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Simultaneous determination of citalopram, fluoxetine, paroxetine and their metabolites in plasma by temperature-programmed packed capillary liquid chromatography with on-column focusing of large injection volumes

P. Molander[a,b,*], A. Thomassen[b], L. Kristoffersen[c], T. Greibrokk[b], E. Lundanes[b]

[a]National Institute of Occupational Health, P.O. Box 8149 Dep., N-0033 Oslo, Norway
[b]Department of Chemistry, University of Oslo, P.O. Box 1033 Blindern, N-0315 Oslo, Norway
[c]National Institute of Forensic Toxicology, P.O. Box 495 Sentrum, N-0105 Oslo, Norway

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Abstract

A miniaturized temperature-programmed packed capillary liquid chromatographic method with on-column large volume injection and UV detection for the simultaneous determination of the three selective serotonin reuptake inhibitors citalopram, fluoxetine, paroxetine and their metabolites in plasma is presented. An established reversed-phase C_8 solid-phase extraction method was employed, and the separation was carried out on a 3.5-μm Kromasil C_{18} 0.32×300 mm column with temperature-programming from 35 (3 min) to 100°C (10 min) at 1.3°C/min. The mobile phase consisted of acetonitrile–45 m*M* ammonium formate (pH 4.00) (25:75, v/v). The non-eluting sample focusing solvent composition acetonitrile–45 m*M* ammonium formate (pH 4.00) (3:97, v/v) allowed injection of 10 μl or more of the plasma extracts. The method was validated for the concentration range 0.05–5.0 μ*M*, and the calibration curves were linear with coefficients of correlation >0.993. The limits of quantification for the antidepressants and their metabolites ranged from 0.05 to 0.26 μ*M*. The within and between assay precision of relative peak height were in the range 2–22 and 2–15% relative standard deviation, respectively. The within and between assay recoveries were in the 61–99 and 54–92% range for the antidepressants, respectively, and between 52–102 and 51–102% for the metabolites. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Temperature programming; Large volume injection; Citalopram; Fluoxetine; Paroxetine

1. Introduction

The interest in packed capillary liquid chromatography (LC) has increased recently due to the numerous advantages offered by this technique [1]. Especially the increased mass sensitivity of the miniaturized columns is attractive with regard to sensitive determination of compounds present at low concentrations in limited sample volumes [1]. Utilization of focusing techniques upon injection of such samples make available introduction of enlarged sample volumes, often allowing total sample volume exploi-

*Corresponding author. Tel.: +47-2285-5573; fax: +47-2319-5206.
E-mail address: pal.molander@stami.no (P. Molander).

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tation [2–4]. Analytes in large sample volumes are traditionally enriched at the LC column inlet using a sample solvent composition of non-eluting properties [2–7], although Molander et al. recently have introduced LC sub-ambient temperature-assisted solute focusing especially suitable for enrichment of hydrophobic compounds that are difficult to dissolve in weak solvents [8,9].

Modern pressurized LC started as a complementary technique to gas chromatography (GC), replacing temperature with solvent strength as elution parameter. Thus, the need for temperature control in LC has appeared to be less urgent, and solvent gradient elution has evolved to be the traditional way to manipulate the elution strength in LC during the chromatographic run. However, this technique requires expensive dedicated micro flow pumping systems when using miniaturized columns, due to the low flow-rates required.

Packed capillary LC is, however, especially well suited for temperature programming, due to the low thermal mass of the small bore columns, and temperature-programmed packed capillary LC has in recent studies proven to be a feasible and elegant alternative to solvent gradients [6–16]. Nonetheless, fears of decomposing analytes or the stationary phases, sometimes unwarranted, have added to the neglect of temperature as a variable in LC, although nearly all physical parameters of importance in LC are a function of temperature [17]. Thus, development of modern, more temperature stable stationary phase materials has to some extent inspired the use of elevated temperatures in LC, resulting in reduced column back pressures, and often enhanced efficiency [5,17] and advantageous selectivity effects [17,18].

Despite the fact that presented results are in favor of conducting separations at elevated temperatures or with temperature programming, temperature-programmed packed capillary LC has only been scarcely explored by a limited number of research groups, mainly with the use of test mixtures and model solutes [19]. Hence, development of validated and applicable temperature-programmed packed capillary LC methods is required in order to demonstrate the potential of this technique.

