A Study on the Peak Separation of Acetone and Acrolein Based on High-Performance Liquid Chromatography (HPLC) Method

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To resolve the differentiation problem of acetone and acrolein in the analysis of carbonyls by high-performance liquid chromatography (HPLC), we investigated the optimum analytical conditions for their separation. Carbonyl compounds were collected by 2,4-dinitrophenylhydrazine (DNPH)-coated cartridges. We examined the influence of three experimental variables: temperature (25, 30, 40, 50 and 60 °C), flow rate (1.0 and 1.2 mL/min), and relative mobile phase composition (among acetonitrile, water and tetrahydrofuran). The experimental results revealed the optimum analytical condition of a flow rate of 1.2 mL/min, temperature of 32 °C and mobile phase composition of acetonitrile: water: tetrahydrofuran = 34 : 52.8 : 13.2. The analysis of indoor air composition indicated that acrolein and acetone comprised 11% and 42% of all aldehydes, respectively.

Key Words: Acetone, Acrolein, Tetrahydrofuran, Peak separation, HPLC

Introduction

People in modern society tend to spend most of their time indoors. As a result, the demand for advanced building materials and living supplies is increasing, with the potential for the release of harmful toxic compounds directly or indirectly into the living environment. Since these materials tend to be the sources of such harmful substances, indoor air pollution should be considered a threat to human health, even at low concentrations.[1]

Formaldehyde is recognized as the most probable carcinogenic source in indoor environments.[2] For example, formaldehyde is the source of carbonyl compounds, which are usually generated indoors from construction materials, furniture or smoking.[3,4] Carbonyl compounds have different characteristics of molecular structure, solubility, chemical reaction and toxicity. Many kinds of carbonyl compound are receptive and are known to stimulate the upper airway's mucosa, visual nerve and the skin.[5] In particular, formaldehyde and acrolein are known to be highly stimulatory, even at low concentration.[6]

Acrolein is classified as a highly toxic substance that causes severe irritation of the mucous membrane, lungs, and skin. EPA integrated risk information system (IRIS) has classified acrolein in Group C, i.e., a potential carcinogen. Acetone can cause breathing problems, pain of the pleura, pulmonary edema, hypoxia, bronchospasm, continuous decline of the lung function, cellulites when exposed to skin, sepsis, contracture, addiction, and osteomyelitis.[5] Exposure to the eyes can induce a burn and festering of the cornea. Oral exposure can lead to a burn of the gullet, a burn of the stomach, pyloristenosis, and gastrointestinal burns.[5]

Korea's indoor air standard standards currently mandate the measurement and management of only formaldehyde among all the carbonyl compounds, whereas most carbonyls are regulated by the standard of odor regulations of working environments. Extensive research has been conducted on formaldehyde in indoor air, but not on carbonyls.

Acrolein and acetone, when analyzed by high-performance liquid chromatography (HPLC), are difficult to differentiate from the analysis of fixed quantity, due to their similar amounts of molecules and retention times (RT). Therefore, the concentrations of other substances can be misinterpreted or even excluded. This study investigates the characteristics of acetone and acrolein with the goal of determining the optimum analytical conditions for their separation by examining the influence of temperature, flow rate, and relative mobile phase composition.

Experimental method

Analytical conditions of HPLC. In this study, an HPLC system (600s model: Waters Corporation, USA) equipped with a Waters 616 pump and a Waters 486 detector (UV/Vis) was used to analyze the carbonyl compounds along with a MIDAS auto sampler. The analytical conditions of HPLC are indicated in Table 1.

Mobile solvent was used by mixing acetonitrile and water (Mix A), or acetonitrile, water and tetrahydrofuran (THF) (Mix B) in isocratic mode. THF was mixed with water at the ratio of 20 : 80. This mixture was used after 24 hr stabilization. An LC-18 column (5 μm × 25 cm × 4.6 mm) was used. The detected wavelength was fixed at 360 nm. The flow rate of the mobile phase was operated at either 1.0 or 1.2 mL/min.

