vertical spacing is governed by the solubilities of the various esters.

At high flow rates, the permeation rates leveled off and became independent of flow in accordance with Eq. 4, wherein the permeation rate becomes only a function of the transport across the membrane. Thus, K and C determined the relative positions of the plateaus in Fig. 3.

In the permeation simulations calculated with Eq. 3, values of D_m and D_w reported previously (2, 4) were assumed to be the same ($D_m = 2.72 \times 10^{-6}$ cm^2/sec and $D_w = 6 \times 10^{-6}$ cm^2/sec) for all members of the series because the change in molecular weight would change the diffusivity insignificantly (6) compared with the order-of-magnitude changes in the other parameters, *e.g.*, solubility and flow rate. Although the diffusivity was not evaluated as an independent parameter in the present work, it has been observed experimentally (7) that the resistance in the aqueous boundary layer film adjacent to a membrane is proportional to the diffusivity raised to the power −0.6. This observation is in reasonably good agreement with Eq. 2.

Equation 3 arises from an assumption that the resistances in the membrane and in the adjacent liquid are additive. This combination permits calculation of the permeation rate under conditions of membrane control or aqueous convective diffusion control or at intermediate points. It is apparent from the permeation data that the predictions were reasonably good. However, in the intermediate region, Eq. 3 should be considered as an interpolation formula rather than as an expression arising directly from basic theory because the instantaneous transport rate decreases somewhat with increasing distance along the membrane in the direction of flow. This decrease is a characteristic of convective diffusion theory. With intermediate cases, there would be a concentration gradient within the membrane in the direction of flow. This gradient is not accounted for in Eq. 3. However, such a gradient would be much smaller than the gradient normal to the membrane because of the relative distances involved and, therefore, may be ignored.

If the data in Fig. 3 are replotted as log permeation rate *versus* log solubility, the curves obtained at different flow rates appear as in Fig. 4. The maximum permeation rate is shifted to the left, *i.e.*, to permeants having a lower solubility, with an increase in flow rate. This is similar to the concepts of Flynn and Yalkowsky (2), who evaluated the effect of fluid flow agitation in terms of a change in the thickness of the stagnant or unstirred layer. The thickness of the unstirred layer is a model-dependent parameter; it cannot be measured independently of permeation experiments. In the present work, the effect of fluid flow is incorporated as the rate of shear adjacent to the membrane. Because hydrodynamic theory permits one to determine the shear rate from the fluid flow rate and the geometry of the cell, the effect of fluid flow on permeation and the position of maximum permeation can be calculated readily *a priori* for a given membrane thickness using Eq. 3.

REFERENCES

(1) K. G. Nelson and A. C. Shah, *J. Pharm. Sci.*, **66**, 137 (1977).

(2) G. L. Flynn and S. H. Yalkowsky, *ibid.*, **61**, 838 (1972).

(3) V. G. Levich, "Physicochemical Hydrodynamics," Prentice-Hall, Englewood Cliffs, N.J., 1962, pp. 43–45.

(4) A. C. Shah and K. G. Nelson, *J. Pharm. Sci.*, **64**, 1518 (1975).

(5) J. Crank, "The Mathematics of Diffusion," Clarendon, Oxford, England, 1956, p. 44.

(6) S. H. Yalkowsky and G. L. Flynn, *J. Pharm. Sci.*, **62**, 210 (1973).

(7) R. E. Beck and J. S. Schultz, *Biochim. Biophys. Acta*, **255**, 273 (1972).

Simultaneous High-Pressure Liquid Chromatographic Determination of Acetaminophen, Guaifenesin, and Dextromethorphan Hydrobromide in Cough Syrup

W. O. McSHARRY *x and I. V. E. SAVAGE ‡

Received April 12, 1979, from the **Quality Control Department, Hoffmann-La Roche Inc., Nutley, NJ 07110,* and ‡*Roche Products Pty. Ltd., Dee Why 2099, Australia.* Accepted for publication August 23, 1979.

