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# **FAST CHROMATOGRAPHIC METHODS FOR DETERMINING ALIPHATIC CARBOXYLIC ACIDS IN BLACK LIQUORS**

BY  
**JAANA KÄKÖLÄ**

Academic Dissertation  
for the Degree of  
Doctor of Philosophy



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## LIST OF ORIGINAL PUBLICATIONS

This thesis consists of an overview and of the following five publications which are referred into the text by Roman numerals (I-V).

I Jaana Käkölä and Raimo Alén, A fast method for determining low-molecular-mass aliphatic carboxylic acids by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *Journal of Separation Science*, 29(13): 1996-2003 (2006).

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II Jaana Käkölä, Raimo Alén, Hannu Pakkanen, Rose Matilainen, and Kaisa Lahti, Quantitative determination of the main aliphatic carboxylic acids in wood kraft black liquors by high-performance liquid chromatography mass-spectrometry, *Journal of Chromatography A*, 1139(2): 263-270 (2007).

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III Jaana Käkölä, Raimo Alén, and Rose Matilainen, A fast analysis method for aliphatic carboxylic acids in alkaline non-wood cooking liquors, *Cellulose Chemistry and Technology*, 42(4-6): 213-222 (2008).

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IV Jaana M. Käkölä, Raimo J. Alén, Jukka Pekka Isoaho, and Rose B. Matilainen, Determination of low-molecular-mass aliphatic carboxylic acids and inorganic anions from kraft black liquors by ion chromatography, *Journal of Chromatography A*, 1190(1-2): 150-156 (2008).

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The experimental work in the publications I-V was performed by the author and the writing was carried out together with co-authors. The planning of the experimental work in publication IV was performed together with Jukka Pekka Isoaho and in publication V together with Professor Raimo Alén. In publication II, Kaisa Lahti made the GC measurements and Hannu Pakkanen provided the data on the cooks and pulps (publication II: Table 1, publication III: Table 1, publication IV: Table 1, publication V: Table 1).

## PREFACE

This thesis is based on experimental work carried out in the Laboratory of Applied Chemistry at the Department of Chemistry, University of Jyväskylä, during the years 2003-2009.

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## ABSTRACT

Two fast quantitative analysis methods and one qualitative analysis method were developed for the analysis of the low-molecular-mass (LMM) aliphatic carboxylic acids present in alkaline spent liquors from wood and non-wood delignification. The methods were applied to monitor the formation of the acid constituents during laboratory-made kraft cooks.

In the first part of the research, a high-performance liquid chromatographic (HPLC) separation method with atmospheric pressure chemical ionization mass spectrometry (APCI-MS) was developed and validated for the analysis of the main LMM aliphatic carboxylic acids in aqueous media. In the second part, the applicability of the method developed was tested for the determination of the most abundant LMM aliphatic mono- and dicarboxylic acids present in softwood (pine kraft and spruce kraft), hardwood (birch kraft and aspen kraft), and non-wood (bamboo soda-AQ (anthraquinone), bamboo kraft, wheat straw soda-AQ, and wheat straw kraft) black liquors.

Even more robust and simpler methods than HPLC-APCI-MS might be useful for industrial applications. Ion chromatography (IC) with conductivity detection (CD) offers a versatile, simple, and rapid method for determining a multitude of their key anions. Therefore, an ion chromatographic method with suppressed conductivity detection was developed and validated for the quantitative determination of several LMM aliphatic mono- and dicarboxylic acids as their carboxylate anions together with some inorganic anions (chloride, sulfate, and thiosulfate). To confirm the identification of some carboxylate anions that lack commercial model substances, a complementary qualitative IC method with suppressed electrospray ionization mass spectrometry (ESI-MS) was also developed. The method validation process for IC-CD was carried out with a mixture of carboxylic acids and inorganic anions in aqueous media and in seven different types of wood and non-wood black liquor samples. The validation results clearly indicated that, with respect to selectivity, linearity, limits of detection and quantification, precision, and accuracy, the IC-CD method showed good applicability in the determination of the LMM aliphatic carboxylic acids in black liquors.

The developed HPLC-APCI-MS and IC-CD methods are potential tools for the fast determination of the acids for many practical purposes. It has been noticed that there is a clear relationship between the acid formation and the progress of delignification. Therefore, the formation of these acid by-products, which are the

main alkaline degradation products of carbohydrates, was investigated. It was observed that a correlation exists between the formation of certain acids and the H-factor. Instead of using absolute amounts, practical information was obtained by plotting the concentration ratios of the selected acid products against the parameters characterizing delignification. This procedure eliminates the difficulty of measuring the absolute concentration (*i.e.*, the use of an internal standard), and means that chromatographic peak areas, being proportional to the concentrations, could be used directly. Acid ratios were measured with HPLC-APCI-MS and IC-CD and no significant difference was observed between the results obtained with these two methods. The correlation between the acid ratio and the total yield and kappa number of the pulp was also examined. When the same acid ratios as in the H-factor case were used, the linear correlations were obtained.



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## ABBREVIATIONS

ACN	Acetonitrile
AHQ	Anthrahydroquinone
AMS	Anthraquinone monosulfonic acid
ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
AQ	Anthraquinone
BSTFA	<i>N,O</i> -bis(trimethylsilyl)trifluoroacetamide
CE	Capillary electrophoresis
CD	Conductivity detector (detection)
CI	Confidence interval
d.s.	Dry solids
E <sub>a</sub>	Activation energy
EA	Effective alkali
ED	Electrochemical detection
EI	Electron impact (ionization)
EIC	Extracted ion chromatogram
EMCC	Extended modified continuous cooking
ESI	Electrospray ionization
EtOH	Ethanol
FA	Formic acid
FIA	Flow injection analysis
FID	Flame ionization detector
GC	Gas chromatography
HA	Hydroxy carboxylic acid
HexA	Hexenuronic acid
H <sub>2</sub> Ph	Phthalic acid
HFBA	Heptafluorobutyric acid
HPICE	High-performance ion-exclusion chromatography
HPIC	High-performance ion-exchange chromatography
HPLC	High-performance liquid chromatography
HMM	High-molecular-mass
IC	Ion chromatography (ion-exchange chromatography)
ICP	Inductively coupled plasma
IE	Ion-exclusion
IEC	Ion-exclusion chromatography
IEPC	Ion-exclusion partition chromatography
IEX	Ion-exchange chromatography

IUPAC	International Union of Pure and Applied Chemistry
KHPh	Potassium hydrogen phthalate
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
LMM	Low-molecular-mass
MCC	Modified continuous cooking
MeOH	Methanol
MS	Mass spectrometry (or MS = monosaccharide in Figures 2, 3, and 5)
MSD	Mass spectrometric detection
MS-MS	Tandem mass spectrometry
<i>m/z</i>	Mass-to-charge ratio
NI	Negative ionization
NP	Normal-phase
NPEs	Non-process elements
PA	Propyl alcohol
PAC	Process analytical chemistry
PAD	Pulsed amperometric detector
PDA	Photo-diode array
PFBA	Perfluorobutyric acid
PS-DVB	Polystyrene-divinylbenzene
PVA	Polyvinyl alcohol
RDH	Rapid displacement heating
RI	Refractive index
RP	Reversed-phase
RSD	Relative standard deviation
SCAN	Scandinavian Pulp, Paper and Board Testing Committee
SIA	Sequential injection analysis
SIM	Selected ion monitoring
S/N	Signal-to-noise ratio
SPE	Solid-phase extraction
TAPPI	Technical Association of the Pulp and Paper Industry
TIC	Total ion chromatogram
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMCS	Trimethylchlorosilane
TMS	Trimethylsilyl
TRIS	Tris(hydroxymethyl)aminomethane
UHQ	Ultra high quality
UV	Ultraviolet
VIS	Visible

# 1 INTRODUCTION

## 1.1 Context description

The purpose of chemical pulping processes is fiber liberation through delignification and the processes can be classified based on the different ways of achieving this aim [Gullichsen, 1999]. The leading alkaline pulping method worldwide is the kraft process in which, the active components are sodium hydroxide (NaOH) and sodium sulfide (Na<sub>2</sub>S). Another important chemical pulping process is the soda process, in which the active component is NaOH without sulfur. This method is mainly used for processing non-wood feedstocks, whereas kraft pulping is the dominant pulping method for wood-based feedstocks [Sjöström, 1993; Alén, 2000].

The spent liquor ("black liquor") obtained after cooking can be considered as the most important by-product of the alkaline cooking process [Sjöström, 1993; Niemelä and Alén, 1999; Alén, 2000]. During kraft pulping roughly half of the wood material is degraded and dissolved. Due to these phenomena, the black liquor is a complex mixture of water, residual inorganic cooking chemicals, and organic material dissolved from feedstock. The main degradation reaction during alkaline delignification of wood polysaccharides (cellulose and hemicelluloses) is the end-wise degradation (*i.e.*, the peeling reaction) with the simultaneous formation of a large number of water/alkali-soluble hydroxy mono- and dicarboxylic acids together with volatile formic and acetic acids. This industrially important type of degradation is mainly responsible for the significant carbohydrate losses that take place during the delignification of chemical pulps. The nature and total amount of carboxylic acids dissolved in the black liquor primarily depend on the raw material and the pulping conditions used.

The driving force behind the investigation into black liquor composition has included a wide range of interests, varying from the very basic pulping chemistry to a number of technical applications. Detailed acid analyses have produced many findings in pulping chemistry, including an arithmetic correlation between the acid formation and the degree of delignification [Alén *et al.*, 1991; Alén, 1997]. In addition, it has been found [Noopila *et al.*, 1991; Alén *et al.*, 1992] that even small changes in the chemical composition of black liquor may considerably influence the burning behavior of black liquor and that the combustion properties of black liquor vary more or less systematically with the delignification. Therefore, the analysis of the low-molecular-mass (LMM) aliphatic carboxylic acids (and "lignin

monomers”), for monitoring the cook and/or predicting the combustion properties of black liquor, has created a need for simpler and more rapid analytical methods, especially for the acid constituents.

The most sensitive methods for the analysis of LMM carboxylic acids are gas chromatography with mass spectrometric detection (GC-MSD) and gas chromatography with flame ionization detection (GC-FID) [Niemelä and Alén, 1999]. However, these GC methods are rather complicated and time-consuming. They usually comprise the extraction of an analyte from a sample matrix and the subsequent acid derivative preparation step prior to their proper separation by chromatographic techniques. If only the main components need to be separated, high-performance liquid chromatography (HPLC), ion chromatography (IC), and capillary electrophoresis (CE) are potential choices for real-time measurements because, in these methods, volatility of the samples is not required and laborious derivatization steps can thus be avoided.

## 1.2 Objectives of the study

The objective of the present work was to develop novel analysis methods for kraft black liquors that can be used as a basis for real-time measurements. More specifically, the aims were:

*To develop novel and fast analytical methods for LMM aliphatic carboxylic acids in aqueous media and validate the methods developed with standard compounds.*

*To apply the methods developed to wood- and non-wood-based black liquors and oxygen delignification filtrates including the method validation with black liquor samples.*

*To analyze black liquor samples from laboratory-made kraft cooks with the methods developed, study the formation of the acids during cooking, and link the data to the parameters that have been previously used to describe the progress of the cook.*

## 1.3 Research methods

Because the main aim was to develop simple analysis methods for industrial processes, sample preparation was limited to filtration and dilution, which can be easily automatized. In this study, a novel HPLC separation method with atmospheric pressure chemical ionization mass spectrometry (APCI-MS) was developed and validated for the analysis of LMM aliphatic carboxylic acids in wood and non-wood black liquors. For industrial applications simpler and more

robust methods than HPLC-APCI-MS could be practical. IC with the conductivity detection (CD) offers an even simpler method than HPLC-APCI-MS for the determination of a multitude of the key anions in black liquors. CD is an unselective detector and therefore a complimentary IC separation method with electrospray ionization mass spectrometry (ESI-MS) was developed to confirm the identification of the carboxylic acids that were not available as commercial chemicals. Once the peak identification has been performed, IC-CD can be used alone because the peak pattern remains unchanged in the same method conditions. An IC-CD method was also thoroughly validated with standard compounds in aqueous media and with various wood and non-wood black liquor samples. Thereafter the HPLC-APCI-MS and IC-CD methods were applied to the analysis of laboratory-made kraft cooks and the results were used to find a correlation between the acid formation and the parameters that have been used to describe the progress of the delignification process.

## 2 LITERATURE REVIEW

### 2.1 Delignification processes

#### 2.1.1 Kraft pulping

Kraft pulping is the dominant method to produce chemical pulp. In 2006, 139 million tons of chemical pulp were produced and kraft pulp accounted for 125 million tons [FAOSTAT, 2008]. The kraft pulping process has several advantages [Sjöström, 1993]. The most significant reasons for the dominance of kraft pulping are the high quality of the fiber product, capability of processing any fibrous material, tolerance to bark, and an efficient recovery of the cooking chemicals. The energy content of the organic material dissolved into the cooking liquor during delignification is high and can be utilized. In addition, side products such as turpentine and tall oil are valuable. These facts together make the kraft method superior to other methods.

The white liquor is mainly composed of sodium hydroxide (NaOH) and sodium sulfide (Na<sub>2</sub>S) in addition to some sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and sodium salts of oxidized sulfur-containing anions [Rydholm, 1965; Mimms *et al.*, 1993; Niemelä and Alén, 1999]. White liquor is used at a high temperature (140–180 °C) to break down and dissolve the lignin that holds the fibers together in wood. The presence of hydrogen sulfide ions facilitates delignification because of their strong nucleophilicity in comparison with hydroxyl ions. Kraft cooking is typically carried out in a batch digester or in a continuous digester. In batch pulping, several digesters are used in parallel (typically 6–8 cooking vessels). In continuous pulping, the chips are fed in at the top of the digester together with the pulping chemicals to form the chip bed inside the digester. The chip bed moves slowly downward inside the digester. The cooking time varies typically from 4 to 6 hours.

The kraft pulping process has been modified to achieve lower lignin contents without extensive reduction in pulp strength and yield [Alén, 2000]. Alkali profiling techniques have proved to be promising methods to extend delignification, leading to reduced bleaching demands and effluent treatment loads. The idea behind the modifications is to divide the charge of the active chemicals during the cook to obtain a more constant concentration profile throughout the process and increase the selectivity of the process. A constant alkali concentration during the cook, higher hydrogen sulfide ion concentration at the



beginning of the cook, low dissolved lignin concentration at the end of the cook, and low temperature profile at the beginning of the cook are examples of the prevailing modification principles [Norden and Teder, 1979; Teder and Olm, 1981; Sjöblom *et al.*, 1983]. For this purpose, modified continuous cooking (MCC), extended modified continuous cooking (EMCC), rapid displacement heating (RDH), and SuperBatch are used. The conventional kraft process can also be improved by using pulping additives such as anthraquinone (AQ) [Holton, 1977; Fleming *et al.*, 1978; Löwendahl and Samuelsson, 1978; Blain, 1992; Goyal *et al.*, 1992; Rajan *et al.*, 1992; Jiang, 1993; Katz, 1993], polysulfides [Kleppe and Kringstad, 1963; 1964; Pekkala, 1982; 1986; Green and Smith, 1983; Blain, 1992; Jiang, 1993; Katz, 1993], and surfactants [Chen, 1994].

After cooking, the black liquor is separated by washing from the pulp [Alén, 2000]. The liquor exiting the digester contains 15–20% solids and it is referred to as weak black liquor. It contains too much moisture to be a reliable fuel and in the recovery stage black liquor is concentrated to 65–80% solids content in a multistage evaporation system. The strong black liquor is then combusted in the recovery boiler for the recovery of the cooking chemicals and the generation of energy. The washed pulp can be delignified further by using oxygen in alkaline conditions. The oxygen delignification can be followed by multistage bleaching to remove the remaining lignin and ensure a sufficient final brightness.

### 2.1.2 Soda pulping

Soda pulping is a sulfur-free pulping method and the active chemical is sodium hydroxide [Gullichsen, 1999; Alén, 2000]. It is generally known that carbohydrate reactions are mainly affected by alkalinity ( $\text{HO}^-$  ions) and hydrogen sulfide ( $\text{HS}^-$  ions) react with lignin. This means that soda pulping (the only active chemical is NaOH) is slower and provides a lower yield and a weaker pulp compared with kraft pulping. To enhance the soda process, AQ or related compounds such as anthraquinone monosulfonic acid (AMS) have been used as additives because these catalysts preserve carbohydrates and simultaneously accelerate delignification. The use of AQ also lowers the effective alkali (EA) requirement. AQ reacts with reducing end groups of carbohydrates, stabilizing them against alkaline peeling and producing the reduced anthrahydroquinone (AHQ), which is alkali-soluble. AHQ reduces lignin, which becomes more reactive, and AQ is formed again and it reacts with carbohydrates. This semicatalytic redox mechanism explains why very small additions of AQ are effective (0.1–0.5% on wood).

The most common chemical pulping method for non-wood raw materials is soda cooking with or without AQ. The soda process is especially suitable for non-wood feedstocks, because non-wood raw materials typically contain less lignin than wood raw materials [Alén, 2000]. However, soda pulping is not the only method for non-wood materials. Kraft cooking is also used for bamboo and reed [Paavilainen, 1998].

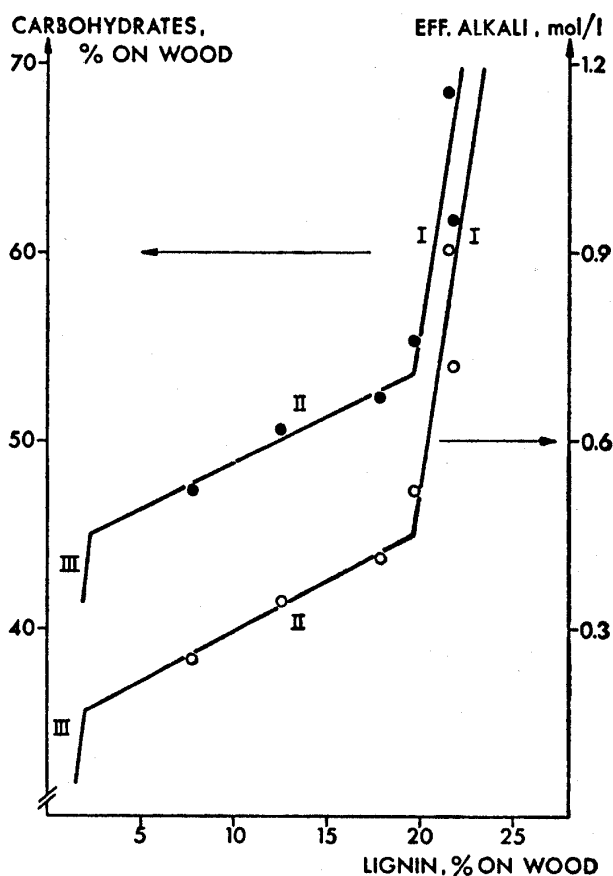
### 2.1.3 Oxygen-alkali delignification

Carbohydrate losses increase considerably near the end of the cooking mainly because of the retarded removal of lignin (*i.e.*, compared to that of carbohydrates) during this final delignification phase. For this reason, there is a practical limit to how far the residual delignification phase can be continued without a significant loss in pulp yield [Sjöström, 1993; Gullichsen, 1999; Alén, 2000]. From a bleachability, selectivity, and yield point of view, it is better to extend the oxygen delignification instead of extending the cook. Oxygen-alkali delignification is a more selective delignification method than the cook to reduce the lignin content of pulp before bleaching. However, this technique is only useful for partial delignification because too extensive removal of lignin can still result in a severe degradation of the polysaccharides. Oxygen delignification fits particularly well with kraft pulping because it can use oxidized white liquor as an alkali source and its spent liquor is counter-currently recoverable with kraft liquor. The main benefit of the oxygen alkali delignification stage is that it reduces emissions from the bleach plant and also decreases the consumption of the bleaching chemicals.

Oxygen delignification can be applied to all pulp types [Gullichsen, 1999; Alén, 2000]. Pulp can also be pretreated before oxygen delignification in order to improve the efficiency of oxygen delignification. Pretreatment can be performed using chlorine, chlorine dioxide, nitrogen dioxide, ozone, or hydrogen peroxide acidified with sulfuric acid [Fossum *et al.*, 1983; Fossum and Marklund, 1988]. Also peroxymonosulfate has been used as such before oxygen delignification [Springer and McSweeney, 1993]. Oxygen delignification process uses an elevated temperature (90–110 °C) and pressure at high (25–30%) or medium consistency (10–14%) [Gullichsen, 1999; Alén, 2000]. The oxygen delignification time is typically 30–60 minutes. Oxygen-alkali delignification can be carried out in single or multiple stages. Additional chemicals such as magnesium salts may be used to improve the process. In the oxygen delignification process, oxygen reacts predominantly with residual lignin through complex radical chain reactions.

### 2.1.4 Reaction kinetics

In kraft cooking, the actual cooking process can be divided into three stages, which are initial delignification, bulk delignification, and residual delignification [Wilder and Daleski, 1965; Kleinert, 1966; Lemon and Teder, 1973; Olm and Tistad, 1979]. In these phases, delignification follows the first-order reaction kinetics, but the slopes are different in various phases. The initial delignification takes place mainly in the impregnation phase, well before the final cooking temperature has been reached (<140 °C) [Olm and Tistad, 1979; Gullichsen, 1999; Alén, 2000]. During this phase, the selectivity of delignification is rather low and the amount of carbohydrates decreases significantly. The lignin content is reduced by only 15–25% of the initial amount, as compared with about 40% of the hemicelluloses. The change between the initial phase and the bulk delignification phase is shown in Figure 1.



**FIGURE 1** Carbohydrate losses and change in the effective alkali during the kraft pulping of softwood [Olm and Tistad, 1979]. I Initial delignification, II bulk delignification, and III residual delignification phases.

In the bulk delignification phase, the rate of delignification increases dramatically when the cooking temperature is elevated above 140 °C [Olm and

Tistad, 1979; Gullichsen, 1999; Alén, 2000]. The rate of delignification becomes controlled by chemical reactions and follows as first-order reaction kinetics. The rate of lignin dissolution remains high during this bulk delignification phase, until about 90% of the lignin is removed. As can be seen from Figure 1, the amount of carbohydrates and the effective alkali concentration in the cooking liquor decrease only slightly. In the residual delignification phase, the removal rate of the lignin is considerably slower and the loss of carbohydrates is increased together with alkali consumption.

## 2.2 Black liquor

Spent liquors derived from alkaline cooking processes are called kraft or soda black liquors, depending on the method used. Black liquor is a complex mixture of water, residual inorganic cooking chemicals, and organic material degraded and dissolved from feedstock [Sjöström, 1993; Alén, 2000]. The organic material in the resulting black liquors after kraft pulping mainly consists of lignin and carbohydrate degradation products in addition to a small fraction of extractives and their reaction products. The typical compositions of the pine and birch black liquors are presented in Table 1. Chemical structures of the acids are presented in Appendix II.

**TABLE 1** Typical composition of the d.s. (dry solids) of pine and birch black liquors (% of total d.s.) [Alén, 1997]

Component	Pine	Birch
Lignin	33	27
High-molecular-mass (> 500 Da)	29	23
Low-molecular-mass (< 500 Da)	4	4
Aliphatic carboxylic acids	31	32
Formic acid	6	4
Acetic acid	4	9
Glycolic acid	2	2
Lactic acid	3	2
2-Hydroxybutanoic acid	1	5
3,4-Dideoxy-pentonic acid	2	1
3-Deoxy-pentonic acid	1	1
Xyloisosaccharinic acid	1	2
Glucisosaccharinic acid	7	3
Others	4	3
Other organics	8	12
Inorganics*	28	29

\*Including sodium bound to organic material; the amount of this sodium in both cases is 12% of the total d.s.

In pine black liquors, the dominating aliphatic carboxylic acids are glucoisosaccharinic, formic, acetic, lactic, glycolic, and 3,4-dideoxy-pentonic acids. In birch black liquors, acetic, 2-hydroxybutanoic, formic, and glucoisosaccharinic acids are the most common aliphatic carboxylic acids. The amount of these compounds depends on the raw material and used pulping method [Niemelä and Alén, 1999].

### 2.2.1 Organics

The organic matter in the black liquor is mainly composed of the degradation products of lignin and polysaccharides in addition to a minor fraction comprising extractives [Sjöström, 1993; Alén, 2000]. The average molecular mass of the lignin fraction in black liquor is relatively high, but also minor amounts of lignin-derived LMM degradation products are present. In addition, small amounts of dissolved polysaccharides, which are not completely degraded during the delignification process, can be found in the final black liquors.

Aliphatic carboxylic acids present in black liquor are formed as a result of the degradation reactions of the polysaccharides (cellulose and hemicelluloses) [Niemelä, 1990a; Sjöström, 1993; Alén, 2000]. Acids can be divided into volatile and non-volatile carboxylic acids. Black liquor's volatile acids consist of acetic and formic acids and non-volatile acids include numerous hydroxy monocarboxylic and hydroxy dicarboxylic acids. Minor amounts of non-hydroxylated di- and tricarboxylic acids are also present. Altogether, nearly 100 hydroxy carboxylic acids have been identified from black liquors [Niemelä and Sjöström, 1986a; Niemelä, 1990a, Niemelä, 1993]. The main classes of carbohydrate-derived carboxylic acids in alkaline pulping liquors are presented in Table 2. In addition, numerous other types of compounds are formed, such as catechols, cyclopentenones, hydrocyclopentenones, thiophenes, and other miscellaneous compounds [Niemelä and Alén, 1999].

Wood extractives consist of resin and fatty acids, which are recovered as tall soap [Sjöström, 1993]. In addition, volatile wood extractives (mainly terpenes) are also found and these compounds are recovered during the kraft pulping process from the digester relief condensates. The amount of these products varies greatly depending on the wood material.

