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Enantiomeric resolution and modeling of DL-alanine-DL-tryptophan dipeptide on amylose stationary phase

Imran Ali[1,2] | Raffat A. Khattab[3] | Zeid A. Alothman[4] | Ahmad Yacine Badjah[4] | Abdulrahman Alwarthan[3]

[1]Department of Chemistry, College of Sciences, Taibah University, Medina, Saudi Arabia

[2]Department of Chemistry, Jamia Millia Islamia, New Delhi, India

[3]Department of Biology, College of Sciences, Taibah University, Medina, Saudi Arabia

[4]Department of Chemistry, College of Sciences, King Saud University, Riyadh, Saudi Arabia

Correspondence
Imran Ali, Department of Chemistry, College of Sciences, Taibah University, Medina, 41477, Saudi Arabia.
Email: drimran.chiral@gmail.com; drimran_ali@yahoo.com

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Abstract

The enantiomeric resolution of DL-alanine-DL-tryptophan dipeptide is described on amylose stationary phase. The eluent used was CH_3OH—CH_3COONH_4 (10mM)—CH_3CN (50: 40, 10) at 0.8-mL/min flow, 230-nm detection, 25-minute run time, and 25°C ± 1°C temperature. The chiral phase was amylose [AmyCoat RP (15 cm × 0.46 cm × 5 micron)]. The magnitudes of the retention factors (k) were 2.71, 3.52, 5.11, and 7.75. The magnitudes of separation factor (α) were 1.19, 1.57, and 1.51 while the resolution factors (Rs) were 3.25, 14.84, and 15.76. The limits of detection and quantitation were of 2.5 to 5.4 and 12.8 to 27.5 μg/mL. The enantiomeric resolution is controlled by hydrogen, hydrophobic, π-π, steric, etc interactions. The elution order of the enantiomer was supported by the modeling data. The described method is fast, reproducible, precise, and selective, which can be used successfully for evaluating the enantiomers of the reported dipeptide.

KEYWORDS

amylose, dipeptide, enantiomeric resolution, HPLC, modeling studies

1 | INTRODUCTION

The enantiomeric resolution is the most important issue in separation science and gaining value continuously.[1,2] It is due to the dissimilar properties of the enantiomers of the drugs, pharmaceuticals, and additional agriculture-related compounds.[3-10] Among some methods, high-performance liquid chromatography (HPLC) is thought as the technique of the option for the enantiomeric resolution.[11-16] Many papers have been published on the racemates with 1 chiral center.[17-23] Though, only a small amount of articles describe the enantiomeric resolution of the racemates with more than 1 chiral center.[24,25] It is due to the fact that the enantiomeric resolution is extremely tricky of the racemates with greater than 1 chiral center, owing to identical properties of the enantiomers. So, there is an immense need of HPLC methods of racemates with more than 1 chiral center.

The dipeptides are very important biological species playing crucial roles in food and medicine industries.[26] Besides, these control many biological activities of the body.[27] Interestingly, the dipeptides are responsible for many stereoselectively biological reactions with big difference of thermodynamic and kinetics because of stereoselectivity. It is important to write here that dipeptides have 2 chiral centers with 4 enantiomers and hence, a challenge for the enantiomeric resolution. Among many dipeptides, aromatic ones have special attention because of the dissimilar interactions of the enantiomers with

other proteins and nucleic acids. During literature survey, only few articles were found to contain enantiomeric resolution of few dipeptides,[22] but no one describes the enantiomeric resolution of very important aromatic dipeptides DL-alanine-DL-tryptophan (Figure 1). So, well-known chiral stationary phase made of amylose was used to achieve enantiomeric resolution of this dipeptide. Computer modeling was also exploited to know the enantiomeric resolution mechanistic.

2 | EXPERIMENTAL

2.1 | Chemicals and reagents

The racemic mixture and optically active enantiomers of alanine-tryptophan were supplied by a USA company (Sean Fisher Peptide 2.0, Inc., Chantilly, Virginia). LiChrosolve MeOH and CH_3CN and ammonium acetate (AR grade) had been obtained from Merck, Bombay, India. Ionized water was prepared by Millipore unit (Milli-Q; Bedford, Massachusetts). One hundred–microgram per milliliter solutions of racemic and enantiomers of dipeptides were made in H_2O—CH_3OH (50: 50, v/v).

