Correct spinosad quantitation strategies

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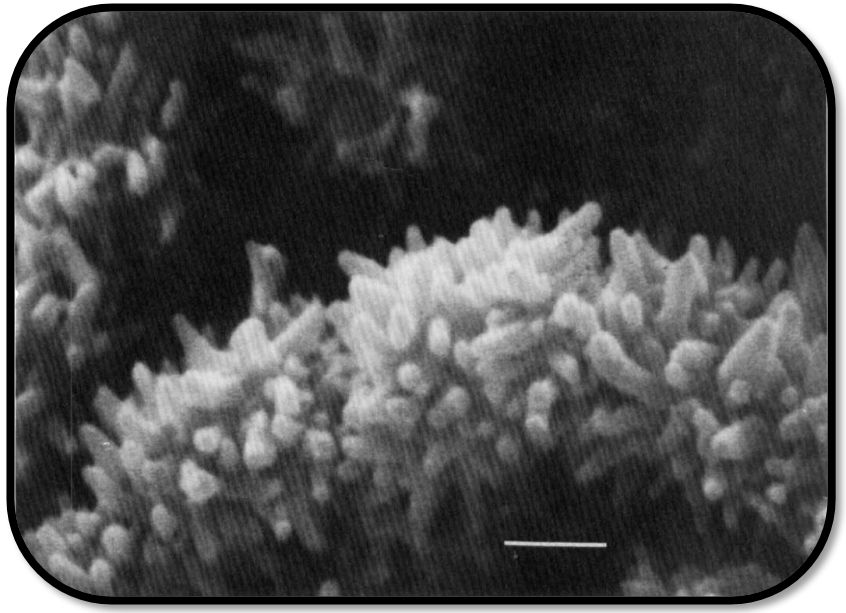
ORIGIN

- Spinosad is a **mixture of two tetracyclic macrolide neurotoxins** of insecticidal action produced naturally by *Saccharopolyspora spinosa* (*S. spinosa*), first reported by Metz and Yao in 1990 in a sugar mill rum still.[1]

- *S. spinosa* naturally occurring spinosyn A and spinosyn D were first isolated from in 1991 by Kirst et al. and subsequently developed as *Spinosad* and patented by Dow AgroSciences as *Tracer®*.[2,3]

- A modification of naturally occurring spinosyns J and L to transform their 3-*O*′-methyl of the rhamnose moiety into 3-*O*′-ethyl group was also developed and patented by Dow AgroSciences as *Pulgus™*.[3] The synthetic spinetoram J and L mixture presents a higher stability and faster insecticide activity compared to spinosad.



Scanning electron micrograph of *S. spinosa*, showing the spiny spore sheath surface. Bar = 1.0 µm.[1]

[1] Metz, F. R.; Yao, R. C. Saccharopolyspora spinosa sp. nov. isolated from soil collected in a sugar mill rum still. *Int. J. Syst. Evol. Microbiol.* **1990**, *40*, 34-39.

[2] Kirst, H. A.; Michel, K. H.; Martin, J. W.; Creemer, L. C.; Chio, E. H.; Yao, R. C.; Nakatsukasa, W. M.; Boeck, L.V. D.; Occolowitz, J. L.; Paschal, J. W.; Deeter, J. B.; Jones, N. D.; Thompson, G. D. A83543A-D, Unique Fermentation-Derived Tetracyclic Macrolides. *Tetrahedron Lett.* **1991**, *37*, 4839-4842.

[3] *Tracer®* and *Pulgus™* are registered trademarks of Dow AgroSciences, now integrated in Corteva Agriscience.

PRICE AND CAS NUMBERS

Compound name	Components		
Spinetoram	**Mixture**	**Spinetoram J**	**Spinetoram L**
	2.90 €/mg 935545-74-7	Not found 187166-40-1	Not found 187166-15-0
Spinosad	**Mixture**	**Spinosyn A**	**Spinosyn D**
	1.51 €/mg 168316-95-8	**21.3** €/mg 131929-60-7	**137.1** €/mg 131929-63-0

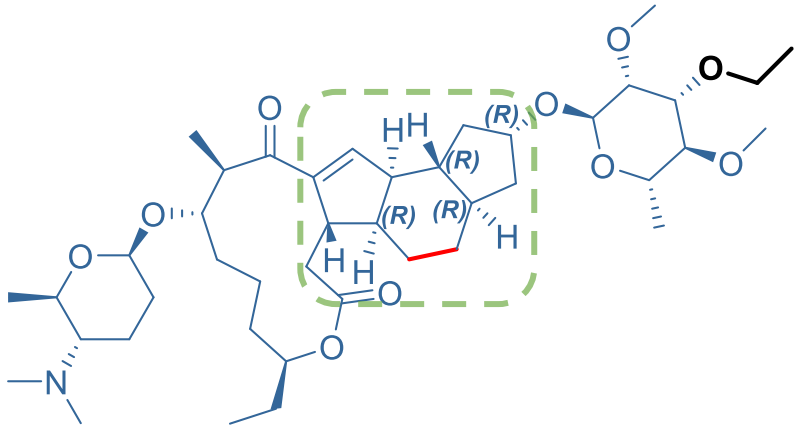
- The **spinosad technical mixture** is comprised of spinosyn A, the major component (50-95 %), and spinosyn D, the minor component (5-50 %).
- Although the **individual standards** are available, relative to the technical mixture, the cost can be between 5 200 % and 10 400 % higher.
- To the best of our knowledge, the individual spinetoram J and spinetoram L standards are not yet commercially available.

