*Article*

# Optimizing Chromatographic Separation with Redosing: Effects on Separation Efficiency of a Model System in Centrifugal Partition Chromatography

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*Supplementary Information 1:*

Adding a sample to the Arizona system brings salicylic acid into contact with methanol – possibly leading to salicylic acid and methanol reacting in an esterification reaction to form methyl salicylate and water. Experiments were carried out with methyl salicylate (98%, supplied by Thermo Fisher Scientific, Waltham, Massachusetts, USA) to proof the component's absence. In addition, absorption maxima and typical retention times were determined to investigate whether methyl salicylate was formed. In the first step, a sample with 200 mg·L$^{-1}$ per component of methyl salicylate and D(+)-carvone was prepared. A separation was then carried out, as discussed in the corresponding paper. The volume flow was set to 10 mL·min$^{-1}$, the rotational speed to 750 rpm, and the setpoint to $Sf^*_{set\text{-}point}$ = 0.72. The cycle time was fixed to $t_{loop}$ = 10 min. Finally, a separation was carried out analogous to the previous experiment, whereby the sample concentration of D(+)-carvone was 200 mg·L$^{-1}$, and the concentration of salicylic acid and methyl salicylate was 100 mg·L$^{-1}$ each. The operating parameters were set identically to the prior experiment.

The spectrum of methyl salicylate is shown in Figure S1. A comparison with the spectra of salicylic acid and D(+)-carvone shows that methyl salicylate has the same maxima as salicylic acid. D(+)-carvone also has a global maximum at 239 nm.

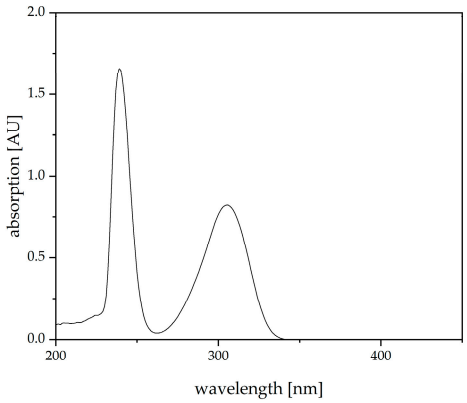


**Figure S1.** Spectrum of 200 mg·L$^{-1}$ methyl salicylate in the lower phase of Arizona N. Measured at a volumetric flow rate of 10 mL·min$^{-1}$ and a temperature of 21 ± 2 °C.

Concerning the absorption maxima, methyl salicylate cannot be distinct from salicylic acid if both components elute simultaneously. Therefore, it is tested when methyl salicylate elutes if it is separated from D(+)-carvone. The resulting chromatogram is given in Figure S2.

The 239 nm peak can be attributed to D(+)-carvone and methyl salicylate, while the 300 nm peak can be attributed to methyl salicylate. Therefore, methyl salicylate elutes shortly after D(+)-carvone.

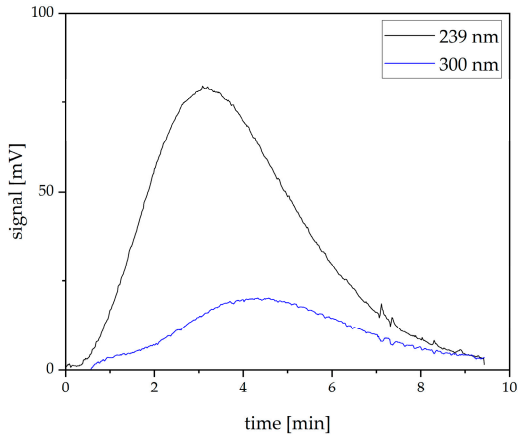


**Figure S2.** Chromatogram of the separation of methyl salicylate and D(+)-carvone in Arizona N in descending mode in the analytical rotor. Volume flow of 10 mL·min$^{-1}$. $Sf^*_{setpoint}$ = 0.72. Measured at 21 ± 2 °C. Injection volume 0.9 mL, concentration of each component $c_{salicylic\ acid} = c_{D(+)\text{-carvone}}$ = 200 mg·L$^{-1}$.

It has already been shown that the retention time in CPC is a function of retention, rotor volume, and the partition coefficient. Therefore, D(+)-carvone and methyl salicylate should have a similar partition coefficient.

A reference chromatogram of the separation of salicylic acid, methyl salicylate, and D(+)-carvone was recorded. The result of this experiment is given in Figure S3. As described before, the first peak with two absorption maxima can be attributed to salicylic acid. The second peak at 239 nm represents D(+)-carvone; the one at 300 nm is methyl salicylate. The absorbance can evaluate the existence of methyl salicylate during the D(+)-carvone elution. A peak at 300 nm would indicate that methyl salicylate is present.

