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Gas–liquid chromatographic and difference spectrophotometric techniques for the determination of benzaldehyde in benzyl alcohol

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Abstract: Simple, sensitive, rapid and selective gas–liquid chromatographic and difference spectrophotometric methods have been described for the determination of benzaldehyde, an oxidation product of benzyl alcohol, in benzyl alcohol intended for use in the manufacture of parenteral dosage forms. The GLC method comprises chromatography of a mixture of benzaldehyde and benzyl alcohol on a CP-Sil5 WSCOT capillary column after dilution with chloroform and addition of 4-chlorocresol as the internal standard, using nitrogen as the mobile phase and a flame ionization detector. The difference spectrophotometric method involves the measurement of the absorbance of benzaldehyde relative to an equimolar solution in which benzaldehyde has been reduced by sodium borohydride. Both methods are accurate, precise and selective for the determination of benzeldehyde in benzyl alcohol in the presence of benzoic acid, a second oxidation product of benzyl alcohol.

Keywords: *Benzyl alcohol; benzaldehyde; benzoic acid; sodium borohydride; difference spectrophotometry; gas–liquid chromatography.*

Introduction

Benzyl alcohol is commonly used as a bacteriostatic agent in various parenteral preparations [1]. Benzaldehyde is the main oxidation product of benzyl alcohol. Oxidation of benzyl alcohol takes place on long-term storage and on autoclaving of parenteral dosage forms containing benzyl alcohol. The limit test for benzaldehyde in the BP 1980 [2] and USP 1990 [3] monographs on benzyl alcohol is non-specific and is designated as a test for aldehydes. It involves the addition of hydroxylamine hydrochloride and titration with sodium hydroxide. However, the BP 1988 [4] monograph for benzyl alcohol states that benzyl alcohol intended for use in the manufacture of parenteral dosage form contains not more than 0.05% of benzaldehyde and describes a GLC method for the determination of benzaldehyde.

The technique of difference spectrophotometry has proved to be particularly useful in eliminating interferences from other UV-absorbing compounds, thereby increasing the selectivity of spectrophotometric analysis [5–10]. The main criterion for applying difference spectrophotometry to the assay of a substance in the presence of one or more other substances is that the change in the spectrum of the substance under assay induced by the addition of one or more reagents is reproducible while the absorbance of each of the interfering substances is not altered by the reagents. Spectral changes could be brought about by change in pH [5], oxidation [6], reduction [7], condensation [8] and ester formation [9].

The difference spectrophotometric method described by Hewala [10], which includes measurement of the difference in absorbance between two equimolar solutions of benzaldehyde, one of which contains sodium bisulphite, is selective for the determination of benzaldehyde in benzyl alcohol. However, the method cannot be applied to samples of benzyl alcohol containing benzoic acid as the optimal pH of the method is very close to the pK_a value of benzoic acid. Consequently, a slight difference in pH between the sodium bisulphite-treated and non-treated benzaldehyde solutions would affect the ratio of dissociated and non-dissociated species of benzoic acid [11], and hence interference due to benzoic acid would not be compensated.

Several GLC procedures have been reported for the determination of benzaldehyde. These include its determination as a product of

toluene oxidation using either Carbowax 20 [12], OV-101 [13], poly(ethanediol adipate) [14], Apiezon [15], or a mixture of poly-(butane-1,3-diol adipate) and Apiezon L [16] as stationary phases. Procedures involving preparation of Schiff base derivatives of benzaldehyde and their use for the GLC determination of benzaldehyde have been described [17, 18]. GLC procedures for the analysis of benzyl alcohol and benzaldehyde include: the use of a Porapak QS column as the stationary phase, with hexanol as the internal standard [19]; 5% SE-60 as the stationary phase, with diethyloxalate as the internal standard [20]; and 3% OV-17 as the stationary phase, with diethylphthalate as the internal standard [4].

This paper reports new GLC and difference spectrophotometric procedures for the determination of benzaldehyde in commercial samples of benzyl alcohol in the presence of benzoic acid, a second oxidation product of benzyl alcohol.

