Hexazinone (DPX-A3674)

Determination of Ethyl Carbamate (IN-08387) in Hexazinone Technical
Headspace Gas Chromatographic (GC) Trace Level Method

I. Principle

The sample is heated in the headspace unit until equilibrium is reached between the solid and the vapor phases. The vapor containing ethyl carbamate (ETC), as well as the other volatile components, is automatically injected into the gas chromatograph. Separation of ethyl carbamate from sample components is accomplished on a J&W DB-Wax column and detected using a flame ionization detector. External standard technique is used for method calibration. The weight percent of ethyl carbamate in each sample is determined by comparison to a calibration curve (peak area vs. concentration) prepared from the analysis of standards prepared by spiking hexazinone technical with ethyl carbamate.

II. Applicability

This method is applicable for the determination of ethyl carbamate in technical hexazinone samples at concentrations ranging from 10 ppm to 50 ppm.

This method was developed to include validation data on linearity, precision (repeatability/reproducibility), accuracy (recovery), limitations and interferences. The data was generated in accordance with U.S. EPA Good Laboratory Practice Standards (40 CFR Part 160), which are consistent with the OECD Principles of Good Laboratory Practice. (Comment 1)

III. Limitations

There are no known chromatographic interferences with ethyl carbamate employing the conditions specified in the method.

Headspace analysis relies on equilibration between the solid, liquid and vapor phase in both the sample and the standard. The standard and sample should have as close to the same weight and chemical make-up as possible.

IV. Statistical Characterization

A. Linearity

Linearity of the calibration curve (area vs. amount) and sensitivity was demonstrated by analysis of preparations of analytical standards at five different levels. The method of linear least squares was used to determine the equation for the straight line that best fits the experimental data. The following values were obtained for the slope, y-intercept, and correlation coefficient:

Amount range (ethyl carbamate): = 10 - 50 μg/g

Slope (sensitivity): = 40.751 (area /amount)

Intercept: = 65.284 (area)

Correlation Coefficient (r): = 0.9988

B. Precision

1. Repeatability

The repeatability of the method for ethyl carbamate was determined utilizing 10 replicate test portions of one sample (DPX-A3674-341) spiked with ethyl carbamate at 25 ppm, analyzed by one analyst on the same day.

The mean range ($\overline{X}$), standard deviation (σ) and relative standard deviation (RSD) for analyses are as follows:

Component	$\overline{X}$ Range	σ	RSD
ethyl carbamate, ppm	26.7	1.5	5.6%

There was 1 outlier in the data set which was removed for statistical analysis.

2. Reproducibility

The reproducibility of the method for ethyl carbamate was determined utilizing 10 replicate test portions of one sample (DPX-A3674-341) spiked with ethyl carbamate at 25 ppm, analyzed in 2 labs on two days. (Comment 1)

The mean range ($\overline{X}$), standard deviation (σ) and relative standard deviation (RSD) for analyses are as follows:

Component	$\overline{X}$ Range	σ	RSD
ethyl carbamate, ppm	28.3	4.6	16.3%

There was 1 outlier in the data set from day 1 which was removed for statistical analysis.

C. Accuracy

Duplicate test portions of hexazinone technical (DPX-A3674-341) were spiked at two levels (nominally 25 ppm and 35 ppm) with a solution containing a predetermined amount of ethyl carbamate. Each sample was then analyzed according to the sample procedure specified in this method. The average recoveries are as follows:

ppm Spiked	ppm Recovered	% Recovery
23.7	21.7	91.7%
24.6	24.6	99.9%
32.6	36.4	111.6%
33.7	36.5	108.3%

The average recovery for the analysis was 102.9%.

D. Limits of Detection and Quantitation

The limit of quantitation of the method for ethyl carbamate was demonstrated utilizing 5 replicate test portions of one sample (DPX-A3674-341) spiked with ethyl carbamate at 10 ppm, analyzed by one analyst on the same day.

The mean range ($\overline{X}$), standard deviation (σ) and relative standard deviation (RSD) for analyses are as follows:

Component	$\overline{X}$ Range	σ	RSD
ethyl carbamate, ppm	10.8	1.3	11.9%

The limit of quantitation for the method has been demonstrated to be at least 10 ppm.

