Supplementary Material

Programmable low-pressure chromatographic sub-90 s assay of parabens in cosmetics with post-column chemiluminescence detection

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Paragraph P1**.** Control and data acquisition, chemiluminescence and UV detectors

A USB-232/2 USB-to-serial card (National Instruments, Austin, TX) was used for the control of the two MilliGAT pumps (via a serial communication protocol). A USB-6008 multi-function DAQ (National Instruments, Austin, TX) was used for the control of the two Minipuls3 pumps (via digital TTL logic control signals), the Cheminert sample injector (via digital TTL logic control signals) as well as for data acquisition from the CL detector (via an analogue-to-digital converter). The control and data acquisition programmes were developed in-house using LabVIEW 8.2 (National Instruments, Austin, TX) and allowed complete automation of the measurement procedure. The chromatograms were saved as ASCII (text) files while processing of data (smoothing, evaluation of the chromatographic parameters) was performed with the LC Solution software (Shimadzu) or with the OriginPro 2019 (OriginLab). Statistical evaluation of the method robustness was performed using Statistica 8 (Statsoft).

The flow-through CL detector was fabricated in-house by placing a miniature photomultiplier (PMT) module (H6780, Hamamatsu Photonics, Japan) in front of Pesrpex flow cell within a light tight-box (Fig. S1). The operating voltage of the PMT was set at at 600 V with the help of a potentiometer connected to the appropriate terminal of the PMT module.

For UV measurements, a U-2000 UV/Vis spectrophotometer (Hitachi) was used operating at 254 nm and equipped with a quartz flow-through cell with 10 mm path length. For comparative

measurements, a LC-20AD HPLC instrument (Shimadzu) was used featuring a column oven (CTO-10AS VP), a degassing unit (DGU-20A$_{5R}$) and a UV/Vis Detector (SPD-20A).

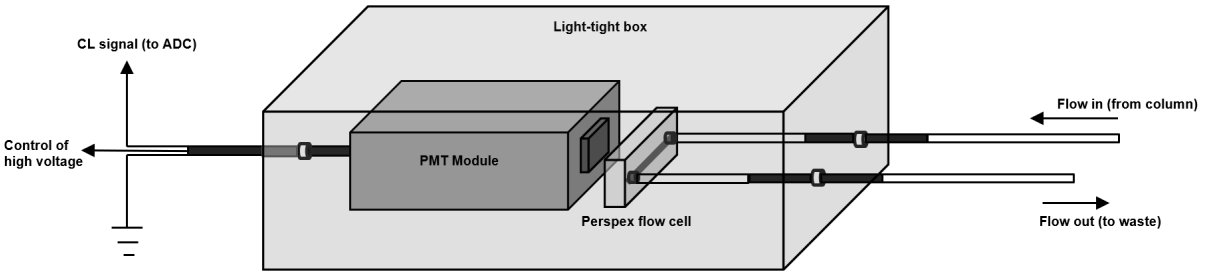

Figure S1. Schematic diagram of the flow-through CL detector.

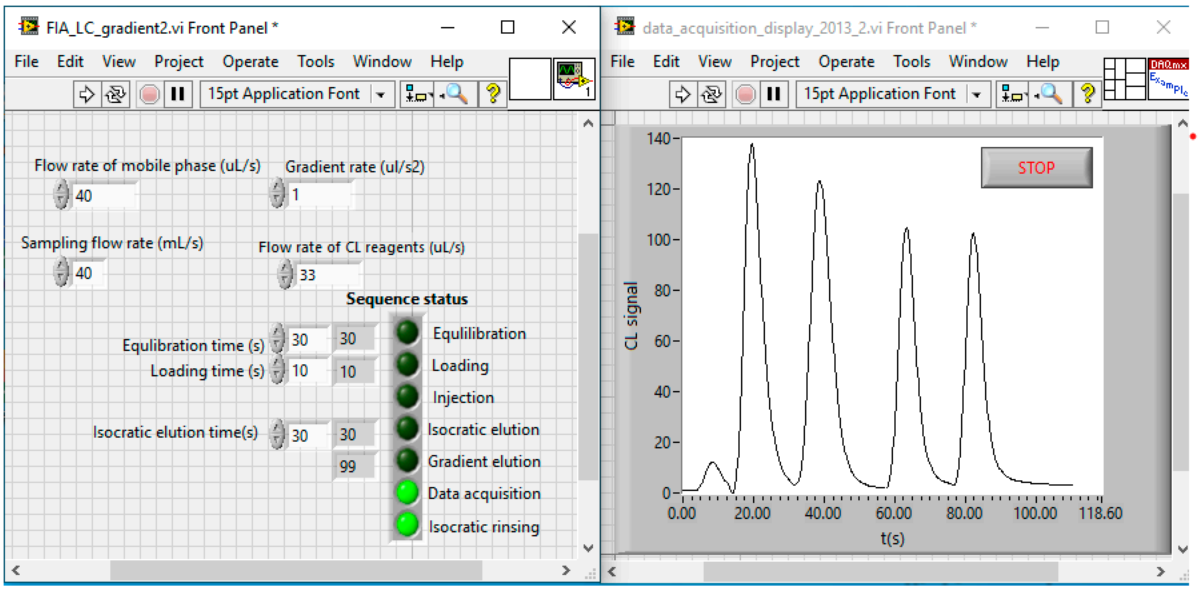

Figure S2. User-interface of the control and data acquisition programs.

