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NOVEL CHIRAL SANGER'S REAGENTS AND CHROMATOGRAPHIC ENANTIOSEPARATION OF A SERIES OF RACEMIC β-BLOCKERS AND DFT OPTIMISATION

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ABSTRACT

An effective and sensitive enantioseparation method was developed to identify the enantiomeric purity of racemic β-blockers. Followed by the substitution, alkylation and acylation, three novel chiral Sanger's reagents were synthesised and characterised with spectroscopic instruments, such as NMR, FT-IR, UV, HRMS and elemental analyser. The diastereomerisation of the β-blockers, a racemic beta receptor blocker drug, was carried out using the active Sanger's reagents. RP-HPLC was then used to perform chromatographic separation on the synthesised diastereomeric pairs. Analysis was done using the eluting phase, which comprises a Methyl nitrile-based buffer solution. In the presence of multiple variables like pH, concentration, and eluting phase ratio, the separation analysis findings were optimised. Additionally, through generating minimised energy structures of the prepared diastereomeric pairings using Gaussian software, the separation procedure, elution sequence, and stable conformer of diastereomeric derivatives were investigated. Validation parameters were determined, and the validation was carried out in accordance with ICH guidelines.

Keywords: Sanger's Reagents, Chiral Separation, β-blockers, Diastereomers, DFT.

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INTRODUCTION

Enantiomers behave differently when introduced to a biological system; thus, a pair of enantiomers in a racemic drug has different pharmacodynamics and pharmacokinetics.[1] Therefore, before approving a new medicine for commercial use, regulatory bodies require comprehensive data on the pharmacodynamics and pharmacokinetics of chiral/racemic drugs, and due to this, the methods for the enantioseparation of racemic compounds are considered very important, especially for pharmaceutical industries.[1,2] Amongst all the β-blockers are the most commonly used racemic drugs and primarily prescribed to lower blood pressure, prevent rapid heart rates, and manage angina pectoris, arrhythmias, and heart failure.[1,3] They work by blocking the action of neurotransmitters epinephrine and norepinephrine at beta-adrenergic receptors, mainly in the heart and blood vessels, resulting in a slowed heart rate and relaxed blood vessels. β-blockers are chiral compounds, commonly possess a stereogenic centre and thus exist as a racemic mixture.[4-6] The therapeutic effect, such as the cardiovascular benefits, resides predominantly in the (*S*)-

enantiomer, which is significantly more potent than its counterpart. For instance, (*S*)-propranolol is much more active than (*R*)-propranolol, and similar chirality-related potency differences are seen in other β-blockers like metoprolol and timolol.[6,8] Despite their chiral nature, these drugs are typically marketed as racemic mixtures containing both enantiomers, and thus, enenatioseparation methods are required to obtained single enantiomer or for trace analysis. Enantioseparation can be achieved using direct and indirect enantioseparation methods. The enantioseparation methods followed by the derivatisation of racemic compounds with chiral reagents are considered better for enantiomer separation due to being easy to handle, robust, inexpensive, fast, reproducible and sensitive.[3, 4] The chiral reagents should be designed in such a way that they must contain an enantiomerically pure chiral centre, an aromatic chromophoric group for highly sensitive UV-Visible detection, and a reactive functional group for derivatisation reactions. Till now, a lots of chiral reagents have been developed for example, succinic and benzotriazole esters of (*S*)-levofloxacine, (*S*)-ketoprofen and (*S*)-neproxene; chiral triazole esters, chiral quinoline esters, etc,[5-9] and have been used in the separation of verity of racemic pharmaceuticals for example, β-blockers, Amino acids, mexiletine, etc.[10-13] Even though a wide variety of commercial chiral reagents are available, the development of new and more efficient reagents is still required to monitor the chiral purities in research laboratories and pharmaceutical industries. In this report, three new chiral Sanger's reagents were prepared by introducing the chiral group (D-Phenylalanine) to the 1-fluoro-2,4-dinitrobenzene and then derivatised to the chiral active acyl reagents (SR_1, SR_2 and SR_3; containing different alkyl substituents). Three newly prepared Sanger's reagents (SRs) were employed to synthesise the diastereomeric derivatives (1a to 8f) of a series of the racemic β blockers, under microwave heating conditions. Following derivatisation, these SRs transform into high molar absorption molecules (*RS*)-β-blockers, enabling extremely sensitive detection using a UV-visible. The methanol or Methylnitrile organic modifier was used with TEAP buffer as a mobile phase for the RP-HPLC separation of the synthesised diastereomeric pairs on the C_{18} column. The role of the molecular geometry of the diastereomeric derivatives of (*RS*)- β -blockers in the chromatographic separation mechanism was established. Additionally, a DFT-based, Gaussian 09 Rev-A.02, was used to generate the energy-minimised optimised structures for the diastereomeric derivatives of (*RS*)-β-blockers and to determine the configuration and elution order. Other than this, Accuracy, linearity, limit of detection (LOD), and limit of quantitation (LOQ) were all evaluated for the current approach.

