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(AIST/JIWA/JSA)

**Testing method for individual
4-nonylphenol isomers in industrial
water and wastewater**

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Foreword

This translation has been made based on the original Japanese Industrial Standard established by the Minister of Economy, Trade and Industry through deliberations at the Japanese Industrial Standards Committee as a result of proposal of establishing a Japanese Industrial Standard from Advanced Industrial Science and Technology (AIST)/Japan Industrial Water Association (JIWA)/Japanese Standards Association (JSA), with a draft being attached, based on the provision of Article 12 Clause 1 of the Industrial Standardization Law.

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JIS K 0450 consists of the following parts.

- JIS K 0450-10-10 *Testing method for bisphenol A in industrial water and waste water*
- JIS K 0450-20-10 *Testing method for alkyl phenols in industrial water and waste-water*
- JIS K 0450-30-10 *Testing method for phthalic acid esters in industrial water and wastewater*
- JIS K 0450-40-10 *Testing method for hexanedioic acid bis(2-ethylhexyl) ester in industrial water and wastewater*
- JIS K 0450-50-10 *Testing method for benzophenone in industrial water and waste-water*
- JIS K 0450-60-10 *Testing method for individual 4-nonylphenol isomers in industrial water and wastewater*

Testing method for individual 4-nonylphenol isomers in industrial water and wastewater

1 Scope

This Japanese Industrial Standard specifies the testing method for the determination of the isomers in table 1 among the isomers of 4-nonylphenol (CAS No. 25154-52-3) in industrial water and wastewater by using gas chromatography/mass spectrometry.

Table 1 List of 4-nonylphenol isomer analytes^{a)}

No.	Name of isomer	Molecular formula	Symbol	CAS No.
1	4-(2,4-dimethylheptan-4-yl) phenol	C ₁₅ H ₂₄ O	NP1	Undecided
2	4-(2,4-dimethylheptan-2-yl) phenol	C ₁₅ H ₂₄ O	NP2	Undecided
3	4-(3,6-dimethylheptan-3-yl) phenol	C ₁₅ H ₂₄ O	NP3	Undecided
4 ^{b)}	4-(3,5-dimethylheptan-3-yl) phenol	C ₁₅ H ₂₄ O	NP4	Undecided
5	4-(2,5-dimethylheptan-2-yl) phenol	C ₁₅ H ₂₄ O	NP5	Undecided
6 ^{b)}	4-(3,5-dimethylheptan-3-yl) phenol	C ₁₅ H ₂₄ O	NP6	Undecided
7	4-(3-ethyl-2-methylhexan-2-yl) phenol	C ₁₅ H ₂₄ O	NP7	Undecided
8	Substance of peak No. 8	—	NP8	Undecided
9	4-(3,4-dimethylheptan-3-yl) phenol	C ₁₅ H ₂₄ O	NP9	Undecided
10	4-(3,4-dimethylheptan-4-yl) phenol	C ₁₅ H ₂₄ O	NP10	Undecided
11	4-(2,3-dimethylheptan-2-yl) phenol	C ₁₅ H ₂₄ O	NP11	Undecided
12	4-(3-methyloctan-3-yl) phenol	C ₁₅ H ₂₄ O	NP12	Undecided
13	Substance of peak No. 13	—	NP13	Undecided
Notes ^{a)} The 4-nonylphenol isomers herein means that which the 4th position (p-position) of phenol is substituted by the isomer of nonane [CH ₃ (CH ₂) ₇ CH ₃] (the sum of number of carbon in straight chain and side chain is nine). The peak number is in order of retention time. ^{b)} Stereoisomer				

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of **JIS K 0450**. The most recent editions of the standards (including amendments) indicated below shall be applied.

JIS K 0050 *General rules for chemical analysis*

JIS K 0094 *Sampling methods for industrial water and industrial wastewater*

JIS K 0101 *Testing methods for industrial water*

JIS K 0102 *Testing methods for industrial wastewater*

- JIS K 0123 *General rules for gas chromatography/mass spectrometry*
- JIS K 0211 *Technical terms for analytical chemistry (General part)*
- JIS K 0215 *Technical terms for analytical chemistry (analytical instrument part)*
- JIS K 0557 *Water used for industrial water and wastewater analysis*
- JIS K 1107 *Nitrogen*
- JIS K 8040 *Acetone for pesticide residue and polychlorinated biphenyl analysis (Reagent)*
- JIS K 8117 *Dichloromethane for pesticide residue and polychlorinated biphenyl analysis (Reagent)*
- JIS K 8150 *Sodium chloride (Reagent)*
- JIS K 8180 *Hydrochloric acid (Reagent)*
- JIS K 8251 *Glass wool (Reagent)*
- JIS K 8825 *Hexane for pesticide residue and polychlorinated biphenyl analysis (Reagent)*
- JIS K 8891 *Methanol (Reagent)*
- JIS K 8987 *Sodium sulfate (Reagent)*
- JIS R 3503 *Glass apparatus for chemical analysis*
- JIS R 3505 *Volumetric glassware*
- JIS Z 0701 *Silicagel desiccants for packaging*

