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Quantitative determination of benzalkonium chloride in treated wood by solid-phase extraction followed by liquid chromatography with ultraviolet detection

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Abstract

Ammoniacal copper quat (ACQ) compound wood preservative is comprised of copper and quaternary ammonium compounds with benzalkonium chloride (BAC) as the active ingredient. Solid-phase extraction (SPE) followed by liquid chromatography with ultraviolet detection (LC–UV) was developed for quantitative determination of BAC in treated wood. Five species of wood were used, Japanese cedar (*Cryptomeria japonica*), Japanese larch (*Larix leptolepis*), Yezo spruce (*Picea jezoensis*), Sakhalin fir (*Abies sachalinensis*), and western hemlock (*Tsuga heterophylla*). BAC used in the present study was composed of 66% C12, 33% C14 and less than 1% C16. BAC was added to each wood species (500 mg) then extracted with HCl–ethanol (20 ml) and quantitatively determined with LC–UV (262 nm). Wood extractives from the heartwood of each species, except western hemlock, interfered with quantitative determination of BAC, but SPE with an Oasis MCX cartridge was effective in preventing this. Using the present methods, BAC homologue peaks were clearly confirmed without interference. Recoveries from wood ranged from 92 to 101% and the limit of quantitation was approximately 240 μg/g wood for the C12 and C14 homologues.

Keywords: Solid-phase extraction; Benzalkonium chloride; Wood preservation

1. Introduction

Wood and wood products are frequently used in residential construction, decking, utility poles, and so on. However, wood is attacked by many organisms, principally fungi and termites, resulting in serious strength loss. Consequently, preservation of wood is necessary for inhibition of attack by these degrading agents, particularly when it is used in frequently wetted areas or placed in ground contact.

In recent years, environmental concerns have drastically changed the active ingredient of wood preservatives, resulting in restricted use of chromated copper arsenate (CCA) in many countries. In Japan, use of CCA in wood preservation drastically decreased in 1997 [1] and it was deleted from the revised Japan Industrial Standard on 20 May 2004 [2]. Alternative copper-based preservatives comprised of a combination of copper and organic biocides are now mainly used for wood preservation in Japan.

Ammoniacal copper quat (ACQ), one such copper-based preservative, is comprised of a combination of copper and quaternary ammonium compounds [2] with benzalkonium chloride (BAC) as the active ingredient. Active ingredients of wood preservatives are specified to guarantee protection, thus accurate quantitative determinations of BAC in wood are needed. In Japan, the amount of BAC in treated wood is regulated by the Japanese Agricultural Standard (JAS) [3] and Japan Housing and Wood Technology Center (HOWTEC) [4]. The methods of JAS [3] and HOWTEC [5] involve BAC extraction from treated wood using acidic ethanol followed

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by removal of the extracted BAC in the resulting solution as an ion-pair complex using orange dye. Ultraviolet (UV) spectrophotometry is then used for quantitative measurements of BAC. On the other hand, the method of the American Wood-Preservers' Association (AWPA) is based on two-phase titration [6].

The technical products of BAC consist of C12, C14 and C16 alkyl chain homologues, which possess different physical, chemical and microbiological properties. Consequently, determination of individual homologues is important not only for assuring preservation of wood, but also for investigations requiring quantitative determination. However, the above methods provide no information on the individual BAC homologues, and furthermore, all are thought to interfere with co-extraction of various compounds, because wood, particularly heartwood, contains a number of extractives [7].

Liquid chromatography (LC) has been used for determination of BAC in various fields [8–16]. LC can successfully separate each homologue allowing them to be determined respectively. However, when BAC is extracted from treated wood with organic solvent, co-extracted components in the sample solution might interfere with analysis using LC with ultraviolet (UV) detection (LC–UV). Furthermore, wood extractives are comprised of many components that differ with the species of wood, thus interference will also vary according to species. On the other hand, using mass spectrometry (MS) and tandem mass spectrometry (MS–MS) as detection methods can avoid such interference, since they are highly selective with regard to information on molecular weight. These methods already been applied to determination of BAC in various fields [13–16]. However, LC–MS and LC–MS–MS methods require more sophisticated laboratory equipment than LC–UV. Thus, quantitative determination of BAC in treated wood using LC–UV could be useful if the selectivity of the methods used was increased. To avoid interference and increase selectivity with LC–UV analysis, sample preparation methods such as liquid–liquid extraction are needed to remove wood extractives from the sample solution.

