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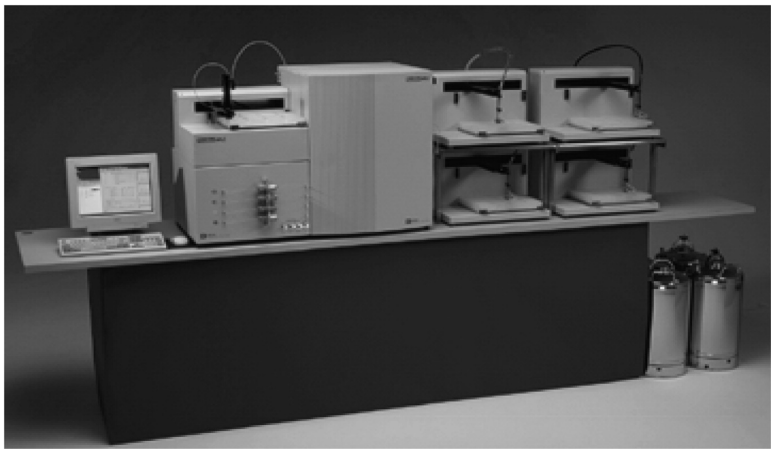

# High-Throughput Purification of Combinatorial Arrays

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Performance and reproducibility of the Biotage Parallex, a high-throughput purification system, was evaluated using known standards. The results indicate that parallel purification is a robust technique for purifying large numbers of compounds. Results from one of the first libraries to be purified on the Biotage Parallex are presented and discussed. Since fractionation by UV can often result in a large number of fractions, threshold trigger versus yield and number of fractions was also investigated. This approach was used to purify an array of 4320 compounds, produced by an 11-step solid-phase synthesis in Irori MicroKans. Ninety-three percent of the compounds were successfully processed, with >90% having purity >95%.

## Introduction

Within the drug discovery process, lead discovery and lead optimization have undergone revolutionary changes over the past decade, driven largely by demand for greater numbers of compounds for high-throughput screening. The widespread introduction of high-throughput chemistry, coupled with application of automation, has had a significant impact on the number of compounds synthesized.[1,2] This dramatic increase in chemistry productivity, coupled with a growing trend toward high quality single compounds, that is, pure, quantified, and characterized, has provided new challenges for analysis, purification and downstream processing.

Often, a purification step is necessary to bring the compounds to acceptable purity. Simple cleanup may be achieved by using scavenger resins,[3] liquid−liquid extraction,[4] or simple solid-phase extraction[5] in which byproducts or excess reagents can readily be removed from solution-phase reactions. All of these techniques are readily automated where they may be incorporated into synthesizers or separate liquid handling robots.

Most large libraries are purified by reversed-phase HPLC, usually in serial mode with fractionation triggered by UV. Throughput of samples can be increased by the use of short columns, rapid gradients, and high flow rates. It has been reported that it is possible to purify 200 samples/day on a single system using this approach.[6] Higher throughput can be achieved by purchase of multiple systems, which often presents issues with data handling and laboratory space.

Mass-directed fractionation has become a popular tool over the past few years, restricting the number of fractions collected to those of selected molecular weights.[7,8] Increased throughput on these systems has been achieved by purifying several products per run[9] or configuring the system to run two columns in parallel.[10] Typical throughput on a commercial prep LC/MS is ∼96 samples/day.[7] This technology is expensive and requires greater expertise than systems using UV detection and is perhaps more appropriate for specialist applications.

The Parallex HPLC was the first high-throughput purification system to be introduced, enabling purification of up to 40 samples/hour running four columns in parallel.[11,12] Although this system can be used for reversed-phase, normal phase, and ion exchange applications, reversed phase is usually preferred, utilizing a few generic methods. The flow rate range of the standard configuration is 5−50 mL/min/flow stream with a pressure limit of 4000 psi, allowing use of a wide range of column sizes and packing materials. Samples are introduced from a deep-well microtiter plate into the four loops sequentially during a method cycle and injected simultaneously at the beginning of the next method cycle to maximize use of time. To minimize precipitation when the samples come into contact with the aqueous solvent conditions at the beginning of the gradient, the samples are sandwiched between a less aqueous solvent in the loop. All columns run with the same binary gradient (U.S. patent 5,089,124), and each flow stream passes through a four-channel flow cell where they are monitored at two wavelengths. Each flow stream has its own intelligent fraction collector where fractionation is triggered by threshold, slope, or both on one or both wavelengths into deep-well microtiter plates or tubes.

This paper presents some basic data to demonstrate the functionality of this four-flow-stream system and how it has been exploited for the high-throughput purification of combinatorial libraries.