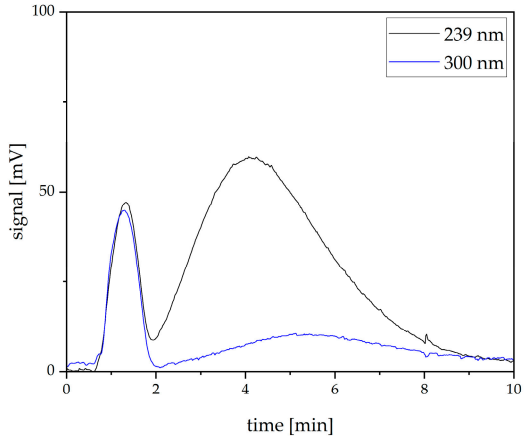


**Figure S3.** Chromatogram of the separation of methyl salicylate, salicylic acid, and D(+)-carvone in Arizona N in descending mode in the analytical rotor. Volume flow 10 mL·min$^{-1}$. $Sf^*_{setpoint}$ = 0.72. Measured at 21 ± 2 °C. Injection volume 0.9 mL, concentration of $c_{D(+)\text{-carvone}}$ = 200 mg·L$^{-1}$ and $c_{methyl\ salicylate} = c_{salicylic\ acid}$ = 100 mg·L$^{-1}$.

The existence of methyl salicylate can also be verified with a heatmap, as given in Figure S4. The absorption of all measured wavelengths is plotted over time. The two maxima at different wavelengths can identify the first peak (timewise) and belong to salicylic acid. In the presence of methyl salicylate, the second peak also consists of maxima at two different wavelengths. The signal that corresponds to methyl salicylate is marked with a black circle.

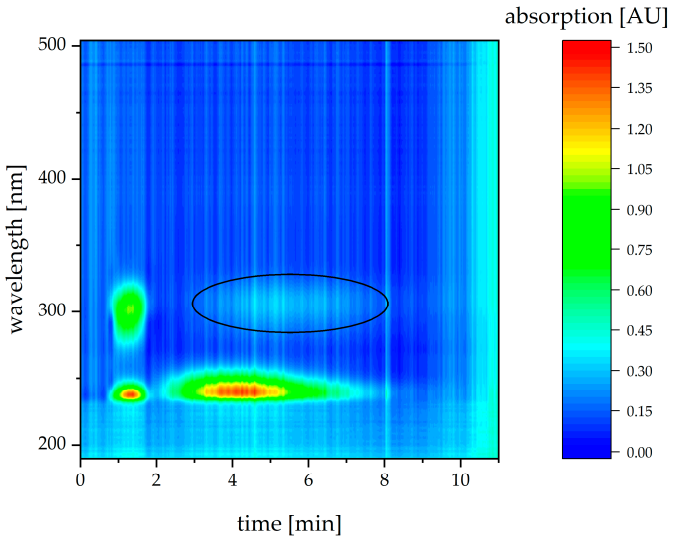


**Figure S4.** Heatmap of the separation of methyl salicylate, salicylic acid, and D(+)-carvone in Arizona N in descending mode in the analytical rotor. Volume flow of 10 mL·min$^{-1}$. $Sf^{*}_{setpoint}$ = 0.72. Measured at 21 ± 2 °C. Injection volume 0.9 mL, concentration of $c_{D(+)\text{-carvone}}$ = 200 mg·L$^{-1}$ and $c_{methyl\ salicylate}$ = $c_{salicylic\ acid}$ = 100 mg·L$^{-1}$. The methyl salicylate signal is marked with a black circle.

These heatmaps were additionally analyzed for all experiments conducted in the paper regarding the presence of methyl salicylate. An example is given in Figure S5. Only the first peak (timewise) shows an absorption maximum at two wavelengths, indicating no methyl salicylate was detected, which is valid for every experimental run.

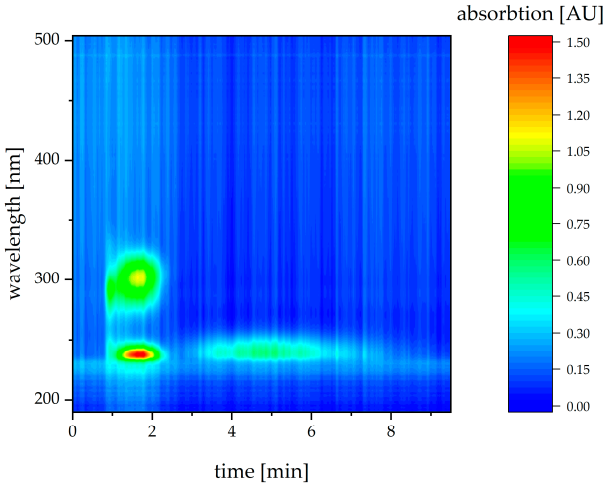


**Figure S5.** Heatmap of the separation of salicylic acid and D(+)-carvone in Arizona N in descending mode in the analytical rotor. Volume flow of 10 mL·min$^{-1}$. $Sf^{*}_{setpoint}$ = 0.72. Measured at 21 ± 2 °C. Injection volume 0.9 mL, concentration of each component $c_{salicylic\ acid}$ = $c_{D(+)\text{-carvone}}$ = 200 mg·L$^{-1}$.