



Quantification of mono- and diaryl compounds in kraft lignins by chromatographic methods

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ABSTRACT

This study describes development of methods for identification and quantification of a set of mono- and diaryl compounds in lignin. The diaryls consist of a set of stilbenes and resinols, while the monoaryls consist of guaiacol, vanillin, acetovanillone and three syringyl counterparts. The methods rely on HPLC-MS and GC-MS. These were quantified in a set of technical lignins and were found to comprise 1 – 11 % of the total mass of the samples.

Keywords: *Mass spectrometry, chromatography, lignin*

INTRODUCTION

Technical lignin isolated from black liquor is an inherently complicated sample matrix, comprising poly- oligo- di- and monomers of various shapes and atomic interconnectivities. A lot of effort has been put into the measurements and understanding of the macromolecular structure of lignin.

It is well established that technical lignins contain various compounds of comparatively low molar mass (< 500 g/mol). For example identification of 4,4'-dihydroxystilbenes with different patterns of methoxylation (compounds **3** – **5**, Figure 2) have been identified from NMR measurements, in which a strong response has been noted on many occasions, by some of the authors of this paper and others (Rönnols *et al.*). Compound **3** has also been investigated as a building block for polymers (Tachibana and Abe), as have the esinol compounds (**1** – **2**) (Janvier *et al.*). Despite the established knowledge of the presence of these compounds, their potential exploitation as a biobased resource and presumed high concentration in lignin samples, we have been unable to find any reports on methods for actual quantification, of these compounds from technical lignins. In this paper we present a quantification method based on HPLC-MS and GC-MS, for 11 low molar mass components of technical lignins.

EXPERIMENTAL

Six samples, A – F, were analysed in this study. Four of them (A – D) were derived from hardwoods and two from softwoods (Table 1).

A triple quadrupole LC/MS system (6400) from Agilent Technologies was used, with ESI source in negative mode. The chromatographic column used was ZORBAX RRHD Eclipse Plus C18, 95Å, 2.1 x 100 mm, 1.8 µm from Agilent Technologies. An injection volume of 5 µl and a flow of 0.4 ml/min was used. Mobile phase A consisted of 5 mM ammoniumformate in 5% acetonitrile and mobile phase B consisted of 95% acetonitrile. The initial percentage was 100% A, with a decrease to 0% in 25 min. Ground lignin samples were dissolved in acetonitrile at a concentration of 1 mg/ml. The dissolved samples were vortex mixed and allowed to stand for



30 min, after which the dissolved fraction was injected. The concentration of the resinols (**1** and **2**) in sample A was calculated from a calibration curve, with an inhouse sample as reference, and the average of six separate measurements. The integrals of the two peaks, corresponding to the three diastereomers, were summarized in the calculations.

Table 1. The lignin samples used in the study

Sample	Species	Treatment
A	Eucalyptus	Kraft + lignoboost
B	Eucalyptus	DCM extract of A
C	Eucalyptus	Retentate from DCM extraction of A
D	Birch	Modified soda cook
E	Scandinavian softwood	Acid precipitation of heat treated black liquor
F	Scandinavian softwood	Kraft + lignoboost

For the GC/MS measurements a 5977B MSD 7890B GC system from Agilent Technologies was used. The system was operated with constant gas flow of helium at 1 ml/min, splitless injection of 1 µl, injector temperature of 280°C and column temperature program: 50°C (1 min), 50-300°C (heating rate 10°C /min) and 300°C (20 min). The chromatographic column used was a BPX5 (30m x 0.25mm x 0.25µm film thickness) from SGE. The MS was operated with an EI source at 70 eV. The source temperature was 230°C and the quadrupole temperature was 150°C. The scan range was 30-700 m/z. The ground lignin samples were dissolved in acetonitrile at a concentration of approximately 1 mg/ml. The dissolved samples were vortex mixed and allowed to stand for approximately 30 min. To a 0.5 ml aliquot of the sample 0.1 ml MSTFA and 0.05 ml internal standard solution was added, and the sample was vortex mixed briefly prior to injection. For quantification of stilbene compounds **3** – **5** 4,4'-Stilbenediol was used as an internal standard. For quantification of the monoaryl compounds **6** – **11** commercial standards were available. Calibration curves utilizing phenanthrene-d₁₀ as internal standard were constructed.

