2,4-ペンタンジオンのマウスを用いた
吸入によるがん原性試験報告書

試験番号：0676

# APPENDICES

# APPENDICES

# APPENDIX 1-1

# IDENTITY OF 2,4-PENTANEDIONE

# IN THE 2-YEAR INHALATION STUDY

IDENTITY OF 2,4-PENTANEDIONE IN THE 2-YEAR INHALATION STUDY

Test Substance : 2,4-Pentanedione (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : DPG1369

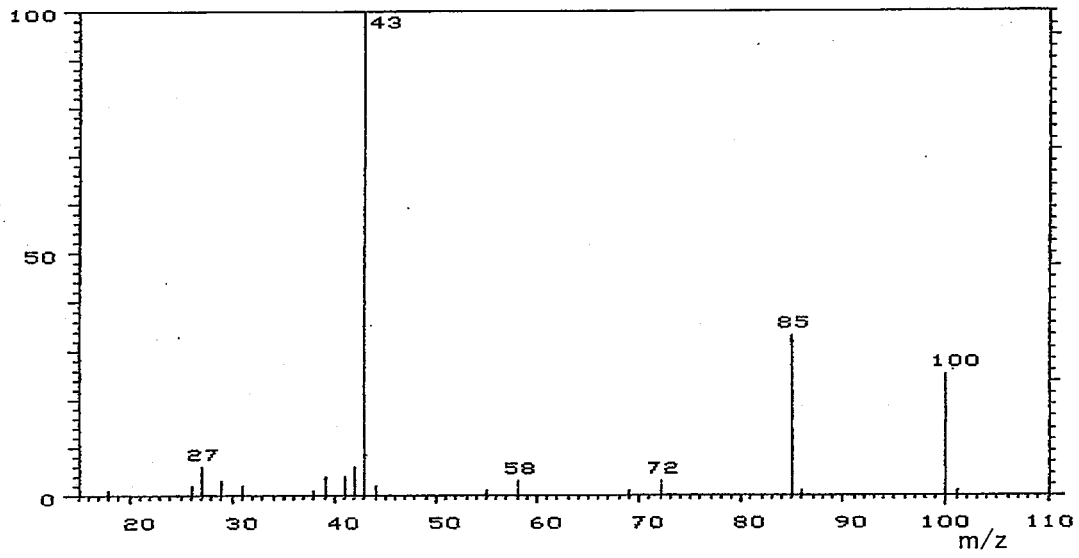
1. Spectral Data

Mass Spectrometry

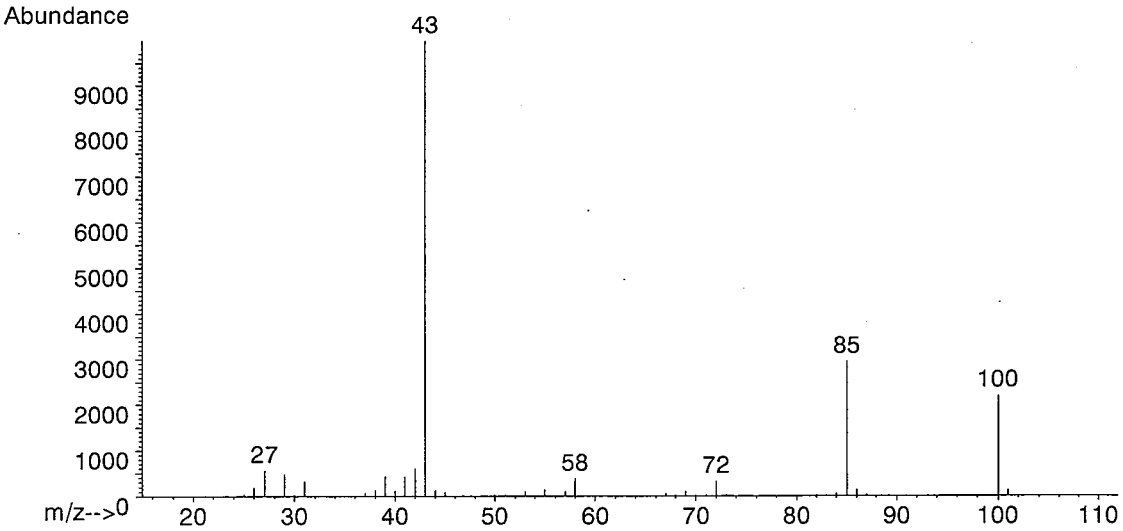
Instrument : Hitachi M-80B Mass Spectrometer

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.

