BASF Corporation

Agricultural Products Division RTP, NC 27709

BASF
The Chemical Company

Final Study Report:

Method Validation of BASF Analytical Method D0203 entitled:

"Method for Determination of Pendimethalin (BAS 455 H) and its Metabolite CL 202347 Residues in Wheat Forage, Hay, Grain and Straw using LC/MS/MS"

BASF Study Number: 198172

Data Requirement:

EPA Residue Chemistry Test Guidelines, OPPTS 860.1340 Residue Analytical Method and SANCO 825/00 rev. 6 – EU Guideline on Residue Analytical Method

Study Director/Author:

Jane Stewart

Study Completion Date:

June 18, 2004

Testing Facility/Performing Laboratory:

BASF Agro Research
26 Davis Drive
Research Triangle Park, NC 27709

This report consists of 75 pages.

BASF Registration Document No. 2004/5000470

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10[d][1][A], [B], or [C]. This claim specifically supercedes any claim or implication of confidentiality contained in this document.

Company: BASF Corporation,
BASF Agro Research
P.O. Box 13528
Research Triangle Park, NC 27709-3528

Company Agent: Rodney Akers

_____ _____

Rodney Akers Date
Global Regulatory Affairs

GOOD LABORATORY COMPLIANCE STATEMENT

The study meets the requirements of 40 CFR part 160, FIFRA Good Laboratory Practices.

Submitter: _____

Sponsor: Samy Abdel-Baky

Study Director: J. A. Stewart

QUALITY ASSURANCE UNIT STATEMENT

Study Number: 198172

Name/Number of Test Substance: BAS 455 H and CL 202347

Type of Study: Method Validation

The quality assurance unit of the testing facility has inspected the study and/or audited the final report and reported the results of these inspections to the study director and management.

Date of Inspection	Date Reported to Study Director and Management
March 17, 2004	March 17, 2004
March 24, 2004	March 24, 2004
March 30, 2004	March 30, 2004
June 10, 2004	June 10, 2004

Signature QAU:

CERTIFICATION

We, the undersigned, hereby declare that this study was performed under our supervision according to the procedure described herein, and that this report provides a true and accurate record of the results obtained.

Author/
Study
Director: J. Stewart

Jane Stewart
BASF Corporation

6/18/04
Study Completion Date

Approved By: Samy Abdel-Baky

Samy Abdel-Baky
Group Leader Environmental and Residue Sciences
BASF Corporation (APC)
(919) 547-2695

6-16-2004
Date:

**Method Validation of BASF Analytical Method D0203 entitled:
"Method for Determination of Pendimethalin (BAS 455 H) and its Metabolite CL 202347 Residues in Wheat Forage, Hay, Grain and Straw using LC/MS/MS"**

Abstract

BASF Method D0203, entitled, "Method for Determination of Pendimethalin (BAS 455 H) and its Metabolite CL 202347 Residues in Wheat Forage, Hay, Grain and Straw using LC/MS/MS" was developed to identify the residues of BASF herbicide BAS 455 H in wheat matrices.

Untreated wheat forage, hay, grain and straw samples were fortified with BAS 455 H and metabolite CL 202347 at the limit of quantitation of the method (0.05 ppm for each compound) and a high fortification level of 0.5 ppm for each compound. The following analyses were conducted for each matrix: one control sample, five fortification replicates at the limit of quantitation for each of the test compounds and five fortification replicates at the higher fortification level for each of the test compounds. In addition, two reagent blanks were analyzed using the extraction procedure for grain, and two reagent blanks were analyzed using the extraction procedure for forage, hay and straw.

The average recoveries for all matrices over all fortification levels for BAS 455 H and CL 202347, respectively, were 88% (%RSD = 11) and 94% (%RSD = 12). Average recoveries for each matrix are summarized in the following table.

Analyte	Matrix	No. of Analyses	Average Recovery (%)	Std Dev.	%RSD
BAS 455 H	Forage	9	81	8	10
	Grain	10	94	8	8
	Hay	10	84	11	14
	Straw	10	92	10	10
	Overall	**39**	**88**	**10**	**12**
CL202347	Forage	10	92	7	8
	Grain	10	107	7	7
	Hay	9	83	10	12
	Straw	10	93	7	7
	Overall	**39**	**94**	**11**	**12**

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CL 202347 Analysis

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GENERAL INFORMATION

BASF Registration Document Number:	2004/5000470
BASF Study Number:	198172
Study Title:	Method Validation of BASF Analytical Method D0203 entitled: "Method for Determination of Pendimethalin (BAS 455 H) and its Metabolite CL 202347 Residues in Wheat Forage, Hay, Grain and Straw using LC/MS/MS"
Test Substances:	
Common Name:	BAS 455 H
Chemical Name:	N-(1-ethylpropyl)-3,-4-dimethyl-2,6-dinitrobenzenamine
Common Name:	CL 202347
Chemical Name:	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitrobenyl alcohol
Sponsor Facility:	BASF Corporation 26 Davis Drive Research Triangle Park, NC 27709
Study Director:	Jane Stewart
Analyst:	John Billotte
Supervisor:	Samy Abdel-Baky
Testing Facility/Performing Laboratory:	BASF Agro Research P.O. Box 13528 26 Davis Drive Research Triangle Park, NC 27709
Study Dates	
Study Initiation Date:	March 18, 2004
Experimental Start Date:	March 23, 2004
Experimental Completion Date:	March 30, 2003

I. INTRODUCTION

A. Purpose of Study

This study was conducted to fulfill EPA Residue Chemistry Test Guidelines, OPPTS 860.1340 Residue Analytical Method and SANCO 825/00 rev. 6 – EU Guideline on Residue Analytical Method. This study was conducted to validate the suitability of Method D0203 for residue analysis.

II. MATERIALS / METHODS

A. Test and Reference Substance:

BAS Code:	**BAS 455 H**
Common Name :	Pendimethalin
Chemical Name	N-(1-ethylpropyl)-3,-4-dimethyl-2,6-dinitrobenzenamine
Reg. No.	900072
Lot Number	AC 12251-83
Purity	99.2%
Exp Date	September 2006

BAS Code	**CL 202347**
Common Name :	5-carboxy-3-hydroxy pyridine imidazolinone
Chemical Name	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitrobenyl alcohol
Reg. No.	4110480
Lot Number	AC 12251-39
Purity	84%
Exp Date	July 2006

The test/reference substances were maintained frozen (<-5° C to <-15° C) until their use in this study. Characterization and purity were determined prior to the substances being used in this study. Details of these determinations are available to BASF and are located at Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany.

The test/reference substances were used in the study to generate data for both instrument and method performance. Quantitation of all samples was achieved using calibration curves calculated by linear regression of instrument responses for the reference substance. The performance of the instrument was evaluated during each injection set. The correlation coefficient of the calibration curve was required to be at or above 0.99.

Test substance solutions for fortifications were refrigerated during their use in this study. BAS 455 H and CL 202347 stock solutions were made fresh in methanol. Mixed fortification solutions of BAS 455 H and CL 202347 were made fresh from a mixed 50 μg/mL standard solution by diluting with methanol.

Reference substance solutions for instrument calibration were refrigerated during their use in this study. Mixed calibration solutions of BAS 455 H and CL 202347 were made fresh from the mixed 0.5 μg/mL fortification solution by diluting in 50/50 MeOH/H_2O.

B. Test System

Untreated wheat forage, hay, grain and straw were obtained from a wheat RAC study (study 82605, Reference 1) for use in this study. Method D0203 was developed for the analysis of residues of BAS 455 H and its metabolite CL 202347 in wheat matrices.

C. Identification of Samples

Untreated wheat samples were obtained from BASF Agro Research Study Number 82605. In the earlier study, the control samples were homogenized and then stored frozen in plastic bags. For this study, the wheat samples were assigned unique residue sample numbers (RSNs): 2004001-1 through 2004001-4.

Each analysis set was uniquely identified with a Master Sheet Number, which consisted of the study number plus a unique number (e.g., 198172-01).

D. Method of Analysis

Fortified wheat samples were analyzed using BASF Analytical Method D0203. A minor modification was made to the centrifuge step (Step 3.2c of the method) that resulted in a rewording of that step to allow a broader range of centrifuge speeds. In all other aspects, the method was followed as written. A brief description of the methodology follows.

Residues of BAS 455 H and its metabolite CL 202347 are extracted from green and dry plant with acidic aqueous methanol and from grain with 10% methanol in dichloromethane. An aliquot is taken from the extract and cleaned using a PolarPlus® C18 Speedisk® solid phase extraction column. The compounds are eluted out of the Speedisk® column using methanol/water. The eluate is diluted with water for analysis via LC/MS/MS. The results are calculated as BAS 455 H and CL 202347 by direct comparison of the sample peak responses to those of external standards. The limit of quantitation of the method for each analyte in all four matrices is 0.05 ppm. The method is attached in Appendix A.

Equations used to calculate residues and recoveries are included in the method. Example calculations are included in Appendix B.

E. Validation of Method

To validate the method, for each matrix, one control sample and five fortification samples at the limit of quantitation (0.05 ppm) and five fortification samples at ten times the limit of quantitation (0.5 ppm) were analyzed for each of the compounds. The method is validated, as per EPA Pesticide Residue Guidelines, when the average recovery at each fortification level is between 70 and 120%, the coefficients of variation are less than 20%, the control values are less than 20% of the LOQ and the correlation coefficient of the standards is at least 0.99.

III. RESULTS

The results of the procedural recoveries for BAS 455 H and CL 202347 are given in the following table.

Table 1. Summary of Results

Matrix	Analyte	Fortification Level (ppm)	Recovery %	Average	Stand. Dev.	Coeff. of Var.[1] (%RSD)
Wheat Forage	BAS 455 H	0.05	89, 75, 84, 80, 92	84	7	8
		0.5	84, 54[2], 67, 82, 74	77	8	10
		Overall (n=9)		81	8	10
	CL 202347	0.05	88, 93, 81, 96, 94	90	6	7
		0.5	83, 99, 90, 104, 90	93	8	9
		Overall (n=10)		92	7	8
Wheat Grain	BAS 455 H	0.05	80, 89, 93, 91, 89	88	5	6
		0.5	97, 100, 107, 101, 90	99	6	6
		Overall (n=10)		94	8	8
	CL 202347	0.05	102, 109, 101, 95, 115	104	8	7
		0.5	111, 100, 111, 116, 105	109	6	6
		Overall (n=10)		107	7	7
Wheat Hay	BAS 455 H	0.05	72, 73, 71, 74, 82	74	4	6
		0.5	101, 85, 88, 87, 102	93	8	9
		Overall (n=10)		84	11	14
	CL 202347	0.05	73, 71, 286[2], 74, 78	74	3	4
		0.5	82, 86, 89, 95, 100	90	7	8
		Overall (n=9)		83	10	12
Wheat Straw	BAS 455 H	0.05	94, 88, 79, 80, 106	89	11	12
		0.5	97, 90, 106, 84, 93	94	8	9
		Overall (n=10)		92	10	10
	CL 202347	0.05	92, 87, 92, 87, 81	88	5	5
		0.5	100, 98, 103, 94, 96	98	3	4
		Overall (n=10)		93	7	7
All Matrices	BAS 455 H (n = 39)			88	10	12
	CL 202347 (n = 39)			94	11	12

[1]Coefficient of Variation (%RSD) = (Standard Deviation ÷ Average Recovery) x 100

[2]These recovery values are considered outliers and are not included in the averages or standard deviations.

There were no residues detected in the control samples above the limit of detection.

Standards used for fortification and calibration were stored no longer than the one month period specified in the method.

Statistical treatment of the data included determinations of averages, standard deviation and coefficients of variation for the procedural recoveries. Sample calculations are included in Appendix B.

IV. DISCUSSION / CONCLUSIONS

BASF Analytical Method D0203 was successfully validated for wheat matrices. Average recoveries over both fortification levels ranged from 81 to 94% for BAS 455 H and 83 to 107% for CL202347. Coefficients of variation (%RSD) for the average recoveries were all less than 15%. There were no residues detected in the control samples above the limit of detection.

Average recoveries at the limit of quantitation (0.05 ppm) for BAS 455 H ranged from 74% in wheat hay to 89% in wheat straw. Average recoveries at 0.5 ppm for BAS 455 H ranged from 77% in forage to 99% in grain. Coefficients of variation at each fortification level (%RSD) were all less than 15% for BAS 455 H.

Average recoveries at the limit of quantitation (0.05 ppm) for CL 202347 ranged from 74% in hay to 104% in grain. Average recoveries at 0.5 ppm for CL 202347 ranged from 90% in hay to 109% in grain. Coefficients of variation at each fortification level (%RSD) were all less than 10% for CL 202347.

Individual recoveries ranged from 67 to 107% for BAS 455 H and 71 to 116% for CL 202347. Two laboratory outliers of 54% recovery of BAS 455 H and 286% recovery of CL 202347 were not included in the statistical analysis.

The method took one analyst eight hours in the laboratory to analyze a set of 12 samples. LC/MS/MS injection was performed overnight.

There were two protocol changes for this study. These changes are described in Appendix C.

Several measures were taken to ensure the quality of the study results. The quality assurance unit at BASF inspected the analytical procedures for compliance with Good Laboratory Practices that included adherence to the protocol. The dates inspected are detailed in the quality assurance unit statement. Study samples and test and reference substances were maintained in secured (i.e. pad-locked) storage with limited access. Freezer temperatures were continuously monitored by electronic means. The final report, protocol, and raw data pertaining to this study are maintained at the BASF Agro Research, 26 Davis Drive, Research Triangle Park, North Carolina.

V. REFERENCES

1. Johnston, R. “Magnitude of BAS 455 H Residues in Wheat”. Reg Doc No. 2003/5000293. 2004.

Table 2. Individual Data for Recoveries of BAS 455 H and CL 202347 in Forage

Fortification Level (ppm)	Filename (index)[1]	Final Volume (mL)	BAS 455 H			CL 202347		
			Peak Area[2]	Net Residue[3] (ppm)	Recovery %	Peak Area	Net Residue[3] (ppm)	Recovery %
Control	040330.wiff(6)	5.0	ND	<0.0125	N/A	ND	<0.0125	N/A
0.05	040330.wiff(7)	5.0	3300	0.045	89	1600	0.044	88
0.05	040330.wiff(9)	5.0	2800	0.038	75	1710	0.047	93
0.05	040330.wiff(10)	5.0	3130	0.042	84	1480	0.041	81
0.05	040330.wiff(11)	5.0	2970	0.040	80	1760	0.048	96
0.05	040330.wiff(14)	5.0	3410	0.046	92	1720	0.047	94
0.5	040330.wiff(15)	50.0	3110	0.42	84	1510	0.41	83
0.5	040330.wiff(16)	50.0	2060	0.27	54[5]	1830	0.50	99
0.5	040330.wiff(19)	50.0	2510	0.34	67	1640	0.45	90
0.5	040330.wiff(20)	50.0	3040	0.41	82	1920	0.52	104
0.5	040330.wiff(21)	50.0	2740	0.37	74	1660	0.45	90
R.Blank[4]	040330.wiff(24)	5.0	ND	--	N/A	ND	--	N/A
R.Blank[4]	040330.wiff(25)	5.0	ND	--	N/A	ND	--	N/A

[1]The filename and index are assigned by the chromatography data system to uniquely identify the samples and standards injected.

[2]N.D. = Nondetect

[3]Net Residue = Residue in Fortified Sample - Residue in Control. There was no control residue detected in the control sample of this matrix.

[4]R. Blank = Reagent Blank

[5]This recovery value is considered an outlier and is not included in the average or standard deviation.

The following data were constant for all samples:

Master sheet = 198172-01
Sample weight (W_s) = 5.0 g
Aliquot (AF) = 0.01
Injection volume (V_i) = 10 μL

Table 3. Individual Data for Recoveries of BAS 455 H and CL 202347 in Hay

Fortification Level (ppm)	Filename (index)[1]	Final Volume (mL)	BAS 455 H			CL 202347		
			Peak Area[2]	Net Residue[3] (ppm)	Recovery %	Peak Area	Net Residue[3] (ppm)	Recovery %
Control	040324.wiff(29)	5.0	ND	<0.0125	N/A	ND	<0.0125	N/A
0.05	040324.wiff(30)	5.0	2880	0.036	72	1530	0.037	73
0.05	040324.wiff(33)	5.0	2920	0.036	73	1490	0.036	71
0.05	040324.wiff(34)	5.0	2860	0.036	71	6050	0.143	286[5]
0.05	040324.wiff(35)	5.0	2990	0.037	74	1550	0.037	74
0.05	040324.wiff(38)	5.0	3300	0.041	82	1630	0.039	78
0.5	040324.wiff(39)	50.0	4050	0.50	101	1730	0.41	82
0.5	040324.wiff(40)	50.0	3410	0.42	85	1810	0.43	86
0.5	040324.wiff(43)	50.0	3540	0.44	88	1880	0.45	89
0.5	040324.wiff(44)	50.0	3480	0.43	87	1990	0.47	95
0.5	040324.wiff(45)	50.0	4110	0.51	102	2100	0.50	100

[1]The filename and index are assigned by the chromatography data system to uniquely identify the samples and standards injected.

