

Development of an enantioselective method by liquid chromatography to monitor 3,4-methylenedioxyprovalerone in culture media from ecotoxicity assays

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Figure S1a. Chromatogram of the enantioseparation of $1.00 \mu\text{g mL}^{-1}$ (*R*)-MDPV (in UPW) in the analytical Lux[®] 3 μm - Cellulose-2 (150 \times 2.0 mm I.D.) column by LC-UV/Vis under reversed elution mode in isocratic mode. LC conditions: mobile phase, 20 mM NH₄OAc in UPW (pH 8.5) and ACN (70:30, v/v); flow rate of 0.3 mL min⁻¹; injection volume of 5 μL ; λ_{max} of 315 nm; column oven temperature set at 25 °C.

Figure S1b. Chromatogram of the enantioseparation of $1.00 \mu\text{g mL}^{-1}$ (*S*)-MDPV (in UPW) in the analytical Lux[®] 3 μm - Cellulose-2 (150 \times 2.0 mm I.D.) column by LC-UV/Vis under reversed elution mode in isocratic mode. LC conditions: mobile phase, 20 mM NH₄OAc in UPW (pH 8.5) and ACN (70:30, v/v); flow rate of 0.3 mL min⁻¹; injection volume of 5 μL ; λ_{max} of 315 nm; column oven temperature set at 25 °C.

Figure S2. Chromatograms of the separation of $.10 \mu\text{g mL}^{-1}$ (*S*)-MDPV (in UPW at different pH) in the analytical Lux[®] 3 μm - Cellulose-2 (150 \times 2.0 mm I.D.) column by LC-UV/Vis under reversed elution mode in isocratic mode. LC conditions: mobile phase, 20 mM NH₄OAc and ACN (70:30, v/v, pH 8.5); flow rate of 0.3 mL min⁻¹; injection volume of 5 μL ; λ_{max} of 315 nm; column oven temperature set at 35 °C. **(a)** time zero; **(b)** 24 h after.

Figure S3. Total ion chromatograms (black) and selected reaction monitoring (m/z 276.00 > 126.15 (pink); m/z 276.00 > 135.05 (blue)) of the MDPV enantiomers in the analytical Daicel[®] 3 μm - CHIRALPAK[®] IF-3 (150 \times 2.1 mm I.D.) column by LC-MS/MS under reversed elution mode in isocratic mode. LC conditions: 5 mM NH₄HCO₃ in UPW (pH 8.8) with ACN (10:90, v/v) as mobile phase; flow rate of 0.3 mL min⁻¹; injection volume of 5 μL ; column oven temperature set at 30 °C. **(a)** (*R,S*)-MDPV at 1000 $\mu\text{g L}^{-1}$ in UPW (sample matrix after SPE); **(b)** (*R,S*)-MDPV at 1000 $\mu\text{g L}^{-1}$ in UPW (standard).

Table S1. e.r of MDPV standards and culture medium samples of *Daphnia magna* enantioselective ecotoxicity assays, analysed by the enantioselective method previously optimized to enantioseparate MDPV by LC-MS/MS in the analytical column Daicel[®] 3 μm - CHIRALPAK[®] IF-3 (150 \times 2.1 mm I.D.) under reversed elution mode in isocratic mode. LC conditions: mobile phase, 5 mM NH₄HCO₃ in UPW (pH 8.8) with ACN (10:90, v/v); flow rate of 0.3 mL min⁻¹; injection volume of 5 μL ; and, column oven temperature set at 30 °C.

