Figure 3—*Average plasma levels of total radioactivity, calculated as unchanged drug, and unchanged metoclopramide (by HPLC) of two subjects who received 10 mg of ^{14}C-metoclopramide. Key: —, radioactivity; and - - -, unchanged drug.*

(VI) also was observed in dog and rat urine, but it had not been observed previously.

A number of other metabolites were isolated and identifed from the urine of dogs and rats. An O-demethylated metabolite was earlier identified in rat urine by Hucker et al. (5). Two metabolites, IV and IX, were identified in the present study. No evidence of the aromatic amine oxidation could be found in these studies, as was noted by Arita et al. (1) for rabbits.

Previous studies showed that metoclopramide is rapidly and well absorbed in lower animals. The results in these studies indicate that this is also true in humans. About 78% was excreted in the urine (Table I) in

the first 24 hr. Maximum blood levels following oral doses were found within the first 2 hr. The half-life of the drug for the 3–8-hr period following dosing was 4.00 hr (*SEM* 0.23) for the six subjects who received 10-mg doses (Fig. 2). The maximum concentration in the blood for these subjects was approximately 40 ng/ml; subjects that received 20 mg in the preliminary study gave twofold higher blood levels (Table III), indicating that the blood level is proportional to dose in this range. Further information concerning pharmacokinetics will appear in another report.

A comparison of the curves in Fig. 3 shows that a considerable amount of the radioactive material in the blood cannot be accounted for as unchanged metoclopramide. Because such a large portion of the drug was excreted as conjugates, samples were analyzed both before and following enzyme[5] hydrolysis of plasma samples from one subject. However, the results indicated that such conjugates were not present in appreciable concentrations. This difference must, therefore, be due to some unidentified metabolite.

REFERENCES

(1) T. Arita, R. Hori, K. Ito, K. Ichikawa, and T. Uesugi, *Chem. Pharm. Bull.*, **18**, 1663 (1970).

(2) O. M. Bakke and J. Segura, *J. Pharm. Pharmacol.*, **28**, 32 (1976).

(3) A. H. Beckett and G. Huizing, *ibid.*, **27**, 42P (1975).

(4) L. Sorrentino, M. DiRossa, and G. De Ruggiero, *Atti Acad. Sci.*, **123**, 52 (1969).

(5) H. B. Hucker, A. Hochberg, E. A. Hoffman, and J. K. Miller, *Pharmacologist*, **8**, 274 (1966).

High-Speed Liquid Chromatographic Analysis of Dapsone and Related Compounds

C. A. MANNAN, G. J. KROL [x], and B. T. KHO

Abstract □ A nonaqueous solvent absorptive support system and an aqueous solvent reversed-phase support liquid chromatographic system for analysis of dapsone and related compounds were investigated. The absorptive support system was more suitable for analysis of dapsone in raw materials, formulations, and tissue residues. The suitability was judged by the relative selectivity, efficiency, precision, and sensitivity of the systems. The adsorptive support system was used for the analysis of trace amounts of raw material impurities and dapsone metabolites. Coupling fluorometric detection to the chromatographic system yielded a 10-pg on-column detection limit for dapsone; the UV detection limit was 250 pg.

Keyphrases □ Dapsone and related compounds—high-speed liquid chromatographic analyses, bulk drug, pharmaceutical formulations, and biological fluids □ High-speed liquid chromatography—analyses, dapsone and related compounds, bulk drug, pharmaceutical formulations, and biological fluids □ Leprostatic antibacterials—dapsone and related compounds, high-speed liquid chromatographic analyses, bulk drug, pharmaceutical formulations, and biological fluids

Dapsone (4,4′-sulfonylbisbenzeneamine) (I), a potent antileprotic drug (1–3), has been commercially available since 1949. Various analytical techniques have been in-

vestigated for its analysis in pharmaceutical preparations and biological fluids.

Colorimetry and titrimetry, the first techniques to be used, were reviewed previously (4). Although these methods are sufficiently sensitive for the analysis of pharmaceutical preparations, they are not sufficiently selective for the determination of chemical stability or for metabolite studies. The current USP method for the analysis of dapsone in tablet formulations is a sodium nitrite titration (5). This method is not specific for dapsone, however, since other arylamino sulfones present as decomposition products and synthetic impurities (4) interfere.

