Communications to the Editor

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High-Performance Liquid Chromatographic Determination of Nitrite Ion by Use of Hydralazine

Hydralazine reacts with nitrite ion under an acidic condition at 37° to form tetrazolo-[5,1-*a*]phthalazine (Tetra-P) almost quantitatively. Without extraction, Tetra-P was determined sensitively by high-performance liquid chromatography. This method is more specific than the conventional methods for the determination of nitrite ion.

Keywords——nitrite determination; hydralazine (HP); tetrazolo[5,1-*a*]phthalazine (Tetra-P); high-performance liquid chromatography; UV detection

In the previous papers, we reported that hydralazine (1-hydrazinophthalazine, HP), an effective depressor for essential hypertention, reacts with nitrite in human saliva under acidic conditions to form tetrazolo[5,1-*a*]phthalazine (Tetra-P) together with an acetylated product, 3-methyl-*s*-triazolo[5,1-*a*]phthalazine (MTP), which were determined by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).[1,2] Tetra–P was also found in rabbit urine when an aqueous solution of sodium nitrite was given before and after the oral administration of HP. The Tetra–P formation from HP and sodium nitrite *in vitro* (Chart 1) was very rapid in acidic buffer solution (pH 1.1, 2.1 and 3.0) at 37°,[1,2] suggesting that the choice of an adequate condition may afford Tetra–P quantitatively in preference to MTP. For nitrite analysis, the colorimetric[3] and GC[4] methods have generally been used. The former, in which sulfanilamide is diazotized by nitrite under acidic conditions and the diazonium salt is coupled with naphthylethylenediamine to give an azo dye, possesses some uncertainty in specificity which is crucially important for a microanalysis. The latter, involving a reaction of nitrite with *o*-phenylenediamine to form 1*H*-benzotriazole, requires complex procedures prior to GC analysis. These facts prompted us to develope a high-performance liquid chromatographic method (HPLC) for the determination of nitrite ion by use of HP as a derivatizing agent.

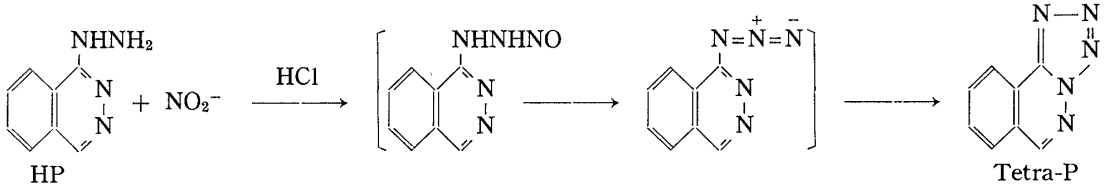

Chart 1

A 100 μl portion of HP hydrochloride (HP–HCl) dissolved in 1 N HCl (70 μg/ml) was added to 1.0 ml of aqueous solution of sodium nitrite, concentration of which was less than 0.10 ppm as nitrite nitrogen. After adjusting the pH to about 1.1, the mixture was incubated at 37° for 1 hr. A 100 μl portion of phenytoin (PHT) methanol solution (120 μg/ml) as an internal standard was added to the reaction mixture, and a 20 μl aliquot was injected directly to HPLC.

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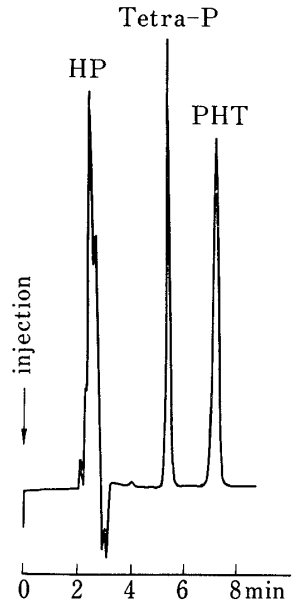

Fig. 1. High-Performance Liquid Chromatogram of the Reaction Mixture of Nitrite Ion (Nitrite Nitrogen: 0.10 ppm) and HP-HCl

conditions: apparatus, HLC-803A (Toyo Soda); detection, UV 228 nm; column, TSK-GEL LS-410 ODS SIL (P-5), 250 mm × 4.6 mm i.d.; mobile phase, 40% acetonitrile in 0.05M phosphate buffer (pH 3.0); flow rate, 1.0 ml/min at ambient temperature; 0.04 a.u.f.s.

Figure 1 shows the HPLC chromatogram of the reaction mixture of nitrite ion (corresponding to 0.10 ppm nitrite nitrogen) and fivefold equivalent of HP–HCl. Under the condition employed, intact HP was eluted together with the reaction solvents 2.5 min after the injection, and the retention times of Tetra–P and PHT were 5.5 and 7.3 min, respectively. Calibration curve was prepared by plotting the peak-area ratio of Tetra–P to PHT against the amounts of nitrite nitrogen. A linear relationship was obtained in range of 0.001 to 0.10 ppm of nitrite nitrogen (nitrite ion: 0.003 to 0.33 ppm) (regression line: $Y=10.10X+0.022$; correlation coefficient: 1.000; recovery: 98.1%).

The present HPLC method using HP is a useful improvement for nitrite determination because of its simplicity, sensitivity, specificity and good reproducibility (coefficients of variation for 0.001, 0.01, 0.02, 0.04, 0.06, 0.08 and 0.10 ppm nitrite nitrogen: 14.4, 2.1, 3.6, 1.4, 0.6, 0.3 and 0.6%; N=5). The studies on nitrite determination from foods and biological fluids are in progress and the details will be reported in the nearest future.

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Stereospecific Inversion of Configuration of 2-(2-Isopropylindan-5-yl)-propionic Acid in Rats

The *d*-tertiary hydroxy metabolite, which was isolated from rat urine after oral administration of 3,3,3-d_3-*l*-2-(2-isopropylindan-5-yl)propionic acid (IIPA), was confirmed