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## IDENTIFICATION OF BASIC DRUGS BY THE THIN-LAYER CHROMATOGRAPHIC PROFILES OF THEIR NINHYDRIN COMPLEXES

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### SUMMARY

Fifty-two common basic drugs produce various colours when spotted with ninhydrin on plastic thin-layer plates and heated. When the plate is then developed in a suitable solvent each of the coloured spots separates into a variety of additional coloured spots and patches, the number of which depends on the temperature of heating. The relative intensity and spatial arrangement form a profile that is highly characteristic of the compounds. The formation of such profiles was investigated at 100 and 160°C.

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### INTRODUCTION

The use of ninhydrin as a spray reagent and in spot tests for the detection of amines, amino acids, aromatic amines, stimulants and other nitrogenous compounds, involving heating at 100–120°C for a few minutes, is well known<sup>1–11</sup>.

We recently reported the use of high concentrations of ninhydrin spray reagent, followed by prolonged heating at selected temperatures, to produce coloured spots for a range of basic drugs<sup>12</sup>. We observed that for several compounds the colours produced on the thin-layer chromatograms were not pure colours but combinations, such as yellow–brown, brown–purple and grey–purple. A similar phenomenon was observed by Kaistha *et al.*<sup>10</sup>.

Investigation of this phenomenon showed that if the compounds were first reacted with ninhydrin by over-spotting on the thin-layer chromatographic (TLC) plate, heated at selected temperatures and then developed, the seemingly two coloured spots separated into a number of spots, bands and patches, forming unique profiles for each compound.

In this study, we aimed to establish, for each of the compounds tested, a profile of their ninhydrin complexes at reaction temperatures of 100 and 160°C to serve as a basis for the identification of unknown compounds with a high level of confidence.

## EXPERIMENTAL

### *Drugs*

A total of 52 basic drugs obtained either from manufacturers or the Singapore Pharmaceutical Department were used without prior tests.

### *Preparation of reference standards*

Standard solutions (1% with reference to the free base) were prepared in appropriate solvents and stored in a refrigerator in 11-ml screw, septum-capped transparent or dark bottles, depending on whether, according to the Merck Index<sup>13</sup>, they decompose on exposure to light.

### *Reagents*

A 1% solution of *p*-nitroaniline (BDH, Poole, Great Britain) in 95% ethanol and a 10% solution of ninhydrin (Merck, Darmstadt, G.F.R.) in 95% ethanol (equivalent to 100  $\mu\text{g}/\mu\text{l}$ ) were prepared. The ninhydrin solution keeps well for up to 1 month if stored in a refrigerator and the *p*-nitroaniline indefinitely if stored likewise.

### *Developing tanks*

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

### *TLC plates*

Merck TLC Plastic Roll pre-coated with silica gel 60 F<sub>254</sub>, layer thickness 0.2 mm, was used. Self-coated silica gel G glass plates were found not to be suitable as they gave unreproducible and often diffuse patterns.

### *Application of solutions and heating*

Amounts of 20  $\mu\text{g}$  of the standard compound were spotted on the baseline (with spots clearly marked with a pencil) using a 5- $\mu\text{l}$  disposable graduated micropipette and cold air, when necessary, to dry the spot. A 1- $\mu\text{l}$  volume of the ninhydrin reagent was then applied on the standard spot and the appearance of any colour noted. Two plates were spotted similarly and one was heated at 100°C and the other at 160°C in a Memmert air oven for 30 min. The plates were then removed and the colour of the spots noted again immediately.

A 1- $\mu\text{l}$  volume of the *p*-nitroaniline reagent was then spotted on another area of the baseline for use as a reference spot to calculate  $R_F$  values. The plate was then developed.

### *Solvent system*

A mixture of chloroform and 95% ethanol (5:1) was used as the mobile phase. This solvent keeps well for up to 1 week. The developing tank was equilibrated for 1 h before use and the solvent front allowed to rise about 6 cm above the baseline.

After development of the plate, the colours of the spots, bands and patches were immediately noted and their  $R_F$  values, relative to the *p*-nitroaniline spot ( $R_{\text{PNA}}$ ), calculated. Also noted were the relative intensities of the colours on a scale of

0–10, with the colour of the most intense spot for the particular compound being assigned a value of 10.

## RESULTS AND DISCUSSION

### Colour profiles

On the initial addition of ninhydrin to the spotted compounds, only a few produced colours at room temperature. On heating at 100 and 160°C almost all of the compounds tested produced characteristic colours which were generally different at the two temperatures. The colours usually changed in hue as the plate cooled and Tables I and II record those observed immediately on removal of the plates from the oven.

On development of the plates, highly characteristic patterns, ranging from a single spot to a remarkable combination of spots, bands and patches, were produced. These also tended to change in hue when exposed to the atmosphere owing to a change in the ninhydrin complex–solvent equilibrium. Again, only colours seen on immediate removal of the plate from the tank are recorded in the tables.

