Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1109 (2006) 267–272
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Qualitative and quantitative determination of extractives in heartwood of Scots pine (*Pinus sylvestris* L.) by gas chromatography

Dag Ekeberg [a,*], Per-Otto Flæte [b], Morten Eikenes [b], Monica Fongen [b], Carl Fredrik Naess-Andresen [a]

[a] *University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, P.O. Box 5003, N-1432 Ås, Norway*
[b] *Norwegian Forest Research Institute, Høgskoleveien 8, N-1432 Ås, Norway*

Received 9 November 2005; received in revised form 5 January 2006; accepted 10 January 2006
Available online 3 February 2006

Abstract

A method for quantitative determination of extractives from heartwood of Scots pine (*Pinus sylvestris* L.) using gas chromatography (GC) with flame ionization detection (FID) was developed. The limit of detection (LOD) was 0.03 mg/g wood and the linear range ($r = 0.9994$) was up to 10 mg/g with accuracy within ±10% and precision of 18% relative standard deviation. The identification of the extractives was performed using gas chromatography combined with mass spectrometry (GC–MS). The yields of extraction by Soxhlet were tested for solid wood, small particles and fine powder. Small particles were chosen for further analysis. This treatment gave good yields of the most important extractives: pinosylvin, pinosylvin monomethyl ether, resin acids and free fatty acids. The method is used to demonstrate the variation of these extractives across stems and differences in north–south direction.

Keywords: Pinewood extractives; Pinosylvin; Resin acids; Free fatty acids; GC–FID; GC–MS

1. Introduction

As the Norwegian Pollution Control Authority has recently proposed the prohibition of traditional wood preservation methods like CCA (copper, chromium, arsenic) or creosote impregnation, there has been increased interest in natural wood durability and other, less hazardous, protecting agents. This has brought interest in the use of heartwood and lightwood as a construction timber with increased natural durability [1]. The heartwood is the inner part of the stem and can often be detected visually by its dark colour (see Fig. 1), caused by increased amounts of extractives [2]. Wood extractives are chemical components like resin acids, free fatty acids and phenolic compounds which exist in the heartwood of Scots pine among other trees. Historically, carpenters have often used Scots pine timber, especially the heartwood, since it is renowned for its structural qualities and durability. In a study on the improvement of natural wood durability, a method for qualitative and quantitative determination of extractives in heartwood of Scots pine (*Pinus sylvestris L.*) was developed using gas chromatography (GC) combined with flame ionization detection (FID) or mass spectrometry (MS). Some of these extractives increase the natural wood durability against destruction by microorganisms and insects. A phenolic compound called pinosylvin, which is classified as a stilbene, has shown a great influence on natural wood durability and resin acids have a restraining effect on fungi [2].

In 1999, Bergstrøm et al. [3] described the changes of concentration and distribution in pinosylvin across sapwood and heartwood. They found that there was no pinosylvin in the sapwood and that the amount of pinosylvin in the inner heartwood was lower than that in the outer. The technique used was Fourier transform near-infrared (FT-NIR) Raman spectroscopy.

Scots pine heartwood and lightwood are water repellent because of large amount of hydrophobic acids which block the hydroxyl groups of the wood cell walls and encrust cell cavities [4,5]. In addition, pinosylvin and pinosylvin methyl ether are considered to be determining factors active against decay [6].

The aim of this work was to develop a method for qualitative and quantitative determination of wood extractives using

* Corresponding author. Fax: +47 64965901.
E-mail address: dag.ekeberg@umb.no (D. Ekeberg).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.01.027

Fig. 1. The selection of 10 samples from trunk discs A and B to measure the distribution of extractives across heartwood and sapwood.

GC–FID or GC–MS. This method will be used to describe the variations in natural durability of Scots pine heartwood.

