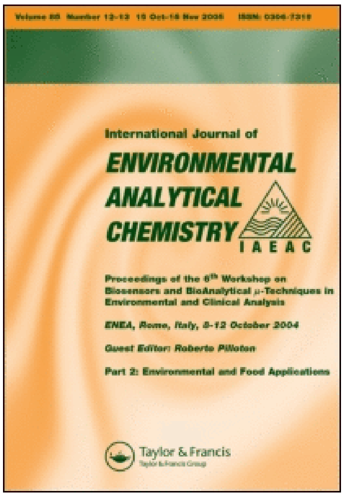

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

The Determination of Formaldehyde in Beer and Soft Drinks by HPLC of the 2,4- Dinitrophenylhydrazone Derivative

J. F. Lawrence[a]; J. R. Iyengar[a]
[a] Food Research Division, Health Protection Branch, Ottawa, Ontario, Canada

To cite this Article Lawrence, J. F. and Iyengar, J. R.(1983) 'The Determination of Formaldehyde in Beer and Soft Drinks by HPLC of the 2,4- Dinitrophenylhydrazone Derivative', International Journal of Environmental Analytical Chemistry, 15: 1, 47 — 52

To link to this Article: DOI: 10.1080/03067318308071912

URL: http://dx.doi.org/10.1080/03067318308071912

Intern. J. Environ. Anal. Chem. 1983, Vol. 15, pp. 47–52
0306-7319/83/1501-0047 $18.50/0

Printed in Great Britain

The Determination of Formaldehyde in Beer and Soft Drinks by HPLC of the 2,4-Dinitrophenylhydrazone Derivative

J. F. LAWRENCE and J. R. IYENGAR

Food Research Division, Health Protection Branch, Ottawa, Ontario, K1A 0L2, Canada

(*Received January 24, 1983*)

A simple procedure was developed for the determination of formaldehyde in samples of beer and soft drinks. A volume of sample was distilled and the aqueous distillate containing the formaldehyde collected directly in a solution of 2,4-dinitrophenylhydrazine. The resulting hydrazone was extracted and analysed by reversed-phase high-performance liquid chromatography with UV absorbance detection. Levels of formaldehyde found were in the low mg/kg range. Detection limits were less than 0.1 mg/kg of sample. Results were confirmed by mass spectrometry (probe) of the derivatives after fraction collection from the HPLC.

KEY WORDS: Formaldehyde, HPLC, 2,4-dinitrophenylhydrazone, beer, soft drinks.

INTRODUCTION

Formaldehyde has received increased attention in recent years due to its suspected carcinogenicity.[1,2] It is used in urea-formaldehyde and phenol-formaldehyde polymers which may be components in home insulation as well as in food contacting materials such as some plastic kitchenwares and the inner lining of cans used for beverages, particularly beer and soft drinks.

Although studies have been carried out on the presence of free formaldehyde in the air of buildings insulated with formaldehyde containing polymers and an expert report prepared,[3] little work has appeared in the literature on the determination of foods which have come in contact with such polymers. It is the point of this study to determine if there is a significant increase in the formaldehyde content of canned beverages due to the plastic inner coating of the metal container.

There have been reports on the leaching of formaldehyde into boiling water from melamine-, phenol-, and urea-formaldehyde plastics.[4–6]. These studies showed that leaching occurs in some cases up to 20–87 mg/l while most results were in the low-to-sub mg/l range.

Since formaldehyde is a normal metabolite of the cell, it has been found to occur naturally in many foods. Möhler and Denbsky[7] carried out a colorimetric determination of formaldehyde in a variety of foods including meat, poultry, fish and fruits and found that the levels were generally in the low mg/kg range (0.5–30 mg/kg). Methodology for formaldehyde in food commodities should, therefore, be capable of detecting less than 1 mg/kg. Several approaches have been used for the determination of formaldehyde including colorimetry,[7–9] gas chromatography,[10–12] and high performance liquid chromatography (HPLC),[13–15] the latter two techniques requiring derivatization usually with 2,4-dinitrophenylhydrazine. The 2,4-dinitrophenylhydrazone derivatives of aldehydes and ketones are strong absorbers of ultraviolet light and are rather stable permitting easy sample handling. This derivatization was particularly attractive for formaldehyde analysis since the compound itself is reactive and volatile and the derivatization offered a means to avoid losses due to these factors. In the present work the liquid samples were distilled directly into a solution of 2,4-dinitrophenylhydrazine. The resulting hydrazone product was determined by HPLC.

EXPERIMENTAL

Apparatus

The HPLC system consisted of an Altex model 110A pump, a Rheodyne syringe-loop injection port and a Hitachi Model-100-10-

variable wavelength detector set at 354 nm (the λ_{max} for formaldehyde-2,4-dinitrophenylhydrazone), and 0.02 absorbance units, full scale (AUFS). The separations were achieved on a 4.6 × 150 mm Ultrasphere ODS column with a mobile phase of 40% acetonitrile in water at a flow rate of 1.0 ml/min.

