Separation of Macrocyclic Lactones (Avermectins) on FLARE C18 MM & FLARE C18+ Columns

Introduction

Avermectins are a series of 16-membered macrocyclic lactone derivatives that are used extensively in animal and crop protection. They occur in nature and can be produced as fermentation by-products by the micro-organism, *streptomyces avermitilis*. Examples of well-known avermectins and derived products include ivermectin, eprinomectin, selamectin, doramectin and abamectin. All the avermectins have high anthelmintic and insecticidal properties even at low dose levels and residues of these veterinary drug components reach the environment through manufacturing and animal waste and may potentially affect terrestrial and aquatic life forms.

Several crop protection companies have strong interest in the synthesis, production, and analysis of these compounds. Therefore, there exists a need to develop a fast and efficient analytical method capable for determining avermectin residues on animals, in food and in the environment. The HPLC methods presented below are fast and efficient and can be used for residual analysis as well as for quality control of avermectins in drug formulations. All the analyses use solvents that are MS-compatible to allow for positive identification of even trace quantities of these samples. The peaks produced are sharp resulting in high sensitivity and signal-to-noise ratios.

HPLC Separation #1 (FLARE C18 MM Short Column)

The FLARE C18 MM column is manufactured with 3.6 µm diamond core-shell particles that have 120 Å pores. The particle geometry and tight particle size distribution contribute to high packing density and column efficiency having reduced plate height, h, of ~2. The stationary phase consists of weak anionic exchange groups close to the support surface and an octadecyl carbon chain which provides reversed phase interactions with analytes. Under neutral pH conditions, when the weak anion exchangers are protonated, the column is particularly efficient at separating different avermectin compounds—even the isomeric types which have very similar chemical structures.

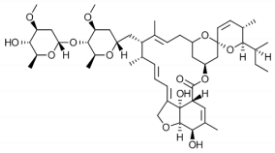
For separation #1, a short FLARE C18 MM, 50 x 4.6 mm ID, was used to quickly resolve avermectin and doramectin. Degradation products along with these major sample constituents are baseline separated with excellent peak shape in under 5 minutes. Detection was done by UV at a wavelength of 244 nm. The mobile phase chosen for this separation consisted on acetonitrile, methanol and water. While gradient elution was chosen, isocratic separations are equally effective.

Column Name:	FLARE C18 MM
Column Dimensions:	4.6 x 50 mm (15698-14-2 TB, 3.6 µm)
HPLC System:	Agilent 1200
Injection Volume:	1.0 µl
Detection:	UV at 244 nm
Flow Rate:	1.0 ml/min
Solvents:	A: 200 ml ACN, 150 ml MeOH, 650 ml H_2O B: 500 ml ACN, 400 ml MeOH, 100 ml H_2O

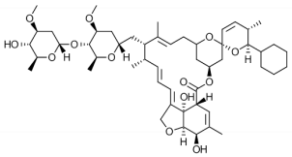
Gradient:

Time (mins)	%A	%B	
0.00	80	20	
5.00	20	80	
5.01	80	20	
10.00	80	20	end

Temperature:	35°C
Analytes:	Avermectin B1a, Doramectin



1. Avermectin B1a



2. Doramectin

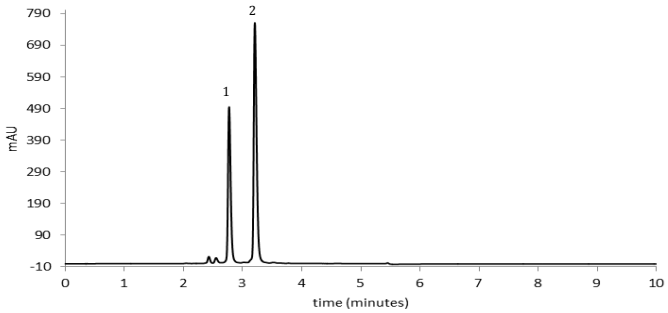
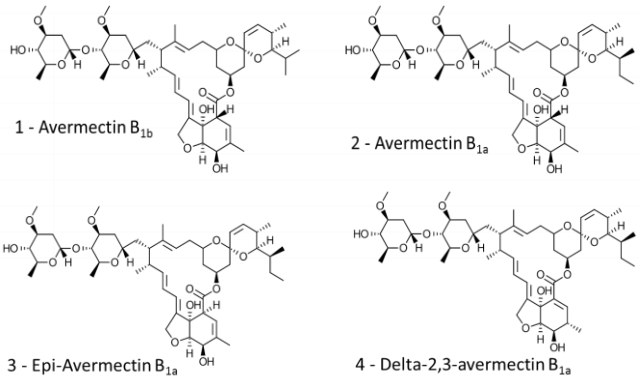


