Electronic Supplementary Material (ESI) for Analytical Methods.


**Electronic Supplementary Information**

# A new flow-injection chromatography method exploiting linear-gradient elution for fast quantitative screening of parabens in cosmetics

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**S1. Considerations for the implementation of linear gradient elution using flow rate modulation**

All the gradient experiments in these work started with isocratic elution with a volumetric ratio of solvent 1:solvent 2 equal to 100:0 % (v/v) (although different starting ratios could be used). Then, the linear gradient was applied in which the solvent 1:solvent 2 volumetric ratio was varied from the starting value of 100:0 % (v/v) to the desired final value under computer control. The rate of change of the volumetric ratio, C%, could be calculated using the flow rate of the mobile phase, $V_{i,}$ and the gradient rate, G, which is actually the rate of change of the flow rates of the 2 solvents:

$$C\ \%\ (v/v\ min^{-1}) = 100 \times G\ (mL\ min^{-2}) / V_i\ (mL\ min^{-1}) \qquad \text{(equation S1)}$$

In this work, the selected flow rate of the mobile phase, $V_{i,}$ was 2.4 mL $min^{-1}$ and the selected rate of change of the flow rates of the 2 solvents, G, was 0.54 mL $min^{-2}$ so that the rate of change of the volumetric ratio, C%, was calculated as 22.5 % (v/v) $min^{-1}$ or 0.375 % (v/v) $s^{-1}$ for both solvents.

The time, $t_f$, required to cover the full linear gradient range solvent 1:solvent 2 volumetric ratio from 100:0 % (v/v) to 0:100 % (v/v) was calculated from:

$$t_f\ (min) = V_i\ (mL\ min^{-1}) / G\ (mL\ min^{-2}) \qquad \text{(equation S2)}$$

In the present work, the time required for a full gradient programme, $t_f$, was calculated as 4.44 min or 267 s. In this case, the flow rate of mG 1 was linearly varied from $V_i$ to 0 while the flow rate of mG 2 was linearly varied from 0 to $V_i$ (i.e. from 2.4 to 0 mL $min^{-1}$ and from 0 to 2.4 mL $min^{-1}$, respectively).

However, for reasons of speed, the linear gradient does not need to cover the full range of the volumetric fraction of solvent 1:solvent 2 from 100:0 % (v/v) to 0:100 % (v/v) but can be terminated after the last peak is eluted. The actual duration of the gradient, $t_s$, can be decided upon visual inspection of the chromatogram. Obviously $t_s < t_f$ and the flow rates of mG1 and mG 2 at $t_s$, $V_{1,s}$ and $V_{2,s}$, respectively, can be calculated from the formulas:

$$V_{1,s}\ (mL\ min^{-1}) = [t_f\ (min - t_s(min)] \times V_i\ (ml\ min^{-1}) / t_f\ (min) \qquad \text{(equation S3)}$$

$$V_{2,s}\ (\text{mL min}^{-1}) = V_i\ (\text{ml min}^{-1}) - [t_f\ (\text{min} - t_s(\text{min})] \times V_i\ (\text{ml min}^{-1}) / t_f\ (\text{min}) \qquad \text{(equation S4)}$$

Obviously, the equality

$$V_{1,s} + V_{2,s} = V_i \qquad \text{(equation S5)}$$

should always hold true. In the present system, the duration of the gradient was at $t_s$ = 3.82 min and $V_{1,s}$ was calculated as 0.33 mL min$^{-1}$ while $V_{2,s}$ was calculated as 2.07 mL min$^{-1}$.

The volumetric fraction of the solvent 1 (% v/v), $S_{1,s}$, in the mobile phase at $t_s$ could be calculated from:

$$S_{1,s}\ \%\ (v/v) = (t_f - t_s)/t_f \times 100 \qquad \text{(equation S6)}$$

while the volumetric fraction of the solvent 2 (% v/v), $S_{2,s}$, in the mobile phase at $t_s$ could be calculated from:

$$S_{1,s}\ \%\ (v/v) = t_s/t_f \times 100 \qquad \text{(equation S7)}$$

In the present work, the volumetric ratio of solvent1:solvent 2 in the mobile phase at $t_s$ was calculated as 14:86 % (v/v).

