Basic Drug Screen and Quantitation of Five Toxic Alkaloids in Milk, Chocolate Milk, Orange Juice, and Blended Vegetable Juice

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A convenient and general method for the determination of alkaloids in processed foods and a screen for basic drugs is presented. The food matrix is removed by mixing the sample with a buffered ion-pair reagent followed by centrifugation. The alkaloids are solid phase extracted from the supernatant. The extracted alkaloids are eluted and then analyzed by HPLC using reversed phase ion-pair chromatography. The run is isocratic. A photodiode array detector allows the simultaneous collection of signals at optimum wavelengths for a series of alkaloids in the same sample. The mean percent recovery across five alkaloids in four matrices was 87%.

Keywords: *Foods; alkaloids; solid phase extraction; HPLC*

INTRODUCTION

The alkaloids are organic–nitrogenous bases. Most are produced by plants, but some are produced by microorganisms and animals (Geissman and Crout, 1969). The remarkable physiological properties of particular alkaloids have made them valuable therapeutics (Geissman and Crout, 1969; Goodman and Gilman, 1975). For instance, eserine (physostigmine) is used in ophthalmology and as an antidote for tricycline antidepressant intoxication (Goodman and Gilman, 1975; Wu et al., 1986). Cinchonidine has been used for the treatment of malaria and fever (Goodman and Gilman, 1975). Historically, even cocaine and strychnine were used in medicine. Cocaine was used as a local anesthetic and the latter as a tonic to improve muscle tone and circulation (Goodman and Gilman, 1975; Hawley, 1981; Baselt and Cravey, 1989).

Alkaloids are also known for their acute toxicity (Geissman and Crout, 1969; Goodman and Gilman, 1975; Wu et al., 1986; Hawley, 1981; Baselt and Cravey, 1989). Alkaloid misuse has led to numerous fatalities (Apple and Roe, 1990). Eserine, nicotine, and strychnine are classified as supertoxic (Gosselin et al., 1984). A fatal dose of nicotine or strychnine is between 15 and 100 mg (Goodman and Gilman, 1975; Hawley, 1981; Baselt and Cravey, 1989; Moffat, 1986; Dreisbach, 1980). A lethal dose of eserine it is only a few milligrams (Gosselin et al., 1984). Cocaine, the most potent of the naturally occurring central nervous stimulators (Hawley, 1981; Baselt and Cravey, 1989), can kill by an ingestion of between 1000 and 1500 mg (Hawley, 1981; Baselt and Cravey, 1989; Apple and Roe, 1990; Moffat, 1986). The legal limit for cinchonidine has been set at 83 μg/g in foods (Furia, 1986), but a dose of 30 mg can, in sensitive individuals, produce headache, dyspnea, nausea, and diarrhea (Dreisbach, 1980).

In practical situations, man has been adding chemicals to foods throughout history for a variety of reasons. One was to preserve, but, unfortunately, other reasons were to swindle and cause physical harm (Doul et al., 1980). The addition of these compounds to processed foods, whether by accident or by a willful illicit act, can cause harm under specified conditions. One of the major efforts of our laboratory is to detect these additions. Analytical screens that can detect multiple analytes in one assay (Logan et al., 1990) are vital for rapid laboratory response. The following procedure is presented as a screen for basic drugs. Foods were selected to present a broad spectrum of analytical matrices. Nicotine, strychnine, eserine, cinchonidine, and cocaine were used during method development.

High-performance liquid chromatography (HPLC) is a proven technique for the analysis of alkaloids (Jacobus et al., 1985; Saito et al., 1989; Roos and Lau-Cam, 1986). Reversed phase HPLC in the isocratic mode maintains constant analytical parameters with maximum sample throughput. This coupled with C_{18} SPE to remove sample matrix combined with diode array detection means basic substances such as nicotine, eserine, strychnine, cocaine, and cinchonidine in 3% milk, chocolate milk, and orange and blended vegetable juice can be measured at harmful levels.

EXPERIMENTAL PROCEDURES

Instrumentation used included the following: Waters Model 602 solvent delivery system, Model 991 diode array/HPLC detector, Model 715 sample processor; NEC PC with Waters LCA-6.22a-01EF 34-53 software; Zymark Benchmate workstation.