At present, solid-phase extraction (SPE) is the most popular sample preparation method [17–19] and accepted in routine analysis as an alternative to liquid–liquid extraction. To develop the sorbents used in SPE, cartridges with various selectivities are available. We previously reported the applicability of SPE with Oasis MCX in avoiding interference from wood extractives during determination of cyproconazole and tebuconazole [20]. Oasis MCX was able to separate these basic biocides and lipophilic co-extractives, and therefore, we speculated that Oasis MCX could separate BAC from the co-extractives interfering with LC–UV analysis.

The purpose of the present work is to confirm interference by extractives from various wood species during quantitative determinations of BAC using LC–UV, and to develop a SPE method to avoid these confirmed interferences. To our knowledge, this is the first report to document the application of SPE in quantitative determination of BAC in treated wood using LC–UV.

2. Materials and methods

2.1. Reagents

BAC was obtained from Sigma–Aldrich (Tokyo, Japan). The alkyl chain homologues of BAC composed 66% C12, 33% C14 and less than 1% C16. A commercial ACQ product was provided by Koshii Preserving Co. Ltd. (Osaka, Japan) and consisted of C12 and C14 homologues (76:24, w/w). These percentages were confirmed using LC–UV (LC conditions are described below), assuming the same UV sensitivity response with each homologue. High-performance liquid chromatography (HPLC) grade methanol and acetonitrile, ammonium formate and formic acid were purchased from Kanto Kagaku (Tokyo, Japan), and aqueous ammonium hydroxide solution (28%) was obtained from Kishida Chemical (Osaka, Japan). Ammonium chloride and hydrochloric acid (HCl) were obtained from Wako Pure Chemical (Osaka, Japan).

2.2. Sample preparation

Heartwoods of Japanese cedar (*Cryptomeria japonica*), Japanese larch (*Larix leptolepis*), Yezo spruce (*Picea jezoensis*), Sakhalin fir (*Abies sachalinensis*) and western hemlock (*Tsuga heterophylla*) were ground with a Wiley mill then BAC (10 or 1 mg/g wood) as methanol solution was added to the resulting wood powder (500 mg) before drying at room temperature. Five hundred milligrams of spiked and non-spiked wood were extracted with 20 ml HCl–ethanol (3/100, v/v) for 2 h in an ultrasonic bath. Amounts of BAC were within a range (7.4–1.8 mg/g) calculated from JAS's retention amounts for K4 (required retention amount: 5.2 kg/m^3 as ACQ) and K2 (1.3 kg/cm^3 as ACQ) [3], assuming that the specific gravity of wood is about 0.35 g/cm^3 and that ACQ contains copper (as CuO) and BAC at a weight ratio of 1:1. The solutions were filtrated under a vacuum then 5 ml of each was applied to SPE. One milliliter of each filtrate was then evaporated to dryness. The residues were dissolved in mobile phase then filtered through a 0.45 μm membrane for HPLC analysis.

2.3. Treatment of wood blocks with ACQ

Blocks of each wood species (20 mm × 20 mm × 10 mm) were dried for 48 h at 60 °C then stored at room temperature until use. Weighted wood blocks were placed in treating solution including about 0.3% (w/w) of BAC then under 90% vacuum for 20 min. They were kept in the solution at atmospheric pressure and temperature for 2 h. Solution uptake by all blocks was determined by weighting before and after treatment. Treated blocks were dried at 60 °C for

48 h then cut into small particles and extracted as described above.

