STUDY TITLE

Analytical Method and Validation for the Determination of Active Ingredient in 1,3-Dichloropropene Technical Grade Active Ingredient

DATA REQUIREMENT

U.S. EPA OCSPP Harmonized Test Guidelines OPPTS series 830.1800-
Enforcement Analytical Method;
EEC Guideline SANCO/3030/99 rev.4

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STUDY COMPLETED ON

November 20, 2015

PERFORMING LABORATORY

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LABORATORY STUDY ID

DAS-AM-G-15-28

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA sec. 10(g).

Company: Dow AgroSciences LLC

Company Agent: Jim Baxter

Title: Regulatory Manager

Signature: J.P. Baxter

Date: 12 November 2015

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STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Analytical Method and Validation for the Determination of Active Ingredient in 1,3-Dichloropropene Technical Grade Active Ingredient

Study Initiation Date: June 4, 2015

This report represents data generated in accordance to the following Good Laboratory Practice Standard.

United States Environmental Protection Agency
Title 40 Code of Federal Regulations Part 160
FEDERAL REGISTER, August 17, 1989

All aspects of this study were conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160, except that one of the test substances that was used for evaluation of interferences was obtained from a commercial supplier, and the GLP status is unknown.

J.P. Baxter — 12 November 2015

Jim Baxter
Sponsor
Dow AgroSciences LLC

Date

J.P. Baxter — 12 November 2015

Jim Baxter
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Date

Rose M Nelson — November 20, 2015

Rose Nelson
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Study Completion Date

Dow AgroSciences Quality Assurance Unit
Good Laboratory Practice Statement Page

Study ID: DAS-AM-G-15-28

Title: Analytical Method and Validation for the Determination of Active Ingredient in 1,3-Dichloropropene Technical Grade Active Ingredient

Study Initiation Date: 4-Jun-2015 **Study Completion Date:** 20-Nov-2015

GLP Quality Assurance Inspections

Date of GLP Inspection(s)	Date Reported to the Study Director and to Management	Phases of the Study which received a GLP Inspection by the Quality Assurance Unit
3-Jun-2015	4-Jun-2015	Protocol
22-Jun-2015	2-Jul-2015	Sample analysis
5, 9-10-Nov-2015	12-Nov-2015	Report and raw data

QUALITY ASSURANCE STATEMENT:

The Quality Assurance Unit has reviewed the final study report and has determined that the report reflects the raw data generated during the conduct of this study.

Patricia Maldonado
Dow AgroSciences, Quality Assurance

20-Nov-2015
Date

SIGNATURES

Rose Nelson — November 20, 2015

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Brandi Thomas
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STUDY PERSONNEL

Title: Analytical Method and Validation for the Determination of Active Ingredient in 1,3-Dichloropropene Technical Grade Active Ingredient

Study Director:	Rose Nelson
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TABLE OF CONTENTS

I. ABSTRACT

This report describes the validation of an analytical method for determination of cis-1,3-dichloropropene and trans-1,3-dichloropropene in 1,3-dichloropropene (1,3-D) technical grade of active ingredient (TGAI). A gas chromatography (GC) method was validated using a DB-1701 column, 60 m x 0.32 mm x 1 µm film thickness. Concentrations were determined using internal standard calibration.

The results for linearity and precision for 1,3-dichloropropene, and linearity for the internal standard are presented below.

Parameter		**Cis-1,3-dichloropropene**	**Trans-1,3-dichloropropene**	**Total 1,3-Dichloropropene**	**Internal Standard**
Method Precision (n=10)	Avg wt %	48.2	49.5	97.7	N/A
	% RSD	0.19	0.14	0.15	
Linearity (n=7)	mg/mL range	26.6 to 103.5	22.8 to 88.7	N/A	22.2 to 90.9
	Equivalent Wt.%	25.3 to 98.4	21.7 to 84.3	N/A	N/A
	Coefficient of Determination (r^2)	1.0000	1.0000	N/A	0.9999

II. INTRODUCTION

A. Scope

This gas chromatography method is applicable to the determination of cis-1,3-D and trans-1,3-D in 1,3-dichloropropene technical grade of active ingredient.

B. Principle

An aliquot of a sample is mixed with chlorobenzene internal standard, diluted with ethyl acetate, and analyzed by GC using a DB-1701 column, 60 x 0.32 mm x 1 µm film, with a thermal conductivity detector (TCD). Quantitation is by internal standard calibration using peak areas.

This study was conducted under protocol DAS-AM-G-15-28. The protocol was amended (Amendment #1) to remove hydrogen as an alternate carrier gas. Validation was conducted using only helium as carrier gas. The validation data for the helium carrier gas are given in this report. Amendment #2 corrected typographical errors in purity and recertification dates of test substances which were used to evaluate interferences. Amendment #3 was written to update the purity and recertification dates for those test substances which expired after the experimental portion of the study was completed. The most current purity data and recertifications dates for all test substances are included in this report.

III. MATERIALS AND METHODS

A. Equipment

1. Analytical balance, capable of measuring to 0.1 or 0.01 mg
2. Agilent 6890 gas chromatography (GC) system equipped with an autosampler, split/splitless injector and thermal conductivity detector
3. Data acquisition and processing system: Agilent EZChrom Elite
4. GC column: DB-1701, 60 m x 0.32 mm x 1µm film thickness
5. Autosampler vials and caps: 2 mL with screw caps
6. Miscellaneous laboratory glassware
7. Restek SKY liners, split/splitless: 4 mm straight with glass wool, part number 23300.5 (pack of 5 for Agilent GCs)
8. Eppendorf pipets and tips
9. Positive displacement pipets and tips

B. Reagents and Standards

1. Test substance:

	Test Substance Number	Purity	Recertification date
1,3-dichloropropene	TSN307909	97.5% cis + trans; 48.1% cis; 49.4% trans	Aug. 31, 2017

Proprietary test substances used for interference evaluation only:

