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Generic systems for the enantioseparation of basic drugs in NACE using single-isomer anionic CDs

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ABSTRACT

The enantioseparation of 10 basic drugs was evaluated in NACE systems using heptakis(2-*O*-methyl-3-*O*-acetyl-6-*O*-sulfo)-β-CD (HMAS-β-CD). For this purpose, a D-optimal design with 21 experimental points was applied. Four antifungal agents (econazole, isoconazole, miconazole, sulconazole), three local anesthetics (bupivacaine, mepivacaine and prilocaine), two sympathomimetics (salbutamol and terbutaline) and one β-blocker (carvedilol) were selected as basic model analytes. The influence on the enantiomeric resolution of anionic CD and BGE anion concentrations as well as the BGE anion nature was investigated. For all studied analytes, the enantiomeric resolution was shown to be significantly influenced by the CD concentration. Based on the observed results, a generic NACE system was recommended, namely 20 mM HMAS-β-CD and 10 mM ammonium camphorSO_3^- in methanol acidified with 0.75 M formic acid. Moreover, this NACE system was compared to previous conditions with heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)-β-CD (HDMS-β-CD) or heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-CD (HDAS-β-CD). Finally, two generic systems using either HDAS-β-CD or HMAS-β-CD were proposed and evaluated for the enantioseparation of ketamine and norketamine after incubation of ketamine in phenobarbital-induced male rat liver microsomes systems.

1. Introduction

Nowadays, many pharmaceuticals bearing chiral centers are still administered as racemic mixtures even if it is well known that in most cases, one of the enantiomers is the most active one while the other one can generate side effects [1]. Therefore, the control of the enantiomeric purity appeared to be crucial in the pharmaceutical development of chiral drugs. Moreover, the elaboration of suitable analytical techniques to separate the enantiomers of chiral drugs is of great importance for pharmaceutical companies [2]. Capillary electrophoresis (CE) was found to be a powerful tool for enantioseparations, principally due to its advantages in terms of high separation efficiency, low operating costs and speed of analysis [3]. During the last decade, the use of organic solvents as background electrolyte (BGE) components has been very successful and widely used. Nonaqueous CE (NACE) may offer various selectivities by extending the range of the solvent parameters such as the dielectric constant, viscosity, polarity and auto protolysis [4–6]. CDs and their derivatives are the most frequently used chiral resolving agents in CE [7]. Besides neutral CDs, charged CD derivatives were also synthesized but randomly substituted sulfated CDs present batch-to-batch variability, and therefore possible problems of reproducibility of the analytical results. With this aim of view, single-isomer charged CDs were introduced [8]. Vigh and coworkers developed a family of highly charged single-isomer β- and γ-CD derivatives, namely heptakis(6-*O*-sulfo)-β-CD and octakis(6-*O*-sulfo)-γ-CD as well as their (2,3-di-*O*-methyl-6-*O*-sulfo) and (2,3-di-*O*-acetyl-6-*O*-sulfo) analogs which are soluble in MeOH [9–14]. Two of these representatives, namely heptakis(2,3-di-*O*-methyl-6-*O*-sulfo) (HDMS-β-CD) and heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-CD (HDAS-β-CD), were successfully used as chiral selectors in the enantiomeric separation of drugs in NACE [15–18]. More recently, additional efforts aiming at expanding the range of the available single-isomer sulfated CDs led to the synthesis

Abbreviations: camphorSO_3^-, camphorsulfonate; HDAS-β-CD, heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-cyclodextrin; HDMS-β-CD, heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)-β-cyclodextrin; HMAS-β-CD, heptakis(2-*O*-methyl-3-*O*-acetyl-6-*O*-sulfo)-β-cyclodextrin; MeOH, methanol; R_s, resolution.

