UGC MAJOR RESEARCH PROJECT

2015-2018

FINAL REPORT

CHIRAL HPLC METHODS FOR THE ANALYSIS OF SELECTED DRUGS IN PHARMACEUTICAL FORMULATION AND BIOLOGICAL MATRICES

By

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PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1.	Name and address of the Principal Investigator	Dr. P.Venkatesan Associate Professor , Department. of Pharmacy, Annamalai University, Annamalai Nagar-608002 (Tamilnadu)
2.	Name and address of the Institution	Department. of Pharmacy, Annamalai University, Annamalai Nagar-608002 (Tamilnadu)
3.	UGC Reference No. & Date	F.42-511/2014(SR) dated 29.12.2015
4.	Date of Implementation	01.07.2015
5.	Tenure of the project	03 years from 01.07.2015 to 30.06.2018
6.	Total Grant Allocated	**Rs. 8,37,000.00**
7.	Grants Received	**Rs. 6,21,600.00**
8.	Final Expenditure	**Rs. 6,21,600.00**
9	Title of the Project	**Chiral HPLC methods for analysis of selected drugs in pharmaceutical formulations and biological matrices**
10.	**Objectives of the Project:** The main objective of the present work is to develop a novel chiral HPLC method and to improve the already reported method for the determination of chiral analytes in formulations and biological matrices. In present work, chemometric tools such as central composite design and derringer's desirability function will be employed.	
11.	**Whether objectives were achieved: Yes**	

12.	**Achievements from the project:** Development and optimization of new as well as improved chiral HPLC methods by employing chemometric protocol are completed. Application of the developed chiral HPLC method in the determination of chiral analytes in formulations and biological matrices completed and published two different combinations with different chemometric protocols. 1. Enantiopurity Assessment of Chiral Switch of Ondansetron by Direct Chiral HPLC 2. D-Optimal mixture design optimization of an HPLC method for simultaneous determination of commonly used antihistaminic parent molecules and their active metabolites in human serum and urine. the developed method reduces overall 30 different methods into one single method. In addition, the organic buffer system be useful for LC–MS studies.
13.	**Summary of the findings:** Approximately 80% of new medicines currently in development are chiral and can exist as mirror-image twins. As medicines, single enantiomers often exhibit greater potency and cause fewer side effects than do more conventional drug molecules, which may be chiral but are often racemic mixtures. of late regulatory agencies, viz. US FDA and European Committee for Proprietary Medicinal Products, demand for enantiospecific pharmacokinetic, pharmacodynamic and toxicological data for racemic chiral pharmaceuticals to be presented for assessing their safety and efficacy. Moreover, *in vivo* a racemic therapeutic is no longer 50:50 due to enantioselective metabolism. Hence tracking individual enantiomers of a chiral drug becomes mandatory and the control of chiral purity is of immense value. As a consequence pharmaceutical companies and research institutes look for chiral analytical methods to quantify component enantiomers of chiral drugs. It is in this context this project if of immense relevance. Enantiopurity Assessment of Chiral Switch of Ondansetron by Direct Chiral HPLC: The present study attempts to develop a direct chiral HPLC method for the enantiomeric separation of OND using Chiral Pak under reversed-phase (RP) mode. The RP mode enantioseparation provides a better solubility for polar analytes, uses nontoxic solvents, and successful HPLC and LC/MS analysis. The chiral selector in Chiral Pak is amylose tris[(S)-α methylbenzylcarbomate] coated on 3 μm silica gel. The separation of enantiomers in this CSP may be attributed to hydrogen bonding interactions, dipole–dipole interactions, and pi–pi interactions. The presence of aromatic functionalities could also provide an additional stabilizing effect on the solute-CSP complex by inclusion of the aromatic group into chiral cavity. This type of mechanism may operate in OND enantiomer separation A simple and rapid direct chiral HPLC method was developed, optimized and validated for the simultaneous estimation of the OND enantiomers in pharmaceutical formulations. The mixture design experiments method provides essential information regarding the effect of solvent variables and their interaction