Separations of acetone and acrolein. The experiment was conducted by varying the flow rate, relative composition of the mobile phase, and temperature. Mix A included 5 different ratios of acetonitrile to water in the mobile phase ((1) 20 : 80, (2) 30 : 70, (3) 35 : 65, (4) 40 : 60, (5) 50 : 50, (6) 60 : 40). Mix B included and fixed up 6 different ratios in the mobile phase (acetonitrile : water : THF = (1) 20 : 64 : 16, (2) 30 : 56 : 14,

Table 1. Analytical Conditions of HPLC

Item	Analysis Condition
Detector	UV/Vis spectrometer (at 360 nm)
Column	Supelco LC-18 column (5 μm × 25 cm × 4.6 mm)
Purge gas	He (99.999)%
Purge gas flow	100 mL/min
Quantity of Injection	20 μL
Run Time	32 min
Elution mode	Isogratic
Mobile Phases	A: Acetonitrile
	B: Water
	C: Tetrahydrofuran
Mobile Phases rate	1) [Mix A] A : B = 20 : 80 ~ 80 : 20
	2) [Mix B] A : B : C = 20 : 64 : 16 ~ 80 : 16 : 64
Column Oven Temperature	25 ~ 60 °C

1) [Mix A] A : B = Acetonitrile : Water. 2) [Mix B] A : B : C = Acetonitrile : Water : Tetrahydrofuran

(3) 35 : 52 : 13, (4) 40 : 48 : 12, (5) 50 : 40 : 10 , (6) 60 : 32 : 8). The optimum condition for peak separation in acetone and acrolein was examined.

Standard solutions (Carb Carbonyl-2,4-dinitrophenylhydrazine (DNPH) Mix) were purchased from Supelco (Bellefonte, PA, USA). The standard contains the following 7 chemicals and concentrations (μg/mL): formaldehyde (215), acetaldehyde (196), acrolein (119), acetone (122), propionaldehyde (122), butyraldehyde (143), and benzaldehyde (185). Table 2 shows the dilution ratio of the standard solution and aldehyde concentration in proportion to the dilution ratio for the calibration curve.

Measurement and analysis of the carbonyl compounds are conform to EPA TO-11A methd that DNPH derivatives (reaction product of carbonyl compounds and 2,4-DNPH (2,4-dinitrophenylhydrazine)) were analyzed by HPLC.

DNPH-silica cartridge is coated with high purity refined 2,4-DNPH in length 4 cm polyprophylen tube (Top trading, Korea). The use of DNPH-coated silica cartridge led to a negative interference on carbonyl concentrations in the presence of ambient ozone. Therefore, cartridge connected to an upstream ozone scrubber (crystalline potassium iodide trap). After collection of the carbonyl compounds on cartridges the DNPH derivatives were separated by HPLC, and keep in cold storage under 4 °C until analyzed.

Results and Discussion

The peaks of acetone and acrolein were not separated in Mix A. However, in Mix B, with the addition of the solvent THF, the acetone and acrolein peaks could be completely separated according to the conditions.

Calibration standard check and detection limit. Figure 1 shows optimum calibration curves when Mixes A and B were used as a mobile phases. The calibration curve was made from 5-step dilution methods (1/200 to 1/4000). Table 3 presents the slope of the calibration curve, R^2, and the standard deviation values. The slope of Mix A was slightly higher than that of Mix B, and both mixtures showed R^2 (0.997).

