

In-line middle-up analysis of monoclonal antibodies by capillary electrophoresis

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Introduction

Monoclonal antibodies (mAbs)



Targeted therapy



Microheterogeneities and biological impact



Need for analytical methods for quality control

- Off-line sample preparation



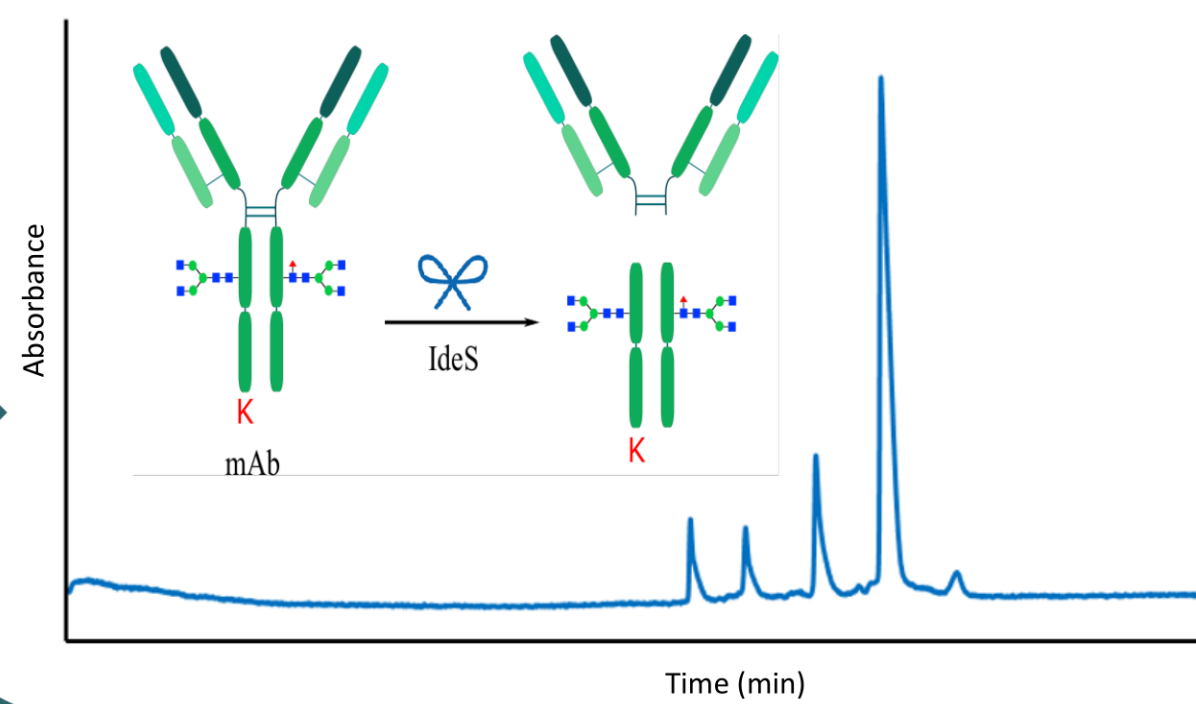
- Multistep
- Time and reactants' consumption
- High contamination risk

- Automated sample preparation

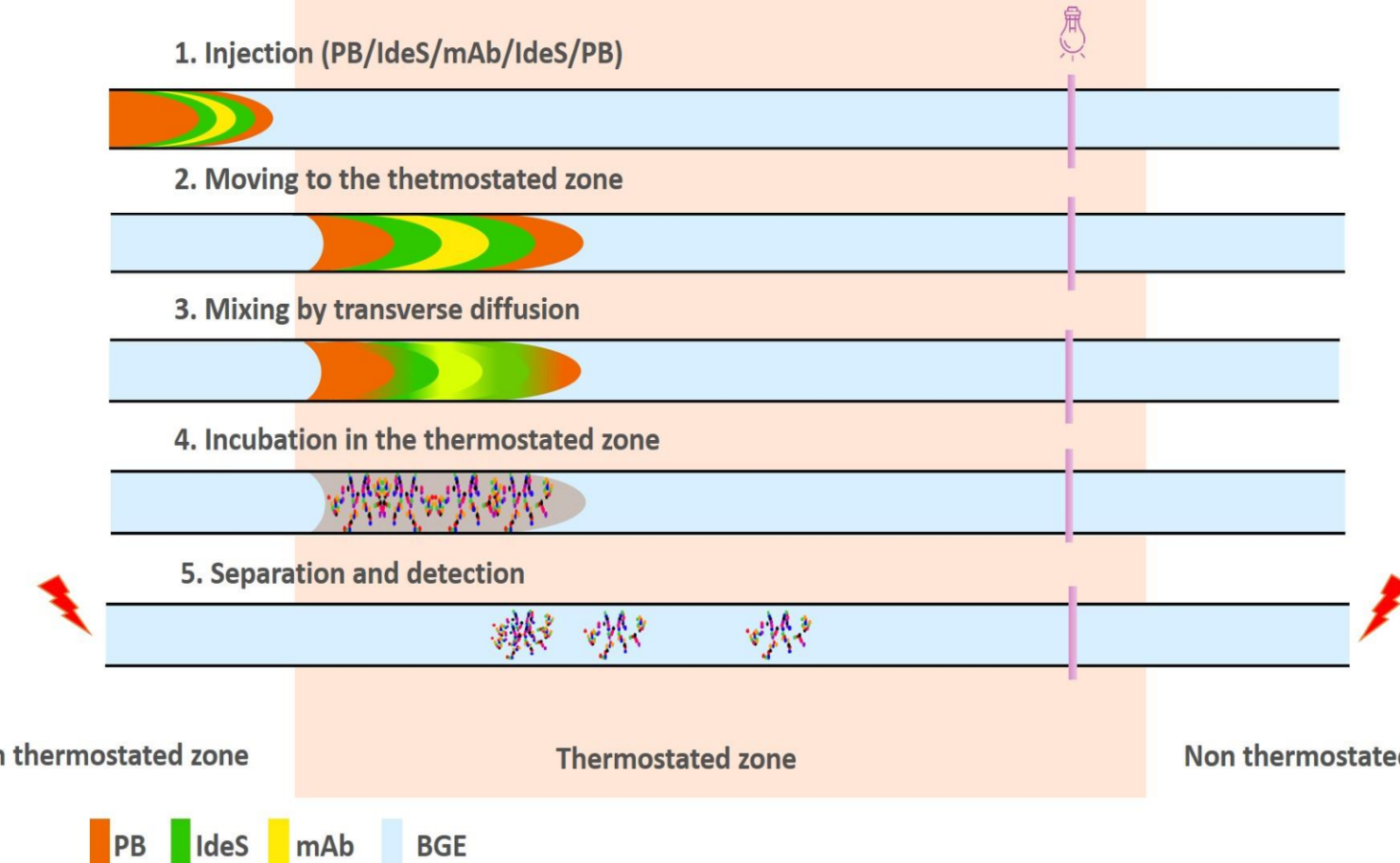
- Fast and controlled conditions
- Low reactants' consumption
- Reduced contamination risk

