Difference Spectrophotometric Determination of *p*-Hydroxybenzoic Acid in Presence of Its Esters

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Abstract □ A difference spectrophotometric analytical method was developed for the selective determination of *p*-hydroxybenzoic acid in the presence of its alkyl esters without prior separation. Based on the spectral shift to a shorter wavelength accompanying carboxyl dissociation, the procedure measures as little as 2% of this acid in mixtures with the alkyl ester preservatives and has an accuracy of 2% mean relative error over the 0.16–12.0 μg of *p*-hydroxybenzoic acid/ml range.

Keyphrases □ *p*-Hydroxybenzoic acid—difference spectrophotometric analysis in presence of alkyl esters □ Spectrophotometry, difference—analysis, *p*-hydroxybenzoic acid in presence of alkyl esters

Previous studies (1, 2) reported that standard procedures for the determination of alkyl *p*-hydroxybenzoate preservatives (parabens) typically require sample isolation to separate *p*-hydroxybenzoic acid prior to measurement. Operations involving derivatization, liquid–liquid partitioning, or chromatographic separation using either thin-layer or column techniques have been used. For example, the interference of *p*-hydroxybenzoic acid in the direct spectrophotometric determination of its esters was eliminated by separation on diatomaceous earth (2); liquid–liquid partitioning also was used to accomplish this same end (3, 4). In addition, instrumental methods using GLC (5–8) and high-pressure liquid chromatography (HPLC) (1, 9) were developed.

This report describes a simple and rapid spectrophotometric method based on an application of the difference spectrophotometric technique (10) for the selective determination of *p*-hydroxybenzoic acid in the presence of its alkyl esters. A pH-induced shift in the spectrum of this acid is used over a range in which the spectra of the esters are pH independent, thus effectively canceling their interferences and obviating any preliminary separation. The primary application is for the stability-indicating measurement of these preservatives through determination of the acid as the hydrolytic degradate.

EXPERIMENTAL

Instrumentation—UV absorption spectra were recorded on a spectrophotometer[1] equipped with 0–0.1 and 0–1.0 absorbance unit full-scale slide wires. The pH measurements were made at 20° with a pH meter[2] and combination electrode[3], calibrated using standard buffers[4].

Materials—*p*-Hydroxybenzoic acid, its methyl ester (methylparaben), and propyl ester (propylparaben)[5] were used without further purification. Buffers, 0.1 *M*, were prepared using anhydrous dibasic sodium phosphate[6] and reagent grade hydrochloric acid[7]. Deionized water was used throughout.

UV Difference Spectrophotometry—Duplicate 5-ml aliquots of aqueous samples and analytical standard were transferred to 50-ml centrifuge tubes; 5 ml of 0.1 *N* HCl was added to one, and 5 ml of pH 5.9 buffer was added to the other. Difference spectra were recorded over the 220–330-nm range with the acidic solution in the sample cell and the pH 5.9-buffered solution in the reference cell after manually adjusting the baseline control to set the pen at $A = 0.4$ with $\lambda = 330$ nm. Absorption difference values, ΔA, were determined from the maxima and minima, $A_{260} - A_{235}$, after applying baseline corrections from the difference spectrum of blank solutions.

Analytical Procedure—Aqueous solutions of *p*-hydroxybenzoic acid and its methyl and propyl esters were prepared. (The propyl ester was first dissolved in a small amount of methyl alcohol.) Dilutions were made with water to obtain the desired concentration, and difference spectra were obtained as described. Values for $\Delta A = A_{260} - A_{235}$ were calculated from each spectrum, and the quantitative determination of the *p*-hydroxybenzoic acid concentration of each sample was obtained by comparison of ΔA to a previously prepared calibration line of ΔA against a known acid concentration.

RESULTS

Absorption Spectra—*Conventional Spectra*—The conventional UV absorption spectra of *p*-hydroxybenzoic acid (3.62×10^{-5} *M*) and of methylparaben (3.29×10^{-5} *M*) in pH 5.9 buffer, 0.1 *N* HCl, and 0.1 *N* NaOH were studied in detail (11) (Fig. 1). The spectrum of the neutral acid in 0.1 *N* HCl was nearly identical to the absorption spectrum of the ester in the same medium; both compounds in this environment exhibited an absorption band at λ_{max} 254 nm, with a molar absorptivity of 1.4×10^4 liters/mole cm at this wavelength and with a width at half-height of 34 nm.

In basic solution (pH > 9), this absorption band shifted to a longer wavelength accompanying the usual dissociation of the phenolic proton. For *p*-hydroxybenzoic acid alone, however, a spectral shift to a higher energy occurred in the pH 5–8 range, representing the monoanionic species. The absorption spectrum of the ester was pH independent in the pH 1–6 range.

