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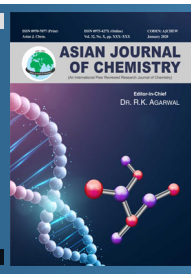

Reverse Phase Chiral HPLC Method for Enantiomeric Excess Determination of 2-Aminobutanamide

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A reverse phase chiral HPLC method was developed for the determination of (*R*)-2-aminobutanamide isomer content in (*S*)-2-aminobutanamide key starting material for levetiracetam drug substance by using a CROWNPAK CR (+) column. Perchloric acid solution (0.05 %) was used as mobile phase and the flow rate was finalized as 0.3 mL/min. UV detection wavelength was 200 nm and column temperature was set as 15 ºC. The limit of detection and limit of quantification were 0.0002 mg/mL and 0.0005 mg/mL, respectively. The linearity calibration curve of (*R*)-2-aminobutanamide was shown good from the range of 0.0005 mg/mL to 0.004 mg/mL. The recovery of (*R*)-2-aminobutanamide isomer was between the range of 93 to 106 % in presence of (*S*)-2-aminobutanamide. The method was validated and found to be precise, accurate and robust. The method can be used for determination of (*R*)-2-aminobutanamide in presence of (*S*)-2-aminobutanamide, which is the key intermediate for preparation of levetiracetam. This method was validated in as per ICH Q2 (R1) and USP validation of compendial methods (1225).

Keywords: 2-Aminobutanamide, Levetiracetam, Enantiomer, Chiral HPLC.

INTRODUCTION

(*S*)-2-Aminobutanamide is the key starting material for the preparation levetiracetam drug substance. Hence, the quantification of (*R*)-2-aminobutanamide is essential and used to treat epilepsy or convulsive seizures. It belongs to an anticonvulsant group [1-3]. Levetiracetam is absorbed completely after oral taking and commonly removed as uncharged drug from the urine [4-7], and may lead to the side effects such as sudden change in the behaviour, irregular movements, sleepiness and hallucinations.

To synthesize one chiral enantiomer from other related enantiomers, the ingredients are also required with enriched in either of enantiomer pair. In some cases only, one enantiomer is essential and having positive effect while the another enantiomer may leads unwanted side effects on human body [8-14]. Most of the drugs available in market are having chiral center and those are marketing as racemic mixtures [15-20]. Even though the enantiomers have similar molecular structure, but those can be differentiated by their biological activities, such as pharmacology, toxicology, pharmakinetics, metabolism, *etc.* [21-23]. It is essential to encourage the chiral separation and its quantification in drug substances, to remove the non-essential enantiomer form the pharmaceutical active ingredient as well as for correct therapeutic control on patients [24-28].

The quantification of (*R*)-2-aminobutanamide in (*S*)-2-aminobutanamide is essential because the presence of (*R*)-2-aminobutanamide will leads to the formation of unwanted isomer of levetiracetam *i.e.* (*R*)-2-(2-oxopyrrolidin-1-yl)butanamide at API stage [29-34].

EXPERIMENTAL

Perchloric acid was procured as Merck grade (Mumbai, India). Samples of (*S*)-2-aminobutanamide were synthesized by process research development of Dr. Reddy's Laboratories, Srikakulam. High purity water was prepared using a Millipore Plus (Millipore, Milford, MA, USA) purification system.

Instrumentation: HPLC system with make Agilent Infinity 1260 series and weights measurements are taken by using analytical balance with make Sartorius and model MSA 225S-100-DA. Data was processed through empower-3 software.

Chromatographic conditions: The analysis was carried out with CROWNPAK CR (+), 150 mm × 4.0 mm, 5µ particle size (Advanced Chromatography Technologies, Scotland) with a mobile phase consisting of 0.05 % perchloric acid solution was used as mobile phase. The mobile phase itself was used as diluent. The column temperature was maintained at 15 ºC. Flow rate was kept at 0.3 mL/min and the column eluent was monitored at 200 nm for 20 min.