Capillary electrophoresis

In-line sample preparation + High resolution of separation



Transverse diffusion of laminar flow profile

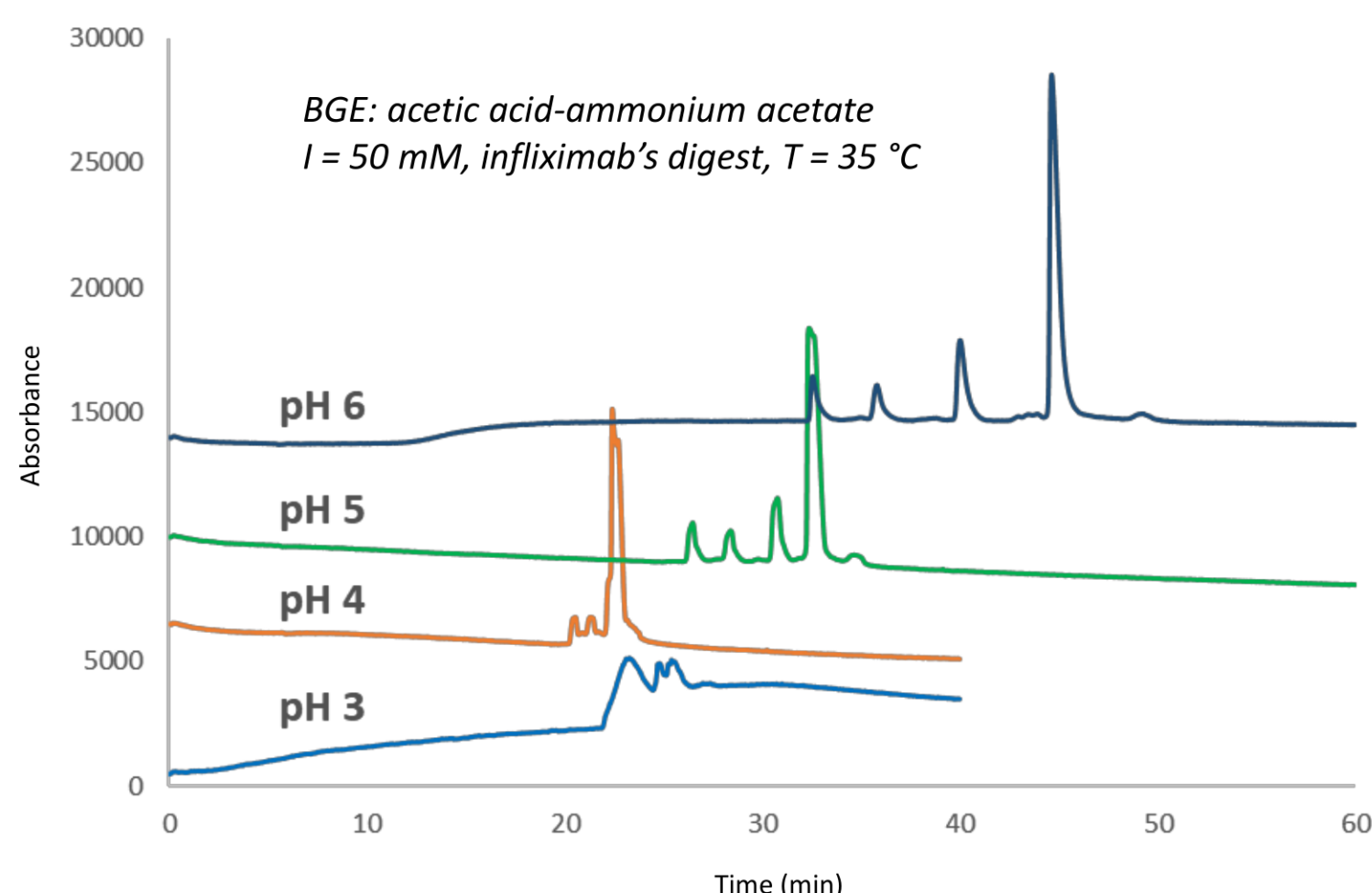


Development of the in-line methodology with temperature control



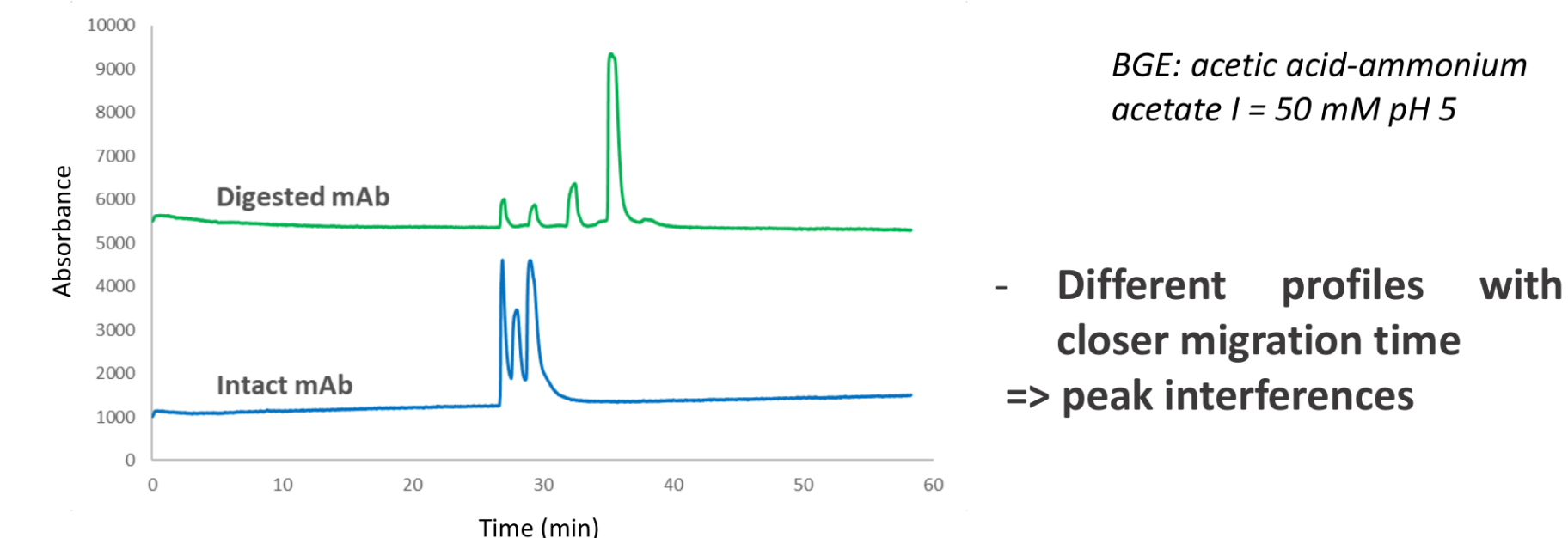
