IJPSR (2012), Vol. 3, Issue 09 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 May, 2012; received in revised form 23 July, 2012; accepted 29 August, 2012

A VALIDATED LC METHOD FOR THE DETERMINATION OF CHIRAL PURITY OF (R)-3-((1-METHYLPYRROLIDIN-2-YL) METHYL)-5-(METHYLSULFONYLMETHYL)-1H-INDOLE: A KEY RAW MATERIAL OF ELITRIPTAN HYDROBROMIDE

M. Sarat, P. Murali Krishna and C. Rambabu*

Acharya Nagarjuna University, Nagarjuna Nagar-522510, Guntur dist, Andhra Pradesh, India

Keywords:
Elitriptan hydrobromide,
Chiral,
HPLC,
Method validation

Correspondence to Author:

C. Rambabu

Acharya Nagarjuna University, Nagarjuna Nagar-522510, Guntur dist, Andhra Pradesh, India

E-mail: msarat_msc@yahoo.com

ABSTRACT

A simple and accurate normal phase liquid chromatographic method was developed for the determination of chiral purity of (R)-3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonylmethyl)-1H-indole, R-isomer used as key starting raw material in the manufacturing of Elitriptan hydrobromide bulk drug. Chromatographic separation between (R)-3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonylmethyl)-1H-indole and its opposite isomer (S)-3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonylmethyl)-1H-indole, S -isomer was achieved using a Chiralpak IA column using a mobile phase containing n-hexane, ethanol, isopropyl alcohol and trifluoro acetic acid (98:1.5:0.5:0.1 v/v/v/v). The resolution between the two isomers was found to be more than 2.0. The limit of detection (LOD) and limit of quantification (LOQ) of the S - isomer was 0.15 and 0.5 µg mL-1, respectively, for 10 µL injection volume. The percentage recoveries of the S-isomer ranged from 96.5 to 105.3 in the samples of (R)-3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonylmethyl)-1H-indole. The test solution and mobile phase was observed to be stable up to 24 h after the preparation. The developed method was validated as per International Conference on Harmonization guidelines in terms of LOD, LOQ, precision, linearity, accuracy, robustness and ruggedness.

INTRODUCTION: Most of the pharmaceutical industries are now concentrating towards the study of the therapeutic effects of isomers of the existing drug molecules. A control and accurate quantification of undesired isomers in pharmaceuticals is essential [1] in this connection and LC is generally opted for this purpose.

Eletriptan, 3-{[(R)-1-methyl-2-pyrrolidinyl] methyl}-5-[2-(phenylsulfonyl)ethyl]indole hydrobromide, is a new orally active 5-HT1B/1D agonist, recently approved by the Food and Drug Administration for the acute treatment of migraine headache [2-5]. The related impurity in bulk drug samples is (R)-5-ethyl-3-(1-methyl-2-pyrrolidinylmethyl)-1H-indole, Control of pharmaceutical impurities is currently a critical issue in the pharmaceutical industry. The International Conference on Harmonization (ICH) has formulated a workable guideline regarding the control of impurities [6].

QUICK RESPONSE CODE

IJPSR:
ICV- 4.57

Website:
www.ijpsr.com

Organic impurities associated with the active pharmaceutical are the unwanted chemicals which are developed during drug synthesis or formulation. The presence of these unwanted chemicals, even in small amounts, may influence the efficacy and safety of the pharmaceutical products. Impurity profiling (identification and quantification) is now receiving increased attention from regulatory authorities.

A number of recent articles [7, 8] described a designed approach and guidance for the isolation and identification of process-related impurities and degradation products. In general, according to ICH guidelines on impurities in new drug products [9], identification of impurities below the 0.1 % level is not considered to be necessary unless the potential impurities are expected to be unusually potent or toxic.

Literature survey revealed that very few analytical methods have been reported for the determination of eletriptan in pure drug, pharmaceutical dosage forms and in biological samples using HPLC[10, 11] and LC-MS [12] techniques.

