Journal of Chromatography, 105 (1975) 182–185

CHROM. 7987

Note

High-resolution liquid chromatography of some psychotropic drugs

K. MACEK and V. ŘEHÁK

3rd Medical Department, Faculty of Medicine, Charles University, and Department of Analytical Chemistry, Faculty of Sciences, Charles University, Prague (Czechoslovakia)

(Received October 1st, 1974)

Recently, a great deal of attention has been paid to the chromatographic behaviour of psychotropic drugs. The main reasons for this interest are firstly that these drugs constitute one of the most widespread groups of drugs and secondly that this group of drugs consists of a great number of structurally closely related compounds for which it is difficult to use classical analytical procedures for their identification and quantitative assay. This study was orientated towards the chromatographic behaviour of some substituted 1,4-benzodiazepines and further towards some compounds containing heterocyclic sulphur. These compounds mostly exhibit the pharmacological properties of tranquillisers, antineurotics and neuroleptics.

Of the available chromatographic techniques, most attention has been paid during recent years to flat-bed procedures, mainly thin-layer chromatography (for reviews, see Macek[1] and Clifford and Smyth[2]). Most of these separations were carried out on silica gel in alkaline media and the drugs were developed in the free base form. Gas chromatography has been applied less frequently because it is necessary to use high temperatures, which involves the risk of the decomposition of the original compound.

Because psychotropic drugs from the toxicological point of view are of considerable importance and because in their assay a rapid analysis is frequently required as well as the possibility of determining trace amounts of these compounds in biological material, we decided to use high-resolution column liquid chromatography (LC) for solving the above problem. Three papers have been published on the separation of 1,4-benzodiazepines by column LC. Scott and Bommer[3] used the chemically bonded phase Durapak OPN (oxydipropionitrile on Porasil C) with *n*-hexane–isopropanol as the mobile phase. A similar arrangement was used by Weber[4], who tried chromatographic separations in a liquid–solid system on Corasil II. For the analysis of chlordiazepoxide, reversed phases were used with chemically bonded octadecyltrichlorosilane on Corasil and acetonitrile–water as the mobile phase[5]. In all instances UV recording was used for detection. Psychotropic drugs with heterocyclic sulphur have not previously been separated by column LC.

EXPERIMENTAL

A Waters Model ALC 202/401 liquid chromatograph with an M 6000 high-

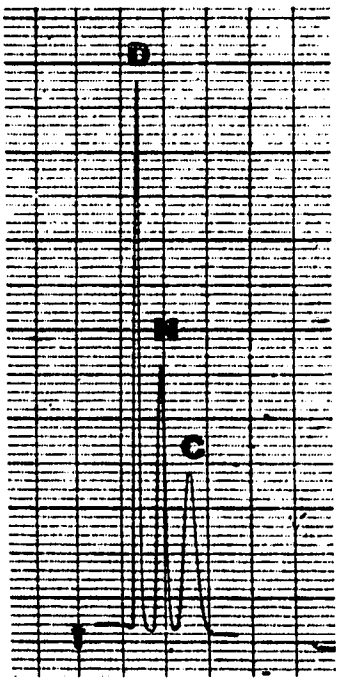
pressure pump, a UV detector operating at a wavelength of 254 nm and a differential refractometer were used. Chromatographic separations were effected using metal columns 1 m long and 2 mm I.D. The sorbents and carriers consisted of the pellicular sorbent Corasil II (Waters Ass., Framingham, Mass., U.S.A.) (grain size 37–50 μm), Zipax (DuPont, Wilmington, Del., U.S.A.) and the chemically bonded phases Carbowax 400 on Corasil and C-18 (octadecyltrichlorosilane) on Corasil (grain size 37–50 μm) (Waters Ass.). Chloroform constituted the base for mobile phase systems, the polarity of which was adjusted by the addition of isopropanol (*e.g.*, chloroform–isopropanol, 98:2), or, alternatively, by the addition of *n*-heptane (*e.g.*, chloroform–*n*-heptane, 9:1). Column pressures of 100–1,000 p.s.i.g. and flow-rates of 0.3–2 ml/min were used. Samples were applied on to the column by the stop–flow technique. The concentrations of the analyzed substances in chloroform were adjusted in such a way that a constant volume of 0.5 μl was injected on to the column.