Abstract □ Acetaminophen (I), guaifenesin (II), and dextromethorphan hydrobromide (III) were separated and quantitated simultaneously in cough syrup by high-pressure liquid chromatography. A chemically bonded octadecylsilane stationary phase was used with a mobile phase of 45% (v/v) aqueous methanol. The mobile phase pH was stabilized to 4.2 by adding formic acid–ammonium formate buffer (~0.4%). The internal standard was *o*-dinitrobenzene. Retention volumes were 4 ml for I, 6 ml for II, 11 ml for the internal standard, and 20 ml for III. Inactive syrup components did not interfere, permitting direct diluted sample injection. Results on active ingredients were essentially 100% of the claim, with standard deviations of ±1.5, 1.2, and 2.1% for I, II, and III, respectively.

Keyphrases □ High-pressure liquid chromatography—simultaneous determination of acetaminophen, guaifenesin, and dextromethorphan hydrobromide, analysis of cough syrup active ingredients □ Pharmaceutical preparations—simultaneous determination of cough syrup active ingredients by high-pressure liquid chromatography □ Acetaminophen—simultaneous determination with guaifenesin and dextromethorphan hydrobromide, high-pressure liquid chromatography □ Guaifenesin—simultaneous determination with acetaminophen and dextromethorphan hydrobromide, high-pressure liquid chromatography □ Dextromethorphan hydrobromide—simultaneous determination with acetaminophen and guaifenesin, high-pressure liquid chromatography

Characterization of numerous drugs (1–4) and analyses of specific dosage forms have been conducted through the use of high-pressure liquid chromatography (HPLC). Recent investigations of pharmaceutical preparations included acetaminophen (paracetamol)–chlorpheniramine maleate–dextromethorphan hydrobromide (5) and codeine phosphate–guaifenesin (glyceryl guaiacolate)–pheniramine maleate–phenylpropanolamine hydrochloride–pyrilamine maleate (6). A liquid chromatographic analysis of 29 drug compounds in acidic, neutral, and alkaline media included syrups containing phenylephrine hydrochloride, acetaminophen, guaifenesin, and dextromethorphan hydrobromide (4). However, it was not clear if the last three compounds could be analyzed using a single mobile phase.

Simultaneous determination of acetaminophan (I), guaifenesin (II), and dextromethorphan hydrobromide (III) in a liquid preparation has not been reported. Routine analysis of a cough syrup was required, and HPLC was the method of choice. A chemically bonded nonpolar-sta-

0022-3549/80/0200-0212$01.00/0

Table I—Typical Results and Precision

Sample	Acetaminophen (I)	Guaifenesin (II)	Dextromethorphan Hydrobromide (III)
1[a]	100.1	99.8	98.3
2[a]	102.1	101.2	101.3
3[a]	100.4	99.7	100.0
Response ratio only, %[b]	±1.3	±1.3	±1.7
Overall procedure, %[b]	±1.5	±1.2	±2.1

[a] Expressed as percent of claim. [b] Standard deviation of a single determination calculated from six replicates.

tionary phase was chosen because it is not adversely affected by the polar components of the syrup, making prior separation unnecessary. The aim was to develop a procedure in which all three components could be determined simultaneously in a single injection.

EXPERIMENTAL

Reagents—Acetaminophen (paracetamol) BP[1] (I), guaifenesin (glyceryl guaiacolate) BPC[1] (II), dextromethorphan hydrobromide[2] (III), and *o*-dinitrobenzene[3] (the internal standard) were used as supplied.

Mobile Phase Preparation—Thirty-four milliliters of 28–30% ammonia[4] was diluted with 30 ml of water. Then 30 ml of formic acid[4] (minimum 98%) was added slowly with stirring. When cool, the solution (pH ~ 3.9) was diluted to 100 ml with water (ammonium formate buffer).

Ammonium formate buffer (10 ml) was added to 450 ml of methanol[4] (ACS grade) and diluted with water to make 1 liter (pH ~ 4.2) at room temperature. The mobile phase was filtered with vacuum.

