Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 475–481

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Enantiomeric separation of antimalarial drugs by capillary electrophoresis using neutral and negatively charged cyclodextrins

Krisztina Németh[a,*], Gábor Tárkányi[b], Erzsébet Varga[c], Tímea Imre[a], Réka Mizsei[b], Róbert Iványi[c], Júlia Visy[a], Julianna Szemán[c], László Jicsinszky[c], Lajos Szente[c], Miklós Simonyi[a]

[a] Institute of Biomolecular Chemistry, Chemical Research Center, Hungarian Academy of Sciences, H-1025 Budapest, Pusztaszeri út 59-67, Hungary
[b] Institute of Structural Chemistry, Chemical Research Center, Hungarian Academy of Sciences, H-1025 Budapest, Pusztaszeri út 59-67, Hungary
[c] CycloLab R&D Ltd., H-1097 Budapest, Illatos út 7, Hungary

ARTICLE INFO

Article history:
Received 28 June 2010
Received in revised form 1 September 2010
Accepted 14 September 2010
Available online 19 September 2010

Keywords:
Antimalarial drugs
Enantioselective analysis
Drug impurity
Cyclodextrin modified capillary zone electrophoresis

ABSTRACT

Capillary electrophoresis (CE) methods for chiral resolution of five antimalarial drugs (primaquine, tafenoquine, mefloquine, chloroquine and quinacrine) were developed by using a wide selection of neutral and anionic cyclodextrin (CD) derivatives. The use of sulfobutyl-β-CD and carboxymethyl-β-CD (CMBCD) resulted in good resolution of quinacrine and tafenoquine, respectively. New results are presented for resolutions of chloroquine and mefloquine. Application of carboxyalkyl- and sulfobutyl-CD derivatives provided improved resolution for primaquine. The impurity in primaquine sample detected by CE was identified as quinocide by MS and NMR. CMBCD provided not only the best separation of primaquine from quinocide but also the simultaneous complete resolution of both compounds.

1. Introduction

Malaria is one of the most important parasitic diseases affecting and killing millions of people throughout the world [1–4]. Recently, beyond other therapeutic approaches the administration of antimalarial drugs remains the main strategy in prophylaxis and in treatment of this illness. Poor financial/economic background, evolution of drug resistance of parasites and serious adverse effects of these pharmaceutical materials are among the unsolved difficulties. Synthetic antimalarial drugs include chloroquine (CLQ), *erythro*-mefloquine (MFQ), primaquine (PRQ), quinacrine (QR) and tafenoquine (TFQ), all administered as racemates. Their structures consist of two or three condensed aromatic rings and aliphatic or alicyclic side chains containing center(s) of chirality and amine group(s). The 8-aminoquinolines PRQ and TFQ, the quinoline methanol MFQ, the 4-aminoquinoline CLQ, and the acridine derivative QR are weakly basic compounds due to their amino functional groups (for structures see Fig. 1).

High performance resolution methods regarding the chiral antimalarial drugs are summarized excellently in the reviews by Brocks et al. and Magalhaes et al. [1,3]. Elaboration of cheap, fast and reproducible analytical methods is still highly needed. Capillary electrophoresis (CE) has some advantages over other chiral separation techniques: great efficiency, high versatility, rapidity and low sample and solvent consumption. Cyclodextrin (CD) derivatives are one of the most commonly used chiral selectors in CE due to their stability and low UV absorption. Furthermore, multiplicity of CD derivatives in the diameter of cavity and the substituents provides the possibility of enantiodiscrimination for a large scale of chiral compounds. Hence, the cyclodextrin-modified capillary zone electrophoresis (CD-CZE) is an appropriate technique to separate enantiomers.

Numerous results were reported on the resolution of antimalarial drugs by CE using various selectors like cyclodextrins [5–28], or other polysaccharide derivatives [29–39] having poor reproducibility due to their heterogeneity. Application of CD selectors with precisely characterized degree of substitution ensures a good reproducibility.

Although, excellent enantiomeric separations have been achieved in case of PRQ [5–13], CLQ [14–24] and MFQ [19–28] further CD-CZE methods are presented here together with novel

* Corresponding author. Tel.: +36 01 4381100; fax: +36 01 4381129.
E-mail address: nemethkr@chemres.hu (K. Németh).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.09.020

