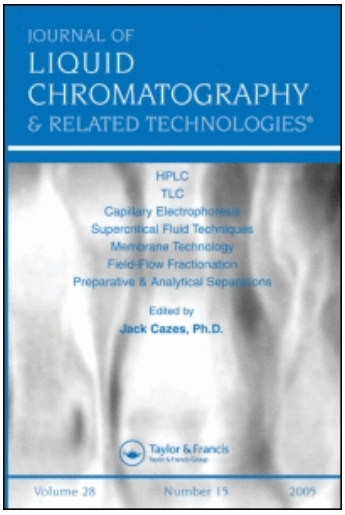

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Determination of Formaldehyde in the Polymerized Ragweed Antigen by HPLC

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DETERMINATION OF FORMALDEHYDE IN THE POLYMERIZED RAGWEED ANTIGEN BY HPLC

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ABSTRACT

Formaldehyde is used in the polymerization of ragweed antigen for the desensitization treatment of hay fever. Because the polymerized ragweed antigen is used as a vaccine which stimulates the production of the desired immunological effects upon injecting into the body, trace formaldehyde in the injected material is a health concern. Two procedures for the determination of formaldehyde were developed. In the direct procedure, formaldehyde in the sample was reacted with 2,4-dinitrophenylhydrazine (2,4-DNP) in solution to yield a strongly UV absorbing derivative. In an alternate approach, formaledhyde in the sample in a sealed vial was allowed to diffuse in the gaseous form into a reaction chamber consisted of a culture tube insert in which formaldehyde was derivatized with 2,4-DNP reagent. Following extraction into methylene chloride, the derivative was injected into the liquid chromatograph, separated on a reversed phase column and detected at UV 254 nm.

INTRODUCTION

Formaldehyde, an industrial toxin, is abundant in the atmosphere. It is present in the exhaust of gasoline and diesel engines as well as in tobacco smoke. Formaldehyde is also released into the air from certain synthetic polymers, i.e.

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phenolic resins, urea formaldehyde resins, and melamine formaldehyde. Formaledehyde is an ingredient in many domestic products. Exposure to formaldehyde results from the use of these resin products in insulators, textile and paper products; and also from a wide variety of household goods, including antiperspirants, wart remedies, mouth wash, and disinfectants, etc.

Exposure to formaldehyde in the concentration range of 1 to 5 ppm can produce primary irritation to the eyes, nose, and throat. Serious exposure can cause asthma and bronchitis (1). Further, contact with formaldehyde may cause skin irritation. The carcinogenicity of formaldehyde, however, is still a disputed issue. Because the high incidence of exposure to formaldehyde in the industrial community, trace level of this air pollutant is a serious enviromental problem. Both GC (2) and HPLC (3) procedures for the monitor of formaldehyde in ambient air have been reported.

Formaldehyde is used in the preparation of certain therapeutic substances. In the production of diphtheria and tetanus vaccines, formaldehyde is used to inactivate the organisms in the bacterial broth. The ragweed antigen, after polymerized with formaldehyde, is used as a vaccine for the desensitization treatment of hay fever. Injected into the body, the ragweed antigen vaccine produces the necessary immunogenicity without the allergenicty (4). However, trace of formaldehyde always remains in these vaccines. As a result of the required treatment with these vaccines, patients may also expose to formaldehyde. A procedure that measures the formaldehyde levels in these therapeutic agents is necessary for health regulatory purpose and consumer protection.

A colorimetric technique for the determination of formaldehyde using 2,4-DNP has been reported (5). However, the procedure is tedious and non-specific. When it is applied to measure the yellowish ragweed preparation, it requires an

accurate blank correction for the sample matrix. In this report, we describe two proceudres for the measurement of formaldehyde in the ragweed preparation. In the direct procedure, the formaldehyde sample was mixed and reacted with the 2,4-DNP solution. In the headspace procedure, formaldehyde in a ragweed sample placed in a sealed vial was allowed to diffuse in the gaseous form into a reaction chamber and derivatized with 2,4-DNP.

EXPERIMENTAL

Instrumentation

The liquid chromatograph consisted of an Altex Model 110A solvent pump and an Altex Model 153 UV detector (254 nm) (Altex Scientific Co., Berkeley, CA.). The chromatogram was recorded on a Linear recorder (Linear Instruments Corp., Irvine, CA). The sample was introduced by a Rheodyne 7125 injection valve onto a Supelcosil® column. The mobile phase consisted of 500 ml H_2O, 400 ml methanol and 0.2 ml H_3PO_4.