Sample preparation: Test samples were prepared with concentration of 2 mg/mL by dissolving in the diluent. System suitability solution was prepared by spiking the solution of (*R*)-2-aminobutanamide isomer at 1 % with respect to the test concentration (*i.e*. 2 mg/mL).

RESULTS AND DISCUSSION

Method development and optimization: To develop the quantitative reverse phase chiral HPLC method for (*R*)-2-aminobutanamide content in (*S*)-2-aminobutanamide, several development trials were taken up by changing the columns, mobile phase and also different temperatures of column. The aim of this HPLC method is to get symmetry in the peaks shape and to separate (*R*)-2-aminobutanamide from (*S*)-2-aminobutanamide with adequate resolution.

The attempts of method development were focused in normal phases solvents such as *n*-hexane, *n*-heptane, 2-propanol, ethyl alcohol, *etc*. Initial method development attempts were made on different stationary phases such as amylose *tris*-(3,5- dimethyl phenyl carbamate), cellulose *tris*-(3,5-dimethyl phenyl) carbamate, cellulose *tris*-(4-methylbenzoate) and crown ether coated on 5 µm silica-gel with different composition of solvent mixtures. In this method development, stationary phase has played a significant role in achieving the resolution between (*R*)-2-aminobutanamide and (*S*)-2-aminobutanamide. Finally, the satisfactory peak shape and resolution were achieved between two isomers on CROWN PAK CR (+) column (150 × 4.0 mm and 5.0 µm particle size) by using perchloric acid solution as mobile phase as well as diluent. These CROWNPAK CR(+) or CROWNPAK CR(-) columns were especially designed to resolve amino acids isomers and also the compounds bearing a primary amino group near the chiral center. In (*S*)-2-aminobutanamide, molecular structure, the primary amine was attached to the chiral carbon hence the separation of isomers was effectively done by using the column of CROWNPAK CR(+). The method development trials are tabulated in Table-1.

Method validation: Results from method validation can be used to judge the quality, reliability and consistency of analytical results, it is an integral part of any good analytical practice. The quality of an analytical method developed is always appraised in terms of suitability for its intended purpose, recovery, requirement for standardization, sensitivity, analyte stability, ease of analysis, skill subset required, time and cost in that order. The described method was meeting all mentioned above parameters such as lesser run time (20 min) and zero usage of solvents which indirectly representing the less cost for the analysis. The described (*R*)-2-aminobutanamide content was validated as per the ICH guidelines.

Limit of detection (LOD) and limit of quantification (LOQ): LOD and LOQ of (*R*)-2-aminobutanamide were established by preparing the solution from the known concentration of stock solution to achieve the signal-to-noise ratio as 3:1 and 10:1. Carried out the LOQ precision by preparing six individual standard solutions with LOQ concentration (R)-2-aminobutanamide. Accuracy at LOQ solutions were prepared by spiking the impurity in (*S*)-2-aminobutanamide test sample at target concentration (*i.e*. 0.0005 mg/mL). The percentage recovery of (*R*)-2-aminobutanamide and recovery results were within the acceptance limit. The results for the precision at LOQ and accuracy at LOQ are given in Table-2 and the chromatograms are shown in Figs. 1 and 2.

TABLE-2
VALIDATION RESULTS

Validation parameter	Criteria	Result
LOD	(*R*)-isomer (mg/mL)	0.0002
LOQ	(*R*)-isomer (mg/mL)	0.0005
Method precision (n = 6)	% RSD for area	1.3
System precision (n = 6)	% RSD for area	1.5
LOQ precision (n = 6)	% RSD for area	3.5
Accuracy at LOQ	% Recovery	94.4
Accuracy	% Recovery at 50 %	102.2
	% Recovery at 100 %	103.0
	% Recovery at 150 %	96.9
Linearity	Correlation coefficient	0.9996
	% Y-intercept	-1.3