The antidepressants citalopram (CIT), paroxetine (PAR) and fluoxetine (FLU) belong to the class of selective serotonin reuptake inhibitors (SSRIs). As compared to the widely used tricyclic antidepressants (TCAs), the SSRIs have a generally more benign tolerability profile, with fewer adverse effects [20]. For treatment over time, however, an SSRI discontinuation syndrome seems to exist [21]. The SSRIs are widely used in the treatment of depression [20], anxiety, obsessive-compulsive disorder, panic disorder and social phobia [22], and they have occasionally been prescribed for pre-menstrual dysphoric disorder [23] and in the treatment of eating disorders [23].

With regard to the use of antidepressants for the treatment of depressions, there is always a risk for suicidal attempts with the prescribed drug [20]. Furthermore, the SSRIs can have a toxic effect in interaction with other drugs [24]. Paucity of information concerning drug intake prior to death is a common problem in forensic toxicology, and there is a need for reliable methods for screening of antidepressants. Such methods should preferably be applicable for as many antidepressants as possible in order to reveal possible drug interactions, in addition to quantifying low concentrations of both the SSRIs and their metabolites in limited volumes of body fluids. In addition, determination of SSRIs and their metabolites in human plasma are of importance in pharmacokinetic studies and in cases where patients do not respond as expected due to interactions with other drugs. Thus, several LC, GC and thin layer chromatographic (TLC) methods have been presented for the determination of CIT, PAR and FLU and their metabolites in human plasma or serum [25–31]. Only a few methods, however, have been published on the simultaneous determination of several of the SSRI compounds and their respective metabolites [32,33].

The aim of this study was to develop and validate a large-volume injection on-column focusing temperature-programmed packed capillary LC screening method especially suitable for the simultaneous determination of low concentrations of CIT, FLU, PAR and their respective metabolites in human plasma, based on a solid-phase extraction (SPE) method for the SSRIs and their metabolites developed by Kristoffersen et al. [33].

2. Experimental

2.1. Reagents and materials

PAR hydrochloride hemihydrate, (−)-*trans* 4-(4-fluorophenyl) - 3 - (3 - hydroxy - 4 - methoxyphenoxy-methyl)piperidine hydrochloride (BRL36583A) and (−)-*trans* 4 - (4 - fluorophenyl) - 3 - (4 - hydroxy - 3-methoxyphenoxymethyl)piperidine hydrochloride (BRL36610A) were kindly provided by SmithKline Beecham (Worthing, UK). CIT hydrobromide, desmethylcitalopram (DCIT) hydrochloride, didesmethylcitalopram (DDCIT) tartrate monohydrate and citalopram N-oxide (CITNO) were likewise gifts from H. Lundbeck (Copenhagen, Denmark). FLU hydrochloride and norfluoxetine (NFLU) hydrochloride were gifts from Eli Lilly (Indianapolis, IN, USA). Analytical grade protriptyline (PRO) hydrochloride was purchased from Sigma (St. Louis, MO, USA). The structures of the SSRIs and their metabolites are shown in Fig. 1.

Analytical grade potassium dihydrogenphosphate (KH_2PO_4) and formic acid (HCOOH) were obtained from Merck (Darmstadt, Germany), while analytical grade ammonium formate (NH_4HCOO) was purchased from Sigma. HPLC grade trifluoroacetic acid (TFA) and acetonitrile (ACN) were obtained from Rathburn (Walkerburn, UK), while methanol (MeOH) of similar quality was purchased from Lab Scan (Dublin, Ireland). Deionized water from a Milli-Q station was used in all procedures (Millipore, Bedford, MA, USA). Carbon dioxide, nitrogen and helium of suitable quality were obtained from AGA (Oslo, Norway). All fused-silica capillaries were received from Polymicro Technologies (Phoenix, AZ, USA). Drug-free citrated plasma was purchased from the Blood Center at Ullevaal Hospital (Oslo, Norway).

2.2. Packed capillary LC instrumentation and chromatographic conditions

The packed capillary LC system consisted of a Merck-Hitachi L-7100 pump, a Hewlett-Packard 5790A gas chromatograph which served as column oven (Wilmington, DE, USA), a Spectrasystem UV-2000 detector (Thermo Separation Products, San Jose, CA, USA) and a Shimadzu C-R6A integrator (Shimadzu, Kyoto, Japan). UV detection was carried out at 230 nm, using a UZ-LI-CAP flow cell with an 8-mm light path (LC Packings, Amsterdam, The Netherlands). A 15-μm I.D. restrictor of 20 cm length was coupled to the outlet of the detector cell, in order to suppress boiling of the mobile phase at elevated temperatures. A Rheodyne model 7725 with external loops of 10 or 50 μl served as injection valve (Cotati, CA, USA), and a 50 μm×20 cm fused-silica capillary connected the packed capillary column to the injection valve, which was operated at ambient temperature. In addition, a Valco Model C4 injection valve with an internal loop volume of 50 nl was utilized during preliminary experiments.

