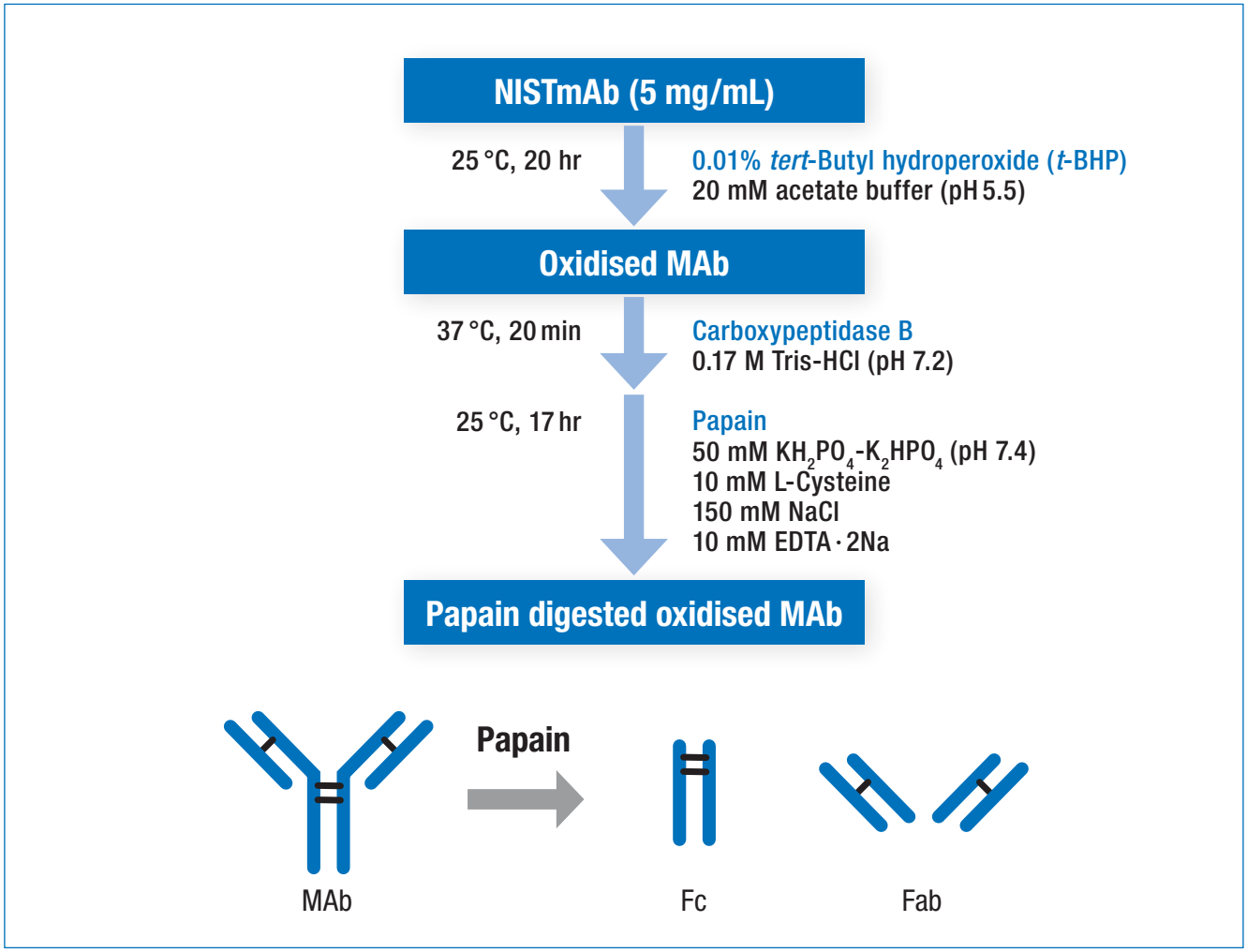
Analysis of oxidised monoclonal antibodies using BioPro HIC BF

During the manufacture and/or storage of biopharmaceuticals, variants with different properties from desired substances are produced by enzyme reactions or physicochemical interactions. Characterisation of these variants is of great importance from the perspective of ensuring efficacy and safety of pharmaceutical products.

Oxidised variants of monoclonal antibodies (MAbs) can be analysed by hydrophobic interaction chromatography (HIC). In this application note, the separation of MAb samples and their oxidised species using YMC's HIC column, BioPro HIC BF, is described. BioPro HIC BF is a more hydrophobic HIC phase dedicated for proteins of low hydrophobicity and oxidised MAb variants.

