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Column switching and high-performance liquid chromatography in the analysis of amitriptyline, nortriptyline and hydroxylated metabolites in human plasma or serum☆

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ABSTRACT

A column-switching system for the direct injection of plasma or serum samples, followed by isocratic high-performance liquid chromatography and ultraviolet detection, is described for the simultaneous quantitation of the tricyclic antidepressant amitriptyline, its demethylated metabolite nortriptyline and the *E*- and *Z*-isomers of 10-hydroxyamitriptyline and 10-hydroxynortriptyline. The method included adsorption of amitriptyline and metabolites on a reversed-phase C_8 clean-up column (10 μm; 20 mm × 4.6 mm I.D.), washing of unwanted material to waste and, after on-line column-switching, separation on a cyanopropyl analytical column (5 μm; 250 mm × 4.6 mm I.D.). The compounds of interest were separated and eluted using acetonitrile–methanol–0.01 *M* phosphate buffer (pH 6.8) (578:188:235, v/v) within less than 20 min. Various drugs frequently co-administered with amitriptyline or other antidepressants did not interfere with the determinations. In plasma samples spiked with 25–300 ng/ml, the recoveries were between 84 and 112% and the inter-assay coefficients of variation were 3–11%. After a minor modification, as little as 5 ng/ml could be quantitated. There were linear correlations ($r > 0.99$) between drug concentrations of 5–500 ng/ml and the detector signal. The method allows routine measurements of amitriptyline, nortriptyline and hydroxylated metabolites in blood plasma or serum of patients treated with amitriptyline or nortriptyline, and enables the results to be reported within 1 h.

INTRODUCTION

Tricyclic antidepressants are widely used in the treatment of patients suffering from depression. Regular determinations of drug concentrations in the blood during psychopharmacotherapy is a cost-effective tool to increase efficacy and safety [1–5]. For precise determination of antidepressants, high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection is the preferred method in most laboratories [6–11]. The determinations require dialysis [8], liquid–liquid [2,6], solid-phase [11,12] or ion-pair [9,12] extraction to remove proteins, lipids or other interfering materials. Such procedures, however, are time-consuming, although recently developed automated processors have minimized the staff requirements. A method that enables complete automation is column-switching with on-line HPLC separation, which has been developed for various drugs [13,14] including antidepressants [7,8,10]. With regard to its application to antidepressant determinations in clinical practice, however, column-switching systems described so

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far [7,8,10] are of limited use: most systems comprise several HPLC pumps and switching valves in addition to the components of a classical isocratic HPLC system. Moreover, gradient elution is required when isomeric hydroxylated metabolites of tricyclic antidepressants are to be analysed together with the parent antidepressants [10]. The metabolites are pharmacologically active [15,16]. Moreover, evidence has been given that the *E* and *Z*-isomers of 10-hydroxynortriptyline contribute substantially to cardiotoxic side-effects of nortriptyline [17–19]. Because of the methodological limitations, most clinical laboratories restrict their routine measurements to the parent tricyclic antidepressants and demethylated metabolites.

This paper describes a simple column-switching system that is suitable for routine determinations of amitriptyline, nortriptyline and the *E*- and *Z*-isomers of the 10-hydroxylated metabolites in human plasma or serum. Pre-extraction on a C_8 reversed-phase clean-up column and on-line isocratic HPLC separation on a cyanopropyl-bonded stationary phase enabled plasma sample processing and reporting of results in less than 1 h.