The aim of the present work is to develop and validate a simple, fast, reliable and appropriate chromato-graphic method with UV detection for the determination of eletriptan in bulk drug and in pharmaceutical formulations. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) guidelines for the determination of eletriptan in bulk sample and in tablet dosage forms.

EXPERIMENTAL:

Chemicals and Reagents: Samples of *S* and *R*-isomers of 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methyl sulfonylmethyl)-1H-indole confirmed by spectral characterization and SOR (specific optical rotation) were obtained from Process Research Department of Emmanthi Laboratories Ltd, Hyderabad, India. HPLC-grade n-hexane was procured from Sigma Aldrich, India. Ethanol and IPA were purchased from Ranbaxy Fine Chemicals, New Delhi, India. Analytical Reagent grade tri-fluoroacetic acid (TFA) was purchased from Fluka.

Instrumentation: Chromatographic system consisted of a Waters Model Alliance 2695 separation module equipped with auto sampler Photodiode array ultraviolet (UV) detector. The data recorded using empower software. The chiral columns used in method development were Chiralcel OD, Chiralpak AD, ChiralcelOJ, Chiralpak IA, Chiralpak IB and Chiralpak IC. All are Daicel make with 5 µm particle size in (250 x 4.6) mm dimension. The other columns Crownpak (Daicel Chemical Industries, Japan), Chiral AGP (Advanced Separation Technologies Inc., New Jersey) and Chirobiotic (Chiron Technologies, Inc., New Jersey) are 5 µm particle sizes in (150 x 4.6) mm dimension.

Preparation of Standard Solutions: The stock solutions of *S* and *R*-isomers of 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonylmethyl)-1H-indole was prepared individually by dissolving an appropriate amount of the substances in diluent of mobile phase. Working solutions were prepared i n mobile phase. The target analyte concentration was fixed as 2.0 mg mL^{-1}.

RESULTS AND DISCUSSION:

Method Development: The objective of this work was to evaluate the isomeric purity of the *R*-isomer of 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonyl methyl)-1H-indole and accurate quantification of the undesired *S*-isomer. The preliminary trails carried out in reverse phase chiral columns were not fruitful in the separation of these isomers. Different chiral stationary phases were employed during the method development namely Chiralpak IA, Chiralpak IB, Chiralpak IC, Chiralpak AD-H, Chiralcel ODH, Crownpak CR (+), Chiral AGP and Chirobiotic T. Different trials were made during the method development and details are mentioned in the **Table 1**.

Chiralpak IA, an amylose based chiral stationary phase was found to be selective for the isomers of 3-((1-methylpyrrolidin-2-yl)methyl)-5-(methylsulfonyl methyl)-1H-indole. Very good resolution was achieved on Chiralpak IA column using mobile phase contains the mixture of n-hexane, ethanol, isopropyl alcohol and TFA (98:1.5:0.5:0.1 v/v/v/v). The addition of IPA and TFA to the mobile phase plays an important role on enhancing the chromatographic efficiency and resolution between the isomers.

