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Note

Analysis of phenols from lignin depolymerization by capillary gas chromatography

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Lignin degradation by hydrogenation/hydrogenolysis in the absence and presence of catalysts as well as alkaline hydrolysis yields a wide variety of monomeric products. Among these are phenols, guaiacol, syringol, catechol, their respective methyl, ethyl, and propyl derivatives, as well as vanillin and syringaldehyde. The number and abundance of these compounds depend primarily on the severity of conditions of depolymerization utilized, especially the catalyst, but more importantly, the temperature and time of reaction.

The complexity of the product mixtures has been a significant problem, particularly in what concerns the separation and quantification of the monomers. Analysis of the monomeric mixtures by gas chromatography has received wide attention with varying degrees of success.

Clark[1] used paper chromatography to separate phenolic mixtures from lignin degradation. However, the inherent loss of some compounds, especially guaiacol, suggested that this technique is poor for quantitative analysis. Schweers[2] analysed phenolic monomers from hydrogenated lignin by gas chromatography using an SE-30 column, Difficulty was reported with both poor resolution and thermal decomposition of a few products. Scaringelli *et al.*[3] separated phenolic compounds on a Tenax column. High column temperatures (150°C initial oven temperature) were utilized, with the column requiring conditioning at 375°C. Good separation and precision were, however, reported by the authors.

Most of the difficulties encountered in the analysis of phenolic mixtures from lignin depolymerization are due to the presence of compounds containing polar functional groups. These have long been known to be either thermally labile at the temperatures required for separation, or to interact with the column high-boiling liquid phases and inert supports usually available. The interactions, caused by the polar nature and hydrogen-bonding ability of these compounds, lead to their adsorption onto the columns and hence incomplete detection and quantification.

To eluviate these restrictions, derivatization, which is essentially a microchemical synthesis, is carried out to convert the protonic functional groups of such com-

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pounds into non-polar and thermally stable derivatives. The most widely used derivatization methods are silylation and acetylation.

Silylation converts the polar hydroxyl groups in phenolics to their alkylsilyl ethers. The reaction is usually carried out by using the reagent trimethylsilylimidazole at elevated temperatures in the presence of trimethylchlorosilane as catalyst. Clark[4] separated phenolic and catechol compounds after converting them to their trimethylsilylated (TMS) derivatives. Due to steric hindrance, a heating time of 2 h at 150°C was found to be necessary for complete derivatization.

Acetylation introduces the acetyl group by replacing the hydrogen atom(s) of the hydroxyl group(s) attached to the aromatic ring. Treatment with acetic anhydride in the presence of catalytic amounts of pyridine at elevated temperatures leads to a rapid derivatization. However, use of the anhydride can lead to undesirable side reactions with sensitive compounds due to the strong acidity of the medium. Schultz *et al.*[5] hydrogenated HCl lignin and separated the acetylated phenolic products on an OV-17 column. Acetylation was achieved by heating the mixture for 2 h at 60°C. The authors identified fifteen compounds and found that these comprised the majority of the peaks in their chromatograms.

The application of capillary columns to the analysis of phenolic compounds from lignin degradation has not been widely exploited. In the separation of complex fuel-related mixtures, such as gasolines and naphthas, as well as in the separation of essential oils in the flavors and fragrances industries, capillary columns have demonstrated one distinct advantage over the more conventional packed columns, namely, their much higher resolution in shorter analysis times.

In this paper a simple gas chromatographic method for the separation and quantification of phenolic compounds from lignin is described which employs a capillary column. Analytical conditions are reported, which enables the analysis of blends having low as well as high concentrations of phenolic compounds. The method is rapid and offers high precision and accuracy and may be recommended for routine analysis of these compounds from various lignin depolymerization procedures.

EXPERIMENTAL

Materials

The following phenolic compounds were obtained from commercial sources: phenol, *o*-cresol, *p*-cresol, 4-ethylphenol, 4-propylphenol, guaiacol, 4-methyl-, 4-ethyl-, 4-*n*-propylguaiacol, catechol, 4-methyl-, 4-ethylcatechol, syringol, *p*-hydroxybenzaldehyde, vanillin, acetovanillone, syringaldehyde, acetosyringone, and 4-ethylresorcinol (standard). Purified diethyl ether, acetic anhydride and pyridine were purchased from Aldrich.

Preparation and treatment of lignin

Glycol lignin was obtained by thermo-mechano-solvolytic treatment of aspen (*Populus deltoides*) wood using ethylene glycol at 220°C for 4–6 min, followed by dilute acidulation of the spent black liquor. The precipitated lignin was air dried at room temperature and used thereafter.

Alkaline hydrolysis was accomplished in a 500-ml magnetically stirred autoclave. About 5 g of lignin, 2 g of sodium hydroxide and 100 ml of distilled water were

added to the reactor. After testing for leaks by purging with nitrogen, the reactor was brought to 300°C in 7 min by immersing in a preheated salt bath. After treatment for 10 min, the reactor was rapidly cooled by immersing in a cold water bath. The products were separated according to the scheme described in one of our papers[6]. The ether-soluble fraction was analysed for phenols by gas chromatography.

