Journal of Chromatography, 195 (1980) 133–138
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,787

Note

Use of ninhydrin as a spray reagent for the detection of some basic drugs on thin-layer chromatograms

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(First received November 22nd, 1979; revised manuscript received February 26th, 1980)

The many reagents commonly used as spray reagents for revealing basic drugs on thin-layer chromatographic (TLC) plates include iodoplatinate, Dragendorff reagent, Marquis reagent, *p*-dimethylaminobenzaldehyde and bromocresol green. Usually these reagents produce one colour or shades of the same colour with basic drugs[1,2]. When they do produce other colours, as with Mandelin's reagent[3], Folins reagent[4] or vanadium pentoxide[5], only a few compounds respond.

Ninhydrin, which is widely used for the detection of amino acids, has also been used to detect pharmaceutical compounds. The colours produced by this reagent on TLC plates or paper chromatograms are violets, blue- and brown-violets and yellows for amines, nitrogenous compounds and stimulants[6–10], purples, yellows and greys for narcotics and CNS stimulants[11], pink for phenmetrazine[12] and methadone[13], violet, grey, pink and yellow fluorescence for amphetamines[10,11,14–16], and mainly purples and greys for several drugs when combined with UV radiation, spraying and heating[17].

This study was aimed at exploring the potential of ninhydrin to produce coloured spots on TLC plates for a wide variety of basic drugs. Various concentrations of ninhydrin, with prolonged heating at various temperatures, were studied.

EXPERIMENTAL

Drugs

Fifty-six basic drugs, obtained either from manufacturers or the Singapore Pharmaceutical Department, were used without any prior tests.

Preparation of reference standards

All compounds were dissolved either in 95% ethanol, ethanol–water mixtures, acetone, methanol, chloroform or ethanol–chloroform mixtures as recommended in the Merck Index[18].

Two standard solutions were prepared for each compound: 1% (equivalent to 10 μg/μl) and 0.1% (equivalent to 1 μg/μl), calculated in each instance with reference to the free base if they were salts. Solutions stated in the Merck Index[18] to decompose in light were stored in brown bottles and the remainder were stored in transparent 11-ml screw septum-capped bottles. All the standard solutions were stored in a

0021-9673/80/0000–0000/$02.25

refrigerator and fresh standards were prepared only when more than one spot was revealed on the TLC plates.

Developing tanks

Rectangular glass tanks (22 × 5.5 × 22.5 cm) lined with chromatographic paper and wetted with solvent were placed in a room at ambient temperature (25–30°C).

TLC plates

Glass chromatoplates (10 × 20 cm) containing a 0.25 mm adsorbent layer of silica gel were made by spreading a mixture of 34 g of the silica gel in 80 ml of distilled water. The plates were dried at room temperature and heated at 100°C for 1 h.

Application of standard solutions

Volumes of 1, 2 and 5 μl of 0.1% standard solution containing 1, 2 and 5 μg of the compound and 1, 2 and 3 μl of the 1% standard solution containing 10, 20 and 30 μg of the compound were spotted with 5-μl disposable graduated micropipettes. When necessary cold air was used for drying the spots.

Solvent system

Ethyl acetate–methanol–concentrated ammonia solution (170:20:16)[19] was used as the mobile phase. Fresh solvents were used every day. The developing tank was equilibrated for 1 h before use and the solvent front was allowed to rise about 9 cm above the baseline.

Ninhydrin spray reagent

A 10% solution of ninhydrin (Merck, Darmstadt, G.F.R.) in 95% ethanol was used. This solution keeps well for up to a month if stored in a refrigerator.

After development the plates were dried in an air-oven at 100°C for 3–4 min to remove ammonia, cooled to room temperature and sprayed generously with the ninhydrin reagent. The plates were observed for the appearance of any coloured spots before proceeding to the next step.

