Three Approaches to the Analysis of Trace Formaldehyde in Bulk and Dosage Form Pharmaceuticals

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Trace-level determinations for the presence of formaldehyde in both bulk and dosage form pharmaceuticals were developed using three innovative strategies. One system adapted the chromotropic acid spot test for formaldehyde. This was accomplished spectrophotometrically over a linear detection range against authentic control samples. The other two chromatographic approaches necessitated rapid derivatization. One derivative was its corresponding oxime, formaldoxime, which was resolved on a gas chromatographic porous polymer column and sensed by a nitrogen-specific detector. The other derivative, sodium formate, was detected and quantified on an ion chromatograph using an anion-exchange column and a conductivity detector. The chromotropic acid technique was sensitive but not specific for formaldehyde. The chromatographic techniques required a high degree of water solubility. All were subject to interferences that could preclude their use for a particular application. None of the tested samples, which included a penicillin analogue, a pharmaceutical dosage form additive, a vitamin, and biological proteins, showed the presence of formaldehyde at trace levels.

KEY WORDS: formaldehyde; analysis; derivatization; gas chromatography; high-performance liquid chromatography; dosage forms.

INTRODUCTION

Although formaldehyde has beneficial antimicrobial activity, it is an irritant (1) and a suspected carcinogen (2), or it may react to form carcinogens (3). Formaldehyde is a relatively abundant pollutant produced by combustion from various sources (4) or by release from organic compounds (5) and from polymers, resins, and plastics that are synthesized from formaldehyde as a precursor (6). Pharmaceutical processing equipment may also utilize formaldehyde-derived plastics or they may have been treated with formaldehyde as a disinfectant. Therefore, it is prudent to test for formaldehyde in finished bulk or dosage form pharmaceuticals. Formaldehyde is a colorless gas at room temperature, and it is a simple molecule difficult to measure. For analysis at the trace level (parts per million or per billion), direct measurement (7–9) or numerous derivatization techniques (10–16) have been employed.

Large-molecule derivatives are made by using reagents such as *o*-(pentafluorobenzyl) hydroxylamine, 2,4-pentanedione + ammonium acetate, 2,4-dinitrophenylhydrazine, and methylbenzothiazolinone hydrochloride. The chromatography of large molecule derivatives may be satisfactory for air sampling applications but problems are likely to occur during pharmaceutical analysis. Interferences from the drug substance or the excipients of the dosage form can be expected. The problem is exacerbated by the necessity to introduce large quantities of sample to the column in order to detect trace amounts of formaldehyde. Furthermore, elution of the derivatizing reagents increases the probability for interferences to occur. Extraction techniques are often not reliable or even possible. Additionally, for pharmaceutical and other applications, large molecule derivatives may not have the volatility that is suitable for gas chromatography. Therefore, modifications to existing methods must be attempted or new approaches must be developed.

In the course of our work we have developed three new strategies for the analysis of trace levels of formaldehyde in pharmaceutical samples. Specifically, we have adapted the chromotropic acid spot test (10) into a spectrophotometric procedure over a linear detection range, developed a gas chromatographic procedure for determination of the formaldoxime derivative, and used ion chromatography to determine formaldehyde after conversion to formate ion.

MATERIALS AND METHODS

Formaldehyde solution (37% in water and containing approximately 10% methanol as a preservative; Fisher Scientific, Fair Lawn, NJ) was used as the primary standard from which further subdilutions were made for all determinations.

Chromotropic Acid Reactions

The reagent was prepared by slowly adding approximately 150 mL of concentrated sulfuric acid to 100 mL of distilled deionized water in a 500-mL Erlenmeyer flask immersed in an ice bath. With the aid of stirring with a glass rod, approximately 1.25 g of dihydroxynaphthalenedisulfonic acid (Eastman Kodak Co., Rochester, NY) was added. Complete dissolving was effected by sonication.

