International Journal of Pharmaceutics, 35 (1987) 13–20
Elsevier

IJP 01165

Determination of benzaldehyde traces in benzyl alcohol by liquid chromatography (HPLC) and derivative UV spectrophotometry

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(Received 25 June 1986)
(Accepted 8 August 1986)

Key words: Reversed-phase HPLC; Derivative UV spectroscopy; Benzyl alcohol; Benzaldehyde trace; UV diode array detection

Summary

Two methods suitable for benzaldehyde trace determination in benzyl alcohol have been developed, based on reversed-phase liquid chromatography (HPLC) and derivative UV spectroscopy. The HPLC method with UV-254 nm detection allowed simultaneous determination of benzyl alcohol and benzaldehyde and represents a convenient alternative procedure to the pharmacopoeial methods. Using a diode array detector the benzaldehyde peak identity and purity were easily confirmed. The described second-order derivative UV spectroscopic method was found highly suited to rapid benzaldehyde traces determination, but attention should be paid to possible interferences from substances (e.g. butylhydroxyanisole) absorbing over the 280–290 nm range. Both the proposed methods allowed a reliable determination of benzaldehyde at concentrations down to 0.05% of the benzyl alcohol level.

Introduction

Benzyl alcohol is an antimicrobial agent widely used as a preservative in pharmaceutical formulations. The product easily undergoes oxidative degradation to benzaldehyde and the presence of this potential impurity need to be checked owing to its reactivity and toxicity. The official procedures for benzaldehyde detection consist of limit tests based on the reaction between benzaldehyde and hydroxylamine hydrochloride (USP XXI—NF XVI, 1985; BP, 1980; DAC, 1979) and 2,4-dinitrophenylhydrazine (Ph. Japan, 1981; Ph. Fr., 1976) or colorogenic tests (Ph. Helv., 1971). These methods are of limited sensitivity and specificity and are unsuitable for the quantitation of benzaldehyde traces. A more specific and sensitive procedure based on gas chromatography has recently been introduced in the Pharmacopée Européenne (1984).

The widespread popularity achieved by the high-performance liquid chromatography (HPLC) in the routine quality control of pharmaceuticals has led us to regard this technique as appropriate for complete benzyl alcohol analysis. HPLC applications in benzaldehyde traces determination in pharmaceutical formulations containing benzyl alcohol have recently been reported (Rego and Nelson, 1982; Floor et al., 1985; Cavrini et al., 1986).

On the other hand, derivative UV spectrophotometry has also proved a useful tool for resolving overlapping spectral bands (O'Haver, 1979; Tal-

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sky et al., 1978; Fell, 1978) and successful applications of this technique in the determination of impurities in drugs have been reported (Fasanmade and Fell, 1985).

In view of this, we have developed two alternative procedures, based on reversed-phase HPLC and derivative UV spectrophotometry, for the specific and sensitive determination of benzaldehyde traces in benzyl alcohol.

Experimental

Apparatus

A Varian HPLC system consisting of a model 5020 liquid chromatograph, a variable wavelength detector (UV-50) and a model 4290 Varian integrator was used. Manual injections were made using a Rheodyne 7125 injectable valve (10 μl loop). A Varian Polychrom 9060 Diode Array detector was used for peak identification and purity determination. All spectrophotometric work was done with a Varian model DMS 90 spectrophotometer equipped with a model 9176 recorder (Varian). The UV spectra were obtained over the range 340–275 nm using 1-cm quartz cells, with a slit width of 1 nm, a scanning speed of 100 nm/min and a recording chart speed of 2 cm/min.

Materials

The benzyl alcohol standard (Fluka guarantee) and 3-butyl-4-hydroxyanisole (BHA) were from Fluka A.G. (Switzerland). Benzaldehyde and the internal standard, methyl paraben, were from C. Erba (Italy). For chromatography, HPLC-grade acetonitrile (C. Erba) and deionized double-distilled water were used. The commercial benzyl alcohol samples analysed in the present work are summarized in Table 4.