	effects on enantioselectivity of OND. The proposed method was validated and found to be linear, sensitive, selective, precise and accurate. The present method offers advantages of being fast (4 min) and efficient nonbuffered reversed phase enantiomeric separation of OND. Adequate retention, better resolution and shorter analysis time of the proposed method demonstrate that it can be applied for chiral impurity profiling of OND chiral switch. D-Optimal mixture design optimization of an HPLC method for simultaneous determination of commonly used antihistaminic parent molecules and their active metabolites in human serum and urine: In the present study, a simple, specific and rapid HPLC method was developed and optimized for the simultaneous determination of four antihistaminic drugs – hydroxyzine, terfenadine, loratadine and rupatadine – and their active metabolites, cetirizine, fexofenadine and desloratadine, respectively, in serum and urine samples. The D-optimal mixture design methodology in this study aided in establishing the ideal mobile phase composition for successful separation of eight analytes with relatively short run time (9.5 min) in the specified biological matrix. In addition, the novel ion-exchange solid-phase extraction procedure employed gave excellent recovery for both the basic and zwitter ionic analytes from serum and urine. The proposed bioanalytical method was validated according to US Food and Drug Administration (2001) guidelines and found to be linear, sensitive, selective, precise and accurate. In conclusion, the proposed isocratic RP-HPLC method could be considered as a suitable chromatographic system for the determination of selected antihistaminic parent compounds and their pharmacologically active main metabolites in serum and urine. Advantages of the proposed method This proposed chromatographic system is a new approach to simultaneous determination of basic hydrophobic HYD and zwitterionic CTZ metabolite in a specified biological matrix. The analysis of hydrophobic basic analytes, viz. LRT and DES, is usually challenging because of the tendency toward peak tailing and poor resolution of these analytes. The separation of these studied analytes was achieved in the same chromatographic system. The proposed method could also be applied to a pharmacokinetic study of RUP and its major metabolite DES in serum and urine. In the present study, an HPLC method was developed intended for routine metabolic and pharmacokinetic studies in a biological matrix where the analysis time needs to be optimized without losing resolution. Furthermore, it was essential to customize the retention factor of the first eluted peak (k1), thus avoiding matrix effects. The D-optimal mixture design methodology in this study aided in establishing the optimum mobile phase composition for successful quality separation of analytes in the specified biological matrix. The proposed method requires only 250 μL of serum for the entire analysis, which could be advantageous when multiple blood sampling is required for studies. The adaptability of the present method for determining four different combinations of active metabolites and their respective parent drugs in biological matrices may be helpful for pharmacologists. The proposed method reduces overall 30 different methods into one single method.

	In addition, the organic buffer system be useful for LC–MS studies
14	**Contribution to the society:** • The developed chiral HPLC methods which are very sensitive and cost-effective could be used for the monitoring of chiral drugs in patients. • As a consequence pharmaceutical companies and research institutes look for chiral analytical methods to quantify component enantiomers of chiral drugs. • One of the developed method reduces overall 30 different methods into one single method. In addition, the organic buffer system be useful for LC–MS studies. • It is in this context this project if of immense relevance. Further these methods can be employed for assay of chiral drugs and routine quality control purposes.
15	**Whether any Ph.D. Enrolled/ Produced out of the Project**: **Nil**
16	**No. of Publications out of the project: 02 (Copy enclosed)** 1. Enantiopurity Assessment of Chiral Switch of Ondansetron by Direct Chiral HPLC, **Chromatographia** volume 80, pages 229–236 (2017). 2. D-Optimal mixture design optimization of an HPLC method for simultaneous determination of commonly used antihistaminic parent molecules and their active metabolites in human serum and urine, **Biomed Chromatography**. Aug;31(8), 2017.

PRINCIPAL INVESTIGATOR

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