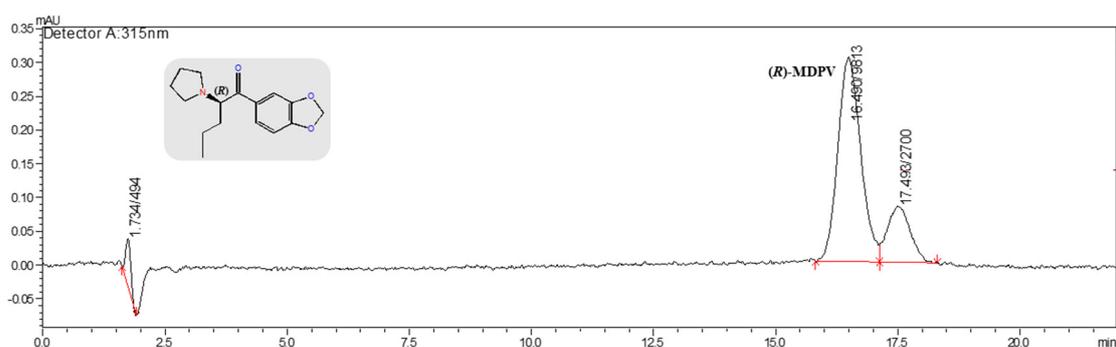


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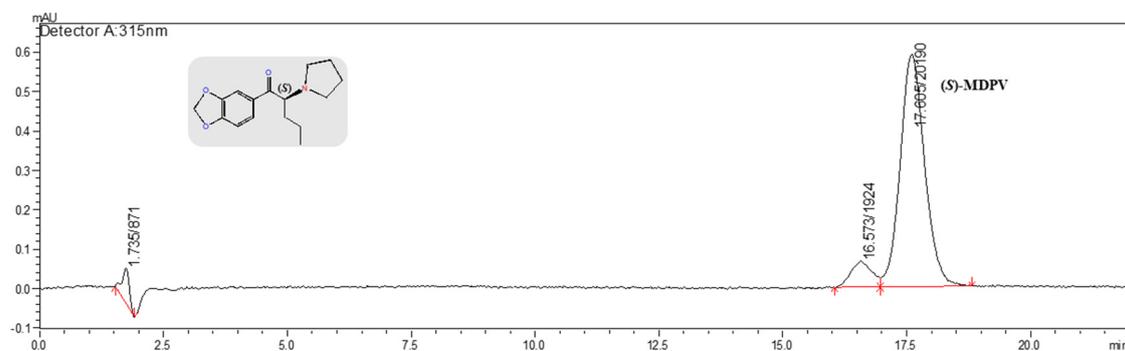


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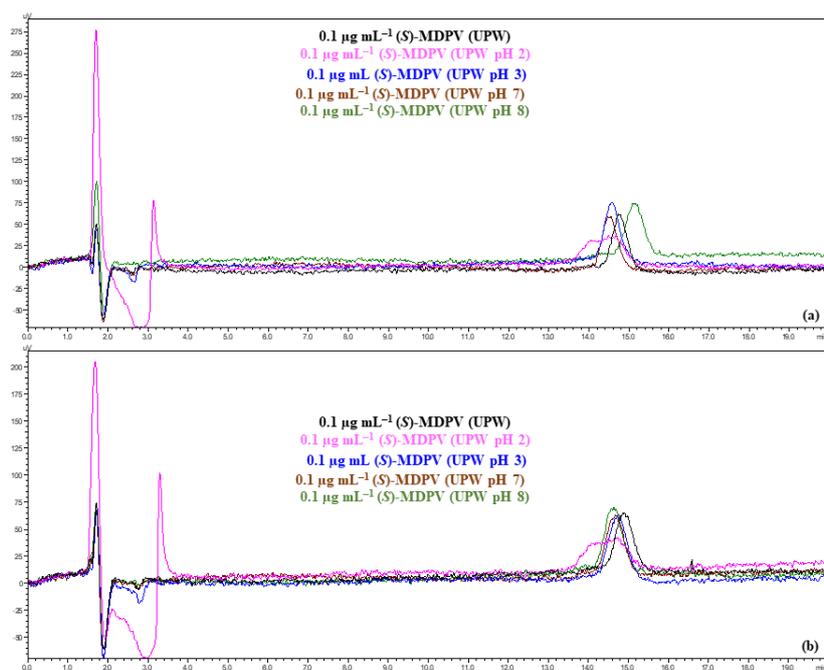


