Note

Selection of mixed ion-pair modifiers for high-performance liquid chromatographic mobile phases

SAMUEL J. COSTANZO

Norwich Eaton Pharmaceuticals, Inc., P.O. Box 191, Norwich, NY 13815 (U.S.A.)

(First received July 2nd, 1984; revised manuscript received August 8th, 1984)

Many cough/cold preparations contain several therapeutic compounds. Very often acidic, basic, and neutral compounds are present in the same formulation. When liquid chromatography (LC) is employed, systems good for one functional group may prove unsatisfactory for another. Neutral and acidic compounds are easily handled by conventional reversed-phase chromatography, whereas amines require either cation-exchange or ion-pairing techniques.

Bidlingmeyer has discussed those aspects of paired-ion chromatography that affect the separations of amines[1]. Lipophilicity of the ion pair varies with the size of the organic portion of the counter-ion. The same author also demonstrated that by using a mixture of different ion-pairing reagents it is possible to control the elution position of amine compounds. For simple chromatographic systems this approach is relatively straightforward. However, the ability to move one or more peaks selectively is important when the chromatogram is complex. Thus, to effectively use the mixed ion-pair concept, it is essential to have a means of optimizing the mobile phase.

A statistical approach based on overlapping resolution mapping (ORM) has been developed for the optimization of normal- and reversed-phase LC systems[2–4]. This method yields excellent results when three organic modifiers plus base solvents are necessary. However, this approach is not suitable when using paired-ion chromatography, since solvent strength calculations are not possible. Recently, Issaq *et al.*[5] have shown the value of the window diagram technique for optimizing binary mobile phases (two organic modifiers plus water). Sachak *et al.*[6] have successfully used this technique to optimize pH and concentration of ion-interaction reagent in selecting a reversed-phase eluent for the separation of nine organic acids. This approach was used in the present work for the optimization of a mixed ion-pair mobile phase, which was needed for the separation of one acid (benzoic acid), two bases (phenylephrine and phenylpropanolamine), and three neutral compounds (guaifenesin, an impurity, and an excipient).

This report describes the simultaneous determination of several species in a cough/cold formulation using a mixed ion-pair mobile phase.

EXPERIMENTAL

Reagents, materials and mobile phase

Phenylephrine hydrochloride and sodium benzoate were supplied by Alba

Chemical (Tenafly, NJ, U.S.A.). Guaifenesin was obtained from Pfaltz & Bauer (Stamford, CT, U.S.A.), and phenylpropanolamine was supplied by Sigma (St. Louis, MO, U.S.A.).

One liter of 0.005 M pentanesulfonic acid (PIC B-5, Waters Assoc., Cat. No. 85110) (I) and one liter of 0.005 M heptanesulfonic acid (PIC B-7, Waters Assoc., Cat. No. 85103) (II) were prepared. Mixed reagent (75% II:25% I) was prepared by mixing 750 ml of II with 250 ml of I. The mobile phase acetonitrile–mixed reagent (15:85, v/v), was prepared by mixing 150 ml of acetonitrile with 850 ml of mixed reagent, filtering through a 0.5-μm Millipore FH filter (Millipore, Bedford, MA, U.S.A.), and degassing under vacuum prior to use. A flow-rate of 2.0 ml/min was used in all experiments.

Sample preparation

The sample solution was prepared by pipetting 10.0 ml of cough/cold preparation into a 100-ml volumetric flask and diluting to volume with distilled water.

Standard preparation

Reference standard phenylpropanolamine hydrochloride, phenylephrine hydrochloride, and sodium benzoate stock solution was prepared by weighing, to the nearest 0.01 mg, 400 ± 10, 100 ± 10, and 100 ± 10 mg of the respective reference standard compounds and transferring into a 100-ml volumetric flask. The flask was then brought to volume with purified water and mixed well.

A standard solution was then prepared by accurately weighing 200 ± 10 mg of reference standard guaifenesin, transferring into a 100-ml volumetric flask, adding 10.0 ml of reference standard stock solution, diluting to volume with purified water, and mixing well.

Instrumentation

A high-performance liquid chromatograph (ALC GC-204; Waters Assoc., Milford, MA, U.S.A.) equipped with an injector (Model 7125; Rainen Instrument, Woburn, MA, U.S.A.), a 20-μl loop, and a Model 6000 reciprocating pump (Waters Assoc.) was used. The LC column was a Whatman Partisil-10, C_8 (Cat. No. 4229-111), 25 cm × 4.6 mm. Integration was accomplished on a Hewlett-Packard 3357 computer.