Paragraph P2. Treatment of samples, recovery experiments, HPLC analysis

Samples containing parabens were purchased from a local drug store. For the recovery experiments, paraben-free samples of wet tissues, liquid soap and face lotion with similar composition to the samples were also purchased. The wet tissues were removed from their package and were left to dry at room temperature for 1 day before extraction. Evaluation of the paraben concentration in the samples was performed by means of external calibration curves.
(a) Recovery experiments. The spiked paraben-free samples were prepared as follows: 1.0 g of each paraben-free sample was weighed, spiked with 1.0 mL of each of the 1.0×10^{-2} mol L^{-1} stock standard solutions of the parabens in MeOH, 16 mL of MeOH was added and the samples were sonicated for 15 min. The extract was diluted to 100 mL with doubly distilled water and 5.0 mL of the diluted extract was further diluted to 50 mL with solvent A (MeOH 20 % (v/v)). The final concentration of the four parabens in the extract was 10 μmol L^{-1}.
(b) Analysis of samples containing parabens. 1.0 g of each sample was weighed and treated with 20 mL of MeOH for 15 min under sonication. The extracts were diluted to 100 mL with doubly distilled water. 1.0 to 5.0 mL of the extract (depending on the sample type) was further diluted to 50 mL with solvent A (MeOH 20 % (v/v)).

All the extracted samples were filtered through cellulose filters of 0.45 mm pore size and ultrasonicated for 5 min before analysis.

The HPLC reference method for the determination of parabens was adapted from [32].

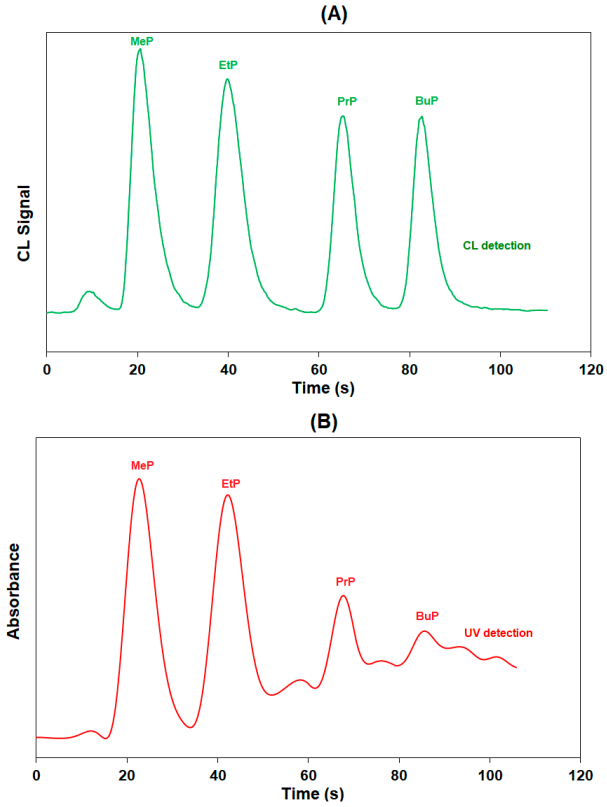

Figure S3. Chromatographs of the four parabens with: (A) CL detection and (B) UV detection using the elution protocol shown in Table 2 of the manuscript.

Table S1. Consumption of reagents per chromatographic run

Reagent (units)	**Consumption**
MeOH (mL)	3.8
Ce(IV) (µmmol)	60
Rho 6G (µmol)	1.5
H_2SO_4 (mmol L)	12

