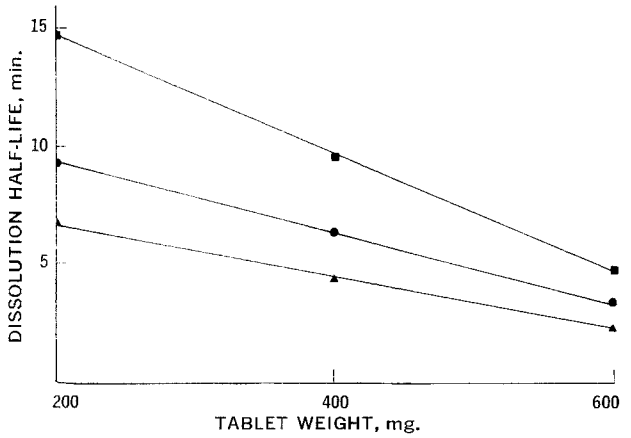

Figure 4—*Relationship of dissolution half-life* ($t_{50\%}$) versus *different tablet sizes compressed at the same pressure. Key:* ▲, $t_{50\%}$, *12,000 p.s.i.;* ●, $t_{50\%}$, *18,000 p.s.i.; and* ■, $t_{50\%}$, *24,000 p.s.i.*

When fabricating a tablet formulation, the research pharmacist many times will select the smallest tablet for a specific quantity of drug. This criterion is both economical and convenient from an oral administration viewpoint. In most cases, if the drug is poorly water soluble, micronization of the drug is utilized to maximize dissolution behavior. The data obtained in this study would recommend a larger tablet than is generally considered appropriate for a 50-mg. quantity of drug. The lesson taught with this poorly water-soluble drug indicates the necessity to consider the ratio of tablet excipient to drug in order to accomplish an optimum dissolution rate.

The force with which the tablet was compressed was also shown to affect dissolution behavior substantially. Figure 4 demonstrates that the larger the tablet size, the less effect compression force has on the dissolution rate.

The linear relationship which existed for tablet size *versus* $t_{50\%}$, when plotted for tablets compressed at 12,000, 18,000, and 24,000 p.s.i., was a good indication of the discrete particle-surface area's dependency on the dissolution characteristics. The discrete particle's total surface area possibly is related as a function of the ratio of diluent to drug when compressed at the same pressure.

The $t_{50\%}$ values reveal that a marked change in dissolution behavior occurs with the smaller tablet when the compression force is changed. This phenomenon is less pronounced as the dilution factor is increased. The dilution of the drug with excipient within each of the discrete particles from disintegration should provide for enhanced *in vivo* dissolution.

In conclusion, it is important to emphasize that when formulating a tablet of a poorly water-soluble drug, the following should be considered: optimum tablet size, hydrophilic nature of diluents, and optimum force of compression.

REFERENCES

(1) A. Morrison and J. Campbell, *J. Pharm. Sci.*, **54,** 1(1965).

(2) J. G. Wagner, *ibid.*, **50,** 359(1961).

(3) J. G. Wagner, *Drug Intel.*, **3,** 198(1969).

(4) G. Levy, J. M. Antkowiak, J. A. Procknal, and D. C. White, *J. Pharm. Sci.*, **52,** 1047(1963).

(5) G. Levy and R. Gumtow, *ibid.*, **52,** 1139(1963).

(6) T. Higuchi, A. N. Rao, L. W. Busse, and J. V. Swintosky, *J. Amer. Pharm. Ass., Sci. Ed.*, **42,** 194(1953).

(7) S. S. Kornblum and J. O. Hirschorn, *J. Pharm. Sci.*, **59,** 606(1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 4, 1970, from the *Pharmacy Research and Development Department, Sandoz Pharmaceuticals, Hanover, NJ 07936*

Accepted for publication October 27, 1970.

The technical assistance of C. Kenworthy and F. Darling is gratefully acknowledged.

* To whom all correspondence should be addressed.

DRUG STANDARDS

TLC Identification of Sulfonamides

HANNAH R. KLEIN and WILLIAM J. MADER

Abstract □ An improved identification procedure for the official sulfonamides is presented. The method described uses TLC for separation; identification is accomplished on the plates using a specific detection reagent and suitable reference standards. By the use of three developing systems, the identity of any individual sulfonamide or the components of a mixture of sulfonamides may be established with certainty.

