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ELECTROANALYSIS

Supporting Information

Electroanalysis of Benzalkonium Chloride in Ophthalmic Formulation by Boron-doped Diamond Electrode

Huda Alghamdi, Majidah Alsaeedi, Alyah Buzid, Jeremy D. Glennon,* and John H. T. Luong*© 2021 The Authors. Electroanalysis published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Electroanalysis of benzalkonium chloride in ophthalmic formulation by boron-doped diamond electrode

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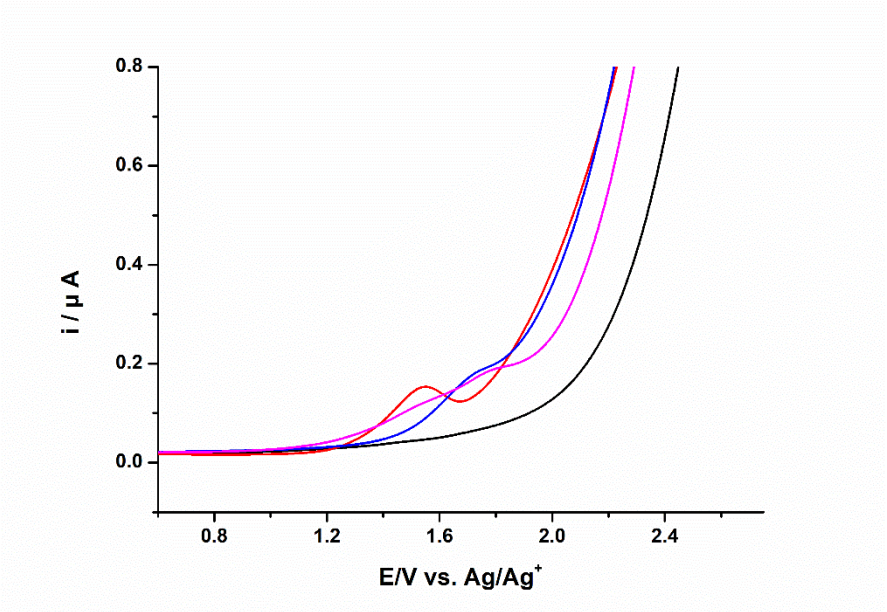


Fig. S1. Overlay of SWV 40 µg/ml of BAK on bare BDD (red), after anodic treatment (blue) and cathodic treatment (pink) on the bare BDD electrode vs. Ag/Ag^+ using 10 mM TBAH/ACN (black).