EXPERIMENTAL

Chemicals

Amitriptyline and nortriptyline were kindly donated by Merck, Sharp and Dohme Research Laboratories (Rahway, NJ, USA), the *E*- and *Z*-isomers of 10-hydroxyamitriptyline and 10-hydroxynortriptyline by H. Lundbeck Laboratory (Copenhagen, Denmark), fluvoxamine by Duphar (Weesp, Netherlands), doxepin and N-desmethyldoxepin by Pfizer (Karlsruhe, Germany), imipramine, 2- and 10-hydroxyimipramine, desimipramine, 2-hydroxydesimipramine, maprotiline, N-desmethylmaprotiline, oxaprotiline, clomipramine, 8-hydroxyclomipramine, N-desmethylclomipramine, 8-hydroxydesmethylclomipramine, trimipramine, N-desmethyltrimipramine and protriptyline by Ciba Geigy (Basel, Switzerland), haloperidol, spiroperidol, pimozide, trifluperidol and fluspirilene by Janssen (Beerse, Belgium), chlordiazepoxide, diazepam, nordiazepam, flurazepam, nitrazepam and oxazepam by Roche (Basel, Switzerland), clobazam and N-desmethylclobazam by Hoechst (Frankfurt, Germany), clozapine and N-desmethylclozapine by Sandoz (Basel, Switzerland) and perazine by Promonta (Hamburg, Germany). Methanol (LiChrosolv, Merck, Darmstadt, Germany) and acetonitrile (LiChrosolv, Merck) were used without further purification. Water was purified and deionized by a Milli-Q water-processing system (Millipore, Eschborn, Germany). All other chemicals were of analytical grade and purchased from commercial sources.

Standards

Drug solutions were prepared by dissolving the pure substances in methanol. The stock solutions contained 1 mg of free base per millilitre. They were diluted using methanol to obtain the final concentrations. The stock solutions could be stored for several months in the dark at −20°C without measurable decomposition. Similar stabilities were observed for plasma or serum samples either spiked with drug or obtained from patients treated with amitriptyline.

Trimipramine, used as the internal standard, was pipetted to plasma or serum samples by the addition of 10 μl of methanolic solution to 990 μl of plasma or serum. The final concentration of trimipramine in the plasma sample was 140 ng/ml. For preparation of plasma samples containing known amounts of drug and to be used for calibration curves, pooled human blank plasma was supplied with 10 μl of standard solution per millilitre, leading to final concentrations of 140 ng of trimipramine and 25–500 ng of all compounds of interest or 1–500 ng of amitriptyline in 1 ml of plasma.

Plasma and serum samples

Blood was withdrawn from the anticubital vein of drug-free healthy volunteers or depressed patients who had been treated daily with amitriptyline for at least seven days. Patients' blood was collected in the morning before the first daily dose. Plasma was obtained by centrifugation of

heparinized blood samples at 3000 g for 10 min and stored at -20°C until analysis. Serum was prepared by the same procedure without inclusion of heparin.

Instrumentation

The chromatographic system (Fig. 1) comprised a Bischoff 2200 HPLC pump (Bischoff, Leonberg, Germany) as the analytical pump and a Constametric III HPLC pump (LDC-Analytical, Gelnhausen, Germany) as the clean-up pump. The solvent-delivery system was equipped with a manual injection valve (Rheodyne 7010), a 100-μl sample loop and a six-port switching valve (Rheodyne 7000). The whole system was fitted to

