Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Imidacloprid

MRID #: 431432-02

Matrix: Water

Analysis: GC/MS

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Appendix 12. Bayer residue method 00200.

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Monheim, May 31, 1990
Dr. We/kdw
RA - 418/90
Method 00200

Method for the determination of total residues of imidacloprid in plant materials and drinking water

Erhard Weber

Summary:

A method for the determination of the total residue of the insecticide imidacloprid is described. This method comprises the residues of imidacloprid and of all metabolites containing the chloropyridine moiety.
Imidacloprid and its metabolites are extracted from plant material with methanol/water. An aliquot of the extract is evaporated to the aqueous remainder. With extracts from sample materials containing fats or waxes a first clean-up is made by partitioning with hexane. Then the residues are adsorbed from the water solution by a column with a polystyrene resin (Amberlite XAD 4). The column is washed with water to remove the matrix compounds and the residues are eluted with methanol. After evaporation of the solvent, the remaining residues are dissolved in water.
Oil samples are dissolved in hexane and the residues are extracted with water. Further clean-up is made with a column with a polystyrene resin.
Water samples are subjected to oxidation without prior clean-up.
Samples of beverages are diluted with water and cleaned up in a column with polystyrene resin.

After clean-up, imidacloprid and its metabolites are oxidized with potassium permanganate to yield 6-chloronicotinic acid. The acid is extracted from water with t-butylmethylether. Then 6-chloronicotinic acid is derivatized with MSTFA. The determination of the derivative is performed by gas chromatography with a mass selective detector.

Recovery rates were in the range of 75 to 113%, when adding amounts of 0.05 to 0.5 mg/kg of imidacloprid to plant material. When adding amounts of 0.05 to 0.5 mg/kg of four different metabolites to four plant materials, recovery rates of 64 to 110% were found. For water samples recovery rates were in the range of 78 to 84%, when adding amounts of 0.05 to 0.1 µg/l imidacloprid. The routine limit of determination was 0.05 mg/kg in plant materials and 0.05 µg/l in water samples.

This method replaces method 00176, since it covers more sample materials.

Appendix 12. (con't.)

1. Introduction

The insecticides Confidor® and Gaucho® contain the active ingredient imidacloprid (proposed common name). Imidacloprid is degraded in the plant to a great number of different metabolites, all of which contain the chloropyridine structure (Dräger, 1989).

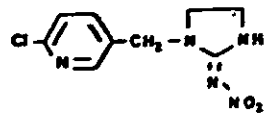
The objective of this method is to determine the residues of the parent compound and its metabolites as a whole after oxidative degradation to 6-chloronicotinic acid. Recoveries were determined with the parent compound for all sample materials being mentioned in this method. Moreover, recovery experiments were conducted with 4 different metabolites for 4 representative sample materials. For cucumbers and eggplants, recovery experiments were also carried out with the addition of 6-chloronicotinic acid.
The method was validated with radioactive residues having developed during the growth of the crops using the sample materials corn, straw; corn, green material; corn, kernels; apple fruit and potato vines (Weber, E., 1990).

Imidacloprid has the following chemical and physical properties:

Chemical designation: 1-((6-Chloro-3-pyridinyl)methyl)-4,5-dihydro-N-nitro-1H-imidazol-2-amine

Chemist code: NTN 33893

Structural formula:



Empirical formula: $C_9H_{10}ClN_5O_2$

Molecular weight: 255.6 g/mole

Appearance: colourless crystals

Melting point: 143.8°C (test sample APF 10038601)

Vapour pressure: at 20°C $8.0 * 10^{-9}$ mbar

at 100°C $3.3 * 10^{-5}$ mbar

Solubility:			
water	0.58		g/l
n-hexane	<0.1		g/l
dichloromethane	50 -	100	g/l
2-propanol	1 -	2	g/l
toluene	0.5 -	1	g/l
fat	0.061		g/100 g

Octanol/water partition coefficient (log P): 0.52

Hydrolytic stability: t 1/2 >> 31 d at pH 5, 7 and 9

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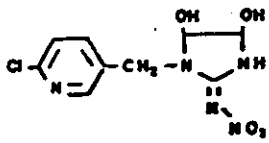
Recovery experiments were conducted with the following metabolites:

WAK 3772

Structural formula:

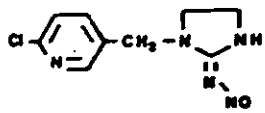


Empirical formula: $C_9H_{10}ClN_5O_4$

Molecular weight: 287.6 g/mole

WAK 3839

Structural formula:

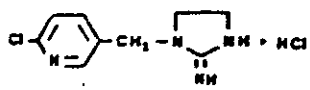


Empirical formula: $C_9H_{10}ClN_5O$

Molecular weight: 239.6 g/mole

WAK 4140

Structural formula:



Empirical formula: $C_9H_{11}ClN_4$ + HCl

Molecular weight: 247.2 g/mole

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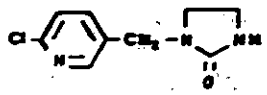
NTN 33519

Structural formula:



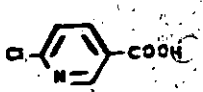
Empirical formula: $C_9H_{10}ClN_3O$

Molecular weight: 211.6 g/mole

Degradation products and derivatives:

6-chloronicotinic acid

Structural formula:

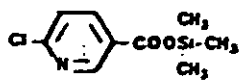


Empirical formula: $C_6H_4ClNO_2$

Molecular weight: 157.6 g/mole

6-chloronicotinic acid-trimethylsilylester

Structural formula:



Empirical formula: $C_9H_{12}ClNO_2Si$

Molecular weight: 229.6 g/mole

For the determination of the total residue of imidacloprid in plant material a method is already available (E. Weber, 1989). The method presented here is an advancement of that method. In this connection a number of additional sample materials were taken into consideration in the description of the clean-up procedures. New clean-up procedures were elaborated for hops (cone), rape (seeds) and for beverages. The oxidation without prior clean-up is proposed for cucumbers and eggplants as 6-chloronicotinic acid occurs in these materials as the predominant metabolite and the clean-up over XAD 4 may result in losses of 6-chloronicotinic acid in the extract. The same variant may also be used for potatoes where 6-chloronicotinic acid is also found as metabolite.

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2. Description of the method

Imidacloprid and its metabolites are extracted from plant materials using a methanol/water mixture. An aliquot of the extract is removed and concentrated by evaporation to the aqueous remainder.

In case of fat- and wax containing sample materials, the aqueous remainder is diluted and extracted with hexane for removal of lipid constituents.

The aqueous phase or the aqueous remainder, resp., is applied to a column containing a polystyrene resin (Amberlite XAD 4) with the residues being adsorbed to the column. For removal of the water soluble matrix constituents, the column is rinsed with water and subsequently the residues are eluted with methanol. After evaporation of the methanol the residues are dissolved in water.

Oil samples are diluted with hexane followed by extraction of the residues with water. Water samples are directly subjected to the subsequent oxidation without prior clean-up.

The aqueous solution of the residues is treated with sodium hydroxide and potassium permanganate. At high temperature imidacloprid and its metabolites containing the chloropyridine moiety are oxidized to 6-chloronicotinic acid. Cooling off is followed by acidification and addition of sodium bisulfite for decomposition of excess permanganate and the manganese dioxide having been formed, until a clear homogeneous solution results. 6-chloronicotinic acid is extracted from the acid water phase by means of t-butylmethylether. The ether phase is dried and the solvent is evaporated. The residues are dissolved in acetonitrile.

An aliquot portion of the acetonitrile solution is treated with a silylation reagent (MSTFA) which results in the formation of 6-chloronicotinic acid-trimethylsilylester. This is determined by GC/MS measurement in the Single Ion Monitoring (SIM) Mode.

3. Instruments

Blender, e.g. Polytron (Kinematica Gmbh)
Laboratory mill or household mixer
Glass bottle, 1 l, wide-necked with ground joint
Vacuum filter flask, 1000 ml
Porcelain suction filter with paper filter, 110 mm i.d.
Round-bottomed flasks 250 ml, 500 ml, 1000 ml
Pear-shaped flask 25 ml
Glass beakers 100 ml, 200 ml, 500 ml
Separatory funnel, 500 ml
Graduated cylinders 100 ml, 250 ml, 500 ml, 1000 ml
Bulb pipettes 2 ml, 5 ml, 25 ml, 50 ml
Graduated pipettes, 2 ml, 5 ml, 10 ml
Calibrated pipettes, 5 ml, 10 ml, 50 ml
Chromatography column, length 25 cm, 10 mm i.d., with glass frit
Chromatography column, length 25 cm, 20 mm i.d., with glass frit
Vacuum rotary evaporator with water bath
Magnetic stirrer
Magnetic stirring bar with wing

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Heating jacket for 500 ml round-bottomed flask
Reflux condenser with NS 29 conical ground joint
Teflon collars for NS 29 conical ground joint
Thermometer
Ice bath
Glass funnel, diameter 80 mm
Gilson Microman pipettes 250 µl
Microliter syringe 250 µl
Microvials 1.5 ml, with septum cap

Gas chromatograph with mass specific detector and automatic sampler, e.g.: Hewlett Packard HP 5890 with HP 7673 sampler and HP 5970 mass specific detector
GC/MS data system, e.g. HP UNIX Workstation

