Quantifying more with less: Implementing charged aerosol detection to improve drug safety

University of Wuerzburg

"With Thermo Fisher Scientific at our side, we always have the opportunity to investigate fundamental questions such as the meaning of the Power-Function-Value and how to best adjust it, thus making the CAD suitable for broader applications."

– Rasmus Walther, Pharmacist and PhD student, University of Wuerzburg

Introduction

The Pharmacy Department of the University of Wuerzburg has a rich history of collaborating with the pharmaceutical industry to help solve analytical problems, to evaluate new technology, and for modernization of legacy methods to ensure drug safety is improved as the technology and techniques evolve.

Professor Dr. Ulrike Holzgrabe has served as an expert on various committees of the German and European Pharmacopoeia for 25 years, all dealing with the development of high-quality analytics of drugs, with a heavy focus on the gold standard high performance liquid chromatography (HPLC). Especially in the last few years, she has become interested in new column resins, such as HILIC and mixed mode columns. For some time now the group has focused on implementing UHPLC-charged aerosol detection (CAD) as a complimentary detector to UV and mass spectrometry to ensure single methods can measure all the components within a drug product without exception.

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Sensitivity

"Due to the narrow-bore capillaries used in the Vanquish system, the peak broadening between the column and CAD is low, which is particularly helpful in the determination of small quantities."

– Rasmus Walther, Pharmacist and PhD student, University of Wuerzburg

Why is charged aerosol detection so powerful?

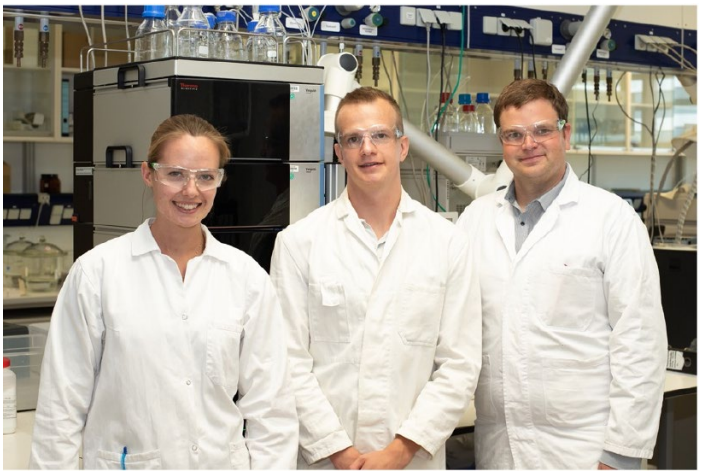
For complex separations where multiple analytes in a sample are not compatible with UV and MS detectors, for instance when compounds lack a chromophore or cannot ionize, liquid chromatographers can turn to evaporative aerosol detectors.

In addition to commonly used UV detection, Prof. Holzgrabe's group initially employed evaporative light scattering detector (ELSD) for APIs and impurities that do not contain a chromophore, or do not readily ionise. However, it was found that ELSD was not specifically suitable for drug purity assessment due to low sensitivity and spike peaks occurring on the tail of the main peak. This led into interest in CAD, which has higher sensitivity than methods based on light scattering or refractive indices, while also offering a higher level of precision.[1]

"One of our fields of focus is the identification of unknown impurities of a drug substance, many of which lack a chromophore," according to Adrian Leistner. "We use the CAD to complement UV detection within hyphenated detection techniques, or for preliminary experiments before identifying the compounds by mass spectrometry."

Both CAD and ELSD can detect non-volatile and many semi-volatile compounds, but how the particles are detected differs between the two technologies. CAD measures particle charge while ELSD measures the ability of the particle to scatter light. Many applications are described in the European and U.S. Pharmacopoeias using ELSD to evaluate the composition of plant extract, however the assessment of a low-amount impurity in the vast majority of drugs is challenging by ELSD due to sigmoidal curve response, which means the content of the impurity can be underestimated. This is not observed with the CAD and thus it makes for a more precise measurement. Laura Backer explains, "We want to make use of CAD technology because we must assume that not all degradation products carry a UV-absorbing structural element. Examples include k-strophanthin ampoules and atropine preparations such as eyedrops and injections."

In the evaluation of older CAD systems compared to the Thermo Scientific ™ Vanquish ™ CAD, the data obtained at the University of Wuerzburg shows a superior sensitivity throughout the whole mass range with the Vanquish CAD model, giving better performance for low level impurities.



