

Separation of 18 standard amino acids

Author

Huiling LU, SinoUnison Technology Co., Ltd. (SUT)

Experimental background

As an important class of nutrients, amino acids are widely studied in the literature. For analyzing amino acids, different methods have been reported by different groups, but not all of them show perfect separation between analytes. The separation efficiency strongly depends on many factors: mobile phases and the gradients, derivating reagent and its usage, column, etc.

In this paper, we used a pre-column derivatization method and SVEA HPLC Column C18 Gold 5 μ m 110 Å 4.6x250mm for a perfect separation of standard amino acid by HPLC. The main advantage of this method that it effectively avoids the use of expensive amino acid-specific analytical columns and amino acid analyzer.

Experimental

Column: SVEA C18 Gold 5 μ m 110Å 4.6*250mm

Serial no: 1801054

Instrument: HPLC

Mobile phase: A: 0.05mol/L Phosphate buffer ;

B: Acetonitrile : water = 1:1

Flow rate: 1.0 mL/min

Column temperature: 40 °C

Detector: UV 360nm

Extractant:

Injection volume: 10 μ L

Analyte: Mixture of 18 common amino acid standards

Chromatogram

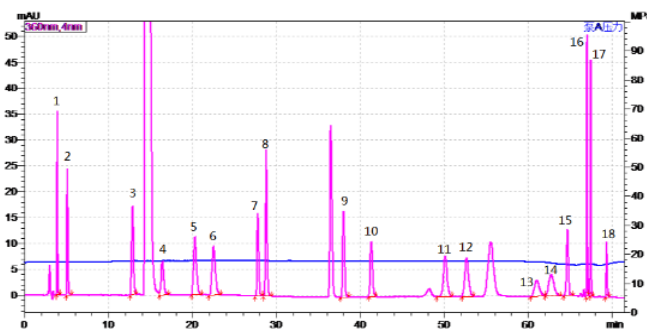


Figure 1: Mixture of 18 standard amino acids

Results

ID#	Compound name	Time	Area	Height	Concentration	Concentration unit
1	Aspartic acid (Asp)	3.884	236030	35443	40	μ L/ml
2	Glutamate (Glu)	5.103	211221	24438	40	μ L/ml
3	Serine (Ser)	12.863	265499	17078	40	μ L/ml
4	Arginine (Arg)	16.420	143516	6553	40	μ L/ml
5	Glycine (Gly)	20.296	271115	11281	40	μ L/ml
6	Threonine (Thr)	22.515	263138	9553	40	μ L/ml
7	Proline (Pro)	27.790	215346	15938	40	μ L/ml
8	Alanine (Ala)	28.788	360930	28199	40	μ L/ml
9	Valine (Val)	38.002	264715	16638	40	μ L/ml
10	Methionine (Met)	41.312	206911	10593	40	μ L/ml
11	Isoleucine (Ile)	50.104	237909	7808	40	μ L/ml
12	Leucine (Leu)	52.653	238304	7452	40	μ L/ml
13	Tryptophan (Try)	61.025	123550	3058	40	μ L/ml
14	Phenylalanine (Phe)	62.723	174995	4142	40	μ L/ml
15	Histidine (His)	64.693	196983	12789	40	μ L/ml
16	Cysteine (Cys)	66.996	289368	50441	40	μ L/ml
17	Lysine (Lys)	67.439	301443	45653	40	μ L/ml
18	Tyrosine (Tyr)	69.343	100042	10715	40	μ L/ml
Total			4101013	317773		

Conclusion

Using SVEA HPLC Column C18 Gold 5 μ m 110Å 4.6x250mm in the analysis of the above 18 amino acid standards, a perfect separation with excellent peak shapes with the tailing factors between 0.9 and 1.2 can be obtained.

