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HPLC Separation of Amino Acids is Appropriate?

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Concerning amino acid analysis, it is so often used post column derivatization method using ninhydrin or ortho-phthal aldehyde with gradient elution. Ninhydrin and ortho-phtal aldehyde are identical. Problem is that 21 sorts of amino acids cannot always baseline separation (Figure 1).

From the Figure, several sorts of amino acids are not base-line separation and require at least Rs (resolution) more than 1.5 for baseline separation. Amino acid chromatographies done by Shimazdu, Hitachi or Nihon Bunko were almost identical. They require baseline separation; otherwise reproducible determination cannot be attained. For that purpose of base-line separation, gradient elution condition must be innovated. Most innovated point is the use of column with higher theoretical plate number. So, I suppose why they do not use capillary-column with higher theoretical plate number.

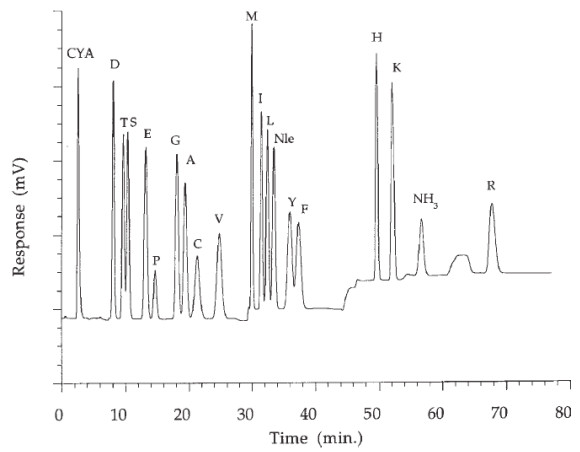

Figure 1: Several sorts of amino acids are not base-line separation and require at least Rs (resolution) more than 1.5 for baseline separation.

The above presented chromatogram is representative chromatogram, so analytical validation is further required and gradient condition and elution condition must be innovated to attain base-line separation chromatogram.

The separation condition obtained chromatogram is presented in Table 1.

The elution order of amino acids is from acidic amino acid, neutral amino acid. and then basic amino acid, indicating cation chromatography-column will be used. This selection is normal, but gradient condition is required innovation for base-line separation. Most of acidic amino acid and neutral amino acid are not separated in based-line. This is. further, requires analytical validation to reproducible analytical results.

Time(min)	Event	Conditions
0.0	Sample injection	Na-E buffer,48°C
8.5	Start temp.gradient	48°C to 60°C in 8 min
24.5	Buffer change	Na-E to Na-F
41.0	Buffer change	Na-F to Na-D
78.0	Reagent pump	Ninhydrin to water
79.0	Buffer change	Na-D to Na-R
80.0	Buffer change	Na-R to Na-E
82.5	Temperature change	60°C to 48°C
84.0	Reagent pump	Water to ninhydrin
97.0	Recycle (Start next run)	

Buffer pump: 16 mL/h
Reagent pump: 8 mL/h

Table 1: Standard amino acid analysis.

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