Multi-Modal Analyte Detection of Cyclodextrin and Ketoprofen Inclusion Complex Using UV and CAD on an Integrated UHPLC System

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Overview

Purpose: To develop a rapid method to simultaneously analyze an inclusion complex containing the non-chromophore compound, cyclodextrin and the non-steroidal anti-inflammatory drug, ketoprophen.

Methods: A long UHPLC column was used to separate the hydrophilic compounds, alpha and beta cyclodextrin and the more hydrophobic drug, ketoprophen. An integrated UHPLC system with a UV and universal charged aerosol detection offering multi-mode detection for the simultaneous analysis of both non-chromophore and chromophore compounds was employed.

Results: A UHPLC method for the determination of cyclodextrins and non-steroidal anti-inflammatory drug analysis using multi-modal UV and charged aerosol detection is described. Multi-modal UV and charged aerosol detection in an integrated system offers a suitable means for the analysis drug inclusion complexes consisting of both chromophore and non-chromophore species. The detectors are orthogonal and complimentary in nature so that you can detect more compounds in the sample. The integrated Vanquish system produces excellent data quality since UHPLC columns packed with smaller particles can be used and operated at maximum efficiencies.

Introduction

Often one HPLC detector is insufficient to reveal all of the compounds that may be present in a specific pharmaceutical formulation. Often with LC detectors one analyte responds more strongly than another, or may not respond at all. What is most desired is the ability to accurately measure a wide range of analytes with consistent response. Absorbance detection is preferred for those compounds that have a chemical structure with a suitable chromophore. However, for those compounds that lack a suitable chromophore structure an alternate detection technique becomes necessary. Alternate detection techniques include universal detectors such as RI, ELSD, CAD or MS.

The new Thermo Scientific™ Vanquish™ UHPLC system is an excellent integrated platform by incorporating a Binary pump, split loop Autosampler, Column compartment and both Diode Array and Charged Aerosol Detectors. This system is standardized with biocompatible fluidics which helps reduce any degradation of labile analytes. The Vanquish System integrates both diode array and charged aerosol detectors which are both orthogonal and complimentary in nature so that you can detect more compounds in the sample. The DAD incorporates LightPipe technology for enhanced sensitivity and signal to noise performance. The system offers sensitive DAD with linearity up to 3 AU for those compounds with suitable chromophores. The CAD is a sensitive universal detector designed for UHPLC and has a wide dynamic range for those compounds that lack a chromophore. Charged Aerosol detection (CAD) is a mass sensitive technique for determining levels of any non-volatile and many semi-volatile analytes after separation by liquid chromatography. This technique produces consistent analyte response independent of chemical characteristics and gives high sensitivity over a wider dynamic range. The presence of chromophoric groups, radiolabels, ionizable moieties, or chemical derivatization is not needed for detection.

Several drug candidates have limited bioavailability or stability due to their inherent chemical properties. Cyclodextrin can form a complex with hydrophobic “guest” molecules like ketoprofen and this inclusion complex drug delivery system (Figure 1) presents several benefits. The main benefits include improved drug solubility, drug stability and dissolution while in the circulatory system. This leads to a more rapid onset of drug action and can reduce certain drug side effects due to time released delivery rates. Examples showing the separation of cyclodextrins and ketoprofen using the Vanquish system presented in Figure 2 illustrate the benefits of multi-mode detection in an integrated system.

Methods

Liquid Chromatography using the Vanquish binary UHPLC system including:

- Binary Pump H (P/N VH-P10-A)
- Split Sampler HT (P/N VH-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Diode Array Detector HL, 320 nm (P/N VH-D10-A)
- Charged Aerosol Detector H, (P/N VH-D20-A)

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Column:	Thermo Scientific™ Vanquish™ Acclaim™ PA2, 2.2 µm, 2.1 x 250 mm
Adiabatic Temperature:	40 ºC
Flow rate:	0.7 mL/min
Mobile Phase A:	water, 0.1% formic acid
Mobile Phase B:	acetonitrile, 0.1% formic acid
Gradient	-2.5 to 0.0 min., 0 %B 0 to 2.0 min., 0 %B 2.0 to 7.0 min., 0 – 90 %B 7.0 to 8 min., hold at 90 %B 8.0 to 8.5 min., 90 – 0 %B
Detector settings:	CAD,10 Hz data rate, 5 s response time, 35 °C evaporation temp.,1.00 PFV DAD, 10 mm, LightPipe, 254 nm, data collection rate 10 Hz, 0.05 s response time, slit 4 nm

Data Analysis
Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 7.2

FIGURE 1. Structures of Analytes: β-Cyclodextrin and Ketoprofen (A) and Inclusion Complex (B).