## Experimental Section

**Chemicals.** *p*-Hydroxy-benzoic acid ethyl and methyl ester (methyl and ethyl paraben) were obtained from Sigma-Aldrich (Poole, U.K.). HPLC-grade solvents were obtained

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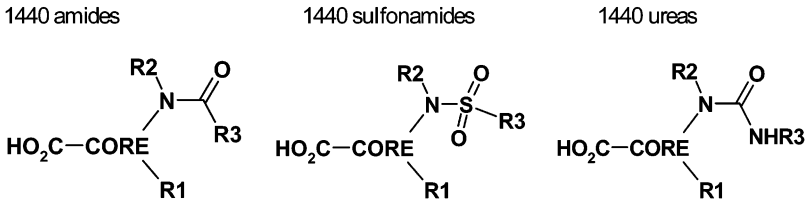
**Chart 1**




from Fischer (Loughborough, U.K.) unless specified. Pure water was obtained from a Milli-Q system (Millipore, Watford, U.K.).

**Recovery of Known Standards.** Four standard solutions containing 5, 10, 20, and 40 mg/mL each of methyl and ethyl parabens were made up in HPLC grade methanol. Each solution was injected (0.5 mL) three times on each of the four flow streams. Samples were separated on four YMC-Pack ODS-AM columns (20 mm i.d. × 100 mm long, 5 $\mu$m; YMC, Germany). Guard columns (20 mm i.d. × 10 mm long; YMC, Germany) packed with 10-$\mu$m ODS-AM were used to protect the columns. The mobile phase was water (A) and acetonitrile (B), both containing 0.1% trifluoroacetic acid. Separation was achieved using a linear gradient starting at 10% B increasing to 90% B over 10 min with 4 min equilibration time. Flow rate was 20 mL/min, and the eluent was monitored at 219 and 254 nm. Fractions were collected in 48 × 5-mL deep-well microtiter plates (Whatman, U.K.). Aliquots of the fractions were removed and analyzed by HPLC as described in "analysis of fractions".

**Optimization of Fractionation.** Stock solutions of the two components were made up in methanol at a concentration of 5.3 mg/mL of each component. Injection volume was 0.5 mL. Samples were separated as described above.

Fractions were collected on the basis of UV threshold at 0.25, 0.5, 0.75, and 1.0 AU (absorbance units) with three replicate injections on each channel. Fractions were analyzed as described below.

**Analysis of Fractions.** Compounds in the fractions were quantified by reversed-phase HPLC using a Waters 490 multichannel detector, a quaternary solvent delivery system, and a WISP autosampler. Samples were separated on a Symmetry C18 column (150 × 4.6 mm i.d., 5-$\mu$m particle size; Waters). An isocratic mobile phase of 40% acetonitrile and 60% water was used at a flow rate of 1 mL/min. Eluent was monitored at 214, 254, and 280 nm. Methyl and ethyl paraben were quantified by external standardization.

**Synthesis of the First Array.** An array of 96 samples was prepared on solid phase using Irori Microkan technology.

**Synthesis of the Second Array.** A multistep synthesis of 4320 compounds was carried out on solid phase using Irori Microkans. The introduction of 12R1, 8R2, and 15R3 variables on a core template gave 3 × 1440 arrays (Chart 1).

**Purification of Synthetic Libraries.** Compounds from the first array were purified on the Parallex HPLC using Zorbax SB-C18 columns (21.2 mm i.d. × 75 mm long; 7-$\mu$m particle size; Hichrom, Theale, U.K.).

Compounds from the second array were purified using four Supelco C18 ABZ+ columns (21 mm i.d. × 75 mm long; 12-$\mu$m particle size; Aldrich, Poole, U.K.). Guard columns packed with 80 SB-C8 (9.4 mm i.d. × 15 mm long; 7-$\mu$m particle size, Agilent Technologies), were used to prolong column life, enabling up to 4000 injections/column. The mobile phase was water (A) and acetonitrile (B), both containing 0.1% trifluoroacetic acid. The sandwich solvent was acetonitrile/water (50/50), and samples were taken up in 0.5 mL of DMSO/methanol (50/50). Compounds were separated using a rapid gradient increasing from 20 to 95% B in 7 min followed by a hold at 95% B for 2 min and then reequilibration for 2.5 min at a flow rate of 20 mL/min. Eluent was monitored at 219 and 254 nm. Decisions for fractionation were initially a threshold of 0.5 AU and a slope of 0.1 AU/s when the threshold was >0.2 AU on both. To reduce the number of fractions, the fractionation decisions were later changed so that collection was based on 254 nm only, with a threshold of 1 AU and a slope of 0.1 AU/s.

