# Alternatives to the *National Formulary* Procedure for Detecting Nitrobenzene in Benzaldehyde

JOHN H. BLOCK*, MATHEWS A. NUNES†, and RICHARD O. MUHLHAUSER

**Abstract** □ A TLC technique has been developed which can be used to detect microgram quantities of nitrobenzene in benzaldehyde. This method, with its sensitivity and simplicity, provides a great advantage over the current detection procedure in the NF XII. A color complex reaction utilizing sodium pentacyanoamine ferroate and a spot test procedure on filter paper were also investigated, and each of these offers specific advantages over the current NF procedure. A mechanism for formation of the color complex is postulated.

**Keyphrases** □ Nitrobenzene detection—benzaldehyde □ TLC—analysis □ UV light—TLC spot visualization □ Colorimetric analysis □ Pentacyanoamine ferroate—color reagent □ Spot testing—nitrobenzene in benzaldehyde

Benzaldehyde has been in the official compendia since 1905 when it became a part of the *United States Pharmacopeia VIII*. In 1936 benzaldehyde was deleted from the USP and became official in the *National Formulary VI*. In 1916, a test for the detection of nitrobenzene became a part of the monograph, and this same test is still in the present revision. The test for nitrobenzene is included because it has an odor similar to benzaldehyde but is extremely toxic, having a lethal dose of 5–50 mg./kg. in man (1). Nitrobenzene is toxic by all routes and is capable of causing methemoglobinemia and destruction of red blood cells (2). The official test involves reduction of nitrobenzene to aniline and then oxidation with potassium dichromate to give a purple color if nitrobenzene is present in the sample (3). A problem which is often encountered is the formation of a precipitate that may make detection of the color difficult if not impossible. Furthermore, since the test is run by different technicians, results may be interpreted differently.

Aromatic nitro compounds have been detected by spot tests (4) and by formation of a colored complex (5). Interpretation of these results, however, is quite difficult for small amounts of nitrobenzene, and the procedures involve many steps. A spectrophotometric procedure was developed in 1949 by Glazko *et al.* (6); although quantitative results are obtained, the procedure is quite involved and lengthy.

To overcome problems associated with interpretation of results and complicated procedures, this paper reports and evaluates three different procedures for the detection of nitrobenzene in benzaldehyde.

## EXPERIMENTAL

**Reagents**—The following chemicals and reagents were used without further purification: benzaldehyde NF and nitrobenzene (Matheson, Coleman & Bell), sodium fluorescein (Eastman Organic Chemicals), sodium pentacyanoamine ferroate (K & K Laboratories), and silica gel G and silica gel HF (Brinkmann Instruments, Inc.). Visualization was made by means of a General Electric purple-X 250 w. UV light.

**Table I**—Reagents and Results Obtained by Color Complexation Procedure

| | | | |
|---|---|---|---|
| Benzaldehyde | Neg. | Butylamine | Neg. |
| Nitrobenzene | Pos. | Dimethylamine | Neg. |
| Aniline[a] | Pos. | Ethylbenzene | Neg. |
| *N*-Methylaniline | Neg. | Xylene | Neg. |
| *N,N*-Diethylaniline | Neg. | 2-Methylnaphthalene | Neg. |
| *N*-Benzylisopropylamine | Neg. | Phenol | Neg. |
| *N,N*-Dibenzylaniline | Neg. | Anisole | Neg. |
| *p*-Aminobenzoic acid | Pos. | *o*-Anisidine | Pos. |

[a] A positive result was obtained also without the reduction step.

**NF Procedure**—Solutions analyzed by the NF XII procedure were nitrobenzene, benzaldehyde, and nitrobenzene in benzaldehyde 5:100 and 1:100. (All dilutions are by volume.)