Experimental

Materials and reagents

Benzaldehyde (Sigma Chemical Co., UK), benzyl alcohol, benzoic acid and 4-chlorocresol (BDH, UK) were of analytical reagent quality. Sodium borohydride (BDH, UK) was of general purpose reagent quality.

Sodium borohydride reagent (0.25%, w/v) was prepared by dissolving 250 mg of sodium borohydride in ethanol and diluting to 100 ml. The solution is stable for 6 days [21].

Buffer solutions were prepared using substances of analytical reagent grade according to the published formulae [22].

Instrumentation

A Perkin–Elmer Model 550 S UV–vis spectrophotometer and a Hitachi Model 561 recorder were used. Absorption spectra and difference absorption spectra of the test and reference solutions were recorded in 1-cm silica quartz cells over the range 320–220 nm. The spectral bandwidth was 2 nm, the response (time constant) was 1 s, the scan speed was 120 nm min^{-1} and the chart speed was 60 nm min^{-1}. The difference absorbance readings of the standard, sample and blank solutions at 247 nm were read from the digital display under non-scanning conditions.

Gas chromatography was performed on a Hewlett–Packard GC system 5890 series II equipped with a flame-ionization detector. A wall-coated superior capacity open tubular (WSCOT) capillary column (50 m × 0.5 mm i.d.) with a polydimethylsiloxane stationary phase (CP-Sil5, film thickness 1 μm) was used. Peak areas were computed by means of an HP 3394 system (Hewlett–Packard). The temperature settings for the oven, the injection port and the detector were 140, 250 and 250°C, respectively. The carrier gas was nitrogen with a flow rate of 10 ml min^{-1} and the auxillary gas flow rate was 15 ml min^{-1}.

Procedures

Gas–liquid chromatographic method.

Standard solutions. Stock solutions of benzaldehyde (**I**), benzyl alcohol (**II**) and 4-chlorocresol (**III**; internal standard) were prepared by carefully weighing about 100 mg into a 100-ml volumetric flask, then dissolving in chloroform. Serial dilutions were prepared from each of the three substances, and then a mixture of the three substances was prepared so that the concentrations of **I**, **II** and **III** were 50, 50 and 100 μg ml^{-1}, respectively. The mixture (0.2 μl) was chromatographed using the previously mentioned conditions.

For the preparation of the calibration graph, solutions containing 25, 50, 75, 100 and 125 μg ml^{-1} of benzaldehyde each with 100 μg ml^{-1} of 4-chlorocresol were chromatographed as described above. The peak-area ratio of benzaldehyde to 4-chlorocresol was related to the concentration of benzaldehyde.

Test solutions. Twenty-five grams of a commercial benzyl alcohol sample was weighed into a 50-ml volumetric flask. An aliquot of a stock solution of 4-chlorocresol was added and the solution was diluted to volume with chloroform so that the final concentration of 4-chlorocresol was 200 μg ml^{-1}. The solution was chromatographed as described above. The concentration of benzaldehyde in the sample was calculated using a reference standard solution chromatographed under the same conditions.

Difference spectrophotometric method.

Standard solution. A standard solution of benzaldehyde was prepared by dissolving 100 mg, accurately weighed, in 100 ml of ethanol (solution I). A 5-ml aliquot of solution I was transferred into a 100-ml volumetric flask and diluted to volume with ethanol (solution

II). A 3-ml aliquot was transferred into each of two 25-ml volumetric flasks. Sodium borohydride reagent (1 ml) was added to one flask and mixed; the contents of both flasks were diluted to 25 ml with buffer (pH 9). The difference absorption spectrum of the untreated solution in the sample cell was recorded relative to that of the solution treated with sodium borohydride, in the reference cell.

For preparation of a calibration graph 1-, 2-, 3-, 4-, 5-, 6- and 7-ml aliquots of solution II were treated as described above.

Test solution. A sample solution of benzyl alcohol was prepared by diluting approximately 2.5 g, accurately weighed, in 25 ml of ethanol. A 1-ml aliquot was transferred to each of two 25-ml volumetric flasks and the procedure was continued as described previously for a standard solution (from the addition of 1 ml of sodium borohydride reagent).

