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Journal of Chromatography A, 736 (1996) 61–75

JOURNAL OF CHROMATOGRAPHY A

On-line Curie-point pyrolysis–high-performance liquid chromatographic–mass spectrometric analysis of lignin polymers

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Received 12 October 1995; revised 11 December 1995; accepted 11 December 1995

Abstract

An on-line Curie-point pyrolysis (Py)–HPLC–MS system has been developed for the analysis of oligomeric and polar compounds which are present in pyrolysis tars. The pyrolysis unit used was designed in such a way that inductive heating of the ferromagnetic sample probe and subsequent trapping of the pyrolysis products is performed inside a glass column, which is part of the analytical system. Cold-trapped compounds are solubilized, concentrated on a precolumn and subsequently separated with conventional RP-HPLC by use of column switching. A frit-electron impact ionization/chemical ionization interface and a continuous flow frit-fast atom bombardment interface are used to couple the chromatographic system directly to a double focusing mass spectrometer. In this study the method is presented, optimized and evaluated for lignin polymers. Results are compared with in-source Py–MS and Curie-point PY–GC–MS data. The first results, presented in this study, clearly demonstrate the feasibility and potential of Py–LC–MS as a reproducible and rapid screening method for the analysis of isomeric and oligomeric structures produced upon pyrolysis of insoluble polymers.

Keywords: Pyrolysis–liquid chromatography–mass spectrometry; Lignins

1. Introduction

Pyrolysis has proven to be a powerful sample preparation method in the compositional and structural analysis of non-volatile materials such as synthetic plastics and naturally occurring polymers [1,2]. The main pyrolysis systems that are currently applied in combination with MS are resistively heated direct probe pyrolysis [3], resistively heated filament pyrolysis [4], inductively heated filament (Curie-point) pyrolysis [5] and laser pyrolysis [6]. When a high-molecular-mass compound or complex particulate matter is subjected to flash pyrolysis, a wide range of thermal degradation products of different polarities and molecular masses may be produced. Also, a large number of isomeric structures may be formed. To obtain detailed information about the nature of the monomers and their mode of linkage, chromatographic methods must be used in combination with mass spectrometry.

On-line Curie-point pyrolysis (Py)–GC–MS is a widely used technique for the investigation of polymers, but only volatile pyrolysis products of a relatively low molecular mass can be analysed and problems may occur for thermally labile and polar compounds [1,2]. Studies revealing larger pyrolysis fragments have been carried out using in-source Py–MS. With this method the sample is pyrolysed

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0021-9673/96/$15.00
SSDI 0021-9673(95)01345-8

directly inside the ion-source of a mass spectrometer. In-source Py–MS has been widely applied as a fingerprinting technique because the method facilitates a fast and collisionless mass transfer and gives a complete inventory of pyrolysis products [2]. However, the complexity of the ion mixture and the transient character of the pyrolysis process present specific difficulties for the identification of isobaric and isomeric compounds by high-resolution MS [7] and Py–MS–MS [8] approaches.

The aim of this study was to develop a novel method for the on-line Py–LC–MS analysis of thermal degradation products of polymers which are not amenable to Py–GC–MS analysis and cannot be resolved by conventional in-source Py–MS. The method is demonstrated and optimized for lignin polymers. The lignin used was isolated by the organosolv pulping process and was previously found to be an ether and carbon-linked copolymer of 2-methoxyphenol (guaiacyl) and 2,6-dimethoxyphenol (syringyl) structural units [9]. Analytical flash-pyrolysis techniques offer a rapid and effective method to dissociate lignin polymers into cleavage products which reflect the origin and structure of the original polymer from which they are derived [6,9]. Furthermore, understanding of the key structural features is of fundamental and economic importance because thermochemical conversion into higher value products is an important first step in the utilization of lignin as a renewable resource [10].

In former applications, the analysis of oligomeric structures produced upon off-line Curie-point pyrolysis involved fraction collection after HPLC separation, evaporation of the solvent, derivatization and subsequent transfer of the analyte to the mass spectrometer by means of a direct insertion probe [11,12]. Therefore, an on-line Py–LC–MS approach, using column-switching techniques, reduces the analysis time and improves the reproducibility of the method by reducing the risk of sample loss and contamination. Furthermore, when mass spectrometric detection is applied, problems with fraction collection are avoided because overlapping elution profiles can be deconvoluted into selected single ion chromatograms.

In this study an evaluation of the feasibility of combining Py on-line with standard LC–MS is presented. This method provides a rapid screening technique of higher-molecular-mass pyrolysis products of lignin polymers without the loss of structural integrity. Results are compared with in-source Py–MS data and Py–GC–MS data.

2. Experimental

2.1. Apparatus

A schematic representation of the Py–HPLC–MS set up used is shown in Fig. 1. The system consisted of two pumps. Pump 1, an isocratic pump (Applied Biosystems Model 400) and pump 2, a ternary gradient pump (LDC/Milton Roy Model CM 4000). An on-line pyrolysis unit was developed for the preparation of the tar fraction of polymers. For the injection of soluble model compounds, the pyrolysis unit was replaced with an injection valve equipped with a 20-μl sample loop (Rheodyne Model 7125). A variable-wavelength UV detector, which was set at 280 nm (Applied Biosystems Model 783), was connected in series with a JEOL SX-102 double focusing mass spectrometer by a direct liquid inlet interface. The system was regulated with a high-pressure ten-port switching valve (Rheodyne Model C10W).

2.2. On-line Curie-point pyrolysis

Fig. 2 shows a schematic diagram of the Curie-point pyrolysis chamber which was developed for on-line HPLC analysis of insoluble polymeric materials. First, a sample suspension (50~100 μg) was applied to a ferromagnetic wire and dried under reduced pressure. The wire was inserted into the glass liner and subsequently placed into the pyrolysis unit. The glass liner used was an empty ChromSep (Chrompack) glass column (50×3 mm I.D.) with one removable frit at the bottom end to prevent the introduction of particles>3 μm in the chromatographic system. A stainless-steel sample holder with standard HPLC connections was developed to fix the ferromagnetic wire inside the glass liner. The ferromagnetic wire was inductively heated within 0.1 s to its Curie-point temperature (510°C) with a FOM-AMOLF-built 200-W self-tuning Rf inductive heating power supply which can deliver a 50-A current at a frequency of 1 MHz to the high-frequency coil in the pyrolysis unit.