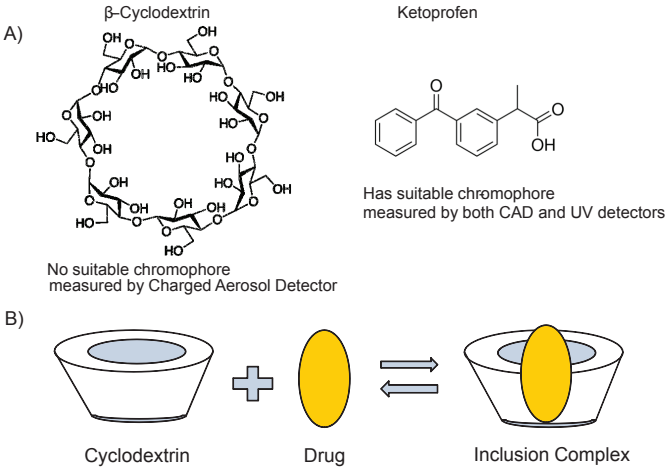
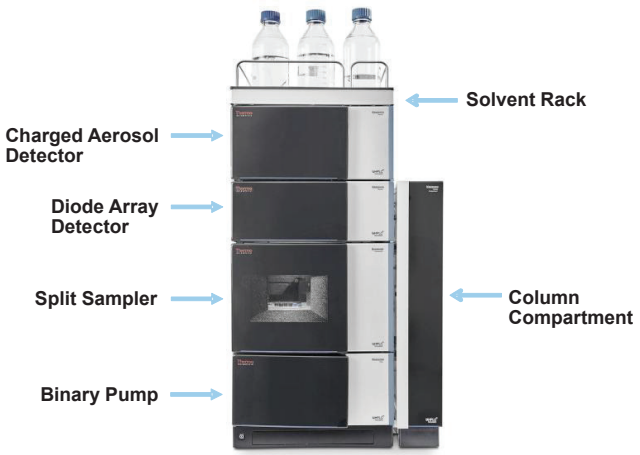


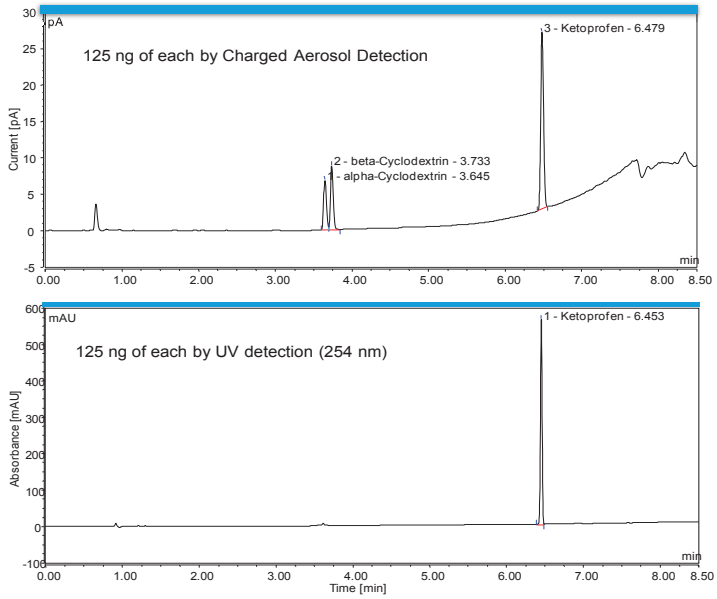
FIGURE 2. Integrated Vanquish UHPLC System with Multi-Modal Analyte Detection.



