HPLC of Monoclonal Antibody in Nonclinical Safety Testing – Degradation Induced by Trace Metal Ions Leaching From the Stainless Steel Surfaces in the Flow Path of Mobile Phase and Its Control

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1 Introduction

New monoclonal antibody (mAb) pharmaceuticals are increasingly being pursued in the pharmaceutical industry, mostly aimed at the treatment of very challenging diseases. In the non-clinical toxicology research of mAb drug candidates, HPLC is the foremost analytical technology applied for both quantitation and characterization (stability and purity, et al). Ions of the major metal components of stainless steel (Table 1) used throughout the flow path in HPLC are potential catalysts for the hydrolysis and/or oxidation of mAbs. We present one of the cases, encountered in our non-clinical toxicology research of biopharmaceuticals, of online degradation of mAb drug candidates, induced by trace metal ions leaching from the steel surface of the injection needle. The degradation fragments of the mAb were separated in the chromatographic profile. The turn-over capacity of the trace metal ions as catalysts for the degradation was investigated for samples over the concentration range of 25 ng/mL to 50 ug/mL. The degradation of the mAb drug candidate was controlled using chelating agents to mask the catalytic metal ions in the sample vials. The established method was validated, then subsequently applied in nonclinical toxicology research of the mAb drug candidate for both quantitative analysis and characterization of samples at concentrations as low as 50 ng/mL.

Table 1. Chemistry of 316/316 L Steel

Metal	Fe	Ni	Cr	Mo	Mn	C	S	P	N
Min (%)	72	10	16	2	--	--	--	--	--
Max (%)	62	14	18	3	2	0.03	0.03	0.05	0.1

2 Investigations and Results

An eluate can constantly encounter trace metal ions leaching from the steel surfaces throughout its chromatographic elution. Because an online degradation induced by such trace metal ions continues over the entire elution time and is usually progressing to a low extent, degradation products elute in a broad band, mostly in front of the parent peak. Figure 1 shows the typical chromatographic profile for an eluate (not the molecule in the present work) undergoing online degradation in its chromatography. Often the intensity of the peak band is low, and an online degradation and its impact on chromatographic analysis can be overlooked. In addition, the investigation of the degradation reaction is difficult with the parent compound and the degradation products in migration.

However, a degradation induced by metal ions leaching from the steel surfaces in HPLC can be demonstrated and investigated in the injection vial. Figure 2 illustrates how metal ions leaching from an injection needle, which is made of the same steel used for connection tubing and column blank, could contaminate the sample in an injection vial, and potentially catalyze degradation of an analyte in the sample.

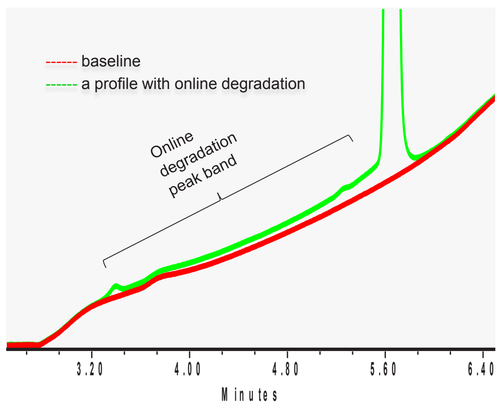

Fig 1. A typical chromatographic profile of online degradation

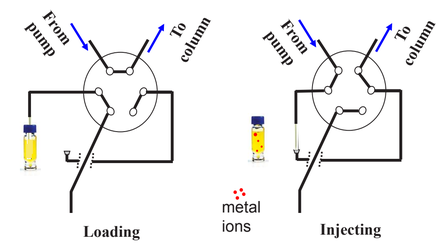

Fig 2. Metal ions leaching from the injection needle, effectively contaminating the sample

An mAb drug candidate from our non-clinical toxicology research was selected for demonstrating the investigation of degradation induced by metal ions leaching from an injection needle. To demonstrate the online degradation induced by metal ions from the needle surface, duplicate aliquots (vial 1 and vial 2) of the same sample solution (50 ug/mL) were transferred into polypropylene injection vials. After one (vial 1) of the duplicate aliquots was injected and chromatographed, both aliquots were stored side by side on a sample tray at 4°C. After storing for the same period of time (~4 hours), both aliquots were injected and chromatographed consecutively. As shown in Figure 3, the sample solution in vial 1 contaminated by the injection needle following the first injection (-------) showed degradation products in the second injection (-------), while the sample solution in vial 2 without prior contamination from the injection needle did not show any evidence of degradation products in the profile (-------).

It was anticipated that the content of metal ions leaching from the needle surface was minimal, limiting their catalytic capacity. To demonstrate the catalytic capacity of the metal ions leaching from the injection needle, sample solutions over the concentration range of 25 ng/mL to 50 ug/mL were first contaminated individually by a single injection for chromatography, followed by storage on the sample tray at 4°C. Following storage for 8, 24 and 48 hours, the samples were re-injected and chromatographed. The extent (%) of degradation was much greater for samples at low concentrations than for those at high concentrations (Figure 4), indicating that the degradation was limited by the capacity of the catalytic metal ions in the samples. In sample solutions at higher concentrations (30 to 50 ug/mL), acceleration of the degradation by accumulated contamination from multiple injections was observed.

