# Chromatographic Separation and Determination of Methyl *p*-Hydroxybenzoate

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**Abstract** □ Methyl *p*-hydroxybenzoate was assayed accurately and precisely by UV spectroscopy after separation from its hydrolytic degradation product, *p*-hydroxybenzoic acid. The complete separation was effected by adsorption chromatography on a column of diatomaceous earth. The method was compared to the USP XVIII GC procedure and to a direct UV spectrophotometric procedure.

**Keyphrases** □ Methyl *p*-hydroxybenzoate—separation from *p*-hydroxybenzoic acid, UV analysis, compared to compendial methods □ Column chromatography—separation of methyl *p*-hydroxybenzoate from *p*-hydroxybenzoic acid, compared to compendial method □ UV spectrophotometry—analysis, methyl *p*-hydroxybenzoate, compared to compendial method

Several methods for assaying methyl *p*-hydroxybenzoate (I) have been reported. Sokol (1) described a colorimetric method, which indicated the percentage of I and *p*-hydroxybenzoic acid (II) in a given mixture. This method is indirect and requires rigorous control of reaction conditions (2). The most common assay method involves either direct UV spectroscopy (3) or adjustment of the sample pH to 7 followed by multiple ether extractions and UV spectroscopy (4). In an ether extraction study, Anderson[1] observed errors in recoveries of I which increased with an increasing concentration of II. One recent method reported for assaying I involved multiple ether extractions of the sample followed by silylation and gas chromatography of the silyl derivative (5).

There appears to be no report in the literature defining the extent to which II interferes with the spectrophotometric assay of I, nor is there any method described that unambiguously separates I from II prior to the assay of I. The following investigation defines the extent to which II interferes with the assay of I and describes a method for separating II from I.

## EXPERIMENTAL

**Reagents**—The following were used: methyl *p*-hydroxybenzoate[2]; *p*-hydroxybenzoic acid[3]; diatomaceous earth[4]; Chromosorb W, AW-DMCS, 80–100 mesh[5]; methyl silicone, SE-30[6]; *N,O*-bis-(trimethylsilyl)-acetamide[6]; ether, absolute, anhydrous[7]; pyridine for Karl Fischer reagent[7]; potassium bromide[8]; and buffer, pH 8 ± 0.1, $NaOH-KH_2PO_4$, prepared as directed in USP XVIII. All other reagents were ACS certified grade. Distilled water was used for the preparation and dilution of all solutions.

**Apparatus**—UV measurements were carried out on a double-beam UV-visible spectrophotometer[9] using matched 1.0-cm. Corex cells and a slit width of 25. IR measurements were made on a Infracord spectrophotometer[10]. A gas chromatograph[11] with a flame-ionization detector was used. The 1.83-m. (6-ft.) glass column [i.d. 0.38 cm. (0.125 in.), o.d. 0.64 cm. (0.25 in.)] was packed with 5% SE-30 on Chromosorb W, AW-DMCS, 80–100 mesh, using a vibrator, and was conditioned for 18 hr. at 200° with $N_2$ gas flowing through the column. A small pledget of glass wool was placed in each end of the column. The flow rate of $N_2$ through the column was 80 ml./min. The column temperature was 150° and the injector port temperature was 200°. A strip-chart recorder[12] with a chart speed of 2.54 cm. (1 in.)/min. was used. All sample volumes were 2.0 μl. delivered from a 10.0-μl Hamilton syringe.

**Standards for UV Spectroscopy**—Weigh 100 mg. of I into a 100-ml. volumetric flask, dilute to volume with water, and mix. Pipet 5.0 ml. into a 100-ml. volumetric flask, dilute to volume with water, and mix. Pipet 3.0-, 4.0-, and 5.0-ml. aliquots of this solution into 50-ml. volumetric flasks; then add 1.0 ml. of approximately 0.1 *N* sodium hydroxide, dilute to volume with water, and mix. These solutions contain 3, 4, and 5 mcg. I/ml., respectively. Determine the absorbance of each solution at 297 nm. against a blank consisting of 2.0 ml. of 0.1 *N* NaOH diluted to 100 ml. with water (6). Calculate the average absorbance per microgram.

**Procedure**—A number of aqueous solutions were prepared containing various ratios of I and II which corresponded to eight degradation levels of I. Each solution was assayed by the following methods.

