Applications of thin-layer chromatography to process control in the pulp and paper field

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Abstract

A thin-layer chromatographic method for analytical separation and detection of the main component groups in extracts of wood, pulp and paper was designed. The method separates fatty acids from resin acids, which has not been reported in earlier studies. Neutral extractives were separated into sterols, triglycerides and steryl esters. The method proved to be useful for troubleshooting and production control and a number of applications are given.

Keywords: Pulp; Wood; Paper; Fats; Fatty acids; Resin acids; Sterols; Terpenes; Phenolic compounds

1. Introduction

Up to 5% of the dry weight of wood consists of materials extractable with organic solvents. The composition of the extractives varies among wood species, and it is a complex mixture of fats (esters), fatty acids, resin acids, sterols and other terpenes as well as phenolic compounds [1]. The extractives can cause problems in the production of pulp and paper, for instance by depositing on the papermaking equipment. A high residual content of extractives can deteriorate the quality of the products, especially the quality of pulp used for hygienic products or paper used for food packaging.

Techniques for group separation of the extractives of wood and pulp are demanded for quality control, troubleshooting and method development. Time-consuming fractionation schemes based on consecutive extractions with

different solvents together with derivatization steps have been reported [2]. A more modern separation of lipid classes in acetone extracts by solid-phase extraction has been described by Chen et al. [3]. However, this method did not separate fatty acids from resin acids.

Thin-layer chromatography (TLC) is an attractive technique for group separations of extracts. Most solvents can be applied on the plates and there is no interference between the sample solvents and the mobile phase. The technique is fast and it allows rough quantification by scanning the developed plates with an appropriate densitometer.

TLC has been used widely for the separation of lipids in the food industry [4]. TLC has also been used in the pulp and paper industry for preparative fractionation of wood extractives. The final determinations have been performed with other techniques. Tall oil [5], a by-product from the kraft pulp process, has been studied with TLC by several authors [6–10]. Paasonen

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used TLC and GC together with MS, IR and NMR in his study of the extractives of birch wood and birch kraft pulp [11]. Ekman studied the composition of the extractives in spruce wood by TLC and GC [12].

The objective of this study was to develop a rapid method for analytical separation and detection of the main component groups in extracts of wood, pulp and paper. The method should be a complement to the more tedious GC methods with derivatization steps [13,14]. The method should allow separation of fatty acids from resin acids, which has not been reported in the earlier studies.

A method based on TLC was developed and applied on the analysis of extracts of wood, pulp, paper as well as of deposits from the pulp and paper mills.

2. Experimental

2.1. Chromatographic conditions

Neutral compounds were eluted on silica-coated HPTLC-plates purchased from Merck, Darmstadt, Germany (Silica Gel 60 F_{254}, 10 × 10 cm or 20 × 10 cm) with a mobile phase consisting of heptane–acetone–ammonia (NH_3 25% aq.) (84.5:15:0.5, v/v/v). The plates were pre-washed with methanol, dried and activated (105°C) for at least 1 h in a laboratory oven, cooled to room temperature before sample application and pre-conditioned with the vapour of the mobile phase for 1 min in a twin trough chamber before development. The migration distance for the solvent front was about 7 cm.

Fatty acids and resin acids were separated on the silica plates described above using dichloromethane–methanol–ammonia (NH_3 25% aq.) (80:19:1, v/v/v) as the mobile phase. The plates were preconditioned with the vapour of the mobile phase for 5 min in a twin trough chamber before development.

Aliquots of the extracts (1 μl, 2 μl) were applied on the plates spotwise, by the use of Nanomat II from CAMAG, Muttenz, Switzerland. The starting line for the spots were usually 10 mm from the edge of the plate and the spots

were applied 10 mm apart. The plates were eluted at ambient temperature and humidity in twin trough chambers (CAMAG 10 × 10 cm or 10 × 20 cm) and evaluated with densitometric scanning in the UV-range using the TLC Scanner II with CATS Software (CAMAG). Standard (calibration and reference) and samples were applied on the same plate.

For gradient elution the AMD (automated multiple development) from CAMAG was used. The chromatograms were developed repeatedly in one direction with increasing migration distances. The polarity of the eluents decreased for each successive run resulting in a stepwise gradient starting with methanol, followed by dichloromethane and methanol mixtures, pure dichloromethane, dichloromethane and hexane mixtures and finally pure hexane. The samples were applied bandwise (usually 5 μl sample in a 5-mm-long band) using Linomat IV from CAMAG.

