REPORT TITLE

Development and Validation of an Analytical Method for the Determination of NF-180 and its Metabolite QP-1-7 in Surface and in Ground Water

STUDY IDENTIFICATION

EAG Laboratories ID: P 4906 G
Sponsor Study Code: JAS-574: Nisso-NF-180

GUIDELINES COVERED

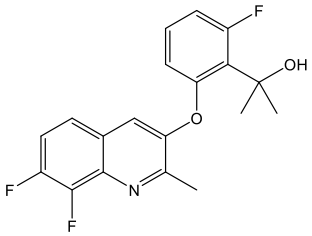
EC Guidance Document on Residue Analytical Methods:
SANCO/825/00 rev. 8.1, 16-Nov-2010
OECD Guidance Document on Pesticide Residue Analytical Methods:
ENV/JM/MONO (2007) 17, 13-Aug-2007
US EPA: OCSPP 860.1340 and OCSPP 850.6100.

1. INTRODUCTION

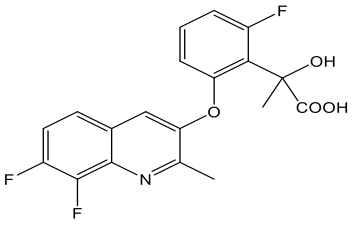
Background and Objective:

To develop and to validate analytical methods for the determination of NF-180 and its metabolite QP-1-7 in surface and ground water with a target limit of quantitation (LOQ) of 0.05 µg/L for each analyte and each water type.

NF-180



QP-1-7



2. GENERAL EXPERIMENTAL

2.1 Test System

Ground water was taken from Buxheim and surface water was obtained from the river Danube, near Leipheim (sampled at Landeswasserversorgung Langenau), Germany. The waters were stored refrigerated until analysis. The analysis data sheets of the 2 water types are attached in Appendix 2. Characteristics of water matrices are listed in the table below:

Parameter	Ground Water	Surface Water
Sampling Date	06-Aug-2018	21-Jun-2018
pH	7.40	8.20
Conductivity at 25 °C in [µS/cm]	827	544
Filterable solids in [mg/L]	0.6	3.6
TOC (total organic carbon) in [mg/L]	1.2	2.1
DOC (dissolved organic carbon) in [mg/L]	1.1	2.0
Magnesium in [mg/L]	17.3	12.7
Calcium in [mg/L]	104	83
Total hardness in [mmol/L]	3.31	2.59
Total german hardness in [°d]	18.5	14.5

2.2 Test/Reference Items

The test/reference items were supplied by the Sponsor, with relevant information concerning identity, molecular mass, purity, and expiration date (see Appendix 1 for Certificate of Analysis).

2.2.1 NF-180

Chemical Name:	2-[2-(7,8-difluoro-2-methylquinolin-3-yloxy)-6-fluorophenyl]propan-2-ol	
Empirical formula:	$C_{19}H_{16}F_3NO_2$	Molar mass: 347.33 g/mol
Lot/Batch No.:	11426- T.Kinoshita	Purity: 98.3 %
Certification:	19-May-16	Expiry Date: 15-June-20
Storage conditions	Keep in a freezer	Date of receipt: 06-May-16

2.2.2 QP-1-7

Chemical Name:	2-[2-(7,8-difluoro-2-methylquinolin-3-yloxy)-6-fluorophenyl]-2-hydroxypropanoic acid	
Empirical formula:	$C_{19}H_{14}F_3NO_4$	Molar mass: 377.31 g/mol
Lot/Batch No.:	31-16149-D.SATO	Purity: 98.1 %
Certification:	09-May-18	Expiry Date: 26-Jun-20
Storage conditions	Keep in a freezer	Date of receipt: 06-Jul-18

2.3 Material and Methods

2.3.1 Equipment and Instrumentation

Balances: Mettler Toledo XS205DU.

Ultrasonic bath:USC 300 TH, VWR international bvba, Belgium.

