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NMR Analysis of Some Alkyl *p*-Hydroxybenzoates

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Abstract □ By applying NMR spectrometry, a number of solutions containing unknown quantities of two or three alkyl *p*-hydroxybenzoates (parabens) in 60% polyethylene glycol in water were analyzed for quantification of the individual parabens. The method was found to be accurate, facile, and rapid and seems to possess a promising potential for applicability in related fields of pharmaceutical analysis.

Keyphrases □ Parabens—NMR analysis □ Alkyl *p*-hydroxybenzoates—NMR analysis □ NMR spectroscopy—analysis

The antibacterial and antifungal properties of esters of *p*-hydroxybenzoic acid (parabens) have been of interest to pharmaceutical scientists for the past few decades. Methyl, propyl, butyl, and benzyl parabens have been investigated individually or in combination in pharmaceutical research and development.

A literature survey revealed that UV spectrophotometry is the most widely used method for a quantitative analysis of the parabens (1). This technique has proved quite satisfactory in the quantification of a single paraben, but it is not sufficient for the analysis of a mixture of parabens in solution. This is due to the fact that most parabens possess nearly identical $\lambda_{max.}$ values.

Procedures such as column chromatography (2) and GLC (3) have been reported for the analysis of parabens. The former procedure is time consuming and laborious. If esters are in an aqueous solution, the silylation technique, which should precede injection onto the column, together with other problems inherent to GLC makes the latter procedure less desirable and casts some doubts upon the accuracy of the results.

An exploratory attempt on the applicability of NMR spectrometry for the analysis of parabens revealed the method to be both rapid and accurate and the procedure quite simple.

EXPERIMENTAL

The parabens used in the experiments were all reagent grade. The general procedure for preparing the standard solutions consisted of placing an accurately weighed quantity of the desired ester in a volumetric flask, dissolving, and adjusting the volume with 60% polyethylene glycol (PEG) 400 in water. This vehicle was also used for preparing the solution of the paraben mixtures. Adoption of this solvent was due to its routine use in some other aspects of the research and to illustrate the point that the analysis does not require deuterated solvents.

The NMR spectra were determined using a Varian A-60 NMR spectrophotometer at an ambient temperature.

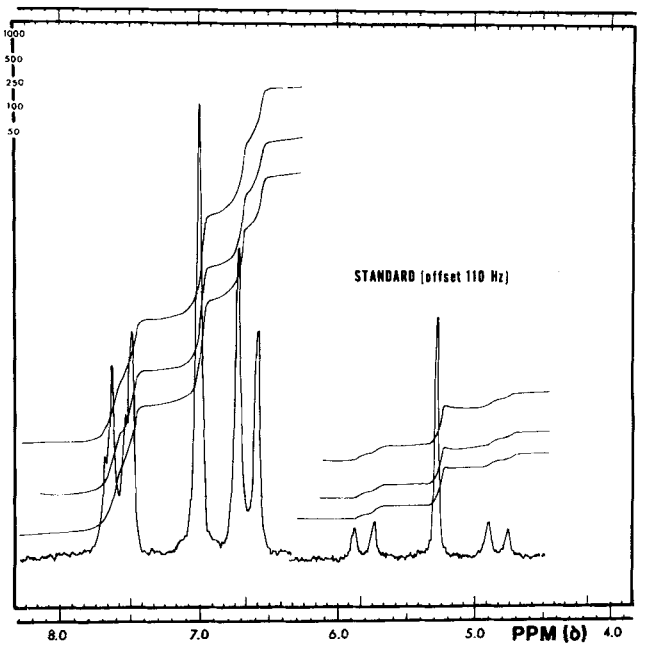
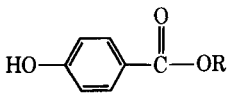


Figure 1—*The NMR spectrum of a mixture of benzyl paraben and methyl paraben in PEG–H_2O. Standard benzyl paraben is depicted at 110 c.p.s. offset.*

RESULTS AND DISCUSSION

Depicted in Fig. 1 is the NMR spectrum of a mixture of benzyl paraben and methyl paraben in 60% PEG in water. Benzyl paraben contains two doublets at δ 6.85 and δ 7.85, which are the results of spin-spin coupling of two pairs of phenyl protons, in two different magnetic environments, in the *p*-hydroxybenzoate moiety of the ester molecule (Structure I). The singlet at δ 7.3 results from the phenyl protons of the benzyl moiety. The two doublets of benzyl paraben superimpose on the doublets of methyl paraben.



$R = CH_3, C_2H_5, C_3H_7, C_4H_9, CH_2Ph$

I

By comparison of the integral values of the peak of the standard to those of the unknown, the concentration of each paraben in the mixture was calculated. The following examples illustrate the calculation of the concentration of individual parabens in mixtures as PEG–water solutions.

The abbreviations used are: standard = st; unknown = u; singlet integral value = SIV; doublet integral value = DIV; triplet integral value = TIV; molarity = *M;* methyl, ethyl, propyl, butyl, and benzyl paraben = MePab, EtPab, PrPab, BuPab, and BzPab, respectively.

