Table I—Effect of Glycocholic Acid, pH, and Ionic Strength on the Intrinsic Factor-Mediated Binding of I to Cholestyramine [a]

	Cyanocobalamin Bound, pg			
	Incubated in Buffer A		Incubated in Buffer B	
Glycocholic Acid Added, μmoles	Washed in Buffer A	Incubated and Washed in Buffer C	Washed in Buffer B	Incubated and Washed in Buffer C
0	2560	1370	1230	280
1.25	2589	1386	1247	262
2.5	3393	1696	1519	487
3.75	3465	2113	1480	674
5.0	3364	2444	605	257
7.5	3210	1806	76	60
10.0	1638	686	54	46

[a] The values given are the arithmetic means of two determinations, each run in triplicate. Incubations were performed as described in the text. The following buffers were used: 0.05 M NaCl, pH 3.0 (Buffer A); 0.1 M NaCl, pH 3.0 (Buffer B); and 0.05 M NaCl, pH 6.8 (Buffer C).

of I to cholestyramine at two different ionic strengths is shown in Table I. At very low concentrations of glycocholic acid, binding of the I–intrinsic factor complex to the resin increased, but when the glycocholic acid concentration was increased, complex binding gradually decreased, which suggests that glycocholic acid blocked the possible binding sites of the I–intrinsic factor complex. A further decrease in the amount of the I–intrinsic factor complex bound was observed when the ionic strength was increased by increasing the sodium chloride concentration from 0.05 to 0.1 M (Table I), which is compatible with the hypothesis that binding at this pH must be due to hydrophobic interactions. However, significant amounts of the complex remained bound to the resin after incubation at pH 6.8 (Table I), which suggests that the forces taking part in the binding also are coulombic.

The results show that binding of the I–intrinsic factor complex was partly reversible at neutral pH as well as at a glycocholic acid concentration similar to that found in the lumen of the small intestine. Hence, it is uncertain whether cholestyramine binds sufficient quantities of the complex in the lumen of the small intestine to impair intestinal vitamin absorption. Furthermore, results of absorption tests are conflicting; *i.e.*, cholestyramine increased as well as decreased intestinal vitamin absorption (11).

REFERENCES

(1) T. B. Van Itallie, S. A. Hashim, R. S. Crampton, and D. M. Tennent, *N. Engl. J. Med.*, **265**, 469 (1961).

(2) S. A. Hashim and T. B. Van Itallie, *J. Am. Med. Assoc.*, **192**, 289 (1965).

(3) R. S. Fisher and S. Cohen, *N. Engl. J. Med.*, **288**, 273 (1973).

(4) H. H. Scudamore, E. E. Eckstam, W. J. Fencil, and C. A. Jaramillo, *Am. J. Gastroenterol.*, **60**, 9 (1973).

(5) R. B. Black, J. Rhodes, G. T. Davies, H. Gravelle, and P. Sweetnam, *Gastroenterology*, **61**, 821 (1971).

(6) "The Pharmacological Basis of Therapeutics," 5th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1975, p. 749.

(7) A. Coronato and G. B. J. Glass, *Proc. Soc. Exp. Biol. Med.*, **142**, 1341 (1973).

(8) R. Gräsbeck, *Acta Med. Scand., Suppl. 314*, **154**, 1 (1956).

(9) S. Lindenbaum and T. Higuchi, *J. Pharm. Sci.*, **64**, 1887 (1975).

(10) R. Gräsbeck, *Acta Chem. Scand.*, **22**, 1041 (1968).

(11) J. G. Spenney and B. J. Hirschowitz, *Gastroenterology*, **58**, 996 (1970).

Simultaneous Programmed Temperature GLC Assay of Phenol, Chloroxylenol, and Lidocaine Hydrochloride in Topical Antiseptic Cream

PHILIP J. PALERMO ˣ and J. BRIAN LUNDBERG

Received October 25, 1977, from *Norwich-Eaton Pharmaceuticals, Norwich, NY 13815.* Accepted for publication February 28, 1978.

