



# Agilent Technologies

1260 infinity LC System



**Poroshell 120 HPH-C18 column**



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Summary Report For:

# Analysis of Amino Acids using Proshell120 HPH-C18

**Ramaprasad Ganni**

Agilent Technologies India Pvt Limited,

Bangalore, INDIA

## Introduction:

Amino Acids analysis was performed using Agilent Amino acids kit and Poroshell120 HPH-C18 column on 1260 Infinity LC system. This study is performed to for Poroshell120 HPH-C18 column life time with this application.



**Figure 5** From left: 1260 Infinity

## Experimental

Analyses were performed by automated online pre-column derivatization using automated liquid sampler, Poroshell120 HPH-C18 column and 1260 Infinity system. The standard preparation, derivatization process, and LC method used was as specified in Agilent Pub.# 5990-4547EN.

### *Mobile Phase Preparation:*

- Mobile Phase A : 1.4 g Na<sub>2</sub>HPO<sub>4</sub> · 10H<sub>2</sub>O + 3.8 g Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> in 1 L Milli-Q Water + 32 mg NaN<sub>3</sub>. Adjust the pH 8.2 with conc HCl.
- Mobile Phase B : Acetonitrile: Methanol: H<sub>2</sub>O (45: 45: 10).
- Injection Diluent : 100 mL of Mobile Phase A + 0.4 mL conc H<sub>3</sub>PO<sub>4</sub>.

### **Online derivatization:**

Online derivatization was performed by programing automatic auto sampler with injection program:

1. Draw 2.5 µL from Borate vial (Agilent P/N: 5061-3339)
2. Draw 1.0 µL from Sample vial
3. Mix 3.5 µL in washport 5 times.
4. Wait 0.2 min.

5. Draw 0.5  $\mu$ L from OPA vial (Agilent P/N: 5061-3335)
6. Mix 4.0  $\mu$ L in washport 10 times with default speed.
7. Draw 0.4  $\mu$ L from FMOC vial (Agilent P/N: 5061-3337)
8. Mix 4.4  $\mu$ L in washport 10 times with default speed.
9. Draw 32  $\mu$ L from injection diluent vial.
10. Mix 20  $\mu$ L in washport 8 times.
11. Inject.
12. Wait 0.2 min.
13. Valve bypass.

**1260 HPLC conditions -**

- Flow rate : 1.5 ml/min
- Column : Poroshell120 HPH-C18; 4.6 mm X 100 mm X 2.7  $\mu$ m; P/N: 695972-702
- Column Temperature : 40°C
- Injection volume : 20  $\mu$ L
- DAD Detector : Signal A: 338 nm, 10 nm band width, ref wave length 390 nm, 20 nm.  
Signal B: 262 nm, 16 nm band width, ref wave length 324 nm, 8 nm.
- Gradient program :

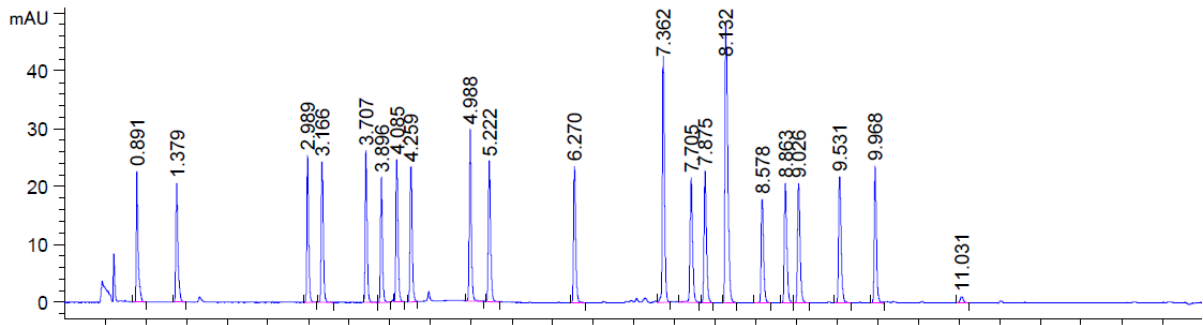
Time in min	% of B
1	2
0.35	2
13.4	100
15.7	100
15.8	2
16	end