2.2 | Instrumentation

An HPLC machine was used with eluent forcing pump (Waters, 510, Milford, Massachusetts), injector (Waters, WISP 710B), (Waters, 484), and integrator (Waters, 740). Amylose chiral stationary phase [AmyCoat RP (15 cm × 0.46 cm × 5 micron), Kromasil, Sweden] was used in this study.

2.3 | Chromatographic conditions

An amount of 20.0 μL of racemic and enantiomeric pure alanine-tryptophan dipeptides were inserted in to an HPLC system. CH_3OH—CH_3COONH_4 (10mM)—CH_3CN (50: 40, 10) was used as the eluent at 0.8-mL/min flow, 230-nm detection, 25-minute run time, and 25°C ± 1°C. The chart rate was stable at 0.1 cm/min. Nitrogen was used to calculate the dead volume.

The chromatography variables such as retention (k), separation (α), and resolution factors (Rs) were determined. The naming of the separated enantiomers of DL-alanine-DL-tryptophan was finalized by match up to the retention times with those of enantiomerically pure enantiomers.

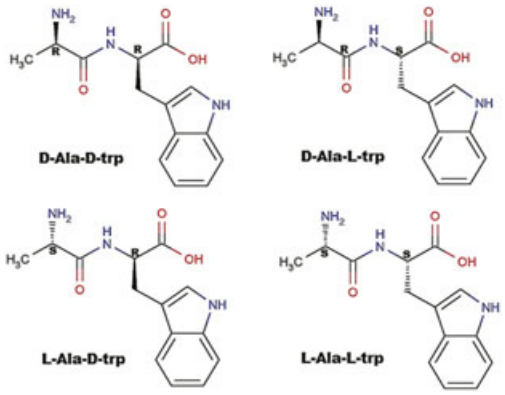

FIGURE 1 The stereomers structures of alanine-tryptophan dipeptide

2.4 | Validation

Validation of chromatographic system was done by determining the linearity, limit of detection (LOD), specificity, limit of quantitation (LOQ), precision, robustness, and accuracy. These were estimated as per the standard procedures described in the literature.[28-30]

2.5 | Modeling

Computer modeling was conducted as per the studied reported elsewhere.[26,27] The ligands used were L-alanine-L-tryptophan, D-alanine-D-tryptophan, D-alanine-L-tryptophan, and L-alanine-D-tryptophan. The receptor was the amylose stationary phase (Figure 2). A computer with Intel(R) Core(TM) i3 CPU (2.3 GHz) with XP-based operating system (Windows 2003) was used for modeling. The other software used were Marvin Sketch (5.8.2 version), AutoDock 4.2 Vina, and PyMol.

3 | RESULTS AND DISCUSSION

3.1 | Chromatographic data

The enantiomeric resolution of the enantiomers of DL-alanine-DL-tryptophan was determined by recoding their retention times. The order of elution was fixed using entiomerically pure isomers, ie, L-alanine-L-tryptophan, D-alanine-D-tryptophan, D-alanine-L-tryptophan, and L-alanine-D-tryptophan. The enantiomers separation of this dipeptide is shown in Figure 3.

The values of the retention times of these 4 enantiomers were 7.42, 8.51, 12.22, and 17.5 minutes. The magnitudes of the retention factors (k) were 2.71, 3.52, 5.11, and 7.75. The magnitudes of separation factor (α) were 1.19, 1.57, and 1.51 while the resolution factors (Rs) were 3.25, 14.84, and 15.76. The LOD and LOQ were of 2.5 to 5.4 and 12.8 to 27.5 μg/mL. These values are summarized in Table 1. All these values are <1, and it means that the enantiomeric resolution was satisfactory. Table 1 also depicts the order of separation of

