酢酸イソプロピルのラットを用いた
吸入によるがん原性試験報告書

試験番号：0610

APPENDICES

APPENDICES

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

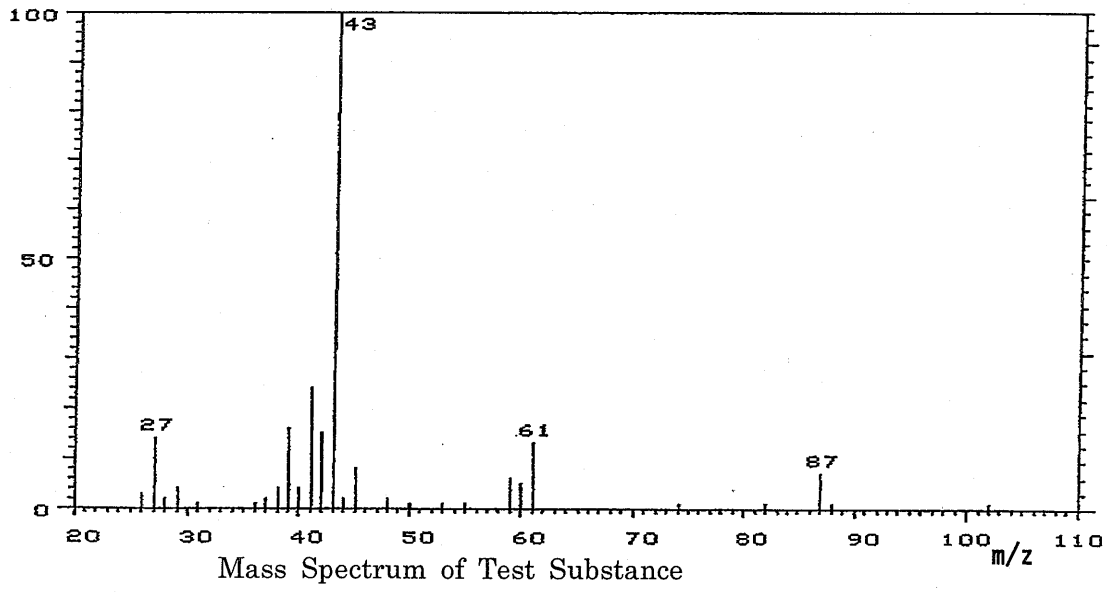
1. Spectral Data

Mass Spectrometry

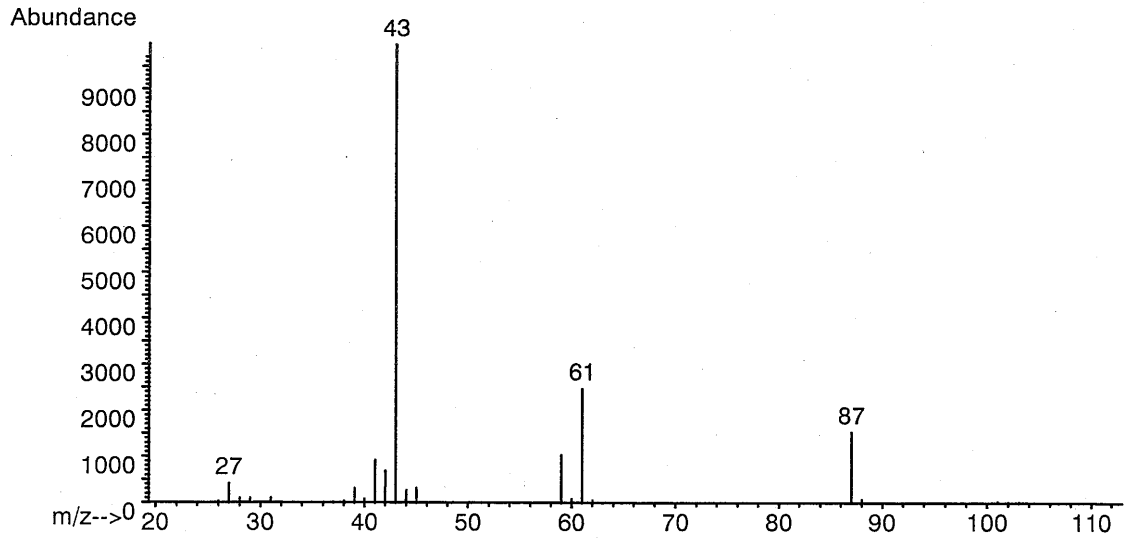
Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

Result: The mass spectrum was consistent with literature spectrum.

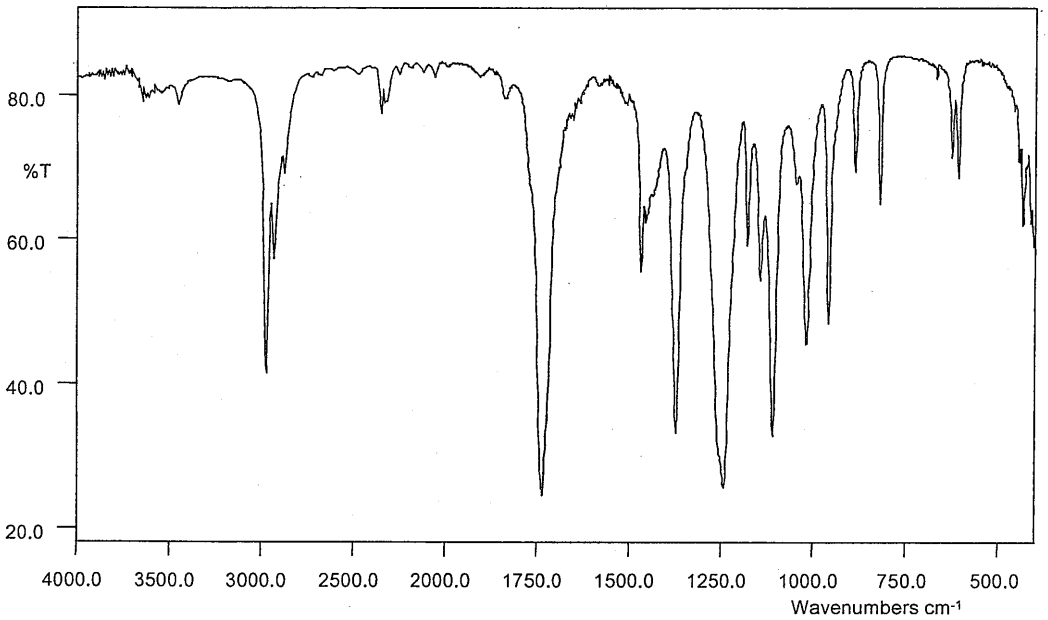
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