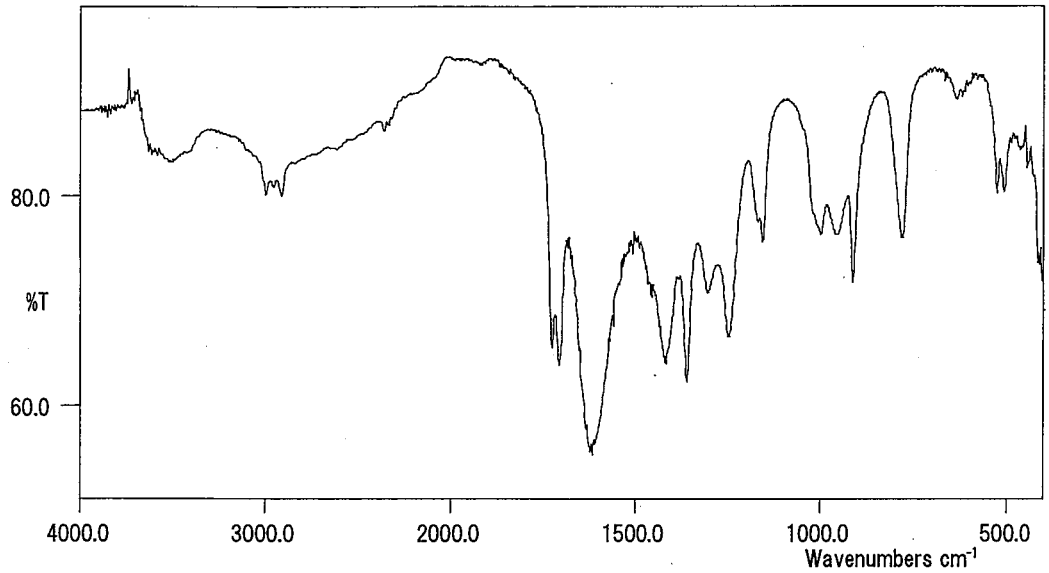
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

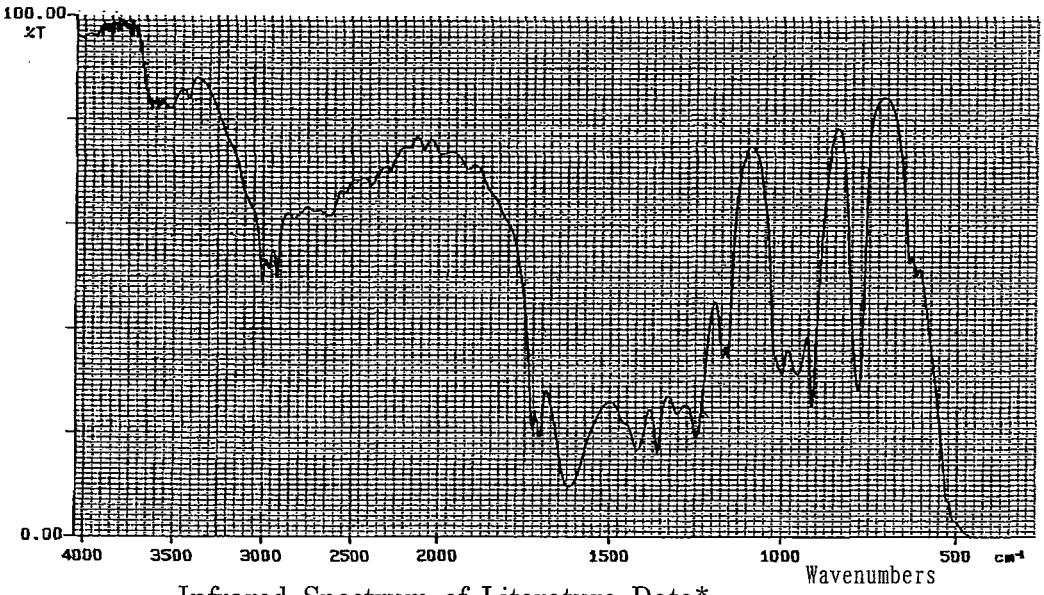
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

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. Conclusion: The test substance was identified as 2,4-pentanedione by mass spectrum and infrared spectrum.

