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# Detection of alkaloids in foods with a multi-detector high-performance liquid chromatographic system

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ABSTRACT

A general screening method for alkaloid drugs in foods is described based on high-performance liquid chromatography with ultraviolet detection at three wavelengths, followed by fluorescence and electrochemical detectors in series. The chromatographic conditions include an ion-pairing reagent, which makes it possible to chromatograph acidic and basic drugs with one screen. Relative response ratios were determined from the peak areas of the alkaloids on the basis of all the detector signals. These ratios were used to create a "fingerprint" of the drugs and to predict the identity of an unknown component in a sample matrix. The fluorescence and electrochemical detectors allowed a detection limit for many of the alkaloids which would not be attainable with the ultraviolet detector alone. Typical detection limits with the electrochemical detector were 5-50 ng/ml and with the fluorescence detector 5–500 ng/ml, while the ultraviolet detector had detection limits of l-20 $\mu$g/ml. The spiking concentrations in the relative response ratio experiments were approximately five times above the lowest detection limit. The extraction method investigated for orange juice yielded recoveries for most alkaloids of 80–100%. A stability study of ergot alkaloids in various food matrices demonstrated degradation, depending on the matrix, temperature, and duration of the experiment.

## INTRODUCTION

The public food supply must be kept safe from accidental or purposeful contamination during product manufacturing and marketing. In several cases, contamination of a food product has caused serious illnes or death to an unsuspecting consumer. Drugs are likely sources of contamination due to their ready availability. This paper focuses on the extraction of alkaloid drugs from orange juice as part of a screening method for alkaloids in foods. This work includes a study which evaluates the stability of ergot alkaloids in a variety of food matrices. The extracts are analyzed by a high-performance liquid chromatographic (HPLC) system which consists of a UV detector that can detect three wavelengths simultaneously, a fluorescence detector, and an electrochemical detector. The use of multiple detectors has been shown to be effective in other forensic laboratories [1–5].

Determinations in which multi-detector systems are used can potentially generate chemical fingerprints of unknowns by using relative response ratios. This "fingerprint" identification is particularly applicable to drugs because most are amenable to UV, fluorescence, and/or electrochemical detection.

A problem which frequently occurs in a forensic laboratory is sample size limitation. In some cases there is only a small amount of sample to begin with and in other cases, several laboratories may have previously handled the sample, diminishing the amount of sample available. Generally, in order to get a conclusive identification of the sample, multiple analyses must be completed. One approach to decreasing the number of individual sample analy-

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ses and conserving sample is to have multiple detectors on line.

A drug data base has been developed on this system using relative response ratios of the three detectors. Using this method, it is possible to make an early prediction of the identity of an unknown component in a sample before confirmation by mass spectrometry can be completed. For example, a sample contains an unknown peak with a retention time equivalent to that for lysergic acid. However, unlike lysergic acid, the unknown does not demonstrate any electrochemical acitivity. Therefore, lysergic acid could be eliminated as a possible contaminant.

## EXPERIMENTAL

### *Reagents*

All of the alkaloids used in this study were obtained from Sigma. HPLC-grade methanol was obtained from J. T. Baker or equivalent. ACS-grade potassium phosphate monobasic was obtained from Fisher Scientific or equivalent. 1-Octanesulfonic acid was obtained from Sigma. ACS-grade potassium chloride was obtained from Fisher Scientific or equivalent. 85% ACS-grade phosphoric acid was obtained from Fisher Scientific or equivalent.

### *Standards*

Stock standards were prepared in concentrations ranging from *ca.* 5000 to 20 000 $\mu$g/ml. The standards were prepared in an 80% methanol solution and the stock standards stored in the refrigerator. On the day of analysis a working standard was prepared by dilution of the stock standard solution with methanol.

### *Sample preparation*

Sample matrices studied included orange juice, vegetable juice, milk, and a cola beverage. To prepare spikes, 2 ml of sample were placed in a polyethylene tube. The spiking solution, which consists of several alkaloids that produce a final concentration of 30–100 $\mu$g/ml of each alkaloid in the sample, was added. In addition, method blanks were prepared that did not contain any spiking solution.