2.2. Chemicals and reference substances

The solvents used were of analytical grade and purchased from Merck (Darmstadt, Germany). Standard substances were purchased from Sigma (St Louis, MO, USA) except abietic acid and dehydroabietic acid which were purchased from Helix Biotech (Richmond, Canada) and sitosterol which was purchased from Merck. A sterol mixture obtained from Kymmene AB in Finland was used as a reference to locate sterols. Lignans were identified by scraping the spot from the plate, extracting the silica with acetone and analyzing the extract with GC–MS after silylation.

2.3. GC analysis of fatty acids and sterols

The fatty acids were determined as methyl esters and sterols as silyl ethers by capillary GC according to Ekman and Holmbom [14].

2.4. Extractions

The extractions were performed in standard Soxhlet equipment with acetone [15] or with dichloromethane (CH_2Cl_2) [16]. The volumes of

the extracts were adjusted to 10 ml for quantitative comparisons.

2.5. Samples

Unbleached chemithermomechanical pulp (CTMP) from spruce (*Picea abies*) and pine (*Pinus sylvestris*), as well as unbleached and bleached kraft pulp from birch (*Betula verrucosa*), were obtained from pulp mills in Sweden.

Dark spots in carton board, 5–10 mm in diameter, were analyzed in order to eliminate the formation of new spots. The spots originated probably from a deposit on the drying cylinders of the board machine. The spots were dissolved in acetone–acetic acid (10:1, v/v) in an ultrasonic bath. The soluble part was analyzed with TLC.

3. Results and discussion

3.1. Chromatography

Neutral compounds were separated into groups with a fairly non-polar solvent mixture containing aqueous ammonia (Fig. 1A). Ammonia shifts the equilibrium of fatty acids and resin acids more to the ionized form, thus keeping the acids in the start spot. The mobile phase was almost saturated with aqueous ammonia and an increase in the concentration of ammonia resulted in an immiscible mixture.

Fatty acids and resin acids were eluted on another TLC plate with a more polar alkaline mobile phase (Fig. 1B). In this system the neutral compounds moved along the solvent front. Fatty acids were separated completely from resin acids, which has not been achieved in earlier studies. The mobile phase pH was optimized for the separation of acids (Table 1).

Using two different eluents on two separate plates for the separation of neutral groups and acids was faster and simpler than to use one gradient elution. However, gradient elution was found to be useful for the separation of neutral compounds with similar polarity in complex mixtures. Squalene and steryl esters, present in birch wood, were separated from each other
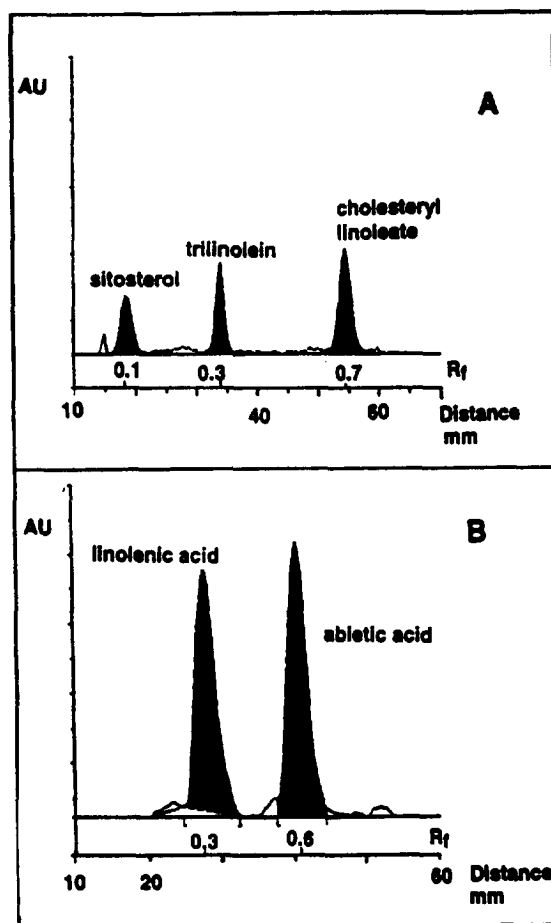

Fig. 1. The separation of neutral extractives (A) and the separation of resin acids and fatty acids (B).

without interferences from other extractive groups with a 25-step gradient.

