

Supplementary Material

A Comprehensive HPTLC-Based Analysis of the Impacts of Temperature on the Chemical Properties and Antioxidant Activity of Honey

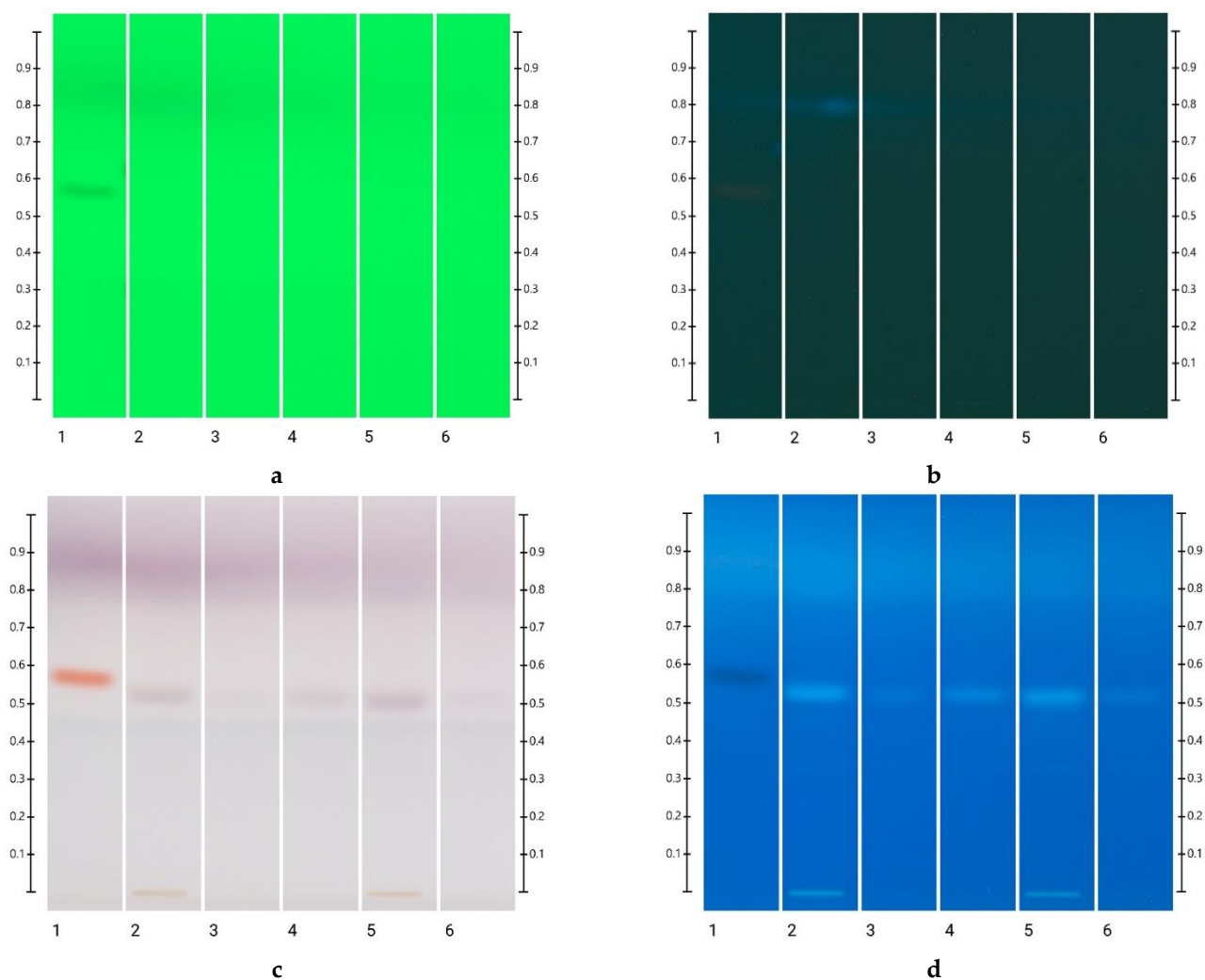
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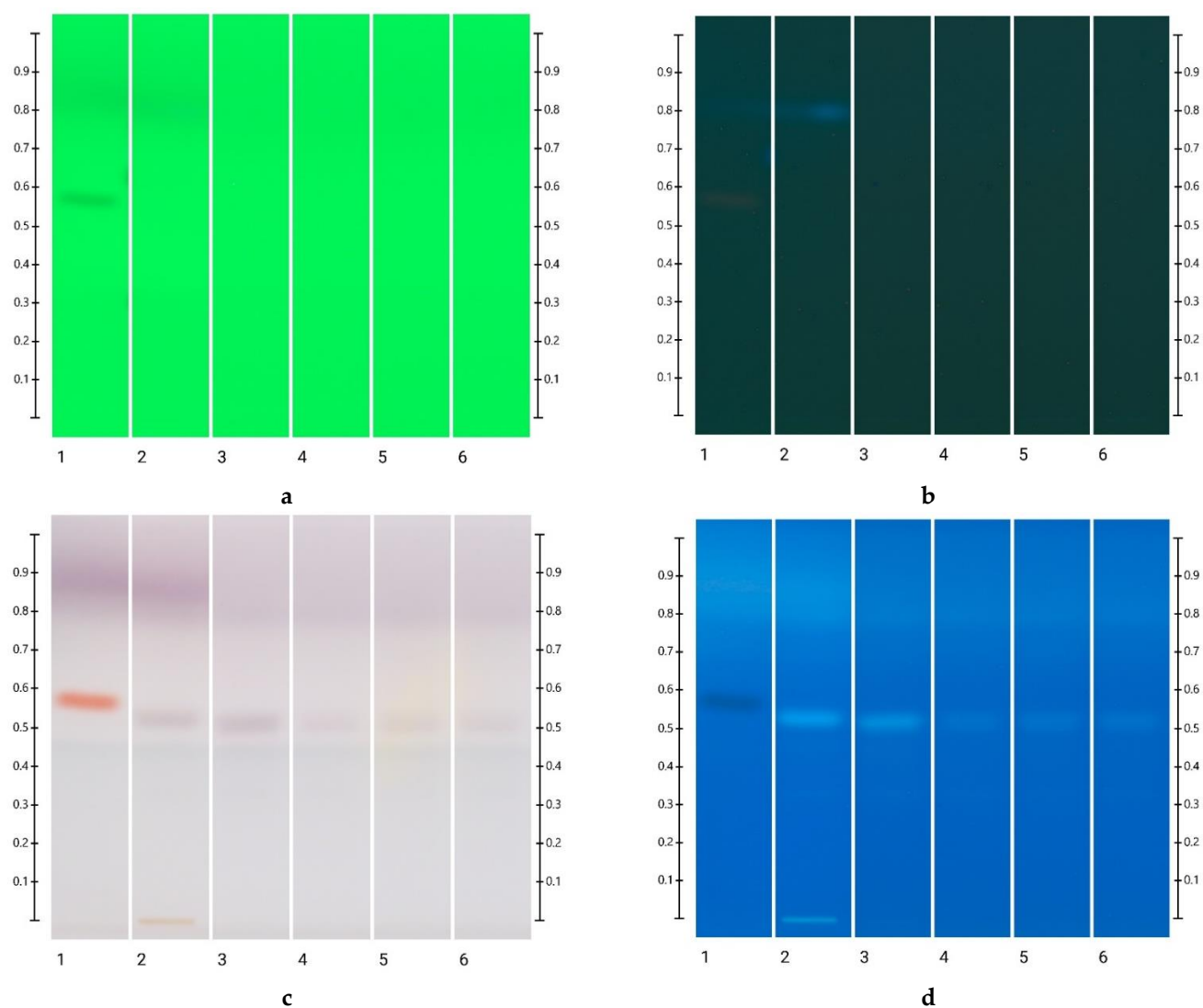
² Division of Pharmacy, School of Allied Health, University of Western Australia, Perth 6009, Australia

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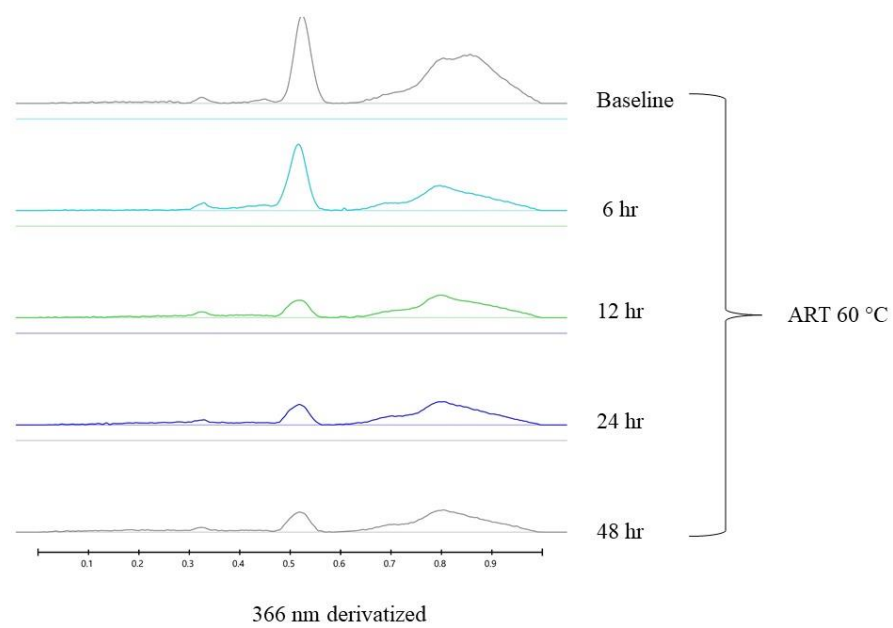
* Correspondence: connie.locher@uwa.edu.au



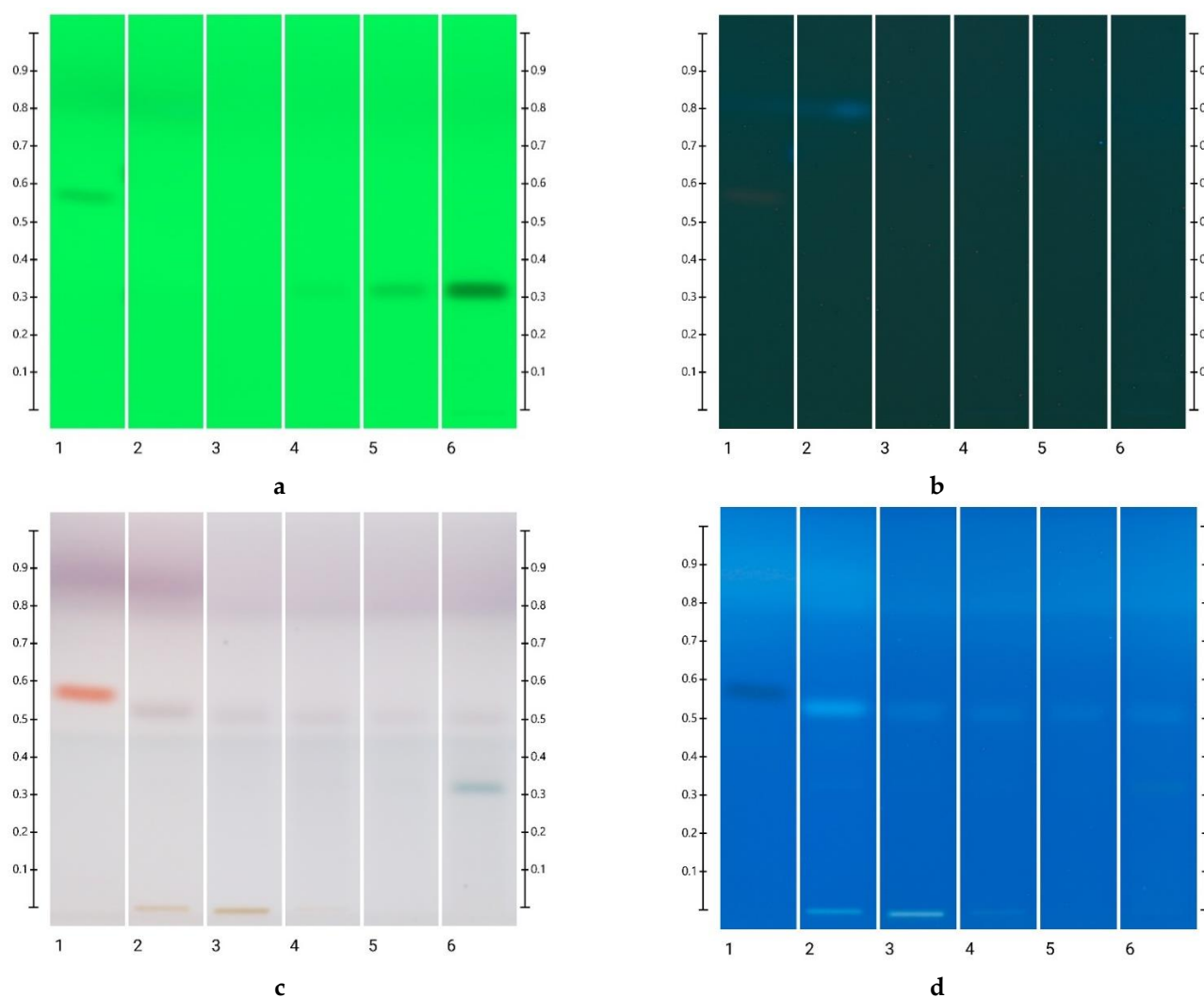
Supplementary Figure S1. ART short-term storage at 40 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1—4,5,7-trihydroxyflavanon, Track 2—0 h, Track 3—6 h, Track 4—12 h, Track 5—24 h, and Track 6—48 h; 5 μ L of each honey extract respectively.



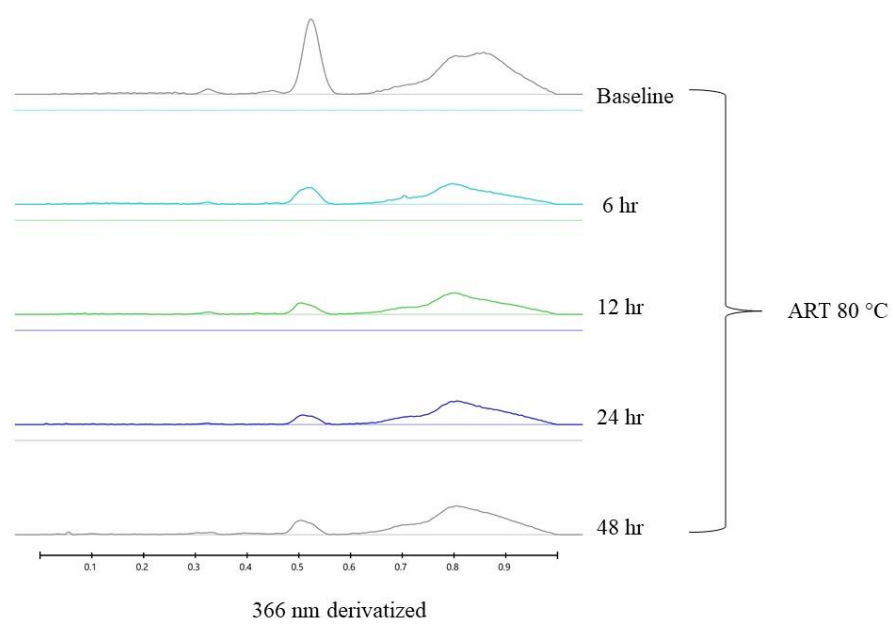
Supplementary Figure S2. ART short-term storage at 60 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanone, Track 2 – 0 h, Track 3 – 6 h, Track 4 – 12 h, Track 5 – 24 h, and Track 6 – 48 h; 5 μ L of each honey extract respectively.



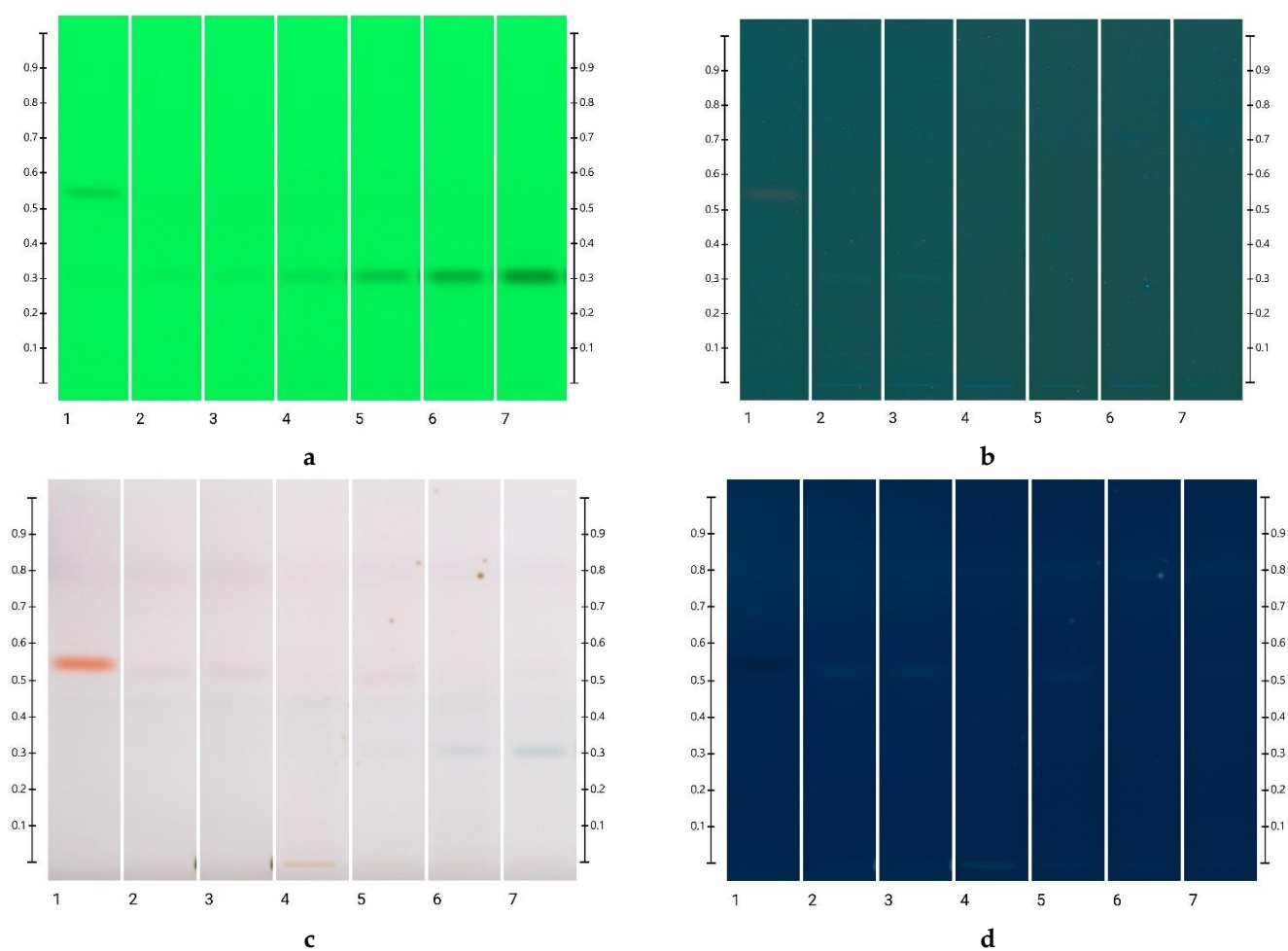
Supplementary Figure S3. Organic honey extract chromatograms (offset Y-axis values).



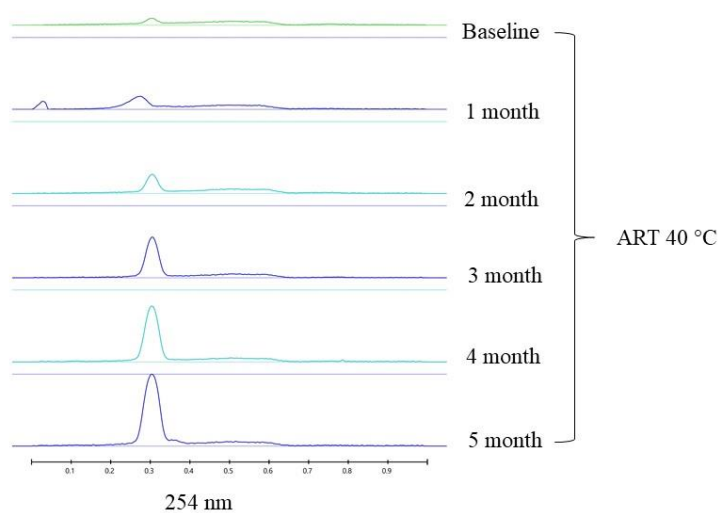
Supplementary Figure S4. ART short-term storage at 80 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanon, Track 2 – 0 h, Track 3 – 6 h, Track 4 – 12 h, Track 5 – 24 h, and Track 6 – 48 h; 5 μ L of each honey extracts respectively.