Abstract □ A simultaneous programmed temperature GLC assay for the active ingredients in a topical antiseptic cream is described. The sample is extracted directly using a dimethyl sulfoxide solution of *p*-cresol, 4-chlorophenol, and 2-amino-4-phenylthiazole as internal standards for phenol, chloroxylenol, and lidocaine hydrochloride, respectively. The resulting solution is chromatographed by temperature programming on an OV-225 column from 90 to 225°. The internal standard calculation is accomplished using peak heights or peak areas. The relative standard deviation of all assays is less than 2%.

Keyphrases □ Phenol—GLC analysis simultaneously with other active ingredients in commercial preparations □ Chloroxylenol—GLC analysis simultaneously with other active ingredients in commercial preparations □ Lidocaine hydrochloride—GLC analysis simultaneously with other active ingredients in commercial preparations □ GLC—simultaneous analyses, phenol, chloroxylenol, and lidocaine hydrochloride in commercial preparations

Existing assay methods for products with multiple active ingredients can often be tedious and time consuming. Current procedures for phenol, chloroxylenol (*p*-chloro-*m*-xylenol), and lidocaine hydrochloride in a topical cream formulation required 2 days of laboratory time and three separate assays for the active ingredients. A simultaneous procedure for the three active ingredients was desirable. In addition, a specific method was necessary for stability-indicating purposes.

GLC has been used successfully to determine lidocaine (1–3), phenol (4–6), and chloroxylenol (7–9). These techniques have inherent specificity qualities. The range of boiling points and polarities of these three components required a programmed temperature method.

EXPERIMENTAL

Materials and Reagents—Phenol and lidocaine hydrochloride meeting USP specifications were used as standards. Chloroxylenol standard material was assayed by a GLC procedure. *p*-Cresol, 4-chlorophenol, and 2-amino-4-phenylthiazole at 99+ % purity were used as received[1]. All other chemicals were ACS reagent grade or equivalent.

The column was 1.8-m × 3-mm silanized stainless steel filled with 3% OV-225 on 80–100-mesh Supelcoport adapted for on-column injection.

[1] Aldrich Chemical Co. and Fairfield Chemical Co.

0022-3549/ 78/ 1100-1627$01.00/ 0

© 1978, American Pharmaceutical Association

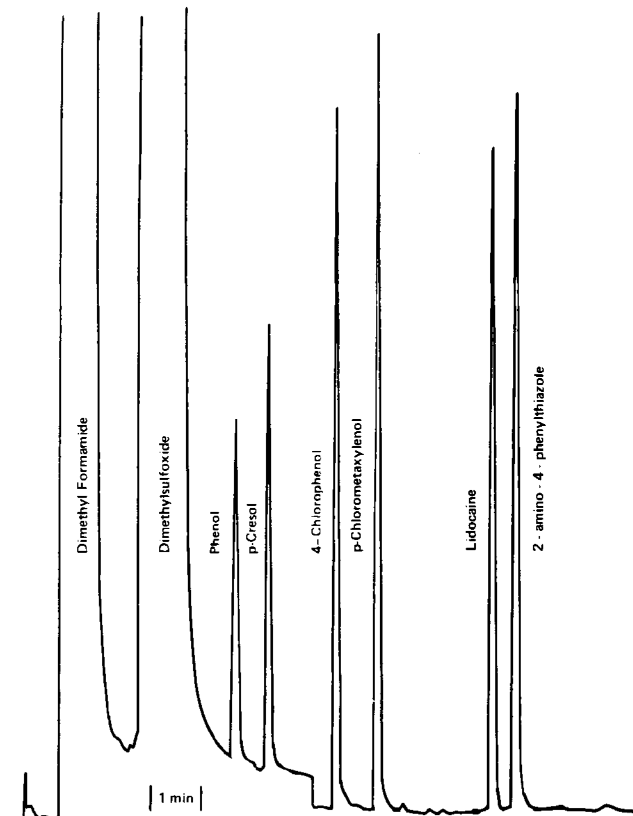

Figure 1—*Chromatogram of standard solution.*

The liquid phase was dissolved in chloroform and coated on the stationary phase by evaporation of the solvent. The column required overnight conditioning at 250° with helium flow.