The packed columns were prepared according to a method described by Trones at al. [15], using neat supercritical carbon dioxide as the slurry medium. Valco ZU1C unions with 2 μm 2SR1 steel screens served as column end fittings and the column body was connected to the end fittings by Valco FS1.4 polyimide ferrules and steel nuts (Valco Instruments, TX, Houston, USA). The column body was of fused-silica (320 μm I.D., 450 μm O.D.) with a polyimide protection layer, and the columns were prepared in various lengths. The stationary phase material was 3.5-μm Kromasil C_{18} particles (Hichrom, Reading, UK) or 5-μm Symmetry C_{18} (Waters, Milford, MA, USA).

The non-eluting focusing solvent composition was ACN–45 m*M* NH_4HCOO (pH 4.00) (3:97, v/v), and the mobile phase consisted of ACN–45 m*M* NH_4HCOO (pH 4.00) (25:75, v/v). HCOOH was used for pH adjustments. The mobile phase flow-rate was 5 μl/min throughout the study, and the mobile phase was helium-degassed for 15 min daily. Injections were performed at a column temperature of 35°C, and a washing procedure consisting of injection of 50 μl water was carried out between the injections. Temperature programming from 35 (3 min) to 100°C (10 min) at 1.3°C/min was utilized for gradient action during the method validation experiments.

In order to correct for effects of rising baseline during temperature programming potentially causing reduced quality of quantification, the chromatograms were digitized using a UN-SCAN-IT software pack-

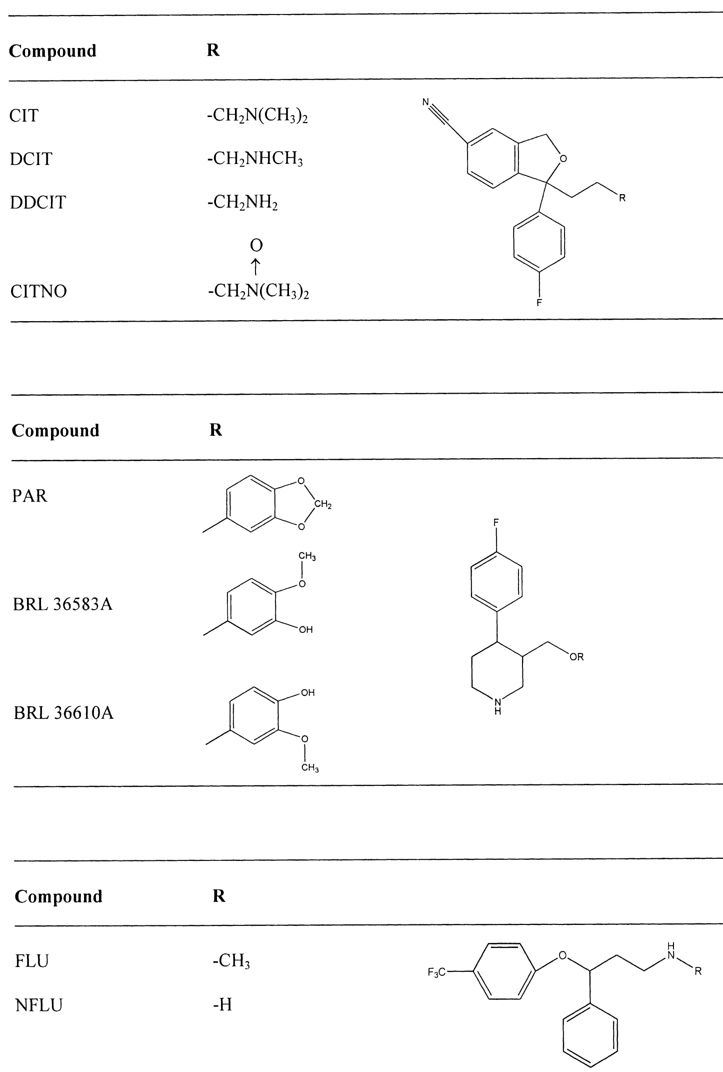

Fig. 1. General structures of the SSRIs and their metabolites.

age (Scientific Software Service, Ober-Olm, Germany) prior to mathematical subtraction of the drifting baseline signal by the use of Microcal Origin 6.0 software (Northampton, MA, USA), according to a procedure previously described [34].

