Journal of Chromatography A, 1217 (2010) 7598–7604

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Simultaneous determination of antidepressants by non-aqueous capillary electrophoresis-time of flight mass spectrometry

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ARTICLE INFO

Article history:
Received 4 June 2010
Received in revised form
29 September 2010
Accepted 1 October 2010
Available online 8 October 2010

Keywords:
Antidepressants
Non-aqueous capillary electrophoresis
Time of flight mass spectrometry

ABSTRACT

Simultaneous determination of 20 antidepressants in plasma samples was carried out by non-aqueous capillary electrophoresis with time of flight mass spectrometry via electrospray ionization, where a mixture of 60 mM ammonium acetate and 1 M acetic acid in acetonitrile, and water, as well as methanol (100:1:0.5, v/v/v) was selected as the background electrolyte. By using time of flight mass spectrometry, accurate mass information was obtained and the background noise was dramatically decreased, thus causing a great improvement in qualitative ability. As for the plasma sample, solid phase extraction with Oasis HLB was used. The limits of detection and quantification were in the range of 0.5–1 and 1–5 ng/ml, respectively. The sensitivity of the present method was found better, i.e. approximately 10–60 folds compared to that using photo diode array detectors because the analyte peak could be clearly distinguished from the background derived from the plasma. The present method was found very useful and practical as regards to routine analysis of plasma samples.

1. Introduction

Tricyclic antidepressants and tetracyclic antidepressants have some side effects and could cause toxic accidents. Recently, it was reported that even selective serotonin reuptake inhibiter (SSRI) with fewer side effects could cause toxic accidents, resulted in arousing aggressive character in the patients. From the viewpoint of forensic science, it was found that poisoning trouble could happen by taking large amounts of any antidepressant; furthermore, many incidents were related to suicides. Thus, fast screening test of these drugs is of crucial need and currently there are a number of commercially available kits in the market for such purpose. For example, Triage® immunoassay test kit is quite often used for preliminary screening of various drug affairs. With this kit, tricyclic antidepressants can be detected, but the detection limit is not sufficient and many other antidepressants are undetectable. Furthermore, it has only been tested using urine specimen and other fluids have not been guaranteed. In reality, it is impossible to take urine samples from all cadavers. Even though plasma samples are detectable, there is a possibility of leading to cross-reaction [1–3]. Therefore, it is necessary to develop alternative simultaneous determination of these antidepressants using instrumental analysis. GC [4–9] and HPLC [10–14] methods for the determination of antidepressants have been reported. But, simultaneous analyses of all important antidepressants by these methods are very difficult in a single run due to their similar properties and structures. Even LC/MS, which is possible for the simultaneous determination of these antidepressants, could accurately determine them only if the number of antidepressants is limited [15–19]. This is because when the peaks were overlapped, the signal could be varied owing to ion suppression or ion enhancement that occurred. It is therefore better to individually separate each peak. Furthermore, hydrophilic venlafaxine is weakly retained in the reversed-phase mode, while the retention of hydrophobic lofepramine is relatively quite strong. Then, it is necessary to change the column or mobile phase for the separation of a wide range of antidepressants by LC.

We then focused our attention on capillary electrophoresis (CE) that has different selectivity and high separation efficiency. CE finds a growing recognition in simultaneous determination of different chemical species in biological samples. In forensic science, automated CE instruments have also become commercially available [20,21]. But, it was not applicable for antidepressants when using normal aqueous buffer due to their hydrophobic nature. The analysis of highly hydrophobic compounds by CE often requires the use of special additives like surfactant or organic solvent [22–28]. On the other hand, non-aqueous capillary electrophoresis (NACE)

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doi:10.1016/j.chroma.2010.10.003

which uses organic solvents and electrolytes, has attracted a great deal of attention too. NACE has been applied to various fields such as food chemistry, environmental chemistry, etc., nowadays [29]. For antidepressants, good results were obtained using NACE as described before [30–32]. The present NACE method can simultaneously determine more number of antidepressants than LC. CE also possesses advantages such as low consumption of the solution and easier conditioning of the separation media, leading to a good screening procedure of antidepressants. Furthermore, CE is useful for cross-checking the determination results done by LC/MS.

