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Application News

High Performance Liquid Chromatograph Nexera™ XR

High-Speed and Simultaneous Analysis of Active Ingredients CPC and GK2 in Mouthwash

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User Benefits

- Within 4 minutes, simultaneous analysis of two types of active ingredients is possible.
- Basic compounds such as quaternary ammonium salts can be analyzed with good repeatability without peak tailing.
- No ion-pairing reagents including perchloric salts (perchlorates) are used in the mobile phase.

■ Introduction

Commercial oral care products (mouthwashes) contain multiple active ingredients, which are generally analyzed by HPLC. When the quaternary ammonium salt CPC (cetylpyridinium chloride) is analyzed with a C18 column, it is known that the CPC will interact with residual silanol groups remaining on the surface of the silica gel packing material, causing adsorption and peak tailing. In recent years, columns which employ end-capping technology to mask residual silanol groups have appeared on the market. However, depending on target compounds, the effect in suppressing residual silanol groups may be slight, and in some cases peak tailing may occur. In such cases, adsorption of target compounds can be suppressed by adding an ion-pairing reagent or perchlorate to an acidic mobile phase. However, using a Shim-pack Arata™ C18 column, interaction with residual silanol groups can be suppressed, and satisfactory peak shapes can be expected without addition of an ion-pairing reagent.

This article introduces a high-speed and simultaneous analysis of CPC and GK2 (dipotassium glycyrrhizinate), which are active ingredients used in mouthwashes.

■ Analysis of Mixed Standard Solution of Cetylpyridinium Chloride and Dipotassium Glycyrrhizinate

CPC and GK2 are added to oral care products, as these compounds are expected to have respective bactericidal and anti-inflammatory effects. Fig. 1 shows the chromatogram of a mixed standard solution of CPC and GK2 (100 mg/L of each compound), and Table 1 shows the analytical conditions. Using a Shim-pack Arata C18 column, a sharp peak of CPC could be obtained, even with a mobile phase which did not contain an ion-pairing reagent or perchlorate. Furthermore, addition of an ion-pairing reagent to the mobile phase will increase retention of CPC, resulting in a longer analysis time. As described in this article, elution of two target compounds was possible within 4 minutes by using a formic acid-based mobile phase, without using any ion-pairing reagents.

Table 1 Analytical Conditions

System	: Nexera XR
Column	: Shim-pack Arata C18*1 (75 mm × 3.0 mm I.D., 2.2 µm)
Flow rate	: 1.0 mL/min
Mobile phase	: A) 0.1% formic acid in water B) 0.1% formic acid in acetonitrile
Time Program	: 40%B (0 min) → 50%B (4.00-4.50 min) → 40%B (4.51-7.00 min)
Mixer	: 180 µL
Column temp.	: 40 ˚C
Injection volume	: 1 µL
Vial	: SHIMADZU LabTotal™ for LC 1.5 mL, Glass*2
Detection (PDA)	: 254 nm (SPD-M40)

*1 P/N: 227-32802-02 *2 P/N: 227-34001-01

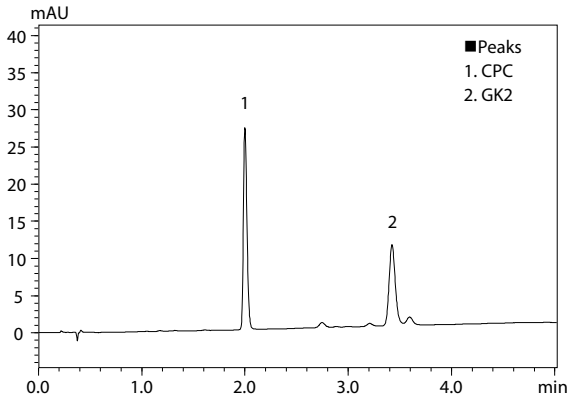

Fig. 1 Chromatogram of Mixed Standard Solution of CPC and GK2 (100 mg/L each)

■ Repeatability

Table 2 shows the repeatabilities of the retention time and the peak area of a 10 mg/L mixed standard solution in 6 repeated analyses. The repeatabilities of the retention time and the peak area of both compounds were 0.6 % or less.

Table 2 Repeatability in 6 Repeated Analyses (%RSD)

Compound	Retention time	Peak area
CPC	0.14	0.52
GK2	0.08	0.31

■ Calibration Curve

Fig. 2 shows the calibration curves (5 to 200 mg/L) of CPC and GK2. Good linearities were obtained for both compounds, with correlation coefficients $r^2 = 0.9999$ or greater.

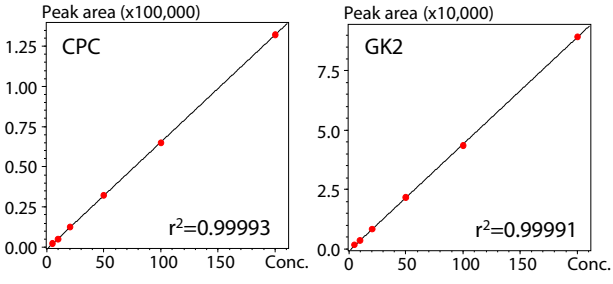

Fig. 2 Calibration Curves

■ Analysis of Mouthwashes

Two types of commercial mouthwashes were used as the samples. Analytes were prepared by diluting the mouthwashes 10 times with ultrapure water and filtration with 0.2 µm membrane filters.