All these values were automatically calculated by the software using the flow rate of the mobile phase, $V_i$, the gradient rate (i.e, the rate of change of the flow rate of the 2 solvents), G, and the duration of the gradient, $t_s$, as input parameters.

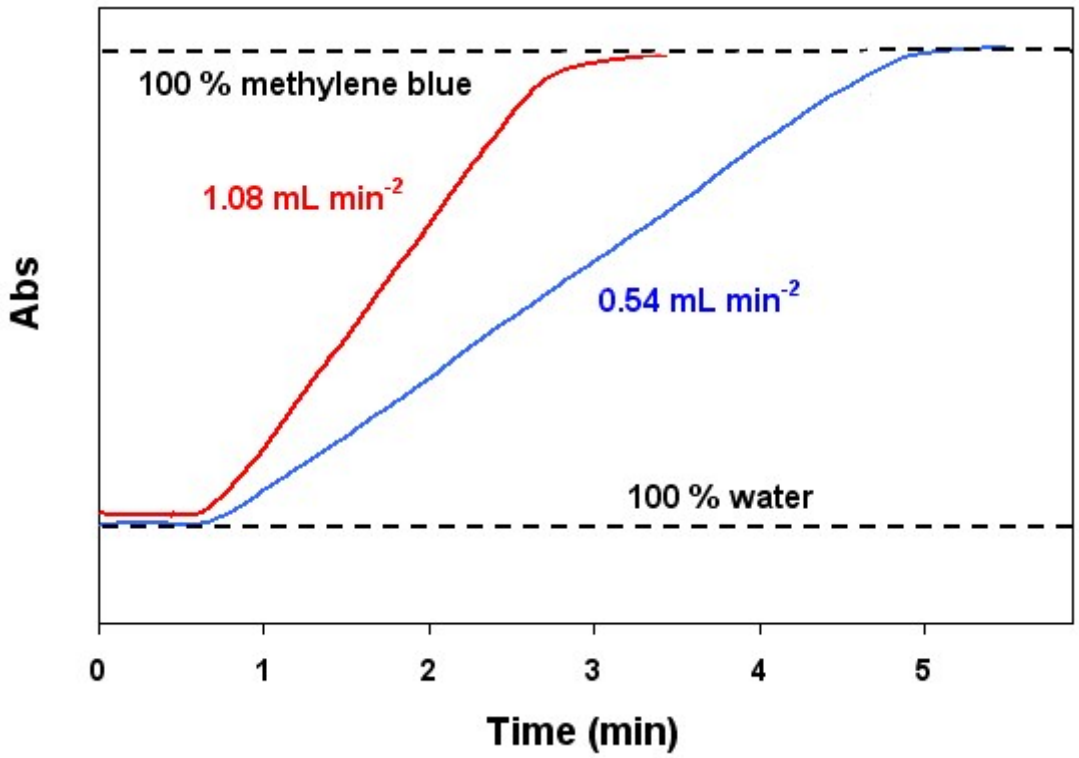


Fig. S1. Absorbance-time profiles recorded at 600 nm for 2 linear solvent gradients using water as solvent 1 and 2.5 μmol $L^{-1}$ methylene blue as solvent 2 in the range 100:0 to 0:100 (v/v %) with gradient rates 0.54 and 1.08 mL $min^{-2}$ at a flow rate of 2.4 mL $min^{-1}$. An isocratic step of 0.66 min preceded the start of the gradient.