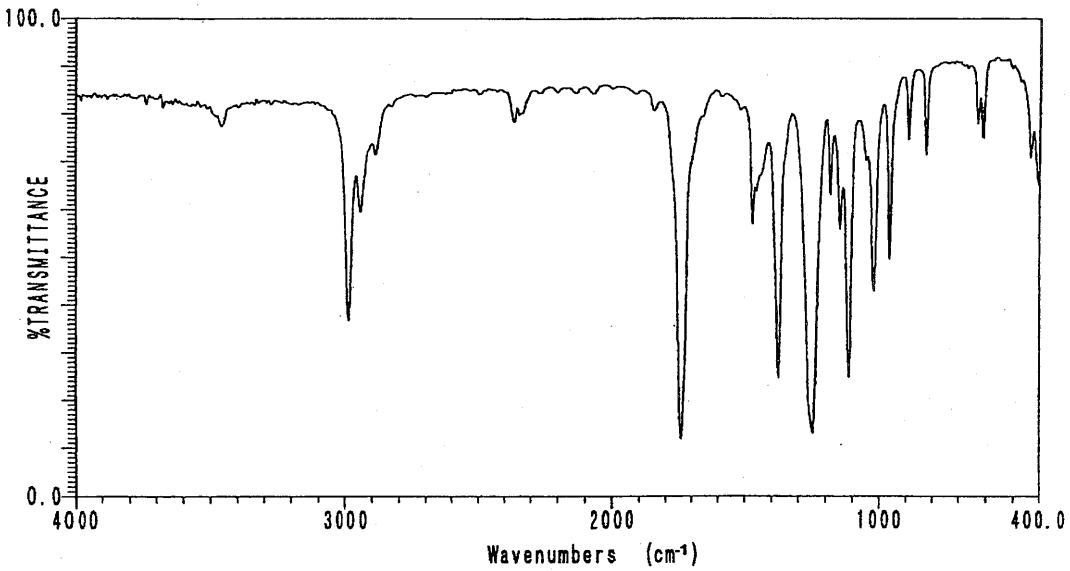
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

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 mmϕ × 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

