Lappeenranta University of Technology

Faculty of Technology

Master's Degree Programme in Chemical and Process Engineering

Sandeep Sharma

DETERMINATION OF SUGARS AND ORGANIC ACIDS IN PULP SAMPLES BY CAPILLARY ELECTROPHORESIS WITH UV DETECTION

Master of Science Thesis

Supervisor: Professor Heli Sirén

Examiner: Maaret Paakkunainen

ABSTRACT

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73 pages, 33 figures, 20 tables, and 9 appendixes

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2010

This MSc work was done in the project of BIOMECON financed by Tekes. The prime target of the research was, to develop methods for separation and determination of carbohydrates (sugars), sugar acids and alcohols, and some other organic acids in hydrolyzed pulp samples by capillary electrophoresis (CE) using UV detection. Aspen, spruce, and birch pulps are commonly used for production of papers in Finland. Feedstock components in pulp predominantly consist of carbohydrates, organic acids, lignin, extractives, and proteins. Here in this study, pulps have been hydrolyzed in analytical chemistry laboratories of UPM Company and Lappeenranta University in order to convert them into sugars, acids, alcohols, and organic acids. Foremost objective of this study was to quantify and identify the main and by-products in the pulp samples.

For the method development and optimization, increased precision in capillary electrophoresis was accomplished by calculating calibration data of 16 analytes such as D-(-)-fructose, D(+)-xylose, D(+)-mannose, D(+)-cellobiose, D-(+)-glucose, D-(+)-

raffinose, D(-)-mannitol, sorbitol, rhamnose, sucrose, xylitol, galactose, maltose, arabinose, ribose, and, α-lactose monohydratesugars and 16 organic acids such as D-glucuronic, oxalic, acetic, propionic, formic, glycolic, malonic, maleic, citric, L-glutamic, tartaric, succinic, adipic, ascorbic, galacturonic, and glyoxylic acid. In carbohydrate and polyalcohol analyses, the experiments with CE coupled to direct UV detection and positive separation polarity was performed in 36 m*M* disodium hydrogen phosphate electrolyte solution. For acid analyses, CE coupled indirect UV detection, using negative polarity, and electrolyte solution made of 2,3 pyridinedicarboxylic acid, Ca^{2+} salt, Mg^{2+} salts, and myristyltrimethylammonium hydroxide in water was used. Under optimized conditions, limits of detection, relative standard deviations and correlation coefficients of each compound were measured.

The optimized conditions were used for the identification and quantification of carbohydrates and acids produced by hydrolyses of pulp. The concentrations of the analytes varied between 1 mg – 0.138 g in liter hydrolysate.

Keywords: capillary electrophoresis, UV detection, sugars, organic acids, pulp samples.

ACKNOWLEDGEMENT

The entire master thesis is dedicated to my late grandfather Kanaiyalal Naudiyal. *"You will always be in our heart."*

First of all, I would like to thank my mother, my father and my lovely sister. Their love and encouragement have carried me through the highs and lows and they have always supported my endeavours. My heartily thanks to my guru Prof. Heli Sirén; she has continued to provide a wealth of knowledge concerning chemistry, her valuable and fruitful discussions and comments leads to achieve me in this position.

I would have never made it alone; my deepest gratitude goes to my examiner Maaret Paakkunainen for her continuous support. With same respect I would like to acknowledge Jaana Ruokonen, Liisa Puro, Helvi Turkia and Anne Marttinen, for technical assistance and helping me throughout my studies.

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My highest appreciation to Anja Kakko and Prof. Mika Mänttäri for provide me pulp samples and sincere thanks to Pekka Teppola for his productive discussion.

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Sandeep Sharma

Lappeenranta, December 01, 2010

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LIST OF SYMBOLS

A	absorbance	mAU
b	absorbance intercept	
c	analyte concentration	$molL^{-1}$
c_i	concentration of a species i	$molL^{-1}$
d	interior diameter of capillary (i.d)	µm
D	diffusion coefficient	cm^2s^{-1}
E	applied electric field	Vcm^{-1}
H	plate height	cm
I_o	initial intensity of light	
I	intensity of light	
I_c	path length of light in solution	m
L_{tot}	capillary total length	cm
L_{det}	capillary detection length	cm
m	slope of regression	
pK_a	acid dissociation constant	
q	ion charge	C
Q_i	amount of species i introduced into the capillary	mol
r	ion radius	cm
r_c	internal radius of capillary	µm
t	time	s
t_{EOF}	migration time of electro-osmotic flow	s
t_m	migration time	s
t_R	migration time of compounds	s
U	voltage	V
v	ion velocity	cms^{-1}

v_{EOF}	velocity of EOF	cms^{-1}
V_{sample}	volume of a sample	cm^3
$w_{h/2}$	temporal peak width at half height of peak	s
x	concentration of sugars	mgL^{-1}

GREEK SYMBOLS

Δv	difference of mobilities of components μ_1 and μ_2	cms^{-1}
$\bar{v}$	mean of migration velocity of components μ_1 and μ_2	cms^{-1}
ΔP	Pressure difference across the capillary	Pa
ΔTA_{mean}	mean value of area occurred on baseline	μm^2
μ_{ep}	electrophoretic mobility	$cm^2V^{-1}s^{-1}$
η	viscosity of solution	$gcm^{-1}s^{-1}$
μ_{eo}	electro-osmotic mobility	$cm^2V^{-1}s^{-1}$
μ_{tot}	total mobility	$cm^2V^{-1}s^{-1}$
ε	dielectric constant	
ζ	zeta potential	V
σ^2	spatial variance	cm^2
Δx	distance between peak centre	cm
μ_1	mobilities of the 1st component	cms^{-1}
μ_2	mobilities of the 2nd component	cms^{-1}
$\bar{\mu}$	Mean of mobilities of 1st and 2nd components	cms^{-1}
μ_i	Mobilities of i^{th} component	cms^{-1}
ε_m	molar absorptivity	m^2mol^{-1}

LIST OF ABBREVIATIONS

ABEE	4-aminobenzoic acid ethyl ester
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BGE	Background electrolyte
CEC	Capillary electrochromatography
CGE	Capillary gel electrophoresis
CIEF	Capillary isoelectric focusing
CITP	Capillary isotachophoresis
CTAB	Cetyl trimethylammonium bromide
CZE	Capillary zone electrophoresis
GC	Gas chromatography
GGM	Glactoglucamannan
HMF	Hydroxymethylfurfural
HPLC	High performance liquid chromatography
LOD	Limit of detection
MEKC	Micellar electrokinetic chromatography
NAD	Nicotinamide adenine dinucleotide
OT-CEC	Open tubular capillary electrochromatography
PDCA	Pyridine-2,6-dicarboxylic acid
PHB	Poly-β-hydroxybutyric acid
RSD	Relative standard deviation
TFA	Trifluoroacetic acid
TMP	Thermo-mechanical pulp
TTAB	Tetradecyl Trimethyl Ammonium Bromide
VRF	Volume reduction factor
UV	Ultra-violet

APPENDICES

1. Introduction

Fermentation is set of chemical reactions that occur in the living organism, whereby breakdown of molecules from e.g. nutrients are transferred into the energy. For basic metabolism living organism needs oxygen, water and food for utilize energy. In the living organism, when the supply of oxygen is low towards the body muscles in that mostly the energy comes from fermentation of glucose and alcohol. This phenomenon is called anaerobic cultivation (without oxygen). Fermentation is supposed to be the first process in the biological history and, most probably, it is the oldest pathway for obtaining energy [1].

In alcoholic fermentation, yeast cell metabolizes sugars, organic acids, and alcohols to obtain energy under anaerobic conditions. In glycolysis, chemical degradation of glucose and lactic acid processes are known to occur [1]. Sugars are the most common substrates of fermentation. Typical examples of fermentation products are ethanol, butyric acid, lactic acid, levulinic acid, formic acid, xylose, and CO_2 [1, 2].

Alcoholic fermentation and glycolysis begin with sugar glucose [1]. As shown in Figure 1 anaerobic energy harvesting mechanism leads to form lactic acids, ethanol, and carbon dioxide. Unlike humans, bacteria and yeast (except lactic acid bacteria, and E. coli in certain conditions) do not ferment glucose to lactate. Instead, they ferment it to ethanol and CO_2 [5].

The process to convert glucose to pyruvate is called glycolysis. Six carbon molecules of glucose are splits into glyceraldehyde-3-phosphates at double cost of adenosine triphosphate (ATP). These molecules are converted to pyruvate creating two ATP fragments per molecule. Two glyceraldehyde-3-phosphates are first broken down and then modified to two pyruvate ions which form four ATP molecules in anaerobic fermentation [4]. The flow chart of glycolysis is a diagram represented in Figure 2. In brief, glycolysis fermentation requires 11 enzymes that degrade glucose to lactic acid. For example the enzymes called lactate dehydrogenase is replaced by one enzyme in glycolysis fermentation as shown in Figure 3 [1].

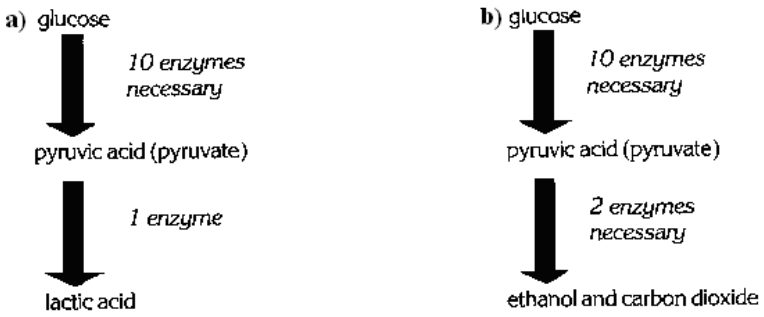


Figure 1. Anaerobic energy-harvesting mechanisms: a) glycolysis and b) alcoholic fermentation [1].

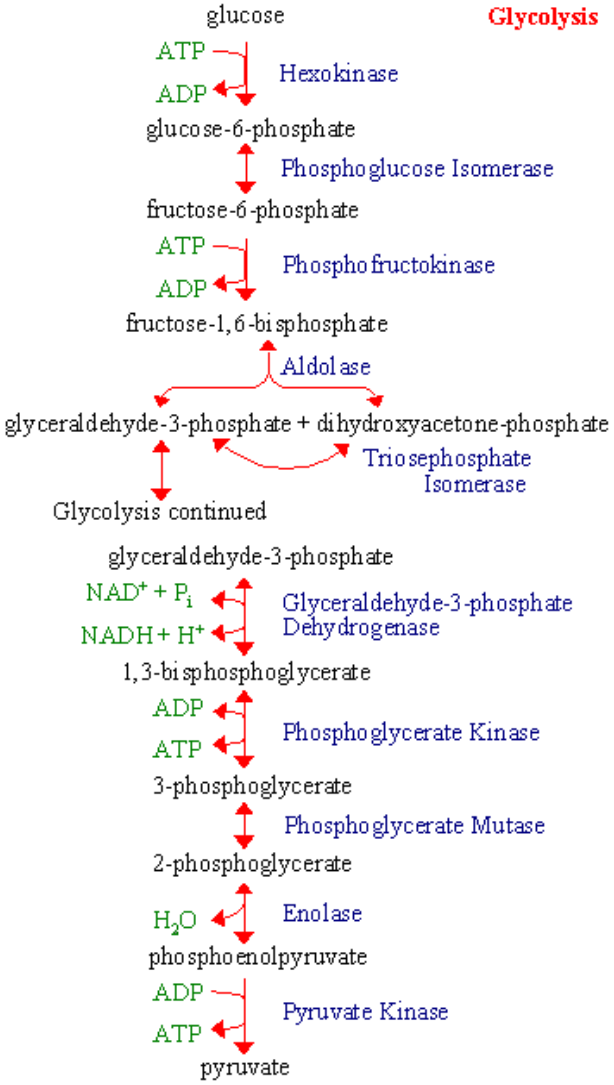


Figure 2. Schematic presentation of glycolysis in presence of pyruvate kinase enzyme [4].

Normally, nicotinamide adenine dinucleotide (NADH) is reoxidized as pyruvate are converted to other compounds (Figure. 3).

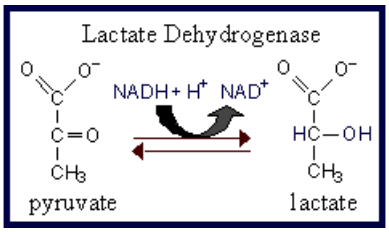


Figure 3. Chemical reaction of pyruvate to lactate under lactate dehydrogenase enzyme [4]

Glycolysis pathway reaction consists of several enzymes like hexokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mustase, enolase, pyruvate kinase, and lactate dehydrogenase [4].

Enzymes are biological catalysts, which enhance the rate of reaction in living organism. In most cases they consist of amino acids, which combine the structure to peptide chains. Such degradation of peptide chains is said to be an anaerobic respiration.

Ethanol plays an important role in an anaerobic process, in which sugars are converted into ethanol and carbon dioxide producing cellular energy (Equation 1).

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \qquad (1)$$

Ethanol is formed from pyruvate in yeast and several other micro-organisms. The first step is decarboxylation of pyruvate (Equation 2). [5]

$$CH_3COCOO^- \xrightarrow{a} CH_3CHO \xrightarrow{b} C_2H_2OH \qquad (2)$$

where

a = H^+ acid added to produce CO_2

b = H^+ added in NADH to produce NAD^+ (Nicotinamide adenine dinucleotide)

The net result of anaerobic process was shown in Equation 3. [5]

$$C_6H_{12}O_6 + 2e^- + 2\ ADP + 2\ H^+ \rightarrow 2C_2H_5OH + 2\ CO_2 + 2\ ATP + 2\ H_2O \qquad (3)$$

Note: It is essential to know that NADH and NAD^+ will not appear in this above equation. Acetaldehyde reduces to ethanol in order to regenerate NAD^+ in reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase. Hence, there is no net oxidation reaction occurred in conversion of glucose into ethanol. [5]

According to Ligor et al. research, scheme of biosynthesis of biacetylene and other useful products were presented and shown in Figure 4. [9]

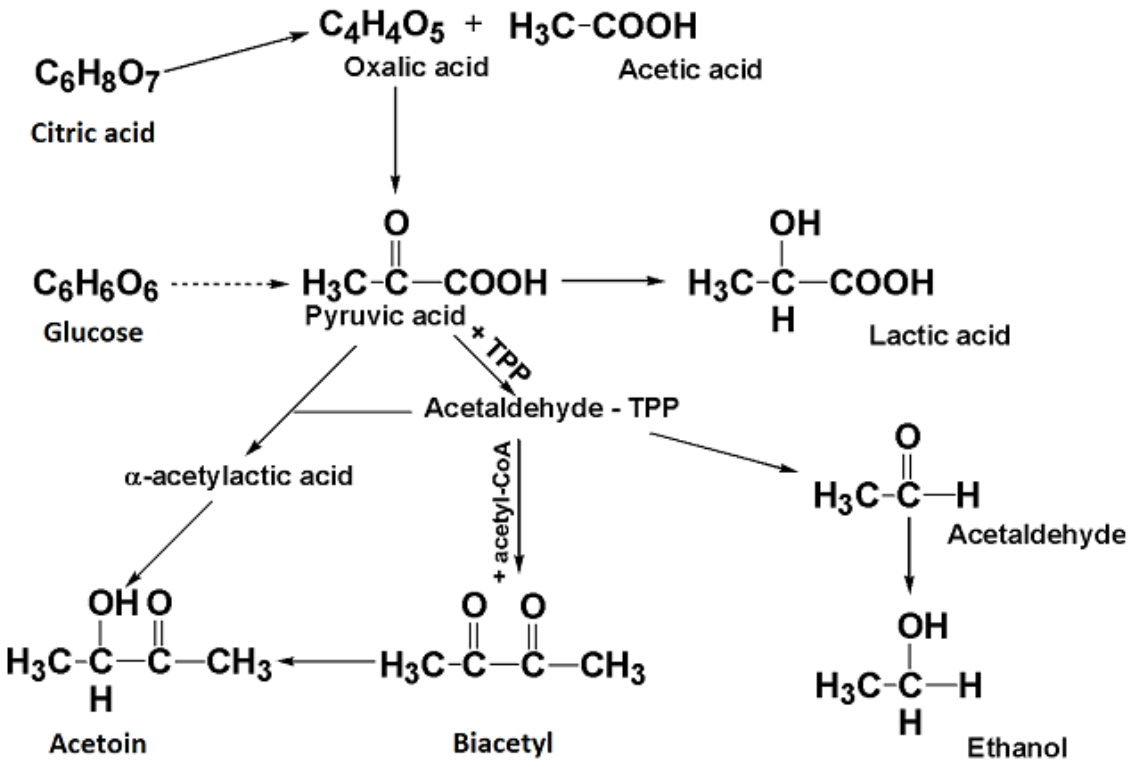


Figure 4. Biosynthesis process of biacetylene and other products [9].

Table 1 shows the list of sugars and organic acids that were used in this study with fermentation process including their molecular formula, molar masses, pK_a values and molecular structural formula.

Table 1: Compounds studied in fermentation process [16,39,42,43]

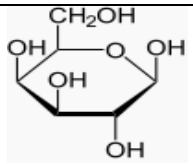
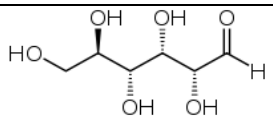
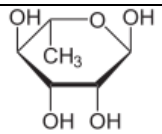
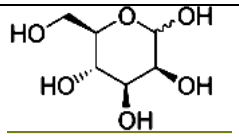
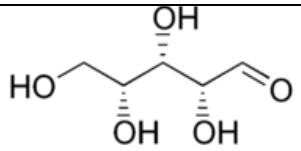
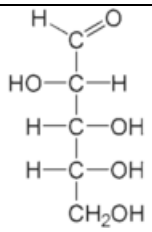
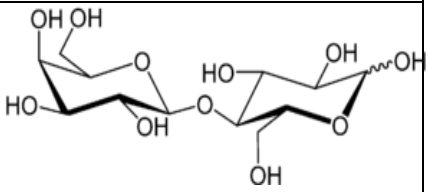
No.	Compounds	Molecular Formula	Molar mass (g/mol)	pK_a	Structural Formula
1	D-(+)-Galactose	$C_6H_{12}O_6$	180.15	12.35	
2	D-(+)-Glucose	$C_6H_{12}O_6$	180.16	12.35	
3	L-Rhamnose	$C_6H_{12}O_5$	164.16	NA[a]	
4	D-(+)-Mannose	$C_6H_{12}O_6$	180.2	12.08	
5	D-(+)-Xylose	$C_5H_{10}O_5$	150.1	12.29	
6	D-(-)-Arabinose	$C_6H_{12}O_6$	180.2	12.35	
7	α-Lactose	$C_{12}H_{22}O_{11}$	342.3	NA[a]	

Table 1. (cont)

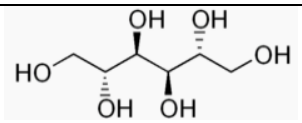
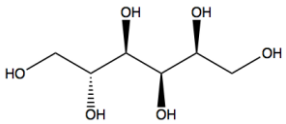
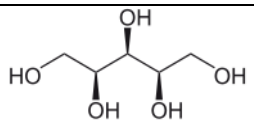
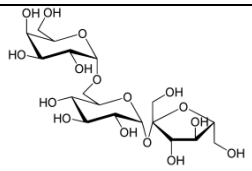
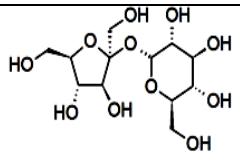
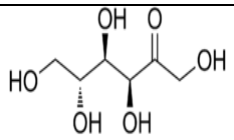
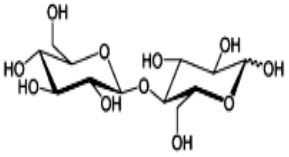
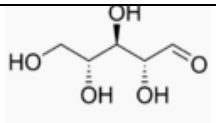
No.	Compounds	Molecular Formula	Molar mass (g/mol)	pK_a	Structural Formula
8	D-(-)-Mannitol	$C_6H_{14}O_6$	182	13.5	
9	Sorbitol	$C_6H_{14}O_6$	182	13.6	
10	Xylitol	$C_5H_{12}O_5$	152	13,8	
11	D-(+)-Raffinose	$C_{18}H_{32}O_{16}$	504.4	12.74	
12	Sucrose	$C_{12}H_{22}O_{11}$	342.3	12.7	
13	D-(-)-Fructose	$C_6H_{12}O_6$	180	6.11	
14	D-(+)-Cellobiose	$C_{12}H_{22}O_{11}$	342.3	NA[a]	
15	Ribose	$C_5H_{10}O_5$	150	NA[a]	

Table 1. (cont)

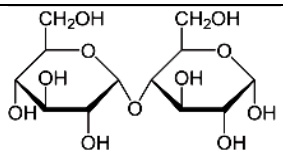
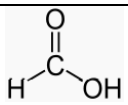
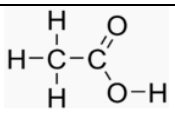
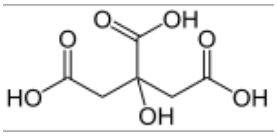
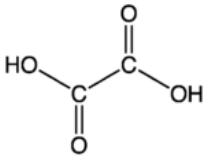
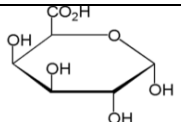
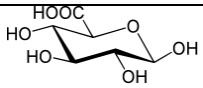
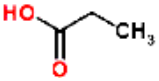
No.	Compounds	Molecular Formula	Molar mass (g/mol)	pK_a	Structural Formula
16	Maltose	$C_{12}H_{22}O_{11}$	342.3	NA[a]	
17	Formic acid	HCOOH	46.025	3.75	
18	Acetic acid	CH_3COOH	60.05	4.75	
19	Citric acid	$C_6H_8O_7$	192.124	pKa_1 = 3.13 pKa_2 = 4.76 pKa_3 = 6.40	
20	Oxalic acid	$C_2H_2O_4$	90.03	pKa_1 = 1.23 pKa_2 = 4.19	
21	Galacturonic acid	$C_6H_{10}O_7$	194.139	3.25	
22	Glucuronic acid	$C_6H_{10}O_7$	194.14	2.93	
23	Propionic acid	$C_3H_6O_2$	74.078	4.88	