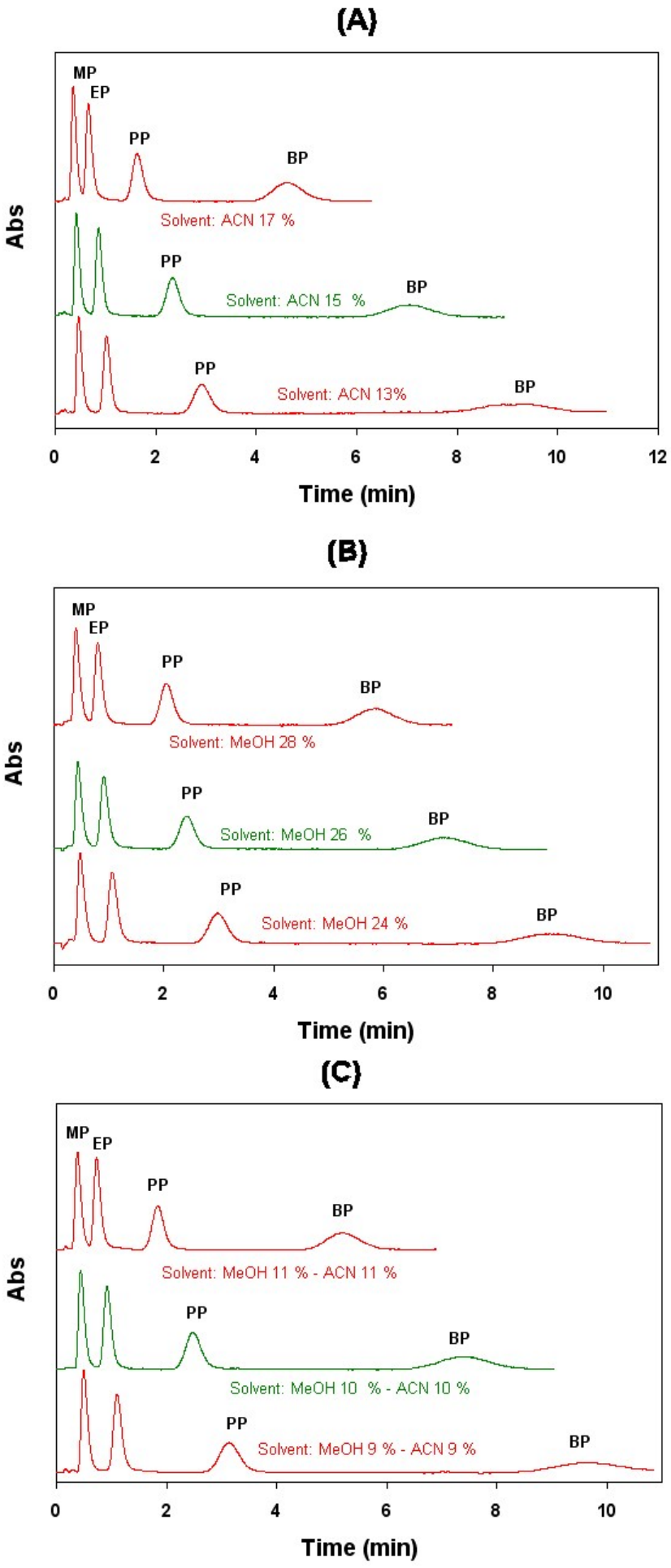


Fig. S2. Isocratic elution chromatograms using different compositions of: (A) ACN, (B) MeOH, (c) mixture of ACN and MeOH. Parabens concentration, 10 μmol $L^{-1}$; flow rate, 2.1 mL $min^{-1}$; sample volume 45 μL. The solvents in the green chromatograms were selected as solvents 1 in subsequent gradient elution experiments.

