4 Analytical method

4.1 Principle of the Analytical Method

4.1.1 Soil

The sample material is extracted with water. An aliquot of the water extract is taken through an anion exchange column for clean-up. The analytes retained on the resin are washed with water and then eluted with formic acid. The eluate is evaporated to complete dryness. The residues containing the active substance (AE F039866) and the metabolites (AE F061517 and AE F064706) are derivatised by refluxing the extract for 4.5 hours with a mixture of acetic acid and trimethylorthoacetate. After derivatisation, the mixture is evaporated to dryness. The residue is transferred with a mixture of toluene/methylacetate on to a silicagel SPE cartridge. The substances are eluted from the cartridge with a mixture of methanol/methylacetate. The chromatographic determination

is performed by gas chromatography using a flame photometric detector (GC-FPD) with a 526 nm phosphorous mode filter.
The limit of quantification (LOQ) in soil is 0.01 mg/kg for each of the three analytes (calculated as Glufosinate-free acid, AE F035956).

4.1.2 Water

AE F039866 (Glufosinate) forms strong complexes with Ca^{2+} and Mg^{2+} ions which do not allow quantitative determination of Glufosinate residues. Removal of these cations before derivatisation is necessary and is achieved with a chelating resin (functional group: amino phosphonic acid) which also forms strong complexes with Ca^{2+} and Mg^{2+} and releases Glufosinate during this process.
AE F039866 and the two metabolites AE F061517 and AE F064619 are eluted under aqueous conditions form the chelating resin.

To concentrate the analytes in the water phase eluted from the cation exchange column, the aqueous eluate is transferred to a highly alkaline anion exchanger (hydroxide form). The analytes are retained on the resin under these conditions. They are subsequently eluted from the anion exchanger using formic acid.

The eluate is evaporated to complete dryness. The residues containing the active substance (AE F039866) and the metabolites (AE F061517 and AE F064706) are derivatised by refluxing the extract for 4.5 hours with a mixture of acetic acid and trimethylorthoacetate. After derivatisation, the mixture is evaporated to dryness. The residue is transferred with a mixture of toluene/methylacetate on to a silicagel SPE cartridge. The substances are eluted from the cartridge with a mixture of methanol/methylacetate. Chromatographic determination is performed by gas chromatography using a flame photometric detector (GC-FPD) with a 526 nm phosphorous mode filter.

The LOQ in water is 0.05 µg/l for each of the three analytes (calculated as Glufosinate-free acid, AE F035956).

4.2 Test commodities, test and reference substances

4.2.1 Soil

For method validation, top soil had been collected on 4 July, 1997 from Assarts Farm in the UK and was shipped to the laboratories of Hoechst Schering AgrEvo, Residues & Consumer Safety in D 65926 Frankfurt. After arrival in the laboratory, the soil was stored deep frozen until analysis.
The soil type is a sandy loam with 5.6 % clay, 12.7 % silt and 81.7 % sand containing 1.35 % organic carbon.

4.2.2 Water

The water used for validation was taken on 25 September, 1997 from a well in Warsop, Nr Mansfield, Nottinghamshire, England. The water was stored in 500 ml plastic bottles in a refrigerator until analysis.
The water contained 83 mg/l Ca^{2+} and 45 mg/l Mg^{2+} ions.

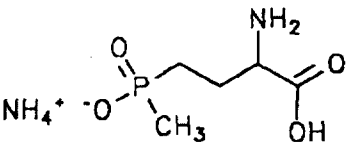
4.3 Test and Reference Substances

4.3.1 AE F039866

AE F039866 in aqueous ammonia solution (0.015 mol/l) in various concentrations.

Chemical name (IUPAC): ammonium-DL-homoalanin-4-yl(methyl)phosphinate
Test and reference substance:

Structural formula:

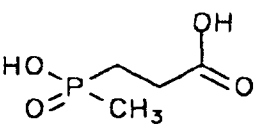


Empirical formula:	$C_5H_{15}N_2O_4P$
Molecular weight:	198.19
Batch code:	AE F039866 00 1B99 0006
Certificate of analysis:	AZ 06064
dated:	13-Sep-1995
issued by:	Hoechst Schering AgrEvo GmbH Entwicklung, Produktanalytik, G 865a D 65926 Frankfurt am Main
purity:	99.2 %
expiry date:	04-Sep-98

4.3.2 AE F061517

AE F061517 in aqueous ammonia solution (0.015 mol/l) in various concentrations.

