

# Development of a Scalable and Sustainable High Performance CounterCurrent Chromatography (HPCCC) Purification for Spinosyn A and Spinosyn D from Spinosad

*Carl DeAmicis,<sup>\*,†</sup> Qiang Yang,<sup>\*,†</sup> Colin Bright,<sup>Ω</sup> Neil A. Edwards,<sup>Ω</sup> Guy H. Harris,<sup>Ω</sup> Sandeep Kaur,<sup>Ω</sup> Philip L. Wood,<sup>Ω</sup> Peter Hewitson,<sup>£</sup> Svetlana Ignatova<sup>£</sup>*

<sup>†</sup>Process Chemistry, Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268, USA

<sup>Ω</sup>Dynamic Extractions, Ltd., Unit 30, Tafarnaubach Industrial Estate, Tredegar, Blaenau Gwent, Wales, NP22 3AA, UK

<sup>£</sup>Advanced Bioprocessing Centre, Brunel University London, Uxbridge, Middlesex, UB8 3PH, UK

## **ABSTRACT**

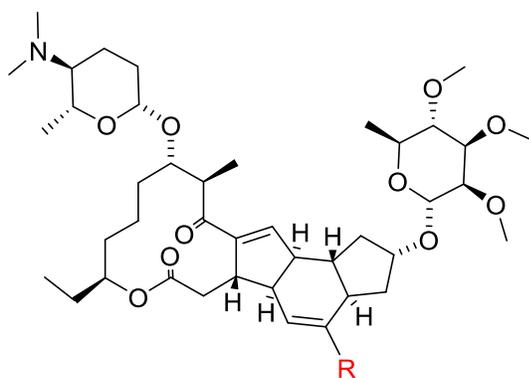
A high performance countercurrent chromatography (HPCCC)<sup>1</sup> process was developed as a more efficient and sustainable alternative to reverse phase high performance liquid chromatography (RP-HPLC) for the pilot scale purification of the naturally occurring fermentation-derived insecticides, spinosyn A and spinosyn D, the major components of

spinosad insecticide. While on pilot scale HPCCC and RP-HPLC both gave >99% purities and comparable combined recoveries of 77% and 83%, respectively, HPCCC was more efficient and sustainable by producing a 60% higher productivity, 11 times higher solute loading, 96% savings in stationary phase costs, and 42% reduction in solvent usage. The increase in productivity and reduction in solvent usage further reduced waste recycle and disposal costs, thus presenting significantly less environmental impact compared to RP-HPLC separations. Use of mixing on demand for solvent system at a preparative scale allowed a complete automation with minimized solvent consumption.

## INTRODUCTION

The spinosyns are members of a class of polyketide derived macrolides which are effective against a broad range of lepidopteran insect pests.<sup>2</sup> The spinosyns have a unique structure, spectrum of activity, mode of action and pose less significant environmental risks than many synthetic pesticides.<sup>3</sup> Spinosad, a mixture of spinosyn A and spinosyn D, is a fermentation-derived insecticide produced by the actinomycete, *Saccharopolyspora spinosa*. Structurally, spinosyn A is distinguished from spinosyn D by a methyl group at the C-6 position (Figure 1). Spinosad received its first US registration in 1997 for use on cotton and turfgrass under EPA's reduced risk pesticide program,<sup>4</sup> and the Presidential Green Chemistry Challenge Award in 1999.<sup>5</sup> As of 2005, spinosad has been approved for use on more than 150 fruit and vegetable crops in the U.S. and also in more than 70 other countries.<sup>4</sup>

**Figure 1.** Structures of Spinosyn A and Spinosyn D



Spinosyn A: R = H  
 Spinosyn D: R = CH<sub>3</sub>

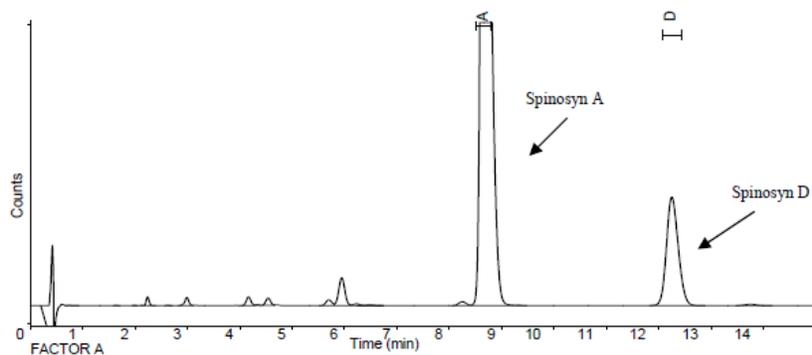
Periodically kilogram quantities of pure spinosyn A and spinosyn D factors are required to produce analytical standards to support regulatory studies and as starting materials for metabolite and process impurity syntheses. Over the years many research groups have reported total synthesis of spinosin A;<sup>6-12</sup> however, these syntheses require >20 steps and thus are not desirable for large scale preparation. Isolation from spinosad remains as an efficient and economical approach to obtain pure spinosyn A and spinosyn D factors. Initial purification attempts via selective precipitation/crystallization, or normal phase silica gel column chromatography were fruitless due to the close physical properties of spinosyn A and spinosyn D. Previously, we have obtained satisfactory purities (>97%) using RP-HPLC; however, large scale RP-HPLC separations are cost prohibitive owing to the high costs of stationary phases, large amount of solvents, and high labor and solvent disposal costs. Recently, HPCCC was shown to increase productivity over RP-HPLC in the pilot scale separation of spinetoram J and spinetoram L from spinetoram insecticide.<sup>13</sup> In an effort to increase productivity and reduce the high cost of spinosyn A and spinosyn D separation on kilogram scales, HPCCC was investigated as a more efficient alternative to RP-HPLC. We found that on similar scales HPCCC provided significantly higher productivity than RP-HPLC for spinosad separation, with much higher loading and lower cost, primarily due to stationary phase savings and reduction in solvent usage

and disposal. Herein, the development of a pilot scale HPCCC purification of spinosyn A and spinosyn D from spinosad is described.

