

HPCCC offers significant advantages compared with RP-HPLC for a 1kg Spinetoram purification

Carl V. Deamicis¹, Neil A. Edwards², Michael B. Giles^{2,*}, Guy H. Harris², Peter Hewitson³, Svetlana Ignatova³, Lee Janaway², Philip L. Wood²

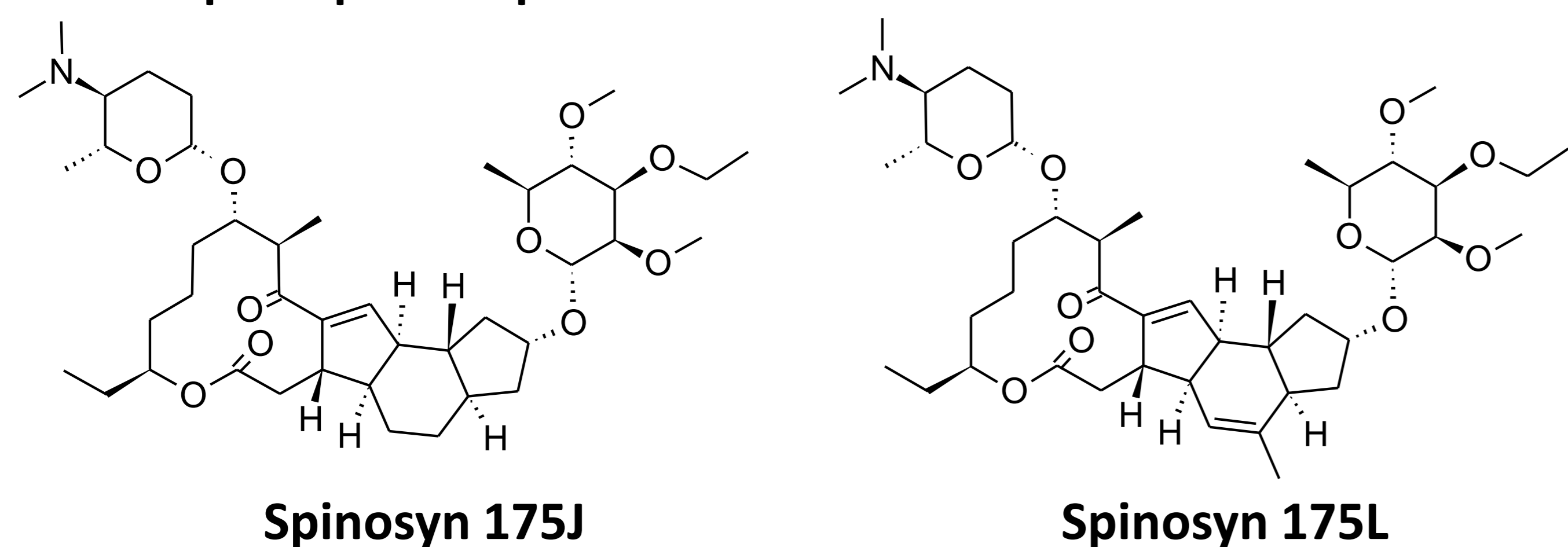
¹Dow AgroSciences LLC, Indianapolis, Indiana, USA

²Dynamic Extractions Ltd, Slough, UK ³Brunel Institute for Bioengineering, Brunel University, UK