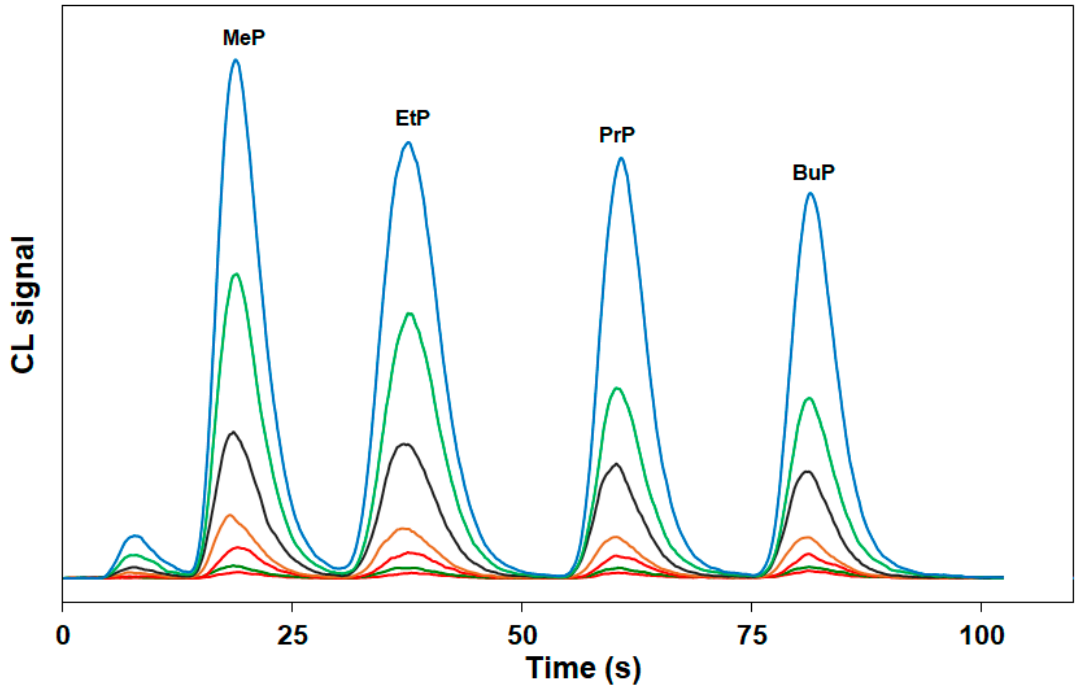

Figure S4. Chromatographs of the four parabens in the concentration range 0.2-20 µmol L^{-1}.

Table S2. Comparison of the existing low pressure separation methods with the present method for the determination of the 4 parabens (all the methods use a 5 mm C18 monolithic column)

Type of manifold*	Automation	Solvent gradient	Assay time	Detection	LOQ	Baseline distortion	Ref
FIC	No	step	~2.5 min	UV	37.4-673 µmol L^{-1}	Yes	23
FIC	No	step	~7 min	UV	0.42-5.1 µmol L^{-1}	Yes	24
SIC-LOV	No	step	~2 min	UV	1.2-13 µmol L^{-1}	Yes	25
FIC	No	linear	~4 min	UV	0.56-1.5 µmol L^{-1}	No	27
MSC	Yes	step	~9 min	CL	0.39-2 µmol L^{-1}	No	29
FIC	No	step	~2.5 min	CL	0.83-0.99 µmol L^{-1}	No	30
FIC	**Yes**	**linear**	**<90 s**	**CL**	**0.2 µmol L^{-1}**	**No**	**present work**

* SIC-LOV, sequential injection chromatography-lab-on-valve; FIC, flow injection chromatography; MSC, multi-syringe chromatography

Table S3. Factors tested and the nominal values and perturbation values ((+)) is the upper perturbation level and (-) is the low perturbation level)

Factor	Central value	(+)	(-)
Gradient rate (mL min^{-2})	3.6	3.8	3.4
MeOH % (v/v) in solvent A	20	22	18
MeOH % (v/v) in solvent B	36	39	33
Isocratic elution time (s)	30	32	28
[Ce(IV)] (mmol L^{-1})	20	22	18
[Rho 6G] (mmol L^{-1})	0.5	0.55	0.45
H_2SO_4 (mol L^{-1})	4	3.8	4.2

Table S4. The combination of factors for the 9 experiments conducted in the 2-level Placket Burman experimental design (experiment No 9 is the central experiment with all the factors at their central values).

No of experiment	Gradient rate (mL min^{-2})	MeOH % (v/v) in solvent A	MeOH % (v/v) in solvent B	Isocratic elution time (s)	[Ce(IV)] (mmol L^{-1})	[Rho 6G] (mmol L^{-1})	H_2SO_4 (mol L^{-1})
1	3.4	18	33	32	22	0.55	3.8
2	3.4	18	39	32	18	0.45	4.2
3	3.4	22	33	32	22	0.45	4.2
4	3.4	22	39	28	18	0.55	3.8
5	3.8	18	33	28	18	0.55	4.2
6	3.8	18	39	28	22	0.45fff	3.8
7	3.8	22	33	32	18	0.45	3.8
8	3.8	22	39	32	22	0.55	4.2
9	**3.6**	**20**	**36**	**30**	**20**	**0.5**	**4.0**

References

[32] Separation of paraben preservatives by Reverse-Phase HPLC, Application Note, Agilent Technologies. Available on line: https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwiT1ejN8K7_AhXLDd4KHVObAFEQFnoECAoQAQ&url=https%3A%2F%2Fwww.agilent.com%2FLibrary%2Fapplications%2F5989-3635EN.pdf&usg=AOvVaw3pLls0aOu9En1ZZDzWtAsi (accessed 6 June 2023)