Specificity: The specificity of an analytical method is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components. The basic criteria to show the specificity is peak angle should be less

TABLE-1
RESULTS OF METHOD DEVELOPMENT

Trial	Column	Dimensions	Stationary phase	Mobile phase	Conclusion
1	Chiral PAK ADH	250 × 4.6 mm × 5.0 µ	Amylose *tris* (3,5-dimethylphenylcarbamate)	*n*-Hexane and IPA	No separation
2	Chiral PAK ODRH	250 × 4.6 mm × 5.0 µ	Cellulose *tris*(3,5-dimethylphenylcarbamate)	*n*-Hexane, 2-propanol and TFA	No separation
3	Chiral PAK OD	250 × 4.6 mm × 5.0 µ	Cellulose *tris* (3,5-dimethylphenylcarbamate)	*n*-Hexane, 2-propanol and TFA	No separation
4	Chiral PAK IA	250 × 4.6 mm × 5.0 µ	Amylose *tris*-(3,5-dimethylphenylcarbamate)	*n*-Hexane, ethanol and TFA	No separation
5	Chiral PAK IA	250 × 4.6 mm × 5.0 µ	Amylose *tris*-(3,5-dimethylphenylcarbamate)	1.0 % $HClO_4$ in water and acetonitrile	No separation
6	CROWN PAK CR (+)	150 × 4.0 mm × 5.0 µ	Chiral crown ether	0.05 % $HClO_4$ and methanol	Slight separation
7	CROWN PAK CR (+)	150 × 4.0 mm × 5.0 µ	Chiral crown ether	0.05 % $HClO_4$	Adequate separation

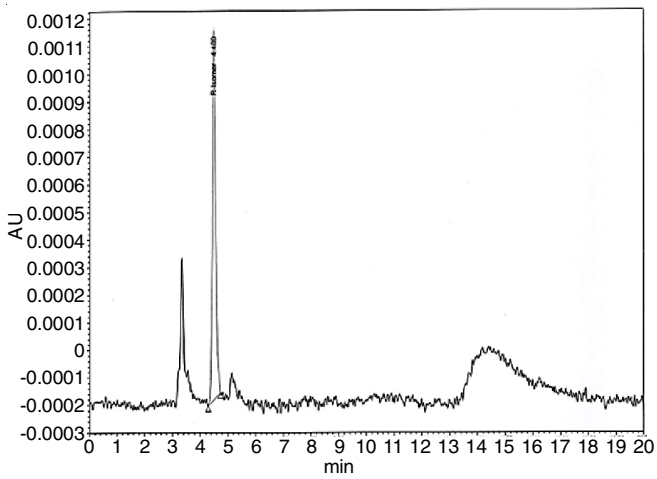

Fig. 1. Typical chromatogram of LOQ solution of (*R*)-2-aminobutanamide

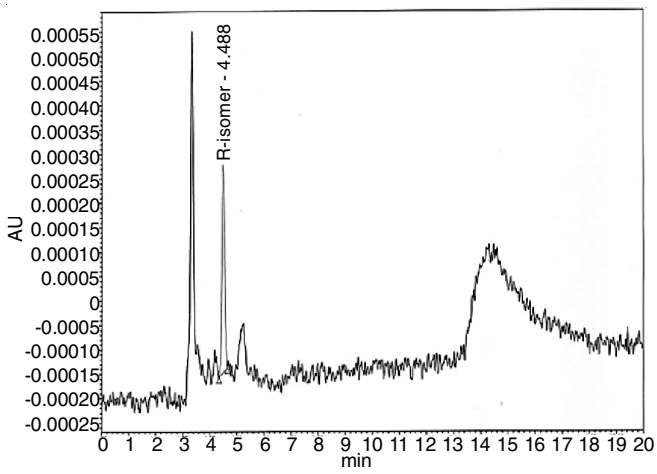

Fig. 2. Typical chromatogram of LOD solution of (*R*)-2-aminobutanamide

than peaks threshold. Peak threshold is a noise level. Always a low level threshold value will enable us to identify the peaks at even low responses. Purity plot for (*R*)-2-aminobutanamide is shown in Fig. 3.