V. Special Apparatus (Equivalent apparatus may be substituted)

1. Gas chromatograph: HP 6890 Series GC, equipped with split/splitless injector, a flame ionization detector and Electron Pressure Control. Hewlett-Packard Company (currently Agilent Technologies), 2850 Centerville Road, Wilmington, DE 19808-1610.
2. Headspace Sampler: HP 7694 equipped with auto-sampler trays and heating oven, Hewlett-Packard Company (currently Agilent Technologies), 2850 Centerville Road, Wilmington, DE 19808-1610. (Comment 2)
3. Analytical column: 30 m x 0.53 mm i.d., 1-µm film thickness, DB Wax, J & W Scientific, Catalog No. 1257032, (J & W Scientific, Folsom, CA 95630).
4. Sample vials: septa and caps, 20 mL, Hewlett Packard, Catalog No. 19395-280020, (Hewlett Packard Co., Valley Forge, PA 19842).
5. Injection liner: split/splitless inlet sleeve for HP 6890 Series GC, SGE FocusLiner manufactured for Agilent, 78.5 X 6.3 X 4 mm, Part No. 210-4004-5, supplied by VWR, Catalog No. 21513-680.
6. Micropipettor: Eppendorf Electronic Pipettor, 4850 Series, 100-µL variable volume, VWR, 53512-207.

VI. Reagents (Reagent grade except as noted)

1. n-Hexane, Fischer Scientific Catalog No. H3021, (Fischer Scientific, Houston, TX 77099).
2. Hexazinone, reference standard grade, (DuPont Crop Protection, Stine-Haskell Research Center, Newark, DE 19714-0030).
3. Ethyl carbamate (urethane), Aldrich Chemical Co. Catalog No. U285-7, (Aldrich Chemical Co. Milwaukee, WI 53201).
4. Nitrogen, Zero grade.

VII. Special Safety Considerations

A. Product Hazards

Hexazinone is corrosive. Exposure to hexazinone by eye contact may cause eye irritation with discomfort, tearing, or blurring of vision. Ingestion may result in abnormal liver function. Avoid contact with eyes and skin. Wear gloves when handling. Wash thoroughly after handling. In case of skin or eye contact, flush with copious amounts of water. Contact a physician. Consult MSDS #M0000071 for detailed information.

B. Procedure Hazards

Ethyl carbamate is a cancer suspect agent. Do not breathe ETC dust. Avoid contact with eyes, skin and clothing. Refer to area MSDS #B0001381 for detailed information.

n-Hexane is a flammable liquid. Avoid skin contact. Refer to area MSDS #L0000758 for detailed information.

VIII. Procedure

A. Operating Conditions

Chromatographic Conditions:

Gas chromatograph	
Type:	HP
Model Number:	6890
Carrier gas:	
Type:	He (Comment 3)
Mode :	Constant flow
Flow rate, mL/min:	6
Injection port:	
Temperature, °C:	250
Injection mode:	Splitless
Purge, min:	0.75
Purge flow, mL/min:	15
Column:	
Length, m:	30
I. D., mm:	0.53
Phase:	DBWAX
Thickness, µm:	1
Temperature limit, °C:	250

Column temperature:	
Initial temperature, °C:	90
Initial hold time, min:	1.0
1st Ramp rate, °C/min:	5
Final temperature, °C:	150
Final hold time, min:	0 (Comment 4)
2nd Ramp rate, °C/min:	30
Final temperature, °C:	220
Final hold time, min:	2.67
Detector:	
Type:	FID
Temperature, °C:	300
H_2, mL/min:	35
Air, mL/min :	450
N_2 (make-up), mL/min:	40 (Comment 5)
Headspace Sampler	
Type	HP
Model Number	7694
Carrier gas	He (Comment 3)
Pressurization gas	He
Sample Loop, mL:	1.0
Oven, °C:	100
Shake:	Low
Loop Temperature, °C:	110
Transfer Line, °C:	110
GC Cycle Time, min:	20
Vial EQ Time, min:	40 (Comment 6)
Pressurization Time, min:	0.2
Loop Fill Time, min:	0.1
Loop EQ Time, min:	0.05
Inject Time, min:	1.00

B. Calibration

Preparation of Standard Solutions (Comment 7)

1. Weigh 0.10 g (± 0.1 mg) of ETC into a 100-mL volumetric flask. Add 90 mL of n-hexane. Sonicate until dissolved. Equilibrate to room

temperature. Dilute to volume with n-hexane. Label the flask “1 mg/mL ETC Stock Solution”.