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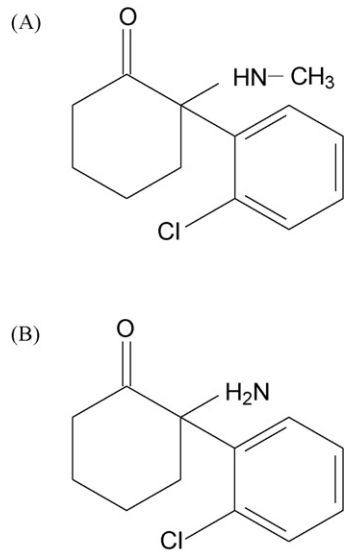

Fig. 1. Chemical structures of (A) ketamine and (B) norketamine.

of an analog carrying non-identical substituents at all C2 and C3 positions, namely heptakis(2-*O*-methyl-3-*O*-acetyl-6-*O*-sulfo)-β-CD (HMAS-β-CD) [19].

The purpose of this study was to provide generic systems for the enantioseparation of various basic chiral drugs using HDMS-, HDAS- or HMAS-β-CD in nonaqueous CE systems. In a previous study, our group evaluated the enantioresolution of 10 β-blockers but only HDMS-β-CD and HDAS-β-CD were tested [18]. Busby et al. evaluated the potential of HMAS-β-CD for the enantioseparation of 24 weak basic pharmaceuticals in acidic aqueous and acidic methanolic BGEs [19]. In this study, the influence of the concentration of HMAS-β-CD as well as the qualitative and quantitative BGE composition on the resolution (R_s) values was studied by means of an experimental design. From this methodology, a generic system based on HMAS-β-CD was proposed. This method was compared to previous conditions using HDAS-β-CD or HDMS-β-CD. Finally, two generic NACE conditions based on either HDAS-β-CD or HMAS-β-CD were recommended and applied to the enantioseparation of ketamine and its primary metabolite, norketamine, after *in vitro* metabolism (cf. Fig. 1). Ketamine is an intravenous analgesic and dissociative anesthetic drug used in clinical practice of man and animals [20–22]. Ketamine is extensively metabolised to N-desmethylketamine, i.e. norketamine [23]. Ketamine and its metabolite are often administered as racemic mixture although each enantiomer exhibits significantly different pharmacodynamic activities [24]. Indeed, S-ketamine showed twice the anesthetic potency of racemic ketamine and S-ketamine is a more potent analgesic agent than R-ketamine [25,26]. However, the post-hypnotic stimulatory properties and agitated behavior are associated with R-ketamine [27]. R- and S-norketamine have similar activity compared to the parent drug, but with a shorter half-life [28]. The CD-NACE potential to study ketamine *in vitro* metabolism was finally demonstrated.

2. Materials and methods

2.1. Instrumentation

All experiments were carried out on a HP^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler, an on-column diode-array detector and a temperature control system (15-60 °C ± 0.1 °C). A CE Chemstation (Hewlett-Packard, Palo Alto, CA, USA) was used for instrument control, data acquisition and data handling. Fused-silica capillaries were provided by ThermoSeparation Products (San Jose, CA, USA). The elaboration of the experimental design and all statistical calculations were performed by means of Modde Software Version 6.0 (Umetri AB, Umeå, Sweden).

2.2. Chemicals and reagents

Prilocaine, carvedilol, terbutaline hemisulfate, econazole, isoconazole, sulconazole nitrate and procain were provided by Sigma–Aldrich (Saint-Louis, MO, USA). Bupivacaine, mepivacaine, salbutamol sulfate and miconazole nitrate were kindly supplied by different pharmaceutical companies. Ketamine was obtained from Australia Government, National Measurement Institute (Pymble, Australia) and norketamine from Cerilliant (Austin, TX, USA) (cf. Fig. 1). All drugs were provided as racemates.

HDMS- and HDAS-β-CD were obtained from Analytical Controls (Rotterdam, the Netherlands) and HMAS-β-CD from Antek Instruments (Houston, TX, USA). Ammonium formate and camphorsulfonate (camphorSO_3^-) were from Sigma–Aldrich. Ammonium acetate was obtained from Acros-Organics (Geel, Belgium). Methanolic BGEs were prepared with 98–100% formic acid (Merck, Darmstadt, Germany) at 0.75 M concentration and ammonium formate, acetate or camphorSO_3^-. Each BGE-CD solution was prepared by adding HDMS-, HDAS- or HMAS-β-CD to the BGE. All reagents were of analytical grade. MeOH (Merck) was of HPLC grade. The BGE and samples solutions were filtered through a Polypure polypropylene membrane filter (0.2 μm) from Alltech (Laarne, Belgium) before use.