Preparation of orange juice and vegetable juice samples involved extraction using Analytichem $C_{18}$ solid-phase extraction (SPE) 1-ml cartridges using a Supelco SPE vacuum manifold. To perform the extraction, 2 ml of 0.005 *M* 1 -octanesulfonic acid (1-octanesulfonic acid allowed for better binding of the alkaloids to the SPE column) were added to 2 ml of juice, followed by vortex-mixing on high speed for 1 min. The SPE column was conditioned with 3 ml of methanol, followed by 3 ml of water. The column was not allowed to go to dryness after the methanol and water conditioning. The sample was applied to the SPE column (avoiding the column to go to dryness) and the column was washed with 3 ml of 10% methanol in water. The column was dried for 5 min after the methanol had washed through the column. The sample was eluted with 3 $\times$ 1 ml of methanol and the column was dried for 2 min after the final methanol wash. The sample was filtered with 0.2-$\mu$m filters from Alltech Assoc. A 5-$\mu$l aliquot was injected onto the HPLC column.

Preparation of milk samples first involved precipitation of the proteins with HCl. To 2 ml of milk, 2 ml of 1 *M* HCI were added, followed by vortex-mixing on high speed for I min. The sample was centrifuged for 10 min. Then l ml of 0.005 A4 1-octanesulfonic acid was added to the supernatant. Extraction was carried out as described above for the juices.

SPE was not necessary to clean up the cola sample. A 2-ml sample was mixed with 1 ml of methanol (I-octanesulfonic acid was not used as the sample was not eluted through an SPE column), followed by vortex-mixing, filtering, and injection of 5 $\mu$l of the sample onto the HPLC column.

### *Instrumentation*

A Hewlett-Packard 1050 HPLC system was employed for these analyses. The 1050 included the 1050 autosampler, 1050 quaternary pumping system and the 1050 multiple-wavelength detector. Three signals can be collected from the multiple wavelength detector. The signals that were used for the analysis were 330,280, and 254 nm. In addition to this detector, an HP 1045A programmable fluorescence detector and an HP 1049A programmable electrochemical detector were used. Each of these detectors were set with 1 V full-scale ranges at the factory and those ranges were not changed in this laboratory. The fluorescence detector was operated with an excitation wavelength of 254 nm and an emission wavelength of 408 nm. The slit width of

the excitation wavelength was 2 mm × 2 mm (equivalent to a 25-nm bandwidth) and the slit width of the emission wavelength was 4 mm × 4 mm (equivalent to a 50-nm bandwidth). The detector was set with a PMT gain of 12 and a response time of 6. The electrochemical detector was operated in the amperometric mode at a potential of 1.2 V *versus* an Ag/AgCl reference electrode with a glassy carbon working electrode. The electrochemical detector was equipped with a solvent thermostat that was maintained at 40°C.

The HPCL system was controlled by HP Chem Station Phoenix software. An HP Vectra 386/25 computer was employed. Since the software could not directly take the signals from the fluorescence and electrochemical detectors, an analog-to-digital converter was used for this purpose. The converter was an HP 35900. The data were printed by an HP Laser Jet III printer.

The column, obtained from Interaction Chemicals (pn 50838-l) was a $C_{18}$ 250 × 4.6 mm I.D., column, 5 $\mu$m particle size. A column heater was set at 30°C.

The mobile phase consisted of phosphate buffer, methanol, and 1-octanesulfonic acid as an ion-pairing reagent. To make 1 1 of mobile phase, 2.0414 g of monobasic potassium phosphate (15 m*M*), 0.8112 g of 1-octanesulfonic acid (3.75 m*M*), and 0.5592 g of potassium chloride (7.5 m*M*) were added to 550 ml of methanol (55%) (potassium chloride was added to the mobile phase for the electrochemical detector which uses a solid-state Ag/AgCl reference electrode). Water, from a Millipore Milli-Q water purification system equipped with a final 0.22-$\mu$m filter, was added to get a final volume of 1 1. The solution was adjusted to pH 4.00 with a 10% solution of phosphoric acid using a Corning 245 pH meter, calibrated with pH 4.00 and pH 7.00 buffers (Fisher Scientific). Finally, the solution was filtered with 0.2-$\mu$m filters from Alltech Assoc. During the analysis, the mobile phase was continous sparged with helium. The flow-rate was 1.0 ml/min.