Table S1. Resolution between the MP and PP peaks using different solvents under isocratic conditions (green shading: selected solvents; red shading: solvents at which the resolution was $R_s$<1.5 with either of the two calculation methods used; yellow shading: solvents with which the MP and EP peaks elute later)

| **Solvent 1 (%v/v)** | **$R_s$ [a]** | **$R_s$ [b]** |
|---|---|---|
| ACN: $H_2O$ 13:87 | 1.9 | 2.4 |
| ACN: $H_2O$ 15:85 | 1.6 | 2.1 |
| ACN: $H_2O$ 17:83 | 1.2 | 1.5 |
| MeOH:$H_2O$ 24:76 | 1.7 | 2.2 |
| MeOH:$H_2O$ 26:76 | 1.6 | 2.0 |
| MeOH:$H_2O$ 28:82 | 1.4 | 1.8 |
| ACN:MeOH:$H_2O$ 9:9:82 | 2.0 | 2.5 |
| ACN:MeOH:$H_2O$ 10:10:80 | 1.7 | 2.1 |
| ACN:MeOH:$H_2O$ 11:11:78 | 1.3 | 1.7 |

[a] $R_s = 2(t_{R2}-t_{R1})/(w_1+w_2)$, where $t_{R1}$, $t_{R2}$ are the retention times and $w_1$, $w_2$ are the peak widths at baseline

[b] $R_s = 1.18(t_{R2}-t_{R1})/(FWHM_1+FWHM_2)$, where FWHM are the peak widths at half maximum peak height.

