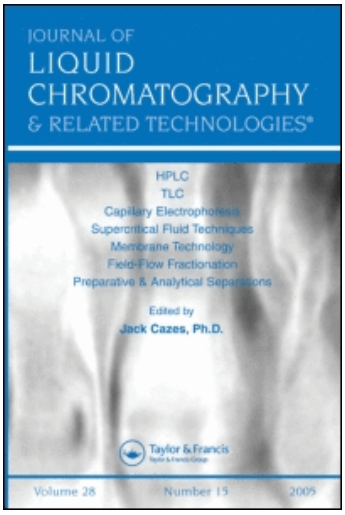

Journal of Liquid Chromatography & Related Technologies

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

Analysis of Cuscohygrine in Coca Leaves by High Performance Liquid Chromatography

Robert L. Glass[a]; Monica B. Johnson[a]
[a] Department of Agriculture Beltsville, Plant Sciences Institute Agricultural Research Service U. S., MD

To cite this Article Glass, Robert L. and Johnson, Monica B.(1996) 'Analysis of Cuscohygrine in Coca Leaves by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 19: 11, 1777 — 1784
To link to this Article: DOI: 10.1080/10826079608014004
URL: http://dx.doi.org/10.1080/10826079608014004

J. LIQ. CHROM. & REL. TECHNOL., 19(11), 1777–1784 (1996)

ANALYSIS OF CUSCOHYGRINE IN COCA LEAVES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Robert L. Glass, Monica B. Johnson

Plant Sciences Institute
Agricultural Research Service
U. S. Department of Agriculture
Beltsville, MD 20705

ABSTRACT

A novel high-performance liquid chromatographic (HPLC) method is described for the determination of the alkaloid cuscohygrine [1,3-bis(1-methyl-2-pyrrolidinyl)-2-propanone] in air-dried leaves of *Erythroxylum coca* var. *coca.* The analysis was performed on a weak cation exchange HPLC column using a mobile phase consisting of MeOH: 0.05 M KH_2PO_4, pH 7 (75:25, v/v) and with UV detection at 220 nm. Cuscohygrine content was determined as 0.21-0.23% in *E. coca* leaves.

INTRODUCTION

The alkaloid cuscohygrine [1,3-bis-(1-methyl-2-pyrrolidinyl)-2-propanone] is found in a variety of plant species and its biosynthetic pathway has been shown to be similar to tropane alkaloids.[1] Alkaloid biosynthesis has become an area of increasing pharmaceutical interest because of the need to find newer and more efficient drugs in plants.

In an earlier article, we reported a high-performance liquid chromatographic (HPLC) method for the determination of hygrine in *Erythroxylum* leaf extracts using a strong cation exchange (SCX) column.[2] Cuscohygrine could not be satisfactorily resolved on this SCX column. As part of our continued interest in using HPLC for the analysis of alkaloids in *Erythroxylum spp.*, the present article describes a new HPLC method for separating cuscohygrine on a weak cation exchange (WCX) column.

EXPERIMENTAL SECTION

Chemicals

Methanol, ethanol and chloroform of HPLC grade were purchased from EM Science (Gibbstown, NJ). All other chemicals were of reagent grade or better. Water used to prepare solutions and mobile phases was initially deionized and was subsequently run through a HP Model 661A water purifier (Hewlett-Packard Co., Avondale, PA).

Cuscohygrine Synthesis

Cuscohygrine was synthesized by the same procedure as reported for hygrine in an earlier paper.[2] N-Methyl pyrrolidone (1.0 g) (Aldrich Chem. Co., Milwaukee, WI), dissolved in ethyl ether, was partially reduced to the aminoaldehyde by refluxing with $LiAlH_4$. The ether in the mixture was removed by rotary evaporation. Acetone dicarboxylic acid (1.0 g) (Aldrich Chem. Co.), dissolved in 0.1 M NaH_2PO_4, was added to the mixture. Cuscohygrine was extracted with chloroform and was then separated by vacuum distillation (10 mm) with the temperature at 85-90°C. GC/MS (mass spectrometric) analysis of the distillate revealed that cuscohygrine (m/z 224) was one of several alkaloids produced in this synthesis. After purifying the distillate by normal column chromatography using acidic alumina, a yield of 10% was obtained for cuscohygrine, density ≈ 1.0 g/mL.