Standard Solutions—*Internal Standard Solution Preparation*—*o*-Dinitrobenzene, 50 mg, was dissolved in 125 ml of methanol and diluted to 250 ml.

Standard Solution Preparation—Separate standard stock solutions were prepared in 50% (v/v) methanol in water containing ~9.0 mg of I/ml, 3.7 mg of II/ml, or 0.4 mg of III/ml. A standard solution of all three components was prepared by pipetting 10.0 ml of I solution, 10.0 ml of II solution, 15.0 ml of III solution, and 5.0 ml of internal standard solution into a 50-ml volumetric flask and diluting to volume with 50% (v/v) methanol in water.

Sample Preparation—A 3-ml sample was transferred to a 50-ml volumetric flask and weighed. Then 5 ml of internal standard solution was added, and the mixture was diluted to volume with 50% (v/v) methanol in water.

Instrumentation—The liquid chromatograph consisted of a constant-flow pump[5], a low pressure injector[6], a 30-cm × 4-mm i.d. octadecylsilane column[7], and an absorbance detector[8] at 280 nm. The flow rate was 2.0 ml/min, and the injection volume was 15 μl. Peak heights were monitored with a strip-chart recorder[9] adjusted for maximum on-scale deflection for the internal standard and III. An electronic integrator[10] was used to monitor peak areas. Settings on the integrator were chosen to minimize noise.

Calculations—Results for I and II were calculated from the integrated area relative to the internal standard area. Results for III were calculated from the peak height relative to the height of the internal standard peak.

RESULTS AND DISCUSSION

The simultaneous analysis of all three components in this cough syrup was difficult for two reasons. First, the wide response range between I and III limited the amount of sample that could be injected without either overloading the detector or losing precision in the smaller component measurement. Second, the wide difference in polarity between I and III made it difficult to separate I and II from interfering impurities while eluting III in a reasonable time.

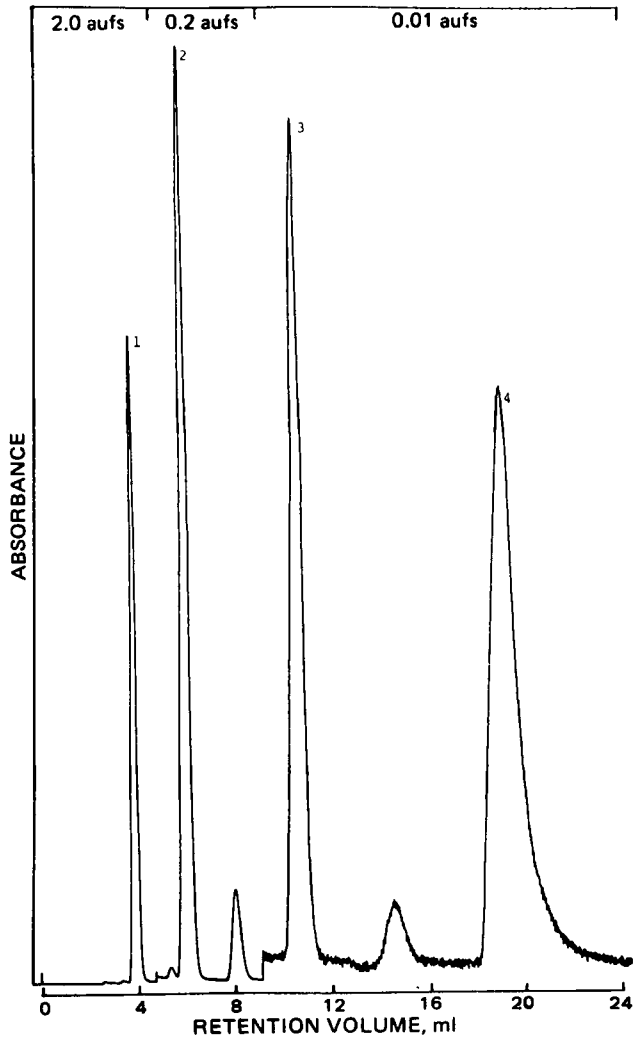

Figure 1—*High-pressure liquid chromatogram of a cough syrup preparation. Peaks 1, 2, and 4 are acetaminophen, guaifenesin, and dextromethorphan hydrobromide, respectively. Peak 3 is the internal standard (o-dinitrobenzene). Other peaks are unidentified components of the syrup. Absorbance units at full scale (aufs) are shown at the top.*