## RESULTS AND DISCUSSION

### *Relative response ratios*

As indicated earlier, relative response ratios can yield a chemical fingerprint. Table I contains a list of alkaloids and their relative response ratios obtained by employing UV detection at three wavelengths (330,280, and 254 nm) and the signals from the fluorescence and electrochemical detectors. This table was developed by taking the smallest detector response and normalizing that to 1 .O. The other detector responses were divided by the smallest detector response to obtain their normalized detector response. For instance, for a 2.5-$\mu$g/ml solution of LSD, the raw data counts were 5, 6, 9, 8771, and 93835 area counts for 280 nm, 330 nm, 254 nm, fluorescence, and electrochemical detection, respectively. Since 5 area counts is the smallest figure it was normalized to 1.0. All of the other detection signals were divided by 5 to yield their normalized detector responses. The raw data given above along with the detector settings given in the *Instrumentation* section could be used as a guide for other researchers to normalize their instruments and obtain a comparable table. Fig. 1 illustrates a fingerprint obtained after extraction from an orange juice matrix for morphine, codeine, eserine, and apomorphine using UV at 254 nm, fluorescence and electrochemical detection. The relative response ratios in the juice sample were not different from those of a standard. The retention times of the alkaloids studied are given in Table II.

The surface of the electrode in the electrochemical detector had to be carefully monitored. At the beginning of each experiment, a standard that consisted of 2.5 $\mu$g/ml of LSD and 10 $\mu$g/ml of ergotamine was injected. These compounds were chosen because they eluted early and late in the chromatogram. The area counts were maintained at least at 50 000 and 100 000 counts for LSD and ergotamine, respectively. If the counts dropped below these, the electrode surface was repolished with a kit obtained from Hewlett-Packard. The electrode surface was repolished every day while data for the relative response ratios were being obtained. Likewise, when an unknown sample is analyzed in this laboratory, the electrode surface is repolished.

This procedure has been used to identify strychnine in a real sample that was sent to this laboratory. The sample contained an unknown component that was not in the control. The unknown eluted at the same time as strychnine and its relative response ratios were the same as those for strychnine. The control was spiked with strychnine and the relative response ratios matched those in the sample. Mass

TABLE I

RELATIVE RESPONSE RATIOS (NORMALIZED DETECTOR RESPONSE RELATIVE TO SMALLEST DETECTOR RESPONSE)

Experimental conditions as described in the text. ND indicates that the compound was not detected.

| Compound | 280 nm | 330 nm | 254 nm | Florescence | Electrochemical |
|---|---|---|---|---|---|
| Anabasine | 1.0 | ND | 30 | ND | 2299 |
| Apomorphine | 1.8 | ND | 1.0 | 29 | 1318 |
| Arecoline | ND | ND | 1.0 | ND | 20 881 |
| Atropine | ND | ND | 1.0 | ND | 149 |
| Berberine | 1.1 | ND | 1.0 | ND | 517 |
| Cocaine | 1.0 | ND | 1.3 | ND | 481 |
| Codeine | 1.0 | ND | 1.0 | | 6050 |
| Colchicine | 1.0 | ND | 4.8 | ND | 775 |
| Dihydroergotamine | 3.1 | ND | 1.0 | 26 | 12 521 |
| Emetine | 8.9 | ND | 1.0 | 8 | 6732 |
| Ergocriptine | 1.0 | 1.6 | 4.1 | 1753 | 18595 |
| Ergonovine | 1.0 | 1.4 | 3.0 | 453 | 54 652 |
| Ergotamine | 1.0 | 1.6 | 4.2 | 1881 | 29 863 |
| Eserine | 1.0 | ND | 1.6 | 99 | 4137 |
| Hydromorphone | 1.4 | ND | 1.0 | ND | 4173 |
| Lobeline | 1.0 | ND | 10 | ND | 462 |
| LSD | 1.0 | 1.2 | 1.8 | 1754 | 18767 |
| Lysergic acid | 1.0 | 1.3 | 2.6 | 419 | 75506 |
| Lysergol | 1.0 | 1.2 | 2.3 | 314 | 47 318 |
| Methylergonovine | 1.0 | 1.4 | 2.9 | 461 | 47 476 |
| Morphine | 1.4 | ND | 1.0 | 10 | 3581 |
| Nornicotine | 1.0 | ND | 3.6 | ND | 317 |
| Oxycodone | 1.3 | ND | 1.0 | ND | 3995 |
| Pentazocine | 9.3 | ND | 1.0 | ND | 21641 |
| Scopolamine | ND | ND | 1.0 | ND | 543 1 |
| Strychnine | 1.0 | ND | 3.6 | ND | 1275 |
| Yohimbine | 1.7 | ND | 1.0 | 82 | 918 |

spectrometry confirmed the unknown to be strychnine.