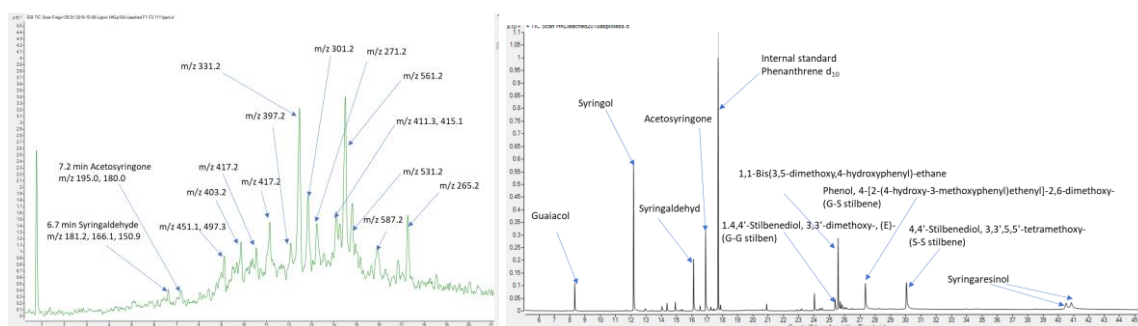


Figure 1. HPLC-MS-chromatogram of the dichloromethane-extract, sample B, with peak annotation of found molar masses (left) and GC-MS-chromatogram of sample A (right).

RESULTS AND DISCUSSION

A full scan HPLC-MS-chromatogram of the dichloromethane extract of a hardwood kraft lignin, sample B, is shown in Figure 1. This was the starting point of these investigations. A large number of peaks were observed, of which several could be tentatively assigned to different



diaryl compounds (e.g stilbenes **3** – **5** and syringaresinol **2**) and smaller compounds (e.g. syringaldehyde **9** and acetosyringone **11**) from their mass numbers. From this initial measurement, LC/MS was chosen as analytical technique for the resinol compounds **1** and **2**. Further tests were evaluated with Sample A in full scan, as well as in SIM and MRM mode, for quantification of the resinols. Syringaresinol was detected as two peaks, resulting from three stereoisomers (to be elaborated on further in an upcoming paper), at 417.2 m/z. No pinoresinol was detected in sample A. With MRM mode a cleaner total ion chromatogram was achieved, compared to SIM mode, thus MRM mode was chosen for quantifications of compounds **1** and **2**.