NAD, NADP and glucose-6-phosphate dehydrogenase were bought from Roche Diagnostics Gmbh (Mannheim, Germany). Glucose-6-phosphate, EDTA, Tris and sucrose were obtained from Sigma. Phenobarbital was provided by Certa (Braine-l'Alleud, Belgium). Acetonitrile was provided by Merck.

Phenobarbital-induced rat liver microsomes were isolated from male Sprague–Dawley rats (±300 g) treated with phenobarbital (50 mg kg^{-1}) administered i.p. daily, for 4 days. Twenty-four hours after the final inducer treatment, rats were sacrificed and their livers excised, blotted dry, weighed, then minced and homogenized in 4 volumes of ice-cold homogenization buffer (0.01 M Tris; 0.25 M sucrose; 0.1 mM EDTA, pH 7.4) using a Potter apparatus. The homogenate was centrifuged (9000 g) for 20 min at 4 °C. The supernatant was isolated and further centrifuged (106,000 g) for 60 min at 4 °C. The pellet was suspended in ice-cold homogenization buffer and the suspension was re-centrifuged (106,000 g) for 40 min at 4 °C. Microsomal pellets were finally re-suspended in 0.1 M Tris buffer (pH 7.4) containing 0.1 mM EDTA to yield a protein concentration of approximately 16–24 mg/ml.

2.3. Electrophoretic technique

Electrophoretic separations were carried out with uncoated fused-silica capillaries having 50 μm internal diameter and 48.5 cm length (40 cm to the detector). At the beginning of each working day, the capillary was washed with MeOH and the BGE for 15 min. Before each injection, the capillary was washed successively with MeOH for 2 min and then equilibrated with the BGE–CD for 2 min. At the end of each working day, the capillary was rinsed for 30 min with 1 M formic acid in MeOH, 30 min with the BGE without CD and 15 min with MeOH. Capillary wash cycles were performed at a pressure of approximately 1 bar. The applied voltage was 25 kV and UV detection was set at 230 nm. Injections were made by applying a pressure of 50 mbar for a period of 3 s (corresponding to 8.8 nL, i.e. 0.9% of the total volume of the capillary) and the capillary was thermostated at 15 °C. The sample solutions were prepared by dis-

solving each analyte at a concentration of ca. 50 μg/mL in MeOH. Resolution (R_s) was calculated according to the standard expression based on peak width at half height [29].

2.4. Metabolism

The *in vitro* biological test system selected to metabolise ketamine was the phenobarbital-induced male rat liver microsomes system. Ketamine was dissolved in MeOH and added directly to the incubation medium in order to reach a final substrate concentration of 100 μM and a final percentage in MeOH lower than 1%. The incubations were performed at 37 °C in a water shaking bath with a final protein content of 1 mg/ml in a total volume of 1 ml. The reactions were initiated by addition of a NADPH regenerating system. The reactions were stopped after an incubation time of 60 min by addition of 1 ml of MeOH and by a subsequent vortexing step. The proteins were then precipitated by addition of 2 ml of ACN. The obtained samples were vigorously stirred for 5 min and were centrifuged at 3939 g for 10 min. The supernatant of 5 samples (5 ml) was further collected and gently evaporated to dryness under an inert nitrogen flux. The residue was finally redissolved in 250 μl of a methanolic solution containing procain (2 μg/ml). The solutions were sonicated for 10 min, filtered through a polypropylene membrane filter and injected into the CE system. Control samples were also prepared. These samples were prepared by addition of MeOH before supplementing the incubation medium with the NADPH regenerating system.