2. Accurately weigh into four sample vials 1.0 g (± 0.1mg) of hexazinone analytical standard.
3. Using a micropipettor, add to the first vial, 10 µL of the 1 mg/mL ETC Stock Solution. Label vial as “10 ppm ETC Standard”.
4. Using a micropipettor, add to the second vial, 25 µL of the 1 mg/mL ETC Stock Solution. Label vial as “25 ppm ETC Standard”.
5. Using a micropipettor, add to the third vial, 50 µL of the 1 mg/mL ETC Stock Solution. Label vial as “50 ppm ETC Standard”.
6. Gently evaporate the n-hexane from the standard vials using a low N_2 purge.
7. After the sample has reached apparent dryness, securely attach the septum and cap to the vial.
8. Equilibrate the gas chromatograph and headspace analyzer.
9. Inject, singly, 1 mL from each standard vial.

NOTE: Due to the nature of the sampling system, the standard and sample should have as close to the same weight and chemical make-up as possible. (See Comment 8)

C. Sampling

Sample should be homogeneous and representative of the bulk material from which it was obtained.

D. Sample Analysis

Preparation of Sample Solutions

1. Accurately weigh 1.0 g (± 0.1 mg) of the sample (in duplicate) into sample vials.
2. Securely attach the septum and cap to each vial.

Analysis

1. Equilibrate the gas chromatograph and headspace analyzer.
2. Inject, singly, 1 mL from each sample vial.

E. Calculations

1. Prepare a calibration curve for ETC by plotting the peak area vs. concentration (ppm), corrected for purity, for each standard. (Comment 9).

2. Using the method of least squares, calculate the equation for the best line fit. The correlation coefficient of the least squares equation should be 0.99, or better.
3. Determine the sample impurity concentration (ppm) of the impurity of interest using the equation for the calibration curve, y = mx + b.

$$\text{Sample result (ppm)} = \frac{\text{peak area - y intercept}}{\text{slope}}$$

IX. Quality Control

1. Obtain a well-characterized sample of test material.
2. Label the sample as “ETC Audit”.
3. Analyze the audit sample according to the documented procedure to determine, employing appropriate SQC techniques, if the analysis is on aim.
4. When SQC analysis indicates the analysis is off aim, appropriate troubleshooting and corrective actions should be taken including recalibration of the method/instrument.

X. Comments

1. Second laboratory data for inter-laboratory reproducibility was not generated under GLP.
2. Initial method development was performed employing an HP 7694 headspace analyzer. Validation of the method was performed employing a Leap Technologies Combi-PAL autosampler with the following parameters.

Headspace Sampler	
Type:	Leap Technologies
Model Number:	Combi-PAL
Injection Volume, mL:	1
Oven, °C:	100
Incubation Time, min.:	40
Sample Shaker, rpm:	250 - 10 sec. On, 20 sec. off
Syringe Temperature, °C:	130
Syringe Fill Speed, µL/sec:	50
Syringe Injection Time, µL/sec:	750
GC Cycle Time, min:	30

3. Hydrogen may be substituted as carrier gas maintaining constant flow (linear velocity). Hydrogen is flammable and explosive in mixtures with air above 4% and below 75% by volume. It is recommended that hydrogen only be utilized as carrier gas employing GC’s equipped with electronic pressure control and safety shutdown devices (e.g., Agilent 6890).
4. Addition of an isothermal hold time at 150ºC may be implemented to allow ethyl carbamate to clear the column prior to the final thermal gradient segment.
5. Use of nitrogen makeup gas for the FID is recommended to optimize performance.
6. Equilibration times for the headspace analyzer may need to be lengthened in some cases to obtain reproducible results.
7. Method validation was performed with a 5-point calibration at 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm. For routine operation the calibration may be reduced to a 3-point calibration curve at 10 ppm, 25 ppm and 50 ppm.
8. Headspace analysis relies on equilibration between the solid, liquid and vapor phase in both the sample and the standard. The standard and sample should have as close to the same weight and chemical make-up as possible. It is recommended for optimal accuracy that calibration be performed utilizing the hexazinone technical sample to be analyzed as the calibration matrix.
9. Peak height may be utilized in place of peak area for this analysis.