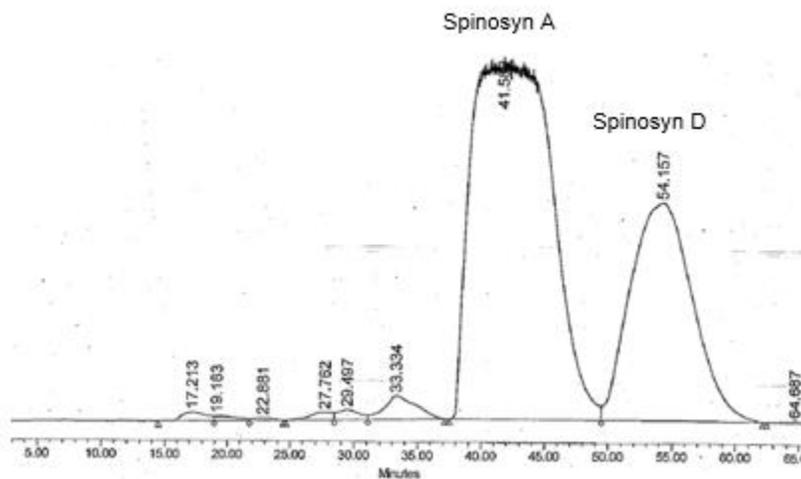
## RESULTS AND DISCUSSION

Previously pure samples of spinosyn A and spinosyn D were obtained via RP-HPLC purification of spinosad. Crude spinosad contains an approximate 5:1 ratio of spinosyn A to spinosyn D. Prior to preparative RP-HPLC purification, the spinosyn D factor in crude spinosad was enriched by extraction with MeOH to give an approximate 2.5:1 ratio of spinosyn A to spinosyn D. The preparative RP-HPLC separation was performed on a 0.50 g scale using a 5 cm × 25 cm Kromasil C18 (10 μm) column and a mobile phase of 85:15 MeOH/10 mM aqueous NH<sub>4</sub>OAc at a flow rate of 40 mL/min with a run time of 70 minutes. Because the D-enriched spinosad had poor solubility in the mobile phase, it was dissolved in a 7:3 mixture of THF/MeCN to give a feedstock solution of 0.1 g/mL. A total of 242 runs were required to process 121 g of D-enriched spinosad. Spinosyn A and spinosyn D were obtained in >97% purity in a single pass and did not require additional chromatography. A total of 48 g of spinosyn A and 36 g of spinosyn D were obtained for a combined recovery of 69% and a productivity of 0.124 g/L. The representative analytical and preparative RP-HPLC chromatograms of spinosad are shown in Figure 2. The major component in the chromatogram is spinosyn A followed by spinosyn D, and the minor polar impurities structurally related to the spinosyns are shown in the chromatograms eluting before the main factors.

**Figure 2.** Typical Chromatograms for RP-HPLC Separation of Spinosyn A and Spinosyn D



a) Analytical RP-HPLC chromatogram: Ascentis Express C18, 4.6 mm × 100 mm, 2.7 μm; 57:14:29 MeCN/MeOH/25 mM pH 6.0 phosphate buffer, 2.0 mL/min, 250 nm.



b) Preparative RP-HPLC chromatogram: Kromasil C18, 5 cm × 25 cm, 10 μm; 85:15 MeOH/10mM aqueous NH<sub>4</sub>OAc, 40 mL/min; 254 nm.

Pilot-scale RP-HPLC separation was performed on a 6.0 g scale using an 11 cm i.d. ProChrom dynamic axial compression column packed with 1.5 kg of Kromasil C18 (10 μm) stationary phase and an isocratic mobile phase of 40:40:15:5 MeOH/MeCN/65 mM aqueous NH<sub>4</sub>OAc/THF at a flow rate of 630 mL/min and a run time of 16.3 minutes (chromatogram not shown). Again, spinosad was not very soluble in the mobile phase and 5% THF was added to the mobile phase

diluent to increase the solubility of the crude spinosad and to give a feedstock solution of 35 g/L spinosad. A total of 67 runs were required to process 402 g of spinosad. The spinosyn A fractions were obtained in >99% purity in a single pass and did not require additional purification before isolation. However, the spinosyn D fractions were obtained in 94% purity from a single pass purification and were recycled one additional time through the purification in order to obtain higher purity. This required an additional 29 runs to obtain spinosyn D in >99% purity. Spinosyn D was much less soluble in the mobile phase than spinosad and 20% THF was added to the mobile phase diluent to dissolve the sample. The second pass feed had a concentration of 20 g/L spinosyn D. The separation of 402 g of crude spinosad required a total of 96 runs and gave 278 g of spinosyn A and 53 g of spinosyn D both in >99% purities, a combined recovery of 83%, and a productivity of 0.377 g/L.