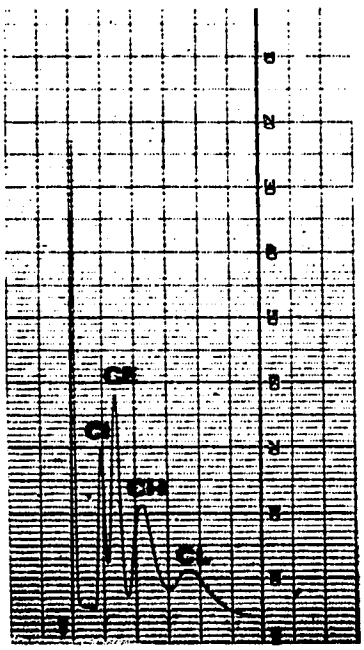
TLC was performed with DC-Plastikfolie Kieselgel F_{254} (Merck, Darmstadt, G.F.R.) using benzene–ethanol–formic acid (95:15:5) as the mobile phase for 1,4-benzodiazepines and benzene–ethanol–diethylamine (95:5:5) for sulphur-containing heterocyclic compounds. Chromatograms were inspected under UV light at 254 nm and an Opton Chromatogramm Spektrophotometer was used for the densitometric tracing. The curves were recorded under reflected light at 240 or 315 nm.

RESULTS AND DISCUSSION

The choice of experimental conditions was based on our previous experience with the flat-bed arrangement, mainly paper chromatography[6], in which better resolution is obtained for psychotropic drugs in liquid–liquid systems. Therefore, in column LC, chemically bonded phases were to be preferred. As an analogy with systems with formamide or propylene glycol as the stationary phase, Carbowax 400 bonded to Corasil was used. With respect to the liquid–solid systems, this arrangement gave a considerably higher reproducibility. Examples of separations of 1,4-benzodiazepines are presented in Fig. 1, while separations of sulphur-containing heterocyclic drugs are presented in Fig. 2. Fig. 3 shows a separation of a mixture of both types of drugs.

Under the experimental conditions specified, the number of theoretical plates was 1000–1500, which is in good agreement with the value expected for the stationary phase used. This value is roughly equal to the number of theoretical plates obtained in paper or thin-layer chromatography (an example of the TLC separation of 1,4-benzodiazepines is presented in Fig. 4). The advantage of the column arrangement is, of course, a considerably higher speed of analysis and higher sensitivity. While the running time in paper chromatography is about 150 min (using formamide systems), in TLC 30–40 min and in GC about 20 min, the running time in the column LC technique is 7–15 min. The detection sensitivity in flat-bed techniques is about 0.5–1 μg, while in column LC the detection limits were 20–300 ng using a UV detector with a constant wavelength of 254 nm. Because the maximum absorbances of the individual drugs assayed are not always coincident with this wavelength, it is likely that there would be a considerable increase in sensitivity if a spectrophotometric detector were used. A differential refractometer was not suitable for detection because of its low sensitivity.