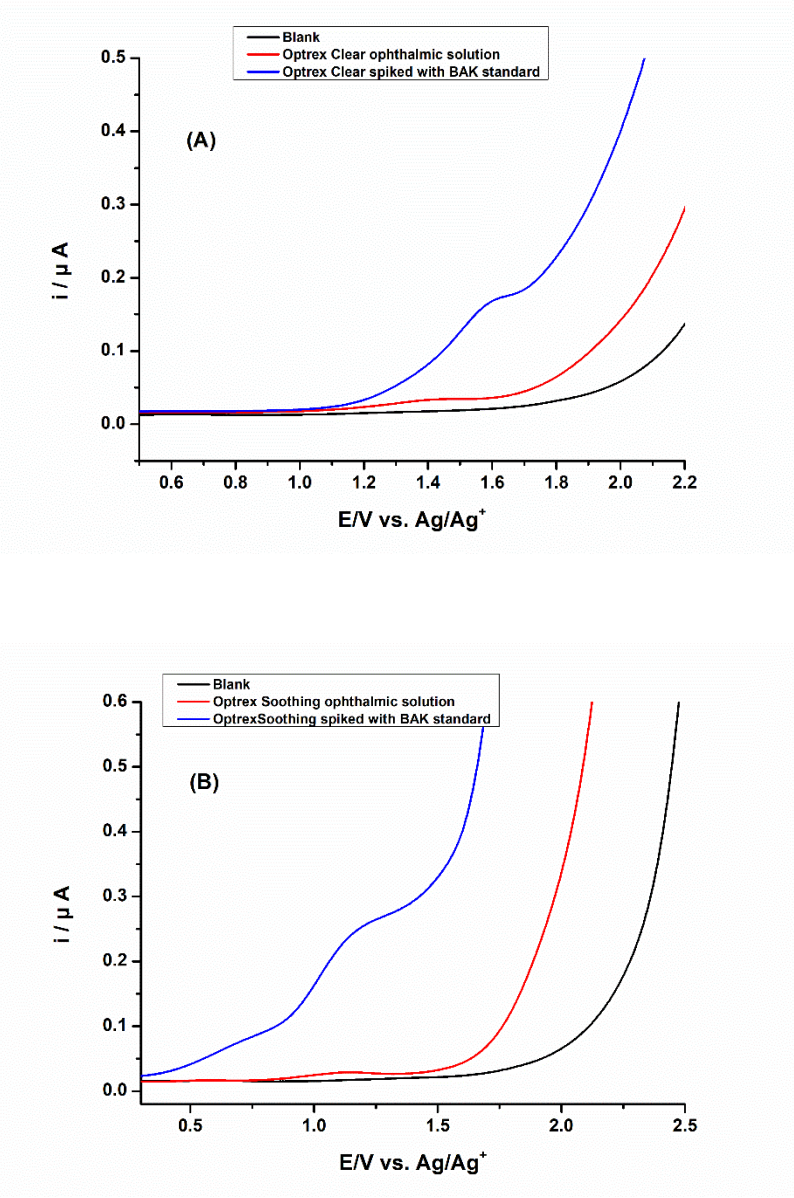
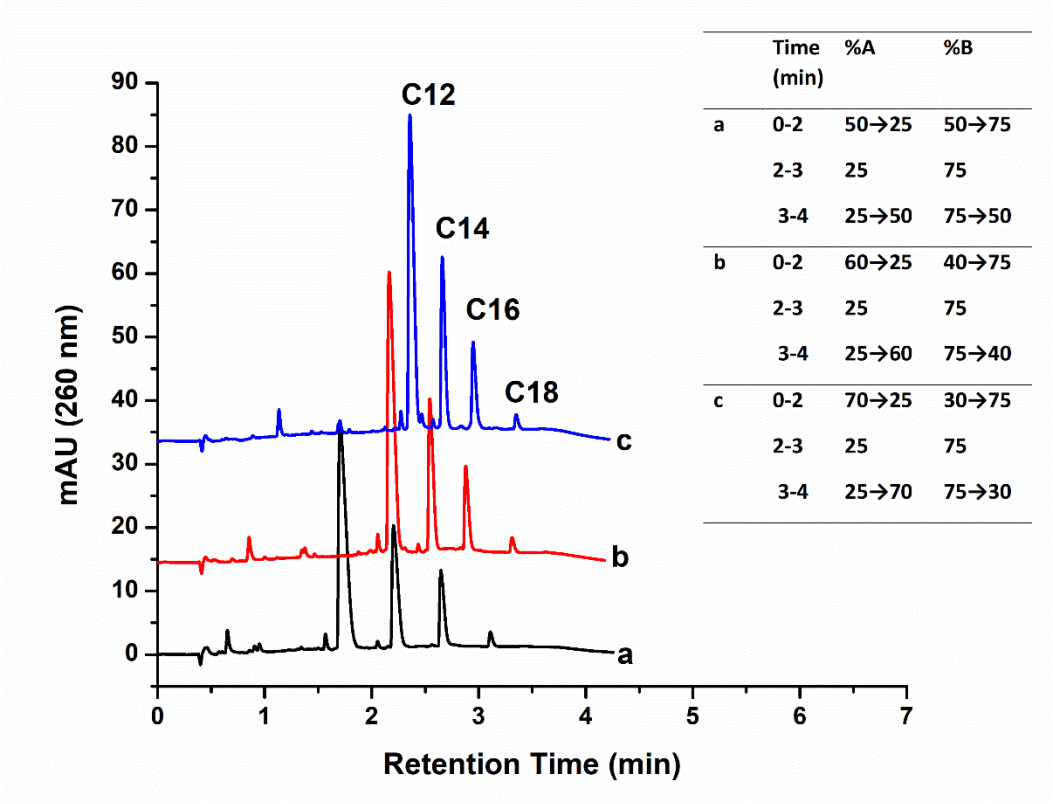


Fig. S2. SWVs of (A) Optrex Clear ophthalmic solution (B) Optrex Soothing ophthalmic solution and spiked with 40 µg/mL of BAK on the bare BDD electrode vs. Ag/Ag+ in 10 mM (TBAH)/ACN.

