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Note

High-performance liquid chromatographic system for the separation of tricyclic antidepressant and related drugs using ODS-Hypersil

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Experience in high-performance liquid chromatography (HPLC) has shown that different commercial packing materials of nominally the same type (*e.g.* ODS-silica) can have very different chromatographic properties. The consequent problems arising from the need to transfer methods between different locations have led Forensic Science Laboratories in the U.K. to standardise on the materials used for routine work[1] and bulk purchases of a silica (Spherisorb S5W) and an ODS-silica (ODS-Hypersil) have been made. These materials have proved to be effective for the vast majority of HPLC separations required for toxicology casework and the concept of using only two types of column has proved to be convenient and economical. Eluents have been developed to use with the standard materials for various drug classes and, in each case, retention data for groups of compounds of forensic interest have been published. This information serves as a guide to the identification of unknown compounds and the quantification of specific drugs using HPLC. At the present time data have been published for barbiturates[2,3], local anaesthetics[4], amphetamines[5], narcotic analgesics[5], ergot alkaloids[6], benzodiazepines[7], analgesic and anti-inflammatory drugs[8] and thiazide diuretics[9]. A further system for basic drug screening on silica has also been developed[10].

In the present paper we describe an eluent for the separation of tricyclic antidepressant and related drugs on ODS-Hypersil, an important group of compounds often encountered in forensic toxicology casework. Numerous HPLC methods for the analysis of specific antidepressants and metabolites have been published and some of the literature has been reviewed[11–13]. The HPLC system finally selected is a modification of a procedure by Kabra *et al.*[14] recommended for the determination of a few antidepressants in plasma or serum. In the present work retention data are presented for 27 drugs.

EXPERIMENTAL

Materials

Acetonitrile (HPLC grade) was obtained from Rathburn Chemicals (Walkerburn, U.K.) and phosphoric acid (AristaR grade) from BDH (Poole, U.K.). Water was distilled in glass in the laboratory. All other chemicals were analytical grade

from BDH except for *n*-nonylamine which came from Aldrich (Gillingham, U.K.).

The HPLC packing material used was 5-μm ODS-Hypersil from Shandon Southern Products (Runcorn, U.K.).

All drugs came from the drug collection of the Central Research Establishment, Home Office Forensic Science Service.

Chromatography

The HPLC equipment consisted of a Waters M6000 pump, a Rheodyne injection valve (Model 7120) fitted with a 20-μl sample loop and a Cecil CE272 variable-wavelength UV detector operated at 230 nm. The stainless-steel column (16 cm × 5 mm I.D., Shandon Southern Products) was packed with ODS-silica using a conventional slurry procedure in which the material was dispersed in isopropanol and pumped with hexane.

The eluent was prepared by mixing acetonitrile (300 ml) with a pH 3 phosphate buffer (700 ml). The aqueous buffer was prepared by adding *n*-nonylamine (0.6 ml) to aqueous sodium dihydrogen phosphate (0.01 *M*, 1000 ml) and then adjusting the pH to 3.0 by the dropwise addition of phosphoric acid[14]. A flow-rate of 2 ml/min was used.

Drug samples were dissolved in acetonitrile–water (30:70, v/v) for injection onto the column. Retention data are expressed as capacity ratios, k', which are defined by $k' = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of the drug and a non-retained compound, respectively. Injections of acetonitrile were used to determine t_0.

RESULTS AND DISCUSSION

The HPLC system chosen for the separation of the tricyclic antidepressant and related drugs was a modification of a published procedure[14]. The original method

TABLE I

HPLC RETENTION DATA FOR TRICYCLIC ANTIDEPRESSANT AND RELATED DRUGS (ARRANGED IN ORDER OF ELUTION)

Compound	*k'*	*Compound*	*k'*
Azatadine*	0	Protriptyline	3.60
Viloxazine	0.17	Cyproheptadine*	4.17
Nomifensine	0.42	Imipramine	4.17
Dibenzepin	0.50	Nortriptyline	4.58
Zimeldine	0.67	Maprotiline	4.92
Triprolidine*	1.17	Amitriptyline	5.42
Oxypertine	1.33	Trimipramine	6.17
Noxiptyline	1.63	Butriptyline	7.33
Opipramol	1.63	Clomipramine	9.92
Mianserin	1.92	Deptropine*	10.40
Doxepin	2.27	Iprindole	10.83
Mebhydrolin*	2.48	Lofepramine	>30
Desipramine	3.60	Trazodone	>30
Dothiepin	3.60		

* Antihistamine drugs.

