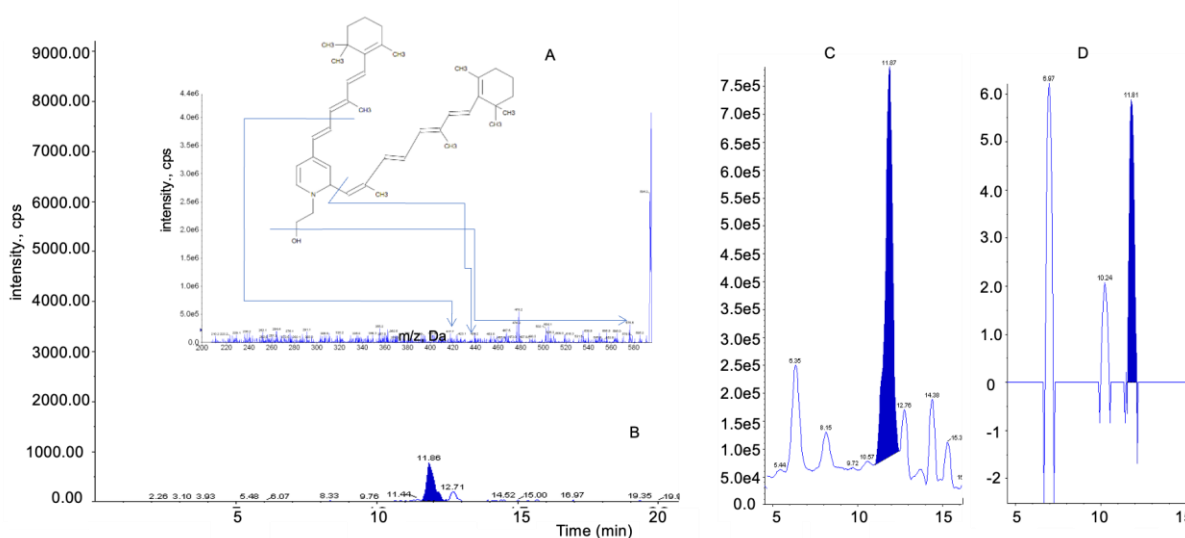


Retinal pigment epithelium in human donor eyes contains higher levels of bisretinoids including A2E in periphery than macula

Ankita Kotnala¹, Srinivasan Senthilkumari², Gong Wu³, Thomas Gordon Stewart³, Christine A. Curcio⁴, Nabanita Halder¹, Sundararajan Baskar Singh⁵, Atul Kumar⁶, Thirumurthy Velpandian^{1*}

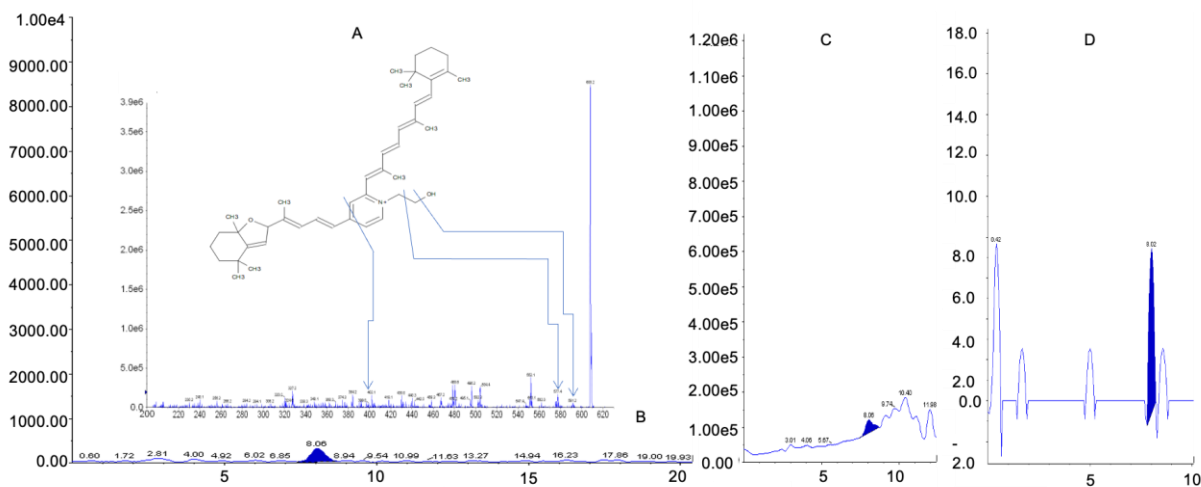
¹Ocular Pharmacology & Pharmacy Division, All India Institute of Medical Sciences, New Delhi, India; ²Department of Ocular Pharmacology, Aravind Medical Research Foundation (AMRF), Dr. G. Venkataswamy Eye Research Institute, #1, Anna Nagar, Madurai -20, Tamilnadu, India; ³Department of Biostatistics, Vanderbilt University Medical Centre, Nashville, TN; ⁴Department of Ophthalmology and Visual Sciences, University of Alabama at Birmingham, Birmingham, AL; ⁵Department of Biophysics, All India Institute of Medical Sciences, New Delhi, India; ⁶Department of Ophthalmology, Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India.