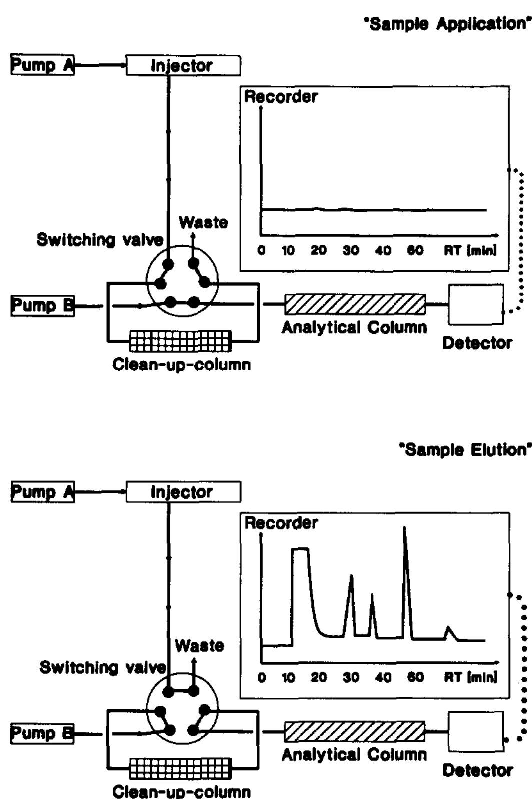

Fig. 1. Column-switching system for direct injection of plasma or serum samples and on-line HPLC analysis of amitriptyline and metabolites. The scheme shows the system during the phases of sample application (upper panel), which includes injection onto the clean-up column and washing of unwanted material to waste by pump A eluent, and sample elution (lower panel), including removal of drug from the clean-up column and subsequent separation on the analytical column by pump B eluent.

an LDC spectromonitor 3000 (LDC-Analytical) of variable wavelength for UV detection, which was set at 214 nm or at 240 nm for the determination of low concentrations of amitriptyline. Chromatograms were recorded and integrated by an MP-3000 integration system (LDC-Analytical).

Mobile phases

The clean-up-solvent from pump A (Fig. 1) was used for loading, washing and re-equilibration of the clean-up column. It consisted of deionized water containing 5% methanol (v/v). The analytical solvent from pump B, used for sample elution from the clean-up column and separation on the analytical column, was acetonitrile–methanol–0.01 M phosphate buffer (pH 6.8) (578:188:235, v/v). Phosphate buffer was prepared by dissolving 0.01 mol of K_2HPO_4 in 1 l of deionized water. The pH was adjusted to 6.8 by the addition of phosphoric acid (85%). Before use, the mobile phases were filtered through 0.22-μm filter elements and gassed with helium to remove oxygen.

Columns and stationary phases

For routine analyses, the chromatographic arrangement (Fig. 1) included a clean-up column (20 mm × 4.6 mm I.D.) filled with C_8 reversed-phase material (Hypersil MOS C_8, Shandon) of 10 μm particle size and a guard column (20 mm × 4.6 mm I.D.) filled with Hypersil CPS CN (Shandon) of 5 μm particle size and placed between the switching valve (not shown in Fig. 1) and the analytical column. The analytical column (250 mm × 4.6 mm I.D.) was filled with cyanopropyl material, Nucleosil 100 CN (Macherey & Nagel) of 5 μm particle size. In addition to Nucleosil 100 CN, Nucleosil 120 CN, LiChrosorb CN (Merck), Hypersil CPS (Shandon) and Spherisorb CN (Merck) were also tested. In the clean-up column, other stationary phases used instead of Hypersil MOS C_8 were spherical, non-modified silica gel, Hypersil phenyl (Shandon) and Nucleosil 100 C_{18} or 100 CN (Macherey & Nagel). All columns were packed and purchased by CTI (Königsstein/Taunus, Germany).

Sample clean-up, column switching and subsequent chromatographic separation

A 100-μl sample of plasma or serum was injected directly onto the Hypersil MOS C_8 clean-up column. Proteins, lipids and other interfering compounds were washed to waste using 5% methanol at a flow-rate of 1.5 ml/min for 15 min. Amitriptyline and metabolites were eluted onto the analytical column in foreflush direction after moving the switching valve from the application to the elution mode (Fig. 1). After 2 min the switching valve was moved back to the application position. After re-equilibration for 5 min, the next sample could be injected onto the clean-up column while the preceding sample was chromatographed on the analytical column at a flow-rate of 1.5 ml/min.

Calculations

From the recorded peak-areas, the ratios of the drug to the internal standard were calculated. Results obtained from spiked plasma samples with known amounts of drug were submitted to linear regression analyses to calculate correlation coefficients, slopes and intercepts. The drug concentrations in blood samples with unknown amounts of drug were calculated on the basis of the computed regression lines.

