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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 μ g/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 μ g/mL of BAK on the bare BDD electrode vs. Ag/Ag+ in 10 mM (TBAH)/ACN.

Analyte	Linear range	Linear regression equation	Correlation coefficient (<i>R</i> 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)	$\begin{array}{l} 0.051 \pm 0.01 \\ mg/mL \\ (0.19 \pm 0.03 \\ mM) \end{array}$	$\begin{array}{l} 0.039 \pm 0.02 \\ mg/mL \\ (0.12 \pm 0.05 \\ mM) \end{array}$
Labelle d BAK	-	-	-	0.1 mg/mL	0.05 mg/mL	N/A ^a

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

^a Not available



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 H₂O, B: 0.05% TFA in (ACN: IPA; 50:50), Column: poroshell EC-C8 (2.1 X 50 mm, 1.9 μ m), flow rate: 0.3 mL/min, temperature 30 °C, injection volume: 1 μ L, (a), (b) and (c). The inserted table represents the gradients conditions.



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 H₂O, B: 0.05% TFA in (ACN: IPA; 50:50)), Column: poroshell EC-C8 (2.1 X 50 mm, 1.9 μ m), flow rate: 0.3 mL/min, temperature 30 °C, injection volume: 1 μ L, (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 H₂O, B: 0.05% TFA in (ACN: IPA; 50:50)), Column: poroshell EC-C8 (2.1 X 50 mm, 1.9 μ m), temperature 30 °C, injection volume: 1 μ L, 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 °C, 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).

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		

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



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 μ m), 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 °C, injection volume: 1 μ L, wavelength at 260nm.

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 $(0.29 \pm 0.03$ mM)	$\begin{array}{c} 0.047 \pm 0.01 \\ mg/mL \\ (0.14 \pm 0.02 \\ mM) \end{array}$	0.035 ± 0.01 mg/mL $(0.10 \pm 0.02$ 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

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

^a Not detected

^b Not available

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 ° HPLC- UV (µg /mL)
C12		1.5-5	y = 121.93C - 10.37	0.990	0.13	0.30	4.6
C14	3-10	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

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

^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

Analyte	Linear range	Correlation coefficient (<i>R</i> 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	$2.8-4.8\ \mu g\ /mL$	0.9997	N.R ^a	[1]
C12 - C14	$50-150\ \mu g\ /mL$	0.999	N.R ^a	[2]
BAK	$20-60\;\mu g/mL$	0.9989	N.R ^a	[3]
C12 - C14 - C16	$1.6-32\ \mu g/mL$	0.9999	N.R ^a	[4]

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

^a N.R, not reported.

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