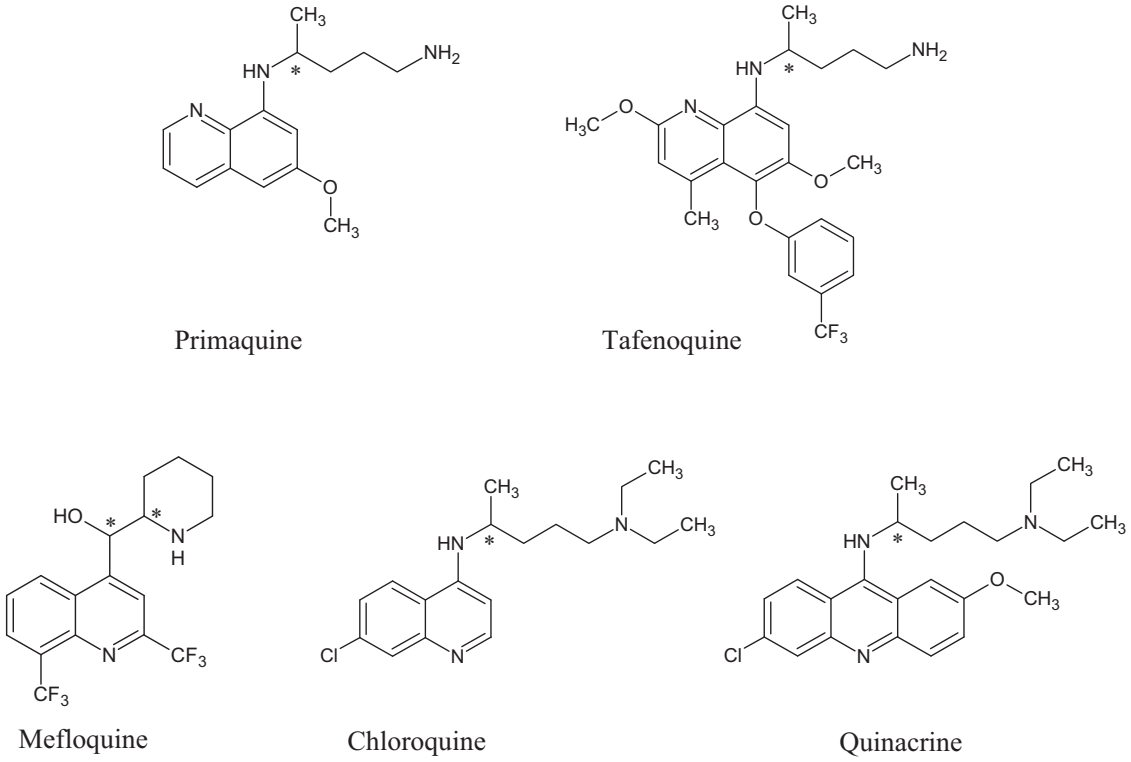

Fig. 1. Structures of antimalarial drugs.

resolutions for QR and TFQ for which scarce data are only available [3].

2. Materials and methods

2.1. Chemicals

Background electrolyte buffer components: *Suprapure* sodium dihydrogene phosphate, sodium hydroxide, methanol were purchased from Merck GmbH (Darmstadt, Germany).

Cyclodextrin derivatives α-CD (ACD), β-CD (BCD), γ-CD (GCD), dimethylated β-CD (DIMEB), permethylated β-CD (heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrin, TRIMEB), randomly methylated β-CD (RAMEB), hydroxypropyl-α-CD (HPACD); hydroxypropyl-β-CD (HPBCD); hydroxypropyl-γ-CD (HPGCD); succinyl-β-CD (SUCCBCD); carboxymethyl-α-CD (CMACD); carboxymethyl-β-CD (CMBCD); carboxymethyl-γ-CD (CMGCD); carboxyethyl-α-CD (CEACD); carboxyethyl-β-CD (CEBCD); carboxyethyl-γ-CD (CEGCD); sulfobutyl-α-CD (SBEACD); sulfobutyl-β-CD (SBEBCD); sulfobutyl-γ-CD (SBEGCD); sulfated α-CD (SACD); sulfated β-CD (SBCD); and sulfated γ-CD (SGCD) were products of CycloLab R&D Ltd. (Budapest, Hungary).

2.2. Antimalarial drugs

(±)Chloroquine diphosphate (Sigma), mefloquine hydrochloride (Sigma, racemic mixture of the (−)-(11*S*,2′*R*)- and (+)-(11*R*,2′*S*)-*erythro* enantiomers), (±)primaquine diphosphate (Aldrich), (±)quinacrine dihydrochloride (Fluka) and tafenoquine (GlaxoSmithKline) were used as supplied. Drug sample stock solutions (1 mg/ml) were prepared in methanol and were further diluted 3-fold with double distilled water on the day of measurements.

2.3. Capillary electrophoresis

CE was performed with an Agilent Capillary Electrophoresis ^{3D}CE system with bare fused silica capillary having 33.5 cm total, 25 cm effective length and 50 μm ID (Agilent Technologies, Santa Clara, CA, USA). On-line UV absorption was detected at 220 nm by DAD UV–vis detector. ChemStation software (rev.A0903, Agilent Technologies, USA) was used for data acquisition and handling. The capillary was thermostated at 20 °C. Sodium phosphate buffer (50 mM) at pH 2.5 (pH adjusted by NaOH) was applied as background electrolyte (BGE). The capillary was rinsed with 1 M HCl, 1 M NaOH, 0.1 M NaOH and distilled water for 2–2 min for each, then with BGE for 5 min between measurements. Samples were injected by 5×10^3 Pa pressure for 3 s. Runs were performed by positive-polarity mode with 20 kV or by negative polarity mode with −15 kV in the presence of 20 mM concentration of anionic CD derivatives. The efficiency of the chiral separations was characterized by resolution (R_s) and selectivity (α) [40].

The amount of impurity in PRQ was evaluated from relative area of the peaks corrected by the corresponding migration times. The possible difference between the molar absorption coefficients was neglected.

2.4. Mass spectrometry

Mass spectrometric measurements were performed using an AB Sciex API-2000 triple quadrupole instrument (Toronto, Canada) equipped with a TurboIonspray Source. A Perkin-Elmer 200 microHPLC pump was used as a solvent delivery system. The flow rate of the acetonitrile eluent (Sigma–Aldrich, St. Louis, USA) was 0.2 ml/min and 10 μl (10 μg/ml) sample was injected directly into this mobile phase. The instrument was controlled by Analyst vs1.5 software (AB Sciex, Toronto, Canada) and operated in positive electrospray ionization mode (ESI-MS). The mass spectrum was scanned in the 50–1000 m/z range with a 0.1 Da step size and the

Table 1
Resolution of antimalarial racemates.