The reproducibility was evaluated for RT (Ed-this abbrevia-

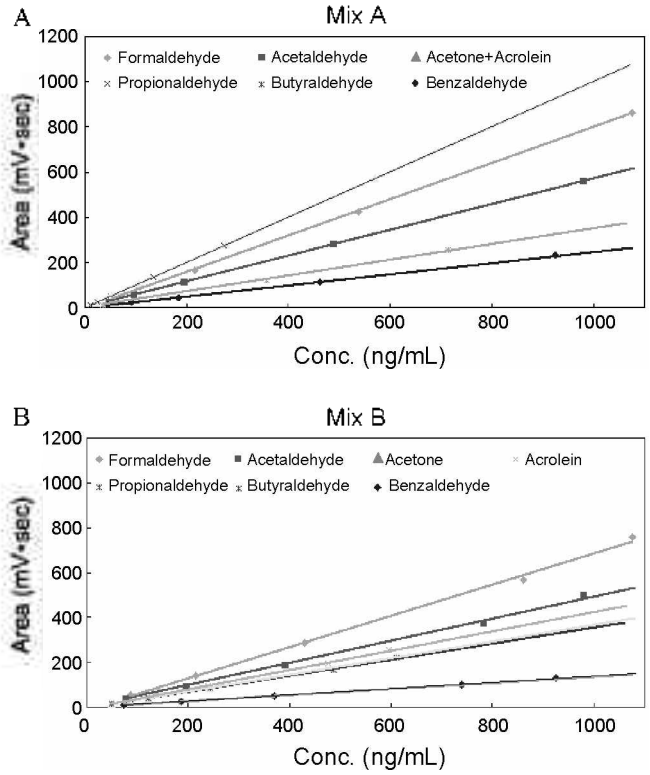


Figure 1. Standard calibration curves of carbonyl compounds for HPLC analysis.

Table 2. Preparation of working standards

Dilution Factor	Primary Standard	Working Standard (ng/mL)						
	ng mL^{-1}	200	250	400	500	1,000	2,500	4,000
Formaldehyde	215000	1,075	860	537.5	430	215	86	53.75
Acetaldehyde	196000	980	784	490	392	196	78.4	49
Acetone	122000	610	488	305	244	122	48.8	30.5
Acrolein	119000	595	476	297.5	238	119	47.6	29.75
Propionaldehyde	122000	610	488	305	244	122	48.8	30.5
Butyraldehyde	143000	715	572	357.5	286	143	57.2	35.75
Benzaldehyde	185000	925	740	462.5	370	185	74	46.25

Table 3. Slopes and determination coefficients of the standard calibration curves for HPLC analysis

	Calibration slope values				Determination coefficients (R^2)			
	Exp 1	Exp 2	Exp 3	Average	Exp 1	Exp 2	Exp 3	Average
1. Mix A								
Formaldehyde	0.803	0.806	0.784	0.798	0.9994	0.9994	0.9999	1.000
Acetaldehyde	0.585	0.586	0.570	0.580	0.9995	0.9993	0.9999	1.000
Acetone	0.472	0.474	0.462	0.469	0.9994	0.9989	0.9996	0.999
Acrolein	0.472	0.474	0.462	0.469	0.9994	0.9989	0.9996	0.999
Propionaldehyde	0.446	0.449	0.438	0.444	0.9991	0.9986	0.9994	0.999
Butyraldehyde	0.353	0.356	0.345	0.352	0.9970	0.9968	0.9990	0.998
Benzaldehyde	0.247	0.248	0.240	0.245	0.9980	0.9966	0.9978	0.997
2. Mix B								
Formaldehyde	0.692	0.685	0.679	0.685	0.9982	0.9941	0.9975	0.997
Acetaldehyde	0.498	0.193	0.486	0.392	0.9982	0.9948	0.9973	0.997
Acetone	0.363	0.362	0.360	0.362	0.9984	0.9958	0.9982	0.997
Acrolein	0.424	0.418	0.417	0.420	0.9984	0.9979	0.9974	0.998
Propionaldehyde	0.361	0.353	0.349	0.354	0.9977	0.9950	0.9968	0.997
Butyraldehyde	0.254	0.243	0.250	0.249	0.9923	0.9864	0.9984	0.992
Benzaldehyde	0.135	0.138	0.136	0.136	0.9974	0.9978	0.9964	0.997

Table 4. The fundamental quality assurance for carbonyl compounds analysis by the HPLC method

[A] Reproducibility of carbonyl compounds analysis by HPLC (n = 3)

