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## Note

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### Rapid and sensitive high-pressure liquid chromatography technique for detecting impurities in benzaldehyde U.S.P.

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Benzaldehyde U.S.P. is used as flavor because of its bitter almond taste. The U.S.P. specifies color tests for hydrocyanic acid, chlorinated compounds and nitrobenzene<sup>1</sup>. The test for hydrocyanic acid is included because benzaldehyde has been obtained from cyanogenic glycosides such as amygdalin. The test for chlorinated compounds is included because benzaldehyde can be obtained by hydrolysis of benzal chloride. The test for nitrobenzene is required because the odor of the very toxic nitrobenzene is, for all practical purposes, indistinguishable from that of benzaldehyde. Interestingly, the U.S.P. does not specify a test for toluene which also is a synthetic precursor of benzaldehyde<sup>2</sup>. Further, the U.S.P. does not stipulate a limit for benzoic acid which quite readily forms by auto-oxidation of benzaldehyde<sup>3</sup>.

Earlier, we described procedures for detecting nitrobenzene which were much more rapid than the U.S.P. procedure and for the most part, more sensitive<sup>4</sup>. These procedures did not detect any of the other possible impurities, nor were they readily quantifiable.

The U.S.P. assay for benzaldehyde, itself, requires several steps. It involves formation of the oxime by reacting benzaldehyde with hydroxylamine hydrochloride and titrating the liberated hydrochloric acid.

It was our goal to develop a procedure that would detect the impurities that could commonly be found in benzaldehyde U.S.P. in one step and, at the same time, the procedure should lend itself to being quantified.

## EXPERIMENTAL

### Reagents and chemicals

Benzaldehyde (Mallinckrodt, St. Louis, Mo., U.S.A.) and benzal chloride (Matheson, Coleman & Bell, Norwood, Ohio, U.S.A.) were redistilled *in vacuo*. Nitrobenzene, analytical-reagent grade (Mallinckrodt) and benzoic acid U.S.P. (Mallinckrodt) were used without further purification. The solvents in the mobile phase were analytical reagent grade and used as received.

### Chromatographic conditions

The mobile phase was 90% cyclohexane and 10% chloroform. After degassing

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the solvents, they were mixed by a Waters Assoc. Model 660 solvent flow programmer. Other instrumentation included two Waters Assoc. Model M-6000 pumps, a Waters Assoc.  $\mu$ Porasil column, a Waters Assoc. U6K universal injector, a Varian Model 635 LC variable wavelength detector and a Soltec 10-in. dual-pen recorder. The flow-rate was 4 ml per min and the UV detector set at 259 nm and 0.1 a.u.

#### *Stock solution*

*Nitrobenzene.* Nitrobenzene was diluted in redistilled benzaldehyde in the following concentrations (v/v): 1:1000, 1:2000, 1:4000, 1:8000, 1:100,000 and 1:200,000.

*Benzoic acid.* Benzoic acid was diluted in redistilled benzaldehyde in the following concentrations: 1:1000, 1:2000, 1:8000 and 1:10,000.

*Benzal chloride.* Redistilled benzal chloride was diluted in redistilled benzaldehyde in the following concentrations (v/v): 1:100, 1:200, 1:400, 1:800, 1:1000 and 1:1600.

#### *Procedure*

Each of the stock solutions was made in duplicate; 1- $\mu$ l injections were made in triplicate.

### RESULTS AND DISCUSSION

The purpose of this study was to evaluate the use of high-pressure liquid chromatography as a rapid qualitative screen for detecting impurities in benzaldehyde U.S.P. Initially, we tried a variety of conditions using gas chromatography. In every situation, the large benzaldehyde peak was so broad and retained so long on the column that it masked any of the potential impurities.

We then decided to examine a reversed-phase high-pressure liquid chromatographic system. This had several deficiencies, including the fact that benzaldehyde was eluted with considerable tailing ahead of nitrobenzene and benzal chloride. The result is that these two impurities are not seen as sharp peaks when dissolved in a large excess of benzaldehyde. Adsorption chromatography worked quite well for all but toluene. The toluene came off with the solvent front. We decided not to pursue the detecting of toluene further since the U.S.P. ignores the presence of this potential impurity. Adsorption high-pressure liquid chromatography does work for the two impurities of importance to the U.S.P. plus benzoic acid which is almost ubiquitous once a bottle of benzaldehyde is opened. Fig. 1 shows the tracings of typical chromatograms.

For benzaldehyde, the chromatographic procedures described previously<sup>4</sup> and in this report were more sensitive than the U.S.P. color test. Using thin-layer chromatography, 12  $\mu$ g of nitrobenzene could be detected in a 10- $\mu$ l sample of benzaldehyde. High-pressure liquid chromatography permitted the detection of 6 ng in a 1- $\mu$ l sample of benzaldehyde. The high-pressure liquid chromatography procedure is rapid, about 5 min, as compared to 1 $\frac{1}{2}$  h for the U.S.P. procedure.

The U.S.P. flame test for benzal chloride is more sensitive than the high-pressure liquid chromatography procedure. In our hands, the flame test produced a nice bright green color at the minimum detectable limits by high-pressure liquid chromatography (0.788  $\mu$ g in a 1- $\mu$ l sample). Benzoic acid, the ubiquitous impurity in benzaldehyde, could be detected at 0.1  $\mu$ g in a 1- $\mu$ l sample. The potential limits of sensitivity for all these substances could be about 20 times greater using a more sensitive ultraviolet detector with 0.005 a.u.

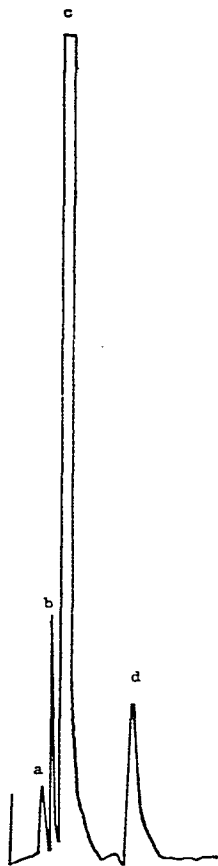


Fig. 1. (a) Benzal chloride, 1.18 min. (b) Nitrobenzene, 1.48 min. (c) Benzaldehyde, 2.21 min. (d) Benzoic acid, 4.43 min, 0-0.1 a.u.

There was no attempt to quantify this procedure by using an internal standard. However, we did note that a simple measurement of peak heights did give a statistically valid linear relationship with concentrations for each of the impurities.

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#### REFERENCES

- 1 *The United States Pharmacopeia, XIX*, United States Pharmacopeial Convention, Rockville, Md., 1975, pp. 551-552.
- 2 *Merck Index*, Merck & Co., Rahway, N.J., 9th ed., 1976, pp. 136-137.
- 3 R. F. Doerge, in C. O. Wilson, O. Gisvold and R. F. Doerge (Editors), *Textbook of Organic Medicinal and Pharmaceutical Chemistry*, J. B. Lippincott, Philadelphia, 7th ed., 1977, p. 964.
- 4 J. H. Block, M. A. Nunes and R. O. Muhlhauser, *J. Pharm. Sci.*, 59 (1970) 1008.