

酢酸イソプロピルのマウスを用いた
吸入によるがん原性試験報告書

試験番号：0611

APPENDICES

APPENDICES

- APPENDIX 1-1 IDENTITY OF ISOPROPYL ACETATE IN THE 2-YEAR
INHALATION STUDY
- APPENDIX 1-2 STABILITY OF ISOPROPYL ACETATE IN THE 2-YEAR
INHALATION STUDY
- APPENDIX 2 ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER
IN THE 2-YEAR INHALATION STUDY OF ISOPROPYL ACETATE
- APPENDIX 3 METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY
AND BIOCHEMISTRY IN THE 2-YEAR INHALATION STUDY OF
ISOPROPYL ACETATE

APPENDIX 1-1

IDENTITY OF ISOPROPYL ACETATE
IN THE 2-YEAR INHALATION STUDY

IDENTITY AND IMPURITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : KLE3931

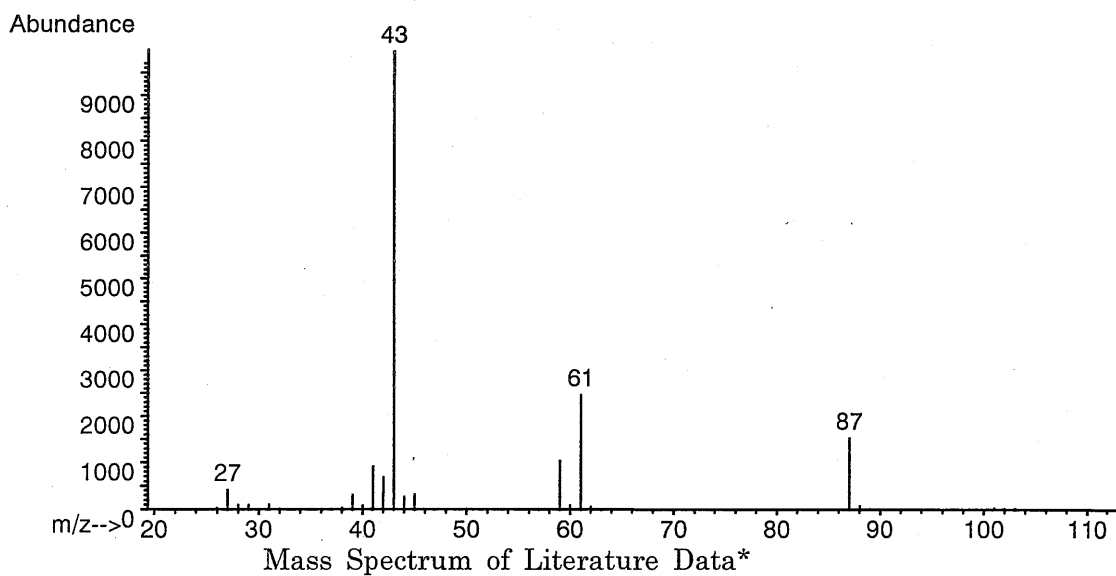
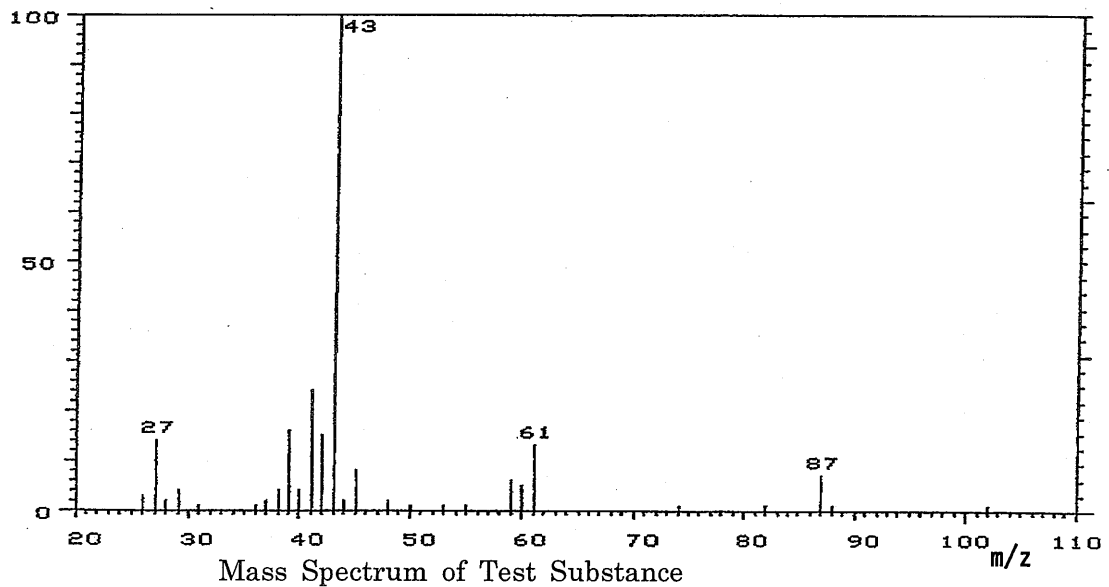
1. Spectral Data

Mass Spectrometry

Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Result: The mass spectrum was consistent with literature spectrum.

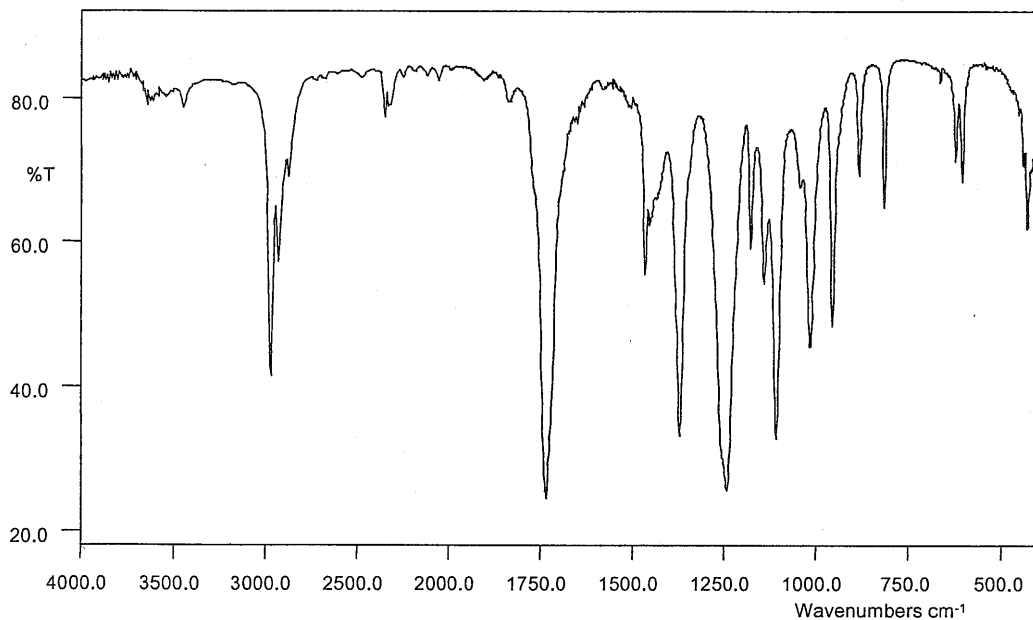
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