Carbonyl compounds	Reproducibility (RSD%)			
	RT		Calibration Slope	
	Mix A[1)]	Mix B[2)]	Mix A[1)]	Mix B[2)]
Formaldehyde	0.972	1.176	1.491	0.256
Acetaldehyde	1.150	1.256	1.540	0.333
Acrolein	0.441	1.542	1.435	0.261
Acetone	0.960	1.529	1.435	0.261
Propionaldehyde	1.700	1.469	1.349	0.407
Butyraldehyde	2.133	1.646	1.601	0.480
Benzaldehyde	1.074	1.983	1.812	0.606

1) Mix A = ACN : Water : THF = 60 : 40 : 0 and 2) Mix B = ACN : Water : THF = 34 : 51.2 : 12.8

[B] Minimum detection limit of carbonyl compounds analyzed by HPLC

Carbonyl compounds	Liq concentration	Absolute mass[a]	Concentration[b]	
	ng/mL	ng	μg/m^3	ppbv
1. Mix A				
Formaldehyde	2.909	0.058	0.808	0.641
Acetaldehyde	4.423	0.088	1.229	0.670
Acetone+Acrolein	3.807	0.076	1.058	0.438
Propionaldehyde	9.557	0.191	2.655	1.099
Butyraldehyde	7.001	0.140	1.945	0.648
Benzaldehyde	13.265	0.265	3.685	0.836
2. Mix B				
Formaldehyde	1.066	0.021	0.296	0.235
Acetaldehyde	2.769	0.055	0.769	0.420
Acetone	2.482	0.050	0.689	0.285
Acrolein	3.295	0.066	0.915	0.392
Propionaldehyde	3.881	0.078	0.078	0.446
Butyraldehyde	2.684	0.054	0.746	0.249
Benzaldehyde	7.033	0.141	1.954	0.443

[a]Estimated from 20 μL sample injection. [b]μg/m^3 is estimated by sampling volume of 18 L and extraction volume of 5 mL, and ppbv is derived by assuming at 25 °C and 1 atm pressure

tion has already been defined above) and calibration slope, which represent the pump performance, to check the level of the errors incurred during the process of aldehyde chemicals analysis. After standard solutions were diluted at 1 mg/L, the relative standard deviations (RSD) were evaluated using the results analyzed in triplicate (Table 4(a)). The RSD values of Mixes A and B for peak RT and calibration slope were 1.9% and 2.2%, respectively. The method detection limit (MDL) of the aldehydes was calculated by multiplying the standard deviation of the diluted standard concentration by 3.14-fold in order to reach the detection limit (DL) level (1/4,000 concentration of standard stock solution). This standard sample analysis process was conducted seven times. The chemical concentrations (ng/mL) in Mixes A and B were formaldehyde (0.9, 0.3), acetaldehyde (1.4, 0.9), acetone (1.2, 0.8), acrolein (1.2, 1.0), propionaldehyde (3.0, 1.2), butyraldehyde (2.2, 0.9), and benzaldehyde (4.2, 2.2), respectively.

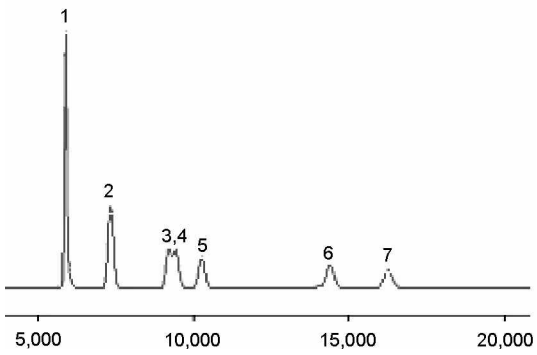
Table 4(b) shows the DL for the aldehyde chemicals of the liquid samples and ambient air concentration based on an actual air sample of 18 L. The reproducibility of Mixes A and B indicated that the RSD of RT was more favorable in the case of Mix A than Mix B, while the reverse was true for the RSD of the linear calibration curve. The MDL of Mix B was lower than that of Mix A.