B. Lot No. : TSK2532

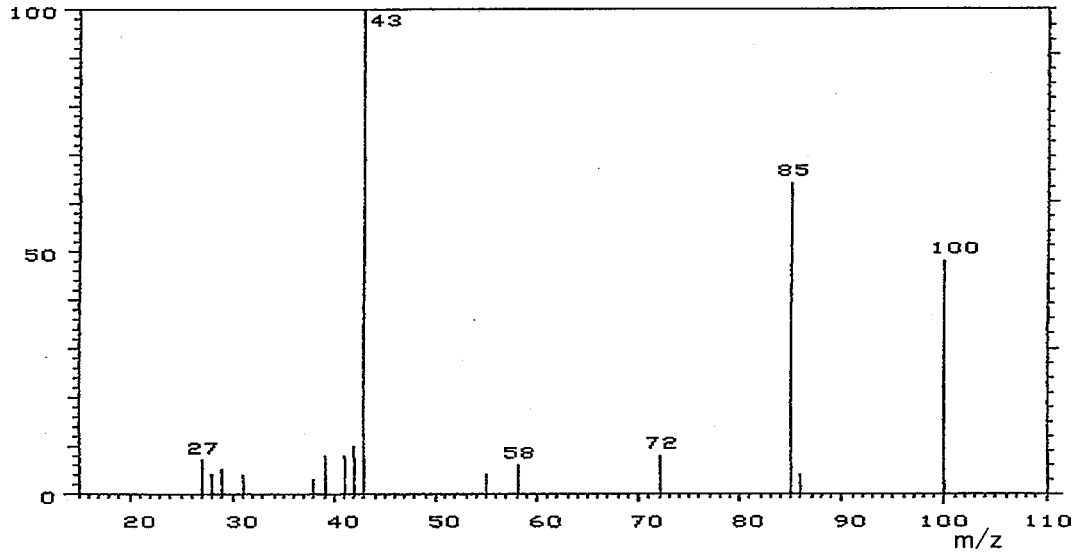
1. Spectral Data

Mass Spectrometry

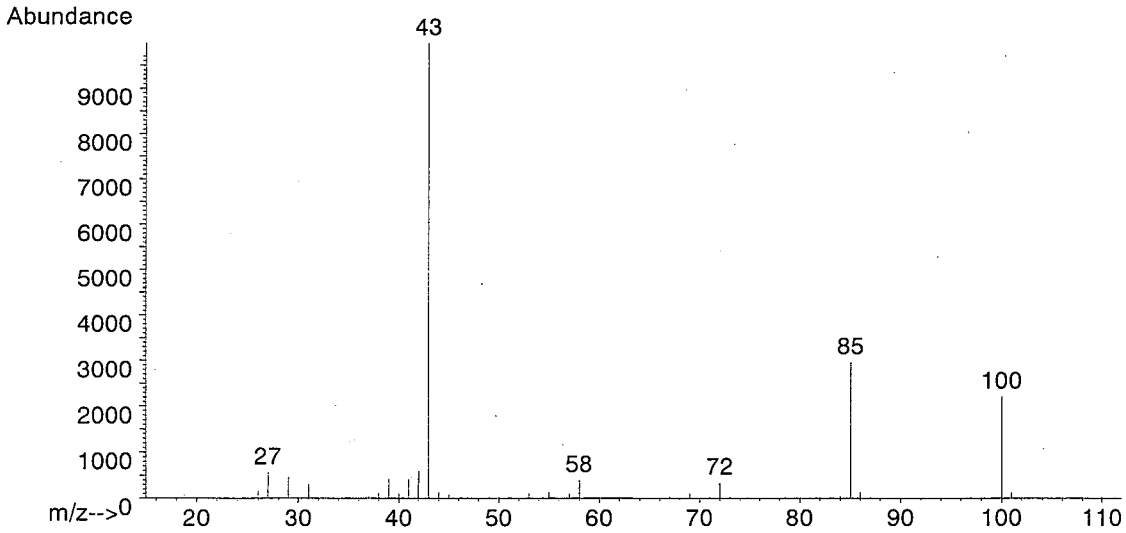
Instrument : Hitachi M-80B Mass Spectrometer

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.

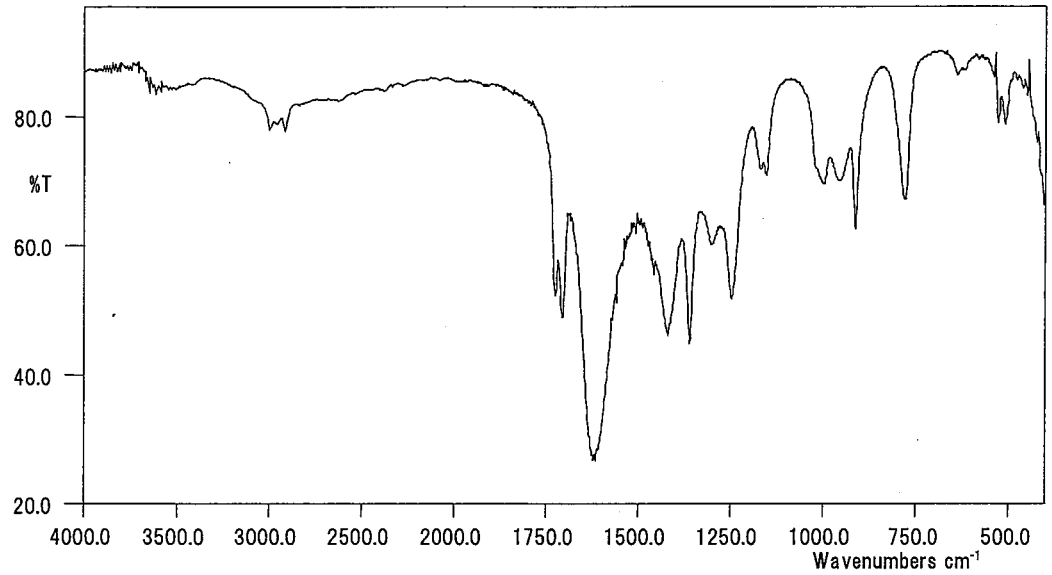
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

