

# GPC CLEANUP OF OLIVE OIL SAMPLES

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## SUMMARY

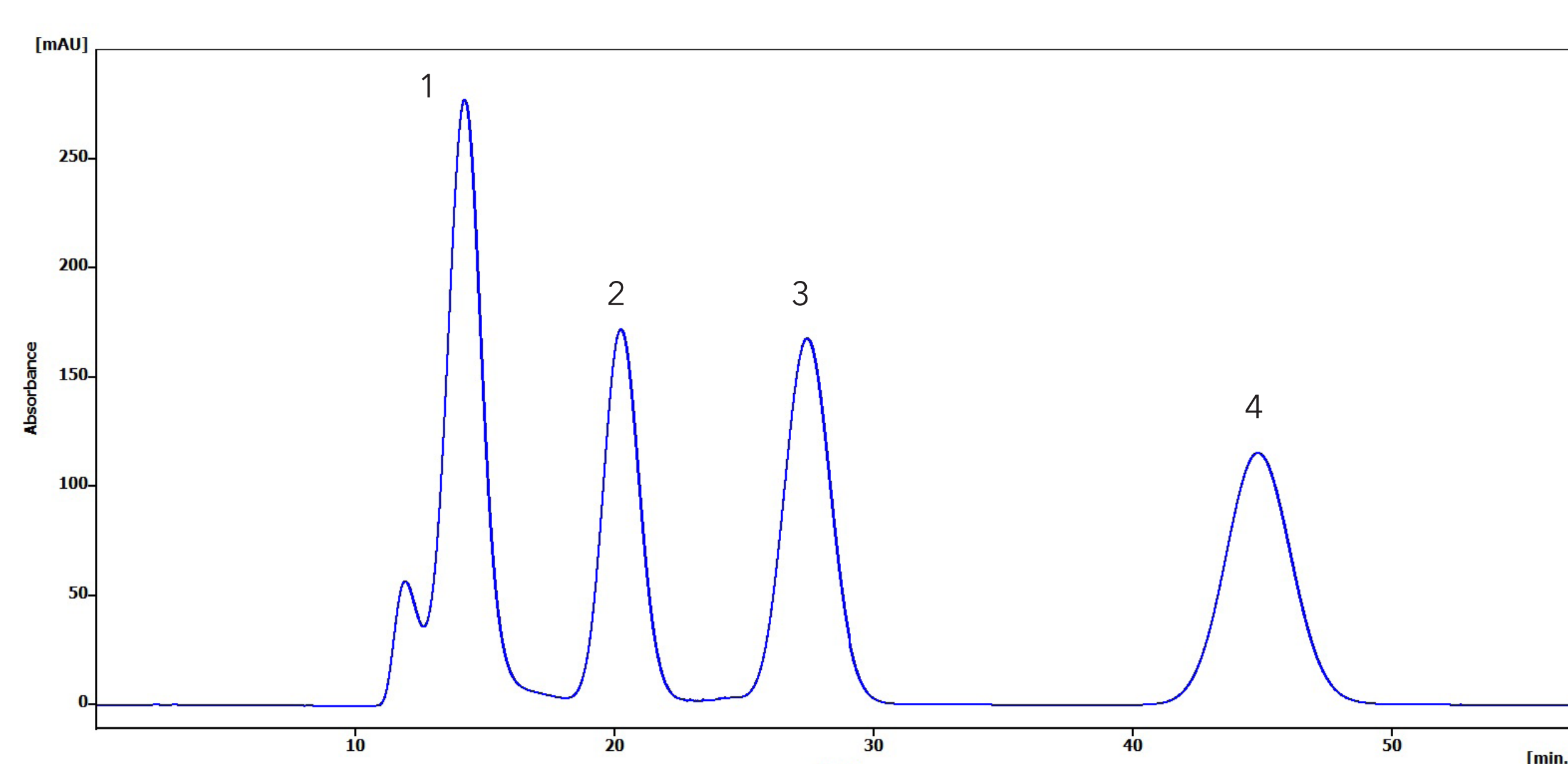
This work describes a sample cleaning method for analyzing pesticide residues in olive oil in preparation for an HPLC analysis. Pesticides were separated from the oil matrix by size exclusion/gel permeation chromatography (GPC) according to US EPA SW-846 method 3640A. The GPC material used in this study was BioBeads SX-3 and the GPC solvent system was cyclohexane/ethyl acetate (1:1, v/v). The optimized GPC purification technique was carried out with a KNAUER AZURA GPC Cleanup System for automated sample cleaning.

