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단 신

공업용 메탄올과 아세토니트릴의 HPLC급으로의 정제에 관한 연구

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Purification of Technical Grade Methanol and Acetonitrile for HPLC Use

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INTRODUCTION

The use of high purity solvent is essential for the successful operation of the high performance liquid chromatography(HPLC), particularly for those with optical detectors.

Solvent purity requirements vary depending upon operation[1,2]. The purity becomes especially important for gradient elution. When the eluent contains UV active and fluorescence impurities that are strongly retained in the column, they are concentrated at the head of the column during the initial stage of the gradient run. As the eluent strength increases, the impurities are moved down the column and sometimes cause base line fluctuations which may interfere with the detection of the sample peaks of interest. Water in the solvent may vary capacity factor and consequently lower the resolution of the analytes. Particulate matter blocks in-line filter and shortens the life time of the column. Other impurities involving acid or base, dissolved oxygen are also controlled for HPLC solvents.

In the present work, technical grades of methanol and acetonitrile, common eluents for HPLC were purified to the grade of HPLC solvent. Adsorptive chromatography[3,4] was utilized for the removal of impurities. The efficiency of purification was primarily evaluated by measuring the UV absorbances, which are most critically controlled in the HPLC solvents.

It appeared that the present method is simple, yet effective for the preparation of HPLC solvents.

EXPERIMENTAL

All the glasswares used in the experiment were cleaned by soaking in the cleaning solution of sulfuric acid and ammonium persulfate for 4~5 hours and sufficiently rinsing with distilled and subboiling waters. They were dried at 200℃ in an oven before use. The fused silica absorption cells were cleaned by soaking in 10% hydrochloric acid for 10 hours and rinsing with subboiling water.

Charcoal(Kanto, 01085-02), silica gel(Wako, gel Q-23), and alumina(Fluka, 507A for neutral and 5016A for basic, Janssen, 2340 for acidic) adsorbents were reactivated by the methods of literatures[4,5] and then cooled in a desiccator containing a drying agent of P_2O_5.

100 g of adsorbent were dry packed in the chro-

matographic column(25 mm×50 cm) with sintered glass filter retaining small adsorbent particles(*Fig.* 1). Three bed volumes of technical grade methanol or acetonitrile distributed by Duk-san chemical company were passed through the column at the flow rate about 0.75 m*l*·min^{-1} regulated by stopcock. The first portion of purified solvent was discarded and the rest of the eluent was successively collected with 20 m*l* portions.

When two adsorbents were employed for purification, 100 g of charcoal(lower bed) and 100 g of alumina(upper bed) were packed in 45 mm×60 cm column. In this case, two and a half bed volumes of the solvent were eluted and 30 m*l* portions of the purified solvents were separately collected.

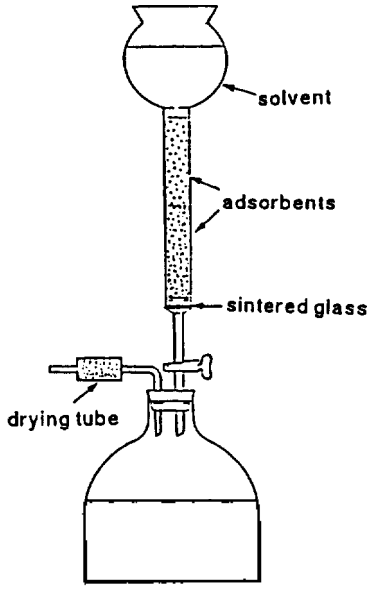

Fig. 1. Apparatus for purification of organic solvents.

Each portion of the eluent was examined by measuring the UV absorbances. Since middle portions of the eluents showed lower absorbances than the first and last ones, the middle portions whose absorbances ranged within 5% were combined and then analyzed.

The UV absorbances were measured by an UV-VIS spectrophotometer(Beckman, model DU-7) with respect to subboiling water as a reference using 1 cm cell. The assay was made by gas chromatograph(Hewlett Packard, Model 5840A) with 50AQ3/BP20 and SPB-1 fused silica capillary column.

Water content was determined by an aquameter (Metrohm, model 630) with Karl Fischer reagent (Merck Cat. No. 9028). Fluorescence impurities were determined on the basis of quinine[6] as a standard by a luminescence spectrometer(Perkin Elmer, model LS-5). The excitation and emission wavelengths chosen for the fluorescence measurements were 255 and 450 nm, respectively. Other impurities such as residue after evaporation, titrable acid and base were determined by JIS or ASTM method.