1 Spinetoram™ (Dow AgroSciences):

- Fermentation-derived semi-synthetic insecticide
- 2 principal components



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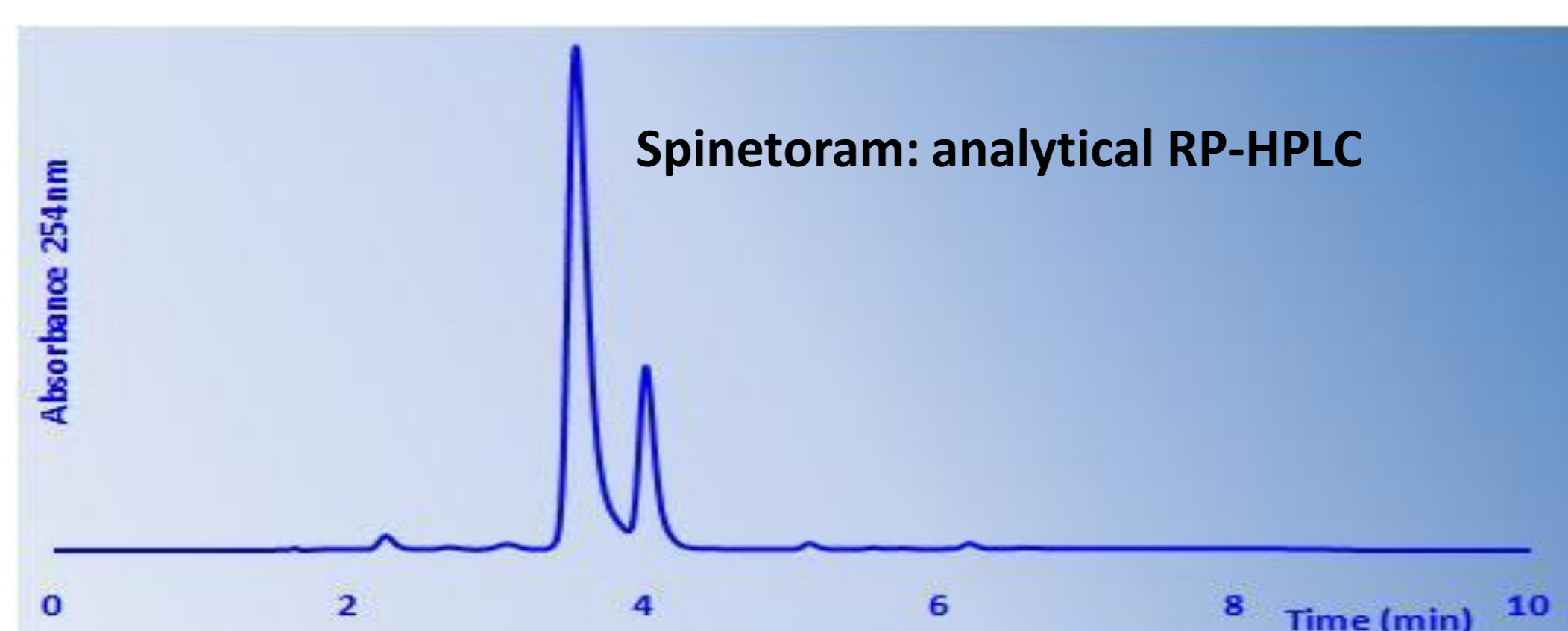
5 CCC Solution

- Dynamic Extractions HPCCC
- Solvent system optimisation: Spectrum Analytical, 23ml column
- Step 1 Scale-up: Spectrum Semi-preparative, 137ml column, 1g load
- Step 2 Scale-up: Midi, 940ml column, 7g load
- Final Scale-up: Maxi, 18L column, 111g load
- Fractions collected and analysed by RP-HPLC
- <98% pure fractions were recycled

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2 The Problem:

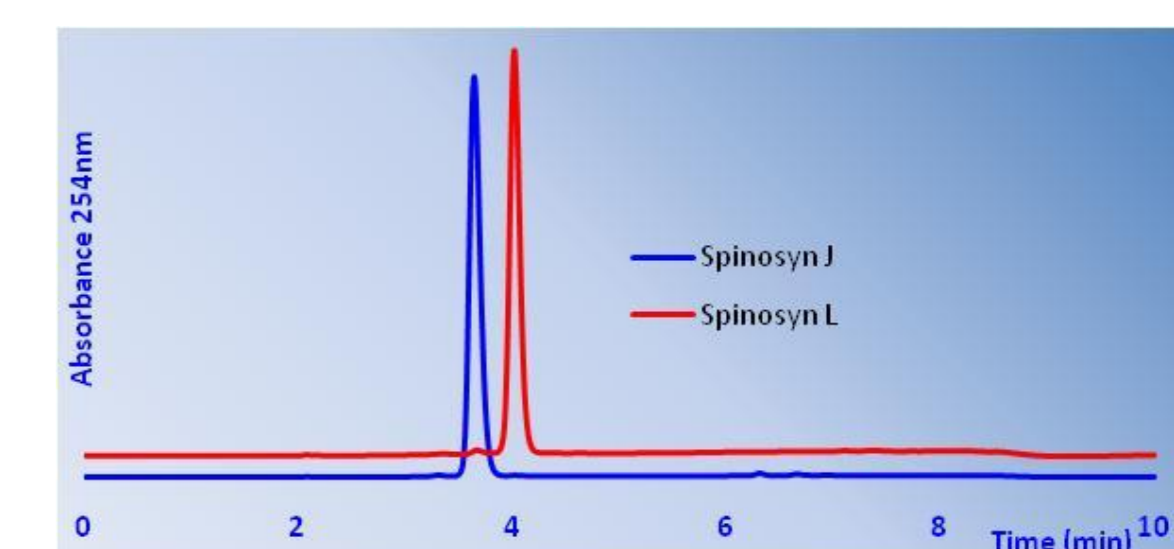
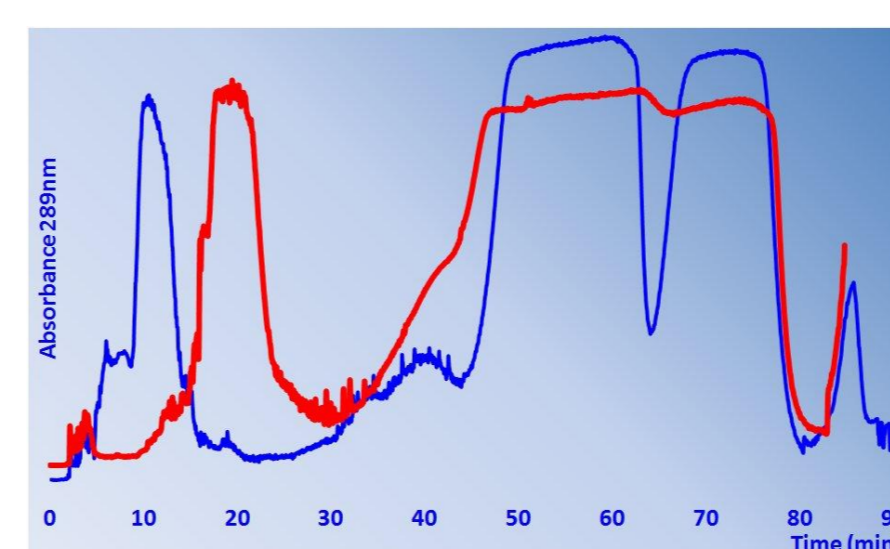
Periodic purification of 1Kg of Spinetoram to provide analytical standards of Spinosyns J and L



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6 Step 1 Scale-up:

- Spectrum Semi-preparative column
- HEMWat 24: (Hexane/Ethyl Acetate/ Methanol/ Water 5:1:5:1)
- Below left: HPCCC chromatograms of 0.5g (blue) and 1.0g (red)
- Increased loading didn't affect purity/recovery of targets
- Below right: analysis of fractions showed high purity