Centrifuge: Hettich Rotanta 460.

Typical glassware and laboratory equipment.

All the glassware was cleaned in a laboratory dishwasher and air-dried before use.

LC-MS/MS Instrumentation:

Agilent 1290 Series LC system (vacuum solvent degasser, binary LC pump), MayLab MistraSwitch column oven and a PAL System HTS-xt Autosampler.

HPLC column: Waters ACQUITY UPLC BEH C_{18} column (length: 100 mm, i.d.: 2.1 mm, particle size: 1.7 µm)

Pre-column: Phenomenex C18 (UPLC).

Applied Biosystems MDS Sciex API 6500+ triple quadrupole LC-MS/MS system with TurboIonspray (ESI) source.

2.3.2 Solvents, Chemicals and Reagents

Acetonitrile, HPLC grade (Promochem).

LC-MS acetonitrile, ≥ 99.9 % (Honeywell).

Millipore water Supply at test facility

LC-MS water, (LC-MS grade, Honeywell).

Glacial acetic acid, (LC-MS grade Sigma-Aldrich).

Citrate extraction tube (Supelco, 55227-U).

Safety Precautions

Each analyst must be acquainted with the potential hazards of the equipment, reagents, products, solvents, and procedures used in this method before commencing laboratory work. Safety information could be obtained from the supplier or literature (e.g. H- and P-phrases). Disposal of waste materials, reagents, reactants, and solvents must be in compliance with applicable governmental requirements.

Acetonitrile and acetic acid are flammable and should be used in well-ventilated areas away from ignition sources. Acetic acid is corrosive.

It is imperative that proper eye and personal protection equipment is worn when handling these reagents.

Safety for reference items: toxicity not specified, usual pre-cautions with toxic compounds.

2.3.3 Preparation of Solutions

Acetonitrile/water (1/1, v/v)

Measure 500 mL Millipore water and add 500 mL of acetonitrile. Mix well.

Water containing 0.1 % acetic acid (mobile phase A)

Measure 1000 mL of water (LC-MS grade). Add 1 mL of concentrated acetic acid (LC-MS grade). Mix well.

Acetonitrile containing 0.1 % acetic acid (mobile phase B)

Measure 1000 mL of acetonitrile (LC-MS grade). Add 1 mL of concentrated acetic acid (LC-MS grade). Mix well.

2.3.4 Preparation of Stock and Fortification Solutions

Separate stock solutions for NF-180 and QP-1-7 were prepared in acetonitrile. During the study each stock solution was prepared two times (2 sets). One set of stock solutions was used for the validation purpose and the second set was used for stability investigations. The concentrations of the NF-180 respectively QP-1-7 were always 0.20 mg/mL.

These concentrations of the stock solutions could be obtained as follows:

Substance name	Weight* [mg]	Dissolve in [mL]	Obtain [mg/mL]
NF-180 (purity 98.3 %)	10.17	50	0.20
QP-1-7 (purity 98.1 %)	10.19	50	0.20

*: Purity taken into account.

Fortification solutions containing both analytes were prepared by accurate dilution into acetonitrile to obtain the following concentrations:

Use solution with	Pipette [mL]	Dilute to [mL]	Concentration of each analyte [µg/mL]
0.20 mg/mL (NF-180 stock solution)	0.050	10	1.0
0.20 mg/mL (QP-1-7 stock solution)	0.050		
1.0 µg/mL (fortification solution)	1.0	10	0.10
0.10 µg/mL (fortification solution)	1.0	10	0.010

All stock and fortification solutions were stored refrigerated and protected from light when not in use.

