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Application News

No. L586

High Performance Liquid Chromatography Nexera™ lite, Nexera XR

Analysis of Active Ingredient Isopropyl Methylphenol in Medicated Soaps by Reversed Phase Chromatography

N. Iwata

User Benefits

- Separation of isopropyl methylphenol (IPMP) and butyl paraben is possible by reversed phase chromatography (resolution of 2 or more).
- IPMP can be detected by both fluorescence and UV-visible absorption at the blending quantity restriction (0.1 to 0.5%) imposed by Japan's Ministry of Health, Labour and Welfare.
- High speed analysis makes it possible to reduce analysis time by approximately 50% and mobile phase consumption by approximately 40%.

■ Introduction

The sale of antibacterial soaps containing triclosan was banned by the European Chemicals Agency (ECHA) in 2015 [1]. Similarly, sale of antibacterial soaps containing 19 compounds including triclosan was also banned by the United States Food and Drug Administration (FDA) in 2017 [2]. Following these measures, Japan's Ministry of Health, Labour and Welfare (MHLW) issued a notification requiring replacement with alternative products [3]. Isopropyl methylphenol (IPMP) is a typical compound adopted for an alternative product.

Referring to the test methods for perfumery and cosmetics in Methods of Analysis in Health Science (The Pharmaceutical Society of Japan, 2015), in case a product contains butyl *p*-hydroxybenzoate (butyl paraben) as a preservative, normal phase chromatography (normal phase mode) should be adopted since there is a possibility of peak-overlapping of butyl paraben and IPMP in reversed phase chromatography (reversed phase mode) [4]. However, it is known that handling is more difficult in the normal phase mode than in the reversed phase mode.

This article introduces analyses in which these compounds were able to be separated with the resolution of 2 or more, even in the reversed phase mode by using a Shim-pack™ GIST C18 column.

■ Analysis of Isopropyl Methylphenol Standard Solution

IPMP is a compound that displays disinfectant, antibacterial, and antioxidative activities and is now used in many products, including quasi-drugs and cosmetics. It is also used as an active ingredient in medicated soaps. A fluorescence detection is suitable for the analysis because the blending quantity of IPMP is restricted from 0.1 to 0.5% by Japan's MHLW [5]. Although medicated soaps are exempted from the scope of the test methods for perfumery and cosmetics provided in Methods of Analysis in Health Science, the fluorescence detection conditions adopted in this experiment were set referring to the above-mentioned MHLW assignment. The test methods also state that a ultraviolet (UV) –visible absorption detector can be used if the blending quantity of IPMP has a concentration of 0.25% or more.

Here, an IPMP analysis based on the test methods for perfumery and cosmetics [4] and its speedup were examined. Table 1 and Table 2 show the conventional and high speed analytical conditions, respectively. Fig. 1 shows the chromatograms obtained with a fluorescence detector (RF-20AXS) and a photo diode array (PDA) detector (SPD-M40). In both figures, the chromatograms in the upper part were obtained using the Shim-pack GIST C18 analytical column of 5 µm particle, and in the lower part using a high speed analytical column of 2 µm particle in the same production column line-up. Approximately 50% and 40% reduction of the analysis time and the mobile phase consumption were confirmed respectively using the high speed analytical column.

