Journal of Chromatography, 156 (1978) 309–316

CHROM. 10,943

SOME ASPECTS OF THE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF FLUPHENAZINE AND ITS ESTERS*

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(Received February 7th, 1978)

SUMMARY

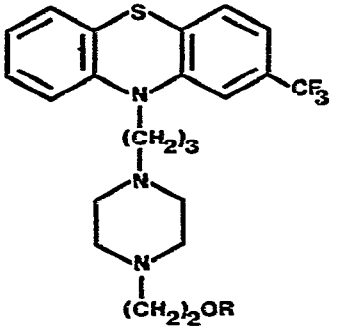
A reversed-phase high-performance liquid chromatography (HPLC) system for the separation of fluphenazine from fluphenazine decanoate is described.

To evaluate the robustness of the proposed procedure the effect of varying some of the chromatographic parameters was determined. The chain length of the alkyl stationary phase, the pH of the system and the ammonium carbonate concentration in the mobile phase were found to be non-critical.

The relationship between mobile phase flow-rate and plate height was investigated and the use of 50-, 100- and 200-mm HPLC columns compared.

INTRODUCTION

Fluphenazine (I) is a member of the class of drugs known collectively as phenothiazines which are widely used as sedatives and tranquillisers.



I R=H, fluphenazine

II $C-(CH_2)_6CH_3$, fluphenazine decanoate

$C-(CH_2)_5CH_3$, fluphenazine enanthate

* Presented in part at the 37th International Congress of Pharmaceutical Sciences (F.I.P.), The Hague (The Netherlands), September 1977.

Substitution of an ester group in the piperazine–ethanol side chain (II) results in a form of the drug with slow release properties. A sustained medication over three weeks is thus possible from administration of a single dose, which is particularly advantageous in the treatment of acute schizophrenia.

The stability of the formulated products has previously been determined using a conventional column chromatographic separation followed by UV spectrophotometric quantitation. However, in order to increase sample throughput the faster technique of high-performance liquid chromatography (HPLC) was investigated.

In the case of fluphenazine decanoate one possible decomposition product is fluphenazine base, which can be predicted by a consideration of the chemistry of the ester. The decomposition product may arise from hydrolysis of the ester when stored under adverse conditions. A stability indicating assay for fluphenazine decanoate must, therefore, be capable of separating the ester from decomposition products such as fluphenazine base.

Preliminary experiments indicated that a suitable HPLC system for this purpose should be comprised of a Partisil-ODS reversed-phase HPLC column together with a mobile phase of methanol–acetonitrile–1 % ammonium carbonate solution (1:1:0.25) (Fig. 1). Ammonium carbonate solution was preferred for the aqueous phase, to ensure the maintenance of alkaline conditions when fluphenazine would be present as the free base. The presence of ammonium carbonate has also been reported to improve the separation process with regard to some antibiotics[1].

Inherent in any chromatographic system are many parameters which, if varied,

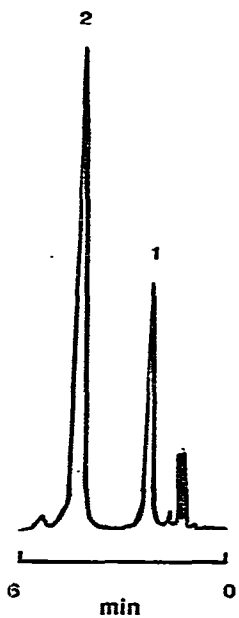


Fig. 1. HPLC separation of fluphenazine and its decanoate ester. Column, Partisil-ODS; mobile phase, methanol–acetonitrile–1 % ammonium carbonate solution (1:1:0.25); flow-rate, 2 ml/min. Peaks: 1 = fluphenazine; 2 = fluphenazine decanoate.

may adversely affect the performance of an assay. In an attempt to define the optimal operating conditions and to determine the limitations of the proposed procedure, the effect of varying some of the chromatographic parameters has been evaluated.

EXPERIMENTAL

Materials and reagents

Analar grade reagents were used wherever possible; similarly, Analar grade methanol (BDH, Poole, Great Britain) and HPLC grade acetonitrile (Rathburn, Walkerburn, Great Britain) were employed as solvents.

Reversed-phase column packing materials

Column packings were prepared using the following procedures.