CD derivatives		Antimalarial racemates											
Name	Conc. (mM)	PRQ		QNC		TFQ		MFQ		CLQ		QR	
		R_s	α	R_s	α	R_s	α	R_s	α	R_s	α	R_s	α
ACD	15												
BCD	15							*0.30*	*1.01*				
GCD	15	*1.02*	*1.06*										
RAMEB	20							2.05	1.06				
TRIMEB	20	*1.03*	*1.02*			0.68	1.02						
HPACD	20	*1.46*	*1.03*					*2.84*	*1.08*				
HPBCD	20			0.95	1.01			*2.34*	*1.06*				
HPGCD	20	*3.24*	*1.07*					*0.84*	*1.02*				
SUCCBCD	20	0.68	1.01	1.07	1.01			*1.35*	*1.03*	1.08	1.01		
CMACD (pH 3)	20	**19.64**	**1.31**	0.45	1.01	1.26	1.03	*3.64*	*1.08*	1.86	1.02		
CMBCD (pH 3)	20	*2.05*	*1.04*	**2.51**	**1.03**	**1.44**	**1.02**	*6.24*	*1.15*	1.09	1.03	1.67	1.06
CMGCD (pH 3)	20	9.18	1.29	1.00	1.01	0.51*(pH 2.5)	1.03	2.85	1.05				
CEACD	20							0.72	1.01				
CEBCD	20			0.95	1.01			1.53	1.03				
CEGCD	20	3.32	1.06					0.73	1.01				
SBEACD	20	1.07	1.03			0.77	1.02	1.18	1.04	1.06	1.03	1.41	1.04
SBEBCD	20	5.87	1.11					*1.70*	*1.06*	*0.5(0.5 mM)*	*1.01*	**2.26**	**1.07**
SBEGCD	20	8.36	1.31					5.55	1.07				
SACD	20	2.38	1.05					*1.65*	*1.05*			1.36	1.03
SBCD	20	*0.86(5 mM)*	*1.01*	0.68	1.01			***9.85***	***1.15***	***3.61***	***1.04***		
SGCD	20	8.09	1.11					*1.90*	*1.05*	0.85	1.02	0.69(5 mM)	1.02

PRQ, primaquine; QNC, quinocide; TFQ, tafenoquine; MFQ, *erythro*-mefloquine; CLQ, chloroquine; QR, quinacrine; R_s, resolution; α, selectivity; BGE, 50 mM phosphate buffer (pH 3.0 in case of CMCD derivatives or pH 2.5 for all the others) complemented with the CD derivatives as indicated. Best resolution for a drug achieved at a CD concentration different from the protocol (first column) are indicated next to the respective R_s value. Empty spaces: no resolution was found in our system. Resolutions comparable with literature data but obtained in this study are indicated by italics in white background. Novel chiral resolutions are indicated by grey. Bold numbers indicate best resolutions for drugs.

scan time was 1 s (Fig. S1). The voltage of the electrospray needle was set to 5 kV, the declustering potential was 30 V. In tandem mass spectrometric (product ion scan) measurements the parameters were the same, collision energy was 30 V. Tandem mass spectrum was recorded in the 50–300 *m*/*z* region (Fig. S2).

2.5. NMR

NMR experiments were carried out on a Varian NMR System™ (399.9 MHz for ^{1}H) using AutoX broadband X{^{1}H} probe (X = ^{31}P–^{15}N). Sample was placed into 5 mm NMR tubes. ^{1}H chemical shifts are referenced to the residual solvent signal (δ_{D2O} = 4.79 ppm). ^{13}C shifts are given relative to the external reference DSS (δ_{DSS} = 0.0 ppm). Deuterium oxide (99.9 D atom%) solvent was purchased from Sigma–Aldrich Inc., Germany. The sample of PRQ was analyzed by ^{1}H NMR (55 °C, 200 mM) with respect to its structural identity and purity. Quinocide content in PRQ was determined according to the integral ratio of the pertinent aliphatic CH resonances at δ 3.75 ppm (PRQ) and δ 3.64 ppm (QNC) (Fig. S3). In the one-dimensional experiments 30 s recycle delay and 16 scans were used to allow accurate integration. The ^{1}H–^{13}C-gHMBC experiment (Fig. S4) was optimized for a 7 Hz long-range proton-carbon *J*-coupling constant.

3. Results and discussion

3.1. Development of CD-CZE method for chiral resolution of aminoquinoline antimalarial drugs

Various (natural, hydroxypropylated, methylated, alkyl-carboxylated, sulfated and alkyl-sulfated) CD selectors were screened for resolution of the antimalarial drugs. Not only the substituents but also the ring size of the CDs were varied using series of α-, β- and γ-CD analogs.

Both the efficiency in resolution and the mobility of the analytes changed according to the charge and the concentrations of CD derivatives applied. The concentrations of the *neutral* selectors were varied in the range of 5–20 mM. Only RAMEB resulted in acceptable resolution for MFQ at 20 mM concentration. The efficiencies of anionic CDs were evaluated in the range of 0.2–20 mM. Only poor or no enantioseparation could be obtained by low concentrations (0.2–5 mM) of the anionic CDs. In order to improve resolution high (20 mM) concentration was chosen.

In order to maintain positive charge on the analytes, measurements were carried out at relatively strong acidic conditions (at pH 2.5 or at pH 3.0). The mobility of electroosmotic flow was very slow and the adsorption of the basic analytes to the silica capillary wall could be neglected since deprotonation of silanol groups are depressed at a pH as low as 2.5. Some of the selectors, e.g. carboxymethyl-, carboxyethyl- and succinyl-CDs are uncharged in our conditions due to their pK_a ~ 4. On the contrary, sulfobutyl- and sulfated-CDs are negatively charged at this pH.