**TABLE 2** The main classes of carbohydrate-derived carboxylic acids and miscellaneous carbohydrate-derived compounds in alkaline pulping liquors, and the number of identified compounds [Niemelä and Alén, 1999]

Compound type	Number
Main classes of carbohydrate-derived carboxylic acids	
Volatile fatty acids	4
Hydroxy monocarboxylic acids	60
Dimeric hydroxy monocarboxylic acids	11
Dicarboxylic acids	10
Hydroxy dicarboxylic acids	30
Hydroxy tricarboxylic acids	3
Miscellaneous carbohydrate-derived compounds	
Catechols	5
Cyclopentenones	7
Hydrocyclopentenones	12
Thiophenes	20
Miscellaneous compounds	10

### 2.2.2 Inorganics

The main inorganic compounds in black liquor are residual cooking chemicals (NaOH and Na<sub>2</sub>S) and conversion products of the cooking chemicals (such as sodium carbonate, sodium sulfate, sodium thiosulfate, polysulfides, and sodium sulfite) [Alén, 2000]. Especially thiosulfate and polysulfides are corrosive in low concentrations and can cause problems for the equipments [Leinonen *et al.*, 2008]. Black liquors also contain various inorganic cations and anions, originating from the wood raw material, equipment, and process water [Alén, 2000]. These are considered as non-process elements (NPEs). NPEs can cause scaling problems in the digester and in the evaporation system. NPEs also increase the load on the recovery furnace. Silicates and calcium salts are considered as the most harmful components. Silicates are not abundant in wood feedstocks, but occur in large amounts in different non-wood raw materials. Pulping liquors also contain a minor amount of chlorides, which can also cause corrosion problems for the equipment.

## 2.3 Formation of the aliphatic carboxylic acids during alkaline delignification

Alkaline delignification is usually carried out under relatively drastic conditions, in which lignin and wood polysaccharides undergo an extremely complex combination of degradation and condensation reactions [Sjöström, 1993; Alén, 2000]. The selectivity of delignification is rather low and more than 30% of

wood polysaccharides are lost during kraft pulping. The degradation and dissolution of carbohydrates during the kraft cooking process are not only of great importance for the yield of the pulp: they also affect the quality of the pulp. The composition of the pulp and the length of the carbohydrate polymers are crucial to the property of the end product, so it is important that the degradation and dissolution of carbohydrates are controlled during the cooking process.

Carbohydrates are attacked even at a comparatively low temperature [Sjöström, 1993; Alén, 2000]. The acid formation is continuous during the cooking but the main part has already been formed when the maximum temperature has been reached [Alén *et al.*, 1985a]. Acids are formed even at temperatures of about 100 °C, but most of the carboxylic acids are formed during the heating-up period. The reactivity of polysaccharides varies depending on their accessibility and structure. Because of the crystalline nature of the cellulose and a high degree of polymerization, cellulose suffers fewer losses than hemicelluloses. In the case of softwood, the total amount of the acids corresponds to 16–18% of the wood mass. Based on this it was concluded that 75–80% of the carbohydrates lost were converted to monomeric aliphatic acids at the end of the cook [Alén *et al.*, 1985a].

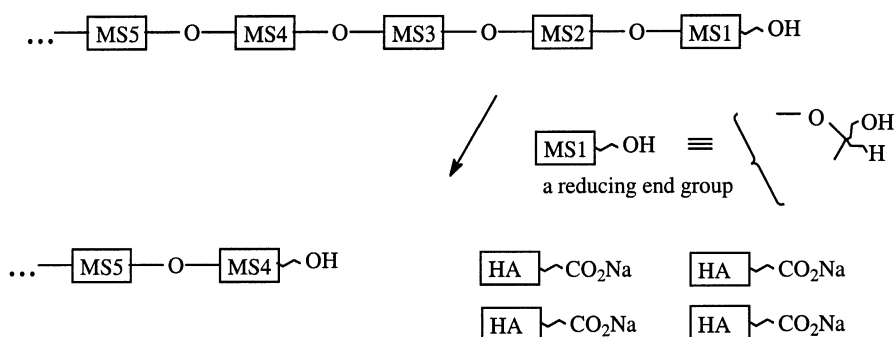
Aliphatic carboxylic acids are also formed during the oxygen delignification when polysaccharides are further degraded and LMM compounds are formed. The formation of the aliphatic carboxylic acids during the oxygen delignification process has been studied in more detail [Alén and Sjöström, 1991a]. The alkali charge and bleaching time affects the formation of the degradation products. In this study, the total amount of carbohydrate-derived aliphatic carboxylic acids in pine kraft black liquor varied between 0.1–1.7% of pulp. Formic, glycolic, acetic, 3-deoxy-pentonic, and deoxy-tetronic acids were the most abundant aliphatic carboxylic acids formed during the oxygen delignification process. Based on the literature review, schematic figures for the formation of the main reaction products during oxygen delignification have recently been presented by Salmela [Salmela, 2007] (see Appendices I a-d).

### **2.3.1 Main reactions of the polysaccharides during alkaline pulping**

In the conventional alkaline processes, the degradation of the polysaccharides is divided into two categories [Sjöström, 1993; Alén, 2000]: peeling and chain cleavage. The peeling reaction (primary peeling) is a stepwise alkaline polymerization of the polymer beginning at the reducing end group. The peeling reaction of polysaccharides involves the elimination of monosaccharide units and simultaneous formation of various carboxylic acids (Figure 2). The prerequisite for the peeling reaction is the presence of a reducing end group (a hemiacetal group)

in the polysaccharide chain. The initial step is the isomerization of a reducing end group to a 2-keto intermediate followed by the  $\beta$ -alkoxy elimination (*i.e.*, of the monosaccharide end unit) leading to a soluble monosaccharide unit and a shortened polysaccharide chain with a new reducing end group.

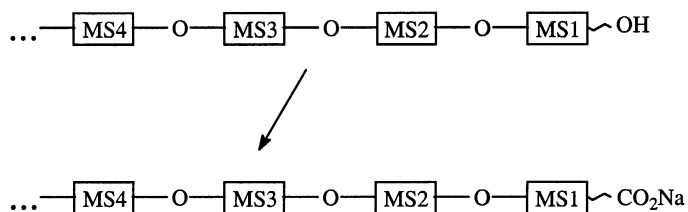
#### PEELING REACTION (PRIMARY PEELING)



**FIGURE 2** Schematic figure of the peeling reaction (primary peeling) [Alén, 2000]. MS = monosaccharide unit, HA = hydroxy carboxylic acid.

As a result of the alkaline degradation of polysaccharides, approximately 1.6 equivalents of acids are formed for every monosaccharide unit peeled from the polysaccharide chain [Sjöström, 1993; Alén, 2000]. The hemicelluloses are peeled much more readily than cellulose and the individual reaction rates depend on the type of hemicelluloses. In the case of cellulose, 50–65 glucose units are expected to be cleaved on average by the peeling reaction until a competing reaction (stopping reaction) takes place and terminates the degradation (Figure 3). In this stopping reaction, the reducing end groups are converted to an alkali stable carboxylic acid group. Without this competitive stopping reaction, it is possible for a whole molecule to be destroyed by peeling.

#### STOPPING REACTION

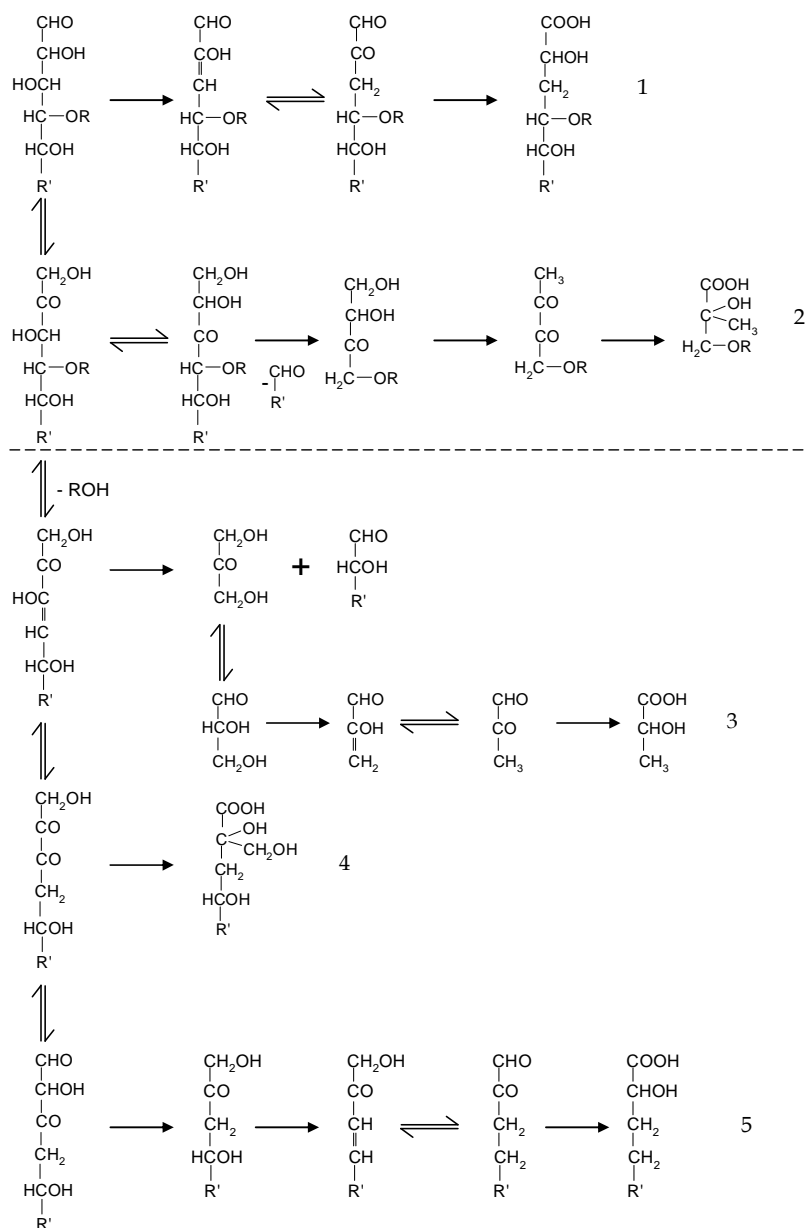


**FIGURE 3** Schematic figure of the stopping reaction [Alén, 2000].

Figure 4 shows a simplified reaction scheme illustrating the formation mechanism of the main degradation products [Sjöström, 1977; 1993]. The main stopping reaction routes are presented in the upper portion of this figure. In the



lower portion is presented the mechanism of formation of the main degradation products.



**FIGURE 4** Peeling and stopping reactions of polysaccharides [Sjöström, 1977]. R = polysaccharide chain and R' = CH<sub>2</sub>OH (cellulose and glucomannans) or H (xylan). Cellulose and glucomannans (R' = CH<sub>2</sub>OH): (1) 3-deoxy-hexonic acid end groups (metasaccharinic acid), (2) 2-C-methylglyceric acid end groups, (3) 3-deoxy-2-C-hydroxymethylpentonic acid (glucoisosaccharinic acid), (4) 2-hydroxypropanoic acid (lactic acid), and (5) 3,4-dideoxypentonic acid (2,5-dihydroxypropanoic acid). Xylan (R' = H): (3) refers to 3-deoxy-2-C-hydroxymethyltetronic acid (xyloisosaccharinic acid), (4) 2-hydroxypropanoic acid (lactic acid), and (5) 2-hydroxybutanoic acid.

In Figure 4, the cleaved monosaccharide unit is rearranged into a 2,3-diulose structure from which either glucoisosaccharinic acid (cellulose and glucomannans) or xyloisosaccharinic acid (xylan) is formed via a benzilic acid rearrangement [Sjöström, 1993]. The diulose structure can also be cleaved by reversed aldol condensation to glyceraldehyde, which is then converted via methylglyoxal to lactic acid. A probable route for the formation of 3,4-dideoxy-pentonic acid proceeds via formic acid elimination from the 3-keto intermediate, followed by a benzilic acid rearrangement. It should be pointed out that only the main reactions are shown in Figure 4.

Alkaline hydrolysis (chain cleavage) of glycosidic bonds leads to the random cleavage of polysaccharide chains and simultaneously new reducing end groups are formed [Alén, 2000]. Chain cleavage reduces drastically the average chain length and creates new reducing end groups. These end groups can be subjected to the secondary peeling (Figure 5). In addition, the deacetylation of acetyl groups in hemicellulose chains takes place, resulting in the formation of the sodium salt of acetic acid.

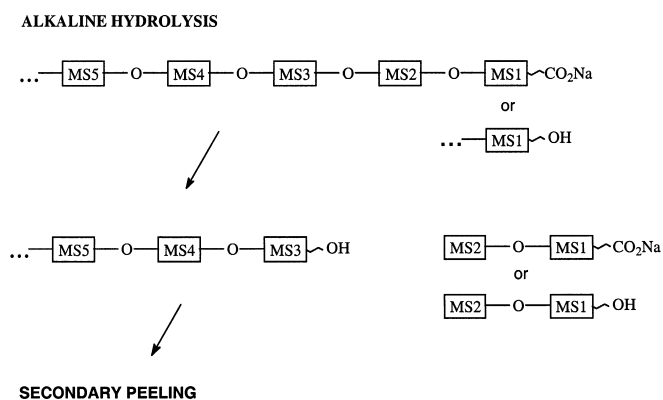


FIGURE 5 Alkaline hydrolysis (chain cleavage) [Alén, 2000].

### 2.3.2 Possible origin of the main aliphatic carboxylic acids

A major part of the carboxylic acids formate via the peeling reaction but it has been noticed that the thermal degradation and recombination reactions give rise to the formation of the acids in minor quantities. The formation routes of the carboxylic acids have been studied, *e.g.*, by treating cotton cellulose with hot sodium hydroxide and by analyzing the degradation products. The formation routes seem to be extremely complex and can not be completely outlined [Niemelä, 1987]. The reactions of the lignin and carbohydrates have also been studied by using model compounds. However, the information that can be gained from such

simplified reactions is not sufficiently comprehensive to make all the pulping reactions understandable. Alkaline pulping may produce a number of unexpected compounds, whereas some anticipated compounds may be missing or occur only in very low concentrations. Most experiments with model compounds have been carried out separately. In addition, the occurrence of joint reactions with lignin and polysaccharides has been recognized [Niemelä, 1990a].

Most of the straight-chain di- and tricarboxylic acids with hydroxyl groups are known to originate from polysaccharides, although some of them, such as tartronic, malic, erythruric, and threonic acids, might also occur as such in wood. Short-chain dicarboxylic acids without hydroxyl groups most probably originate from various polysaccharides, but some of them may be degradation products of lignin or even natural constituents of wood [Niemelä, 1990a]. More detailed information is available in the literature about the origin and the composition of the carbohydrate degradation products in various black liquors as well as for their possible formation routes during cooking [Alfredsson and Samuelson, 1968; Kolmodin and Samuelson, 1973; Malinen and Sjöström, 1975; Löwendahl *et al.*, 1976a; 1976b; Samuelson and Sjöberg, 1978; Alén *et al.*, 1985a; Niemelä *et al.*, 1985; Niemelä and Sjöström, 1986b; Niemelä, 1990a; 1990b; Sjöström, 1993]. Table 3 summarizes the possible origins of the main aliphatic carboxylic acids in black liquor.

**TABLE 3** Possible origins of the main aliphatic carboxylic acids in black liquor

Compound	Origin		
	Cellulose	Glucomanan	Xylan
Volatile acids			
Formic acid	● <sup>1</sup>	● <sup>1</sup>	● <sup>1</sup>
Acetic acid		● <sup>2</sup>	● <sup>2</sup>
Hydroxy acids			
Glycolic acid	● <sup>1</sup>	● <sup>1</sup>	● <sup>1,4</sup>
Lactic acid	● <sup>1,3</sup>	● <sup>1,3</sup>	● <sup>3,4</sup>
2-Hydroxybutanoic acid			● <sup>4,5</sup>
2-Deoxy-tetronic acid	● <sup>1</sup>	● <sup>1</sup>	
3-Deoxy-tetronic acid	● <sup>1</sup>	● <sup>1</sup>	● <sup>1,4,5</sup>
3-Deoxy-pentonic acid	● <sup>1</sup>	● <sup>1</sup>	● <sup>4</sup>
3,4-Dideoxy-pentonic acid	● <sup>5</sup>	● <sup>5,6</sup>	
Glucosaccharinic acid	● <sup>1,3</sup>	● <sup>1,3</sup>	
Xylosaccharinic acid			● <sup>1,3,4</sup>
Dicarboxylic acids			
Oxalic acid	● <sup>1</sup>	● <sup>6</sup>	● <sup>4</sup>
Tartronic acid	● <sup>1</sup>	● <sup>6</sup>	
Succinic acid		● <sup>6</sup>	● <sup>4</sup>
Malic acid		● <sup>6</sup>	● <sup>4</sup>

<sup>1</sup>Löwendahl *et al.* [1976a], <sup>2</sup>Alén [2000], <sup>3</sup>Sjöström [1993], <sup>4</sup>Niemelä [1990b], <sup>5</sup>Niemelä *et al.* [1985], <sup>6</sup>Niemelä and Sjöström [1986c]

Glucosaccharinic acid is formed from glucomannan and cellulose and xylosaccharinic acid from xylan [Sjöström, 1993; Alén, 2000]. Glucosaccharinic and xylosaccharinic acids are formed when a cleaved monosaccharinic unit is rearranged to a dicarbonyl compound and formed further via a benzilic acid rearrangement. The disaccharide structure can also be cleaved by reverse aldol condensation to glyceraldehyde, which is then converted via methylglyoxal to lactic acid. 2-Hydroxybutanoic acid is the most abundant hydroxy acid in the birch kraft black liquor and it is formed from the xylan, whereas 3,4-dideoxy-pentonic acid originates from cellulose and glucomannan [Niemelä *et al.*, 1985]. A probable route for the formation of 3,4-dideoxy-pentonic acid and 2-hydroxybutanoic acids proceeds via formic acid elimination from the 3-keto intermediate, followed by the benzilic acid rearrangement.

## 2.4 Monitoring of delignification and pulping models

Good process control requires accurate, fast, and practical methods of analysis. However, it is difficult to measure accurately the parameters that affect the delignification process. There are several reasons for this. In the cooking vessel, high pressure and temperature are used, and the cooking conditions are highly alkaline. The cooking vessels are typically very large and the content of the cooking vessel is heterogeneous. Variations in the wood raw material are notable and during the delignification process various chemical reactions occur simultaneously. This makes the controlling of the cooking extremely challenging. Online continuous measurement is preferable but not always possible. Properties such as uniformity of pulping, residual lignin content in the pulp, and estimates of carbohydrate degradation or fiber length distribution cannot be measured easily in online mode.

The process variables affecting the end result of pulping can be divided into raw materials and to process-condition-related properties. Raw material properties cannot be easily controlled, but should be taken into account when setting operating conditions. Process-condition-related variables such as chemical charge, cooking liquor composition, cooking time, and temperature are adjusted by process control.

The controlling of kraft pulping has been approached by modeling the pulping process. The kraft pulping kinetic has been investigated from various points of view and several pulping models exist. Comprehensive reviews of the main reaction kinetics models are available in the literature [Andersson, 2003; Rantanen, 2006]. In this chapter, earlier models are briefly mentioned and the model based on the degradation of carbohydrates is described in more detail

because it is further developed and used in the experimental part. Also, the Vroom's H-factor model [Vroom, 1957] is explained in more detail because it is used as one parameter in the experimental part.

Empirical models for batch digesters using the H-factor include the Vroom's model [Vroom, 1957] and the Hatton's model [Hatton, 1973; 1976]. The Hatton's model relates the pulp yield and kappa number to the H-factor and effective alkali charge. One of the earliest complete models of kraft cooking kinetics is the Purdue model developed by Smith and Williams [Smith, 1974; Smith and Williams, 1974]. In the Purdue model, the digester was considered by a series of continuous stirred tank reactors with external flows entering and exiting those reactors. Each reactor was assumed to contain three phases: solid wood mass, entrapped liquor, and free liquor. The Purdue model sets the framework in which the digester model exists and can be further developed. The Purdue model has been modified during the last decades by Christensen *et al.* [1982] and Wisniewski *et al.* [1997]. Improvements made by Christensen *et al.* [1982] were related to kinetic parameters and reactant consumption. The wood species were divided into hardwood and softwood, and a rate multiplier was used to adjust the kinetics for the different species. Wisniewski *et al.* [1997] developed a model of a continuous digester. The Wisniewski's model was still further developed and integrated into dynamic process simulation software [Savolainen, 2004].

Batch-digester models have also been presented by Olm and Tistad [1979], Paulonis and Krishnagopalan [1988], Lee and Datta [1994], and Vanchinathan and Krishnagopalan [1997]. The effects of liquor penetration, diffusion, and chip size on the reaction kinetics in kraft pulping have been examined in detail by Gustafson *et al.* [1983], Jiménez *et al.* [1989], and Agarwal and Gustafson [1997]. Gustafson *et al.* [1983] presented rate equations for carbohydrate degradation for initial, bulk, and residual phases separately. The Gustafson's theoretical model was further developed by Pu *et al.* [1991]. Improvements were made to control the alkali consumption and carbohydrate degradation more accurately.

Most systems for the control of delignification are based on the H-factor [Vroom, 1957; Kerr, 1970]. The Vroom's H-factor model combines temperature (T) and time (t) into a single numerical variable representing the extent of the cooking. The overall rate of the bulk delignification in kraft pulping follows pseudo-first-order kinetics with the following equation:

$$-\frac{dL}{dt} = kL \quad (1)$$

where  $L$  is the lignin content of wood residue at time  $t$  and  $k$  is the rate constant. Based on experimental data of  $k$  at varying temperatures, the value of the activation energy  $E_a$  can be calculated from the Arrhenius equation:

$$\ln k = \ln A - \frac{E_a}{RT} \quad (2)$$

where  $T$  is the absolute temperature (K),  $R$  is the gas constant ( $8.3145 \text{ J K}^{-1} \text{ mol}^{-1}$ ), and  $A$  is a constant. The activation energy  $E_a$  has been determined to be  $130\text{--}150 \text{ kJ mol}^{-1}$  for the bulk phase and  $50\text{--}120 \text{ kJ mol}^{-1}$  for the initial and final phases. In the H-factor model, the system is simplified and a value of  $134 \text{ kJ mol}^{-1}$  has been used for  $E_a$  and a temperature of  $100 \text{ }^\circ\text{C}$  is chosen and all other temperatures and rates are related to this standard [Sjöström, 1993]. The time integral of the relative reaction rate is called the H-factor:

$$H = \int_0^t \exp\left(43.2 - \frac{16,113}{T}\right) dt \quad (3)$$

A normal heating period contributes to the H-factor by 150–200 and 1500–2000 are needed for a complete kraft cook. Within the bulk delignification phase, the relative reaction rate is doubled when the temperature is increased by about  $8 \text{ }^\circ\text{C}$  [Sjöström, 1993]. Modern digester control systems automatically compute and accumulate the H-factor during the cook, thus monitoring the degree of delignification [Alén, 2000].

The H-factor model does not take into account the effect of chemical concentration. A prerequisite for the use of the H-factor is that the cooking conditions are exactly known and kept strictly constant since the prediction of the end point and the cooking time needed to reach the target kappa number are based only on temperature measurements [Alén, 2000]. The basic H-factor model has been refined over the years. The most popular way to model kraft pulping is by dividing the cook into three phases: the initial, bulk, and residual phases. Time and temperature correlations are given for each phase separately as a function of liquor alkalinity. Despite the modifications and refining of the H-factor model, it still has disadvantages. There still exist uncontrollable variations in cooking conditions, such as moisture content, quality of chips, and concentrations of cooking chemicals.

Other methods to measure the extent of the delignification are kappa number and chlorine number determinations [Holmbom and Stenius, 2000]. The kappa number is traditionally obtained by titration with potassium permanganate. The kappa number is defined as the volume in mL of a  $0.1 \text{ N}$  ( $0.02 \text{ M}$ ) potassium

permanganate solution that is consumed by one gram of moisture-free pulp under specified conditions. The hexenuronic acid (HexA) units can give a significant contribution to the kappa number [Li and Gellersted, 1997] because HexA units are sensitive to oxidation by the permanganate. The significant drawback in using kappa or chlorine numbers to determine the extent of delignification is that these measurements give only the ratio of lignin structures to the mass of the pulp sample. Ultraviolet (UV) spectrophotometric measurements have been used to follow the changes in the total lignin concentration [Williams, 1968; Paulonis and Krishnagopalan, 1988]. The challenge with this UV method seems to be that concentration increase is very small toward the end of the cook.

The degradation product analysis method brings a new way to approach the cooking control. The formation of LMM compounds during alkaline delignification has been found to correlate with the degree of delignification [Alén *et al.*, 1991]. Based on this finding, an approach to the process control of kraft pulping has been presented. In this method, a number of important degradation products can be measured simultaneously and a single chromatographic run gives fingerprint information for controlling the process conditions. It was noticed that it is practical to use the concentration ratios of the selected hydroxy carboxylic acids. This eliminates the difficulty of measuring the absolute concentrations. The prerequisites for a suitable acid concentration ratio were that the data had to be reproduced with sufficient precision and accuracy. One of the acid ratios was found to be  $(A+B)/C$ , where the components were, for example, 3,4-dideoxypentonic acid (A), anhydroglucoisosaccharinic acid (B), and 2-hydroxybutanoic acid (C) [Alén *et al.*, 1991].

The degradation product analysis method was tested by a series of laboratory experiments in which both the alkali charge and sulfidity were changed [Alén *et al.*, 1991]. Also some mill-scale experiments were carried out. It was noticed that the fragmentation pattern was different for softwood and hardwood cooks. The method proposed could be a more accurate tool for cooking prediction because it also takes into account the variations in the wood compositions in the digester. This degradation analysis method has also been tested for the monitoring of oxygen delignification [Alén, 1993] of pine kraft pulp. Because the concentration of some of these acids is systematically changed as the treatment proceeds, the progress of oxygen-alkali delignification can be followed on the basis of the analytical data.

## 2.5 Low-molecular-mass acids and black liquor combustion properties

As shown above, black liquor is a complex mixture of water, inorganic salts, and organic material. The composition of black liquors plays a role in determining the thermochemical properties of black liquor [Noopila *et al.*, 1991; Frederick *et al.*, 1991; Alén *et al.*, 1992; Frederick and Hupa, 1994; Alén, 1997; 2004]. The combustion process of individual black liquor droplets sprayed into the furnace can be divided into four stages [Hupa *et al.*, 1987]: drying, devolatilization, char burning, and smelt coalescence. Understanding the possible relationships between the main organic constituents and the different combustion stages of a black liquor droplet is important when considering the combustion properties of different black liquors.