Humidity, temperature and the presence of ammonia or acid fumes in the atmosphere did not produce significant changes in the colours. Further heating of the plate did not produce any significant additional colours for any of the compounds.

A schematic diagram of a few profiles is shown in Fig. 1.

The intensity of colour within a profile was highly characteristic of a compound and added to the discriminatory power for characterization. As estimation of the relative intensities tended to be subjective, the figures listed in the tables are intended to serve only as a guide. Barely visible colours have been omitted from the tables.

TABLE I  
COLOUR PROFILES OF NINHYDRIN COMPLEXES AT 100°C

| Compound                    | Colour of spot before TLC development |              | Colour after TLC development* | $R_{FVA}$ | Relative intensity |
|-----------------------------|---------------------------------------|--------------|-------------------------------|-----------|--------------------|
|                             | Room temperature 100°C                |              |                               |           |                    |
| Amethocaine hydrochloride   | Pale yellow                           | Grey         | Blue-purple (B)               | 0.54      | 10                 |
| Amitriptyline hydrochloride | Nil                                   | Yellow-grey  | Pink (B)                      | 0.49      | 3                  |
|                             |                                       |              | Purple (B)                    | 0.68      | 4                  |
|                             |                                       |              | Grey-yellow (P)               | 1.39      | 10                 |
| Amphetamine sulphate        | Pale brown                            | Red-brown    | Red (B)                       | 0.30      | 7                  |
|                             |                                       |              | Purple (P)                    | 0.53      | 3                  |
|                             |                                       |              | Orange (P)                    | 1.29      | 8                  |
|                             |                                       |              | Purple (P)                    | 1.38      | 10                 |
| Antazoline hydrochloride    | Purple                                | Brown-purple | Purple (P)                    | 0.45      | 10                 |
|                             |                                       |              | Orange** (P)                  | 0.73      | 5                  |
| Atropine sulphate           | Nil                                   | Beige        | Nil                           |           |                    |
| Benzhexol hydrochloride     | Nil                                   | Grey-violet  | Purple-grey (B)               | 0.87      | 3                  |
|                             |                                       |              | Blue-purple (B)               | 1.25      | 3                  |
|                             |                                       |              | Purple (P)                    | 1.42      | 10                 |

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TABLE I (continued)

| Compound                      | Colour of spot before TLC development |               | Colour after TLC development* | $R_{FNA}$ | Relative intensity |
|-------------------------------|---------------------------------------|---------------|-------------------------------|-----------|--------------------|
|                               | Room temperature 100°C                |               |                               |           |                    |
| Brucine                       | Pale yellow                           | Yellow-pink   | Purple (B)                    | 0.41      | 4                  |
|                               |                                       |               | Pink (P)                      | 1.30      | 10                 |
| Bucizine hydrochloride        | Nil                                   | Pink-brown    | Grey-green (P)                | 1.38      | 10                 |
| Chlordiazepoxide              | Nil                                   | Yellow-violet | Purple (B)                    | 0.27      | 10                 |
| Chloroquine phosphate         | Nil                                   | Violet-grey   | Purple (P)                    | 0.39      | 10                 |
| Chlorpheniramine maleate      | Nil                                   | Pink-violet   | Pink-violet (S)               | 1.39      | 10                 |
| Chlorpromazine hydrochloride  | Yellow                                | Pale brown    | Blue-purple (B)               | 0.63      | 10                 |
| Clonazepam                    | Nil                                   | Beige         | Nil                           |           |                    |
| Cocaine hydrochloride         | Nil                                   | Beige         | Nil                           |           |                    |
| Codeine phosphate             | Nil                                   | Grey-blue     | Purple-grey (B)               | 0.34      | 4                  |
|                               |                                       |               | Blue (S)                      | 1.40      | 10                 |
| Diazepam                      | Nil                                   | Beige         | Yellow (P)                    | 1.47      | 10                 |
| Diphenhydramine hydrochloride | Nil                                   | Brown         | Purple-grey (B)               | 0.55      | 10                 |
| Emetine hydrochloride         | Yellow-grey                           | Pink-violet   | Yellow (P)                    | 0.09      | 6                  |