Validation procedure

The final method was validated using a diagnostic technique published recently by Cardone[7]. A sample curve was prepared by pipetting 2–20 ml of sample into a 100-ml volumetric flask and diluting to volume with distilled water. Standard curves were generated at levels of 20 to 200% of nominal. The method of standard addition was used at sample levels of 40% of nominal by adding standard at levels from 20 to 160% of nominal.

Solvent optimization

Solvent optimization was accomplished using the window diagram technique[8,9]. A third-order binomial regression analysis was used to draw the best lines.

RESULTS AND DISCUSSION

Chromatographic optimization

     The technique of using mixed ion-pair reagents was first described by Bidling-meyer[10]. This approach was shown to be useful for the separation of thiamine, pyridoxine, niacinamide, and riboflavin, using pentanesulfonate and heptanesulfonate counter-ions. When only pentanesulfonate was used as the counter-ion, insufficient retention was observed. However, by using water–methanol (50:50) containing 2.5 mM each of pentanesulfonate and heptanesulfonate, a satisfactory separation was obtained. Even though four different species were separated, this problem was simple enough so that acceptable results might have been obtained with only hexane- or heptanesulfonate as a single counter-ion.

     The chromatographic space in the system reported here is much more restricted than in the above situation; hence, a suitable optimization technique is needed. Initially, a mobile phase of acetonitrile–water (0.005 M pentanesulfonic acid) (15:85, v/v) was determined to be satisfactory for the separation of guaifenesin, phenylpropanolamine, sodium benzoate, excipient, and impurity. However, this system was deficient since phenylephrine eluted close to the solvent breakthrough peak. Attempts to remedy this problem by increasing the size of the alkyl group of the counter-ion
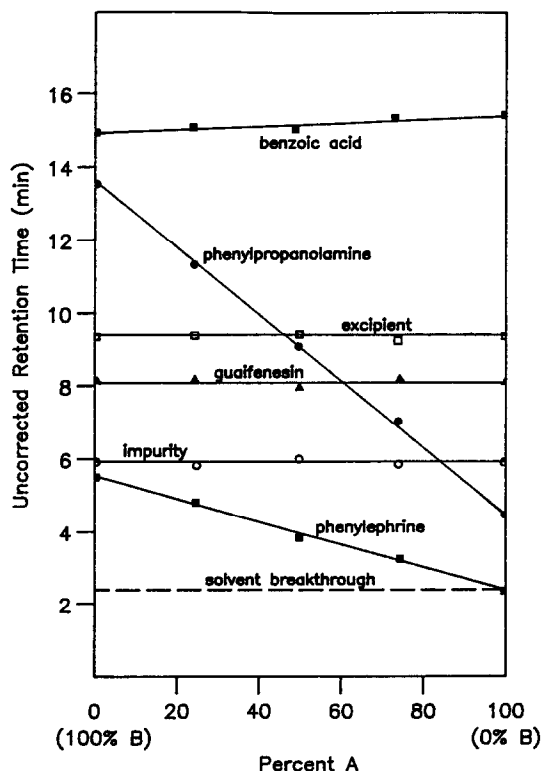

Fig. 1. Retention time *vs.* mixed ion-pair composition. System A is 15% acetonitrile in pentanesulfonic acid (0.005 M); system B is 15% acetonitrile in heptanesulfonic acid (0.005 M).
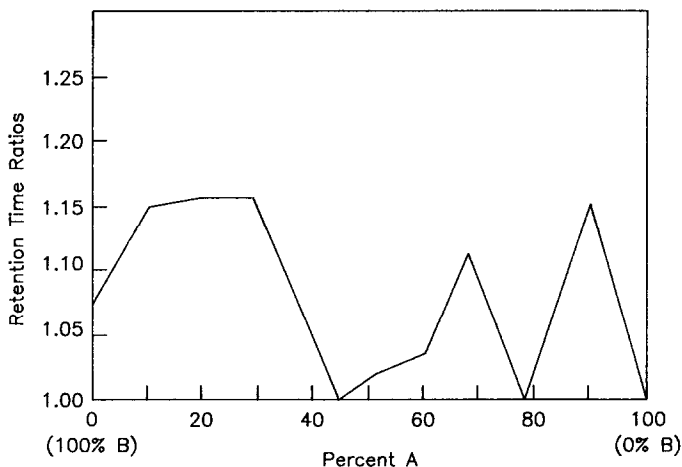
Fig. 2. Window diagram for all eluting peaks, calculated from Fig. 1. See Fig. 1 for systems A and B.