EXPERIMENTAL

Material and Methods

Racemic β -blockers, 1-Fluoro-2,4-dinitrobenzene, D-Phenylalanine, Iodomethane, Iodoethane, 2-Iodopropane, oxalyl chloride, 4-DMAP, Na_2CO_3, K_2HPO_4 and NMR solvents were ordered from Sigma-Aldrich. Hyma Chemicals (India) and TIC (Japan) supplied this investigation's solvent and other reagents.

Instruments and Equipment

Analysis was conducted using a HPLC system (Shimadzu) with a 20µL manual injector, a C_{18} achiral steel column, and a PDA detector. LC solution software was used to process the generated results. In addition, materials were characterised using a 500 MHz NMR spectrometer (Jeol 500 MHz), a pH meter, a FT-IR instrument (Agilent), an HR-MS (Bruker HD), a UV-visible spectrometer (Shimadzu), and an elemental analyser.

Synthesis of SR_{SA}

1-fluoro-2,4-dinitrobenzene (930 mg, 5 mmol) was dissolved in 40 mL THF, and a solution of D-phenylalanine (825 mg, 5 mmol) and 1 mL TEA in THF was added dropwise. The reaction mixture was allowed to stir for 1 hour at room temperature, and the reaction progress was monitored using TLC.[12] After removing the solvent at lower pressure, ice-cold water was added. The yellow precipitate was vacuum-dried after being cleaned with ice-cold water (yield 92%; Fig.-1; Table-1).

Synthesis of SR_{TA}, SR_1, SR_2 and SR_3

Monohydrated caesium hydroxide (330 mg, 2 mmol, 1 equiv) was dissolved in anhydrous DMF (10 mL) and allowed to stir for one hour at RT, and then the solution of SR_{SA} (660 mg, 2 mmol, 1 equiv) in DMF was poured into the earlier reaction.

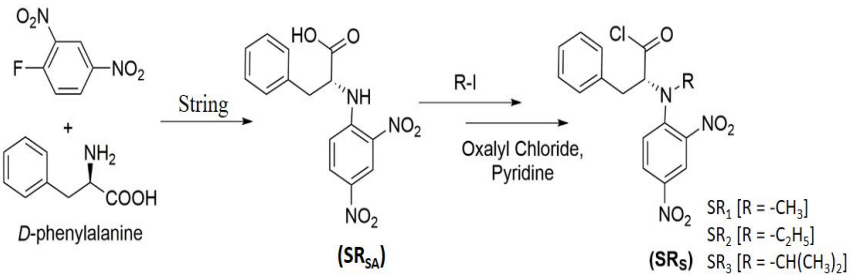

Fig.-1: Synthesis of the Chiral Sanger's Reagents (SR_1, SR_2 and SR_3)

