times more permeable to hydrocortisone than human skin. Assignment of an exact factor to this difference is made difficult, as the representativeness of the human epidermal value is uncertain, with there being no statistical data provided, nor are the temperature sensitivities of the permeation of hydrocortisone through the tissues known (but they can be assumed to be large). Nevertheless, this appears to be the first clear demonstration that to some permeating species, intact human and hairless integuments offer quantitatively different mass transfer resistances, with human skin being at least one logarithmic order less permeable. Previous diffusion cell-generated data for the alkanols (13, 14, 16) and phenol (19) have left the impression that the two skin types were, for practical purposes, identical in permeability.

Biphasic Character in Some Permeability Plots—A most unusual feature of some of these data is the biphasic quality of the permeability plots obtained with mice 5 and 35 d old. At 35 d of age, this aspect of the data was unequivocal. Like other researchers (8), we at first assumed that the skin membranes were deteriorating. However, when the permeability coefficient of a single section of skin was repetitively assessed approximately every 12 h over a 100-h period, using a previously outlined "hydration" protocol (16), the permeability coefficient remained constant at 2.5×10^{-4} cm/h. Subsequently, additional evidence has been gathered on skin of older animals which shows these same qualities (21), and it now appears that if a run is long and continuous, two distinct phases of permeation will be evident, with the second, more permeable, phase being as reproducible in its time of onset and permeability rate as the first. However, the onset of the second phase is at >40 h with the older skin sections, which is, by chance, the arbitrarily selected duration for the experiments reported here and which explains why the second phase was not apparent with the older skin sections. Also, the permeability coefficient of the second phase seems to increase dramatically with mouse age (consider the data for 5- and 35-d-old mice presented here). We suspect it was not evident after 5 d but not until 35 d because permeability in the early phase dominated events at 15 and 20 d. The physicochemical implication that this represents two separate and parallel permeability pathways through the stratum corneum with different dependencies on animal age is clear. This concept is mechanistically important and is thus being put to rigorous experimental testing.

REFERENCES

(1) F. D. Malkinson, *J. Soc. Cosmet. Chem.*, **11**, 146 (1960).

(2) F. D. Malkinson and E. H. Ferguson, *J. Invest. Dermatol.*, **25**, 281 (1955).

(3) R. J. Feldman and H. I. Maibach, *Arch, Dermatol.*, **91**, 661 (1965).

(4) C. S. Livingood, *J. Invest. Dermatol.* **31**, 27 (1958); in discussion of Ref. 5.

(5) F. D. Malkinson, *J. Invest. Dermatol.* **31**, 19 (1958).

(6) R. C. Wester, P. K. Noonan, and H. I. Maibach, *Arch. Dermatol.*, **113**, 620 (1977).

(7) R. C. Wester, P. K. Noonan, and H. I. Maibach, *Arch. Dermatol.*, **116**, 186 (1980).

(8) A. E. Solomon and N. J. Lowe, *Br. J. Dermatol.*, **100**, 717 (1979).

(9) H. Shinkai and I. Tanaka, *Yakugaku Zasshi*, **89**, 1283 (1969).

(10) H. Shinkai, *Yakugaku Zasshi*, **89**, 365 (1969).

(11) C. A. Schlagel, *Adv. Biol. Skin*, **12**, 339 (1972).

(12) R. J. Scheuplein, I. H. Blank, G. J. Brauner, and D. J. MacFarlane, *J. Invest. Dermatol.*, **52**, 63 (1969).

(13) H. Durrheim, G. L. Flynn, W. I. Higuchi, and C. R. Behl, *J. Pharm. Sci.*, **69**, 781 (1980).

(14) G. L. Flynn, H. Durrheim, and W. I. Higuchi, *J. Pharm. Sci.*, **70**, 52 (1981).

(15) C. R. Behl, G. L. Flynn, T. Kurihara, W. Smith, O. Gatmaitan, W. I. Higuchi, N. F. H. Ho, and C. L. Pierson, *J. Invest. Dermatol.*, **75**, 340 (1980).