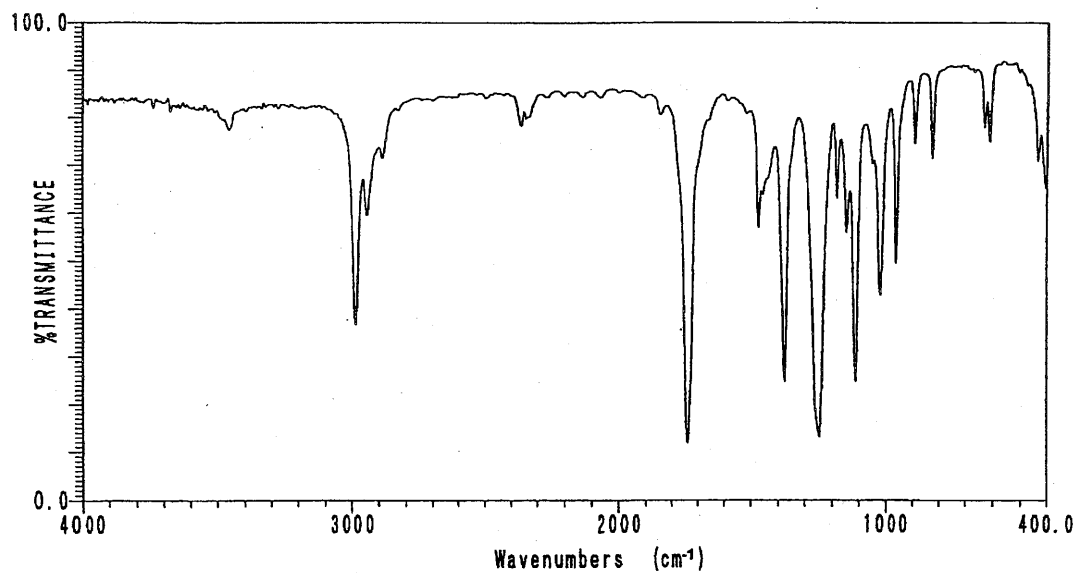
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 4 cm⁻¹



Infrared Spectrum of Test Substance



Infrared Spectrum of Literature Data*

Result: The infrared spectrum was consistent with literature spectrum.

(*Performed by Wako Pure Chemical Industries, Ltd.)

2. Impurity

Instrument : Agilent Technologies 5890A Gas Chromatograph
Column : Methyl Silicone (0.53 mm ϕ \times 60 m)
Column Temperature: 80° C
Flow Rate : 15 mL/min
Detector : FID (Flame Ionization Detector)
Injection Volume : 1 μ L

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.031	2-Propanol
Test Substance	2	99.969	Isopropyl acetate

Result: Gas chromatography indicated one major peak (peak No. 2) and one impurity. The impurity (peak No. 1) was identified as 2-propanol by comparing GC-MS with that of standard sample. The amount of 2-propanol in the test substance was 0.031% (The quantity value by the standard sample was 0.031%.) with a gas chromatograph.

3. Conclusion: The test substance was identified as isopropyl acetate by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (isopropyl acetate) and one impurity. The impurity was 2-propanol in the test substance.

IDENTITY AND IMPURITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

B. Lot No. : EWH6219

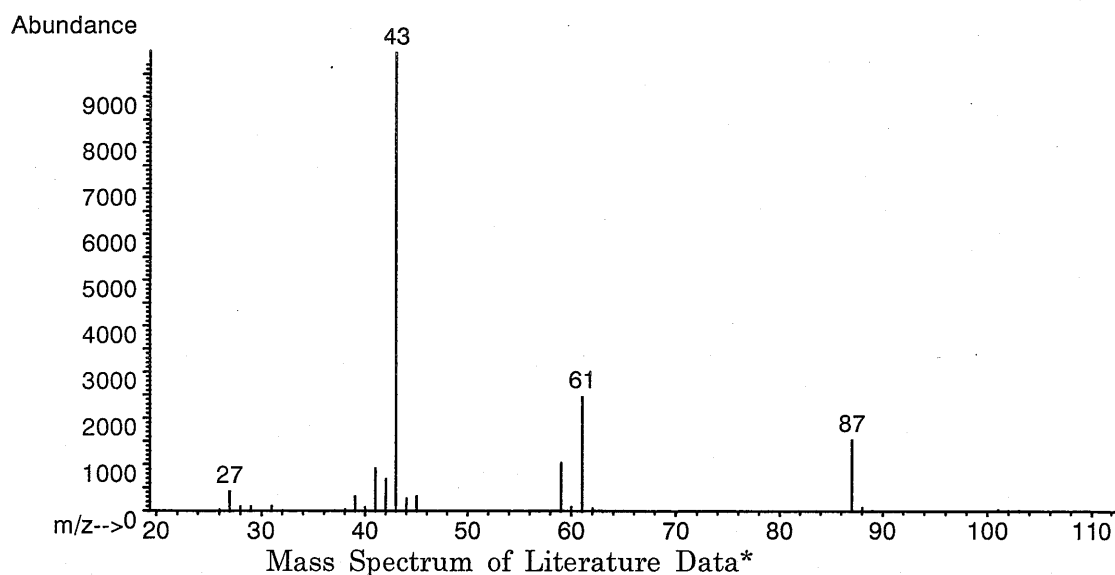
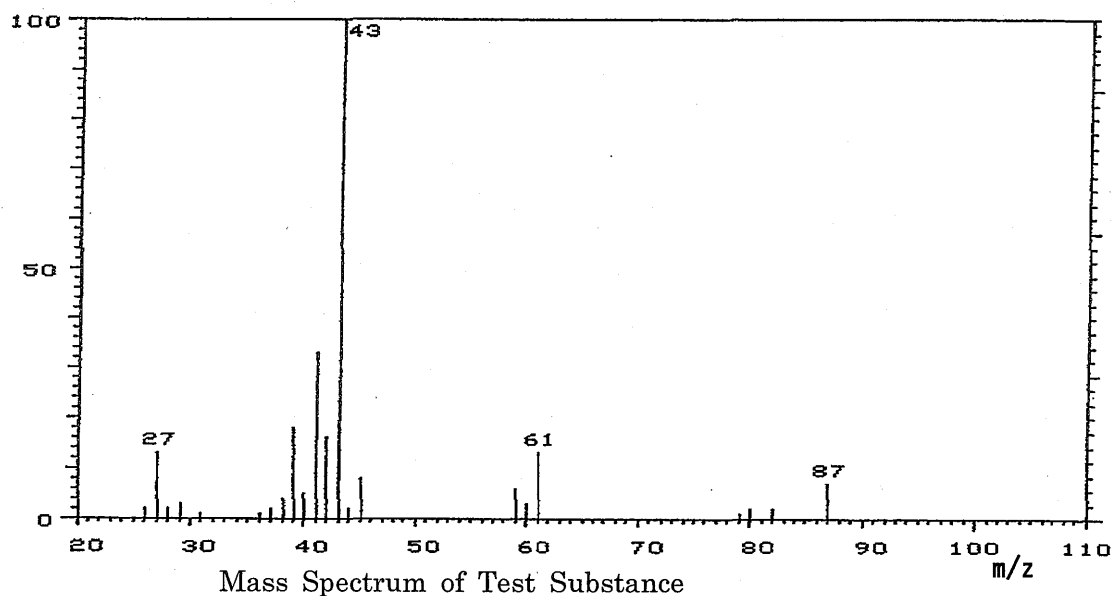
1. Spectral Data

Mass Spectrometry

Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Result: The mass spectrum was consistent with literature spectrum.