The diagrammatic structures of 20 major antidepressants are shown in Fig. 1. A photodiode array detector was previously used for studying the simultaneous determination of antidepressants by NACE method. As a result, 20 antidepressants could be analyzed at once [32]. However, a few issues still remained in the case of the photodiode array detector. The first issue is its low detection sensitivity due to the short path length. Although the sensitivity could be

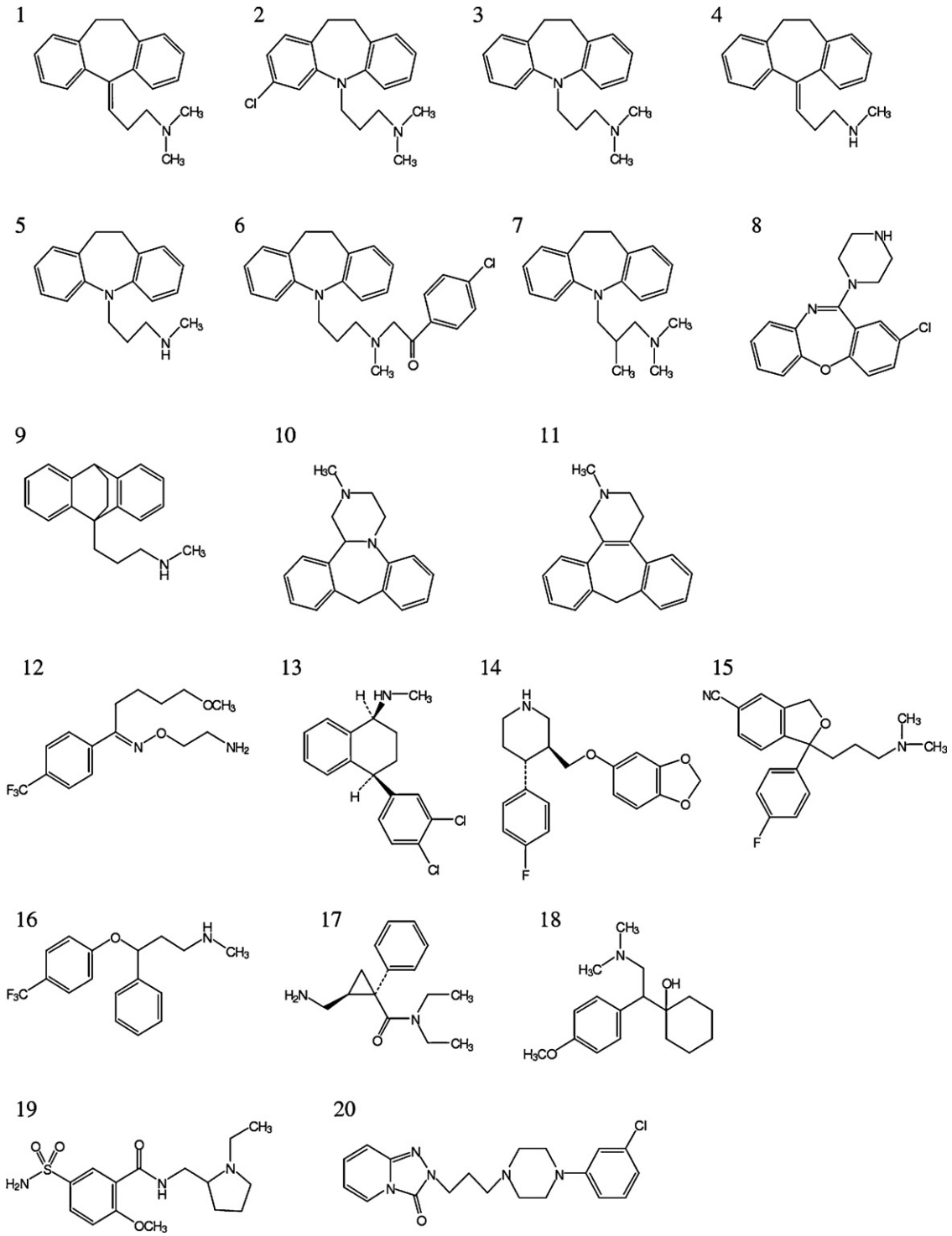

Fig. 1. Structures of the twenty antidepressants. (1) Amitriptyline, (2) clomipramine, (3) imipramine, (4) nortriptyline, (5) desipramine, (6) lofepramine, (7) trimipramine, (8) amoxapine, (9) maprotiline, (10) mianserin, (11) setiptiline, (12) fluvoxamine, (13) sertraline, (14) paroxetine, (15) citalopram, (16) fluoxetine, (17) milnacipran, (18) venlafaxine, (19) sulpiride, (20) trazodone.

improved by using a bubble cell fused silica capillary, the sensitivity of detectable antidepressants in plasma was still very low. Another concern is the weak identification capability. Previously, the identification was judged from the migration time and the absorption spectrum. But, some of the compounds may lack in characteristic absorption spectrum. For example, three representative analytes, i.e. citalopram, sertraline and setiptiline have absorption in the vicinity of 210 nm, and citalopram has characteristic absorption at 237 nm. Accordingly, citalopram can be identified qualitatively with ease, but this is not applicable to sertraline and setiptiline. The other analytes have similar absorption with each other, too. So, when using a photodiode array detector, the identification capability was rather weak. In order to overcome this drawback, a mass spectrometer (MS) was used in the present study.

