Determination of Phenol, Cresol isomers in urine by GC-MS

Stephan Drothler

I. BACKGROUND

In this lab course, artificial urine is investigated for phenol, o-cresol and p-cresol. An external calibration curve is used for the quantification of all three analytes.

II. METHODS AND MATERIALS

A. MATERIALS

1) Tools:

- eppendorff-pipettes (200 μL, 1000 μL)
- glas-flask (12 *mL*)
- GC-MS (HP 6890 (GC) HP 5973 (MS))
- Capillary (HP-5 MS, 30 m0.25 mm I.D., 0.25 m film thickness)
- vacuum chamber
- SPE column (Bond Elut 100 mg C18/OH, Varian)

2) Chemicals:

- methanol (provided by the institue)
- milliQ water (provided by the institute)

B. METHODS

The injector temperature was set to 250°C with 1 μL of splitless injection volume while the oven temperature was set to 60°C for 3 min, then increased by 5 $\frac{^\circ C}{min}$ to 80°C and then by 25$\frac{^\circ C}{min}$ to 250°C; final temperature: 250°C for 1 min with He as a carrier gas at a flow-rate of 2.5 $\frac{mL}{min}$ (*Analytical Chemistry* 2019). A standard solution mix of phenol, o-cresol and p-cresol [100 $\frac{\mu g}{mL}$] was already prepared by the supervisor. The working standards were prepared in methanol in a 1 mL GC-vial at final concentrations of 0,1 $\frac{\mu g}{mL}$, 0,25 $\frac{\mu g}{mL}$, 0,5 $\frac{\mu g}{mL}$, 0,75 $\frac{\mu g}{mL}$ and 1 $\frac{\mu g}{mL}$ of the phenolic compounds. A 10 μg/mL solution was used to determine the retention times and the appropriate fragments for the SIM mode *Analytical Chemistry* 2019). The data processing was performed via R studio (V 1.2.1335) and visualized via the graphrobot web tool (R Core Team 2019, Wang 2019).

III. RESULTS

The calibration curves of o-cresol (Fig. 2) p-cresol (Fig. 3) and phenol (Fig. 4) provide the variables to the linear equation 1, with m = slope and t = intersept. For the following calculations x = analyte concentration and y = peak area.

$$y = mx + t \tag{1}$$

The sample data (peak area) from Tab III is used to determine the concentration via Equation 1.

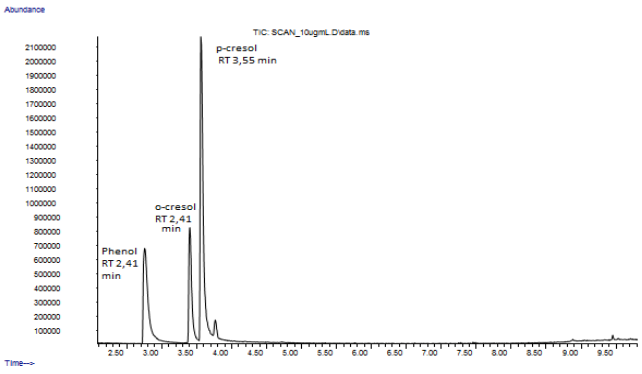

Fig. 1: Chromatogram of the three analytes (10 $\frac{\mu g}{mL}$)

Analyte	RT[min]	Fragment ion [m/z]
phenol	2,7	65, 66, 94
o-cresol	3,41	77, 107, 108
p-cresol	3,55	77, 107, 108

TABLE I: Retention times and fragment ion ratios of the three analytes (Fig. 1).

c [mg/L]	peak number	retention time	Area	analyte
1	1	2,902	48726	phenol
	2	3,474	21211	o-cresol
	3	3,775	23485	p-cresol
25	1	2,849	216298	phenol
	2	3,451	94760	o-cresol
	3	3,635	169866	p-cresol
50	1	2,831	711996	phenol
	2	3,443	326199	o-cresol
	3	3,613	742563	p-cresol
75	1	2,814	1426140	phenol
	2	3,433	645475	o-cresol
	3	3,598	1705651	p-cresol
100	1	2,811	2092602	phenol
	2	3,434	965613	o-cresol
	3	3,594	2653595	p-cresol

TABLE II: Data for the external calibration of phenol, o-cresol and p-cresol

analyte	RT	area	c [mg/L]
phenol	2,822	1229404	0,66
o-cresol	3,434	596134	0,69
p-cresol	3,602	1317470	0,61

TABLE III: Concentration of the analytes in sample 1 via Equation 1.

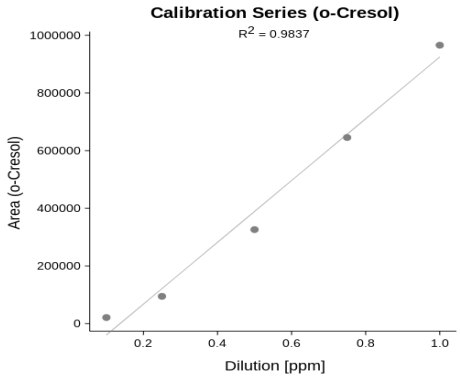

Fig. 2: External calibration of o-cresol: Slope 1071173.9024, Intercept -146358.8293

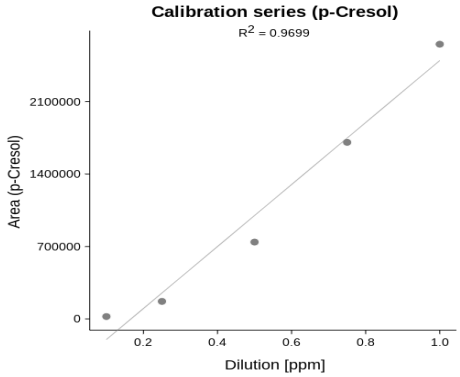

Fig. 3: External calibration of p-cresol: Slope 2993333.1144, Intercept -497501.2195

IV. DISCUSSION

The analytes within the sample 1 have a concentration of c_{phenol} = 662 $\frac{ng}{ml}$, $c_{o\text{-}cresol}$ = 693 $\frac{ng}{ml}$ and $c_{p\text{-}cresol}$ = 606 $\frac{ng}{ml}$.

V. APPENDIX

A. Experimental environment

The experiment was performed at TNF Turm JKU, Linz, Austria 06.19 under the supervision of Franz Mlynek.

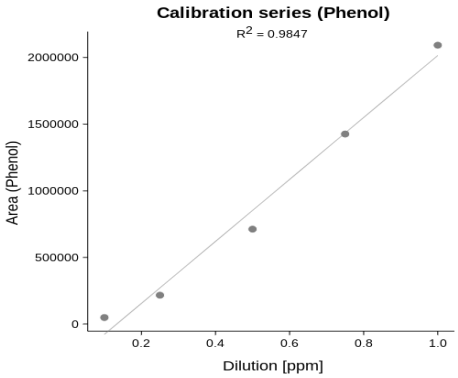

Fig. 4: External calibration of phenol: Slope 2325245.5159, Intercept -309975.2683

REFERENCES

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