Heptanesulfonic acid and pentanesulfonic acid were tried as ion-pairing agents but retained III at methanol concentrations sufficiently low to separate I and II. Ammonium acetate buffer significantly reduced III retention without affecting I and II. Ammonium formate finally was chosen because it further reduced III retention, again without affecting I and II.

In all mobile phases tried, III tailed badly. Due to the tailing and low concentration of III, electronic integration was not reproducible (coefficient of variation ±5%). Therefore, peak height measurement was used. The possibility of interfering peaks was checked by injecting: a blank syrup preparation containing all ingredients except I, II, and III; a syrup preparation with no internal standard added; and the internal standard only, at a concentration 10 times that used in the analysis.

None of the inactive syrup components coeluted with the active ingredients or internal standard, nor did the internal standard contain any impurities that coeluted with the active ingredients (Fig. 1). Separation of I from its potential hydrolysis product, *p*-aminophenol, was confirmed by injecting a freshly prepared solution of *p*-aminophenol, which eluted separately from I. Detector response was linear within ±2% for all components between 80 and 110% of the claim.

Altogether nine samples were analyzed (Table I). Method precision was evaluated in two ways. The precision of the response ratio mea-

[1] Rhone-Poulenc Industries, Paris, France.
[2] F. Hoffmann-La Roche, Basel, Switzerland.
[3] British Drug Houses, Poole, England.
[4] Ajax Chemicals, Sydney, Australia.
[5] Model 6000 A, Waters Associates, Milford, Mass.
[6] Model U6K, Waters Associates, Milford, Mass.
[7] μBondapak C_{18}, Waters Associates, Milford, Mass.
[8] Model 440, Waters Associates, Milford, Mass.
[9] Series 5000, Fisher.
[10] Model CRS-204, Infotronics.

surement was determined by making six replicate injections of a single standard preparation. The overall precision was evaluated by making one injection for each of six separate sample preparations and calculating the result by comparison with the six replicate single injections. Most of the error resulted from the response ratio measurement (Table I), which can be reduced by replicate injections.

Instrument setup and preparation of the standard required ~2 hr. Individual analyses, including calculations, can be carried out at a rate of more than one sample per hour.

REFERENCES

(1) P. J. Twitchett and A. C. Moffat, *J. Chromatogr.*, **111**, 149 (1975).

(2) R. G. Achari and E. E. Theimer, *J. Chromatogr. Sci.*, **15**, 320 (1977).

(3) I. L., Honigberg, J. T. Stewart, and A. P. Smith, *J. Pharm. Sci.*, **63**, 766 (1974).

(4) H. Y. Mohammed and F. F. Cantwell, *Anal. Chem.*, **50**, 491 (1978).

(5) A. Menyharth, F. P. Mahn, and J. E. Heveran, *J. Pharm. Sci.*, **63**, 431 (1974).

(6) V. D. Gupta and A. G. Ghanekar, *ibid.*, **66**, 895 (1977).

ACKNOWLEDGMENTS

The authors thank Michael Meade of Roche Products Pty. Ltd. for the blank syrup preparation.

Work was performed at Roche Products Pty. Ltd., Dee Why, N.S.W. Australia.

