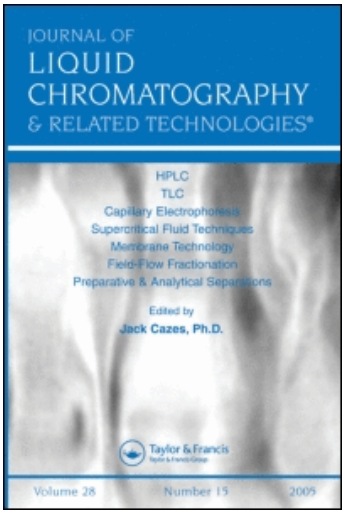

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatographic Determination of Emetine in Corn

Vipin K. Agarwal[a]
[a] The Connecticut Agricultural Experiment Station, Connecticut

To cite this Article Agarwal, Vipin K.(1991) 'High Performance Liquid Chromatographic Determination of Emetine in Corn', Journal of Liquid Chromatography & Related Technologies, 14: 13, 2601 — 2608
To link to this Article: DOI: 10.1080/01483919108049341
URL: http://dx.doi.org/10.1080/01483919108049341

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(13), 2601-2608 (1991)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF EMETINE IN CORN

VIPIN K. AGARWAL
The Connecticut Agricultural Experiment Station
Box 1106
New Haven, Connecticut 06504

ABSTRACT

A High performance liquid chromatographic (HPLC) method for the quantitative analysis of emetine in corn is described. Corn kernels are removed from the ears, blended, and extracted with methanol. The extract is filtered through a 0.45 um Acrodisc filter and analyzed by HPLC using a LC-18-DB column. For detection, a fluorescence detector set at excitation of 285 and emission at 316 nm is used. The recoveries of samples fortified between 0.1 ppm to 20 ppm with emetine ranged from 95.5% to 103.3%.

INTRODUCTION

Emetine, a pharmocologically important alkaloid is derived from the plant species, Cephaelis Ipecacuanha (1). Emetine hydrochloride has effectively been used for the treatment of intestinal amebiosis and amoebic hepatitis (2). Emetine with other ipecac alkaloids is also used as ipecac syrup to induce vomiting following ingestion of poison (3).

A number of High performance liquid chromatographic methods have been developed for the quantitative analysis of emetine in pharmaceutical preparation and in biological samples (4-8). For the analysis of emetine at low levels in

biological matrices such as plasma, organic extracts are subjected to mild mercuric acetate oxidation to convert emetine to a fluorescent product (7). Another method requires extraction with n-butyl chloride followed by back extraction into 0.01M HCL and then analysed by HPLC with fluorometric detection (8).

Scientists at The Connecticut Agricultural Experiment Station have been investigating the efficiency of applying emetine to corn to control raccoon predation. Treatment of food with emetine dihydrochloride not only averted captive raccoons from treated food but also caused them to generalize the aversion to same food when untreated (9). Emetine hydrochloride is known for its toxicity and a dose of 50 mg of emetine hydrochloride caused vomiting and diarrhea in captive raccoons but all recovered in one to two days. In the field, corn ears were treated with emetine and then placed on the ground around the perimeter of a growing stand of unripened corn to modify the behaviour of raccoons. In order to determine the amount of emetine remaining on the corn during a specific period, a method for the quantitative analysis of emetine was required.

A simple HPLC method for the quantitative analysis of emetine is described. This method does not require any chemical treatment of emetine or complicated extraction before HPLC analysis.

METHOD

Reagents and standards:

a) Chemicals.- Emetine hydrochloride (Sigma Chemical Co., St. Louis, MO), Cephaeline hydrobromide (USPC, Inc., Rockville, MD), Disodium hydrogen phosphate and HPLC grade methanol (Fisher Chemical Co., Fairlawn, NJ)

b) Mobile phase for HPLC.- A 0.025M disodium hydrogen phosphate buffer was

prepared. Mobile phase was prepared with 250 mL of buffer and 750 mL methanol. The final pH of the mobile phase was adjusted to 8.5 with phosphoric acid.

c) Standard solutions.- Standard solutions of emetine and cephaeline were prepared by dissolving 20 mg of each separately in 500 mL methanol. This stock solution was used for spiking the corn samples.