One milliliter of formaldehyde solution was dissolved in 100 mL of distilled deionized water. This was then diluted 1.35 mL to 500 mL to give a formaldehyde concentration of 10 ng/μL (standard stock solution).

A 10% solution of methanol in water was prepared, from which a 1.0- to 100-mL subdilution was made. This was further subdiluted to 1.35 to 500 mL (blank stock solution).

Two milliliters of reagent was added to a series of 4-mL screw-capped vials. Individual working standards were made by combining microsyringe volumes of standard stock and blank stock solutions totaling 100 μL. Each standard was prepared in duplicate.

All samples (lyophilized proteins) were contained in small, crimped-cap bottles with rubber septa. Two milliliters of reagent and 100 μL of blank stock were injected into each bottle through the rubber septum. Five sample preparations of each lot were made.

Each standard vial and sample bottle was immersed in an 80°C constant-temperature water bath for 30 min. Each

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was allowed to cool for 30 min before absorbance readings were made.

All solutions were transferred to microcuvettes (10 × 4 × 45 mm) and placed inside a Shimadzu UV-VIS Model 160A recording spectrophotometer (Shimadzu Inc., Princeton, NJ). Absorbance readings were made at 570 nm.

Oxime Derivatization and Gas Chromatography

The reagent was prepared by weighing approximately 200 mg of hydroxylamine hydrochloride (MC&B, East Rutherford, NJ) into a 100-mL volumetric flask and dissolving in and diluting to volume with deionized distilled water.

Formaldehyde solution was subdiluted with water several times: 1 to 100, 1 to 100: 1 to 100, 1 to 100, 1 to 10 and 1 to 100, 1 to 100, 1 to 50. One milliliter of each reference standard solution was transferred to vials containing 1 mL of hydroxylamine hydrochloride reagent solution. The vials were then swirled and placed inside a 70°C water bath for 5 min.

Approximately 100 mg of a penicillin-type antibiotic sample was dissolved in 1 mL of deionized distilled water. One milliliter of hydroxylamine hydrochloride reagent solution was transferred to the vial, which was then swirled and placed inside a 70°C water bath for 5 min.

Blanks were also prepared by adding 1 mL of water to 1 mL of hydroxylamine hydrochloride reagent and heating at 70°C for 5 min.

Instrument and Conditions

A Varian Model 3700 gas chromatograph (Varian Instruments, Sunnyvale, CA) containing a TSD (nitrogen-specific detector) was used. A 6-ft × 2-mm-i.d. silanized glass column containing Tenax-GC, 60/80-mesh packing was set isothermally at a temperature of 70°C and at a carrier gas flow rate of 20 mL/min nitrogen. The detector was set at a temperature of 270°C, with the bias voltage and bead current set at 9 and 350, respectively. Detector gas flow rates were 5 mL/min for hydrogen and 300 mL/min for air. Five-microliter on-column injections were made at an injector temperature of 250°C. The electrometer was set at 256× at a range of 10^{-12} A/mV. Formaldoxime elutes at approximately 7 min.

Sodium Formate Derivatization and Ion Chromatography

One milliliter of formaldehyde solution was added to 5 mL of 1 *N* NaOH in a 100-mL volumetric flask, which was then placed inside a 70°C water bath for 10 min. After cooling, the level was brought to volume with deionized distilled water. This solution was then subdiluted 0.5 to 250 mL to give an approximate level of 125 ppm. Additional subdilutions were made to the 1-ppm level.

Approximately 100 mg of sample [one was a dosage form ingredient (mannitol), and the other a vitamin (riboflavin)] was dissolved in 1 mL of deionized distilled water to which was added 1 mL of 5 m*M* NaOH. The contents were placed inside a water bath at 70°C for 10 min.

Blanks were also prepared by combining 1 mL of deionized distilled water with 1 mL of 5 m*M* NaOH and heating at 70°C for 10 min.