**Analysis of Library Fractions before and after Purification.** Crude products were analyzed by HPLC with UV and mass detection (HP 1100 with diode array and Micromass ZMD detector) to confirm mass and determine purity. Those products below the target purity were purified. Compounds were separated on a Zorbax SB-C18 cartridge column (2.1 mm i.d. × 30 mm long; 3.5-$\mu$m particle size; Agilent, Stockport, U.K.). The mobile phase was water (A) and acetonitrile (B), both containing 0.1% TFA. Samples were separated using a rapid gradient increasing from 5 to 100% B in 2 min at a flow rate of 1.5 mL/min at 40 °C. UV was monitored from 210 to 220 nm, and *m*/*z* was monitored from 181 to 897 amu.

Fractions from the Parallex were selected for analysis on the basis of UV absorbance and retention, as compared to initial QC of the crude product, using custom software (Winnow).[13] Selected fractions were analyzed as described above, but the mass detector was a Finnigan aQa (Thermoquest, U.K.).

## Results and Discussion

**Recovery of Methyl and Ethyl Paraben from Parallex.** Different concentrations of methyl and ethyl parabens were recovered in high yields across all four channels of the Parallex HPLC, as shown in Table 1. Where percentage recovery was slightly high, this was most likely due to minor evaporation while they were on the autosampler.

**Optimization of Fractionation.** As expected, there was a reduction in yield of both standards as the threshold for triggering collection was increased (Table 2). The recoveries on channel D (Table 2) were lower than the other three as a result of column degradation and tailing. However, triggering fractionation at a higher threshold resulted in one fraction being collected, as opposed to two, at the lower threshold (Figure 1). This has serious implications on the number of

**Table 1.** Yield of Methyl and Ethyl Paraben Using Parallex HPLC

| | | yield of parabens at increasing concns mg ± SD (% recovery) | | | |
|---|---|---|---|---|---|
| | | 2.5 mg | 5.0 mg | 10 mg | 20 mg |
| channel A | methyl | 2.58 ± 0.075 (101) | 5.08 ± 0.065 (101) | 9.62 ± 1.06 (96) | 20.84 ± 0.71 (104) |
| | ethyl | 2.33 ± 0.062 (93) | 4.74 ± 0.043 (95) | 9.57 ± 0.26 (96) | 19.26 ± 0.27 (96) |
| channel B | methyl | 2.50 ± 0.057 (100) | 4.76 ± 0.31 (95) | 10.33 ± 0.19 (103) | 20.67 ± 0.47 (103) |
| | ethyl | 2.21 ± 0.035 (89) | 4.54 ± 0.17 (91) | 9.49 ± 0.19 (95) | 20.05 ± 0.77 (100) |
| channel C | methyl | 2.42 ± 0.01 (97) | 4.60 ± 0.15 (92) | 9.79 ± 0.32 (98) | 20.72 ± 1.01 (103) |
| | ethyl | 2.28 ± 0.08 (91) | 4.46 ± 0.07 (90) | 9.82 ± 0.53 (98) | 19.20 ± 1.04 (96) |
| channel D | methyl | 2.38 ± 0.11 (95) | 4.66 ± 0.15 (93) | 10.12 ± 0.44 (101) | 20.15 ± 0.27 (101) |
| | ethyl | 1.99 ± 0.12 (89) | 4.45 ± 0.33 (90) | 9.37 ± 0.56 (94) | 19.38 ± 1.43 (97) |

**Table 2.** Yield of Methyl and Ethyl Paraben When Fractionation Was Triggered at Increasing Threshold

| | | yield of parabens when collected, threshold values mg ± SD (% recovery) | | | |
|---|---|---|---|---|---|
| | | 0.25 AU | 0.5 AU | 0.75 AU | 1.0 AU |
| channel A | methyl | 2.45 ± 0.06 (92) | 1.97 ± 0.15 (74) | 1.64 ± 0.12 (62) | 1.39 ± 0.06 (52) |
| | ethyl | 2.40 ± 0.03 (91) | 1.81 ± 0.12 (68) | 1.63 ± 0.11 (62) | 1.24 ± 0.37 (47) |
| channel B | methyl | 2.41 ± 0.02 (91) | 1.89 ± 0.08 (71) | 1.59 ± 0.19 (60) | 1.53 ± 0.03 (58) |
| | ethyl | 2.31 ± 0.07 (87) | 1.78 ± 0.10 (67) | 1.60 ± 0.07 (60) | 1.49 ± 0.06 (56) |
| channel C | methyl | 2.40 ± 0.04 (91) | 1.86 ± 0.09 (70) | 1.88 ± 0.02 (71) | 1.57 ± 0.02 (59) |
| | ethyl | 2.47 ± 0.07 (93) | 1.81 ± 0.10 (68) | 1.75 ± 0.16 (66) | 1.39 ± 0.08 (52) |
| channel D | methyl | 2.51 ± 0.09 (95) | 1.77 ± 0.22 (67) | 1.40 ± 0.05 (53) | 0.90 ± 0.24 (34) |
| | ethyl | 2.31 ± 0.09 (87) | 1.65 ± 0.12 (62) | 1.43 ± 0.10 (54) | 0.94 ± 0.17 (35) |