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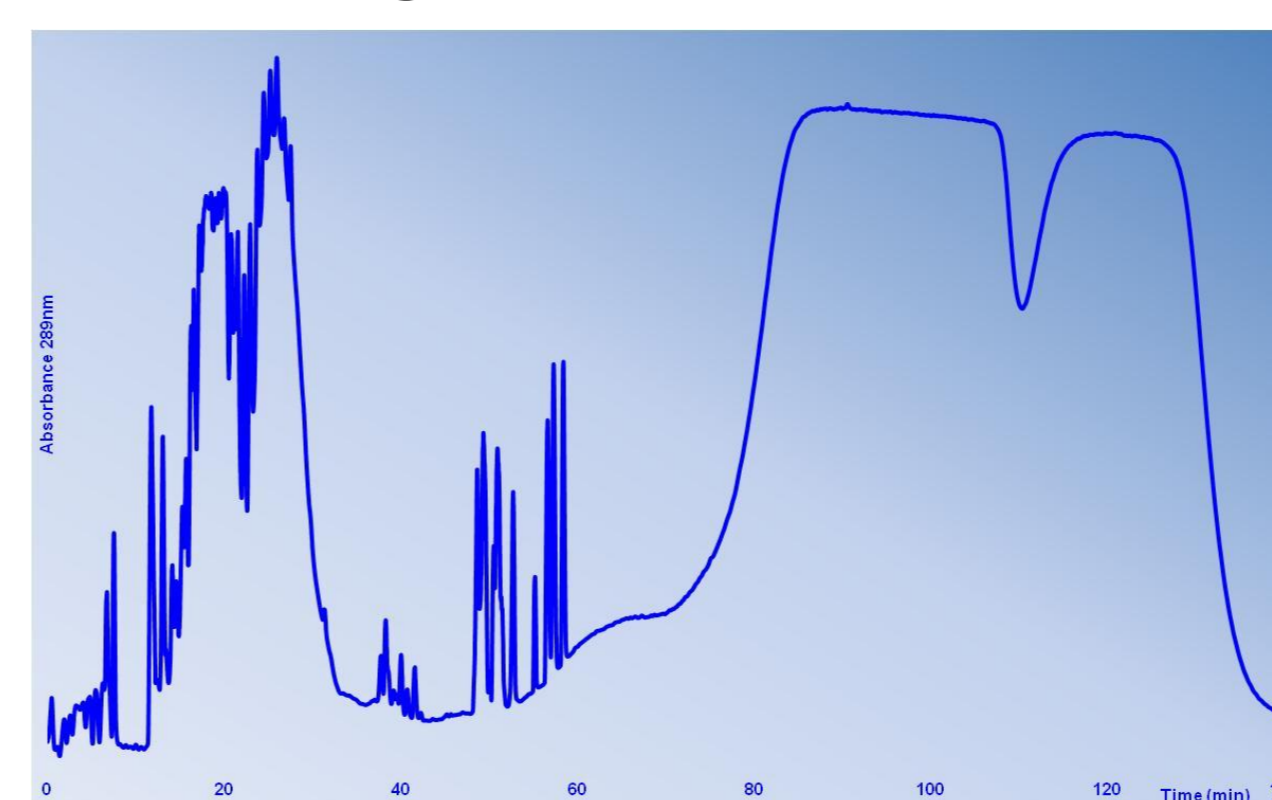
3 Traditional Solution

- Preparative RP-HPLC
- 11 x 25cm bed (1200g) Waters Xterra, C8, 10μ
- 40:60 v/v, 10 mM NH₄OH – MeCN, 800 mL/min
- Load: 2.5g Spinetoram in 10ml MeCN
- Stacked injection every 8 minutes
- Fractions collected and analysed by RP-HPLC
- <98% pure fractions were recycled

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7 Step 3 Scale-up:

- Maxi column
- HEMWat 24: (Hexane/Ethyl Acetate/ Methanol/ Water 5:1:5:1)
- 111g load



- Good recovery
- High Purity

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4 Preparative Scale HPLC Summary

Total Runs/Kg	400
Duration (min/Kg)	3200
Total Mobile Phase Volume (L/Kg)	2560
Pooled Fraction Volume (L/Kg)	1196
Recovery 175J (%)	63
Purity 175J (%)	99
Recovery 175L (%)	15
Purity 175L (%)	99
Total Recovery (%)	78

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8 Preparative Scale HPCCC Summary

Total Runs/Kg	9
Duration (min/Kg)	1972
Total Mobile Phase Volume (L/Kg)	730
Pooled Fraction Volume (L/Kg)	156
Recovery 175J (%)	63
Purity 175J (%)	97
Recovery 175L (%)	15
Purity 175L (%)	98
Total Recovery (%)	78

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www.dynamicextractions.com

www.dowagro.com

www.brunel.ac.uk/about/acad/bib

9 Preparative Scale Chromatography Comparison

	HPLC	HPCCC	HPCCC/HPLC
Total Runs/Kg	400	9	0.02
Duration (min/Kg)	3200	1972	0.62
Total Mobile Phase Volume (L/Kg)	2560	730	0.29
Pooled Fraction Volume (L/Kg)	1196	156	0.13

- Recovery and purity equivalent
- HPCCC is faster
- HPCCC used 70% less solvent
- HPCCC fraction volume to be worked up was 87% less

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