2.4. Solid-phase extraction

Solid-phase extraction (SPE) was carried out using a 3 ml cartridge packed with 60 mg of 30 μm Oasis MCX (Waters, Tokyo, Japan). The cartridges were set on a 12-position SPE vacuum manifold (Supelco, Tokyo, Japan) and conditioned with 2 ml methanol followed by 2 ml distilled water. Next, 5 ml samples were loaded after dilution with 10 ml distilled water then the cartridges were washed with 3 ml distilled water followed by 3 ml methanol. Finally, the retained compounds were eluted with 4 ml HCl–methanol (3/97, v/v). All SPE steps were performed at a flow rate of ca. 2 ml/min. The eluate was evaporated to dryness then the residue was dissolved in mobile phase and filtered through a 0.45 μm membrane filter. The filtrate was analyzed by LC–UV. NH_4OH (as 28% aqueous solution)–methanol (5/95, v/v) and NH_4Cl (as 28% aqueous solution)–methanol (5/95, v/v) were also used as eluents.

2.5. LC analysis

All LC analyses were performed with an HPLC system consisting of a LC-10AD pump (Shimadzu, Kyoto, Japan), DIL-10AXL auto sample injector (Shimadzu), CTO-10AC column oven (Shimadzu), and SPD-M10AV photodiode array (PDA) detector (Shimadzu). Results were calculated from chromatograms obtained at 262 nm. Peak identification was carried out with confirmation of the UV spectrum (230–300 nm) of the peak. CLASS LC-10 was used for system control, data acquisition, and analysis. The LC column used was an Xtera C18 (5 μm, 150 mm × 4.6 mm I.D. column; Waters, Tokyo, Japan) with a column oven temperature set at 40 °C. The mobile phase was acetonitrile–100 mM ammonium formate buffer, pH 3.5 (70: 30 v/v), with a flow rate of 1.0 ml/min. All samples (20 μl) were injected via the auto sample injector.

2.6. Identification of BAC peaks and quantitative analysis

BAC in the sample solutions was identified by comparing the retention time and UV spectra with that of standard solution. To construct calibration curves, BAC composed of a mixture of C12 and C14 homologues in the mobile phase were prepared at concentrations of 500, 250, 100, 50, 25 and 10 μg/ml. The calibration curves were based on the peak areas of the C12 and C14 homologues at 262 nm, since preliminary experiments showed that BAC in the purchased reagents and commercial ACQ product were mainly comprised of C12 and C14. Standard solutions in mobile phase were filtered through a 0.45 μm membrane filter then the resulting filtrates were analyzed by LC–UV. Correlations between the peak areas of the C12 and C14 homologues and their determined concentrations were determined by linear regression as $r^2 = 0.9999$ and 0.9998, respectively.

3. Results and discussion

3.1. Interference of wood extractives

To confirm the interference of wood extractives in quantitative determination of BAC by LC–UV, we used heartwood because most extractives are located here [7]. The quantitatively determined amounts of BAC co-existing with extractives of each wood are shown in Table 1. At a BAC level of 10 mg/g, the ratios of the quantitative amounts of C12 and C14 homologues to the spiking amounts in extractives of each wood species ranged from 96 to 118% and from 79 to 112%, respectively. The C14 homologue from Sakhalin fir showed the lowest results. These findings show that quantitative determinations of BAC are less interfered with by extractives from each wood species, except Sakhalin fir, at this spiking level.

At a spiking level of 1 mg/g, interference was apparent (Table 1), and miss quantification or high SD values were obtained, except with western hemlock. Matrix or overlapping peaks caused insufficient results as shown in Fig. 1. At

Table 1
Quantitative determination of benzalkonium chloride in wood ($n = 3$ for each species)

	Spiking level, 10 mg/g				Ratio (C12/C14)[b]	Spiking level, 1 mg/g				Ratio (C12/C14)[b]
	C12 homologue		C14 homologue			C12 homologue		C14 homologue		
	Ratio (%)[a]	SD	Ratio (%)[a]	SD		Ratio (%)[a]	SD	Ratio (%)[a]	SD	
Japanese cedar	103	7.8	108	3.7	1.9	72	14.1	166	23.1	0.8
Japanese larch	112	0.7	106	2.9	2.1	122	0.7	91	11.2	2.7
Yezo spruce	118	0.4	103	0.2	2.2	225	9.7	88	8.3	5.0
Sakhalin fir	96	2.2	79	1.6	2.4	132	14.0	81	20.1	3.4
Western hemlock	110	1.4	112	0.8	1.9	111	4.4	116	6.8	1.8

SD, standard deviation.
[a] Ratio of the quantitative amount to the spiking amount.
[b] Calculated with the obtained amounts of C12 and C14.

