Force Degradation of an Oligonucleotide – A Case Study

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ABSTRACT

Antisense oligonucleotides (ASO) stability-indicating test demand has increased significantly within the CRO industry. The demand is specifically for the most commonly used method to monitor the purity and impurity profiles of an oligonucleotide. The IP-RP-HPLC-UV-MS method, which uses ion-pairing (IP) reversed-phase (RP) chromatography coupled with both ultraviolet (UV) and mass spectrometry (MS) detections in parallel. The purity and impurity profile of these oligonucleotides are relatively stable and typically show an increase of common degradants throughout the course of a stability study. These common degradants coelute with the main component making quantitation an important focus. Herein we examine a "real life" scenario for the forced degradation study on the effects of heat and oxidation on antisense oligonucleotides with analysis by IP-RP-HPLC-UV-MS.

INTRODUCTION

The ASO drugs are synthetic ribonucleic acid (RNA) that complement the target RNA. These drugs are designed to "turn off" disease causing proteins. The ASO drug market is growing in popularity because the drugs are smart, specific and life-saving. To get the drugs for commercial use, full method validation of the most commonly used quantitative method to determine the assay and impurity profile, is known as the IP-RP-HPLC-UV-MS method. The UV portion of the IP-RP-HPLC-UV-MS method detects the main peak for assay and purity value, whereas the MS portion of the method provides specificity (m/z) to resolve different impurities species co-eluting with the main component(s). These impurities such as P=O, n-1, etc., can easily be distinguished by MS. The ability to detect these impurity peaks helps provide a more accurate purity value for the main component and can be a useful tool to monitor impurity quantity change over time, during stability storage or across different manufacturing lots.

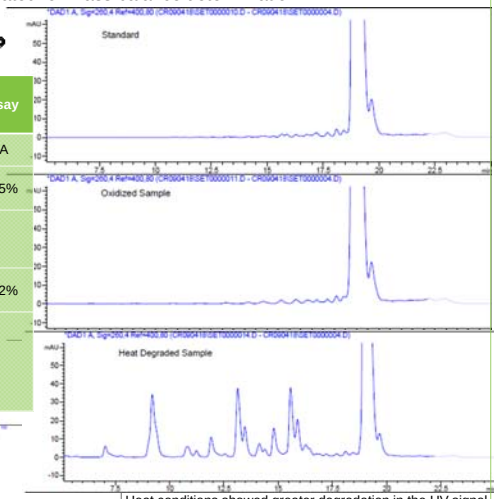
Method validation for assay and impurity profile need to demonstrate the method is stability indicating, by the ability to detect changes in degradation. This requires drug product, substance and related placebo to be force stressed against a non-stressed control, commonly known as Forced Degradation. It is important to perform during validation, when all related substances are not available, as it can help determine the impurity profile over a short amount of time, which can give useful insight on what effects an analyte. The stress conditions for testing typically include the effects of light, heat, humidity, oxidation, and acid/base hydrolysis. ICH guidelines require each stress condition to be 5-20% reduction of the main analyte peak. The level of degradation is determined by calculating the analyte mass balance as a percent degraded vs the non-stressed control.

PROCEDURE AND RESULTS

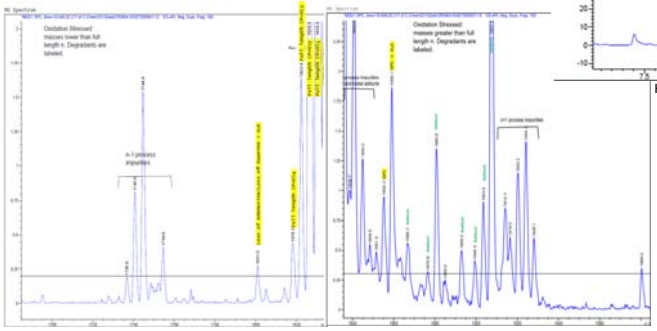
The oligonucleotide sequence is A*UGAUG*MeC*A*T* MeC*A*T* MeC*A*T*A*UGAUG*A (obtained from Sigma Aldrich) where "*" denotes phosphorothioate linkages, underlined bases are 2'-OMethyl RNA . The oligonucleotide was stressed per the conditions below. The MS and UV impurity profile was evaluated for mass balance determination.

$$\text{Mass Balance} = \frac{\text{Assay of Stressed Sample} + \text{Total Reportable Impurities}}{\text{Assay of Control Sample} + \text{Total Reportable Impurities}} \times 100$$

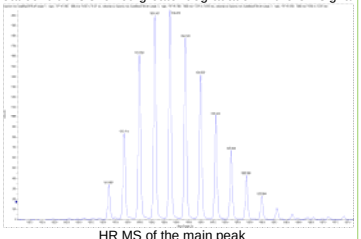
Condition	Parameters	MS Purity	UV Purity	Total Impurities	Assay
Nominal	NA	88.65%	92.5%	18.0%	NA
Heat	1 mg/mL solution in oven at 80C for 4 days then diluted to 0.1 mg/mL	78.78%	62.6%	55.1%	45.5%
		11.1% Degradation	31.4% Degradation	106% Mass Balance	
Oxidation	1 mg/mL solution exposed for 20 minutes to 30% H2O2 solution, desalted by exchange column	69.76%	91.7%	36.0%	60.2%
		21.3% Degradation	0.9% Degradation	96.2% Mass Balance	



Heat conditions showed greater degradation in the UV signal



Oxidative conditions showed greater degradation in the MS signal



HR MS of the main peak

CONCLUSION

A forced degradation method validation study can show main peak degradation under stress conditions, giving a useful insight into the stability of the drug. Furthermore, making it necessary to asses both the UV and MS for ASO drugs as different stress conditions effect the drug, detectable only in the UV or MS signal. Quantitation of both signals will provide an accurate MS purity, which is representative of both MS and UV signals.