Apparatus:

a) High Performance Liquid Chromatograph.- A ConstaMetric III pump (LDC/Milton Roy, Riviera Beach, FL) with a Rheodyne 7105 injector was used for solvent delivery. A LS-40 Fluorescence detector (Perkin Elmer, Norwalk, CT) was used for detection.

b) HPLC Columns.- (1) LC-18-DB, 25cm x 4.6 mm, 5um particle size (Supelco Inc., Bellefonte, PA). (2) Ultrasphere ODS 25 cm x 4.6 mm, 5um particle size (Rainin Instrument Co., Woburn, MA)

c) Acrodisc 0.45um filters (Gelman Sciences, Ann Arbor, MI).

Fortification of corn with emetine:

Corn kernels were ground in an electric blender and 10 gms were spiked with 0.025 mL, 0.125 mL, 0.250 mL, 1.0 mL, 2.5 mL, and 5 mL of stock solution of emetine to provide corn samples spiked at 0.1, 0.5, 1, 4, 10 and 20 ppm levels. All samples were also spiked with appropriate amounts of cephaeline used as an internal standard to calculate the recoveries of emetine.

Sample Preparation:

A 10 gm portion of the ground corn kernels were placed in an Erlenmeyer flask and 50 mL HPLC grade methanol was added to the flask. The flask was

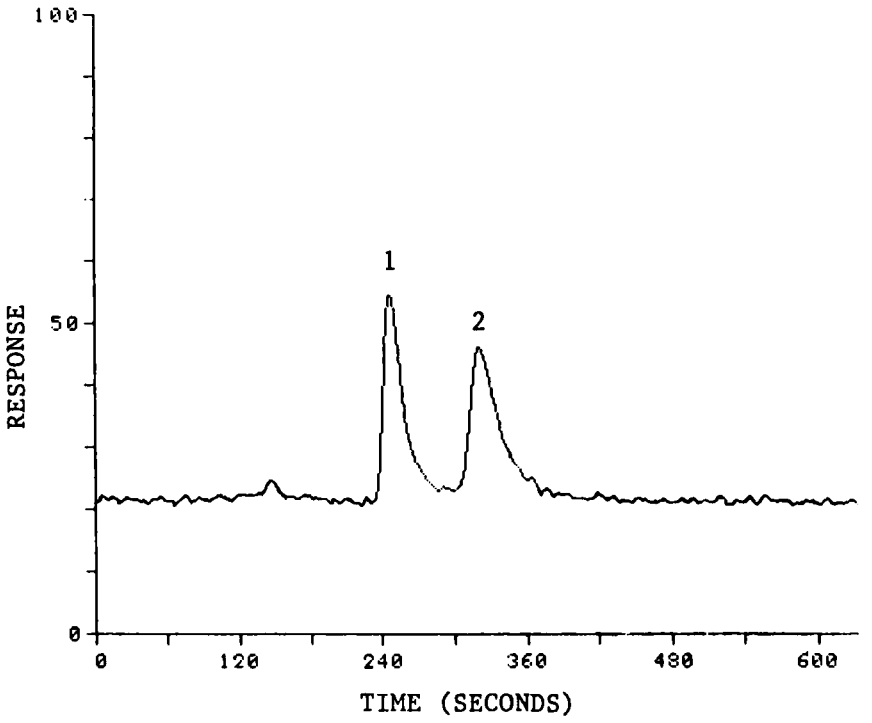

Figure 1. Chromatogram of standard Cephaeline (1) and Emetine (2).

covered with aluminum foil and shaken on a wrist action shaker for 30 minutes at 300 rev/min. After shaking, the contents of the flask were filtered through Whatman #2 filter paper and washed twice with 10 mL of methanol each time. The volume of the combined filtered extract was finally made to 100 mL with methanol and filtered through an Acrodisc filter before injecting into the HPLC.

