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Pre-column Derivatization and Separation of Diastereomeric-Derivatives of Racemic Mexiletine and Confirmation of Elution Order and Molecular Configuration

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Present study describes the synthesis of cyanuric chloride based four active chiral reagents (ACRs) and their application in the enantiomeric separation of (*RS*)-mexiletine. Herein, four cyanuric chloride-based ACRs were prepared by introducing L-proline derivatives under nucleophilic substitution reaction. The synthesized ACRs were characterized by different spectroscopic techniques. Racemic mexiletine hydrochloride was used for the enantio-recognition study. All the four ACRs were used to convert (*RS*)-mexiletine into related diastereomeric derivatives and then separated on the C_{18}-column of RP-HPLC. The different parameters such as sample amount, the concentration of mobile phase, organic modifier and pump pressure were varied to optimize separation conditions. The energy-minimized structures of synthesized diasteromeric derivatives (DDs) were developed using DFT calculations. The validation study was conducted for the developed method and correlation-coefficient, calibration range, LOD and LOQ calculated. The stability and recovery were calculated by inter and intraday assay.

Keywords: Cyanuric chloride, Activated chiral reagents, Mexiletine, Diastereomeric derivatives, RP-HPLC, Configuration.

INTRODUCTION

Cyanuric chloride or trichloro-triazine is an affordable and widely accessible chemical that belongs to an agrochemical active ingredient [1]. It's systematic chemical name is 2,4,6-trichloro-1,3,5-triazine. Cyanuric chloride, as a backbone, have been used in the synthesis of verities of the herbicides such as cyanazine, propazine, simazine, atrazine, *etc*. [1]. Also, the cyanuric-chloride has been explored extensively in the verities of organic reactions as the catalyst and environmental applications [2], synthesis of the porous and microporous polymer [3], preparation of photoelectric materials [4-7], organic synthesis [8,9], preparation of polymer and nanoparticles for energy storage [4,10-12], preparation of chiral derivatizing reagents [13-17], preparation of chiral stationary phase [18,19] and synthesis of pharmaceuticals [1,20]. The cyanuric chloride modified with enantiomerically pure amines and amino acids has been used widely to separate enantiomers of different racemic pharmaceuticals or compounds [13-17]. Literature on cyanuric chloride based chiral stationary phases (CSPs) shows its remarkable capacity to separate a wide range of racemic compounds.

Due to the presence of the three active chlorine atoms (act as leaving groups), cyanuric chloride shows trifunctionality. In presence of the appropriate nucleophiles (thiols, amines, alcohols, *etc.*), cyanuric chloride undergoes in the managed and sequential displacement of three Cl^- atoms. Due to multiple nucleophilicities, cyanuric chloride is considered a valuable source for introducing chromophore into low UV-Visible absorbing compounds. The cyanuric chloride based chiral reagents have been widely utilized for racemic amines, amino acids and amino alcohols *via* an indirect approach [13-17].

(*RS*)-Mexiletine (l-(2,6-dimethylphenoxy)-2-aminopropane (Fig. 1) is a non-selective voltage-gated sodium channel blocker and classified as IB antiarrhythmic class drug. Mexiletine

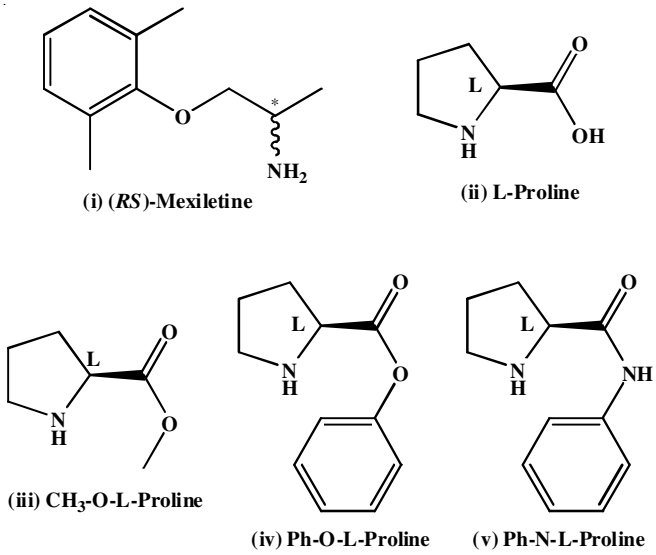

Fig. 1. Molecular structures of (*RS*)-mexiletine (i) and derivatives of L-proline (ii-v)

is used to treat chronic pain, muscle stiffness and abnormal heart rhythms [20-25]. Mexiletine is marketed and administered as racemic mixture (*S*- and *R*-enantiomers), but according to literature the desirable action of mexiletine is only associated with (*R*)-enantiomer. The (*R*)-enantiomer shows better/higher binding with human serum proteins compared to (*S*)-enantiomer [26,27]. Due to the different therapeutic activity of (*S*) and (*R*) enantiomers of mexiletine, the enantioseparation of mexiletine is important for biological and pharmaceutical applications [20]. The separation of the enantiomers of the (*RS*)-mexiletine has been achieved using direct and indirect enantioseparation (covalent derivatization) and for this purpose different chiral reagent and chiral stationary phases (CSPs) have been used [20,26,27].

