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(71) Applicant: **Shimadzu Corporation**
Kyoto-shi, Kyoto 604-8511 (JP)

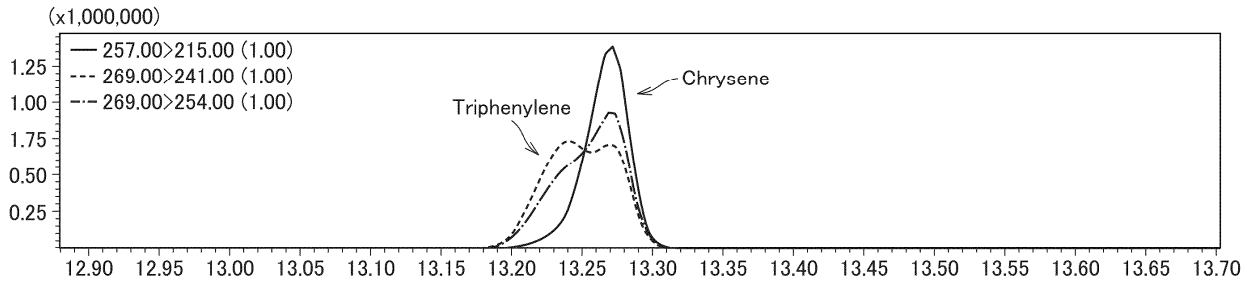
(72) Inventor: **ISHII, Toshinari**
Kyoto-shi, Kyoto 604-8511 (JP)

(74) Representative: **Müller-Boré & Partner**
Patentanwälte PartG mbB
Friedenheimer Brücke 21
80639 München (DE)

(54) METHOD FOR DETECTING PRESENCE OR ABSENCE OF POLYCYCLIC AROMATIC HYDROCARBONS

(57) A method can be provided that can individually detect some PAHs and speed up analysis by a method for detecting the presence or absence of a predetermined PAH (molecular formula: M), the method including: step 1 of chromatographically separating a sample containing the PAH; step 2-1 of generating at least one of $[M+CH_3]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$ as a precursor ion by ionization of the PAH using a chemical ionization method with introduction of methane gas, or step 2-2 of generating at least one of $[M+CH]^+$, $[M+HNC_2]^+$, and $[M+H_2NC_2]^+$ as a precursor ion by ionization of the PAH using a solvent mediated chemical ionization method with introduction of acetonitrile; step 3 of generating a fragment ion derived from the precursor ion; and step 4 of detecting the presence or absence of the PAH based on the presence or absence of the fragment ion.

FIG.1



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Description

TECHNICAL FIELD

[0001] The present invention relates to a method for detecting the presence or absence of a polycyclic aromatic hydrocarbon.

BACKGROUND ART

[0002] Polycyclic Aromatic Hydrocarbons (PAHs) are a group of compounds in which two or more benzene rings are fused. They are unintentionally generated by incomplete combustion or thermal decomposition of organic matter and are contained in food and environmental water due to food processing/cooking processes or contamination from the environment. Benzo[a]pyrene, a representative substance, has carcinogenicity, and some other PAHs are also suspected of having carcinogenicity (see, for example, the Ministry of Agriculture, Forestry and Fisheries website [searched April 19, 2023], Internet <URL: https://www.maff.go.jp/j/syouan/seisaku/risk_analysis/priority/hazard_chem.html> (Non-Patent Literature 1)).

[0003] For these reasons, several types of PAHs are regulated in Japan and abroad, and separation analysis methods by Gas Chromatography-Mass spectrometry (GC/MS) and Liquid Chromatography-Mass spectrometry (LC/MS) are mainstream. Many PAHs have similar structures, and their physical properties such as mass, polarity, and boiling point are close. Due to these characteristics, mass separation by ionization is insufficient, and it is common to separate them by chromatogram using several different types of columns.

[0004] PAHs have multiple isomers due to differences in the fusion position of the benzene rings. For these isomers, ionization methods such as Electron Ionization (EI), Positive Chemical Ionization (PCI), and Atmospheric Pressure Chemical Ionization (APCI) do not produce differences in their mass spectra. As mentioned above, the physical properties that characterize the compounds are also similar, making chromatographic separation difficult and hindering the quantification of regulated components.

[0005] Here, FIG. 7(a) shows the results of chromatographic separation of triphenylene and chrysene by a conventional GC method. In particular, chrysene and triphenylene, which have a molecular weight of 228, are considered difficult to separate, and it is necessary to use different columns suitable for their respective separation. Chrysene is regulated in various countries in the food and environmental fields, but triphenylene is not, making their separation extremely important to prevent misidentification of chrysene. Furthermore, FIG. 7(b) shows the mass spectra of triphenylene and chrysene by a conventional EI method. Triphenylene and chrysene not only co-elute chromatographically but also have the same fragment ions when ionized by EI, PCI, APCI, etc., making the separation of isomers using a mass spectrometer difficult. No differences are found in their Ultraviolet (UV) absorption wavelengths, excitation wavelengths, or Infrared (IR) absorption spectra, and isomers cannot be distinguished by spectroscopic methods either.

CITATION LIST

NON-PATENT LITERATURE

[0006]

[Non-Patent Literature 1] Ministry of Agriculture, Forestry and Fisheries website [searched April 19, 2023], Internet <URL: https://www.maff.go.jp/j/syouan/seisaku/risk_analysis/priority/hazard_chem.html>

[Non-Patent Literature 2] John Oostdijk, Agilent Technologies, Inc., Application News SI-002959 "Fast Separation of EU and US EPA Regulated PAHs on Agilent J&W Select PAH GC Columns", 2010.

[Non-Patent Literature 3] Yoshio Iida et al., "Quantification of Trace Impurities in Benzene by Gas Chromatography-Chemical Ionization Mass Spectrometry," Mass Spectrometry, Vol. 24, No. 4, 1976

[Non-Patent Literature 4] Kenji Arikawa et al., "Ion-Molecule Reactions of CH5+, C2H5+, C3H5+ with Halogenated Benzenes in an Ion Trap Mass Spectrometer," Journal of the Mass Spectrometry Society of Japan, (2011), doi: 10.5702/massspec. 11-21

[Non-Patent Literature 5] Colleen K. Van Pelt et al., "Studies of structure and mechanism in acetonitrile chemical ionization tandem mass spectrometry of polyunsaturated fatty acid methyl esters | Journal of the American Society for Mass Spectrometry", J Am Soc Mass Spectrom 1999, 10, 1253-1262

[Non-Patent Literature 6] Xiang Zhang., "Gas-phase cleavage of the novel iminium ions [R1-CH+-N=CH-R2←→R1-CH=N+=CH-R2]: An experimental and computational study", Journal of Molecular Structure 1056-1057 (2014) 219-226

SUMMARY OF INVENTION

TECHNICAL PROBLEM

[0007] The present invention has been made to solve the above problems, and an object thereof is to provide a method that can individually detect some PAHs and can speed up analysis.

[SOLUTION TO PROBLEM]

[0008] A first aspect of the present invention relates to a method for detecting the presence or absence of a predetermined polycyclic aromatic hydrocarbon (molecular formula: M), the method including:

step 1 of chromatographically separating a sample containing the polycyclic aromatic hydrocarbon,
step 2-1 of generating at least one of $[M+CH_3]^+$, $[M+C_2H_3]^+$, and $[M+C_3H_5]^+$ as a precursor ion by ionization of the polycyclic aromatic hydrocarbon using a chemical ionization method with introduction of methane gas,
or,
step 2-2 of generating at least one of $[M+CH]^+$, $[M+HNC_2]^+$, and $[M+H_2NC_2]^+$ as a precursor ion by ionization of the polycyclic aromatic hydrocarbon using a solvent mediated chemical ionization method with introduction of acetonitrile,
step 3 of generating a fragment ion derived from the precursor ion, and
step 4 of detecting the presence or absence of the polycyclic aromatic hydrocarbon based on the presence or absence of the fragment ion.