Results

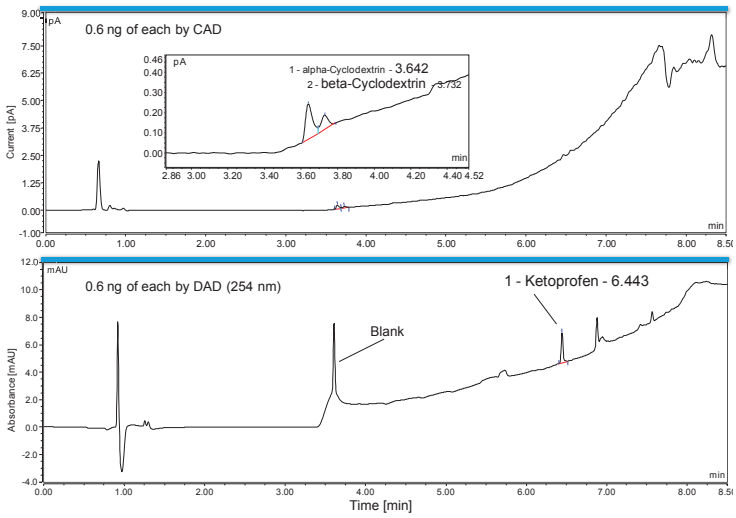
In the current study, an Acclaim PA2, 2.2 µm, 2.1 x 250 mm UHPLC column was employed so that baseline separation of alpha and beta cyclodextrin could be obtained even though the cycle time of the run was less than 9 minutes. To separate alpha and beta cyclodextrin a long UHPLC column and a mobile phase containing no organic solvent for the first two minutes was used. The gradient was then rapidly adjusted to elute the ketoprofen standard (125 nanograms on column) within 6.5 minutes by increasing the amount of organic solvent from 0% to 90% as shown in Figure 3. Elevated column pressures were observed due to flow rate and solvent viscosity. Therefore, the column compartment was operated using the still air mode since this contributes to optimal column efficiencies when operating at elevated UHPLC pressures by minimizing issues related to frictional heat.[3]

FIGURE 3. Detection of Cyclodextrins and Ketoprofen by CAD and UV.



Picogram sensitivity was obtained on both charged aerosol and diode array detectors and this is illustrated in Figure 4. Additional cyclodextrin products like 2-hydroxypropyl-β-cyclodextrin can also be assayed using a similar approach (data not shown).

FIGURE 4. Sensitive Detection of Cyclodextrins and Ketoprofen by CAD and UV.



Calibration data shown in Figure 5 illustrates the quality of data even with the non-linear response trend of the charged aerosol detector with increasing analyte concentrations. Calibration curves for both alpha and beta Cyclodextrin show excellent correlations having an R^2 of 0.999 and good precision with 1.1% RSD. Table 1 shows LOQ values obtained for each cyclodextrin species were in the mid picogram range. The calibration curves for ketoprofen also exhibit excellent correlations with the R^2 of 0.999 for both the UV and charged aerosol detectors. The LOQ for ketoprofen was better using the UV than CAD. With UV detection low picogram levels of Ketoprofen could be detected while mid picogram levels were detected using the charged aerosol detector.

FIGURE 5. Calibration Data for Cyclodextrins and Ketoprofen by CAD.

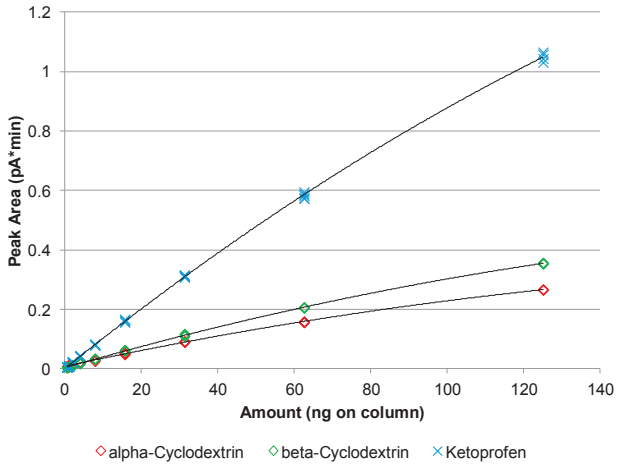
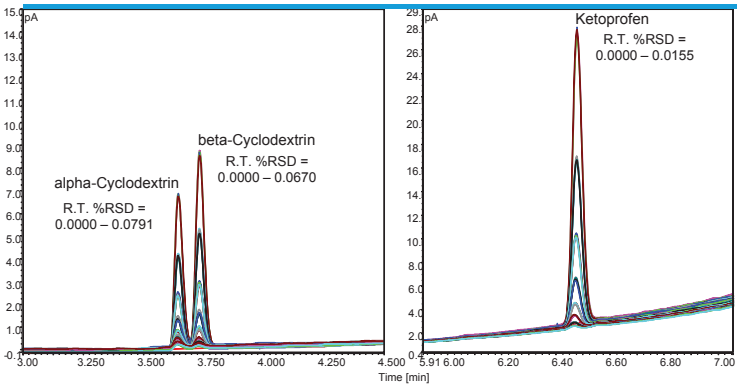