Test Substance/Lot Number	Purity	Recertification date
AGR277102	99%	12-Aug-2017
AGR238091	97%	30-Aug-2017
TSN030278-0001	96%	02-Aug-2016
TSN106505	98%	11-Aug-2018
TSN028018-0001	100%	12-Aug-2017
TSN303599	77%	24-Aug-2016
TSN303946	83%	14-Aug-2016
AGR238090	99.5%	30-Aug-2017
TSN301451	97%	02-Aug-2019
AGR238088	94%	03-Aug-2017
TSN307901	91%	02-Aug-2017
AGR238086	88%	2-Aug-2017
TSN030579-0001	97%	7-Aug-2017
TSN106329	94%	3-Aug-2017
TSN304464	95%	7-Aug-2017
TSN303759	92%	28-Aug-2017
TSN303032	100%	28-Aug-2017
TSN303341	97%	14-Aug-2016
MKBG8560V*	99.9%	27-Mar-2017

*Obtained from a commercial supplier; GLP status is unknown

2. Reference substance:

	Reference Substance Number	Purity	Recertification date
1,3-dichloropropene	TSN306293	99.0% cis + trans; 53.3% cis-1,3-D, 45.7% trans 1,3-D	Aug. 12, 2016

3. Test System: Ethyl acetate, 99.7%, Sigma Aldrich

4. Internal standard: Chlorobenzene, 99.9%, Aldrich

C. Safety

Each analyst was acquainted with potential hazards of the reagents, products and solvents before beginning laboratory work. Sources of information included: safety data sheets, literature and other related data. Disposal of reagents, reactants, and solvents were in compliance with local, state and federal laws and regulations.

D. Analytical Procedures

1. Preparation of the Calibration Solutions
   A capped 1 ounce jar was placed on an analytical balance, and the balance was tared. The cap was removed, and using an Eppendorf pipet, approximately 1 mL of the chlorobenzene internal standard was added. The container was capped and the weight was recorded. The balance was then tared. The cap was removed, and using an Eppendorf pipet, approximately 2 mL of the analytical standard was added into the container. The jar was capped, placed on the balance, and the weight was recorded. The jar was then uncapped, and using a graduated cylinder, approximately 20 mL of ethyl acetate was added. The jar was capped and the solution was shaken by hand. Aliquots were transferred into autosampler vials for GC analysis.

2. Calibration Procedure
   The calibration solutions were injected at least twice into the gas chromatograph, using the conditions summarized in Section III.E., and the response factors for cis and trans-1,3-dichloropropene were calculated using the equations given in Section III.F. The average of the response factors was used for calibration. A typical chromatogram of the calibration solution is shown in Figure 1.

3. Sample Preparation and Analysis
   A capped 1 ounce jar was placed on an analytical balance, and the balance was tared. The cap was removed, and using an Eppendorf pipet, approximately 1 mL of the chlorobenzene internal standard was added. The container was capped and the weight was recorded. The balance was then tared. The cap was removed, and using an Eppendorf pipet, approximately 2 mL of the sample was added into the container. The jar was capped, placed on the balance, and the weight was recorded. The jar was then uncapped, and using a graduated cylinder, approximately 20 mL of ethyl acetate was added. The jar was capped and the solution was shaken by hand. Aliquots were transferred into autosampler vials for GC analysis.

4. Preparation of Precision Samples
   Five precision samples were prepared as detailed in section III.D.3 and analyzed on each of two days to evaluate method precision. Single injections of the samples were analyzed using the conditions given in Section III.E. The day one precision samples were re-analyzed four days after preparation to evaluate stability of the analytes in the prepared standards and samples. An additional precision sample (Prec Day 1-1) was used to evaluate system precision by injecting a total of five times. A typical chromatogram of a sample solution is shown in Figure 2.

5. Preparation of Linearity Solutions
   Seven standard and internal standard linearity solutions were prepared by adding chlorobenzene internal standard and 1,3-dichloropropene analytical standard into seven 25 mL volumetric flasks and diluting to volume with ethyl acetate.

6. Preparation of Samples for Evaluation of Interferences
   Analytical Standard: A solution containing only the analytical standard was prepared by adding a 2 mL aliquot of 1,3-D analytical standard into a 1 ounce jar. Using a graduated cylinder, 20 mL of ethyl acetate was added to the jar, which was shaken by hand to mix.

   Internal Standard: A solution containing only the internal standard was prepared by adding a 1 mL aliquot of chlorobenzene to a 1 ounce jar followed by 20 mL of ethyl acetate. The solution was shaken by hand to mix.

   Impurity Standards: Solutions of individual 1,3-D impurity reference compounds were prepared by adding 20 µL of each reference standard into separate jars, each containing 20 mL of ethyl acetate. The solutions were shaken by hand to mix.

   An aliquot of the solvent blank was also analyzed.

E. Instrumentation

The following analytical conditions describe the analysis of samples for cis and trans-1,3-dichloropropene in 1,3-dichloropropene technical grade active ingredient.

Chromatograph:	Agilent 6890	
Column:	DB-1701 60 m x 0.32 mm x 1 µm film	
Flow rate:	1.9 mL/min helium (constant flow mode)	
Inlet:	Split at 130°C	
Inlet liner:	Restek Sky 4 mm straight liner with glass wool	
Inlet conditioning:	Make at least 10 injections of a standard solution prior to instrument calibration and sample analysis	
Injection volume:	1 µL, split ratio 38:1, one injection per vial	
Syringe wash solvent:	Ethyl acetate	
Injector parameters:	Syringe type: 5 or 10 µL with metal plunger Note: Do NOT use syringes with a Teflon tipped plunger Sample washes: 3; sample pumps: 3 Syringe wash: 3 for wash solvent A; 3 for wash solvent B Syringe speed: fast; Viscosity delay: 1 second	
Temperature program:	40°C hold for 2 minutes; 5°C/minute to 80°C, hold for 7.5 minutes; 5°C/minute to 110°C, hold for 1 minute; 25°C/minute to 270°C, hold 0 minutes	
Detector:	TCD at 280 °C	
Make-up:	5 mL/min helium	
Reference:	15 mL/min helium	
Run Time:	30.9 minutes	
Integrator:	Agilent EZChrom Elite	
Approximate Retention Times:	Cis-1,3-dichloropropene	~14.2 min
	Trans-1,3-dichloropropene	~16.7 min
	Chlorobenzene (internal standard)	~20.6 min

Approximate time to prepare and analyze sample: 2 hours

F. Methods of Calculation

1. Calculation of the weight of cis-1,3-D or trans-1,3-D in the calibration solutions:

$$Wt_x = Wt_{AS} \times Purity_{AS}$$

where:

Wt_x = Weight of cis-1,3-D or trans-1,3-D in the calibration standard solution, g
Wt_{AS} = Weight of the analytical standard added to the calibration solution, g
$Purity_{AS}$ = Purity of the analytical standard, expressed as a decimal

The following is an example calculation for cis-1,3-D in calibration solution STD 2 – Prec Day 1:

$$Wt_X = 2.42111\ g \times 0.533 = 1.290\ g$$

where:

Wt_x = Weight of cis-1,3-D in the calibration standard solution (1.290 g)
Wt_{AS} = 2.42111 g
$Purity_{AS}$ = 53.3% = 0.533

2. Calculation of the response factor for cis-1,3-D or trans-1,3-D in the calibration solutions:

$$Rf_x = \frac{Wt_x \times A_{ISTD}}{Wt_{ISTD} \times A_x}$$

where:

Rf_x = Response factor for cis or trans-1,3-D
Wt_x = Weight of cis-1,3-D or trans-1,3-D in standard solution, g
A_x = Area of cis-1,3-D or trans-1,3-D peak obtained during analysis of the calibration solution
A_{ISTD} = Area of the internal standard peak obtained during analysis of the calibration solution
Wt_{ISTD} = Weight of internal standard in calibration solution, g

The following is an example calculation for cis-1,3-D in calibration solution STD 2 – Prec Day 1 (\\elntrd12\EZChrom\Projects\202gc104\DAS-AM\DAS-AM-G-15-28\Precision Day 1\005 STD 2 - Prec Day 1.dat):

$$Rf_x = \frac{1.290 \times 584235}{1.11431 \times 684463}$$

where:

Rf_x = Response factor for cis-1,3-D (0.988146)
Wt_x = 1.290 g
A_x = Area of cis-1,3-D in standard solution (684463)
Wt_{ISTD} = 1.11431 g
A_{ISTD} = 584235

The response factors from all standard injections during a run sequence were averaged to calculate $RF_{(avg)}$. The $RF_{(avg)}$ for cis-1,3-D in run sequence (\\elntrd12\EZChrom\Projects\ 202gc104\DAS-AM\DAS-AM-G-15-28\Precision Day 1\DAS-AM-G-15-28 Precision Day 1.seq) was 0.989189.

3. Calculation of the weight % of cis-1,3-D or trans-1,3-D in the sample:

$$Wt\%_x = \frac{A_x \times RF_{(avg)x} \times Wt_{ISTD}}{A_{ISTD} \times Wt_{sample}} \times 100$$

where:

$Wt\%_x$ = Weight % of cis-1,3-D or trans-1,3-D in the sample
A_x = Area of cis-1,3-D or trans-1,3-D obtained during analysis of the sample solution
A_{ISTD} = Area of the internal standard obtained during analysis of the sample solution
Wt_{ISTD} = Weight of internal standard in sample solution, g
$RF_{(avg)x}$ = Average response factor for cis-1,3-D or trans-1,3-D
Wt_{sample} = Weight of sample in sample solution, g

The following is an example calculation for cis-1,3-D in sample solution "Prec Day 1-1" (\\elntrd12\EZChrom\Projects\202gc104\DAS-AM\DAS-AM-G-15-28\Precision Day 1\007 Prec Day 1-1.dat):

$$Wt\%_x = \frac{611490 \times 0.989189 \times 1.10959}{574909 \times 2.41288} \times 100 = 48.383\ \%$$

where:

$Wt\%_x$ = Weight % of cis-1,3-D isomer in the sample ()
A_x = Area of cis-1,3-D (611490)
Wt_{ISTD} = Weight of internal standard (1.10959 g)
A_{ISTD} = Area of internal standard (574909)
$RF_{(avg)}$ = Average response factor (0.989189)
Wt_{sample} = Sample weight (2.41288 g)

4. Calculation of the weight % 1,3-D (cis-1,3-D + trans-1,3-D) in the sample:

$$Wt\%\ 1,3\text{-D} = Wt\%\ cis\text{-}1,3\text{-D} + Wt\%\ trans\text{-}1,3\text{-D}$$

The following is an example calculation for cis-1,3-D and trans-1,3-D in sample solution "Prec Day 1-1" (\\elntrd12\EZChrom\Projects\202gc104\DAS-AM\DAS-AM-G-15-28\Precision Day 1\007 Prec Day 1-1.dat):

$$Wt\%\ 1,3\text{-D} = 48.383\% + 49.624\% = 98.0\%$$

where:

Wt% 1,3-D = Weight % of cis-1,3-D + trans 1,3-D in the sample (98.0%)
Wt% cis-1,3-D = 48.383%
Wt% trans-1,3-D = 49.624%

5. Statistical Methods

The statistical methods used in this study were means, standard deviations, relative standard deviations, Horwitz equation for acceptable repeatability, two sample t-test assuming equal variances, and regression analysis.

6. Spreadsheet and Data System Calculations

Response factors and weight percent values were calculated by the EZChrom Elite data system. Other calculations (e.g. linearity and precision) were performed using a spreadsheet program. Due to rounding and the significant figures used by the spreadsheet program or data system, minor discrepancies may occur between reported values and values obtained by hand calculation.

IV. RESULTS AND DISCUSSION

A. Precision

The precision of the method was evaluated by preparing and analyzing five aliquots of 1,3-D technical grade active ingredient on each of two days. Fresh standard solutions were prepared for each group of samples. At average concentrations of 48.2% cis-1,3-D, 49.5% trans-1,3-D and 97.7% total 1,3-dichloropropene (cis + trans), the RSDs were 0.19%, 0.14%, and 0.15%, respectively (Table I). Since the experimental RSD was less than the Horwitz RSD_r for cis-1,3-D, trans-1,3-D and total 1,3-D, the precision of the method was acceptable. Typical chromatograms of a calibration solution and sample solution are shown in Figures 1 and 2, respectively.

System precision was determined by analyzing a prepared sample of 1,3-D TGAI five times. The RSD of peak area for cis-1,3-D, trans-1,3-D and internal standard were 1.03%, 1.17% and 1.12%, respectively. The RSD of the peak area ratios of cis-1,3-D to internal standard and trans-1,3-D to internal standard were 0.11% and 0.11%, respectively (Table II).