Partisil-TMS. 10 g Partisil-10 (Reeve Angel, Clifton, N.J., U.S.A.) was dried at a temperature of 120° before refluxing for an hour with a solution of trimethylchlorosilane (TMCS) in xylene (5% v/v). The TMCS solution was then decanted off, replaced by a fresh 5% solution of TMCS in xylene and the mixture refluxed for a further hour. The resulting Partisil-TMS was separated by filtration, washed with methanol and finally dried under vacuum.

Partisil-ODS. A similar procedure to that described above for the preparation of Partisil-TMS was adopted except that a 5% v/v solution of octadecyltrichlorosilane (ODTCS) (Aldrich, Milwaukee, Wisc., U.S.A.) was substituted for the first reflux. The second refluxing with TMCS solution was retained in order to block the remaining active sites on the silica which are sterically inaccessible to ODTCS reagent[2].

Column packing procedure

A carbon tetrachloride slurry of each bonded-phase packing material was prepared (20%, w/v, as recommended by Bar *et al.*[3]) and, to ensure complete dispersion in the solvent, the slurry was subjected to ultrasonic treatment for a few minutes. 50-, 100- and 200-mm stainless-steel columns, 4.6 mm I.D., were then packed with the required material at a pressure of 4600 p.s.i. using a Haskel air-driven fluid pump. Packed columns were finally washed with 500 ml methanol to remove all traces of carbon tetrachloride.

HPLC equipment

Most of the experimental work was carried out using a gas pressure pump and a Cecil 212 variable-wavelength UV detector set at 260 nm. Where accurate constant-flow conditions were required the gas pump was replaced by an Altex Model 110 reciprocating pump.

RESULTS AND DISCUSSION

Effect of stationary phase alkyl chain length

The chromatographic system originally developed employed a Partisil-10 support with a bonded octadecyl stationary phase. To assess the influence of the stationary phase alkyl chain length on the chromatographic separation of fluphenazine

and fluphenazine decanoate, the ODS was replaced by a stationary phase composed of trimethyl groups, again bonded to a Partisil-10 support. A suitable separation of the compounds under investigation was achieved on the Partisil-TMS column by simply altering the ratio of the mobile phase components. Hence it would appear that, in this case, the stationary phase alkyl chain length is not critical provided that the correct mobile phase is carefully chosen.

Influence of pH

The partition characteristics of some fluphenazine esters between heptane and buffer solutions of various pH values have been reported[4] which demonstrates the critical nature of the pH in this system. By analogy, pH could thus be expected to influence the chromatography of fluphenazine when using the proposed system of methanol acetonitrile and aqueous ammonium carbonate. Table I shows the effect of the mobile phase pH on the capacity factors of fluphenazine, fluphenazine enanthate and fluphenazine decanoate for columns of Partisil-TMS and Partisil-ODS. Over the pH range 11–7 little change in the capacity factors of the esters was observed but below pH 7, a decrease in mobile phase pH resulted in a decrease in the capacity factor.

TABLE I

INFLUENCE OF pH ON CAPACITY FACTORS k' OF FLUPHENAZINE ESTERS

pH of mobile phase	*Partisil-TMS column*			*Partisil-ODS column*		
	Fluphenazine	*Fluphenazine enanthate*	*Fluphenazine decanoate*	*Fluphenazine*	*Fluphenazine enanthate*	*Fluphenazine decanoate*
11	1.60	4.20	8.00	—	—	—
10	—	—	—	1.73	3.93	7.86
9	1.56	4.20	8.00	—	—	—
8	—	—	—	1.94	4.11	8.11
7	1.56	4.23	7.79	1.44	3.13	4.50
6	1.21	3.33	6.13	0.94	2.25	4.25
4	1.07	2.91	5.39	0.91	2.00	3.63
2	0.42	1.40	2.51	0.38	0.94	1.63

As the pH of the proposed mobile phase is approximately 8, this parameter may be considered to be non-critical.

Effect of ammonium carbonate concentration

A previous paper[5] describing a reversed-phase HPLC assay for nortriptyline, reported that an increase in the ammonium carbonate concentration of the mobile phase aqueous component resulted in a corresponding decrease in the retention time of nortriptyline. A similar investigation with the present chromatographic system proposed for fluphenazine, failed to show any noticeable effect when the ammonium carbonate concentration was varied over the range 0.1–1%.

