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Note

Detection of diuretic and oral hypoglycemic drugs on thin-layer plates using π-acceptors as spray reagents

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The reported methods for the detection of diuretics on thin-layer plates include UV light (254 nm), Bratton–Marshall reagent[1,2], *p*-dimethylaminobenzaldehyde[2,3] and metal-ion-containing reagents[4]. Wallace *et al.*[5] used iodoplatinate, a saturated solution of 1,2-naphthoquinone-4-sulphonate in ethanol–water (1:1) and ceric sulphate (5% solution in dil. sulphuric acid) as spray reagents for the identification of several antihypertensive drugs, including thiazide diuretics in biological fluids. Stumph and Noall[6] have recently described a method for the detection of hydrochlorthiazide in human urine by thin-layer chromatography (TLC).

The detection of oral hypoglycemic drugs on thin-layer plates has been carried out using UV light at 254 nm[7] or solutions of ninhydrin, sodium nitroprusside–aq. sodium hydroxide–aq. hydrogen peroxide, ammoniacal copper sulphate or 1% ethanolic fluorescein[8,9]. Spray reagents containing cobalt(II), iron(III), copper(II) and cerium(IV) were also found to be useful in the identification of commonly used hypoglycemic drugs[4].

The use of π-acceptors as spray reagents for some alkaloids, steroids and penicillins was reported[10–12]. Here, we report the use of several π-acceptors as suitable detection reagents for fifteen diuretic and seven oral hypoglycemic drugs. The method is also applicable to the detection of these drugs in their dosage forms or when present in urine.

EXPERIMENTAL

Materials

Pure samples of acetazolamide, bendrofluazide, benzthiazide, chlorthalidone, cyclopenthiazide, cyclothiazide, ethoxzolamide, furosemide, hydrochlorthiazide, hydroflumethiazide, methazolamide, methyclothiazide and polythiazide were obtained from commercial sources. Pure samples of acetohexamide, carbutamide, chlorpropamide, glibenclamide, gliclazide, tolazamide and tolbutamide were similarly obtained. Dosage forms of several of the above drugs were also made available. *p*-Coranil, *p*-fluoranil and 2,5-dichloro-*p*-benzoquinone were from Pfaltz and Bauer, 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% *p*-chloranil in diox-

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TABLE I

REACTIONS OF DIURETIC AND ORAL HYPOGLYCEMIC DRUGS WITH SPRAY REAGENTS

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

Drug	*Trade name (dosage form)*	*p-Chloranil + DMF*		*p-Fluoranil*		*2,5-Dichloro-p-benzoquinone + DMF*		*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)*
Diuretics									
Acetazolamide	Diamox®	Purple (+ +)	1.5	Pink* (+)	2.0	Purple (+)	2.0	Purple (+ + +)	1.0
Amiloride HCl	Moduretic®	–		Purple (+)	2.0	–		–	
Bendrofluazide	Naturetin®	Purple (±)	4.0	Purple (+)	2.0	Purple (+)	2.0	Violet (+ +)	1.5
Benzthiazide		Pink (+ +)	1.5	Purple (+)	2.0	Orange (+ + + +)	0.5	Brown (+ +)	1.5
Chlorthalidone	Hygroton®	Purple (+)	2.0	Purple (+)	2.0	–		Violet (+)	2.0
Cyclopenthiazide	Navidrex®	Purple (+)	2.0	Purple (+)	2.0	Purple (+)	2.0	Violet (+ + +)	1.0
Cyclothiazide		Purple (+)	2.0	Purple (+)	2.0	Purple (+)	2.0	Violet (+ +)	1.5
Ethoxolamide		Purple (+ + +)	1.0	Pink* (+)	2.0	Purple (+)	2.0	Violet (+ +)	1.5
Furosemide**	Lasix®	Purple (+)	4.0	–		Purple (+)	4.0	–	
Hydrochlorthiazide	Esidrex® Moduretic®	Purple (+ + +)	1.0	Purple* (+ + +)	1.0	Purple (+ + +)	1.0	Purple (+ + + +)	0.5

