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**DEVELOPMENT OF FAST ANALYSIS METHODS FOR EXTRACTIVES  
IN PAPERMAKING PROCESS WATERS**

BY

**PIIA VALTO**

Academic Dissertation for the Degree of  
Doctor of Philosophy

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## ABSTRACT

A fast analysis method with an online sample enrichment technique for the analysis of resin and fatty acids present in papermaking process waters was developed. This method was based on atmospheric pressure chemical ionization (APCI) and mass spectrometry (MS) for the detection of  $[M-H]^-$  ions of compounds of interest. The method was applied to the control of the most prevalent resin (dehydroabietic and abietic) and fatty (palmitic, stearic, oleic, and linolenic) acid levels in papermaking process waters. Continuous data on these acids are of practical importance when predicting possible deposit-derived problems in the paper machine.

The first part of the study was focused on the development of an online sample enrichment system for the analysis equipment used. The method was partially validated and tested for the selected resin and fatty acids in aqueous media and papermaking process waters. The online sample enrichment step was connected to the analytical system through a standard high-performance liquid chromatography (HPLC) precolumn and time-controlled column switching valves. In the second part, the feasibility of the method developed was tested by means of three different applications: laboratory-scale online sample enrichment studies, enrichment of resin and fatty acids when increasing water reuse, and the development of a faster offline laboratory method with HPLC column separation. The column separation was validated by means of selectivity, repeatability, precision, and limits of detection (LOD). These validation results clearly indicated that the HPLC-APCI-MS method with column separation was applicable for this purpose.

The analysis system developed provides a potential tool for monitoring selected resin and fatty acids for many applications of practical importance. It was noticed that measuring only the resin acid concentration level, for example, may offer a possible indirect control method for papermaking. The results also indicated that the fast offline HPLC-APCI-MS technique with column separation of the compounds of interest is a useful alternative to other traditional analysis methods such as gas chromatography (GC). The main benefits of this new method are simplicity of use, absence of the need for multistage sample pretreatment, and rapidity of measurement.

## PREFACE

This doctoral thesis is based on experimental work carried out in the Laboratory of Applied Chemistry at the Department of Chemistry, University of Jyväskylä, during 2005-2010.

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Jyväskylä, August 2011

Piia Valto

## LIST OF ORIGINAL PUBLICATIONS

This thesis consists of an overview and of the following five publications, referred to in the text by Roman numerals (I-V).

I Piia Valto, Juha Knuutinen, Raimo Alén, Resin and fatty-acid analysis by solid-phase extraction coupled to atmospheric pressure chemical ionization-mass spectrometry, *Intern. J. Environ. Anal. Chem.*, 87(2): 87-97 (2007).

II Piia Valto, Juha Knuutinen, Raimo Alén, Fast analysis of relative levels of dehydroabiatic acid in papermaking process waters by on-line sample enrichment followed by atmospheric pressure chemical ionization-mass spectrometry (APCI-MS), *Intern. J. Environ. Anal. Chem.*, 88(13): 969-978 (2008).

III Piia Valto, Juha Knuutinen, Raimo Alén, Evaluation of resin and fatty acid concentration levels by online sample enrichment followed by atmospheric pressure chemical ionization-mass spectrometry (APCI-MS), *Environ. Sci. Pollut. Res.*, 16(3): 287-294 (2009).

IV Piia Valto, Juha Knuutinen, Raimo Alén, Mikko Rantalankila, Jani Lehmonen, Antti Grönroos, Juha Houni, Analysis of resin and fatty acids enriched in papermaking process waters, *BioResources*, 5(1): 172-186 (2010).

V Piia Valto, Juha Knuutinen, Raimo Alén, Direct injection analysis of fatty and resin acids in papermaking process waters by HPLC/MS, *J. Sep. Sci.*, 34(8): 925-930 (2011).

In all publications, the planning of the experimental work and writing the manuscripts were carried out by the author and the manuscripts were completed with co-authors. In publication I the laboratory work was carried out with Heidi Ahkola, in publication II with Marjut Lindh, and in publication III with Jaana Käkölä. In publication IV, the experimental work was carried out with Mikko Rantalankila and the samples were provided by the other co-authors. In publication V, the experimental work was partly based on HPLC separation method developed by Jaana Käkölä and author performed the laboratory work and validation.

*Other related publications by the author*

Juha Knuutinen, Raimo Alén, Piia Valto, Extraction and analysis of paper industry process waters and waste waters, especially for fatty and resin acids and esters, Finn. (2003). 10 pp. Patent written in Finnish. FIXXAP FI 111416 BI 20030715.

Piia Valto, Juha Knuutinen, Raimo Alén, A fast determination of resin and fatty acid levels in papermaking process waters, 15<sup>th</sup> International Symposium on Wood, Fiber and Pulping Chemistry - ISWFPC, Oslo, Norway, June, (2009).

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## ABBREVIATIONS

ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionization
ASE	Accelerated solvent extraction
ATR	Attenuated total reflection
BOD	Biological oxygen demand
BSTFA	<i>N,O</i> -bis(trimethylsilyl)trifluoroacetamide
CE	Capillary electrophoresis
COD	Chemical oxygen demand
CTMP	Chemi-thermomechanical pulping
DCM	Dissolved and colloidal material
DCS	Dissolved and colloidal substances
DHA	Dehydroabietin
DHAA	Dehydroabietic acid
ECF	Elemental chlorine-free
EIC	Extracted ion current
ESI	Electrospray ionization
FIA	Flow injection analysis
FID	Flame ionization detector
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
HP	Hewlett Packard
HPLC	High-performance liquid chromatography
LC	Liquid chromatography
LIF	Laser induced fluorescence
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
MSD	Mass spectrometric detection
MTBE	Methyl <i>tert</i> -butyl ether
NI	Negative ionization
NPE	Non-process element
NTU	Nephelometric turbidity unit
PFB	Pentafluorobenzyl
RF	Response factor
RP	Reversed-phase
RSD	Relative standard deviation

SCD	Short circulation device
SE	Size-exclusion
SEC	Size-exclusion chromatography
SIM	Selected ion monitoring
S/N	Signal-to-noise ratio
SPE	Solid phase extraction
THR	Tetrahydroretene
TLC	Thin layer chromatography
TMCS	Trimethylchlorosilane
TMP	Thermomechanical pulping
TMS	Trimethylsilyl
TOC	Total organic carbon
UHQ	Ultra high quality
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet
VIS	Visible
WO	Wet oxidation
XAD	Macroreticular, non-ionic resin

# 1 INTRODUCTION

## 1.1 Background

The pulp and paper industry is responsible for a large amount of water usage throughout the world. Environmental legislation and national economic situations are leading to an increase in water circulation closure and a decrease in fresh water usage [Ali and Srekrishnan, 2001; Latorre *et al.*, 2005]. Paper mills have answered new, tighter regulations by upgrading or replacing, for example, bleaching facilities with elemental chlorine-free (ECF) bleaching or adding extended delignification in pulping. The emissions of various oxygen-demanding substances have been reduced, and the use of highly chlorinated substances has been eliminated. Although water usage is essential to papermaking, ideas for reduction in fresh water use and further recycling of effluents have been presented [Gavrilescu *et al.*, 2008]. The non-process elements (NPEs) entering the pulp mill with the wood have been suggested to be potential air and water contaminants and they possibly contribute to the solid waste.

Due to the water circulation closure, the papermaking industry has had to encounter a new challenge caused by the possible concentration of harmful substances, *i.e.*, wood resin constituents in water circulation [Lacorte *et al.*, 2003]. Resin and fatty acids are one of the most important wood resin constituents because of their important role in several process problems, such as lower pulp quality, foaming, odor, and effluent toxicity [Holmbom, 1999a; Sitholé, 2007]. To prevent these compounds from causing pitch deposits, one possibility is to bind soaps formed by resin and fatty acids to the mechanically pulped fibers by adding complex forming additives, thus binding pitch droplets to the fiber surface through the presence of the complex.

The role of analytical chemistry in resolving problems caused by wood extractives is also vital. Monitoring resin acids during storage time, for example, plays an important role in the further success of the papermaking process. The most common method for the analysis of resin and fatty acids in papermaking process waters is GC with flame ionization detector (FID) [Örså and Holmbom, 1994]. This technique and many other current methods are rather time-consuming and include a complicated pretreatment, such as solvent extraction and derivatization of the evaporated samples, before actual chromatographic analysis. The use of HPLC with mass spectrometric detection (MSD) provides a useful alternative with excellent repeatability and without complicated sample

pretreatment steps, thus ensuring faster analysis with almost real-time results for process control.

## 1.2 Objectives of the study

The main purpose of the study was to develop a fast analytical method for the analysis of wood extractives in industrial process waters by using the HPLC-MS technique. At the same time, the aim was to increase the general knowledge of the behavior of resin and fatty acids in the paper manufacturing process by also analyzing these compounds through traditional methods like GC. The more detailed objectives were to:

- *develop an online sample enrichment technique and connect it with HPLC-MS*
- *evaluate the suitability of the method developed to analyze selected resin and fatty acids in papermaking process waters and to compare the results with those of traditional analysis methods*
- *apply the methods developed to different applications such as laboratory-scale online measurements and enrichment studies and develop a faster offline technique for laboratory purposes*

## 1.3 Research methods

The analytical procedure included the online connection of sample enrichment (solid phase extraction (SPE)) with the HPLC-MS using APCI in a negative ionization (NI) mode. Traditional solvent extraction (liquid-liquid extraction, (LLE)) combined with GC analysis was also used in order to compare the results obtained by analysis methods used. The method quality parameters were measured and the performance of the enrichment columns with different sorbent phases was tested by means of these partial validation parameters.

The online sample enrichment APCI-MS method developed was applied to the analysis of the most prevalent resin and fatty acids in papermaking process waters. These compounds were selected based on their occurrence in the process and also their characteristic properties, such as toxicity and degradability. The applicability of the online sample enrichment APCI-MS method was tested by conducting laboratory-scale online studies with a large amount of process water sample recycled through a ceramic filter and passed to analysis with time-controlled switching valves. The buildup of resin and fatty acids in process waters was demonstrated by recycling process waters through a Short Circulation Device

(SCD), a special pilot-scale installation for the demonstration of paper machine water circulation and the chemical state of the papermaking process. The validation of a faster offline HPLC-MS method for selected resin and fatty acid separation with a commercial HPLC column was also performed with standards in aqueous media and process waters from different stages of the wet-end environment of a paper machine.

## 2 LITERATURE REVIEW

### 2.1 Wood extractives and pitch

Wood extractives can be defined as lipophilic compounds that are soluble in neutral organic solvents [Sjöström, 1993; Back and Ekman, 2000]. Water-insoluble lipophilic extractives can also be called wood resin or pitch and comprise mainly free fatty acids, resin acids, waxes, fatty alcohols, steryl esters, sterols, glycerides, ketones, and other oxidized compounds. The composition and the content of wood extractives in the tree vary depending on the different parts of the tree (heartwood and sapwood), the wood species, age, the growth conditions, and environmental factors [Levitin 1970; Hillis, 1971; Alén, 2000a].

Extractives are considered a major characteristic of wood species although they constitute, depending on the wood species, only 2-5% of the total dry matter [Sjöström, 1993; Sjöström and Westermarck, 1999; Alén, 2000a]. Resin acids occur only in softwoods, and the composition of individual resin acids depends on the wood species [Holmbom, 1999a; Back and Ekman, 2000]. The composition of fatty acids also differs significantly according to the wood species and climate. Trees in warm climates produce a higher amount of saturated fatty acids but show less seasonal variability. In addition, wood extractives affect the wood's odor, color, and physical properties and play a significant role in the protection of wood from a biological attack. Examples of the chemical structures of typical wood extractives-derived compounds are presented in Figure 1.

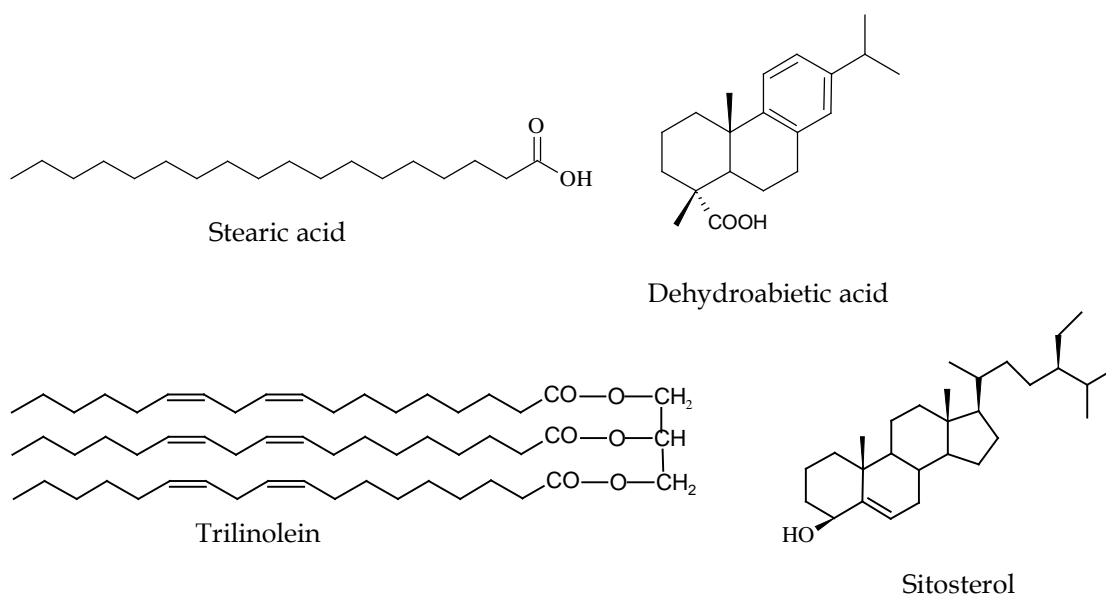


FIGURE 1. Structures of typical wood resin compounds.

The extractives have an important role in pulping and papermaking because they can produce negative effects such as process problems and lower paper quality, but they can also be useful raw materials as by-products, for example, in the form of tall oil in kraft pulping and as a source of the further production of biodiesel fuel [Holmbom, 1977; Quinde and Paszner, 1991; Sitholé, 1993; Lee *et al.*, 2006].

### 2.1.1 Wood extractives in papermaking

The increasing amount of recirculation waters - for example, white waters of the paper machine - is leading to an accumulation of a large number of harmful substances, mainly organic materials called dissolved and colloidal substances (DCS) that interfere with the papermaking system [Ricketts, 1994; Holmberg, 1999b; Holmbom and Sundberg, 2003; Latorre *et al.*, 2005; Gavrilescu *et al.*, 2008]. These substances are anionic and can often disturb the function of papermaking chemicals. DCS are known to be released especially during mechanical, chemi-mechanical, and sulfite pulping [Dorado *et al.*, 2000], and high levels of them are associated with different process problems such as the formation of pitch deposits [Laubach and Greer, 1991; Back, 2000a] and effluent toxicity [Holmbom, 1999a; Ali and Sreekrishnan 2001; Lacorte *et al.*, 2003; van Beek *et al.*, 2007]. The papermaking process itself causes the accumulation of organic compounds in the process (Fig. 2). The relations of substances present in the papermaking process depend greatly on the raw materials, additives, and energy sources used.