The concentration of benzaldehyde in the sample solution, and hence in benzyl alcohol, was calculated from the proportional relationship that exists between the difference absorbance at 247 nm and the concentration of benzaldehyde.

Results and Discussion

Development and validation of the GLC method

Chromatography of a mixture of benzaldehyde and benzyl alcohol on a CP-Sil5 WSCOT capillary column using the experimental conditions described in the Experimental section showed complete resolution of the peaks (Fig. 1). 4-Chlorocresol was chosen as the internal standard as its peak and the peak due to benzyl alcohol were completely resolved.

The precision of the injection was investigated by chromatography ($n = 10$) of 0.2 μl of a mixture of benzaldehyde (100 μg ml^{-1}) and 4-chlorocresol (100 12g ml^{-1}; $n = 10$). The mean peak-area ratio of benzaldehyde to 4-chlorocresol was 0.82 and the RSD was 0.73%, indicating good precision of injection.

Linearity graphs showed that there was a proportional relationship between the peak-area ratio of benzaldehyde–4-chlorocresol and the concentration of benzaldehyde. The linear regression equation was $y = 0.0082x + 0.0012$, where y is the peak-area ratio and x μg ml^{-1} is the concentration of benzaldehyde ($n = 6$; correlation coefficient = 0.9997).

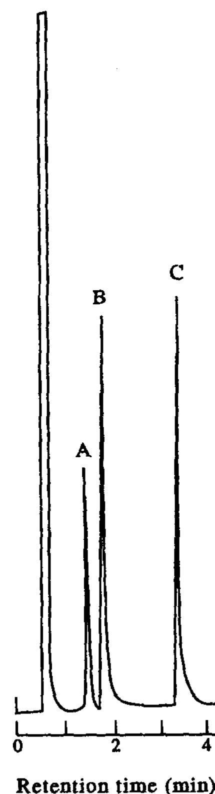

Figure 1
GLC chromatogram of a standard mixture of benzaldehyde (50 μg ml^{-1}), benzyl alcohol (50 μg ml^{-1}) and 4-chlorocresol (100 μg ml^{-1}). (A) Benzaldehyde, (B) benzyl alcohol and (C) 4-chlorocresol.

The precision of the GLC procedure was tested by analysing ($n = 10$) a mixture containing 50 μg ml^{-1} of benzaldehyde, 50 μg ml^{-1} of benzoic acid, 50 μg ml^{-1} of benzyl alcohol and 100 μg ml^{-1} of 4-chlorocresol. The mean percentage recovery of benzaldehyde was 100.81 and the SD was 0.63, indicating that the GLC procedure has good precision.

The limit of detection was taken as the concentration of benzaldehyde corresponding to the peak area or height larger than the uncertainty associated with it [23]. It was calculated as the peak area or height equivalent to three times the largest peak obtained from the chromatogram of solvent blank and was found to be 0.01 μg ml^{-1}.

Development and validation of the difference spectrophotometric method

Figure 2 shows the UV absorption spectra of benzaldehyde in the presence and absence of sodium borohydride, and the difference absorption spectrum obtained by recording the spectrum of the solution of unreduced benz-

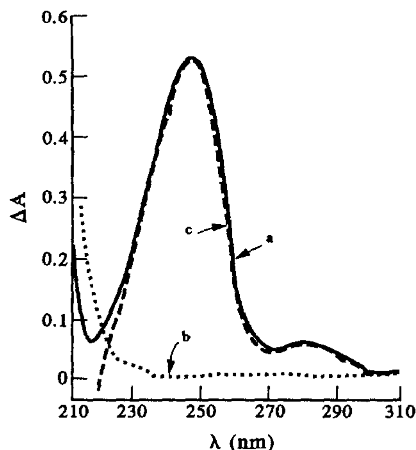

Figure 2
UV absorption spectra of benzaldehyde (4.25 μg ml^{-1}) in buffer (pH 9) solution (a), in sodium borohydride (pH 9) solution (b) and the difference absorption spectrum (c) of solution (a) relative to solution (b).