TABLE 1. Goodness of fit and LOQ metrics for analysis of Cyclodextrins and Ketoprofen by CAD and UV.

Compound	Detector	Coefficient of Determination (R^2)	Lower Limit of Quantification (LOQ)
Alpha-Cyclodextrin	CAD	0.9992	440 pg
Beta-Cyclodextrin	CAD	0.9995	530 pg
Ketoprofen	CAD	0.9996	350 pg
Ketoprofen	DAD	1.00	55 pg

The Vanquish system offers high precision performance. The %RSD on retention time was quite low and ranged from 0 to 0.08 for the earliest eluting compound and 0 to 0.016 for ketoprofen as indicated in Figure 6. All eight calibration levels were made with 5 injections at each level.

FIGURE 6. Retention Time Precision of Cyclodextrins and Ketoprofen using the Vanquish System (5 injections at each level).



Discussion

When UHPLC columns are packed with smaller particles the maximum flow rate is determined by the upper pressure rating of the UHPLC system. The Vanquish system, with optimized fluidics that brings extra-low system dispersion for maximum resolution, and delivers pressures up to 1500 bar (22,000 psi). The Vanquish autosampler dispenses maximum injection volume accuracy and precision, due to the design of the metering system used for precise volumetric fluid displacement. The column compartment was operated using the “still air” mode, which produces optimal column efficiencies to compensate for the frictional heat observed with elevated UHPLC pressures. One of the key benefits of the Vanquish system is the excellent data quality it provides.

Multi-mode detection is implemented by Vanquish diode array and charged aerosol detectors which are both orthogonal and complimentary in nature. This offers better coverage of all sample analytes that may be present. The diode array detector incorporates LightPipe technology for enhanced sensitivity and signal to noise performance. This DAD achieves the best signal-to-noise performance through the combination of lowest baseline noise, a very long light-path, and minimum peak dispersion. Data collection rates as high as 200 Hz means excellent compatibility with UHPLC methods. The sensitive DAD with linearity up to 3 AU is used for those compounds that exhibit suitable chromophores. The CAD is a sensitive universal detector designed for UHPLC and has a wide dynamic range. The charged aerosol detector is used for those compounds that lack a chromophore.

The formation of a non-covalent inclusion complexes with hydrophobic “guest” molecules is used to alter the bioavailability of certain drugs.. Beta Cyclodextrin is a ring structure of seven sugars as illustrated in Figure 1. Since it is comprised of carbohydrate molecules it does not possess a suitable chromophore for UV detection and is only detected with the charged aerosol detector. The non-steroidal anti-inflammatory drug, Ketoprofen can be detected by both the DAD and CAD detectors since it is non-volatile and possesses a suitable chromophore. Multi-mode detection used in this work supplies a means of detecting both types of compounds with excellent sensitivity.

Conclusions

- The described UHPLC method for cyclodextrins and non-steroidal anti-inflammatory drug analysis using multi-modal UV and charged aerosol detection is described.
- Multi-modal UV and charged aerosol detection in an integrated system serves as a suitable means for the analysis drug inclusion complexes consisting of both chromophore and non-chromophore species. The detectors are orthogonal and complimentary in nature so that you can detect more compounds in the sample.
- The integrated Vanquish system offers excellent data quality since UHPLC columns packed with smaller particles can be used and operated at maximum efficiencies.

References

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