Chemical name (IUPAC): 3-methylphosphinico-propionic acid
Test and reference substance:
Structural formula:

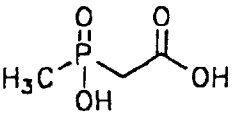


Empirical formula: $C_4H_9O_4P$

Molecular weight:	152.10
Batch code:	AE F061517 00 1C97 0002
Certificate of analysis:	AZ 06324
dated:	20-Feb-1996
issued by:	Hoechst Schering AgrEvo GmbH Entwicklung Produktanalytik, G 865a D-65926 Frankfurt am Main
purity:	97.9 %
expiry date:	16-Feb-00

4.3.3 AE F064619

AE F064619 in aqueous ammonia solution (0.015 mol/l) in various concentrations.
Chemical name (IUPAC): 2-methylphosphinico-acetic acid
Test and reference substance:
Structural formula:



Empirical formula:	$C_3H_7O_4P$
Molecular weight:	138.07
Batch code:	AE F064619 00 1B99 0001
Certificate of analysis:	AZ 06557
dated:	06.08.1996
issued up by:	Hoechst Schering AgrEvo GmbH Entwicklung Produktanalytik, G 865a D-65926 Frankfurt am Main
purity:	99.4 %
expiry date:	30-Jul-98

Solutions of AE F039866, AE F061517 and AE F064619 in ammonia solution (0.015 mol/l) were used for fortifying the procedural recoveries.

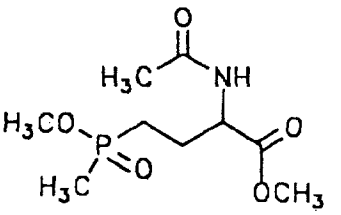
4.3.4 AE F064706

AE F064706 is dissolved in methanol, while dilutions are made with methyl acetate to obtain various concentrations.

Chemical name (IUPAC): methyl-4-(methoxy)(methyl)phosphinoyl-2-acetamido-butyrate

Reference substance:

Structural formula:

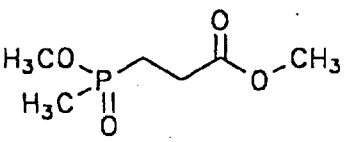


Empirical formula:	$C_9H_{18}NO_5P$
Molecular weight:	251.25
Batch code:	AE F064706 00 1B99 0002
Certificate of analysis:	AZ 05979
dated:	30-May-1995
issued by:	Hoechst Schering AgrEvo GmbH Entwicklung Produktanalytik, G 865a D-65926 Frankfurt am Main
purity:	99.1 %
expiry date:	22-May-98

4.3.5 AE F070951

AE F070951 is dissolved in methanol, while dilutions are made with methyl acetate to obtain various concentrations.

Chemical name (IUPAC): methyl-3-(methoxy)(methyl)phosphinoylpropionate
Reference substance:
Structural formula:

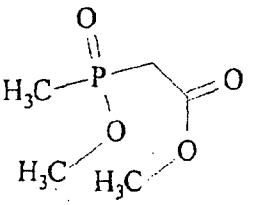


Empirical formula:	$C_6H_{13}O_4P$
Molecular weight:	180.16
Batch code:	AE F070951 00 1B99 0001
Certificate of analysis:	AZ 06101
dated:	05-Sep-1995
issued by:	Hoechst Schering AgrEvo GmbH Entwicklung Produktanalytik, G 865a, D-65926 Frankfurt am Main
purity:	98.8 %
expiry date:	29-Aug-99

4.3.6 AE F096432

AE F096432 is dissolved in methanol, while dilutions are made with methyl acetate to obtain various concentrations.
Chemical name (IUPAC): methyl-(methoxy)(methyl)phosphinoylacetate
Reference substance:

Structural formula:



Empirical formula:	$C_5H_{11}O_4P$
Molecular weight:	166.13
Batch code:	AE F096432 00 1B99 0001
Certificate of analysis:	AZ 05969
dated:	24-May-95
drawn up by:	Hoechst Schering AgrEvo GmbH Entwicklung Produktanalytik, G 865a, D-65926 Frankfurt am Main
purity:	97.1 %
expiry date:	20-May-98

Solutions of AE F064706, AE F070951 and AE F096432 in methyl acetate were used for quantification of the derivatized analytes during gas chromatographic measurement.

