

REASON FOR AMENDED REPORT

This report has been revised to remove the Sponsor Representative/Study Monitor Signature prompt from Page 5 of the original report, as it is unnecessary for submission requirements.

1 INTRODUCTION

1.1 Scope of the Method

BASF Method 477/0 was developed to determine the residues of BAS 083 W AI (mepiquat-chloride) in water using ion chromatography (IC) and LC/MS/MS at BASF in Limburgerhof, Germany. The method was validated at BASF and independently validated for LC/MS/MS at ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia, Missouri.

The independent lab validation was conducted using two fortification levels (0.05 and 0.5 ppb) for drinking water. For each fortification level, five replicates were analyzed. Additionally, two replicates of unfortified samples and a reagent blank were examined.

1.2 Principle of the Method

The residues of BAS 083 W are extracted from water (200 g) using dichloromethane extractions in conjunction with partitioning and Al_2O_3 column cleanup. The final determination was conducted using LC-MS/MS in positive ion mode.

In drinking water, the method has a limit of quantitation of 0.05 ppb and a limit of detection for the analyte is set to 0.005 ppb.

1.3 Specificity

BAS 083 W (mepiquat-chloride) was identified and quantified.

2 MATERIALS AND METHODS

2.1 Test systems

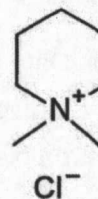
The following test system was considered in this study:
Drinking (Tap) Water, ABC Laboratories, Columbia, MO

2.2 Test / Reference Substance

2.2.1 BAS 083 W (Mepiquat-chloride)

BASF Reg. No.	85559
Molecular Formula	$C_7H_{16}ClN$
Molecular Weight	149.7
IUPAC Name	1,1-dimethylpiperidinium chloride
Batch No.	L74-34
Purity (%)	99.8
Storage Advice	Room Temperature (typically 25 °C)
GLP	Yes
Expiration Date	March 1, 2015

Chemical structure:



2.3 Materials and Methods

2.3.1 Equipment

Equipment	Size, Description	Manufacturer
Balance	Analytical	Mettler XP205DR
Balance	Analytical	Mettler BB2440
Balance	Analytical	Mettler PB5001-S
Scintillation Vials	10-mL	Fisher
Volumetric, pipettes	Various sizes	Fisher
Positive-displacement, pipettes	Various sizes	Gilson
Air-displacement, pipettes	Various sizes	Hamilton
Flasks, Volumetric	Various sizes	Various
Separatory Funnel	500-mL	Various
Plastic Centrifuge Tubes	50-mL	Fisher
Rotary Evaporator	N-Evap	Meyer
Pear Shaped Flasks	50-mL	Various
Flat Bottom Flasks	125, 250-mL	Various
Liquid Chromatographic System	Waters Acquity UHPLC	Waters
Mass Spectrometer	AB Sciex API5000	AB Sciex
HPLC Column	Alltima HP C18 3 μ m, 100 \times 4.6 mm I.D.	Alltech

2.3.2 Reagents

2.3.2.1 Chemicals

Chemical	Grade	Manufacturer/Supplier
Acetonitrile (ACN)	Optima	Fisher
Alumina	Deactivated	Acros
Dichloromethane (DCM)	HPLC Grade	Fisher
Formic acid	N/A	Fisher
Hydrochloric acid (HCl)	ACS Plus	Fisher
Methanol	Optima	Fisher
Na-tetraphenyl borate	N/A	Chem-impex
Water	HPLC Grade	Fisher

2.3.2.2 Solutions and Solvent Mixtures

Description	Code	Composition
Elution Solvent	S1	ACN:MeOH, 90:10 (v/v)
Na-tetraphenylborate Solution	S4	Dissolve 2 g of Na-tetraphenylborate in a 100-mL volumetric flask and dilute to the mark with H ₂ O.

2.3.2.3 Standard Solutions

Stock Solutions

A 1.0-mg/mL stock solution was prepared by weighing an appropriate amount of BAS 083 W (mepiquat-chloride) into a 10-mL volumetric flask and adding the required volume of water (10.0-mL).