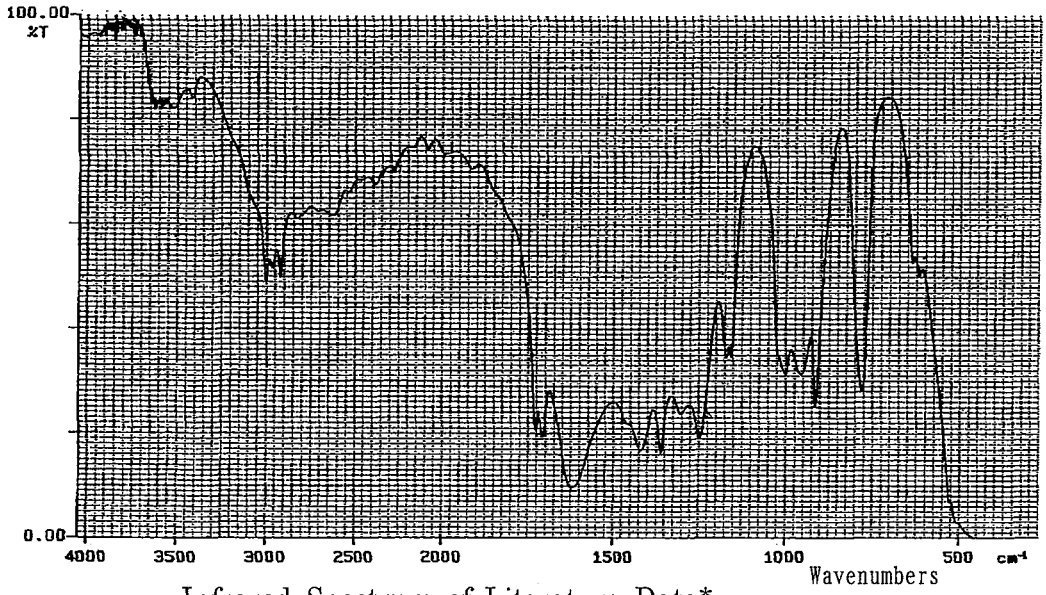
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

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. Conclusion: The test substance was identified as 2,4-pentanedione by mass spectrum and infrared spectrum.