Standard Solutions

Purified cuscohygrine (50 μL) was dissolved in 50 mL of methanol to give a standard solution of concentration 1.0 mg/mL. Standards ranging in concentrations from 0.5 to 0.05 mg/mL were prepared by serial dilutions using methanol. These standards were stored in amber vials at 0°C.

HPLC Analysis

A Model 8800 ternary gradient HPLC pump (Spectra-Physics, San Jose, CA) was used with a Model 7125 Rheodyne valve (Cotati, CA) fitted with a 5-μL loop. Cuscohygrine was separated on an Synchropak CM100 weak cation exchange (WCX) column (10.0 cm x 4.6 mm i.d., 5 μm; SynChrom, Inc., Lafayette, IN). The WCX column was used without a guard column. The mobile phase consisted of methanol: 0.05 M KH_2PO_4, pH 7.0 (75:25, v/v) delivered isocratically at 1.2 mL/min resulting in a column head pressure of about 1250 psi. Detection was made with a Model UV2000 dual wavelength detector (Thermo Separation Products, Fremont, CA) operated at 220 nm (0.05 AUFS).

Calibration Curve

The area counts of the individual peaks and the corresponding concentrations were used to construct the standard curve for cuscohygrine. The curve followed Beer's law in the range 0.1 to 1.0 mg/mL.

Extraction Procedure

Cuscohygrine was extracted from coca leaves, which were collected from plants grown under greenhouse conditions, using two separate procedures that are designated as Methods A and B. In Method A, air-dried leaves (1.0-4.0 g) were crushed by hand and refluxed in 95% ethanol (50-200 mL) at 70°C for 30 min. The extract was passed through filter paper. The solvent was removed by rotary evaporation at 60°C under vacuum (2-10 mm). The residue was re-dissolved in 50 mL of chloroform and then transferred to a separatory funnel.

The chloroform extract was shaken separately with two 25-mL volumes of 1.5% citric acid in water (w/v) which were then combined in a beaker containing a magnetic stirring bar. The aqueous layer, which was slowly stirred, was adjusted to pH 5.5 using powdered $NaHCO_3$ and was subsequently shaken with two 25-mL aliquots of chloroform. This partition step assisted in removing cocaine and other interfering alkaloids from the aqueous phase. The aqueous layer was then adjusted to pH 8.8 with 10% NH_4OH in water. Cuscohygrine was partitioned into chloroform by mixing the aqueous layer with two 25 mL volumes of chloroform in a separatory funnel. The chloroform layer was collected in an erlenmeyer flask over anhydrous Na_2SO_4 in order to remove any traces of water. The chloroform layer was then transferred to a round-bottomed flask and was subsequently reduced to a volume of 0.5 - 1.0 mL on the rotary evaporator. The extract was diluted to 8 mL with methanol for HPLC analysis.

In Method B, powdered leaf tissue (1-4 g), which was prepared by grinding the dry leaves in a Wiley mill, was combined with 50-100 mL of 95% ethanol and then was stirred for 20 min at room temperature. The extract was passed through filter paper and was handled thereafter as in Method A.

Fortified Samples

To 25-mL subsamples of a crude *E. coca* leaf extract, aliquots of a working stock solution of cuscohygrine (8 mg/mL) were added to give 1.0, 5.0, and 10.0 mg of cuscohygrine per subsample. A minimum of two replicates were made of each fortification level.

The crude leaf extract was prepared as follows: refluxed 10.0 g of dry *E. coca* leaves with 400 mL of 95% ethanol for 30 min; filtered the extract; made up the final volume of the extract to 600 mL. The mean cuscohygrine content found in the 25 mL subsamples was 0.4 mg using Methods A and B. The recovery (%) of cuscohygrine (Cusco.) was calculated as follows:

$$\text{Recovery (\%)} = \frac{\text{Amt. Cusco. (mg) Found - 0.4 mg in Subsample}}{\text{Amt. Cusco. (mg) Added}} \times 100\%$$

RESULTS AND DISCUSSION

HPLC Analysis

The chromatograms in Figure 1 show the resolution of cuscohygrine in a standard solution of hygrine (0.5 mg/mL), on (A) the SCX column and (B) the WCX column. The retention time for cuscohygrine on the WCX column was about 4.5 min at a flow rate of 1.2 ml/min. The calculated number of theoretical plates (N) was 733 plates/m. The high pH of the mobile phase, which was primarily due to the phosphate solution (pH 7), appeared to have adversely affected the WCX column by causing occasional ghost peaks and excessive baseline drift.