Table 1. (cont)

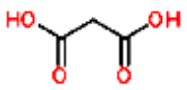
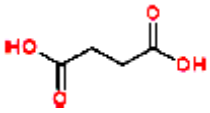
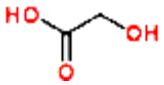
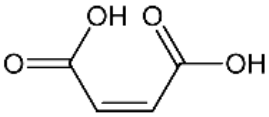
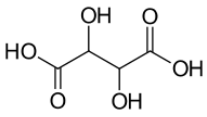
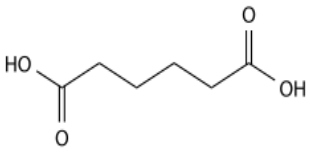
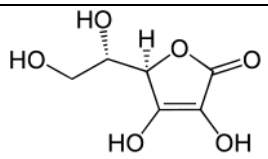
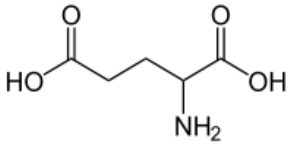
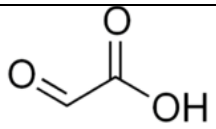
No.	Compounds	Molecular Formula	Molar mass (g/mol)	pK_a	Structural Formula
24	Malonic acid	$C_3H_4O_4$	104.06	pKa_1 = 2.83 pKa_2 = 5.70	
25	Succinic acid	$C_4H_6O_4$	118.09	pKa_1 = 4.2 pKa_1 = 5.6	
26	Glycolic acid	$C_2H_4O_3$	76.05	3.83	
27	Maleic acid	$C_4H_4O_4$	116.07	pKa_1 = 1.84 pKa_2 = 6.07	
28	Tartaric acid	$C_4H_6O_6$	150.09	pKa_1 = 2.98 pKa_1 = 4.34	
29	Adipic acid	$C_6H_{10}O_4$	146.14	pKa_1 = 4.43 pKa_2 = 5.41	

Table 1. (cont)

No.	Compounds	Molecular Formula	Molar mass (g/mol)	pK_a	Structural Formula
30	Ascorbic acid	$C_6H_8O_6$	176.12	4.10	
31	Glutamic acid	$C_5H_9NO_4$	147.13	pKa_1 = 2.31 pKa_2 = 4.1	
32	Glyoxylic acid	$C_2H_2O_3$	74.04	3.32	

[a] Not available

2. Capillary electrophoresis

Capillary electrophoresis (CE) is expected to be fastest-growing analytical technique. It consists of sub-techniques such as:

1. Capillary zone electrophoresis (known as “Free Zone” or Capillary Electrophoresis)
2. Capillary isoelectric focusing (CIEF)
3. Micellar electrokinetic chromatography (MEKC)
4. Capillary electrochromatography (CEC)
5. Capillary gel electrophoresis (CGE)
6. Capillary isotachophoresis (CITP)
7. Open tubular capillary electrochromatography (OT-CEC)

Theory of CE composed of electrophoresis terms like migration time, buffer solution, electropherogram, capillary, theoretical plates, electro-osmotic flow, efficiency, resolution, and electric field.

2.1 Basic electrophoretic separation modes

The basic method of CE includes zone electrophoresis (ZE), isotachophoresis (ITP), and isoelectric focusing (IEF). Moving boundary electrophoresis was the first electrophoretic method, which was successfully applied to the separation of charged compounds in free solution [6]. Basic principles of ZE, ITP, and IEF are described below with relevant applications.

2.1.1 Capillary zone electrophoresis

Capillary zone electrophoresis encompassed of capillary, detector, applied voltage source, injector, sample vials and running buffer solution. CZE analytes migrates in EOF (Chapter 3.2 and 3.3) but separate into bands due to change in their electrophoretic mobilities μ_e. Anionic and cationic sample solutions are introduced into the continuous buffer system at one end of capillary along with solvent (Figure 5). The electrode reservoirs are filled with background electrolyte, which conducts electric current and provides buffer capacity. Two electrodes are connected to D.C. source, under high electric field analytes migrates towards electrodes and hence difference in μ_e of each

analyte does proceed to separation into bands. If precisely electro-osmotic flow exists, both cations and anions move through the detector, which records individual zones [6]. The electro-osmotic flow phenomenon is described on Chapter of electro-osmosis flow (Chapter 3.2).

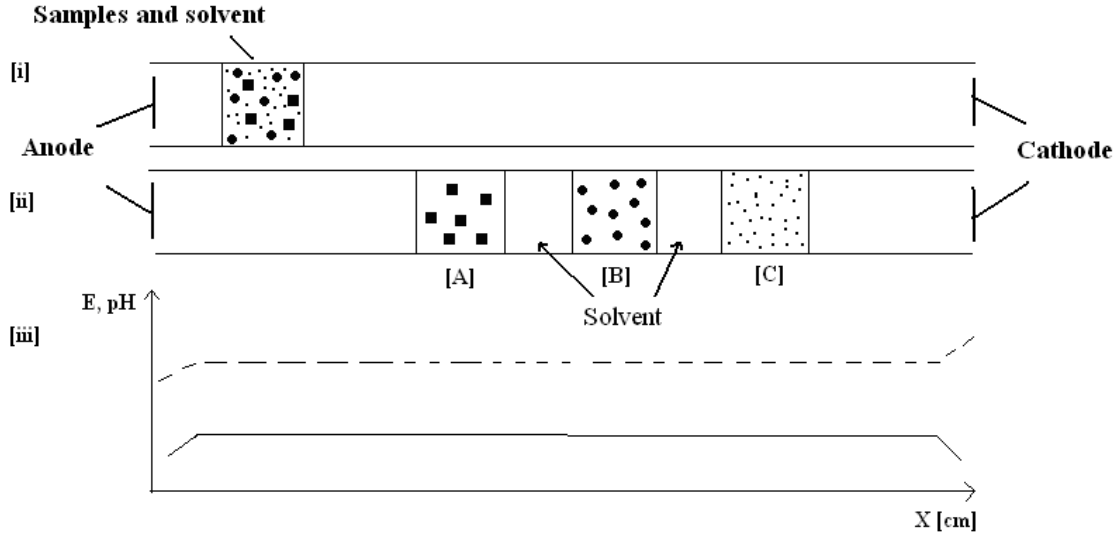


Figure 5. Plot of capillary zone electrophoresis separation mode [i] initial-state at t = 0, [ii] steady-state at t > 0, [iii] profile of field strength (plain line) and pH (dashed line) across separation capillary, [A], [B], and [C] are sample zones [6, 24].

Application areas includes analyse amino acids, peptides, enantiomers, sugar acids, organic acids and numerous other ionic species [24].

2.1.2 Isotachophoresis

Isotachophoresis (ITP) is a powerful electrophoretic technique using discontinuous electrical field to create sharp boundaries between sample constituents [6]. In ITP multianalyte samples are introduced between leading electrolyte (L) and terminating electrolyte (T). Two conditions are concerned in ITP phenomenon that the sample components must have the same polarity. They have lower electrophoretic mobilities than the leading ion, but higher than the terminating ion. Under high electric field, the fast moving cationic compound will migrate earlier than that with lower mobility as shown in Figure 6. The principle of ITP has been applied as a preconcentration step prior to CZE, MEKC, or CGE [6].

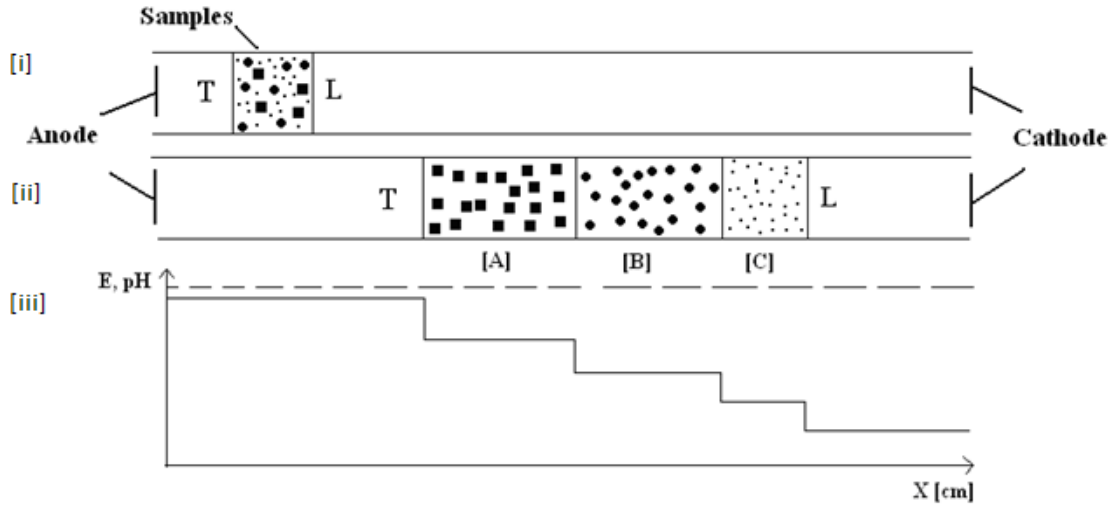


Figure 6. Schematic representation of isotachophoresis separation mode [i] initial-state at t = 0, [ii] steady-state at t > 0, [iii] profile of field strength (plain line) and pH (dashed line) across separation capillary, T = terminating electrolyte; L = leading electrolyte; [A], [B], and [C] are sample zones [6, 24].

2.1.3 Isoelectric focusing

In capillary isoelectric focusing (CIEF), the pH gradient is formed by using amphoteric substance. Amphoteric systems contain molecules that are composed of acidic and basic moiety [24]. The pH of sample component has zero net electrical charge. The pH point is said to be an isoelectric point (pI) and due to differences in zwitterions in range of pI, which leads to molecules to migrate quickly. The anode reservoir is filled with an acidic solution, whereas that of cathode contains a basic solution. Under applied electric field, naturally, positive ions migrate to cathode and negative ions to anode. Moreover, ampholytes will migrate according to their pI values towards the respective electrodes.

At first, capillary is filled with amphoteric substance, which creates pH gradient. Later, samples are injected through pressure to the system, which pushes the whole solution through the capillary (Figure 7 [i]). Each ampholyte migrates towards the position, where the pH value is equal to pI. Constant voltage is applied on the system, which avoids band broadening. In Figure 7 [ii] as velocity becomes zero, the sample will be concentrated into the narrow end, and it will be detected with the detector. Most

commerical carrier ampholytes are polyamino polycarboxylic acids, which are polymerized of both acrylic acid and polyethylene polyamines in water [6].

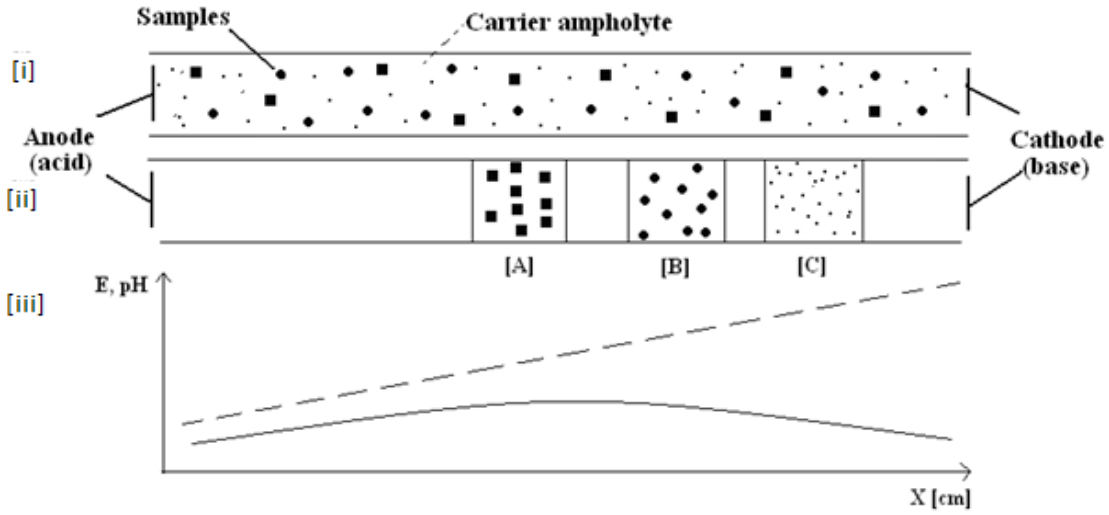


Figure 7. Plot represents isoelectric focusing separation [i] under generation of pH gradient and samples introduction, [ii] steady-state at t > 0, [iii] profile of field strength (plain line) and pH (dashed line) across separation capillary. [A], [B], and [C] are sample zones [6, 24].

3. Capillary zone electrophoresis

Capillary zone electrophoresis (CZE) is a separation technique where charged compounds are separated from each other in a solution under a high electric field. The velocity of an ion can be represented by

$$v = \mu_e E \tag{4}$$

where v = ion velocity (cms^{-1})

μ_{ep} = electrophoretic mobility ($cm^2V^{-1}s^{-1}$)

E = applied electric field (Vcm^{-1})

The electrophoretic mobility can be defined as ratio of electric forced applied on the capillary to the frictional force of molecules.

$$\mu_{ep} = \frac{Electric\ \ force\,(F_E)}{Frictional\ \ force\,(F_F)} = \frac{qE}{-6\pi\eta rv} \quad (5)$$

where q = ion charge (C)

η = viscosity of solution ($gcm^{-1}s^{-1}$)

r = ion radius (cm)

In sum up, it is well known that ions that are negatively charged will migrate toward anode and vice versa for positive charged ions.

3.1 Rate of migration

The main reason that CE is an efficient method in component separation is that in every aqueous solution, ions will move at different velocities under electric field, which provide better separation efficiency. Main phenomena are based on charge compared to relative hydrodynamic molecule size, which is affined to mass of molecules [22]. Therefore, migration of charged colloidal particles or molecules under the influence of an applied electric field is provided by immersed electrodes called "electrophoretic mobility" [22]. Electrophoretic mobility is generally dependent on dissociation constant (pK_a) and composition of the electrolyte solution.

Ionic mobility is taken into account in presence of ions in aqueous solution. Ionic mobility is dependent on the viscosity of solvents and applied voltages (equation 6). The trend is: The more voltage, the more attraction will occur. The total mobility is a summation of electrophoretic mobility and electro-osmotic mobility are shown in equation 7.

$$\mu_{ep} = \frac{L_{tot} \cdot L_{\det}}{U \cdot t_m} \quad (6)$$

$$\mu_{tot} = \mu_{ep} + \mu_{eo} \quad (7)$$

where μ_{eo} = electro-osmotic mobility ($cm^2V^{-1}s^{-1}$)

μ_{ep} = electrophoretic mobility ($cm^2V^{-1}s^{-1}$)

U = voltage (V)

L_{tot} = capillary total length (cm)

L_{det} = capillary detection length (cm)

t_m = time of migration (s)

μ_{tot} = total mobility ($cm^2V^{-1}s^{-1}$)

3.2 Electroosmotic flow

Electroosmosis is a basic phenomenon in all electrophoretic separation processes. In general, electroosmotic flow (EOF) is the motion of ions in liquids induced by fixed charged surface caused by an electric field. The velocity and magnitude of electroosmotic flow is depending on the behaviour of solution and material of the capillary. A schematic cross sectional view and inside view of capillary tube are shown in (Figure 8).

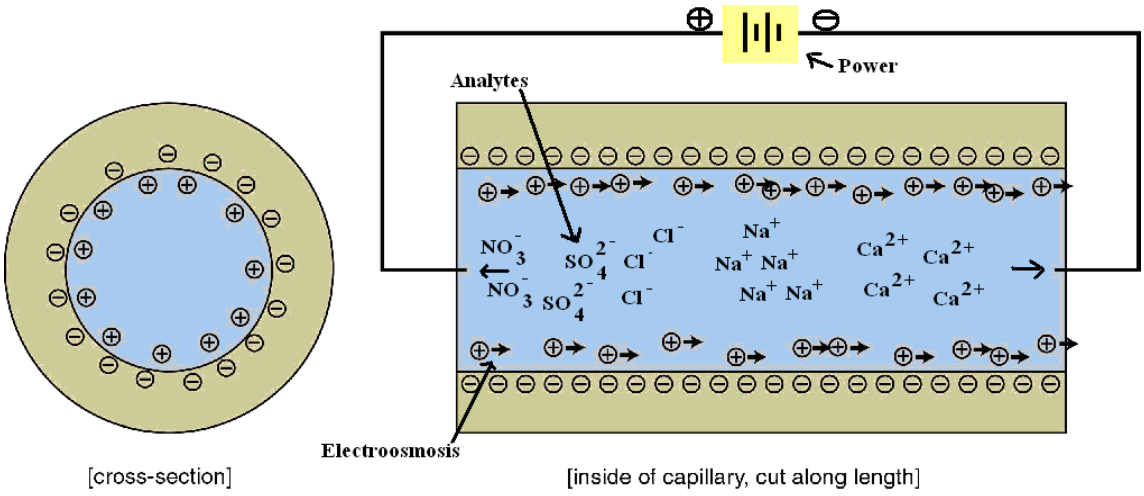


Figure 8. Cross section and an inside view of a capillary tube under the separation of charged compounds according to their electroosmotic flow mobilities [23].

The magnitude of EOF can be represented in terms of mobility by Equation 8 [24].

$$v_{eof} = (\varepsilon \zeta / \eta) E \quad (8)$$

where $v_{eof} = \mu_{EOF}$ = velocity (cms^{-1})

ε = dielectric constant

ζ = zeta potential (V)

η = Newtonian viscosity ($gcm^{-1}s^{-1}$)

E = applied electric field (Vcm^{-1})

3.3 Electroosmotic flow in CE

The ions of electrolyte solution conduct electric field, and provide sufficient current in CE. Currents are evenly distributed over the entire capillary boundaries, and the process of EOF occurs gradually. As shown in Figure 9 the sample ions in background electrolyte (BGE) migrate toward cathode in even manner by showing plug shape. These phenomena occur due to flat front, even flows and extreme high theoretical plate counts are possessed by CE. The Figure 9 shows the difference between plug flow profile of CE and the parabolic flow of pressure induced flow in high performance liquid chromatography (HPLC) [22]. In HPLC, component ions were adsorption with the sorbent and Eddy diffusions formed between particles.

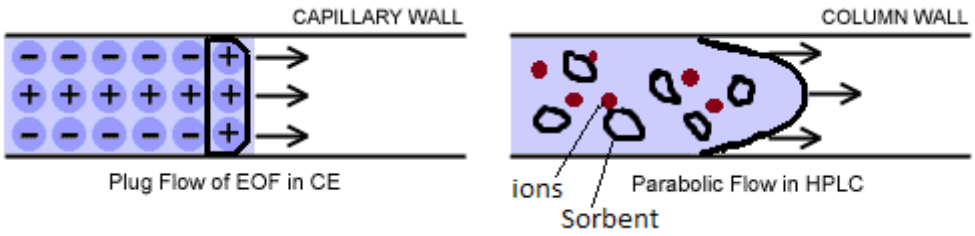


Figure 9. Difference between plug flow and parabolic flow occurred in CE and HPLC, respectively [22].

In CE, due to electro-osmosis, analytes migrate evenly which forms flat front profile, whereas in HPLC process has parabolic shape flow due to the longitudinal and Eddy diffusions [46].

3.3 Performance criteria

3.3.1 Efficiency

The number of theoretical plates (N) achieved in capillary are called the efficiency of electrophoretic system. From the electropherogram shown in Figure 10, N can be calculated as presented on Equation 9.

$$N = 5.545 \left(\frac{t_m}{w_{h_{/2}}} \right)^2 \qquad (9)$$

where N = number of theoretical plates per meter

t_m = migration time of components (s)

$w_{h_{/2}}$ = temporal peak width at half-height (s)

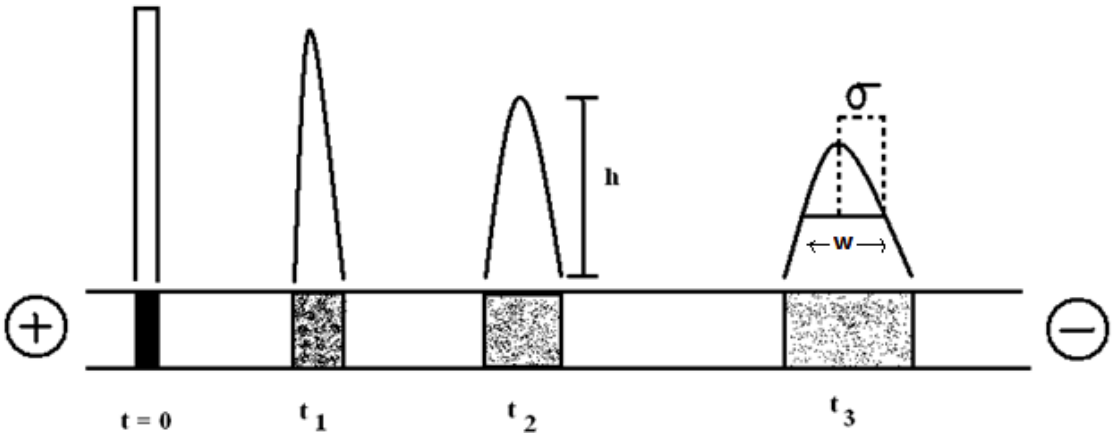


Figure 10. Electropherogram of analytes in capillary electrophoretic. Change in concentration distribution with time, σ = half width with half height of peak, w = width of peak, and h = height of peak [6].

