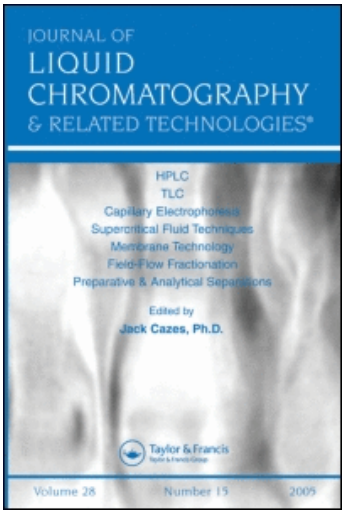

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Identification of Adenine in Cacao Products

B. A. Kiefer[a]; R. M. Sheeley[a]; W. Jeffrey Hurst[b]; R. A. Martin Jr.[b]
[a] Dickinson College, Carlisle, PA [b] Hershey Foods Technical Center, Hershey, PA

To cite this Article Kiefer, B. A. , Sheeley, R. M. , Hurst, W. Jeffrey and Martin Jr., R. A.(1983) 'The Identification of Adenine in Cacao Products', Journal of Liquid Chromatography & Related Technologies, 6: 5, 927 — 930
To link to this Article: DOI: 10.1080/01483918308067014
URL: http://dx.doi.org/10.1080/01483918308067014

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(5), 927-930 (1983)

THE IDENTIFICATION OF ADENINE IN CACAO PRODUCTS

Bruce A. Kiefer,[1] Richard M. Sheeley,[1]
W. Jeffrey Hurst,[2] and Robert A. Martin, Jr.[2]

[1] Dickinson College
Carlisle, PA, 17013
and
[2] Hershey Foods Technical Center
Hershey, PA, 17033

ABSTRACT

High performance liquid chromatography has been employed to separate and identify adenine in aqueous extracts of defatted chocolate liquor. The purine was identified by comparison of its behavior in a variety of solvent systems with that of an authentic sample, and through absorbance ratios at 254 and 235 nm.

INTRODUCTION

Recently we reported the identification of 7-methylxanthine in defatted cacao liquor through HPLC methodology (1), and suggested its implication in the biogenesis of the xanthine bases, particularly caffeine, theobromine, and theophylline, by analogy to Ogutuga and Northcote's work on tea (2). This has encouraged us to extend this search to other compounds that are potential intermediates in such pathways. In this study HPLC has been employed to separate and identify another minor component, adenine, in aqueous extracts of defatted chocolate liquor.

0148-3919/83/0605-0927$3.50/0

Sample Preparation

Samples of chocolate liquor, obtained from the Hershey Chocolate Company, were finely divided, and the sample (10g) was defatted by extracting with two portions of petroleum ether (75ml) and allowed to dry. When no residual solvent was evident, an aliquot of the sample (2.50g) was dispersed in HPLC grade water (50g), and the mixture was boiled gently, with stirring, for 30 minutes. When the sample had cooled to room temperature it was brought to the original weight which is sample weight plus the weight of the HPLC water by addition of water. After filtration through Whatman No. 41 paper (or the equivalent) an aliquot was filtered through a Swinney syringe system using a 0.45μ filter, and the resulting filtrate was used for analysis.

Chromatography

The chromatographic system consisted of an M6000A Solvent Delivery System, U6K Injector, as well as a 10 mv recorder, M440 Ultraviolet Detector (254nm), all from Waters Associates, for the work involving comparison of retention times in various solvent systems. For absorbance ratio determinations a Beckman 165 Variable Wavelength Detector was used in place of the Waters Model 440 Ultraviolet Detector. The following 300 x 4.0mm (I.D.) columns were used: μBondapack C_{18} (10μ particle size, Waters Associates) and Spherisorb ODS (10μ particle size, HPLC Technology, Inc.). The mobile phases used consisted of 0.01M H_3PO_4 and HPLC grade tetrahydrofuran, adjusted to various pH values by careful addition of NaOH solution (aqueous, 50%) (w/v).

Standards

Standard adenine (Sigma Chemical Co.) was dissolved in HPLC water at a concentration of 0.1 mg/ml. The solution was filtered through a Swinney syringe system using a 0.45 filter prior to use.

Analysis

Standard and extract solutions were injected successively into the HPLC unit using mobile phases at various pH values. Absorbance ratios at 235/254 nm

Table 1

Retention Times for Standard and Samples

Column	Mobile Phase	pH	Retention Time, minutes Standard	Sample
μ Bondapak C_{18}	0.5% THF in 0.01M aq. phosphate	3.00	11.81	11.87
μ Bondapak C_{18}	0.5% THF in 0.01M aq. phosphate	4.50	27.40	27.83
μ Bondapak C_{18}	0.5% THF in 0.01M aq. phosphate	6.00	36.14	36.22
Spherisorb ODS	2.0% THF in 0.01M aq. phosphate	4.50	43.42	46.93

Table 2

Absorbance Ratios for Standard and Extract at pH 4.5

Sample	Absorbance Ratio 235/254nm
Adenine	1.12
Defatted Liquor Extract	1.16

were determined at pH 4.50 (±0.01). The flow rate throughout the analysis was 0.5ml/minute.

Results

The results indicate that the peak of interest is due to adenine. The data in Table 1 shows the retention time for baseline resolution of standard and sample at various pH values.

Absorbance ratioing (3) for the standard and sample shows the peak of interest to be adenine.

CONCLUSION

The presence of adenine in aqueous extracts of defatted chocolate liquor has been established by HPLC. These data support the contention of Ogutuga and Northcote (2) that adenine may be implicated in the metabolic pathway leading to caffeine.

Further studies are underway involving the identification and quantitation of other purines in similar liquor extracts.

REFERENCES

1. Aleo, M.D., Sheeley, R.M., Hurst, W.J., and Martin, R.A., Jr., The Identification of 7-Methylxanthine in Cacao Products, Journal of Liquid Chromatography, 5(5), 939, 1982.

2. Ogutuga; D.B.A., and Northcote, D.H., Biosynthesis of Caffeine in Tea Callus Tissue, Biochem, J, 117, 715, 1970.

3. Yost, R; Stoveken, J. & McLean, W., Positive Peak Identification in Liquid Chromatography using Absorbance Rationing with a Variable-Wavelength Spectrophotometric Detector, J. Chromatography, 134, 73, 1977.