Assessing Pressurized Liquid Extraction for the High-Throughput Extraction of Marine-Sponge-Derived Natural Products[⊥]

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In order to compare the utility of standard solvent partitioning (SSP) versus accelerated solvent extraction (ASE), a series of experiments were performed and evaluated. Overall yields, solvent consumption, processing time, and chemical stability of the fractions obtained by both methods were compared. Five marine sponges were selected for processing and analysis containing 12 structurally distinct, bioactive natural products. Extracts generated using SSP and ASE were assessed for chemical degradation using comparative LC MS-ELSD. The extraction efficiency (EE) of the ASE apparatus was 3 times greater than the SSP method on average, while the total extraction yields (TEY) were roughly equivalent. Furthermore, the ASE methodology required only 2 h to process each sample versus 80 h for SSP, and the LC MS-ELSD from extracts of both methods appeared comparable. These results demonstrate that ASE can serve as an effective high-throughput methodology for extracting marine organisms to streamline the discovery of novel and bioactive natural products.

Early milestone discoveries in marine natural products chemistry can be traced back to the seminal research conducted during the 1970s by Prof. Richard E. Moore on the metabolites of marine cyanobacteria.[1−3] Today, descriptions of nearly 20 000 marine-derived compounds[4] can be found in the literature and/or in commercial databases. Some of these structures are extremely significant, and examples to underscore this point include ziconotide (Prialt)[4] and ET-743 (trabectedin or Yondelis),[4] which are now available as clinical therapeutics. There are numerous other marine-derived lead compounds undergoing clinical evaluation[5] with dozens more undergoing advanced preclinical studies.[6]

The classical way to work up natural-product-containing extracts is often labor intensive and time-consuming.[4] Many discovery programs based on screening of extract libraries, bioassay-guided isolation, and dereplication/structure elucidation now use high-throughput screening (HTS) as an important filter.[7] Surprisingly, few investigators have explored a high-throughput approach for generating extracts. Several years ago we began to de-emphasize the classic Kupchan extraction scheme,[8,9] which involves standard solvent partitioning (SSP), in favor of the pressurized liquid extraction system[10] called accelerated solvent extraction (ASE).[11] The ASE apparatus is now widely used in marine environmental studies[12−15] and terrestrial-based natural products research,[10] as several comparative studies have validated its use as being both time and cost-effective.[16−19] However, there are no reports specifically describing the benefits or problems of employing ASE for the rapid discovery of bioactive marine natural products.

We can cite several successful examples from our recent research that have employed ASE as the first step in the isolation of novel sponge-derived natural products possessing varying functional groups.[20−24] Examples of new structures obtained using ASE are shown in Figure 1 and consist of a variety of biosynthetic classes ranging from terpenoids to alkaloids and peptides as well as compounds assembled from mixed biosynthetic origins. The specific examples shown include isojaspic acid (**1**),[20] aignopsanoic acid A (**2**),[21] plakinidine E (**3**),[22] psymbamide A (**4**)[23] and CTP-431 (**5**).[24] One early concern in employing the ASE apparatus during these and subsequent studies was that the high temperature (∼100 °C) and pressure (∼1700 psi) conditions utilized would alter or cause decomposition of the metabolites being isolated. Alternatively, the cycle time for extraction was minutes rather than the hours or days associated with standard extraction protocols. It seemed important to rigorously evaluate the ASE method from the viewpoint of comparing extraction efficiency (EE) and total extraction yields (TEY) to that associated with the traditional protocols.[9] We now report our experimental results of SSP versus ASE with an evaluation that considers overall extraction yields, solvent consumption, extraction time, and chemical stability.

Results and Discussion

Using collections housed in our repository we developed a test bed of diverse sponges and metabolites to guide this study and validate the advantages of using ASE versus SSP. Our evaluation consisted of five parts: (a) a comparison of extraction efficiencies (coded as EE = total organic extract/organic solvent use), (b) a comparison of total extract yields (coded as TEY = total organic extract/specimen weight × 100%), (c) the determination of whether a second or third pass through the ASE system was necessary based on the percentages of the overall extraction yields, (d) a comparison of the EE and TEYs for equivalent samples extracted using the ASE apparatus at 100 °C and at room temperature (∼22 °C), and (e) an analysis of LC MS-ELSD chromatograms to probe for chemical degradation. A total of five marine sponges (*Cacospongia mycofijiensis*, *Auletta* cf. *constricta*, *Zyzzya fuliginosa*, *Fascaplysinopsis reticulata*, and *Jaspis coriacea*) were selected for processing and evaluated. Eleven major metabolites have previously been reported from these aforementioned sponges and, similar to compounds **1**−**4** discussed above, possess a diverse array of bioactive structural motifs. These metabolites are outlined in Figure 2 and include fijianolide B (syn. laulimalide, **6**),[25] latrunculin A (**7**),[26] mycothiazole (**8**),[27] milnamide C (**9**),[28,29] jasplakinolide[28,30] (syn. jaspamide, **10**),[31] makaluvamines C, H, D, and J (**11**−**14**),[32,33] fascaplysin (**15**),[34,35] and bengamides A (**16**) and B (**17**).[36,37] These structures provided an excellent starting point to validate our previous observations that high-temperature and pressure conditions of ASE do not lead to the chemical degradation of the compounds being isolated.