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

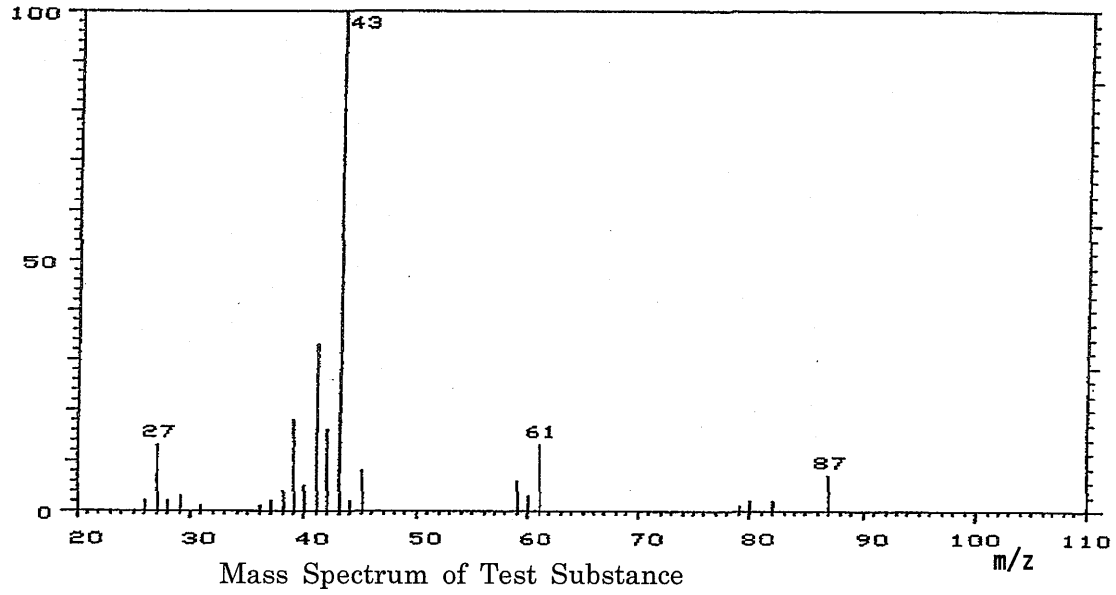
1. Spectral Data

Mass Spectrometry

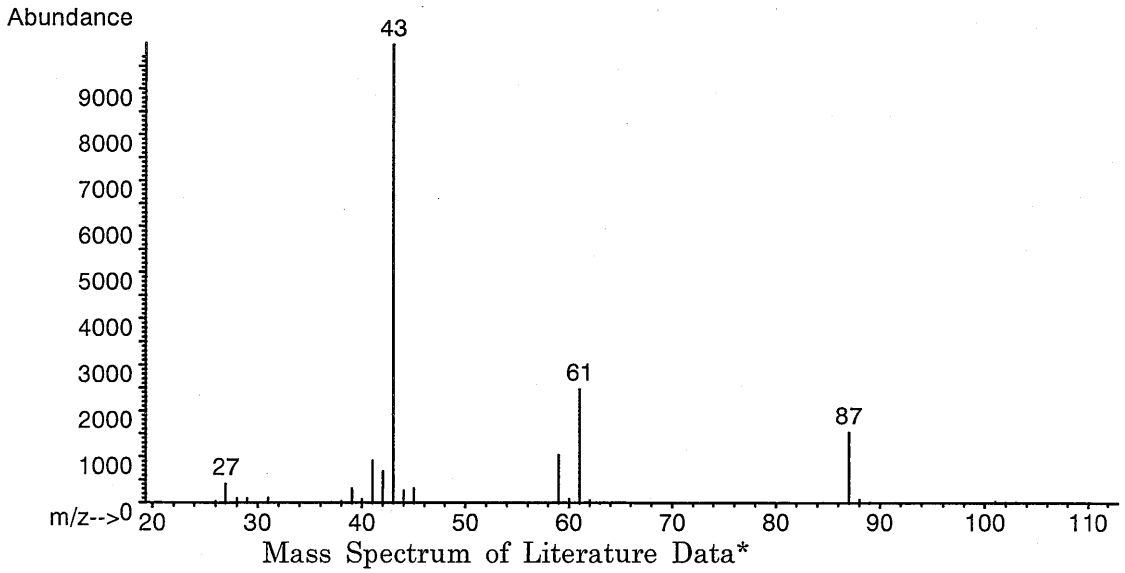
Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

Result: The mass spectrum was consistent with literature spectrum.

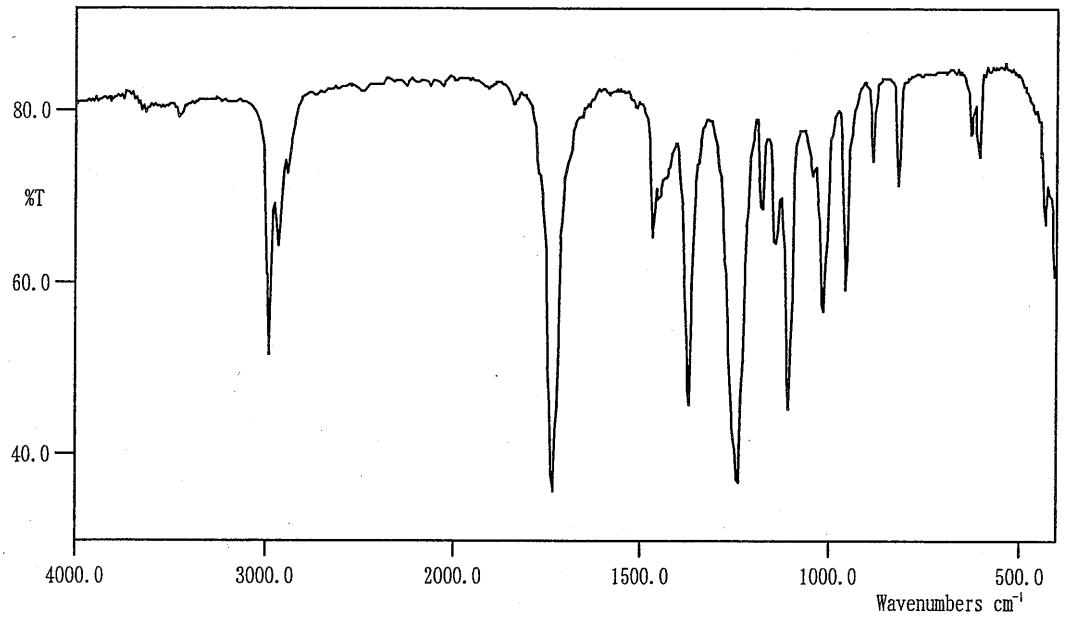
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

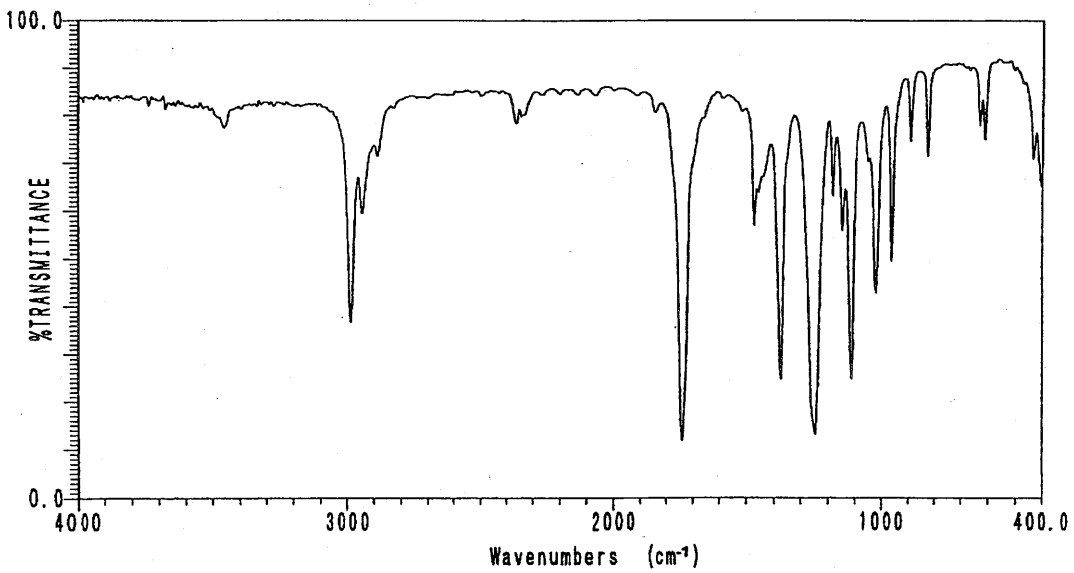
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