CAS number

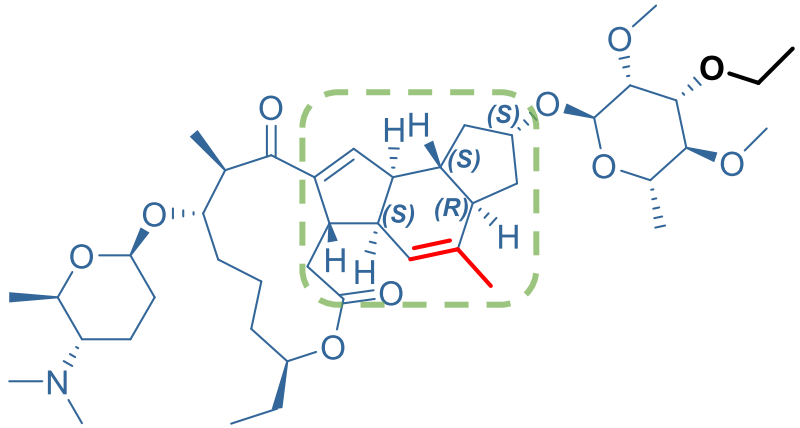
In the past, **wrong CAS numbers** were detected in the main companies selling pesticide standards

- Check the CAS number of the component before purchasing
- Re-check the CAS number in the certificate of analysis after arrival

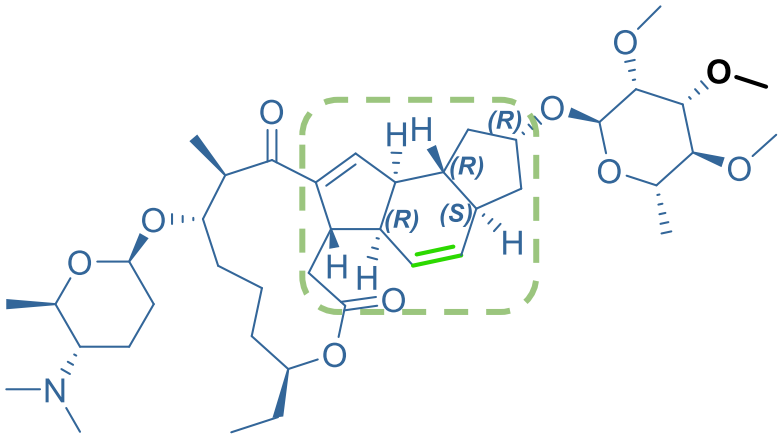
Spinosyns



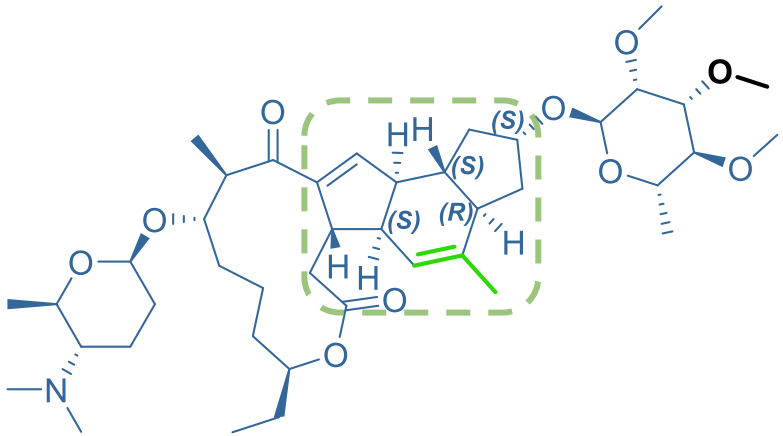
Spinetoram J
Chemical Formula: $C_{42}H_{69}NO_{10}$
Exact Mass: 747.4921 Da



Spinetoram L
Chemical Formula: $C_{43}H_{69}NO_{10}$
Exact Mass: 759.4921 Da



Spinosyn A
Chemical Formula: $C_{41}H_{65}NO_{10}$
Exact Mass: 731.4608 Da



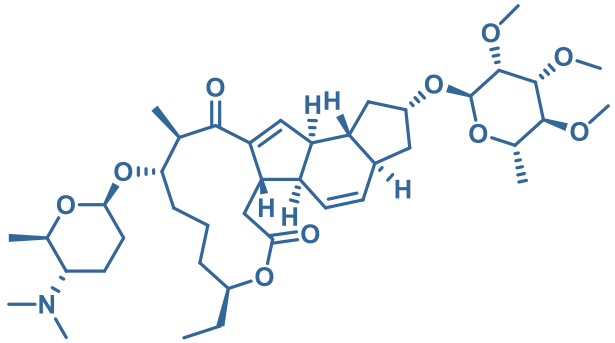
Spinosyn D
Chemical Formula: $C_{42}H_{67}NO_{10}$
Exact Mass: 745.4765 Da