The results displayed in Tables 1 and 2 from parallel ASE and SSP processing of an individual specimen of *C. mycofijiensis* (coll.

⊥ Dedicated to the late Dr. Richard E. Moore of the University of Hawaii at Manoa for his pioneering work on bioactive natural products.

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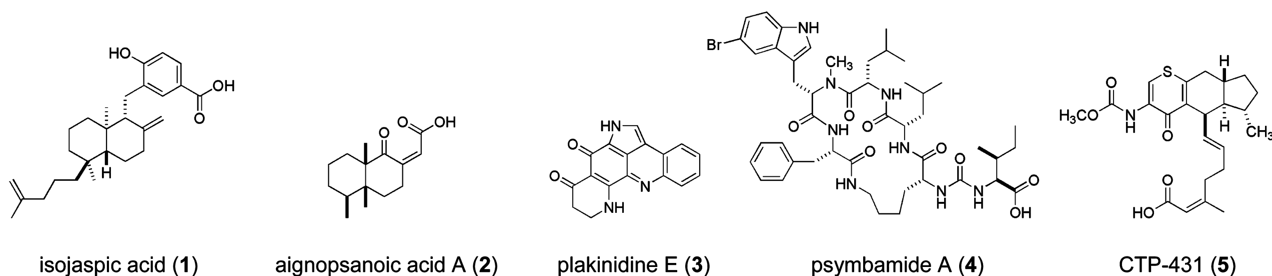

Figure 1. Examples of different marine natural product scaffolds isolated using accelerated solvent extraction (ASE).

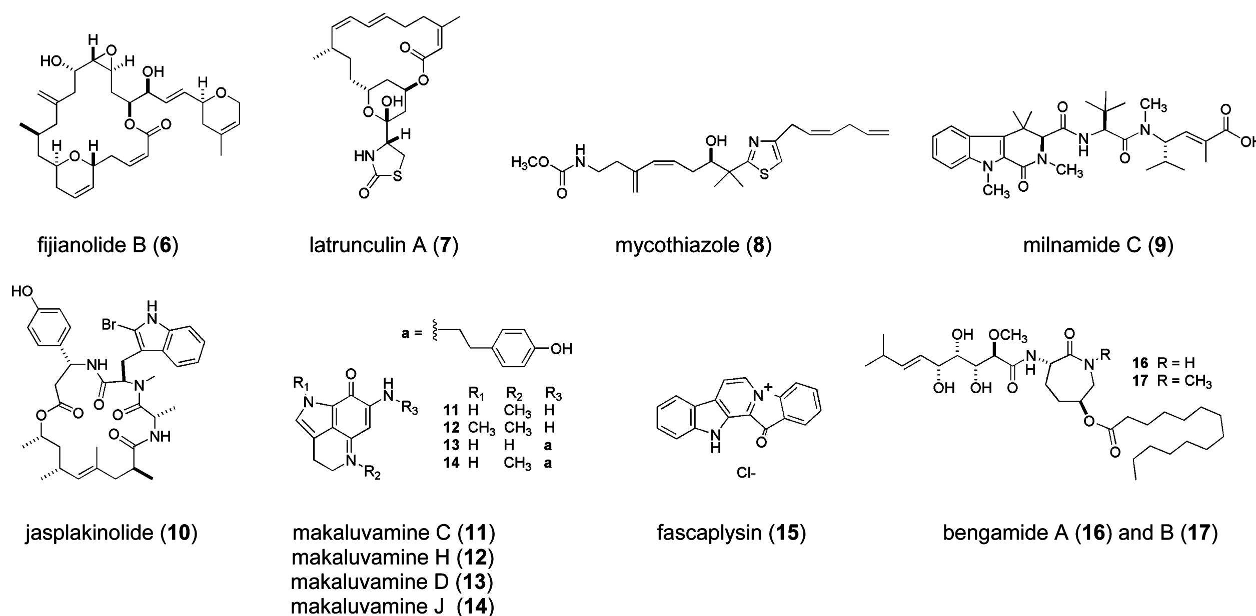

Figure 2. Summary of structurally distinct marine natural products from sponges selected for comparative processing using standard solvent partitioning (SSP) and accelerated solvent extraction (ASE).

