1. Introduction and Summary

1.1 Purpose of Study

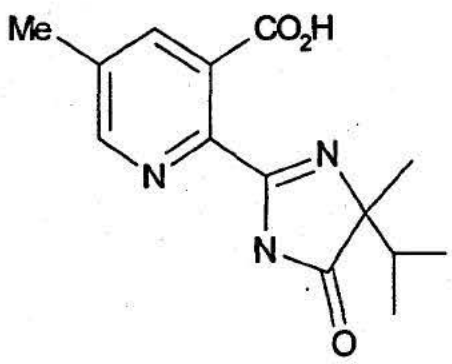
BAS 715 H is a herbicide utilized in a variety of crops. To support the registration of the herbicide and for establishing a procedure to test for and quantitate BAS 715 H and its metabolite CL 354825 in groundwater, a residue analytical method with a limit of quantitation of 0.05 µg/L for the active ingredient and its major metabolite was developed. Method D 0304 allows the determination of BAS 715 H and
CL 354825 with the required limit of quantitation in groundwater. The ability to monitor multiple ions allows this LC MS/MS procedure also acts as a confirmatory technique.

2. Materials/Methods

2.1 Test and Reference Substances

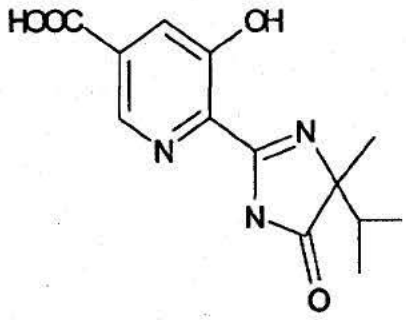
BASF Code Name:	BAS 715 H (CL 263,222)
CAS Number:	81334-60-03
Chemical Name:	Nicotinic acid, 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methyl
Common Name:	Imazapic®
Appearance:	White Powder
Molecular Weight:	275 g/mole
Purity:	99.3%
Lot Number:	AC9918-101/AC10606-119
Stability:	Expected Stable for Five Years
Expiration Date:	2/21/06

Structural Formula:



BASF Code Name:	None
BASF Registry Name:	CL 354825
Chemical Name:	Nicotinic acid, 5-hydroxy-6-(isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)
Common Name:	5-carboxy-3-hydroxy pyridine imidazolinone
Molecular Weight:	277.3 g/mole
Appearance:	Yellow Powder
CAS Number:	None
Lot Number:	AC 9918-101
Purity:	90.0 %
Stability:	Expected Stable for Five Years
Expiration Date:	10/17/06

Structural Formula:



Reference Standards (used for calibration)

Same as fortification compounds (section 2.1

2.2 Standard Solutions

2.2.1 Standard Solution Storage Stability

Standard solutions are kept refrigerated in amber glassware. The storage stability of standard solutions made in methanol and water was established during the course of BASF study number 97799. For this procedure BASF recommends that stock solutions (1 mg/mL in methanol) be made fresh every three months. Dilution of stock solutions should be stored refrigerated no longer than one month or according to their established storage stability in a particular solvent.

2.4.4 Solution Stability

Test and reference substances were refrigerated during their use in this study. Stock solutions (0.5 mg/mL or 1.0 mg/mL) were made fresh every three months and further diluted to a proper concentration. Dilutions of the stock solutions were made fresh every month.

a. Test System

The test system of untreated water samples obtained from trial sites of BASF Study 97799 (Prospective Groundwater Study) conducted in the US was used to validate method D0304.

b. Sample Storage and Handling

The water samples were stored frozen (< -5°C) before use in analysis. All samples were allowed to reach room temperature and shaken to ensure proper homogenization before use.

c. Experimental Design

Control water samples were fortified by applying standard solution directly to the water prior to extraction with the aid of an volumetric pipette. The fortified control samples were analyzed to determine recoveries of BAS 715 H and its metabolite CL354825. The validation sets consisted of two untreated control samples, five control fortified samples at the 0.05 PPB (LOQ) level and five control fortified samples at the 0.5 PPB level. Two analytical sets were completed.

d. Method of Analysis

BASF Analytical Method D0304 was developed to determine the residues of BAS 715 H and its metabolite CL354825 in groundwater matrices using LC MS/MS. The method was designed to complete analyses required for both well water and lysimeter water collected in a prospective groundwater monitoring study.

The LC-MS/MS quantitation was based on the following transitions in the table below:

Analyte	Transitions
BAS 715 H	m/z 276.1 → 231.0
CL 354825	m/z 278.1 → 232.9

4. Protocol Changes

Amendment one eliminated the use of reagent blanks in the study ran concurrently with fortified samples. Because this method was a filter, dilute and inject procedure it was not required. Control samples and solvent blanks included with each analytical set were used and the data included.

5. Conclusion

The study has shown that BASF Method D0304 is adequate for measuring residues of BAS 715 H and its metabolite CL 354825 in groundwater samples at an LOQ of 0.05 PPB.