The calibration curve for cuscohygrine (not shown) was fitted by the regression equation $f(x) = 6.52x + 0.28$, with a coefficient of regression (r^2) of 0.99. The detection limit (signal/noise = 3) for cuscohygrine was determined as 0.05 mg/mL, or 250 ng/injection at the 0.05 AUFS sensitivity level.

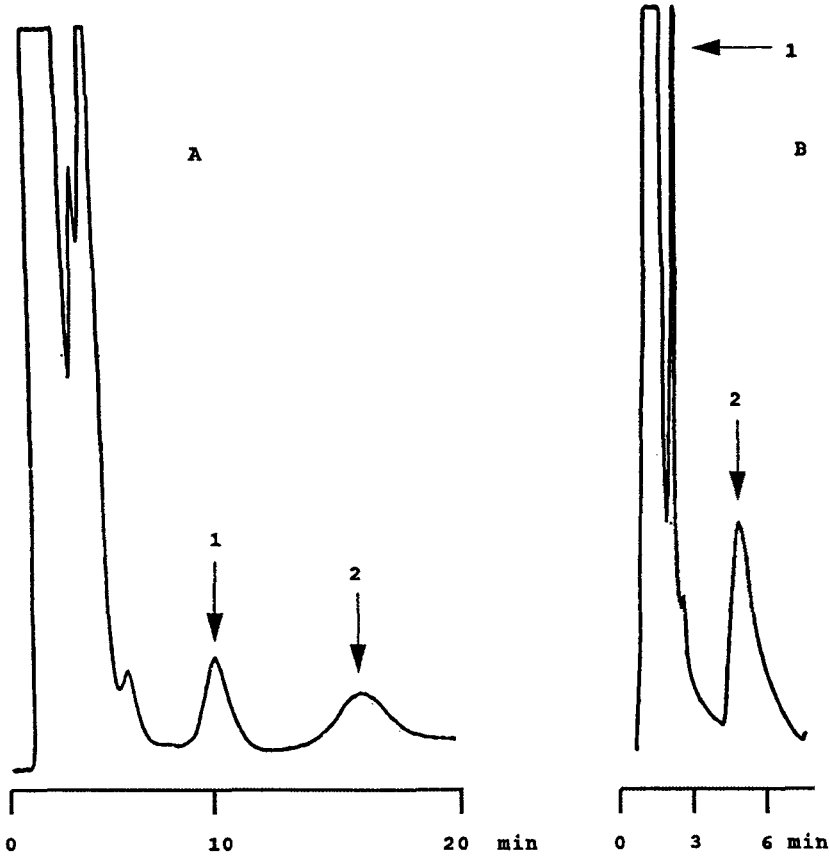

Figure 1. Chromatograms obtained from a standard solution of cuscohygrine (0.5 mg/mL) separated on (A) Column #1, the strong cation exchange (SCX) column and (B) Column #2, the weak cation exchange (WCX) column. Peaks 1 and 2 are hygrine and cuscohygrine, respectively.

Analysis of Leaves

The mean recoveries of cuscohygrine were 38.11 ± 0.18% and 64.4 ± 0.18% from fortified *E. coca* leaf extracts using Methods A and B (Table 1), respectively. The precision was similar for the Methods A and B with C.V.'s of 6.7% and 5.6%, respectively. The smaller recovery of cuscohygrine and the lower precision in Method A are attributed to the refluxing heat, which presumably caused hydrolysis of cuscohygrine in the ethanolic solution. In a

similar study involving the reflux of coca leaves in ethanolic solutions,[3] the recovery of cocaine and the precision of the method suffered significantly by longer refluxing times (i.e., 15 min or more), resulting in the hydrolysis of the alkaloid.

