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Note

High-performance liquid chromatographic separation of the N- and S-oxides of fluphenazine and fluphenazine decanoate*

W. F. HEYES* and J. R. SALMON
Squibb Institute for Medical Research, International Development Laboratory, Moreton, Merseyside L46 1QW (Great Britain)
and
W. MARLOW
School of Pharmacy, The Polytechnic, Liverpool (Great Britain)
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Oxidation of the phenothiazine group of drugs is a well documented feature of these compounds. The N- and S-oxides produced may be formed metabolically[1] or by an ageing process which is often the result of photooxidation. The fluphenazine molecule contains four potential oxidation sites, thus a total of fifteen oxides are theoretically possible.

To study the oxidation of fluphenazine and fluphenazine decanoate in various media, a suitable chromatographic method was required which would separate the individual components of the expected mixture. For this purpose the use of reversed-phase high-performance liquid chromatography (HPLC) was investigated.

EXPERIMENTAL

Reagents

AnalaR grade methanol (BDH, Poole, Great Britain) and HPLC grade acetonitrile (Rathburn, Walkerburn, Great Britain) were used as solvents. Ammonia, ammonium carbonate and potassium chloride (BDH) were of reagent grade.

Apparatus

HPLC apparatus was assembled from commercially available components and was comprised of a Cecil 212 variable-wavelength detector and an Altex 110 HPLC pump.

The column (200 × 4.6 mm) of SAS-Hypersil (Shandon Products, Runcorn, Great Britain) was prepared by slurry-packing the material from isopropanol as recommended by the manufacturers. A Haskel air-driven fluid pump at a pressure of 4600 p.s.i. was utilised for the packing procedure.

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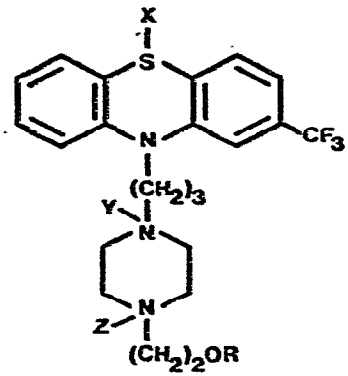
TABLE I

OXIDES OF FLUPHENAZINE AND FLUPHENAZINE DECANOATE



Compound	*X*	*Y*	*Z*	*R*
1	—	—	O	H
2	—	O	—	H
3	O	—	—	H
4	—	O	O	H
5	O	—	O	H
6	O	O	—	H
7	O	O	O	H
8	—	—	O	COC_9H_{19}
9	—	O	—	COC_9H_{19}
10	O	—	—	COC_9H_{19}
11	—	O	O	COC_9H_{19}
12	O	—	O	COC_9H_{19}
13	O	O	—	COC_9H_{19}
14	O	O	O	COC_9H_{19}

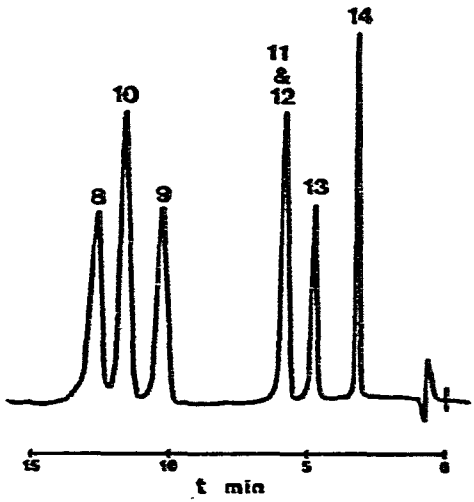


Fig. 1. Separation of fluphenazine decanoate oxides (Table I). Column, SAS-Hypersil; mobile phase, methanol–acetonitrile–1% ammonium carbonate solution (1:1:1).

Preparation of oxides

All compounds (Table I) were prepared by standard chemical oxidation techniques and identified spectroscopically. No evidence for the oxidation of the phenothiazinyl nitrogen atom was observed during the synthetic work.

RESULTS AND DISCUSSION

Fluphenazine decanoate oxides

A mobile phase of methanol–acetonitrile–1% aqueous ammonium carbonate (1:1:1), which was originally devised for the separation of fluphenazine from fluphenazine decanoate[3], resulted in the separation of six of the seven oxides (Fig. 1). Compounds 11 and 12 chromatographed together in this mobile phase. The ratio of methanol to acetonitrile was found to be critical, the best resolution being obtained with the two organic components in the ratio of 1:1.

For the complete separation of all seven compounds an alternative mobile phase comprising of methanol–10% aqueous ammonia, in the ratio of 2:1, was developed (Fig. 2). The presence of ammonia was essential to prevent tailing.

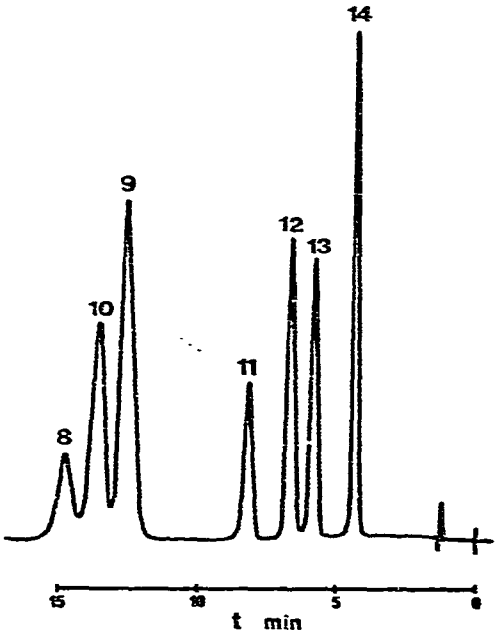


Fig. 2. Separation of fluphenazine decanoate oxides (Table I). Column, SAS-Hypersil; mobile phase, methanol–10% aqueous ammonia (1:1).