[2]N.D. = Nondetect

[3]Net Residue = Residue in Fortified Sample - Residue in Control. There was no control residue detected in the control sample of this matrix.

[4]R. Blank = Reagent Blank

[5]This recovery value is considered an outlier and is not included in the average or standard deviation.

The following data were constant for all samples:

Master sheet = 198172-21
Sample weight (W_s) = 5.0 g
Aliquot (AF) = 0.01
Injection volume (V_I) = 10 μL

Table 4. Individual Data for Recoveries of BAS 455 H and CL 202347 in Grain

Fortification Level (ppm)	Filename (index)[1]	Final Volume (mL)	BAS 455 H			CL 202347		
			Peak Area[2]	Net Residue[3] (ppm)	Recovery %	Peak Area	Net Residue[3] (ppm)	Recovery %
Control	040330a.wiff(6)	5.0	ND	<0.0125	N/A	ND	<0.0125	N/A
0.05	040330a.wiff(7)	5.0	2520	0.040	80	2010	0.051	102
0.05	040330a.wiff(9)	5.0	2830	0.045	89	2150	0.054	109
0.05	040330a.wiff(10)	5.0	2940	0.046	93	2000	0.051	101
0.05	040330a.wiff(11)	5.0	2900	0.046	91	1870	0.048	95
0.05	040330a.wiff(14)	5.0	2830	0.045	89	2280	0.058	115
0.5	040330a.wiff(15)	50.0	3090	0.49	97	2190	0.55	111
0.5	040330a.wiff(16)	50.0	3180	0.50	100	1970	0.50	100
0.5	040330a.wiff(19)	50.0	3390	0.53	107	2190	0.55	111
0.5	040330a.wiff(20)	50.0	3190	0.50	101	2290	0.58	116
0.5	040330a.wiff(21)	50.0	2870	0.45	90	2070	0.53	105
R.Blank[4]	040330a.wiff(24)	5.0	ND	--	N/A	ND	--	N/A
R.Blank[4]	040330a.wiff(26)	5.0	ND	--	N/A	ND	--	N/A

[1]The filename and index are assigned by the chromatography data system to uniquely identify the samples and standards injected.

[2]N.D. = Nondetect

[3]Net Residue = Residue in Fortified Sample - Residue in Control. There was no control residue detected in the control sample of this matrix.

[4]R. Blank = Reagent Blank

The following data were constant for all samples:

Master sheet = 198172-11
Sample weight (W_s) = 5.0 g
Aliquot (AF) = 0.01
Injection volume (V_i) = 10 µL

Table 5. Individual Data for Recoveries of BAS 455 H and CL 202347 in Straw

Fortification Level (ppm)	Filename (index)[1]	Final Volume (mL)	BAS 455 H			CL 202347		
			Peak Area[2]	Net Residue[3] (ppm)	Recovery %	Peak Area	Net Residue[3] (ppm)	Recovery %
Control	040324.wiff(29)	5.0	ND	<0.0125	N/A	ND	<0.0125	N/A
0.05	040324.wiff(30)	5.0	2860	0.047	94	1470	0.046	92
0.05	040324.wiff(33)	5.0	2860	0.044	88	1380	0.044	87
0.05	040324.wiff(34)	5.0	2450	0.040	79	1460	0.046	92
0.05	040324.wiff(35)	5.0	2470	0.040	80	1380	0.044	87
0.05	040324.wiff(38)	5.0	3170	0.053	106	1280	0.041	81
0.5	040324.wiff(39)	50.0	2940	0.49	97	1620	0.50	100
0.5	040324.wiff(40)	50.0	2760	0.45	90	1570	0.49	98
0.5	040324.wiff(43)	50.0	3180	0.53	106	1650	0.51	103
0.5	040324.wiff(44)	50.0	2580	0.42	84	1490	0.47	94
0.5	040324.wiff(45)	50.0	2820	0.46	93	1540	0.48	96

[1]The filename and index are assigned by the chromatography data system to uniquely identify the samples and standards injected.

[2]N.D. = Nondetect

[3]Net Residue = Residue in Fortified Sample - Residue in Control. There was no control residue detected in the control sample of this matrix.

[4]R. Blank = Reagent Blank

The following data were constant for all samples:

Master sheet = 198172-32
Sample weight (W_s) = 5.0 g
Aliquot (AF) = 0.01
Injection volume (V_i) = 10 µL

Table 6. Summary of Standard Data

Master Sheet Number (198172-)	Analyte	Peak Area				Calibration Curve Data[1]		
		2.5 pg / 10 µL	5.0 pg / 10 µL	10.0 pg / 10 µL	20.0 pg / 10 µL	Slope	Intercept	Corr. Coeff.[2]
01	BAS 455 H	2110 2120 1760	4110 3850 3420	7120 7060 6640	14300 14500 14800	714	117	0.9982
	CL 202347	846 990 1020	2020 1730 1720	3930 3730 3350	7730 7510 7900	388	-98	0.9976
11	BAS 455 H	1560 1370 1740	2620 3330 3560	5620 6900 6690	11400 13300 13500	641	-29.8	0.9905
	CL 202347	928 976 1160	2140 1940 1790	3550 4280 3920	8100 8380 8170	413	-91.5	0.9974
21	BAS 455 H	1910 2100 2310	4130 3900 4110	6800 8170 8570	16300 16300	809	-23.8	0.9962
	CL 202347	1060 950 1070	2060 1980 2180	4680 4130 4100	8770 8140	426	-25.1	0.9969
32	BAS 455 H	1380 1960 1580	3210 3060 2590	6030 5510 6050	10600 11400	539	321	0.9961
	CL 202347	921 889 812	1470 1590 1550	3040 3220 3410	7350 6770	356	-170	0.9958

[1]The standard curves were constructed using the following equation:

$$\text{Analyte (pg)} = \frac{\text{Peak Area - Intercept}}{\text{Slope}}$$

[2]Corr. Coeff. = Correlation coefficient

Figure 1. Sample Standard Chromatogram for BAS 455 H, 0.25 ng/mL (2.5 pg) Master sheet 198172-01: Filename 040330.wiff(1)

Sample Name: "ERS-04-214" Sample ID: "2.5pg Standard" File: "040330.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "ST BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	1	
Sample Type:	Standard	
Concentration:	2.50	
Acq. Date:	03/30/04	
Acq. Time:	09:33:47 AM	
Modified:	Yes	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.75	min
Area:	2110.	counts
Height:	4.58e+002	cps
Start Time:	1.61	min
End Time:	1.93	min

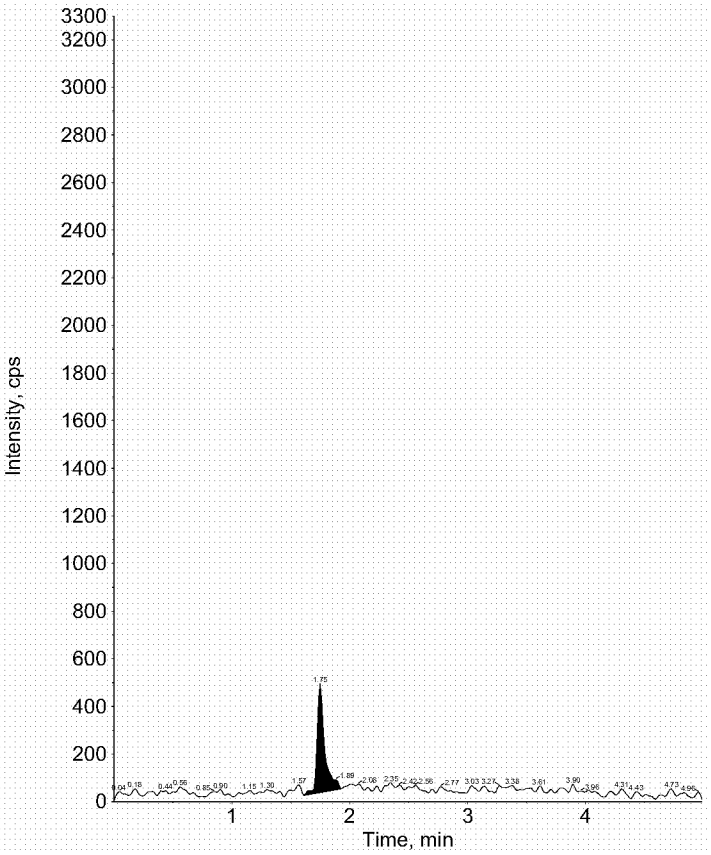


Figure 2. Sample Standard Chromatogram for BAS 455 H, 0.50 ng/mL (5.0 pg) Master sheet 198172-01: Filename 040330.wiff(2)

Sample Name: "ERS-04-213" Sample ID: "5.0pg Standard" File: "040330.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "ST BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	2	
Sample Type:	Standard	
Concentration:	5.00	
Acq. Date:	03/30/04	
Acq. Time:	09:39:28 AM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	30.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.77	min
Area:	4110.	counts
Height:	8.31e+002	cps
Start Time:	1.60	min
End Time:	2.01	min

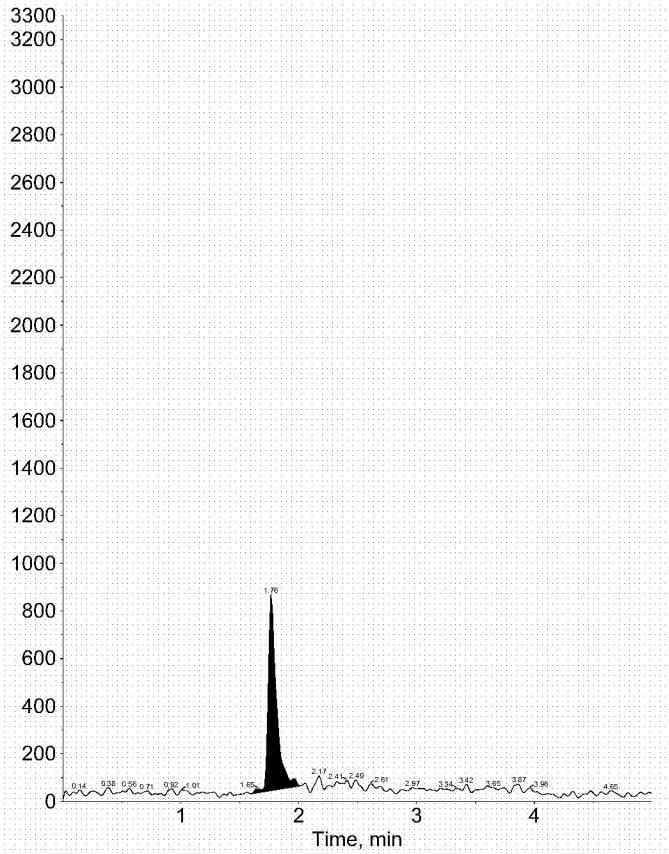


Figure 3. Sample Standard Chromatogram for BAS 455 H, 1.0 ng/mL (10 pg) Master sheet 198172-01: Filename 040330.wiff(3)

Sample Name: "ERS-04-212" Sample ID: "10.0pg Standard" File: "040330.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "ST BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	3	
Sample Type:	Standard	
Concentration:	10.0	
Acq. Date:	03/30/04	
Acq. Time:	09:45:14 AM	
Modified:	Yes	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.76	min
Area:	7120.	counts
Height:	1.50e+003	cps
Start Time:	1.68	min
End Time:	2.04	min

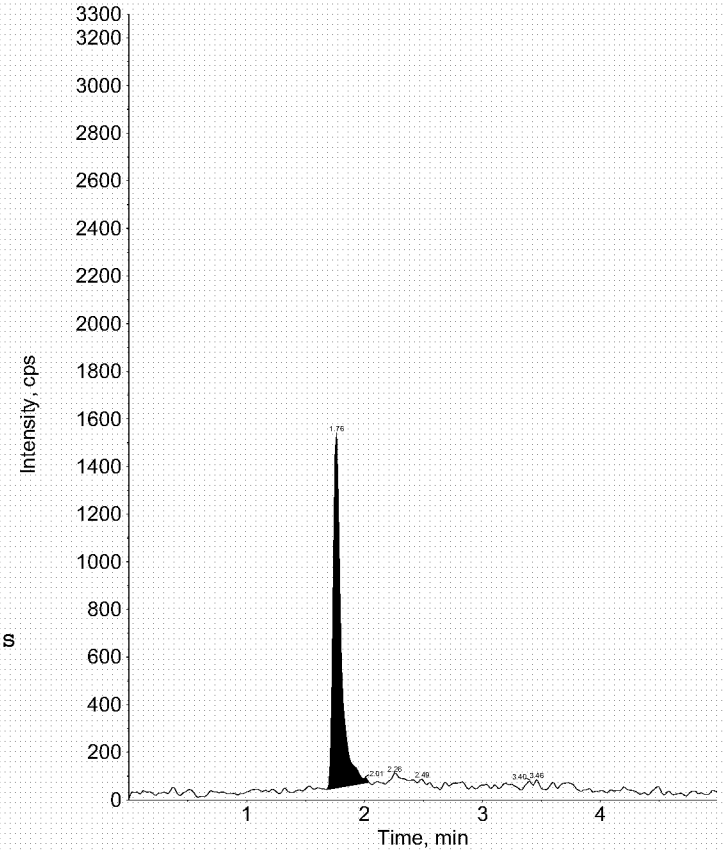


Figure 4. Sample Standard Chromatogram for BAS 455 H, 2.0 ng/mL (20 pg) Master sheet 198172-01: Filename 040330.wiff(4)

Sample Name: "ERS-04-211" Sample ID: "20.0pg Standard" File: "040330.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "ST BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	4	
Sample Type:	Standard	
Concentration:	20.0	
Acq. Date:	03/30/04	
Acq. Time:	09:50:59 AM	
Modified:	Yes	
Bunching Factor:	2	
Noise Threshold:	80.00	
Area Threshold:	600.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.76	min
Area:	14300.	counts
Height:	3.26e+003	cps
Start Time:	1.68	min
End Time:	1.95	min

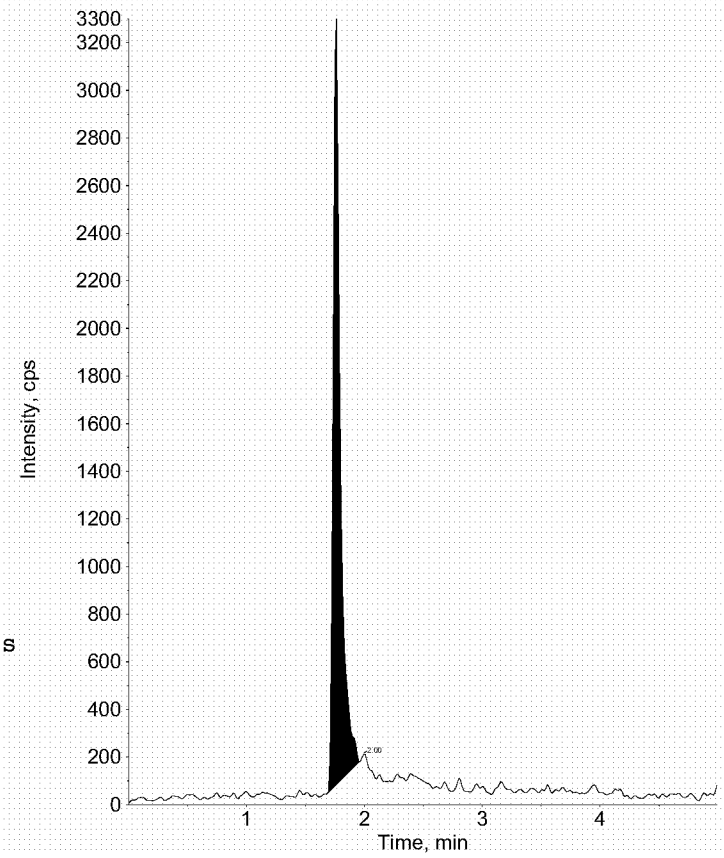


Figure 5. Representative Standard Curve for BAS 455 H
Master sheet 198172-01

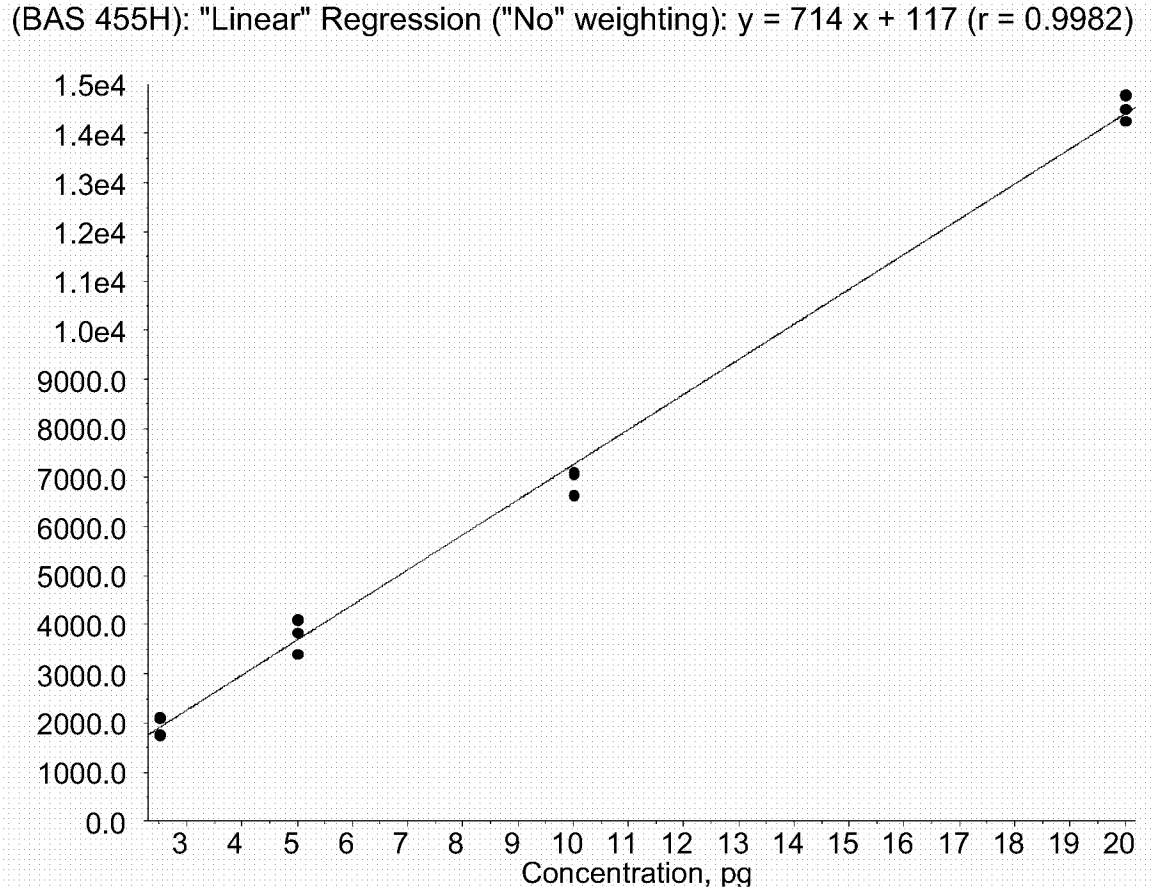


Figure 6. Representative Control Sample for Wheat Grain (BAS 455 H Analysis) Master sheet 198172-11: Filename 040330a.wiff(6)