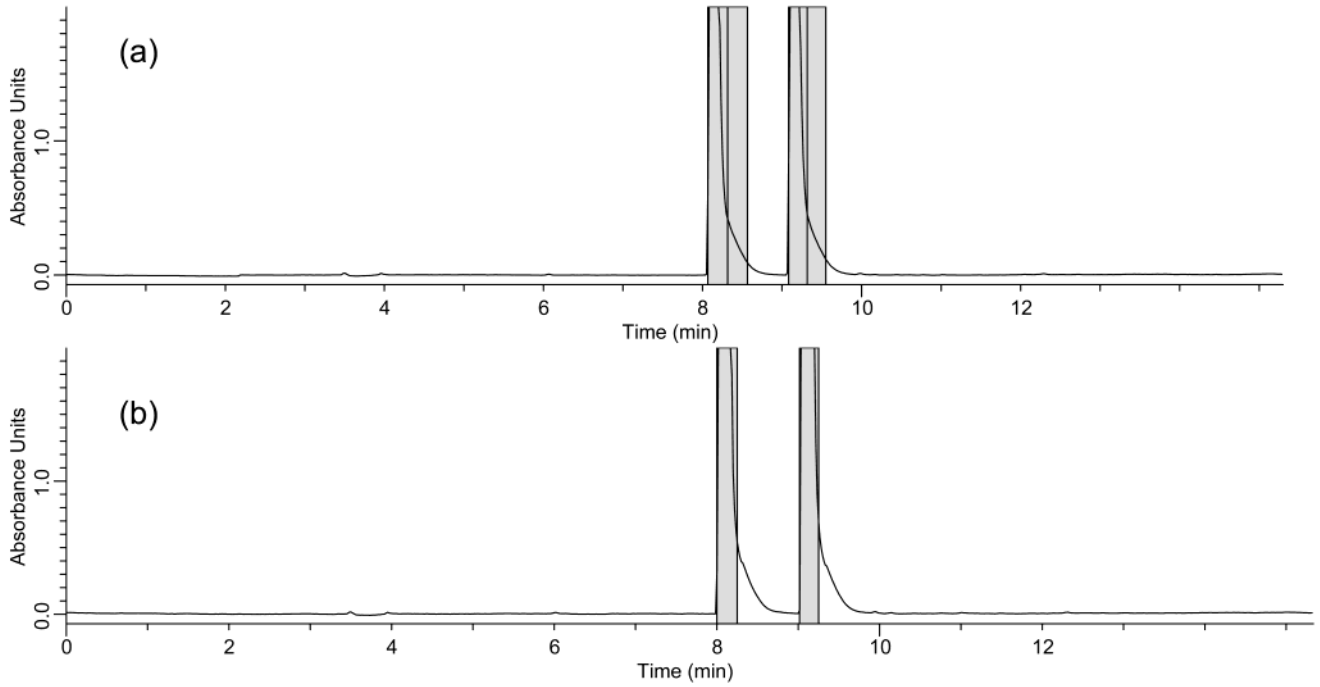



**Figure 1.** Separation of methyl and ethyl parabens where fractionation was triggered at a threshold of 0.25 (a) and 1 AU (b).

fractions collected per sample in a high-throughput environment. In such situations, a small sacrifice of yield to ensure high quality fractions, increased capacity of the fraction collector, and hence, the number of samples processed. As the concentration of compounds is increased, the percentage of compound lost due to collection at high threshold, is reduced. Table 3 shows recovery of methyl and ethyl parabens (10 mg each injected) when fraction collection was triggered at 1 AU.

**Purification of First Array.** Table 4 shows one-half of the results from the first array. "Fail" indicates samples that were below the acceptable purity threshold or were not mass-confirmed. Eighty-one percent of the compounds were >80% pure, and most were >90% pure, despite the fact that this was one of the first arrays to be purified and the selected columns were not ideal. Although compound yields were generally good, several samples were significantly lower than expected (e.g., 18.5 and 23.9%), opening up one of the most debated topics in purification: Why does the amount of purified material often not correspond to the anticipated yield? Working with large numbers of compounds with diverse chemistry, it is sometimes difficult to prove exactly what proportion of the crude sample is actually product, given that no single method of detection is universal for all

