*Journal of Chromatography*, 362 (1986) 303–307
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 692

## Note

# Visualization of sulphonamide drugs on thin-layer plates using π-acceptors as spray reagents

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(First received February 3rd, 1986; revised manuscript received April 2nd, 1986)

Antibacterial sulphonamides, alone or in combination with trimethoprim, are still widely used in the treatment of infection[1]. Methods for the visualization of these drugs on thin-layer plates have been reviewed[2,3]. The use of fluorescamine, either as a spray reagent[4] or by dipping the developed plate quickly in a solution of reagent in acetone, has found application both in qualitative detection and in quantitative determination of these drugs when present in low concentration, *e.g.* in animal tissues[5–9]. Fluorescamine derivatization is slow (15–30 min)[7,10] and the fluorescent product has limited stability (2–3 h)[5]. Touchstone *et al.*[10] recommend the use of dimethyl sulphoxide (DMSO) or dimethylformamide (DMF) as solvent for fluorescamine in order to improve the stability of fluorescence.

The use of π-acceptors as spray reagents for the detection of alkaloids, triterpenoids, steroids, penicillins, diuretics and hypoglycemic drugs on thin-layer plates has been reported[11–14]. The use of several π-acceptors in the visualization of eighteen sulphonamides and a related drug (trimethoprim) on thin-layer plates is reported here.

## EXPERIMENTAL

### *Materials*

Acetylsulphisoxazole, phthalylsulphathiazole, succinylsulphathiazole monohydrate, sulphacetamide, sulphadiazine, sulphadimethoxine, sulphadoxine, sulphaethidole, sulphaguanidine, sulphamerazine, sulphamethazine, sulphamethizole, sulphamethoxazole, sulphanilamide, sulphapyridine, sulphathiazole, sulphisomidine, sulphisoxazole, and trimethoprim were either reference standards or pure samples supplied by the manufacturer and used as such. *o*-Chloranil, *p*-chloranil, *p*-fluoranil and 2,5-dichloro-*p*-benzoquinone were from Pflätz and Baüer and were used as received. Solvents and other chemicals were of reagent grade.

### *Spray reagents*

The following spray reagents were freshly prepared: (I) 0.5% *o*-chloranil in dioxane; (II) 0.5% *p*-chloranil in dioxane; (III) 0.5% *p*-fluoranil in dioxane; (IV)–(VI) 0.5% 2,5-dichloro-*p*-benzoquinone in dioxane, DMSO or DMF, respectively.

0021-9673/86/$03.50 

TABLE I

REACTIONS OF SULPHONAMIDE DRUGS WITH SPRAY REAGENTS

Order of increasing response: ±, +, ++, +++.

| *Drug* | *I, o-Chloranil** | | *II, p-Chloranil*** | | *III, p-Fluoranil**** | | *V, 2,5-Dichloro-p-benzoquinone (in DMSO)*§ | |
|---|---|---|---|---|---|---|---|---|
| | *Colour (response)* | *Detection limit (μg)* | *Colour (response)* | *Detection limit (μg)* | *Colour (response)* | *Detection limit (μg)* | *Colour (response)* | *Detection limit (μg)* |
| Acetylsulphisoxazole | Purple (+) | 2.0 | Green (±) | 4.0 | Yellow (±) | 4.0 | Yellowish green (+) | 2.0 |
| Phthalylsulphathiazole | Purple (+) | 2.0 | Green (±) | 4.0 | – | | Pink (+) | 2.0 |
| Succinylsulphathiazole | Purple (+) | 2.0 | Green (±) | 4.0 | – | | Pink (+) | 2.0 |
| Sulphacetamide | Purple (++) | 1.5 | Green (+) | 2.0 | Yellow (+) | 2.0 | Yellowish green (+) | 2.0 |
| Sulphadiazine | Purple (+) | 2.0 | Green (++) | 1.5 | Yellow (+) | 2.0 | Orange (+) | 2.0 |
| Sulphadimethoxine | Purple (+) | 2.0 | Green (+) | 2.0 | Yellow (±) | 4.0 | Orange (+) | 2.0 |
| Sulphadoxine | Purple (+) | 2.0 | Green (±) | 4.0 | Yellowish green (±) | 4.0 | Yellowish green (+) | 2.0 |
| Sulphaethidole | Purple (+) | 2.0 | Green (+) | 2.0 | Yellow (±) | 2.0 | Pink (+) | 2.0 |
| Sulphaguanidine | Purple (+) | 2.0 | Green (++) | 1.5 | Yellow (+) | 2.0 | Yellowish green (±) | 4.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sulphamerazine | Purple (+) | 2.0 | Green (+) | 2.0 | Yellow (+) | 2.0 | Orange (++) | 1.5 |
| Sulphamethazine | Purple (+) | 2.0 | Grey (++) | 1.5 | Yellow (+) | 2.0 | Grey (+) | 2.0 |
| Sulphamethizole | Purple (+) | 2.0 | Green (+) | 2.0 | Yellow (+) | 2.0 | Pink (+) | 2.0 |
| Sulphamethoxazole | Purple (+) | 2.0 | Green (+) | 2.0 | Yellow (+) | 2.0 | Orange (++) | 1.5 |
| Sulphanilamide | Purple (+) | 2.0 | Green (+) | 2.0 | Yellow (+) | 2.0 | Grey (+) | 2.0 |
| Sulphapyridine | Purple (+) | 2.0 | Green (++) | 1.5 | Yellow (+) | 2.0 | Orange (+++) | 1.0 |
| Sulphathiazole | Purple (+) | 2.0 | Green (++) | 1.5 | Yellow (+) | 2.0 | Orange (++) | 1.5 |
| Sulphisomidine | Purple (+) | 2.0 | Green (+) | 2.0 | Yellow (+) | 2.0 | Grey (+) | 2.0 |
| Sulphisoxazole | Purple (+) | 2.0 | Green (+) | 2.0 | Grey (+) | 2.0 | Grey (+) | 2.0 |
| Trimethoprim | Purple (++) | 1.5 | Purple (+) | 2.0 | Brown (+) | 2.0 | Purple (+++) | 1.0 |