Instrument and Conditions

A Dionex Model 4000i ion chromatograph (Dionex Corp., Sunnyvale, CA) was used for the investigations. A Dionex HPIC-AS4A anion-exchange column was connected to an HPIC-AG4A guard column. The eluant was 0.75 m*M* sodium bicarbonate + 2.2 m*M* sodium carbonate at a flow rate of 1 mL/min. An anion micromembrane suppressor using 0.025 *N* sulfuric acid as regenerant optimized the ionization signal. A conductivity detector spanning a range from 3 to 100 μsiemens was used. Injections were made from a 50-μL loop. The formate ion elutes at approximately 2 min.

RESULTS AND DISCUSSION

The adduct produced by the chromotropic acid–formaldehyde reaction shows an absorbance maximum at 570 nm. The intense purplish color has made this reaction useful as a spot test. At this wavelength duplicate determinations of formaldehyde standard solution ranging from 100 to 1000 ng of formaldehyde were made. The regression line of the linearity study had a correlation coefficient of 0.9993 and a *y* intercept of 0.00096. Since the total sample weight in each vial containing lyophilized protein is approximately 17 mg, the minimum formaldehyde detection range corresponds to 6 to 60 ppm. Table I shows recovery data for the method. Results appear to be less reliable at the lower end, probably due to absorbance contributions from the sample.

Testing consisted of four lots of one and two lots of another protein concentrate in sealed vials. Each preparation was blanked against a control lot known to be formaldehyde free because exposure to formaldehyde was not possible. Five vials of each lot were reacted and their respective absorbances were read at 570 nm. The results of all lots tested showed none to have an average absorbance reading greater than zero. Therefore, none of the tested samples had detectable formaldehyde to at least the minimum detection level of 100 ng or 6 ppm.

The conversion of aldoses to oximes using hydroxylamine hydrochloride on an analytical scale has been reported (17). Formaldehyde can be converted to its corre-

Table I. Recovery Data Obtained for Protein Samples Spiked with Formaldehyde and Reacted with the Chromotropic Acid Reagent Versus a Formaldehyde Standard

Wt of formaldehyde added to protein sample (ng)	Absorbance		% recovery
	Theoretical	Experimental	
100	0.043	0.057	132.6
200	0.086	0.095	110.5
400	0.171	0.185	108.2
600	0.257	0.254	98.8
800	0.342	0.315	92.1
1000	0.428	0.399	93.2

sponding oxime, formaldoxime, by reaction with hydroxylamine HCl.

$$\begin{matrix}H\\H\end{matrix}\!\!>\!C{=}O + H_2NOH \cdot HCl \xrightarrow[5\ min]{70°C}$$

$$\begin{matrix}H\\H\end{matrix}\!\!>\!C{=}N\text{-}OH + H_2O + HCl$$

Formaldoxime is more responsive than formaldehyde to the flame ionization detector. The nitrogen-specific detector increases the responsiveness of formaldoxime even further. Retention of this volatile analyte is achieved through the use of an adsorbent-type porous polymer column which also traps the sample. Several lots of a penicillin-type antibiotic were tested for possible formaldehyde contamination. Figure 1 shows an offset mapped chromatographic display of these lots, none of which indicates the presence of formaldehyde as compared to the blank, the standards, and a spiked sample. The level of detection is good to a minimum of 7 ppm or approximately 4 ng.

A third approach toward trace formaldehyde determinations in pharmaceuticals utilizes the Cannizzaro reaction:

$$2HCHO + NaOH \xrightarrow[10\ min]{70°C} CH_3OH + HCOONa$$

Sodium formate can then be analyzed for formate ion by ion chromatography.

The elution position for formate ion was confirmed by coelution with a peak produced by injection of dilute formic acid. Blanks were prepared corresponding to the levels of formaldehyde standard and the strength of sodium hydroxide in each. Formate elution positions in the blanks were free of peak interferences but a later-eluting peak at approximately 3 min was observed. This peak could be chloride ion, which sometimes is present as a system contaminant. The primary standard was prepared based upon the amount of sodium hydroxide required to convert 1 mL of formaldehyde solution, which is 5 mL of 1 *N* for approximately 370 mg of formaldehyde.

