ELECTRONIC SUPLEMENTARY INFORMATION

Synergistic effect of hydrophobic deep eutectic solvent based on terpenoids and carboxylic acids for tetracyclines microextraction

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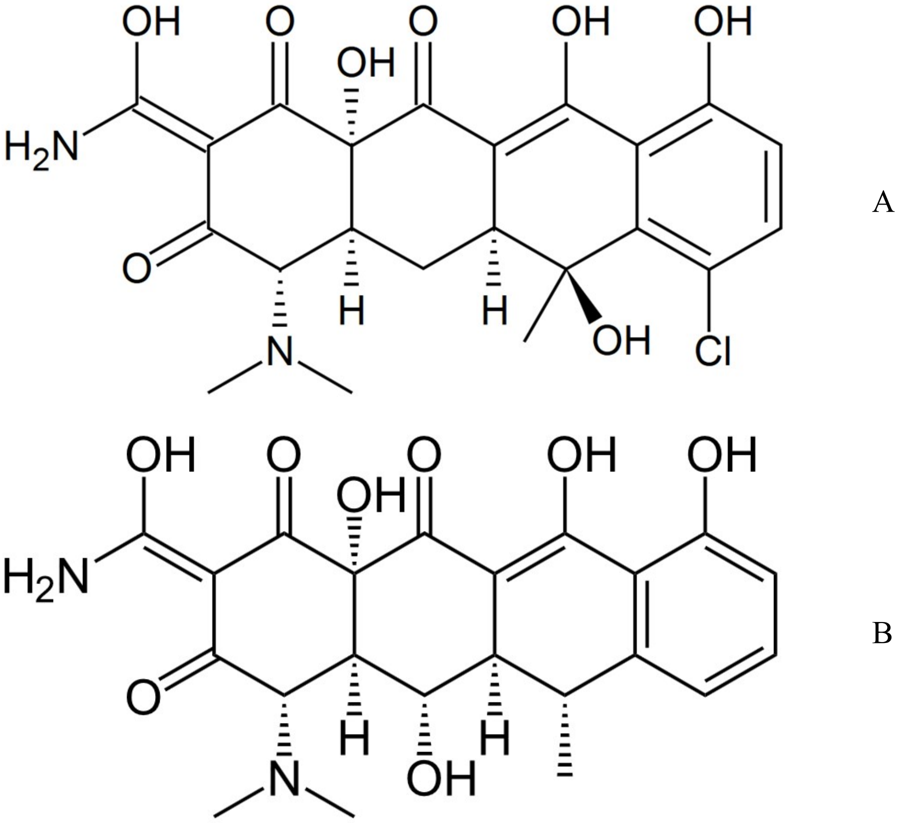
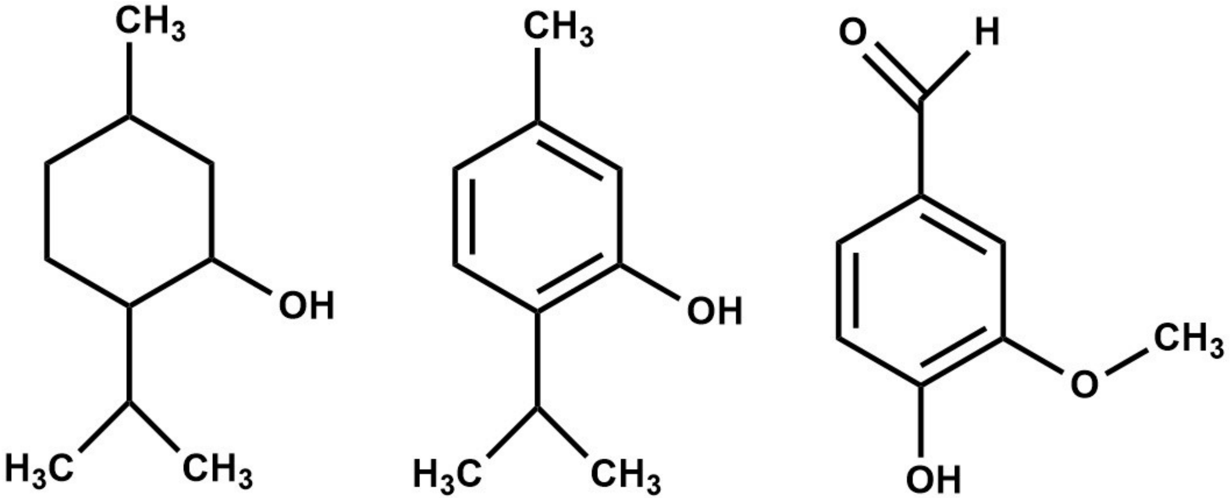