Liquid chromatographic (HPLC) method

Chromatographic conditions

Chromatographic separations were performed at ambient temperature on a reversed-phase (Hypersil RP-8, 5 μm) column (250 × 4.0 mm i.d.) using a mobile phase consisting of acetonitrile–water 38 : 62 at a flow-rate of 1.0 ml/min. The detector wavelength was set at 254 nm, for the simultaneous determinations of benzyl alcohol and benzaldehyde, and 282 nm for the selective analysis of benzaldehyde. The integrator attenuation values were 128 for benzyl alcohol analysis and 32–16 for the benzaldehyde traces determination.

Calibration curves

Stock solutions of benzyl alcohol (10 mg/ml), benzaldehyde (50 μg/ml or 125 μg/ml for determinations at 254 nm or 282 nm, respectively) and methyl paraben (the internal standard; 200 μg/ml) were made in acetonitrile.

For benzyl alcohol determination working standard solutions containing 1.5–3.0 mg/ml of the compound and 20 μg/ml of internal standard were prepared in acetonitrile. Peak height was measured and the peak height ratios of benzyl alcohol to internal standard were plotted against the benzyl alcohol concentration to obtain the calibration curve. Similarly, for benzaldehyde analysis at 254 nm working standard solutions containing 1.25–7.5 μg/ml of benzaldehyde and 20 μg/ml of internal standard were prepared in acetonitrile and a calibration curve was constructed. When the benzaldehyde determination was performed at 282 nm, standard solutions containing 6.25–37.5 μg/ml of benzaldehyde and 20 μg/ml of internal standard were used to obtain the calibration curve.

Simultaneous benzyl alcohol and benzaldehyde determination (λ = 254 nm)

A 1.0-ml volume of commercial benzyl alcohol was diluted 1 : 100 with acetonitrile. A 2.0-ml aliquot of the resulting solution was transferred into a 10-ml volumetric flask, then 1.0 ml of internal standard (methyl paraben) solution was added and the volume adjusted with acetonitrile. A 10-μl volume was injected in triplicate into chromatograph and the integrator attenuation was changed (128 to 32) after internal standard peak elution. The sample solutions were chromatographed concurrently with the appropriate standard solutions and peak height ratios (benzyl alcohol and benzaldehyde to internal standard) were determined for quantitation of both compounds.

Benzaldehyde determination ($\lambda = 282$ nm)

Method A. An appropriate aliquot (1.0–2.5 ml) of benzyl alcohol sample was transferred into a 50-ml volumetric flask, 5.0 ml of internal standard solution were added and the volume was adjusted with acetonitrile. A 10-μl volume was then injected in triplicate. The ratios of peak heights (benzaldehyde to internal standard) were determined and the amount of benzaldehyde in each of the analysed samples was calculated by interpolating the calibration curve.

Method B. Sample aliquots (1.0 ml) were transferred into separate 20.0-ml volumetric flasks spiked with varying amounts (2.0–5.0 ml) of benzaldehyde stock solution, 2.0 ml of internal standard solution were added and the contents were diluted to volume with acetonitrile. A 10-μl volume of each solution was injected in triplicate. The regression line of peak height ratio of benzaldehyde/internal standard versus the amount of benzaldehyde added was then constructed. The observed x-intercept of the regression line was used to calculate the benzaldehyde content in the sample.

Derivative UV spectrophotometry

Calibration curves

A benzaldehyde stock solution (c = 1.046 mg/ml) was prepared in methanol–water 1 : 1. Standard solutions containing 1.0 ml of benzaldehyde-free benzyl alcohol (Fluka A.G.) and increasing amounts (0–5.0 ml) of benzaldehyde stock solution in 20 ml of methanol–water 1 : 1 were prepared. The second-order UV spectra of these solutions were recorded over the range 345–275 nm. The amplitudes peak-zero D_{296} and peak-to-peak $D_{296,281}$ were measured (millimeters) and plotted against the benzaldehyde concentration to obtain the corresponding calibration curves.

Benzaldehyde determination

Method A. An aliquot of the benzyl alcohol sample was diluted 1 : 20 with methanol–water 1 : 1 and the second-order UV spectrum of the resulting solution was recorded. The amplitudes D_{296} and $D_{296,281}$ were measured and by interpolating the corresponding calibration curve, the benzaldehyde content in the benzyl alcohol sample was determined.