Table 1. Extraction Yields of *Cacospongia mycofijiensis* (coll. no. 02600) Using SSP[a]

fraction code (volume)	MeOH extraction[b]	solvent partition/ evaporate	total
TPE	ND	1371.4 mg	1371.4 mg
W (100 mL)	ND	1289.3 mg	1289.3 mg
F (100 mL)	ND	80.1 mg	80.1 mg
WW (100 mL) "salts"	ND	1213.4 mg	1213.4 mg
WB (100 mL)	ND	75.1 mg	75.1 mg
FH (300 mL)	ND	21.1 mg	21.1 mg
FD (180 mL)	ND	20.7 mg	20.7 mg
FM (100 mL)	ND	38.2 mg	38.2 mg
total organic extract	ND	154.9 mg	154.9 mg
organic solvent use	1.5 L	0.68 L	2.18 L
process time	72 h	8 h	80 h
extraction efficiency (EE)[c]	ND	ND	71.0 mg/L
total extraction yield (TEY)[d]	ND	ND	1.3%

[a] Sample processed using an 11.5 g weight specimen. [b] Three successive extractions using MeOH (500 mL each) were performed and decanted after 24 h. [c] Total organic extract (mg)/solvent used (L). [d] Total organic extract (mg)/specimen weight (mg) × 100%. Codes: ND = not determined; TPE = total polar extract; W = water-soluble; F = fat-soluble; WW = water-soluble/water; WB = water-soluble/butanol; FH = fat-soluble/hexanes; FD = fat-soluble/dichloromethane; FM = fat-soluble/methanol.

no. 02600, 23.1 g wet wt) collected from Vanuatu were encouraging. The SSP extract (11.5 g wt weight) showed that the expected three major metabolites **6**−**8**[25] were present, as illustrated in the upper panel of Figure 3. The initial focus on *C. mycofijiensis* was motivated by the circumstance that **6**[38] and **8**[39] have been previously shown to be labile and rearrange or decompose under relatively mild conditions. Thus, the ability to observe these compounds in an extract processed by the ASE procedure represents a rigorous test. Processing of the SSP sample involved a modified Kupchan extraction scheme[9] (see Chart S1 and Experimental in the Supporting Information), and the results of this overall extraction are summarized in Table 1. The EE = 71.0 mg/L, and TEY = 1.4% and 80 h were required to carry out the SSP [including overnight methanol extractions (72 h), solvent partitioning (±8 h, depending on the formation of emulsions), but not including rotatory evaporation of the final extracts]. The ASE workup (on 11.6 g wet wt, see Table 2) began with an aqueous extraction (three successive exposures to afford samples coded XWW I = 1064.2 mg, XWW II = 43.5 mg, and XWW III = 34.4 mg) to selectively remove residual inorganic salts, often encountered in large concentrations from marine extracts.[40] The organic extraction was a bit involved, employing three independent runs (coded ASE run I, etc.) of three successive solvents: (a) hexanes (sample coded XFH I = 20.5 mg, XFH II = 4.4 mg, XFH III = 2.3 mg), (b) dichloromethane (sample coded XFD I = 43.3 mg, XFD II = 8.2 mg, XFD III = 2.1 mg), and (c) methanol (sample coded XFM I = 83.5 mg, XFM II = 14.2 mg, XFM III = 9.6 mg). The EE for the first run (ASE I = 246.0 mg/L) was three times greater than that observed for the SSP sample (71 mg/L). The TEY = 1.3% was roughly equivalent to the SSP sample (1.4%) yet required only 2 h to generate versus 80 h for the traditional method. Finally EE = 105.0 mg/L was the total for the combined ASE runs, which translates to a TEY = 1.6% (total time for processing = 6.0 h). In summary, the first extraction generated the highest percent yield of the total organic extract, which was 78%. Only small quantities of the total organic