3.2. Quantification

In order to choose the best wavelength for quantification, UV-spectra were recorded for different resin acids, for free fatty acids with different numbers of double bonds in the carbon chain, as well as for triglycerides and steryl esters of the different fatty acids. The spectra were established in solutions as well as densitometrically on TLC plates. The spectra showed that 200 nm was a sensitive wavelength for scanning most compounds of interest. They also showed that the molar absorptivity was, as expected,

Table 1
Resolution between fatty acids (FA) and resin acids (RA) obtained on silica plates with different mobile phases

Mobile phase	Rs (FA and RA)	R_F for RA
CH_2Cl_2–CH_3OH–HAc (94.5:3:2.5)	ca. 1.5 RA asymmetric	0.5
CH_2Cl_2	no separation	0.1
CH_2Cl_2–CH_3OH–NH_3 (25%) (80:19:1)	≥3	0.6
CH_2Cl_2–CH_3OH–NH_3 (25%) (80:16:4)	ca. 1	0.4

dependent on the number of double bonds in the carbon chain.

The fatty acid distribution in different pulp samples was studied with capillary GC. The average number of double bonds was determined from the GC analysis and correction factors for different types of samples and different standards, to be used when scanning densitometrically at 200 nm, were calculated. The concentration of the standard solutions were multiplied with the correction factor when quantifying the samples (Table 2). Response factors are strongly dependent on the wood raw material, the pulping process and pulp conditions and figures in Table 2 should be regarded as examples.

Resin acids have similar absorptivities within the group except from dehydroabietic acid. By detecting the resin acids at two different wavelengths the amount of dehydroabietic acid and the amount of other resin acids can be calculated (Eqs. 1, 2). The absorbance of dehydroabietic acid at 240 nm can be neglected. The equations below are valid only in the linearity range, or within a narrow part of the standard curve, which can be approximated as linear. Hence, the standard and sample areas should be within such a narrow range.

μg abietic acid in the sample ≈

$$SA_{240} \times a_{Ab240} \qquad (1)$$

μg dehydroabietic acid in the sample ≈

$$(SA_{200} - \mu g \text{ abietic acid}/a_{Ab200}) \times a_{Deab200} \qquad (2)$$

where: SA_{240} = measured sample area (TLC) at 240 nm; SA_{200} = measured sample area (TLC) at 200 nm; a_{Ab240} = response factor at 240 nm for abietic acid (μg/area) calculated from the abietic acid standard; a_{Ab200} = response factor at 200 nm for abietic acid (μg/area) calculated from the abietic acid standard; $a_{Deab200}$ = response factor at 200 nm for dehydroabietic acid (μg/area) calculated from the dehydroabietic acid standard. The amount of abietic acid calculated includes all the other resin acids except dehydroabietic acid.

Table 2
Examples of correction factors used for scanning at 200 nm to compensate for the deviation in saturation between standard and sample (the standard concentration should be multiplied with the correction factors before quantifying the samples)

Standard		Sample	
Component group	Standard substance	Unbleached CTMP	Unbleached softwood kraft pulp
Fatty acids	Oleic acid	0.6	0.8
	Linoleic acid	1.4	1.8
Triglycerides	Triolein	0.4	0.4
	Trilinolein	1.0	1.1
Steryl esters	Cholesteryl stearate	0.4	0.4
	Cholesteryl linoleate	1.0	1.0

Different sterols were separated from each other on the plate eluted with the non-polar solvent mixture. For quantification standards were chosen depending on the appearance of the chromatogram. Sitosterol, sterol mixture and betulinol were used without any correction factors.

The densitometric standard curves were linear only within a narrow range. Thus, when single level standards were used the standard and samples were at the same concentration level. The curve was approximated to be linear in the range between the sample and standard concentration. Non-linear standard curves for a wider concentration range were established with the CATS software. Typical standard amounts on the plate were 0.2-2 μg.

The relative standard deviation for eight applications of linoleic acid (1.7 μg) analyzed on the same plate was 2.2%. The relative standard deviation for samples depends on the composition of the extracts.