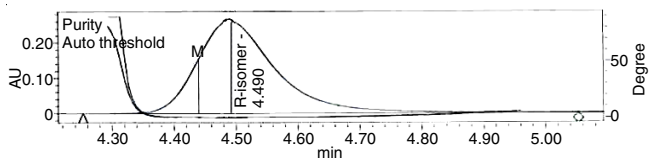

Fig. 3. Purity graph for (*R*)-2-aminobutanamide

Method precision: Precision measures of how close individual measurements are to each other. Method precision was carried out by preparing the six individual solutions with spiking of (*R*)-2-aminobutanamide at 0.002 mg/mL in each preparation. The precision results are given in Table-2 and its typical precision chromatogram is shown in Fig. 4.

Linearity: The linearity of an analytical method is the ability to elicit test results which are directly proportional to the concentration of analyte in samples within a given range. Linearity was carried out by preparing (*R*)-2-aminobutanamide solutions at different concentration levels (*i.e.* LOQ, 50, 75, 100, 150 and 200 %) in the diluent. The peak area *versus* concentration data was plotted for linear regression analysis. The

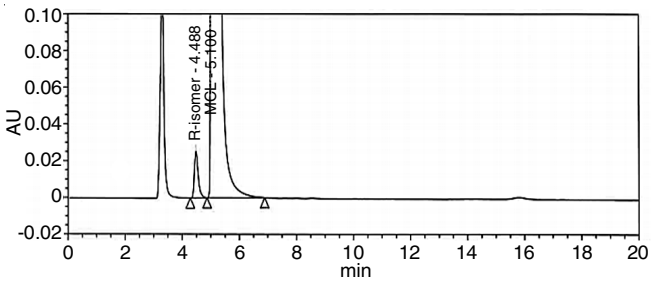

Fig. 4. Typical chromatogram of method precision

correlation coefficients of regression, slope, intercept and percent y-intercept of the calibration curves were computed. The linearity results are given in Table-2.

Accuracy: The most common technique for determining accuracy in several chemical studies is the spike recovery method, in which the amount of a target compound is determined as a percentage of theoretical amount present in the matrix. In a spike recovery experiment, a measured amount of the constituent of interest is added to a matrix (spiked) and then the analysis is performed on the spiked material, from the sample preparation through chromatographic determination. A comparison of the amount found *versus* the amount added provides the recovery of the method, which is an estimate of accuracy of the method. The accuracy of the method was carried out by triplicate preparation at three different concentration levels of (*R*)-2-aminobutynamide (*i.e.* 0.001, 0.002 and 0.003 mg/mL). The accuracy results are shown in Table-2, while its typical chromatogram is shown in Fig. 5.

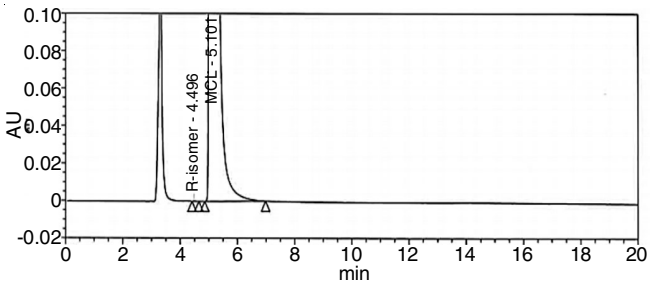

Fig. 5. Typical test sample chromatogram

Ruggedness: To determine the robustness of the method, chromatographic parameters are modified and evaluated the change of resolution between (*S*) and (*R*)-isomers. Experiments are conducted by varying column temperature by ± 5 ºC, wavelength by + 5.0 nm and mobile phase strength ± 10 %. Resolution details are shown in Table-3.