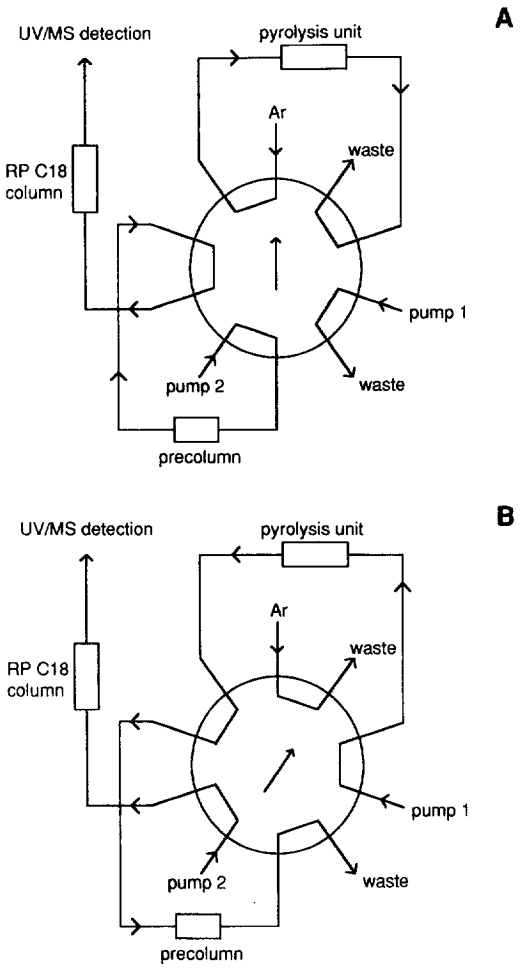

Fig 1. Schematic representation of the Curie-point Py–LC–MS system. In position A, the sample is pyrolysed in an argon atmosphere while the analytical column is equilibrated by solvent 2. When the switching valve is rotated to position B, the cold-trapped fraction on the glass liner is washed out with solvent 1 and the analytes are trapped on the precolumn. When the switching valve is rotated back to position A, solvent 2 back-flushes the retained compounds from the precolumn to the analytical column where they are separated by gradient elution before UV detection and subsequent mass spectrometric detection.

2.3. Columns and mobile phases

The precolumn (10×3 mm I.D.) was dry-packed with a Hypersil ODS-C_{18}, 30-μm particles or a phenyl stationary phase (Chrompack). A 10% acetonitrile in water solution (solvent 1) was used as the washing solvent for the glass liner after pyrolysis. A LiChroCART system (Merck) with a RP-C_{18} column

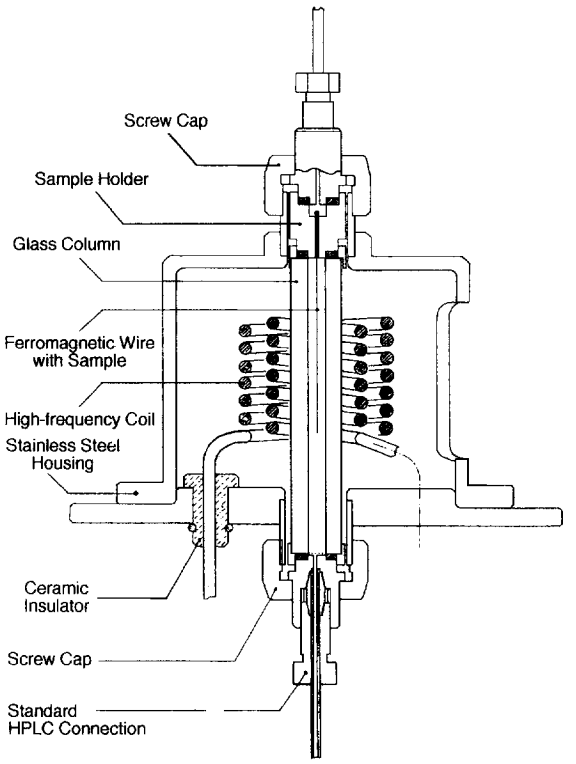

Fig. 2. Schematic diagram of the Curie-point pyrolysis unit for on-line Py–LC–MS analysis.

(250×4 mm I.D.) packed with 5-μm spherical particles was used as the analytical column. The analytical separation was performed by linear gradient elution using an initial composition of 95% 0.01 *M* phosphate buffer (pH 2) and 5% acetonitrile (solvent 2) at a flow-rate of 0.8 ml/min. The acetonitrile concentration was raised to a final concentration of 70% in 50 min. To re-equilibrate the column, the initial conditions were restored via a 1-min step gradient, and held for 10 min before the next analysis was started. All solutions were filtered (0.45 μm, Millipore) and purged with helium before use. The chromatographic separation was performed at ambient temperature.

2.4. Column-switching operation

First, with the switching valve in position A, the glass column containing the ferromagnetic wire with the sample was flushed with argon for 1 min to remove air (Fig. 1). Subsequently, the ferromagnetic

wire was inductively heated to its Curie-point temperature (510°C) at which it was held for 4 s. Volatile pyrolysis fragments are flushed to waste by the argon carrier gas and the tar fraction is cold-trapped on the glass column. At the same time, solvent 1 is pumped to waste and the analytical column is being re-equilibrated for gradient elution by solvent 2. When the switching valve is rotated to position B, the cold-trapped fraction on the walls of the glass liner is washed out with solvent 1 and the analytes are trapped on the precolumn. At the same time the analytical column is still re-equilibrated by solvent 2 and the argon flow can be switched off. Different times for flushing the precolumn were investigated in order to optimize the selectivity and the sensitivity but, in general, the switching valve was turned back to position A after 1.1 min. When the switching valve is rotated back to position A, solvent 2 back-flushes the retained compounds from the precolumn to the analytical column where the compounds are separated by gradient elution before UV detection and subsequent mass spectrometric detection. At the same time the pyrolysis unit can be prepared for the next analysis.

2.5. Mass spectrometry

The HPLC system was connected to a JEOL SX-102 double focusing mass spectrometer (B/E geometry) by a JEOL frit-electron impact ionization (EI)/chemical ionization (CI) LC–MS interface consisting of a fused-silica capillary tube (1 m×60 μm I.D.) which is led directly to a porous stainless-steel frit [13]. Positive fast atom bombardment (FAB) mass spectra were obtained with a JEOL frit-FAB LC–MS interface. During routine Py–LC–MS analysis, the nonvolatile phosphate buffer is removed and a gradient of water–acetonitrile was used to prevent clogging of the frit. During FAB-ionization measurements, 1% (v/v) glycerol (Merck, 98%) was added to the mobile phase. The effluent from the column was split by a JEOL pneumatic splitter to reduce the flow volume before introduction into the mass spectrometer and to allow the connection of standard HPLC columns. A split ratio of approximately 1/100 was used throughout the experiments at a flow-rate of 0.8 ml/min corresponding to an amount of effluent introduced to the frit of approximately 8 μl/min. The temperature of the ion source was maintained in the range between 250–300°C during EI/CI measurements and at 60°C during FAB measurements. The ion source pressure was maintained in the range between $5 \cdot 10^{-3}$–$2 \cdot 10^{-2}$ Pa. Evaporated products were ionized under 20- or 70-eV electron impact conditions, accelerated to 6 kV and mass analyzed over a m/z range of 120–800 with a scan cycle time of 1 s. Mass calibration was obtained using Ultramark 2000. FAB-ionization was obtained with a primary xenon beam (6 kV, 10 mA). Ions were accelerated to 8 kV and mass analyzed over a m/z range of 100–1000 with a scan cycle time of 1.1 s. Mass calibration was obtained using the glycerol background spectrum. A liquid-nitrogen trap was used to trap the solvent vapours in the ion source.

2.6. In-source Py–MS

Experiments were performed on a JEOL JMS-SX102 double focusing mass spectrometer (B/E mode). A 5-μg aliquot of a lignin suspension in water was applied to a Pt/Rh filament (0.1 mm diameter, 10% Rh) and dried in vacuo. After insertion of the probe into the ion source (10^{-4} Pa, 180°C), the filament was heated resistively at a rate of 16°C/s to a final temperature of 800°C. Pyrolysis and evaporated products were ionized under 16-eV electron impact conditions, accelerated to 10 kV and mass analyzed over a m/z range of 20–800 with a scan cycle time of 1.0 s.

