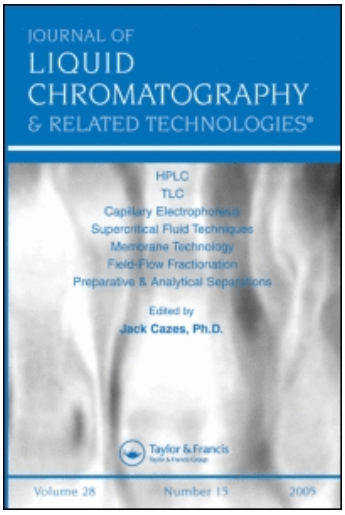

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High Performance Liquid Chromatographic Determination of Formaldehyde in Milk

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF FORMALDEHYDE IN MILK

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ABSTRACT

An HPLC procedure was developed for the determination of formaldehyde in milk. The analytical procedure consisted of the two-phase derivatisation reaction with 2,4-dinitrophenylhydrazine (2,4-DNPH) and elution of formaldehyde-2,4-dinitrophenylhydrazone (DNPF) from Lichrosorb RP8 column with acetonitrile-water (2:3) as mobile phase.

INTRODUCTION

Formaldehyde is a normal product of intermediary metabolism in mammals (1). It is also used in ruminant feeding to protect dietary protein from ruminal degradation and as a preservative in silage (2). The use of formaldehyde in animal feedstuffs requires monitoring of its content in milk.

Quantitative discrepancies in formaldehyde determination in milk have been pointed out (3, 4).

The most widely used method is the AOAC colorimetric method based on the formation of coloured derivative with chromotropic acid (5). With this method, the determined levels of formaldehyde may vary from 0 mg/kg (6) to a high of 0.2 mg/kg (7) in the milk of cows fed diets containing no formaldehyde. Colorimetric method is also prone to interferences from other low-molecular weight carbonyl compounds and from phenol (8).

The present study has developed more specific and sensitive method. The method employs direct single step formation-extraction of formaldehyde-2,4-dinitrophenylhydrazone (DNPF) which is then separated by HPLC (9).

EXPERIMENTAL

Apparatus and Materials

The instrument used for HPLC separations was a Varian Vista 5500 equipped with a Varian 2050 variable wavelength UV detector.

The samples were applied to an Alltech Lichrosorb RP8 10U column (250 mm x 4.6 mm).

All solvents were HPLC grade (Fisher Scientific). 2,4-DNPH was purchased from Sigma, formaldehyde as 37% formalin from Baker.

Commercial milk (2% M.F., partly skimmed, pasteurized) was used for experiments.

2,4-DNPF was synthesised according to established method (10).

Procedure

2 mL of 2,4-DNPH solution (0.14% in 2N HCl) was added to a vigorously stirred mixture of 2 mL of milk and 4 mL of hexane.

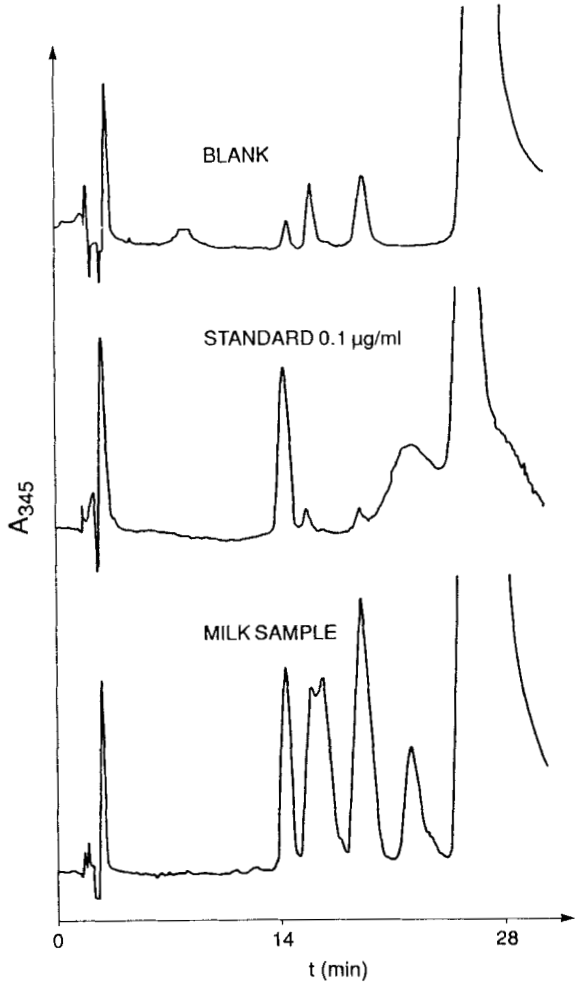

FIGURE 1.

HPLC separation of the formaldehyde-2,4-dinitrophenylhydrazone (DNPF). The reagent blank, the standard solution of formaldehyde (0.1 µg/mL) and the milk sample have been extracted as described in the experimental section.

TABLE 1.

Formaldehyde concentrations μg/mL in commercial milk samples (2% M.F., partly skimmed, pasteurized).

Sample #	Formaldehyde (μg/mL)*
1	0.182 ± 0.011
2	0.147 ± 0.010
3	0.243 ± 0.036
4	0.080 ± 0.008
5	0.110 ± 0.009
6	0.131 ± 0.011
7	0.221 ± 0.017
8	0.164 ± 0.014
9	0.255 ± 0.027
10	0.075 ± 0.004
11	0.232 ± 0.019
12	0.132 ± 0.009

* Each figure is a mean value of three replicates.

After 30 min, the two phases were separated and the aqueous phase extracted twice with 6 mL of hexane. The combined hexane extracts were washed 3 times with 10 mL of water and evaporated to dryness under a gentle stream of nitrogen. The residue containing the DNPF was dissolved in 1 mL of acetonitrile. 20 μL aliquots were injected to the HPLC with detector set at 345 nm. Acetonitrile/water (40:60, v/v) was used as the mobile phase at a flow rate of 1 mL/min.

RESULTS AND DISCUSSION

The quantitation was based on a calibration by series of dilutions from primary standard. The dependence between the area of the peak and the amount of DNPF was determined. The regression analysis of triplicate calibration data has shown linear relationship in the 0.005 to 0.1 μg range of DNPF in 20 μL injection volume, with the r = 0.998.

In order to estimate the efficiency of the recovery the milk samples were spiked with 0.1 and 1 μg/mL of formaldehyde. The recovery was 89.9 ± 3.9% and 96.9 ± 2.8%, respectively. The yields of extraction are the mean of three replicate experiments.

Typical HPLC chromatogram for the formaldehyde determination in standard and milk samples are given in Fig. 1.

In the present study, 12 random samples of commercial milk, bought during the month of March 1992, were analyzed for formaldehyde content. The results are summarized in Table 1.

Formaldehyde level found in this study in milk destined for human consumption varies from 0.075 to 0.255 μg/mL. These amounts are much smaller than those reported for beverage products such as beer and soft drinks (11) and coffee (12).

CONCLUSION

In this paper, an attempt was made to present a new method for the formaldehyde determination in milk. The simplicity and short time of analysis make it a convenient alternative over earlier methods. Reproducibility was found to be very good, and the limit of detection was estimated to be 0.0089 μg/mL.

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