Post run time : 3.0min

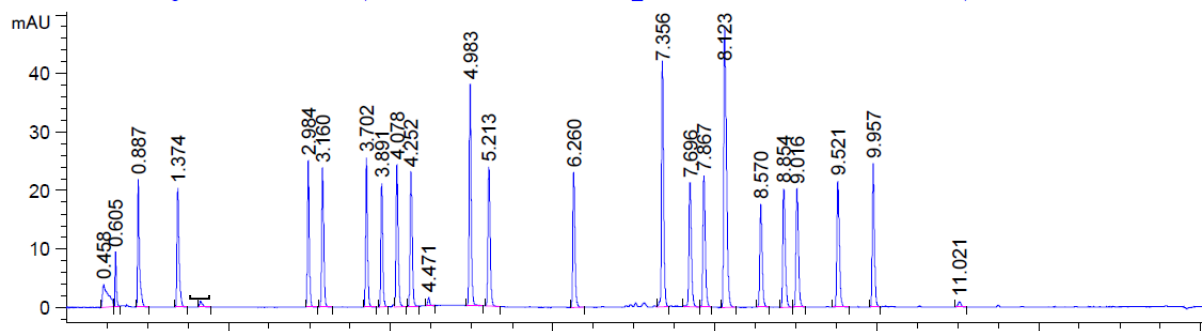
## Results and Discussion

### Chromatograms of different Injections: 225 pmol/ul.

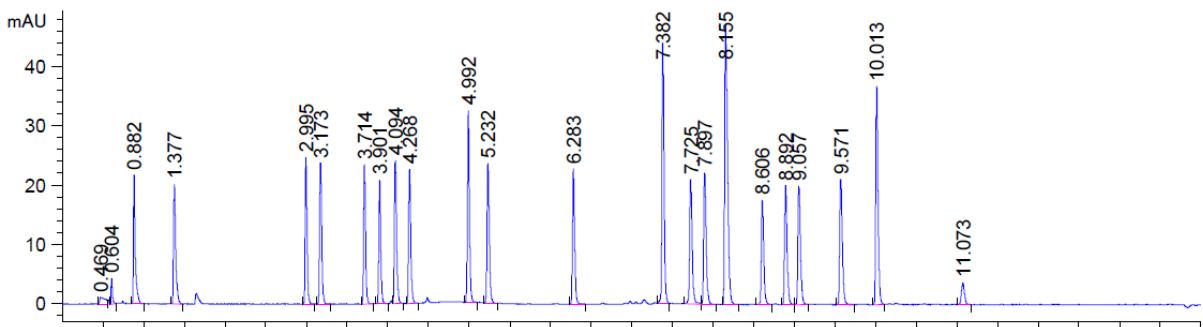
#### Injection 1:



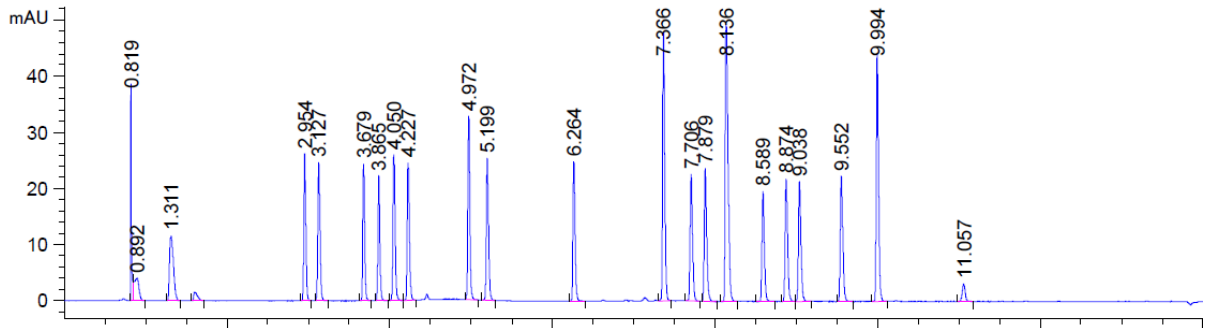
#### Injection 100:



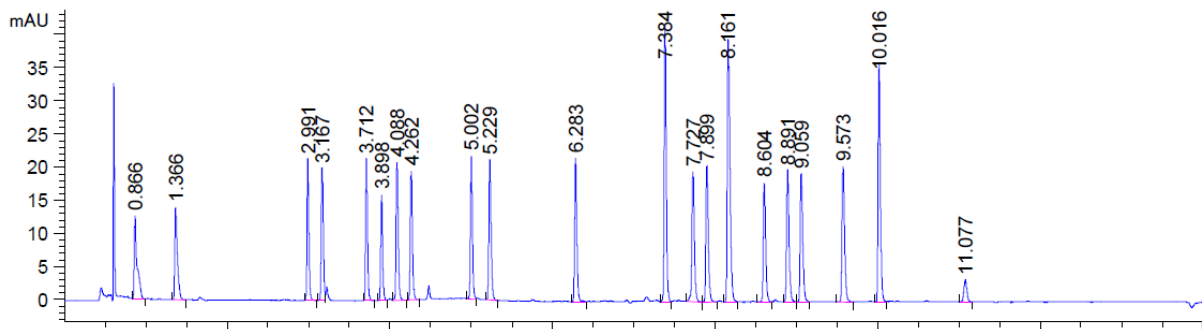
#### Injection 200:



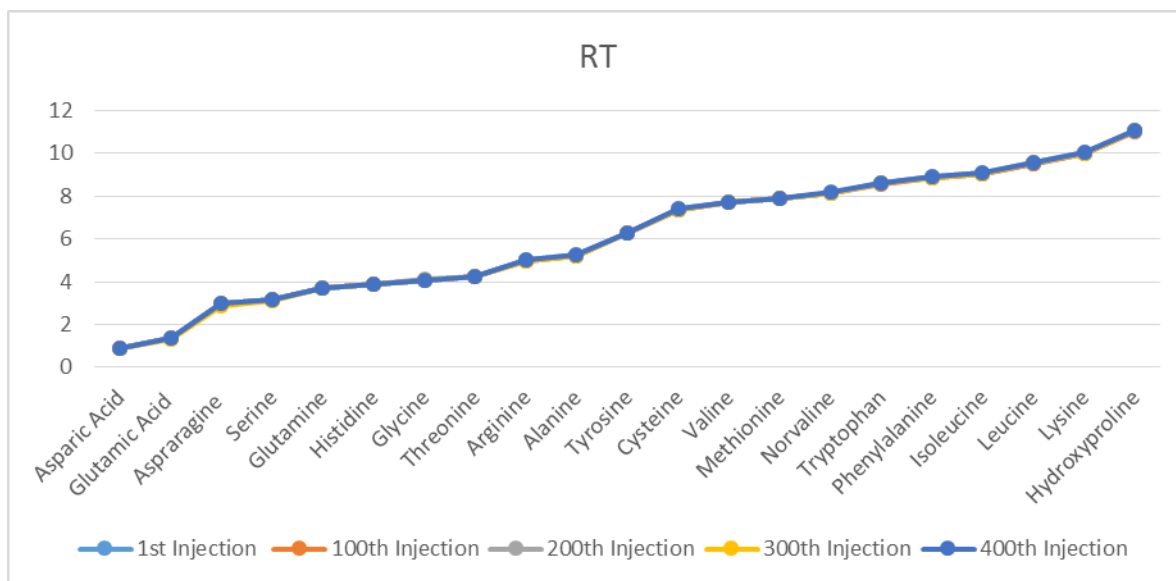
**Injection 300:**



**Injection 400:**

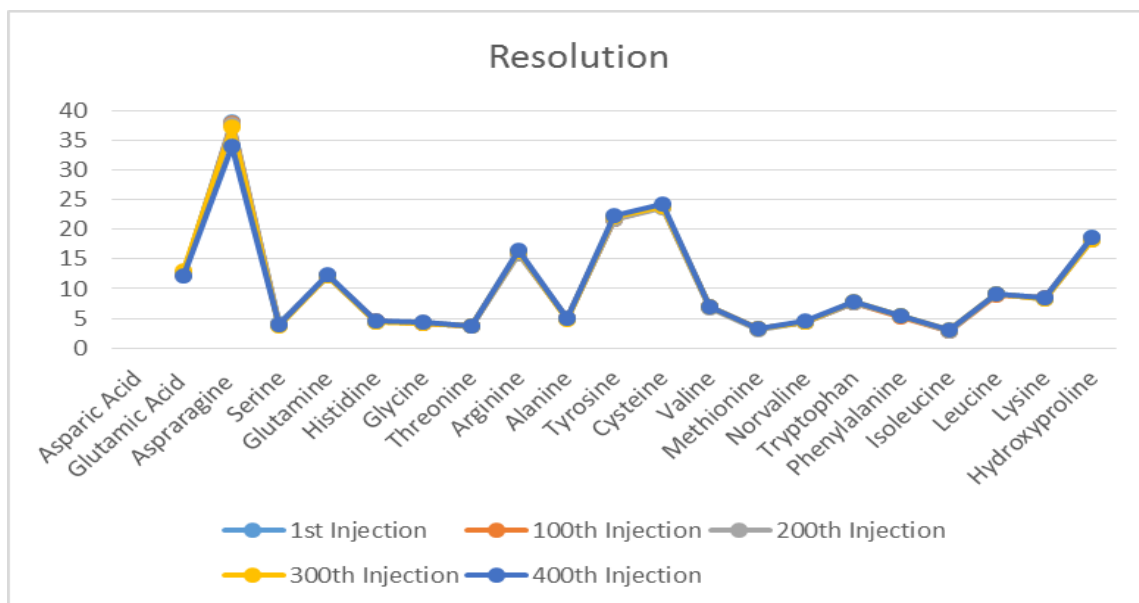


**Retention Times (RT's) for all Amino Acids in different Runs @ Sig: 338, 10; Ref: 390, 20.**



S. No	Analytes	1 <sup>st</sup> Injection	100 <sup>th</sup> Injection	200 <sup>th</sup> Injection	300 <sup>th</sup> Injection	400 <sup>th</sup> Injection	%RSD
1	Asparic Acid	0.891	0.887	0.882	0.892	0.866	1.199
2	Glutamic Acid	1.379	1.374	1.377	1.311	1.366	2.101
3	Asprargine	2.989	2.984	2.995	2.85	2.991	2.114
4	Serine	3.166	3.161	3.173	3.127	3.167	0.579
5	Glutamine	3.707	3.702	3.714	3.679	3.712	0.381
6	Histidine	3.896	3.891	3.901	3.865	3.898	0.374
7	Glycine	4.085	4.078	4.094	4.05	4.088	0.422
8	Threonine	4.259	4.252	4.268	4.227	4.262	0.375
9	Arginine	4.988	4.983	4.992	4.972	5.002	0.222
10	Alanine	5.222	5.213	5.232	5.199	5.229	0.256
11	Tyrosine	6.27	6.26	6.283	6.264	6.283	0.170
12	Cysteine	7.362	7.356	7.382	7.366	7.384	0.168
13	Valine	7.705	7.696	7.725	7.706	7.727	0.176
14	Methionine	7.875	7.867	7.897	7.879	7.899	0.178
15	Norvaline	8.132	8.123	8.155	8.136	8.161	0.197
16	Tryptophan	8.578	8.57	8.606	8.589	8.604	0.184
17	Phenylalanine	8.863	8.854	8.892	8.874	8.891	0.189
18	Isoleucine	9.026	9.016	9.057	9.038	9.059	0.209
19	Leucine	9.531	9.521	9.571	9.552	9.573	0.244