Heating

A Memmert air-oven with temperature control was used. The temperature of the oven was first adjusted to 80°C and the sprayed plates were heated at this temperature for 1/2 h. The plates were then removed and the colours of the spots and the limits of detection were immediately noted. Some colours tended to change on cooling but, for this study, such changes were ignored.

The temperature of the oven was then increased to 120°C, the plates were heated at this temperature for 1/2 h and again the colours and limits of detection were immediately noted. The temperature of the oven was finally increased to 160°C, the plates were heated for 1/2 h and the colours and limits of detection were immediately noted. After the final heating the R_F values were measured. To prevent contamination of the oven with ninhydrin, which tends to vapourise from the plate, we found it convenient to pack the plates in a suitably sized tin box.

RESULTS AND DISCUSSION

Colours

A wide variety of colours, on a white background, were produced by 52 of the 56 bases studied. In most instances different colours were produced at each of the selected temperatures (80, 120 and 160°C). With amphetamine, for example, the colours produced were pink-violet, grey-brown and red-brown, respectively. For oxyphencyclimine they were grey-purple at room temperature and purple, pink and dark orange at 80, 120 and 160°C, respectively. In some instances, however, the colours remained the same at all temperatures (see Table I).

Incomplete removal of ammonia from the plates before spraying with the ninhydrin reagent causes a brownish background which tends to mask the colour of the spots.

In describing the colours, some difficulty was experienced in differentiating shades of the same colour. Also, it must be appreciated that colour interpretation tends to be subjective. Consequently, the colours listed should be taken only as a guide. When identifying an unknown compound, a selection of standard compounds, with similar colour combinations, should be run side-by-side for actual comparison of colours.

Non-reacting compounds

The following compounds showed no response to the technique described: clonazepam, flunitrazepam, hyoscine N-methyl bromide and meprobamate.

Concentration of ninhydrin spray reagent

A study of the concentration of the ninhydrin spray showed that greater sensitivity and distinction of colours for a particular drug, when moving from one temperature to the next, was achieved with increasing ninhydrin concentration. The limited solubility of ninhydrin in ethanol at 4°C (refrigerator) dictated the choice of a 10% solution.

Over-spraying

The effect of over-spraying the plates with iodoplatinate was studied, and it was noted that whilst some compounds showed a weak response, others merely gave a blend of the colour of the ninhydrin complex and the pink colour of the iodoplatinate. Over-spraying was, therefore, not seriously pursued.

Colour–structure relationship

No consistent relationship was noted between a class of compounds, with respect to their structure, and the colours obtained.

R_F values

The R_F values listed in Table I are average values of five determinations and, as expected, vary from some of the values published previously[19]. As the aim of this study was only to study colours, other solvent systems were not investigated. The method should, however, be applicable with any solvent system that does not include non-volatile alkalis.

TABLE I

COLOURS PRODUCED WITH 10% NINHYDRIN SPRAY REAGENT AT DIFFERENT TEMPERATURES

The figures in parentheses indicate the sensitivity in micrograms.

