HORIBA

Explore the future

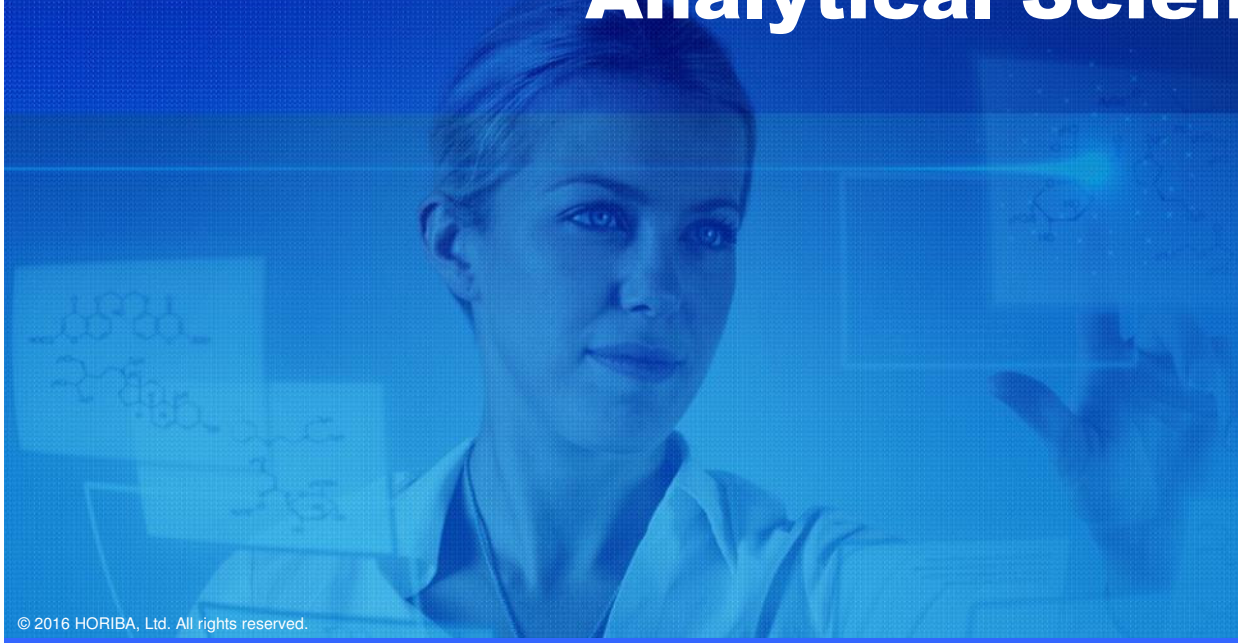
HORIBA Scientific

In Partnership with deltaDOT Ltd.

Dr. Stuart Hassard

Label Free Capillary Electrophoresis can be a Powerful Tool in Analytical Science

April 2016



Introduction to the HPCE-512

Superior Capillary Electrophoresis

HPCE-512 technology selling into the biomedical, biopharmaceutical and analytics markets. The company has a sold nearly 30 systems so far.

At its core is the proprietary Label Free Intrinsic Imaging (LFII®) approach. LFII® has significant advantages over other approaches.

It has a wide spread of applications and customers in many sectors including biotech, academic, industrial and government sectors.

This technology addresses the PAGE, HPLC and to some extent the Mass Spec markets.

- **Biopharmaceutical production, QC/QA**
- **Vaccine Development**
- **Diagnostics – Human & Veterinary**
- **Food & Drink**
- **Proteomics**
- **General Biotech**
- **Analytical Chemistry**
- **Small Molecules**

Solving Regulatory Challenges

US Food and Drug Administration (FDA) definition of Process Analytical Technology (PAT):

"A mechanism to design, analyze, and control pharmaceutical manufacturing processes through the measurement of Critical Process Parameters (CPP) which affect Critical Quality Attributes."

FDA requirements for PAT:

- Define and monitor CPP
- Do so in a timely manner that can effect outcomes
- In-line and/or at line
- Reduce processing time (e.g. by ameliorating bias)
- Improve consistency
- Minimize or rapidly identify rejects

Multivariant Capabilities

Low bias and label independent molecule analysis allow true multi-analyte analysis on a single platform.

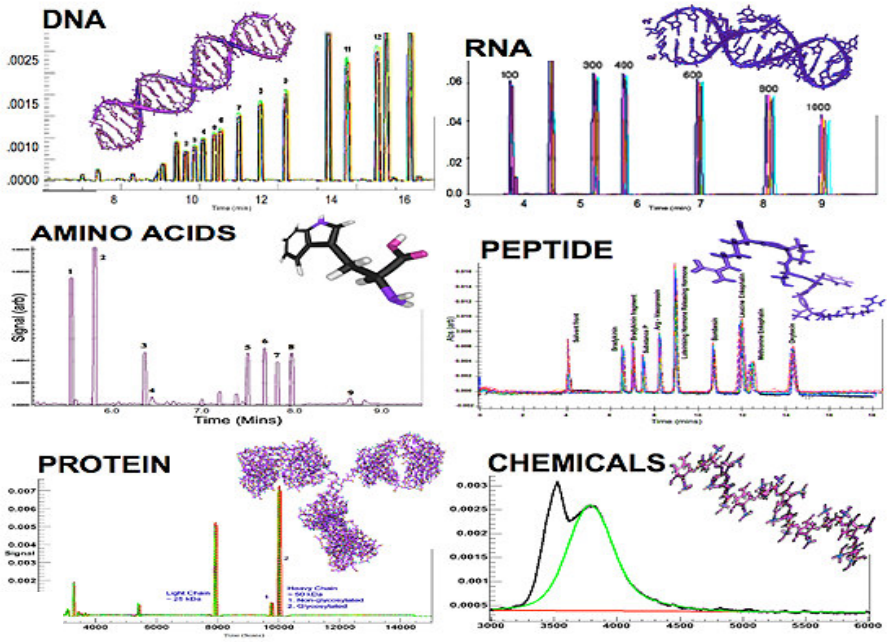
deltaDOT HPCE-512 in the PAT workflow

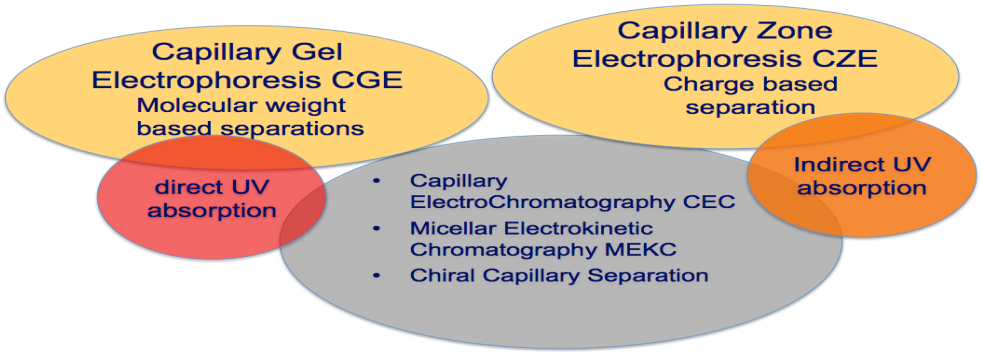
	STARTING MATERIALS	UPSTREAM METHOD DEVELOPMENT	MIDSTREAM	DOWNSTREAM	QA/QC MS TRIAGE	FORMULATION	BATCH RELEASE
WATER	QUALITY			WASTE		QUALITY	
AMINO ACIDS	QUALITY	USAGE OPTIMISATION	USAGE MONITORING SUPPLEMENTATION	USED MEDIA ANALYSIS			
VITAMINS	QUALITY	USAGE OPTIMISATION	USAGE MONITORING SUPPLEMENTATION	WASTE			
CARBOHYDRATES	QUALITY	USAGE OPTIMISATION	USAGE MONITORING SUPPLEMENTATION	WASTE			
CONTAMINATION			LACTIC ACID ENDOTOXIN	RUBISCO NICOTINE	NICOTINE		
DNA		CLONING	CONTAMINATION	CONTAMINATION			
PROTEIN EXPRESSION			QUANTIFICATION QUALITY	QUANTIFICATION QUALITY	QUANTIFICATION QUALITY		
PROTEIN PURIFICATION			QUANTIFICATION QUALITY	QUANTIFICATION QUALITY	POST-TRANSLATIONAL MODIFICATIONS	AGGREGATION	
PROTEIN FRAGMENTATION			COLUMN SHEAR	PRODUCT STABILITY	PRODUCT STABILITY	PRODUCT STABILITY	
PROTEIN FOLDING		PROCESS EFFICIENCY	EXPRESSION EFFICIENCY	PRODUCT STABILITY		PRODUCT STABILITY	
BIOPOTENCY				TARGET AFFINITY		PRODUCT EFFICIENCY	PRODUCT STABILITY
STABILITY						FRAGMENTATION AGGREGATION	PRODUCT FIDELITY

Multivariant Capabilities

Label independent analysis allows true multi-analyte analysis on a single platform.

- Proteins / Antibodies
- Nucleic Acids
- Amino Acids
- Interaction e.g. DAR
- Drugs / Small Molecules
- Vitamins
- Carbohydrates
- Inorganic Acids
- Fatty acids
- Nucleotides

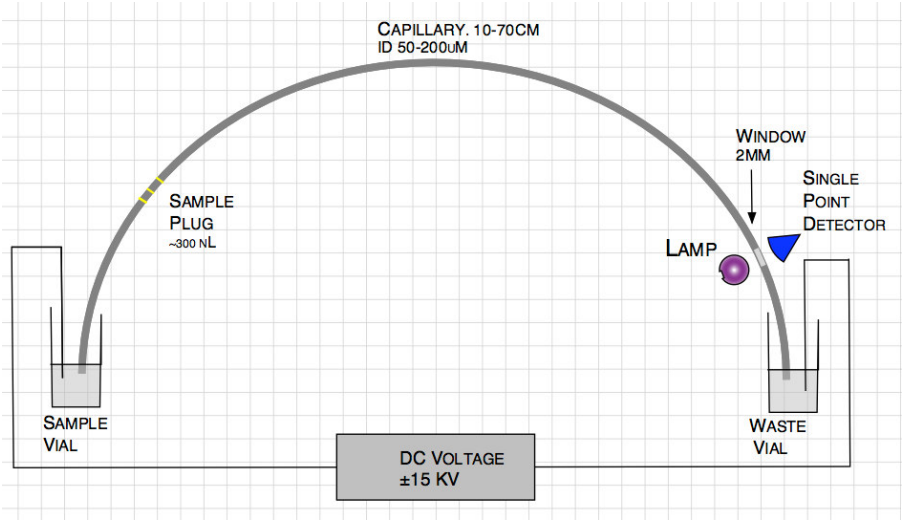




Other Featuers of LFII®

- Novel **Label Free** data acquisition:
  - No potential effects of fluorophores on molecules behaviour.
  - Reduce the costs of analysis
- Highest CE specs for resolution, sensitivity & reproducibility providing the customer with the best possible data:
  - Resolution (CGE – ~200Da, <10bp DNA)
  - Sensitivity (~1.0 µg/ml protein - LOQ)
  - Repeatability (<4% RSD)
  - Quantitative (<2% RSD for simple mixtures, 5% for complex lysates etc.)
  - Linear Dynamic Range (5 orders concentration, 4 in MW)
- UV detection 214 / 254 / 280nm enabling the analysis of Protein, DNA, RNA virus particles and small molecules.