|                               |                                       |               | Red (B)                       | 0.18      | 6                  |
|                               |                                       |               | Pink-violet (P)               | 0.36      | 10                 |
|                               |                                       |               | Purple-grey (P)               | 0.64      | 5                  |
|                               |                                       |               | Pink (B)                      | 0.79      | 4                  |
|                               |                                       |               | Pink-orange (P)               | 1.37      | 5                  |
| Ephedrine hydrochloride       | Nil                                   | Pink-violet   | Orange (B)                    | 0.32      | 3                  |
|                               |                                       |               | Purple (P)                    | 0.50      | 4                  |
|                               |                                       |               | Orange-brown (P)              | 1.37      | 10                 |
|                               |                                       |               | Purple (P)                    | 0.63      | 10                 |
| Ergometrine maleate           | Grey-yellow                           | Dark purple   | Purple (P)                    | 0.37      | 5                  |
|                               |                                       |               | Purple (P)                    | 0.63      | 10                 |
|                               |                                       |               | Pink-purple (P)               | 0.76      | 7                  |
| Flunitrazepam                 | Nil                                   | Beige         | Nil                           |           |                    |
| Flurazepam                    | Nil                                   | Violet-brown  | Purple (B)                    | 0.76      | 3                  |
|                               |                                       |               | Orange-brown (P)              | 1.39      | 10                 |
| Heroin hydrochloride          | Nil                                   | Blue          | Grey-purple (B)               | 0.57      | 3                  |
|                               |                                       |               | Blue (S)                      | 1.45      | 10                 |
| Hyoscine N-methyl bromide     | Nil                                   | Beige         | Nil                           |           |                    |
| Imipramine hydrochloride      | Nil                                   | Violet-yellow | Orange-yellow (B)             | 0.40      | 6                  |
|                               |                                       |               | Purple (B)                    | 0.62      | 10                 |
| Iproniazid phosphate          | Nil                                   | Violet-brown  | Purple (B)                    | 0.31      | 10                 |
| Isoniazid                     | Yellow                                | Orange-brown  | Purple (B)                    | 0.28      | 7                  |
|                               |                                       |               | Orange (P)                    | 1.30      | 10                 |
|                               |                                       |               | Orange (B)                    | 0.12      | 7                  |
| Lignocaine hydrochloride      | Nil                                   | Violet        | Purple (P)                    | 0.43      | 10                 |
|                               |                                       |               | Pink (P)                      | 0.71      | 4                  |
|                               |                                       |               | Orange (B)                    | 0.71      | 3                  |
|                               |                                       |               | Orange (B)                    | 0.76      | 3                  |
| Meprylcaine hydrochloride     | Nil                                   | Pink-violet   | Purple (B)                    | 0.84      | 4                  |
|                               |                                       |               | Brown-orange (P)              | 1.38      | 10                 |
|                               |                                       |               | Purple (B)                    | 0.39      | 3                  |
|                               |                                       |               | Yellow (P)                    | 0.80      | 10                 |
| Mepyramine maleate            | Nil                                   | Beige         | Yellow (B)                    | 0.32      | 3                  |
|                               |                                       |               | Purple-grey (B)               | 0.48      | 3                  |
| Methadone hydrochloride       | Nil                                   | Red-brown     | Pink (B)                      | 0.81      | 3                  |
|                               |                                       |               | Pink (S)                      | 1.29      | 10                 |
|                               |                                       |               | Green-brown (S)               | 1.43      | 5                  |
|                               |                                       |               |                               |           |                    |