ADVANTAGEOUS EFFECTS OF INVENTION

[0009] According to the method of the present invention, it is possible to individually detect some PAHs and speed up the analysis. In particular, the temperature ramping time required for the separation of chrysene and triphenylene can be shortened. Furthermore, when using the solvent mediated chemical ionization method with the introduction of acetonitrile, phenanthrene and anthracene can also be separated, enabling further efficiency improvement in analysis. Moreover, the probability of false positives due to misidentification can be reduced even for polycyclic aromatics in food samples with complex matrices. Additionally, by applying the ratio of specific precursor ions derived from aromatics, qualitative analysis of compounds having aromatic rings becomes possible.

BRIEF DESCRIPTION OF DRAWINGS

[0010]

[FIG. 1] FIG. 1 shows the results of analyzing a mixed standard of chrysene (10 ppm) and triphenylene (10 ppm) by Chemical Ionization (CI)-MS/MS method with introduction of methane gas.
[FIG. 2] FIG. 2 shows the results of analyzing a mixed standard of chrysene (10 ppm) and triphenylene (10 ppm) by Solvent Mediated Chemical Ionization (SMCI)-MS/MS method with introduction of acetonitrile.
[FIG. 3] FIG. 3 shows the results of analyzing a mixed standard of phenanthrene (10 ppm) and anthracene (10 ppm) by SMCI-MS/MS method with introduction of acetonitrile.
[FIG. 4] FIG. 4 shows an example in which the method according to the present invention is applied to the analysis method using the J&W Select PAH column described in John Oostdijk, Agilent Technologies, Inc., Application News SI-002959 "Fast Separation of EU and US EPA Regulated PAHs on Agilent J&W Select PAH GC Columns", 2010. (Non-Patent Literature 2). The boxed sections highlight a "temperature ramp for separation of anthracene and phenanthrene" and an "isothermal period for separation of chrysene and triphenylene," indicating where a "Speed-up possible" can be achieved.
[FIG. 5] FIG. 5 shows the results of analyzing a mixed standard of benzo[b]fluoranthene (10 ppm), benzo[k]fluoranthene (10 ppm), and benzo[j]fluoranthene (10 ppm) by CI-MS/MS method with introduction of methane gas, where FIG. 5(a) shows the results for 281.0>266.0, FIG. 5(b) for 281.0>225.0, and FIG. 5(c) for 281.0>253.0.
[FIG. 6] FIG. 6 shows the results of analyzing a mixed standard of benzo[b]fluoranthene (10 ppm), benzo[k]fluoranthene (10 ppm), and benzo[j]fluoranthene (10 ppm) by SMCI-MS/MS method with introduction of acetonitrile, where FIG. 6(a) shows the results for 288.0>276.0, FIG. 6(b) for 288.0>265.0, and FIG. 6(c) for 306.0>289.0.
[FIG. 7] FIG. 7(a) shows the results of chromatographic separation of triphenylene and chrysene by a conventional GC method, and FIG. 7(b) shows the mass spectra of triphenylene and chrysene by a conventional EI method.

DESCRIPTION OF EMBODIMENTS

[0011] A first aspect of the present invention relates to a method for detecting the presence or absence of a predetermined PAH (molecular formula: M), the method including:

step 1 of chromatographically separating a sample containing the PAH,
step 2-1 of generating at least one of $[M+CH_3]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$ as a precursor ion by ionization of the PAH using CI with introduction of methane gas,
or,
step 2-2 of generating at least one of $[M+CH]^+$, $[M+HNC_2]^+$, and $[M+H_2NC_2]^+$ as a precursor ion by ionization of the PAH using SMCI with introduction of acetonitrile,
step 3 of generating a fragment ion derived from the precursor ion, and
step 4 of detecting the presence or absence of the PAH based on the presence or absence of the fragment ion.

[0012] The first aspect of the present invention utilizes a CI-MS/MS method to generate characteristic fragment ions for each PAH isomer and separates the PAHs by mass separation. This makes it possible to individually detect some PAHs and speed up the analysis, and in

particular, the temperature ramping time required for the separation of chrysene and triphenylene can be shortened. The first aspect of the present invention is broadly divided into the case of using the CI-MS/MS method with the introduction of methane gas (via step 1, step 2-1, step 3, and step 4) and the case of using the SMCI-MS/MS method with the introduction of acetonitrile (via step 1, step 2-2, step 3, and step 4).

[1] CI-MS/MS method with introduction of methane gas

[0013] FIG. 1 shows the results of analyzing a mixed standard of chrysene (10 ppm) and triphenylene (10 ppm) by a Chemical Ionization (CI)-MS/MS method (more specifically, a PCI-MS/MS method) with the introduction of methane gas. As shown, by using the CI-MS/MS method with the introduction of methane gas, individual detection of chrysene and triphenylene, which was conventionally difficult, becomes possible. The CI-MS/MS method with the introduction of methane gas itself is a known technique and can be performed under appropriate conditions using commercially available products (for example, GCMS-TQ™ 8050 NX (manufactured by Shimadzu Corporation), GCMS-TQ™ 8040 NX (manufactured by Shimadzu Corporation)) based on known literature such as Yoshio Iida et al., "Quantification of Trace Impurities in Benzene by Gas Chromatography-Chemical Ionization Mass Spectrometry," Mass Spectrometry, Vol. 24, No. 4, 1976 (Non-Patent Literature 3) and Kenji Arikawa et al., "Ion-Molecule Reactions of CH3+, C2H5+, C3H5+ with Halogenated Benzenes in an Ion Trap Mass Spectrometer," Journal of the Mass Spectrometry Society of Japan, (2011), doi: 10.5702/massspec. 11-21 (Non-Patent Literature 4). FIG. 1 shows the results of CI-MS/MS performed with the introduction of methane gas under the following conditions:

- Vaporization chamber temperature: 275°C
- Carrier gas control: Column flow rate (1.00 mL/min)
- Column: Rxi-PAH (60.0 m × 0.25 mm I.D. × 0.10 μm)
- Column temperature: 200°C (1 min) → (10°C/min) → 300°C (10 min)
- Transfer line temperature: 250°C
- Ion source temperature: 230°C
- Collision energy: 10 eV

[0014] It should be noted that the above conditions are merely an example and are, of course, not limited thereto.

[0015] In step 1, a sample containing PAHs is first chromatographically separated. In this case, suitable examples of PAHs include, but are not limited to, chrysene, triphenylene, benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[j]fluoranthene. Suitable examples of the column used for chromatographic separation in step 1 include, but are not limited to, the aforementioned Rxi-PAH (manufactured by Restek Corporation), as well as SH-I-XLB (manufactured by Shimadzu Corporation), SH-I-17Sil MS (manufactured by Shimadzu Corporation), and the like.

[0016] In step 2-1, at least one of $[M+CH_3]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$ is generated as a precursor ion by ionization of a PAH (molecular weight: M) using a CI method with the introduction of methane gas. The CI method is a softer ionization compared to the EI method, and using this technique, precursor ions such as $[M+H]^+$ and $[M+CH_3]^+$ are observed. When methane gas is introduced, $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$ are observed as precursor ions in addition to $[M+H]^+$ and $[M+CH_3]^+$.

[0017] The precursor ion generated in step 2-1 is preferably at least one of $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$, and particularly preferably $[M+C_2H_5]^+$. In this case, as the PAH, chrysene, which generates a fragment ion with a mass number of 215 in step 3 after step 2-1 and can be detected individually from triphenylene, is preferable. Also preferable as PAHs are benzo[b]fluoranthene, which generates a fragment ion with a mass number of 266, benzo[k]fluoranthene, which generates a fragment ion with a mass number of 225, and benzo[j]fluoranthene, which generates a fragment ion with a mass number of 253, in step 3 after step 2-1.