Chemicals and Reagents

Methanol and 37% formalin solution were purchased from Polyscientific Corp., Bayshore, NY. 2,4-Dinitrophenylhydrazine was obtained from Eastman Kodak Co., Rochester, NY. Phosphoric acid was bought from J.T. Baker Chemical Co., Phillipsburg, N.J. Methylene Chloride was obtained from Fisher Scientific Co., Fairlawn, N.J.

Procedure

A working derivatization reagent was prepared by dissolving 40 mg of 2,4-DNP in 25 ml of 2.5 N HCl. The direct determination of formaldehyde was accomplished by adding 25 ul of sample to 100 ul of 2,4-DNP working reagent in a 10 x 75 mm test tube. After

vortexing for several seconds, the reaction product was extracted into 200 ul of methylene chloride. A 25 ul aliquot of the extract was injected into the liquid chromatograph and the derivative was separated on a reversed phase column. The retention time of the formaldehyde 2,4-dinitrophenylhydrazone was 4.6 minutes. A calibration curve was constructed from a set of formaledhyde standards. The concentration of the sample corresponding to the peak height was obtained from the calibration curve.

The reaction set-up is shown for the headspace method (Figure 1). It consisted of a 4 ml screw cap vial with a 6x35 mm culture tube insert which served as the reaction chamber. One ml of the sample or standard to be analyzed was pipetted in the outer well and one hundred ul of 2,4-DNP solution in the inner. The vial was capped tightly and shaken gently to speed up the gaseous diffusion and the reaction of formaldehyde in the 2,4-DNP solution. A set of standards was run under the same experimental conditions. After 14 hours, 3.5% of the total formaldehyde in the sample reacted with the 2,4-DNP reagent in the inner well. At the end of the experiment, the reaction chamber was removed from the screw cap vial and 100 ul of methylene chloride was added to extract the hydrazone. A 25 ul aliquot of the extract was injected into the liquid chromatograph and analyzed as in the direct procedure.

RESULTS AND DISCUSSION

The determination of formaldehye by gas chromatography is difficult because it is polar and reactive, and could be irreversibly adsorbed onto the solid supports. Although the unmodified formaldehyde can be monitored by the thermoconductivity detector, the sensitivity is inadequate for trace analysis

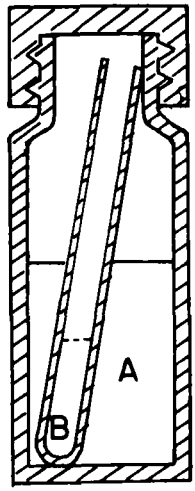

Figure 1. Set up of the headspace reaction vial. (A). Sample well - 4 ml screw cap vial. (B). Reagent well - 6x35 mm culture tube insert.

studies. With hydrogen flame ionization detectors, the signal responses to formaldehyde is insignificant because the absence of methyl carbon on the molecule. To enhance the detectability of formaldehyde by the flame detectors, derivatization, for example with 2,4-DNP, is necessary. However, among other problems associated with the derivatization technique (6), the major difficulty is thermal decomposition at the high temperature required to vaporize the modified formaldehyde.

Because its 2,4-DNP derivative absorbs strongly in UV, determination of formaldehye by HPLC is a simple procedure. Formaldehyde reacts rapidly with 2,4-DNP in acidic solution, and forms a precipitate. The precipitate is extracted into methylene chloride with an efficiency of 96%. Although an equally good extraction solvent, the use of ethyl acetate, which may contain traces of aldehyde or ketone which interferes with the assay, is discouraged. A 25 ul aliquot of the organic phase can be