4.4 Statement concerning the code of the test and reference substances

With respect to the Company information system, the internal code of the test and reference substances had to be amended in the course of time. This does not imply any change in the composition or properties of the test and reference substances.
Herewith, we expressly confirm the identity of the test and reference substances if one of the following codes is mentioned in any document. The codes may have additional digits for the identification of the batch.

AE F039866	Current Code
Hoe 039866	Former Code
AE F061517	Current Code
Hoe 061517	Former Code
AE F064619	Current Code
Hoe 064619	Former Code

AE F064706	Current Code
Hoe 064706	Former Code
AE F096432	Current Code
Hoe 096432	Former Code

4.4.1 Standard Solutions

Stock solutions are prepared from the standard substances AE F039866, AE F061517 and AE F064619 (weighed out on an analytical balance) in 0.015 M ammonia solution at a concentration of approx. 1 mg AE F035956-equivalents/ml.
Dilutions are prepared from these stock solutions at the concentrations required for fortifications.

Stock solutions of approx. 1 mg AE F035956-equivalents/ml are prepared in **methanol** from the GC-derivatives AE F064706, AE F070951 and AE F096432. From these stock solutions, dilutions are prepared in **methyl acetate** at the concentrations required for quantification.

4.5 Reagents and Materials

Deionised water
formic acid 100 %
methyl acetate (GC)
glacial acetic acid 100 % (e.g. Baker)
trimethylorthoacetate 99 % (e.g. Aldrich)
methanol HPLC grade
toluene HPLC grade
hydrochloric acid 36 - 38%
sodium hydroxide - pellets p.a.
silica gel SPE cartridges, 500 mg with 10 ml reservoir

Chelating resin (e.g. Duolite C-467 from Supelco, Order No. 1-0354)
Chelating resin:
100 g Chelating resin is washed to a neutral stage with deionised water. A 4 fold quantity (ml) of 1 N hydrochloric acid is then added to the resin, the solution is stirred for 2 min and allowed to stand for 30 min. The hydrochloric acid is decanted and the resin is washed with deionised water until a neutral pH is reached. Subsequently, a 4 fold volume of 1 N sodium hydroxide solution is added to the resin, the solution stirred for 2 min and allowed to stand for another 30 min. The sodium hydroxide solution is decanted, drawn off via a frit and the resin is washed with deionised water until the pH is neutral.

Anion exchange resin AG 1X8, 50 - 100 dry mesh (e.g. Bio Rad or equivalent)
Anion exchanger:
100 g Anion exchanger is washed with deionised water until a neutral state is reached. The anion exchanger is decanted into a large vessel together with 1 litre 1 N sodium hydroxide solution, the solution is mixed thoroughly for approx. 2 min and then allowed to stand for 30 min. The sodium hydroxide solution is separated by filtering, and the resin is washed using deionised water until a neutral pH is reached. 3 - 5 l water are normally required for 100 g resin. The anion exchanger is now ready for use.

The following dilutions must be prepared:
Sodium hydroxide solution c(NaOH)= 1 mol / l = 40 g sodium hydroxide - tablets in 1 l deionised water
Formic acid $c(CH_2O_2)$= 50 % = make up 500 ml deionised water to 1 l with 100 % formic acid.
Ammonia $c(NH_3)$= 0.015 mol/l = make up 1 ml ammonia solution ca. 28 - 30 % NH_3 to 100 ml with deionised water.
Hydrochloric acid c(HCl)= 1 mol/l = make up 100 ml (W=37%) to 1000 ml with deionised water.