Zone broadening caused by diffusion can be recognized by Einstein's equation 10 [6]:

$$\sigma^2 = 2 \cdot D \cdot t \qquad (10)$$

where D = diffusion coefficient (cm^2s^{-1})

t = time (s)

The plate height H can be represented as in equation 11.

$$H = \frac{\sigma^2}{x} = \frac{2 \cdot D}{v} \tag{11}$$

where σ^2 = spatial variance (cm^2)

v = velocity (cms^{-1})

D = diffusion coefficient (cm^2s^{-1})

3.3.2 Resolution

Resolution (R) of two peaks is defined as mean of distance between peak centres based on Δx and 4σ (Equation 12) [6].

$$R = \frac{\Delta x}{4\sigma} \tag{12}$$

where Δx = distance between two peak center (cm)

σ = half width of a peak (cm)

However, Δx is directly proportional to the incremental migration velocity Δv and inversely proportional to mean of migration velocity of two components $\bar{v}$. Resolution can be written as:

$$R = \frac{\bar{x}}{4\sigma} \cdot \frac{\Delta v}{\bar{v}} \tag{13}$$

where Δv = difference of mobilities of components μ_1 and μ_2 (cms^{-1})

$\bar{v}$ = mean of migration velocity of components μ_1 and μ_2 (cms^{-1})

Nevertheless, $H = \sigma^2 / x$ and $N = x / H$ resolution related to plate number N as shown [6]

$$R = \frac{\sqrt{N}}{4} \cdot \frac{\Delta v}{\bar{v}} \tag{14}$$

Moreover, the ratio of difference in mobilities of two separated components is the mean of migration and can be presented as:

$$\frac{\Delta v}{\bar{v}} = \frac{\mu_1 - \mu_2}{\bar{\mu}} \tag{15}$$

In presence of electroosmosis, equation 15 can be modified to

$$\frac{\Delta v}{\bar{v}} = \frac{\mu_1 - \mu_2}{\bar{\mu} + \mu_{eo}} \tag{16}$$

where μ_1 = mobility of 1st component (cms^{-1})

μ_2 = mobility of 2nd component (cms^{-1})

$\bar{\mu}$ = mean of mobilities of 1st and 2nd components (cms^{-1})

μ_{eo} = mobilities of electro-osmosis (cms^{-1})

By replacing equation 13 and 10 into equation 16, the resolution (R) of two coumpounds can be calculated as:

$$R = \frac{1}{4}\sqrt{\frac{(\bar{\mu} + \mu_{eo}) \cdot U}{2 \cdot D}} \cdot \frac{\mu_1 - \mu_2}{\bar{\mu} + \mu_{eo}} \tag{17}$$

where $\bar{\mu}$ = mean of mobilities of components 1 and 2 (cms^{-1})

μ_{eo} = mobility of electro-osmosis (cms^{-1})

D = diffusion coefficient ($cm^{2} \cdot s^{-1}$)

U = voltage (kV)

3.4 Injection

Injection is used to introduce the certain amount of sample solution into the capillary. Injection can be carried out by utilizing two different techniques.

1) Hydrodynamic injection (vacuum or pressure)

2) Electrokinetic injection

Hydrodynamic injection is the most commonly used method. Vacuum injection is handled from the outlet vial (Figure 11 b), whereas pressure is operated from the inlet vial (Figure 11 a) [24]. The pressure differential between two sides of capillary moves the liquid. The volume of sample loaded particularly for pressure injection can be determined by using Hagen-Poiseuille's law for liquid flow through a circular tube shown in Equation 18.

$$V_{sample} = \frac{\Delta P d^4 \pi t}{128 \eta L_{tot}} \tag{18}$$

where ΔP = pressure difference across the capillary (Pa)

d = interior diameter of capillary (μm)

t = time (s)

L_{tot} = total length of capillary (cm)

η = viscosity of electrolyte ($gcm^{-1}s^{-1}$)

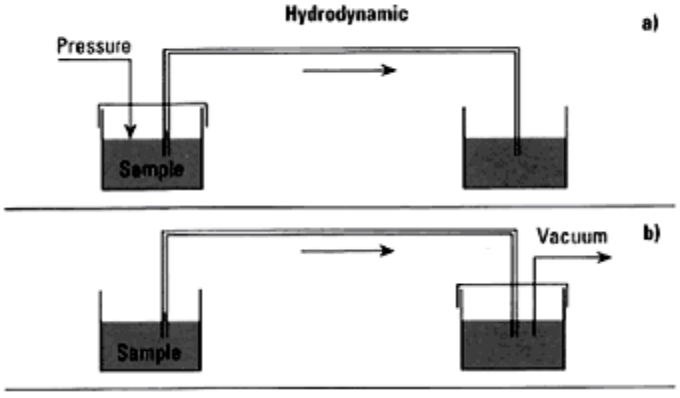


Figure 11. Schematic diagram represents the hydrodynamic injection. Where, a) Hydrodynamic pressure injection flow, and b) vacuum injection flow [24].

Electrokinetic, or electromigration injection performs by replacing the injection end reservoir with sample vial as shown in Figure 12 [24]. The main advantage of electrokinetic injection in CE is trace enrichment. In low medium of EOF, through electrokinetic injection it is possible to inject little amount of ions into capillary.

The main disadvantages of electrokinetic injection technique are the control and accuracy.

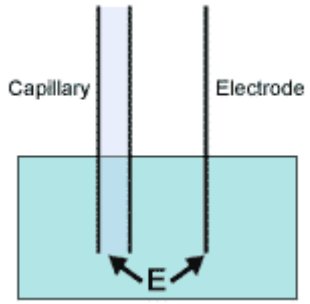


Figure 12. Schematic presentation of electrokinetic injection used in CE, E is electrokinetic injection.

In electrokinetic injection the volume of liquid introduced into the capillary is: [24]

$$Q_i = \frac{(\mu_i + \mu_{eo}) \cdot \pi \cdot r_c^{\,2} \cdot U \cdot c_i \cdot t}{L_{tot}} \qquad (19)$$

where Q_i = amount of species i introduced into the capillary (mol)

U = voltage (V)

c_i = concentration of species i (molL^{-1})

L_{tot} = total length of capillary (cm)

μ_i = mobility of i component (cm^{2}V^{-1}s^{-1})

μ_{eo} = mobility of electro-osmosis (cm^{2}V^{-1}s^{-1})

r_c = inner radius of the capillary (μm)

t = time (s)

3.5 Capillary electrophoresis instrument

The main components of a capillary electrophoresis are capillary, injector, data acquisition, high-voltage power supply, detector, buffer and waste reservoirs, and sample vials [6].

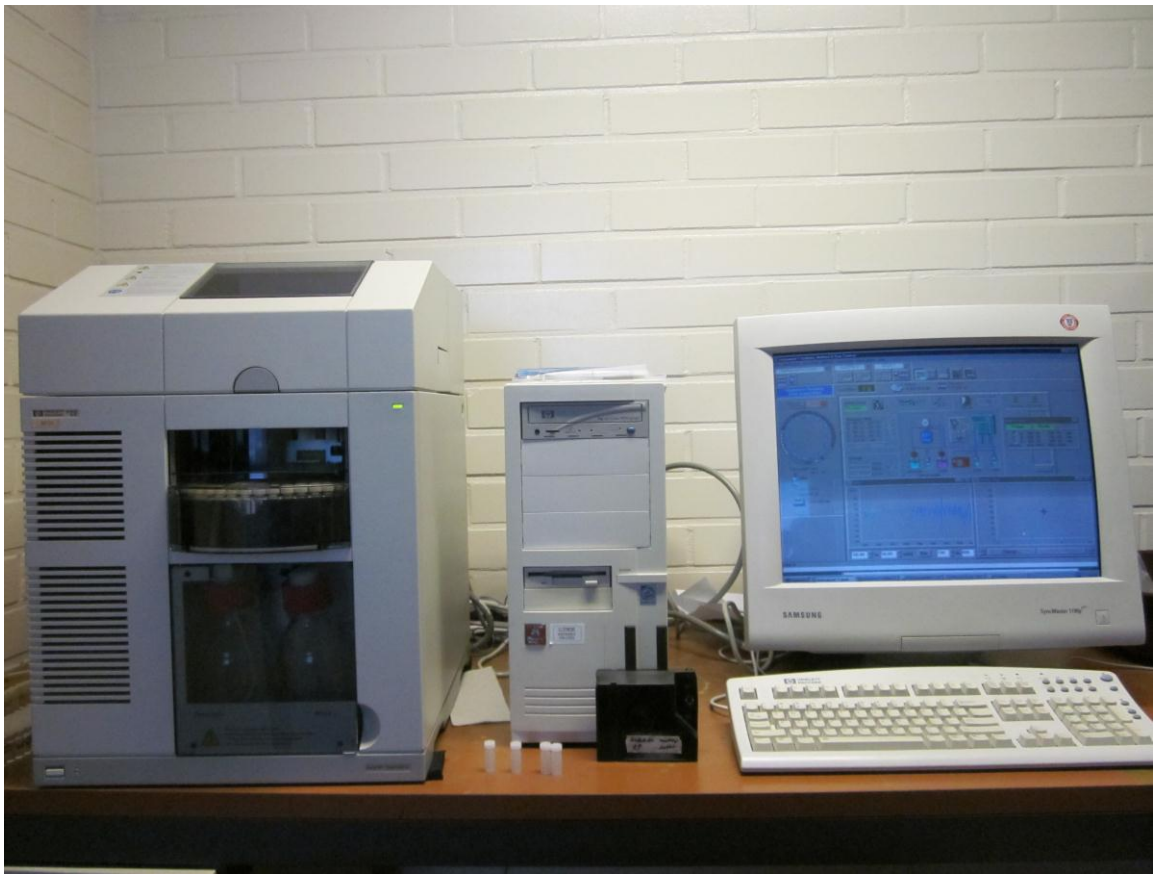


Figure 13. Instrumental set up of capillary electrophoresis system.

The buffer reservoirs and capillary are filled with an electrolyte (buffer) solution. Later, introduction of sample are carried out, the capillary inlet is set into a sample containing vial, and returned to source vial by siphoning effect. Under high electric field on both end of electrodes, migration of analytes are initiated. For once, it is necessary to note down moving direction of all ions, positive or negative pulled over same direction by electro-osmotic flow [6].

So going over further, analytes separate gradually due to their electrophoretic mobility. They are detected near to the outer end of capillary. Brief note on detector is explained in Chapter (3.6). The results from detector are sent to data acquisition device, such as a

computer and displayed as an electropherogram [6]. Distinct compounds appear as area of peaks with different migration times in the electropherogram.

3.6 Detector

Many detectors are used in CE. The mass detection and quantification limits are shown in Table 2.

Table 2. Methods of detection in CE [24]

Method	**Mass detection limit M (mol/l) range**	**Quantification limit M (mol/l) range**	**Benefits**
UV-Vis absorption spectrophotometry	$10^{-13} – 10^{-16}$	$10^{-5} – 10^{-8}$	• Universal • Diode array offers spectral information
Mass spectrometry	$10^{-16} – 10^{-17}$	$10^{-8} – 10^{-9}$	• Sensitive • Offers structural information
Fluorescence spectrometry	$10^{-15} – 10^{-17}$	$10^{-7} – 10^{-9}$	• Sensitive • Sample derivatization required
Laser-induced fluorescence spectrometry	$10^{-18} – 10^{-20}$	$10^{-14} – 10^{-16}$	• Extremely sensitive • Expensive
Amperometry spectrometry	$10^{-18} – 10^{-19}$	$10^{-10} – 10^{-11}$	• Sensitive • Requires special electronics and capillary modification
Indirect UV spectrophotometry	10 – 100	Not found	• Universal • Lower sensitivity than direct UV

UV-Vis absorbances are used when the analytes have functional groups, like double bonds and aromatic rings. In chromatography Beer-Lambert law may be stated as intensity of absorbed light in dependence of analyte concentration c and of the optical path length of light through detection cell [7].

$$A = \varepsilon \; c \, I \tag{20}$$

where A = absorbance (AU)

I = path length of the light in the sample

ε = molar extinction coefficient of absorptivity ($molcm^{-1}$)

c = analyte concentration ($molL^{-1}$)

Experimentally, the wavelengths are examined in pulp samples by

- Direct UV-detection at 270 nm for sugars.
- Indirect UV-detection at 460 nm to 270 nm for organic compounds.

The main parts of an UV-Vis spectrophotometer are a light source, a monochromator, a holder (for sample), and detector. As shown in Figure 14, the UV-Vis light emits its radiation and reflects on diffraction grating. Then the light is passes through an aperture and the sample whereby a detector identifies the analytes.

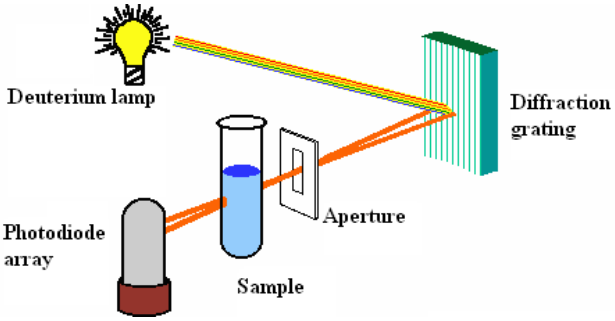


Figure 14. Scheme of wavelength path of single-beam used in an UV-Vis spectrophotometer [25]

3.7 Capillary

A CE capillary is considered as core of the CE system. Basic properties of capillary material include being transparent, flexible, robust, and inexpensive [24]. Fused silica owes intrinsic properties. The cross sectional view of fused silica capillary is shown in Figure 15.

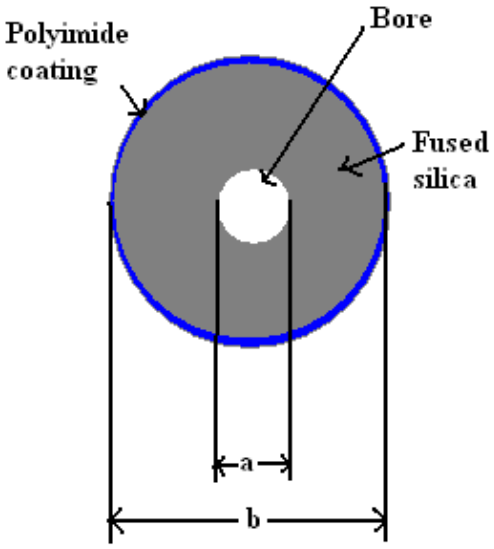


Figure 15. Cross-sectional view of fused silica capillary used in CE, a = interior diameter (µm) and b = exterior diameter (µm) [6]. Fused silica capillaries have a) 25-75 µm internal diameter, b) 350 - 400 µm outer diameter, and 60 cm length was employed.

Washing of capillary is an important process, which removes absorbed compounds and refreshes the surface of inner wall by deprotonation of silanol groups [24]. Most common washing is made by 1 M NaOH, strong acids, organic acids like dimethyl sulfoxide (DMSO), or detergents. Often in our studies capillary has washed by 0.1 M NaOH, Milli-Q water (purified water), and buffer solution respectively.

4. Hydrolysis process

The degradation of molecules with inorganic chemicals such, as H_2SO_4, is called hydrolysis. In degradation, catalysts such as palladium, carbon, silicon dioxide, and titanium dioxide are often used in process chemical industry. Concentrated sulphuric acid (H_2SO_4), trifluoroacetic acid (TFA), and sodium hydroxide (NaOH) are often used as hydrolysis agents in pulp and paper process waters for degradation of cellulose [18, 37].

4.1 Hydrolysis of cellulose

Clean-up of pulp is a crucial step, and it depends upon the research development's requirement. Usually, for research analysis process, for example hydrolysis (degradation), cultivation/enzymatic fermentation (enzymatic hydrolysis in nonaerobic medium), and extraction (transfer of analytes) are highly employed [27, 30, 37]. Basically, there are two different approaches for acid catalyzed hydrolysis of cellulose. First, the procedure applies high concentrate of mineral acids, like sulfuric acid, hydrochloric acid or nitric acid as degradation agents at low operating temperature [33]. In the second approach, highly diluted acids are used at high operating temperature [34].

Acid hydrolysis of polysaccharides occurs by destruct of hydroxium ion (H_3O^+) at poly mode linkage [31]. Hydrolysis process follows the kinetic of a first order reaction. Its value depends on the concentration of acid, and temperature. Hydrolysis process leads to break cellulose chain into smaller oligosaccharides and monosaccharides [31]. For instance, cellulose maintains its original fibrous shape. Under the influence of acid it decreases physical strength and viscosity of fibers, in results fibers reduce its fragility and are converted into hydrate powder [31].

For example, degradation of α-1,4-glycosidic bond α-maltose(disaccharides) is hydrolysed by H_2SO_4. In that case, the strong bindings are split into α-D-glucose (a monosaccharide) as shown in (Figure 16). Condensation of two monosaccarides (α-D-glucose) forms disaccharides. As to all disaccharides, mostly the compound hydrolysed is sucrose. The mixture of glucose and fructose is called an invert sugar [31, 32].

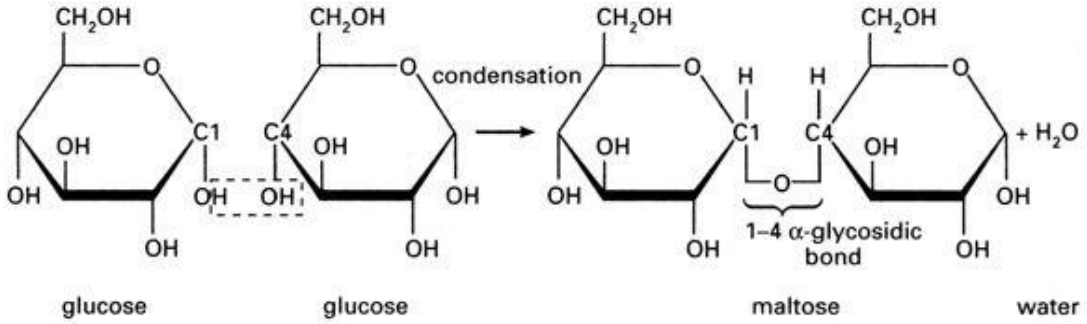


Figure 16. Hydrolysis process of α-maltose to α-D-glucose [31].

In another example, it has been explained by S. Willför et al. [32] that extraction and isolation of galactoglucomannas (GGM) can be well achieved from wood or thermo-mechanical pulp (TMP) (Figure 17). First, distilled water was added into acetone-extracted wood meal or TMP, and vigorously stirred at (23 ± 2) ºC for 3 hours. Suspension was vacuum filtered on paper machine wire, and the resulting fiber pad was washed with distilled water. The suspension was vacuum evaporated and the concentrate was then filtered to eliminate the colloidal substances. Supernatant was further concentrated to 0.3 L by vacuum evaporation. Then the concentrated supernatant was added to ethanol (volume 90%) and polysaccharides were permitted to settle for 24 hr, and analysed by gas chromatorgraphy (GC) [32].

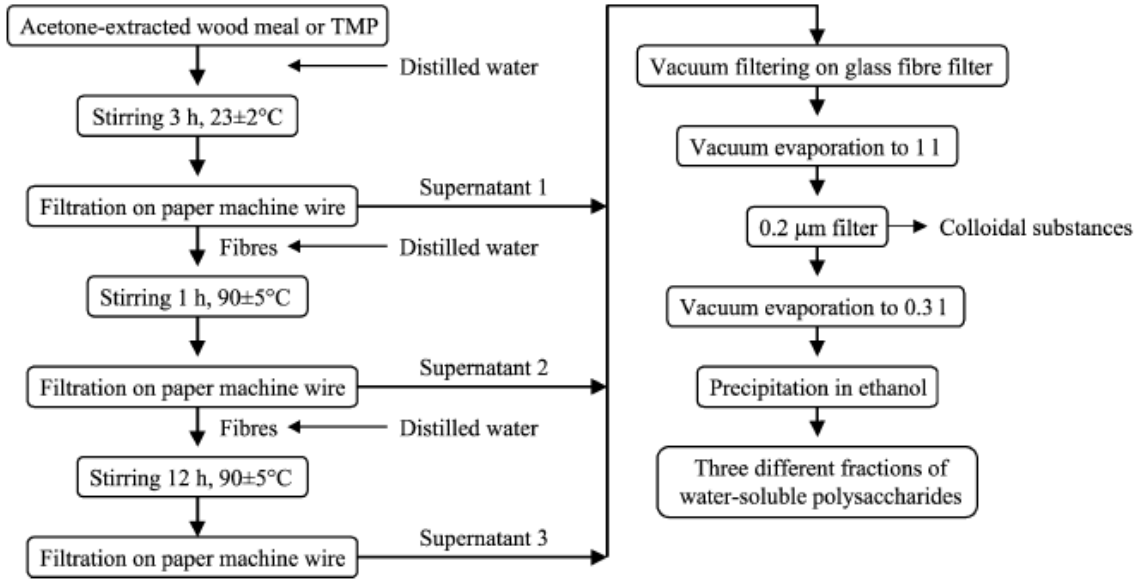


Figure 17. Flow diagram of production of galactoglucomannas from ground wood and TMP [32]

According to Ales et al. [27] analysis of galactoglucomannas from spruce wood can be done by capillary electrophoresis. The acid hydrolysis process of achieving hydrolytes of galactoglucomannas (GGM) was demonstrated. A volume of 8 ml GGM solution [32] was added into 2.04 ml of 0.5*M* H_2SO_4 followed by an autoclave step for 40 min at 120 ºC. Then after, hydrolytes were cooled down at room temperature. By Dahlman et al.[26] derivatization of hydrolytes of GGM was performed through reductive amination by using sodium cyanoborohydride. The stock solution was prepared by adding 100 mg/L aminobenzoic acid ethyl ester (ABEE) and 100 mg/L of acetic acid in methanol. Derivatizing solution was prepared by adding 10 mg of sodium cyanoborohydride to 1L of stock solution to obtain ABEE reagent solution. The reaction of monosaccharide with ABEE is shown in Figure 18 [27].