C. Lot No. : ALQ3659

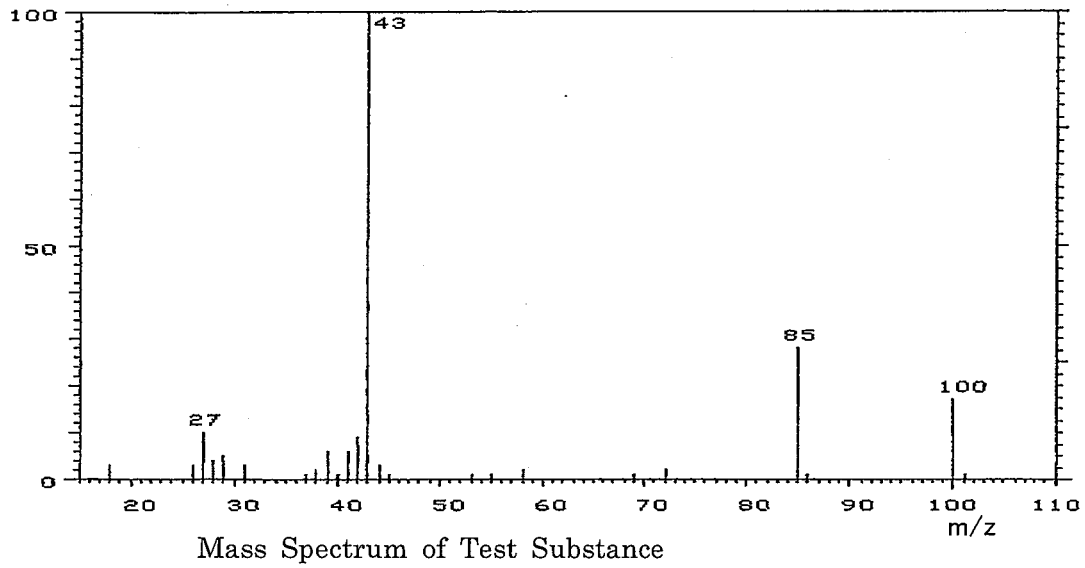
1. Spectral Data

Mass Spectrometry

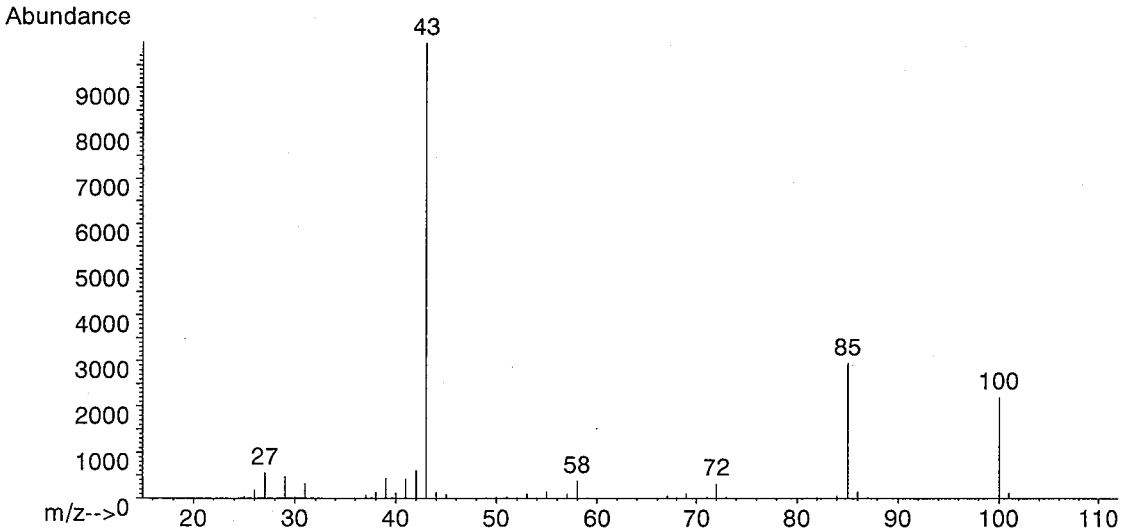
Instrument : Hitachi M-80B Mass Spectrometer

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.

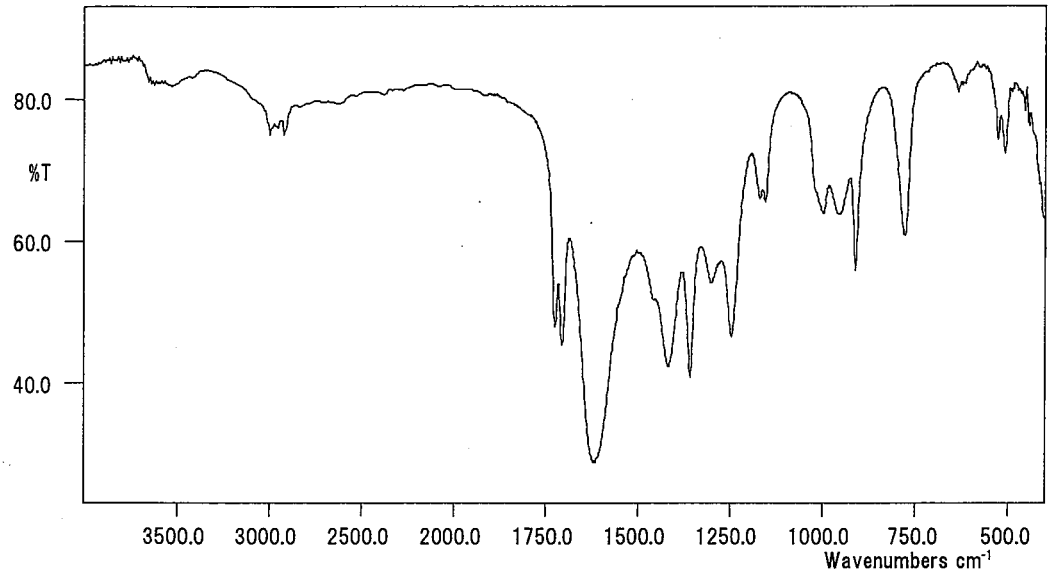
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

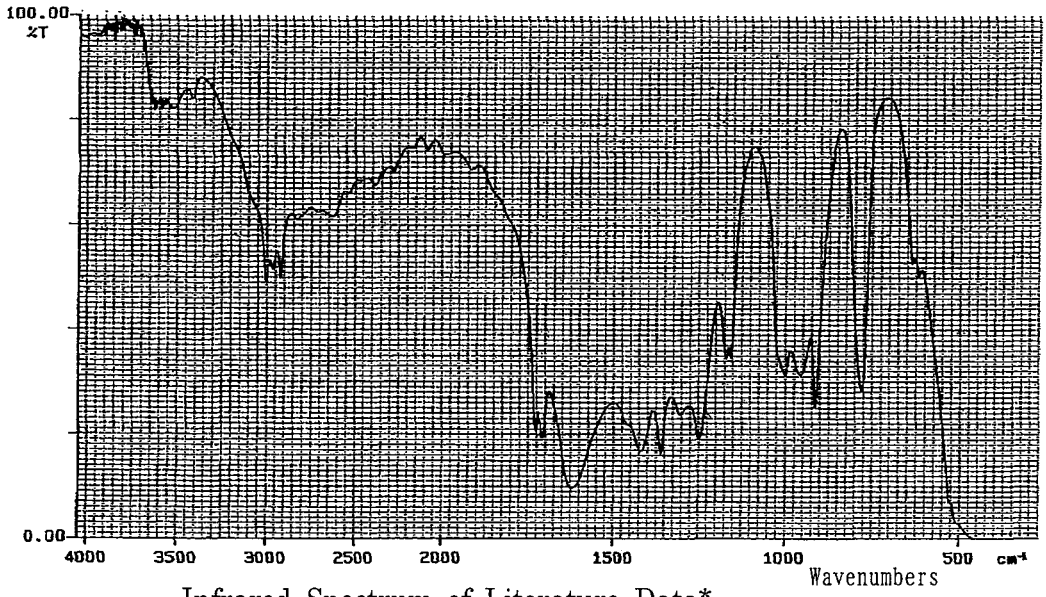
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

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. Conclusion: The test substance was identified as 2,4-pentanedione by mass spectrum and infrared spectrum.