This work described a successful report on the synthesis of active chiral reagents (ACRs) and diastereomeric derivatives (DDs) of racemic mexiletine followed by enantioseparation on RP-HPLC. Cyanuric chloride based four ACRs were prepared *via* nucleophilic substitution with L-proline based derivatives. All the prepared ACRs were characterized by spectroscopic techniques. The ACRs were used to convert racemic mexiletine into high molar absorbing DDs pairs *via* nucleophilic substitution reaction under microwave irradiation (MWI) conditions. The ACRs moiety as the part of DDs of (*RS*)-mexiletine makes them very sensitive for UV-Visible detection. The DDs pair of mexiletine were separated on the C_{18} column of the RP-HPLC (indirect approach of enantioseparation). The mobile phase for separation was investigated by varying the organic modifier (acetonitrile and methanol) in the mobile phase. The organic solvent was used in combination with triethylammonium phosphate (TEAP) in gradient mode. Additionally, the separation mechanism, elution order and absolute configuration were established for DDs of (*RS*)-mexiletine by preparing the energy minimized structures using Gaussian software (09 Rev. A.02; DFT calculations). The developed method was validated for accuracy, linearity, the limit of detection (LOD) and the limit of quantification (LOQ).

EXPERIMENTAL

All of the chemicals and reagents used in this study were obtained from Sigma-Aldrich (St Louis, USA) and analytical and HPLC grade solvents were obtained from E. Merck (Mumbai, India).

Chromatographic system and equipment: Shimadzu HPLC system with SPD-M20A PDA detector and LC solution and DAO-3.5 operating software (Shimadzu, Japan) with C_{18}-column (L × I.D. 25 cm × 4.6 mm, 5 µm particle size; LiChrospher), Microwave-Multiwave 3000 (800 W, Perkin-Elmer, USA), Milli-Q system of Millipore (Bedford, MA, USA), FT-IR spectrometer (Nicolet-6700, Thermo Scientific, USA), elemental analyzer (Vario EL III, Hanau, Germany), NMR spectrometer 400 MHz (JEOL Inc., Peabody, USA) and UV-2450 (Shimadzu, Japan).

Preparation of stock solutions: The stock solutions of 0.1 M (*RS*)-mexiletine, 0.1 M ACRs (1-4) and 0.1 M $NaHCO_3$ were prepared by dissolving the calculated amount of compounds, respectively in the methanol, acetonitrile and water. Triethylammonium phosphate buffer (TEAP, 10 mM) was prepared in purified water.

Synthesis of L-proline derivatives

Phenyl ester of L-proline (Ph-O-L-proline): Phenol (200 mg, 2 mmol) and boc-L-proline (440 mg, 2 mmol) were dissolved in 8 mL dry dichloromethane under inert atmosphere conditions. The solution of EDC (360 mg, 2.1 mmol) in dry dichloromethane was then added dropwise in the above solution. The reaction was allowed to stir for 3 h. After the completion, 1 N HCl solution was added to the reaction and extracted with dichloromethane. The dichloromethane was removed under reduced pressure [28-31]. The obtained product was then treated with 1 N HCl solution in order to deprotect Boc-group and then extracted with dichloromethane [32,33]. The dried solid product was characterized with spectroscopic techniques as given below.

A similar approach was used to prepare phenyl amide (Ph-N-L-proline) and methyl ester of L-proline (CH_3-O-L-proline) by using aniline and methanol, respectively instead of phenol. The structures of the prepared L-proline derivatives are shown in Fig. 1.

CH_3-O-L-Proline: Yield: 98%, colour: white, $[\alpha]_D^{25}$ = (-)80° (*c* = 0.5, methanol), UV (nm, in acetonitrile): 222 (λ_{max}), IR (KBr, ν_{max}, cm^{-1}): 2980, 2892, 2850, 1740, 1470, 1330, 1180 and 857. ^{1}H NMR ($CDCl_3$): δ 3.71 (3H, s), 3.56-3.64 (2H, m), 3.2 (2H, m), 1.94 (1H, m), 1.85 (1H, m) and 1.72 (2H, m). HRMS for $C_6H_{11}NO_2$: 130.1012 (M^++H). Anal. calcd. (found) % for $C_6H_{11}NO_2$: C, 55.80 (55.68); N, 10.84 (10.92); H, 8.58 (8.26).

Ph-O-L-Proline: Yield: 92%, colour: white, $[\alpha]_D^{25}$ = (-)72° (*c* = 0.5, methanol), UV (nm, in acetonitrile): 230 (λ_{max}), IR (KBr, ν_{max}, cm^{-1}): 3040, 2985, 2830, 1748, 1608, 1485, 1465, 1320, 1180, 1020, 986 and 864. ^{1}H NMR ($CDCl_3$): δ 7.65 (1H, t), 7.12 (2H, dd), 7.45 (2H, t), 3.80 (2H, m), 2.95-3.10 (2H, m), 2.01-2.06 (1H, m) and 1.70-1.90 (3H, m). HRMS for $C_{11}H_{13}NO_2$: 192.1124 (M^++H). Anal. calcd. (found) % for $C_{11}H_{13}NO_2$: C, 69.09 (68.85); N, 7.32 (7.12); H, 6.85 (6.34).