3 Common matters

3.1 General rules

The general matters common to chemical analysis shall be in accordance with **JIS K 0050**.

3.2 Terms and definitions

For the purposes of this part of **JIS K 0450**, the definitions given in **JIS K 0101**, **JIS K 0102**, **JIS K 0211** and **JIS K 0215** apply.

3.3 Gas chromatography/mass spectrometry method

The general matters common to gas chromatography/mass spectrometry shall be in accordance with **JIS K 0123**.

3.4 Name of compound

The name of compound of analyte shall be in accordance with table 1.

NOTE : For the name of compound of analyte, the nomenclature of compounds [based on the nomenclature of organic chemistry of International Union of Pure and Applied Chemistry (IUPAC)] established by the Chemical Society of Japan is used and mentioned as NP1 to NP13 in symbol.

3.5 Water

The water shall be A1 to A4 water specified in **JIS K 0557**.

3.6 Reagents

The reagents shall be as follows.

- a) The reagents, which are specified in **JIS** and do not hinder the test, shall be used. When there are no specifications in **JIS**, those which do not hinder the test shall be used.
- b) The concentration of the mixed solution of liquid reagent A and liquid reagent B shall be expressed by $A - B (a + b)$. This expression indicates that A and B are mixed in the rate of a ml and b ml.
- c) The designations of reagents are basically conformed to the nomenclature of compounds and the designations of **JIS** reagents established by the Chemical Society of Japan based on the nomenclature of inorganic chemistry and the nomenclature of organic chemistry of the International Union of Pure and Applied Chemistry (IUPAC).
- d) Care shall be taken of the room pollution, the inhalation and adherence to human body due to reagents and waste liquids. For handling reagents and wastewater, care shall be sufficiently taken to ensure that the relate laws and regulations shall be observed.
- e) The concentration of standard solution shall be expressed by the mass in 1 ml (mg/ml, μ g/ml or ng/ml).
- f) The concentration indicated in parentheses behind the name of standard solution means that it is a correct concentration.
- g) It is recommended to use the reagents to be used for the preparation of standard solution of which the traceability is secured in the national metrological standard (Article 134 of the Measurement Law). When they are not available, the standard products of which the purity and uncertainty are clarified shall be used among the products on the market.

3.7 Glasswares

The common matters of glasswares shall be as follows.

- a) Unless otherwise specified, the glasswares specified in **JIS R 3503** and **JIS R 3505** shall be used. When heating operation is accompanied, the borosilicate glass-1 specified in **JIS R 3503** shall be used.

For the desiccant to be used in desiccator, unless otherwise specified, Type A and Class 1 of the silicagel for packaging specified in **JIS Z 0701** shall be used.

- b) The glasswares shall be washed with water of **5.2 a)** before using, and then furthermore washed with acetone (of the quality of concentration 300 or more, specified in **JIS K 8040**), and acetone shall be volatilized by leaving it as it is. Afterward, they shall be heated about at 200 °C about for 2 h and be allowed to cool at a pollution-free place.

3.8 Working curve

For the preparation of working curve, the standard solution shall be taken so that the determination range is divided into 4 stages to 6 stages and the amount of 4-nonylphenol introduced to gas chromatograph mass spectrometer shall coincide with the amount of determination range of each isomer.

4 Sample

4.1 Sampling

4.1.1 Sample container

For the sample container, a 1 000 ml glass bottle with grounding stopper shall be used. A screw cap (with a tetrafluoroethylene packing) may be used instead of a grounding stopper.

4.1.2 Sampling operation

The sampling operation shall be as follows.

- a) **Sampling of surface water** In accordance with 4.1.1 or 4.1.2 of JIS K 0094.
- b) **Sampling of water at each depth** In accordance with 4.1.4 of JIS K 0094.
- c) **Sampling from piping apparatus** In accordance with 4.3 of JIS K 0094.