4. Reagents

Methanol for residue analysis
Water, purest, e.g.: from Millipore Milli Q unit
Methanol/water mixture 3:1 (v/v)
n-Hexane for residue analysis
t-Butylmethylether for analysis
Acetonitrile for HPLC
Dichloromethane for residue analysis
Filter aid, e.g. Celite 545
Amberlite XAD 4, 20 - 50 mesh, FLUKA AG, CH-9470 Buchs
Glass wool
Potassium permanganate solution for analysis, 50 g/l
Sodium hydroxide solution, purest, 32%
Sulfuric acid 10%, for analysis
Sodium bisulfite water free, for analysis
pH paper
Sodium sulfate water free, for analysis
GC capillary column i.d. 0.2 mm with dimethylsilicone phase, e.g.: ULTRA 1, Hewlett Packard Co.
6-chloronicotinic acid, FLUKA AG, CH-9470 Buchs, Order no. 25345
N-methyltrimethylsilyltrifluoroacetamide (MSTFA), Macherey & Nagel Co., D-5190 DÜren, FRG

Standard solutions in water (for recovery experiments)
Stock solution: 500 mg/l (dissolve substances in acetonitrile)

Dilutions: 10 and 1 mg/l
Stock solutions to be stored in the refrigerator, solutions of imidacloprid, WAK 3772, WAK 4140 and NTN 33519 are stable for at least 4 weeks
Solutions of WAK 3839 must be stored in the refrigerator under exclusion of light (brown glass). Stability 1 week

Standard solution in dichloromethane (for recovery experiments with oil)
Stock solution: 500 mg imidacloprid per 1
Dilutions: 10 and 1 mg/l

Standard solutions in acetonitrile (for GC/MS measurement)
Stock solution 1000 mg 6-chloronicotinic acid per 1
Dilutions: 100, 10, 1.25, 0.125 and 0.0625 mg/l
Stability at least 3 months

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5. Sampling

The analytical samples are taken and prepared according to the instructions of Chapters VIII and X of the DFG collection of methods for residue analysis of plant protectants (see Deutsche Forschungsgemeinschaft = German Society for the Advancement of Scientific Research, 1987). If immediate analysis of the samples is not possible, they are stored in the deep-freezing cabinet at -20°C.

6. Performance of the analysis

6.1 Extraction and sample clean-up

6.1.A Water containing and dry sample materials*, which do not contain fats or waxes

For analysis, 50 g plant material (G) are mixed with 300 ml of a methanol/water mixture (3:1, v:v) in a 1000 ml storage flask and allowed to soak for 30 minutes.

The sample is homogenized and sucked through a porcelain suction filter with fast filter paper and 10 g filter aid. The flask and the suction filter are flushed with 100 ml methanol/water mixture. The filter solids are discarded. The filtrate is transferred into a graduated cylinder, filled up with methanol to a total volume of 500 ml and homogenized by agitation. An aliquot corresponding to the 10 g sample weight is removed from this solution (100 ml in case of 50 g weight), transferred into a 1000 ml round-bottomed flask and concentrated on the vacuum rotary evaporator (bath temperature 60°C) to the aqueous remainder (final volume about 20 ml). Then the aqueous remainder is further cleaned up in a column with 10 g XAD 4 resin, as described in 6.2.A.

6.1.B Dry sample materials* which contain fats or waxes

For analysis, 50 g plant material (G) are mixed with 300 ml of a methanol/water mixture (3:1, v:v) in a 1000 ml storage flask and allowed to soak for 30 min. In case of straw samples, 25 g are treated with 350 ml methanol/water mixture. The sample is homogenized and sucked off through a porcelain suction filter with fast filter paper and 10 g filter aid. Flask and suction filter are flushed with 100 ml methanol/water mixture. The filter solids are discarded. The filtrate is transferred into a graduated cylinder, filled up with methanol to a total volume of 500 ml and homogenized by agitation. An aliquot corresponding to 10 g sample weight is removed from this solution (100 ml in case of 50 g weight, 200 ml in case of 25 g weight), transferred into a 1000 ml round-bottomed flask and concentrated to the aqueous remainder (final volume about 20 ml, about 40 ml in case of straw) on the vacuum rotary evaporator (bath temperature 60°C).

* Sample materials are listed in Table 1

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The aqueous remainder is diluted with 100 ml water (straw extract with 80 ml) and transferred into a 500 ml separatory funnel. The aqueous solutions are extracted 3 times with 100 ml n-hexane and the hexane phases are discarded.

After extraction, the aqueous phase is returned into the 1000 ml round-bottomed flask and again concentrated on the vacuum rotary evaporator for removal of the remaining hexane. Then the extract is further cleaned up in a column with 25 g XAD 4 resin as described in 6.2.B.