Multipixel Detection Improves CE

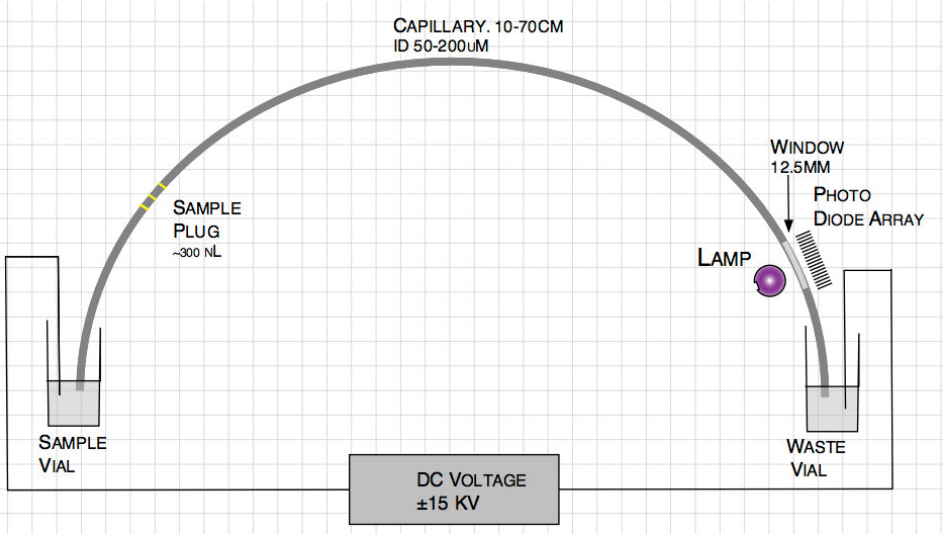


Conventional single point detection systems have a poor reputation due to poor data quality, especially repeatability over different analyses of the same samples.

They measure each analyte only **once** at a single point in time. This reduces the amount of data points available for analysis.

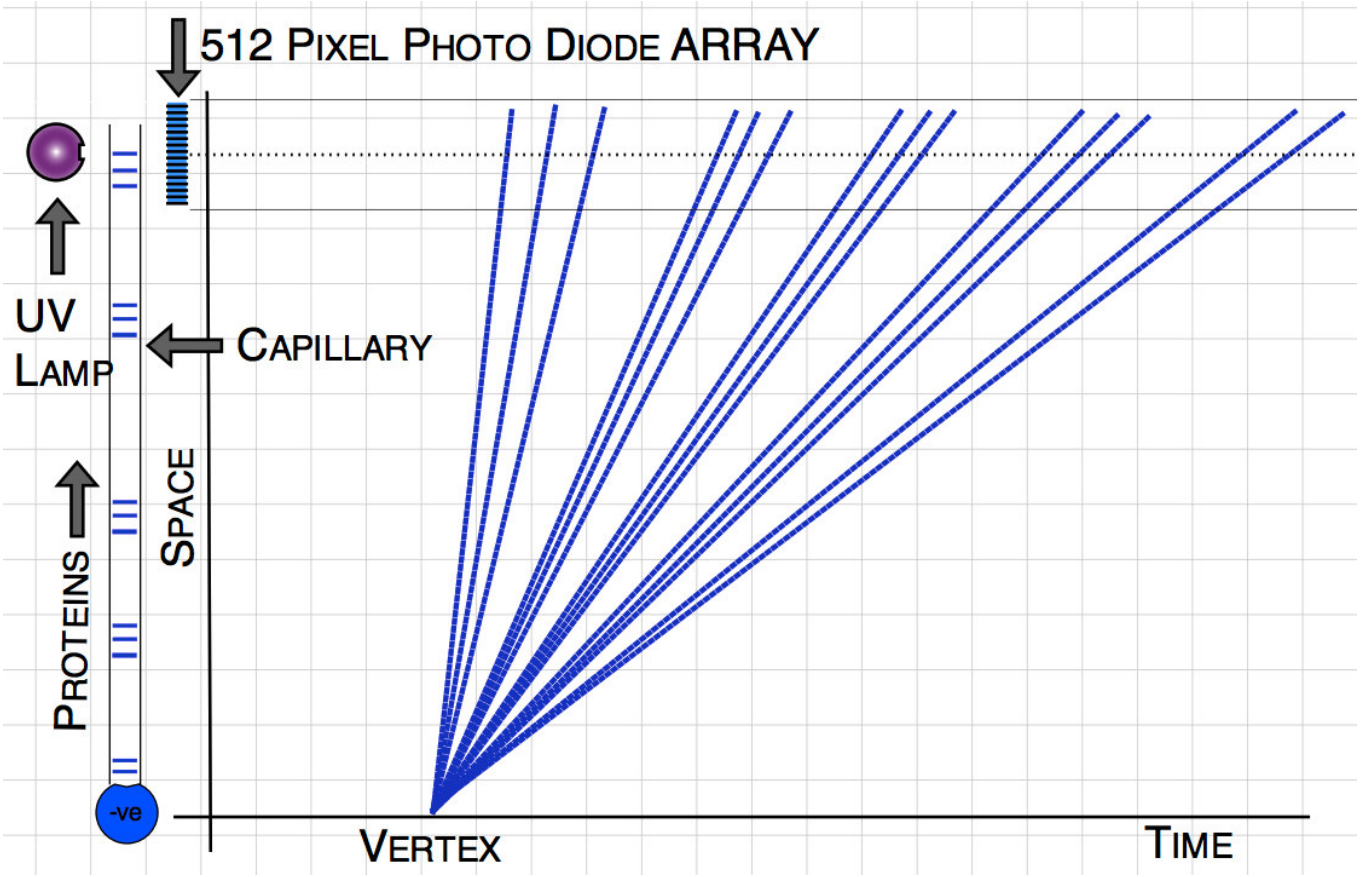
HPCE technology images each analyte band **512** times across a photo diode array at ~**10** times a second.

This results on ~**100 000** data points on a typical analysis including data on both time and band position.



Separations are rapidly performed in a thermally efficient capillary

Multipixel CE Systems

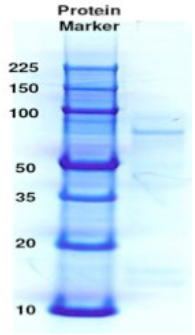


- The slope of the line leading to the vertex is dependent on protein size.
- The straightness of the line indicates the fidelity of the separation conditions.
- These factors improve data quality and repeatability.

Advantages of HPCE

Traditional I-Dimensional Polyacrylamide gel electrophoresis

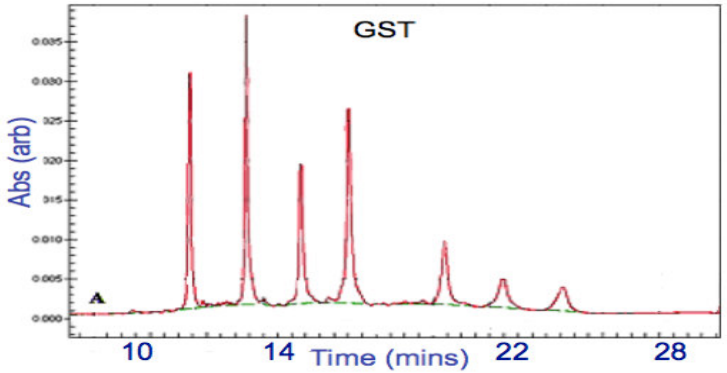
- 1D gels are 1950's Technology.
- They take hours to run, stain, de-stain, dry, analyse and document.
- **They are expensive in time, they are expensive in money**



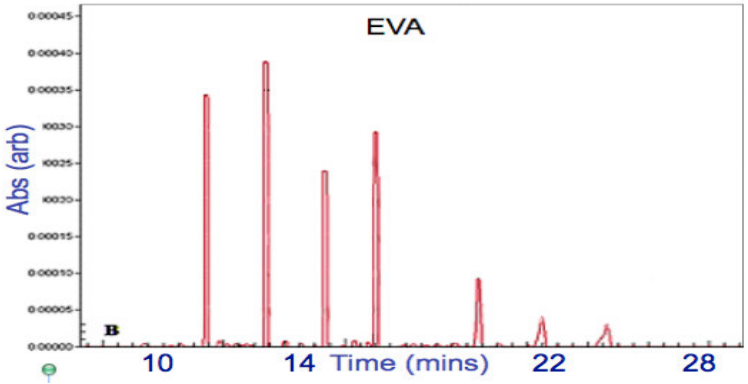
Label Free Intrinsic Imaging gel electrophoresis

- Fast, accurate, inherently digital, quantitative, repeatable and high resolution.
- LFII® has two major signal processing algorithms :-

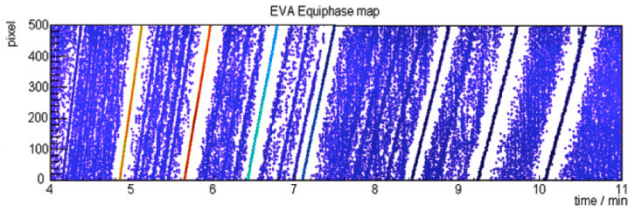
Generalised Separation Transform



Equiphase Vertexing Algorithm



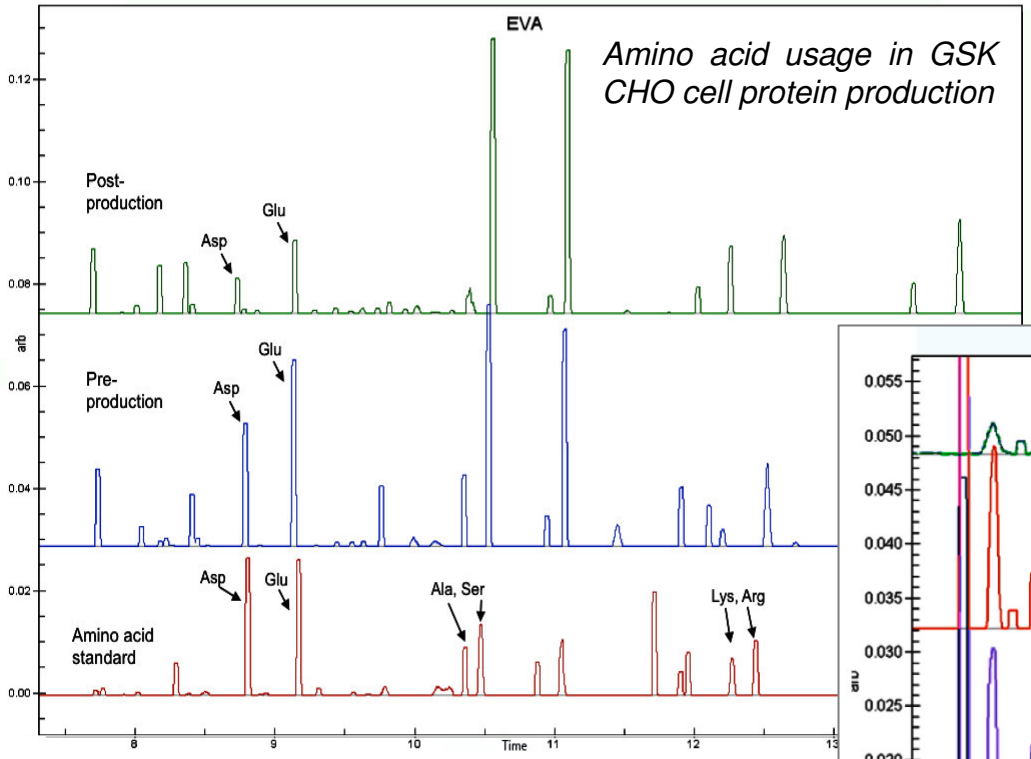
The algorithms produce the **Equiphase** map, an internal self check on the **repeatability** of the system