B. Linearity

The linearity of detector response was evaluated by preparing seven solutions containing the 1,3-D analytical standard and chlorobenzene internal standard. The relationship between peak area and concentration was linear for cis-1,3-D, trans-1,3-D and internal standard, r^2 = 1.0000, 1.0000 and 0.9999, respectively (Figures 3 - 5). A linear detector response was obtained for cis-1,3-D over the range of 26.6 to 103.5 mg/mL, which is equivalent to a sample containing to 25.3% to 98.4% cis-1,3-D. A linear detector response was obtained for trans-1,3-D over the range of 22.8 to 88.7 mg/mL, which is equivalent to a sample containing 21.7 to 84.3% trans-1,3-D. The linear range of the chlorobenzene internal standard was 22.2 to 90.9 mg/mL, which is equivalent to 0.46 to 1.9 times the typical concentration of the internal standard in a prepared solution.

C. Stability

Stability was determined by analyzing Day 1 method precision samples and standards four days after the initial analysis. Fresh standard solutions were prepared for the re-analysis.

The average concentrations at time zero were 48.2% cis-1,3-D and 49.5% trans-1,3-D (total of 97.7% 1,3-D). The average concentrations after 4 days of ambient storage were 48.2% cis-1,3-D and 49.6% trans-1,3-D (total of 97.8% 1,3-D) (Table III) . The t-test was used to compare the results and indicated that concentrations for cis-1,3-D, trans-1,3-D and total 1,3-D were statistically equivalent at the 95% confidence interval.

The 1,3-D analytical standard contains 53.3% cis-1,3-D and 45.7% trans-1,3-D. Analysis of the day 1 precision standards (STD 1 – Prec Day 1 and STD 2 – Prec Day 1) as unknowns using the day 2 precision standards resulted in values of 53.3% for cis-1,3-D and 45.7% for trans-1,3-D. Recovery values were 100% for both isomers, indicating that the standard solutions are stable for at least four days when stored at lab ambient temperature. (Table IV).

D. Interferences

No significant interferences (>3%) were observed from the solvent blank, 1,3-D analytical standard, chlorobenzene internal standard, and 1,3-D impurity standards in the analytical method. Chromatograms of the solvent blank, internal standard and analytical standard are shown in Figure 6. Chromatograms of the individual impurity standards are stored in the raw data file.

V. CONCLUSIONS

This method is applicable for the determination of cis-1,3-D and trans-1,3-D in 1,3-D technical grade of active ingredient. The linearity and precision data have shown this method to be acceptable for the assay of 1,3-dichloropropene in the technical grade active ingredient. If the method is used with another set of equipment, it is suggested that method precision and linearity be re-determined. This report accurately reflects what was done during the course of the study and includes all amendments and/or deviations to the protocol.

The databook(s), raw data and the original copy of the final report for this study will be stored in the Dow AgroSciences LLC test facility archives at 9330 Zionsville Road, Indianapolis, Indiana.

VI. TABLES

Table I. Method Precision Data for 1,3-Dichloropropene in 1,3-Dichloropropene Technical Grade Active Ingredient, TSN307909

Date	Sample ID	Cis-1,3-D Weight %	Trans 1,3-D Weight %	Cis and trans-1,3-D Weight %
6/18/15	Prec Day 1-1	48.4	49.6	98.0
	Prec Day 1-2	48.2	49.5	97.8
	Prec Day 1-3	48.1	49.6	97.7
	Prec Day 1-4	48.2	49.4	97.6
	Prec Day 1-5	48.2	49.5	97.7
6/22/15	Prec Day 2-1	48.1	49.5	97.7
	Prec Day 2-2	48.3	49.7	98.0
	Prec Day 2-3	48.3	49.6	97.9
	Prec Day 2-4	48.1	49.5	97.6
	Prec Day 2-5	48.2	49.5	97.6
Overall Average		48.2	49.5	97.7
Std. Dev.		0.089	0.071	0.15
Overall RSD		0.19	0.14	0.15
Horwitz RSD_R		2.23	2.22	2.01
Horwitz RSDr		1.50	1.49	1.34
Acceptable (Overall RSD<Horwitz RSDr)?		Acceptable	Acceptable	Acceptable

Table II. System Precision Data for Cis and Trans-1,3-Dichloropropene and Internal Standard

Sample	Peak Area Internal standard	Peak Area Cis 1,3-D	Peak Area Ratio Cis/ISTD	Peak Area Trans 1,3-D	Peak Area Ratio Trans/ISTD
System Prec Day 1-1 inj 1	584162	618478	1.059	630918	1.080
System Prec Day 1-1 inj 2	571509	605882	1.060	616430	1.079
System Prec Day 1-1 inj 3	583356	617790	1.059	631067	1.082
System Prec Day 1-1 inj 4	584329	617701	1.057	630682	1.079
System Prec Day 1-1 inj 5	572890	607101	1.060	618826	1.080
Average	579249	613390	1.059	625585	1.080
Std. Dev.	6464	6320	0.00117	7314	0.00119
RSD	1.12	1.03	0.11	1.17	0.11

Table III. Stability Data for 1,3-Dichloropropene in 1,3-Dichloropropene TGAI

	Weight %			
	Cis 1,3-dichloropropene		Trans 1,3-dichloropropene	
Sample ID	**6/18/2015**	**6/22/2105**	**6/18/2015**	**6/22/2015**
Prec Day 1-1	48.383	48.307	49.624	49.620
Prec Day 1-2	48.222	48.193	49.536	49.498
Prec Day 1-3	48.116	48.291	49.554	49.649
Prec Day 1-4	48.157	48.269	49.445	49.503
Prec Day 1-5	48.164	48.167	49.535	49.533
Average	48.2	48.2	49.5	49.6
Std. Dev	0.1047	0.0619	0.0639	0.0695
SD^2	0.01096	0.00383	0.00408	0.00484
t Stat	-0.68		-0.52	
t Critical two-tail	2.31		2.31	
Statistical outcome (Acceptable if |tStat| ≤ tcritical)	Acceptable		Acceptable	

	Weight %	
	Cis + trans-1,3-dichloropropene	
Sample ID	**6/18/2015**	**6/22/2105**
Prec Day 1-1	98.007	97.927
Prec Day 1-2	97.758	97.691
Prec Day 1-3	97.670	97.940
Prec Day 1-4	97.602	97.772
Prec Day 1-5	97.699	97.700
Average	97.7	97.8
Std. Dev	0.156	0.121
SD^2	0.0242	0.0146
t Stat	-0.67	
t Critical two-tail	2.31	
Statistical outcome (Acceptable if |tStat| ≤ tcritical)	Acceptable	

If absolute value tStat < tCritical, then results are statistically equivalent. Conclusion: Results are statistically equivalent at the 95% confidence level after 4 days.

