APPLICATION NOTE

Analysis of commercial monoclonal antibodies by microLC-MS/MS

Monoclonal antibodies (MAbs) are high-potency agents mainly used in cancer therapy or to combat auto-immune diseases. Even small amounts of these substances can have severe effects even when taken accidently.

Therefore, it is of interest to analyse different kinds of samples which could contain these antibodies, e.g. solutions prepared for patient application (usually a dilution of the dosage form). By use of microLC-MS/MS, only a very small amount of this valuable solution is required to identify and quantify the monoclonal antibody in very short run times.

In addition, microLC-MS/MS can be used to monitor residues on surfaces or materials in hospitals, pharmacies or laboratories that prepare solutions for patient application. An easy way to monitor residues is by wipe sampling followed by microLC-MS/MS [1]. This workflow could be applied to MAbs as well.

The ideal selectivity of YMC-Triart Bio C4 for intact monoclonal antibodies has been presented already in several applications. In this application note, YMC-Triart Bio C4 was used in YMC capillary column format to analyse and identify intact commercial MAbs, such as rituximab or bevacizumab, by microLC-MS/MS. An elevated temperature of 75 °C and formic acid as additive was applied.

Table 1: Chromatographic conditions

Column:	YMC-Triart Bio C4 (30 nm, 3 µm) 100 x 0.3 mm ID, 1/16" end fittings
Part No.:	TB30S03-10H0AU
Eluent:	A) H_2O + 0.1 % formic acid
	B) acetonitrile + 0.1% formic acid
Gradient:	20 % B (0–2.5 min), 20 %–100 % B (2.5–4 min), 100 % B (4–5 min), 20 % B (5–7 min)
Flow rate:	15 µL/min
Temperature:	75 °C, 4 °C Autosampler
Detection:	Shimadzu LCMS-9030 QTOF
Injection:	0.1 µL
Sample:	Rituximab dosage form (10 mg/mL, diluted to 0.1 µg/mL)
	Bevacizumab dosage form (10 mg/mL, diluted to 0.1 µg/mL)
	Bevacizumab solution for patient application (3 mg/mL, diluted to 0.03 µg/mL)
LC system:	Shimadzu Nexera Mikros

The chromatograms of intact monoclonal antibodies, Rituximab and Bevacizumab, both in dosage form were obtained by direct injection. The aged solution (about 6 month old) for patient application showed an additional peak derived from the used dilution buffer, but the MAb is still intact. This demonstrates that use of microLC-MS is possible to monitor amount and stability of, for example, patient solutions for injection.

[1] T. Hetzel, et al., Micro-liquid chromatography mass spectrometry for the analysis of antineoplastic drugs from wipe samples, Anal. Bioanal. Chem. (2016)

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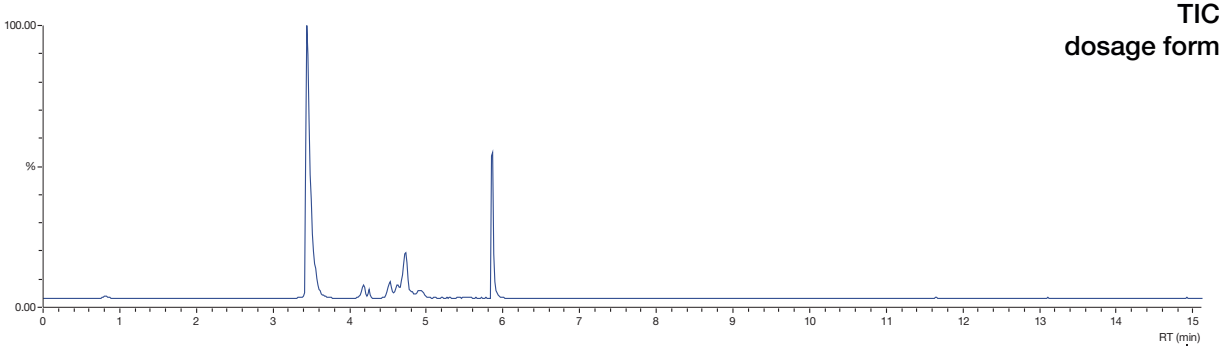
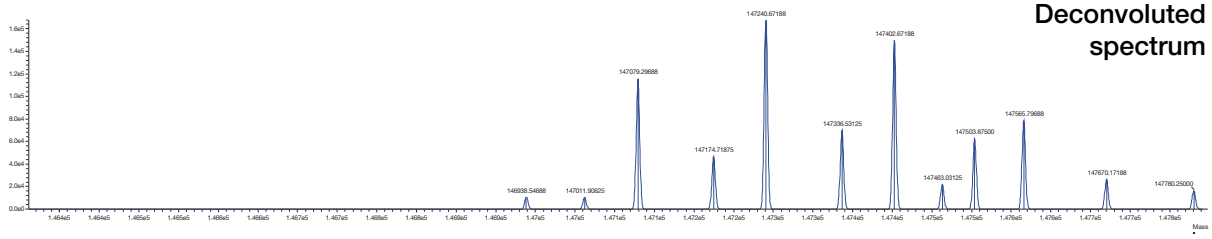
Rituximab (MabThera®)