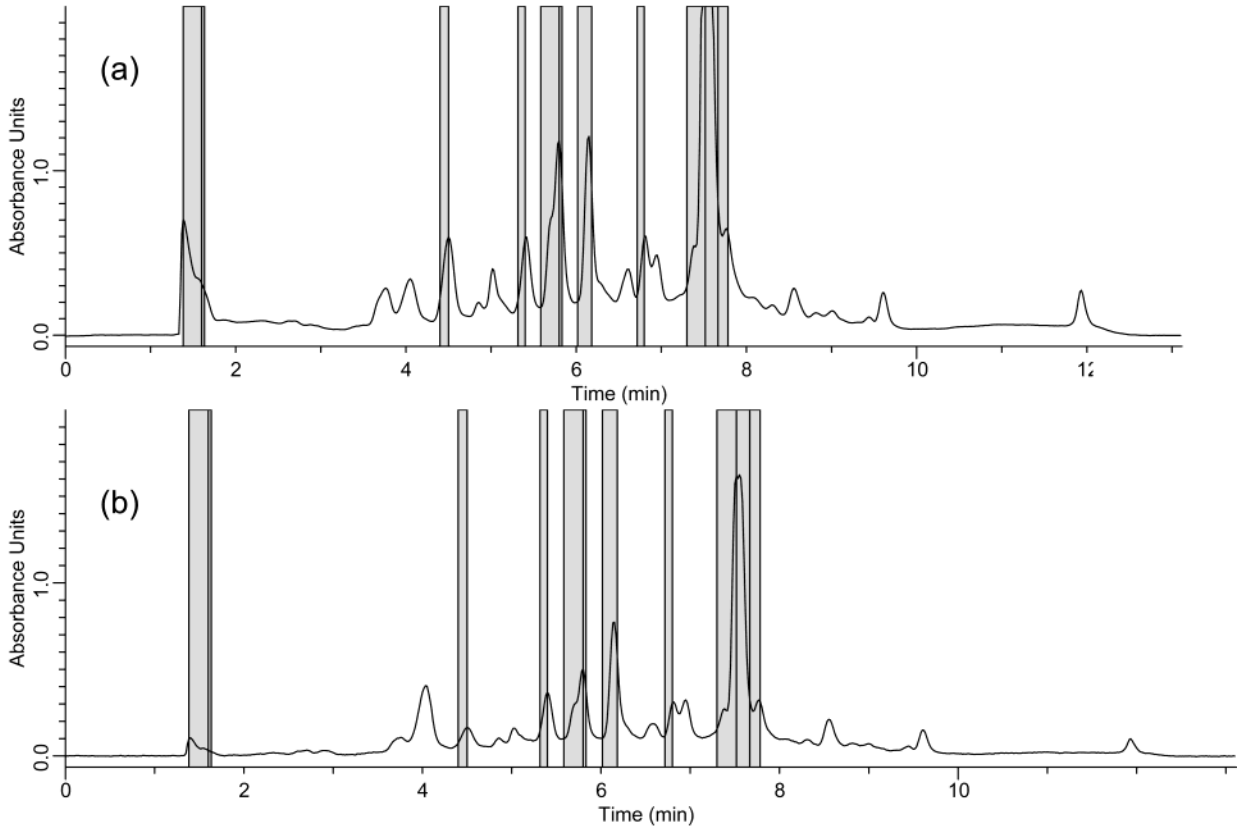



**Figure 2.** Separation and fractionation of representative sample from amide library where fractionation was triggered at 0.5 AU at 220 (a) or 254 nm (b).