Apparatus—The method was validated on two commercial gas chromatographs[2] using a flame-ionization detector. The instrument parameters were: column temperature, 90–225°; program rate, 20°/min; postinjection time, 2 min; upper limit time, 4 min; injector temperature, 250°; detector temperature, 300°; carrier gas flow rate (helium), 30 ml/min; hydrogen gas flow rate, 25 ml/min; air flow rate, 500 ml/min; attenuation, 4 for phenol and *p*-cresol and 8 for subsequent peaks; range, 1000; and recorder[3] chart speed, 1.25 cm/min.

Standard Solutions—The internal standard was prepared to contain about 0.35 g of *p*-cresol, 1.5 g of 4-chlorophenol, and 1.8 g of 2-amino-4-phenylthiazole/100 ml of dimethyl sulfoxide. (Exact weights are not necessary but should be within 20% of the recommended amounts.) A standard solution of the analytes was prepared by accurately weighing (±0.1 mg) and diluting 0.12 g of phenol, 0.48 g of chloroxylenol, and 0.43 g of lidocaine to 100 ml with dimethylformamide. The standard for injection was made by pipetting exactly 10.00 ml of internal standard and 25.00 ml of the standard analyte solution and diluting to 50 ml with dimethylformamide.

Sample Solution—Six grams of the sample was introduced into a 40-ml glass-stoppered centrifuge tube, and 10.0 ml of internal standard solution was added. This tube was then swirled in a vortex-type mixer and shaken vigorously until the sample was completely homogenized. Dimethylformamide, 10 ml, was added, and the sample was shaken briefly again. The resulting suspension was centrifuged, and the solution was decanted and diluted to 50 ml with dimethylformamide. A portion of this mixture was centrifuged.

The sample and standard solutions were injected into the gas chromatograph (Figs. 1 and 2). The injection volume was about 1 μl. This volume corresponds to about 0.7 μg of phenol, 0.8 μg of *p*-cresol, 2.7 μg of chloroxylenol, 3.0 μg of 4-chlorophenol, 2.3 μg of lidocaine, and 4.0 μg of 2-amino-4-phenylthiazole injected.

The response curve characteristics are shown in Table I for peak heights.

As an additional test of the method's viability, a solution containing

[2] Hewlett-Packard model 5750B and Perkin-Elmer model 3920.
[3] Hewlett-Packard model 7128 A.

Table I—Linear Regression Analysis

	Phenol	Chloro-xylenol	Lidocaine
Amount injected, μg	0–1	0–4	0–4
Slope, cm/μg	14.8	8.4	7.4
Correlation coefficient	0.9992	0.9996	0.9999
y-Intercept, cm	−0.92	−0.90	0.05
Standard error of estimate (Sy/x)	0.11	0.17	0.05
Percent variation $[(Sy/x)/\bar{y}] \times 100$	1.24	0.79	0.32

all internal standards and active drugs was prepared. This solution was used for adding known amounts (spiking) of analytes and internal standards to an appropriate amount of placebo material. The resulting chromatogram showed no significant difference in the peak height ratios from a portion of the solution not treated with placebo within the precision of the method.

RESULTS AND DISCUSSION

Because the phenolic compounds are acids and lidocaine is a base, quantitative partition of all compounds from a diphasic system was not investigated. Rather, a leaching procedure that leaves the excipient residue behind was employed. The excipient base consisted mainly of a saturated polyethylene glycol ether and white petrolatum. Dimethylformamide and dimethyl sulfoxide were the best solvents for breaking up the cream and getting the analytes into solution. Dimethyl sulfoxide extracted fewer excipients but eluted closer to phenol than did dimethylformamide. To keep the dimethyl sulfoxide to a minimum, a compromise procedure was to extract the sample with dimethyl sulfoxide and to dilute it to a workable volume with dimethylformamide. Quantitative recovery of the leach liquor was not necessary since the internal standards were incorporated into the dimethyl sulfoxide solution so that the ratios of each analyte to its internal standard were fixed at this point.