A number of researches had reported on connecting CE to MS [33–40] due to the fact that CE is compatible to electrospray ionization (ESI). NACE is especially more compatible to ESI because it uses an organic solvent which has high volatilization. In this study, a time of flight mass spectrometer (TOF-MS) was selected as the detector. TOF-MS is useful for screening unknown samples because it can provide accurate mass information of the compounds. TOF-MS was formerly unsuitable for quantitative determination owing to its narrow dynamic range. However, TOF-MS has become more advanced in recent years and has achieved a dynamic range comparable to quadrupole MS. Furthermore, the scan speed of TOF-MS is extremely faster than that of quadrupole MS, and thus it is pretty useful when quantifying any amount of compounds simultaneously [41–44]. Besides, drawing a mass electropherogram of accurate mass could increase the S/N ratio, and therefore an increase in the detection sensitivity can be expected. Qualitative capability is also expected to improve compared to photodiode array detector because spectra of accurate mass and match with those of isotopic pattern.

2. Experimental

2.1. Chemical

Amitriptyline, trimipramine, imipramine, desipramine, clomipramine, nortriptyline, amoxapine, maprotiline, mianserin, fluvoxamine, milnacipran, trazodone and sulpiride were purchased from Sigma–Aldrich (St. Louis, MO, USA). Paroxetine, sertraline, flurazepam, methanol (LC/MS grade), acetonitrile (LC/MS grade), acetic acid (LC/MS grade), sodium acetate and ammonium acetate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Citalopram, fluoxetine and venlafaxine were purchased from LKT Laboratories Inc. (St. Paul, MN, USA). Lofepramine and setiptiline were obtained free-of-charge from Daiichi-Sankyo Co., Ltd. (Tokyo, Japan) and Mochida Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively. Oasis HLB extraction cartridges were purchased from Waters (Milford, MA, USA). All other common chemicals were commercially available and of reagent grade.

2.2. Instrumentation and CE/TOF-MS procedures

All analyses were carried out with a P/ACE MDQ (Beckman Coulter Inc., Billerica, MA, USA), which is modified for hyphenation with MS. The Agilent CE ESI-MS Sprayer Kit (Agilent Technologies, Palo Alto, CA, USA) was used for the coupling which provides both a coaxial sheath liquid make-up flow and a nebulizer gas to assist droplet formation. The TOF-MS utilized was a micrOTOF II (Bruker Daltonics Inc., Billerica, MA, USA). A single-syringe infusion pump (KD Scientific Inc., and Harvard Apparatus Inc., Holliston, MA, USA) equipped with a 2.5-ml or 500-μl Hamilton gastight syringe (Hamilton, Reno, NV, USA) delivered the sheath liquid and calibrant solution.

CE was performed in the normal capillary zone electrophoresis mode. The applied voltage was 30 kV. The temperature of capillary was controlled at 25 °C by a coolant liquid from 3 to 50 cm of capillary inlet side, and the other part was exposed to the room temperature. Samples were introduced hydrodynamically at 1 psi (69 mbar) for 15 s. The background electrolyte (BGE) was a mixture of 60 mM ammonium acetate and 1 M acetic acid in acetonitrile, water, and methanol (100:1:0.5, v/v/v) as the non-aqueous separation medium. The capillary was kept dry (inside) when it was not used for a long time. A new capillary was conditioned with acetonitrile/methanol (1:1, v/v) for 10 min. Before every injection, the capillary was conditioned with acetonitrile/methanol (1:1, v/v) as well as with the running buffer for 3.0 min each.

The composition of the sheath liquid was methanol/water (9:1, v/v). The flow rate of the sheath liquid was 3 μl/min. The nebulizing gas pressure was 0.3 bar. The drying gas flow rate was 4 l/min at a temperature of 180 °C. Drying and nebulizer gases were nitrogen. The spectrometer was scanned from m/z 50 to 1000 at 1 scan/s.

The ESI with the positive mode gave the overall best responses. The capillary voltage was set at 4000 V using a capillary exit voltage of 100 V. All compounds were detected as their respective protonated molecular ions. Mass drift calibration and determination of accurate masses were carried out with a sodium acetate solution.

The sheath liquid was introduced by ESI-MS sprayer kit using the syringe pump via a six-port valve. The calibrant of sodium acetate solution was supplied to the sample loop, which was attached to the six-port valve, using a syringe pump. In 0.1 min after starting migration (i.e. after the current starts to flow), the calibrant was injected by switching the valve. Then, the valve was reinstated in 0.2 min after. The calibrant was continuously flowed at 1 μl/min through the sample loop until the following measurement.

The aspiration effect, which is caused by the nebulization gas, makes the MS side of capillary a negative pressure, and so the BGE solution was pulled, thus resulting in shorter detection time compared to the case using a photodiode array detector.