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 mmϕ × 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

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

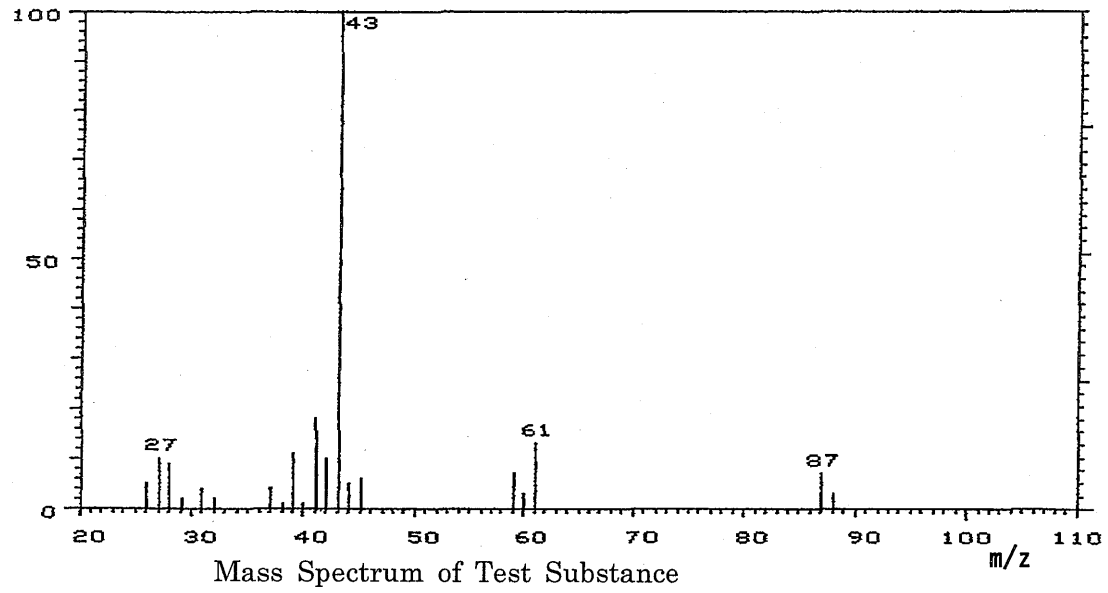
1. Spectral Data

Mass Spectrometry

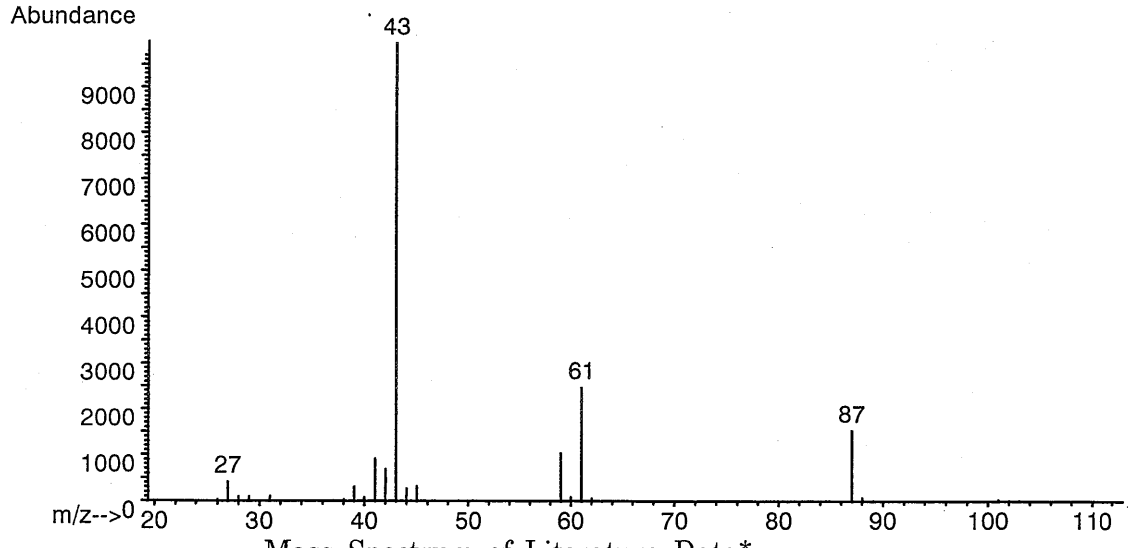
Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

Result: The mass spectrum was consistent with literature spectrum.