Reagents and Materials. A 1:1 mixture of 2-propanol and methanol and a 4:1 mixture of methanol and distilled water were used. All solvents were of HPLC grade or better. Ion-pairing reagent (IPR) was an aqueous solution containing sodium octyl sulfonate (0.01 M) and citric acid (0.05 M), adjusted to pH 3.00 with dilute potassium hydroxide. The HPLC mobile phase was 55% IPR and 45% methanol.

Stock standards were 2.00 mg/mL. (Caution: Alkaloids are toxic substances. Do not breathe, ingest or have skin contact. Reduce the risk of skin contact by wearing gloves. Prepare all stock solutions in a hood.) Stock solutions were prepared by dissolving 0.108 g of nicotine sulfate solution (37% solution), 0.040 g of eserine, 0.0473 g of strychnine hemisulfate, 0.0450 g of cocaine hydrochloride, and 0.0450 g of cinchonidine hydrochloride in methanol (20 mL). The alkaloids were obtained from Sigma Chemical Co, St. Louis, MO. The standard solutions used for calibration were prepared by diluting the stock solutions (2.00 mg/mL) of nicotine, eserine, strychnine, cocaine, and cinchonidine in methanol. Five dilutions were prepared for each alkaloid. The nominal concentrations were 5, 40, 80, 120, and 160 μg/mL. For each standard solution, five replicate injections were made and analyzed under stated analytical conditions.

Spiked samples were prepared in chocolate milk, orange juice, blended vegetable juice, and 3% milk that were obtained from a local grocery. Fifteen milliliters of juice or milk was

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Table 1. Linear Regression of Data Obtained from Standards Analyzed in Methanol

alkaloid	λ (nm)	m [AU/(μg/mL)]	b (AU)	SE intercept	correl coeff
cinchonidine	232	0.00098	−0.00071	0.000896	0.998
cocaine	232	0.00091	−0.00032	0.000907	0.999
eserine	246	0.00060	−0.000306	0.00047	0.999
nicotine	259	0.00039	−0.00197	0.000466	0.997
strychnine	254	0.00065	−0.0004	0.000604	0.998

Table 2. Percent[a] of Alkaloid Spike Recovered from 3% Milk, Chocolate Milk, Orange Juice, and Blended Vegetable Juice (40 μg/mL Spikes)

alkaloid	3% milk	chocolate milk	orange juice	vegetable juice
cinchonidine	91	70	95	89
cocaine	97	85	99	81
eserine	94	93	97	95
nicotine	94	84	86	95
strychnine	91	73	105	83

[a] Mean of five replicate determinations.

Table 3. Percent[a] of Alkaloid Spike Recovered from 3% Milk, Chocolate Milk, Orange Juice, and Blended Vegetable Juice (200 μg/mL Spikes)

alkaloid	3% milk	chocolate milk	orange juice	vegetable juice
cinchonidine	81	68	91	88
cocaine	87	78	95	87
eserine	86	80	97	95
nicotine	78	76	89	89
strychnine	83	65	93	86

[a] Mean of five replicate determinations.

Table 4. Precision of Recovery Data Expressed as Percent Relative Standard Deviation (n = 10)

alkaloid	3% milk	chocolate milk	orange juice	vegetable juice
cinchonidine	2.5	3.5	3.7	8.9
cocaine	1.6	2.6	4.5	9.1
eserine	2.1	1.4	6.0	8.3
nicotine	2.8	5.5	2.0	9.1
strychnine	1.4	1.7	4.2	9.2

spiked with 1.50 or 0.30 mL of stock standard. The spike levels were 200 or 40 μg/mL of nicotine, eserine, strychnine, cocaine, and cinchonidine. Five replicates were prepared at each spiking level.

