Asian Journal of Chemistry Vol. 22, No. 6 (2010), 4945-4946

NOTE

Chiral Separation of Aspartic Acid by Thin-Layer Chromatography

PRASHANT K. KATIYAR* and RAVI KABRA WALA
Kanpur Institute of Technology and Pharmacy, A-1,
UPSIDC Industrial Area, Rooma, Kanpur-208 001, India
E-mail: pkatiyar1981@gmail.com

In this study, chiral separation of D/L-aspartic acid was achieved by thin layer chromatography using L-arginine as a chiral separator using the mobile phase as butanol:glacial acetic acid:water (70:10:20) and the visualizing agent is ninhydrin and butanol (20:10).

Key Words: Chiral separation, Aspartic acid, Arginine, Thin-layer chromatography.

Chiral separation is an important method, because enantiomers can have different activities when exposed to a natural environment[1]. Enantiomer separation can either be carried out *via* direct or indirect methods. An indirect method consists of adding an enantiomeric pure derivatization reagent to the sample, which forms diastereomers with the enantiomers present in the sample[2].

Current methods of enantiomeric analysis include such non-chromatographic techniques as polarimetry, nuclear magnetic resonance, isotopic dilution, calorimetry and enzyme techniques. The disadvantages of these techniques are the need for pure samples and no separation of enantiomers are involved. Quantitation, which does not require pure samples and separation of enantiomers can be done simultaneously by either gas chromatography or high performance liquid chromatography[3-5].

Amino acid analysis is traditionally performed using HPLC[6], GC/GC-MS[7,8] or more recently capillary electrophoresis-electrospray mass spectrometry[9] and electrospray mass spectrometry[10], but these analytical methods do not provide the low instrument mass and analysis volume characteristics desired for current *in situ* space exploration. In this paper, TLC as an analytical tool applied for the separation of D/L-aspartic acid. For this, L-arginine as a chiral separator is selected using the mobile phase as butanol:glacial acetic acid:water (70:10:20) and the visualizing agent is ninhydrin and butanol (20:10).

Preparation of chromatographic plate: The TLC plates were prepared by spreading method. The thin layer (2.5 μm) of stationary phase (silica gel-G) on the plate was prepared by spreading slurry (silica gel-G:water; 1:2) with the help of spreader. After preparing the plates, allow it to air dried for some time. For activation put it in an oven at 100-105 ºC for 1 h.

Preparation of Arginine impregnated TLC plates: TLC was made on a glass plates with silica gel-G slurry containing 10 % of L-arginine as a chiral selector. After preparing the plates allow it to air dried for some time. For activation put it in an oven at 100-105 ºC for 1 h.

Preparation of mobile phase: Butanol:glacial acetic acid:water, 70:10:20 (v/v) maintaining the pH 4 ± 0.2 by using 10 % sodium hydroxide solution.

Saturation of mobile phase: Line the walls of the chromatographic tank with filter paper. Pour into the chromatographic tank. A sufficient quantity of the mobile phase for the size of the chromatographic tank, layer of appropriate depth related to the dimension of the plate to be used. For saturation of the chromatographic tank, replace the lid and allow to stand at 20 to 25 °C for 1 h. Unless otherwise indicated, the chromatographic separation is performed in a saturated tank.

Preparation of sample solution: For TLC analysis the solution of aspartic acid was prepared in 17 % ammonia solution (prepared by diluting ammonium hydroxide, 6 mL in 10 mL). Dissolve 100 mg of aspartic acid in 2 mL of 17 % of ammonium hydroxide solution and make the volume up to 10 mL with water and mix. From that volume 5 µL sample is applied to the TLC plate.

Development of chromatograms: Development of TLC plate of DL-aspartic acid was performed at room temperature. The plates were spotted with 5 µL solution D/L-aspartic acid. The spotting was done at 2 cm from the bottom edge of the plates. After spotting the plates were put in the development chamber, containing mobile phase butanol:glacial acetic acid:water, 70:10:20 (v/v) having the pH 4 ± 0.2. Then plates were allowed to run up to 75 % of the plate height. After development of the chromatograms remove the plates from the development chamber and allow it to air dried.

Polorimetry: For polorimetry the solution of aspartic acid was prepared by dissolving 80 mg in 1 mL of 6 N HCl. The specific rotation and R_f values are given in the Table-1.

TABLE-1
R_f VALUE AND SPECIFIC ROTATION OF ASPARTIC ACID

Aspartic acid	R_f value				Optical rotation $[\alpha]_D$
	(1)	(2)	(3)	Average	
D/L-Aspartic acid*	0.63	0.65	0.63	0.63	+0.15 – +1.64
D- Aspartic acid	0.23	0.24	0.23	0.23	-16.80 – -17.40
L-Aspartic acid	0.56	0.56	0.56	0.56	+25.30 – +26.20

*Run on TLC plate not impregnated with L-arginine.

REFERENCES

1. F. Qin, C. Xie, Z. Yu, L. Kong, M. Ye and H. Zou, *J. Sep. Sci.*, **29**, 1332 (2006).
2. M. Lämmerhofer, F. Svec, J.M.J. Fréchet and W. Lindner, *Trends Anal. Chem.*, **20**, 676 (2000).
3. V.A. Davankov, *Chromatographia*, **27**, 475 (1989).
4. R. Cirilli, R. Ferretti, B. Gallinella, L. Zanitti and F. La Torre, *J. Chromatogr. A*, **1061**, 27 (2004).
5. L. He and T.E. Beesley, *J. Liq. Chromatogr. Rel. Tech.*, **28**, 1075 (2005).
6. F.R. Antoine, C.I. Wei, R.C. Littell and M.R. Marshall, *J. Agric. Food Chem.*, **47**, 5100 (1999).
7. E. Gil-Av, B. Feibush and R. Charles-Sigler, *Tetrahedron Lett.*, **10**, 1009 (1966).
8. H. Frank, G.J. Nicholson and E. Bayer, *J. Chromatogr.*, **146**, 197 (1978).
9. T. Soga and D.N. Heiger, *Anal. Chem.*, **72**, 1236 (2000).
10. L.W. Beegle, I. Kanik, L. Matz and H.H. Hill Jr., *Anal. Chem.*, **73**, 3028 (2001).

(*Received*: 26 May 2009; *Accepted*: 2 March 2010) AJC-8496