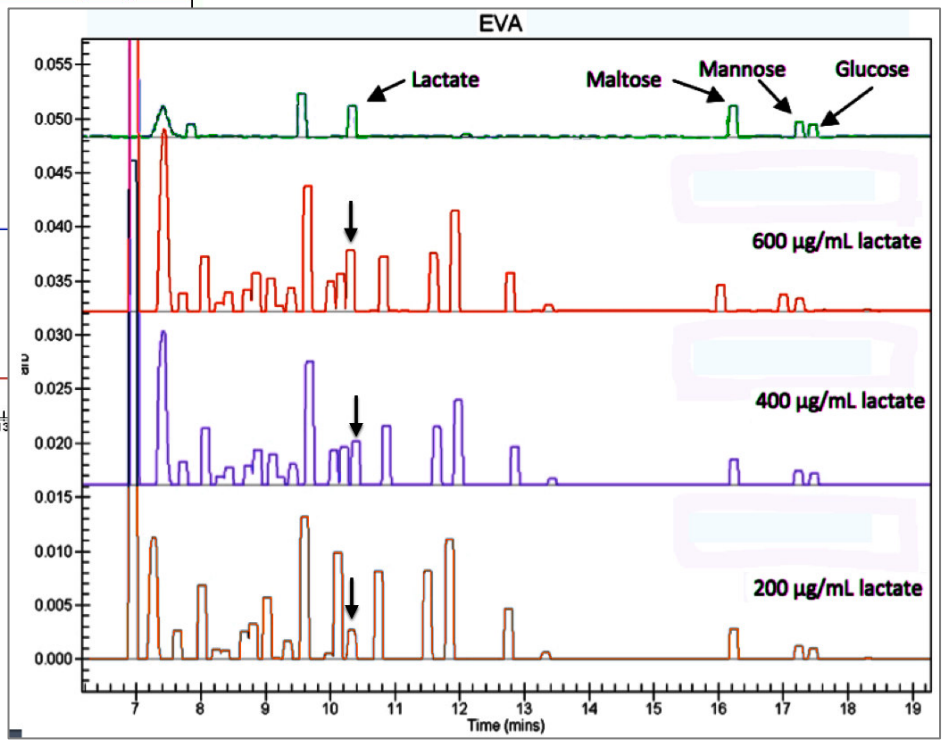
The HPCE-512 out performs Gels and HPLC and can approach MS resolutions

Core Application #1 | Media Quality

Amino acid and sugar analysis in media



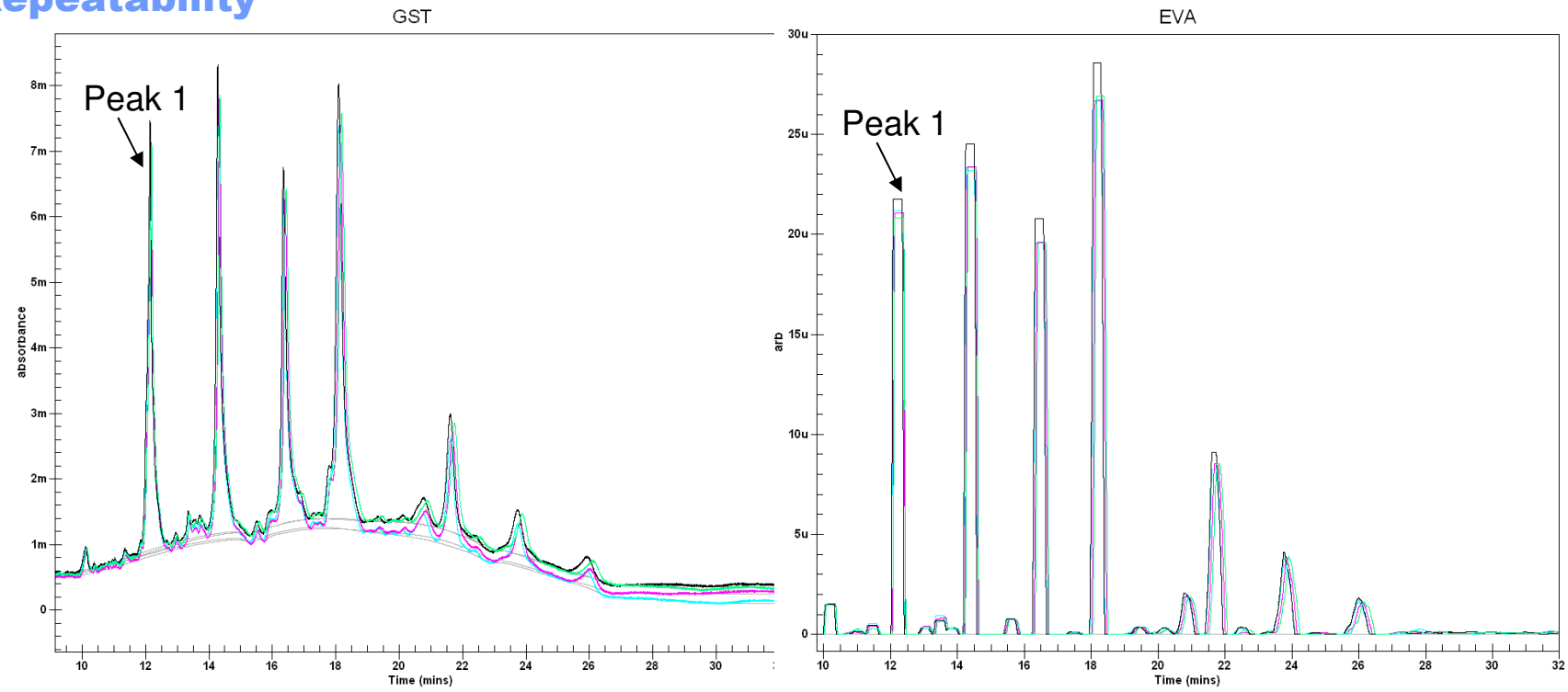
The difference in amino acid levels before and after a protein vaccine is produced can indicate the efficiency of the process. It will also allow timely addition of a heavily used amino acid to increase production.



The technology can also monitor the uptake of sugars such as Mannose, Maltose and Glucose and harmful by-products such as Lactic Acid (Lactate), which can ruin a production run.

Core Application #2 | General Protein Analysis

Repeatability

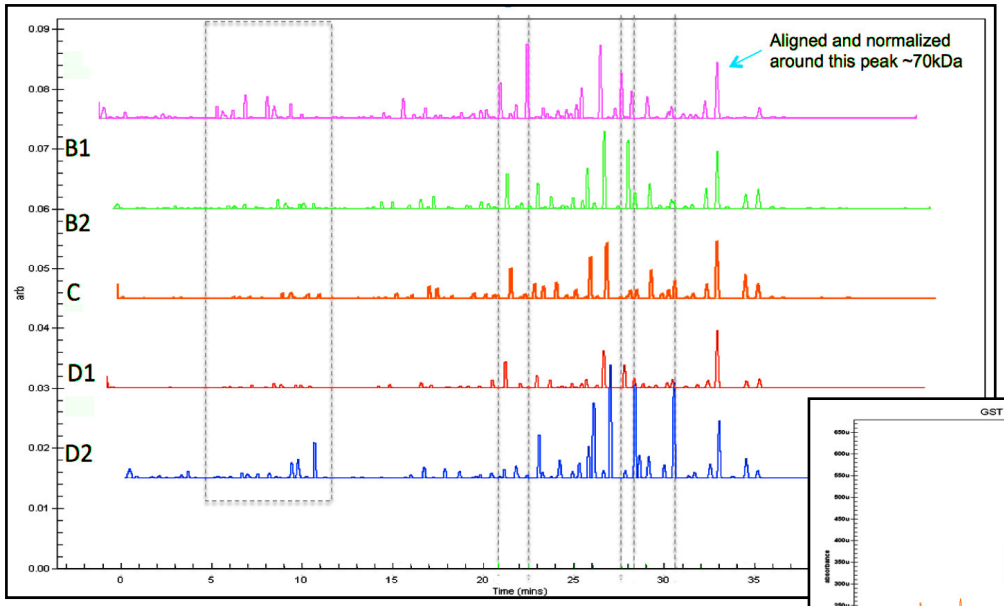


GST electropherogram and EVA trace depicting the separation of the molecular weight ladder. The 7 protein peaks are readily resolved. Repeatability was assessed by calculating relative standard deviation (RSD) of peak migration time and peak area for peak 1.
Excellent repeatability of **0.2%** for peak time and **1.99%** for peak area were obtained.

This system software prompts the user to create a calibration curve from this ladder allowing molecular weight assignments to 2 decimal places for protein identification.

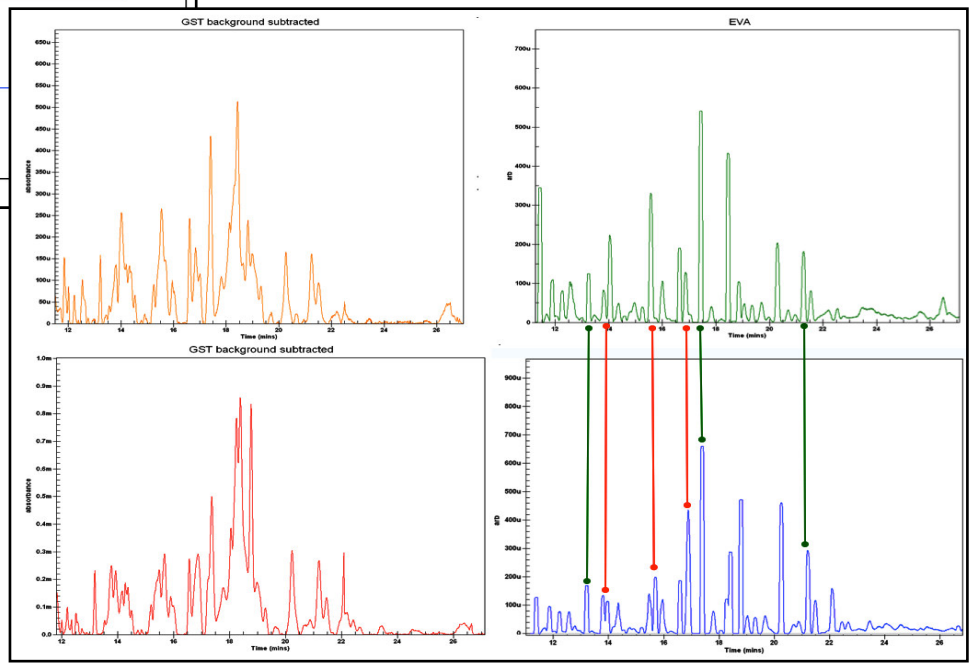
Core Application #2 | General Protein Analysis

Production / Purification Monitoring Technology



Expression level comparisons - bacteria wild type and genetically engineered strains.

The EVA format allows easy identification of regions where differential expression levels are observed. These are highlighted with grey dashed lines/boxes.