TABLE I (continued)

| Compound                        | Colour of spot before TLC development |               | Colour after TLC development* | $R_{PNA}$ | Relative intensity |
|---------------------------------|---------------------------------------|---------------|-------------------------------|-----------|--------------------|
|                                 | Room temperature 100°C                |               |                               |           |                    |
| Methoxyphenamine hydrochloride  | Nil                                   | Grey          | Purple (P)                    | 0.48      | 5                  |
|                                 |                                       |               | Pink (P)                      | 1.36      | 10                 |
| Morphine hydrochloride          | Nil                                   | Blue          | Purple-grey (B)               | 0.28      | 10                 |
|                                 |                                       |               | Purple-grey (B)               | 0.96      | 10                 |
| Nalorphine hydrobromide         | Nil                                   | Blue-purple   | Blue (B)                      | 0.95      | 10                 |
|                                 |                                       |               | Yellow (P)                    | 0.36      | 7                  |
| Narcotine                       | Pale yellow                           | Red-orange    | Red-orange (B)                | 0.88      | 10                 |
|                                 |                                       |               | Pink** (P)                    | 1.13      | 7                  |
| Nitrazepam                      | Nil                                   | Beige         | Nil                           |           |                    |
| Oxyphencyclimine hydrochloride  | Nil                                   | Beige         | Nil                           |           |                    |
| Papaverine hydrochloride        | Nil                                   | Orange-yellow | Orange-yellow (S)             | 1.34      | 10                 |
| Pethidine hydrochloride         | Nil                                   | Grey-blue     | Grey (B)                      | 0.60      | 3                  |
|                                 |                                       |               | Purple-blue (S)               | 1.42      | 10                 |
| Pholcodine                      | Nil                                   | Grey          | Purple (B)                    | 0.27      | 10                 |
|                                 |                                       |               | Pink (S)                      | 1.27      | 10                 |
| Physostigmine salicylate        | Nil                                   | Violet-brown  | Blue (S)                      | 1.45      | 4                  |
|                                 |                                       |               | Purple (B)                    | 0.45      | 3                  |
| Procaine hydrochloride          | Yellow                                | Pink-violet   | Pink (P)                      | 0.69      | 4                  |
|                                 |                                       |               | Brown-violet (P)              | 1.31      | 10                 |
| Prochlorperazine dimaleate      | Yellow                                | Yellow-brown  | Purple (P)                    | 0.47      | 10                 |
|                                 |                                       |               | Pink (P)                      | 1.38      | 8                  |
| Promethazine                    | Pale yellow                           | Grey-violet   | Purple (B)                    | 0.57      | 4                  |
|                                 |                                       |               | Purple-grey (B)               | 0.92      | 4                  |
| Quinidine sulphate              | Nil                                   | Yellow-grey   | Green (S)                     | 1.31      | 7                  |
|                                 |                                       |               | Yellow (S)                    | 1.40      | 10                 |
| Quinine sulphate                | Nil                                   | Yellow        | Purple (B)                    | 0.65      | 10                 |
|                                 |                                       |               | Orange (B)                    | 0.73      | 3                  |
| Reserpine                       | Brown                                 | Orange-brown  | Grey-brown (P)                | 1.38      | 3                  |
|                                 |                                       |               | Purple (B)                    | 0.70      | 5                  |
| Ritalin                         | Nil                                   | Dark violet   | Yellow-orange (P)             | 1.23      | 7                  |
|                                 |                                       |               | Orange-brown (P)              | 1.39      | 10                 |
| Strychnine hydrochloride        | Nil                                   | Pink-brown    | Yellow (P)                    | 0.72      | 10                 |
|                                 |                                       |               | Purple (B)                    | 0.83      | 3                  |
| Trifluoperazine dihydrochloride | Nil                                   | Yellow-brown  | Purple (B)                    | 1.18      | 7                  |
|                                 |                                       |               | Purple (B)                    | 1.26      | 7                  |
| Trimethoprim                    | Nil                                   | Beige         | Purple (S)                    | 1.43      | 8                  |
|                                 |                                       |               | Purple (B)                    | 0.39      | 4                  |
| Yohimbine hydrochloride         | Yellow                                | Orange-brown  | Pink** (P)                    | 1.31      | 10                 |
|                                 |                                       |               | Purple (B)                    | 0.56      | 3                  |
| Yohimbine hydrochloride         | Yellow                                | Orange-brown  | Purple (B)                    | 0.98      | 5                  |
|                                 |                                       |               | Yellow (P)                    | 1.46      | 10                 |
| Yohimbine hydrochloride         | Yellow                                | Orange-brown  | Nil                           |           |                    |
|                                 |                                       |               | Purple (B)                    | 0.33      | 10                 |
| Yohimbine hydrochloride         | Yellow                                | Orange-brown  | Pink (B)                      | 1.10      | 5                  |
|                                 |                                       |               | Pink** (P)                    | 1.33      | 5                  |

\* Ninhydrin produces a faint orange-yellow colour ( $R_{PNA}$  about 0.18) and a yellow colour ( $R_{PVA}$  1.02). These have been omitted from the profiles. (P) denotes a patch; (B) denotes a band; (S) denotes a spot.

\*\* Colour does not appear consistently.