Ph-N-L-Proline: Yield: 94%, colour: white, $[\alpha]_D^{25}$ = (-)76° (*c* = 0.5, methanol), UV (nm, in acetonitrile): 233 (λ_{max}), IR (KBr, ν_{max}, cm^{-1}): 3391, 3060, 2950, 2868, 282, 1689, 1620, 1540, 1440, 1350, 1240, 1160, 1070, 878 and 736. ^{1}H NMR ($CDCl_3$): δ 8.8 (1H, s), 7.46 (4H, m), 7.14-7.18 (1H, m), 3.78-3.81 (1H, m), 3.64-3.68 (1H, m), 2.85-3.04 (2H, m), 2.04-2.08 (1H, m) and 1.71-1.94 (3H, m). HRMS for $C_{11}H_{14}NO_2$: 191.1020 (M^++H). Anal. calcd. (found) % for $C_{11}H_{14}NO_2$: C, 69.45 (69.07); N, 14.73 (14.52); H, 7.42 (7.72).

Synthesis of active chiral reagents (ACRs): Under the stirring condition, a solution of 368 mg (2 mmol) cyanuric chloride in 12 mL was added into the solution containing 260 mg (2 mmol) CH_3-O-L-proline and 0.5 g Na_2CO_3 in 22 mL acetone. The resultant reaction mixture was then allowed to stir at 20 ºC for 1 h. After the reaction completion, 15 mL water was added to quench the reaction. Acetone was removed under low pressure and the desired product was obtained as crystals. The product obtained from the reaction was filtered and washed several times with chilled water [13,34]. The filtrate was extracted using dichloromethane and concentrated to obtain pure ACR-2, under reduced pressure. A similar approach was used to prepare ACRs (1, 3 and 4) with derivatives of L-proline (Ph-N-L-proline, Ph-O-L-proline and L-proline) (Fig. 2).

ACR-1: Yield: 94%, colour: white, $[\alpha]_D^{25}$ = (-)68° (*c* = 0.5, methanol), UV (nm, in acetonitrile): 232 (λ_{max}), IR (KBr, ν_{max}, cm^{-1}): 3300, 3000, 2936, 2872, 2812, 1710, 1660, 1480, 1460, 1410, 1340, 1260, 1070, 905 and 730. ^{1}H NMR ($CDCl_3$): δ, 4.30-4.35 (1H, m, -N-CH_1-), 3.75-3.95 (2H, m, -CH_2-), 2.25-2.33 (1H, m, -CH-) and 2.0-2.15 (3H, m, -CH_2-). HRMS for $C_8H_8Cl_2N_4O_2$: 263.9205 (M^++H); Anal. calcd. (found) % for $C_8H_8Cl_2N_4O_2$: C, 36.52 (36.82); N, 21.30 (21.25); H, 3.07 (3.11).

ACR-2: Yield: 96%, colour: white, $[\alpha]_D^{25}$ = (-)59° (*c* = 0.5, methanol), UV (nm, in acetonitrile): 234 (λ_{max}), IR (KBr, ν_{max}, cm^{-1}): 3290, 2970, 2910, 2880, 2830, 1770, 1480, 1460, 1370, 1320, 1180, 980 and 745. ^{1}H NMR ($CDCl_3$): δ 4.32-4.36 (1H, m, -N-CH_1-), 3.75-3.95 (2H, m, -CH_2-), 3.72 (3H, s, -O-CH_3), 2.27-2.34 (1H, m, -CH-) and 2.0-2.15 (3H, m, -CH_2-). HRMS for $C_9H_{10}Cl_2N_4O_2$: 278.1168 (M^++H); Anal. calcd. (found) % for $C_9H_{10}Cl_2N_4O_2$: C, 39.01 (38.89); N, 20.22 (20.55); H, 3.64 (3.54).

ACR-3: Yield 92%, colour: white, $[\alpha]_D^{25}$ = (-)62° (*c* = 0.5, methanol), UV (nm, in acetonitrile): 233 (λ_{max}), IR (KBr, ν_{max}, cm^{-1}): 3050, 2970, 2900, 2850, 1760, 1610, 1585, 1490, 1465, 1320, 1240, 1210, 1089, 989, 857 and 750. ^{1}H NMR ($CDCl_3$): δ 7.65 (1H, t, Ar), 7.4 (2H, t, Ar), 7.1 (2H, dd, Ar), 4.29-4.38 (1H, m, -N-CH_1-), 3.70-3.92 (2H, m, -CH_2-), 2.32-2.38 (1H, m, -CH-) and 1.96-2.21 (3H, m, -CH_2-). HRMS for $C_{14}H_{12}Cl_2N_4O_2$: 339.0854 (M^++H); Anal. calcd. (found) % for $C_{14}H_{12}Cl_2N_4O_2$: C, 49.58 (48.98); N, 16.52 (16.85); H, 3.57 (3.71).

