Journal of Chromatography, 441 (1988) 454–457
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 373

Note

Thin-layer chromatographic separation of some sulpha drugs using acetoacetanilide as a coupling agent

RAJEEV JAIN* and ASHA BHATIA
School of Studies in Chemistry, Jiwaji University, Gwalior-474011 (India)
(Received January 26th, 1988)

Sulpha drugs have been separated by various chromatographic procedures, *e.g.* paper chromatography[1–5] and thin-layer chromatography (TLC)[6–19]. Poethke and Kize[20] employed two-dimensional TLC on alumina plates but all the sulpha drugs investigated could not be separated. Wehrli[21] reported the separation of sulpha drugs using N-(1-naphthyl)ethylenediammonium dichloride as a coupling agent. Sulpha drugs in amounts up to 4 μg have been quantitatively estimated by Wagner and Wandel[22] using chloroform–*n*-butanol–light petroleum (b.p. 60–80°C) (3:30:30). Twenty sulphonamides were separated on thin layers of ion exchangers in eighteen solvents[23]. Various new chromogenic reagents for the detection of sulphonamides on TLC plates have been reported[24]. Srivastava *et al.*[25] separated ten closely related sulpha drugs on silica gel impregnated with cadmium acetate.

The present paper describes the resolution and identification of some sulpha drugs in the form of their acetoacetanilide derivatives. Thus a mixture of sulpha drugs was directly converted into the coupled products on thin-layer plates and subsequently chromatographed. In a mixture of twelve sulpha drugs it was possible to characterize 0.1–0.2 μg of each drug in the form of its coupled product.

EXPERIMENTAL

All the solvents used were freshly dried and distilled. Glass plates (20 cm × 20 cm) were used. An ascending irrigation technique was employed.

Preparation of coupled products

A sulpha drug (0.01 mol) was dissolved in a mixture of concentrated hydrochloric acid (4 ml) and water (5 ml) and cooled to 0°C in an ice-bath. A cold aqueous solution of sodium nitrite (0.01 mol, 0.69 g) was added. The diazonium salt so obtained was filtered into a cooled mixture of sodium acetate (10.0 g) and acetoacetanilide (0.01 mol) in ethanol (25 ml). The product so precipitated was filtered off, thoroughly washed with water and finally recrystallized from ethanol as yellow crystals.

The melting points of the coupled products are as follows: sulphanilamide, 210°C; sulphamethoxazole, 190°C; sulphamethizole, 212°C; metacalfin, 145°C; sulphacetamide, 230°C; sulphathiazole, 170°C; sulphaguanidine, 240°C; sulphadiazine,

190°C; phthalylsulphathiazole, 110°C; sulphaphenazole, 177°C; sulphasomidine, 230°C; sulphadimidine, 190°C.

Adsorbents

The following adsorbents were employed: A, silica gel G (E. Merck, throughout); B, silica gel G with 20% calcium carbonate; C, silica gel G with 1% tetrabutylammonium bromide; D silica gel G with 1% Triton X-100. E, silica gel G with 1% sodium lauryl sulphate; F, silica gel G with 1% cupric acetate.

Preparation, spotting and irrigation of plates

The TLC plates (thickness 0.5 mm) were prepared by means of a Stahl applicator. A slurry of the above adsorbents, in distilled water was applied. The plates were dried for 24 h at 60 ± 1°C. The solutions of the diazotized sulpha drugs (each containing 1–2 μg) in acetone were spotted with a fine glass capillary, and at the same positions a solution of acetoacetonilide (equivalent to 1.1 mol of sulpha drug) in acetone was applied. Authentic coupled products of the sulpha drugs were also spotted alongside. The plates were left for 1 h at room temperature, after which they were irrigated with suitable solvents. It took about 30 min for the solvent system to migrate 16 cm. Yellow spots could be observed without any visualizing agent. However, for dilute solutions below 0.1 μg, the spots could be visualized by exposure to iodine vapour. Table I gives the R_F values of the various coupled products.

TABLE I

$R_F \cdot 100$ VALUES OF SOME SULPHA DRUGS AS THEIR COUPLED PRODUCTS WITH ACETOACETANILIDE

Solvent systems: 1 = 100 ml dichloromethane; 2 = 45 ml dichloromethane + 50 ml carbon tetrachloride + 5 ml methanol; 3 = 50 ml dichloromethane + 50 ml chloroform; 4 = 90 ml benzene + 10 ml ethyl acetate.