MAb oxidation with *t*-BHP treatment

tert-Butyl hydroperoxide (*t*-BHP) was used as a chemical oxidant to promote oxidation of methionine residues of NISTmAb. Subsequently, papain was used for the preparation of two Fab fragments and one Fc fragment [1].



Analysis of oxidised MAbs

When analysing the oxidised NISTmAb using the ammonium sulphate (a), four peaks appeared at earlier elution times compared to the peak of the non-oxidised MAb, presumably due to the conformational changes via the oxidisation of the methionine residues.

Applying sodium chloride (b), eight peaks appeared at earlier elution times compared to the peak of the non-oxidised MAb. The better resolution was further achieved within shorter analysis time compared to the usage of ammonium sulphate (a).

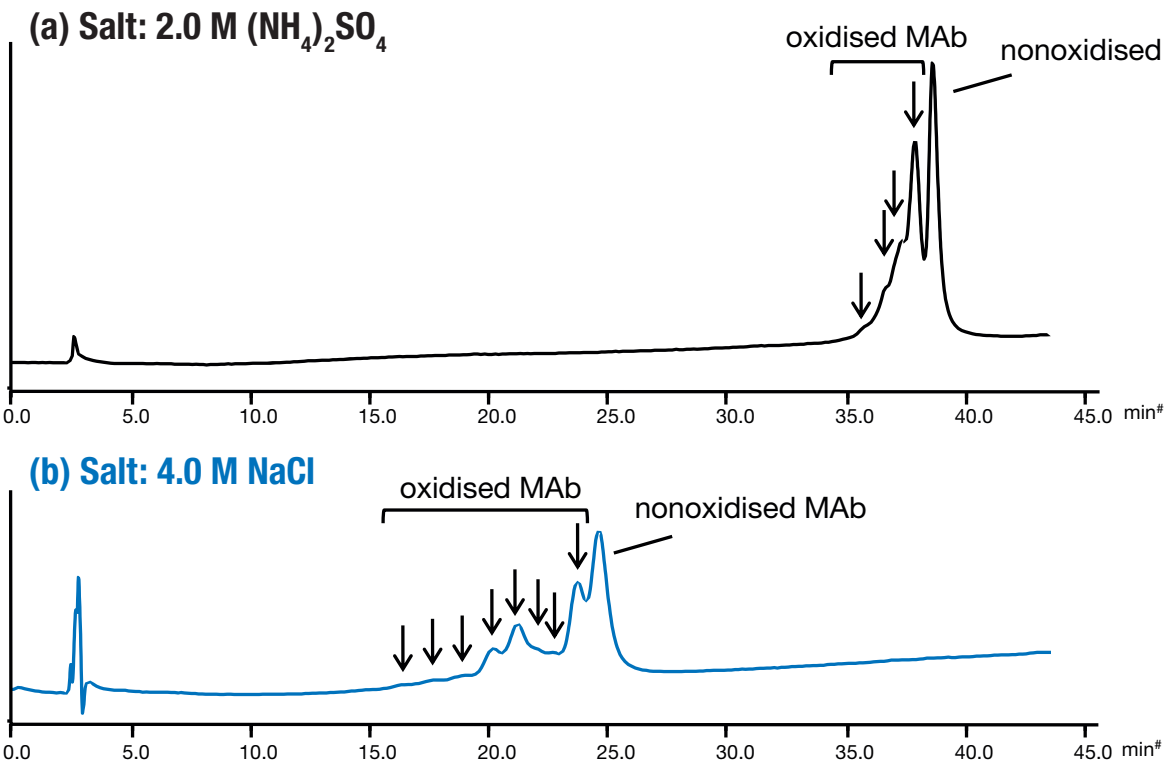


Table 1: Chromatographic conditions

Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Part No:	BHB00S04-1046WT
Eluent:	A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing salt
	B) 100mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)
Gradient:	40–80%B (0–40 min), 80%B (40–45 min)
Flow rate:	0.3 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	5 µL (1.0 mg/mL)

Analysis of papain-digested oxidised MAb

The papain digests of NISTmAb samples with and without oxidisation were analysed. The Fab and Fc fragments were characterised from the chromatogram of the papain digested MAb.
In the chromatogram of the papain digested oxidised MAb, multiple peaks appeared at earlier elution times compared to the peaks assigned to the Fab and Fc fragments. These peaks correspond to oxidised fragments according to a previous report [2], describing that oxidised fragments elute earlier than the non-oxidised ones.

