Degradation Products Generated by Sonication of Benzyl Alcohol, a Sample Preparation Solvent for the Determination of Residual Solvents in Pharmaceutical Bulks, on Capillary Gas Chromatography

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Benzyl alcohol used as the sample preparation solvent in the determination of residual solvents in pharmaceutical bulks yielded benzene, toluene, and benzaldehyde on capillary gas chromatography (GC) by sonication. The factors responsible for compounds generated are discussed. The quality of benzyl alcohol and the type of sonicator were not involved in the generation of benzene, toluene, and benzaldehyde, whereas matrix contributions were observed. The degradation profiles of benzyl alcohol and its analogous compounds obtained by pyrolysis-GC/mass spectrometric analysis were similar to those obtained by sonication, suggesting that benzyl alcohol is degraded by the high local heat generated by sonication. Consequently, no matter how long it may take to dissolve bulk substances in benzyl alcohol completely, we do not recommend the use of a sonicator in sample preparation for the determination of residual solvents in pharmaceutical bulks.

Key words benzyl alcohol; residual solvent; pharmaceutical bulk; capillary gas chromatography; sonication; pyrolysis-GC/mass spectrometric analysis

The determination of residual solvents in pharmaceutical bulks is very important for the development of pharmaceuticals due to the toxicity of some types of solvent. In addition to this main reason, residual solvents may affect the physicochemical properties and stability of pharmaceutical products. For the determination of residual solvents, gas chromatography (GC) as described in standards USP 24 and EP 3 has been widely used as the most appropriate of a number of generalized methods. In the ICH guidelines, limits for residual solvents in pharmaceutical bulks are described in detail, although the determination method is not described.[1] Furthermore, JP 13 Supplement No. 2, newly revised to include a residual solvent test, does not include a definitive determination method.

Sampling techniques in GC contain direct injection (USP) and headspace sampling (USP and EP). Direct injection is the preferred method because it is simple and requires standard GC equipment. Dissolution of a bulk substance in a suitable solvent and direct injection of this solution onto the GC column are both rapid and convenient, and can readily be automated. Benzyl alcohol is generally used as a solvent because its boiling point is higher than that of the objective residual solvents used as analytes.[2—5]

To dissolve bulk substances into benzyl alcohol rapidly, they are usually irradiated with ultrasonic waves. We have found that benzene and toluene are generated by sonicating benzyl alcohol solution of bulk substances in the residual solvent test. This appears to be a serious hindrance for the performance of the test.

This report describes the identification of compounds generated by the sonication of benzyl alcohol and speculation on the mechanism by which these compounds are generated based on the degradation profile obtained by pyrolysis-GC/mass spectrometric analysis.

Experimental

Materials and Reagents All pharmaceutical bulks (compounds A—E) were prepared in-house by Takeda Chemical Industries, Ltd. (Osaka, Japan). All solvents were of reagent grade and obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan) unless stated otherwise. Benzyl alcohol obtained was of 97.0 and 99.5% purity as determined by GC, in addition to the reagent grade (99.0%).

Gas Chromatographic Analysis Quantitative analysis was performed on a Shimadzu Model GC-15A gas chromatograph equipped with a flame ionization detector and a Shimadzu Model AOC-17 auto injector. Samples were injected into the gas chromatograph in the direct mode. The column used was a 30 m×0.53 mm i.d. fused silica capillary column coated with 5.0 μm film of 5% diphenyl, 95% dimethylsiloxane (SPB-5, Supelco Co., Ltd., Bellefonte, PA, U.S.A.) coupled to a 5 m×0.53 mm i.d. fused silica column coated with 0.5 μm film of SPB-5 as a guard column. The carrier gas was helium, and the average column linear velocity determined by injections of methane was about 35 cm/sec. Nitrogen was used as the make-up gas at a flow rate of 50 ml/min. The injector and detector temperatures were 140 °C and 260 °C, respectively. The column temperature was programmed at 35 °C for 10 min, increased to 175 °C at 4 °C/min, then to 260 °C at 35 °C/min, and finally maintained at 260 °C for at least 16 min. The injection volume was 1.0 μl.

