Ravi Bhushan et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-031

HPLC enantioseparation of (RS)-isoprenaline and enhanced detection in human plasma and commercial sample: Establishment of configuration and elution order in the absence of pure isomer

Ravi Bhushan[1] and **V K Vashistha**[2]
[1]Indian Institute of Technology Roorkee, India
[2]GLA University, India

There has been an increasing awareness that the two enantiomers of a chiral drug have different pharmaceutical responses in the human body and other living systems. Therefore, the importance of enantioseparation remains as established and challenging. (RS)-isoprenaline (Ipn) is a non-selective β-adrenergic agonist used in the treatment of bradycardia and heart block. The (R)-Ipn is approximately 90 times more potent than its (S)-enantiomer, therefore, determination of enantiomeric purity of this compound is essential for diagnostic purposes and therapeutic drug monitoring. With the above background and lack of literature on the work focused herein, we developed a validated analytical method for separation of enantiomers of (RS)-isoprenaline and pharmaceutical formulations. Ipn contains a reactive amino functional group (only one in close proximity to the stereogenic centers) suitable for quantitative transformation with a functionally compatible chiral reagent. Cyanuric chloride (CC, trichloro-s-triazine) was chosen for its tri-functionality and high molar absorptivity and D-phenyl glycine (D-Phg) was introduced as chiral auxiliary by substitution of one of the Cl atoms in CC. The second Cl atom was replaced with piperidinyl moiety as the achiral unit resulting into a structurally new CDR because such a CDR with combination of these two moieties has not yet been reported. The CDR was characterized. Diastereomeric derivatives of (RS)-Ipn were synthesized under microwave irradiation when the third Cl atom of CC was substituted. The separation of these diastereomeric derivatives was achieved using LiChrospher C18 (IxI.D. 25 cm×4.6 mm, 5 um particle size) column with mobile phase consisting of MeCN and TFA under gradient elution at flow rate of 1.0 mL min^{-1} and UV detection at 254 nm. The conditions for synthesis and separation were optimized by extensive experimentation with many variations at different stages. The method was successfully applied for detection and separation of enantiomers of (RS)-Ipn in human plasma and from the commercial sample. Limit of detection values were found to be 24.6 and 26.8 ng mL-1 for the two diastereomeric derivatives. Geometry optimized lowest energy structures of diastereomeric derivatives of (RS)-Ipn were developed using a standard Gaussian software program which helped in establishing the configuration and elution order of the diastereomeric derivatives. The method can be used not only for control of enantiomeric purity of Ipn in industrial/commercial samples but also for enantioseparation and determination of other structurally similar pharmaceutically important molecules for routine analysis as well.

Biography

Ravi Bhushan has an expertise in "Enantiomeric resolution of compounds of pharmaceutical importance using liquid chromatography". So far, he has guided 28 doctoral and 50 masters' theses, published more than 230 research papers in international refereed journals and chapters in books and encyclopedia. He edited four Special Issues of Biomedical Chromatography on chiral resolutions as Guest Editor. He received Alexander von Humboldt fellowship of Germany in 1988, and European Economic Community Fellowship in 1992. He is an elected Fellow of the Royal Society of Chemistry, London, and Fellow of National Academy of Sciences India, (FNASc). He received 'Outstanding Teacher Award' of the year 2007 at IIT Roorkee, and Khosla Research Prize and Silver Medal of University of Roorkee. His current research interest includes enantioseparation with both achiral phases of chromatography in the absence of any external chiral species.

rbushfcy54@gmail.com