TABLE 1: RESULTS OF VARIOUS TRIALS AND SYSTEM SUITABILITY TEST

S. No	LC Condition	Retention Factor	Remarks
1	Column: chiralpak-IA 250 x 4.6 mm, 5 µm, Mobile phase: n-hexane, ethanol, IPA, TFA (98:1.5:0.5:0.1, v/v/v/v), Flow rate: 1.0 mL min^{-1}	R- 7.5 S – 8.1	Resolution between the pair of isomers >2
2	Column: chiralpak-IB 250 x 4.6 mm, 5 µm, Mobile phase: n-hexane, IPA, TFA (98:2:0.1, v/v/v), Flow rate: 1.0 mL min^{-1}	R- 6.3 S – 6.3	R and S are co
3	Column: chiralpak-IC 250 x 4.6 mm, 5 µm, Mobile phase: n-hexane, IPA, TFA (98:2:0.1, v/v/v),Flow rate: 1.0 mL min^{-1}	R- 5.6 S – 5.6	R and S are co
4	Column: chiralpak AD-H 250 x 4.6 mm, 5 µm, Mobile phase: n-hexane, IPA and TEA (80:20:0.1, v/v/v), Flow rate: 1.0 mL min^{-1}	R- 6.0 S – 6.0	R and S are co
5	Column: chiralcel OD-H 250 x 4.6 mm, 5 µm, v/v/v), Flow rate, Mobile phase: n-hexane, IPA and TEA (80:20:0.1, : 1.0 mL min^{-1}	R- 4.8 S- 4.8	R and S are co
6	Column: crownpak CR (+) 150 x 4.6 mm, 5µm, Mobile phase: 0.1 M HClO4, Flow rate: 1.0 mL min^{-1}	R- 2.5 S- 2.9	R and S are co
7	Column: chiral AGP 150 x 4.6 mm, 5 µm, Mobile phase: 0.01 M KH2PO4 :Methanol (50:50, v/v), pH:4.0, Flow rate: 1 mL min^{-1}, Column temperature: 25 °C	R- 6.2 S- 6.2	R and S are co
8	Column: chirobiotic T 150 x 4.6 mm, 5 µm, Mobile phase: 0.02 M KH2PO4 : Methanol (90:10, S- 5.5 v/v), pH : 4.0, Flow rate: 1 mL min^{-1}, Column temperature: 25 °C	R- 5.5 S- 5.5	R and S are co

Optimized Chromatographic Conditions: Chromatographic base to base separation was achieved only on a Chiralpak IA (250 x 4.6 mm, 5 microns particle size) chiral column using the mobile phase, which contains the mixture of nhexane, ethanol, isopropyl alcohol and TFA (98:1.5:0.5:0.1 v/v/v/v). The flow rate of the mobile phase was 1.0 mL min^{-1}. The column temperature was maintained at 25°C and the detection wavelength was 215 nm. The injection volume was 10 µL. The total analysis time for each run was 20 min. Very good separation was observed within short runtime on Chiralpak IA column (resolution >2.0). The typical retention times of *S* and *R*-isomers of 3-((1-methylpyrrolidin-2-yl)methyl)-5-(methylsulfonyl methyl)-1H-indole are 7.7 and 8.4. The USP tailing factor (T) was found to be 1.2 for both *S* and *R*-isomers of 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methyl sulfonylmethyl)-1H-indole. The system suitability results were given in **Table 2**.

TABLE 2: SYSTEM SUITABILITY TEST RESULTS

Name	Retention time	Resolution	USP Tailing factor
R – Isomer	7.7	---	1.05
S – Isomer	8.4	2.0	1.15

The structure and configurations of *R* and *S*-isomers of 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonyl methyl)-1H-indole are displayed in **Fig. 1**.

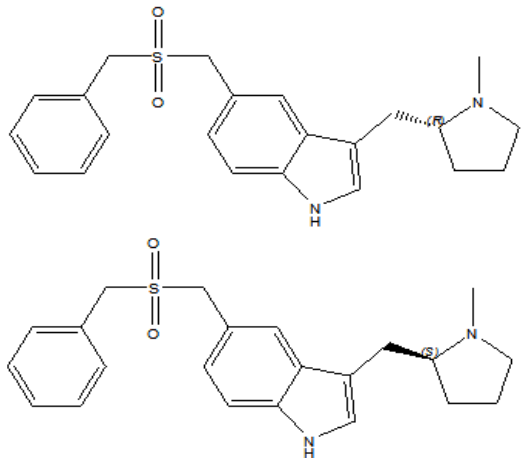

FIG. 1: THE STRUCTURE AND CONFIGURATIONS OF *R* AND *S*-ISOMERS OF 3-((1-METHYLPYRROLIDIN-2-YL) METHYL)-5-(METHYLSULFONYLMETHYL)-1H-INDOLE

The chromatogram of the *S*-isomer spiked with *R*-isomer is displayed in **Fig. 2**.

