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Note

Determination of L-glutamine in preparations using high-performance liquid chromatography

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L-Glutamine is an important medicine which has been used for the treatment of peptic ulcers, especially in Japan[1]. The determination of L-glutamine has been carried out by means of, *e.g.*, ion-exchange chromatography[2–9], gas chromatography[10–13], fluorimetric methods[14,15], methods for measuring the amount of ammonia liberated by the actions of enzymes[16–21] or acids[22,23] on L-glutamine, and various other methods[24–27]. In this paper, the determination of L-glutamine in preparations using high-performance liquid chromatography is described.

EXPERIMENTAL

Reagents

0.02 *M* Potassium dihydrogen phosphate was prepared by weighing 2.72 g of potassium dihydrogen phosphate in sufficient water to make 1000 ml. 0.2 *M* Sodium hydroxide was prepared by weighing 0.8 g of sodium hydroxide in sufficient water to make 100 ml. Phosphate buffer solution was adjusted to pH 5.0 by mixing 0.02 *M* potassium dihydrogen phosphate and 0.2 *M* sodium hydroxide, filtered through a membrane filter (Toyo, TM-2, 0.45 μm; Toyo Roshi, Tokyo, Japan) and used as a mobile phase after degassing. L-Glutamine (Ajinomoto, Tokyo, Japan) was dried at 80° for 3 h, *ca.* 50 mg accurately weighed and dissolved in sufficient water to make exactly 50 ml, and used as a standard solution. An amount of sample, equivalent to 30 mg of L-glutamine, was weighed accurately in a centrifuge tube with a stopper, 30 ml of water was added, it was shaken for 10 min, filtered through Toyo No. 5 filter paper 10 ml of first filtrate was removed, 5 ml of following clear filtrate was collected and used as a sample solution. All other reagents were commercially available products of analytical reagent grade.

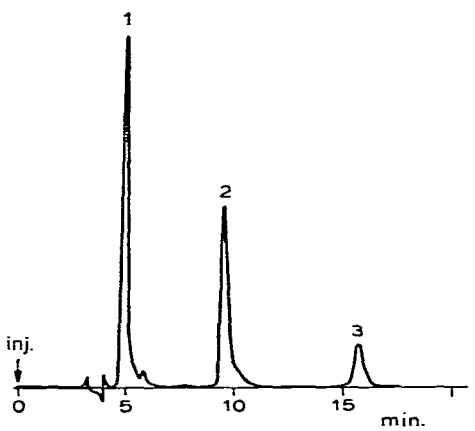
Apparatus and procedure

The chromatograph consisted of an Altex pump (Model 110-A; Berkeley, Calif., U.S.A.) operated at a flow-rate of 0.8 ml/min. The column effluent was monitored by a Shimazu UV variable-wavelength detector (Model 202; Kyoto, Japan) with a 20-μl flow-through cell. The detector was operated at 210 nm ($32 \cdot 10^{-2}$ a.u.f.s.). The column consisted of a stainless-steel tube (25 cm × 4.6 mm I.D.) packed

with Nucleosil 10 SB (Macherey, Nagel & Co., Düren, G.F.R.) by the balanced viscosity and dispersion slurry-packing technique. All experiments were carried out at room temperature. A 20-μl volume of standard or sample solution was injected accurately by using a syringe loading sample injector (Model 7120; Rheodyne, Berkeley, Calif., U.S.A.). The chromatogram was recorded at a chart speed of 5 mm/min. The L-glutamine content in the sample was calculated by peak height measurements relative to standard solutions.

RESULTS

Under these chromatographic conditions, L-glutamine was separated from nicotinic amide and calcium pantothenate; these have been prescribed frequently together (Fig. 1). L-Glutamine is not very stable in water, and decomposes forming pyrrolidonecarboxylic acid[28] or glutamic acid[29] by the action of moisture, and basic compounds, such as sodium bicarbonate, calcium carbonate, magnesium carbonate, magnesium oxide etc., in preparations stored for long periods of time. L-Glutamine was easily separated from these by-products (Fig. 2). The recovery of L-glutamine was 98.5% and the coefficient of variation was 0.98% for 5 analyses. These authors believe that this chromatographic method is very useful for the determination of L-glutamine in routine preparations, because the procedure is performed easily and rapidly.