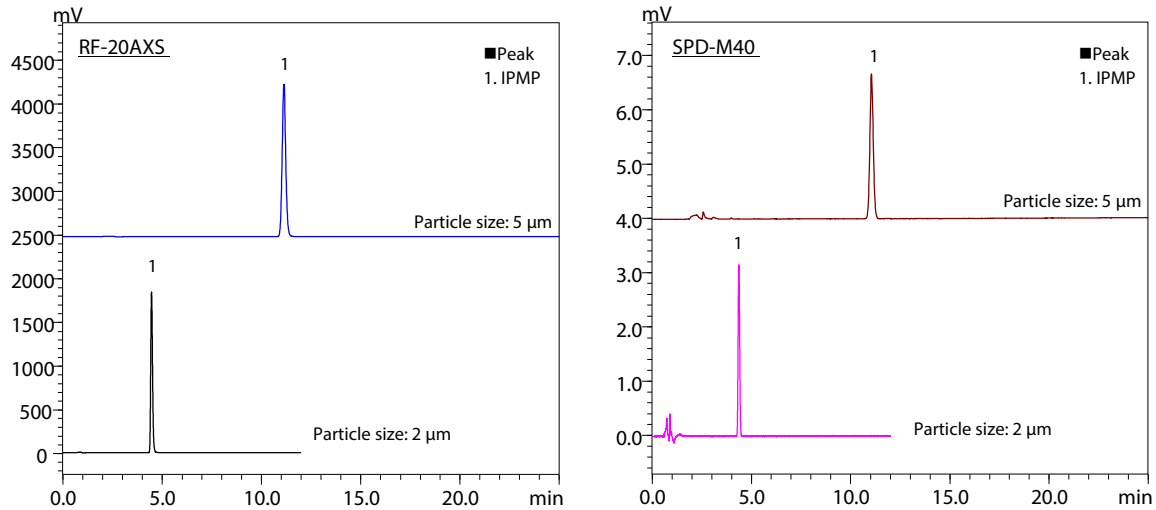

Fig. 1 Chromatograms of IPMP Standard Solution (5 mg/L)

Table 1 Conventional Analytical Conditions (Particle Size: 5 µm)

System	: Nexera lite
Column	: Shim-pack GIST C18 *1 (250 x 4.6 mm I.D., 5 µm)
Flow rate	: 1.0 mL/min
Mobile phase	: A) water B) acetonitrile B. Conc: 50%
Column temp.	: 40 ˚C
Injection volume	: 10 µL
Vial	: SHIMADZU LabTotal™ for LC 1.5 mL, Glass *2
Detection (PDA)	: SPD-M40 at 278 nm
Detection (FL)	: RF-20AXS Em: 280 nm, Ex: 305 nm

*1 P/N: 227-30017-08 *2 P/N: 227-34001-01

Table 2 High Speed Analytical Conditions (Particle Size: 2 µm)

System	: Nexera XR
Column	: Shim-pack GIST C18 *3 (150 x 3.0 mm I.D., 2 µm)
Flow rate	: 0.8 mL/min
Mobile phase	: A) water B) acetonitrile B. Conc: 50%
Mixer	: MR 180 µL II
Column temp.	: 40 ˚C
Injection volume	: 4 µL
Vial	: SHIMADZU LabTotal for LC 1.5 mL, Glass
Detection (PDA)	: SPD-M40 at 278 nm
Detection (FL)	: RF-20AXS Em: 280 nm, Ex: 305 nm

*3 P/N: 227-30002-05

■ Separation of IPMP and Butyl Paraben

Referring to the above-mentioned test method for perfumery and cosmetics, the normal phase mode should be adopted in case a product contains butyl *p*-hydroxybenzoate (butyl paraben) because there is a possibility of inadequate separation of the two compounds in the reversed phase mode. However, it is known that handling in the normal phase mode is not as easy as in the reversed phase mode since column equilibration requires considerable time and stabilization of the baseline is difficult. Fig. 2 shows the chromatogram of a mixed standard solution of IPMP and four parabens including butyl paraben obtained with an RF-20AXS fluorescence detector. Separation of these two compounds with resolution of 2 or more was possible even in the reversed phase mode using the Shim-pack GIST C18 (Table 3).

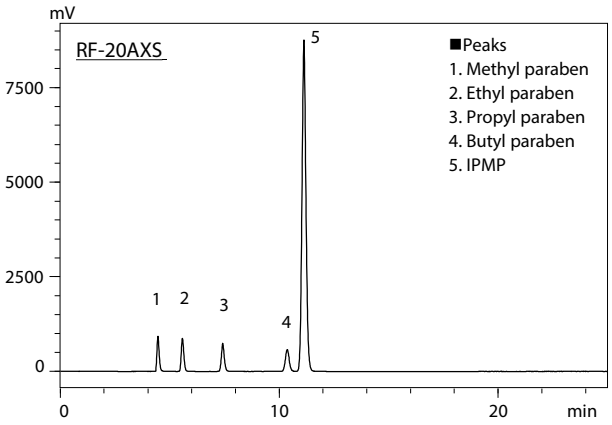

Fig. 2 Chromatogram of Mixed Standard Solution of IPMP and Four Parabens (25 mg/L each)

Table 3 Resolution of IPMP and Butyl Paraben

Column	Detector	Resolution
Shim-pack GIST C18 (particle size: 5 µm)	RF-20AXS	2.3
	SPD-M40	2.3
Shim-pack GIST C18 (particle size: 2 µm)	RF-20AXS	2.0
	SPD-M40	2.2

■ Calibration Curves

Fig. 3 shows the calibration curves for concentrations from 0.5 to 10 mg/L in analyses using the various combinations of columns and detectors. This concentration range is equivalent to contents of 0.025 to 0.5% in a real sample. Excellent linearities were obtained in all cases, as the contribution rates were $r^2 = 0.9999$ or more.

