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# THE LIQUID CHROMATOGRAPHIC PROPERTIES OF PHENOTHIAZINES

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## SUMMARY

The high-pressure liquid chromatographic behaviour of different groups of phenothiazines (drug substances) has been investigated on 10-$\mu$m silica gel particles. Variation of the ammonia concentration between 0.5 and 1.5% in an isopropanol–diisopropyl ether (15:85) mixture permits the adjustment of the solvent system to fit the different basicities of the various groups of interest. While the capacity factors ($k'$) for pairs of compounds in two homologous oxidation series vary considerably owing to differences in basicity, there is reasonably good agreement between the relative retention ($\alpha$) values. This fact can be utilized in order to identify phenothiazine homologues of series that have not previously been studied. Elutropic diagrams in connection with $\alpha$ values can be used to predict the chromatographic behaviour of new groups of phenothiazines.

## INTRODUCTION

Many phenothiazine derivatives are widely known for their pharmaceutical properties[1]. In order to control pharmaceutical formulations and to study the metabolic breakdown pattern, it is important that suitable analytical procedures with adequate selectivity and sensitivity be made available. Two recent reviews have surveyed the present state of methods of analysis for phenothiazine drugs[1,2].

Ultraviolet (UV) spectrophotometry has been widely used, as the presence of an extensive conjugated $\pi$-system makes these compounds strongly UV active[3]. The quantitation of some phenothiazines and oxidation products by spectrofluorimetry has been reported[2].

In order to achieve the required specificity when mixtures of these compounds are analyzed for individual components, their chromatographic properties are utilized and many paper, thin-layer and gas chromatographic methods have been described[1,2].

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In thin-layer chromatography, UV and fluorescence properties have been used for the evaluation of the chromatographic zones. Some recent work[4] showed that it is possible to combine UV and fluorescence densitometry for the determination of phenothiazines and oxidation products. Oxidation of the zones was effected with a hydrogen peroxide solution similar to a method described by Ragland and Kinross-Wright[3,5]. More than a ten-fold increase in sensitivity can be achieved by this approach.

Several gas chromatographic methods have also been reported[1,2]. Retention times for sulphoxides were generally too high (40–60 min) to permit sensitive and efficient determinations[6]. Volatility and stability problems persist in many instances and it was for this reason that high-speed liquid chromatography (HSLC) was chosen as an alternative method. With the excellent chromophores of the phenothiazines, both UV and, for some oxidation products also fluorescence detection, seemed appropriate for this type of work.

The first HSLC work on phenothiazines was reported by Muusze and Huber[7], who utilized a number of liquid–liquid systems. In our work, attention was focused on solid–liquid systems, which are generally more suitable for routine investigations. A comparison of the chromatographic behaviour of homologous series was attempted in order to facilitate the identification of derivatives and to predict the behaviour of other groups of phenothiazines.

## EXPERIMENTAL

### *Apparatus*

All the studies were carried out with a Hewlett-Packard 1010A liquid chromatograph. The UV detectors (Perkin-Elmer Model 250) and the LDC monitor, both with detection at 254 nm, were used together with a Philips PM 8221 recorder. The chromatographic columns were made of stainless steel, 25 cm long and 3.0 mm I.D. Dacron sail-cloth served to close the column at the end. Swagelok fittings were used throughout the system.

The samples were injected directly on to the column into a plug of PTFE wool by means of a 10-$\mu$l Hamilton syringe or a 10-$\mu$l Scientific Glass Engineering Corp.

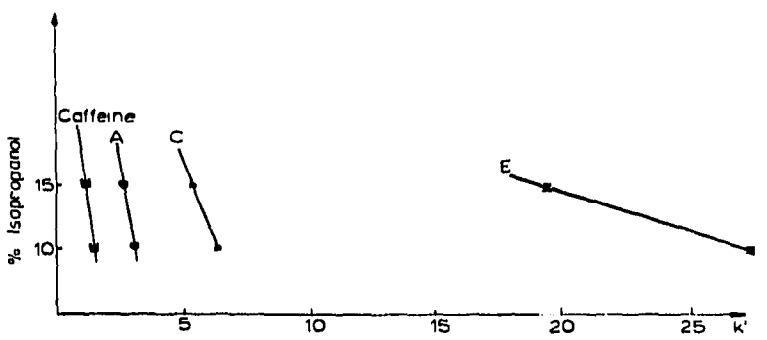



Fig. 1. Variations of $k'$ values for homologues of the thioridazine series as a function of the isopropanol content. Solvent: isopropanol + diisopropyl ether + 0.5% ammonia. For A, C and E, see Table I.