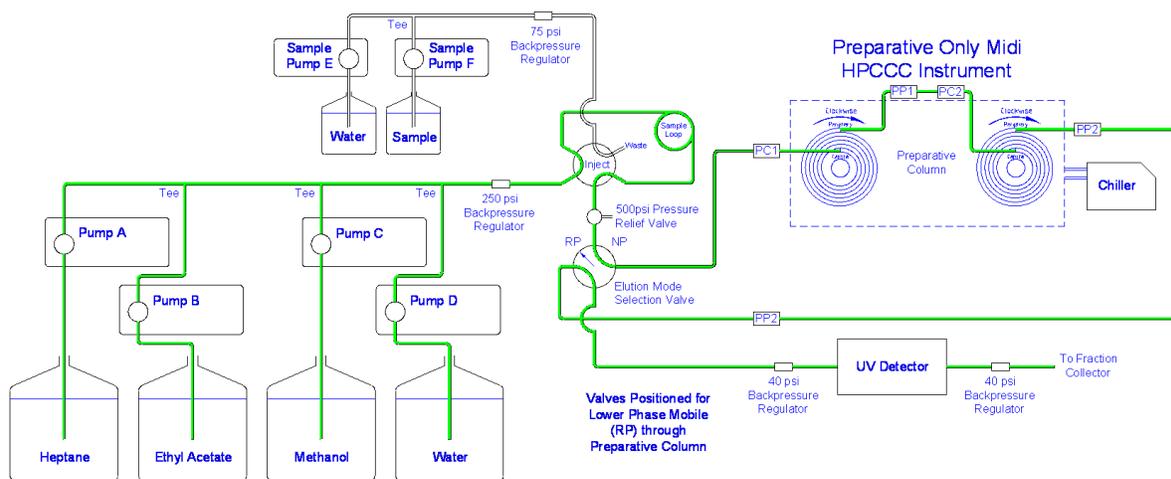
Although hundred-gram quantities of materials were obtained via RP-HPLC purification, large scale RP-HPLC separations are not desirable owing to the high cost. Previous studies showed that HPLC separations significantly increased the productivity and scalability over RP-HPLC in the pilot scale separation of spinetoram J and spinetoram L,<sup>13</sup> thus the feasibility of HPLC purification of spinosyn A and spinosyn D factors was evaluated. A successful HPLC separation relies on the choice of an appropriate immiscible solvent system. Compared to solid-support chromatography, the selection of HPLC solvent systems is equivalent to simultaneously choosing both the solid column matrix and the mobile liquid phase. There are a variety of appropriate immiscible solvent systems available and one of the most commonly used is a mixture of heptane, EtOAc, MeOH, and water in different ratios, often referred to as the HEMWat system<sup>14</sup> and the Arizona system.<sup>15</sup>

Previous studies with the structurally related compound spinetoram identified solvent system 25 in the HEMWat series (heptane/EtOAc/MeOH/water, 6:1:6:1, v:v:v:v) as an optimal solvent system providing distribution ratios for spinetoram J and spinetoram L of approximate unity.<sup>13</sup> Therefore, as a starting point solvent system 25 in the HEMWat series was used for spinosad separation method development. Because spinosad is less lipophilic than spinetoram, solvent scouting proceeded from solvent system 25 towards the less lipophilic end of the HEMWat system and quickly solvent system 21 (heptane/EtOAc/MeOH/water, 5:2:5:2, v:v:v:v)<sup>14</sup> was identified as optimum yielding distribution ratios for spinosyn A and spinosyn D of 3.65 and 3.97, respectively, as per ratio of HPLC peak areas. The separation was first performed on analytical scale using a 24 mL column and a flow rate of 1.0 mL/min. The separation of 83 mg of spinosad using solvent system 21 in reversed phase mode (lower phase as the mobile phase) resulted in a separation of spinosyn A (69 min) and spinosyn D (76 min) with the stationary phase retention as 73%. Acidic modifiers were detrimental to the quality of the separation, while ammonia offered no significant advantage. All further development and scale-up work was performed without the use of pH modifiers and in reversed phase mode (aqueous phase mobile, non-polar phase stationary).

Since scale-up of HPLCCC is direct, linear, and volumetric if performed on similarly designed columns operating at identical centrifugal field,<sup>16</sup> further development of this separation was carried out on a semi-preparative scale column, which had a volume of 134 mL and a flow rate of 6.0 mL/min. The separation of 0.50 g of crude spinosad using this solvent system was very similar to the analytical scale separation results. In the semi-preparative scale separation, the stationary phase retention was 78%. The semi-preparative separation was further scaled to a preparative column using a Dynamic Extractions Midi instrument (Figure 3) with a volume of

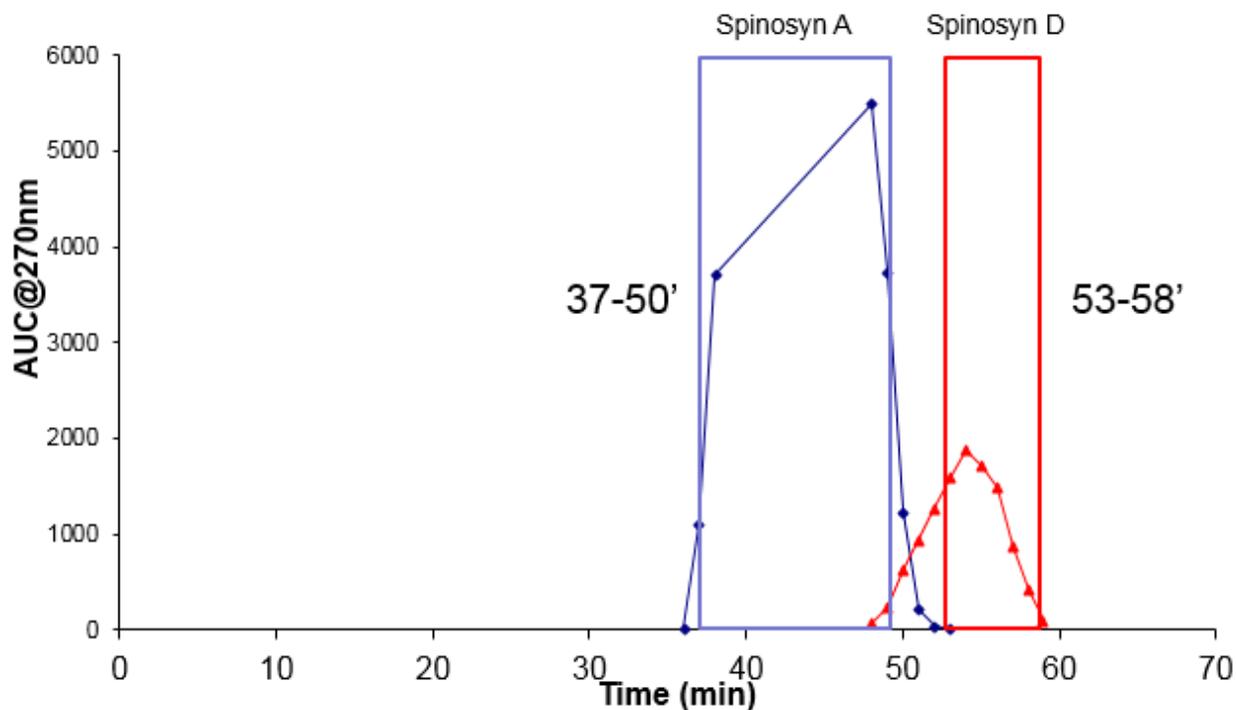
1007 mL, and a scaling factor of 7 $\times$ . Using the same solvent system, both the sample loading and mobile phase flow rate were further increased to 6 g and 67.5 mL/min, respectively. The results from the preparative separation compared well with those of the small scale purifications, and the stationary phase retention was 77%. The separation of 444 g of crude spinosad required a total of 74 runs, and provided 288 g of spinosyn A and 37.7 g of spinosyn D with purities of >99%, a combined recovery of 73%, and a productivity of 0.765 g/L (Figure 4). The separation demonstrated excellent reproducibility throughout the runs (Figure 5).