Apparatus

All the data were obtained with a Hewlett-Packard Model 5890 gas chromatography unit equipped with a flame ionization detector. The capillary column, 60 m × 0.25 mm I.D., was a bonded polydimethylsiloxane phase DB-5 (J&W Scientific) purchased from Chromatographic Specialties (Brockville, Canada). The split-injector mode had a split ratio of 120:1 and was maintained at 240°C. The detector temperature was also held at 240°C. High-purity helium, at a constant flow-rate of 1.2 ml/min, was the carrier gas. The recorder connected was a Hewlett-Packard Model 3392 A integrator. A Hamilton 7000 series No. 7001 (Hamilton, Reno, NV, U.S.A.) 1.0-μl syringe was employed for sample injection.

Preparation of sample blends

Phenolic compounds and standard were weighed into a 2.5-ml opaque-colored reaction vial. Derivatization consisted of the following steps: (1) addition of 1.5 ml of acetic anhydride and 1–2 drops of pyridine; (2) sealing the vial and shaking vigorously; (3) heating at 70°C for 1 h in a stirred water bath. The mixture was then cooled to room temperature and directly injected into the gas chromatograph.

Adequacy of the method was verified by the absence of extraneous peaks other than those attributed to the acetylated products in the chromatogram.

Chromatographic procedures

Before injecting a sample, the column was pre-equilibrated with acetic anhydride for 1 h (blank run). After pre-equilibrium, an aliquot of 0.3 μl of the solute mixture was injected into the system thus ensuring that there was no saturation of the column and detector due to large concentrations of phenols which may be present.

The initial column temperature was set at 65°C with no initial temperature hold. The oven temperature programme was as follows: heating rate 6°C/min, hold at 140°C for 10 min, then heated at 4°C/min to 240°C, hold for 10 min, and cooled to 65°C. Duplicate analyses were carried out for each sample.

RESULTS AND DISCUSSION

Qualitative analysis

The acetate derivatives of the phenolic compounds were chromatographed by using several different analysis conditions. A chromatogram of 18 acetylated compounds and the standard obtained under the conditions described is shown in Fig. 1A. These conditions were found to effect the best separation for the compounds considered. Fig. 1B shows the chromatogram of acetylated products obtained from the alkaline hydrolysis of glycol lignin. All the acetylating reagents eluted within 7.8 min.

No peak tailing was evident for all the compounds. However, the peaks corre-

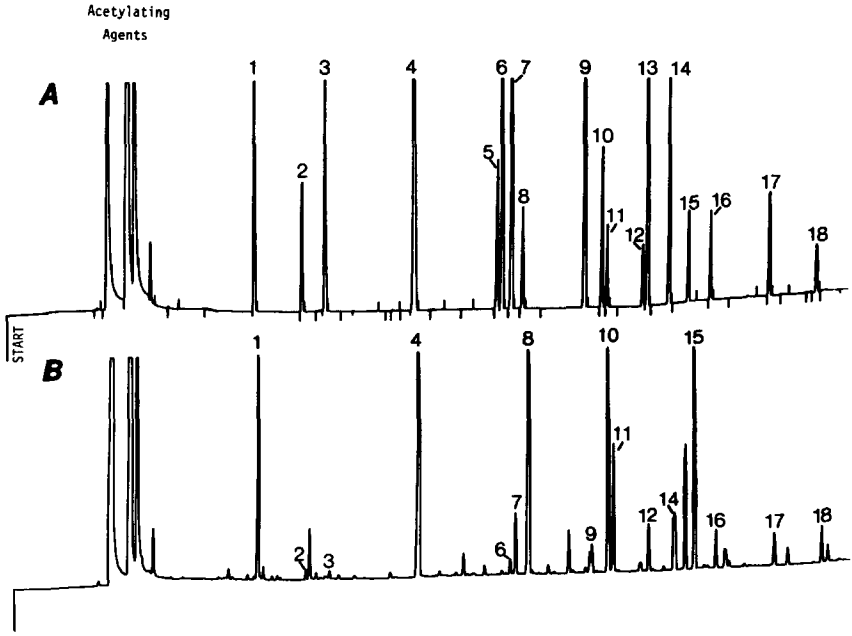

Fig. 1. Chromatograms of (A) standard mixture, and (B) acetylated phenolic products obtained from the hydrolysis of glycol lignin at 300°C, 10 min reaction time in 2% sodium hydroxide solution. Peaks (acetates): 1 = Phenol; 2 = *o*-cresol; 3 = *p*-cresol; 4 = 4-ethylphenol and/or guaiacol; 5 = *p*-hydroxybenzaldehyde; 6 = 4-*n*-propylphenol; 7 = 4-methylguaiacol; 8 = catechol; 9 = 4-ethylguaiacol; 10 = syringol; 11 = 4-methylcatechol; 12 = vanillin; 13 = 4-*n*-propylguaiacol; 14 = 4-ethylcatechol; 15 = 4-ethylresorcinol (standard); 16 = acetovanillone; 17 = syringaldehyde; 18 = acetosyringone.

sponding to the acetates of guaiacol and 4-ethylphenol overlapped. For subsequent quantification of these two compounds, standard blends were separately prepared and chromatographed for each one. Increased resolution may be feasible by adjusting the programmed temperature rate and/or the initial oven temperature. Tests indicated that changing the initial oven temperature is more effective. Due to apparatus limitations, our initial oven temperature could not be adjusted to lower than 65°C.

