Poster Number 810-1

Maleate Salt Detection Using UV versus CAD Detection

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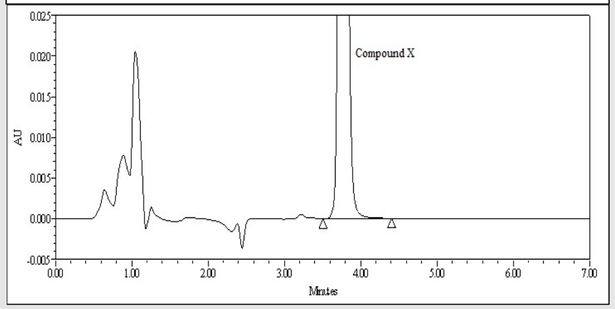
INTRODUCTION

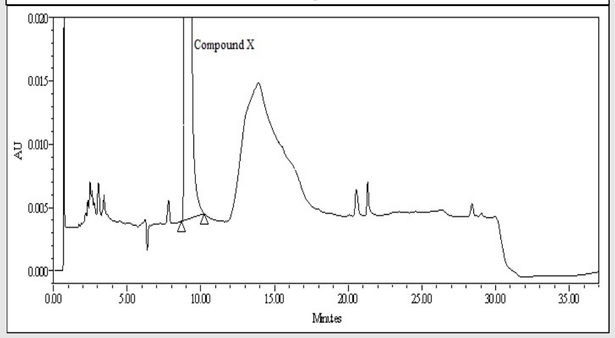
A validated HPLC method for the assay and identification of Compound X by UV detection was provided to Metrics Contract Services (MCS) for the release testing of Phase II clinical trial material. The method demonstrated consistent chromatography with the validating laboratory and met the requirements for precision and linearity. During UV analysis a single peak was observed with no impurities detected. Review of the chemical structure of Compound X showed that no major chromophores were present and that the compound was a maleate salt. This observation partnered with the lack of impurities sparked interest into analysis using charged aerosol detection (CAD). The analysis using CAD generated the presence of two peaks suggesting the peak identified as Compound X in the original method using UV detection was the Maleate salt, not the active ingredient. The identity of these peaks were later confirmed by HPLC-MS.

INITIAL DATA

Chromatography from Initial Assay Method Using UV Detection @ 210 nm



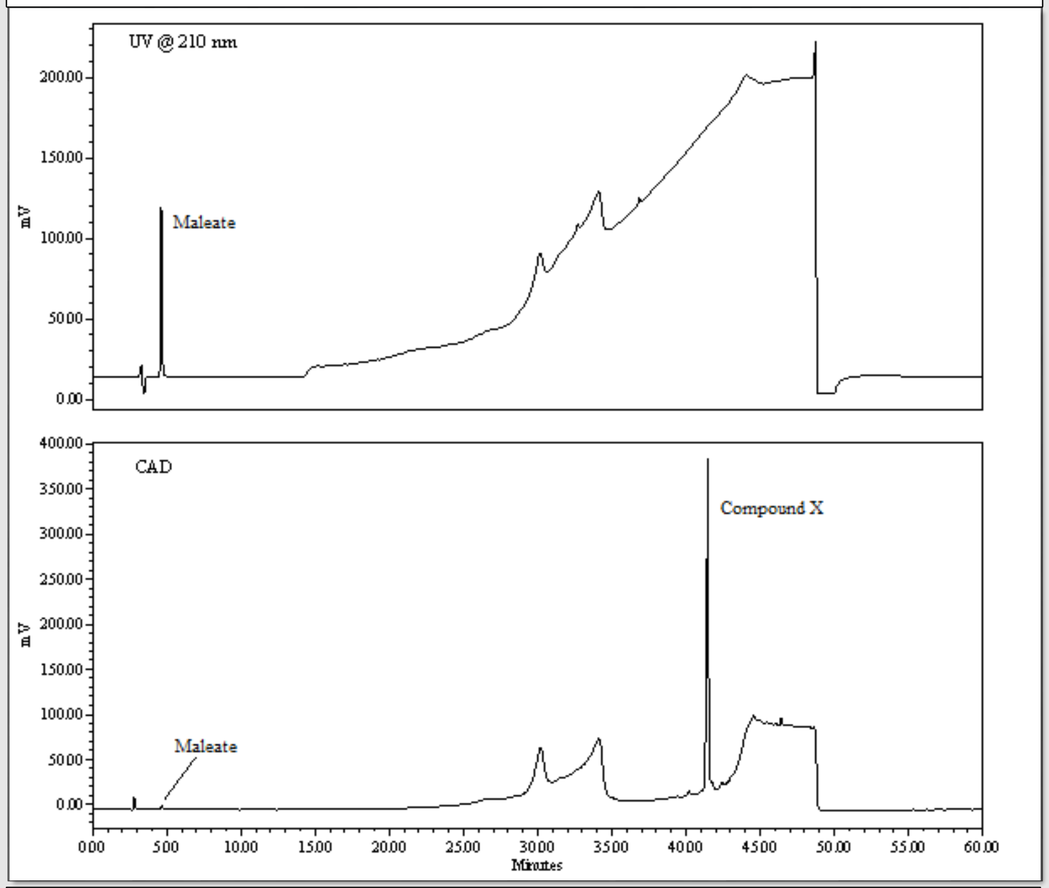
Chromatography from Initial Related Substances Method Using UV Detection @ 210 nm



Original chromatography for the Assay and Related Substances methods for Compound X using UV detection at 210 nm.