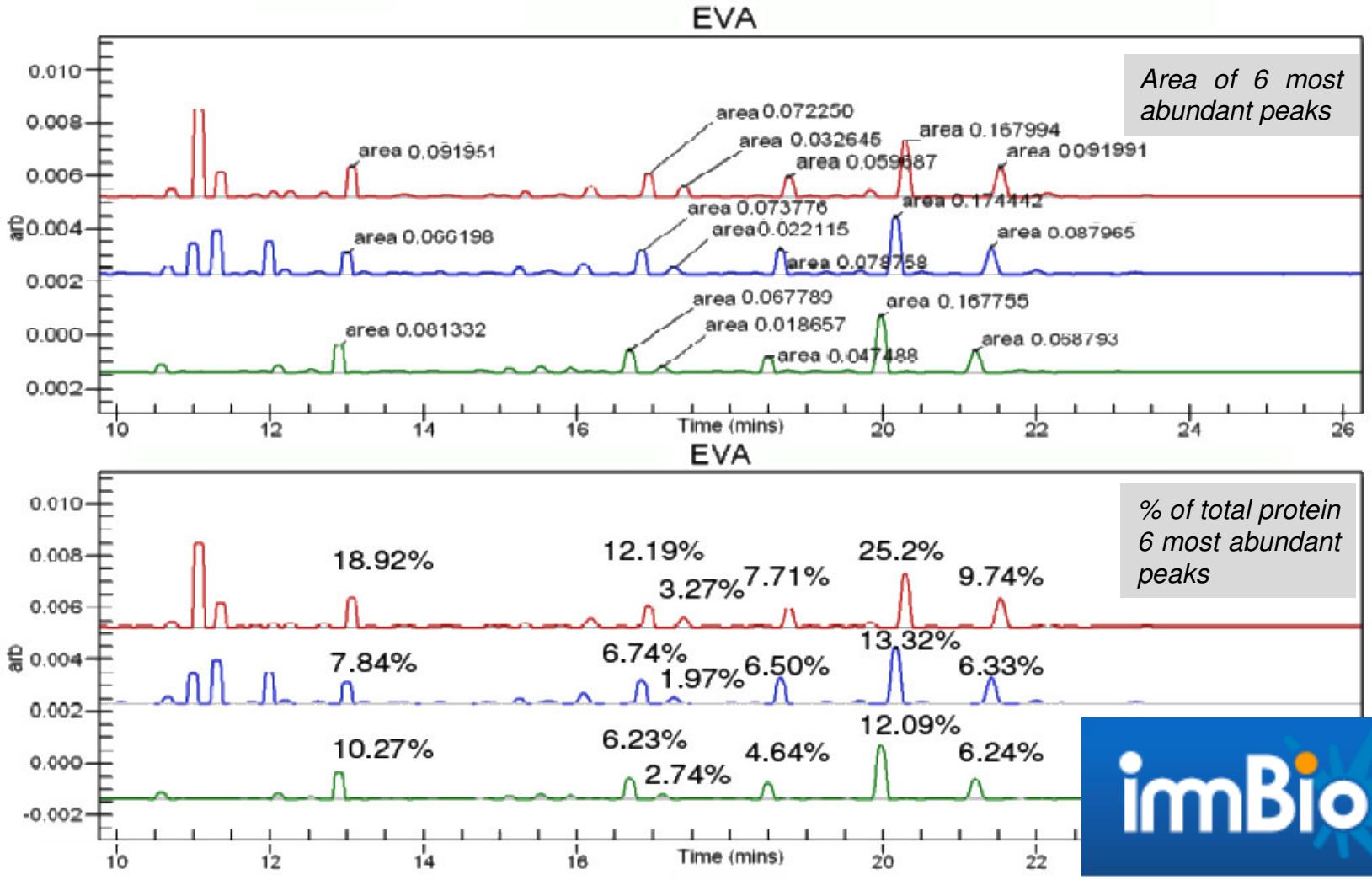


Stem cell lysate analysis

The two related stem cell lysates shown both significant differences and strong similarities in the expression profiles.

Core Application #2 | General Protein Analysis

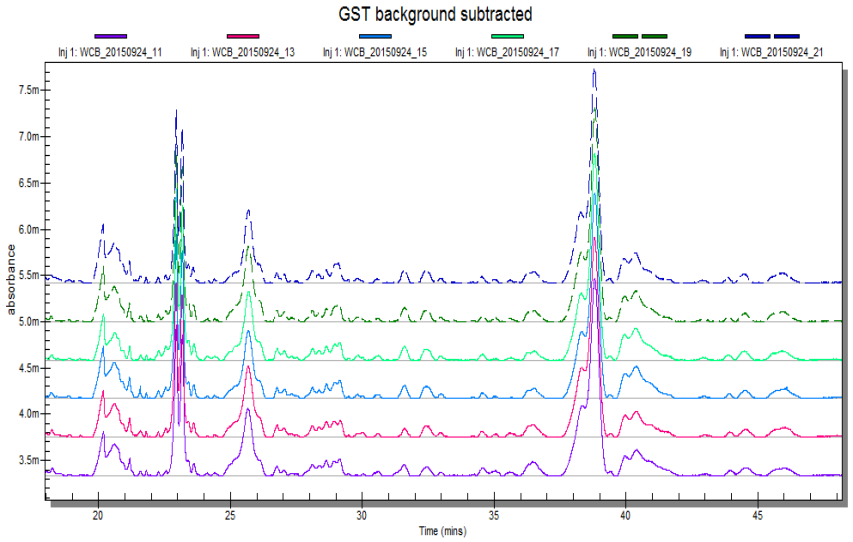
Formulation, Vaccine Stability Analysis



Analysis of Quantitative Fingerprint Data

Student's T-test - Methodology

Step 1: Perform six consecutive runs of each pair sample batch and record the % peak area of each fingerprint region.



	batch 1 run 1	batch 1 run 2	batch 1 run 3	batch 1 run 4	batch 1 run 5	batch 1 run 6
Peak 1	5.07	4.85	5.32	5.35	5.76	5.09
Peak 2	6.64	6.23	6.59	6.66	7.03	6.61
Peak 3	10.01	10.52	10.56	10.48	10.32	10.47
Peak 4	4.94	5.35	5.29	5.41	4.94	5.19
Peak 5	39.56	39.30	39.88	40.28	39.09	39.73
Peak 6	2.50	2.41	2.13	2.29	2.83	2.31
Region A	9.29	8.25	8.18	8.34	8.88	8.82
Region B	4.92	4.02	3.91	3.91	3.44	3.97
Region C	11.16	13.19	13.27	11.89	12.28	12.72
Region D	1.43	1.35	1.39	1.43	1.77	1.55
Region E	4.49	4.52	3.47	3.96	3.65	3.54

Step 2: perform Student's T-test for each quantitative fingerprint region between each pair of sample batches to determine the p-value - the probability of obtaining a result equal to or more extreme than what was actually observed.

Step 3: Define a threshold value (also known as significance level α, traditionally 5% or 1%).
If the p-value is equal to or smaller than the significance level (α), it suggests that the observed data are inconsistent with the assumption that the null hypothesis is true and thus that hypothesis must be rejected.

Analysis of Quantitative Fingerprint Data

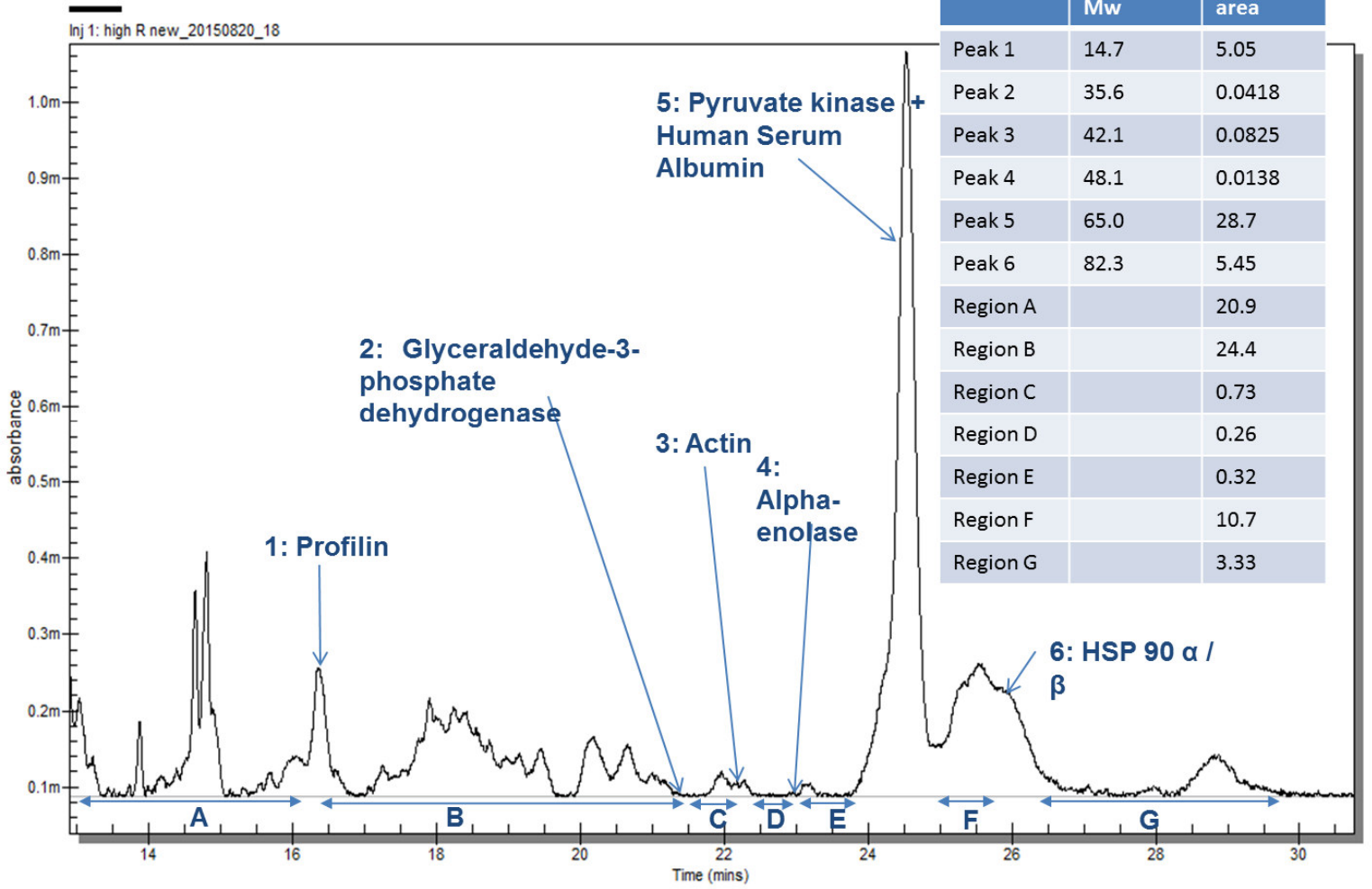
Student's T-test – Between Sample Batch 1 & Batch 2

	batch 1 run 1	batch 1 run 2	batch 1 run 3	batch 1 run 4	batch 1 run 5	batch 1 run 6	batch 2 run 1	batch 2 run 2	batch 2 run 3	batch 2 run 4	batch 2 run 5	batch 2 run 6	*p-value*
Peak 1	5.07	4.85	5.32	5.35	5.76	5.09	10.735	10.366	11.085	11.999	11.172	10.545	1.21E-09
Peak 2	6.64	6.23	6.59	6.66	7.03	6.61	9.435	9.449	9.413	10.112	9.635	9.651	2.15E-09
Peak 3	10.01	10.52	10.56	10.48	10.32	10.47	12.521	13.765	11.972	12.339	13.356	12.064	2.40E-05
Peak 4	4.94	5.35	5.29	5.41	4.94	5.19	7.94	7.375	7.051	7.5	7.394	7.877	4.58E-08
Peak 5	39.56	39.30	39.88	40.28	39.09	39.73	21.461	20.875	20.65	21.003	20.798	21.116	7.21E-16
Peak 6	2.50	2.41	2.13	2.29	2.83	2.31	2.967	2.85	3.599	3.663	3.304	3.152	4.90E-04
Region A	9.29	8.25	8.18	8.34	8.88	8.82	10.13	11.04	12.43	9.29	10.93	12.94	1.70E-03
Region B	4.92	4.02	3.91	3.91	3.44	3.97	4.79	4.05	3.96	4.34	4.52	3.80	4.07E-01
Region C	11.16	13.19	13.27	11.89	12.28	12.72	13.03	13.29	12.44	12.33	11.97	12.14	7.79E-01
Region D	1.43	1.35	1.39	1.43	1.77	1.55	1.58	1.86	2.19	2.33	2.18	2.21	1.49E-03
Region E	4.49	4.52	3.47	3.96	3.65	3.54	5.41	5.09	5.20	5.10	4.74	4.51	9.98E-04

With an user defined threshold of 0.01, the p-values of 9 out of 11 fingerprint regions are < 0.01. The null hypothesis is rejected. The two sample batches are therefore considered statistically different from each other.