Method B. A fixed aliquot (1.0 ml) of benzyl alcohol sample was placed in separate 20-ml volumetric flasks, increasing amounts (0–3.0 ml) of benzaldehyde stock solution were added and dilution to volume was made with methanol–water 1 : 1. The amplitudes $D_{296,281}$ as well as D_{296} from the second derivative of the UV spectra of the resulting solutions were plotted against the benzaldehyde amounts added. The x-intercepts of the two regression lines obtained were both used for the benzaldehyde quantitation in each benzyl alcohol sample.

Results and Discussion

Both the proposed HPLC and derivative UV methods for the benzaldehyde determination in benzyl alcohol were based on the spectral differences between the compounds. Fig. 1 shows the zero-order UV spectra of solutions of benzyl alcohol and benzaldehyde in methanol–water (1 : 1). The significantly higher ($\approx 10^2$) absorbtivity value of benzaldehyde (at $\lambda = 254$ nm) makes an HPLC method with UV-254 detection sensitive enough for benzaldehyde traces determination. On the other hand, the additional absorption maximum at about 283 nm (absent in the benzylalcohol UV spectrum) was the rationale to develop specific HPLC and UV procedures for benzaldehyde determination.

HPLC analysis

Under the described chromatographic conditions, an adequate separation between benzyl alcohol ($t_r = 4.54$), the internal standard, methyl paraben ($t_r = 5.54$) and benzaldehyde ($t_r = 7.74$) was achieved. Figs. 2 and 3 show representative chromatograms obtained with detection at 254 nm and 282 nm, respectively. The identification of the benzaldehyde peak at $t_r = 7.74$, was obtained by comparing the peak height ratios 254/282 nm in the sample chromatograms with the corresponding ratios from benzaldehyde standard chromatograms. This agreement was primarily verified by a conventional variable wavelength detector and

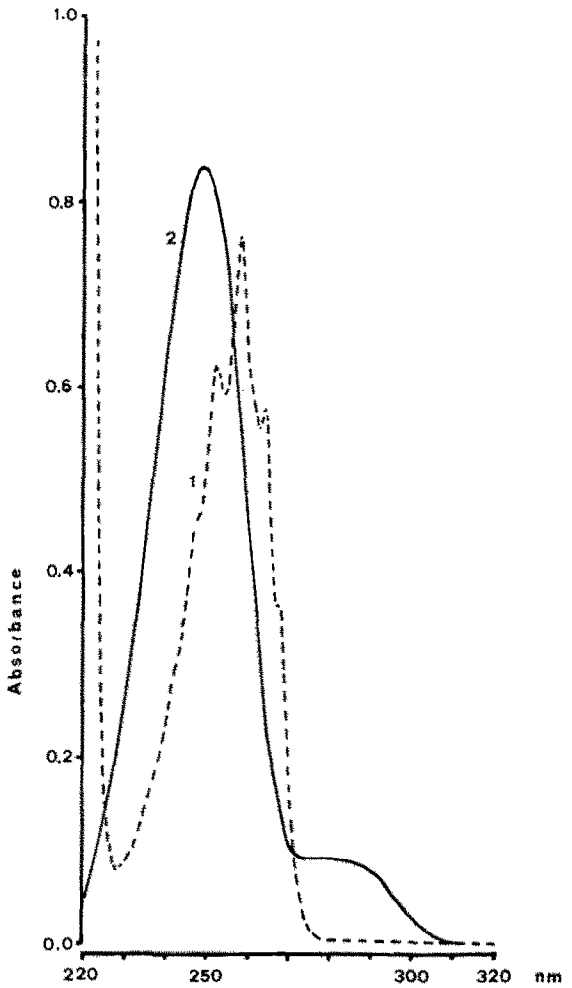

Fig. 1. Normal UV spectra of benzyl alcohol (1, c = 400 μg/ml) and benzaldehyde (2, c = 6 μg/ml) in methanol-water (1:1). Slit: 1 nm; scan speed: 20 nm/min.