(16) C. R. Behl, G. L. Flynn, T. Kurihara, N. Harper, W. Smith, W. I. Higuchi, N. F. H. Ho, and C. L. Pierson, *J. Invest. Dermatol.*, **75**, 346 (1980).

(17) C. R. Behl, G. L. Flynn, T. Kurihara, W. M. Smith, N. Bellantine, O. G. Gatmaitan, C. L. Pierson, W. I. Higuchi, and N. F. H. Ho, *J. Soc. Cosmet. Chem.*, **35**, 237 (1984).

(18) C. R. Behl, M. Barrett, G. L. Flynn, T. Kurihara, K. A. Walters, O. G. Gatmaitan, N. Harper, W. I. Higuchi, N. F. H. Ho, and C. L. Pierson, *J. Pharm. Sci.*, **71**, 229 (1982).

(19) C. R. Behl, E. E. Linn, G. L. Flynn, C. L. Pierson, W. I. Higuchi, and N. F. H. Ho, *J. Pharm. Sci.*, **72**, 391 (1983).

(20) E. E. Linn, Ph.D. Thesis, "Thermotropic Effects on Skin Permeability," University of Michigan, Arbor, Mich., 1982.

(21) W. M. Smith, Ph.D. Thesis, "An Inquiry into the Mechanism of Percutaneous Absorption of Hydrocortisone and its 21-*n*-alkyl Esters," University of Michigan, Ann Arbor, Mich., 1982.

ACKNOWLEDGMENTS

This work was presented in part at the 127th Annual Meeting of the American Pharmaceutical Association, Washington, D.C., April 1980 (Basic Pharmaceutics Section Abstract No. 100).

Determination of Minoxidil in Bulk Drug and Pharmaceutical Formulations by Ion-Pairing High-Performance Liquid Chromatography

P. A. ASMUS ˣ, J. B. LANDIS, M. E. GRANT, and H. A. HAVEL

Received June 8, 1983, from *Control Analytical Research and Development, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication September 23, 1983.

Abstract ☐ An ion-pairing liquid chromatographic method with UV detection is described for the determination of minoxidil in bulk drug, compressed tablet, and topical solution formulations. The chromatographic system consists of a microparticulate octadecylsilica column and a mobile phase composed of sodium dioctylsulfosuccinate in aqueous methanol (pH 3). The bulk drug and the topical solution samples are prepared by the dissolution of the drug in internal standard solution. Sample preparation for the compressed tablet formulation involves dissolving the drug from an aliquot of pulverized sample and centrifuging to remove insoluble excipients. Quantitative recovery of minoxidil from formulation excipients was demonstrated; assay precision was <1% *CV*.

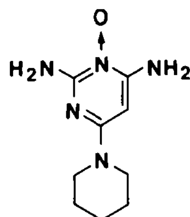
Keyphrases ☐ Minoxidil—compressed tablets, topical solution and bulk drug, HPLC ☐ HPLC—minoxidil, compressed tablets, topical solution, and bulk drug

Minoxidil (I), 2,4-diamino-6-piperidinopyrimidine 3-oxide, a potent, orally active antihypertensive agent, is commercially available as a compressed tablet formulation[1]. There are, as yet, no published methods for the determination of minoxidil in bulk drug or dosage forms. A simple, precise, and specific assay method for minoxidil in these matrices was needed for quality control activities. For use in accelerated stability studies, potential decomposition products of minoxidil must be separated from the drug peak. Minoxidil is quite stable, decomposing only at high temperatures to form 2,4-di-

[1] Loniten, The Upjohn Co., Kalamazoo, Mich.

0022-3549/ 84/ 0900-1290$01.00/ 0

© 1984, American Pharmaceutical Association



amino-6-piperidinopyrimidine (the deoxy product).

