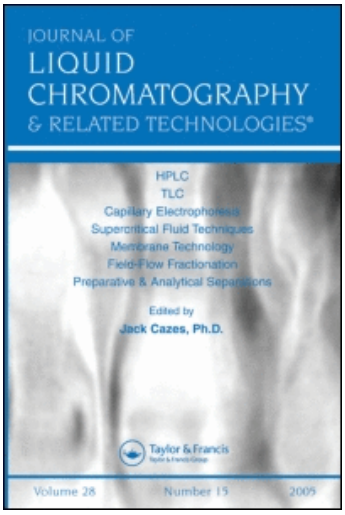

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The HPLC Analysis of Caffeine and Theobromine in Animal Diets

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The HPLC Analysis of Caffeine and Theobromine in Animal Diets

by

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An HPLC method is described for the analysis of added caffeine and theobromine in animal diets using HPLC with samples extracted in $CHCl_3$ and interferences eliminated with a Sep-pak™. This method has good accuracy and precision but is not suitable for matrixes where the methylxanthines exist as a integral part of the matrix (e.g., foods).

The HPLC Analysis of Caffeine and
Theobromine in Animal Diets

An animal diet is a complex matrix of many components consisting of various amount of protein, fat, carbohydrate vitamins and minerals used as a carrier for a wide variety of drugs and food components. The matrix by virtue of its complexity presents a formidable analytical challenge for the analyst. HPLC allows the analysis of a large variety of potential components with great accuracy and precision in a short analysis time.

A method is described for the rapid accurate analysis of the pure added methylxanthines) caffeine and theobromine in animal diet using HPLC with sample cleanup accomplished using a commercially available Sep-pak™.

Methods and Materials

Extraction of caffeine and theobromine. Place 2.5 g ± 0.1 (weighed to 0.01 g) of animal diet into a flask add 100 ml of $CHCl_3$ and heat the solution to 60°C for 30 min. Cool extract to room temperature and bring up to weight with $CHCl_3$. Withdraw 10 ml of $CHCl_3$ extract and run through a Silica Sep-pak™. Elute caffeine or theobromine with 15 ml of CH_3OH. Depending on the level of the compound of interest the sample can be injected directly from the CH_3OH or concentrated to an appropriate volume.

The analysis was accomplished by HPLC using a Water's Radial Compression Module (RCM) with a C_{18} (Radial Pak A) cartridge. The mobile phase was 74/25/1 $H_2O/CH_3OH/HOAC$ at a flow rate of 3.0 ml/min. Detection was at 280 nm. Typical analysis for both compounds was less than 10 minutes. Standard concentrations were 0.5 µg/µl for theobromine and .025 µg/µl for caffeine. Figures 1 and 2 show sample chromatograms for standards and samples respectively.

Results

Precision studies of the method show a %Cv of less than 1% for standards (n = 10) and less than 2% for sample (n = 10). Recovery studies of additions to diet are summarized in Table 1.

An additional study of multiple extractions (n = 4) of a spiked sample (60 mg/100 g theobromine) gave excellent data with data showing differences to be not significant at a 95% confidence level.

An examination of two rabbit diets with added theobromine gave data that can be seen in Table 2.

This method provides a fast accurate analysis of added pure methylxanthines in animal diets. Although it is not suitable for use in systems where the methylxanthine is in a bound form (i.e. like a food system) because the energy input from the $CHCl_3$ is not sufficiently high as to extract