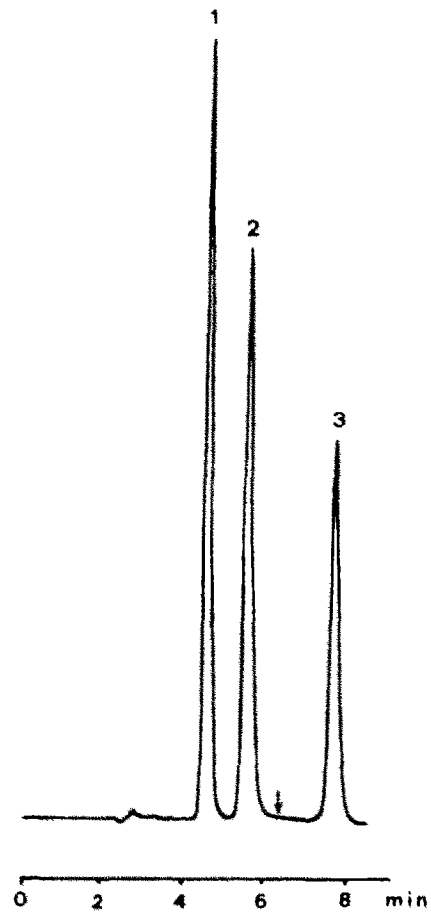

Fig. 2. Typical HPLC separation between benzyl alcohol (1, c = 1.6 mg/ml), the internal standard methyl paraben (2, c = 20 μg/ml) and benzaldehyde (3, c = 5.8 μg/ml, equivalent to 0.36% of benzyl alcohol). Column: Hypersil RP-8 (5 μm); mobile-phase: acetonitrile–water (38:62) at a flow-rate of 1 ml/min. Detection: UV-254 nm. The arrow indicates an attenuation variation from 128 to 32.

then confirmed using a Varian Polychrom diode array detector which provided instantaneous spectra and absorbance ratios acquisition. As a feature, the Polychrom detector also offers a purity parameter, derived from instantaneous mathematic evaluation of the spectral data for peak purity determination. The absorbance ratios (254/282) and the purity parameter values determined at the apex and the inflection points of the methyl paraben (the internal standard) and benzaldehyde peaks in the sample chromatogram (Table 2), compared to the corresponding values obtained for the standards (Table 1), confirmed the benzaldehyde peak identity and showed that there was no interference. Accordingly, the UV spectrum of the peak apex at $t_r = 7.74$ for the sample was found to overlap that of the benzaldehyde standard. Therefore, the described chromatographic conditions allowed specific benzaldehyde traces analysis in benzyl alcohol and methyl paraben was an appropriate interference-free internal standard.

In the present work, the benzyl alcohol from

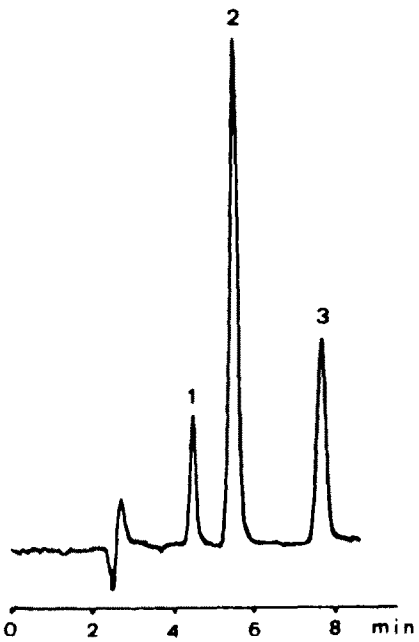

Fig. 3. Typical HPLC separation between benzyl alcohol (1, c = 20.84 mg/ml), methyl paraben (2, c = 20 μg/ml) and benzaldehyde (3, c = 13.21 μg/ml, equivalent to 0.063% of benzyl alcohol) with UV-282 detection (att. 32). Chromatographic conditions as in Fig. 2.