6.1.C Hops

For analysis 10 g hop cones (G) are mixed with 400 ml of a methanol/water mixture (3 : 1, v : v) in a 1000 ml storage flask and allowed to soak for 30 minutes. The sample is homogenized and sucked off through a porcelain suction filter with fast filter paper and 10 g filter aid. Flask and suction filter are flushed with 200 ml methanol/water mixture. The filter solids are discarded.

The filtrate is transferred into a 1000 ml round-bottomed flask and concentrated to the aqueous remainder (final volume about 150 ml) on the vacuum rotary evaporator (bath temperature 60°C). The solid parts of the sample having precipitated during this process are then removed by sucking off through a porcelain suction filter with fast filter paper and filter aid. The filter is flushed with 50 ml water and the aqueous phase is transferred into a 500 ml separatory funnel. The aqueous solutions are extracted three times with 100 ml of n-hexane and the hexane phases are discarded.

After extraction the aqueous phase is returned into the 1000 ml round-bottomed flask and again concentrated on the vacuum rotary evaporator for removal of remaining hexane. Then, the extract is further cleaned up via a column with 25 g XAD 4 resin as described in 6.2.B. After column clean-up this is dissolved in 50 ml water and 100 ml potassium permanganate solution are added for oxidation.

6.1.D Rape, seeds

For analysis, 10 g rape seeds (G) are ground in a mill together with about 20 g dry ice. The resulting powder is filled into a 1000 ml storage flask and the dry ice is allowed to evaporate. Then, 150 ml of a methanol/water mixture (3 : 1, v : v) are added and the mixture is allowed to soak over night. The sample is sucked off through a porcelain suction filter with fast filter paper and 10 g filter aid. Flask and suction filter are flushed with 100 ml methanol/water mixture. The filter solids are discarded. The extract is transferred into a 1000 ml round-bottomed flask and concentrated to the aqueous remainder (final volume about 80 ml) on the vacuum rotary evaporator (bath temperatures 60°C). The aqueous remainder is transferred into a 500 ml separatory funnel. The aqueous solutions are extracted three times with 100 ml n-hexane and the hexane phases are discarded.

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After extraction, the aqueous phase is returned into the 1000 ml round-bottomed flask and again concentrated on the vacuum rotary evaporator for removal of remaining hexane. Then, the extract is further cleaned up via a column with 25 g XAD 4 resin as described in 6.2.B.

6.1.E Cucumbers and eggplants

Extraction and processing of the samples are made as described in 6.1.A. A clean-up by column-chromatography is not made, however. For oxidation the aqueous remainder of the extracts is diluted with 30 ml water and treated with 100 ml potassium permanganate solution.

6.1.F Oil

10 g oil (G) are dissolved in 100 ml n-hexane and extracted 3 times with 50 ml water. The water phases are collected in a 500 ml round-bottomed flask. After the third extraction the hexane phase is discarded.

If a foamy intermediate phase develops during shaking, this is left in the separatory funnel together with the hexane phase and finally also discarded.

The collected aqueous phases are concentrated on the vacuum rotary evaporator for removal of the remaining hexane and subsequently subjected to oxidation according to 6.3.

6.1.G Beverages

10 g beverage (G) are mixed with 30 ml water and homogenized by shaking. This is cleaned up via a column with 10 g XAD 4 resin as described in 6.2.B.

6.1.H Water

250 ml water are applied into a 500 ml round-bottomed flask and oxidized immediately according to 6.3.

6.2 Clean-up by column-chromatography at XAD 4 resin

6.2.A Column with 10 g resin

10 g Amberlite XAD 4 are suspended with 30 ml methanol in a 100 ml glass beaker and allowed to settle. Then the turbid supernatant is decanted. This procedure is repeated and subsequently the XAD 4 resin is re-suspended and filled into a chromatography column having an inner diameter of 10 mm. The methanol is allowed to trickle to the upper level of the column packing and a glass wool plug is placed on top. Subsequently the column is rinsed with 50 ml methanol and with 50 ml water.

The aqueous solution from 6.1 (20-30 ml) is applied onto the column and allowed to trickle slowly (dripping rate about 2 ml/min). The flask is rinsed with 20 ml water and the rinsing solution is applied onto the column. Then the column is again flushed with 20 ml water (elution rate about 5 ml/min). All aqueous eluates are discarded. The residues are eluted with

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100 ml methanol. This eluate is collected in a 500 ml round-bottomed flask and concentrated almost to dryness (about 1 ml aqueous remainder) on the vacuum rotary evaporator (bath temperature 60°C). Make sure to completely distil off the methanol!

The residue is dissolved in 100 ml water.

6.2.B Column with 25 g XAD 4 resin

25 g Amberlite XAD 4 are suspended with 50 ml methanol in a 100 ml glass beaker and allowed to settle. Then the turbid supernatant is decanted. This procedure is repeated and subsequently the XAD 4 resin is re-suspended and filled into a chromatography column having an inner diameter of 20 mm . The methanol is allowed to trickle to the upper level of the column packing and a glass wool plug is placed on top of the column packing. Subsequently the column is rinsed with 100 ml methanol and with 100 ml water.