Methods using different solvents for extraction of pinosylvin and pinosylvine methyl ether have been published. In 2000, Ingram et al. [7] published a method where they used dichlomethane and 1,4-dichlorobenzene in a Soxhlet extraction. Earlier benzene has been used [8]. Since benzene has been shown to be carcinogenic and halogenated solvents have been shown to be mutagenic, there is a need to use alternative solvents. Several workers have found acetone to be a suitable solvent for the analysis of extractives in pulp and paper samples [9 and references therein]. Acetone in combination with other solvents as e.g. water has also been used. In 2004, Venäläinen et al. [10] published a method where acetone/water 80/20 v/v was used.

The work consisted of estimating the limit of detection, determining linear range, accuracy and precision and identifying the wood extractives. To obtain the highest yield of wood extractives from the samples, studies were performed on how to process the wood samples before extraction. The last part of this work was done to see how the wood extractives were distributed across the heartwood and sapwood, and in the north–south and east–west directions of the heartwood.

2. Experimental

2.1. Chemicals and standards

The standards used for calibration and identification where pinosylvin and pinosylvin monomethyl ether (CAS number 22139-77-1 and 35302-70-6, respectively, Apin chemicals, Abingdon, UK), palustric acid (90–95%, CAS number 1945-53-5), pimaric acid (75–80%, CAS number 127-27-5), levopimaric acid (95%, CAS number 79-54-9) and neoabietic acid (>99%, CAS 471-77-2) all Helix Biotech, New Westminster, Canada). Heptadecanoic acid (99%, CAS number 506-12-7, Fluka, Steinheim, Germany) and diethylstilbestrol (99%, CAS number 56-53-1, Sigma, Steinheim, Germany) were used as internal standard and for evaluation of performance.

Acetone (analytical-reagent grade, Merck, Darmstadt, Germany) was used to prepare stock solutions of the internal standard and for the extraction procedure. The extracts were redissolved in absolute dry pyridine (puriss, $H_2O < 0.05\%$, Fluka) and silylated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, ≥99%, CAS number 25561-30-2, Sigma) which was kept at 4 °C under argon atmosphere.

Stock solutions of the heptadecanoic acid and diethylstilbestrol were prepared by dissolving 140 and 200 mg in 50 mL acetone, respectively. Both stock solutions were kept in the dark at 4 °C.

2.2. Wood samples

The samples were taken from five different trees, A–E. All samples were from Scots pine, *P. sylvestris* L. The stem of a Scots pine tree consists of sapwood and heartwood. All wood samples used in this study were prepared from cross sectional sample discs taken from mature Scots pine trees at a trunk height of about 5 m above the base. The average height of the trees was 21 m and the average stem diameter at ground level was 41.5 cm.

2.3. Extraction of wood samples

Ground wood samples (200 mg) were placed in cellulose filters (10 mm × 50 mm Munktell Filters, Grycksbo, Sweden), which were then sealed with wads of cotton wool. Both the filters and the cotton wads were washed in acetone prior to use. Acetone (pro analysis, 25 mL) and stock solution of internal standard (100 μL) were added to the extraction bottles. The filters and the bottles were placed in a Soxhlet extraction unit (FexIKA 50, IKA Werke, Staufen, Germany). The extraction unit was heated to 78 °C and kept there for 4 min. The solvent was then cooled to 40 °C. The heating and cooling cycle were repeated 36 times with a total time of 9 h. The extractions were started in the afternoon and the solvent was removed by vacuum evaporation in a rotary evaporator (RV06-ML, IKA Werke) at 50 °C the next day. The extractives were then redissolved in absolute dry pyridine (1 mL) and silylated with addition of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (500 μL) and left at ambient temperature for minimum 1 h before analysis by GC–FID or GC–MS.

2.4. Evaluation of sampling

A piece of Scots pine heartwood was divided into se 3 × 3 equal sized sub-samples (10 mm × 5 mm × 30 mm) in the longitudinal and tangential directions. Three sub-sets of three samples were chosen so that none of the samples within a sub-set had an origin next to each other in the original sample. The three sub-sets were then extracted as (a) solid wood, (b) small