In both of the above systems the oxides were well separated from, and eluted prior to the parent compound fluphenazine decanoate.

Fluphenazine oxides

Some difficulty was encountered in chromatographing this series of compounds. Using a similar system to that described above (methanol–10% aqueous

ammonia) but in the ratio of 1:1, a partial resolution of fluphenazine oxides (compounds 1–7) was accomplished. The presence of ammonia reduced the degree of tailing which was particularly noticeable with compounds 1 and 4 but did not completely eliminate this effect. In a recent study[4] of the role of electrolytes in the HPLC of phenothiazines, the addition of potassium chloride has been recommended to improve the chromatographic resolution. The beneficial effect of incorporating potassium chloride in the mobile phase for use with the oxides of fluphenazine is shown in Fig. 3. Subsequent experiments demonstrated that both ammonia and potassium chloride were required for the satisfactory chromatography of this series of oxides.

By partially replacing the methanol component of the mobile phase by acetonitrile an improved resolution of compounds 1–7 was achieved (Fig. 4). Again the

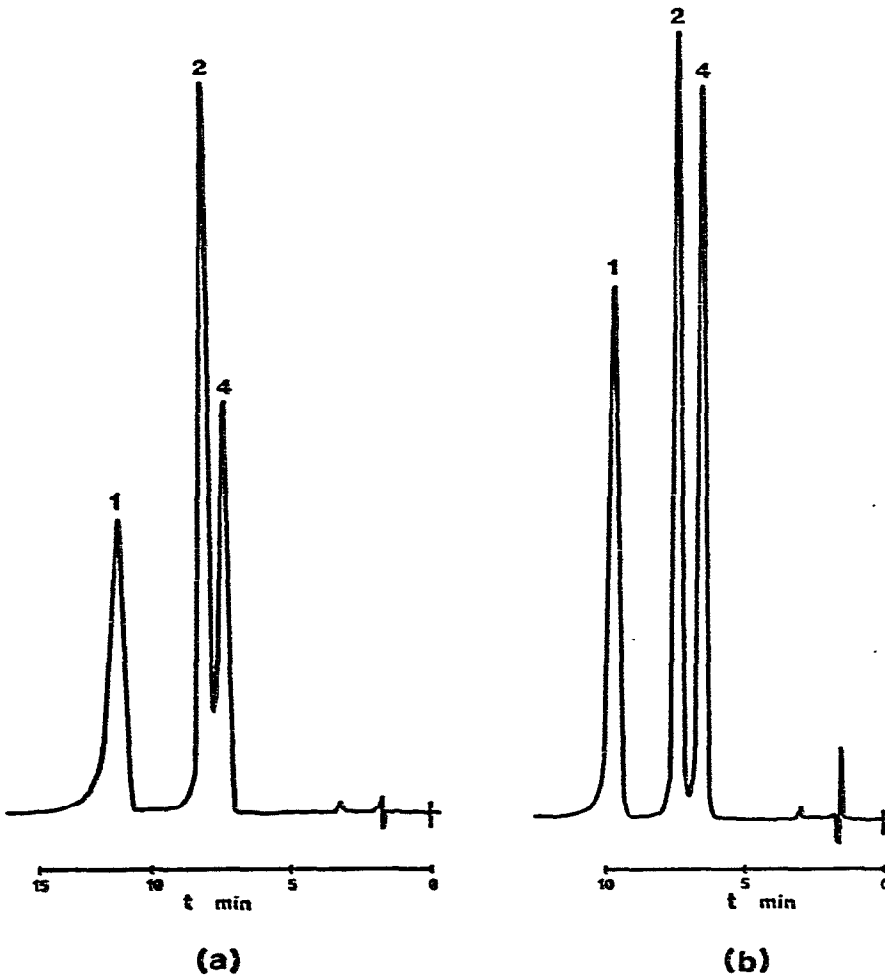


Fig. 3. Effect of potassium chloride on resolution of fluphenazine N-oxides. (a) Mobile phase, methanol–10% aqueous ammonia (1:1); (b) mobile phase, methanol–10% aqueous ammonia containing 1% potassium chloride (1:1).

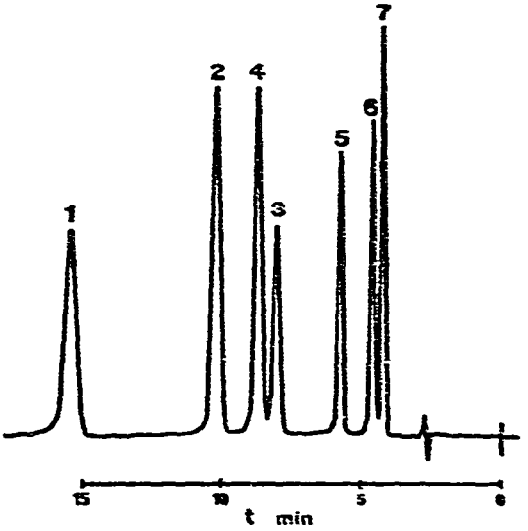


Fig. 4. Separation of fluphenazine oxides (Table I). Column, SAS-Hypersil; mobile phase, methanol–acetonitrile–10% aqueous ammonia containing 1% potassium chloride (1:1:3).

methanol–acetonitrile ratio was found to be critical. Optimum resolution was obtained with the two organic components present in the ratio of 1:1.

As expected, the oxides were eluted prior to the parent compound fluphenazine (capacity factor, $k' = 6.5$).

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