TOOLS FOR EFFECTIVE IMPURITY ISOLATION

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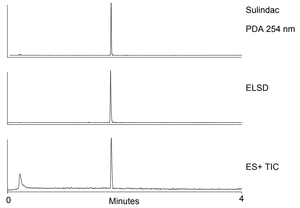
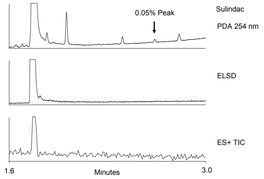
OVERVIEW

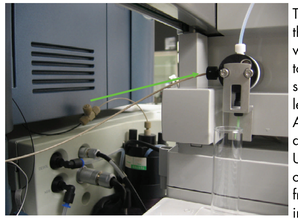
The proper characterization of impurities that may be present in health care products are required by regulatory agencies throughout the world. In order to ensure that an impurity is not missed, complementary detector techniques such as UV/vis, evaporative light scattering and mass spectrometry, have been coupled together with liquid chromatography for the analysis of samples originating from various phases of the commercialization process. With the introduction of a new class of liquid chromatography, Ultra Performance Liquid Chromatography (UPLC), increased chromatographic resolution and sensitivity along with increased sample throughput are now possible. UPLC, coupled with multiple detectors, now provides the ability to resolve components that were not previously separated nor possibly detected, resulting in improvements for older analytical methods as well as the development of appropriate new analytical methods. In those instances where a new peak is detected, it may be desirable to preparatively collect this new peak of interest from a UPLC system for further characterization without significant changes to the system. Because of the increased sample throughput afforded by UPLC, such micro-preparative isolation work can be performed over a short period of time. This poster illustrates a preparative scale-up strategy for the isolation of a 0.05% impurity from a drug substance using Ultra Performance Liquid Chromatography with the least invasive changes in the ACQUITY™ UPLC instrumentation so as to minimize the disruption of the flow of work in the analytical laboratory.



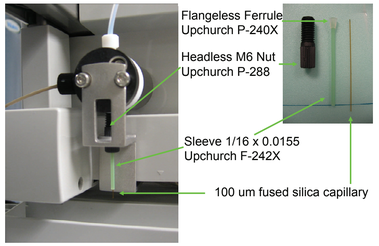
An example of a UPLC separation using triple detectors for the analysis of a drug substance (Sulindac) utilizing a gradient of 5 to 95% acetonitrile with 0.05% TFA over 4 minutes at a flow rate of 0.84 mL/min. on an ACQUITY UPLC™ BEH C18 column (2.1 x 50 mm, 1.7µm) is shown. The sample is prepared at a concentration of 1 mg/mL in DMSO with an injection volume of 0.5 µL using a 2 µL loop.



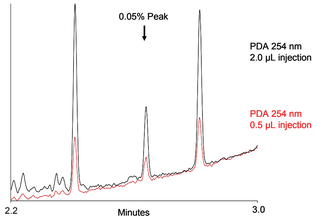
Closer examination of the three chromatograms shows the presence of multiple impurities in the UV/vis chromatogram not seen with either the ELS or mass spectrometric detectors. A peak of interest has been identified at a 0.05% concentration relative to the major drug substance peak for preparative isolation. The experimental boundaries that will direct the micro-preparative work will be as follows: 1. The overall intent is to obtain a small quantity of the contaminant peak for further work on structural characterization.; 2. We do not want to change to a preparative HPLC system from our ACQUITY™ UPLC system because it is important for us to "track" this new peak of interest using its retention time and it's relative position to other peaks in the chromatogram. ; 3. The concentration of the stock solution is to be fixed at the concentration of the received drug substance of 1.0 mg/ml in DMSO.



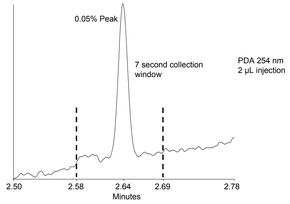
The easiest solution to collect the 0.05% contaminant peak was to add a fraction collector to the ACQUITY™ UPLC system and to do a timed collection of the peak of interest. A fraction collector was added to the ACQUITY™ UPLC system by means of disconnecting the PDA outlet line from the microtee and attaching it directly to the inlet of the modified low flow diverter valve of the Waters Fraction Collector III.



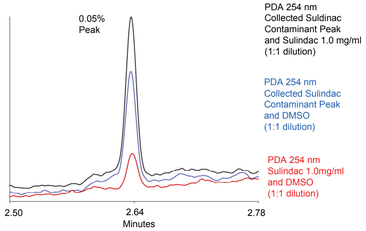
In order to handle the small peak volumes to be collected, the standard nozzle of the low volume diverter valve was replaced with a new nozzle as illustrated.