As indices characterizing HPLC separation, capacity factors (k') and resolution factors (R_s) were calculated as follows:

$$k' = (t_R - t_M)/t_M$$

where t_R is the retention time of the compound of interest and t_M the hold-up time and

$$R_s = (t_{R1} - t_{R2}) / \sigma_t$$

where t_{R1} is the retention time of the first compound, t_{R2} that of the second one and σ_t is the peak width at 61% of peak height.

RESULTS

Chromatographic separations

The choice of an appropriate packing material for the separation of amitriptyline, nortriptyline and the *Z*- and *E*-isomers of the 10-hydroxylated metabolites was essential. Separation of the analytes required stationary phases that exhibited sufficiently high resolution for qualitative and quantitative determination of the compounds of interest. Optimal results were obtained using Nucleosil 100 CN material. Amitriptyline and nortriptyline, the isomeric hydroxylated metabolites and the internal standard, trimipramine, were well separated within 15 min. The separation of the *E*- and *Z*-isomers was occasionally better using Nucleosil 120 CN (7 μm). High batch-to-

TABLE I

RETENTION TIMES, CAPACITY FACTORS AND RESOLUTION FACTORS FOR SEPARATION OF AMITRIPTYLINE (AMI) AND NORTRIPTYLINE (NOR) ON A COLUMN-SWITCHING SYSTEM USING DIFFERENT STATIONARY PHASES IN THE CLEAN-UP COLUMN

All stationary phases were of 5 μm particle size; for calculation of the capacity and resolution factors see Experimental.

Conditions	Retention time (min)		Capacity factor (k')		Resolution factor (R_s) (AMI/NOR)
	AMI	NOR	AMI	NOR	
Without clean-up column	5.9	10.5	6.0	11.6	9.1
With clean-up column[a]					
Silica	6.5	11.2	7.8	14.1	10.2
Phenyl	8.0	13.6	9.7	17.4	5.6
Cyanopropyl	6.4	11.2	6.0	11.3	9.5

[a] Stationary phases: unmodified silica, Hypersil phenyl, or Nucleosil 100 CN.

batch variabilities of this material, however, prohibited its use for routine analyses.

Other cyanopropyl-bonded stationary phases, LiChrosorb CN, Hypersil CPS and Spherisorb CN, or C_{18} reversed-phase materials exhibited insufficient capacities or resolutions, especially with regard to the separation of isomeric metabolites. On C_{18} stationary phases, amitriptyline and nortriptyline were only poorly resolved.

Column-switching

In pilot studies, several clean-up columns (20 mm × 4.6 mm I.D.) filled with different stationary phases (5 μm particle size) were coupled to the analytical column and used in a foreflush mode as shown in Fig. 1. The retention times of amitriptyline and nortriptyline increased by 9–36 and 6–29%, respectively (Table I). Moreover, the capacity factors were also increased by column-switching compared with direct injection. The calculated resolution factors indicated that the chromatographic separation was impaired if a phenyl-bonded phase was applied (Table I). The retention times of 10-*E*-hydroxyamitriptyline increased by 10% (Nucleosil 100 C_{18}), 16% (Hypersil phenyl) or 13% (Nucleosil 100 CN).

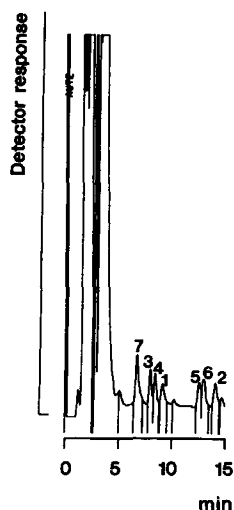

Fig. 2. Representative chromatogram of a standard solution containing amitriptyline (1), nortriptyline (2), *E*-10-hydroxyamitriptyline (3), *Z*-10-hydroxyamitriptyline (4), *E*-10-hydroxynortriptyline (5) and *Z*-10-hydroxynortriptyline (6) at concentrations of 25 ng/ml. Separation was performed on Nucleosil 100 CN (5 μm) material. Trimipramine (7) was used as internal standard at a final concentration of 140 ng/ml.