Sample Name: "2004001-3" Sample ID: "qc" File: "040330a.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-11 Wheat Grain JCB" Annotation: ""

Sample Index:	6	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	09:52:42 PM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	30.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	No Peak	
Retention Time:	N/A	min
Area:	N/A	counts
Height:	N/A	cps
Start Time:	N/A	min
End Time:	N/A	min

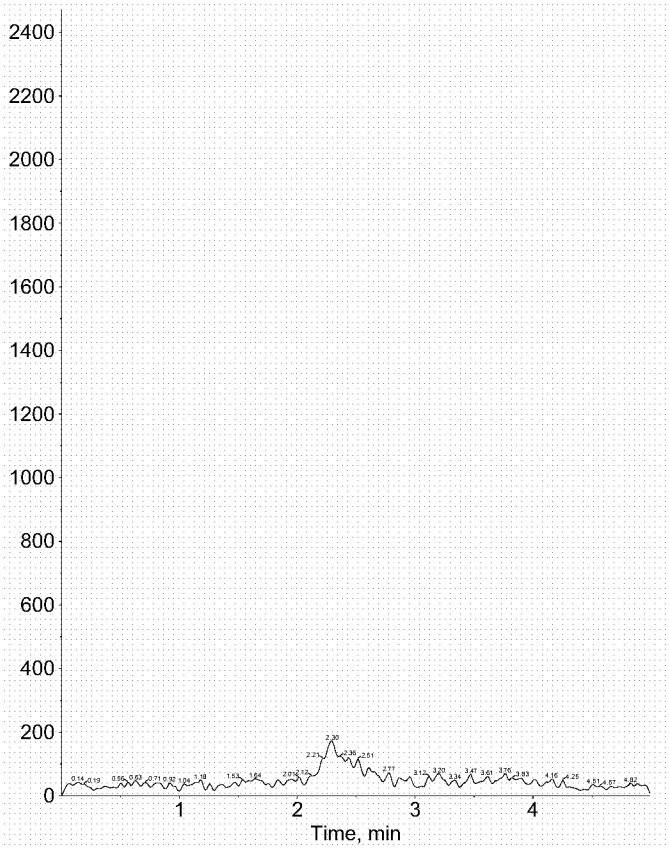


Figure 7. Representative 0.05 ppm Fortification (LOQ) Sample for Wheat Grain (BAS 455 H Analysis)
Master sheet 198172-11: Filename 040330a.wiff(10)

Sample Name: "2004001-3" Sample ID: "low fort" File: "040330a.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-11 Wheat Grain JCB" Annotation: ""

Sample Index:	10
Sample Type:	QC
Concentration:	0.00
Acq. Date:	03/30/04
Acq. Time:	10:15:39 PM
Modified:	No
Bunching Factor:	2
Noise Threshold:	30.00
Area Threshold:	300.00
Num. Smooths:	2
RT Window:	30.0 sec
Expected RT:	1.91 min
Sep. Width:	0.20
Sep. Height:	0.01
Exp. Peak Ratio:	5.00
Exp. Adj. Ratio:	4.00
Exp. Val. Ratio:	3.00
Use Relative RT:	No
Int. Type:	Base To Base
Retention Time:	1.76 min
Area:	2940. counts
Height:	6.35e+002 cps
Start Time:	1.68 min
End Time:	1.93 min

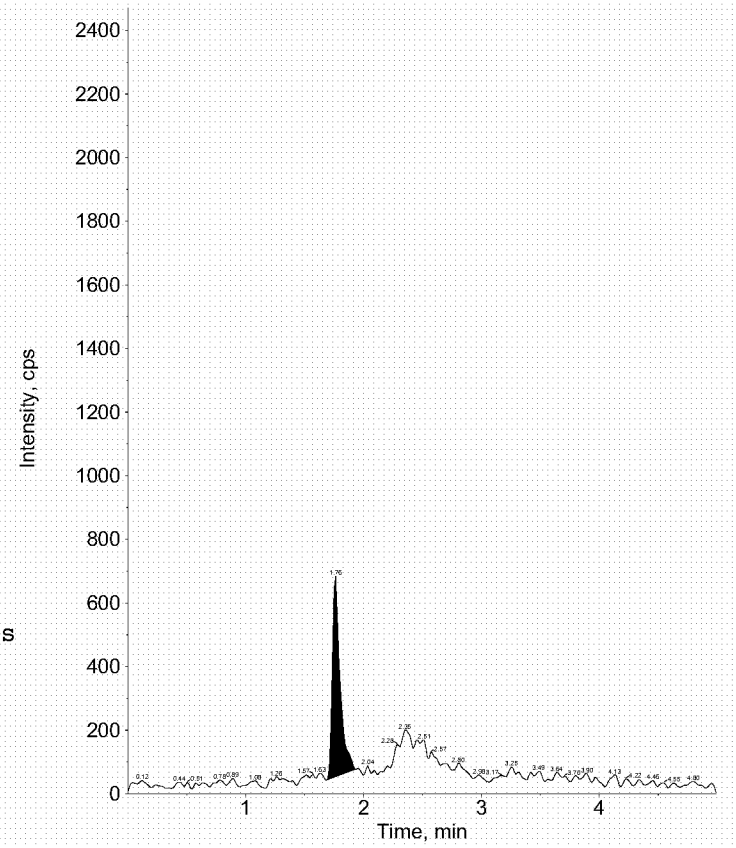


**Figure 8. Representative 0.5 ppm Fortification Sample for Wheat Grain (BAS 455 H Analysis)
Master sheet 198172-11: Filename 040330a.wiff(20)**

Sample Name: "2004001-3" Sample ID: "high fort" File: "040330a.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-11 Wheat Grain JCB" Annotation: ""

Sample Index:	20	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	11:13:04 PM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	30.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.76	min
Area:	3190.	counts
Height:	7.42e+002	cps
Start Time:	1.67	min
End Time:	1.93	min

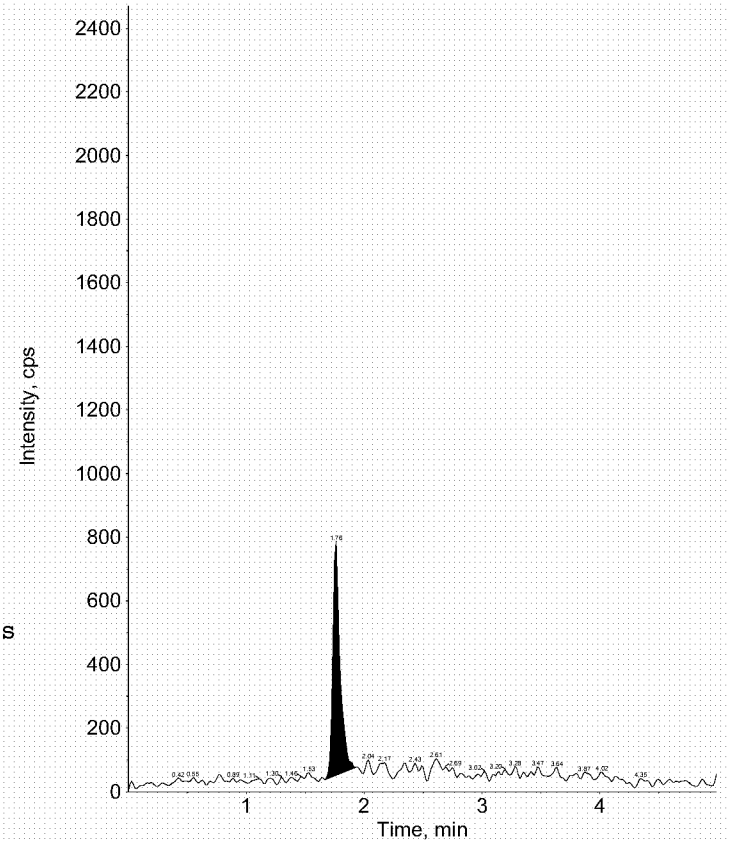


Figure 9. Representative Control Sample for Wheat Straw (BAS 455 H Analysis) Master sheet 198172-32: Filename 040326.wiff(6)

Sample Name: "2004001-4" Sample ID: "control" File: "040326.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-32 Wheat Straw JCB" Annotation: ""

Sample Index:	6	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/26/04	
Acq. Time:	04:43:12 PM	
Modified:	Yes	
Bunching Factor:	2	
Noise Threshold:	10.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.71	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	No Peak	
Retention Time:	N/A	min
Area:	N/A	counts
Height:	N/A	cps
Start Time:	N/A	min
End Time:	N/A	min

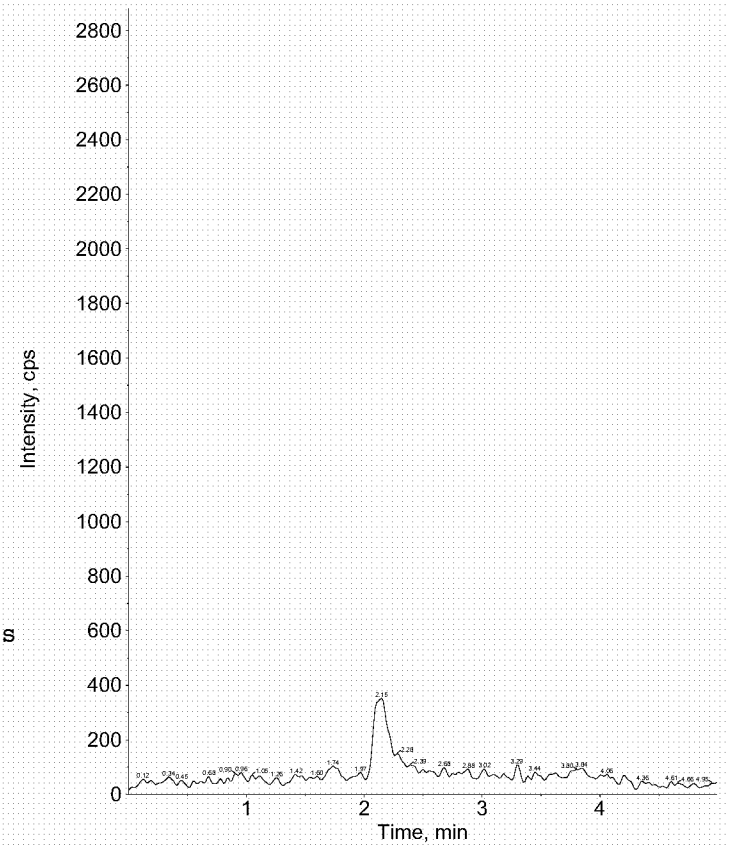


Figure 10. Representative 0.05 ppm Fortification (LOQ) Sample for Wheat Straw (BAS 455 H Analysis)
Master sheet 198172-32: Filename 040326.wiff(11)

Sample Name: "2004001-4" Sample ID: "low fort" File: "040326.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-32 Wheat Straw JCB" Annotation: ""

Sample Index:	11	
Sample Type:	QC	
Concentration:	5.00	
Acq. Date:	03/26/04	
Acq. Time:	05:11:54 PM	
Modified:	Yes	
Bunching Factor:	2	
Noise Threshold:	40.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.74	min
Area:	2450.	counts
Height:	7.06e+002	cps
Start Time:	1.63	min
End Time:	1.87	min

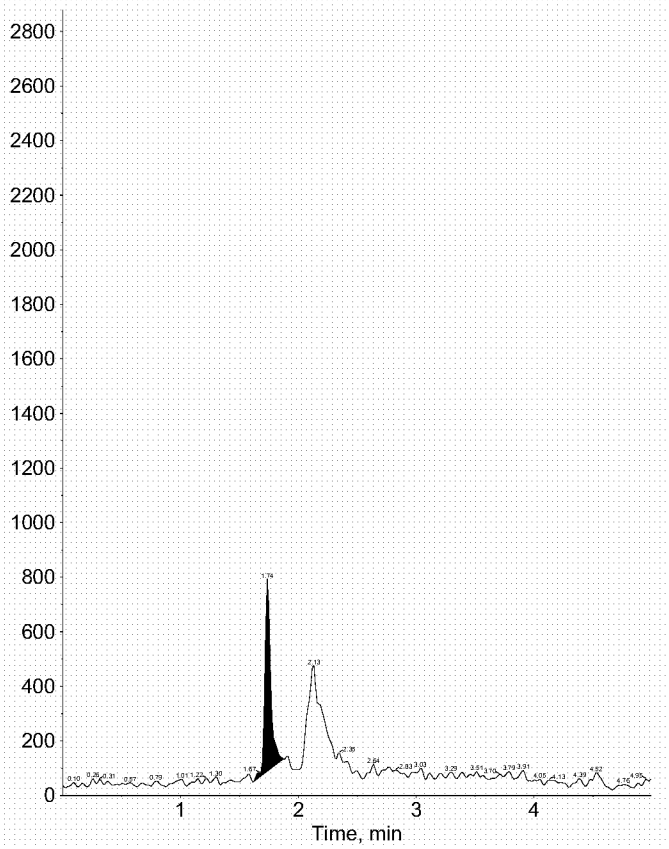


**Figure 11. Representative 0.5 ppm Fortification Sample for Wheat Straw (BAS 455 H Analysis)
Master sheet 198172-32: Filename 040326.wiff(16)**

Sample Name: "2004001-4" Sample ID: "high fort" File: "040326.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-32 Wheat Straw JCB" Annotation: ""

Sample Index:	16	
Sample Type:	QC	
Concentration:	5.00	
Acq. Date:	03/26/04	
Acq. Time:	05:40:32 PM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	30.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.75	min
Area:	2940.	counts
Height:	7.11e+002	cps
Start Time:	1.68	min
End Time:	1.90	min

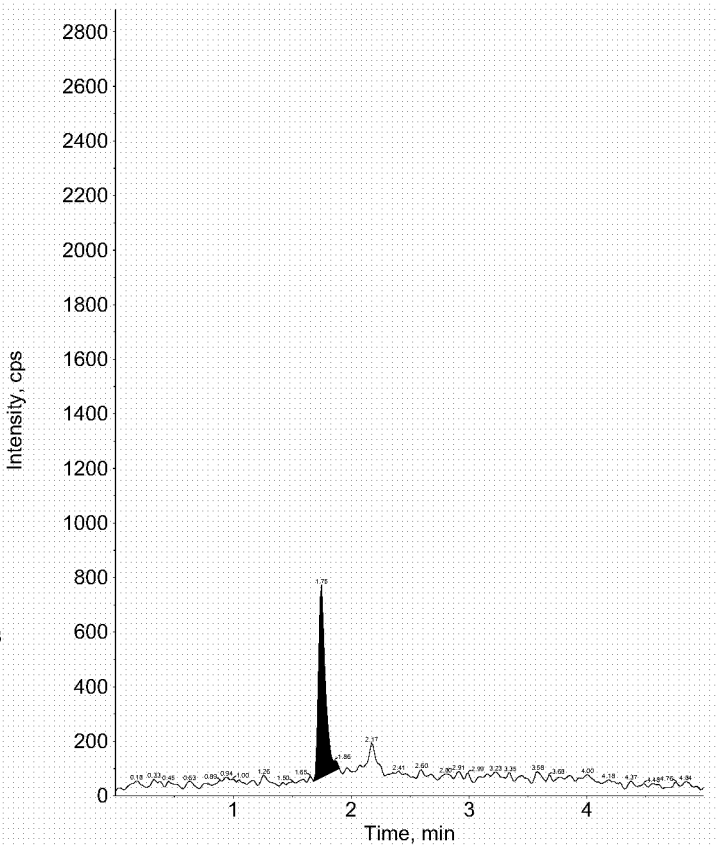


Figure 12. Representative Control Sample for Wheat Forage (BAS 455 H Analysis) Master sheet 198172-01: Filename 040330.wiff(6)