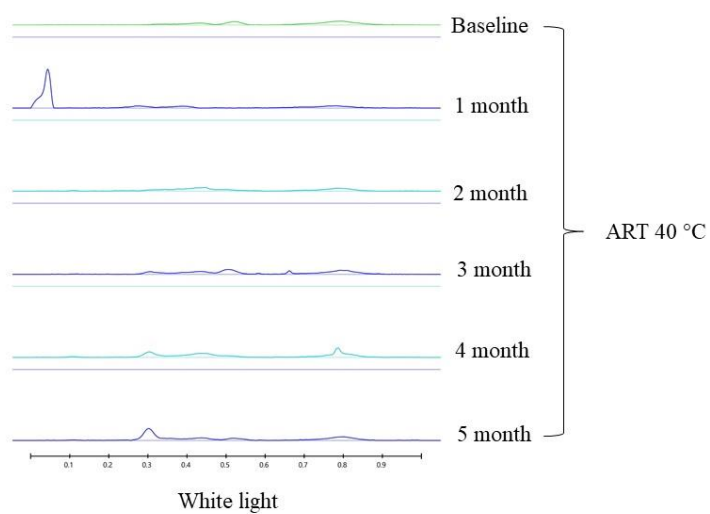
Supplementary Figure S5. Organic honey extract chromatograms (offset Y-axis values).



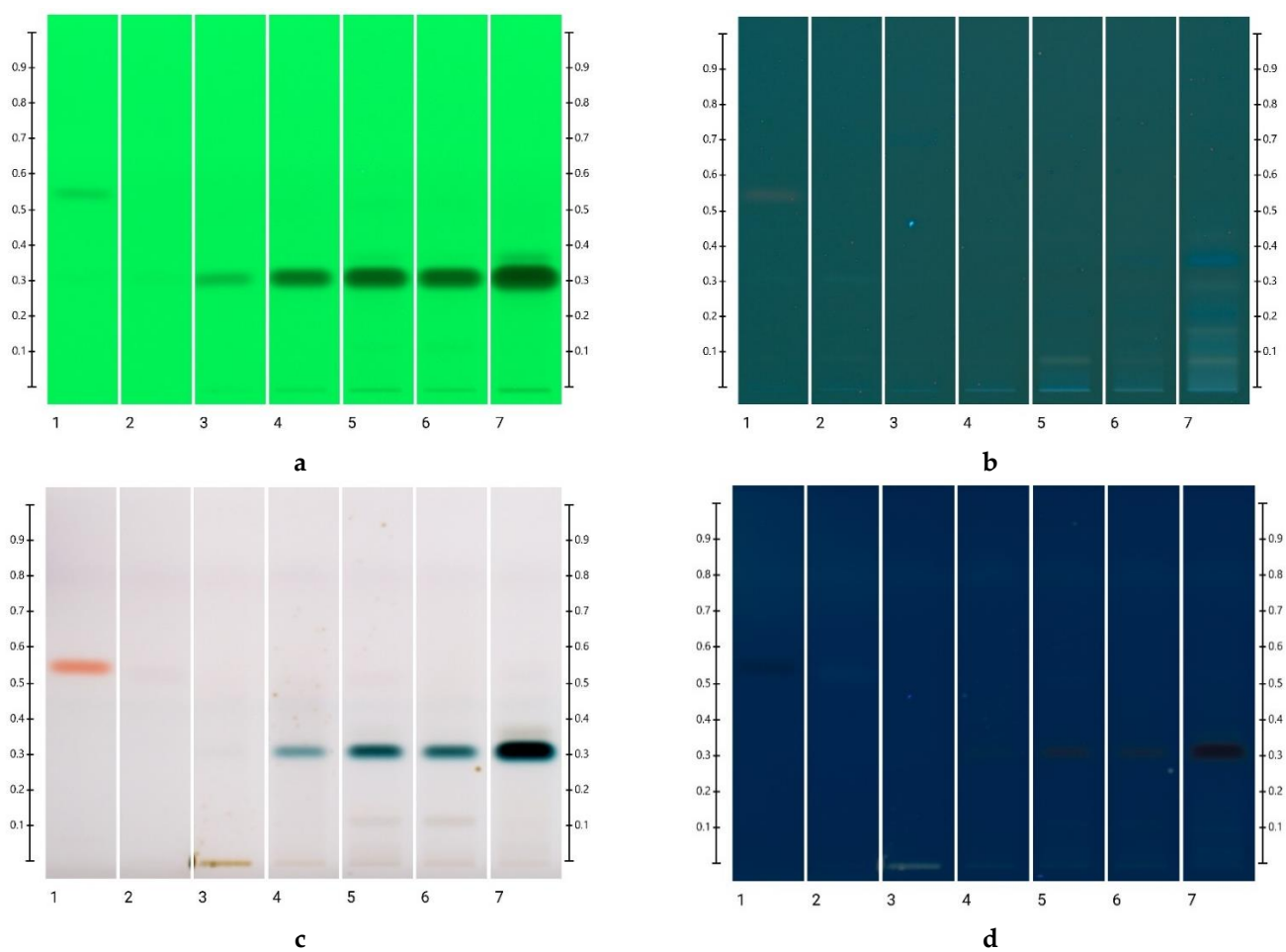
Supplementary Figure S6. ART long-term storage at 40 °C Images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1—4,5,7-trihydroxyflavanon, Track 2— 0 h, Track 3— 1 month, Track 4— 2 month, Track 5— 3 month, Track 6— 4 month, and Track 7— 5 month; 5 μ L of each honey extract respectively.



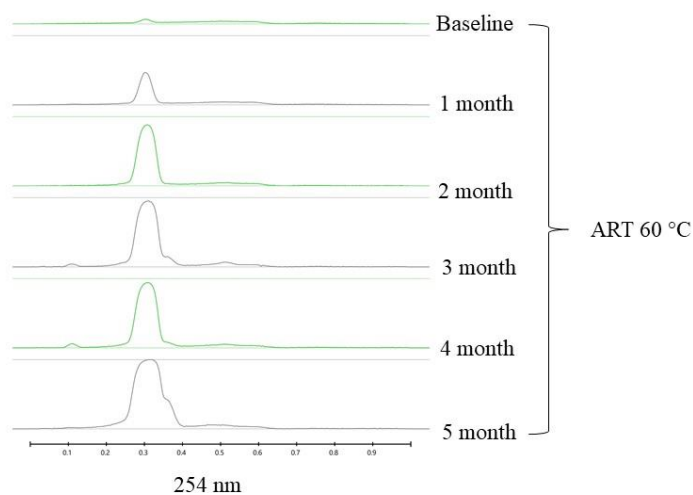
Supplementary Figure S7. Organic honey extract chromatograms.



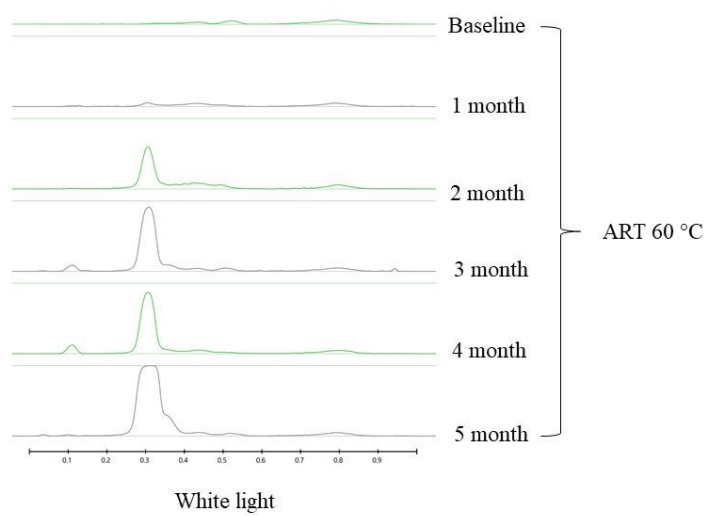
Supplementary Figure S8. Organic honey extract chromatograms.



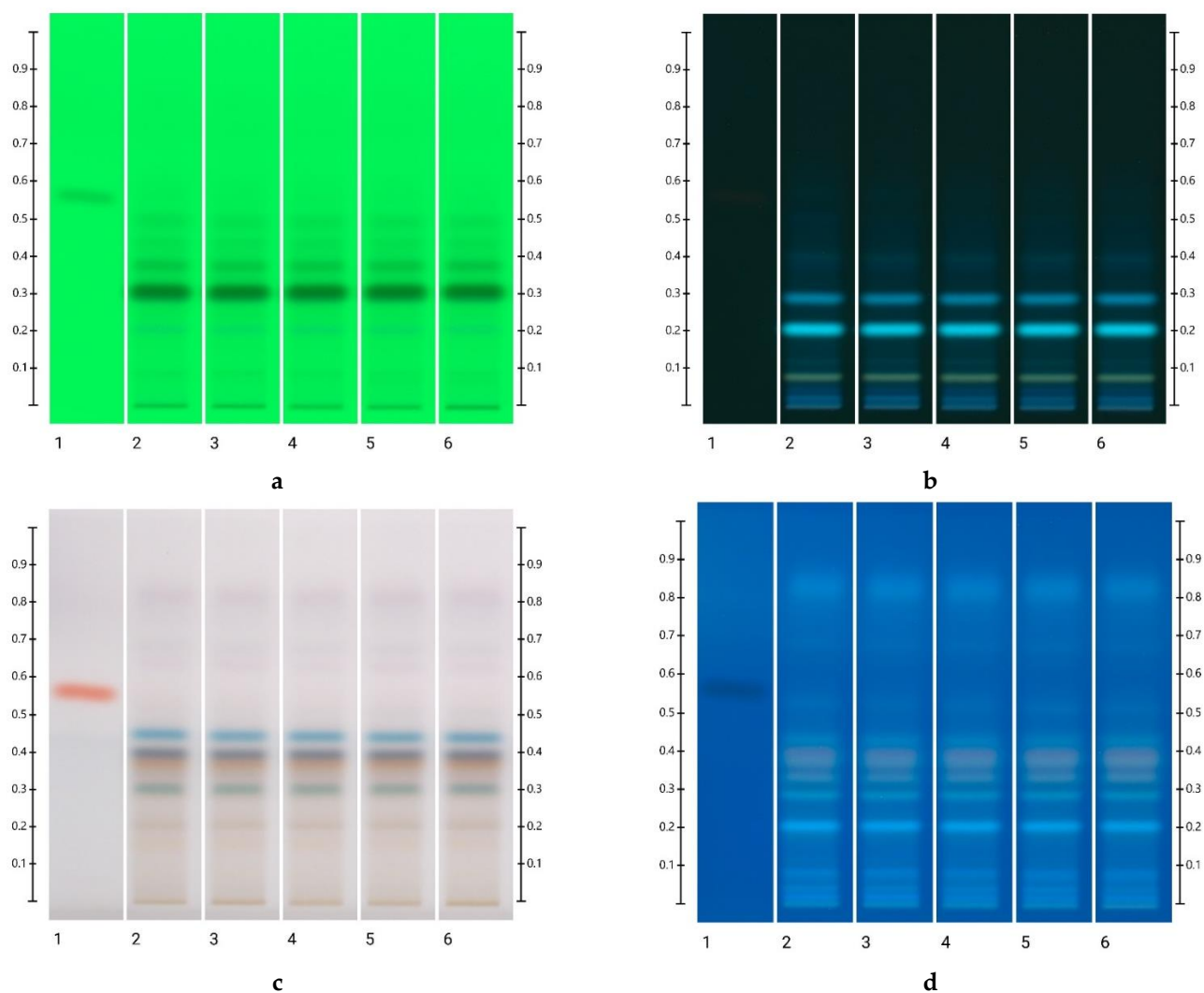
Supplementary Figure S9. ART long-term storage at 60 °C Images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1—4,5,7-trihydroxyflavanon, Track 2— 0 h, Track 3— 1 month, Track 4— 2 month, Track 5— 3 month, Track 6— 4 month, and Track 7— 5 month; 5 μ L of each honey extract respectively.



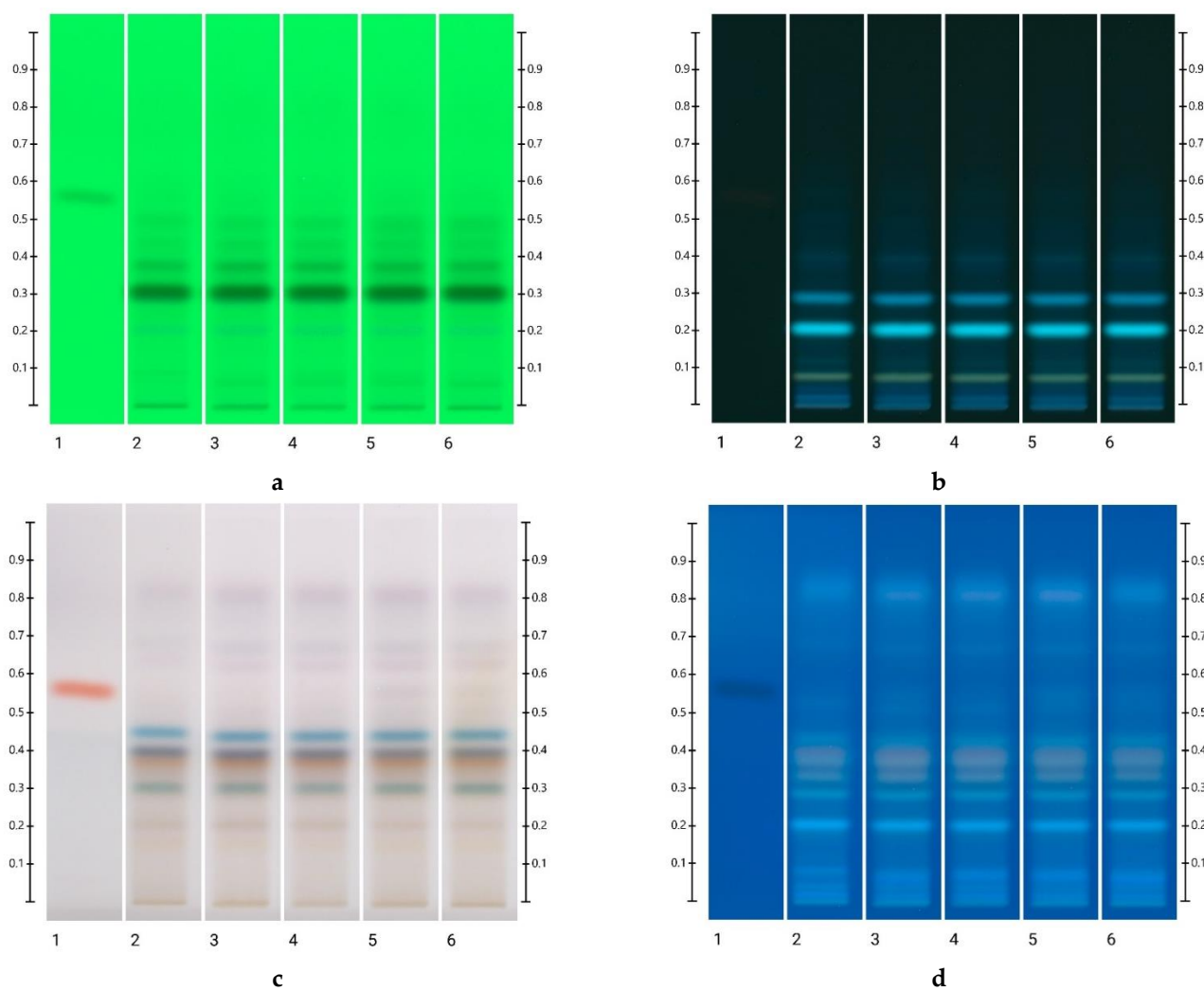
Supplementary Figure S10. Organic honey extract chromatograms (offset Y-axis values).



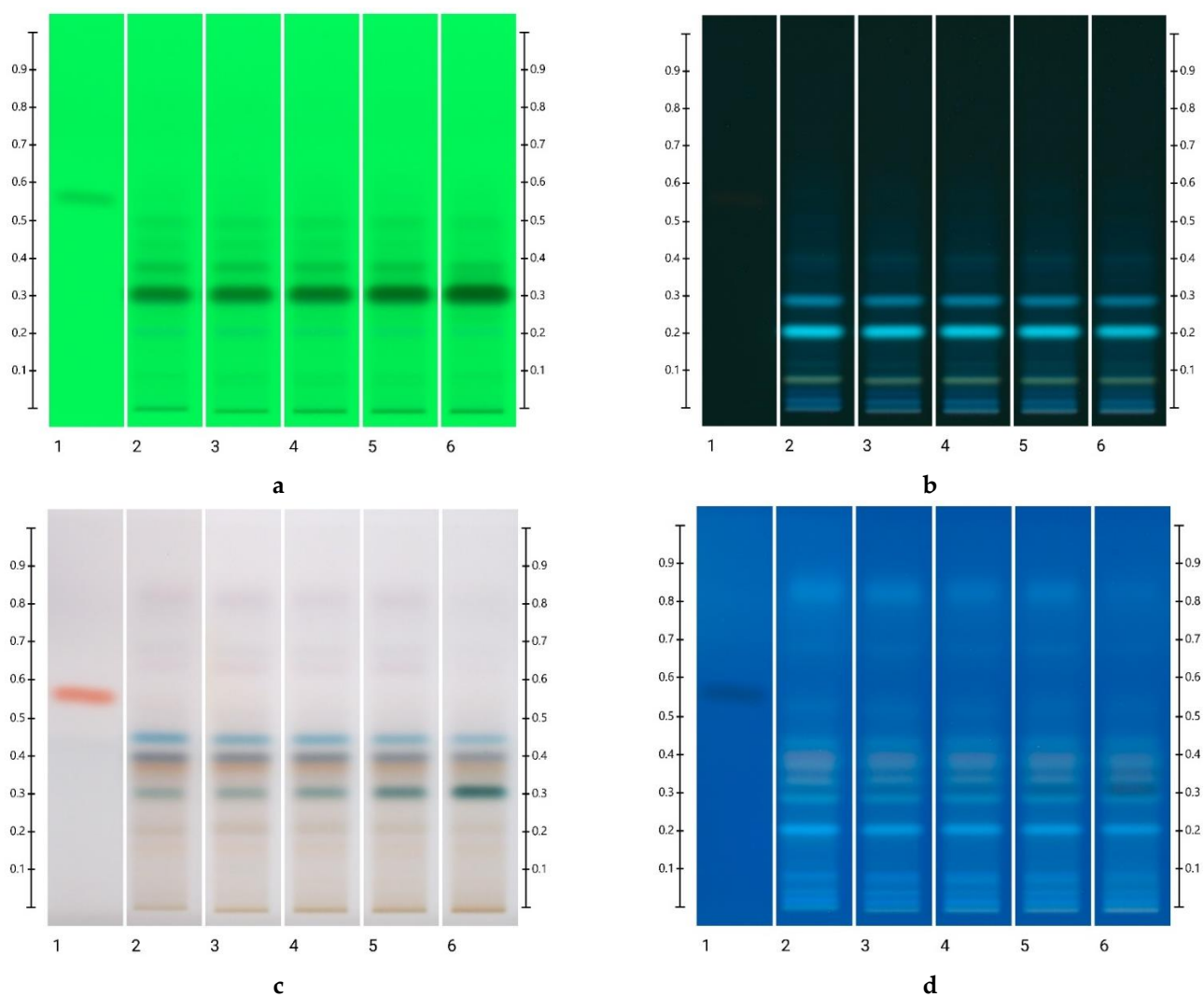
Supplementary Figure S11. Organic honey extract chromatograms (offset Y-axis values).



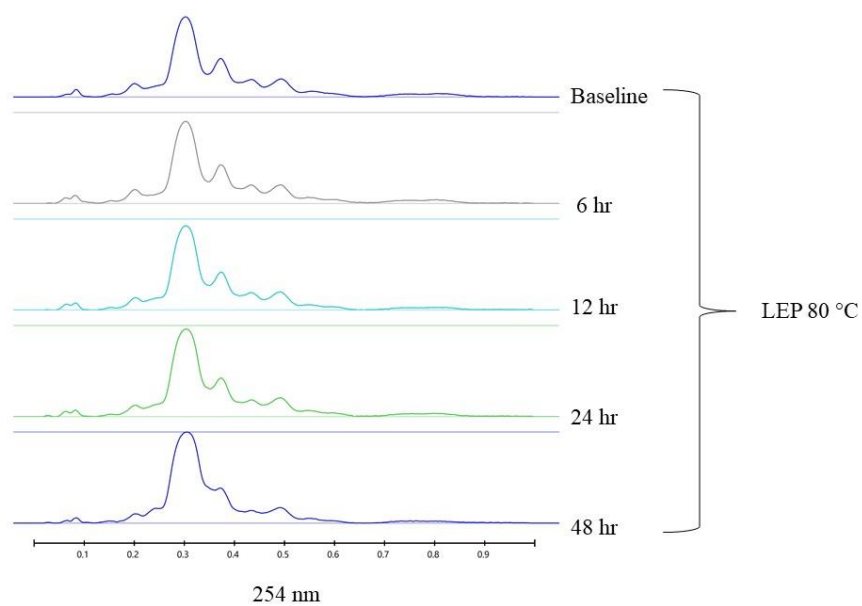
Supplementary Figure S12. LEP short-term storage at 40 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanone, Track 2 – 0 h, Track 3 – 6 h, Track 4 – 12 h, Track 5 – 24 h, and Track 6 – 48 h; 5 μ L of each honey extract respectively.