2.7. Samples

An ALLCELL lignin (sample no. ADI/L920) from Repap Technologies (Valley Forge, PA, USA) was isolated from mixed hardwoods (maple, birch and poplar) by an organosolv process using aqueous ethanol. This experimental lignin was prepared for a world-wide two-dimensional Round Robin on lignin analysis, organized by the National Renewable Energy Laboratory (NREL, USA) [14].

The model compounds coniferyl alcohol [2-methoxy-4-(1′-hydroxyprop-2′-enyl)phenol] (4) (Aldrich) and *trans*-4-(prop-2′-enyl)guaiacol (Jansen Chimica) were used without further purification. *p*-Coumaric acid (4′-hydroxycinnamic acid) (5) and

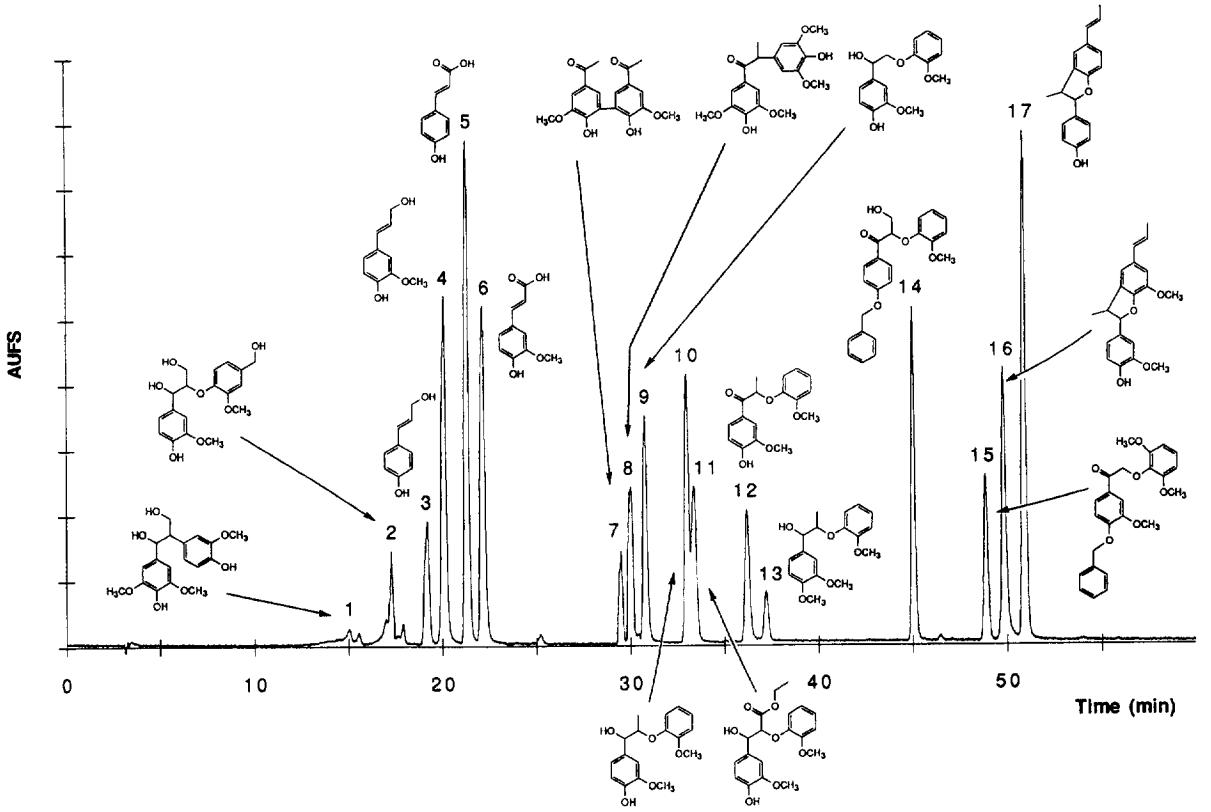

Fig. 3. HPLC chromatogram of lignin model compounds 1–17 (UV detection 280 nm).

ferulic acid (4′-hydroxy-3′-methoxy cinnamic acid) (6) were gifts from R.A. Hartley. Compounds 1 and 2, *p*-coumaryl alcohol [4-(1′-hydroxyprop-2′-enyl)phenol] (3) and 7–17 (Fig. 3) were generous gifts from Dr. O. Faix, Institut für Holzchemie und Chemische Technologie des Holzes, Hamburg, Germany. Solutions of the samples were prepared in ethanol (0.01 *M*) and used without further purification.

3. Results and discussion

3.1. Reversed-phase HPLC–MS of lignin model compounds

A typical HPLC chromatogram of a mixture of lignin reference compounds, containing different interunit linkages and different substituents on the aromatic rings and aliphatic side chains, is shown in Fig. 3. The sample was introduced by means of an injection valve. The influence of the substituents on the elution order of these phenolic compounds is clearly demonstrated. The introduction of hydroxyl groups on the aliphatic side chain dramatically decreases the retention time as is shown e.g. for compounds 1 and 2 compared to 7–12 whereas the introduction of aldehyde and methyl groups increases the retention time. The addition of a methyl group to the aliphatic side chain also results in a stronger interaction with the stationary phase as is observed for compounds 9 and 10.

The effects of substitution on the aromatic ring are also apparent. The introduction of a methoxy group, *ortho* to the phenolic –OH, causes an increase in retention time as is demonstrated by compounds 3–6: coumaryl alcohol (3) elutes before coniferyl alcohol (4) and *p*-coumaric acid (5) elutes before

ferulic acid (6). The alcohols are eluted faster than the corresponding acids, probably as a result of intermolecular hydrogen bonding of the acidic protons which disturbs the interaction with the mobile phase [15]. To reduce peak tailing of some of the more polar substances such as phenolic acids, the pH value of the buffer should be between pH 1.9 and 2.0 because higher values lead to a shift of the equilibrium to favor the free acid over its conjugate base. Omission of the phosphate buffer from the eluent results in broader peaks and longer elution times and compounds 3–6 could not be completely separated when an acetonitrile–water gradient was used at pH>2.0. Methylation of the weakly acidic phenolic –OH leads to longer retention times as is demonstrated by compounds 10 and 13. When a methoxy group is introduced in β-5 linked structures such as 16 and 17, a decrease of retention time is observed as a result of the increased interactions with the mobile phase.