were unsuccessful. Although some improvement was noted with hexanesulfonic acid (0.005 M) as the counter-ion, the capacity factor of phenylephrine was still too low. The next higher homologue, heptanesulfonic acid (0.005 M) moved phenylephrine well away from the solvent breakthrough, but almost on top of the impurity peak. Simple direct attempts to control the retention volume of the phenylephrine peak with a mixed ion-pair mobile phase pointed out the need for a more systematic optimization approach. For example, a 50:50 mixture of pentanesulfonic acid and heptanesulfonic acid (0.0025 M each) within the 85% aqueous phase moved phe-nylpropanolamine well away from the solvent breakthrough peak; however, phe-nylpropanolamine and excipient co-eluted. The well known window diagram tech-nique of Laub and Purnell[8] proved to be a satisfactory means of finding the proper ratio of ion-pairing reagents. Fig. 1 shows a plot of the uncorrected retention time for all six compounds vs. percent pentanesulfonic acid (used in the 85% aqueous
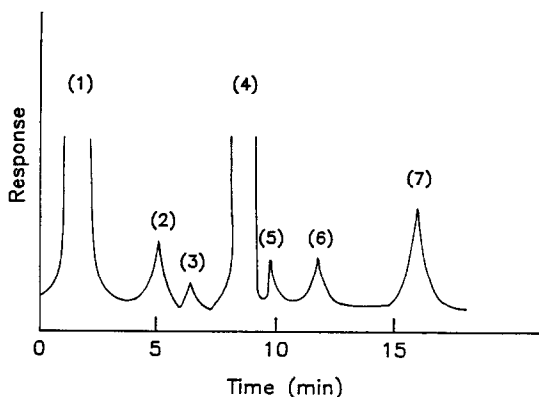

Fig. 3. Chromatogram of (1) solvent breakthrough, (2) phenylephrine hydrochloride, (3) impurity, (4) guaifenesin, (5) excipient, (6) phenylpropanolamine hydrochloride, and (7) sodium benzoate using a mixed ion pair: acetonitrile–water (0.00125 M pentanesulfonic acid–0.00375 M heptanesulfonic acid) (15:85, v/v). See Fig. 2 for optimized ratio of ion-pair reagents.

TABLE I

METHOD PERFORMANCE DATA GENERATED FROM CORRIGIBLE ERROR CALCULATIONS

See text for discussion.

Parameter	Phenylephrine	Phenyl-propanolamine	Guaifenesin	Sodium benzoate
Standard curve assay* (mg/ml)	0.0965 (0.0975)	3.89 (3.79)	19.43 (19.23)	0.980 (1.000)
R.S.D. (%)	1.07	2.30	0.36	0.92
% Constant error	1.2	−1.8	0.7	0.8
% Proportional error	99.6	100.2	97.8	100.3
Slope ratio assay (mg/ml)	0.0967	3.89	19.52	0.981
Reciprocal sample size assay (mg/ml)	0.0972	3.85	19.78	0.981
Method of standard addition assay* (mg/ml)	0.0942	3.85 (3.71)	19.56 (20.74)	0.975

* Uncorrected results in parentheses.

portion). The window diagram drawn from this plot is shown in Fig. 2. This diagram reveals that the best conditions for complete separation of all peaks is 20–30% pentanesulfonic acid and 80–70% heptanesulfonic acid (Fig. 3). The total concentration of the mixed ion-pairing reagent remains at 0.005 M for all mixtures. It is possible to obtain a satisfactory separation in a different area of the window. For example, a ratio of 90% pentanesulfonic acid: 10% heptanesulfonic acid results in a satisfactory separation. When the window diagram is examined, it becomes obvious that a slight deviation in solvent composition on either side of this ratio significantly worsens the quality of the separation. However, the window diagram reveals that a ratio of 25% pentanesulfonic acid: 75% heptanesulfonic acid is preferable because slight variation in the ratio of the mixed ion-pair will not significantly alter the separation.