2.3. SPE procedure

An automated SPE procedure developed by Kristoffersen et al. for sample preparation of the SSRIs and their metabolites was employed in the present study using manual operation with vacuum suction. The original method is described in detail elsewhere [33]. Fifty μl of the 0.5 μM solution of the internal standard PRO were added to a 0.5-ml volume of spiked plasma, followed by 15 min treatment in an ultrasonic bath and addition of 5.0 ml 0.1 M KH_2PO_4 (pH 7.00)–MeOH (90:10, v/v). The sample was subsequently mixed using a whirlmixer and centrifuged at 2000 rev./min for 10 min, and the supernatant was applied to the C_8 non-endcapped SPE columns (3 ml, 100 mg) (International Sorbent

Technology, Mid Glamorgan, UK), which previously had been conditioned with 2 ml MeOH, 1 ml water and 1 ml 0.1 *M* KH_2PO_4 (pH 7.00). The SPE columns were rinsed with 2 ml water, 2 ml 0.1 *M* KH_2PO_4 (pH 7.00) and 1 ml ACN–water (50:50, v/v), prior to elution with 0.75 ml MeOH–TFA (98:2, v/v). Finally, the eluates were evaporated to dryness under a stream of nitrogen at 40°C, followed by redissolving in 100 μl of the predetermined non-eluting sample solvent composition ACN–45 m*M* NH_4HCOO (pH 4.00) (3:97, v/v).

2.4. Preparation of standard solutions

Stock solutions of CIT, DCIT, DDCIT, CITNO, PAR, BRL36610A, BRL36583A, FLU and NFLU as well as of the internal standard PRO were prepared in methanol (MeOH) at a concentration of 2500 μ*M* at the National Institute of Forensic Toxicology (Oslo, Norway), and stored at −78°C. Predetermined volumes of each stock solution were evaporated to dryness under a stream of nitrogen at 40°C, prior to in-house redissolving in similar volumes of ACN–45 m*M* NH_4HCOO (pH 4.00) (10:90, v/v) to give 2500 μ*M* working solutions of each analyte. These solutions were stored at −18°C.

For robustness testing of the large volume injection temperature-programmed packed capillary LC method, a solution of CIT, DCIT, DDCIT, CITNO, PAR, BRL36610A, BRL36583A, FLU and NFLU (each 2.5 μ*M*), were prepared in ACN–45 m*M* NH_4HCOO (pH 4.00) (3:97, v/v). The concentration of the internal standard PRO was 0.25 μ*M* in the solution, which was stored at 4°C.

For method validation experiments, LC calibration solutions containing all analytes were prepared in ACN–45 m*M* NH_4HCOO (pH 4.00) (3:97, v/v) at concentrations of 0.600 (0.250 μ*M* regarding CIT, DCIT, DDCIT and CITNO), 2.50, 12.5, 17.5 and 25.0 μ*M* of each component, which corresponds to plasma concentrations of 0.120 (0.0500 μ*M* regarding CIT, DCIT, DDCIT and CITNO), 0.500, 2.50, 3.75 and 5.00 μ*M* of each component. The concentration of the internal standard PRO was 0.250 μ*M* in the LC calibration solutions, corresponding to 0.0500 μ*M* in plasma.

Drug-free plasma was spiked with appropriate aliquots of the working solutions in order to obtain concentrations of 0.120 (0.0500 μ*M* regarding CIT, DCIT, DDCIT and CITNO), 2.50, 12.5, 18.8 and 25.0 μ*M* of each analyte after the SPE procedure, evaporation and re-dissolving in ACN–45 m*M* NH_4HCOO (pH 4.00) (3:97, v/v). The plasma extracts were stored at 4°C prior to analysis.

2.5. Quantification

Relative peak height ratios were used for quantification and for method validation. The recovery of the analytes from spiked plasma samples were obtained by external standard quantification using the LC calibration solutions.

3. Results and discussion

The use of temperature programming in miniaturized LC has only been scarcely explored, mainly with the use of test mixtures and model solutes. Promising results have been presented, but only a limited number of recent papers have presented practical solutions to real-life applications utilizing temperature-programmed packed capillary LC [6–8,35,36]. Thus, there is an absolute need for further thorough evaluation of this technique in order to establish proofs of performance beyond the level of forecasts of its usefulness. Hence, this paper presents, to the authors' knowledge, the first validated temperature-programmed packed-capillary LC method, using antidepressants from human plasma as a biological real-life model application. The eminent mass sensitivity of packed capillary columns combined with the often limited volumes of biological samples containing analytes at low concentrations, encouraged the implementation of on-column focusing large volume injection, making the need for thorough validation expedient.