The following mixtures must be prepared:
Methanol HPLC grade / methyl acetate HPLC grade 1 / 1 (v / v)
Methanol HPLC grade / toluene HPLC grade 1 / 1 (v / v)

4.6 Apparatus

Analytical balance (e.g. Mettler)
Buchner funnel
precision balance (e.g. Mettler)
Hamilton microliter syringes of various capacities
round-bottom and tapered flasks of various capacities
volumetric flasks and graduated cylinders of various capacities
beakers of various capacities
Rotavapor (e.g. Büchi)
ultrasonic bath (e.g. Bransonic 52)

heating rack for round-bottom flasks (e.g. Gerhardt, SV3)
high-efficiency condenser, NS 29 with drying tube NS 29 filled with silica gel with moisture indicator
bulb pipettes of different sizes
Pasteur pipettes, length ca. 15 and 23 cm
disposable syringes 10 ml
steel syringe needle, length ca. 10 cm, flattened tip
disposable syringe needle, length ca. 5 cm
chromatography columns made of glass (type 1), 20 mm i.d. with reservoir (e.g. 500 ml)
chromatography columns made of glass (type 2), 10.5 mm i.d. with reservoir (e.g. 250 ml)
quartz wool
0.5 µm disposable filter (e.g. Millex SR, Millipore)
pH-paper

4.7 GC-Conditions

Determination by gas chromatography is carried out using a flame-photometric detector (FPD) in the phosphorus mode. Typical GC conditions are described below.

Equipment:	Carlo Erba 8000, FPD 800 (526 nm), micro PTV, splitless mode	
Column:	15 m 0.5 µm AT-Wax Alltech, i.d. 0.32mm	
Injector	SL time 90 sec, 60°C, 40 sec, 15°C /sec, 280°C, 2 min	
Temperature:	detector (base)	280 °C
	detector (body)	170°C
	column	80°C, 1.5/min, 60°C/min 175°C , 2 min, 10°C/min 185°C, 1 min 79°C/min 280°C, 5 min
Gases:	carrier gas	He 3.6 ml/min (constant flow)
	detector gases	air: 140 ml/min, H_2: 87 ml/min
Injection volume:		5 µl
Approximate retention time:	AE F096432	5.5 min
	AE F070951	6.9 min
	AE F064706	10.6 min

4.8 Laboratory Steps

4.8.1 Preparation of chelating resin/anion exchange column

Cation exchange with chelating resin:
A chromatography column (type 1) with an internal diameter of 20 mm is used for the cation exchange clean- up with the chelating resin. The column is plugged with glass wool at the lower end. 10 ml water is filled into the column and the top level of the water column is marked on the glass wall. The chelating resin is then poured into the column , slurry- packing with water to assist in packing the resin in the column up to the mark on the chromatography tube. Prior to further use, the resin in the column is washed with approx. 100 ml deionised water.

Anion exchange:
A chromatography column (type 2) with an internal diameter of approx. 10 mm is used in preference. The column is plugged with glass wool at the lower end. 12 ml water is filled into the column and the top level of the water column is marked on the glass wall Before slurry-packing the resin as described above. Prior to further use, the column is washed to a neutral stage using approx. 100 ml deionised water.

4.8.2 Extraction of residues from soil samples

25 g of soil are weighed out for analysis and stirred with 100 ml water for 30 min. The mixture is filtered using a Buchner funnel. The extraction of the soil is repeated with another 100 ml of water. The soil/water mixture is stirred for 30 min. then the mixture is filtered and the 2 filtrates are combined and mixed. The volume obtained for calculation is considered to be 225 ml. An aliquot of 100 ml is taken for clean-up. on the anion exchange column (type 2). The further clean-up procedure is described under point 4.8.4 of this method.

4.8.3 Remove of Ca^{2+}, Mg^{2+} from water samples

The chromatography tube (type 1) which is filled with the chelating resin (cation exchange) is installed above the anion exchange column. The water sample (ca. 500 ml) is transferred to the reservoir of the cation-exchange column so that the water flowing through the resin runs onto the anion exchange column. After the sample has passed through the column, the chelating resin is rinsed with 100 ml distilled water. The water flow should be regulated that 1-2 drops per second may pass through the resin.

4.8.4 Further sample workup for water and soil samples

Water samples

The total amount of water including the amount for rinsing the chelating resin is allowed to run onto the anion exchange column. The water then passes through the anion exchange column and the retained substances from the residues in soil or water are washed with 100 ml of water.

Soil samples
From soil samples a 100 ml aliquot of the aqueous soil extract is passed through the anion exchange resin for clean-up.