**Spot Tests (4)**—Samples analyzed were nitrobenzene, benzaldehyde, and nitrobenzene in benzaldehyde 1:100 and 1:1000. Two drops of each solution was placed on a piece of filter paper, and 1–2 drops of 10% stannous chloride in ethanol saturated with hydrogen chloride gas was added. After absorption, 1 drop of 35% cuprous chloride in concentrated hydrochloric acid was added and allowed to be absorbed. Six drops of pyridine was added and a green color developed. Two drops of carbon tetrachloride was then added and the characteristic colors formed in 10–30 sec.

**Color Complex (5)**—Samples analyzed were nitrobenzene, benzaldehyde, and nitrobenzene in benzaldehyde 1:100, 1:1000, and 1:10,000. One-tenth milliliter of the solution to be analyzed was dissolved in 3 ml. of ethanol, previously heated on a hot water bath, in a 10-cm. (4-in.) test tube. Six drops of 10% calcium chloride solution and about 50 mg. of zinc dust were added, and the solution was heated to boiling in a hot water bath. The excess zinc was removed by filtration, and the cooled filtrate was treated with 1 drop of a 1% solution of sodium pentacyanoamine ferroate. A colored flocculent precipitate formed in about 1 min., the time required depending upon the amount of nitrobenzene present. Best results were obtained when the solution was not stirred. Dilute samples required 2–3 hr. if the solution was stirred after the addition of the sodium pentacyanoamine ferroate solution.

Additional samples were also tested to learn something about the nature of the complex formed. The samples used and results appear in Table I.

**Thin-Layer Chromatography**—TLC was carried out on 5 × 20-cm. chromatoplates prepared according to Stahl, utilizing silica gel G, with 0.04% sodium fluorescein, and silica gel HF. Eight solvent systems were investigated (7–9) (Table II); however, only the visualization technique of Berei and Vasaros (7) was employed.

The other adsorbent was investigated with the 2.5% acetone in benzene solvent system.

Samples analyzed were nitrobenzene, benzaldehyde, and nitrobenzene in benzaldehyde 1:100, 1:1000, and 1:10,000. In all of the initial chromatograms, normal spotting procedures using a capillary tube were employed. The sensitivity of this procedure was also evaluated; for this test, 10 μl. of the solution was spotted *via* a calibrated micropipet.

## RESULTS AND DISCUSSION

**NF Procedure**—The 1:100 solution gave a negative result for nitrobenzene using this procedure. The 5:100 solution did give positive results; however, another analyst may well have judged the results to be negative when compared with a control sample.

**Spot Tests**—The pure sample of benzaldehyde gave a predominantly blue spot, and the nitrobenzene produced a definite red-orange color. However, problems were encountered in trying to

Table II—Solvent Systems Investigated Using Silica Gel G with 0.04% Sodium Fluorescein

| |
|---|
| Benzene–methanol–acetic acid, 45:8:4 |
| Petroleum ether–ethyl acetate, 4:1 |
| Diethyl ether |
| Acetone (2.5%) in benzene |
| *n*-Hexane–diisopropyl ether, 6:4 |
| Cyclohexane–diethyl ether–diisopropyl ether, 2:1:1 |
| Cyclohexane–diethyl ether, 2:1 with 10% silicone oil |
| Benzene–ethyl acetate, 5:1 |

distinguish the characteristic orange color produced by the nitrobenzene from the yellowish discoloration of the filter paper due to the other chemicals. The 1:100 dilution was definitely positive; but with the 1:1000 dilution, it was not possible to determine whether the test was positive when compared with a control sample.

**Color Complex**—In this test, pure benzaldehyde produced a yellow precipitate whereas pure nitrobenzene produced a deep-purple precipitate. As the amount of nitrobenzene in the sample was reduced, the intensity of the color faded through blue to a blue-gray color for the 1:1000 dilution. No bluish hue was distinguishable for the 1:10,000 dilution.

The basis of these tests is the reduction of nitrobenzene and subsequent reaction with an appropriate reagent to produce a characteristic color. In the NF XII procedure the reduction is carried out with zinc and sulfuric acid, followed by the addition of potassium dichromate to yield a blue-green colored product if nitrobenzene was originally present. In the spot test procedure, stannous chloride and HCl reduce the nitro group to the primary aromatic amine. The color formed is most likely due to the formation of a complex between the amine and the cuprous ion (4).

