CHROMTECH

SPDE™ the Magic Needle

Application Note SP301e

Determination of Amitraz in Honey with *SPDE* – GC-MS/MS

Method of the Chemisches und Veterinäruntersuchungsamt Sigmaringen (Hedingerstraße 2/1, 72488 Sigmaringen), H. Hahn, A. Nothhelfer und S. Preuß

1. Introduction:

Amitraz is a wideley used non-systemic acaricide against mites on fruit trees because of its exeptional miticidal activity towards mites of pears, apples, and citrus fruits. In the apiculture amitraz is used against the honey bee parasitic mite, Varroa jacobsoni.

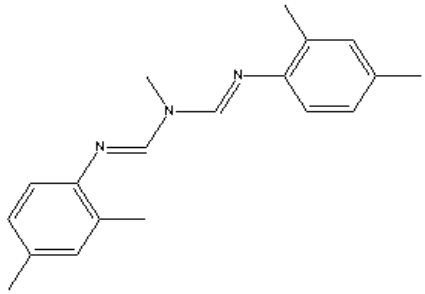
The tolerance for amitraz residues in food in Germany is define in the „Rückstandshöchstmengenverordnung“. The permitted level for amitraz in honey is 200 µg/kg.

Honey is regularly tested for amitraz within the scope of the „Nationalen Rückstandskontrollplan“. For this the relative costly analytical determination according to § 35 LMBG (1) was applied up to now. Here the indirect gaschromatographic determination takes place after alkaline hydrolysis of amitraz and subsequent isolation of the reaction product 2,4-Dimethylaniline (Fig. 1) through Clevenger distillation.

This elborate analysis method is vastly improved by combination of the automated SPDE-Method (eg. Fig. 2) with mass-selective detection in MS/MS-mode (SPDE-GC/MS/MS). In the following a method is described, which reduces the determination of amitraz at a fraction of the previously required analysis time.

4-Chloro-2-methylaniline is used as internal standard. Qualification was based on matrix-calibration with spiked honey samples, eg. honey samples without remains are spiked with amitraz.

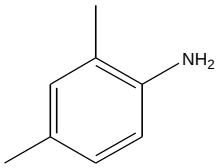
Thus, the recovery figures will include both the physical recovery of the analyte plus any matrix suppression or enhancement on the ion signal within the surce of the mass spectrometer.



Amitraz

(1,5-Di(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene)
Synonyms: Ovasyn; Mitac; Baam; Triazid; Taktic; 1,5-Di(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene; Formamidine, N-methyl-N'-2,4-xylyl-N-(N-2,4-xylylformimidoyl)-; Methyl-bis(2,4-xylyliminomethyl)amine

Molecular formula: $C_{19}H_{23}N_3$
CAS-No.: 33089-61-1
Molecular weight: 293,41 g/mol



2,4-Dimethylaniline

Molecular formula: $C_8H_{11}N$
CAS-No.: 95-68-1
Molecular weight: 121,18 g/mol

Fig. 1: Chemical data of Amitraz and the reaction product 2,4-Dimethylanilin

2. Materials and reagents

- Amitraz, from Promochem (C 102300)
- 4-Chloro-2-methylaniline as internal standard (ISTD) from Aldrich (C5,110-5)
- sodium hydroxide solution: 5 % (v/v)

3. Solutions

Stock solutions

- Amitraz: 500 mg/L solution in aceton.

Spiking Solutions:

1:50 solutions prepared from stock solution in aceton resulting in concentration of 10 mg/L.

Calibration:

Ascending volumes of the amitraz spiking solution as well as the internal standard solution (10 mg/mL) were added to honey samples (2 g) without remains prior to extraction:

	Sample	Blank matrix	S I	S II	S III	S IV	S V
Amitraz-Spiking solution, µL	-	-	20	40	60	100	200
Amitraz, µg	-	-	0,2	0,4	0,6	1	2
Conc. Of Amitraz in sample, µg/kg		-	100	200	300	500	1000
ISTD spiking solution, µl	60	60	60	60	60	60	60
Conc. Of ISTD, µg/kg	300	300	300	300	300	300	300

Samples and stock solutions were stored in a freezer (less than -20°C).

4. Instrumentation

20 ml Headspace vials

Steel vial caps with silicone/PTFE-septa.

SPDE-System from Chromtech GmbH in combination with the CTC autosampler (enrichment on inner-coated syringe needle, Figure 2).