Optimization of subunits separation

Effect of BGE pH on infliximab's subunits separation

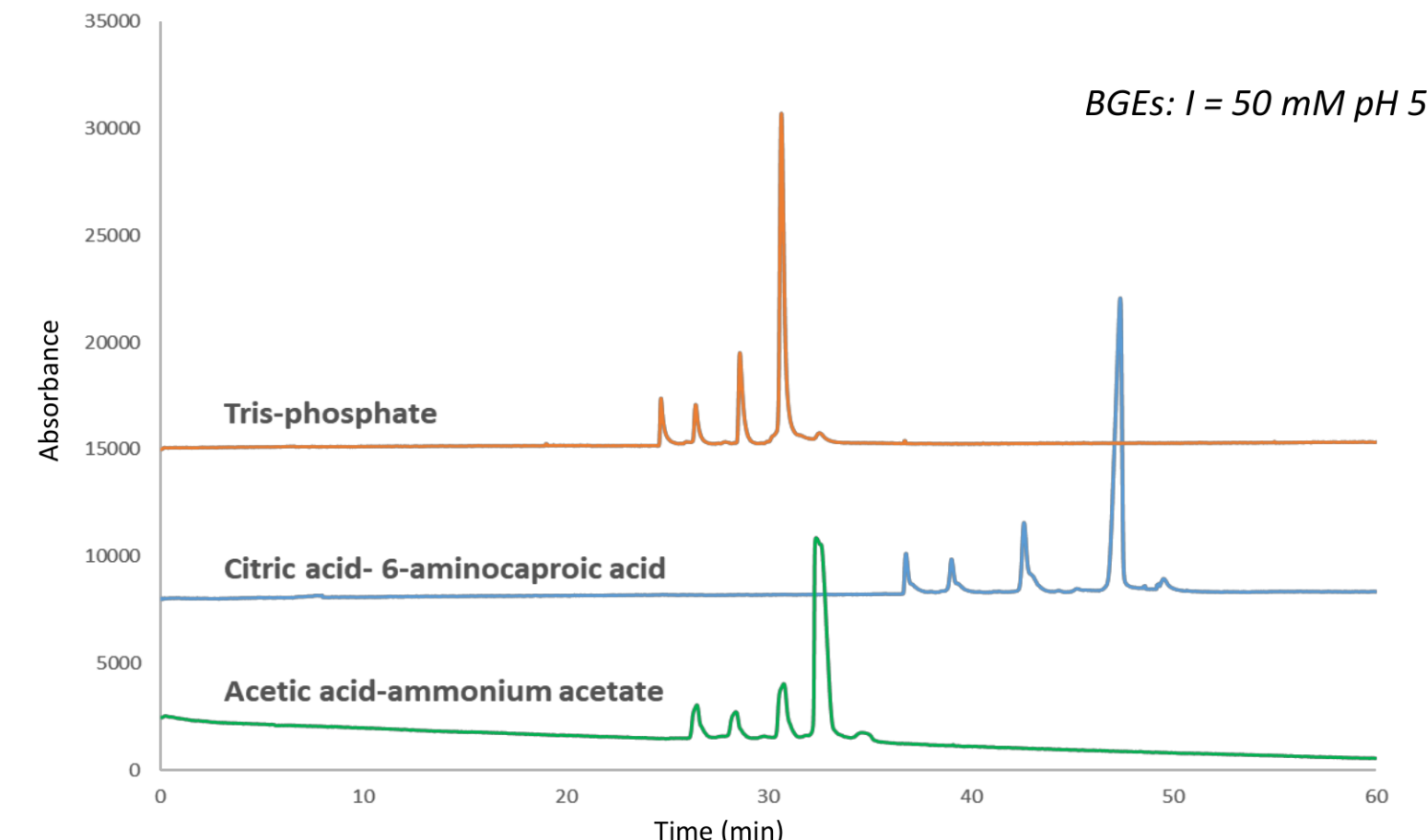


- High resolution of infliximab's subunits separation at pH above pH 5

Comparison of intact and digested infliximab's electrophoretic profiles