TABLE-3
(*R*)-2-AMINO BUTANAMIDE VALIDATION RESULTS FOR ROBUSTNESS

Parameter	Condition	Resolution
Column temperature-actual	15 °C	1.33
Lower temperature	10 °C	1.37
Higher temperature	20 °C	1.26
Wavelength-actual	200 nm	1.33
Higher wavelength	205 nm	1.30
Mobile phase-actual	0.05 % $HClO_4$ solution	1.33
Lower concentration	0.045 % $HClO_4$ solution	1.35
Higher concentration	0.055 % $HClO_4$ solution	1.31

Conclusion

The developed reverse phase chiral HPLC method for (*R*)-2-aminobutynamide content in (*S*)-2-aminobutynamide hydrochloride is precise, accurate, linear and specific. The validated parameters met the pre-requirements of ICH guidelines, hence it can be used for the quality control analysis of (*S*)-2-aminobutynamide samples and also used for (*R*)-2-aminobutynamide and (S)-2-aminobutynamide isomer content analysis in levetiracetam drug substance and drug product samples.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. B.M. Rao, R. Ravi, B.S. Sundar Reddy, S. Sivakumar, I. Gopi Chand, K.P. Kumar, P.V.R. Acharyulu, G.O. Reddy and M.K. Srinivasu, *J. Pharm. Biomed. Anal.*, **35**, 1017 (2004); https://doi.org/10.1016/j.jpba.2004.03.015.
2. H. Klitgaard, A. Matagne, J. Gobert and E. Wülfert, *Eur. J. Pharmacol.*, **353**, 191 (1998); https://doi.org/10.1016/S0014-2999(98)00410-5.
3. R. Berkecz, A. Sztojkov-Ivanov, I. Ilisz, E. Forró, F. Fülöp, M.H. Hyun and A. Péter, *J. Chromatogr. A*, **1125**, 138 (2006); https://doi.org/10.1016/j.chroma.2006.06.064.
4. R. Heydari, M. Shamsipur, *J. AOAC. Int.*, **98**, 1529 (2015); https://doi.org/10.5740/jaoacint.15-016.
5. M.K. Srinivasu, B.M. Rao, R. Ravi, K.I. Gopi Chand, C.M. Reddy, S.M. Rao, G.O. Reddy, *Indian Drugs*, **41**, 226 (2004).
6. V.A. Davankov, *Pure Appl. Chem.*, **69**, 1469 (1997); https://doi.org/10.1351/pac199769071469.
7. R. Nishioka and S. Harada, *Chromatography*, **37**, 65 (2016); https://doi.org/10.15583/jpchrom.2015.036.
8. S. Einarsson, B. Josefsson, P. Moeller and D. Sanchez, *Anal. Chem.*, **59**, 1191 (1987); https://doi.org/10.1021/ac00135a025.
9. A. Rahman, M.R. Haque, MM. Rahman and M.A. Rashid, *Dhaka Univ. J. Pharm. Sci.*, **16**, 165 (2017); https://doi.org/10.3329/dujps.v16i2.35253.
10. G. Blaschke, H.P. Kraft, K. Fickentscher and F. Koehler, *Arzneim.-Forsch.*, **29**, 1640 (1979).
11. Y. Zhang, W. Watts, L. Nogle and O. McConnell, *J. Chromatogr. A*, **1049**, 75 (2004); https://doi.org/10.1016/S0021-9673(04)01266-X.
12. C. Roussel and P. Piras, *Pure Appl. Chem.*, **65**, 235 (1993); https://doi.org/10.1351/pac199365020235.
13. M.D. Palamareva and L.R. Snyder, *Chromatographia*, **19**, 352 (1984); https://doi.org/10.1007/BF02687768.
