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Reuse of HPLC Guard Column by Ultrasonic Cleaning

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Abstract : This study investigated reusability of costly guard column by ultrasonic. It also investigated various effects that affect to guard column generation by ultrasonic. When investigated 30 KHz of frequency, area of ascorbic acid is 73.0% compared to unused guard column. As a result of investigation of effect of pH, guard column by ultrasonic is effective at alkali area. As a result of investigation of solvent effect, when ethanol is used, generation rate is 81.9% as of peak area compared to the case of analysis in un used column. From the result, it indicates that regenerated guard by ultrasonic is reusable.

Keywords : Ultrasonic, reuse, guard column, frequency, pH, solvent, optimal irradiation condition

1. Introduction

High Performance Liquid Chromatography, HPLC universal analysis machine that is even currently used in almost every university's natural science and engineering laboratory in Korea. Also, government funded research center and private analysis institution use HPLC a lot. HPLC is used consumables called guard column a lot[1]. This guard column protects this after elevated column for extending life span for main purpose. Life span of guard column directly affects to not only increase of analysis cost but also HPLC analysis degree. Guard column is very complex mechanism that works physical, chemical and biological factors complexly[2-4]. Also, particular guard column is largely affected by physical factors such as HPLC analysis condition, kind of used eluent, the velocity of moving fluid, and pressure. As the effort to get rid of pollution causing material, research about various cleaning products was researched through laboratory and field experiment, and most of them are influenced by spacer[5]. When minute amount of suspended solids in eluent flows between spacers, this study analyzes phenomenon moving on surface of film, and research about optimization of separation membrane module design and water flow was also carried out[6]. Also, dissolved inorganic material exceeds solubility in process that is condensed from guard column, and when it becomes deposit in colloid form and is cumulated on surface of guard column, this study researched phenomenon that penetration ratio is reduced through systematic analysis of what colloid size, electronic characteristic influence on pollution[7]. Since polluting phenomenon of organic material depends on physical/chemical sociability between polluting material and guar column material, research to

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develop proper guard column material to particular liquid has been carried out. Especially, method to introduce functional group on existing surface of guard column that restrains pollution has been used[8-10]. Meanwhile, desorption technology caused by ultrasonic (cleaning) has been researched for decades, and it has been currently applied in various areas[11-13]. Cleaning by ultrasonic has high cleaning efficiency, and low processing cost, and it is applicable to various polluting mediums, which is known as advantages. Therefore, this study pays attention to the regenerating method that reuses polluted guard column by ultrasonic.

2. Materials and Methods

2.1. Materials

The materials used were as follows: ethanol, hexane, acetonitrile, ascorbic acid, and KH_2PO_4 at 99% purity, respectively (Waco); H_2SO_4 and NaOH (Samchun) at 98% purity.

2.2. regeneration experiment and regenerating guard column function evaluation method

About regeneration method, it investigated ultrasonic after fill up 50mL of distilled water after put guard column into 100 mL volume beaker. For evaluation method of guard column function, the fact that it does not affect to this after column 's function (resolving power) when generated guard column is used was proved through performance test with standard material. Also, when regenerated guard column is used, it is compared to whether there is pressure within HPLC pump's permissible range and when new column is used, and was carried out performance test. When regenerated guard column is used, performance test was carried out after comparing to standard material's resolving power and detection time, secure safety of HPLC base line, and when un used column is used. Performance test was carried out along with comparing to when unused column is used.

3. Results and Discussion

3.1. Regenerating effect of guard column by ultrasonic investigation strength

Guard column used for this study, guard column holder, and HPLC bone column used general prototype, and appeared in Fig. 1.

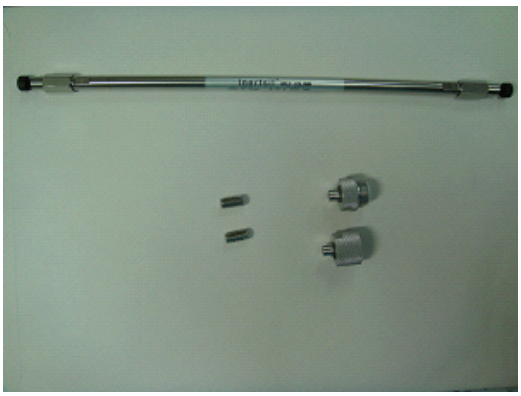

Fig. 1. HPLC main column (upper), guard column (below left), and guard column holder (below right).

It researched effect about polluting material desorption from guard column by ultrasonic strength (frequency). In order to analyze regeneration effect of guard column by frequency change, applied HPLC analysis condition is appeared in below Table 1.

The regenerated result of polluted guard column as changes ultrasonic frequency is summarized in below Table 2. HPLC bone column used intertsil ODS-3[14] of GL science.

When 30 KHz of frequency is researched, peak area (Fig. 2) of ascorbic acid represents 73.0% of area when guard column (Fig. 3) was used when regenerated guard column was used.