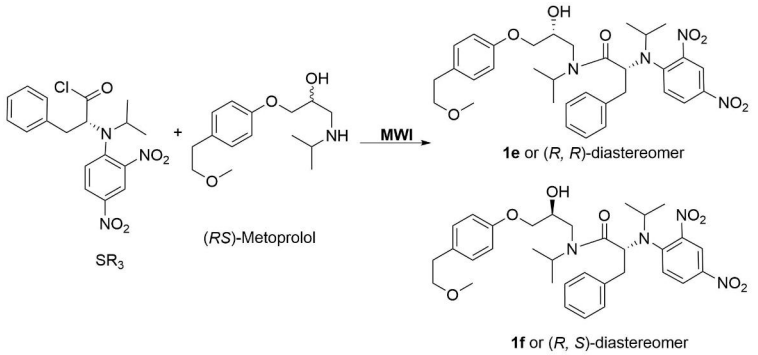

Fig.-2: Synthesis of the Diastereomeric Derivatives of (*RS*)-metoprolol (1e and 1f) with SR_3

Under the stirring, DMF solution of iodomethane (0.23 mL, 2.2mmol, 1.2equiv) was added to the reaction and let it proceed for the next twenty hours at RT.[19] The reaction solution was filtered, and DMF was removed over crushed ice, and then extracted using 1 N NaOH and ethylacteate. The extracted organic layer was then dried under reduced pressure and yielded (87%) a yellow solid **(SR_{TA})**. The obtained compound (690 mg, 2 mmol) was dissolved in 30 mL dry DCM and dropwise, 8 mL oxalyl chloride was added to the solution, and a catalytic amount of 4-DMAP was added. The reaction was stirred for two hours at RT.[8,9] The excess of oxalyl chloride and DCM was removed under reduced pressure and yielded (98%; pale-yellow solid) SR_1. Similar methodology was used to prepare the SR_2 and SR_3, but iodoethane and 2-iodopropane were used instead of iodomethane, respectively.

Synthesis of Diasteromeric Derivatives of (*RS*)-β-blockers

Under microwave heating conditions, the diastereomeric derivatives of the (*RS*)- β -blockers were produced.[10, 11] The reaction mixture containing 55 µL (50 nmol) (*RS*)-metoprolol, 55 µL (50 nmol) SR_3 and 10 µL triethylamine was microwave heated for 45 seconds to prepare diastereomeric derivatives (Figure 2). An aliquot of 12 µL was extracted from the reaction solution and diluted with Methylnitrile 20 times. RP-HPLC analysis was then performed on the filtered diluted solution of diastereomeric derivatives. Similarly, SR_1, SR_2 and SR_3 were used to synthesise other diastereomeric derivatives of (*RS*)-β-blockers. Synthesis conditions were optimised in the presence of elements such as pH, excess of the reagent, reaction duration, and MWI-heating timings.

RP-HPLC and Optimisation of the Conditions

The synthesised diastereomeric derivatives of (*RS*)-β-blockers were subjected to gradient mode RP-HPLC analysis, which employed Methylnitrile and triethylaminephosphate buffer as an eluting phase in the following ratios: 45-55%, 40-60%, 30-70%, and 20-80% (in a linear gradient). Before using the RP-HPLC system, the solvents and eluting phase underwent pretreatment, which involved filtering and degassing them on a sonicator. The diastereomeric derivatives were detected at a wavelength of 296 nm, and the eluting phase flow rate was maintained at 1 mL/min. Similarly, the HPLC conditions were optimised using methanol as an organic modifier.

