High performance ion-exchange chromatography of amino-acids in biological fluids using Chromakon 500 – performance of the apparatus

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Introduction

The separation and quantitative measurement of amino-acids by ion-exchange chromatography has been in use for many years [1]. However, the value of this method is limited by the time required for the analysis: 6 to 23 h. Other methods, such as gas chromatography, give results more rapidly, but do not allow the separation of all amino-acids [2]. The development of cation-exchange resins, capable of resisting high pressure, considerably shortens the time required to perform an analysis. In this study the performance of the Chromakon 500 (Kontron, Switzerland) with a cation-exchange resin (Kontron AS-70 [3]) is evaluated; 39 amino-acids and derivatives can be separated in less than 130 min (170 min including the regeneration time).

Material and methods

All the reagents necessary for the ion-exchange chromatography were supplied by Merck (Darmstadt, FR Germany). Stabilized lyophylized serum (set Hw) was provided by Bio Merieux (Charbonnières les Bains, France). The 40 amino-acid standard solution was supplied by Pierce and was supplemented with glutamine (final concentration 250 μmol/l) from Calbiochem (San Diego, California, USA). Amino-acids were separated on the Chromakon 500 equipped with an automatic injection loop, a 15 cm column packed with Kontron AS70 resin (diameter 7 μ) and with five citrate buffers (see table 1). A simplified diagram of the apparatus is given in figure 1. The elution program was a slightly modified version of that given by Kontron (table 2) with modifications which improved the separation of the following amino-acids: cysteine and methionine, cystathionine and isoleucine, 3- methyhistidine and anserine. Detection was performed by colorimetry at 570 + 440 nm with the ninhydrin reaction.

The apparatus was coupled to a Shimadzu CR 1 B integrator and amino-acid concentrations were calculated by the method of pic areas. Prior to analysis, stabilized serum was deproteinized by sulphasaliclyc acid (50 mg/ml serum) and half diluted in buffer 1.

Results and discussion

The stability of retention times was studied by 10 consecutive injections of the calibration solution. Amino-acids were adequately separated (a typical chromatogram is shown in figure 2). Retention times are constant: CVs ranged from 0.1 to 1.5% (table 3) and are rather better than those provided by liquid HPLC [4]. When the apparatus is stopped, it is interesting to note that a 1 h equilibration time with buffer 1 is required before performing the first analysis in order to obtain constant retention times.

Table 1. Composition of buffers.

|               | 1    | 2    | 3    | 4    | 5    |
|---------------|------|------|------|------|------|
| Lithium hydroxide monohydrate (g) | 5.05 | 5.45 | 8.4  | 8.4  | 8.4  |
| Citric acid monohydrate (g)       | 17.4 | 17.4 | 17.4 | 17.4 | 14.7 |
| Lithium chloride (g)              | 0    | 0    | 16   | 35   |      |
| Chlorhydric acid (ml)             | 19   | 15   | 20   | 8    |     |
| Phenol (ml)                       | 2    | 2    | 2    | 2    |     |
| Methanol (ml)                     | 70   | 50   | —    | —    |     |
| Purified water, quantity for 1 l  | 2.60 | 3.10 | 3.75 | 4.0  | 5.25 |

The buffers were filtered, degassed, and left for 12 h before adjusting the pH to: 2.60 3.10 3.75 4.0 5.25
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Figure 2. A typical chromatogram obtained with the standard solutions supplemented with glutamine. Where: PHS = phosphate; TPR = taurine, POE = phosphoethanolamine, URE = urea, ASP = aspartic acid, HYP = hydroxyproline, THR = threonine, SER = serine, ASN = asparagine, GLU = glutamic acid, GLN = glutamine, ALA = alanine, SAR = sarcosine, AAA = alphaaminoadipic acid, PRO = proline, GLY = glycine, VAL = valine, CYS = cysteine, MET = methionine, CYST = cystathionine, ILE = isoleucine, LEU = leucine, TYR = tyrosine, PHE = phenylalanine, bALA = betaalanine, BABA = b-aminoisobutyric acid, GB = 2-aminobutyric acid, TRP = tryptophane, OHLYS = hydroxylysine, NH = ammonium, ORN = ornithine, LYS = lysine, HIS = histidine, 1MeHIS = 1-methylhistidine, 3MeHIS = 3-methylhistidine, ANS + CAR = anserine + carnitine.

Figure 3. Elution of amino-acids.

| Buffer | pH | Temperature | Time (min) |
|--------|----|-------------|------------|
| 1      | 2.50 | 35°C | 0-60 |
| 2      | 3.00 | 62°C | 11-13 |
| 3      | 3.75 | 70°C | 43-58 |
| 4      | 4.00 | 77°C | 97-100 |
| 5      | 5.25 | 88°C | 135-140 |
| 6      | 6.37 | 37°C | 3-7 |

N.B. Times given are from the beginning of the analysis.