One of the most important phenomena in the thermochemical processing of black liquor is swelling. The swelling of spent liquor droplets during combustion has been studied earlier and the results have clearly indicated that the liquor composition has a significant effect on the swelling behavior of black liquor [Hupa *et al.*, 1987; Frederick *et al.*, 1991; Noopila *et al.*, 1991; Alén *et al.*, 1992; Alén, 1994]. It was found that aliphatic carboxylic acids are the primary source of the volatile degradation products necessary for swelling [Alén, 1994] and aliphatic carboxylic acids have a significant influence on the drying rate of black liquor droplets due to the possibility of forming intermolecular hydrogen bonds with water molecules [Alén, 2000]. Because the composition of the black liquor has a marked influence on the burning behavior and the combustion properties of black liquor vary more or less systematically with the delignification [Noopila *et al.*, 1991; Alén *et al.*, 1992], it has been concluded that there is a correlation between the formation of various LMM organic compounds released during delignification and the combustion properties of black liquor [Alén, 1997]. The combustion properties of black liquor have been investigated by measuring LMM degradation products (aliphatic carboxylic acids and lignin monomers) with GC [Alén, 1997]. The results from the laboratory-made conventional batch cooking of softwood and hardwood indicated that it was possible to predict the primary combustion properties of black liquor by analyzing the chemical composition of the LMM compounds in black liquor.

More efficient recovery methods of energy from black liquor have been studied extensively. These studies are mainly based on black liquor gasification. Because the thermal properties of black liquor are related to black liquor composition, it can be concluded that the analysis of black liquor components would benefit the developing and controlling of black liquor combustion and gasification. Simpler and faster analysis methods would help to gain more data on the correlation between the black liquor thermal properties and black liquor



composition. In addition, a good understanding of the properties of black liquor may improve the efficient running of the mill and help to design further a mill's recovery unit.

## 2.6 Separation and utilization of the aliphatic carboxylic acids

The recovery of the aliphatic carboxylic acids from black liquor is an interesting alternative instead of using them as a fuel [Alén and Sjöström, 1985; Alén *et al.*, 1986]. The average heat value of the fraction of aliphatic acids is only 50% or less than that of lignin. Because of the low heating value of the aliphatic carboxylic acids, the interest has been directed at the possibility of recovering the aliphatic carboxylic acids formed during the delignification [Sjöström, 1993]. In addition, more heat is generated than is needed for the process. Therefore, considerable amounts of carboxylic acids could be recovered without disturbing the energy balance.

LMM carboxylic acids represent a potentially interesting group of compounds since many of them (such as formic, acetic, glycolic, and lactic acids) are commercially important chemicals and currently are produced by alternative methods [Alén and Sjöström, 1985; Alén, 2000]. Hydroxy carboxylic acids can be converted into corresponding derivatives by reduction, oxidation, or esterification. For example, glucoisosaccharinic acid can be reduced to a polyalcohol [Alén and Sjöström, 1979], oxidized to a dicarboxylic acid [Alén, 1987] or esterified with fatty acids [Reintjes and Cooper, 1984]. The reaction products can be used as raw materials and additives in various applications and they are also suitable as starting materials for the synthesis of chemicals [Alén and Sjöström, 1991b]. So far, the potential utilization of the other alkaline degradation products from cellulose and hemicelluloses (such as 2-hydroxybutanoic, 3,4-dideoxy-pentonic, xyloisosaccharinic, and glucoisosaccharinic acids) has been studied to a very limited extent. However, these chemicals represent a potential raw material source in the future and further studies on their utilization are necessary [Alén and Sjöström, 1985; Alén, 2000].

The main obstacle is the lack of simple methods of separating acids from lignin and other constituents and a need to recover the inorganic chemicals [Alén, 1981; Alén *et al.*, 1984a]. To avoid separation problems, process streams containing less lignin than is present in black liquors has been suggested for use because the most extensive degradation of carbohydrates takes place during the heating-up period [Sjöström, 1993]. Pretreatment of the chips with NaOH alone has been studied for this purpose [Alén *et al.*, 1984a]. For this reason, one of the suggested possibilities for the acid separation was based on the withdrawal of the pulping

liquor from the digester at the end of the heating-up period [Alén *et al.*, 1986]. In this study, the effect of the aliphatic acid recovery system on the inorganic pulping chemical cycle and the black liquor heating value were investigated. One approach to isolate carboxylic acids from black liquor is based on the lignin precipitation with carbon dioxide and sulfuric acid. After recovery of the volatile acids from the evaporation condensates, a crude fraction composed mainly of hydroxy carboxylic acids is obtained. However, the separation and purification of the crude hydroxy carboxylic acid fraction is a difficult problem. Possible methods include distillation under reduced pressure or special chromatographic techniques such as ion-exclusion chromatography [Alén *et al.*, 1991]. The recovery of organic acids from pulping liquors presents a complicated separation problem, which has not been solved satisfactorily [Alén, 1981; 2000], and still only the heat produced by aliphatic carboxylic acids is utilized [Sjöström, 1983a; 1983b]. Fast analysis methods of the carboxylic acids would be useful tools if the separation problem will be further investigated.

## 2.7 Analysis of the low-molecular-mass aliphatic carboxylic acids

### 2.7.1 Gas chromatography

The most sensitive methods for the analysis of LMM carboxylic acids are GC-MSD and GC-FID [Niemelä and Alén, 1999]. The determination of the main volatile acids (formic and acetic acids) by GC usually requires a sample pretreatment procedure such as esterification and/or chemical derivatization. Due to the high volatility of these acids, significant losses during sample preparation may occur. Volatilization of the formic and acetic acids have been prevented by the preparation of benzyl esters via *tert*-butyl ammonium salts [Bethge and Lindström, 1974; Alén *et al.*, 1985b; Niemelä and Alén, 1999]. This procedure has also been used to analyze volatile acids in alkaline pulping liquors. In an alternative potential method, non-volatile triethylamine adducts may be prepared from acetic and formic acids, which are subsequently *tert*-butyldimethylsilylated [Kim *et al.*, 1987; Niemelä and Alén, 1999]. This method has not been applied to black liquor.

GC-MS is the only instrument that allows a successful separation of all the significant hydroxy carboxylic acids and during the past 30 years, nearly 100 such compounds have been identified [Malinen and Sjöström, 1975; Alén *et al.*, 1984b; Niemelä and Sjöström, 1986a; Niemelä, 1990a; 1993; Niemelä and Alén, 1999]. A detailed description of the GC analysis of hydroxy carboxylic acids is given in the literature [Niemelä and Alén, 1999]. In the separation method developed by Alén *et al.* [1984b], a sample of black liquor is passed through a cation exchange ( $\text{NH}_4^+$ )

column and the resulting aqueous solution of ammonium carboxylates is then evaporated to dryness, per(trimethylsilyl)ated (*i.e.*, the preparation of trimethylsilyl (TMS) derivatives) with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, containing a small percentage of trimethylchlorosilane (TMCS)), and analyzed by GC-MS. By using this procedure only small black liquor volumes are usually required, typically 0.1–0.5 mL. The main benefit of the use of ammonium salts is that the complete or partial lactonization of certain hydroxy carboxylic acids can be avoided. If only the main hydroxy carboxylic acids need to be analyzed, there are even simpler procedures that can be applied, since it is possible to per(trimethylsilyl)ate aqueous solutions of sodium carboxylates.

Hydroxy carboxylic acids can also be derivatized without converting them into their ammonium salts [Malinen and Sjöström, 1975], which means that the acids are trimethylsilylated either as the free acids or as the lactones. The interpretation of the chromatograms may be problematic since many hydroxy carboxylic acids are only partially lactonized. However, many of these compounds will be completely lactonized if hydrochloric acid is added. The main disadvantage of this procedure is that some compounds will remain totally undetected due to the volatility of their lactones. Also, many compounds are only partially lactonized, thus producing several GC peaks [Niemelä and Alén, 1999].

The extraction of black liquor has been also used as a sample treatment in the determination of LMM organic compounds including carboxylic acids [Silvestre *et al.*, 2000]. The extraction method included sequential extraction with diethyl ether at pH 12, 7, and 2, followed by extraction with ethyl acetate at pH 2. This method was tested for eucalypt samples and GC-MS analysis was performed after the extraction procedure.

For the detection of the minor compounds, sample concentration may be needed [Niemelä and Alén, 1999]. Since the high-molecular-mass (HMM) materials (lignin and polysaccharides) are also present in the sample, more concentrated samples cannot always be prepared simply by increasing the black liquor volume. Instead, higher liquor volumes are suggested to be freed from lignin and polysaccharides by mixing diluted samples with a strong cation exchange resin (in H<sup>+</sup>-form), followed by filtration. The subsequent addition of ammonia solution to the filtrate prior to evaporation ensures that ammonium carboxylates are obtained for trimethylsilylation.

The use of *tert*-butyldimethylsilylation has become more popular in carboxylic acid determination [Niemelä and Alén, 1999]. The main advantage is that these derivatives are significantly more resistant towards hydrolysis than the TMS derivatives are. However, their suitability for black liquor analysis is

questionable because of the difficulty of derivatization of polyhydroxy compounds and because the molecular masses increase significantly.

Derivatized mixtures of the carboxylic acids can be analyzed using various chromatographic conditions. The initial oven temperature typically varies from 80 °C to 130 °C and the program heating rate varies from 5 °C to 15 °C. Reference chromatograms have been published and can be used as guidelines [Alén *et al.*, 1984b; Niemelä and Sjöström, 1986a; Niemelä, 1988]. Also, relative retention times have been published for carboxylic acids in black liquor [Pettersson, 1977]. For the quantitative determination, the addition of an internal standard is necessary. There are numerous suitable internal standards available, such as aldonic acids and alditols [Niemelä and Alén, 1999].

Electron impact (EI) ionization mass spectrometry (70 eV) has been a commonly used detection method for GC analyses of the black liquor acids. Mass spectra of the TMS derivatives of black liquor carboxylic acids are presented in the literature [Niemelä and Sjöström, 1986a; Niemelä, 1990a]. Additional reference acid mixtures can be obtained by the alkaline treatment of selected carbohydrates, such as rhamnose, glucose, cellobiose, and xylan as well as pectic acids. These carbohydrate samples cover typically all the precursors of the black liquor hydroxy carboxylic acids [Niemelä and Alén, 1999]. Reference chromatograms are available in the literature [Niemelä and Sjöström, 1985; Niemelä and Sjöström, 1986c; 1986d; Niemelä, 1990b]. In addition to mass spectrometry, FID is used for quantification purposes.

## 2.7.2 Liquid chromatography

Liquid chromatography (LC) is an important analysis method, because most compounds are not sufficiently volatile for GC and sample preparation for GC is often very laborious. HPLC uses high pressure to force solvents through columns containing very fine particles that give high resolution separations. IUPAC [IUPAC, 2008] classifies chromatographic techniques according to mechanism of separation into five categories: adsorption, partition, ion-exchange, ion-exclusion, and affinity chromatography. The liquid chromatographic separation of organic acids may utilize different chromatographic techniques such as normal-phase (NP), reversed-phase (RP), ion-pair, ion-exclusion (IE), or ion-exchange chromatography (IEX or IC).

### 2.7.2.1 Separation mechanisms

In general, three primary characteristics of chemical compounds can be used to create LC separations, which are polarity, electrical charge, and molecular size. In this chapter, the separation mechanisms used in liquid chromatographic analysis of the carboxylic acids, are shortly described. NP chromatography is a traditional separation mode based on adsorption/desorption of the analyte [Dong, 2006]. In an NP separation (a polar stationary phase and a non-polar mobile phase), the least polar analyte spends proportionally less time in the polar stationary phase and is the first analyte to elute from the column [Harvey, 2000]. Typical stationary phases for NP chromatography are silica and alumina. Also polar functional groups such as amino- or cyano-moiety are often bonded to the silanol groups [Dong, 2006]. In RP chromatography (a non-polar stationary phase and a polar mobile phase), the order of the elution is reversed, with the most polar analyte being the first to elute. Separation mechanism is very complex involving a great variety of interactions. The most important of them are analyte-analyte interactions in both the mobile and stationary phases, analyte-solvent and solvent-solvent interactions in the mobile and possibly on the stationary phase, and interactions of the alkyl-chains of the stationary phase among themselves and with the analyte and solvent molecules [Vailaya, 2005; Nikitas and Pappa-Louisi, 2009].

Ion-exclusion chromatography (IEC) is based on the separation of partially ionized species on strong anion- or strong cation-exchange stationary phases. In IEC, the basic separation mechanism is the Donnan exclusion effect, caused by the ionic repulsion between the charged resin and the partially charged analytes. Further influences on the separation of the LMM acids are derived from size-exclusion effects, RP interactions, and van der Waals' forces. The analytical column used in IEC separations of anionic analytes is usually packed with fully sulfonated polystyrene-divinylbenzene (PS-DVB) co-polymer [Pecina *et al.*, 1984; Fischer *et al.*; 1995; Novic and Haddad, 2006].

The terminology is not uniform in all publications and IEC is sometimes mixed with ion-exchange chromatography. The term IEC has been generally used for ion-exclusion chromatography. In some publications, ion-exchange chromatography is also referred to as IEC and mixed with ion-exclusion chromatography. Ion-exclusion chromatography has been described by a variety of alternative names, which reflect the continuous search for the exact separation mechanism of the technique [Haddad and Jackson, 1990; Novic and Haddad, 2006]. Examples include: ion-exclusion partition chromatography (IEPC), Donnan exclusion chromatography, and ion-moderated partition chromatography. The term HPICE is used for high-performance ion-exclusion chromatography [Weiss, 1986] and for ion-exchange chromatography, terms IEX and HPIC (high-

performance ion-exchange chromatography) are used. Present day ion-exchange chromatography on small particle high efficiency columns and usually utilizing conductometric or spectroscopic detectors is often referred to as ion chromatography (IC) [International Union of Pure and Applied Chemistry, IUPAC, 2008]. In this work, the term IEC is used for ion-exclusion chromatography and the term IC refers to the ion-exchange chromatography.

IEC is based on the separation of partially ionized species on strong anion- or strong cation-exchange stationary phases, with Donnan exclusion of the analytes from the charged stationary phase being the basic separation mechanism [Novic and Haddad, 2006]. Novic and Haddad [2006] listed several parameters that influence to the retention behavior of an analyte, such as the degree of ionization of the analyte, the molecular size and structure of the analyte, the eluent concentration and its pH value, the presence of organic solvents in the eluent, the ionic strength of the eluent, the temperature of the column, the material comprising the ion-exchanger and its hydrophobicity, the type of ion-exchange functional group on the stationary phase, the degree of cross-linking of the polymer used in the stationary phase, the ion-exchange capacity, and the ionic form of the resin. Novic and Haddad [2006] also proposed an alternative retention mechanism for IEC, based on the presence of a potential well at the surface of the fully functionalized PS-DVB stationary phase. Based on their findings, analytes diffuse into the potential well under the effects of concentration gradients, and undergo repulsion effects from the fixed charges inside the pores. The net contributions of these two opposing processes determine the degree to which an analyte is retained on the stationary phase.

In ion-exchange chromatography, the separation is based mainly on differences in the ion-exchange affinities of the sample components and ion-exchange process occur between the mobile phase and ion-exchange groups bonded to the support material [Weiss, 1986; Small, 1989; Fritz and Gjerde, 2000]. In highly polarizable ions, additional non-ionic adsorption processes contribute to the separation mechanism. The stationary phase typically consists of polystyrene, ethylvinylbenzene, or methacrylate resins co-polymerized with divinylbenzene and modified with ion-exchange groups. For anion-exchange, the resins of the stationary phase are usually bonded to a quaternary ammonium base acting as the exchange function. The types of separation interaction rarely exist in a pure form. If more than one mode of separation is used at the same time, then the separation method is mixed-mode separation. Due to mixed-mode separation, it is difficult to explain comprehensively the elution order of the compounds and optimal separation conditions are achieved experimentally.

### 2.7.2.2 Detection methods

The most popular LC detectors are based on spectroscopic measurements, including UV/VIS (ultraviolet/visible) absorption and fluorescence [Harris, 1999; Harvey, 2000]. Another common group of LC detectors are those based on electrochemical measurements such as amperometry, voltammetry, coulometry, and conductivity. The IC analyte detection is normally performed by using refractive index (RI), UV, conductivity, or MS detection. IC has been coupled with inductively coupled plasma mass spectrometry (ICP-MS), particle beam MS, atmospheric pressure ionization (API) operated in the ESI or APCI modes, and electrospray tandem mass spectrometry (MS-MS) [Buchberger, 2000]. The combination of IC with MS has several important applications in the analysis of inorganic and organic species and these techniques have been comprehensively reviewed by Buchberger [2000].

Carboxylic acids are commonly monitored by RI, CD, or UV detection (UV detection at the wavelength of 210 nm) [Casella and Gatta, 2002]. A major problem with the detection of aliphatic organic acids is that the chromophoric properties of a carboxyl group are weak, resulting in relatively poor sensitivity and selectivity of detection. Because the analytes do not exhibit a sufficiently high UV absorptivity, indirect absorbance, suppressed conductivity, and electrochemical detection (ED) methods have been used for the detection of these compounds. To achieve more sensitive and selective detection, several pre-column derivatization methods have also been developed. However, most of these derivatization methods are not suitable for adequate quantitative determination and they also require a fairly long analysis time and/or a rigorous sample clean-up procedure. MS offers major advantages for specific applications due to its potential to combine high sensitivity with mass selectivity. In this respect, ESI and APCI are particularly attractive techniques, since they allow soft ionization in both the positive and negative modes. ESI is most suitable for compounds that exist as ions in solution or molecules that can be ionized at a certain pH in solution [Harris, 1999; Harvey, 2000]. Electrospray ejects preexisting ions from solution into the gas phase, but does not create new ions. The APCI technique is probably the most versatile interface between LC and MS because it can handle a variety of analytes and can accept conventional chromatography flow rates up to 2 mL min<sup>-1</sup>. There is usually little fragmentation of ions in the mass spectrum from APCI. The spectra are easy to interpret, but provide little structural information other than molecular weight.

Suppressors are used with conductivity detectors to improve detection sensitivity. The key feature of suppressed IC is the removal of unwanted electrolyte ions prior to conductivity measurement because conductivity detectors respond to all ions [Harris, 1999]. Continuously regenerated membrane

suppressors and intermittently regenerated packed-bed suppressors are commonly used for anion analysis. Electrochemical and chemical regeneration have been used with both suppressor types [Saari-Nordhaus and Anderson, 2002]. In suppressed IC eluent conductivity is reduced to near zero by the suppression step. Suppression also allows us to use eluent concentration gradients [Harris, 1999]. RI detection is characterized by low sensitivity and so it is generally suitable only for detecting the major compounds. Furthermore, UV detection is also very sensitive to many interfering compounds, which make inconvenient preparatory stages necessary in sample preparation. The combination of nonselective detection with IC (*e.g.*, indirect UV or CD) requires that all the target analytes are resolved from each other (and from any other interfering matrix components) in order to provide positive confirmation. The use of a more selective detector, such as MS, can eliminate these problems [Corr and Anacleto, 1996]. This selectivity eases the requirements for sample preparation or complete chromatographic separation of the components of a mixture.

One of the major difficulties encountered when coupling IC to ion spray has been the incompatibility of the separation buffers with the ionization process [Corr and Anacleto, 1996]. Typically employed eluents for ion-exchange chromatography are in many cases regarded as incompatible with ESI-MS because of contamination and plugging of the interface. Therefore, the eluents recommended for coupling LC to MS generally contain volatile compounds as eluent additives. For this reason, in anion-exchange chromatography, the possible electrolytes are more or less limited to formate and acetate. The disadvantage of these anions is their low elution strength compared with other organic and inorganic anions. Buffer incompatibilities with the ionization process were overcome by using postcolumn suppression for IC separations. In particular, in IC-ESI-MS, the use of a membrane ion suppressor has been proved to remove effectively the sodium ions from the mobile phase. Sodium ions can cause the formation of uncharged species (*i.e.*, organic/inorganic anion and sodium ion as counter-ion) in the MS interface. This decreases the transfer efficiency from the atmospheric part of the interface to the vacuum region causing, in turn, a decrease of MS detection sensitivity. Therefore, the use of an ion suppressor makes it possible to combine the advantages of the unique selectivity offered by IC with the specificity and structural elucidation capability of MS [Corr and Anacleto, 1996; Buchberger and Ahrer, 1999; Buchberger, 2000; Mascolo *et al.*, 2005].

There is a trend towards reduction in the diameters of IC separation columns in accordance with the increase usage of various sensitive and selective spectroscopic techniques which can be hyphenated with IC [Haddad *et al.*, 2008]. Robust capillary ion chromatographic systems or microchip based separation



approaches at microscale level are better suited for interfacing with *e.g.*, ESI without the need for flow splitters.

The simplest way to use MS as a chromatographic detector is to measure the total ion chromatogram (TIC) from all the ions reaching the detector [Harris, 1999]. In this mode, a mass spectrometer simply detects everything from the column that can be ionized. Alternatively, the spectrometer can be selective by measuring the ion current for one or more particular fragment ions (selected ion monitoring, SIM).

### 2.7.2.3 Applications

The methods used for the LC analysis of LMM aliphatic carboxylic acids are collected in Table 4. The list of aliphatic carboxylic acids analyzed in these studies is presented in Table 5. The methods presented in Table 4 contain various sample matrices and a wide range of the aliphatic carboxylic acids. Numerous straightforward methods are currently used for the determination of different aliphatic acids, but so far only limited data on the utilization of these methods, such as in black liquor or related investigations, are available. The criterion for the reference selection was that the method was used for the analysis of some of the acids that can also be found in the black liquor samples.

The earliest liquid chromatographic determinations of the LMM carboxylic acids were made in the 1960s and early 1970s by Alfredsson *et al.* [1960; 1962; 1963], Alfredsson and Samuelson [1968], and Carlson and Samuelson [1970]. In these studies, different hydroxy carboxylic acids formed during the alkaline degradation of cellulose and hemicelluloses were separated by using anion-exchange chromatography. Detection was carried out mainly by using colorimetric detection (titration) and also by using an Auto Analyzer.

IC has been used for the analysis of pulping and bleaching spent liquors [Franklin, 1982; Franklin and Fitchett, 1982; Easty *et al.*, 1985] because IC-CD offers a versatile and rapid method for determining a multitude of their key anions such as sulfide, sulfite, sulfate, thiosulfate, hypochlorite, chlorite, chloride, chlorate, and oxalate. The Technical Association of the Pulp and Paper Industry (TAPPI) has also issued a test method, T699 "Analysis of pulping liquors by suppressed ion chromatography" [TAPPI, 2000]. However, these methods applied to pulping and bleaching liquors mainly deal with the analysis of inorganic ions by using IC. In Franklin's research [Franklin, 1982], some organic acids were also separated by using ion-exclusion chromatography and ion-exchange chromatography was used

only for the analysis of oxalic acid. In the study carried out by Easty *et al.* [1985] only inorganic compounds were analyzed.

IEC has been recognized as a useful technique for the separation of various weak acids and a great number of investigations into the use of IEC without MS for this purpose have been published [Fischer *et al.*, 1995; 1997; Lodi and Rossin, 1995; Tanaka *et al.*, 1995; 1997; 1999; 2002a; 2002b; 2003; Ohta *et al.*, 1996; 2003a; 2003b; 2003c; Eiteman and Chastain, 1997; Sharma *et al.*, 1998; Chen and Adams, 1999; Ohta and Tanaka, 1999; Ohta, 2001; Casella and Gatta, 2002; Chinnici *et al.*, 2002; Qui and Jin, 2002; Ohta and Ohashi, 2003; Ito *et al.*, 2004; Chi and Huddersman, 2007]. A comprehensive review article of the environmental analysis of aliphatic carboxylic acids by IEC has been written by Fischer [2002]. Also, RP separation without MS detection has been used for the analysis of LMM acids. RP liquid chromatography with a photo-diode array (PDA) detector was used by Cawthray [2003] for the separation and quantification of LMM organic acids in plant root exudates. Destandau *et al.* [2005] have presented a proper quantitative RPLC method with UV detection for the determination of carboxylic acids in industrial reaction mixtures.

Only a few studies have been published on analyzing LMM carboxylic acids with IEC-MS or RPLC-MS. Pacholec *et al.* [1986] used ion-exclusion partition chromatography (IEPC) with thermospray MS to characterize a mixture of aliphatic carboxylic acids. Separation was performed by using HCl solution as an eluent, which is not recommended for the MS. Johnson *et al.* [1997] analyzed LMM carboxylic acids with an HPLC-IEC-ESI-MS method. A mixture of trifluoroacetic acid (TFA) and propanol was used as an eluent. Frauendorf and Herzsuh [1998] examined the RP separation of carboxylic acids with ESI-MS. However, the standard compounds in their study were mainly aromatic acids and included only a few aliphatic acids. Ahrer and Buchberger [1999] have been able to separate a mixture of carboxylic acids by using an IEC-API-MS system with 0.5 mM ammonium citrate and 50% acetonitrile as an eluent. Helaleh *et al.* [2002] have created a qualitative HPLC-IEC-APCI-MS analysis method for carboxylic acids by using 0.85 mM benzoic acid and 10% aqueous methanol (MeOH). Gamoh *et al.* [2003] developed the HPLC-ESI-MS analysis method for aliphatic carboxylic acids by using 0.1% aqueous formic acid (FA). Only one extracted ion chromatogram (EIC) was presented, the peak shapes were poor for all the compounds, and most of the peaks overlapped. Bylund *et al.* [2007] developed an analysis method for LMM aliphatic carboxylic acids present in natural waters. The method is based on separation with IEC and detection with ESI tandem mass spectrometry (MS-MS). In this study, aqueous FA with MeOH was used as an eluent. The compounds included in these studies are listed in detail in Table 4.

Several applications for the analysis of LMM organic acids by ion-exchange chromatography without MS detection have been published [Ammann and Rüttimann, 1995; Mongay *et al.*, 1996; Révész *et al.*, 1996; Bruzzoniti *et al.*, 1997; Ding *et al.*, 1997; Jaffrezo *et al.*, 1998; Sharma *et al.*, 1998; Alcazar *et al.*, 2003; Cassidy *et al.*, 2004; Chi and Huddersman, 2007; Wei *et al.*, 2007; Yoshikawa *et al.*, 2007; Zhong *et al.*, 2007; Geng *et al.*, 2008]. Saccani *et al.* [1995] and Chen [1996] analyzed LMM carboxylic acid by using mixed mode columns (Dionex OmniPac), which combine the ion-exchange mechanism and RP separation. However, only a few studies have focused on the analysis of LMM mono- and dicarboxylic acids with IC-MS [Xiang *et al.*, 1996; Ahrer and Buchberger, 1999; Mascolo *et al.*, 2005; Meyer *et al.*, 2007].