Difference Spectrum—Figure 2 presents the difference absorption spectrum of *p*-hydroxybenzoic acid recorded as described at six concentrations in the 2.0–12.0-μg/ml range. This spectrum exhibited a maximum at 260 nm, a minimum at 235 nm, and isosbestic points at 221 and 246 nm.

Calibration Line—Eleven aqueous solutions of *p*-hydroxybenzoic acid were prepared in the 0.32–24.0-μg/ml range, and their difference spectra were recorded as described. After dilution, the concentrations of the analytical solutions were 0.16–12.0 μg/ml; some representative difference spectra are shown in Fig. 2.

A calibration line was obtained from the 11 difference spectra by plotting the difference between the maximum and minimum of each spectrum ($\Delta A = A_{260} - A_{235}$) against concentration after applying corrections for the blank solutions. The correlation coefficient of the resulting line was 1.000, and the standard error of estimate from a linear regression analysis was 0.024, demonstrating excellent linearity over this concentration range.

Analysis—Recovery experiments for *p*-hydroxybenzoic acid over the 0.20–10.0-μg/ml range in mixtures with its esters are summarized in Table I. These mixtures were prepared in the aqueous solution and analyzed by the described procedure. Different amounts of methylparaben and propylparaben were added to simulate their combination use.

The results demonstrate that the esters did not interfere with the determination of *p*-hydroxybenzoic acid over the concentration ranges examined (Table I). As little as 2% of the acid was detected accurately in the presence of its esters; excellent results were obtained as well for solutions in which the acid was the predominant species. These results had a mean relative error of 1.9% ($SD = 3.9\%$).

Similar results were obtained using the 0–0.1 absorbance unit full-scale slide wire for solutions containing up to about 1.2 μg of *p*-hydroxybenzoic

[1] Cary 15.
[2] Corning model 12.
[3] Corning 476050.
[4] Corning 477070.
[5] Eastman Kodak Co.
[6] Baker analyzed reagent.
[7] Reagent, Merck.

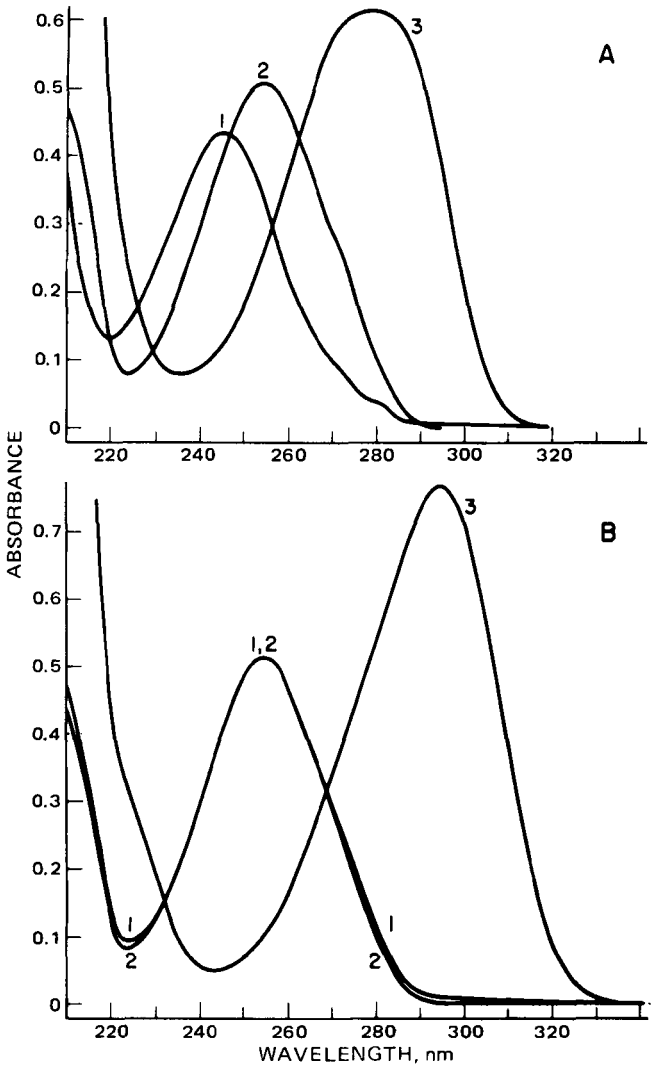

Figure 1—*Absorption spectra of 3.62 × 10^{-5} M p-hydroxybenzoic acid (A) and 3.29 × 10^{-5} M methylparaben (B) in pH 5.9 buffer (1), 0.1 N HCl (2), and 0.1 N NaOH (3).*