Keyphrases □ Sulfonamides, individual, mixed—identification procedure □ Chromatographic systems (TLC)—sulfonamide identification □ TLC—separation, identification

The USP and NF monograph procedures for identification of the official sulfonamides rely principally on the classical methods of organic chemistry for the recognition of a particular material. While used as identity tests, these procedures are extremely difficult and time consuming and often do not provide absolute proof in distinguishing individual substances of a class of compounds such as the sulfonamides.

The identity tests used in both compendia illustrate the difficulty in using these methods to identify and distinguish the sulfonamides. Roughly 80% of the methods used for official identity tests are based on visual observation of one of the following: heat decomposition, diazotization and coupling with β-naphthol, color or precipitate formed with cupric sulfate, color or fluorescence with resorcinol or phenol, ferric chloride, sodium bicarbonate solubility, or reduction. All of these procedures are subject to interferences, and none of them effectively distinguishes individual sulfonamides. The UV spectra of some compounds are used; and while they may be of considerable quantita-

Table I—Systems Examined

No.	Thin Layer	Solvents	Reference
S-1	Silica gel G	Ethyl acetate–methanol–25% ammonium hydroxide (17:6:5)	12
S-2	Silica gel G	Petroleum ether–chloroform–*n*-butanol (1:1:1)	13
S-3	Silica gel	Chloroform–methanol (95:5)	14
S-4	Silica gel G	Chloroform–acetone–methanol–6 *N* ammonia (60:10:25:0.5)	5
S-5	Silica gel G	*n*-Propanol–0.05*N* hydrochloric acid (8:2)	5
S-6	Silica gel G	Butyl acetate–*n*-butanol–acetone–10% ammonia (3:3:4:1)	6
S-7	Silica gel G	Chloroform–*n*-butanol–acetone–85% formic acid (8:2:2:2)	6, 8
S-8	Silica gel G	Chloroform–methanol–*n*-butanol–2% ammonia (80:10:9:1)	7
S-9	Silica gel G	Chloroform–methanol (10:1)	8
S-10	Silica gel G	Chloroform–methanol–*n*-butanol–2% ammonia (8:10:10:1)	8
S-11	Alumina H	Ethyl acetate–water, buffered with ammonia (80:1)	9
S-12	Silica gel G	Ether–chloroform–*n*-butanol (1:1:1)	10
S-13	Silica gel G	Chloroform–methanol (9:1)	11
S-14	Alumina G	Ethyl acetate–methanol–25% ammonium hydroxide (17:3:3)	12
S-15	Silica gel G	Methyl isobutyl ketone–acetone–25% ammonium hydroxide (1:4:1)	12
S-16	Polyamide	Methyl isobutyl ketone–acetone–25% ammonium hydroxide (5:20:1)	12
S-17	Polyamide	Ethyl acetate–methanol–25% ammonium hydroxide (17:3:1)	12
S-18	Silica gel	Chloroform–methanol–acetic acid (94:5:1)	14
S-19	Silica gel	Chloroform–methanol–acetic acid (90:5:5)	14
S-20	Silica gel	Chloroform–*p*-dioxane (8:2)	14
S-21	Silica gel G	Chloroform–ethanol–heptane (1:1:1)	11
S-22	Alumina G	Methanol–chloroform (3:7)	15

tive value, they are of relatively little qualitative value since the spectra of all the sulfonamides are quite similar. In three instances the IR spectrum is used, and this will be discussed in the following paragraph. The only other official tests that could be used in identification are the melting points. Since many of the sulfonamides melt with decomposition and, consequently, over a wide range, this procedure must be used with caution.

Serious drawbacks in the IR characteristics of the sulfonamides prevent the use of IR spectra for identification. These drawbacks are: (*a*) the high incidence of polymorphism (1), giving rise to different spectra for different crystalline forms of the same compound; and (*b*) the low solubility of the sulfonamides in nonpolar solvents which prevents the use of solution spectra. Reproducible conversion between different forms of the sulfonamides is difficult to achieve and cannot be guaranteed by using similar solvent treatment on more than one form. The same can be said of recrystallization which, in addition, is undesirable since impurities may be excluded. Lyophilization of the sample, which has been reported to be successful using other compounds (2), was applied to separate forms of one of the sulfonamides but was unsuccessful.