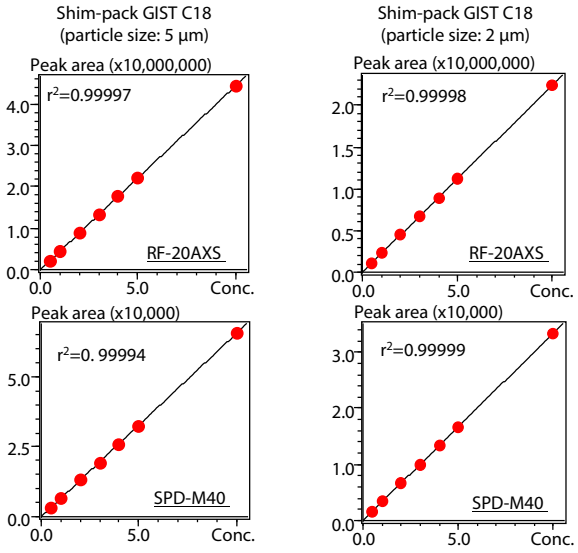

Fig. 3 Calibration Curves

■ Comparison of Sensitivity of RF-20AXS and SPD-M40

Table 4 shows the S/N ratio of IPMP (2 mg/L) calculated by the ASTM method for the RF-20AXS and SPD-M40 detectors. Fig. 4 shows a comparison of the chromatograms obtained with these detectors. The concentration corresponds to the content of 0.1% that is equivalent to the lowest allowable content in real sample specified in the MHLW assignment. In comparison with the SPD-M40, IPMP was detected with approximately 20 times higher sensitivity by the RF-20AXS. Moreover, even using the SPD-M40, the limit of quantitation calculated with the obtained result was lower than the concentration corresponding to the lowest allowable content in MHLW assignment.

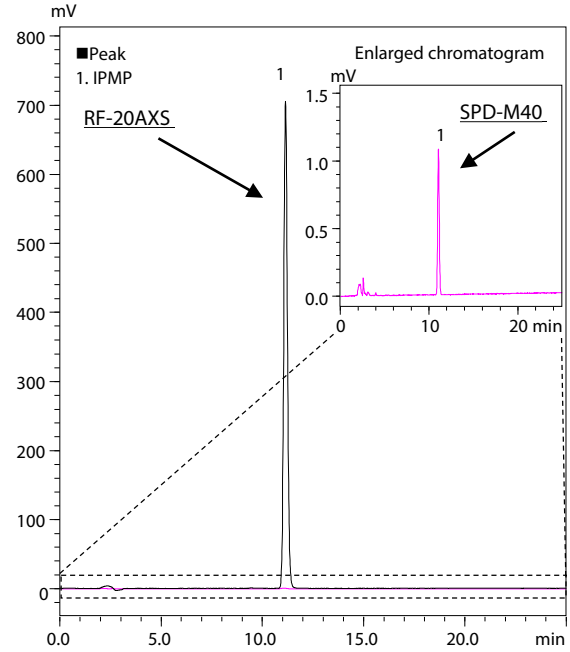

Fig. 4 Comparison of Chromatograms of IPMP Standard Solution (2 mg/L)

Table 4 Comparison of S/N Ratio of IPMP (2 mg/L)

Column	Detector	S/N*4
Shim-pack GIST C18 (particle size: 5 µm)	RF-20AXS	6289
	SPD-M40	317

*4 ASTM, Range: 15-25 min, Interval: 0.5 min

■ Repeatability

Table 5 shows the relative standard deviation (%RSD) of the retention time and peak area based on six repeated analyses of a 1 mg/L standard solution on the two columns that have different particle sizes. In all the analyses, %RSDs of 1% or less were obtained for both the retention time and peak area. Additionally, it is noteworthy that %RSD of the peak area of the RF-20AXS, which enables high sensitivity detection, was superior to that of the SPD-M40.