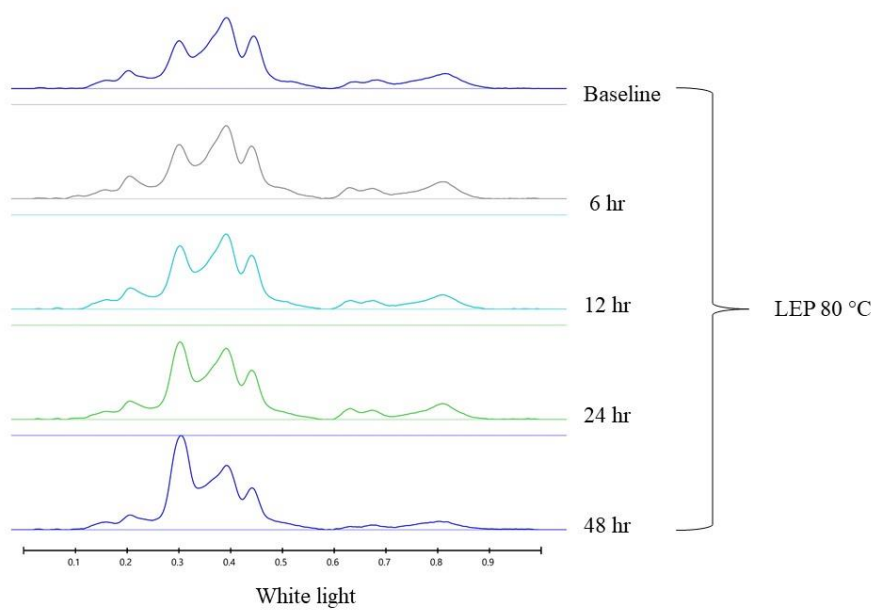
Supplementary Figure S13. LEP short-term storage at 60 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanon, Track 2– 0 h, Track 3– 6 h, Track 4– 12 h, Track 5– 24 h, and Track 6– 48 h; 5 μ L of each honey extract respectively.



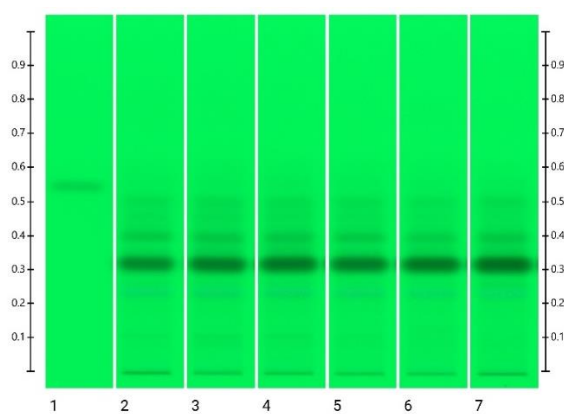
Supplementary Figure S14. LEP short-term storage at 80 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanon, Track 2– 0 h, Track 3– 6 h, Track 4– 12 h, Track 5– 24 h, and Track 6– 48 h; 5 μ L of each honey extract respectively.



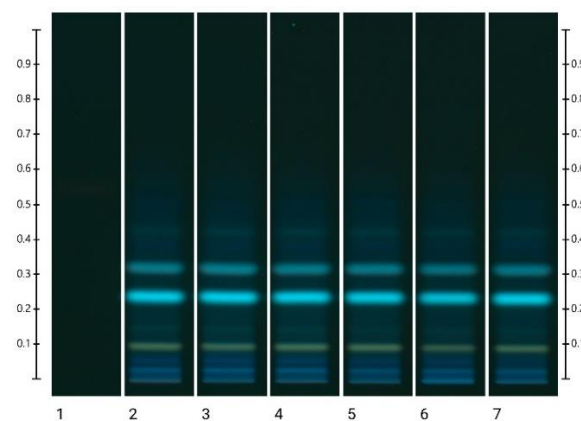
Supplementary Figure S15. Organic honey extract chromatograms (offset Y-axis values).



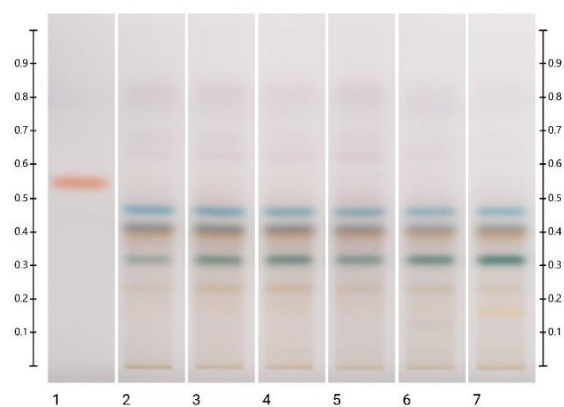
Supplementary Figure S16. Organic honey extract chromatograms (offset Y-axis values).



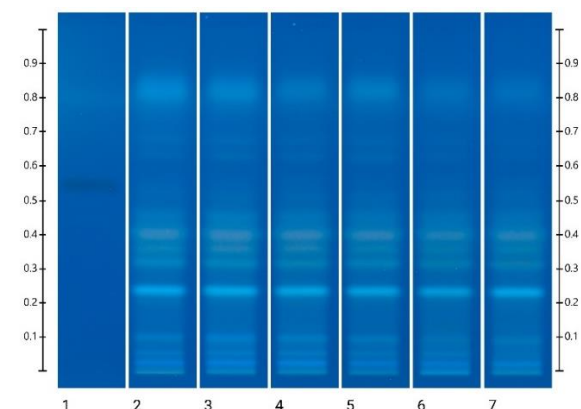
a



b

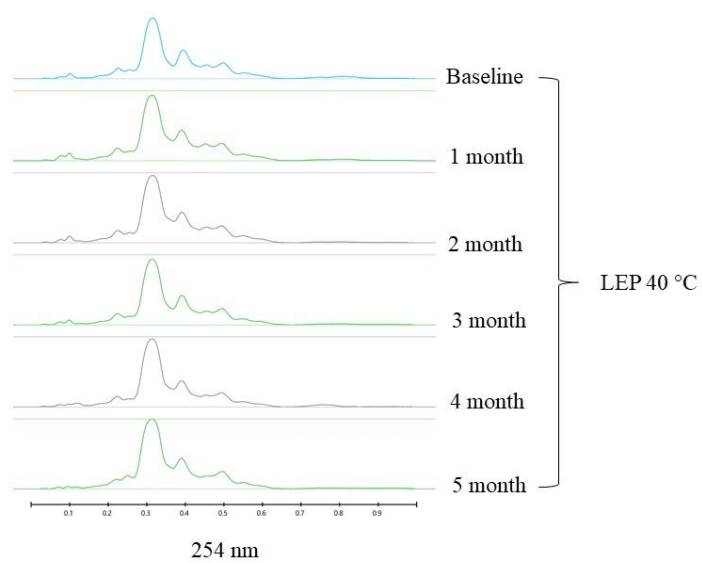


c

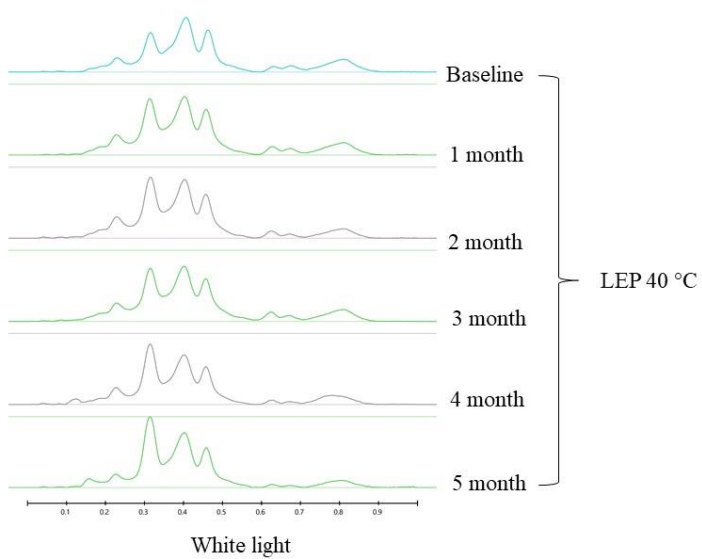


d

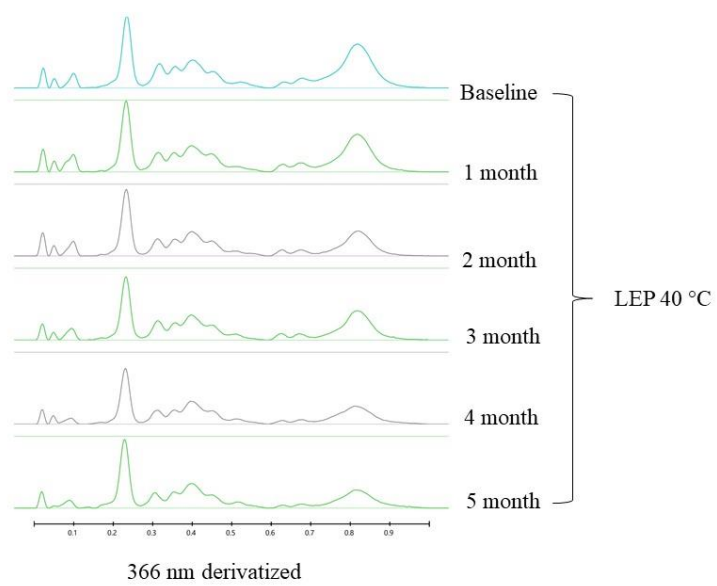
Supplementary Figure S17. LEP long-term storage at 40 °C Images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1—4,5,7-trihydroxyflavanon, Track 2— 0 h, Track 3— 1 month, Track 4— 2 month, Track 5— 3 month, Track 6— 4 month, and Track 7— 5 month; 5 μ L of each honey extract respectively.



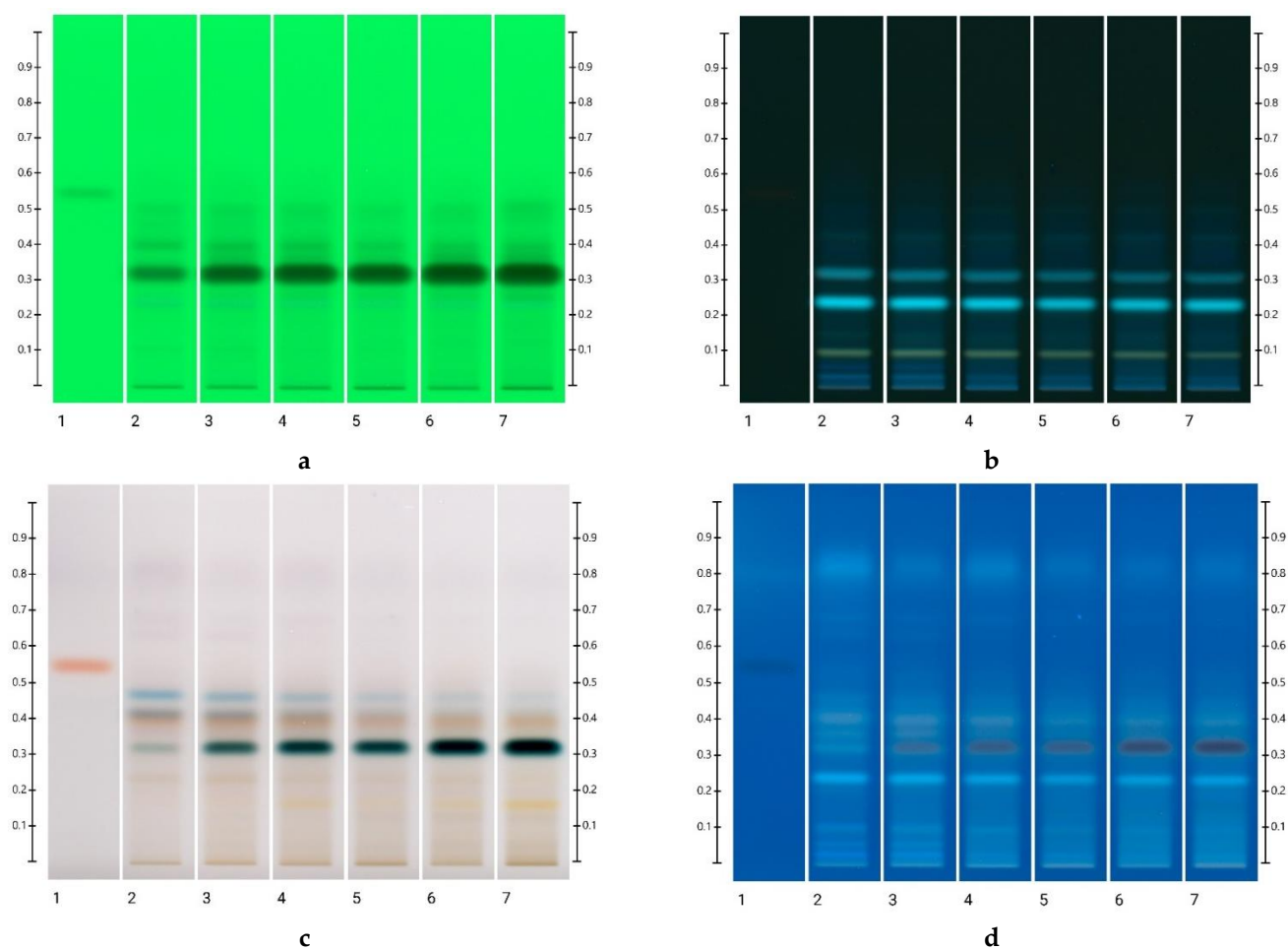
Supplementary Figure S18. Organic honey extract chromatograms (offset Y-axis values).



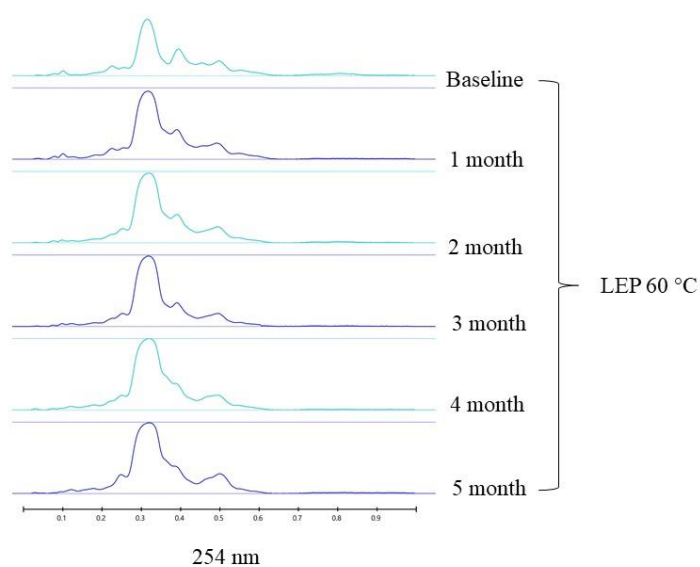
Supplementary Figure S19. Organic honey extract chromatograms (offset Y-axis values).



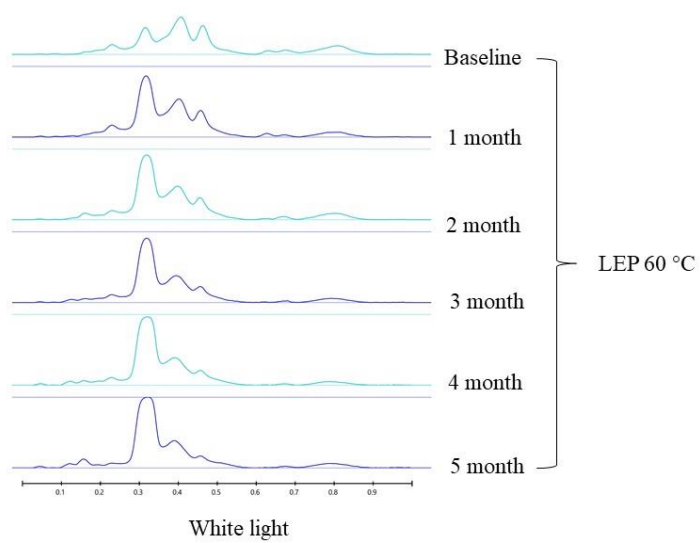
Supplementary Figure S20. Organic honey extract chromatograms (offset Y-axis values).



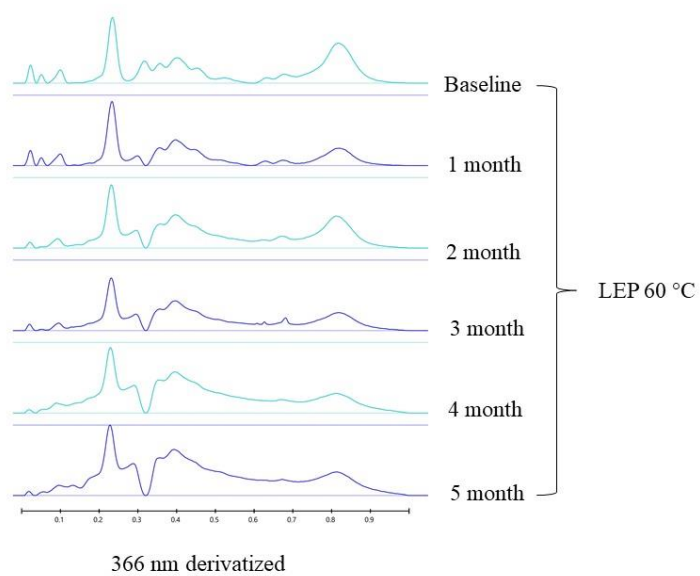
Supplementary Figure S21. LEP long-term storage at 60 °C Images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanon, Track 2 – 0 h, Track 3 – 1 month, Track 4 – 2 month, Track 5 – 3 month, Track 6 – 4 month, and Track 7 – 5 month; 5 μ L of each honey extract respectively.



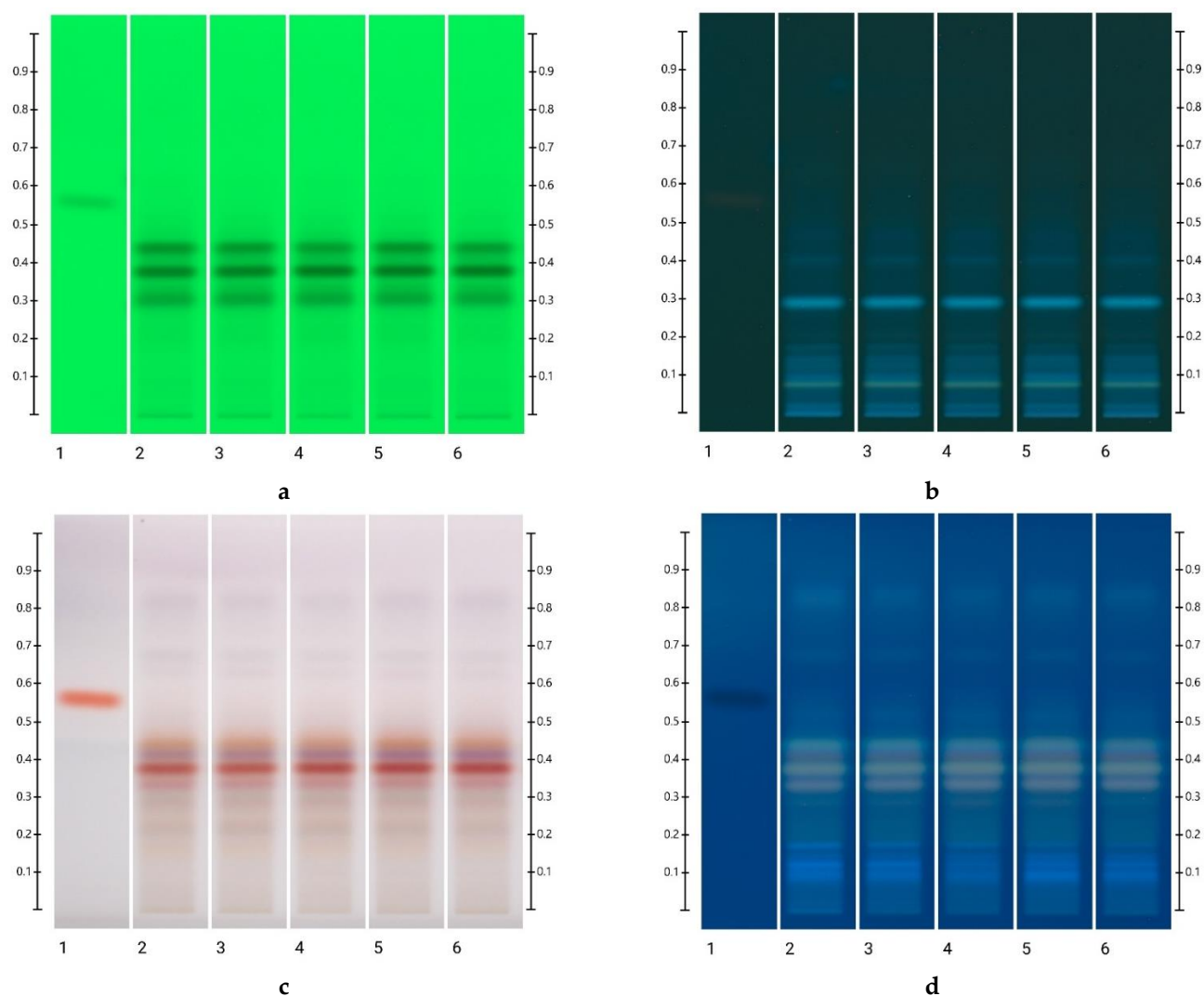
Supplementary Figure S22. Organic honey extract chromatograms (offset Y-axis values).