XI. References

Hewlett Packard HP7694 Headspace Sampler Operating and Service Manual. (Hewlett Packard Co., Valley Forge, PA 19842.)

XII. Appendix

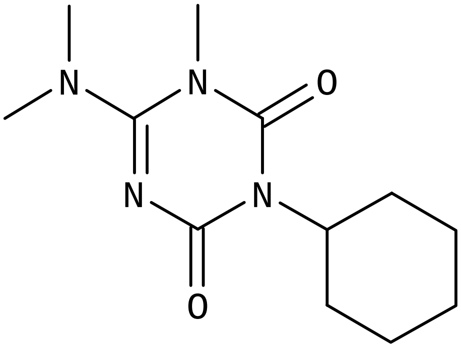
A. Chemical Structures

B. Chromatograms

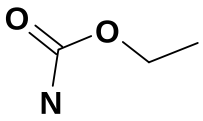
C. Calibration Curve

APPENDIX A

Chemical Structures



Hexazinone



Ethyl Carbamate

APPENDIX B

Chromatograms

GC Chromatogram of a Hexazinone Standard Containing 25.0 ppm of Ethyl Carbamate.

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                                  Exygen Research

Gas Chromatography          Ethyl Carbamate Analysis               Data Report
                            Dataset ~~051705~~ 052305
Sample: 25 ppm std                    062005 Acq On:5/23/05 8:47:16->5/23/05 8:4->
Misc.:  P1397, L5310-1, C72645        CC
        DPX-A3674-341

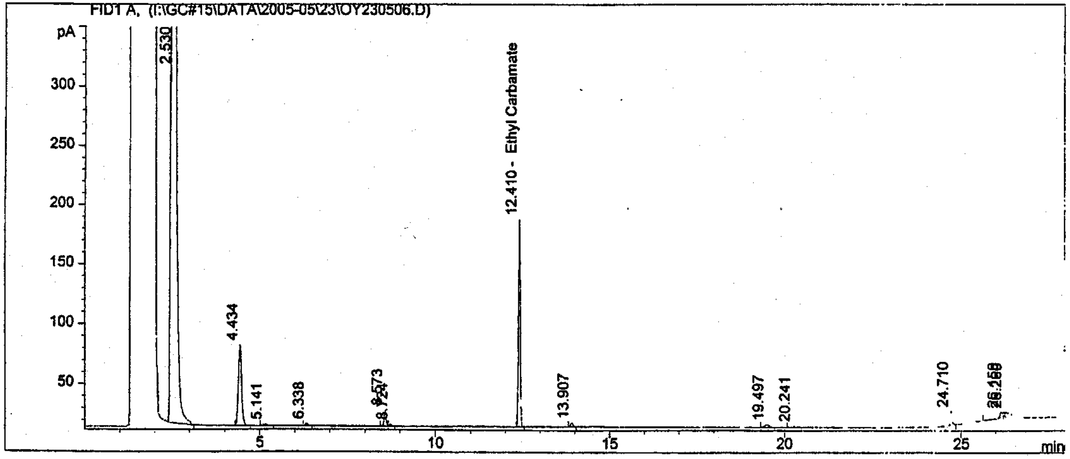

                        FID1 A,

Inst:                   PC03043
Operator:               DJarrell
Raw Data Filename:      I:\GC#15\DATA\2005-05\23\OY230506.D
Aquisition Method:      P1397.M
Analysis Method:        I:\GC#15\METHODS\P1135.M