ACR-4: Yield 94%, colour: pale white, UV (nm, in methanol): 232 (λ_{max}), IR (KBr, ν_{max}, cm^{-1}): 3385, 3300, 2940, 2810, 1730, 1610, 1580, 1530, 1440, 1356, 1240, 1110, 980, 885 and 735. ^{1}H NMR ($CDCl_3$): δ 8.89 (1H, s, -NH-CO-), 7.42-7.47 (4H, m, Ar), 7.15 (1H, m, Ar), 4.29-4.38 (1H, m, -N-CH_1-), 4.62-4.65 (1H, m, -CH-), 3.69-3.87 (2H, m, -CH_2-), 2.31-2.39 (1H, m, -CH-) and 2.08-2.19 (3H, m, -CH_2-). HRMS for $C_{14}H_{13}Cl_2N_5O$: 338.2011 (M^++H); Anal. calcd. (found) % for $C_{14}H_{13}Cl_2N_5O$: C, 49.72 (49.22); N, 20.71 (20.98); H, 3.87 (3.76).

Synthesis of diastereomeric derivatives of (*RS*)-mexiletine: The MWI conditions were applied to synthesize the DDs of (*RS*)-mexiletine. The reaction mixture containing ACR-4 (140 µL, 1 mM), (*RS*)-mexiletine (100 µL, 1mM) and TEA (20 µL) was microwave irradiated for 60s (800 W, 80%) [35] (Figs. 3 and 4). Similarly, the rest of the DDs of (*RS*)-mexiletine were prepared with ACR-1, ACR-2 and ACR-3.

The experimental conditions for the synthesis of DDs of (*RS*)-mexiletine were optimized by modifying the variable such as MWI (microwave irradiation) time (30-100s), pH (9-11), ACRs:(*RS*)-mexiletine ratio (1:1 to 3:1). Under the optimal RP-HPLC separation conditions, the reaction progress and completion time were monitored by recording the peak area of DDs chromatograms (Fig. 5).

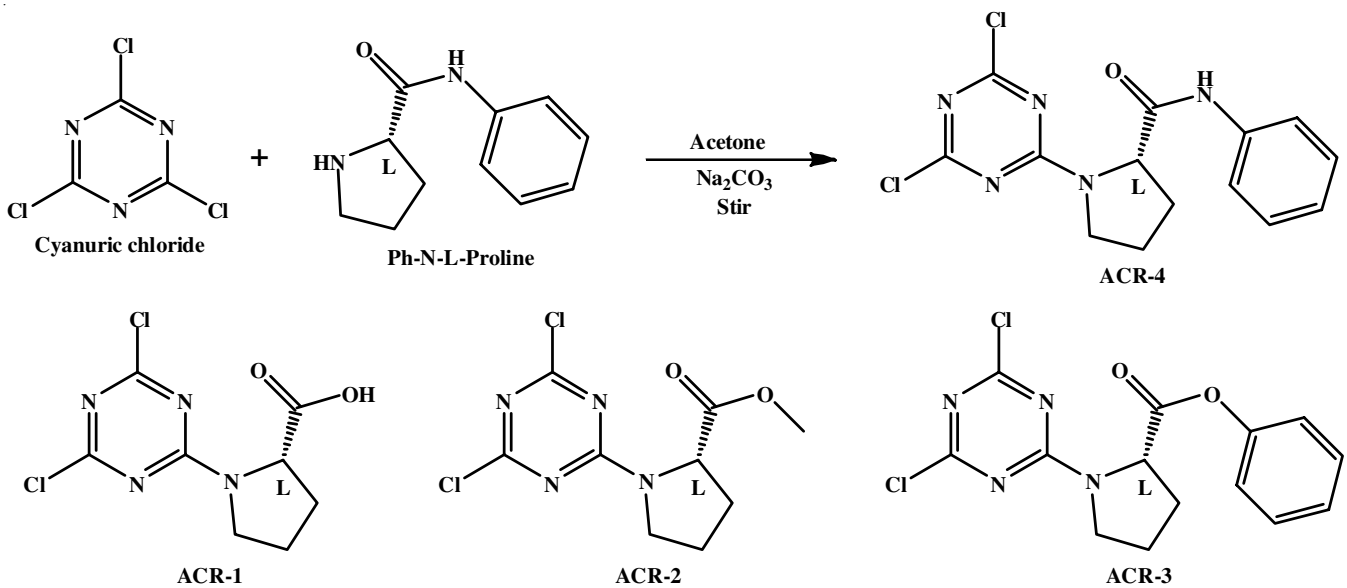

Fig. 2. Synthesis of active chiral reagents (ACRs)