Supplemental Information



Supplemental Figure 1. A) Shows the fragmentation pattern for A2DHPE at [M+H]⁺ 594.4, B) representative peak from ESI-MRM mode of A2DHPE at [M+H]⁺ 594.4/576.5, C) representative chromatogram from ESI-SIM mode of quantification [M+H]⁺ 594.4, D) APCI-MRM chromatogram for A2DHPE at [M+H]⁺ 594.4/576.5.

“Reprinted from J Chromatogr B Analyt Technol Biomed Life Sci, 1073, A Kotnala, S Senthilkumari, N Halder, A Kumar, T Velpandian, Microwave assisted synthesis for A2E and development of LC-ESI-MS method for quantification of ocular bisretinoids in human retina, 10-18, Copyright (2018), with permission from Elsevier.”



Supplemental Figure 2. A) Shows the fragmentation pattern for MFA2E at [M] 608.2, B) representative peak from ESI-MRM mode of MFA2E at [M] 608.2/ 591.2, C) representative chromatogram from ESI-SIM mode of quantification at [M] 608.2, D) APCI-MRM chromatogram for MFA2E at [M] 608.2/ 591.2 .

“Reprinted from J Chromatogr B Analyt Technol Biomed Life Sci, 1073, A Kotnala, S Senthilkumari, N Halder, A Kumar , T Velpandian, Microwave assisted synthesis for A2E and development of LC-ESI-MS method for quantification of ocular bisretinoids in human retina, 10-18, Copyright (2018), with permission from Elsevier.”

Supplemental Table 1: Macula vs peripheral retina comparison of bisretinoids (measured as EEEQ)

| Variable | Level | N | Median (IQR) | <i>p</i> (KW) |
|--|--------------|----------|---------------------|----------------------|
| A2E | P | 252 | 0.27 (0.13, 0.63) | < 0.0001 |
| | M | 241 | 0.05 (0.03, 0.09) | |
| A2GPE | P | 251 | 2.10 (1.44, 3.12) | < 0.0001 |
| | M | 238 | 0.35 (0.24, 0.49) | |
| A2DHPE | P | 254 | 0.43 (0.21, 1.08) | < 0.0001 |
| | M | 240 | 0.05 (0.03, 0.10) | |
| Monofuran-A2E | P | 253 | 2.30 (1.37, 3.58) | < 0.0001 |
| | M | 240 | 0.29 (0.14, 0.57) | |
| periphery; M, macula; IQR, interquartile range; KW, Kruskal-Wallis | | | | |

Supplemental Table 2: Levels of bisretinoids (measured as EEEQ), in two age groups

| Bisretinoid | Overall | | <60 years | | ≥60 years | |
|--------------------------|----------------|---------------------|---------------------|---------------------|------------------|---------------------|
| | N | Median (IQR) | N | Median (IQR) | N | Median (IQR) |
| A2E | 234 | 5.0 (2.7, 10.2) | 124 | 3.8 (2.2, 6.8) | 110 | 7.5 (3.6, 16.5) |
| A2GPE | 230 | 5.9 (3.5, 9.2) | 120 | 4.8 (3.0, 7.5) | 110 | 7.1 (4.5, 10.7) |
| A2DHPE | 233 | 8.3 (4.5, 16.6) | 122 | 6.4 (3.6, 10.7) | 111 | 12.0 (6.1, 27.5) |
| Monofuran-A2E | 233 | 8.8 (3.6, 17.5) | 123 | 8.0 (2.4, 21.0) | 110 | 9.6 (5.2, 14.4) |
| IQR, interquartile range | | | | | | |

Supplemental Table 3: Left eye vs right eye levels of bisretinoids (measured as EEEQ)

| Variable Eye | Level | N | Median (IQR) | <i>p</i> (KW) |
|---|--------------|----------|---------------------|----------------------|
| A2E | LE | 228 | 0.12 (0.05, 0.30) | 0.5888 |
| | RE | 265 | 0.11 (0.04, 0.30) | |
| A2GPE | LE | 225 | 0.79 (0.36, 2.14) | 0.6621 |
| | RE | 264 | 0.85 (0.34, 2.17) | |
| A2DHPE | LE | 228 | 0.16 (0.05, 0.47) | 0.6150 |
| | RE | 266 | 0.15 (0.05, 0.44) | |
| monofuran-A2E | LE | 227 | 0.90 (0.33, 2.27) | 0.9805 |
| | RE | 266 | 0.97 (0.28, 2.61) | |
| LE, left eye; RE, right eye; IQR, interquartile range; KW, Kruskal-Wallis | | | | |

Supplemental Table 4: Male vs Female comparison of bisretinoids (measured as EEEQ)

| Variable | Level | N | Median (IQR) | <i>p</i> (KW) |
|--|--------------|----------|---------------------|----------------------|
| Sex | M | | | |
| | F | | | |
| A2E | M | 280 | 0.11 (0.05, 0.27) | 0.0540 |
| | F | 213 | 0.14 (0.05, 0.38) | |
| A2GPE | M | 278 | 0.82 (0.35, 2.13) | 0.8826 |
| | F | 211 | 0.82 (0.35, 2.20) | |
| A2DHPE | M | 279 | 0.14 (0.05, 0.39) | 0.0709 |
| | F | 215 | 0.20 (0.05, 0.56) | |
| monofuran-A2E | M | 277 | 0.89 (0.31, 2.39) | 0.5221 |
| | F | 216 | 1.13 (0.29, 2.53) | |
| M, Male; F, Female; IQR, interquartile range; KW, Kruskal-Wallis | | | | |