The alkaloids were extracted from the spiked samples using this process. Juice or milk (2 mL) was added to a 20 mL plastic scintillation vial. IPR, 8 mL, was added, and the vial's contents were shaken vigorously for 30 s. The vial was centrifuged for 10 min at 7000 rpm and the supernatant decanted. Occassionly, some solids did remain in the supernatant. In those cases, it was necessary to pass the supernatant through a 0.45 μm filter. The SPE steps were conducted using the Zymark BenchMate workstation. A Bond Elute plastic SPE column (C_{18}, 3 cm^3/500 mg) (Varian, Harbor City, CA) was conditioned by passing through the column in sequence the following reagents: methanol (5 mL), distilled water (5 mL), and IPR (10 mL). The supernatant was passed through the conditioned extraction column, and then the column was washed with distilled water (10 mL). The excess moisture was removed by blowing air through the column for 5 min. The column was then rinsed with isooctane (1 mL), and the residual isooctane was expelled by passing air through the column for 2 min. The extracted alkaloids were eluted from the column by introducing 1 mL of methanol/2-propanol (1:1), followed by 2 mL of methanol/water (4:1). The combined column eluate was diluted to 3 mL with methanol. The sample was then chromatographed, and the alkaloid levels were measured.

Instrumental Conditions. The HPLC column was a Supelco reversed phase microparticulate silica base with a 12−13% carbon loading (pKb-100), particulate size −5 μm, 4.6 × 250 mm. The mobile phase flow rate was 1 mL/min. The injection volume was 20 μL. The diode array detector was set to monitor wavelengths between 230 and 300 nm.

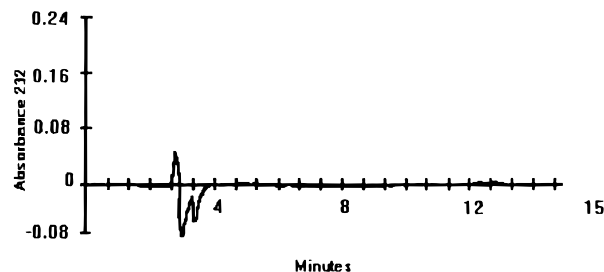

Figure 1. Chromatogram of unspiked 3% milk at 232 nm.

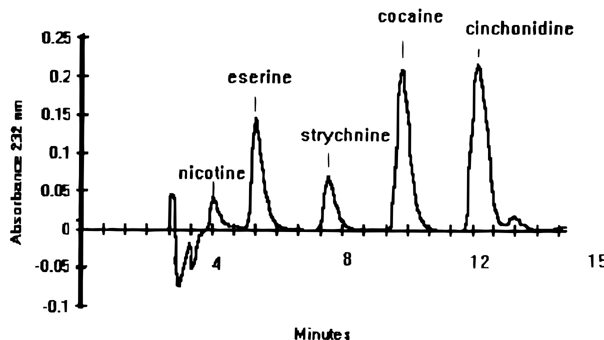

Figure 2. Chromatogram of alkaloid-spiked 3% milk at 232 nm (200 μg/mL).

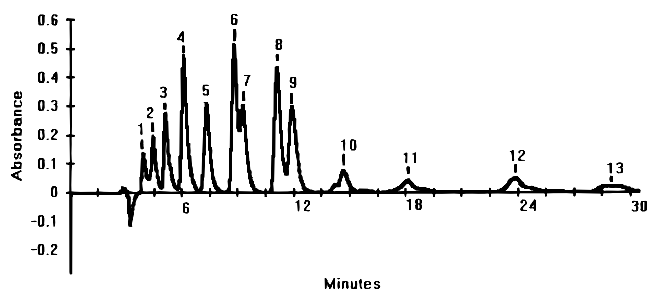

Figure 3. Thirteen alkaloids chromatographed at 232 nm: (elution order 1−13) nicotine, anabasine, eserine, colchicine, strychnine, thebaine, cocaine, berberine, cinchonidine, yohimbine, lobeline, emetine, and aconitine.

Table 5. Mean Percent Recovery of Alkaloids from Processed Squid (40 μg/g Spike)

alkaloid	no. of spikes	% recovered	alkaloid	no. of spikes	% recovered
nicotine	4	90	colchicine	3	70
strychnine	5	65	lysergic acid	3	65
cocaine	3	82	thebaine	3	75
eserine	3	78	berberine	3	30

RESULTS AND DISCUSSION

This procedure offers the application of SPE and ion-pair reversed phase HPLC to the analysis of alkaloids in processed foods. Sample preparation is rapid and direct because it is conducted in an aqueous environment without the need for a liquid−liquid extraction. Mixing with a buffered ion-pair reagent, followed by centrifugation and solid phase extraction, is all that is required to remove the alkaloids from the matrix. Each chromatographic run was isocratic and required only 15 min. Standard curves were constructed by applying linear regression to data obtained by chromatographing the standard solutions for each alkaloid. The properties