When the frit-EI/CI LC–MS interface is employed, the ionization mechanism observed for producing ions is a mixture of EI and CI in which the mobile phase acts as the reagent gas. The CI spectra, which are superimposed on the EI spectra, facilitate structural identification by the formation of characteristic ions [16]. In the LC–MS spectra of *p*-coumaryl alcohol (3) and coniferyl alcohol (4) intense $[M+H-H_2O]^+$ elimination ions are observed while hydroxycinnamic acids (5 and 6) and α-carbonyl phenylalkyl phenyl ethers (i.e. 7, 8 and 12) show intense $[M+H]^+$ ions (Fig. 4A). However, a disadvantage of the frit-EI/CI LC–MS interface, used in this study, is that no molecular ions are observed for model compounds that contain a benzylic hydroxyl group (i.e. 1, 2, 10 and 11, Fig. 5A) as a result of the relatively high temperature of the frit (250–300°C). For these compounds only intense $[M+H-H_2O]^+$ elimination ions and $[M-H_2O]^+$ fragment ions are observed. This problem is minimized when continuous flow frit-FAB is used because this method does not require extensive heating of the probe to volatilize the analytes. Stable signals were obtained when the ion source temperature was kept at 60°C. All α-carbonyl phenylalkyl phenyl ether model compounds showed $[M+H]^+$ ions upon FAB ionization (i.e. 14, Fig. 4B). However, the α-hydroxy phenylalkyl phenyl ether compounds (i.e. 1, 2, 10 and 11) show $[M]^{+\cdot}$ ions because the $[M+H]^+$ ions undergo an almost complete protonation initiated elimination reaction yielding the $[M+H-H_2O]^+$ ions as the base peak (i.e. 11, Fig. 5B).

Comparable chromatographic resolution was achieved in the UV chromatograms and the LC–MS chromatograms indicating that little dead volume exists between the column outlet and the frit. However, a decrease in chromatographic resolution was observed during frit-FAB measurements, compared to EI/CI LC–MS measurements, because of the glycerol matrix which was added to the mobile phase.

3.2. Py–LC–MS analysis of organosolv lignin

Flash pyrolysis of lignin produces a variety of phenolic products which consist of guaiacyl and syringyl structural units with different functional groups on the aliphatic side chain on position 1 of the aromatic ring. These compounds are predominantly produced by thermolytic cleavage of the β-alkyl aryl ether interunit linkage which accounts for 50–70% of the total linkages in lignin [17]. Upon Py–GC–MS analysis it was observed that higher molecular mass fractions condense as a liquid tar fraction on the glass liner which is used in the interface. The on-line Py–LC unit used in this study (Fig. 2) was developed to analyse this tar fraction.

After pyrolysis of the organosolv lignin, the switching valve is rotated to position B to wash out the cold-trapped fraction (Fig. 1). The tar fraction could not be completely removed from the glass liner when water was used as the only washing solvent indicating that a large fraction of less polar compounds is formed. Different solvent mixtures were investigated to optimize the dissolution of the tar fraction. The addition of organic solvents, such as ethanol or acetonitrile, reduces the time needed to flush all of the tar components onto the precolumn but it also reduces the retention of these compounds. It was found that most compounds could be dissolved and preconcentrated using a 10% (v/v) acetonitrile in water solution. Two different packings have been tested as a stationary phase for the precolumn, Hypersil ODS-C_{18} (30 μm) and a phenyl stationary phase. The retention of the investigated

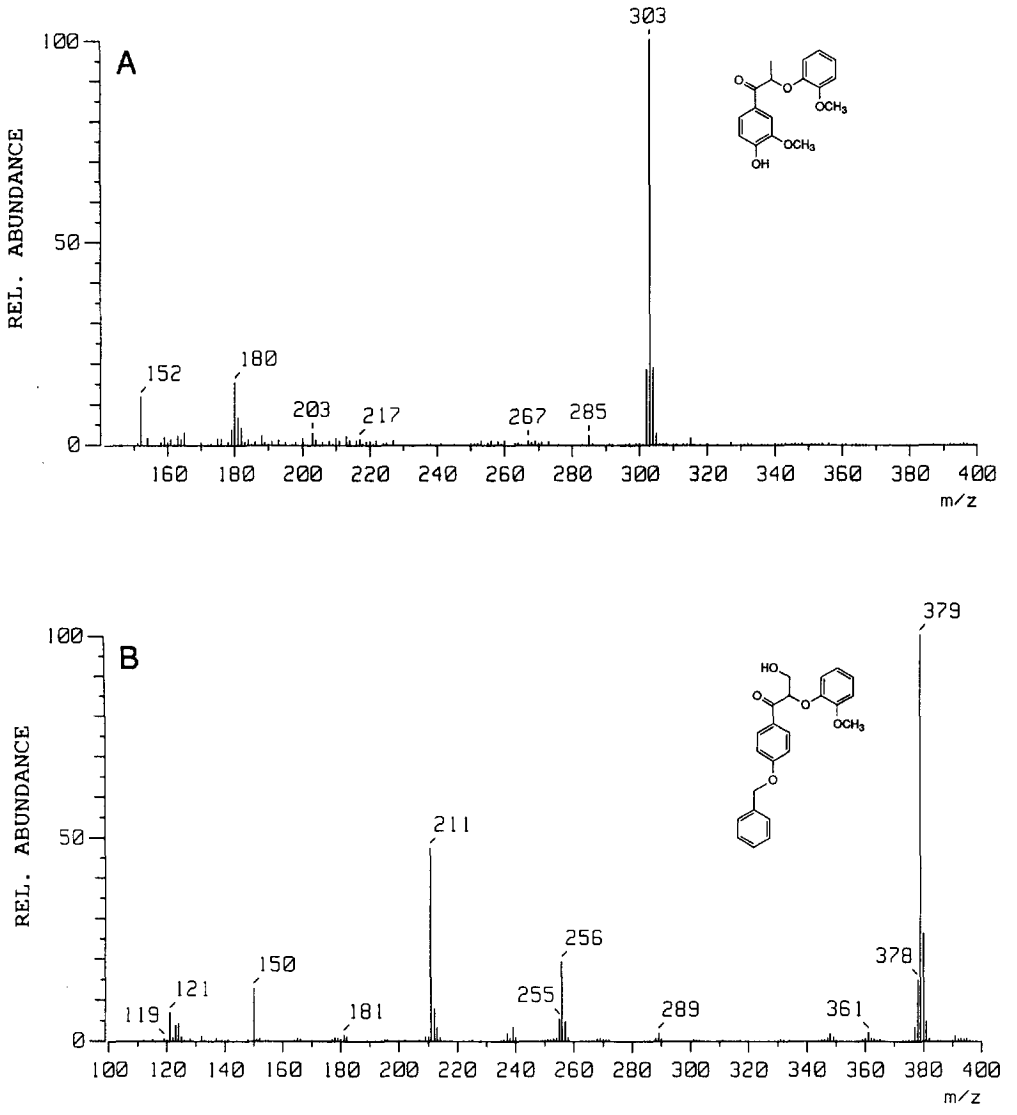

Fig. 4. (A) Frit-EI/CI mass spectrum (20 eV) of model compound 12 with a molecular mass of 302 and (B) frit-FAB mass spectrum of model compound 14 with a molecular mass of 378.

compounds did not differ significantly on these stationary phases and the C_{18} packing was used throughout this study. With model compounds, recoveries of approximately 50% were obtained for polar compounds such as coumaryl alcohol and ferulic acid and recoveries of more than 95% were obtained for relatively nonpolar compounds such as *trans*-4-(prop-2′-enyl)guaiacol. Quantitation of the recoveries of the compounds trapped in the tar fraction is not possible yet, since UV and mass spectrometric response factors for most of these compounds are unknown.

