minor, application of the present approach could be extended to other protein species.

The method has been used in conjunction with an existing analytical method. Some advantages are that the reagents are stable and that the method requires no special equipment other than the osmometer and it is efficient. Above all, its accuracy is comparable to that achieved by atomic absorption spectrometry.

## REFERENCES


(1) "Rules and Regulations," *Fed. Regist.*, **42**, 27575 (1977).

(2) E. J. Cohn, E. L. Strong, W. L. Hughes, D. J. Mulford, J. N. Ashworth, M. Mein, and H. L. Taylor, *J. Am. Chem. Soc.*, **68**, 459 (1946).

(3) P. K. Ng, J. L. Lundblad, and G. Mitra, *Sep. Sci.*, **11**, 499 (1976).

(4) P. K. Ng, G. Mitra, and J. L. Lundblad, *J. Pharm. Sci.*, **67**, 531 (1978).

(5) G. Scatchard, A. C. Batchelder, and A. J. Brown, *Clin. Invest.*, **23**, 458 (1944).

(6) B. W. Zweifach and M. Intaglietta, *Microvasc. Res.*, **3**, 83 (1971).


# Quantitative Determination of Hexylresorcinol in Commercial Antiseptic Solution by High-Pressure Liquid Chromatography


**JOSEPH D. MUSTO, JAYANT N. SANE, and VICTOR D. WARNER** ˣ



Received July 5, 1977, from the *Department of Medicinal Chemistry and Pharmacology, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA 02115.* Accepted for publication June 21, 1978.



**Abstract** □ High-pressure liquid chromatography was used with a 5-μm silica gel column to quantitate hexylresorcinol in a commercial antiseptic solution following extraction with methylene chloride. This method shows linearity to at least 0.025% hexylresorcinol. A mobile phase consisting of 63% heptane, 34% chloroform, and 5% methanol was used with a UV detector (254 nm) and a flow of 3 ml/min. No interfering substances were observed.



**Keyphrases** □ Hexylresorcinol—high-pressure liquid chromatographic analysis in commercial preparations □ High-pressure liquid chromatography—analysis, hexylresorcinol in commercial preparations


Methods for the quantitative analysis of phenolic derivatives utilize bromination (1, 2), GLC (3, 4), NMR spectroscopy (5), and high-pressure liquid chromatography (HPLC) (6). This report describes a rapid, quantitative HPLC method for the analysis of hexylresorcinol in a commercial antiseptic solution[1].

## EXPERIMENTAL

**Chemicals and Reagents**—All chemicals and reagents were USP, NF, ACS, or chromatographic grade.

**Chromatography**—The high-pressure liquid chromatograph[2] was equipped with a UV detector (254 nm) and a recorder. Septum injection was utilized. The column[3] (15 cm × 6.3 mm o.d.) was packed with 5-μm silica.

The chromatographic solvent consisted of 63% heptane, 34% chloroform, and 3% methanol. The temperature was ambient, and the solvent flow rate was 3.0 ml/min (at an inlet pressure of ~70.3 kg/cm²). The detector was set at a sensitivity of 0.08 aufs, and the chart speed was 5.08 cm/min (30.48 cm/hr). The elution order was phenol (2.7 min) and hexylresorcinol (4.7 min); total analysis time was 6 min.

**Preparation of Solutions**—All solutions of hexylresorcinol and phenol (internal standard) were prepared (weight per volume) in methylene chloride by a simple solution method. An aqueous solution of hexylresorcinol was prepared and used as a primary standard. This aqueous standard was run with each group of samples analyzed. The commercial antiseptic solution was purchased from a local pharmacy.

[1] ST 37 (Calgon).
[2] Waters ALC 202 equipped with a separation injector.
[3] Hi Eff Micropart column (0.5-μm silica), Applied Science Laboratories.