Table 2. Extraction Yields of *Cacospongia mycofijiensis* (coll. no. 02600) Using ASE[a]

fraction code (volume)	ASE run I	ASE run II	ASE run III	total
XWW (200 mL) "salts"	1064.2 mg (93%)	43.5 mg (4%)	34.4 mg (3%)	1145.5 mg
XFH (200 mL)	20.5 mg (75%)	4.4 mg (16%)	2.3 mg (8%)	27.2 mg
XFD (200 mL)	43.3 mg (80%)	8.2 mg (15%)	2.1 mg (4%)	53.6 mg
XFM (200 mL)	83.5 mg (78%)	14.2 mg (13%)	9.6 mg (9%)	107.3 mg
total organic extract	147.3 mg (78%)	26.8 mg (14%)	14.0 mg (8%)	188.1 mg
organic solvent use	0.6 L	0.6 L	0.6 L	1.8 L
process time	2.0 h	2.0 h	2.0 h	6.0 h
extraction efficiency (EE)[b]	245.5 mg/L	44.7 mg/L	23.3 mg/L	104.5 mg/L
total extraction yield (TEY)[c]	1.3%	0.2%	0.1%	1.6%

[a] Sample processed using an 11.6 g weight specimen with percent yield in parentheses. [b] Total organic extract (mg)/solvent used (L). [c] Total organic extract (mg)/specimen weight (mg) × 100%. Codes: X = ASE; XWW = water-soluble/water; XFH = fat-soluble/hexanes; XFD = fat-soluble/dichloromethane; XFM = fat-soluble/methanol. Note: Processing time using ASE/solvent ≈30 min not including rotatory evaporation.

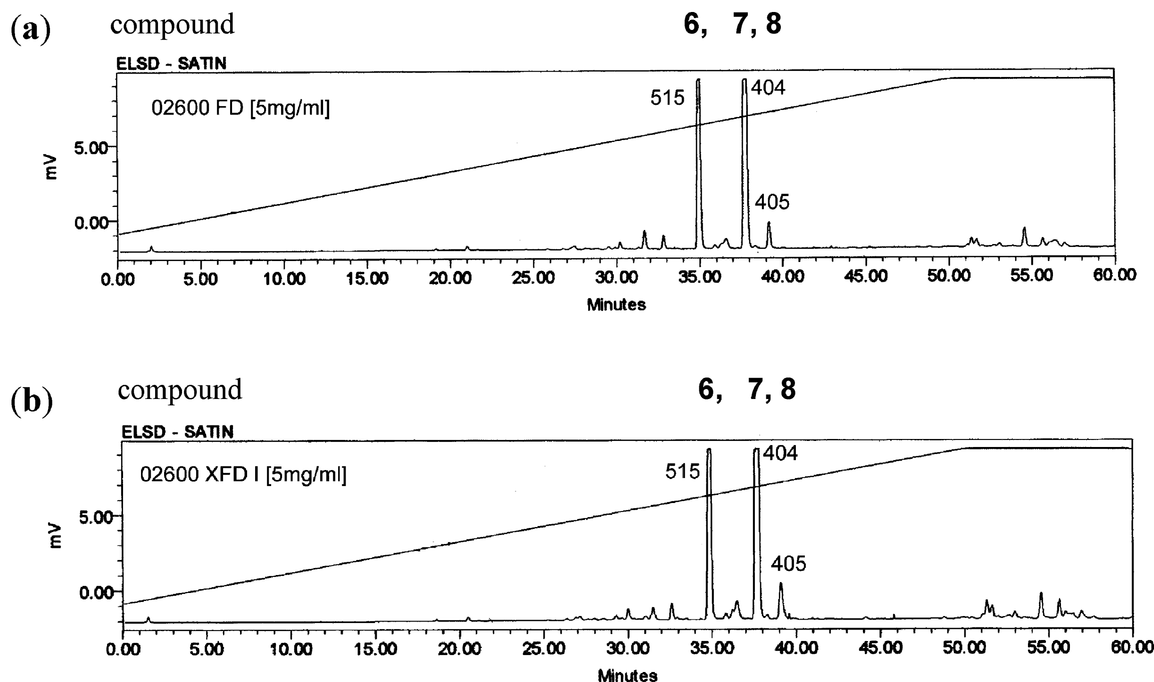

Figure 3. Examples of an ELSD analysis of *C. mycofijiensis* (coll. no. 02600) extracts processed using (a) SSP (FD) vs (b) ASE (XFD I) with annotations of m/z ions (fijianolide B (**6**), m/z = 515; $[M + H]^+$; isotopic molecular weight (IMW) = 514 amu; latrunculin A (**7**), m/z = 404; $[M - H_2O + H]^+$; IMW = 421 amu; mycothiazole (**8**), m/z = 405; $[M + H]^+$; IMW = 404 amu).