**Figure 3.** Flow Diagram of Midi HPCCC Processor

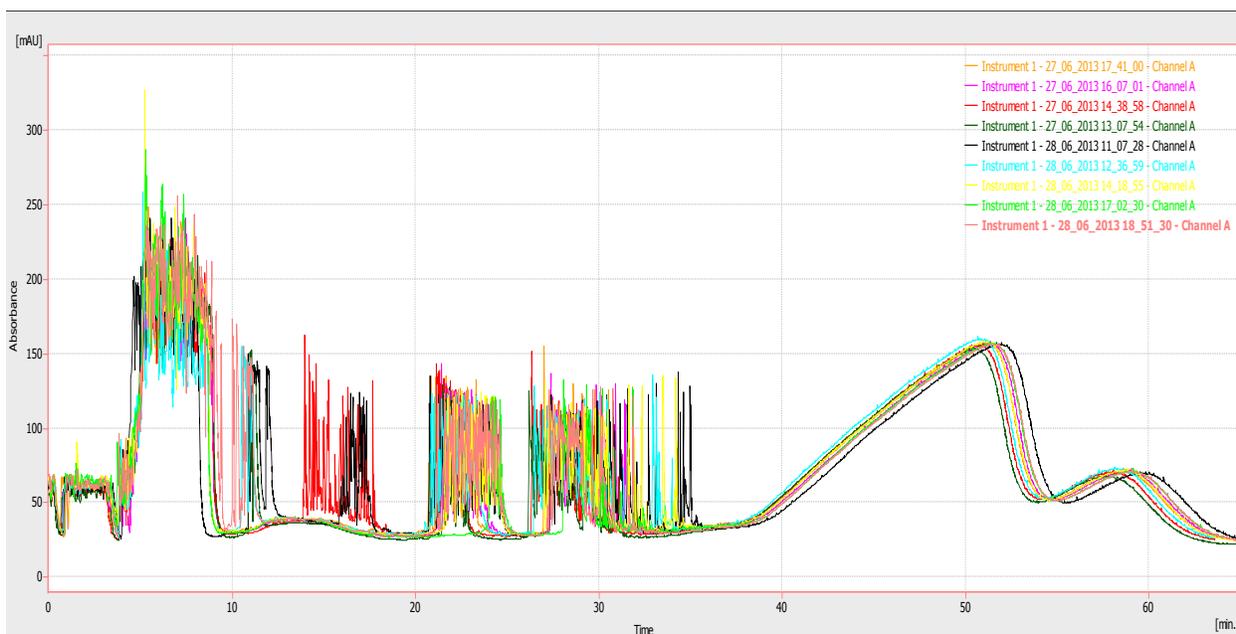


Using 4 pumps allows for mixing both phases on demand. Clarity software was used to control all the stages in this separation process from initial solvent mixing and sample loading to collecting fractions and controlling the HPCCC processor.<sup>17</sup>

**Figure 4.** Typical Results for Preparative Scale HPCCC Separation



**Figure 5.** Reproducibility of Preparative Scale HPLC Separation



The separation of spinosyn A and spinosyn D was then attempted on a pilot scale using an 18 L column. In this case, a true volumetric scaling was not possible since the pilot centrifuge was

only capable of operating at  $120 \times g$  rather than  $240 \times g$  as the column was not designed to the same geometry as the preparative and semi-preparative scale instruments. However, since the distribution ratios of spinosyn A and spinosyn D remain constant on scale-up, probe experiments at  $120 \times g$  on the semi-preparative column combined with simple empirical experience guided the flow rate selection as well as the loading. A conservative approach was adopted and the sample was purified in batches of 67 g/injection and a flow rate of 400 mL/min. A total of 8 runs (536 g of crude spinosad) were performed with cycle times of 183 minutes/run. Typically, spinosyn A was collected between 90–120 minutes and spinosyn D between 130–150 minutes (Figure 6). The stationary phase retention was 77% for the pilot scale run. The total recovery was increased by a minimal amount of reprocessing of mixed fractions eluting between spinosyn A and spinosyn D (amounted to approximately 10% of the crude material processed). This purification produced 348 g of spinosyn A and 62.5 g of spinosyn D each with purities >99% in a combined recovery of 77% and a productivity of 0.604 g/L. Reproducibility of the separation on this scale as shown in Figure 7 is different from that of Midi due to difference in sample concentration (40 mg/mL for Midi and 73 mg/mL for Maxi separation), although the peak resolution for spinosyn A and spinosyn D remained the same. Also, variability of elution time for spinosyns can be explained by inconsistency of the crude sample (variable ratio between components), which caused additional stripping of a stationary phase. However, it had no impact on a purity or recovery of spinosyns at pilot scale.