Later, fluorometric methods were developed; they are more sensitive and specific and, thus, are especially suitable for biological fluid metabolite studies (4, 6). However, these methods are not very accurate because of quenching and other complications, and they also involve complicated extraction and cleanup steps.
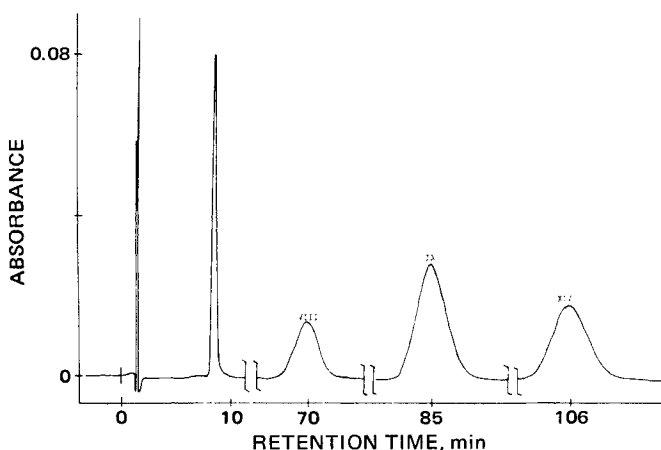
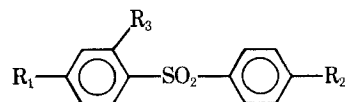These limitations led to the development of TLC (7),
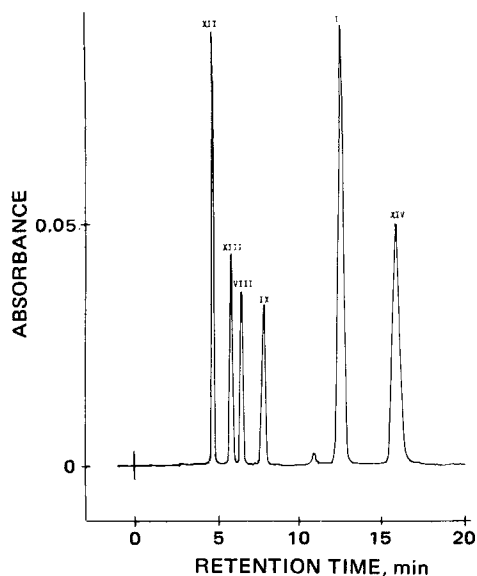
Figure 1—*System 1 chromatogram of dapsone and related compounds. The flow rate was 1.0 ml/min. Detection was in the UV at 295 nm, 0.1 absorbance unit full-scale (aufs).*

I: $R_1 = R_2 = NH_2$, $R_3 = H$
II: $R_1 = CH_3CONH$, $R_2 = NH_2$, $R_3 = H$
III: $R_1 = R_2 = CH_3CONH$, $R_3 = H$
IV: $R_1 = NH_2$, $R_2 = R_3 = H$
V: $R_1 = H$, $R_2 = R_3 = NH_2$
VI: $R_1 = NH_2$, $R_2 = Cl$, $R_3 = H$
VII: $R_1 = NH_2$, $R_2 = OH$, $R_3 = H$
VIII: $R_1 = NH_2$, $R_2 = NO_2$, $R_3 = H$
IX: $R_1 = CH_3CONH$, $R_2 = NO_2$, $R_3 = H$

X

XI: $R_1 = R_2 = NH_2$
XII: $R_1 = NH_2$, $R_2 = NO_2$
XIII: $R_1 = CH_3CONH$, $R_2 = NO_2$
XIV: $R_1 = R_2 = CH_3CONH$

paper chromatographic (8), and GLC (9, 10) methods for dapsone analysis. However, in view of its relative simplicity and accuracy, current emphasis is on the use of liquid chromatography for the analysis of dapsone and its metabolites. Initially, Gordon and Peters (11) applied microbore silica gel-packed column chromatography to the separation of dapsone from 4-aminophenyl 4-acetamidophenyl sulfone (II) and bis(4-acetamidophenyl) sulfone (III). This procedure was later applied to the quantitation of dapsone tablet preparations in the presence of small amounts of the possible raw material impurities: 4-aminophenyl phenyl sulfone (IV), 2-aminophenyl 4-aminophenyl sulfone (V), and 4-aminophenyl 4-chlorophenyl sulfone (VI) (12). Subsequently, this system was modified to incorporate fluorometric detection (13, 14), which decreased the detection limit of dapsone to about 100 pg.
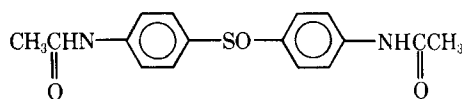
The objective of this work was to develop a fast, sensitive, and selective liquid chromatographic system suitable for the quantitation of dapsone in pure drugs, pharmaceutical formulations, and biological fluids. Comparison of the relative selectivity and efficiency of a reversed-phase
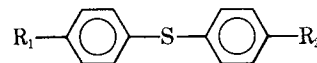
system and a microparticle adsorptive system led to selection of the adsorptive support and a low viscosity nonaqueous solvent system. Both UV and fluorometric detection methods were investigated.