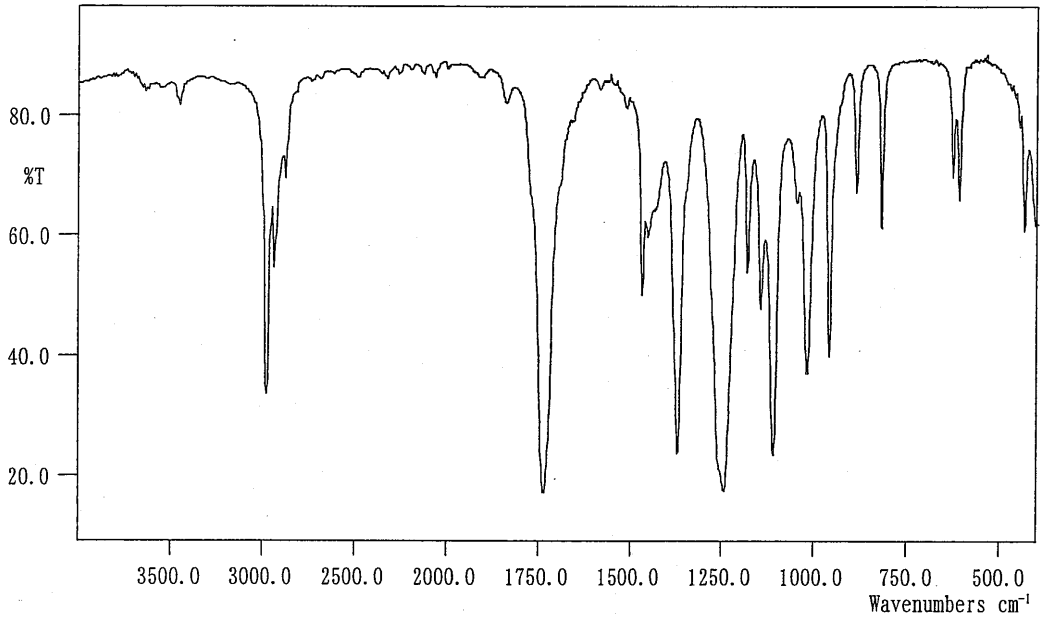
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

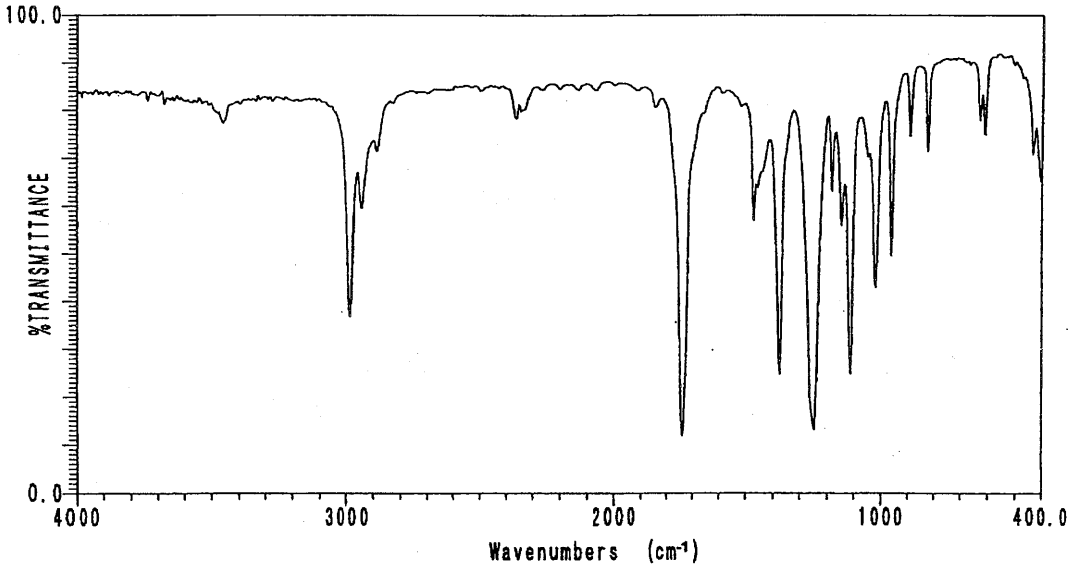
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

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 mmϕ × 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