The aqueous solution from 6.1 is applied on to the column and allowed to trickle slowly (dripping rate about 5 ml/min). Then the column is flushed with 50 ml water (elution rate about 5 ml/min). All aqueous eluates are discarded. The residues are eluted with 250 ml methanol. This eluate is collected in a 500 ml round-bottomed flask and concentrated almost to dryness (aqueous remainder, about 5 ml) on the vacuum rotary evaporator (bad temperature 60°C). Make sure to completely distil off the methanol!

The aqueous remainder is dissolved in 100 ml water (hop samples are dissolved in 50 ml water).

6.3 Oxidation to 6-chloronicotinic acid

5 ml caustic soda solution (32%), 50 ml potassium permanganate solution (50 g/l) and a magnetic stirring bar are added to the aqueous solutions from 6.2 (100 ml potassium permanganate solution are added to samples of hops and samples from processing according to 6.2.E). The flask is immediately placed into a heating jacket being placed on a magnetic stirrer and fitted with a reflux condenser. The ground joint is sealed with a teflon collar in order to avoid that it gets sticky. This is heated at the highest state of the heating jacket under strong agitation so that the sample boils under reflux after about 10 min. 15 min after starting the heating, the heating jacket is switched off and immediately removed. Then about 50 ml water are added through the reflux condenser in order to rinse possible splashes of sample back into the flask and to terminate the boiling of the sample.

The reflux condenser is removed and the hot flask is transferred into a prepared ice bath. There it is allowed to cool down under agitation to an inner temperature of 15°C (about 10 min). 50 ml sulfuric acid (10%) are added under further cooling and agitation. Then about 1 g each of solid sodium bisulfite are added under cooling and agitation at intervals of about 1 min until the solution is completely clear and colourless (about 5 portions are needed for this purpose).

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The aqueous solution is checked for its pH-value. If the pH-value is >1, additional sulfuric acid is added until the pH-value is <= 1. Subsequently, the aqueous solution is transferred into a 500 ml separatory funnel and extracted three times with 50 ml t-butylmethylether. The organic phases are filtered into a 250 ml round-bottomed flask via a funnel with cotton pad and about 30 g sodium sulfate. After the last extraction the aqueous phase is discarded. The sodium sulfate is rinsed again with 30 ml t-butylmethylether. The organic phase is concentrated to dryness on the vacuum rotary evaporator at a bath temperature of 40°C. The flask should not remain longer than necessary in the water bath on the rotary evaporator as otherwise losses of parent compound may occur. The remaining solvent is evaporated in the nitrogen stream.

The residue is dissolved in 2 ml acetonitrile (V_{END}). This solution is stored in the refrigerator if no immediate analysis is possible.

For analysis, 250 µl of the acetonitrile solution are removed with a syringe or a positive displacement pipette and passed into a 1.5 ml sampler vial. 250 µl HSTPA are added to the sample; the vial is closed with a cap and shaken vigorously.

Residues of water samples are transferred into a 25 ml pear-shaped flask using t-butylmethylether. The solvent is evaporated on the vacuum rotary evaporator. Then the sample is dissolved in 250 µl acetonitrile (V_{END}) and 250 µl HSTPA are added.

Diluted solutions of 6-chloronicotinic acid in acetonitrile are correspondingly reacted as standard sample. The reaction is completed after 1 hour and the gas chromatographic analysis can start. The derivatives having been formed are stable at room temperature for at least 3 days.

6-chloronicotinic acid is determined gas chromatographically with a mass specific detector in the SIM mode (Single Ion Monitoring).

6.4 Gas chromatographic measurement

Gas chromatograph:	HP 5890 with sampler HP 7673
Column:	12 m HP ULTRA1 (dimethyl silicone) quartz capillary i.d. 0.2 mm, layer thickness 0.33 µm
Carrier gas:	Helium, delivery pressure 80 KPa
Sample injection:	Capillary injection system in splitless mode Split flow rate 30 ml/min, valve on 0.6 min Injector temperature 250°C 1 µl sample injected (V_{INJ})

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Temperature program:	100°C, 1 min isothermal 15°C/min up to 180°C 30°C/min up to 300°C 300°C, 3 min isothermal
Detector:	HP 5970 mass specific detector in the Single Ion Monitoring (SIM) Mode Detection on ion 214 Detection may be made on ion 170 in addition for verification of the analysis. The detector is switched on after 3 min only, as solvents, MSTFA and degradation products of MSTFA elute at the beginning of the temperature program.
Retention time:	under the given conditions 6-chloronicotinic acid-trimethylsilylester elutes from the GC column after about 4.6 min. If the peak form becomes worse after analysis of a larger number of samples, the glass insert of the injection block is cleaned and the first 50 cm of the GC column are cut off. This results in a reduction of the retention time by about 0.3 minutes.