The preconcentrated components on the precolumn are back-flushed onto the analytical column by rotating the switching valve back to position A (Fig. 2). The reversed-phase separation of the organosolv lignin pyrolysate tar fraction is shown in Fig. 6. When EI ionization was applied, a useful total ion current could be obtained. With FAB ionization only single ion currents could be used after back-

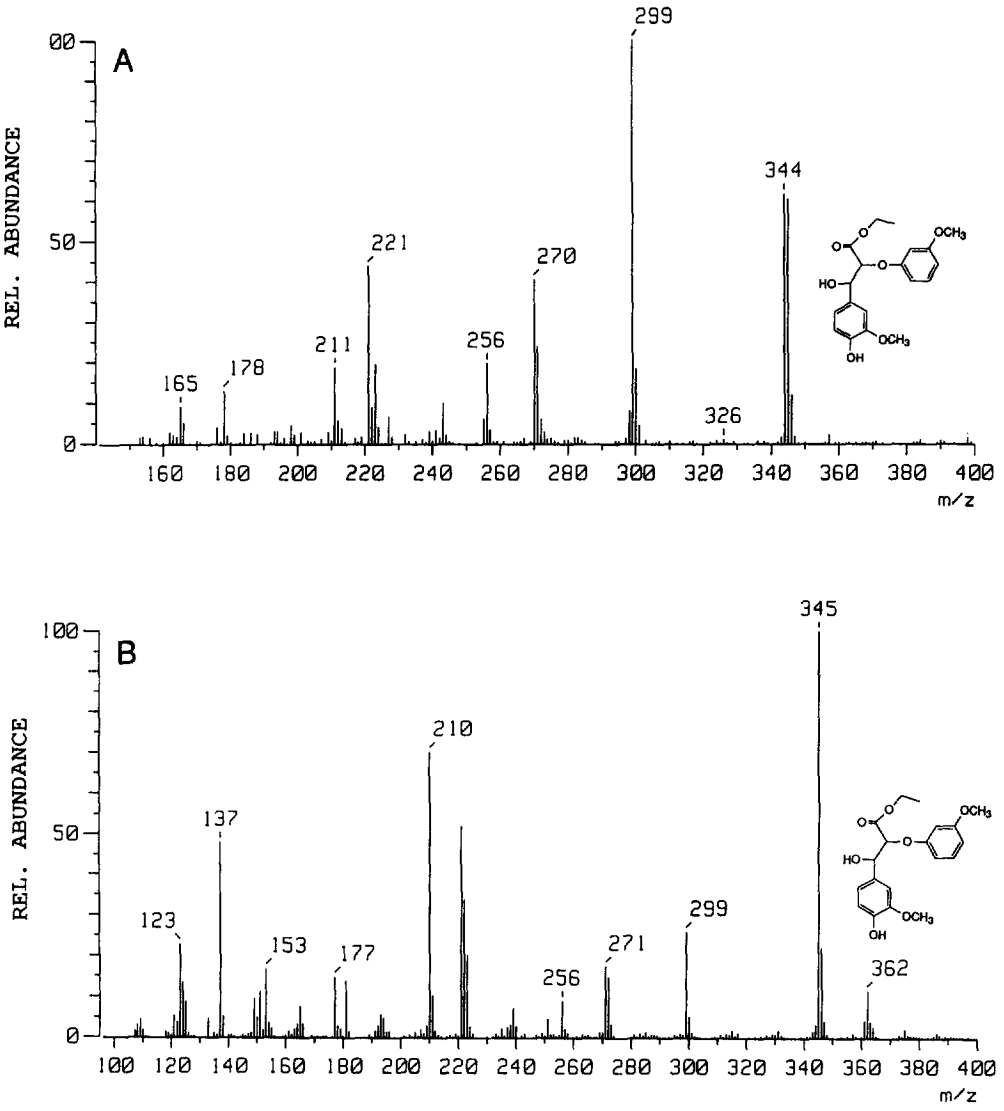

Fig. 5. (A) Frit-EI/CI mass spectrum (20 eV) of model compound 11 with a molecular mass of 362 and (B) frit-FAB mass spectrum of model compound 11.

ground substraction as a result of the matrix background signal. In general, FAB ionization yields $[M+H]^+$ ions of phenolic compounds present in the pyrolysis tar. However, a disadvantage of FAB ionization is that the ionization efficiency is dependent on the matrix used and the proton affinity of the analyte. This complicates on-line analysis of the multicomponent pyrolysate mixture which contains a wide range of compounds with different proton affinities. Py–LC–MS analysis revealed that most peaks observed in the chromatogram obtained with UV detection are composed of several overlapping compounds. The retention times and the measured masses of some compounds of interest are listed in Table 1.

Interestingly, predominantly dimethoxyphenol monomer compounds are trapped in the tar fraction. This is probably explained by the higher volatility of the monomethoxy compounds produced upon pyrolysis which allows these compounds to be

