

MILLENNIUM APPLICATION

BRIEF

TOPIC: Calculation of ~~Amino Acid Residues~~ Using the Millennium Custom Calculation Feature

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Introduction:

Recently a customer using our ~~Amo~~ system was interested in being able to calculate the number of residues of each amino acid present in their original peptide or protein sample. This calculation is very easy to do using the millennium custom calculation feature. This calculation is simple the average number of all amino acids present in the sample divided into each amino acid amount. To do this in millennium you first need to make sure that you have set the AMQ and NH3 peaks to none for quantitate by in the integration method. Second, you need to create a custom field for the average amount present by creating a custom result field (Avg_Amt) as shown below.

The screenshot shows the 'Custom Field: Avg_Amt' dialog box with the following settings:

- Field Type:** Result (selected)
- Data Source:** Calculated (selected), ave(Amount)
- Data Type:** Real (0.0) (selected)
- Limits:** Width: 2, Precision: 3, Min Value: -9999999.999, Max Value: 10000000.000
- Field Order:** 4
- Default Value:** (empty text box)
- Help Text:** (empty text box)
- Order field will be processed in:** (empty text box)

Next, you need to set up the average amount calculation by selecting formula and entering the following formula.

Edit Custom Field Formula

Avg_Amt `ave(Amount)`

Fields

- % Amount
- % Area
- % Height
- % Time Corr. in Area
- % Poly < MVM1

Operations

- +
-
- *
- /
- **

OK Cancel Help

Finally, you need to now set up a peak custom field which divides each peak amount by the Avg_Amt field you just created. Shown below is the field and formula used.

Custom Field: Residues
File Edit Help

Field Type

- ☐ Sample
- ☐ Residue
- ☒ Peak

Data Source

- ☐ Keyboard ☐ Entry Required ☐ Allow Suffix
- ☒ Calculated `Amount/Avg_Amt`
- ☐ External ☐ Calibration Field ☐ Avg Col Points

Data Type

- ☐ Integer (0)
- ☒ Real (0.0)
- ☐ Text
- ☐ True / False
- ☐ Date

Limits

Width:

Precision:

Min Value:

Max Value:

Field Order

Default Value:

Help Text:

Order field will be processed in

Edit Custom Field Formula

Residues Amount/Avg_Amt

Fields

% Amount	↑
% Area	
% Height	
% Time Corr. In Area	
% Poly < MWM1	↓

Operations

+	↑
-	
*	
/	
**	↓

OK
Cancel
Help

Attached is an example report which incorporates this new field in the report showing not only pmol of each amino acid present but also the residue numbers. Once set up this field can be used for all future projects.