Laura Backer, Rasmus Walther, and Adrian Leistner, pharmacists and PhD students, University of Wuerzburg

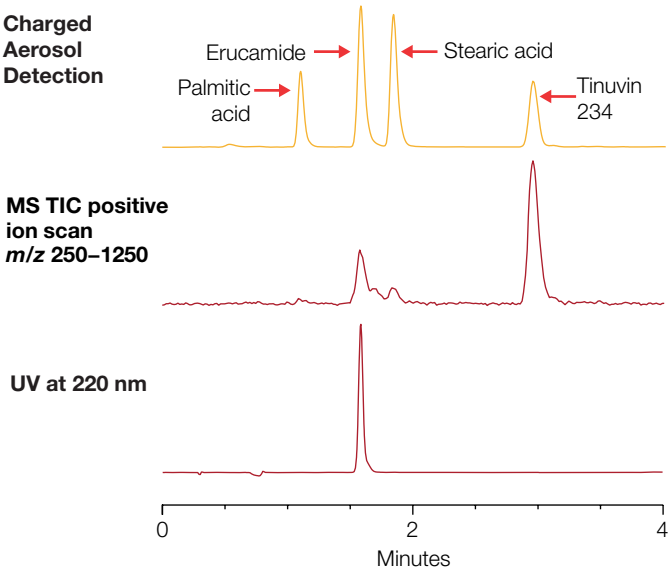


Figure 1. Mix of standards demonstrating differences in detection methods. Unlike mass spectrometry and UV, the CAD can measure all semi- and non-volatile analytes in the sample. Mass spectrometry (MS) requires the analyte to form gas phase ions, while response by a UV detector depends upon the nature of the chromophore.

Ease of use, minimal user training

"After a brief introduction to the system and the software, the basic operation is clear. It is rather easy to find the way around the new user interface as it follows a clear, application-oriented structure."

– Laura Backer, Pharmacist and PhD student, University of Wuerzburg

Quality analysis of drugs is challenging because impurities are often present in very low quantities. Hence the impurities must be quantified in the presence of a large amount of the drug. This is only possible when drugs and their impurities are baseline separated. In addition, high sensitivity is needed to assess the small amounts of the impurities. "Due to the narrow-bore capillaries used in the Vanquish system, the peak broadening between column and CAD is low, which is particularly helpful in the determination of small quantities," according to Rasmus Walther, co-worker in Prof. Holzgrabe's team.

Beside the analysis of drugs, the quality of the excipients must also be assessed, which very often do not contain a chromophore. Currently, many excipients are only characterized by bulk parameters, such as acid value, iodine value, saponification value, and others. Those parameters are not very specific. Here, the UHPLC analysis can take advantage of the charged aerosol detection to measure their concentration and any additional impurities originating from the excipient manufacture.

How easy is it to implement charged aerosol detection?

"After a brief introduction to the system and the software, the basic operation is clear," says Laura Backer. "It is easy to navigate the new user interface, as it follows a clear, application-oriented structure. Therefore, it is also possible for newcomers to carry out simple operations after a short training. This saves a lot of time and enables the quick start of new projects also by scientists who are not yet fully familiar with the equipment and the software. Besides, the software is also characterized by a very user-friendly generation and transfer of, for example, result tables and chromatograms. This option was missed in the previously known software of other suppliers and provides a great relief in the data organization and presentation."

For gradient-based separations, the Thermo Scientific™ Vanquish™ Duo UHPLC system can be implemented to make use of the inverse gradient configuration. This ensures that uniform detector response can also be achieved with gradient methods, as the mobile phase composition is kept constant at the detector inlet. In parallel to the CAD, and within the same injection, the diode array detector (DAD) can be inline so that, for example, an assessment of peak purity is possible for UV-active substances and co-elution can be detected more easily.