## INTRODUCTION

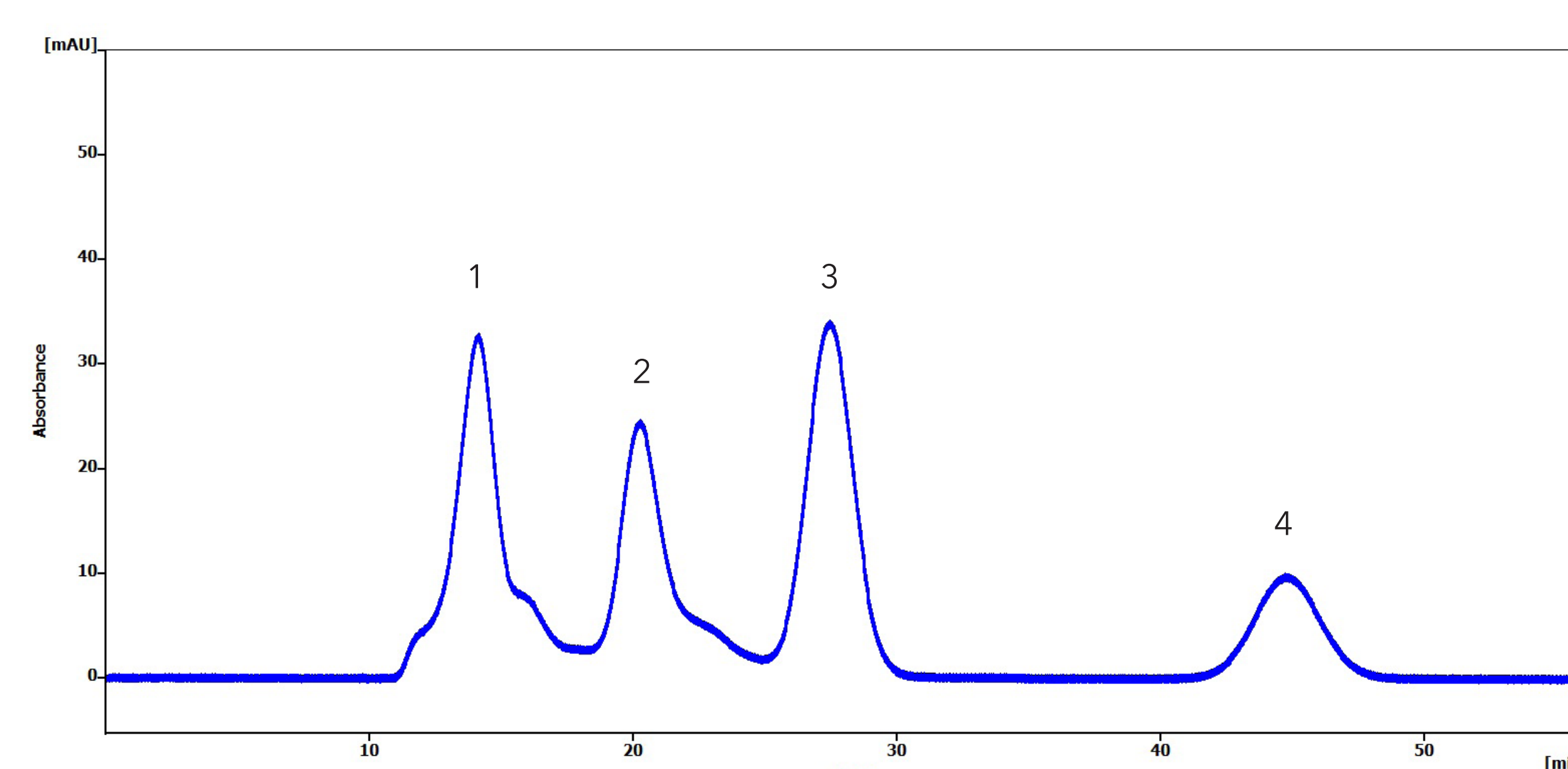
GPC is extensively used as an effective post-extraction cleanup procedure for removing high molecular weight interferences such as lipids, proteins, and polymers from sample extracts. The efficiency of BioBeads SX-3 with an organic solvent to separate multi-pesticide residues has been extensively documented [1-3]. The GPC technique is appropriate for both polar and non-polar analytes so it can be effectively used to cleanup extracts containing a broad range of compounds. To demonstrate the flexibility of the sample cleaning method, the olive oil samples investigated were spiked with different types of compounds.

## RESULTS

**Fig. 1** shows the chromatogram of the GPC calibration standard eluted with cyclohexane/ethyl acetate (1:1, v/v). The three detected pesticides were baseline separated and could be identified easily. **Fig. 2** shows the elution profile of one olive oil sample containing different types of pesticides. It can be seen that all pesticides were detected with the US EPA method 3640A. Compared to the measurement of the standard solution, the spiked sample showed less matrix effects. This means that all interfering high molecular elements were removed during clean up. The recovery for all of these compound classes was higher than 70 %.



**Fig. 1** Chromatogram of US EPA method 3640A calibration standard containing 1. Corn oil matrix, 2. Bis-(2-ethylhexyl)phthalate, 3. Methoxychlor, 4. Perylene



**Fig. 2** Chromatogram of olive oil sample, spiked with pesticides: 1. Olive oil matrix, 2. Bis-(2-ethylhexyl)phthalate, 3. Methoxychlor, 4. Perylene

## MATERIALS AND METHOD

This study used the KNAUER AZURA GPC Cleanup System which automates the GPC cleanup process. The system comprising of the two AZURA ASM 2.1L Assistant modules with different valves, a pump, and a UV detector. The compounds were detected at 254 nm wavelength with the AZURA UVD 2.1S UV detector with 10 Hz data rate. The two 16-port multiposition valves used here enabled the loading of up to 15 oil samples (1 mL or 5 mL samples loops). Moreover, the pesticide fraction was collected in a round-bottomed flask between the elution of corn oil by a third 16-port multiposition valve.