Table 5 %RSD based on Six Repeated Analyses

Column	Detector	Retention time	Peak area
Shim-pack GIST C18 (particle size: 5 µm)	RF-20AXS	0.04	0.13
	SPD-M40	0.04	0.79
Shim-pack GIST C18 (particle size: 2 µm)	RF-20AXS	0.12	0.17
	SPD-M40	0.13	0.49

■ Analysis of IPMP in Medicated Soaps

The samples used here were four different commercially-available medicated soaps. Sample preparation was done referring to the above-mentioned test method for perfumery and cosmetics. Fig. 5 shows the sample preparation protocol. The medicated soaps were extracted with methanol, filtered with a 0.2 µm membrane filter, diluted with methanol, and then injected into the HPLC.

Fig. 6 and Fig. 7 show the chromatograms obtained using the columns of 5 µm particle and 2 µm particle, respectively. Here, only the results obtained with the RF-20AXS are shown. In all analyses, IPMP was separated from the other compounds. In the case of medicated soap C, methyl paraben and ethyl paraben were detected.

Next, the contents of IPMP in the medicated soaps were calculated from the quantitative analytical results. Table 6 and Table 7 summarize the contents of IPMP in the medicated soaps obtained with the columns of 5 µm particle and 2 µm particle, respectively. All the executed analyses provided almost identical IPMP content of 0.1 g/100 g or 0.1%, resulting that there were no significant inter-method and inter-detection differences under above-mentioned conditions.

Table 6 Content of IPMP in Medicated Soaps
Shim-pack GIST C18 (particle size: 5 µm)

	Concentration		Content			
	mg/L		g/ 100 g		%*5	
	RF-20AXS	SPD-M40	RF-20AXS	SPD-M40	RF-20AXS	SPD-M40
Medicated soap A	2.04	2.04	0.100	0.100	0.1	0.1
Medicated soap B	2.08	2.08	0.102	0.102	0.1	0.1
Medicated soap C	2.05	2.06	0.102	0.102	0.1	0.1
Medicated soap D	2.06	2.05	0.101	0.101	0.1	0.1

*5 Allowable content by MHLW of Japan: 0.1 to 0.5%

Table 7 Content of IPMP in Medicated Soaps
Shim-pack GIST C18 (particle size: 2 µm)

	Concentration		Content			
	mg/L		g/ 100 g		%	
	RF-20AXS	SPD-M40	RF-20AXS	SPD-M40	RF-20AXS	SPD-M40
Medicated soap A	2.04	2.04	0.100	0.100	0.1	0.1
Medicated soap B	2.09	2.08	0.103	0.102	0.1	0.1
Medicated soap C	2.05	2.04	0.101	0.101	0.1	0.1
Medicated soap D	2.05	2.05	0.100	0.100	0.1	0.1

Weigh 1 g of medicated soap in 100 mL of volumetric flask

↓

Add 70 mL of methanol, then sonicate

↓

Add methanol to make 100 mL

↓

Filtrate with 0.2 µm membrane filter (discard initial flow)

↓

Transfer 5 mL of the sample solution in 25 mL of volumetric flask, add methanol to make 25 mL, then mix well