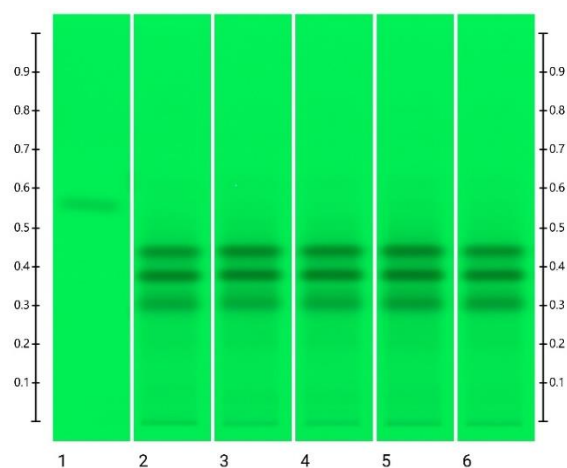
Supplementary Figure S23. Organic honey extract chromatograms (offset Y-axis values).



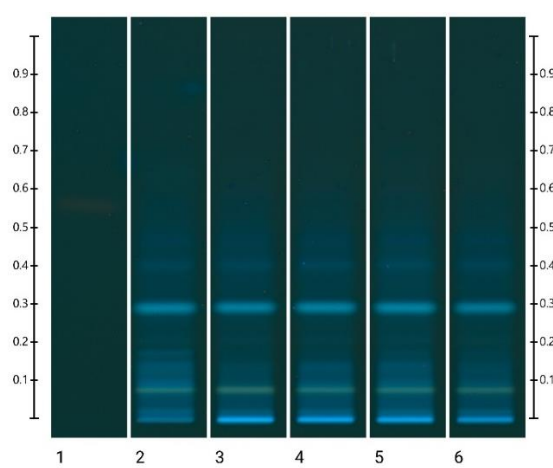
Supplementary Figure S24. Organic honey extract chromatograms (offset Y-axis values).



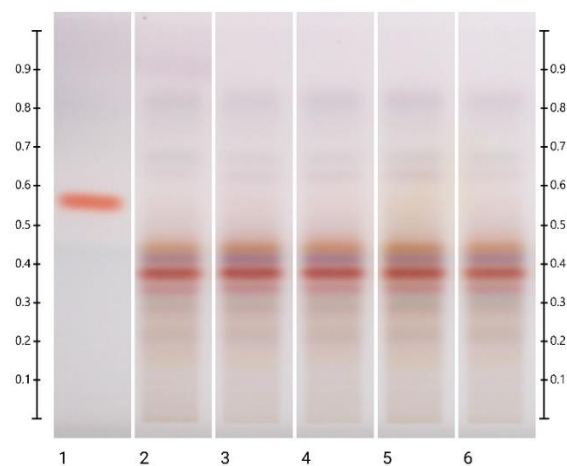
Supplementary Figure S25. MAR short-term storage at 40 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanon, Track 2 – 0 h, Track 3 – 6 h, Track 4 – 12 h, Track 5 – 24 h, and Track 6 – 48 h; 5 μ L of each honey extract respectively.



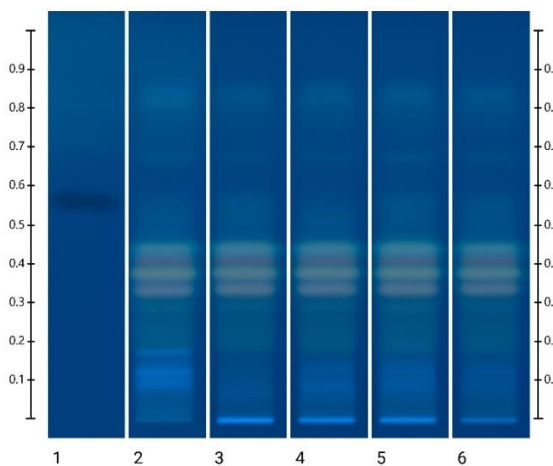
a



b

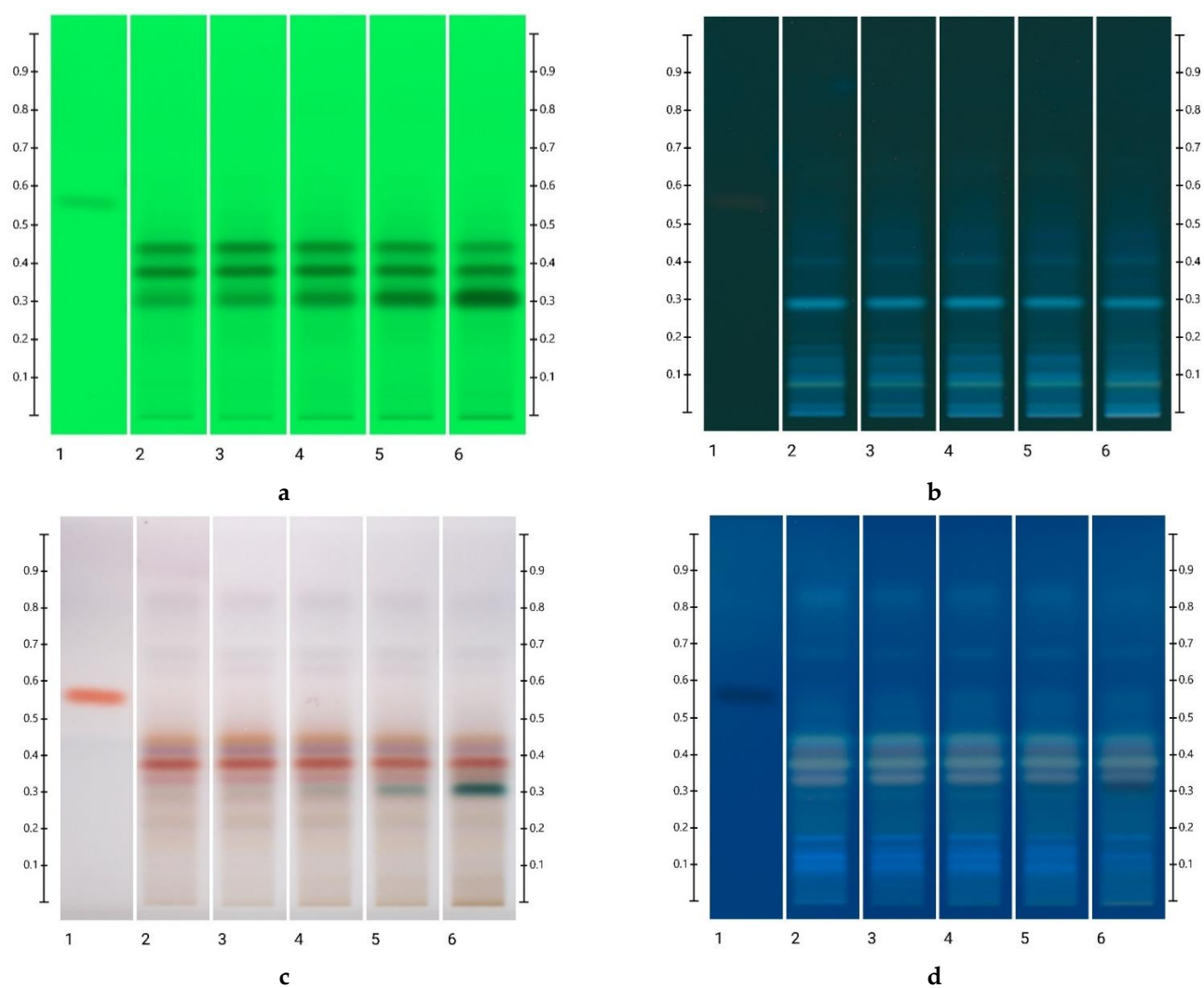


c

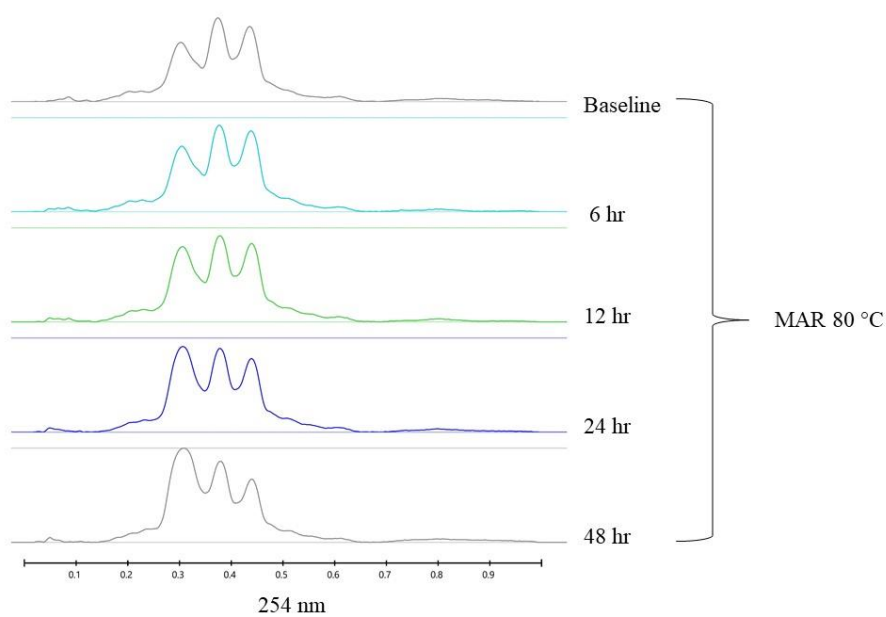


d

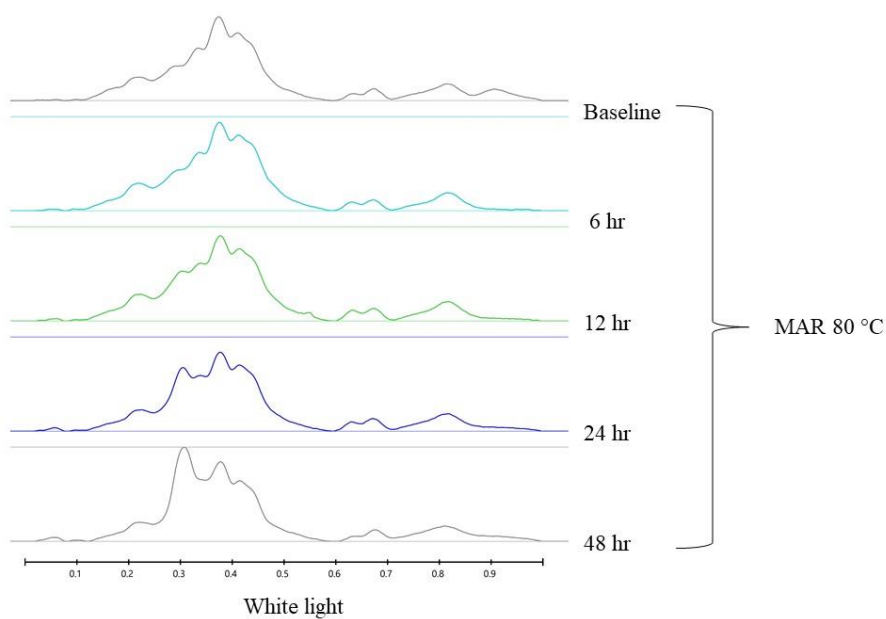
Supplementary Figure S26. MAR short-term storage at 60 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanon, Track 2— 0 h, Track 3— 6 h, Track 4— 12 h, Track 5— 24 h, and Track 6— 48 h; 5 µL of each honey extract respectively.



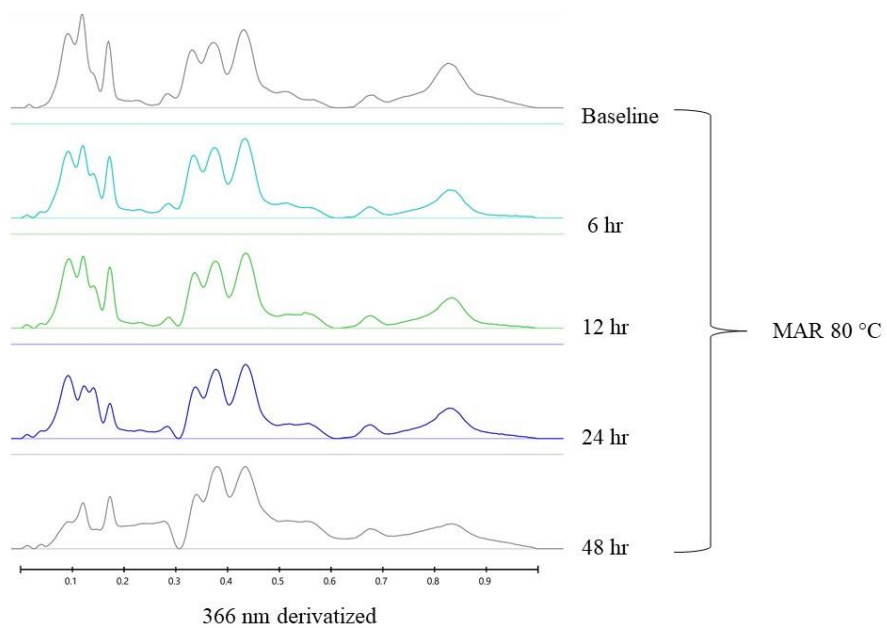
Supplementary Figure S27. MAR short-term storage at 80 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanon, Track 2 – 0 h, Track 3 – 6 h, Track 4 – 12 h, Track 5 – 24 h, and Track 6 – 48 h; 5 μ L of each honey extract respectively.



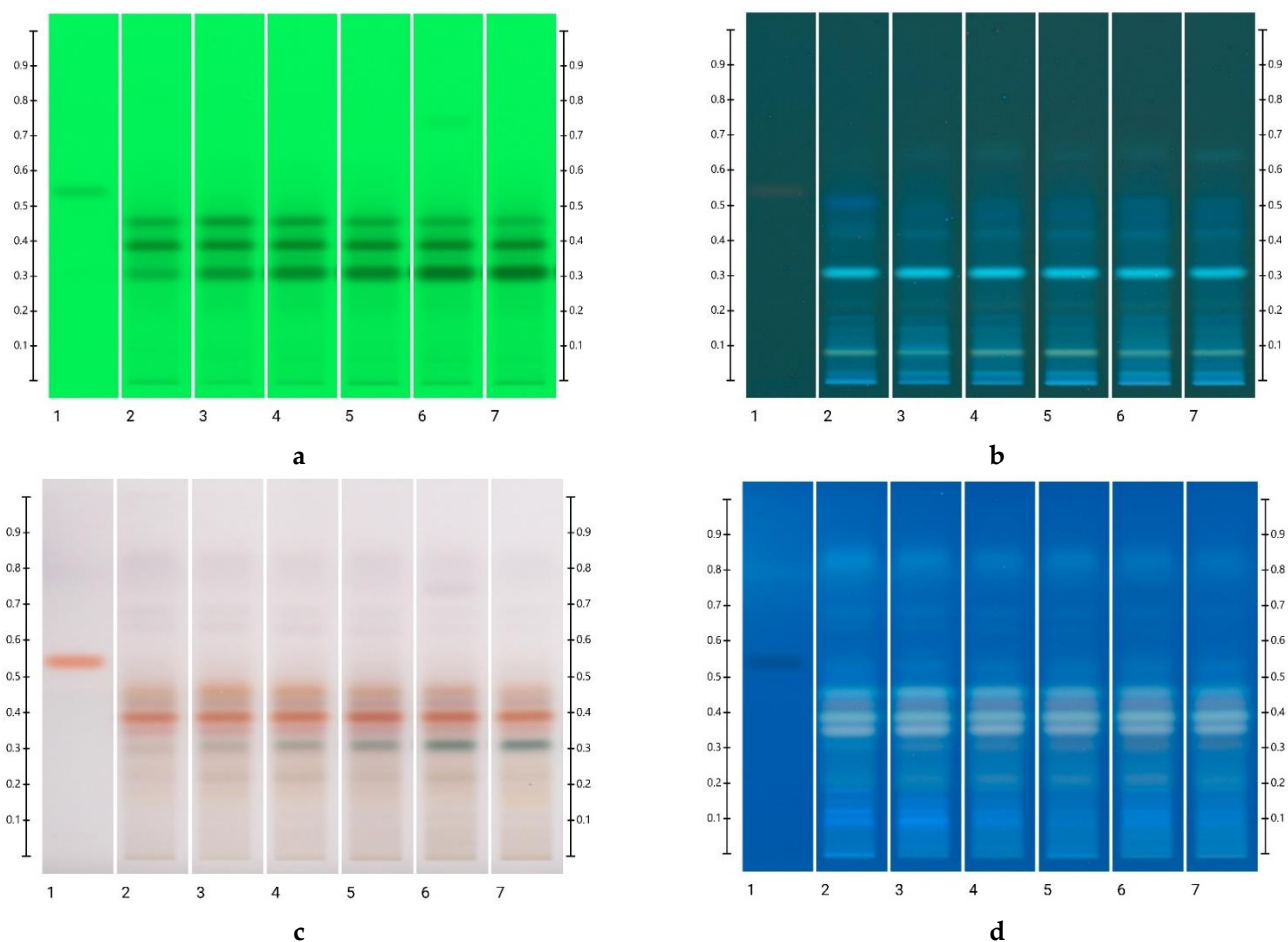
Supplementary Figure S28. Organic honey extract chromatograms (offset Y-axis values).



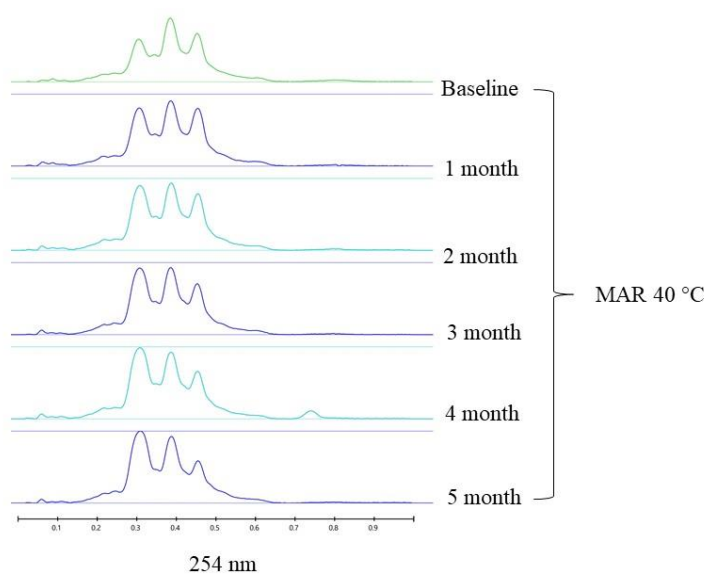
Supplementary Figure S29. Organic honey extract chromatograms (offset Y-axis values).



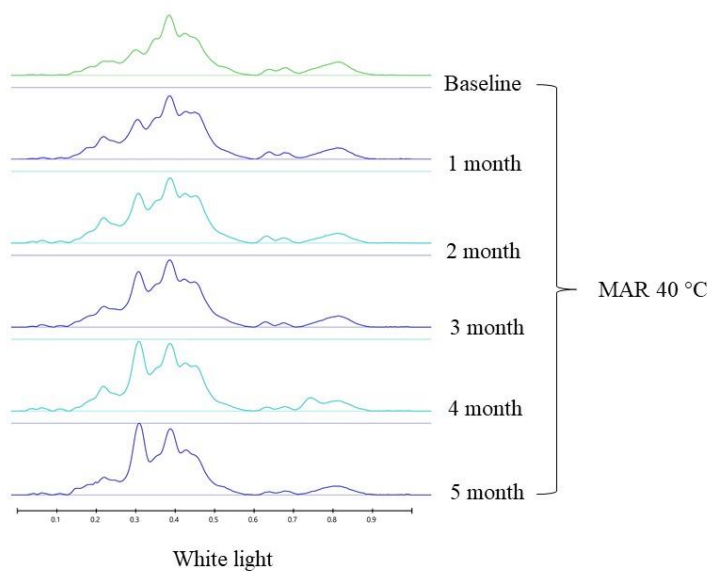
Supplementary Figure S30. Organic honey extract chromatograms (offset Y-axis values).



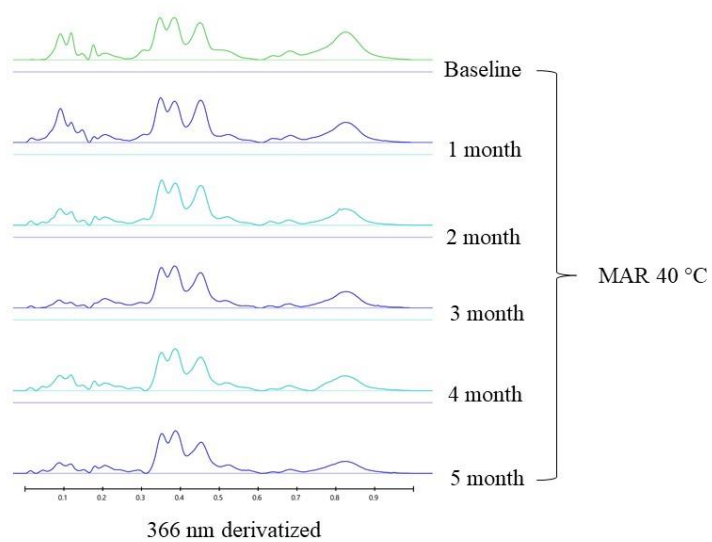
Supplementary Figure S31. MAR long-term storage at 40 °C Images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1—4,5,7-trihydroxyflavanon, Track 2— 0 h, Track 3— 1 month, Track 4— 2 month, Track 5— 3 month, Track 6— 4 month, and Track 7— 5 month; 5 μ L of each honey extract respectively.