TABLE II

HPLC RETENTION DATA FOR TRICYCLIC ANTIDEPRESSANT AND RELATED DRUGS (ARRANGED IN ALPHABETICAL ORDER)

Compound	*k'*	*Compound*	*k'*
Amitriptyline	5.42	Mebhydrolin*	2.48
Azatadine*	0	Mianserin	1.92
Butriptyline	7.33	Nomifensine	0.42
Clomipramine	9.92	Nortriptyline	4.58
Cyproheptadine*	4.17	Noxiptyline	1.63
Deptropine*	10.40	Opipramol	1.63
Desipramine	3.60	Oxypertine	1.33
Dibenzepin	0.50	Protriptyline	3.60
Dothiepin	3.60	Trazodone	>30
Doxepin	2.27	Trimipramine	6.17
Imipramine	4.17	Triprolidine*	1.17
Iprindole	10.83	Viloxazine	0.17
Lofepramine	>30	Zimeldine	0.67
Maprotiline	4.92		

* Antihistamine drugs.

used an ODS-Ultrasphere column and an eluent containing 21% acetonitrile with a pH 3 phosphate buffer containing *n*-nonylamine. An identical aqueous buffer was used in the present work but an increase in the concentration of acetonitrile, to 30%, was found to be necessary with the ODS-Hypersil column to achieve an appropriate retention range for the compounds studied. Basic drugs often give poor peak shapes on ODS-silica columns and require the addition of amine modifiers to the eluent to control peak shapes[15]. The *n*-nonylamine used in the aqueous buffer was found to

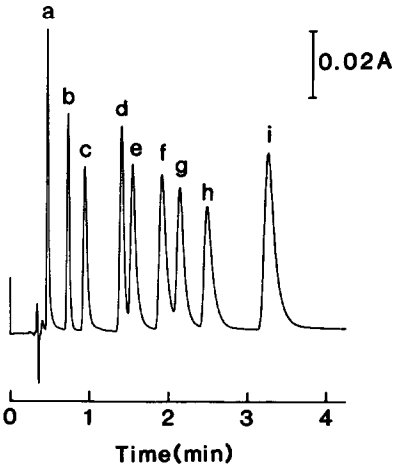

Fig. 1. Chromatography of tricyclic antidepressant drugs on ODS-silica. Column: ODS-Hypersil, 5 μm (16 cm × 5 mm I.D.); eluent: 30% acetonitrile containing a phosphate buffer (pH 3) and nonylamine; flow-rate: 2 ml/min; detection: UV absorbance (230 nm). Peaks: a = zimeldine; b = noxiptyline; c = doxepin; d = protriptyline; e = imipramine; f = amitriptyline; g = trimipramine; h = butriptyline; i = clomipramine.

be a very satisfactory additive, maintaining symmetrical peak shapes for the tricylic antidepressants on ODS-Hypersil.

Table I gives the retention data (k' values) for 27 drugs arranged in their order of elution. The list includes 22 tricyclic antidepressant drugs with the remaining five compounds being antihistamine drugs, selected as having chemical structures similar to those of the antidepressants. The antidepressant drugs include all 17 such compounds available for prescription in the U.K. in June 1986 along with five others (dibenzepin, nomifensine, noxiptyline, opipramol and zimeldine).

Table I indicates that most drugs show some retention within the range $k' = 0$ to 11 with the exceptions of azatadine which is not retained at all, and lofepramine and trazodone which show very long retention times with the eluent used. Table II presents the same data arranged by alphabetical order of the drug names to facilitate the rapid retrieval of information for a specific compound. The good peak shapes obtained for the present system are demonstrated in Fig. 1, which shows the separation of nine tricyclic antidepressant drugs on the 16-cm column.

No attempt has been made in the present note to demonstrate the use of the HPLC system for the determination of tricyclic antidepressants in biological fluids although such applications are feasible. The original paper by Kabra *et al.*[14] includes a description of an extraction procedure for serum and plasma which may be directly applicable. Further work is required to explore the range of drugs which may be analysed using this HPLC system.

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