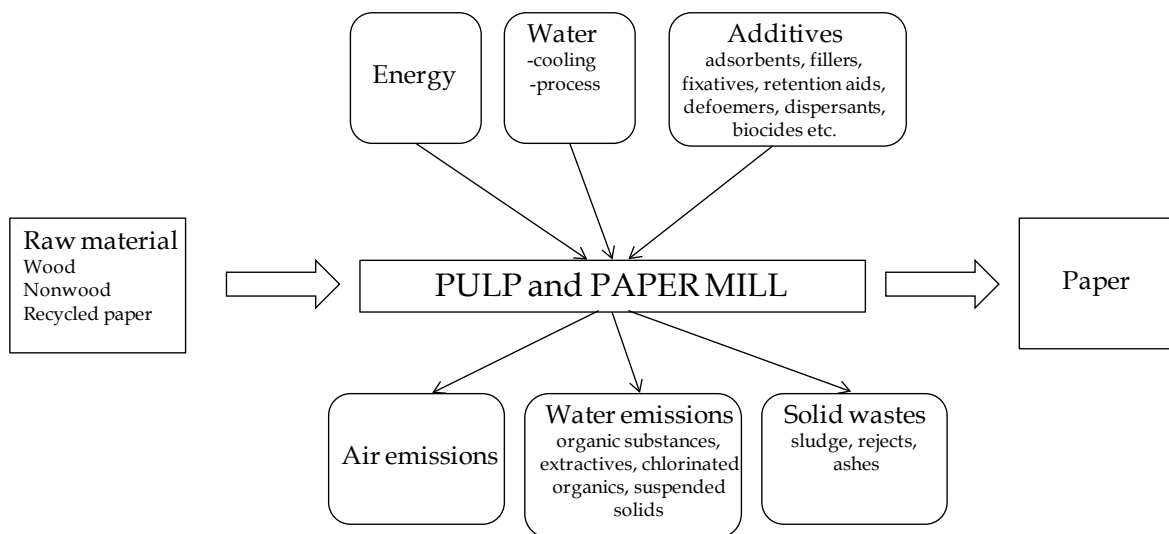


FIGURE 2. Overview of the papermaking process mass stream [Lacorte *et al.*, 2003].

In general, most studies have focused mainly on wood extractives and their role and effect on effluents [Koistinen *et al.*, 1998; Latorre *et al.*, 2005]. Due to modern waste water treatment technology, a major part of these compounds can be removed from the effluent waters, but even at low concentration levels, they can have negative effects on aquatic life and on rats, when bioaccumulating in liver, bile, and plasma [Fåhræus-Van Ree and Payne, 1999; Kostamo and Kukkonen, 2003; Rana *et al.*, 2004]. An effective effluent treatment system enables the recycling of these waters back to the paper mill, thus decreasing fresh water usage [Gavrilescu *et al.*, 2008].

The main components of the DCS are hemicelluloses, wood extractives, lignans, and lignin-related substances. They can be classified by their lipophilic and hydrophilic properties (Table 1). The extent of the problems caused by these DCS compound groups depends greatly on the wood species, the pulping process, and the degree of water circulation closure. These substances also cause considerable damage to the receiving waters if they are not treated before discharge.

TABLE 1. The main components of the DCSs [Holmberg, 1999b]

	Lipophilic/Hydrophobic	Hydrophilic
Fatty acids	X	
Flavonoids		X
Phenols		X
Resin acids	X	
Salts		X
Sterols	X	
Steryl ester	X	
Sugars		X
Tannins		X
Triglycerides	X	

### 2.1.2 Resin and fatty acids in papermaking

The role of resin and fatty acids in papermaking process waters has been studied extensively [Ali and Sreekrishnan, 2001; Lacorte *et al.*, 2003]. These compounds originate from raw materials and also from additives such as surfactants. The papermaking process releases these compounds during debarking, pulping, bleaching, washing, and with the final product, paper. Each paper manufacturing process is a unique combination of these different steps; the levels of resin and fatty acids in the process depend on the process performance. In particular, the pH of the process strongly affects the behavior of resin and fatty acids [Ström, 2000]. At high pH values, these acids dissociate and dissolve in waters, depending on the temperature and the metal ion concentrations. The metal soaps formed can either go on to form soluble aggregates or precipitate as metal salts. Therefore the  $pK_a$  values (see Table 2) of resin and fatty acids play an important role in predicting and resolving possible problems caused by these compounds [McLean *et al.*, 2005].

The most commonly found resin acids in papermaking process waters are divided into two compound groups: the abietanes (abietic, levopimaric, palustric, and neoabietic acids along with dehydroabietic acid) and the pimaranes (pimaric, isopimaric, and sandaracopimaric acids) [Sjöström, 1993; Ekman and Holmbom, 2000; Serreqi *et al.*, 2000]. Due to the chemical structure of a combination of a hydrophobic skeleton and a hydrophilic carboxyl group, they work as good solubilizing agents in the process. Dehydroabietic acid is the most common and stable (the aromatic nature of ring in structure) resin acid found in papermaking process waters and effluents [Chow and Shepard, 1996]. It also accounts for the majority of the waste water toxicity because it can transform into more toxic compounds such as retene (Fig. 3) [Judd *et al.*, 1996; Liss *et al.*, 1997; Hewitt *et al.*, 2006]. Detrimental effects of dehydroabietic acid to fish have also been reported [Bogdanova and Nikinmaa, 1998; Peng and Roberts, 2000a]. In addition,



dehydroabietic acid is the most soluble acid among the resin acids, whereas the pimaric type acids are the least soluble [Peng and Roberts, 2000a].

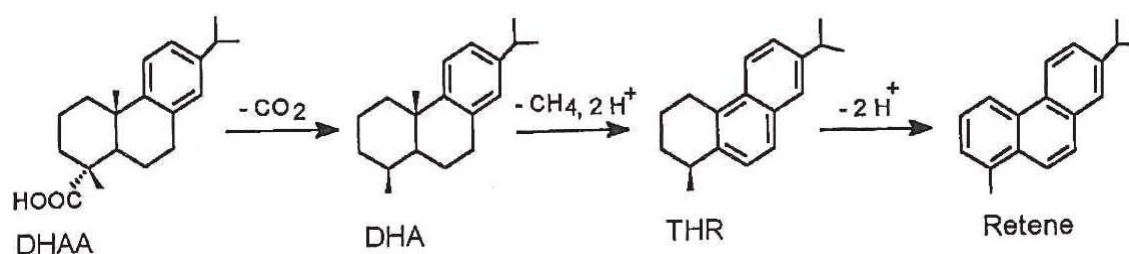


FIGURE 3. Isomerization path of dehydroabietic acid to retene [Judd *et al.*, 1996; Leppänen and Oikari, 1999]. DHAA = dehydroabietic acid, DHA = dehydroabietin, THR = tetrahydroretene.

Palustric, abietic, and neoabietic acids have a structure with conjugated diene structures, thus facilitating the isomerization process. On the other hand, pimaranes have a similar thermodynamic stability to that of dehydroabietic acid with non-conjugated double bonds, which are not significantly isomerized [Quinde and Paszner, 1991; Morales *et al.*, 1992]. The isomerization path of neoabietic and palustric acids to abietic acid is presented in Figure 4.

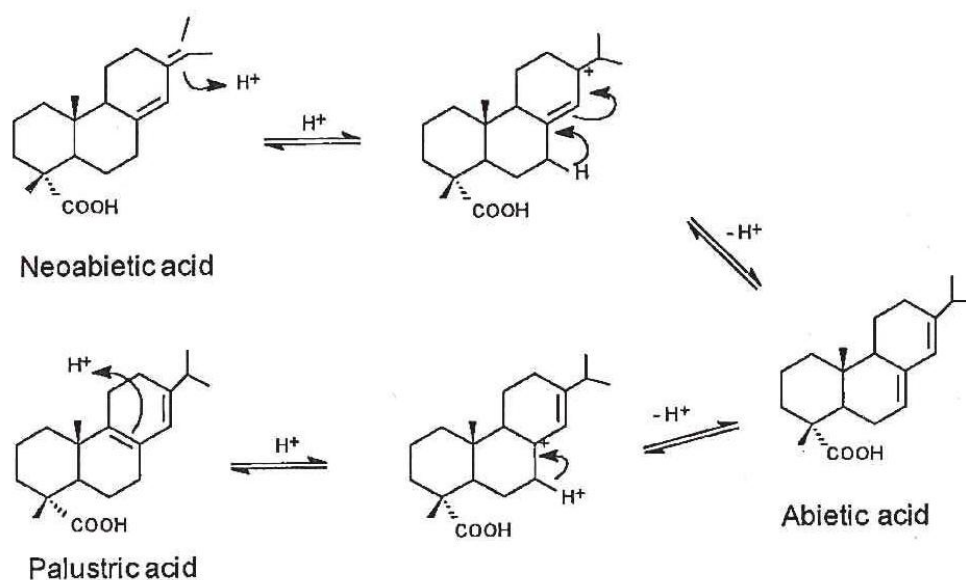
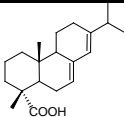
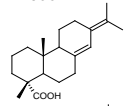
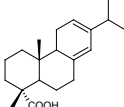
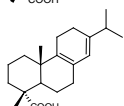
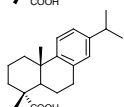


FIGURE 4. Isomerization path of neoabietic and palustric acids to abietic acid [Morales *et al.*, 1992].

Fatty acids exist as both free fatty acids and neutral esterified fatty acids in triglycerides and steryl esters, which are the esters of a fatty acid and a sterol. The compounds originate from the parenchyma cells in wood; the most common unsaturated fatty acids are oleic, linoleic, and linolenic acids, depending on the wood species [Alén, 2000a; Ekman and Holmbom, 2000; Björklund Jansson and Nilvebrant, 2009]. These acids dominate in pine and spruce (between 75 and 85% of the fatty acids), but only 3% and 10% of the fatty acids in pine and spruce, respectively, are saturated fatty acids such as palmitic and stearic acids. In birch, linoleic acid dominates (59%). The toxicity of unsaturated fatty acids like oleic, linoleic, and linolenic acids to fish has to be considered when evaluating the effect of these compounds on aquatic biota [Ali and Srekrishnan, 2001]. Table 2 represents the typical resin and fatty acids present in papermaking process waters. The determinations of so-called colloidal pK<sub>a</sub> values were made at 50 °C (normally 20 °C), which was a temperature representative of the actual papermaking process [McLean *et al.*, 2005].

TABLE 2. The most common resin and fatty acids in pine and spruce [Alén, 2000a; Back and Ekman, 2000; Ström, 2000]

Name	Formula	Molar mass [g mol <sup>-1</sup> ]	pK <sub>a</sub>
Fatty acids:			
Palmitic	C <sub>15</sub> H <sub>31</sub> COOH	256.42	5.1 <sup>a</sup> , 8.6 <sup>c</sup>
Linolenic	C <sub>17</sub> H <sub>29</sub> COOH	278.43	8.3 <sup>b</sup> , 6.3 <sup>c</sup>
Linoleic	C <sub>17</sub> H <sub>31</sub> COOH	280.45	9.2 <sup>b</sup> , 7.8 <sup>c</sup>
Oleic	C <sub>17</sub> H <sub>33</sub> COOH	282.46	5.0 <sup>a</sup> , 9.9 <sup>b</sup> , 8.3 <sup>c</sup>
Stearic	C <sub>17</sub> H <sub>35</sub> COOH	284.48	10.1 <sup>b</sup> , 9.3 <sup>c</sup>
Resin acids:			
	Structure		
Abietic		302.45	6.4 <sup>a</sup> , 6.2 <sup>c</sup>
Neoabietic		302.45	6.2 <sup>c</sup>
Levopimaric		302.45	-
Palustric		302.45	-
Dehydroabietic		300.44	5.7 <sup>a</sup> , 6.2 <sup>c</sup>

a=Ström, 2000; b= Kanicky and Shah, 2002; c= McLean *et al.*, 2005

## 2.2 Problems caused by wood extractives

The extent of the pitch problems and environmental issues depends greatly on the pulp (chemical or mechanical) manufacturing process and the degree of water circulation closure [Holmberg, 1999a; Manner *et al.*, 1999; Allen, 2000]. Paper mills with integrated pulp mills have more problems because DCS originating from the pulping and bleaching process will remain in the further processing of the pulp. In the alkaline process, the total wood extractives content may not be as relevant as the composition of these extractives [Dunlop-Jones *et al.*, 1991]. Saponification of fats and waxes is involved in the process, and resin and fatty acids create soluble soaps that are removed from the process in an early segment of the cooking stage [Alén, 2000b]. Sterols and some waxes do not form a soluble soap under alkaline conditions and therefore have a tendency to deposit and cause pitch problems, whereas in neutral and acidic processes like mechanical pulping (pH ~5), it is difficult to remove lipophilic extractives. In addition, extractives that are not retained in the wet web will accumulate in the white water system and finally end up in the final effluent, thus giving rise to possible toxicity problems [Peng and Roberts, 2000a; Rigol *et al.*, 2004].

The aim of the thermomechanical pulping (TMP) process is to separate the fibers from the wood matrix with minimum damage through high temperature and pressure. The beneficial TMP process also preserves the lignin, hemicelluloses, and wood extractives in the fibers and fines produced [Kangas and Kleen, 2004]. This enables only small material losses (1-5%); the composition of the pulp in the process differs only slightly from that of the original wood [Manner *et al.*, 1999; Sundholm, 1999]. Compared to other pulping methods such as chemical pulping, a high yield up to 97-98% is achieved and more paper can be produced from limited wood resources. However, during the TMP process the harmful lipophilic extractives in the parenchyma cells and softwood resin canals are released and accumulated in the papermaking water system because mechanical pulp is not usually washed [Ekman *et al.*, 1990; Laubach and Greer, 1991]. For example, in the bleaching stage, which consists of several intermediate washing cycles using oxygen and various chemicals like hydrogen peroxide and ozone, the importance of the pulp washing must be considered because removal of the wood resin components and metal salts is not efficient in the closed bleaching process, *i.e.*, recycling the bleaching effluents [Basta *et al.*, 1998]. TMP pulping also causes dissolution of high-charge-density pectic acids in the waters, thus constituting a major part of the anionic charge in waters, consuming the cationic retention chemicals and forming aggregates with cations such as sodium ( $\text{Na}^+$ ), magnesium

(Mg<sup>2+</sup>), and calcium (Ca<sup>2+</sup>) present in the process [Bertaud *et al.*, 2002; Saarimaa *et al.*, 2007].

Pitch deposition results in low-quality pulp and can cause the shutdown of mill operations [Pelton *et al.*, 1980; Sundberg *et al.*, 2000]. Economic losses associated with pitch problems in kraft mills often amount to 1-2% of sales. The main cost components of pitch in pulp mills are the loss of money as a result of contaminated pulp, lost production, and the cost of pitch control additives. Pitch present in contaminated pulp is the source of many problems in paper machine operation, including spots and holes in the paper, sheet breaks, and technical shutdowns [Allen, 2000]. The main substance group in the pitch deposits has been identified as hydrophobic wood extractives, composed mainly of free fatty (~6%) and resin acids (~10%), sterols, steryl esters, and triglycerides [Qin *et al.*, 2003]. The impacts of the resin and fatty acids are summarized in Table 3.

TABLE 3. The effects of resin and fatty acids in papermaking

Component groups	Effect	Reference
Resin acids	Paper machine runnability, deposits	Holmbom, 1999a; Zhang <i>et al.</i> , 1999; Rigol <i>et al.</i> , 2003a
	Odor	Tice and Offen, 1994; Holmbom, 1999a
	Allergic reactions (oxidized products)	Holmbom, 1999a
	Effluent and sediment toxicity	Holmbom, 1999a; Peng and Roberts, 2000a; Ali and Sreekrishnan, 2001; Rigol <i>et al.</i> , 2003a,2004; Lahdelma and Oikari, 2005; Rämänen <i>et al.</i> , 2010
Fatty acids	Paper machine runnability, deposits	Zhang <i>et al.</i> , 1999; Holmbom, 1999a
	Odor	Blanco <i>et al.</i> , 1996; Holmbom, 1999a
	Lower sheet strength, friction	Holmbom, 1999a; Sundberg, 1999; Tay, 2001; Kokkonen <i>et al.</i> , 2002; Kokko <i>et al.</i> , 2004
	Toxicity (unsaturated fatty acids)	Ali and Sreekrishnan, 2001; Rigol <i>et al.</i> ,2004
Resin and fatty acid soaps	Foaming	Holmbom, 1999a
	Deposits	Holmbom, 1999a; Rigol <i>et al.</i> , 2003a

Due to their stable structure (tricyclic diterpenoid acids), resin acids resist chemical degradation and easily survive the pulping and whole papermaking process, thus tending to form pitch deposits in white waters [Dethlefs and Stan, 1996] and end up in industrial sediments [Leppänen *et al.*, 2000; Lahdelma and Oikari, 2005; Rämänen *et al.*, 2010]. This might also lead to resin acid being transformed into resin acid-derived base neutrals such as dehydroabietin and tetrahydroretene that in turn accumulate in fish and freshwater mussels [Tavendale *et al.*, 1997].