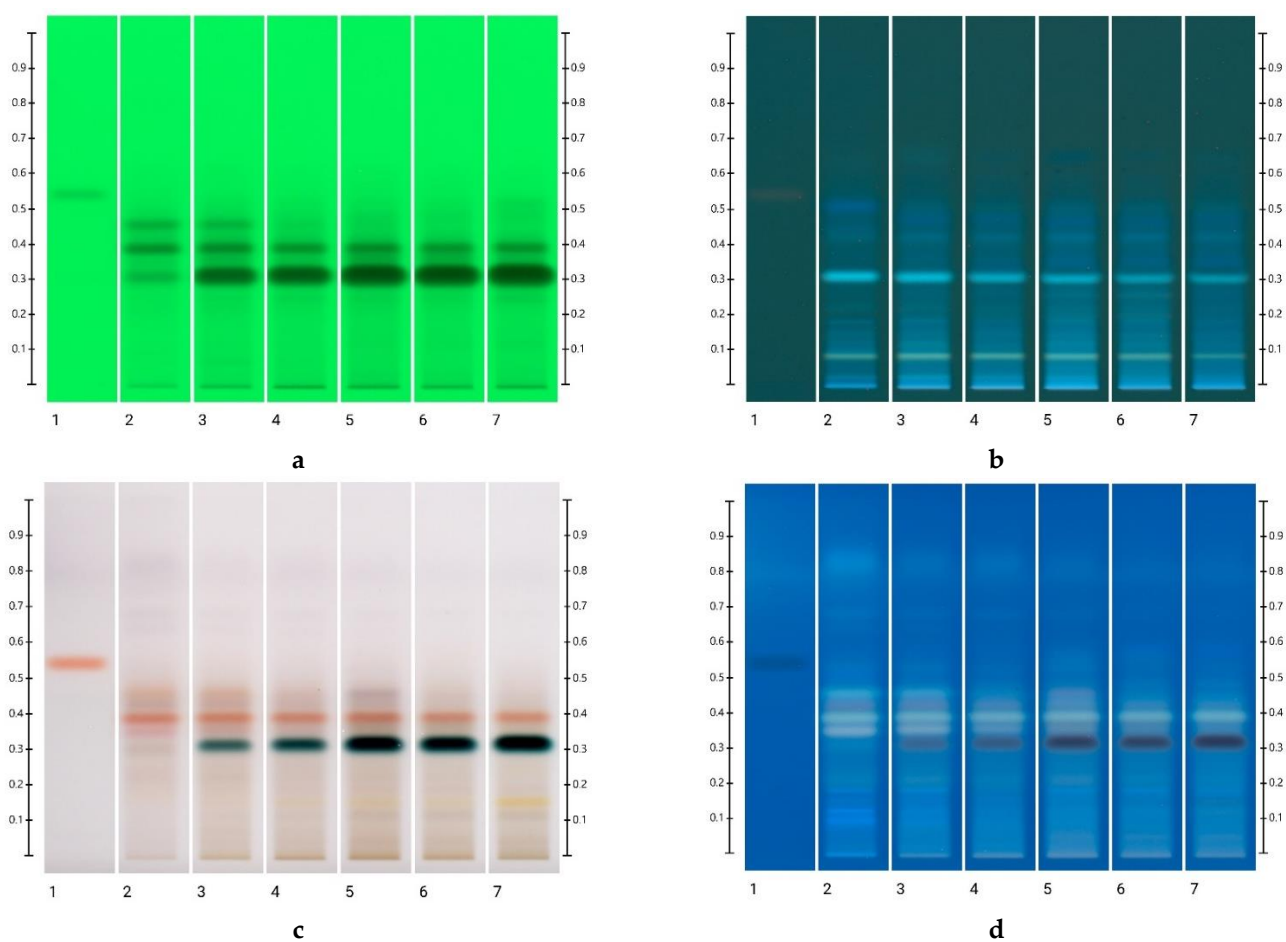
Supplementary Figure S32. Organic honey extract chromatograms (offset Y-axis values).



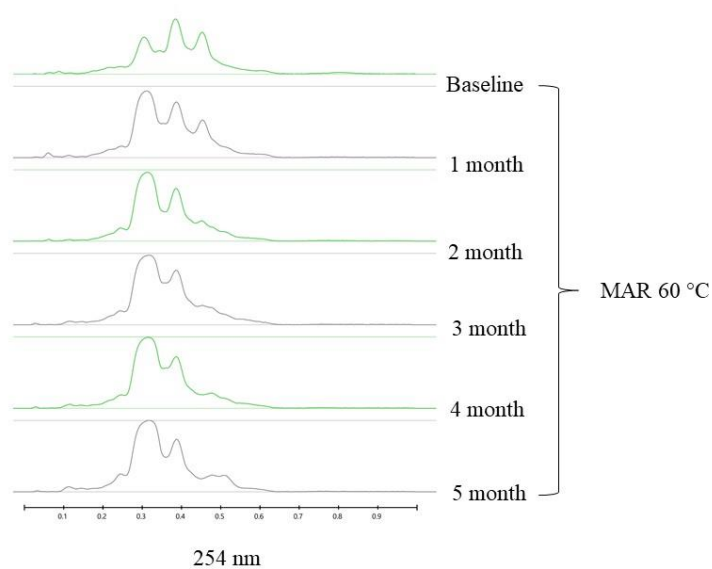
Supplementary Figure S33. Organic honey extract chromatograms (offset Y-axis values).



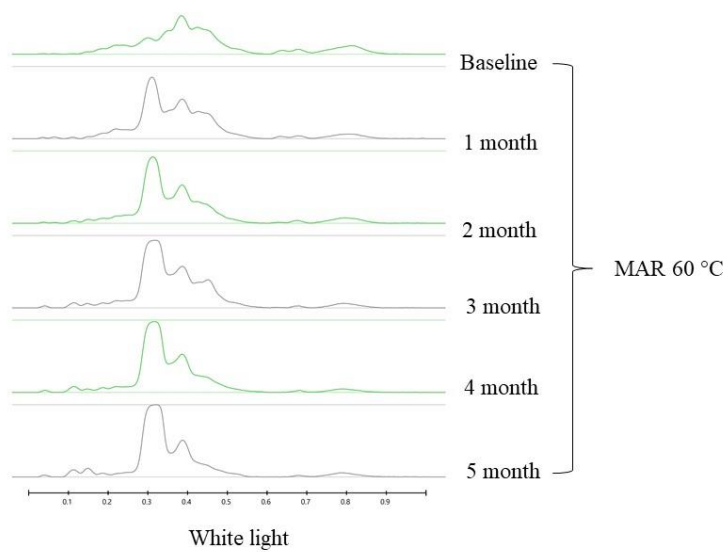
Supplementary Figure S34. Organic honey extract chromatograms (offset Y-axis values).



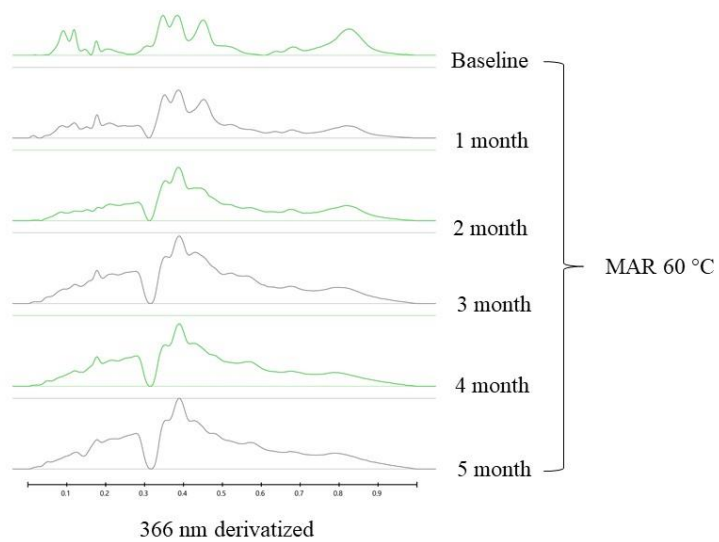
Supplementary Figure S35. MAR long-term storage at 60 °C Images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1—4,5,7-trihydroxyflavanon, Track 2— 0 h, Track 3— 1 month, Track 4— 2 month, Track 5— 3 month, Track 6— 4 month, and Track 7— 5 month; 5 μ L of each honey extract respectively.



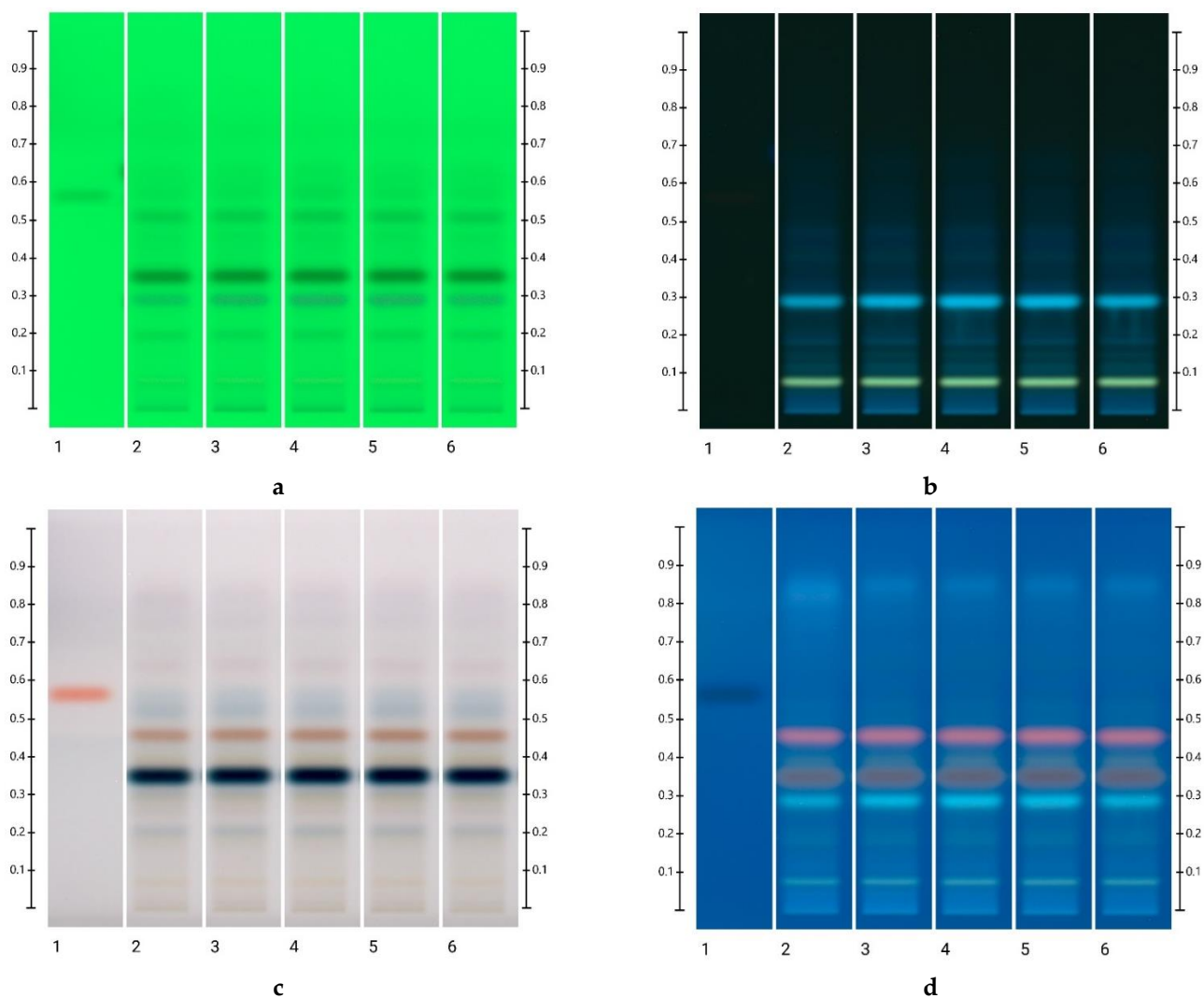
Supplementary Figure S36. Organic honey extract chromatograms (offset Y-axis values).



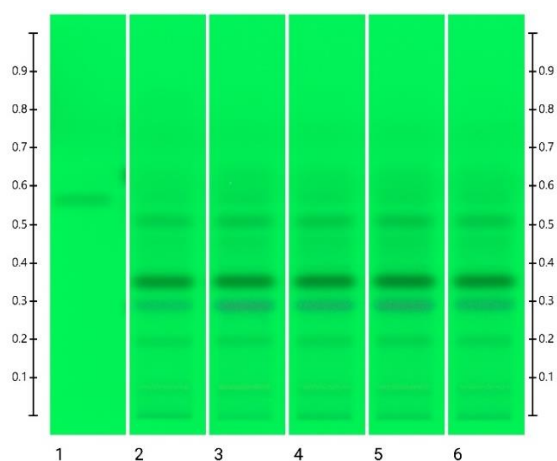
Supplementary Figure S37. Organic honey extract chromatograms (offset Y-axis values).



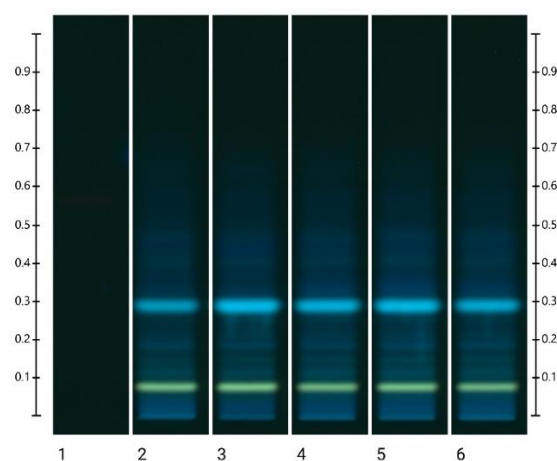
Supplementary Figure S38. Organic honey extract chromatograms (offset Y-axis values).



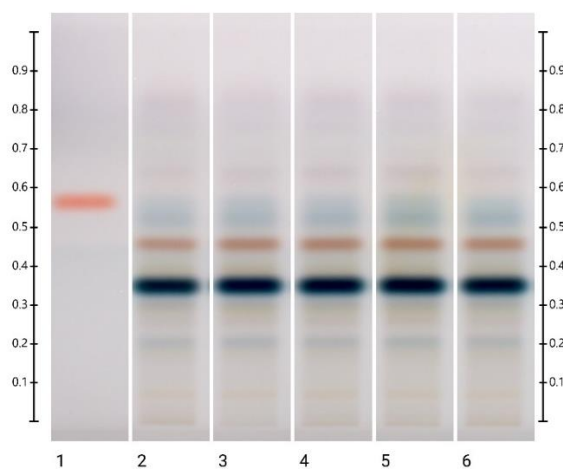
Supplementary Figure S39. PEP short-term storage at 40 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanon, Track 2– 0 h, Track 3– 6 h, Track 4– 12 h, Track 5– 24 h, and Track 6– 48 h; 5 μ L of each honey extract respectively.



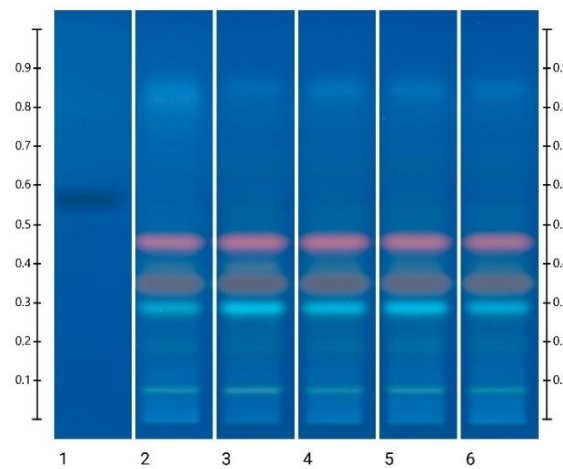
a



b

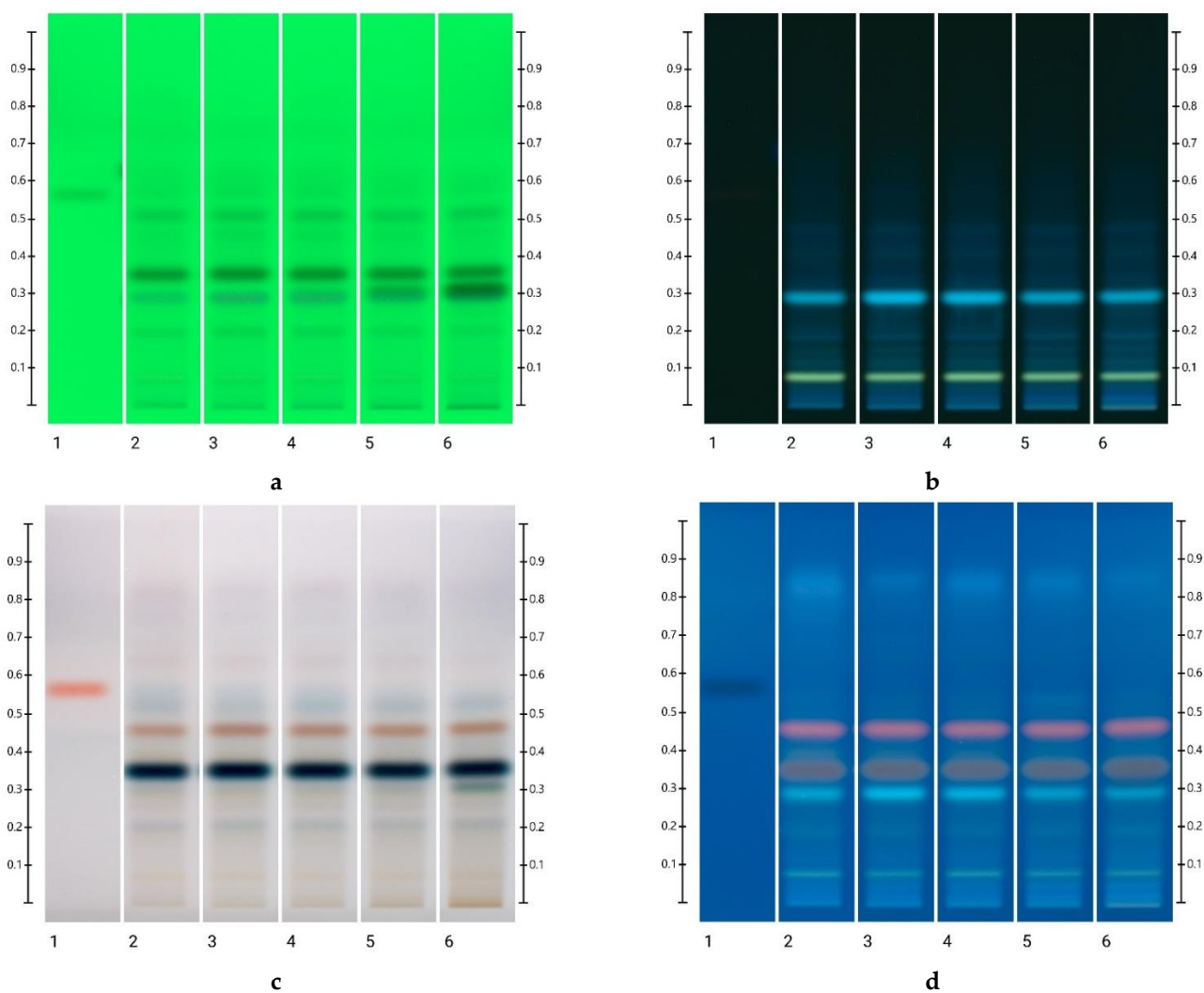


c

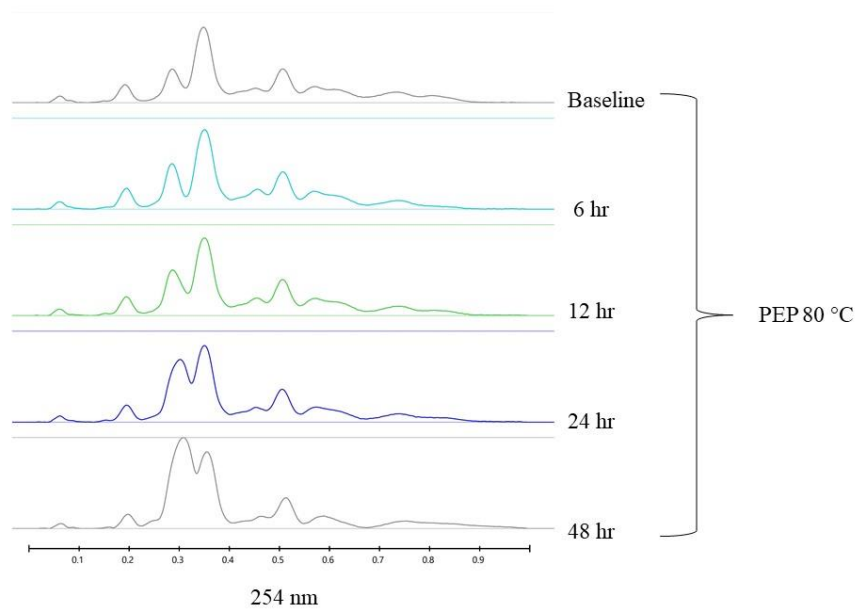


d

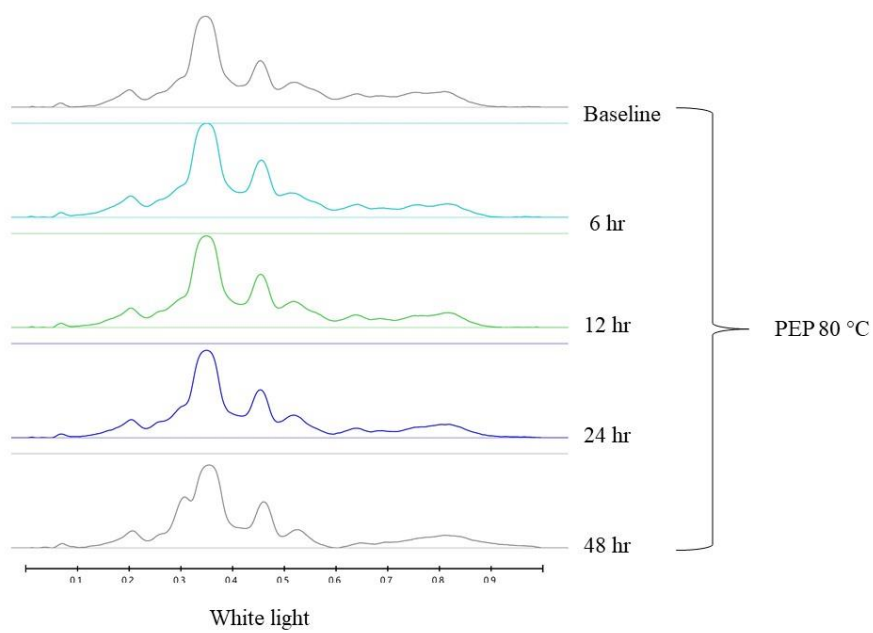
Supplementary Figure S40. PEP short-term storage at 60 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanone, Track 2 – 0 h, Track 3 – 6 h, Track 4 – 12 h, Track 5 – 24 h, and Track 6 – 48 h; 5 μ L of each honey extract respectively.