*GC*—Follow the procedure for I described in USP XVIII (5). Peak heights were used to quantitate I since they gave more precise results than peak areas measured with a planimeter. (See Fig. 1.)

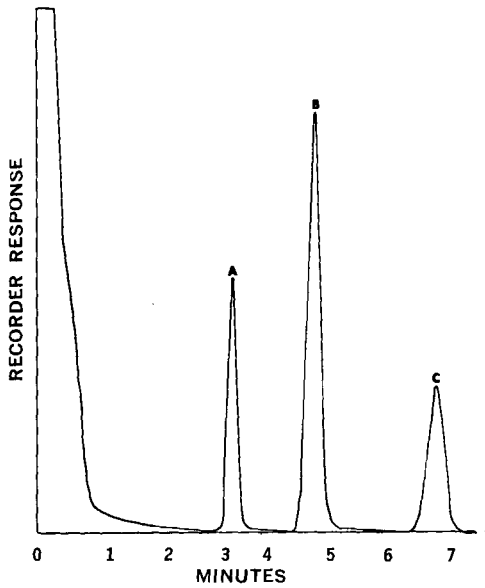


**Figure 1**—*Typical GC of: A, methyl* p*-hydroxybenzoate; B, 2-naphthol; and C,* p*-hydroxybenzoic acid.*

[1] Personal communication from Dr. Jon C. Anderson, Research Laboratories, Astra Pharmaceutical Products, Inc., Worcester, MA 01606

[2] Heyden-Newport Co. This reagent met USP XVIII specifications for methylparaben.

[3] Eastman Organic Chemicals; m.p. 212–213.5°, assay 99.6%.

[4] Celite 545, AW, Johns-Manville.

[5] Wilkens Instrument and Research, Inc.

[6] BSA, Pierce Chemical Co.

[7] Matheson, Coleman and Bell.

[8] Isomet Corp.

[9] Perkin-Elmer model 202.

[10] Perkin-Elmer model 137B.

[11] Perkin-Elmer model 811.

[12] Bristol Dynamaster.

Table I—Recovery of Methyl *p*-Hydroxybenzoate (I) from Aqueous Solutions Containing Methyl *p*-Hydroxybenzoate and *p*-Hydroxybenzoic Acid (II)

| Solution Number | Percent Degradation | mg. II/ml. | mg. I/ml. | I Recovered, mg./ml.[a] | | |
|---|---|---|---|---|---|---|
| | | | | Direct UV Method | GC | Column Chromatography |
| 1 | 0 | 0.000 | 1.000 | 0.997 (0.3)[b] | 1.03 (3.0) | 0.996 (0.4) |
| 2 | 10 | 0.091 | 0.900 | 0.962 (6.9) | 0.949 (5.4) | 0.923 (2.6) |
| 3 | 20 | 0.182 | 0.800 | 0.890 (11.3) | 0.841 (5.1) | 0.815 (1.9) |
| 4 | 30 | 0.272 | 0.700 | 0.848 (21.0) | 0.752 (7.4) | 0.697 (1.1) |
| 5 | 50 | 0.454 | 0.500 | 0.736 (47.2) | 0.503 (0.6) | 0.518 (3.6) |
| 6 | 70 | 0.635 | 0.300 | 0.634 (111.3) | 0.299 (0.3) | 0.299 (0.3) |
| 7 | 90 | 0.817 | 0.100 | 0.552 (452.0) | 0.101 (1.0) | 0.105 (5.0) |
| 8 | 100 | 0.908 | 0.000 | 0.473 (∞) | 0.000 (0.0) | 0.000 (0.0) |
| *SD* | | | | | ±0.044[c] | ±0.028[d] |

[a] Based on an average of a minimum of three assays. [b] All numbers in parentheses are percent error. [c] Calculated from four separate assays of the 50% degradation solution. [d] Calculated from seven separate assays of the 50% degradation solution.

*Direct UV Spectrophotometry*—Pipet 5.0 ml. of sample solution containing 1.0 mg. I/ml. (or its equivalent in degradation products) into a 100-ml. volumetric flask, dilute to volume with water, and mix. Pipet 4.0 ml. of this solution into a 50-ml. volumetric flask, add 1.0 ml. of approximately 0.1 *N* sodium hydroxide, dilute to volume with water, and mix. Determine the absorbance of this solution at 297 nm. against a blank consisting of 2.0 ml. of 0.1 *N* NaOH diluted to 100 ml. with water. The calculation is:

$$\frac{A \times 100 \times 50}{B \times 5 \times 4 \times 1000} \text{ or } \frac{A}{B \times 4} = \text{mg. I/ml.} \qquad \text{(Eq. 1)}$$

where $A$ = absorbance of the sample, and $B$ = average absorbance per microgram of the standard.

