Application Note

Pharma-Biopharma, Small Molecules

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Entire Workflow from Dissolution Sampling, LIMS, HPLC Measurement to Result

Analysis of dissolution samples by HDR HPLC detection and a barcode sample ID workflow

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Abstract

Pharmaceutical quality control labs generate a high volume of dissolution samples with a great range of active pharmaceutical ingredient (API) content. This application note describes the use of an Agilent 1290 Infinity III LC system equipped with a high dynamic range (HDR) detector for the measurement of samples from a dissolution run with multidrug capsules. The capsules comprise a combination of four APIs over a broad concentration range, which are all quantifiable in one analysis by means of HDR, saving time and cost. The samples were filled in barcoded vials and introduced into a laboratory information management system (LIMS). For sample tracking, the built-in Agilent InfinityLab sample ID reader of the Agilent 1290 Infinity III multisampler was used. This setup saves time, provides improved ease-of-use, and reduces the sources or error for possible sample mix-ups.

Introduction

In pharmaceutical quality control testing, the three major criteria of content uniformity, assay, and drug release need to match the set specifications for the batch release of most finished drug products. Moreover, dissolution testing is an important performance criterion throughout the life cycle of product development, from R&D to final quality control. For dissolution testing, the finished drug product (e.g. tablet, capsule, powder, or suspension) is exposed to a defined amount of media under light stirring and a temperature of 32 to 37 °C. The release of API at a defined time is monitored by drawing samples at defined time points with subsequent analysis, mainly by UV spectroscopy or HPLC.

Compound separation in UV spectroscopic analysis can be compromised by excipients or the presence of several APIs in the formulation. Also, the broad range in concentration of the different APIs can challenge the sensitivity and must be taken into consideration. A possible solution is the analysis of the samples by HPLC, which separates multiple compounds and detects them separately. If the concentration of multiple APIs varies over a broad range, the Agilent HDR solution will detect them with the best possible sensitivity.

This application note demonstrates a dissolution experiment of a pharmaceutical product comprising four APIs present at a broad concentration range, followed by HPLC separation. To detect the highly concentrated APIs in the same run as the low-abundant compounds, Agilent HDR detection solution was used. To confirm that all samples have been analyzed, a 1290 Infinity III multisampler was equipped with a sample ID reader, and barcoded vials were used.[1,2] The complete workflow, from sampling into barcoded vials, introduction to the LIMS, forwarding to measurement, and reporting can be done in an effective time- and cost-saving manner.

Experimental

HPLC instrumentation

- Agilent 1290 Infinity III high-speed pump (G7120A)
- Agilent 1290 Infinity III hybrid multisampler (G7167B) equipped with a sample ID reader (G4756A or option #110 of all 1260/1290 Infinity III multisamplers).
- Agilent 1290 Infinity III multicolumn thermostat (G7116B)
- 2x Agilent 1290 Infinity III diode array detector (G7117B) with 60 mm Max-Light cartridge cell (G4212-60007) and 3.7 mm Max-Light cartridge cell (G4212-60032)

Dissolution instrumentation

Agilent 708-DS Dissolution Apparatus with 850-DS autosampler (G7910A, G7930A, G7913A)

Software

Agilent OpenLab CDS revision 2.8, or later version with Agilent Sample Scheduler for OpenLab

Column

Agilent ZORBAX Eclipse Plus C18, RRHD, 2.1 × 100 mm, 1.8 µm (part number 959758-902)

LC method

Parameter	Value
Solvents	A) Water B) Acetonitrile
Flow Rate	0.5 mL/min
Gradient	Time (min) %B 0 15 5 65 5.1 95 Stop time: 6 min Post time: 2 min
Injection Volume	5 µL
Needle Wash	3 s in Solvent A
Column Temperature	50 °C
Detection	254/4 nm, Ref. 360/16 nm, data rate 20 Hz

Dissolution method

Parameter	Value
Media	0.1 N HCL, 900 mL
Stirring Rate	75 rpm
Temperature	37 °C
Sampling Time Points	10, 20, 40, 60, 75 min
Sinker Basket	Japanese sinker (p/n 12-3070)

Additional materials

- Vial, screw, amber, write on, certified, 2 mL with bottom barcode (part number 5182-0716-ID)
- Vial screw caps, PTFE/rubber (part number 5184-5917)
- 40-vial tray with bottom holes for barcode reading (part number 5401-0068)
- Sample tray holder with open bottom for barcode reading (G7167-60205)
- USB hand-held barcode scanner (part number 5018-0003)
- Filtration of dissolution samples: 70 µm full-flow filters (17- 4020) and 8-channel filter plates 0.45 µm, nylon (Cytiva 7707-3100)