Ammann and Rüttimann [1995] examined the separation of LMM carboxylic acids in environmental samples. Also,  $F^-$ ,  $H_3CSO_3^-$ ,  $Cl^-$ ,  $NO_2^-$ ,  $CO_3^{2-}$ ,  $PO_4^{3-}$ ,  $Br^-$ ,  $NO_3^-$ ,  $SO_3^{2-}$ , and  $SO_4^{2-}$  were analyzed. They used a step gradient procedure with a borate eluent on a Dionex AS11 low-capacity column. Some of the compounds co-eluted and also an isocratic separation method with a Dionex AS10 column was developed to identify and separate co-eluting compounds. In this case, borate was used as an eluent. Saccani *et al.* [1995] developed an ion chromatographic method to separate and determine LMM carboxylic acids. The method allowed the separation of organic anions on a Dionex OmniPac PAX-500 column by NaOH gradient elution and conductometric detection. Chen [1996] developed an analysis method for aliphatic carboxylic acids in industrial streams. In this study, the separation column was the same as in Saccani's study: a Dionex OmniPac PAX-500. An NaOH gradient elution was used to separate compounds. During the same analysis, inorganic ions and aromatic carboxylic acids were also analyzed.

Mongay *et al.* [1996] determined organic acids by using a Shimpack IC-AI column for the separation and 0.975 mM phthalic acid as an eluent. Révész *et al.* [1996], Bruzzoniti *et al.* [1997], Ding *et al.* [1997], and Jaffrezo *et al.* [1998] used a latex-based strong anion-exchange resin (Dionex AS4A-SC) for the separation of the LMM carboxylic acids. Bruzzoniti *et al.* [1997] have studied the analysis of carboxylic acids together with chloride and sulfate. In Ding's study [Ding *et al.*, 1997], several inorganic ions ( $H_2PO_4^-$ ,  $Cl^-$ ,  $NO_2^-$ ,  $Br^-$ ,  $NO_3^-$ ,  $SO_4^{2-}$ ) were determined in addition to carboxylic acids. A mixed eluent of potassium hydrogen phthalate (KHPH) and phthalic acid ( $H_2Ph$ ) was used and detection was carried out by CD. Jaffrezo *et al.* [1998] analyzed a mixture of carboxylic acids and, during the same run, also chloride, nitrate, and sulfate ions were determined. The eluent in their study was 0.5 mM  $NaHCO_3$ .

Sharma *et al.* [1998] analyzed a mixture of LMM organic acids by using a Dionex AS-11 anion-exchange column with an NaOH gradient elution and conductivity detection. In addition to organic acids, also nitrite and nitrate were separated. An ion chromatographic method for the simultaneous determination of carboxylic acids in addition to chloride and phosphate ions was described by Alcazar *et al.* [2003]. In this study, separation was performed on an anion-exchange column by an isocratic elution with 0.6 mM aqueous KPh solution containing acetonitrile (ACN) as an eluent and detection was carried out with a conductivity detector. Cassidy *et al.* [2004] developed an IC-CD analysis method for carboxylic acids in addition to inorganic fluoride, chloride, nitrite, bromide, nitrate, carbonate, sulfate, and phosphate. KOH was used for gradient elution. Chi and Huddersman [2007] used IC-CD as a complimentary method for the IEC. In their IEC method, oxalic and maleic acids were not separated and IC-CD was used for the quantitative analysis. Wei *et al.* [2007] analyzed LMM carboxylic acids in addition to chloride, nitrate, sulfate, and phosphate by using an NaOH gradient elution in an IonPac AS-11 analytical column and conductivity detection. Yoshikawa *et al.* [2007] used a cetyltrimethylammonium coated graphite carbon column to separate a mixture of carboxylic acids. The acids were directly detected by a non-suppressed IC coupled with a conductivity detector. In this work, the separation behaviors of chloride, nitrite, nitrate, and sulfate were also examined. The best results were achieved by using 2.0 mM benzoic acid-1.2 mM tris(hydroxymethyl) aminomethane (TRIS) as a mobile phase. The main benefit of using a graphite carbon column is that the column is very durable and it has excellent chemical and physical resistance. Zhong *et al.* [2007] developed an IC method for the simultaneous separation and determination of carboxylic acids in addition to fluoride, chloride, and sulfate in Bayer liquors. A Dionex IonPac AS11-HC column was used for the separation with a KOH gradient eluent and detection was carried out with CD. Geng *et al.* [2008] developed an IC analysis method for a great variety of aliphatic carboxylic acids and inorganic anions. In their study, 19 aliphatic carboxylic acids (listed in Table 4), in addition to 10 inorganic ions ( $F^-$ ,  $Cl^-$ ,  $Br^-$ ,  $I^-$ ,  $SCN^-$ ,  $NO_2^-$ ,  $NO_3^-$ ,  $SO_4^{2-}$ ,  $S_2O_3^{2-}$ , and  $PO_4^{3-}$ ) were analyzed using a Dionex IonPac AG11-HC column and an NaOH/MeOH gradient elution.

In the literature, only a few studies dealing with the IC-MS analysis of LMM carboxylic acids were available. Xiang *et al.* [1996] developed an IC-ESI-MS analysis method for four carboxylic acids. Simultaneously, chloride and sulfate were also analyzed. In their study, a Dionex IonPac AS5A column was used with an NaOH gradient elution. Ahrer and Buchberger [1999] examined IC-MS analysis of carboxylic acids. Ion-exchange chromatography of organic anions was performed using of a mixture of 1 mM ammonium citrate (pH 4.8) and ACN (50:50, v/v) as the mobile phase. The analysis of organic acids was performed with a micro column packed with dimethylamino-modified Nucleosil particles. Mascolo

*et al.* [2005] developed an IC-ESI-MS method for a mixture of carboxylic acids. Chloride, nitrate, nitrite, and sulfate were analyzed together with several by-products formed during the 2,4-dichlorophenol degradation. They used a Dionex IonPac AS-11 analytical column and an NaOH gradient elution for the separation. An ASRS suppressor was operated at 100 mA in external water mode and the detection was carried out with negative ionization (NI) ESI-MS. Meyer *et al.* [2007] developed an anion-exchange chromatography-ESI-MS analysis method for 18 aliphatic polyhydroxy carboxylic acids including sugar and uronic acids. The separation column was a Dionex IonPac AS11-HC and both NaOH and KOH gradient elutions were tested. The eluent was modified with borate, 2-morphinoethanesulfonic acid, or an alkyl glycoside to improve separation. Despite the incomplete separation, reproducible and sensitive identification and quantification was possible with MS detection in the SIM mode. They also applied an internal standard for the compensation of the sensitivity drift of the detector.

The main problem with the LC analysis of the LMM acids in black liquors seems to be detection. UV detection is not an appropriate choice for black liquor samples because samples include many interfering chromophoric compounds and carboxylic acids contain only a weak chromophoric group. Black liquor samples also contain a variety of acids that are not available as commercial chemicals and the detection of those acids with a non-selective detector alone is not possible. Most of the reviewed methods were based on separation with inorganic buffers and UV or CD detection alone and were therefore unsuitable. MS was the only possible detection method for the organic acid analysis in black liquor samples. IEC is one of the prime techniques for the separation of organic acids and has the advantage of being compatible with ESI-MS. However, the technique also has a rather low peak capacity and thus exhibits a relatively large risk for co-elution of isobaric compounds [Fischer, 2002]. Even if all the studies were performed with pure standard compounds, the separation of the carboxylic acids with an IEC column and organic buffers did not work well in most of the above-reviewed studies. These studies also lacked proper method validation and only a few acids could be separated from the same mixture during the same run. However, the use of IEC-MS has also shown promising results [Ahrer and Buchberger, 1999] and IEC has also been tested in the experimental part. Ion-exchange chromatography faces the same detection problems as ion-exclusion or RP chromatography. CD is sensitive but if it is used for the analysis of the black liquor samples, the acids must be identified with MS due to the highly complex sample matrix of black liquor and the lack of commercial model substances. After peak identification, IC-CD can be used alone because the elution order of the peaks remains unchanged in same method conditions.

**TABLE 4** Determination of aliphatic carboxylic acids by liquid chromatography. For compounds, see Table 5

Reference	Sample	Column (commercial name)	Separation mechanism	Eluent	Detection	Compounds
Alfredsson <i>et al.</i> , 1960	Standard compounds	Dowex 1-X 8 resin	Anion-exchange	0.07 M Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub>	Titration (with K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> )	2-3, 3-3, 3-6, 4-14, 5-12, 6-10, 6-11, 6-12
Alfredsson <i>et al.</i> , 1962	Standard compounds	Dowex 1-X 8 resin	Anion-exchange	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , various concentrations	Colorimetric	2-3, 3-3, 3-6, 4-14, 6-11
Alfredsson <i>et al.</i> , 1963	Standard compounds	Dowex 1-X 8 resin	Anion-exchange	CH <sub>3</sub> COONa, various concentrations	Colorimetric	1, 2-3, 3-3, 3-4, 4-15, 4-16
Alfredsson and Samuelson, 1968	Hydrolyzed cotton	Dowex 1-X 8 resin	Anion-exchange	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , various concentrations	Colorimetric	2-3, 3-3, 4-14, 4-15, 5-10, 5-12, 5-13, 6-11, 6-12, 6-13
Franklin, 1982	Pulping and bleaching liquors	IEC column and Ion-exchange (not specified)	Ion-exclusion	NaOH	CD	1, 2-1, 2-2, 3-3
Pacholec <i>et al.</i> , 1986	Standard compounds	Dionex IonPac ICE-AS6	Ion-exclusion	1 mM HCl	Thermospray MS	2-1, 2-3, 3-1, 4-1
Ammann and Rüttimann, 1995	Environmental samples	Dionex IonPac AS10/ Dionex IonPac AS11	Anion-exchange Anion-exchange	Borate, various concentrations	CD	1, 2-1, 2-2, 2-3, 3-1, 3-3, 3-5, 4-1, 4-10, 4-11, 5-1
Fischer <i>et al.</i> , 1995	Standard compounds	Merck Polyspher OA-HY	Ion-exclusion	H <sub>2</sub> SO <sub>4</sub> , various concentrations	UV, 210 nm	1, 2-1, 2-3, 2-4, 3-1, 3-2, 3-3, 3-4, 4-1, 4-3, 5-3, 6-3, 6-8, 6-9
Lodi and Rossin, 1995	Sugar factory products	Dionex IonPac ICE-AS1/ Bio-Rad Aminex HPX-87H	Ion-exclusion Ion-exclusion	2 mM HCl 5 mM H <sub>2</sub> SO <sub>4</sub>	CD UV, 210 nm	1, 2-1, 3-1, 3-3, 4-1, 4-4, 5-1,
Saccani <i>et al.</i> , 1995	Juices	Dionex OMNI PAC PAX-500	Mixed mode	NaOH + EtOH	CD	2-1, 3-3, 4-4, 4-5, 6-4, 6-5
Tanaka <i>et al.</i> , 1995	Standard compounds	TSKgel SCX	Ion-exclusion	MeOH-H <sub>2</sub> O (10:90, v/v)+0.15 M sucrose	CD	1, 2-1, 3-1, 4-1, 5-1
Chen, 1996	Industrial samples	Dionex OMNI PAC PAX-500	Mixed mode	NaOH gradient	CD	1, 2-1, 2-2, 3-1, 3-2, 4-1, 5-1,6-2
Mongay <i>et al.</i> , 1996	Wine	Shim-pack IC-A1	Anion-exchange	0.975 mM phthalic acid	CD	2-2, 3-3, 3-5, 4-3, 4-4, 4-5, 4-8, 6-4
Ohta <i>et al.</i> , 1996	Standard compounds	Unmodified silica gel	Ion-exclusion	0.5 mM H <sub>2</sub> SO <sub>4</sub> + 0.05% heptanol	UV, 220 nm	1, 2-1, 3-1, 4-1, 5-1, 6-1, 7-1, 8-1, 9-1, 10-1
Révész <i>et al.</i> , 1996	Standard compounds	Dionex IonPac AS4A-SC	Anion-exchange	NaOH gradient	CD	1, 2-1, 2-2, 3-1,3-2, 3-3, 3-5, 4-3, 4-5, 4-7, 4-8
Xiang <i>et al.</i> , 1996	Pharmaceuticals	Dionex IonPac AS5A	Anion-exchange	NaOH gradient	CD + ESI-MS	1, 2-2, 2-3, 3-3
Bruzzoniti <i>et al.</i> , 1997	Standard compounds	Dionex IonPac AS4A-SC	Anion-exchange	H <sub>2</sub> CO <sub>3</sub> , various concentrations	CD	1, 2-1, 2-2, 3-1, 3-2, 3-3, 3-5, 4-3, 4-5, 4-7, 4-8
Ding <i>et al.</i> , 1997	Tea	Shim-pack IC-A1	Anion-exchange	0.75 mM KHPH + 0.25 mM H <sub>2</sub> Ph	CD	2-1, 3-2, 4-3, 4-4, 4-5, 6-4
Eiteman and Chastain, 1997	Fermentation samples	Coregel 64H	Ion-exclusion	7-16 mN (3.5-8 mM) H <sub>2</sub> SO <sub>4</sub>	UV, 210 nm	1, 2.1, 3-1, 3-3, 3-5, 4-1, 4-2, 4-3, 4-7, 4-8, 5-4, 6-4
Fischer <i>et al.</i> , 1997	Composting and fermentation plant effluents	Merck Polyspher OA-HY/ Dionex IonPac ICE-AS6	Ion-exclusion Ion-exclusion	H <sub>2</sub> SO <sub>4</sub> , various concentrations PFBA	UV, 210 nm + CD	1, 2-1, 2-2, 2-3, 2-4, 3-1, 3-2, 3-3, 3-4, 3-5, 3-8 4-1, 4-2, 4-3, 4-4, 4-5, 4-10, 4-11, 4-13, 5-1, 5-2, 5-3, 5-6, 5-7, 6-3, 6-4, 6-5
Johnson <i>et al.</i> , 1997	Standard compounds	Dionex IonPac ICE-AS6	Ion-exclusion	0.4 mM TFA	ESI-MS	1, 2-2, 2-4, 4-7, 4-13
Tanaka <i>et al.</i> , 1997	Standard compounds	TSKgel SCX	Ion-exclusion	10% MeOH-H <sub>2</sub> O + 0.2% PVA	CD	1, 2-1, 3-1, 4-1, 5-1
Frauendorf and Herzs Schuh, 1998	Standard compounds	RP-C <sub>18</sub>	Reversed-phase	0.01% FA + ACN	ESI-MS	3-2, 4-7, 5-5, 6-3
Jaffrezo <i>et al.</i> , 1998	Environmental samples	Dionex IonPac AS4A-SC	Anion-exchange	0.5 mM NaHCO <sub>3</sub>	CD	1, 2-1, 2-2, 2-3, 3-1, 3-3, 4-3, 4-7, 5-3
Sharma <i>et al.</i> , 1998	Waste samples	Dionex IonPac AS11/ Dionex IonPac ICE-AS6	Anion-exchange Ion-exclusion	NaOH gradient 0.4 mM HFBA	CD	1, 2-2, 2-3, 6-4
Ahrer and Buchberger, 1999	Water samples	YMS ODS column/ Bio-Rad Fast Acid HPAH/ Nucleosil 100-5 N(CH <sub>3</sub> ) <sub>2</sub>	Reversed-phase Ion-exclusion Ion-exchange	1 mM TFA/5 mM FA 3 mM FA 1 mM ammonium citrate + 50% ACN	API-MS	3-1, 3-2, 3-3, 4-1, 4-3, 4-4, 4-5, 8-1, 4-8, 5-3
Chen and Adams, 1999	Aqueous plant tissue extracts	Bio-Rad Aminex HPX-87H	Ion-exclusion	5 mM H <sub>2</sub> SO <sub>4</sub> + 7.5% ACN	UV, 200 nm	1, 2-1, 2-2, 4-3, 4-4, 4-8, 4-8, 6-4
Ohta and Tanaka, 1999	Standard compounds	Silica gel modified with Al	Ion-exclusion	0.5 mM H <sub>2</sub> SO <sub>4</sub> + various alcohols	UV, 220 nm	1, 2-1, 3-1, 4-1, 5-1, 6-1, 7-1, 8-1

**TABLE 4** Continues

Reference	Sample	Column (commercial name)	Separation Mechanism	Eluent	Detection	Compounds
Tanaka <i>et al.</i> , 1999	Standard compounds, wine	TSKgel OApak-A	Ion-exclusion	H <sub>2</sub> SO <sub>4</sub> , various concentrations + MeOH	CD	1, 2-1, 2-2, 3-1, 3-2, 3-3, 4-1, 4-3, 4-4, 4-5, 5-1, 5-3, 6-1, 6-3, 6-4
Ohta, 2001	Standard compounds	Silica gel modified with Zr	Ion-exclusion	0.2 mM pyromellitic acid + 0.15% heptanol	CD	1, 2-1, 3-1, 4-1, 5-1, 6-1, 7-1, 8-1
Casella and Gatta, 2002	Food and beverages	Bio-Rad Aminex HPX-87H	Ion-exclusion	50 mM HClO <sub>4</sub>	ED (PAD)	1, 2-1, 3-3, 4-4, 4-5, 4-7, 6-4
Chinnici <i>et al.</i> , 2002	Musts and wines	Bio-Rad Aminex HPX-87H	Ion-exclusion	0.01 N H <sub>3</sub> PO <sub>4</sub> + 4% THF + 5% <i>n</i> -PA ( <i>n</i> -propyl alcohol)	UV, 215 nm + RI	2-1, 3-3, 4-3, 4-4, 4-5, 4-8, 6-4
Helaleh <i>et al.</i> , 2002	Standard compounds, beverages	TSKgel OApak-A	Ion-exclusion	0.85 mM benzoic acid + 10% MeOH	UV, CD, APCI-MS	1, 2-1, 3-1, 4-2, 5-1, 5-2, 6-2
Qiu and Jin, 2002	Tobacco	Bio-Rad Aminex HPX-87H	Ion-exclusion	0.8-1.8 mM HFBA	CD	1, 2-1, 3-2, 3-3, 4-3, 4-4, 6-4
Tanaka <i>et al.</i> , 2002a	Standard compounds	TSKgel OApak-A	Ion-exclusion	Mixture of carboxylic acids as eluent	CD	1, 2-1, 2-2, 3-1, 4-1, 5-1
Tanaka <i>et al.</i> , 2002b	Standard compounds	TSKgel SCX	Ion-exclusion	Mixture of carboxylic acids as eluent	CD	1, 2-1, 2-2, 3-1, 4-1, 5-1
Alcazar <i>et al.</i> , 2003	Coffee and tea	Hamilton PRP-X110	Anion-exchange	0.6 mM KH <sub>2</sub> PO <sub>4</sub> + 4% ACN	CD	2-1, 4-3, 4-7, 6-4
Cawthray, 2003	Plant root exudates	Alltima C <sub>18</sub>	Reversed-phase	93% 25 mM KH <sub>2</sub> PO <sub>4</sub> + 7% MeOH	PDA, 210 nm	2-1, 3-2, 3-3, 4-3, 4-4, 4-7, 4-8, 6-4, 6-6-, 6-7
Gamoh <i>et al.</i> , 2003	Standard compounds	ULTRON PS-80H	Ion-exclusion	0.1% FA	ESI-MS	2-1, 2-4, 3-1, 3-2, 3-3, 4-1, 4-3, 4-5, 4-10, 5-3, 6-4
Ohta and Ohashi, 2003	Standard compounds	TSKgel SCX	Ion-exclusion	3-Methyl- <i>n</i> -valeric, <i>iso</i> -caproic, and caproic acids	CD	1, 2-1, 3-1, 4-1, 4-2, 5-1, 5-2
Ohta <i>et al.</i> , 2003a	Standard compounds	TSKgel SCX	Ion-exclusion	0.5 mM H <sub>2</sub> SO <sub>4</sub> + 0.025% heptanol	CD	1, 2-1, 3-1, 4-1, 4-2, 5-1, 5-2, 5-8, 5-9, 6-1, 6-2, 6-14, 6-15
Ohta <i>et al.</i> , 2003b	Standard compounds	TSKgel CM-5PW	Ion-exclusion	0.05 mM H <sub>2</sub> SO <sub>4</sub>	CD	1, 2-1, 3-1, 4-1, 5-1, 5-2, 6-1, 6-2, 6-14, 7-1
Ohta <i>et al.</i> , 2003c	Standard compounds	TSKgel SCX	Ion-exclusion	1 mM 5-methylhexanoic acid	CD	1, 2-1, 3-1, 4-1, 4-2, 5-1, 5-2, 6-1, 6-2
Tanaka <i>et al.</i> , 2003	Standard compounds	TSKgel OApak-A	Ion-exclusion	0.05 mM H <sub>2</sub> SO <sub>4</sub> + 10 mM β-cyclodextrin	CD	1, 2-1, 2-2, 3-1, 3-3, 4-1, 4-3, 4-4, 4-5, 5-1, 6-4
Cassidy <i>et al.</i> , 2004	Pharmaceutical ingredients	Dionex IonPac AS11-HC	Anion-exchange	KOH gradient	CD	1, 2-1, 2-2, 3-1, 4-7
Ito <i>et al.</i> , 2004	Anaerobic digestion process waters	TSKgel OApak-A/ TSKgel Super IC-A/C	Ion-exclusion Ion-exclusion	Benzoic acid, various concentrations	CD	1, 2-1, 3-1, 4-1, 4-2, 5-1, 5-2
Destandau <i>et al.</i> , 2005	Industrial reaction mixtures	BetaMax neutral C <sub>18</sub>	Reversed-phase	1 mM HClO <sub>4</sub> + 5% ACN	UV, 220 nm	1, 2-1, 3-1, 4-1, 5-1, 5-3, 6-1, 6-3
Mascolo <i>et al.</i> , 2005	Industrial wastewater	Dionex IonPac AS11	Anion-exchange	NaOH gradient	ESI-MS	1, 2-1, 2-2, 2-3, 3-2, 4-5, 4-8
Bylund <i>et al.</i> , 2007	Natural waters	Supelcogel C610-H	Ion-exclusion	0.01% formic acid + 10% MeOH	MS-MS	2-2, 3-2, 3-3, 3-5, 3-9, 4-3, 4-4, 4-5, 4-7, 4-8, 5-3, 5-4, 6-4, 6-5, 6-6, 6-7
Chi and Huddersman, 2007	Fenton oxidation reaction products	Metrosep Organic acid/ Dionex IonPac AS9-SC	Ion-exclusion Anion-exchange	0.38 mM H <sub>2</sub> SO <sub>4</sub> NaHCO <sub>3</sub> + Na <sub>2</sub> CO <sub>3</sub>	CD	1, 2-1, 2-2, 3-1, 3-2, 3-7, 4-1, 4-3, 4-7, 5-3, 6-3
Meyer <i>et al.</i> , 2007	Environmental samples	Dionex IonPac AS11-HC	Anion-exchange	NaOH gradient and KOH gradient	CD + ESI-MS	2-3, 3-3, 3-4, 4-4, 4-5, 4-10, 4-12, 5-6, 5-11, 6-4, 6-5, 6-8, 6-9, 6-10, 6-16
Wei <i>et al.</i> , 2007	Xylem sap of oilseed rape	Dionex IonPac AS11	Anion-exchange	NaOH gradient	CD	1, 2-1, 2-2, 4-4, 6-4
Yoshikawa <i>et al.</i> , 2007	Food	Carbon IC BI-01	Anion-exchange	2.0 mM benzoic acid + 1.2 mM TRIS	CD	2-1, 3-3, 4-3, 4-4, 4-5, 6-4
Zhong <i>et al.</i> , 2007	Bayer liquors	Dionex IonPac AS11-HC	Anion-exchange	KOH	CD	1, 2-1, 2-2, 3-1, 4-3, 5-3
Geng <i>et al.</i> , 2008	Standard compounds, cell cultural broth	Dionex IonPac AG11-HC	Anion-exchange	NaOH + MeOH gradient	CD	1, 2-1, 2-2, 3-1, 3-3, 3-5, 3-9, 4-1, 4-3, 4-4, 4-5, 4-7, 4-8, 5-3, 5-4, 6-4, 6-5, 6-6, 6-7

**TABLE 5** List of detected aliphatic carboxylic acids. For analysis methods, see Table 4

Number and compound		
1 Formic	4-8 Fumaric	5-13 3-Deoxy- <i>erythro</i> -pentonic
2-1 Acetic	4-9 Methylmalonic	6-1 Caproic
2-2 Oxalic	4-10 2-Hydroxybutanoic	6-2 Isocaproic
2-3 Glycolic	4-11 3-Hydroxybutanoic	6-3 Adipic
2-4 Glyoxylic	4-12 4-Hydroxybutanoic	6-4 Citric
3-1 Propionic	4-13 2-Hydroxyisobutanoic	6-5 Isocitric
3-2 Malonic	4-16 2-Deoxy-tetronic	6-6 <i>cis</i> -Aconitic
3-3 Lactic	4-15 3-Deoxy-tetronic	6-7 <i>trans</i> -Aconitic
3-4 Glyceric	4-16 2,3-Dihydroxybutanoic	6-8 Gluconic
3-5 Pyruvic	5-1 <i>n</i> -Valeric	6-9 Glucaric
3-6 2-C-Methylglyceric	5-2 Isovaleric	6-10 Glucosaccharinic
3-7 Acrylic	5-3 Glutaric	6-11 Glucoisosaccharinic
3-8 Tartronic	5-4 2-Oxoglutaric	6-12 Glucometasaccharinic
3-9 Citraconic	5-5 Methylsuccinic	6-13 Anhydroglucoisosaccharinic
4-1 <i>n</i> -Butanoic	5-6 2-Hydroxyisovaleric	6-14 2-Methylcaproic
4-2 Isobutanoic	5-7 2-Hydroxy- <i>n</i> -valeric	6-15 5-Methylcaproic
4-3 Succinic	5-8 2-Methylvaleric	6-16 2-Hydroxycaproic
4-4 Malic	5-9 2,2-Dimethyl- <i>n</i> -valeric	7-1 <i>n</i> -Heptanoic acid
4-5 Tartaric	5-10 2,5-Dihydroxyvaleric	8-1 Caprylic
4-6 Threonic	5-11 Xylonic	9-1 Pelargonic
4-7 Maleic	5-12 3-Deoxy- <i>threo</i> -pentonic	10-1 Capric

### 2.7.3 Capillary electrophoresis

CE has been used also for the determination of LMM acids. In this chapter CE methods are described very briefly, because CE was not used in the experimental part of this work. However, there are a few studies in which CE analysis has been applied for the analysis of LMM acids from black liquor samples [Masselter *et al.*, 1996; Volgger *et al.*, 1998; Sullivan and Douek, 2004] or other wood-derived sample matrices [Sirén *et al.*, 2000; Kokkonen *et al.*, 2004; Dupont *et al.*, 2007]. Masselter *et al.* [1996] developed a CE analysis method for oxalic acid and various inorganic anions such as sulfate, sulfite, thiosulfate, hydroxide, chloride, and carbonate in kraft pulping process liquors. Volgger *et al.* [1998] presented two methods for the quantitative CE determination of phenolic lignin degradation compounds as well as of inorganic anions (hydroxide, thiosulfate, chloride, sulfate, sulfide, sulfite, and carbonate) and organic acids (oxalic, formic, acetic, propionic, and butyric acids) in kraft black liquors. Inorganic ions, which are of importance to the pulping process, were determined by simply diluting the black liquors after sampling and subsequent analysis with a chromate electrolyte system and indirect UV detection at 185 nm. LMM aliphatic carboxylic acids were determined simultaneously during the same run and within two minutes. A short separation time was obtained by adding a polycationic electroosmotic flow modifier (hexadimethrine bromide) to the electrolyte, which reversed the electroosmotic flow. Sullivan and Douek [2004] developed an analysis method for the determination of hydroxide, sulfide, thiosulfate, sulfate, sulfite, chloride, oxalate, and formate in green, white, and black liquors by CE. Analytes were quantified by



indirect UV-detection at 275 nm. Sirén *et al.* [2000] and Kokkonen *et al.* [2004] used CE for online monitoring of paper mill process waters. Sirén *et al.* [2000] determined oxalic, formic, and acetic acids in addition to chloride, sulfate, potassium, calcium, sodium, magnesium, and aluminium ions. Kokkonen *et al.* [2004] analyzed oxalic, formic, and acetic acids in addition to chloride, thiosulfate, sulfate, sulfite, hydrogen sulfide, carbonate, and phosphate by using an ion-specific separation system in CE. Dupont *et al.* [2007] used CE for the analysis of formic, acetic, glycolic, succinic, and lactic acids found in aqueous extracts of ligno- and holocellulosic papers.