4.2 Handling of sample

The test shall be carried out immediately after sampling. When it is unable, it shall be preserved at a dark place of 0 °C to 10 °C and be tested as quickly as possible. When it is preserved at a cool place, it shall be made so as not to be frozen.

5 Pretreatment of sample

5.1 Summary of pretreatment of sample

Hydrochloric acid is added to sample to adjust its pH at about 3.5 and ¹³C labelling 4-nonylphenol (ring-¹³C₆) is added as an internal standard material. The analyte is adsorbed by passing water according to pressurization method or depressurization method by using a solid phase column or a solid phase disc, and afterwards it is eluted with acetone and dichloromethane. It is dehydrated and concentrated and made to be a definite amount.

NOTE 1 The pressurization method means the method that sample is fed under pressurized condition.

NOTE 2 The depressurization method means the method that sample is fed under depressurized condition. It is difficult in this method to keep the flow rate at constant rate due to the dispersion in liquid-flow resistance of solid phase, the suspended matter in sample and the like, however there are advantages that the plenty of samples are able to be treated by using a simple apparatus at the same time and the like.

The apparatus of pressurization method consists of the regulation part and pump part. The regulation part controls the time of passing sample and the flow rate of

water flow, and the pump part is based on the method using syringe and the like. The tube used for introducing sample is made of tetrafluoroethylene resin.

The apparatus of depressurization method consists of the piping part for suction and suction pump or aspirator. When plenty of suspended matters are contained in sample, the solvent extraction method in Annex A or the removing method for suspended matters in Annex C may be used as the pretreatment method.

5.2 Reagents

The reagents shall be as follows.

- a) **Water** For the water to be used for the preparation of reagents, operation and blank test, use the water of A4 or A3 in **3.5**. Use the water immediately after purified. When the confirmation of water quality is required, carry out the operation of Annex B before using, and confirm that the peak is not found at the position corresponding to the retention time of 4-nonylphenol. When the purification is additionally required, treat by the method according to remarks 6 in clause 4 of **JIS K 0557** or treat the water of A4 or A3 by activated carbon and the like.
- b) **Hydrochloric acid (1 mol/L)** Prepare by using the hydrochloric acid specified in **JIS K 8180**.
- c) **Sodium sulfate** Heat the sodium sulfate specified in **JIS K 8987** at about 600 °C for about 60 min, and then allow it to cool in a desiccator.
- d) **Acetone** The acetone of 300 or more in concentration specified in **JIS K 8040**. Before using, carry out the operation of **A.4 e)** and **f)** by using 10 ml of acetone [provided that the addition of phenanthrene-*d*₁₀ internal standard solution (1 µg/ml) is not carried out]. Next, carry out the operation of **6.4 c)** by using this solution and confirm that the peak is not found at the position corresponding to the retention time of 4-nonylphenol. After breaking the seal, preserve it at a pollution-free place.
- e) **Dichloromethane** The dichloromethane of 300 or more in concentration specified in **JIS K 8117**. Before using, carry out the operation of **A.4 e)** and **f)** by using 100 ml of dichloromethane [provided that the addition of phenanthrene-*d*₁₀ internal standard solution (1 µg/ml) is not carried out]. Next, carry out the operation of **6.4 c)** by using this solution and confirm that the peak is not found at the position corresponding to the retention time of 4-nonylphenol. After breaking the seal, preserve it at a pollution-free place.
- f) **¹³C labelling 4-nonylphenol internal standard solution (10 µg/ml)** Take 1 ml of ¹³C labelling 4-nonylphenol standard solution (100 µg/ml) on the market, transfer it to a 10 ml volumetric flask containing 2 ml to 5 ml of acetone, and add acetone up to the marked line. Preserve it at a dark place of 5 °C or lower in temperature, when necessary.

Deuterated 4-nonylphenol (4-nonylphenol-*d*₄) may be used as the internal standard material.
- g) **¹³C labelling 4-nonylphenol internal standard solution (0.1 µg/ml)** Take 1 ml of ¹³C labelling 4-nonylphenol internal standard solution (10 µg/ml), transfer

it to a 100 ml volumetric flask containing 5 ml to 10 ml of acetone, and add acetone up to the marked line. Preserve it at a dark place of 5 °C or lower in temperature, which necessary.