Table 1 HPLC condition used for ascorbic acid analysis

Pump	Hitachi L-6000
Detector	Shimadzu UV-VIS SPD-10Avp
Injector	Hitachi 655A-40 auto sampler
Integrator	Hitachi D-2500
Solvent	Acetonitrile: 10 mM KH_2PO_4 (85:15, v/v)
Column temperature	20℃
Flow rate	0.8 mL/min
Injection volume	10 μL
Detection wavelength	254 nm

Table 2 Regeneration performance evaluation by frequency (GL science intertsil ODS-3)

Frequency (KHz)	Peak area	Retention time(min)	Pressure (100 kg/cm^2)	Base line stability
unused guard column	7335361	4.424	47	very stable
10	5002982	4.581	51	little unstable
20	5135180	4.958	55	stable
30	5357204	5.219	58	very stable
40	5195840	5.201	58	little unstable

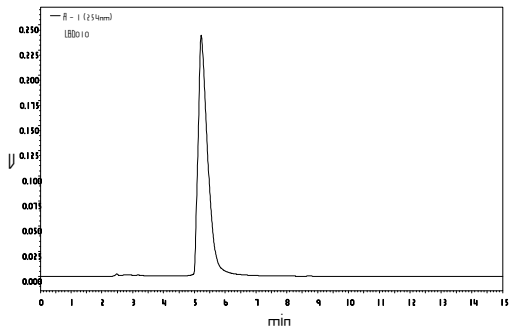

Fig. 2. HPLC chromatogram when regenerated guard column used at 30 KHz (GL science intertsil ODS-3).

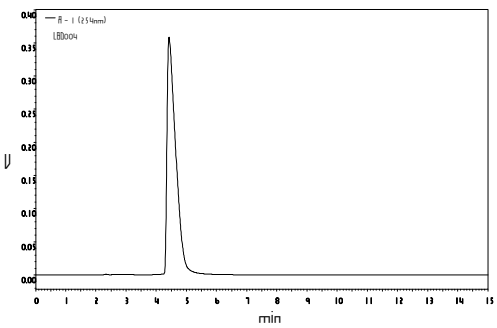

Fig. 3. HPLC chromatogram when unused guard column used (GL science intertsil ODS-3).

At 20 and 40 KHz, it appears little smaller ascorbic acid peak compared to at 30 KHz, and it shows the best regeneration effect at 30 KHz. For Retention time, as frequency increases, retention time of ascorbic acid tends to increase slightly, and when 30 KHz of frequency is researched, it appears 5.219 minutes. Comparing to when it is unused, retention time extends 0.8 minute or more. This is permissible range in general analysis. Seeing HPLC pump's pressure, as frequency increases, it shows that it increases 11×100 kg/cm^2 or more compared to unused. This phenomenon is cause that increases pump's

pressure because unremoved polluted material is left in guard column. Though base line on chromatogram of HPLC was little unstable at 20 and 40KHz, it is permissible range, and it gets stable at 10 and 30 KHz.

In order to prove regeneration effect of guard column by ultrasonic more clearly, changed HPLC bone column to Tosoh's TSK-GEL ODS-80T[15], and summarized result of same experiment in below Table 3. When researched frequency 20 (Fig. 4) and 30 KHz, 70.8% of area is appeared compared to unused guard column if ascorbic acid's peak area (Fig. 5) compares to unused guard column. At frequency 10, peak area is appeared 4487302, which is inadequate regeneration effect. It shows little smaller area at 40 KHz compared to 20 and 30 KHz, it appeared the best regeneration effect at 20 and 30 KHz. Seeing retention time, as frequency increases, retention time of ascorbic significantly increases, and when researched 30 KHz, it shows 5.510 minutes, and retention time extends 2.64 minutes or more. Though this retention time difference is permissible at general analysis, change of retention time may affect to precision degree of analysis so caution is required for analysis. Seeing pressure of HPLC pump, as frequency increases, it appears pressure increases 6×100 kg/cm^2 or more compared to unused. This pressure increase is judged that it is in change of pressure at HPLC analysis. Baseline on chromatogram of HPLC is stable at 20, 30, 40 KHz, and is unstable at 10 KHz.

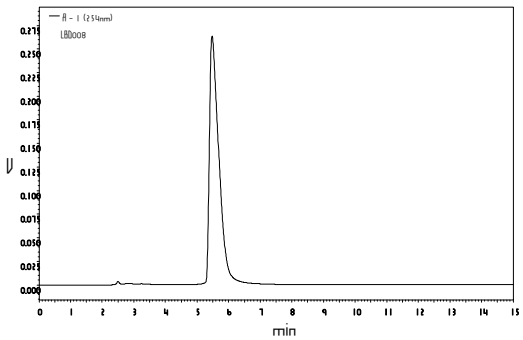

Fig. 4. HPLC chromatogram at 20 KHz of frequency when regenerated guard column used (Tosoh TSK-GEL ODS-80T).