20	Lysine	9.968	9.957	10.013	9.994	10.016	0.265
21	Hydroxyproline	11.031	11.021	11.073	11.057	11.077	0.226

### Resolution for all Amino Acids in different Runs:



S. No	Analytes	1 <sup>st</sup> Injection	100 <sup>th</sup> Injection	200 <sup>th</sup> Injection	300 <sup>th</sup> Injection	400 <sup>th</sup> Injection
1	Asparic Acid					
2	Glutamic Acid	12.56	12.47	12.49	13.09	12.09
3	Aspraragine	38.09	37.89	37.78	37.23	34.05
4	Serine	4.05	4.02	4.03	3.74	4.01
5	Glutamine	12.19	12.21	12.13	12.19	12.44
6	Histidine	4.5	4.5	4.44	4.44	4.55
7	Glycine	4.23	4.18	4.28	4.11	4.33
8	Threonine	3.65	3.65	3.62	3.66	3.73
9	Arginine	15.96	15.96	15.74	16.22	16.42
10	Alanine	5.01	4.95	5.12	4.87	4.93
11	Tyrosine	21.76	21.71	21.61	22.04	22.22
12	Cysteine	23.85	23.78	23.62	23.85	24.22
13	Valine	6.94	6.87	6.84	6.87	7.05
14	Methionine	3.19	3.19	3.18	3.24	3.27
15	Norvaline	4.37	4.38	4.34	4.37	4.63
16	Tryptophan	7.72	7.71	7.71	7.81	7.89
17	Phenylalanine	5.37	5.34	5.35	5.38	5.46
18	Isoleucine	2.96	2.95	2.96	2.99	3.09
19	Leucine	9.07	9.01	9.08	9.2	9.2
20	Lysine	8.51	8.45	8.33	8.36	8.46
21	Hydroxyproline	18.61	18.35	18.17	18.3	18.65

## Conclusions

- Poroshell120 HPH-C18 column was used at high pH 8.2 and temperature of 40 °C for the analysis of 21 Amino Acids.
- The % RSD of RT's for 400 injections is between 0.168 and 2.114.