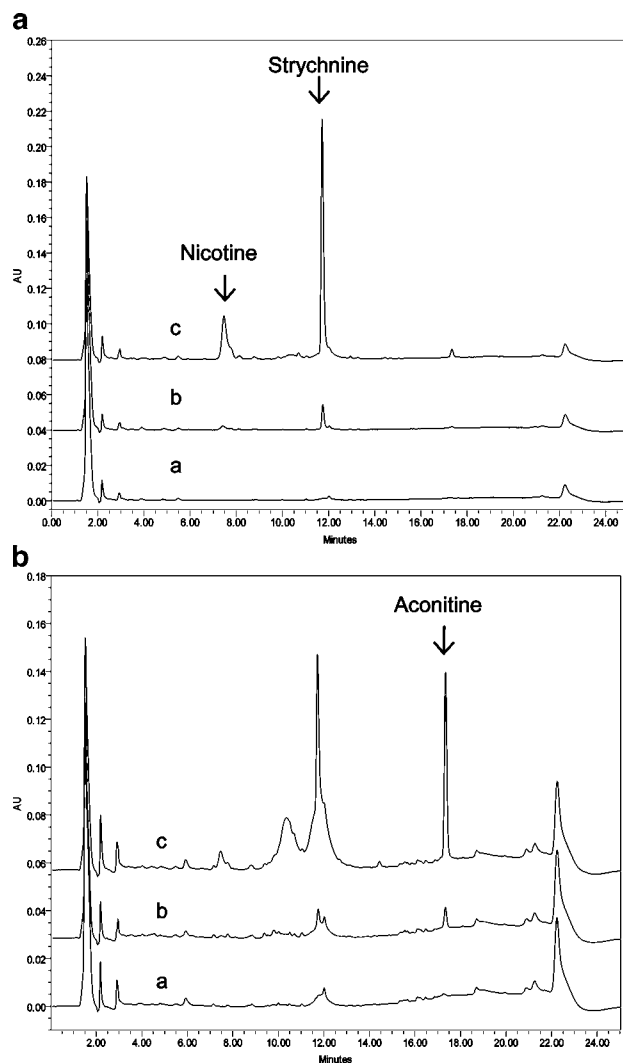


Figure 4. (a) Cream samples, nicotine and strychnine at 260 nm: chromatograms of (a) control, (b) 0.2 ppm spike, (c) 2.0 ppm spike. (b) Cream samples, nicotine and aconitine at 232 nm: chromatograms of (a) control, (b) 0.2 ppm spike, (c) 2.0 ppm spike.

Table 1. Summary of Validation: Recovery of Nicotine, Strychnine, and Aconitine from Whole Milk Spiked between 0.1 and 10.0 ppm

analyte	N	mean recovery %	RSD %	range
nicotine	10	89.2	9.0	79.0–104.2
strychnine	10	75.7	16.2	59.0–103.1
aconitine	10	85.1	14.9	73.0–118.1

Table 2. Summary of Validation: Recovery of Nicotine, Strychnine, and Aconitine from Skim Milk Spiked between 0.1 and 10.0 ppm

analyte	N	mean recovery %	RSD %	Range
nicotine	7	72.1	14.1	56.0 – 85.3
strychnine	7	78.2	4.0	72.0 – 81.0
aconitine	7	82.9	10.1	70.2 – 89.9

and strychnine, $\alpha = 0.05$, $F_{crit} = 3.368$. Recovery of nicotine did show significant difference between matrices, $F = 6.129$.

Recoveries were corrected for background found in concurrently analyzed control samples. In some control samples, small matrix background peaks were detected in the retention window of nicotine. These control nicotine values were subtracted from the nicotine quantitated in spiked samples. Most of the nicotine

Table 3. Summary of Validation: Recovery of Nicotine, Strychnine, and Aconitine from Cream Spiked between 0.2 and 20.0 ppm

analyte	N	mean recovery %	RSD %	Range
nicotine	12	88.0	18.0	69.8 – 121.5
strychnine	12	76.8	14.4	53.0 – 95.0
aconitine	12	82.0	10.9	63.0 – 101.0

Table 4. Method Validation: Recovery of Nicotine, Strychnine, and Aconitine from Whole Milk Samples on Two Separate Days, Spiked between 0.1 and 10.0 ppm

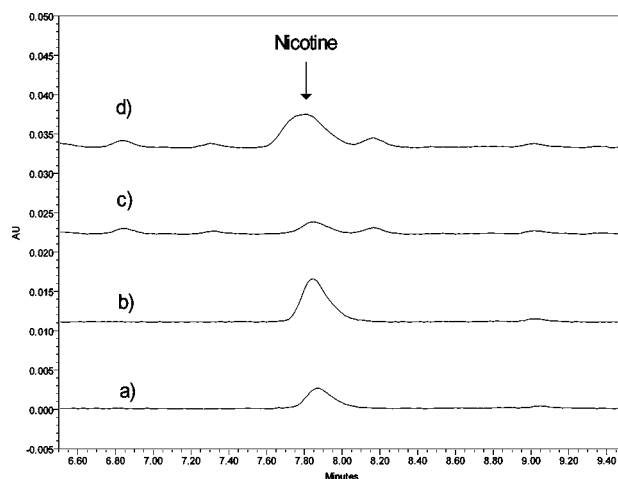
sample ID	spike level (ppm)	aconitine (%)	nicotine (%)	strychnine (%)
1015-2	0.1	73.0	91.0	60.0
1015-3	0.1	75.0	79.0	59.0
1117-2	0.1	83.0	93.0	78.0
1117-3	0.1	87.0	100.0	78.0
	mean	79.5	90.8	68.8
	RSD	8.3	9.6	15.5
1015-4	1.0	76.7	81.1	69.3
1117-4	1.0	86.6	86.8	79.0
1117-5	1.0	87.3	86.0	80.5
	mean	83.5	84.6	76.3
	RSD	7.1	3.6	8.0
1015-5	10.0	82.9	84.5	74.8
1015-6	10.0	81.6	86.0	75.4
1117-6	10.0	118.1	104.2	103.1
	mean	94.2	91.6	84.4
	RSD	22.0	12.0	19.1