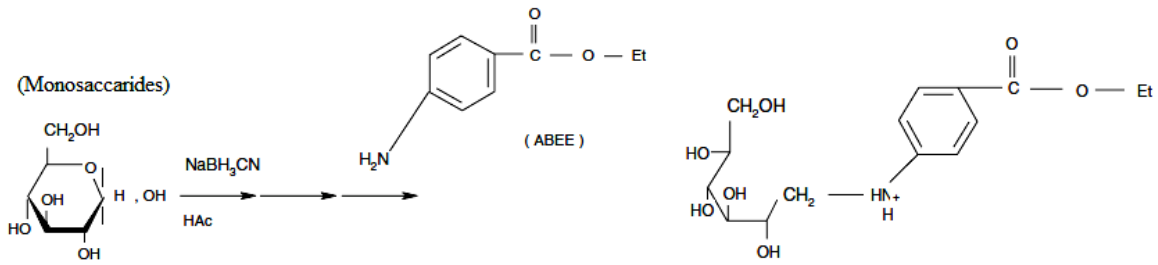


Figure 18. Reductive amination of monosaccharides with ABEE

4.2 Fermentation

Fermentation is a metabolic process, whereby breakdown of molecules from nutrients are transferred into energy. In anaerobic medium bacteria like *Escherichia Coli* and *Salmonella* use mixed acid ferment and produce ethanol, lactic acid, succinic acid, acetic acid, CO_2, and H_2 [31]. In the Table 3 below, the list of chemical derivate through microorganism are shown.

Table 3. Microbial production of chemicals during fermentation [31].

Chemical	Process		Microogranism
Ethanol	$C_6H_{12}O_6$	C_2H_2OH	Saccharomyces cerevisiae
Ethylene		$CH_2=CH_2$	Zymomonas mobilis
1,3-Butadiene		$CH_3=CH-CH=CH_2$	
Ethylene glycol		CH_2OH-CH_3OH	
Acetic acid	$C_6H_{12}O_6$	CH_3COOH	Clostridium thermoaceticum
	C_3H_2OH	CH_3COOH	Acetobacter aceti
Acetone	$C_6H_{12}O_6$	CH_3COCH_3	Clostridium acetobutylicum
Butanol		$CH_3(CH_2)_2CH_2OH$	
Isopropyl alcohol	$C_6H_{12}O_6$	$(CH_3)_2CHOH$	Clostridium aurianticum
Adipic acid	$CH_3(CH_2)_4CH_3$	$HOOC(CH_2)_4COOH$	Pseudomonas species
Acrylic	$C_6H_{12}O_6$	$CH_3CH(OH)COOH$	Lactobacillus bulgaricus
Methyl ethyl ketone	$C_6H_{12}O_6$	$CH_3CH(OH)CH(OH)CH_3$	Klebsiella pneumonia
Glycerol	$C_6H_{12}O_6$	$CH_2OHCH(OH)CH_2OH$	Saccharomyces cerevisiae
	$H_2O + CO_2$	$CH_2OHCH(OH)CH_2OH$	Dunaliella sp.
Citric acid	$C_6H_{12}O_6$	$CH_2(COOH)(OH)C(COOH)CH_2(COOH)$	Aspergillus niger

In the decomposition of sugars, acid catalyzed hydrolysis was carried out of cellulose to breakdown the molecules into glucose. Glucose was then decomposed to 5-hydroxymethylfurfural (5-HMF). Girisuta et al. [2] reported that 5-HMF further converted into levulinic acid, acetic acid, and formic acid. Dias et al. [36] studied that 5-HMF converted into C_5-sugars like xylose. By *Saccharomyces cerevisiae* it has been reported that cellulose hydrolyzates highly immunized with budding yeast to ferment high yield of glucose [36].

In preparation of poly-β-hydroxybutyric acid Jin et al. [10] had constructed fermentation broth based on *Bacillus thuringiensis* gene engineering strain. Bacterial strain was cultivated on pigment medium (PM) at 30 ºC for 16 h in shaken flasks at 230 rpm. PM medium consists of tryptone 10 g/l, yeast extract 2 g/l, glucose 5 g/l, KH_2PO_4 1 g/l, $MgSO_4{\cdot}7H_2O$ 0.3 g/l, $FeSO_4{\cdot}7H_2O$ 0.02 g/l, $ZnSO_4{\cdot}7H_2O$ 0.02 g/l, $MnSO_4$ 0.02 g/l at pH 7.2. Centrifugation was carried out and samples were proceeding toward

fermentation. Fermentation broth was treated with ultrasonic bath at 100 W for 5 min, and precipitated samples were suspended in 10 *M* H_2SO_4.

Later, the mixture was shaken for several time and then $Ba(OH)_2 \cdot 10H_2O$ powder are added for neutralizing. Evaluation of poly-β-hydroxybutyric acid Jin et al. [10] were carried out by CZE with indirect UV detection.

Appendix II lists about fermentation used in the determination of sugar acids and organic acids by capillary electrophoresis. Overview about hydrolysis process and fermentation among articles based on pulp and paper industries are shown in Appendix III.

GEA liquid processing Inc renowned liquid processing industry had developed distinct micro-organism and cell fermentation systems as shown in Figure 19, which could be further used in pulp and paper fermentation. Main advantages of GEA liquid processing are highly accurate temperature controls, longstanding sterility during medium, aeration and venting, and stirrers adapted to provide optimum oxygen transfer rates and cell densities [44].

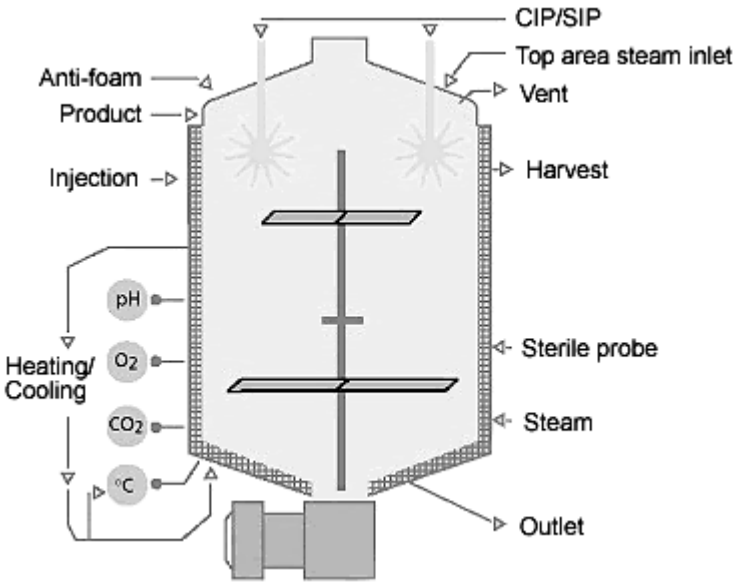


Figure 19. A Typical fermenters from GEA liquid processing which developed different micro-organism and cell fermentation system[44].

5. Overview about research articles

The literature overview is made from articles published on the year 2000 to 2010. Several experiments have been carried out for determinations of sugar acids, organic acids and carbohydrates. The article based on fermentation processes are listed in Appendix I.

In brief, information related to all article's parameters like electrolyte solution, separation voltage, ultraviolet wavelength detection, reaction time, and temperature are listed also in Appendix I.

The overview is made by using articles on such studies that are most potential ones for real use in process technology.

First article is dealing with determination of poly-β-hydroxybutyric acid (PHB) in *Bacillus thuringiensis* and it was written by He et al. [10]. This method can be used to detect the PHB contents from fermentation broth and single colony in *Bacillus thuringiensis* directly without drying sample. It has been noticed that the detection limit for β-hydroxybutyrate was 0.2 µg/ml, which was two to three orders of magnitude lower than of gas chromatography (GC). Hence, CE has greater advantages over GC method, when the detection is made with indirect UV. Experimental conditions include a carrier electrolyte that is made of 5 m*M* p-hydroxybenzate with 0.5 m*M* TTAB at pH 8.0. Separation voltage with negative polarity and temperature were -15 kV (see table 4) and 30 ºC, respectively. Indirect UV detection was at 254 nm, and injection pressure mode was 0.5 psi for 5 sec. For hydrolysis conditions at first, 10.0 ml of fermentation broth was treated with ultrasonic at 100 W and centrifuged for 5 min, respectively. Later, the precipitation was suspended in 10.0 ml of 10 *M* H_2SO_4 at 100 ºC for 2 h. At room temperature the mixture was washed several times with 50 ml of water and neutralized with $Ba(OH)_2 \cdot 10\ H_2O$ powder until pH 7.0 – 8.0. More fermentation and hydrolysis conditions are listed on Appendix II.

The main reason of considering p-hydroxybenzoate as the organic buffer solution in experiments was: 1) To get high molar absorptivity which allows sensitive in indirect UV detection and 2) To provide differences between the mobility of analytes and that of BGE, which leads to better sepearation efficiency and peak area of analytes. Furthermore, it has been noticed that migration of anions was opposite to electro-

osmotic flow (EOF), in order to get reverse EOF and to avoid addition of cationic surfacts like TTAB or CTAB was added in buffer solution.

He et al. showed that under electrophoretic conditions with indirect UV detection, limit of detection of poly β-hydroxybutyrate was 0.2 µg/ml and relative standard deviation of migration time and peak area were less than 1.0 %. The electropherogram of PHB molded from fermentation broth at 16 h is shown in Figure 20. To affirm precision of CZE on migration time and separation efficiency, series of experiment were carried out on separation voltage from -5.0 to -25.0 kV to get optimize voltage.

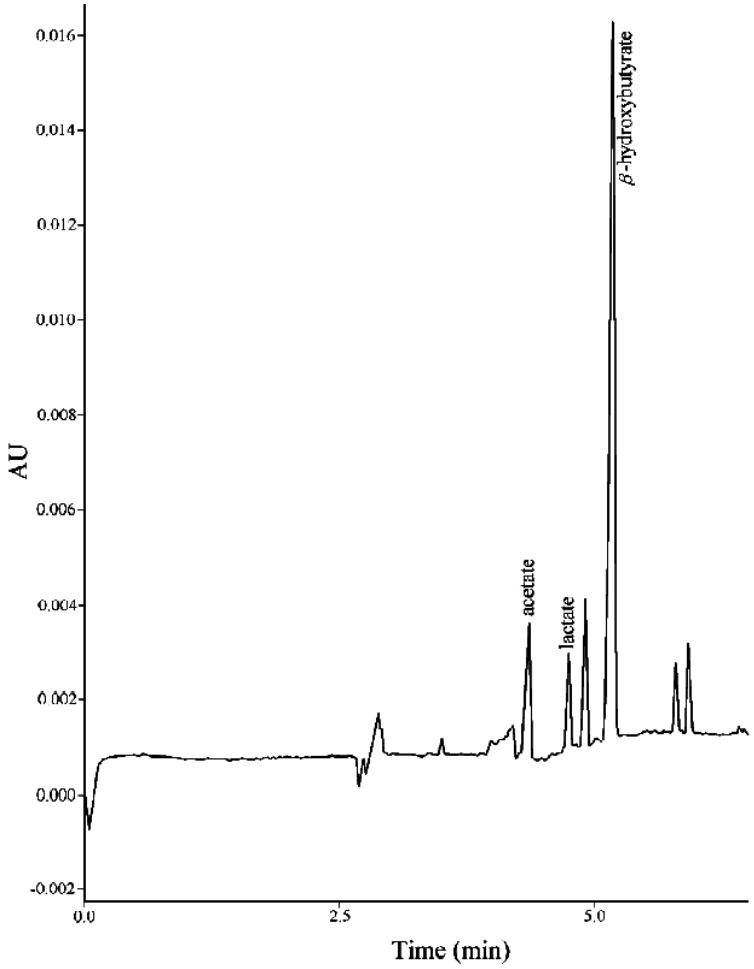


Figure 20. Electropherogram of PHB formed (from fermentation broth at 16 h) by CZE separation mode. Experimental condition: Separation voltage with negative polarity was -15 kV, Optimum temperature was 30 °C, Injection pressure of 0.5 psi for 5 sec and indirect UV detection was at 254 nm. [10]

In Table 4 the effects of separation voltage on migration time and separation efficiency is shown. It can be confirmed that -15 kV gave the best results, which compromise between analysis time and separation efficiency.

Table 4. For precision of CZE on migration time and separation efficiency, series of different voltages were experimented [10].

	Separation voltage (kV)								
	−5.0	−7.5	−10.0	−12.5	−15.0	−17.5	−20.0	−22.5	−25.0
Migration time (min)	14.8	10.0	7.5	6.0	5.2	4.3	3.8	3.2	3.0
Number of theoretical plates ($\times 10^3$/m)	181	217	230	207	203	185	173	158	134

Another article focused here is concerning the monitoring of 18 carboxylic acids hydrolyzed from *Gluconobacter oxydans* by Turkia et al [14]. Moreover, it was shown that *G. oxydans* have unique capacity to transform carbohydrates to carboxylic acid, which could be used as ligands, buffer chemicals, and dispersants. The BGE solution was made of ammonia, 2,3-pyridinedicarboxylic acid, Ca^{2+} and Mg^{2+} salts, but also myristyltrimethylammonium hydroxide was added as a dynamic capillary coating reagent. Detection limits for standard compounds were between 2 and 5 mg/l. It was reported that standard deviations of migration times were less than 1.6%, and in peak areas between 1.0% and 5.9%. Product yield was 96% for the leading analytes gluconic and glucose, and 45% for the least analytes of xylonic acid and xylose. In Table 5, the results show the concentrations of organic acids found in wheat straw hydrolyzates. The electropherograms of different interval of time of fermentation are shown in Figure 21 [14]. Analysis conditions includes of injection pressure by using 0.5 psi for 15 sec, reversed polarity with separation voltage of -20 kV at constant temperature of 25 °C. Indirect UV detection was at 254 nm.

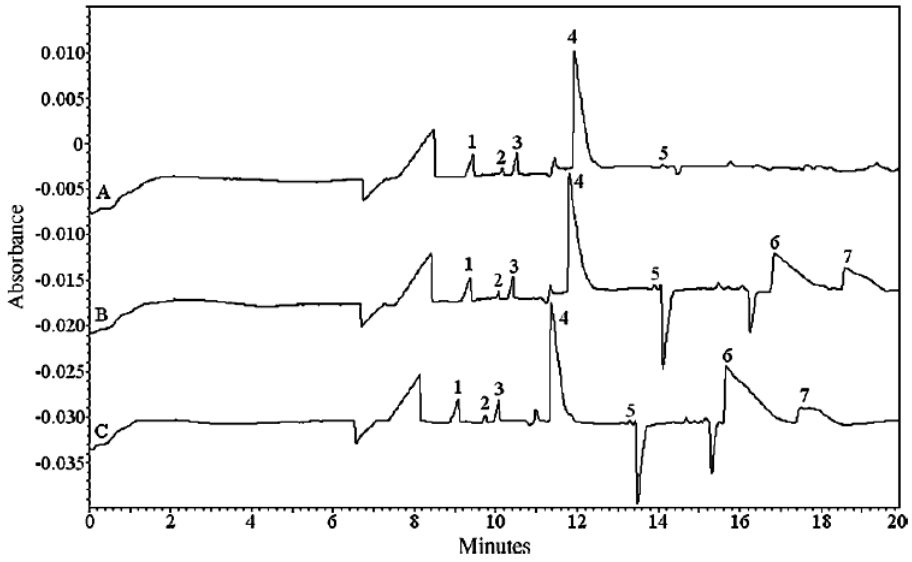


Figure 21. Electrophoretic separation of carboxylic acids in wheat straw hydrolytes and fermentation time A) 0 h; B) 19.8 h; and C) 119.1 h. Peak assignments: 1, formic acid; 2, succinic acid; 3, malic acid; 4, acetic acid; 5, lactic acid; 6, xylonic acid, and 7, gluconic acid [14]. Experimental conditions contain of injection pressure of 0.5 psi for 15 sec. Separation was carried out at -20 kV (negative polarity) at constant temperature of 25 °C and indirect UV detection was at 254 nm.

Table 5. Performance of method under optimized condition in CE instrument [14].

	Formic acid	Malonic acid	Maleic acid	α-Ketoglutaric acid	Succinic acid	Oxalic acid
Migration time (intraday)[a]	0.74	0.78	0.81	0.81	0.82	0.84
Migration time (interday)[b]	3.01	3.12	3.15	3.20	3.21	3.34
Peak area (intraday)[a]	5.34	5.91	4.79	5.11	3.02	4.01
Peak area (interday)[b]	9.34	6.93	7.70	9.00	5.71	6.64
Calibration equation	$y=77.85x+138.79$	$y=76.522x+97.892$	$y=53.912x-10.12$	$y=53.702x-155.72$	$y=82.747x+723.29$	$y=73.784x-77.377$
Calibration correlation coefficient	0.9996	0.9951	0.9984	0.9966	0.9987	0.9921
Detection limit (mg/l)	2	2	2	2	2	2
Quantification limit (mg/l)	5	5	5	5	5	5

	Malic acid	Isocitric acid	Galactaric acid	Acetic acid	Glycolic acid	Propionic acid
Migration time (intraday)[a]	0.85	0.94	0.99	1.01	1.01	1.13
Migration time (interday)[b]	3.37	3.41	3.49	3.55	3.62	3.78
Peak area (intraday)[a]	2.22	5.71	5.76	5.34	2.61	4.30
Peak area (interday)[b]	7.27	7.23	8.12	8.12	6.44	6.81
Calibration equation	$y=63.709x+71.634$	$y=57.933x-172.07$	$y=48.6x-82.184$	$y=124.07x+419.46$	$y=92.3x-60.602$	$y=89.502x+76.24$
Calibration correlation coefficient	0.9992	0.9992	0.9989	0.9968	0.9993	0.9987
Detection limit (mg/l)	2	2	5	2	2	2
Quantification limit (mg/l)	5	5	10	5	5	5

	Lactic acid	Citric acid	Xylonic acid	Arabonic acid	Gluconic acid	Galacturonic acid
Migration time (intraday)[a]	1.14	1.45	1.46	1.46	1.56	1.61
Migration time (interday)[b]	3.91	3.98	4.71	4.73	4.94	5.01
Peak area (intraday)[a]	4.28	4.79	5.89	3.76	5.18	0.99
Peak area (interday)[b]	7.26	7.31	6.43	7.95	7.04	6.30
Calibration equation	$y=97.321x+921.61$	$y=93.506x-278.23$	$y=64.405x-206.7$	$y=70.445x-90.896$	$y=54.059x-166.4$	$y=62.454x-313.05$
Calibration correlation coefficient	0.9988	0.9980	0.9937	0.9995	0.9988	0.9985
Detection limit (mg/l)	2	5	5	5	5	5
Quantification limit (mg/l)	5	10	10	10	10	10

[a] Percentage of relative standard deviation, $n=8$.
[b] Percentage of relative standard deviation, $n=4$.

The calibration data, repeatabilities, correlation coefficients, and reproducibilities of the method are summarized in Table 5. In BGE composition, Ca^{2+} and Mg^{2+} metals are used, because it provide better separation efficiency between organic acids.

In the other article Tahkoniemi et al. [8] monitoring of bioaccumulation of acetic acid, citric acid, Cu, Zn, Co, and Cd in *Rhodococcus sp* are described [8]. The method was applied to monitor bioaccumulation of heavy metals and organic acids isolated from highly polluted soil of Estonia. With CZE technique, it was possible to analysis of heavy metals and organic acid accumulation in on-line mode in a closed reactor. In experimental studya buffer solution made of by pyridine-2,6-dicarboxylic acid (PDCA), and cetyltrimethylammonium bromide (CTAB) was used. Separation voltage with negative polarity and temperature was -24 kV and 25 °C respectively. UV detection was at 230 nm. Electrolytes in the study were: A) 20 m*M* PDCA - 4m*M* CTAB solution at pH 5.70, and B) 17 m*M* PDCA - 4m*M* CTAB solution at pH 6.50 (Figure 22). The results of migration times and peak areas (interday and intraday) with detection limit are listed in Table 6. Detection limits of Cu, Zn, Co, and Cd were 0.46, 0.37, 1.2, and 0.84 mM, respectively.

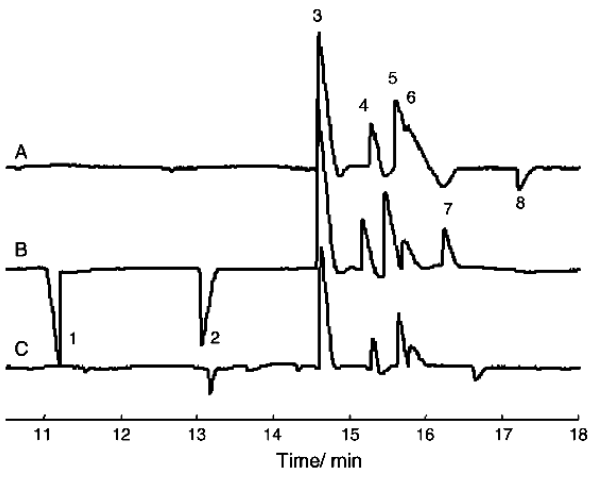


Figure 22. Electropherograms of on-line analyse using (A) real sample, separation not optimized, (B) standard sample with optimum conditions and (C) real sample with optimum condition. Peaks: 1, acetic acid; 2, citric acid; 3, copper; 4, zinc; 5, cadmium; 6, cobalt; 7, calcium, and 8, unknown element. Analysis conditions compose separation voltage with reversed polarity and optimum temperature was -24 kV and 25 °C respectively. UV detection was at 230 nm [8].

