High Performance Liquid Chromatograph Nexera™ XR

Application News

High-Speed and High Sensitivity Analysis of Active Ingredients in Mouthwash

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User Benefits

◆ Enables high sensitivity analysis of three types of active ingredients within 4 minutes.

◆ Basic compounds such as quaternary ammonium salts can be analyzed with good repeatability without peak tailing.

◆ The photodiode array (PDA) detector allows chromatograms of any wavelength to be obtained with a single analysis.

■ Introduction

Commercial oral care products (mouthwashes) contain multiple active ingredients, which are generally analyzed by HPLC[1]. When the quaternary ammonium salt CPC (cetylpyridinium chloride) is analyzed with a C18 column, interactions between the CPC and residual silanol groups remaining on the surface of the silica gel packing material are known to cause adsorption and peak tailing. Application News No. 01-00116A describes high-speed and simultaneous analysis of active ingredients CPC and GK2 (dipotassium glycyrrhizinate) in mouthwash using a Shim-pack Arata™ C18 column. It was shown that using that column, satisfactory peak shapes were obtained without addiing an ion-pairing reagent or perchlorate to an acidic mobile phase.

Furthermore, if high sensitivity analysis is desired, it is effective to use a mobile phase with low UV absorption. This article describes high-speed and high sensitivity analysis of CPC, GK2, and isopropyl methylphenol (IPMP) in mouthwash using a phosphate buffer-based mobile phase.

■ Analysis of Mixed Standard Solution

CPC, GK2, and IPMP are added to oral care products due to purported bactericidal and anti-inflammatory effects. Fig. 1 shows the chromatograms from a mixed standard solution of CPC, GK2, and IPMP (5 mg/L of each, prepared with 50 % methanol solution). Table 1 shows the analytical conditions. With a typical C18 column, adsorption of basic compounds can be suppressed by using an acidic mobile phase that contains an ion-pairing reagent or perchlorate. However, with a Shim-pack Arata C18 column, the addition of ion-pairing reagents to the mobile phase was unnecessary and the three target compounds could be analyzed quickly.

Table 1 Analytical Conditions

System:	Nexera XR
Column:	Shim-pack Arata C18[*1]
	(75 mm × 3.0 mm I.D., 2.2 μm)
Flowrate:	0.8 mL/min
Mobile Phase:	A) 20 mmol/L (Sodium) phosphate buffer (pH 2.6)
	B) Methanol
Time Program:	60 % B (0 min)→80 % B (4.00-4.50 min)
	→60 % B (4.51-7.00 min)
Mixer:	180 μL
Column Temp.:	45 °C
Injection Volume:	5 μL
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass[*2]
Detection (PDA):	250 nm (GK2), 258 nm (CPC), 280 nm (IPMP)
	(STD cell for SPD-M40)

*1 P/N: 227-32802-02   *2 P/N: 227-34001-01
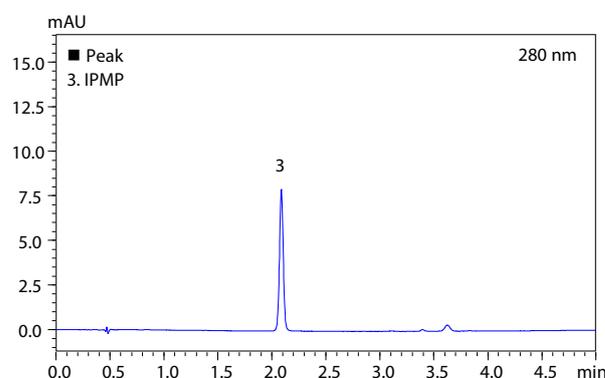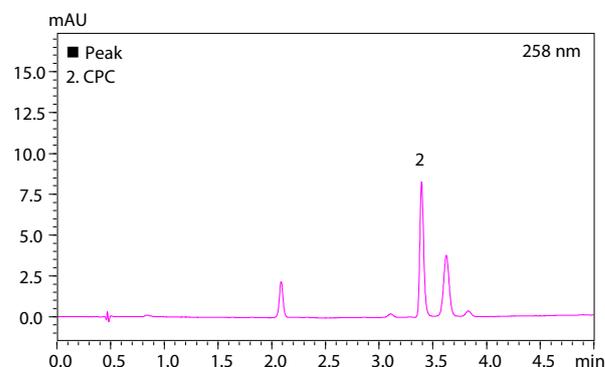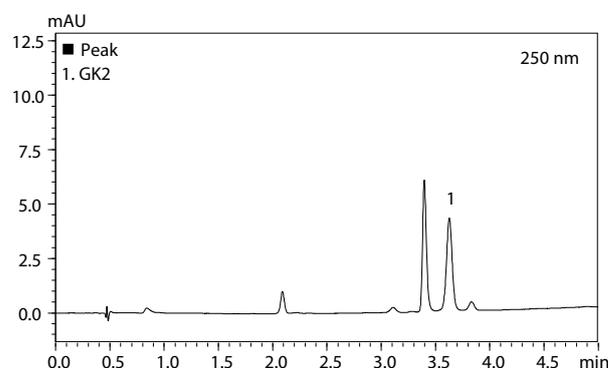
Fig. 1 Chromatograms of Mixed Standard Solution (5 mg/L each)

■ Repeatability

Table 2 shows the repeatability (%RSD) of the retention time and the peak area in six repeated analyses of a mixed standard solution (0.5 mg/L each). The repeatability of the retention time and the peak area was good for all compounds.