INVESTIGATION

Overlay of Compound X API UV at 210 nm vs. CAD



The chromatography above displays the UV analysis at 210 nm versus CAD.

- Single peak detected at 210 nm was assumed to be Compound X
- No Impurities detected under these conditions.
- Due to the lack of impurities, as well as the lack of chromophores in the chemical structure, analysis using charged aerosol detection (CAD) was employed.

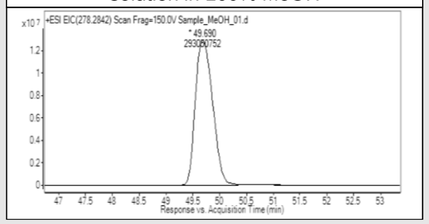
Due to the lack of chromophores in the chemical structure, the peak eluting at approximately 5 min under UV detection was not believed to be Compound X. UV detection requires the molecule to be analyzed to have chromophores that absorb UV light energy in order to be detected. CAD, however, does not rely on chromophores but rather on the initial mass concentration of the analyte in the droplets formed upon nebulization of the sample. The droplets are dried then charged and it's these charged analytes that are detected as seen in the CAD chromatogram above.

- Hypothesized that the peak at approximately 5 min was a maleate salt and the peak at approximately 42 min was Compound X based on the data.
- Further identity of both peaks was performed using LC-MS analysis.

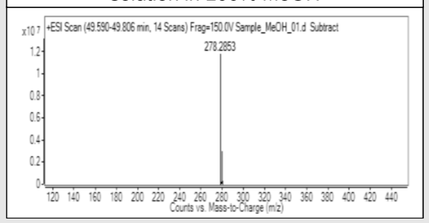
RESULTS

To aid in peak ID of the maleate salt peak, maleic acid solutions at varying concentrations were analyzed using UV/CAD detection. The retention time of the maleic acid peak corresponded with the peak detected at ~ 5 min as seen in the UV/CAD overlay. Identification of both eluting peaks was further confirmed using LC-MS. Solutions of Compound X Maleate reference standard and Compound X sample were used for analysis. The ESI scans of each solution showed a mass accuracy of within 5 ppm for both Compound X and the Maleate salt.

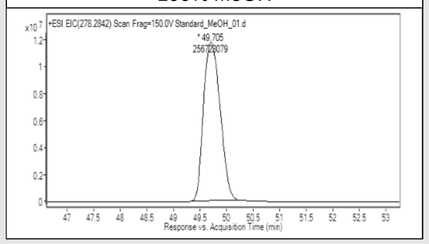
EIC of Compound X in a 0.01 mg/mL sample solution in 100% MeOH



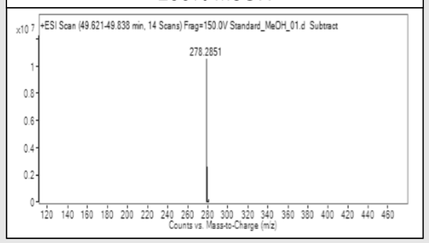
ESI of Compound X in a 0.01 mg/mL sample solution in 100% MeOH



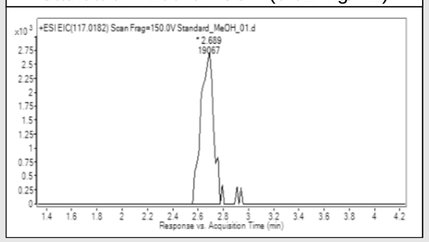
EIC of Compound X in Reference Standard in 100% MeOH



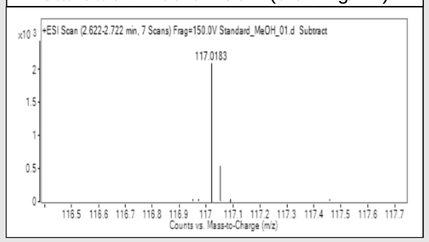
ESI of Compound X in Reference Standard in 100% MeOH



EIC of Maleic Acid in Compound X Reference Standard in 100% MeOH (0.01 mg/mL)



ESI of Maleic Acid in Compound X Reference Standard in 100% MeOH (0.01 mg/mL)



CONCLUSIONS

A thorough investigation into the structure and chromatography of Compound X showed that UV detection was not a suitable means for quantifying the main compound or the impurities. Analysis performed using charged aerosol detection (CAD) displayed the presence of two peaks, which drew concern to the initial identification of Compound X by UV detection. Proper identification of these peaks was confirmed via LC-MS and as a result of these findings, HPLC-CAD methods for assay, identification, related substances, and dissolution of Compound X were developed and validated prior to the release testing of Phase II clinical trial material to ensure the safety and efficacy of the clinical trial material.