Table 6. Performance data in optimum conditions in CE instrument [8].

	Acetic acid	Citric acid	Cu	Zn	Cd	Co
Migration time (interday)[a]	1.67	1.63	1.09	1.39	1.01	0.99
Migration time (intraday)[a]	1.64	1.65	1.73	1.95	1.82	2.08
Peak area (interday)[a]	2.97	3.73	3.53	6.60	6.21	8.09
Peak area (intraday)[a]	2.99	3.75	3.69	7.29	7.19	11.11
b_1[b]	278063	298982	847378	198678	670252	180046
b_0[b]	443563	218162	853840	109611	−116498	−107690
CC[c]	0.985	0.872	0.987	0.956	0.933	0.900
Detection limit (mM)	0.21	0.19	0.46	0.37	1.2	0.84

[a] Percentage of relative standard deviation.
[b] Parameter of calibration line: peak area = $b_0 + b_1 \times$ concentration.
[c] CC, calibration correlation coefficient.

Supplementary according to Dahlman et al [20] analyses of carbohydrates in wood and pulp employs enzymatic hydrolysis and subsequent capillary zone electrophoresis [26]. The idea was to develop supreme method for analyzing the carbohydrate composition of extractive-free delignified wood and pulp. Chemical pulp is effectively hydrolyzed into saccharides using enzymatic hydrolysis. Quantification of saccharides is achieved with high precision to level as low as 0.1%. The electropherogram of separation of 4-aminobenzoic acid ethyl ester (ABEE) derivatives of saccharides by CZE is shown in Figure 23. Experiment conditions include of injection pressure and separation power was of 0.3 psi for 5 sec and 2500 mW respectively. Electrolyte solution made of 438 mmol/L borate at pH 11.5.

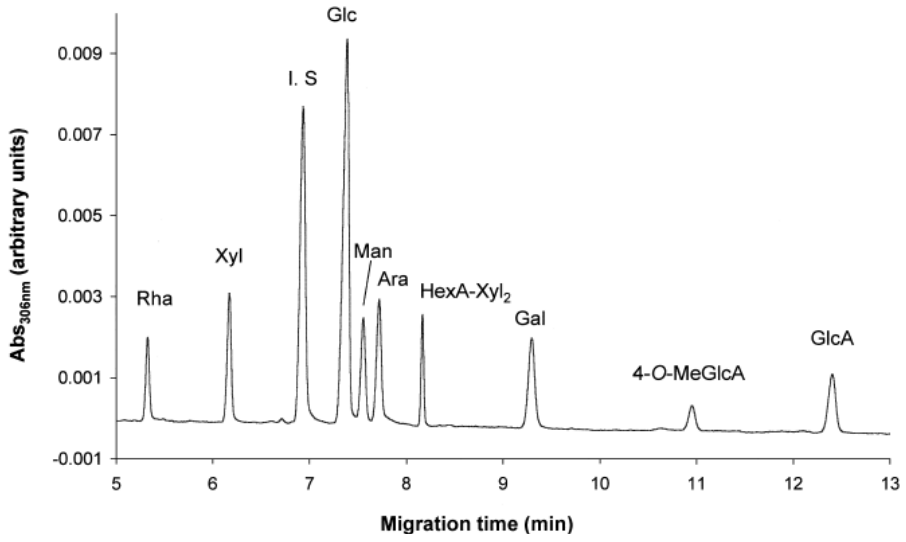


Figure 23. Electropheorgram of the separation of ABEE derivatives of saccharides by CZE technique. Peak identification: Rha = rhamnose, Xyl = xylose, I.S = internal standard, Glc = glucose, Man = mannose, Ara = arabinose, HexA-Xyl_2 = HexA-xylobiose, Gal = galactose, 4-O-MeGlcA = 4-O-methyl-glucuronic acid, GlcA =

glucuronic acid [26]. Experiment conditions include a buffer solution made of 438 mmol/L borate at pH 11.5, injection pressure was 0.3 psi for 5 sec, and separation power employed at 2500 mW.

Table 7 shows repeatability of CZE producers with eight monosaccharides and organic acids.

Table 7. Repeatability of CZE procedure: Quantitation and regression equations of eight monosaccharides by CE [26].

Monosaccharide	Quantitation: RSD (%) at a concentration of		Regression	
			Equation ($y=kx+m$)	Coefficient (r^2)
	$2\cdot10^{-6}$ mol l^{-1}	$5\cdot10^{-3}$ mol l^{-1}		
Rhamnose	8.8	3.5	$y=1.497x-0.001$	0.997
Xylose	2.7	2.4[b]	$y=1.157x-0.096$	0.999
Glucose	4.4[a]	1.4[b]	$y=0.931x-0.109$	0.999
Mannose	4.9	5.4	$y=0.820x+0.026$	0.999
Arabinose	6.0	4.3	$y=0.825x+0.046$	0.999
Galactose	9.6	4.3	$y=0.652x+0.057$	0.998
Glucuronic acid	11.8	2.6	$y=0.935x+0.038$	0.995
Galacturonic acid	2.6	6.7[c]	$y=0.949x+0.011$	0.998

[a] $9\cdot10^{-6}$ mol l^{-1}.
[b] $25\cdot10^{-3}$ mol l^{-1}.
[c] $0.2\cdot10^{-3}$ mol l^{-1}.

Sjöberg et al. [29] made an optimized CZE method for analysis of a mono and oligomeric aldose mixture. The scope of the research work was to achieve an ideal separation of 4-aminobenzoic acid ethyl ester (ABEE) derivatives of all aldopentose, aldohexose and xylose. Linear calibration curves were in the limit of 0.1 – 5 mM (upto 50 mM for glucose and xylose). The electropherogram of mono and oligomeric aldose mixture are presented in Figure 24. Experimental condition for separation of aldose mixture includes a fused silica capillary of 48.5 cm x 30 µm, buffer solution of 450 mM borate at pH 9.7, separation current of 100 µA and injection pressure of 30 mbar for 5 sec. Detection and quantification of derivate carbohydrates was at 305 nm wavelength. For aldopentose the optimized conditions includes of fused silica capillary with internal diameter of 30 µm and total length of 48.5 cm with the same buffer as used in aldohexoses. In order to analyse hexoses the analysis condition contains capillary with internal diameter of 50 µm and total length of 64.5 cm.

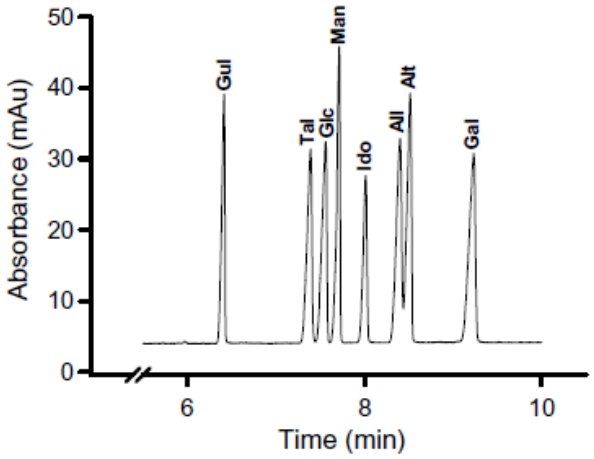


Figure 24. Separation of aldohexoses as their ABEE derivates under optimized CE conditions. Peak identification: gulose (Gul); talose (Tal); glucose (Glc); mannose (Man); idose (Ido); allose (All); altrose (Alt); and, galactose (Gal) [29]. For aldohexoses analysis conditions includes of pressure injection 30 mbar for 5 sec. Buffer solution was made of 450 mM borate at pH 9.7, separation current of 100 µA, and detection signal was at 305 nm.

In results, the electrophoretic mobilities and resolution of aldohexoses, aldopentose, and hexoses are shown on Table 8.

Table 8. Electrophoretic mobility and resolution between adjacent peak of ABEE derivatives of aldopentose and hexose in fused capillary using 450 mM borate buffer at pH 9.7 [29].

No	Carbohydrate	Abbrev.	Electrophoretic mobility,[a] μ_e ($cm^2/V\,s$)		Efficiency,[b] N		Resolution,[c] R_s	
			$\times 10^{-4}$	RSD	$\times 10^5$	RSD		RSD
P1	Lyxose	Lyx	−1.54	0.15	1.5	14	6.4	6.4
H1	Gulose	Gul	−1.67	0.18	1.8	7.7	3.2	3.5
P2	Xylose	Xyl	−1.72	0.19	1.8	8.9	8.4	4.1
P3	Ribose	Rib	−1.85	0.22	1.4	7.1	3.9	3.3
H2	Talose	Tal	−1.92	0.21	0.9	4.3	1.8	1.9
H3	Glucose	Glu	−1.96	0.20	1.3	6.6	2.1	3.5
H4	Mannose	Man	−1.99	0.19	1.0	7.3	1.0	3.1
P4	Arabinose	Ara	−2.01	0.22	2.1	5.3	3.8	6.1
H5	Idose	Ido	−2.06	0.14	1.6	5.7	4.9	3.3
H6	Allose	All	−2.13	0.16	1.1	7.0	1.2	2.9
H7	Altrose	Alt	−2.15	0.16	1.4	6.2	8.3	3.4
H8	Galactose	Gal	−2.27	0.12	1.5	14	6.4	6.4

Averages and relative standard deviations from 10 consecutive runs (injection pressure: 30 mbar for 5 s, applied current: 100 µA) are presented.
[a] $\mu_e = lLV^{-1}\,(t_m^{-1} - t_{EOF}^{-1})$, where l = length to detector, L = total length, V = applied voltage, t_m = migration time, t_{EOF} = EOF time.
[b] $N = 5.54(tw_{1/2})^2$, where $w_{1/2}$ = peak width at half height.
[c] $R_s = 0.5N^{1/2}(\mu_2 - \mu_1)(\mu_2 + \mu_1)^{-1}$ for two adjacent peaks 1 and 2.[14]

There are also articles based on sugars analysis, determination of monosaccharide composition in plant fiber materials by capillary zone electrophoresis that was presented by Rovio et al. [16]. In this study, the CZE method was carried out for monitoring of galactose, glucose, rhamnose, mannose, arabinose, and xylose by using a high alkaline electrolyte solution containing sodium phosphate. The method is fast and accurate, since only sample dilution is required in sample preparation. Analysis condition composed of separation voltage of +17 kV, UV detection at 270 nm with direct mode, injection pressure of 0.5 psi for 6s, total length of capillary of 60 cm, detection length of capillary of 50 cm, separation temperature of 20 ºC were used. Electrolyte solution was made of 130 m*M* NaOH and 36 m*M* $Na_2HPO_4 \cdot 2H_2O$ at pH 12.6. Electropherogram of six monosaccarides used in the experiment on CE instrument is shown on Figure 25.

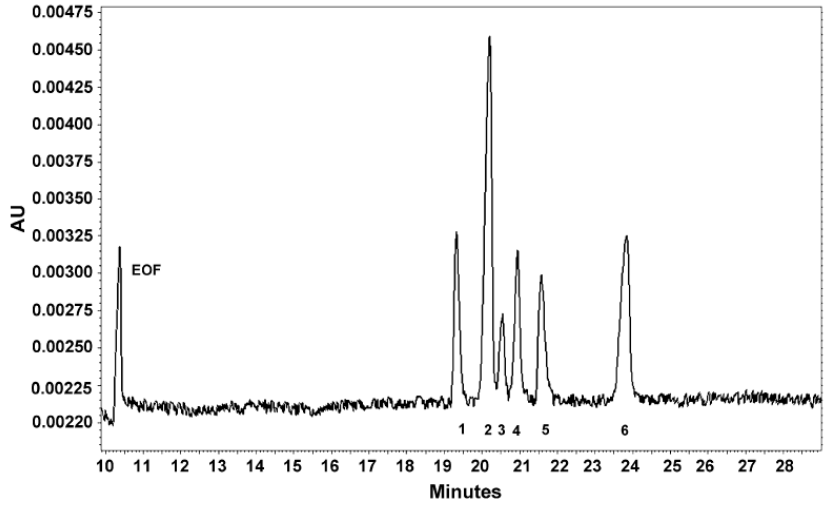


Figure 25. Electropherogram of six monosaccharides. Peak identification: 1, galactose; 2, glucose; 3, rhamnose; 4, mannose; 5, arabinose: 6, xylose. Experimental conditions consist of direct UV detection at 270 nm, optimum temperature and separation voltage was of 20 ºC and 17 kV respectively [16].

The samples were hydrolyzed before used. Acid hydrolysis of extractive-free wood and pulp was two-step hydrolysis with sulfuric acid. Firstly for pre-hydrolysed, oligosaccharides were formed from lignocellulose matrix. Secondly, as per requirement cleaves the bonds between sugars in released oligosaccharides.

Limits of detection, migration times, peak area regressions of all six compounds are listed on Table 9.

Table 9. Mean values, average relative standard deviations (RSD %), regression equations and concentration range of monosaccharaides [16].

Monosaccharide	Concentration range (mg/L)		Migration time[a]		Peak area[a] (Regression)		
			Mean (minute)	Average RSD %	Equation ($y=ax+b$)	Coefficient (r^2)	Average RSD %
D-(+)-Galactose	10–150	Day 1	19.41	0.6	$y=109.42x+231.73$	0.9964	5.7
		Day 5	19.35	0.5	$y=116.06x+186.59$	0.9964	3.3
D-(+)-Glucose	50–400	Day 1	20.13	0.4	$y=85.064x+3251.9$	0.9946	4.8
		Day 5	20.18	0.3	$y=86.935x+4995.4$	0.9971	3.7
L-Rhamnose	10–150	Day 1	20.48	0.4	$y=45.623x-128.12$	0.9967	11.8
		Day 5	20.54	0.3	$y=60.465x-341.51$	0.9906	9.0
D-(+)-Mannose	10–150	Day 1	20.90	0.6	$y=98.478x+23.839$	0.9974	10.1
		Day 5	20.96	0.4	$y=105.55x-23.801$	0.9997	7.9
D-(−)-Arabinose	10–150	Day 1	21.63	0.7	$y=90.125x+103.54$	0.9985	6.9
		Day 5	21.62	0.6	$y=101.04x+249.04$	0.9944	8.3
D-(+)-Xylose	50–400	Day 1	23.71	0.5	$y=49.027x+2724.7$	0.9907	5.1
		Day 5	23.80	0.4	$y=60.512x+1928.2$	0.9906	5.6

With injection of 6 s at 0.5 psi, the limit of detection (LOD) was 5 mg/L for all of the analytes.
[a] Seven concentration levels of standards, two injections/concentration level.

6. Objectives of present study

Specifically, the aim of study was:

1) To develop optimize method for sugars and organic acids in CE.

2) To apply optimize methods for monitoring of sugars and organic acids in the pulp samples obtained from two laboratories that made the fermentation.

3) Quantification and identification of compounds presented in the samples.

7. Experimental

Experimental part consists of instrumentation, chemicals, samples and standards, measurement of pH, capillary conditions, and cleaning. A fused-silica based capillary (50 µm ID and 375 µm OD) was used. For Effective length of fused silica capillary was 70 cm, and detection window length of 61.5cm. For calibration, concentrations of the mix standard sugars and organic acids were prepared from stock solution by diluting with Milli-Q water. Concentrations of 25, 20, 15, 10, 5, 4, 3, 2, and 1 mgL^{-1} were used.

7.1 Instrumentation

List of all instrumentation used in the experiments are shown in Table 10. Throughout the studies a CE 3D (Hewlett Packard, Germany) instrument coupled with diode array detection at wavelength range of 190 - 600 nm was executed. Digital electric power supply up to 30 voltages, an auto sampler (with 48 vials) and HP 3D Chemstation data analysis software were employed.

Table 10: List of instruments and materials along with manufactures details used in the experiment

Sr No	Instruments and materials	Manufactures
1	HP 3D CE Series no A0129	Hewlett Packard
2	Computer (OS Window NT workstation)	Pentium (microprocessor)
2	pH meter	ORION model 410A U.S.A
3	pH electrode	VMR Finland
3	Magnetic stirrer and heater	Amroh Company
4	Ultra-sonication bath	Finnsonic Finland
6	A fused-silica based capillary (50 µm ID and 375 µm OD)	Teknolab AS Trollasen Norway
7	Glass vials (Volume 2.00 ml)	Agilent technology Germany

7.2 Chemicals

All sugars, organic acids and other chemicals used in the experiments are listed in Table 11.

Table 11: List of all chemicals used in the CE experiments

Sr No	Compounds	Suppliers and Countries
	Sugars	
1	D-(-)-Fructose	Sigma Aldrich Germany
2	D(+)-Xylose	Fluka Chemie Switzerland
3	D(+)-Mannose	Fluka Chemie Switzerland
4	D(+)-Cellobiose	Fluka Chemie Switzerland
5	D-(+)-Glucose	AnalaR Normapur Leuven
6	D-(+)-Raffinose	Fluka Chemie Switzerland
7	D(-)-Mannitol	Riedel-de Haen Germany
8	Sorbitol	Sigma Chemical U.S.A
9	Rhamnose	Sigma-Aldrich Germany
10	Sucrose	Sigma-Aldrich Germany
11	Xylitol	Fluka Chemie Switzerland
12	Galactose	Merck Germany
13	Maltose	Merck Germany
14	Arabinose	Sigma-Aldrich Germany
15	Ribose	Merck Germany
16	α-Lactose Monohydrate	Sigma-Aldrich Germany

Table 11. (cont)

Sr No	Compounds	Suppliers and Countries
	Organics	
17	D-Glucuronic	Sigma Chemical U.S.A
18	Oxalic	J.T. Baker Chemical Holland
19	Acetic	J.T. Baker Chemical Holland
20	Propionic	Fluka Chemie Switzerland
21	Formic	Merck Germany
22	Glycolic	Fluka Chemie Switzerland
23	Malonic	Merck Germany
24	Maleic	Sigma-Aldrich Germany
25	Citric	J.T. Baker Chemical Holland
26	L-Glutamic	Sigma-Aldrich Germany
27	Glyoxylic	Merck Germany
28	Tartaric	Sigma-Aldrich Germany
29	Succinic	Fluka Chemie Switzerland
30	Adipic	Fluka Chemie Switzerland
31	Ascorbic	Merck Germany
32	Galacturonic	Sigma-Aldrich Germany
	Other chemicals	
33	Methanol	Merck Germany
34	Ammonium Hydroxide	J.T. Baker Chemical Holland
35	Di-sodium hydrogen phosphate-2-hydrate	Riedel-de Haen Germany
36	Magnesium chloride 6-hydrate	Riedel-de Haen Germany
37	Calcium chloride	VWR International Finland
38	2,3 - pyridinedicarboxylic acid	Sigma-Aldrich Germany
39	Myristyltrimethylammonium hydroxide	Waters Milford
40	Sodium hydroxide	Riedel-de Haen Germany

7.3 Samples

Various fermented pulp samples were collected from UPM pulp and paper company (Kajaani, Finland). Acidic fermentation samples were produced by Membrane technology department at Lappeenranta University (Lappeenranta, Finland) during May and June in 2010. Samples are listed on Table 13 with detail description.

Table 13: Pulp samples with description used in the CE experiments

Sample No	Description
UPM samples	
5 UPM	LPR Cap Red Extract, birch, 10% NaOH, 100 ml bottle
6 UPM	LPR White Cap extract, coniferous, 10% NaOH, 100 ml bottle
7 UPM	TP 11 / 3, the extract, 20% NaOH, hot
8 UPM	Extract, NaOH 30%, 5% pulp
9 UPM	Conc. BEF. DF NaOH in 20% of the hot-birch
10 UPM	Conc. BEF. DF NaOH in 20% of the hot-birch
11 UPM	TP 4 / 5, extract, hot water birch
12 UPM	TP 15 / 6, extract, hot water birch
13 UPM	Extract, coniferous, 10% NaOH
14 UPM	Feed, enriched with 3.5, six, GGM
15 UPM	Process in the future, 3.5, six, GGM
16 UPM	Concentrate 3.5, six, GGM
17 UPM	TP3, extract, NaOH 30% of the birch
21 UPM	Birch, cooking liquor
22 UPM	Havu, the cooking liquor
LUT samples	
I	Original hot-water extraction solution UC030I (prefiltration)
VII	UC030I (concentrate, end, VRF = 5.19)
J	UC030II (concentrate,VRF = 6.44)
12	UC030 PCD (concentrate, VRF = 9.23)
17	RC70PPII (final concentration)

7.4 Measurement of pH

The pH value of background electrolyte buffer solution was measured by ORION model 410A with pH electrode of VMR. It was calibrated with commercial buffer solutions of pH 4.00 (±0.01), 7.00 (±0.01) and 10 (±0.01) (Oy FF-Chemicals AB (Finland)) at temperature of 30°C (± 1) and 25°C (± 1) for sugar and organic buffer solution, respectively.

7.5 Capillary conditioning

A fused-silica based capillary was rinsed with 0.1 *M* NaOH, Milli-Q water, and background electrolyte solution for 20 min each. In order to avoid migration time drift between the analyses, preconditioning of capillary was provided. For sugar analysis, capillaries were rinsed with 0.1 *M* NaOH, Milli-Q water, and the electrolyte solution for 1 min, 2min, and 7 min, respectively.

7.6 Cleaning and other issues

Before starting the analysis, buffer solution was ultrasonicated for 20 min at room temperature and filtrated through 0.25µm disposable membrane filters. Buffer precipitates, salts or dust materials on the electrodes and prepuncher can cause current leakage and can give bad separation results. Cleaning of electrodes and prepunchers were made in ultrasonication bath for 20 min in methanol-water (30:70, v/v). Then after, electrodes and prepunchers were thoroughly dried by air dryer.