**Figure 6.** Typical Results for Pilot Scale HPCCC Separation

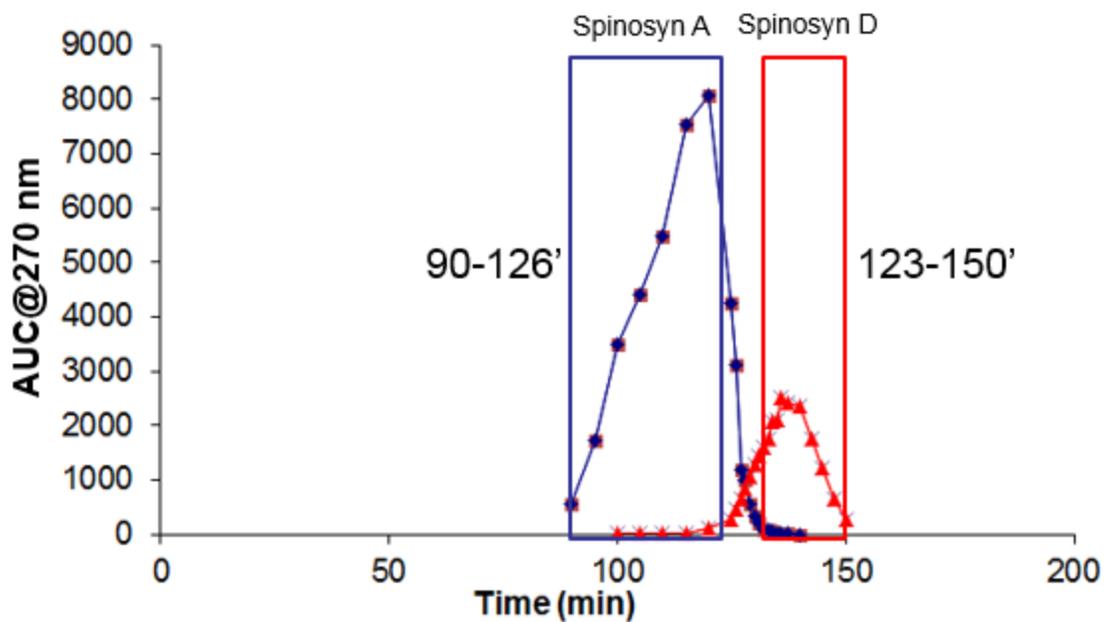
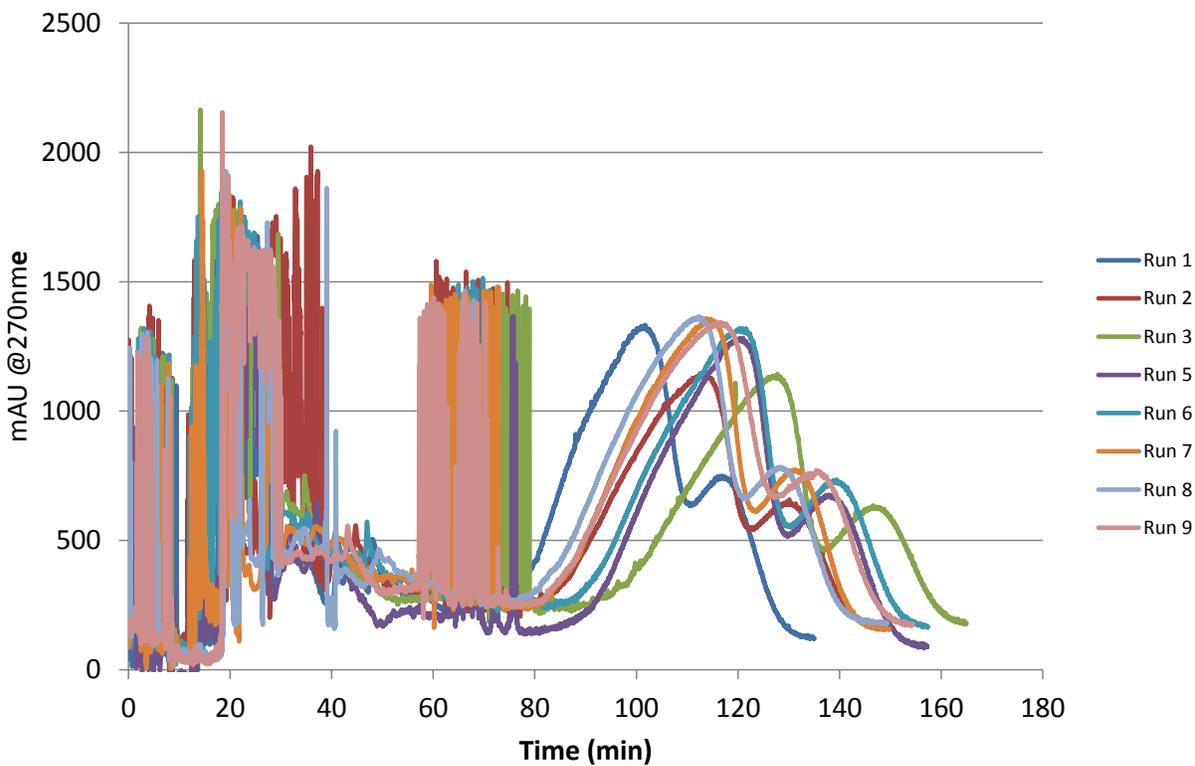


Figure 7. Reproducibility of Pilot Scale HPCCC Separation



In this study, both RP-HPLC and the HPCCC were optimized for the separation of spinosad components on pilot scales. Table 1 shows that on pilot scale HPCCC afforded 96% reduction on stationary phase cost. The loadings were 11× higher and solvent usage was 42% less compared to similar scale RP-HPLC. Since the stationary phase in HPCCC is solvent, one can replace or change the stationary phase with very little effort or cost. Similarly, the stationary phase is much larger in HPCCC, which leads to much greater loadings compared to RP-HPLC. This is particularly valuable for samples with poor solubility like spinosyn D, which did not require additional solvent to solubilize during the HPCCC separation. Other advantages of HPCCC include linear scaling and better tolerance to particulates in the mobile phase.