## 2.8 Online analysis and process analytical chemistry

The demands to improve process efficiency and process control have brought along an increased interest in developing real-time measurement systems and process analytical chemistry (PAC) has developed in response to the needs of industry [Workman *et al.*, 1999; Rice, 2001]. Real-time measurements will almost always result in more valued data about the process and many industrial processes have benefited from the value of applying laboratory-based technologies in manufacturing. Direct chemical and physical information describing the composition of a process stream enables the optimization and control to be implemented in real time. Improved monitoring capabilities help to fine-tune the chemistry to maximize process efficiency and reduce plant operating costs. Today, industrial processes often rely upon process analytical measurements for such benefits as enhanced process and product quality management in addition to environmental control and monitoring.

Process measurements have traditionally included simple parameters like temperature, pressure, and flow rate. Advances in technology for process instrumentation generally lag several years behind laboratory instrumentation. Typically, the concepts are researched and proven in the laboratory and then must be developed to meet the process requirements such as safety and reliability [Beebe *et al.*, 1993]. The value of discriminating information from complex online process analyzers has been recognized. The development of instrumental analysis has led to the automation of many laborious analytical techniques, providing greater throughput, accuracy, and reproducibility. Rapid developments have occurred in sampling, miniaturization of the analytical systems, data processing, and computer technology. The technology base continues to expand with new analysis techniques and applications, and more robust designs of existing technologies as well as increasingly powerful data handling [Workman *et al.*, 1999; 2003].

Every process analysis technique requires a sampling system and obtaining a representative sample is a challenging task. A majority of process analytical problems can be attributed to problems in the sampling system and, consequently, a lot of effort has been put into improving the state of sampling system technology and design [Workman *et al.*, 1999]. Sampling techniques, designs, and components are generic to many industries, processes, and types of process analytical measurements. Online sampling systems have been used successfully in the pulp and paper industry. Sirén *et al.* [2000] and Kokkonen *et al.* [2004] used an online sampling system for the monitoring of water-soluble ions in pulp and paper machine waters by capillary electrophoresis. The sampling system was fully automated and it enabled the filtration and dilution of the samples.

Chromatography continues to enjoy wide usage in the field of PAC. Complete characterization with multicomponent analysis provides more information than single component or bulk property measurements [Workman *et al.*, 1999]. Although GC is the most mature of the multicomponent process analyzers and dominates the field of separation techniques used for monitoring environmental and production streams, other methodologies such as LC is becoming more widely practiced. The development of more rugged and reliable sampling equipment is largely responsible for this diversification. Sampling systems for LC include, for example, online solid-phase extraction (SPE), automatic sample filtration and dilution, and online dialysis. IC in particular shares commonalities with LC and has also been used in process monitoring. Automated sample preparation techniques for IC have been described in the literature [Montgomery *et al.*, 1998].

Chromatographic data treatment systems have experienced developments and more selective information can be obtained from the process streams [Workman *et al.*, 1999]. However, the increased use of chemical analyzers and instrumentation has led to the generation of huge amounts of process data. Chemometric tools have been used to handle this information. Process chemometrics can provide additional insights into the chemical processes through monitoring, modeling, and control.

Flow injection analysis (FIA) and sequential injection analysis (SIA) can be viable methods in process analytical applications [Workman *et al.*, 1999]. In FIA or SIA, samples and reagents are manipulated fluidically by the pumps, valves, and reactors that comprise the flow system. The flow system's sample line can be situated in a production reactor vessel, process line, or flask containing the same material. Once sampling occurs, the automated fluid handling coupled with flow-through detection is the same regardless of the origin of the sampled volume.

MS is particularly appealing for the analysis of process streams, due to its inherent speed, sensitivity, and molecular selectivity [Workman *et al.*, 1999]. The historical limitations in the implementation of MS were typically seen to be size, cost, and complexity. These impediments are gradually being removed because of extensive ongoing research into the miniaturization of every kind of mass spectrometer (ion trap, ion cyclotron resonance, time of flight, magnetic sector, and quadrupole) as well as continuing developments in computer technology and software.

FIA-MS can speed up the analysis compared with traditional chromatographic separation [Workman *et al.*, 1999]. In FIA-MS analysis, there is no separation prior to MS detection and the separation of ions will be accomplished within the mass spectrometer. If the sample is very complex and some compounds in the mixture have same mass-to-charge ( $m/z$ ) value, it is not possible to use FIA-MS for those compounds and chromatographic separation is still needed.

In this work, the goal was to achieve robust and accurate analysis of complex black liquor samples. This was a challenging task since the analytes were present over a large concentration range and the matrix may cause disturbances in the detection. When considering a fast and simple analysis method, the sample preparation should be minimal and the separation and detection time should be as short as possible. In the method optimization, it becomes important to make the right compromises when choosing experimental settings. The literature review has shown that there are several possible methodologies that can be implemented after modifications for the fast analysis of the LMM organic acids in black liquor samples. In the experimental section, the developed novel chromatographic analysis methods are described in detail as well as the method validation results.

## 3 EXPERIMENTAL

### 3.1 Chemicals, materials, and apparatus

The chemical structures of the aliphatic carboxylic acids are presented in Appendix II and the chemicals and materials used in this research are listed in Appendix III. The dilution and use of the standard compounds are described in detail in papers I-V. The instruments and columns used in studies I-V are listed in Appendix III.

The HPLC instrument was used for the separation in studies I-III and V. The detection was carried out using a PDA detector (I) and a single quadrupole MS, equipped with an APCI interface operating in NI mode (I-III, V). An HP Series 1100 binary pump, a vacuum degasser, a thermostatted column compartment, and an autosampler were used for the analysis. The separation column was a Waters Atlantis dC<sub>18</sub> column combined with a guard column of the same packaging material. In paper I, several other columns were also tested and those are listed in Appendix III. Data were stored and processed using an HP ChemStation (A06.03) data system.

GC separations were performed using an Agilent 6850 Series GC equipped with an HP-5 column and FID. The identification of the individual acids was performed by an Agilent 6890 Series GC system equipped with an HP-5 column and an Agilent 5973 mass-selective detector. The data collection and processing were handled by an HP ChemStation (A06.03) chromatography data system.

IC-CD determinations were carried out by a Dionex chromatography system equipped with an AS50 autosampler, an LC25 chromatography oven, an EG40 eluent generator, and an IC25 ion chromatograph. A Dionex ASRS Ultra II self-regenerating suppressor was operated at 149 mA in the AutoRegen mode. The system was equipped with an anion trap column (IonPac ATC-1) and the separation column was an IonPac AS 11-HC analytical column combined with an AG11-HC guard column from Dionex. The data were stored and processed using a Dionex Chromeleon (6.50) data system.

For IC-ESI-MS determinations, an HP Series 1100 binary pump, a vacuum degasser, a thermostatted column compartment, and an autosampler were used in the chromatographic analysis. The same columns were used as in the IC-CD system. A Dionex ASRS Ultra II self-regenerating suppressor (4 mm) was used in

pressurized bottle mode to remove the sodium ions. A T-piece was used to split the flow from the suppressor and an external pump was used to ensure the operation of the suppressor. Detection was carried out with an HP 1100 Series single quadrupole MS equipped with an ESI interface operating in the NI mode. Data collection and processing were handled by an HP ChemStation (A06.03) chromatography data system.

### 3.2 Black liquor samples and pretreatments

Wood black liquor samples were obtained from the conventional laboratory scale kraft cooks of industrial wood chips from birch (*Betula pendula/B. pubescens*), aspen (*Populus tremula*), and softwoods consisting of 75% Scots pine (*Pinus sylvestris*) and 25% Norway spruce (*Picea abies*). The non-wood black liquor samples analyzed were obtained from the laboratory-scale alkaline cooks of chips originating from Vietnamese bamboo (*Bambusa procera*) and cultivated Finnish wheat straw (*Triticum aestivum*). The bamboo chips used for pulping were screened according to standard SCAN-CM 40:94 [SCAN, 1994]. Chips with knots and bark residues were eliminated. Wheat straw was cut to a length distribution of 3 cm to 5 cm and both knots and leaves were removed. Conventional alkaline pulping was performed in an oil bath digester (CRS CAS 420) equipped with 1.25-L rotating stainless steel autoclaves. Data on the cooking conditions are presented in Table 6.

**TABLE 6** Cooking conditions

Feedstock/method	Effective alkali <sup>a</sup>	Sulfidity (%)	Cooking temperature (°C) <sup>b</sup>	Liquor-to-feedstock ratio (L kg <sup>-1</sup> )
Aspen	20	35	160 <sup>b</sup>	4.5
Birch	19	35	162 <sup>b</sup>	4.5
Softwood	19	35	170 <sup>b</sup>	4.5
Bamboo/kraft	15	35	165 <sup>c</sup>	4.0
Bamboo/soda (AQ 0.05%)	21	-	165 <sup>c</sup>	5.0
Wheat straw/kraft	15	30	145 <sup>c</sup>	5.0
Wheat straw/soda (AQ 0.05%)	15	-	145 <sup>c</sup>	5.0

<sup>a</sup> EA% (as NaOH) on o.d. feedstock

<sup>b</sup> Time from 25 °C to this max. temperature was 90 min.

<sup>c</sup> Initial cooking temperature 80 °C and heating-up rate 1 °C min<sup>-1</sup>.

The black liquor samples were stored at -18 °C and were warmed up to room temperature and mixed carefully before dilution. Because the aim was to develop simple analysis methods for industrial processes, the sample preparation for HPLC-APCI-MS, IC-CD, and IC-ESI-MS analyses was limited to filtration and dilution, which can be easily automatized. Prior to HPLC-APCI-MS and IC-ESI-MS analyses, the samples were diluted from a stock solution with ultra high quality (UHQ) water and filtered in a vacuum through 0.2-µm membrane filters. Prior to

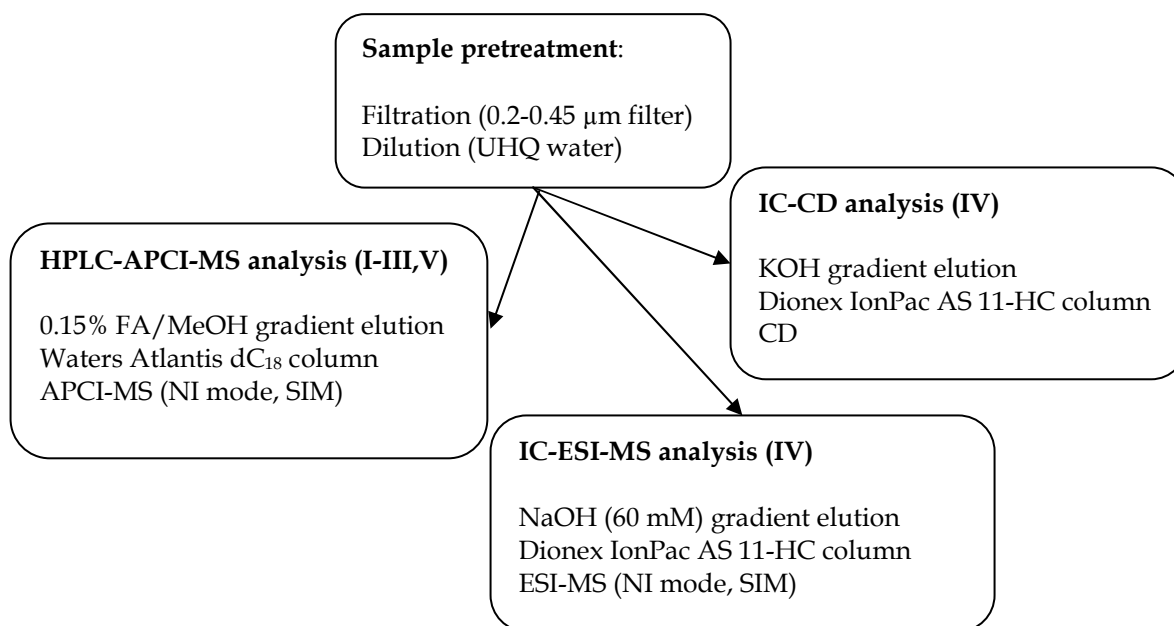
IC-CD analysis, the samples were diluted with degassed UHQ water and filtered through 0.45- $\mu\text{m}$  nylon filters.

Sample preparation for the GC-FID and GC-MS analyses were adapted from Alén *et al.* [1984b] with slight modifications. The black liquor samples were diluted (1:10) from the stock solutions with UHQ water. The diluted sample (1 mL) and the internal standard (1 mL of xylitol solution, concentration 0.1 mg L<sup>-1</sup>) was passed through a column filled with weakly acidic cation-exchange resin (4 mL, Amberlite IRC-50, 16-50 mesh, NH<sub>4</sub><sup>+</sup>-form). The column was then washed with water to obtain an effluent volume of 30-40 mL. The effluent was evaporated with a rotary vacuum evaporator at 35 °C. For derivatization, 1 mL pyridine and 0.5 mL BSTFA containing 1% of TMCS were added to the evaporation residue. Finally, the mixture was shaken for 30 min at room temperature.

## 4 RESULTS AND DISCUSSION

### 4.1 Development of the analysis methods

In the method development stage, the interest was to find the combination of parameter settings that gave the best analytical results for the main LMM aliphatic carboxylic acids. In Figure 6 is presented a simplified scheme of the methods developed.



**FIGURE 6** Scheme of the sample pretreatments and analytical methods for the determination of the LMM aliphatic carboxylic acids in alkaline delignification spent liquors.

#### 4.1.1 HPLC-APCI-MS

In HPLC methods, carboxylic acids are commonly monitored by UV detection at about 210 nm. A major problem with the detection of aliphatic organic acids with UV is that the chromophoric properties of a carboxyl group are weak, resulting in relatively poor sensitivity of the detection. It was also noticed that black liquor samples had other chromophoric groups which interfered with the UV detection. Some of the major acids were not available as commercial chemicals and therefore the use of MS detection was necessary. Peak identification was based on the retention times,  $m/z$  values, and abundances. All samples were also measured

with GG-FID and GC-MS and results were compared to HPLC-APCI-MS measurements.

The MS detection set limitations to the chromatographic separation because the inorganic buffers normally used for the separation of the organic acids were excluded. The separation of the acids with organic buffers was difficult because all these acids were LMM compounds and their  $pK_a$  values were close to each other. In the HPLC-APCI-MS method development stage (I), two different analytical IEC columns were tested: a PRP-X300 ion-exclusion column with a sulfonated PS-DVB phase combined with a PRP-X300 guard column and two Waters Fast Fruit Juice columns joined in series. In addition, four RP columns were tested. Two of them were Agilent SB-AQ (Stable Bond Aqua) columns with an alkyl RP-bonded phase. The third one was an Agilent SB-C<sub>18</sub> column, and the fourth one was a Waters Atlantis dC<sub>18</sub> column with a guard column. The Atlantis dC<sub>18</sub> column is a difunctionally bonded, silica based C<sub>18</sub> RP-column that is designed to retain polar compounds. These columns were tested with organic buffers (formic and acetic acids and TFA) at various concentrations and temperatures and together with organic modifiers (ACN, MeOH, and EtOH). For black liquor samples, a Waters Atlantis dC<sub>18</sub> RP column provided the best separation when aqueous formic acid (0.15% v/v, pH 2.5, solvent A) and MeOH (solvent B) were used in various proportions (for the gradient program, see Table 7).

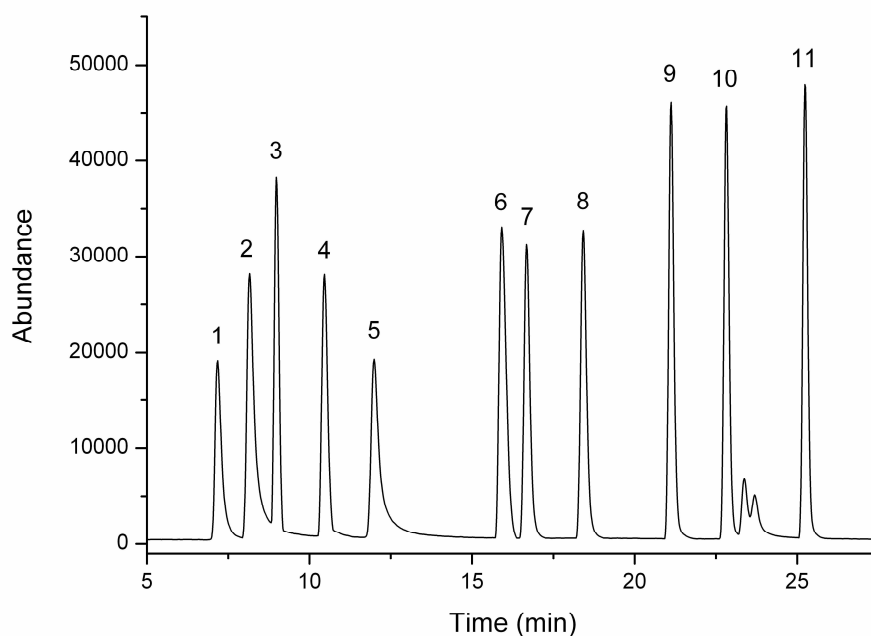
At the beginning, both UV and MS detections were used. It would have been an ideal situation if the peak order had been confirmed with MS and quantitative detection carried out with the UV detector. However, it was noticed already with the standard compounds that the UV detection was not sensitive enough. Some compounds had very weak chromophoric properties and were not detected properly within the required concentration range. When the black liquor samples were analyzed using UV detection, interfering compounds made the interpretation of the UV chromatograms impossible.

MS was selected as a main detector for both qualitative and quantitative purposes. After defining the chromatographic separation conditions, MS parameters such as needle temperature, cone voltage, drying gas temperature, nebulizer pressure, fragmentor value, and corona current were optimized (I). For this purpose, flow injection analysis was used. The highest MS detection sensitivity was achieved with a fragmentor value of 50, capillary voltage of 2250 V, and nebulizer pressure of 40 psig. The optimal drying gas (N<sub>2</sub>) flow was 4.0 L min<sup>-1</sup> at the temperature of 300 °C. A value of 325 °C was found to be optimal for the vaporizer temperature and a value of 16 μA for the corona current. Different compounds have dissimilar responses to the changes in operating conditions and setting the operating parameters for the MS detector was a compromise.

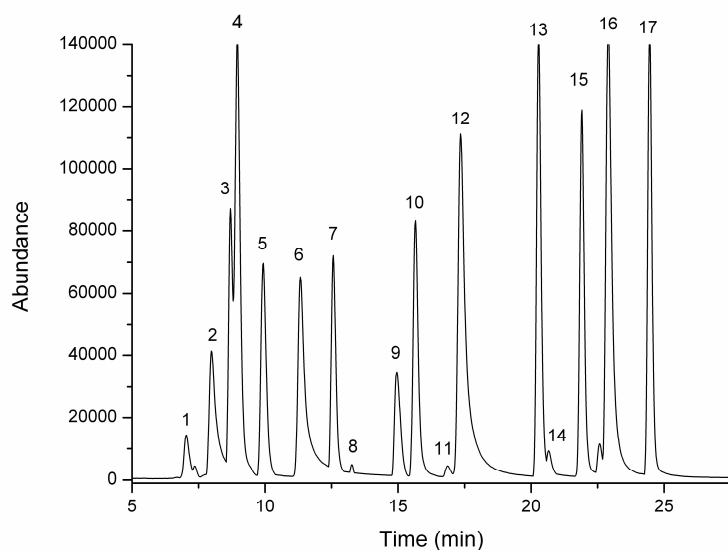


It was noticed that the MSD was quite sensitive to changes and the peak areas varied from day to day. To solve this problem, internal standards were used. Fumaric, glutaric, and adipic acids were chosen as the internal standards. It has been reported earlier [Niemiälä, 1990a] that glutaric acid can be present in black liquors. However, these acids were not found in the tested black liquor samples and they did not overlap with other compounds. Adipic acid proved to be the most suitable internal standard for the standard compounds in aqueous media (I). The validation results for the wood (II) and non-wood black liquors (III) later showed that glutaric acid was a more appropriate internal standard for alkaline spent liquors.

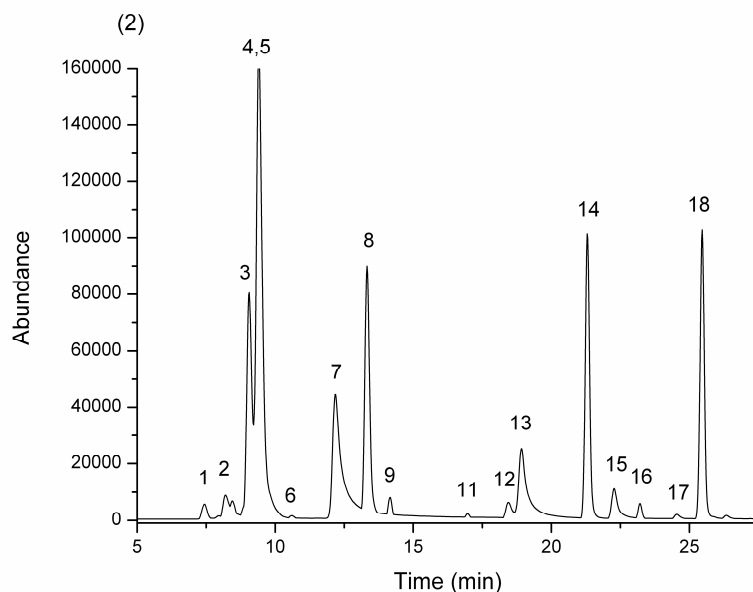
An SIM chromatogram of the separation of the acid standard compounds in aqueous media is presented in Figure 7 (I). Figure 8 shows an SIM chromatogram of acids in softwood black liquor after the addition of the standard compounds (I). In Figure 9 is presented an SIM chromatogram of the acids in softwood black liquor (dilution 1:75, v/v) without the addition of the standard compounds (II).



**FIGURE 7** HPLC-APCI-MS (SIM) chromatogram of the separation of the acid standard compounds on a Waters Atlantis dC<sub>18</sub> column at pH 2.5 with 0.15% (v/v) formic acid and MeOH (for the gradient program, see Table 7) (I): oxalic (1), glycolic (2),  $\alpha$ -glucoisosaccharinic (3), malic (4), lactic (5), maleic (6), succinic (7), fumaric (8), glutaric (9), methylsuccinic (10), and adipic (11) acids.



**FIGURE 8** HPLC-APCI-MS (SIM) chromatogram of acids in softwood black liquor after addition of the standard compounds (II): oxalic (1), glycolic (2),  $\alpha$ -glucoisosaccharinic (3),  $\beta$ -glucoisosaccharinic and xyloisosaccharinic (4), malic (5), lactic (6), 3,4-dideoxy-pentonic (7), 2-hydroxyglutaric (8), maleic (9), succinic (10), 4-hydroxybutanoic (11), 2-hydroxybutanoic (12), glutaric (13), 2-hydroxy-4-pentenoic (14), methylsuccinic (15), 2-hydroxy-2-methylbutanoic (16), and adipic (17) acids.



**FIGURE 9** HPLC-APCI-MS (SIM) chromatogram of the acids in softwood black liquor (dilution 1:75) (II). Compounds: oxalic (1), glycolic (2),  $\alpha$ -glucoisosaccharinic (3),  $\beta$ -glucoisosaccharinic (4) xyloisosaccharinic (5), malic (6), lactic (7), 3,4-dideoxy-pentonic (8), 2-hydroxyglutaric (9), succinic (11), 4-hydroxybutanoic (12), 2-hydroxybutanoic (13), glutaric (14), 2-hydroxy-4-pentenoic (15), methylsuccinic (16), 2-hydroxy-2-methylbutanoic (17), and adipic (18) acids.