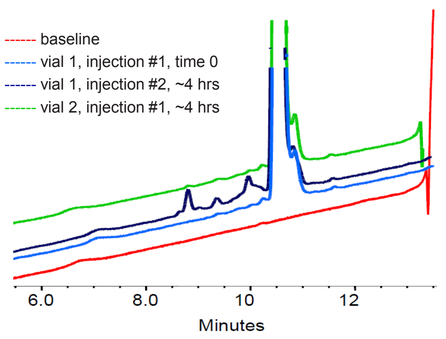

Fig 3. Peak profiles for sample aliquots with and without prior contamination of metal ions from the injection needle (samples in an acidic diluent of water/acetonitrile/TFA/Tween 20)

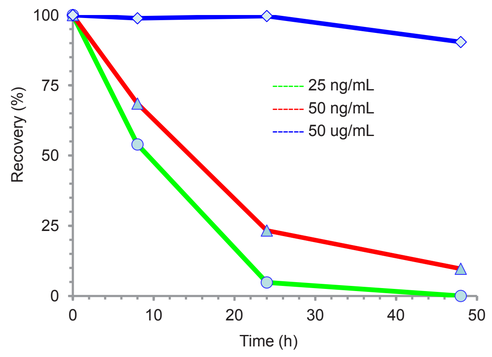

Fig 4. Degradation in samples after contamination by a single injection (samples in an acidic diluent of water/acetonitrile/TFA/Tween 20)

Solution pH was considered a factor which could significantly affect both leaching of metal ions from the needle surface and the reaction rate of the degradation. Stability of sample solutions at neutral pH and acidic pH (0.05% trifluoroacetic acid) were assessed. Without prior contamination by the injection needle, the sample solutions under both pH conditions were stable. However, with prior contamination by the injection needle in a single injection, while the recovery in re-injections (48 hrs) was reduced for all sample solutions, the losses were very different between the sample solutions under the two different pH conditions (Figure 5). The degradation in the acidic solutions was much greater than that observed in the sample solutions at neutral pH, particularly for solutions at lower concentrations. This difference in degradation could be attributed to differences in the content of the metal ions leaching from the injection needle and/or in the reaction rate of the degradation, at the different pHs.

It was understood that the ions of the main metal components (Table 1) leaching from the injection needle could readily be masked by chelating agents. This work used EDTA as a chelating agent in sample solutions. Sample solutions were prepared in a diluent containing 5 mM EDTA at pH 4 to 5. After the initial injection, the solutions were all stored in a sample tray at 4°C, and re-injected approximately 20 and 48 hours later. Figure 6 demonstrates the stability data for solutions at concentrations of 25 ng/mL to 50 ug/mL. As compared to the data in Figure 4, the stabilization of the analyte by EDTA, masking the catalytic metal ions in solutions, is clearly evident, which allows for the accurate and repeatable analyses of the samples.

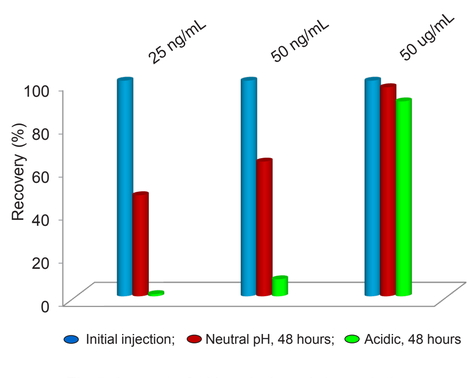

Fig 5. Impact of pH on induced degradation

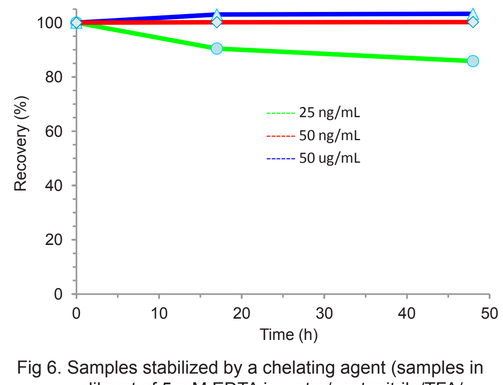

Fig 6. Samples stabilized by a chelating agent (samples in a diluent of 5 mM EDTA in water/acetonitrile/TFA/ Tween 20, ~pH 4.5)

3 Summary

The investigations demonstrated the degradation of an mAb drug candidate induced by metal ions leaching from the steel surface of an injection needle in the injection vial. As anticipated, the amount of catalytic metal ions leaching from the steel surface was minimal, limiting the catalytic capacity for the degradation of the analyte in solution. The degradation could be effectively controlled by chelating agents masking the catalytic metal ions. Based on the understanding of the metal ion induced degradation of the analyte from these investigations in the injection vial, a method was successfully established, validated, and applied to the quantitation and characterization of the drug candidate, in support of its non-clinical safety testing.

4 Acknowledgement

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