High-Performance Liquid Chromatographic Determination of Components in Eye Lotion Using Methoxy(3-morpholinopropyl)silanediyl Modified Silica Gel Column

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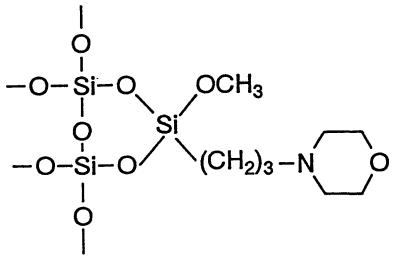
Synopsis. A recently developed column packing material, methoxy(3-morpholinopropyl)silanediyl modified silica gel, was successfully applied to the separation and determination of components (pyridoxine, flavin adenine dinucleotide, cyanocobalamin, chlorpheniramine, naphazoline, and neostigmine) in an eye lotion, in a single analysis by a single buffer elution with UV detection.

Quite recently, we reported on the development of new column packing materials for the high-performance liquid chromatographic (HPLC) separation of water-soluble vitamins[1] as well as such substances[2] as inosinic, xanthylic, and guanylic acids related to umami. It was consequently confirmed that methoxy(3-morpholinopropyl)silanediyl modified silica gel (MPS), similar to an ion-exchange resin, is suitable for separating water-soluble compounds. On the other hand, in ophthalmologic or pharmaceutical studies, a simple and rapid determination of components in eye lotions should be useful. Although the determination of a single component, such as boric acid[3] or thimerosal,[4] in an eye lotion by HPLC is known, HPLC-analysis of multicomponents has until now been scarcely reported. In this study, we thus tried to expand on the application of MPS to the separation and determination of components in eye lotions using UV detection.



Formula.

Experimental

Material. MPS was prepared according to a previous report.[1] Pyridoxine (PY) and flavin adenine dinucleotide (FAD) were obtained from Wako Pure Chemicals Co., Ltd. Cyanocobalamin (CC), chlorpheniramine (CP) (as its maleate), naphazoline (NA), and neostigmine (NE) (as its methyl sulfate) were purchased from Sigma Chemical Co. All other reagents used were of analytical grade (Wako).

Apparatus. The chromatograph system comprised a Tosoh CCPD pump with a Rheodyne-type injector (7125) with a 100 μl loop, a Tosoh UV8011 variable-wavelength UV monitor and a Hitachi Model 561 recorder. The eluates were monitored at 270 nm.

HPLC Column and Eluent. The gel (MPS) was packed into a stainless-steel tube (150 mm×6.0 mm I.D.) using a slurry-packing technique [glycerol : methanol, 4 : 6 (v/v)] and applied to the HPLC separation at a flow rate 1.0 ml min^{-1} of 0.1 M (1 M=1 mol dm^{-3}) phosphate buffer (pH 2.3) (column temperature, 30 °C).

Preparation of a Standard Solution of the Components Contained in an Eye Lotion. Six components (PY, FAD, CC, CP, NA, and NE) in a commercially available eye lotion were separately dissolved in the eluent [0.1 M phosphate buffer (pH 2.3)]; the solutions were then arranged to 5×10^{-4} M for PY, CP, and NA, 5×10^{-5} M (CC), and 10^{-3} M (NE).

Determination. Each component was determined by the standard addition method, as follows: To a commercially available eye lotion was added a series of known concentrations of standard solution, and diluted ten times with an eluent. Aliquots (100 μl) were injected onto the HPLC.

Results and Discussion

In the separation of water-soluble components with MPS, a phosphate buffer as an eluent has been studied in detail and found to be useful.[1] We therefore used a phosphate buffer for an eluent. First of all, the effect of the eluent pH was examined in order to separate the six components (PY, FAD, CC, CP, NA, and NE). The use of a 0.1 M phosphate buffer (pH 2.3) gave a relatively good peak separation. Almost no effect was observed after the addition of sodium 1-octanesulfonate. Under the above-mentioned HPLC conditions, it was demonstrated that their water-soluble substances, including maleic acid liberated from CP maleate, can be well separated (except for CP and NA) within 15 min in a single analysis (Fig. 1). The calibra-

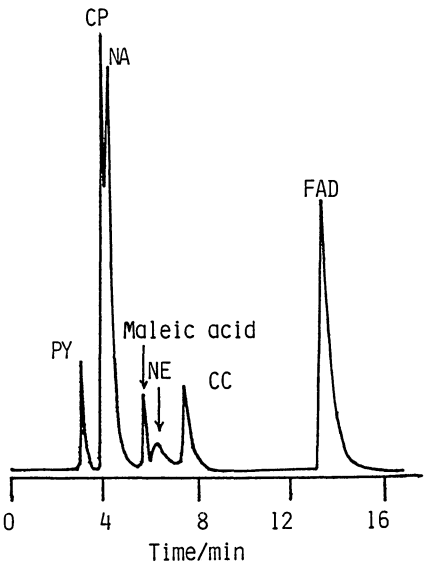


Fig. 1. Chromatogram of the components of an eye lotion.

