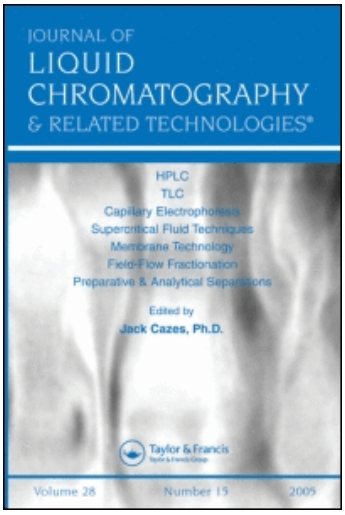

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HPLC Determination of Oxalic Acid in Cocoa

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HPLC DETERMINATION OF OXALIC ACID IN COCOA

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Abstract

An HPLC method is described for the determination of oxalic acid in cocoa and milk chocolate. Samples are extracted using 6N HCl; after extraction the pH of an aliquot is adjusted to 6.0 and interfering substances are eliminated through the use of a C_{18} Sep-pak®. The final HPLC determination uses a monolayer reversed phase column with an ion-pairing mobile phase and electrochemical detection. The results indicate excellent accuracy and precision.

Introduction

Analytical methods for the determination of oxalic acid in various matrices have included physical methods such as optical microscopy, x-ray, diffraction, electron diffraction and electron microprobe. These methods

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require equipment that is not within the scope of many analytical laboratories. Other methods that have been used include several colorimetric assays which involve the reactions of oxalic acid with phenylhydrazine (2), or reaction with 2,7-dihydroxynaphthalene and chromotropic acid (3). Volumetric methods have relied on titration with permanganate (4,5). The fluorometric assay involves the quenching of the fluorescence of a 1:1 zirconium-flavanol chelate with oxalate (16). Enzymatic methods using oxalate decarboxylase (EC 4.1.1.2) (7,8), ion selective electrodes techniques (9) and GLC (10,11) have also been used.

HPLC can be used for the determination of various organic acids in samples of food (12) and has been successfully applied to the determination of oxalic acid in samples of spinach.(13) The use of HPLC coupled with the electrochemical detector allows the specific determination of oxalic acid in many sample types; it has also been applied to the determination of oxalic acid in samples of urine.(14)

This paper reports a rapid accurate method for the determination of oxalic acid in samples of cocoa.

Experimental

Equipment

The HPLC equipment used consisted of an M6000A Solvent Delivery System (Waters Associates), a Model 7120 Loop Injector equipped with a 50 µl loop (Rheodyne Instruments) and a Model LC-4 Electrochemical Detector equipped with a glassy carbon electrode (Bioanalytical System). Column temperature was maintained by using an electronic column heater set at 24°C (Jones Chromatography). The HPLC column was a MC-18 reversed phase (4.6 mm I.D. x 25 mm) (E S Industries). The detector was set at +1.25 volts vs. AgCl in the oxidative mode @ 50 nafs. Data acquisition and analysis was accomplished through use of a CR-2A Data Unit (Shimadzu Scientific).

HPLC Mobile Phase

The HPLC mobile phase was made by adding 1 ml of a 25% solution of hexadecyltrimethylammonium chloride (Fisher Scientific) and 7.5 g of

KH_2PO_4 to 500 ml of water. After these components are in solution, 500 ml of HPLC grade methanol was added; this mobile phase was mixed thoroughly, filtered, and degassed prior to use.

Extraction of Samples

Weigh 1.0 g of cocoa to the nearest mg into a 100 ml beaker. Add 75 ml of distilled water and adjust the pH to 1.7±0.1 with 6N HCl; heat to 50°C±5° in a water bath for 30 minutes and cool to room temperature. Transfer the solution to a 100 ml volumetric flask and dilute to volume with water. Filter the resulting solution through S&S 58 prepleated filter paper or equivalent. Pipette 1.0 ml of the filtered solution into a 150 ml beaker and add about 75 ml of water and adjust the pH to 6.0±0.1 with 0.1% NaOH. After the pH adjustment, transfer the resulting solution to a 100 ml volumetric flask and dilute to volume with water.

Sample Cleanup

A C_{18} Sep-pak (Waters Associates) was used for removal of contaminants and interferences. It was prepared for use by rinsing with 3 ml of CH_3OH followed by 5 ml of H_2O. Ten ml of the final solution obtained from sample preparation were passed through the Sep-pak. The first 2 ml were discarded and the next 8 ml were used for the resulting analysis.

Standard

Twenty mg of oxalic acid (Sigma Chemical Co.) were placed in a 100 ml volumetric and dissolved in water for a final concentration of 0.2 mg/ml. This standard was stored at 4°C and replaced weekly when peak shape of the standard deteriorated or when multiple peaks were evident in the chromatogram of the standard.