8. Sugar analysis

8.1 Reagents and standard preparation

At first, stock solution mixture (10.000 mgL^{-1}) of 16 sugars were prepared into Milli-Q water and stored in refrigerator at temperature of +5 °C. The background electrolyte solution was made of 36 m*M* disodium hydrogen phosphate dihydrate and the pH was adjusted to 12.6 by grout pulverised 130 m*M* sodium hydroxide according to reference [38]. Ionic strength of the buffer solution was 0.238. Main purpose of the use of disodium hydrogen phosphate in the buffer solution was the sodium cations that have intensity to interact with enediolate anions produced to the carbohydrates structures [16]. The carbohydrates were determined and identified as enolate anions.

8.2 Optimized conditions and calibration of sugars

Identification of all sugars was carried out individually based on their migration times. The study shows that sugars alcohols such as like sorbitol and xylitol, cellobiose and lactose, maltose and galactose, and also arabinose and rhamnose migrate closely to each other. Therefore, for best separation efficiency of 16 sugar alcohols and the main sugars, the analyte quantification was split into two groups. The groups are shown in Table 14.

Table 14: List of the analytes groups used in the experiments in the study

Group A					Group B		
Sugar alcohol	Mono saccaride	Di saccharide	Deoxy Sugar	Tri saccharide	Mono saccaride	Di saccharide	Sugar alcohol
xylitol	glucose	sucrose	rhamnose	raffinose	arabinose	cellobiose	sorbitol
mannitol	mannose	lactose			fructose	maltose	
	galactose						
	ribose						
	xylose						

8.2.1 Conditions in capillary electrophoresis analysis

The separation of sugars standards and the analyses of the samples were carried out with an HP 3D capillary electrophoresis (Hewlett Packard) instrument. The separation temperature was 25 °C. Standard solutions were injected at a pressure of 50 mbar for 8

sec with the help of external nitrogen pressure. The outlet pressure of nitrogen gas cylinder was 7.5 bars. Total analysis time was 20 min. The effective separation voltage with positive polarity was 16 kV. Moreover, it was examined that the best photodiode array UV detector wavelength was 270 nm with bandwidth of 20 nm. By online Beckman CE Expert software [41] the hydraulic volume of capillary was calculated to be 9.84 nL.

8.2.2 Calibration

The main purpose of computing calibration data was to determine concentration of substance in unknown samples by comparing with standard solution of known concentration [40]. Key reasons behind calibration were:

1) To calculate standard deviation, average mean and relative standard deviation of all sugar alcohols and sugars with respect to migration time and peak area.
2) To calculate LODs (limit of detection) of each sugars for monitoring sensitivity of CE instrument.
3) To estimate peak area regression equations and co-efficient of each sugars.

The 25 mgL^{-1} sugars mixture groups A (xylitol, raffinose, mannitol, sucrose, lactose, galactose, glucose, rhamnose, mannose, xylose, and ribose), and B (sorbitol, cellobiose, maltose, arabinose, and fructose) electropherogram are illustrated below (Fig. 26 and 27). The results of calibration are based on three sequence run of experiment.

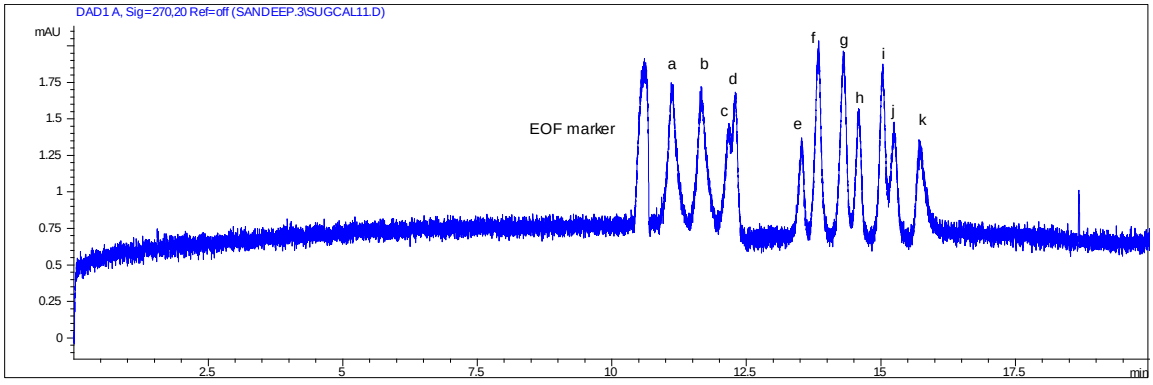


Figure 26. Peak identification: a, xylitol; b, raffinose; c, mannitol; d, sucrose; e, lactose; f, galactose; g, glucose; h, rhamnose; i, mannose; j, xylose; k, ribose experimented on CE.

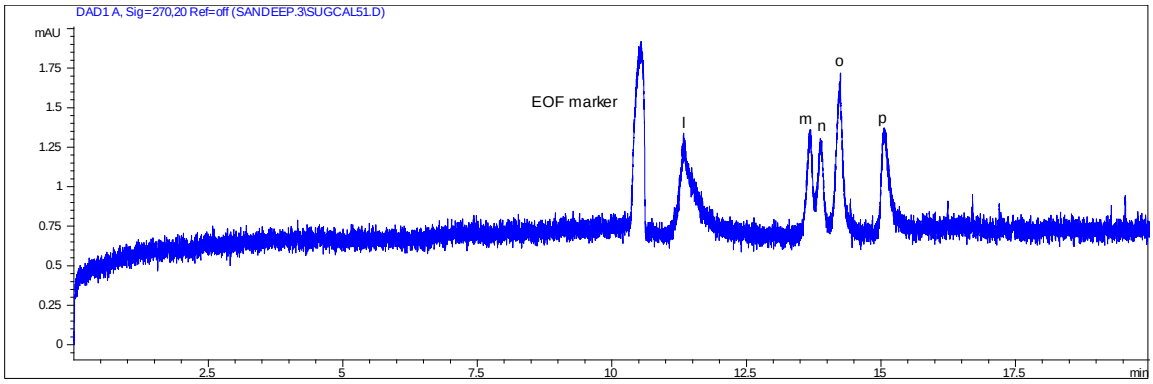


Figure 27. Peak identification: l, sorbitol; m, cellobiose; n, maltose; o, arabinose; p, fructose performed on CE.

The best way to calibrate all analytical data is to use linear regression or least squares analysis [40]. Linear regression can be presented as:

$$A = kx + b \qquad (21)$$

Where: x = concentration (mgL^{-1})

A = peak area (mAUs)

k = slope of line

b = intercept

The standard calibration curves of each mono and disaccharides sugar are shown in Appendix VI. Limit of detection (LOD) was determined by injecting a low concentrate of the working standard solution to obtain a signal about greater than equal to two times of the signal-to-noise ratio. Relative standard deviations were measured from standard deviations of migration times and the peak areas.

Results of linearity data including of limit of detection, mean migration time, migration relative standard deviation, peak area regression equation, peak area standard deviation, and co-efficient of standard solution and samples analysis are shown in Chapter (8.3.1).

8.2.3 Sample preparation for sugar analysis

Birch kraft pulp and coniferous wood were fermented and hydrolysed at UPM (Kajaani, Finland). LUT samples were treated in Lappeenranta University of Technology, Membrane Technology laboratory (Lappeenranta, Finland). All samples are listed in Experimental 7.3. For analysis, all samples were analysed both without dilution and with dilution. Dilution with Milli-Q water was depending upon the quantification of monosaccarides. In most cases, the samples were diluted. Furthermore, spiking method (see Chapter 8.3.2) was used. Detailed information of dilution ratio of each sample is shown in Table 13. It was studied that filtered samples contained less sugars. Therefore, all samples were employed without filtration. The results of samples are based on two sequences.

8.3 Results and discussion

Several experiments were performed to achieve stability of the two methods used in the study. The quantitative results for sugar alcohols and main sugars are compiled in Table 15. In Appendix VI,3 brief details of calibration data are presented.

8.3.1 Calibration data of sugars

Integration of results can be done by calculating peak height or peak areas. However, here calibration calculation was computed by using peak areas. Calibration curve for 16 sugars in a mixture were ranging in concentration from 1 to 25 mgL^{-1}. Figure 28 shows the calibration curve of D-(+)-glucose having correlation coefficient R^2 0.997 and detection limit of 1 mgL^{-1}. Limit of detection (LOD) were calculated by using equation 22.

$$LOD = \frac{A}{\Delta TA_{mean}} \qquad (22)$$

where A = area formed by individual component peak.

ΔTA_{mean} = mean value of area occurred on baseline.

LOD value signal should be greater than that equal to two times of the signal-to-noise ratio.

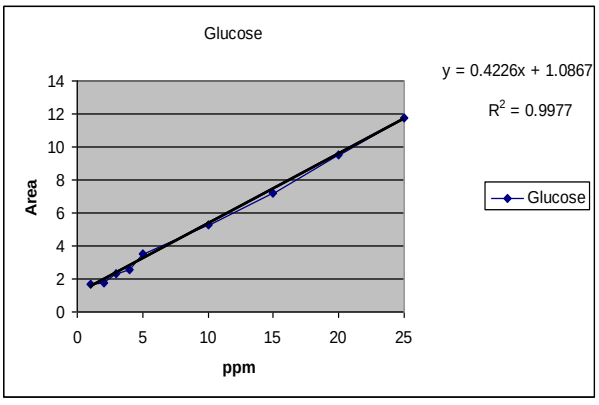


Figure 28. Schematic calibration curve of D-(+)-glucose in CE with optimized conditions (8.2.1).

The average effective mobility μ_e, for each sugar was calculated by using equation 23 [38]:

$$\mu_e = \frac{L_{\det} L_{tot}}{t_R U} - \frac{L_{\det} L_{tot}}{t_{EOF} U} \qquad (23)$$

where L_{det} = length of capillary to detector (m)

L_{tot} = total length of capillary (m)

t_R = migration time of analyte (s)

t_{EOF} = migration time of electro-osmotic marker (s)

U = applied voltage (kV)

Results in Table 12 show that the detection limits of all sugars are quite low (<1 mgL^{-1}) except sorbitol, maltose, and ribose. Relative standard deviations for migration time of the sugars were less than 1.29 % and those for peak areas between 52 % and 73 %. Correlation coefficients for the calibration curves were of the sugars were between 0.971 and 0.998. To attain precision in CE is very essential and several approaches are shown in Chapter 10.

Table 15: Calibration data entail of concentration range, migration time, peak area regression and average mobility of all sugars under optimized conditions shown in Chapter (8.2.1).

Sugars	Limit of detection* range 1-25 mg/l	Migration time	Peak area regression			Average mobility
	(*mg/l*)	*RSD%*	*Equation (y = kx+b)*	R^2	*RSD%*	$(10^{-10} m^2 V^{-1} s^{-1})$
EOF	-	0.48	-	-	2.28	0
Xylitol	1	0.49	y = 0.46x + 1.35	0.99	65.48	-0.20
Raffinose	1	0.56	y = 0.47x + 0.93	0.99	73.98	-0.37
Mannitol	1	0.38	y = 0.20x + 1.17	0.99	56.07	-0.53
Sucrose	1	0.39	y = 0.23x + 0.66	0.99	70.42	-0.54
Lactose	1	0.51	y = 0.18x + 1.33	0.99	52.04	-0.90
Galactose	1	0.60	y = 0.41x + 1.33	1.00	68.55	-0.98
Glucose	1	0.56	y = 0.42x + 1.08	1.00	72.29	-1.09
Rhamnose	1	0.63	y = 0.26x + 0.97	0.99	66.01	-1.15
Mannose	1	0.61	y = 0.34x + 1.39	0.99	63.49	-1.24
Xylose	1	0.70	y = 0.24x + 1.24	1.00	59.46	-1.28
Ribose	3	0.79	y = 0.40x + 0.25	0.97	70.29	-1.37
Sorbitol	3	0.17	y = 0.59x - 0.21	0.99	67.82	-0.26
Cellobiose	1	1.18	y = 0.24x + 0.62	0.97	59.95	-0.98
Maltose	2	1.00	y = 0.18x + 1.06	0.99	56.65	-1.04
Arabinose	1	1.14	y = 0.37x + 0.69	0.98	64.64	-1.11
Xylose	1	1.29	y = 0.28x + 1.41	0.99	61.61	-1.31

* *LOD values with tolerance ±0.10 with signal noice ratio 2.*

8.3.2 Analyses of sugars in real samples

Results of the pulp samples show that xylose, arabinose and glucose are dominant sugars, whereas the amount of maltose and rhamnose are very low. However, in sample 11 any monosaccarides or disaccarides were detected. In LUT samples arabinose, fructose and sucrose were the main monosaccharides and maltose, galactose and xylose were the least existing sugars. Figure 29 presents the electropherogram of the sample 13 (Extract, coniferous, treated with 10% NaOH) with dilution (sample: water 10:90 v/v). The analytes in the samples were identified by comparing their migration times with those of the standard. Figure 29 shows the separation of xylitol, raffinose, sucrose, glucose and xylose. For assurance, spiking method was carried out by injecting low concentration of sugars (xylitol, raffinose, sucrose, glucose and xylose) into samples as shown in Figure 30.

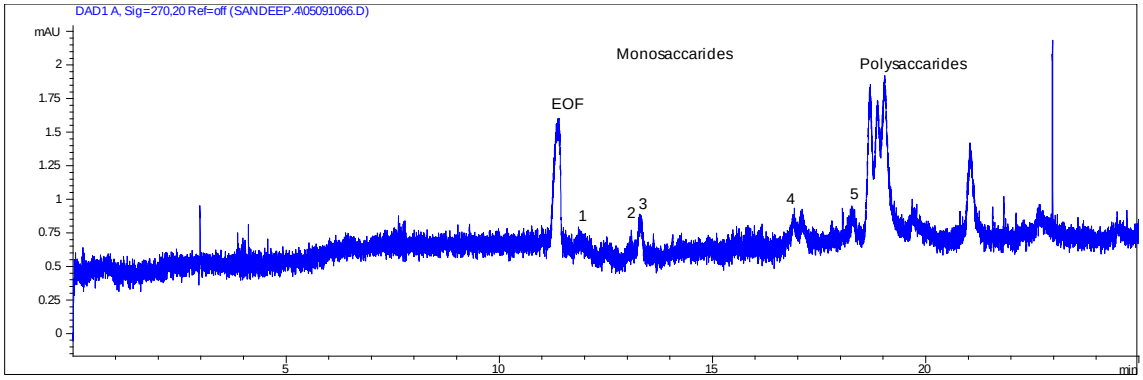


Figure 29. Electropherogram of the diluted sample 13 (Extract, coniferous, treated with 10% NaOH) under optimized condition (Chapter 3.2.1). Peak identification: EOF marker, 1, xylitol; 2, raffinose; 3, sucrose; 4, glucose and 5, xylose.

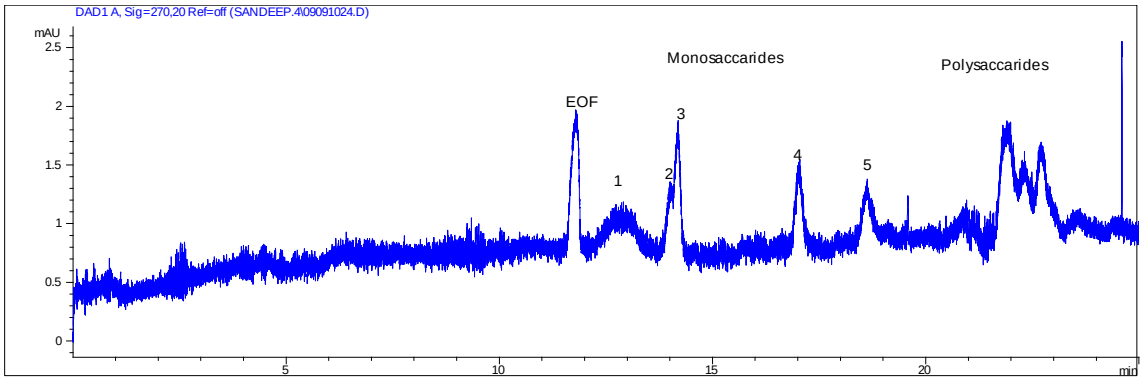


Figure 30. Electropherogram of spiked sample 13 (Extract, coniferous, treated with 10% NaOH). Peak identification: 1, xylitol; 2, raffinose; 3, sucrose; 4, glucose and 5, xylose. Separation conditions: +16 kV, UV-detection at 270 nm (signal) 20 nm (bandwith), injection pressure 50 mbar for 8 sec, capillary 70/61.5 cm (L_{tot}/L_{det}), separation temperature 25 °C.

It was noticed that analytes each of the sugar influences on the separation profile, due to different ionic strength and migration times of sugars in samples. However, EOF marker peak remained stable in timing and assisted to identify the sugar alcohols and main sugars. The identification was confirmed by spiking method. Preparation of spiked solution concentration accomplished deliberately. Total concentration of the sugars was monitored in different samples with respect to the relative standard deviation (RSD) of peak areas and migration times are that shown in Table 16. All samples electropherogram of sugar analysis are shown in Appendix VIII. The total concentrations of sugars in samples were calculated in the original samples and not in diluted samples. It is worth noting that in the pulp samples, sample 8 (Extract, treated with NaOH 30 %, 5 % pulp) there was minor amount of sugars detected whereas, in sample 7 (TP 11/3, the extract, that was treated with 20 % NaOH in hot water) and in the sample 14 (Feed, enriched with 3.5, six, GGM) there was an excessive amount of sugars.

Over again, it is vital to note in LUT samples, sample J (UC030II, concentrate, VRF = 6.44) have a huge amount of sucrose and fructose detected and sample VII (UC0301, concentrate, end, VRF = 5.19) there was an excessive amount of sugars.

Table 16: Total concentration of sugars determined in different samples

Sample	Sugars	Dilution ratio in the analysis *(v/v)*	Peak area *RSD (%)*	Migration time *RSD (%)*	Total concentra-tion in original sample *(ppm)*
5	Xylitol	1:1	30.41	0.93	2
	Raffinose		29.80	1.14	2
	Glucose		18.97	1.27	2
	Ribose		13.21	1.06	2
6	Raffinose	1:5	37.93	2.51	5
	Mannitol		56.18	2.86	7
	Sucrose		58.27	3.08	8
	Arabinose		46.39	2.32	8
7	Sucrose	1:25	56.26	1.32	30
	Mannose		3.43	2.70	5
	Fructose		56.03	1.90	38
	Xylose		42.03	1.86	28
8	Mannose	1:1	32.40	0.79	1
	Xylose		55.12	0.80	2
9	Mannose	1:30	9.61	7.55	24
10	Xylitol	1:30	52.47	0.16	137
	Xylose		5.70	0.47	15
	Ribose		24.3	0.7	41
11	Not detected		Not detected	Not detected	Not detected
12	Xylitol	1:30	8.18	3.39	16
	Maltose		20.02	4.55	27
	Mannose		21.94	5.22	46
13	Xylitol	1:10	39.08	0.74	10
	Raffinose		32.13	1.31	8
	Sucrose		41.88	1.42	11
	Glucose		35.87	2.21	10
	Xylose		29.70	3.09	9
14	Sucrose	1:10	33.45	0.31	9

Table 16. (cont)

Sample	Sugars	Dilution ratio in the analysis (v/v)	Peak area RSD (%)	Migration time RSD (%)	Total concentra-tion in original sample (ppm)
	Cellobiose		27.14	0.33	10
	Rhamnose		17.69	0.32	13
	Xylose		29.01	0.29	12
15	Raffinose	1:2	20.21	1.52	3
	Sucrose		23.29	1.54	3
	Galactose		30.90	1.87	4
	Mannose		20.98	2.11	2
16	Galactose		27.40	0.11	16
	Glucose		25.07	0.37	22
	Mannose		33.29	0.07	20
17	Xylitol	1:2	24.81	1.73	4
	Galactose		25.79	0.78	5
	Mannose		10.47	1.95	2
	Ribose		16.79	1.80	3
21	Xylose	1:40	12.18	2.30	10
	Ribose		19.79	2.33	47
22	Xylose	1:40	15.57	2.75	62
	Ribose		26.15	2.13	47
LUT-I	Galactose	1:10	16.09	1.74	12
	Glucose		30.43	1.80	13
	Xylose		54.74	1.55	18
LUT-VII	Maltose	1:3	10.17	1.56	14
	Arabinose		15.82	1.40	2
	Mannose		32.43	1.36	5
	Xylose		23.14	1.57	2
	Ribose		43.44	1.22	4
LUT-J	Sucrose	1:30	46.36	5.76	47

Table 16. (cont)

Sample	Sugars	Dilution ratio in the analysis *(v/v)*	Peak area *RSD (%)*	Migration time *RSD (%)*	Total concentra-tion in original sample *(ppm)*
	Fructose		67.50	10.08	42
LUT-12	Maltose	1:10	18.02	0.53	5
	Arabinose		19.68	0.47	10
	Xylose		19.85	0.55	13
LUT-17	Arabinose	1:10	19.47	6.12	8
	Mannose		30.39	6.41	7

9. Analysis of organic acids

9.1 Reagents and standard preparation

A stock solution (1000 mgL^{-1}) of 16 organic acids (listed in appendix IV, 1) was prepared in Milli-Q water and stored at 5 °C. For capillary coating buffer solution of 20 m*M* 2,3 pyridinedicarboxylic acid, 30 mgL^{-1} Ca^{2+}, 30 mgL^{-1} Mg^{2+}, and 0.3 m*M* myristyltrimethylammonium hydroxide, in methanol : water solution (10:90, v/v) was composed and pH of 9.0 was adjusted with ammonia (25 %, v/v). Combination of two metals Ca^{2+} and Mg^{2+}, executed because to increase the high separation efficiency [14].

9.2 Optimized conditions and calibration of organic acids

Identification of organic acids was performed by migration times of individual organic acids. In method development, the difficult task was to optimize conditions, and to separate malonic acid from formic acid. Because malonic acid migrates very near to formic acid, better separation efficiency was obtained by increasing the capillary length. In this study it was increased with 10 cm. Figure 31 illustrates an electropherogram of the 16 organic acids at concentration of 25 mgL^{-1}.