                             Analysis Report

|  Retention          Compound          Peak       Peak    |
|  Time  (min)          Name            Width      Area    |
|_______________ ____________________ ________ ____________|
|        2.530                           0.118    7517.554|
|        4.434                           0.096     435.514|
|        5.141                           0.108      13.688|
|        6.338                           0.079      11.969|
|        8.573                           0.063      54.778|
|        8.724                           0.067       7.159|
|       12.410 Ethyl Carbamate            0.055     615.433|
|       13.907                            0.076      16.977|
|       19.497                            0.111      19.810|
|       20.241                            0.116      10.344|
|       24.710                            0.044      31.873|
|       26.159                            0.042      10.398|
|       26.260                            0.034       3.385|
----------------------------------------------------------
                                              Total Area
                                                8748.883
```

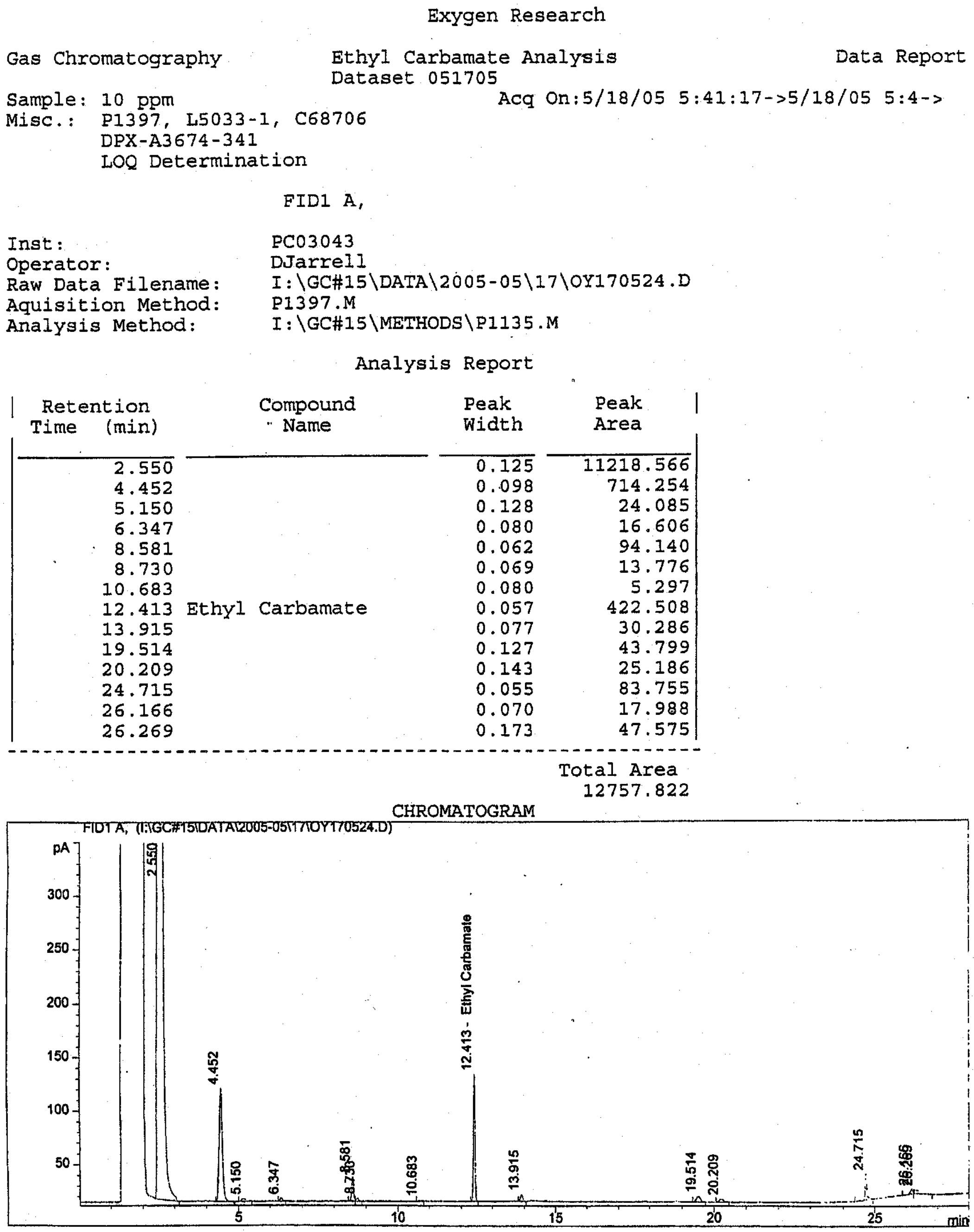
CHROMATOGRAM



APPENDIX B (Cont.)

UChromatograms

GC Chromatogram of a Hexazinone Sample (DPX-A3674-341) Containing 10 ppm of Ethyl Carbamate.



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                                      Exygen Research

Gas Chromatography          Ethyl Carbamate Analysis              Data Report
                            Dataset 051705
Sample: 10 ppm                         Acq On:5/18/05 5:41:17->5/18/05 5:4->
Misc.:  P1397, L5033-1, C68706
        DPX-A3674-341
        LOQ Determination

                           FID1 A,

Inst:                     PC03043
Operator:                 DJarrell
Raw Data Filename:        I:\GC#15\DATA\2005-05\17\OY170524.D
Aquisition Method:        P1397.M
Analysis Method:          I:\GC#15\METHODS\P1135.M

                                 Analysis Report

|  Retention             Compound             Peak        Peak    |
|  Time   (min)           Name                Width       Area
|
|         2.550                               0.125   11218.566|
|         4.452                               0.098     714.254|
|         5.150                               0.128      24.085|
|         6.347                               0.080      16.606|
|         8.581                               0.062      94.140|
|         8.730                               0.069      13.776|
|        10.683                               0.080       5.297|
|        12.413  Ethyl Carbamate              0.057     422.508|
|        13.915                               0.077      30.286|
|        19.514                               0.127      43.799|
|        20.209                               0.143      25.186|
|        24.715                               0.055      83.755|
|        26.166                               0.070      17.988|
|        26.269                               0.173      47.575|
------------------------------------------------------------------
                                                     Total Area
                                                      12757.822
                                 CHROMATOGRAM
```