Peak separation by temperature condition. Firstly, the temperature (25 - 60 °C), and mobile phase composition were controlled equally for both A and B, while the reproducibility of the peak separation was investigated. With Mix A, peak separation didn't occur in any condition, whereas the peaks were separated with reproducibility *via* the appropriate control of temperature, flow rate, and mobile phase composition with Mix B.

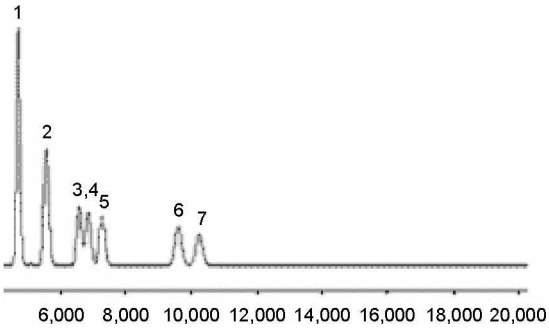
Peak separation by mobile phase condition. Mix A condition (Acetonitrile + Water): Figure 2 shows the chromatography results with Mix A. Figure 2 (a) presents the chromatogram of the standard samples analyzed under HPLC, a method often used as to analyze the general indoor air quality in Korea. The peaks of acetone (#3) and acrolein (#4) were overlapped.

Figure 2(b) ~2(d) presents a typical case of the altered

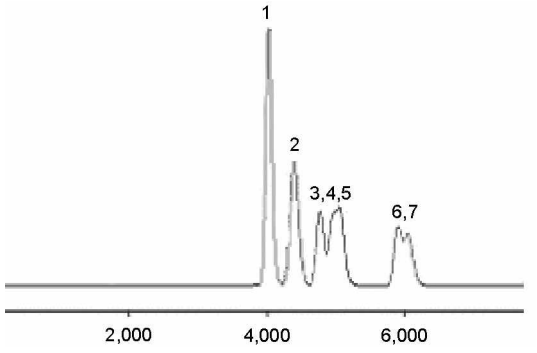
a) Acetonitrile-Water (60 : 40) at 1.0 mL/min, 25 °C



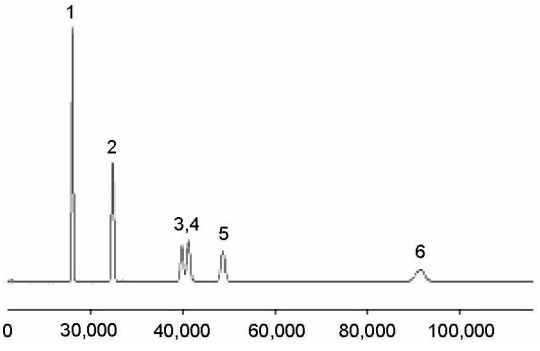
b) Acetonitrile-Water (60 : 40) at 1.0 mL/min, 60 °C



c) Acetonitrile-Water (80 : 20) at 1.0 mL/min, 25 °C



d) Acetonitrile-Water (40 : 60) at 1.0 mL/min, 25 °C



1. Formaldehyde, 2. Acetaldehyde, 3. Acrolein, 4. Acetone, 5. Propionaldehyde, 6. Butyraldehyde, 7. Benzaldehyde

Figure 2. Chromatogram obtained from a standard solution of carbonyl compounds by Mix A types (Acetonitrile + Water).

analytical condition of Figure 2(a). When temperature was higher than of the Figure 2(a) condition, peak separation performance improved, although complete peak isolation was not possible. When the composition rate of acetonitrile was too high (Figure 2(c)), peaks that had been previously isolated became overlapped due to the short RT. With increasing water rate (Figure 2(d)), the analysis process was not completed and the two peaks were not isolated in spite of the expanded RT to 120 min.

As mentioned above, the non-isolated peaks of acetone and acrolein prevent objective analysis of the HPLC findings, depending on individuals. Furthermore, identifying the status of these two substances can be difficult due to their underestimation or overestimation.