Fig 1: Separation of Avermectin B1a and Doramectin

HPLC Separation #2 (FLARE C18 MM Long Column)

For separation #2, 3 important mectin compounds were analyzed individually and as a mixture of approximately 0.2 mg/ml concentration of each analyte. The mixture solvent was methanol.

Abamectin is a natural fermentation product which consists of avermectin B_{1b} (minor component) and avermectin B_{1a} (major component). The two compounds differ by a single methyl group. The second mixture component was an epimer of avermectin B_{1a} (ie. epi-avermectin B_{1a})—the two molecules differ in the orientation of a single hydrogen atom. The final major mixture component was Delta-2,3-avermectin B_{1a}, an isomer of epi-avermectin B_{1a}. The last two compounds differ in the position of a double bond. The chemical structures of these compounds are presented below.



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As demonstrated in Fig 5 and Fig 6, the mixture can be baseline separated in about 18 minutes using gradient elution with an acetonitrile/methanol/water mobile phase. The chromatograms corresponding to individual analytes is also presented. What is even more interesting is that up to 10 degradation products can be seen and monitored in the combined chromatogram.

Column Name:	FLARE C18 MM			
Column Dimensions:	4.6 x 150 mm (15698-14-2 TB, 3.6 µm)			
HPLC System:	Agilent 1200			
Injection Volume:	5.0 µl, ca. 0.2 mg/ml in MeOH			
Detection:	UV at 244 nm			
Flow Rate:	1.0 ml/min			
Solvents:	A: 50 ml ACN, 100 ml MeOH, 850 ml H_2O			
	B: 600 ml ACN, 200 ml MeOH, 200 ml H_2O			
Gradient:	Time (mins)	%A	%B	
	0.00	90	10	
	10.00	10	90	
	10.01	90	10	
	18.00	90	0	end
Temperature:	35°C			

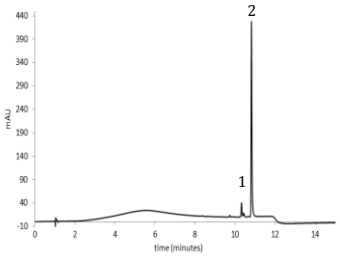

Fig 2: Abamectin: Mixture of avermectin B1a (~90%) and avermectin B1b (~10%)