It is readily apparent that there is currently no one procedure that will identify and differentiate all the official sulfonamides. Equally apparent is the desirability of replacing the numerous qualitative tests now employed by the USP and NF with a single procedure which could identify any of the official sulfonamides. In recent years, a number of reports (3, 4) dealt with TLC separation of various mixtures of sulfonamides. If a system could be found that would effectively separate all the sulfas, then a TLC separation coupled with a specific detection agent would provide an improved identification test.

In an attempt to find such a procedure, 22 separate TLC systems, which have been employed for some sulfonamides and on which data have been published, were examined. From these investigations, three separate solvent systems were found that will provide complete separation of the sulfonamides when used in conjunction. Complete separation cannot be achieved with less than three systems.

EXPERIMENTAL

Apparatus—Desaga-Brinkmann equipment for TLC was used throughout.

Adsorbents—The following were used: silica gel G and alumina H[1], polyamide[2], alumina and silica gel precoated glass plates, and aluminum-foil backed sheets[1].

Preparation of Layers—Glass plates, 20 × 20 cm., were coated with a 0.25-mm. thick layer of adsorbent as a slurry, air dried overnight, activated at 105° for 30 min., and allowed to cool to room temperature in a desiccator.

Solvent Systems—The 22 systems evaluated are listed in Table I. All solvents were reagent grade.

Samples—The sulfonamides tested were all either USP or NF grade. USP and NF reference standards were used where available. Solutions of each compound were prepared in acetone to contain 2 mg./ml. of the test substance.

Detection Reagents—The following were used:

1. *p*-Dimethylaminobenzaldehyde, 1% in 5% HCl.
2. Bratton–Marshall reagent: (*a*) 1 *N* HCl; (*b*) 5% sodium nitrite; (*c*) 100 mg. *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 100 ml. water. Spray with (*a*), then spray with (*b*), dry briefly at 100°, and then spray with (*c*).

Chromatographic Procedure—Chromatography chambers, lined with filter paper, were filled with the appropriate solvent mixture to a depth of about 1 cm. and allowed to equilibrate at least 1 hr. Thin-layer plates, prepared as previously described, were spotted with 2 μl. of each test solution at a point 1.5 cm. from the bottom of the plate and about 1 cm. apart. The spots were air dried, and the plate was placed in an equilibrated chamber and allowed to develop until the solvent front traveled 15 cm. above the point of application of the spots. The developed plates were air dried and sprayed with the detecting reagent.

RESULTS AND DISCUSSION

Evaluation of the solvent systems listed in Table I revealed that the best resolution could be achieved by using Solvents S-1, S-2, and S-3 in conjunction. None of the solvents, used individually, would separate all the compounds tested. The R_f values of the 16 official sulfonamides obtained with each of these three solvents are listed in Table II. The values represent an average of at least four determina-

[1] E. Merck AG, Darmstadt, West Germany.
[2] Woelm.

Table II—TLC R_f Values of Sulfonamides[a]

Compound	$R_f \times 100$ S-1	S-2	S-3
1. Acetylsulfisoxazole NF	56 ± 4	90 ± 2	54 ± 2
2. Phthalylsulfacetamide NF[b]	49 ± 2, and 29 ± 1	38 ± 2, and 3 ± 1	8 ± 2 and origin
3. Phthalylsulfathiazole NF	37 ± 4	62 ± 1	No migration
4. Succinylsulfathiazole USP	28 ± 1	5 ± 1	No migration
5. Sulfacetamide NF	44 ± 2	59 ± 3	9 ± 2
6. Sulfadiazine USP	42 ± 2	55 ± 3	24 ± 1
7. Sulfadimethoxine NF	59 ± 3	87 ± 2	36 ± 2
8. Sulfaethidole NF	57 ± 3	78 ± 6	17 ± 3
9. Sulfaguanidine NF	66 ± 1	18 ± 2	2 ± 1
10. Sulfamerazine USP	47 ± 2	67 ± 3	29 ± 2
11. Sulfamethazine USP	55 ± 2	68 ± 2	31 ± 2
12. Sulfamethizole NF	53 ± 5	71 ± 4	19 ± 1
13. Sulfamethoxazole NF	54 ± 3	84 ± 1	26 ± 2
14. Sulfanilamide USP	79 ± 1	42 ± 4	8 ± 2
15. Sulfapyridine USP	62 ± 2	54 ± 2	23 ± 2
16. Sulfisoxazole USP	56 ± 2	80 ± 2	14 ± 2