3.1. Chromatography

A 3.5-μm Kromasil C_{18} stationary phase was employed in the present study, due to its good stability and performance in aqueous environment at elevated temperatures. The 5-μm Symmetry C_{18} stationary phase material was also evaluated, resulting in less retention, poorer resolution, increased

peak tailing and reduced efficiency as compared to the Kromasil material. A column length of 30 cm was required in order to separate CIT and its metabolites from the UV absorbing matrix front, resulting in relatively long analysis time. The mobile phase composition was established by careful optimization of the ratio between ACN and 45 m*M* NH_4HCOO (pH 4.00). Different amounts of ACN ranging from 20 to 35% were evaluated, and 25% ACN resulted in an optimized compromise between peak resolution and analysis time when operating isothermally at 45°C. Consequently, a mobile phase composition of ACN–45 m*M* NH_4HCOO (pH 4.00) (25:75, v/v) was used for the rest of the study. The isothermal separation of the SSRIs and their metabolites using these conditions is shown in Fig. 2. ACN was chosen as the organic modifier in the mobile phase due to its low viscosity and negligible UV absorption at 230 nm. A mobile phase pH of 4.00 resulted in optimized separation of the first eluting components, in accordance with results presented by Kristoffersen et al. using multivariate analysis [33].

As shown in Fig. 2, the analysis time when operating isothermally at 45°C was unacceptably long, and the last eluting peaks were severely broadened, illustrating the need for a gradient method. Thus, temperature programming was explored in order to achieve gradient action. Unfortunately, effects of rising baseline during temperature programming occurred, which potentially can preclude quantitative measurements. However, mathematical baseline correction was performed prior to the peak height measurements in order to reduce such effects, utilizing a procedure previously described by Bruheim et al. [34]. As illustrated in Fig. 3, an initial temperature of 35°C was required in order to separate the first eluting components from the matrix front of the plasma samples, and a temperature program starting at 35°C (3 min) prior to a ramp of 1.3°C/min to 100°C (10 min) resulted in a 50% reduction of the retention time of the last eluting peak, FLU, as compared to the isothermal separation at 45°C. By the use of a gradient of 2°C/min, a 75% reduction of the retention time of FLU was achieved. However, this gradient resulted in a steeper baseline as compared to the ramp of 1.3°C/min, making mathematical baseline correction troublesome. A final temperature of 110°C during temperature programming was also explored, resulting in further reduction of the total analysis time. However, a final

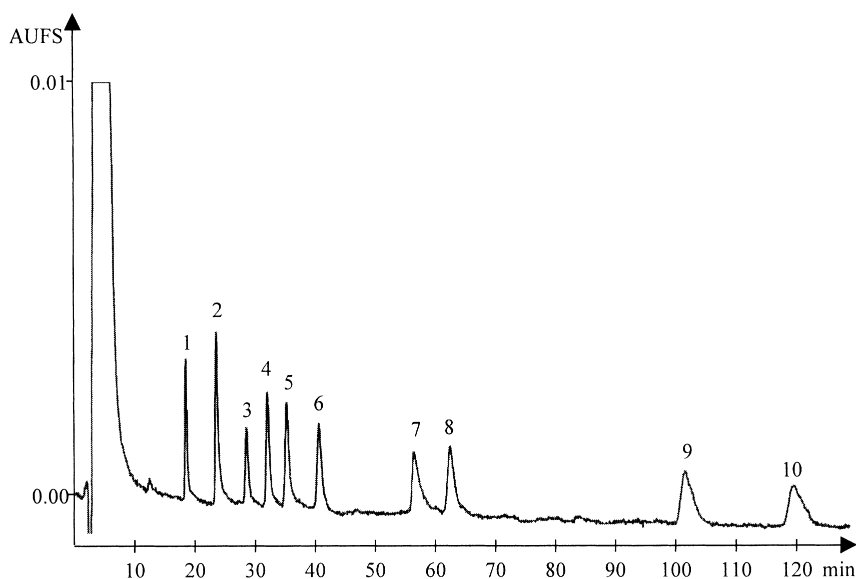

Fig. 2. Separation of the SSRIs and their metabolites at 45°C using a 300×0.32 mm 3.5-μm Kromasil C_{18} column and a mobile phase consisting of ACN–45 m*M* NH_4HCOO (pH 4.00) (25:75, v/v). Peak identification: BRL36610A (1), BRL36583A (2), DDCIT (3), DCIT (4), CIT (5), CITNO (6), PAR (7), PRO (8), NFLU (9) and FLU (10).