TABLE II

LINEAR REGRESSSION DATA FOR STANDARD SOLUTIONS OF PHENYLEPHRINE, PHENYLPROPANOLAMINE, GUAIFENESIN, AND SODIUM BENZOATE

	Phenylephrine	Phenylpropa-nolamine	Guaifenesin	Sodium benzoate
Range (μg/injection)	0.4–4.0	1.6–16	8–80	0.48–4.8
Nominal sample (μg)	2	8	40	2.4
Correlation coefficient	0.9999	0.9997	0.9999	0.9999
Slope (area/μg)	1.12×10^6	3.97×10^5	1.45×10^6	3.59×10^6
y-Intercept (area)	4.65×10^2	4.07×10^3	4.64×10^4	7.61×10^2
Standard error of estimate (area)	5.77×10^2	2.47×10^3	1.84×10^4	2.44×10^3
% Variation $\left(\dfrac{\text{standard error of estimate}}{\bar{y}} \times 100\right)$	0.51	1.87	0.55	0.56
Relative y-intercept $\left(\dfrac{y\text{-intercept}}{\bar{y}} \times 100\right)$ (%)	0.41	0.03	1.38	0.18

From Fig. 1, it can be seen that the retention time for both amines increases linearly from 0 to 100% heptanesulfonic acid. Further, benzoic acid, guaifenesin, the excipient, and the impurity are all independent of the amount of ion-pairing reagent. This is expected for non-basic species which cannot form ion pairs with alkylsulfonic acids. This information is useful in helping to identify unknown peaks.

Method validation

Method validation was accomplished using a diagnostic technique capable of revealing constant and proportional errors[7]. Table I lists both the corrected and uncorrected assay results, and shows the magnitude of both types of errors. Clearly any bias remains well within the normal variation of the method.

All performance characteristics are satisfactory, and are typical for this type of methodology. The relative standard deviations range from 1 to 2.5%; while the recoveries (percent proportional error) remain close to 100%.

Linear regression data for phenylephrine, phenylpropanolamine, guaifenesin, and sodium benzoate is listed in Table II. All four compounds have acceptable correlation coefficients, low percent variations, and *y*-intercepts close to zero. The good linearity and small relative *y*-intercept show that calculations can be done using a single-point ratio procedure[11]. In this situation the error in measurement of sample response remains acceptably small when using one standard.

Finally, additional verification of the method performance results is provided by three supplementary calculations: the method of standard addition assay (MOSA); the slope ratio assay; and the reciprocal sample size assay. See Table I and ref. 7.

In summary, by using an optimization procedure such as the window diagram technique, it is possible to use a mobile phase containing a mixed ion pair. This technique works well even with relatively crowded chromatographic space. In the present system, six peaks were adequately resolved by selectively moving phenylephrine and phenylpropanolamine with a mixed ion-pair reagent.

ACKNOWLEDGEMENT

REFERENCES

1 B. A. Bidlingmeyer, *J. Chromatogr. Sci.*, 18 (1980) 525.
2 J. L. Glajch, J. J. Kirkland and K. M. Squire, *J. Chromatogr.*, 199 (1980) 57.
3 J. L. Glajch, J. J. Kirkland and L. R. Snyder, *J. Chromatogr.*, 238 (1982) 269.
4 H. J. Issaq, J. R. Klose, K. L. McNitt, J. E. Haky and G. M. Musckik, *J. Liq. Chromatogr.*, 4 (1981) 2091.
5 H. J. Issaq, G. M. Musckik and G. M. Janine, *J. Liq. Chromatogr.*, 6 (1983) 259.
6 B. Sachak, R. C. Kong and S. N. Deming, *J. Chromatogr.*, 199 (1980) 317.
7 M. J. Cardone, *J. Ass. Offic. Anal. Chem.*, 66 (1983) 1283.
8 R. J. Laub and J. H. Purnell, *J. Chromatogr.*, 112 (1975) 71.
9 R. J. Laub, *Amer. Lab.*, 13 (1981) 47.
10 B. A. Bidlingmeyer, *The Second International Symposium on Column Liquid Chromatography, Wilmington, DE, May 18, 1976.*
11 M. J. Cardone, P. J. Palermo and L. B. Sybrandt, *Anal. Chem.*, 52 (1980) 1187.