Fluka, which proved to be essentially benzaldehyde-free, was used as standard for all calibrations. At first, an HPLC method suitable for the simultaneous determination of benzyl alcohol and benzaldehyde traces was devised. Accordingly, the UV-254 detection was chosen, and the procedure involved the use of a single internal standard (methyl paraben) for both benzyl alcohol and benzaldehyde assay and an attenuation variation (128 to 32) after the internal standard peak elution. For a selective determination of benzaldehyde traces, UV-282 detection with a fixed 32 or 16 attenuation value was used. Under the experimental conditions described, linear calibration curves were obtained by both the proposed HPLC procedures. Table 3 reports the regression and suitability system data.

The methods were applied to the analysis of some commercial benzyl alcohols and the results obtained are summarized in Table 4. As can be seen, benzaldehyde levels lower than the pharmacopeia limit values (0.2% of the benzyl alcohol) were found in all the samples analysed with the exception of sample A. However, only samples B, D and F showed a benzaldehyde content lower than 0.05% as required (Ph. Eur., 1984) when benzyl alcohol is used for parenteral preparations. The different HPLC procedures provided results in good agreement and showed adequate precision. Quantitative recoveries were obtained when benzyl alcohol solution spiked with known benzaldehyde quantities were analysed.

Derivative UV spectrophotometric analysis

For comparison purposes, the HPLC analysis results were compared to those obtained by a second-order UV derivative spectrophotometric method. Spectrophotometric procedures have been reported for benzaldehyde determination in ben-

TABLE 1

Absorbance ratios and purity parameter values from standard methylparaben and benzaldehyde chromatogram

Time (min)	Type	Absorbance ratio [a]	Purity parameter [b]	Compound
5.45	Peak upslope	8.841	254.14	methylparaben
5.52	Peak apex	8.834	254.08	methylparaben
5.60	Peak downslope	8.433	254.04	methylparaben
7.63	Peak upslope	7.651	245.88	benzaldehyde
7.71	Peak apex	7.590	245.82	benzaldehyde
7.80	Peak downslope	7.688	245.70	benzaldehyde

[a] Absorbance ratio 254/282 nm.
[b] Wavelength range 220–311 nm.

TABLE 2

Absorbance ratios and purity parameter values from sample a chromatogram

Time (min)	Type	Absorbance ratio [a]	Purity parameter [b]	Compound
5.46	Peak upslope	8.734	254.16	methylparaben
5.54	Peak apex	8.881	254.18	methylparaben
5.62	Peak downslope	8.854	254.06	methylparaben
7.65	Peak upslope	7.613	245.91	benzaldehyde
7.74	Peak apex	7.508	245.84	benzaldehyde
7.83	Peak downslope	7.554	245.82	benzaldehyde

[a] Absorbance ratio 254/282 nm.
[b] Wavelength range 220–311 nm.

TABLE 3

Data for the calibration curves and the system suitability

Compound	Internal standard	Detector wavelength	Linear regression parameters [a]			System suitability		
			Slope	Intercept	Correlation coefficient	RSD% [b]	Resol. [c]	Detection limit [d] (μg/ml)
Benzyl alcohol	Methylparaben	254	0.596	0.0120	0.9999	0.51	2.22	20.0
Benzaldehyde	Methylparaben	254	0.144	0.0009	0.9999	0.40	4.0	0.1
Benzaldehyde	Methylparaben	282	0.395	0.0176	0.9998	0.46	4.0	1.0

[a] Peak height ratio of analyte to internal standard on the y-axis versus concentration on the x-axis.
[b] From 6 replicate injections of a single standard solution.
[c] Resolution factor between the peaks from analyte and internal standard.
[d] Based on a signal-to-noise ratio of 5 : 1.