extract were obtained from the next two runs, 14% and 8%, respectively, which is consistent with results in the literature.[18] Shown in the bottom panel of Figure 3 is the LC MS-ELSD of the ASE crude extract, demonstrating parallel results observed for SSP. These results are also consistent with previous reports that have shown no evidence for thermal degradation of compounds during ASE extractions.[11,16,41−44]

A second fresh specimen of *C. mycofijiensis* (coll. no. 07327-O, 22.3 g wet wt), from a collection previously reported to contain compounds **2** and **6**−**8**,[21] was selected for further EE and TEY comparative processing. This involved using the ASE at room temperature (∼22 °C) and at 100 °C, and these results are summarized in Table 3. The EE = 86 mg/mL and TEY = 0.5% for the sample extracted at 22 °C was forty percent of the sample extracted at 100 °C (EE = 213 mg/mL, TEY = 1.2%). Shown in Figure 4 are the comparative LC MS-ELSD traces of the crude extracts known to contain compounds **2** and **6**−**8**, demonstrating that parallel results were observed for ASE processing at 22 and 100 °C.

The four additional sponges selected for comparative processing also gave encouraging but not quite parallel results. These organisms, all preserved according to our standard laboratory procedures, afforded constituents as follows: *A. constricta* (coll. no. 03505), **9** and **10**; *Z. fuliginosa* (coll. no. 03501), **11**−**14**; *F. reticulata* (coll. no. 05417), **15**; and *J. coriacea* (coll. no. 00102), **16** and **17**. Two

Table 3. Extraction Yields of *Cacospongia mycofijiensis* (coll. no. 07327 O) Using ASE[a] at 22 and 100 °C

temperature fraction codes (volume)	22 °C	100 °C
XWW (200 mL) "salts"	254.7 mg	992.4 mg
XFH (200 mL)	10.3 mg	25.0 mg
XFD (200 mL)	14.1 mg	35.2 mg
XFM (200 mL)	27.8 mg	67.8 mg
total organic extract	51.5 mg	128.0 mg
organic solvent use	0.6 L	0.6 L
process time	2.0 h	2.0 h
extraction efficiency (EE)[b]	85.8 mg/L	213.0 mg/L
total extraction yield (TEY)[c]	0.5%	1.2%

[a] Sample processed using 10.5 g wet weight specimens. [b] Total organic extract (mg)/solvent used (L). [c] Total organic extract (mg)/specimen weight (mg) × 100%. Codes: X = ASE; XWW = water-soluble/water; XFH = fat-soluble/hexanes; XFD = fat-soluble/dichloromethane; XFM = fat-soluble/methanol. Note: Processing time using ASE/solvent ≈ 30 min not including rotatory evaporation.

identical samples (100 g wet wt) were divided equally and processed by SSP and ASE according to the methods outlined above. The results of these overall extractions are summarized in Table 4 (as entries 2−5) and Tables S1−S4 in the Supporting Information. In every case the EE was greater for ASE I versus that for SSP total. The results pertaining to TEY % fell into two categories: ASE I yielding TEYs > SSP total for entries 2 and 3, and vice versa for entries 4 and 5. An inspection of the percent yield of the total

