1 INTRODUCTION

1.1 Scope of the method

BAS 083 W (mepiquat-chloride) is used as plant growth regulator on cotton, certain vegetables and other crops. The compound belongs to the chemical class of quaternary ammonium salts. For registration of the compound and for monitoring purposes a residue analytical method for the active ingredient in tap and surface water with a limit of quantitation of 0.05 µg/kg is needed.

The described method No. 477/0 allows the determination of the active ingredient with the required limit of quantitation in tap and surface water.

This method was developed at BASF Aktiengesellschaft, Agricultural Center Limburgerhof, Germany.

The purpose of this study was to demonstrate the validity of the method by performing recovery trials with spiked water samples.

The recovery trials were carried out with two types of water (tap water and surface water).

The spiking levels were 0, 0.05, 0.5 and 5.0 µg/kg. The fortified samples were analyzed in 5 replicates. The analyses were performed by one person, with the same equipment, in the same laboratory, within a short interval of time.

In the following we report the design and results of the study.

1.2 Principle of the method

A 1000 g sample of water is first extracted with DCM to remove any non-polar components. The ion-pairing reagent, Na-tetraphenyl borate is added to the aqueous phase and then BAS 083 W is partitioned into DCM. BAS 083 W is re-extracted from the DCM phase with 2 M-hydrochlorid acid. The HCl phase is reduced to dryness, the residue re-dissolved in acetonitrile / methanol (90+10 / v+v) and further cleaned using an Al_2O_3 column. The eluate is reduced to dryness, dissolved in ultra pure water and quantified by ion chromatography.

An HLPC-MS/MS method is described as confirmatory technique.

1.3 Specificity

The method allows the specific determination of BAS 083 W ai in tap water and surface water.

2 MATERIALS AND METHODS

2.1 Test system water

Two different types of water were used: Tap water from Limburgerhof and surface water taken from a lake in the palatinate forest. (For more details see Appendices 6.1 and 6.2).
The tap (drinking) water was directly taken from the water pipe in Li 445 for analysis. The surface water was stored in a polyethylene container in a refrigerated room (ca. 4 °C) before analysis.

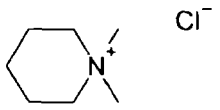
2.2 Test and reference items

2.2.1 Test item

(used for fortifications)

2.2.1.1 BAS 083 W ai (mepiquat-chloride)

Substance identification:	Reg. No. 085559
Common name:	Mepiquat-chloride (Internal LIMS code: MPIQAC)
Chemical name:	1,1-dimethylpiperidiniumchloride
Structural formula:	



Empirical formula:	$C_7H_{16}NCl$
Molecular weight:	149.67
Purity:	99.3 %, homogeneous
Lot. No.:	39-153-1; PCP01315, Reanalysis: PCP04698 supplied by BASF, APD/FC, Li 444
Stability:	expected to be stable at least for 7.5 years at room temperature or cooler; hygroscopic (keep away from humidity)

2.2.2 Reference item

(used for calibration)

Same item as test item, see 2.2.1.1.

2.3 Stability of standard solutions

Test/reference item

Standard solutions are kept refrigerated at 4°C. A study concerning the storage stability of mepiquat-chloride standard solutions found no decomposition of solutions made in water when stored refrigerated (4°C) in the dark for 119 days [1].

2.4 Materials and instruments

Materials, instrumentations and instrument methods were used as described in the technical procedure (see Appendix 6.4).

Deviations from technical procedure:

The surface water validation of method 477/0 has been performed with the instrument and conditions described below:

IC instrument:	Dionex DX-500 (Dionex Corporation, US)
Auto sampler:	AS 3500 (Thermo Electron Corporation, US)
Suppressor:	Dionex CSRS-Ultra 4-mm
Injection volume:	200 µL
Detector:	Dionex ED 50
Pumps:	2 x Dionex GP 50
Column oven:	Dionex LC 30
Column switching valve:	Dionex
Column 1 (pre-column):	Stainless steel, PRP-1, 150 x 4.1 mm, 10 µm, Hamilton
Column 2 (analytical-column):	Stainless steel, PRP-1, 250 x 4.1 mm, 10 µm, Hamilton
Data management software:	Dionex Chromeleon 6.20

2.5 Analytical procedure

The procedure described in the technical procedure was followed.