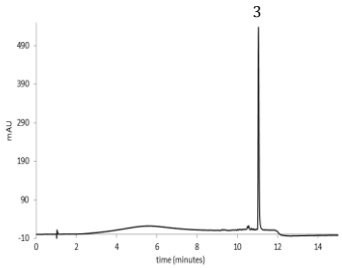

Fig 3: Epi-avermectin B1a

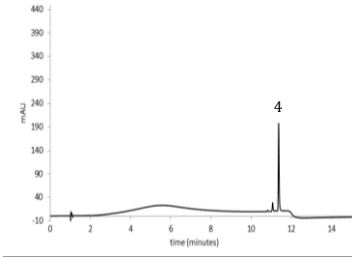

Fig 4: 2,3-didehydro-5-O-dimethyl-3,4-dihydro-(4s)-Avermectin A1a

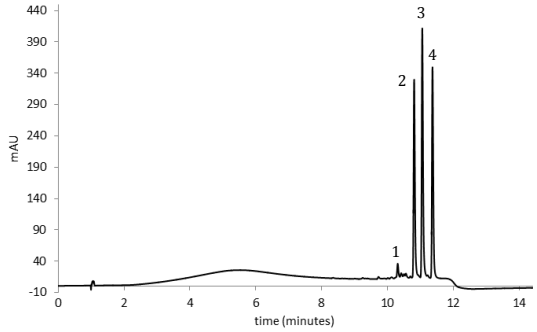

Fig 5: Separation of a mixture of compounds 1, 2, 3 and 4 above

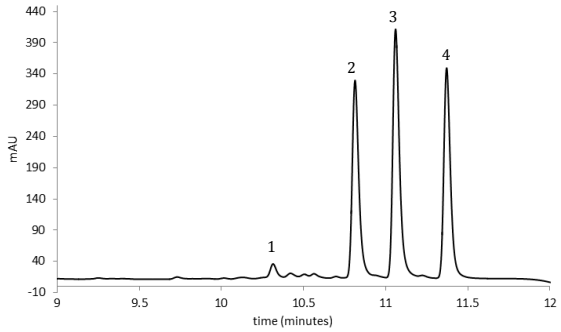

Fig 6: Separation of a mixture of compounds 1, 2, 3 and 4 above (exploded view)

HPLC Separation #3 (FLARE C18+ Long Column)

Like the FLARE C18 MM phase, the FLARE C18+ column also exhibits ion exchange properties along with hydrophobic interactions. However, the C18+ phase has a permanent positive charge on the support surface which is unaffected by the pH of the mobile phase making the C18+ columns about half as retentive as their C18 MM equivalents. These columns are particularly efficient when analyzing very hydrophobic molecules which may be overly retained on a traditional C18 phase. Even while

decreasing the strength of the mobile phase compared to Separation #2, retention time was reduced by about 10% on the C18+ phase.

Finally, in the separations below, the effect of tetramethylammonium hydroxide (t-MeAmOH) additive was investigated to see how pH may affect resolution when using the C18+ phase. As can be seen, comparing Fig. 8 and Fig. 10, the effect of t-MeAmOH on separation efficiency and resolution of the avermectins was quite minimal.

Separation of avermectin mixture without t-MeAmOH additive

Column Name:	FLARE C18+
Column Dimensions:	4.6 x 100 mm (115889-4-2, 3.6 µm)
HPLC System:	Agilent 1200
Injection Volume:	5.0 µl, ca. 0.2 mg/ml in MeOH
Detection:	UV at 244 nm
Flow Rate:	1.0 ml/min
Solvents:	A: 50 ml ACN, 100 ml MeOH, 850 ml H_2O B: MeOH C: ACN

Gradient:

Time (mins)	%A	%B	%C	
0.00	80	10	10	
10.00	40	25	35	
10.01	80	10	10	
18.00	80	10	10	end

Temperature:	35°C
Analytes:	Same as in Separation #2

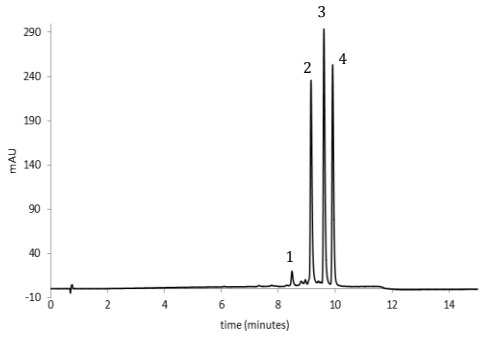

Fig 7: Separation of a mixture of compounds 1, 2, 3 and 4 on C18+ phase without t-MeAmOH additive

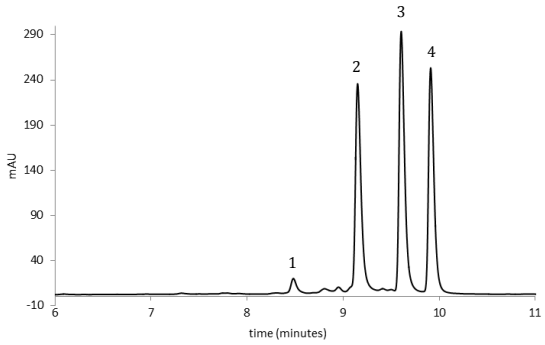

Fig 8: Separation of a mixture of compounds 1, 2, 3 and 4 on C18+ phase without t-MeAmOH additive (exploded view)

Separation of avermectin mixture with t-MeAmOH additive

Column Name:	FLARE C18+
Column Dimensions:	4.6 x 100 mm (115889-4-2, 3.6 µm)
HPLC System:	Agilent 1200
Injection Volume:	5.0 µl, ca. 0.2 mg/ml in MeOH
Detection:	UV at 244 nm
Flow Rate:	1.0 ml/min
Solvents:	A: 50 ml ACN, 100 ml MeOH, 850 ml H_2O B: 500 ml ACN, 500 ml MeOH, 4ml t-MeAmOH C: MeOH D: ACN