The glass column with BioBeads SX-3 was flushed with cyclohexane/ethyl acetate (1:1, v/v) for an extended period at a flow rate of 5 mL/min. To determine the elution profile of the GPC column, a calibration solution was prepared in cyclohexane/ethyl acetate containing the following analytes: corn oil (25 g/L), bis(2-ethylhexyl)phthalate (1 g/L), methoxychlor (0.2 g/L), and perylene (0.02 g/L). The calibration solution was injected after solvent flow and column pressure were established. The eluates were collected based on the UV traces of the four eluates. For further analysis purposes with GC, DC or HPLC techniques (not described here), the various oil sample fractions collected were carefully evaporated under a nitrogen stream, dispensed in 1 mL of a suitable solvent and filtered using a 0.45 µm syringe filter.

## CONCLUSION

GPC sample preparation is a useful tool for separating small amounts of pesticides from high molecular weight matrices such as olive oil. The KNAUER AZURA GPC Cleanup System is particularly well-suited for sample preparation in pesticide analysis but can also be easily adapted to other laboratory procedures to perform a large variety of GPC sample preparation tasks. The arrangement of the 15 sample loops and one wash loop avoids cross contamination hence allowing a robust sample preparation procedure.

## REFERENCES

- [1] Chamberlain, S.J. Determination of multi-residues in cereal products and animal feed using gel-permeation chromatography. Analyst (1990), 115:1161-1165
- [2] Di Muccio, A.; Ausili, a.; Versori, L; Camoni, I; Dommarco, t; Ganibetti, L; santillo, A and Versori, F. Single-step multi-residue clean-up for organophosphate pesticide residue determination in vegetable oil extracts by gas chromatography. Analyst (1990), 115: 1167-1169
- [3] Guardia-Rubio, M; Fernandez-De Cordova, M.L.; Ayory-Canada, M.J. and Ruiz-Medina, A. Simplified pesticide multi-residue analysis in virgin olive oil by gas chromatography with thermoionic specific, electron-capture and mass spectrometric detection. J. of Chrom. A (2006) Volume 1108, 231-239

Additional information:



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## ADDITIONAL MATERIALS AND METHODS

Tab. A1 Sample preparation

| Standards                     | Prepared and diluted with Cyclohexane/ethyl acetate (1:1, v/v) |
|-------------------------------|----------------------------------------------------------------|
| 1. Corn oil                   | 25 g/L                                                         |
| 2. Bis-(2-ethylhexyl)phtalate | 1 g/L                                                          |
| 3. Methoxychlor               | 0.2 g/L                                                        |
| 4. Perylene                   | 0.02 g/L                                                       |

Tab. A2 Method parameters

|                      |                                      |                 |           |
|----------------------|--------------------------------------|-----------------|-----------|
| Eluent A             | Cyclohexane/ethyl acetate (1:1, v/v) |                 |           |
| Isocratic            | Time [min]                           | % A             | % B       |
|                      | 0                                    | 100             | 0         |
|                      | 60                                   | 100             | 0         |
| Flow rate            | 5 mL/min                             | System pressure | 0.6 bar   |
| Column temperature   | 25°C                                 | Run time        | 60 min    |
| Injection volume     | 1 mL                                 | Injection mode  | Full loop |
| Detection wavelength | 254 nm                               | Data rate       | 10 Hz     |
|                      |                                      | Time constant   | 0.10s     |

Tab. A3 System configuration & data

| Instrument       | Description                                                                                                                                                                                                     | Article No. |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| Assistant 1      | AZURA ASM 2.1L,<br><b>left:</b> single variable wavelength UV detector<br><b>middle:</b> 6 port 2 position injection valve, 1/16" connectors<br><b>right:</b> Pump with pressure sensor, 10 mL pump head, SSt   | AYCAEABA    |
| Assistant 2      | AZURA ASM 2.1L,<br><b>left:</b> 16 port multi position valve, 1/16" connectors<br><b>middle:</b> 16 port multi position valve, 1/16" connectors<br><b>right:</b> 16 port multi position valve, 1/16" connectors | AYGAGAGA    |
| Flow cell        | UV, 3mm, 2 µL                                                                                                                                                                                                   | A4042       |
| GPC tubing guide | 16 sample loops with 1 ml                                                                                                                                                                                       | A5329-2     |
| Software         | ClarityChrom                                                                                                                                                                                                    | A1670-9     |
| Column           | BioBeads SX-3                                                                                                                                                                                                   | B41         |
| Injection valve  | Manual injection valve<br>6-Port/3-position, 1/16" connectors                                                                                                                                                   | AVI26BC     |