2.6 Example of calculation

Calculation of recovery of sample no. 815000 (tap water fortified with 0.5 µg/kg of mepiquat-chloride)

Queue file: PS00005 (analysis date: Nov-16-2000)

Calibration curve:

Type	= linear
Peak height	= slope x concentration + intercept
Slope	= 164675
Intercept	= - 1673
Correlation coefficient	= 0.9998

e.g. sample no. 815000:

Concentration of analyte [µg/mL] = (Peak height - intercept) / slope
= (70153 + 1673) / 164675
= 0.4362

Data required for calculation of residues (control sample no.: 810000)

Sample no.:	810000	815000
Sample weight:	1.0 kg	1.0 kg
Fortification:	0 µg/kg = untreated	0.5 µg/kg mepiquat-chloride
Final volume (V_{end}):	1.0 mL	1.0 mL
Peak height:	0	70153
Conc. of analyte (C):	0 µg/mL	0.4362 µg/mL
Aliquot of extract [%]:	100%	100%

Equation:

$$R\,[\mu g/kg] = \frac{V_{end} \times C}{S_M \times AI}$$

R	=	Residue in the water sample [µg/kg]
V_{end}	=	End volume of the extract after all dilution steps [mL]
C	=	Concentration of analyte in the injection volume as read from the calibration curve [µg/mL]
S_M	=	Weight of the water sample extracted [kg]
AI	=	Aliquot of water extract taken for analysis [%]

$$R\,(\text{untreated sample}) = \frac{1.0 \times 0}{1.0 \times 100\%} = 0\ \mu g/kg$$

$$R\,(\text{fortified sample}) = \frac{1.0 \times 0.4362}{1.0 \times 100\%} = 0.4362\ \mu g/kg$$

$$\%\ \text{Recovery} = \frac{R\,(\text{found, fortified}) - R\,(\text{found, untreated})}{R\,(\text{fortified})} \times 100$$

$$= \frac{0.4362 - 0}{0.5} \times 100 = 87.2$$

Appendix 6.4 continued: Technical procedure (Non-GLP)

1 INTRODUCTION

Mepiquat-chloride is used as plant growth regulator on cotton, certain vegetables and other crops. The compound belongs to the chemical class of quaternary ammonium salts.
For registration of the compound and for monitoring purposes a residue analytical method for the active ingredient in tap and surface water with a limit of quantitation of 0.05 µg/kg is needed.
The described method No. 477/0 allows the determination of the active ingredient with the required limit of quantitation in tap and surface water.
This method was developed at BASF Aktiengesellschaft, Agricultural Center Limburgerhof, Germany.

2 PRINCIPLE OF THE METHOD

A 1000 g sample of water is first extracted with DCM to remove any non-polar components. The ion-pairing reagent Na-tetraphenyl borate is added to the aqueous phase and then BAS 083 W is partitioned into DCM. BAS 083 W is reextracted from the DCM phase with 2 M-hydrochlorid acid. The HCl phase is reduced to dryness, the residue re-dissolved in acetonitrile / methanol (90+10 / v+v) and further cleaned using an Al_2O_3 column. The eluate is reduced to dryness, dissolved in ultra pure water and quantified by ion chromatography.

The method has a limit of quantitation of 0.05 µg/kg in water.

An HLPC-MS/MS method is described as confirmatory technique.