Soil and water samples
The analytes are eluted from the anion exchange column, using 100 ml 50 % formic acid. The eluate is collected in a 250 ml round-bottom flask and concentrated to dryness with a rotary evaporator (60°C). The rotary evaporator is operated at a moderately low speed which avoids excessive distribution of the sample over the walls of the flask. 10 ml water is added to the dried sample extract and concentrated to dryness as described above until no traces of formic acid are apparent. This procedure may be repeated if necessary. 5-10 ml methanol are added to the dried sample extract and concentrated as described above. This procedure is repeated once with 5-10 ml methanol.

4.8.5 Derivatisation

3 ml glacial acetic acid are added to the sample residue in the round-bottom (from section 4.8.4). The solution is ultrasonicated at ambient temperature for approx. 1 min until all visible sample residues are dissolved or dislodged from the wall of the flask. 12 ml of trimethylorthoacetate and a few glass beads are added. The solution is then mixed by swirling with ultrasonication. When mixing is complete, the reaction mixture is refluxed for 4.5 h.

The sample is then allowed to cool to room temperature. The procedure may be stopped at this point, e.g. derivatisation overnight , controlled by a time switch.
After derivatisation, the flask is removed from the reflux condenser and 15 ml toluene is added. The procedure may also be stopped at this point (the flask is closed and stored at room temperature).

The sample is evaporated to a final volume of approx. 1-2 ml using a rotary evaporator. Care should be taken to ensure that the water bath temperature does not exceed 40 °C, since in particular the derivatives AE F070951 and AE F096432 could evaporate.
2 portions of 15 ml toluene are each added successively and the evaporation procedure is repeated. The mixture is evaporated each time to an approximate volume of 1-2 ml to remove all traces of the derivatisation solution. It is important that the solution is not evaporated to dryness during the working-up procedure.

4.8.6 Silica gel SPE cartridge clean-up

Prior to analysis by GC/FPD, the derivatised sample is cleaned-up using commercially available silica gel solid phase cartridges.

Before use, the SPE cartridges are conditioned by passing ~10 ml degassed (ultrasonicated) methyl acetate/toluene (1:1 v/v) through each cartridge. The use of a degassed solvent mixture allows conditioning without causing air bubbles in the cartridges. The cartridges must not be allowed to become dry. If this happens, the cartridge must be conditioned again as described above.

To load the SPE cartridge, a 5 cm disposable syringe needle is attached to a 10 ml disposable syringe.
The remaining toluene in the round bottom flask is drawn into the syringe, dissolving the sample in the flask with aid of an ultrasonic bath.
The volume in the syringe is made up to 4 ml with toluene.
4 ml methyl acetate are added to the flask and the solution is again ultrasonicated to dislodge any residual sample material which may adhere to the flask.
The methyl acetate is drawn into the syringe. The final volume in the syringe is adjusted with metylacetate to 8.0 ml, achieving a 1:1 ratio of methyl acetate/toluene. The empty flask is retained.

The syringe is inverted (plunger end down), the needle is removed and saved. A 0.5 µm disposable filter is attached to the tip of the syringe and the sample is passed through the disposable filter onto the cartridge.

The (retained) empty flask is washed with 5 ml methyl acetate and 5 ml toluene. The solution is ultrasonicated and also drawn into the disposable syringe (filter was removed and saved) via the re-attached syringe needle. After removal of the syringe needle, the filter is reconnected and the wash solution applied to the SPE cartridge. After the wash solution is eluted, the cartridge is dried using a slight vacuum suction. The total washings from cartridge clean-up are discarded.

For elution of the derivatives, the silica gel cartridge is eluted using not less than 7 ml, but not more than 10 ml of the solvent mixture consisting of methanol/methyl acetate (1:1 v/v). The eluate is directly collected in a test tube.