3. Results and discussion

3.1. Experimental design

In a previous paper, the influence of the nature of both anionic CD derivative (HDAS- or HDMS-β-CD) and BGE anion (acetate, camphorSO_3^- or formate) as well as their concentrations in NACE was studied by means of a multivariate approach using 10 β-blockers as model compounds [18]. The lowest level of the CD concentration was settled at 5 mM and the highest one at 40 mM. HMAS-β-CD could not be included in the previous study since it was not soluble at concentrations higher than 27 mM. Therefore, another experimental design was carried out in order to study the influence of the concentration of this chiral selector (from 1.5 to 20 mM) as well as the nature of the BGE anion (formate, acetate or camphorSO_3^-). The lowest level of BGE concentration was settled at 10 mM and the highest one at 50 mM, this range being optimal with respect to conductivity. The experimental design involved three levels for each quantitative factor in order to estimate the quadratic effects. The other factors were kept constant, such as the BGE cation (NH_4^+), the temperature, the voltage and the formic acid concentration (see Sections 2.2 and 2.3). Four antifungal agents (econazole, isoconazole, miconazole and sulconazole), three local anesthetics (bupivacaine, mepivacaine and prilocaine), two sympathomimetics (salbutamol and terbutaline) and one β-blocker (carvedilol) were selected as basic model compounds.

As the optimal conditions obtained by modelling R_s values were found to be the same compared to those resulting from the modelisation of mobility difference and selectivity, confirming the validity of our approach based on enantioresolution [18], only the latter was selected as response. Considering that *i* refers to the BGE anion nature, the following equation corresponds to the quadratic regression model selected to define the relationship between the response and the factors:

$$Y_i(X_1, X_2) = \beta_0 + \beta_{0,i} + \beta_1 X_1 + \beta_{1,i} X_1 + \beta_2 X_2 + \beta_{2,i} X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon$$

Table 1
Experimental design.

Trial	Factors		
	BGE anion nature	CD concentration (mM)	BGE concentration (mM)
1	Formate	1.5	10
2	Formate	20	10
3	Formate	1.5	50
4	Formate	20	50
5	Formate	10.75	30
6	CamphorSO_3^-	1.5	10
7	CamphorSO_3^-	20	10
8	CamphorSO_3^-	1.5	50
9	CamphorSO_3^-	20	50
10	CamphorSO_3^-	1.5	30
11	CamphorSO_3^-	20	30
12	CamphorSO_3^-	10.75	10
13	CamphorSO_3^-	10.75	50
14	Acetate	1.5	10
15	Acetate	20	10
16	Acetate	1.5	50
17	Acetate	20	50
18	Acetate	10.75	30
19	Acetate	10.75	30
20	Acetate	10.75	30
21	Acetate	10.75	30

where *Y* is the enantioresolution, X_1 is the CD concentration (mM); X_2 is the BGE anion concentration (mM), and ε is the error term. The following statements have to be considered: $\sum_{i=1}^{3} \beta_{0,i} = 0$; $\sum_{i=1}^{3} \beta_{1,i} = 0$ and $\sum_{i=1}^{3} \beta_{2,i} = 0$.

A D-optimal design was applied. Twenty-one experiments were carried out in a random order with three replicates (trials 19, 20 and 21) at the center point (cf. Table 1). The Modde software was used to elaborate this design and to perform all statistical calculations.

3.2. Evaluation of the effects of the factors

Among all coefficients of the model, the most interesting ones with their statistical significance are presented in Table 2. The *p*-value is the probability of getting a result as extreme or more extreme than the one observed if the proposed null hypothesis is correct. If $p < 0.05$, the effect is considered as significant. As can be seen in Table 2, for all compounds, the concentration of HMAS-β-CD produces a significant effect on enantioresolution. A favourable effect of a high chiral selector concentration on the response was observed. The coefficient of the main effect of the BGE concentration is significant in most cases (except for terbutaline, econazole and sulconazole). A low BGE concentration is favourable to the enantiomeric resolution, as previously observed for ten β-blockers [18]. Concerning the nature of the BGE anion, camphorSO_3^- significantly increases the enantioresolution of three model compounds (bupivacaine, sulconazole and carvedilol) (cf. Table 2). Formate produces a significant decrease of the response for bupivacaine, econazole and carvedilol. Finally, the enantiomeric resolution of mepivacaine increases when formate is used and decreases in the presence of acetate.