**Table 1.** Comparison of Performance Parameters of HPCCC and RP-HPLC.

Parameter	Laboratory Scale		Pilot Scale	
	RP-HPLC	HPCCC	RP-HPLC	HPCCC
Stationary phase	0.1 kg	1.0 L	1.5 kg	18 L
Stationary phase cost	\$12,200 <sup>18</sup>	\$30	\$14,640 <sup>19</sup>	\$540
Crude processed (g)	121	444	400	536
Loading/run (g)	0.5	6	6	67
Number runs	242	74	96	8
Total cycle time (h)	282	97	26	24
Solvent (L)	677	426	877	680
Solvent usage/g spinosad (L)	5.60	0.96	2.19	1.27
Recovery A (g/%)	48/39.7	288/64.9	278/69.5	348/64.9
Purity A (%)	>97	>99	>99	>99
Recovery D (g/%)	36/29.8	37.7/8.5	52.5/13.1	62.5/11.7

Purity D (%)	>97	>99	>99	>99
Combined recovery (%)	69.5	73.4	82.6	76.6
Productivity (A+D/solvent, g/L)	0.124	0.765	0.377	0.604

---

## CONCLUSIONS

We have developed a scalable alternative to RP-HPLC using HPCCC for the pilot scale separation of spinosad. Compared to RP-HPLC separation, HPCCC separation process is much more efficient and poses a much smaller sustainability footprint by improving the productivity by 60%, increasing the sample loading by 11 fold, and reducing stationary phase cost by 96% and solvent usage by 42%. In addition, waste recycle and disposal costs were also significantly reduced as a result of solvent usage reduction. The reported process represents a solid base for further development of a separation process suitable for larger scale purification of spinosyn A and spinosyn D. HPCCC should be the method of choice for pilot-scale separations of complex natural products such as the spinosyns.

## EXPERIMENTAL SECTION

**General.** The spinosad sample used in this study contained a ratio of approximately 5:1 of spinosyn A and spinosyn D with an estimated purity of 90–92%. Other components within the sample were structurally related minor factors from fermentation. NMR spectra were obtained using a Bruker AVANCE 400 MHz spectrometer, operating at 400.13 MHz ( $^1\text{H}$ ) and 100.62 MHz ( $^{13}\text{C}$ ).  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra were obtained using composite pulse decoupling. Mass spectra were obtained using a Waters Micromass ZQ mass spectrometer. The following HPLC method was used for the initial analysis of crude spinosad and for the analysis of preparative

HPCCC derived fractions. Phenomenex Gemini NX, 4.6 × 150 mm, solvent A: 0.1% v/v TFA in water; solvent B: 0.1% v/v TFA in MeCN, flow rate 1 mL/min; gradient 75–100% B/4 min; hold at 100% B for 2 min then 4 min at 75% B.

### **RP-HPLC Separations:**

**Analytical HPLC separation of spinosad.** Analytical analysis was performed on a Perkin-Elmer Series 200 HPLC system (Shelton, CT) equipped with an Ascentis Express C18, 2.7 μm, 4.6 × 100 mm column (Sigma-Aldrich, St Louis Mo.) and a mobile phase of 57:14:29 MeCN/MeOH/25mM pH 6 phosphate, and using a UV detector at 250 nm. A typical injection was 20 μL with a run time of 15 min.

**Preparative RP-HPLC separation of spinosad.** Preparative HPLC separation of spinosad was performed using an Agilent preparative HPLC 1200 series (Santa Clara, California) equipped with a 5 cm × 25 cm Kromasil C18, 10 μm column (AkzoNobel, Bophus, Sweden) operating at a flow rate of 40 mL/min, an eluent of 85:15 MeOH/10 mM aqueous NH<sub>4</sub>OAc, and using a photodiode detector at 254 nm. The feed solution was prepared by suspending crude spinosad (300 g) in MeOH (600 mL) and stirring for 2 h at 50 °C. The mixture was cooled to 25 °C and the solids collected by vacuum filtration and washed with cold MeOH (100 mL). The sample was dried to constant weight to give 146 g of D-enriched spinosad, which contained a 2.5:1 ratio of spinosyn A/D with a purity of 96%. The D-enriched spinosad was dissolved in 30:70 MeCN/THF (1460 mL) to give a 0.10 g/mL solution as the feed stock. A typical injection consisted of 5 mL (0.50 g) with a run time of 70 min.

**Pilot scale RP-HPLC separation of spinosad.** Pilot scale RP-HPLC separation of spinosad was conducted using a high performance preparative liquid chromatography system (Prochome,

Champigneulle, France) consisting of a solvent delivery system and column skid (LC110.VE.700.70SP). The solvent delivery module consisted of three solenoid actuated solvent inlets, a manifold mixing chamber, a LEWA FC solvent duplex piston-diaphragm pump (LEWA, Leonberg, Germany) operating with flow range of 25–1000 mL/min, a 10 port solenoid valve fraction collector, a variable wavelength detector, and a sample injection pump (1–35 mL/min). The column module consisted of an LC110.VE.700.70SP column skid dynamic axial compression i.d. 11 cm column. (Prochrom SA, France). The column was packed with 1.5 kg Kromasil C18, 10  $\mu\text{m}$  (Lot No. DT0109, AkzoNobel, Separation Products, Bophus, Sweden) to produce a bed height of approximately 23 cm and a bed volume of 2.2 L. The system was operated at a flow rate of 630 mL/min with an isocratic mobile phase of 40:40:15:5 MeOH/MeCN/65 mM aqueous  $\text{NH}_4\text{OAc}$ /THF. Detection was by UV at 260 nm and the run time was 16.3 minutes. The feed sample solution was prepared by dissolving the crude spinosad (400 g) in DCM (10 L) and filtering through a Hyflo Super Cel filter aid to remove fine particles. The solvent was removed by rotary evaporation and the residue solids dissolved in the mobile phase to give a concentration of 35 g/L solution. The sample injection volume for each run was 170 mL which contained 6.0 g crude spinosad. The collected fractions were analyzed by HPLC, and fractions that were >98% pure at 254 nm were pooled. Fractions of <98% purity were pooled and resubmitted to purification. The pooled fractions were concentrated under reduced pressure by rotary evaporation to remove most of the MeCN to produce an opaque aqueous suspension which was extracted with DCM. The DCM extract was evaporated under reduced pressure by rotary evaporation to give the purified components as white solids.