Samples

- Multi-API capsules containing vitamin C (150 mg), paracetamol (200 mg), caffeine (25 mg), and chlorphenamine (2.5 mg)
- Capsules were introduced to the dissolution vessels of an Agilent dissolution tester (708-DS) in Japanese sinkers in an Agilent Dissolution Apparatus with six vessels. Samples were drawn from each vessel at 10, 20, 40, 60, and 75 minutes.

Relative calibration

A capsule was dissolved completely with strong stirring for 90 minutes in the same solvent used for the dissolution experiment. The measured area values were used as 100% for a relative calibration.

Solvent and chemicals

- Agilent InfinityLab acetonitrile gradient grade for LC* (part number 5191-5100) was used for all analyses. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak).
- 0.1 N HCl was purchased from VWR, Germany.

Results and discussion

Barcoded vials were scanned into the LIMS and linked with sample information such as dissolution vessel number and time points. Sample information was then combined into an Agilent OpenLab CDS sequence within the LIMS. From the dissolution run, samples of 1 mL were withdrawn at the defined time points and automatically filtrated and filled into the barcoded vials arranged accordingly in the Agilent 850-DS autosampler. The LIMS sample information was directly sent to the Agilent Sample Scheduler software. After moving the dissolution samples from the 850-DS autosampler to the multisampler, the sample ID reader scanned the barcodes of the vials and compared them to the obtained sequence. Then, the sequence was submitted from the Sample Scheduler to the LC instrument for measurement. The complete workflow is shown schematically in Figure 1 and a detailed description of the workflow is described in an Agilent white paper.[3]