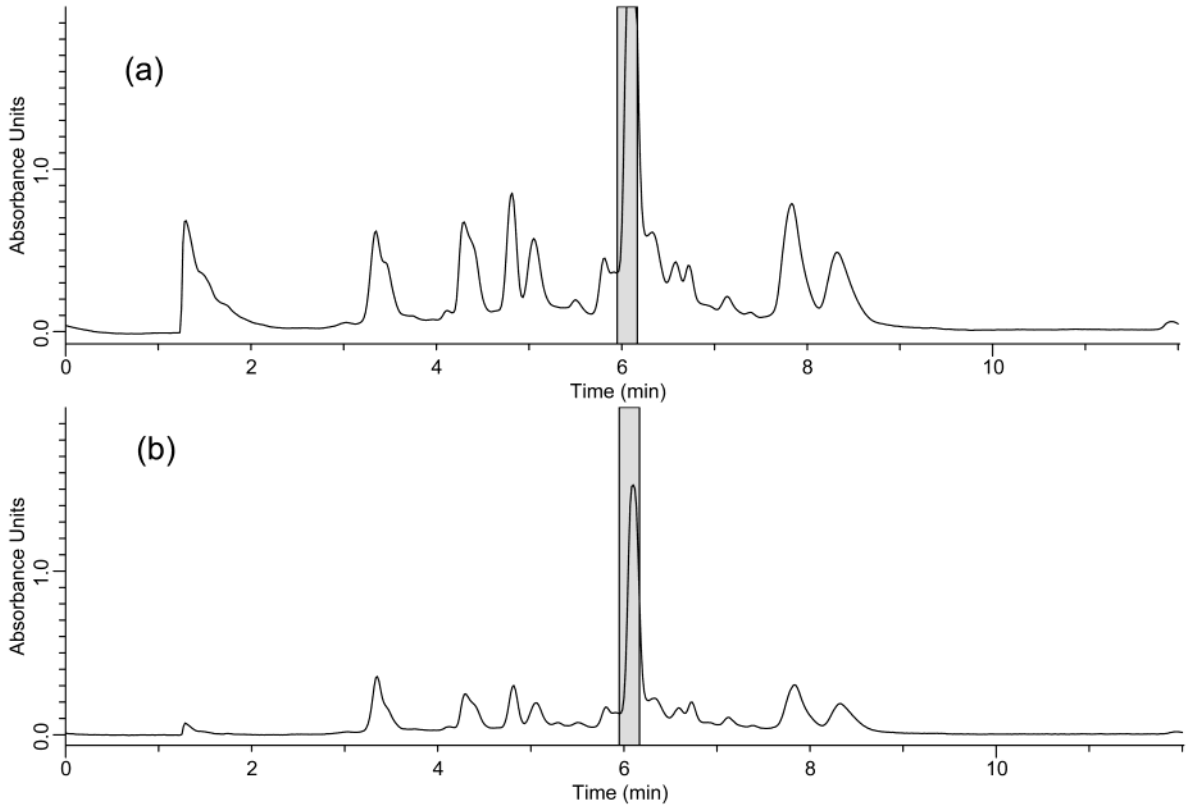



**Figure 3.** Separation and fractionation of library sample at 220 (a) and 254 nm (b) where fractionation was triggered at 1 AU on 254 nm only.

compounds. The favored analytical approach is a combination of detectors, usually electrospray mass spectrometry, UV, and often evaporative light scattering (ELSD), although purity is most often based on UV data. Moisture and inorganics in samples in the 5−20-mg range can also contribute to inaccurate weights.

**Table 3.** Yield of Parabens with Threshold Trigger at 1 AUFS at 254 nm

| | | yield of parabens with threshold trigger at 1AU at 254 nm mg ± SD (% recovery) |
|---|---|---|
| channel A | methyl | 7.61 ± 0.59 (76) |
| | ethyl | 7.82 ± 0.52 (78) |
| channel B | methyl | 8.41 ± 0.39 (84) |
| | ethyl | 7.44 ± 1.54 (74)[a] |

[a] Second fraction containing sample was lost.