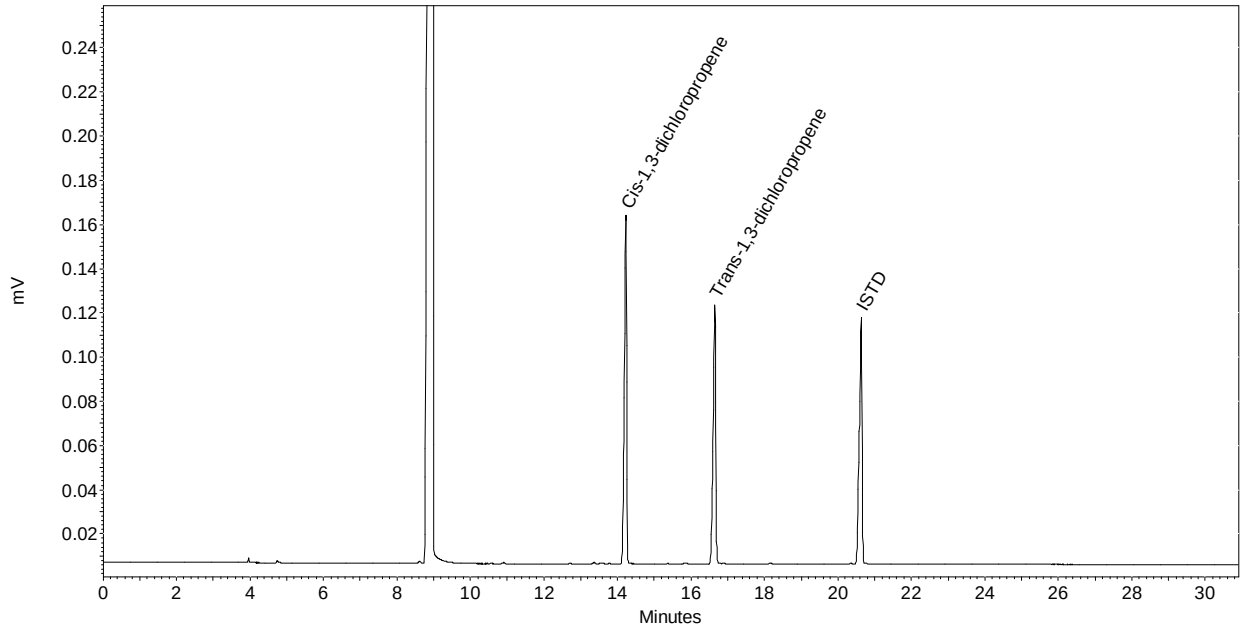
Table IV. Stability Data for 1,3-Dichloropropene in Standard Solution

Standard ID	Cis-1,3-Dichloropropene			
	Wt% found	Theoretical Wt%	% Recovery	Average % Recovery
STD 1 – Prec Day 1	53.3	53.3	100	100
STD 1 – Prec Day 1	53.4	53.3	100	
STD 2 – Prec Day 1	53.4	53.3	100	100
STD 2 – Prec Day 1	53.2	53.3	99.9	
Overall average	53.3			100

Standard ID	Trans-1,3-Dichloropropene			
	Wt% found	Theoretical Wt%	% Recovery	Average % Recovery
STD 1 – Prec Day 1	45.7	45.7	99.9	100
STD 1 – Prec Day 1	45.7	45.7	100	
STD 2 – Prec Day 1	45.7	45.7	100	99.9
STD 2 – Prec Day 1	45.7	45.7	99.9	
Overall average	45.7			100

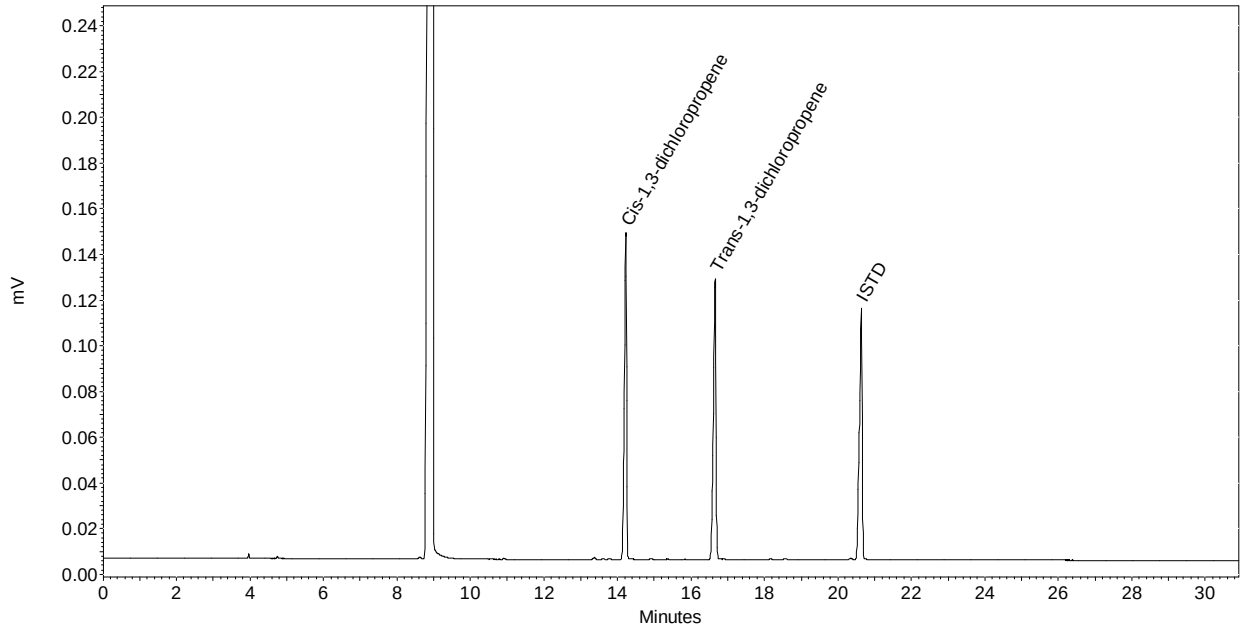
VII. FIGURES

Figure 1. Representative Chromatogram of a Calibration Solution of 1,3-Dichloropropene