D. Lot No. : ALL1711

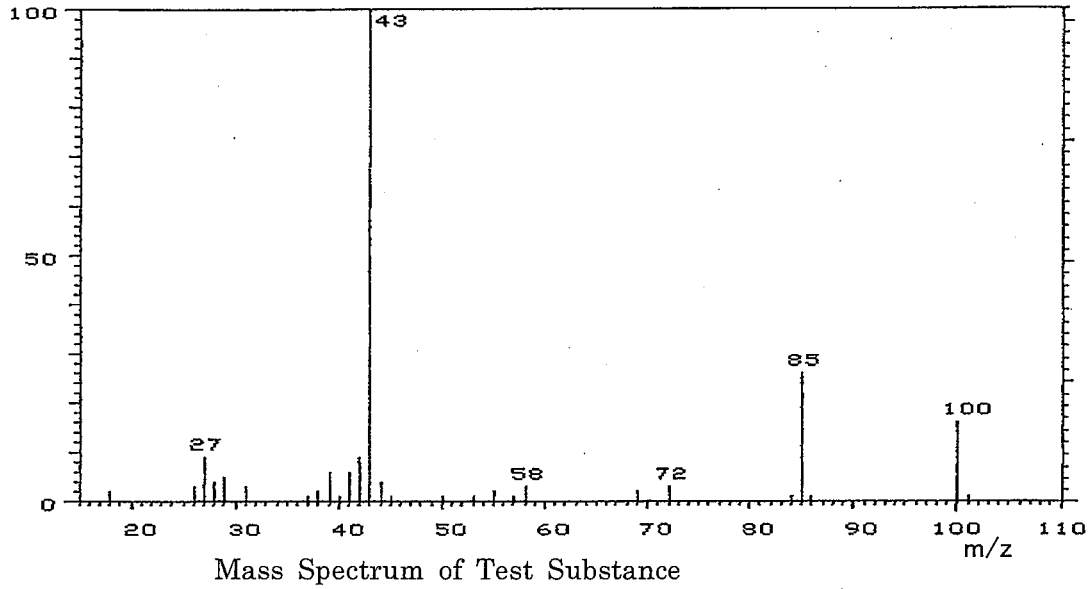
1. Spectral Data

Mass Spectrometry

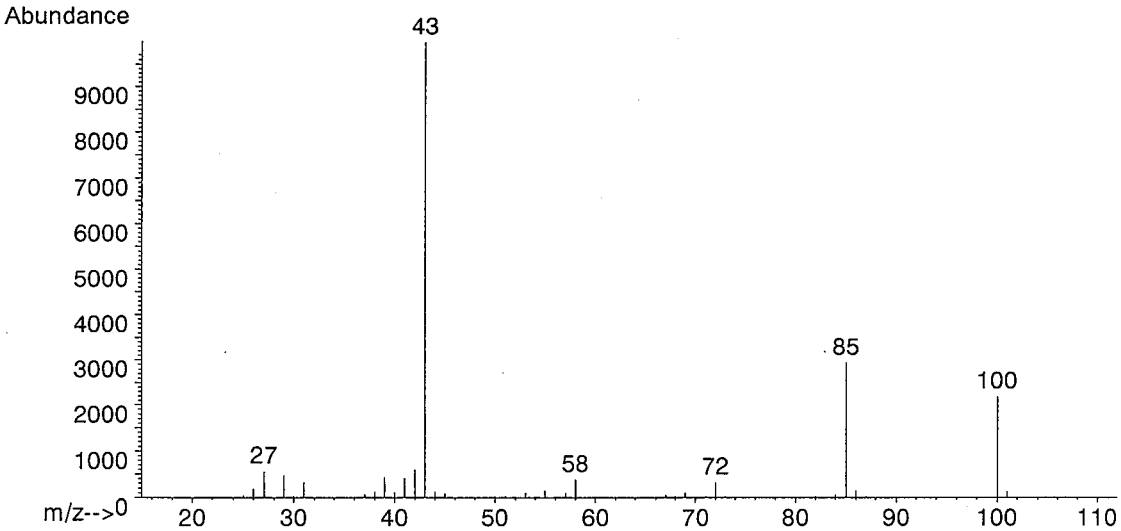
Instrument : Hitachi M-80B Mass Spectrometer

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.