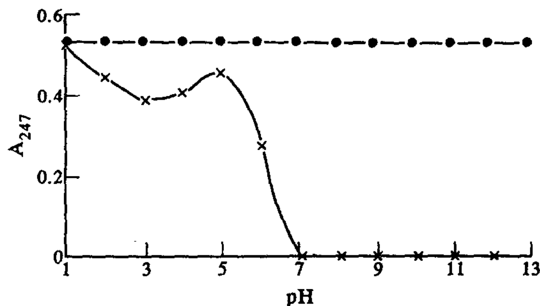

Figure 3
The effect of pH on the absorbance of benzaldehyde (4.25 μg ml^{-1}) in the presence (–*–) and absence (–●–) of sodium borohydride (0.1 mg ml^{-1}).

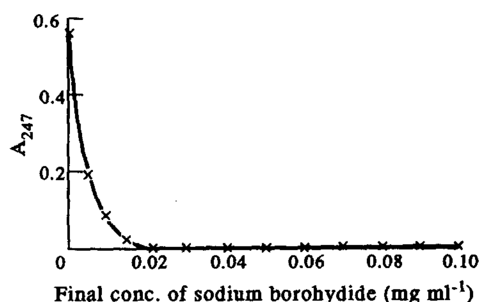

Figure 4
The effect of the concentration of sodium borohydride on the absorbance of benzaldehyde (4.25 μg ml^{-1}) at pH 9.

aldehyde against the equimolar solution of reduced benzaldehyde in the reference cell. Reduction of benzaldehyde by sodium borohydride eliminates the strong and weak absorption bands of benzaldehyde near 247 and 280 nm, respectively. The difference absorption spectrum shows a λ_{max} at 247 nm and an isoosbestic point at 223 nm. The change in spectral properties of benzaldehyde by reduction is due to the destruction of the dienone chromophore of benzaldehyde to yield the diene chromophore of benzyl alcohol.

The effect of pH on the reduction of benzaldehyde was investigated by measuring the absorbance at 247 nm of a solution of benzaldehyde that had been buffered to various pH values in the range 1–10. Figure 3 shows that the A_{247} is not affected by pH and that a pH value higher than 6 is necessary for complete reduction of benzaldehyde. The buffer (pH 9) was chosen to be sure that benzoate, if present, will be completely dissociated. Figure 4 shows the effect of varying the concentration of freshly prepared sodium borohydride reagent on the reduction of benzaldehyde in buffer (pH 9). Complete reduction was obtained with all concentrations above 0.02 mg ml^{-1}. The concentration of the sodium borohydride reagent was selected to be 0.25% (w/v) in ethanol. An aliquot of that reagent was added to the solution so that the final concentration of sodium borohydride was 0.1 mg ml^{-1}.

The proportionality of the measured ΔA_{247} values and the concentration of benzaldehyde was examined by using a calibration series of solutions (1, 2, 3, 4, 5, 6 and 7 μg ml^{-1}) of benzaldehyde in the absence and presence of benzyl alcohol (4 mg ml^{-1}), benzyl chloride (50 μg ml^{-1}), benzoic acid (10 μg ml^{-1}) and a mixture of benzyl alcohol, benzyl chloride and benzoic acid (4 mg ml^{-1}, 50 μg ml^{-1} and 10 μg ml^{-1}, respectively). The results (Table 1) show that the ΔA_{247} is proportional to the concentration of benzaldehyde in the range 1–7 μg ml^{-1}, and that the presence of the second oxidation product of benzyl alcohol, i.e. benzoic acid, does not interfere with the measured ΔA_{247} values. The precision of the procedure was determined by assaying a sample of commercial benzyl alcohol 10 times. The mean concentration of benzaldehyde in benzyl alcohol was 178.2 μg g^{-1} and the SD was 1.13 μg g^{-1} (RSD = 0.64%) showing that the procedure has good precision.

The specificity of the procedure was tested by comparing the curves of log ΔA versus the wavelength for standard benzaldehyde solution and commercial benzyl alcohol samples. It was observed that the curves were superimposed over a wavelength range of 280–225 nm. This

indicates that the substance being determined by the proposed difference spectrophotometric procedure is benzaldehyde.