From the pilot studies it could also be seen that all compounds of interest were eluted from any clean-up column tested within 5 min.

When plasma or serum had to be analysed, the best results were obtained using C_8 Hypersil MOS of 10 μm particle size in the clean-up column. All compounds of interest were well separated (Fig. 2). The separation of the *E*- and *Z*-hydroxy metabolites was even improved when comparing chromatographic runs with and without inclusion of a clean-up column. The C_8 phase exhibited optimal stability for long-term use in the analysis of plasma samples.

Analyses of plasma or serum samples

When a 100-μl sample of plasma or serum was injected onto the column-switching system, the drugs were retained on the Hypersil MOS clean-up column. Unwanted material, such as proteins or lipids, was removed by the 5% methanol wash within 15 min without loss of the drugs to be analysed. At a flow-rate of 1.5 ml/min, using acetonitrile–methanol–phosphate buffer (578:188: 235, v/v), all substances were eluted after switching the system from the application to the elution mode (Fig. 1) within 2 min and separated within 20 min (Fig. 2).

Various healthy, non-medicated volunteers did not contain measurable interfering compounds in their plasma (Fig. 3). Minor peaks were registered occasionally, but their retention times differed from those of amitriptyline and its metabolites. Serum could be analysed as well as plasma with similar precision without a decrease in recovery, sensitivity or specificity.

Interferences with various other psychotropic drugs that are frequently administered to patients in addition to or instead of amitriptyline were minimal (Table II).

Linearity

The calibration curves for each analyte in plasma revealed that the detector response was linear from 0 to at least 500 ng/ml. The correlation coefficients calculated by linear regression analy-

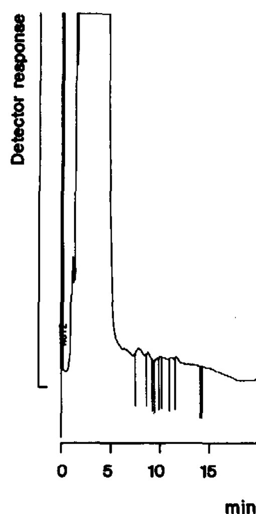

Fig. 3. Representative chromatogram obtained from the analysis of a blank plasma sample of a non-medicated healthy subject by column-switching and subsequent separation on Nucleosil 100 CN.

ses of the relative peak areas ranged between 0.994 for *E*-10-hydroxyamitriptyline and 0.998 for amitriptyline. The curve intercepts were close to 0, ranging between −0.04 and 0.24, which was close to the lower limit of detection.

Sensitivity and detection limit

Using equivalent concentrations of the internal standard, trimipramine, and of the drug to be quantified, the relative detector responses registered at 214 nm ranged between 2.6 (*E*-hydroxynortriptyline) and 2.9 (*Z*-hydroxynortriptyline). When 100-μl plasma samples were injected, the lower detection limit was 5–10 ng/ml. It could be decreased to 1 ng/ml when the clean-up column was used in an enrichment mode. After three injections of 100-μl plasma samples on the clean-up column, each followed by a 15-min washing period to remove interfering substances, as little as 1 ng/ml could be detected. In the enrichment mode the lower limit of quantification was *ca.* 5 ng/ml (Fig. 5). Blank plasma samples spiked with 5 ng/ml revealed day-to-day coefficients of variation (C.V.) between 22% (amitriptyline) and 5% (*E*-hydroxyamitriptyline).

TABLE II

RETENTION TIMES OF SELECTED PSYCHOTROPIC DRUGS

The retention times were determined by analysing plasma samples spiked with 200 ng of pure drug substances per millilitre.