3.3. Optimization

Table 3 presents the optimum operating conditions, the predicted R_s values and those observed experimentally under these optimum conditions. This table also shows the confidence intervals in order to assess the agreement between predicted and observed resolutions. As can be seen in this table, all observed responses are inside the confidence intervals, which demonstrates the suitability of the proposed models. Except for terbutaline, all analytes were completely resolved and the best CD concentration for the ten model drugs was 20 mM HMAS-β-CD. Concerning the BGE anion,

Table 2
Coefficients of the model and their *p*-value[a].

Parameter	R_s salbutamol		R_s terbutaline		R_s bupivacaine		R_s mepivacaine		R_s prilocaine	
	Coef.	*p*-Value	Coef.	*p*-Value	Coef.	*p*-Value	Coef.	*p*-Value	Coef.	*p*-Value
β_1	0.233	**0.003**	0.318	**0.001**	4.290	**<0.0001**	5.863	**<0.0001**	3.079	**<0.0001**
β_2	−0.293	**0.0007**	0.025	0.723	−0.947	**0.007**	−1.015	**0.002**	−0.907	**0.0009**
$\beta_{0,i(camphorSO3^-)}$	0.063	0.074	0.009	0.088	1.841	**0.0005**	0.613	0.058	0.193	0.441
$\beta_{0,i(acetate)}$	0.028	0.068	−0.148	0.081	−0.102	0.757	−1.967	**<0.0001**	0.213	0.358
$\beta_{0,i(formate)}$	−0.091	0.065	0.139	0.078	−1.739	**0.0003**	1.354	**0.002**	−0.406	0.085
Parameter	R_s econazole		R_s isoconazole		R_s miconazole		R_s sulconazole		R_s carvedilol	
	Coef.	*p*-Value	Coef.	*p*-Value	Coef.	*p*-Value	Coef.	*p*-Value	Coef.	*p*-Value
β_1	0.329	**0.004**	0.366	**0.0001**	0.561	**<0.0001**	0.721	**<0.0001**	3.025	**<0.0001**
β_2	−0.163	0.091	−0.164	**0.024**	−0.256	**0.003**	−0.093	0.360	−0.629	**0.005**
$\beta_{0,i(camphorSO3^-)}$	0.205	0.095	−0.050	0.532	0.108	0.221	0.222	**0.002**	0.804	**0.005**
$\beta_{0,i(acetate)}$	0.123	0.255	−0.059	0.426	0.255	−0.060	−0.186	0.134	−0.018	0.932
$\beta_{0,i(formate)}$	−0.328	**0.008**	0.109	0.141	0.008	−0.048	−0.036	0.775	−0.786	**0.003**

[a] Values significant at the 5% level are printed in bold.

Table 3
Predicted and observed R_s values under the optimum operating conditions.

	Operating conditions		R_s	
	HMAS-β-CD (mM)	BGE	Predicted (conf. interval)	Observed
Salbutamol	20	10 mM camphorSO$_3^-$	1.7 (1.4–2.0)	1.6
Terbutaline	20	10 mM camphorSO$_3^-$	0.9 (0.7–1.1)	1.0
Bupivacaine	20	10 mM camphorSO$_3^-$	15.4 (13.4–17.4)	16.9
Mepivacaine	20	10 mM formate	19.9 (18.3–21.5)	19.5
Prilocaine	20	10 mM formate	10.2 (8.8–11.6)	11.1
Econazole	20	10 mM camphorSO$_3^-$	2.6 (2.2–3.0)	2.5
Isoconazole	20	10 mM camphorSO$_3^-$	2.2 (1.9–2.5)	2.5
Miconazole	20	10 mM camphorSO$_3^-$	2.1 (1.7–2.5)	2.4
Sulconazole	20	10 mM camphorSO$_3^-$	2.3 (1.9–2.7)	2.2
Carvedilol	20	10 mM camphorSO$_3^-$	9.1 (7.8–10.4)	9.3

10 mM ammonium camphorSO$_3^-$ was the optimum for all compounds except for mepivacaine and prilocaine for which 10 mM ammonium formate was found to be more appropriate.