7. Evaluation

7.1 Evaluation procedure

The quantitative evaluation was made with a data system by determination of the peak areas of the analytical solutions (F_A) and comparison with the peak areas of standard solutions (F_{St}). In the range of the routine limit of determination, standard solutions should be used which contain control sample extract of the respective sample material in the same concentration as the analytical solutions, if at all possible. The detector used shows for 6-chloronicotinic acid a linear correlation between peak area and amount of substance in the range from 30 to 2500 pg.

7.2 Recoveries and lowest determined concentration

The recoveries were determined in fortif... ... experiments in which defined amounts of imidacloprid being a.ssolved in 1 to 2.5 ml water were added to control samples prior to extraction. In some representative sample materials, recoveries were also determined under addition of different metabolites.

In case of oil samples, 250 µl of a solution of imidacloprid in dichloromethane were added. The results of the recovery experiments are summarized in Table 1.

The lowest determined concentration amounted to 0.1 mg/kg for hops, 0.05 mg/kg for all other plant materials and 0.05 µl/l for water (drinking water). The blank values were 30% of the lower limit of determination in case of all sample materials.

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7.3 Calculation of the residues

The residue level in mg/kg is calculated as follows:

$$R = \frac{F_A * V_{END} * W_{St} * F_U}{F_{St} * V_{INJ} * G * Al}$$

where:

G = weight of the analytical sample in g

V_{END} = final volume of the acetonitrile solution of 6.2 in ml

V_{INJ} = volume injected into the gas chromatograph in µl

F_A = peak area resulting from V_{INJ} for the analytical solution

F_{St} = peak area resulting from W_{St} for the standard solution

W_{St} = amount of 6-chloronicotinic acid injected with the standard solution in ng

Al = aliquotation factor
(1 for oil, water, hops and rape seeds, 0.4 for straw, 0.2 for other sample materials)

F_U = Conversion factor 6-chloronicotinic acid → imidaclo-prid
F_U = 1.622

The following factors apply for recoveries of metabolites

WAK 4140	F_U = 1.569
WAK 3772	F_U = 1.825
WAK 3839	F_U = 1.520
MTN 33519	F_U = 1.343
6-chloronicotinic acid	F_U = 1.000

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Table 1:

Recoveries (recovery rates) for imidacloprid
Two experiments were conducted for each concentration

Sample material	Variant*	Fortification (mg/kg)	Recoveries in per cent Range	Mean
Barley				
Green material	A	0.05	85 - 89	87
		0.5	74 - 82	78
Grain	A	0.05	80 - 85	82
		0.5	81 - 83	82
Straw	B	0.05	90 - 98	94
		0.5	94 - 96	95
Oats				
Green material	A	0.05	73 - 90	82
		0.5	97 - 99	98
Grain	A	0.05	70 - 74	72
Straw	B	0.05	79 - 83	81
		0.5	106 - 107	107
Corn				
Green material	A	0.05	85 - 90	88
		0.5	80 - 85	83
Kernels	A	0.05	82 - 91	87
		0.5	80 - 85	83
Straw	B	0.05	86 - 92	89
		0.5	80 - 87	84
Wheat				
Green material	A	0.05	86 - 90	88
		0.5	84 - 96	90
Grain	A	0.05	73 - 82	78
Straw	B	0.05	88 - 103	96
		0.5	100 - 113	107
Grass	A	0.05	80 - 88	84
		0.5	79 - 85	82

* Concerning processing (see 6.1 Extraction and sample clean-up)

Appendix 12. (con't.)

Table 1: (continued)

Recoveries (recovery rates) for imidacloprid
Two experiments were conducted for each concentration

Sample material	Variant*	Fortification (mg/kg)	Recoveries in per cent Range	Mean
Potato				
Tuber	A	0.05	93 - 99	96
		0.5	99 - 100	100
	E	0.5	103 - 103	103
Sugarbeet				
Green material	A	0.05	107 - 109	108
		0.5	98 - 99	99
Foliage	A	0.05	85 - 88	86
		0.5	93 - 95	94
Root	A	0.05	95 - 95	95
		0.5	94 - 96	95
Field bean				
Bean	A	0.05	98 - 107	103
		0.5	86 - 97	92
Pea				
Seed	A	0.05	83 - 84	84
		0.5	79 - 88	84
Pod	A	0.05	70 - 80	75
		0.5	92 - 94	93
Eggplant	E	0.05	70 - 83	76
Cucumber	E	0.05	73 - 78	75
Paprika	A	0.05	105 - 122	113
		0.5	89 - 89	89
Lettuce	A	0.05	107 - 113	110
		0.5	98 - 98	98
Tomato	A	0.05	97 - 105	101
	E	0.05	79 - 81	80
	E	0.5	79 - 82	81

* Concerning processing (see 6.1 Extraction and sample clean-up)

Appendix 12. (con't.)