APPENDIX B (Cont.)

Chromatograms

GC Chromatogram of a Hexazinone Sample (DPX-A3674-341) Unfortified.

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                                         Exygen Research

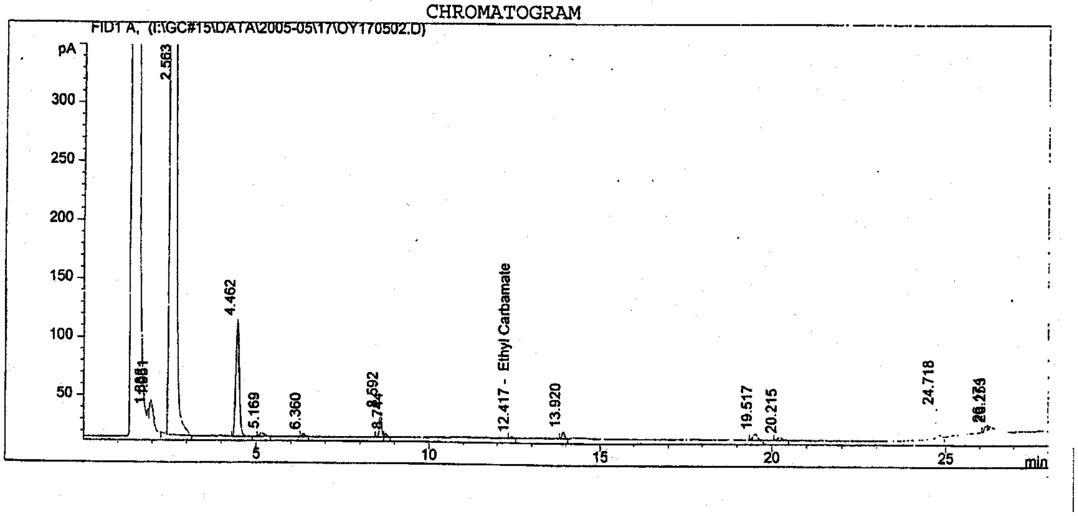
Gas Chromatography          Ethyl Carbamate Analysis               Data Report
                            Dataset 051705
Sample: Blank                             Acq On:5/17/05 6:23:19->5/17/05 6:2->
Misc.:  P1397, L5033-1, C68706
        DPX-A3674-341