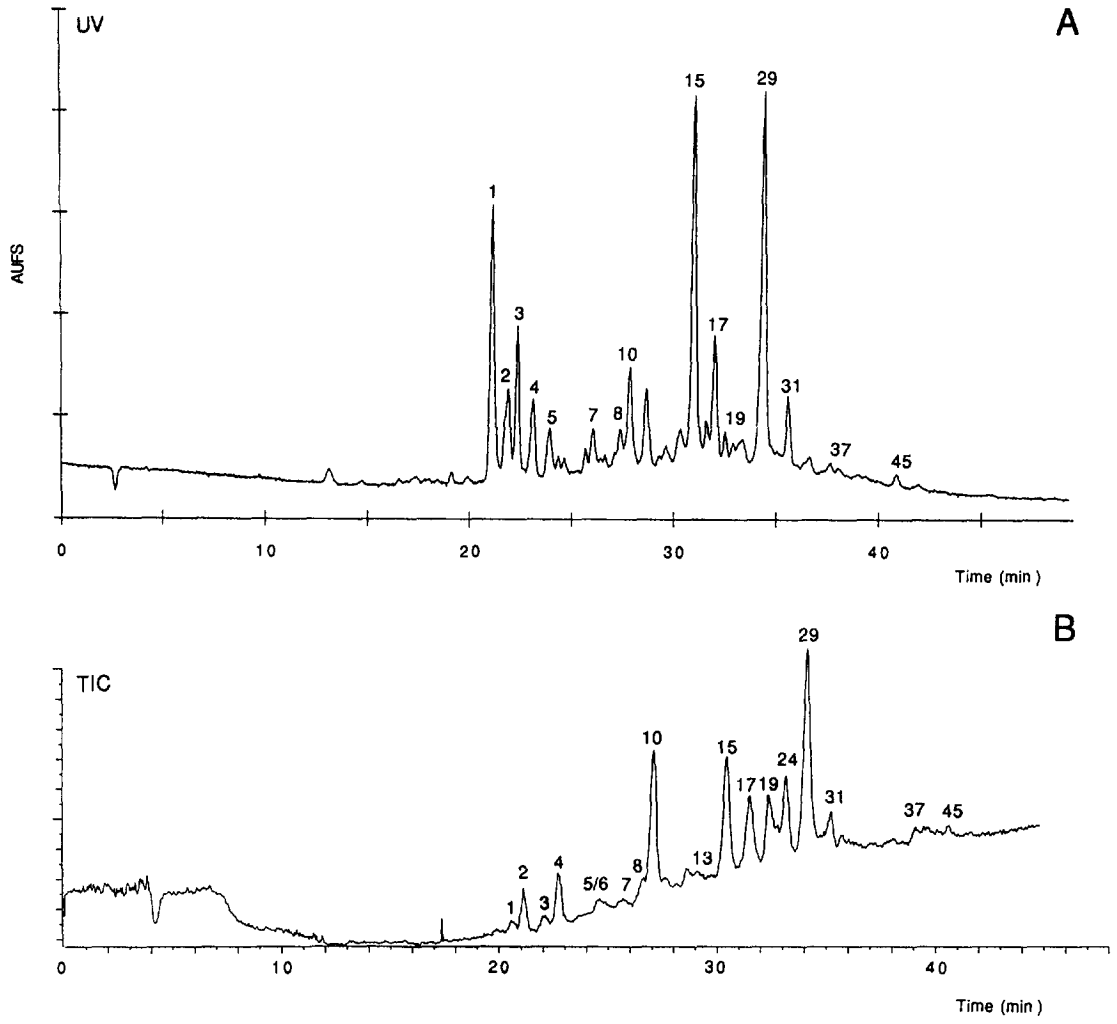

Fig. 6. Py–LC chromatogram of organosolv lignin with (A) UV detection and (B) MS detection (EI, 20 eV).

flushed away in the argon flow before they can be cold-trapped on the glass liner. Differences in aliphatic side chain types, observed in the Py–LC–MS data, are an important criterion for the degree of modification of the lignin polymer structure during the isolation procedure [18]. Both coniferyl alcohol and sinapyl alcohol are observed upon Py–LC–MS, indicating that a part of the polymer structure is depolymerized into the monomers most likely by thermal dissociation of ether interunit linkages. Also, relatively high abundances of *trans*-4-(prop-2′-enyl)guaiacol and *trans*-4-(prop-2′-enyl)syringol are observed in the Py–LC–MS data. These structures are most likely introduced in the lignin polymer during the isolation procedure. However, it is known that Curie-point pyrolysis at atmospheric pressure may lead to some thermally induced dehydration of the aliphatic side chains [9].

Several homologous series of higher molecular mass compounds with $\Delta m/z$ 30 are observed in the Py–LC–MS chromatogram (Fig. 7). The ion series at m/z 260, 290, 320 and m/z 272, 302, 332 are assigned to 1,1-di(methoxyphenol)methane and methoxyhydroxystilbene types of structures, respectively, which differ only by the mass of a methoxy group. This observation confirms the occurrence of

Table 1
Structures identified by on-line Py–LC–MS analysis

Peak	Retention time (min)	*m/z*	Compound
1	20.5	212	4-(1-Hydroxypropyl)syringol
2	20.8	182	4-Formylsyringol
3	20.9	196	4-Acetylsyringol
4	22.5	154	Syringol
5	24.1	180	*trans*-Coniferyl alcohol
6	24.5	210	*trans*-Sinapyl alcohol
7	25.5	208	4-(Prop-1-en-3-one)syringol
8	26.6	212	Vanillic acid methyl ester
9	26.7	384	Unknown
10	27.1	168	4-Methylsyringol
11	27.4	418	Syringaresinol
12	28.7	418	Episyringaresinol
13	29.2	320	1,1-(Disyringyl)methane
14	30.2	290	1,1-(Guaiacyl,syringyl)methane
15	30.4	180	4-Vinylsyringol
16	31.3	260	1,1-(Diguaiacyl)methane
17	31.4	182	4-Ethylsyringol
18	32.2	356	Dihydroconiferyl alcohol
19	32.3	332	3,3′,4,4′-Tetramethoxy-4,4′-dihydroxystilbene (*trans*)
20	32.6	344	Unknown
21	32.9	332	3,3′,4,4′-Tetramethoxy-4,4′-dihydroxystilbene (*cis*)
22	33.1	314	Unknown
23	33.2	194	4-(Prop-2-enyl)syringol (*cis*)
24	33.3	302	3,3′,4-Trimethoxy-4,4′-dihydroxystilbene (*trans*)
25	33.4	384	Unknown
26	33.5	314	Unknown
27	33.8	388	Medioresinol
28	33.9	284	Unknown
29	34.0	194	4-(Prop-2-enyl)syringol (*trans*)
30	34.1	302	3,3′,4-Trimethoxy-4,4′-dihydroxystilbene (*cis*)
31	34.2	272	3,3′-Dimethoxy-4,4′-dihydroxystilbene (*trans*)
32	34.3	164	4-(Prop-2-enyl)guaiacol (*cis*)
33	34.9	272	3,3′-Dimethoxy-4,4′-dihydroxystilbene (*cis*)
34	35.2	164	4-(Prop-2-enyl)guaiacol (*trans*)
35	35.7	196	4-(Propane)syringol
36	36.7	384	Unknown
37	38.1	386	Dihydro-dehydro-coniferylaldehyde- sinapylaldehyde isomer
38	38.5	386	Dihydro-dehydro-coniferylaldehyde-sinapylaldehyde isomer
39	39.1	386	Dihydro-dehydro-coniferylaldehyde-sinapylaldehyde isomer
40	39.2	388	Unknown
41	39.6	356	Dihydro-dehydro-diconiferylaldehyde isomer
42	39.8	386	Dihydro-dehydro-coniferylaldehyde-sinapylaldehyde isomer
43	40.1	356	Dihydro-dehydro-diconiferylaldehyde isomer
44	40.5	356	Dihydro-dehydro-diconiferylaldehyde isomer
45	40.7	334	Unknown

considerable amounts of these structures in organosolv lignin, which are most likely formed via aryl-group migrations of ether-linked structural units during the technical processing of the sample [19]. The *cis* and *trans* isomers of the methoxyhydroxystilbenes are clearly separated with the *trans* structure eluting faster (Fig. 7). The homologous series observed at *m/z* 284, 314 and 344 indicate the