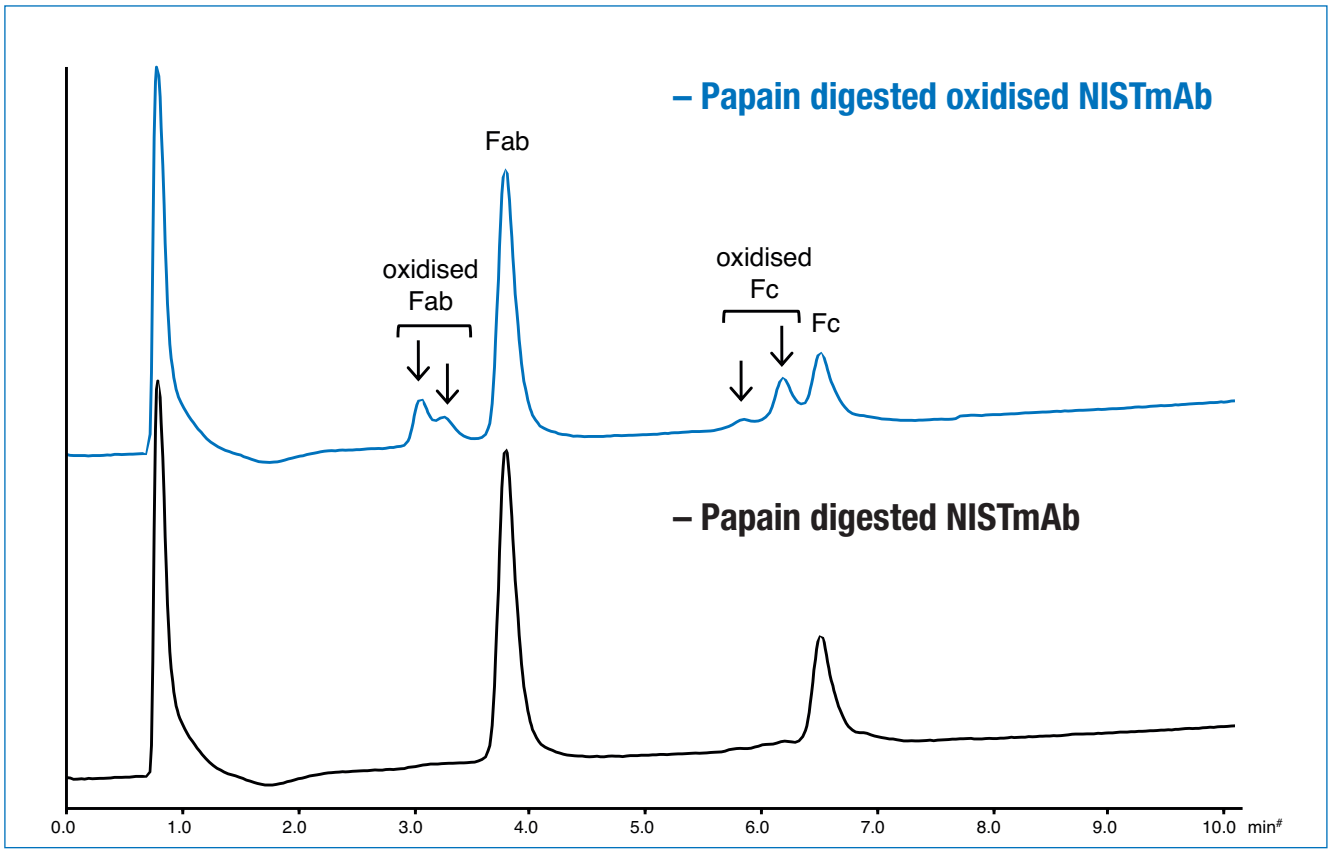


Table 2: Chromatographic conditions

Column:	BioPro HIC BF (4 µm)100 x 4.6 mm ID
Part No:	BHB00S04-1046WT
Eluent:	A) 100 mM NaH_2PO4-Na_2HPO_4 (pH 7.0) containing 2.0 M $(NH_4)_2SO_4$
	B) 100mM NaH_2PO4-Na_2HPO_4 (pH 7.0)
Gradient:	40–80% B (0–10 min)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	5 µL (0.5 mg/mL)

[1] Techniques in Protein Chemistry Volume 7, 1996, 275-284.
[2] Journal of Chromatography A, 2008, 1214, 81-89.