Resin acids are also thought to be the main contributors to effluent toxicity in softwood pulping effluents [Patoine *et al.*, 1997; Peng and Roberts 2000a; Makris and Banerjee, 2002; Rigol *et al.*, 2004]. However, even low concentrations of unsaturated fatty acids and sterols can also have long-term effects [Ali and Sreekrishnan, 2001]. For instance, toxic effects of resin acids together with unsaturated fatty acids occur at a concentration of only 20  $\mu\text{g L}^{-1}$  [Kostamo *et al.*, 2004]. The influence of resin acid toxicity to fish has been studied extensively for decades [Oikari *et al.*, 1980,1984; Meriläinen *et al.*, 2007; Hewitt *et al.*, 2008]. The effluent constituents can accumulate in the fish and affect reproduction. Furthermore, sterols have been reported to affect the development, reproduction, and growth of fish [Nakari and Erkonmaa, 2003; Lahdelma and Oikari, 2006].

The metal soaps formed by the free resin and fatty acids present in the process waters with metal ions such as  $\text{Mg}^{2+}$ ,  $\text{Al}^{3+}$ , or  $\text{Ca}^{2+}$  are connected to tackiness problems in papermaking [Allen, 1988; Laubach and Greer, 1991; Sihvonen *et al.*, 1998; Ström, 2000; Hubbe *et al.*, 2006]. However, higher pH values increased the stability of the deposits in a colloidal pitch solution with  $\text{Al}^{3+}$  [Dai and Ni, 2010]. The high ionic concentrations of sodium salts made some resin and fatty acids like oleic and abietic acids insoluble, which implied the possible deposition problem of normally water-soluble sodium soaps of wood resin in closed water circulations [Palonen *et al.*, 1982]. Metal ion concentrations are expected to increase in a closed paper mill because of the usage of different process chemicals in various stages, *e.g.*, bleaching, in mill operations.

The effect of temperature and pH on wood pitch deposition in the papermaking process depends on the chemistry of the wood compounds and the operating conditions. Unexpected pH changes with temperature changes can destabilize the colloidal pitch, thus causing pitch deposition [Allen, 1979; Back, 2000a]. The polymerization of wood resin components with increasing temperature can form material with low solubility in common solvents or alkali [Raymond *et al.*, 1998; Dai and Ni, 2010]. This pitch polymerization has an important role in promoting pitch deposition, and it is obvious that wood chips

could enhance pitch polymerization, whereas storing wood as logs could be beneficial for reducing this phenomenon. Low temperature at neutral pH caused minimal deposition of the resin acid pitch, whereas deposition of the fatty acid pitch increased significantly in the same conditions [Dreisbach and Michalopoulos, 1989; Dai and Ni, 2010].

The relationship between pH and  $pK_a$  values strongly affects the deposition of acidic lipophilic extractives such as resin and fatty acids. It has been found that due to the low solubility of these compounds in water, they can appear as suspended colloids in the process [Ström, 2000; Nylund *et al.*, 2007]. At a pH near the  $pK_a$  values, resin acids especially tend to combine with colloidal particles, whereas at pH values higher than  $pK_a$  values, the amounts of these compounds in water can rise to a higher level. The composition of colloidal pitch changes and less deposition is expected when resin and fatty acids start to dissolve ( $pH > 6$ ); more free acids could work as emulsifiers in the process [Sihvonen *et al.*, 1998; Lehmonen *et al.*, 2009]. This might also influence the adsorption behavior of the wood resin onto a surface. However, in real processes the presence of  $Ca^{2+}$  causes a high tendency toward the formation of insoluble Ca-soaps with free acids [Otero *et al.*, 2000].

## 2.3 Solutions to the pitch problems

### 2.3.1 Removal of wood extractives from the process

The extractive content is considered to be an important quality parameter for papermaking, especially for pulp production [Alén, 2000a]. The formation of extractives-derived pitch deposits is unavoidable, but a series of procedures has been developed to study and reduce this problem [Ekman *et al.*, 1990; Laubach and Greer, 1991; Fischer, 1999; Allen, 2000; Alén and Selin, 2007; Sitholé *et al.*, 2010]. Basically, the wood resin components (*i.e.*, DCS) need to leave with the final paper product, or the closed water circulation system should have facilities to handle the enrichment/increased concentrations of the wood resin compounds in the white waters (*i.e.*, internal cleaning) or, finally, in the effluents and discharges (Fig. 5).

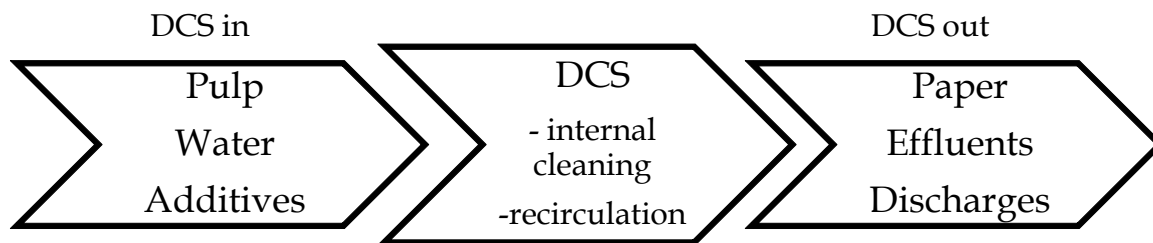


FIGURE 5. The schematic flow of the DCS in papermaking [Sundberg *et al.*, 2000].

Especially in the mechanical pulping process, the variability of process waters parameters (*e.g.*, pH, temperature, bleaching type, and process chemicals) could have an influence on the tendency towards pitch deposition [Holmberg, 1999a; Alén and Selin, 2007; Nylund *et al.*, 2007; Gantenbein *et al.*, 2010]. Process temperature and pH changes have a great impact on wood resin removal during pulping processes [Ekman *et al.*, 1990; Allen and Lapointe, 2003]. For example, increasing white water temperature due to circulation closure decreases pitch problems because the higher temperature reduces resin viscosity, thus preventing resins from accumulating on metal surfaces [Allen, 2000; Back, 2000a]. The problems resulting from sudden pH or temperature changes in the process might be rapid pitch deposition on foils, suction boxes, and press rolls as well as an increase in the amount of soap anions in white waters.

Traditionally, pitch deposits in pulping processes have been reduced by debarking, seasoning logs, and wood chips [Allen *et al.*, 1991; Sjöström, 1993; Farrell *et al.*, 1997; Allen, 2000]. The storage of wood in the form of chips reduces pitch problems considerably because oxidation occurs faster. Wood seasoning and storage are an effective way to reduce wood resin compounds in papermaking systems, especially in mechanical pulping processes [Quinde and Paszner, 1991]. In practice, the efficiency of seasoning is highly dependent on the weather; *i.e.*, in a cold winter the rate of hydrolysis decreases with the decreasing temperature. However, wood storage can also produce negative effects, such as reduced pulp yield, a loss of brightness, and a low pulp quality due to the uncontrolled action of microorganisms. Moreover, isomerization of resin acids has been detected (Fig. 4).

In kraft pulping processes, pulp washing plays an important role in pitch deposition behavior [Laubach and Greer, 1991; Fleet and Breuil, 1998; Back, 2000b; Ström, 2000]. Good washing of unbleached pulp will decrease the wood resin amount in the bleaching and paper manufacturing stages. However, closing water

circulation will increase concentrations of wood resin and metal ions in the processes, thus resulting in poor washing of the pulp [Ström *et al.*, 1990]. Besides pulp washing, the stock system purification has a positive influence on preventing pitch deposition [Holmberg, 1999b; Allen, 2000]. In bleaching, deresination can be achieved by removing the desorpted resin from fibers through washing liquors, by dissolving it with bleaching liquors followed by removal by proper washing, especially in alkaline conditions or oxidation of resin into more water-soluble forms. The bleaching technique used also has an influence on the wood resin components. For example, ozone significantly decreases the amount of sterols in eucalyptus pulp [Freire *et al.*, 2006]. On the other hand, the peroxide bleaching stage effectively oxidizes the resin, thus producing complex oxidized products [Holmbom *et al.*, 1991; Bergelin and Holmbom, 2003].

The formation of pitch deposits is also connected to the disturbance of colloidal stability and aggregation of the pitch droplets [Dreisbach and Michalopoulos, 1989; Hubbe *et al.*, 2006]. In unbleached TMP process waters, colloidal extractives are usually sterically and electrostatically stabilized, which inhibits aggregation even with high concentrations of electrolytes [Sundberg *et al.*, 1994]. However, resin droplets are usually only electrostatically stabilized in bleached TMP; therefore, aggregation with electrolytes is possible [Willför *et al.*, 2000].

Both polysaccharides and wood extractives are released during mechanical pulping, giving rise to the importance of these polysaccharides to the deposition problems in the form of complexes of anionic polysaccharides and cationic polymers. These water-soluble polysaccharides in mechanical pulping have also been considered a source of bioactive polymers [Willför *et al.*, 2005] or barrier film production [Persson *et al.*, 2007]. Many researchers have shown that a small amount of galactoglucomannans decreased the deposition tendency and affected the stability and character of the colloidal pitch and also had a positive effect on paper strength [Sundberg *et al.*, 1993,2000; Sihvonen *et al.*, 1998; Otero *et al.*, 2000; Johnsen *et al.*, 2004; Alén and Selin, 2007].

Process additives have been used for pitch control [Allen, 2000; Hubbe *et al.*, 2006]. Alén and Selin [2007] categorized deposit control according to the chemicals needed to solve the problem: adsorbents, fixatives, retention aids, dispersants, surfactants, chelants, solvents, and enzymes. For example, talc has been used to stabilize dissolved and colloidal material (DCM) and to avoid agglomeration through the reactions of a talc hydrophobic surface with the hydrophobic surface of the tacky material, thus reducing its potential to form deposits [Monte *et al.*, 2004; Guéra *et al.*, 2005; Gantenbein *et al.*, 2010]. Kaolin affects the stability of DCM, resulting in a decrease in the amount of lipophilic extractive droplets in the



dispersion [Nylund *et al.*, 2007]. In addition, retention aids such as cationic polymers have been used to make wood extractives substantive to fibers, solving the precipitation problems and reducing rates of accumulation of these compounds on the papermaking equipment [Sundberg, 1999; Allen, 2000; Hubbe *et al.*, 2009].

The degradation of wood extractives has been conducted with enzymes and microorganisms in the water phase as well as in the wood chips or pulp by wood-inhabiting fungi, to eliminate the possibility of lipophilic extractives leaching into process waters [Farrell *et al.*, 1997; Burnes *et al.*, 2000; Dorado *et al.*, 2001; Kallioinen *et al.*, 2003; Gutiérrez *et al.*, 2006,2009; van Beek *et al.*, 2007; Dubé *et al.*, 2008; Widsten and Kandelbauer, 2008]. Such treatment can take from several hours to several days; the degradation of the extractives with enzymes/microorganisms is a very selective reaction when DCS is to be eliminated. These biotechnological products have been successfully used for the selective removal of pitch problems caused by sterols, triglycerides, and resin acids [Gutiérrez *et al.*, 2001]. So-called biopulping, *i.e.*, wood chip pretreatment with white-rot fungi capable of selectively degrading lignin and some extractives, also enables cost savings in the form of lower energy consumption, in mechanical pulp production. Especially promising results have been achieved by using a novel surfactant (non-ionic alkyl diethanolamide) with a lipase enzyme treatment that can reduce a wide range of wood extractive compounds in pulp and process waters [Dubé *et al.*, 2008].

The ozonation process can be used to prevent the accumulation of wood extractive compounds in the TMP circulation waters, which improves product quality and functionality of the paper machine [Laari *et al.*, 1999]. Ozonation is based on the sensitivity of lipophilic wood extractives to oxidation. However, a relatively high dose of ozone is needed to reach complete degradation, which makes the cost of the treatment relatively high. It has also been found that ozone selectively oxidizes resin acids, decreasing total resin acid concentration by over 90 % [Korhonen and Tuhkanen, 2000; Ledakowicz *et al.*, 2006]. Therefore, it is beneficial to use ozone in the post-treatment process of white waters before the actual treatment process in order to reduce the amounts of organic compounds, chemical oxygen demand (COD), and toxicity [Latorre *et al.*, 2007]. The high oxidizability of pimarane type resin acids was also achieved, rather than in the abietanes type, as had been expected. Ozone selectively affects the toxicity of the effluents; ozone treatment increases the toxicity of resin acids because more toxic intermediates are formed. However, an overall decrease in the toxicity of fatty acids was achieved [El-Din *et al.*, 2006].

### 2.3.2 Removal of wood extractives in waste water treatment

The papermaking industry is one of the largest water consumers in the world. Its consumption depends greatly on the type of paper being produced and the degree of recycling in the process [Thompson *et al.*, 2001; Garvilescu *et al.*, 2008]. Debarking and bleaching are the main sources of the wood extractives in pulp and paper mill waste water. The waste water treatment processes have become more intensive and important due to the trend of water circulation closure [Latorre *et al.*, 2005]. A huge amount of solid waste, including sludge, mud, ash, and wood processing residuals, is also generated from papermaking [Monte *et al.*, 2009]. The paper mills have started an attempt to lower water consumption and discharges to the environment for both environmental and economic reasons. The characteristic properties of the waste waters depend greatly on the type of papermaking process, wood materials, recirculation degree of the waters and effluents, and the process technology applied to the papermaking and waste water treatment. New and efficient waste water treatment techniques are being developed constantly; in addition, economic and social aspects need to be taken into account [Burkhard *et al.*, 2000; El-Ashtoukhy *et al.*, 2009]. There are several categories of waste water treatment methods: Table 4 breaks down the most widely used techniques for waste water treatment in papermaking.

TABLE 4. The different technologies for waste water treatment [Pokhrel and Viraraghavan, 2004]

Physicochemical	Biological
Air flotation	Activated sludge process
Ion exchange	Aerobic biological reactors
Adsorption	Anaerobic treatment
Membrane filtration	Fungal and enzymes treatments
Chemical oxidation	
Ozonation	
Sedimentation	

Physicochemical processes are in general quite expensive but achieve the beneficial removal of high-molecular-mass lignins, color, toxicants, suspended solids, and COD. On the other hand, waste waters after biological treatments still contain lignin, color, and some COD. To attain optimal biological temperature and pH conditions, biological treatment systems may also require rather extensive modifications of environmental conditions such as cooling [Latorre *et al.*, 2005]. Different kinds of solutions have been tested to overcome these problems, for example, separating the white water and effluent treatments. However, these solutions have not been enough to solve all the problems. While the secondary

waste water treatments have successfully decreased the toxicity of the effluent, these effluents still have a negative effect on aquatic organisms [Kovacs and Voss, 1992; Pokhrel and Viraraghavan, 2004]. The activated sludge treatment removes up to 90% of wood extractives. However, the transformation of resin acids to more persistent forms, such as retenes, will create new challenges to the environment in the form of new toxic compounds in the sediments [Kostamo *et al.*, 2004]. Chemical pulping effluents are especially problematic for the environment, since they may contain, even after biotreatment, compounds that are resistant to biological treatment and can thus cause changes in the physiology and biochemistry of fish.