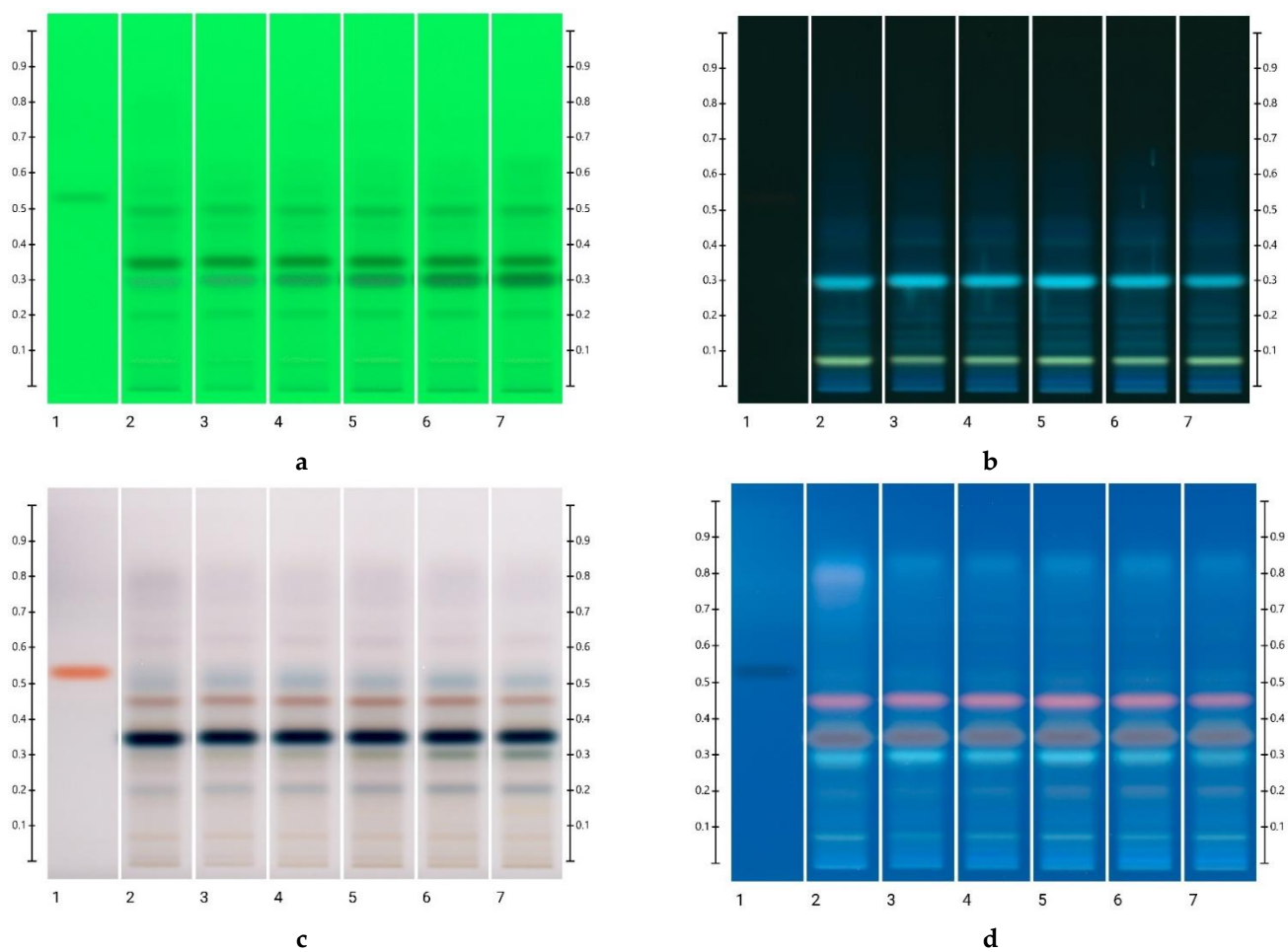
Supplementary Figure S41. PEP short-term storage at 80 °C; images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanon, Track 2 – 0 h, Track 3 – 6 h, Track 4 – 12 h, Track 5 – 24 h, and Track 6 – 48 h; 5 μ L of each honey extract respectively.



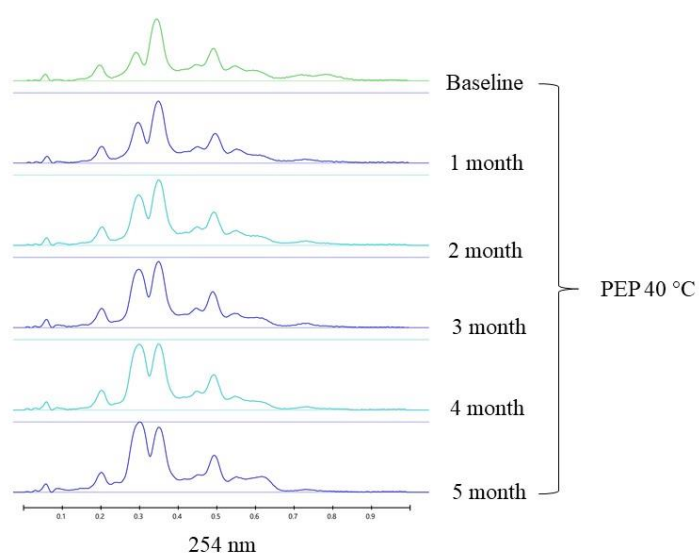
Supplementary Figure S42. Organic honey extract chromatograms (offset Y-axis values).



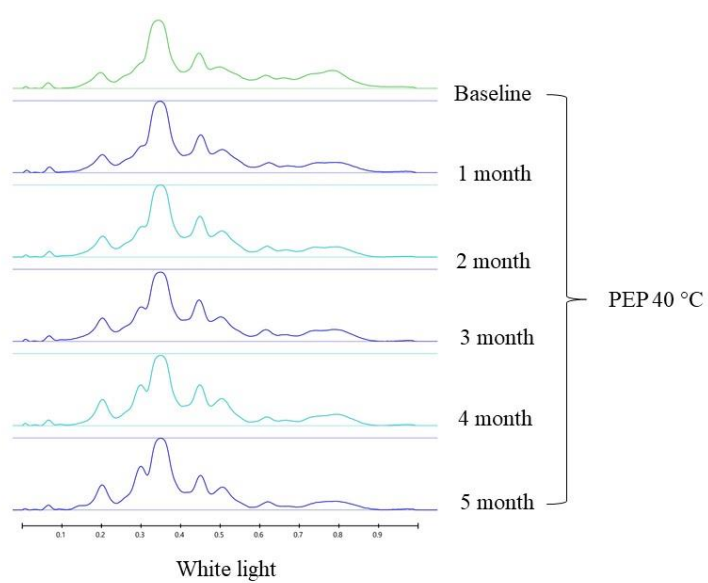
Supplementary Figure S43. Organic honey extract chromatograms (offset Y-axis values).



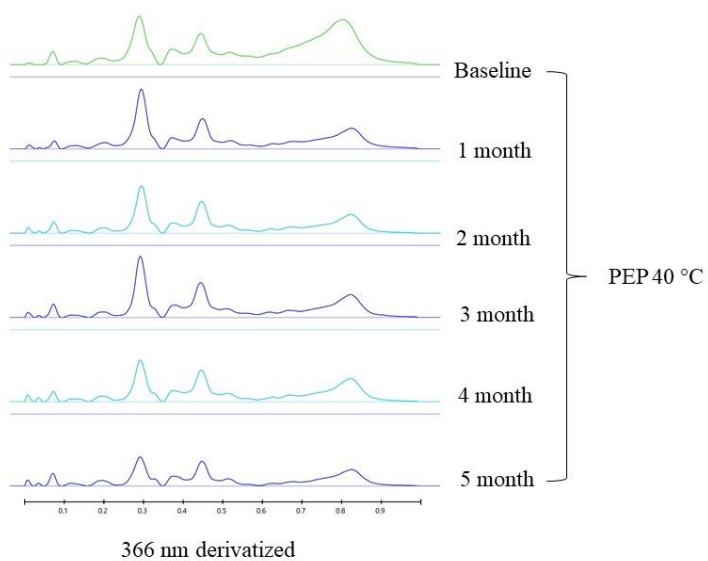
Supplementary Figure S44. PEP long-term storage at 40 °C Images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 – 4,5,7-trihydroxyflavanon, Track 2 – 0 h, Track 3 – 1 month, Track 4 – 2 month, Track 5 – 3 month, Track 6 – 4 month, and Track 7 – 5 month; 5 μ L of each honey extract respectively.



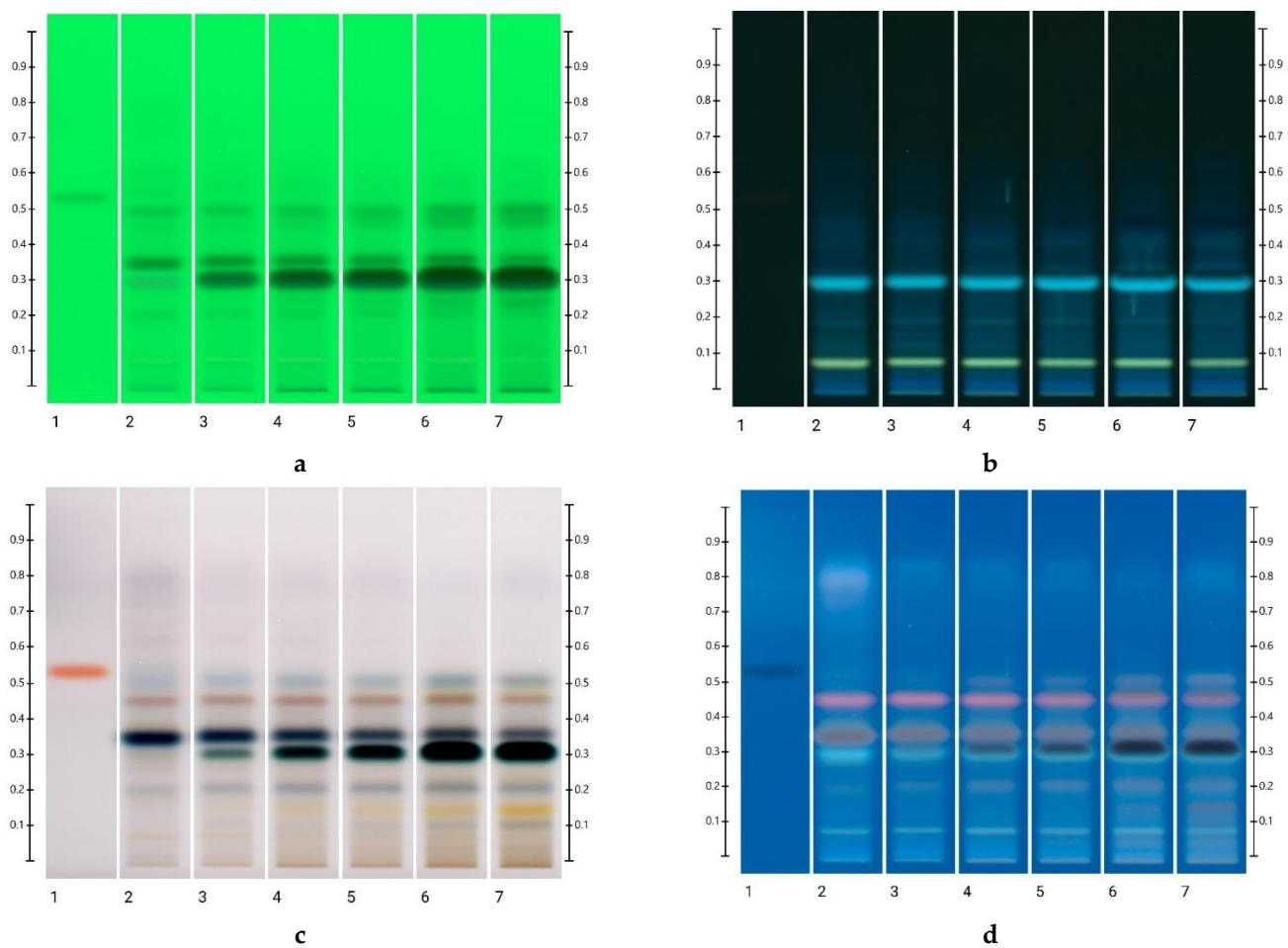
Supplementary Figure S45. Organic honey extract chromatograms (offset Y-axis values).



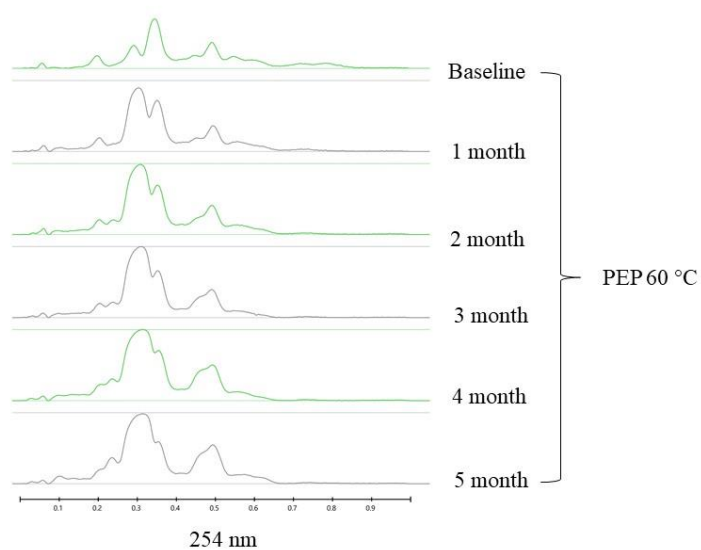
Supplementary Figure S46. Organic honey extract chromatograms (offset Y-axis values).



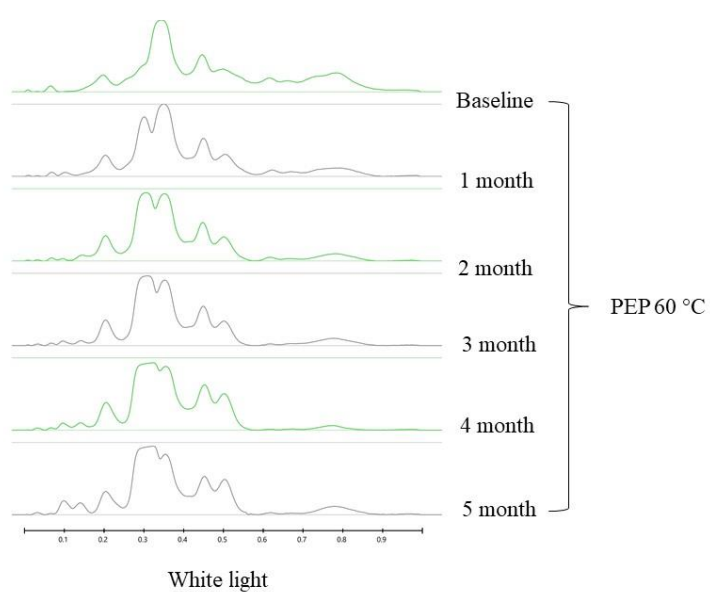
Supplementary Figure S47. Organic honey extract chromatograms (offset Y-axis values).



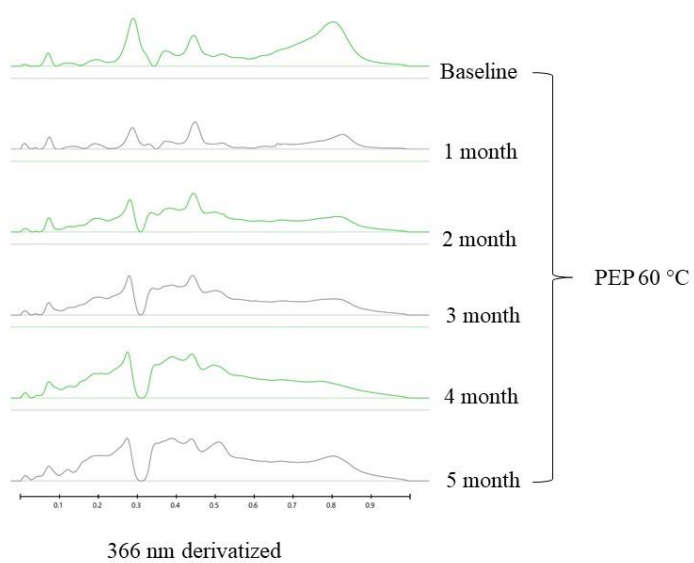
Supplementary Figure S48. PEP long-term storage at 60 °C Images taken at (a) 254 nm; (b) 366 nm; (c) White light after derivatisation and (d) 366 nm after derivatisation with vanillin reagent; Track 1 — 4,5,7-trihydroxyflavanon, Track 2 — 0 h, Track 3 — 1 month, Track 4 — 2 month, Track 5 — 3 month, Track 6 — 4 month, and Track 7 — 5 month; 5 μ L of each honey extract respectively.



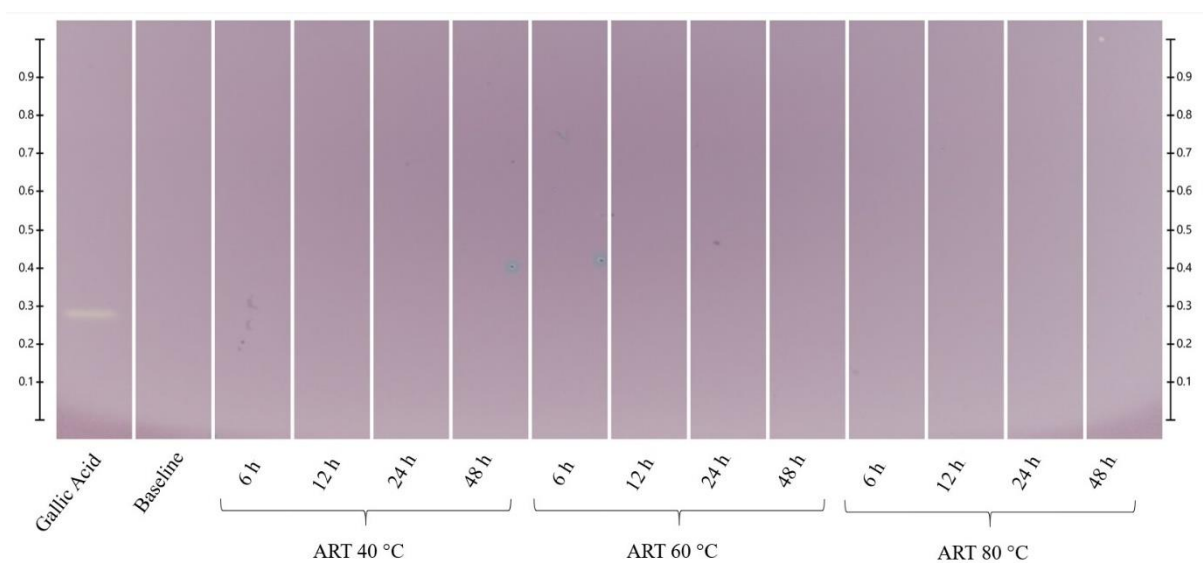
Supplementary Figure S49. Organic honey extract chromatograms (offset Y-axis values).