Compound	*Colour of spot*				R_F
	Room temperature	*80°C*	*120°C*	*160°C*	
Amethocaine hydrochloride	Yellow (20)	Dirty yellow (5)	Orange-brown (5)	Red-brown (5)	0.65
Amitriptyline hydrochloride	Nil	Nil	Light brown (10)	Brown (5)	0.76
Amphetamine sulphate	Nil	Pink-violet (5)	Grey-brown (5)	Red-brown (5)	0.49
Antazoline hydrochloride	Bright purple (5)	Bright purple (1)	Red-brown (5)	Red-purple (5)	0.46
Atropine sulphate	Nil	Nil	Beige (20)	Light orange (10)	0.17
Benzhexol hydrochloride	Nil	Nil	Grey (10)	Orange-brown (5)	0.72
Brucine	Nil	Nil	Pink (5)	Pink-violet (1)	0.23
Buclizine hydrochloride	Nil	Pink-violet (10)	Brown (5)	Orange-brown (5)	0.76
Chlordiazepoxide	Nil	Pink-violet (10)	Orange-yellow (5)	Orange-brown (5)	0.57
Chloroquine phosphate	Nil	Nil	Pink-violet (5)	Red-brown (5)	0.37
Chlorpheniramine maleate	Nil	Pink-violet (10)	Pink-violet (5)	Pink-violet (1)	0.54
Chlorpromazine hydrochloride	Yellow (10)	Yellow-brown (10)	Yellow-brown (5)	Green-blue (1)	0.66
Cocaine hydrochloride	Nil	Nil	Beige (10)	Pale brown (5)	0.67
Codeine phosphate	Nil	Nil	Blue (5)	Light brown (5)	0.41
Cyclizine hydrochloride	Nil	Nil	Green-yellow (5)	Brown (5)	0.67
Diazepam	Nil	Nil	Pink-violet (5)	Grey (5)	0.64
Diphenhydramine hydrochloride	Nil	Nil	Light brown (5)	Beige (5)	0.66
Emetine hydrochloride	Nil	Grey-purple (5)	Red-violet (5)	Brown-violet (5)	0.45
Ephedrine hydrochloride	Nil	Bright purple (1)	Light red (5)	Orange-brown (5)	0.26
Ergometrine maleate	Nil	Bright purple (1)	Dark purple (1)	Dark purple (1)	0.47
Flurazepam	Nil	Yellow-grey (10)	Brown-violet (5)	Brown (5)	0.64
Heroin hydrochloride	Nil	Nil	Blue (10)	Beige (5)	0.61
Homatropine hydrobromide	Nil	Nil	Beige (10)	Beige (10)	0.27

Imipramine hydrochloride	Nil	Brown (10)	Yellow-brown (10)	Orange-brown (5)	0.66
Iproniazid phosphate	Nil	Nil	Brown-violet (5)	Brown (5)	0.46
Isoniazid	Yellow (5)	Yellow-brown (5)	Orange (5)	Orange (5)	0.23
Lignocaine hydrochloride	Nil	Purple (20)	Pink-violet (5)	Orange-brown (5)	0.73
Meprylcaine hydrochloride	Nil	Nil	Yellow-brown (5)	Orange-yellow (5)	0.71
Mepyramine maleate	Nil	Nil	Yellow (5)	Orange (5)	0.59
Methadone hydrochloride	Nil	Nil	Red-brown (1)	Red-brown (1)	0.65
Methamphetamine hydrochloride	Nil	Pink-purple (1)	Grey (5)	Brown (1)	0.38
Methoxyphenamine hydrochloride	Nil	Purple (5)	Pink-violet (5)	Dark pink (5)	0.30
Morphine hydrochloride	Nil	Grey (10)	Blue (10)	Brown (10)	0.23
Nalorphine hydrobromide	Nil	Blue (5)	Blue (1)	Brown (5)	0.43
Narcotine	Nil	Yellow (5)	Light orange (1)	Brown (5)	0.70
Nitrazepam	Nil	Yellow (20)	Yellow (20)	Yellow (10)	0.48
Oxyphencyclimine hydrochloride	Grey-purple (5)	Purple (5)	Pink (1)	Dark orange (1)	0.10
Papaverine hydrochloride	Nil	Orange-yellow (1)	Orange (1)	Orange (1)	0.64
Pethidine hydrochloride	Nil	Nil	Purple-blue (5)	Dark beige (5)	0.63
Pholcodine	Nil	Grey-purple (5)	Dark grey (5)	Dark brown (5)	0.29
Physostigmine salicylate	Nil	Purple (20)	Brown-purple (1)	Red-brown (1)	0.44
Procaine hydrochloride	Yellow (10)	Pink (5)	Pink-violet (5)	Brown-red (5)	0.65
Prochlorperazine dimaleate	Nil	Nil	Brown (10)	Grey (20)	0.62
Promethazine	Nil	Grey-purple (10)	Blue-grey (10)	Green-blue (1)	0.67
Quinidine sulphate	Nil	Nil	Yellow (20)	Brown (10)	0.45
Quinine sulphate	Nil	Nil	Yellow-brown (5)	Violet-brown (1)	0.53
Reserpine	Brown (5)	Violet (1)	Orange-brown (5)	Brown (1)	0.64
Ritalin	Nil	Nil	Dark-purple (1)	Black (1)	0.69
Strychnine hydrochloride	Nil	Nil	Pink (10)	Red-violet (5)	0.35
Trifluoperazine dihydrochloride	Yellow (20)	Yellow (10)	Brown (5)	Grey (5)	0.70
Trimethoprim	Nil	Nil	Yellow (10)	Yellow (5)	0.42
Yohimbine hydrochloride	Nil	Brown (5)	Orange (5)	Brown (5)	0.62