The use of ozone to treat different types of industrial waste water was reviewed comprehensively by Rice [1997], who found that ozone bleaching has a strongly positive effect on the plant's influent waters in that it allows water reuse in bleaching and lowers the cost of waste water discharge. Basically, ozone is used to enhance the biodegradability of the effluents before further reduction with biological treatment. New techniques with great potential, such as a gas-induced ozone reactor for highly complex industrial waste water treatment with ozone [Lin and Wang, 2003] or an integrated anaerobic bioreactor and ozone treatment system [Chaparro *et al.*, 2010] have been developed. Moreover, wet oxidation (WO) [Laari *et al.*, 1999; Verenich *et al.*, 2004] has been successfully used to degrade lipophilic wood extractives from TMP waste waters. In the WO process, the organic matter in the water phase reacts with oxygen at high temperature and pressure to produce carbon dioxide and water [Collyer *et al.*, 1997].

The removal of wood extractives in waste water is a challenging process that also requires the development of faster analysis methods for these compounds. The resin acids are the main contributors to the toxicity of the effluent; therefore, their removal by waste water treatment plays an important role. Biological treatment has been considered to be the most effective way of removing large amounts of organic matter from waste waters [Lacorte *et al.*, 2003; Latorre *et al.*, 2007]. It has also been used for removal of resin acids, as have anaerobic reactors [Ali and Sreekrishnan, 2001; Kostamo and Kukkonen, 2003]. In particular, the use of secondary treatment in an aerobic lagoon was found to remove over 90% of the influent resin acids. However, the system does not take into account the possible process variations that can cause effluent biological oxygen demand (BOD), COD, and toxicity values to rise to unacceptable levels. Moreover, pH strongly affects the toxicity and solubility of resin acids in waste waters [Ali and Sreekrishnan, 2001]. It must be also noted that a reduction in resin acid levels may not have a direct correlation with the reduction of toxicity or COD values, because of the possible modification of these compounds in the water treatment process [Liss *et al.*, 1997; Fähræus-Van Ree and Payne, 1999]. The fatty acids can be degraded anaerobically,

but their concentration levels should be kept below elevated levels to prevent them from inhibiting the anaerobic bacteria.

## 2.4 Analysis of extractives

The analysis of wood extractives in water samples has been extensively studied [Holmbom 1999a,b; Ekman and Holmbom, 2000; Holmbom and Stenius 2000; Baeza and Freer, 2001; Lacorte *et al.*, 2003; Rigol *et al.*, 2003a; Douek, 2007]. The use of component group analysis of wood extractives, using techniques such as extraction and gravimetric determination, has been preferred. However, these analytical methods provide little detailed information about the composition of different wood extractive groups such as resin and fatty acids. Alternative analysis procedures have also been presented in the literature. For example, somewhat simple turbidity measurements have been used to evaluate DCS levels in process streams in paper mills [Tornberg *et al.*, 1993; Sundberg *et al.*, 1994; Mosbye *et al.*, 2003; Ravnjak *et al.*, 2003; Saarimaa *et al.*, 2006]. In model systems, the correlation between turbidity and colloidal wood pitch seems to be applicable. However, in the real process, fibers and fines affect turbidity measurement and disturb this correlation.

A potentially interesting study involves a 5-component analytical system in which typical TMP water constituents such as carbohydrates, extractives, lignans, lignin, and low-molecular-mass components were measured; the results were controlled by the use of COD or total organic carbon (TOC) measurement [Lenes *et al.*, 2001]. The results showed that these five components summed up only 75-90% of the COD values measured directly. Traditionally, a simple COD measurement has been used to study organic compounds, *i.e.*, the total concentration of particulate and dissolved components in pulp mills, or evaluate the efficiency of white water treatment [Latorre *et al.*, 2007]. Clearly, the analysis of organic compounds like wood extractives in papermaking process waters must include compromises between simple and less accurate and more sophisticated and accurate methods.

Wood extractives have often been isolated from the sample matrix through extraction techniques. The choice of the extraction method is vital for the further success of the analysis [Lacorte *et al.*, 2003; Rigol *et al.*, 2003a; Latorre *et al.*, 2005]. To remove harmful matter like suspended solids and particles from process water samples, centrifugation (2000 rpm for 20 min) or filtration through 0.45  $\mu\text{m}$ , 0.7  $\mu\text{m}$ , and 1  $\mu\text{m}$  filters is necessary. Filter extraction is also recommended because apolar compounds of the sample may remain on the filters. Centrifugation is preferred to filtration because dissolved and colloidal particles are taken into account with supernatant, whereas in filtration an unmeasured amount of lipophilic droplets

and colloidal particles may remain on the filter or the fiber mat formed [Örså and Holmbom, 1994]. Most methods use LLE with an organic solvent such as hexane, acetone, dichloromethane, or methyl *tert*-butyl ether (MTBE). The use of diethyl ether has also been presented by Ekman and Holmbom [1989] in their analytical scheme for extractives in water samples. The extraction studies have shown that a mixture of selected solvents will give better recovery results than will single solvents [Peng and Roberts, 2000b]. These solvent fractions are collected and dried by evaporation. Prior to chromatographic analysis, the samples are exposed to chemical derivatization with methylation (*i.e.*, diazomethane-ether solution), formation of pentafluorobenzyl (PFB) esters or per(trimethylsilyl)ated (*i.e.*, the preparation of trimethylsilyl (TMS) derivatives) with *N,O*-bis(trimethylsilyl)-trifluoroacetamidi (BSTFA) containing trimethylchlorosilane (TMCS).

SPE have also been used to isolate resin and fatty acids from papermaking process waters and effluents [Richardson *et al.*, 1992; Dethlefs and Stan 1996; Mosbye *et al.*, 2000; Rigol *et al.*, 2003a]. SPE uses a solid and a liquid phase to isolate analytes from a solution. Typically, silica-based liquid chromatography type stationary phases with a special functional group, such as hydrocarbon chain, amino groups, sulfonic acid, or carboxyl group resin, are packed in a glass or disposable plastic column with a frit. The samples are passed through these columns and analytes retained to the stationary phases are flushed with organic solvents [Fritz, 1999]. Various solvents or combinations, such as hexane, chloroform, and diethylether, were used in these applications, for example, to isolate different groups of lipophilic extractives present in wood and pitch deposits [Gutiérrez *et al.*, 1998]. The SPE technique has clear advantages with a short analysis time, low solvent need, and possibility to connect online with different chromatographic techniques such as HPLC [Hennion, 1999]. Compared to LLE, SPE also minimizes the formation of emulsions. However, the overall efficiency of SPE was shown to be highly dependent on the sorbent type, amount, and column size. Chen *et al.* [1994] used the SPE technique to separate different groups of extractives in the fractions; quantitative results were achieved by weighing the fractions.

### 2.4.1 Gas chromatography

The most widely used analytical method for wood extractives analysis is GC with high-resolution capillary columns. This method has been used for the analysis of resin and fatty acids present in tall oil since the 1970s [Holmbom, 1977]. The analysis procedure of wood extractives includes the separation of wood extractives from a sample matrix with extraction, derivatization of the samples, and finally, GC analysis combined with mass spectrometer (MS) or FID [Holmbom, 1999b;

Knuutinen and Alén, 2007]. The sensitive and reliable FID detector has a wide linear range and good responses for different organic compounds.

The critical points of the analysis have been the choice of the extraction method, extraction solvent, and the pH of the water samples. LLE extraction has proven to efficiently extract organic compounds from particulate and dissolved fractions, whereas the SPE may suffer losses of some of the compounds through adsorption [Lacorte *et al.*, 2003]. Resin acids have also been extracted from TMP effluent by adsorption onto XAD resin with subsequent analysis by GC [Richardson and Bloom, 1982]. However, this technique is rather time-consuming as it involves long sample preparation and analysis time.

The effect of the sample pH value on extraction efficiency has been studied extensively; pH values varying from 2 to 12 have been used in the analysis. Voss and Rapsomatiotis [1985], followed by several other studies [Lee *et al.*, 1990; Dethlefs and Stan, 1996; Serreqi *et al.*, 2000; Gutiérrez *et al.*, 2001], used a basic pH to prevent isomerization and binding problems of the resin acid. Several researchers have reported extraction with organic solvents with an acidic pH because this reduced the formation of emulsions and thus microbial growth during sample storage could be achieved [Ekman and Holmbom, 1989; Morales *et al.*, 1992; Örså and Holmbom, 1994]. For example, a medium-acidic pH increases the isomerization of some resin acids, such as palustric acid, to abietic acid. In addition, low solubility of resin acids in aqueous systems may require higher pH values (*i.e.*, above  $pK_a$  values). For example, due to structural differences, dehydroabietic acid is the most soluble ( $\sim 5 \text{ mg L}^{-1}$ ) in water at pH 7, twice the solubility of other resin acids [Peng and Roberts, 2000a]. Mosbye *et al.* [2000] preferred the original sample pH value ( $\sim 5$ ), which is representative for the real papermaking process. A better recovery was also observed with the original pH 5 than with the acidic (pH 3) or basic (pH 12) conditions tested.

The derivatization of the sample is commonly recommended in GC analysis to improve separation and ensure quantitative reliability [Lacorte *et al.*, 2003]. Derivatization to methyl esters or PFB derivatives has also been reported [Lee *et al.*, 1990], and the formation of TMS ethers has been shown to be beneficial for the analysis procedure [Holmbom, 1999b]. The main problem is caused by the shortened lifespan of the derivatized extract and also the possible long-term effects on GC-MS performance. In particular, the TMS derivatives are susceptible to hydrolysis, and the analysis time of the derivatized sample is limited to 12-24 hours. The use of internal standards is also preferred in order to improve the quantification of the compounds. Several possible internal standards, such as heptadecanoic acid [Ekman and Holmbom, 1989] or heneicosanoic acid [Örså and Holmbom, 1994], are available to aid in quantification of the resin and fatty acids.

A detailed method of analysis for wood extractives as groups (free fatty acids, resin acids, lignans, steryl esters, and triglycerides) was introduced by Örså and Holmbom [1994]. They used an effective MTBE solvent to separate out both lipophilic extractives and hydrophilic lignans in mechanical pulping process waters. The use of non-split on-column injection is preferred to achieve reliable results, and several different internal standards with different volatilities ensured the possible differences between responses with an FID detector. The analysis of individual free resin and fatty acids, fatty alcohols, and sterols can be conducted by a standard 15-30 m long capillary column with different polarities. Fused-silica, non-polar (methyl silicone), phenyl methyl siloxane, and HP-5 columns have been used for this purpose [Holmbom, 1999b; Rigol *et al.*, 2003a].

GC-MS has also been used for the analysis of resin and fatty acids in waters, sediments, and fish bile samples since the 1970s [Morales *et al.*, 1992; Gutiérrez *et al.*, 2001; Rigol *et al.*, 2002]. The MS detection provides the spectra with molecular fragment ions, which provides useful information about the ionized compound and unequivocal peak identification in the complex mixtures as well as good sensitivity and reliability with excellent LOD values of 3-9  $\mu\text{g L}^{-1}$  [Rigol *et al.*, 2002]. The compounds can be easily identified by comparing them using databases with spectra involving a wide range of common extractive compounds. The combination of GC-FID analysis with GC-MS provides the best overall component identification.

#### **2.4.2 High-performance liquid chromatography**

HPLC provides an important alternative for the difficult analysis of wood extractives with non-volatile requirements for the compounds [Holmbom, 1999b; Rigol *et al.*, 2003a,b]. The method uses high pressure to force solvent through packed columns to provide resolution of the compounds of interest. HPLC can utilize, for example, the reversed-phase (RP) technique, thus providing a good separation of wood resin component groups; the size-exclusion (SE) technique can also be used to fractionate the sample for the further analysis of individual components [Suckling *et al.*, 1990]. Mixtures of water and acetonitrile or methanol with an acid modifier are used to elute the compounds. One of the major challenges in the HPLC analysis of resin acids is the difficulty in separating the various resin acid isomers in a mixture by common C8 or C18 columns because of the hydrophobic analyte-column interactions. Recently, ultra-performance liquid chromatography (UPLC), which provides good chromatographic separation with shorter analysis time, has been introduced for water analysis of pharmaceuticals [Nováková *et al.*, 2006; Van De Steene and Lambert, 2008]. The UPLC technique has

a beneficial low solvent consumption, and its systems are designed to withstand high system back-pressures. However, this technique has not yet been used to analyze extractives in papermaking process waters.

Ultraviolet (UV) and fluorescence detection have been used to analyze dehydroabietic acid and total resin acids in effluent samples [Richardson *et al.*, 1983,1992]. The analysis of other resin acids such as abietic acid through fluorescence detection is not possible because of the non-aromatic structures of these acids. The faster direct injection technique of an untreated effluent sample has shown quite similar results with dehydroabietic acid. A slightly modified direct injection HPLC method by Chow and Shepard [1996] showed an excellent possibility of using dehydroabietic acid as an indicator for assessing total resin acid concentration in paper mill effluents. Screening for toxicity in effluents can also be done with this fast detection technique.

The analysis of wood extractives involves a series of limitations because the detection range must be wide and a large number of different compounds must be analyzed. The limitations of UV detection are based on the absence of chromophores in all resin compounds, whereas the refractive index and infrared detectors are not compatible with gradient elution [Suckling *et al.*, 1990]. However, light scattering and mass detectors are compatible with gradient elution and thus suitable for analysis of methylated resin and fatty acids as well as partially separated triglycerides.

The derivatization of the extracts in HPLC analysis will also improve the separation of the compounds [Holmbom, 1999b]. The conversion of resin acids in effluent samples to different types of coumarin esters has been presented by Volkman *et al.* [1993] and Luong *et al.* [1999a,b], who also found that the HPLC method was suitable for routine monitoring of total resin acids and dehydroabietic acid in process effluents. However, the changes in the composition of individual resin acids in water were better evaluated by the GC-MS method for environmental purposes.

#### **2.4.3 High-performance liquid chromatography-mass spectrometry**

The use of the HPLC-MS method for the analysis of environmental or pharmaceutical residuals in water samples has been widely published [Zwiener and Frimmel, 2004a,b; Petrović *et al.*, 2005]. However, only limited information on the utilization HPLC-MS have been reported regarding the analysis of resin and fatty acids in papermaking waters, effluents, or river water samples [McMartin *et al.*, 2002; Latorre *et al.*, 2003; Rigol *et al.*, 2003b,2004]. Due to the high sensitivity and selectivity of HPLC-MS, the main advantage of analysis is that samples can be



directly injected into a column without the need for a derivatization step and that ionization of the compounds takes place in an interface without the need for any post-column addition. Thus, the problem of the decomposition of silylated samples during their possible storage, as in GC analysis, could be avoided.

In general, APCI and electrospray ionization (ESI) provide suitable interfaces for analysis. These techniques allow soft ionization in both negative and positive modes, but APCI seems to be the most versatile in that it provides clear mass spectra with little fragmentation [Willoughby *et al.*, 1998; Kostianen and Kauppila, 2009]. ESI has been used for large biomolecules, but also for small polar organic compounds, whereas the APCI provides a useful choice especially for non-polar compounds. The benefits of APCI technique over ESI are that it tolerates higher salt and additive concentrations, polar and non-polar solvents can be used, and the ionization of neutral and less polar compounds is also possible. The selectivity and sensitivity of HPLC-MS analysis depends also upon the HPLC technique. RP technique is the most commonly used, but ion-change and SE chromatography (SEC) have also been used. The column diameter and solid-phase material affect the HPLC separation efficiency and analysis time. The common solvents consist of mixtures of acetonitrile or methanol and water. The additives must be volatile because non-volatile additives can cause background noise and contamination of the ion source. However, compromises between chromatographic separation and ionization efficiencies must often be made when selecting the eluent composition.