### **HPCCC Separations:**

Upper and lower phases of solvent systems for HPCCC separation were prepared according to the experimentally determined composition. The HPCCC column was filled with the upper stationary phase. A specified centrifugal field was applied to the column and mobile phase was pumped into the center of the column while the displaced stationary phase was collected from the peripheral column outlet. The volume of displaced stationary phase was used to calculate the stationary phase retention ( $S_f$ ) for each separation, which is ratio of a stationary phase to a total column volume. Spinosad was dissolved in lower phase, typically 1 mL for analytical, 10 mL for semi-preparative, and 150 mL for preparative scale separations. Mobile phase flow rates were 1 mL/min, 6 mL/min, and 67.5 mL/min, respectively. The separations were monitored by UV absorbance at 270 nm.  $S_f$  was 0.73 for the analytical scale, 0.78 for the semi-preparative scale, and 0.77 for preparative scale separations.

**HPCCC analytical and semi-preparative scale separation of spinosad.** HPCCC analytical and semi-preparative scale experiments were performed on a Dynamic Extractions Spectrum instrument (Tredegar, UK) which was fitted with an analytical scale column with a volume of 22 mL, an i.d. of 0.8 mm, and a  $\beta$ -value range from 0.64–0.81 and a semi-preparative scale column with a volume of 134 mL, an i.d. of 1.6 mm and a  $\beta$ -value range of 0.52–0.86. The Spectrum HPCCC was interfaced to a SSI Q-grad pump (State College, PA), a Model 500 Lab Alliance UV detector, manually controlled fluidic and sample injection valves, and the entire system was controlled using EZChrom chromatography software (Agilent).

**HPCCC preparative scale separation of spinosad.** HPCCC preparative scale experiments were performed on a Dynamic Extractions Midi instrument (Tredegar, UK) which was fitted with a preparative scale column with a volume of 1007 mL, an i.d. of 4 mm, and a  $\beta$ -value range of 0.58–0.87. The Midi HPCCC was interfaced to four SSI Prep 300 pumps (State College, PA),

an ECOM Flash 06 DAD detector (Prague, Czech Rep.), and the system was controlled using Clarity software (Prague, Czech Rep.). Fluidics were controlled using Valco high pressure actuated valves. The sample injection was remotely controlled using a Valco 2-position-6-port valve with a sample loop.

**HPCCC pilot scale separation of spinosad.** HPCCC pilot scale experiments were performed on a Dynamic Extractions Maxi instrument (Tredegar, UK), which was fitted with two bobbin-mounted coils giving the 17510 mL total column volume with an i.d. of 10 mm and a  $\beta$ -value range of 0.54–0.92. The centrifuge was connected to an Armen Industrial CCC Control Unit pumping system (Armen Instrument, Vannes, France) with in-built Knauer UV spectrophotometer and preparative flow cell (Knauer, Berlin, Germany). The injection was manual using a sample loop. The two separate coils which form the Maxi column were filled with stationary phase (upper phase) at 2000 mL/min in parallel. The instrument was rotated at 600 rpm ( $120 \times g$ ) at a temperature of 30 °C. The coils were equilibrated in reversed phase mode (lower phase mobile) from head-center to tail-periphery at 700 mL/min in parallel. Following equilibration, the flow was changed to serial through the two coils at 400 mL/min.  $S_f$  was 0.79 for the pilot scale separations. The sample was injected via a 920 mL manual sample loop, the UV signal was monitored at 280 nm and fractions were collected manually for analysis by HPLC.

**Spinosyn A:** mp 110–126 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.77 (s, 1H), 5.88 (d,  $J = 9.8$  Hz, 1H), 5.80 (dt,  $J = 9.8, 2.9$  Hz, 1H), 4.85 (d,  $J = 1.7$  Hz, 1H), 4.71–4.63 (m, 1H), 4.43 (d,  $J = 6.2$  Hz, 1H), 4.31 (q,  $J = 6.9$  Hz, 1H), 3.68–3.59 (m, 1H), 3.58–3.42 (m, 5H), 3.56 (s, 3H), 3.50 (s, 3H), 3.49 (s, 3H), 3.34–3.23 (m, 1H), 3.18–3.08 (m, 2H), 3.01–2.96 (m, 1H), 2.87 (ddt,  $J = 11.3, 8.6, 2.6$  Hz, 1H), 2.40 (dd,  $J = 13.3, 3.2$  Hz, 1H), 2.31–2.10 (m, 4H), 2.24 (s, 6H), 2.02–1.71 (m,

4H), 1.61–1.22 (m, 10H), 1.28 (d,  $J = 6.3$  Hz, 3H), 1.26 (d,  $J = 6.2$  Hz, 3H), 1.18 (d,  $J = 6.7$  Hz, 3H), 0.99–0.85 (m, 1H), 0.82 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  202.83, 172.49, 147.41, 144.16, 129.31, 128.83, 103.47, 95.54, 82.30, 81.11, 80.65, 77.77, 76.67, 76.12, 73.67, 67.96, 64.88, 60.87, 58.99, 57.69, 49.45, 47.67, 47.64, 46.06, 41.55, 41.18, 40.68, 37.40, 36.31, 34.34, 34.18, 30.97, 30.16, 28.39, 21.60, 18.95, 18.47, 17.80, 16.16, 9.32. HRMS (ESI) calcd for  $\text{C}_{41}\text{H}_{66}\text{NO}_{10}$   $[(\text{M}+\text{H})^+]$  732.4687, found 732.4685 m/z.