In the colored-complex procedure, reduction is accomplished with zinc dust and the calcium chloride solution. The color formed is probably due to the formation of a complex between the reduced nitro group and the $Fe(CN)_5^{-3}$ moiety of the sodium pentacyanoamine ferroate, $Na_3[Fe(CN)_5NH_3]$. Two possibilities exist: (*a*) that there is a replacement of the $NH_3$ ligand by the $—NH_2$ portion of the aromatic amine or (*b*) that a charge transfer complex exists between iron and $\pi$ electrons of the phenyl ring (10). On the basis of data in Table I, it seems reasonable to postulate that the complexation consists of a replacement of $NH_3$ ligand by the primary aromatic amine. If a charge transfer complex formed, all the aromatic compounds with strongly electron-donating groups would have been expected to form the complex. The data also indicate that a primary aromatic amine is necessary for complexation. The differences in inductive effects of aniline and *N*-methylaniline are so small that a charge transfer complex should have been formed with both. The methyl group of *N*-methylaniline, in addition to providing steric hindrance for ligand replacement, prevents overlap of the unshared *p*-orbital electrons of the nitrogen with the $\pi$ electrons of the phenyl ring. It is this delocalization of electrons that is probably of primary importance in color change, as pointed out by the fact that the aliphatic amines tested did not delocalize and gave negative results. It would seem that phenol, which also has an unshared pair of electrons, should also form the complex. However, Basolo and Johnson have pointed out that the greater the base strength of a ligand, the greater the stability of the complex formed (11). Thus, aniline, which has a greater basicity than phenol, will form a more stable complex.

**Thin-Layer Chromatography**—Of the eight solvent systems examined, none gave better separation than that of 2.5% acetone in benzene. The solvent systems of benzene–methanol–acetic acid and of diethyl ether provided very poor separation, and the other systems used gave results similar to the 2.5% acetone in benzene system. This acetone–benzene system had the added advantage of being one of the faster systems, requiring only about 40 min. for the solvent front to travel 15 cm.

Using the silica gel G with 0.04% sodium fluorescein, the 1:100 dilution showed a red-violet band ($R_f$ 0.93) which appeared purple under UV light. This band was not visible without irradiation at 1:1000 but was clearly seen with the aid of UV light. At a dilution of 1:10,000, this band was barely visible even with UV light, and the limit of the test was taken to be the 1:1000 dilution. Pure samples were also spotted for comparison, and the appearance of the chromatograms is shown in Fig. 1. Since aldehydes are easily

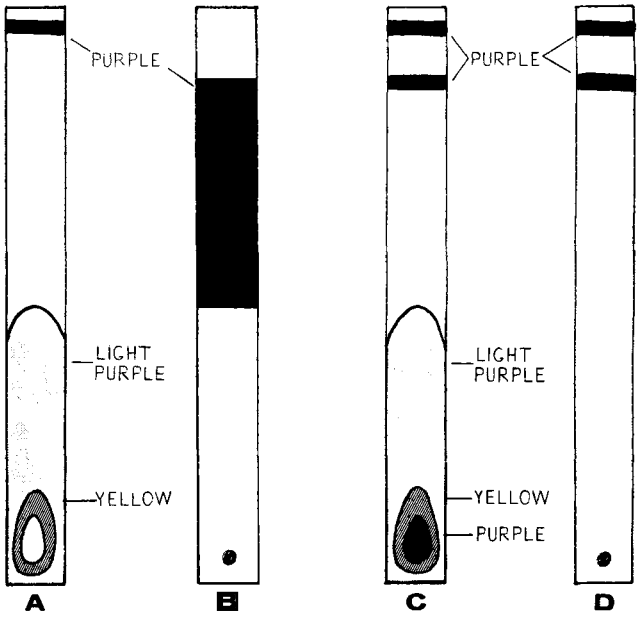


**Figure 1**—*Thin-layer chromatograms. Solvent system: 2.5% acetone in benzene. Plates: A, B, and C, silica gel G with 0.04% sodium fluorescein; D, silica gel HF. Key: A, benzaldehyde; B, nitrobenzene; and C and D, nitrobenzene in benzaldehyde, 1:1000.*

oxidized and possibly reduced, benzyl alcohol and benzoic acid were also chromatogramed and found not to interfere.