This paper reports the development of a reversed-phase ion-pairing high-performance liquid chromatographic (HPLC) assay method for minoxidil in several formulations and bulk drug. Since the work of Schill and co-workers (1, 2), Haney *et al.* (3, 4), and Knox *et al.* (5, 6), many workers have found that reversed-phase ion-pairing with alkylsulfates of alkylsulfonates is effective in reducing tailing and also for adjusting retention and selectivity (7-14). The method is simple, precise, and specific for minoxidil in the presence of possible decomposition products and several synthetic intermediates.

EXPERIMENTAL SECTION

Materials—Minoxidil[2], 2-*N*-dicyano-*N*′,*N*′-pentamethylene acetoamidine[2], 2,4-diamino-6-chloropyrimidine 3-oxide[2], 2,4-diamino-6-chloropyrimidine[2], 2,4-diamino-6-piperidinopyrimidine[2], and sodium dioctylsulfosuccinate[3] were >95% pure by HPLC and used as received. The internal standard, medroxyprogesterone acetate, was USP-NF quality. The methanol[4] and water used in the mobile phases were distilled. All other chemicals were reagent grade.

Apparatus—Experiments were conducted on a modular HPLC consisting of a reciprocating piston pump[5], a loop injection valve[6], a UV detector[7] (254 nm), and a recorder[8]. For quantitation, data were collected and processed by a digital computer[9].

Mobile Phase—The mobile phase was prepared as follows. Sodium dioctylsulfosuccinate (3.0 g) and 10 mL of glacial acetic acid were dissolved in 700 mL of methanol. Stirring was continued and 300 mL of water was added. The apparent pH was adjusted to 3.0 with concentrated perchloric acid. The solution was filtered through a 0.45 μm filter[10] before use.

The separation between minoxidil and the internal standard, medroxyprogesterone acetate, could be increased without affecting selectivity by decreasing the methanol concentration of the mobile phase.

The volume of the mobile phase in the column, V_m, which is equal to the retention volume of an unretained compound, was estimated by the retention volume of sodium nitrate (20 mg/mL in the mobile phase). The retention times of the compounds studied were measured using 1 mg/mL solutions of authentic standards. Capacity factors, k', were determined using the equation: $k' = V_R - V_m/V_m$, where V_R is the retention volume of the compound being measured. The separation factors were calculated by taking the ratio of the capacity factor of the compound to the capacity factor of minoxidil.

Column—The microparticulate octadecylsilane column[11] (10-μm particles, 300 × 3.9 mm) used was washed with methanol-water (80:20, v/v) and stored in the same solvent when not in use. The column temperature was ambient, the column pressure was ~1500 psi, the injection volume was ~10 μL, and the flow rate was 1.0 mL/min.

Sample Preparation—Standards and bulk drug samples were prepared by accurately weighing ~5 mg of the sample and dissolving it in 20 mL of internal standard solution (~0.2 mg/mL medroxyprogesterone acetate in mobile phase). For compressed tablet samples, 10 tablets were weighed and then pulverized to a fine powder. An accurately weighed aliquot of the powder containing ~5 mg of minoxidil was dissolved in 20 ml of internal standard solution, and insoluble excipients were separated by centrifugation. The clear supernatant was separated for analysis. Topical solution samples were prepared by accurately measuring an amount of solution containing ~50 mg minoxidil and diluting to 50 mL with mobile phase. Five milliliters of this solution was dissolved in 20 mL of the internal standard solution.