2.3. Sample preparation

The 20 antidepressants were individually stocked in acetonitrile at −4 °C, where they were stable for 6 months. But, when these standard solutions were mixed up, e.g. 200 μg/ml of each compound in acetonitrile, lofepramine was degraded via reaction with the coexisting substances in a few days after. If this standard solution (20 antidepressants) was stored at −20 °C, it would stay stable for a month. Human plasma samples were spiked with the standard solution. The plasma sample was prepared by solid phase extraction using Oasis HLB (hydrophilic–lipophilic balance type, cartridge size = 3 ml, sorbent mass = 60 mg). The cartridges were activated with methanol (1 ml), followed by washing with water (1 ml). It was then loaded with 1 ml of human plasma spiked with 10 μl of a standard solution, 10 μl of the internal standard (flurazepam, 1 μg/ml) and 20 μl of phosphoric acid. For the loading and eluting steps, flow rates were lower than 1 ml/min. In all other steps, the flow rate was increased up to 10 ml/min. The cartridge was then washed with 5% (v/v) methanol (1 ml), followed by eluting with methanol. The eluate was then dried under a stream of nitrogen gas while heating at 40 °C. The residue for assay was finally dissolved in 100-μl BGE, which was diluted 10 times with acetonitrile.

3. Results and discussions

3.1. Comparison of NACE/MS and aqueous CE/MS

NACE could be connected with TOF-MS simply using the ESI sprayer kit. All of the antidepressants were easily detected as

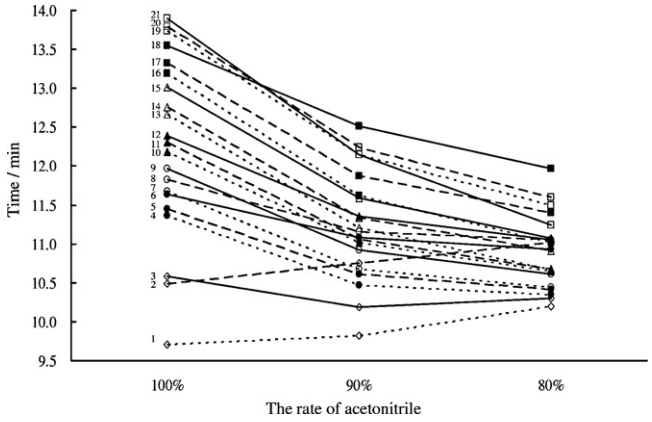

Fig. 2. Variation of the migration times in different composition of acetonitrile/water (100:0, 90:10, 80:20, v/v, and contained 50 mM ammonium acetate and 1 M acetic acid, respectively). Capillary: fused silica capillary, 50 μm i.d., 375 μm o.d., 100 cm of total length. Applied voltage: 30 kV. Sample injection: hydrodynamic injection, 15 s at 1 psi (69 mbar). Sheath liquid: methanol/water (9:1, v/v). Nebulizing gas pressure: 0.3 bar. Analytes: 1 venlafaxine (open diamond, dotted line), 2 sulpiride (open diamond, dashed line), 3 milnacipran (open diamond, solid line), 4 trimipramine (closed circle, dotted line), 5 amitriptyline (closed circle, dashed line), 6 lofepramine (closed circle, solid line), 7 imipramine (open circle, dotted line), 8 flurazepam (I.S., open circle, dashed line), 9 clomipramine (open circle, solid line), 10 nortriptyline (closed triangle, dotted line), 11 desipramine (closed triangle, dashed line), 12 citalopram (closed triangle, solid line), 13 setiptiline (open triangle, dotted line), 14 maprotiline (open triangle, dashed line), 15 fluoxetine (open triangle, solid line), 16 sertraline (closed square, dotted line), 17 fluvoxamine (closed square, dashed line), 18 trazodone (closed square, solid line), 19 paroxetine (open square, dotted line), 20 amoxapine (open square, dashed line) and 21 mianserin (open square, solid line).

protonated ions. The detection sensitivity and resolution as regards to antidepressants were investigated for both NACE and aqueous CE coupled to MS.

The addition of water into the non-aqueous media was also investigated, as demonstrated in Fig. 2. It can be seen from the figure that the resolution becomes poor with increasing water composition. Different organic solvents other than acetonitrile were not studied in the present work because acetonitrile achieved the best resolution among methanol, isopropanol and acetonitrile in the previous study [32]. The conductivity of the BGE increased with increasing water content in the BGE. This means that with increasing water composition in the BGE, the electric field in the sample zone increases while the electric filed in the BGE decreased. This leads to the decrease in the migration time of analytes owing to the increase in the electroosmotic flow, and this also leads to the decrease in the efficiency owing to the decrease in the electric filed of the BGE zone. These are the reasons for the decrease in the resolution with increasing water composition in the BGE.