GC/Mass Spectrometry (MS) A Shimadzu Model QP5050A GC/MS system with an electron-impact ion source was used. The column used was a 30 m×0.25 mm i.d. fused silica capillary column coated with a 5.0 μm film of 5% diphenyl, 95% dimethylsiloxane (DB-5, J&W Scientific Co., Ltd., Folsom, CA, U.S.A.). The split-injection mode was used with an approximate splitting ratio of 1 : 50. The carrier gas, avarage column linear velocity, and injection temperature were the same as in the GC (FID) conditions described above. The column temperature was programmed at 35 °C for 5 min, increased to 175 °C at 8 °C/min, then to 260 °C at 35 °C/min, and finally maintained at 260 °C for at least 16 min. Mass spectra were recorded under an electron-impact (EI) ionization mode at 70 eV from *m/z* 35 to 200, and the ion source was maintained at 280 °C.

Pyrolysis-GC/MS A vertical furnace-type Double Shot Pyrolyser (Frontier Lab Co., Ltd., Koriyama, Japan) mounted on top of the GC/MS injection port mentioned above was used. The GC and MS conditions were similar to those in the GC/MS analysis mentioned above. Several tens of micrograms of solvents placed inside the sample holder were pyrolysed at 500 °C for 1 min.

Determination of Residual Solvents Approximately 0.1 g of a bulk substance weighed accurately was placed into a 5-ml volumetric flask, dissolved in and diluted to volume with benzyl alcohol, and mixed. Standard

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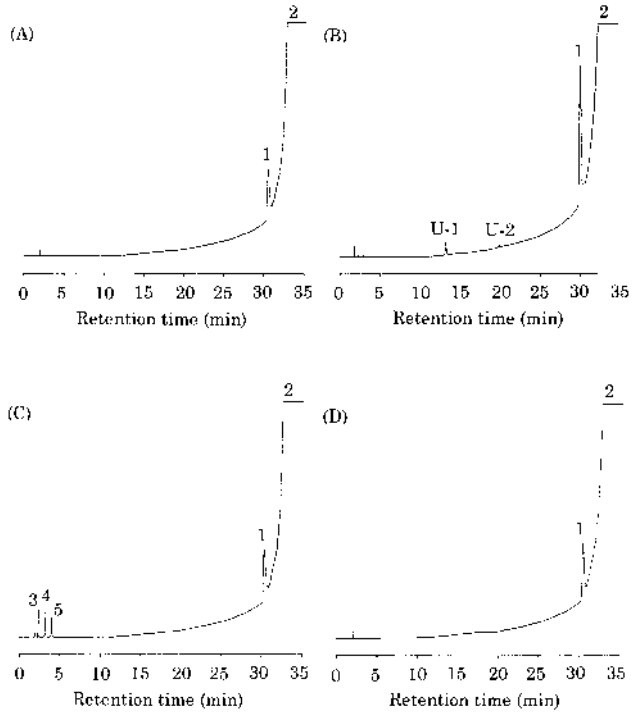

Fig. 1. Gas Chromatographic Profiles from Determination of Residual Solvent in Pharmaceutical Bulk

(A) Sample prepared by dissolving pharmaceutical bulk in benzyl alcohol with shaking; (B) sample prepared with sonication; (C) standard containing methanol, ethanol, and 2-propanol 250 ppm each; (D) benzyl alcohol (sample preparation solvent). 1: Benzaldehyde; 2: benzyl alcohol; 3: methanol; 4: ethanol; 5: 2-propanol.

solvents were prepared by accurately weighing approximately 1.0 g of appropriate solvents into a 20-ml volumetric flask, diluting to volume with benzyl alcohol, and mixing. These solutions were accurately diluted 10000 times with benzyl alcohol, which corresponds to solvent level of 250 ppm by weight in the bulk substance. An equal volume (about 1 μl) of the sample and standard was separately subjected to GC or GC/MS analysis.