3.3. Applications

Comparison of extraction techniques

TLC is a simple technique for evaluation of extraction techniques. The selectivity of the TLC methods allowed free choice of extraction solvent (Fig. 2). Dichloromethane, which used to be the standard solvent for extractions of wood, pulp and paper, has been replaced by acetone because of health and environmental reasons. Acetone is a less selective solvent, giving extracts which include more polar extractives such as hydroxymatairesinol and other phenolic compounds referred to as lignans [17]. Thus, weighing the extract does not necessarily reveal the amount of compounds of interest, and simple group separation techniques such as TLC are desired.

Pulp washing and pulp bleaching

Pulp washing is important for the cleanliness of the products, especially for food packaging materials and tissues. It is also important to minimize the amount of extractives reaching the bleaching plant in order to reduce the consump-
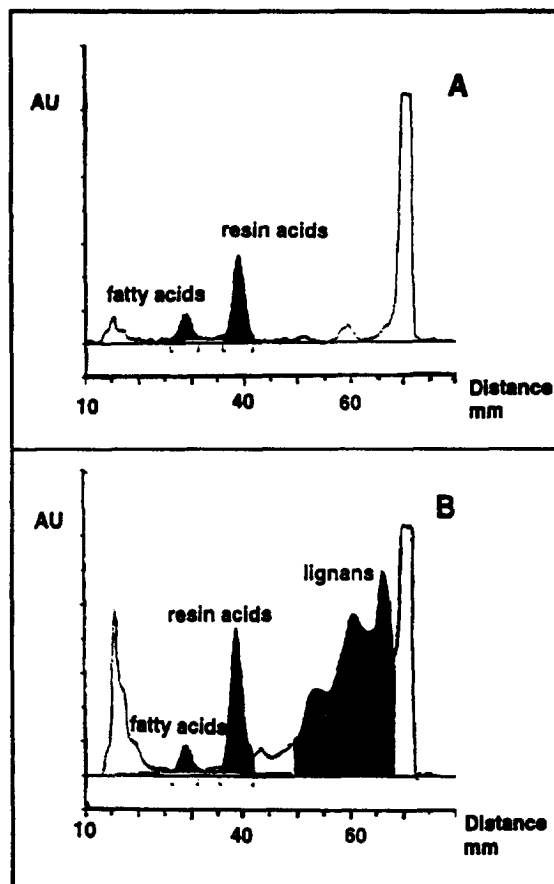

Fig. 2. Dichloromethane-extractives (A) and acetone-extractives (B) of unbleached CTMP.

tion of bleaching chemicals. Chlorination of extractives during chlorine (Cl_2) bleaching and to a minor extent during chlorine dioxide (ClO_2) bleaching might lead to increased emissions of organic chlorine compounds to the bleaching effluents and mill recipients [18,19].

TLC was used to study the influence of washing-water pH on the extractives of unbleached birch kraft pulp. The analysis showed the importance of keeping the pH of the washing water high (Fig. 3). Neutral compounds are solubilized by fatty acid soaps which explains the influence of pH on the removal of neutral compounds. Resin acids are not found in hardwoods. Sterols were determined separately by GC.
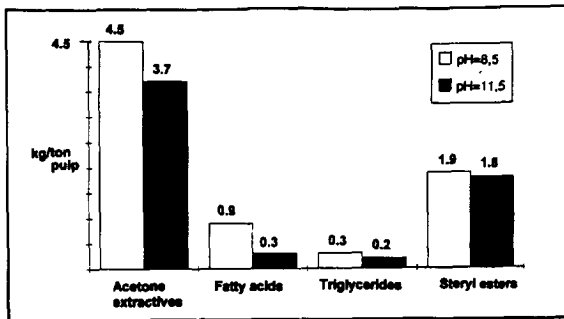
The chromatograms of unbleached and ClO_2-

Fig. 3. The influence of washing-water pH on the amount of extractives in unbleached birch kraft pulp.

bleached birch kraft pulps showed that the composition of extractives is changed during bleaching (Fig. 4). It appears that especially sitosterol and steryl esters react with ClO_2. The compound before sitosterol, probably sitostanol, seemed unaffected during bleaching [20].