4.8.7 Reconstitution

The eluate in the test tube is transferred into a round bottom flask. The tube is washed with 10 ml methyl acetate and the washing solution is also transferred into the round bottom flask. The solution is concentrated using a rotary evaporator (40°C) to an approximate volume of 1-2 ml. The sample is adjusted with methylacetate to its final volume.
Final volume: Soil samples: 5 ml, water samples: 3 ml

Normally, a final volume of 3 ml or 5 ml provides adequate sensitivity for determination. The solution is ready for quantification with GC/FPD

4.8.8 Critical steps of the analytical method

Care should be taken when solutions containing the GC-derivatives are evaporated using a rotary evaporator. The bath temperature should not exceed 40°C. The round bottom flasks should never run dry!

Soil samples with a high content of Ca^{2+} and Mg^{2+} ions could result in low recoveries below 60%.
In these cases, the aqueous soil extracts should be cleaned- up with the chelating resin as described in the method for water.

5 Calculation

To establish a calibration curve or one-point calibration, test solutions with known amounts of AE F064706, AE F070951 or AE F096432 in methylacetate are injected as a mixture of all three derivatives into the GC-system.

Calibration curve

Peaks heights measured by electronic integration are plotted against the amount of AE F039866, AE F061517 or AE F064619. Normally the calibration curve follows the general equation $y = a + b \cdot x$. However, a large range of concentrations can be described only by a function such as $y = a + b \cdot x + c \cdot x^2$. The amount of AE F039866, AE F061517 or AE F064619 in different matrices can be calculated directly from these calibration curves.

One -point calibration

Peak heights are measured by electronic integration. The concentrations in the sample are calculated against the peak heights of a calibration sample.

Residue concentrations of AE F039866, AE F061517 and AE F064619 are calculated as follows:

Determination of residues and apparent residues

$$R \text{ or } A = \frac{C_S\,[pg/\mu L] \bullet V_1\,[mL] \bullet f}{W \bullet 1000}$$

with $$C_S\,[pg/\mu L] = \frac{A_S\,[pg]}{T_{inj}\,[\mu L]} \qquad f = \frac{V_2\,[mL] \bullet V_3\,[mL] \bullet V_4\,[mL]}{T_1\,[mL] \bullet T_2\,[mL] \bullet T_3\,[mL]}$$

C_S can be calculated from the calibration curve or one -point calibration with the following equation:

$$C_S\,[pg/\mu L] = \frac{C_T\,[pg/\mu L] \bullet F_S\,[counts]}{F_T\,[counts]} = \frac{A_T\,[pg] \bullet F_S\,[counts]}{T_{inj}\,[\mu L] \bullet F_T\,[counts]}$$

Determination of the recovery efficiency

$$\text{Recovery } [\%] = \left(\frac{C_S\,[pg/\mu L] \bullet V_1\,[mL] \bullet f}{W \bullet 1000} - A\right) \bullet \frac{100}{W_R}$$

List of symbols

R	residue in treated sample [mg/kg or µg/l]
A	apparent residue in the control sample [mg/kg or µg/l]
A_S	amount of analytical target in the injection volume T_{inj} of the sample solution [pg]
A_T	amount of analytical target in the injection volume T_{inj} of the test solution [pg]
C_S	amount of AE F039866 obtained from the calibration curve or one- point calibration [pg/µL]
C_S	amount of AE F061517 obtained from the calibration curve or one- point calibration [pg/µL]
C_S	amount of AE F064619 obtained from the calibration curve or one- point calibration [pg/µL]
C_T	amount of AE F064706 in the test solution [pg/µL]
C_T	amount of AE F070951 in the test solution [pg/µL]
C_T	amount of AE F096432 in the test solution [pg/µL]
F_S	peak area or peak height of analytical target in the injection volume T_{inj} of the sample solution [counts]
F_T	peak area or peak height of analytical target in the injection volume T_{inj} of the test solution [counts]
f	dilution factor
V_1	volume of the final solution [mL]
V_2	volume of the final solution after refill of T_1 [mL]
V_3	volume of the final solution after refill of T_2 [mL]
V_4	volume of the final solution after refill of T_3 [mL]
W	weight of the analytical sample [g or l]
W_R	amount of AE F039866 added per sample weight [mg/kg or µg/l]
W_R	amount of AE F061517 added per sample weight [mg/kg or µg/l]
W_R	amount of AE F064619 added per sample weight [mg/kg or µg/l]
T_{inj}	injection volume [µL]
T_1	aliquot of V_1 [mL]
T_2	aliquot of V_2 [mL]
T_3	aliquot of V_3 [mL]

All masses and concentrations are calculated and expressed as Glufosinate free acid equivalents (AE F035956).