Wang *et al.* presented the results of 13 cigarette smoke samples to optimize the HPLC analytical condition for carbonyl compounds analysis. The proposed optimum analytical conditions were Mix A (with a water : acetonitrile : THF : iso-propanol composition of 59 : 30 : 10 : 1) and Mix B (the rate of acetonitrile : water is 65 : 35). The efficiency of Mix B as an efficient analytical condition was not ascertained due to insufficient graphs and explanations, while Mix A was judged to be efficient. However, Wang *et al.*'s study did not include temperature, one of the crucial factors in analytical conditions, that also needs to be carefully considered.[7]

Accordingly, the present experimental focus on the mobile phase composition and column temperature profile has determined the optimum HPLC condition. This finding should be useful for other research. In addition, the concentrations of acetone and acrolein, which were not determined through the existing mobile phase method, were compared quantitatively under the optimum condition of the mobile phase composition of acetonitrile, water and THF.

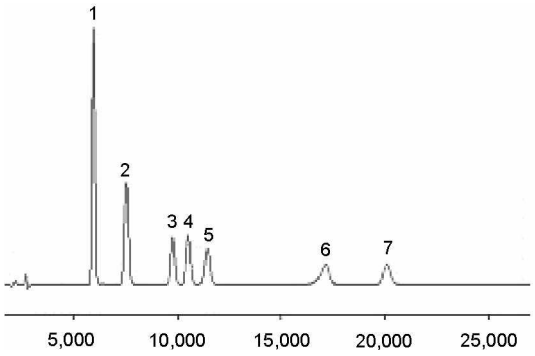
Mix B condition (Acetonitrile+Water +Tetrahydrofuran): Figure 3 represents a chromatogram for Mix B showing that the isolation of acetone and acrolein was done up to the baseline using the three-solvent mobile phase. This separation attributed the result to the improved solubility with the addition of a small amount of THF, leading to minimized RT and the tailing effect.[7]

The sophisticated reproducibility of the resulting chromatogram with the three-solvent mobile phase enabled the optimum condition to be determined as follows : acetonitrile : water : THF composition of 34 : 52.8 : 13.2, temperature of 32 °C, and flow rate of 1.2 mL/min. The isolation efficiency of the acetone and acrolein peaks, which couldn't be separated otherwise, was enhanced by the addition of the solvent THF.

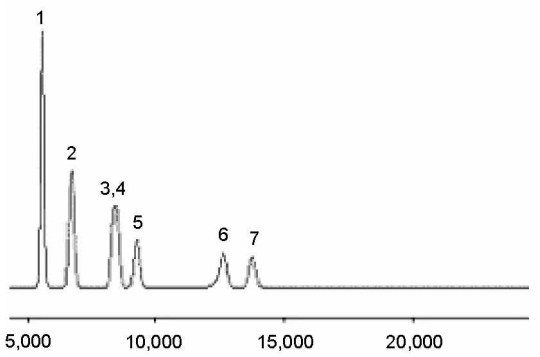
Figure 3(b) ~ 3(d) shows the isolation characteristics at the optimum conditions according to the temperature and mobile phase composition matrix. At the Figure 3(a) condition, the RT was shortened with increasing temperature, as was the case in Figure 3(b), but the acetone and acrolein peaks were overlapped again. This resulted from the highly reduced RT due to the excessively high acetonitrile composition (Figure 3(c)). Additionally, a higher water composition (Figure 3(d)) afforded complete peak isolation, while its efficiency decreased due to prolonged analysis time.