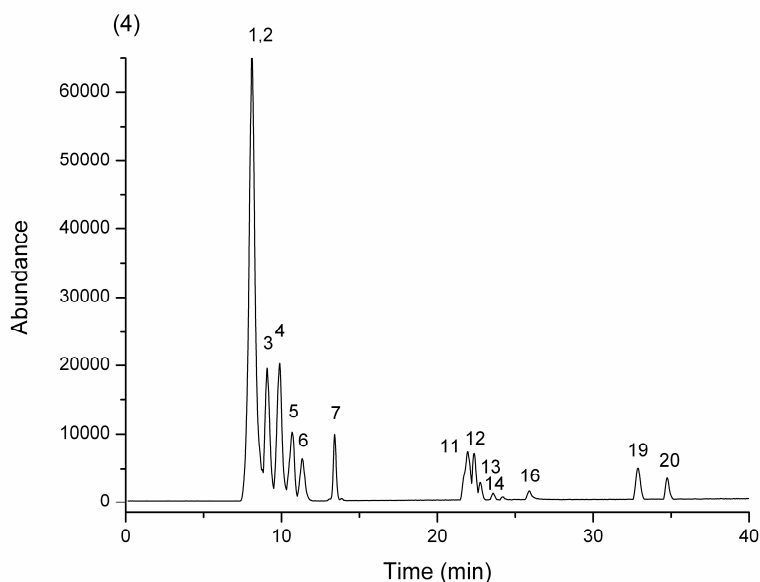
### 4.1.2 IC-ESI-MS

Ion chromatography with the conductivity detection offers a simple method for the determination of the carboxylic acids in black liquors. The conductivity detector is an unselective detector and therefore a complimentary IC-ESI-MS method was first developed to confirm the identification of the carboxylic acids that were not available as commercial chemicals (IV). Once the peak identification was complete, IC-CD was used alone because the elution order of the peaks remained unchanged (when same column was used with NaOH or KOH eluent).

In the IC-ESI-MS method (IV), the binary solvent system consisted of aqueous sodium hydroxide (60 mM, solvent A) and UHQ water (solvent B). The gradient program is presented in Table 7. After a suppression step, the flow from the chromatographic system was split into the ratio 3:10, by means of a zero dead volume T-piece to allow 30% of the flow to enter an MS equipped with an ESI interface operating in the NI mode. A portion (70%) of the flow was returned to the self-regenerating suppressor and, to ensure the proper operation of the suppressor (external pressurized bottle mode), external water circulation (flow 1.5 mL min<sup>-1</sup>) was added to the suppressor to compensate for the removal of 30% of the flow.

The ESI-MS parameters such as drying gas flow and temperature, nebulizer pressure, capillary voltage, and fragmentor value were optimized by using FIA. The highest MS detection sensitivity was achieved with a fragmentor value of 50, capillary voltage of 3750 V, and nebulizer pressure of 30 psig. The optimal drying gas (N<sub>2</sub>) flow was 10.0 L min<sup>-1</sup> at the temperature of 300 °C.

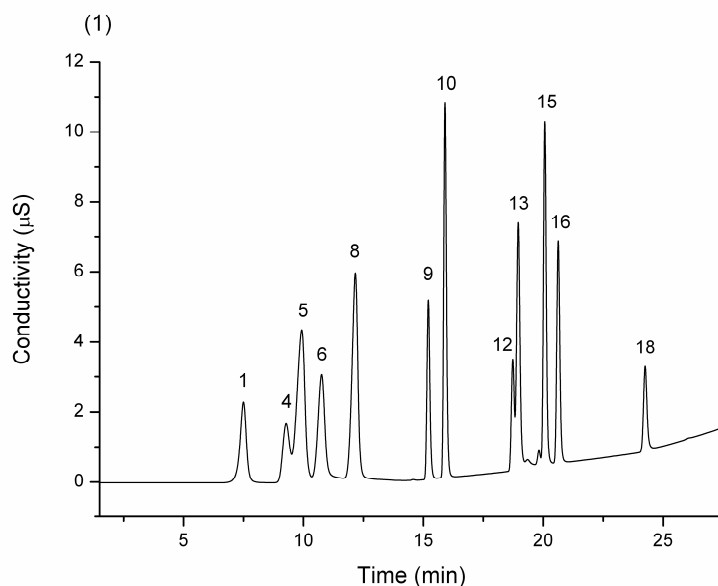
Figure 10 shows the IC-ESI-MS chromatogram of the carboxylate anions in softwood black liquor (dilution 1:50).



**FIGURE 10** IC-ESI-MS (SIM) chromatogram of acids in softwood black liquor (dilution 1:50) (IV). Carboxylate anions:  $\alpha$ - and  $\beta$ -glucoisosaccharinate (1), xyloisosaccharinate (2), 3,4-dideoxy-pentionate (3), lactate (4), acetate and glycolate (5), 2-hydroxybutanoate (6), 2-hydroxy-4-pentenoate (7), glucoisosaccharinarate (11), methylsuccinate (12), malate and succinate (13), 2- and 3-deoxy-tetronate (14), oxalate (16), unknown peaks (19,20).

#### 4.1.3 IC-CD

The UHQ water used for the preparation of the mobile phase was degassed via ultrasonic treatment for approximately 15 min prior to use. The required concentration for the mobile phase was made in the eluent generator module in the IC system. The samples were injected via a 25- $\mu$ L loop and were eluted at a flow rate of 1.0 mL min<sup>-1</sup>. The gradient program and method conditions are presented in Table 7. The gradient program was first optimized with standard compounds. The conductivity chromatogram obtained for the mixture of the standard compounds in aqueous media is presented in Figure 11 (IV). The acids and inorganic anions were selected on the basis of their presence in kraft black liquors. In addition, it was possible, during the same run, to separate other carboxylates (*e.g.*, citrate and pyruvate) and inorganic anions (*e.g.*, phosphate and sulfite), but they were not present in significant amounts in the kraft black liquors.

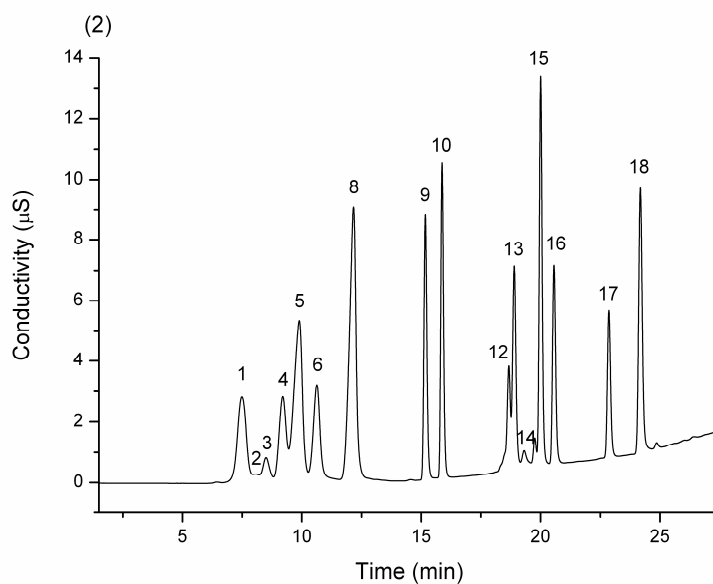


**FIGURE 11** IC-CD chromatogram of the carboxylates and inorganic anions as pure standards in aqueous media (IV). Detected anions:  $\alpha$ -glucoisosaccharinate (1), lactate (4), acetate and glycolate (5), 2-hydroxybutanoate (6), formate (8), chloroacetate (9), chloride (10), methylsuccinate (12), malate and succinate (13), sulfate (15), oxalate (16), and thiosulfate (18).

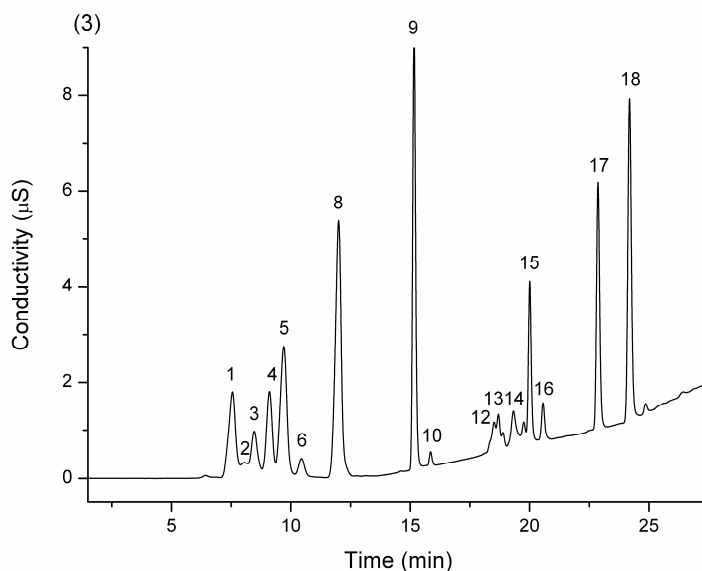
Figure 12 shows the IC chromatogram of the softwood black liquor after the addition of the standard compounds in the same concentrations as those in Figure 11. Figure 13 shows the same black liquor sample with the internal standards (chloroacetate and phosphate) but without the controlled addition of other acids and inorganic anions. By means of these figures, it was confirmed that the method developed was capable of separating the target compounds from the black liquor sample.

Dual peaks for acetate and glycolate as well as for malate and succinate could not be avoided by this system, although the gradient program was modified several times. It should be pointed out that it was now possible to determine formate (peak 8 in Figure 11), which was used as a mobile phase in the HPLC-APCI-MS method developed earlier (I-III).

For quantitative purposes, phosphate ion, chloroacetate, and pyruvate were tested as internal standards. Since the method validation showed that chloroacetate gave the best results, all the method validation calculations were based on the use of this compound as an internal standard.



**FIGURE 12** IC-CD chromatogram of the carboxylates and inorganic anions in softwood black liquor (dilution 1:1000) after the addition of the standard compounds (IV). Detected anions:  $\alpha$ - and  $\beta$ -glucoisosaccharinate (1), xyloisosaccharinate (2), 3,4-dideoxy-pentionate (3), lactate (4), acetate and glycolate (5), 2-hydroxybutanoate (6), formate (8), chloroacetate (9), chloride (10), methylsuccinate (12), malate and succinate (13), 2- and 3-deoxy-tetronate (14), sulfate (15), oxalate (16), phosphate (17), and thiosulfate (18).



**FIGURE 13** IC-CD chromatogram of the carboxylates and inorganic anions in softwood black liquor (dilution 1:1000) (IV). Detected anions:  $\alpha$ - and  $\beta$ -glucoisosaccharinate (1), xyloisosaccharinate (2), 3,4-dideoxy-pentionate (3), lactate (4), acetate and glycolate (5), 2-hydroxybutanoate (6), formate (8), chloroacetate (9), chloride (10), methylsuccinate (12), malate and succinate (13), 2- and 3-deoxy-tetronate (14), sulfate (15), oxalate (16), phosphate (17), and thiosulfate (18).

**TABLE 7** Method conditions

Parameter	HPLC-APCI-MS (I-III, V)		IC-MS (IV)		IC-CD (IV)	
Injection volume (μL)	10-20		5		25	
Analytical column	Waters Atlantis dC <sub>18</sub> (250 mm × 4.6 mm)		IonPac AS 11-HC (250 mm × 4 mm)		IonPac AS 11-HC (250 mm × 4 mm)	
Guard column	Waters Atlantis dC <sub>18</sub> (20 mm × 4.6 mm)		IonPac AG11-HC (50 mm × 4 mm)		IonPac AG11-HC (50 mm × 4 mm)	
Ion trap column	-		IonPac ATC-1 (24 mm × 9 mm)		IonPac ATC-1 (24 mm × 9 mm)	
Column temperature (°C)	25		25		25	
Solvent	0.15% formic acid (v/v) (A)/MeOH (B)		UHQ water (A)/NaOH (B)		KOH/UHQ water	
Flow rate (mL min <sup>-1</sup> )	0.5		1		1	
Suppressor	-		Dionex ASRS Ultra II Self-Regenerating (4 mm)		Dionex ASRS Ultra II Self-Regenerating (4 mm)	
Suppressor current (mA)	-		max. 300		149	
Detection	MS		MS, suppressed, pressurized bottle mode		CD, suppressed, AutoRegen mode	
Ionization mode:	APCI, NI mode		ESI, NI mode			
Drying gas (N <sub>2</sub> , L min <sup>-1</sup> )	4.0		10.0			
Nebulizer pressure (psig)	40		30			
Gas temperature (°C)	300		300			
Capillary voltage (V)	2250		3750			
Fragmentor	50		50			
Vaporizer temperature (°C)	325					
Corona current (μA)	16					
Gradient	Time	(%A, 0.15% FA)	Time	(%B, 60 mM NaOH)	Time	mM KOH
	0.01	100.00	0.01	1.70	0.01	1.00
	7.50	90.00	1.00	12.50	2.00	1.00
	30.00	20.00	17.50	25.00	10.00	4.00
	35.00	100.00	27.50	50.00	20.00	35.00
			37.50	100.00	24.00	60.00
			37.51	1.70	27.00	60.00
			40.00	1.70	27.20	1.00
					35.00	1.00

## 4.2 Quality parameters of the methods

### 4.2.1 Evaluation of the separation

The resolution factor describes the ability of a column to separate chromatographic peaks. A value of 1 is considered to be the minimum for a measurable separation to occur and to allow good quantitation. A value of 0.6 is required to discern a valley between two equal-height peaks and a value of 1.5 is considered sufficient for baseline resolution for two peaks of equal height. Values of 1.7 or greater are generally desirable for rugged methods. Resolution values were calculated to evaluate the separation of the peaks. In the HPLC-APCI-MS method, the values were  $>1.0$  for all the acids (I). The peaks for glycolic and acetic acids were tailing but the resolution for lactic acid was high (5.72). In the IC-CD method, the resolution values were  $>1.0$  for the selected anions except for methylsuccinate (0.90) (IV).

### 4.2.2 Linearity

Linearity studies were performed to determine the linear ranges of the analytes and to verify that the sample solutions were in a concentration range where the analyte response was linearly proportional to the concentration. In the HPLC-APCI-MS method, the linearity of the method was first evaluated with the standard compounds in aqueous media (I). The results were very good for all the acids except for the  $\alpha$ -glucoisosaccharinic acid. The slopes were close to 1 and the intercepts were close to zero. Correlation coefficients (R values) were  $>0.99$ . The linearity of the HPLC-APCI-MS method in the different black liquor samples was evaluated with the standard addition technique. The standard additions were made to diluted black liquors samples (II, III). The R values for acids in wood and non-wood black liquors were  $>0.99$ .

The linearity of the IC-CD method was evaluated for the standard compounds in aqueous media (IV). After this, the linearity of the method was evaluated in black liquor samples with the standard addition technique (IV). The results show no matrix effects in the determination of carboxylate anions and inorganic anions as the target values of 1 for slope and 0 for intercept were achieved. The R values for all the compounds in all the sample matrices were 1.

The results for  $\alpha$ -glucoisosaccharinic acid were not as good as for other acids, probably due to the equilibrium between its open and cyclic structures.



Glucosisosaccharinic acid has a corresponding enantiomeric lactone conformation and the conformation is pH-dependent with the glucoisosaccharinic form dominating at a higher pH, and the lactone form at a lower pH [Sowden, 1957; Ekberg *et al.*, 2004]. This lactone formation was noticed when a higher amount of the standard compounds was added to the black liquor sample.

### 4.2.3 Accuracy

To evaluate the accuracy of the HPLC-APCI-MS method, mixtures of the carboxylic acids were spiked at different concentrations. For standard compounds in aqueous media, the results comparing the introduced and measured concentrations were in good agreement and for most of the acids the error was less than 5% (I). Only for  $\alpha$ -glucosisosaccharinic acid the accuracy was not really satisfactory (the recovery % varied from 93.2% to 96.0%). The accuracy of the HPLC-APCI-MS method was also tested with black liquor samples. A mixture of the standard compounds was added to diluted black liquor samples at various concentrations (II, III). For most of the acids, recovery values close to 100% were achieved and relative standard deviations (RSDs) were less than 5%. However, the RSDs for oxalic acid in wood black liquors were systematically relatively high (up to 9%). Also, the recovery percentage for the 2-hydroxybutanoic acid from the wheat straw sample was high, indicating that in some cases one response factor did not necessarily cover fully the whole concentration range of the standard addition.

Accuracy evaluation was also performed for the IC-CD method with standard compounds in aqueous media and with standard additions to black liquor samples. Recoveries were close to 100% for all compounds but it should be pointed out that the RSDs for methylsuccinate and for thiosulfate were relatively high. Oxidation reactions affect the accuracy results of the thiosulfate [Sullivan and Douek, 2004]. However, if the sample storage time is relatively long, oxidation reactions can be avoided by using stabilizing agents such as carbonyl and alcohol compounds [Lindgren *et al.*, 1984]. Also, the addition of reduced glutathione during black liquor dilution had been shown to stabilize sulfide, sulfite, and thiosulfate [Sullivan and Douek, 2004]. For methylsuccinate, the reason may be that the peak was not separated well enough (resolution 0.90), which may cause interference in the analysis.

Accuracy determinations were made by using several concentrations (low and high concentration levels). A decrease of the sample pH affected negatively the accuracy results. It was obvious that when more acid standard was added the pH decreased significantly. The pH values in the black liquor samples were over

10 after the first acid addition and, after the last addition (the highest amount of the standard added), the pH was close to 5. As a result, for example, the recoveries for  $\alpha$ -glucoisosaccharinic acid were unsatisfactory. For this reason, it was concluded that it is important to control the initial pH values of the samples. When measuring black liquor samples from the process streams, this will not be a problem because the samples will be analyzed without adding acidic compounds and the sample pH values are typically  $>10$ .

These accuracy results also included an error from the intra-day and day-to-day analyses as well as an error resulting from the variation in the concentrations used, because the measurements were made during a long period. In addition, it was noticed that, when hundreds of samples were analyzed, the efficiency of the HPLC column gradually decreased, leading to slightly shorter retention times. When the gradient program was used, the proportion of methanol had a marked influence on ionization and abundances: the higher the proportion of methanol, the smaller the response factors were. For this reason, all the response factors, especially in the HPLC-APCI-MS method, should be controlled from time to time.

Statistical analyses were also performed to verify that there was no systematic error in the results. The null hypothesis was that the accuracy results did not significantly differ from 100%. A two-sided t-test was performed to evaluate the HPLC-APCI-MS and IC-CD methods separately. The data consisted of five compounds (oxalic, 2-hydroxybutanoic,  $\alpha$ -glucoisosaccharinic, lactic, and methylsuccinic acids). Recovery measurements ( $n = 10$ ) were made at various concentrations during different days. All the measurements were made for seven sample matrices, except for  $\alpha$ -glucoisosaccharinic acid, for which six sample matrices were used ( $n_{tot} = 340/\text{method}$ ). For both methods, the obtained t-values (1.942 for HPLC-APCI-MS and 0.997 for IC-CD) were below the critical t-value ( $t_{\text{critical two-tail}}=1.963$ ) at the confidence interval (CI) level of 95%.

Single factor analysis of variance (ANOVA) was used to evaluate the differences among the seven sample matrices. A single factor ANOVA was performed for oxalic, 2-hydroxybutanoic, glucoisosaccharinic, lactic, and methylsuccinic acids separately and analyses were carried out for both methods. In the case of the HPLC-APCI-MS method, the calculated F-values for 2-hydroxybutanoic,  $\alpha$ -glucoisosaccharinic, lactic, and methylsuccinic acids were higher than the critical F-values at the CI level of 95%. It was observed that the variances were high in wheat straw soda-AQ samples (for 2-hydroxybutanoic,  $\alpha$ -glucoisosaccharinic, and lactic acids) compared with other sample matrices. In the case of the IC-CD method, all the calculated F-values were below the critical value. Detailed test results are presented in the Appendix IV.

#### 4.2.4 Limits of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) values with the HPLC-APCI-MS method were obtained by the selected ion monitoring of the parent anions of the carboxylic acids studied (II, III). The LOD and the LOQ values were based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. The noise magnitude was taken from the total ion chromatograms. For the black liquor samples, the results ranged between 20  $\mu\text{g L}^{-1}$  and 1000  $\mu\text{g L}^{-1}$  for LOD and between 100  $\mu\text{g L}^{-1}$  and 1700  $\mu\text{g L}^{-1}$  for LOQ. It should be pointed out that the LOD values are subjected to change when more  $m/z$  values are included in the SIM program or when the scanning program is used to determine unknown acids at other  $m/z$  values.

The LOD and LOQ values with the IC-CD method were evaluated similarly to the HPLC-APCI-MS method. The results for standard compounds in aqueous media were between 1 and 8  $\mu\text{g L}^{-1}$  for LOD and between 3 and 27  $\mu\text{g L}^{-1}$  for LOQ (IV). Depending on the analyte, the limits of detection and quantification were in black liquor samples, between 6 and 106  $\mu\text{g L}^{-1}$ , and between 14 and 148  $\mu\text{g L}^{-1}$ , respectively.

#### 4.2.5 Precision

The precision of the HPLC-APCI-MS method was evaluated at a low and high concentration level by making repeated analyses on different days. The results for the standard compounds in aqueous media (I) were less than 4.7% for intra-day precision. The day-to-day precision repeatability values were less than 4.9% for all the acids except for  $\alpha$ -glucoisosaccharinic acid, which had a range from 6.4% to 12.0%. Intermediate precisions were less than 5.4%. Precision was also evaluated for the acids in black liquor samples (II, III). In these tests, a mixture of the standard compounds was added to diluted black liquor samples. The dilution ratios were kept constant and only the concentrations for the added standard compounds were changed. In wood black liquors, RSDs for the intra-day, day-to-day, and intermediate precisions ranged from 0.1% to 7.0%. The repeatability values were less than 5% for all the acids in wood black liquor sample matrices with the exception of the  $\alpha$ - and  $\beta$ -glucoisosaccharinic acids, for which the values ranged from 0.3% to 6.7%. The probable reason for these high values was the earlier-mentioned equilibrium between their open and cyclic structures; however, black liquors also contain a minor amount of anhydroisosaccharinic acid [Niemelä and Alén, 1999], which may cause some interference in the determination. The RSDs for intra-day, day-to-day, and intermediate precisions in non-wood black liquors were below 5% for most of the compounds (III). For the 4-hydroxybutanoic

acid, the intermediate precision was over 5%, while intra-day and intermediate precisions were of 5% for the 2-hydroxyglutaric acid, which was probably due to the fact that the concentration values for the 2-hydroxyglutaric acid were very close to those of LOD.

Compared with the results obtained for standard compounds in aqueous media (I), the repeatability values for the HPLC-APCI-MS method were even better for acids analyzed from real samples. In the black liquor samples, the pH varied between 11 and 12, and these relatively strong alkaline conditions probably stabilized, for example, glucoisosaccharinic acids. It was noticed that, after standard addition, the pH decreased to a value as low as 5-6, and part of the  $\alpha$ -glucoisosaccharinic acid was transformed into the corresponding lactone. This transformation was not as fast for  $\beta$ -glucoisosaccharinic acid. The differences in precision values between the various black liquors were negligible.

Precision measurements were performed similarly for the IC-CD method (IV). In aqueous media, the RSDs for the intra-day, day-to-day, and intermediate precisions for standard compounds were less than 5% for all the compounds. In black liquor samples, the intra-day precision values ranged from 0.9% to 5%. Day-to-day and intermediate precision values were less than 5% for all the other compounds except sulfate and thiosulfate. Higher values resulted because of the oxidation reactions of thiosulfate. The variability in the thiosulfate and sulfate results is due in large part to the oxidation of sulfide and thiosulfate, respectively. Sulfide oxidation increases with increasing dissolved oxygen concentration and is catalyzed by quinone-type structures present in lignin, which is dissolved in the black liquor [Sullivan and Douek, 2004].

#### 4.2.6 Application to black liquor samples

The HPLC-APCI-MS and IC-CD methods were applied to determine the actual concentrations of the main carboxylic acids in black liquors. In Appendix V are the results for the softwood and birch black liquors. Measurements were performed by using HPLC-APCI-MS, GC-FID, and IC-CD. When the results of HPLC-APCI-MS were compared with those of IC-CD, some concentration levels seemed to vary to some extent. Basically, this was due to the relative response factors for acid components not being available as standard compounds were assumed to be 1.0 in relation to the internal standard. Also, HPLC-APCI-MS and IC-CD methods were only partially capable of determining the same compounds. The restricting factor was the separation efficiency of the columns. The compounds that were possible to determine with both methods were xyloisosaccharinic, 3,4-dideoxy-pentonic, lactic, 2-hydroxybutanoic acids. The comparison of the absolute

amounts of the glucoisosaccharinic acids was impossible because in the HPLC-APCI-MS method the response factor for  $\beta$ -glucoisosaccharinic was assumed to be 1. The same applies to xyloisosaccharinic, 3,4-dideoxy-pentonic, 4-hydroxybutanoic, 2-hydroxyglutaric, and 2-hydroxy-4-pentenoic acids, which were not available as standard compounds. For 2-hydroxybutanoic and lactic acids, it was possible to determine response factors and these were also detected from the black liquor samples. The concentrations for 2-hydroxybutanoic were similar in wood black liquors but in non-wood samples IC-CD gave systematically higher concentration values. For lactic acid, the obtained concentrations were slightly higher in wood black liquor samples when measured with the HPLC-APCI-MS method. Because the samples were obtained from laboratory-made cooks, the actual concentrations were not known and it was not possible to compare the obtained results with actual values. It should be mentioned that the time between these measurements was over two years and this may also have affected the results. The preservation of the samples should have been studied in more detail and conclusions regarding which method gives more reliable results cannot be drawn without further research.

The disadvantage of these methods was that minor acids could not be determined. This was mainly due to the concentrations for the major acids being high and the samples needing to be diluted. Without dilution, the separation capacity of the column was insufficient and major compounds eluted as a group or peak shapes tailed. However, the aim of this work was to determine the main acids, and both methods fulfilled this requirement.

### **4.3 Laboratory-made cooks and monitoring of kraft pulping**

The main aim was to resolve to a sufficient extent the complex mixture of aliphatic carboxylic acids into single components using the HPLC-APCI-MS and IC-CD methods. Since most of the significant degradation products can be measured simultaneously, a single chromatographic run gives fingerprint data for monitoring the progress of delignification. The degradation product analysis method is based on the idea that there is a clear relationship between acid formation and the progress of delignification. The suitability of these methods was tested for monitoring delignification in a series of conventional kraft cooking experiments in which the feedstock material (softwood, hardwood, and wheat straw) was changed. The experiments using the HPLC-APCI-MS method are reported in Paper V. The results obtained with the IC-CD method have not been published before.