EXPERIMENTAL

Reagents and Chemicals—All reagents were spectrograde distilled-in-glass solvents[1]. USP dapsone reference standard was used as received[2]. Compounds II, III, and VII–XIV were prepared in this laboratory[3]. Compounds IV–VI were used as received[4].

Chromatographic Systems—*System 1*—The mobile phase, consisting of isopropyl alcohol–ethyl acetate–acetonitrile–*n*-pentane (1:1:1:7 v/v), was pumped through a 30-cm × 4-mm i.d. stainless steel column packed with 10-μm silica microparticles[5]. The pump was a constant-flow rate screw-driven syringe[6].

System 2—The mobile phase was water–acetonitrile (84:16 v/v). A 15-cm × 4-mm i.d. stainless steel column was packed with a 5-μm non-
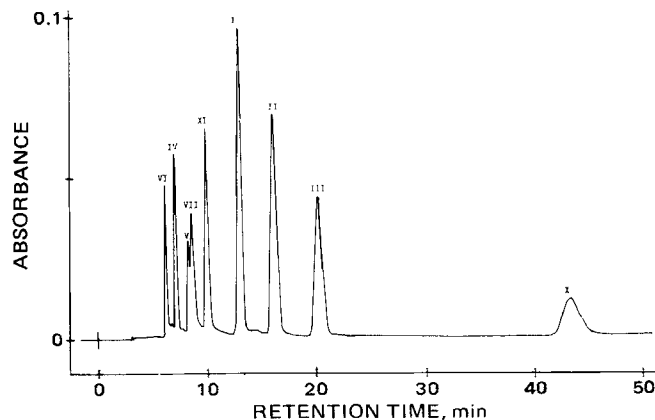
Figure 3—*System 1 chromatogram of dapsone and related compounds. The flow rate was 1.0 ml/min. Detection was in the UV at 295 nm.*

Figure 2—*System 2 chromatogram of dapsone and related compounds. The flow rate was 1.2 ml/min. Detection was in the UV at 295 nm.*

[1] Burdick and Jackson Laboratories, Muskegon, Mich.
[2] Lot 0371-F-3.
[3] By C. E. Orzech and F. Gemmill, Jr.
[4] Provided by G. R. Gordon, Stanford Research Institute, Menlo Park, Calif.
[5] μPorasil, Waters Associates, Milford, Mass.
[6] Model 314 metering pump, Instrumentation Specialties Co., Lincoln, Neb.

Table I—Comparison of Chromatographic Parameters[a] for Systems 1 and 2

Compound	System 1			System 2		
	RT^b	α^c	R^d	RT	α^c	R^d
I	1.00	—	—	1.00	—	—
II	1.21	1.2	2.2	1.43	1.5	4.5
III	1.54	1.7	5.7	2.07	2.3	7.7
IV	0.52	2.7	8.6	>20	—	—
V	0.62	2.0	2.2	>20	—	—
VI	0.46	3.1	10.4	>20	—	—
VIII	0.51	2.7	12.4	8.40	9.4	11.5
IX	0.62	2.0	9.0	10.45	11.7	14.2
XII	0.36	5.1	15.6	>20	—	—
XIII	0.45	3.2	12.9	>20	—	—
XIV	1.27	1.4	4.5	12.80	14.5	16.0

[a] All parameters are relative to dapsone. [b] Relative retention time. [c] Selectivity parameter. [d] Resolution parameter.

spherical octadecyltrichlorosilane bonded support[7]. A dual-piston reciprocating constant-flow rate pump[8] was used.

Sample Preparation—Compounds to be chromatographed were first prepared as 1-mg/ml stock solutions in chloroform or methanol. The solutions were diluted to the appropriate concentration with chromatographic solvent before chromatography. Samples were injected with a high-pressure six-port rotary valve with a 10-µl external loop[9].

Detection—The column eluate was monitored in the UV at 254[10] or 295[11] nm and by fluorometry[12] with excitation at 285 nm and emission above 340 nm.

Recording and Integration—Chromatographic peaks were recorded on a 25.4-cm strip-chart recorder[13]. Quantitation was based on either mechanical disk[14] or electronic integration[15].