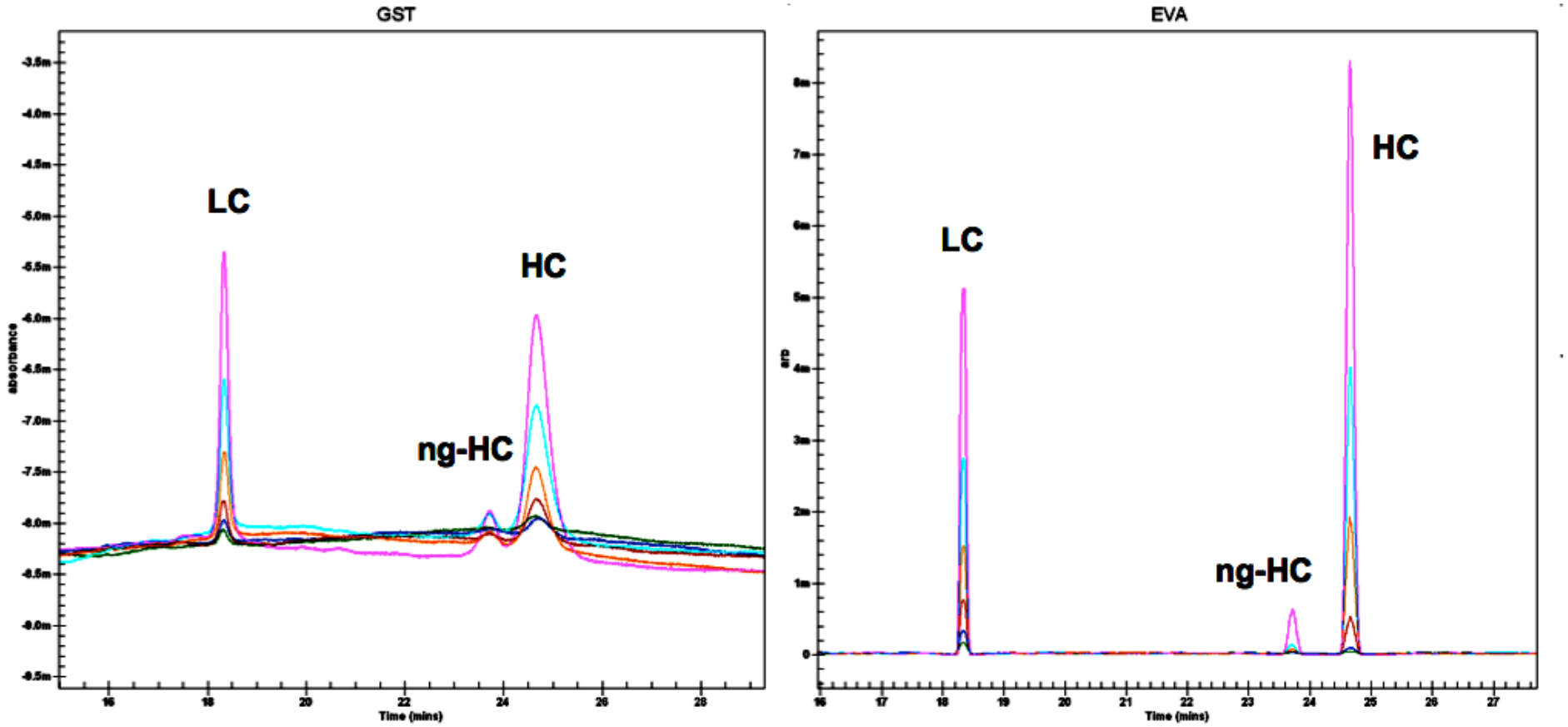
Defining Fingerprint Regions | Sample Batch 1

- 30cm separation length, 24kV separation voltage, 15s injection
- Based on 6 peaks of interest



	Estimated Mw	% peak area
Peak 1	14.7	5.05
Peak 2	35.6	0.0418
Peak 3	42.1	0.0825
Peak 4	48.1	0.0138
Peak 5	65.0	28.7
Peak 6	82.3	5.45
Region A		20.9
Region B		24.4
Region C		0.73
Region D		0.26
Region E		0.32
Region F		10.7
Region G		3.33

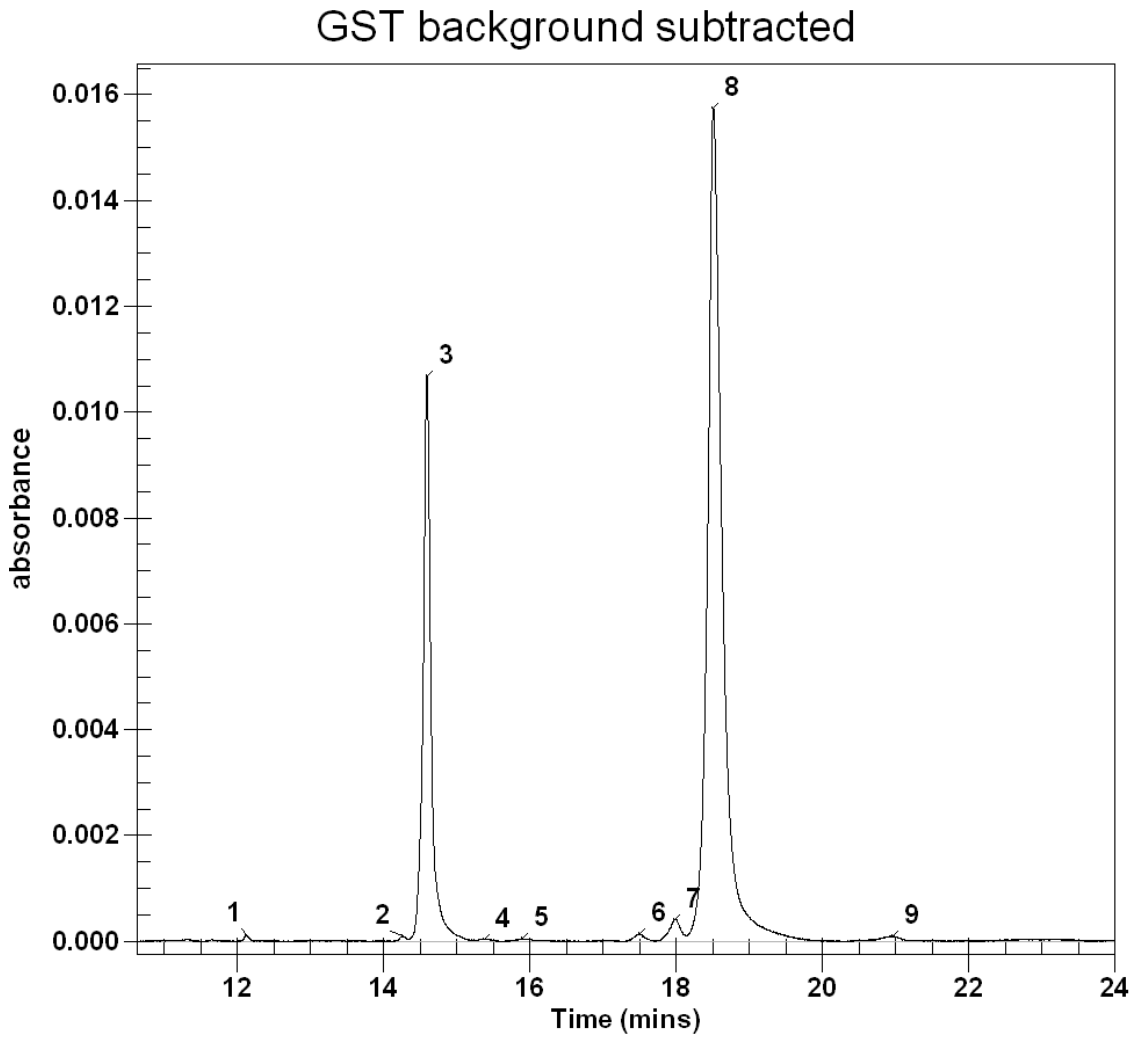
Core Application #3 | Antibody Analysis



GST electropherogram and EVA trace depicting the separation of a standard IgG sample at dilutions of 0.5mg/ml to 0.0156mg/ml. The light chain (LC), heavy chain (HC) and non-glycosylated heavy chain (ng-HC) are clearly resolved and the data is very repeatable.

mAbX | Stock

Reduced

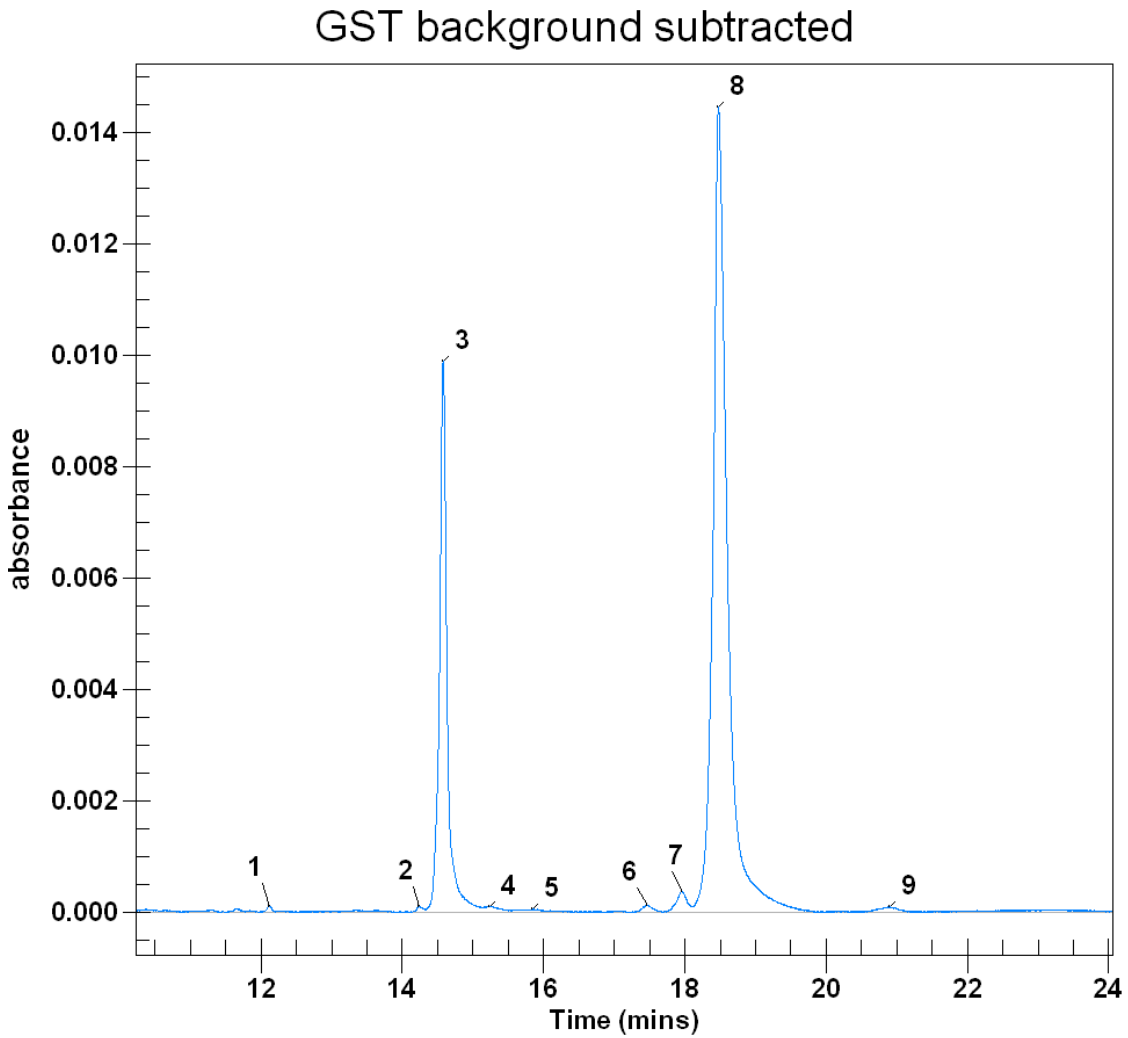


Peak ID	Molecular weight	% Peak Area
1	13.3	0.285
2	20.6	0.228
3	22.2	30.55
4	27.0	0.192
5	30.8	0.296
6	45.7	0.457
7	51.4	1.346
8	58.3	66.059
9	102	0.588