Evaluation of the performance of acetic acid-ammonium acetate BGE

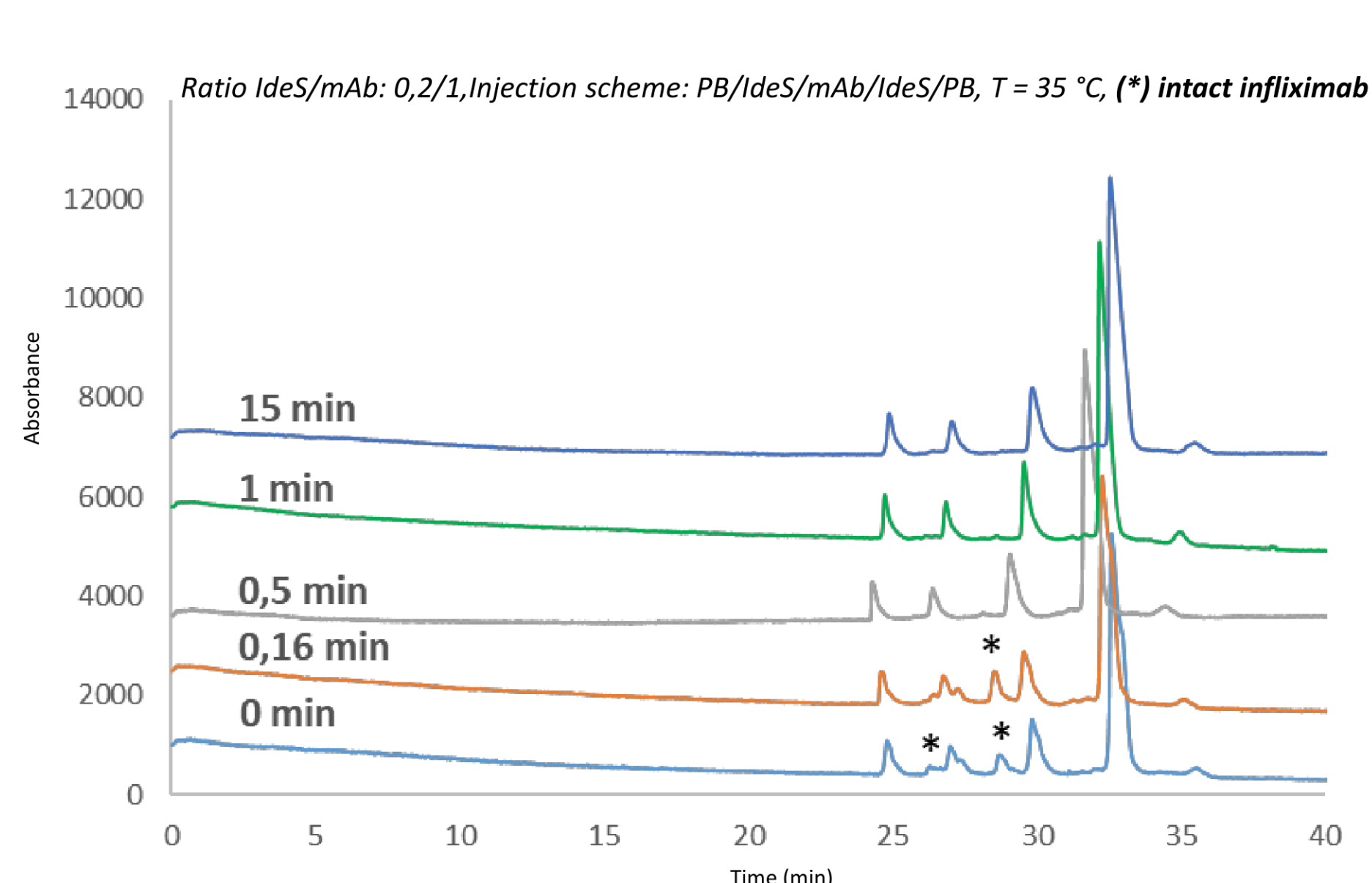


- Good peak efficiencies
- Compatibility with MS analyses
- Acceptable analysis time of acid-ammonium acetate BGE



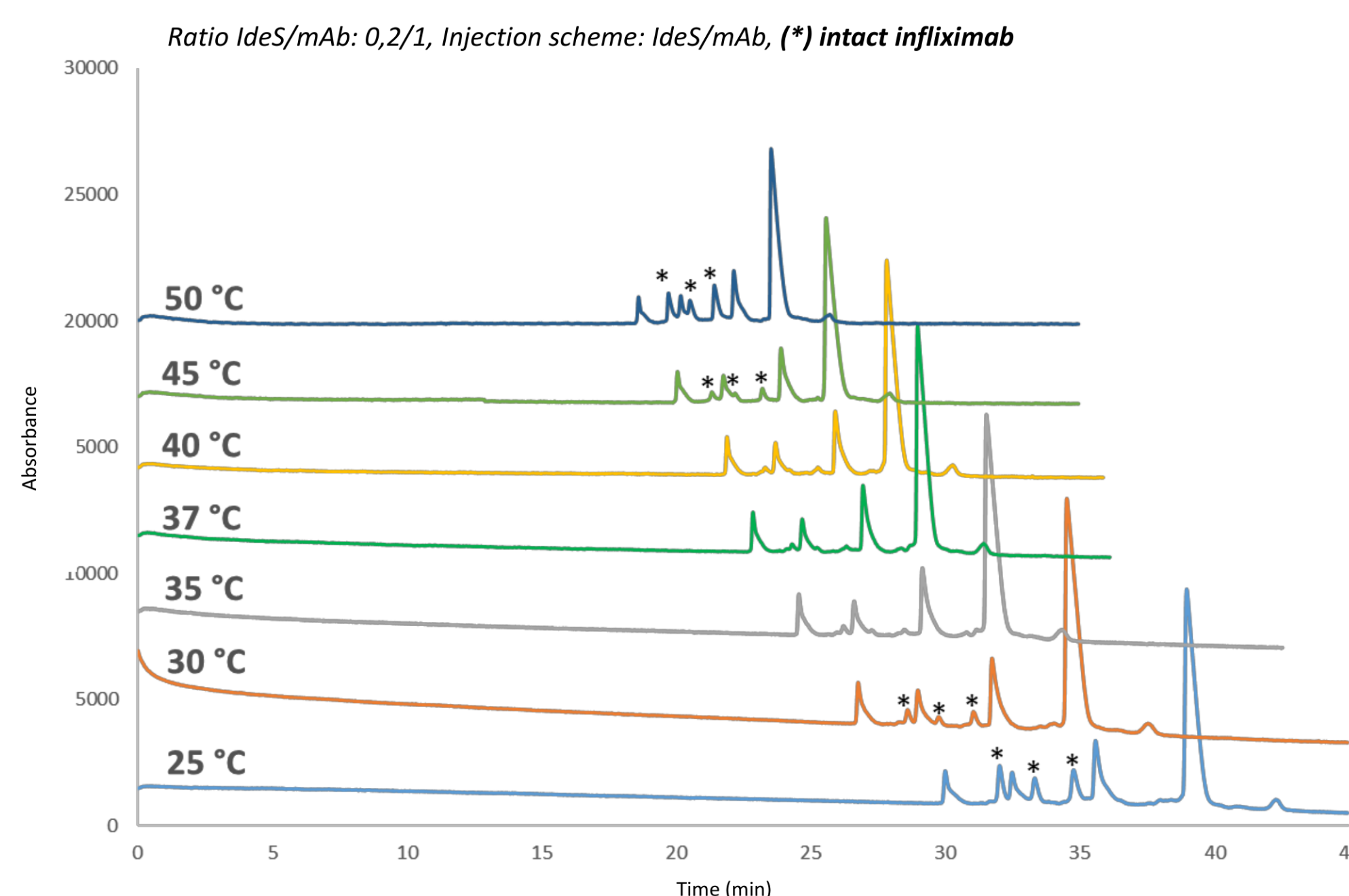
Optimization of in-line sample preparation