Appendix I:

Flow diagram (soil)

Extraction ***AE F039866*** ***AE F061517*** ***AE F064619***	25 g soil were extracted 2 x with 100 ml of water. Filtration of extracts and combination. 100 ml aliquot for further analysis
Clean-up	100 ml aliquot on anion exchange column Wash column with 100 ml water and discard washing.
Isolation	Elution with 100 ml formic acid (50 %). Collect eluate.
	Rotavap eluate to complete dryness (60°C) until no traces of acid are left. Add 10 ml water and rotavap. 2 * 5-10 ml methanol.
Derivatisation	Dissolve residue with 3 ml acetic acid (Ultrasonic). Add 12 ml trimethylorthoacetate and some glass beads. Mix (Ultrasonic). Reflux for 4,5 h (Overnight and timer!).
Attention: never let sample go dry!	Add 1 x 15 ml Toluol. Concentrate to 1-2 ml using rotavap (**max 40°C**). Add 2 x 15 ml toluene and concentrate each time to 1-2 ml.
Clean-up Silicagel (SPE) cartridge	Conditioning of column with 10 ml (methylacetate/toluene; 1+1; V+V). Draw up toluene in disposable syringe and fill up to 4 ml with toluene. Dissolve residue in round bottom flask (Ultrasonic) in 4 ml methylacetate and draw up in syringe. Filtration of solvent mixture (disposible filter) onto cartridge. Dissolve residue with 5 ml methylacetate and 5 ml toluene (Ultrasonic). Draw up in same syringe without filter. Filtration (filter) onto cartridge. Discard toluene/methylacetate. Dry cartridge with vacuum. Elution with minimum 7 ml maximum 10 ml (Methanol/methylacetate; 1+1; V+V)

Attention: never let sample go dry!

Transfer eluate to round bottom flask
and rinse residue in tube with 10 ml methylacetate
into round bottom flask.
Concentrate in rotavap (**max 40°C**)

GC

Final volume
5 ml methylacetate (soil samples)
3 ml methylacetate (water samples)
GC-FPD (526 nm)

Appendix I:

Flow diagram (water)

Removal of cations ***AE F039866*** ***AE F061517*** ***AE F064619***	Pour 500 ml water onto the chelating resin column and collect water flowing through resin.
Enrichment and clean-up	Let water run through anion exchange column. Wash column with 100 ml water and discard washing.
Isolation	Elution with 100 ml formic acid (50 %). Collect eluate.
	Rotavap eluate to complete dryness (60°C) until no traces of acid are left. Add 10 ml water and rotavap. 2 * 5-10 ml methanol.
Derivatisation	Dissolve residue with 3 ml acetic acid (Ultrasonic). Add 12 ml trimethylorthoacetate and some glass beads. Mix (Ultrasonic). Reflux for 4,5 h (Overnight and timer!).
Attention: never let sample go dry!	Add 1 x 15 ml Toluol. Concentrate to 1-2 ml using rotavap (**max 40°C**). Add 2 x 15 ml toluene and concentrate each time to 1-2 ml.
Clean-up Silicagel (SPE) cartridge	Conditioning of column with 10 ml (methylacetate/toluene; 1+1; V+V). Draw up toluene in disposable syringe and fill up to 4 ml with toluene. Dissolve residue in round bottom flask (Ultrasonic) in 4 ml methylacetate and draw up in syringe. Filtration of solvent mixture (disposible filter) onto cartridge. Dissolve residue with 5 ml methylacetate and 5 ml toluene (Ultrasonic). Draw up in same syringe without filter. Filtration (filter) onto cartridge. Discard toluene/methylacetate. Dry cartridge with vacuum. Elution with minimum 7 ml, maximum 10 ml (Methanol/methylacetate; 1+1; V+V)

Attention: never let sample go dry!

Transfer eluate into round bottom flask
and rinse residue in tube with 10 ml methylacetate
into round bottom flask.
Concentrate in rotavap (**max 40°C**)

GC

Final volume
5 ml methylacetate (soil samples)
3 ml methylacetate (water samples)
GC-FPD (526 nm)