Table S1. The content of BAK homologs in three ophthalmic solutions (n=3) with BDD

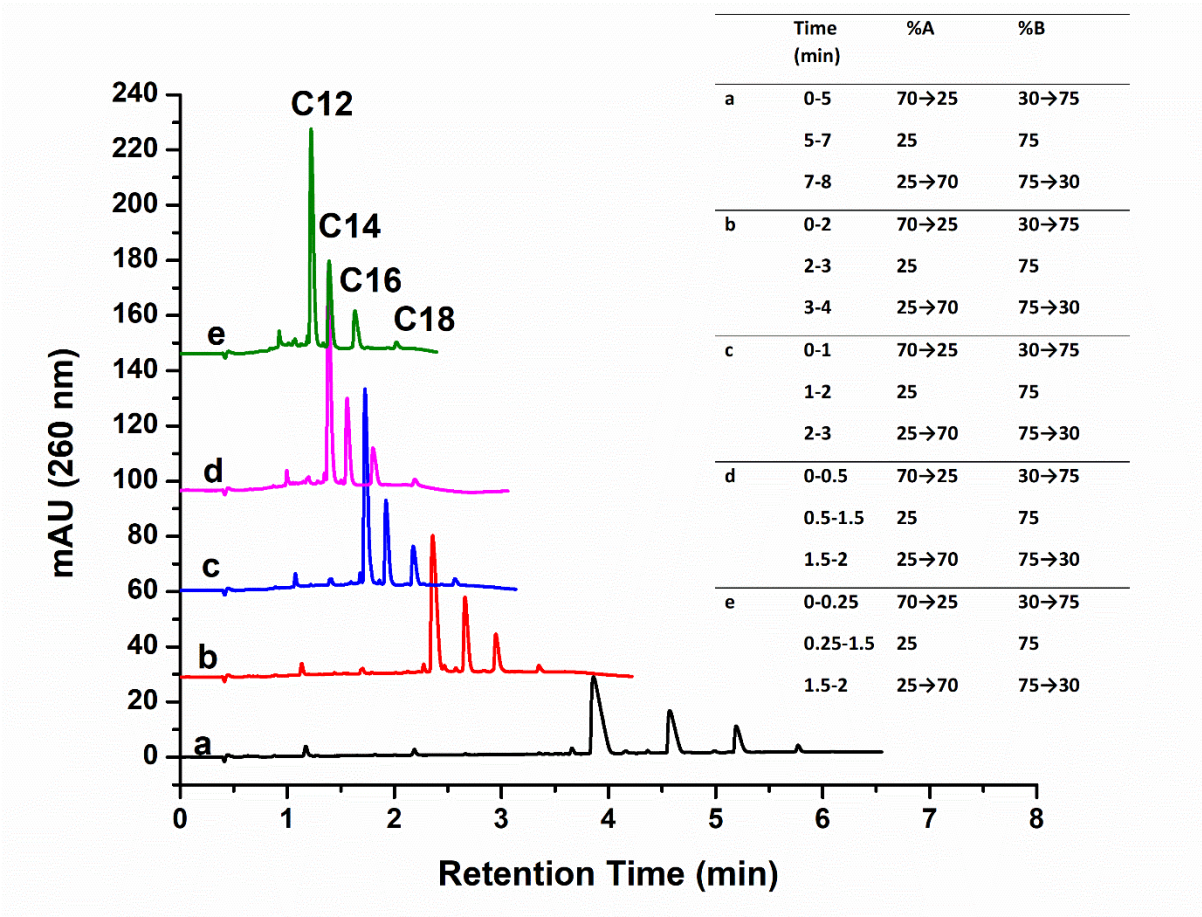
Analyte	Linear range	Linear regression equation	Correlation coefficient (R^2)	Detected in Murine	Detected in Optrex Clear	Detected in Optrex Soothing
BAK	0.001 – 0.3 mg/mL (0.003 – 0.8 mM)	y = 0.0027x + 0.0166	0.9947	0.1 ± 0.03 mg/mL (0.33 ± 0.07 mM)	0.051 ± 0.01 mg/mL (0.19 ± 0.03 mM)	0.039 ± 0.02 mg/mL (0.12 ± 0.05 mM)
Labelled BAK	-	-	-	0.1 mg/mL	0.05 mg/mL	N/A[a]