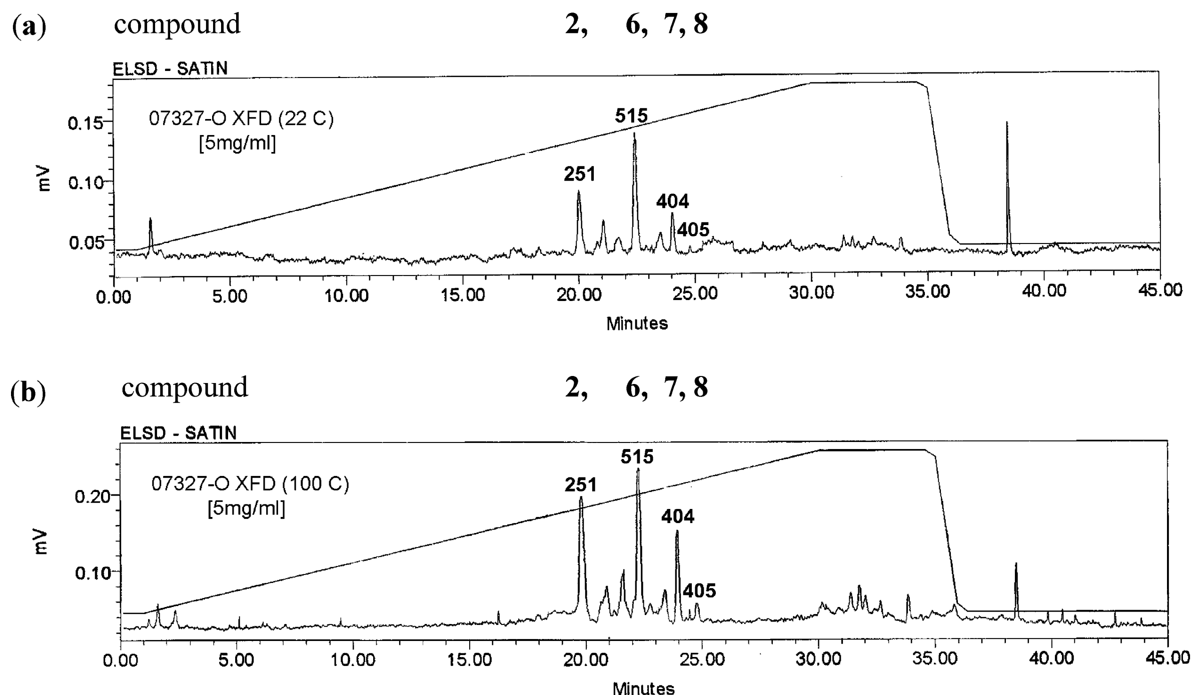

Figure 4. Examples of an ELSD analysis of *C. mycofijiensis* (coll. no. 07327-O) extracts processed using (a) ASE 22 °C (XFD) vs (b) ASE 100 °C (XFD) with annotations of m/z ions (aignopsanoic acid A (**2**), m/z = 251; $[M + H]^+$; isotopic molecular weight (IMW) = 250 amu; fijianolide B (**6**), m/z = 515; $[M + H]^+$; IMW = 514 amu; latrunculin A (**7**), m/z = 404; $[M - H_2O + H]^+$; IMW = 421 amu; mycothiazole (**8**), m/z = 405; $[M + H]^+$; IMW = 404 amu).

Table 4. Summary of Extraction Efficiency (EE)[a] and Total Extraction Yield (TEY)[b] Using SSP and ASE (100 °C) of Five Marine Sponges

			SSP total		ASE I		ASE II		ASE III		ASE total	
entry	sample	coll. no.	EE	TEY	EE	TEY	EE	TEY	EE	TEY	EE	TEY
1	*C. mycofijiensis*	02600	71 mg/L	1.3%	246 mg/L	1.3%	45 mg/L	0.2%	23 mg/L	0.1%	105 mg/L	1.6%
2	*A. constricta*	03505	349 mg/L	1.5%	1,398 mg/L	1.7%	249 mg/L	0.3%	121 mg/L	0.1%	589 mg/L	2.1%
3	*Z. fuliginosa*	03501	375 mg/L	1.6%	1,607 mg/L	1.9%	303 mg/L	0.4%	158 mg/L	0.2%	688 mg/L	2.5%
4	*F. reticulata*	05417	423 mg/L	1.8%	596 mg/L	0.7%	210 mg/L	0.2%	127 mg/L	0.2%	311 mg/L	1.1%
5	*J. coriacea*	00102	216 mg/L	0.9%	481 mg/L	0.6%	264 mg/L	0.3%	117 mg/L	0.1%	287 mg/L	1.0%
	average totals		287 mg/L	1.4%	866 mg/L	1.5%	214 mg/L	0.3%	109 mg/L	0.2%	396 mg/L	1.6%
	process time		80 h		2 h		2 h		2 h		6 h	

[a] EE = total organic extract (mg)/solvent used (L). [b] TEY = total organic extract (mg)/specimen weight (mg) × 100%.

Table 5. Summary of Extraction Efficiency (EE)[a] and Total Extraction Yield (TEY)[b] Using ASE at 22 and 100 °C of Five Marine Sponges

			temperature			
			22 °C	22 °C	100 °C	100 °C
entry	sample	coll. no.	EE	TEY	EE	TEY
1	*C. mycofijiensis*	07327	86 mg/L	0.5%	213 mg/L	1.2%
2	*A. constricta*	03505	531 mg/L	0.6%	1,288 mg/L	1.5%
3	*Z. fuliginosa*	03501	449 mg/L	0.5%	1,543 mg/L	1.9%
4	*F. reticulata*	05417	373 mg/L	0.4%	632 mg/L	0.8%
5	*J. coriacea*	00102	349 mg/L	0.4%	505 mg/L	0.6%
	average totals		357 mg/L	0.5%	836 mg/L	1.3%
	process time		2 h		2 h	