3 TEST AND REFERENCE ITEMS

3.1 Test Item

(used for fortifications)

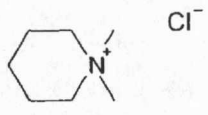
3.1.1 BAS 083 W ai

Substance identification: Reg. No. 085559

Common name: Mepiquat-chloride (Internal LIMS code: MPIQAC)

Chemical name: 1,1-dimethylpiperidiniumchloride

Structural formula:



Empirical formula: $C_7H_{16}NCl$

Molecular weight: 149.67

Appendix 6.4 continued: Technical procedure (Non-GLP)

Purity:	99.3 %, homogeneous
Lot. No.:	39-153-1; PCP01315, Reanalysis: PCP04698 supplied by BASF, APD/FC, Li 444
Stability:	expected to be stable at least for 7.5 years at room temperature or cooler; hygroscopic (keep away from humidity)

3.2 Reference Item

(used for calibration)

Same items as test items, see 3.1

Appendix 6.4 continued: Technical procedure (Non-GLP)

4 MATERIALS AND INSTRUMENTS

4.1 Equipment for Extraction and Sample Clean-up

Note: The materials, chemicals, and the equipment specified below were used for method development. They are specified as examples only and may be substituted by supplies with similar specifications. If the use of supplies other than stated is intended, applicability to this method must be confirmed prior to method validation and/or routine analysis.

Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
Balance	Top load, U 4100 S	Sartorius (Germany)	
Balance	Analytical, RC 250 S	Sartorius (Germany)	
Rotary evaporator	R 124	Büchi (Switzerland)	
Vacuum pump/ controller	Diaphragm pump/ B161	Vacuubrand (Germany)	
Water bath	B480	Büchi (Switzerland)	
Ultrasonic bath	T460	Hans Schmiedbauer (Germany)	
Glass columns	L = 400 mm, d = 18 mm L = 300 mm, d = 12 mm		
Glass funnels	d = 35 mm, d = 70 mm, d = 100 mm		
Powder funnels	d = 60 mm, d = 100 mm		
Separatory funnels	500 mL, 1 L		
Round bottom flasks	250 mL, 500 mL		
Beakers	50 mL, 100 mL		
Volumetric flasks	10 mL, 20 mL, 100 mL, 500 mL, 1 L, 2 L		
Tapered flasks	25 mL		
Cylinders, graduated	50 mL, 100 mL, 250 mL, 1 L, 2 L		
Volumetric pipets	Various sizes, 0.5 – 5 mL		
Transfer pipets	5 mL	Gilson Abimed (Germany)	
Pipet tips	P 5000	Gilson Abimed (Germany)	10860
Measuring pipets	10 mL, 25 mL		
Pasteur pipets	L =150 mm	Fortuna (Germany)	3.525
Vials	2 mL		
Vial caps	Teflon®-lined caps		
Glass stoppers	10/19, 14/23, 19/26, 29/32		
Cotton wool			
Lab spoon			

Appendix 6.4 continued: Technical procedure (Non-GLP)

4.2 Reagents

4.2.1 Chemicals

Note: Equivalent chemicals from other suppliers may be substituted but all chemicals used must be at least of "analytical grade" or must meet equivalent specifications.

Chemical	Grade	Manufacturer/ Supplier	Catalog Number
Acetonitrile (ACN)	Gradient grade for LC, LiChrosolv	Merck, Germany	1.00030
Dichloromethane (DCM)	GC grade, SupraSolv	Merck, Germany	1.06054
Methanol	GC grade, SupraSolv	Merck, Germany	1.06011
Ultra pure Water, in this method referred to as H_2O	High Purity	prepared with Millipore apparatus	Millipore (France)
Hydrochlorid acid	p.a. 37%	Riedel-de-Haen, Germany	30721
Na-tetraphenyl borate		Merck, Germany	1.06669.0100
Hexanesulfonic acid sodium salt for ion chromatography		Fluka, Buchs (Switzerland)	52863
Tetrabutylammonium hydroxide solution 40 % in water		Riedel-de-Haen, Germany	65341
Dowex cation exchange resin, 50 W X 4, 50-100 mesh, H^+ - Form		Fluka, Buchs (Switzerland)	44475
Alumina, acidic, ICN Aluminia A, activity I, reagent grade, 50-200µm, (70-290 mesh)	1)	ICN Biomedicals, Eschwege (Germany)	02105

1) Acitivity adjustment of alumina to activity level II

In order to adjust the standardized activity according to Brockmann, 97 g of alumina of activity I are vigorously shaken for 60 seconds in a capped flask with 3 g of H_20, until no lumps are left. The alumina is then allowed to stand for at least 12 hours (preferably over night) in a tightly sealed flask. Before use, shake the flask vigorously. Do not store the deactivated alumina for more than three days before use.