Fig. 3 demonstrates the mass electropherograms at 278.190 ± 0.050 obtained by using BGEs with different water content, where 20 antidepressants are injected. The peak shapes of venlafaxine, amitriptyline and mianserin, which were obtained by using the BGE consisting of a mixture of acetonitrile/water (80:20, v/v) with ammonium acetate and acetic acid, were poor compared to those obtained by using acetonitrile with ammonium acetate and acetic acid as the BGE. The content of 20% (v/v) water led to poor peak shapes for some analytes. The reason for poor peak shape may be caused by poor solubility of hydrophobic antidepressants in aqueous solution, while the poor sensitivity may be caused by the ion suppression. Comparing with aqueous CE/MS, NACE/MS showed better resolution and peak shape as well as higher detection sensitivity for highly hydrophobic antidepressants, as shown in Fig. 4. For example, CE/MS and NACE/MS achieved an asymmetry factor of 7.3 and 1.8, a S/N ratio of 720 and 1452, and a plate number of 24,000 and 28,000, respectively,

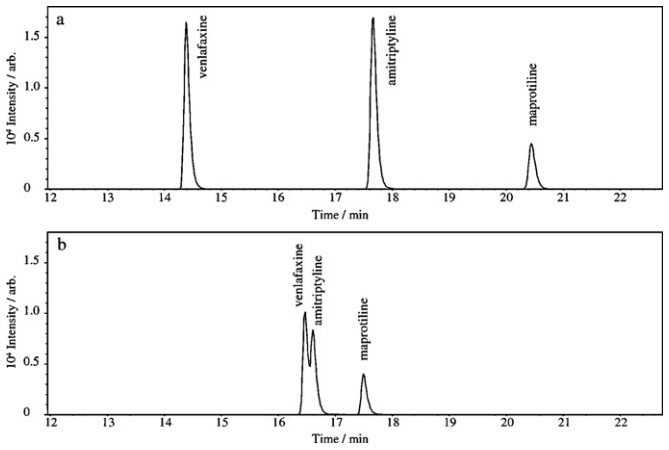

Fig. 3. Comparison of mass electropherograms at m/z 278.190 ± 0.050 obtained with different migration solutions. Migration solutions: (a) the BGE, i.e. 50 mM ammonium acetate and 1 M acetic acid in acetonitrile; (b) a mixture of acetonitrile/water (80:20, v/v) containing 50 mM ammonium acetate and 1 M acetic acid. Capillary: fused silica capillary, 50 μm i.d., 375 μm o.d., 120 cm of total length. Applied voltage: 30 kV. Sample injection: hydrodynamic injection, 15 s at 1 psi (69 mbar). Sheath liquid: methanol/water (9:1, v/v). Nebulizing gas pressure: 0.3 bar.

for setiptiline under the conditions in Fig. 4. Since an accurate mass could be measured by using TOF-MS, compounds having the same mass number with a different molecular formula could be distinguished, and qualitative performance was improved spectacularly as against to when using a photodiode array detector (e.g. amitriptyline and venlafaxine have the same mass number, but the molecular formula are different).

3.2. The sheath liquid

The composition of the sheath liquid was investigated, and it was found that high sensitivity was achieved at high content of organic solvent in the sheath liquid. The sensitivity was almost the same for methanol and acetonitrile. When no water was added into the BGE, i.e. when 100% of organic solvent was used as the sheath liquid, the peak shape of some analytes was enormously worse. This is because water plays an important role in providing protons for ionization. However, when the BGE contained about 1% of

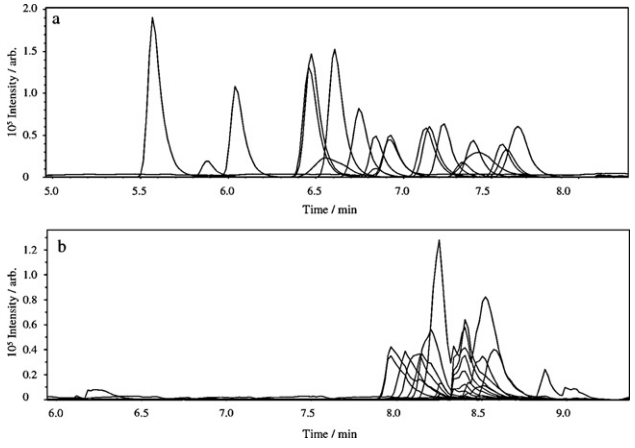

Fig. 4. Comparison between non-aqueous CE and aqueous CE. BGE, (a) 50 mM ammonium acetate and 1 M acetic acid in acetonitrile, (b) 1 M formic acid in water. Capillary: fused silica capillary, 50 μm i.d., 375 μm o.d., 78 cm of total length. Applied voltage: 30 kV. Sample injection: hydrodynamic injection, 15 s at 1 psi (69 mbar). Sheath liquid: methanol/water (9:1, v/v). Nebulizing gas pressure: 0.3 bar. Sheath flow rate: 3 μl/min. Sample, 0.1 μg/ml of each prepared in BGE which was diluted 10 times with acetonitrile.