The cooking conditions used (effective alkali, sulfidity, maximum cooking temperature, and liquor-to-feedstock ratio) are presented in Table 6. The H-factor range, final kappa number, total yield range, and residual alkali range are presented in Table 8. For softwood, birch, and wheat straw kraft black liquor samples, the initial cooking temperature was 80 °C and the heat-up rate was 1 °C min<sup>-1</sup>. In each cook, 12 H-factor points were chosen and autoclaves were removed from the oil bath and cooled in running cold tap water when the required H-factor target was achieved. Black liquor was separated from the delignified feedstock by pressing in a nylon-woven fabric bag. The black liquor samples were stored in a freezer at -18 °C. Some of the feedstock samples that had a short cooking time (H-factor <200 for hardwoods and H-factor <400 for softwoods) were not sufficiently pulped and thus were not screened and their kappa numbers were not determined. Kappa numbers were determined according to standard SCAN-C 1:00 [SCAN, 2000]. Three replicates of each black liquor sample were analyzed with HPLC-APCI-MS and IC-CD and an average of the measurement results was calculated.

**TABLE 8** Cooking data

Parameter	Softwood	Birch	Wheat straw
H-factor range	4 → 2500	7 → 1030	1 → 97
Final kappa number	20	18	11.5
Total yield range (%)	78 → 43	82 → 51	56 → 45
Residual alkali range (g NaOH L <sup>-1</sup> )	22 → 9	21 → 9	13 → 7.5

The experiments were based on the finding that, instead of using absolute measures of acid contents, practical information can also be obtained by plotting the concentration ratios of the selected acids against the parameters characterizing pulping [Alén *et al.*, 1991; Alén, 1997]. This eliminates the need to measure the absolute concentration (*i.e.*, the use of an internal standard) and simplifies the analysis. In this study, the chromatographic peak areas were used directly to calculate acid ratios and several acid ratios were tested. Examples of the ratios were the sum of the peak areas of the isosaccharinic acids (*i.e.*,  $\alpha$ - and  $\beta$ -glucoisosaccharinic and xyloisosaccharinic acids) divided by the peak area of lactic acid for wood black liquors or by the peak area of 3,4-dideoxy-pentonic acid for non-wood black liquors.

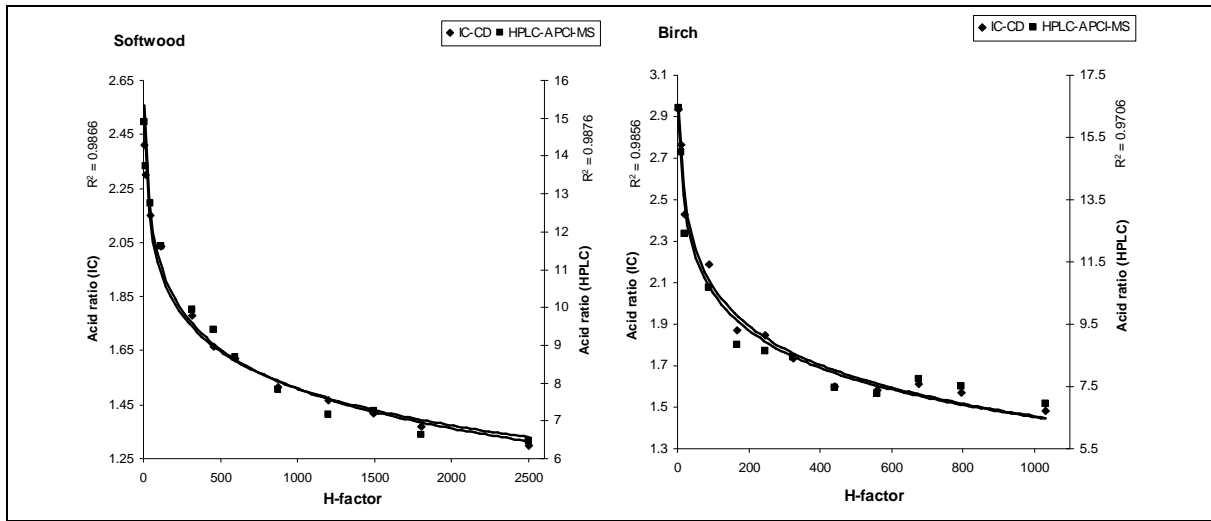
It was noticed earlier that the mass spectrometer was sensitive to changes and the peak areas varied from day to day. Also, the signal levels changed after the maintenance cleaning, tuning, and calibration of the mass spectrometer. In the method development stage, the method validation procedures were performed by using an internal standard and, with the experiments based on acid ratios, the internal standard was eliminated. Therefore, the precision of the acid ratios was evaluated by performing repeated analyses. The sample size for intra-day

precisions was six and all the samples were analyzed during three different days. The acid ratios were also changed (by adding  $\alpha$ -glucoisosaccharinic and lactic acids) and repeated analyses were made. These experiments were carried out to study whether changes in acid concentrations have an effect on the acid ratio. Precision measurement procedures were carried out for seven black liquor types. The results were calculated as an average of these experiments and are presented in Table 9. The RSDs for the intra-day, day-to-day, and intermediate precisions for both acid ratios were less than 5% in all the samples. It was concluded that the decrease (or increase) in signal intensity in the mass spectrometer was systematical and did not affect the acid ratios. The acid ratios were also stable regardless of the concentration of the single acid components.

**TABLE 9** RSDs (%) of the analysis of acid ratios for intra-day (A,  $n = 6$ ), day-to-day (B,  $n_1 = 3$ ), and intermediate precision (C)

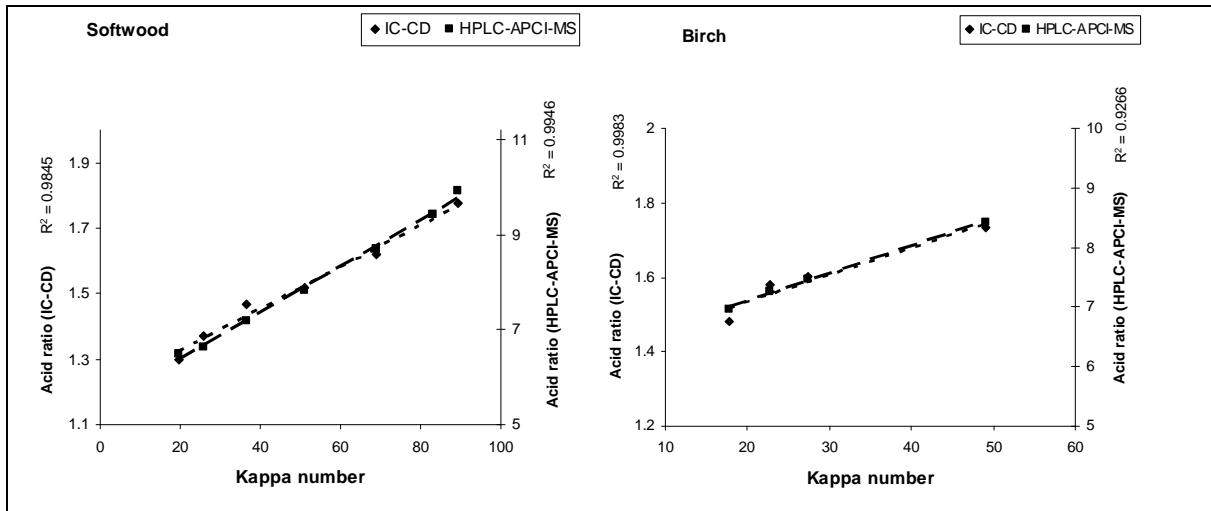
Compound	A	B	C	A	B	C
	Isosaccharinic acids/ lactic acid			Isosaccharinic acids/ 3,4-dideoxy-pentonic acid		
HPLC-APCI-MS						
Softwood	1.6	0.8	1.9	2.3	2.5	3.6
Birch	2.4	1.2	2.7	3.2	1.1	3.2
Aspen	1.8	3.2	3.7	1.9	1.9	2.9
Bamboo kraft	2.0	0.8	2.0	1.6	0.5	1.6
Bamboo soda-AQ	2.8	0.9	2.8	3.1	1.1	2.9
Wheat straw kraft	2.9	2.3	3.8	3.2	1.5	3.5
Wheat straw soda-AQ	4.2	0.7	4.5	4.2	1.5	4.2
IC-CD						
Softwood	1.9	0.3	1.8	1.5	0.6	1.8
Birch	1.1	0.7	1.3	2.9	3.8	4.9
Aspen	2.2	0.6	2.1	2.8	1.1	3.0
Bamboo kraft	2.4	1.0	2.3	2.1	1.1	2.3
Bamboo soda-AQ	1.9	0.8	2.0	2.5	0.3	2.5
Wheat straw kraft	1.7	0.6	1.7	2.2	1.7	2.9
Wheat straw soda-AQ	2.7	1.0	2.8	4.7	1.2	4.5

It was confirmed (V) that a correlation exists between the formation of certain acids and the H-factor. In these measurements, acid concentrations ( $\text{g L}^{-1}$ ) were used. Acid ratios were measured with both the HPLC-APCI-MS and the IC-CD methods. Figure 14 shows the correlation between the acid ratio and H-factor in the case of softwood (pine and spruce) and hardwood (birch) pulping. Both methods give very similar results and the change in acid ratio in relation to the H-factor is almost identical. The difference between the scales results from the two different detectors. The MS detector gives a different response to the CD and, because internal standard methods were not used, the detector response has a direct effect on the acid ratios.



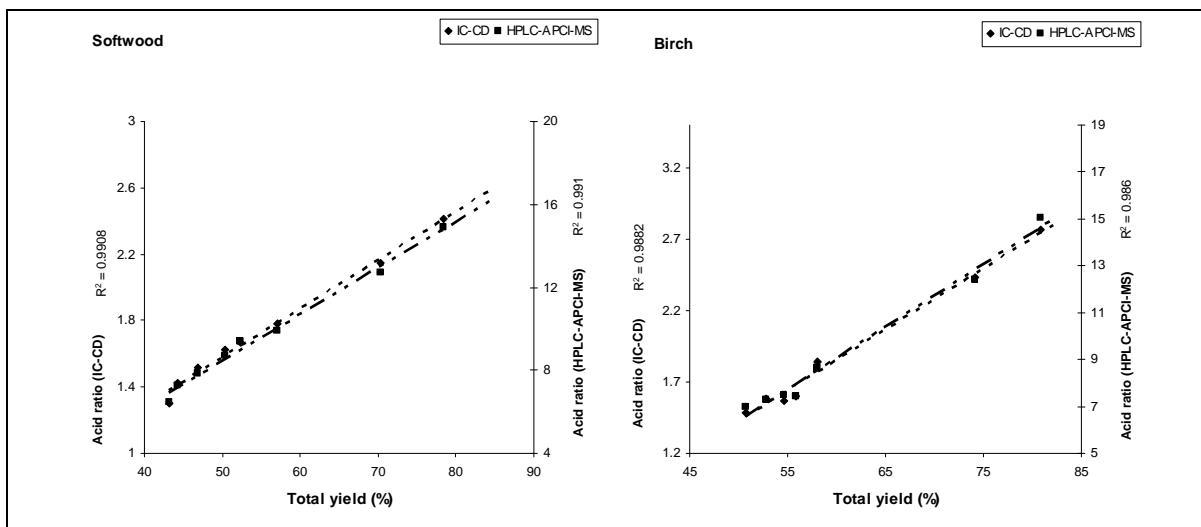
**FIGURE 14** The acid ratio as a function of the H-factor during softwood and hardwood (birch) pulping.

The correlations between the acid ratio and the total yield and kappa number of the pulp were examined. When the same acid ratio as in the H-factor case was used, the linear correlations were obtained. In Figure 15 is presented the correlation of the acid ratio and kappa number and Figure 16 shows the correlation between the acid ratio and total yield (%) in softwood and hardwood pulping. Figure 17 shows the relation between the acid ratio and cooking time. Acid ratios were measured with the HPLC-APCI-MS method and the IC-CD method. In all the cases, a clear relationship was detected and changes in the acid ratio were nearly identical regardless of the analysis method.

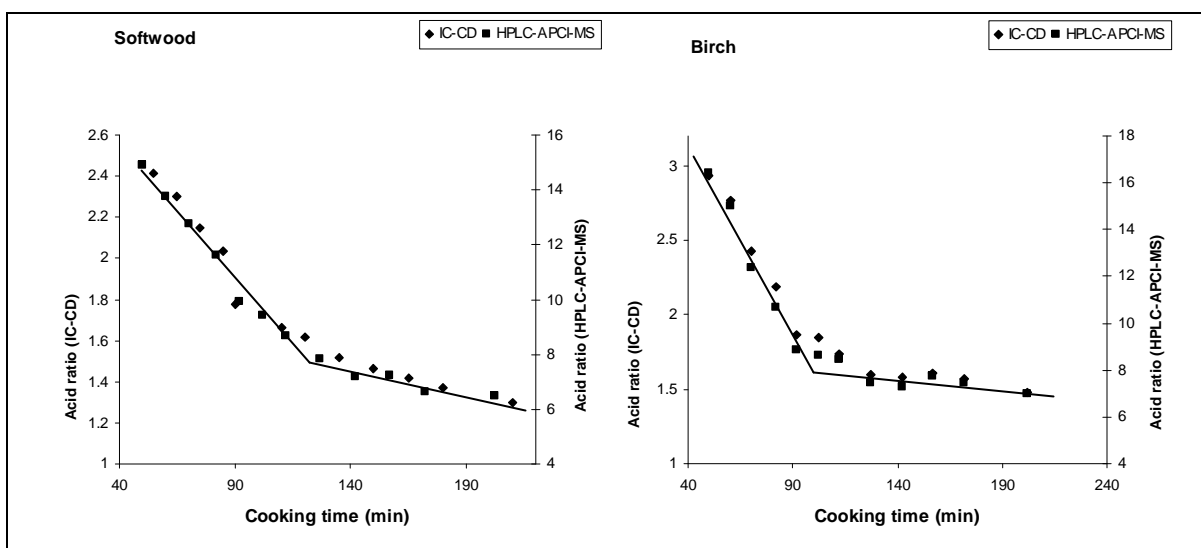


**FIGURE 15** The acid ratio as a function of the kappa number during the softwood and hardwood (birch) pulping.





**FIGURE 16** The acid ratio as a function of the total yield (%) during the softwood and hardwood pulping.



**FIGURE 17** The acid ratio as a function of the cooking time during the softwood and hardwood pulping.

The results clearly indicated that there is a correlation between various parameters that are used to describe the progress of the cooking. Acid ratios were discovered to be an appropriate tool to describe the changes in the acid concentrations. These findings also support the original aim, which was to develop a fast chromatographic analysis method for the main acid components. If the analysis can be performed without standard additions, it is simplified significantly. Modification of the method to an online process analytical tool is easier if samples can be analyzed without standard additions.

## 5 CONCLUDING REMARKS

### 5.1 Summary and conclusions

The results showed that the developed HPLC-APCI-MS, IC-ESI-MS, and IC-CD analysis methods are simple and useful tools for the analysis of LMM (C<sub>2</sub>-C<sub>6</sub>) aliphatic carboxylic acids in alkaline spent liquors. The main benefits of the methods are simplicity of use, absence of the multistage sample treatment, and rapidity of measurement. These methods create a basis for an online analysis application, which can be used for monitoring the alkaline delignification process.

Proposed monitoring method for the alkaline delignification process is based on the systematic formation of these acid by-products, which are the main alkaline degradation products of feedstock carbohydrates. The suitability of the HPLC-APCI-MS and IC-CD methods for the monitoring of delignification was investigated. The results indicated that the progress of delignification can be monitored by the fast chromatographic methods developed in this study. These methods are capable of generating useful data on both wood and non-wood pulping. It was found that acid ratios *i.e.*, the sum of the peak areas of the isosaccharinic acids ( $\alpha$ - and  $\beta$ -glucoisosaccharinic and xyloisosaccharinic acids) divided by the peak area of lactic acid for wood black liquors or by the peak area of 3,4-dideoxy-pentonic acid for non-wood black liquor correlated with reasonable accuracy with the progress of the delignification. The use of an acid ratio eliminates the difficulty of measuring the absolute concentration (*i.e.*, the use of an internal standard), and means that chromatographic peak areas, being proportional to the concentrations, could be used directly. The use of the acid ratio instead of absolute amounts simplifies the analysis process significantly and facilitates method deployment in online applications.

The acid ratios were linked to the H-factors, total yields, cooking times, and kappa numbers in laboratory-scale cooks of different wood and non-wood feedstocks (birch, softwood, and wheat straw). The results of preliminary tests carried out with kraft black liquors from the conventional laboratory-scale cooking looked promising: the analytical data correlate well with the progress of the delignification, and the cooking time can be optimized in real time with reasonable accuracy. It was also found that the acid ratio was stable and changes in analysis conditions had little or no impact on it.

The main contribution of this research was to develop the fast chromatographic analysis methods so they can be used as a good basis for online analysis in industrial applications. This research also supports and continues earlier researches, in which have been presented innovative approaches to control delignification process and combustion of black liquors by using an acid ratio model.

## 5.2 Suggestions for the future research

The chromatographic analysis methods developed in this study offer a tool for monitoring the alkaline delignification process based on the systematic formation of acid by-products. However, these methods can be further improved and simplified. Automatic dilution and filtration systems are commercially available and should be tested with these methods. A process sample pretreatment in online introduction to the HPLC-APCI-MS and IC-CD instruments need to be studied by building up a dilution and filtration apparatus first in the laboratory environment. Before transferring the analysis methods to mill-scale, long term laboratory tests should be carried out. In addition, an online sample clean-up method based on the sample enrichment technique is also an interesting option for an online sample pretreatment. The basic idea behind this method is that analytes are first trapped into the precolumn and with automatic back flushing ejected into the detector. Furthermore, it seems possible to shorten the analysis time by focusing only on certain acid components (*e.g.*, isosaccharinic, lactic, and 3,4-dideoxy-pentonic acids). In those cases, where components have different  $m/z$  values, separation can be carried out within the MS in the SIM mode. Flow injection analysis without using a column will take approximately 2 minutes. When a conductivity detector is deployed, the analysis without a column is not possible. However, the analysis time with CD can be shortened by modifying the gradient program. Acid components that have been used to calculate the acid ratios eluted within 10 minutes.

For full-scale utilization, more experiments need to be made to validate the "acid ratio model". More detailed data should be collected on the correlation between the acid ratios and the progress of delignification. Also more information, especially about the effect of variations in the process conditions on the acid concentration ratios is still needed. In addition to laboratory-made cooks, samples from the pulp mills should be included and the methods developed should be tested in mill-scale processes (batch and continuous cooking). An interesting research area is oxygen-alkali delignification. LMM acids are formed during oxygen-alkali delignification and the suitability of the methods developed should be tested for the oxygen-alkali delignification process.

Earlier results have shown that even small changes in the chemical composition of black liquor may considerably influence the burning behavior of black liquor. Fast chromatographic analysis methods developed in this study facilitate the analysis of LMM acids and make the analysis fast and easy. These methods could be utilized to find a correlation between the combustion properties and black liquor acid composition. An online analysis method might be a useful tool also for the optimization of black liquor combustion process.

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## APPENDICES

APPENDIX I a: Examples of proposed reaction paths for the formation of the main reaction products of hexosans (cellulose and glucomannan) during oxygen delignification.

APPENDIX I b: Examples of proposed reaction paths for the formation of the main reaction products of pentosans (xylan) during oxygen delignification.

APPENDIX I c: Examples of proposed degradation routes of intermediate end groups in alkaline oxidation of cellulose.

APPENDIX I d: Examples of proposed degradation routes of intermediate end groups in alkaline oxidation of xylan.

APPENDIX II: Carboxylic acids used in this study and their structures.

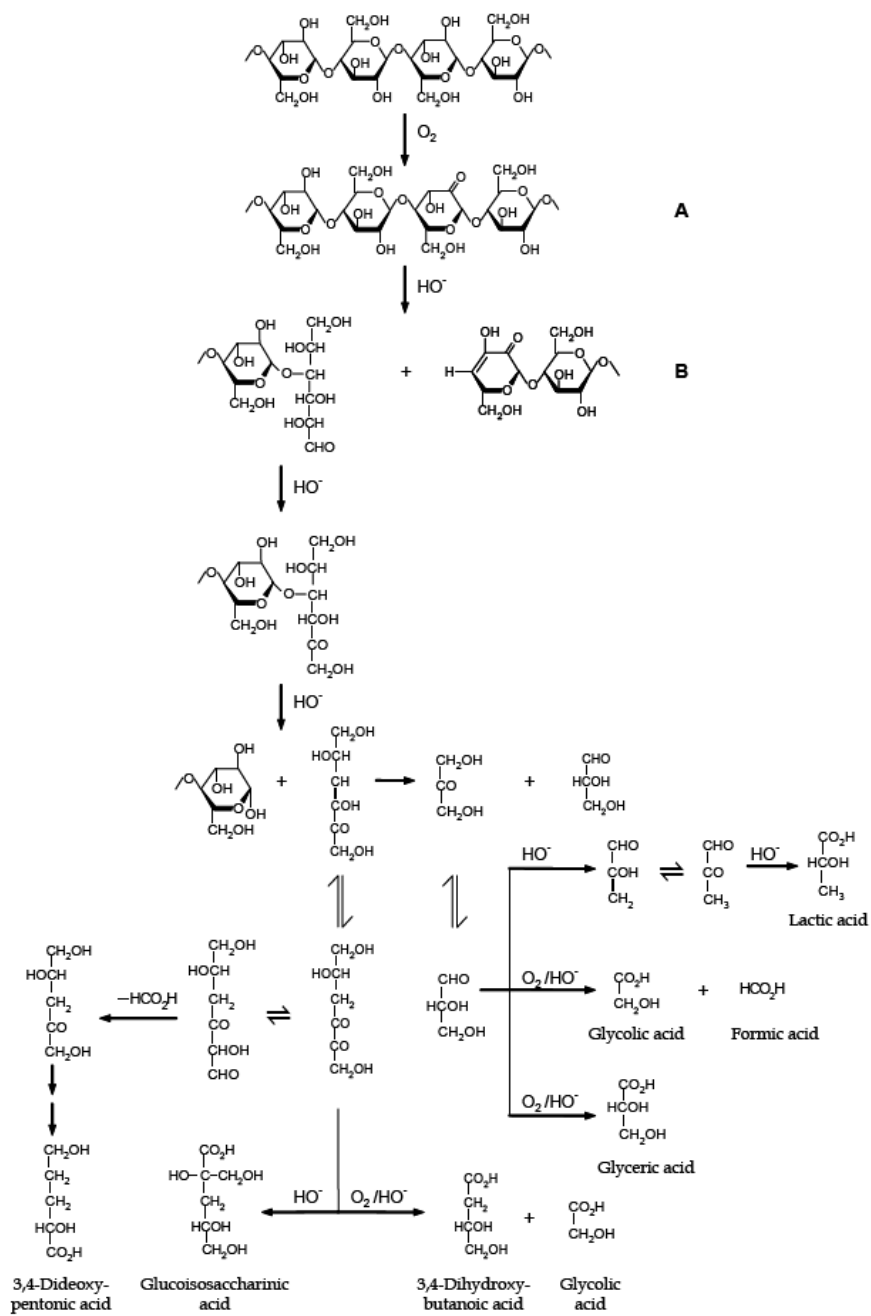
APPENDIX III: Lists of chemicals, materials, instruments, and columns used in this study.

APPENDIX IV: Single factor ANOVA test results.

APPENDIX V: Acid concentrations in softwood and birch black liquors.

## APPENDIX I a

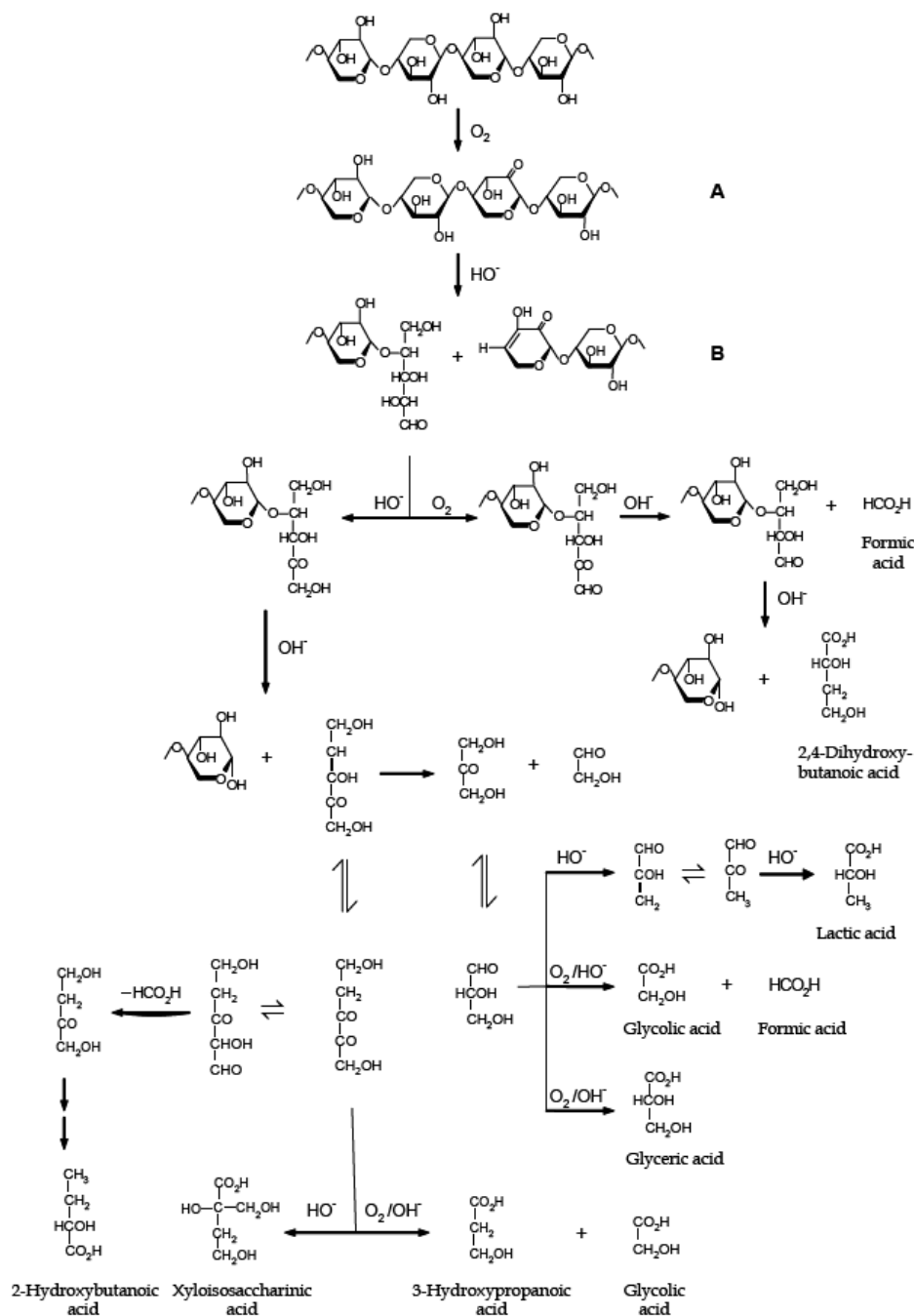
Examples of proposed reaction paths for the formation of the main reaction products of hexosans (cellulose and glucomannan) during oxygen delignification. Reprinted from the doctoral dissertation of Salmela [2007] with permission. Original references: [Ericsson *et al.*, 1973; Löwendahl and Samuelson, 1974; Malinen, 1974; 1975; Sjöström, 1993].