TABLE II  
COLOUR PROFILES OF NINHYDRIN COMPLEXES AT 160°C

| <i>Compound</i>               | <i>Colour of spot before TLC development</i> | <i>Colour after TLC development*</i> | <i>R<sub>FNA</sub></i> | <i>Relative intensity</i> |
|-------------------------------|--|--------------------------------------|------------------------|---------------------------|
| Amethocaine hydrochloride     | Red-brown                                    | Orange-brown (P)                     | 0.41                   | 3                         |
|                               |  | Purple (P)                           | 0.53                   | 7                         |
|                               |  | Pink (P)                             | 0.86                   | 3                         |
|                               |  | Orange-brown (P)                     | 1.41                   | 10                        |
| Amitriptyline hydrochloride   | Brown  | Yellow (P)                           | 0.53                   | 3                         |
|                               |  | Purple (B)                           | 0.77                   | 6                         |
|                               |  | Orange-brown (P)                     | 1.34                   | 10                        |
|                               |  | Black (P)                            | 1.44                   | 10                        |
| Amphetamine sulphate          | Red-brown                                    | Red (B)                              | 0.36                   | 6                         |
|                               |  | Purple (P)                           | 0.55                   | 6                         |
|                               |  | Yellow (P)                           | 0.81                   | 4                         |
|                               |  | Pink (P)                             | 0.92                   | 3                         |
|                               |  | Orange-violet (P)                    | 1.41                   | 10                        |
| Antazoline hydrochloride      | Red-brown                                    | Grey-blue** (B)                      | 0.33                   | 3                         |
|                               |  | Purple (P)                           | 0.48                   | 7                         |
|                               |  | Orange (P)                           | 0.73                   | 6                         |
|                               |  | Orange-brown (P)                     | 1.43                   | 10                        |
| Atropine sulphate             | Yellow-brown                                 | Orange (B)                           | 0.23                   | 10                        |
| Benzhexol hydrochloride       | Brown  | Orange (B)                           | 0.21                   | 3                         |
|                               |  | Purple (B)                           | 0.88                   | 3                         |
|                               |  | Brown-orange (P)                     | 1.45                   | 10                        |
| Brucine                       | Brown-purple                                 | Purple (P)                           | 0.48                   | 7                         |
|                               |  | Pink/brown (P)                       | 1.27                   | 10                        |
|                               |  |                                      |                        |                           |
| Buclizine hydrochloride       | Red-brown                                    | Purple (P)                           | 0.49                   | 3                         |
|                               |  | Bright yellow (P)                    | 0.94                   | 8                         |
|                               |  | Purple-brown/orange-yellow (P)       | 1.43                   | 10                        |
| Chlordiazepoxide              | Orange-brown                                 | Orange-yellow (P)                    | 0.94                   | 10                        |
|                               |  | Orange-yellow (P)                    | 1.40                   | 10                        |
|                               |  |                                      |                        |                           |
| Chloroquine phosphate         | Purple-grey                                  | Brown-purple (P)                     | 0.65                   | 3                         |
|                               |  | Grey (P)                             | 0.93                   | 4                         |
|                               |  | Brown-orange (P)                     | 1.41                   | 10                        |
|                               |  |                                      |                        |                           |
| Chlorpheniramine maleate      | Purple                                       | Pink-violet (B)                      | 0.33                   | 5                         |
|                               |  | Purple (B)                           | 0.42                   | 5                         |
|                               |  | Orange** (P)                         | 0.58                   | 4                         |
|                               |  | Pink-orange (P)                      | 0.84                   | 4                         |
|                               |  | Violet (P)                           | 1.35                   | 10                        |
|                               |  |                                      |                        |                           |
| Chlorpromazine hydrochloride  | Brown/green-blue                             | Pink (P)                             | 0.53                   | 3                         |
|                               |  | Blue-purple (B)                      | 0.68                   | 5                         |
|                               |  | Red-orange (P)                       | 0.90                   | 4                         |
|                               |  | Orange-brown/green-black (P)         | 1.44                   | 10                        |
|                               |  | Nil                                  |                        |                           |
| Clonazepam                    | Orange-brown                                 |                                      |                        |                           |
| Cocaine hydrochloride         | Grey-brown                                   | Purple**                             | 0.31                   | 3                         |
|                               |  | Yellow (P)                           | 0.65                   | 8                         |
|                               |  | Violet-orange (P)                    | 1.45                   | 10                        |
|                               |  |                                      |                        |                           |
| Codeine phosphate             | Purple-brown                                 | Brown-purple (P)                     | 0.39                   | 4                         |
|                               |  | Violet-brown (P)                     | 1.40                   | 10                        |
|                               |  |                                      |                        |                           |
| Diazepam                      | Brown-purple                                 | Orange-brown (P)                     | 1.41                   | 10                        |
| Diphenhydramine hydrochloride | Red-brown                                    | Yellow-green (B)                     | 0.39                   | 3                         |
|                               |  | Purple (B)                           | 0.58                   | 5                         |
|                               |  | Violet-orange (P)                    | 1.41                   | 10                        |

TABLE II (continued)

