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i-Series LC-2050C 3D, High Performance Liquid Chromatograph

Application News

Simultaneous Analysis of Dipotassium Glycyrrhizinate and Tranexamic Acid

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User Benefits

- Simultaneous analysis of dipotassium glycyrrhizate and tranexamic acid in cosmetics can be performed with simple pretreatment.
- Photodiode array (PDA) detector provides reliable identification performance for even heavily contaminated samples using UV-visible absorption spectral information.

■ Introduction

Glycyrrhizic acid is a medicinal compound found in Chinese crude medicines such as licorice and is generally known to have pharmacological effects such as anti-allergy, anti-inflammation, and detoxification. Dipotassium glycyrrhizate (GK2), a salt from glycyrrhizic acid is widely used for products such as cosmetics, shampoos, and toothpastes, in addition to over-the-counter drugs. Tranexamic acid (TA), an artificially synthesized amino acid, is generally known for its anti-inflammatory, hemostatic, and other pharmacological effects. Additionally, it is also used in cosmetics as an active whitening agent.

In general, it is difficult to retain TA on C18 columns due to its high polarity, and it often show tailing peak shape due to its basicity. On the other hand, GK2 is a compound that is relatively well retained on C18 columns. Therefore, this article introduces of TA and GK2 in cosmetics employing sodium perchlorate as a component of mobile phase for simultaneous analysis of these two compounds.

■ Analysis of Mixed Standard Solution

Fig. 1 shows the chromatogram of a mixed standard solution of GK2 and TA (GK2: 200 mg/L, TA: 4,000 mg/L, prepared in ultrapure water). Table 1 shows its analytical conditions. TA was sufficiently retained with suppressed peak tailing by adding sodium perchlorate to the mobile phase. Sodium perchlorate provides rapid stabilization of baseline condition compared to typical ion-pair reagents such as sodium alkyl sulfonate, and can be used with the mobile phase containing large portion of organic solvent.

Fig. 2 shows the UV spectra obtained by analyzing standard solution. In addition to retention time, UV spectrum obtained with PDA detector is effective for the peak identification especially in the analysis of complicated sample such as cosmetics that have many unknown compounds.

Table 1 Analytical Conditions

System	:	i-Series LC-2050C 3D
Column	:	Shim-pack™ VP-ODS, 5 µm*1 (150 mm×4.6 mm I.D., 5 µm)
Flow rate	:	1.0 mL/min
Mobile phase	:	A) 100 mM $NaClO_4$ in 10 mmol/L (Sodium) phosphate buffer (pH 2.6) B) 100 mM $NaClO_4$ in acetonitrile
Time Program	:	2%B (0 min)→90%B (9.00 -12.00 min) →2%B (12.01-15.00 min)
Mixer	:	40 µL
Column temp.	:	40 ℃
Injection volume	:	5 µL
Vial	:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*2
Detection (PDA)	:	250 nm (GK2), 220 nm (TA)

*1 P/N: 228-34937-91
*2 P/N: 227-34001-01

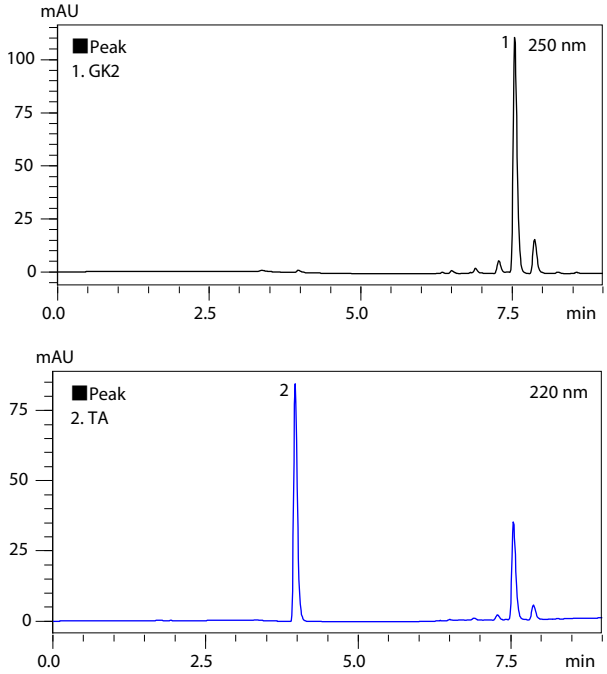

Fig. 1 Chromatograms of mixed standard solution

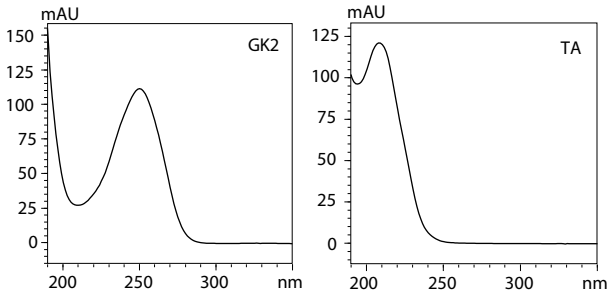

Fig. 2 UV spectra of separated standard compounds

■ Repeatability

The repeatabilities of retention times and peak areas for the two compounds of interest were confirmed by five times repeated analyses. Table 2 and Table 3 show the repeatabilities of retention times and peak areas in terms of relative standard deviations (%RSD) respectively. Good repeatabilities were obtained for both.