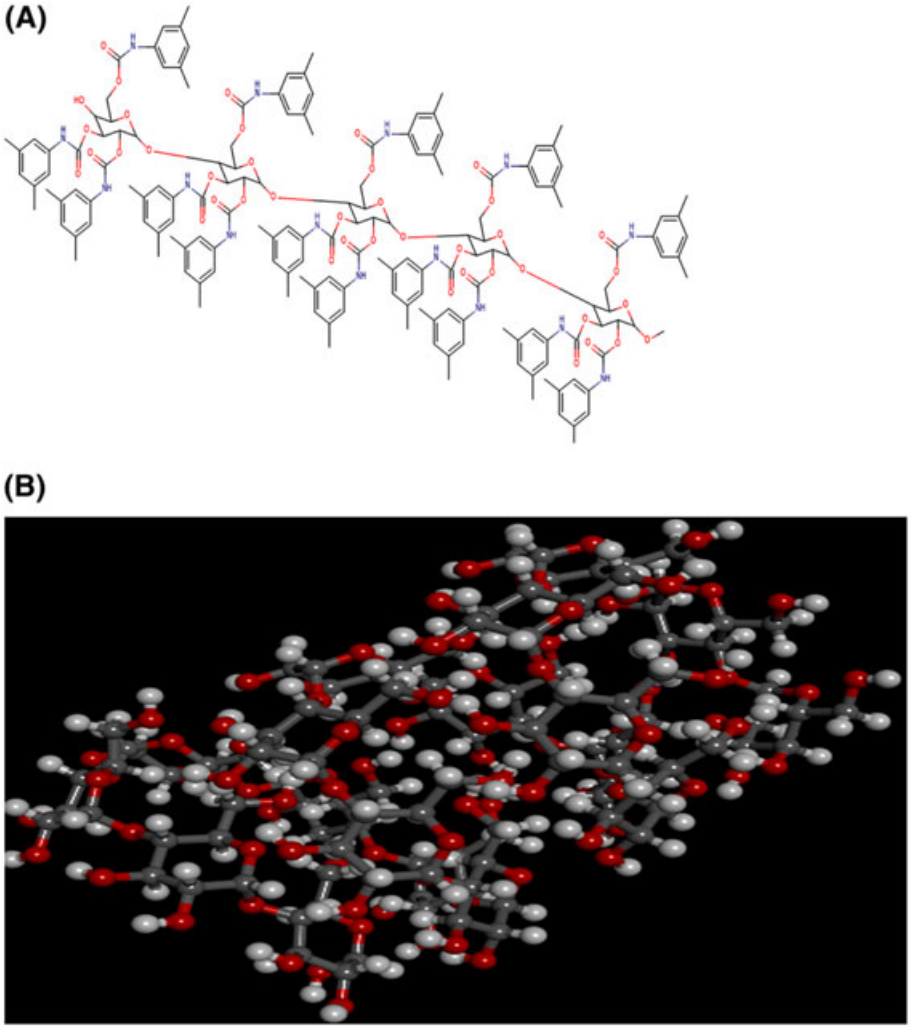

FIGURE 2 A, 2-D and B, 3-D structures of tris-(3,5-dimethylphenyl carbamate) amylose chiral selector

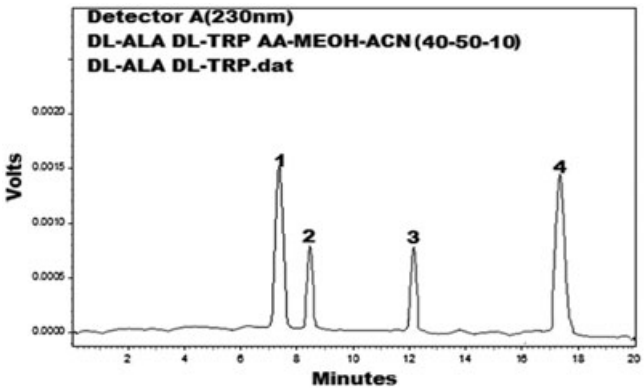

FIGURE 3 Chromatograms of the chiral separation of DL-alanine-DL-tryptophan on AmyCoat column

these enantiomers. This separation order was L-alanine-L-tryptophan > D-alanine-D-tryptophan > D-alanine-L-tryptophan > L-alanine-D-tryptophan.