[0018] In step 3, a fragment ion derived from the precursor ion is generated. In the case of step 3 after step 2-1, in the cases described above, a fragment ion with a mass number of 215 is generated when the PAH is chrysene, a fragment ion with a mass number of 266 is generated when the PAH is benzo[b]fluoranthene, a fragment ion with a mass number of 225 is generated when the PAH is benzo[k]fluoranthene, and a fragment ion with a mass number of 253 is generated when the PAH is benzo[j]fluoranthene.

[0019] In step 4, the presence or absence of the PAH is detected based on the presence or absence of the fragment ion. Here, when the PAHs are chrysene and triphenylene, the presence or absence of chrysene or triphenylene may be detected in step 4 based on the presence or absence of the fragment ion. In this case, in step 4, if the fragment ion is present, it may be determined that at least chrysene is present, or if the fragment ion is absent, it may be determined that at least chrysene is not present.

[2] SMCI-MS/MS method with introduction of acetonitrile

[0020] FIG. 2 shows the results of analyzing a mixed standard of chrysene (10 ppm) and triphenylene (10 ppm) by an SMCI-MS/MS method with the introduction of acetonitrile. As shown, by also using the SMCI-MS/MS method with the introduction of acetonitrile, individual detection of chrysene and triphenylene, which was conventionally difficult, becomes possible. Furthermore,

FIG. 3 shows the results of analyzing a mixed standard of phenanthrene (10 ppm) and anthracene (10 ppm) by an SMCI-MS/MS method with the introduction of acetonitrile. When using the SMCI-MS/MS method with the introduction of acetonitrile, individual detection, which was difficult even with the CI-MS/MS method with the introduction of methane gas, also becomes possible. The SMCI-MS/MS method with the introduction of acetonitrile is also a known technique and can be performed under appropriate conditions using commercially available products (for example, GCMS-TQ™ 8050 NX (manufactured by Shimadzu Corporation)) based on known literature such as Colleen K. Van Pelt et al., "Studies of structure and mechanism in acetonitrile chemical ionization tandem mass spectrometry of polyunsaturated fatty acid methyl esters | Journal of the American Society for Mass Spectrometry", J Am Soc Mass Spectrom 1999, 10, 1253-1262 (Non-Patent Literature 5) and Xiang Zhang., "Gas-phase cleavage of the novel iminium ions [R1-CH+-N=CH-R2←→R1-CH=N+=CH-R2]: An experimental and computational study", Journal of Molecular Structure 1056-1057 (2014) 219-226 (Non-Patent Literature 6). FIG. 2 and FIG. 3 show the results of SMCI-MS/MS performed with the introduction of acetonitrile under the following conditions:

- Vaporization chamber temperature: 275°C
- Carrier gas control: Column flow rate (1.00 mL/min)
- Column: Rxi-PAH (60.0 m × 0.25 mm I.D. × 0.10 μm)
- Column temperature: 110°C (1 min) → (30°C/min) → 210°C → (3°C/min → 250°C (6 min) → 350°C (5 min)
- Transfer line temperature: 250°C
- Ion source temperature: 230°C
- Collision energy: 10 eV

[0021] It should be noted that the above conditions are merely an example and are, of course, not limited thereto. Furthermore, similar results are obtained even if the SMCI-MS/MS method with the introduction of acetonitrile is performed under the conditions exemplified for the CI-MS/MS method with the introduction of methane gas.

[0022] Step 1 may be performed in the same manner as described above for step 1 in the case of the CI-MS/MS method with the introduction of methane gas. However, in the case of the SMCI-MS/MS method with the introduction of acetonitrile, suitable examples of PAHs also include phenanthrene and anthracene, in addition to chrysene, triphenylene, benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[j]fluoranthene.

[0023] In step 2-2, at least one of $[M+CH]^+$, $[M+HNC_2]^+$, and $[M+H_2NC_2]^+$ is generated as a precursor ion by ionization of the PAH using an SMCI method with the introduction of acetonitrile. The SMCI method is an ionization method that uses a solvent instead of a flammable gas, and when acetonitrile is used as this solvent, an ion called MIE is generated in the ion source. MIE has the property of undergoing a 2+2 cycloaddition reaction with double bonds. Therefore, it is known that compounds such as olefins containing a double bond generate an MIE adduct ion with a mass number increased by 54 from the mass number M. In the method according to the present invention, it was found that for compounds having a benzene ring, not only the MIE adduct ion but also other specific precursor ions are generated. These precursor ions are formed by the cleavage of the MIE adduct ion centered on the bonding site, and three types ($[M+CH]^+$, $[M+HNC_2]^+$, $[M+H_2NC_2]^+$) are generated. This has been confirmed for 36 types of PAHs regulated in EPA 2260a, as well as for benzene, toluene, and xylene. Since aromatics have a stable structure, it is considered that the MIE bonding part, which is less stable than the basic skeleton, causes fragmentation. Furthermore, since one of the three generated ion types is thought to be formed by abstracting a hydrogen from the basic skeleton, the ease of ion generation changes depending on the steric structure of the basic skeleton. Also, since it changes the stability of the basic skeleton itself, the ease of fragment ion generation also changes depending on the location from which the hydrogen is abstracted.

[0024] In step 3, a fragment ion derived from the precursor ion is generated. In the case of step 3 after step 2-2, in the cases described above, a fragment ion with a mass number of 215 is generated when the PAH is chrysene, a fragment ion with a mass number of 241 is generated when the PAH is triphenylene, a fragment ion with a mass number of 266 is generated when the PAH is benzo[b]fluoranthene, a fragment ion with a mass number of 225 is generated when the PAH is benzo[k]fluoranthene, a fragment ion with a mass number of 253 is generated when the PAH is benzo[j]fluoranthene, a fragment ion with a mass number of 201 is generated when the PAH is phenanthrene, and a fragment ion with a mass number of 192 is generated when the PAH is anthracene.

[0025] In step 4, the presence or absence of the PAH is detected based on the presence or absence of the fragment ion. In the case of step 4 following step 3 which has passed through step 2-2, PAHs can be individually detected by the mass number of the fragment ion.

[0026] According to the method of the present invention including [1] the CI-MS/MS method with the introduction of methane gas and [2] the SMCI-MS/MS method with the introduction of acetonitrile as described above, it becomes possible to individually detect some PAHs and to speed up and improve the efficiency of the analysis. Here, FIG. 4 shows an example in which the method according to the present invention is applied to the analysis method using the J&W Select PAH column described in John Oostdijk, Agilent Technologies, Inc., Ap-

plication News SI-002959 "Fast Separation of EU and US EPA Regulated PAHs on Agilent J&W Select PAH GC Columns", 2010. (Non-Patent Literature 2). FIG. 4 is based on Figure 1 of Non-Patent Literature 2, but by applying the method according to the present invention, the time required for the parts enclosed by the two rectangles shown in the figure can be shortened, and further speed-up is possible. More specifically, the rectangle on the left side of the page in FIG. 4 is the temperature ramp for separating anthracene and phenanthrene, and this can be shortened by applying [2] the SMCI-MS/MS method with the introduction of acetonitrile. Also, the rectangle on the right side of the page in FIG. 4 is the isothermal period for separating chrysene and triphenylene, and this can be shortened by applying either [1] the CI-MS/MS method with the introduction of methane gas or [2] the SMCI-MS/MS method with the introduction of acetonitrile.

[0027] With such a method according to the present invention, the probability of false positives due to misidentification can be reduced even for polycyclic aromatics in food samples with complex matrices. Furthermore, in the method according to the present invention, qualitative analysis of compounds having aromatic rings becomes possible by applying the ratio of specific precursor ions derived from aromatics.