The limit of detection of the assay [24, 25], calculated as the concentration of benzaldehyde giving a ΔA_{247} equal to twice the SD (n = 10) of the ΔA_{247} of a solution containing pure benzyl alcohol (4 mg ml^{-1}) in buffer (pH 9) relative to an equimolar solution of pure benzyl alcohol containing sodium borohydride (0.1 mg ml^{-1}) in buffer (pH 9) was 0.023 μg ml^{-1}. The lower limit of determination, calculated as the concentration of benzaldehyde giving a ΔA_{247} reading of 0.050 was 0.42 μg ml^{-1}.

Assay results

The procedures were applied to the determination of the concentration of benzaldehyde in four different batches of commercial benzyl alcohol (pharmaceutical grade) intended for use in the manufacture of parenterals (Table 2). The GLC chromatogram of commercial benzyl alcohol showed a small peak of the same retention time as that of benzaldehyde [Fig. 5(a)]. The enrichment of that peak on the addition of standard benzaldehyde [Fig. 5(b)] confirms that the substance determined by the proposed GLC procedure is benzaldehyde. In comparison with the GLC methods [4, 19, 20], the main advantages of the proposed method

Table 1
Calibration data for standard benzaldehyde in the presence of benzyl alcohol, benzyl chloride and/or benzoic acid using the ΔA method

Composition of solutions	Regression equation*	r
Benzaldehyde	$y = 0.1219x - 0.001$	0.9999
Benzaldehyde + benzyl alcohol (4.0 mg ml^{-1})	$y = 0.1216x - 0.001$	0.9992
Benzaldehyde + benzyl chloride (0.05 mg ml^{-1})	$y = 0.1216x - 0.002$	0.9997
Benzaldehyde + benzoic acid (0.01 mg ml^{-1})	$y = 0.1220x - 0.001$	0.9998
Benzaldehyde + benzyl alcohol (4.0 mg ml^{-1}) + benzyl chloride (0.05 mg ml^{-1}) + benzoic acid (0.01 mg ml^{-1})	$y = 0.1221x - 0.004$	0.9991

* $y = \Delta A_{247}$, x = concentration of benzaldehyde (μg ml^{-1}), and r = correlation coefficient (n = 7).

Table 2
Determination of benzaldehyde in commercial samples of benzyl alcohol

Sample	Approximate age (months)	Concentration of benzaldehyde mean % (w/w) ± SD*		
		ΔA_{247} method†	GLC method†	BP method†
A	New	0.0153 ± 0.0001 (2.00) 0.25 RSD = 0.65%	0.0154 ± 0.0003 (0.62) 2.25 RSD = 1.95%	0.0155 ± 0.0002 RSD = 1.29%
B	3	0.0185 ± 0.0002 (1.86) 2.25 RSD = 1.08%	0.0186 ± 0.0002 (1.24) 2.25 RSD = 1.08%	0.0188 ± 0.0003 RSD = 1.60%
C	8	0.0212 ± 0.0001 (2.00) 4 RSD = 0.47%	0.0209 ± 0.0003 (0.62) 0.44 RSD = 1.44%	0.0210 ± 0.0002 RSD = 0.95%
D	15	0.0301 ± 0.0003 (0.53) 1 RSD = 1.00%	0.0303 ± 0.0002 (1.86) 2.25 RSD = 0.65%	0.0300 ± 0.0003 RSD = 1.00%

*Mean of five determinations ± SD.

†The figures in parentheses are the calculated t-values for which the corresponding theoretical value (P = 0.95) is 2.31 for $n_1 = n_2 = 5$. The underlined figures are the calculated F-values for which the corresponding theoretical value (P = 0.95) is 6.39 for $n_1 = n_2 = 5$.