McMartin *et al.* [2002] reported the use of the liquid chromatography-electrospray-mass spectrometric (LC-ESI-MS) method for the analysis of four resin acids in river water. The method used external standard calibration and provided a highly sensitive analysis of dehydroabietic acid and coelution of three structural resin acid isomers (abietic, isopimaric, and pimaric acids). In other studies, LLE has been used for sample preparation, but the use of direct analysis techniques with only sample dilution has been tested [Rigol *et al.*, 2003b]. C8 and C18 columns have been used in separations of the resin acids with acetonitrile-water and methanol-water (25 mmol L<sup>-1</sup>, CH<sub>3</sub>COONH<sub>4</sub>, pH 7) as the mobile phase. Owing to the low polarity of resin and fatty acids, high percentages of organic solvent were necessary for their elution. In addition, the use of acetonitrile caused high background noise since carbon deposits were produced in the corona of the APCI interface. Dehydroabietic acid was able to separate from the non-aromatic acids that coeluted in the McMartin *et al.* [2002] study. On the other hand, Rigol *et al.* [2003b] doubled the analysis sensitivity by using APCI and partly separating the coeluting non-aromatic resin acids with a C8 column and adding isopropanol in the mobile phase. The intensive [M-H]<sup>-</sup> ion was obtained, even with high fragmentor voltage values, for the resin and fatty acids, and identification could be

made by means of a single ion by comparing retention time against a standard or a standard addition technique [Lacorte *et al.*, 2003].

The HPLC-MS technique with an RP-18 column attained good linearity, repeatability, and LOD values with recovery values higher than 70% [Rigol *et al.*, 2003b]. LOD values of 0.2-1.3  $\mu\text{g L}^{-1}$  and 0.5-3.1  $\mu\text{g L}^{-1}$  were achieved for MTBE extraction and direct sample introduction, respectively. Through the use of a direct injection technique, somewhat higher LOD values were obtained compared to MTBE extraction, but they were still below the levels of the target compounds encountered in the paper industry effluent samples tested. Although coelution of the non-aromatic resin acids occurred, the direct injection technique presented was demonstrated to be a useful technique for unequivocal quality control during papermaking.

#### 2.4.4 Applications of the methods

Examples of the most commonly used methods for the analysis of wood extractives in water matrix are shown in Table 5. The references were selected on the basis of utility for water analysis related to papermaking. Rigol *et al.* [2003a] have reviewed in detail the analysis of resin acids in process waters; other researchers have also listed various analytical methods for resin and fatty acids [Holmbom, 1999b; Peng and Roberts, 2000b]. Several other studies have been done on the analysis of wood extractives in other sample matrices such as wood, pulp, black liquors, or wood resin deposits [Sjöström, 1990; McGinnis, 1998; Holmbom, 1999a; Sitholé, 2000; Bergelin *et al.*, 2003; Hubbe *et al.*, 2006; Douek, 2007]. These analysis procedures utilize the same analytical methods as the water analysis, with the exception of sample pretreatment like wood grinding or pulp extraction with soxhlet [Holmbom, 1999a] or accelerated solvent extraction (ASE) [Thurbide and Hughes, 2000].

The most commonly used and most accurate analysis of wood extractives in papermaking process waters is based on traditional LLE extraction with MTBE and GC analysis of the silylated extracts [Örså and Holmbom, 1994] (Table 5). The development of a different kind of faster analytical technique has been presented for resin acids in TMP/chemi-thermomechanical pulping (CTMP) process waters [Serreqi *et al.*, 2000]. GC-FID analysis was used to determine individual and total resin acid content of a series of in-mill process waters; correlation coefficients of the results were determined. They used one or two resin acids as markers for analysis of total resin acid content and found that especially abietic and isopimaric acids, but not dehydroabietic acid, were useful. However, the opposite results were also obtained by research that found dehydroabietic acid to be a good marker for pulp mill effluent samples [Chow and Shepard, 1996]. These results showed that the

sampling location of the process water plays an important role when using individual resin acids as a marker for total resin content. In addition, Monte *et al.* [2004] developed a procedure in which destabilization of DCM with cationic polymer addition, deposit collection, and quantification with image analysis were used to predict the tendency of the material to form deposits. The applicability of the procedure was tested with adhesives, coated and recycled papers, and deinking soaps. The results showed good reproducibility, and the procedure proved to be suitable for the evaluation of DCM and deposit tendency.

The other techniques reported for analysis included capillary electrophoresis (CE) and thin layer chromatography (TLC) [Zinkel and Rowe, 1964]. In general, CE is not preferred to the analysis of resin acids, since these compounds have a relatively apolar structure with low strength chromophores [Rigol *et al.*, 2003a]. However, Luong *et al.* [1999a,b] successfully used cyclodextrin-modified CE technique for the analysis of the derivatized resin acids. In this research, they succeeded in separating methoxycoumarin esters of resin acids with laser-induced fluorescence (LIF) detection with model compounds. TLC was used for preparative isolation of component groups with silica plates prior to further, more detailed individual component analysis by GC or HPLC [Holmbom, 1999a]. Quantitative analysis is also possible with appropriate internal standards for each component group. Moreover, the application of TLC use has been presented by Yusiasih *et al.*, [2003], who use TLC to screen wood extractives by using cellulose TLC plate separation and application directly to bioassays.

The importance of analyzing wood extractives in waste waters and receiving effluent will arise in closing water circulations [Stratton *et al.*, 2004; Gavrilescu *et al.*, 2008]. A variety of studies have been conducted concerning waste water treatment in the pulp and paper industry [Ali and Sreekrishnan, 2001; Pokhrel and Viraraghavan, 2004]. The problems encountered in treatment are often caused by the diversity of the possible ecologically problematic contaminants present, such as bleaching agents, salts, and organics. The analysis of interfering substances, *i.e.*, wood extractives or resin and fatty acids, in waste waters is basically performed with the same methods as is process water analysis. The analysis of resin acids has been used to study and to confirm the efficiency of the waste water treatment process as well as the quality of the waste water [Laari *et al.*, 1999; Ledakowicz *et al.*, 2006].

TABLE 5. Determination of wood extractives in water matrix

Sample	Sample preparation/ pH	Compounds	Detection method	Reference
Effluent	Dichloromethane extraction/-	Dehydroabietic acid	LC-UV	Symons, 1981
Effluent	Adsorption to XAD resin	Resin acids	GC	Richardson and Bloom, 1982
Effluent	Extraction/pH 12	Dehydroabietic acid	HPLC-UV, HPLC- Fluorescence	Richardson <i>et al.</i> , 1983
Effluent	MTBE extraction/pH 9	Resin and fatty acids	GC-FID	Voss and Rapsomatiotis, 1985
Mechanical pulp waters	Diethyl ether extraction/ pH 3.5	Wood extractives	GC-MS GC-FID	Ekman and Holmbom, 1989
Pulp mill effluent	MTBE extraction/pH 8	Resin and fatty acids	GC-MS	Lee <i>et al.</i> , 1990
Water	MTBE extraction/ Dichloromethane/pH 5	Resin and fatty acids	GC-MS	Morales <i>et al.</i> , 1992
Effluent, water	SPE/pH 9	Total resin acids	HPLC-UV HPLC- Fluorescence	Richardson <i>et al.</i> , 1992
Effluents, river waters	SPE/pH 8	Resin acids	GC-MS	Volkman <i>et al.</i> , 1993
Process waters, effluents	MTBE extraction/pH 3.5	Wood extractives	GC-FID	Örså and Holmbom, 1994
Bleaching effluents	MTBE extraction, SPE/pH 8-9	Resin acids	GC-MS	Dethlefs and Stan, 1996
Primary effluent	Dichloromethane extraction SPE/pH 2	Fatty acid esters	GC-MS	Koistinen <i>et al.</i> , 1998
TMP circulation water	MTBE extraction/pH 3.5 Hexane extraction	Wood extractives Total relative wood extractives	GC-FID UV-VIS	Laari <i>et al.</i> , 1999
Model waters	Model compounds solution	Resin acids	CE-UV HPLC-UV CE-LIF	Luong <i>et al.</i> , 1999a,b

White water	MTBE extraction	Total extractives, extractive groups	Gravimetric GC-FID	Zhang <i>et al.</i> , 1999
White water	MTBE extraction/pH 3.5, 9 SPE extraction/pH 5	Resin and fatty acids, sterols	GLC-FID	Mosbye <i>et al.</i> , 2000
Process water, effluent	SPE (solvent mixture)/pH 10	Resin acids	GC-FID	Peng and Roberts, 2000b
Process waters (eucalypt)	Hexane:acetone extraction	Lipophilic extractives	GC GC-MS	Gutiérrez <i>et al.</i> , 2001
Paper-recycling process waters	MTBE extraction/original	Resin and fatty acids	GC-MS	Rigol <i>et al.</i> , 2002; Latorre <i>et al.</i> , 2003
River water	-/original	Dehydroabietic acid, abietic acid isomers	LC-ESI-MS	McMartin <i>et al.</i> , 2002
White waters, effluents, process waters, river water	MTBE extraction, direct injection/pH 6	Resin and fatty acids	LC-APCI-MS	Rigol <i>et al.</i> , 2003b, 2004
Waste water	Hexane:ethanol/original	Wood extractives	GC-MS	Kostamo <i>et al.</i> , 2004
Model solution	Trichloromethane-diethyl ether	Resin acids	GC-FID GC-MS	Ledakowicz <i>et al.</i> , 2006
White water	MTBE extraction/pH 6	Resin and fatty acids	LC-MS	Latorre <i>et al.</i> , 2007
Model water (fish exposure)	Hexane:acetone/pH 3.5	Resin acids	GC-MS	Meriläinen <i>et al.</i> , 2007
Kraft mill effluent	Dichloromethane extraction/pH 9	Sterols	GC-FID	Vidal <i>et al.</i> , 2007
Pulp and paper mill waste water	MTBE extraction/pH 3.5	Wood extractives	GC-MS	Leiviskä <i>et al.</i> , 2009

-= not reported

## 2.5 Online analysis and process chemistry

The closing of water circulation during the papermaking process will increase the need for sensitive and rapid online measurements. The increasing consumer demand for product and the use of papermaking additives for paper machines have created the need for real-time monitoring of the wet-end chemistry [Tornberg *et al.*, 1993; Holmbom, 1999b; Rice, 2001; Holmbom and Sundberg, 2003]. The general understanding and rapid developments in sampling, analytical procedures, miniaturization, and data processing have helped satisfy the need for useful and current information provided by real-time analysis [Workman *et al.*, 2003,2009]. In addition, the automation of laborious analytical techniques has improved analytical quality parameters such as accuracy and reproducibility of the analytical methods.

Particularly, online/real-time chemical measurements are focused on monitoring basic summative parameters with simple and rapid techniques to measure pH, temperature, conductivity, turbidity, and charge [Scott, 1996; Boegh *et al.*, 2001]. The use of COD and total organic carbon (TOC) for the analysis of the total organic compounds in the process waters has been preferred in paper mills [Holmberg, 1999a; Manner *et al.*, 1999; Knuutinen and Alén, 2007]. However, these measurements produce little or no information about the chemical behavior of the individual compounds present in the process waters. Also, for example, the optimal consumption of process chemicals in paper production will be more difficult to control without specific identification techniques such as chromatographic measurements. Controlling the chemical additives will improve the stability of paper machines and reduce costs. The most promising techniques are based on laboratory chromatographic analytical equipment such as continuous-flow extraction [Rice *et al.*, 1997] and flow cytometry [Vähäsalo *et al.*, 2003,2005]. Fourier transform infrared spectroscopy (FTIR) to measure DCS in wet-end with a continuously operating centrifuge separator has also been tested [Tornberg *et al.*, 1993]. This measurement was compared with COD and also with pitch ball accounting, and a good correlation was observed. The sensitivity of commercial FTIR equipment was found to be a critical factor in the analysis of online mill trials.

The problems with the sampling system can be considered the most challenging task in developing online measurements for process control. Therefore, a lot of research has been concerned the improving the state of sampling systems [Workman *et al.*, 1999,2005]. The main purpose of sampling is to provide a representative sample to a process analyzer. Flow injection analysis (FIA) can be useful for sampling in developing process analytical methods. In this procedure,

the sample is treated fluidically through the pumps, valves, and reactors that comprise the flow system. Once sampling occurs, the automated fluid handling, coupled with detection, is the same regardless of the origin of the sampled volume. This useful FIA procedure has been used, for example, in monitoring online COD or kraft pulping liquors [Kuban and Karlberg, 2000]. Modification of the conventional FIA extraction mode for high-pressure flow extraction has also been studied [Rice *et al.*, 1995,1997]. The tangential flow filtration method was developed to remove coarse fibers from pulp slurry; it showed good reliability in mill trials.

The problems arising from automated sampling in the pulp and paper industry have successfully been solved by using CE with UV detection for the analysis of soluble anions (*e.g.*, chloride, oxalate, formate, and acetate) in paper mill waters [Sirén *et al.*, 2000,2002; Kokkonen *et al.*, 2004]. This online procedure has been applied in several process machines at pulp and paper mills. This technique enabled the simultaneous separation and determination of the monitored ions, and a good correlation was obtained between changes in process conditions and ion concentrations. In addition, Chai *et al.* [2002] have developed an attenuated total reflection (ATR) UV-sensor, which could replace the online automatic titration systems by online monitoring of sulfide, hydroxide, and carbonate ions in kraft pulp white liquors. However, most of the current potential online analytical systems have suffered from high maintenance requirements. This has made them unlikely to be suitable for mill applications; in addition, many of these sensors can provide only single-component measurements.

In this research, the main purpose was to develop a fast and simple analysis system for certain interfering substances in complex papermaking process waters. Sampling is one of the challenging tasks in developing online analytical methods by adapting equipment normally used in laboratory conditions to paper mill use. The process water sample matrix caused problems in the development work because papermaking process waters usually contain large amounts of  $> 0.2 \mu\text{m}$  particles like colloidal lipophilic extractives droplets ( $0.1\text{-}2 \mu\text{m}$ ) [Holmbom *et al.*, 1991]. The HPLC and MS are highly sophisticated analysis equipments, which are generally not suitable for samples containing particles such as fines, normally found in papermaking process waters. In this research, the problem was solved by the use of an online sample enrichment technique, which enriched the sample and the possible particles to the enrichment column (standard HPLC precolumn). As shown in the literature review, the wood extractives that impact the papermaking process are rather complicated and therefore extensive studies have been conducted to prevent and solve problems caused by these compounds. Several different kinds of analytical procedures are in fact available for developing a

simpler and faster method to analyze the wood extractives in papermaking process waters. However, the use of HPLC-MS technique in the analysis of process waters has not been widely reported. The following experimental section of the work presents the analytical methods developed as well as the applications of the method with quality parameters.



### 3 EXPERIMENTAL

#### 3.1 Chemicals and equipment

The chemicals used in this study are listed in Appendix I. The structures of the most common resin and fatty acids occurring in papermaking process waters are presented in Appendix II.

All standard stock solutions were prepared by dissolving an accurate amount of a pure standard in a solvent. For GC analysis, all solutions were made with MTBE and for APCI-MS analysis with methanol. Ultra high quality (UHQ) water (I-IV) or methanol (V) was used for dilutions.

The GC separations were performed by an HP 5890 Series II Plus System equipped with an HP 7673 injector and a Programmable Cool On-Column inlet (Hewlett Packard, Palo Alto, CA, USA). The individual resin and fatty acids were analyzed with the above equipment (I,II) and with an Agilent 6850 Series instrument (IV). Identification of the peaks was made with an Agilent 6890 GC-5973 MSD or Hewlett Packard 5890 Series II-HP 5972 MSD instruments. Experimental details are shown in Table 6.