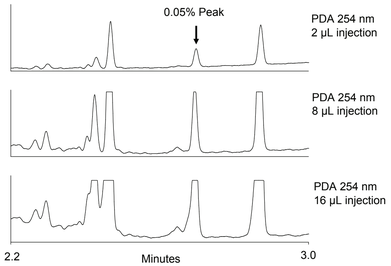
The first micro-preparative plan was to make multiple 0.5 µL injections based upon the analytical chromatographic method. With the concentration of the sample at 1.0 mg/mL, a 0.5 µL injection would result in the loading of 0.5 µg of the drug substance and theoretically 0.25 ng of the 0.05% impurity peak. Since the objective was to obtain approximately 250 ng of the impurity peak, a total of 1000 injections was required. This would utilize the ACQUITY™ UPLC instrumentation for four days. Because tying up the use of the instrument for four continuous days would not be desirable, a second micro-preparative strategy was devised. In this second micro-preparative plan the 0.5 µL analytical injection was increased to the full volume of the loop, 2 µL. No adverse effect on the chromatography was observed by increasing the injection volume. Because it was possible to increase the injection volume 4 fold, only 250 injections were required (one day) to obtain the desired 250 ng quantity of material.



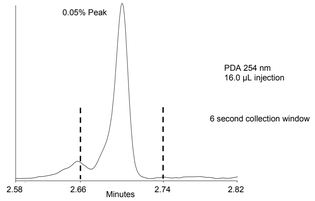
A seven second collection window (98 µL collection volume) was selected for the preparative runs.



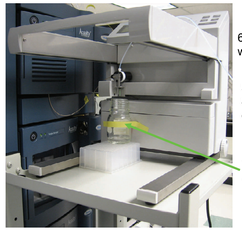
After all 250 injections were completed, the combined fractions were taken to dryness under a stream of nitrogen and dissolved in 25 µL of DMSO. This resulted in a 20 fold increase in concentration. Dilutions of the collected fraction with DMSO and the starting drug substance soution as compared against a dilution of the starting drug substance solution diluted with DMSO showed that the correct material had been collected. However, this amount of material was still not enough to allow for the acquisition of a UV/vis or mass spectrum of the contaminant peak using the UPLC triple detection system.



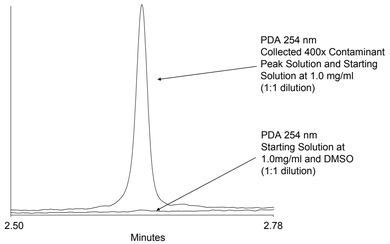
A third micro-preparative plan was devised that required changing of the 2 µL loop and the 100 µL sample syringe to a 50 µL lop and a 250 µL sample syringe. With these changes completed, a loading study was performed to determine the amount of the 1.0 mg/mL concentration of drug substance in DMSO could be loaded without compromising the resolution of the 0.05% contaminant peak. Doubling injection volumes of 2, 4, 8, 16 and 32 µL were investigated. Comparisons of the 2 µL injection volume with the 8 and 16 µL injection volumes were compared and the decision made to utilize the 16 µL injection volume for the next micro-preparative collection.



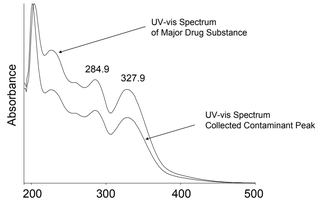
With the concentration of the drug substance sample at 1.0 mg/mL, a 16 µL injection resulted in the loading of 16 µg of the drug substance and theoretically 8.0 ng of the 0.05% impurity. The decision was made to inject a total of 500 injections over a weekend. It was felt that the collection of 4 µg of material would be sufficient for further work. A six second collection window (84 µL collection volume) was selected for the preparative runs.



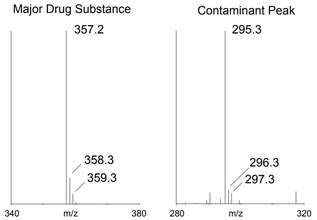
Because the injection volume exceeded the volume of a 18 x 150 mm test tube previously used, a 100 mL wide mouth jar was used for collection.



After all 500 injections were completed, the combined fractions were taken to dryness under a stream of nitrogen and dissolved in 20 µL of DMSO. This resulted in a 400 fold increase in concentration. Dilutions of the collected fraction with DMSO and the starting material showed that the correct material had been collected.



The concentrated solution of the 0.05% contamination peak allowed a UV/vis spectrum to be collected as well as the mass spectrum revealing that the contaminant peak was related to the parent compound with a similar UV/vis spectrum but with a different molecular weight.



CONCLUSIONS

- A 0.05% contaminant peak was preparatively collected so that:
  - a UV/vis spectra was obtained showing that the 0.05% contaminant peak is structurally related to the parent compound peak.
  - a mass spectra was obtained showing that the 0.05% contaminant peak is structurally different form the parent compound peak.
- The preparative process did not require disruption of the ACQUITY™ UPLC system other than the addition of a larger loop, sample syringe and a fraction collector.
- The preparative collection was completed unattended over a weekend.

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