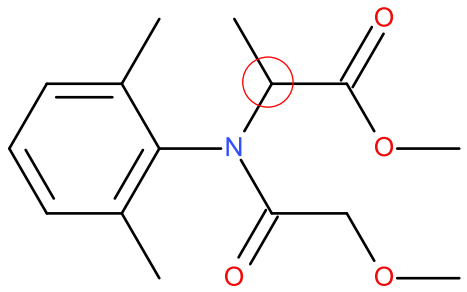
Metalaxyl-M

Assay determination by chiral HPLC

Metalaxyl and Metalaxyl-M



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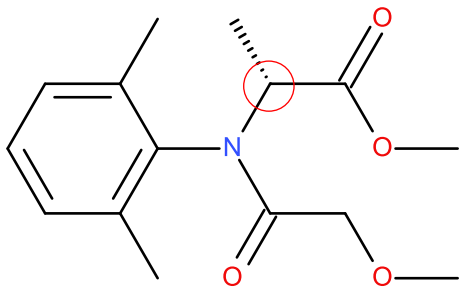

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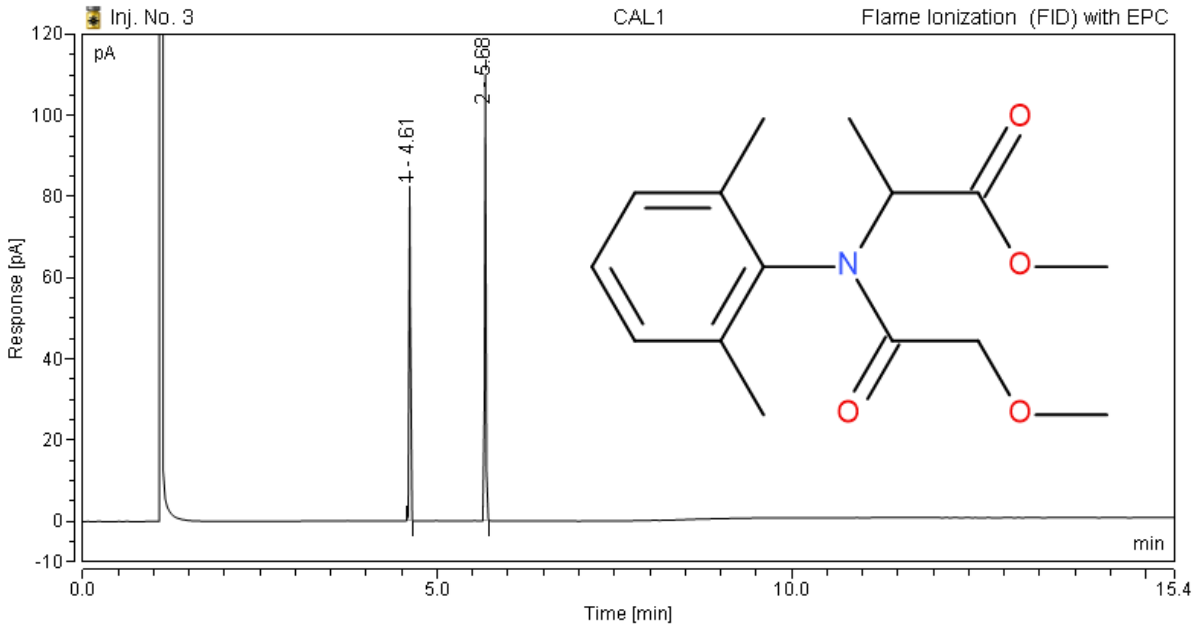
GC Method

Column fused silica, length 30 m x 0.25 (i.d.) mm, film thickness: 0.25 µm, coated with crosslinked dimethyl polysiloxane (DB-5 MS or equivalent). Carrier gas: Hydrogen

Injector	split injection
Detector	flame ionization
Temperatures	
Injector	250 ºC
Detector	300 ºC
Oven program	
temp 1	160 ºC,
ramp	rate 10 ºC/min
temp 2	230 ºC,
ramp	rate 50 ºC/min
temp 3	300 ºC, hold 7 min