ESM Fig. 1. Chemical structures of chlortetracycline (A) and doxycycline (B).

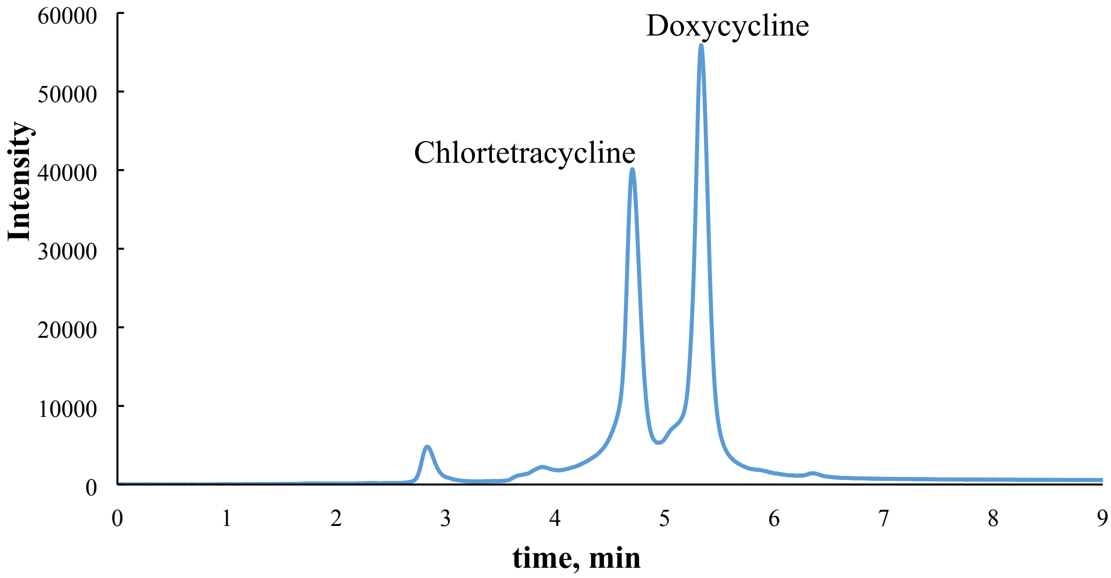


Menthol Thymol Vanilin

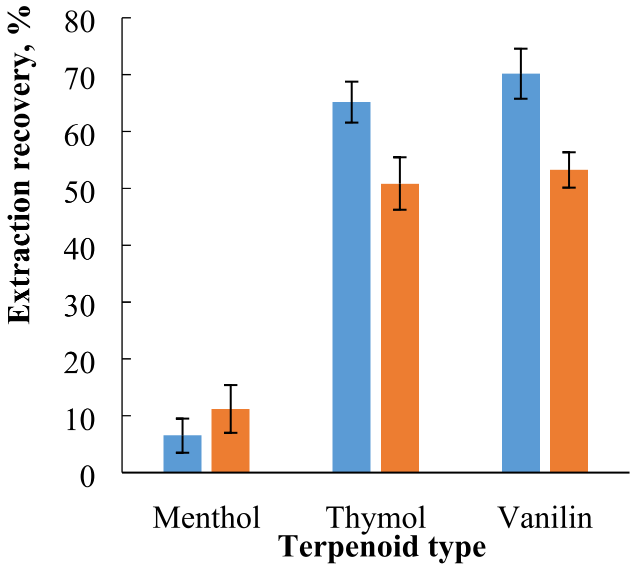
ESM Fig. 2. Chemical structures of terpenoids.