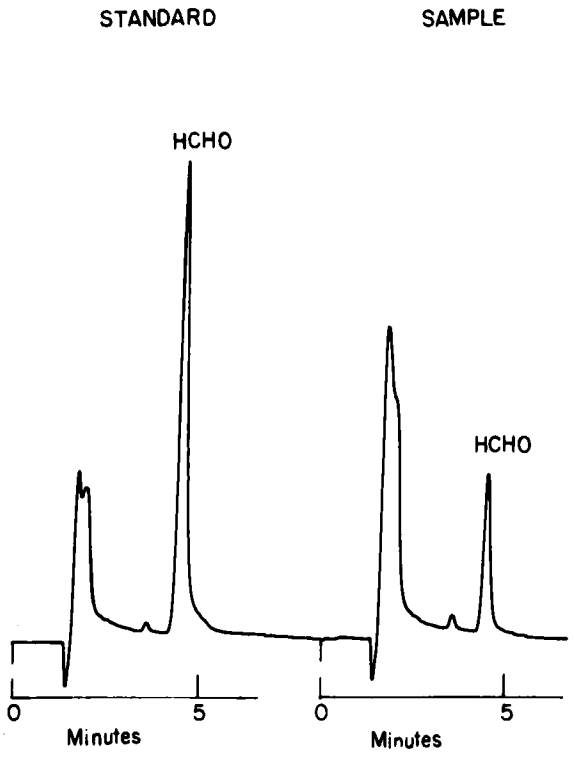

Figure 2. Chromatogram of formaledehyde, (A) in standard; and (B) in ragweed antigen sample.

injected into the liquid chromatograph without pre-concentration. The chromatograms of a formaldehyde standard and a ragweed antigen sample are shown (Figure 2). Low molecular weight carbonyls, acetaldehyde and acetone, evidently not interfere in this procedure are separated from formaldehyde (Figure 3). A calibration curve of formaldehyde at a ten inch full scale absorbance of 0.8 A.U. is shown (Figure 4). The limit of detection of 1 nanogram with a signal to noise ratio of 5 was achieved. By concentrating the organic extract, the detection limit can be improved at least by an order of magnitude. In the repeated analysis of 8 samples, a coefficient of variation of 2.5% was obtained.

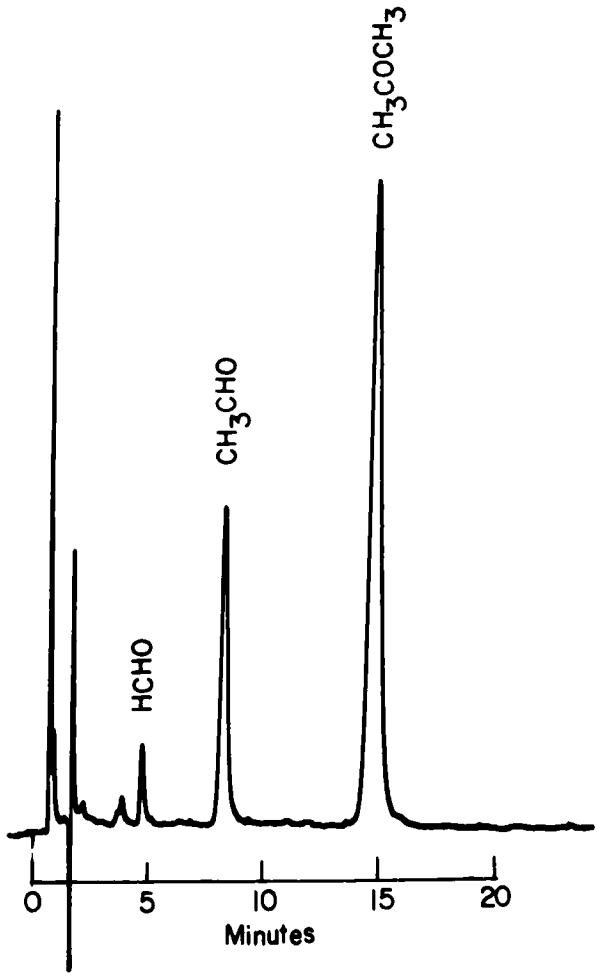

Figure 3. Chromatogram of low molecular weight carbonyls.

Since the ragweed sample may be sensitive to acid hydrolysis in the derivatization solution, formaldehyde which is used for the polymerization of the ragweed antigen could be released to give a falsely high result. A headspace procedure for the determination of formaldehyde was devised to investigate the possibility. The gas phase reaction set-up is shown (Figure 1).