**Preparation of Linearity Curve**—A 1% hexylresorcinol solution was prepared using methylene chloride containing phenol (0.025%) as the internal standard. Aliquots of this solution were diluted with the methylene chloride containing phenol (0.025%) to yield five standard solutions consisting of 0.025, 0.05, 0.07, 0.10, and 0.125% hexylresorcinol with phenol (0.025%) as the internal standard. A volume of 5–10 μl of each standard was injected, and the peak height ratio of hexylresorcinol to phenol was calculated and plotted *versus* the hexylresorcinol concentration.

The plotted data (Table I) indicated that the hexylresorcinol concentration *versus* the peak height ratio ($y$) was linear from 0.025 to 0.125% hexylresorcinol ($x$) ($y = 8.960x + 0.011$; $r = 0.999$), and the straight line intersected zero. Three data points were determined for each concentration. The standard deviations are given in parentheses.

**Assay**—A 5-ml aliquot of the commercial antiseptic solution was extracted by adding 1 ml of 1 *N* HCl, 0.5 ml of internal standard (0.5% phenol in methylene chloride), and 4.5 ml of methylene chloride. The mixture was hand shaken for 1–2 min and centrifuged; the aqueous supernate was discarded by aspiration with a Pasteur pipet. A 10-μl sample of the extract was injected into the chromatograph. The aqueous standard containing 0.05% hexylresorcinol was carried through the same extraction procedure at the same time as the sample. A 0.1% hexylresorcinol solution in methylene chloride containing phenol (0.025%) was regularly injected during analysis to monitor instrument stability.

**Calculation**—The following formula was used for calculating concentrations:

$$\% \text{ sample} = \frac{R_s}{R_{std}} \times \% \text{ std} \qquad \text{(Eq. 1)}$$

where % sample is the percent hexylresorcinol in the sample, % std is the percent hexylresorcinol in the standard, $R_{std}$ is the ratio of the peak heights of hexylresorcinol to phenol in the standard, and $R_s$ is the ratio of the peak heights of hexylresorcinol to phenol in the commercial antiseptic.

**Table I—Data for Linearity Curve**

| Hexylresorcinol Standard, % | Peak Height Ratios |
|---|---|
| 0.025 | 0.232 (0.009) |
| 0.050 | 0.463 (0.015) |
| 0.070 | 0.637 (0.014) |
| 0.100 | 0.905 (0.019) |
| 0.125 | 1.130 (0.010) |




*0022-3549/79/0200-0240$01.00/0*

**Table II—Assay Results on Commercial Antiseptic (Percent Concentration of Hexylresorcinol)** [a]

| Assay[b] | Percent Found | Percent Reported[c] |
|---|---|---|
| 1 | 0.097 | 0.1 |
| 2 | 0.107 | 0.1 |
| 3 | 0.106 | 0.1 |
| 4 | 0.107 | 0.1 |

[a] Average deviation = 0.004, *SD* = 0.005, and coefficient of variation = 4.8%. [b] Replicate assays of same sample. [c] Data from "Handbook of Nonprescription Drugs," 5th ed., American Pharmaceutical Association, Washington, D.C., 1977, p. 263.

## DISCUSSION

The results (Table II) show that a commercial antiseptic solution can be assayed for hexylresorcinol by using a simple HPLC method. No interfering peaks were observed from the other listed component (glycerin) in the commercial product analyzed. Recovery studies were performed on the aqueous hexylresorcinol solution (0.05%), and the extraction efficiency was 74.9%. The extraction and HPLC analyses of aqueous standard solutions gave a linear response with hexylresorcinol concentrations between 0.025 and 0.125%.

This analysis utilized an aqueous standard carried through the exact procedure as the samples. Since the aqueous standard closely simulated the samples, the concentrations were calculated by comparing peak heights of the aqueous standard and the unknown sample. Extraction efficiency affects both samples and standards equally and, therefore, need not be considered in the calculation of concentrations.

A control also was run with each batch to monitor instrument conditions. This procedure minimizes assay errors due to technique. The total analysis time was less than 10 min/sample.