The chromatographic separation

The chromatographic separation was carried out on a SUPELCO C18 column (250 mm×4.6 mm, 5 µm), mobile phase consisted of solvent A (0.5% formic acid) and solvent B (acetonitrile and methanol at a ratio of 2:1, v/v) were used for the determination of chlortetracycline and doxycycline. The determination was carried out in isocratic elution mode. The concentration of solvent A was 55%, whereas the concentration of solvent B was 45%. The column was washed between the sample injections by solvent B for 4 min to remove thymol residues. The flow-rate was 0.75 mL min^{-1}. The wavelength of a diode-array detector was 355 nm.



ESM Fig. 3. Chromatogram of chlortetracycline and doxycycline (C (TCs) = 20 mg L^{-1}).



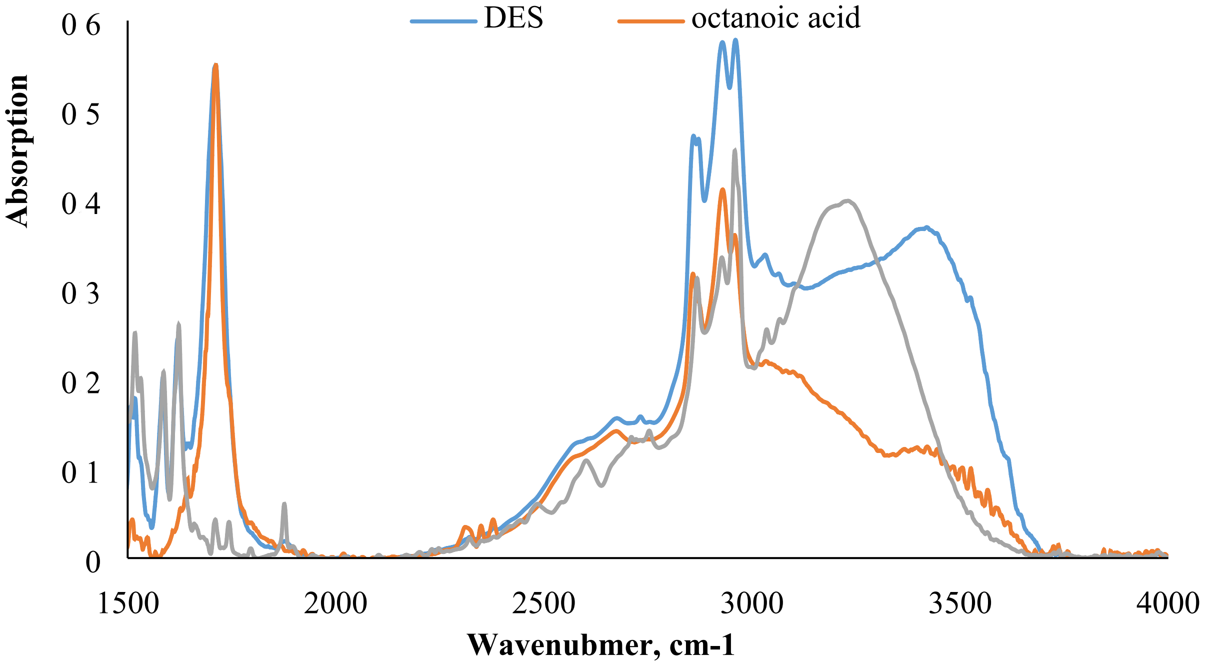
ESM Fig. 4. Effect of terpenoids: C_{TCs} = 20 mg L^{-1}, $V_{organic\ phase}$ = 100 µL (n=3). chlortetracycline■ doxycycline■.

