Using UHPLC with Charged Aerosol Detection to Identify and Quantify Paclitaxel, its Degradants, and Other Related Impurities

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Cancer is one of the leading causes of death worldwide [1], prompting broad interest in developing innovative drug treatments for improved patient care. One of these drugs, paclitaxel, is used for chemotherapy treatment of different cancer types [2]. In order to develop a drug like paclitaxel that is injected directly into a vein, stringent drug testing is performed to ensure impurities and related substances are not incorporated into the final drug formulation. However, common monitoring methods such as UHPLC with UV/Vis detection can cause uncertainty in quantification of products in stability and degradation studies due to lack of detection or varying response [3]. Meeting ICH guidelines [4] for reporting, identification, and qualification of compounds can be better achieved using combined methods such as UHPLC-UV-CAD.

Introduction

Given that nearly 1 in 6 deaths worldwide is caused by cancer and an estimated 23.6 million new cases of cancer will exist each year by 2030 [1,5], the search for novel therapies that are more effective than current treatments is a top priority for many pharmaceutical companies and cancer researchers. Treatment types are numerous and can vary with cancer type and progression, including chemotherapy, radiation, immunotherapy, hormone treatments, and targeted therapies. For example, the chemotherapy drug paclitaxel is a standard treatment given for a broad range of cancers. Even though it was discovered in the 1960s when isolated from the bark of a Pacific Yew tree, it now has become a reliable mitotic inhibitor that can reduce the cancer load in patients [2].

In any drug lifecycle, monitoring impurities that have the potential to turn a drug from safe and effective to one with severe side effects is extremely important. Impurities in drug substances are tolerated only at extremely low level, therefore highly sensitive analysis methods are required to assess the purity of drugs. Not only this, but impurities can also be very similar to the active pharmaceutical ingredient (API), and the chromatographic separation can be a challenge. Regardless of the complexity of a sample, ICH guidelines Q3A-Q3D require that impurities be accurately reported, identified, and qualified to prevent issues with the final drug formulation [4].

ICH guidelines for impurities target those which might arise as degradation products of the drug substance, potential additions during the manufacturing process, or from interactions between a drug substance and packaging material components [6]. These guidelines are set for analysis based on the determination of any observed impurities and provide threshold values based on the maximum daily dose of the active drug component supplied in the final product.

Standard methods for the monitoring of impurities throughout the drug development lifecycle include the use of ultra-high performance liquid chromatography (UHPLC) with UV/Vis detection [7]. UV/Vis detection is easy-to-use, sensitive, and provides reasonable specificity relative to its targets. While this detection method is currently preferred for drug substance analysis, UV response factors may vary widely among compounds, and some of them may even lack a chromophore making their detection and quantitation impossible [8]. These obstacles impede successful guideline compliance due to uncertainties in quantification. In addition, calibration standards are not always available for the early stages of drug development, yet are needed for quantitation. By incorporating an approach where analysis can be performed with a single calibration and elicit uniform responses, many of these challenges can be overcome.

As an alternative approach to UV/Vis, charged aerosol detection (CAD) can measure the amount of all non-volatile and many semi-volatile analytes in a sample by generating dried charged aerosol particles, which are detected using an electrometer. Instead of relying on an inherent property that not all molecules have, such as a chromophore requirement for UV/Vis detection, CAD measures the charge of aerosol particles, which is in direct proportion to the mass concentration of the analyte, providing a response independent of chemical structure [9]. This benefit makes CAD compatible with complex pharmaceutical samples and specific, even in variable conditions. Though the method shows sensitivity in response to mobile phase composition and change in gradient elution, the use of a compensation gradient can be used to overcome this and ensures a

universal and uniform response [10].

In this work, in order to separate paclitaxel from related compounds and impurities generated during a thermal degradation experiment, UHPLC with both UV/Vis and CAD were applied. Calibrations were performed using paclitaxel and two other known related impurities as standards to estimate the quantities of other unknown impurities present in the sample.