ID	Purity	Weight (mg)	Concentration (mg/mL)
BAS 083 W	99.8 %	10.06	1.00

Fortification Solutions

Fortifications Solution Preparation

A fortification solution was prepared by serial dilution of the stock solution with water.

Take Solution (µg/mL)	Volume (mL)	Final Volume (mL)	BAS 083 W Concentration (µg/mL)
Stock (~1000 µg/mL)	1.0	100	10
10	5.0	10	5.0
10	0.5	10	0.50
10	0.05	10	0.050

Calibration Standard Solutions

Intermediate Calibration Standard Solutions Preparation

Intermediate solutions were prepared by serial dilution of the stock solution with 0.1% formic acid (aq) in appropriate containers.

Solution Used	Volume Taken (mL)	Final Volume (mL)	BAS 083 W Concentration (ng/mL)
10 µg/mL	0.25	100	25.0

Calibration Standard Solutions Preparation

Calibration solutions were prepared by dilution of the intermediate solutions with 0.1% formic acid (aq) in appropriate containers.

Solution Used (ng/mL)	Volume Taken (mL)	Final Volume (mL)	BAS 083 W Concentration (ng/mL)
25	2.00	10	5.0
25	1.00	10	2.5
25	0.50	10	1.25
25	0.40	10	1.0
25	0.30	10	0.75
25	0.20	10	0.5

All standard solutions were stored refrigerated when not in use.

3. Analytical Procedure

3.1 Weighing and Fortification

Thirteen samples were prepared for drinking water: two control samples, one reagent blank, five samples treated at the LOQ and five treated at 10 times the LOQ. For each, 200 g of water was weighed into a 500-mL separatory funnel and spiked with the appropriate fortification solution.

The following scheme was used:

Sample Type	Sample Weight	Concentration of Spiking Solution [µg/mL]	Volume of Spiking Solution [mL]	Level of Fortification [µg/kg]
Control	200 g	-	-	0.00
Fortification (LOQ)	200 g	0.05	0.2	0.05*
Fortification (10× LOQ)	200 g	0.5	0.2	0.5

* Limit of quantification

3.2 Extraction of Sample Material

The sample was weighed and fortified, if appropriate, in a 500-mL separatory funnel. 50 mL of dichloromethane were added to the funnel. The funnel was shaken for 60 seconds. The organic and aqueous layers were allowed to separate and the DCM layer was discarded. 1 mL of Na-tetraphenylborate solution (S4) was added and partitioned with 50 mL of DCM. The funnel was shaken for 60 seconds. The organic and aqueous layers were allowed to separate and the DCM layer was collected into a flask. The aqueous layer was extracted an additional time with DCM and both organic layers were collected into the same flask then poured into a separatory funnel. 50 mL of 2 M HCl were added to the funnel. The funnel was shaken for 60 seconds. The organic and aqueous layers were allowed to separate and the DCM and 2 M HCl layers were collected into separate flasks. The DCM was poured back into the separatory funnel and 50 mL of 2 M HCl were added. The funnel was shaken for 60 seconds. The organic and aqueous layers were allowed to separate and the DCM layer was discarded. The 2 M HCl layer previously collected was poured back into the separatory funnel to create a pooled extract, and 25 mL of DCM were added. The funnel was shaken for 60 seconds. The organic and aqueous layers were allowed to separate and the DCM layer was discarded.

3.3 Preparation for Measurement

Each pooled extract was brought to dryness in a water bath set to ~60 °C using a rotary evaporator. An additional 25 mL portion of water was added and evaporated to dryness again to ensure removal of the HCl.

3.4 Al₂O₃ Column Purification

The residue was dissolved in 10 mL of elution solution (S1) and poured onto the preconditioned Al₂O₃ column material. The flask was rinsed with 5 mL of S1, the analyte was eluted from the column with 85 mL of S1, and the eluate was reduced to dryness using a rotary evaporator with waterbath set at 60 °C. The residue was transferred with 3 x 5 mL of methanol into a 50-mL pear-shaped flask and reduced to dryness.