TABLE 4

Assay results for benzaldehyde determination in some commercial benzyl alcohol samples

Sample [a]	Benzyl alcohol [b]		Benzaldehyde [b,c]							
	HPLC		HPLC (254 nm)		HPLC (282 nm)		UV — $D_{296,281}$		UV — D_{296}	
	Found%	RSD%	Found	RSD%	Found	RSD%	Found	RSD%	Found	RSD%
A	99.10	0.86	0.370	1.60	0.367	0.80	0.460	0.65	0.470	0.70
B	100.30	0.90	0.009	2.60	0.008	1.83	0.025	1.70	0.027	1.20
C	100.60	1.10	0.065	1.85	0.063	1.65	0.066	1.50	0.068	1.35
D	100.05	1.05	0.021	2.82	0.019	2.10	0.019	2.10	0.021	1.80
E	99.80	0.80	0.116	1.90	0.116	1.70	0.119	1.07	0.120	1.10
F	99.60	0.65	0.036	2.30	0.035	1.50	0.035	1.90	0.037	1.75

[a] Samples: A, C. Erba ASA-NF-BP; B, C. Erba RPE, containing about 0.02% BHA; C, Merck DAC, BP, NF; D, Serva research grade; E, Baker Erg. B6, Ph. Eur.; F, Prolabo for analysis.
[b] Average of 5 determinations.
[c] Expressed as a percentage of benzyl alcohol.

zyl alcohol (Rees and Anderson, 1949; Gorog and Suto, 1976), but to our knowledge derivative UV spectroscopy has not been applied to this topic.

Fig. 4 shows the zero-order spectrum of benzyl alcohol standard (c = 52.1 mg/ml) containing 0.05% of benzaldehyde, while Fig. 5 shows the

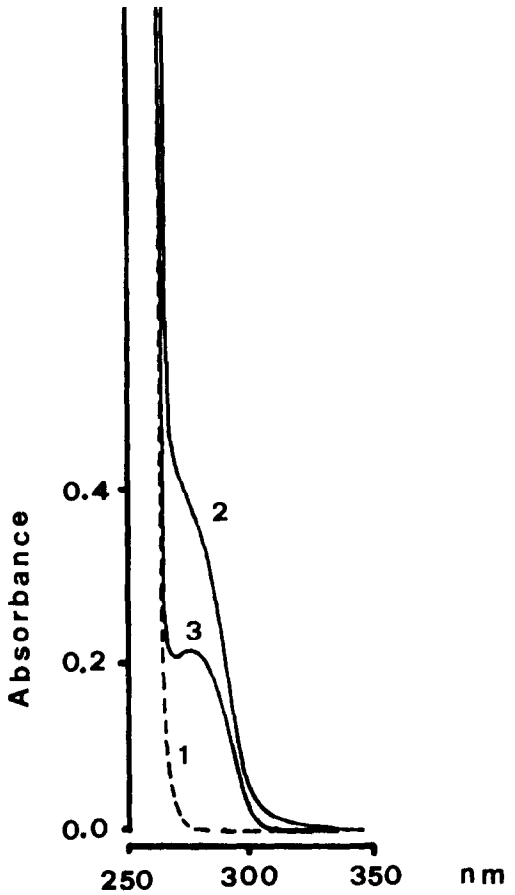

Fig. 4. Normal UV spectra of benzyl alcohol standard (1), benzyl alcohol containing 0.05% of benzaldehyde (2) and benzyl alcohol containing 0.02% of BHA (3). Benzyl alcohol concentration: 50 mg/ml in methanol–water (1 : 1). Scan speed: 50 nm/min.