A limiting factor in these analyses is that excessive presence of hydroxide ions could significantly interfere with the formate ion elution, producing a peak followed by a dip. This phenomenon is analogous to water being detected by a flame ionization detector due to the disturbance it creates. Therefore, samples should be reacted with only the amount of sodium hydroxide necessary for the conversion of 100 ppm (or less) formaldehyde, or approximately 1 mL of 5 m*M* sodium hydroxide per 100 mg of sample. Figure 2 is a multiplot of formaldehyde concentration levels from 0.37 to 0.000148 mg/mL. Figure 3 shows a series of ion chromatograms of standards, samples, and also a blank. The samples which are of a vitamin (riboflavin) and a dosage form ingredient (mannitol) show the absence of formaldehyde at these levels. Table II provides the recovery data obtained for a sample spiked with formaldehyde at levels between 10 and 200 ppm. A dosage form of a protein pharmaceutical, however, could not be analyzed because of the overwhelming contribution of one of the excipients, sodium chloride.

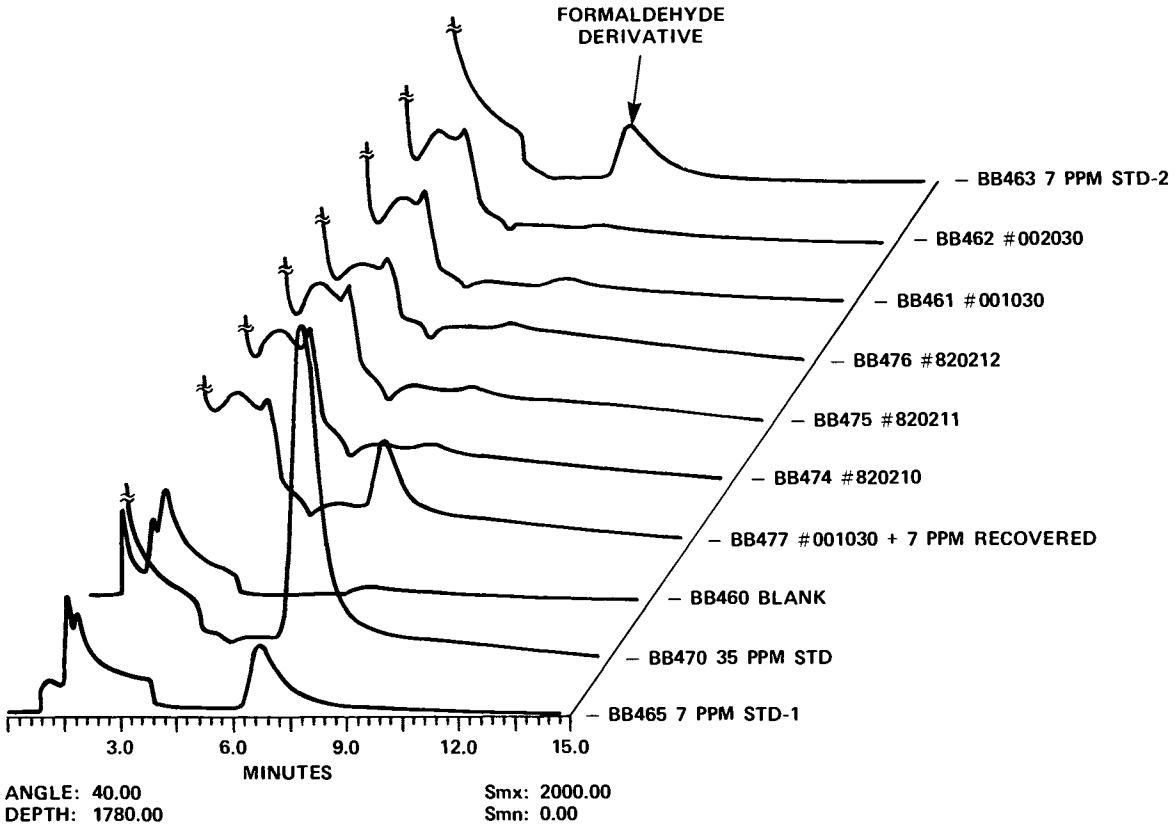

Fig. 1. Multiple gas chromatograms of the formaldehyde–hydroxylamine hydrochloride reaction product showing standards and several antibiotic samples tested for the presence of formaldehyde.