Results of resolutions of antimalarial racemates are summarized in Table 1. In order to obtain comparable data, selectivity of CD derivatives described previously in literature was investigated in our setup, as well (white background in Table 1). Our novel resolution results (indicated by grey) provided additional data referring to the series of selectors. The best resolutions corresponding to individual drugs are emphasized in bold.

3.1.1. Separation of PRQ from impurity

Primaquine has lethal effect on the dormant form of the parasites. In pharmaceutical products of PRQ an impurity, quinocide (QNC) is found that is an isomer with altered constitution in the alkyl side-chain [41]. The maximal amount of this impurity may be 3% of the main component, as approved by the European and British Pharmacopoeias [42,43]. According to the literature [13], QNC as a minor peak migrates after the main component in CZE *without selectors*. To check the purity of PRQ, the sample was subjected to ESI-MS analysis (positive ion mode). Molecular ion (*m*/*z* ($M+H^+$) = 260 Da) and products of in-source fragmentation processes could be detected in the normal ESI-MS spectrum of PRQ

Table 2
Separation of primaquine (PRQ) from its main impurity quinocide (QNC).

CD derivatives		Resolution of PRQ from QNC		
Name	Conc. (mM)	R_{sc}	α_c	Migration order
Without CD		*1.85*	*1.03*	*PRQ/QNC*
ACD	15	*1.62*	*1.04*	*PRQ/QNC*
BCD	15	*2.00*	*1.03*	*QNC/PRQ*
GCD	15			
RAMEB	20			
TRIMEB	20	6.47	1.09	$PRQ_1/PRQ_2/QNC$
HPACD	20			
HPBCD	20	1.82	1.03	$QNC_1/QNC_2/PRQ$
HPGCD	20	*1.66*	*1.03*	*$PRQ_1/QNC/PRQ_2$*
SUCCBCD	20	11.29	1.16	$QNC_1/QNC_2/PRQ_1/PRQ_2$
CMACD (pH 3)	20	1.15	1.02	$QNC_1/QNC_2/PRQ_1/PRQ_2$
CMBCD (pH 3)	20	**21.79**	**1.40**	**$QNC_1/QNC_2/PRQ_1/PRQ_2$**
CMGCD (pH 3)	20	1.03	1.02	$PRQ_1/QNC_1/QNC_2/PRQ_2$
CEACD	20	0.67	1.02	PRQ/QNC
CEBCD	20	6.99	1.16	$QNC_1/QNC_2/PRQ$
CEGCD	20			
SBEACD	20			
SBEBCD	20	1.14	1.02	$QNC/PRQ_1/PRQ_2$
SBEGCD	1	1.93	1.09	$PRQ_1/PRQ_2/QNC$
SACD	20	1.99	1.03	$PRQ_1/PRQ_2/QNC$
SBCD	20	0.87	1.01	$PRQ/QNC_1/QNC_2$
SGCD	20	4.26	1.05	$PRQ_1/QNC/PRQ_2$

R_{sc}, critical resolution is the lowest R_s value calculated for the peaks of the main and the impurity components; α_c, critical selectivity is the lowest α value calculated for the peaks of PRQ and QNC; migration order, migration order of the subsequent peaks resolved; 1 and 2 in lower index, indicate the related enantiomers; BGE, 50 mM phosphate buffer (pH 3.0 in case of CMCD derivatives or pH 2.5 for all the others) complemented with the CD derivatives as indicated. Empty spaces, no separation was found in our system. Resolutions comparable with literature data but obtained in this study are indicated by italics in white background. Novel resolutions are indicated by grey. Bold numbers indicate best resolutions for the constitutive isomers

sample (Fig. S1). These fragments were confirmed by tandem mass spectrometric (product ion scan) analysis (Fig. S2). Measured m/z ratios of fragments confirmed the results of Brondz et al. [41]. All of the peaks observed in the ESI-MS spectrum are in agreement with the theoretical mass of PRQ and QNC. The structural verification of the impurity was based on NMR experiments (Figs. S3 and S4). In the two-dimensional ^{1}H–^{13}C-gHMBC NMR spectrum different *aliphatic–aromatic* long-range ***H***–C–N–***C*** correlations were established for PRQ and QNC (Fig. S4) [44].

According to Elbashir et al. [8,13] PRQ could be separated from QNC by CE using BCD, HPGCD or dual system of HPGCD and SBCD. In case of HPGCD selector, QNC migrated between the enantiomers of PRQ and it was not resolved chirally in our measurements. The main difficulty in this kind of electropherogram is to evaluate whether the single minor peak corresponds to the racemic QNC or only to one of the enantiomers of QNC while the other one is hidden in the peak of PRQ.

In case of investigating the mixture of the two racemates, the number of peaks varied from one to four depending on the CD selector applied. We characterized the efficiency of the separation of the main component from the impurity by "critical resolution" (R_{sc}) value (see Table 2) which was given as the calculated R_s value of the nearest peaks between PRQ and QNC. Several kinds of migration orders were detected while using various CD derivatives (cf. Table 2).