TABLE 6. GC analysis of wood extractives

Parameter	GC/FID (groups)	GC-FID (individual)	GC-MS (individual)	
Equipment	HP5890	HP 5890/Agilent 6890	HP 5890	Agilent 6890
Column	HP-1	Rtx-5/Supelco Equity-5	ZB-5HT Inferno	HP5-MS/Rtx-5/ Supelco Equity-5
-length (m)	7.5	30	30	30
-i.d. (mm)	0.53	0.32	0.25	0.25
-film thickness ( $\mu\text{m}$ )	0.15	0.25	0.25	0.25
Temperature program	1 min at 90 °C, 12 °C min <sup>-1</sup> to 320 °C, and 10 min at 320 °C	1.5 min 100 °C, 6 °C min <sup>-1</sup> to 180 °C, and 4 °C min <sup>-1</sup> to 290 °C for 20 min	Same as GC-FID (individual)	Same as GC-FID (individual)
Injector temperature program (°C)	90 °C, raised to 320 °C at a rate of 200 °C min <sup>-1</sup>	290	290	290
Detector temperature (°C)	290	300	290	290
Carrier gas	N <sub>2</sub>	N <sub>2</sub>	He	He

For APCI-MS studies, an HP 1100 liquid chromatography-mass spectrometer from Hewlett Packard (Palo Alto, CA, USA) including a binary pump, a vacuum degasser, and a thermostatted column compartment with a six-port switching valve was used.

In papers I-II and IV, the sample was introduced by a Waters 501 pump (Waters, Milford, MA, USA), and an HP 1100 pump was used to deliver the mobile phase (methanol) at a flow rate of 0.5 mL min<sup>-1</sup>. The sample was enriched for one minute and the sample flow was directed into the waste with a six-port switching valve (HP 1100 thermostatted column compartment). The analytes trapped in the precolumn were flushed in the backflush mode and transferred online to the MS. A Waters column switching valve (Milford, MA, USA) controlled by an HP 35900E Interface (Palo Alto, CA, USA) was used to switch the flow coming from the pump either to the waste (sample enrichment) or to the MS.

In paper III, the samples were filtered with a ceramic filter and the sample was introduced to the system by a centrifugal pump. The overflow of the sample from the filter and the sample collection were recycled to a sample container. In addition, a Waters column switching valve controlled by an HP 35900E Interface was used to switch the flow from the pump either to the waste (sample enrichment) or to the MS. In paper V, the compounds were separated with a Waters Atlantis dC<sub>18</sub> column connected to the guard column of the same packaging material; an autosampler was used to inject the sample.

Detection was carried out using an HP 1100 Series single quadrupole MS (Hewlett Packard, Palo Alto, CA, USA), equipped with an APCI interface operating in the negative ion mode. The MS parameters were optimized in papers I and II. HP ChemStation software (version A.06.03) was used for data collection and processing.

### **3.2 Process water samples and sample preparation**

The process water samples used in this study are listed in Table 7. All process water samples (I-III,V) and unbleached TMP (IV) were taken from different stages of the papermaking process from a paper mill using TMP. Before analysis, samples were stored at either 4 °C (II,III) or -20 °C (I,IV,V).

TABLE 7. Process water samples used

Paper	Sample	Information
I	1-4	Different sampling times, grinding zone
I	5	Pulp section of a paper mill
I	6-8	Modified TMP model waters
II	1-3,5	Different places, grinding zone
II	4	Paper machine
III	1	Grinding zone (laboratory-scale online experiments)
IV	1-5	Pilot-scale installation (SCD)
V	1-6	Paper machine process waters

Prior to analysis, the samples were allowed to warm up and then mixed carefully. For GC analysis, the sample preparation procedure reported earlier by Örså and Holmbom [1994] was used with the following minor changes: betulinol (I,II), heneicosanoic acid (I), cholesteryl heptadecanoate (I), and margaric acid (II,IV) were used as internal standards (Table 8).

TABLE 8. Sample preparation for the GC-FID analysis

Paper	I		II	IV
Pretreatment	Centrifuged	Centrifuged	No prefiltration or 0.45/1.2 $\mu\text{m}$ filter	Filtration 8 $\mu\text{m}$ ceramic filter
Sample amount (mL)	4-20	5-20	2-10	5
Sample extraction	MTBE <sup>a</sup>	SPE	MTBE <sup>a,I</sup>	MTBE <sup>a,I</sup>
Internal standards	Betulinol, cholesteryl heptadecanoate, heneicosanoic acid	Betulinol, heneicosanoic acid	Betulinol, margaric acid	Margaric acid

a= Örså and Holmbom, 1994

For APCI-MS analysis, sample preparation was limited to filtration and dilution in order to keep the developed method as fast and simple as possible, except in paper I, in which centrifugation was also used (Table 9). In paper IV, the TMP process water was recycled through the SCD and the samples were taken from the filtrate flow of the first vacuum box at different recycling times. After sampling, turbidity measurements were made and samples were filtered through an 8  $\mu\text{m}$  ceramic filter before APCI-MS analysis.

TABLE 9. Sample preparation for the APCI-MS analysis

Paper	I	II	III	IV	V
Pretreatment	Centrifugation	No prefiltration or 0.45/1.2 $\mu\text{m}$ filter	Online sample filtration (0.2 $\mu\text{m}$ )	Filtration 8 $\mu\text{m}$ ceramic filter	Dilution 1.2 $\mu\text{m}$ filter
Sample flow rate (mL min <sup>-1</sup> )	0.2	0.2	0.3	0.2	-
Sample enrichment time (min)	1	1	2	1-2	-
Sample amount (mL)	0.2	0.2	0.6	0.2-0.4	1:1
Enrichment column	Resolve C18	Hypersil ODS Atlantis dC <sub>18</sub> Guard-Pak RCSS CN, Guard-Pak $\mu$ Bondapak NH <sub>2</sub>	Resolve C18	Fusion-RP (Phenomenex)	-
Separation column	-	-	-	-	Waters Atlantis dC <sub>18</sub>
Quantification method	External standard calibration	Internal standard (margaric acid)	External standard calibration	Internal standard (margaric acid)	Internal standard (myristic acid)

- = not used

## 4 RESULTS AND DISCUSSION

### 4.1 Development of the analytical method

The main purpose of the method development work was to find a simple procedure to analyze selected resin and fatty acids present in papermaking process waters. The scheme of the research structure is presented in Figure 6. At the beginning of the sample pretreatment studies, the efficiency of the LLE and SPE techniques for the isolation of resin and fatty acids from papermaking process waters was compared and SPE was connected online to the MS system with an HPLC arrangement due to technical interface obstacles (I). Secondly, several different precolumns were tested for sample cleanup and enrichment (II, see also Appendix III). Quality parameters such as precision, linearity, and accuracy were determined for the precolumns; finally, the NH<sub>2</sub> precolumn was applied to the samples of process waters.

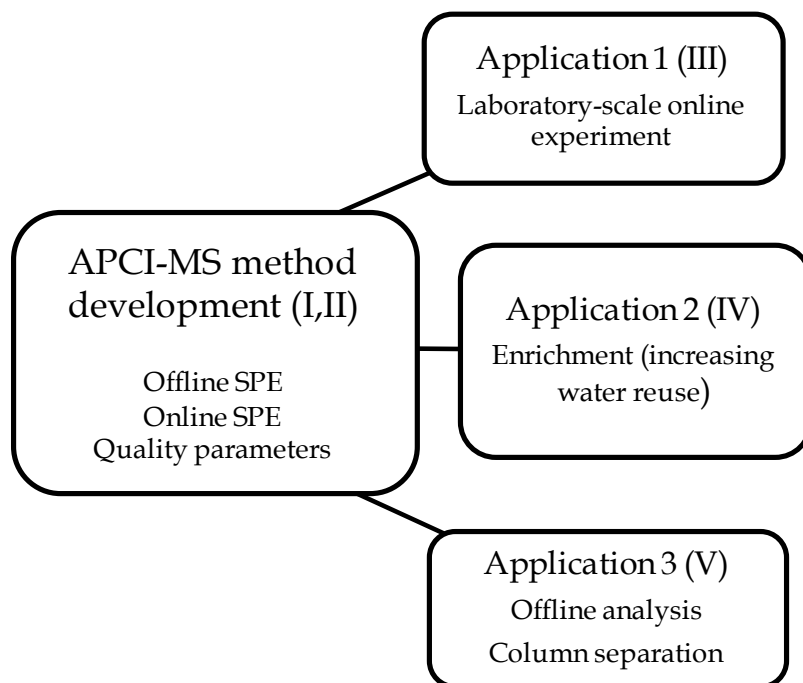


FIGURE 6. Schematic diagram of the research.

After the above pretreatment studies (I,II), the research work continued by testing the analytical procedure in three different applications. The laboratory-scale online enrichment study (Application 1, III) provided useful data for possible online use of the analytical procedure. The installation of the analytical procedure is presented in Figure 7. The sample flow was switched by time-controlled valves and the overflow of the sample from the filter and sample collection was recycled to a sample container. In Application 2 (IV), the enrichment of the wood extractives in the process waters was demonstrated by reusing the process water in a pilot-scale water circulation environment. Finally, the faster laboratory HPLC-MS method with Waters Atlantis dC<sub>18</sub> column separation combined with a guard column of the same packaging material was developed in order to fulfill the need for faster laboratory measurements (V).

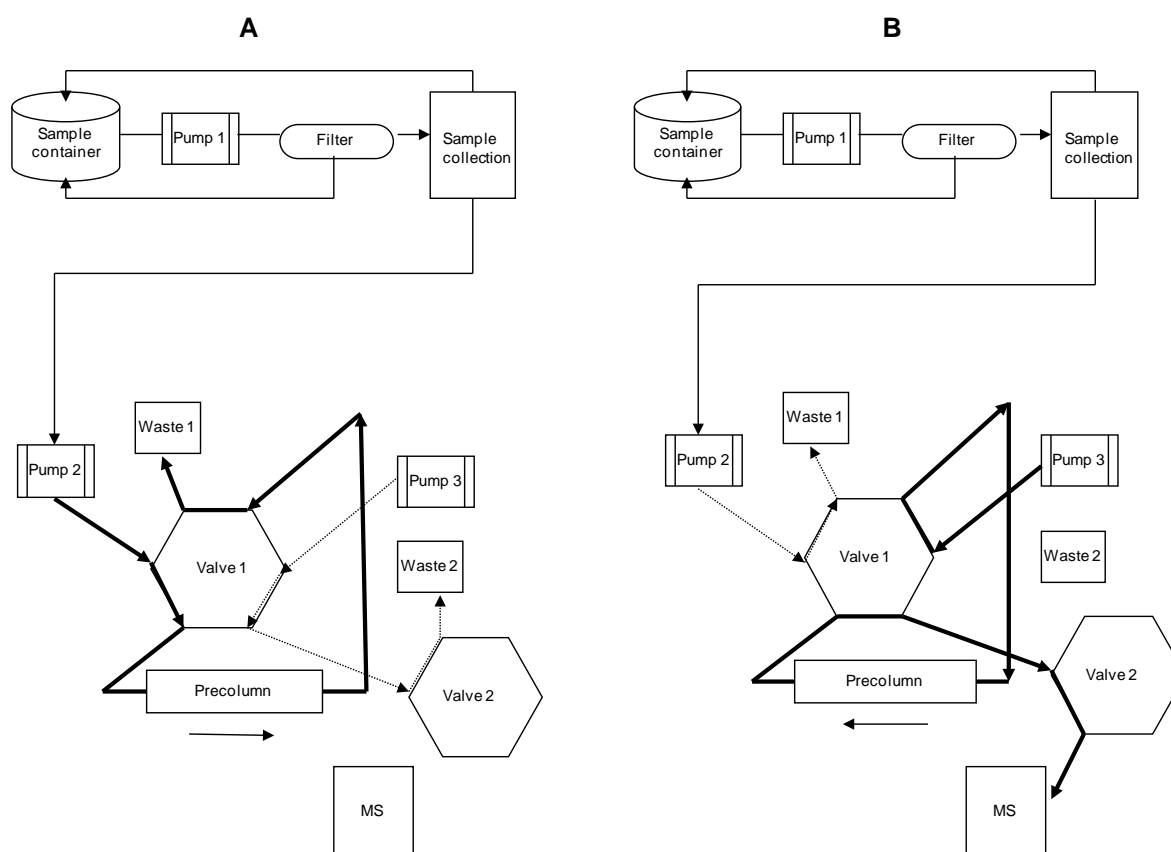


FIGURE 7. Schematic diagram of the research (Application 1, III). A: Sample enrichment; B: sample elution to MS.

Before the online sample enrichment connection to the MS, the efficiency of traditional sample preparation methods such as LLE and SPE extraction was compared; the sample pH effect on the extraction results was also evaluated (I). The results supported the aim of the study, to analyze process water samples without pH adjustment, as has been stated in other studies [Mosbye *et al.*, 2000]. The comparisons showed that both of the extraction methods have almost equal extraction efficiency, but a beneficially short separation time and low organic solvent consumption could be achieved with SPE. The online connection of SPE for sample enrichment purposes was completed by using standard HPLC precolumns and time-controlled switching valves before MS detection. The best overall material for the enrichment precolumns was further studied with four different standard HPLC precolumns for quality parameters (*i.e.*, precision, linearity, and accuracy) (II).

The APCI ionization parameters, such as cone voltage, nebulizer pressure, fragmentor value, and corona current, were optimized through the FIA technique using no separation or sample enrichment column (I,II). In the negative APCI selected ion monitoring (SIM) technique, only the intensive  $[M-H]^-$  ions of the compounds of interest were generated, with no fragment ions produced even at high cone voltage or corona current values. The highly resistant resin acids with cyclic structure set limitations on the ionization conditions, so compromises had to be made when setting the MS parameters. The highest MS detection sensitivity values were achieved with a nebulizer temperature of 350 °C, a vaporizer temperature of 325 °C, a nebulizer pressure of 60 psig, and a drying gas ( $N_2$ ) flow of 3 L  $min^{-1}$ . The value 16  $\mu A$  was set to the corona current, and capillary and fragmentor voltage were found to be optimal at 3500 V and 100 V respectively.

The results of APCI-MS analysis were quantified by either the external calibration method (I,III) or the internal standard method (II,IV,V). In the first phase of the research, the use of external calibration was preferred, but it was also noted that MS ionization was susceptible to daily variations. A comparison of the internal standard, external standard, and response factors (RF) confirmed that the use of the internal standard method improved the reliability of the results (II). Therefore, possible daily variations, *i.e.*, peak areas, in MS ionization were eliminated through the use of internal standards. Heneicosanoic acid has been used as an internal standard in GC-FID analysis [Örså and Holmbom, 1994] and margaric acid in GC-MS and HPLC-MS analysis [Rigol *et al.*, 2003a]. The small aliphatic carboxylic acids such as fumaric, glutaric, and adipic acids have also been tested as internal standards. Margaric acid was proven to be the most suitable internal standard in online sample enrichment studies, whereas the use of myristic acid was preferred with column separation HPLC-MS analysis (V). Myristic acid

provided a valid alternative because trace amounts of margaric acid can be present in some process waters.

## 4.2 Quality parameters of the method

Quality parameters such as accuracy, linearity, and precision were evaluated by measuring a series of replicate injections of the analytes on different measurement days. The extent of the validation measurements depended on the focus of the research. In the method development phase (I,II) the method was roughly evaluated for precision (*i.e.*, intra-day, day-to-day, and intermediate precision), repeatability, LOD, and linearity evaluations. In laboratory-scale online measurement, the main interest was sample enrichment and the increase in analyte concentrations (III,IV). Therefore, daily variations and repeatability of the different online enrichment studies were measured. The method was completely validated in paper V, in which the compounds were separated by an HPLC column.

### 4.2.1 Accuracy

The accuracy of the online sample enrichment APCI-MS method was measured by spiking a mixture of the compounds analyzed at different concentrations. Repeated injections ( $n=7$ ) were performed and the introduced and measured concentrations were compared (II). For standard compounds in aqueous media, the results varied between 87 and 112, depending on the compound and precolumn studied. The best overall recovery was achieved by using a  $\text{NH}_2$  precolumn with values of 99 and 101 for the low and high concentration levels, respectively.

The accuracy of the HPLC-APCI-MS method was evaluated by standard addition in both aqueous media and process water samples (V). The acids were added to the samples at varying concentrations, and there was good consistency in the results of the spiked and measured concentrations. It must be pointed out that compared with the results obtained by standard compounds in aqueous media, the best overall results were actually achieved in process water samples, except for stearic acid, with a recovery value of 107.8%. This might be due to the limited solubility of stearic acid in methanol [Heryanto *et al.*, 2007]. The relative standard deviation (RSD) values varied between 1.2% and 3.3%. However, it must be noted that the RSD value was 6.9% for palmitic acid, indicating that the separation of the palmitic acid might be disturbed by near eluting oleic acid.



