Complementary Dual LC as Alternative to Multi Heart-Cut LC for Samples of medium Complexity resulting in improved Precision, Sensitivity and Productivity

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ABSTRACT

Purpose: Experimental evaluation of chromatographic performance of complementary dual LC compared to multi heart-cut LC for a moderately complex samples.

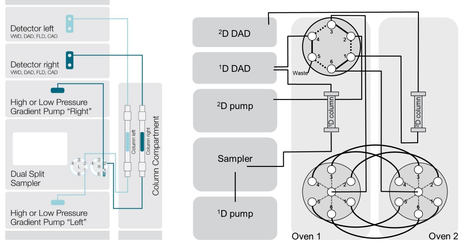
Methods: The two fluidic setups of complementary dual LC and multi heart-cut LC were realized with a Thermo Scientific™ Vanquish™ Duo UHPLC system.

Results: In the tested scenario a clear performance advantage was seen for the complementary dual LC. It outperformed the multi heart-cut approach in terms of peak area precision, sensitivity and productivity and significantly reduced the solvent consumption.

INTRODUCTION

The analysis of highly complex samples with 100-1000 components is commonly recommended to be executed by comprehensive multi-dimensional LC techniques. However, as the complexity is reduced to a level of <100 components multi heart-cut 2D-LC becomes a viable alternative to transfer unresolved sample zones from the 1st into an orthogonal 2nd dimension.[1] Either technique amplifies the available separation space but on the other hand requires an advanced instrumentation and sophisticated software control. Analysis times usually are longer than in one dimensional LC and accurate compound quantification can be challenging due to peak segmentation from the 1st dimension. In contrast dual LC is primarily utilized to increase analysis throughput in independent parallel or tandem setups but its potential to enhance a separation by employing methods of different selectivity is often missed.[2-4] At sufficient orthogonality and moderate sample complexity the probability is high that non-resolved peaks of one method are well resolved by the other and vice versa.[5] Thus the combined information from two one-dimensional LC runs would allow the quantification of all components from the respective resolving method. If the experimental setup enables simultaneous injection of a sample and parallel analysis on two independent flow paths, like it is the case with the Vanquish Duo UHPLC system for Dual LC, the analysis time is equivalent to a one-dimensional separation and challenging aspects of two-dimensional methods resulting from peak cutting, fraction transfer and dilution can be avoided. An experimental evaluation is shown here for a model mixture of 22 polyphenolic compounds.

Figure 1. Fluidic setup of dual LC (left) and multi heart-cut 2D-LC (right)



MATERIALS AND METHODS

Samples

Stock solutions of single compounds and mixed calibration standards (1, 5, 10, 25, 50, 100 and 200 µg/mL) of 22 polyphenolic compounds (see Figure 2) were prepared in methanol/water (25/75, v/v).

Instrumentation

Both fluidic setups are shown in Figure 1. The chromatographic conditions are given in Table 1. Method 1 and 2 were employed in parallel in the Dual LC approach. For the multi heart-cut 2D-LC approach method 1 served as 1st dimension (1D), method 3 as 2nd dimension (2D). Fractions were collected in 200 µL loops and were transferred directly after the 1D run was finished. Each injection was repeated 3 times.
System control and data analysis was performed with Thermo Scientific™ Chromeleon™ 7.2.9 CDS software.

Table 1. Chromatographic conditions:

	Method 1			Method 2			Method 3		
Column	Thermo Scientific™ Accucore™ Polar Premium LC Column, 2.6 µm, 2.1 × 100 mm			Thermo Scientific™ Hypersil GOLD™ aQ C18 Polar Endcapped LC Column, 1.9 µm, 2.1 × 100 mm			Thermo Scientific™ Acclaim™ Phenyl-1 LC Column, 3 µm, 3 × 100 mm		
Eluents	A–0.1% formic acid in water B–0.1% formic acid in methanol								
Gradient	min	% A	% B	min	% A	% B	min	% A	% B
	0	97	3	0	90	10	0	95	5
	2.5	97	3	1	90	10	3.5	40	60
	8.83	40	60	4.75	65	35	3.9	0	100
	10	0	100	7.35	35	65	4.3	0	100
	11	0	100	8	0	100	4.35	95	5
	11.1	97	3	8.5	0	100	5.85	95	5
	14.1	97	3	8.6	90	10			
				11.6	90	10			
Flow rate	400 µL/min			400 µL/min			1.3 mL/min		
Col. temp.	40 ° C			40° C			50° C		
Inj. vol.	1 µL			1 µL			up to 150 µL		
Detection	260 nm, 280 nm, 20 Hz, 0.2 s resp. time								