Without CD selectors or in the presence of ACD, TRIMEB, CEACD, SACD and SBCD, the QNC peak(s) followed the peak(s) of PRQ. QNC migrated in the separation window of PRQ enantiomers using γ-ring containing CD derivatives (namely SGCD, HPGCD and CMGCD). More precise evaluations of the amount of impurity can be achieved when QNC peak(s) migrate before PRQ one(s) and, can be attained by using BCD, HPBCD, SUCCBCD, CEBCD, SBEBCD, CMACD and

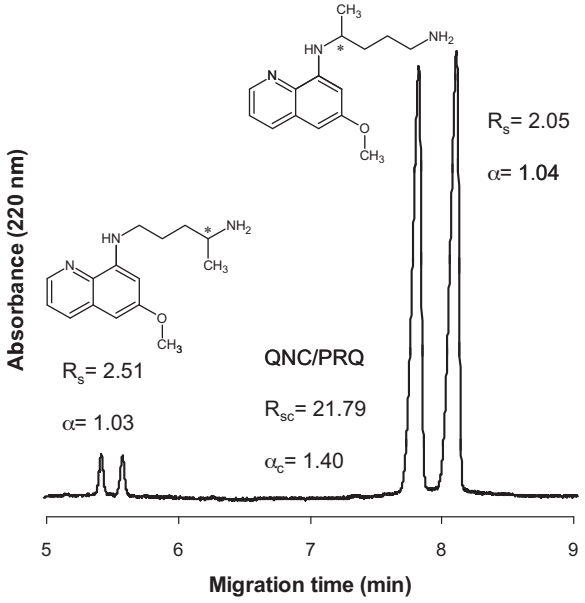

Fig. 2. Simultaneous separation of primaquine from quinocide and their enantiomeric resolution by 20 mM CMBCD at pH 3.0; for experimental details see Section 2.3.

CMBCD. In other words, complexes of QNC formed by these CD derivatives are less stable than those of PRQ. The best separation was achieved by CMBCD (Fig. 2).

The content of QNC in our sample is approximately 6.7% as determined from CE experiments. This is in good agreement with the ^{1}H NMR measurements (Fig. S3) yielding 6.9%. This value is higher than the amount approved by pharmacopoeias and the chemical is sold only for *in vitro* R&D.

3.1.2. Resolution of PRQ and QNC enantiomers

Resolution efficiencies of PRQ by CMACD, CMBCD, SUCCBCD, CEGCD, SBEACD, SBEGCD, SACD and SGCD are listed in Table 1. Application of CMACD provided excellent chiral resolution (Fig. 3) exceeding the selectivity of other CD derivatives reported previously [5–13].

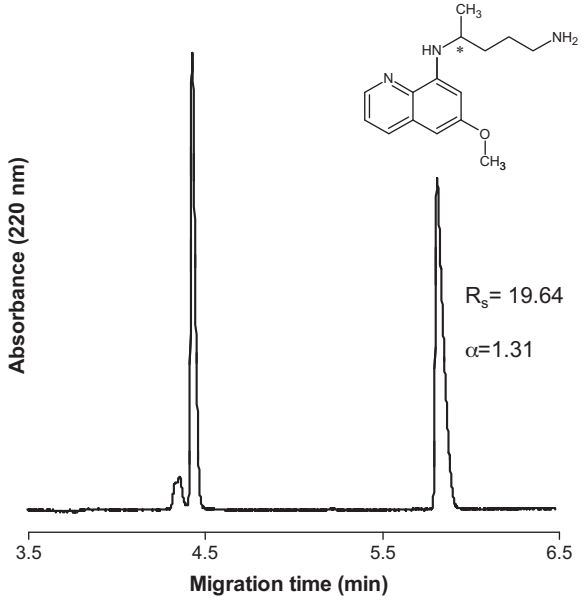

Fig. 3. Chiral resolution of primaquine by 20 mM CMACD at pH 3.0; for experimental details see Section 2.3.

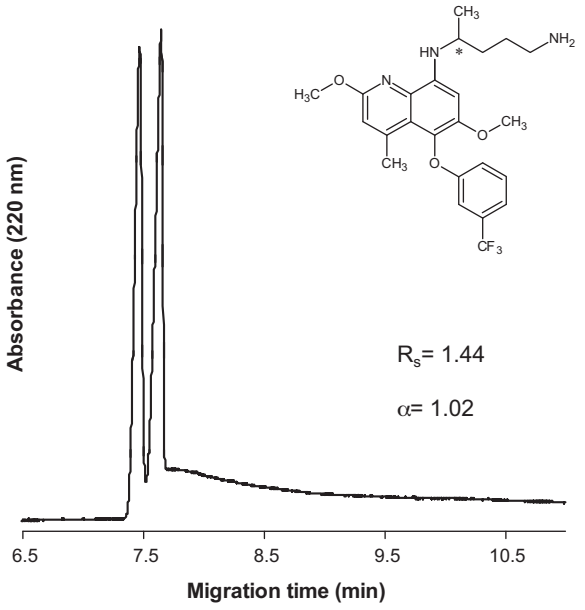

Fig. 4. Chiral resolution of tafenoquine by 20 mM CMBCD at pH 3.0; for experimental details see Section 2.3.

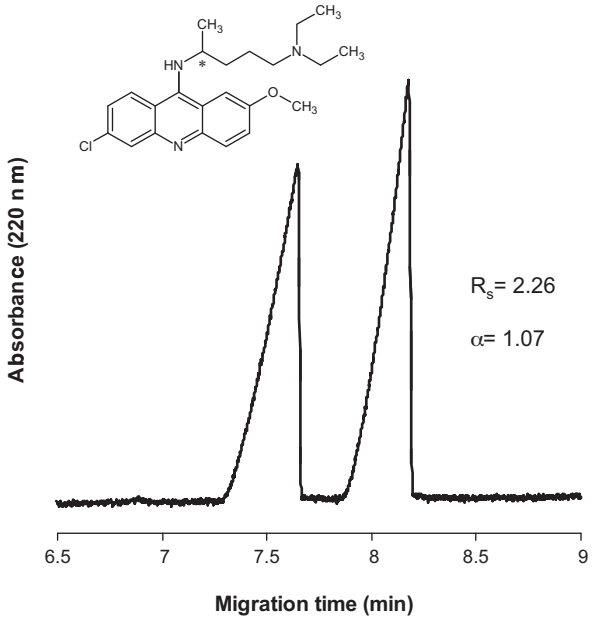

Fig. 5. Chiral resolution of quinacrine by 20 mM SBEBCD at pH 2.5; for experimental details see Section 2.3.