Ultrasonic Irradiation Five milliliters solvent or solution of bulk substances was placed into a Pyrex test tube clamped in the center of the sonicators (SONO Cleaner Za 200 [38 kHz, 200 W], Za 100a [38 kHz, 100 W], or Za 100 [25 kHz, 100 W], Kaijo Co., Ltd., Tokyo, Japan) and irradiated with ultrasonic waves. The level of the solution in the tube was maintained at the same as that of water in the sonicator to obtain reproducible sonochemical yields. The temperature of the water in the sonicator was maintained at approximately 25 °C. Standard samples were prepared in the same manner described for the determination of residual solvents, which corresponds to a solvent level of 250 ppm by weight in the bulk substance. An equal volume (about 1 μl) of the sonicated sample or solvent and standard was separately subjected to GC or GC/MS analysis. The amount of generated compounds corresponding to the concentration (ppm) in the bulk substance was calculated using the following equation:

$$\text{concentration of compounds generated (ppm)} = \frac{A_{Ti}}{A_{Si}} \times W_{Si} \times 250$$

where A_{Ti} and A_{Si} represent the peak area responses of the compounds in the sample and standard, respectively. W_{Si} is the sampling amount of intended compounds in the standard.

Results and Discussion

Figure 1 shows typical gas chromatograms of samples and standard along with benzyl alcohol as the sample preparation solvent. Figures 1A and 1B are chromatograms of samples prepared by dissolving a pharmaceutical bulk in benzyl alcohol with shaking and sonicating (38 kHz, 200 W), respectively. Figure 1C is a chromatogram of the standard containing methanol, ethanol, and 2-propanol used for purification and recrystallization of the bulk. Although none of these solvents in the standard was detected in either sample, two unknown peaks, U-1 and U-2, were observed in the sonicated sample. This phenomenon was also seen when benzyl alcohol was sonicated alone, as shown in Fig. 2.

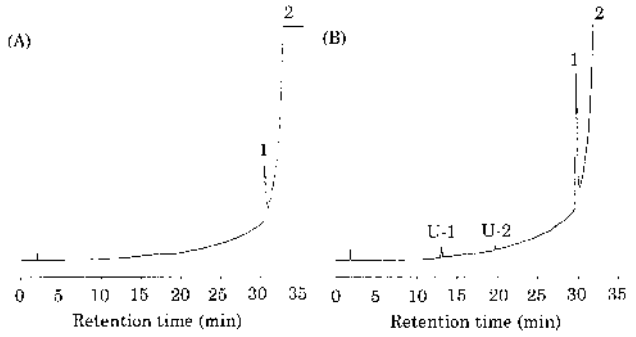

Fig. 2. Gas Chromatographic Profiles of Benzyl Alcohol

(A) Intact; (B) sonicated. 1: Benzaldehyde; 2: benzyl alcohol.

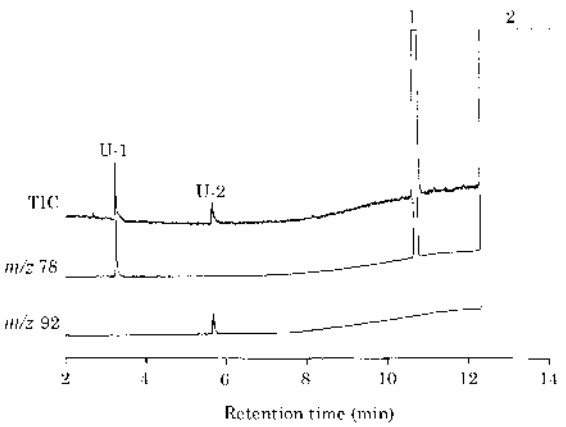

Fig. 3. Mass Chromatograms of Total Ions, *m/z* 78, and *m/z* 92 for Sonicated Benzyl Alcohol

1: Benzaldehyde; 2: benzyl alcohol.

The chemical structures of U-1 and U-2 were elucidated by EI-MS. The mass spectra showed characteristic ion peaks due to ionization and fragmentation, and U-1 and U-2 were easily identified as benzene and toluene, respectively. Furthermore, these mass spectra were in good agreement with those of the benzene and toluene standard, respectively (Figs. 3 and 4).

Figure 5 shows the time courses of concentrations of these compounds generated by the sonication of benzyl alcohol. Five replicate experiments revealed that benzene, toluene, and benzaldehyde were generated almost in proportion to the sonication period and reached 110 ppm, 38 ppm, and 1030 ppm, respectively, at 30 min. Therefore, to study the generation of these compounds as described below, the operating conditions were set at 38 kHz and 200 W for 30 min as standard conditions.

Factors in Generation Initially, the effect of the purity of benzyl alcohol on the amount of compounds generated by sonication was examined. As shown in Table 1, no difference in the amount of compound generated was observed by sonication of three different commercially available grades of benzyl alcohol under the standard conditions.