Table 2 Evaluation of retention time repeatability (n=5)

Compound	Average of retention time (min)	%RSD
GK2	7.5	0.03
TA	4.0	0.06

Table 3 Evaluation of peak area repeatability (n=5)

Compound	Average of peak area	%RSD
GK2	509,962	0.3
TA	350,576	0.1

■ Calibration Curve

Fig. 3 shows the calibration curves for the two compounds of interest. Good linearities were obtained for both with the coefficients of determination of $r^2 = 0.9999$ or higher.

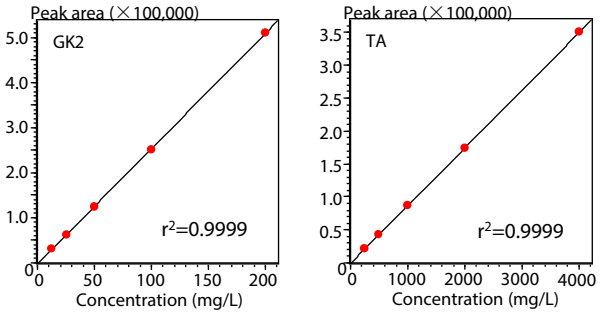

Fig. 3 Calibration curves of GK2 and TA

■ Analysis of Cosmetics

One mL of commercially available lotion was diluted ten times with ultrapure water, filtered through 0.45 µm membrane filter, then subjected to HPLC (Fig. 4).

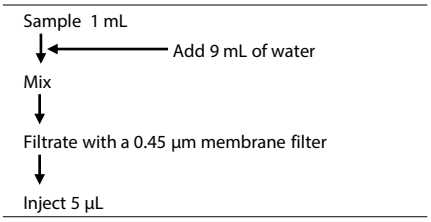

Fig. 4 Pretreatment procedures for lotion

Fig. 5 shows the chromatogram of the lotion sample. Fig. 6 shows UV- spectra at the retention times of GK2 and TA. Table 4 shows the similarities when compared to the UV- spectra of the standard compounds. Comparing the retention times and the related UV spectra simultaneously, it was confirmed that the respective peaks are GK2 and TA, and that the unknown contaminations were also separated.

Table 4 Obtained results

Compound	Similarity
GK2	0.97
TA	0.99

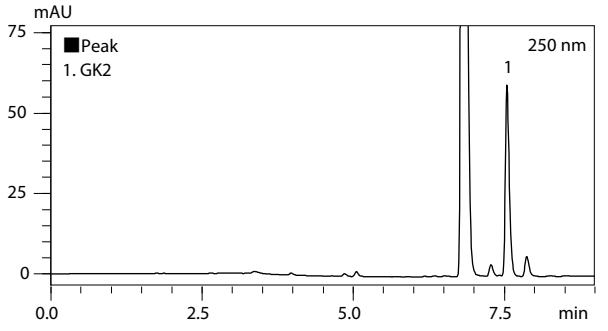

Fig. 5 Chromatograms of lotion

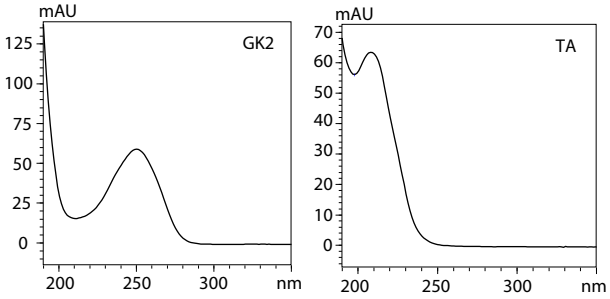

Fig. 6 UV spectra of GK2 and TA in lotion

The obtained concentrations of GK2 and TA in the lotion after pretreatment and their recovery rates are shown in Table 5. The recovery rates were calculated through three times repeated analyses of the lotion sample added with GK2 and TA to make the increases of 100 mg/L and 2,000 mg/L in concentration respectively.

Table 5 Obtained results

Compound	Average of concentration (mg/L)	%RSD	Average of recovery (%)
GK2	106.8	0.6	98.1
TA	1,970	0.5	97.7

■ Conclusion

Simultaneous analysis of GK2 and TA, the active ingredients in cosmetics, was successfully performed. By adding sodium perchlorate to the mobile phase, TA was able to be retained on the C18 column, resulting in simultaneous analysis of these two compounds. In addition to the retention times, the UV spectra from PDA detector enabled reliable identifications of the target compounds in cosmetics.

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