[0028] The present invention will be described in more detail below with reference to experimental examples, but the present invention is not limited thereto.

<Experimental Example 1>

[0029] A mixed standard of chrysene (10 ppm) and triphenylene (10 ppm), as well as solutions of benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[j]fluoranthene each prepared at 10 μg/mL (= 10 ppm) were used as analysis samples. Using a GCMS-TQ™ 8050 NX (manufactured by Shimadzu Corporation), a CI-MS/MS method with the introduction of methane gas was performed under the following conditions.

[0030]

- Vaporization chamber temperature: 275°C
- Carrier gas control: Column flow rate (1.00 mL/min)
- Column: Rxi-PAH (60.0 m × 0.25 mm I.D. × 0.10 μm)
- Column temperature: 200°C (1 min) → (10°C/min) → 300°C (10 min)
- Transfer line temperature: 250°C
- Ion source temperature: 230°C
- Collision energy: 10 eV

[0031] FIG. 1 shows the results for the mixed standard of chrysene and triphenylene, where a fragment ion (mass number: 215) was confirmed only for chrysene. Furthermore, FIG. 5 shows the results for benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), and benzo[j]fluoranthene (B[j]F), where FIG. 5(a) shows the results for 281.0>266.0, FIG. 5(b) for 281.0>225.0, and FIG. 5(c) for 281.0>253.0.

<Experimental Example 2>

[0032] A mixed standard of chrysene (10 ppm) and triphenylene (10 ppm), a mixed standard of phenanthrene (10 ppm) and anthracene (ppm), and solutions of benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[j]fluoranthene each prepared at 10 μg/mL (= 10 ppm) were used as analysis samples. Using a GCMS-TQ™ 8050 NX (manufactured by Shimadzu Corporation), an SMCI-MS/MS method with the introduction of acetonitrile was performed under the following conditions.

[0033]

- Vaporization chamber temperature: 275°C
- Carrier gas control: Column flow rate (1.00 mL/min)
- Column: Rxi-PAH (60.0 m × 0.25 mm I.D. × 0.10 μm)
- Column temperature: 110°C (1 min) → (30°C/min) → 210°C → (3°C/min → 250°C(6 min) → 350°C (5 min)
- Transfer line temperature: 250°C
- Ion source temperature: 230°C
- Collision energy: 10 eV

[0034] FIG. 2 shows the results for the mixed standard of chrysene and triphenylene, where a fragment ion (mass number: 227) was confirmed for chrysene, and a fragment ion (mass number: 226) was confirmed for triphenylene. Furthermore, FIG. 3 shows the results for the mixed standard of phenanthrene and anthracene, where a fragment ion (mass number: 201) was confirmed for phenanthrene, and a fragment ion (mass number: 192) was confirmed for anthracene. Furthermore, FIG. 6 shows the results for benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), and benzo[j]fluoranthene (B[j]F), where FIG. 6(a) shows the results for 288.0>276.0, FIG. 6(b) for 288.0>265.0, and FIG. 6(c) for 306.0>289.0.

[0035] It is understood by those skilled in the art that the exemplary embodiments described above are specific examples of the following aspects.

(Item 1)

[0036] A method for detecting the presence or absence

of a predetermined polycyclic aromatic hydrocarbon (molecular formula: M) according to one aspect includes step 1 of chromatographically separating a sample containing the polycyclic aromatic hydrocarbon, step 2-1 of generating at least one of $[M+CH_3]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$ as a precursor ion by ionization of the polycyclic aromatic hydrocarbon using a chemical ionization method with introduction of methane gas, or step 2-2 of generating at least one of $[M+CH]^+$, $[M+HNC_2]^+$, and $[M+H_2NC_2]^+$ as a precursor ion by ionization of the polycyclic aromatic hydrocarbon using a solvent mediated chemical ionization method with introduction of acetonitrile, step 3 of generating a fragment ion derived from the precursor ion, and step 4 of detecting the presence or absence of the polycyclic aromatic hydrocarbon based on the presence or absence of the fragment ion.

[0037] According to the method described in item 1, it is possible to individually detect some PAHs and speed up the analysis, and in particular, the temperature ramping time required for the separation of chrysene and triphenylene can be shortened. Moreover, the probability of false positives due to misidentification can be reduced even for polycyclic aromatics in food samples with complex matrices. Additionally, by applying the ratio of specific precursor ions derived from aromatics, qualitative analysis of compounds having aromatic rings becomes possible.

(Item 2)

[0038] In the method described in item 1, the precursor ion generated in the step 2-1 is at least one of $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$.

[0039] According to the method described in item 2, characteristic fragment ions can be generated to individually detect chrysene and triphenylene.

(Item 3)

[0040] In the method described in item 1, the polycyclic aromatic hydrocarbon is chrysene.

[0041] According to the method described in item 3, chrysene can be individually detected.

(Item 4)

[0042] In the method described in item 3, the precursor ion generated in the step 2-1 is $[M+C_2H_5]^+$.

[0043] According to the method described in item 4, characteristic fragment ions can be generated to individually detect chrysene and triphenylene.

(Item 5)

[0044] According to the method described in item 3 or item 4, the fragment ion generated in the step 3 after the step 2-1 has a mass number of 215.

[0045] According to the method described in item 5, a fragment ion with a mass number of 215 is confirmed only for chrysene, allowing chrysene to be individually detected.

(Item 6)

[0046] In the method described in item 1, the polycyclic aromatic hydrocarbons are chrysene and triphenylene, and in the step 4 after the step 2-1 and the step 3, the presence or absence of chrysene or triphenylene is detected based on the presence or absence of the fragment ion.

[0047] According to the method described in item 6, chrysene and triphenylene can be individually detected, and the analysis can be sped up.

(Item 7)

[0048] In the method described in item 6, in the step 4 after the step 2-1 and the step 3, if the fragment ion is present, it is determined that at least chrysene is present.

[0049] According to the method described in item 7, the temperature ramping time required for the separation of chrysene and triphenylene can be shortened.

(Item 8)

[0050] In the method described in item 6, in the step 4 after the step 2-1 and the step 3, if the fragment ion is absent, it is determined that at least chrysene is not present.

[0051] According to the method described in item 8, the temperature ramping time required for the separation of chrysene and triphenylene can be shortened.

(Item 9)

[0052] In the method described in item 1, the polycyclic aromatic hydrocarbons are benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[j]fluoranthene.

[0053] According to the method described in item 9, benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[j]fluoranthene can also be individually detected.

(Item 10)

[0054] In the method described in item 9, the precursor ion generated in the step 2-1 is $[M+C_2H_5]^+$.

[0055] According to the method described in item 10, characteristic fragment ions can be generated to individually detect benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[j]fluoranthene.

(Item 11)

[0056] In the method described in item 10, the fragment ions generated in the step 3 after the step 2-1 have mass numbers of 266 for benzo[b]fluoranthene, 225 for benzo

[k]fluoranthene, and 253 for benzo[j]fluoranthene.

[0057] According to the method described in item 11, benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo [j]fluoranthene can be individually detected.

(Item 12)

[0058] In the method described in item 1, the polycyclic aromatic hydrocarbons are phenanthrene and anthracene, and in the step 4 after the step 2-2 and the step 3, the presence or absence of phenanthrene or anthracene is detected based on the presence or absence of the fragment ion.

[0059] According to the method described in item 12, when SMCI with the introduction of acetonitrile is used, phenanthrene and anthracene can also be separated, enabling further efficiency improvement in analysis.

