

Determination of the amino acids in Cell Culture Media



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Introduction

The aim is that the validation of the analytical method should demonstrate that it is suitable for the determination of amino acids in cell culture media.

Sample Preparation and Analysis

Validation/Precision:

100 μL of the sample was mixed with 100 μL internal standard norleucine (10 $\mu\text{mol}/\text{mL}$) and 600 μL sample dilution buffer. 150 μL of precipitation solution were added and deposit in the refrigerator for 20 min for the protein precipitation. The sample was centrifuged for 5 min at 14000 rpm. Afterwards the supernatant was filtered with a membraSpin by centrifugation at 14000 rpm for five minutes. The particle free solution was used for the injection. For the analysis 100 μL of sample were diluted with 900 μL sample dilution buffer (dilution 1:100).

Accuracy/Recovery:

For the analysis 2 mL of sample and 2 mL of sample dilution buffer (including norleucin, 10 $\mu\text{mol}/\text{mL}$) were mixed and diluted with sample dilution buffer 1:50. The obtained stock solution was used for the dilution series of corresponding to 100 %, 80 %, 60 %, 40 %, 20 % and 10 %.

Table 1: Dilution series of stock solution for the validation of accuracy.

Run	stock solution	dilution buffer	%
1	1000 μL	-	100
2	800 μL	200 μL	80
3	600 μL	400 μL	60
4	400 μL	600 μL	40
5	200 μL	800 μL	20
6	100 μL	900 μL	10

The samples were analyzed by the Amino Acid Analyzer ARACUS, manufactured and distributed by membraPure GmbH worldwide. ARACUS is using the classic routine analysis of amino acids by post-column derivatization with ninhydrin and the detection at 440 nm and 570 nm.



Figure 1: Amino Acid Analyzer ARACUS

Validation/ Precision

The precision of the method was determined by repeatability (intra-day) (Table 2 & Table 3) and intermediate precision (inter-day) (Table 4 & Table 5). The intra-day precision was calculated as the relative standard deviation (RSD) of results from ten runs of the sample, during the same day, and the inter-day precision was studied by the sample preparation on ten different days. Ten sample solutions were prepared, and the standard

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deviation (SD) and RSD (coefficient of variation CV %) were calculated.

Table 2: Intra-day precision of amino acids in cell media concentration in nmol/g (n=10)

Amino Acid	Mean \bar{x}	SD	RSD / %
Asp	13024	± 436	3.34
Thr	11475	± 250	2.17
Ser	17914	± 353	1.97
Asn	11420	± 217	1.9
Glu	7903	± 210	2.65
Gly	8444	± 153	1.81
Ala	21745	± 769	3.53
Val	14681	± 244	1.66
Cys2	1830	± 82	4.48
Met	4295	± 70	1.62
Ile	8921	± 147	1.64
Leu	14115	± 244	1.72
Tyr	3248	± 106	3.26
Phe	6938	± 166	2.39
His	2807	± 40	1.42
Trp	4063	± 64	1.57
Lys	7893	± 130	1.64
NH ₄	20747	± 435	2.09
Arg	6697	± 207	3.09
Pro	3348	± 82	2.44

Table 3: Intra-day precision of amino acids in cell media concentration in mg/g (n=10)

Amino Acid	Mean \bar{x}	SD	RSD / %
Asp	1.733	± 0.058	3.34
Thr	1.366	± 0.0297	2.17
Ser	1.882	± 0.037	1.97
Asn	1.508	± 0.0286	1.9
Glu	1.162	± 0.0308	2.65
Gly	0.634	± 0.0114	1.8
Ala	1.937	± 0.0685	3.53
Val	1.719	± 0.0285	1.66
Cys2	0.221	± 0.0099	4.48
Met	0.641	± 0.0104	1.62
Ile	1.17	± 0.0192	1.64
Leu	1.859	± 0.032	1.72
Tyr	0.588	± 0.0192	3.26
Phe	1.146	± 0.0274	2.39
His	0.435	± 0.0062	1.42
Trp	0.829	± 0.013	1.57
Lys	1.153	± 0.019	1.64
NH ₄	0.374	± 0.0078	2.09
Arg	1.166	± 0.0361	3.09
Pro	0.385	± 0.0094	2.44