Table 2 shows the results of effect of bulk matrices in ben-

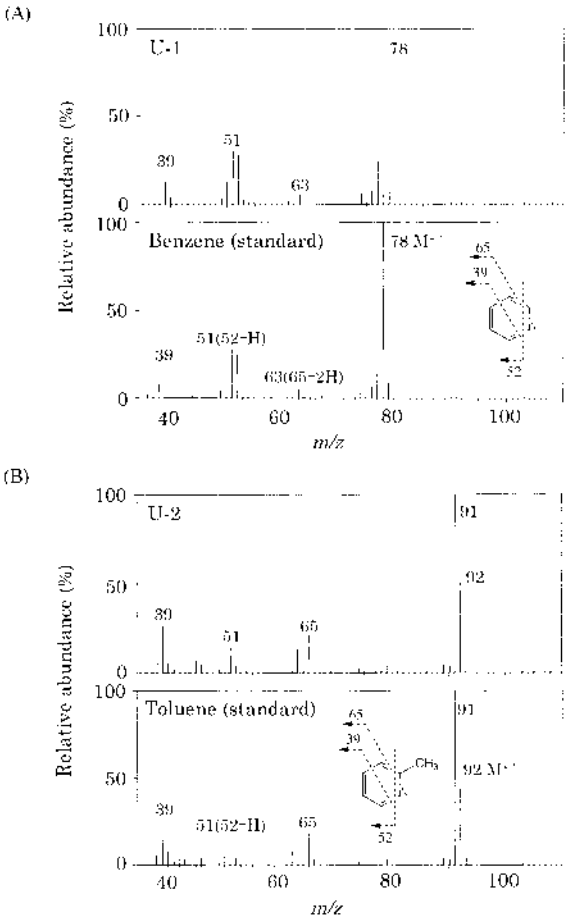

Fig. 4. Mass Spectra of U-1 (A) and U-2 (B) Generated by Sonication of Benzyl Alcohol

zyl alcohol on the amount of compounds generated. The amount of compounds generated by the sonication of benzyl alcohol in the presence of bulk substances tended to decrease compared with that in the absence of drug matrix. In addition, the amount of compounds generated was independent of the type of sonicator with power of 28 to 38 kHz and 100 to 200 W, as shown in Table 3.

Generation Mechanism Commercially available benzyl alcohol may contain impurities undetected by GC, with the exception of benzaldehyde. Therefore several approaches were used to determine whether benzyl alcohol itself or its impurities degrade into benzene and toluene. The sonication of benzaldehyde under the standard conditions yielded neither benzene nor toluene. Furthermore, the amount of compounds generated by the sonication of benzyl alcohol containing 1% benzaldehyde was not greater than that of benzyl alcohol alone. These results indicate that degradation products were not derived from benzaldehyde (Chart 1). Additionally, when benzyl alcohol containing 1% toluene was sonicated, the amount of compounds generated was not greater than that of benzyl alcohol alone, indicating that benzene is not a second degradation product *via* toluene but a primary degradation product of benzyl alcohol (Chart 1). Table 4 summarizes the amount of each degradation product generated by the sonication of some of the solvents mentioned above.

It is accepted that ultrasonic irradiation of a liquid medium

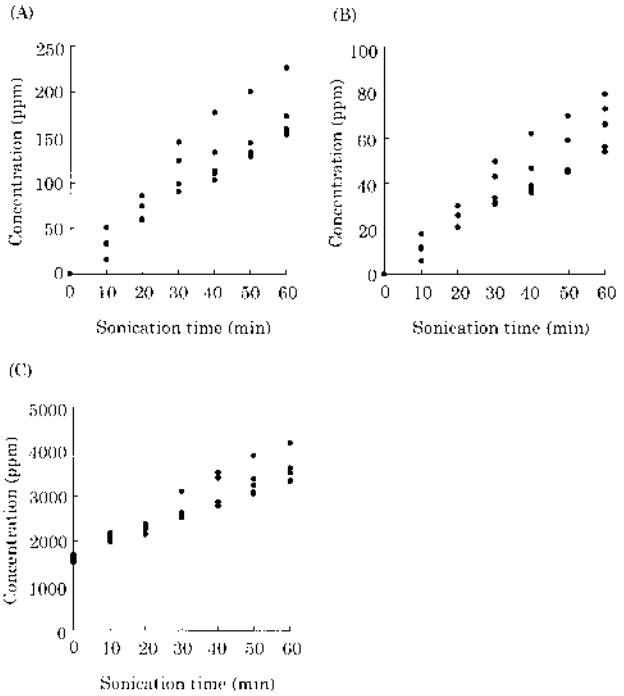

Fig. 5. Time Course of Concentration of Generated Compounds
(A) Benzene; (B) toluene; (C) benzaldehyde.