water (v/v), the peak shape was excellent for all the analytes even when the sheath liquid was consisted of a pure organic solvent. In this case, many analytes had the best detection sensitivity when pure methanol was used as the sheath liquid, while some analytes (lofepramine, paroxetine, etc.) showed a decrease in sensitivity. Therefore, methanol/water (9:1, v/v) was selected as the composition of the sheath liquid. Although the addition of ammonium acetate in the sheath liquid as the ionization accelerator was also checked, it did not improve the detection sensitivity of analytes. Thus, ammonium acetate was added only in the BGE. In addition, it was reported that a sheath liquid without any salt could also be used for ESI-CE/MS [37].

The flow rate of sheath liquid was also examined in the region between 1 and 10 μl/min in terms of the detection sensitivity, the signal repeatability and the current stability. Although the detection sensitivity of analytes increased with decreasing sheath liquid flow rate, the background signal could not be observed at the flow rate of 1 or 2 μl/min because these flow rates were too low to get stable spray. Contrarily, since the repeatability of the peak signal and the current stability were nearly the same at higher flow rate, 3 μl/min was chosen as the sheath liquid flow rate.

3.3. The nebulizer gas

During the operation of NACE using the present system, the current was sometimes unstable. The current must be kept constant in order to obtain reproducible migration, but the current sometimes stopped immediately after injection of the sample solutions. This problem did not occur when a photodiode array detector was used. It was suggested that air could be brought into the capillary in the case of MS detection. During sample injection, the capillary end at the sample injection side was exposed to the atmosphere. Since the BGE was pulled out to the MS side by an aspiration effect, the air was taken into the capillary. Then, the air segment stopped the flow of current, or suction of a vaporizing solution into capillary might be induced by Joule heating because the resistance value on the air segment had become so high that a high voltage was applied unexpectedly. This aspiration effect was more severe when low-viscous acetonitrile solution was used, compared to aqueous solution (viscosity of acetonitrile at 25 °C = 0.34 cP; viscosity of water at 25 °C = 0.89 cP [45]). It was necessary to stop the nebulizing gas during the injections in order to prevent the aspiration effect. But, the sheath liquid cannot be stopped automatically for the present instrument in order to prevent the sheath liquid from accumulating in the ion source and causing contamination. It was found that the reduction of the nebulizer gas pressure could decrease the amount of air entering into the capillary. When the nebulizer gas pressure was more than 0.5 bar, the current was stopped, while when the pressure was reduced to 0.3 bar, the measurement could be carried out stably. Thus, the nebulizer gas pressure was applied at 0.3 bar. By comparison, according to the manufacturer, recommended nebulizing gas pressure is 0.4–1.0 bar at 1–10 μl/min sheath flow rate.

It is clear that when NACE is hyphenated to ESI, the problem caused by the aspiration effect must be taken into account. If possible, the nebulizer gas flow should be switched off when the CE capillary is filled with the BGE solution and the sample is injected.

Under these optimized conditions, simultaneous separation of 20 antidepressants was demonstrated in Fig. 5. All analytes were identified easily by drawing their mass electropherograms. Venlafaxine, amitriptyline and maprotiline appeared in the same mass electropherogram. Both amitriptyline and maprotiline have the same molecular formula, but it was possible to distinguish them by the migration time. It was possible to identify venlafaxine by accurate mass in addition to the migration time.