Peak ID	% Peak Area
Light Chain	30.55
Heavy chain	66.059
Others	3.391

mAbX | Preparation in Saline Drip

Reduced

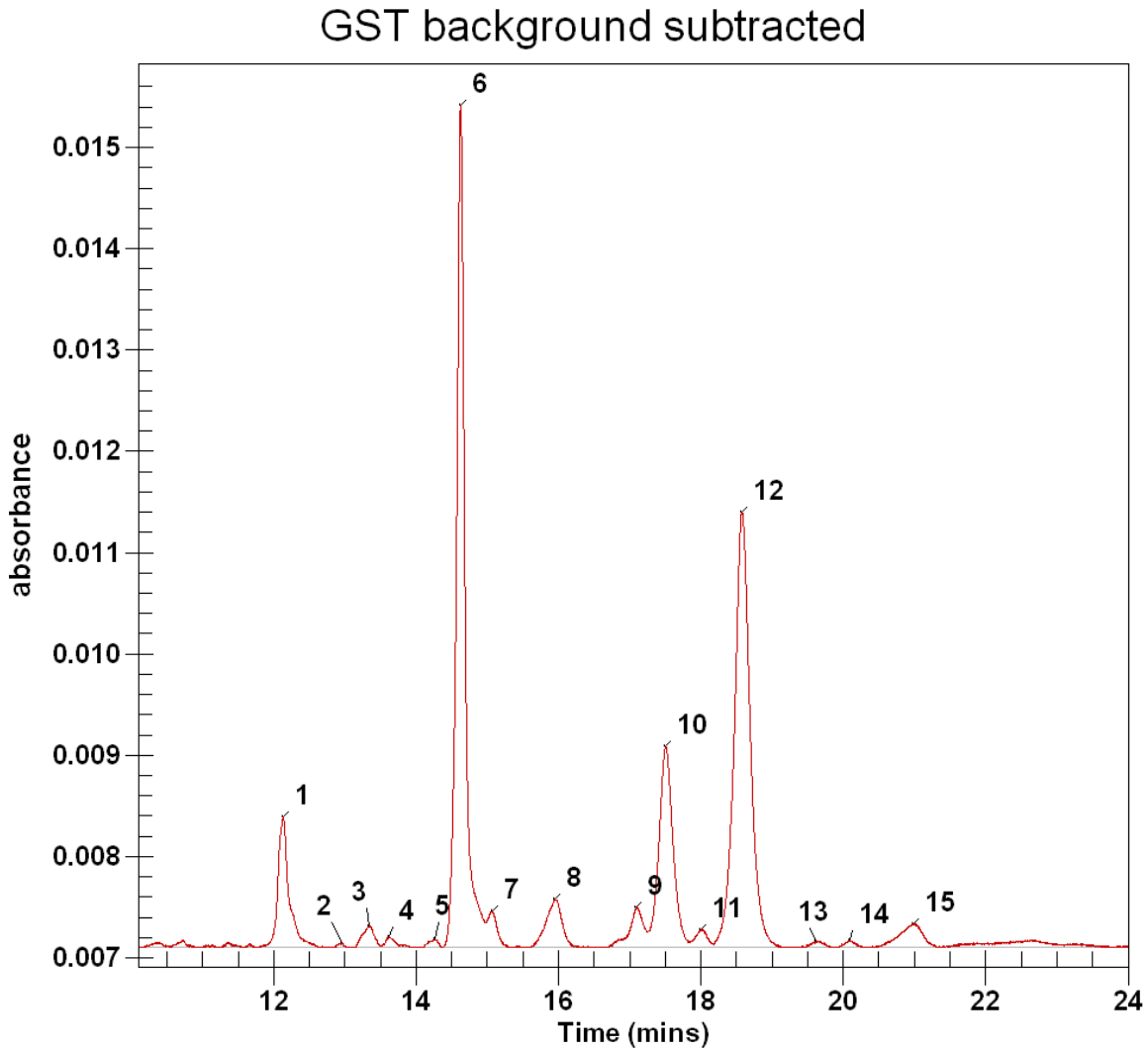


Peak ID	Molecular weight	% Peak Area
1	13.3	0.359
2	20.4	0.246
3	22.1	30.138
4	26.0	0.649
5	30.3	0.51
6	45.4	0.431
7	51.1	1.254
8	57.9	62.891
9	101	0.52

Peak ID	% Peak Area
Light Chain	30.138
Heavy chain	62.891
Others	6.971

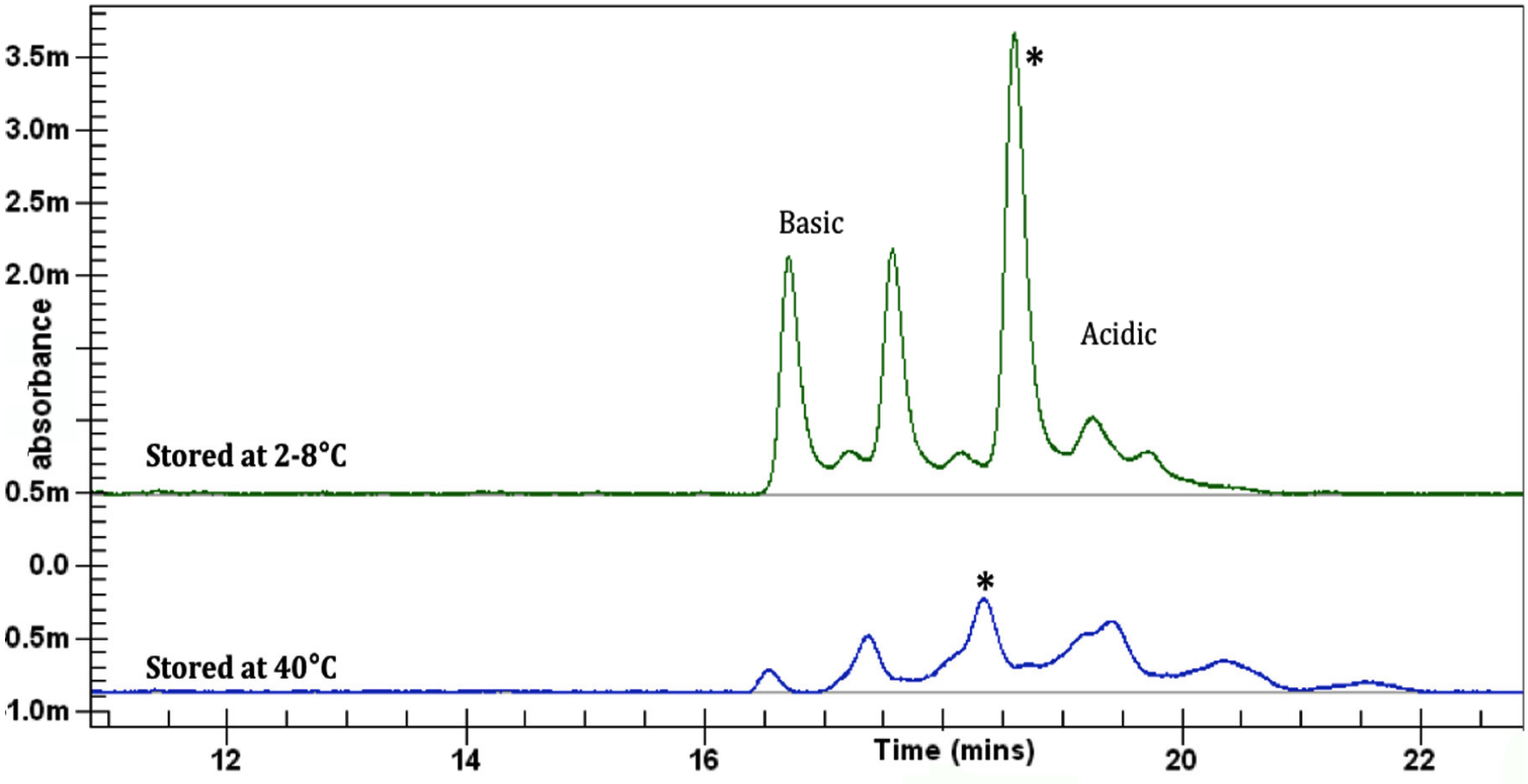
mAbX | Forced Degraded

Reduced



Peak ID	Molecular weight	% Peak Area
1	13.4	8.991
2	15.4	0.159
3	16.7	1.433
4	17.8	0.623
5	20.5	0.412
6	22.4	36.884
7	25.0	1.826
8	31.1	3.629
9	41.3	2.468
10	45.8	12.186
11	51.7	0.92
12	59.4	27.863
13	75.6	0.319
14	84.3	0.278
15	103	2.01