Sample analysis

A 100 ml volume of cold (*ca* 4°C) beverage was carefully poured into a 500 ml distillation flask containing 100 ml distilled water, 1 ml anti-foam (Dow–Corning) and 1 ml of 1*N* H_3PO_4, and the condenser immediately attached in the same manner as described earlier.[9] A heating mantle was placed around the flask and the temperature raised for distillation. The exit tip of the condenser was immersed in 20 ml of a solution of 2,4-dinitrophenylhydrazine (DNPH), 2.5 g/l in 6*N* hydrochloric acid. On top of this solution was added 25 ml of hexane to create a two-phase system. The distillation of the sample was continued until 100 ml of distillate were collected in the reagent solution. The solution was then permitted to react for 1 hr at 35°C. After this time the contents were transferred to a 500 ml separatory funnel and the hexane layer removed and retained. The aqueous phase was then extracted twice with 25 ml volumes of hexane and the organic phases combined. The organic solution was dried by the addition of about 1 g of anhydrous Na_2SO_4, then decanted and evaporated just to dryness by rotary vacuum evaporation at 30°C. The residue was dissolved in 50 ml of acetonitrile, 1 ml of which was diluted ten fold for soft drinks and 5 fold for beer for HPLC analysis. Normally, 20 μl were injected.

Mass spectrometric confirmation

For confirmation by mass spectrometry, 100 μl of the initial concentrated sample extract was injected into the HPLC system with the detector attenuation reduced to 0.2 AUFS. The fraction corresponding to the formaldehyde-DNPH derivative was collected in a 5 ml graduated centrifuge tube and the solution evaporated to dryness then dissolved in a small volume of acetonitrile for mass spectrometric identification by probe using a Varian MAT 311 A mass spectrometer at 67 ev, an emission current of 2000 mA and a resolution of 1000. The ion source temperature was 175°C.

RESULTS AND DISCUSSION

Initial preparation of formaldehyde-DNPH was done on a preparative scale[16] and purified by recrystallization from ethanol. Solutions of the pure derivative were used for development of the HPLC methodology.

For beverage analysis, the derivatization was carried out in essentially the same manner as described by Selim[17] where a two phased reaction system was used to improve reaction yields at trace levels of carbonyl compounds.

Figures 1 and 2 show typical chromatograms of some beverage samples. The chromatograms of the samples contained other peaks that were not identified which eluted close to but later than formaldehyde-DNPH. Neither of these were attributed to acetaldehyde-DNPH since authentic acetaldehyde derivative eluted much later than those peaks observed in the chromatograms. The

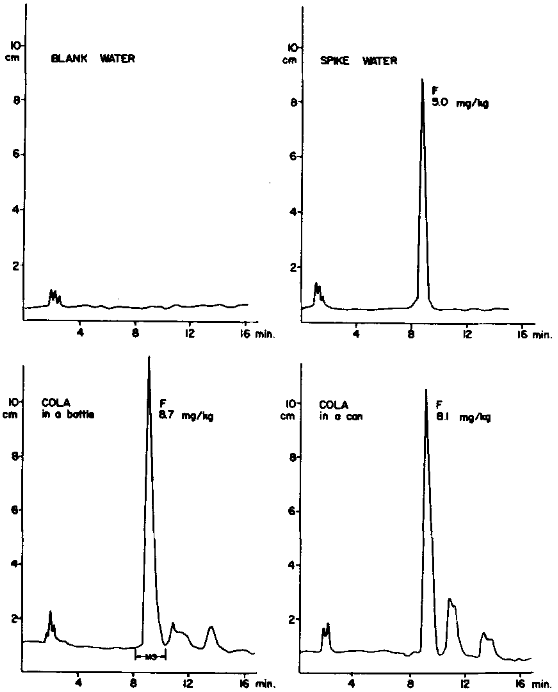

FIGURE 1 Chromatograms of *A*. 100 ml water blank and 100 ml of water spiked with 5 mg/kg of formaldehyde. *B*. Cola beverage analysis in bottle and can. *F* = formaldehyde-DNPH peak. Detector sensitivity, 0.5 AUFS. MS indicates the region collected for mass spectrometric analysis.