ESM Table 1. Condition for preparation of thymol-based deep eutectic solvents.

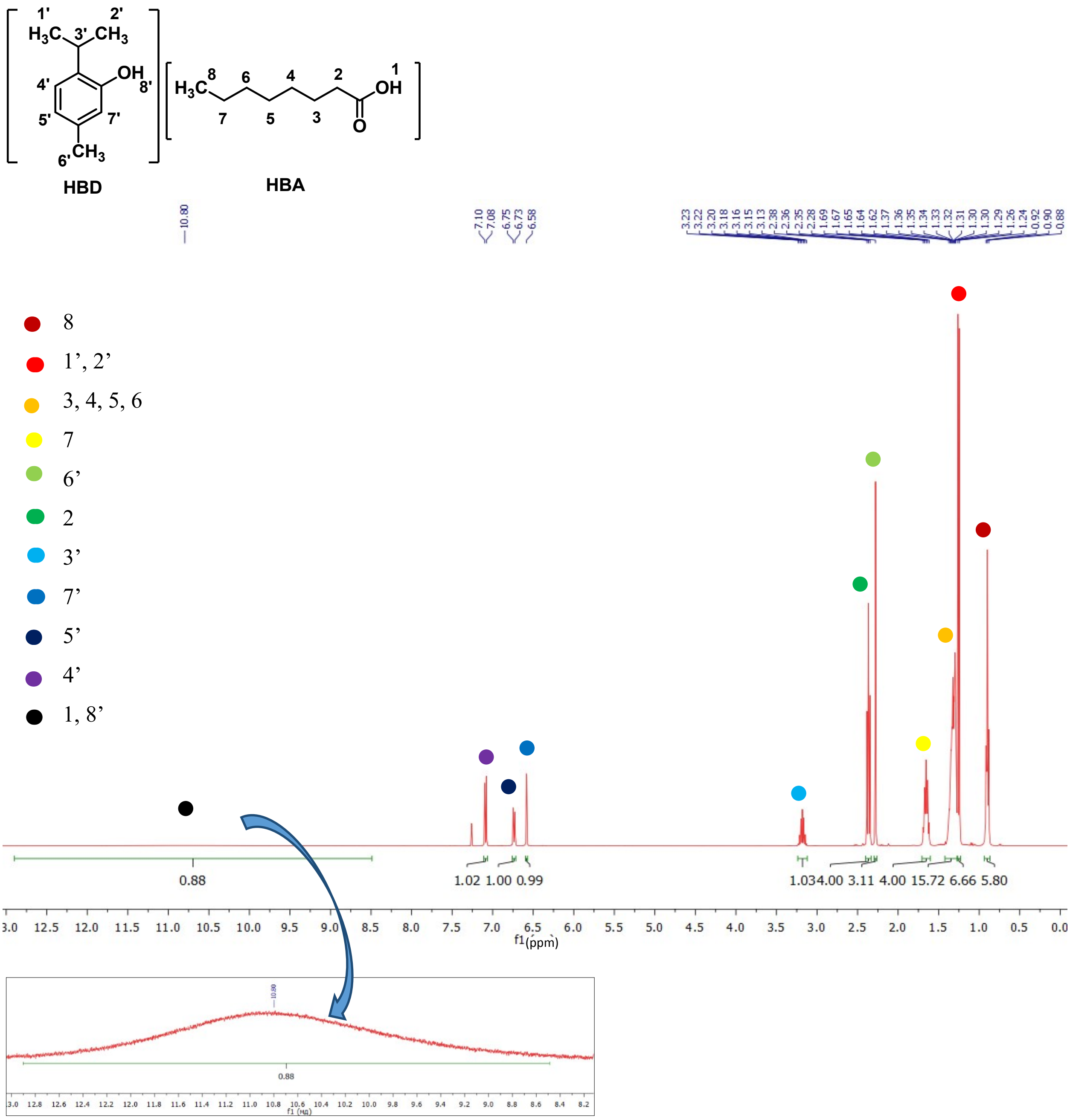
Hydrogen bond donor	Molar ratio (thymol:donor)	Thymol mass, g	Hydrogen bond donor mass, g	Synthesis temperature, °C
Hexanoic acid	0.50:0.50	0.7511	0.5808	50
Octanoic acid	0.20:0.80	0.2066	0.7934	50
Octanoic acid	0.50:0.50	0.5102	0.4898	50
Octanoic acid	0.35:0.65	0.3594	0.6406	50
Nonanoic acid	0.50:0.50	0.4850	0.5130	50
Oleic acid	0.50:0.50	0.3472	0.6528	50
Propenoic acid	0.50:0.50	0.6758	0.3242	50
Tetradecanoic acid	0.50:0.50	0.7511	1.1419	59
Octadecanoic acid	0.50:0.50	0.7511	1.4224	70