Appendix 6.4 continued: Technical procedure (Non-GLP)

4.2.2 Solvent Mixtures

Code	Solvent mixture
S 1	ACN + methanol, 90 + 10 (v + v)
S 2	**Mobile phase for ion chromatography** To condition the column: Swell approximately 500 g of Dowex cation exchange resin for at least 24 hours in H_2O. Keep the H_2O level above the resin during the swelling process. Place the resin in a glass column (d = 18 mm). Fill to a height of approximately 30 cm. Wash the exchange resin with 200 mL 2 M-HCl and then with 500 mL H_2O. This cation exchange material can be used again by reconditioning with the 2 M-HCl / water process. To prepare the concentrated mobile phase: To the column carefully add a solution of hexanesulfonic acid sodium salt, 4.12 g for the monohydrate or 3,76 g for the anhydrous, dissolved in 100 mL H_2O. Elute the solution dropwise into a 500 mL volumetric flask. Rinse the column further with 200 mL H_2O and combine both eluates. Dilute to the mark with H_2O. To prepare the ready-to-use mobile phase (eluent): Transfer 100 mL of the concentrated eluent into a 2 L volumetric flask, add the appropriate amount (100 mL for 5 %) of acetonitrile, and dilute to the mark with H_2O. The final concentration of the hexanesulfonic acid should be approximately 2 mM. This mobile phase is used for both the analytical and pre-columns.
S 3	**Regenerant** In a 2 L volumetric flask dissolve 32 g of tetrabutylammonium hydroxide solution in H_2O and dilute to the mark with H_2O.
S 4	**Na-tetraphenylborate solution** Dissolve 2g of Na-tetraphenyl borate in H_2O in a 100 mL volumetric flask and dilute to the mark with H_2O.

Note: Degas the eluent for ion chromatography specified in **S 2** for approximately 5 minutes by bubbling helium gas through the solution.

Appendix 6.4 continued: Technical procedure (Non-GLP)

4.3 Standard Solutions

4.3.1 Standard Solution Storage and Stability

Standard solutions are kept refrigerated at 4°C. A study concerning the storage stability of mepiquat-chloride standard solutions found no decomposition of solutions made in water when stored refrigerated (4°C) in the dark for 119 days [1].

At BASF stock solutions (1 mg/mL water) and diluted working solutions are prepared freshly every month.

Note:
Use amber bottles with Teflon®-lined screw caps as storage containers for standard solutions. Suggested standard concentrations are listed below. A different concentration scheme may be used and additional standards may be prepared as needed.

4.3.2 Standard Solutions for Fortifications and Calibration

Stock solution for Fortifications

Prepare a 1.0 mg/mL stock solution by weighing the appropriate amount of mepiquat-chloride into a volumetric flask. Dissolve with ultra pure water and dilute to mark. For example, to prepare a 10 mL stock solution, place 10 mg of the compound into a 10 mL volumetric flask. Dissolve and dilute to mark with H_2O.

Diluted Standard Solutions for Fortifications

Prepare a standard solution containing 10 µg/mL of mepiquat-chloride by pipetting 1 mL of the corresponding stock solution into a 100 mL volumetric flask. Dilute to mark with H_2O. Prepare serial solutions of this solution with H_2O as needed. Suggested concentrations of standard solutions are 5 µg/mL (for 5 ppb spiking), 0.5 µg/mL (for 0.5 ppb spiking) and 0.05 µg/ml (for 0.05 ppb spiking), in H_2O.

Standard Solutions for Calibration

Starting from the 10 µg/mL solution described above working solutions are prepared by dilution with H_2O as needed.
Suggested concentrations of standards for calibration are 0.05 µg/mL, 0.1 µg/mL, 0.25 µg/mL, 0.5 µg/mL and 1.0 µg/mL. Other concentration schemes and different or additional standard concentrations may be used if required.