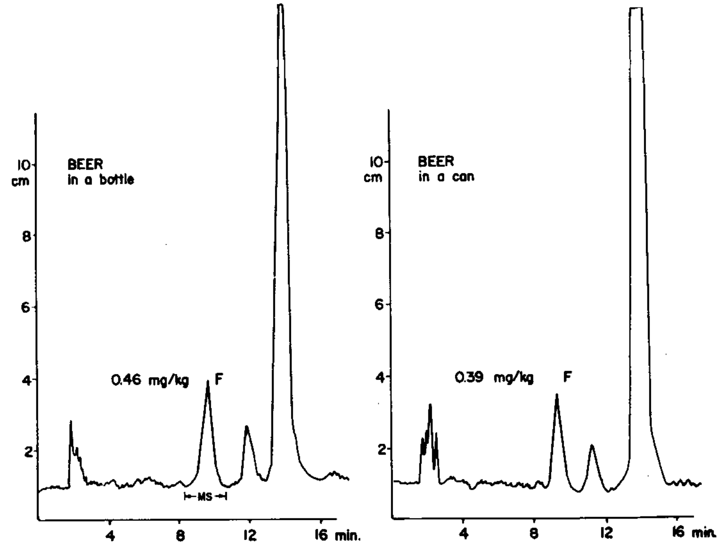

FIGURE 2 Chromatograms of two beer samples (bottle and can). *F* = formaldehyde-DNPH peak. Detector sensitivity, 0.02 AUFS. MS indicates the region collected for mass spectrometric analysis.

samples contained a great quantity of carbonyl containing compounds other than formaldehyde. This was indicated by the much deeper yellow color of the final solution after derivatization, compared to a water blank containing only added formaldehyde at 5–10 mg/kg. Also, upon washing the column after a series of analyses with 100% acetonitrile, a large quantity of unknown less polar DNPH derivatives were eluted. This is not surprising since carbonyl compounds are produced in the brewing process and also can result naturally from oxidation in other types of samples.

For quantitation, sample results were compared to a known standard dissolved in water and carried through the complete analytical procedure. Recovery values over the range of 0.5–5 mg/kg were 75 ± 5% with detection limits of less than 0.1 mg/kg.

In a survey of nine commercial beer samples the average levels found were 0.54 mg/kg (range 0.1–1.5 mg/kg) for canned samples and 0.70 mg/kg (range 0.4–1.4 mg/kg) for bottled beer. The results of two samples each of a commercial cola soft drink showed values of 7.4 and 8.5 mg/kg (can) and 8.7 and 8.1 mg/kg (bottle). The results for the beer are in the range of values normally expected. The soft drink samples were significantly higher in formaldehyde content than the beer although they were comparable to results obtained earlier in other food commodities.[7]

From the above results it is clear that no significant levels of formaldehyde are leached from polymer lined cans containing formaldehyde-resins. The samples analysed included the major beverage brands available in Canada.

Results in both selected beer and soft drink samples were confirmed by mass spectrometry. The mass spectrum of authentic formaldehyde-DNPH was similar to that obtained elsewhere,[5] exhibiting a strong M^+ ion at 210 m/e and other characteristic fragments at 180, 152, 122, 91, 79, 78, 63 and 51 m/e. Mass spectrometric identification of formaldehyde in the samples was easily carried out at levels of 1 mg/kg or greater. For lower levels, a repeated HPLC purification of the collected fraction was required to reduce the hydrocarbon background in the resulting mass spectrum.

References

1. U.S. National Institute of Occupational Safety and Health, Current Intelligence Bulletin No. 34 (April 15, 1981).
2. ECETOC Technical Report No. 2. European Chemical Industry, Ecology and Toxicology Center, Brussels, Belgium (May 13, 1981).
3. Final Report of the Department of National Health and Welfare Expert Advisory Committee on Urea-Formaldehyde Foam Insulation, Health and Welfare Canada (April, 1981).
4. H. Piekacz et al., *Loczniki Panst. Zaklady Higeny* **24,** 325 (1973).
5. Y. Watanabe, K. Sato, R. Yoshida and F. Endo, *Ann. Rep. Tokyo Res. Lab. P.H.* **31,** 195 (1980).
6. Y. Wada, S. Watanabe and T. Takahasi, *Shokuhin Eiseigaku Zasshi* **15,** 434 (1974).
7. K. Möhler and G. Denbský, *Zeitschrift Lebens, Unters, Forsch.* **42,** 109 (1970).
8. J. C. Underwood, *J. Assoc. Offic. Anal. Chem.* **47,** 548 (1964).
9. E. Kruger, *Monats, fur Brauerei* **21,** 155 (1968).
10. L. J. Papa and L. P. Turner, *J. Chromatogr. Sci.* **10,** 747 (1972).
11. H. Kallio, R. R. Linko and J. Kaitaranta, *J. Chromatogr.* **65,** 355 (1972).
12. Y. Hoshika and Y. Takata, *J. Chromatogr.* **120,** 379 (1976).
13. M. P. Maskarinec, D. L. Manning and P. Oldham, *J. Liq. Chromatogr.* **4,** 31 (1981).
14. K. Fund and D. Grosjean, *Anal. Chem.* **53,** 168 (1981).
15. K. Kuwata, M. Uebori and Y. Yamasaki, *J. Chromatogr. Sci.* **17,** 264 (1979).
16. R.L. Shriner, R. C. Fuson and D. Y. Curtin, *The Systematic Identification of Organic Compounds*, Wiley, New York, 5th ed. (1965) p. 253.
17. S. Selim, *J. Chromatogr.* **136,** 271 (1977).