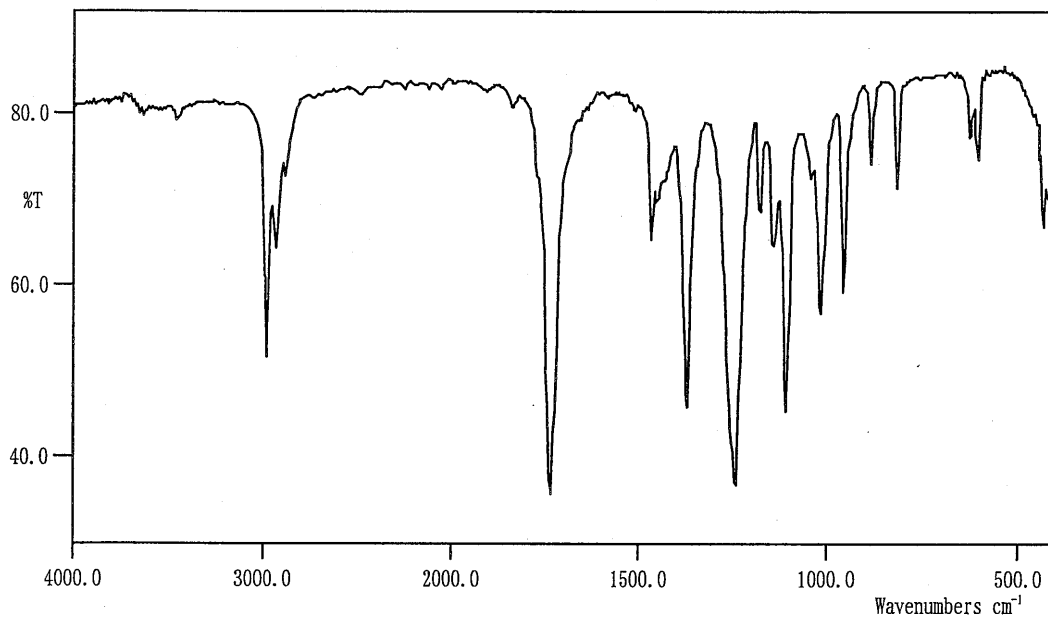
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

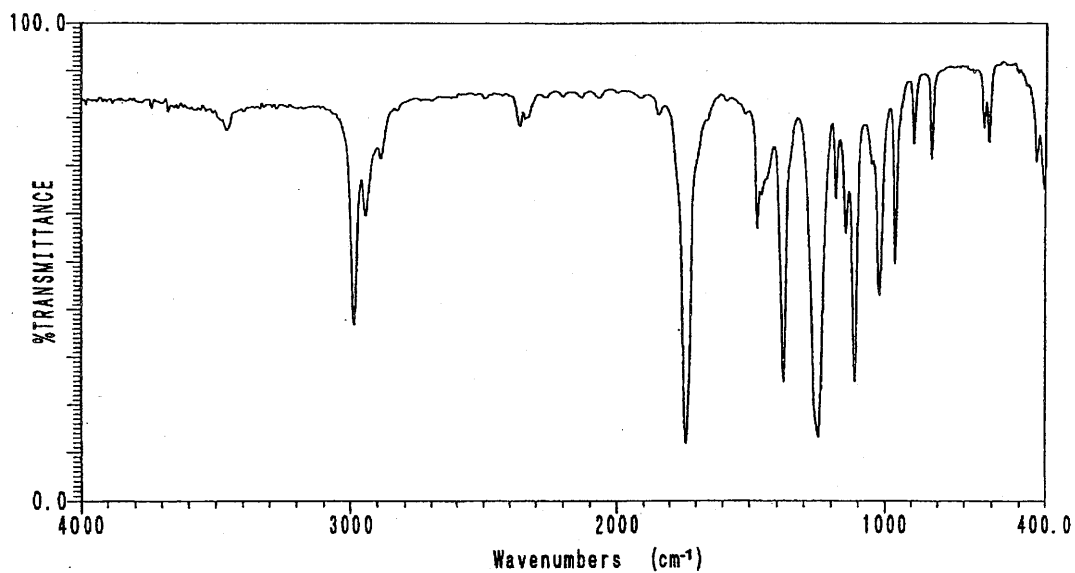
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 4 cm^{-1}



Infrared Spectrum of Test Substance



Infrared Spectrum of Literature Data*

Result: The infrared spectrum was consistent with literature spectrum.
(*Performed by Wako Pure Chemical Industries, Ltd.)

2. Impurity

Instrument : Agilent Technologies 5890A Gas Chromatograph
Column : Methyl Silicone (0.53 mm ϕ \times 60 m)
Column Temperature: 80° C
Flow Rate : 15 mL/min
Detector : FID (Flame Ionization Detector)
Injection Volume : 1 μ L

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.039	2-Propanol
Test Substance	2	99.961	Isopropyl acetate

Result: Gas chromatography indicated one major peak (peak No. 2) and one impurity. The impurity (peak No. 1) was identified as 2-propanol by comparing GC-MS with that of standard sample. The amount of 2-propanol in the test substance was 0.039% (The quantity value by the standard sample was 0.032%.) with a gas chromatograph.

3. Conclusion: The test substance was identified as isopropyl acetate by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (isopropyl acetate) and one impurity. The impurity was 2-propanol in the test substance.