Table 1. Concentration of Compounds Generated by the Sonication of Various Grades of Benzyl Alcohol

Grade	Compound generated (ppm)		Av. (min.—max.)[a]
	Benzene	Toluene	Benzaldehyde
97.0% Purity	115 (69—193)	33 (24—49)	712 (443—1187)
99.0% Purity	110 (91—145)	38 (31—50)	1029 (816—1473)
99.5% Purity	130 (60—254)	37 (18—74)	771 (380—1146)

a) Results of five determinations.

Table 2. Concentration of Compounds Generated by the Sonication of Benzyl Alcohol Containing Various Drug Substances

Matrix	Compound generated (ppm)		Av. (min.—max.)[a]
	Benzene	Toluene	Benzaldehyde
No bulk substance	110 (91—145)	38 (31—50)	1029 (816—1473)
Bulk substance A	100 (83—135)	29 (25—37)	602 (464—842)
Bulk substance B	78 (58—122)	32 (23—61)	458 (353—573)
Bulk substance C	71 (53—116)	22 (17—27)	459 (288—550)
Bulk substance D	68 (42—95)	23 (19—28)	622 (501—775)
Bulk substance E	62 (47—84)	26 (20—34)	422 (316—503)

a) Results of five determinations.

Table 3. Concentration of Compounds Generated by the Sonication of Benzyl Alcohol with Various Sonicators

Sonicator	Compound generated (ppm)		Av. (min.—max.)[a]
	Benzene	Toluene	Benzaldehyde
38 kHz, 200 W	110 (91—145)	38 (31—50)	1029 (816—1473)
38 kHz, 100 W	110 (68—135)	31 (22—38)	1079 (716—1359)
28 kHz, 100 W	93 (66—115)	29 (25—33)	1186 (747—1609)

a) Results of five determinations.

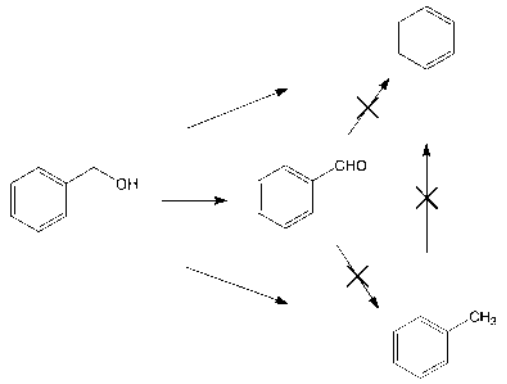

Chart 1. Estimated Degradation Pathway of Benzyl Alcohol by Sonication

Table 4. Concentration of Compounds Generated by the Sonication of Various Solvents

Solvent	Compound generated (ppm)		Av. (min.—max.)[a]
	Benzene	Toluene	Benzaldehyde
Benzyl alcohol	110 (91—145)	38 (31—50)	1029 (816—1473)
Benzaldehyde	0 (0—0)	0 (0—0)	—
1% Benzaldehyde[b]	68 (39—101)	18 (9—32)	—
1% Toluene[b]	22 (12—32)	—	1362 (1110—1850)

a) Results of five determinations. *b*) Added to benzyl alcohol. Data indicated by — are not applicable.