It is useful for the analysts to have generic methods at their disposal for a rapid and efficient enantioseparation. With this aim in view, from the applied experimental design, a generic system made up of 20 mM HMAS-β-CD and 10 mM ammonium camphorSO$_3^-$ in MeOH acidified with 0.75 M formic acid seems to be particularly interesting. Indeed, all tested compounds were completely enantioseparated, except terbutaline (cf. Table 3). In our previous work dealing with the enantioseparation of β-blockers using HDAS- or HDMS-β-CD, a generic NACE system was proposed, namely 40 mM HDAS-β-CD and 10 mM ammonium acetate in MeOH acidified with 0.75 M formic acid [18]. Nevertheless, HDMS-β-CD was found to provide better results in two cases. These conditions were also tested with the 10 basic compounds. R_s values and analysis times obtained were then compared to HMAS-β-CD system (cf. Table 4). As can be seen in this table, when HDAS-β-CD gave low R_s values (i.e. with bupicaine, isoconazole, sulconazole and carvedilol), HMAS-β-CD always provided a complete enantioseparation, with a better enantioresolution power than HDMS-β-CD which was found to give the shortest analysis times. Based on the observed results, two generic systems, namely 40 mM HDAS-β-CD and 10 mM ammonium acetate or 20 mM HMAS-β-CD and 10 mM ammonium camphorSO$_3^-$ in MeOH acidified with 0.75 M formic acid, were recommended. Fig. 2 shows the electropherograms under the best generic conditions for terbutaline, bupivacaine, isoconazole and carvedilol.

Table 4
R_s values observed and migration times of the last peak.

Analyte	HDMS-β-CD[a]		HDAS-β-CD[b]		HMAS-β-CD[c]	
	R_s observed	MT (peak 2) (min)	R_s observed	MT (peak 2) (min)	R_s observed	MT (peak 2) (min)
Salbutamol	5.4	13.5	14.5	22.8	1.6	17.2
Terbutaline	9.5	14.7	16.6	21.0	1.0	16.8
Bupivacaine	10.0	11.3	5.8	15.6	16.9	19.3
Mepivacaine	24.6	17.4	26.9	31.2	17.6	25.7
Prilocaine	9.8	10.9	30.7	27.8	9.3	23.3
Econazole	1.9	12.5	6.3	24.2	2.5	14.8
Isoconazole	1.5	10.6	0.5	14.7	2.5	13.7
Miconazole	1.3	12.1	4.1	22.7	2.4	15.2
Sulconazole	1.4	11.4	0.7	17.3	2.2	14.5
Carvedilol	4.5	13.3	1.9	22.6	9.3	23.5

[a] 40 mM HDMS-β-CD and 10 mM ammonium acetate in MeOH acidified with 0.75 M formic acid.
[b] 40 mM HDAS-β-CD and 10 mM ammonium acetate in MeOH acidified with 0.75 M formic acid.
[c] 20 mM HMAS-β-CD and 10 mM ammonium camphorSO$_3^-$ in MeOH acidified with 0.75 M formic acid.