Temperature Protection—To reduce detector noise due to ambient temperature fluctuations at the high detector sensitivity setting, the column, flowcell housing, monochromator, and light source for System 1 were kept in a constant-temperature box[16] at 25°.
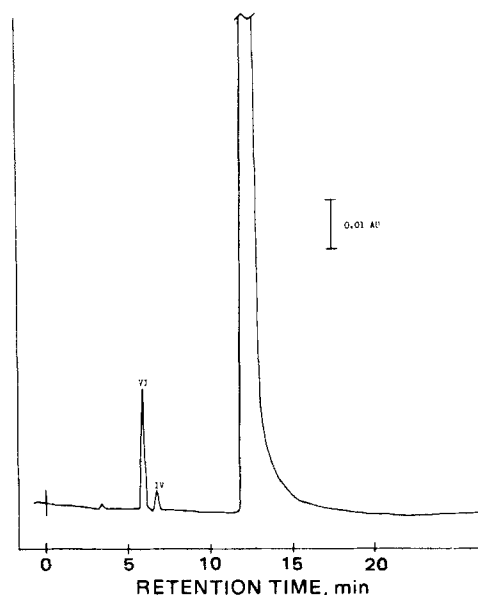
Figure 4—*System 1 chromatogram of USP dapsone reference standard. The flow rate was 1.0 ml/min. Detection was in the UV at 295 nm.*

[7] Merck Lichrosorb RP 18, EM Laboratories, Elmsford, N.Y.
[8] Spectra-Physics model 740, Spectra-Physics Inc., Santa Clara, Calif.
[9] Model CV-UHPA-C-20, Valco Instruments Co., Houston, Tex.
[10] Chromatronix model 220 UV absorbance monitor, Spectra-Physics Inc., Santa Clara, Calif.
[11] Model SF-770 variable spectroflow monitor, Schoeffel Instruments Corp., Westwood, N.J.
[12] Model FS-970, LC fluorescence detector, Schoeffel Instruments Corp., Westwood, N.J.
[13] Model 282 (10 in.), Linear Instruments Corp., Irvine, Calif.
[14] Series 200 Disc integrator, Disc Instruments, Santa Ana, Calif.
[15] Autolab System I computing integrator, Spectra-Physics Inc., Santa Clara, Calif.
[16] A 71 × 51 × 30-cm (28 × 20 × 15-in.) stainless steel cork-lined box, held at constant temperature with a Yellow Springs Instruments Co. model 74 temperature controller powering Watlow model 7424 250-w heating strips.

Precision and Accuracy for Tablet Formulations—The tablets[17] (25 and 100 mg) were assayed over 1 month. The precision was established as follows. Twenty tablets were ground to powder. The equivalent of one tablet was weighed from the bulk, and dapsone was extracted with 200 ml of methanol in an ultrasonic bath. The mixture was centrifuged, and an aliquot was diluted to approximately 25 µg/ml for analysis.

The instrument was standardized at a 1.2-ml/min flow rate with two to four separate injections of a USP reference standard solution of known concentration. The sample solution was injected a minimum of two times. The average area of the sample peaks was then compared to the average area of the standard peaks[18].

Assay accuracy was determined by spiking blank tablet material with known amounts of dapsone. Subsequently, the spiked sample was extracted, chromatographed, and quantitated as described.

Tablet Uniformity Analysis—Dapsone tablets (10 single 25 mg) were extracted by adding 0.5 ml of water to each tablet in a 200-ml volumetric flask. The flask was placed in an ultrasonic bath briefly to disintegrate the tablet. Methanol was then added to volume, and the flask was again placed in the ultrasonic bath for about 10 min. After centrifugation and dilution, an aliquot of the sample was chromatographed at a flow rate of 3.0 ml/min for fast sample turnover.

RESULTS AND DISCUSSION

Selection of Chromatographic System—Separation of dapsone from possible synthetic impurities and related compounds was studied using an adsorptive system and a reversed-phase system. The adsorptive system consisted of a microparticulate silica support and a nonaqueous solvent mobile phase. The reversed-phase system consisted of octadecyltrichlorosilane bonded to a microparticulate silica support and an aqueous solvent mobile phase.

The complete separation of dapsone from VIII, IX, and XII–XIV using
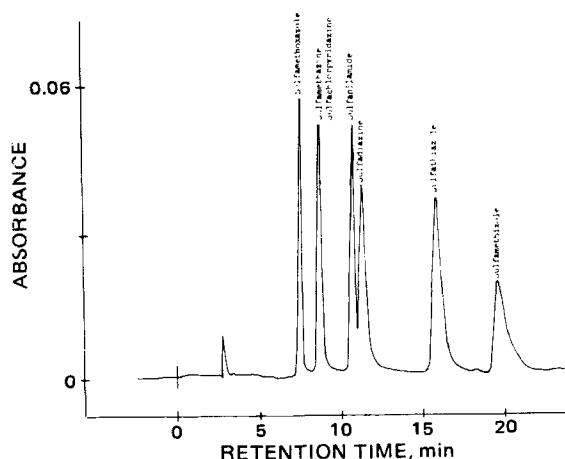
Figure 5—*System 1 chromatogram of sulfa drugs. The flow rate was 1.0 ml/min. Detection was in the UV at 254 nm.*

[17] Avlosulfon tablets, Ayerst Laboratories.
[18] These data were obtained with a Chromatronix model 3100 liquid chromatograph monitored at 254 nm and disk integration.