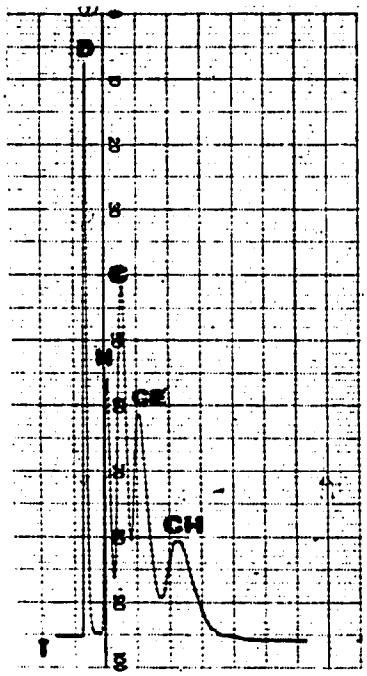


Fig. 1. High-resolution column LC of some 1,4-benzodiazepines. D = diazepam; N = nitrazepam; C = chlordiazepoxide. Carbowax 400; mobile phase, chloroform; pressure, 400 p.s.i.g.; flow-rate, 1 ml/min; running time, 7 min.

Fig. 2. High-resolution column LC of some heterocyclic sulphur-containing drugs. Cl = clothiapin; CE = clothepin; CH = chlorprothixen; CL = clopenthixol. Carbowax 400; mobile phase, chloroform–isopropanol (98:2); pressure, 900 p.s.i.g.; flow-rate, 2 ml/min; running time, 22 min.

Fig. 3. High-resolution column LC of a mixture of psychotropic drugs. Abbreviations as in Figs. 1 and 2. Carbowax 400; mobile phase, chloroform–*n*-heptane (9:1); pressure, 350 p.s.i.g.; flow-rate, 1 ml/min; running time, 15 min.

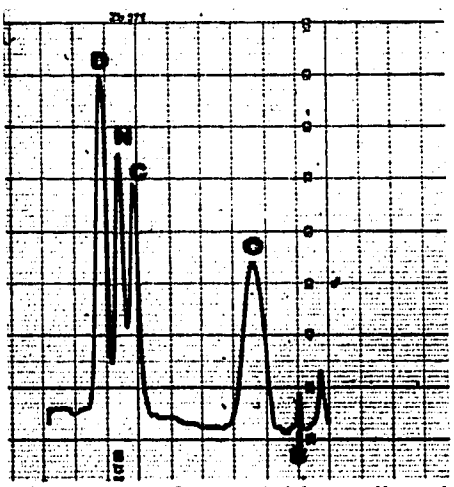


Fig. 4. TLC of some 1,4-benzodiazepines (densitometry tracing). D = diazepam; N = nitrazepam; C = chlordiazepoxide; O = oxazepam; S = start. DC-Plastikfolie Kieselgel F_{254}; mobile phase, benzene–ethanol–formic acid (95:15:5). Remission measurement at 240 nm.

Preliminary experiments with reversed phases using C-18/Corasil and methanol–water (45:55) as the mobile phase indicated that this system could be used for the separation of 1,4-benzodiazepines. In this instance, however, it is necessary to use a much higher column overpressure (about 1,500 p.s.i.g.) in order to obtain flow-rates of 1–2 ml/min. The separation of diazepam and chlordiazepoxide was not so good as when Carbowax 400 was used.

ACKNOWLEDGEMENTS

The authors are obliged to Waters Messtechnik, Frankfurt, G.F.R., for supplying the Model ALC 202/401 liquid chromatograph. Equally, thanks are due to Dr. U. Hezel of Opton Feintechnik, Oberkochen, G.F.R., for measuring the remission spectra and for the TLC densitometric tracings.

REFERENCES

1 K. Macek (Editor), *Pharmaceutical Applications of Thin-Layer and Paper Chromatography*, Elsevier, Amsterdam, 1972.
2 J. M. Clifford and W. F. Smyth, *Analyst (London)*, 99 (1974) 241.
3 C. G. Scott and P. Bommer, *J. Chromatogr. Sci.*, 8 (1970) 446.
4 D. J. Weber, *J. Pharm. Sci.*, 61 (1972) 1797.
5 *Analysis of Pharmaceutical Products*, Waters Ass., Framingham, Mass., 1973, p. 11.
6 K. Macek, J. Večerková and J. Stanislavová, *Pharmazie*, 20 (1965) 605.