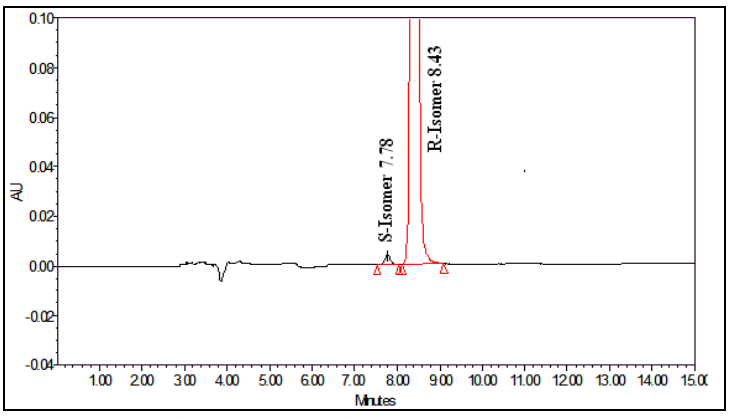

FIG 2. THE CHROMATOGRAM OF THE *S*-ISOMER SPIKED WITH *R*-ISOMER

Method Validation:

Precision: The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions. The system and method precision for the *S*-isomer were checked at its specification level (i.e. 0.5% with respect to analyte concentration, 2.0 mg mL-1). The percentage RSD of method repeatability and system repeatability for the *S*-isomer were found to be 6.3 and 4.1, respectively, which confirms good precision of the method.

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. The linearity of the method for the *S*-isomer was checked at six concentration levels, i.e. from limit of quantitation (LOQ) (0.05%) to 200% of the undesired *S*-isomer specification level (0.5%), which is with respect to of (R) -3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonyl methyl)-1H-indole. The coefficient of regression of the calibration curve was found to be 0.999, thus confirming the excellent correlation between the peak area and concentration of the *S*-isomer.

Limit of Detection and Limit of Quantitation: The limit of detection (LOD) represents the concentration of analyte that would yield a signal tonoise ratio of 3. The limit of detection for the *S*-isomer was found to be 0.15 µg mL^{-1} for 10 µL of injection volume. The LOQ represents the concentration of analyte that would yield a signal-to-noise ratio of 10. The limit of quantitation for the *S*-isomer was found to be 0.5 µg mL^{-1} for the 10 µL of injection volume.

Ruggedness and Robustness: The ruggedness of a method was defined as degree of reproducibility of results obtained by analysis of the same sample under a variety of normal test conditions such as different laboratories, different analysts, different instruments and different days. The standard addition and recovery experiments carried out for the *S*-isomer in (*R*)- 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonyl methyl)-1H-indole bulk samples at the same concentration levels tested in Laboratory A were again carried out at laboratory B using a different instrument and analyst. The data obtained from Laboratory B was well in agreement with the results obtained in Laboratory A, thus proving the method ruggedness. The robustness of an analytical procedure is measured by its capability to remain unaffected through small, but deliberate, variations in method parameters and provide an indication of its reliability during normal usage. In the varied chromatographic conditions like flow rate, mobile phase ratio and column temperature, the resolution between the peaks of *S* and *R*-isomers of 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonyl methyl)-1H-indole was found to be >2.0 illustrating the robustness of the method.

Quantitation of (R)-Isomer: Standard addition and recovery experiments were conducted to determine the accuracy of the present method, for the quantification of the (*S)*-isomer in samples of (*R*)- 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonylmethyl)-1H-indole. The study was carried out at 0.25, 0.50 and 0.75% of target analyte concentration of (*R*) - 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonylmethyl)-1H-indole. The percentage recoveries of the *S* - isomer ranged from 96.5 to 105.3 in samples of (*R*) - 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonylmethyl)-1H-indole.