Table 1

Recovery (%) of Cuscohygrine from Fortified Coca Extracts[a]

Cuscohygrine (mg) Added		Recovery[b] (%)	±S.D.	C.V.%
		Method A		
1.0		24.5	0.02	6.1
5.0		50.0	0.08	3.2
10.0		39.8	0.43	10.8
	Means	38.1	0.18	6.7
		Method B		
1.0		62.0	0.04	6.4
5.0		63.0	0.01	3.03
10.0		68.3	0.40	7.23
	Means	64.4	0.18	5.57

[a] Cuscohygrine was added to 25 mL aliquots of a working stock extract which was prepared by refluxing 10.0 g of air-dried *E. coca* leaves in 600 mL of 95% ethanol. [b] Results are the mean of two to four replicates (n), standard deviation, and the coefficient of variation.

Cuscohygrine extraction required about 80 min per sample using Method A, whereas Method B required only 60 min.

The mean cuscohygrine content in unfortified *E. coca* leaves was 0.23 ± 0.03% and 0.21 ± 0.02% for Methods A and B (Table 2), respectively. There was no difference in the amounts of cuscohygrine found in unfortified samples using methods A and B as compared to the fortified samples (Table 1). Other plant components, such as lipids, amino acids, proteins, and other alkaloids, presumably inhibit the rate of hydrolysis of cuscohygrine, resulting in smaller

losses of cuscohygrine in the unfortified leaf extract. In fortified samples, there are fewer components to protect cuscohygrine from undergoing hydrolysis during reflux in ethanolic solution. The cuscohygrine content of 0.21-0.23% determined here by HPLC was slightly smaller than the 0.31% value reported for the capillary gas chromatographic (CGC) method using greenhouse-cultivated *E. coca* leaves.[4]

Table 2

Cuscohygrine Content of Air-Dried *E. Coca* Leaves

Average Dry Weight		Cuscohygrine[a] Content (%)	±S.D.	C.V.%
		Method A		
2.0 g		0.22	0.03	13.6
4.0 g		0.24	0.03	12.5
	Means	0.23	0.03	13.1
		Method B		
2.0 g		0.21	0.02	9.5
4.0 g		0.20	0.02	3.0
	Means	0.21	0.02	6.3

[a] Results are the mean of two to four replicates (n), standard deviation, and the coefficient of variation.

In conclusion, the new HPLC method described here is a simple and efficient technique for analyzing cuscohygrine and provides a reliable alternative to the capillary gas chromatographic method. In addition, the extraction procedures presented in this report allow for the specific isolation and separation of cuscohygrine and hygrine from the tropane alkaloids, e.g., cocaine and cinnamoyl-cocaine, in *E. coca* extracts.

ACKNOWLEDGMENTS

We wish to thank Dr. James Avery of the Insect Chemical Ecology Laboratory of ARS for the GC/MS analysis of the cuscohygrine that was synthesized for this study.

Mention of companies or commercial products does not imply recommendation or endorsement by the United States Department of Agriculture over others that are not mentioned.

REFERENCES

1. M. L. Newquist, T. W. Abraham, T. W., E. Leete, E., "Biosynthetic Incorporation of Ethyl (RS) [2,13-^{13} C,3-^{14}-C]-4-(1-Methyl-2-pyrrolidinyl)-3-oxobutanoate into cuscohygrine in *Erythroylum coca*," Phytochem., **46**, 254 (1993).

2. R. L. Glass, "Analysis of Hygrine in Coca Leaves using a Novel High-Performance Liquid Chromatographic Method," J. Liq. Chromatogr., **18**, 2877 (1995).

3. C. E. Turner, C. Y. Ma, M. A. Elsohly, "Constituents in Erythroxylum Coca. I: Gas Chromatographic Analysis of Cocaine from Three Locations in Peru," Bull. Narcotics **31**, 71 (1979).

4. J. M. Moore, J. F. Casale, R. F. X. Klein, D. A. Cooper, J. Lydon, "Determination and In-Depth Chromatographic Analyses of Alkaloids in South American and Greenhouse-Cultivated Coca Leaves," J. Chromatogr. A, **659**, 163 (1994).

Received November 11, 1995
Accepted January 25, 1996
Manuscript 4018