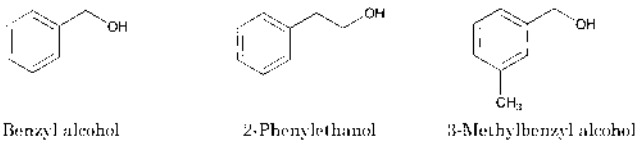

Chart 2. Structure of Benzyl Alcohol Analogues

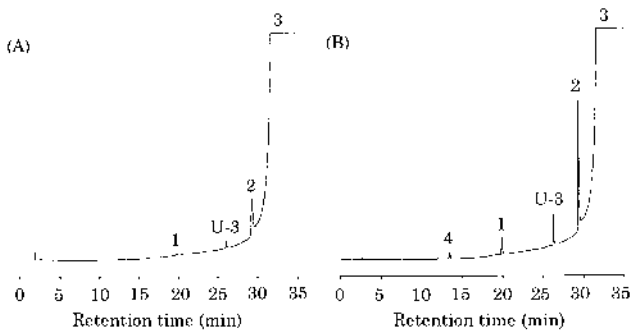

Fig. 6. Gas Chromatographic Profiles of 2-Phenylethanol

(A) Intact; (B) sonicated. 1: Toluene; 2: benzaldehyde; 3: 2-phenylethanol; 4: benzene.

causes acoustic cavitation: the formation, growth, and implosive collapse of bubbles. When the cavity implodes, an enormous amount of local heat energy is generated, and peak temperatures of several thousands of degrees Celsius have been predicted.[6—8] Many synthetic or degradation studies of organic compounds in liquid medium using this energy have been reported,[9—12] although there are only a few reports on the degradation of organic solvents themselves.[13]

To investigate the orientation of substances, 2-phenylethanol and 3-methylbenzyl alcohol (Chart 2), which have structures similar to that of benzyl alcohol, were sonicated under the standard conditions. When 2-phenylethanol was sonicated under the standard conditions, toluene, benzaldehyde, and the unknown compound U-3 in trace amounts were observed, as shown in Fig. 6. U-3 was identified as styrene by its mass spectrum (Fig. 7). On the other hand, sonication of 3-methylbenzyl alcohol yielded only toluene, which is

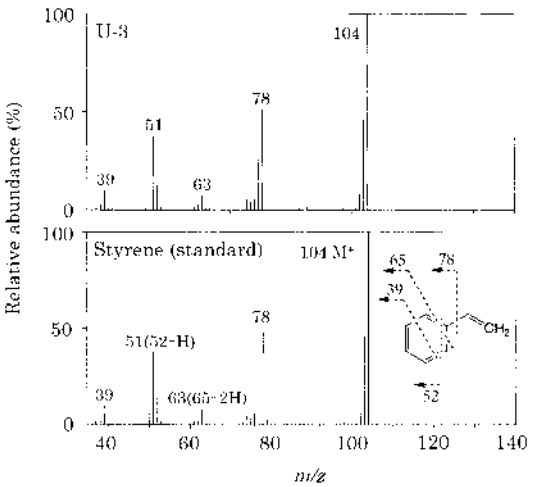

Fig. 7. Mass Spectrum of U-3 Generated by Sonication of 2-Phenylethanol

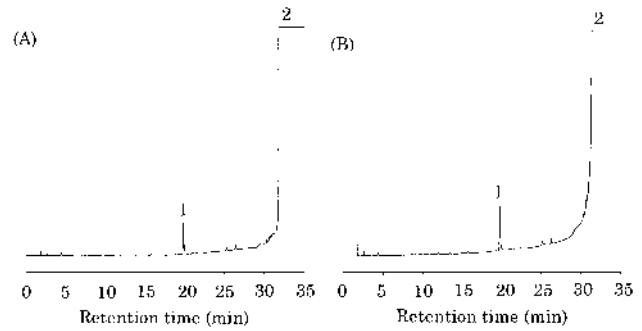

Fig. 8. Gas Chromatographic Profiles of 3-Methylbenzyl Alcohol

(A) Intact; (B) sonicated. 1: Toluene; 2: 3-methylbenzyl alcohol.

Table 5. Concentration of Compounds Generated by the Sonication of 2-Phenylethanol and 3-Methylbenzyl Alcohol

Solvent	Compound generated (ppm)		Av. (min.—max.)[a]	
	Benzene	Toluene	Styrene	Benzaldehyde
2-Phenylethanol	127 (87—161)	229 (142—300)	145 (54—220)	203 (115—309)
3-Methylbenzyl alcohol	0 (0—0)	51 (29—84)	0 (0—0)	—

a) Results of five determinations. Datum indicated by — is not applicable because the benzaldehyde peak overlapped with the 3-methylbenzyl alcohol peak.