Table II—Chromatographic Efficiency *versus* Flow Rate Dependence Observed with Related Compounds

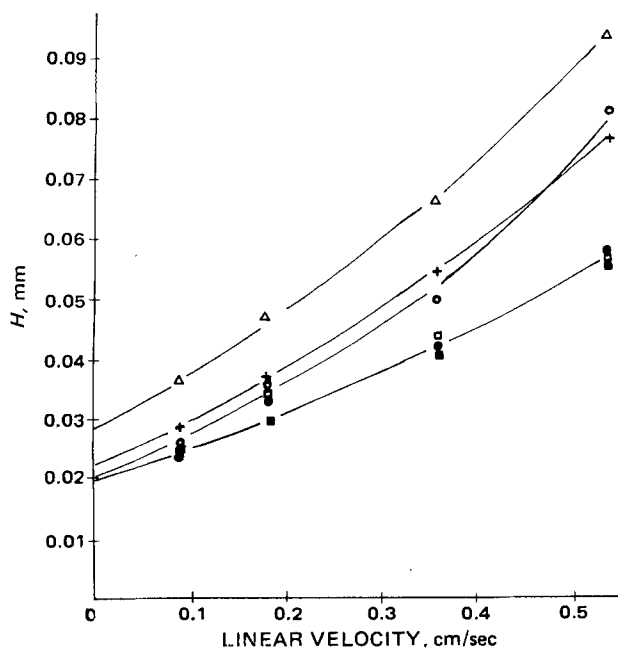| Compound	Plate Count at Decreasing Linear Velocity[a]			
	0.54	0.36	0.18	0.09
XII	5250	7130	9640	12,310
XIII	5300	6920	8860	11,640
VIII	5370	7300	9860	12,710
IX	3890	5460	8220	10,720
Dapsone	4710	5960	8660	11,730
XIV	3220	4550	6320	8,350

[a] Linear velocity in centimeters per second; 1 cm/sec = 5.56 ml/min.

the adsorptive system is shown in Fig. 1. Chromatography of some of these compounds with the reversed-phase system is shown in Fig. 2; XII and XIII were not eluted from the column. Table I lists the retention time, resolution, and selectivity of the adsorptive and reversed-phase systems. The differences in retention time are reflected by the selectivity values (α) relative to dapsone.

Compound XIV had a longer retention time than dapsone with the adsorptive system and would be expected to precede dapsone using the reversed-phase system. Instead, it was retained by the reversed-phase column considerably longer than dapsone. A similar observation applied to II and III.

The apparent lack of correlation between the observed order of elution on the adsorptive and reversed-phase supports may be attributed to the fact that the two systems use different solvents as well as supports. Since the aqueous solvent used with the reversed-phase support can influence the relative polarity of the solute by induction of dipoles and ionization, the relative polarity of a molecule in a nonaqueous solvent may be different from its relative polarity in an aqueous solvent. The effect of pH on the relative retention times of ionizable molecules chromatographed on a reversed-phase support was reported previously (15).

Although the elution pattern obtained with the reversed-phase system showed greater selectivity than that obtained with the adsorptive support system, the adsorptive support system was selected for routine analysis to minimize the time necessary to separate and quantitate dapsone and several related compounds. The reversed-phase system exhibited excessive elution times for possible impurities and metabolites of dapsone. Furthermore, since possible impurities in dapsone raw material (IV–VI) are not eluted on the reversed-phase support unless dapsone elutes at the solvent front or a gradient solvent system is utilized, the reversed-phase system is not suitable for simultaneous analysis of dapsone and its impurities. In contrast, the nonaqueous solvent–adsorptive support system yields adequate separation and short retention times for dapsone
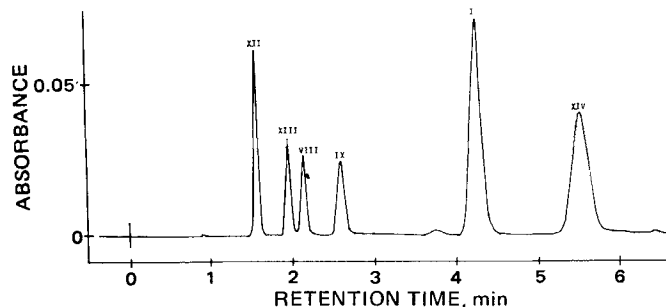
Figure 7—*System 1 chromatogram of dapsone and possible raw material impurities. The flow rate was 3.0 ml/min. Detection was in the UV at 295 nm, 0.1 aufs.*
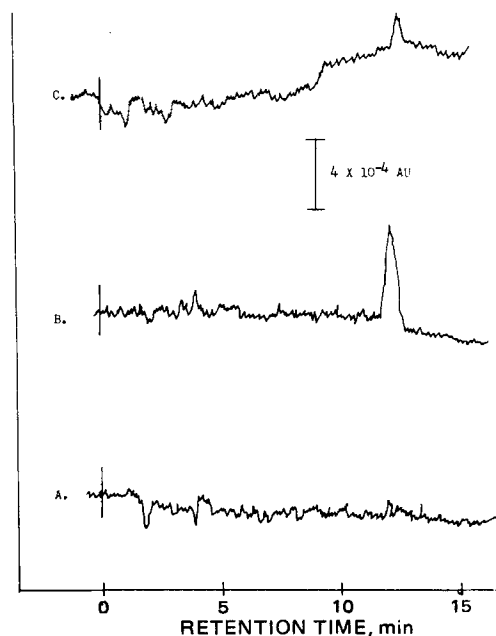
impurities and, hence, sharp impurity peaks (Figs. 1 and 3) that can be quantitated accurately even at trace amount levels. Figure 4 illustrates a chromatogram obtained with USP dapsone reference standard. The observed impurities, IV and VI, were present at 0.4 and 0.08%, respectively.