3.2 | Chromatographic optimization

To finalize the chromatographic settings, various combinations of CH_3OH—CH_3COONH_4—CH_3CN (50: 40, 10) were tested. The different ratios tested were 60: 30: 10, 70: 20: 10, 40: 50: 10, 30: 60: 10, 50: 45, 5, 60: 35: 15, 70: 25: 5, 40: 55: 5, and 30: 65: 5. Besides, low and high pHs of these combinations were also tested. It was observed that these variations resulted into a wide range of results. But these combinations could not give good results. At high amount of CH_3CN, the peaks of L-alanine-L-tryptophan and D-alanine-D-tryptophan merged into each other. Low amount of CH_3CN led to tailing of the peaks. Similarly, high amount of methanol gave partial resolution of L-alanine-L-tryptophan and D-alanine-D-tryptophan. Contrarily, low amount of CH_3OH gave broad peaks, but the tailing magnitude was low in comparison to low amount of CH_3CN. The effect of the amount of CH_3COONH_4 was also evaluated on the enantiomeric

TABLE 1 Chiral high-performance liquid chromatography parameters of DL-alanine-DL-tryptophan dipeptide

Dipeptide	Retention Factors (k)				Separation Factors (α)			Resolution Factors (Rs)		
	k_1	k_2	k_3	k_4	α_1	α_2	α_3	Rs_1	Rs_2	Rs_3
DL-Alanine-DL-tryptophan	2.71	3.52	5.11	8.67	1.19	1.57	1.70	3.25	14.84	21.22

k_1 = LL enantiomer; k_2 = DD enantiomer; k_3 = DL enantiomer; k_4 = LD enantiomer

resolution. High amount of CH_3COONH_4 gave disturbed peaks of L-alanine-L-tryptophan and D-alanine-D-tryptophan while low amount of this constituent gave broad peaks. However, 50: 40: 10 combination could give only good result as represented in Figure 3 and Table 1.

3.3 | Chromatographic validation

The chromatography method was validated involving various parameters, ie, counting linearity, LOD, specificity, LOQ, precision, and accuracy.[31]

3.4 | Linearity

Linearity was evaluated by regression analysis (least squares) of the curve. Linearity of standardizing peak areas vs concentrations for all the 4 enantiomers were tested in 1.0- to 100-μg/mL amount ranges. The plots were linear in the concentration ranges (n = 5) for all the 4 enantiomers. The peak areas of the 4 enantiomers were graphed aligned with their individual concentrations. Linear regression analysis was achieved on the consequential curves. The correlation coefficients of the establishment were 0.9997 to 0.9999 for the 4 enantiomers (n = 5). The %RSDs and confidence limits were varied from 0.50 to 1.00 and 98.0 to 98.5.

3.5 | LOD and LOQ

Limits of detection and quantitation were considered from the calibration plots of all the 4 enantiomers as 3 and 5 multiplication of the noise level for LOD and LOQ.[32] The values of LOD of L-alanine-L-tryptophan, D-alanine-D-tryptophan, D-alanine-L-tryptophan, and L-alanine-D-tryptophan were 5.1, 2.5, 2.6, and 5.4 μg/mL. The values of LOQ of L-alanine-L-tryptophan, D-alanine-D-tryptophan, D-alanine-L-tryptophan, and L-alanine-D-tryptophan were 25.6, 12.8, 13.1, and 27.5 μg/mL, respectively. The %RSD, correlation coefficients, and confidence levels for LOD were 0.80 to 1.00, 0.9996 to 0.9998 and 97.6 to 98.0, respectively. Similarly, the %RSD, correlation coefficients, and confidence levels for LOQ were 0.78 to 0.95, 0.9996 to 0.9997, and 98.0 to 98.4, respectively.

3.6 | Specificity

The technique is a pretty good exact as evident from Figure 3. Retention times of all the 4 enantiomers were approximately alike in both standard solution and enantiomerically pure enantiomers. Even a small impurity added could not disturb the optimized peaks. The LOD, peak retention times, and LOQ were similar. The %RSD, correlation constants, and confidence stages were 0.68 to 0.79, 0.9996 to 0.9997, and 98.1 to 98.6, respectively. These discoveries indicated a fine specific method.