Effect of incubation time in the thermostated zone



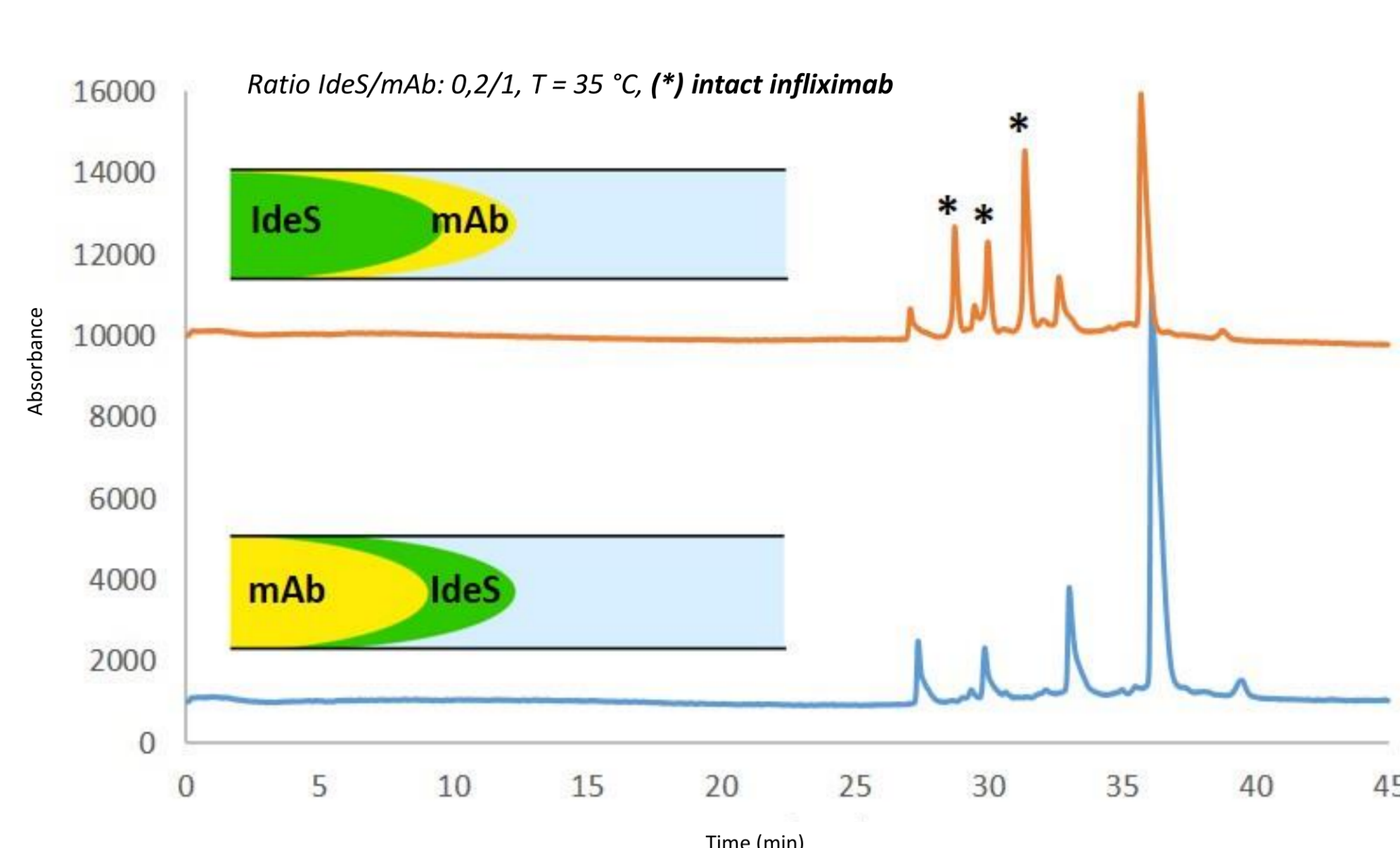
- Selection of 1 min => total incubation time 6,5 min
- Reduction of incubation time by a factor of 4,7