Claims

1. A method for detecting the presence or absence of a predetermined polycyclic aromatic hydrocarbon (molecular formula: M), the method comprising:

step 1 of chromatographically separating a sample containing the polycyclic aromatic hydrocarbon;
step 2-1 of generating at least one of $[M+CH_3]^+$, $[M+C_2H_3]^+$, and $[M+C_3H_5]^+$ as a precursor ion by ionization of the polycyclic aromatic hydrocarbon using a chemical ionization method with introduction of methane gas,
or,
step 2-2 of generating at least one of $[M+CH]^+$, $[M+HNC_2]^+$, and $[M+H_2NC_2]^+$ as a precursor ion by ionization of the polycyclic aromatic hydrocarbon using a solvent mediated chemical ionization method with introduction of acetonitrile;
step 3 of generating a fragment ion derived from the precursor ion; and
step 4 of detecting the presence or absence of the polycyclic aromatic hydrocarbon based on the presence or absence of the fragment ion.

2. The method according to claim 1, wherein the precursor ion generated in the step 2-1 is at least one of $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$.

3. The method according to claim 1, wherein the polycyclic aromatic hydrocarbon is chrysene.

4. The method according to claim 3, wherein the precursor ion generated in the step 2-1 is $[M+C_2H_5]^+$.

5. The method according to claim 3 or 4, wherein the fragment ion generated in the step 3 after the step 2-1 has a mass number of 215.

6. The method according to claim 1, wherein

the polycyclic aromatic hydrocarbon is chrysene and triphenylene, and
in the step 4 after the step 2-1 and the step 3, the presence or absence of chrysene or triphenylene is detected based on the presence or absence of the fragment ion.

7. The method according to claim 6, wherein in the step 4 after the step 2-1 and the step 3, if the fragment ion is present, it is determined that at least chrysene is present.

8. The method according to claim 6, wherein in the step 4 after the step 2-1 and the step 3, if the fragment ion is absent, it is determined that at least chrysene is not present.

9. The method according to claim 1, wherein the polycyclic aromatic hydrocarbon is benzo[b]fluoranthene, benzo[k]fluoranthene, or benzo[j]fluoranthene.

10. The method according to claim 9, wherein the precursor ion generated in the step 2-1 is $[M+C_2H_5]^+$.

11. The method according to claim 10, wherein the fragment ion generated in the step 3 after the step 2-1 has a mass number of 266 for benzo[b]fluoranthene, 225 for benzo[k]fluoranthene, or 253 for benzo[j]fluoranthene.

12. The method according to claim 1, wherein

the polycyclic aromatic hydrocarbon is phenanthrene and anthracene, and
in the step 4 after the step 2-2 and the step 3, the presence or absence of phenanthrene or anthracene is detected based on the presence or absence of the fragment ion.

FIG.1

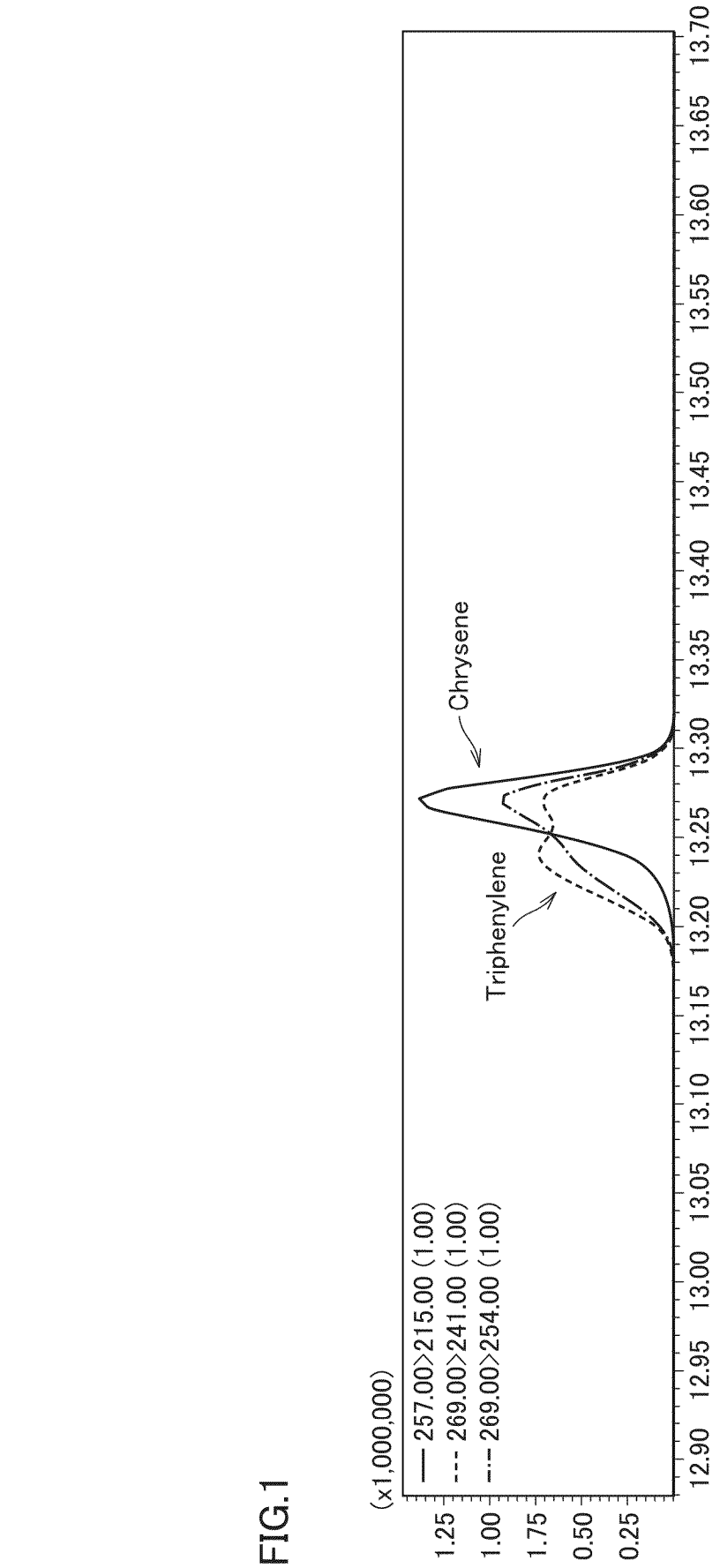

(x1,000,000)
1.25
1.00
1.75
0.50
0.25
257.00>215.00 (1.00)
269.00>241.00 (1.00)
269.00>254.00 (1.00)
Chrysene
Triphenylene
12.90
12.95
13.00
13.05
13.10
13.15
13.20
13.25
13.30
13.35
13.40
13.45
13.50
13.55
13.60
13.65
13.70

FIG.2

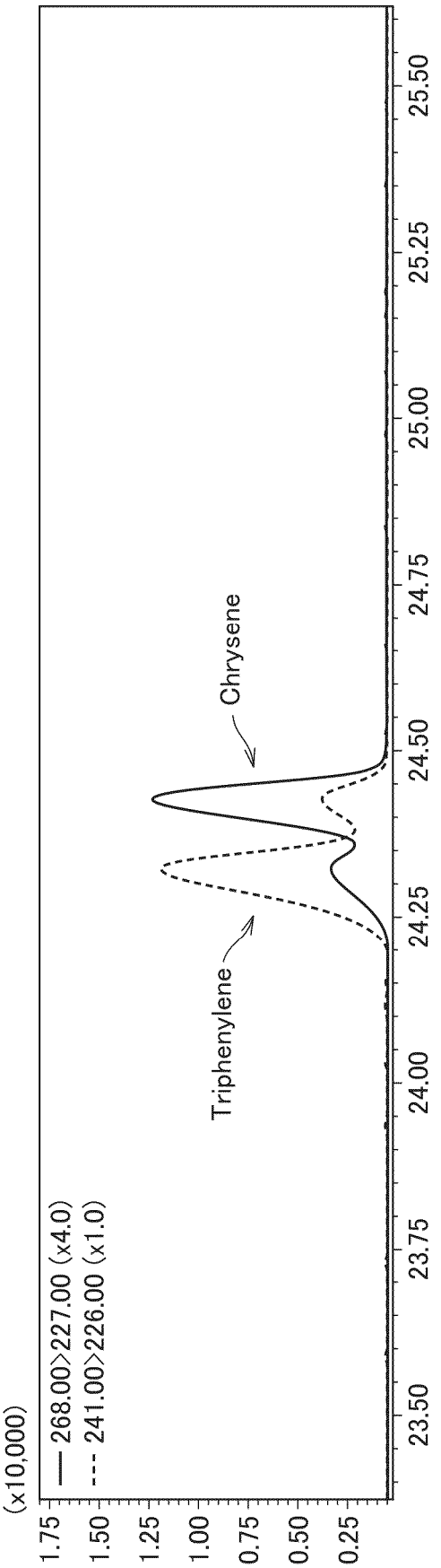

(x10,000)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
268.00>227.00 (x4.0)
241.00>226.00 (x1.0)
Chrysene
Triphenylene
23.50
23.75
24.00
24.25
24.50
24.75
25.00
25.25
25.50