- h) **Phenanthrene- d_{10} internal standard solution (10 µg/ml)** Take 0.100 g of the standard product of phenanthrene- d_{10} (C_{14} - d_{10}), transfer it to a 100 ml volumetric flask containing 5 ml to 10 ml of dichloromethane, and add dichloromethane up to the marked line. Take 1 ml of this solution in a 100 ml volumetric flask and add dichloromethane up to the marked line. Preserve it at a dark place of 5 °C or lower in temperature, when necessary.
- i) **Phenanthrene- d_{10} internal standard solution (1 µg/ml)** Take 1 ml of phenanthrene- d_{10} internal standard solution (10 µg/ml) in a 10 ml volumetric flask, and add dichloromethane up to the marked line. Preserve it at a dark place of 5 °C or lower in temperature, when necessary.
- j) **Nitrogen** Nitrogen, Class 2, specified in **JIS K 1107**.

5.3 Implements

The implements shall be as follows.

- a) **Graduated test tube with grounding stopper** 10 ml to 20 ml in capacity and has the graduation at 0.5 ml and 1 ml.
- b) **Solid phase column** The cartridge of 10 mm in inside diameter and 30 mm to 50 mm in length. The column packing shall be the silicagel bonded chemically with reversed phase based compound or the one filled with synthetic adsorbent. The synthetic adsorbent shall be porous styrene-divinylbenzene copolymer or the one having the same performance as it. Before using, wash it by passing about 10 ml of acetone and next about 10 ml of water.

For the solid phase, there is a disc type one in the market, and it may be used. In such a case, confirm the flow rate of sample and the required amount of eluting solvent beforehand.

NOTE : For the solid phase, the following is available.

Aqsis PLS-3, Excelpak SPE-ENV/124, InertSep RP-1, OasisTMHLB, Sep-Pak PS-2 Cartridge, and the like.

5.4 Operation

The operation shall be as follows.

- a) After shaking to mix the sample taken in **4.1.2** to unify, take the adequate amount (for example, 1 L) of it, adjust pH at about 3.5 by adding hydrochloric acid (1 mol/L), add 1 ml of ¹³C labelling 4-nonylphenol internal standard solution (0.1 µg/ml), and then pass the sample through a solid phase column at 5 ml/min to 10 ml/min by the pressurization method or the depressurization method.

When plenty of suspended matters are contained in the sample, carry out the operation of **C.4**, join these solutions together and carry out the operation from the addition of hydrochloric acid (1 mol/L). For the operation in the case of using solid phase disc, carry out the operation of **H.3**.

- b) After washing a solid phase column by letting 10 ml of water flow through it, suck it for about 30 min and separate the water content to remove.

NOTE : When sucking for a long time, there is the possibility that the recovery rate is decreased. Care shall be taken.

- c) Elute the analyte so that 5 ml of dichloromethane is slowly passed from the upper end of solid phase column, and receive it in a graduated test tube with grounding stopper.

Confirm beforehand that the amount of dichloromethane is the sufficient amount to elute the analyte. And, make the flow rate of eluting to be the degree that the drops of eluate from the column do not continue.

- d) After adding 1 ml of phenanthrene-*d*₁₀ internal standard solution (1 µg/ml) in a graduated test tube with grounding stopper, spray nitrogen gently to the eluate as heating in the water bath at about 40 °C and concentrate it until dichloromethane becomes about 1 ml. Successively, dehydrate it by adding about 0.3 g of sodium sulfate. However, sodium sulfate shall not be separated by filtration. Take this solution as the solution for measurement.

When the operation of **6.4** is not carried out immediately, preserve this concentrated solution at a dark place at -20 °C. Also when the interfering substance coexists, carry out the operation of **D.4** and **E.4**. In the operation of spraying nitrogen, take care so that the concentrated solution does not scatter. Regulate the flow rate of nitrogen to the degree that the moving of the surface of concentrated solution is barely visible. When it is exsiccated, the analyte may be volatilized by spraying nitrogen in some cases. Therefore, care shall be taken.

- e) For the blank test, carry out the operations of **a)** to **d)** by using the same amount of water as the sample, and take it as the solution for blank test.

6 Gas chromatography/mass spectrometry

6.1 Summary of gas chromatography/mass spectrometry

A definite amount of solution for measurement is introduced in a gas chromatograph mass spectrometer, 4-nonylphenols are measured by using the selected ion detection method (SIM method) or the total ion detection method (TIM method), and 4-nonylphenols are determined according to the working curve method or the sensitivity coefficient method of Annex G.

NOTE : The isomer of analyte, its determination range and repeat analytical accuracy (coefficient of variation) are shown in table 2 (In any case, the determination range is different depending on apparatus and measurement conditions). The repeat analytical accuracy is the approximate value of coefficient of variation (%) obtained in the repeated tests by using the standard solution.