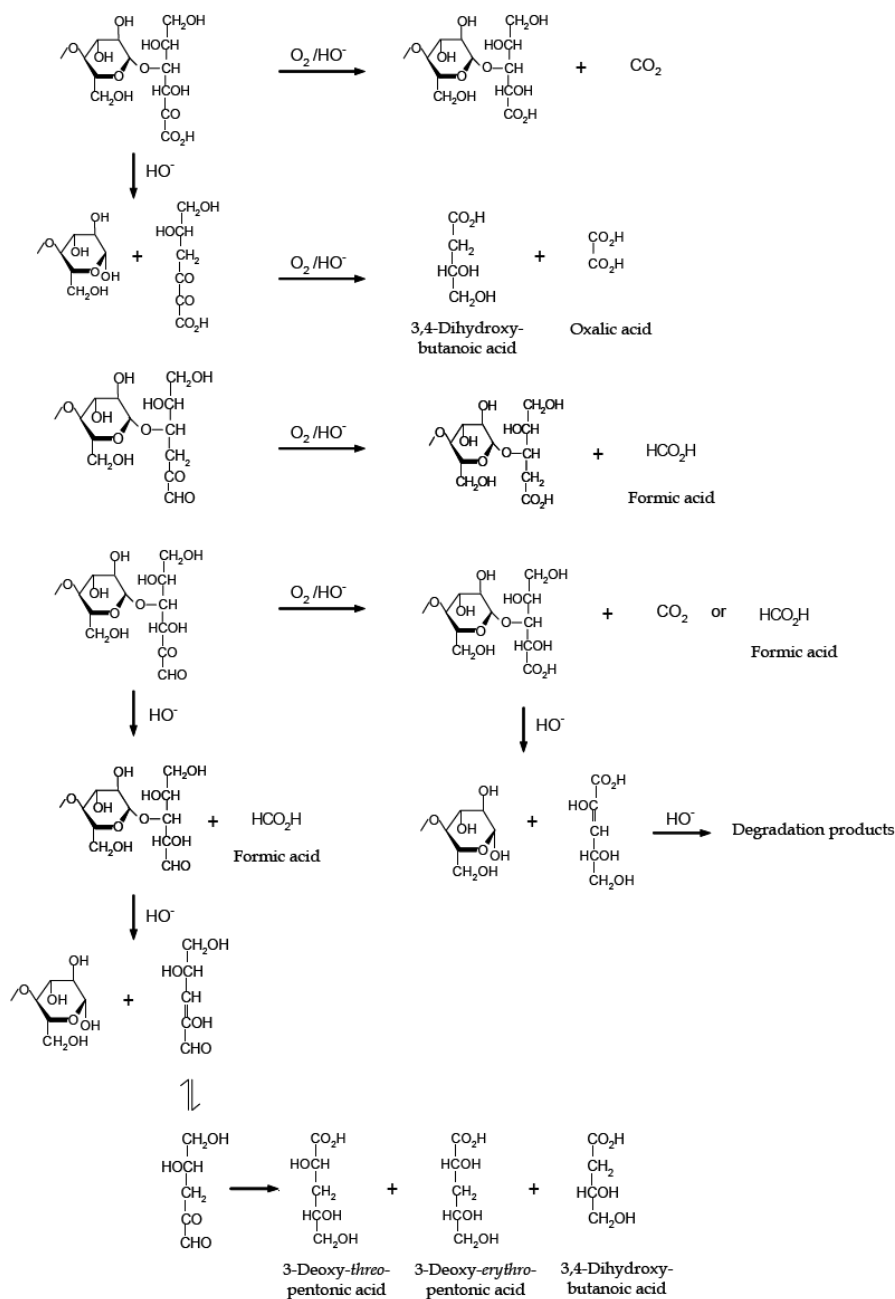
## APPENDIX I b

Examples of proposed reaction paths for the formation of the main reaction products of pentosans (xylan) during oxygen delignification. Reprinted from the doctoral dissertation of Salmela [2007] with permission. Original references: [Kolmodin and Samuelson, 1971; 1973; Malinen 1974; 1975; Sjöström, 1993].



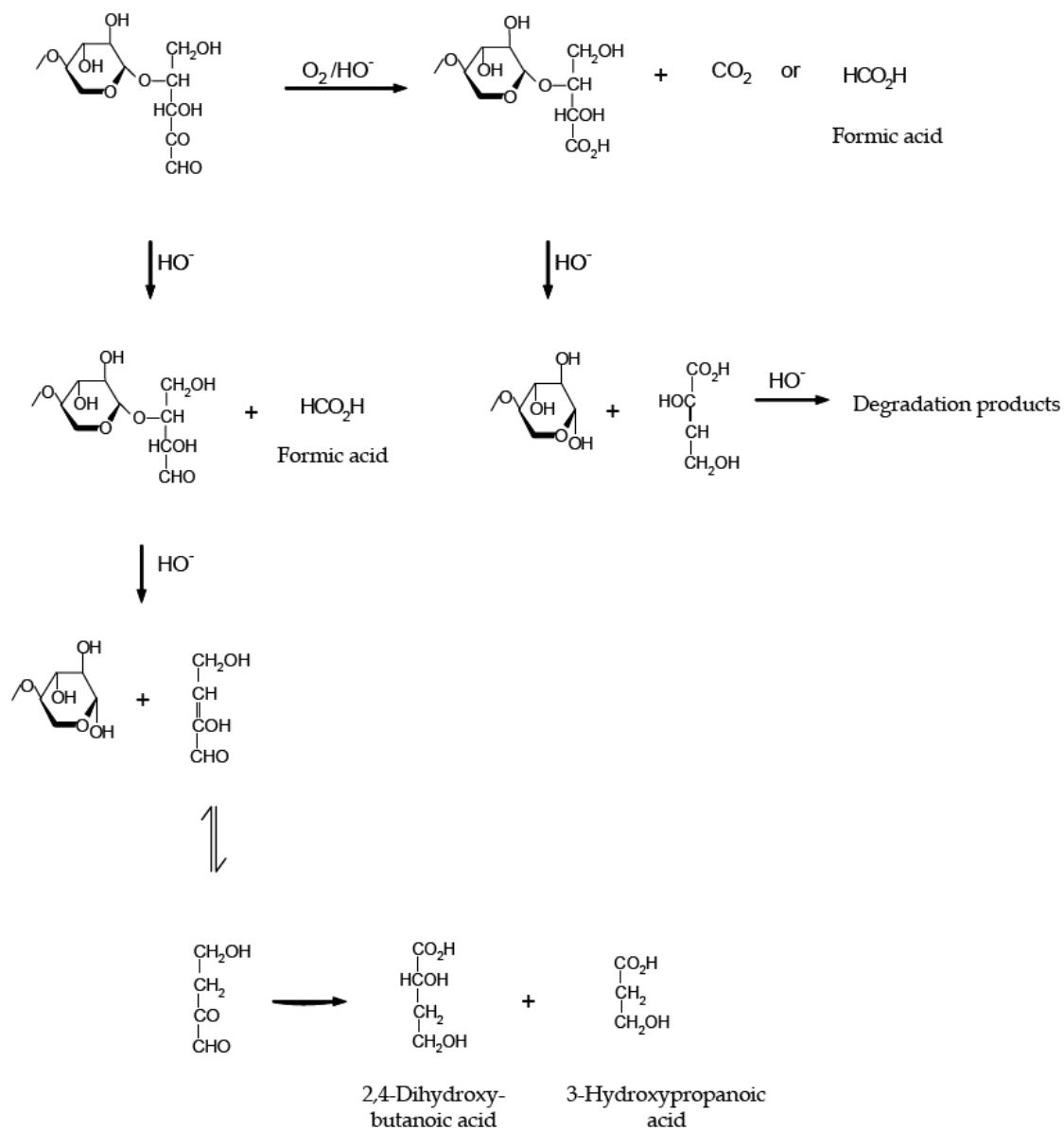
## APPENDIX I c

Examples of proposed degradation routes of intermediate end groups in alkaline oxidation of cellulose. Reprinted from the doctoral dissertation of Salmela [2007] with permission. Original references: [Kolmodin and Samuelson, 1972; Ericsson *et al.*, 1973; Löwendahl and Samuelson, 1974; Malinen, 1974; 1975].



## APPENDIX I d

Examples of proposed degradation routes of intermediate end groups in alkaline oxidation of xylan. Reprinted from the doctoral dissertation of Salmela [2007] with permission. Original references: [Malinen, 1974; 1975].



## APPENDIX II

### Carboxylic acids used in this study and their structures

Compound	Chemical structure
Acetic acid	$\text{HO}_2\text{C}-\text{CH}_3$
Adipic acid <sup>a</sup>	$\text{HO}_2\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CO}_2\text{H}$
Chloroacetic acid <sup>a</sup>	$\text{HO}_2\text{C}-\text{CH}_2\text{Cl}$
2-Deoxy-tetronic acid	$\text{HO}_2\text{C}-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2(\text{OH})$
3-Deoxy-tetronic acid	$\text{HO}_2\text{C}-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}_2(\text{OH})$
3,4-Dideoxy-pentonic acid	$\text{HO}_2\text{C}-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}_2-\text{CH}_2\text{OH}$
Formic acid	$\text{HCO}_2\text{H}$
Glucosaccharinic acid	$\text{HO}_2\text{C}-\text{C}(\text{OH})(\text{CH}_2\text{OH})-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2\text{OH}$
Glutaric acid <sup>a</sup>	$\text{HO}_2\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CO}_2\text{H}$
Glycolic acid	$\text{HO}_2\text{C}-\text{CH}_2\text{OH}$
2-Hydroxybutanoic acid	$\text{HO}_2\text{C}-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}_3$
4-Hydroxybutanoic acid	$\text{HO}_2\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_2\text{OH}$
2-Hydroxyglutaric acid	$\text{HO}_2\text{C}-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}_2-\text{CO}_2\text{H}$
2-Hydroxy-2-methylbutanoic acid	$\text{HO}_2\text{C}-\text{C}(\text{OH})(\text{CH}_3)-\text{CH}_2-\text{CH}_3$
2-Hydroxy-4-pentenoic acid	$\text{HO}_2\text{C}-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}=\text{CH}_2$
3-Hydroxypropanoic acid	$\text{HO}_2\text{C}-\text{CH}_2-\text{CH}_2\text{OH}$
Lactic acid	$\text{HO}_2\text{C}-\text{CH}(\text{OH})-\text{CH}_3$
Maleic acid	$\text{HO}_2\text{C}-\text{CH}=\text{CH}-\text{CO}_2\text{H}$
Malic acid	$\text{HO}_2\text{C}-\text{CH}(\text{OH})-\text{CH}_2-\text{CO}_2\text{H}$
Methylsuccinic acid	$\text{HO}_2\text{C}-\text{CH}(\text{CH}_3)-\text{CH}_2-\text{CO}_2\text{H}$
Oxalic acid	$\text{HO}_2\text{C}-\text{CO}_2\text{H}$
Succinic acid	$\text{HO}_2\text{C}-\text{CH}_2-\text{CH}_2-\text{CO}_2\text{H}$
Xyloisaccharinic acid	$\text{HO}_2\text{C}-\text{C}(\text{OH})(\text{CH}_2\text{OH})-\text{CH}_2-\text{CH}_2\text{OH}$

<sup>a</sup> used as an internal standard

## APPENDIX III

### Chemicals and materials used in this study

Chemical/material	Manufacturer/supplier	Purpose	Paper
$\alpha$ -Glucosaccharino-1,4-lactone	Laboratory made	standard	I-III, IV
Formic acid	Riedel de Haën	eluent, standard	I-V
Oxalic acid	Riedel de Haën	standard	I-III, IV
Succinic acid	Riedel de Haën	standard	I-IV
Glycolic acid	Aldrich	standard	I-IV
2-Hydroxy-2-methylbutanoic acid	Aldrich	standard	I-III
Methylsuccinic acid	Aldrich	standard	I-IV
Maleic acid	Merck	standard	I-III
DL-malic acid	Merck	standard	I-IV
Glutaric acid	Merck	internal standard	I-III, V
Trifluoroacetic acid (TFA)	Fluka	eluent	I
Adipic acid	Fluka	internal standard	I-III
Fumaric acid	Fluka	internal standard	I
2-Hydroxybutanoic acid	TCI Europe	standard	I-IV
Lactic acid	BDH Chemicals	standard	I-IV
Methanol	Rathburn, J.T. Baker	eluent	I-III, V
Membrane filter (0.2 $\mu$ m), ME24	Schleicher & Schuell	filtration	I-IV
BSTFA (1 % TMCS)	Regis	derivatization	II, III
Pyridine	Riedel de Haën	solvent	II, III
Xylitol	Fluka	internal standard	II, III
NaOH	J.T. Baker	eluent	IV
KOH (EGC II KOH cartridge)	Dionex	eluent	IV
Chloroacetic acid	J.T. Baker	internal standard	IV
Sodium thiosulfate	Fluka	standard	IV
Sodium chloride	Merck	standard	IV
Acetic acid	Merck	eluent/standard	I, IV
Amberlite IRC-50 (NH <sub>4</sub> <sup>+</sup> -form)	Fluka	cation-exchange resin	II, III
Nylon filter (0.45 $\mu$ m)	Rotilabo	filtration	IV

### List of instruments and columns used in this study

Instrument	Manufacturer	Model	Data collection and processing		Paper
HPLC-APCI-MS	Hewlett Packard	HP1100	HP ChemStation (A06.03)		I-III, V
GC-FID	Agilent	GC6850	HP ChemStation (A06.03)		II, III
GC-MS	Agilent	GC6890, MS5973	HP ChemStation (A06.03)		II, III
IC-ESI-MS	Hewlett Packard	HP1100	HP ChemStation (A06.03)		IV
IC-CD	Dionex	IC25	Dionex Chromeleon (6.50)		IV
Column	Column type	Dimensions	Particle size	Manufacturer	Paper
PRP-X300 column	analytical	250 mm $\times$ 2.1 mm	7 $\mu$ m	Hamilton	I
PRP-X300 column	guard	-	-	Hamilton	I
Fast Fruit Juice column	analytical	150 mm $\times$ 7.8 mm	7 $\mu$ m	Waters	I
SB-C <sub>18</sub>	analytical	150 mm $\times$ 3.0 mm	5 $\mu$ m	Agilent	I
SB-C <sub>18</sub>	analytical	250 mm $\times$ 3.0 mm	5 $\mu$ m	Agilent	I
Atlantis dC <sub>18</sub>	analytical	250 mm $\times$ 4.6 mm	5 $\mu$ m	Waters	I-III, V
Atlantis dC <sub>18</sub>	guard	20 mm $\times$ 4.6 mm	5 $\mu$ m	Waters	I-III, V
HP-5	analytical	30 m $\times$ 0.32 mm	0.25 $\mu$ m <sup>a</sup>	J&W Scientific	II, III
IonPac ATC-1	anion trap	30 mm $\times$ 0.32 mm		Dionex	IV
IonPac AS 11-HC	analytical	250 mm $\times$ 4.0 mm	9 $\mu$ m	Dionex	IV
IonPac AS 11-HC	guard	24 mm $\times$ 9.0 mm	9 $\mu$ m	Dionex	IV

<sup>a</sup>Film thickness

## APPENDIX IV

Single factor ANOVA test results. Data in columns are: 1=softwood, 2=birch, 3=aspen, 4=bamboo kraft, 5=bamboo soda-AQ, 6=wheat straw kraft, and 7=wheat straw soda-AQ.

HPLC-APCI-MS		Oxalic acid				
Anova: Single Factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
Column 1	10	1011.12	101.11	4.83		
Column 2	10	991.06	99.11	4.77		
Column 3	10	1017.73	101.77	6.95		
Column 4	10	1006.00	100.60	10.56		
Column 5	10	988.80	98.88	35.85		
Column 6	10	990.37	99.04	103.96		
Column 7	10	1006.34	100.63	20.33		
ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	79.36	6.00	13.23	0.49	0.81	2.25
Within Groups	1685.32	63.00	26.75			
Total	1764.68	69.00				

HPLC-APCI-MS		Glucosiosaccharinic acid				
Anova: Single Factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
Column 1	10	981.80	98.18	16.12		
Column 2	10	979.36	97.94	19.30		
Column 3	10	1000.01	100.00	4.92		
Column 4	10	999.94	99.99	4.71		
Column 5	10	1012.05	101.20	6.24		
Column 6	10	1043.89	104.39	29.80		
ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	278.02	5.00	55.60	4.11	0.00	2.39
Within Groups	729.76	54.00	13.51			
Total	1007.79	59				

HPLC-APCI-MS		Lactic acid				
Anova: Single Factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
Column 1	10	979.74	97.97	3.67		
Column 2	10	996.02	99.60	2.30		
Column 3	10	990.56	99.06	10.07		
Column 4	10	980.36	98.04	29.12		
Column 5	10	1086.43	108.64	52.94		
Column 6	10	1024.40	102.44	32.47		
Column 7	10	959.31	95.93	41.09		
ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	1058.23	6.00	176.37	7.19	0.00	2.25
Within Groups	1545.05	63.00	24.52			
Total	2603.28	69.00				

IC-CD		Oxalic acid				
Anova: Single Factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
Column 1	10	1028.30	102.83	37.95		
Column 2	10	979.67	97.97	15.53		
Column 3	10	999.79	99.98	24.73		
Column 4	10	993.93	99.39	11.24		
Column 5	10	1003.96	100.40	16.14		
Column 6	10	998.98	99.90	33.91		
Column 7	10	990.46	99.05	20.65		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	135.54	6.00	22.59	0.99	0.44	2.25
Within Groups	1441.38	63.00	22.88			
Total	1576.91	69.00				

IC-CD		Glucosiosaccharinic acid				
Anova: Single Factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
Column 1	10	1016.97	101.70	15.50		
Column 2	10	999.28	99.93	5.00		
Column 3	10	1002.94	100.29	2.17		
Column 4	10	988.72	98.87	16.52		
Column 5	10	1008.91	100.89	14.38		
Column 6	10	1014.58	101.46	13.16		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	55.21	5.00	11.04	0.99	0.43	2.39
Within Groups	600.57	54.00	11.12			
Total	655.778	59				

IC-CD		Lactic acid				
Anova: Single Factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
Column 1	10	1035.44	103.54	29.28		
Column 2	10	976.04	97.60	78.95		
Column 3	10	1024.89	102.49	52.61		
Column 4	10	993.34	99.33	45.45		
Column 5	10	1016.89	101.69	105.59		
Column 6	10	967.00	96.70	61.04		
Column 7	10	1017.55	101.76	29.30		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	403.71	6.00	67.28	1.17	0.33	2.25
Within Groups	3620.01	63.00	57.46			
Total	4023.72	69.00				

**HPLC-APCI-MS**                      **2-Hydroxybutanoic acid****Anova: Single Factor****SUMMARY**

Groups	Count	Sum	Average	Variance
Column 1	10	996.54	99.65	1.59
Column 2	10	999.70	99.97	4.75
Column 3	10	994.27	99.43	6.22
Column 4	10	976.92	97.69	16.22
Column 5	10	1034.66	103.47	27.19
Column 6	10	1140.75	114.07	31.27
Column 7	10	1098.09	109.81	47.25

**ANOVA**

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	2291.72	6.00	381.95	19.88	0.00	2.25
Within Groups	1210.37	63.00	19.21			
<b>Total</b>	<b>3502.09</b>	<b>69.00</b>				

**HPLC-APCI-MS**                      **Methylsuccinic acid****Anova: Single Factor****SUMMARY**

Groups	Count	Sum	Average	Variance
Column 1	10	969.31	96.93	1.64
Column 2	10	989.57	98.96	1.66
Column 3	10	990.53	99.05	0.61
Column 4	10	1000.55	100.06	0.77
Column 5	10	998.76	99.88	1.28
Column 6	10	972.09	97.21	1.77
Column 7	10	985.23	98.52	2.93

**ANOVA**

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	87.82	6.00	14.64	9.61	0.00	2.25
Within Groups	95.97	63.00	1.52			
<b>Total</b>	<b>183.79</b>	<b>69.00</b>				

**IC-CD**                                      **2-Hydroxybutanoic acid****Anova: Single Factor****SUMMARY**

Groups	Count	Sum	Average	Variance
Column 1	10	1023.34	102.33	7.60
Column 2	10	984.31	98.43	13.63
Column 3	10	1017.16	101.72	9.73
Column 4	10	995.21	99.52	3.83
Column 5	10	1007.96	100.80	23.21
Column 6	10	993.81	99.38	59.67
Column 7	10	991.06	99.11	43.56

**ANOVA**

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	126.62	6.00	21.10	0.92	0.49	2.25
Within Groups	1451.07	63.00	23.03			
<b>Total</b>	<b>1577.70</b>	<b>69.00</b>				

**IC-CD**                                      **Methylsuccinic acid****Anova: Single Factor****SUMMARY**

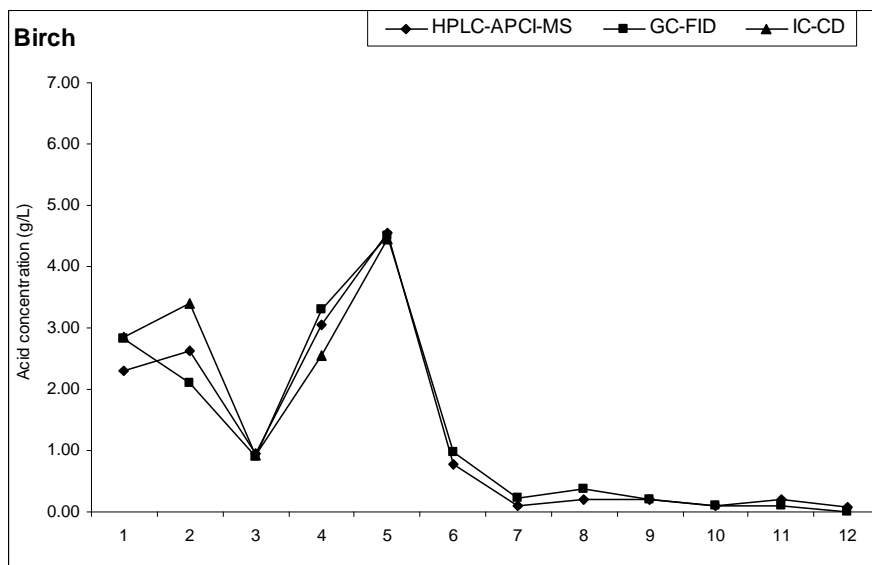
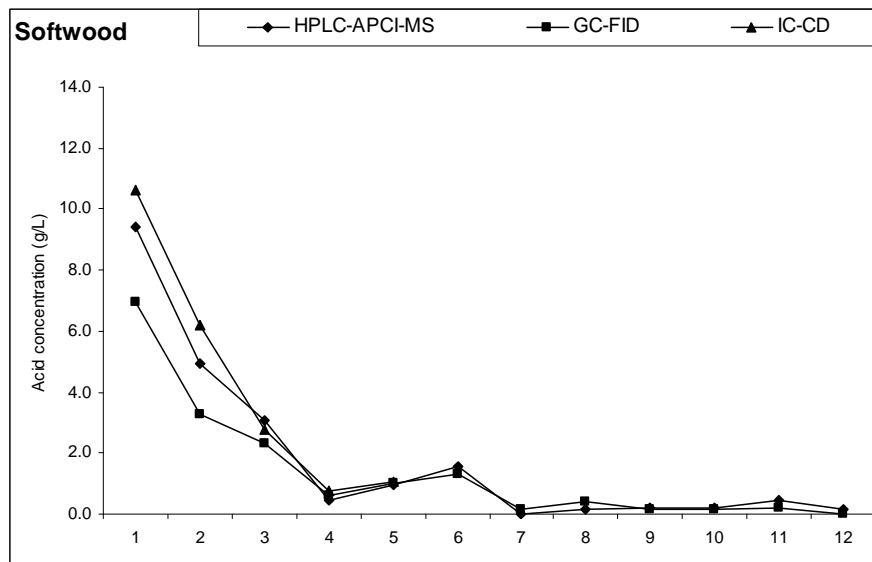
Groups	Count	Sum	Average	Variance
Column 1	10	1033.32	103.33	80.31
Column 2	10	970.86	97.09	65.09
Column 3	10	1018.61	101.86	50.07
Column 4	10	994.73	99.47	95.85
Column 5	10	1044.45	104.45	98.32
Column 6	10	997.24	99.72	69.44
Column 7	10	979.62	97.96	52.48

**ANOVA**

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	451.72	6.00	75.29	1.03	0.41	2.25
Within Groups	4604.08	63.00	73.08			
<b>Total</b>	<b>5055.80</b>	<b>69.00</b>				

## APPENDIX V

Acid concentrations in softwood and birch black liquors. Measurements were performed by using HPLC-APCI-MS, GC-FID, and IC-CD. Carboxylic acids:  $\alpha$ - and  $\beta$ -glucoisosaccharinic (1), lactic (2), 3,4-dideoxy-pentonic (3), xyloisosaccharinic (4), 2-hydroxybutanoic (5), glycolic (6), malic (7), 2-hydroxyglutaric (8), succinic (9), 4-hydroxybutanoic (10), 2-hydroxy-4-pentenoic (11), and methylsuccinic (12) acids.





## PAPER I

<https://doi.org/10.1002/jssc.200600106>

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## PAPER II

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### PAPER III

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## PAPER IV

<https://doi.org/10.1016/j.chroma.2008.02.096>

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## PAPER V

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