Drug	Retention time (min)
Antidepressants and metabolites	
Amitriptyline	8.54
E-10-Hydroxyamitriptyline	6.97
Z-10-Hydroxyamitriptyline	7.38
Nortriptyline	14.54
E-10-Hydroxynortriptyline	12.29
Z-10-Hydroxynortriptyline	12.63
Imipramine	9.65
2-Hydroxyimipramine	7.60
Desipramine	15.81
2-Hydroxydesipramine	13.33
Clomipramine	8.54
8-Hydroxyclomipramine	7.60
N-Desmethylclomipramine	15.35
8-N-Desmethylclomipramine	13.33
Doxepin	8.26
N-Desmethyldoxepin	13.86
Maprotiline	17.04
N-Desmethylmaprotiline	13.86
Fluvoxamine	6.19
Neuroleptics	
Haloperidol	N.D.[a]
Spiroperidol	N.D.
Pimozide	N.D.
Fluspirilene	N.D.
Trifluperidol	N.D.
Perazine	N.D.
Clozapine	N.D.
N-Desmethylclozapine	6.19
Tranquilizers	
Chlordiazepoxide	N.D.
Clobazam	N.D.
N-Desmethylclobazam	N.D.
Diazepam	N.D.
Nordiazepam	N.D.
Flurazepam	N.D.
Nitrazepam	N.D.
Oxazepam	N.D.

[a] N.D. = not detected; drugs with retention times of greater than 20 min or less than 5 min were considered to be not detectable.

Analytical recovery and precision

Table III summarizes day-to-day variations in

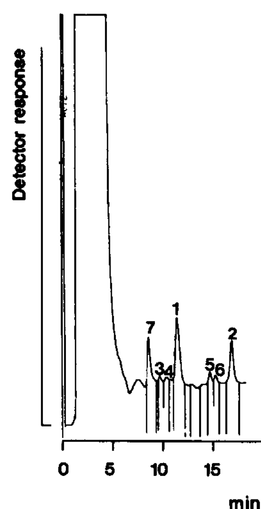

Fig. 4. Representative chromatogram of a plasma sample obtained from a patient who had received daily oral doses of 150 mg of amitriptyline for at least seven days and analysed by the HPLC column-switching system (Fig. 1). To the sample 140 ng of trimipramine (7) were added as internal standard (140 ng/ml) before injection on the clean-up column and subsequent separation on Nucleosil 100 CN. Peak labelling as in Fig. 2.

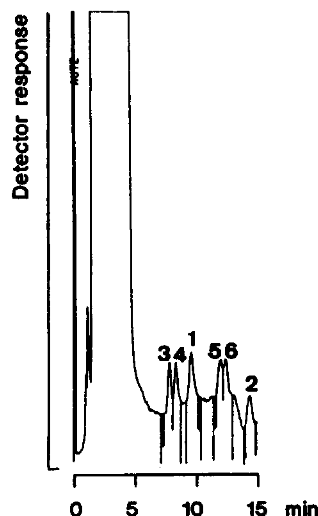

Fig. 5. Chromatogram of a blank plasma sample spiked with 5 ng each of amitriptyline (1) and metabolites (2–6), using the clean-up column for sample enrichment. The clean-up column was loaded three times with 0.1 ml of plasma sample, each followed by a 15-min washing period (5% methanol) before elution, chromatographic separation and detection. Peak labelling as in Fig. 2.

the determination and recovery rates for amitriptyline, nortriptyline and the hydroxylated metabolites. The C.V. determined at four different concentrations in spiked plasma samples ranged between 1 and 14%. The analysis of solutions containing pure drug substances of 50 or 300 ng/ml without column switching and comparison of the results with those obtained from the analyses of plasma samples spiked with 50 or 300 ng revealed recovery rates of 84–112% (Table III).

Routine determinations

The applicability of the method to routine use was tested by analysing various plasma samples of patients who had received amitriptyline (Fig. 4). The steady-state concentrations of amitriptyline and its metabolites were highly variable, especially those of the hydroxylated metabolites. The concentrations of the *E*-isomers always exceeded those of the *Z*-isomers (Table IV).