ESM Table 2. Distribution coefficients and synergy coefficients for DESs based on thymol and octanoic acid.

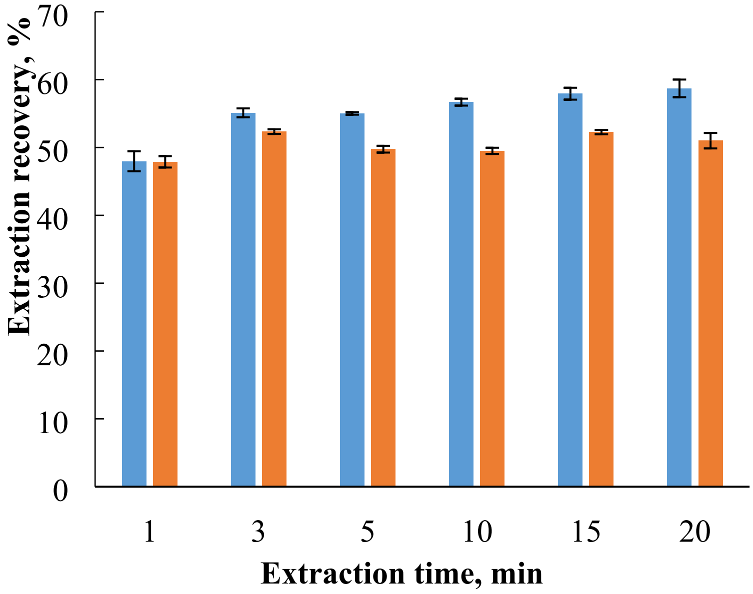
χ (octanoic acid)	D_{exp}		D_{add}		k	
	Chlortetracycline	Doxycycline	Chlortetracycline	Doxycycline	Chlortetracycline	Doxycycline
0	332	330	332	330	-	-
0.50	507	524	221	240	0.36	0.34
0.65	606	632	188	213	0.51	0.47
0.80	507	524	155	186	0.52	0.45
1.00	110	150	110	150	-	-



ESM Fig. 5. FT-IR spectra of thymol, octanoic acid and thymol-octanoic acid eutectics.



ESM Fig. 6. ^{1}H-NMR spectrum of DES based on thymol and octanoic acid ($CDCl_3$).



ESM Fig. 7. Effect of extraction time: C_{TCs} = 20 mg L^{-1}, $V_{organic\ phase}$ = 100 µL (n=3). chlortetracycline🟦 doxycycline🟧.

ESM Table 3. Quantitative features of the proposed procedure.