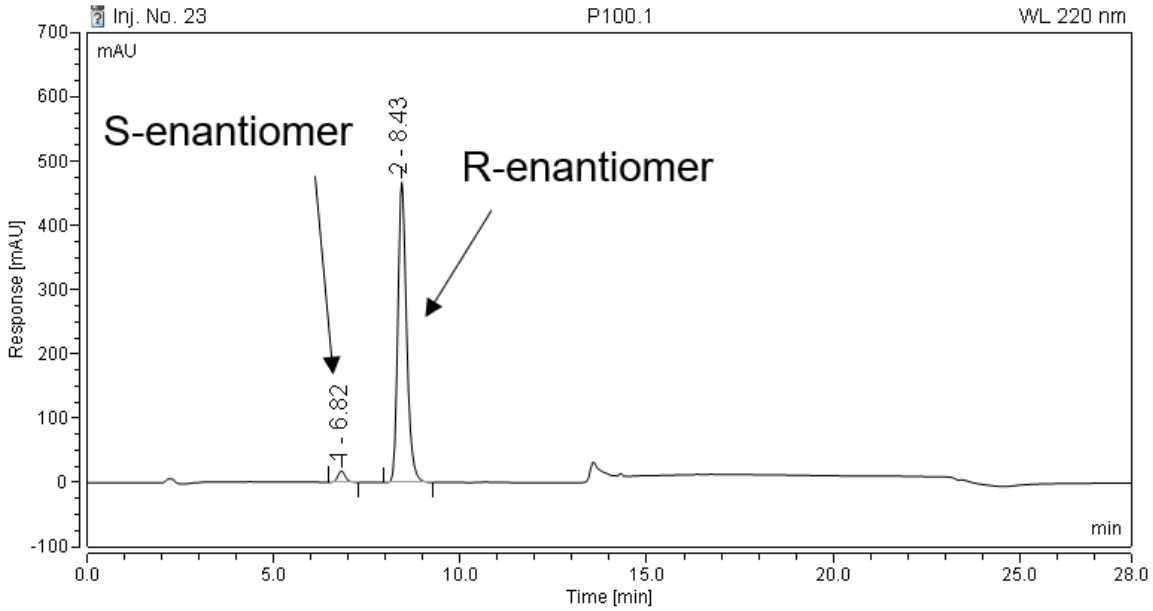
TBME or Acetone as solvent, Benzyl benzoate as internal standard

LC Method

Column	150 x 4.6 mm (i.d.) packed with Chiralpak IB, 5 µm particle size
Temperature	40 °C
Flow	0.8 ml/min
Solvent	Acetonitril
Injection volume	10 µl
Wavelength	220 nm
Run time	28 min

Eluent A	Wasser/Acetonitril (65/35, v/v)
Eluent B	Acetonitril
Gradient	

Zeit (min)	A	B
0	100	0
10	100	0
11	20	80
20	20	80
21	100	0
28	100	0



As this is an area% method the overall assay was set for all samples to eliminate errors from assay determination.

Collaborative trial

5 Samples have been send together with Metalaxyl-M reference and internal standard Benzyl benzoat.

- Metalaxyl TC (racemic; Assay 995 g/kg)
- Metalaxyl-M, TC (enantiomerically enriched; Assay: 980 g/kg)
- SL 480 (Assay 460 g/kg)
- ES 350 (Assay 320 g/kg)
- WG 4 (Assay 40 g/kg)

Current set up vs direct chiral determination

Current method

- GC sample preparation
- GC Instrument
- GC run time 15.4 minutes per sample
- LC sample preparation
- LC Instrument
- LC Run time 28 minutes per sample

Proposed set up

- LC sample preparation
- LC run time 28 minutes per sample
- Less solvent, no hydrogen carrier gas
- Only one instrument

More waste and more elaborative but also more precise?

Greener and faster solution but less precise?

Aim of the chiral assay method

- Use of existing data
- Initial trial was with given assay and only chiral separation was assess
- Calibration was only used for identification
- One point calibration only
- Feedback from the participants indicated that there is interference

Who is using chiral LC for direct assay determination?