Nonvariance of LD_{50} Values of Drugs in Gravid and Nongravid Mice

JAMES A. RUSSELL, MICHAEL P. MAHALIK, JOSEPH M. McDEVITT, and RONALD F. GAUTIERI ˣ

Received April 5, 1979, from the *Department of Pharmacology, School of Pharmacy, Temple University, Philadelphia, PA 19140.* Accepted for publication August 22, 1979.

Abstract □ This study showed that the LD_{50} values for morphine sulfate, cobalt chloride, and phenytoin sodium did not vary significantly on Day 9 of gestation in CF-1 mice when compared to values of nongravid animals.

Keyphrases □ LD_{50} testing—gravid and nongravid mice, morphine sulfate, cobalt chloride, phenytoin sodium, teratological study □ Teratological study—gravid and nongravid mice, morphine sulfate, cobalt chloride, phenytoin sodium, LD_{50} testing □ Morphine sulfate—LD_{50} testing, gravid and nongravid mice □ Phenytoin sodium—LD_{50} testing, gravid and nongravid mice □ Cobalt chloride—LD_{50} testing, gravid and nongravid mice

The LD_{50} value is the dose of a drug that proves lethal to 50% of a population. Despite the frequent use of this parameter to determine dosage schedules in teratological studies, it is often utilized with reservation due to its variance in gravid mice.

Therefore, to determine the LD_{50} variance in gravid and nongravid mice, it was decided to utilize drugs from different classes (morphine sulfate, cobalt chloride, and phenytoin sodium) and to subject the mice to a typical LD_{50} screen. Gravid mice were challenged on Day 9 of gestation, which corresponds to the midpoint in mouse pregnancy and also with the appearance of overt birth defects (1).

EXPERIMENTAL

Male and female CF-1 mice[1], ≥25 g, were utilized. Males were caged individually, and females were maintained in groups of 10 in large aggregate cages until exposure to the males. All mice were allowed water and food[2] *ad libitum*.

For morphine sulfate[3] and cobalt chloride[4] testing, 40 nongravid females were assigned to one of five groups of eight using a table of random numbers. Fifty-five animals, 11 per group, were used for phenytoin sodium testing[5] in the same manner. Dosage levels then were assigned to the groups (Tables I–III). Each mouse was weighed to the closest 0.1 g, caged individually, and administered the respective drug subcutaneously on the medial side of the right inguinal region.

Morphine sulfate (2%) and cobalt chloride (1 or 2%) were prepared daily in double-distilled water; phenytoin sodium (1%) was prepared in 70% propylene glycol in 0.9% NaCl. If the injected volume exceeded 1 ml, it was divided into two equal portions and was injected in separate inguinal regions. After injection, each mouse was observed for 24 hr, at which time the fatalities were observed (Tables I–III).

Breeding was accomplished as follows. Two females were placed

Table I—Observed Deaths [a] with Morphine Sulfate after 24 hr

Mice	Dosage, mg/kg				
	250	400	450	500	750
Nongravid	0/8	2/8	2/8	5/8	8/8
Gravid	0/8	1/8	2/8	5/8	7/8

[a] Deaths per number of mice tested.

Table II—Observed Deaths [a] with Cobalt Chloride after 24 hr

Mice	Dosage, mg/kg				
	100	125	150	200	300
Nongravid	0/8	2/8	4/8	6/8	8/8
Gravid	0/8	1/8	3/8	5/8	8/8

[a] Deaths per number of mice tested.

Table III—Observed Deaths [a] with Phenytoin Sodium after 24 hr

Mice	Dosage, mg/kg				
	200	250	300	325	400
Nongravid	0/11	3/11	7/11	10/11	11/11
Gravid	0/11	6/11	9/11	9/11	11/11

[a] Deaths per number of mice tested.

[1] Charles River Breeding Laboratories, Wilmington, Mass.
[2] Purina Laboratory Chow.
[3] USP, Merck & Co., Rahway, N.J.
[4] Lot 713262, Fisher Scientific, Fair Lawn, N.J.
[5] Lot PE338, Parke-Davis Co., Detroit, Mich.

0022-3549/80/0200-0214$01.00/0