Figure 1. Flow Diagram of BAS 715 H and CL 354825 Analytical Procedure

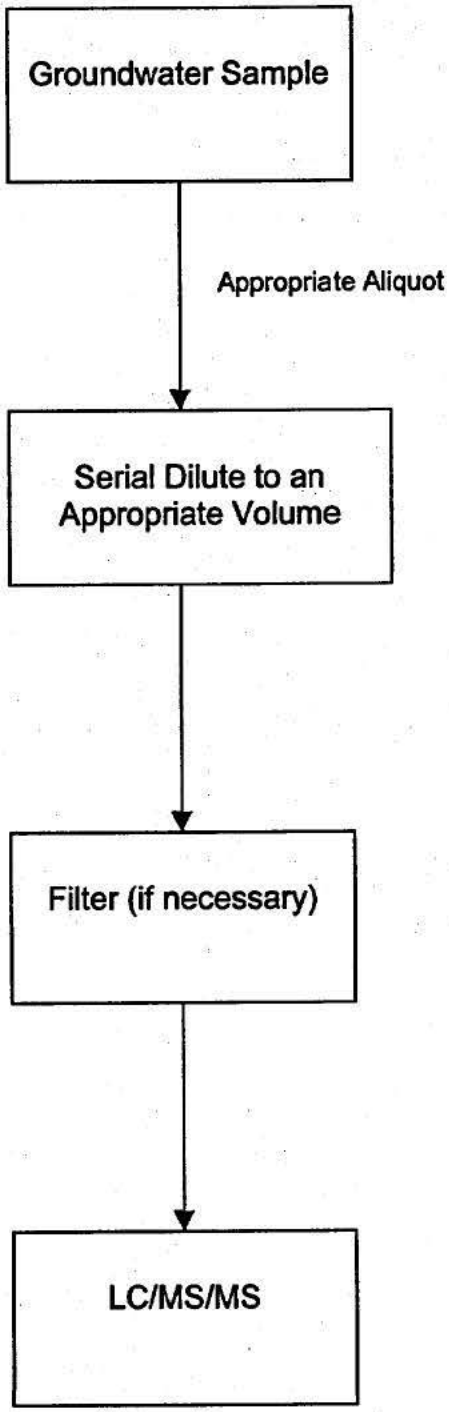


Figure 2. Sample Calculation of Recovery of Sample A121737-03 : A 0.05 PPB BAS 715 H/CL 354825 Procedural Fortification Sample.

BASF Sample Number A121737-03, 0.05 PPB Procedural Fortification of BAS 715 H.

pg= value interpolated from the standard curve.
BAS715 H: Intercept = 470.0 Slope = 750.0

BAS 715 H Calculation:

$$\text{Standard Curve} = \frac{\text{Peak area} - (\text{Intercept})}{\text{Slope}} = \frac{3386.0 - (470.0)}{750.0} = \mathbf{3.89\ pg}$$

$$\text{PPB} = \frac{\text{pg (curve)} \times \text{Final Volume}}{\text{Wt.} \times \text{inj Vol.}} \times \frac{1000\ \text{uL}}{1\ \text{mL}} \times \frac{1\ \text{ng}}{1000\ \text{pg}}$$

$$\text{PPB} = \frac{(3.89\ \text{pg})(5\ \text{mL})}{(4\ \text{mL})(100\ \text{uL})} \times \frac{1000\ \text{uL}}{1\ \text{mL}} \times \frac{1\ \text{ng}}{1000\ \text{pg}} = \mathbf{0.04863\ ng/mL}$$

$$\text{Percent (\%) Recovery} = \frac{\text{Amount (PPB) detected}}{\text{Amount Fortified(PPB)}} = \frac{0.04863}{0.0500} = \mathbf{97.3\ \%}$$

BASF Sample Number A121737-03, 0.05 PPB Procedural Fortification of CL 354825

pg = value interpolated from standard curve
CL 354825 Intercept: -689.0 Slope = 1230.0

CL 354825 Calculation

$$\text{Standard Curve} = \frac{\text{Peak Area} - (\text{Intercept})}{\text{Slope}} = \frac{3737 - (-689)}{1230.0} = \mathbf{3.60\ pg}$$

$$\text{PPB} = \frac{\text{pg (curve)} \times \text{Final Volume}}{\text{Wt.} \times \text{inj Vol.}} \times \frac{1000\ \text{uL}}{1\ \text{mL}} \times \frac{1\ \text{ng}}{1000\ \text{pg}}$$

$$\text{PPB} = \frac{(3.60\ \text{pg})(5\ \text{mL})}{(4\ \text{mL})(100\ \text{uL})} \times \frac{1000\ \text{uL}}{1\ \text{mL}} \times \frac{1\ \text{ng}}{1000\ \text{pg}} = \mathbf{0.0450\ ng/mL}$$

$$\text{Percent (\%) Recovery} = \frac{\text{Amount (PPB) detected}}{\text{Amount Fortified(PPB)}} = \frac{0.0450}{0.0500} = \mathbf{90.0\ \%}$$