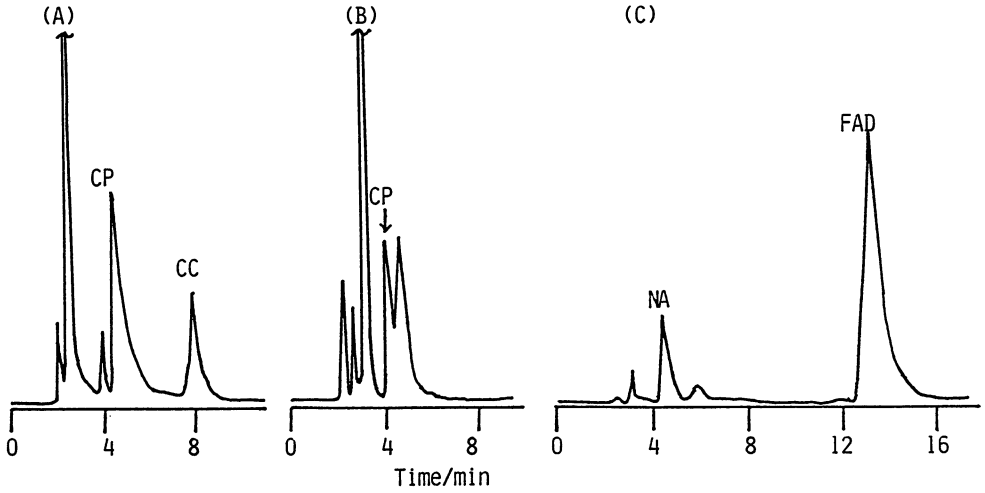


Fig. 2. Chromatograms of a commercially available eye lotion (A—C).

Table 1. Calibration Curves for the Components in Eye Lotion

Compound	Equation[a)]	r	RSD(%), n=5 (concn)
PY	Y=0.19X+0.01	0.999	3.20 (10 nmol)
FAD	Y=3.34X+0.15	0.999	1.32 (5 nmol)
CC	Y=2.24X−0.03	0.999	1.03 (1 nmol)
CP	Y=1.54X−0.08	0.999	1.28 (5 nmol)
NE	Y=0.09X−0.04	1.000	2.42 (20 nmol)
NA	Y=0.78X+0.70	0.999	1.22 (25 nmol)

a) Y=Peak height (cm), X=Concentration (nmol/injection).

Table 2. Analyses of Eye Lotion

Sample	Found (M)[a)] (w/v%)	Indicated (M) (w/v%)
Eye lotion A		
CC	7.5×10^{-5} (0.01)	7.4×10^{-5} (0.01)
CP	2.2×10^{-4} (0.01)	2.6×10^{-4} (0.01)
Eye lotion B		
CP	2.4×10^{-3} (0.09)	5.1×10^{-4} (0.02)
Eye lotion C		
FAD	5.1×10^{-4} (0.04)	4.2×10^{-4} (0.033)
NA	1.8×10^{-4} (0.004)	1.2×10^{-4} (0.003)

a) Mean of duplicate assay.

tion curves for these components obtained by using the corresponding standard solutions were linear in the nmol range of 1.75—14 (PY), 0.98—7.9 (FAD), 0.18—1.4 (CC), 0.88—7 (CP), 3.6—29 (NE), and 6.25—50 (NA). The linear regressions are shown in Table 1 with the correlation coefficients (r). As the application work, three commercially available eye lotions were assayed. Typical chromatograms are shown in Fig. 2. The peaks were confirmed by co-chromatography. Compounds CC, CP, FAD, and NA in commercially available eye lotions (A—C) were determined by the standard addition method (Table 2). Compounds PY and NE could not be determined under the experimental conditions. On the other hand, it was observed that the separation capacity of MPS gradually decreased with increasing the sample loading. This might be caused by the hydrolysis of MPS with a lower pH mobile phase. This point must be improved and is now under study.

In conclusion, MPS could be further applied to the analysis of components in commercially available eye lotions. This method might be useful for pharmaceutical studies on the stability of the components contained in eye lotions.

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