f: Number of the animals of each group.

$$s^2 = \frac{\sum y^2 - \frac{Y}{f}}{n}$$

 Σy^2 : The sum of squares of y_1 , y_2 , y_3 and y_4 in each

group.

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

$$n = 4 (f - 1)$$

$$L = 2\sqrt{(C-1)(CM^2 + 0.09062)}$$

$$C = \frac{Y_b^2}{Y_b^2 - 4fs^2t^2}$$

 t^2 : Value shown in the following table against n used to calculate s2.

n	$t^2=F$	n	$t^2=F$	n	$t^2 = F$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers. Storage—Not exceeding 8°C.

Enflurane

エンフルラン

C₃H₂ClF₅O: 184.49

(RS)-2-Chloro-1,1,2-trifluoroethyl difluoromethyl ether [13838-16-9]

Description Enflurane is a clear, colorless liquid.

It is slightly soluble in water.

It is miscible with ethanol (95) and with diethyl ether.

It is a volatile, and not an inflammable.

It shows no optical rotation. Boiling point: 54 - 57°C

Identification (1) Take 0.05 mL of Enflurane, and prepare the test solution as directed to the Oxygen Flask Combustion Method using 40 mL of water as the absorbing liquid. The test solution responds to the Qualitative Tests for chloride and fluoride.

(2) Determine the infrared absorption spectrum of Enflurane as directed in the liquid film method under the Infrared

Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index n_D^{20} : 1.302 – 1.304

Specific gravity d_{20}^{20} : 1.520 – 1.540

Purity (1) Acid or alkali—To 60 mL of Enflurane add 60 mL of freshly boiled and cooled water, shake for 3 minutes, separate the water later, and use the layer as the sample solution. To 20 mL of the sample solution add one drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide TS: the color of the solution is purple. To 20 mL of the sample solution add one drop of bromocresol purple TS and 0.06 mL of 0.01 mol/L hydrochloric acid TS: the color of the solution is yellow.

- (2) Chloride—To 20 g of Enflurane add 20 mL of water, shake well, and separate the water layer. Take 10 mL of the water laver add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid (not more than 0.001%).
- (3) Nonvolatile residue—Evaporate exactly 65 mL of Enflurane on a water bath to dryness, and dry the residue at 105°C for 1 hour: the of the residue is not more than 1.0 mg.
- Related substances—Proceed the test with 5 µL of (4) Enflurane as directed under the Gas chromatography according to the following conditions. Determine each peak area other than the peak of air which appears soon after the injection of the sample by the automatic integration method, and calculate the amount of each peak by the area percentage method: the amount of the substances other than enflurane is not more than 0.10%.

Operating conditions—

Detector: A thermal conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μ m in particle diameter, coated with diethylene glycol succinate ester for gas chromatography in the ratio of 20%.

Column temperature: A constant temperature of about 80°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of enflurane is about 3 minutes.

Selection of column: Mix 5 mL of Enflurane and 5 mL of 1,2-dichloroethane. Proceed with 1 μ L of this mixture under the above operating conditions, and calculate the resolution. Use a column giving elution of enflurane and 1,2dichloroethane in this order with the resolution between these peaks being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of enflurane from 5 μ L of Enflurane is not less than 50% of the full scale.

Time span of measurement: About three times as long as the retention time of enflurane.

Water Not more than 0.10% (10 g, direct titration).

Containers and storage Containers—Tight containers. Storage—Not exceeding 30°C.