A wide range of resolution efficiencies of QNC from weak to baseline resolutions was found in our conditions (Table 1). The excellent resolution properties of CMBCD (Fig. 2) can be attributed to its good enantio-selectivity for both substances resulting in four peaks in a single run.

CM-derivatives containing shorter alkyl chain were more efficient than their respective CECD pairs in both cases. The efficiency of enantio-discrimination of CDs depended on the ring size and was different for PRQ and QNC. As seen from Table 1, ACD and GCD derivatives are more efficient than BCD members of the corresponding CDs series for resolutions of PRQ. On the contrary, improved chiral resolution for QNC was demonstrated by the corresponding BCD derivative.

3.2. Chiral resolution of TFQ

As a potential antimalarial drug tafenoquine is under investigation. According to the best of our knowledge, no enantiomeric separation by CE of TFQ has been published yet. From all of the CDs investigated CMBCD provided the most selective recognition of TFQ enantiomers (Table 1, Fig. 4). Carboxyethyl-CDs having longer alkyl chain were inefficient.

3.3. Chiral resolution of MFQ

Mefloquine has been applied widely for decades against malaria. Although, the 4-methanol-quinoline MFQ has two centers of asymmetry only the *erythro* pair of enantiomers is administered as a drug. The purity of our *erythro*-MFQ sample concerning the diastereomer impurities was proved by CE using DIMEB according to Fanali and Camera [27] and by HPLC according to Qiu et al. [45]. Fortunately, among CD derivatives investigated in our setups there were a few new ones apparently being as good as the others reported earlier (cf. Table 1). The best resolutions include: SBEGCD (Fig. S5), CMGCD and RAMEB (one of the cheapest CD derivatives). Interestingly, while the enantioselectivity of TRIMEB for MFQ seemed to be very weak the randomly methylated form of BCD demonstrated improved resolution.

In general, best resolution was found with the β ring size ($R_{S(CMGCD)} < R_{S(CMACD)} < R_{S(CMBCD)}$, $R_{S(CEACD)} = R_{S(CEGCD)} < R_{S(CEBCD)}$ and $R_{S(SACD)} < R_{S(SGCD)} < R_{S(SBCD)}$), with the exception of HPACD and SBEGCD. The characteristic structural feature of MFQ is the additional piperidyl ring with a size that may match the cavity of BCD derivatives. The remaining parts of the molecule might prefer complexation with CD selectors of other ring sizes. Accordingly, the order of efficiency in enantio-recognition by different CDs in the corresponding series could vary.

3.4. Chiral resolution of CLQ

CLQ is one of the first synthetic antimalarial drugs administered for a long time. Table 1 lists our resolutions obtained by SUCCBCD, CMACD, CMBCD, SBEACD and SGCD. Usage of CMACD resulted in a good resolution (Fig. S6) however, its efficiency did not exceed that of reported earlier for SBCD [16–18] being the best selector of CLQ.

The presence of carboxyl or sulfate functional groups on CD derivatives is advantageous for the resolution of CLQ enantiomers. We could not correlate the ring size of CDs with the efficiency of resolutions.

3.5. Chiral resolution of QR

Quinacrine is a structural homologue of CLQ. The difference between these two molecules is an additional aromatic ring bound to the quinoline ring of CLQ. According to the best of our knowledge, no data regarding the chiral separation of QR based on CD-CZE is available in the literature although its weak enantioseparation by heparin with CE was reported [39]. As a novelty, using CMBCD, SBEACD, SBEBCD, SACD and SGCD resulted in resolution of enantiomers of QR (Table 1). Best efficiency with baseline resolution could be achieved by SBEBCD (Fig. 5).

In conclusion, the presence of acidic functional groups in the CD molecules may provide enantio-selectivity both for CLQ and QR presumably due to their capabilities to form polar interaction(s). No correlation was found between chiral resolution efficiencies and ring size of CDs in cases of CLQ or QR.

4. Conclusions

The present work demonstrates improved effective chiral resolutions of five antimalarial racemates by CE using properly selected CD variants. Our novel results supplements series of enantioseparations for CLQ, MFQ reported elsewhere [14–28]. In addition, baseline or improved resolutions of QR and TFQ by CD-CZE could be demonstrated. Although good methods for enantioseparations of PRQ can be found in the literature here we demonstrated a superior one using CMACD. This study represents excellent separation of PRQ from its major impurity QNC, where the impurity migrated before the main component by applying CMBCD. Additionally, usage of CMBCD resulted in successful chiral resolutions of both PRQ and QNC racemates in a single run.

Surveying efficiency of a large series of CD selectors the best resolutions for these basic analytes could be achieved by "anionic" CD derivatives according to literature data [46]. In conclusion, structural similarity of these antimalarial drugs determined the type of anionic CD providing the best efficiency in enantioselection. Accordingly, carboxymethyl-CDs seemed to be the best selectors for 8-aminoquinolines, on the other hand sulfated or sulfoalkyl-CDs were the best ones for the analogues of 4-substituted quinolines (quinacrine involved).