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Table 4: Inter-day precision of amino acids in cell media concentration in nmol/g (n=10)

Amino Acid	Mean \bar{x}	SD	RSD / %
Asp	12494	± 720	5.76
Thr	11007	± 625	5.67
Ser	17175	± 976	5.68
Asn	10852	± 739	6.8
Glu	7687	± 320	4.15
Gly	8109	± 447	5.51
Ala	20265	± 1397	6.89
Val	13533	± 962	7.11
Cys2	1733	± 138	7.96
Met	4151	± 193	4.64
Ile	8625	± 395	4.57
Leu	13679	± 586	4.28
Tyr	3133	± 202	6.44
Phe	6695	± 331	4.94
His	2683	± 155	5.77
Trp	3859	± 286	7.41
Lys	7722	± 431	5.58
NH ₄	21860	± 1389	6.35
Arg	6486	± 451	6.95
Pro	2982	± 132	4.42

Table 5: Inter-day precision of amino acids in cell media concentration in mg/g (n=10)

Amino Acid	Mean \bar{x}	SD	RSD / %
Asp	1.661	± 0.0957	5.76
Thr	1.309	± 0.0743	5.67
Ser	1.803	± 0.1024	5.68
Asn	1.432	± 0.0975	6.8
Glu	1.129	± 0.047	4.15
Gly	0.608	± 0.0335	5.51
Ala	1803	± 0.1243	6.89
Val	1.583	± 0.1125	7.11
Cys2	0.21	± 0.0167	7.96
Met	0.618	± 0.0287	4.64
Ile	1.129	± 0.0517	4.57
Leu	1.791	± 0.0767	4.28
Tyr	0.567	± 0.0365	6.44
Phe	1.104	± 0.0546	4.94
His	0.416	± 0.024	5.77
Trp	0.787	± 0.0583	7.41
Lys	1.127	± 0.0629	5.58
NH ₄	0.393	± 0.025	6.35
Arg	1.128	± 0.0784	6.95
Pro	0.342	± 0.0151	4.42

Accuracy (Recovery)

In this validation study, accuracy of the method has been investigated by calculating the recovery values obtained by analyzing the sample prepared with norleucine standard (100 µl/mL) corresponding to 100%, 80%, 60%, 40%, 20% and 10% (Table 6).

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Table 6: Recovery values for dilution series for each amino acid, which were analysed in cell culture media.

Cell media	100%	80%	60%	40%	20%	10%
Run	1 [nmol/g]	2 [nmol/g]	3 [nmol/g]	4 [nmol/g]	5 [nmol/g]	6 [nmol/g]
Nle						
Mean \bar{x}	9889	8232	6209	4280	2109	1028
SD	± 153	± 135	± 148	± 65	± 74	± 49
RSD %	1.54	1.63	2.38	1.51	3.5	4.76
Discovery %	98.9	83.2	62.7	43.2	21.3	10.3
Asp						
Mean \bar{x}	12911	10163	7551	4937	2484	1219
SD	± 320	± 117	± 166	± 55	± 71	± 32
RSD %	2.47	1.15	2.19	1.11	2.85	2.62
Discovery %	100	78.7	58.4	38.2	19.2	9.4
Thr						
Mean \bar{x}	11330	8992	6721	4465	2246	1218
SD	± 179	± 160	± 186	± 119	± 94	± 54
RSD %	1.57	1.77	2.76	2.66	4.18	4.43
Discovery %	100	79.3	59.3	39.4	19.8	10.7
Ser						
Mean \bar{x}	17866	14341	10693	7104	3661	1897
SD	± 353	± 149	± 137	± 128	± 82	± 39
RSD %	1.97	1.03	1.28	1.8	2.23	2.05
Discovery %	100	80.2	59.8	39.7	20.4	10.6
Asn						
Mean \bar{x}	12380	9975	7341	4826	2436	1252
SD	± 407	± 217	± 143	± 146	± 96	± 57
RSD %	3.28	2.17	1.94	3.02	3.94	4.55
Discovery %	100	80.5	59.2	38.9	19.6	10.1
Glu						
Mean \bar{x}	9207	7423	5381	3748	1926	1084
SD	± 369	± 180	± 129	± 102	± 57	± 26
RSD %	4.01	2.42	2.39	2.72	2.95	2.39
Discovery %	100	80.6	58.4	40.7	20.9	11.7