745.4765 (100.0%)
746.4799 (45.4%)
747.4832 (10.1%)

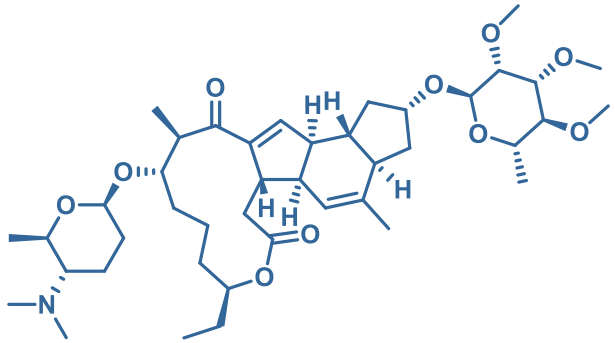
Spinosad

European Commission

Spinosad (spinosad, sum of spinosyn A and spinosyn D)

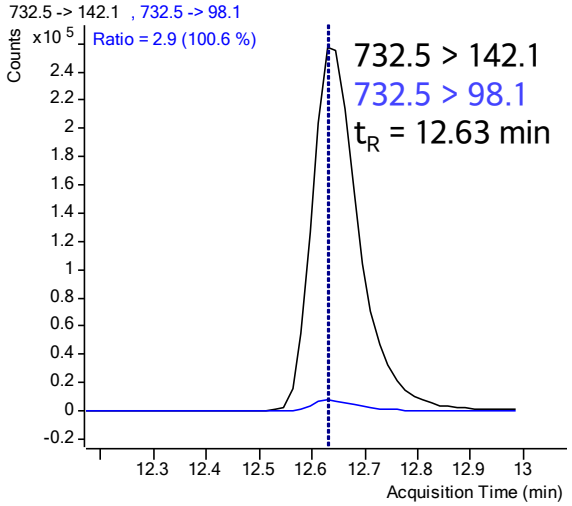


Spinosyn A

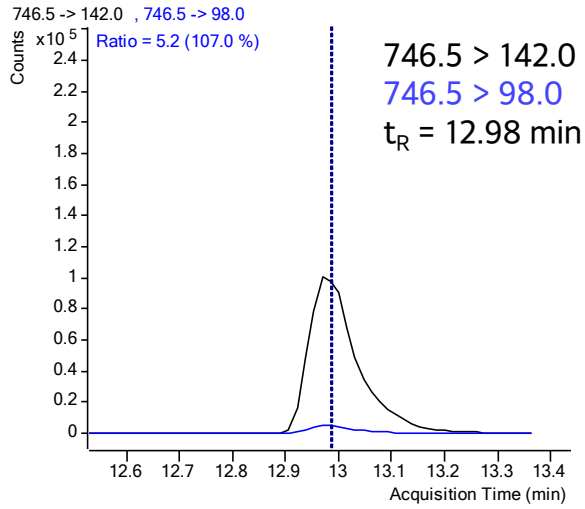


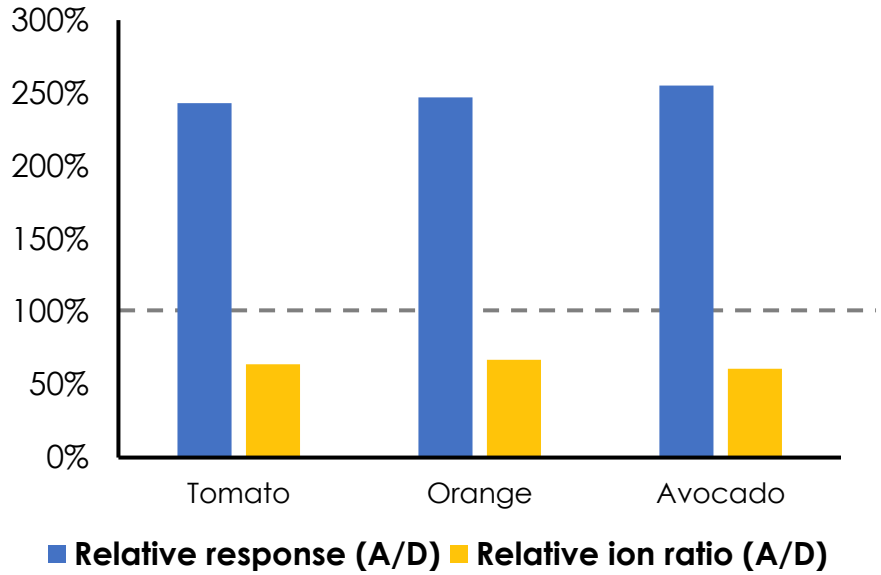
Spinosyn D

Spinosyn A



Spinosyn D





Spinosad

Potential quantitation strategies

1. Use of the spinosad technical mixture
   A. Assume equal concentrations of spinosyn A and D.
      i. Quantitate independently and sum the concentrations.
      ii. Quantitate with the sum of spinosyn A and D areas.
      iii. Quantitate with the average of spinosyn A and D areas.