Table 1: (continued)

Sample material	Variant*	Fortification (mg/kg)	Recoveries in per cent Range	Mean
Apple Fruit	A	0.05	108 - 109	109
		0.5	87 - 90	89
Juice	G	0.05	104 - 107	106
Orange Pulp	A	0.05	108 - 112	110
		0.5	87 - 91	89
Peel	A	0.05	102 - 103	103
		0.5	88 - 89	89
Pear	A	0.05	77 - 89	83
		0.5	78 - 84	81
Peach	A	0.05	86 - 90	88
		0.5	98 - 108	103
Cotton Seed	B	0.05	83 - 98	90
Oil	F	0.05	82 - 83	83
Rape Seed	D	0.05	86 - 91	89
Straw	B	0.05	72 - 91	81
		0.5	107 - 109	108
Sunflower Seed	B	0.05	83 - 95	89
Hop Cone	C	0.2	82 - 87	85
		2.0	77 - 79	78
Beer	G	0.05	78 - 81	80

* Concerning processing (see 6.1 Extraction and sample clean-up)

Appendix 12. (con't.)

Table 1: (continued)

Sample material	Variant*	Fortification (mg/kg)	Recoveries in per cent Range	Mean
Tobacco	A	0.05	78 - 79	79
		0.5	99 - 108	104
Drinking water (corrected for blank value)	H	0.05 µg/l	76 - 91	84
	H	0.1 µg/l	75 - 80	78

* Concerning processing (see 6.1 Extraction and sample clean-up)

Table 2:

Recoveries (recovery rates) for metabolites of imidacloprid
Two experiments were conducted for each concentration

Sample material added metabolite	Variant*	Fortification (mg/kg)	Recoveries in per cent Range	Mean
Corn Straw				
WAK 4140	B	0.05	67 - 69	68
		0.5	82 - 85	83
WAK 3772	B	0.05	79 - 86	83
WAK 3839	B	0.05	73 - 75	74
NTN 33519	B	0.05	72 - 73	73
Sugarbeet Root				
WAK 4140	A	0.05	70 - 76	73
		0.5	80 - 82	81
WAK 3772	A	0.05	74 - 78	76
WAK 3839	A	0.05	62 - 66	64
NTN 33519	A	0.05	109 - 112	110

Appendix 12. (con't.)

Table 2: (continued)

Sample material added metabolite	Variant*	Fortification (mg/kg)	Recoveries in per cent Range	Mean
Apple Fruit				
WAK 4140	A	0.05	96 - 100	98
		0.5	79 - 87	83
WAK 3772	A	0.05	104 - 107	106
WAK 3839	A	0.05	78 - 90	84
WAK 33519	A	0.05	106	106
Cotton Seed				
WAK 4140	B	0.05	65 - 65	65
WAK 3772	B	0.05	85 - 90	88
WAK 3839	B	0.05	68 - 70	69
WTN 33519	B	0.05	75 - 85	80
Eggplant Fruit				
6-chloro-nicotinic acid	E	0.05	80 - 80	80
Cucumber Fruit				
6-chloro-nicotinic acid	E	0.05	73 - 75	74
Potato Tuber				
6-chloro-nicotinic acid	E	0.05	101 - 106	104

* Concerning processing (see 6.1 Extraction and sample clean-up)

Appendix 12. (con't.)

8. Special hints:

Water being deionized by means of ion exchanger must not be used by any means for the purification of XAD 4, for column chromatography at XAD 4 and also for all other operating steps! This water contains a large amount of organic impurities which are concentrated on XAD 4 and interfere with oxidation or determination of residues. Even water being treated with ion exchanger and subsequently distilled, is still contaminated. If no purest water is available, the work should be done with pure tap water.

During extraction of samples with hexane according to Chapter 6.1.B, difficulties may be encountered in the phase separation due to the formation of a foam-like intermediate phase. In this case the foam-like intermediate phase is separated off together with the hexane phase in each case and the homogeneous water phase is further extracted. The hexane phases are combined and put again into the separatory funnel. Then wait until the phase separation is as complete as possible. Subsequently the lower water phase together with an intermediate phase possibly still being present are drained off and combined with the majority of the water phase from the extraction. Hexane still being present is removed by concentration on the rotary evaporator. Make sure that all the hexane is distilled off as otherwise the subsequent column separation may be disturbed! If the phase separation is very difficult, it may be achieved by centrifugation of the sample (4000 rpm).