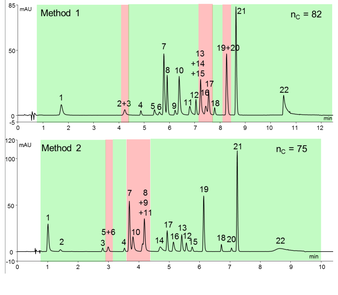
The Vanquish Duo UHPLC system for Dual LC consisted of:

- System Base Vanquish Duo for Dual LC (VF-S02-A-02)
- 2x Binary Pump Horizon (VH-P10-A-02)
- Dual Split Sampler HT (VH-A40-A-02)
- Column Compartment H (VH-C10-A-02)
- 2x Diode Array Detector FG (VF-D11-A-01) with semi-micro flow cell (6083.0550)

for the multi heart-cut setup only the left sampling unit of the Dual sampler was fluidically connected and the following modules were added:

- 2nd Column Compartment H (VH-C10-A-02)
- Valve 2-p 6-p 150MPa bio (6036.1560)
- 2x Valve 6-p 7-p 150MPa bio (6036.1570)
- 4x Sample loop 200 µL Viper, ACC-3000 (5830.2418)

Figure 2. Complementary dual LC chromatograms of the 25 µg/mL standard. The table indicates which method provided baseline separation ($R_s \geq 1.5$) for which compound



#	compound	1	2
1	Gallic acid	✔	✔
2	Gallocatechine	✖	✔
3	Tyrosol	✖	✔
4	Homovanillyl alcohol	✔	✔
5	Epigallocatechine	✔	✖
6	Catechine	✔	✖
7	Vanillic acid	✔	✖
8	Syringic acid	✔	✖
9	Epicatechine	✔	✖
10	Caffeic acid	✔	✖
11	Epigallocatechine gallate	✔	✖
12	Sinapic acid	✔	✔
13	Ferulic acid	✖	✔
14	Gallocatechine gallate	✖	✔
15	Benzoic acid	✖	✔
16	Epicatechine gallate	✖	✔
17	p-Coumaric acid	✖	✔
18	Oleuropein	✔	✔
19	o-Coumaric acid	✖	✔
20	Pinoresinol	✖	✔
21	Cinnamic acid	✔	✔
22	Apigenin	✔	✔

RESULTS

The compound mixture utilized in the current study is an artificial set of 22 polyphenolic compounds selected from tea and olive oil antioxidants. It was compiled as a medium complex sample that was not fully resolvable by a one dimensional UHPLC method. The best separation achieved in preliminary experiments (data not shown) generated 20 peaks with only 16 compounds baseline resolved (resolution Rs≥1.5). Thus two methods (method 1 and 2) with complementary separations were developed. Figure 2 shows the respective chromatograms of the two complementary LC methods run in the dual setup for a standard of reasonable concentration (25 µg/mL). The table in that figure provides information which compound is sufficiently resolved (Rs≥1.5) in which chromatogram. For some compounds this is the case in either chromatogram, so they could be quantified with either method, but for the majority of the components reasonable quantification is feasible from only one of the LC methods.

Compared to that the chromatographic result of the multi heart-cut approach for the same concentration standard is depicted in Figure 3. Method 1 was kept as the starting point for the development of the heart-cut method. Four fractions of unresolved sample zones in method 1 were transferred online to 200 µL loops and stored until the end of the 1st dimension run. After that run was finished the respective four runs of the 2nd dimension were executed by switching the loops consecutively into the flow path of the 2nd dimension. The peaks that were quantifiable from the 1st dimension were the same as in the dual LC approach, 9 compounds needed to be quantified from the 2nd dimension due to complete or partial co-elution in the 1st dimension. The 2nd dimension column was wider in its inner diameter than the 1st dimension column as it is common practice in 2D-LC because of the high volumes that are transferred.[1] This is especially true for heart-cut approaches with quantification purpose, were the 1st dimension flow has to be transmitted over the complete peak width. The fraction volumes of the current application are indicated in Figure 3. High organic content of the fraction solvent thus can cause solvent mismatches and peak distortions in the 2nd dimension due to insufficient focusing as it is also looming for compound 17 in Figure 3.

Figure 3. Multi heart-cut 2D-LC chromatograms of the 25 µg/mL standard.

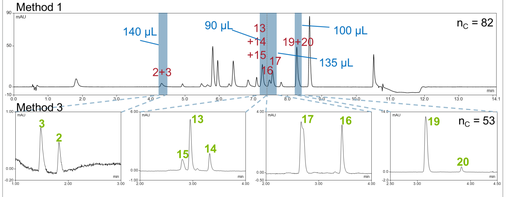


Figure 4. Low concentration injection (1 µg/mL standard) in complementary dual LC and exemplary calibration curves for each method