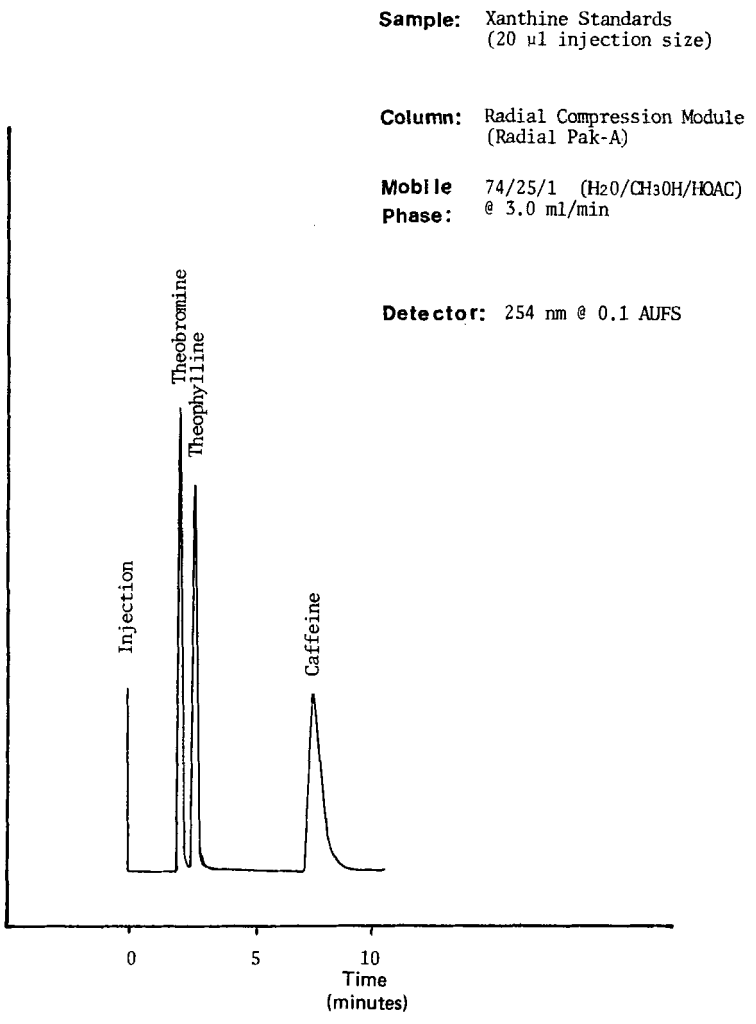

Figure 1. Analysis of Xanthine Standards, 20 ul inj. size; Column: Radial Compression Module, Rad-Pak-A; Mobile Phase: 74/25/1 (H_2O/CH_3OH/HOAc) @ 3.0 ml/min; Detector: UV/254 nm @ 0.1AUFS.

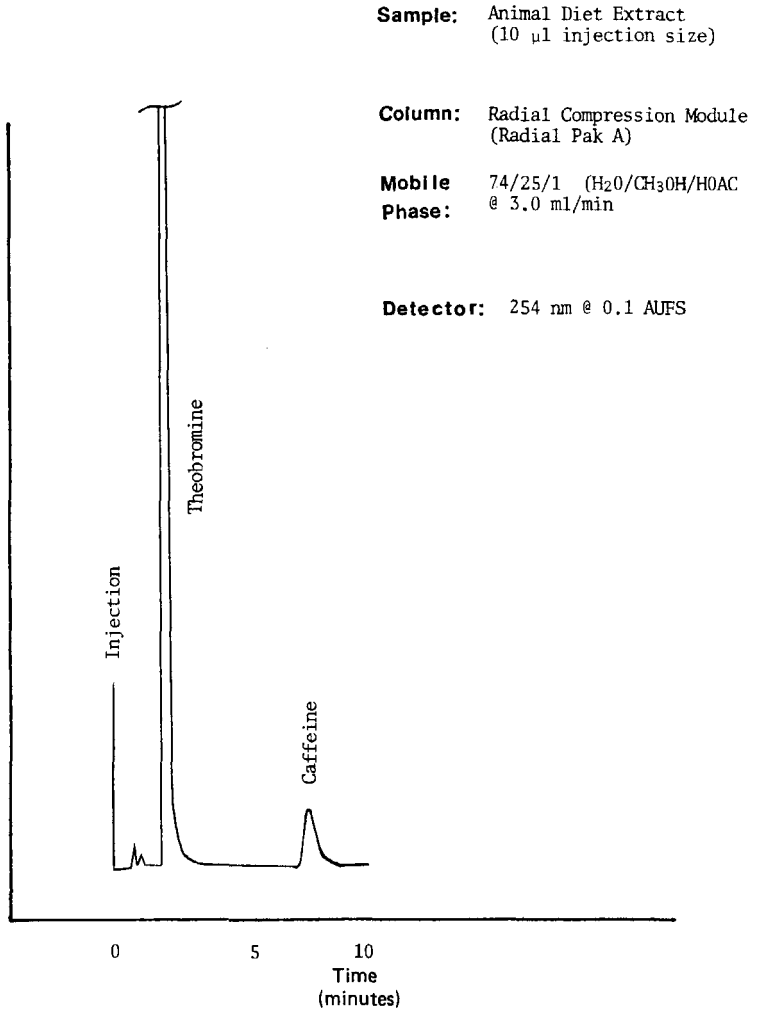

Figure 2. Analysis of Animal Diet Extract, 10 ul inj. size; Operating conditions as in Figure 1.

Table 1

Recovery Study of Added Caffeine and

Theobromine to Animal Diets

n = 2

% Theobromine Added	% Theobromine Rec.	% Recovery
+.027	.025	95.6
+.068	.067	98.6
+.135	.134	99.3
		x = 97.8

% Caffeine Added	% Caffeine Rec.	% Recovery
+.002	.0019	95.0
+.004	.003875	93.8
+.008	.00795	99.4
		x = 96.1

Table 2

Amount Added Theobromine	Amount Analyzed Theobromine
60 mg/100 g	57.6 mg/100 mg
120 mg/100 g	114 mg/100 mg

all of the bound xanthines. This method will extract 70-75% of the total compounds in the food system.

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