3.7 | Accuracy

Accuracy of the system was experienced by inserting standards of the enantiomers at a variety of amounts. Accuracy was fixed by interpolation of replicate (n = 5) chromatogram areas of 25.0-, 50.0-, and 100.0-μg/mL amount standards. In every run, percent error was calculated and found to be 0.6% to 1.0%. The %RSD, correlation constants, and confidence stages were 0.61 to 0.77, 0.9997 to 0.9999, and 98.3 to 98.8, respectively. This array specified a fine accuracy of method.

3.8 | Precision

Precision figures were deliberated by 3 concentrations of all the enantiomers (25.0, 50.0, and 100.0 μg/mL). Five chromatographic experiments were done for 3 amounts. The %RSD, correlation coefficients, and confidence levels were 0.53 to 0.62, 0.9998 to 0.9999, and 98.6 to 99.1, respectively, indicating a good precised HPLC method.

3.9 | Modeling data

The modeling data of the ligands (L-alanine-L-tryptophan, D-alanine-D-tryptophan, D-alanine-L-tryptophan, and L-alanine-D-tryptophan) with receptor (amylose) is given in Table 2. This table describes binding affinity (kcal/mol), total hydrogen bonds, and residues participated in hydrogen bondings and hydrophobic interactions. The various residues involved in hydrogen and hydrophobic interactions are clear from Table 2. It is also evident that there is only 1 hydrogen bond between each ligand and receptor. The binding affinity

TABLE 2 Modeling data of DL-alanine-DL-tryptophan on amylose chiral selector

DL-Ala-DL-trp	Binding Affinity (kcal/mol)	No. of H Bonds	Residues Involved in H-Bonding	Residues Involved in Hydrophobic Interactions
L-Ala-L-trp	−3.1	1	UNK236/O::HO3 of NH group	Unk402::C2,C5,C9,C10
D-Ala-D-trp	−3.3	1	UNK186/HO5:: O of CO group*(3.5)*	Unk402::C2 C3&C5 Unk403::O2
D-Ala-L-trp	−3.7	1	UNK186/HO5:: O of CO group*(3.4)*	Unk402::C8
L-Ala-D-trp	−4.3	1	UNK236/O::HO3 of NH group	Unk386::C5,C8,C9,C10 &N2 Unk 403::C6

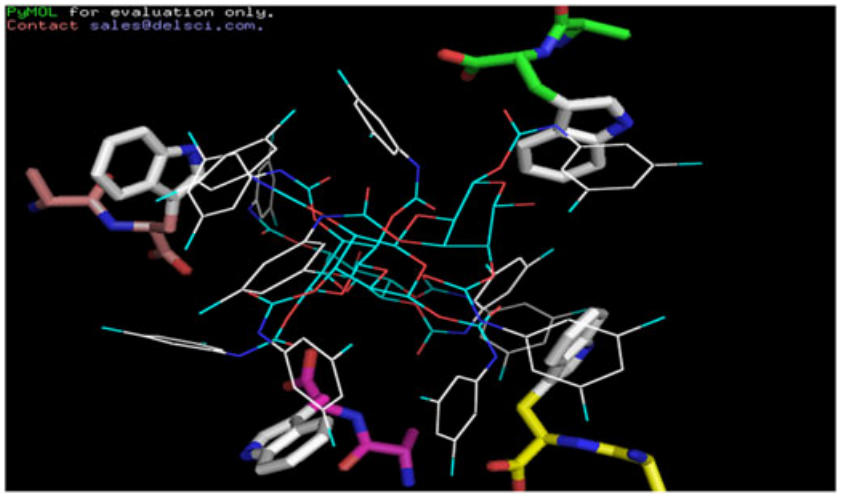

FIGURE 4 Docking model of all the enantiomers of DL-alanine-DL-tryptophan dipeptide on amylose stationary phase

of L-alanine-L-tryptophan, D-alanine-D-tryptophan, D-alanine-L-tryptophan, and L-alanine-D-tryptophan were −3.1, −3.3, −3.7, and −4.3 kcal/mol. These binding affinities clearly supported the order of elution of the enantiomers as discussed in chromatographic data section. The docking model of all the enantiomers of DL-alanine-DL-tryptophan dipeptide on amylose stationary phase is given in Figure 4.