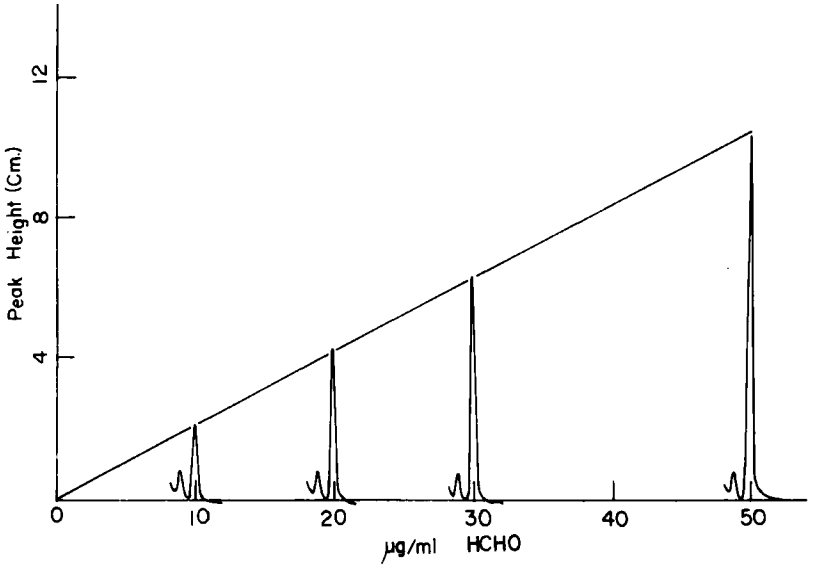

Figure 4. Standard curve by the direct procedure.

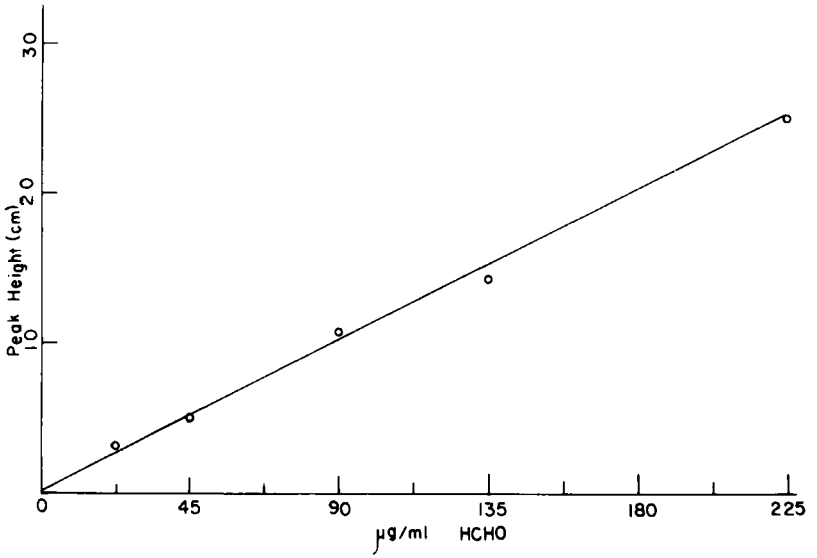

Figure 5. Standard curve by the indirect headspace procedure.

Because less sensitive in the headspace procedure due to incomplete reaction, the detector attenuation was set at 0.01 A.U.F., whereby a concentration of 1 ug/ml could be easily detected. A linear calibration curve that relates the concentration of formaldehyde to the peak height is shown (Figure 5). The headspace procedure gave results that agreed with the direct procedure and assured the integrity of the polymerized ragween antigen that the bonding was not disrupted under the 2.5N HCl derivatization condition.

In conclusion, two procedures for the determination of formaldehyde are described. The direct procedure is fast and sensitive while the diffusion procedure is more specific. The headspace procedure is an excellent alternative when the sample is sensitive to the derivatization reagent, or contains potential non-volatile interferences as in the determination of volatile aldehydes and ketones in serum which is abundant in non-volatile carbonyls such as sugars and ketoacids (7).

REFERENCES

1. D.K. Harris, British J. Medicine, 10, 255 (1953).

2. Y.U. Yokouchi, T. Fujh, Y. Ambe and K. Fuwa, J. of Chromatogr., 180, 133 (1979).

3. K. Fung and D. Grosjean, Anal. Chem. 53, 168 (1981).

4. W.A. Check, JAMA 243, 1703 (1980).

5. M.B. Jacods, E.L. Eastman and D.L. Shepard, J. Am. Pharm. Assoc., XL, 365 (1951).

6. L.J. papa and L.P. Turner, J. Chromatogr. Science, 10, 744 (1972).

7. A.R. Stowell, K.E. Crow, R.M. Greenway and R.D. Batt, Anal. Biochem., 84, 384 (1978).