Fig. 3 and Fig. 4 show the chromatograms of the two mouthwashes, and Table 3 shows the concentrations of the two compounds in the mouthwashes. Note that these values are the concentrations after sample preparation.

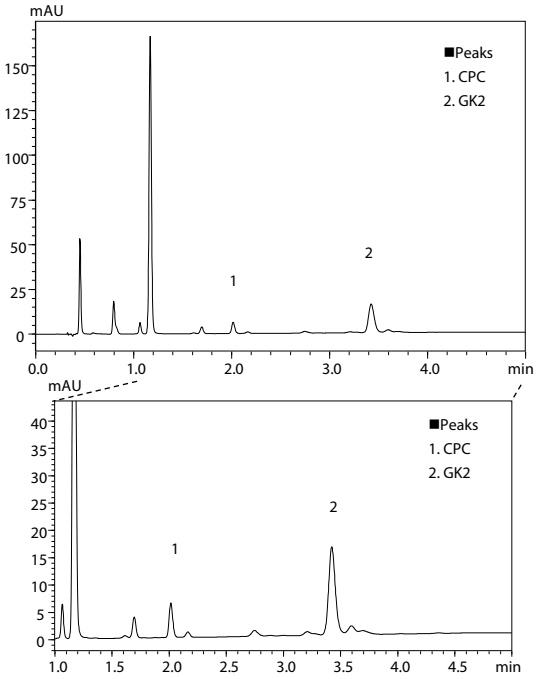

Fig. 3 Chromatogram of Mouthwash A

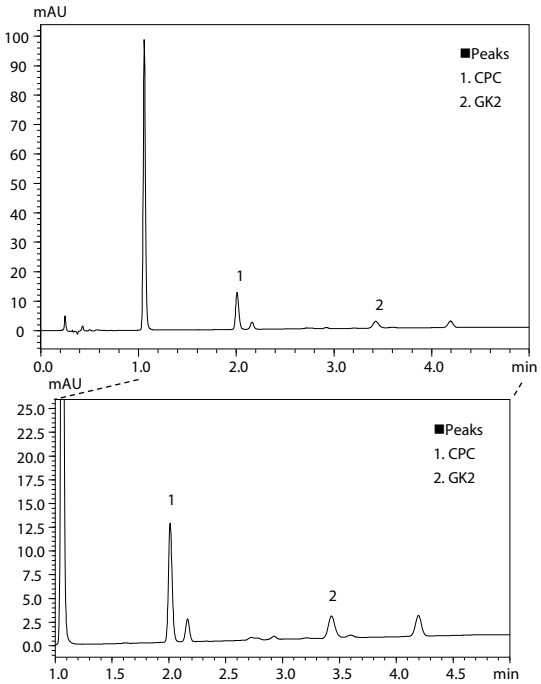

Fig. 4 Chromatogram of Mouthwash B

Table 3 Analysis Results

Compound	Concentration (mg/L)	
	Mouthwash A	Mouthwash B
CPC	23.1	44.7
GK2	143.6	22.4

■ Verification by UV Spectrum

In addition to the peak retention time identification, qualitative analysis is also possible based on the similarity accordance of UV spectrum to that of a standard solution by a photodiode array (PDA) detector.

Fig. 5 shows the overlay of the UV spectra of the CPC reference standard and the peak observed at approximately 2 minutes in mouthwash A in Fig. 3. The respective UV spectra shown here were normalized for comparison. The peak of mouthwash A at approximately 2 minutes was able to be identified as CPC because the maximum absorption wavelengths were determined at 212 nm and 258 nm for both mouthwash A and the standard. Similarly, the UV spectrum of the peak at approximately 3.5 minutes in mouthwash A was able to be identified as GK2 because the maximum absorption wavelength was determined at 250 nm in Fig. 6.

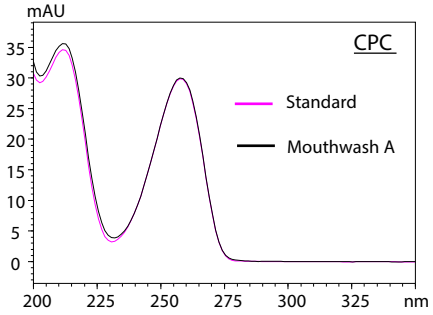

Fig. 5 Comparison of UV Spectra (CPC)

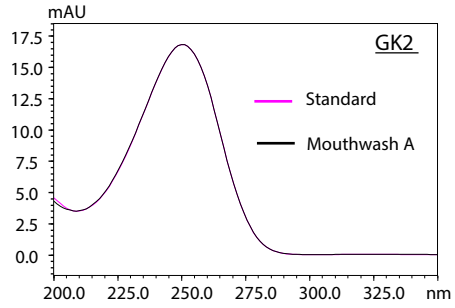

Fig. 6 Comparison of UV Spectra (GK2)

■ Conclusion

A high-speed and simultaneous analysis of CPC and GK2, which are active ingredients in mouthwashes, was conducted. Tailing of the peak of basic compounds such as CPC was able to be suppressed by using a Shim-pack Arata C18 column. It was possible to analyze the two target compounds by a short time method because an ion-pairing reagent or perchloric salt was not used in the mobile phase.

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