The selectivity and efficiency of the adsorptive support system are also reflected by the observed resolution of dapsone from a known dapsone metabolite (II), a dapsone prodrug derivative (III), three previously identified impurities (IV–VI), and three structurally related compounds (VII, X, and XI). Figure 3 illustrates the separation obtained. Excluding X, all compounds were resolved within 22 min.

The adsorptive support system also was applied to the separation of several sulfa drugs. Although this system was not optimized for the separation of sulfa compounds, only sulfamethazine and sulfachlorpyridazine were unresolved (Fig. 5). However, the adsorptive support system does not appear to be as suitable for the separation of sulfa compounds as it is for the separation of dapsone and its analogs.

Efficiency of Nonaqueous Solvent–Adsorptive Support System—The selection of pentane as the major component of the nonaqueous solvent was based primarily on its low viscosity. The relationship between solvent viscosity and chromatographic efficiency was investigated previously (16).

As expected, an increased flow rate decreased column efficiency (Table II and Fig. 6). With few exceptions, essentially linear relationships between the flow rate and the width of the theoretical plate were observed. However, the slope of the plate width *versus* the flow rate line was dependent on the structure of the molecule. Thus, the chromatographic efficiency of the more polar molecule (*e.g.*, XIV, Fig. 6) decreased more

Figure 6—*VanDeemter plot based on System 1 chromatograms. Key: O, I; ●, XII; □, XIII; ■, VIII; +, IX; and △, XIV; H = HETP (height equivalent to a theoretical plate).*

Figure 8—*System 1 chromatograms at high UV and fluorescence detector sensitivity settings. Key: A, 50 pg of dapsone, UV detector, 295 nm; B, 50 pg of dapsone, fluorescence detector; and C, 250 pg of dapsone, UV detector, 295 nm.*

Table III—Accuracy and Precision Data for Dapsone Tablets

|		25-mg Tablets		100-mg Tablets	
Run	Date	Precision[a], mg/Tablet	Accuracy[b], %	Precision[a], mg/Tablet	Accuracy[b], %
1	Feb. 10, 1976	24.6	101.9	100.6	100.8
2	Feb. 11, 1976	24.3	98.3	99.2	100.9
3	Feb. 12, 1976	24.5	99.3	98.3	97.1
4	Feb. 13, 1976	24.5	99.0	97.5	99.7
5	Feb. 18, 1976	24.7	100.6	99.8	101.3
6	Feb. 19, 1976	23.0	100.9	97.6	98.5
7	Feb. 20, 1976	—	—	100.2	103.6
8	Feb. 20, 1976	—	—	99.3	97.5
9	Feb. 25, 1976	24.8	98.1	100.6	100.2
10	Mar. 1, 1976	24.1	97.2	99.8	100.8
11	Mar. 2, 1976	24.4	97.6	96.2	99.2
12	Mar. 3, 1976	23.8	101.7	102.4	99.4
$\overline{X}$		24.3	99.5	99.3	99.9
S		0.5	1.7	1.7	1.8
CV, %		2.2	1.7	1.7	1.8

[a] Twenty tablets were used for each run. [b] A ground placebo was spiked with a known amount of sample corresponding to claim. Number presented in table represents percent recovered.

with the flow rate than the chromatographic efficiency of a less polar molecule (e.g., XII, Fig. 6). This trend may be attributed to the fact that the relative contribution of the mass transfer term to the overall column efficiency increased with the flow rate. Since the mass transfer process involves mobile and stationary phase interactions, the structure of the molecules chromatographed will influence the kinetics of mass transfer.