[a] EE = total organic extract (mg)/solvent used (L). [b] TEY = total organic extract (mg)/specimen weight (mg) × 100%.

organic extract of the ASE runs I−III (see Tables S1−S4 in the Supporting Information) indicated that the majority of the extract is generated in the first ASE extraction for entries 2−5 as reported above for coll no. 02600. Comparisons using LC MS-ELSD of the crude extracts previously reported to contain the known major components of all these sponges, **9**−**16** (Figures S1−S4 in the Supporting Information), were made with those of SSP and ASE and all exhibited comparable patterns of elution time, percent composition, and the detection of m/z ions for the major metabolites, indicating there was no detectable chemical degradation.

Additional specimens of the above four sponges were also selected for EE and TEY comparative processing using the ASE at room temperature (∼22 °C) and 100 °C. These results are summarized in Table 5 (and Supporting Information Tables S5−S8). The average EE (357 mg/L) and TEY (0.5%) for entries 2−5 of samples extracted at 22 °C were considerably lower than for those extracted at 100 °C (EE = 836 mg/mL, TEY = 1.3%). These data are consistent with reports that have shown that 100 °C is an optimal temperature for generating maximum yields during ASE.[10,45] The LC MS-ELSD analysis of the five crude extracts known to contain

compounds **9**−**16** showed that all could be observed (see Supporting Information, Figures S1−S4) using the ASE apparatus set at either 22 or 100 °C.

Our experience using ASE has shown us that it can function as a robust high-throughput approach that can be both highly efficient and rewarding.[20,22−24] A particular advantage we have found to employing this method resides in its ability to be incorporated prior to the production of 96-well plate peak libraries to streamline HTS bioassay evaluation.[46] More recently we have appreciated added benefits from using ASE to rapidly extract a large colony of individual sponges (15 single-organism specimens) of *C. mycofijiensis* for LC MS-ELSD chemical profiling that culminated in the discovery of a novel class of sesquiterpenes.[21] These results continue to provide us with further stimulation to incorporate this added high-throughput methodology into our marine natural products discovery pipeline.

In conclusion, a number of noteworthy outcomes have emerged from our pilot survey involving comparative extractions of five marine sponges using SSP and ASE and are summarized in Tables 4 and 5. First, the average total EE (287 mg/L) of SSP samples is much lower than just one extraction using the ASE apparatus (ASE I, 866 mg/L). The average TEYs are roughly equivalent, ∼1.5%, for both methods; however, the ASE I processing time (2 h) is considerable less than for SSP (80 h). Second, a single pass (ASE I, 866 mg/mL, 1.5%) through the ASE system appears sufficient, as the average EE and TEY of the second (ASE II, 214 mg/L, 0.3%) or third (ASE III, 109 mg/L, 0.2%) run were much less than the first extraction ASE I. Also noteworthy is that the ASE and SSP extractions displayed varying organic extract yields depending on the sponge specimen processed, thereby indicating neither method was optimal for obtaining maximum yields. Furthermore, the average EE (357 mg/mL) and TEY (0.5%) obtained using ASE at 22 °C are clearly lower than those generated at 100 °C (EE = 836 mg/mL, TEY = 1.3%). However, the former approach can be applied to samples suspected of containing thermally labile compounds, while the yields obtained are sufficient to allow for LC MS profiling and the preparation of peak libraries.[47,48] A final important observation is that the chemical stability of 100 °C ASE extracts using LC MS-ELSD analysis appeared comparable to those generated using SSP or ASE at room temperature. Overall, these results demonstrate that employing ASE to process marine sponges can serve as an effective high-throughput methodology for the rapid discovery of novel and bioactive marine natural products.

Experimental Section

General Experimental Procedures. Analytical LC MS analysis was performed on all samples at a concentration of approximately 5 mg/mL, using a reversed-phase 150 × 4.60 mm 5 μm C_{18} Phenomenex Luna column. Samples were injected onto the column using a volume of 15 μL, with a flow rate of 1 mL/min that was monitored using a Waters model 996 photodiode array UV detector. The elution was subsequently split (1:1) between a SEDERE model 55 evaporative light scattering detector (ELSD) and an Applied Biosystems Mariner electrospray ionization time-of-flight (ESI-TOF) mass spectrometer.