↓

Inject to HPLC

Fig. 5 Sample Preparation Protocol

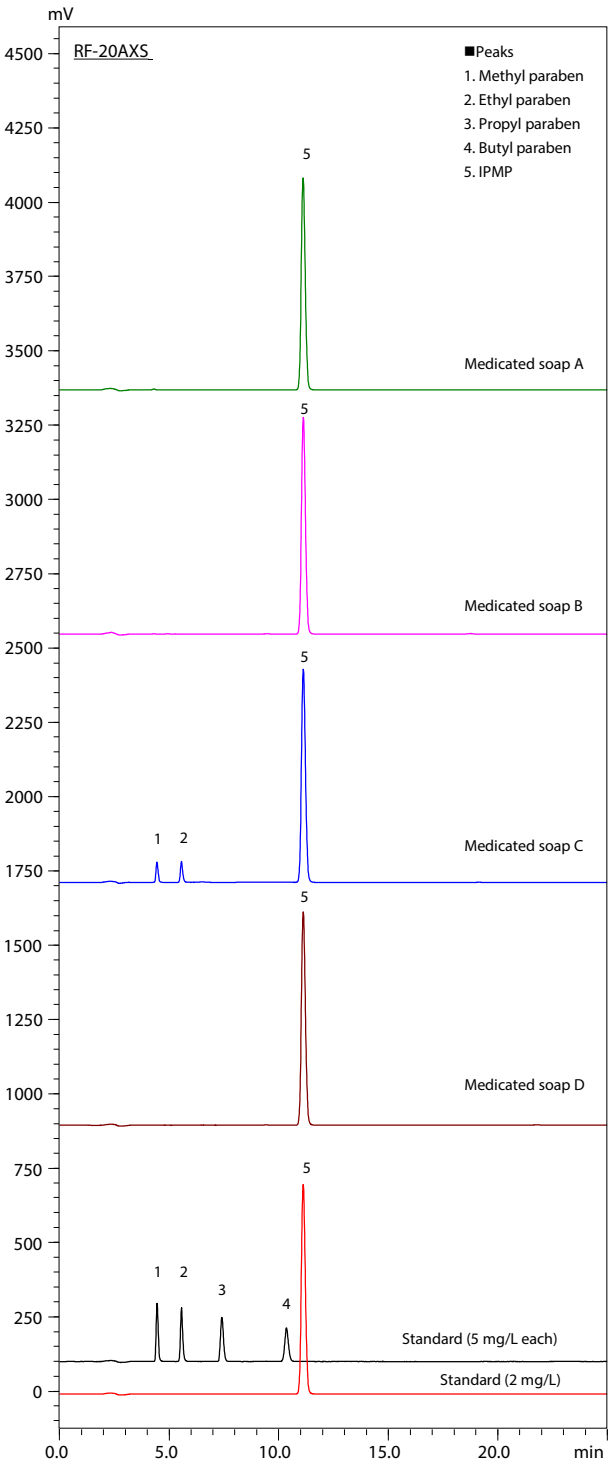

Fig. 6 Chromatograms of Medicated Soaps
(Particle size 5 µm column, RF-20AXS)

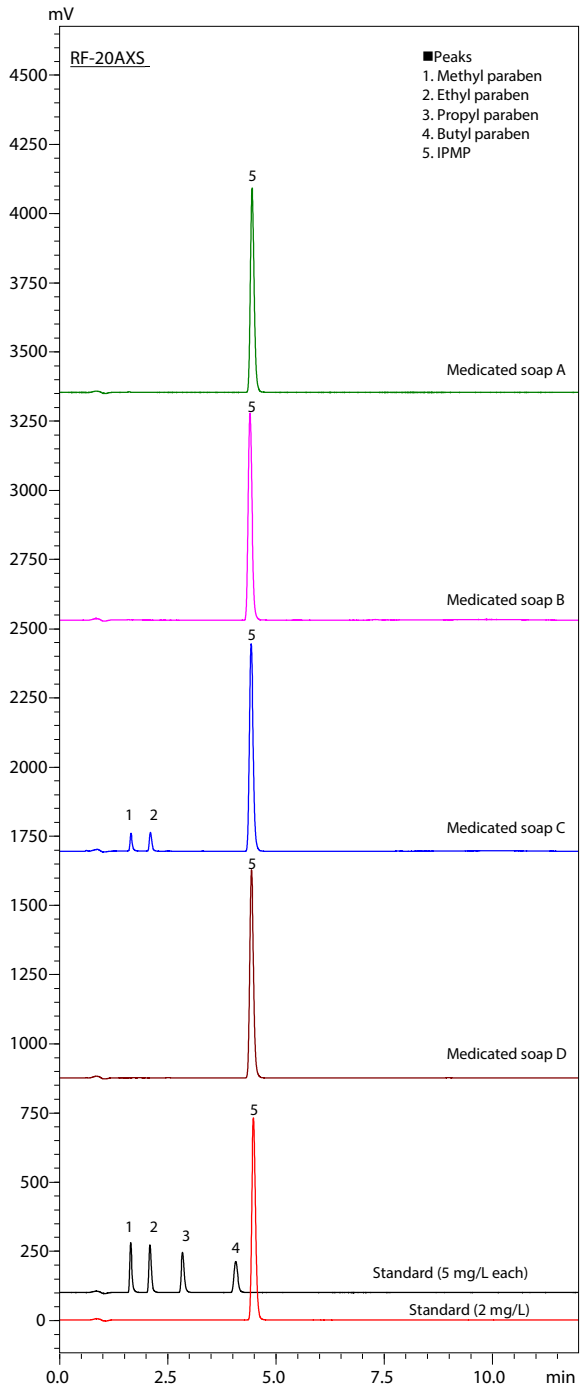

Fig. 7 Chromatograms of Medicated Soaps
(Particle size 2 µm column, RF-20AXS)