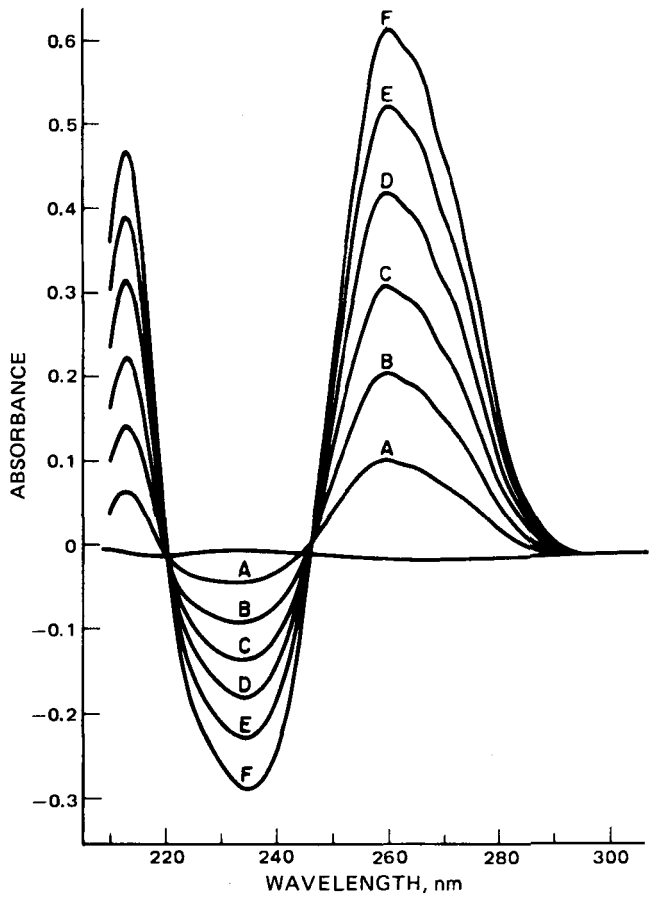

Figure 2—*Difference spectrum of p-hydroxybenzoic acid as a function of concentration. Sample solutions were prepared in 0.1 N HCl; reference solutions were prepared in pH 5.9 phosphate buffer. Concentrations are 2.0 (A), 4.0 (B), 6.0 (C), 8.0 (D), 10.0 (E), and 12.0 (F) μg of p-hydroxybenzoic acid/ml.*

acid/ml. For either slide wire, however, it was necessary to keep the total concentration of acid plus ester below about 30 μg/ml to allow sufficient light to pass to the detector on the instrument; above this concentration, the large slit width required precluded accurate measurements.

DISCUSSION

Difference spectroscopy was discussed previously (10). This technique is appropriate for analyses of formulations because other UV-absorbing species can be tolerated if they do not exhibit a spectral shift with the analate perturbation. This particular difference spectrophotometric application was indicated previously (12). It was suggested that two measurements at pH 1 and 7 could be used to determine *p*-hydroxybenzoic acid and its ester in the presence of each other.

The principles of this method are apparent from a consideration of the spectra. Unlike the esters, the acid can exist in three acid–base species. A red shift of the long wavelength band with dissociation is a property common to many *meta*- and *para*-substituted phenols (11, 13, 14). The absorption spectra of the neutral acid and the ester are nearly identical, demonstrating that the proton on the carboxyl group creates the same delocalized electronic structure as the alkyl group; these compounds are indistinguishable at pH < 4.

In neutral solution (pH 5–8), the absorption spectrum of the acid shifts to a shorter wavelength—from λ_{max} 254 to 244 nm. This blue shift accompanying dissociation of the carboxyl group (at pH 4.5) appears regularly for aromatic rings with this substituent. As discussed (11) and examined (15) previously, both of these spectral shifts reflect the electron donor and acceptor tendencies of the protonated species and their corresponding conjugate bases.

The spectral change occurring with the first ionization forms the basis for the determination of this acid by difference spectroscopy. In this technique, the reference cell contains the acid in the monoanionic form and the sample cell contains a solution with the same concentration of the analate in the neutral conjugate acid form. The recorded spectrum is the difference between the spectra of these two species. This difference spectrum exhibited a maximum at 260 nm, corresponding to net absorption by the sample, and a minimum at 235 nm, corresponding to net absorption by the reference. The difference in absorbance between the maximum and the minimum was directly proportional to the *p*-hydroxybenzoic acid concentration and obeyed Beer's law to at least 12 μg of *p*-hydroxybenzoic acid/ml, with an average absorptivity in this concentration range of 7.88 × 10^{-2} ml/cm μg for the 11 calibration measurements.

This technique is potentially useful for the analyses of formulations because it compensates for interferences in the sample by having the same interferences (*e.g.*, the esters) present at equal concentration in both the sample and reference cells. Because the absorption spectrum of the ester is pH independent in the pH 1–5.9 range, it does not interfere.