RESULTS AND DISCUSSION

UV Absorptivity. The UV absorbances of puri-

Table 1. UV absorptivity of various methanol

Sample \ Wavelength(nm)	Absorbance[a]			
	225	254	280	350
HPLC grade MeOH(J. T. Baker)				
Specification	<0.15	<0.01	<0.01	<0.01
Measured	0.156	0.017	0.009	0.000
Technical MeOH(Duk-San, Korea)	0.227	0.039	0.019	0.004
Purified MeOH(from Duk-San, Korea)				
Acidic alumina[b]	0.239	0.132	0.035	0.029
Neutral alumina[b]	0.167	0.013	0.003	0.005
Silica gel[b]	0.187	0.033	0.018	0.012
Charcoal[b]	0.199	0.014	0.008	0.001
Neutral alumina+charcoal[b]	0.137	0.010	0.002	0.002

[a] Used 1 cm cell, reference : subboiling water, [b] Adsorbents.

Table 2. UV absorptivity of various acetonitrile

Sample \ Wavelength(nm)	Absorbance[a]			
	200	220	254	280
HPLC grade Acetonitrile				
Specification(J. T. Baker)	<0.05	<0.01	<0.01	<0.01
Measured				
Burdick & Jackson	0.026	0.003	0.002	0.001
Merck	0.025	0.003	0.002	0.002
Technical Acetonitrile(Duk-San, Korea)	0.982	0.215	0.033	0.010
Purified Acetonitrile(from Duk-San, Korea)				
Acidic alumina[b]	0.604	0.132	0.039	0.029
Basic alumina[b]	0.770	0.212	0.059	0.053
Neutral alumina[b]	0.537	0.093	0.011	0.005
Silica gel[b]	0.910	0.211	0.031	0.017
Charcoal[b]	0.387	0.053	0.007	0.015
Neutral alumina+charcoal[b]	0.027	0.014	0.008	0.004

[a] Used 1 cm cell, reference : subboiling water, [b] Adsorbents.

fied methanol at several wavelengths are shown in *Table* 1. The absorbances were decreased with an order of acidic alumina, charcoal, silica gel and neutral alumina. However, the UV absorbances of the purified methanol were higher than that of the specifications for HPLC grade solvent[7,8].

Since the eluotropic strengths of solvents on alumina and charcoal are reverse[9], the adsorbent pair of neutral alumina and charcoal was employed for the removal of UV impurities. The UV absorbances of the methanol by the two adsorbents were markedly decreased. It was decreased to 0.137 at 225 nm, which was lower than the specification of the HPLC solvent. The purified amount of the methanol was about 55% of the total eluent volume.

The purification efficiency of the acetonitrile showed slightly different trends from that of the methanol. Charcoal had the highest purification efficiency and the neutral alumina showed the next. When two of the adsorbent, neutral alumina and charcoal, were employed, the UV absorbance of acetonitrile at 200 nm was lowered to 0.027. About 60% of purified acetonitrile could be recovered with the adsorbent pair. The UV absorbances of acetonitrile purified by various adsorbents are summarized in *Table* 2.

Fluorescence Impurities. *Fig.* 2 shows the fluorescence spectra of methanol. The fluorescence impurities measured as an equivalent of quinine in the technical grade methanol corresponded to about 1 ppb. The impurities decreased to below 0.1 ppb after it was purified by neutral alumina and charcoal. Fluorescence impurities in acetoni-

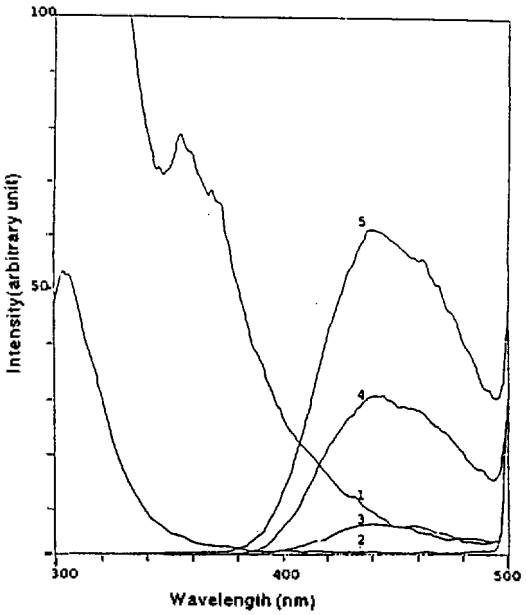

Fig. 2. Fluorescence spectra of methanol.
1. Technical grade, 2. Purified, 3. 1.0 ppb quinine, 4. 5.0 ppb quinine, 5. 10.0 ppb quinine.

trile were decreased from 8 ppb to 0.27 ppb after purification. Fluorescence impurities in methanol and acetonitrile observed at 450 nm and fluorescence maximum wavelengths are listed in *Table* 3.