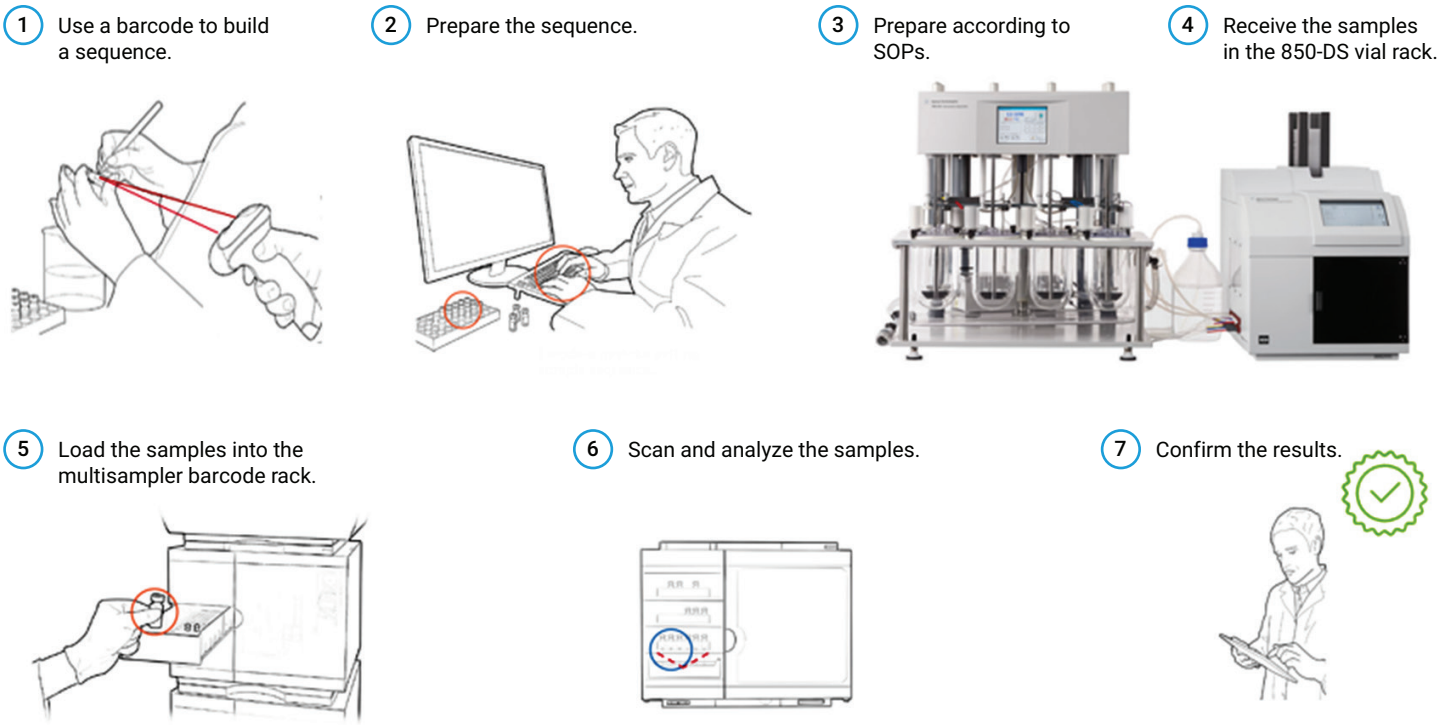

Figure 1. Possible workflow for the processing of dissolution samples with LIMS and the Agilent Sample Scheduler Software.

* Only available in select countries.

During the measurement of the submitted sequence, progress can be seen in the sample scheduler window (Figure 2). The status of the individual samples is given by a color code.

The applied Agilent HDR detection solution provides a combined chromatogram of both the high- and low-abundant API compounds in one run[4] without exceeding the dynamic range of the detector. Figure 3 displays the HDR signal showing all compounds, high-abundant vitamin C, and paracetamol, as well as the low-abundant chlorphenamine (see insert of Figure 3), acquired in one run.

Name / LIMS ID1	State	Project	Barcode	Data file	Acq. method	Proc. method	Instrument / Vial
	In Progress	HDR-4APIs					HDR-LC1
Sample-V1-t10 ep0000072001	Ended		36130101FA	2024-11-13 15-08-57 (GMT +01-00)_01	Acq-HDR-4APIs.amx	DA-HDR-4APIs.pmx	Vial:
Sample-V1-t20 ep0000072002	Ended		36130101EG	2024-11-13 15-08-57 (GMT +01-00)_02	Acq-HDR-4APIs.amx	DA-HDR-4APIs.pmx	Vial:
Sample-V1-t40 ep0000072003	In Progress		36130101EJ	2024-11-13 15-08-57 (GMT +01-00)_03	Acq-HDR-4APIs.amx	DA-HDR-4APIs.pmx	Vial:
Sample-V1-t60 ep0000072004	Submitted		36130101FB	2024-11-13 15-08-57 (GMT +01-00)_04	Acq-HDR-4APIs.amx	DA-HDR-4APIs.pmx	Vial:
Sample-V1-t75 ep0000072005	Submitted		36130101EM	2024-11-13 15-08-57 (GMT +01-00)_05	Acq-HDR-4APIs.amx	DA-HDR-4APIs.pmx	Vial:

Figure 2. Progress of the sample measurement displayed in the Agilent Sample Scheduler window. The displayed part of the sequence shows samples drawn from dissolution vessel 1 at time points of 10, 20, 40, 60, and 75 minutes (green = measured, blue = measurement in progress, purple = submitted, not yet measured).