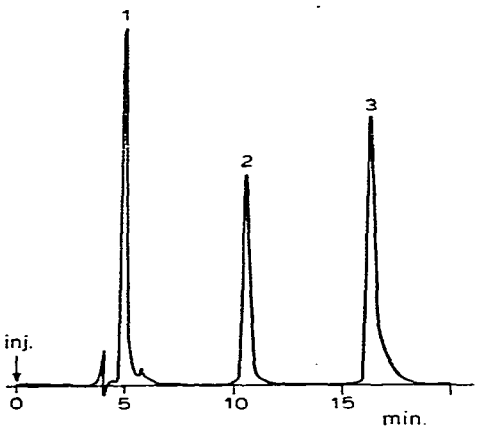


Fig. 1. Chromatogram of L-glutamine (1), nicotinic amide (2) and pantothenate (3).

Fig. 2. Chromatogram of L-glutamine (1), pyrrolidonecarboxylic acid (3) and glutamic acid (2).

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REFERENCES

1 Japan Pharmaceutical Information Center, in M. Horioka, T. Saito, K. Takagi, M. Takasugi and N. Motohashi (Supervisors), *Drugs in Japan, Ethical Drug Edition*, Yakugyo Jiho, Tokyo, 1975, p. 192.

2 I. Oreskes, F. Cantor and S. Kupfer, *Anal. Chem.*, 37 (1965) 1720.
3 H. Lorenz, *J. Chromatogr.*, 27 (1967) 267.
4 T. L. Perry and S. Hansen, *Clin. Chim. Acta*, 25 (1969) 53.
5 A. Vega and P. B. Nunn, *Anal. Biochem.*, 32 (1969) 446.
6 S. Tartarini, *J. Pharm. Sci.*, 61 (1972) 960.
7 J. L. Young and M. Yamamoto, *J. Chromatogr.*, 78 (1973) 349.
8 E. H. F. McGale and I. F. Pye, *J. Chromatogr.*, 89 (1974) 350.
9 P. T. Ozand, R. L. Hawkins, R. M. jun. Collins, J. T. Tildon and M. Cornblath, *Biochem. Med.*, 14 (1975) 170.
10 H. Hediger, R. L. Stevens, H. Brandenberger and K. Schmid, *Biochem. J.*, 133 (1973) 551.
11 J. M. L. Mee and B. Halpern, *Anal. Lett.*, 9 (1976) 605.
12 F. S. Collins and G. K. Summer, *J. Chromatogr.*, 145 (1978) 456.
13 P. Hušek and V. Felt, *J. Chromatogr.*, 152 (1978) 546.
14 S. R. Nahorski, *Anal. Biochem.*, 42 (1971) 136.
15 R. E. Miller, *Anal. Biochem.*, 75 (1976) 91.
16 F. L. Iber and J. Bruton, *Clin. Chem.*, 2 (1956) 413.
17 M. E. A. Ramadan and D. M. Greenberg, *Anal. Biochem.*, 6 (1963) 144.
18 H. G. Preuss, B. B. Bise and G. E. Schreiner, *Clin. Chem.*, 12 (1966) 329.
19 M. Kikuchi, *Anal. Biochem.*, 59 (1974) 83.
20 P. J. Buttery and E. V. Rowsell, *Anal. Biochem.*, 39 (1971) 297.
21 D. Coony and R. Davis, *Anal. Biochem.*, 40 (1971) 312.
22 T. P. Whitehead and S. R. F. Whittaker, *J. Clin. Pathol.*, 8 (1955) 81.
23 P. R. Henderlong and R. R. Schmidt, *Plant Physiol.*, 41 (1966) 1102.
24 E. M. Olsen, D. C. Hill and H. D. Branion, *Can. J. Biochem. Physiol.*, 40 (1962) 381.
25 E. B. Robertson, B. D. Sykes and H. B. Dunford, *Anal. Biochem.*, 9 (1964) 158.
26 H. Kluge, H. Hermann and V. Wieczorek, *Z. Klin. Chem.*, 5 (1967) 86.
27 P. van der Harst, J. D. Baars and A. J. P. F. Lombarts, *Clin. Chim. Acta.*, 50 (1974) 439.
28 H. B. Vickery, G. W. Pucher and H. E. Clark, *Biochem. J.*, 29 (1935) 2710.
29 E. Schulze and G. Trier, *Chem. Ber.*, 45 (1912) 257.