Communications to the Editor

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF FORMALDEHYDE ACCOMPLISHED USING HYDRALAZINE

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A reaction of formaldehyde with hydralazine (HP) under weakly acidic conditions resulted in a rapid, almost constant yield of s-triazolo[3,4-a]-phthalazine (Tri-P) which could be determined directly and sensitively by high-performance liquid chromatography without extraction. This procedure is believed to be a useful method for determining formaldehyde in aqueous samples.

KEYWORDS ——— formaldehyde; hydralazine; s-triazolo[3,4-a]phthalazine (Tri-P); high-performance liquid chromatography; UV detection; fluorometric detection

In previous papers,[1-3] we reported that hydralazine (1-hydrazinophthalazine, HP), a third-choice depressor of hypertension, reacts with nitrite ion in acidified human saliva to give tetrazolo[5,1-a]phthalazine (Tetra-P; Fig. 1). This transformation could be applied to the development of a simple and sensitive method for the determination of nitrite ion in ambient waters, foods and biological fluids by means of high-performance liquid chromatography (HPLC).[4-6] s-Triazolo[3,4-a]phthalazine (Tri-P), the formylation product from HP, was also found in an incubation mixture of HP in human saliva, together with the acetylation product, 3-methyl-s-triazolo-

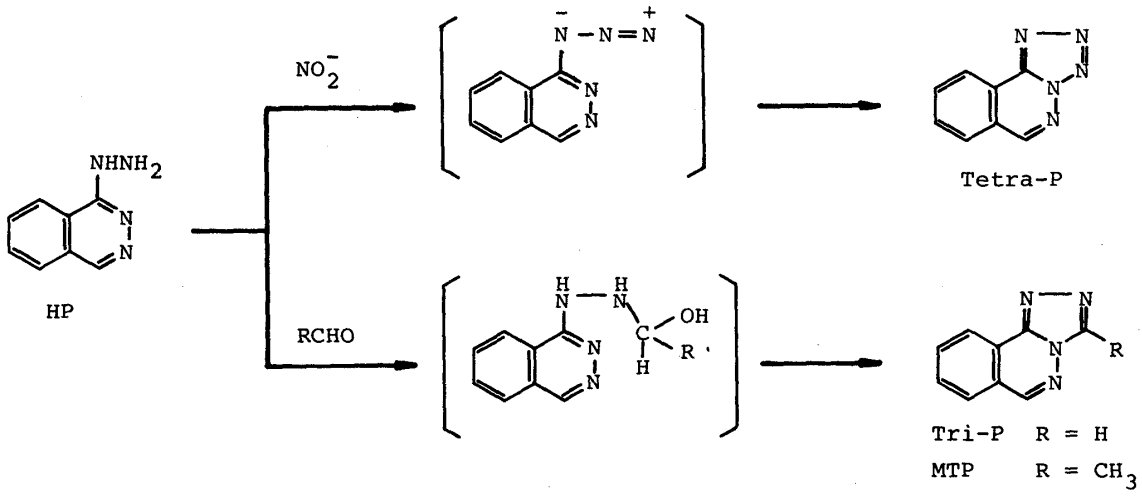

Fig. 1.

[3,4-a]phthalazine (MTP; Fig. 1).[1-3] Both products, as well as Tetra-P, were easily detected by HPLC in extracts from the urine of a patient on HP treatment.[7] On the other hand, for the analysis of formaldehyde, colorimetric determination has generally been used by measuring the condensation products with acetylacetone-ammonium acetate,[8] 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole[8,9] or chromotropic acid.[8,10] The colorimetry is not necessarily reliable because the samples are sometimes opaque and colored. A gas chromatographic method using 2,4-dinitrophenylhydrazine[11] or cyanide ion (cyanohydrin formation),[12] and HPLC of the condensates with acetylacetone-ammonium acetate[13] or with 2,4-dinitro-phenylhydrazine[14] have also been reported. But these require rather complex pretreatments such as the extraction of a derivative, drying and concentration of the solution prior to the analysis. So we attempted to develop a simple but precise HPLC method to determine formaldehyde in environmental samples using HP as a derivatizing agent.