[a] Not available



	Time (min)	%A	%B
a	0-2	50→25	50→75
	2-3	25	75
	3-4	25→50	75→50
b	0-2	60→25	40→75
	2-3	25	75
	3-4	25→60	75→40
c	0-2	70→25	30→75
	2-3	25	75
	3-4	25→70	75→30

Fig. S3. Optimize initial %B of 3 mg/mL BAK homologs analysed by gradient HPLC-UV at 260 nm. Mobile phase: A: 0.05% TFA in H_2O, B: 0.05% TFA in (ACN: IPA; 50:50), Column: poroshell EC-C8 (2.1 X 50 mm, 1.9 µm), flow rate: 0.3 mL/min, temperature 30 ℃, injection volume: 1µL, (a), (b) and (c). The inserted table represents the gradients conditions.



	Time (min)	%A	%B
a	0-5	70→25	30→75
	5-7	25	75
	7-8	25→70	75→30
b	0-2	70→25	30→75
	2-3	25	75
	3-4	25→70	75→30
c	0-1	70→25	30→75
	1-2	25	75
	2-3	25→70	75→30
d	0-0.5	70→25	30→75
	0.5-1.5	25	75
	1.5-2	25→70	75→30
e	0-0.25	70→25	30→75
	0.25-1.5	25	75
	1.5-2	25→70	75→30

Fig. S4. Optimize the analysis time of 3 mg/mL BAK homologs analysed by gradient HPLC-UV at 260 nm. Mobile phase: A: 0.05% TFA in H_2O, B: 0.05% TFA in (ACN: IPA; 50:50)), Column: poroshell EC-C8 (2.1 X 50 mm, 1.9 µm), flow rate: 0.3 mL/min, temperature 30 °C, injection volume: 1µL, (a), (b), (c), (d) and (e). The inserted table represents the gradients conditions.

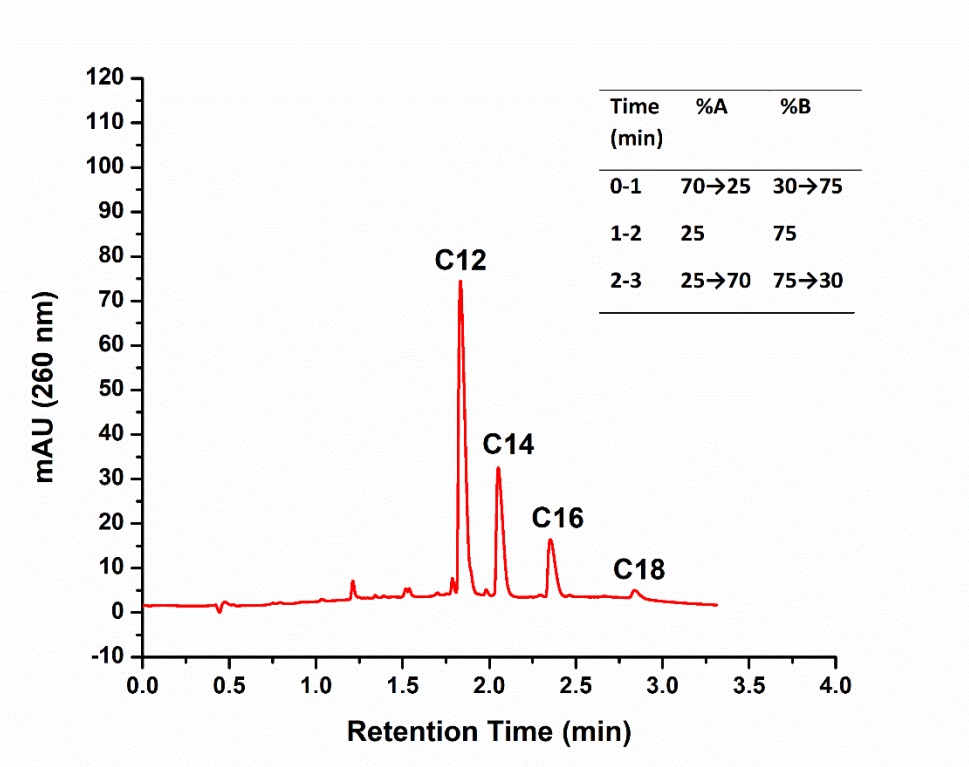


Fig. S5. Chromatogram of 3 mg/mL BAK homologs analysed by gradient HPLC-UV, the inserted table represents the gradient condition. Mobile phase: A: 0.05% TFA in H_2O, B: 0.05% TFA in (ACN: IPA; 50:50)), Column: poroshell EC-C8 (2.1 X 50 mm, 1.9 µm), temperature 30 °C, injection volume: 1µL, UV detection: 260 nm, flow rate: 0.3 mL/min.