Current approach: statistical evaluation with GC Assay and chiral LC

Assay:

Sample	x_m [g/kg]	L	N	s_r	s_L	s_R	r	R	RSD_R	RSD_R (Hor)	HorRat
Metalaxyl	994.66	12	24	8.32	10.59	13.47	23.30	37.72	1.35	2.00	0.68
Metalaxyl-M	971.46	12	24	9.88	13.17	16.47	27.67	46.10	1.69	2.01	0.84
SL 480	459.14	13	25	6.10	7.44	9.62	17.08	26.95	2.10	2.25	0.93
ES 350	318.71	13	25	3.83	5.91	7.04	10.73	19.71	2.21	2.38	0.93
WG 4	39.16	13	26	0.74	0.83	1.11	2.08	3.11	2.84	3.26	0.87

Chiral:

Sample	x_m [g/kg]	L	N	s_r	s_L	s_R	r	R	RSD_R	$RSD_{R\ (Hor)}$	HorRat
Metalaxyl	497.84	10	20	0.76	0.94	1.21	2.14	3.39	0.24	2.22	0.11
Metalaxyl-M	949.18	10	20	1.88	2.14	2.85	5.27	7.97	0.30	2.02	0.15
SL 480	443.95	10	20	0.90	0.86	1.24	2.51	3.47	0.28	2.26	0.12
ES 350	308.22	10	20	0.31	0.99	1.03	0.88	2.90	0.34	2.39	0.14
WG 4	38.48	10	20	0.51	0.40	0.65	1.43	1.81	1.68	3.27	0.52

Statistical evaluation chiral assay determination

Sample	x_m [g/kg]	L	N	s_r	s_L	s_R	r	R	RSD_R	$RSD_{R\ (Hor)}$	HorRat
Metalaxyl	507.39	9	17	12.49	10.47	16.30	34.97	45.64	3.21	2.22	1.45
Metalaxyl-M	940.08	10	19	30.18	0.00	30.18	84.51	84.51	3.21	2.02	1.59
SL 480	449.24	10	19	12.19	5.86	13.52	34.12	37.86	3.01	2.26	1.33
ES 350	317.77	10	20	27.73	15.04	31.55	77.64	88.33	9.93	2.38	4.18
WG 4	41.49	9	18	4.61	0.00	4.61	12.91	12.91	11.11	3.23	3.44

Comparison of the Assay as obtained in trials

Sample	Assay (including S-enantiomer)	Chiral LC (R-enantiomer)	Chiral Assay (R-enantiomer)
Metalaxyl racemic	994.66	497.84	507.39
Metalaxyl-M	971.46	949.18	940.08
SL 480	459.14	443.95	449.24
ES 350	318.71	308.52	317.77
WG 4	39.16	38.60	41.49

Conclusion and Outlook

Chiral assay determination is useful and possible if

- The number of samples is limited
- The content of TC in the sample is relatively high
- The matrix allows a simple sample preparation and chromatography
- The result is well in spec and there is no doubt about the material
- In case of doubt could be backed up with GC Assay determination
- material specific, with a solid or a liquid AI weighing is much easier

Source:
https://www.google.com/imgres?q=kristallisierter%20honig%20wieder%20fl%C3%BCssig%20machen&imgurl=https%3A%2F%2Fhonicum.at%2Fwp-content%2Fuploads%2Fkandiert.jpg&imgrefurl=https%3A%2F%2Fhonicum.at%2Fblog%2Fhonig-wieder-fluessig-machen%2F&docid=hunpxf71eMvoEM&tbnid=0U9Wpt6xNpJuyM&vet=12ahUKEwj74bKImIeOAxXvTEEAHSyJFg0QM3oECFsQAA..i&w=800&h=533&hcb=2&ved=2ahUKEwj74bKImIeOAxXvTEEAHSyJFg0QM3oECFsQAA

What if we ask for a collaborative trial based on SFC:
who would be ready to participate in a trial?


Thanks for your kind attention and to all laboratories and their staff participating in this collaborative trial and sending back the results in time
syngenta