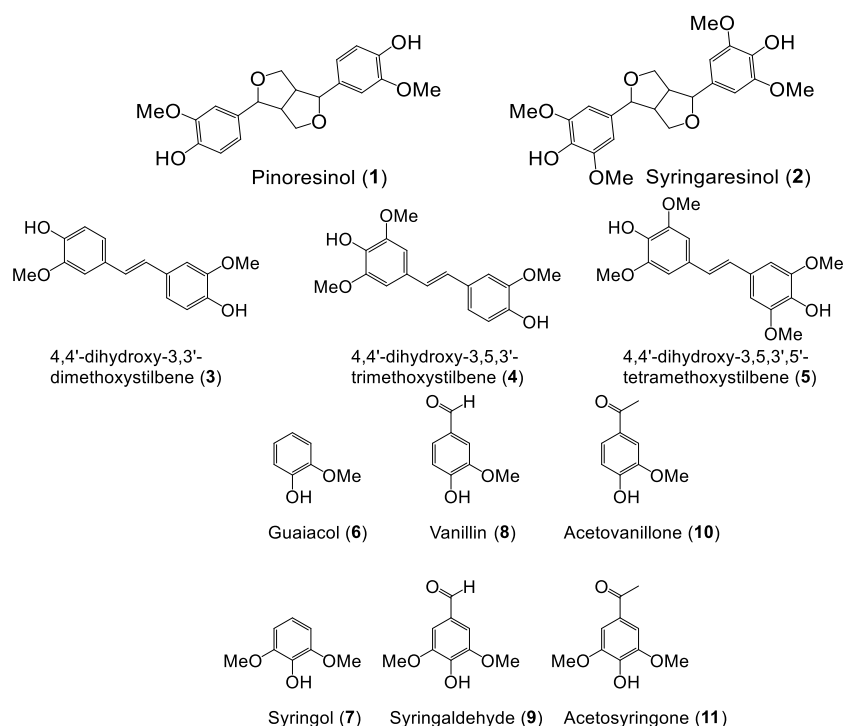


Figure 2. Molecular structures of compounds 1 - 11.

The stilbene compounds **3-5** would appear to be suitable to measure upon with LC/MS, however, the chromatographic separation between the internal standard 4,4'-Stilbenediol and compound **5**, was insufficient and the fragmentation of the ions was poor. GC/MS was investigated as an alternative analytical technique. Derivatization of the samples with MSTFA and running the measurements in scan mode gave sufficient separation and sensitivity, and was thus used. The monoaryls were analyzed with the same method as the stilbenediols. An example chromatogram is displayed in Figure 1.

Measurements on a set of lignin samples, A-F (Table 1) from different sources and treatment methods were performed. The concentrations of compounds **1** – **11** are presented in Table 2. Key takeaways in comparing the results for the different samples: Pinoresinol (**1**) was only detected in sample F. The compounds were enriched upon dichloromethane extraction, by factors 2.8 – 3.7, where the highest enrichment was seen for the stilbene compounds **3** and **4**.



Table 2. Amounts of diarylic (1 – 5) and monoarylic compounds (6 – 11) in lignin samples A-F. The compound identities are specified in Figure 2.

Sample	Compound, % (w/w)										
	1	2	3	4	5	6	7	8	9	10	11
A	n.d ^a	0.60	0.21	0.45	1.16	0.09	0.50	0.05	0.27	0.05	0.29
B	n.d	1.66	0.78	1.67	4.1	0.27	1.35	0.09	0.57	0.08	0.63
C	n.d	0.26	0.03	0.06	0.14	n.d	0.07	n.d	0.12	n.d	0.09
D	n.d	0.73	0.14	0.14	0.22	n.d	0.06	0.15	0.49	n.d	0.21
E	n.d	- ^b	1.06	-	-	n.d	-	0.07	-	n.d	-
F	0.09	-	1.30	-	-	0.24	-	0.10	-	0.11	-

^an.d = Not detected. ^b- = Not detected and not expected in softwood lignin.

The stilbene concentration was reduced by a factor 7 in the retentate sample C. Sample B contained 8.2 w/w % of identified diaryl compounds and 3 w/w % of identified monoaryl compounds. The softwood lignin samples (E and F) had lower content of diaryl and monoaryl compounds than the hardwood lignin samples. The content of guaiacol and syringol (6 and 7) fluctuated within the range of samples. No guaiacol was recorded above the detection limit (approximately 0.05 w/w %) in samples C – E. The birch lignin, sample D, contained higher amounts of aldehydes, compounds 8 and 9, compared to sample A, but lower amounts of the other measured compounds. The guaiacol content recorded for sample F was notably high compared to the authors experience on what has been recorded with headspace SPME GC/MS for other lignin samples.

CONCLUSIONS

These measurements present a novel way of quantifying low molar mass compounds in lignin and can be expanded upon to other compounds, upon identification. The methods generated both qualitative and quantitative results for a complex sample matrix. The methods were found to be repeatable, selective and sensitive enough for the intended use. If improved sensitivity is needed, the possibility to increase sample amounts and/or to concentrate the sample extract is available. Investigating the quantities of compounds 1 - 11 in a small series of lignins of different origins and pre-treatment methods showed that the amounts of these compounds clearly varies with dependence on pretreatment. This constitutes a good base for further investigations.

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