   B. Apply a correction factor.
      i. Check the relative purity of spinosyn A and D in the technical mixture. Quantitate independently and sum the individually corrected concentrations.

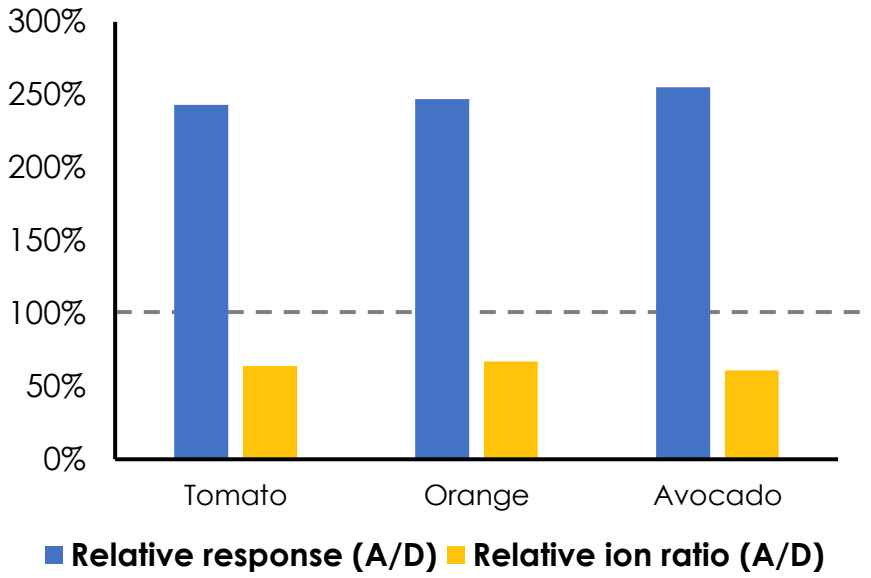
2. Use individual spinosyn A and spinosyn D standards
   A. Quantitate independently and sum the concentrations.
   B. Quantitate with the sum of spinosyn A and D areas.
   C. Quantitate with the average of spinosyn A and D areas.

Spinosad

Correct quantitation strategies

1. Use of the spinosad technical mixture
   A. Assume equal concentrations of spinosyn A and D.
      ~~i. Quantitate independently and sum the concentrations.~~
      ~~ii. Quantitate with the sum of spinosyn A and D areas.~~
      ~~iii. Quantitate with the average of spinosyn A and D areas.~~

   B. Apply a correction factor.
      i. Check the relative purity of spinosyn A and D in the technical mixture. Quantitate independently and sum the individually corrected concentrations.



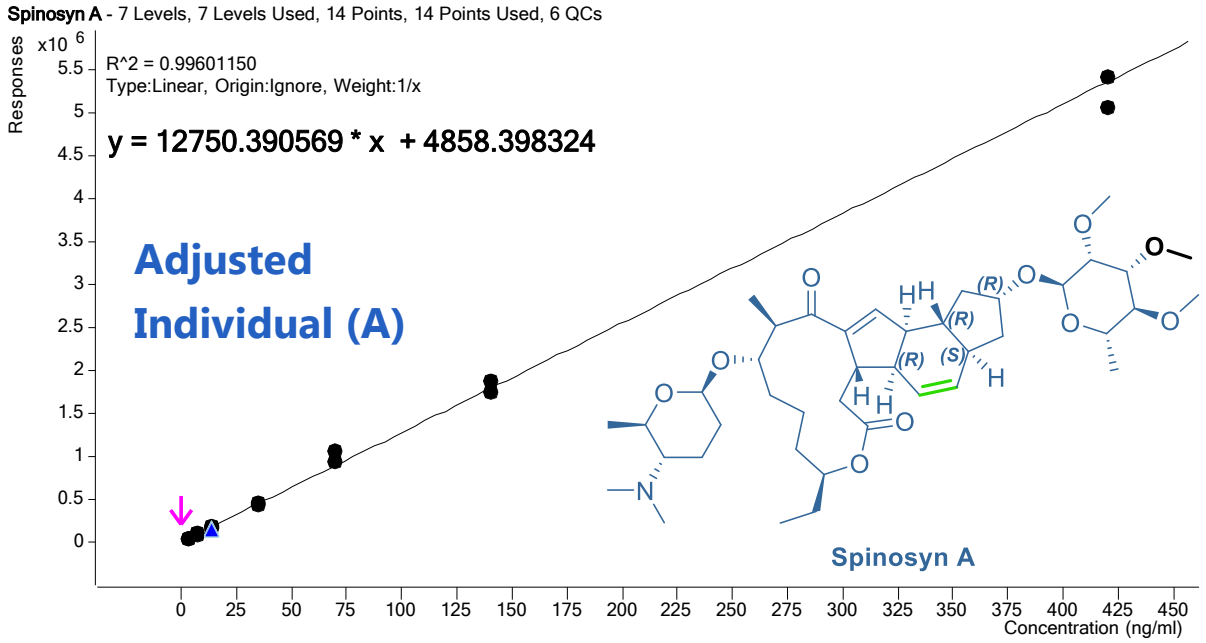
Instrumental behaviour is not equal for spinosyn A and D