TLC is not the method of choice when analyzing extractives from Cl_2-bleached kraft pulps. Cl_2 causes extensive chlorination of the extractives and no study of chlorinated extractives has been done with this method. Chlorinated organic compounds formed during Cl_2-bleaching and their effects on the mill recipients is the reason why North-European kraft pulp mills have replaced Cl_2 by oxidative bleaching agents [18,19].
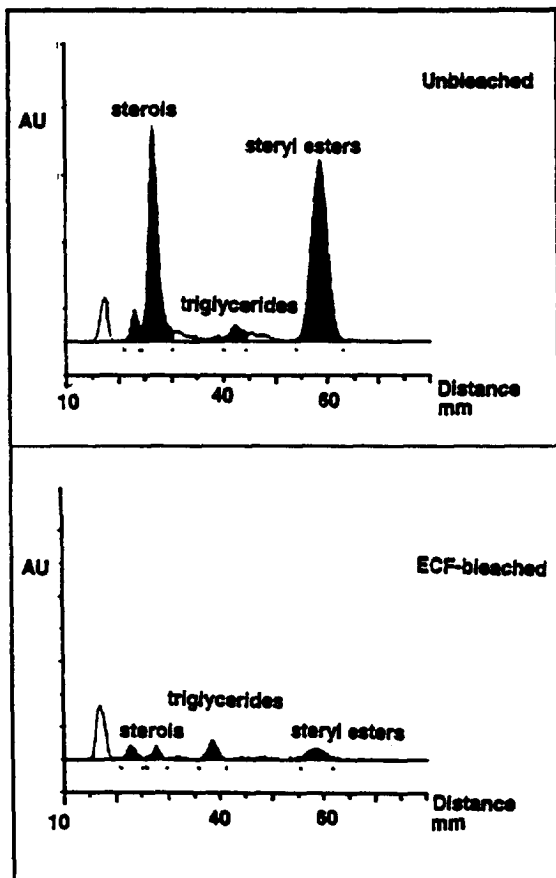

Fig. 4. Analysis of neutral extractives in unbleached and ClO_2-bleached birch kraft pulp (ECF). The chromatograms show that ClO_2 reacts with sterols and steryl esters.
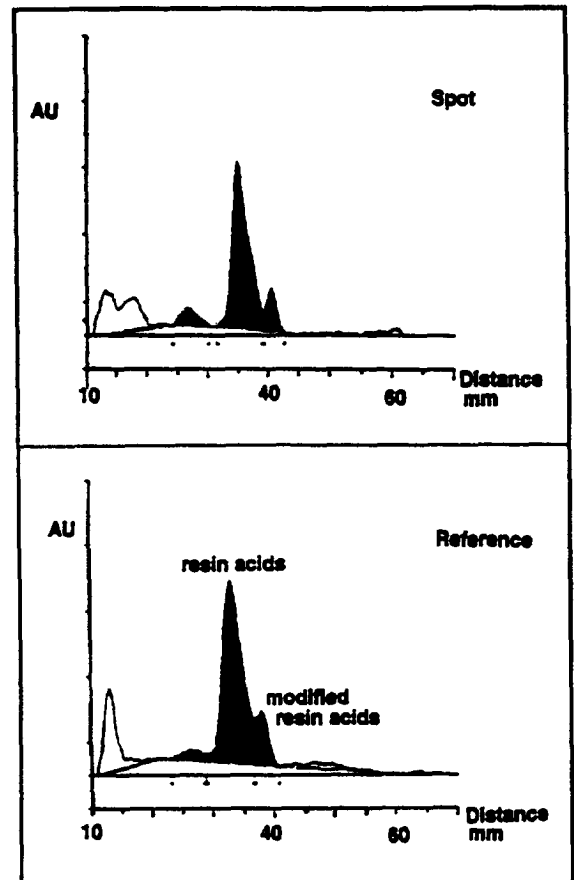

Fig. 5. Analysis of spots in carton board. The analysis of the extract shows that the deposit consists mainly of resin acids and the chromatogram is almost identical with the chromatogram of an extract of rosin size.

Analysis of deposits.

Spots in paper, probably originating from deposited material on the drying cylinders, were analysed after dissolution in acetone–acetic acid. The extract was compared with pulp extractives as well as with chemicals used in the paper production. The TLC analysis showed that the spots consisted mainly of rosin size (Fig. 5). Rosin size contains resin acids as the main component and it is used to increase the hydrophobicity of the paper.

4. Conclusions

A rapid and simple TLC-method was developed for the analysis of extractives in different samples from the pulp and paper industry. The method separates the extracts into the main component groups, and it is useful for troubleshooting and production control.

Acknowledgement

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