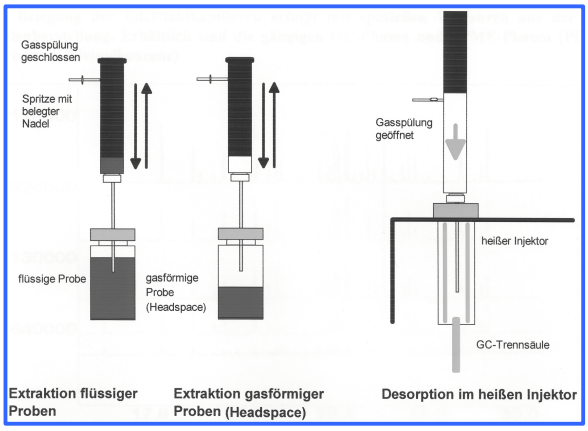


Fig. 2 Principle of the automatic SPDE extraction. Sample enrichment on the needle coating via pumping with the gastight headspace syringe inside of the sample vial.

5. Sample preparation

2.00 g honey are weighted in 20 mL Headspace vials. Afterwards 5 mL of 5% (v/v) sodium hydroxide solution are added.

The crimp-capped vials are conditioned at 95 °C for 1 hour in a thermoblock. After cooling down the resulting reaction product 2,4-Dimethylanilin is being analysed with SPDE-GC/MS/MS.

6. Instrumental conditions

Following instruments and settings are proved:

Instruments: GC: Thermo-Finnigan Trace 2000 MS/MS (Iontrap): Thermo-Finnigan Polaris Q Autosampler: CTC-Combipal with Chromtech SPDE option (special 2.5 ml Headspace syringe with inner-coated needle (80 mm ID x 50 µm PDMS (Code Nr.: SPDE-1-50-80)

GC-Column: DB-1701 / 45 m / 0,32 mm i.D. / 1 µm

Flow: 1.2 ml/min Helium

Oven program: 50 °C – 1.5 min hold - 20 °C/min – 120 °C - 0 min hold – 15 °/min – 200 °C – 0 min hold 10 °C/min – 270 °C - 10 min hold

Temperatures: Injector: 250°C; Interface: 270 °C

SPDE-parameters:

Pre Incubation Time (sec):	40
Needle Flush time (sec):	10
Syringe Temp (°C)	40
Incubation Temp (°C):	40
Agitator Speed (rpm):	500
Agitator On Time (sec):	10
Agitator Off Time (sec):	2
Sample Penetration (mm):	35
Extraction Strokes (Anzahl der Hübe)	25
Extraktion volume per stroke (µl)	2000
Desorption Gas Volume (µl):	750
Injector Penetration (mm):	50
Desorption Flow Speed (µl/sec):	50
Pre Desorprion Time (sec):	15
GC-Runtime (min):	30
Needle conditioning temperature (°C)	260
Needle conditioning time (min)	3

MS/MS - parameters:

Electron ionisation: (+EI),

Electron energy: 70 eV

CID voltage: 1,2 V

q-value: 0,45

Group 1 (2,4-Dimethylaniline): RT 10.0 –13.2 min, Precursor: m/z 120.5 ± 1, m/e, product ion scan: m/z 60-110

Group 2 (4-Chloro-2-methylaniline): RT 13.2-17.0 min, Precursor: m/z 106 ± 0.7, product ion scan: m/z 53-107

7. Results and Discussion

Quantitation takes part by means of internal standard method. For the creation of the calibration curve area ratios of 2,4-Dimethylaniline und 4-Chloro-2-methylaniline are spread against the concentrations of amitraz.

Amitraz concentrations of the samples are calculated from area ratios and the regression parameters.

The calculated concentration is already related to the net weight of 2 g honey. Yet it has to be calculated to the weighted sample.

$$\text{Residue concentration (µg/kg) amitraz} = \frac{C_{A\ calib} \times 2}{\text{tare}}$$

(calculated as „Total amount of amitraz and all related metabolites, wich contain a 2,4-Dimethylaniline functional group, collective calculated as amitraz“)