Effect of incubation temperature



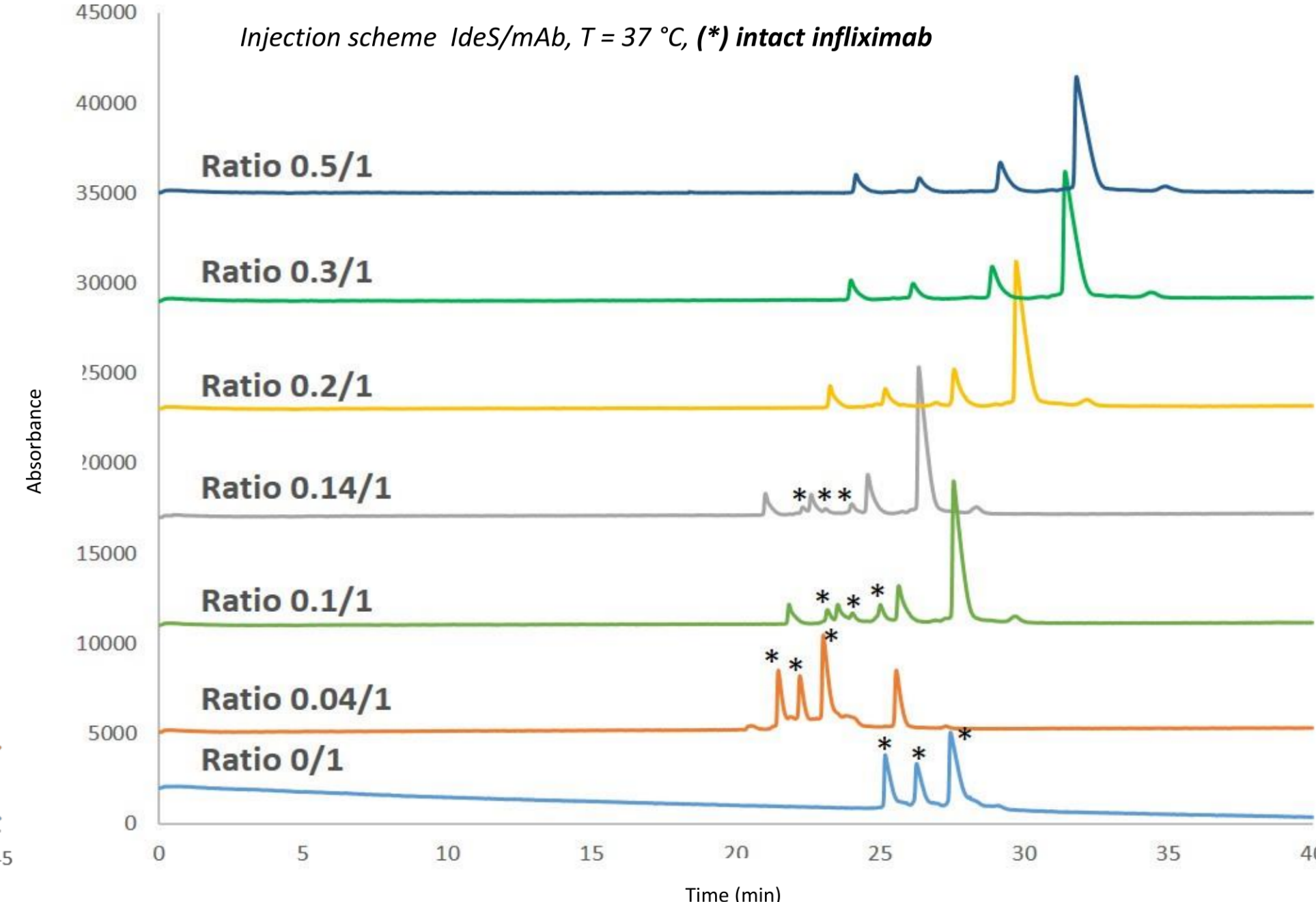
- Advanced enzymatic reaction in the temperature range of IdeS activity
- Successful temperature control of enzymatic reaction

Effect of injection scheme



- Dependence of digestion efficiency on the order of the reactants injection
- Compatibility of the BGE with the enzymatic reaction

Effect of IdeS/mAb ratio (w/w)



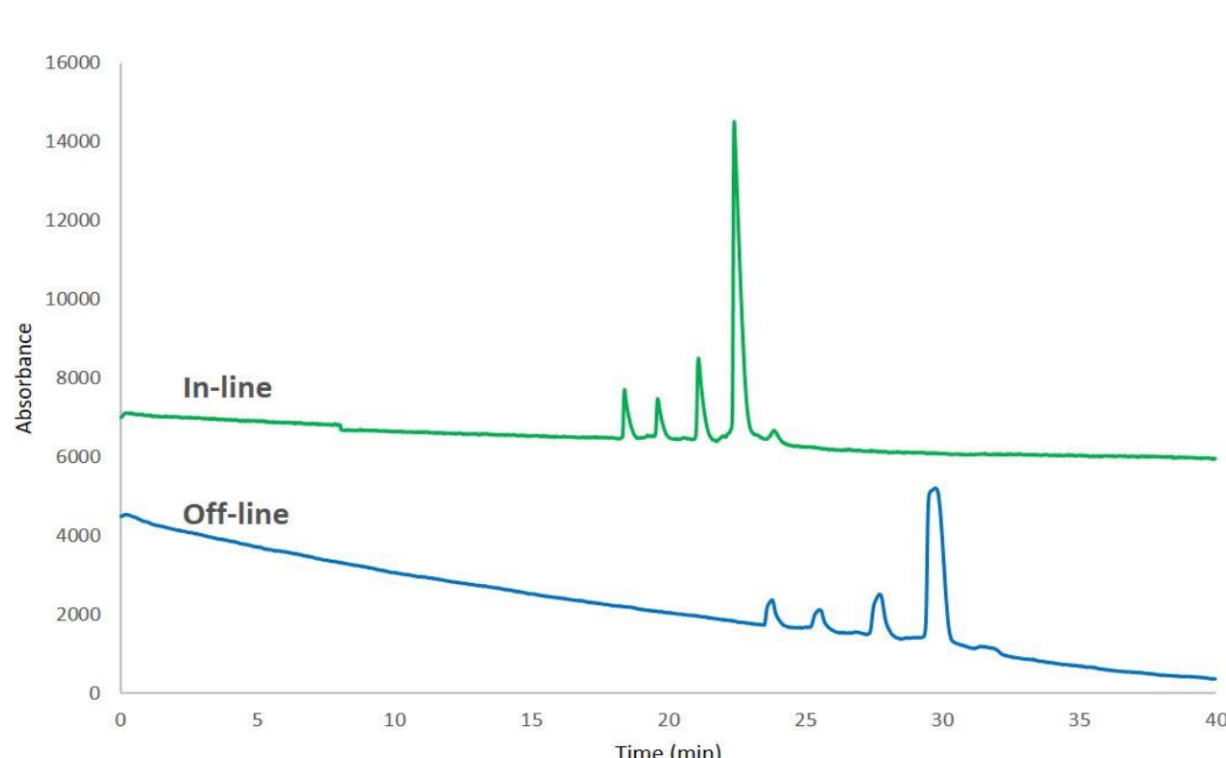
- Entire enzymatic reaction from 0,2/1 to 0,5/1 (w/w) ratio
- Selection of the ratio 0,3/1 (w/w)