Quantitative analysis

For quantitative analysis, 4-ethylresorcinol was used as internal standard. The choice of internal standard was primarily based on its absence in products from lignin depolymerization by alkaline hydrolysis (which we verified), as well as from published work on lignin degradation that we have seen to date. Also, for the DB-5 column the peak of the acetate derivative of 4-ethylresorcinol was seen to be well separated from the other peaks with no overlapping, its retention time fairly centered in relation to the other compounds.

Calibration curves were prepared by chromatographing sample mixtures of increasing ratio of phenolic concentration to standard (w/w) and then plotting this ratio *versus* the ratio of the peak areas of phenols to standard. The chromatographing conditions used were the same as those described above. Curves were linear in the concentration range used.

TABLE I

RELATIVE RETENTION TIME (t_R), SLOPE (m), INTERCEPT (c) AND CORRELATION COEFFICIENT (r) OF CALIBRATION CURVE OF ACETYLATED COMPOUNDS ON DB-5 (60 m) CAPILLARY COLUMN

Internal standard used: 4-ethylresorcinol.

Compound	t_R	m	c	r
Phenol	0.358	0.4901	0.8975	0.987
o-Cresol	0.429	1.0586	−0.1766	0.999
p-Cresol	0.463	0.5334	0.6760	0.998
4-Ethylphenol	0.594	1.0177	−0.1468	0.999
4-Propylphenol	0.725	0.5373	1.5716	0.999
Guaiacol	0.594	1.3292	−0.3666	0.999
4-Methylguaiacol	0.738	0.7230	1.5849	0.999
4-Ethylguaiacol	0.846	0.8583	0.9689	0.997
4-*n*-Propylguaiacol	0.940	0.9453	0.4050	0.999
Catechol	0.754	1.2534	−0.4625	0.950
4-Methylcatechol	0.879	1.2820	−0.3780	0.996
4-Ethylcatechol	0.971	0.9840	0.1123	1.000
Syringol	0.871	1.1575	0.2554	1.000
p-Hydroxybenzaldehyde	0.718	0.9797	0.2897	0.999
Vanillin	0.933	1.8553	−0.3680	0.983
Acetovanillone	1.033	1.4751	−0.1090	0.968
Syringaldehyde	1.120	2.6669	−0.6089	0.998
Acetosyringone	1.900	1.3980	0.1711	0.987

TABLE II

ACCURACY OF CAPILLARY GAS CHROMATOGRAPHIC METHOD SHOWN THROUGH OBSERVED VALUES *vs.* ACTUAL VALUES IN CALIBRATION COMPOSITE SAMPLE

Compound	*Actual values(mg)*	*Observed values(mg*	*Relative deviation(%)*
Phenol	7.1	7.2	1.41
o-Cresol	9.4	10.0	6.38
p-Cresol	7.3	7.3	0.00
4-Ethylphenol	6.5	6.4	1.54
4-Propylphenol	12.2	12.3	0.82
Guaiacol	27.8	29.5	6.12
4-Methylguaiacol	14.8	14.8	0.00
4-Ethylguaiacol	14.6	14.7	0.68
4-*n*-Propylguaiacol	12.9	13.4	3.88
Catechol	10.2	10.5	2.94
4-Methylcatechol	8.5	8.6	1.18
4-Ethylcatechol	6.1	6.1	0.00
Syringol	16.3	16.4	0.61
p-Hydroxybenzaldehyde	9.2	9.1	−1.09
Vanillin	14.3	14.9	4.20
Acetovanillone	8.1	8.3	2.47
Syringaldehyde	8.8	9.0	2.27
Acetosyringone	9.7	9.4	−3.09

The appropriate equation $y = mx + c$ was found to be applicable. For each compound, the slope m and intercept c were calculated from duplicate analyses of the reference blends. The calibration curve, relative retention time (t_R) and correlation coefficient (r) are shown in Table I. As seen from the values of the correlation coefficient, all the data closely approximate a straight line.

In order to define the accuracy of the method used, a standard mixture was prepared and analysed. The concentration of phenolic compounds ranged from 6 to 28 mg/ml, representing a typical concentration range of these compounds from conventional lignin degradation processes. The mixture was analysed under the conditions reported previously, and the results are reported in Table II. The data indicate high accuracy, deviating in the range of -1 to 6.4% from the true value, in most cases not exceeding 2%. The precision and accuracy of the data in Table II imply that the method should be applicable in analysing phenolic mixtures containing the compounds considered from lignin degradation.

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