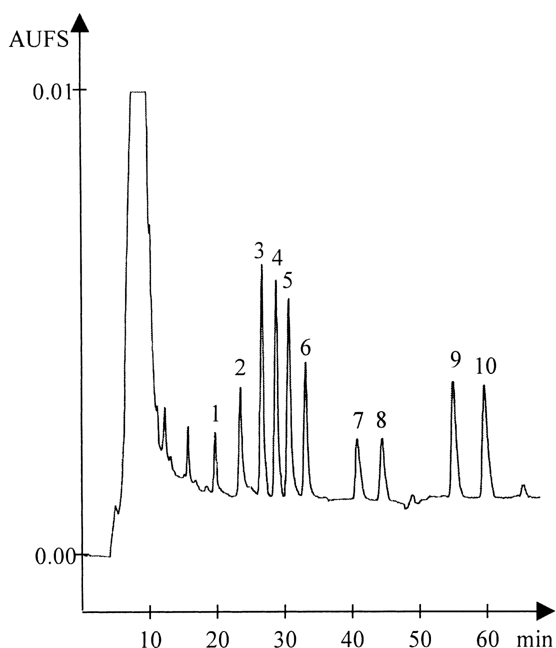

Fig. 3. Separation of the SSRIs and their metabolites in a spiked plasma sample using a temperature program of 35 (3 min)–100°C (10 min) at 1.3°C/min. All other conditions as described in Fig. 2. Peak identification: BRL36610A (1), BRL36583A (2), DDCIT (3), DCIT (4), CIT (5), CITNO (6), PAR (7), PRO (8), NFLU (9) and FLU (10).

temperature of 100°C was preferred due the reduced risk of stationary phase hydrolysis and increased long term stability. Hence, the temperature program of 35 (3 min)–100°C (10 min) at 1.3°C/min was used throughout the rest of the study (Fig. 3). A 10-min temperature equilibrium period was required in between the analyses, in which a simultaneous washing procedure consisting of injection of 50 μl water was carried out.

PRO was selected as internal standard because this drug showed no interference with endogenous compounds in plasma samples, and PRO was easily baseline separated from the other analytes. It is also of importance that PRO is not used therapeutically in Norway, eliminating problems related to co-medication.

3.2. On-column focusing large volume injection

In preliminary experiments an injection volume of 50 nl was used, revealing the need for a more sensitive method. In order to improve the concentration limit of detection (cLOD) by several orders of magnitude, the use of on-column focusing of larger sample volumes was utilized. The non-eluting sample solvent composition was established by measuring the peak width at half the peak height of the first eluting component, BRL36610A, when varying the ratio of ACN–45 m*M* NH_4HCOO (pH 4.00) between 0.5 and 20% ACN, always injecting 10 μl of a standard solution of 12.5 μ*M* BRL36610A at 35°C. The peak width increased when the amount of ACN in the solvent mixture exceeded 3%, while being invariant in the range 0.5–3% ACN. Hence a non-eluting sample solvent composition of ACN–45 m*M* NH_4HCOO (pH 4.00) (3:97, v/v) was used for the large volume injection experiments. Furthermore, a 50-μl sample volume was injected using the established sample solvent composition, displaying no differences as compared to the 10-μl injections, which supported a theory of sufficient analyte focusing, making the band broadening during loading independent of the sample volume applied. Nevertheless, an injection volume of 10 μl was sufficient for therapeutic monitoring, and was consequently used during later experiments.

The plasma SPE procedure used in the present study was originally designed for use in combination with a conventional LC method where no focusing effect was employed, and thus the evaporated extracts were redissolved in the mobile phase [33]. In the present study, however, the dry, evaporated extracts were redissolved using the non-eluting solvent composition ACN–45 m*M* NH_4HCOO (pH 4.00) (3:97, v/v) in order to inject large sample volumes without column overload.

3.3. Method validation

3.3.1. Linearity

The concentration range was selected according to plasma concentration levels after normal drug intake; 0.12–0.60, 0.060–0.58 and 0.16–1.5 μ*M* of CIT [37], PAR [38] and FLU [38], respectively. The metabolites are generally present at lower concentrations as compared to the parent compounds. Whole blood is traditionally used for forensic analysis, and Kristoffersen et al. have previously shown that the employed SPE method is applicable for both

plasma and whole blood samples [33]. Although only plasma samples were investigated in the present study, it was a goal that the LC method also could be implemented for forensic analysis of whole blood extracts after SSRI overdoses. Thus, the method was validated for a wider concentration range than usually employed [33], adjusted to cover both therapeutic drug monitoring of the SSRIs and their metabolites and forensic analysis after SSRI overdoses (0.05–5.0 μ*M* in plasma). Fig. 4a shows the large volume injection temperature-programmed packed capillary LC separation of the SSRIs and their metabolites in a spiked plasma extract (0.05 μ*M* of each), while Fig. 4b similarly shows the chromatographic profile of a drug-free plasma extract.