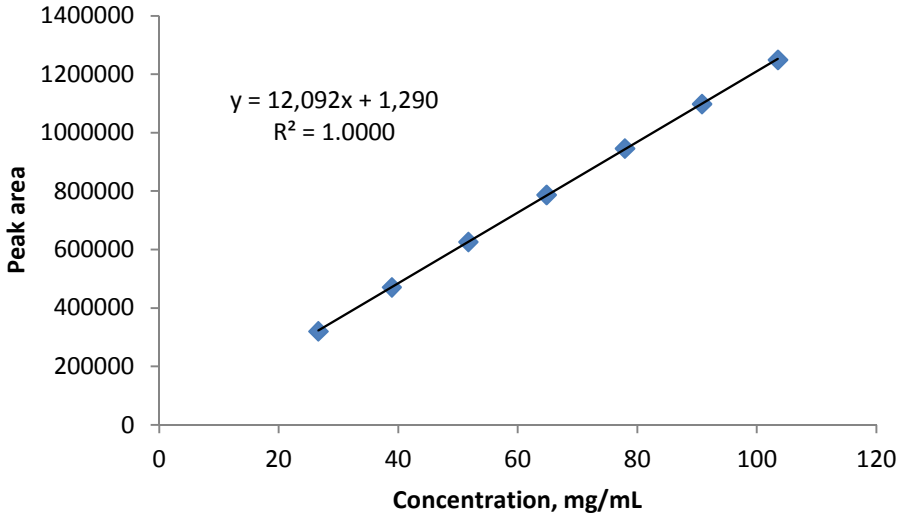
Data file Path: \\elntrd12\ezchrom\Projects\202gc104\DAS-AM\DAS-AM-G-15-28\Precision Day 1\003 STD 1 - Prec Day 1.dat

Figure 2. Representative Chromatogram of a Sample Solution of 1.3-Dichloropropene Technical Grade Active Ingredient



Data file Path: \\elntrd12\ezchrom\Projects\202gc104\DAS-AM\DAS-AM-G-15-28\Precision Day 1\ 007 Prec Day 1-1.dat

Figure 3. Linearity Plot for Cis-1,3-Dichloropropene

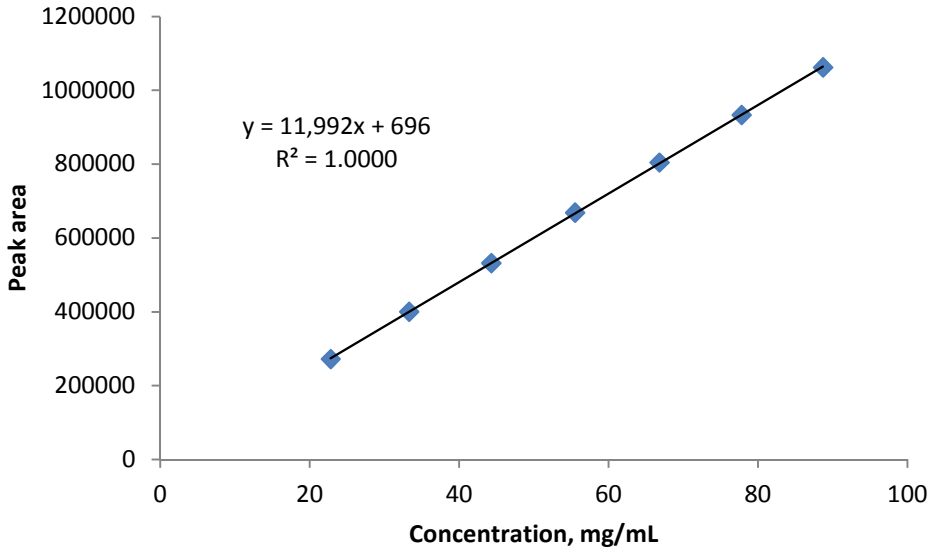


Sample ID	Concentration (mg/mL)	Peak Area*	Equivalent Weight %**
Linearity A	26.6	320684	25.3
Linearity B	38.9	471242.5	37.0
Linearity C	51.7	626727.5	49.2
Linearity D	64.8	787728	61.6
Linearity E	77.9	946403.5	74.0
Linearity F	90.8	1098536.5	86.3
Linearity G	103.5	1249776	98.4

*Average of two injections

**Equivalent wt% = (Concentration, mg/mL x 23 mL)/(2.42 g x 1000 mg/g) x 100%

Figure 4. Linearity Plot for Trans-1,3-Dichloropropene

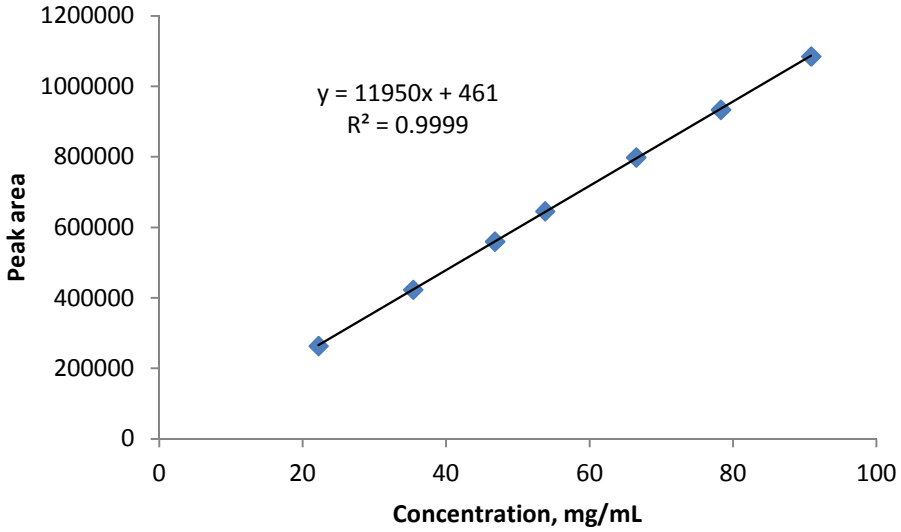


Sample ID	Concentration (mg/mL)	Peak Area*	Equivalent Weight %**
Linearity A	22.8	272130	21.7
Linearity B	33.3	400178.5	31.7
Linearity C	44.3	531896	42.1
Linearity D	55.5	668521.5	52.8
Linearity E	66.8	804344.5	63.5
Linearity F	77.8	933109.5	74.0
Linearity G	88.7	1062030.5	84.3