3.10 | Modeling mechanism support

The arrangement of elution of the 4 enantiomers was L-alanine-L-tryptophan > D-alanine-D-tryptophan > D-alanine-L-tryptophan > L-alanine-D-tryptophan. This order was ascertained by comparing the retention times of the standard enantiomers. This order of elution is supported by the binding affinity of the enantiomers. The values of the binding affinities of the 4 enantiomers were in the order of L-alanine-L-tryptophan (−3.1) < D-alanine-D-tryptophan (−3.3) < D-alanine-L-tryptophan (−3.7) < L-alanine-D-tryptophan (−4.3). This is the reason that the above discussed elution order was observed. The more binding energy resulted to the more strong retention, resulting into late elution.

Our earlier research work and literature research papers[33-40] are clearly in the support that chiral recognition is controlled by hydrogen, hydrophobic, π-π, steric interactions, etc. These interactions are among amino, amide, and carboxylic group of dipeptides and amide, amino, oxide, and hydroxyl groups of amylose. Hence, the reported 4 enantiomers get fitted enantioselectively in the asymmetric grooves of amylose. The enantiomers are fixed well because of the above-discussed interactions. It is essential to report here that π-π interactions are dominant among the enantiomers of aromatic molecules (like this dipeptide) and amylose.[28,41-48] Hence, π-π interactions may chief contributors for the enantiomeric resolution of the reported dipeptide. In a nut shell, the competition between eluent and these bondings resulted into the elution of the enantiomers at the different time intervals.

4 | CONCLUSION

The successful, fast, reproducible, precise, and selective method of the enantiomeric resolution of DL-alanine-DL-tryptophan dipeptide is described on amylose

stationary phase. The enantiomers got resolved successfully within 18 minutes. The enantiomeric resolution is controlled by hydrogen, hydrophobic, π-π, steric interactions, etc. The modeling data supported the experimental results and hence, was used to describe the enantiomeric resolution mechanism. Therefore, the elution order of the enantiomer was supported by the modeling data. The described method is successful for evaluating the enantiomers of the reported dipeptide in any sample.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