IDENTITY AND IMPURITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

C. Lot No. : DPP3664

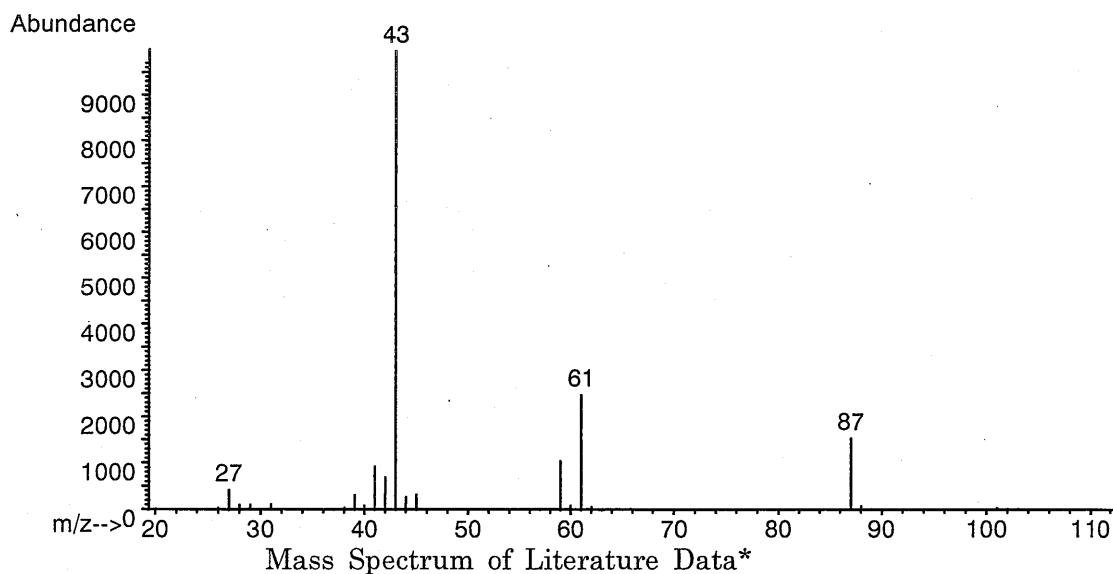
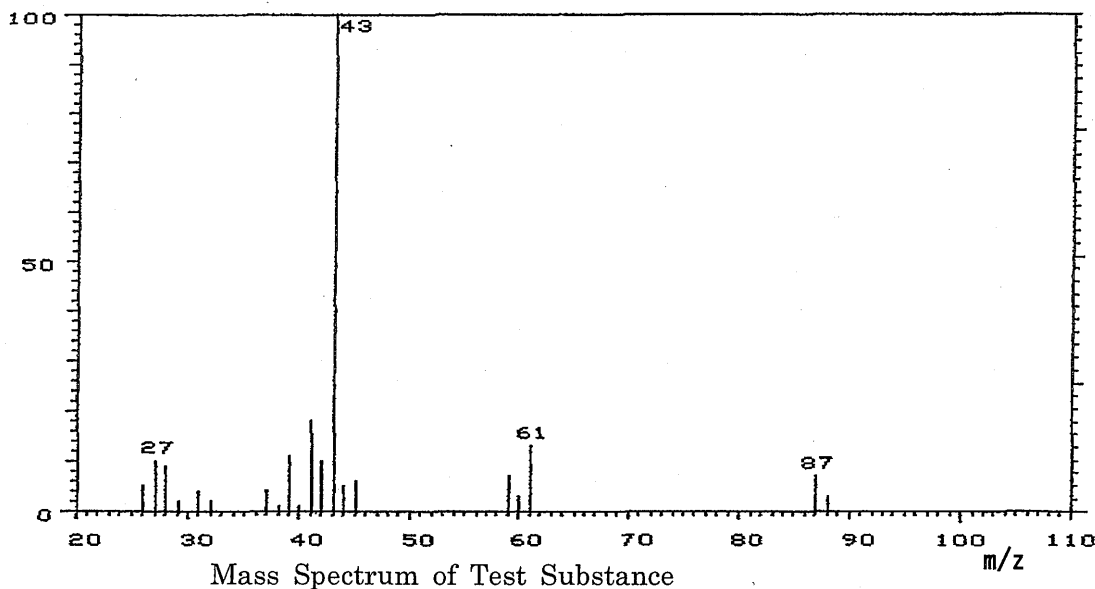
1. Spectral Data

Mass Spectrometry

Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Result: The mass spectrum was consistent with literature spectrum.

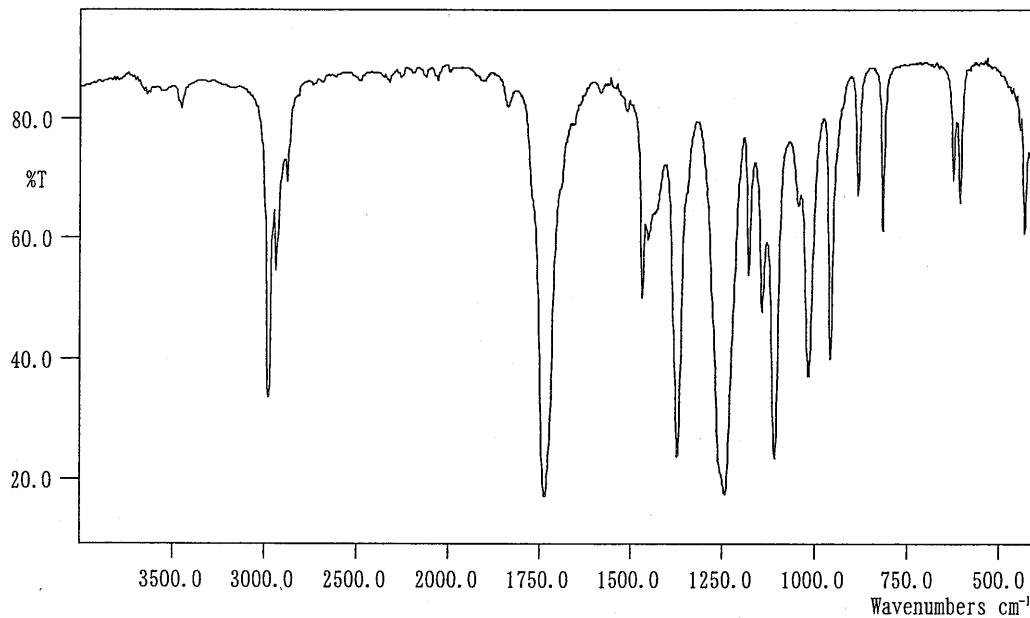
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

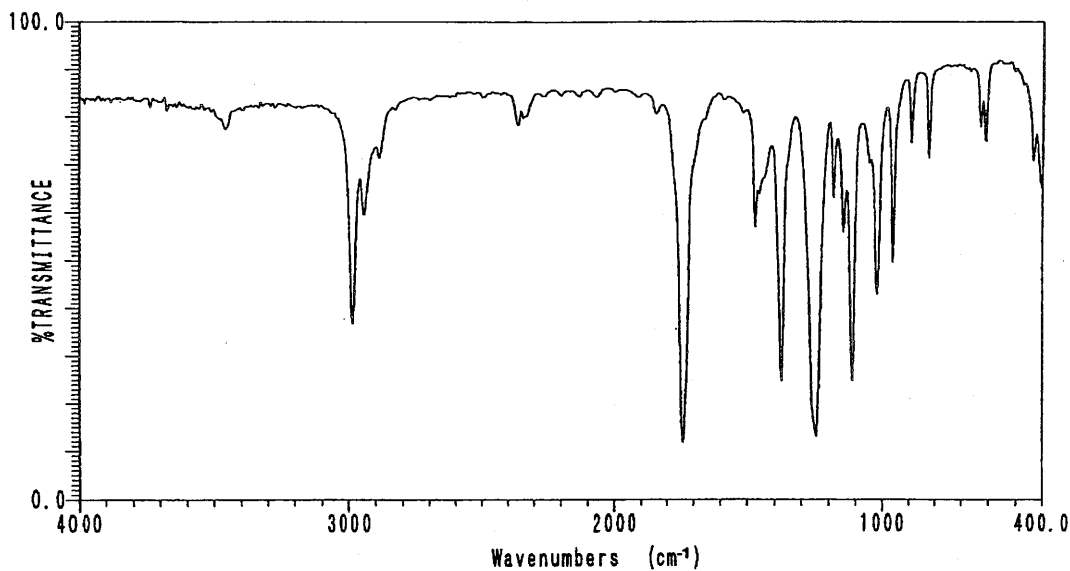
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 4 cm⁻¹



Infrared Spectrum of Test Substance



Infrared Spectrum of Literature Data*

Result: The infrared spectrum was consistent with literature spectrum.