Sample Name: "2004001-1" Sample ID: "qc" File: "040330.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	6	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	10:02:30 AM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	30.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	No Peak	
Retention Time:	N/A	min
Area:	N/A	counts
Height:	N/A	cps
Start Time:	N/A	min
End Time:	N/A	min

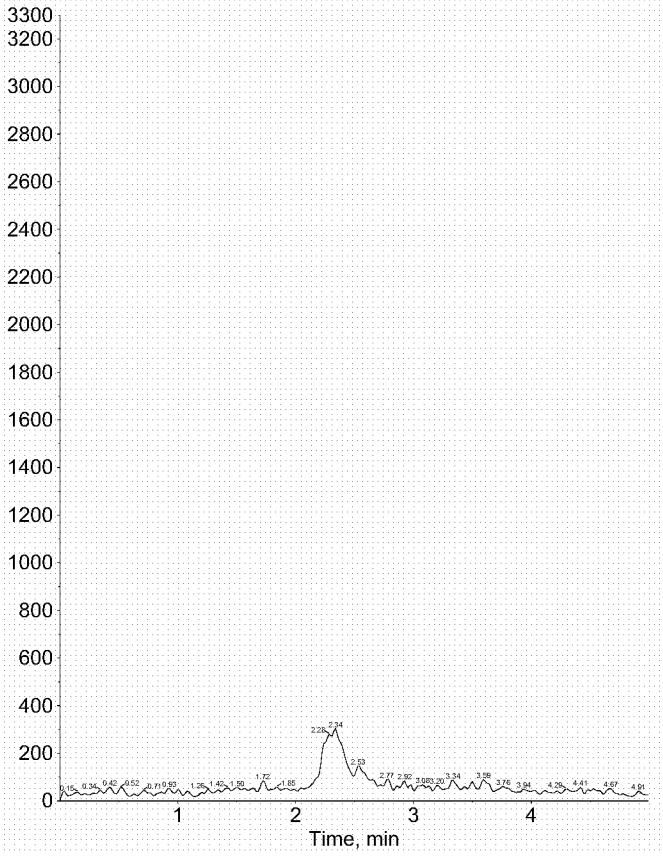


Figure 13. Representative 0.05 ppm Fortification (LOQ) Sample for Wheat Forage (BAS 455 H Analysis)
Master sheet 198172-01: Filename 040330.wiff(11)

Sample Name: "2004001-1" Sample ID: "low fort" File: "040330.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	11	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	10:31:13 AM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	30.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.76	min
Area:	2970.	counts
Height:	7.23e+002	cps
Start Time:	1.68	min
End Time:	1.93	min

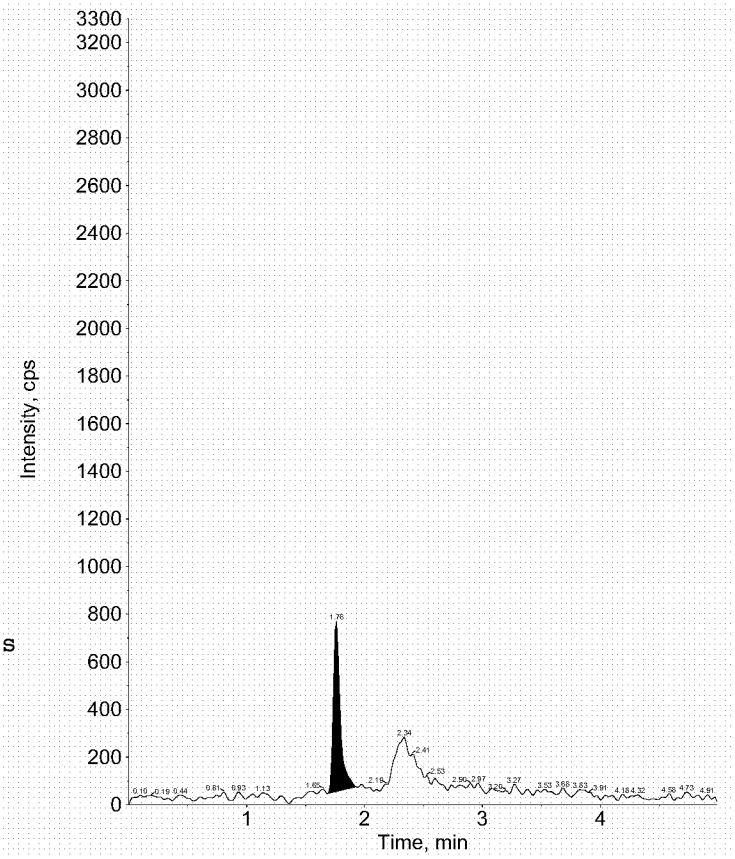


Figure 14. Representative 0.5 ppm Fortification Sample for Wheat Forage (BAS 455 H Analysis)
Master sheet 198172-01: Filename 040330.wiff(15)

Sample Name: "2004001-1" Sample ID: "high fort" File: "040330.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	15
Sample Type:	QC
Concentration:	0.00
Acq. Date:	03/30/04
Acq. Time:	10:54:05 AM
Modified:	No
Bunching Factor:	2
Noise Threshold:	30.00
Area Threshold:	300.00
Num. Smooths:	2
RT Window:	30.0 sec
Expected RT:	1.91 min
Sep. Width:	0.20
Sep. Height:	0.01
Exp. Peak Ratio:	5.00
Exp. Adj. Ratio:	4.00
Exp. Val. Ratio:	3.00
Use Relative RT:	No
Int. Type:	Base To Base
Retention Time:	1.77 min
Area:	3110. counts
Height:	6.61e+002 cps
Start Time:	1.65 min
End Time:	1.97 min

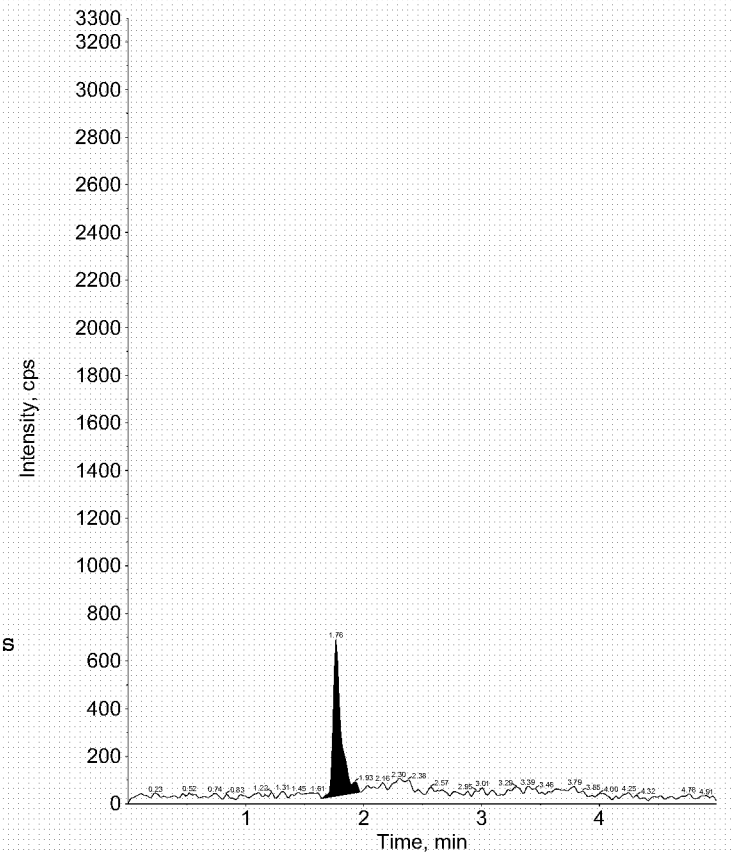


Figure 15. Representative Control Sample for Wheat Hay (BAS 455 H Analysis) Master sheet 198172-21: Filename 040324.wiff(29)

Sample Name: "2004001-2" Sample ID: "control" File: "040324.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-21 Wheat Hay JCB" Annotation: ""

Sample Index:	29	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/24/04	
Acq. Time:	07:02:58 PM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	10.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	No Peak	
Retention Time:	N/A	min
Area:	N/A	counts
Height:	N/A	cps
Start Time:	N/A	min
End Time:	N/A	min

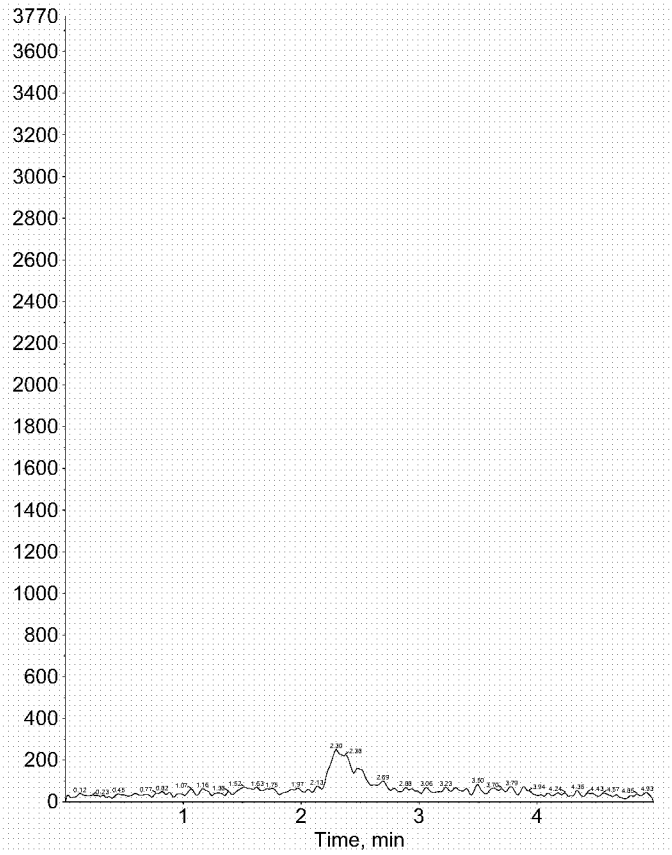


Figure 16. Representative 0.05 ppm Fortification (LOQ) Sample for Wheat Hay (BAS 455 H Analysis)
Master sheet 198172-21: Filename 040324.wiff(33)

Sample Name: "2004001-2" Sample ID: "0.05" File: "040324.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-21 Wheat Hay JCB" Annotation: ""

Sample Index:	33	
Sample Type:	QC	
Concentration:	5.00	
Acq. Date:	03/24/04	
Acq. Time:	07:25:55 PM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	10.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.76	min
Area:	2920.	counts
Height:	6.86e+002	cps
Start Time:	1.61	min
End Time:	1.90	min

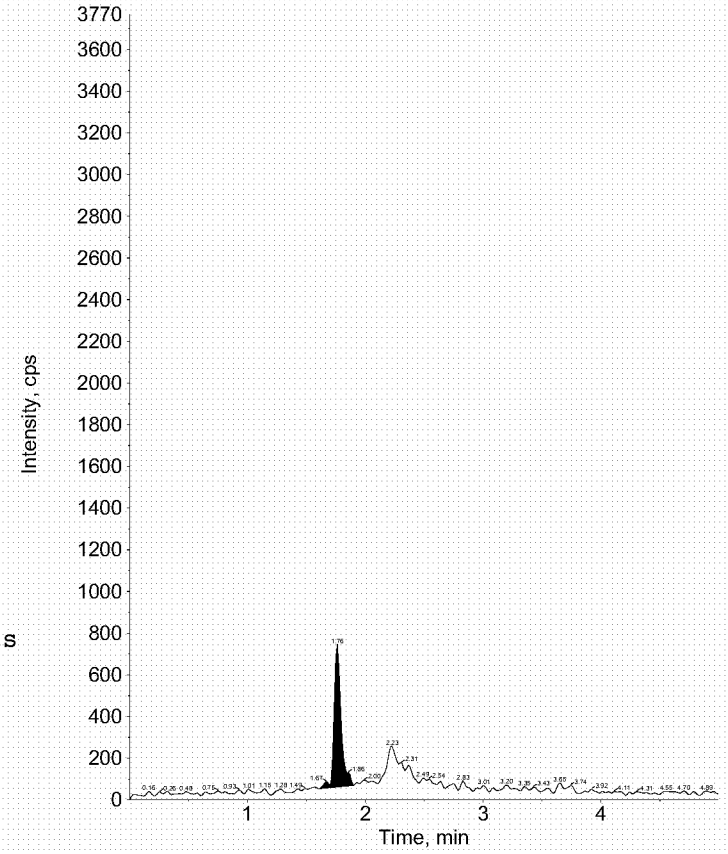


**Figure 17. Representative 0.5 ppm Fortification Sample for Wheat Hay (BAS 455 H Analysis)
Master sheet 198172-21: Filename 040324.wiff(39)**

Sample Name: "2004001-2" Sample ID: "0.5" File: "040324.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-21 Wheat Hay JCB" Annotation: ""

Sample Index:	39	
Sample Type:	QC	
Concentration:	5.00	
Acq. Date:	03/24/04	
Acq. Time:	08:00:12 PM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	10.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.75	min
Area:	4050.	counts
Height:	9.16e+002	cps
Start Time:	1.63	min
End Time:	2.05	min

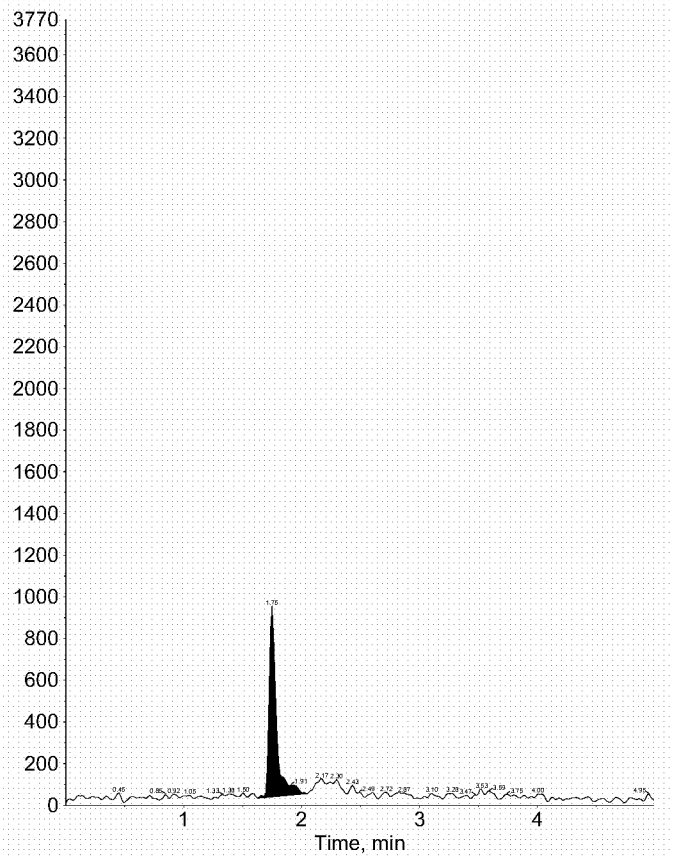


**Figure 18. Representative Reagent Blank (BAS 455 H Analysis)
Master sheet 198172-01: Filename 040330a.wiff(25)**

Sample Name: "Reagent Blank" Sample ID: "qc" File: "040330.wiff"
Peak Name: "BAS 455H" Mass(es): "282.1/212.0 amu"
Comment: "QC BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	25	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	11:51:35 AM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	30.00	
Area Threshold:	300.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.91	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	No Peak	
Retention Time:	N/A	min
Area:	N/A	counts
Height:	N/A	cps
Start Time:	N/A	min
End Time:	N/A	min

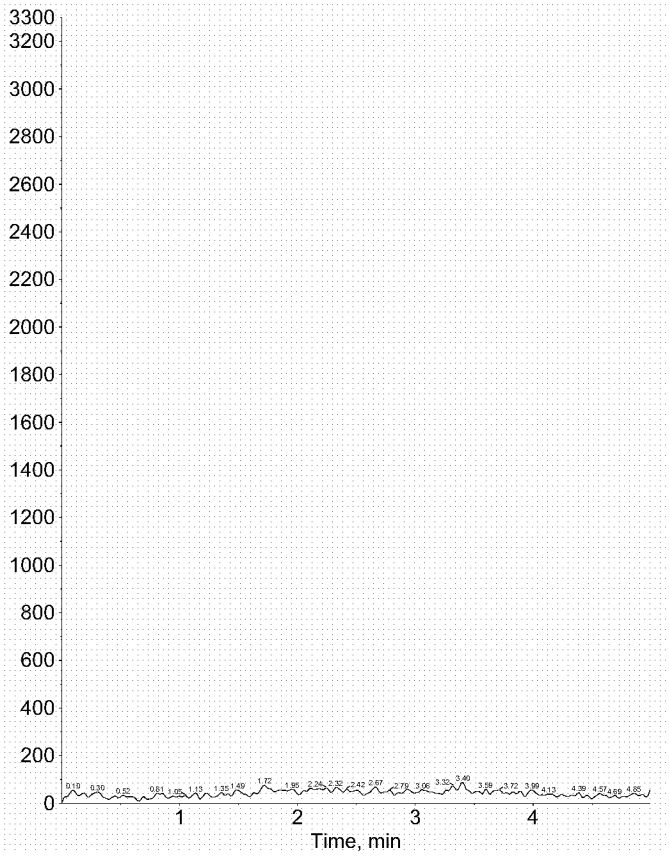


Figure 19. Sample Standard Chromatogram for CL 202347, 0.25 ng/mL (2.5 pg) Master sheet 198172-01: Filename 040330.wiff(1)