background levels were in the range of 0.01–0.02 ppm. Chromatographic background interference due to sample matrix was minimized when the protein filtration step was added to the method.

Recovery of aconitine was improved significantly by using two SPE elution steps separated by a drying step. When a single elution with 3 or 4 mL of SPE elution solvent was used instead, aconitine recoveries were in the 40–50% range. Nicotine recovery was not reproducible until the volume of sample concentrated after the hexane partition was consistently reduced to under 30 mL. Good recoveries of strychnine and aconitine were obtained in samples concentrated to volumes higher than 30 mL, but nicotine recovery was often low (less than 50%) in these samples.

The 0.1 ppm spike for whole and skim milk is the lower limit of method validation (LLOMV). This was the lowest spike level run with the method. The 0.1 ppm sample fortification is equivalent in peak size to the 0.5 $\mu\text{g}/\text{mL}$ standard. The lowest-level calibration standard injected was 0.2 $\mu\text{g}/\text{mL}$. The signal-to-noise (S/N) ratios of analyte peaks in the 0.2 $\mu\text{g}/\text{mL}$ standard (**Figure 1**, parts **a** and **b**, chromatogram **b**) were nicotine 23, strychnine 64, and aconitine 41. These values are well above the generally accepted ratio of 10 for limit of quantitation. **Figure 5** shows an enlarged view of the nicotine peaks in (a) 0.2 $\mu\text{g}/\text{mL}$ standard, (b) 0.5 $\mu\text{g}/\text{mL}$ standard, (c) whole milk control, and (d) whole milk 0.1 ppm spike from a validation set of pasteurized whole milk. The peaks in the control and spiked samples represent 0.018 and 0.118 ppm of nicotine, respectively. The background-corrected nicotine recovery in the spiked sample was 100%.

The practical limit of quantitation used to measure background residues was set to 0.01 ppm on the basis of the calibration standard S/N and typical chromatographic background interference encountered. The LLOMV for cream is 0.2 ppm since a sample of smaller sample size (2.5 g) was used for cream. We did not attempt to measure recoveries at lower levels

**Figure 5.** Nicotine at 260 nm: chromatograms of (a) 0.2 g/mL, (b) 0.5 $\mu\text{g}/\text{mL}$; (c) whole milk control; (d) whole milk spiked at 0.1 ppm.**Table 5.** Retention Times of Nicotine, Strychnine, and Aconitine

set	mean retention time (min) + RSD		
	nicotine	strychnine	aconitine
1	7.81 + 0.42%	11.89 + 0.32%	17.53 + 0.08%
2	7.57 + 1.51%	11.74 + 0.18%	17.34 + 0.06%
3	7.56 + 1.49%	11.57 + 0.18%	17.42 + 0.07%

in any of the matrices. Background interference and broad peak shape make it difficult to lower the LLOMV for nicotine. The goal of this method was to measure analyte residues at LLOMV values of 0.1–0.2 ppm. Additional recoveries and controls need to be analyzed to establish a method LOD (limit of detection) and LOQ (limit of quantitation) based on more rigorous methodologies (30, 31).

HPLC-UV methods rely on retention time to identify components. It is essential that retention time be highly reproducible for HPLC-UV methods. **Table 5** shows mean retention times in minutes and RSD values for three analytical sets of 15–20 injections. The highly reproducible retention times allow narrow retention windows to be used with the data system. Narrow retention windows reduce the chance that background peaks are identified as an analyte. Over 1000 injections were made on the analytical column for this study; the guard column was changed one time. Very little, if any, loss of chromatographic resolution and efficiency was observed through the course of the study. Ion-pairing mobile phases are frequently used for HPLC analysis of nicotine. These mobile phases can present special problems with artifact peaks, slow column equilibration, and poor peak shape (32). Even under optimized ion-pair conditions, nicotine can exhibit a broad shape (33). The absence of ion-pairing reagents from the mobile phase allows the method described in this work to be adapted for LC-MS.

The method described should be useful for measurement of other nitrogen alkaloids in milk, provided they have basic pK_a values and some UV absorbance. The OASIS HLB SPE columns retained nicotine, strychnine, and aconitine at pH 10, and they afforded significant sample cleanup prior to HPLC. The method will be used to measure the partitioning of nicotine, strychnine, and aconitine into cream and milk phases after addition to raw milk. Additional sample analyses are required to validate this method according to rigorous standards (31). However, this procedure serves as a good model for those who need to analyze dairy matrices for basic nitrogen alkaloids by HPLC without ion-pairing reagents.

SAFETY

Nicotine, strychnine, and aconitine are extremely toxic. Wear gloves and perform transfer of the neat compound in a hood. Crystalline strychnine and aconitine can develop static charges. Treat glassware and utensils for transfer and weighing of solid strychnine and aconitine with antistatic devices.

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