DISCUSSION

When using HPLC for routine determinations of psychotropic or other drugs, a major problem is the long time required for blood-sample processing. Available immunoassays are more rapid, but these methods lack sufficient specificity, *e.g.* they can hardly distinguish between a parent antidepressant and its metabolites. Using column-switching and on-line HPLC separation as described here, the plasma levels of antidepressants and their metabolites could be rapidly quantified with sufficient specificity, sensitivity and precision.

For drug monitoring with high specificity, chromatographic separations of the compounds to be quantified are required with minimal interference from other endo- or exogenous substances. Among the various stationary phases tested here, only cyanopropyl-bonded materials exhibited satisfactory properties. On Nucleosil 100 CN, amitriptyline and its five demethylated and/or 10-hydroxylated metabolites were well separated. Neverthelesss, each new batch of Nucleosil 100 CN had to be tested before use.

Retention on cyanopropyl columns is ascribed

TABLE III

ASSAY RELIABILITY AS FOUND FOR INDEPENDENT ANALYSES OF AMITRIPTYLINE AND ITS METABOLITES IN PLASMA SAMPLES

Values given are the mean ± S.D. of three or four determinations on different days. Recovery rates were calculated for 50- and 300-ng samples by analysing standard solutions without column switching, and spiked plasma samples submitted to column switching and subsequent HPLC separation (data represent means from three determinations).

Drug	Given (ng/ml)	Found (ng/ml)	C.V. (%)	Recovery (%)
Amitriptyline	25	25 ± 2	4.2	N.D.[a]
	50	45 ± 2	4.4	92 ± 7
	100	102 ± 5	4.9	N.D.
	300	309 ± 3	1.0	84 ± 4
Nortriptyline	25	23 ± 2	8.7	N.D.
	50	45 ± 2	4.4	112 ± 4
	100	104 ± 8	7.7	N.D.
	300	316 ± 6	1.9	95 ± 3
E-10-Hydroxyamitriptyline	25	24 ± 3	12.5	N.D.
	50	49 ± 3	6.1	92 ± 3
	100	105 ± 9	8.6	N.D.
	300	318 ± 6	1.9	91 ± 2
E-10-Hydroxynortriptyline	25	21 ± 2	9.5	N.D.
	50	45 ± 3	6.7	97 ± 6
	100	107 ± 3	2.8	N.D.
	300	326 ± 5	1.5	93 ± 3
Z-10-Hydroxyamitriptyline	25	21 ± 3	14.3	N.D.
	50	43 ± 1	2.3	91 ± 6
	100	100 ± 7	7.0	N.D.
	300	309 ± 3	1.0	95 ± 5
Z-10-Hydroxynortriptyline	25	21 ± 2	9.5	N.D.
	50	45 ± 3	6.7	96 ± 6
	100	107 ± 8	7.5	N.D.
	300	326 ± 5	1.5	93 ± 2

[a] N.D. = not determined.

to the slightly acidic residual silanols, π-electrons and the moderately basic properties of the CN group [20,21]. For the separation of amitriptyline and its metabolites, it seemed likely that lipophilic π–π electron interactions were more relevant than retention due to basic properties. This was concluded from the sequence of elution of the different drugs. The secondary amine nortriptyline, which is more basic than the tertiary amine amitriptyline, was retained longer in the analytical column than the tertiary amine. When the hydrophobicity of amitriptyline was decreased by the introduction of a hydroxy group into the molecule, the retention time decreased. Drug retention due to π–π electron interactions is further supported by the results obtained when different modified stationary phases were tested in the clean-up column. Connecting a clean-up column filled with a phenyl-bonded phase increased the number of π electrons in the separating material. The manipulation decreased the elution of both amitriptyline and nortriptyline, indicating that tricyclic antidepressants were sensitive to π–π electron interaction. These effects were less pro-

TABLE IV

STEADY-STATE CONCENTRATIONS OF AMITRIPTYLINE AND METABOLITES

Samples taken from eight depressed patients treated with 150 mg daily of amitriptyline for at least 7 days. *E*-OH-Ami = *trans*-10-hydroxyamitriptyline; *Z*-OH-Ami = *cis*-10-hydroxyamitriptyline; AMI = amitriptyline; *E*-OH-NOR = *trans*-10-hydroxynortriptyline; *Z*-OH-NOR = *cis*-10-hydroxynortriptyline; NOR = nortriptyline. N.D. = not detected (< 1 ng/ml).