Table-1: Characterisation Data of the Synthesised Sanger's Reagents

S/N	Compounds	Characterisation Data
1.	SR_{SA}	**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 9.05 (s, Ar, 1H), 8.34-8.56 (d, Ar, 1H), 7.85-7.86 (d, -NH, Ar, 1H), 7.24-7.36 (m, Ar, 6H), 4.43 (q, 1H), 2.94 and 3.21 (dm, 2H). Anal. calcd. (found) % for $C_{15}H_{13}N_3O_6$: C, 54.68 (54.38); H, 3.78 (3.96); N, 12.11 (12.68). HRMS: 332.09 ($M+H^+$).
2.	**SR**	**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.82 (s, Ar, 1H), 8.28-8.46 (d, Ar, 1H), 7.20-7.33 (m, Ar, 6H), 4.52 (q, 1H), 3.24 (m, 1H), 3.12 (s, 3H, $-CH_3$) and 2.95 (m, 1H). Anal. calcd. (found) % for $C_{16}H_{14}ClN_3O_5$: C, 52.11 (52.83); H, 3.79 (3.88); N, 11.89 (11.55). HRMS: 365.06 ($M+H^+$).
3.	SR_2	**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.83 (s, Ar, 1H), 8.30-8.48 (d, Ar, 1H), 7.21-7.35 (m, Ar, 6H), 4.38 (q, 1H), 3.50-3.68 (m, 2H), 3.22 (m, 1H), 2.95 (m, 1H) and 1.28 (t, 3H). Anal.calcd. (found) % for $C_{17}H_{16}ClN_3O_5$: C, 54.27 (54.05); H, 4.09 (4.27); N, 10.89 (11.12). HRMS: 378.09 ($M+H^+$).
4.	SR_3	**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.79 (s, Ar, 1H), 8.27-8.28 (d, Ar, 1H), 7.32 (d, 1H), 7.20-7.31 (m, Ar, 5H), 4.72 (t, 1H), 4.42 (m, 1H), 3.19 (3, 1H), 2.93 (m, 1H), 1.29 (d, 3H) and 1.19 (d, 3H). Anal. calcd. (found) % for $C_{18}H_{18}ClN_3O_5$: C, 54.77 (55.18); H, 4.26 (4.63); N, 10.98 (10.72). HRMS: 392.11 ($M+H^+$).

Method's Validation

As a representative, the validation was carried out using diastereomeric derivatives of (*RS*)-metoprolol synthesised with SR_3 in accordance with ICH specifications.[20] MS Excel software was used to compute the parameters for the validation (graphical plot, coefficient of correlation, and slope calibration) using the least squares approach. A 20–2000 ng/mL concentration range was employed for the validation.

RESULTS AND DISCUSSION

Synthesis of Chiral Sanger's Reagents and Diastereomers

1-fluoro-2,4-dinitrobenzene-based reagents are commonly known as Sanger's reagents. The activated acyl chloride-based three new chiral Sanger's reagents (SR_1, SR_2 and SR_3) were prepared in this work. The D-phenylalanine was introduced during the synthesis of Sanger's reagent as a chiral moiety, which makes the SR_S chiral. The fluorine atom of the 1-fluoro-2,4-dinitrobenzene acts as an excellent leaving group due to the high electron-withdrawing characteristics of the aromatic nitrobenzene;[12] thus, it was easily substituted by the amino group of the D-phenylalanine and formed a secondary amine (SR_{SA}; Fig.-1). The nucleophilic nature of the secondary amine was suppressed by modifying the secondary amine group to the tertiary amine groups (SR_{TA1}, SR_{TA2} and SR_{TA3}) in the presence of the iodo-alkyl (Iodomethane, Iodoethane and 2-Iodopropane) and with the caesium hydroxide monohydrate (CeOH).[19] Ce bind with the secondary amino group and reduces its activity; thus, single alkylated amines (tertiary amine) are obtained in a high yield and prevent the formation of quaternary salts.[19] Under S_N2 substitution, chlorinating agents (pyridine and oxalyl chloride) turn the carboxylic group of SR_{TA} (1, 2, and 3) into acyl chloride.[9,10] The chlorinating reagent, the catalytic amount of pyridine with an excess of oxalyl chloride, is regarded as a good reagent,[8, 9] and produces over 100% of the required product. Acid chloride is an extremely powerful nucleophilic assault agent. In the presence of appropriate nucleophiles, it facilitates the facile synthesis of an ester or amide bond during the substitution process and produces a considerable amount of products.[9]

Diastereomerisation

The synthesized chiral Sanger's reagents (SRs, chiral acyl chlorides) are unstable and very reactive when exposed to a selective nucleophile; thus, SRs produce desired amides when treated with the amino group of the (*RS*)-β-blockers with a high yield, in a substitution reaction.[2 8,13] Although the reaction is fast to make synthesis more accurate and greener, the microwave heating was applied for the synthesis of diastereomeric derivatives of (*RS*)-β-blockers, and 45 Seconds found to be sufficient to complete the reaction with high yield. During the synthesis of diastereomeric pairs (*RS*)-β-blockers, no kinetic resolution and racemisation was observed because the reaction wasn't taking place on chiral centres. RP-HPLC examination further validated the diastereomeric pairs' purity (Fig.-3); the chromatogram's two

equal-intensity peaks indirectly prove that racemisation or kinetic-resolution did not happen during the microwave synthesis.