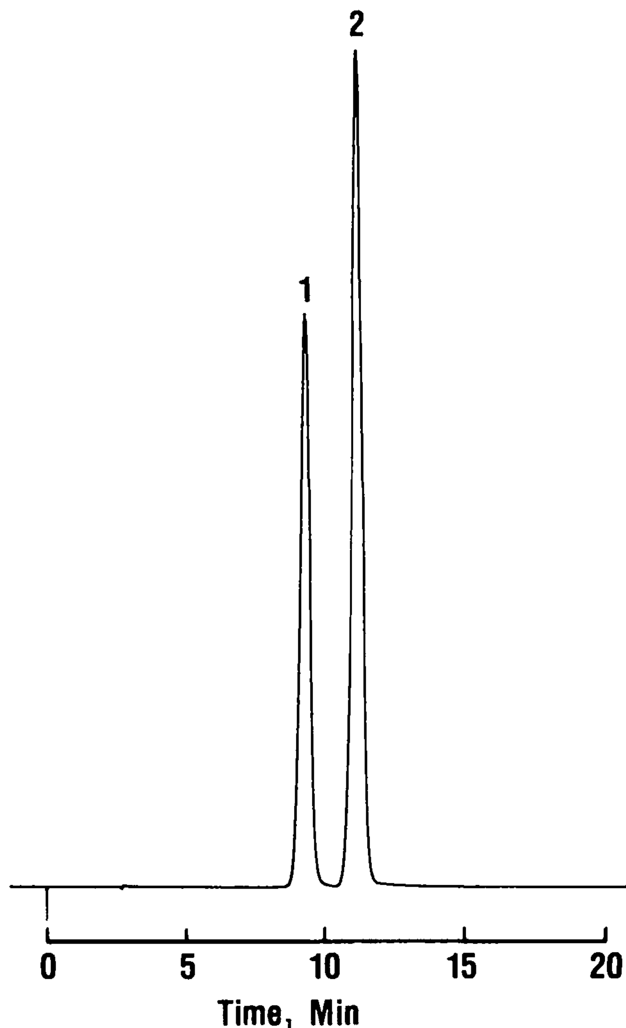


Figure 1—*Chromatogram of a compressed tablet preparation of minoxidil. The mobile phase was 6.7 mM sodium dioctylsulfosuccinate and 175 mM acetic acid in 70% methanol, pH 3.0; the flow rate was 1.0 mL/min; the temperature was ambient; the attenuation was 0.512 AUFS. Key: (1) medroxyprogesterone acetate (internal standard); (2) minoxidil.*

RESULTS AND DISCUSSION

A typical chromatogram of a minoxidil compressed tablet sample preparation is shown in Fig. 1. No excipient peaks are observed even though no sample clean-up steps were employed. The minoxidil peak was symmetrical and an adequate number of theoretical plates (3500) were generated. Retention and selectivity data for minoxidil and related compounds are shown

Table I—Retention and Selectivity Data for Minoxidil and Related Compounds

Compound	Retention Time, min	Capacity Factor	Separation Factor
2-*N*-Dicyano-*N*′,*N*′-pentamethylene acetoamidine	3.41	0.34	0.12
2,4-Diamino-6-chloropyrimidine 3-oxide	4.13	0.63	0.22
2,4-Diamino-6-chloropyrimidine	6.25	1.46	0.51
Medroxyprogesterone acetate (internal standard)	8.57	2.37	0.82
Minoxidil	9.87	2.89	1.00
2,4-Diamino-6-piperidinopyrimidine	12.4	3.88	1.35

[2] The Upjohn Co.
[3] Eastman Kodak Co., Rochester, N.Y.
[4] Burdick & Jackson Laboratories, Muskegon, Mich.
[5] Model 110A; Altex Corp., Berkeley, Calif.
[6] Model 7125; Rheodyne Inc., Cotati, Calif.
[7] Model UVIII; Laboratory Data Control, Riviera Beach, Fl.
[8] Model XKR; Sargent Welch Co., Skokie, Ill.
[9] PDP 11; Digital Equipment Co., Maynard, Mass.
[10] Type HA; Millipore Corp., Bedford, Mass.
[11] μ-Bondapak C_{18}, Waters Associates, Milford, Mass.