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Gly						
Mean \bar{x}	8242	6663	5005	3328	1779	891
SD	± 158	± 197	± 107	± 91	± 37	± 24
RSD %	1.91	2.95	2.13	2.73	2.07	2.69
Discovery %	100	80.8	60.7	40.3	21.5	10.8
Ala						
Mean \bar{x}	20253	16412	12318	8186	4244	2148
SD	± 434	± 186	± 307	± 93	± 79	± 56
RSD %	2.14	1.13	2.49	1.13	1.86	2.6
Discovery %	100	81	60.8	40.4	20.9	10.6
Val						
Mean \bar{x}	14336	11278	8415	5493	2720	1317
SD	± 347	± 155	± 194	± 120	± 68	± 41
RSD %	2.42	1.37	2.3	2.18	2.5	3.11
Discovery %	100	78.6	58.6	38.3	18.9	9.1
Cys2						
Mean \bar{x}	1672	1308	1046	643	326	151
SD	± 109	± 46	± 61	± 42	± 17	± 9
RSD %	6.51	3.51	5.83	6.53	5.21	5.96
Discovery %	100	78.2	62.5	38.4	19.4	9
Met						
Mean \bar{x}	4169	3288	2513	1674	914	430
SD	± 152	± 116	± 78	± 53	± 48	± 17
RSD %	3.64	3.52	3.1	3.16	5.25	3.95
Discovery %	100	78.8	60.2	40.1	21.9	10.3
Ile						
Mean \bar{x}	8690	6901	5254	3508	1783	984
SD	± 118	± 73	± 112	± 52	± 36	± 28
RSD %	1.35	1.05	2.13	1.48	2.01	2.84
Discovery %	100	79.4	60.4	40.3	20.5	11.3

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Leu						
Mean \bar{x}	14786	11617	8495	5693	2795	1387
SD	± 227	± 196	± 163	± 131	± 74	± 49
RSD %	1.53	1.68	1.91	2.3	2.64	3.53
Discovery %	100	78.5	58.1	38.5	18.9	9.3
Tyr						
Mean \bar{x}	2958	2347	1743	1209	573	312
SD	± 138	± 129	± 69	± 52	± 23	± 14
RSD %	4.66	5.49	3.95	4.3	4.01	4.48
Discovery %	100	79.3	58.9	40.8	19.3	10.5
Phe						
Mean \bar{x}	7083	5672	4238	2721	1393	694
SD	± 109	± 138	± 78	± 63	± 36	± 27
RSD %	1.53	2.43	1.84	2.31	2.58	3.89
Discovery %	100	80.1	59.8	38.4	19.6	9.7
His						
Mean \bar{x}	2736	2164	1613	1085	546	249
SD	± 71	± 53	± 48	± 43	± 18	± 12
RSD %	2.59	2.44	2.97	3.96	3.29	4.81
Discovery %	100	79.1	58.9	39.6	19.9	9.1
Trp						
Mean \bar{x}	3780	2934	2236	1579	728	348
SD	± 83	± 88	± 105	± 63	± 21	± 15
RSD %	2.19	2.99	4.69	3.98	2.88	4.31
Discovery %	100	77.8	59.1	41.7	19.2	9.2
Lys						
Mean \bar{x}	8369	6736	5012	3312	1670	822
SD	± 91	± 84	± 75	± 49	± 32	± 43
RSD %	1.08	1.24	1.49	1.47	1.91	5.23
Discovery %	100	80.4	59.8	39.5	19.9	9.8