Other Impurities. The amount of other impurities i.e., water content, titrable acid and base, and residue after evaporation were below the specifications of HPLC solvent, after they were purified by neutral alumina and charcoal. The analytical results are shown in *Table* 4 and 5.

Table 3. Quinine equivalent fluorescence impurities in methanol and acetonitrile (Unit: ppb)

Solvent	Wavelength, nm	
	450	maximum fluorescence
Methanol		
Specification[a]	<0.3	<1.0
Technical	1.00± 0.01	1.90± 0.01
Purified	<0.1	<0.1
Acetonitrile		
Specification[a]	<0.3	<1.0
Technical	7.80± 0.05	1.79± 0.02
Purified	0.17± 0.01	0.85± 0.01

[a] J. T. Baker Inc. for HPLC, Used 1 cm cell.

Organic Impurities were Determined by GC. Technical grade methanol contained 210 ppm of ethanol, 10 ppm of isoamylacetate, and trace of toluene. Technical grade acetonitrile contained 540 ppm of methanol and two unidentified impurities which were estimated to be roughly less than 1000 ppm. Therefore, no further attempt to increase the purity of solvent was made. The assays of methanol and acetonitrile were above 99.9% after water removal by adsorption.

In conclusion, adsorptive chromatography is an effective method for purification of organic solvents for HPLC uses. The method can circumvent tedious chemical pretreatment steps for removal of specific impurities and distillation operation.

REFERENCES

1. E. L. Johnson and R. Stevenson, "Basic Liquid Chromatography", Varian Associates Inc., p. 320, Palo Alto, CA, U.S.A. (1978).
2. S. R. Bakalyar, *Int. Lab.*, **8**, 83(1978).
3. B. Buszewski, R. Lodkowski and J. Trocewicz, *J. of High Res. Chrom. and Chrom. Communications*, **10**, 527(1987).

Table 4. Assay and impurities in methanol

Methanol / Item(unit)	Specification[a]	Technical	Purified
Assay(%)	>99.8	99.85 ± 0.03	99.94 ± 0.03
Water(%)	<0.05	0.068 ± 0.002	0.032 ± 0.002
Acidity[b](meq/g)	<0.0003	0.00040± 0.00002	0.00021± 0.00002
Residue after evaporation(ppm)	<1.0	5.0 ± 0.1	1.0 ± 0.03

[a] J. T. Baker Inc. for HPLC, [b] Determined as an equivalent of HCOOH.

Table 5. Assay and impurities in acetonitrile

Acetonitrile / Item(unit)	Specification[a]	Technical	Purified
Assay(%)	>99.8	99.51 ± 0.03	99.92 ± 0.03
Water(%)	<0.01	0.349 ± 0.006	0.008 ± 0.001
Acidity[b](meq/g)	<0.0008	0.00033± 0.00002	0.00020± 0.00002
Residue after evaporation(ppm)	<1.0	0.90 ± 0.03	0.88 ± 0.03

[a] J. T. Baker Inc. for HPLC, [b] Determined as an equivalent of CH_3COOH.

4. D. D. Perrin and W. L. R. Armarego, "Purification of Laboratory Chemicals", 3rd ed., p. 20, Pergamon Press, Oxford U.K. (1988).
5. J. W. Vogh and J. S. Thomson, *Anal. Chem.*, **53**, 1345(1981).
6. R. A. Valapoldi and K. D. Mielenz, "A Fluorescence Standard Reference Material: quinine sulfate dihydrate", Natl. Meas. Lab. N.B.S., Washington DC. U.S.A., Gov. Rep. Announce Index(U.S.), 80(8), 1293(1980).
7. J. T. Baker Inc., "Laboratory Reagents and Chromatographic Products", 1989/90 Catalog, Phillipsburg, N. J., U.S.A. (1989).
8. Burdick and Jackson Laboratory Co., "High Purity Solvent Guide", p. 74(1984).
9. L. R. Snyder, "Principles of Adsorption Chromatography", Marcel Dekker Inc., p. 192, New York, U.S.A. (1968).