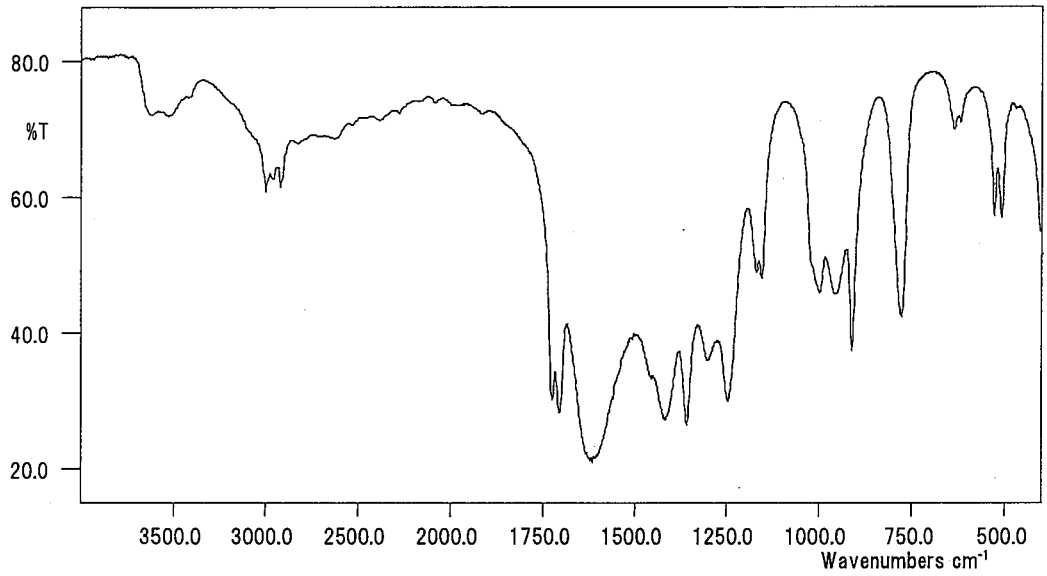
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

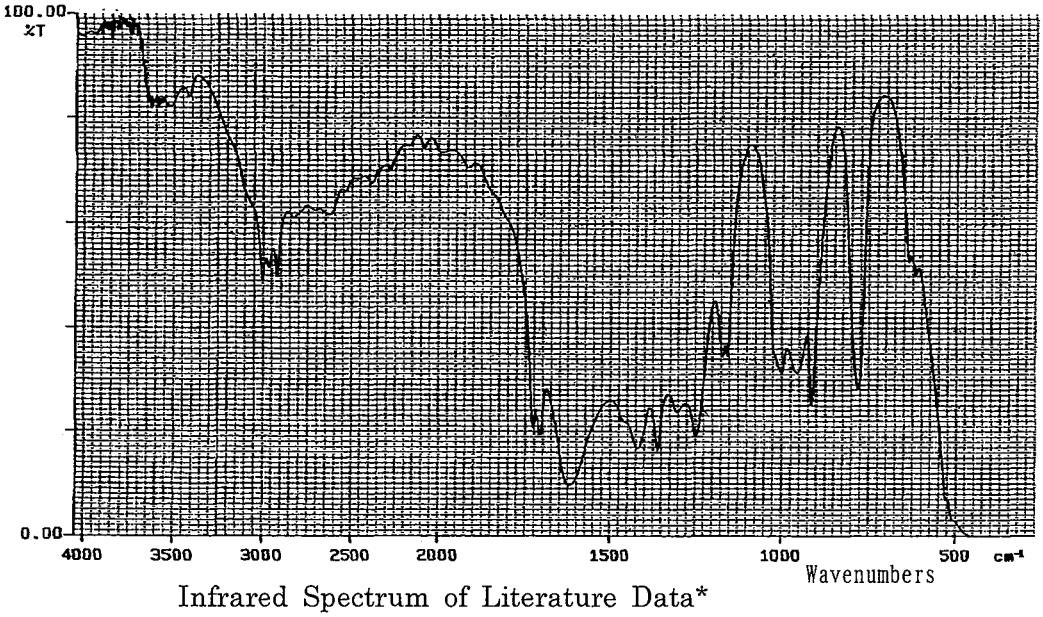
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

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. Conclusion: The test substance was identified as 2,4-pentanedione by mass spectrum and infrared spectrum.

# APPENDIX 1-2

# STABILITY OF 2,4-PENTANEDIONE

# IN THE 2-YEAR INHALATION STUDY

STABILITY OF 2,4-PENTANEDIONE IN THE 2-YEAR INHALATION STUDY

Test Substance : 2,4-Pentanedione (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : DPG1369

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX ( 0.53 mm$\phi$ × 60 m)

Column Temperature: 150° C

Flow Rate : 3 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

| Date (date analyzed) | Peak No. | Retention Time (min) | Area (%) |
|---|---|---|---|
| 2007.01.09 | 1 | 5.807 | 100 |
| 2007.09.14 | 1 | 5.736 | 100 |

Result: Gas chromatography indicated one major peak (peak No.1) analyzed on 2007.1.9 and one major peak (peak No.1) analyzed on 2007.9.14.
No new trace impurity peak in the test substance analyzed on 2007.9.14 was detected.