* Initial purple colour changes to grey (rapid) and then to yellowish green (slow).
** No immediate visualization. Colours develop slowly after several hours.
*** Colours develop in *ca.* 2 min.
§ Full colour development takes 2–5 min.

*Thin-layer chromatography*

The drugs were dissolved in either acetone or methanol. The sample was applied to silica gel G (0.2 mm) thin-layer plates and, after development in ethyl acetate–methanol (90:10), the plates were air-dried and sprayed. Where necessary the plates were oversprayed with DMSO or DMF.

## RESULTS

Table I gives the results of colour reactions of sulphonamide drugs with various spray reagents. *o*-Chloranil in dioxane (I) produced an immediate visualization of all drugs as purple spots against an orange (the same as colour of reagent) background. The initial intense purple colour, however, fades rapidly to grey while the background becomes purple. The spots, which later turn into yellowish green (against a white background), are clearly visible even after several weeks. Spraying of the plate with DMSO either prior to or after spraying with *o*-chloranil did not improve the visualization. The use of *p*-chloranil in dioxane (II) was unsatisfactory as none of the drugs could be seen immediately after spraying with this reagent. After the sprayed plates have been stored overnight the drugs appears as green spots (grey for sulphamethazine and purple for trimethoprim). The use of DMSO did not accelerate the reaction, except that the drugs appeared as yellow or yellowish green when the plate was sprayed with DMSO either before or after spraying with *p*-chloranil.

The use of fluoranil in dioxane (III) resulted in prompt visualization of all drugs as yellow spots, except for sulphisoxazole which appeared as grey. Phthalylsulphathiazole and succinylsulphathiazole could not be visualized by this reagent. The use of DMSO was found to have no effect. 2,5-Dichloro-*p*-benzoquinone in dioxane (IV) gave a light grey colour with all the drugs, except sulphisomidine and sulphisoxazole which appeared as purple. The reaction was very slow as the colours developed only after overnight storage of the sprayed plate. The use of either DMSO (V) or DMF (VI) as solvent for 2,5-dichloro-*p*-benzoquinone resulted in the immediate visualization of sulphoamide drugs as given in Table I. The use of 2,5-dichloro-*p*-benzoquinone in DMSO (V) as spray reagent is to be preferred as the colours produced are sharper and more stable. Other reagents tried included 0.5% chloranilic acid in dioxane, and 2,3-dichloro-5,6-dicyanobenzoquinone in dioxane, but these did not give any noticeable colour reaction immediately.

In conclusion, the use of 2,5-dichloro-*p*-benzoquinone in DMSO (V) is recommended as a spray reagent for the prompt visualization of sulphonamide drugs on silica gel G thin-layer. The reaction between the reagent and drugs is rapid and produces sharp colours, which are highly stable.

## ACKNOWLEDGEMENTS

The author is grateful to various pharmaceutical companies for the generous supply of authentic samples of drugs and in particular to Roche (Nigeria) Ltd. for the supply of sulphadoxine, trimethoprim and dosage forms containing these drugs. This work was supported by Senate Research Grant Committee, University of Nigeria (Grant No. 00354/79).

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