The silica gel HF showed the same sensitivity as the silica gel G but had the advantage of not showing the spots at the lower end of the plate which are attributed to the benzaldehyde. Using silica gel G and spraying with 0.04% sodium fluorescein failed to differentiate between the nitrobenzene and the benzaldehyde.[1]

Unlike the previous procedures, TLC does not require the reduction of nitrobenzene. Thus, it is possible to detect smaller amounts of nitrobenzene, since the other procedures are sensitive to only that portion of the nitrobenzene that has been reduced.

## SUMMARY

A TLC technique has been developed which can be used to detect small amounts of nitrobenzene. The estimated limit of this test is $10^{-5}$ ml. or 12 micrograms of nitrobenzene. This test is 100 times as sensitive as the color complex procedure. In addition, there are fewer mechanical steps involved and, therefore, less chance of error when compared with the NF procedure.

The spot test procedure has approximately the same sensitivity as the NF procedure, but it takes much less time. It requires only about 5 min. as compared to about 1.5 hr. for the monograph procedure.

The color complex reaction is much more sensitive than either the NF or the spot test procedure. Nitrobenzene was detectable in 0.1 ml. of the 1:100 dilution which corresponded to 1.2 mg. of nitrobenzene. This procedure is also quite simple and requires less than 0.5 hr. total.

On the basis of this investigation, it can be stated that the color complex and the TLC procedures are superior to the present NF procedure, both from the standpoint of sensitivity and time required for routine use. The thin-layer procedure is the simpler and more sensitive of the two and could presumably be adapted to obtain quantitative results.

## REFERENCES

(1) M. N. Gleason, R. E. Gosselin, and H. C. Hodge, "Clinical Toxicology of Commercial Products," Williams and Wilkins,

[1] Two commercial precoated silica gel products with fluorescent indicator failed to show spots with or without UV light.

Baltimore, Md., 1963, sec. II, inside front cover, and p. 85.
(2) S. Locket, "Clinical Toxicology," C. V. Mosby, St. Louis, Mo., 1957, p. 505.
(3) "National Formulary," 12th ed., Mack Publishing Co., Easton, Pa., 1965, p. 48.
(4) Y. D. Mogilyanskii, *Tr. Kom., Anal. Khim., Akad. Nauk SSSR*, **13**, 418(1963); through *Chem. Abstr.*, **60**, 1901d(1964).
(5) F. Feigl, "Spot Tests in Organic Analysis," 7th ed., Elsevier, New York, N. Y., 1966, p. 297.
(6) A. J. Glazko, L. M. Wolf, and W. A. Dill, *Arch. Biochem.*, **23**, 411(1949).
(7) K. Berei and L. Vasaros, *J. Chromatogr.*, **26**, 301(1967).
(8) K. Randerath, "Thin Layer Chromatography," Academic, New York, N. Y., 1963, p. 179.
(9) L. Frishbein, *J. Chromatogr.*, **27**, 368(1967).
(10) J. Nibler, personal communication.
(11) F. Basolo and R. Johnson, "Coordination Chemistry," W. A. Benjamin, New York, N. Y., 1964, p. 125.

ACKNOWLEDGMENTS AND ADDRESSES

Received September 2, 1969, from the *Departments of Pharmaceutical Chemistry and Pharmaceutical Sciences, School of Pharmacy, Oregon State University, Corvallis, OR 97331*

Accepted for publication January 19, 1970.

* To whom all correspondence should be addressed.