(*Performed by Wako Pure Chemical Industries, Ltd.)

2. Impurity

Instrument : Agilent Technologies 5890A Gas Chromatograph
Column : Methyl Silicone (0.53 mm ϕ \times 60 m)
Column Temperature: 80° C
Flow Rate : 15 mL/min
Detector : FID (Flame Ionization Detector)
Injection Volume : 1 μ L

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.038	2-Propanol
Test Substance	2	99.962	Isopropyl acetate

Result: Gas chromatography indicated one major peak (peak No. 2) and one impurity. The impurity (peak No. 1) was identified as 2-propanol by comparing GC-MS with that of standard sample. The amount of 2-propanol in the test substance was 0.038% (The quantity value by the standard sample was 0.038%) with a gas chromatograph.

3. Conclusion: The test substance was identified as isopropyl acetate by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (isopropyl acetate) and one impurity. The impurity was 2-propanol in the test substance.

IDENTITY AND IMPURITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

D. Lot No. : DPF2284

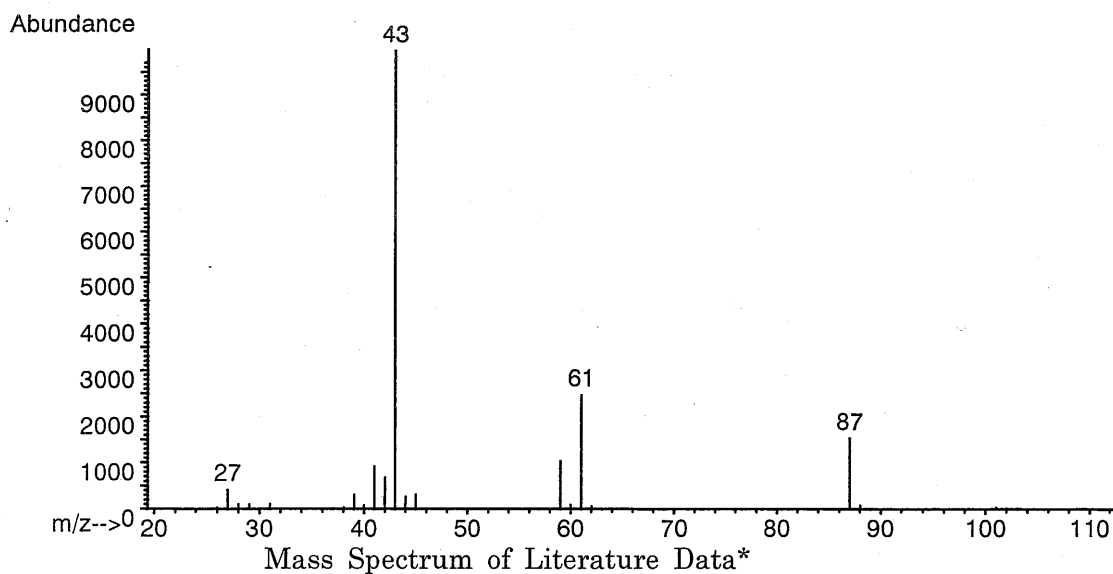
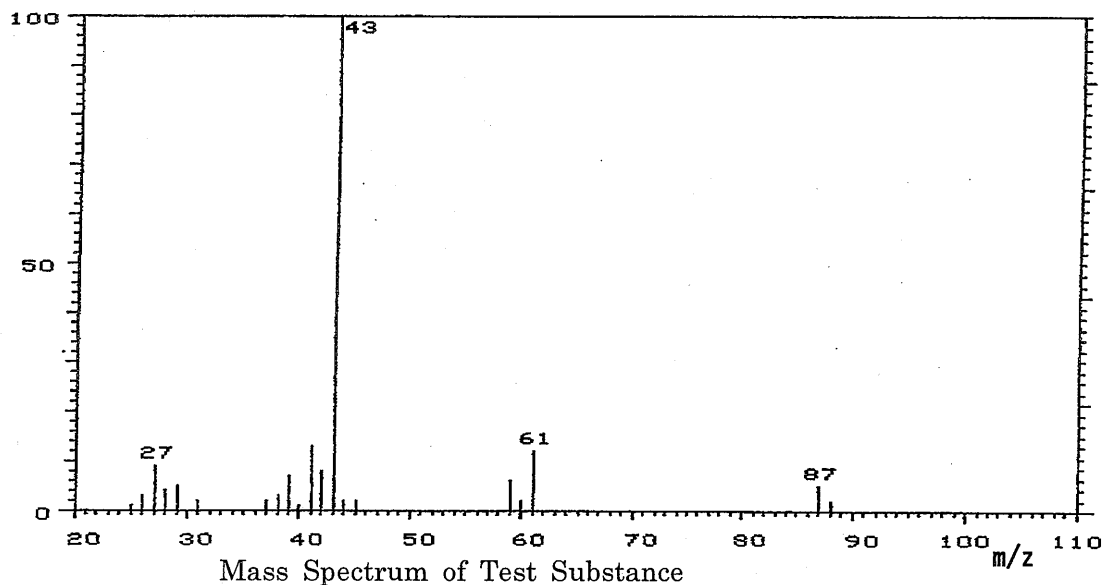
1. Spectral Data

Mass Spectrometry

Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Result: The mass spectrum was consistent with literature spectrum.