Solution Stability and Mobile Phase Stability: Solution stability was studied by keeping the test solution in tightly capped volumetric flasks at room temperature on a laboratory bench for 24 h. The content of (*S)*-isomer was checked for every 6 h interval and compared with freshly prepared solution. No variation was observed in the content of the (*S)*-isomer for the study period and it indicates (*R*)- 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonyl methyl)-1H-indole sample solutions prepared in diluent were stable up to 24 h.

Mobile phase stability was carried out by evaluating the content of (*S)*-isomer in (*R*)- 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonylmethyl)-1H-indole sample solutions, which were prepared freshly at every 6 h interval for 24 h. The same mobile phase was used during the study period. No variation was observed in the content of (*S)*- isomer for the study period and it indicated the prepared mobile phase was stable up to 24 h.

CONCLUSION: A simple and accurate normal phase chiral LC method was developed for the quantitative determination of the (*S)*-isomer in (*R*) - 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonyl methyl)-1H-indole, a key starting material of Elitriptan hydrobromide. Chiralpak IA, an amylose based chiral stationary phase was found to be selective for the isomers of 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonylmethyl)-1H-indole. The method was completely validated showing satisfactory data for all the method validation parameters tested.

The developed method can be used for the quantitative determination of the undesired (*S)* -isomer in 3-((1-methylpyrrolidin-2-yl) methyl)-5-(methylsulfonyl methyl)-1H-indole samples.

ACKNOWLEDGMENTS: The authors wish to thank the management of Emmathi laboratories limited, Hyderabad for supporting this research work. Authors wish to acknowledge the Process Research group for providing the samples for our research.

REFERENCES:

1. Milton K.A., Scott N.R., Allen M.J., Abel S., Jenkins V.C., James G.C., Rance D.J., Eve M.D., J Clin Pharmacol, 42,2002;528.
2. The Merck Index 13th Edition, Merck Research Laboratories Division of Merck Co. Ind.,Whitehouse Station, NJ, 2001.
3. K. L. Hoskin, G. A. Lambert, C. Donaldson, A. S. Zagami, Brain Research 998, 2004; 91.
4. P. L. Durham, P. X. Dong, K. T. Belasco, J. Kasperski, W. W. Gierasch, L. Edvinsson, D. D. Heistad, F. M. Faraci, A. F. Russo, Brain Research 997, 2004; 103.
5. http://www.medicinenet.com/eletriptan/article.htm.
6. ICH Q3A(R) Draft Revised Guidance on Impurities in New Drug Substances, Federal Register 65 (140), 2000; 45085.
7. S. Gorog, Identification and Determination of Impurities in Drugs, Elsevier, Amsterdam, 2000.
8. K. M. Alsante, T. D. Hatajik, L. L. Lohr, T. R. Sharp, American Pharmaceutical Review 4, 2001; 70.
9. ICH Q3B(R) Draft Revised Guidance on Impurities in New Drug Products, Federal REgister 65 (139) (2000;44791.
10. Zecevic M., Jocic B., Agatovonic-Kustrin S., Zivanovic L., J. Serb. Chem. Soc., 71, 2006; 1195.
11. Cooper J.D.H., Muirhead D.C., Taylor J.E., J. Pharm. Biomed. Anal., 21, 1999; 787.
12. Jocic B., Zecevic M., Zivanovic L., Protic A., Jadranin M., Vajs V., J. Pharm. Biomed. Anal., 50, 2009; 622.

How to cite this article:
Sarat M., Murali P. Krishna and Rambabu C. A validated lc method for the determination of chiral purity of (r)-3-((1-methylpyrrolidin-2-yl)methyl)-5-(methylsulfonylmethyl)-1h-indole: a key raw material of elitriptan hydrobromide. *Int J Pharm Sci Res*, 2012; Vol. 3(9): 3151-3155.