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| 5      | 5.25 | 88°C | 135-140 |
| 6      | 6.37 | 37°C | 3-7 |

In conclusion, this apparatus gives precise results for physiological amino-acids in a reasonably short time, so that the use of this high-performance ion-exchange chromatography is very attractive.

The determination of the amino-acids from the standard solutions supplemented with glutamine provided a useful feature, and the analysis program can be modified at all times. In particular, the creation of a new program is very simple, and it is particularly useful in the amino-acids field. Repeatability assays were performed on consecutive serum samples (HIV +/+ and HIV -/-) using the same analytical conditions. The results were comparable, and the precision of the method was similar to that of other liquid-liquid chromatographic methods. The results obtained with this apparatus are comparable to those obtained by gas chromatography [4] and liquid chromatography [5].

The system is robust and reliable, and it has been continuously used for more than 18 months. Finally, it is worth noting that the equipment is more reliable if it is in continuous use.
Table 3. Stability of retention time, repeatability and reproducibility assays.

| Amino-acid | Retention time (minutes) | Repeatability (N = 10) | Reproducibility (N = 10) |
|------------|--------------------------|------------------------|--------------------------|
|            | x ± SD                   | CV%                    | x ± SD                   | CV%                    |
|            | (µmol/l)                 |                        | (µmol/l)                 |                        |
|            |                          |                        |                          | CV%                    |
| PHS        | 2.17 ± 0.02              | 0.9                    | 25 ± 0.9                 | 3.6                    | 29 ± 2                    | 8.4 |
| TAU        | 3.72 ± 0.02              | 1.5                    | 91 ± 2.3                 | 2.5                    | 72 ± 15                   | 11.1 |
| ASP        | 17.83 ± 0.21             | 1.2                    | 58 ± 1.1                 | 1.8                    | 40 ± 5                    | 12.5 |
| HYP        | 20.63 ± 0.16             | 0.2                    | <5                      |                        |                         |     |
| THR        | 24.55 ± 0.15             | 0.6                    | 77 ± 2.8                 | 3.6                    | 78 ± 7                    | 9.3 |
| SER        | 27.27 ± 0.15             | 0.6                    | 56 ± 2.3                 | 4.1                    | 70 ± 7                    | 9.5 |
| ASN        | 38.46 ± 0.27             | 0.7                    | <5                      |                        |                         |     |
| GLU        | 39.53 ± 0.33             | 0.8                    | 107 ± 2.8                | 2.6                    | 137 ± 14                  | 10.2 |
| GLN        | 49.26 ± 0.24             | 0.6                    | 29 ± 1.0                 | 3.4                    | 72 ± 8                    | 11.1 |
| PRO        | 49.85 ± 0.34             | 0.7                    | 75 ± 1.8                 | 2.4                    | 90 ± 16                   | 17.6 |
| GLY        | 51.45 ± 0.36             | 0.7                    | 304 ± 2.5                | 0.8                    | 335 ± 31                  | 9.3 |
| ALA        | 53.13 ± 0.48             | 0.9                    | 306 ± 5.2                | 1.7                    | 271 ± 22                  | 7.9 |
| CIT        | 54.45 ± 0.38             | 0.7                    | 53 ± 2.0                 | 3.5                    | 55 ± 5                    | 10.0 |
| VAL        | 59.83 ± 0.45             | 0.8                    | 232 ± 4.5                | 1.9                    | 213 ± 20                  | 9.4 |
| CYS        | 62.99 ± 0.36             | 0.6                    | <5                      |                        |                         |     |
| MET        | 63.61 ± 0.35             | 0.5                    | 31 ± 0.4                 | 1.3                    | 29 ± 3                    | 10.3 |
| ILE        | 65.31 ± 0.85             | 1.3                    | 93 ± 2.0                 | 2.2                    | 78 ± 8                    | 10.0 |
| LEU        | 67.21 ± 0.36             | 0.5                    | 199 ± 2.8                | 1.3                    | 162 ± 13                  | 8.2 |
| TYR        | 68.26 ± 0.11             | 0.2                    | 41 ± 1.8                 | 4.4                    | 43 ± 5                    | 11.6 |
| PHE        | 72.83 ± 0.45             | 0.2                    | 63 ± 5.0                 | 8.3                    | 62 ± 5.5                  | 8.9 |
| TRP        | 91.80 ± 0.52             | 0.6                    | 39 ± 0.7                 | 1.8                    | 36 ± 3                    | 8.5 |
| ORN        | 106.27 ± 0.50            | 0.5                    | 114 ± 1.5                | 1.3                    | 86 ± 10                   | 11.6 |
| LYS        | 108.51 ± 0.43            | 0.4                    | 133 ± 2.3                | 1.7                    | 118 ± 15                  | 12.7 |
| HIS        | 110.79 ± 0.21            | 0.2                    | 86 ± 1.4                 | 1.6                    | 78 ± 7                    | 9.6 |
| ARG        | 126.73 ± 0.06            | 0.1                    | 169 ± 4.6                | 2.7                    | 140 ± 11                  | 7.6 |

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