AccQ*Tag Method Sample Information

Sample Name: AMH 210

Vial: 3

Sample Type: Unknown

Injection: 1

Run Time: 45.0 min

Volume: 10.00

Channel: SATIN

Set Name 17

Channel Descr. 474 ex 250nm em395

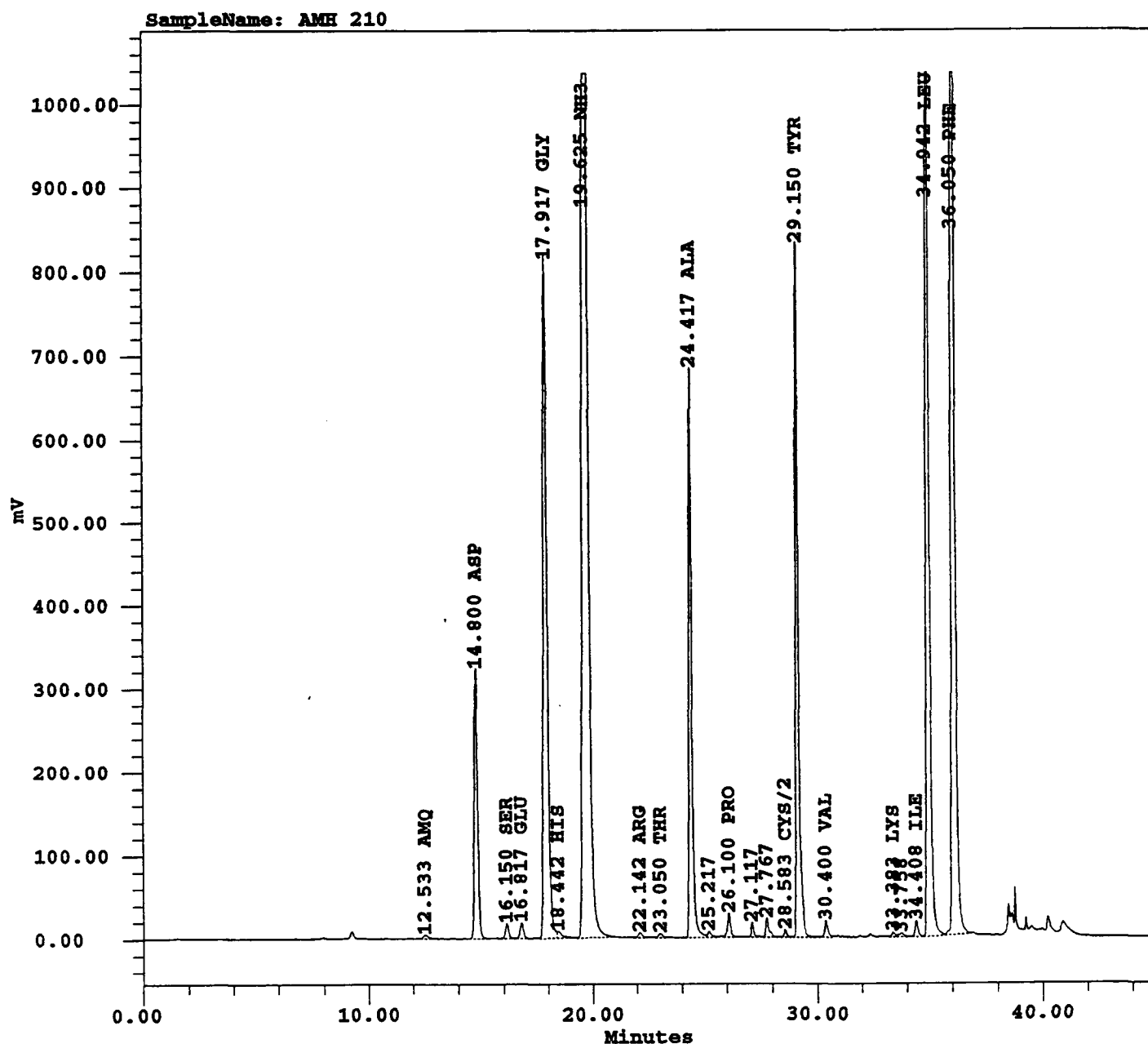
Project Name: AccQTag_NE

Acq Meth Set: AccQ_Tag_MS2

Date Acquired: 03/17/95 11:42:52 AM

Processing Method: AccQ_Tag_PM3a

Date Processed: 03/20/95 11:14:09 PM



Result Table Information

SampleName AMH 210

Vial 3 Injection 1

Set Name 17

Processing Method: AccQ_Tag_PM3a

Peak Results

#	Amino Acid	Ret Time (min)	Area (uV*sec)	Height (uV)	Pmol	Residues
1	AMQ	12.53	58397	4128		
2	ASP	14.80	3317675	327406	158.03	2.172
3	SER	16.15	184833	17435	6.40	0.088
4	GLU	16.82	194659	18439	8.10	0.111
5	GLY	17.92	9576438	822847	354.88	4.877
6	HIS	18.44	105973	7454	2.58	0.035
7	NH3	19.62	21934737	1035023		
8	ARG	22.14	65913	5786	1.77	0.024
9	THR	23.05	54192	4834	1.55	0.021
10	ALA	24.42	6049472	700452	166.45	2.288
11		25.22	86275	7375		
12	PRO	26.10	271478	28778	14.24	0.196
13		27.12	120273	17261		
14		27.77	207457	23159		
15	CYS/2	28.58	64802	8832	6.87	0.094
16	TYR	29.15	6633751	844483	167.88	2.307
17	VAL	30.40	189566	19812	2.77	0.038
18	MET	30.88				
19	LYS	33.38	47356	5529	1.54	0.021
20		33.76	56088	4047		
21	ILE	34.41	169008	18886	1.94	0.027
22	LEU	34.94	11840549	1034862	140.13	1.926
23	PHE	36.05	13619184	1033248	129.02	1.773