The relatively high efficiency of the nonaqueous chromatographic solvent, even at high flow rates, makes possible an accurate and specific analysis within a relatively short time. This possibility is an important consideration in uniformity and dissolution assays of tablets where the analysis of numerous samples is involved. Thus, dapsone was completely resolved from five related compounds even at a 3-ml/min flow rate (Fig. 7).

Detection—Although the quantitation of dapsone and related compounds with the aid of a variable wavelength UV detector is applicable to the analysis of pure drug and pharmaceutical formulations, fluorometric detection is more sensitive and, thus, more suitable for the analysis of tissue residue samples. This study utilized a novel spectrofluorometric liquid chromatographic detector. The unique design of the spectrofluorometric detector flowcell and optics made possible the detection of subnanogram amounts of dapsone and related compounds. Figure 8b illustrates a chromatogram of dapsone close to the fluorometric detection limit, approximately 10 pg. In contrast, 50 pg cannot be detected by UV (Fig. 8a); the detection limit of the UV detector is about 250 pg (Fig. 8c).

Figure 9 illustrates a chromatogram of dapsone and related compounds,
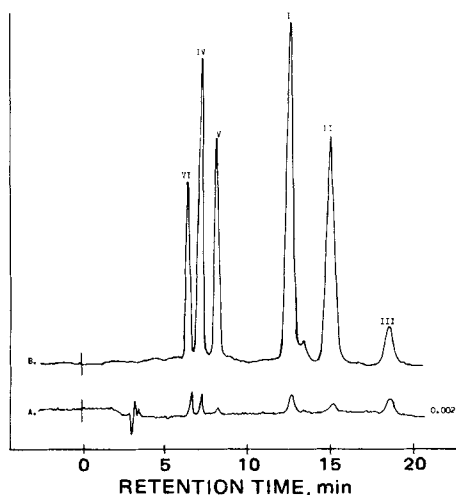
obtained with UV and fluorometric detectors connected in series to monitor the same chromatographic elution. The patterns indicate that the UV and fluorometric detection limits vary with the compound. However, except for dapsone, no attempt was made to select the optimum cutoff filters for each compound. Therefore, the fluorometric detection limits of compounds other than dapsone are uncertain.

Precision and Accuracy—The accuracy and precision of the adsorptive support system are presented in Table III. Since the tablet formulations were analyzed on different days, the observed results include the day-to-day contribution to assay variability. Since individual tablet content uniformity assay involves a relatively high volume of samples, the procedure for the determination of tablet potency was modified. The flow rate was increased to yield less than a 6-min retention for dapsone with marginal sacrifice of assay accuracy, selectivity, and precision.

REFERENCES

(1) C. C. Shepard, Ann. Rev. Pharmacol., 9, 37 (1969).
(2) R. L. Degowin, R. B. Eppes, P. E. Carson, and R. D. Powell, Bull. WHO, 34, 671 (1966).
(3) D. Willerson, Jr., K. H. Rickmann, L. Kass, P. E. Carson, H. Frischer, and J. E. Bowman, Am. J. Trop. Med. Hyg., 21, 138 (1972).
(4) C. E. Orzech, N. G. Nash, and R. D. Daley, in "Analytical Profiles of Drug Substances," vol. 5, K. Florey, Ed., Academic, New York, N.Y., 1976, pp. 88–114.
(5) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 118, 119.
(6) A. J. Glazko, W. A. Dill, J. Baukema, and P. E. Thompson, Int. J. Lepr., 36, 511 (1968).
(7) L. M. S. Armasecu and M. Manda, Farmacia (Bucharest), 22, 165 (1974).
(8) S. Tsutsumi, Chem. Pharm. Bull., 9, 432 (1961).
(9) H. P. Burchfield, E. E. Storrs, R. J. Wheeler, V. K. Bhat, and L. L. Green, Anal. Chem., 45, 916 (1973).
(10) E. Sawicki, H. Johnson, and K. Kosinski, Microchem. J., 10, 72 (1966).
(11) G. R. Gordon and J. H. Peters, J. Chromatogr., 47, 269 (1970).
(12) G. R. Gordon, D. C. Ghaul, and J. H. Peters, J. Pharm. Sci., 64, 1205 (1975).
(13) J. F. Murray, Jr., G. R. Gordon, and J. H. Peters, J. Lab. Clin. Med., 78, 464 (1971).
(14) J. F. Murray, Jr., G. R. Gordon, C. C. Gulledge, and J. H. Peters, J. Chromatogr., 107, 67 (1975).
(15) P. J. Twitchett and A. C. Moffat, ibid., 111, 149 (1975).
(16) G. J. Krol, C. A. Mannan, F. Q. Gemmill, Jr., G. E. Hicks, and B. T. Kho, ibid., 74, 43 (1972).