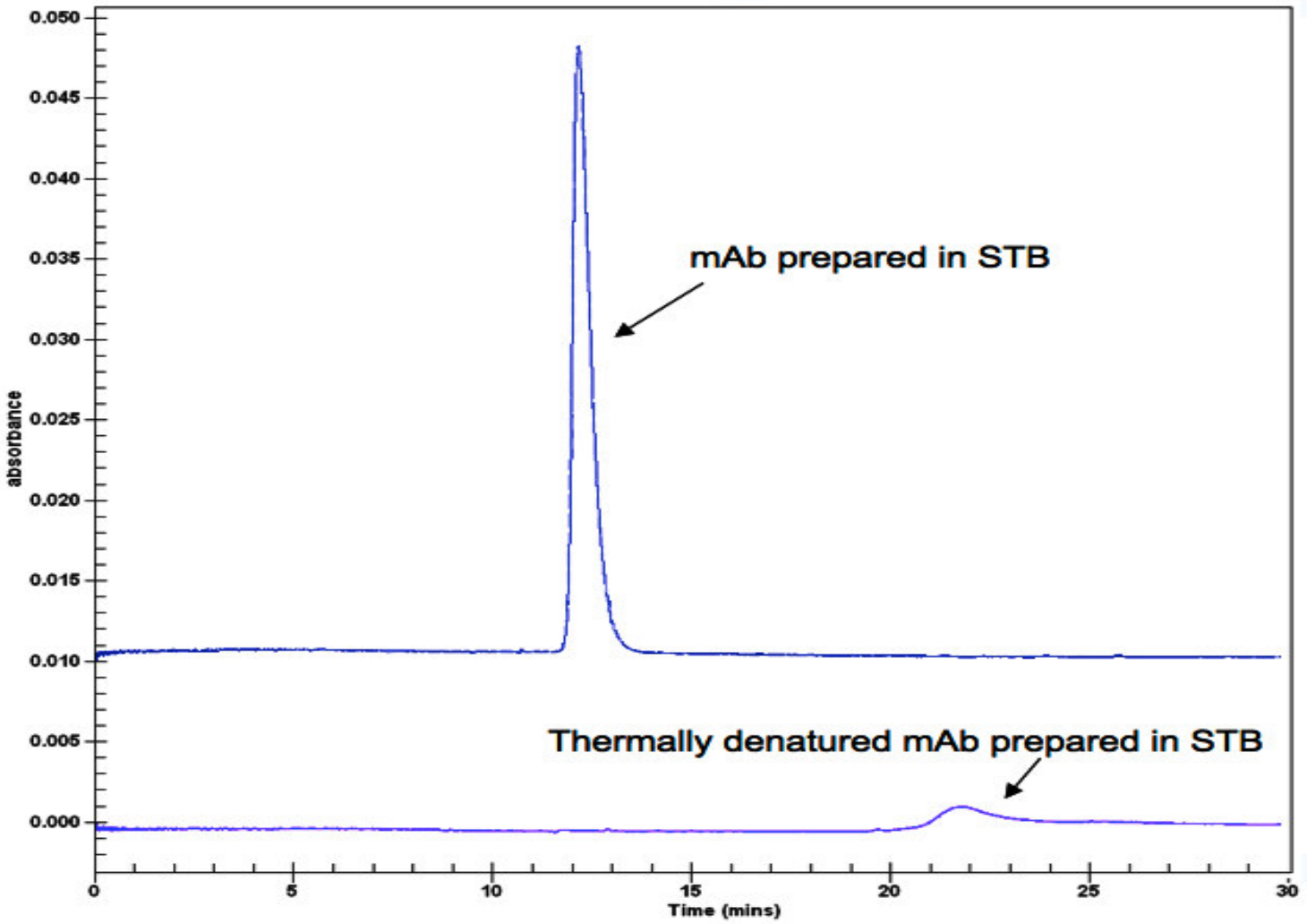
mAb Charge Heterogeneity by CZE



GST electropherogram showing the separation of a 1mg/ml sample the main peak is marked * and the basic and acidic charge variants are shown.