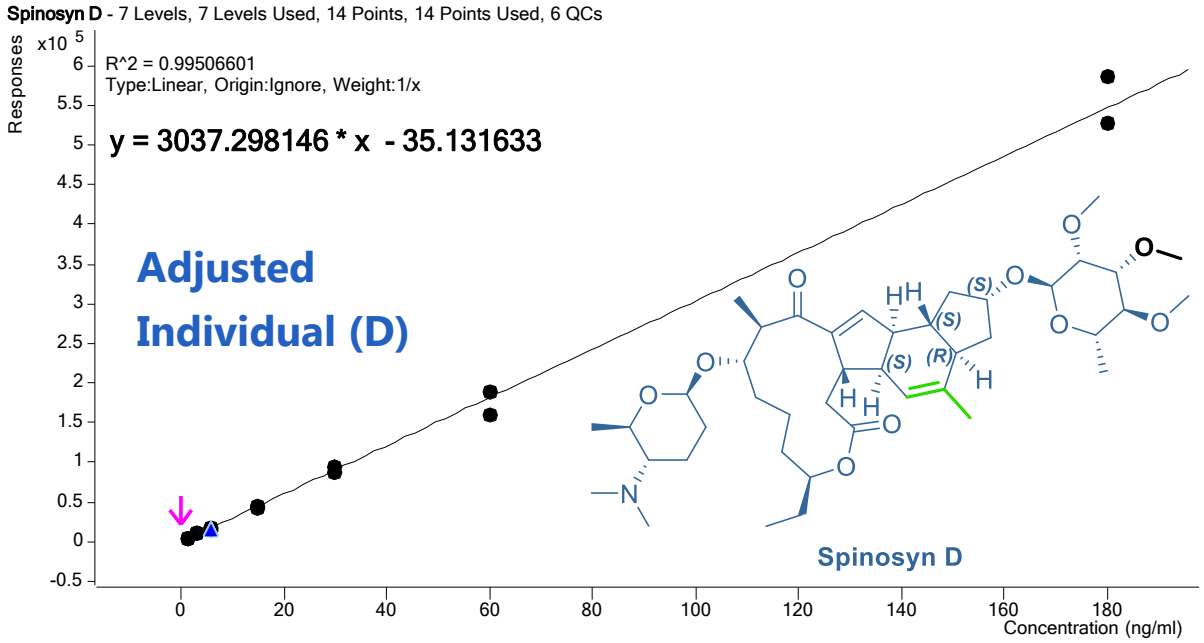
2. Use individual spinosyn A and spinosyn D standards
   A. Quantitate independently and sum the concentrations.
   ~~B. Quantitate with the sum of spinosyn A and D areas.~~
   ~~C. Quantitate with the average of spinosyn A and D areas.~~

Spinosad

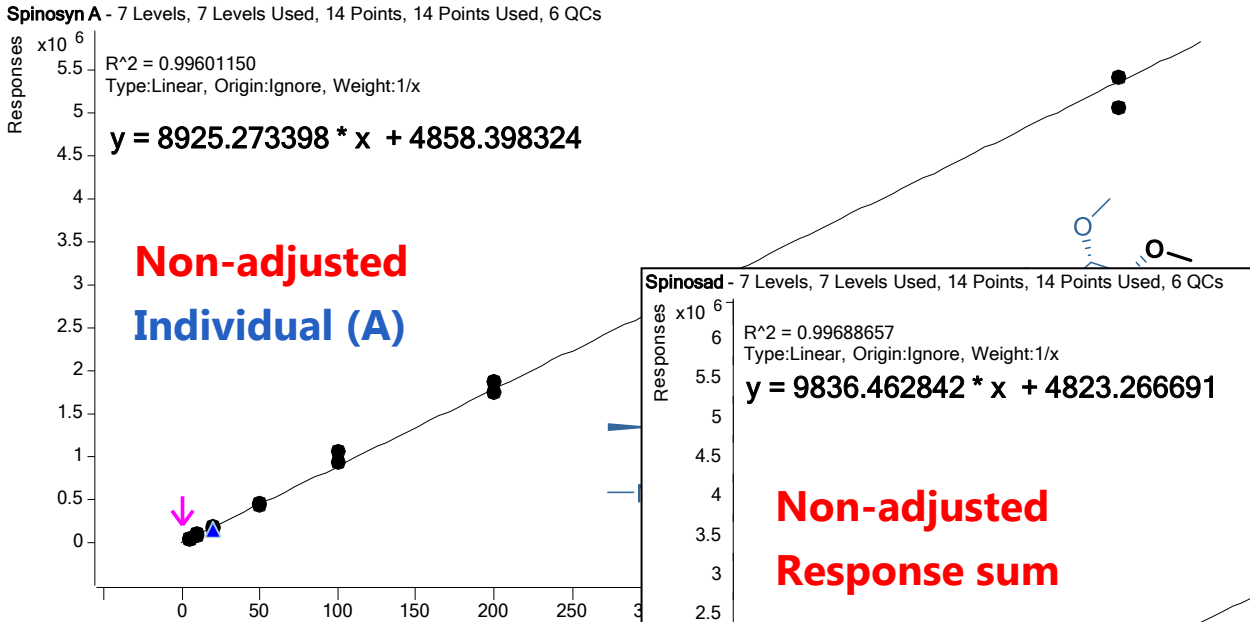
Practical examples: different quantitation approaches