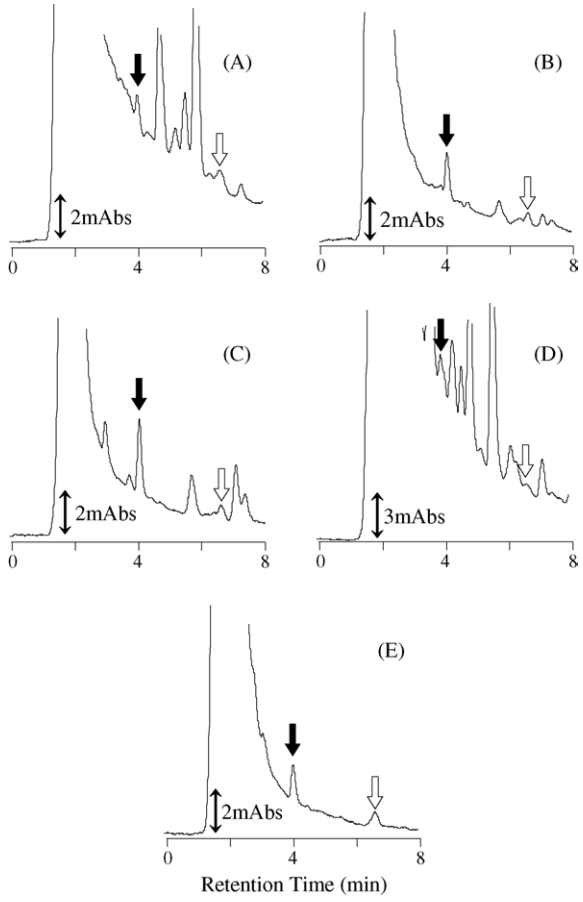

Fig. 1. HPLC chromatograms of HCl–ethanol extracts of (A) Japanese ceder, (B) Japanese larch, (C) Yezo spruce, (D) Sakhalin fir, and (E) western hemlock, which were spiked with benzalkonium chloride at a concentration 1 mg/g. Black arrow: C12, white arrow: C14.

this spiking level, wood extractives interfered with quantitative determination of BAC using LC–UV.

3.2. Clean-up with SPE

Various SPE methods for isolation of BAC have been reported, and in these reports, C18 or PLRP cartridges were used [9,13,14]. However, as mentioned above, determination of BAC is interfered with by wood extractives during LC–UV analysis with the ODS column. Thus, we believed separation of BAC and wood extractives with C18 or PLRP cartridges to be difficult since they also retain compounds with reversed-phase mechanisms. SPE methods employing cation-exchange resin were previously developed for isolation of quaternary ammonium compounds from water or crop samples [21] since they are cationic in nature. BAC is also a quaternary ammonium compound; however, on the other hand, most wood extractives are anionic or neutral. Thus, we examined the use of Oasis MCX cartridges, which contain a mixed mode sorbent with both reversed-phase and strong cation exchange functionalities [18,19], in separation of BAC and wood extractives.

However, BAC was not retained on the MCX sorbent when loaded as an HCl–ethanol solution. The MCX sorbent retains compounds using reversed-phase and cation exchange mechanisms, but retention with these mechanisms is destroyed with organic solvents, such as ethanol, and counter cations, such as H^+, respectively. Thus, BAC dissolved in HCl–ethanol resulted in poor retention. Consequently, to achieve successful retention of the BAC on MCX, the HCl–ethanol solution was diluted with twice the volume of water before loading. Levels of 10 and 1 mg/g BAC showed 101 (SD = 1.2%, $n = 3$) and 98% C12 (SD = 1.1%, $n = 3$) and 101 (SD = 1.8%, $n = 3$) and 95% C14 (SD = 1.7%, $n = 3$) recovery, respectively. These results indicate that dilution with H_2O results in successful retention of BAC on MCX. Thus, dilution with twice the volume of H_2O was used for SPE before loading.