The extraction of oil is performed as described in 6.1.F and the intermediate phase is discarded at the end.

The water phase applied to the XAD 4 column must no longer contain any organic solvent as otherwise parent compound and metabolites are not adsorbed quantitatively. Therefore, the water phases must be concentrated prior to application to the column so that water has been distilled over for some time. The aqueous solution must percolate only slowly into the column in order to achieve as quantitative an adsorption of the residues to the XAD 4 as possible.

It is absolutely necessary to pre-clean the XAD 4 resin in the column as described. If the column is directly prepared with XAD 4 and water and then the sample is applied, the amount of impurities being eluted with methanol from the XAD 4 is so large that the potassium permanganate is completely used up in the subsequent oxidation and the residues are only incompletely oxidized.

If many analyses are performed, a larger batch of XAD 4 may be pre-cleaned as follows:
500 g XAD 4 resin are digested with about 500 ml methanol in a 2 l Erlenmeyer flask and allowed to settle. This is followed by decantation from the turbid supernatant. This procedure is repeated three times. Two portions of resin treated in this way (= 1000 g) are applied to a 4 l glass suction filter with frit; the frit is standing on a 6 l vacuum filter flask. Four times about 1.5 l methanol are applied to the resin and the methanol is allowed to slowly percolate through the resin without suc-

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tion. After the last addition, methanol is briefly sucked off until no more solvent elutes. Subsequently this procedure is repeated with toluene, again with methanol and with water. After addition of water the resin is sucked dry. The resin cleaned in this way is stored in a carefully closed glass bottle. The washing with toluene is absolutely necessary; washing with methanol alone does not result in a sufficient cleaning effect.

The elution volume of the XAD 4 column of 100 ml methanol (or 250 ml resp.) must not be reduced as otherwise the metabolite WAK 4140 is not completely eluted.

The methanol eluate of the XAD 4 column must be strongly concentrated almost to dryness so that finally water distils over. If not all the methanol is distilled off from the methanol eluate of the XAD 4 column, the permanganate will be completely used up during oxidation and the oxidation of the residues will be only incomplete.

Make sure that sufficient permanganate is still present at the end of the oxidation (violet colour of the solution)! If the solution is brown and a very voluminous precipitate is present, all of the permanganate has been used up. Possible causes are: incomplete removal of methanol prior to oxidation, use of insufficiently clean XAD 4 resin or contaminated water.

Under these conditions the residues might have been incompletely oxidized. In that case the analysis must be repeated.

6-chloronicotinic acid is only moderately stable in the heat in the strongly alkaline medium. Under the indicated conditions of oxidation, it is re-decomposed already after about 30 min. Therefore, the indicated times for the reaction must be observed. After the reaction the sample is quickly cooled down in the ice bath in order to avoid the decomposition under the oxidation conditions.

If the oxidation is performed with another experimental arrangement (e.g. other heating system), the kinetics of the oxidation reaction and of the stability of 6-chloronicotinic acid under these conditions should be measured. On the basis of these kinetics the reaction time should then be fixed. In case of oxidation in heavily charged matrix the reaction time should be prolonged for some minutes.

In the acid medium in the heat, 6-chloronicotinic acid is very unstable. Therefore, cooling down is absolutely necessary before the reaction solution is acidified. Also sodium bisulfite must be added slowly as this results in a re-warming of the solution.

On account of this instability of 6-chloronicotinic acid, the processing after oxidation must be done steadily.

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For quantitative extraction of 6-chloronicotinic acid from water, the pH-value must be <= 1. Besides t-butylmethylether, ethyl acetate may also be used for extraction. It is not possible to achieve quantitative extraction with dichloromethane.

Evaporation of the solution in t-butylmethylether for too long a time or at too great heat may result in losses of 6-chloronicotinic acid. It is best to concentrate the solution almost to dryness and allow the rest of the solvent to evaporate.

During elaboration of the method, interference peaks were found in individual cases which resulted from the chemicals used. It is therefore recommendable to conduct a control experiment without additive and without matrix in order to be able to eliminate such interferences.

In each series of analyses a sample of acetonitrile with derivatization reagent should be co-analyzed in order to be able to recognize possible interfering components originating from the reagents.

A prolonged interruption of the analysis is only possible on the step of the methanol eluate after column clean-up.

The direct oxidation of sample extracts with clean-up via XAD 4 resin according to chapter 6.1.E should also be possible in case of other sample materials with a high water content. In general, however, this procedure results in lower recoveries (see values for tomatoes in Table 1) and the chromatogram shows more interference peaks from the matrix.

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9. Literature

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Translations for these references can be found on page 128

Signed: Dr. E. Weber

Thanks are expressed to Mrs. H. Arndt and Mr. R. Schöning for the elaboracion of this method.

Appendix 12. (con't.)

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