FIG.3

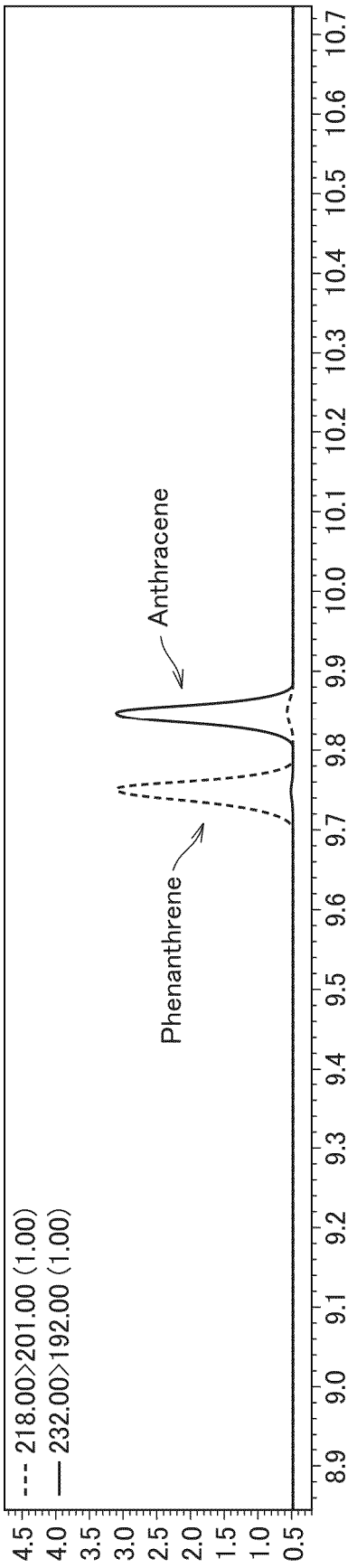

--- 218.00>201.00 (1.00)
— 232.00>192.00 (1.00)
Anthracene
Phenanthrene
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
8.9
9.0
9.1
9.2
9.3
9.4
9.5
9.6
9.7
9.8
9.9
10.0
10.1
10.2
10.3
10.4
10.5
10.6
10.7

FIG.4

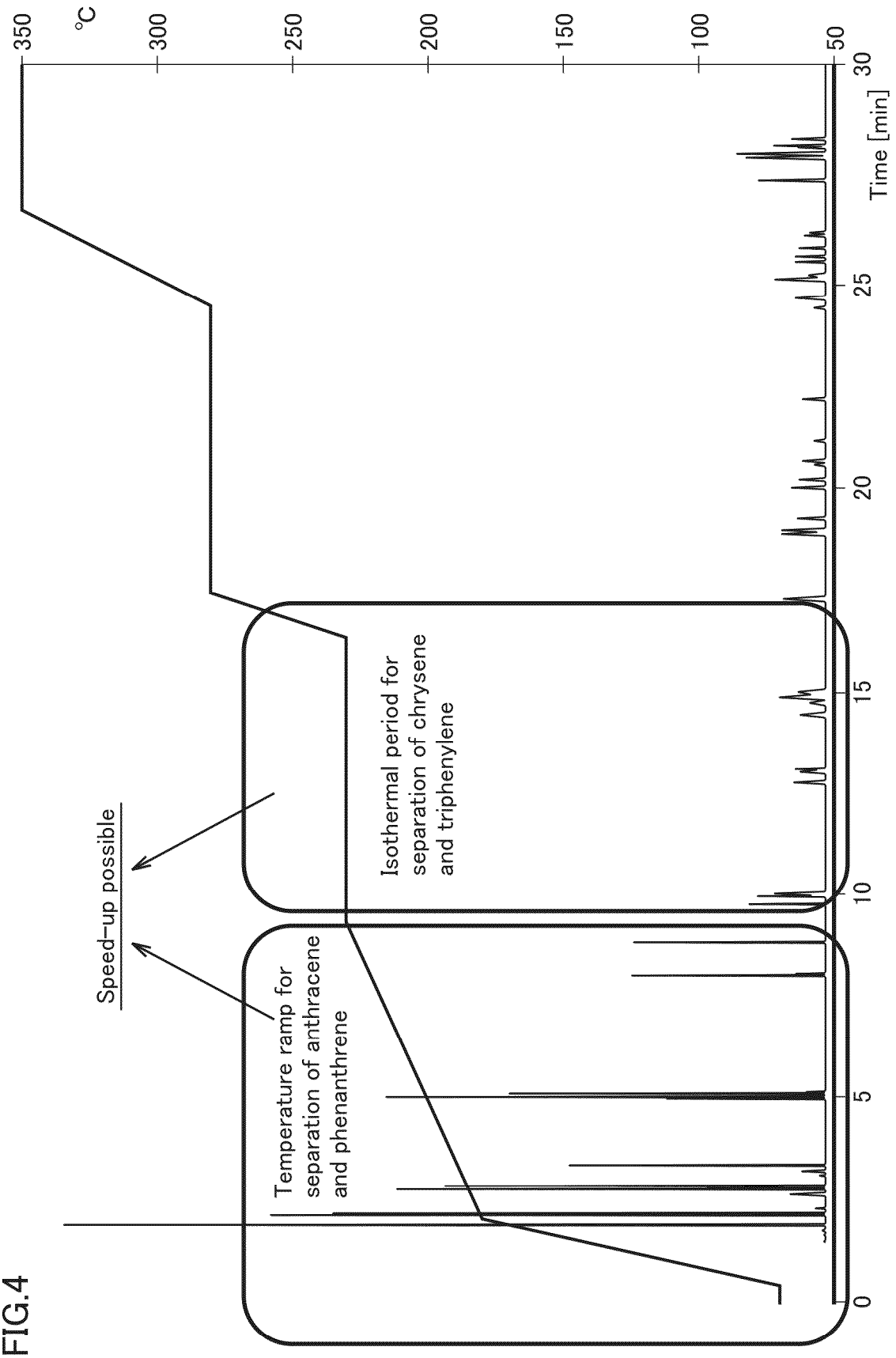

350
°C
300
250
200
150
100
50
30
Time [min]
25
20
15
10
5
0
Speed-up possible
Temperature ramp for separation of anthracene and phenanthrene
Isothermal period for separation of chrysene and triphenylene