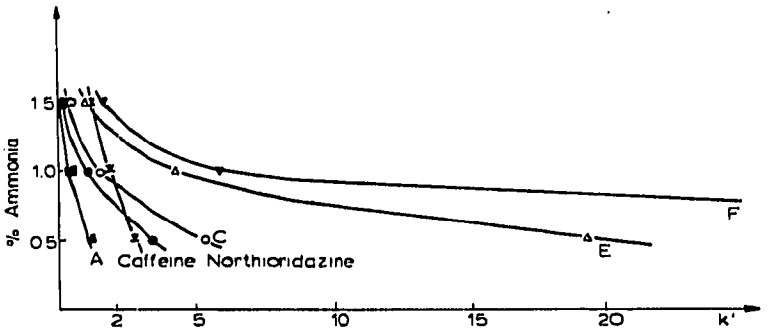



Fig. 2. Variations of $k'$ values for homologues of the thioridazine series as a function of the ammonia content. Solvent: isopropanol–diisopropyl ether (15:85) + ammonia.

Model B syringe. The injection volumes were 2–5 $\mu$l; the substances were dissolved in mobile phase. Septum-injection systems of Varian and Portmann Ltd. (Basle, Switzerland) and the Hewlett-Packard injection device were used. The columns were packed with silica gel of particle size 10 $\mu$m according to a procedure described earlier[8].

*Reagents*

Merckosorb SI-60 (Merck, Darmstadt, G.F.R.) with an average particle size of 10 $\mu$m and pore openings of 60 Å were used. The pre-treatment was carried out as described earlier[8], the equal-density mixture consisting of reagent-grade tetrabromoethane and tetrachloroethylene (Fluka, Buchs, Switzerland). Re-distilled reagent-grade diisopropyl ether (Fluka) and analytical-reagent grade isopropanol (Merck) were used as chromatographic solvents. Analytical-reagent grade ammonia solution (25%, w/w) (Merck) was added.

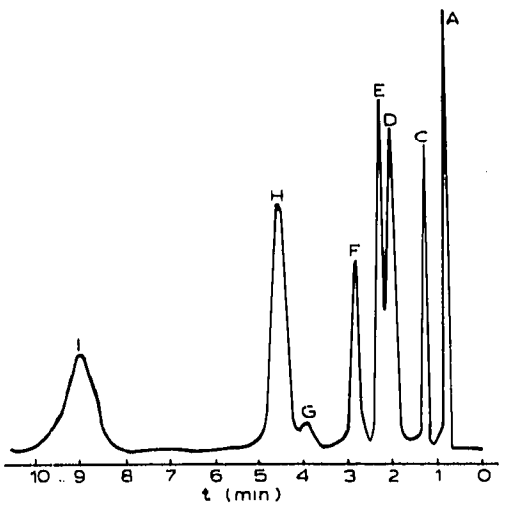


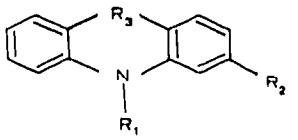

Fig. 3. Typical pattern for the homologues of the thioridazine series (Table I). Column dimensions, 25 × 2.4 mm I.D.; silica gel, 10-$\mu$m particle size; mobile phase, ammonia–diisopropyl ether–isopropanol (1.5:85:15); flow-rate, 1.74 ml/min.

## RESULTS AND DISCUSSION

Of the many solvent systems tested in our laboratories, the most suitable one was a mixture of isopropanol, diisopropyl ether and ammonia. Variation of the isopropanol content with fixed proportions of diisopropyl ether and ammonia did not result in a significant improvement in the separation pattern (Fig. 1), whereas small variations in the ammonia concentration gave improved separations (Fig. 2). From Fig. 2 it can be seen that at ammonia concentrations between 0.5 and 1.5%, almost any desired separation in an oxidation series can be achieved in such a way as to fit the internal standard (caffeine) between the other peaks and still obtain the most efficient separation. A typical separation with a solvent mixture containing 1.3% of ammonia is shown in Fig. 3. Similar separations of other groups of phenothiazines can be achieved by adjusting slightly the ammonia concentration or the basicity of the mobile phase depending on the basic nature of the phenothiazines under investiga-

TABLE I

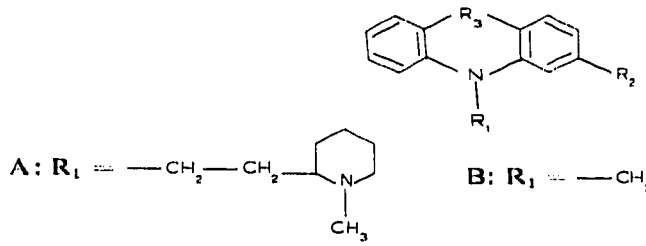
OXIDATION HOMOLOGUES OF THE THIORIDAZINE SERIES

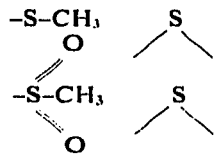
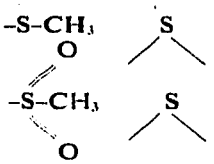
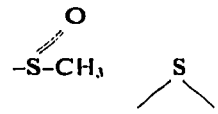
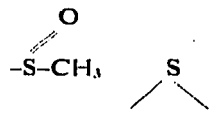
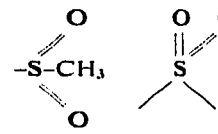
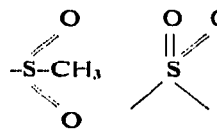
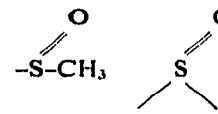
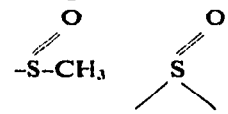