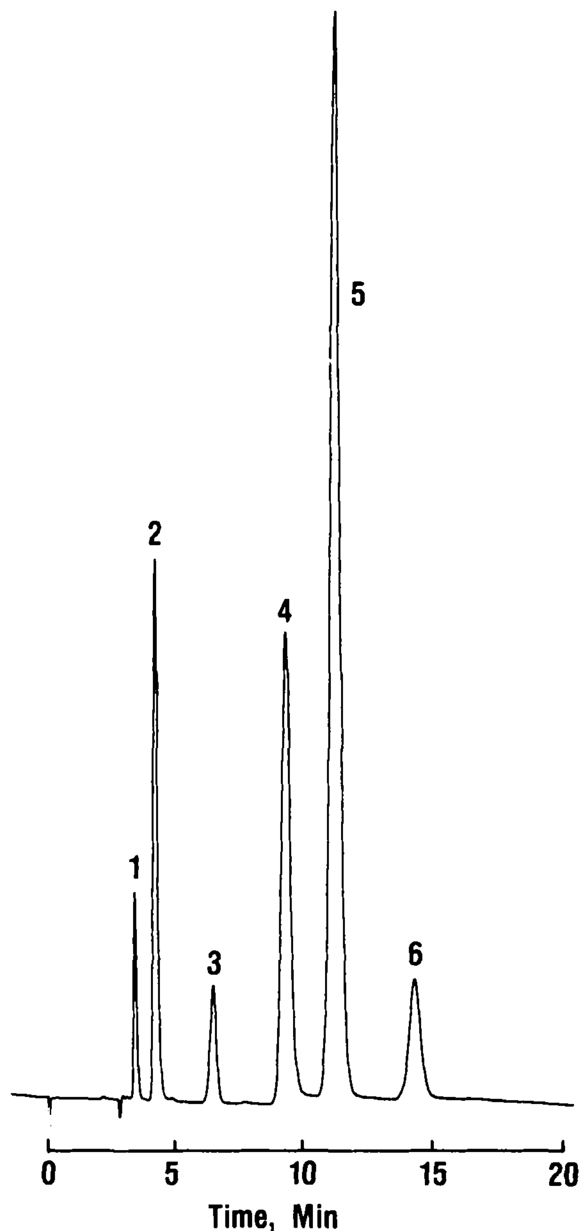


Figure 2—*Chromatogram of minoxidil and related compounds. Key: (1) 0.065 μg 2-N-dicyano-N′,N′-pentamethylene acetoamidine; (2) 1.5 μg 2,4-diamino-6-chloropyrimidine 3-oxide; (3) 0.5 μg 2,4-diamino-6-chloropyrimidine; (4) 0.9 μg medroxyprogesterone acetate; (5) 2.3 μg minoxidil; (6) 0.4 μg 2,4-diamino-6-piperidinopyrimidine. See Fig. 1 for mobile phase composition. Attenuation was 0.256 AUFS.*

in Table I. The sample preparation involved dissolution of the drug from a powdered tablet using the mobile phase which contained the internal standard. Insoluble excipients were separated by centrifugation and the supernatant was chromatographed.

The specificity of the chromatographic system was evaluated by chromatographing a mixture of minoxidil and its possible impurities. Figure 2 shows the very good resolution obtained between all components. This degree of specificity was achieved by manipulation of the mobile phase constituents, *i.e.*, the ion-pairing reagent concentration, the organic modifier concentration, and the pH.

Sodium dioctylsulfosuccinate was chosen as the ion-pairing reagent after several alkylsulfates and alkylsulfonates were tried. The dioctylsulfosuccinate anion is a large hydrophobic sulfonic acid containing 20 carbon atoms, of which 18 can be considered alkyl chain carbons. It has, therefore, a very large affinity for the alkyl groups on the packing material surface. Ion-pairing reagents with smaller carbon chain lengths allowed the minoxidil peak to tail. Sodium dioctylsulfosuccinate has been successfully used as an ion-pairing reagent in several other HPLC assay methods (10-14).