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

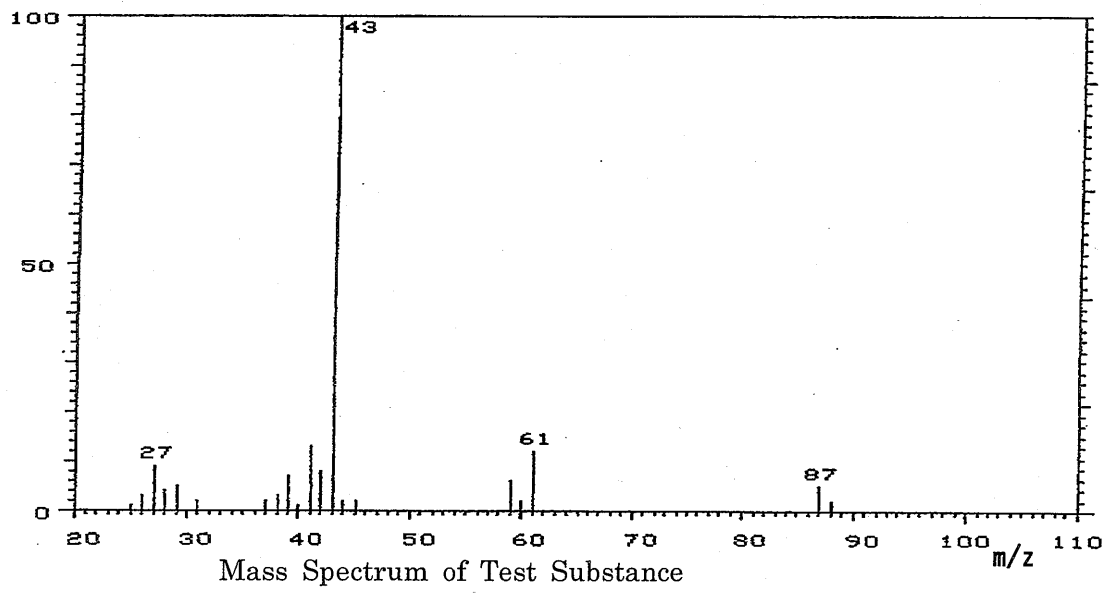
1. Spectral Data

Mass Spectrometry

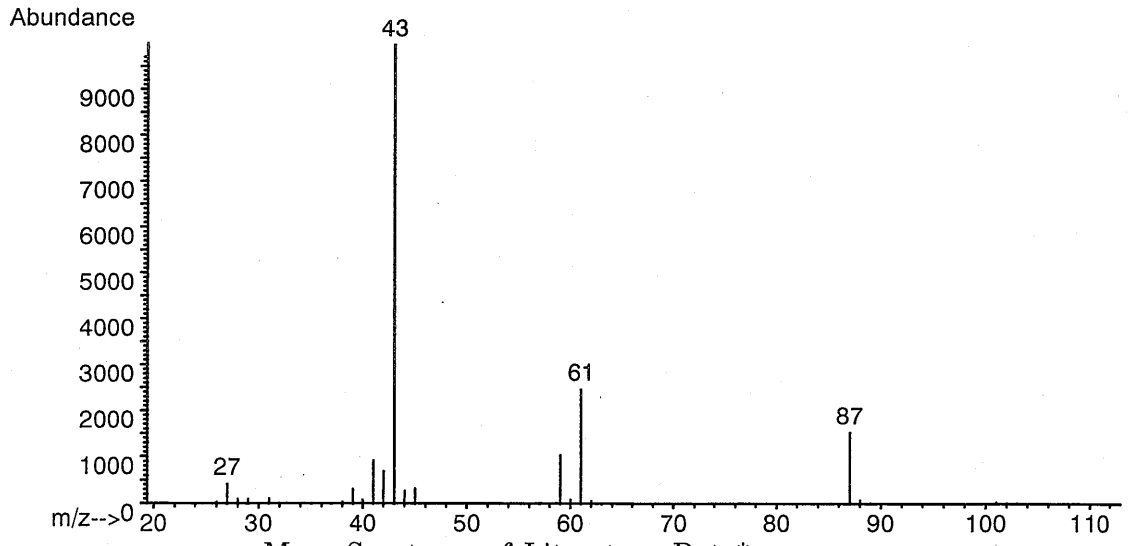
Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

Result: The mass spectrum was consistent with literature spectrum.

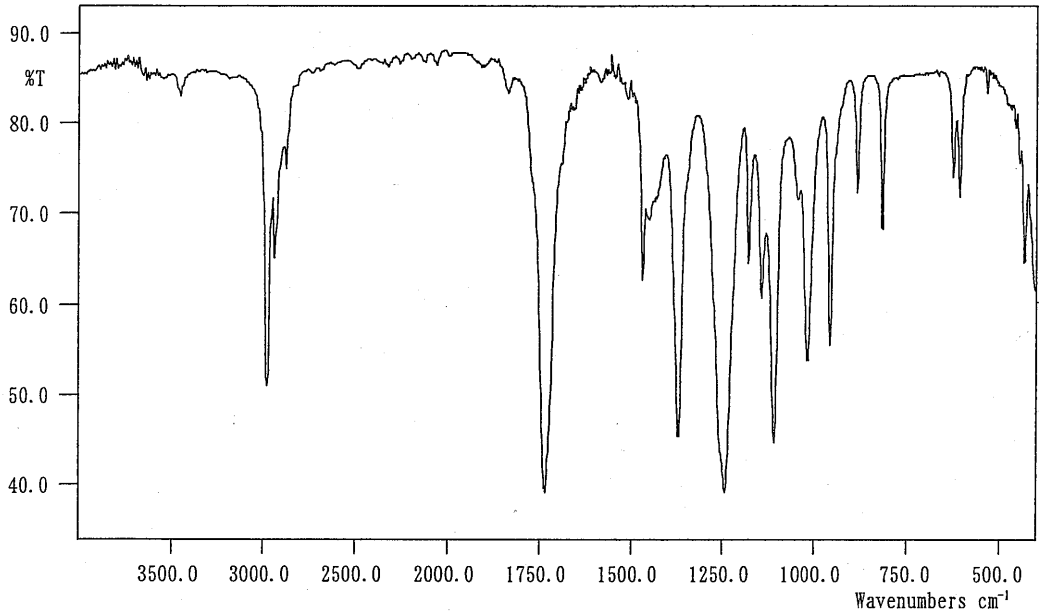
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

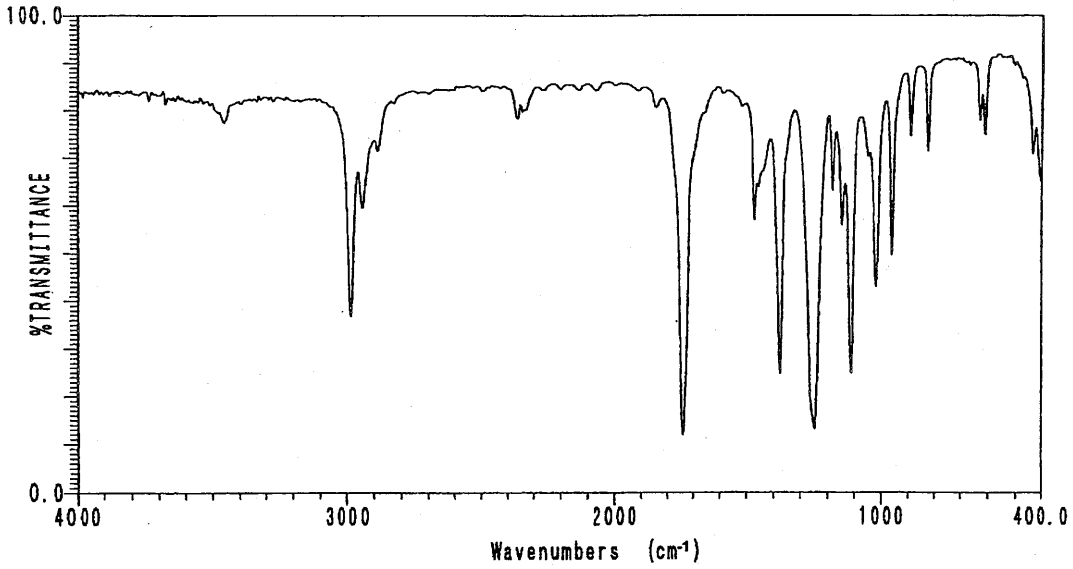
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