Evaluation of the in-line methodology



Comparison between off-line and in-line methodology:

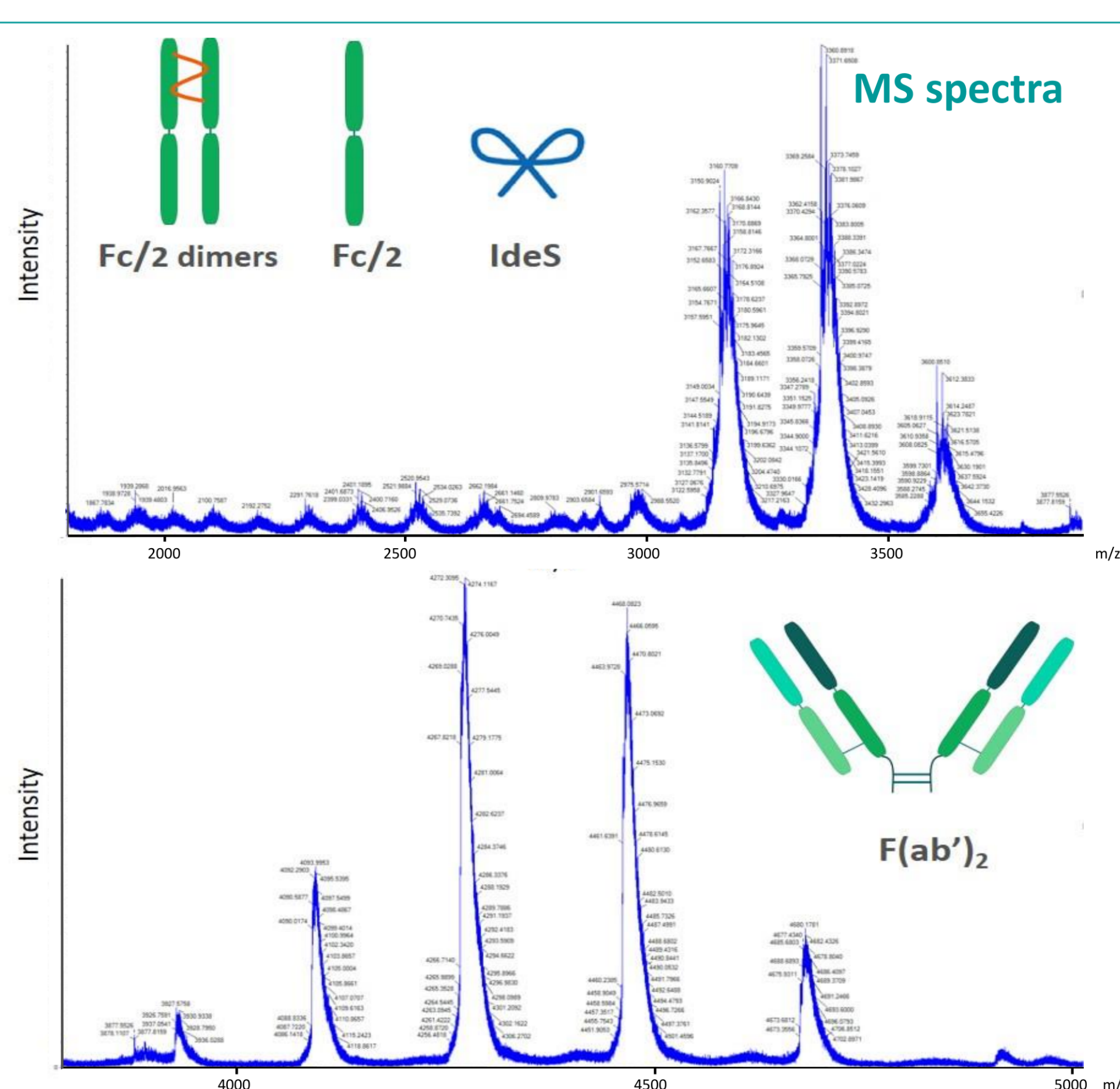
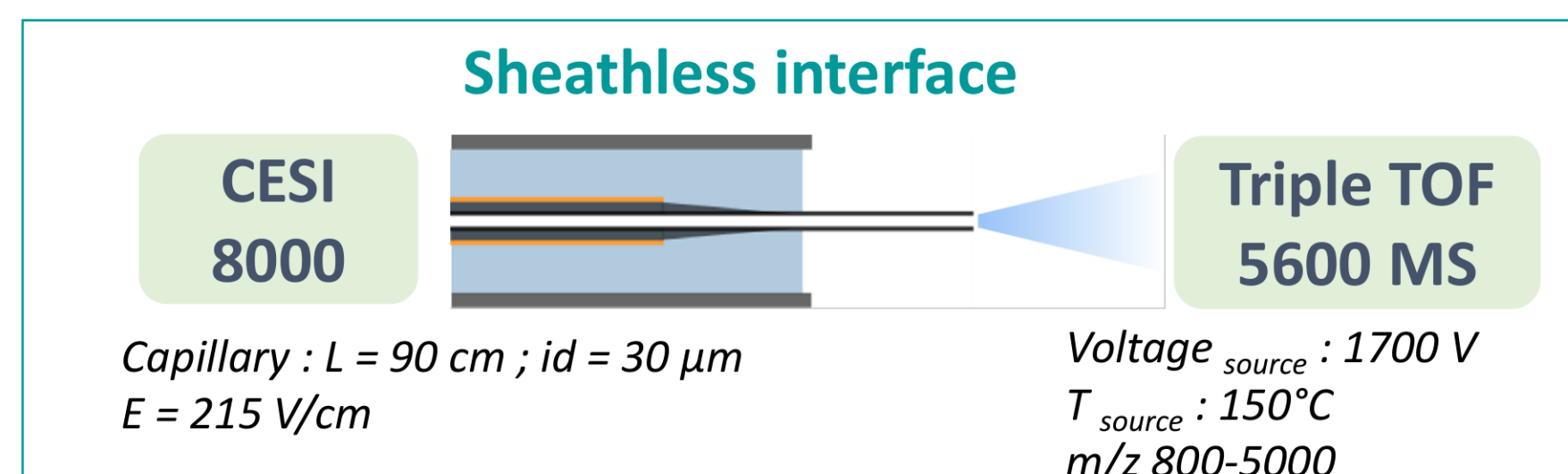