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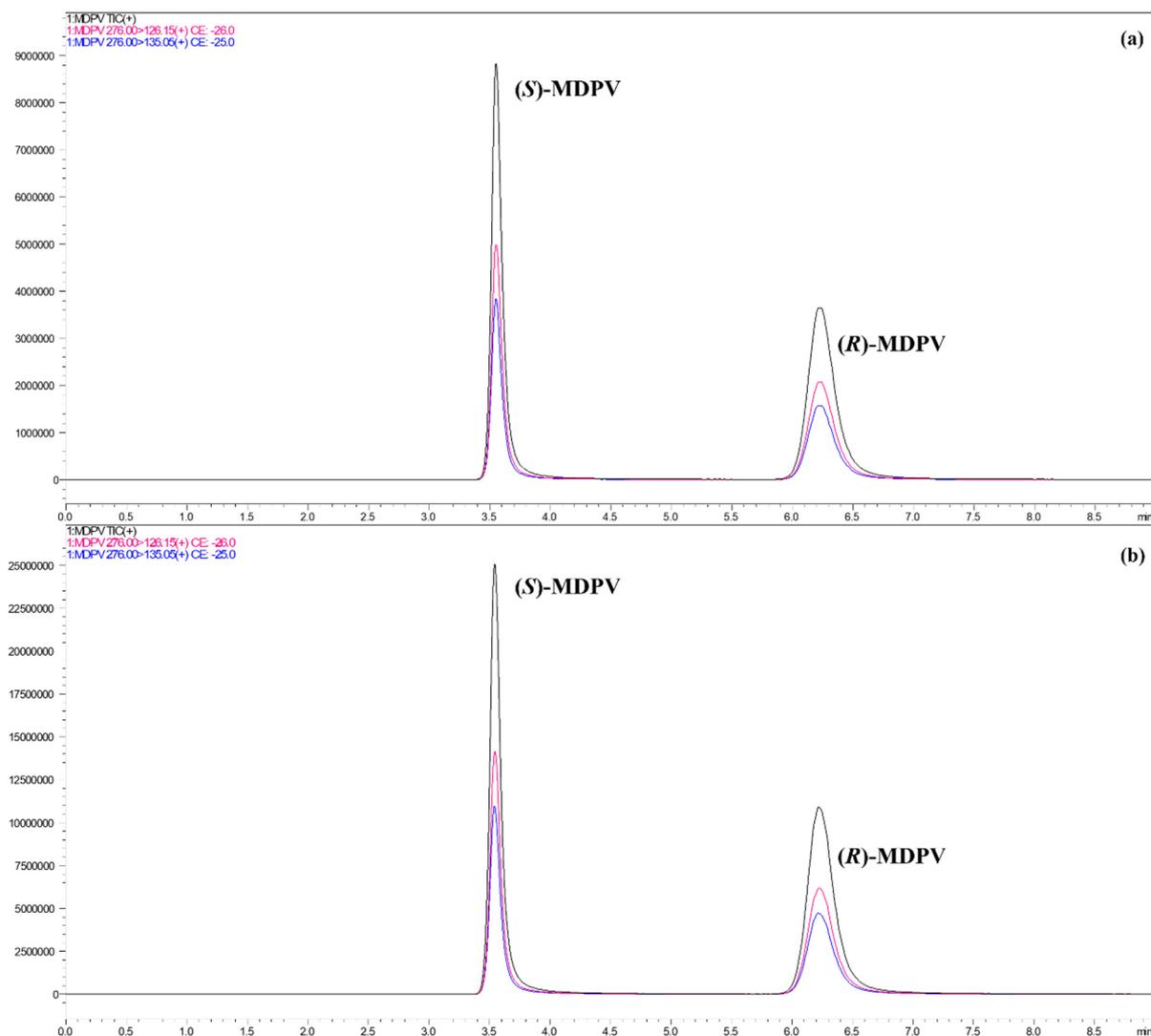


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Sample (Average)	e.r. of (<i>S</i>)-MDPV	e.r. of (<i>R</i>)-MDPV
(<i>R,S</i>)-MDPV ^(a)	49.5	50.5

(S)-MDPV ^(a)	96.4	3.6
(R)-MDPV ^(a)	15.6	84.4
(R,S)-MDPV ^(b) C1	48.9	51.1
(R,S)-MDPV ^(b) C2	48.2	51.8
(R,S)-MDPV ^(b) C3	48.5	51.5
(R,S)-MDPV ^(c) C1	49.5	50.5
(R,S)-MDPV ^(c) C2	49.2	50.8
(R,S)-MDPV ^(c) C3	49.7	50.3
(S)-MDPV ^(b) C2	75.0	25.0
(S)-MDPV ^(b) C3	81.5	18.5
(S)-MDPV ^(c) C2	75.5	24.5
(S)-MDPV ^(c) C3	80.1	19.9
(R)-MDPV ^(b) C1	28.0	72.0
(R)-MDPV ^(b) C2	25.8	74.2
(R)-MDPV ^(b) C3	30.7	69.3
(R)-MDPV ^(c) C1	31.7	68.3
(R)-MDPV ^(c) C2	30.4	69.6
(R)-MDPV ^(c) C3	23.3	76.7

(a) MDPV Standards at 1.00 $\mu\text{g L}^{-1}$ (in UPW); **(b)** culture medium samples spiked at 1.00 $\mu\text{g L}^{-1}$ (in UPW); **(c)** MDPV samples at 1.00 $\mu\text{g L}^{-1}$ (in UPW); **(C1)** Sample Collection 1; **(C2)** Sample Collection 2; **(C3)** Sample Collection 3.