We examined the following eluents in the elution step of SPE: NH_4OH (28% aqueous solution)–methanol (5/95, v/v) and NH_4Cl (28% aqueous solution)–methanol (5/95 v/v), in addition to HCl–methanol, to confirm recovery of BAC with each eluent from MCX loaded with 5 ml methanol solutions containing 1 mg BAC. NH_4OH–methanol solutions are commonly used to elute cationic compounds from cation exchangers [18] and in the case of quaternary ammonium herbicides such as paraquat and diquat, ammonium chloride or HCl–methanol are used as eluents for various cation exchange resins [21]. We confirmed that NH_4OH–methanol could not elute BAC from MCX (less than 1% recovery was observed with 5 ml of this eluent), although the HCl–methanol and NH_4Cl–methanol solutions, on the other hand, eluted BAC from MCX with C12 and C14 homologue recoveries of >98%. In the present study, we therefore used HCl–methanol (3/97) in the elution step of the SPE method.

HCl–methanol and NH_4Cl–methanol achieved successful elution of BAC from MCX. However, on the other hand, NH_4OH–methanol could not completely elute BAC, although the selectivity of common sulfonated resin to NH_4^+ is higher than to H^+, and 28% NH_4OH contains a higher amount of NH_4^+ than 28% NH_4Cl. Thus, Cl^- involved in the mobile phase was thought to be an important factor for recovery of BAC from MCX.

To confirm behaviors of the matrix components, HCl–ethanol extracts of all wood species without BAC were applied to SPE on the MCX cartridge. Most of the interfering components could be eluted from the sorbents by washing the MCX with distilled water followed by methanol; the resulting chromatograms are shown in Fig. 2. The interfering peaks disappeared in all these chromatograms. Fig. 2 also shows the chromatograms of HCl–ethanol extracts of each wood species with BAC at a spiking level of 10 mg/g after SPE. C12 and C14 homologue peaks were identified by comparing the UV spectrum with those of standard BAC homologues. The UV spectra of these peaks matched those of C12 and C14 homologues of standard BAC (Fig. 3), and for all five species, the peaks of the C12 and C14 homologues were clearly confirmed. Fig. 4 shows the chromatograms obtained

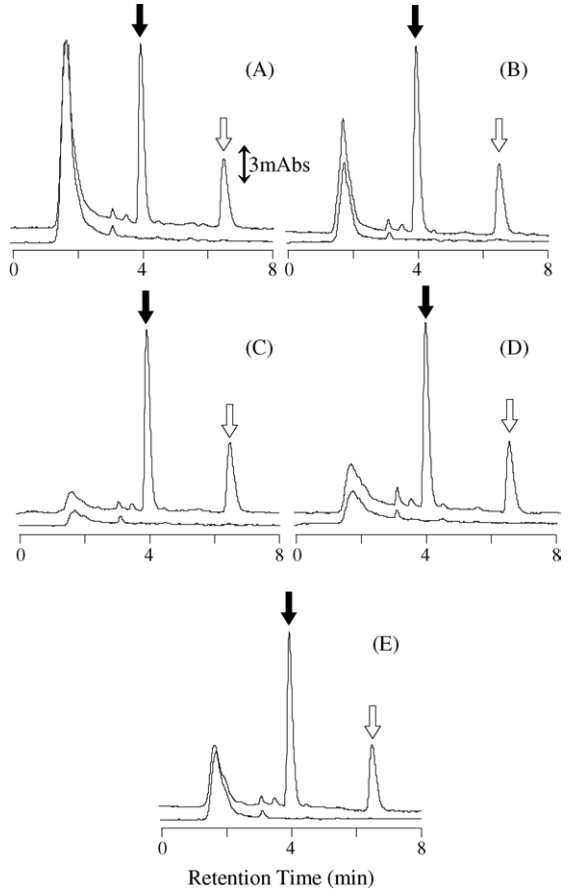

Fig. 2. HPLC chromatograms of HCl–ethanol extracts of wood, and of wood spiked with benzalkonium chloride at a concentration of 10 mg/g, after SPE with MCX. Black arrow: C12, white arrow: C14. See Fig. 1 for key to (A–E).