2.3.5 Preparation of Calibration Solutions in Solvent

Calibration solutions containing NF-180 and QP-1-7 as mix were prepared by accurate dilution into acetonitrile/water (1/1 v/v) to obtain the following concentrations:

Solution Used [ng/µL]	Pipette [µL]	Dilute to [mL]	Concentration of each analyte [ng/mL]
200 (NF-180 stock solution)	500	10	10000
200 (QP-1-7 stock solution)	500		
10 (calibration solution)	100	10	100
0.10 (calibration solution)	1000	10	10
0.10 (calibration solution)	100	10	1.0
0.010 (calibration solution)	250	10	0.25
0.010 (calibration solution)	100	10	0.10
0.0010 (calibration solution)	250	10	0.025
0.0010 (calibration solution)	100	10	0.010
0.0010 (calibration solution)	50	10	0.0050

All calibration solutions were stored refrigerated and protected from light when not in use.

2.3.6 Preparation of Calibration Solutions in Matrix

Preparation of Matrix-Matched Standards

One matrix-matched standard solution containing NF-180 and QP-1-7 at 0.025 ng/mL was prepared for both water types. 0.450 mL of final blank extract was mixed with 0.050 mL of the 0.25 ng/mL calibration solution (containing NF-180 and QP-1-7). These solutions were used to establish the matrix-effect on LC-MS/MS response.

2.3.7 LC-MS/MS Determination

The final extracts were analyzed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) using a Sciex API 6500+ mass spectrometer:

LC System	Agilent 1290 Series LC system (vacuum solvent degasser, binary LC pump), MayLab MistraSwitch column oven and a PAL System HTS-xt Autosampler.
LC Column	Pre-column: Phenomenex C_{18} (UPLC). Column: Waters ACQUITY UPLC BEH C18 column (length: 100 mm, i.d.: 2.1 mm, particle size: 1.7 µm). Column temperature: 40 °C.
LC Injection Volume	10 µL.
LC Method	Solvent A: Water + 0.1 % acetic acid Solvent B: Acetonitrile + 0.1 % acetic acid Mobile Phase Composition: Time (min) / Flow rate (mL/min) / % A / % B 0.00 / 0.30 / 80 / 20 5.00 / 0.30 / 10 / 90 6.00 / 0.30 / 10 / 90 6.10 / 0.30 / 80 / 20 8.00 / 0.30 / 80 / 20
Retention time	≈ 4.9 min for NF-180. ≈ 3.8 min for QP-1-7.
MS/MS System	Applied Biosystems MDS Sciex API 6500+ triple quadrupole LC-MS/MS system with TurboIonspray (ESI) source.
Ion Source Conditions ESI Positive Polarity	Source temperature: 400°C Gas supply (GS 1): 60 (arbitrary units) Gas supply (GS 2) 60 (arbitrary units) Curtain gas: 35 (arbitrary units) CAD gas: 10 Entrance potential: 10 V Declustering potential (DP): 41 V IonSpray voltage: 5500 V Resolution: Q1: Unit, Q3: Unit
MS/MS Conditions for NF-180 and QP-1-7	*NF-180:* *MS/MS transition for quantification*: *348 m/z > 330 m/z* Collision energy (CE): 31V Cell exit potential (CXP): 22 V Dwell time: 200 ms *MS/MS transition for confirmation*: *348 m/z > 180 m/z* Collision energy (CE): 39 V Cell exit potential (CXP): 12 V Dwell time: 200 ms *QP-1-7:* *MS/MS transition for quantification:* *378 m/z > 332 m/z* Collision energy (CE): 31 V Cell exit potential (CXP): 22 V Dwell time: 200 ms *MS/MS transition for confirmation:* *378 m/z > 314 m/z* Collision energy (CE): 43 V Cell exit potential (CXP): 20 V Dwell time: 200 ms