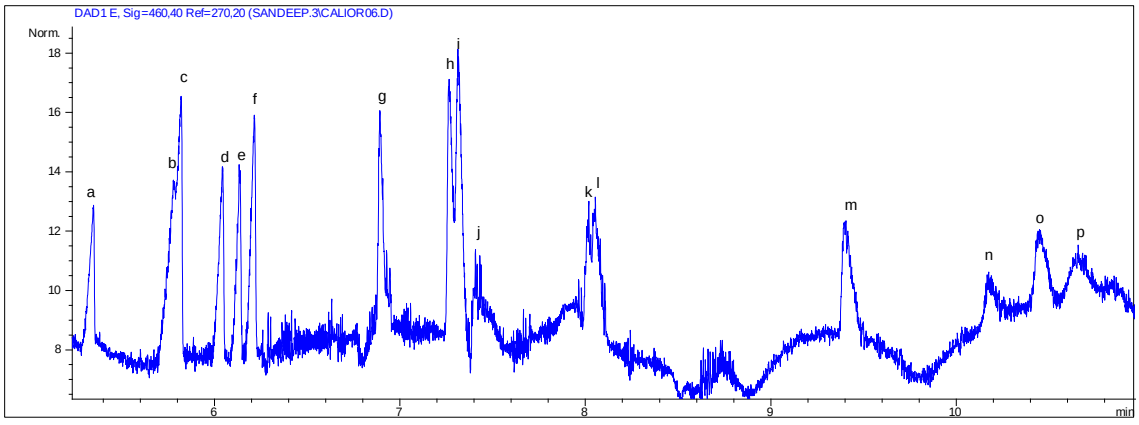


Figure 31. CE electropherogram of 16 organic acids under optimize conditions Chapter (9.3.1). Peak identification: a, oxalic acid; b, malonic acid; c, formic acid; d, maleic acid; e, tartaric acid; f, succinic acid; g, adipic acid; h, glyoxylic acid; i, acetic acid; j, citric acid; k, glycolic acid; l, propionic acid; m, glutamic acid; n, ascorbic acid; o, glucuronic acid, and p, galacturonic acid.

9.3.1 Capillary electrophoresis conditions

All organic acid separations were made with an automated HP 3D capillary electrophoresis (Hewlett Packard) instrument. Effective length of a fused silica capillary was 60 cm, detection length of 51.5 cm and internal diameter of 50 µm. For selectivity of organic acids, various buffer solutions, electrophoretic voltages, wavelengths and temperatures were tested. Finally, the optimum temperature was 20 °C. Injection of samples and standard solutions were at a pressure of 50 mbar for 20 sec with the help of external nitrogen. Best separation voltage with negative polarity was +25 kV. Photodiode array with indirect UV detection during gave the best separation efficiency at signal wavelength of 460 nm (bandwidth 40 nm) to signal wavelength 270 nm (bandwidth of 20 nm). In capillary the hydraulic volume was 17.54 nL [41]. Total analysis time was 15 min (neglecting EOF generating peak, which occurs at 35 min). Preconditioning were conducted by rinse of capillaries with 0.1 M NaOH, Milli-Q water and background buffer solution for 2 min, 3 min and 6 min, respectively. However, at certain time on repetition of analysis with same buffer solution methods provide inaccurate results. To avoid inaccuracy, the capillary was thoroughly rinsed with Milli-Q water for 20 min in reversed flow direction.

9.3.2 Calibration data of organic acids

Calibration data were determined with a standard of the organic acids (see Chapter 8.3.1). The concentrations of 1, 2, 3, 4, 5, 10, 15, 20, and 25 mgL^{-1} of the organic acids were made from the stock solution by diluting it with Milli-Q water. The linear regression represented in Equation 21. The statistical data of calibration includes of the 16 organic acids, limit of detection, migration and relative standard deviation of peak areas, correlation co-efficient of each organic acids that are shown in result Chapter (9.3.1).

9.3.3 Sample preparation for analyses of organic acid

Sample preparations for organic analyses are as mentioned in sugar analysis Chapter. Details on dilution factors and samples are shown on table 17.

9.3 Results and discussion

9.3.1 Calibration data of organic acids

Here calibration data are obtained from 1-25 mgL^{-1} concentration of all organic acids. To achieve precision in CE particularly in organic acids Chapter was crucial part and was done deliberately (see Chapter 10). Limit of dection of most of organic acids were quite low (<1 mg/l) except ascorbic acid, glucuronic acids, and glycolic acid.

Table 17: Calibration data involve of concentration range, migration time, peak area regression and average mobility of all organic acids under optimized conditions (Chapter 9.3.1)

Organic acids	Limit of detection* range 1-25 mg/l	Migration time	Peak area regression			Average mobility
	(*mg/l*)	*RSD%*	*Equation (y = kx+b)*	R^2	*RSD%*	$(10^{-10}m^2V^{-1}s^{-1})$
EOF	-	4.61	-	-	-	0
Oxalic	1	1.38	y = 0.56x + 2.03	0.99	64.53	-4.33
Malonic	1	1.14	y = 1.44x - 0.48	1.00	90.04	-4.05
Formic	1	1.23	y = 0.70x + 2.33	0.99	62.52	-4.03
Maleic	1	1.39	y = 0.75x + 1.97	0.99	69.75	-3.89
Tartaric	1	1.47	y = 0.66x + 1.43	0.99	72.23	-3.84
Succinic	1	1.41	y = 0.87x + 2.18	0.99	70.39	-3.80
Adipic	1	1.81	y = 0.69x + 1.98	0.97	68.99	-3.47
Glyoxylic	1	2.03	y = 0.70x + 3.80	0.96	58.07	-3.31
Acetic	1	2.05	y = 1.89x - 2.26	0.98	99.61	-3.29
Citric	2	2.62	y = 0.48x + 4.79	0.99	40.78	-3.26
Glycolic	5	1.52	y = 0..569x-0.88	0.97	52.22	-3.08

Table 17. (cont)

Organic acids	Limit of detection* range 1-25 mg/l	Migration time	Peak area regression			Average mobility
	(mg/l)	*RSD%*	*Equation (y = kx+b)*	R^2	*RSD%*	$(10^{-10}m^2V^1s^{-1})$
Propionic	1	1.82	y = 0.97x + 1.28	0.99	77.51	-3.04
Glutamic	2	2.36	y = 1.05x + 0.48	0.98	69.59	-2.68
Ascorbic	6	1.36	y = 0.60x - 1.60	0.98	38.77	-2.55
Glucuronic	2	2.44	y = 0.79x + 1.04	0.98	62.43	-2.46
Galacturonic	3	2.17	y = 0.86x + 0.29	0.97	52.29	-2.43

* *LOD values with tolerance ±0.30 with signal noice ratio 2.*

9.3.2 Analyses of organic acids in real samples

In most cases, sample preparation techniques are critical and one has to do it precisely. Good sample preparation method can make difference between accurate results and adjustable results [6]. Specifically, while experimenting of organic acids in the samples, it has been noted that due to long runs at the same buffer solution drastically changes the migration times of compounds. In the consequence, capillary rinsed with Milli-Q water for 20 min by vacuum pressure. As shown in Figure 32 (sample 21, birch, cooking liquor) with dilution of 1:50 (v/v), the peaks were identified by comparison of migration times of compounds in the samples with those in standard organic solutions. In Figure 33, for assertion, spike method of organic acids was implemented with concentration of 25 mgL^{-1}.

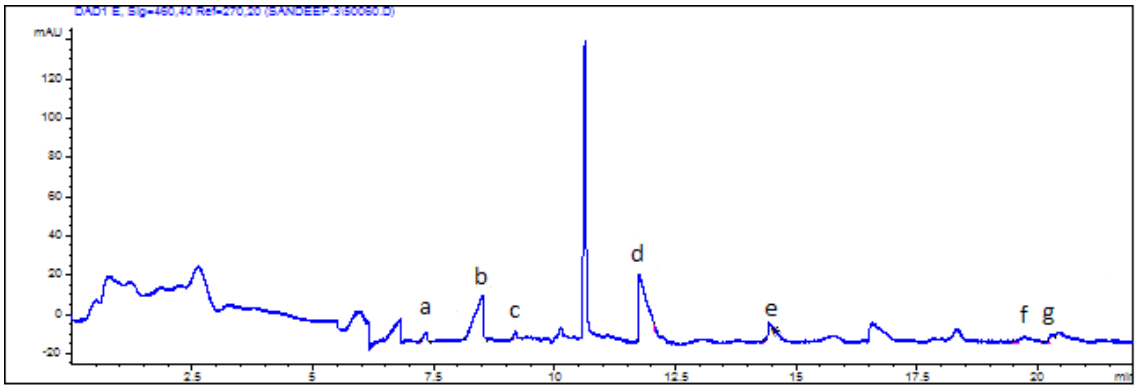


Figure 32 Electropherogram of the sample 21(Birch, cooking liquor) that was diluted in 1:50 (v/v). Peak identification: a, oxalic; b, formic; c, succinic; d, acetic; e, propionic; f, ascorbic, and glucuronic acid.

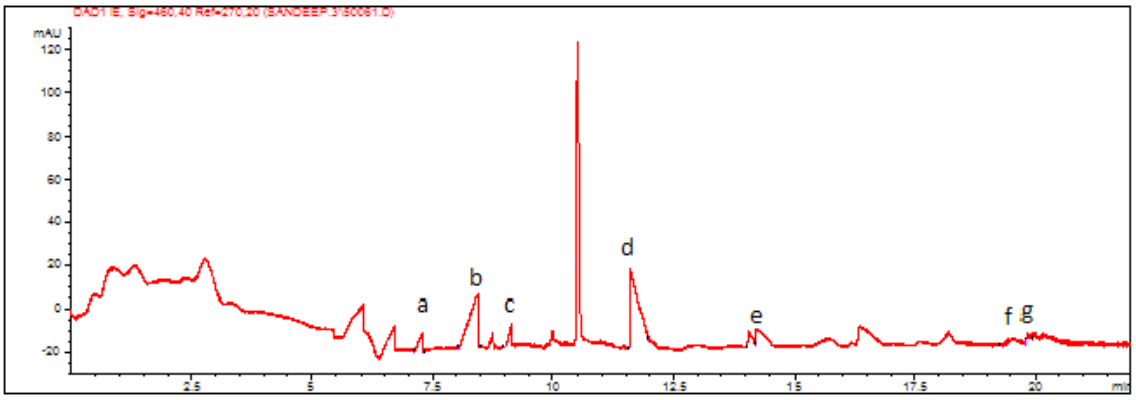


Figure 33. Electropherogram of the sample 21 that was diluted in 1:50 (v/v). Identification with spiking of seven organic acids at concentration of 25 mgL^{-1}. Peak identification: a, oxalic; b, formic; c, succinic; d, acetic; e, propionic; f, ascorbic, and glucuronic acid.

In most of the pulp samples fine peak profiles of unknown organic acids were formed. Most carboxylic acids, like formic, oxalic, acetic, succinic, propionic, and glucuronic were the dominant organic acids whereas citric and galacturonic acids were the minor organic acids. It is worth to note, that the sample 9 (Conc. BEF. DF treated with NaOH in 20% of the hot-birch) and the sample 22 (softwood, cooking liquor) have large amount of organic acids, whereas the sample 16 (concentration 3.5, six, galactoglucamannas) has the least concentration of the analytes.

The LUT samples were an identified and quantified by comparison of migration times with those of real standards solution. The main organic acids determined in the LUT samples were oxalic, formic, propionic and glucuronic acids. The sample LUT 12 (UC030 PCD, concentrate, VRF = 9.23) has a high amounts of organic acids (Table 18).

Table 18: Total concentration of sugars determined in different samples

Sample	Organic acids	Dilution ratio in the analysis *(v/v)*	Peak area *RSD*	Migration time *RSD*	Total concentration in original sample *(ppm)*
5	Oxalic	1:50	54.46	0.30	55
	Formic		47.91	0.26	51
	Acetic		5.58	0.63	29
	Propionic		64.58	0.89	65
6	Oxalic	1:50	32.83	0.45	30
	Formic		28.81	0.41	24
	Acetic		17.99	0.30	23
	Propionic		22.65	0.47	10
	Glucuronic		26.43	1.15	21
7	Oxalic	1:50	28.19	1.13	43
	Formic		10.83	1.42	38
	Tartaric		40.24	1.32	43
	Acetic		5.06	2.10	64
	Propionic		11.77	2.54	47
	Glucuronic		5.85	3.18	27
8	Oxalic	1:10	43.18	0.23	17
	Formic		28.02	0.16	6
	Propionic		49.40	1.11	16
	Glutamic		30.99	0.61	6
	Glucuronic		6.52	0.69	11
9	Oxalic	1:100	44.57	3.33	97
	Formic		40.50	3.68	102
	Succinic		48.29	3.89	101
	Acetic		29.51	4.57	94
	Glutamic		47.40	5.31	120
	Glucuronic		56.23	5.69	128
	Galacturonic		56.48	6.01	132

Table 18. (cont)

Sample	Organic acids	Dilution ratio in the analysis *(v/v)*	Peak area *RSD*	Migration time *RSD*	Total concentration in original sample *(ppm)*
10	Oxalic	1:50	Not detected	Not detected	20
	Acetic		Not detected	Not detected	137
11	Oxalic	1:10	30.46	0.33	8
	Formic		20.53	0.32	6
	Acetic		28.86	0.39	8
	Propionic		15.27	0.25	7
	Glutamic		8.47	0.34	11
	Glucuronic		29.09	0.48	10
12	Formic	1:30	14.73	0.44	10
	Acetic		19.12	0.64	42
	Propionic		23.11	0.71	11
	Glucuronic		22.09	0.93	35
13	Oxalic	1:30	33.94	0.31	23
	Formic		20.85	0.37	15
	Succinic		35.64	0.45	23
	Acetic		3.31	0.91	1
	Glucuronic		20.26	1.69	9
14	Acetic	1:20	65.24	0.69	30
	Propionic		37.57	1.07	28
	Glutamic		70.99	0.61	30
15	Oxalic	1:10	18.92	0.95	11
	Acetic		31.40	0.66	10
	Glucuronic		36.46	1.53	11
	Galacturonic		19.82	1.34	8
16	Acetic	1:30	56.39	1.11	39
17	Formic	1:10	39.80	2.73	19
	Acetic		6.77	2.91	10
21	Oxalic	1:50	25.04	0.10	35
	Formic		10.38	0.15	48

Table 18. (cont)

Sample	Organic acids	Dilution ratio in the analysis *(v/v)*	Peak area *RSD*	Migration time *RSD*	Total concentration in original sample *(ppm)*
	Succinic		43.05	0.18	52
	Acetic		10.45	0.66	46
	Propionic		49.51	0.99	52
	Glucuronic		48.68	0.78	73
22	Oxalic	1:50	39.68	0.34	53
	Formic		18.08	0.23	67
	Succinic		45.05	0.38	53
	Acetic		16.25	0.46	52
	Propionic		39.07	0.69	46
	Glutamic		23.69	0.14	74
	Glucuronic		52.53	0.41	84
LUT-I	Oxalic	1	Not detected	Not detected	24
	Formic				1
	Propionic				2
LUT-VII	Oxalic	1	Not detected	Not detected	30
	Succinic				31
	Acetic				6
LUT-J	Oxalic	1:1	Not detected	Not detected	19
	Malonic				2
	Formic				4
LUT-12	Oxalic	1:0.5	Not detected	Not detected	37
	Formic				46
	Acetic				69
	Glucuronic				103
LUT-17	Oxalic	1:0.5	Not detected	Not detected	16
	Malonic				4
	Formic				3
	Acetic				18
	Propionic				4
	Glutamic				6

All electropherogram of organic acid samples are shown in appendix IX.

10. Precision in CE

For method development and optimization of capillary electrophoresis, several aspects are important. Apparently, there are two major aspects of precision which leads to get precise results in CE [24, 45].

- Migration times
- Peak areas

For achieve precise data of migration time variation, list of troubleshooting are shown in Table 19.

Table 19: List of all troubleshooting occurred with respect to migration time in CE instrument [24, 45].

Sr No	Source	Troubleshooting
1	Overload sample	Dilute sample
2	Wall effects	Use new coated capillaries Reverse wash techniques Increase in preconditioning
3	Buffer problem	Check pH value of BGE Ultra-sonicate BGE daily
4	Temperature	Use constant temperature

To avoid variable results of peak areas, list of remedies are shown in Table 20.

Table 20: List of all remedies formed to improve resolution of peak areas in CE instrument [24, 45].

Sr No	Source	Troubleshooting
1	Injection	Injection time (increase or decrease as per requirement) Rinse of capillary, reduce sample carry over Ultra-sonicate buffer solution
2	Sample evaporation	Use clean caps and vial (avoid salts formation on caps) Clean electrode and prepunchers
3	Sample depletion	Use fresh samples on analysing at room temperature

11. Conclusion

The MSc study shows that capillary electrophoresis (CE) is a separation technique that has not only potential in inorganic metal and species studies, but also in studies of organic compounds with polar and non-absorbing properties. It is a technique that in many cases may be more efficient than high pressure liquid chromatography (HPLC) or gas chromatography (GC). The capacity in CE to separate both charged and non-charged analytes is one of its best advantages of CE over the chromatographic separation technique. Capillary electrophoresis also provides high separation selectivity and rapid analysis times. Moreover, it requires minimal amount of solvents and samples, which enlarge its use in broad fields of chemical and pharmaceutical industries, in bio-chemistry, and in monitoring of environment.

The present study shows that the work to determine selected amount carbohydrates (sugars), sugar alcohols, sugar acids and organic acids in hydrolyzed pulp samples with CE-indirect-UV was fulfilled. The methods used in the study were developed in our earlier studies but here further optimized to get better separation efficiency between coeluting analytes, like formic acid and malonic acid.

For sugar analysis, the method provides an easy and cost-effective determination methodology with UV-detection, low cost electrolyte solutions, and fast analysis time. Under optimized conditions with a photodiode array detector at 270 nm the carbohydrates could be monitored sensitively due to their structure rearrangement during separation in alkaline electrolyte solution under applied electric field. In case or acids, better selectivity could be obtained with addition of metal salts to form complexes with the anlytes acids in the system.

In the report, the limits of detection, co-efficient correlations, regression equations, and relative standard deviations of migration times and peak areas of each sugars and sugar alcohols are well described based on the repetitions, intra and interday analyses. The methods were successfully applied in determination of hydrolyzed pulp samples, and also in spiking method to identify of the targeted analytes.

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Referred articles based on fermentation with details of buffer solution and analysis condition. Appendix I, 1

	Determination	Compounds	Buffer Solution	UV (nm)	pH B.S	Rxn Time (min)	Fermentation	Separation voltage (kV)	Temp (°C)
1	Poly-B-Hydroxybutyric acid in Bacillus thuringiensis Hydrolyzed by H_2SO_4 and $Ba(OH)_2$	Organic acid	5 mM p-hydroxybenzoate 0.5 mM TTAB	254	8 8	6	Bacterial strain was cultivated on PM medium	-15	30
2	Yogurt along with Biacetyl and Carboxylic acid(formic, acetic, citric and lactic)	Organic acid	20 mM PDC 0.5 mM CTAB	220	5.65		Lactobacillus bulgaricus streptococcus thermophilus	-10	25
3	Phenazine-1-carboxylic acid 2-hydroxyphenazine and Phenazine	Hydrocarbon Organic acid	10mM phsophate	0.38 μgmL^{-1} 0.47μgmL^{-1}	7.3	2	Pseudomonas Chlororaphis GP72	Not given	25
4	Glyconic acid, acetic acid, sulfate and phosphate	Sugar acid Organic acid	Benzoic acid, ethylene glycol, disodium tetraborate decahydrate, and hexadecyl-trimethylammonium bromide	220	5.4	120	Pseudomonas sp	-15	Not given
5	Galacturonic, gluconic and glucuronic acid.	Sugar acid	5 mM B-resorcylic acid 1 mM TTAOH	214	3	8	Bacterial oxidation of glucose	-20	200
6	18 carboxylic acid	Organic acid Sugar acid	2,3-PDC acid MTAH	254	8 -- 10	3	G. Oxydans E97003 Wheat straw hydrolyzate	-20	25
7	Shikimic acid, Tartaric acid, Malic acid, Acetic acid and Citric acid	Organic acid Sugar acid	2 mM TTAB p-aminobenzoic acid	260 213	7	4	N.A	-30	70
8	D-(+)-galactose D-(+)-glucose L-rhamnose D-(+)-mannose D-(-)-arabinose D-(+)-xylose	Hydrocarbon	130 mM NaOH 36 mM $Na_2HPO_4.2H_2O$ 450 mM disodium hydrogen-phosphate dihydrate	270	12.6	5	N.A	17	20

9	Oxalic acid malonic acid tartaric acid malic acid	Organic acid	5 mM potassium hydrogen phthalate 0.5 mM CTAB	300	6	Not given	white-rot fungi	-25	10
10	Arabinose galactose glucose manose xylose	Hydrocarbon	CTAB 20 mM phosphate	224	12.1	Not given	N.A	-30	Not given
11	DL-tartaric citric acid malic acid succinic acid	Organic acid	DNB CTAB	254	3.6	20	N.A	-25	Not given
12	Chloride, Nitrate, Sulfate, Oxalate, Formate, Malate, Citrate, Succinate, Pyruvate, Acetate, Lactate, Sorbate, Phosphate and Benzoate	Organic acid Anions	0.1N NaOH	N.G	N.G	8	Fermentation Broth	-30	25
13	D-Glucose, glucose oxidase, L-glutamate oxidase, L-glutamic acid, catechol, dopamine, PDDA and PSS	Hydrocarbon Enzymes	0.01 M NaH2PO4 0.01 M Na2HPO4 0.05 M KCL	N.A.	N.G.	N.G.	Aspergillus Niger Streptomyces	N.G.	N.G.
14	Acetic acid, citric, copper, zinc, cadmium, cobalt, calcium and unknown elemt	Organic acid Elements	17-20 mM PDCA 4 mM CTAB	230	4.5-6.5	3	Rhodococcus sp Bacterial strain	-24	25