**Table 4.** Results of Library Purification

| full tag | init purity[a] (%) | wt (mg) | theor content (mg) | final purity[a] (%) | final wt[b] (mg) | recovery (%) |
|---|---|---|---|---|---|---|
| A1-B1-C4 | 65.0 | 12.5 | 8.1 | 100.0 | 1.5 | 18.5 |
| A2-B1-C4 | 71.0 | 12.5 | 8.9 | 100.0 | 7.0 | 78.9 |
| A3-B1-C4 | 65.0 | 12.4 | 8.1 | 87.0 | 5.8 | 72.0 |
| A4-B1-C4 | 73.0 | 13.3 | 9.7 | 96.0 | 7.6 | 78.3 |
| A5-B1-C4 | 44.0 | 10.1 | 4.4 | 94.0 | 3.9 | 87.8 |
| A6-B1-C4 | 63.0 | 9.9 | 6.2 | fail | fail | fail |
| A7-B1-C4 | 44.0 | 11.7 | 5.1 | 85.0 | 4.6 | 89.4 |
| A8-B1-C4 | 78.0 | 11.6 | 9.0 | 88.0 | 5.1 | 56.4 |
| A9-B1-C4 | 62.0 | 12.6 | 7.8 | 94.0 | 2.9 | 37.1 |
| A10-B1-C4 | 67.0 | 12.1 | 8.1 | 91.0 | 6.6 | 81.4 |
| A11-B1-C4 | 65.0 | 10.3 | 6.7 | 93.0 | 3.4 | 50.8 |
| A12-B1-C4 | 61.0 | 13.3 | 8.1 | 96.0 | 6.6 | 81.4 |
| A1-B2-C4 | 63.0 | 14.2 | 8.9 | 88.0 | 6.9 | 77.1 |
| A2-B2-C4 | 66.0 | 11.4 | 7.5 | 98.0 | 5.3 | 70.4 |
| A3-B2-C4 | 64.0 | 13.0 | 8.3 | fail | fail | fail |
| A4-B2-C4 | 69.0 | 13.3 | 9.2 | 90.0 | 5.2 | 56.7 |
| A5-B2-C4 | 44.0 | 11.4 | 5.0 | 98.0 | 5.0 | 99.7 |
| A6-B2-C4 | 59.0 | 12.2 | 7.2 | fail | fail | fail |
| A7-B2-C4 | 50.0 | 12.7 | 6.4 | 88.0 | 5.1 | 80.3 |
| A8-B2-C4 | 74.0 | 18.7 | 13.8 | 80.0 | 9.1 | 65.8 |
| A9-B2-C4 | 62.0 | 13.2 | 8.2 | 97.0 | 6.1 | 74.5 |
| A10-B2-C4 | 71.0 | 11.8 | 8.4 | 100.0 | 5.8 | 69.2 |
| A11-B2-C4 | 65.0 | 11.5 | 7.5 | 100.0 | 3.7 | 49.5 |
| A12-B2-C4 | 62.0 | 11.3 | 7.0 | 96.0 | 6.3 | 89.9 |
| A1-B3-C4 | 62.0 | 14.1 | 8.7 | 92.0 | 8.3 | 94.9 |
| A2-B3-C4 | 63.0 | 10.0 | 6.3 | 96.0 | 3.9 | 61.9 |
| A3-B3-C4 | 60.0 | 11.4 | 6.8 | 94.0 | 3.6 | 52.6 |
| A4-B3-C4 | 65.0 | 13.5 | 8.8 | 94.0 | 4.8 | 54.7 |
| A5-B3-C4 | 39.0 | 13.0 | 5.1 | 93.0 | 4.7 | 92.7 |
| A6-B3-C4 | 57.0 | 12.1 | 6.9 | fail | fail | fail |
| A7-B3-C4 | 44.0 | 11.1 | 4.9 | 87.0 | 2.8 | 57.3 |
| A8-B3-C4 | 79.0 | 8.1 | 6.4 | 83.0 | 2.6 | 40.6 |
| A9-B3-C4 | 59.0 | 12.7 | 7.5 | 100.0 | 5.4 | 72.1 |
| A10-B3-C4 | 65.0 | 11.6 | 7.5 | 86.0 | 5.0 | 66.3 |
| A11-B3-C4 | 63.0 | 10.6 | 6.7 | 100.0 | 4.2 | 62.9 |
| A12-B3-C4 | 57.0 | 12.2 | 7.0 | fail | fail | fail |
| A1-B4-C4 | 60.0 | 14.6 | 8.8 | 92.0 | 8.3 | 94.7 |
| A2-B4-C4 | 61.0 | 14.2 | 8.7 | 85.0 | 4.6 | 53.1 |
| A3-B4-C4 | 59.0 | 13.6 | 8.0 | 92.0 | 5.4 | 67.3 |
| A4-B4-C4 | 66.0 | 15.6 | 10.3 | fail | fail | fail |
| A5-B4-C4 | 40.0 | 12.9 | 5.2 | 97.0 | 4.7 | 91.1 |
| A6-B4-C4 | 57.0 | 13.3 | 7.6 | fail | fail | fail |
| A7-B4-C4 | 44.0 | 11.9 | 5.2 | 82.0 | 4.0 | 76.4 |
| A8-B4-C4 | 75.0 | 12.6 | 9.5 | fail | fail | fail |
| A9-B4-C4 | 59.0 | 12.0 | 7.1 | 100.0 | 5.3 | 74.9 |
| A10-B4-C4 | 65.0 | 14.8 | 9.6 | 100.0 | 2.3 | 23.9 |
| A11-B4-C4 | 63.0 | 10.6 | 6.7 | 100.0 | 4.5 | 67.4 |
| A12-B4-C4 | 59.0 | 10.9 | 6.4 | fail | fail | fail |

[a] Based on analysis by reversed-phase HPLC at 215 nm. [b] Fractions were dried in preweighed vials and weighed using an automated weigh station (Bhodan).

**Purification of the Second Array.** The amide array was purified using fractionation criteria of 0.5-AU threshold and a slope of 0.1 AU/s. Table 5 shows that 15 740 fractions were collected, an average of >10 fractions/sample. However, only 2469 fractions were analyzed by LC/MS on the basis of selection using Winnow. Winnow utilizes information in the Parallex database, such as UV absorbance data, as well as original LC/MS data of the crude sample. Although 87% of the compounds in the amide array were submitted for high-throughput screening, the fact that so many fractions were collected makes liquid handling and subsequent processing more challenging. Collecting in this way reduces the capacity of the fraction collector and restricts the number of samples that can be purified in a single batch.

By changing the fractionation parameters to trigger collection at 1 AU of 0.1 AU/s at threshold >1 of 254 nm only, the number of fractions was significantly reduced, as shown in Table 6, where 5225 fractions were collected for the second array. Purification of the urea array gave similar results (Table 7).