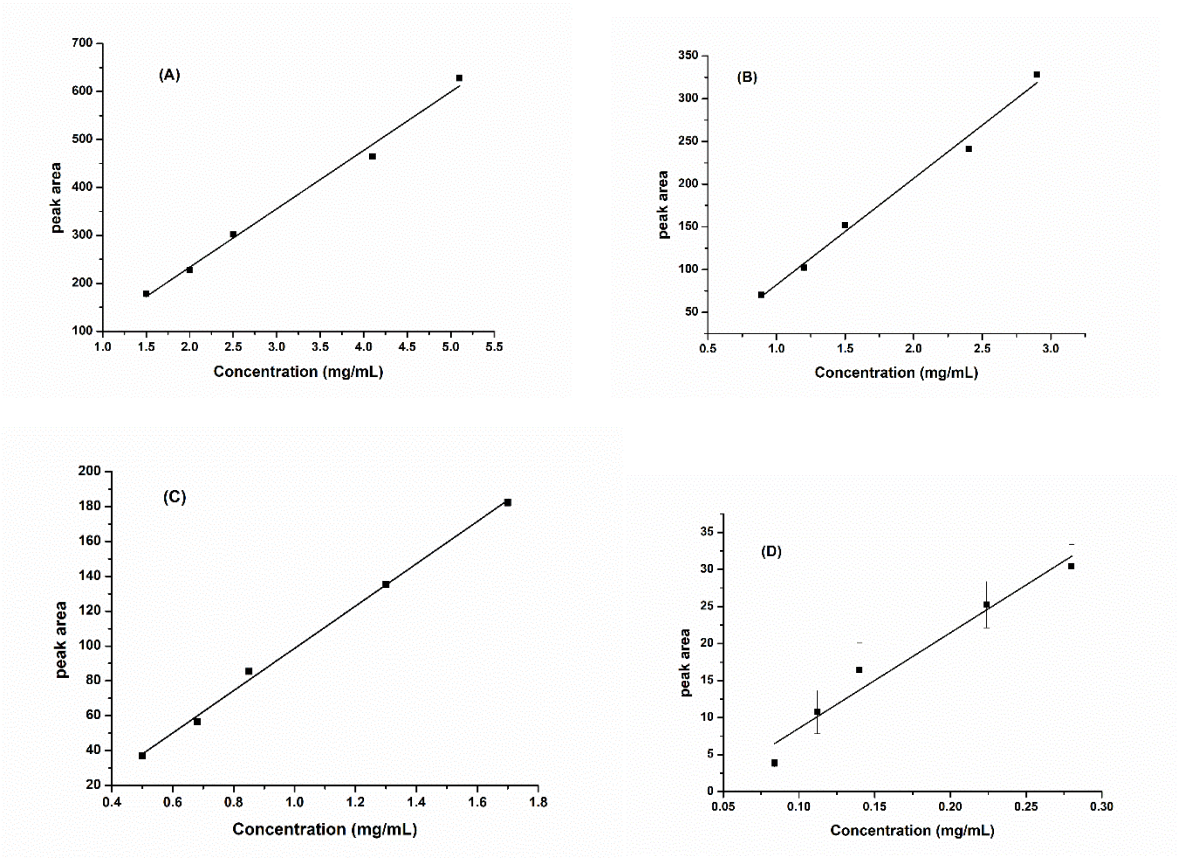


Fig. S6. Calibration plot of BAK over the concentration range 3-10 mg/mL (A) C_{12} homolog; (B) C_{14} homolog; (C) C_{16} homolog and (D) C_{18} homolog analyzed by gradient HPLC-UV at 260 nm. Mobile phase: A: 0.05% TFA in H2O, B: 0.05% TFA in (ACN:IPA; 50:50)), Column: poroshell EC-C8 (2.1 X 50 mm, 1.9 μm), flow rate: 0.3 mL/min, temperature 30 ℃, injection volume: 1μL. All measurements were made in triplicate, using the peak area of the analyte response on the HPLC-UV. The error bars present as a mean ± standard deviation (SD).

