



SeaHARRE Technical Reports

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Method Summary Information

To ensure proper documentation of the methods used, each participant needs to submit summary information regarding method parameters following the tables used in the SeaHARRE-2 and SeaHARRE-3 reports.

Table 6. A summary of the extraction specifications for each of the methods. The volume of solvent added is given in milliliters. Each filter was disrupted for the indicated amount of time, allowed to soak for the specified number of hours, and then clarified.

<i>Meth. Code</i>	<i>Volume Added</i>	<i>Extraction Solvent</i>	<i>Internal Standard</i>	<i>Mode and Time of Disruption</i>	<i>Soak Time [h]</i>	<i>Clarification</i>
<i>C</i>	4.2	97.5% Acetone	Vitamin E acetate	Sonicating bath 15 min	15–18	Centrifuge and 0.2 μm Teflon syringe filter
<i>D</i>	3.099	100% Acetone	Vitamin E acetate	Sonicating bath ~ 10 min	24	0.45 μm Teflon syringe filter
<i>H</i>	1.832	95% Acetone	Vitamin E acetate	Sonic probe ~ 15 s	4	0.45 μm Teflon syringe filter
<i>J</i>	1.5	100% Acetone	<i>trans</i> - β -apo-8'-carotenal	Grinder 30 s	24	0.45 μm Teflon syringe filter
<i>L</i>	3.0	100% Methanol	Vitamin E acetate	Sonic probe ≤ 10 s	1 [†]	1.3 μm GF/C filter
<i>M</i>	2–6	100% Acetone	<i>trans</i> - β -apo-8'-carotenal	Sonic probe 30 s	0.5	Centrifuge 10 min (3,500 rpm)
<i>S</i> ₈	4.0	100% Acetone	<i>trans</i> - β -apo-8'-carotenal	Sonic probe <15 s	≥ 24	Centrifuge 4 min (5,100 rpm) [‡]
<i>S</i> ₁₈	4.0	100% Acetone	<i>trans</i> - β -apo-8'-carotenal	Sonic probe <15 s	≥ 24	Centrifuge 4 min (5,100 rpm) [‡]

[†] The sum of soaking for 0.5 h, sonicating, and then soaking for another 0.5 h.

[‡] Plus a 0.2 μm Teflon membrane filter.



Method Summary Information (*cont.*)

Table 7. A summary of the HPLC separation procedures used by the SeaHARRE-3 methods. Column particle size (P_s) is in units of micrometers, and column length (L_c) and diameter (D_c) are given in millimeters. Column temperature is denoted by T_c , and wavelength is denoted as λ .

Meth. Code	Stationary Phase	Column				Detector and Monitoring Wavelength	
		P_s	L_c	D_c	T_c	Manufacturer and Model	λ [nm]
<i>C</i>	C ₈	3.5	150	4.6	55°C	Waters PDA 996	436
<i>D</i>	C ₈	3.5	150	4.6	60°C	Shimadzu SPD-M10A VP-DAD	436, 450, and 665
<i>H</i>	C ₈	3.5	150	4.6	60°C	Agilent 1100	222†, 450, and 665
<i>J</i>	C ₁₈	5.0	250	4.6	Room	Agilent 1100	436 and 405
<i>L</i>	C ₈	3.5	150	3.0	60°C	Agilent 1100	440 and 667
<i>M</i>	C ₈	3.0	100	4.6	25°C	ThermoQuest UV6000	440 and 665
<i>S</i> ₈	C ₈	3.5	150	4.6	60°C	ThermoQuest UV6000	436 and 450
<i>S</i> ₁₈	C ₁₈	5.0	250	4.6	Room‡	ThermoQuest UV6000	436 and 450

† Used for monitoring vitamin E (the internal standard).

‡ Maintained at 18°C by a specialized air conditioner.

Table 8. A summary of the HPLC solvent systems used with the SeaHARRE-3 methods: MeCN is acetonitrile, NH₄Ac is ammonium acetate, EtOAc is ethyl acetate, MeOH is methanol, and TbAA is tetrabutyl ammonium acetate. The flow rate is in units of milliliters per minute.