HPLC Analysis

A 50 µl aliquot of the solution obtained from the Sep-pak cleanup was injected onto the HPLC with the mobile phase flowing at 2.0 ml/min. The concentration of oxalic acid was calculated by comparison of the peak area of sample with peak areas of previously run external standards of oxalic acid.

Results and Discussion

Representative chromatograms of an oxalic acid standard and cocoa extract are seen in Figures 1 and 2. The method previously described was evaluated for precision and accuracy. Table 1 outlines the results of a precision study using a 1 gram cocoa matrix.

The results indicate that the assay exhibits good precision with a Cv of 3.15% for five replicate determinations.

The linear range of the oxalic acid assay was from 0.5-50 µg/ml with a calculated regression coefficient (r^2) of 0.99 for this range. The sensitivity was found to be 5 µg/g based on original sample weight at 2 times S/N.

The accuracy of the assay was evaluated and Table 2 summarizes the accuracy data in the cocoa matrix.

The cocoa recovery indicates excellent accuracy.

A small survey of available imported cocoa liquors was conducted to obtain information about the relative concentrations of oxalic acid in samples of cocoa liquor obtained from diverse geographic locations. These data are summarized in Table 3.

Table 1

Cocoa Precision Study

Cocoa Aliquot #	Concentration of Oxalic Acid (mg/g)
1	6.73
2	6.98
3	6.40
4	6.60
5	6.66
	$\bar{x}$ = 6.67

Column: MC-18 (E S Industries)

Detector: Electrochemical with
Glassy Carbon Electron
@ +1.25 volts vs. AgCl

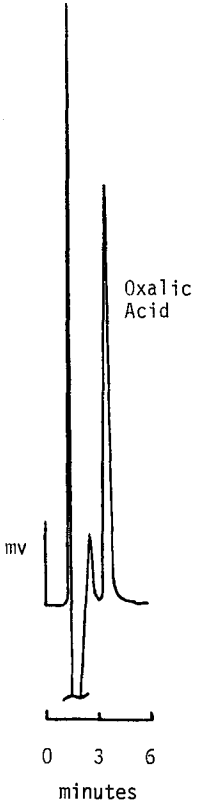

Figure 1

Chromatogram of Oxalic Acid Standard

Column: MC-18 (E S Industries)

Detector: Electrochemical with
Glassy Carbon Electron
@ +1.25 volts vs. AgCl

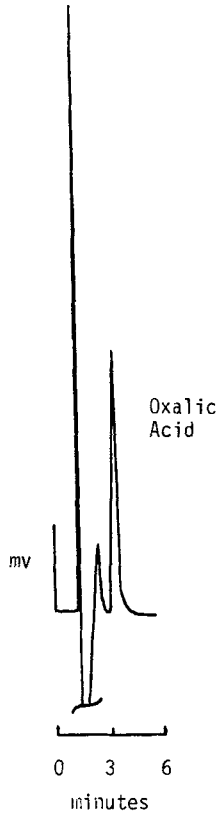

Figure 2

Chromatogram of Cocoa Extract

Table 2

Cocoa Recovery Study

n = 2

1 gram sample size

Amt. Added (mg)	Amt. Recovered (mg)	% Recovery
(6.67 mg/g) no add	-	-
5	5.16	103.2
10	10.19	101.9
15	14.27	95.1
20	19.81	101.1
		$\bar{x}$ = 100.3

Table 3

Survey of Cocoa Liquors for Oxalic Acid Content

Liquor Type	Concentration of Oxalic Acid (mg/g)
Trinidad	3.62
Bahia	3.26
New Guinea	2.75
Ivory Coast	3.20
Hispaniola	3.20

These results indicate an average oxalic acid concentration in these five samples of 3.21 mg/g. The narrow spread of this data is somewhat surprising considering the wide geographical area covered by these samples.

Electrochemical detection allows the use of the unique electrochemical character of oxalic acid and its subsequent selective and sensitive detection. Oxalic acid is a strong acid with a first Pka of 1.23. The oxalic

acid did not exhibit the passivating effect on the detector as was reported by other researchers when evaluating this detection mechanism for the determination of oxalic acid in urine.(14) One can only surmise that the use of a judicious sample cleanup in this assay eliminated many compounds which might have fouled the glassy carbon electrode.

The data generated in these studies compared well with data from the literature (1) where cocoa was reported to have contained 623 mg of oxalic acid per 100 grams. In this study, the average oxalic acid content of a 10-12% fat cocoa would be 667 mg per 100 grams.

In summary, an HPLC method has been developed to allow the determination of oxalic acid in samples of chocolate and cocoa with good accuracy and precision.

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