Calculation of Concentration of Methyl Paraben and Benzyl Paraben Present in Unknown Quantities as a Mixture in PEG–Water—See Fig. 1. The standard was a known concentration of benzyl paraben solution.

$$\frac{(SIV)_{BzPab}}{(SIV)_{st}} \times (M)_{st} = (M)_{BzPab} \quad \text{(Eq. 1)}$$

$$4/5 \times (SIV)_{BzPab} = (DIV)_{BzPab} \quad \text{(Eq. 2)}$$

$$\text{total } (DIV)_u - (DIV)_{BzPab} = (DIV)_{MePab} \quad \text{(Eq. 3)}$$

$$\frac{(DIV)_{MePab}}{(DIV)_{st}} \times (M)_{st} = (M)_{MePab} \quad \text{(Eq. 4)}$$

Quantification of a mixture of any alkyl paraben mixed with benzyl paraben can be carried out by a similar procedure.

Calculation of Concentration of Methyl Paraben and Propyl Paraben Present in Unknown Quantities as a Mixture in PEG–Water—The standard was a known concentration of methyl paraben solution.

$$4/3 \times (TIV)_{PrPab} = (DIV)_{PrPab} \quad \text{(Eq. 5)}$$

$$\text{total } (DIV)_u - (DIV)_{PrPab} = (DIV)_{MePab} \quad \text{(Eq. 6)}$$

$$\frac{(DIV)_{MePab}}{(DIV)_{st}} \times (M)_{st} = (M)_{MePab} \quad \text{(Eq. 7)}$$

$$\frac{(DIV)_{PrPab}}{(DIV)_{st}} \times (M)_{st} = (M)_{PrPab} \quad \text{(Eq. 8)}$$

Calculation of Concentration of Benzyl Paraben, Methyl Paraben, and Propyl Paraben Present in Unknown Quantities as a Mixture in PEG–Water—The standard was a known concentration of benzyl paraben solution.

$$\frac{(SIV)_{BzPab}}{(SIV)_{st}} \times (M)_{st} = (M)_{BzPab} \quad \text{(Eq. 9)}$$

$$4/5 \times (SIV)_{BzPab} = (DIV)_{BzPab} \quad \text{(Eq. 10)}$$

$$\text{total } (DIV)_u - (DIV)_{BzPab} = (DIV)_{MePab + PrPab} \quad \text{(Eq. 11)}$$

$$4/3\ (TIV)_{PrPab} = (DIV)_{PrPab} \quad \text{(Eq. 12)}$$

$$(DIV)_{MePab + PrPab} - (DIV)_{PrPab} = (DIV)_{MePab} \quad \text{(Eq. 13)}$$

$$\frac{(DIV)_{PrPab}}{(DIV)_{st}} \times (M)_{st} = (M)_{PrPab} \quad \text{(Eq. 14)}$$

$$\frac{(DIV)_{MePab}}{(DIV)_{st}} \times (M)_{st} = (M)_{MePab} \quad \text{(Eq. 15)}$$

Table I—Results of NMR Analyses of Parabens in PEG–H_2O

Alkyl Group	Concn., mole/l., Prepared	Concn., mole/l., Calculated Using NMR Peak Integral Values
	Single	
1. CH_3	0.2500	0.2497
2. C_2H_5	0.3000	0.3000
	Mixture	
3. CH_3 / n-C_3H_7	0.5000 0.3000	0.5006 0.3000
4. CH_3 / n-C_3H_7	0.7500 0.5000	0.7511 0.4989
5. CH_3 / CH_2Ph	0.7500 0.3000	0.7484 0.3000
6. CH_3 / n-C_3H_7 / CH_2Ph	0.5000 0.2500 0.1500	0.5000 0.2516 0.1500

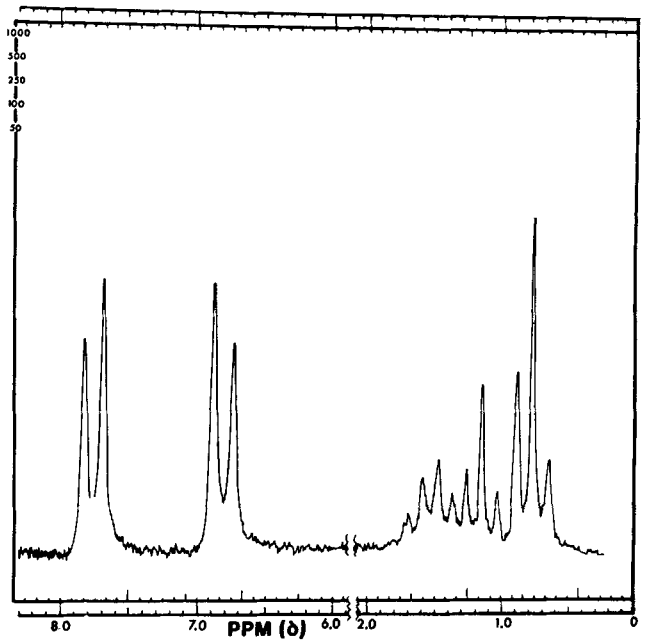


Figure 2—*The NMR spectrum of a mixture of ethyl paraben and propyl paraben in PEG–H_2O.*

A summary of the precision of the experimental procedure is depicted in Table I.

As long as one of the two parabens in a mixture contains a discernible characteristic resonance peak, the individual esters can be determined quantitatively by NMR. Analysis of a mixture of ethyl and propyl paraben, whose triplet peaks are not superimposed, can be cited as an example and is depicted in Fig. 2.

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