Appendix 6.4 continued: Technical procedure (Non-GLP)

5 ANALYTICAL PROCEDURE

5.1 Sample Preparation and Storage

Filter turbid water samples through paper filter. Store water samples in clean amber glass bottles in a refrigerator at ca. 4 °C.

5.2 Spiking of Samples for Recovery Experiments

Weigh 1000 g of untreated water into a 1 L separatory funnel. Add 1 mL of the appropriate spiking solutions to the samples. The correlation between the concentration of the spiking solution and the resulting final analyte concentration in the sample is shown below:

Sample Weight	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
1000 g	0.0 µg/mL	1 mL	0.00 µg/kg
1000 g	0.05 µg/mL	1 mL	0.05 µg/kg *
1000 g	0.5 µg/mL	1 mL	0.5 µg/kg
1000 g	5.0 µg/mL	1 mL	5.0 µg/kg

* proposed limit of quantitation (LOQ)

5.3 Extraction of the Sample Material

5.3.1 DCM Partition

Extract the 1000 g water samples once with 100 mL of DCM in a 1 L separatory funnel. The DCM phase is discarded.

5.3.2 Extraction into the organic Phase with DCM / Na-tetraphenylborate

Add 1 mL of the Na-tetraphenylborate solution **S4** and partition with 2 x 100 mL of DCM (shake for approx. 60 s), drain the DCM into a 500 mL round bottom flask containing 100 mL of 2 M-HCl. The aqueous phase is discarded.

5.3.3 Re-extraction into aqueous Phase

The DCM/HCl mixture from step 5.3.2 is shaken in the round bottom flask for 60 s. The two phases are separated in a separatory funnel, the DCM phase is discarded and the aqueous phase extracted again with 50 mL DCM. The DCM is discarded and the aqueous phase reduced to dryness (60°C starting with 150 mbar and reducing pressure further to full vacuum). To make sure that the HCl is removed completely, 25 mL of H_2O are added to the dry residue and the solution is reduced to dryness again.

Note:
Care should be taken that HCl is removed completely.

Appendix 6.4 continued: Technical procedure (Non-GLP)

5.4 AL_2O_3 Column Purification

The Al_2O_3-material is preconditioned as described in 4.2.1. A glass column (d = 12 mm) is filled with 10 g of Al_2O_3. After approx. 15 min the sample can be added:
The residue of 5.3.3 is dissolved in 10 mL **S1** and poured onto the Al_2O_3 column material. The round bottom flask is rinsed with 5 mL **S1**. This solution is also loaded onto the Al_2O_3 column and is allowed to penetrate until the surface of the column bed is reached.

The analyte is eluated from the column with 85 mL **S1** and the eluate is reduced to dryness using a rotary evaporator (60°C, 150 mbar). The residue is transferred with 3 x 5 mL methanol into a 25 mL tapered flask and reduced to dryness using a rotary evaporator.
(When rather low residue levels are expected (e.g. concentrations at LOQ) the residue is transferred into 10 mL tapered flasks using 3 x 2.5 mL methanol and reduced to dryness.

Note: Use transfer pipets for methanol solvent transfer.

5.5 Preparation of the Final Volume for Ion Chromatography Quantitation

The dry residue of step 5.4 is re-dissolved in ultra pure water (=V_{End}). If necessary this process can be enhanced by ultra sonication.
For quantification at the limit of quantitation (LOQ) of the method (0.05 µg/kg) a volume of 0.5 mL should be used. In case of higher residues dilute with appropriate amounts of water (e.g. at a residue level of ca. 5.0 ppb a final volume of 10 mL may be used). An aliqot of this solution is injected for analysis (see below).

Note: Use transfer pasteur-pipets for solvent transfer into vials.

5.6 Quantitation

From the final volume V_{End} 100 µL (=V_i) is injected into the ion chromatograph for quantitation.