Mobile phase component ratio

The mobile phase chosen for use with a 200-mm column of Partisil-TMS was

methanol–acetonitrile–1% ammonium carbonate solution (1:1:1). Varying the ratio of methanol to acetonitrile whilst keeping the total volume of organic phase constant was shown to have little influence on the separation of fluphenazine from its decanoate ester. A change in the ratio of total organic phase component–aqueous phase component had a profound effect, as would be expected. Reduction of the amount of aqueous component present in the mobile phase caused a corresponding reduction in the capacity factor of the esters. By this means sharp symmetrical peaks were obtained for the higher esters of the homologous series and by careful choice of the mobile phase organic component–aqueous component ratio separation of any given esters in the homologous series, from acetate to stearate, may be achieved. Fig. 2 illustrates a typical separation of the myristate, palmitate and stearate esters and shows the resulting chromatogram to be suitable for quantitation.

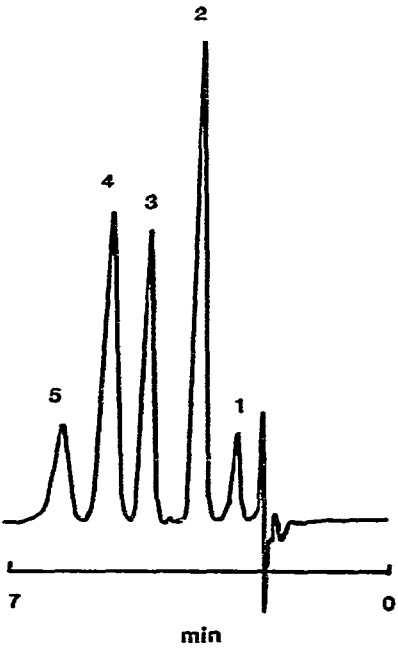


Fig. 2. Separation of some fluphenazine esters. Column, Partisil-TMS; mobile phase, methanol–acetonitrile–1% ammonium carbonate solution (1:1:0.3). Peaks: 1 = fluphenazine; 2 = fluphenazine decanoate; 3 = fluphenazine myristate; 4 = fluphenazine palmitate; 5 = fluphenazine stearate.

Mobile phase velocity

The dependance of the plate height (and hence the efficiency) of a column on the eluent velocity is a well documented relationship[6]. To assess this effect with regard to the chromatographic system proposed for fluphenazine, the efficiency of a 200-mm Partisil-TMS column was determined at different mobile phase flow-rates. The results obtained (Fig. 3) demonstrated that it was possible to double the column efficiency by reducing the eluent velocity from 2.5 to 0.5 ml/min. This increase in efficiency was larger than expected so to investigate further, values for the reduced plate height and reduced velocity were calculated and the logarithm functions plotted

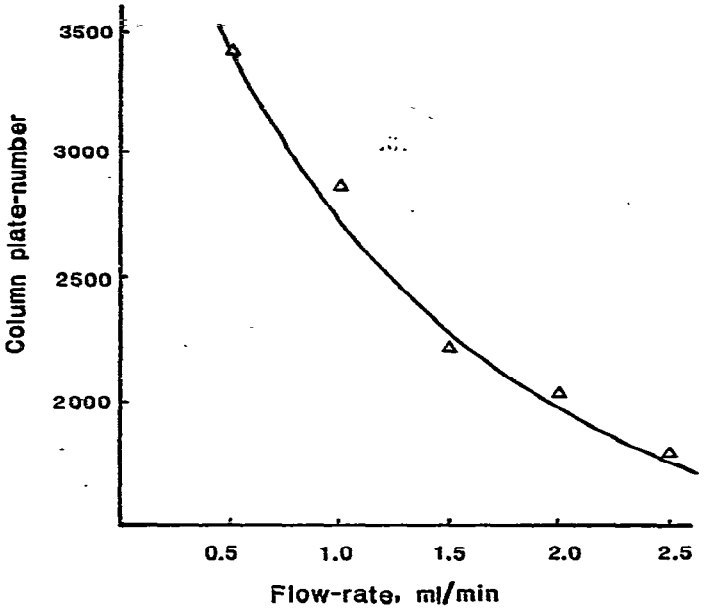


Fig. 3. Influence of mobile phase flow-rate on efficiency of fluphenazine enanthate peak. Column, Partisil-TMS; mobile phase, methanol–acetonitrile–1% ammonium carbonate solution (1:1:1).

according to the procedure of Bristow and Knox[7]. The curve obtained for fluphenazine enanthate indicated that chromatography was being performed under non-ideal conditions and that the optimum potential of the system was only approached at a flow-rate of 0.5 ml/min. Similar observations were noted when using the 200-mm column of Partisil-ODS (Fig. 4).