The pKa values of the phenolic and carboxylic groups of *p*-hydroxybenzoic acid are 9.5 and 4.5, respectively (16), establishing that the optimum sensitivity of the difference spectrophotometric technique will be realized with a pH 7.0 buffer, where the fraction of the acid existing as the monoanion (Fig. 1A, spectrum 1) is maximized (>99%). However, at this pH, the alkyl *p*-hydroxybenzoate esters interfere by contributing to the difference spectrum. Because the pKa values of the parabens are in the 8.4–8.5 range (17), approximately 3% of these esters are ionized at pH 7. Hence, pH 5.9, where only 0.3% of the esters are ionized, was selected to reduce this interference. At this pH, some sensitivity is sacrificed to achieve this selectivity; *i.e.*, 96% of the acid exists as the monoanionic species, causing a 4% reduction in sensitivity.

Each difference spectrum of *p*-hydroxybenzoic acid recorded in the presence of the esters intersected zero at 246 and 221 nm, the isosbestic

Table I—Determination of *p*-Hydroxybenzoic Acid in the Presence of Its Alkyl Esters

p-Hydroxybenzoic Acid Taken, μg/ml	Ester Added, μg/ml		ΔA	*p*-Hydroxybenzoic Acid Found, μg/ml
	Propylparaben	Methylparaben		
0.20	0	10.0	0.020	0.22
0.40	0	10.0	0.033	0.39
0.80	0	4.0	0.069	0.86
	0	8.0	0.070	0.88
	0	10.0	0.068	0.85
	0	12.0	0.062	0.77
1.20	0	10.0	0.096	1.22
1.60	0	10.0	0.122	1.56
			0.125	1.60
2.00	0	4.0	0.166	2.14
	0	8.0	0.162	2.09
	0	10.0	0.157	2.02
	4.0	4.0	0.165	2.13
	8.0	0	0.155	2.00
3.20	0	10.0	0.242	3.14
4.00	0	8.0	0.310	4.04
		10.0	0.305	3.97
	4.0	4.0	0.308	4.01
	8.0	0	0.310	4.04
6.00	0	8.0	0.458	5.99
	4.0	4.0	0.460	6.02
10.00	0	8.0	0.762	9.99
	4.0	4.0	0.766	10.0
	8.0	0	0.762	9.99

points between the two species. This result is additional confirmation, as described previously (10), that the esters do not interfere at these points. Moreover, in this example of difference spectroscopy, the interferences were not at low concentration levels but were present at up to 50 times the analate concentration. In addition, as Table I demonstrates, this method is applicable in the presence of mixtures of alkyl paraben esters, giving the total amount of hydrolysis. To determine the relative decomposition of individual esters in mixtures, further direct measurements would be required.

This method is convenient since it avoids the usual preliminary separations and is more rapid than the other methods previously reported. This procedure is especially useful in kinetic studies to monitor the paraben degradation under selected conditions. It possesses the usually sought analytical attributes of simplicity, short analysis time, selectivity, stability of the measurement, linearity, sensitivity, accuracy, and precision.

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Simultaneous Determination of Procainamide and *N*-Acetylprocainamide in Plasma by High-Performance Liquid Chromatography

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Abstract □ A sensitive, specific, high-performance liquid chromatographic procedure is described for the simultaneous determination of procainamide and its metabolite, *N*-acetylprocainamide, in plasma. Basic plasma (2.0 ml), containing pheniramine maleate as an internal standard, is partitioned with methylene dichloride. The organic extract is concentrated to between 0.3 and 0.5 ml, and 100-μl aliquots are chromatographed on a microparticulate silica gel column using 0.1% acetic acid–20% 0.1 *M* ammonium acetate in acetonitrile as the mobile phase. With a fixed-wavelength (254-nm) UV detector, both compounds can be quantitated in the 0.1–8.0-μg/ml of plasma range.

Keyphrases □ Procainamide—high-performance liquid chromatographic analysis simultaneously with *N*-acetylprocainamide in plasma □ *N*-Acetylprocainamide—high-performance liquid chromatographic analysis simultaneously with procainamide in plasma □ High-performance liquid chromatography—simultaneous analyses, procainamide and *N*-acetylprocainamide in plasma □ Cardiac depressants—procainamide, high-performance liquid chromatographic analysis simultaneously with *N*-acetylprocainamide in plasma

Several spectrofluorometric and colorimetric procedures were reported (1–5) for the determination of procainamide in plasma; however, these procedures lack specificity because of interference either from *N*-acetylprocainamide, the active metabolite of procainamide (6), or from endogenous materials. Several specific GLC procedures were reported (7–11) for procainamide, but not for *N*-acetylprocainamide, in plasma. One GLC procedure was re-