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 mmϕ × 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

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

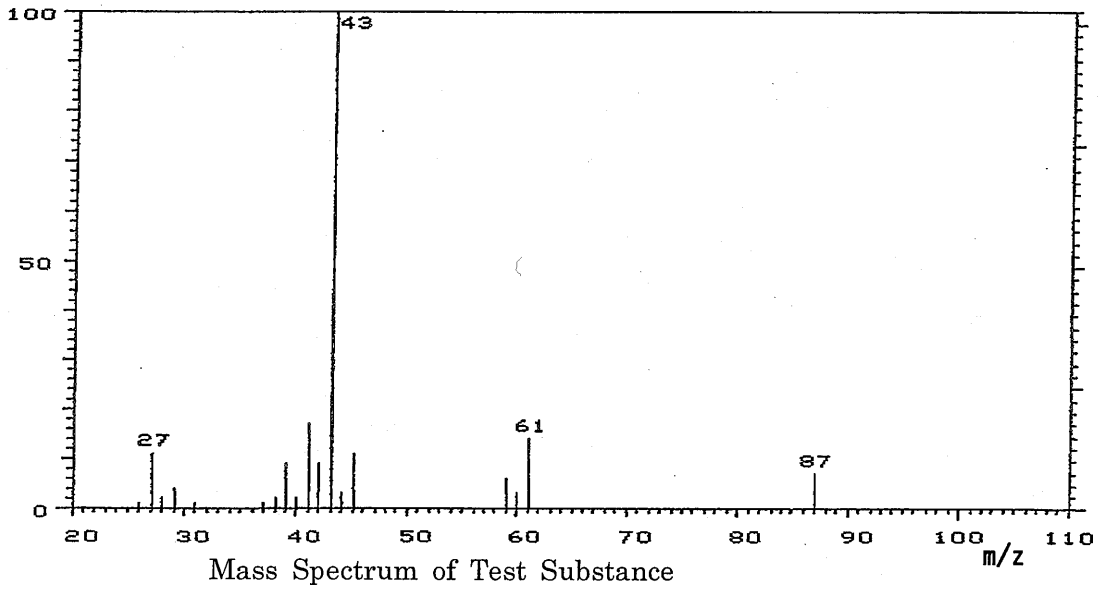
1. Spectral Data

Mass Spectrometry

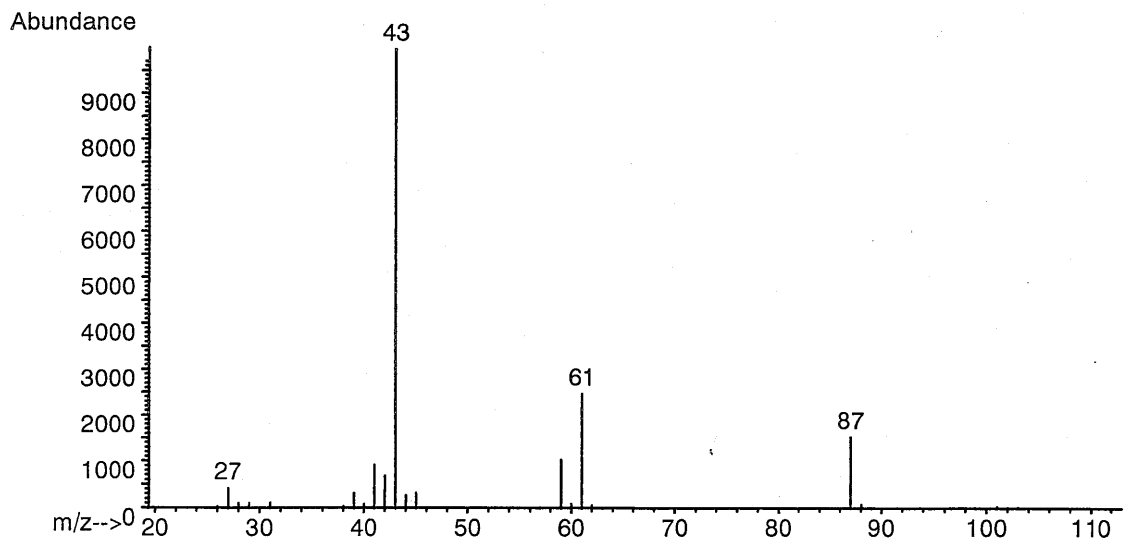
Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

Result: The mass spectrum was consistent with literature spectrum.