### 4.2.2 Linearity

Linearity studies were performed to verify that the analyte responses were linearly proportional to the concentrations. The linearity of the method was evaluated with the standard model compounds of dehydroabietic, abietic, palmitic, and stearic acids in aqueous media (I). The results were good and correlation coefficients (R-values) were  $>0.99$ , except for dehydroabietic acid, whose value was 0.97. The linearity of the four precolumns tested was also measured with dehydroabietic acid in aqueous media: the R-values varied between 0.97 and 0.99, depending on the precolumn (II). The best overall linearity was achieved with the  $\text{NH}_2$  precolumn (peak area), and the other precolumns also showed satisfactory R-values ( $>0.9$ ).

In the sample enrichment studies, five samples were taken from the enrichment process and spiked with three different concentrations of palmitic, stearic, and dehydroabietic acids (IV). Several replicate injections were made and the linearity response of the enrichment was then measured by analyzing the data through linear regression. The R-values varied between 0.94 and 0.99, with RSDs between 2.2% and 9.8%. The method showed excellent sensitivity to the increase in analyte concentrations.

The linearity of the HPLC-APCI-MS method with column separation was evaluated by a standard addition method (V). The R-values were 0.96-0.99 and 0.97-0.99 for aqueous media and process water samples, respectively. The satisfactory slope and intercept values indicated no matrix effects in the determination of resin and fatty acids.

### 4.2.3 Precision

The precision of the method and possible daily variations were measured at low and high concentration levels by making repeated analysis measurements on different days. A single factor analysis of variance (ANOVA) was used to evaluate the intra-day, day-to-day, and intermediate precisions (II,III,V). In most of the measurements, the values were satisfactory, thus indicating good precision of the method developed.

When the four different precolumns were tested, the RSDs for the intra-day, day-to-day, and intermediate precision for dehydroabietic acid standard solution varied between 1.3% and 23.3%, depending on the precolumn material used (II). As in the linearity measurements, the  $\text{NH}_2$  precolumn gave the best RSD values, even though the day-to-day repeatability value for low concentration levels was

15.3%. The performance of the precolumn at these concentration levels may suffer some matrix interference in first measurements. The day-to-day precision repeatability values for laboratory-scale online measurements varied between 2.8% and 10.7%, depending on the dilution degree (III). The total precision of the measurement was evaluated by intermediate precision calculations. These values were somewhat higher than intra-day values, which varied between 3.0% and 12.2%.

In the fast offline HPLC-APCI-MS method (Application 3, Fig. 6), the results for the intra-day and day-to-day precisions of standard compounds were less than 3.4%, except for the stearic acid, whose day-to-day value was 5.9% (V). The intermediate precision was also higher than 5% for stearic acid (7.2%). The higher precision values for stearic acid might be due to the possible matrix effects or ion suppression in the MS instrument as a result of a small amount of interfering fines occurring in the samples after filtration through a 1.2  $\mu\text{m}$  filter.

#### 4.2.4 LOD

The LOD values of the online sample enrichment APCI-MS were measured by the SIM ions of the selected resin and fatty acids studied (I). The values were based on a signal-to-noise ratio (S/N) of 3 and 10 for LOD and limits of quantification (LOQ), respectively. The LOD value of 124  $\mu\text{g L}^{-1}$  was achieved for dehydroabietic acid in laboratory-scale online measurements (III). The values agreed well with values reported earlier [Rigol *et al.*, 2003b]; they were in fact expected to be somewhat higher than those obtained by a traditional analysis method with sample pretreatment steps. In the HPLC column separation study (V), the results for standard compounds in aqueous media were between 0.01 and 0.12  $\mu\text{g L}^{-1}$  for LOD and between 0.02 and 0.40  $\mu\text{g L}^{-1}$  for the LOQ, depending on the analyte.

#### 4.2.5 Column separation tests

The separation efficiency of the chromatographic column was evaluated by measuring the resolution values of the peaks (V). In the ideal cases, values  $> 1.0$  are considered to be a minimum for acceptable separation to occur and to give a good quantification [Braithwaite, 1996]. In the present HPLC-APCI-MS analysis, the values were  $> 1.0$  except for the linolenic acid, whose value was 0.78. The asymmetry values were close to 1, thus indicating a satisfactory separation of the compounds, even though no sample pretreatment was done.

### 4.3 Application to the process waters

The method developed was tested by means of three different applications to measure relative concentrations of the selected resin and fatty acids in papermaking process waters (III-V). As reported earlier, the overall process condition can be evaluated by measuring only one or two important compounds in process streams [Chow and Shepard, 1996]. The results of the online sample enrichment APCI-MS method compared to the more traditional GC-FID method may vary to some extent, but the internal standard can be used to balance the variation of the results. Obviously, this is due to the presence of interfering substances, such as fines, in process waters because no sample pretreatment was conducted. However, the difference between the results obtained from different analytical techniques is not significant if the main interest is to follow only possible changes in the concentration of resin and fatty acids in the process streams.

The online sample enrichment APCI-MS method was tested to monitor changes in the concentration levels of dehydroabietic acid in papermaking process waters (I,II,IV). Based on these results, dehydroabietic acid can be considered a good specific indicator for the total concentration level of resin acids (Fig. 8). A good correlation of the results was achieved with R-values  $>0.96$ , and in case of significant changes, traditional analysis with GC-FID or HPLC with a sample pretreatment could be used to verify the results. The use of palmitic, stearic, oleic, or linolenic acids as an indicator for total concentration levels of fatty acids was also tested. The enrichment of these compounds in the process was not as straightforward as that of resin acids, but some trend ( $R>0.9$ ) was also found with increased concentrations of fatty acids measured by APCI-MS and turbidity methods (IV).

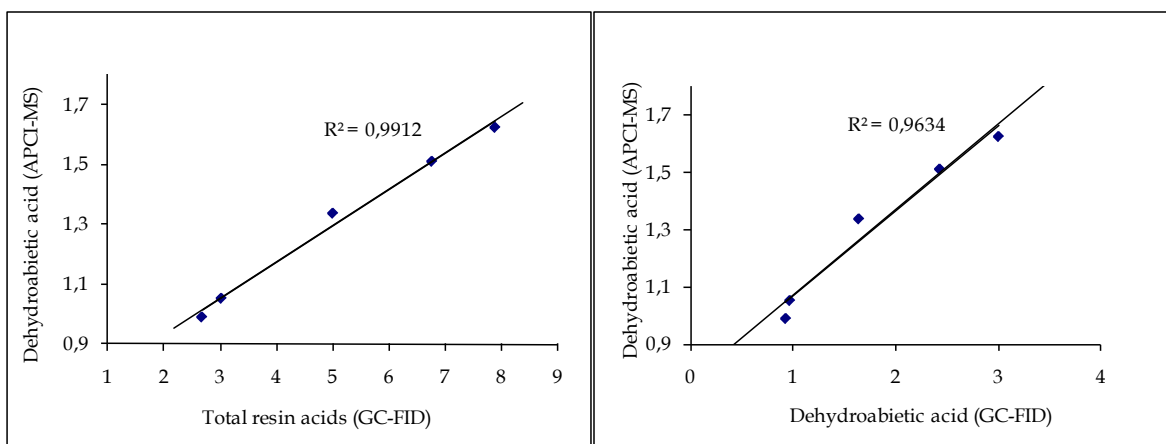


FIGURE 8. Correlation of dehydroabietic acid and total resin acids analyzed by APCI-MS and GC-FID methods (IV).

### 4.3.1 Laboratory-scale online measurement

A centrifugal pump and a ceramic filter were connected to the APCI-MS system in order to demonstrate the possible online use of the method developed in the paper machine environment (III). The sample flow was switched with time-controlled column switching valves and the sensitivity of the method to increase in analyte concentrations was evaluated by performing several repeated injections at 5-minute intervals. The time-controlled valves were used to control the time between sampling. The use of the method for the continuous analysis of selected acids was also measured by changing the time of the injection intervals. Several replicate injections (8-10) were made with time intervals of 10, 20, and 28 minutes; the repeatability of these measurements was <9%. The best overall repeatability was achieved at the 28-minute interval, which was probably because the longer flushing time of the enrichment column ensured the proper cleaning and stabilization of the enrichment column. The results of the repeated injections of dehydroabietic, oleic, and stearic acids showed that the system developed can be used to continuously evaluate the selected interfering substances in papermaking process waters (Fig. 9).

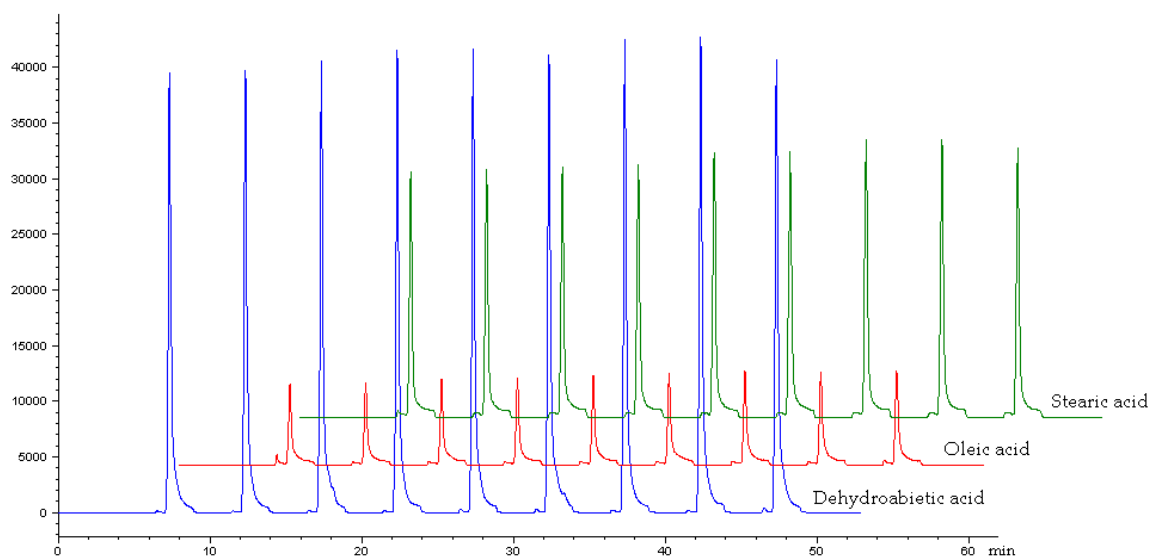


FIGURE 9. Chromatogram of the laboratory-scale online sample enrichment studies (III).

#### 4.3.2 Pilot-scale installation studies

The pilot-scale installation studies were performed by recirculating the TMP process water in SCD, demonstrating paper machine water circulation that could enrich selected resin and fatty acids (IV). The results showed that enrichment of the resin and fatty acids was linear, with R-values of  $>0.9$ . Also, conductivity increased after each recycling, indicating an increase in anionic trash in the process. The profiles of the different analytical methods (Fig. 10) confirmed that the total resin acid content in process streams can be evaluated by analyzing only the dehydroabietic acid level.

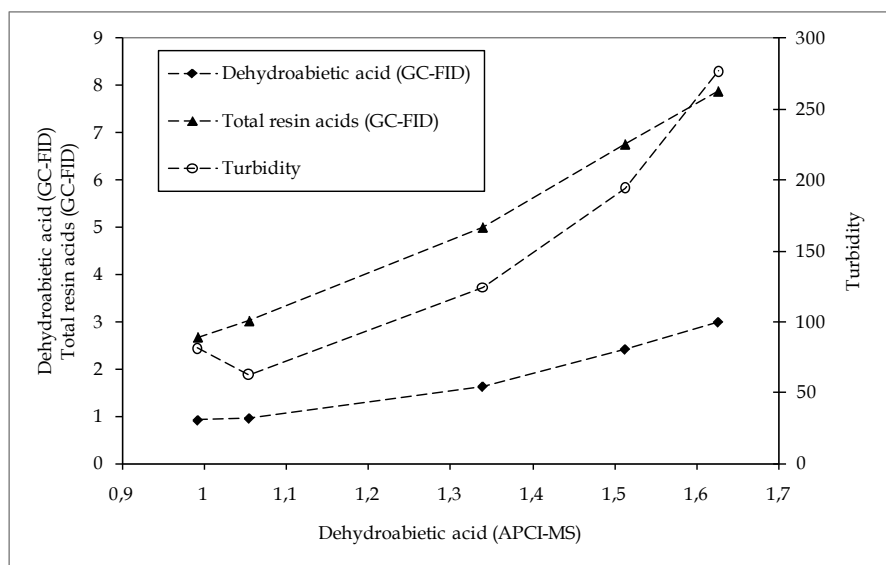


FIGURE 10. Correlations of dehydroabietic acid levels ( $\text{mg L}^{-1}$ ) analyzed by APCI-MS and GC-FID and total resin acid concentration ( $\text{mg L}^{-1}$ ) analyzed by GC-FID and turbidity (NTU) in recycled process water samples.

The analysis of selected resin and fatty acids by GC-FID, turbidity, and online sample enrichment APCI-MS methods was compared (IV). All these methods have a series of beneficial properties; the choice of the analytical method depends on the information needed. The comparison of the analytical methods commonly used for the analysis of wood extractives or selected extractive component is presented in Figure 11. More detailed information can be achieved by analyzing the samples with the traditional LLE and GC-FID methods, whereas turbidity measurement provides a rough evaluation of the process condition when estimating wood resin in process streams. The comparison of the methods clearly showed that significant time and cost savings can be achieved by using faster and simpler analytical methods that can provide sufficient information about the process state and possible oncoming problems without full analytical processing.

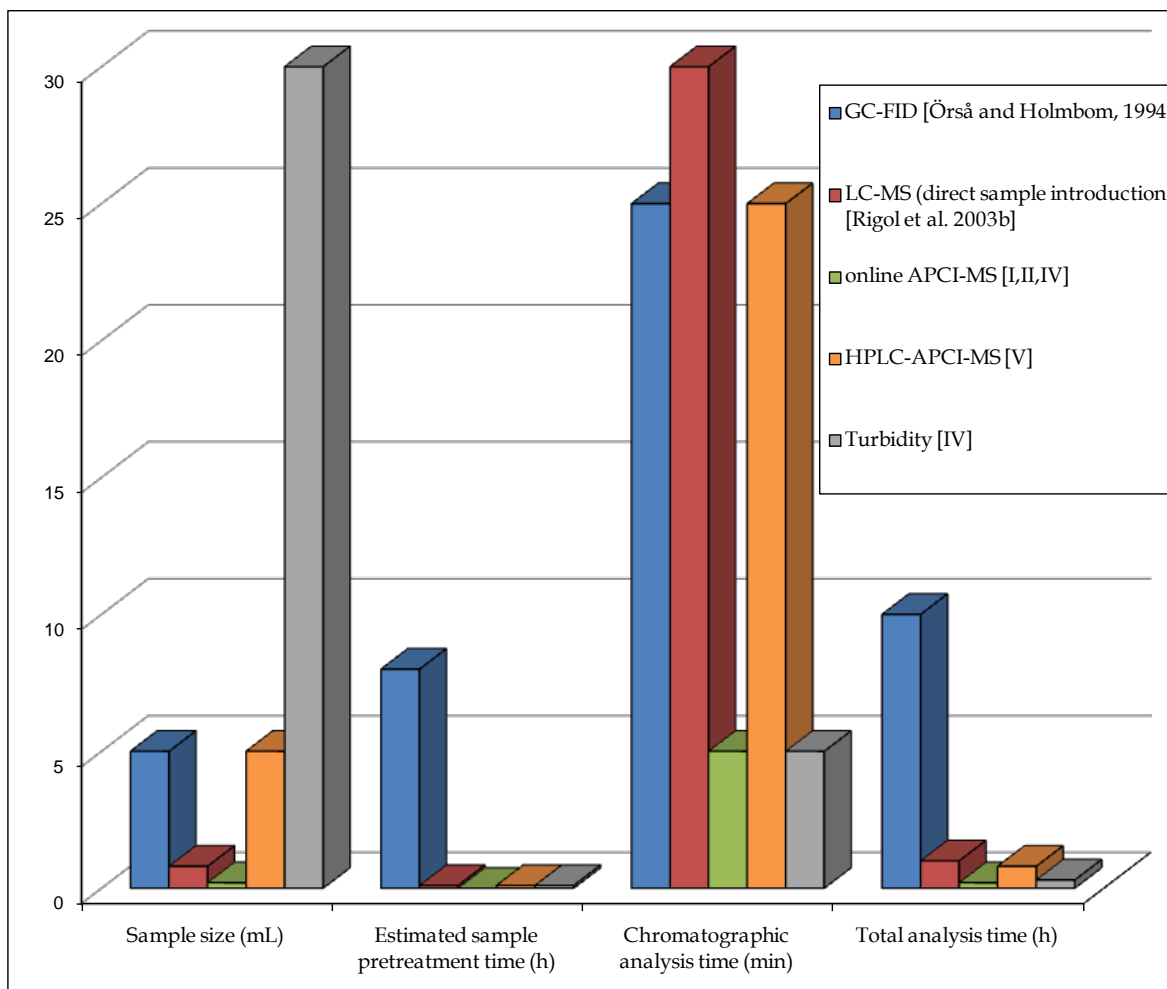


FIGURE 11. Comparison of the analytical methods.