Meth. Code	Injection Buffer	Flow Rate	Mobile Phase Solvent			Initial Conditions
			A	B	C	
<i>C</i>	TbAA:MeOH†	1.1	70:30 28 mM TbAA:MeOH	MeOH		95% A:5% B
<i>D</i>	TbAA:MeOH†	1.1	70:30 28 mM TbAA:MeOH	MeOH		95% A:5% B
<i>H</i>	TbAA:MeOH†	1.1	70:30 28 mM TbAA:MeOH	MeOH		95% A:5% B
<i>J</i>	1.0 M NH ₄ Ac	1.0	80:20 MeOH:0.5 M NH ₄ Ac	90:10 MeCN:Water	EtOAc	100% A
<i>L</i>	TbAA:MeOH†	0.55	70:30 28 mM TbAA:MeOH	MeOH		95% A:5% B
<i>M</i>	1.0 M NH ₄ Ac	1.0	70:30 MeOH:1.0 M NH ₄ Ac	MeOH		75% A:25% B
<i>S</i> ₈	TbAA:MeOH†	1.1	70:30 28 mM TbAA:MeOH	MeOH		95% A:5% B
<i>S</i> ₁₈	Water	1.0	80:20 MeOH:0.5 M NH ₄ Ac	90:10 MeCN:Water	EtOAc	100% A

† 28 mM TbAA:MeOH in a 90:10 (v:v) mixture, and the TbAA has a 6.5 pH.



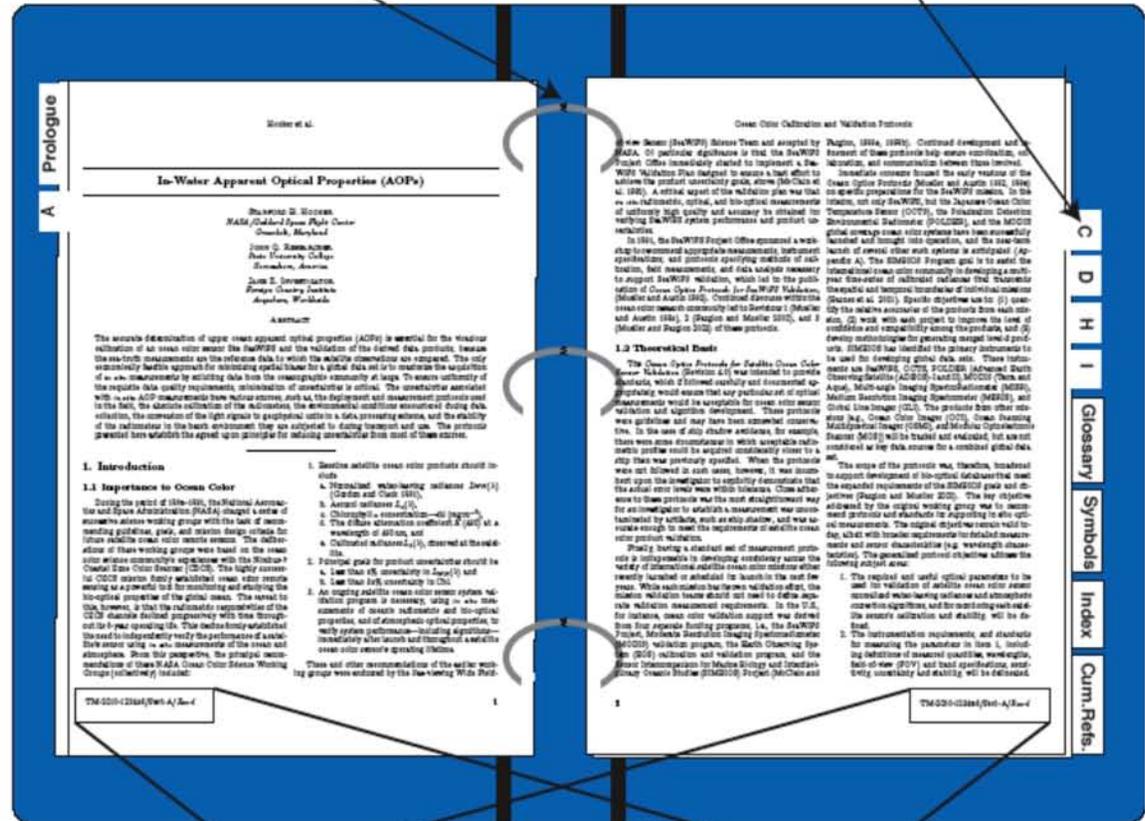
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A representation of the Protocols placed in a looseleaf binder with tabbed dividers for the glossary and symbols sections, in addition to the specialized sections for a high-level index and a cumulative references section.

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