Biological Material, Collection, and Identification. The sponges profiled for these experiments were obtained using scuba at depths of 15−30 m. Specimens of *Cacospongia mycofijiensis* (coll. no. 02600, 23 g wet wt, and 07327-O, 20 g wet wt) were collected in 2002 from Mele Bay, Vanuatu,[25] and Kimbe Bay, Papua New Guinea.[21] Samples of *Auletta* cf. *constricta* (coll. no. 03505; 100.3 g wet wt) and *Zyzzya fuliginosa* (coll. no. 03501; 100.3 g wet wt) were acquired in 2003 from Milne Bay, Papua New Guinea. Specimens of *Jaspis coriacea* (coll. no. 00102; 100.5 g wet wt) were collected in 2000 from the Beqa Lagoon, Fiji, while samples of *Fascaplysinopsis reticulata* (coll. no. 05417, 100.8 g wet wt) were obtained in 2005 from the Rabaul Province in Papua New Guinea. Taxonomic identifications were based on comparison of the biological features to other voucher samples in our repository. The secondary metabolite chemistry is also consistent with these identifications.[25,28,32,34,36] Voucher specimens and underwater photos are available.

Extraction and Isolation. Samples were preserved in the field by being immersed in a 50:50 MeOH/H_2O solution. After approximately 24 h this solution was decanted and discarded. The damp organisms were placed in collection bottles (Nalgene) and shipped back to UCSC at ambient temperature and then stored at 4 °C until further processed. Individual specimens of each sponge (100 g wet wt) were bifurcated into equal portions (approximately 50 g each unless otherwise specified) and processed by SSP using a modified Kupchan extraction scheme (see Supporting Information, Chart S1) or four times using ASE (see Supporting Information, Chart S2). Samples undergoing SSP were first extracted using 100% methanol three successive times for 24 h. The solvent was evaporated at room temperature, and the resulting oil was partitioned between water (sample coded "W") and dichloromethane (sample coded "F" for fats). The W fraction was next partitioned between water (sample coded "WW"), which contained mostly inorganic salts, and *sec*-butyl alcohol (sample coded "WB"). The concentrated F was then partitioned between hexanes three times (sample coded FH), to remove unwanted lipids and steroid components, and 10% aqueous methanol. The methanol layer was adjusted to 50% aqueous methanol, and an equal volume of dichloromethane was added. The dichloromethane fraction (coded "FD") and the methanol fraction (coded "FM") were evaluated separately.

ASE samples were processed using a Dionex model 100 ASE.[11] The experimental settings of the ASE model 100 used in this study are outlined in the Supporting Information. Samples were extracted after being preserved, and stored according to the method described above. On the basis of the hydroscopic nature of the sponge, samples were processed immediately as damp specimens (02600, 07327, 03505, 00102) or dried in a fume hood for 12 h (03501, 05417) prior to extraction. During each ASE extraction, samples were exposed to 200 mL of solvent for around 30 min at 100 or 22 °C under a pressure of ∼1700 psi (using nitrogen) based on successful experimental parameters reported by others.[10,45] The samples were initially extracted using distilled H_2O (sample coded XWW) to obtain the aqueous extract and to remove residual inorganic salts. The organic extraction involved three successive passes with solvents of (a) hexanes to remove unwanted lipids (samples coded as XFH, I−III), (b) dichloromethane (samples coded as XFD, I−III), and (c) methanol (samples coded as XFM, I−III). The XFD and XFM extracts were evaluated separately. Between each separate solvent extraction (water, hexanes, dichloromethane, and methanol) a rinse step was employed by removing the sample cell from the apparatus and replacing it with a "rinse cell" for approximately 3 min to flush the system of residual solvents between runs. The sample cell was then reinserted into the apparatus, and subsequent extractions were performed as described above. Samples did not need to be removed from the cell to be dried or washed with a miscible solvent prior to going from extractions with water to hexanes, followed by dichloromethane, and methanol. Our experience with the ASE system processing marine sponges of sample size ≥50 g would occasionally lead to added back-pressure (e.g., preventing the solvent from filling the sample cell), requiring the operator to abort the method, delaying overall processing times and complicating the extraction process. Furthermore, we saw no evidence that yields were increased if specimens were blended into a fine powder versus specimens processed as dice size whole organisms. In actuality, samples prepared as an amorphous powder using the blender method posed additional complications related to system back-pressure as noted above.

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Supporting Information Available: Two charts, eight tables, and four figures are provided. These data include the general experimental procedures, schematics of the experimental procedures, and the comparative extract yields, solvent consumption, extraction times, and LC MS-ELSD analysis of coll. nos. 03505, 03501, 05417, and 00102 using SSP and ASE processing methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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