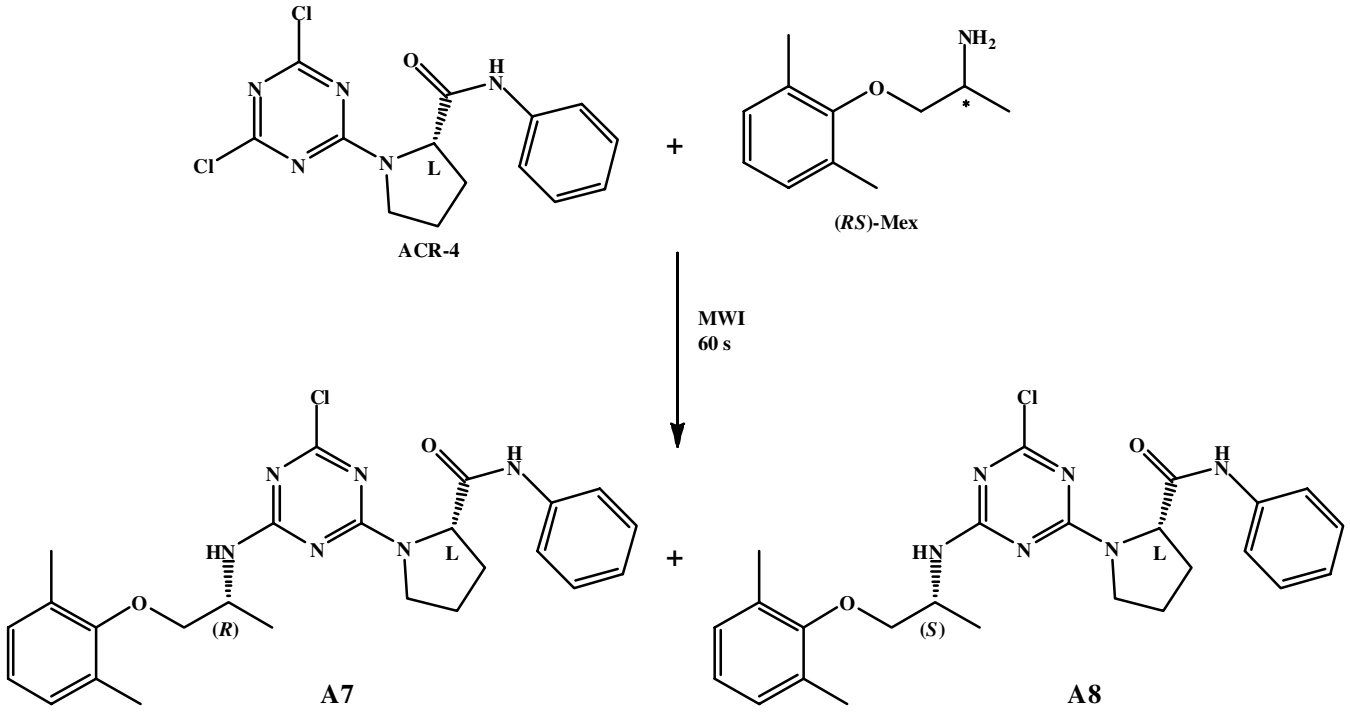

Fig. 3. Synthesis of DDs of (*RS*)-mexiletine by ACR-4

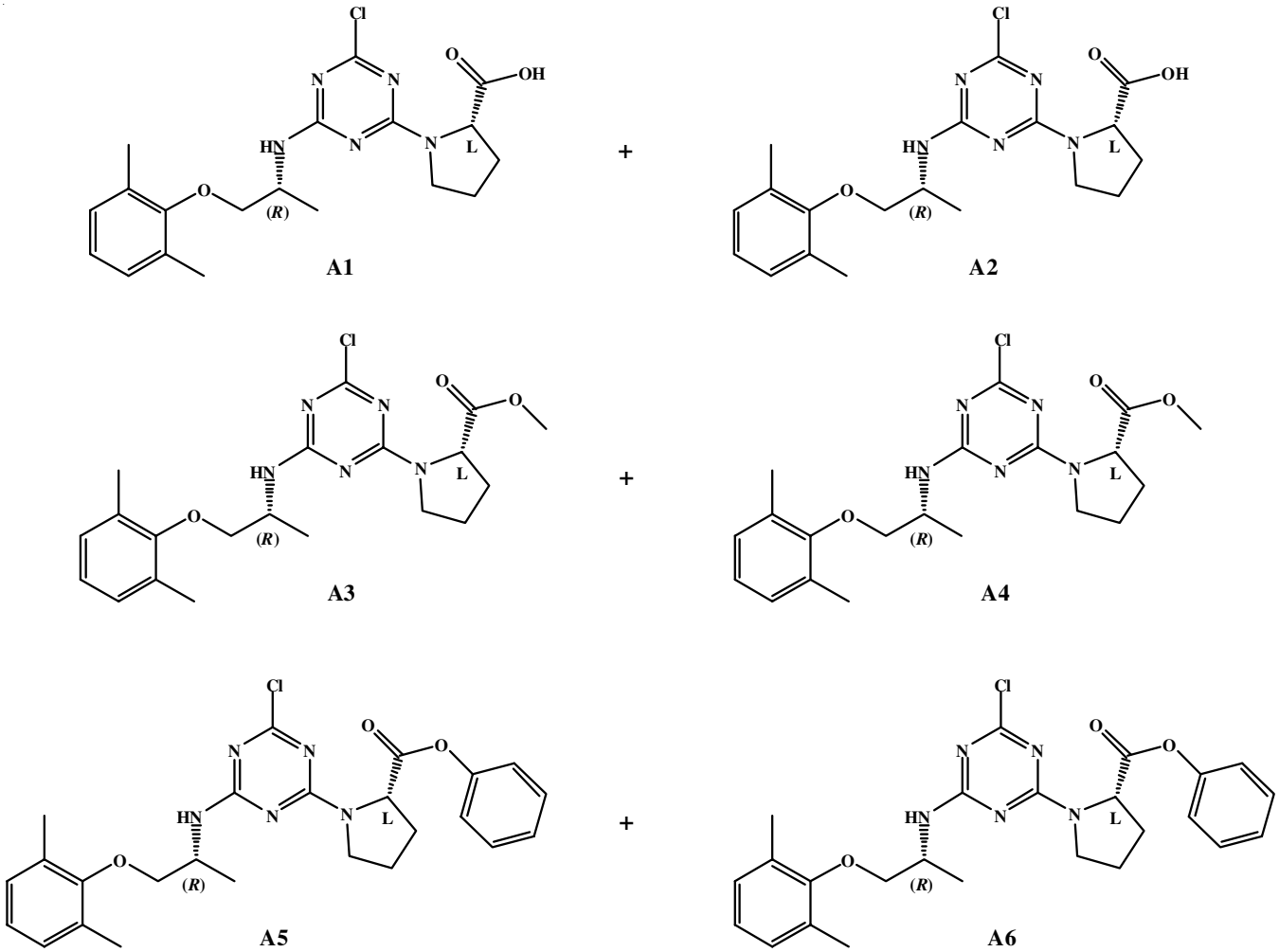

Fig. 4. Structures of DDs (A1-A6) prepared by ACR-1, ACR-2 and ACR-3

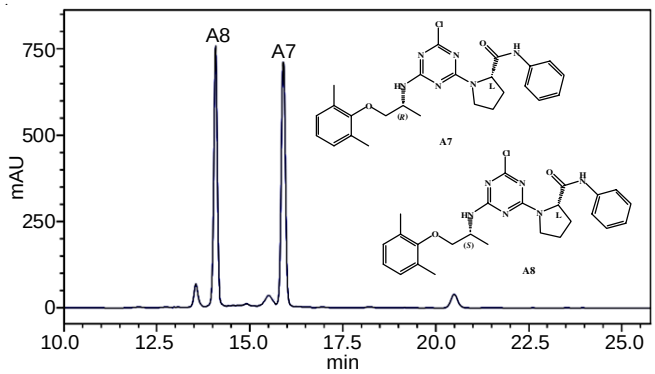

Fig. 5. RP-HPLC chromatogram of A7 and A8

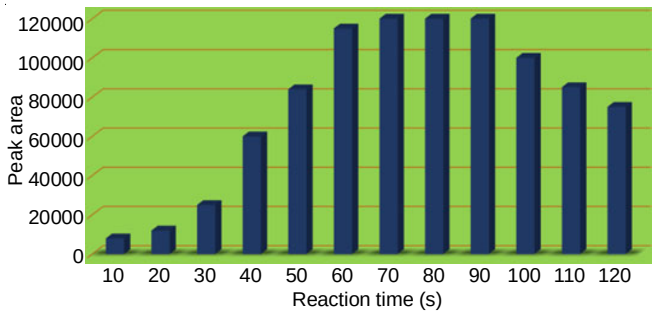

Fig. 6. Effect of microwave irradiation time on synthesis of the DDs of mexiletine

RESULTS AND DISCUSSION

Activated chiral reagents (ACR) and DDs of (*RS*)-mexiletine: Four optically pure derivatives (esters) of L-proline were prepared by converting the carboxylic group in the related esters (ii-iv) in the presence of the dehydrating (coupling) reagent EDC. EDC removes –OH from the carboxylic group and –H from alcoholic groups and gives an easy synthesis of the esters [20]. In this report, four activated chiral reagents based on cyanuric chloride were prepared by introducing derivatives of L-proline (i-iv). The ACRs were prepared under MW irradiation conditions. The reactivity toward nucleophilic substitution for cyanuric chloride is very high and provides an easy synthesis of ACRs by removing a chlorine atom from its structure [13]. The amino group of derivatives of L-proline acted as a strong nucleophile and displaced the one chlorine atom easily/quickly from cyanuric chloride in order to prepare ACRs (1-4). These ACRs are also known as DCT (dichloro-*s*-triazine) due to the presence of two chlorine atoms in their structure [14]. Due to the high molar absorbance and high reactivity, DCTs have been used extensively to separate enantiomers [13-16].