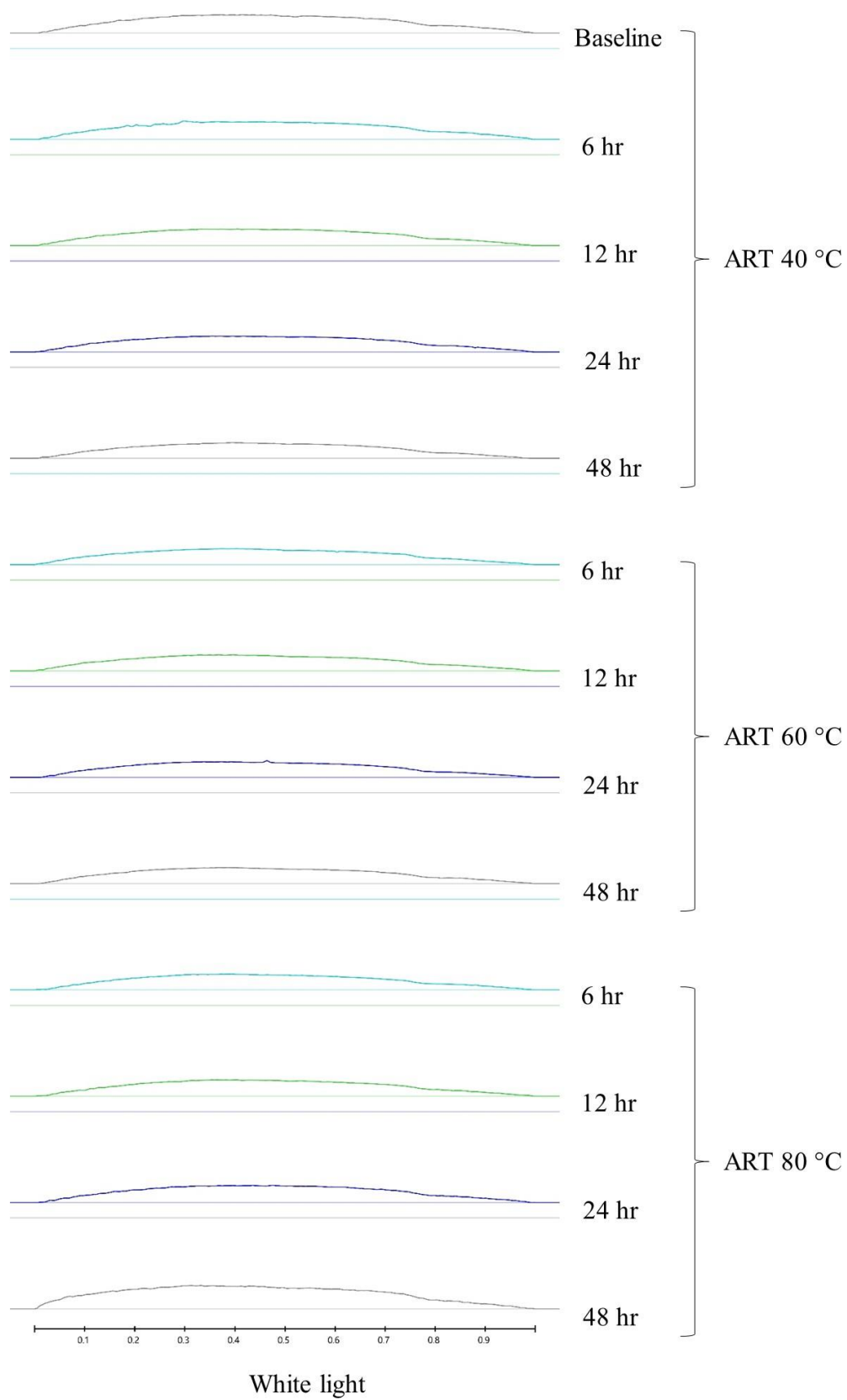
Supplementary Figure S50. Organic honey extract chromatograms (offset Y-axis values).



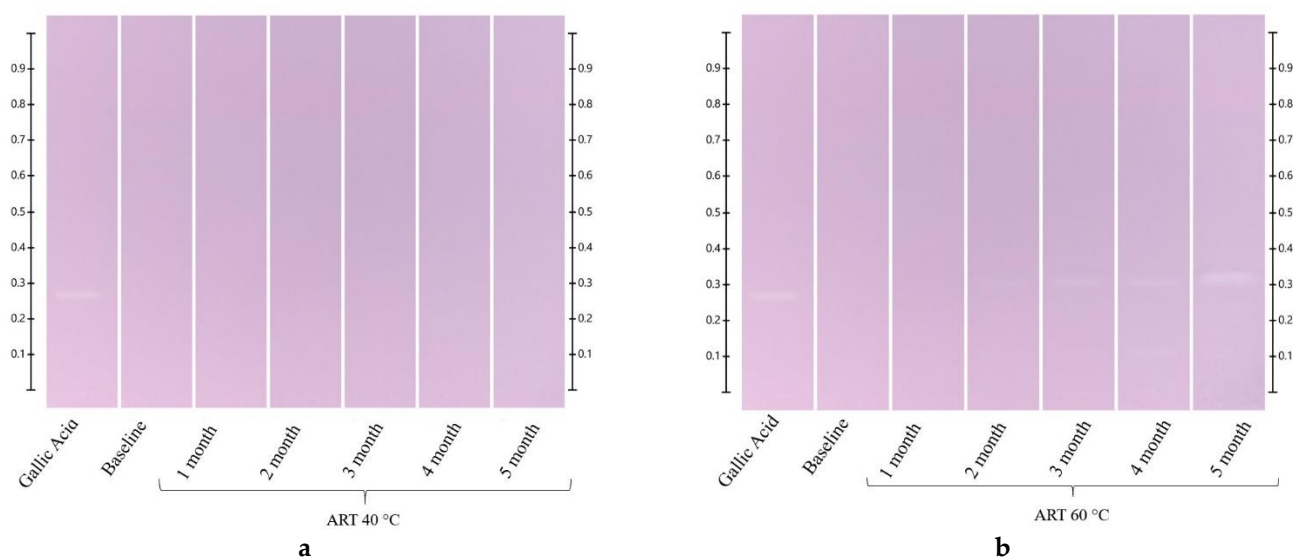
Supplementary Figure S51. Organic honey extract chromatograms (offset Y-axis values).



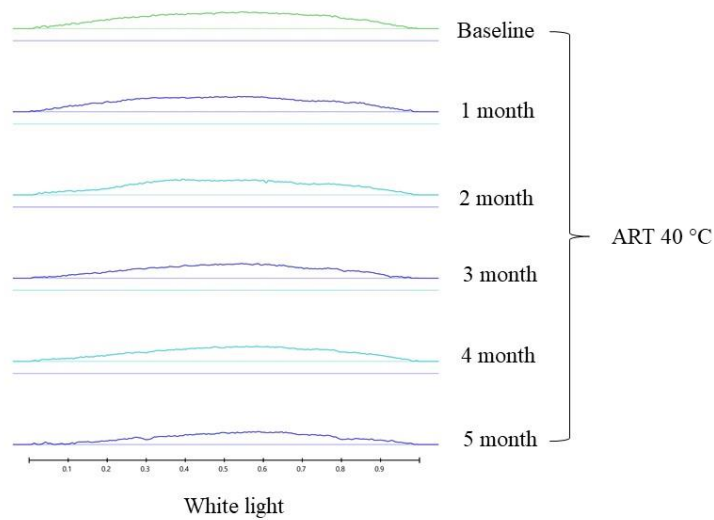
Supplementary Figure S52. HPTLC-DPPH fingerprints of ART stored at 40 °C, 60 °C and 80 °C for up to 48 h. Images of HPTLC plate were taken at White light after 60 min of derivatization with DPPH* reagents; Gallic acid (4 µL) and honey extracts (5 µL) respectively.



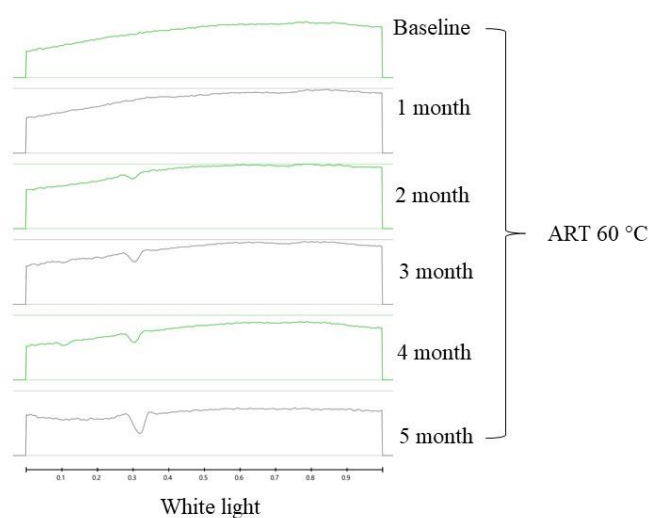
Supplementary Figure S53. Organic honey extract chromatograms (offset Y-axis values).



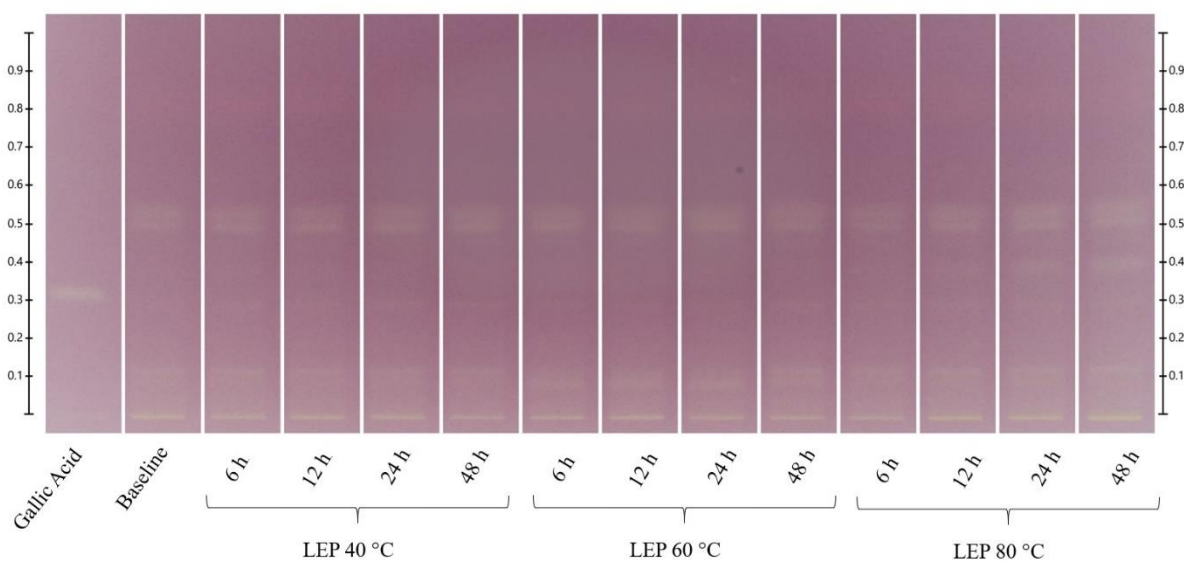
Supplementary Figure S54. HPTLC-DPPH fingerprints of ART stored at 40 °C (**a**), and at 60 °C (**b**) for up to 5 months. Images of HPTLC plate were taken at White light after 60 min of derivatization with DPPH* reagents; Gallic acid (4 μ L) and honey extracts (5 μ L) respectively.



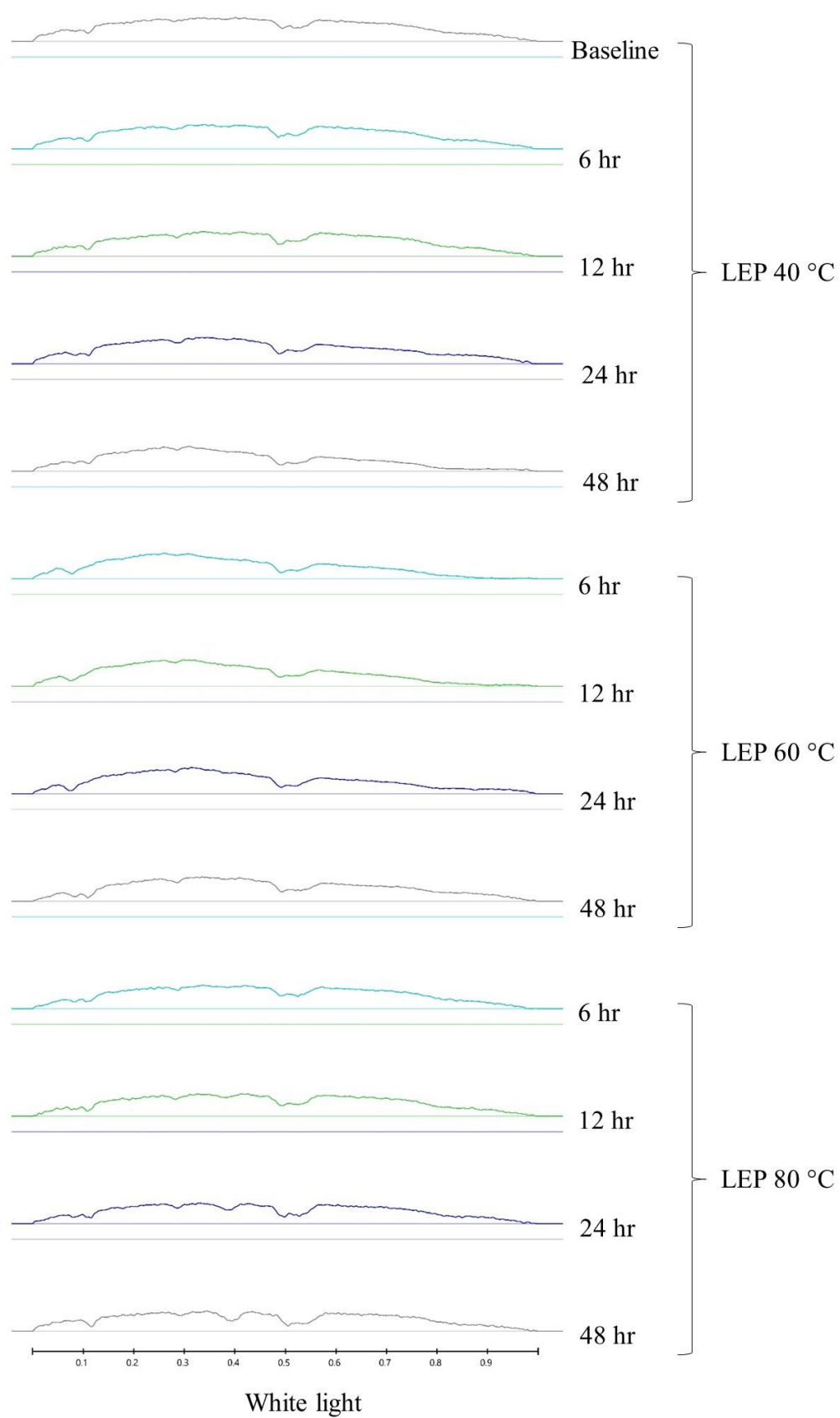
Supplementary Figure S55. Organic honey extract chromatograms (offset Y-axis values).



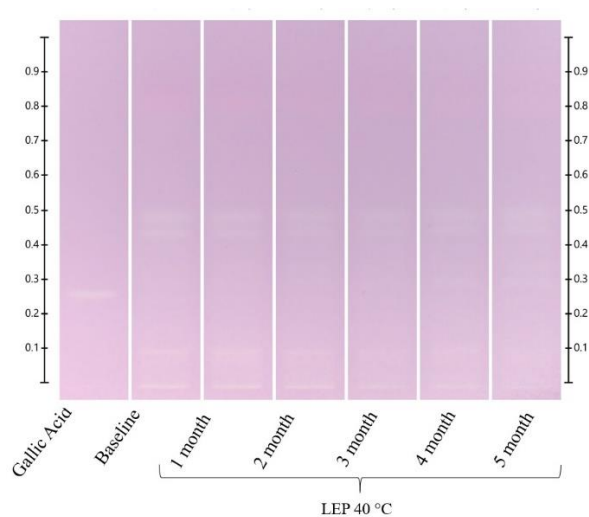
Supplementary Figure S56. Organic honey extract chromatograms (offset Y-axis values).



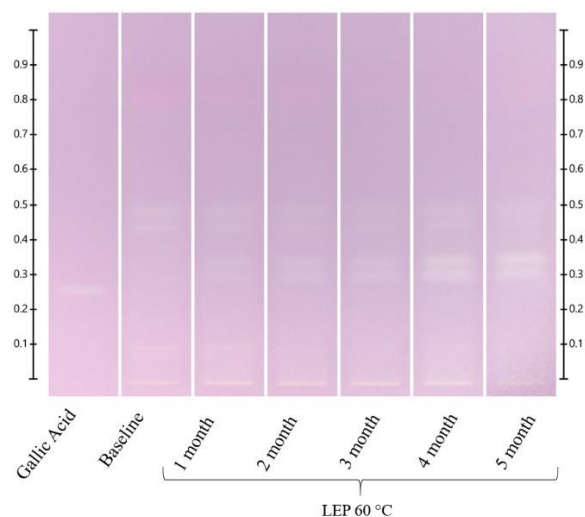
Supplementary Figure S57. HPTLC-DPPH fingerprints of LEP honey stored at 40 °C, 60 °C and 80 °C for up to 48 hours. Images of HPTLC plate were taken at White light after 60 min of derivatization with DPPH* reagents; Gallic acid (4 μ L) and honey extracts (5 μ L) respectively.



Supplementary Figure S58. Organic honey extract chromatograms (offset Y-axis values).

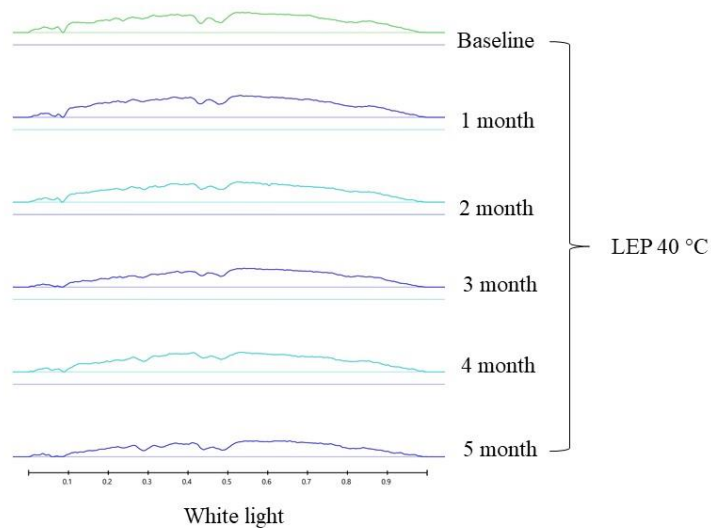


a

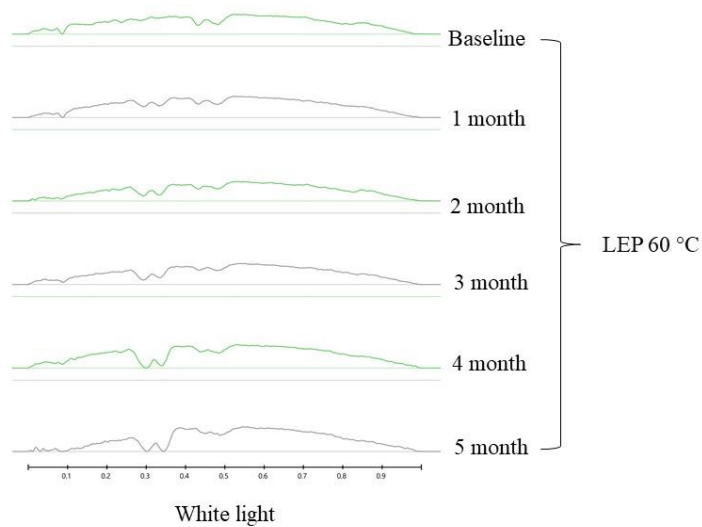


b

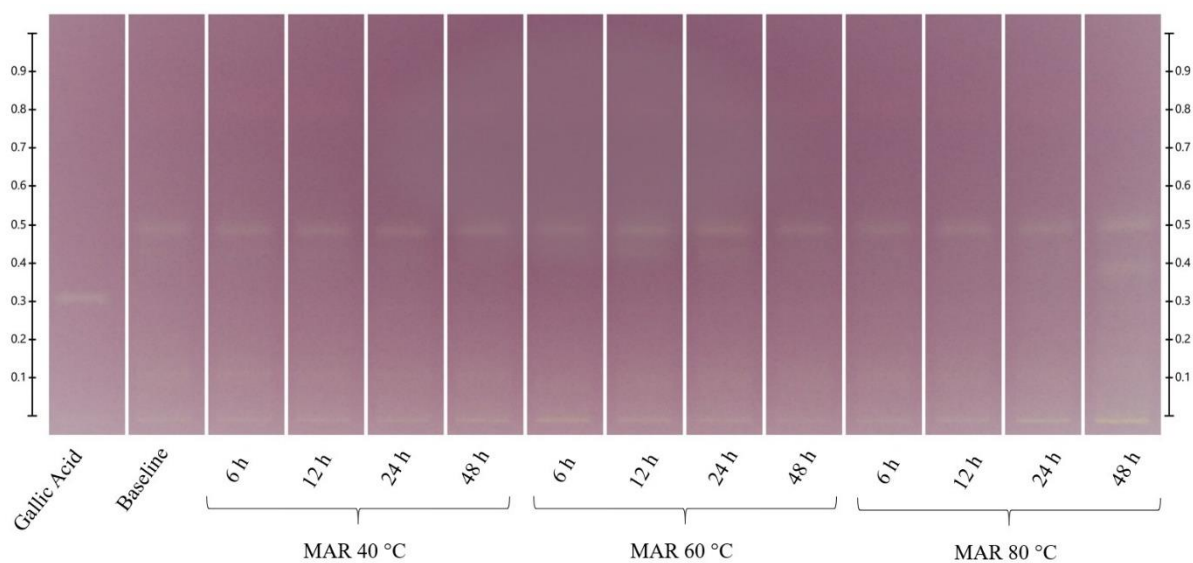
Supplementary Figure S59. HPTLC-DPPH fingerprints of LEP honey stored at 40 °C (**a**), and at 60 °C (**b**) for up to 5 months. Images of HPTLC plate were taken at White light after 60 min of derivatization with DPPH* reagents; Gallic acid (4 μ L) and honey extracts (5 μ L) respectively.