5.6.1 IC-Measurement

The quantitation of the residues is performed with a Dionex Ion Chromatograph with suppressed conductivity detection, a Dionex Switching valve, an auto sampler injection unit and a data system.

5.6.1.1 IC-Instrumentation

IC instrument:	Dionex DX-300 (Dionex Corporation, US)
Auto sampler:	AS 3500, 200 µL loop (Thermo Electron Corp., US)
Detector:	Dionex ED 40
Pumps:	Dionex GP 40
	Waters 590 (Waters Corporation, US)
Column switching valve:	Dionex
Column 1 (pre-column):	Stainless steel, PRP-1, 150 x 4.1 mm, 10 µm (Hamilton, US)
Column 2 (analytical-column):	Stainless steel, PRP-1, 250 x 4.1 mm, 10 µm (Hamilton, US)
Data management software:	Dionex PeakNet 5.1

Appendix 6.4 continued: Technical procedure (Non-GLP)

Note:	The equipment listed above may be substituted by instruments with similar specifications. Columns with equivalent stationary phases and similar specifications may be available from other sources. If the use of material with specifications other than those stated is intended, applicability of the new equipment for this method must be confirmed.

5.6.1.2 Chromatographic Conditions

Injection volume: 100 µL
Flow rate pre-column: 1mL/min
Flow rate analytical column: 1mL/min
Mobile phase for pre-column and analytical column: **S2**
Regenerant: **S3**

5.6.2 Calibration Procedures

Calibration curves are generated by plotting peak area or height versus the amount of the analytes measured by direct injection of reference standards containing known amounts of mepiquat-chloride. The linear least squares working curve in the form y = bx + c is used for the construction of the calibration curve.
A typical curve could cover a range from 0.05 µg/mL to 1.0 µg/mL. In a given analytical series, the same injection volume is used for all samples and standards.
In a measuring series standards and samples are injected alternately to show the stability of the detection response during the whole series.
For each series, the set should begin and end with standard injections. Each standard level should be injected at least in duplicate.

5.7 Limit of Quantitation and Limit of Detection

The limit of quantitation is defined as the lowest fortification level successfully tested. For water, the limit of quantitation is 0.05 µg/kg.
The limit of detection for mepiquat-chloride is 5 ng. It is here defined as the absolute amount of analyte injected into the ion chromatography instrument using the lowest standard of the calibration curve.

Appendix 6.4 continued: Technical procedure (Non-GLP)

6 CALCULATION OF RESULTS

6.1 Principle

Calculation of results is based on calibration curves recorded within each analytical series. Peak area or peak height is plotted versus the amount of analyte. The residue of mepiquat-chloride is calculated from its calibration curve and the equations are shown in section 6.2.

6.2 Calculation of Residues

The residue (R) in the water sample in µg/kg is calculated as shown in the following equation:

$$R = \frac{V_{End} \times C}{S_M \times AI}$$

R = Residue in the water sample [µg/kg]
V_{End} = End volume of the extract after all dilution steps [mL]
C = Concentration of analyte in the inj. vol. as read from the calibration curve [µg/mL]
S_M = Weight of the water sample extracted [kg]
AI = Aliquot of water extract taken for analysis [%]

If residue data are to be corrected for loss of analyte during sample extraction and clean-up procedures the residue [R] has to be corrected with the results of the procedural recoveries as shown in the following equation II:

$$R_{RC} = R \times R_{FE}$$

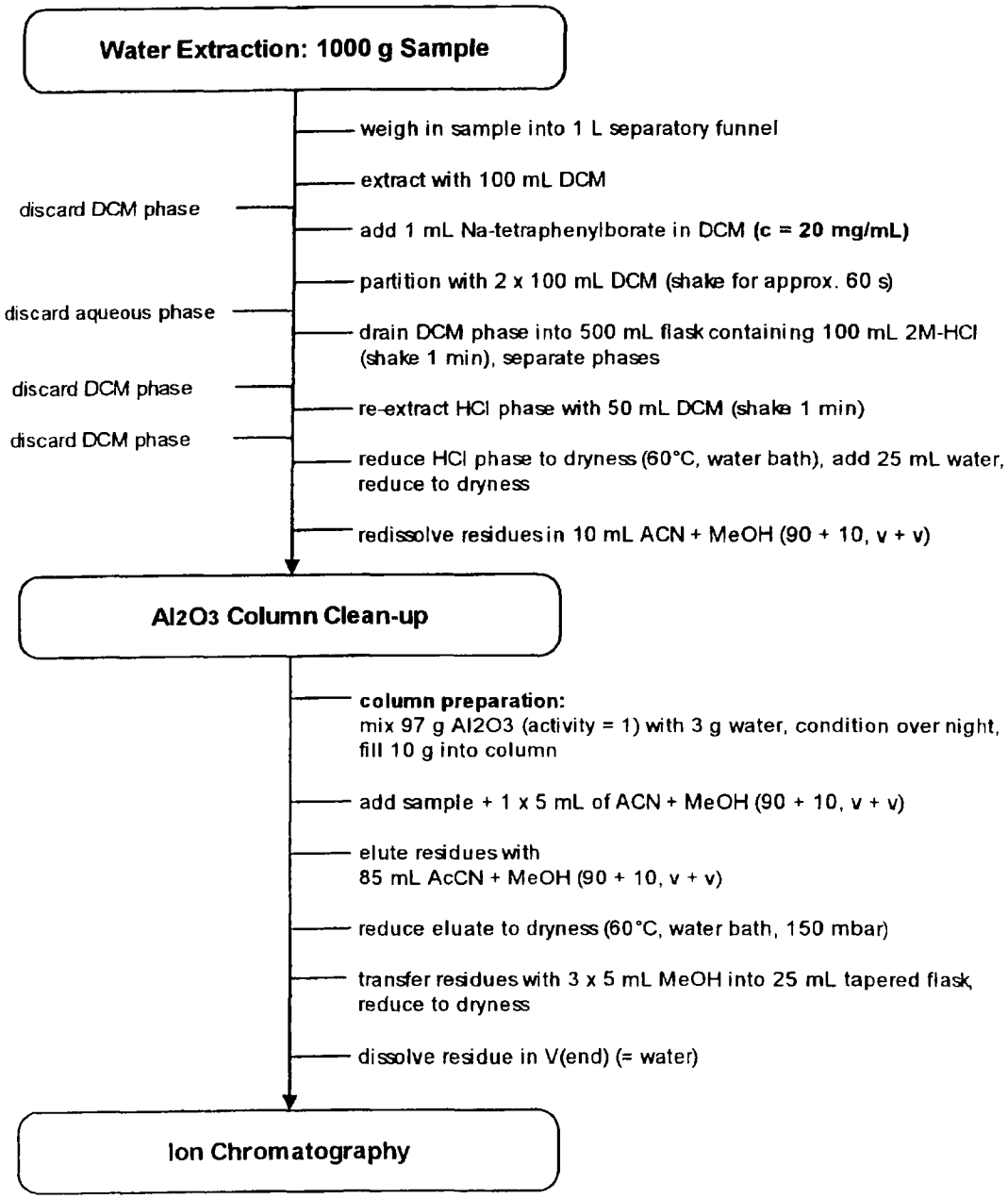
R_{RC} = Residue concentration of the analyte in the sample corrected with the procedural recovery of the analyte in fortification experiments [µg/kg sample material]
R_{FE} = Procedural recovery of the analyte as determined from fortification experiments performed in parallel to the sample analysis

$$= \frac{100\ \%\ (\text{level of fortification})}{\%\ \text{recovery}}$$

Note: For routine analysis requirements residue data should not be corrected for procedural recoveries. Results of fortification experiments should be listed individually.

Appendix 6.4 continued: Technical procedure (Non-GLP)

7 FLOW CHART OF METHOD 477/0



Appendix 6.4 continued: Technical procedure (Non-GLP)

8 RECOVERIES

Recovery data will be provided in the validation part of the analytical method 477/0. The study code of the validation is 58417.