Sample Name: "ERS-04-214" Sample ID: "2.5pg Standard" File: "040330.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "ST BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	1	
Sample Type:	Standard	
Concentration:	2.50	
Acq. Date:	03/30/04	
Acq. Time:	09:33:47 AM	
Modified:	Yes	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.59	min
Area:	846.	counts
Height:	2.19e+002	cps
Start Time:	1.52	min
End Time:	1.72	min

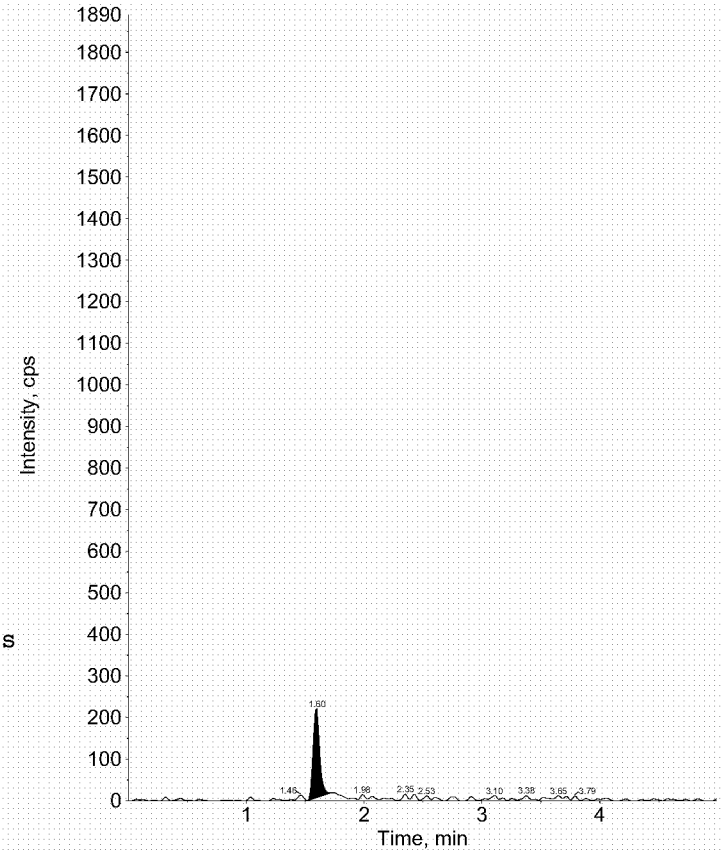


Figure 20. Sample Standard Chromatogram for CL 202347, 0.50 ng/mL (5.0 pg) Master sheet 198172-01: Filename 040330.wiff(2)

Sample Name: "ERS-04-213" Sample ID: "5.0pg Standard" File: "040330.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "ST BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	2	
Sample Type:	Standard	
Concentration:	5.00	
Acq. Date:	03/30/04	
Acq. Time:	09:39:28 AM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.60	min
Area:	2020.	counts
Height:	4.64e+002	cps
Start Time:	1.52	min
End Time:	1.76	min

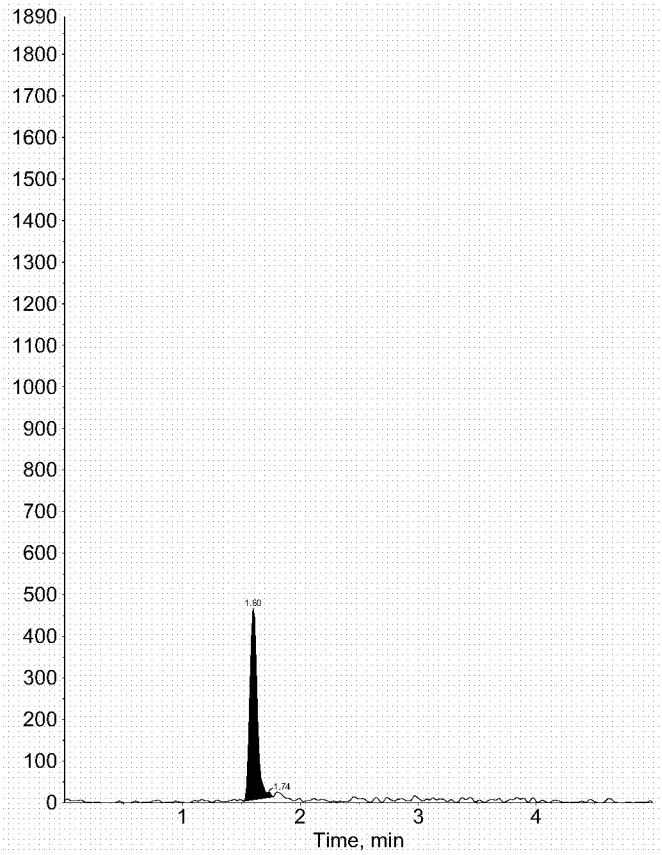


Figure 21. Sample Standard Chromatogram for CL 202347, 1.0 ng/mL (10 pg) Master sheet 198172-01: Filename 040330.wiff(3)

Sample Name: "ERS-04-212" Sample ID: "10.0pg Standard" File: "040330.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "ST BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	3	
Sample Type:	Standard	
Concentration:	10.0	
Acq. Date:	03/30/04	
Acq. Time:	09:45:14 AM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.59	min
Area:	3930.	counts
Height:	8.71e+002	cps
Start Time:	1.38	min
End Time:	1.79	min

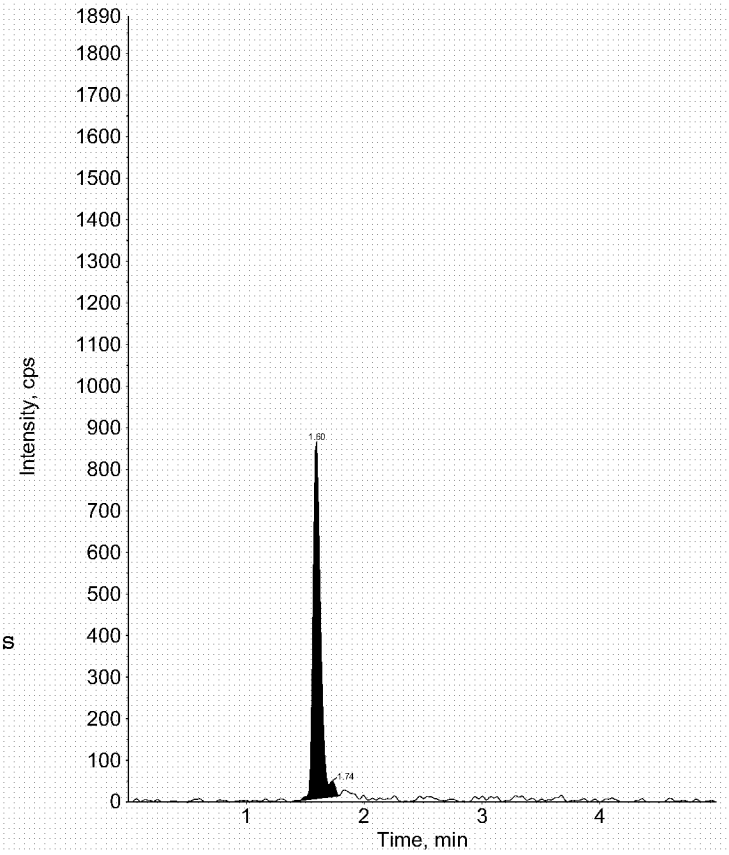


Figure 22. Sample Standard Chromatogram for CL 202347, 2.0 ng/mL (20 pg) Master sheet 198172-01: Filename 040330.wiff(4)

Sample Name: "ERS-04-211" Sample ID: "20.0pg Standard" File: "040330.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "ST BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	4	
Sample Type:	Standard	
Concentration:	20.0	
Acq. Date:	03/30/04	
Acq. Time:	09:50:59 AM	
Modified:	Yes	
Bunching Factor:	2	
Noise Threshold:	40.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.59	min
Area:	7730.	counts
Height:	1.88e+003	cps
Start Time:	1.52	min
End Time:	1.76	min

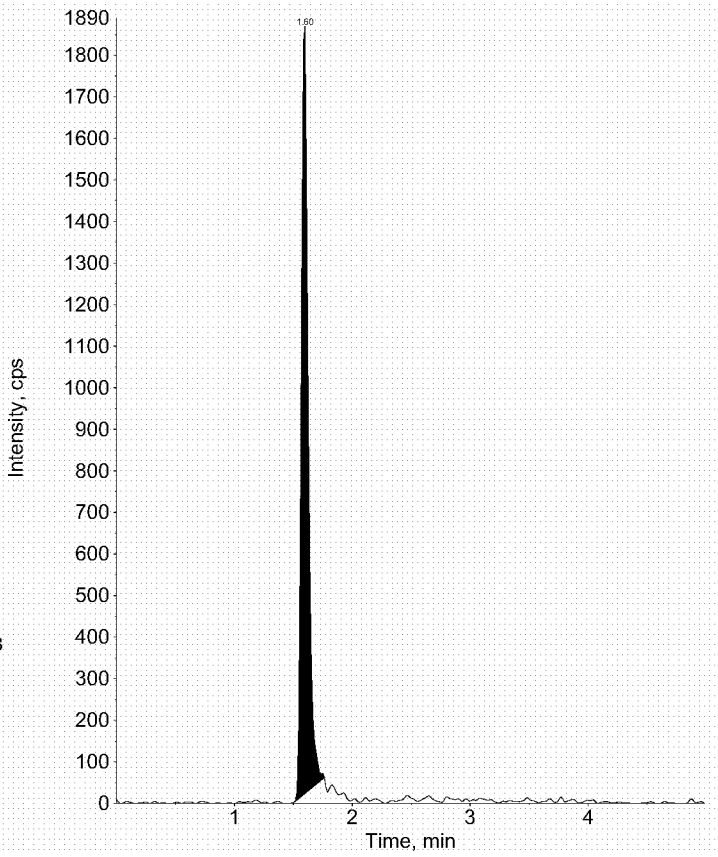


**Figure 23. Representative Standard Curve for CL 202347
Master sheet 198172-01**

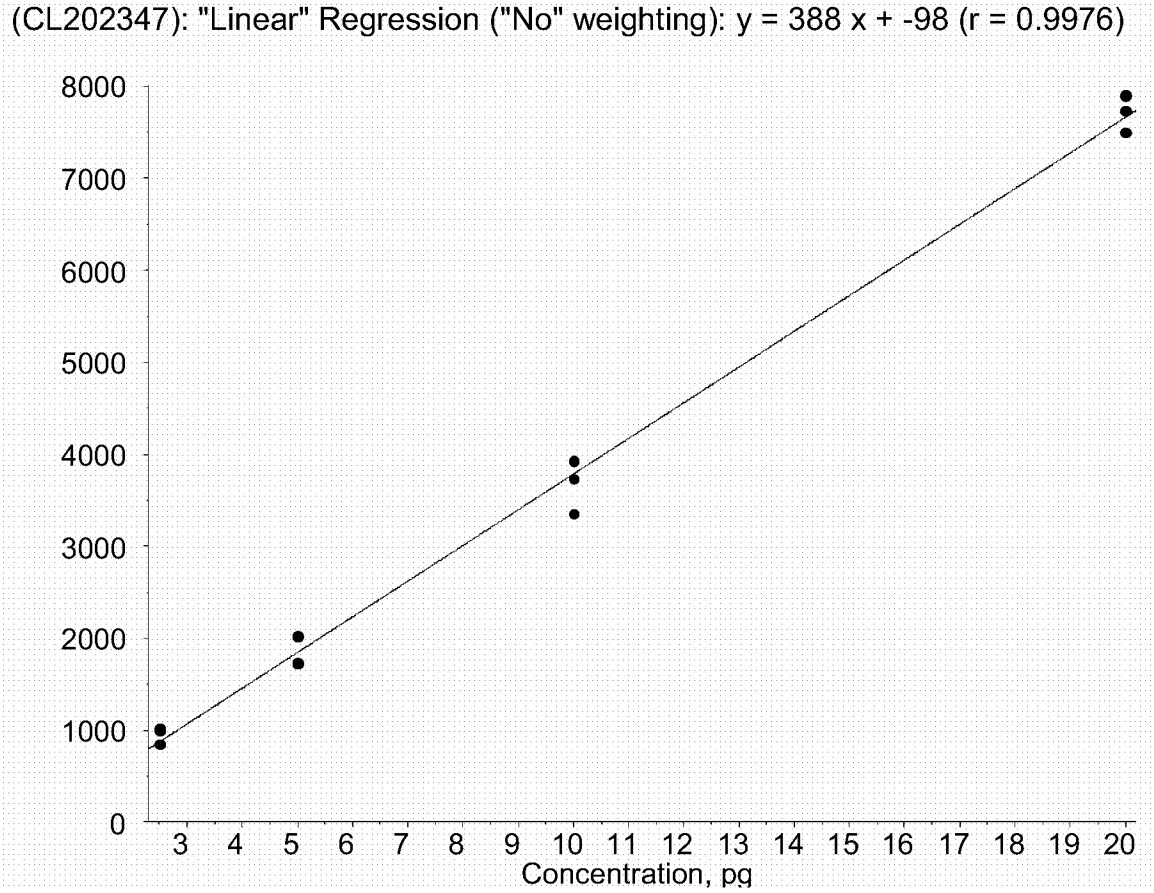


Figure 24. Representative Control Sample for Wheat Grain (CL 202347 Analysis) Master sheet 198172-11: Filename 040330a.wiff(6)

Sample Name: "2004001-3" Sample ID: "qc" File: "040330a.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-11 Wheat Grain JCB" Annotation: ""

Sample Index:	6	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	09:52:42 PM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	No Peak	
Retention Time:	N/A	min
Area:	N/A	counts
Height:	N/A	cps
Start Time:	N/A	min
End Time:	N/A	min

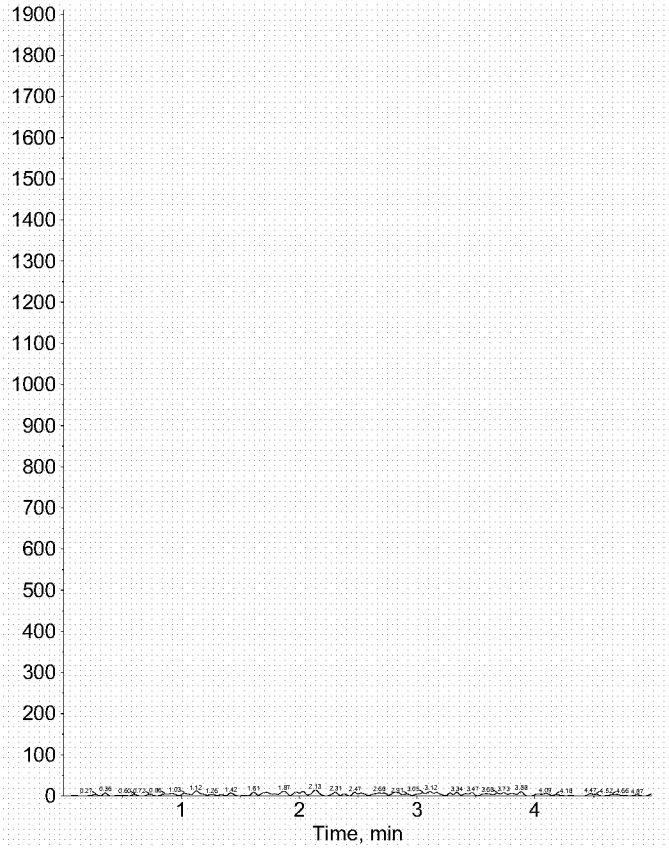


Figure 25. Representative 0.05 ppm Fortification (LOQ) Sample for Wheat Grain (CL 202347 Analysis)
Master sheet 198172-11: Filename 040330a.wiff(10)

Sample Name: "2004001-3" Sample ID: "low fort" File: "040330a.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-11 Wheat Grain JCB" Annotation: ""

Sample Index:	10	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	10:15:39 PM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.60	min
Area:	2000.	counts
Height:	5.09e+002	cps
Start Time:	1.49	min
End Time:	1.76	min