| <i>Compound</i>           | <i>Colour of spot before TLC development</i> | <i>Colour after TLC development*</i> | $R_{F,N,A}$ | <i>Relative intensity</i> |
|---------------------------|--|--------------------------------------|-------------|---------------------------|
| Emetine hydrochloride     | Violet                                       | Bright pink (B)                      | 0.32        | 8                         |
|                           |  | Orange (B)                           | 0.53        | 5                         |
|                           |  | Brown-violet (P)                     | 0.67        | 5                         |
|                           |  | Red-orange (P)                       | 1.09        | 10                        |
|                           |  | Brown-violet (P)                     | 1.36        | 8                         |
| Ephedrine hydrochloride   | Violet-brown                                 | Purple (P)                           | 0.67        | 6                         |
|                           |  | Violet (P)                           | 1.37        | 10                        |
|                           |  | Orange (P)                           | 1.46        | 10                        |
|                           |  | Pink (5 bands)                       | 0.50        | 7                         |
| Ergometrine maleate       | Blue-purple                                  | to                                   | 1.03        |                           |
|                           |  | Pink** (B)                           | 1.24        | 4                         |
| Flunitrazepam             | Yellow-brown                                 | Brown-violet (P)                     | 1.43        | 10                        |
|                           |  | Yellow (P)                           | 1.00        | 10                        |
| Flurazepam                | Red-brown                                    | Purple** (P)                         | 0.54        | 4                         |
|                           |  | Orange-brown (P)                     | 0.67        | 6                         |
| Heroin hydrochloride      | Grey-brown                                   | Orange-brown (P)                     | 1.40        | 10                        |
|                           |  | Purple** (P)                         | 0.44        | 10                        |
|                           |  | Orange-yellow (P)                    | 0.63        | 10                        |
|                           |  | Pink** (P)                           | 0.94        | 10                        |
|                           |  | Orange (B)                           | 0.25        | 10                        |
| Hyoscine N-methyl bromide | Yellow-brown                                 | Pink (B)                             | 0.58        | 3                         |
|                           |  | Purple (B)                           | 0.70        | 4                         |
| Imipramine hydrochloride  | Violet-brown                                 | Brown** (B)                          | 0.80        | 3                         |
|                           |  | Pink (P)                             | 0.90        | 3                         |
|                           |  | Orange-brown (P)                     | 1.43        | 10                        |
|                           |  | Brown (B)                            | 0.21        | 3                         |
|                           |  | Purple (P)                           | 0.34        | 3                         |
| Iproniazid phosphate      | Violet                                       | Orange (P)                           | 0.94        | 3                         |
|                           |  | Orange-brown (P)                     | 1.44        | 10                        |
|                           |  | Purple (P)                           | 0.47        | 10                        |
|                           |  | Red-orange (B)                       | 0.14        | 5                         |
| Isoniazid                 | Violet                                       | Green (B)                            | 0.33        | 5                         |
|                           |  | Purple (P)                           | 0.49        | 4                         |
| Lignocaine hydrochloride  | Violet-brown                                 | Violet-brown (P)                     | 0.68        | 6                         |
|                           |  | Pink (B)                             | 0.77        | 5                         |
|                           |  | Violet-brown/black (P)               | 1.45        | 10                        |
|                           |  | Pink** (B)                           | 0.70        | 3                         |
|                           |  | Purple (P)                           | 1.09        | 4                         |
| Meprylcaine hydrochloride | Red-brown                                    | Orange-brown (P)                     | 1.42        | 10                        |
|                           |  | Blue (B)                             | 0.25        | 3                         |
| Mepyramine maleate        | Grey-brown                                   | Yellow (P)                           | 0.37        | 6                         |
|                           |  | Purple (B)                           | 0.48        | 3                         |
|                           |  | Yellow (P)                           | 0.88        | 8                         |
|                           |  | Pink** (P)                           | 0.90        | 3                         |
|                           |  | Orange (P)                           | 1.30        | 8                         |
|                           |  | Violet-brown (P)                     | 1.43        | 10                        |
|                           |  | Blue (B)                             | 0.30        | 3                         |
| Methadone hydrochloride   | Violet-brown                                 | Purple (B)                           | 0.58        | 3                         |
|                           |  | Orange** (B)                         | 0.70        | 3                         |
|                           |  | Orange (P)                           | 0.91        | 4                         |
|                           |  | Red-orange (P)                       | 1.32        | 6                         |
|                           |  | Brown (P)                            | 1.42        | 10                        |
|                           |  |                                      |             |                           |

(Continued on p. 274)

TABLE II (continued)