Feng *et al.* performed an experiment on the mobile phase

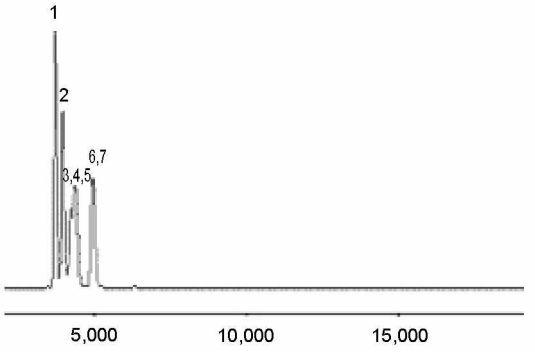
a) Acetonitrile-Water-THF (34 : 52.8 : 13.2) at 1.2 mL/min, 32 °C



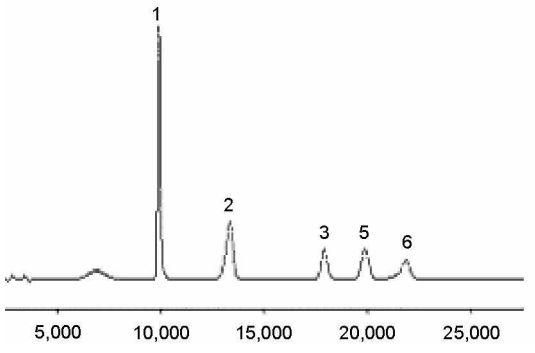
b) Acetonitrile-Water-THF (34 : 52.8 : 13.2) at 1.2 mL/min, 60 °C



c) Acetonitrile-Water-THF (80 : 16 : 4) at 1.0 mL/min, 35 °C



d) Acetonitrile-Water-THF (30 : 56 : 14) at 1.0 mL/min, 30 °C



1. Formaldehyde. 2. Acetaldehyde. 3. Acetone. 4. Acrolein. 5. Propionaldehyde. 6. Butyraldehyde. 7. Benzaldehyde

Figure 3. Chromatogram obtained from a standard solution of carbonyl compounds by Mix B types (Acetonitrile + Water + Tetrahydrofuran).

optimum condition by comparing the results achieved with analyzed capillary electrochromatography (CEC) and HPLC. The acrolein results did not show linearity between using HPLC and gas chromatography (GC), while the CEC method showed good acrolein peak separation efficiency from acetone and propionaldehyde, demonstrating the superiority of CEC.[8]

The present research showed, however, that satisfactory separation efficiency can be achieved by controlling only mobile phase composition (acetonitrile, water, THF) and temperature. This validates the practicality of HPLC performance for carbonyl compounds analysis in indoor air pollutants.

Composition ratio check: Figure 4 shows the peak isolation characteristics of both isolated and non-isolated peaks. This shows that the averaged value of the diluted standard samples (1/200) was analyzed twice. The analyzed results were not significantly affected by the addition of THF, implicating that there was almost no difference in total peak area. Furthermore, the use of THF made the acetone peak appear earlier than that of acrolein in the initial stage of the experiments. Before the peak separation, the peak area and ratio of the combined materials of acetone and acrolein were determined, while the peak area and ratio of the respective material was identified after the separation. The ratio of acetone and acrolein before separation accounted for the 21% of the total area, compared with the ratio of acrolein at 11% and acetone at 10%.

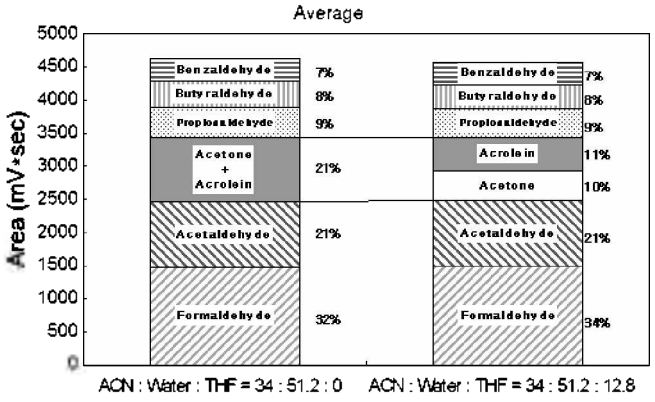


Figure 4. Proportion of carbonyl compounds in standard stock solution for isolated peak and non-isolated peak.