The $[M+H]^+$ ion of NF-180 at 348 m/z was used as parent ion for MS/MS detection. The MS/MS transition to the daughter ion at 330 m/z was used for quantification of the analyte. The transition 348 m/z-> 180 m/z was used as confirmation transition.
The $[M+H]^+$ ion of QP-1-7 at 378 m/z was used as parent ion for MS/MS detection. The MS/MS transition to the daughter ion at 332 m/z was used for quantification of the analyte. The transition 348 m/z-> 314 m/z was used as confirmation transition.
Calibration functions as exemplified in Figure 1 and Figure 2 were established with standards in solvent ranging from 0.0050 to 1.0 ng/mL for both analytes (≥ 5 levels) by injecting 10 µL of standard solutions in solvent. The LC-MS/MS calibration functions were calculated and plotted by regression analysis, using the LC-MS/MS software. The correlation coefficients (r) were always > 0.99. Matrix interference on LC-MS/MS response must be always examined for each analyte / matrix and if significant, compensated by the use of matrix-matched standard calibration solutions. Matrix effect observed in the course of the present study were not significant (≤ 20 %).

2.3.8 Calculation

The following equation was used to calculate the residues R of the analytes in µg/L:

$R = C_{End} \times [(V_{Ex} \times V_{End}) / (V_1 \times V_{sample})$
$= C_{End} \times$ Multiplier M

R: Residue in µg/L.
C_{End}: Final concentration of analyte in extract in ng/mL.
(where multiple injections were evaluated: mean).
V_{Ex}: Extraction volume: 10 mL of acetonitrile.
V_1: Aliquot of V_{Ex} used for preparation of final extract: 0.50 mL
V_{End}: Final volume of extract: 1.0 mL.
V_{sample} Volume of water used for extraction: 10 mL (equivalent with 10 g).

Residues R of the analytes are calculated in µg/L. The values reported in the tables are calculated with full precision, but displayed rounded. Minor/insignificant discrepancies may be observed when recalculated.
Recoveries (Rec.) were calculated for the fortified specimens as follows:
$Rec. = (R / R_{fortified}) \times 100$ %
The calculation of the residue R is demonstrated with a surface water specimen, which was fortified at the LOQ level (0.05 µg/L) with NF-180 and QP-1-7 and assigned with the specimen ID P 4906-56. The final extract was examined by LC-MS/MS (P4906API6#076, see Table 1 and Figure 6 top).
The daughter ion chromatogram of the final extract was evaluated by the instrument software using external calibration functions.

NF-180 elutes at 4.9 min and gives upon integration a peak area of 1.13 x 10^5 counts (quantified using the primary transition 348 m/z > 330 m/z), resulting in a concentration C_{End} of 0.0216 ng/mL.

The residue was calculated with:

R = C_{End} x [(V_{Ex} x V_{End}) / (V_1 x V_{sample})]
= C_{End} x Multiplier M
= 0.0216 ng/mL x [(10 mL x 1.0 mL) / (0.50 mL x 10 mL)]
= 0.0216 ng/mL x 2 = 0.0432 ng/mL = 0.0432 µg/L)

Recoveries (Rec.) were calculated for the fortified specimen as follows:

Rec. = (R / $R_{fortified}$) x 100 % = (0.0432 µg/L / 0.050 µg/L) x 100 %
= 86 %

3. METHOD

3.1 Extraction Procedure for Surface and Ground Water

1. Weigh 10 g (equivalent with 10 mL) of surface or ground in a 50 mL centrifuge tube.
2. Accurately fortify recovery control specimens with fortification solutions. For LOQ recoveries pipette 50 µL of the 0.010 µg/mL fortification solution containing NF-180 and QP-1-7. For the higher-level recoveries, add 50 µL of the 0.10 µg/mL fortification solution containing NF-180 and QP-1-7.
3. Add 10 mL of acetonitrile and shake vigorously for 1 minute.
4. Add contents of Citrate Extraction Tube* (Supelco, 55227-U). Shake vigorously for 1 minute and centrifuge for 5 minutes at 4000 rpm.
5. Dilute the extract (acetonitrile layer) by dilution factor (DF 2) i.e. by mix 500 µL of acetonitrile layer with 500 µL milli-pore water to obtain a 1.0 mL extract in acetonitrile/water (1/1, v/v).