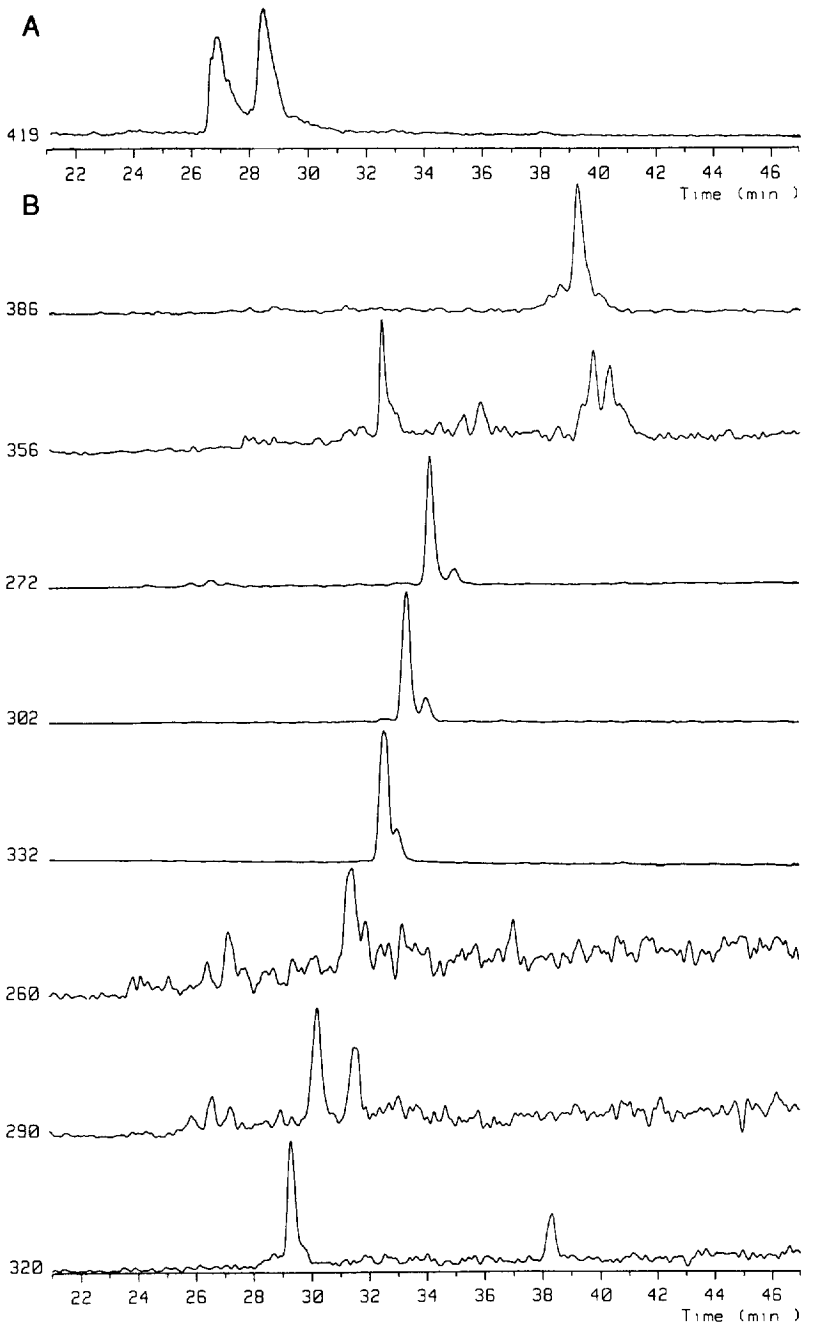

Fig. 7. (A) Single ion chromatogram of the $[M+H]^+$ ion of m/z 418 obtained by FAB ionization and (B) single ion chromatograms obtained by Py–LC–MS (EI, 20 eV) of homologous ion series of m/z 260, 290, 320 and m/z 272, 302, 332 and m/z 356, 386.

presence of methoxyhydroxyphenanthrene types of structures [6,20] but these could not be identified unequivocally yet.

Two important higher-molecular-mass pyrolysis products are observed at m/z 356 and 386. A number of corresponding isomeric structures is clearly re-

solved upon Py–LC–MS analysis (Fig. 6) and the main peaks are assigned to the phenylcoumaran structures dihydro-dehydro-diconiferylaldehyde (*m/z* 356) and dehydro-dihydro-sinapylaldehyde-coniferylaldehyde (*m/z* 386) (Fig. 8A). These types of compounds contain a β-5 interunit linkage and may account for more than 10% of the total interunit linkages formed during the dehydrogenative polymerization of lignin [17]. Three 2,6-diaryl-3,7-dioxabicyclo[3.0.0]octane (resinol) types of structures are identified: medioresinol (*m/z* 388) and two syringaresinol isomers (*m/z* 418, Fig. 6A and Fig. 8B). Pinoresinol (*m/z* 358) was only detected in trace amounts which confirms the view that this β–β linked compound is rare in lignin polymers derived from hardwood [17]. Polymerization of *trans*-coniferyl alcohol monomers yields β-5 linked structures while polymerization of sinapyl alcohol monomers

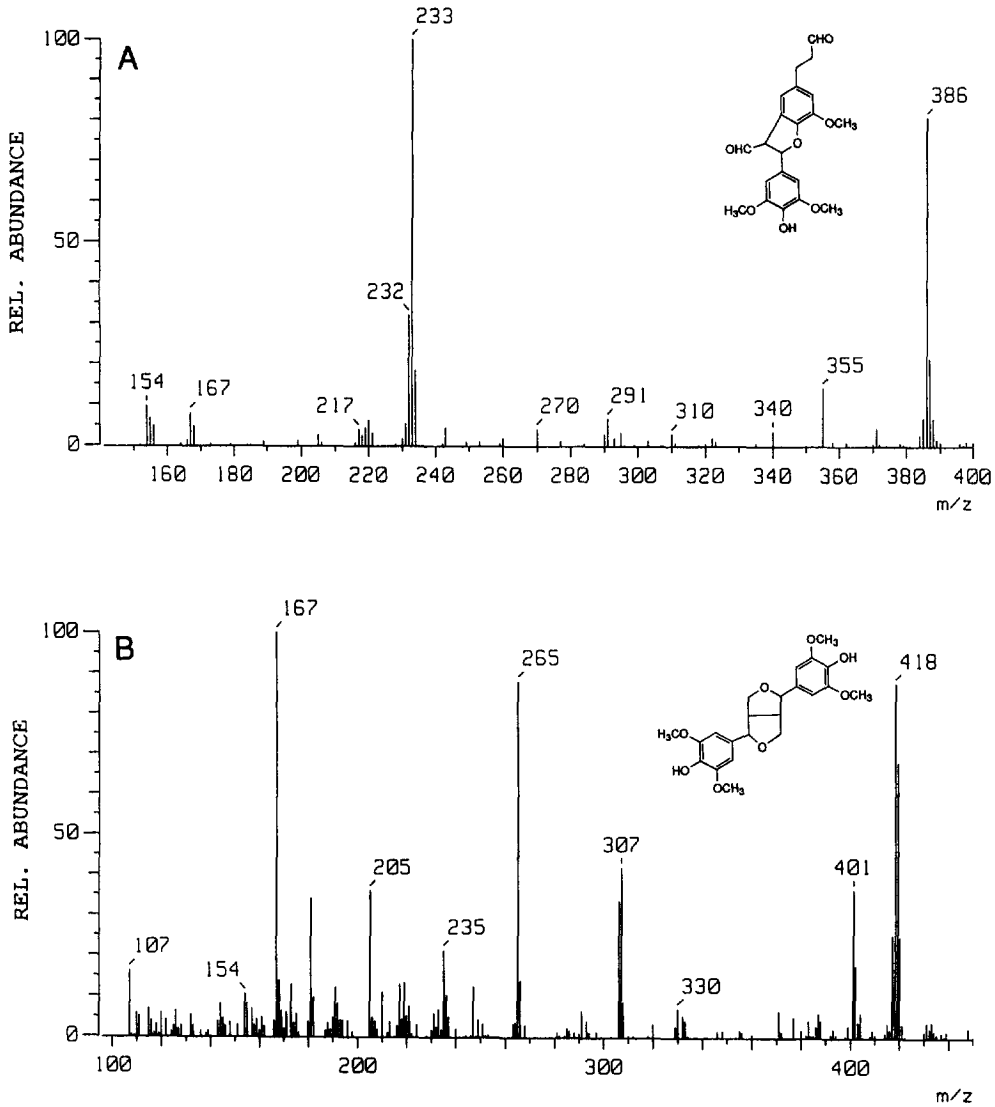

Fig. 8. Mass spectra obtained by Py–LC–MS of (A) dehydro-sinapylaldehyde, coniferyl alcohol (EI, 20 eV) and (B) syringaresinol (FAB ionization).

yields predominantly β–β linked structural units because both *ortho* positions are methoxylated and β-5 coupling is not possible. The molecular ions of the resinols were only observed when FAB ionization was applied demonstrating that these types of structures are fragmented upon frit-EI/CI analysis.