The nucleophilicity of the cyanuric chloride decreases after replacing one chlorine atom; thus, the second substitute of chlorine atom requires more energy/temperature as compared to the first substitution and similarly, the third substitution needs comparatively more energy for nucleophilic substitution [13]. ACRs have two chlorine atoms, so at the time of the synthesis of DDs, only one chlorine atom replaces *via* nucleophilic substitution, thus no side product from during reaction [14]. The reaction was completed within the 60s under MWI (80%, 800W). Further heating the reaction lead to producing side products (Fig. 6). High pH fever the synthesis quickly and smoothly compared to low pH conditions. During the synthesis, racemization didn't appear because the reaction didn't take place on the stereo centre [28,35]. The prepared ACRs were tested for their stability by modifying the different parameters, such as temperature, pH and storage time. Under the neutral conditions, the ACRs were found stable for more than six months, but in the high pH conditions, these readily react and deactivate.

Fig. 3 shows the chemical structures of the DDs of (*RS*)-mexiletine synthesized with ACR-4, as representative. Fig. 4 shows the structures of the remaining DDs of (*RS*)-mexiletine synthesis with ACRs (1-3). The prepared DDs of (*RS*)-mexiletine with ACRs (1-4) are designated as A1-A8, respectively.

RP-HPLC separation of prepared DDs of (*RS*)-mexiletine: Two mobile phase were optimized for separation of DDs of (*RS*)-mexiletine, (i) MeCN-TEAP (pH 3.5) and (ii) MeOH-TEAP (pH 4.0). Both mobile phases were used in gradient mode (20-80%) and 1 mL min^{-1} flow rate was maintained. For the separation of DDs of (*RS*)-mexiletine both mobile phase found successful in terms of reproducibility and selectivity. The values for separation factor (α), retention factor (k) and resolution (*Rs*) were calculated under the optimized chromatographic conditions for the separation of (*RS*)-mexiletine is given as Table-1. Fig. 5 shows sections of chromatograms of the resolution A7 and A8 (DDs prepared with ACR-4), as representative.

TABLE-1
CALCULATED SEPARATION DATA OF THE DDs

Activated chiral reagents	Separation data of DDs of (*RS*)-mexiletine					
	t_1 (min)	t_2 (min)	k_1	k_2	α	Rs
ACR-1	10.1	12.6	5.73	7.40	1.29	9.91
ACR-2	9.7	12.9	5.46	7.60	1.39	12.80
ACR-3	13.0	14.7	7.66	8.80	1.15	8.50
ACR-4	13.6	15.9	8.6	9.60	1.19	11.20

The organic modifier MeCN in the mobile phase provides a sharper peaks compared to MeOH in mobile phase, under the same RP-HPLC conditions. Thus, MeCN based mobile phase providing better resolution and lower retention time. The DDs shows better solubility in MeCN due to low viscosity (0.38 cP) and high polarity of MeCN compared to MeOH (0.59 cP). Also, MeCN has low UV-Visible cutoff and low chemical reactivity and low acidity; hence, as an organic modifier MeCN provides sharp peaks with lower retention factor [11,13,36]. The PDA detector was used to capture UV-Vis spectrum corresponding to first and second eluting DDs at 13.67 and 15.98 min for A8 and A7, as a representative. These were found to be identical.

DFT optimization, separation mechanism and elution order: The DFT structures of synthesis DDs were developed for the lowest energy using Gaussian 09 rev. A.02 to investigate the absolute configuration, separation mechanism and elution order of the prepared DDs (as representative A7 and A8; Fig. 7). The L-proline molecule in the DDs cause to have dissimilar configurations in between pair of DDs. The optimized struct-