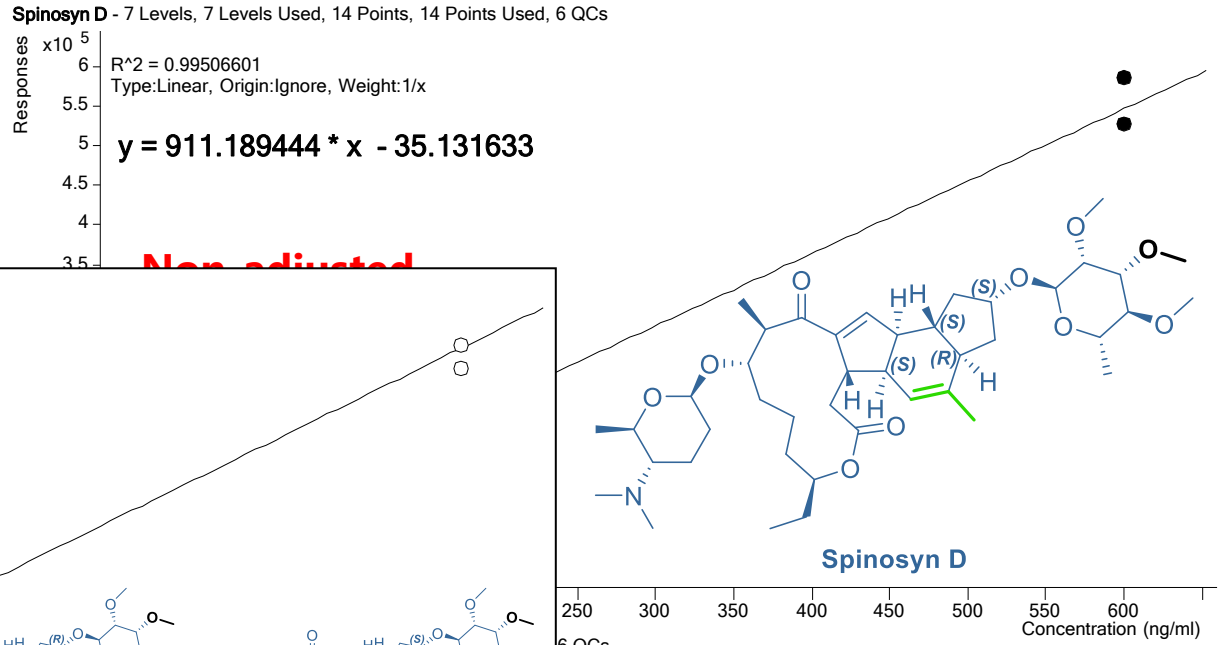
1. Use of the spinosad technical mixture (e.g. 70:30, w/w, A:D)
   A. Appropriately **adjust the concentration** of each calibration point to the actual spinosyn A or D concentration.
   B. In such a 0.100 mg/L spinosad **calibration point** (here in avocado) there are 0.070 mg/L of spinosyn A and 0.030 mg/L of spinosyn D.

