



Reliable Quantitative Results for Main and Trace Compounds in Complex Matrixes Using the Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System

Technical Overview

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Abstract

The Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System provides a 30-fold increase in linear dynamic range compared with a traditional diode array detector. By combining the signals from two diode array detectors with different path length Max-Light flow cells, the 1200 Infinity Series HDR-DAD Impurity Analyzer enables detection and quantification of main and trace compounds in a single run.



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Introduction

Using conventional Diode Array Detectors (DAD) in UHPLC, the analysis of main and trace compounds often requires two injections to quantify both with good area precision. Trace compounds often need manual integration to obtain reliable area results, especially if the baseline shows drift.

Both issues can be addressed by using the Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer. The detector design enables analysis of main and trace compounds simultaneously in one run. Further, the need for manual integration of compounds is avoided mainly due to improved signal-to-noise (S/N) ratios for trace compounds.

Experimental

Compounds

Sample provided by customer

Instrumentation

| Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System | | |
|---|--|-------------|
| G4212A | Agilent 1290 Infinity DAD with 60-mm cell | } Clustered |
| G4212A | Agilent 1290 Infinity DAD with 3.7-mm cell | |
| G2199A | High Dynamic Range DAD Kit | |
| G1316C | Agilent 1290 Infinity Thermostatted Column Compartment | |
| G4226A | Agilent 1290 Infinity Autosampler | |
| G1330B | Agilent 1290 Infinity Thermostat | |
| G4204A | Agilent 1290 Infinity Quaternary Pump | |

Chromatographic conditions

| | |
|-------------|--|
| Column | Sub-2 μm particle column, provided by customer |
| Temperature | 40 °C |
| Solvent A | 10 mM NH_4OAc |
| Solvent B | Acetonitrile |
| Solvent C | Methanol |
| Flow rate | 0.5 mL/min |
| Gradient | 24 % to 76 % acetonitrile and 6 to 19 % methanol in 17 minutes |
| DAD | 250/4 nm, Ref: 450/80 nm, 20 Hz |

Acquisition and evaluation software

OpenLAB CDS ChemStation Rev. C.01.05

Results and Discussion

The 1200 Infinity Series HDR-DAD Impurity Analyzer provides a 30-fold increased linear dynamic range relative to traditional DAD detectors. By combining the signals from two diode array detectors with different path length Max-Light flow cells, it enables detection and quantification of components with significantly different concentrations in a single run¹.

The separation of compounds in complex matrixes often needs time-consuming method development using different columns, mobile phases, gradients, and temperatures. Figure 1 shows the optimized separation for a complex naturally-derived sample. Typically, if one main compound and several trace compounds are present in a chromatogram, two different injection volumes must be used. The detection of a main compound with a peak height of 5,700 mAU, as shown in the chromatogram in Figure 1, exceeds the linear range of a conventional UV-detector, but still permits the detection of trace compounds. However, these trace compounds might disappear into the noise level if the injected sample amount is reduced to where the main peak is within the linear range of the conventional UV-detector.

Using the 1200 Infinity HDR-DAD, all compounds can be detected from very low to extremely high peak heights in one run.

The trace-level target compound in this example was only 0.04 % of the peak height of the main peak. Four consecutive runs were evaluated (Figure 2).

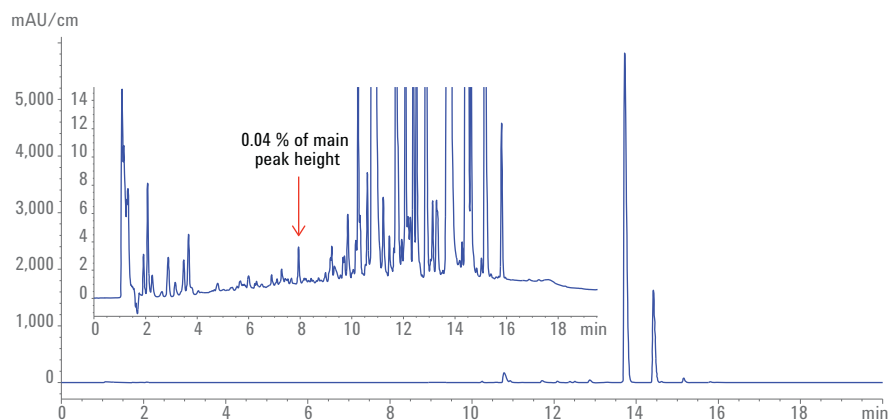


Figure 1. Optimized separation of complex sample.

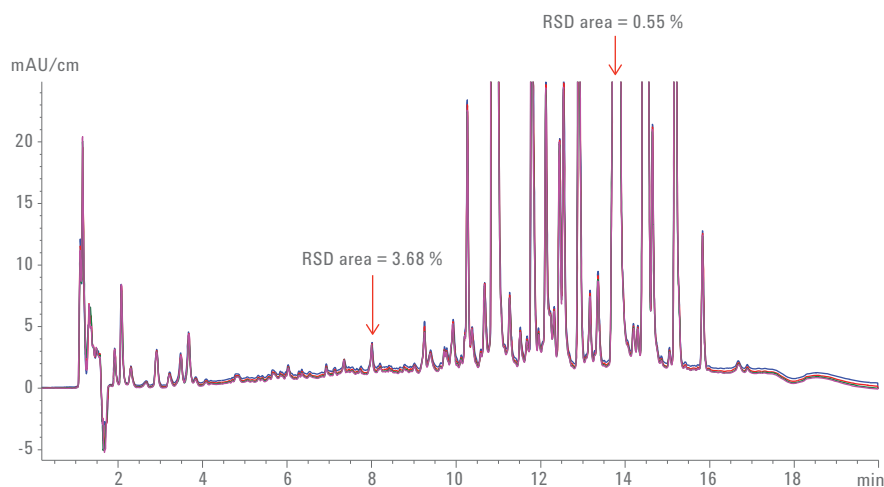


Figure 2. Overlay of four consecutive runs.

The relative standard deviations (RSD) for the trace compound (with approximately nine area counts) and for the main peak (with approximately 26,000 area counts) were 3.68 % and 0.55 % respectively. The integration was done using the standard integration parameters. No manual

integration parameters were used. Using manual integration, the area precision was 3.9 % RSD for the impurity. The retention time precision for the trace compound was 0.064 % RSD, and for the main compound 0.004 % RSD.

Conclusion

The Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer allowed the analysis of main and trace compounds in one run without exceeding the linear range for the main compound, which showed a peak height of approximately 5,700 mAU. The peak height of trace compound was only 0.04 % that of the main compound. The area precision for the trace compound was < 4 % even in the complex matrix of the analyzed sample.

Reference

1. Huesgen, A.G., Comparison of Sensitivity and Linearity of the Agilent 1200 Infinity Series High dynamic Range Diode Array Detector Solution and the Conventional Agilent 1290 Infinity DAD, *Agilent Technologies Technical Overview*, publication number 5991-3877EN, **2014**.

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