*Orange juice extraction*

Selected alkaloids were extracted from orange juice (Table III). The number of determinations in all cases was 7 with three method blanks. The blanks were averaged together and subtracted from the spikes should any matrix interference occur. To calculate the total number of analyses, however, 7 was multiplied by the number of detector signals that detected the alkaloid, i.e., a solution of LSD in methanol was detected at all three UV wavelengths, and by the fluorescence and electrochemical detectors. Therefore, the actual number of determinations for LSD was 35. However, a solution of strychnine in methanol was only detected by UV at 280 and 254 nm, and the electrochemical detector. Therefore, the actual number of determinations for strychnine is 21. Each of these determinations were averaged and the response was compared with that obtained for a standard in order to calculate the extraction efficiency for each alkaloid. The extraction efficiencies between detectors was comparable.

*Ergot alkaloid matrix stability study*

One of the concerns of this laboratory is an estimation of the stability of a poison in a particular product. This information may then be used to assess the inherent risk in a real product contamina-

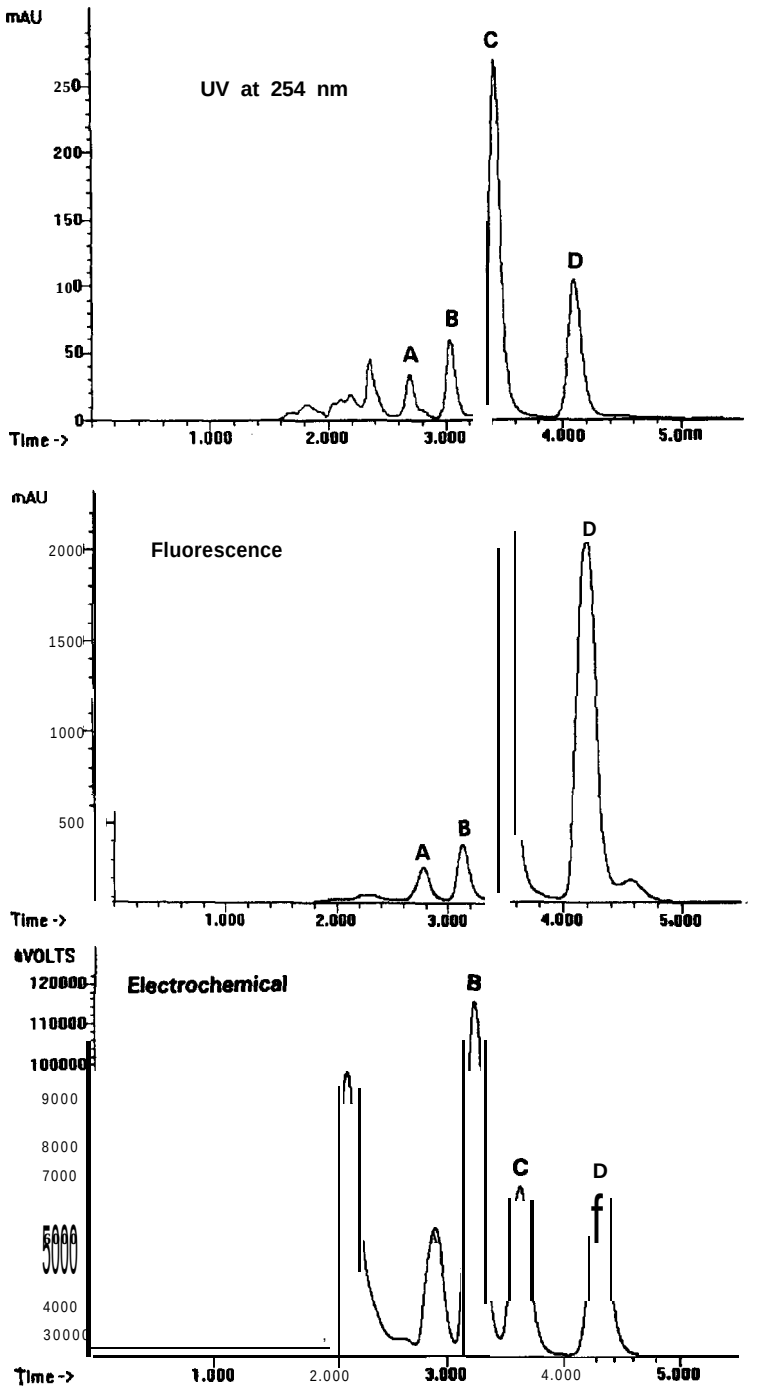


Fig. 1. Chromatograms of spiked orange juice samples. Conditions as described in the text. Peaks: A = morphine; B = codeine; C = eserine; D = apomorphine.