ORCID

Imran Ali http://orcid.org/0000-0001-6511-8374

REFERENCES

1. Ali I, Aboul-Enein HY. *Chiral Pollutants: Distribution, Toxicity and Analysis by Chromatography and Capillary Electrophoresis*. Chichester, UK: John Wiley & Sons; 2004.
2. Aboul-Enein HY, Ali I. *Chiral Separations by Liquid Chromatography and Related Technologies*. New York, USA: Marcel Dekker, Inc.; 2003.
3. Aboul-Enein HY, Ali I. Macrocyclic antibiotics as effective chiral selectors for enantiomeric resolution by liquid chromatography and capillary electrophoresis. *Chromatographia*. 2000;52:679-691.
4. Ali I, Kumerer K, Aboul-Enein HY. Mechanistic principles in chiral separations using liquid chromatography and capillary electrophoresis. *Chromatographia*. 2006;63:295-307.
5. Ali I, Gaitonde VD, Aboul-Enein HY, Hussain A. Chiral separation of β-adrenergic blockers on CelluCoat column by HPLC. *Talanta*. 2009;78:458-463.
6. Ali I, Aboul-Enein HY. Impact of immobilized polysaccharide chiral stationary phases on enantiomeric separations. *J Sep Sci*. 2006;29:762-769.
7. Ali I, Saleem K, Hussain I, Gaitonde VD, Aboul-Enein HY. Polysaccharides chiral stationary phases in liquid chromatography. *Sepn & Purifn Rev*. 2009;38:97-147.
8. Aboul-Enein HY, Ali I. Studies on the effect of alcohols on the chiral discrimination mechanisms of amylose stationary phase on the enantioseparation of nebivolol by HPLC. *J Biochem & Bioanl Meth*. 2001;48:175-188.
9. Ali I, Gupta VK, Aboul-Enein HY, Singh P, Sharma B. Role of racemization in optically active drugs development. *Chirality*. 2007;19:453-463.
10. Ali I, Naim L, Ghanem A, Aboul-Enein HY. Chiral separations of piperidine-2, 6-dione analogues on Chiralpak IA and Chiralpak IB columns by using HPLC. *Talanta*. 2006;69:1013-1017.
11. Ali I, Gupta VK, Aboul-Enein HY. Chirality: a challenge for the environmental scientists. *Curr Sci*. 2003;84:152-156.
12. Aboul-Enein HY, Ali I. Comparative study of the enantiomeric resolution of chiral antifungal drugs econazole, miconazole and sulconazole by HPLC on various cellulose chiral columns in normal phase mode. *J Biochem & Bioanl Meth*. 2002;27:441-446.
13. Ali I, Aboul-Enein HY. Enantioseparation of some clinically used drugs by HPLC using cellulose Tris (3, 5-dichlorophenylcarbamate) chiral stationary phase. *Biomed Chromatogr*. 2003;17:113-117.
14. Aboul-Enein HY, Ali I. HPLC enantiomeric resolution of nebivolol on normal and reversed amylose based chiral phases. *Pharmazie*. 2001;56:214-216.
15. Ali I, Aboul-Enein HY, Ghanem A. Enantioselective toxicity and carcinogenesis. *Cur Pharm Anal*. 2005;1:109-125.
16. Aboul-Enein HY, Ali I, Gübitz G, Simons C, Nicholls PJ. HPLC enantiomeric resolution of novel aromatase inhibitors on cellulose-and amylose-based chiral stationary phases under reversed phase mode. *Chirality*. 2000;12:727-733.
17. Aboul-Enein HY, Ali I. Comparison of the chiral resolution of econazole, miconazole, and sulconazole by HPLC using normal-phase amylose CSPs. *Fresenius J Anal Chem*. 2001;370:951-955.
18. Aboul-Enein HY, Ali I. Normal phase chiral HPLC of methylphenidate: comparison of different polysaccharide-based chiral stationary phases. *Chirality*. 2002;14:47-50.
19. Ali I, Al-Othman ZA, Hussain A, Saleem K, Aboul-Enein HY. Chiral separation of β-adrenergic blockers in human plasma by SPE-HPLC. *Chromatographia*. 2001;73:251-256.
20. Ali I, Singh P, Aboul-Enein HY, Sharma B. Chiral analysis of ibuprofen residues in water and sediment. *Anal Let*. 2009;42:1747-1760.
21. Aboul-Enein HY, Ali I. Enantiomeric resolution of some imidazole antifungal agents on chiralpak WH chiral stationary phase using HPLC. *Chromatographia*. 2001;54:200-202.
22. Ali I, Al-Othman ZA, Al-Warthan A, Asnin L, Chudinov A. Advances in chiral separations of small peptides by capillary electrophoresis and chromatography. *J Sep Sci*. 2014;37:2447-2466.
23. Ali I, Aboul-Enein HY, Gaitonde VD, Singh P, Rawat MSM, Sharma B. Chiral separations of imidazole antifungal drugs on AmyCoat RP column in HPLC. *Chromatographia*. 2009;70:223-227.
24. Ali I, Suhail M, Lone MN, AL-Othman ZA, Alwarthan A. Chiral resolution of multi-chiral centre racemates by different modalities of chromatography. *J Liq Chromatogr & Rel Technol*. 2016;39:435-444.
25. Ali I, Suhail M, AL-Othman ZA, Alwarthan A, Aboul-Enein HY. Enantiomeric resolution of multiple chiral centres racemates by capillary electrophoresis. *Biomed Chromatogr*. 2016;30:683-694.
26. Ali I, Sahoo DR, Al-Othman ZA, Al-Warthan A, Asnin L, Larsson B. Validated chiral HPLC separation method and

simulation studies of dipeptides on amylose chiral column. *J Chromatogr A*. 2015;1406:201-209.