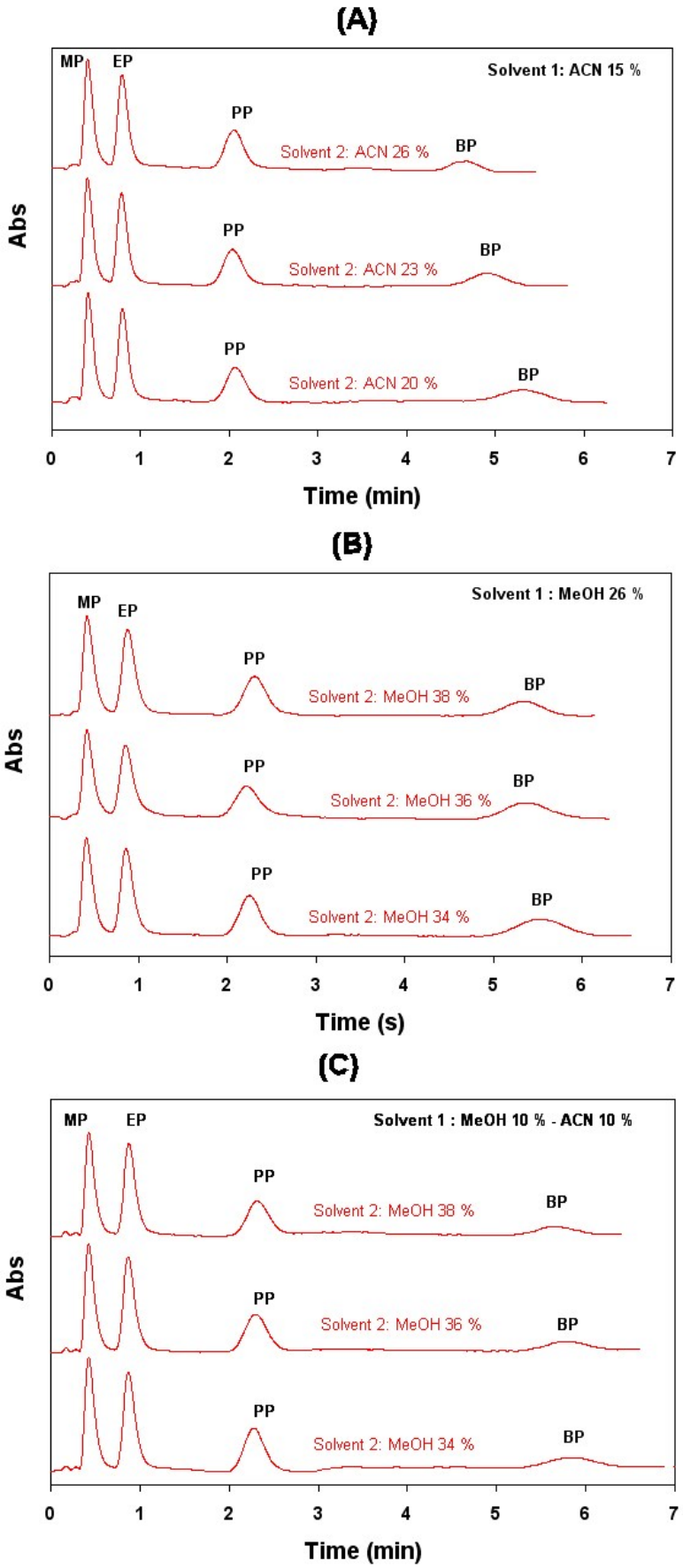


Fig. S3. Gradient elution chromatograms using: (A) ACN as solvents 1 and 2, (B) MeOH as solvents 1 and 2, (C) mixture of ACN and MeOH as solvent 1 and MeOH as solvent 2. Parabens concentration, 10 μmol $L^{-1}$; flow rate, 2.1 mL $min^{-1}$; sample volume 45 μL; isocratic step, 1.25 min.

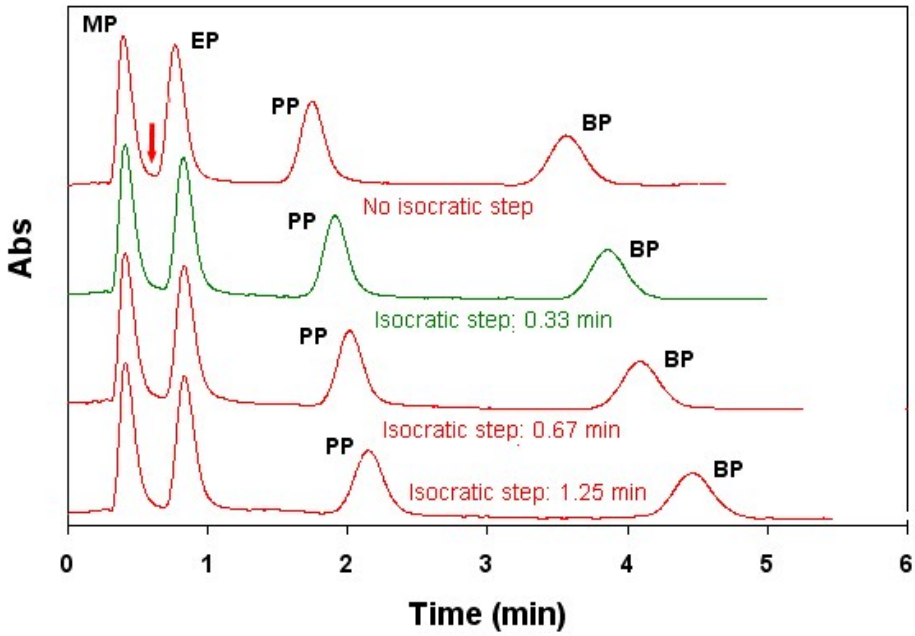


Fig. S4. Linear gradient elution chromatograms using ACN:MeOH:$H_2O$ 10:10:20 (% v/v) as solvent 1 and ACN:$H_2O$ 24:76 (% v/v) as solvent 2 with initial isocratic steps of different durations. Parabens concentration, $1.0\times10^{-5}$ mol $L^{-1}$; flow rate, 2.1 mL $min^{-1}$; sample volume, 45 μ; gradient rate, 0.40 mL $min^{-2}$. The isocratic step in the green chromatogram (0.33 min) was selected for subsequent gradient elution experiments.