TABLE II

RETENTION TIMES OF ALKALOIDS

| Compound | Retention time (min) |
|---|---|
| Morphine | 2.8 |
| Arecoline | 2.9 |
| Hydromorphone | 3.0 |
| Nornicotine | 3.0 |
| Anabasine | 3.2 |
| Lysergic acid | 3.2 |
| Codeine | 3.3 |
| Oxycodone | 3.5 |
| Eserine | 3.8 |
| Ergonovine | 4.0 |
| Scopolamine | 4.2 |
| Lysergol | 4.3 |
| Apomorphine | 4.6 |
| Methylergonovine | 4.8 |
| Strychnine | 4.8 |
| Atropine | 5.0 |
| Colchicine | 5.5 |
| Cocaine | 7.3 |
| Yohimbine | 9.2 |
| LSD | 9.9 |
| Pentazocine | 10.2 |
| Berberine | 10.3 |
| Emetine | 12.5 |
| Lobeline | 14.8 |
| Ergotamine | 20.1 |
| Dihydroergotamine | 21.4 |
| Ergocriptine | 37.0 |

TABLE 111

EXTRACTION OF SELECTED ALKALOIDS FROM ORANGE JUICE

Experimental conditions as described in the text.

| Compound | Spiking concentration ($\mu$g/ml) | Extraction efficiency[a] (%) |
|---|---|---|
| Apomorphine | 10 | 68 |
| Arecoline | 200 | 94 |
| Codeine | 10 | 111 |
| Dihydroergotamine | 30 | 94 |
| Ergocriptine | 30 | 106 |
| Ergonovine | 5 | 100 |
| Ergotamine | 30 | 100 |
| Eserine | 10 | 100 |
| LSD | 0.125 | 84 |
| Lysergic acid | 5 | 98 |
| Lysergol | 5 | 101 |
| Methylergonovine | 5 | 100 |
| Morphine | 10 | 99 |
| Oxycodone | 10 | 98 |
| Scopolamine | 200 | 80 |
| Strychnine | 10 | 86 |

[a] Efficiency = (sample area counts)/(standard area counts) (3/2) (100%); the equation contains the variable 3/2 because in all cases the initial sample volume was 2 ml and the final sample volume was 3 ml.

tion situation. The results of a study such as this are only definitive for the set of conditions used during the course of the study and may not apply to other sets of conditions. In this work, the stability of ergot alkaloids (lysergic acid, ergonovine, lysergol, methylergonovine, LSD, ergotamine, dihydroergotamine, and ergocriptine) was evaluated in orange juice, vegetable juice, milk, and a cola beverage under two different sets of storage conditions (with the exception of milk that was stored in only one condition). These matrices represented a fairly uniform or homogeneous sample matrix. The time period over which the stability was evaluated was two weeks.

Seven spiked samples and three blank drinks were analyzed on day 0 of the study. Seven spikes and three blanks that were refrigerated and seven spikes and three blanks that were stored at room temperature were analyzed on day 7 (with the exception of milk, that was stored only at refrigerated temperatures). This was repeated on day 14. The samples stored at room temperature were left in the dark. A working standard containing the eight ergot alkaloids listed above at their spiking concentration levels (0.125 $\mu$g/ml of LSD; 5 $\mu$g/ml of lysergic acid, ergonovine, lysergol, and methylergonovine; and 30 $\mu$g/ml of ergotamine, dihydroergotamine, and ergocriptine) was prepared on the first day of analysis. This standard was kept in the refrigerator during the experiment, and used as the standard on days 7 and 14. The percentage recovery from all five signals was determined. These recoveries were in good agreement with each other and were averaged. Tables IV-VII contain the stability study results in the orange juice, vegetable juice, milk, and cola beverage, respectively. The recoveries listed in the tables are the average of the five signals with the exception of dihydroergotamine in the cola beverage because it was not detected and