The LC calibration curves were linear in the investigated concentration range, with coefficients of correlation (r^2)>0.998. The SPE–LC method was also linear within the investigated concentration range (r^2>0.993) for all analytes.

3.3.2. Detection limits

The mass limit of detection (mLOD) and mass limit of quantification (mLOQ) of the method were defined using a signal-to-noise ratio of 3:1 and 10:1, respectively. The mLOD, mLOQ, concentration LOD (cLOD) and concentration LOQ (cLOQ) of the SSRIs and their respective metabolites ranged between 53.0–260 and 170–870 pg and 0.020–0.080 and 0.050–0.30 μ*M*, respectively, as summarized in Table 1. Thus, the method is suitable for therapeutic drug monitoring of the SSRIs [33]. Furthermore, the method is capable of detecting and quantifying far lower concentrations of the analytes by focusing larger volumes than the 10 μl upon injection or by employing other suitable and more sensitive de-

Table 1

Mass and concentration limits of detection and quantification for the SSRIs and their metabolites in plasma

Analyte	mLOD (pg)	cLOD (μ*M*)	mLOQ (pg)	cLOQ (μ*M*)
CIT	55.0	0.020	180	0.050
DCIT	53.0	0.020	170	0.060
DDCIT	53.0	0.020	170	0.060
CITNO	65.0	0.020	210	0.070
PAR	260	0.080	870	0.30
BRL36610A	130	0.040	410	0.10
BRL36583A	130	0.040	410	0.10
FLU	100	0.030	340	0.10
NFLU	92.0	0.030	310	0.10

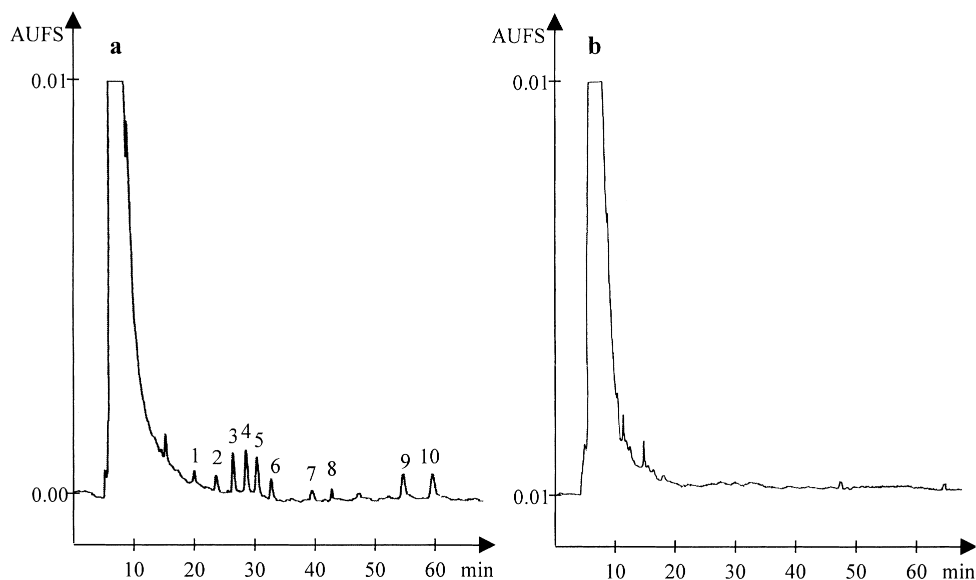

Fig. 4. Chromatographic profiles of a spiked plasma sample containing the SSRIs and their metabolites at the lowest concentration in the investigated linear range (0.05 μ*M* of each) (a) and of a blank plasma sample (b). All other conditions as described in Figs. 2 and 3. Peak identification: BRL36610A (1), BRL36583A (2), DDCIT (3), DCIT (4), CIT (5), CITNO (6), PAR (7), PRO (8), NFLU (9) and FLU (10).

tection principles, such as MS or fluorescence detection. However, UV detection and an injection volume of 10 μl were a good compromise between the required sensitivity and loading time when working with plasma samples of 0.5 ml containing the SSRIs at their typical concentration levels.