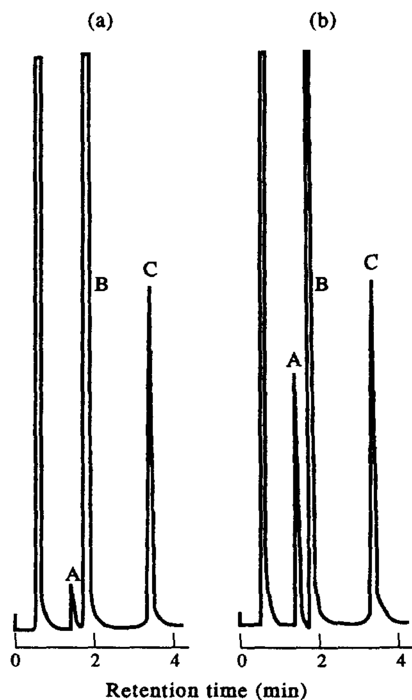

Figure 5
Typical GLC chromatograms of commercial sample of benzyl alcohol, (a) before and (b) after addition of standard benzaldehyde (50 μg ml^{-1}). (A) benzaldehyde, (B) benzyl alcohol and (C) 4-chlorocresol.

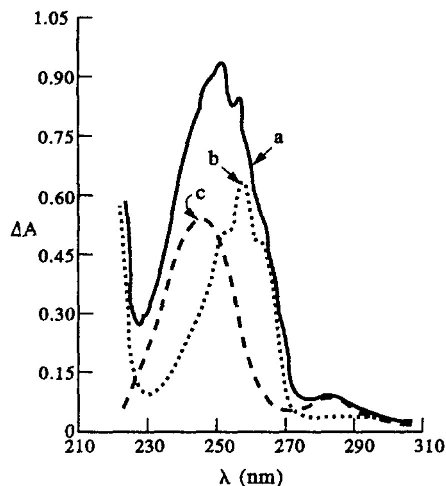

Figure 6
UV absorption spectra of a stored commercial sample of benzyl alcohol (0.35 mg ml^{-1}) in buffer (pH 9) solution (a) and in sodium borohydride (0.1 mg ml^{-1}) solution (pH 9) (b) and the difference absorption spectrum (c) of solution (a) relative to solution (b).

are the high resolution between the peaks due to benzaldehyde and benzyl alcohol and its small limit of detection. Moreover, using the described capillary GLC system would allow the analyst to use small concentrations of benzyl alcohol sample, thus decreasing the overlap between the peaks of benzaldehyde and benzyl alcohol during the analysis of commercial benzyl alcohol by the BP method [4].

Figure 6 shows the UV absorption spectra of a stored sample of commercial benzyl alcohol before and after treatment with sodium borohydride and their difference absorption spectrum. The spectra show that a direct spectrophotometric assay of benzaldehyde is subject to interference from benzyl alcohol and that the difference spectrophotometric assay is selective for benzaldehyde. For comparison, the samples were also assayed by the official GLC method [4]. The results (Table 2) show no significant difference between the reproducibility (F-test) and the accuracy (t-test) of the proposed methods and the official method since the calculated values did not exceed the theoretical values.

The limit test for benzaldehyde in the USP monograph on benzyl alcohol, which involves the addition of hydroxylamine hydrochloride and titration with sodium hydroxide, was carried out for all the provided samples of benzyl alcohol and was found to conform with the stated specifications. To investigate whether the USP non-specific limit test for benzaldehyde would be useful in the detection of samples of benzyl alcohol in which the concentration of benzaldehyde is beyond the BP limit (0.05%), two batches of commercial benzyl alcohol (A and C, Table 2) were modified by the addition of benzaldehyde in a concentration to exceed the BP permitted limit. The modified samples were analysed using the USP, the proposed GLC and the proposed ΔA methods. It was found that both modified samples conformed with the USP stated specifications. However, the concentrations of benzaldehyde using the proposed GLC and ΔA methods were 0.067 and 0.062% for samples A and C, respectively. Consequently, the two modified samples would not fulfil the requirements of the BP as expected but would fulfil the specification of the USP. This indicates the necessity for applying the BP method or one of the proposed methods for determination of the exact content of benzaldehyde in benzyl alcohol intended for use in the manufacture of parenterals.

The difference spectrophotometric method is simple, direct and of good accuracy and

reproducibility; it could be recommended for detection and determination of benzaldehyde in benzyl alcohol intended for use in the manufacture of parenterals.

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