TABLE IV

PERCENT RECOVERIES OF ERGOT ALKALOIDS IN ORANGE JUICE

Experimental conditions as described in the text. ND indicates that the compound was not detected

| Compound | Day 0 | Day 7/Refr.[a] | Day 7/RT[b] | Day 14/Refr.[a] | Day 14/RT[b] |
|---|---|---|---|---|---|
| Dihydroergotamine | 94 | 68 | 64 | 88 | 51 |
| Ergocriptine | 106 | 68 | 80 | 121 | 91 |
| Ergonovine | 98 | 84 | 41 | 74 | 37 |
| Ergotamine | 100 | 70 | 80 | 111 | 79 |
| LSD | 84 | 61 | 86 | ND | ND |
| Lysergic acid | 100 | 94 | 88 | 91 | 72 |
| Lysergol | 101 | 96 | 85 | 93 | 16 |
| Methylergonovine | 100 | 92 | 85 | 89 | 76 |

[a] Refrigerated temperature.
[b] Room temperature.

TABLE V

PERCENT RECOVERIES OF ERGOT ALKALOIDS IN VEGETABLE JUICE

Experimental conditions as described in the text.

| Compound | Day 0 | Day7/Refr.[a] | Day 7/RT[b] | Day 14/Refr.[a] | Day 14/RT[b] |
|---|---|---|---|---|---|
| Dihydroergotamine | 23 | 21 | 17 | 27 | 14 |
| Ergocriptine | 21 | 17 | 20 | 24 | 23 |
| Ergonovine | 42 | 27 | 36 | 43 | 55 |
| Ergotamine | 21 | 18 | 15 | 23 | 15 |
| LSD | 23 | 26 | 21 | 21 | 19 |
| Lysergic acid | 29 | 31 | 28 | 35 | 26 |
| Lysergol | 17 | 23 | 15 | 31 | 18 |
| Methylergonovine | 26 | 38 | 21 | 39 | 31 |

[a] Refrigerated temperature.
[b] Room temperature.

TABLE VI

PERCENT RECOVERIES OF ERGOT ALKALOIDS IN MILK

Experimental conditions as described in the text.

| Compound | Day 0 | Day 7 | Day 14 |
|---|---|---|---|
| Dihydroergotamine | 20 | 14 | 20 |
| Ergocriptine | 23 | 10 | 11 |
| Ergonovine | 30 | 29 | 34 |
| Ergotamine | 19 | 11 | 13 |
| LSD | 49 | 59 | 49 |
| Lysergic acid | 76 | 71 | 80 |
| Lysergol | 29 | 25 | 38 |
| Methylergonovine | 30 | 33 | 33 |

lysergic acid and lysergol in vegetable juice due to matrix interferences. Fluorescence and electrochemical chromatograms of vegetable juice, milk, and the cola beverage are presented in Figs. 2 and 3, respectively.

The recoveries in vegetable juice and milk are much poorer than those in the orange juice and cola beverage. One explanation for the poor recovery of alkaloids from the milk matrix could be the coprecipitation with the proteins. Also note that the recoveries appear to increase with time in the vegetable juice samples. This result is possibly due to a mold growth which occurred in these samples both at room temperature and under refrigeration. The sample preparation was sometimes difficult because