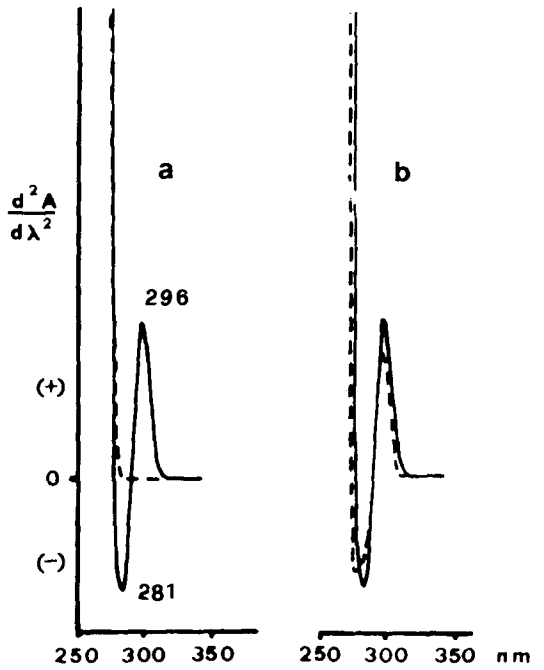

Fig. 5. Second-order derivative UV spectra of: (a) benzyl alcohol standard (- - - - -) and benzyl alcohol containing 0.05% of benzaldehyde (———); (b) benzyl alcohol containing 0.05% of benzaldehyde (———) and benzyl alcohol containing 0.02% of BHA (- - - - -). Benzyl alcohol concentration as in Fig. 4. Scan speed: 100 nm/min.

corresponding second-order derivative of the spectrum. As can be seen, the shoulder near to 282 nm in the normal spectrum, due to benzaldehyde, is converted in a new two peaks ($\lambda = 296$ nm and 281 nm) profile when the second-order derivative is recorded. The easily measurable peak-to-peak amplitude $D_{296,281}$ and peak-zero amplitude D_{296} appear unaffected by benzyl alcohol. In fact, when increasing quantities of benzaldehyde were added to benzyl alcohol standard (Fluka A.G.) and the measured amplitudes $D_{296,281}$ and D_{296} were plotted against the benzaldehyde amounts added ($c = 0.05 - 0.5\%$ of benzyl alcohol) linear calibration curves were obtained passing close to the origin:

$D_{296,281} = 71.146c - 0.082$; $r = 0.9999$; $n = 7$;

$D_{296} = 39.0c + 0.035$; $r = 0.9999$; $n = 7$.

The relative standard deviations of the amplitudes, derived from replicate ($n = 8$) recording of the second-derivative spectrum, all fell in the range of 0.54 – 1.00%.

The calibration curves were used (Method A) to analyse benzyl alcohol samples (A and E) containing a high level of benzaldehyde, while the standard-addition method (Method B) was applied to analyse samples having a very low benzaldehyde content. Following this procedure, the regression lines obtained by both the amplitudes $D_{296,281}$ and D_{296} were essentially parallel to the corresponding calibration curves and the observed x-intercepts were then used for the benzaldehyde quantitation. The results obtained (Table 4) were

in good agreement with those from the HPLC procedures. For the sample B, however, a significantly higher benzaldehyde content was found, indicating that some interference was present. Sample B, in effect, was benzyl alcohol stabilized with 0.02% of butylhydroxyanisole (BHA) and this additive proved to interfere with the benzaldehyde determination. This is illustrated in Fig. 5b, showing the second-order derivative UV spectrum of a benzyl alcohol standard solution containing 0.02% of BHA. To a slight extent, a similar interference effect could be also involved in the sample A analysis.

Conclusion

Liquid chromatography with UV detection at 254 nm constitutes a rapid and sensitive method for simultaneous determination of benzyl alcohol and benzaldehyde, its oxidative degradation product. The analysis can be carried out using an inexpensive UV-254 detector and the proposed method could be used as a convenient alternative to official procedures involving a time-consuming acetylation reaction for the benzyl alcohol assay (Ph. Jap., 1981, Ph. Eur., 1984, Ph. Helv., 1971) and poorly specific tests for the benzaldehyde detection. Moreover, a variable wavelength detector and, even better, a diode array detector allow confirmation of the identity and the purity of the analytes peaks. Working with UV-282 detection, a specific benzaldehyde traces determination was performed, but a lower sensitivity was achieved.

A method based on derivative (second-order) UV spectroscopy proved convenient for a rapid and easy benzaldehyde traces determination in benzyl alcohol, but interferences from substances absorbing near to 290 nm (e.g. butylhydroxyanisole) were found to give an inflated benzaldehyde content. Both the chromatographic and spectroscopic methods allow benzaldehyde determination at concentrations down to 0.05% of the benzyl alcohol level, the limit value required (Ph. Eur., 1984) for parenteral dosage forms.

Acknowledgements

The authors wish to thank Miss Barbara Giammattei for skilful technical assistance. This work was supported by the Ministero della Pubblica Istruzione (40%), Italy.

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