TIC
dosage form

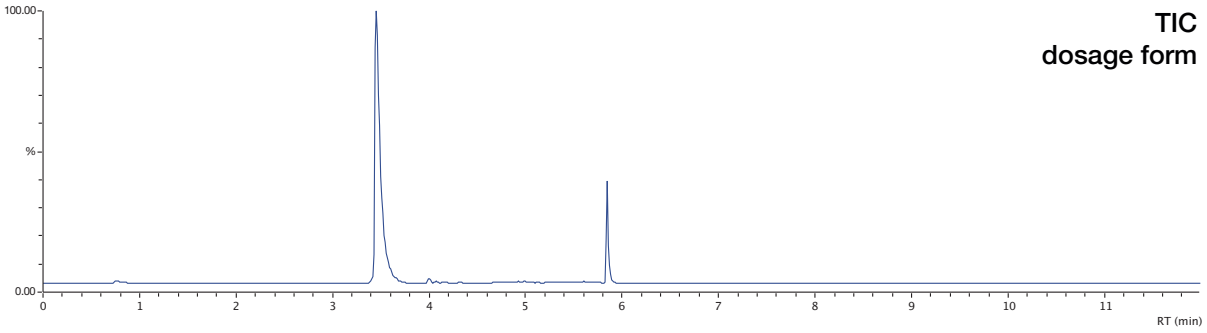


Deconvoluted
spectrum

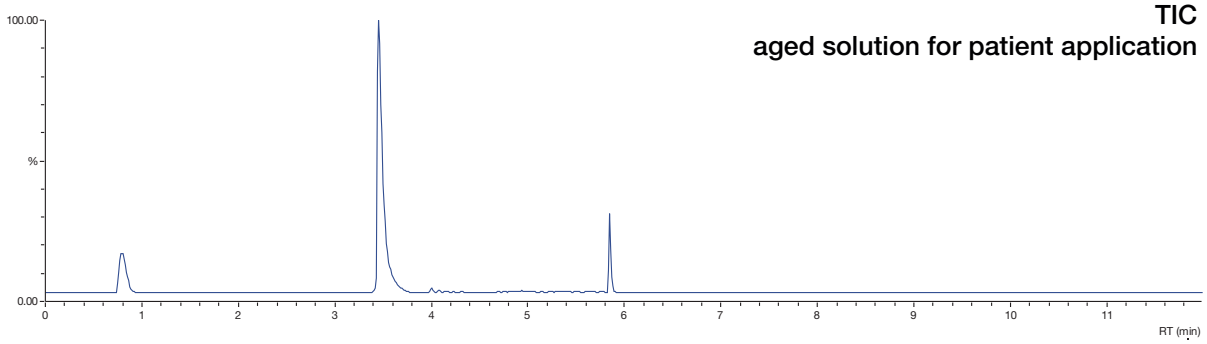


Bevacizumab (Avastin®)

TIC
dosage form



TIC
aged solution for patient application



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