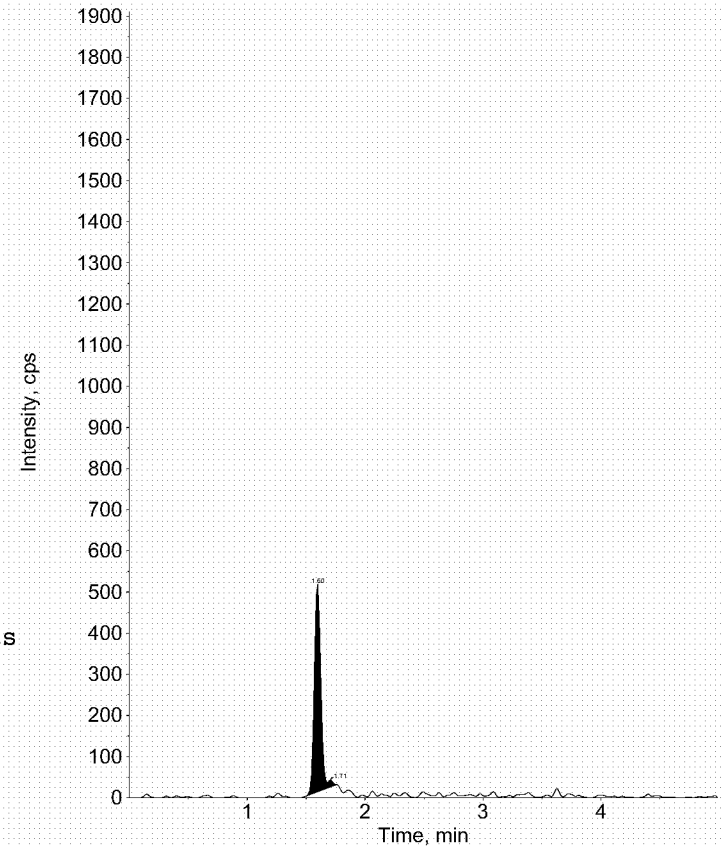


Figure 26. Representative 0.5 ppm Fortification Sample for Wheat Grain (CL 202347 Analysis) Master sheet 198172-11: Filename 040330a.wiff(20)

Sample Name: "2004001-3" Sample ID: "high fort" File: "040330a.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-11 Wheat Grain JCB" Annotation: ""

Sample Index:	20	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	11:13:04 PM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.59	min
Area:	2290.	counts
Height:	5.14e+002	cps
Start Time:	1.52	min
End Time:	1.76	min

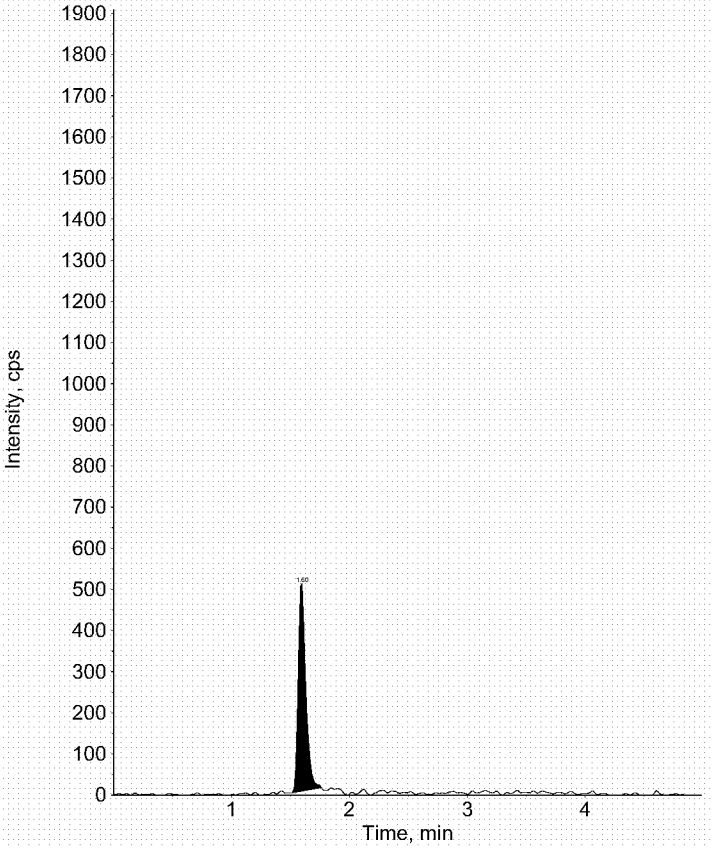


Figure 27. Representative Control Sample for Wheat Straw (CL 202347 Analysis) Master sheet 198172-32: Filename 040326.wiff(6)

Sample Name: "2004001-4" Sample ID: "control" File: "040326.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-32 Wheat Straw JCB" Annotation: ""

Sample Index:	6	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/26/04	
Acq. Time:	04:43:12 PM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	No Peak	
Retention Time:	N/A	min
Area:	N/A	counts
Height:	N/A	cps
Start Time:	N/A	min
End Time:	N/A	min

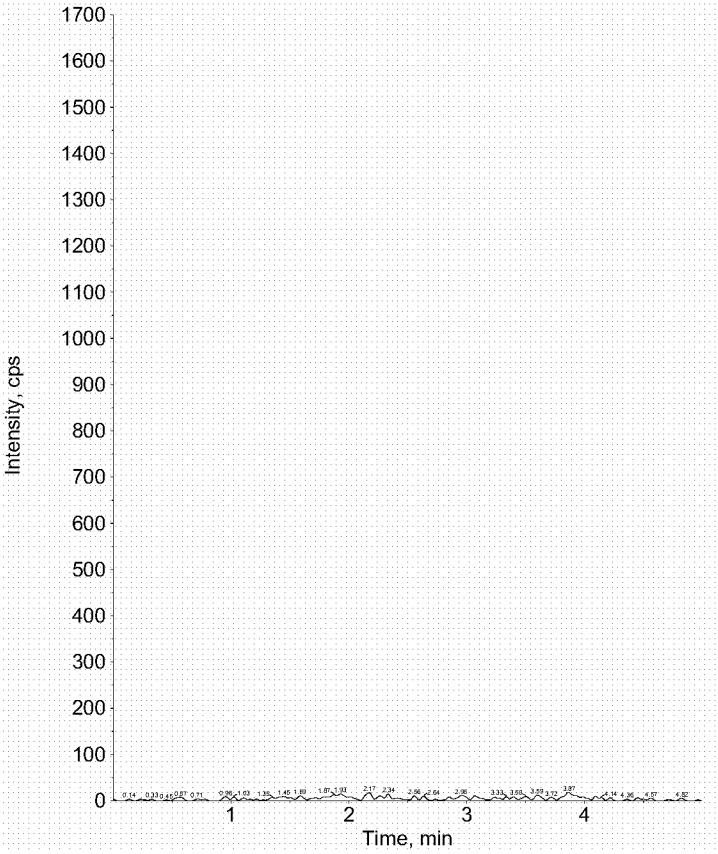


Figure 28. Representative 0.05 ppm Fortification (LOQ) Sample for Wheat Straw (CL 202347 Analysis)
Master sheet 198172-32: Filename 040326.wiff(12)

Sample Name: "2004001-4" Sample ID: "low fort" File: "040326.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-32 Wheat Straw JCB" Annotation: ""

Sample Index:	12	
Sample Type:	QC	
Concentration:	5.00	
Acq. Date:	03/26/04	
Acq. Time:	05:17:40 PM	
Modified:	Yes	
Bunching Factor:	2	
Noise Threshold:	5.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.53	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.57	min
Area:	1380.	counts
Height:	3.02e+002	cps
Start Time:	1.45	min
End Time:	1.80	min

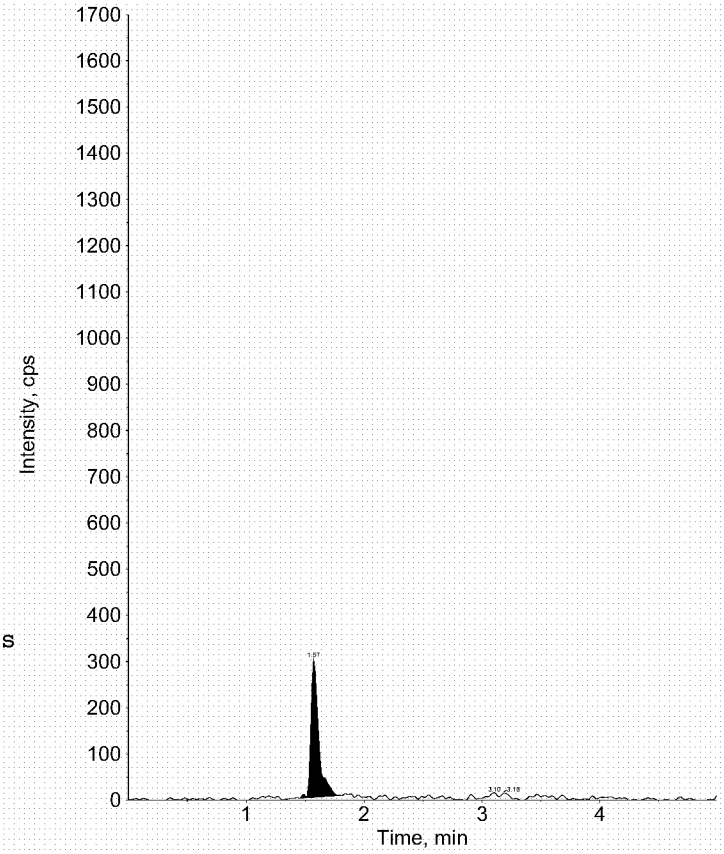


**Figure 29. Representative 0.5 ppm Fortification Sample for Wheat Straw (CL 202347 Analysis)
Master sheet 198172-32: Filename 040326.wiff(16)**

Sample Name: "2004001-4" Sample ID: "high fort" File: "040326.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-32 Wheat Straw JCB" Annotation: ""

Sample Index:	16	
Sample Type:	QC	
Concentration:	5.00	
Acq. Date:	03/26/04	
Acq. Time:	05:40:32 PM	
Modified:	Yes	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.53	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.58	min
Area:	1620.	counts
Height:	4.63e+002	cps
Start Time:	1.50	min
End Time:	1.74	min

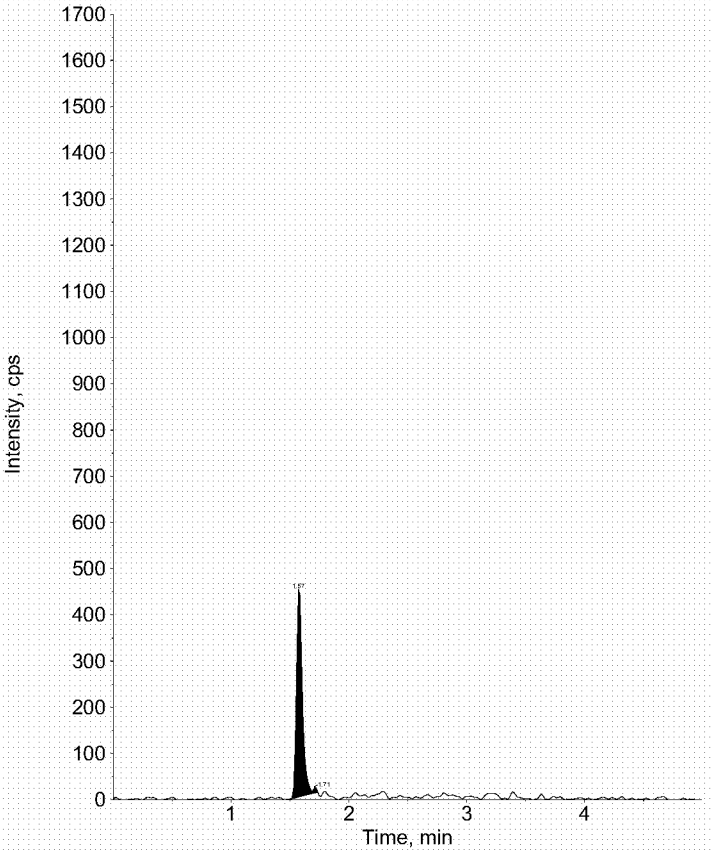


Figure 30. Representative Control Sample for Wheat Forage (CL 202347 Analysis) Master sheet 198172-01: Filename 040330.wiff(6)

Sample Name: "2004001-1" Sample ID: "qc" File: "040330.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	6	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	10:02:30 AM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	No Peak	
Retention Time:	N/A	min
Area:	N/A	counts
Height:	N/A	cps
Start Time:	N/A	min
End Time:	N/A	min

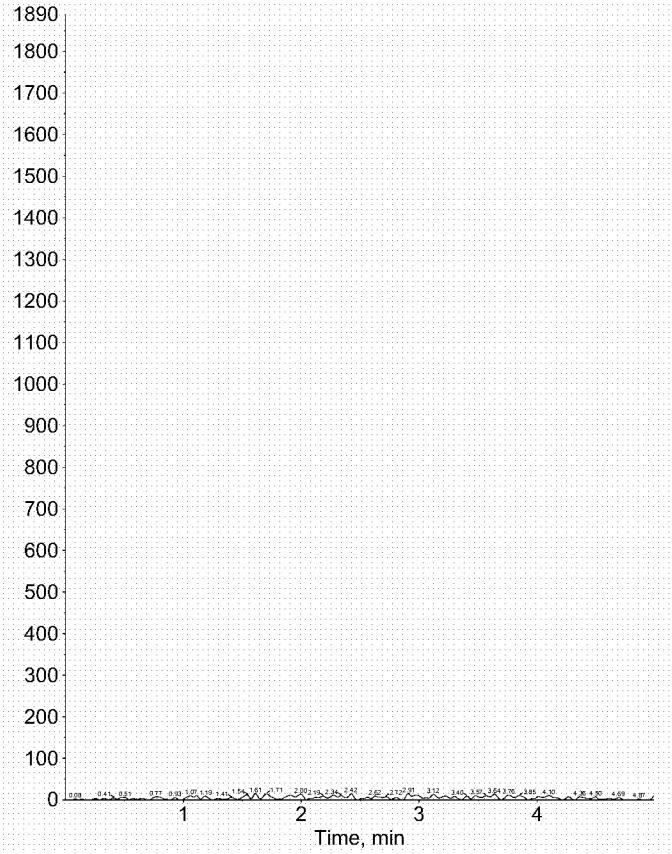


Figure 31. Representative 0.05 ppm Fortification (LOQ) Sample for Wheat Forage (CL 202347 Analysis)
Master sheet 198172-01: Filename 040330.wiff(11)

Sample Name: "2004001-1" Sample ID: "low fort" File: "040330.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	11	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	10:31:13 AM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.59	min
Area:	1760.	counts
Height:	3.88e+002	cps
Start Time:	1.46	min
End Time:	1.79	min

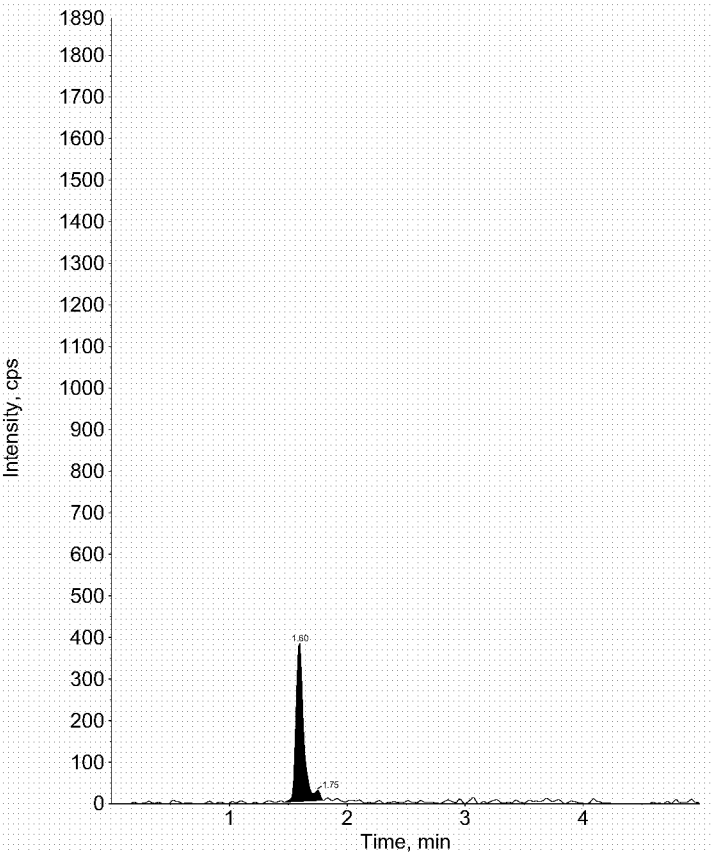


**Figure 32. Representative 0.5 ppm Fortification Sample for Wheat Forage (CL 202347 Analysis)
Master sheet 198172-01: Filename 040330.wiff(15)**

Sample Name: "2004001-1" Sample ID: "high fort" File: "040330.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	15	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	10:54:05 AM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.60	min
Area:	1510.	counts
Height:	3.28e+002	cps
Start Time:	1.53	min
End Time:	1.82	min