Samples were reconstituted with 2.0 mL 0.1% formic acid (aq). The final concentration of each sample, as prepared, is presented below:

Sample Type	BAS 083 W Concentration (ng/mL)
Control	0.0
Fortification (LOQ)	0.05
Fortification (10× LOQ)	0.50

4 Instrumentation and Conditions

UHPLC-MS/MS Conditions for BAS 083 W (Primary Transition) and BAS 083 W a (Confirmatory Transition)

		Parameter	
Chromatographic System	MDS Sciex API 5000		
Analytical-column	Alltima HP C18 100mm x 4.6 mm, 3 µm		
Column Temperature	40 °C		
Injection Volume	2-10 µL		
Mobile Phase	A: 0.1% formic acid (aq) B: 0.1% formic acid in methanol		
Gradient	Total Time (min)	Mobile Phase	
		A%	B%
	0.00	100	0
	0.10	100	0
	4.60	10	90
	9.00	10	90
	12.00	100	0
Flow Rate	0.6 mL/min		
Detection System	AB Sciex 5000 Mass Spectrometer		
Ionization	Turbo Spray		
Ionization Temperature	500 °C		
Analyte	Transitions	Polarity	Approximate Retention Time
BAS 083 W (Primary)	114 → 98	Positive	2.8
BAS 083 W a (Confirmatory)	114 → 58		

4.1 Calibration Procedures

Calculation of results is based on peak area measurements using a calibration curve. The calibration curve is obtained by direct injection of the calibration standards containing known amounts of BAS 083 W in the range of 0.5 ng/mL to 1.25 ng/mL. Linear regression was used for calibration, which is shown in Figures 10.1.2 and 10.1.3. To demonstrate reliability of the calibration, plots showing the response factor (peak area divided by nominal concentration) versus the nominal concentration for all calibration points are given in Figure 10.1.4.

4.2 Calculation of Residues and Recoveries

Calculation of results was based on area measurements. The recoveries of both analytes were calculated relative to the linearity curve generated with each set. Due to residues found in the

control samples, the residues were averaged and the recovery values were corrected for this average. This was applicable for the post extraction spike as well.

I. **Concentration [ng/mL]** = $\frac{\text{Peak Area} - \text{Intercept}}{\text{Slope}}$

Sample concentration results were calculated by Analyst Software version 1.5.1 (validated system).

II. **Concentration [ppb]** = $\frac{\text{ng/mL found} \times \text{Final Volume} \times \text{Dilution Factor}}{\text{Sample Weight}}$

The recoveries of spiked compounds are calculated according to equation III:

III. **Recovery % PE (cfc)** = $\frac{\text{ppb found (cfc)}}{\text{ppb added} \times 100}$

Recovery corrected for the average residues of three control samples analyzed in Trial 2.

Example: Example: BAS 083 W, 114 → 98; Drinking Water 80506-128 fortified at 0.05 µg/kg:

The following values were used in this calculation:

Response of fortified sample	11894
Response of control sample	4744
Slope:	14091.92
Intercept:	-487.8712
Final Volume (mL):	2
Dilution Factor:	6
Sample Weight (g):	200

I. **Concentration (ng/mL)** = $(11894 + 487.8712) / 14091.92 = 0.879 \text{ ng/mL}$

II. **Concentration (ppb)** = $0.879 \text{ ng/mL} \times 2 \text{ mL} \times 6 / 200 \text{ g} = 0.0527 \text{ ppb}$

III. **Recovery %** = $0.0527 \text{ ppb} / 0.05 \text{ ppb} \times 100 = 72\%$

Note: Unrounded ng/mL values calculated by Analyst Software were pasted into an Excel 2010 spreadsheet to determine concentrations in ppb. Hand calculated results may differ as a result of rounding.