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

B. Lot No. : TSK2532

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX ( 0.53 mm$\phi$ × 60 m)

Column Temperature: 150° C

Flow Rate : 3 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

| Date (date analyzed) | Peak No. | Retention Time (min) | Area (%) |
|---|---|---|---|
| 2007.09.11 | 1 | 5.736 | 100 |
| 2008.04.11 | 1 | 5.667 | 100 |

Result: Gas chromatography indicated one major peak (peak No.1) analyzed on 2007.9.11 and one major peak (peak No.1) analyzed on 2008.4.11. No new trace impurity peak in the test substance analyzed on 2008.4.11 was detected.

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

C. Lot No. : ALQ3659

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX ( 0.53 mm$\phi$ × 60 m)

Column Temperature: 150° C

Flow Rate : 3 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

| Date (date analyzed) | Peak No. | Retention Time (min) | Area (%) |
|---|---|---|---|
| 2008.04.04 | 1 | 5.762 | 100 |
| 2008.08.26 | 1 | 5.767 | 100 |

Result: Gas chromatography indicated one major peak (peak No.1) analyzed on 2008.4.4 and one major peak (peak No.1) analyzed on 2008.8.26.
No new trace impurity peak in the test substance analyzed on 2008.8.26 was detected.

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

D. Lot No. : ALL1711

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX ( 0.53 mm$\phi$ × 60 m)

Column Temperature: 150° C

Flow Rate : 3 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

| Date (date analyzed) | Peak No. | Retention Time (min) | Area (%) |
|---|---|---|---|
| 2008.08.08 | 1 | 5.751 | 100 |
| 2009.01.15 | 1 | 5.758 | 100 |

Result: Gas chromatography indicated one major peak (peak No.1) analyzed on 2008.8.8 and one major peak (peak No.1) analyzed on 2009.1.15.
No new trace impurity peak in the test substance analyzed on 2009.1.15 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 2,4-PENTANEDIONE

ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER IN THE 2-YEAR INHALATION STUDY OF 2,4-PENTANEDIONE

| Group Name | Temperature (℃) Mean ± S.D. | Humidity (%) Mean ± S.D. | Ventilation Rate (L/min) Mean ± S.D. | Air Change (time/h) Mean |
|---|---|---|---|---|
| Control | 23.0 ± 0.0 | 57.3 ± 0.7 | 743.7 ± 5.2 | 12.1 |
| 100 ppm | 23.0 ± 0.0 | 54.2 ± 1.1 | 741.5 ± 4.9 | 12.0 |
| 200 ppm | 23.0 ± 0.0 | 55.0 ± 1.1 | 744.1 ± 4.9 | 12.1 |
| 400 ppm | 23.0 ± 0.0 | 53.9 ± 1.2 | 739.6 ± 5.0 | 12.0 |

# APPENDIX 3

# METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR INHALATION STUDY OF 2,4-PENTANEDIONE

# METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR INHALATION STUDY OF 2,4-PENTANEDIONE

| 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 | Light scattering method [1)] | % | 0 |
| Biochemistry | | | |
| Total protein(TP) | Biuret method [2)] | g/dL | 1 |
| Albumin (Alb) | BCG method [2)] | g/dL | 1 |
| A/G ratio | Calculated as Alb/(TP−Alb) [2)] | − | 1 |
| T-bilirubin | Azobilirubin method [2)] | mg/dL | 2 |
| Glucose | GlcK·G-6-PDH method [2)] | mg/dL | 0 |
| T-cholesterol | CE·COD·POD method [2)] | mg/dL | 0 |
| Triglyceride | MGLP·GK·GPO·POD method [2)] | mg/dL | 0 |
| Phospholipid | PLD·ChOD·POD method [2)] | mg/dL | 0 |
| Aspartate aminotransferase (AST) | JSCC method [2)] | IU/L | 0 |
| Alanine aminotransferase (ALT) | JSCC method [2)] | IU/L | 0 |
| Lactate dehydrogenase (LDH) | JSCC method [2)] | IU/L | 0 |
| Alkaline phosphatase (ALP) | JSCC method [2)] | IU/L | 0 |
| $\gamma$-Glutamyl transpeptidase ($\gamma$-GTP) | JSCC method [2)] | IU/L | 0 |
| Creatine kinase (CK) | JSCC method [2)] | IU/L | 0 |
| Urea nitrogen | Urease·GLDH method [2)] | mg/dL | 1 |
| Sodium | Ion selective electrode method [2)] | mEq/L | 0 |
| Potassium | Ion selective electrode method [2)] | mEq/L | 1 |
| Chloride | Ion selective electrode method [2)] | mEq/L | 0 |
| Calcium | OCPC method [2)] | mg/dL | 1 |
| Inorganic phosphorus | PNP·XOD·POD method [2)] | mg/dL | 1 |

1) Automatic blood cell analyzer (ADVIA120 : Siemens Healthcare Diagnostics Inc.)
2) Automatic analyzer (Hitachi 7080 : Hitachi,Ltd.)