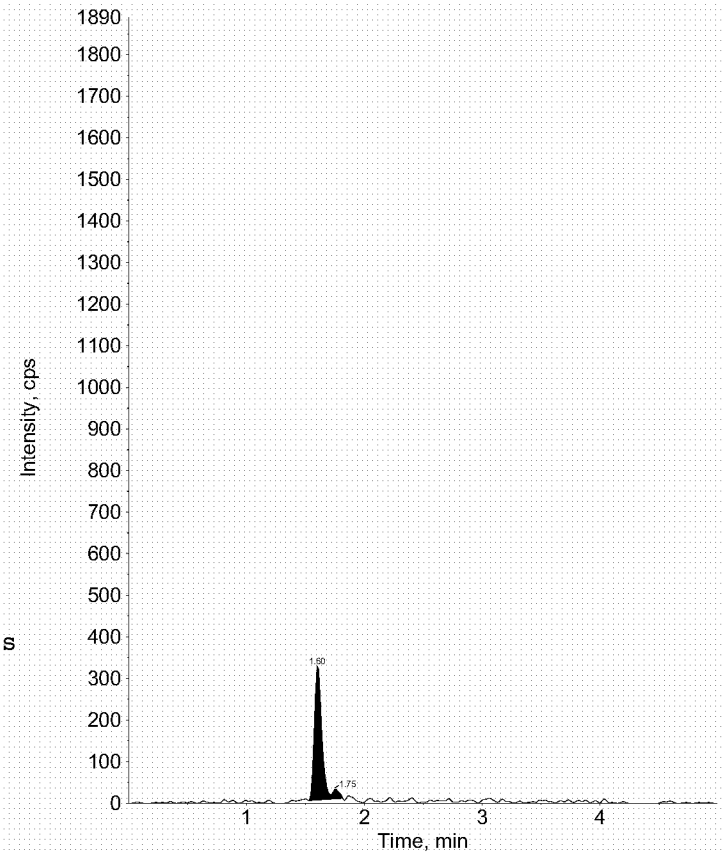


Figure 33. Representative Control Sample for Wheat Hay (CL 202347 Analysis) Master sheet 198172-21: Filename 040324.wiff(29)

Sample Name: "2004001-2" Sample ID: "control" File: "040324.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-21 Wheat Hay JCB" Annotation: ""

Sample Index:	29	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/24/04	
Acq. Time:	07:02:58 PM	
Modified:	No	
Bunching Factor:	1	
Noise Threshold:	10.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	No Peak	
Retention Time:	N/A	min
Area:	N/A	counts
Height:	N/A	cps
Start Time:	N/A	min
End Time:	N/A	min

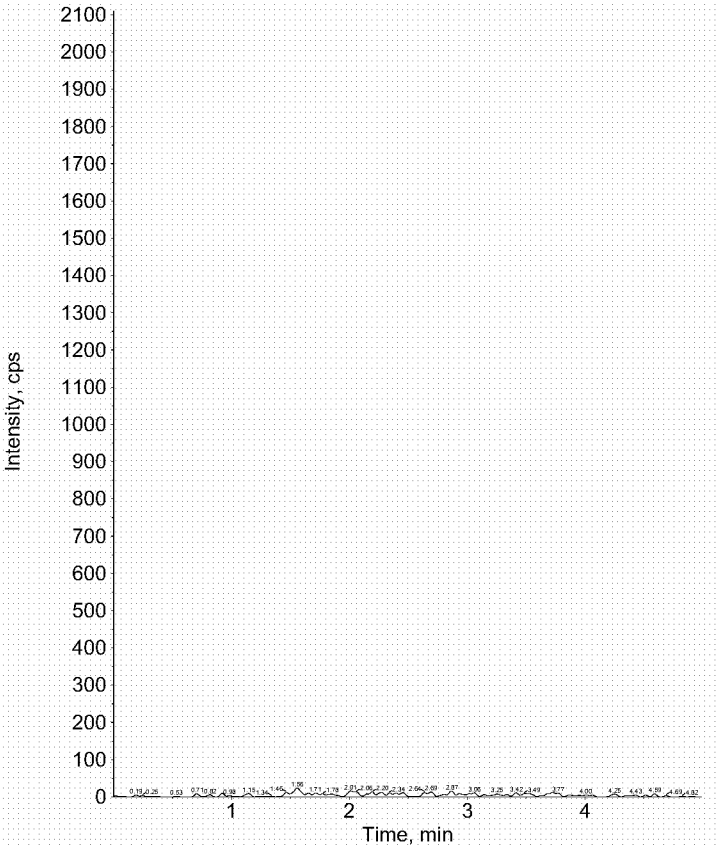


Figure 34. Representative 0.05 ppm Fortification (LOQ) Sample for Wheat Hay (CL 202347 Analysis)
Master sheet 198172-21: Filename 040324.wiff(33)

Sample Name: "2004001-2" Sample ID: "0.05" File: "040324.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-21 Wheat Hay JCB" Annotation: ""

Sample Index:	33	
Sample Type:	QC	
Concentration:	5.00	
Acq. Date:	03/24/04	
Acq. Time:	07:25:55 PM	
Modified:	Yes	
Bunching Factor:	2	
Noise Threshold:	10.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.60	min
Area:	1490.	counts
Height:	3.22e+002	cps
Start Time:	1.50	min
End Time:	1.82	min

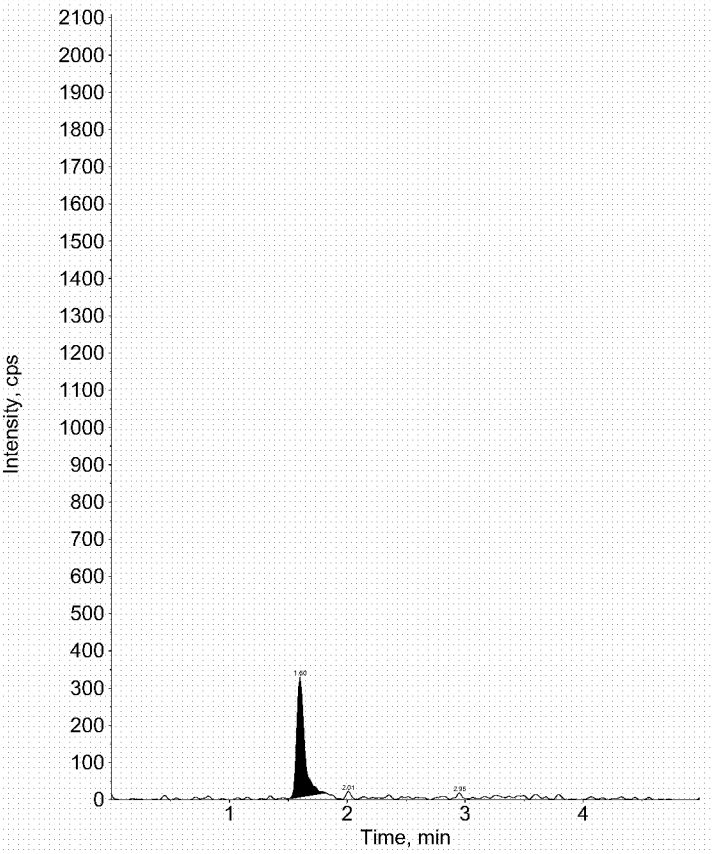


**Figure 35. Representative 0.5 ppm Fortification Sample for Wheat Hay (CL 202347Analysis)
Master sheet 198172-21: Filename 040324.wiff(39)**

Sample Name: "2004001-2" Sample ID: "0.5" File: "040324.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-21 Wheat Hay JCB" Annotation: ""

Sample Index:	39	
Sample Type:	QC	
Concentration:	5.00	
Acq. Date:	03/24/04	
Acq. Time:	08:00:12 PM	
Modified:	Yes	
Bunching Factor:	2	
Noise Threshold:	10.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	Base To Base	
Retention Time:	1.59	min
Area:	1730.	counts
Height:	4.39e+002	cps
Start Time:	1.52	min
End Time:	1.76	min

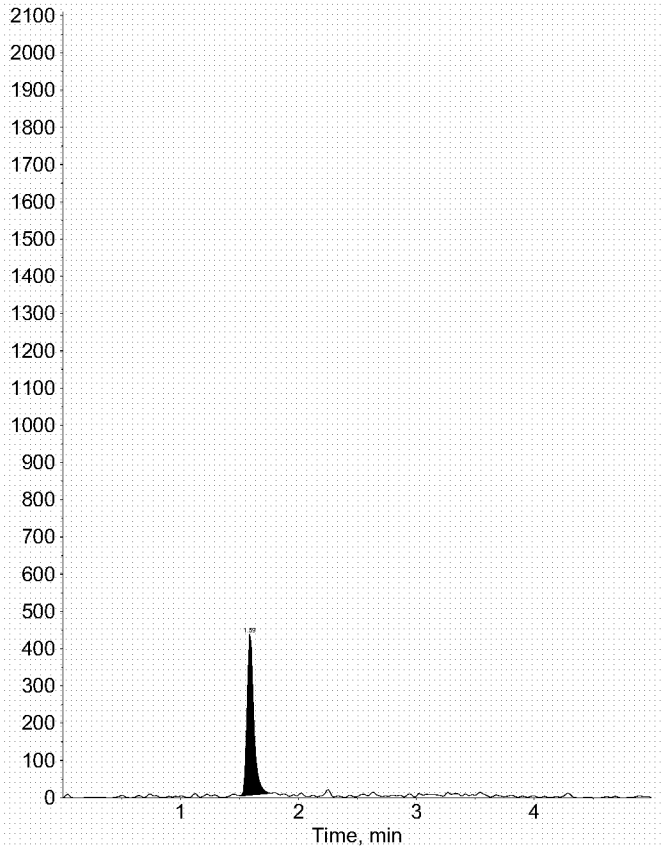
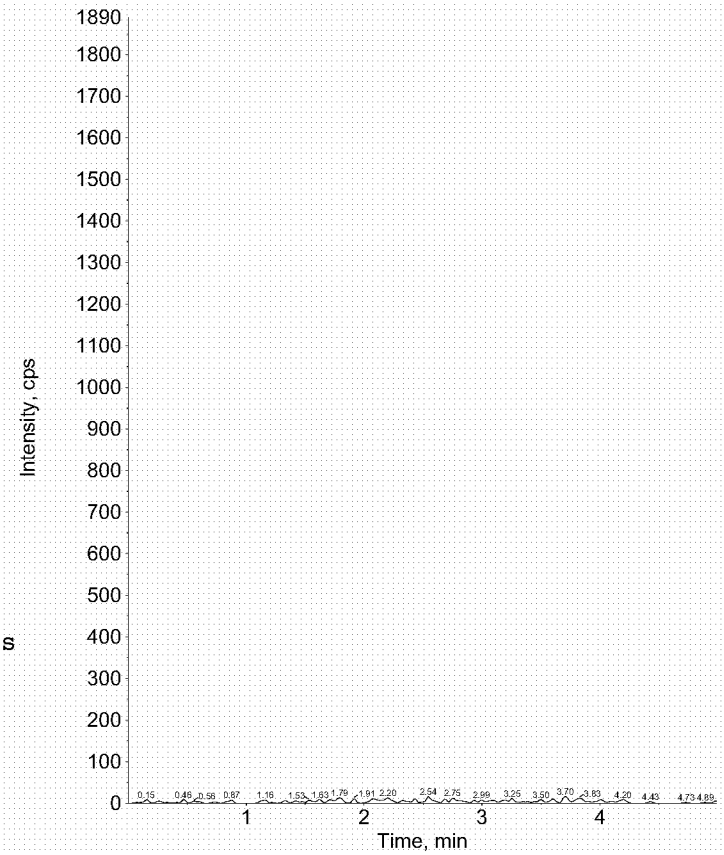


**Figure 36. Representative Reagent Blank (CL 202347 Analysis)
Master sheet 198172-01: Filename 040330a.wiff(25)**

Sample Name: "Reagent Blank" Sample ID: "qc" File: "040330.wiff"
Peak Name: "CL202347" Mass(es): "298.1/228.1 amu"
Comment: "QC BASF1132 ND 198172-01 Wheat Forage JCB" Annotation: ""

Sample Index:	25	
Sample Type:	QC	
Concentration:	0.00	
Acq. Date:	03/30/04	
Acq. Time:	11:51:35 AM	
Modified:	No	
Bunching Factor:	2	
Noise Threshold:	20.00	
Area Threshold:	200.00	
Num. Smooths:	2	
RT Window:	30.0	sec
Expected RT:	1.83	min
Sep. Width:	0.20	
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	3.00	
Use Relative RT:	No	
Int. Type:	No Peak	
Retention Time:	N/A	min
Area:	N/A	counts
Height:	N/A	cps
Start Time:	N/A	min
End Time:	N/A	min



APPENDIX A

BASF Analytical Method D0203 entitled "Method for Determination of Pendimethalin (BAS 455 H) and its Metabolite CL 202347 Residues in Wheat Forage, Hay, Grain and Straw using LC/MS/MS"

BASF Corporation
Agricultural Products Division RTP, NC 27709

BASF
The Chemical Company

Technical Procedure:

Method for Determination of Pendimethalin (BAS 455 H) and its Metabolite CL 202347 Residues in Wheat Forage, Hay, Grain and Straw using LC/MS/MS

BASF Method Number D0203

May 2004

Authors:

Jane Stewart
John C. Billotte

BASF Corporation
Agro Research
26 Davis Drive
P.O. Box 13528
Research Triangle Park, NC 27709-3528
USA

This technical procedure consists of 15 pages

Method for Determination of BAS 455 H and Its Metabolite CL 202347 Residues in Wheat Forage, Hay, Grain and Straw using LC/MS/MS

ABSTRACT

Analytical Method D0203 was developed in order to determine residues of BAS 455 H and its metabolite CL 202347 in wheat forage, hay, grain and straw. Residues of BAS 455 H and its metabolite CL 202347 are extracted from green and dry plant with acidic aqueous methanol and from grain with 10% methanol in dichloromethane. An aliquot is taken from extract and then cleaned using a PolarPlus® C18 Speedisk® solid phase extraction column. The compounds are eluted out of the Speedisk® column using methanol/water. The eluates are diluted with water for analysis via LC/MS/MS. The limit of quantitation of the method for the analytes in all matrices is 0.05 ppm. This method was developed at BASF Agro Research, Research Triangle Park, N.C., USA.

Analytical Method Number D0203 is suitable for measuring residues of BAS 455 H and its metabolite CL 202347 in wheat forage, hay, grain and straw to a quantitation limit of 0.05 ppm.

TABLE OF CONTENTS

1 INTRODUCTION

BAS 455 H is an herbicide used for the control of grassy weeds in several crops including winter wheat. Prior wheat metabolism investigations showed that relevant residues in wheat commodities consist of the unchanged parent and predominant identifiable hydroxyl metabolite CL 202347. The current analytical method determines these residues in wheat samples, including forage, hay, grain and straw. This method may be used for other plant matrices.

2 MATERIALS

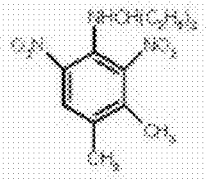
Standard substances are stored in a refrigerator at around 4 °C until use. Information on the characterization of these substances is available from BASF Aktiengesellschaft, Agricultural Center, Limburgerhof, Germany.

2.1 LIST OF ABBRIVIATIONS

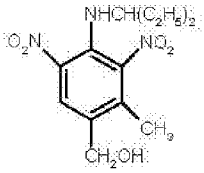
DCM	Dichloromethane
HPLC	High Performance Liquid Chromatography
LOQ	Limit of Quantitation
LOD	Limit of Detection
MeOH	Methanol
MS	Mass Spectrometry
SPE	Solid Phase Extraction

2.2. FORTIFICATION/REFERENCE SUBSTANCES

Parent

BASF Code Name	BAS 455 H Pendimethalin
Chemical Name	N-(1-ethylpropyl)-3,-4-dimethyl 2,6-dinitrobenzamine
Molecular Formula	$C_{13}H_{19}N_3O_4$
Molecular Weight	281.3
Structural Formula	

Metabolite CL 202347

BASF Code Name	CL 202347
Chemical Name	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitrobenzyl alcohol
Molecular Formula	$C_{13}H_{19}N_3O_5$
Molecular Weight	297.3
Structural Formula	

2.3 Equipment -- Suggested Sizes/Suppliers, Manufacturers

Method Step	Equipment	Size, Description	Manufacturer / Supplier	Catalog Number [1)]
Various	Spatula	Various	VWR	
3.2	Wide-Mouth Centrifuge Bottle	250 mL	Nalge	3121-0250
Various	Volumetric Flasks	5, 10, 50, 100	VWR	
Various	Pipettes	0.5mL,1mL, 5mL,	VWR	
2.5.2	Analytical Balance	AT261 DeltaRange®	Mettler Toledo	
3.2	Top-loading balance	PG5002 DeltaRange®	Mettler	
Various	Culture tubes	16 X 100	VWR	47729-576
3.2	Polytron homogenizer	PT 3100	Kinamatica AG	
Various	Sample Tube Vortexer	Vortex-Genie 2™	VWR	
3.3	SPE Vacuum Manifold		IST	
Various	Centrivap Concentrator	78100	Labconco	7810002
3.3	PolarPlus® C18 Speedisk® SPE Column	100-mg, 3-mL column	J.T. Baker	8153-06
3.5	LC/MS/MS	LCQ Deca Mass Analyzer	Finnigan	
3.5	LC/MS/MS	SciEx API 3000	Perkin Elmer	
Various	general laboratory supplies	Various	Various	

NOTE: Equivalent equipment from other suppliers may be substituted.