Experimental

Sample preparation

The calibration standards for this study include paclitaxel and its related impurities, Impurity C and cephalomannine [European Pharmacopiea (Strasbourg, France) & United States Pharmacopeia (Rockville, MS, USA)], Baccatin III [Sigman-Aldrich (Schnelldorf, Germany)]. The calibration standards were weighed and prepared to a final concentration of 0.1 mg/mL with methanol. The three substances were mixed to achieve a 10 µg/mL stock solution with methanol. Then 10, 5, 1, and 0.5 µg/mL calibration standards were produced by a dilution series with methanol. Each calibration standard was analysed in three consecutive runs with blank injections in between the different concentrations. As the standards displayed limited stability in solution, they were prepared directly prior to analysis.

Forced degradation was carried out on 100 µL volume of 1 mg/mL paclitaxel solution diluted with 350 µL methanol and 50 µL dimethyl sulphoxide (DMSO). The solution was incubated at 65°C for two hours and analysed immediately.

Instrumentation

A Thermo Scientific™ Accucore™ Pentafluorophenyl (PFP) column was used for compound separation. Chromatographic conditions are presented in Figure 1. Detection was performed using the Thermo Scientific™ Vanquish™ Flex Variable Wavelength Detector followed by the Thermo Scientific™ Vanquish™ Flex Charged Aerosol Detector.

Inverse Gradient Workflow

The Thermo Scientific™ Vanquish™ Flex Duo UHPLC system for Inverse Gradient (Figure 2) included a System Base Vanquish Flex (P/N VF-S01-A-02), Dual Pump F (P/N VF-P32-A-01), Split Sampler FT (P/N VF-A10-A-02) with a 25 µL sample loop,

UHPLC Experimental Conditions	
Column:	Accucore PFP 2.1 × 150 mm, 2.6 µm
Mobile phase:	A: Water, ultra-pure (18.2 MΩ·cm at 25°C) B: LC-MS grade acetonitrile
Analytical gradient:	23–60% B in 25 minutes; 0.3 mL/min
Compensation gradient:	23–60% A in 25 minutes, 0.3 mL/min
Temperature:	Forced air 35°C; Active pre-heater 35°C
Injection volume:	1 µL
UV detection:	227 nm, 5 Hz, response time 1 s
CAD:	Evaporation temp. 50°C, 5 Hz, Filter 3.6

Figure 1: Chromatographic conditions used.

Column Compartment H (P/N VH-C10-A-02), Charged Aerosol Detector F (P/N VF-D20-A), Variable Wavelength Detector F (P/N VF-D40-A), and a Vanquish Duo for Inverse Gradient Kit (P/N 6036.2010).

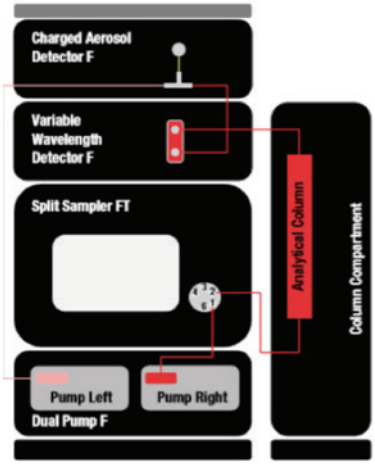

Figure 2: Fluidic scheme of the Vanquish Duo Inverse Gradient Workflow.

Data Analysis

The Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.2.8 was used for data acquisition and evaluation.

Results and Discussion

Uniform Response with Gradient Compensation

CAD begins with the pneumatic nebulisation of the mobile phase from the analytical column to form an aerosol. Solvent is evaporated from the smaller droplets to form particles. Diffusion charging of the particles, by collision with an opposing ion jet formed via corona discharge, occurs in the mixing chamber. The aggregate charge of aerosol particles is measured using an electrometer [11].

The charge on the dried particle is proportional to the particle diameter, which depends on the mass concentration of the analyte. Since CAD is a nebulisation technique, changes in solvent composition, such as gradient elution methods, will affect the nebulisation efficiency and therefore detector response [11]. To address this effect, an inverse gradient is applied post-column that mirrors the analytical gradient composition; in this way, a uniform solvent composition in the nebuliser will be maintained throughout the method. In an inverse gradient workflow, a second pump can be used to deliver a mirrored gradient to the analytical gradient post column using a T-piece to neutralise the effect of changing organic content.