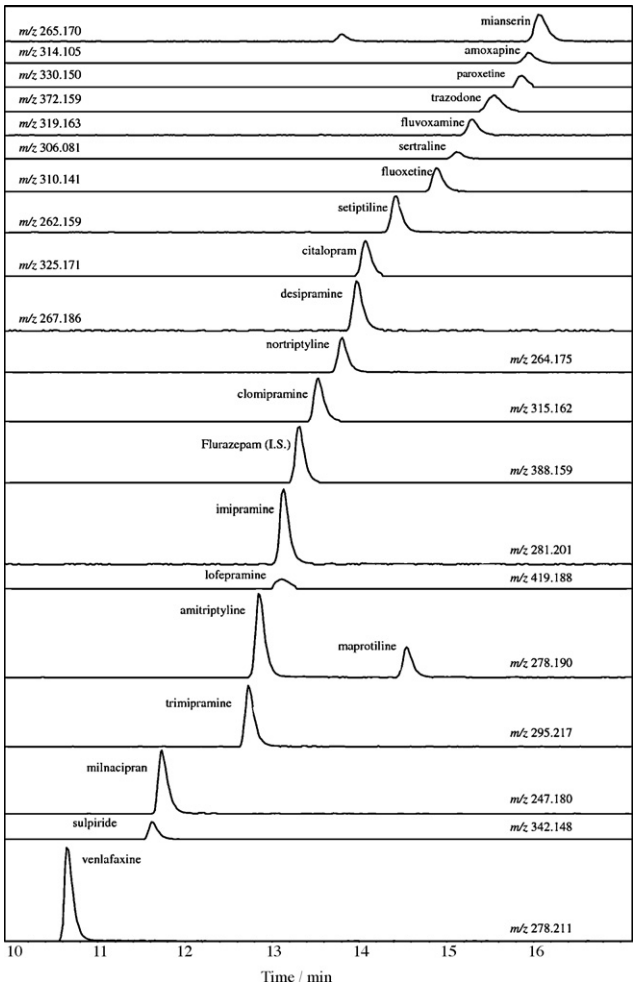

Fig. 5. Mass electropherograms of a test mixture of 20 antidepressants (0.1 μg/ml each). Capillary: fused silica capillary, 50 μm i.d., 375 μm o.d., 100 cm of total length. Applied voltage: 30 kV. The electric current: about 9 μA. Sample injection: hydrodynamic injection, 15 s at 1 psi (69 mbar). Sheath liquid: methanol/water (9:1, v/v). Nebulizing gas pressure: 0.3 bar. Sheath flow rate: 3 μl/min. Drying gas flow rate: 4 l/min (180 °C). BGE solution: 1 M acetic acid and 60 mM ammonium acetate in acetonitrile:water:methanol (100:1:0.5, v/v/v). Internal standard (I.S.): flurazepam.

3.4. Plasma sample

3.4.1. Solid phase extraction

Biological plasma samples were tested in this study. Even when the analyte peaks were overlapped with other coexisting biological components, it was possible to extract only the analyte peaks by choosing the accurate mass. However, the analyte peak sensitivity became extremely poor when ion suppression, which was caused by excessive overlapping components, occurred [46]. Nevertheless, the ion suppression due to coexisting components could be improved by using solid phase extraction with Oasis HLB. The recovery of each analyte is shown in Table 1.

3.4.2. Migration time and peak area optimization using an internal standard

Migration time and peak area were calibrated by flurazepam as the internal standard. Relative standard deviation (RSD) of migration times and peak areas are also shown in Table 2. Despite the aspiration effect, this system seemed to have almost the same error for the migration times and peak areas in comparison to those obtained by using the photodiode array detector [32]. The RSD of migration times when using internal standard indicated satisfactory values of less than 0.4%. Therefore, the identification ability of

Table 1
Recovery (0.1 μg/ml plasma sample), limits of detection (LODs), limits of quantification (LOQs), treatment and toxic plasma concentrations [47].

Analyte	Recovery (%, $n=6$)	RSD (%)	LODs (μg/ml)	LOQs (μg/ml)	Treatment level (μg/ml)	Toxic level (μg/ml)	LODs (μg/ml) by DAD[a]	LOQs (μg/ml) by DAD[a]
Venlafaxine	93.7	8.0	0.0005	0.001	0.25–0.75	>1	0.03	0.1
Sulpiride	77.8	18.1	0.001	0.005	0.40–0.60	38	0.01	0.03
Milnacipran	80.5	6.6	0.0005	0.001	0.15		0.01	0.03
Trimipramine	96.9	3.8	0.0005	0.001	0.015–0.051	>1	0.01	0.03
Amitriptyline	98.5	3.9	0.0005	0.001	0.05–0.24	0.55–16.1	0.01	0.03
Lofepramine	68.2	14.9	0.001	0.005	0.04–0.14		0.03	0.1
Imipramine	102.7	4.0	0.0005	0.001	0.01–0.08	0.8–13	0.01	0.03
Clomipramine	102.5	6.8	0.0005	0.001	0.02–0.07	>0.4	0.01	0.03
Nortriptyline	101.0	6.6	0.0005	0.001	0.05–0.15	>0.25	0.01	0.03
Desipramine	117.6	8.4	0.0005	0.001	0.02–0.88	>10	0.01	0.03
Citalopram	100.6	5.7	0.001	0.005	0.02–0.2	0.5	0.01	0.03
Setiptiline	94.4	7.8	0.0005	0.001	0.0035<	0.78–1.8	0.01	0.03
Maprotiline	95.9	6.5	0.0005	0.001	0.05–0.24	6.2	0.01	0.03
Fluoxetine	96.5	8.1	0.0005	0.001	0.15–0.5	1.3–6.8	0.01	0.03
Sertraline	106.5	8.3	0.0005	0.001	0.05–0.25	2.9	0.01	0.03
Fluvoxamine	83.6	9.1	0.001	0.005	0.05–0.25	0.16–1.4	0.01	0.1
Trazodone	99.4	9.7	0.0005	0.001	0.49–2.3	15	0.01	0.03
Paroxetine	89.6	5.8	0.001	0.005	0.01–0.075	>0.4	0.03	0.1
Amoxapine	75.4	8.9	0.001	0.005	0.02–0.09	0.26–6.7	0.01	0.03
Mianserin	102.9	10.0	0.0005	0.001	0.015–0.07	>0.5	0.01	0.03

[a] Cited from Ref. [32].