Infliximab, IdeS/mAb ratio: 0,3/1 (w/w), Injection scheme: IdeS/mAb, T = 37 °C

- Decrease of reactant's consumption
- Increase of peak efficiencies
- RSD < 2,2% for migration times
- RSD < 3,2% for corrected peak areas inless the last peak (11,8%) due to its low efficiency

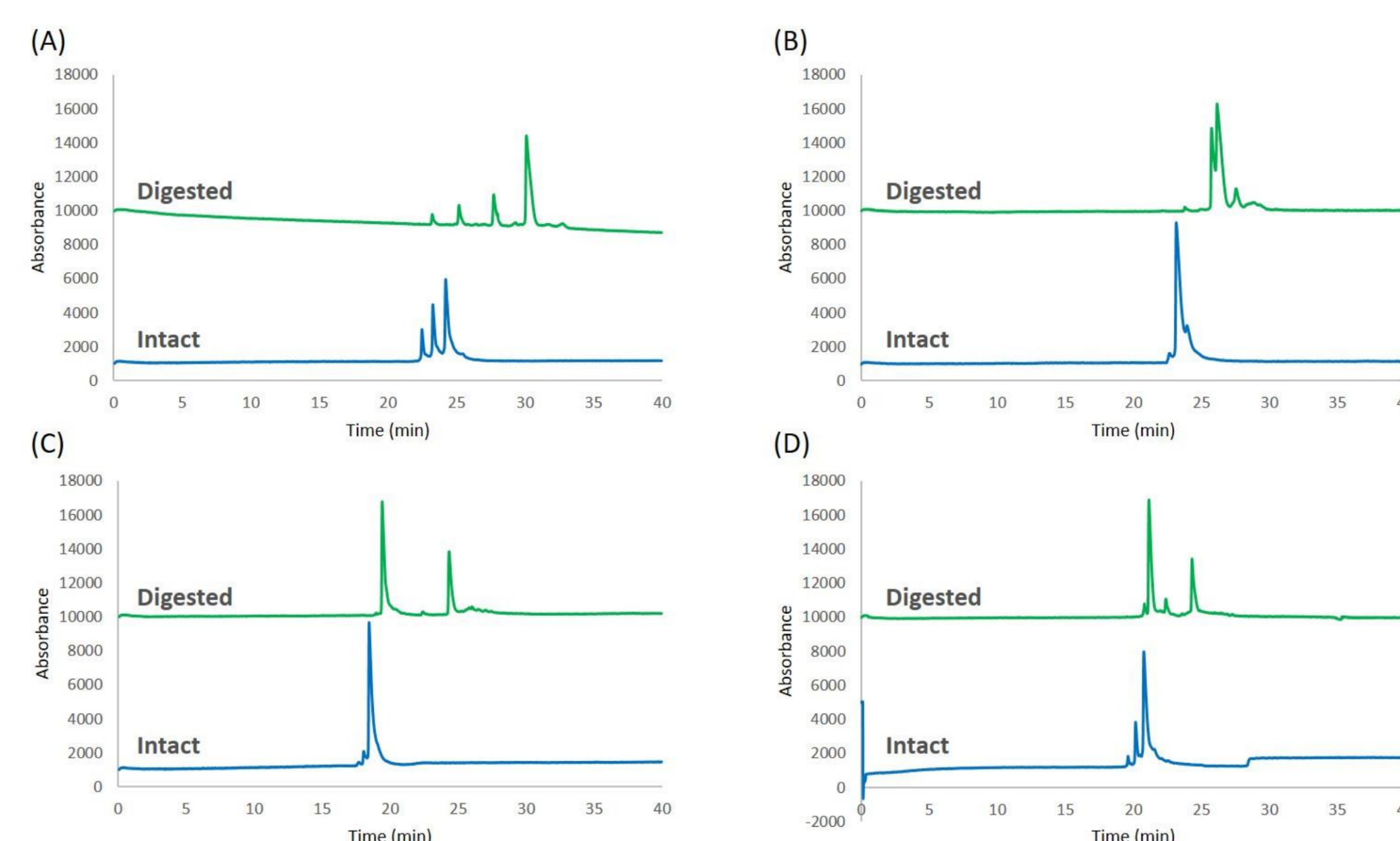


CE-ESI-MS analysis:



Applicability:

(A) Infliximab's biosimilar, (B) Belimumab, (C) Tocilizumab, (D) Adalimumab
IdeS/mAb ratio: 0,3/1 (w/w), Injection scheme: IdeS/mAb, T = 37 °C



- Different electrophoretic profiles between intact and digested mAbs



Conclusion

In-line methodology for middle-up analysis of mAbs

- Transverse diffusion of laminar flow profile (TDLFP) mixing
- Control of temperature

- ✓ High resolution of infliximab's subunits separation
- ✓ Increase of peaks' efficiencies by a factor of 2
- ✓ Decrease of reactants' consumption by a factor of 1000
- ✓ Good repeatability
- ✓ Identification of infliximab's subunits by CE-ESI-MS
- ✓ Successful application to the analysis of other mAbs



References

1. B. Beyer *et al.*, *Biotechnol. J.*, 2018, 13, 1700476.
2. M. Dadouch *et al.*, *Separation*, 2021, 8, 4.
3. M. Dadouch *et al.*, *Analyst*, 2020, 145, 1759.