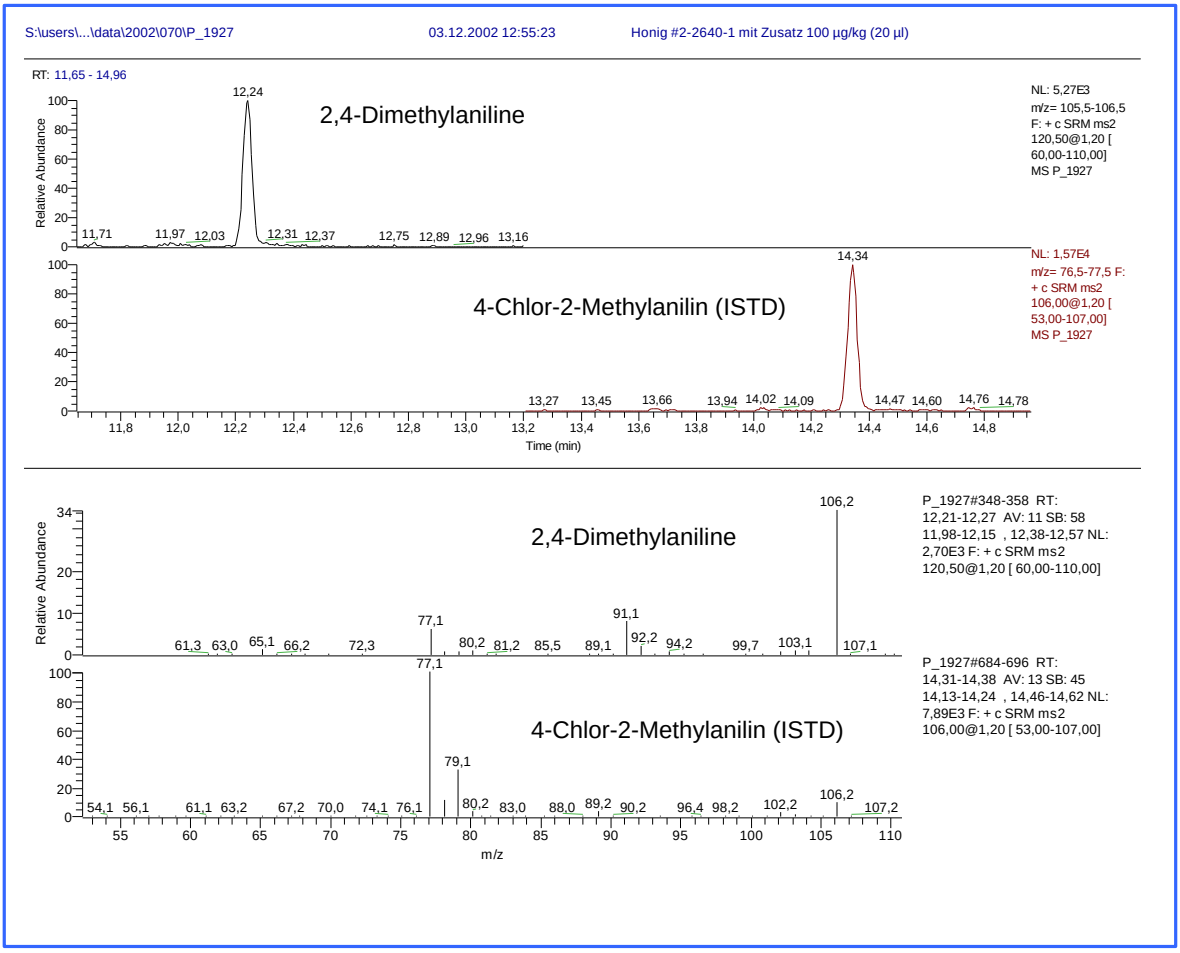
$C_{A\ calib}$ = Concentration of amitraz in µg/kg (calculated via calibration curve)

Tare = weighted sample in g

8. Validation

Based on determination of spiked samples a calculated LOD (limit of detection, according to the procedure of the DFG) of 140 µg/kg amitraz and a limit of quantitation (LOQ) of 200 µg/kg honey was determined. The recovery is about 87% to 99%. A mass spectrometrical identification is possible down to a concentration of 15 µg/kg amitraz (S/N 5:1). So a screening approach could be easily established. For legal reasons all concentrations in the range of 200 µg/kg honey have to be confirmed according to the procedure of § 35-LMBG (1).

9. Chromatograms and Spectra



10. Literature

(1) Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG L 00.00-58: Gaschromatographische Bestimmung von Amitraz und Vinclozolin sowie ihren 2,4-Dimethylanilin bzw. 3,5-Dichloranilin enthaltenden Metaboliten in Lebensmitteln, Stand Juli 2000, Beuth-Verlag.

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