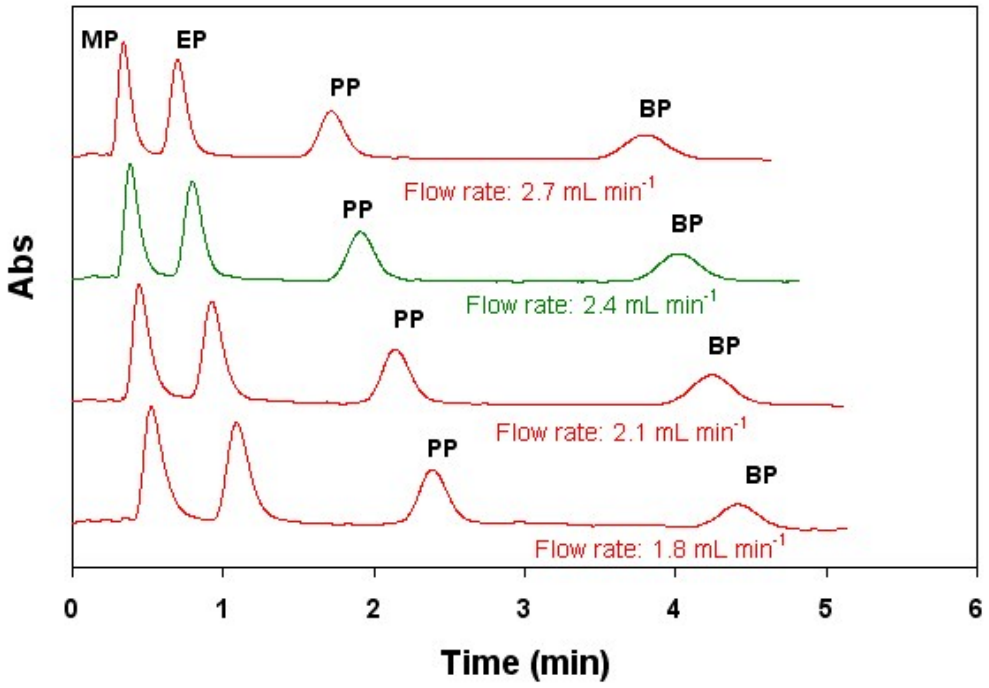


Fig. S5. Linear gradient elution chromatograms using ACN:MeOH:$H_2O$ 10:10:80 (% v/v) as solvent 1 and ACN:$H_2O$ 24:76 (% v/v) as solvent 2 at different flow rates. Parabens concentration, 10 μmol $L^{-1}$; sample volume, 45 μL; isocratic step, 20 s; gradient rate, 0.40 mL $min^{-2}$. The flow rate in the green chromatogram was selected for subsequent gradient elution experiments.