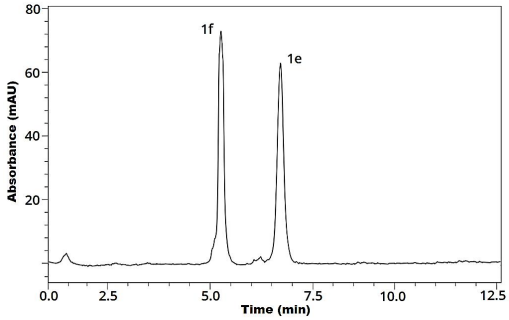

Fig.-3: RP-HPLC Chromatogram of Enantioseparation of Diastereomers of (*RS*)-metoprolol (1e and 1f)

Table-2: Chromatographic Separation Data of Diastereomeric Derivatives of (*RS*)-β-Blockers Prepared with SRs (* Represent the First Eluted Diastereomeric Derivative)

S/N	β-blockers	Sanger's Reagent	Abbreviations		Separation data for diastereomers of β-blockers			
			(*R*,*R*)	(*S*,*R*)	k_1	k_2	α	*Rs*
1.	Metoprolol	SR_1	1a	1b*	2.83	4.07	1.43	5.84
		SR_2	1c	1d*	3.05	4.19	1.37	6.45
		SR_3	1e	1f*	3.48	4.63	1.34	5.39
2.	Propranolol	SR_1	2a	2b*	3.35	4.80	1.43	7.11
		SR_2	2c*	2d	3.62	5.11	1.34	6.81
		SR_3	2e*	2f	3.26	4.23	1.29	7.56
3.	Atenolol	SR_1	3a	3b*	3.74	5.37	1.44	4.21
		SR_2	3c	3d*	3.65	5.18	1.42	5.17
		SR_3	3e	3f*	4.01	5.60	1.39	5.62
4.	Carvedilol	SR_1	4a	4b*	3.54	5.04	1.42	7.18
		SR_2	4c	4d*	3.740	5.24	1.41	7.87
		SR_3	4e*	4f	3.74	5.13	1.37	6.89
5.	Salbultamol	SR_1	5a	5b*	3.56	4.93	1.38	7.41
		SR_2	5c*	5d	3.68	5.09	1.34	7.23
		SR_3	5e	5f*	3.43	4.81	1.40	8.56
6.	Esmolol	SR_1	6a	6b*	2.42	4.04	1.66	6.94
		SR_2	6c	6d*	2.59	3.94	1.52	7.32
		SR_3	6e	6f*	2.87	4.25	1.48	7.68
7.	Sotalar	SR_1	7a	7b*	2.48	4.12	1.66	8.18
		SR_2	7c*	7d	2.65	4.27	1.61	8.43
		SR_3	7e	7f*	2.74	4.47	1.63	7.97
8.	Isoprenaline	SR_1	8a	8b*	2.72	3.68	1.35	6.29
		SR_2	8c	8d*	2.80	3.96	1.41	7.05
		SR_3	8e	8f*	3.03	4.20	1.38	7.41