The relationship between retention and the dioctylsulfosuccinate concentration in the mobile phase was studied for minoxidil and related compounds (Fig. 3). In accordance with well known reversed-phase ion-pairing behavior, retention of all the basic compounds was proportional to the dioctylsulfosuccinate concentration. The concentration of dioctylsulfosuccinate was 6.7 mM, since adequate retention and resolution was observed at that level. Selectivity was not affected enough to cause peak reversal over a 1-25 mM dioctylsulfosuccinate concentration range.

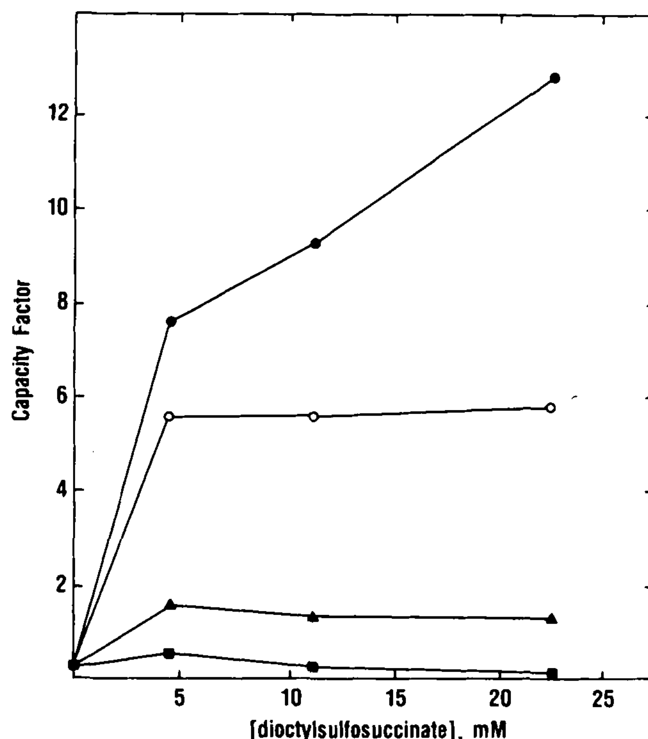


Figure 3—*Effect of mobile phase dioctylsulfosuccinate concentration on retention. Key: (■) 2-N-dicyano-N′,N′-pentamethylene acetoamidine and 2,4-diamino-6-chloropyrimidine 3-oxide; (▲) 2,4-diamino-6-chloropyrimidine; (○) minoxidil; (●) 2,4-diamino-6-piperidinopyrimidine. The mobile phase was 175 mM acetic acid in 70% methanol, pH 3.0.*

The mobile phase contained methanol as the organic modifier. The dependence of retention on its concentration was studied, and the results are

Table II—Recovery of Minoxidil Added to Compressed Tablet Placebo

Amount Added, mg	Amount Found, mg	Recovery, %
1.388	1.401	100.9
2.964	2.973	100.3
4.213	4.286	101.7
6.039	6.061	100.4
9.852	9.757	99.0
Mean		100.5

Table III—Analyses of Minoxidil Formulations

Lot No.	Labeled Amount, mg	Assay Result, mg
Tablets		
A	0.3	0.30
B	1.0	1.01
C	2.5	2.45
D	5.0	4.97
E	10.0	9.83
F	15.0	15.0
G	25.0	24.6
Topical Solutions[a]		
A	10.0	10.3
B	30.0	31.3
C	50.0	52.2

[a] Expressed as mg/mL for topical solutions.