The sample dosage levels were: phenol, 0.5%; chloroxylenol, 2.0%; and lidocaine hydrochloride, 2.0%. A 6-g sample extracted and diluted to 50 ml gave a good response. The sample was extracted with 10 ml of dimethyl

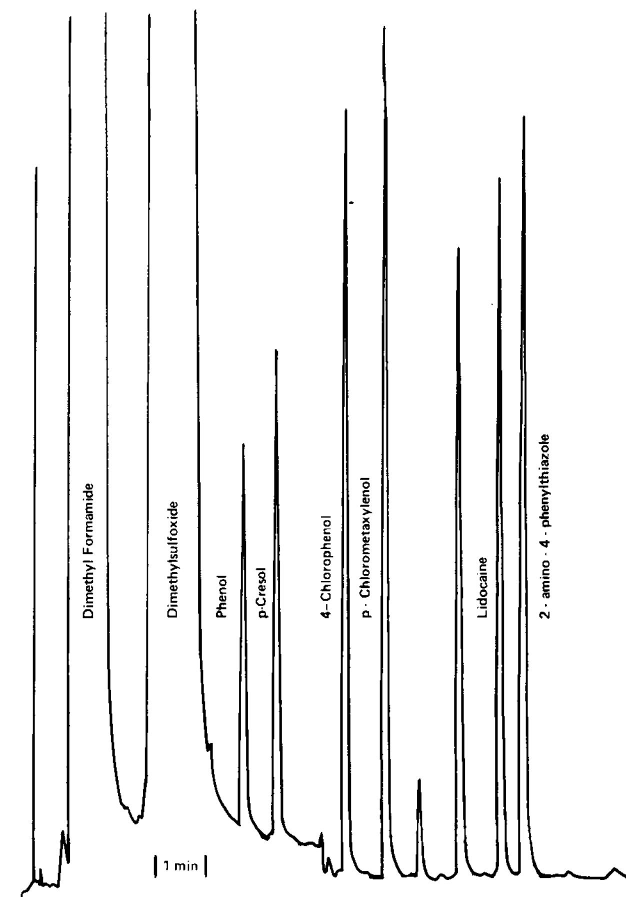

Figure 2—*Chromatogram of sample solution.*

Table II—Interlaboratory Collaborative Evaluation

Run	First Laboratory	Second Laboratory
Phenol, %	0.499	0.509
	0.490	0.519
	0.505	0.503
$\overline{X}$	0.498	0.510
RSD, %	1.5	1.6
Chloroxylenol, %	1.95	1.98
	2.01	1.97
	2.02	2.01
$\overline{X}$	2.00	1.99
RSD, %	1.9	0.9
Lidocaine hydrochloride, %	2.04	2.02
	2.04	2.03
	2.06	2.01
$\overline{X}$	2.04	2.02
RSD, %	0.33	0.68

sulfoxide internal standard solution and then diluted with dimethylformamide.

An internal standard for each analyte was preferable because of the temperature programming. Comparisons of all three drugs to any one of the internal standards did not yield acceptable precision for all of them. Phenol and *p*-cresol eluted during the initial constant-temperature period, chloroxylenol and 4-chlorophenol eluted at about 180° during the program, and lidocaine and 2-amino-4-phenylthiazole eluted during the final upper level temperature (225°). The attenuation was lowered for the first part of the chromatogram because of the lower dosage of phenol.

Two synthetic samples were prepared at the label values and assayed 10 times by two analysts on 4 different days. The sample size was varied from 80 to 120% of the recommended amount. Since the response curves are linear over the range of interest and have acceptably low *y*-intercepts, the calculations may be performed using a single-point ratio of peak response. There was no statistical difference in results between calculations using peak heights or areas. The ratios (*R*) are calculated as the peak response (heights or areas) of the analyte divided by the peak response of its internal standard.

The final calculation for each component is:

$$\text{percent drug} = \frac{R_{sample}}{R_{std}} \times \frac{g_{std}}{g_{sample}} \times K \quad \text{(Eq. 1)}$$

where *K* is a constant incorporating dilution factors and, for lidocaine, the molecular weight ratio of lidocaine hydrochloride to lidocaine.