Table 2
Linearity parameters and the repeatability (0.01 μg/ml plasma sample).

Analyte	Linearity range (μg/ml)	Coefficience of correlation	Intraday RSD (%) ($n=6$)		Interday RSD (%) ($n=6$)	
			Migration time	Area	Migration time	Area
Venlafaxine	0.001–0.5	0.9962	0.34	7.5	0.48	9.7
Sulpiride	0.005–0.5	0.9997	0.27	9.2	0.32	11.7
Milnacipran	0.001–0.5	0.9938	0.20	7.9	0.27	8.4
Trimipramine	0.001–0.5	0.9972	0.10	5.7	0.11	6.5
Amitriptyline	0.001–0.5	0.9973	0.09	3.8	0.09	9.6
Lofepramine	0.005–0.5	0.9981	0.11	9.7	0.17	13.6
Imipramine	0.001–0.5	0.9976	0.07	3.9	0.05	7.8
Clomipramine	0.001–0.5	0.9986	0.04	3.3	0.05	3.6
Nortriptyline	0.001–0.5	0.9971	0.07	5.9	0.12	7.0
Desipramine	0.001–0.5	0.9987	0.09	5.5	0.13	5.9
Citalopram	0.005–0.5	0.9981	0.10	3.2	0.15	5.2
Setiptiline	0.001–0.5	0.9973	0.15	3.6	0.23	3.1
Maprotiline	0.001–0.5	0.9994	0.15	3.2	0.26	4.2
Fluoxetine	0.001–0.5	0.9990	0.22	5.3	0.33	7.3
Sertraline	0.001–0.5	0.9999	0.25	5.3	0.37	4.8
Fluvoxamine	0.005–0.5	0.9975	0.28	6.0	0.40	4.5
Trazodone	0.001–0.5	0.9988	0.30	4.3	0.43	6.4
Paroxetine	0.005–0.5	1.0000	0.35	4.8	0.50	7.3
Amoxapine	0.005–0.5	0.9984	0.34	3.4	0.51	8.3
Mianserin	0.001–0.5	0.9993	0.36	7.4	0.56	10.1

this developed system was improved remarkably by combining the information obtained from the accurate migration time as well as accurate mass. However, the error of peak area was slightly larger in comparison to photodiode array detector.

3.4.3. *The linearity, limits of detection (LOD), and limits of quantification (LOQ)*

The linearity of the peak area was tested for the spiked plasma sample. The peak areas were taken from mass electropherograms of respective analytes ($m/z = [M+H]^+ \pm 0.010$). Every measurement was calibrated with sodium acetate in each running in order to minimize the errors of m/z. The linear regression equations obtained using the least squares method and the coefficients of correlation are presented in Table 2. A good linearity over the range from 0.001 (0.005) to 0.5 μg/ml (plasma sample) for each analyte was observed under the optimized conditions. The peak areas became a plateau at concentrations greater than 1 μg/ml (plasma sample) for some analytes, thus high concentration samples therefore need to be diluted. The present detection sensitivity was found more than 10 folds better than when using a photodiode array detector.

This method was successfully applied to simultaneously analyze 20 antidepressants in plasma samples. The concentration of the treatment level and toxic level for antidepressants are summarized in Table 1 [47]. At the concentration of treatment level, all of the antidepressants could be determined quantitatively except for setiptiline. It can be seen that the sensitivity achieved by TOF-MS is better than that of the photodiode array detector (DAD) [32] for the examined analytes.

4. Conclusions

The coupling of NACE method to ESI-TOF-MS for simultaneous determination of 20 antidepressants was developed. Even though the available electrolyte was limited, except for this inconvenience, NACE/TOF-MS was found to be a very powerful technique for analysis of hydrophobic compounds like antidepressants. Since TOF-MS can measure accurate mass, the S/N ratio and qualitative power were improved significantly in comparison to the use of photodiode array detector. The sensitivity was prominent especially for

biological samples. Therefore, the NACE/TOF-MS method is very useful for screening the antidepressants in plasma. This method is also expected to be able to apply for other kinds of hydrophobic drugs.

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