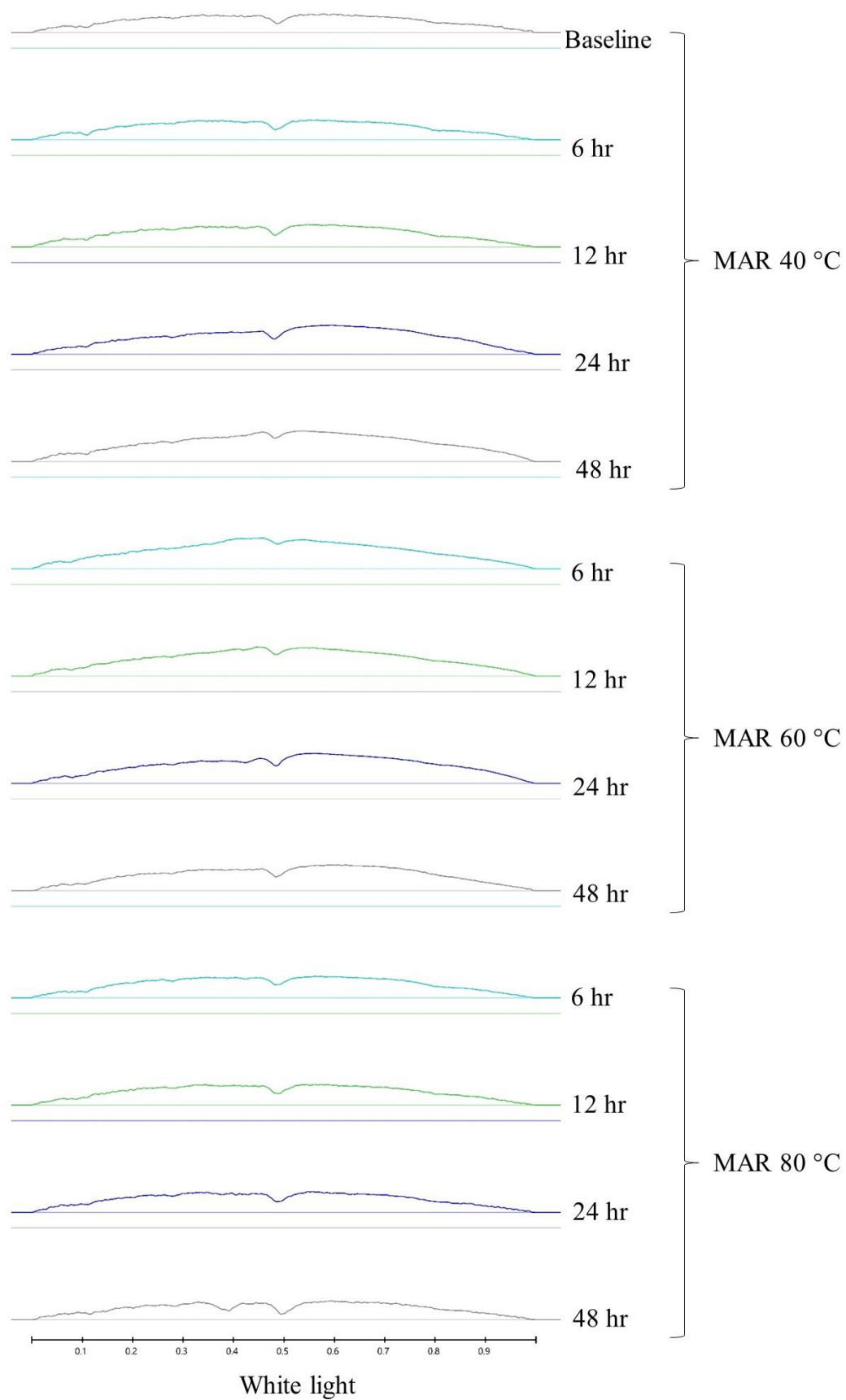
Supplementary Figure S60. Organic honey extract chromatograms (offset Y-axis values).



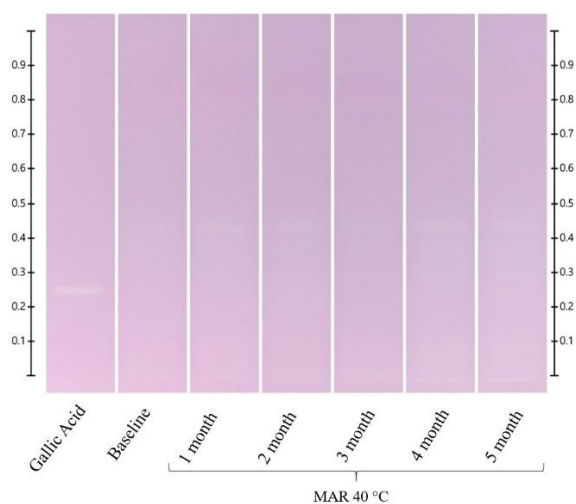
Supplementary Figure S61. Organic honey extract chromatograms (offset Y-axis values).



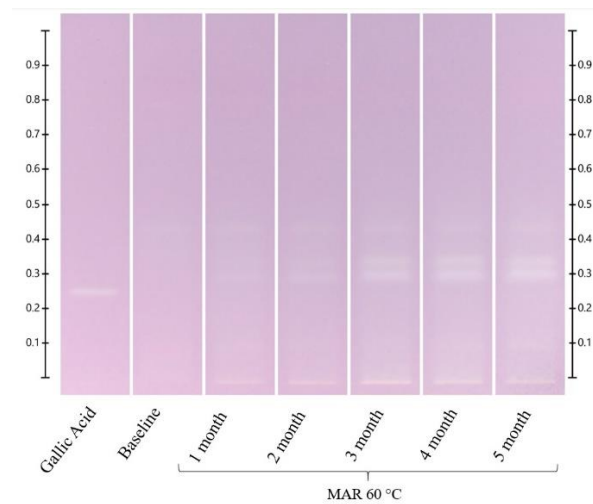
Supplementary Figure S62. HPTLC-DPPH fingerprints of MAR stored at 40 °C, 60 °C and 80 °C for up to 48 hours. Images of HPTLC plate were taken at White light after 60 min of derivatization with DPPH* reagents; Gallic acid (4 μ L) and honey extracts (5 μ L) respectively.



Supplementary Figure S63. Organic honey extract chromatograms (offset Y-axis values).

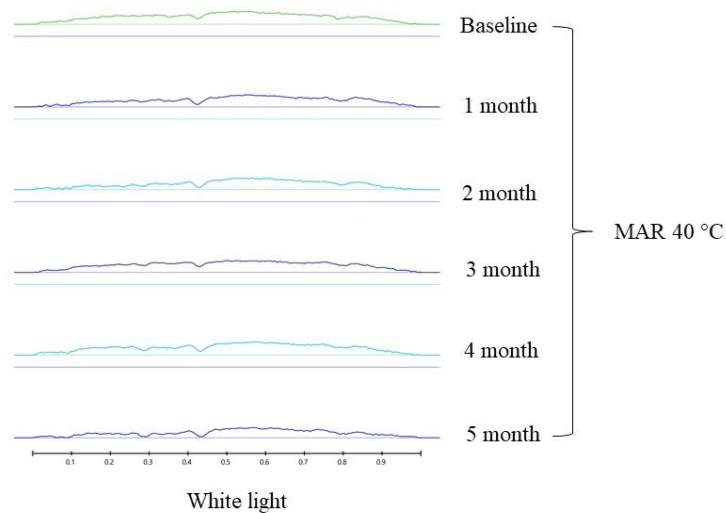


a

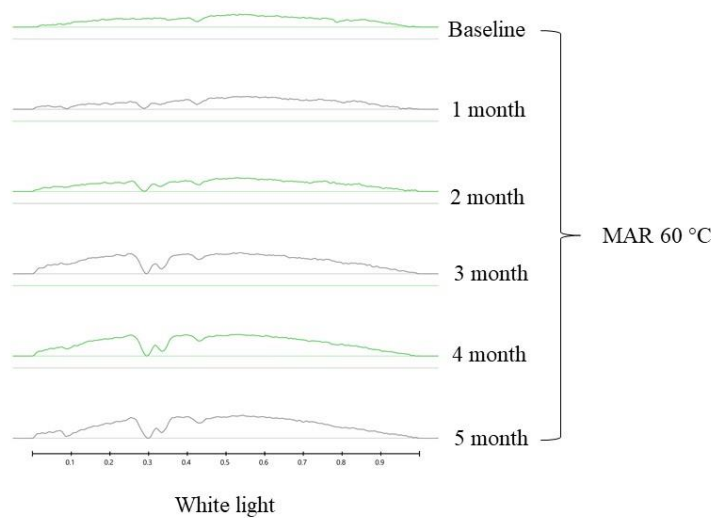


b

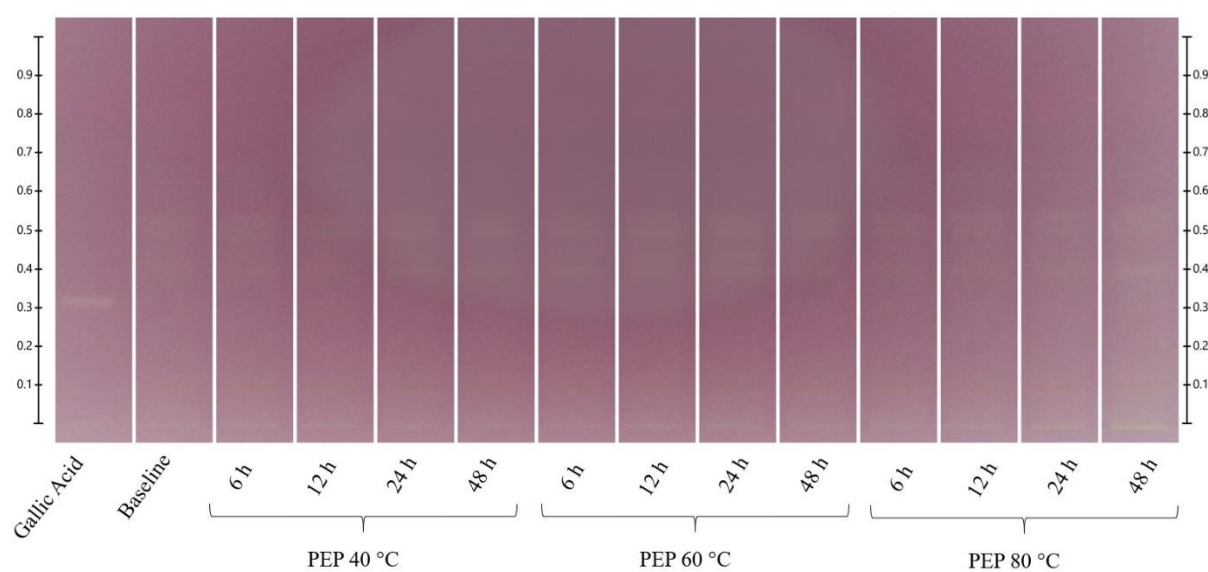
Supplementary Figure S64. HPTLC-DPPH fingerprints of MAR honey stored at 40 °C (**a**), and at 60 °C (**b**) for up to 5 months. Images of HPTLC plate were taken at White light after 60 min of derivatization with DPPH* reagents; Gallic acid (4 μ L) and honey extracts (5 μ L) respectively.



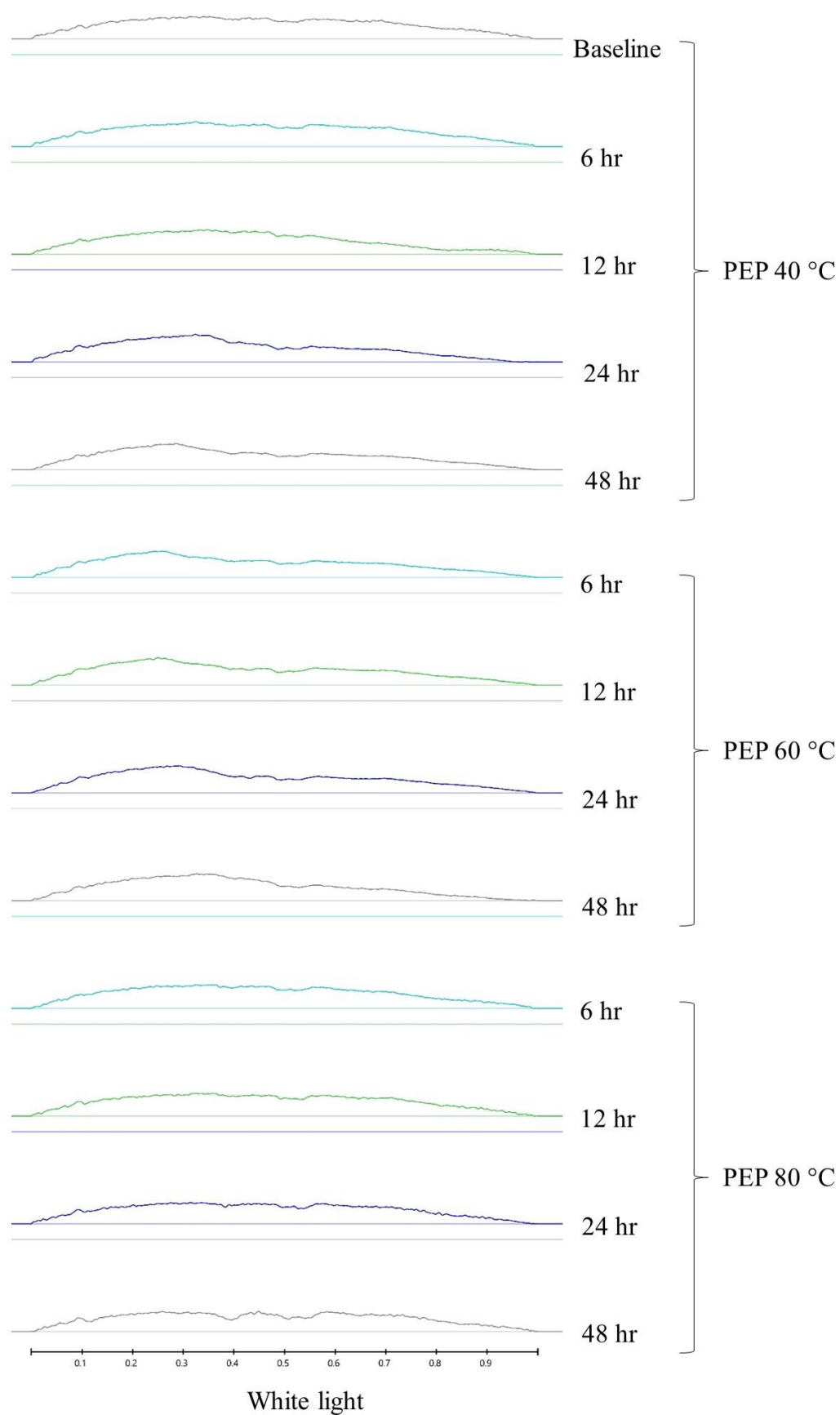
Supplementary Figure S65. Organic honey extract chromatograms (offset Y-axis values).



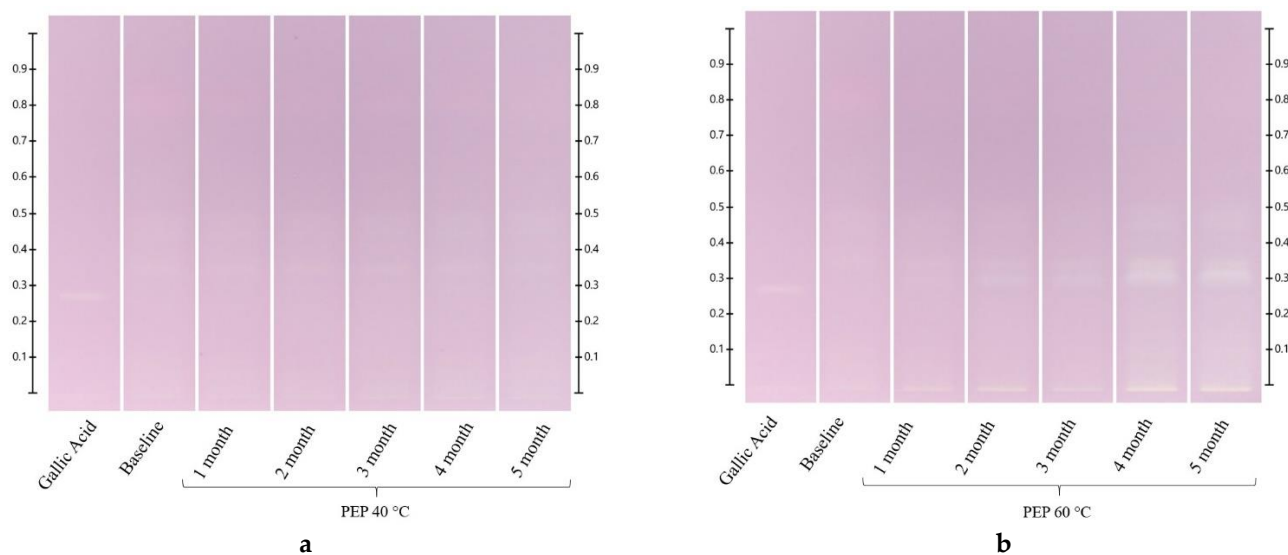
Supplementary Figure S66. Organic honey extract chromatograms (offset Y-axis values).



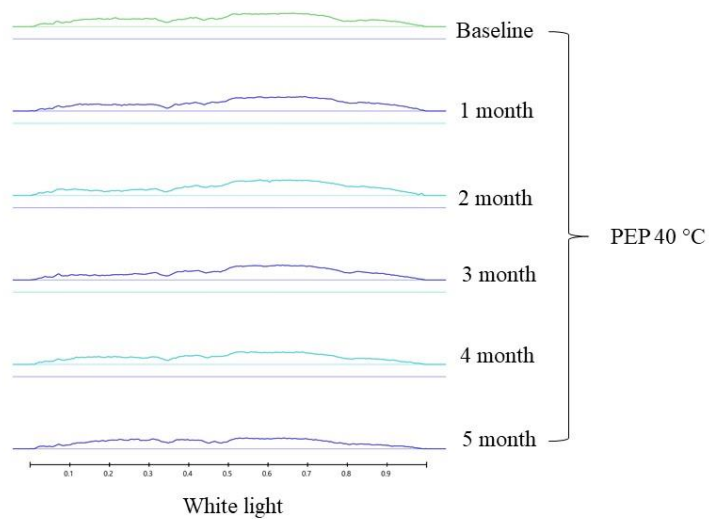
Supplementary Figure S67. HPTLC-DPPH fingerprints of PEP stored at 40 °C, 60 °C and 80 °C for up to 48 hours. Images of HPTLC plate were taken at White light after 60 min of derivatization with DPPH* reagents; Gallic acid (4 μ L) and honey extracts (5 μ L) respectively.



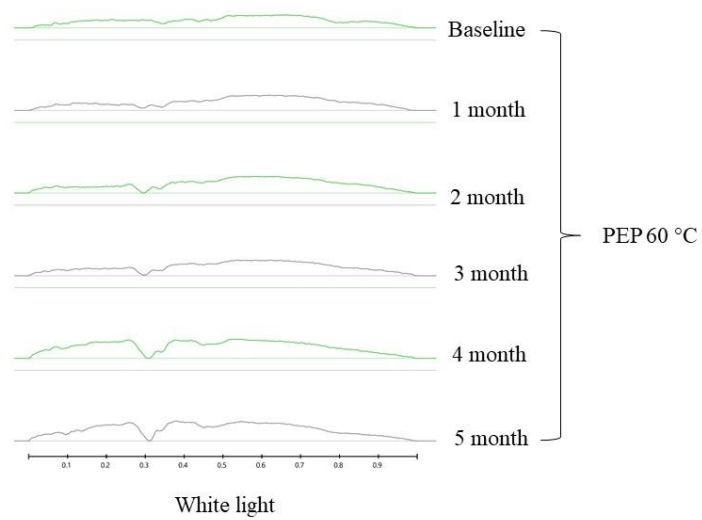
Supplementary Figure S68. Organic honey extract chromatograms (offset Y-axis values).



Supplementary Figure S69. HPTLC-DPPH fingerprints of PEP honey stored at 40 °C (**a**), and at 60 °C (**b**) for up to 5 months. Images of HPTLC plate were taken at White light after 60 min of derivatization with DPPH* reagents; Gallic acid (4 μ L) and honey extracts (5 μ L) respectively.



Supplementary Figure S70. Organic honey extract chromatograms (offset Y-axis values).



Supplementary Figure S71. Organic honey extract chromatograms (offset Y-axis values).