                          FID1 A,

Inst:                     PC03043
Operator:                 DJarrell
Raw Data Filename:        I:\GC#15\DATA\2005-05\17\OY170502.D
Aquisition Method:        P1397.M
Analysis Method:          I:\GC#15\METHODS\P1135.M

                               Analysis Report

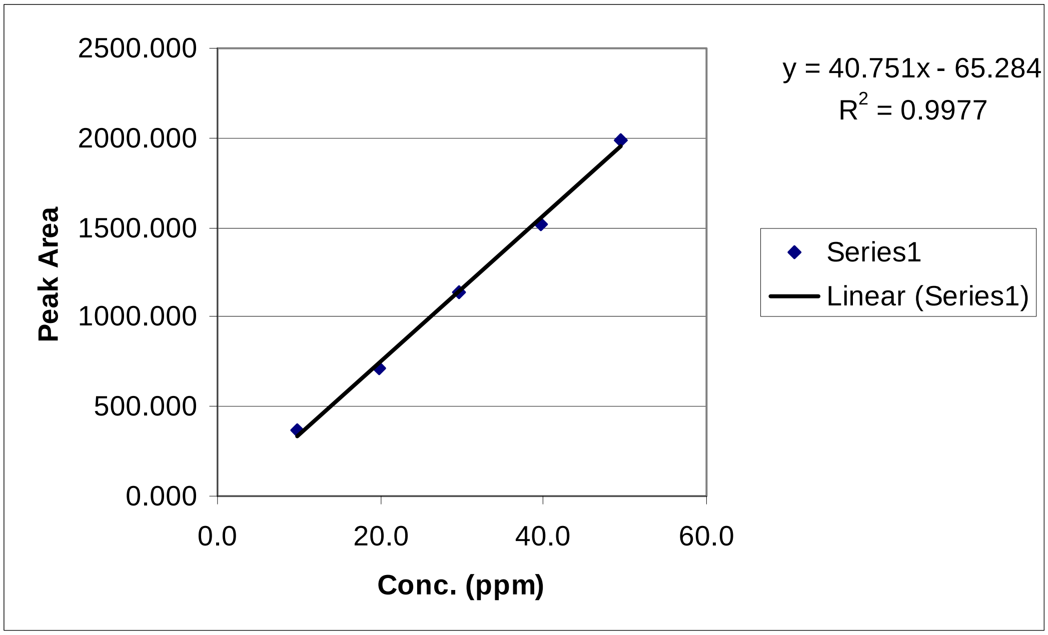
| Retention          Compound             Peak       Peak     |
 Time  (min)           Name               Width      Area
|____________  ____________________   _________  ____________ |
       1.885                              0.050       22.041
       1.951                              0.086       94.203
       2.563                              0.122    10667.997
       4.462                              0.101      663.104
       5.169                              0.110       25.014
       6.360                              0.083       14.599
       8.592                              0.064       87.717
       8.744                              0.066       13.260
      12.417 Ethyl Carbamate              0.057        4.954
      13.920                              0.077       28.704
      19.517                              0.134       44.841
      20.215                              0.139       23.109
      24.718                              0.059       97.477
      26.174                              0.081       17.342
      26.253                              0.026        2.894
-----------------------------------------------------------
                                                Total Area
                                                 11807.255
                                  CHROMATOGRAM
```



APPENDIX C

Calibration Curve

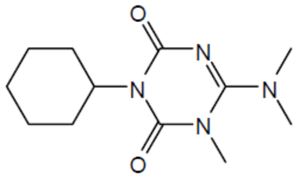
Representative calibration curve (5-point) for determining ethyl carbamate obtained by plotting the ethyl carbamate peak area vs. the concentration of ethyl carbamate. Slope = 40.751; y-intercept = 65.284; Correlation coefficient (r) = 0.9988.



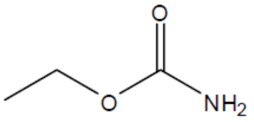
4. Appendices

Appendix 1 Analytical Method of Hexazinone TGAI for Ethyl Carbamate (NO. NC2009038C)

1. Introduction

Test Item:	**Hexazinone TGAI**
Classification:	Herbicide
Active Ingredient:	Hexazinone
Empirical Formula:	$C_{12}H_{20}N_4O_2$
Molecular Weight:	252.3
CAS Register Number:	51235-04-2
IUPAC Name:	3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-(1H,3H)-dione
Structure Formula:	

Impurity Information		
Impurity Name	**Empirical Formula**	**Structure**

Impurity I	$C_3H_7NO_2$	

2. Summary

Limit Test of Impurity I in Hexazinone TGAI is performed through HPLC-MSD system, separated on a ZORBAX XDB C8 reverse phase column, detected by MS detector in SIM mode with extract ion of 90 and 112, and analyzed by comparing with external standard.

3. Determination of Impurity I in Hexazinone TGAI

3.1 Apparatus

Test system: HPLC-MSD 1100 system (Agilent Company).

Glassware: Volumetric;

Balance: Mettler Toledo XS 205;

Vials: 2 ml volume.