† Present address: School of Pharmacy, University of California Medical Center, San Francisco, CA 94122

# Spectrophotometric Determination of Chlorpromazine in Pharmaceutical Dosage Forms

**B. S. R. MURTY and R. M. BAXTER**

**Abstract** □ A rapid and convenient spectrophotometric method for the determination of chlorpromazine hydrochloride and its unit dosage forms is described. Extensive separation and extraction of the active ingredient are not required. The microsensitive color response ($\lambda_{max}$, 520 m$\mu$) with Van Urk's reagent is the basis of the analytical technique. The results are reproducible.

**Keyphrases** □ Chlorpromazine dosage forms—analysis □ Colorimetric analysis—spectrophotometry □ Van Urk's reagent—color formation

Chlorpromazine hydrochloride (CPZH), a phenothiazine derivative, is a widely used psychopharmacological agent. Chlorpromazine hydrochloride and some of its unit dosage forms are official in BP 1968 (1), USP XVII (2), and Ph.I. (3). A number of gravimetric, titrimetric, opticometric, electrometric, and chromatographic methods for the quantitative determination of phenothiazines have been reported in the literature, each one claiming individual advantages. These have been reviewed by Blazek (4), Blazek *et al.* (5), and Gyenes (6). Blake and Agarwal (7) recently reported a photometric titration of phenothiazines with ceric sulfate. The current pharmacopeias recognized nonaqueous titrimetry for determining the drug, while the unit dosage forms containing the drug—*viz.*, tablets, injections, *etc.*, are determined by methods based on the UV absorption properties of the phenothiazine base. However, these methods involve a series of extractions of the active ingredient from the unit dosage forms.

The authors have observed that a color ($\lambda_{max.}$, 520 m$\mu$) results when CPZH is treated with Van Urk's reagent. The color-producing reaction with phenothiazines has not been previously reported in the literature. This investigation is primarily directed toward the evaluation of the observed novel reaction as a quantitative measure of chlorpromazine in its various dosage forms.

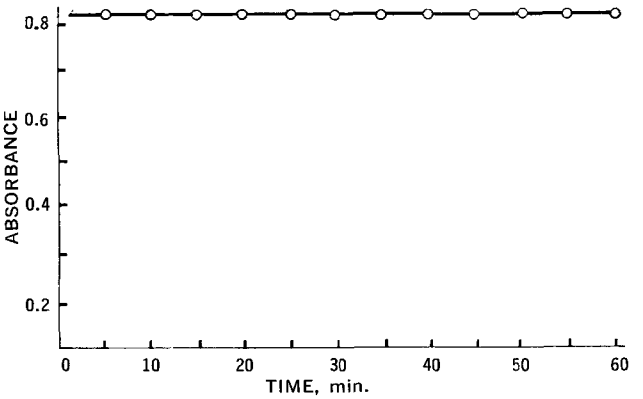


**Figure 1**—*Effect of time on the stability of color with Van Urk's reagent and CPZH.*

## EXPERIMENTAL

**Instrumentation**—Beckman DU spectrophotometer (1-cm. cell) was used.

**Materials**—Van Urk's reagent, BP 1968 (8), was used. Chlorpromazine hydrochloride and the various dosage forms were obtained from commercial sources. All reagents were analytical grade. Glass-distilled water was used throughout this work.

**Standard Reference Solution**—Chlorpromazine hydrochloride (50 mg.), previously dried, in distilled water (250 ml.) was used.

**Sample Preparation**—*Tablets*—Twenty tablets were weighed and reduced to a fine powder. An accurately weighed portion of the powder, equivalent to 50 mg. of the drug, was transferred to a 250-ml. volumetric flask. The flask was shaken thoroughly for 10–15 min. after adding 100 ml. of water and was made to volume. The contents of the flask were filtered.

*Injections*—An equivalent volume, representing 50 mg. of the drug, was measured and diluted with water to 250 ml.

*Suppositories*—Suppositories representing 100 mg. of the drug were placed in a 500-ml. volumetric flask and melted by heating on a