*: Citrate extraction tubes (12 ml) containing 4 g $MgSO_4$, 1 g NaCl, 0.5 g NaCitrate dibasic sesquihydrate and 1 g NaCitrate tribasic dehydrate equivalent to QuEChERS[1] (EN 15662:2008 or EN 15662:2009-02) were used for clean-up of extracts.

3.2 Effects of Matrix on Analyte Response

Matrix effects were tested by comparing LC-MS/MS response of a standard in neat solvent with a standard in matrix at 0.025 ng/mL concentration (see Table 3).

No significant matrix (< 20%) effect was observed for NF-180 or QP-1-7 in both surface and ground water matrices. Therefore, calibration standards prepared in solvent (acetonitrile/water (1/1, v/v)) were used to quantify the final extracts.

3.3 Stability in Working Solutions and Final Extracts

Stability of stock solutions in acetonitrile was proven by comparing the areas of 10 ng/mL solutions prepared by diluting the aged and the freshly prepared stock solution.

[1] EN 15662:2009-02: Foods of plant origin –Determination of pesticide residues using GC-MS and/or LC/MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE – QuEChERS-method.

The stock solutions of NF-180 and QP-1-7 prepared in acetonitrile were found to be stable for at least 28 days when stored refrigerated.

Stability of the calibration solutions containing NF-180 and QP-1-7 were proven by comparing the area of original, aged calibration solutions with a dilution made from a freshly prepared stock solution. The calibration solutions prepared in acetonitrile/water (1/1, v/v) were stable for at least 27 days when stored refrigerated. Details concerning the stability of working solutions are shown in Table 4.

Selected final extracts for both water matrices were re-injected after storage for 7 or 9 days under refrigerated conditions to demonstrate stability. These selected final extracts were quantified using newly prepared calibration solutions. The results obtained showed small differences (always less than 20 %) in comparison to the initial analysis. Thus it can be concluded that NF-180 and QP-1-7 are stable in final extracts for at least 7 days under refrigerated storage conditions. Results of re-analysis are given in Table 5 and Table 6.

3.4 Time Required for Analysis

The time required for extraction of 13 samples is about 2 hours. LC-MS/MS analyses can be performed unattended overnight and requires about 8 min per run. Thus, a set of 13 specimens injected interspersed with calibration solution needs about 3-4 hours. Evaluation of the LC-MS/MS results need about 3 hours.
Thus, a complete set of 13 samples can be completed in approximately one and a half working days.

3.5 Extraction Efficiency

In the current study, extraction efficiency was not conducted experimentally because the radiolabeled samples with incurred residues were not available. The determination of extraction efficiency was not a subject/objective of the present study

4. RESULTS AND DISCUSSION

The objective of this study was to develop and validate analytical methods for the determination of NF-180 and its metabolite QP-1-7 in surface water and ground water at a target limit of quantitation (LOQ) of 0.05 µg/L and at 10-fold LOQ (0.50 µg/L) for each analyte and each water type.

4.1 LC-MS/MS Selectivity and Sensitivity

The highly specific LC-MS/MS method used at least two mass transitions for each of the two analytes:

- *For NF-180*: The transition 348 m/z-→ 330 m/z was used for quantitation and transition 348 m/z → 180 m/z was used for confirmation for all matrices.

- *For QP-1-7:* The transition 378 m/z-→ 332 m/z was used for quantitation and transition 378 m/z → 314 m/z was used for confirmation for all matrices.

LC-MS/MS using the commonly available Sciex API6500 + instrument allows the detection of NF-180 and its metabolite QP-1-7 at concentrations as low as 0.0050 ng/mL with 10-µL injections, therefore providing sufficient sensitivity to determine and to confirm residues of both analytes in the extracts.

4.2 Specificity and Limit of Detection

Final specimen extracts were analyzed by the highly specific LC-MS/MS technique, monitoring at least two mass transitions (MRMs) per analyte.