with a low spiking level of BAC (1 mg/g); peak identification was carried out by comparing their retention times and UV spectra. The recoveries of BAC calculated from these chromatograms are shown in Table 2. At both spiking levels, successful recovery was achieved without breakthrough of BAC from the MCX sorbent, even at the higher spiking level. Based on the results obtained from the lower concentration of BAC, the limit of quantification for C12 and C14 homologues using these methods is approximately 240 μg/g wood.

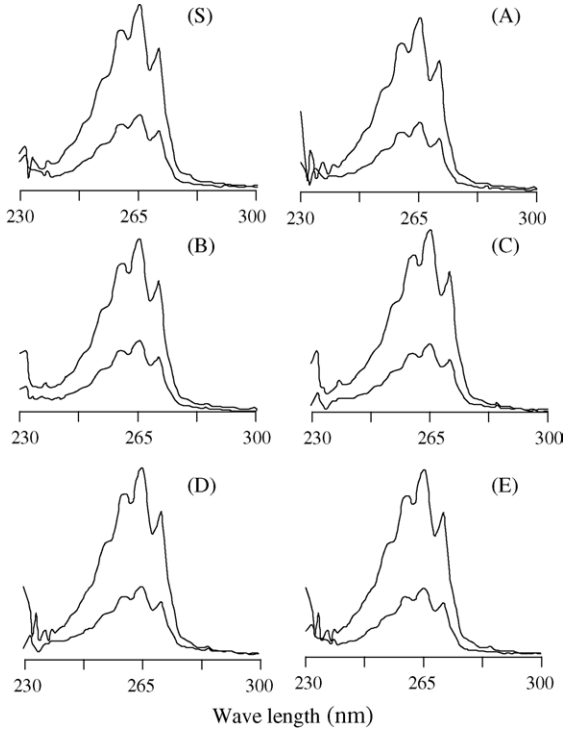

Fig. 3. UV spectra of C12 (upper) and C14 (lower) homologues in each sample solution from benzalkonium chloride (10 mg/g)-spiked wood after SPE. S, standard of benzalkonium chloride. (A) Japanese cedar, (B) Japanese larch, (C) Yezo spruce, (D) Sakhalin fir, and (E) western hemlock.

3.3. Recovery from treated wood blocks

We applied the presented SPE methods to quantitative determination of BAC in wood treated with ACQ using a vacuum procedure (detailed in Section 2.2). Western hemlock was not used in this experiment because its wood extractives scarcely interfered with LC–UV analysis. The peaks of each homologue of BAC were easily confirmed in each chromatogram and the resultant quantitative determination is shown in Table 3. The amounts of BAC in penetrated wood were as follows: Japanese cedar > Sakhalin fir > Yezo spruce > Japanese larch. These results reflect ACQ solution uptake and the difference in uptake between species are perhaps attributed to differences in permeability [22].

Table 2
Quantitative determination of benzalkonium chloride in wood using SPE with MCX ($n = 3$ for each species)

	Spiking level, 10 mg/g				Ratio (C12/C14)[b]	Spiking level, 1 mg/g				Ratio (C12/C14)[b]
	C12 homologue		C14 homologue			C12 homologue		C14 homologue		
	Ratio (%)[a]	SD	Ratio (%)[a]	SD		Ratio (%)[a]	SD	Ratio (%)[a]	SD	
Japanese cedar	98	1.4	96	2.4	2.0	101	5.1	98	6.9	2.0
Japanese larch	100	1.0	99	2.9	2.0	92	3.0	98	6.0	1.9
Yezo spruce	99	0.8	98	3.2	2.0	94	4.7	96	4.0	1.9
Sakhalin fir	101	1.3	98	2.9	2.1	95	3.9	92	3.6	2.0
Western hemlock	98	1.4	96	2.9	2.0	92	2.9	94	4.4	1.9

SD, standard deviation.
[a] Ratio of the quantitative amount to the spiking amount.
[b] Calculated with the obtained amount of C12 and C14.