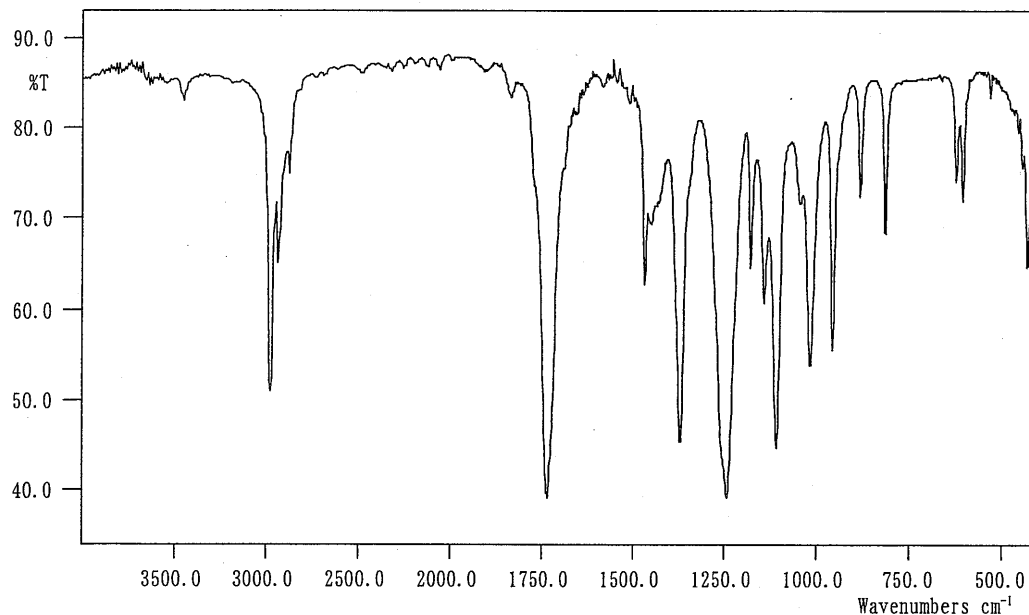
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

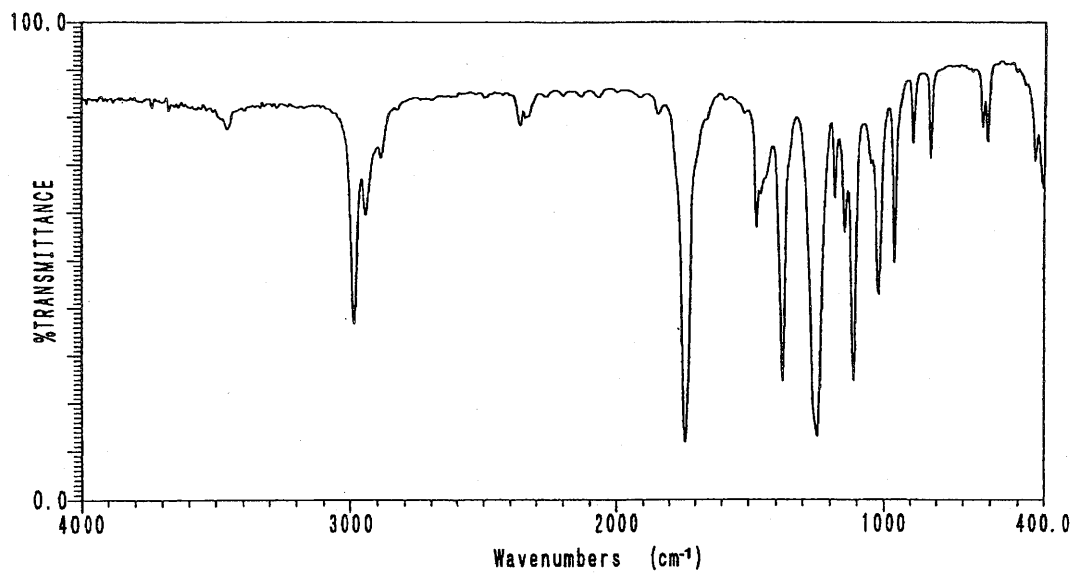
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 4 cm^{-1}



Infrared Spectrum of Test Substance



Infrared Spectrum of Literature Data*

Result: The infrared spectrum was consistent with literature spectrum.

(*Performed by Wako Pure Chemical Industries, Ltd.)

2. Impurity

Instrument : Agilent Technologies 5890A Gas Chromatograph
Column : Methyl Silicone (0.53 mm ϕ \times 60 m)
Column Temperature: 80 $^{\circ}$ C
Flow Rate : 15 mL/min
Detector : FID (Flame Ionization Detector)
Injection Volume : 1 μ L

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.044	2-Propanol
Test Substance	2	99.956	Isopropyl acetate

Result: Gas chromatography indicated one major peak (peak No. 2) and one impurity. The impurity (peak No. 1) was identified as 2-propanol by comparing GC-MS with that of standard sample. The amount of 2-propanol in the test substance was 0.044% (The quantity value by the standard sample was 0.044%.) with a gas chromatograph.

3. Conclusion: The test substance was identified as isopropyl acetate by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (isopropyl acetate) and one impurity. The impurity was 2-propanol in the test substance.

IDENTITY AND IMPURITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

E. Lot No. : TSK3141

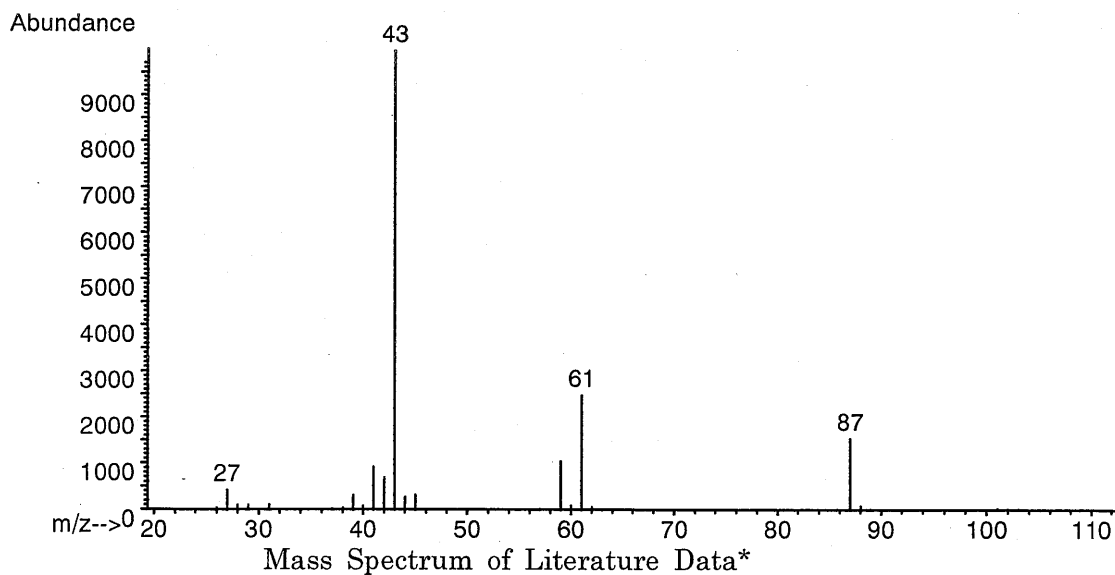
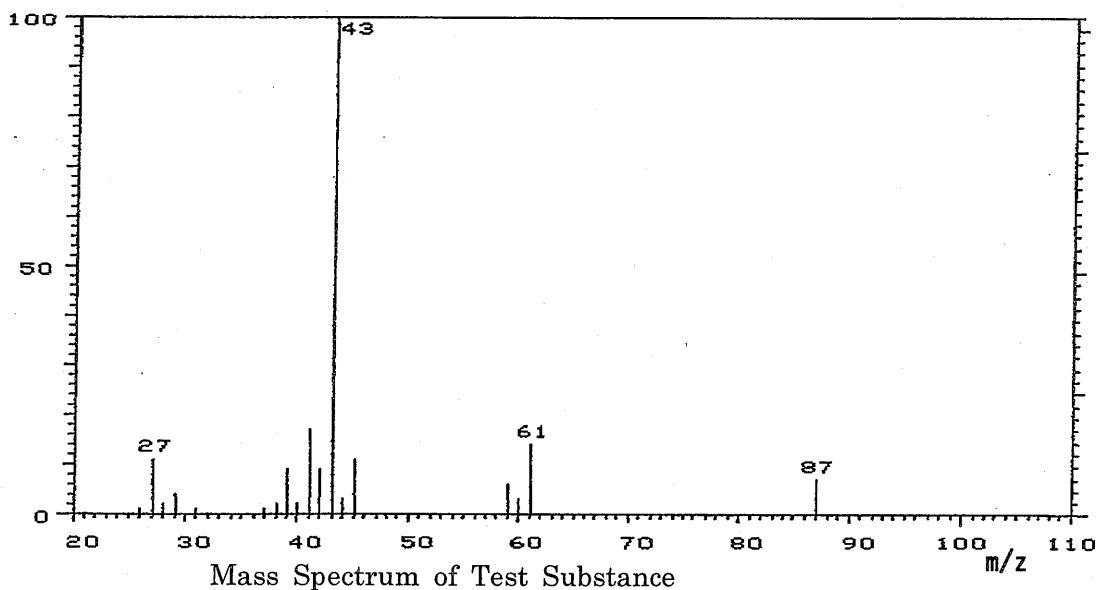
1. Spectral Data