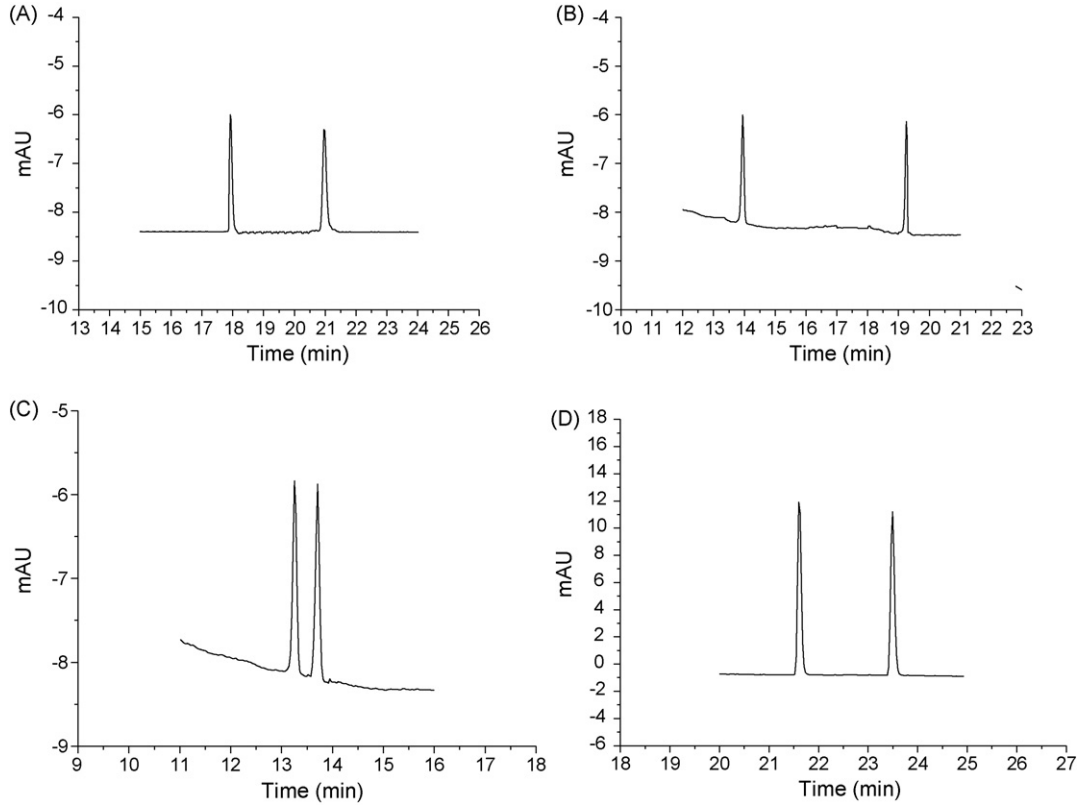

Fig. 2. Electropherograms obtained under the optimum conditions. BGEs: (A) terbutaline: 40 mM HDAS-β-CD and 10 mM ammonium acetate in MeOH acidified with 0.75 M formic acid. (B) bupivacaine, (C) isoconazole and (D) carvedilol: 20 mM HMAS-β-CD and 10 mM ammonium camphorSO_3^- in MeOH acidified with 0.75 M formic acid. Applied voltage: 25 kV; UV detection: 230 nm; temperature: 15 °C. Other conditions as described in Section 2.

3.4. Application

The two proposed generic systems, using either HDAS-β-CD or HMAS-β-CD, were applied to the enantioseparation of ketamine and its main metabolite, norketamine, after *in vitro* metabolism. No enantioresolution of ketamine and only a partial separation of norketamine enantiomers (R_s value: 0.8) were observed using the generic system based on HDAS-β-CD. On the contrary, a complete enantioresolution of ketamine (R_s value: 6.9) and of its main metabolite (R_s value: 4.1) could be obtained with HMAS-β-CD. Fig. 3 shows a typical electropherogram obtained under the optimum operating conditions, i.e. 20 mM HMAS-β-CD and 10 mM ammonium camphorSO_3^- in MeOH acidified with 0.75 M formic acid.

Biotransformation of ketamine was performed by incubation of the compound in the presence of phenobarbital-induced male rat liver microsomes and a NADPH generating system. Following the incubation step, the samples were analysed by NACE. Metabolites highlighting was made possible by comparing electropherograms