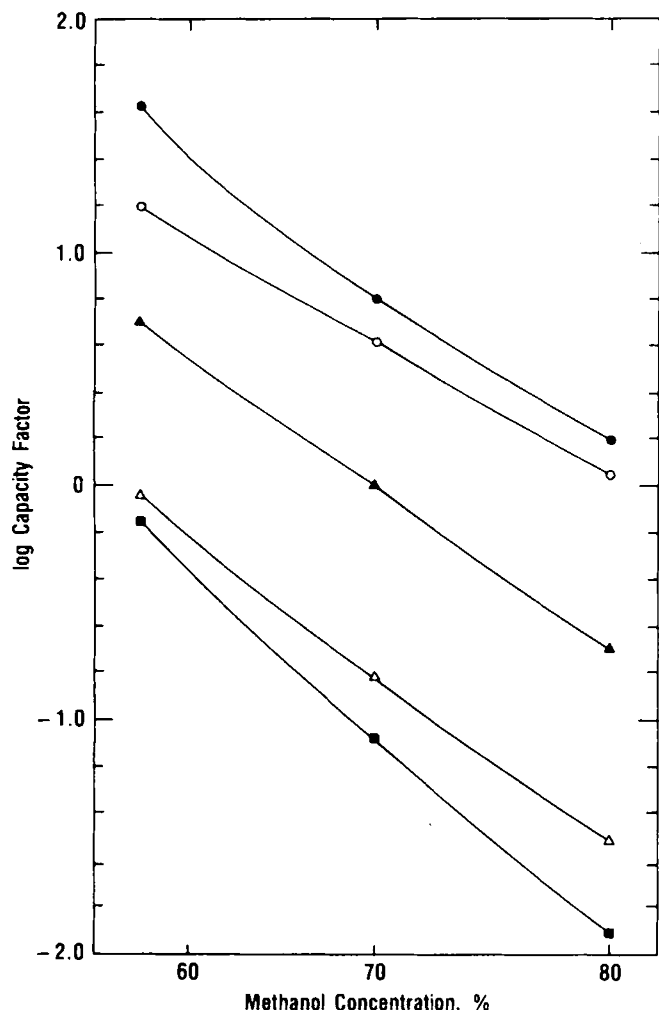


Figure 4—*Effect of mobile phase methanol concentration on retention. Key: (■) 2-N-dicyano-N′,N′-pentamethylene acetoamidine; (△) 2,4-diamino-6-chloropyrimidine 3-oxide; (▲) 2,4-diamino-6-chloropyrimidine, (○) minoxidil, (●) 2,4-diamino-6-piperidinopyrimidine. The mobile phase was 6.7 mM sodium dioctylsulfosuccinate and 175 mM acetic acid, pH 3.0.*

shown in Fig. 4. As expected, retention varies linearly with the log of the methanol concentration. It can be seen that selectivity does not change as the methanol concentration varies. The mobile phase methanol concentration can, therefore, be reduced to compensate for reduced retention as the column ages.

In reversed-phase ion-pairing liquid chromatography, the mobile phase pH is an important variable. The retention behavior of the compounds was studied as a function of the apparent pH of the mobile phase (Fig. 5). From this experiment, an apparent pH of 3.0 was chosen for the best separation of all compounds. At that pH, the ion-pairing reagent was ionized and the solutes were fully protonated. In the pH region of 4–7, the retention of minoxidil decreased due to deprotonation of the molecule, which decreased the magnitude of the interaction between minoxidil and dioctylsulfosuccinate. Glacial acetic acid and perchloric acid were added to the mobile phase to obtain an apparent pH of 3. The effect of the acetic acid concentration on retention is minimal.

Linearity of the assay for bulk drug was determined over a range of 75–125% of the amount called for in this procedure. The regression equation for the line was: minoxidil found = (1.014 ± 0.038) (minoxidil added) + (−0.102 ± 0.196). Tablet placebo samples were spiked with minoxidil, mixed in the solid state, and analyzed by this method to measure recovery. Quantitative recovery of minoxidil was observed over the range of ~1.4–9.9 mg/0.13 g of compressed tablet placebo (Table II). This range corresponds roughly to 30–200% of the label claim for a 10 mg compressed tablet formulation. Replicate analyses of a single lot of 5-mg compressed tablets gave a method precision of 0.6% *RSD*. Results from the analyses of several lots of compressed tablets and topical solution formulations of various strengths are shown in Table III. Good agreement between assay results and the label claim amount was observed in all cases.