Acknowledgements

We gratefully acknowledge Mrs. Ilona Kawka for the technical support and Dr. Ferenc Zsila for TFQ samples. The authors thank for financial support from the following grant: Jedlik Ányos Grant NKFP_A3-2008-0211.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2010.09.020.

References

[1] D.R. Brocks, R. Mehvar, Stereoselectivity in the pharmacodynamics and pharmacokinetics of the chiral antimalarial drugs, Clin. Pharmacokinet. 42 (2003) 1359–1382.
[2] D.R. Brocks, M. Vakily, R. Mehvar, Chapter 6. Stereospecific pharmacokinetics and pharmacodynamics: selected classes of drugs, in: I.K. Reddy, R. Mehvar (Eds.), Chirality in Drug Design and Development, Marcel Dekker, New York, 2004, pp. 191–280.
[3] I.R.S. Magalhaes, P.S. Bonato, Enantioselective analysis of antimalarial drugs and their metabolites, Curr. Pharmaceut. Anal. 6 (2010) 15–29.
[4] J.C. Leffingwell, Chirality & bioactivity. I: Pharmacology, Leffingwell Rep. 3 (2003) 1–27.
[5] H. Nishi, K. Nakamura, H. Nakai, T. Sato, S. Terabe, Enantiomeric separation of trimetoquinol, denopamine and timepidium by capillary electrophoresis and HPLC and the application of capillary electrophoresis to the optical purity testing of the drugs, Chromatographia 40 (1995) 638–644.
[6] H. Nishi, Y. Kokusenya, T. Miyamoto, T. Sato, Chiral separation of drugs using cyclodextrins in capillary zone electrophoresis, J. Chromatogr. A 659 (1994) 449–457.
[7] H. Nishi, K. Nakamura, H. Nakai, T. Sato, Chiral separation of drugs by capillary electrophoresis using beta-cyclodextrin polymer, J. Chromatogr. A 678 (1994) 333–342.
[8] A.A. Elbashir, B. Saad, A.S.M. Ali, M.I. Saleh, H.Y. Aboul-Enein, Enantioselective analysis of primaquine and its impurity quinocide by capillary electrophoresis, Biomed. Chromatogr. 23 (2009) 464–471.
[9] Y. Tanaka, M. Yanagawa, S. Terabe, Separation of neutral and basic enantiomers by cyclodextrin electrokinetic chromatography using anionic cyclodextrin derivatives as chiral pseudo-stationary phases, J. High. Resol. Chromatogr. 19 (1996) 421–433.
[10] Y. Tanaka, Method development of enantiomer separations by affinity capillary electrophoresis cyclodextrin electrokinetic chromatography and capillary electrophoresis–mass spectrometry, Chromatography 23 (2002) 13–24.
[11] E.G. Yanes, S.R. Gratz, R.M.C. Sutton, A.M. Stalcup, A comparison of phosphated and sulfated β-cyclodextrins as chiral selectors for capillary electrophoresis, Fresenius J. Anal. Chem. 369 (2001) 412–417.
[12] A.M. Stalcup, H.G. Kyung, Application of sulfated cyclodextrins to chiral separations by capillary zone electrophoresis, Anal. Chem. 68 (1996) 1360–1368.
[13] A.A. Elbashir, B. Saad, A.S.M. Ali, M.I. Saleh, H.Y. Aboul-Enein, Determination of quinocide as impurity in primaquine tablets by capillary electrophoresis, Biomed. Chromatogr. 23 (2009) 295–301.
[14] B. Chankvetadze, K. Lomsadze, N. Burjanadze, J. Breitkreutz, G. Pintore, M. Chessa, K. Bergander, G. Blashke, Comparative enantioseparations with native β-cyclodextrin, randomly acetylated β-cyclodextrin and heptakis-(2,3-di-O-acetyl)-β-cyclodextrin in capillary electrophoresis, Electrophoresis 24 (2003) 1083–1091.
[15] Y. Dong, A. Huang, Z. Sun, Chiral separation of chloroquine and pemoline by capillary zone electrophoresis with sulfobutyl ether β-cyclodextrin as buffer additive, Chromatographia 48 (1998) 310–313.
[16] L.J. Jin, S.F.Y. Li, Comparison of chiral recognition capabilities of cyclodextrins for separation of basic drugs in capillary zone electrophoresis, J. Chromatogr. B 708 (1998) 257–266.
[17] G.S. Yang, D.M. Chen, Y. Yang, B. Tang, J.J. Gao, H.Y. Aboul-Enein, B. Koeppenhoefer, Enantioseparation of some clinically used drugs by capillary electrophoresis using sulphated β-cyclodextrin as a chiral selector, Chromatographia 62 (2005) 441–445.
[18] M. Zandkarimi, A. Shafaati, S.M. Foroutan, Chiral separation basic and zwitterionic drugs by highly sulphated cyclodextrins using short-end injection capillary electrophoresis, Iranian J. Pharmaceut. Res. 7 (2008) 275–281.
[19] B. Lin, Y. Ji, Y. Chen, U. Epperlein, B. Koppenhoefer, Separation of drug enantiomers by capillary electrophoresis: α-cyclodextrin as chiral solvating agent, Chromatographia 42 (1996) 106–110.
[20] B. Lin, X. Zhu, S. Wuerthner, U. Epperlein, B. Koppenhoefer, Separation of enantiomers of drugs by capillary electrophoresis. Part 8. β-Cylodextrin as chiral solvating agent, Talanta 46 (1998) 743–749.