FIG.5

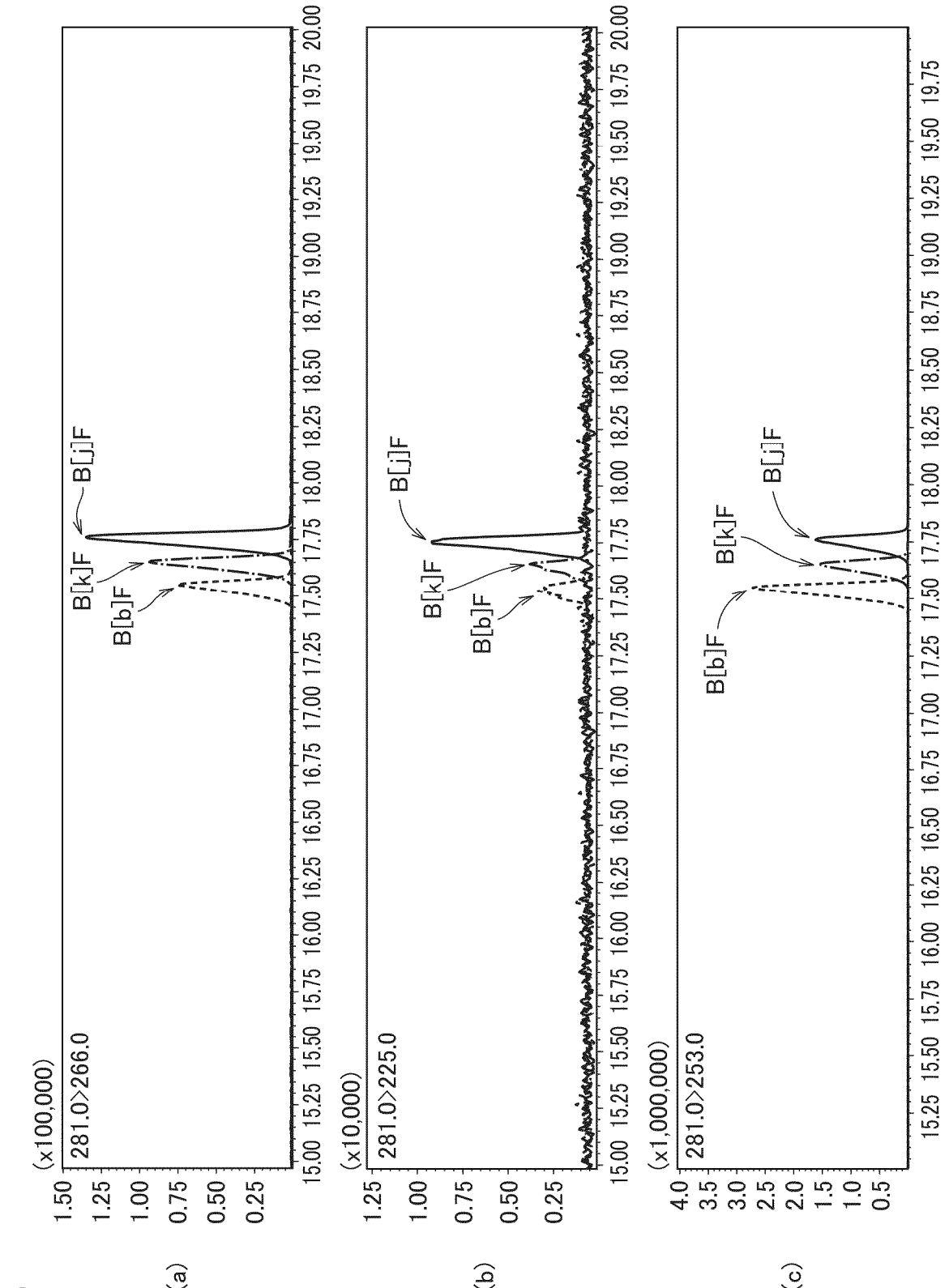

(a)
(x100,000)
281.0>266.0
B[j]F
B[k]F
B[b]F
(b)
(x10,000)
281.0>225.0
B[j]F
B[k]F
B[b]F
(c)
(x1,000,000)
281.0>253.0
B[k]F
B[j]F
B[b]F

FIG.6

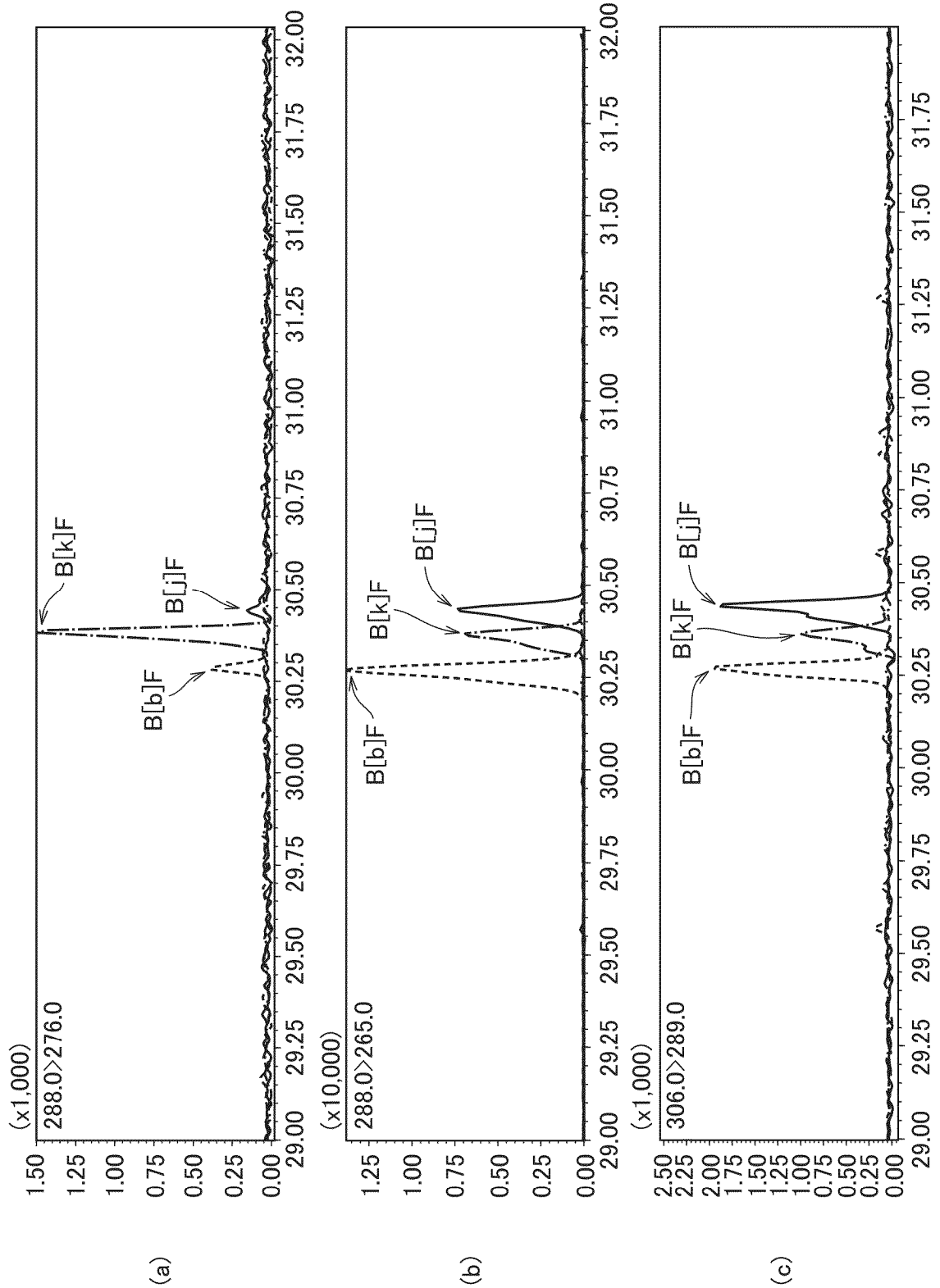
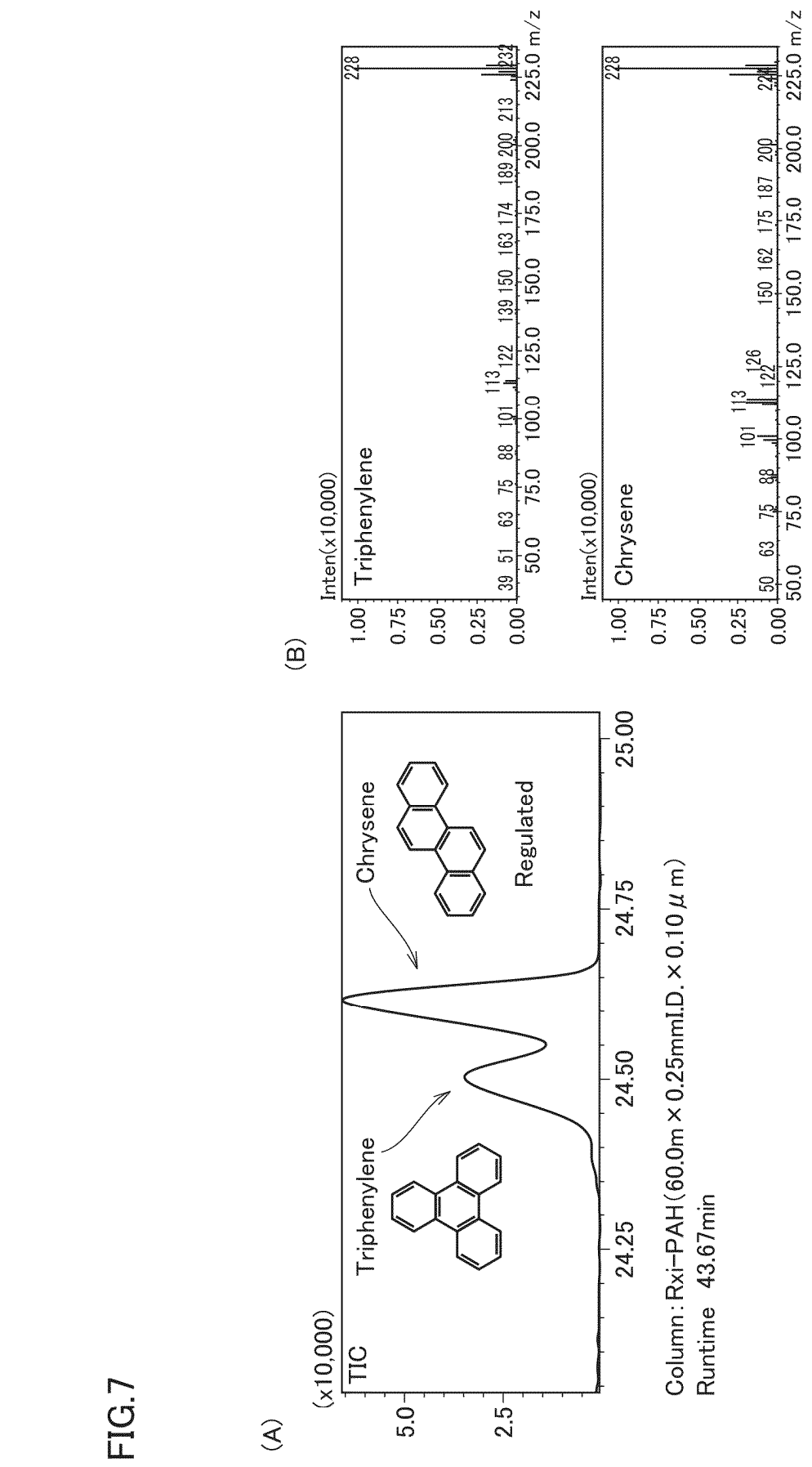