[a] Thin layer = silica gel G. Solvents = see Table I. Development distance = 15 cm. [b] Phthalylsulfacetamide produces two spots, apparently due to hydrolysis.

tions and are given with the mean deviation. The four official sodium salts of Compounds 1, 5, 6, and 10 are not separated from the free drug and can be identified as the corresponding sulfonamide. Since the R_f values of the salts are identical with those of the drugs, they are not listed separately. Examination of these R_f values shows that, by the use of appropriate reference standards, the identity of any individual sulfonamide, or the components of a mixture of sulfonamides, may be established with certainty.

Since the purpose of this method is both to separate and identify sulfonamides, the use of a reagent that gives a characteristic color reaction with sulfonamides is a valuable aid to identification in addition to R_f values. Visualization of the spots on the plate was studied with two specific reagents: (*a*) Bratton–Marshall, and (*b*) *p*-dimethylaminobenzaldehyde. Since the Bratton–Marshall reagent is useful only for N-4 unsubstituted sulfonamides and is the least sensitive of the two reagents, the *p*-dimethylaminobenzaldehyde reagent is preferred. This reagent produces bright-yellow spots on spraying with most of the compounds, but heating at 100° is necessary with some of the compounds before the spots are visible. Thin layers incorporating UV fluorescent indicators were not used, because they lack specificity and are not as sensitive as either of the spray reagents.

REFERENCES

(1) R. J. Mesley and E. E. Houghton, *J. Pharm. Pharmacol.*, **19,** 295(1967).

(2) U. Schiedt, *Appl. Spectros.*, **7,** 75(1953).

(3) J. G. Kirchner, in "Thin-Layer Chromatography, Techniques of Organic Chemistry," vol. XII, A. Weissberger, Ed., Interscience, New York, N. Y., 1967, pp. 518–523.

(4) "Thin-Layer Chromatography, A Laboratory Handbook," 2nd ed., E. Stahl, Ed., Springer-Verlag, New York, N. Y., 1969, pp. 541–547.

(5) W. Kamp, *Pharm. Weekbl.*, **101,** 181(1966); through *Anal. Abstr.*, **14,** 4255(1967).

(6) J. Zarnack and S. Pfeifer, *Pharmazie*, **19,** 216(1964); through *Chromatogr. Data*, **18,** D20(1965).

(7) E. A. Moreira, *Trib. Farm.*, **31,** 53(1964); through *Chromatogr. Data*, **18,** D20(1965).

(8) J. L. Kiger and J. G. Kiger, *Ann. Pharm. Franc.*, **24,** 599 (1966).

(9) Z. Margasinski, R. Danielak, and H. Rafalawska, *Acta Polon. Pharm.*, **22,** 423(1965); through *Chem. Abstr.*, **64,** 9509c (1966).

(10) C. H. Pao, *Yao Hsueh Hsueh Pao*, **13,** 67(1966); through *Anal. Abstr.*, **14,** 3540(1967).

(11) K. C. Guven and O. Pekin, *Eczacilik Bul.*, **8,** 146(1966); through *Chem. Abstr.*, **65,** 19928b(1966).

(12) M. T. Von der Venne and J. B. T'Siobbel, *Chromatogr. Symp., 2nd Brussels*, 196(1962).

(13) N. Karpetschka, *Microchim. Acta*, **1963,** 157.

(14) M. Gajdos, *Cesk. Farm.*, **14,** 71(1965); through *Chromatogr. Data*, **26,** D7(1967).

(15) L. R. Alexander and E. R. Stanley, *J. Ass. Offic. Agr. Chem.*, **48,** 278(1965).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 7, 1970, from the *Drug Standards Laboratory, 2215 Constitution Ave., N. W., Washington, DC 20037*

Accepted for publication October 6, 1970.

The Drug Standards Laboratory is jointly sponsored by the American Medical Association, the American Pharmaceutical Association, and the United States Pharmacopeial Convention, Inc.