High Performance Liquid Chromatography:

The following operating conditions were used; Mobile phase as described above was used with a flow rate of 1.1 mL/minute. The peaks were quantitated

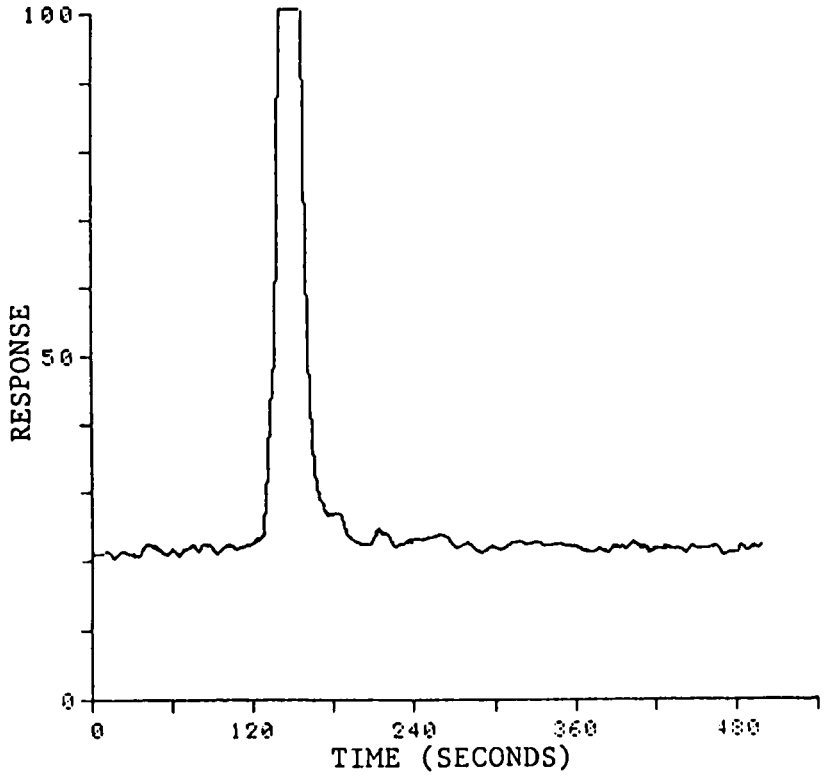

Figure 2. Chromatogram of blank corn sample.

by peak heights. The LS-40 Fluorescence detector was set at excitation 285, emission 316, factor 8, and response 5.

RESULTS AND DISCUSSIONS

Figure 1 shows a representative chromatogram of standard cephaeline and emetine with retention times of 4.1 and 5.3 minutes, respectively. A blank sample of corn was prepared and analyzed by HPLC (Figure 2). No interferring peaks were present at the retention times of cephaeline and emetine. Similarly, a corn