Patient No.	Plasma level (ng/ml)					
	E-OH-AMI	*Z*-OH-AMI	AMI	*E*-OH-NOR	*Z*-OH-NOR	NOR
1	5	8	153	18	15	82
2	15	3	60	66	5	29
3	11	N.D.	152	93	4	100
4	17	5	99	83	10	65
5	6	5	39	67	9	75
6	21	16	61	136	37	86
7	3	4	88	15	4	19
8	21	N.D.	78	278	N.D.	55

nounced for the hydroxylated metabolites. Good separations on Nucleosil 100 CN, however, should not only be ascribed to π–π electron interactions. Addition of a phenylated stationary phase in the clean-up column decreased the resolution factor calculated for the separation of amitriptyline and nortriptyline. The resolution factor increased when adding unreacted silanols to the silica support, which was done by coupling a column filled with silica material. This material, however, was inappropriate for routine use because of its incompatibility with the clean-up solvent. It must therefore be concluded that the cyanopropyl stationary phase used together with the described analytical solvent provided an optimal mixture of lipophilic π electrons and slightly acidic silanols for satisfactory chromatographic resolution of amitriptyline and the five metabolites that are formed *in vivo*.

The lower limit of quantitation was 10–15 ng/ml for all compounds of interest. If necessary, this limit can be easily decreased by using the clean-up column in the enrichment mode [8]. As little as 5 ng/ml could be accurately determined when the plasma sample was injected three times on the clean-up column before elution and subsequent detection. The method is therefore more sensitive than others [7,8,10].

With regard to the stability of column-switching systems, intermittent reversal of the eluent flow is recommended to wash out impurities from the clean-up column, especially when analysing serum or plasma samples [13]. Such operations, however, complicate the configuration of the system [10,13]. The column-switching system described here did not need flow reversal. As shown in Fig. 1, drug desorption was carried out in the foreflow direction using the acetonitrile–methanol–phosphate buffer eluent, and deionized water containing 5% methanol was sufficient for washing and re-equilibration of the clean-up column. In our hands, this mode allowed the analysis of more than 100 plasma samples without having to change the clean-up column. Only occasionally, when the back-pressure exceeded 28 bar, it was necessary to exchange more frequently the clean-up column or the sieve protecting it.

In depressed patients treated with amitriptyline, plasma levels of 150–300 ng/ml of amitriptyline plus nortriptyline are considered optimal [1,4,5]. At lower levels poor responses may result, and at concentrations exceeding 300 ng/ml the risk of toxic side-effects increases [1–5]. The column-switching method developed by us enabled the determination of therapeutically relevant concentrations of amitriptyline, nortriptyline and

their hydroxylated metabolites. The latter are considered important with regard to drug safety, especially cardiotoxic side-effects [17–19].

In comparison with other reported methods [6–11] the column-switching system described here seemed advantageous. Using a rather simple configuration, consisting of an HPLC pump and a switching valve, in addition to the components of a classical isocratic HPLC system, the parent antidepressant and its demethylated and hydroxylated metabolites were extracted from plasma, separated and recorded. Plasma or serum samples can be processed and results reported in less than 1 h after blood has been collected from the patient. From the finding that a lot of psychotropic drugs were detectable and well separated from amitriptyline and the metabolites, it can be concluded that the method described here may easily be adapted to the determination of various other drugs.

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