Gradient:

Time (mins)	%A	%B	%C	%D	
0.00	80	10	5	5	
10.00	40	10	20	30	
10.01	80	10	5	5	
18.00	80	10	5	5	end

Temperature:	35°C
Analytes:	Same as in Separation #2

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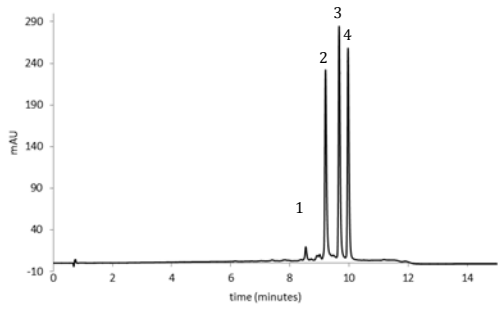

Fig 9: Separation of a mixture of compounds 1, 2, 3 and 4 on C18+ phase with t-MeAmOH additive

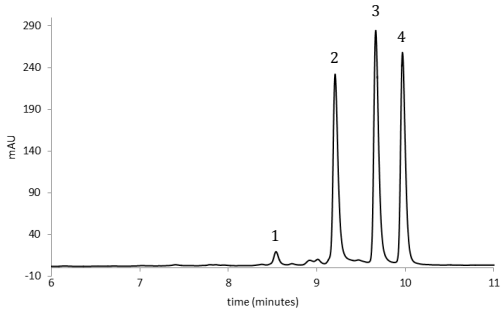

Fig 10: Separation of a mixture of compounds 1, 2, 3 and 4 on C18+ phase with t-MeAmOH additive (exploded view)

Conclusion

It is evident from the separations above that the FLARE C18MM and C18+ columns, long or short, are capable of resolving the most important macrocyclic lactones (avermectins). These compounds can be fully resolved in a reliable and sensitive method in a short time using MS-compatible mobile phases. Additionally, a host of degradation products can be studied in the same separation. In particular, in the mixture of 4 avermectin products considered above, up to 10 separate degradation products could be identified.

References

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Acknowledgement: David A. Hunt, Syngenta

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About Diamond Analytics

Diamond Analytics, a US Synthetic company, expands the existing range of analytical capabilities in separation science by providing diamond-based solutions that allow for the exploration of novel chemistries. The company is a pioneer in the creation of non-silica diamond core-shell particles that are chemically modified for use in High Performance Liquid Chromatography (HPLC).

As a result of breakthroughs in diamond technology, Diamond Analytics' HPLC columns offer expanded pH range capability (1-13), elevated temperature ranges (up to 100 °C), increased longevity, novel selectivity and uncompromising efficiency—leading to a longer, more productive life and increased total cost savings. All Diamond Analytics columns are LC-MS compatible and can operate from 0% to 100% organic/aqueous mobile phases. Particle manufacturing uses 99.999% pure diamonds to minimize secondary interactions with metals.

The parent company, US Synthetic, was founded in 1978. The Diamond Analytics division was created in 2005 with over 50 years of combined experience in chromatography.

Column Phases

FLARE C18 MM (Mixed-Mode)
*O*perates primarily in a reversed-phase and anion exchange mode depending on the pH of the mobile phase. At low to medium pH, the column is great for analysis of neutral compounds, acids and anionic compounds. At high pH, the column is great for analysis of bases with good peak shape.

FLARE C18+
*E*xhibits a permanent positive charge regardless of the pH of the mobile phase. It is good for the analysis of polar molecules (including carbohydrates, sugars and steroids) and anionic species. It also works for very hydrophobic compounds where low retention is desired.

FLARE HILIC
*H*as a hydrophilic amino-diol surface and is able to retain very polar molecules at all pH conditions. Works well for the analysis of sugars, nuclotides, and polar APIs.

Column Dimensions

ID/Length	33 mm	50 mm	75 mm	100 mm	150 mm
2.1 mm		x	x	x	x
4.6 mm	x	x	x	x	x

For more information or to order a column,

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