[21] B. Koppenhoefer, U. Epperlein, B. Christian, Y. Ji, Y. Chen, B. Lin, Separation of enantiomers of drugs by capillary electrophoresis. I. γ-Cylodextrin as chiral solvating agent, J. Chromatogr. A 717 (1995) 181–190.
[22] B. Koppenhoefer, U. Epperlein, R. Schlunk, X. Zhu, B. Lin, Separation of enantiomers of drugs by capillary electrophoresis. V. Hydroxypropyl-α-cylodextrin as chiral solvating agent, J. Chromatogr. A 719 (1998) 153–164.
[23] B. Lin, X. Zhu, U. Epperlein, M. Schwierskott, R. Schlunk, B. Koppenhoefer, Separation of enantiomers of drugs by capillary electrophoresis. Part 6. Hydroxypropyl-β-cylodextrin as chiral solvating agent, J. High Resol. Chromatogr. 21 (1998) 215–224.
[24] B. Koppenhoefer, A. Jakob, X. Zhu, B. Lin, Separation of enantiomers of drugs by capillary electrophoresis. Permethyl-gamma-cylodextrin as chiral solvating agent, J. High Resol. Chromatogr. 23 (2000) 413–429.
[25] M. Heuermann, G. Blaschke, Chiral separation of basic drugs using cyclodextrins as chiral pseudo-stationary phasesin capillary electrophoresis, J. Chromatogr. A 648 (1993) 267–274.
[26] B. Chankvetadze, G. Endresz, G. Blaschke, About some aspects of the use of charged cyclodextrins for capillary electrophoresis enantioseparation, Electrophoresis 25 (1994) 804–807.
[27] S. Fanali, E. Camera, Use of cyclodextrins in the capillary electrophoresis separation of *erythro*- and *threo*-mefloquine enantiomers, J. Chromatogr. A 745 (1996) 17–23.
[28] B. Chankvetadze, N. Burjanadze, G. Blaschke, Enantioseparation of *erythro*-mefloquine and its analogues in capillary electrophoresis, J. Pharm. Biomed. Anal. 32 (2003) 41–49.
[29] A.M. Stalcup, N.M. Agyel, Heparin: a chiral mobile-phase additive for capillary zone electrophoresis, Anal. Chem. 66 (1994) 3054–3059.
[30] H. Nishi, S. Izumoto, K. Nakamura, H. Nakai, T. Sato, Dextran and dextrin as chiral selectros in capillary zone electrophoresis, Chromatographia 45 (1996) 617–630.
[31] H. Nishi, Enantiomer separation of drugs by electrokinetic chromatography, J. Chromatogr. A 735 (1996) 57–76.
[32] Y. Du, A. Taga, S. Suzuki, W. Liu, S. Honda, Colominic acid: a novel chiral selector for capillary electrophoresis of basic drugs, J. Chromatogr. A 962 (2002) 221–231.
[33] R. Gotti, V. Carvini, V. Andrisano, G. Mascellani, Dermatan sulfate as useful chiral selector in capillary electrophoresis, J. Chromatogr. A 814 (1998) 205–211.
[34] K.W. Phinney, L.A. Jinadu, L.C. Sander, Chiral selectors from fruit: application of citrus pectins to enantiomer separations in capillary electrophoresis, J. Chromatogr. A 857 (1999) 285–293.
[35] C. Zhang, C. Zhu, X. Lin, F. Ghao, Y. Wei, Enantiomeric separation primaquine, an anti-malarial drug, by cyclodextrin-modified micellar electrokinetic capillary chromatography, Anal. Sci. 18 (2002) 595–597.
[36] H Nishi, Enantioselectivity in chiral capillary electrophoresis with polysaccharides, J. Chromatogr. A 792 (1997) 327–347.
[37] K.W. Phinney, L.C. Sander, Enantioselective separations in capillary electrophoresis with dextran sulphate as the chiral selector, Anal. Bioanal. Chem. 375 (2003) 763–768.
[38] R. Bortocan, P.S. Bonato, Enantioselective analysis of primaquine and its metabolite carboxyprimaquine by capillary electrophoresis, Electrophoresis 25 (2004) 2848–2853.
[39] F.E. Stanley, A.M. Stalcup, Modeling circular dichroism for quinacrine in the presence of heparin, Biochem. Biophys. Res. Commun. 394 (2010) 628–632.
[40] S. Fanali, Controlling enantioselectivity in chiral capillary electrophoresis with inclusion-complexation, J. Chromatogr. A 792 (1997) 227–267.
[41] I. Brondz, D. Ekeberg, D.S. Bell, A.R. Annino, J.A. Hustad, R. Svedsen, V. Vlachos, P. Oakley, G.J. Langley, T. Mohini, C.-G. Amaury, F. Mikhalitsyn, Nature of the main concomitant in the drug primaquine diphosphate: SCF and SCF-MS methods of analysis, J. Pharmaceut. Biomed. Anal. 43 (2007) 937–944.

[42] European Pharmacopoeia, Method of Analysis 2.2.47, Council of Europe, Strassbourg, 2005, pp. 1061–1063.
[43] British Pharmacopoeia, vol. 2, HSMO, London, 2003, pp. 1557–1558.
[44] J.D. McChesney, S. Srinivasin Saranga, Rapid aromatic hydrogen exchange in the antimalarial primaquine, Pharmaceut. Res. 4 (1984) 184–186.
[45] Y. Qui, S. Kitamura, K.J. Guillory, A high-performance liquid chromatographic method for the quantitative enantioselective analysis of mefloquine stereoisomers, Pharmaceut. Res. 9 (1992) 1640–1643.
[46] B. Chankvetadze, Separation of enantiomers with charged chiral selectors in CE, Electrophoresis 30 (2009) S211–S221.