Hydroflumethiazide		Purple (+++)	1.0	Purple* (+++)	1.0	Purple (+++)	1.0	Purple (++)	1.0
Methazolamide	Neptazane®	Purple (+++)	1.0	Orange* (+++)	1.0	Purple (+++)	1.0	Purple (++++)	0.5
Methyclothiazide	Enduron® Diutensen® Aquatensen®	Purple (+)	2.0	Purple (++)	1.5	Purple (+)	2.0	Violet (++)	1.5
Polythiazide	Renese®	Purple (+)	2.0	Purple (+)	2.0	Purple (+)	2.0	Violet (++)	1.5
Quinethazone	Hydromox®	Yellow*** (++)	1.5	Pink (±)	4.0	Purple (++)	1.5	Purple*** (+)	3.0
Oral hypoglycemics									
Acetohexamide	Dymelor®	–		–		–		Violet (+)	2.0
Carbutamide	Nadisan®	–		–		Purple (+)	2.0	Brown (+++)	1.0
Chlorpropamide	Diabenese®	–		Yellow (+)	2.0	Purple (+)	2.0	Violet (++)	1.5
Glibenclamide	Euglucon®	–		–		Purple (±)	4.0	Violet (++)	1.5
Gliclazide	Diamicron®	Orange yellow (++++)	0.5	–		Yellow (+)	2.0	Yellowish green (+++)	1.0
Tolazamide		–		Silvery white (+++)	1.0	Silvery white (+)	2.0	Silvery white (+++)	1.0
Tolbutamide	Orinase®	–		Silvery white (++)	1.5	Purple (+)	2.0	Violet (++)	1.5

* Colour intensifies (3–4 times) on overspraying the plate with DMF.
** Tailing at origin.
*** Colour fades with time.

ane; II, 0.5% *p*-fluoranil in dioxane; III, 0.5% 2,5-dichloro-*p*-benzoquinone in dioxane and IV, 0.5% 2,5-dichloro-*p*-benzoquinone in dimethyl sulphoxide (DMSO).

TLC

The drugs were dissolved in methanol or acetone. Tablets were first crushed to a fine powder and an aliquot accurately weighed and suspended in methanol or acetone. After thorough shaking, the suspended excipients were allowed to settle and the supernatant was used. The sample was applied to silica gel G (0.2 mm) TLC plates and, after development in chloroform–methanol (90:10), the plates were air-dried and sprayed. Where necessary, the plates were oversprayed with dimethylformamide (DMF) after the primary spraying.

RESULTS

The results of the reaction of diuretic and oral hypoglycemic drugs with the various spray reagents is given in Table I. When 0.5% chloranil in dioxane was sprayed on the developed thin-layer plate, no reaction could be observed. However, overspraying of the plate with DMF resulted in a gradual (*ca.* 2 min) development of coloured spots with virtually all of the diuretics and with gliclazide which appeared as an intense orange yellow spot. Diuretics gave a purple colour, with the exception of quinethazone (yellow and fading with time) and amiloride hydrochloride which did not yield any colour. The use of 0.5% fluoranil alone was quite successful with diuretic drugs, as they gave either a purple or a pink colour. Furosemide did not react with this reagent. Overspraying of the plate with DMF resulted in a 3–4-fold intensification of colour with the following drugs: acetazolamide, ethoxzolamide, hydrochlorthiazide, hydroflumethiazide and methazolamide. In the case of oral hypoglycemics, only chlorpropamide, tolazamide and tolbutamide showed reaction.

Significantly better results were obtained when the plates were sprayed with a solution of 0.5% 2,5-dichloro-*p*-benzoquinone in dioxane followed by DMF. This reacted with almost all of the drugs studied, giving mostly a purple colour. Benzthiazide gave a characteristic orange colour, whereas gliclazide and tolazamide appeared as yellow and silvery white, respectively. Amiloride, chlorthalidone and acetohexamide failed to give any colour. Further improvements could be noticed with the use of a solution of 2,5-dichloro-*p*-benzoquinone (0.5%) in DMSO, which reacted with all of the drugs studied with the exception of furosemide. Colours produced by this reagent are sharper and quite stable. However, as far as possible, the reagent should be prepared fresh, though it can still be used within 48 h of storage at room temperature. Further storage leads to a darkening of colour. Storage in a refrigerator is inadvisable as this can change the solution into a gel-like mass.

The method was applied to the detection of drugs when present in dosage forms. Table I includes the trade names of dosage forms used in this study. In all cases, the drug could easily be identified with the use of 2,5-dichloro-*p*-benzoquinone in DMSO as spray reagent.

Preliminary experiments seem to indicate that the method could be applied to the detection of drugs in biological fluids as well. Hydrochlorthiazide and ethoxzolamide were added to fresh urine and extraxted according to the procedure of Wallace *et al.*[5]. The ethyl acetate extract was applied to thin-layer plates, developed and then

sprayed with 2,5-dichloro-*p*-benzoquinone in DMSO. Both drugs yielded the characteristic purple–violet colour.

In conclusion, the use of 2,5-dichloro-*p*-benzoquinone in DMSO is recommended as the spray reagent in the detection of diuretic and oral hypoglycemic drugs on thin-layer plates.

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