14. J. Zhuang, S. Kumar and A. Rustum, *J. Chromatogr. Sci.*, **54**, 1813 (2016); https://doi.org/10.1093/chromsci/bmw162.
15. R. Dappen, H. Arm and V.R. Meyer, *J. Chromatogr. A*, **373**, 1 (1986); https://doi.org/10.1016/S0021-9673(00)80205-8.
16. P. Piras, C. Roussel and J. Pierrot-Sanders, *J. Chromatogr. A*, **906**, 443 (2001); https://doi.org/10.1016/S0021-9673(00)00948-1.
17. C. Perrin, V.A. Vu, N. Matthijs, M. Maftouh, D.L. Massart and Y. Vander Heyden, *J. Chromatogr. A*, **947**, 69 (2002); https://doi.org/10.1016/S0021-9673(01)01573-4.
18. X. Wang, J.T. Lee and T.E. Beesley, *LC-GC*, **18**, 626 (2000).
19. P. Madhavan, B.M. Rao, Pravin, Abhishek, P.R. Kumar, M. Sreenivasulu and K.B. Chandrasekhar, *Chromatographia*, **65**, 81 (2007); https://doi.org/10.1365/s10337-006-0110-9.
20. M. Dousa, P. Lehnert, H. Adamusová and Z. Bosakova, *J. Pharm. Biomed. Anal.*, **74**, 111 (2013); https://doi.org/10.1016/j.jpba.2012.10.017.
21. V. Rane and D. Shinde, *J. Chromatogr. Sci.*, **46**, 772 (2008); https://doi.org/10.1093/chromsci/46.9.772.
22. I.W. Wainer, R.M. Stiffin and T. Shibata, *J. Chromatogr. A*, **411**, 139 (1987); https://doi.org/10.1016/S0021-9673(00)93965-7.
23. A. Van Overbeke, W. Baeyens, W. Van Den Bossche and C. Dewaele, *J. Pharm. Biomed. Anal.*, **12**, 911 (1994); https://doi.org/10.1016/0731-7085(94)E0012-P.
24. J. Caldwell, A.J. Hutt and S. Fournel-Gigleux, *Biochem. Pharmacol.*, **37**, 105 (1988); https://doi.org/10.1016/0006-2952(88)90762-9.
25. A. Ito and Y. Mori, *Res. Commun. Chem. Pathol. Pharmacol.*, **70**, 131 (1990).
26. B. Kasprzyk-Hordern, V.V.R. Kondakal and D.R. Baker, *J. Chromatogr. A*, **1217**, 4575 (2010); https://doi.org/10.1016/j.chroma.2010.04.073..
27. K.R. Chimalakonda, V. Gudala, M. Gutta, S. Polisetty and S.V.S. Koduri, *Am. J. Anal. Chem.*, **3**, 478 (2012); https://doi.org/10.4236/ajac.2012.37063.
28. J. Caldwell, *J. Chromatogr. A*, **719**, 3 (1996); https://doi.org/10.1016/0021-9673(95)00465-3.
29. S. Singh, B. Singh, R. Bahuguna, L. Wadhwa and R. Saxena, *J. Pharm. Biomed. Anal.*, **41**, 1037 (2006); https://doi.org/10.1016/j.jpba.2006.01.030.
30. International conference on Harmonization Guideline on Impurities in New Drug Substance and Products, Q3 B (R2) (2006).
31. E.J. Ariens, *Eur. J. Clin. Pharmacol.*, **26**, 663 (1984); https://doi.org/10.1007/BF00541922.
32. S.C. Stinson, *Chem. Eng. News*, **79**, 45 (2001); https://doi.org/10.1021/cen-v079n020.p045.
33. A. Venkateshwarlu, A.V.R. Rao and K. Mukkanti, *Am. J. Anal. Chem.*, **3**, 470 (2012); https://doi.org/10.4236/ajac.2012.37062.
34. J. Dancey and A. Eisenhauer, *Br. J. Cancer*, **74**, 327 (1996); https://doi.org/10.1038/bjc.1996.362.