## REFERENCES


(1) "The National Formulary," 14th ed., Mack Publishing Co., Easton, Pa., 1975, p. 317.

(2) D. G. Garrett, "The Quantitative Analysis of Drugs," 3rd ed., Chapman and Hall Ltd., London, England, 1964, pp. 513–518, 550.

(3) P. A. Hedin, J. P. Minyard, Jr., and A. C. Thompson, *J. Chromatogr.*, **30**, 43 (1967).

(4) V. Kusy, *ibid.*, **57**, 132 (1971).

(5) V. D. Gupta and L. A. Cates, *J. Pharm. Sci.*, **63**, 93 (1974).

(6) V. D. Gupta, *ibid.*, **65**, 1706 (1976).


## ACKNOWLEDGMENTS


Supported in part by National Institutes of Health Grant RR07143.


# High-Pressure Liquid Chromatographic Assay of Folic Acid: A Collaborative Study


**WILLIAM O. McSHARRY** ˣ **and FRANK P. MAHN**



Received April 17, 1978, from the *Quality Control Department, Hoffmann-La Roche, Inc., Nutley, NJ 07110.* Accepted for publication June 28, 1978.



**Abstract** □ A collaborative study of the USP high-pressure liquid chromatographic assay for folic acid was performed. Two samples were analyzed in duplicate by 14 participating laboratories. Relative standard deviations for a single determination (*RSDS*) ranged from ±0.40 to ±2.39%. Based on an analysis of variance, it was concluded that the method of peak measurement was a major determinant of reproducibility and that graphical measurement was associated with a high standard deviation. Adequate resolution was obtained using a variety of columns and operating conditions. The interlaboratory *RSDS* was ±1.8%.



**Keyphrases** □ Folic acid—high-pressure liquid chromatographic analysis in prepared samples, collaborative study □ High-pressure liquid chromatography—analysis, folic acid in prepared samples, collaborative study □ Vitamins—folic acid, high-pressure liquid chromatographic analysis in prepared samples, collaborative study


A recently developed high-pressure liquid chromatographic (HPLC) method (1) for the assay of folic acid bulk substance was introduced into the USP (2) to replace the spectrophotometric method (3). In a subsequent supplement (4), the new method was revised slightly.

The HPLC method is more specific and accurate than the previous one. A collaborative study[1] was undertaken to validate the precision and practical applicability of this HPLC method on a wide scale.

## EXPERIMENTAL

Four vials containing 100 mg each of two folic acid test samples (A and B), 75 mg of folic acid USP reference standard, and 20 mg of leucovorin calcium[2] (calcium formyltetrahydrofolate) were sent to 19 collaborators, along with a copy of the method (5) and auxiliary instructions for performing the determination and reporting results. Collaborators were requested to make duplicate injections of each of two weighings of Samples A and B and the folic acid reference standard and to report raw data and results, calculated on the dry basis, using the average response from the duplicate injections in the calculation.

The method as written allowed the individual collaborator considerable latitude in choice of column and operating conditions. However, a system suitability test required that the operator obtain a resolution factor of at least 3.6 between folic acid and leucovorin and a relative standard deviation no greater than ±2% in the responses from six to 10 replicate injections of the standard preparation.

The amounts of folic acid provided were sufficient for two 30-mg weighings by each collaborator plus equipment optimization and checkout. The water content of the folic acid was determined (duplicate 50-mg samples in methanol) before subdivision by the titrimetric method and reported to the collaborators (reference standard, 7.7%; Sample A, 7.6%; and Sample B, 7.8%). Collaborators were requested to report, in addition to raw data and assay results, results of the system suitability test and certain information about instrument and operating conditions.

Raw data from all collaborators were entered into a time-shared computer, and results were calculated by a user-written program.

[1] A subcommittee of the PMA-QC Section, appointed by Dr. H. Hammer and chaired by Dr. J. Sheridan, was responsible for carrying out this study.

[2] Provided by Lederle Laboratories.


*0022-3549/79/0200-0241$01.00/0*