■ Verification by UV Spectrum

In case an SPD-M40 or other PDA detector is used, quantitative analysis is also possible based on the similarity accordance of UV spectrum to that of a standard solution of IPMP, in addition to the retention time identification.

Fig. 8 shows the chromatograms of medicated soap A and a standard solution detected by the SPD-M40.

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Fig. 9 shows the overlay of the UV spectra of the standard and the compound corresponding to the peak at about 11 min of the lower chromatogram of medicated soap A in Fig.8. Here, the UV spectra are normalized for comparison. The peak of medicated soap A at about 11 min was able to be identified as IPMP because the maximum absorption wavelength was determined as 279 nm for both medicated soap A and the standard.

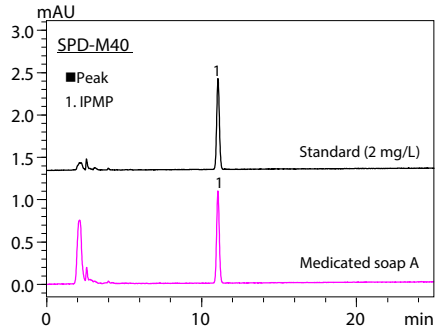

Fig. 8 Chromatogram of Medicated Soap (SPD-M40)

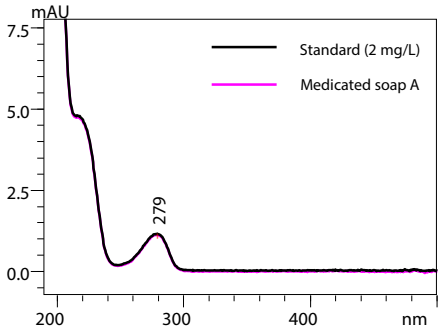

Fig. 9 Comparison of UV Spectra (SPD-M40)

■ Conclusion

Detection of IPMP in medicated soap samples by the reversed phase mode was evaluated by two methods (fluorescence detection and UV detection by PDA detector), and the possibility of high speed analysis was examined. Separation of IPMP and butyl paraben was investigated using a mixed standard solution because none of the samples analyzed in this study contained butyl paraben. As a result, separation with resolution of 2 or more was accomplished using the Shim-pack GIST C18. IPMP was detected with approximately 20 times higher sensitivity by fluorescence detection in comparison with UV detection. In addition, the results confirmed that quantitative analysis was also possible by UV detection at a concentration of 0.1% that was the lowest allowable concentration of IPMP specified by Japan's MHLW assignment, and the separation of IPMP from other compounds was confirmed based on the UV spectra. Thus, both qualitative analysis with enhanced accuracy using the PDA detector and high sensitivity analysis using the fluorescence detector were able to be executed. It was also possible to reduce analysis time and mobile phase consumption by approximately 50% and 40%, respectively, while maintaining the equivalent separation by using a high speed analytical column in the same production column line-up as the adopted conventional column of 5 µm particle.

<References>

(1) ECHA, Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products (2015)
(2) FDA, FDA issues final rule on safety and effectiveness of antibacterial soaps (2016)
(3) Ministry of Health, Labour and Welfare (MHLW), Concerning the handling of medicated soaps, etc. (Notification *Yakuseiyakushin* 0930 No. 4 and Notification *Yakuseian* 0930 No. 1, MHLW, issued September 30, 2016)
(4) The Pharmaceutical Society of Japan Eds., Methods of Analysis in Health Science 2015, 692-693, 2015, Kanehara & Co., Ltd.
(5) MHLW, Concerning items to note in examinations for approval of medicated soaps (Notification *Yakuseiyakushin* 0329 No. 13, MHLW, issued March 29, 2018)

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