Table 3
Quantitative determination of benzalkonium chloride in wood blocks penetrated with ACQ using SPE with MCX ($n = 4$ for each species)

	C12 homologue			C14 homologue			Ratio (C12/C14)[a]	Solution uptake (g/block)[c]
	Analyzed (mg/cm^3)	SD	Calculated[b] (mg/cm^3)	Analyzed (mg/cm^3)	SD	Calculated[b] (mg/cm^3)		
Japanese cedar	3.2	0.05	2.0	0.9	0.01	0.6	3.6	3.23
Japanese larch	1.9	0.08	1.3	0.5	0.02	0.4	3.6	2.01
Yezo spruce	2.4	0.07	1.4	0.6	0.01	0.4	3.8	2.26
Sakhalin fir	3.0	0.18	1.9	0.8	0.05	0.6	3.8	3.08

[a] Calculated with the obtained amount of C12 and C14. The ratio of the ACQ treatment solution was 3.3.
[b] Calculated from solution uptake.
[c] Determined by weighting before and after treatment.

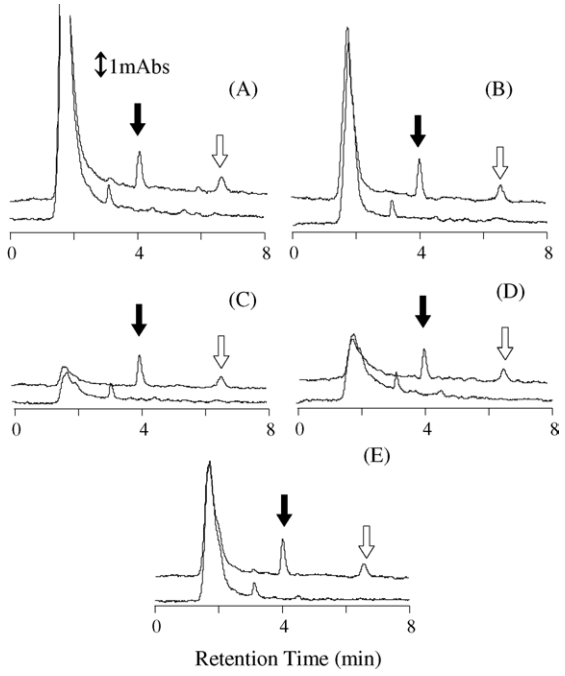

Fig. 4. HPLC chromatograms of HCl–ethanol extracts of wood, and of wood spiked with benzalkonium chloride at a concentration of 1 mg/g, after SPE with MCX. Black arrow: C12, white arrow: C14. See Fig. 1 for key to (A–E).

The amounts of C12 and C14 homologues in all species of wood were higher than the amounts calculated based on ACQ solution uptake. Jin and Preston [23] reported that the affinities of quaternary ammonium compounds towards cellulose, hemicellulose and lignin, major components of wood, are different. Furthermore, each homologue of BAC possesses different physical and chemical properties, and thus, differences in the ratio of these components between or within species [24] might have affected retention of BAC, resulting in these higher resultant amounts of C12 homologues. We are currently in the process of conducting further work using the present SPE methods to confirm these differences further.

4. Conclusion

Interference by extractives of five wood species on quantitative determination of BAC using LC–UV analysis was confirmed, except with western hemlock, particularly at a spiking level of 1 mg/g. Interference was avoided using SPE with MCX, and HCl–ethanol extraction and solid-phase extraction with MCX achieved successful recovery of BAC from ACQ-penetrated wood blocks. With the present methods, BAC homologues (C12 and C14) in preserved wood could be quantitatively determined up to 240 μg/g. Thus, this method seems to be applicable in quality control of preserved wood. In the future, we aim to apply this LC–MS method to quantitative determination of treated wood samples with lower levels of BAC.

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