RP-HPLC Analysis

The obtained diastereomeric pairs (1a to 8f) from the reactions were diluted, filtered, and subjected to RP-HPLC analysis in order to be separated. As an example, Figure-3 displays the chromatogram of the pair of diastereomers (1e and 1f) of (*RS*)-metoprolol synthesised with SR_3. All diastereomeric pairings were successfully separated on the C_{18}-column (non-polar and achiral). The chromatographic data values were determined using the separation result and are shown in Table-2. In contrast to diastereomeric derivatives containing (*R*)-β-blockers, the separation data indicate that the diastereomers containing (*S*)-β-blockers often elute first (Table-2 lists the elution order of diastereomers). It was discovered that the eluting phase, which included Methylnitrile (80%) and triethylaminephosphate buffer (20%) in a linear-gradient mode, was adequate to separate all synthesised diastereomeric pairs. The solution's pH of 3.5 was kept acidic to promote effective separation. To maximise the separation efficiency of the developed method, several eluting phase variables were adjusted, including the concentration of the organic phase, the pH of the

eluting solvent, the rate of flow, and the buffer concentration. Another organic phase, methanol, was also explored for separation. Methylnitrile's greater polarity, high volume, and low UV-Visible cut-off made it a superior organic phase for the eluting solvent. Also, in the presence of Methylnitrile, the peaks that appear in chromatograms were sharper.[3,5,6] The eluting phase at 3.5 pH gives better separation than the lower or higher acidic eluting phase. A 1 mL/min flow rate of the eluting phase for HPLC was found suitable for separation, which provides low system pressure and produces high resolution.

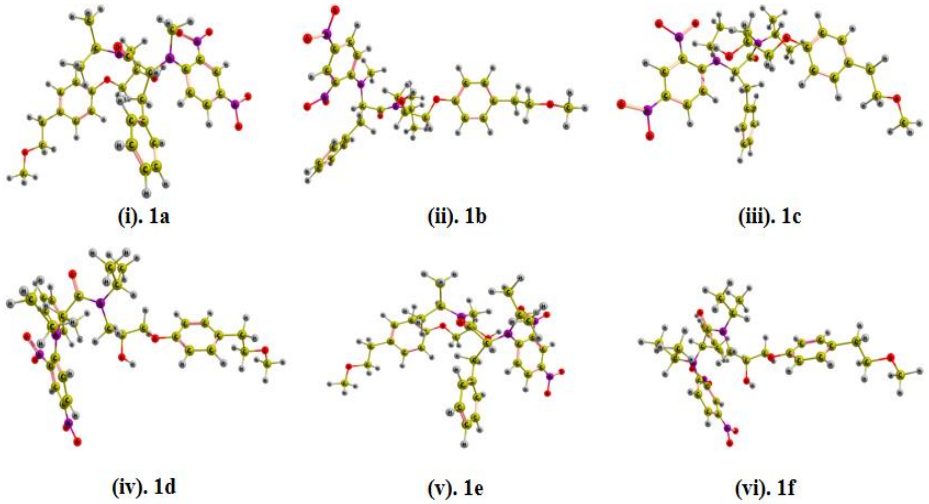

Fig.-4: Optimised 3D Structures of Diastereomeric Derivatives of (*RS*)-metoprolol Prepared by SR_1, SR_2, and SR_3

Elution Sequencing and DFT Optimisation

Gaussian software was used to perform DFT calculations to verify the outcomes of the separation procedure and elution sequence. A non-polar C_{18}-column was utilised to investigate the chromatographic interaction and the molecular geometry of the diastereomeric derivatives of (*RS*)-metoprolol (**1a** to **1f**) prepared with chiral Sanger's reagents using their 3D optimised structures. Figure-4 displays a typical 3D optimised structure of the diastereomeric pair **1e** and **1f** as a representative. Compared to the **1e** molecule (another diastereomer), the spatial arrangement of the **1f** diastereomeric molecule makes it appear larger (18.98 A°). As a result, the **1f** molecule exhibits higher hydrophilic characteristics and has a larger surface area to be exposed to the eluting solvent. The **1f** diastereomeric derivative elutes first because of a stronger polar-polar interaction with the eluting phase. The **1e** diastereomeric derivative (17.47 A°) exhibits greater hydrophobic characteristics and has less surface area to expose to the eluting phase. Because of this, it interacts more with non-polar column material and elutes last.[4,13] In addition, the substituent groups that were attached to the D-phenylalanine molecule (dinitrobenzene and different alkyl groups) demonstrated a significant impact on the elution time. The dinitrobenzene substituent, an aromatic ring with highly polar groups, strengthens the polarised engagement between the eluting phase and the diastereomeric derivatives. Therefore, in diastereomeric pairings, the dinitrobenzene substituent shortens the elution duration. In contrast, a diastereomer's hydrophobic aliphatic substituent enhances its hydrophobic interactions with non-polar column material. As a result, diastereomeric pairings with aliphatic substituents have a slightly longer elution duration and eventually elute. Each produced diastereomeric derivative (1a to 8f) was subjected to a similar hypothesis, and the diastereomeric pairs' elution order was determined. In every instance, the larger diastereomer in a diastereomeric pair exhibits greater polarity, dissolves more in the eluting phase, and elutes faster. Table-2 provides the order of elution for the produced diastereomeric compounds.