(a)
(x1,000)
288.0>276.0
B[k]F
B[j]F
B[b]F
(b)
(x10,000)
288.0>265.0
B[b]F
B[k]F
B[j]F
(c)
(x1,000)
306.0>289.0
B[b]F
B[k]F
B[j]F

FIG.7


(A)
(x10,000)
TIC
5.0
2.5
Triphenylene
Chrysene
Regulated
24.25
24.50
24.75
25.00
Column : Rxi-PAH (60.0m × 0.25mmI.D. × 0.10 μ m)
Runtime 43.67min
(B)
Inten(x10,000)
Triphenylene
1.00
0.75
0.50
0.25
0.00
50.0
75.0
100.0
125.0
150.0
175.0
200.0
225.0 m/z
Inten(x10,000)
Chrysene

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2024/020336

A. CLASSIFICATION OF SUBJECT MATTER

G01N 27/62(2021.01)i
FI: G01N27/62 V; G01N27/62 C

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N27/60-G01N27/70

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2024
Registered utility model specifications of Japan 1996-2024
Published registered utility model applications of Japan 1994-2024

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

JSTPlus/JMEDPlus/JST7580 (JDreamIII); Scopus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SIMONSICK, JR. W. J. HITES, R. A. Analysis of Isomeric Polycyclic Aromatic Hydrocarbons by Charge-Exchange Chemical Ionization Mass Spectrometry. ANALYTICAL CHEMISTRY. 1984, vol. 56, no. 14, pp. 2749-2754 entire text, all drawings	1-12
A	HAWTHORNE, S. B. MILLER, D. J. SUPERCRITICAL-FLUID CHROMATOGRAPHY-MASS SPECTROMETRY OF POLYCYCLIC AROMATIC HYDROCARBONS WITH A SIMPLE CAPILLARY DIRECT INTERFACE. Journal of Chromatography. 1989, vol. 468, pp. 115-125 entire text, all drawings	1-12
A	JP 2008-516242 A (UNIVERSITY OF VIRGINIA PATENT FOUNdATION) 15 May 2008 (2008-05-15) entire text, all drawings	1-12
A	US 4721858 A (THE UNITED STATES OF AMERICA AS REPRESENTED BY THE UNITED STATES DEPARTMENT OF ENERGY) 26 January 1988 (1988-01-26) entire text, all drawings	1-12

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06 August 2024	**20 August 2024**
Name and mailing address of the ISA/JP **Japan Patent Office (ISA/JP)** **3-4-3 Kasumigaseki, Chiyoda-ku, Tokyo 100-8915** **Japan**	Authorized officer Telephone No.

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/JP2024/020336

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
JP 2008-516242 A	15 May 2008	US 2008/0044915 A1 entire text, all drawings GB 2434028 A WO 2006/042187 A2 CA 2582578 A1 CN 101073012 A	
US 4721858 A	26 January 1988	(Family: none)	

Form PCT/ISA/210 (patent family annex) (July 2022)

REFERENCES CITED IN THE DESCRIPTION

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- Fast Separation of EU and US EPA Regulated PAHs on Agilent J&W Select PAH GC Columns. **JOHN OOSTDIJK**. Application News SI-002959. Agilent Technologies, Inc., 2010 **[0006] [0010] [0026]**
- **YOSHIO IIDA et al.** Quantification of Trace Impurities in Benzene by Gas Chromatography-Chemical Ionization Mass Spectrometry. *Mass Spectrometry*, 1976, vol. 24 (4) **[0006] [0013]**
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- **XIANG ZHANG**. Gas-phase cleavage of the novel iminium ions [R1-CH+-N=CH-R2←→R1-CH=N+=CH-R2]: An experimental and computational study. *Journal of Molecular Structure*, 2014, vol. 1056-1057, 219-226 **[0006] [0020]**
- **KENJI ARIKAWA et al.** Ion-Molecule Reactions of CH3+, C2H5+, C3H5+ with Halogenated Benzenes in an Ion Trap Mass Spectrometer. *Journal of the Mass Spectrometry Society of Japan*, 2011, 11-21 **[0013]**