*Average of two injections

**Equivalent wt% = (Concentration, mg/mL x 23 mL)/(2.42 g x 1000 mg/g) x 100%

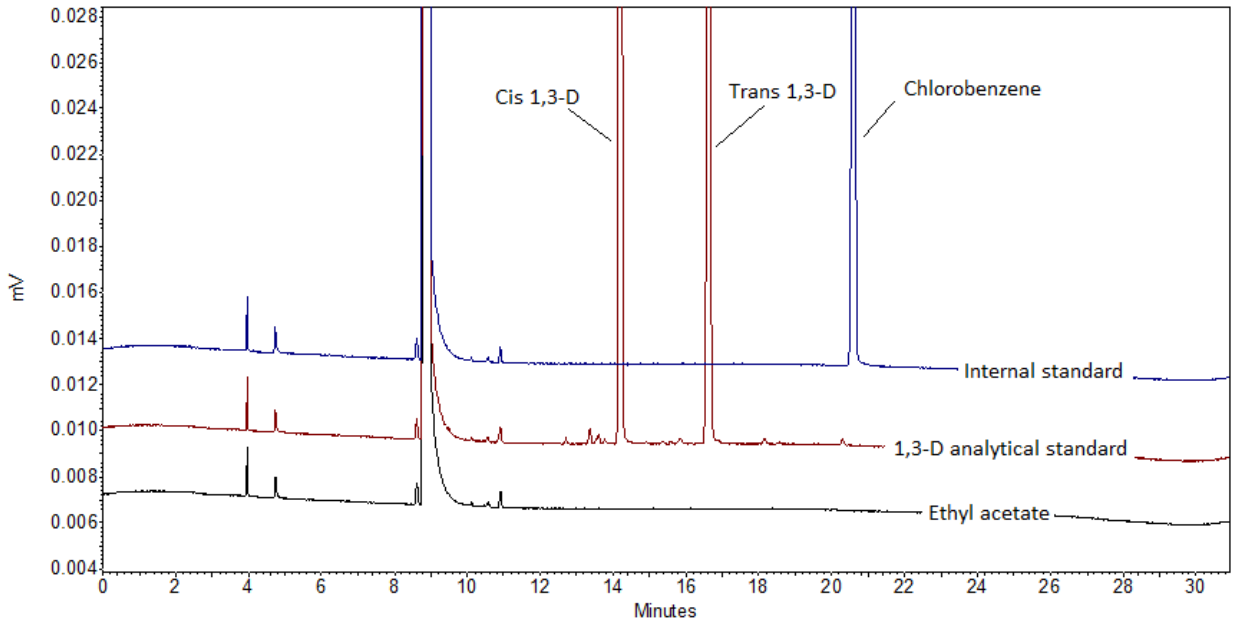
Figure 5. Linearity Plot for Chlorobenzene Internal Standard



Sample ID	Concentration (mg/mL)	Peak Area*	Fraction of nominal**
Linearity A	22.2	263633	0.46
Linearity B	35.4	423291.5	0.74
Linearity C	46.8	559835	0.98
Linearity D	53.8	645839	1.12
Linearity E	66.5	798571.5	1.39
Linearity F	78.3	933881.5	1.64
Linearity G	90.9	1085099	1.90

*Average of two injections
**Nominal concentration = 1.1 g/23 mL x 1000 mg/g = 47.8 mg/mL
Fraction of nominal = (Concentration, mg/mL) / (47.8 mg/mL)

Figure 6. Chromatograms of Solvent Blank, Test Substance and Internal Standard



Data file Path: \\elntrd12\ezchrom\Projects\202gc104\DAS-AM\DAS-AM-G-15-28\Interferences\

Trace	Sample	Filename
A	Ethyl acetate (solvent blank)	003 Solvent blank – Int.dat
B	1,3-D analytical standard	004 STD – Int.dat
C	Internal Standard	005 ISTD – Int.dat

APPENDIX I. ANALYTICAL METHOD SUMMARY

**Quantitative Analysis for 1,3-Dichloropropene
in 1,3-Dichloropropene Technical Grade Active Ingredient**

1. Preparation of calibration solutions

   Tare an appropriate size capped container, remove the cap, and using an Eppendorf pipet, add approximately 1 mL of the chlorobenzene internal standard. Cap the container and record the weight (~ 1.1 grams). Tare the capped container, remove the cap, and using an Eppendorf pipet, add approximately 2 mL of the analytical standard into the container. Cap and record the weight (~ 2.4 grams). Uncap the container, and using a graduated cylinder or repipettor, quickly add approximately 20 mL of ethyl acetate, cap and mix well.

2. Preparation of sample solutions

   Tare an appropriate size capped container, remove the cap, and using an Eppendorf pipet, add approximately 1 mL of the chlorobenzene internal standard. Cap the container and record the weight (~ 1.1 grams). Tare the capped container, remove the cap, and using an Eppendorf pipet, add approximately 2 mL of the sample into the container. Cap and record the weight (~ 2.4 grams). Uncap the container, and using a graduated cylinder or repipettor, quickly add approximately 20 mL of ethyl acetate, cap and mix well.