3.3.3. Precision

The within and between assay precisions ($n=4$) of the relative peak height ratio between the analytes and the internal standard are given in Table 2, and the relative standard deviations (RSD) were in the range 2.0–22% and 2.3–15%, respectively. The analyses were repetitively performed by the same analyst over a period of 3 weeks in order to obtain the between assay precision data. As shown in Table 2, the within and between assay precision was satisfactory at the higher concentrations, while at concentrations close to the LOQ, the RSDs of the relative peak height ratios were not surprisingly comparatively higher regarding some of the analytes.

3.3.4. Recovery

The within and between assay recovery ($n=4$) of CIT, PAR and FLU were in the range 61–99% and 54–92%, respectively, and between 52–102% and 51–102%, respectively, with regard to their metabolites (Table 2), in accordance to that obtained by Kristoffersen et al. [33].

3.3.5. Robustness

The column preparation method has previously proven to produce columns with an RSD of less than 3% with regard to column-to-column retention time reproducibility [39]. Hence, identically prepared capillary columns produced identical chromatographic performance in the present study ($n=3$). The column that was used during method validation

Table 2
Within- and between assay recovery and -precision of the SSRIs and their metabolites in plasma

Analyte	Concentration in plasma (μM)	Within assay recovery (RSD, $n=4$)	Within assay precision (%, $n=4$)	Between assay recovery (%, $n=4$)	Between assay precision (RSD, $n=4$)
CIT	0.125	91.6	2.4	90.0	3.6
	2.50	89.6	5.2	88.2	2.5
	5.00	85.3	2.0	86.6	9.4
DCIT	0.125	59.4	18	75.0	13
	2.50	99.0	5.4	94.6	5.8
	5.00	85.7	5.5	84.5	2.3
DDCIT	0.125	54.6	14	56.0	13
	2.50	102	6.0	102	3.8
	5.00	87.6	1.7	86.9	2.9
CITNO	0.125	83.6	14	72.0	20
	2.50	83.2	7.5	85.7	3.5
	5.00	70.0	4.1	69.8	4.0
PAR	0.600	63.3	21	53.5	13
	2.50	93.4	7.1	92.1	5.3
	5.00	80.7	8.9	84.6	3.5
BRL36610A	0.600	54.9	22	51.5	7.4
	2.50	94.6	6.3	95.3	6.4
	5.00	69.5	3.0	68.5	7.2
BRL36583A	0.600	51.5	19	51.3	15
	2.50	94.3	4.6	88.1	5.2
	5.00	90.6	9.6	90.8	3.5
FLU	0.600	61.3	8.5	62.5	3.3
	2.50	98.7	13	91.5	12
	5.00	83.6	9.5	84.0	8.5
NFLU	0.600	58.6	20	60.8	11
	2.50	97.6	5.2	97.5	6.5
	5.00	84.9	5.6	89.9	7.6

experiments was extensively used at elevated temperatures over a period of 7 weeks, and did not show any signs of degradation during that period of time. The within and between assay precision of retention times in temperature-programmed mode were always below 6%, and the peak resolution was invariant throughout the study. LC calibration solutions and spiked plasma SPE extracts stored at 4°C were consecutively analyzed over a period of 6 weeks, displaying no quantitative or qualitative differences within this period. The automated SPE method developed by Kristoffersen et al. [33] was proven to be robust also when being subject to manual operation.

4. Conclusions

The present study has shown that large-volume injection temperature-programmed packed capillary LC is a suitable technique for sensitive determination of SSRIs in plasma, demonstrating comparable characteristics to that of a conventional gradient elution reference LC method. Improved LODs are easily accessible by loading larger injection volumes or by using more sensitive detectors, such as MS, fluorescence or electrochemical detection.

The development of an analytical technique is a three-phase process. In the first phase, a proof of principle is established by using well characterized standards. In the second phase, the robustness of the technique must be demonstrated with real samples, which is often a major stumbling block, while in the third phase of development, the technique is converted into a commercialized entity. The present study has proven that temperature-programmed packed capillary LC elegantly can be utilized for screening of antidepressants from plasma, providing satisfactory results that are comparable to that of an established method [33]. Thus, temperature-programmed packed capillary LC has presently entered the second phase of the technique development process. However, there is still a need for further demonstration of the feasibility and robustness of this technique with real-life samples, and further emphasis will be directed towards applications where temperature-programmed packed capillary LC provides improved performance as compared to established techniques.

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