Validation

The accuracy, robustness, linearity, and precision of the current approach were confirmed in accordance with ICH criteria.[20-24] The proposed approach, as stated in the literature, was validated using the diastereomeric pair (1e and 1f) of (*RS*)-metoprolol produced using SR_3 as a representative.[3-8] Relative standard deviation (RSD), linearity, accuracy, precision, detection limit (LOD), and quantification limit

(LOQ) were determined using the sample concentration, which was between 20 and 2000 ng/mL. The RP-HPLC system's peak area was used to analyse and quantify the method's recoveries and stabilities. For both intra-day and inter-day assays, the diastereomeric pair's obtained recovery was greater than 98% (98.87 and 99.11% for the intraday test and 99.55 and 98.80% for the interday assay). The current method's lower LOD and LOQ values (0.206 ng/mL and 0.618 ng/mL, respectively) demonstrated an impressive sensitivity for the identification of diastereomeric pairings. In comparison to the previously published work on enantioseparation, the results [separation factor (1.29-1.66), resolution (4.21-8.44), elution time (4.41-8.32 min), and retention factor (2.42-5.60)] obtained by the devised approach for the separation of diastereomeric derivatives of (*RS*)-β-blockers were found to be superior. Additionally, compared to other research on the direct chiral separation of (*RS*)-β-blockers, the findings are superior to those of the current report.