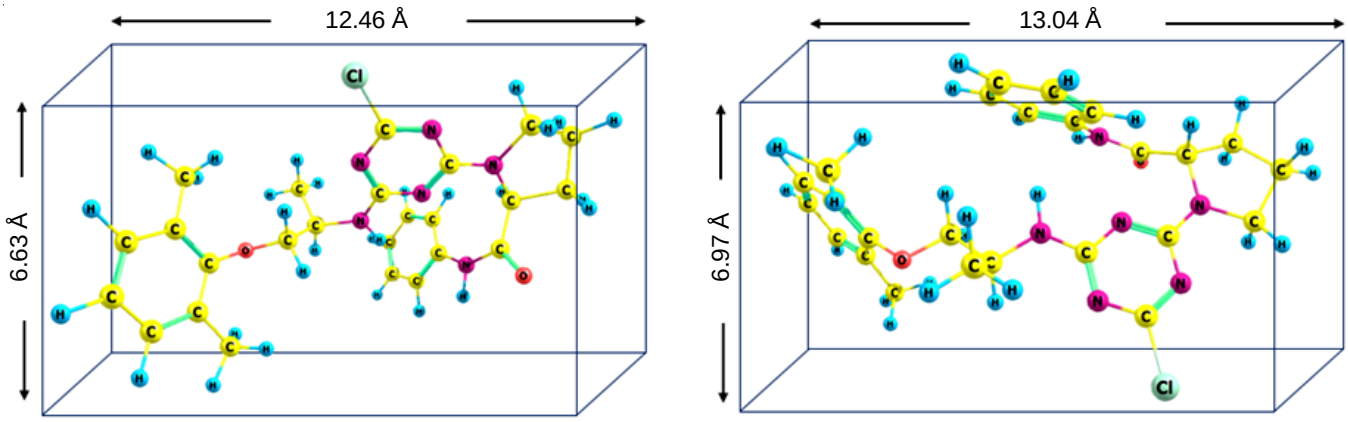

Fig. 7. DFT optimized structure of the A7 and A8

ures (A7 and A8) show the different gaps between the aromatic rings of mexiletine and L-proline. The structures of A7 (*R*,*L*-DD) and A8 (*S*,*L*-DD) show the clean size difference due to the gaps of the aromatic rings of mexiletine and L-proline. A8 has a bigger size (13.04 Å), while A7 has a smaller size (12.46 Å). According to the literature [20], the bigger DD is having more polarity due to high surface area. So it has more solubility in the polar mobile phase than small DD. Thus, it elutes first from the column. The smaller DD (A7) shows more hydrophobic properties; therefore, it stays longer on C_{18}-column and elutes in the last. A similar study was applied to the rest of the DDs to determine elution order. Among all DDs, A2 and A1 take the lowest time to elute (respectively, 10.11 and 12.56), while A8 and A7 take the highest time to elute (respectively, 13.67 and 15.98; Table-2). It was observed that the higher carbon ratio in DDs leads to high elution time, while polar groups lead to low elution time (low hydrophobic interaction with column material).

TABLE-2
DFT AND ELUTION ORDERS OF THE DDs

ACRs	Size of the optimized DDs (terminal C-C distance in Å		First eluded DD
	(*L*,*S*)-DD	(*D*,*R*)-DD	
ACR-1	9.15 (A1)	12.60 (A2)	A2
ACR-2	9.48 (A3)	12.21 (A4)	A4
ACR-3	9.75 (A5)	12.49 (A6)	A6
ACR-4	12.46 (A7)	13.04 (A8)	A8

Validation: ICH guidelines [37] were used to validate the current method and the linearity, accuracy, precision, relative standard deviation (RSD), the limit of detection (LOD) and the limit of quantification (LOQ) were determined for DDs A7 and A8 (concentration range 200-2000 ng/mL), as repre-

TABLE-3
VALIDATION STUDY OF THE A7 AND A8

Linearity	First eluting DD (A8)			Second eluting DD (A7)		
Range (ng mL^{-1})	100-1000			100-1000		
Slope	3.289			3.824		
Intercept	9.21			8.46		
Correlation coefficient (R^2)	0.998			0.999		
Accuracy and precision						
Conc. of each DDs (ng mL^{-1})	First eluding DD (A8)			Second eluding DD (A7)		
	Found conc. Mean ± SD (ng mL^{-1})	Recovery (%)	RSD (%)	Found conc. Mean ± SD (ng mL^{-1})	Recovery (%)	RSD (%)
Intra-day precision						
100	98.87 ± 0.35	98.87	0.58	98.01 ± 0.29	98.01	0.67
250	245.8 ± 0.41	98.32	1.09	249.8 ± 0.46	99.92	1.18
500	492.1 ± 3.10	98.42	1.12	498.1 ± 2.54	99.62	1.24
750	744.2 ± 7.37	99.22	1.28	749.9 ± 6.61	99.85	1.52
1000	991.1 ± 11.04	99.11	1.74	997.2 ± 9.21	99.72	1.68
Mean		98.78	1.16		99.42	1.25
Inter-day precision						
100	98.54 ± 0.24	98.54	0.31	100.1 ± 0.31	100.10	0.72
250	246.5 ± 0.38	98.60	0.98	248.2 ± 0.64	99.28	1.11
500	494.6 ± 1.42	98.92	1.12	498.5 ± 1.21	99.70	1.38
750	742.5 ± 5.53	99.00	1.41	746.3 ± 3.56	99.51	1.62
1000	997.2 ± 8.91	99.72	1.78	1000.2 ± 7.51	100.02	1.86
Mean		98.95	1.12		99.72	1.34

Sensitivity = LOD (ng mL^{-1}): 0.306; LOQ (ng mL^{-1}): 0.918
[n (=5) is the number of replicates, SD = Standard deviation, RSD = relative standard deviation]

sentative. The interday and intraday assays were performed to investigate the robustness of the developed method and validation (Table-3). The recovery of the DDs were found more than 99% and the method showed a susceptible detection of DDs using a PDA detector (LOD = 0.306 ng mL^{-1} and LOQ = 0.918 ng mL^{-1}). The robustness of the validation were optimized by applying different variables such as temperature, eluting solvents and flow rate and detection wavelength. The validation results were found stable on changing the above parameters.

Conclusion

The current work is an excellent report on the synthesis of diastereomeric derivatives (DDs) of (*RS*)-mexiletine, under microwave irradiation conditions, using cyanuric chloride based chiral reagents. The determination of enantiomeric purity of (*RS*)-mexiletine with current method was found simple, precise and accurate with short retention time, high *Rs* and low LOD and LOQ values. The separation conditions were optimized and acetonitrile with TEAP buffer was found a better eluting medium for RP-HPLC separation. The mechanism of separation, elution order and absolute configurations of DDs were confirmed using DFT optimized energy minimized structures. This approach can be applied to detect trace levels of the amino group containing compounds to control the enantiomeric purity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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