Parameter	Analyte	
	Chlortetracycline	Doxycycline
Linear range, µg L^{-1}	15-5000	30-5000
Correlation coefficient (n = 3)	0.998	0.999
Limit of detection, µg L^{-1}	5	10
Limit of quantification, µg L^{-1}	15	30
RSD (%) (n = 3)		
Intra-day (C = 30 or 5000 µg L^{-1})	3/2	3/2
Inter-day (C = 30 or 5000 µg L^{-1})	10/7	10/8
Extraction recovery, %	80	78
Enrichment factor	48	47

ESM Table 4. Figure of merits for the proposed method and reported methods based on liquid-liquid extraction for the determination of tetracyclines in milk.

Method	Sample preparation	Extractant	Extractant volume, mL	Sample amount	Extraction time, min	LOD	Linear range	RSD, %	Extraction recovery, %	Ref.
HPLC-DAD	HF-DLLME	octanol containing 20% ionic liquid (methyl trioctyl ammonium chloride, Aliquat-336)	-	3 mL	60	1.5 μg L^{-1} for TC; 2.1 μg L^{-1} for OTC; 3.6 μg L^{-1} for CTC; 0.95 μg L^{-1} for DC	5-1000 μg L^{-1} for TC, DC; 10-2500 μg L^{-1} for OTC;` 20-2500 μg L^{-1} for CTC	2-5	97	1
UHPLC-MS/MS	extraction, evaporation	0.05 mol L^{-1} EDTA in water, 0.1% formic acid in acetonitrile	20	1 g	-	-	0.5-100 μg kg^{-1}	2.3-28	≈99	2
HPLC-ED	LLE, evaporation	acetonitrile	40	10 mL	15	12.5 μg L^{-1} for OTC; 20 μg L^{-1} for TC; 25 μg L^{-1} for CTC, DC; 10 μg L^{-1} for MTC	50-1000 mg L^{-1}	0.5-4.3	-	3
UHPLC-MS/MS	SALLME, evaporation	EDTA-McIlvaine buffer, acetonitrile	4.7	1 g	>25	0.05-0.13 μg kg^{-1} for TC, OTC; CTC, DC, MTC	0.5-100 μg kg^{-1}	3.9-9.3	75	4
HPLC–DAD	LLME	DES based on thymol and octanoic acid	0.1	6 mL	<10	5 μg L^{-1} for CTC; 10 μg L^{-1} for DC	15-5000 μg L^{-1} for CTC; 30-5000 μg L^{-1} for DC	2-3	78-80	This work

Methods: HPLC-DAD – high-performance liquid chromatography with diode-array detection, UHPLC-MS/MS – ultrahigh-performance liquid chromatography-tandem mass spectrometry, HPLC-ED – high-performance liquid chromatography with a coulometric electrode array system; **Analytes**: TC – tetracycline, OTC – oxytetracycline, CTC – chlortetracycline, DC – doxycycline, MTC – methacycline. **Sample preparation methods**: HF-DLLME – hollow fiber membranes-based dynamic liquid-liquid microextraction; LLE – liquid–liquid extraction; SALLME – salting-out liquid-liquid microextraction, LLME – liquid-liquid microextraction.

References

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2. H. Zhao, J. Zulkoski, K. Mastovska, Development and validation of a multiclass, multiresidue method for veterinary drug analysis in infant formula and related ingredients using UHPLC-MS/MS, Journal of agricultural and food chemistry 65(34) (2017) 7268-7287. https://doi.org/10.1021/acs.jafc.7b00271.
3. F. Zhao, X. Zhang, Y. Gan, Determination of tetracyclines in ovine milk by high-performance liquid chromatography with a coulometric electrode array system, J. of Chrom. A 1055(1-2) (2004)109-114. https://doi.org/10.1016/j.chroma.2004.08.131.
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Buffer solution preparation

Acetic buffer solution ($pH = 6.5$) was prepared by dissolving 4.65 g of disodium ethylenediaminetetraacetate, 2.57 g of calcium chloride, 6.80 g of sodium acetate and 2.9 mL of acetic acid (85%) in 500 mL of ultra pure water, pH value was adjusted by $1\ \mathrm{mol\ L^{-1}}$ HCl or NaOH addition.