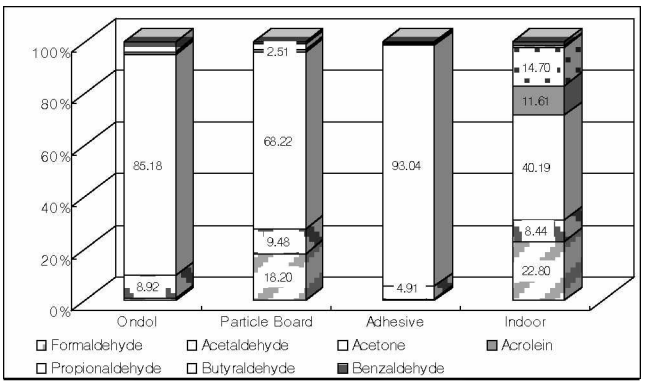


Figure 5. Results of carbonyl compounds emission rates in samples using the DNPH method.

Application for indoor air quality analysis. Figure 5 presents the results of the emission rates of three building materials, Ondol (Korean underfloor heating), particleboard, and adhesive, and of the indoor emission rate, using the DNPH method.

The chemical emission rate ($\mu g/m^3$) was estimated by analyzing the results of 2 ~ 5 samples of 4 materials. The small chamber method was used for emission rate testing (Korea Air Cleaning Association). The collected indoor air sample was 167 mL/min, and the total sample volume was 5 L. Ondol, particleboard, and adhesives, which are available in any market, were used for the experiments. The results are presented according to the averaged values of analysis of 5 Ondol samples, two particleboards and two adhesives. Allowing for the differences in the aldehyde emission characteristics between the various building materials and indoor spaces, the characteristics of two samples collected in an indoor space were compared.

The indoor samples were collected in the 3rd floor of the UOS Engineering Building, with the average value of the two sample results being determined.

The results of measuring the indoor air quality of new apartment houses nationwide in Korea, performed in 2005 by the National Institute of Environmental Research (NIER), Korea, showed that the acetone-acrolein composition comprised the greatest portion in the carbonyl compounds in 801 household samples from 63 buildings. The next highest level was formaldehyde, followed by butyraldehyde.

As presented in Figure 3, in the average carbonyl compositions emitted from the adhesives and flooring materials, acetone occupied the greatest portion, as previously reported in the results of the research in 2005.[1]

In indoor spaces, a greater variety of aldehyde compounds was emitted than in building materials. Similarly in building materials, however, acetone comprised the highest portion, followed by formaldehyde. Acetone and acrolein compositions accounted for about 42% and 11%, respectively. These results suggest that the use of data analysis to gain an understanding of chemical compositions will be very complicated in non-peak separation.

The building materials – Ondol, particleboard, and adhesives – used in the indoor space and 801 samples collected in the indoor space were carefully analyzed, and the applicability of the analytical condition for HPLC was fully considered. The proposed methods showed a more decent shape and RT than the existing mobile phase method did.

Conclusion

In this study, the optimum condition for acrolein and acetone peak separation was determined to be a mobile phase composition of acetonitrile : water : THF of 34 : 52.8 : 13.2, a temperature of 32 °C, and a flow rate of 1.2 mL/min. The calibration curve evaluation result showed decent linearity with R^2 of 0.997 in the optimum condition. From the reproducibility analysis using the slope of the calibration curve and peak retention area, excellent reproducibility was ascertained on the basis of the RSD values. The area and ratio of the standard samples showed little variation in both peak separation and non-separation cases of acetone and acrolein. The exact peak area was determined by the process of peak separation. Of the total integrated area, acrolein accounted for 11% and acetone for 10%.

An indoor air sample was collected on the 3rd floor of the UOS Engineering Building using the DNPH cartridge for analysis. In the result, acrolein accounted for about 11% and acetone 42% out of the total. This confirmed the necessity of separating the acetone and acrolein peaks in order to understand the precise status of each element in the air. Further research with more diverse materials in more various spaces will be required to confirm the accuracy of the method.

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