The limits of detection (LOD) are estimated (based on the signal obtained for the lowest calibration) to be 0.0050 ng/mL corresponding to 0.01 µg/L (20 % of the LOQ). No interfering signals > LOD (0.01 µg/L) were detected at the relevant retention times in the blank control specimens.

4.3 Linearity

Linear calibration functions (standard calibrations in solvent) with 1/x weighting were calculated and plotted by regression analysis, using the Analyst software (for calibration curves exemplified see Figure 1 and Figure 2. Calibration functions ranging from 0.0050 to 1.0 ng/mL for both analytes (≥ 5 levels) were established by injecting 10 µL of the calibration solutions in solvent. The correlation coefficients (r) for both analytes were always > 0.99. Calibration data used for water sample sets are shown in Table 7 to Table 10.

4.4 Validation Results for Analytical Method

The method was validated for both water types at the 0.050 µg/L LOQ level and at 10-fold LOQ (0.50 µg/L) level for both NF-180 and QP-1-7.

APPENDIX 5 STUDY PLAN

Study Plan

1.0 Title: Development and Validation of an Analytical Method for the Determination of NF-180 and its Metabolite QP-1-7 in Surface Water

2.0 Study Identification: EAG Laboratories ID: P 4906 G

3.0 Objectives:

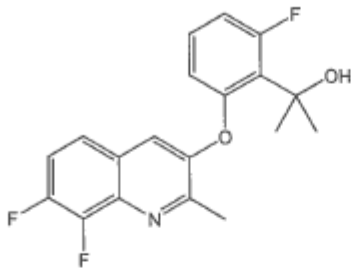
To develop and to validate analytical methods for the determination of NF-180 and its metabolite QP-1-7 in surface water with a target limit of quantitation (LOQ) of 0.05 µg/L for each analyte.

Guidelines/Guidance:

SANCO/825/00 rev. 8.1, 16/11/2010
OECD ENV/JM/MONO(2007)17
US EPA OCSPP 850.6100
US EPA OCSPP 860.1340
German GLP Standards «Grundsätze der Guten Laborpraxis (GLP).
OECD Principles of Good Laboratory Practice.

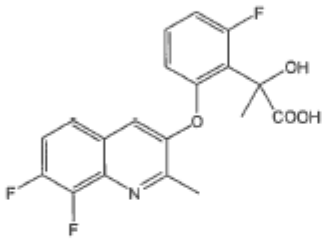
7.0 Analytical Test/Reference Items (Provided by the Sponsor):

7.1 NF-180:



Empirical formula: $C_{19}H_{16}F_3NO_2$ Molar mass: 347.3 g/mol

7.2 **QP-1-7**



Empirical formula: $C_{19}H_{14}F_3NO_4$ Molar mass: 377.3 g/mol

The test/reference item(s) will be supplied by the Sponsor, with relevant information concerning identity, purity, and expiration date. Information on the analytical standard(s) as provided will be included in the final report.

Safety: Usual pre-cautions with chemicals.

8. 0 Test System Description, Application and Study Design:

8.1 Test Systems:

Surface (pond or river) obtained locally. Sampling site of the water type will be reported. Water will be characterized for physical and chemical properties, including pH, conductivity, total hardness, DOC (dissolved organic carbon) and silt content (filtered particles). Some characterization parameters will be performed (non-GLP, DIN/EN methods) in environmental laboratory Institut Alpha (Ulm).

8.2 Method Validation:

Water will be mixed with acetonitrile, filtered, diluted (when necessary) and injected into LC-MS/MS.

If LC-MS/MS is not sensitive enough a pre-concentration step e.g. via liquid/liquid extraction (LLE) or solid phase extraction will be used.

The target limit of detection will be at 20 % of the LOQ.

8.3 Justification, Identification, and Statistical Methods:

A validated method for the determination of residues in water samples is required for the

authorities responsible for environmental and consumer exposure. Solutions, specimens and extracts will be uniquely identified with test facility study ID and individual identifications. Average, relative standard deviation and regression calculations will be used as appropriate.