9 LIMIT OF DETERMINATION, BLANK VALUES

The limit of determination (quantitation) was defined as the lowest fortification level successfully tested = 0.05 µg/kg.
The tested untreated water samples showed no interfering peaks at the retention time of the analytes.

10 TYPICAL CHROMATOGRAMS

Typical chromatograms will be provided in the validation part of the analytical method 477/0. The study code of the validation is 58417.

11 CONFIRMATORY TECHNIQUE (LC-MS/MS METHOD)

For confirmatory purposes the following LC-MS/MS method can be used. The sample preparation is adapted from the sample preparation described in chapter 5 according to the reduced sample weigh-in required for LC-MS/MS analysis.

11.1 Sample Preparation

11.1.1 DCM Partition

Extract the 200 g water sample once with 50 mL of DCM in a separatory funnel.
The DCM phase is discarded.

11.1.2 Extraction into the organic Phase with DCM / Na-tetraphenylborate

Add 1 mL of the Na-tetraphenylborate solution **S4** and partition with 2 x 50 mL of DCM (shake for approx. 60 s), drain the DCM into a round bottom flask containing 50 mL of 2 M-HCl. The aqueous phase is discarded.

11.1.3 Re-extraction into aqueous Phase

The DCM/HCl mixture from step 11.1.2 is shaken in the round bottom flask for 60 s. The two phases are separated in a separatory funnel, the DCM phase is discarded and the aqueous phase extracted again with 25 mL DCM. The DCM is discarded and the aqueous phase reduced to dryness (60°C starting with 150 mbar and reducing pressure further to full vacuum). To make sure that the HCl is removed completely, 25 mL of H_2O are added to the dry residue and the solution is reduced to dryness again.

Note:
Care should be taken that HCl is removed completely.

11.1.4 AL_2O_3 Column Purification

The Al_2O_3-material is preconditioned as described in 4.2.1. A glass column (d = 12 mm) is filled with 10 g of Al_2O_3. After approx. 15 min the sample can be added:
The residue of 11.1.3 is dissolved in 10 mL **S1** and poured onto the Al_2O_3 column material. The round bottom flask is rinsed with 5 mL **S1**. This solution is also loaded onto the Al_2O_3 column and is allowed to penetrate until the surface of the column bed is reached.

Appendix 6.4 continued: Technical procedure (Non-GLP)

The analyte is eluated from the column with 85 mL **S1** and the eluate is reduced to dryness using a rotary evaporator (60°C, 150 mbar). The residue is transferred with small amounts of methanol into a 10 mL or 25 mL tapered flask and reduced to dryness using a rotary evaporator.

11.1.5 Preparation of the Final Volume for LC-MS/MS Quantitation

The dry residue of step 11.1.4 is re-dissolved in solvent A (water/formic acic, 1000/1, v/v) ($=V_{End}$).
For quantitation at the LOQ of the method (0.05 µg/kg) a volume of 2.0 mL should be used. In case of higher residues dilute with appropriate amounts of solvent A.

11.1.6 Quantitation

From the final volume V_{End} 25 µL ($=V_i$) is injected into the LC-MS/MS system for quantitation.

11.2 Chromatographic Conditions

HPLC system:	Agilent 1100 LC binary pump
Autosampler:	PE 200
Injection volume:	25 µL
HPLC column:	Altima C18, 100 x 4.6 mm ID, 3 µm
Column temperature:	RT
Mobile phase:	Solvent A – water/formic acid, 1000/1 (v/v)
	Solvent B – methanol/formic acid, 1000/1 (v/v)

Gradient:

Time (min)	Composition (% A)
0	100
0.1	100
4.6	10
9.0	10
9.1	100
12.1	100

Flow rate:	0.6 mL/min	
Retention times:	mepiquat-chloride:	approx. 3.6 min
Run time:		approx. 12 min

11.3 Mass Spectrometric Conditions

Mass spectrometer:	PE Sciex API 3000 triple stage quadrupole	
Interface:	ESI (+)	
Ion mode:	MRM	
Transitions:	mepiquat-chloride:	114.1 -> 98.1 and 114.1 -> 58.0