A 1.0-ml aliquot of HP hydrochloride (HP-HCl) solution (100 μg in 1 ml of 0.2M KH_2PO_4) was added to 1.0 ml of an aqueous solution of formaldehyde, the concentration of which was less than 0.4 ppm. The mixture was heated in a boiling water-bath for 15 min. To the cooled reaction mixture, a 1.0 ml-volume of tetrazolo[5,1-a]isoquinoline (Tetra-I) in acetonitrile (5 μg/ml) was added as a reference standard (RS). A 10-μl sample was injected for HPLC. A typical example of a HPLC chromatogram of the reaction mixture of formaldehyde (0.2 ppm) and 76 equivalents of HP-HCl is shown in Fig. 2. Under the analytical condition described in the legend, the excess of HP was first eluted along with the reaction medium (t_R = 2.2 min). After that two sharp peaks corresponding to Tri-P and RS (Tetra-I) appeared (t_R = 4.1 and 12.6 min, respectively). A calibration curve was made by plotting the peak-height ratio of Tri-P and RS against the amounts of formaldehyde. An excellent linear relationship with good reproducibility existed between 0.02 and 0.4 ppm of the aldehyde: <UV detection> regression line, Y = 3.91X + 0.110; correlation coefficient, r = 0.9997; the coefficient of variation for 0.02, 0.05, 0.1, 0.2 and 0.4 ppm of formaldehyde was 4.1, 2.5, 1.2, 2.3 and 0.7% (n=5), respectively. <FL detection> regression line, Y = 5.36X + 0.138; correlation coefficient, r = 0.9997; the coefficient of variation for 0.02, 0.05, 0.1, 0.2 and 0.4 ppm of formaldehyde was 3.7, 2.5, 1.1,

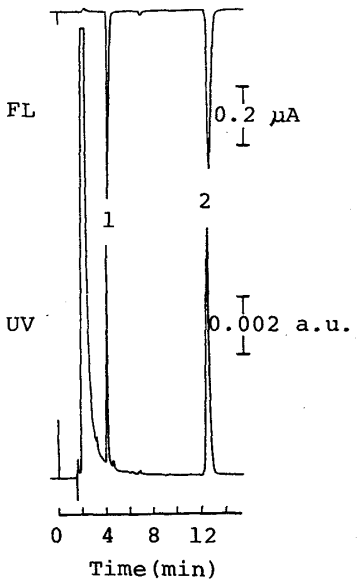

Fig. 2. High-Performance Liquid Chromatogram of the Reaction Mixture of Formaldehyde (0.2 ppm) and HP-HCl

Conditions: apparatus, HLC-803D (Toyo Soda); column, TSKgel ODS-120A, 15 cm x 4.6 mm I.D.; mobile phase, 30% acetonitrile in 0.05 M KH_2PO_4; flow-rate, 1.0 ml/min at ambient temperature. Detection by UV: 237 nm, 0.02 a.u.f.s.(bottom). Detection by fluorescence, excitation = 237 nm, emission > 370 nm, 2.0 μA f.s.(top). peak: 1 = Tri-P, 2 = Tetra-I (reference standard).

2.0 and 0.6% (n=5), respectively.

The HPLC method described here for the determination of formaldehyde in environmental samples (food, drinking water, clothes, drug preparations, containers, etc.)is convenient, reliable and widely applicable. Further studies are in progress for the development of a general method for the determination of carbonyl compounds. The details will be reported in the near future.

REFERENCES

1) A.Noda, K.Matsuyama, S.-H.Yen, N.Otsuji, S.Iguchi, and H.Noda, Chem. Pharm. Bull., 27, 1938 (1979).
2) A.Noda, K.Matsuyama, S.-H.Yen, K.Sogabe, Y.Aso, S.Iguchi, and H.Noda, Chem. Pharm. Bull., 27, 2820 (1979).
3) H.Noda, A.Noda, K.Matsuyama, S.-H.Yen, and S.Iguchi, J. UOEH (Sangyo-idai Zasshi), 1, 339 (1979).
4) H.Noda, M.Minemoto, A.Noda, K.Matsuyama, S.Iguchi, and T.Kohinata, Chem. Pharm. Bull., 28, 2541 (1980).
5) H.Noda, M.Minemoto, T.Asahara, A.Noda, and S.Iguchi, J. Chromatogr., 235, 187 (1982).
6) H.Noda, M.Minemoto, S.Eto, A.Noda, and K.Matsuyama, Yakugaku Zasshi, 104, 409 (1984).
7) A.Noda, H.Noda, M.Minemoto, K.Zaitsu, Y.Ohkura, and S.Iguchi, Chem. Pharm. Bull., 29, 2683 (1981).
8) Standard Methods of Analysis for Hygienic Chemists, with Commentary, 1980, Pharmaceutical Society of Japan (ed.), Kanehara, Tokyo, 1980, pp. 80-81, 1131-1133.
9) G.Avigad, Anal. Biochem., 134, 499 (1983).
10) L.Chafetz, W.Hong, D.C.Tsilifonis, A.K.Taylor, and J.Philip, J. Pharm. Sci., 73, 1186 (1984).
11) K.Kido, and T.Sakuma, Eisei Kagaku, 25, 39 (1979).
12) C.Improta, G.Nota, C.Ferretti, and U.Papale, J. Chromatogr., 285, 385 (1984).
13) M.Okamoto, and F.Yamada, Yakugaku Zasshi, 101, 378 (1981).
14) J.F.Lawrence, and J.R.Iyengar, Int. J. Environ. Anal. Chem., 15, 47 (1983).

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