**Table 5.** Purification of Amide Array before Optimization of Fractionation

| compd | fractions collected | fractions analyzed (LC/MS)[a] | compds submitted |
|---|---|---|---|
| 96 × R3(1) | 878 | 134 | 78 |
| 96 × R3(2) | 1043 | 137 | 83 |
| 95 × R3(3) | 1469 | 166 | 79 |
| 96 × R3(4) | 1254 | 199 | 90 |
| 96 × R3(5) | 945 | 231 | 90 |
| 96 × R3(6) | 1314 | 243 | 90 |
| 96 × R3(7) | 1171 | 149 | 84 |
| 96 × R3(8) | 1278 | 163 | 82 |
| 96 × R3(9) | 1266 | 126 | 75 |
| 96 × R3(10) | 881 | 147 | 90 |
| 96 × R3(11) | 787 | 138 | 80 |
| 96 × R3(12) | 1022 | 123 | 80 |
| 96 × R3(13) | 814 | 184 | 85 |
| 96 × R3(14) | 923 | 180 | 75[b] |
| 96 × R3(15) | 695 | 149 | 86 |
| 1440 | 15 740 | 2469 | 1247 |

[a] Analyzed by Finnigan aQa. [b] Fractions analyzed by MicroMass Diversity.

**Table 6.** Purification of Sulfonamide Library after Fractionation Was Optimized

| compd | fractions collected | fractions analyzed (LC/MS) | compds submitted |
|---|---|---|---|
| 96 × R3(1) | 595 | 152 | 96 |
| 96 × R3(2) | 277 | 135 | 83 |
| 96 × R3(3) | 295 | 133 | 90 |
| 96 × R3(4) | 420 | 161 | 96 |
| 96 × R3(5) | 414 | 231[a] | 141 |
| 96 × R3(6) | 432 | 243[a] | 119 |
| 96 × R3(7) | 291 | 143 | 95 |
| 96 × R3(8) | 369 | 136 | 88 |
| 96 × R3(9) | 364 | 144 | 94 |
| 96 × R3(10) | 316 | 139 | 94 |
| 96 × R3(110 | 308 | 149 | 95 |
| 96 × R3(12) | 166[b] | 100[b] | 61[b] |
| 96 × R3(13) | 328 | 140 | 91 |
| 96 × R3(14) | 266 | 107 | 90 |
| 96 × R3(16) | 384 | 138 | 90 |
| 1440 | 5225 | 2251 | 1423 |

[a] Potentially, 2 × 96 products due to incomplete reaction of initial sulfonamide and subsequent addition of a second reagent. [b] UV detector lamp failed.

**Table 7.** Purification of Urea Library

| compd | fractions collected | fractions analyzed (LC/MS) | compds submitted |
|---|---|---|---|
| 96 × R3(1) | 333 | 139 | 95 |
| 96 × R3(2) | 322 | 143 | 93 |
| 96 × R3(3) | 294 | 142 | 92 |
| 96 × R3(4) | 305 | 159 | 96 |
| 96 × R3(5) | 223 | 133 | 88 |
| 96 × R3(6) | 263 | 156 | 91 |
| 96 × R3(7) | 486 | 199 | 93 |
| 96 × R3(8) | 381 | 172 | 77 |
| 96 × R3(9) | 281 | 154 | 86 |
| 96 × R3(10) | 433 | 180 | 89 |
| 96 × R3(11) | 451 | 176 | 91 |
| 96 × R3(12) | 257 | 132 | 93 |
| 96 × R3(13) | 515 | 184 | 94 |
| 96 × R3(14) | 382 | 175 | 89 |
| 96 × R3(15) | 300 | 129 | 85 |
| 1440 | 5226 | 2373 | 1352 |

The final result was that 4012 compounds were submitted for screening, an overall success rate of 93%, and more than 90% of the compounds had purity >95%.

## Conclusion

Parallex HPLC has been shown to be a robust system for high-throughput purification. At least 200 samples can be purified in 10 h using the methods described. This could be further increased by a small reduction in cycle time. Fraction collection can readily be tailored to collect only the largest UV adsorbing component(s). However, if the desired component represents only a small percentage of the sample, then more fractions would need to be collected and analyzed offline. In some instances, such samples may be better suited to mass-triggered fractionation.

There is a great deal of controversy over the use of a "universal" gradient. Our experience suggests that it is more practical to have a handful of methods that can be selected on the basis of information obtained on reaction analyses. Most reports in the literature seem to favor this approach.[13−15]

Minimal reequilibration was used in all separations. This did not appear to affect chromatographic performance or column lifetime. Other approaches include column switching to enable one column to reequilibrate while a separation occurs on the second column.[7]

Solubility is a crucial issue that is rarely addressed in the literature but is important in the purification of a broad range of chemically diverse compounds. Our approach using a sample diluent of DMSO/methanol (50/50) in conjunction with the "sandwich solvent" minimizes solubility and precipitation problems. Selection of an appropriate method for the polarity of the samples will also minimize risk of precipitation and blockages. Alternative purification technologies are being investigated to increase throughput on a wider scale, for example, the introduction of SFC to reduce solvent consumption and eliminate the need for removal of large volumes of solvent from fractions;[16] however, parallel HPLC with UV detection remains the method of choice for purification of large combinatorial arrays.

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