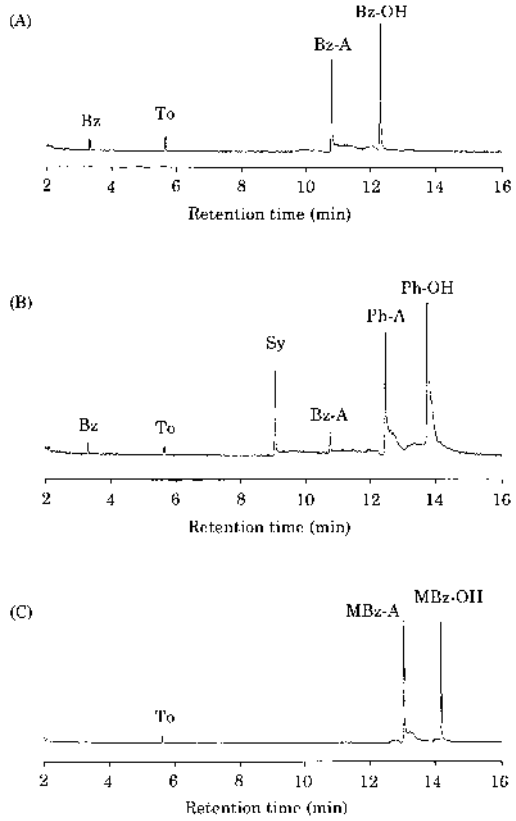

Fig. 9. Pyrograms of Benzyl Alcohol (A), 2-Phenylethanol (B), and 3-Methylbenzyl Alcohol (C)

Bz: Benzene; To: toluene; Bz-A: benzaldehyde; Bz-OH: benzyl alcohol; Sy: styrene; Ph-A: phenylacetaldehyde; Ph-OH: 2-phenylethanol; MBz-A: 3-methyl benzaldehyde; MBz-OH: 3-methylbenzyl alcohol.

contained initially (Fig. 8). The amount of each degradation product is summarized in Table 5.

These results indicate that not only benzyl alcohol but also 2-phenylethanol and 3-methylbenzyl alcohol yield degradation products characterized by each parent structure. This suggests that benzene, toluene, and benzaldehyde generated by the sonication of benzyl alcohol are degradation products of benzyl alcohol itself.

Pyrolysis-GC/MS Figure 9 shows the pyrograms for benzyl alcohol, 2-phenylethanol, and 3-methylbenzyl alcohol. The mass spectra of each peak revealed that the pyrolytic products of benzyl alcohol were benzene, toluene, and benzaldehyde (Fig. 9A), which were identical with the products generated by the sonication of benzyl alcohol. In addition, the pyrolysis of 2-phenylethanol and 3-methylbenzyl alcohol yielded the corresponding products by sonication, expect for phenylacetaldehyde and 3-methyl benzaldehyde, which overlapped with 2-phenylethanol and 3-methylbenzyl alcohol, respectively, in the GC (FID) conditions (Figs. 9B and 9C). The good agreement between the products generated by sonication and pyrolysis suggests that the enormous amount of local heating generated by sonication is one factor responsible for the degradation of benzyl alcohol as well as of 2-phenylethanol and 3-methylbenzyl alcohol.

Conclusions

Sonochemical degradation of benzyl alcohol, the sample preparation solvent for the determination of residual solvents in pharmaceutical bulks, was studied using capillary GC. Benzene, toluene, and benzaldehyde were generated at about 110 ppm, 38 ppm, and 1030 ppm, respectively, by the sonication of benzyl alcohol at 38 kHz and 200 W for 30 min. Although the amount of these compounds was independent of the quality of benzyl alcohol and the type of sonicator (28 to 38 kHz and 100 to 200 W), it increased in proportion to the length of the sonication period and decreased slightly in the presence of bulk matrices. Good agreement between the degradation products of the sonication and pyrolysis of benzyl alcohol as well as its structurally analogous compounds was observed, suggesting that these compounds are degraded by the enormous local heat generated by sonication. According to the ICH guidelines for residual solvents,[1] benzene belongs to the Class 1 toxic solvents which should be avoided, and its limit is set at 2 ppm. Consequently, no matter how long it may take to dissolve bulk substances in benzyl alcohol completely, we do not recommend the use of a sonicator for the sample preparation in the determination of residual solvents in pharmaceutical bulks.

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References and Notes

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