| <i>Compound</i>                | <i>Colour of spot before TLC development</i> | <i>Colour after TLC development*</i> | <i>R<sub>FNA</sub></i> | <i>Relative intensity</i> |
|--------------------------------|--|--------------------------------------|------------------------|---------------------------|
| Methoxyphenamine hydrochloride | Violet                                       | Pink** (B)                           | 0.19                   | 3                         |
|                                |  | Green** (B)                          | 0.23                   | 3                         |
|                                |  | Pink** (B)                           | 0.30                   | 3                         |
|                                |  | Purple (P)                           | 0.45                   | 3                         |
|                                |  | Orange** (B)                         | 0.59                   | 4                         |
|                                |  | Green-blue (B)                       | 0.68                   | 4                         |
|                                |  | Yellow (P)                           | 0.83                   | 6                         |
| Morphine hydrochloride         | Grey   | Purple/red (P)                       | 1.39                   | 10                        |
|                                |  | Purple (P)                           | 0.34                   | 3                         |
|                                |  | Orange-brown (B)                     | 0.58                   | 3                         |
|                                |  | Violet-brown (P)                     | 1.39                   | 10                        |
| Nalorphine hydrobromide        | Dark grey                                    | Red-brown (B)                        | 0.25                   | 3                         |
|                                |  | Purple (P)                           | 0.35                   | 3                         |
|                                |  | Orange (P)                           | 0.66                   | 8                         |
|                                |  | Purple (P)                           | 1.05                   | 10                        |
| Narcotine                      | Dark orange                                  | Blue (B)                             | 0.29                   | 3                         |
|                                |  | Yellow-orange** (P)                  | 0.37                   | 4                         |
|                                |  | Yellow-orange** (P)                  | 0.42                   | 3                         |
|                                |  | Red-orange (P)                       | 1.04                   | 10                        |
|                                |  | Red-brown (P)                        | 1.42                   | 10                        |
| Nitrazepam                     | Pale brown                                   | Nil                                  |                        |                           |
| Oxyphencyclimine hydrochloride | Red-brown                                    | Purple (B)                           | 0.29                   | 10                        |
| Papaverine hydrochloride       | Orange-brown                                 | Orange (P)                           | 0.28                   | 5                         |
|                                |  | Green (P)                            | 0.59                   | 3                         |
|                                |  | Grey (P)                             | 1.21                   | 3                         |
|                                |  | Yellow-orange/black (P)              | 1.37                   | 10                        |
|                                |  | Purple (B)                           |                        |                           |
| Pethidine hydrochloride        | Violet-brown                                 | Purple (B)                           | 0.59                   | 3                         |
|                                |  | Pink** (P)                           | 1.02                   | 3                         |
|                                |  | Brown-orange (P)                     | 1.40                   | 10                        |
| Pholcodine                     | Violet                                       | Purple (P)                           | 0.39                   | 4                         |
|                                |  | Brown-orange (P)                     | 0.60                   | 3                         |
|                                |  | Orange-violet (P)                    | 1.34                   | 10                        |
| Physostigmine salicylate       | Red-brown                                    | Red-orange (B)                       | 0.20                   | 4                         |
|                                |  | Purple (B)                           | 0.48                   | 4                         |
|                                |  | Pink** (P)                           | 0.80                   | 3                         |
|                                |  | Pink (P)                             | 0.97                   | 3                         |
|                                |  | Brown-orange (P)                     | 1.23                   | 9                         |
|                                |  | Brown-orange (P)                     | 1.40                   | 10                        |
|                                |  | Brown-orange (B)                     | 0.12                   | 5                         |
| Procaine hydrochloride         | Brown-violet                                 | Brown (B)                            | 0.26                   | 3                         |
|                                |  | Purple (P)                           | 0.45                   | 5                         |
|                                |  | Brown-orange (B)                     | 0.68                   | 4                         |
|                                |  | Pink** (B)                           | 0.81                   | 3                         |
|                                |  | Orange (P)                           | 0.87                   | 7                         |
|                                |  | Orange-brown (P)                     | 1.36                   | 10                        |
|                                |  | Pink (B)                             | 0.37                   | 3                         |
| Prochlorperazine dimaleate     | Red-brown                                    | Purple (B)                           | 0.53                   | 3                         |
|                                |  | Pink (B)                             | 0.63                   | 4                         |
|                                |  | Yellow (P)                           | 0.77                   | 5                         |
|                                |  | Red-orange (P)                       | 0.94                   | 5                         |
|                                |  | Orange-brown (P)                     | 1.44                   | 10                        |
|                                |  |                                      |                        |                           |



TABLE II (continued)

| Compound                        | Colour of spot before TLC development | Colour after TLC development* | $R_{PNA}$ | Relative intensity |
|---------------------------------|---------------------------------------|-------------------------------|-----------|--------------------|
| Promethazine                    | Red-brown/green-blue                  | Brown-orange (B)              | 0.14      | 4                  |
|                                 |                                       | Purple (B)                    | 0.42      | 3                  |
|                                 |                                       | Brown-orange (B)              | 0.66      | 3                  |
|                                 |                                       | Red-orange** (P)              | 0.82      | 4                  |
|                                 |                                       | Orange-brown/green-black (P)  | 1.46      | 10                 |
| Quinidine sulphate              | Grey-violet                           | Purple (B)                    | 0.68      | 4                  |
|                                 |                                       | Brown-orange (P)              | 1.41      | 10                 |
| Quinine sulphate                | Grey-violet                           | Purple (B)                    | 0.66      | 3                  |
|                                 |                                       | Violet-orange (P)             | 1.43      | 10                 |
| Reserpine                       | Dark-brown                            | Brown-orange (P)              | 1.45      | 10                 |
| Ritalin                         | Dark grey                             | Purple (B)                    | 0.32      | 5                  |
|                                 |                                       | Purple (P)                    | 0.80      | 3                  |
|                                 |                                       | Blue (P)                      | 1.17      | 10                 |
|                                 |                                       | Black (P)                     | 1.46      | 10                 |
| Strychnine hydrochloride        | Brown-violet                          | Grey-purple (B)               | 0.49      | 3                  |
|                                 |                                       | Brown/pink (P)                | 1.31      | 10                 |
| Trifluoperazine dihydrochloride | Brown                                 | Purple (P)                    | 0.45      | 3                  |
|                                 |                                       | Orange (P)                    | 0.90      | 4                  |
|                                 |                                       | Orange-brown (P)              | 1.40      | 10                 |
| Trimethoprim                    | Yellow                                | Nil                           |           |                    |
| Yohimbine hydrochloride         | Dark brown                            | Purple (P)                    | 0.40      | 3                  |
|                                 |                                       | Violet-brown (P)              | 1.34      | 10                 |

\* Ninhydrin produces a faint grey colour ( $R_{PNA}$  0.26), a faint yellow colour ( $R_{PNA}$  1.00), a yellow colour ( $R_{PNA}$  1.16) and an orange colour ( $R_{PNA}$  1.45). These have been omitted from the profiles. (P) denotes a patch; (B) denotes a band.