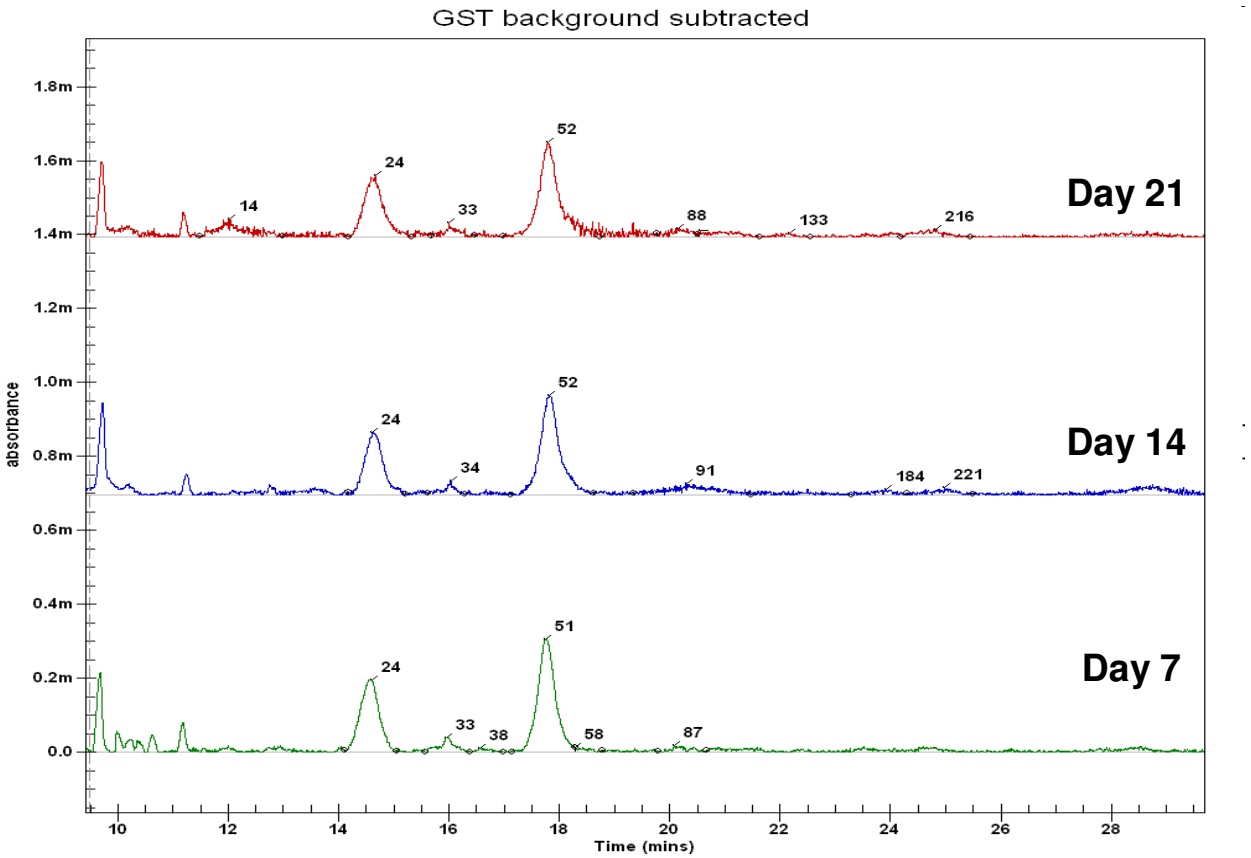
Detection and Analysis of Aggregates

Large Proteins



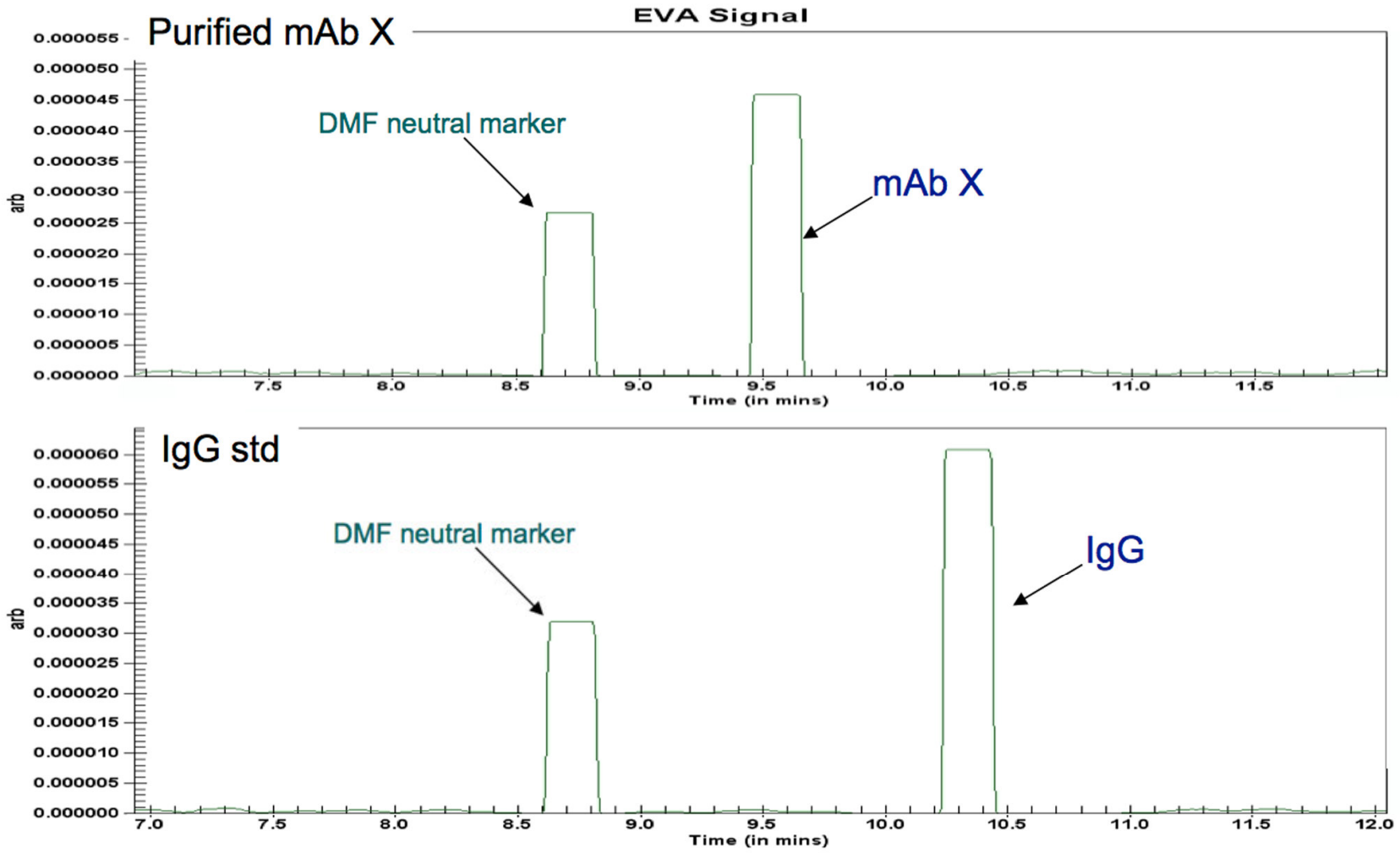
Detection and Analysis of Aggregates

Large Proteins



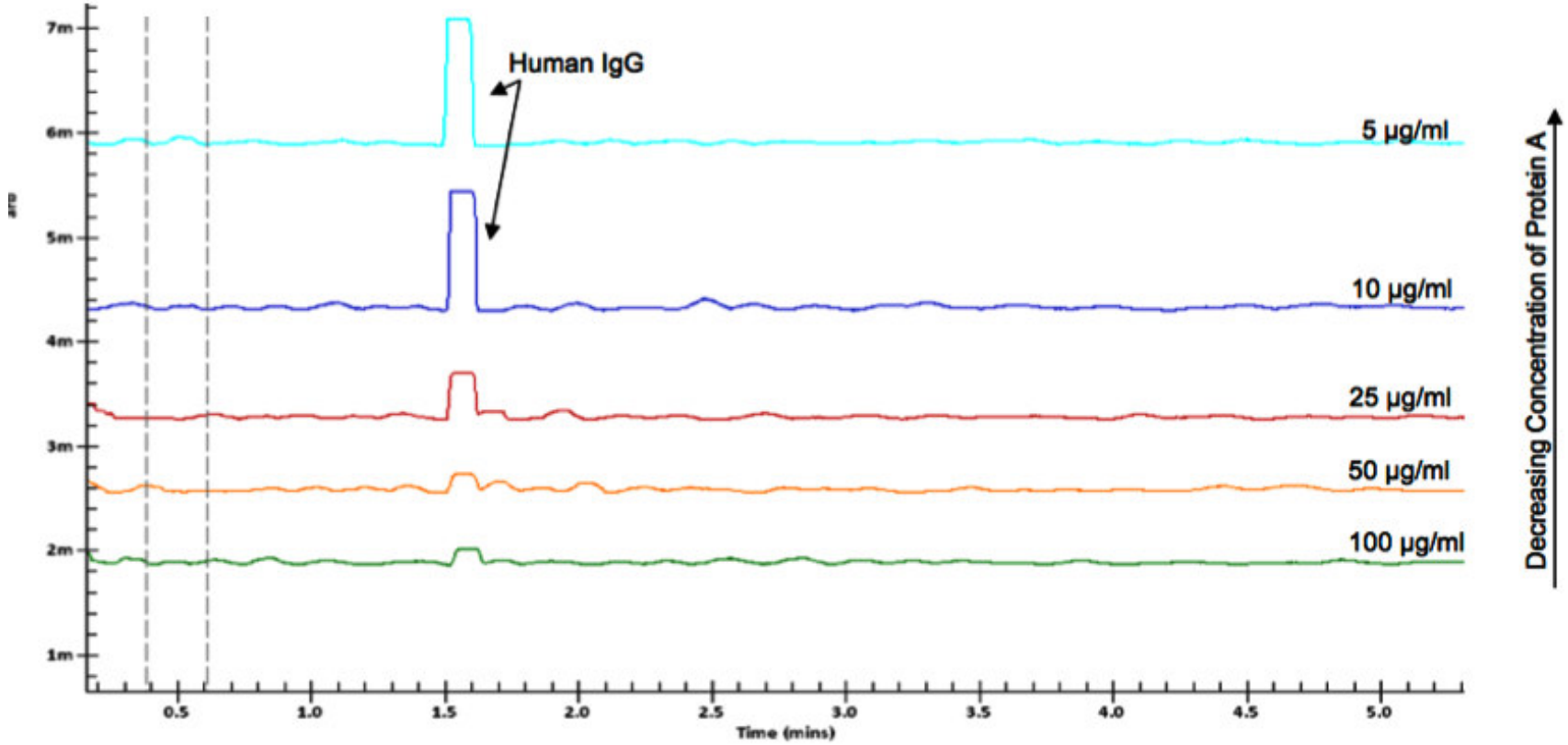
Separation of base treated mAbs. A comparison between the samples analysed at 7, 14 and 21 days is shown. An increased number of very large peaks is observed in the samples analysed on days 14 and 21 compared to the sample analysed on day 7.

Antibody – Non reduced CZE Analysis



Charge and hydrodynamic radius induced migration shift

CZE Analysis | Protein A Titration at pH 8

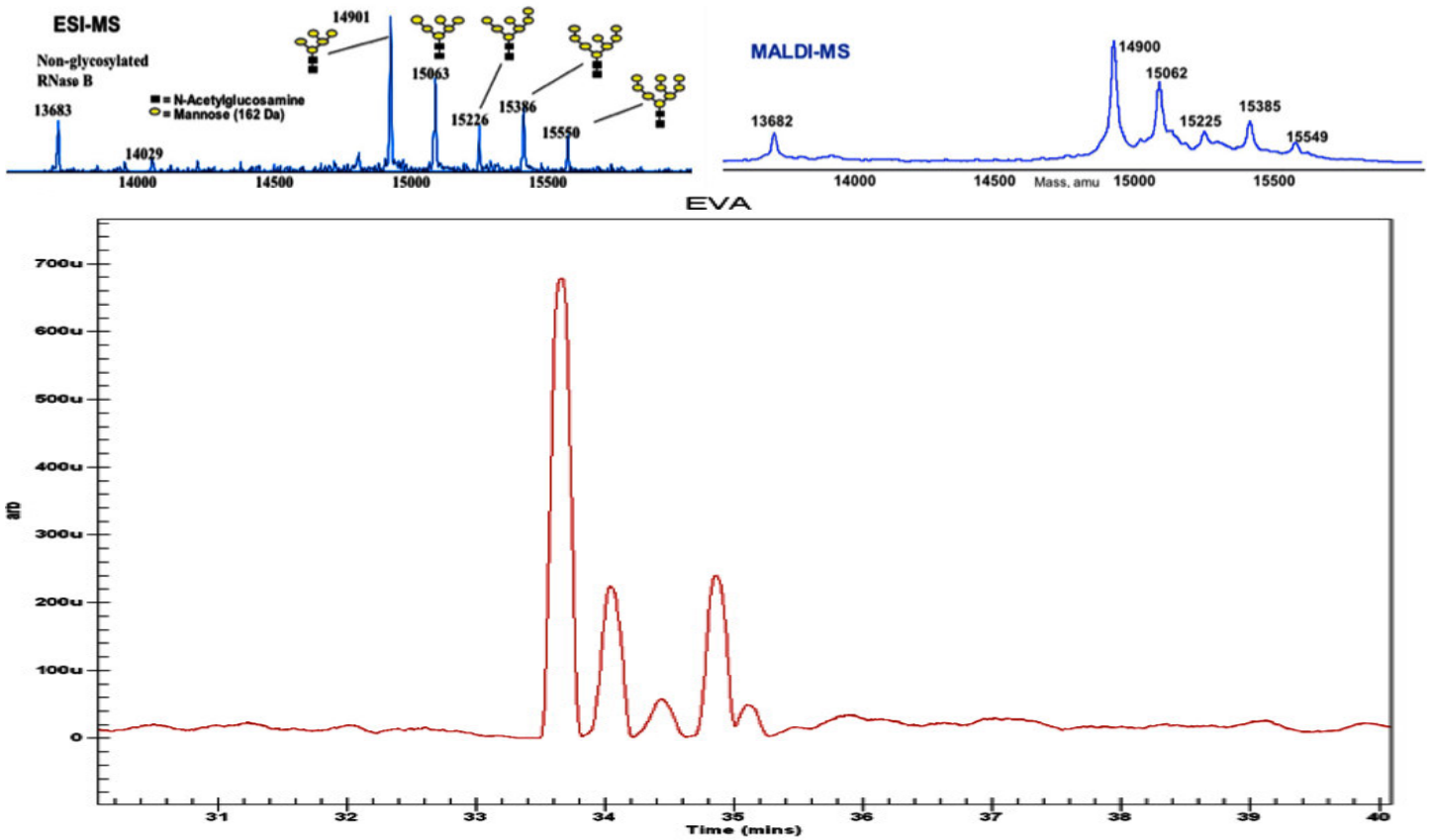


Protein A and IgG were prepared as a mixture in a pH 8 buffer. The concentration of IgG was kept constant at 280 µg/ml while the concentration of Protein A was decreased from 100 µg/ml to 5 µg/ml.

A clear label free stoichiometric interaction is observed leading to the potential to use proteins as biosensors to “count” their ligands.

High Resolution Protein Analysis

Comparison to Mass Spectrometry

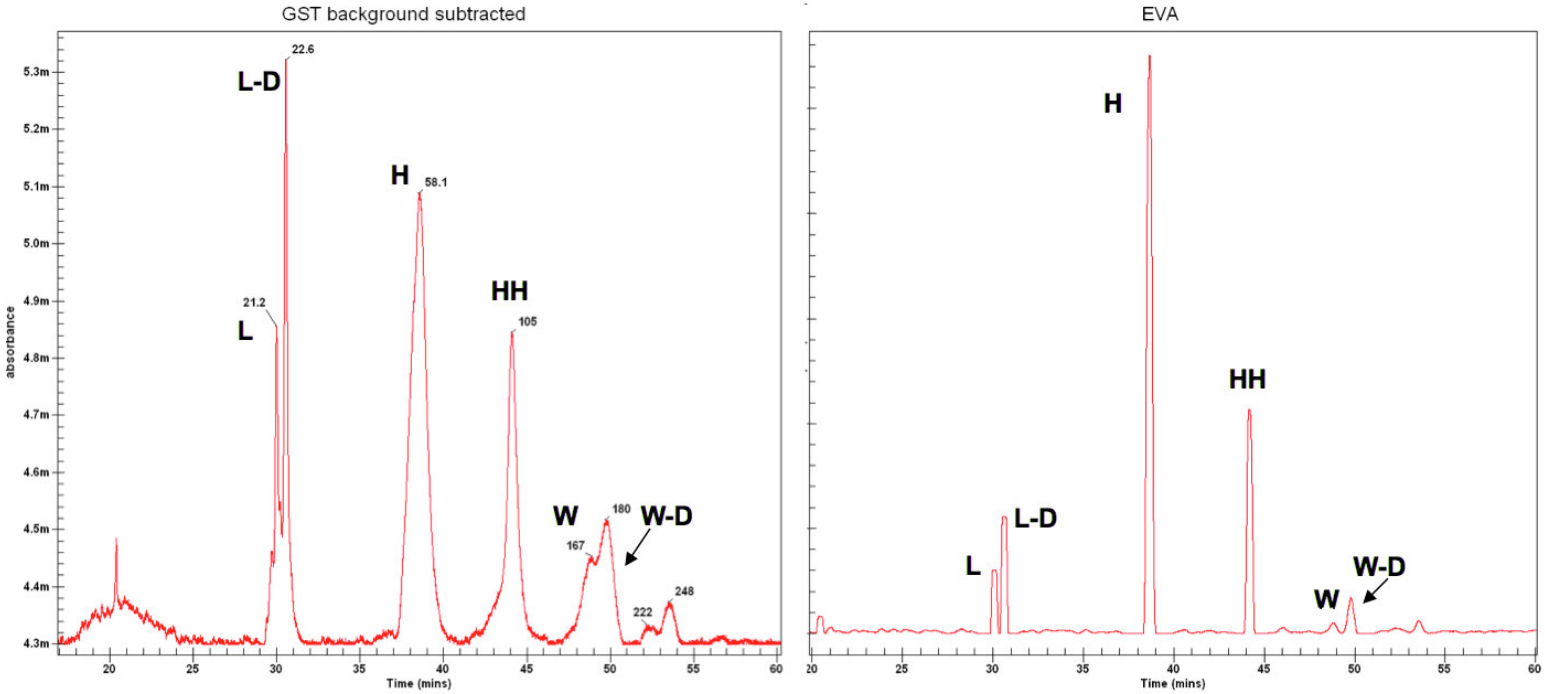


Ribonuclease B Glycoforms can be successfully analysed of HPCE-512 technology comparing favourably to Electron Spray Ionisation MS (ESI-MS $1M) and MALDI-MS ($350K).

HPCE-512 technology can replace MS in some applications.

High Resolution Protein Analysis

Drug Antibody Complexes



GST electropherogram and EVA trace depicting the separation of non-reduced sample.. Possible assignments have been made for each of the peaks; light chain (L), light chain + drug (L-D), heavy chain (H), heavy-heavy (HH), whole antibody (W), whole antibody + drug molecules (W-D).

The higher molecular weight species may be aggregates (222 and 248kDa).

N.B. Peaks with molecular weights above 225kDa are outside the calibration range and are therefore approximations.

Addressed Analytical Targets

- **Media QA/QC**
  - Goods received
  - In process -when to supplement Amino Acid X?
  - Post process - how efficient was my bioprocess?

- **Protein Analysis**
  - Protein expression
  - Protein purification
  - Protein quantification
  - Protein Aggregation
  - Protein Fragmentation
  - Protein charge heterogeneity
  - Protein interactions
  - Formulation and Batch release

HORIBA Scientific

Thank you very much for your attention.

Thank you

Omoshiro-okashiku

Joy and Fun

眞峰

감사합니다

Cảm ơn

ありがとうございました

Dziękuję

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Grazie

Merci

谢谢

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ขอบคุณครับ

Obrigado

Σας ευχαριστούμε

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Tack ska ni ha

Большое спасибо

Danke

Gracias