### 4.3.3 HPLC-APCI-MS

The faster laboratory method with HPLC column separation (Application 3, V) was tested for analysis of selected resin and fatty acids in papermaking process waters. The minimum sample pretreatment with only filtration and dilution enabled a fast analysis of dehydroabietic, palmitic, stearic, oleic, and linolenic acids in different parts of the paper machine wet-end. The main contribution of the method was to offer a new solution for the time-consuming sample pretreatment and chromatographic analysis. The method provided a clear advantage, with a total chromatographic analysis time of less than 25 minutes. An SIM chromatogram of the separation of the selected resin and fatty acids in a process water sample after addition of the standard compounds is presented in Figure 12

(V). The individual peaks were separated by their extracted ion current (EIC) chromatograms from the SIM ions. In the EIC chromatogram, only the ions of a particular molecular mass are considered when extracting mathematically specific information (EIC) from the crude SIM data.

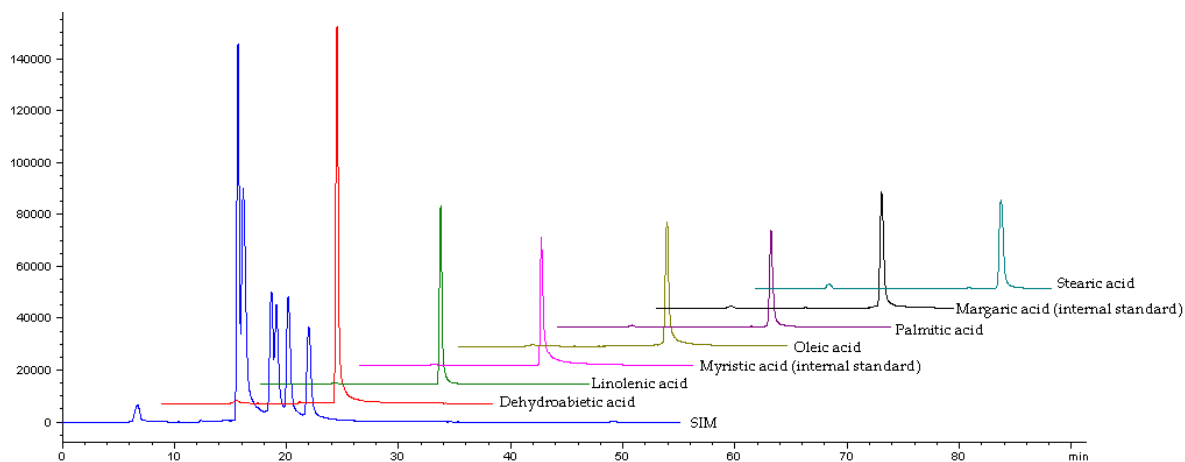


FIGURE 12. SIM and EIC chromatograms of a process water sample after the addition of target compounds (V).



## 5 CONCLUDING REMARKS

The results showed that the present procedure based on online sample enrichment followed by APCI-MS analysis is suitable for determining the concentration levels of resin and fatty acids in papermaking process waters with reasonably high accuracy and repeatability. Compared to the conventional methods commonly used for this purpose, the main benefits of the method are rapidity of measurement, simplicity of use, and absence of the need for multistage and time-consuming sample pretreatment. For this reason, this method is suitable for a rapid monitoring of papermaking by analyzing individual resin and fatty acid concentrations, which can be used to evaluate the total concentration of the interfering extractive substance groups in process waters. The use of concentration levels of the compounds eliminates the difficult and time-consuming analysis of absolute concentrations. This means that peak areas proportional to the concentration levels could be used directly when monitoring possible changes in the process streams at the same sampling places.

The result also showed that the APCI-MS method provides several possible applications in which faster analysis methods are needed for a rough evaluation of important and harmful substances in papermaking processes. Based on this research, the multistage analytical scheme presented in Figure 13 can be recommended for the analysis of resin and fatty acids in papermaking process waters. In the first stage, a process water sample can be quickly screened with an online sample enrichment method and the further analytical procedure can be determined based on these results. In the next step of the procedure, the research supports the use of conventional analytical methods such as LLE and GC-FID. This is necessary when detailed structural information is needed from all abietic acid isomers, for example. The fast HPLC-MS method can be used for individual resin and fatty acid analysis. The possible coeluting compounds from HPLC-MS analysis can be confirmed using HPLC combined with tandem mass spectrometric detection (MS/MS). The proposed analytical procedure can be used to optimize the sampling by fast screening of the sample and deciding on further analysis based on the positive or negative results. The main users for this analytical procedure can be found in the papermaking industry and papermaking chemical suppliers. However, many other chemical industries that use organic chemical compounds may also find this procedure useful.

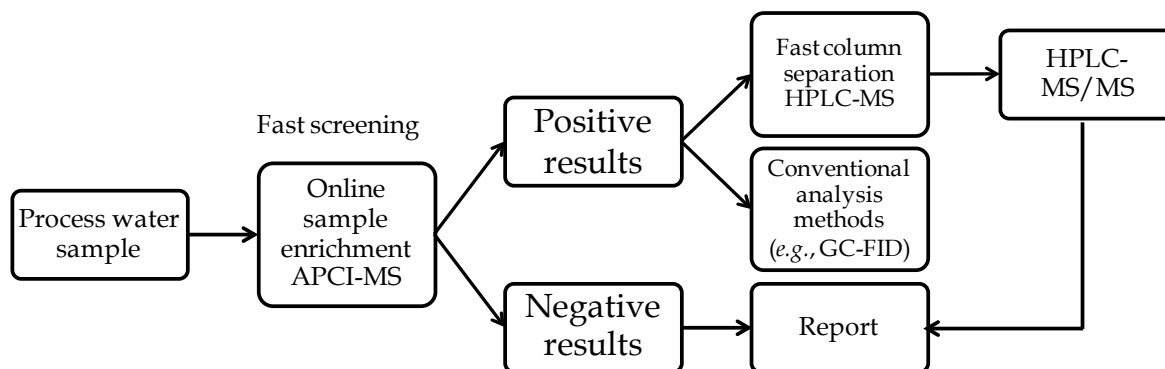


FIGURE 13. Proposed scheme of the analytical procedures used for analysis of resin and fatty acids in papermaking process waters.

## 6 SUGGESTIONS FOR FUTURE RESEARCH

The developed method with online sample enrichment provides a basic tool for monitoring the papermaking process. However, it can be further improved and simplified. The automation of the sampling process has been successfully tested in laboratory conditions (III), but it should be also tested in real process conditions. For total utilization of the analytical procedure, the main challenge in this experiment is protecting sensitive MS equipment from process heat and other disturbances.

Many reports have emphasized the importance of knowing the concentration of resin and fatty acids present in the papermaking process. The analysis of these compounds in the papermaking process is important due to the current trend to close more effectively process water circulations, aiming at a drastic decrease in the waste water load. However, there are still several areas in the papermaking process in which water usage could be further reduced. Principally, the APCI-MS technique could also be easily modified for analyzing not only resin and fatty acids, but also other chemical compounds, such as biocides or carboxylic acids, and many environmental pollutants.

Tall oil soap, comprising mainly the sodium salts of resin and fatty acids, is an important byproduct of the kraft pulping process and can be recovered from spent black liquor for further processing. The fast analysis of black liquor carboxylic acids and resin and fatty acids could be achieved with the same analysis procedure, which provides excellent information about the removal of soap from the liquor at various stages of the process. This would be a further benefit for the whole recovery operation.

In the process monitoring, the use of process chemicals could be one possible alternative application. The evaluation of the need for process additives such as pitch controlling agents could be achieved by measuring the selected resin and fatty acid levels in process waters; these levels have been shown to be excellent indicators for total extractives content. An interesting environmental research area would also be the effluents from the debarking of wood.

The procedure developed basically uses MS with the SIM mode, in which the selected ions are detected based on the molecular mass of the compounds. For the so-called fingerprint analysis of the samples, the MS can be used in full scan mode with a wider selected mass area. The samples can be evaluated by comparing these

different fingerprint chromatograms, which are typical for each sampling location. This fingerprint technique can also be utilized when analyzing different kinds of precipitates, for example, in paper machine wires, cylinders, and felts.

In the laboratory, the need for faster offline analysis methods is also a challenge. The fast HPLC-APCI-MS method developed here could be further modified by using the UPLC technique, which has not yet been used for analysis of resin and fatty acids. The UPLC technique provides a beneficial lower solvent consumption and fast resolution of compounds of interest. Fast chromatographic analysis with HPLC-MS techniques provides a valid alternative to time-consuming GC analysis, thus decreasing the sampling need and analysis time substantially.

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## **APPENDICES**

APPENDIX I: List of chemicals used in this study.

APPENDIX II: The structure of the most common resin and fatty acids present in papermaking process waters.

APPENDIX III: Precolumns tested for sample enrichment.



## APPENDIX I

### Chemicals used in this study

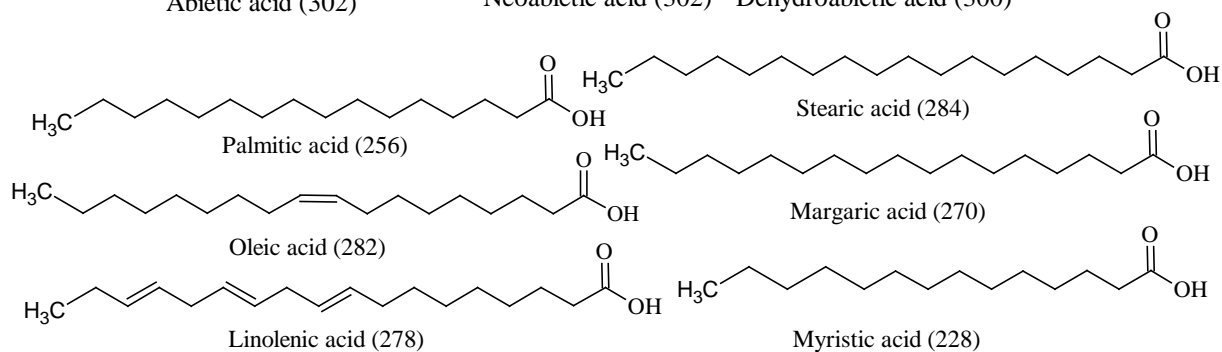
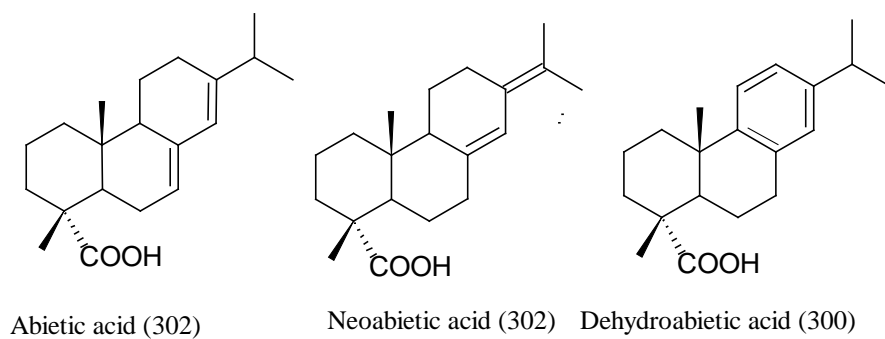
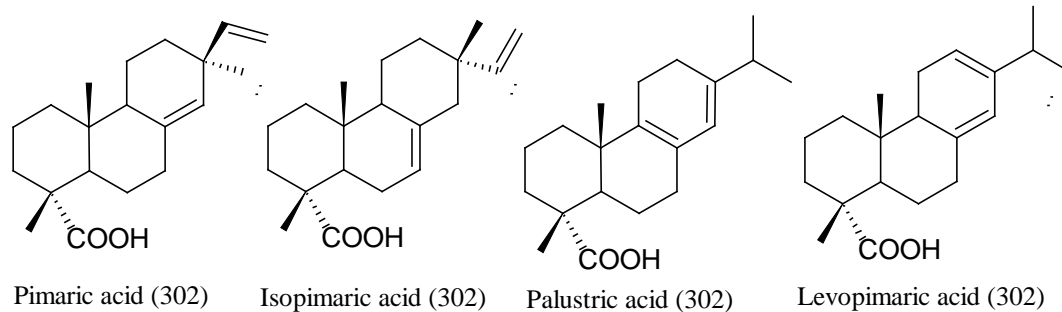
Chemical	Supplier	Purity (%)	Paper
Abietic acid	Sigma	85	I
Betulinol	Sigma	98	I, IV
Cholesteryl heptadecaonate	Sigma	>98	I
	TCI	>97	IV
DHAA	ICN	>98	I,II,III
	Helix Biotech	>99	IV,V
1,3-Dipalmitoyl-2-oleyl-glycerol	Sigma	99	IV
Heneicosanic acid	Sigma	99	I
Linolenic acid	Sigma	99	IV,V
Margaric acid	Aldrich-Europe	97	II,IV,V
Myristic acid	Fluka	97	V
Oleic acid	Merck	>60	III,V
Palmitic acid	Fluka	97	I,IV,V
Stearic acid	Fluka	99.5	I,III,IV,V

### Solvents used in this study

Chemical	Supplier	Purity (%)	Paper
Acetone	Riedel de Haën	Analytical grade	I
BSTFA	Regis Technologies	99	I,IV
Dichloromethane	Rathburn	HPLC-grade	I
Methanol	Rathburn	HPLC-grade	I-III
	J.T. Baker	HPLC-grade	IV,V
MTBE	Lab-Scan	99.8	I,II,IV
TMCS	Regis Technologies	99	I,IV

## APPENDIX II

The structure of the most common resin and fatty acids present in papermaking process waters (molecular mass in parentheses)



## APPENDIX III

The precolumns used for sample enrichment

Precolumn	Manufacturer	Paper
Resolve C18 10 $\mu\text{m}$	Waters	I
Hypersil ODS 5 $\mu\text{m}$	Agilent Technologies	II
Atlantis dC <sub>18</sub> 5 $\mu\text{m}$	Waters	II
Guard-Pak RCSS CN 10 $\mu\text{m}$	Waters	II
Guard-Pak $\mu$ Bondapak NH <sub>2</sub> 10 $\mu\text{m}$	Waters	II
Resolve C18 10 $\mu\text{m}$	Waters	III
Fusion-RP 4*2.0 mm	Phenomenex	IV

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