Sensitivity

The response of compounds to the method varied from 20 μg at room temperature to 1 μg at 160°C.

CONCLUSION

This study has shown that ninhydrin has considerably more potential in forming coloured complexes with basic drugs than has been realised. Low concentrations of ninhydrin and low temperatures of heating, for short durations, probably accounted for this. The formation of numerous colours at different temperatures adds a new dimension to the identification of basic drugs on TLC plates.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the following companies for their donation of pharmaceutical compounds: Burroughs Wellcome (London, Great Britain), Ciba-Geigy (Basle, Switzerland), May & Baker (Dagenham, Great Britain), Roche Pharmaceuticals (Nutley, NJ, U.S.A.) and Smith Kline & French (Philadelphia, PA, U.S.A.).

REFERENCES

1 G. Zweig and J. Sherma, *Handbook of Chromatography*, Vols. I and II, CRC Press, Cleveland, OH, 1972.
2 I. Sunshine, *Handbook of Analytical Toxicology*, CRC Press, Cleveland, OH, 1969.
3 W. W. Fike and I. Sunshine, *Anal. Chem.*, 37 (1965) 127.
4 C. P. Stewart and A. Stolman, *Toxicology*, Vol. II, Academic Press, London, 1961, p. 548.
5 M. Malaiyandi, J. P. Barrette and M. Lanouette, *J. Chromatogr.*, 101 (1974) 155.
6 A. Wickstrom and B. Salvsen, *J. Pharm. Pharmacol.*, 4 (1952) 631.
7 J. Borecky, *J. Chromatogr.*, 28 (1967) D1.
8 L. Reio, *J. Chromatogr.*, 13 (1964) 475.
9 L. Reio, *J. Chromatogr.*, 47 (1970) 60.
10 A. H. Beckett, G. T. Tucker and A. C. Moffat, *J. Pharm. Sci.*, 19 (1967) 273.
11 K. K. Kaistha and J. H. Jaffe, *J. Pharm. Sci.*, 61 (1972) 679.
12 K. K. Kaistha and J. H. Jaffe, *J. Pharm. Sci.*, 61 (1972) 305.
13 K. G. Blass, R. J. Thibert and T. F. Draisey, *J. Chromatogr.*, 95 (1974) 75.
14 S. J. Mulé, *J. Chromatogr.*, 39 (1969) 302.
15 B. Davidow, N. L. Petri and B. Quame, *Amer. J. Clin. Pathol.*, 50 (1968) 714.
16 A. N. Masoud, *J. Pharm. Sci.*, 65 (1976) 1585.
17 K. K. Kaistha, R. Tadrus and R. Janda, *J. Chromatogr.*, 107 (1975) 359.
18 *The Merck Index*, Merck, Rahway, NJ, 9th ed., 1976.
19 B. Davidow, N. L. Petri and B. Quame, *Tech. Bull. Reg. Med. Technol.*, 38 (1968) 298; *Anal. Abstr.*, 18 (1970) 2599.