Figure 9—System 1 chromatograms of dapsone and related compounds monitored by UV (at 295 nm) (A) and fluorescence (B) detectors. Amounts of samples injected were: IV and VI, 0.08 ng; V, 0.05 ng; I and II, 2.5 ng; and III, 4.0 ng. The UV detector setting was 0.02 aufs.

NOTES

Preparation and Skin-Photosensitizing Activity of Substituted Psoralens

MOHAMMED A. LOUTFY ˣ and HAMED A. ABU-SHADY *

Abstract ☐ 8-Monosubstituted and 5,8-disubstituted psoralen derivatives were prepared, and their skin-photosensitizing activity was evaluated. The results were correlated in terms of molecular configuration, and 8-allyloxypsoralen can be considered as a new agent of potent photodynamic activity.

Keyphrases ☐ Psoralens, substituted—synthesized, evaluated for skin-photosensitizing activity ☐ Photosensitizing activity, skin—various substituted psoralens evaluated ☐ Structure–activity relationships—various substituted psoralens evaluated for skin-photosensitizing activity

In a continuation of previous studies (1–5), this report describes the preparation and skin-photosensitizing activity of substituted psoralens. The synthesis and photosensitizing activity of many psoralen (I) derivatives have been investigated extensively (6, 7), but little has been published concerning the 5,8-disubstituted psoralens. For example, while 5-methylpsoralen and 8-methylpsoralen are potent skin photosensitizers (8), nothing has been reported about 5,8-dimethylpsoralen. Furthermore, it was observed (9) that allyloxycoumarins have weak, but definite, photodynamic activity. Nevertheless, the effect on the photodynamic activity of introducing allyl, allyloxy, or dimethylallyl substituents in the psoralen molecule has not been studied.

On the other hand, the structure–activity relationships in the furocoumarin series demonstrated clearly the following. 2-Thiopsoralen derivatives and oxazolocoumarins do not exhibit any photodynamic activity (6). There is great variation in the potency of the skin-photosensitizing activity of the derivatives, determined by the number and the position of methyl groups (8). Methyl substitution at the 5'-carbon of psoralen does not alter the activity, but methyl substitution at the 4'-position results in a considerable loss of activity (9). Substitution with a methoxy group at the 5- or 8-carbon results in decreased activity, while nitro or amino group substitution renders the compound completely inactive (9). The fact that 2,6,8-tri-methylbenzodifuran (10) and some other coumarin derivatives (9) do elicit photosensitized responses suggests that the furocoumarin structure is not essential for photosensitizing activity.

Further investigation is needed to reveal definite points in the structure–activity relationship in the furocoumarin series.

DISCUSSION

Previously, the isolation of xanthotoxin (II) and imperatorin (III) from the fruits of the umbellifer *Ammi majus* (L.)[1] was reported (1). These compounds were readily transformed into xanthotoxol (IV) according to a reported (4) procedure. The identity of xanthotoxin, imperatorin, and xanthotoxol was established through mixed melting-point, UV, IR, and NMR spectral comparisons with authentic samples[1]. For the preparation of derivatives V–VII, the procedure followed was essentially the same as that described previously (3).

It was reported (11) that imperatorin underwent Claisen rearrangement to afford alloimperatorin (IX) by heating in a vacuum. In the present work, to induce the allyl ethers to undergo Claisen rearrangement, the ethers were heated under reflux for 6 hr in ethylene glycol. In this way, 5-allyl-8-hydroxypsoralen (VIII) and IX were obtained in low yields (65%). Better results or even quantitative yields were obtained by refluxing the allyl ethers in *N,N*-diethylaniline for 1 hr. In the latter method, Claisen rearrangement products were of higher purity; 1 hr of refluxing was sufficient.

Methylation, acetylation, and allylation reactions were carried out according to the general method of etherification and esterification (3). However, allylation of VIII ended in failure. The identity of the new psoralen derivatives was ascertained by spectral (IR and NMR) analysis.

Compounds V–XIV (Table I) were prepared and screened as dermal photosensitizing agents. Two tests were performed with albino guinea pig skin. The preliminary test, employing very strong conditions (high amount of substance and high amount of radiation), was used for selecting the active compounds, even if weakly active, from the inactive ones. In a second test, only the active compounds were tested in very mild conditions and their photosensitizing activities were compared with that of psoralen.

The relative activity was evaluated by comparing the erythema re-

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[1] Provided by Memphis Chemical Co., Cairo, Egypt.