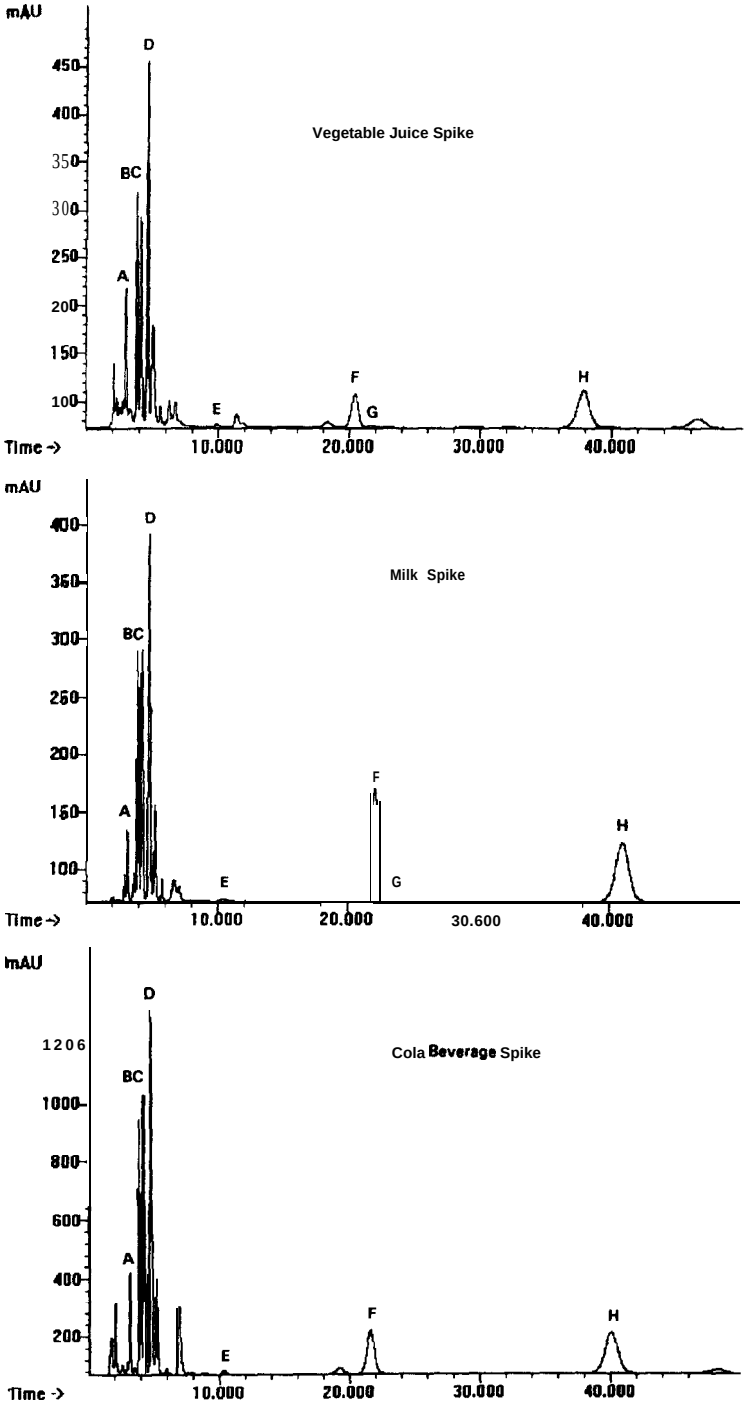


Fig. 2. Fluorescence chromatograms of ergot alkaloids in vegetable juice, milk, and a cola beverage. Peaks: A = lysergic acid; B = ergonovine; C = lysergol; D = methylergonovine; E = ergotamine; G = dihydroergotamine; H = ergocriptine.