NH4						
Mean \bar{x}	27864	22820	16914	11677	5642	2832
SD	± 364	± 242	± 441	± 408	± 218	± 140
RSD %	1.3	1.06	2.6	3.49	3.86	4.94
Discovery %	100	81.8	60.7	41.9	20.2	10.1
Arg						
Mean \bar{x}	7328	5905	4265	2853	1532	785
SD	± 175	± 88	± 137	± 108	± 38	± 53
RSD %	2.38	1.49	3.21	3.78	2.48	6.75
Discovery %	100	80.5	58.3	38.9	20.9	10.7
Pro						
Mean \bar{x}	3386	2715	1962	1364	646	314
SD	± 113	± 147	± 68	± 56	± 29	± 17
RSD %	3.33	5.41	3.46	4.1	4.48	5.41
Discovery %	100	80.1	57.9	40.2	19	9.2

Linearity

The linearity is important for the confirmation of the method's sensitivity for the analysis of the amino acid concentration within a defined range.

Linearity is the ability to obtain test results which are directly proportional to the concentration of the amino acid. Linearity was determined by five injections of six different concentrations (injection volume 5 µl, 10 µl, 20 µl, 30 µl, 40 µl, 50 µl). The average peak areas were plotted against concentrations. Then linearity was evaluated using the calibration curve to calculate coefficient of correlation. Table 7). In general a value of correlation coefficient (R^2) \gg 0.990 is considered as the evidence of an acceptable fit for the data to the regression line.

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Table 7: Resulted linearity of plotting average peak area against different concentrations of amino acids.

Amino Acid	R ²
Asp	0.9999
Thr	0.9996
Ser	0.9999
Asn	0.9999
Glu	0.9997
Gly	0.9995
Ala	0.9996
Val	0.9994
Cys2	0.9989
Met	0.9996
Ile	0.9994
Leu	0.9998
Nle	0.9990
Tyr	0.9991
Phe	0.9991
His	0.9993
Trp	0.9999
Lys	0.9996
NH ₄	0.9999
Arg	0.9997
Pro	0.9968

Table 8: Limit of detection and limit of qualification for each amino acid, analysed in cell culture media.

Amino Acid	LOD nmol/g	LOQ nmol/g
Asp	1.9	5.7
Thr	1.4	4.2
Ser	1.8	5.4
Asn	1.9	5.7
Glu	1.2	3.6
Gly	1.6	4.8
Ala	1.7	5.1
Val	1.1	3.3
Cys2	1.4	4.2
Met	1.5	4.5
Ile	1.8	5.4
Leu	1.3	4.2
Tyr	1.4	4.2
Phe	1.7	5.1
His	1.5	4.5
Trp	1.3	3.9
Lys	1.6	4.8
NH ₄	2.1	6.3
Arg	1.5	4.4
Pro	1.8	5.4

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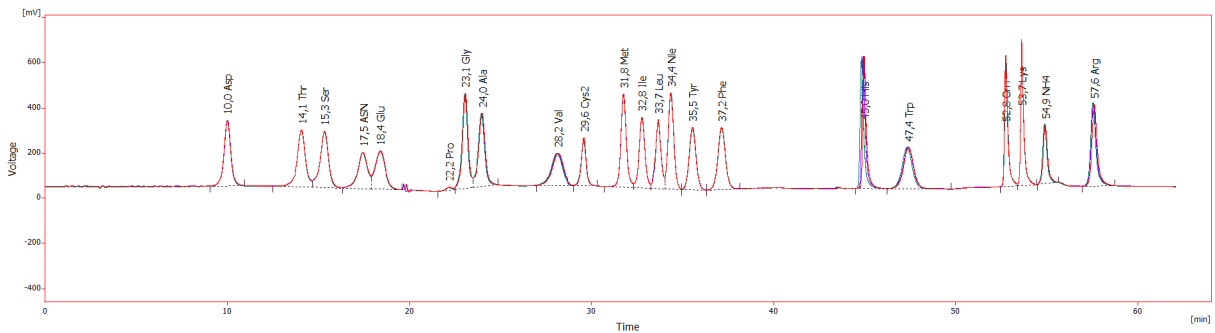