| *Order of peaks* | $R_2$ | $R_3$ | $k'$ |
|---|---|---|---|
| A | $-S(=O)-CH_3$ | S | 0.11 |
| C | $-S(=O)-CH_3$ | S | 0.63 |
| D | $-S-CH_3$ | $S(=O)$ | 1.5 |
| E | $-S-CH_3$ | S | 1.72 |
| F | $-S(=O)-CH_3$ | $S(=O)_2$ | 2.5 |
| H | $-S(=O)-CH_3$ | $S(=O)$ | 4.89 |
| I | $-S(=O)-CH_3$ | $S(=O)$ | 9.44 |

TABLE II

COMPARISON OF $\alpha$ VALUES OF OXIDATION HOMOLOGUES OF THE THIORIDAZINE AND NORTHIORIDAZINE SERIES




| *Order of peaks* | *A* | | | *B* | | |
|---|---|---|---|---|---|---|
| | $R_2$ | $R_3$ | $\alpha$ | $R_2$ | $R_3$ | $\alpha$ |
| a |  | | 5.70 |  | | 5.41 |
| b |  | | 15.48 |  | | 13.26 |
| c |  | | 22.5 |  | | 19.3 |
| d |  | | 85 |  | | 79 |

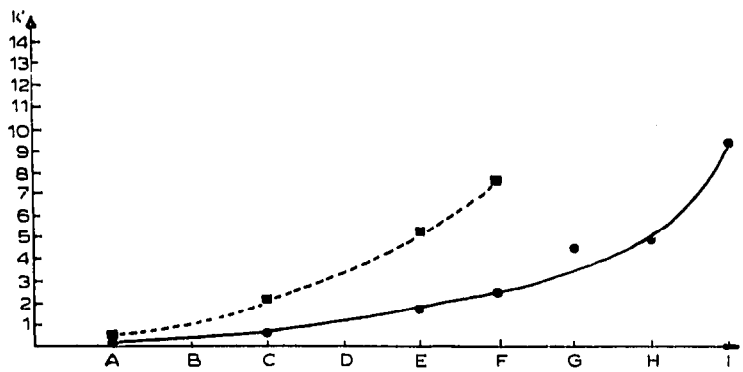



Fig. 4. Trend of $k'$ values for the homologues of the thioridazine (●) and northioridazine (■) series as a function of the separation order.

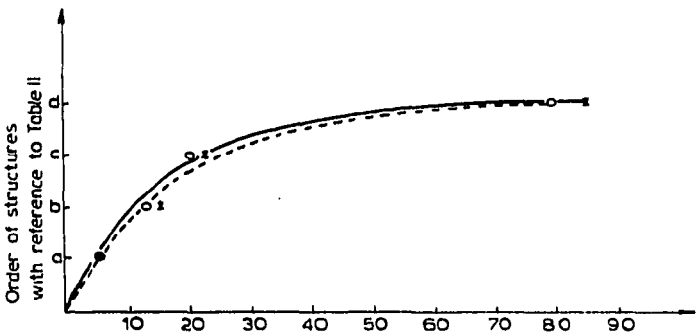



Fig. 5. Comparison and trend of $\alpha$ values for the thioridazine (*) and northioridazine (○) series.

of chromatographic peaks in the two series investigated (Tables I and II) is shown in Fig. 4.

A different series of phenothiazines (Table II, B) was used for comparison. Four oxidation products were available and a similar separation trend was observed (Fig. 5). The $k'$ values* were determined under the same chromatographic conditions but, owing to the higher basicity of the compounds of this series (N-unsubstituted piperidines), the retention was stronger.

The $\alpha$ values** were determined with respect to the non-oxidized compounds in each series. A comparison of the $\alpha$ values for the two series of phenothiazines demonstrates, however, that the chromatographic characteristics are indeed transferable (see Table II). The $\alpha$ values for the corresponding oxidation products agree to within about 5–15%, as can also be seen in Fig. 5. Knowledge of the $\alpha$ values under well defined chromatographic conditions should therefore permit the characterization of unknown peaks of similar homologous series. In addition, with a knowledge of the chromatographic behaviour of one series, one could predict separation patterns of similar homologous series.

## ACKNOWLEDGEMENTS

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* $k'$ = capacity factor = $(t_R - t_0)/t_0$, where $t_R$ = net retention time and $t_0$ = non-sorbed time.
** $\alpha$ = relative retention = $k'_2/k'_1$.