Table S2. The proportions of homologs in the BAK standard solutions

Concentration of BAK standard (mg/mL)	Proportion %			
	C12 Homolog	C14 Homolog	C16 Homolog	C18 Homolog
3	61.54	24.37	12.78	1.32
4	57.37	25.74	14.19	2.70
5	54.35	27.37	15.34	2.95
8	53.60	27.86	15.63	2.91
10	53.71	28.09	15.59	2.60

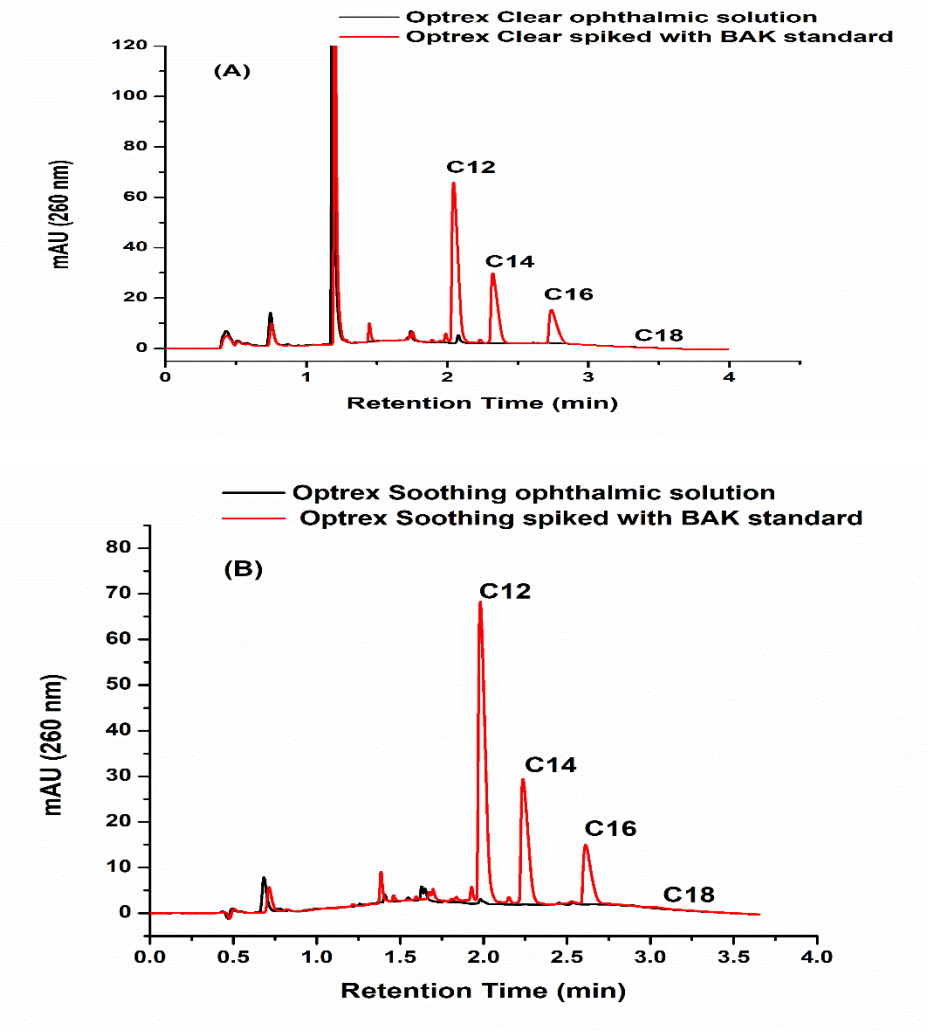


Fig. S7. HPLC-UV gradient analysis of (A) Optrex Clear ophthalmic solution (B) Optrex Soothing ophthalmic solution and spiked with 3 mg/mL of BAK analyzed by gradient HPLC-UV. Separation conditions: Column: poroshell EC-C8 (2.1 X 50 mm, 1.9 µm), Mobile phase: A: 0.05% TFA in H2O, B: 0.05% TFA in (ACN:IPA; 50:50), flow rate: 0.3 mL/min, temperature 30 ℃, injection volume: 1µL, wavelength at 260nm.