Mass Spectrometry

Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Result: The mass spectrum was consistent with literature spectrum.

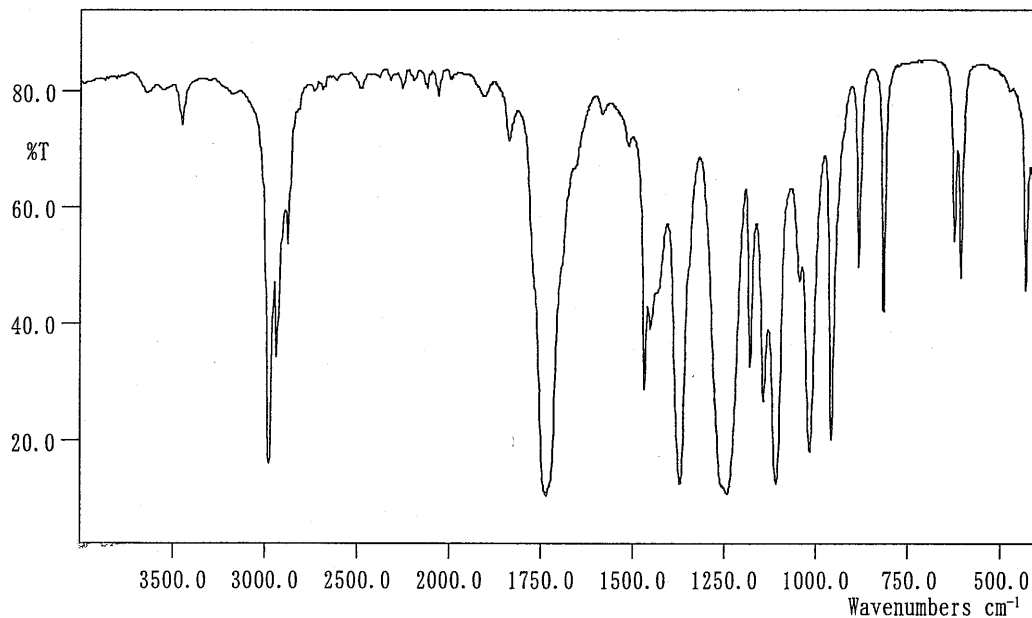
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

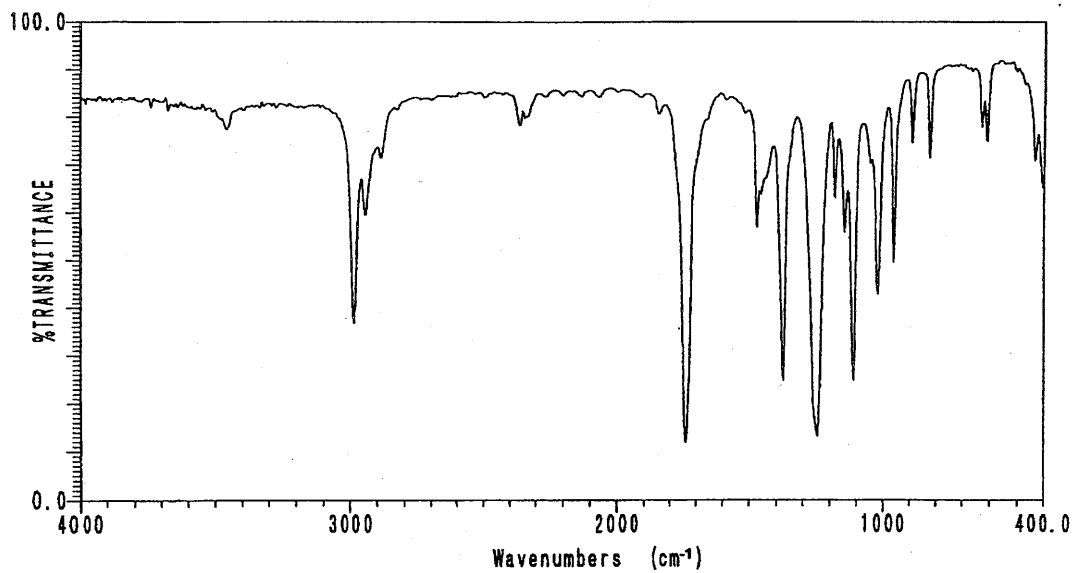
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 4 cm⁻¹



Infrared Spectrum of Test Substance



Infrared Spectrum of Literature Data*

Result: The infrared spectrum was consistent with literature spectrum.

(*Performed by Wako Pure Chemical Industries, Ltd.)

2. Impurity

Instrument : Agilent Technologies 5890A Gas Chromatograph
Column : Methyl Silicone (0.53 mm ϕ \times 60 m)
Column Temperature: 80° C
Flow Rate : 15 mL/min
Detector : FID (Flame Ionization Detector)
Injection Volume : 1 μ L

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.044	2-Propanol
Test Substance	2	99.956	Isopropyl acetate

Result: Gas chromatography indicated one major peak (peak No. 2) and one impurity. The impurity (peak No. 1) was identified as 2-propanol by comparing GC-MS with that of standard sample. The amount of 2-propanol in the test substance was 0.044% (The quantity value by the standard sample was 0.029%.) with a gas chromatograph.