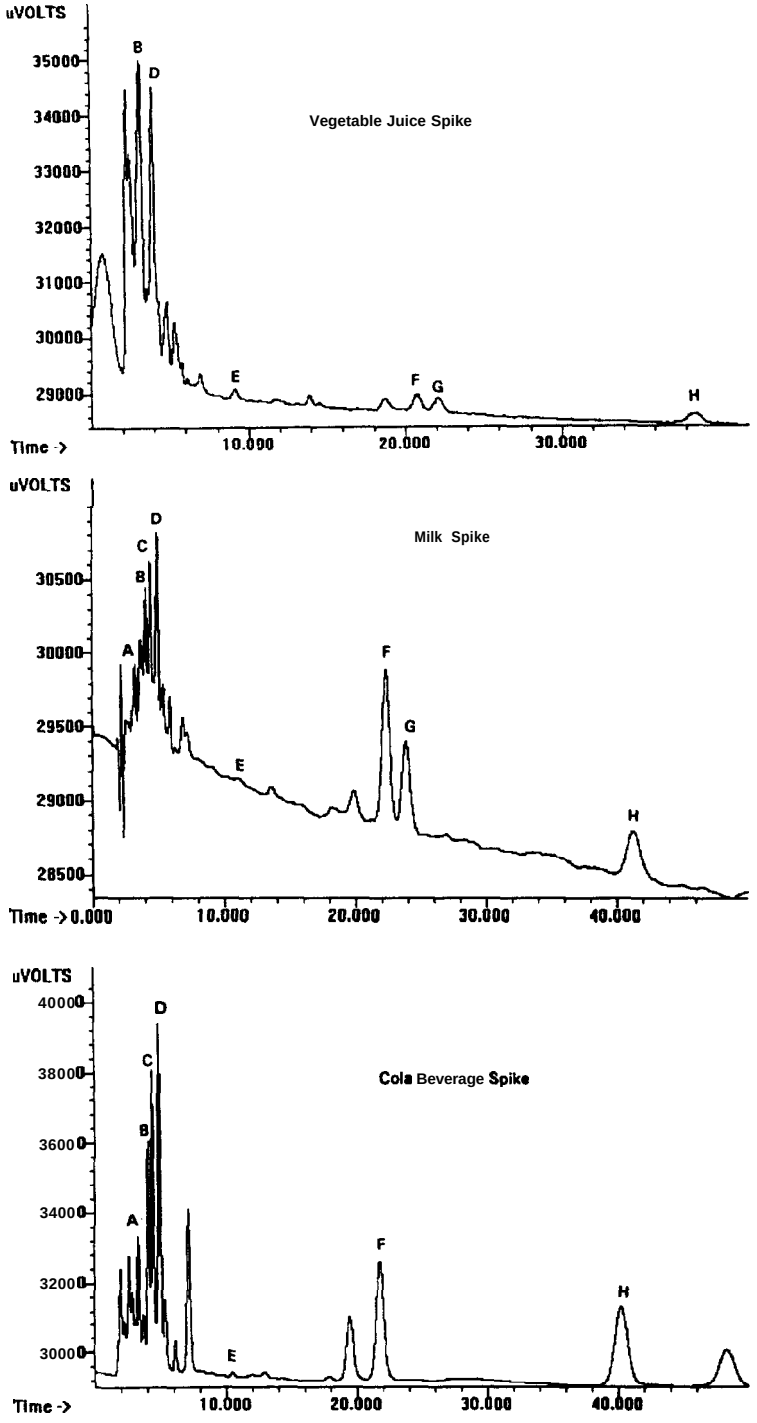


Fig. 3. Electrochemical chromatograms of ergot alkaloids in vegetable juice, milk, and a cola beverage. Peaks: A = lysergic acid; B = ergonovine; C = lysergol; D = methylergonovine; E = LSD; F = ergotamine, G = dihydroergotamine; H = ergocriptine.

TABLE VII

PERCENT RECOVERIES OF ERGOT ALKALOIDS IN COLA BEVERAGES

Experimental conditions as described in the text. ND indicates that the compound was not detected

| Compound | Day 0 | Day 7/Refr.[a] | Day 7/RT[b] | Day 14iRefr." | Day 14/RT[b] |
|---|---|---|---|---|---|
| Dihydroergotamine | 45 | ND | ND | ND | ND |
| Ergocriptine | 64 | 64 | 52 | 69 | 46 |
| Ergonovine | 105 | 85 | 73 | 80 | 65 |
| Ergotamine | 39 | 30 | 25 | 39 | 25 |
| LSD | 113 | 78 | 57 | 78 | 57 |
| Lysergic acid | 105 | 87 | 58 | 89 | 67 |
| Lysergol | 103 | 87 | 65 | 88 | 66 |
| Methylergonovine | 101 | 87 | 62 | 80 | 63 |

[a] Refrigerated temperature.
[b] Room temperature.

not all of the mold could be scraped from the sample and this would hinder during the SPE step. Likewise, the recoveries increase for ergocriptine and ergotamine on day 14 in the orange juice. A matrix interference that could not be resolved from these alkaloids occurred on day 14 which made analyses difficult.

The remaining alkaloids either remained constant over the course of the experiment or slightly decreased with the exception of LSD in orange juice and dihydroergotamine in cola beverage. These components were not detected by the end of the study. It is believed that these components were completely metabolized in these matrices.

## CONCLUSIONS

The general HPLC method is a good method for analyzing acidic and basic drugs due to the ion-pairing reagent in the mobile phase. This is demonstrated by the determination of lysergic acid and lysergol during the same chromatographic run. By using multiple detectors, a chromatographic fingerprint of an unknown drug in a sample can be obtained. This method can be used for the analysis of extracts of drugs from food matrices. Although not all compounds can be detected by each detector or wavelength, it has been shown that each compound will be detected by at least one of the detectors.

Work is continuing is this area. Stability studies are being conducted in other matrices and other classes of drugs are being investigated.

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