The API sample was analysed with and without gradient compensation with the Vanquish Duo System for Inverse Gradient, which utilised the Vanquish Flex Dual Pump, illustrated in Figure 2. While both experiments resulted in the same number of peaks, there was a clear difference in peak response. Since the inverse gradient creates a more uniform analyte response over the course of a gradient elution, it can provide a more accurate and unbiased analysis of each impurity in the complex sample [10].

Comparing CAD response with and without applying inverse gradient compensation highlighted the variability in response based on gradient elution and its dependency on solvent composition. Without the gradient compensation, analyte quantities eluting before the API were underestimated, while analytes eluting after the API were overestimated, as shown in Figure 3 at peak at approximately 26 min. Applying gradient compensation brings this imbalance to light by providing more even analyte quantitation across the gradient before and after the API, as shown in Figure 3, when comparing the blue peaks to the red peaks. A greater than ten percent difference was observed in combined peak area between using and

not using gradient compensation and thus in determined impurity content. Combined peak area for all impurities using gradient compensation was 53.8% of the API, while the same peak area was 63.9% of the API when not using gradient compensation (Figure 3). This large variation demonstrates the influence of solvent composition on CAD response and successful correction using inverse gradient compensation.

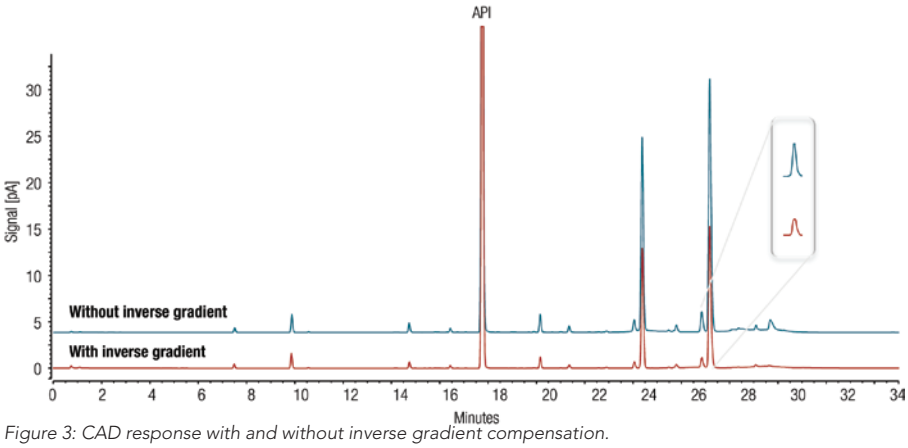

Figure 3: CAD response with and without inverse gradient compensation.

Three-standards Linear Calibration

Figure 4 shows the advantages of using CAD with inverse-gradient compared to UV for accurate quantitation. Since the response of the CAD depends on solvent composition and does not depend on chemical structure, the calibration lines obtained for the three standards overlaps. Looking at the measurements of the standards at 10 µg/mL level, the CAD delivers an almost identical normalised response for all three compounds, whereas UV response varies up to 63%.

The calibration curve with CAD detection and inverse gradient shows a linear relationship of peak area to analyte concentration for all compounds, illustrating minimal response variation across compounds. This gives us the confidence to measure levels of impurities even when we do not have standards available for those components.

Multi-detector Approach for Degradation Analysis

UV/Vis and CAD used with UHPLC each have strengths that can be leveraged in the quantification of unknown compounds such as those observed in pharmaceutical mixtures. Some compounds can lack a chromophore necessary for UV absorption and cannot be detected using UV8, seen with CAD peaks at 24.6 and 27 minutes in Figure 5. Likewise, if a compound is too volatile it cannot be nebulised or detected by CAD. The analysis of the degradation products of paclitaxel showed that one of the degradation products of interest was only detectable with UV. The two main impurities identified were only detectable using CAD (Figure 5). As such, each method provides relevant data that one method alone would not have detected.

Combining the two approaches offers a comprehensive analysis of complex samples where unknown impurities might be too volatile for CAD but detected in UV, or undetectable by UV but easily seen using CAD. Complementary methods such as these provide a more accurate analysis when quantifying compounds using a single calibration curve [12]. Applying both methods enables detection of all impurities and related compounds, providing a robust multi-detector approach that is well-suited for stability studies.

