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1 In-line Fourier-Transform Infrared Spectroscopy as a
2 Versatile Process Analytical Technology for Preparative
3 Protein Chromatography

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10 **Abstract**

11 Fourier-Transform Infrared Spectroscopy (FTIR) is a well-established spec-
12 troscopic method in the analysis of small molecules and protein secondary struc-
13 ture. However, FTIR is not commonly applied for in-line monitoring of protein
14 chromatography. Here, the potential of in-line FTIR as a Process Analyti-
15 cal Technology (PAT) in downstream processing was investigated in three case
16 studies addressing the limits of currently applied spectroscopic PAT methods.
17 A first case study exploited the secondary structural differences of monoclonal
18 antibodies (mAbs) and lysozyme to selectively quantify the two proteins with
19 Partial Least Squares Regression (PLS) giving Root Mean Square Errors of
20 Cross Validation (RMSECV) of 2.42 g/l and 1.67 g/l, respectively. The corre-
21 sponding Q^2 values are 0.92 and, respectively, 0.99, indicating robust models in
22 the calibration range. Second, a process separating lysozyme and PEGylated
23 lysozyme species was monitored giving an estimate of the PEGylation degree of
24 currently eluting species with RMSECV of 2.35 g/l for lysozyme and 1.24 g/l for
25 PEG with Q^2 of 0.96 and 0.94, respectively. Finally, Triton X-100 was added
26 to a feed of lysozyme as a typical process-related impurity. It was shown that
27 the species could be selectively quantified from the FTIR 3D field without PLS
28 calibration. In summary, the proposed PAT tool has the potential to be used
29 as a versatile option for monitoring protein chromatography. It may help to
30 achieve a more complete implementation of the PAT initiative by mitigating
31 limitations of currently used techniques.

32 *Keywords:* chromatography, proteins, process analytical technology (PAT),
33 fourier-transform infrared spectroscopy (FTIR), downstream processing

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