**Spiniosyn D:** mp 171–175 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.76 (s, 1H), 5.49 (s, 1H), 4.86 (d,  $J = 1.7$  Hz, 1H), 4.78–4.73 (m, 1H), 4.72–4.61 (m, 1H), 4.42 (d,  $J = 7.4$  Hz, 1H), 4.30 (q,  $J = 6.9$  Hz, 1H), 3.67–3.59 (m, 1H), 3.56 (s, 3H), 3.54–3.37 (m, 3H), 3.51 (s, 6H), 3.36–3.21 (m, 1H), 3.19–3.16 (m, 2H), 3.03–2.91 (m, 1H), 2.78 (ddt,  $J = 11.2, 8.6, 2.5$  Hz, 1H), 2.40 (dd,  $J = 13.3, 3.3$  Hz, 1H), 2.33–2.11 (m, 3H), 2.24 (s, 6H), 2.10–1.76 (m, 4H), 1.73 (s, 3H), 1.60–1.22 (m, 12H), 1.28 (d,  $J = 6.3$  Hz, 3H), 1.26 (d,  $J = 6.1$  Hz, 3H), 1.18 (d,  $J = 6.7$  Hz, 3H), 1.09–0.94 (m, 1H), 0.82 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  202.95, 172.57, 147.62, 144.44, 136.20, 122.49, 103.49, 95.60, 82.33, 81.15, 80.63, 77.79, 76.69, 75.83, 73.71, 67.99, 64.93, 60.90, 59.05, 57.73, 49.09, 47.85, 47.79, 46.01, 44.47, 42.09, 40.71, 37.77, 34.87, 34.36, 34.06, 30.99, 30.13, 28.42, 21.70, 20.67, 18.99, 18.50, 17.82, 16.15, 9.34. HRMS (ESI) calcd for  $\text{C}_{42}\text{H}_{68}\text{NO}_{10}$   $[(\text{M}+\text{H})^+]$  746.4843, found 746.4848 m/z.

## AUTHOR INFORMATION

### Corresponding Authors

Carl DeAmicis: cdeamicis@butler.edu

Qiang Yang: qyang1@dow.com

## Present Address

Carl DeAmicis: Department of Chemistry, Butler University, 4600 Sunset Ave., Indianapolis, IN 46208, USA

## Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENT

The authors thank Dr. Belgin Canturk for helpful discussions during the preparation of this manuscript, Dr. Quanbo Xiong for collecting the HRMS data, and the Dow AgroSciences Spinosad Production Plant for the generous supply of sample. Dow AgroSciences is acknowledged for support of this work.

## REFERENCES

1. Keay, D.; Wood, P. *GIT Lab. J. Eur.*, **2007**, *11*, 33.
2. Dripps, J. E.; Boucher, R. E.; Chloridis, A.; Cleveland, C. B.; DeAmicis, C. V.; Gomez, L. E.; Paroonagian, D. L.; Pavan, L. A.; Sparks, T. C.; Watson, G. B. *Green Trends in Insect Control*, RSC Green Chemistry No. 11, The Royal Society of Chemistry, London, **2011**, 163.
3. Mayes, M. A.; Thompson, G. D.; Husband, B.; Miles, M. M. *Reviews of Environmental Contamination and Toxicology*, **2003**, *179*, 37-71.
4. Racke, K. D. *ACS Symposium Series* **2007**, *947*, 92.
5. <https://www.epa.gov/greenchemistry/presidential-green-chemistry-challenge-1999-designing-greener-chemicals-award> (accessed July 18, 2017).
6. Evans, D. A.; Black, W. C. *J. Am. Chem. Soc.* **1993**, *115*, 4497.

7. Paquette, L. A.; Gao, Z.; Ni, Z.; Smith, G. F. *J. Am. Chem. Soc.* **1998**, *120*, 2543.
8. Paquette, L. A.; Collado, I.; Purdie, M. *J. Am. Chem. Soc.* **1998**, *120*, 2553.
9. Mergott, D. J.; Frank, S. A.; Roush, W. R. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 11955.
10. Winbush, S. M.; Mergott, D. J.; Roush, W. R. *J. Org. Chem.* **2008**, *73*, 1818.
11. Kim, H. J.; Choi, S.-H.; Jeon, B.-S.; Kim, N.; Pongdee, R.; Wu, Q.; Liu, H.-W. *Angew. Chem., Int. Ed.* **2014**, *53*, 13553.
12. Bai, Y.; Shen, X.; Li, Y.; Dai, M. *J. Am. Chem. Soc.* **2016**, *138*, 10838.
13. DeAmicis, C; Edwards, N. A.; Giles, M. B.; Harris, G. H.; Hewitson, P. Janaway, L.; Ignatova, S. *J. Chromatogr. A*, **2011**, *1218*, 6122-6127.
14. Oka, F.; Oka, H.; Ito, Y. *J. Chromatogr.* **1991**, *538*, 99.
15. Margraff, R. in: Foucault, A. (Ed.), *Centrifugal Partition Chromatography* (Chromatographic Science Series, vol. 68), Marcel Dekker, New York, **1995**, 331.
16. Ignatova, S.; Wood, P.; Hawes, D.; Janaway, L.; Keay, D.; Sutherland, I. A. *J. Chromatogr. A*, **2007**, *1151*, 20.
17. This is the first time, to our knowledge, that the Dynamic Extractions Midi processor has been used in a fully automated 24/7 way.
18. <http://www.sigmaaldrich.com/catalog/product/supelco/k08670628?lang=en&region=US> (accessed July 18, 2017).
19. <http://www.sigmaaldrich.com/catalog/product/supelco/k08971149?lang=en&region=US> (accessed July 18, 2017).

TOC Graph:

### Pilot Scale HPLC Separation of Spinosyn A and D