3.2 Reagents

Impurity I standard: known purity;

Hexazinone TGAI for analysis:

Acetic acid: Analytical grade;

Acetonitrile: HPLC grade;

Water: Redistilled;

4. Experimental Conditions for Chromatography

The following conditions have been established using an Agilent 1100 HPLC/MSD system. Chromatographic conditions may be changed to obtain satisfactory performance with other instruments provided adequate resolution and sensitivity are achieved.

Equipment: Agilent HPLC-MSD 1100 system.

Column: ZORBAX C8 150 x 4.6 mm, 5 micron

Mobile phase:

Time (min)	Acetonitrile (%)	Acetic acid aqueous solution (pH = 2.8 - 3.2)
0	10	90
3	10	90
10	90	10

Column Temperature: 30℃

Sample size injected: 5.0 µl

Stop time: 10.0 min

Post time: 5 min

Drying gas flow N_2: 11.0 L/min,

Drying Gas Temperature: 350 ^{0}C

Ionization Mode: API-ES

Polarity: Positive

Neb Press: 45 psig

Capillary voltage: 4000 V

Fragmentor: 70 V for SIM run

Gain EMV: 1.0

Actual Dwell: 289

SIM ions: 90 and 112 m/Z

Retention Time: **Impurity I:** *ca 3.0* min

5 Limit test of Impurity I

5.1 Preparation of stock Impurity I standard solutions

Accurately weigh about 10 mg analytical standard grade Impurity I into a 100 ml volumetric flask, dissolve and make to volume with acetonitrile.

5.2 Preparation of Impurity I LOQ standard solution

Prepare the Impurity I standard solution at acceptable LOQ concentration obtained from the method validation of LOQ by diluting certain amount stock Impurity I standard solution 5.1 with acetonitrile.

5.3 Preparation of sample solution

Prepare in duplicate per batch.

Approximately 100 mg of Hexazinone TGAI is accurately weighed into a 10 mL volumetric flask, dissolved and made to volume with acetonitrile. The flask is mixed thoroughly.

Another approximately 100 mg of Hexazinone TGAI with same batch is accurately weighed into a 10 mL volumetric flask, dissolved and made to volume with Impurity I LOQ standard solution 5.2. The flask is mixed thoroughly.

5.4 Preparation of blank solution

An acetonitrile blank solvent solution is prepared.

5.5 Analysis

When the instrument gets to equilibrium, inject the blank solution. Then, inject Impurity I LOQ standard solution and sample solutions in the following sequence: Impurity I LOQ standard solution, twice non-spiked sample solution, twice spiked sample solution, Impurity I LOQ standard solution. Determine the area of the Impurity I peak.

5.6 Result

Compare Peak Area of Impurity I with LOQ standard solution. The corrected area in spiked samples is calculated as the subtraction of peak area in the spiked sample with the average peak area of the 2 nearest injections of the impurity I LOQ standard solution.

If both the area in TGAI and the corrected area in spiked sample are less than the area of LOQ standard solution, the content of Impurity I in the Hexazinone TGAI is given a limit test result of < LOQ (equivalent to W/W in Test Item). Otherwise, the quantitation method for Impurity I will be developed.