2.4 REAGENTS AND CHEMICALS -- SUGGESTED SOURCES

2.4.1 Chemicals

Chemical	Grade	Manufacturer	Catalog No.
Water	HPLC	B & J	365-4
Dichloromethane	HPLC	B & J	300-4
Methanol	HPLC	B & J	230-4
Hydrochloric Acid	36.5%	EM Science	HX 0603-3
Formic Acid	98.4%	Sigma	F-4636

NOTE: Equivalent reagents and chemicals from other suppliers may be substituted.

2.4.2 Solvent Mixtures

Acidic aqueous methanol Step 3.2 e.g. add 20 mL of concentrated HCl and 200 mL of DI water into 1000-mL graduated cylinder, make up volume to 1000 mL with MeOH
10/90 MeOH/DCM Step 3.2 e.g. add 100 mL of MeOH to a 1000-mL graduated cylinder and adjust to 1000 mL with DCM
50/50 MeOH/H_2O Step 3.3 & 3.4 e.g. add 250 mL of MeOH to 500-mL graduated cylinder and adjust volume to 500 mL with HPLC H_2O
90/10 MeOH/H_2O Step 3.3 & 3.4 e.g. add 450 mL of MeOH to 500-mL graduated cylinder and adjust volume to 500 mL with HPLC H_2O
0.1% Formic acid in water (or methanol) Step 3.6 e.g. add 1 mL formic acid into 1000 mL HPLC H_2O (or methanol)

2.5 Standard Solutions

2.5.1 Standard Solution Storage and Stability

Standard solutions are kept refrigerated. BASF recommends that stock solutions (1.0 mg/mL in methanol) be made fresh every three months. Dilutions of stock solutions should be used no longer than one month.

NOTE: Suggested standard concentrations are listed below. A different concentration scheme may be used and additional standards may be prepared as needed.

2.5.2 Standard Solutions of BAS 455 H and CL 202347 for Fortifications

BAS 455 H

Prepare a 1.0 mg/mL BAS 455 H stock solution by weighing an appropriate amount of BAS 455 H into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 10 mL stock solution, place 10.0 mg of BAS 455 H into a 10 mL volumetric flask. Dissolve and dilute to mark with methanol.

CL 202347

Prepare a 1.0 mg/mL CL 202347 stock solution by weighing an appropriate amount of CL 202347 into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 10 mL stock solution, place 10.0 mg of CL 202347 into a 10 mL volumetric flask. Dissolve and dilute to mark with methanol.

Mixed Standards of BAS 455 H and CL 202347 for Fortification

Prepare a 50 µg/mL mixed standard solution for fortification by combining 5 mL of each of the BAS 455 H and CL 202347 stock solutions (1mg/mL) into a 100 mL volumetric flask. Dilute to mark with methanol. Prepare serial dilutions of this combined solution as needed. Suggested concentrations (in methanol) of mixed standards for fortifications are 5.0 µg/mL (for 0.5 ppm spiking) and 500 ng/mL (for 0.05 ppm spiking).

2.5.3 Standard Solutions of BAS 455 H and CL 202347 for LC/MS/MS Analysis

Mixed Standards of BAS 455 H and CL 202347 for LC/MS/MS Analysis

Prepare a 5.0 ng/mL mixed standard solution by transferring 1.0 mL of the 500 ng/mL fortification solution into a 100 mL volumetric flask. Dilute to mark with 50/50 MeOH/HPLC H_2O. Prepare serial dilutions of this combined solution as needed. Suggested concentrations of mixed standards in 50/50 MeOH/HPLC H_2O for LC/MS/MS analyses are: 0.25 ng/mL, 0.5 ng/mL, 1.0 ng/mL, and 2.0 ng/mL. Other concentration schemes may be used if required.

3 ANALYTICAL PROCEDURE

3.1 SAMPLE PREPARATION

Individual wheat samples were processed with dry ice using a Stephans Vertical Cutter for hay, straw and forage and Wylie Mill for grain. Wheat samples were kept cold throughout the procedure via the addition of dry ice. The dry ice was allowed to sublime prior to sampling.

3.2 FORTIFICATION AND EXTRACTION

a) Weigh 5 g (± 0.1g) of the wheat forage, hay, grain or straw into a 250 mL Teflon® bottle. For the fortification samples, add an appropriate volume of BAS 455 H and CL 202347 mixture standard solution to the respective control sample by volumetric pipette. For example, for a 0.05 ppm fortification sample, pipette 0.5 mL of the 500 ng/mL mixed standard solution of BAS 455 H and CL 202347 onto a control sample.

b) Use 100-mL graduated cylinder to add exactly 100 mL of appropriate extraction solvent (use acidic aqueous methanol for forage, hay, and straw; use 10% methanol in DCM for grain). Polytron is used to homogenize/macerate sample at 10,000 rpm for 5 min.

c) Filter the extract, using vacuum, through glass fiber filter paper supported by a Buchner funnel. Filtration may be replaced by centrifugation. If centrifuging, use adequate speed and duration to form a pellet on the bottom of the bottle.

d) For grain sample, remove 1 mL (1%) of the filtrated (or centrifuged) extract into an 11 mL culture tube and concentrate to dryness on a Centrivap concentrator at 35 °C and vacuum. Add 1 mL of MeOH to culture tube and sonicate briefly, then add 2 mL of HPLC water and mix well. For forage, hay and straw, take an aliquot of 1mL (1%) of the filtrated (or centrifuged) extract into a culture tube, add 2 mL water and mix well.

NOTE: For hay and straw samples, higher Polytron homogenizer speed may be required to macerate sample into very fine particles.
If samples are pulverized very well by sample preparation (section 3.1), polytron homogenization can be replaced by vortex or shaking for 5 min (**section 3.2.b**).

NOTE: BAS 455 H is extremely volatile, **do not** use nitrogen for evaporation or **do not** over dry the samples on the centrivap.

3.3 POLARPLUS C18 SPEEDDISK SPE CLEANUP

a) Condition PolarPlus® C18 Speedisk® SPE cartridges with 3 mL methanol followed by 3 mL water.

b) Load sample on the preconditioned C18 cartridge using disposable Pasteur pipette, rinse sample tube with 2 mL of HPLC water and load the rinse to the same C18 cartridge, discard the eluant.

c) Wash the C18 cartridge with 2 mL of 50/50 MeOH/H_2O and discard the eluant.

d) Elute the BAS 455 H and its metabolite CL 202347 out of the C18 cartridge with 2 mL of 90/10 MeOH/H_2O and collect the eluant into a culture tube.

3.4 PREPARATION OF THE FINAL VOLUME FOR LC/MS/MS QUANTITATION

Bring up volume of the elution solution from **Step 3.3.d** to 5 mL using HPLC water. Transfer an aliquot to an HPLC vial and proceed to LC/MS/MS analysis in **Step 3.6**. Further dilution should be made using 50/50 MeOH/H_2O.

3.5 LC/MS/MS INSTRUMENTATION AND CONDITIONS

The following instrument parameters are suggested for a Sciex API 3000 mass spectrometer:

Instrument:	Sciex API 300 LC/MS/MS Mass Spectrometer		
HPLC System	PE LC-200 Micro Pump		
Column:	Keystone Prism RP, 2.0 mm X 50 mm, 5 µ particle size		
Injection:	10 µL		
Gradient	Solution A: 4mM ammonium formate + 0.1% formic acid in water Solution B: 0.1% formic acid in water		
	Time (minutes)	A (buffer solution)	B (acidic water)
	0.0	75	25
	0.4	10	90
	3.0	10	90
	3.1	75	25
	5.0	75	25
Flow Rate:	0.4 mL/minute		
Expected Retention Times	**BAS 455 H:** About 1.75 minutes		**CL 202347:** About 1.58 minutes
Ionization Mode:	Positive Mode		
Transitions:	**BAS 455 H:** 282.1→212.0 282.1→194.0 (alternate)		**CL 202347:** 298.1→228.1 298.1→192.0 (alternate)

The following instrument parameters are suggested for a Finnegan LCQ Deca mass spectometer.

Instrument:	Finnigan LCQ Deca Mass Spectrometer Detector		
HPLC System	Agilent HPLC 1100 Series		
Column:	TosoHass TSK Super-ODS C18, 4.6mm X 50 mm, 2 µ particle size		
Injection:	100 µL		
Gradient	Solution A: 0.1% formic acid in water Solution B: 0.1% formic acid in methanol		
	Time (minutes)	A (Aqueous solution)	B (MeOH)
	0.0	50	50
	8	10	90
	10	10	90
	10.5	50	50
	12	50	50
Flow Rate:	1.0 mL/minute		
Expected Retention Times	**BAS 455 H:** About 5.7 minutes		**CL 202347:** About 7.6 minutes
Ionization Mode:	APCI, Positive Mode		
Transitions:	**BAS 455 H:** Segment 1, 0.0-6.5 min, 282.3→212.0, parent ion IsoW 1.5, CE 35%		**CL 202347:** Segment 2, 6.5-10 min, 298.3→228.0, parent ion IsoW 1.5, CE 35%
Divert Valve	0.0 min to waste, 3.0 min to source, 8.2 min to waste		
More MS Conditions	Sheath gas flow: 80 mL/min, Aux gas flow: 4 mL/min, Vaporizer temperature: 450 ^{0}C, Capillary temperature: 150 ^{0}C, Injection waveform: off, MSn microscans: 3, MSn maximum ion time: 100 ms, MSn AGC target: 2 x 10^{7}		

NOTE: Suggested LC/MS/MS operating conditions could be modified if necessary. Additionally, other LC/MS/MS instrumentation may be used.

3.6 CALIBRATION PROCEDURES

Calculation of results is based on peak area measurements (or peak height) using a calibration curve. The standard curve is obtained by direct injection of the mixed BAS 455H and CL 202347 standards into LC/MS/MS in the range of 0.25 ng/mL to 2.0 ng/mL. In a given injection run, the same volume is used for all samples and standards. Typical standard amounts injected on-column (10 µL) range as follows: 2.5, 5.0, 10, and 20 pg. Other concentrations may be used as needed. The calibration curves are obtained by plotting peak area or height, monitoring transitions m/z 282→212 for BAS 455 H, and transitions m/z 298 → 282 for CL 202347. The linear least squares working curve in the form $y = bx + c$ is used for the construction of the calibration curve. For each injection set, the set should begin and end with standard injections, and each standard level should be injected at least in duplicate.

3.7 LIMIT OF QUANTITATION AND LIMIT OF DETECTION

The limit of quantitation is defined as the lowest fortification level successfully tested. The limit of quantitation (LOQ) of the method for all analytes in all matrices is 0.05 ppm. The estimated limit of detection (LOD) is 12.5 pg/100 µL (concentration on the LC column) which is 50% of the lowest standard and has signal/noise > 3.

4 CALCULATION OF RESULTS

4.1 PRINCIPLE

Calculation of results is based on peak area (or height) measurements. The residues of BAS 455 H and CL 202347 are calculated from the calibration curve and the equations shown in Section 4.2 as BAS 455 H equivalents.

4.2 CALCULATION OF RESIDUES

The residues of **BAS 455 H** in µg/g (ppm) are calculated with the following formula and expressed as BAS 455 H equivalents.

$$\text{Residue (ppm)} = \frac{V_E \times W_A \times MWCF}{G \times A_F \times V_I \times 1000}$$

V_E	=	Final Volume after dilutions (mL)
W_A	=	Amount of analyte from calibration curve (pg)
G	=	Sample weight extracted (g)
A_F	=	Aliquot factor (e.g. 1% = 0.01)
V_I	=	Injection volume (µL)
1000	=	Factor remaining after all unit conversions

MWCF (Molecular Weight Conversion Factor):
= 1.0 for BAS 455 H
= 0.9462 to convert, CL 202347 to BAS 455 H

4.3 CALCULATION OF RECOVERIES

The recoveries of spiked BAS 455 H and CL 202347 are calculated with the following formula:

$$\text{Residue (ppm)} = \frac{V_E \times W_A}{G \times A_F \times V_I \times 1000}$$

V_E	=	Final Volume after dilutions (mL)
W_A	=	Amount of analyte from calibration curve (pg)
G	=	Sample weight extracted (g)
A_F	=	Aliquot factor
V_I	=	Injection volume (µL)
1000	=	Factor remaining after all unit conversions

Recovery % is calculated as follows:

$$\text{Recovery \%} = \frac{\text{Residue in fortified sample (ppm) - Residue in control (ppm)}}{\text{Amount analyte fortified (ppm)}} \times 100$$

5 TIME REQUIREMENT FOR ANALYSIS

The laboratory time required for a set of 12 samples, 2 recoveries and a control is approximately 8 person-hours. This does not include LC/MS/MS analysis and calculation times, and also does not account for any special problems that may arise, such as matrix interference.

6 CONFIRMATORY TECHNIQUES

The method allows for the determination of BAS 455 H and CL 202347 by LC/MS/MS, which is a highly selective detection technique, therefore, no confirmatory technique is required.

7 SAFETY AND HEALTH CONSIDERATIONS

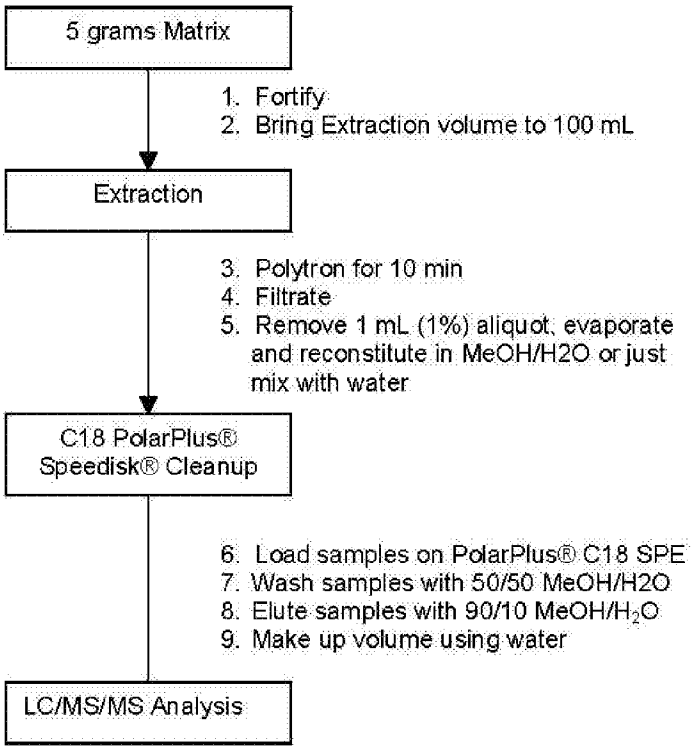
All procedures involving organic solvents should be performed under a well-ventilated hood. Personal protective equipment (gloves, eye protection, and lab coats) should be worn while performing this method. Heed all label statements and precautions.

8 REFERENCES

Blinn, C. Roger "CL 92,553: Metabolism XIV. Uptake and Residues of Radioactivity in Winter Wheat Grown in Soil Treated with Carbon-14 Labeled PROWL® Herbicide", American Cyanamid Report Number PN-640-007.

APPENDIX

FIGURE 1: FLOW CHART FOR THE ANALYTICAL METHOD



APPENDIX B

Example Calculations

The recoveries of spiked BAS 455 H and CL 202347 are calculated with the following formula:

$$\text{Residue (ppm)} = \frac{V_E \times W_A}{G \times A_F \times V_I \times 1000}$$

V_E	=	Final Volume after dilutions (mL)
W_A	=	Amount of analyte from calibration curve (pg)
G	=	Sample weight extracted (g)
A_F	=	Aliquot factor
V_I	=	Injection volume (µL)
1000	=	Factor remaining after all unit conversions

Recovery % is calculated as follows:

$$\text{Recovery \%} = \frac{\text{Residue in fortified sample (ppm) - Residue in control (ppm)}}{\text{Amount analyte fortified (ppm)}} \times 100$$

Example: Wheat Straw Sample from master sheet 198172-32 – 0.05 ppm Fortification [lab sample #5, filename (index) 040324.wiff(35)]

V_E	=	5.0 mL
W_A	=	3.98 pg
G	=	5.0 g
A_F	=	0.01
V_I	=	10 µL

Residue in corresponding control (ppm) = ND (0.00)

$$\text{Residue (ppm)} = \frac{5.0 \times 3.98}{5.0 \times 0.01 \times 10 \times 1000} = 0.0398 = 0.040 \text{ ppm}$$

$$\text{Recovery \%} = \frac{0.040 - 0.00}{0.050} \times 100 = 80\%$$

APPENDIX C

Protocol Changes

There were two changes to the protocol for this study.

Protocol deviation #1 to the Analysis Phase stated that one control sample (instead of two) was analyzed for each matrix.

Protocol deviation #2 to the Analysis Phase indicated that in method step 3.2c, the analyst centrifuged the samples at a speed of approximately 3600 rpm instead of 10,000 rpm. Since there was no adverse affect, the draft method was updated to permit a broader range of centrifuge speeds.