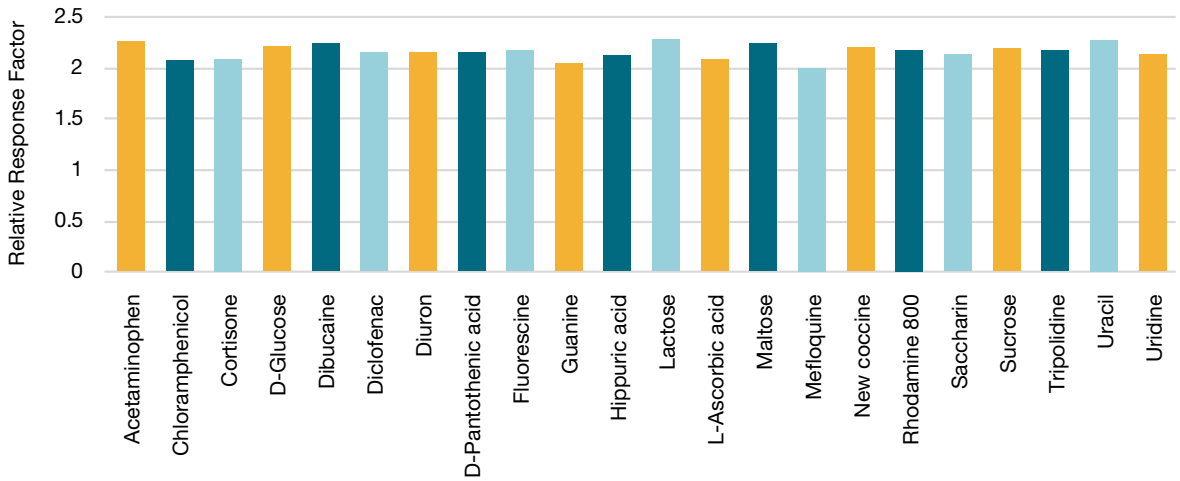


Figure 2. The CAD shows near-uniform response (<5% RSD variation) for all non-volatile analytes (0.5 µg each; flow injection analysis).

Collaborative approach enables advancements on testing strategies

The quality of medicinal products is a prerequisite for their safe use by patients. To ensure this in the best possible way, it is necessary to know the impurity profile precisely and to develop correspondingly precise methods.

One option to maximize the detection of impurities in a given drug substance is to employ a multidetector strategy, such as using traditional optical detectors in conjunction with alternate detection techniques. One such orthogonal technique relies on the recent advances in HPLC aerosol-based detection, such as evaporative light scattering detector and charged aerosol detectors. The University of Wuerzburg invested in one of the first CAD detectors for assessment against ELSD to determine the pros and cons. Since the CAD is a near-universal detector, many drugs with weak and no chromophore could easily be analyzed, providing an opportunity to be at the forefront of the technology. With further collaboration with Thermo Fisher Scientific, they were able to take full advantage as the technique evolved.

Adrian Leistner and Laura Backer, University of Wuerzburg

Recently, the group upgraded to the Thermo Scientific™ Vanquish™ Charged Aerosol Detector H, which allows flexible adjustments of the evaporation temperature and power function, allowing further method optimization. To be as flexible as possible within method development, the use of a Thermo Scientific™ Vanquish™ Flex UHPLC allows the autosampler, pumps, diode array detector, and CAD to be integrated into a single stack. The Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) is used for data acquisition and data analysis.

Further reading

For more in-depth discussion of the application and benefits of CAD when applied to pharmacopoeia methods and impurity profiling, see list of resources.

Resources

1. Holzgrabe, U.; Schilling, K.; Pawellek, R.; Scherf-Clavel, O. With united forces against impurities: Complementary detectors in pharmacopoeia analysis, Wiley Analytical Science Magazine, 7 May 2021. https://analyticalscience.wiley.com/do/10.1002/was.000600106
2. Pawellek, R.; Schilling, K.; Holzgrabe, U. Impurity profiling of l-aspartic acid and glycine using high-performance liquid chromatography coupled with charged aerosol and ultraviolet detection. *J. Pharm, Biomed, Anal.* **2020** May 10; 183, 113149. doi: 10.1016/j.jpba.2020.113149. Epub 2020 Feb 5. PMID: 32058290.
3. Walther, R.; Holzgrabe, U. Simplification of pharmacopoeial liquid chromatography methods for related substances of statins by hyphenated ultraviolet and charged aerosol detection. *J. Pharm. Biomed. Anal.* **2023** Feb 20, 225, 115218. doi: 10.1016/j.jpba.2022.115218. Epub 2022 Dec 29. PMID: 36608427.
4. Pawellek, R.; Holzgrabe, U. Influence of the mobile phase composition on hyphenated ultraviolet and charged aerosol detection for the impurity profiling of vigabatrin. *J. Pharm. Biomed. Anal.* **2021** Jul 15, 201, 114110. doi: 10.1016/j.jpba.2021.114110. Epub 2021 May 3. PMID: 33971590.
5. Pawellek, R.; Muellner, T.; Gamache, P.; Holzgrabe, U. Power function setting in charged aerosol detection for the linearization of detector response - optimization strategies and their application. *J. Chromatogr, A.* **2021** Jan 25, 1637, 461844. doi: 10.1016/j.chroma.2020.461844. Epub 2020 Dec 25. PMID: 33445033.

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