CONCLUSION

Racemic β-blockers have been diastereomerized using the established approach, which outlines an effective synthesis of three novel chiral reagents based on Sanger's acyl chloride. β-blocker's amino groups react rapidly with the synthesised chiral Sanger's reagents (SR_S), forming an amide bond that is crucial for the resolution of synthetic pairs of the diastereomeric derivatives. Microwave conditions allowed for the rapid and effective synthesis of the diastereomeric pairs. An effective polar eluting phase comprising methylnitrile and triethylamine-phosphate buffer allowed a well-resolved separation of the produced diastereomeric pairs on RP-HPLC with a short duration. In diastereomers, the devised method's detection sensitivity was shown to be extremely high (LOD=0.206 ng/mL and LOQ=0.618 ng/mL) when dinitrobenzene was present as a fluorophore. Using energy-minimised 3D structures, elution duration, separation, and molecule interaction were described. Because it is easy to synthesise diastereomeric derivatives and has high sensitivity, short elution time, and high resolution, this approach is suitable for enantioseparation of the racemic β-blockers. This technique can be utilised to detect enantiomeric impurities in trace analysis of racemic substances with an amine functional group in their structure.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. V. Alwera, S. Sehlangia and S. Alwera, *Separation Science and Technology,* **56(13)**, 2278(2021), https://doi.org/10.1080/01496395.2020.1819826
2. S. Alwera, V. Alwera and S. Sehlangia, *Biomedical Chromatography*, **34**, e4943(1-9)(2020), https://doi.org/10.1002/bmc.4943
3. S. Alwera and R. Bhushan, *Biomedical Chromatography***,** **30(8)**, 1223(2016), https://doi.org/10.1002/bmc.3671
4. V. Alwera, S. Sehlangia, and S. Alwera, *Journal of Liquid Chromatography and Related Technologies,* **43(17)**, 742(2020), https://doi.org/10.1080/10826076.2020.1798250
5. S. R. Bireddy, T. R. Bantu, V. K. Vashistha, S. Kumar and S. Yadav, *Research Journal of Chemistry and Environment*, **26(2)**, 1(2022), https://doi.org/10.25303/2602rjce0108
6. S. Alwera and R. Bhushan, *Biomedical Chromatography*, **30**, 1772(2016), https://doi.org/10.1002/bmc.3752
7. V. K. Vashistha, A. Kumar, D. K. Das, S. Sethi, R. Pullabhotla and H. Nagar, *Journal of planner chromatography-Modern TLC*, **34**, 211(2021), https://doi.org/10.1007/s00764-021-00109-5
8. Raffiunnisa, N. Jaishetty, P. Ganesh, M. S. Patel, V. S. Talismanov and S. Yadav, *Asian Journal of Chemistry*, **35(8)**, 1855(2023), https://doi.org/10.14233/ajchem.2023.28037
9. M. Solanki, V. S. Talismanov, A. Damayanthi, M. S. Patel, S. Shrivastava and S. Kumar, *Asian Journal of Chemistry*, **36(2)**, 404(2024), https://doi.org/10.14233/ajchem.2024.30926
10. N. K . Sharma, L. Kaur, S. Kumar, V. S. Talismanov, A. K. Tripathi and A. Mondal, *Asian Journal of Chemistry*, **36(6)**, 1334(2024), https://doi.org/10.14233/ajchem.2024.31487
11. S. Alwera, V. Alwera and S. Sehlangia, *Biomedical Chromatography*, **34(11)**, e4943(2020), https://doi.org/10.1002/bmc.4943
12. R. Bhushan and V. K. Vashistha, *Journal of Chromatography A*, **1379**, 43(2015), http://dx.doi.org/10.1016/j.chroma.2014.12.033
13. S. Alwera, *ACS Sustainable Chemistry and Engineering*, **6**, 11653(2018), https://doi.org/10.1021/acssuschemeng.8b01869
14. P. P. Majalekar and P. J. Shirote, *Current Drug Targets*, **21(13)**, 1354(2020), https://doi.org/10.2174/1389450121666200621193355
15. R. H. Drew and H. A. Gallis, *Pharmacotherapy*, **8(1)**, 35(1988), https://doi.org/10.1002/j.1875-9114.1988.tb04063.x
16. P. Wierzbiński, J. Hubska, M. Henzler, B. Kucharski, R. Bieś and M. Krzystanek, *Pharmaceuticals,* **16(8)**, 1105 (2023), https://doi.org/10.3390/ph16081105
17. J. Bertino Jr. and D. Fish, *Clinical Therapeutics*, **22(7)**, 798(2000), https://doi.org/10.1016/S0149-2918(00)80053-3
18. D. N. Fish, *Pharmacotherapy*, **21(10)**, 253S(2001), https://doi.org/10.1592/phco.21.16.253S.33993
19. R. N. Salvatore, A. S. Nagle and K. W. Jung, *The Journal of Organic Chemistry*, **67,** 674 (2002), https://doi.org/10.1021/jo010643c
20. ICH. Q2B Document: Validation of Analytical Procedures, *International Conference of Harmonisation: Geneva*, **1996.**
21. N. Jaishetty, V. S. Talismanov, M. S. Patel, S. Sehlangia and S. Kumar**,** *Letters in Applied NanoBioScience*, **12(4)**, 139(2023), https://doi.org/10.33263/LIANBS124.139
22. M. Sujatha, K. M. Devi, A. K. Tripathi, A. Damayanthi, Alimuddin, G. S. Sundari and R. Mukherjee, *Rasayan Journal of Chemistry*, **18(3)**, 1451(2025), http://doi.org/10.31788/RJC.2025.1839309
23. R. Mukherjee, L. Kaur, Raffiunnisa, V. N. Agme, A. Tewari and A. K. Tripathi, *Rasayan Journal of Chemistry*, **18(1)**, 373-379(2025), http://doi.org/10.31788/RJC.2025.1819128
24. S. Alwera, V. Alwera and D. Domyati, *Biointerface Research in Applied Chemistry*, **13(2)**, 1(2022), https://doi.org/10.33263/BRIAC132.179

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