3. Instrumentation and Conditions:

Chromatograph:	Agilent 6890, or equivalent
Column:	DB-1701 60 m x 0.32 mm x 1 µm film
Flow rate:	1.9 mL/min helium (constant flow mode)
Inlet:	Split at 130°C
Inlet liner:	Restek Sky 4 mm straight liner with glass wool
Inlet conditioning:	Make at least 10 injections of a standard solution prior to instrument calibration and sample analysis*
Injection volume:	1 µL, split ratio 38:1, one injection per vial
Syringe wash solvent:	Ethyl acetate
Injector parameters:	Syringe type: 5 or 10 µL with metal plunger Note: Do NOT use syringes with a Teflon tipped plunger Sample washes: 3; sample pumps: 3 Syringe wash: 3 for wash solvent A; 3 for wash solvent B Syringe speed: fast; Viscosity delay: 1 second
Temperature program:	40°C hold for 2 minutes; 5°C/minute to 80°C, hold for 7.5 minutes; 5°C/minute to 110°C, hold for 1 minute; 25°C/minute to 270°C, hold 0 minutes
Detector:	TCD at 280 °C
Make-up:	5 mL/min helium
Reference:	15 mL/min helium
Run Time:	30.9 minutes
Integrator:	Agilent EZChrom Elite, or equivalent

Approximate Retention Times:	Cis-1,3-dichloropropene	~14.2 min
	Trans-1,3-dichloropropene	~16.7 min
	Chlorobenzene (internal standard)	~20.6 min

*Equilibration of inlet liner to be performed after a liner change.

Approximate time to prepare and analyze sample: 2 hours

4. Calculations:

a. Calculation of the weight of cis-1,3-D or-trans 1,3-D in the calibration solutions:

$$Wt_x = Wt_{AS} \times Purity_{AS}$$

where:
Wt_x = Weight of cis-1,3-D or trans-1,3-D in the calibration standard solution, g
Wt_{AS} = Weight of the analytical standard added to the calibration solution, g
$Purity_{AS}$ = Purity of the analytical standard, expressed as a decimal

b. Calculation of the response factor for cis-1,3-D or trans-1,3-D from the calibration solutions:

$$Rf_x = \frac{Wt_x \times A_{ISTD}}{Wt_{ISTD} \times A_x}$$

where:
Rf_x = Response factor for cis-1,3-D or trans-1,3-D
Wt_x = Weight of cis-1,3-D or trans-1,3-D in standard solution, g
A_x = Area of cis-1,3-D or trans-1,3-D peak obtained during analysis of the calibration solution
A_{ISTD} = Area of the internal standard peak obtained during analysis of the calibration solution
Wt_{IS} = Weight of internal standard in calibration solution, g

c. Calculation of the weight % of cis-1,3-D or trans-1,3-D in the sample:

$$Wt\%_x = \frac{A_x \times RF_{(avg)x}}{A_{ISTD} \times Wt_{sample}} \times 100$$

where:
$Wt\%_x$ = Weight % of cis-1-3,D or trans-1,3-D in the sample
A_x = Area of cis-1,3-D or trans-1,3-D obtained during analysis of the sample solution
A_{ISTD} = Area of the internal standard obtained during analysis of the sample solution
Wt_{ISTD} = Weight of internal standard in sample solution, g
$RF_{(avg)x}$ = Average response factor for cis-1,3-D or trans-1,3-D
Wt_{sample} = Weight of sample in sample solution, g

d. Calculation of the weight % of cis-1,3-D and trans-1,3-D in the sample:

$$\text{Wt\% 1,3-D} = \text{Wt\% cis-1,3-D} + \text{Wt\% trans-1,3-D}$$

Example chromatograms are attached.

Additional details are provided in the body of the report.

Figure 1. Representative Chromatogram of a Calibration Solution

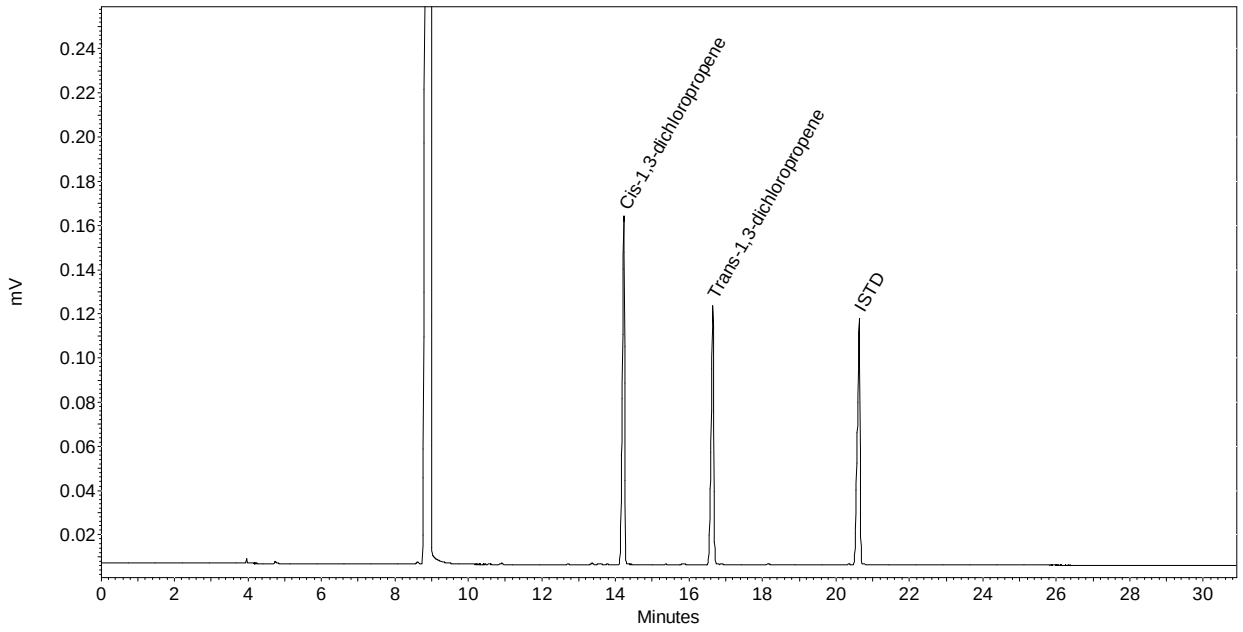


Figure 2. Representative Chromatogram of a Sample Solution of 1,3-Dichloropropene TGAI