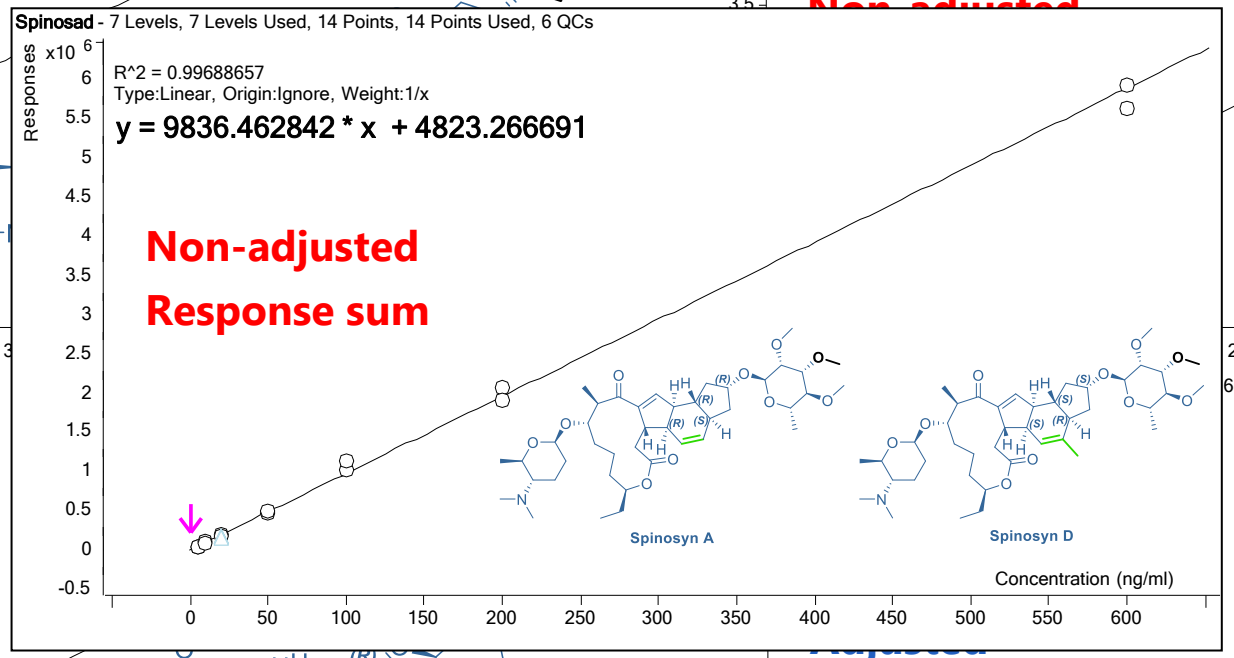


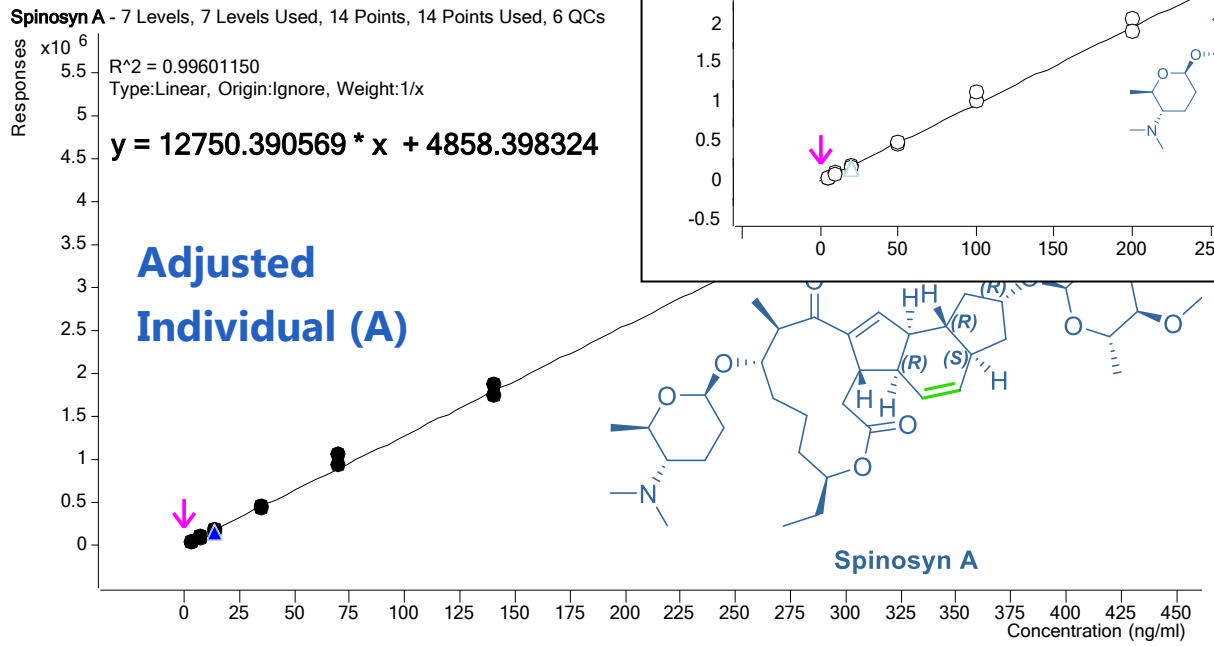


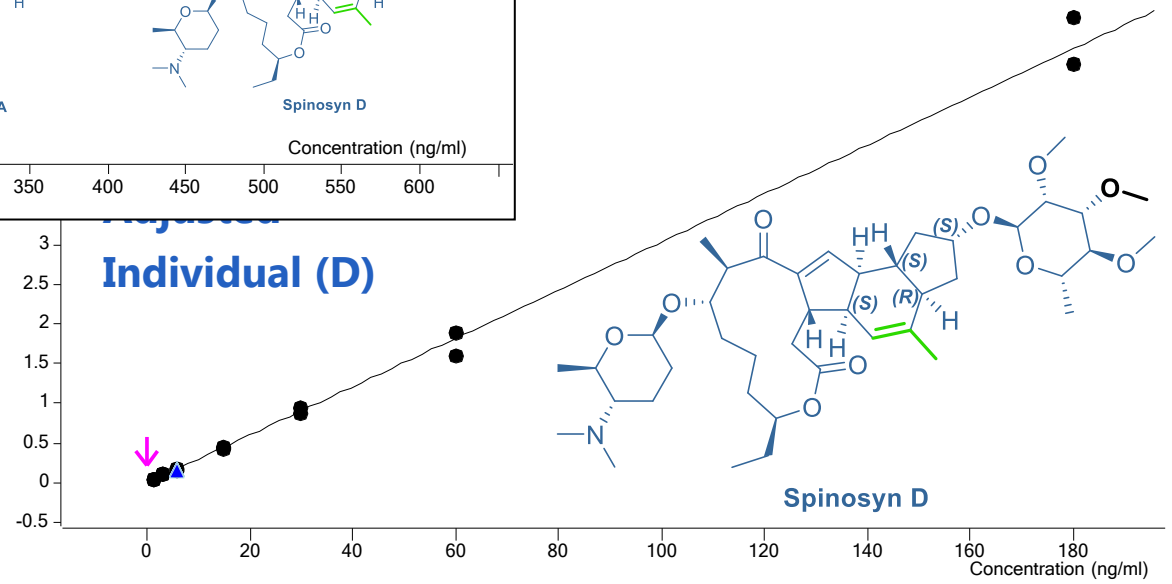
Spinosad


Spinosyn A - 7 Levels, 7 Levels Used, 14 Points, 14 Points Used, 6 QCs
Responses
x10 6
R^2 = 0.99601150
Type:Linear, Origin:Ignore, Weight:1/x
y = 8925.273398 * x + 4858.398324
Non-adjusted
Individual (A)


Spinosyn D - 7 Levels, 7 Levels Used, 14 Points, 14 Points Used, 6 QCs
Responses
x10 5
R^2 = 0.99506601
Type:Linear, Origin:Ignore, Weight:1/x
y = 911.189444 * x - 35.131633
Spinosyn D
Concentration (ng/ml)


Spinosad - 7 Levels, 7 Levels Used, 14 Points, 14 Points Used, 6 QCs
Responses
x10 6
R^2 = 0.99688657
Type:Linear, Origin:Ignore, Weight:1/x
y = 9836.462842 * x + 4823.266691
Non-adjusted
Response sum
Spinosyn A
Spinosyn D
Concentration (ng/ml)


Spinosyn A - 7 Levels, 7 Levels Used, 14 Points, 14 Points Used, 6 QCs
Responses
x10 6
R^2 = 0.99601150
Type:Linear, Origin:Ignore, Weight:1/x
y = 12750.390569 * x + 4858.398324