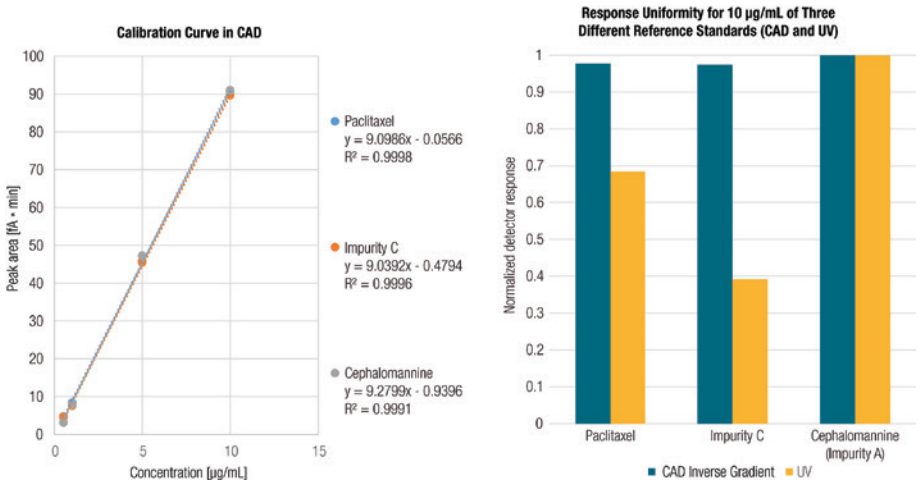

Figure 4: Similar calibration curves for all three standards demonstrate uniform response using CAD and the inverse gradient workflow (left). Comparison of CAD and UV response across the three standards (right).

Conclusion

The ability to resolve and quantify impurities and degradants in complex mixtures by LC-UV is significant to pharmaceutical products' efficacy and safety monitoring. Quantitation of impurities and degradants can be challenging as standards are typically unavailable and UV response can vary significantly between analytes. To overcome these issues a CAD-UV approach was developed. However, CAD response is affected by gradient elution. Thus an inverse gradient compensation approach was adopted. Gradient compensation was evaluated and shown to be of significant benefit for CAD response. Therefore the CAD approach using single calibrant quantification could be successfully used to estimate levels of impurities where external standards are unavailable.

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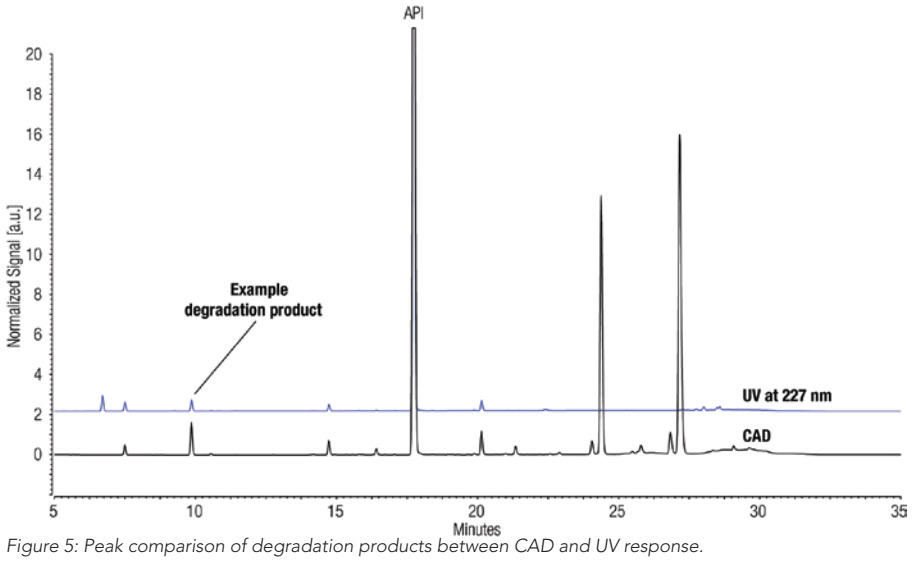

Figure 5: Peak comparison of degradation products between CAD and UV response.

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