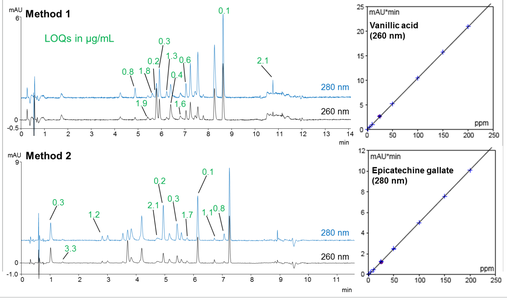
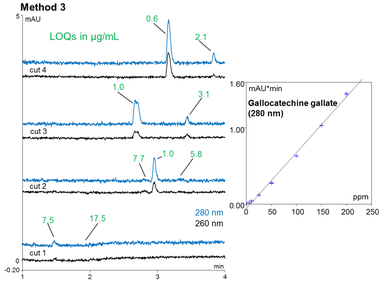
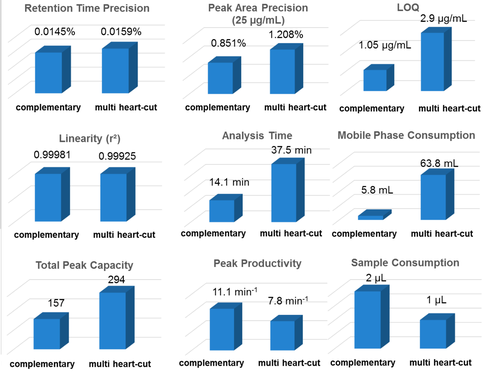


Figure 5. Low concentration injection (1 µg/mL standard) in multi heart-cut 2D-LC and exemplary calibration curve for a compound to be quantified from the 2nd dimension



The performance of the compared workflows in terms of quantification is indicated in Figures 4 and 5. Both figures show the chromatograms of the lowest concentrated standard (1 µg/mL) and exemplary calibration curves. In sum the linearity of calibration curves is equivalent for both workflows (see also Figure 6) but detection sensitivity differs significantly. Although the limits of quantification (LOQs, signal-to-noise ratio >10) for compounds resolved by method 1 are equivalent in both setups, the LOQs of the remaining compounds depend on the selected workflow. Because of dilution effects the LOQs of method 3 as the 2nd dimension of the heart-cut approach are distinctly higher than in method 2 in the dual approach. Thus the average LOQ is higher for the 2D approach compared to dual LC (Figure 6). That difference in sensitivity is also underlined by the chromatograms of the 1 µg/mL standard. Each compound is detectable by one of the detection wavelengths at this concentration by complementary dual LC but not by multi heart-cut 2D-LC. .

Figure 6. Performance comparison of the two workflows



More performance comparisons are given in Figure 6. A slight increase in the relative standard deviation (%RSD) of the retention times over 3 injections was noticeable for peaks in the 2nd dimension compared to one dimensionally eluted peaks. But the effect on the average retention time precision was low. However, the effect was more pronounced for the peak area precision as 2nd dimension peaks were less reproducible than one dimensional ones.
The analysis time of the dual methods run in parallel was equivalent to a single one dimensional analysis and thus distinctly shorter than under 2D-LC conditions. The solvent consumption was more than 10fold with the multi heart-cut setup due to the longer run time and high flow rate in the 2nd dimension. Sample consumption is doubled with dual LC though, because of injecting twice. The peak capacities (n_C) of the workflows are indicated in Figures 2 and 3. The repetition of the 2nd dimension causes an increased peak capacity of the 2D-LC. However, the produced excess is hardly utilized and thus the peak productivity is lower than for dual LC.

CONCLUSIONS

- Complementary parallel LC is a convenient alternative to heart-cut 2D-LC workflows and is prone to be advantageous for quantitative analysis of medium complex samples comprising 15-30 compounds.
- On a dual channel UHPLC system it can be accomplished in the same analysis time like a respective single channel one dimensional UHPLC separation.
- In terms of quantification precision, LOQ, throughput and solvent consumption, it clearly outperforms the alternative workflow of multi heart-cut 2D-LC. The spent sample amount is double though.

REFERENCES

1. Stoll, D.R., Carr, P.W.; Two-dimensional liquid chromatography: a state of the art tutorial, Anal. Chem. (2017), 89, 519-531
2. Grosse, S., De Pra, M., Steiner, F., Doubling the throughput of long chromatographic methods by using a novel Dual LC workflow, Thermo Scientific Application Note 72601 (2018),
3. Grosse, S., De Pra, M., Steiner, F., Simultaneous determination of water- and fat-soluble vitamins in tablets and energy drinks by using a novel Vanquish Flex Duo system for Dual LC, Thermo Scientific Application Note 72592 (2018)
4. Samonig, M. et al., Tandem UHPLC operation for high-throughput LC-MS peptide mapping analyses, Thermo Scientific Application Note 72203 (2018)
5. Vivo-Truyols, G., Towards the optimization of complementary systems in reversed-phase liquid chromatography, Chromatographia (2002), 56, 699-707

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