9.0 Analytical Study Design:

9.1 Assessment of Method Procedures:

Calibrations should extend over a range covering nominal concentrations of the analytes between ≤ 20 % of LOQ and ≥ 120 % of 10xLOQ. 5 or more different standard concentrations will be used to span the required calibration range, a single injection of each standard concentration is sufficient. As much as possible, linear calibration functions will be used rather than quadratic functions which require to be justified by a scientific reason. Plots showing response factors versus the concentration for all calibration points will be reported. LC-MS/MS will be established (spectra, linearity, sensitivity) for the analytes. Matrix effects on response will be demonstrated, matrix-matched standards may be used, if necessary.

9.2 Method Validation:

Fortifications will be performed with diluted solutions of the analytes (Objective: To assess repeatability). Fortifications will be performed to obtain fortification levels at the target LOQ (0.05 µg/L) and at 10xLOQ (0.50 µg/L) for each analyte. The analytes should be fortified together in the samples. Sets of specimens will be processed to obtain the following (specimens per matrix): 1 reagent blank, 2 blank controls, 5 specimens fortified at the LOQ and 5 specimens fortified at the higher fortification level. Two ion transitions will be monitored and evaluated. Results from the validation attempts will be reported to the Sponsor Monitor.

9.3 Acceptance Criteria for Method Validation:

Average recoveries (overall, and per level):	70 % to 120 %.
Individual recoveries	70 % to 120 %.
Relative standard deviation (overall, and per level):	≤ 20 %.
Signal / interference in blank controls:	≤ 20 % of LOQ.

Recoveries and relative standard deviations outside these ranges may be acceptable in certain justified cases, but require approval of the Study Monitor.
If validation criteria are not met, further validation set(s) may become necessary. In this case additional set(s) of specimens may be processed.
Recovery data will be calculated for two ion transitions.

9.4 Stability of Solutions and Extracts:

Stability of selected working solutions and final sample extracts (if applicable) will be assessed after refrigerated storage for at least 5 days.

10.0 Reporting and Evaluation of Data:

The final report will include but not be limited to:
- a description of the analytical method(s) and procedures,
- time required for one sample set,
- calculations (including typical calibration curves, and response factor over concentration

diagrams) and results obtained for fortified and blank control specimens,
- appropriate (product ion) mass spectra of the analyte(s),
- typical chromatograms of calibrations, of fortified specimens and of blank controls/reagent blank,
- a statement with respect to the suitability of the method for the determination of the analyte(s).

A draft report in English language will be prepared for review. The final report will include QAU statement, statement of compliance with GLP and a copy of the GLP certificate.

11.0 Records and Samples to be Maintained:

All data and observations will be recorded on data sheets and/or study files dedicated to this study. All raw data are the property of the Sponsor. The Sponsor (via representative/monitor) will receive a certified unbound copy of the final report and appropriate Word and pdf-A files.

Upon issue of the final report, all raw data, the study plan, any amendments and the original report will be archived in the Test Facility GLP archive for the period required by current German GLP regulations (15 years). The Test Facility will archive sample(s) of the test/reference item(s) and the quality assurance reports for the period required by GLP regulations. No specimens will be archived.

12.0 Quality Assurance:

This study will be conducted under the criteria of "The Principles of Good Laboratory Practice" with regulations in force at study initiation. The test facility's Quality Assurance Unit will monitor the progress, results and the report of the study to assure the compliance with GLP regulations, study plan and standard operating procedures.

13.0 Study Plan Amendments and Deviations:

If modifications of this study plan are deemed necessary, all change(s) with reason(s) will be expressed as written amendment(s), signed and dated by the Study Director, Study Monitor and the QA. Deviations of the study plan will be documented and acknowledged by the Study Director. If there is suspected an important impact on the study, deviations will be reported to the Study Monitor and the Management of the test facility within 3 working days of such signature.