3. Conclusion: The test substance was identified as isopropyl acetate by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (isopropyl acetate) and one impurity. The impurity was 2-propanol in the test substance.

APPENDIX 1-2

STABILITY OF ISOPROPYL ACETATE
IN THE 2-YEAR INHALATION STUDY

STABILITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : KLE3931

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : Methyl Silicone (0.53 mm ϕ \times 60 m)Column Temperature: 80 $^{\circ}$ C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μ L

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2005.12.13	1	1.872	0.031
	2	3.817	99.969
2006.01.18	1	1.868	0.030
	2	3.818	99.970

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2005.12.13 and one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2006.1.18. No new trace impurity peak in the test substance analyzed on 2006.1.18 was detected.

2. Conclusion: The test substance was stable for the period that the test substance had been used for the study.

STABILITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

B. Lot No. : EWH6219

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : Methyl Silicone (0.53 mm ϕ \times 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μ L

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2006.01.10	1	1.866	0.039
	2	3.820	99.961
2006.07.21	1	1.863	0.039
	2	3.794	99.961

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2006.1.10 and one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2006.7.21. No new trace impurity peak in the test substance analyzed on 2006.7.21 was detected.

2. Conclusion: The test substance was stable for the period that the test substance had been used for the study.

STABILITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

C. Lot No. : DPP3664

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : Methyl Silicone (0.53 mm ϕ \times 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μ L

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2006.07.14	1	1.859	0.038
	2	3.789	99.962
2007.03.05	1	1.858	0.038
	2	3.767	99.962

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2006.7.14 and one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2007.3.5. No new trace impurity peak in the test substance analyzed on 2007.3.5 was detected.

2. Conclusion: The test substance was stable for the period that the test substance had been used for the study.

STABILITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

D. Lot No. : DPF2284

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : Methyl Silicone (0.53 mm ϕ \times 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μ L

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2007.02.27	1	1.858	0.044
	2	3.789	99.956
2007.10.11	1	1.881	0.055
	2	3.983	99.945

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2007.2.27 and one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2007.10.11. No new trace impurity peak in the test substance analyzed on 2007.10.11 was detected.

2. Conclusion: The test substance was stable for the period that the test substance had been used for the study.

STABILITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

E. Lot No. : TSK3141

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : Methyl Silicone (0.53 mm ϕ \times 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μ L

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2007.10.01	1	1.881	0.044
	2	3.980	99.956
2008.01.07	1	1.881	0.043
	2	3.984	99.957

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2007.10.1 and one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2008.1.7. No new trace impurity peak in the test substance analyzed on 2008.1.7 was detected.

2. Conclusion: The test substance was stable for the period that the test substance had been used for the study.

APPENDIX 2

ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER IN THE 2-YEAR INHALATION STUDY OF ISOPROPYL ACETATE

ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER IN THE 2-YEAR
INHALATION STUDY OF ISOPROPYL ACETATE

Group Name	Temperature (°C)	Humidity (%)	Ventilation Rate (L/min)		Air Change (time/h)	
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.* ¹	Mean ± S.D.* ²	Mean* ¹	Mean* ²
Control	23.1 ± 0.2	56.3 ± 1.3	375.0 ± 4.9	741.0 ± 7.2	6.1	12.0
1000 ppm	23.0 ± 0.1	54.6 ± 1.8	372.4 ± 4.4	740.4 ± 6.0	6.0	12.0
2000 ppm	23.0 ± 0.1	52.8 ± 2.2	372.9 ± 4.5	739.7 ± 7.1	6.0	12.0
4000 ppm	23.0 ± 0.2	52.0 ± 2.6	369.7 ± 4.8	745.0 ± 7.0	6.0	12.1

* 1: Exposure period * 2: After exposure period

APPENDIX 3

METHODS, UNITS AND DECIMAL PLACE FOR
HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR
INHALATION STUDY OF ISOPROPYL ACETATE

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY
IN THE 2-YEAR INHALATION STUDY OF ISOPROPYL ACETATE

Item	Method	Unit	Decimal place
Hematology			
Red blood cell (RBC)	Light scattering method ¹⁾	$\times 10^6/\mu\text{L}$	2
Hemoglobin(Hgb)	Cyanmethemoglobin method ¹⁾	g/dL	1
Hematocrit(Hct)	Calculated as $\text{RBC} \times \text{MCV}/10$ ¹⁾	%	1
Mean corpuscular volume(MCV)	Light scattering method ¹⁾	fL	1
Mean corpuscular hemoglobin(MCH)	Calculated as $\text{Hgb}/\text{RBC} \times 10$ ¹⁾	pg	1
Mean corpuscular hemoglobin concentration (MCHC)	Calculated as $\text{Hgb}/\text{Hct} \times 100$ ¹⁾	g/dL	1
Platelet	Light scattering method ¹⁾	$\times 10^3/\mu\text{L}$	0
Reticulocyte	Light scattering method ¹⁾	%	1
White blood cell(WBC)	Light scattering method ¹⁾	$\times 10^3/\mu\text{L}$	2
Differential WBC	Pattern recognition method ²⁾ (Wright staining)	%	0
Biochemistry			
Total protein(TP)	Biuret method ³⁾	g/dL	1
Albumin (Alb)	BCG method ³⁾	g/dL	1
A/G ratio	Calculated as $\text{Alb}/(\text{TP} - \text{Alb})$ ³⁾	—	1
T-bilirubin	Azobilirubin method ³⁾	mg/dL	2
Glucose	GlcK·G-6-PDH method ³⁾	mg/dL	0
T-cholesterol	CE·COD·POD method ³⁾	mg/dL	0
Triglyceride	MGLP·GK·GPO·POD method ³⁾	mg/dL	0
Phospholipid	PLD·ChOD·POD method ³⁾	mg/dL	0
Aspartate aminotransferase (AST)	JSCC method ³⁾	IU/L	0
Alanine aminotransferase (ALT)	JSCC method ³⁾	IU/L	0
Lactate dehydrogenase (LDH)	SFBC method ³⁾	IU/L	0
Alkaline phosphatase (ALP)	GSCC method ³⁾	IU/L	0
γ -Glutamyl transpeptidase (γ -GTP)	JSCC method ³⁾	IU/L	0
Creatine kinase (CK)	JSCC method ³⁾	IU/L	0
Urea nitrogen	Urease·GLDH method ³⁾	mg/dL	1
Sodium	Ion selective electrode method ³⁾	mEq/L	0
Potassium	Ion selective electrode method ³⁾	mEq/L	1
Chloride	Ion selective electrode method ³⁾	mEq/L	0
Calcium	OCPC method ³⁾	mg/dL	1
Inorganic phosphorus	PNP·XOD·POD method ³⁾	mg/dL	1

1) Automatic blood cell analyzer (ADVIA120 : Siemens Healthcare Diagnostics Inc.)

2) Automatic blood cell differential analyzer (MICROX HEG-120NA : OMRON Corporation)

3) Automatic analyzer (Hitachi 7080 : Hitachi,Ltd.)