*Column Chromatography*—Insert a pledget of glass wool into the bottom of the chromatography column[13]. Mix 2.0 ml. of pH 8 buffer solution with 3.0 g. of diatomaceous earth, transfer to the column, and compress the material into a uniform mass by tamping with a rod. Pipet 5.0 ml. of sample solution containing 1 mg. I/ml. (or its equivalent in degradation products) into a 25-ml. volumetric flask and dilute to the mark with distilled water. Mix 2.0 ml. of the diluted solution with 3.0 g. of diatomaceous earth in a beaker, transfer to the column, and lightly tamp with a rod. Add 1.0 g. of diatomaceous earth and 0.5 ml. water to the beaker, mix, and transfer to the column. Tamp the column lightly with a rod and insert a pledget of glass wool.

Elute the column with 60 ml. of water-saturated ether and collect the eluate in a 100-ml. beaker. Evaporate the ether and take up the residue in 25 ml. of water and 2.0 ml. of 0.1 *N* NaOH. Quantitatively transfer the contents of the beaker to a 100-ml. volumetric flask and dilute to the mark with water. Determine the absorbance at 297 nm. using a blank consisting of 2 ml. of 0.1 *N* NaOH diluted to 100 ml. with water. Use the calculation described under *Direct UV Spectrophotometry*.

**Separation Efficiency of Diatomaceous Earth**—To determine the suitability of diatomaceous earth as a means of separating I and II, duplicate 2.0-ml. aliquots of a solution containing 0.2 mg. II/ml. were treated as follows: one aliquot was subjected to the column chromatography procedure, while the other was subjected to the direct UV method.

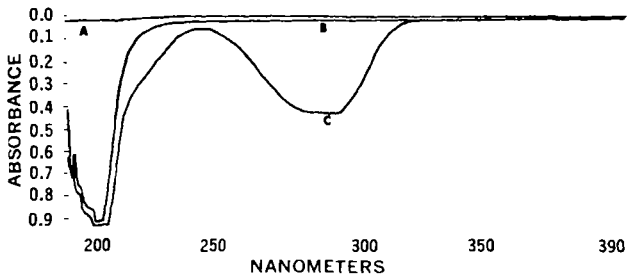


**Figure 2**—*UV spectra. Curve A = water* versus *water. Curve B = 0.2 mg. II/ml. subjected to column chromatograpy. Curve C = 0.2 mg. II/ml. assayed by the direct UV method after dilution to 4.0 mcg./ml.*

[13] The glass chromatography column was 25 × 200 mm. (Scientific Glass Apparatus Co., Inc., catalog No. 576466).

**Stability of I on Diatomaceous Earth**—To ensure that I remained intact throughout the column chromatography procedure, an aqueous solution containing 1 mg. I/ml. was subjected to the column chromatography procedure. The eluate was evaporated and an IR spectrum of the residue was run as a 0.2% KBr pellet. This spectrum was compared to the IR spectrum of an authentic sample of I.

## RESULTS AND DISCUSSION

**Assay Results**—Table I is a summary of the assay results for solutions containing various ratios of I and II. The results clearly indicate that large errors will be made when I is quantitated by direct UV spectroscopy in the presence of II. Although the standard deviation of the GC results was higher than that of the column chromatographic results, a *t*-test based on the standard deviation data in Table I indicated that there was no significant difference between the means at the 5% level.

**Separation Efficiency of Diatomaceous Earth**—The UV curves in Fig. 2 clearly indicate that II is trapped on the column and is not eluted under the conditions used in this study.

**Stability of I on Diatomaceous Earth**—The IR spectrum of the column eluate was identical to an authentic sample of I. This indicated that I remained intact throughout the column chromatographic procedure.

Although the reported investigation was restricted to one acid–ester pair, it seems likely that the column chromatographic procedure could be used for the separation and accurate quantitation of other acid–ester pairs. This method would be suitable for raw material evaluation and for ester metabolism studies after ensuring that no interfering materials were present.

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