No. Sulpha drug	*Silica gel G*		*Silica gel G + 1% tetrabutyl ammonium bromide*			*Silica gel G + 20% $CaCO_3$*		*Silica gel G + 1% cupric acetate*	
	1	*2*	*2*	*3*	*4*	*2*	*4*	*1*	*2*
1 Sulphanilamide	47	75	56	80	22	16	97	42	28
2 Sulphamethoxazole	53	95	79	73	26	1	17	7	18
3 Sulphamethizole	74	68	68	88	8	3	1.7	0	0
4 Metacalfin	19	88	96	00	14	45	82	32	42
5 Sulphacetamide	98	61	51	38	6	6	4	4	6
6 Sulphathiazole	47	90	47	40	11	8	10	9	1
7 Sulphaguanidine	15	27	21	6	90	21	2	4	2
8 Sulphadiazine	64	2	90	63	94	2	87	2	9
9 Phthalylsulphathiazole	0	89	86	33	91	4	86	53	46
10 Sulphaphenazole	2	18	82	39	21	48	14	0	4
11 Sulphasomidine	4	40	60	7	76	80	3	2	11
12 Sulphadimidine	10	84	90	78	36	96	50	65	14

RESULTS AND DISCUSSION

When the sulpha drugs were spotted as mixtures or individually and were then converted on the plates into their coupled products with acetoacetanilide, on subsequent irrigation they were distinctly resolved. Thus, a mixture of twelve sulpha drugs in the form of their coupled products was readily resolved (Table I), and their R_F values correspond to those of the authentic coupled products.

It was found that silica gel impregnated with 1% tetrabutylammonium bromide was the best adsorbent for these compounds. On the other hand, on silica gel impregated with Triton X-100, sodium lauryl sulphate and cupric acetate, good separation could not be achieved. With these adsorbents, either there was no clear separation or profuse tailing occurred with the majority of the drugs.

From Table I, it is seen that the best solvent system was 45 ml dichloromethane + 50 ml carbon tetrachloride + 5 ml methanol, in which all but one of twelve coupled products of sulpha drugs could be separated. The coupled products could be observed directly as yellow spots and there was no necessity for a spray reagent as in the case of the sulpha drugs alone.

Mixtures of polar solvents when employed for irrigation of the plates resulted in either long tailing or highly diffuse spots. Non-polar solvents and their mixtures produced excellent resolution of the compounds.

Thin coatings of the adsorbents produced good resolution. Silica gel G and silica gel impregnated with 1% tetrabutylammonium bromide gave good results. In general, the mobility of compounds on silica gel G (impregnated with 20% calcium carbonate) in 100 ml dichloromethane, 50 ml dichloromethane + 50 ml chloroform, 40 ml dichloromethane + 60 ml carbon tetrachloride and 90 ml benzene + 10 ml ethyl acetate solvent systems was reduced when compared to that on silica gel G.

ACKNOWLEDGEMENT

We thank Professor R. P. Bhatnagar, Head, Chemistry Department, Jiwaji University, Gwalior for providing the facilities to carry out this work.

REFERENCES

1 G. Thomas, J. Ransy, P. Roland and A. Vander Bulcke, *J. Pharm. Belg.*, 15 (1950) 263.
2 R. Robinson, *Nature (London)*, 168 (1951) 512.
3 A. E. Steel, *Nature (London)*, 168 (1951) 877.
4 G. L. San and A. J. Ulteetr, *Nature (London)*, 169 (1952) 586.
5 H. G. Bray, H. J. Lake and W. V. Thorpe, *J. Biochem (Tokyo)*, 48 (1951) 400.
6 E. Adam and C. L. Lapiero, *J. Pharm. Belg.*, 19 (1964) 79.
7 K. Adank and U. W. Hammer Schmidt, *Chimia*, 18 (1964) 361.
8 A. R. Alha and U. R. Lindfers, *Ann. Med. Exp. Penn.*, 37 (1959) 149.
9 W. Awe and U. H. G. Tracht, *Pharm. Ztg. (Frankfurt)*, 108 (1963) 1365.
10 Br. Bachler, *Helv. Chim. Acta*, 45 (1962) 309.
11 Br. Nachler, *Pharm. Acta Helv.*, 29 (1964) 457.
12 Br. Bachler, *Chimia*, 17 (1963) 257.
13 I. Bayer, *J. Chromatogr.*, 16 (1964) 237.
14 K. H. Byer, *Angew. Chem.*, 75 (1963) 1136.
15 S. Klein and B. T. Kho, *J. Pharm. Sci.*, 51 (1962) 967.
16 J. Zarnack and U. S. Pfeifer, *Pharmazie*, 19 (1964) 216.

17 U. R. Cieri, *J. Chromatogr.*, 45 (1969) 421.
18 U. R. Cieri, *J. Chromatogr.*, 49 (1970) 493–502.
19 L. Lepri, P. Cs. Desideri and O. Heimler, *J. Chromatogr.*, 169 (1979) 271–278.
20 W. Poethke and U. W. Kize, *Pharm. Zentralhalle*, 103 (1964) 95.
21 A. Wehrli, *Can. Pharm. J. Sci. Sect.*, 97 (1964) 208.
22 G. Wagner and U. J. Wandel, *Pharmazie*, 21 (1966) 105.
23 L. Lepri, P. G. Ocsideri, M. Landimi and G. Tanturli, *J. Chromatogr.*, 109 (1975) 365.
24 R. R. Krishna, P. Siraj amd C. S. P. Sastry, *Acta Cienc. Indica Chem.*, 7 (1981) 180–183.
25 S. P. Srivastava, V. K. Dua, R. N. Mehfotra and R. C. Saxene, *J. Chromatogr.*, 176 (1979) 145–147