Table 2 Repeatability (%RSD) in Six Repeated Analyses

| Compound	Retention time	Peak area
GK2	0.11	1.01
CPC	0.12	0.48
IPMP	0.12	0.64

■ Calibration Curves

Fig. 2 shows the calibration curves of the three target compounds for concentrations from 0.1 to 10 mg/L each. Excellent linearities were obtained for all compounds, with coefficients of determination (r^2) of 0.999 or greater. In addition, high sensitivity analysis was achieved by using a phosphate buffer as a mobile phase with low UV absorption. Table 3 shows the limit of quantitation calculated from the lowest calibration curve concentrations for the three target compounds (calculation method: ASTM).
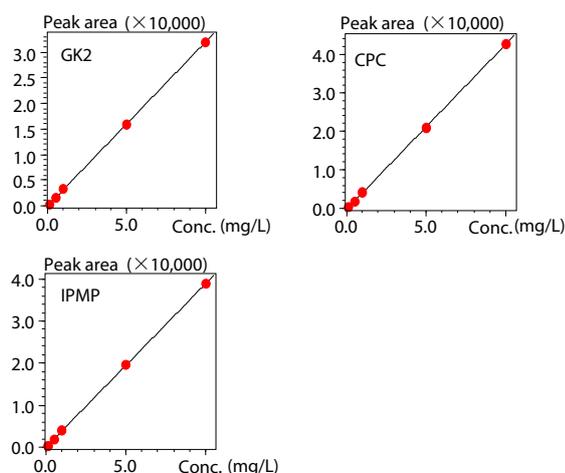
Fig. 2 Calibration Curves

Table 3 Limit of Quantitation

Compound	LOQ (mg/L)
GK2	0.079
CPC	0.076
IPMP	0.053

■ Analysis of Mouthwash

A commercial mouthwash was used as the sample. Analytes were prepared by diluting the mouthwash 100-fold with 50 % methanol solution and filtration with a 0.2 μm membrane filter. Fig. 3 shows the chromatograms of the mouthwash, and Table 4 shows the concentrations of the three compounds in the mouthwash. Note that these concentrations shown in Fig. 4 are average concentrations obtained from six repeated analyses of samples after pretreatment.

Table 4 Analysis Results (n=6)

Compound	Concentration (mg/L)	%RSD
GK2	1.65	0.27
CPC	4.25	0.24
IPMP	2.92	0.15
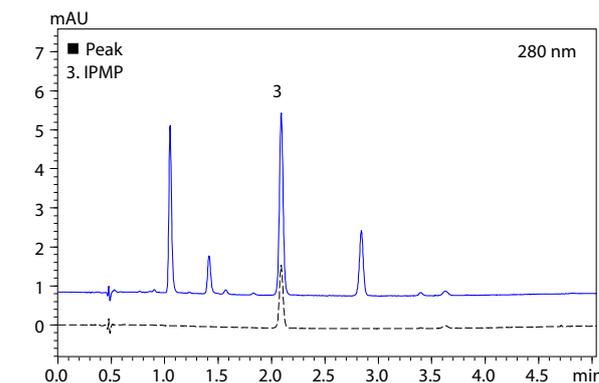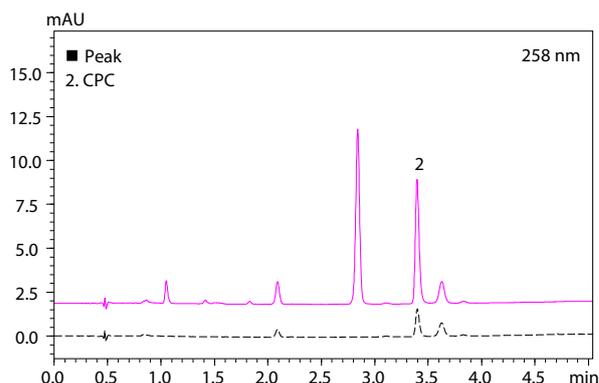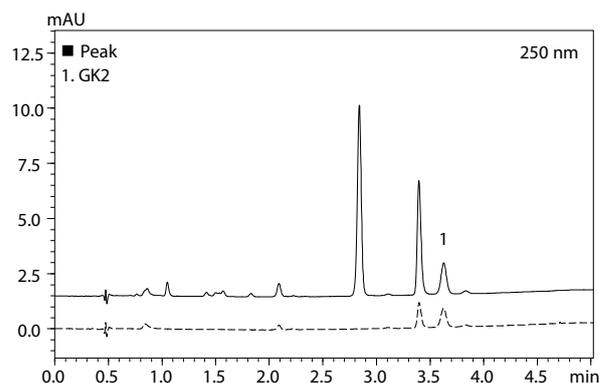
Fig. 3 Chromatograms of Mouthwash
(Solid line: Sample, Broken line: Standard solution)

■ Conclusion

A high-speed and high sensitivity method for analyzing CPC, GK2, and IPMP, which are active ingredients in mouthwash, was developed. In combination with a Shim-pack Arata C18 column and a phosphate buffer as the mobile phase, tailing of the peaks of basic compounds was suppressed, enabling the quantification of trace compounds in a short period of time.

References

1) U. S. Pharmacopeia 41-NF 36, 2017, Official Monographs "Cetylpyridinium Chloride"

Related Applications

1. High-Speed and Simultaneous Analysis of Active Ingredients CPC and GK2 in Mouthwash, Application News No. 01-00116A

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