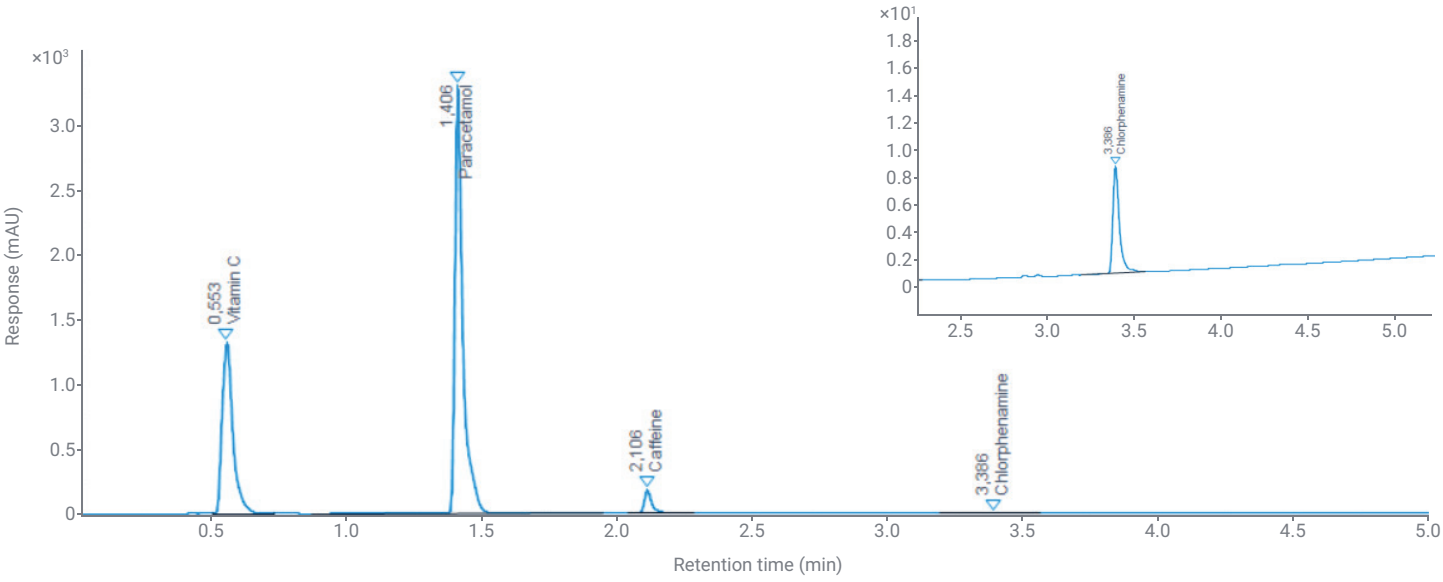

Figure 3. Chromatogram of the HDR signal combined from the measurement of high-abundant compounds by means of a short path length DAD cell and the low-abundant compounds with a long path length DAD cell.

Because of the ability to separate the four APIs by HPLC analysis and detect the wide concentration range of the APIs with similar sensitivity by HDR, it was possible to establish release profiles for the four APIs paracetamol, vitamin C, caffeine, and chlorphenamine. Over the time frame of 10 to 75 minutes, a maximum release of 100% was encountered for all four APIs (Figure 4).

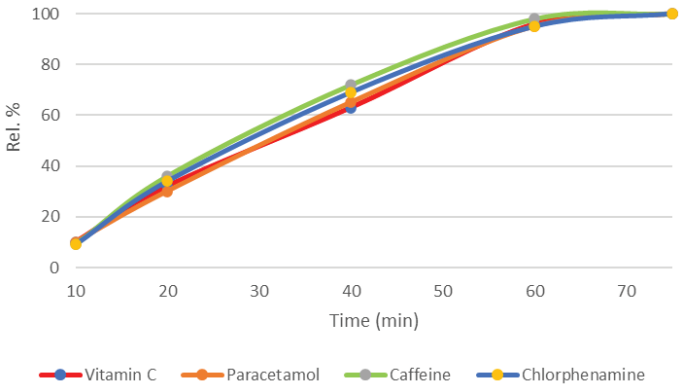

Figure 4. Dissolution curves of the four APIs at different timepoints.

Conclusion

This application note demonstrates an entire workflow from a dissolution run over the sample measurement by means of an HPLC instrument equipped with a sample ID reader, to achieve dissolution results. The samples were directly filled into barcoded vials after scanning and passed through the workflow by a LIMS system. The samples were automatically assigned to an HPLC system and handled by Agilent Sample Scheduler software for measurement and results reporting. Moreover, the HDR detector was used to analyze the wide concentration range of the four APIs, making it a valuable alternative to UV spectroscopy analysis in the analysis of complex API mixtures.

References

1. Confirmation of Sample Position – Using the Agilent 1290 Infinity III Multisampler with Agilent InfinityLab Sample ID Reader – Part 1 of 2. *Agilent Technologies technical overview*, publication number 5994-7568EN, **2024**.
2. Sample Position Identification and Measurement Confirmation – Using the Agilent 1290 Infinity III Multisampler with Agilent InfinityLab Sample ID Reader – Part 2 of 2. *Agilent Technologies technical overview*, publication number 5994-7569EN, **2024**.
3. Agilent Advanced Sample Linking – A Complete Workflow from Any Laboratory Information Management System to the Vial and Analytical Results. *Agilent Technologies white paper*, publication number 5994-7570EN, **2024**.
4. Schneider, S. Impurity Testing of Fixed-Dose Combination Drugs Using the Agilent 1290 Infinity II HDR-DAD Impurity Analyzer Solution. *Agilent Technologies application note*, publication number 5991-5743EN, **2015**.

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