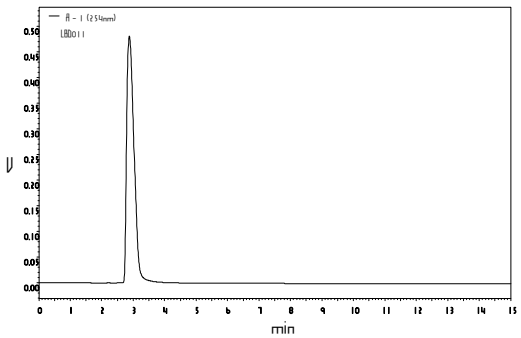

Fig. 5. HPLC chromatogram when unused guard column used (Tosoh TSK-GEL ODS-80T).

Table 3. Regeneration performance evaluation of guard column by frequency (Tosoh TSK-GEL ODS-80T).

Frequency (KHz)	Peak area	Retention time (min)	Pressure (100 kg/cm^2)	Base line stability
unused guard column	7923015	2.870	59	very stable
10	4487302	4.148	67	little unstable
20	5607948	5.473	65	stable
30	5528923	5.510	65	very stable
40	5105950	3.608	65	stable

Table 4. Evaluation of regeneration performance of guard column by pH (Tosoh TSK-GEL ODS-80T)

pH	Peak area	Retention time	Pressure (100 kg/cm^2)	Base line stability
unused guard column	7335361	4.424	47	very stable
4	5048800	5.224	57	stable
6	5117220	5.229	57	stable
8	5500844	5.117	54	stable
10	5950120	4.750	49	stable

Table 5. Evaluation of regeneration performance of guard column by solvent

Solvent	Peak area	Retention time	Pressure (100 kg/cm^2)	Base line stability
unused guard column(distilled water)	7335361	4.424	47	very stable
ethanol	6005208	4.501	48	stable
hexane	5368200	4.860	49	stable

Meanwhile, at optimum frequency 30 KHz, researched changing temperature of water to find out effect that the temperature affects to regeneration of guard column. However, as temperature increases, since increase of peak area of ascorbic acid is not enough, it is judged that regeneration effect of guard column is not that high.

As pH increases, peak area of ascorbic acid increases. Especially at pH 10, increase in area is appeared high. In alkali area, see regeneration of guard column by ultrasonic has effect.

3.2. Review of regeneration effect by adding solvent

In order to detach polluted material more effectively from guard column by ultrasonic, researched regeneration effect of guard column with using well-melting solvent instead of used distilled water.

For solvent, representative water friendly ethanol and representative hydropathy hexane were used.

When uses ethanol as solvent for ultrasonic research, regeneration rate as of peak area is appeared 81.9% compared to analysis of use of unused guard column. Meanwhile, comparing to case that used unused guard column for analysis, regeneration rate was appeared 73.2% when hexane is used. H_2O_2 was used in order to detach polluted material more effectively from guard column by ultrasonic. As far as we know, H_2O_2 generates strong oxidizing powered OH radical in water with small amount of catalyst (Fe^{2+} and others). The radical dissolves polluted material on guard column, and was applied to increase guard column's regeneration rate. Concentration of H_2O_2 was experimented at

0.04, 0.1, 0.2M, which is considered its characteristic. However, regeneration effect as of acid peak area is appeared not enough. Since various polluted material was pressed on guard column for long time, removing OH radical pressed polluted material is limited.

4. Conclusions

In order to reuse elevated guard column, researched ultrasonic at various range of Hz on polluted guard column. Also, about various effect (temperature, pH, solvent and oxidizer), received following results as deduced optimized condition.

1. When researched 30 KHz, peak area of ascorbic acid was 73.0% compared to unused guard column. At 20, 40, and 30 KHz, it shows slightly smaller peak area, and showed the best regeneration effect at 30 KHz.
2. As a results of review of effect that affects to regeneration effect of guard column through ultrasonic research, as temperature increases, increase of ascorbic acid's peak area is not enough. Judged that regeneration effect is not high by increase temperature.
3. As result of review of the pH effect that affects to regeneration effect of guard column caused by ultrasonic research, as pH increases, peak area of ascorbic acid increases. Especially at pH 10, area increases remarkably. At alkali, regeneration of guard column by ultrasonic seems to be effective.
4. According to the result of review of effect that affects to regeneration, regeneration rate is 81.9% as of peak area compared to analysis with unused guard column when ethanol is used. Regeneration rate as of peak area is appeared 81.9%
5. The oxidizing agent is hydrogen peroxide to effect a playback Reviewing the impact guard column reproduction effect by the ultrasonic wave irradiation, based on the ascorbic acid peak area with the addition of hydrogen peroxide was found insufficient.

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