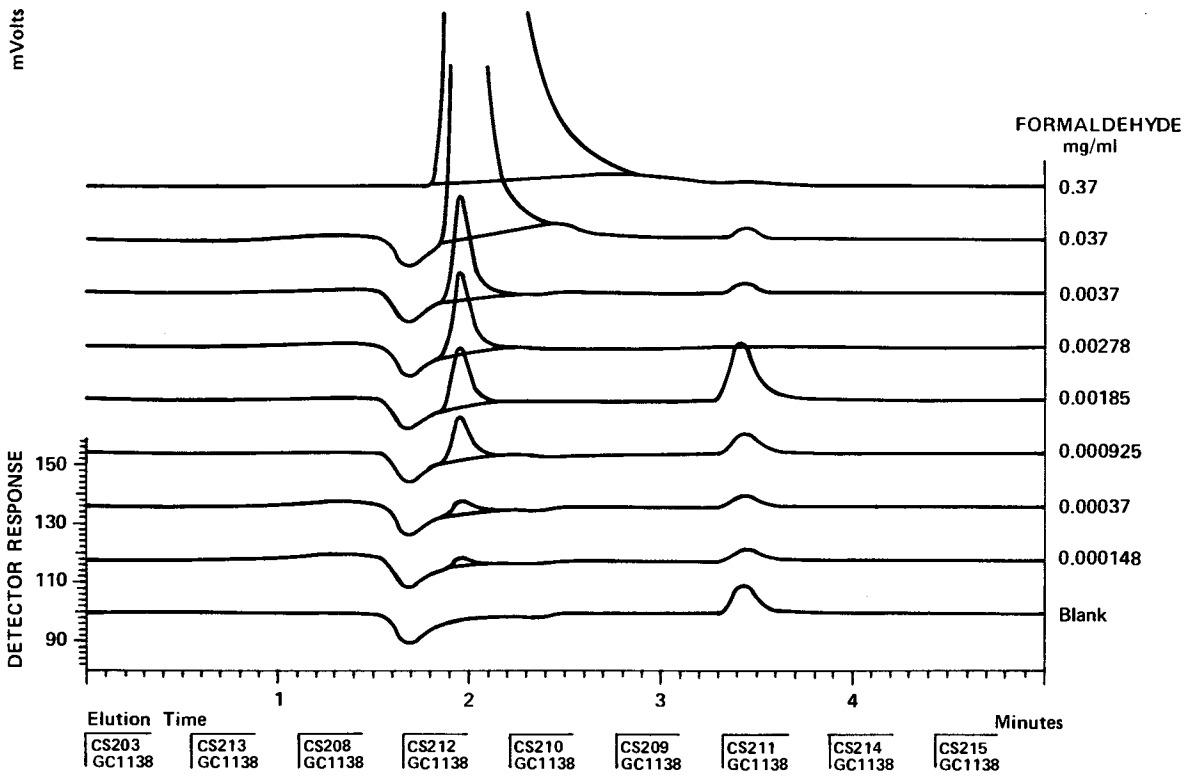

Fig. 2. Ion chromatograms of formaldehyde as formate ion covering the concentration range from 0.000148 to 0.37 mg/mL.