Adjusted
Individual (A)
Spinosyn A
Concentration (ng/ml)

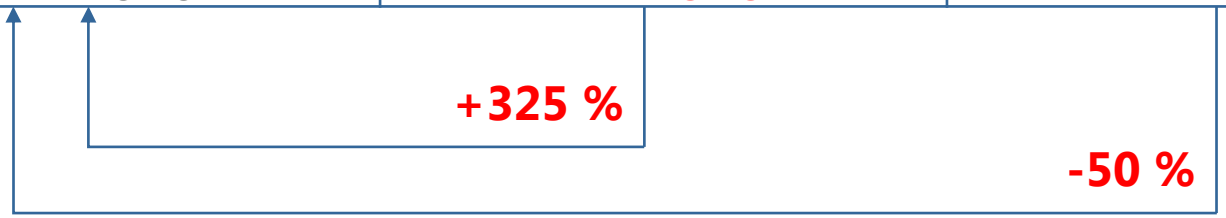

Individual (D)
Spinosyn D
Concentration (ng/ml)

Spinosad

Practical examples: different quantitation approaches

1. Use of the spinosad technical mixture (e.g. 70:30, w/w, A:D)
   A. Appropriately **adjust the concentration** of each calibration point to the actual spinosyn A or D concentration.
   B. In such a 0.100 mg/L spinosad **calibration point** (here in avocado) there are 0.070 mg/L of spinosyn A and 0.030 mg/L of spinosyn D.

Results for an instrumental response of 5.0E5 counts for each spinosyn A and spinosyn D			
Analyte	**Adjusted, individual calibration curves. Sum of spinosyn A and D**	**Non-adjusted, individual calibration curves. Sum of spinosyn A and D**	**Response sum calibration curve**
Spinosyn A	0.039 mg/kg	0.055 mg/kg	-
Spinosyn D	0.16 mg/kg	0.59 mg/kg	-
Spinosad	**0.20 mg/kg**	**0.65 mg/kg**	**0.10 mg/kg**



FURTHER READING: EURLFV-2020-M41