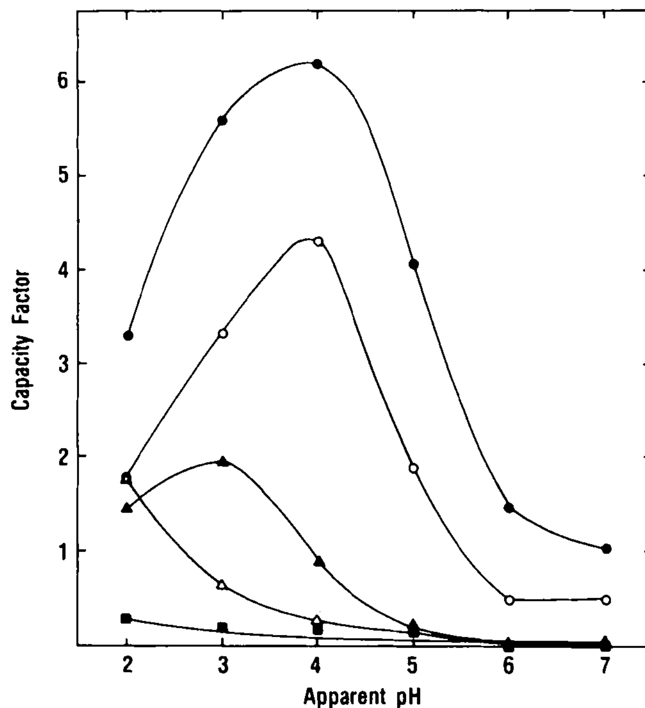


Figure 5—*Effect of mobile phase apparent pH on retention. See Fig. 3 for the key. The mobile phase was 6.7 mM sodium dioctylsulfosuccinate and 175 mM acetic acid in 70% methanol; pH from 2.0 to 3.5 adjusted with perchloric acid and from 3.5 to 7.0 adjusted with ammonium hydroxide.*

REFERENCES

(1) G. Schill, R. Modin, and B. Persson, *Acta Pharm. Suec.*, **2**, 119 (1965).

(2) G. Schill, *Acta Pharm. Suec.*, **2**, 13 (1965).

(3) D. P. Wittmer, N. O. Nuessle, and W. G. Haney, Jr., *Anal. Chem.*, **47**, 1422 (1975).

(4) S. P. Sood, L. E. Sartori, D. P. Wittmer, and W. G. Haney, Jr., *Anal. Chem.*, **48**, 796 (1976).

(5) J. H. Knox and G. R. Laird, *J. Chromatogr.*, **122**, 17 (1976).

(6) J. H. Knox and J. Jurrard, *J. Chromatogr.*, **125**, 89 (1976).

(7) C. Olieman, *J. Chromatogr.*, **133**, 385 (1977).

(8) H. F. Proeless, H. J. Lohmann, and D. G. Miles, *Clin. Chem.*, **24**, 1948 (1978).

(9) S. L. Pierson, J. J. Hanigan, R. E. Taylor, and J. E. McClurg, *J. Pharm. Sci.*, **68**, 1550 (1979).

(10) J. T. Stewart, I. L. Honingberg, J. P. Brant, W. A. Murray, J. L. Webb, and J. B. Smith, *J. Pharm. Sci.*, **65**, 1536 (1976).

(11) S. Sood, D. P. Wittmer, S. A. Ismaiel, and W. G. Haney, *J. Pharm. Sci.*, **66**, 40 (1977).

(12) P. A. Hartman, *J. Assoc. Off. Anal. Chem.*, **62**, 1099 (1979).

(13) E. J. Kubiak and J. W. Munson, *J. Pharm. Sci.*, **69**, 1380 (1980).

(14) G. W. Halstead, *J. Pharm. Sci.*, **71**, 1108 (1982).