Figure 2: Overlap of three chromatograms of the amino acid standard.

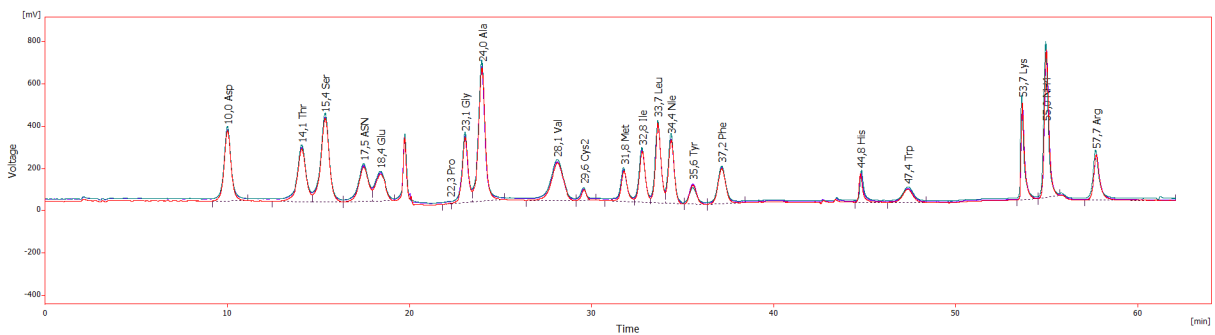


Figure 3: Overlap of three chromatograms of one cell media sample.

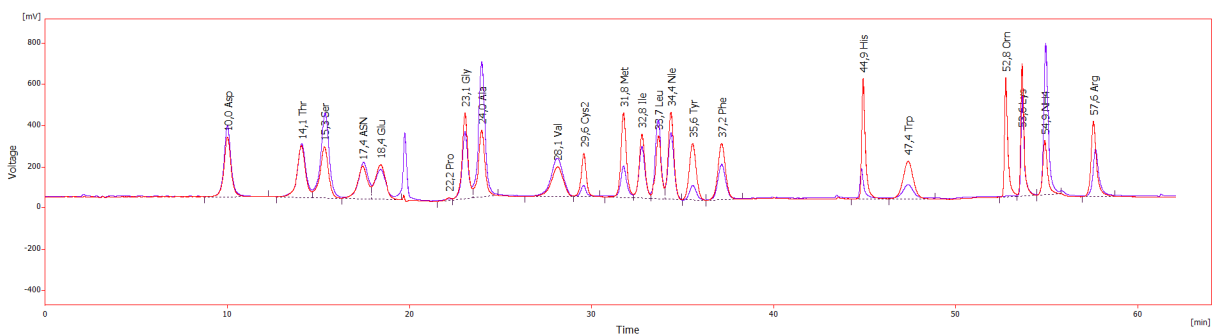


Figure 4: Overlap of one chromatogram of the amino acid standard and one chromatogram of the cell media sample.

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Conclusion

The method was validated by using various validation parameters like accuracy, precision and linearity. All the validation parameters were found to be well within the acceptance criteria. It is shown, the method was precise, accurate, linear, reproducible and repeatable. These results show the method could find practical application for the analysis of the amino acids in cell culture media.

References

- Salazar, M. Keusgen, J. von Hagen, Amino Acids in the cultivation of mammalian cells, Amino Acids (2016) 48:1161-1171.

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