In total, more than 50 structures were detected, with a molecular mass in the range of *m/z* 250–500 of which only the most abundant are listed in Table 1. In addition, the presence of more than 20 compounds with obscure molecular ions were observed. A full identification of all compounds present in the pyrolysis tar will require the availability of appropriate model compounds or the application of additional techniques such as tandem mass spectrometry or high-resolution mass spectrometry in combination with Py–LC.

3.3. Applicability of the on-line Py–LC–MS method

A complete inventory of all products obtained upon pyrolysis of the lignin polymer under investigation is obtained by in-source Py–MS analysis (Fig. 9). A series of 2-methoxyphenol (i.e. *m/z* 124, 138, 150, 164, 178, 180) and 2,6-dimethoxyphenol (i.e. *m/z* 154, 168, 180, 194, 208, 210) derivatives is observed, characteristic for lignin polymers derived from angiosperm wood (hardwood) [4,6,9]. Also, a wide variety of pyrolysis products is observed in the range from *m/z* 250–500. In a previous study, it was shown that Curie-point Py–GC–MS provides the characterization of most of the monomeric compounds but mass-transport limitations prevented a detailed identification of compounds in the range from *m/z* 250–500 [9]. The major peaks in the Py–MS spectrum were assigned to guaiacol (*m/z* 124), 4-methylguaiacol (*m/z* 138), syringol (*m/z* 154), *trans*-4-(prop-2′-enyl)guaiacol (*m/z* 164), 4-methylsyringol (*m/z* 168), 4-vinylsyringol (*m/z* 180), *trans*-coniferyl alcohol (*m/z* 180), *trans*-4-(prop-2′-enyl)syringol (*m/z* 194), and *trans*-sinapyl alcohol (*m/z* 210).

Most masses, which are observed in the range from *m/z* 250 to 500 in the in-source Py–MS spectrum (Fig. 8), are also detected upon Py–LC–MS analysis (Table 1). These compounds are of particular interest because they contain information about interunit linkages between the structural units of the polymer system. In this study, it is demonstrated that these compounds are readily accessible by on-line Py–LC–MS analysis. Furthermore, Py–LC–MS analysis revealed that nominal masses obtained by in-source Py–MS analysis (Fig. 9) may represent several isomeric and isobaric compounds which are separated by RP-HPLC.

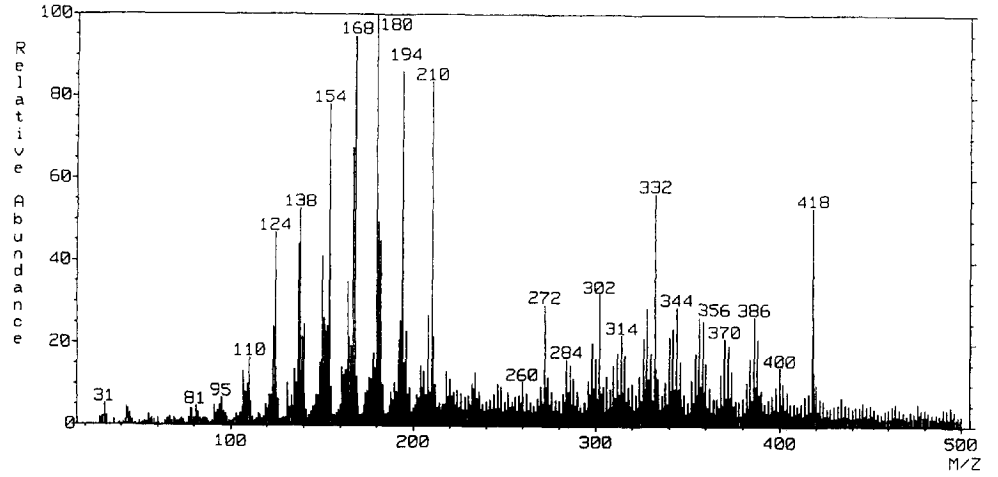

Fig. 9. In-source Py–MS of an organosolv lignin polymer (160–640°C) measured on a double focusing (B/E) mass spectrometer under 16 eV electron impact conditions.

3.4. Reproducibility and limits of detection

The sensitivity of the LC–MS system, using the frit-EI/CI interface, was determined by measuring a series of successive dilutions of *trans*-4-(prop-2′-enyl)guaiacol standard solutions in ethanol. The samples were introduced by means of an injection valve. Calibration curves were linear over a range from 50 μg to 2 μg which corresponds to 500 ng to 20 ng after the splitter (split ratio~1/100). The LOD ($S/N=3$) obtained for *trans*-4-(prop-2′-enyl)guaiacol was 0.8 μg which corresponds to 8 ng (49 pmol) after the splitter. The LOD ($S/N=3$) obtained for the continuous flow frit-FAB method was 1.5 μg, corresponding to 15 ng (91 pmol) of *trans*-4-(prop-2′-enyl)guaiacol after the splitter.

The reproducibility of the LC–MS system was established by multiple injections ($n=5$) of a 1000 ppm *trans*-4-(prop-2-enyl)guaiacol solution in ethanol. The relative standard deviation obtained in the peak area and peak height were 4.8% and 5.5%, respectively, when the frit-EI/CI LC–MS interface was used. Similar results were obtained with the frit-FAB interface.

4. Conclusions

An on-line Py–LC–MS method is developed which provides a rapid and reproducible screening method for the routine analysis of various polar and oligomeric products which are cold-trapped as the pyrolysis tar of polymers. Py–LC–MS analysis of an organosolv lignin polymer reveals that many isomeric and isobaric compounds are formed upon pyrolysis which can be resolved by RP-HPLC separation, prior to MS analysis. The results obtained confirm the view that intermolecular ether linkages in the lignin polymer have been subjected to considerable cleavage during the organosolv pulping process. Predominantly 1,2-diarylpropane, 1,1-diarylmethane, phenylcoumaran and resinol types of structures were detected in the pyrolysis tar.

The frit-EI/CI LC–MS interface used in this study provides a general means of ionization of methoxyphenols but extensive fragmentation is observed for compounds containing benzylic hydroxyl groups. The frit-FAB LC–MS interface provides a relatively soft ionization of methoxyphenols and molecular ions are observed even for α-hydroxy phenylalkyl phenyl ether compounds. However, optimum ion formation for FAB analysis is dependent on the matrix used to form protonated molecular ions and consequently this method provides a less general means of ionization for multicomponent mixtures which contain compounds with a wide range of proton affinities.

Acknowledgments

The authors gratefully acknowledge J.W. Marinissen (Chrompack International B.V.), Dr. C.G. de Koster and J.B. Pureveen for technical assistance and helpful discussions and Dr. T.L. Weeding for critically reading the manuscript. This work is part of the research program of the Foundation for Fundamental Research of Matter (FOM) with financial support from the Dutch Organisation for Scientific Research (NWO).

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