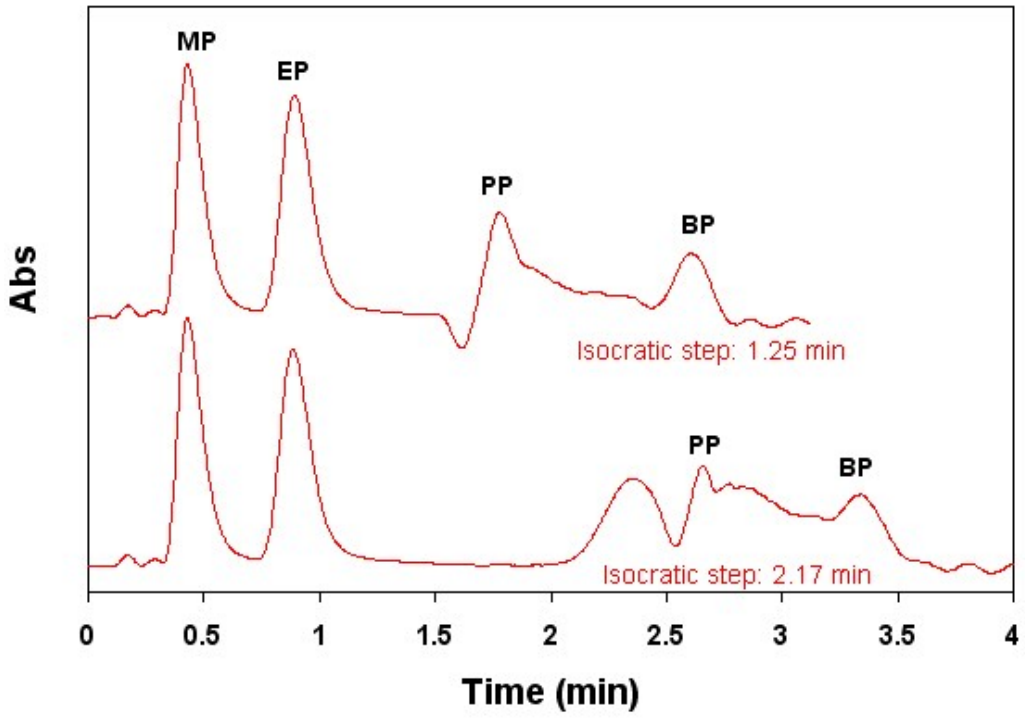


Fig. S6. Chromatograms using step gradient from ACN:MeOH:$H_2O$ 10:10:80 % (v/v) to ACN-$H_2O$ 24:76 % (v/v). Parabens concentration, 10 μmol $L^{-1}$; flow rate, 2.4 mL $min^{-1}$; sample volume 45 μL.

Table S2. Repeatability and between-days reproducibility of the 4 parabens. The repeatability is expressed as the $RSD_r$ % of peak areas (n=6) in a single day and the between-days reproducibility is expressed as the $RSD_R$ % of concentrations calculated from the calibration plots (n=9) over 3 days.

| **Concentration (µmol $L^{-1}$)** | **MP** | | **EP** | | **PP** | | **BP** | |
|---|---|---|---|---|---|---|---|---|
| | **$RSD_r$ %** | **$RSD_R$ %** | **$RSD_r$ %** | **$RSD_R$ %** | **$RSD_r$ %** | **$RSD_R$ %** | **$RSD_r$ %** | **$RSD_R$ %** |
| **1.0** | 3 | 4 | 3 | 4 | 5 | 5 | – [a] | – [a] |
| **2.0** | 1 | 3 | 3 | 3 | 4 | 4 | 5 | 4 |
| **5.0** | 1 | 2 | 1 | 2 | 2 | 3 | 3 | 4 |
| **10** | 0.6 | 0.9 | 1 | 2 | 1 | 3 | 1 | 3 |
| **20** | 0.5 | 0.7 | 0.6 | 1 | 0.9 | 2 | 1 | 2 |
| **40** | 0.5 | 0.6 | 0.6 | 1 | 0.9 | 0.8 | 0.9 | 1 |
| **50** | 0.5 | 0.7 | 0.5 | 1 | 0.8 | 1 | 0.9 | 1 |

[a] For BP, 1 µmol $L^{-1}$ is below the LOQ

Table S3. Parameters tested and their numerical values for the robustness study

| Variable | Low level (-) | Nominal value (0) | High level (+) | $I_{exp}$ [a] |
|---|---|---|---|---|
| **Flow rate (mL $min^{-1}$)** | 2.28 | 2.4 | 2.52 | [2.28, 2.52] |
| **MeOH (% v/v) in solvent 1** | 9.5 | 10 | 10.5 | [9.5, 10.5] |
| **ACN (% v/v) in solvent 1** | 9.5 | 10 | 10.5 | [9.5, 10.5] |
| **ACN (% v/v) in solvent 2** | 23 | 24 | 25 | [23, 25] |
| **Isocratic elution time (min)** | 0.30 | 0.33 | 0.36 | [0.30, 0.36] |
| **Gradient rate (mL $min^{-2}$)** | 0.504 | 0.54 | 0.576 | [0.504, 0.576] |
| **Detection wavelegth (nm)** | 252 | 254 | 256 | [252, 254] |

[a] $I_{exp}$ = [Low level (-), High level (+)]