The average percent recovery and relative standard deviations (%) were: phenol, 100.6 ± 1.4; chloroxylenol, 100.3 ± 1.1; and lidocaine hydrochloride, 99.1 ± 1.4.

Eighteen analyses were performed on a 2-year-old actual sample by one analyst on 4 different days. The relative standard deviations of the assays were: phenol, ±0.9%; chloroxylenol, ±0.8%; and lidocaine hydrochloride, ±1.2%.

This method was further evaluated by simultaneous interlaboratory collaborative analyses on identical samples (Table II).

This simultaneous three-assay procedure represents a significant saving in time. Approximately eight samples (24 assays) can be performed per worker day.

REFERENCES

(1) A. Cometti, G. Bagnasco, and N. Maggi, *J. Pharm. Sci.*, **60,** 1074 (1971).

(2) N. D. Greenwood and I. W. Guppy, *Analyst*, **99,** 313 (1974).

(3) N. Benowitz and M. Rowland, *Anesthesiology*, **39,** 639 (1973).

(4) C. C. Douglas, *J. Assoc. Offic. Anal. Chem.*, **55,** 610 (1972).

(5) J. Hrivnak and J. Macak, *Anal. Chem.*, **43,** 1039 (1971).

(6) J. B. Johnson and V. S. Venturello, *Bull. Parenter. Drug Assoc.*, **25,** 239 (1971).

(7) S. Husain, *Indian J. Technol.*, **6,** 94 (1968).

(8) H. Koenig, *Fresenius Z. Anal. Chem.*, **266,** 119 (1973).

(9) I. Takemura, *Bunseki Kagaka*, **20,** 61 (1971).

Drug Resistance Studies with Topical Antiseptics

HERBERT N. PRINCE ˣ, WALTER S. NONEMAKER, RICHARD C. NORGARD, and DANIEL L. PRINCE

Received January 23, 1978, from the *Research Department, Gibraltar Biological Laboratories, Inc., Fairfield, NJ 07006.* Accepted for publication March 6, 1978.

Abstract ☐ Species of *Proteus*, *Serratia*, and *Pseudomonas* became resistant to chlorhexidine after five to eight transfers *in vitro*. Cross-resistance to benzalkonium chloride also was detected. Resistance to povidone-iodine was not encountered. Chlorhexidine resistance was stable after drug-free transfers of *Serratia* and *Pseudomonas* but was transitory for *Proteus*.

Keyphrases ☐ Chlorhexidine gluconate—resistance by various microorganisms *in vitro* ☐ Povidone-iodine—resistance by various microorganisms *in vitro* ☐ Resistance—various microorganisms to chlorhexidine gluconate and povidone-iodine *in vitro* ☐ Antiseptics, topical—chlorhexidine gluconate and povidone-iodine, resistance by various microorganisms *in vitro*

Chlorhexidine, *N,N''*-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide, first described in 1954 by Davies *et al.* (1), has been used extensively in England and Europe as a preservative, disinfectant, and topical antiseptic. It recently was introduced in the United States for use in hospitals as a topical antimicrobial cleanser.

Resistant strains of *Proteus mirabilis* were isolated from postoperative urinary infections and in paraplegics undergoing catheterization of the bladder following repeated use of chlorhexidine for cleansing the external genitalia (2–4). More recently, Stickler (5) examined 104 clinical isolates of *P. mirabilis* for sensitivity to chlorhexidine and found minimum inhibitory concentrations

Table I—Baseline *In Vitro* Activity of Three Topical Antiseptics against Parent Gram-Negative Rods

	MIC, μg/ml (in Dubos Broth, 48 hr at 35°)		
Organism	Chlorhexidine Gluconate	Benzalkonium Chloride	Available Iodine from Povidone-Iodine
P. mirabilis	8	16	8
Ps. aeruginosa	8	128	8
Ps. cepacia	1	1000	16
Ser. marcescens	8	16	8
Ser. rubidae	32	512	32
Sal. enteritidis	8	32	16

0022-3549/ 78/ 1100-1629$01.00/ 0

© 1978, American Pharmaceutical Association