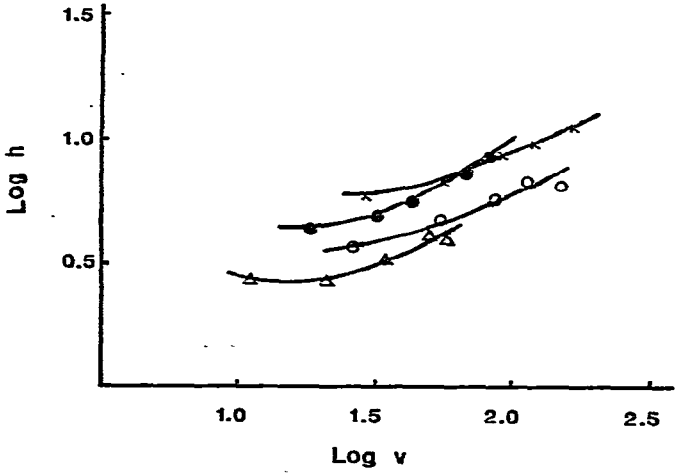


Fig. 4. Comparison of log h–log v curves for 50-, 100- and 200-mm columns of Partisil-TMS and a 200-mm column of Partisil-ODS. Mobile phase, methanol–acetonitrile–1% ammonium carbonate solution [(1:1:1) for TMS stationary phase and (1:1:0.5) for ODS stationary phase]. ●, 200-mm Partisil-ODS; ×, 200-mm Partisil-TMS; ○, 100-mm Partisil-TMS; △, 50-mm Partisil-TMS.

The dependance of the reduced plate height, h, upon the reduced velocity, v, is described by the equation

$$h = B/v + Av^{0.33} + Cv$$

and the shape of the curve obtained for fluphenazine enanthate indicated that the values for h were mainly influenced by the factor C. It has been reported[7] that the C term in the above equation represents the contribution to the reduced plate height due to the slow equilibration of the solute between mobile and stationary phases. For a bulky molecule, such as fluphenazine enanthate, slow equilibrium between the mobile and stationary phase would appear to be a reasonable explanation of the observed effect.

Operating the system under optimum conditions (mobile phase flow-rate 0.5 ml/min) results in a retention time of 27 min for fluphenazine enanthate. From a practical point of view such as retention time is unsatisfactory because a fast sample throughput is essential in an industrial laboratory. Consequently we feel justified in performing the chromatography under non-ideal conditions as in practice this does not interfere with the separation of fluphenazine from fluphenazine enanthate.

Effect of column length

The present trend in HPLC towards the use of shorter columns, and hence lower operating pressures led us to evaluate the chromatographic properties of 50- and 100-mm columns as well as the 200-mm column previously used. To ensure a valid comparison all columns were packed under identical conditions and with packing material from the same batch of Partisil-TMS. Reduced plate height values were then determined over a range of mobile phase flow-rates and a log h/log v curve constructed for each column (Fig. 4). A comparison of the curves showed that the 50-mm column had the lowest minimum reduced plate height indicating this to be the most efficient of the three column lengths under investigation. The reason for this observation is probably in the packing of the columns, our particular packing procedure being more suitable for shorter column lengths. If the plate numbers of the three columns are now compared (Table II), the highest value is observed with a

TABLE II

COMPARISON OF PLATE HEIGHTS FOR 50-, 100- AND 200-mm COLUMNS OF PARTISIL-TMS

Mobile phase velocity (ml/min)	*Plate number*		
	50-mm column	*100-mm column*	*200-mm column*
2.5	—	1518	1800
2.0	—	1449	2027
1.5	—	1724	2218
1.0	1229	2089	2867
0.8	1198	—	—
0.6	1527	—	—
0.5	—	2705	3412
0.4	1852	—	—
0.2	1794	—	—

200-mm column operated at a flow-rate of 0.5 ml/min and to obtain maximum resolution it will be necessary to use the 200-mm column under these conditions. This will only be necessary if the two compounds to be separated have similar capacity factors (*e.g.* two homologues such as fluphenazine enanthate and fluphenazine octanoate). For the proposed separation of fluphenazine from fluphenazine decanoate or enanthate the resolution of the 50-mm column is more than adequate.

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