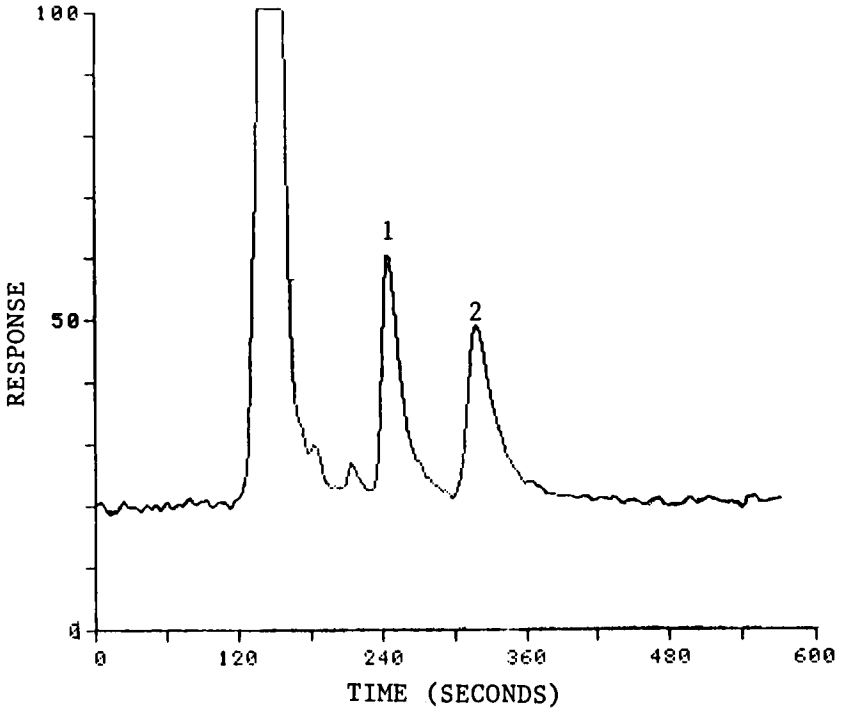

Figure 3. Chromatogram of corn sample spiked with cephaeline (1) and emetine (2), at 1 ppm.

sample spiked with emetine at 1 ppm was prepared and analyzed by HPLC (Figure 3). Several corn samples spiked with emetine ranging from 0.1 ppm to 20 ppm were prepared and analyzed by HPLC. The recoveries of emetine were calculated, based on cephaeline as an internal standard, and ranged from 95.5% to 103.3% (Table 1).

For the analysis of emetine in biological samples at low levels two approaches have been taken. In one report, a chemical reaction was necessary to convert emetine to a more fluorescent active compound before HPLC analysis (7). In another report, emetine was extracted with n-butyl chloride followed by

Table 1

Recoveries of Emetine From Spiked Corn

Spiking Levels PPM	Recovery % Average± SD
0.1	95.5 ± 3.2
0.5	98.3 ± 2.1
1.0	98.0 ± 1.9
4.0	100.3 ± 2.6
10.0	101.0 ± 1.8
20.0	103.3 ± 1.5

back extraction into 0.01M HCL (8). In the present method described, however, a simple extraction with methanol was sufficient for complete extraction of emetine from corn and no further cleanup was required before HPLC analysis.

Initially for HPLC analysis, a 28%, 0.025 M disodium hydrogen phosphate buffer (pH 8) and 72% methanol as a mobile phase with an Ultrasphere ODS column as described by Crouch et al (8) was used. The peaks for cephaeline and emetine were, however, very broad. Therefore certain modifications were made in the mobile phase. It was observed that the pH of the mobile phase played a significant role on the retention of cephaeline and emetine. By adjusting the final pH of the mobile phase to 8.5 with phophoric acid and increasing the methanol concentration to 75% resulted into sharper peaks. Also the use of a LC-18-DB column gave results superior than with an Ultrasphere ODS column.

In conclusion, the method described here is a fast, simple, and precise method for the quantitative analysis of emetine in corn to a low level of 0.1 ppm.

REFERENCES

1. Feyns, L.V., and Grady, L.T.: Emetine Hydrochloride in Analytical Profiles of Drug Substances, 289-335, 1981.
2. Rogers, L.: Indian Medical Gaz. 47, 42, 1912.
3. Physician Desk Reference, 37 Ed. Medical Economics Company, 1141, 1983.
4. Frei, R.W., Santu, W., and Thomas, M.: J. Chromatogr. 116, 365-377, 1976.
5. Sahu, N.P., Mahato, S.B.: J. Chromatogr. 238, 525-529, 1982.
6. Teshima, D., Ikeda, K., Shimomura, K, and Aoyama, T: Chem. Pharm. Bull. 37(1), 197-199, 1989.
7. Bannister, S.J., Stevens, J., Musson, D., and Sternson, L.A.: J. Chromatogr. 176, 381-390, 1979.
8. Crouch, D.J., Moran, D.M., Finkle, B.S., and Peat, M.A.: J. Analytical Toxicology, 8, 63-65, 1984.
9. Conover, M. R., Wildl. Soc. Bull. 17, 430-435, 1989.