\*\* Colour does not appear consistently.

#### $R_{PNA}$ values

As  $R_F$  values are, *per se*, seldom reproducible their values relative to that of *p*-nitroaniline ( $R_{PNA}$ ) were used. *p*-Nitroaniline was chosen as it is coloured and has an  $R_F$  value of about 0.50, thereby providing a well spread range of  $R_{PNA}$  values for the spots, etc., making up the profiles. It is applied just prior to the development of the plates as it tends to vapourise when heated.

The  $R_{PNA}$  values recorded in the tables are the average of five determinations and in most instances were fairly reproducible. These values may vary to some extent for a particular colour appearing commonly for a particular compound at 100 and 160°C. This could be due to changes in plate properties when heated at 100 and 160°C, or to different admixtures of compounds formed at the two temperatures and having different effects on the mobility of the spots.

#### Operating conditions

The highest concentration of ninhydrin possible without its precipitation at refrigerator temperature is 10% (w/v), and this concentration was chosen to limit overspotting on the compounds to one or two applications. Amounts of 20  $\mu$ g of drugs were selected so as to produce a reasonable range of intensities in the profiles. Larger amounts were found to distort the stronger bands. Studies were restricted to

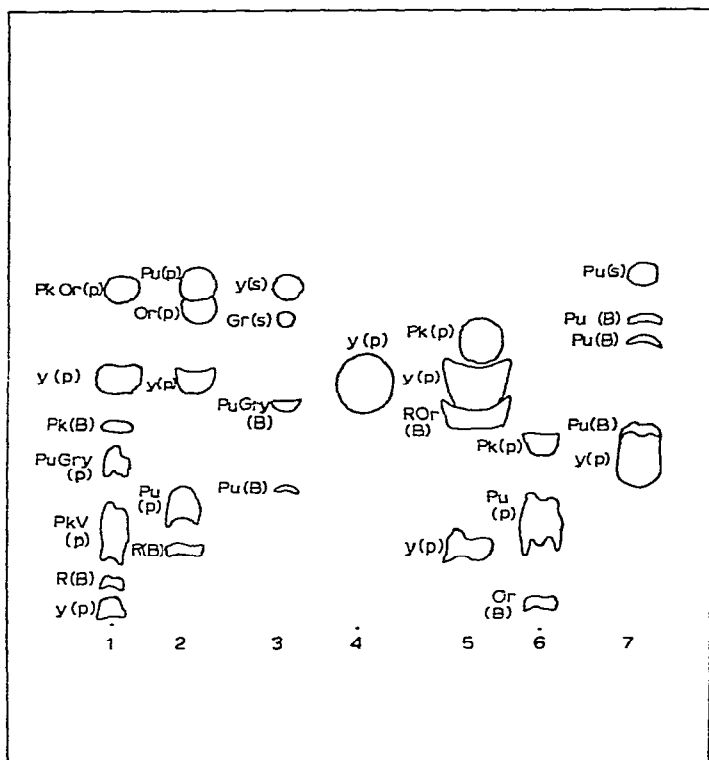


Fig. 1. Schematic diagram of a chromatogram showing profiles of ninhydrin complexes of a few compounds formed at 100°C. 1 = Emetine hydrochloride; 2 = amphetamine sulphate; 3 = prochlorperazine dimaleate; 4 = *p*-nitroaniline; 5 = narcotine; 6 = lignocaine hydrochloride; 7 = ritalin. Gr = Green; Gry = grey; Or = orange; Pk = pink; Pu = purple; R = red; V = violet; Y = yellow. (B) = Band; (p) = patch; (s) = spot.

100 and 160°C as significant differences in colour were not observed at intermediate temperatures, and poor quality spots were observed at temperatures above 160°C. Optimal results were obtained when the heating time was 30 min.

#### *Discriminative power*

A combination of colours, patterns,  $R_{\text{PNA}}$  values and relative intensities provides an excellent means of identifying a compound on just one plate. Of the 49 compounds that gave colours, no two compounds had identical features. When only limited amounts of compounds are available for identification, the method promises to be the best first step in a series of tests.

For identifying an unknown compound, it would be advisable to spot various concentrations of the unknown compound with 20- $\mu\text{g}$  amounts of standards and to compare the colours produced at every step of the procedure.

#### *Nature of the ninhydrin complexes*

Ninhydrin reacts with organic compounds in a variety of ways, ranging from simple addition and condensation reactions to Diels-Alder, redox, free radical and

cleavage reactions and fused ring formation<sup>14</sup>. An explanation of the reaction between phenethylamines and ninhydrin was recently attempted<sup>11</sup>. We also attempted to correlate colour profiles with structures, but no correlation could be found.

## CONCLUSION

This study has shown that by simply overspotting a wide range of basic-drugs with ninhydrin and heating, a wide variety of coloured complexes, in varying amounts are formed. These are easily resolved by TLC development and form profiles that are highly characteristic of the compound and specific enough for the preliminary identification of such compounds.

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