Table S3. The content of BAK homologs in three ophthalmic solutions (n=3) with HPLC-UV

Analyte	Linear range	Linear regression equation	Correlation coefficient (R^2)	Detected in Murine	Detected in Optrex Clear	Detected in Optrex Soothing
C12	0.02 – 0.15 mg/mL (0.05 – 0.4 mM)	y = 112.38x + 4.8744	0.9835	0.097 ± 0.01 mg/mL (0.29 ± 0.03 mM)	0.047 ± 0.01 mg/mL (0.14 ± 0.02 mM)	0.035 ± 0.01 mg/mL (0.10 ± 0.02 mM)
C14	0.1 – 0.2 mg/mL (0.3 – 0.6 mM)	y = 40.289x + 0.5462	0.9945	ND [a]	ND [a]	ND [a]
C16	0.06 – 0.1 mg/mL (0.1 – 0.3 mM)	y = 50.071x + 6.993	0.9681	ND [a]	ND [a]	ND [a]
C18	0.01 – 0.02 mg/mL (0.03 – 0.06 mM)	y = 31.978x – 0.6015	0.964	ND [a]	ND [a]	ND [a]
Labelled BAK	-	-	-	0.1 mg/mL	0.05 mg/mL	N/A[b]

[a] Not detected

[b] Not available

Table S4. Linear regression parameters of calibration curves, and precision data on HPLC-UV at 260 nm.

Homolog	Linear range of BAK mixture (mg/mL)	Linear range of each homolog mg/mL	Linear regression equation	Correlation coefficient (R^2)	Intra-day (%) [a]	Inter-day (%) [b]	LOD [c] HPLC-UV (µg /mL)
C12	3-10	1.5-5	y = 121.93C - 10.37	0.990	0.13	0.30	4.6
C14		0.9-2.9	y = 124.56C - 42.43516	0.988	0.19	0.51	2.9
C16		0.5-1.7	y = 121.39C- 22.81	0.996	0.19	0.62	1.7
C18		0.08-0.28	y = 128.99C - 4.34	0.950	0.35	0.75	8.4

[a] Intra-day (%) calculated from triplicate measurements within one experiment for the retention time at 3 mg/mL of BAK.

[b] Inter-day (%) calculated from triplicate measurements within three experiments for the retention time at 3 mg/mL of BAK.

[c] LOD (S/N=3) at 260 nm.

C is the concentration

Table S5. Comparison of the developed method for the detection of BAK homologs on the HPLC-UV with the literature methods.

Analyte	Linear range	Correlation coefficient (R^2)	Detection limit	Reference
C12	1.5 −5 mg/mL (4 −14 mM)	0.9901	4.6 µg /mL (13.5 µM)	Present work
C14	0.9 −2.9 mg/mL (2.4 − 8.1 mM)	0.9878	2.9 µg /mL (8 µM)	Present work
C16	0.5 −1.7 mg/mL (1.4 − 4.7 mM)	0.9962	1.7 µg /mL (4.3 µM)	Present work
C18	0.08 −0.28 mg/mL (0.2 – 0.7 mM)	0.9503	8.4 µg /mL (20 µM)	Present work
C12 − C14	2.8 − 4.8 µg /mL	0.9997	N.R [a]	[1]
C12 − C14	50 – 150 µg /mL	0.999	N.R [a]	[2]
BAK	20 – 60 µg/mL	0.9989	N.R [a]	[3]
C12 – C14 – C16	1.6 – 32 µg/mL	0.9999	N.R [a]	[4]

[a] N.R, not reported.

References

[1] K. L. N. Rao, K. P. Reddy, K. S. Babu, K. S. Raju, K. V. Rao, J. V. Shaik, *Int. J. Res. Pharm. Sci.* **2010**, *1*, 473-480.

[2] H. K. Trivedi, M. C. Patel, *J. Chem.* **2010**, *7*, 1514-1522.

[3] M. Jovović, N. Kostić, B. Jančić-Stojanović, A. Malenović, *J. Liq. Chromatogr. Rel. Technol.* **2012**, *35*, 231-239.

[4] L.-P. Labranche, S. N. Dumont, S. Levesque, A. Carrier, *J. Pharm. Biomed. Anal.* **2007**, *43*, 989-993.