27. Alajmi MF, Hussain A, Suhail M, et al. Chiral HPLC separation and modeling of four stereomers of DL-leucine-DL-tryptophan dipeptide on amylose chiral column. *Chirality*. 2016;28:642-648.
28. Ali I, Saleem K, Gaitonde VD, Aboul-Enein HY, Hussain I. Chiral separations of some β-adrenergic agonists and antagonists on AmyCoat column by HPLC. *Chirality*. 2010;22:24-28.
29. Aboul-Enein HY, Ali I. Chiral resolution of clenbuterol, cimaterol, and mabuterol on Chirobiotic V, T, and TAG columns. *J Sep Sci*. 2002;25:851-855.
30. Ali I, Aboul-Enein HY. Immobilized polysaccharide CSPs: an advancement in enantiomeric separations. *Curr Pharm Anal*. 2007;3:71-82.
31. United States Pharmacopeia. United States Pharmacopeial Convention Inc., Rockville, MD, USA (2005).
32. ICH. Draft guidelines on validation of analytical procedures: definitions and terminology. *Fed Regist*. 1995;60, IFPMA, Switzerland:
33. Morris GM, Goodsell GS, Halliday RS, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J Comput Chem*. 1998;19:1639-1662.
34. Ali I, Alam SD, Al-Othman ZA, Farooqi JA. Recent advances of chiral drugs by SPE-HPLC in biological samples. *J Chromatogr Sci*. 2013;51:645-654.
35. Ali I, Saleem K, Hussain I, Gaitonde VD, Aboul-Enein HY. Polysaccharides chiral stationary phases in liquid chromatography. *Sep & Purif Rev*. 2009;38:1-51.
36. Aboul-Enein HY, Ali I. Applications of polysaccharide based chiral stationary phases for resolution of different compound classes. *Methods Mol Biol*. 2004;243:183-196.
37. Aboul-Enein HY, Ali I. Optimization strategies for HPLC enantioseparation of racemic drugs using polysaccharides and macrocyclic glycopeptide antibiotic chiral stationary phases. *IL Farmaco*. 2002;57:513-529.
38. Aboul-Enein HY, Ali I. Studies on the effect of alcohols on the chiral discrimination mechanisms of amylose stationary phase on the enantioseparation of nebivolol by HPLC. *J Biochem & Biophys Methd*. 2001;48:175-188.
39. Ali I, Sanagi MM, Aboul-Enein HY. Advances in chiral separations by non-aqueous capillary electrophoresis in pharmaceutical and biomedical analysis. *Electrophoresis*. 2014;35:926-936.
40. Ali I, Hussain A, Saleem K. Determination of stereo-selective bindings of racemic propranolol with β2-AD-GPCR in human plasma. *J Liq Chromatogr & Rel Technol*. 2013;36:792-806.
41. Aboul-Enein HY, Ali I, Laguerre M, Felix G. Molecular modeling of enantiomeric resolution of methylphenidate on different polysaccharide based chiral stationary phases. *J Liq Chromatogr & Rel Technol*. 2002;25:2739-2748.
42. Ali I, Hussain A, Aboul-Enein HY, Bazylak G. Supramolecular systems-based HPLC for chiral separation of β-adrenergics and β-adrenolytics in drug discovery schemes. *Curr Drug Discov Technol*. 2007;4:255-274.
43. Ali I, Al-Othman ZA, Hussain A, Saleem K, Aboul-Enein HY. Chiral separation of β-adrenergic blockers in human plasma by SPE-HPLC. *Chromatographia*. 2011;73:251-256.
44. Ali I, Hussain I, Saleem K, Aboul-Enein HY. Enantiomeric resolution of ibuprofen and flurbiprofen in human plasma by SPE-chiral HPLC methods. *Comb Chem High Throughput Screen*. 2012;15:509-514.
45. Ali I, Haque A, Hussain A, Sanagi MM, Hussain I, Aboul-Enein HY. Supramolecular chiro-biomedical aspect of β-blockers in drug development. *Curr Drug Targets*. 2014;15:729-741.
46. Al-Othman ZA, Al-Warthan A, Ali I. Advances in enantiomeric resolution on chiral monolithic phases in liquid chromatography and electrochromatography. *J Sep Sci*. 2014;37:1033-1057.
47. Bhushan R, Ali I. Resolution of enantiomeric mixtures of phenylthiohydantoin amino acids on (+)-tartaric acid-impregnated silica gel plates. *J Chromatogr A*. 1987;392:460-463.
48. Basheer A. *Chemical Chiral Pollution: Impact on the Society and Science and Need of the Regulations in the 21st Century*. Chirality: In Press; 2017.

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