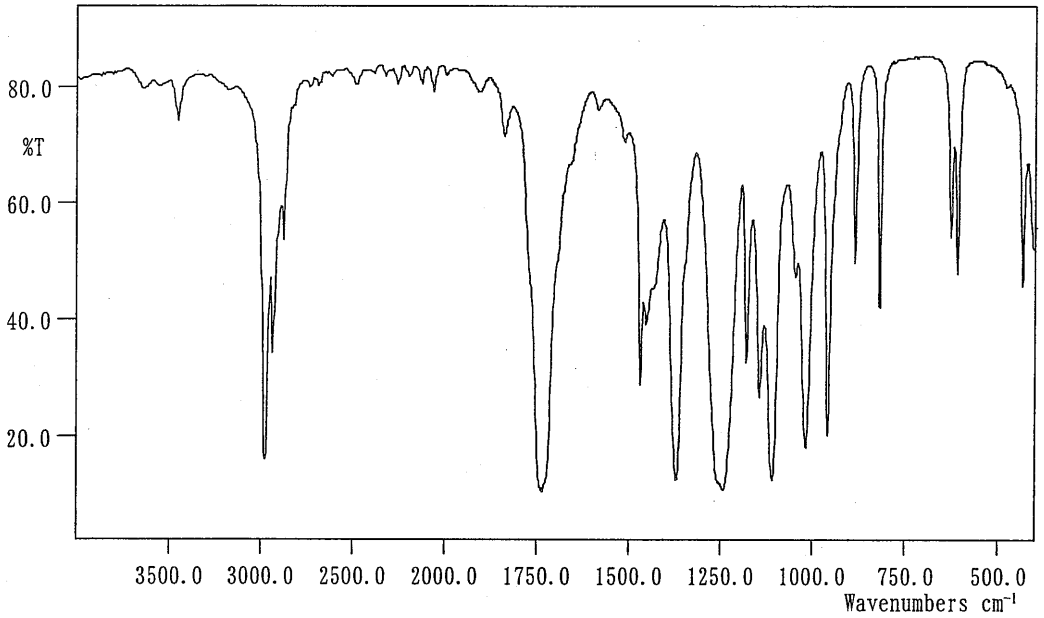
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

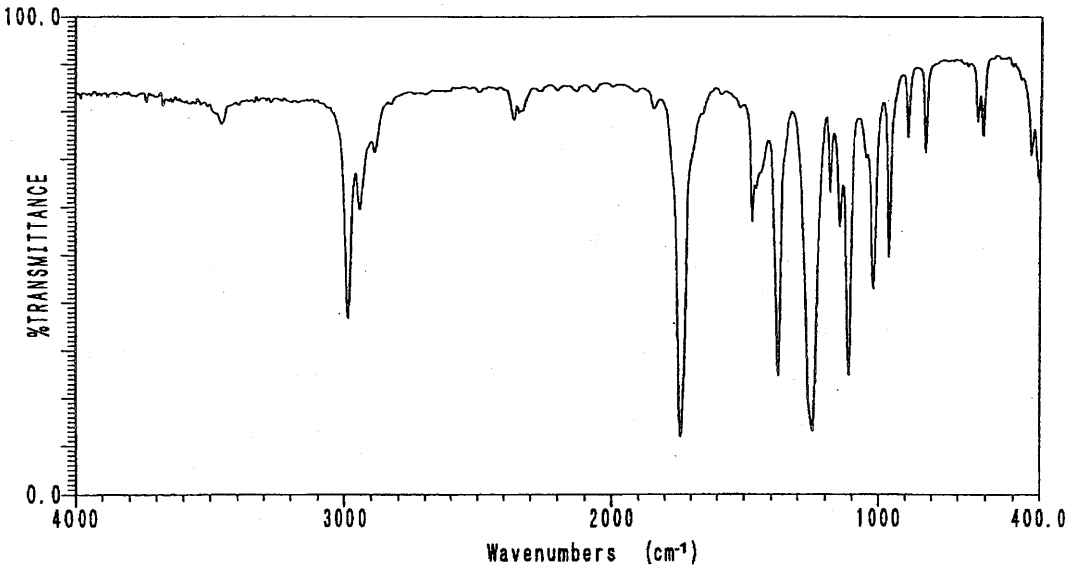
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

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 mmϕ × 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Sample Name	Peak No.	Area (%)	Peak Name
Test Substance	1	0.044	2-Propanol
	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 mmϕ × 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

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 mmϕ × 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

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 mmϕ × 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

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 mmϕ × 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

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 mmϕ × 60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

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 (℃) Mean ± S.D.	Humidity (%) Mean ± S.D.	Ventilation Rate (L/min) Mean ± S.D.*1	Ventilation Rate (L/min) Mean ± S.D.*2	Air Change (time/h) Mean*1	Air Change (time/h) Mean*2
Control	23.0 ± 0.2	54.8 ± 2.5	763.3 ± 5.2	1514.3 ± 18.9	6.0	12.0
1000 ppm	23.0 ± 0.2	54.6 ± 1.8	764.5 ± 3.9	1520.7 ± 16.6	6.0	12.0
2000 ppm	23.0 ± 0.2	53.3 ± 2.1	764.0 ± 4.4	1521.5 ± 17.8	6.0	12.0
4000 ppm	23.0 ± 0.2	53.1 ± 2.5	765.1 ± 3.6	1517.0 ± 16.4	6.0	12.0

＊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]	$\times 10^6/\mu L$	2
Hemoglobin(Hgb)	Cyanmethemoglobin method [1]	g/dL	1
Hematocrit(Hct)	Calculated as RBC×MCV/10 [1]	%	1
Mean corpuscular volume(MCV)	Light scattering method [1]	fL	1
Mean corpuscular hemoglobin(MCH)	Calculated as Hgb/RBC×10 [1]	pg	1
Mean corpuscular hemoglobin concentration (MCHC)	Calculated as Hgb/Hct×100 [1]	g/dL	1
Platelet	Light scattering method [1]	$\times 10^3/\mu L$	0
Reticulocyte	Light scattering method [1]	%	1
White blood cell(WBC)	Light scattering method [1]	$\times 10^3/\mu L$	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 Alb/(TP−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
Creatinine	Jaffé 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.)