CONCLUSIONS

The chromotropic acid spectrophotometric analysis is usable if control samples are available that indicate the absence of interferences. The limitation is the nonspecificity of the method. For the chromatographic techniques, the samples must be highly water soluble. Interferences coming from the sample matrix, such as might be caused by thermal instability, residual solvents, or excipients, could doom the use of these approaches. The ion chromatograph, in partic-

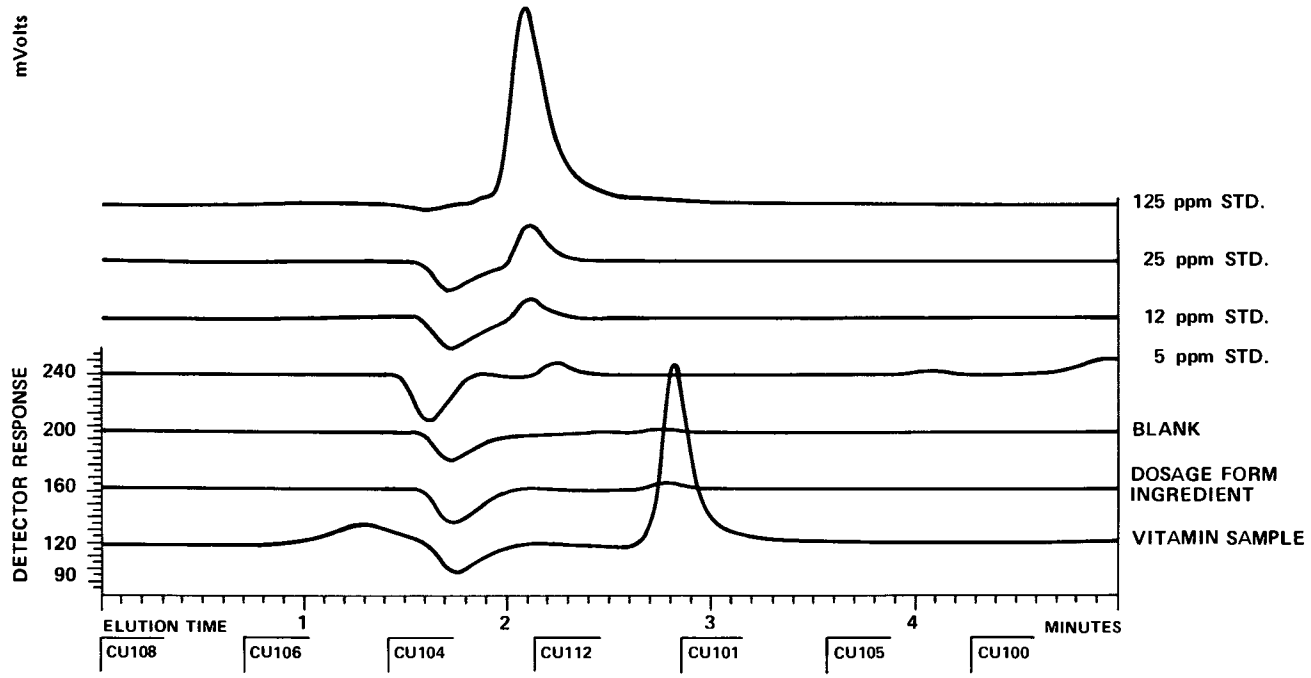

Fig. 3. Ion chromatograms of the formaldehyde–sodium hydroxide reaction product showing standards, blank, and samples of a dosage form ingredient and a vitamin.

Table II. Recovery Data Obtained for Mannitol Samples Spiked with Formaldehyde and Reacted with 5 m*M* NaOH Versus a Formaldehyde Standard[a]

PPM formaldehyde added to sample	Area units		
	Theoretical	Experimental	% Recovery
10	20	10	50.0
20	40	19	47.5
50	101	72	71.3
100	202	166	82.2
200	404	334	82.7

[a] The sample shows the presence of formate ion which is below the 10 ppm level and whose area is subtracted from the other peaks.

ular, is very susceptible to interfering ions that may be present in the water, glassware, or from the sample itself. Hydrochloride salts, for example, would swamp the formate elution region with a massive dose of chloride ion.

Our investigations have demonstrated, however, that a suitable procedure can be found among these for several widely different types of sample applications.

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