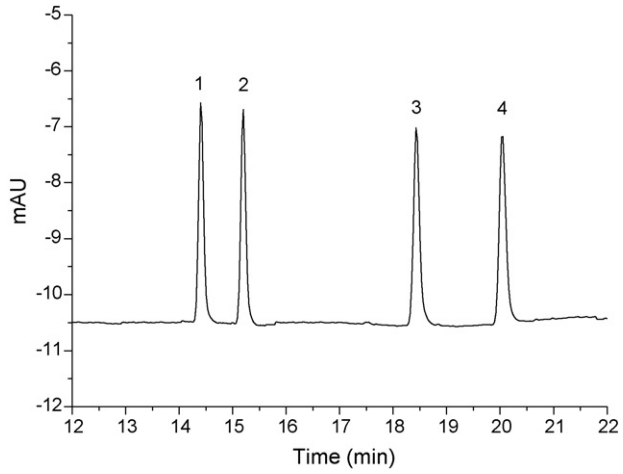

Fig. 3. Electropherogram obtained under the selected generic conditions. BGE: 20 mM HMAS-β-CD and 10 mM ammonium camphorSO_3^- in MeOH acidified with 0.75 M formic acid. Other conditions as described in Section 2. Peaks: 1, norketamine (enantiomer 1); 2, norketamine (enantiomer 2); 3, ketamine (enantiomer 1); 4, ketamine (enantiomer 2).

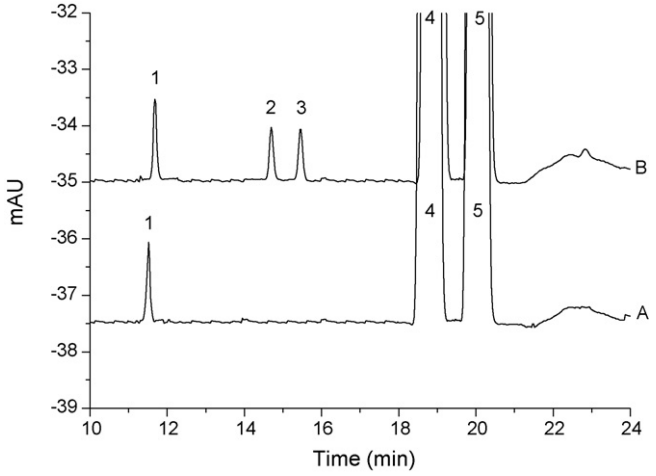

Fig. 4. (A) Electropherogram of a control sample. Peaks: 1, procain; 4, ketamine (enantiomer 1); 5, ketamine (enantiomer 2). (B) Electropherogram resulting from *in vitro* metabolism of ketamine. Peaks: 1, procain; 2, norketamine (enantiomer 1); 3, norketamine (enantiomer 2); 4, ketamine (enantiomer 1); 5, ketamine (enantiomer 2). *In vitro* metabolism and electrophoretic conditions as described in Section 2.

of samples having undergone or not (control samples) a biotransformation mediated by rat liver microsomes (cf. Fig. 4). Control samples were prepared like the other samples but CYPs were inactivated by addition of MeOH before starting the biotransformation reactions. As shown in Fig. 4A, no significant interfering peak was observed at the migration times of the peaks corresponding to the enantiomers of norketamine, which demonstrates the method selectivity as well the stability of the parent compound under these conditions. As expected, norketamine was detected in the samples after incubation of ketamine in the phenobarbital-induced male rat liver microsomes systems.

4. Concluding remarks

The influence on the enantiomeric resolution of the concentration of HMAS-β-CD and the BGE anion as well as the nature of BGE anion was studied in NACE for ten drug substances. Optimal conditions were deduced from the multivariate approach in order to obtain the highest R_S values. Moreover, a generic NACE sytem based on HMAS-β-CD was proposed and compared to both previous conditions using HDAS-β-CD or HDMS-β-CD. HMAS-β-CD was found to be particularly useful for the enantioseparation of analytes when HDAS-β-CD gave rise to R_S values lower than HDMS-β-CD. Therefore, two NACE systems seemed to be interesting to resolve the enantiomers of basic drugs, namely 40 mM HDAS-β-CD and 10 mM ammonium acetate or 20 mM HMAS-β-CD and 10 mM ammonium camphorSO_3^- in MeOH acidified with 0.75 M formic acid. Finally, a fast method development strategy based on these principles was applied to the enantioseparation of ketamine and norketamine after *in vitro* metabolism.

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