Table 6. Range of Retention Times For Alkaloids and Basic Compounds Successfully Chromatographed under Stated Condition

3–5 min	5–7 min	7–9 min	9–11 min	16–30 min
arecoline	nortriptyline	papaverine	theobromine	ergotamine
codeine	brucine	atropine	clenbuterol	aconitine
heroin	ephedrine	scopolamine	berberine	emetine
morphine	selegiline	pentazocine		
oxymorphone	procaine			
caffeine	procainamide			
nornicotine				

of each alkaloid's standard curve are listed in Table 1. The detection limit, defined as the mean square error divided by the slope, is 3 μg/mL for nicotine, eserine, cocaine, and cinchonidine, but for strychnine the limit of detection is 5 μg/mL. The limit of quantitation is defined as twice the detection limit.

The versatility of the method is demonstrated by its ability to measure the concentration of five alkaloids in four different processed foods with widely different matrices at levels between 6 and 200 μg/mL. Tables 2 and 3 show spike recovery data obtained from the analyses of samples at low-spike (40 μg/mL) and high-spike (200 μg/mL) levels. The data in Tables 2 and 3 show that in most cases the extraction removes >80% of nicotine, eserine, strychnine, cocaine, and cinchonidine that was spiked into milk, chocolate milk, orange juice, and blended vegetable juice. The exceptions are strychnine and cinchonidine in chocolate milk. The mean recovery is 69%. The explanation may be in the character of chocolate, which is a mixture of a lipophilic compounds. The food's fat content seems to reduce alkaloid recovery. This conclusion is based on observations for spiked 3% milk, for which initial recoveries were only 50–60%. An isooctane column wash improved alkaloid recovery for 3% milk to the levels reported. However, increasing the isooctane wash volume for alkaloid-spiked chocolate milk did not improve recoveries, but actually reduced them. For nicotine, strychnine, cocaine, cinchonidine, and eserine in orange or vegetable juice and in 3% milk, the suggested (1 mL) isooctane wash was necessary for maximum alkaloid recovery. We have not determined what sample fat content can be tolerated, but this study showed that 3% milk presented no special problems.

Table 4 shows the precision data expressed as relative standard deviation (RSD). All RSDs are 6% or less except for vegetable juice, which has RSDs from 8.3 to 9.2%. The cause may be coextractables in the matrix, the extraction of which may be more variable than that of the alkaloids. The coextractables produce variable chromatographic baselines and detector noise which are manifested as greater RSD.

The analysis of a control 3% milk is presented as an example (Figure 1). Notice that from 4 to 15 min after injection there is a clear analytical window void of prominent peaks even at 232 nm. This is also true for the wavelengths at 254 and 280 nm. Therefore, wavelengths between 230 and 300 nm could be used to monitor alkaloid levels. Thus, nicotine (RT ~ 4.00 min), eserine (RT ~ 5.15 min), strychnine (RT ~ 7.50 min), cocaine (RT ~ 10.00 min), and cinchonidine (RT ~ 11.85 min) can be analyzed in a clear analytical window (Figure 2).

Under the same chromatographic conditions, other alkaloids can be chromatographed and their levels monitored at wavelengths between 230 and 300 nm. Figure 3 is a chromatogram of a solution containing 13 alkaloids in methanol. The alkaloids in solution were nicotine, anabasine, colchicine, eserine, thebaine, strychnine, cocaine, papaverine, berberine, cinchonidine, yohimbine, lobeline, aconitine, and emetine. This procedure has also been used to measure other alkaloids in other matrices different from those presented here. Table 5 lists eight alkaloids and associated spike recoveries from processed squid matrix. Table 6 lists additional compounds, and approximate retention times, that have been chromatographed under the same instrumental conditions.

This procedure is offered as an opportunity to screen and measure basic drugs and alkaloids in some common processed foods. The method has demonstrated acceptable precision and accuracy for our purposes for the compounds and matrices studied. The method could be automated since all analysis parameters remain fixed. This analysis provides UV spectra between 230 and 300 nm and retention time data. The combination of retention times and UV spectra has proven to be a useful identification marker for sample screens. The procedure could be applied to other basic compounds and matrices.

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