Sr No	Determination	Fermenation	Chemicals	Quantity (g/l)	pH	Time (hr)	Temp (°C)	other
1	Poly-B-Hydroxybutyric acid in Bacillus thuringiensis Hydrolyzed by H_2SO_4 and $Ba(OH)_2$	Bacterial strain was cultivated on PM medium	Tryptone Yeast Glucose KH2PO4 $MgSO_4.7H_2O$ $FeSO_4.7H_2O$ $ZnSO_4.7H_2O$ $MnSO_4$	10 2 5 1 0.3 0.02 0.02 0.02	7.2	16	30	Shaken flask (230 rpm)
2	Yogurt along with Biacetyl and Carboxylic acid(formic, acetic, citric and lactic)	*Lactobacillus bulgaricus streptococcus thermophilus*	Milk sample	800 mL	6.98	8	43	Milk sample was mixed with bacteria.
3	Phenazine-1-carboxylic acid 2-hydroxyphenazine and Phenazine	Pseudomonas *Chlororaphis GP72*	King's B medium Pigment producing medium (PPM)	250 mL 150 mL		10 84	28 28	
4	Glyconic acid, acetic acid, sulfate and phosphate	Phosphorus-solubilizing *bacteria Pseudomonas sp. Lactococcus lactis*	Agar medium Peptone Yeast extract D-(+)-glucose salts medium	 12.5 6.25 2.5	N.G.	22	20	
5	Galacturonic, gluconic and glucuronic acid.	Bacterial oxidation of glucose	Not given	N.G	N.G	N.G	N.G	N.G

6	18 carboxylic acid	*G. Oxydans E9700B* Wheat straw hydrolyzate	Yeast $(NH_4)_2SO_4$ $K_2HPO_4.3H_2O$ KH_2PO_4 $MgSO_4.7H_2O$ glycerol glucose xylose biomass	5 5 2 1 0.5 8 1 1 0.1	5.6	48	30	Aeration agitation
7	Oxalic acid malonic acid tartaric acid malic acid	white-rot fungi *Phanerochaete chrysosporium* *Phlebia radiata 79* *Ceriporiopsis subvermispora* *A. Biennis* *B. Adusta*	Low nitrogen asparagine ammonium nitrate dimethylsuccinate medium	250 mL	4.6	N.G	28	
8	Acetic acid, citric, copper, zinc, cadmium, cobalt, calcium and unknown elemt	Rhodococcus sp Bacterial strain	Tryptose Yeast extract glucose agar	5 2.5 1 15	N.G	144	25	Phenol degraded

N.G – Not given

Sr No	Determination	Hydrolyzis Chemical	Pulp or Chemical Sample	Hydrolyzed process	Purpose
1	Poly-B-Hydroxy butyric acid in *Bacillus thuringiensis*	H_2SO_4 $Ba(OH)_2$	Poly-B-Hydroxy-butyric acid	After extraction and purification of PHB from bacteria, the precipitation was suspended in 10.0 ml of 10 *M* H_2SO_4 solution. $Ba(OH)_2$ is used as neutralizer	To get precipitation of PHB. For maintaing pH 7.0 - 8.0.
2	D-(+)-galactose D-(+)-glucose L-rhamnose D-(+)-mannose D-(-)-arabinose D-(+)-xylose	H_2SO_4	Oat spelt, wheat straw, spruce thermomechanical pulp, aspen steam-wood and bleached birch kraft pulp	All pulp samples are extracted with acetone-water mixture(95:5, v/ Sample are treated with 70 %(v/v) H_2SO_4 at 30 °C for 1 hr, then dilu with water and hydrolysis continued in 2.4% at 120 °C for 50 min.	To remove lipophilic. To get glucose, xylose and monosaccharides
3	HexA-xylobiose, galactose, Rhamnose, xylose, glucose, mannose, arabinose, 4-O-methyle-glucuronic acid	Cellulases Hemi-cellulases $C_2H_3NaO_2$	Wood chips and mechanical pulp	10mg wood pulp are added 0.5 ml of enzyme mixture and 0.5 ml sodium acetate buffer stirred 30h at 40 °C at pH 4.0	To get saccharide solution (salts).
4	Arabinose galactose glucose manose xylose	H_2SO_4 or Trifluoro-acetic acid (TFA)	*Picea Abies*, Norway spruce, Isothermal cooking (ITC)	TFA preffered most over to H_2SO_4 because it giving high yield in degradation of carbohydrates. 31 mg of ITC was dissolved in 4 mL 100% TFA for 3 h at 25 °C. Sol diluted twice with 1mL of water and refluxed for 50 min. 700 µL sample of hydrolysate was extracted with 40 µL MTBE for 30 min.	To remove cellulose and hemicellulose To remove lignin residues.

5	galactoglucomannans	H_2SO_4	Thermomechanical pulp.	2.04 mL of 0.5 *M* H_2SO_4 to 8 ml of GGM solution at 120 °C for 40 min and autoclave step. After that hydrolysates are cooled down to room temperature and diluted to final concentration of GGM	To formed GGM salts.
6	Copper and Iron	EDDS acid (Ethylenedi-aminedi succinic acid)	soft wood pulp	20g of pulp are treated with first deionized water and later 8 mM [S,S]-EDDS was added at 45 °C for 3 h. Continous stirr caused the pulp to disintegrate and release metals into aq phase. For maintain pH 7.0, 1.0 MNaOH used.	For disintegrate metal in aq phase
7	Lyxose,gulose, ribose talose, glucose,allose, mannose, arabinose, idose, galactose altrose, xylose.	CH_3COOH, CH_3OH, Cyanoboro-hydride borate buffer	4-aminobenzoic acid ethyl ester (ABEE)	ABEE and acetic acid are added in methanol with ratio of 100 mg/mL. Cyanoborohydride 10 mg/mL was added immediately before mixing 1:1 (v/v) with aq sample containing the reducing sugars at 1 h at 80 °C. Precipitated of ABEE was removed by adding 1 parts of borate buffer.	Aldose are derivated with ABEE
8	Hemicellulose, Hexenuronic acid, 4-O-methyle-glucuronic acid	NaOH Cellulases Hemi-cellulases	Wood chips and mechanical pulp	10mg wood pulp are added 0.5 ml of enzyme mixture and 0.5 ml sodium acetate buffer stirred 30h at 40 °C at pH 4.0. 1 ml of 17% aq NaOH was sucked into the packed sample of pulp (100mg) and extraction allowed to proceed at room temperature for 3 h.	To get saccharide solution (salts) To extract hemicellulose

List of all compounds used in experiment and with manufactures details. Appendix IV, 1

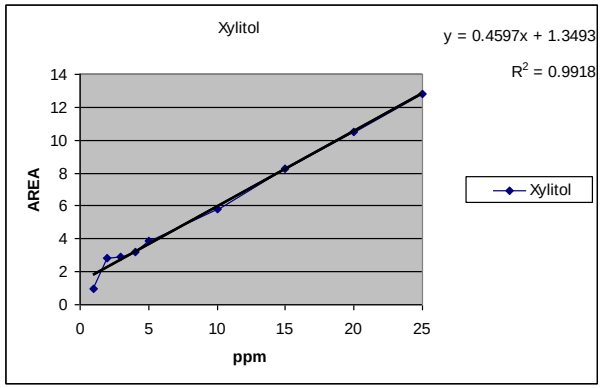
Sr No	Compounds	Manufactures or supplier and Country
	Sugars	
1	D-(-)-Fructose	Sigma Aldrich Germany
2	D(+)-Xylose	Fluka Chemie Switzerland
3	D(+)-Mannose	Fluka Chemie Switzerland
4	D(+)-Cellobiose	Fluka Chemie Switzerland
5	D-(+)-Glucose	AnalaR Normapur Leuven
6	D-(+)-Raffinose	Fluka Chemie Switzerland
7	D(-)-Mannitol	Riedel-de Haen Germany
8	Sorbitol	Sigma Chemical U.S.A
9	Rhamnose	Sigma-Aldrich Germany
10	Sucrose	Sigma-Aldrich Germany
11	Xylitol	Fluka Chemie Switzerland
12	Galactose	Merck Germany
13	Maltose	Merck Germany
14	Arabinose	Sigma-Aldrich Germany
15	Ribose	Merck Germany
16	α-Lactose Monohydrate	Sigma-Aldrich Germany
	Organics	
17	D-Glucuronic	Sigma Chemical U.S.A
18	Oxalic	J.T. Baker Chemical Holland
19	Acetic	J.T. Baker Chemical Holland
20	Propionic	Fluka Chemie Switzerland
21	Formic	Merck Germany
22	Glycolic	Fluka Chemie Switzerland
23	Malonic	Merck Germany
24	Maleic	Sigma-Aldrich Germany
25	Citric	J.T. Baker Chemical Holland
26	L-Glutamic	Sigma-Aldrich Germany
27	Glyoxylic	Merck Germany

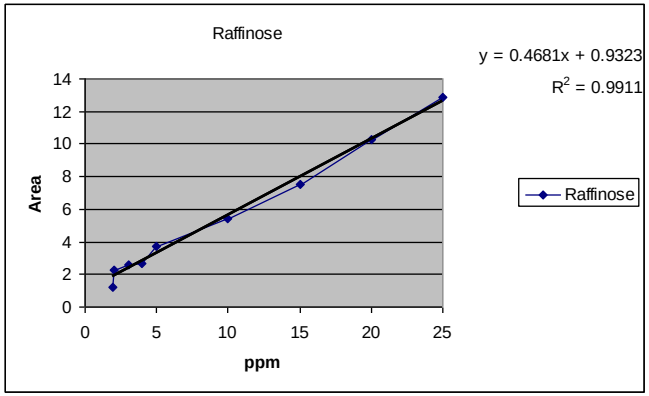
28	Tartaric	Sigma-Aldrich Germany
29	Succinic	Fluka Chemie Switzerland
30	Adipic	Fluka Chemie Switzerland
31	Ascorbic	Merck Germany
32	Galacturonic	Sigma-Aldrich Germany
	Other chemicals	
33	Methanol	Merck Germany
34	Ammonium Hydroxide	J.T. Baker Chemical Holland
35	Di-sodium hydrogen phosphate-2-hydrate	Riedel-de Haen Germany
36	Magnesium chloride 6-hydrate	Riedel-de Haen Germany
37	Calcium chloride	VWR International Finland
38	2,3 - pyridinedicarboxylic acid	Sigma-Aldrich Germany
39	Myristyltrimethylammonium hydroxide	Waters Milford
40	Sodium hydroxide	Riedel-de Haen Germany

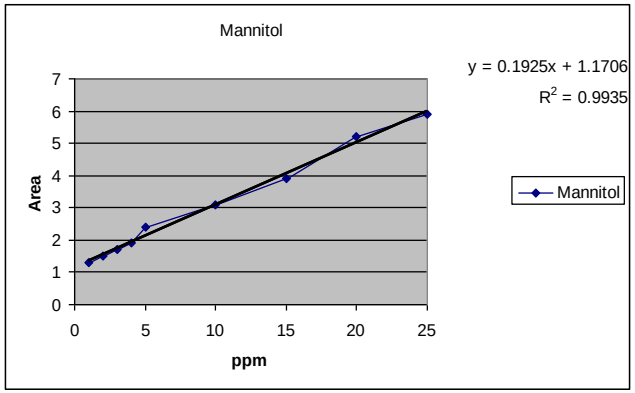
Samples details with description for analysis used in experiment Appendix V, 1

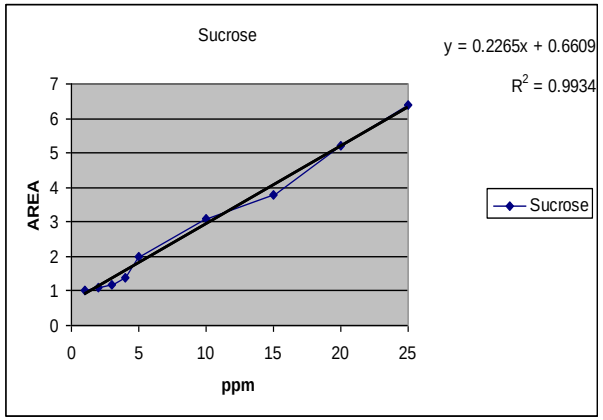
Sample No	Description
UPM samples	
5 UPM	LPR Cap Red Extract, birch, 10% NaOH, 100 ml bottle
6 UPM	LPR White Cap extract, coniferous, 10% NaOH, 100 ml bottle
7 UPM	TP 11 / 3, the extract, 20% NaOH, hot
8 UPM	Extract, NaOH 30%, 5% pulp
9 UPM	Conc. BEF. DF NaOH in 20% of the hot-birch
10 UPM	Conc. BEF. DF NaOH in 20% of the hot-birch
11 UPM	TP 4 / 5, extract, hot water birch
12 UPM	TP 15 / 6, extract, hot water birch
13 UPM	Extract, coniferous, 10% NaOH
14 UPM	Feed, enriched with 3.5, six, GGM
15 UPM	Process in the future, 3.5, six, GGM
16 UPM	Concentrate 3.5, six, GGM
17 UPM	TP3, extract, NaOH 30% of the birch
21 UPM	Birch, cooking liquor
22 UPM	Havu, the cooking liquor
LUT samples	
I	Original hot-water extraction solution UC030I (prefiltration)
VII	UC030I (concentrate, end, VRF = 5.19)
J	UC030II (concentrate,VRF = 6.44)
12	UC030 PCD (concentrate, VRF = 9.23)
17	RC70PPII (final concentration)

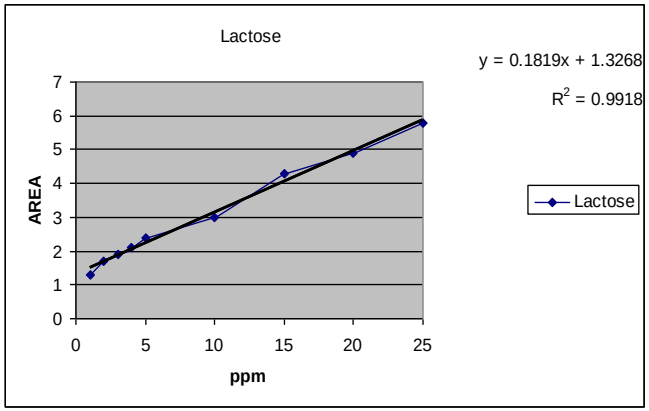
Calibration curve with area regression equation and co-efficient of each sugars

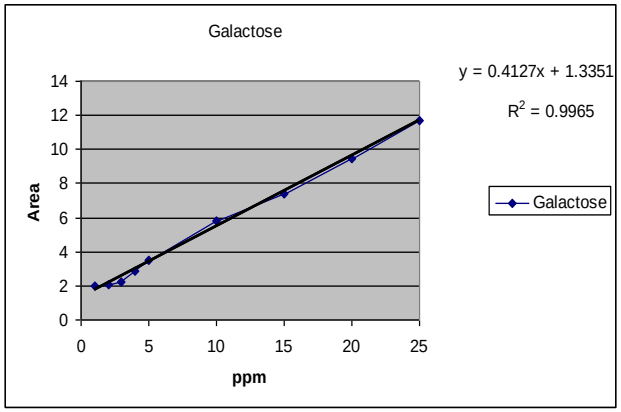


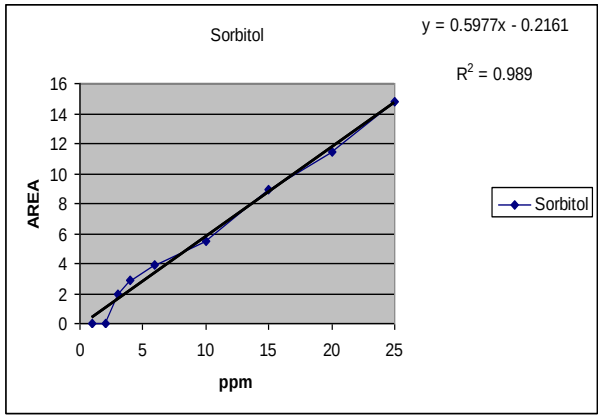


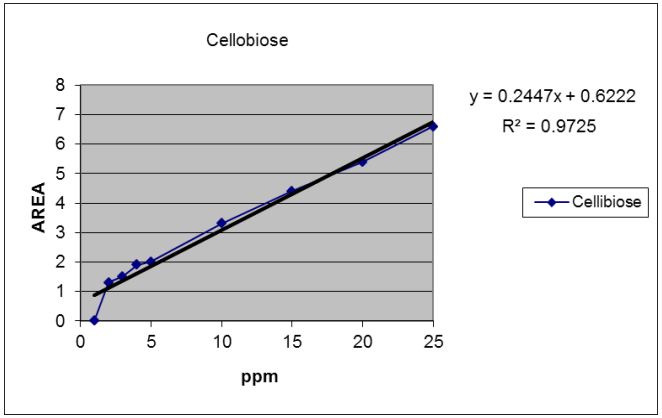


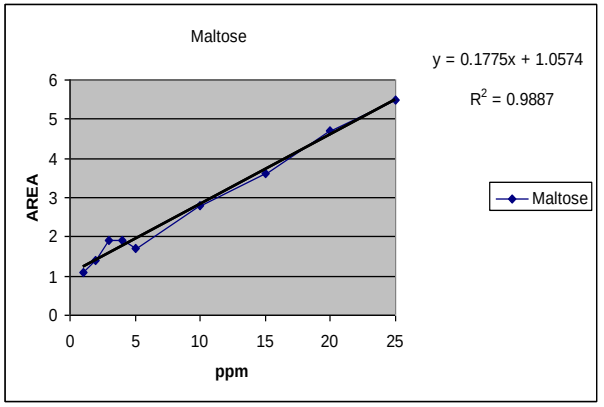


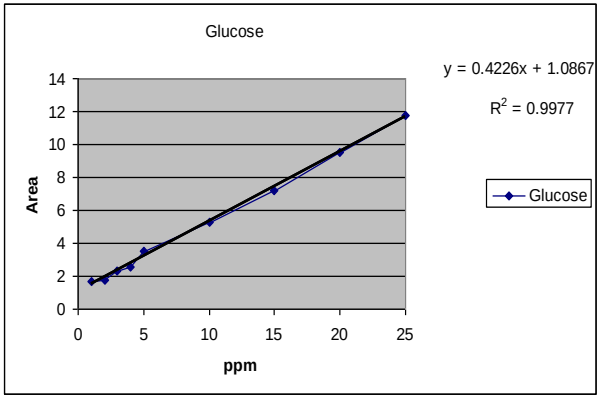


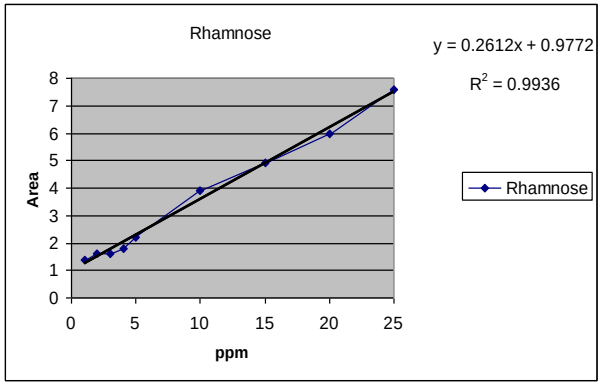


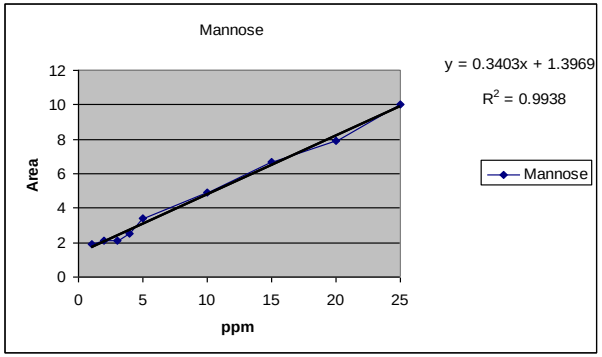


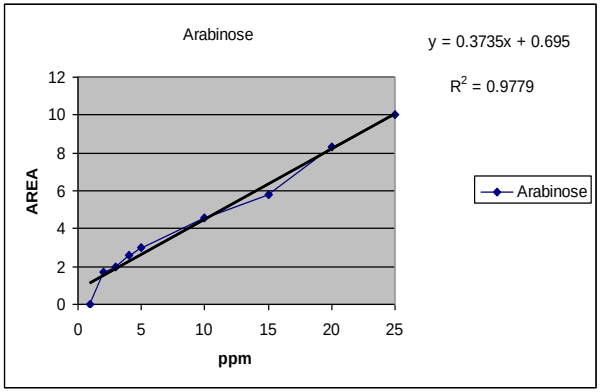


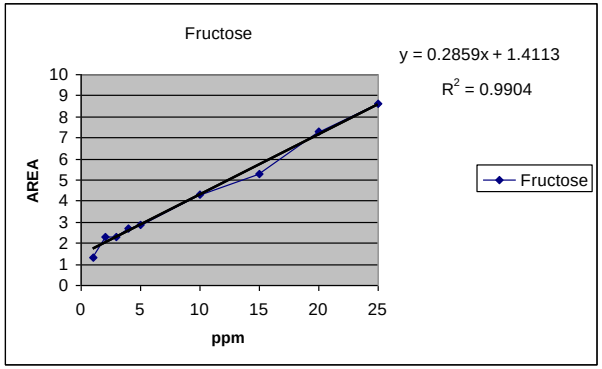


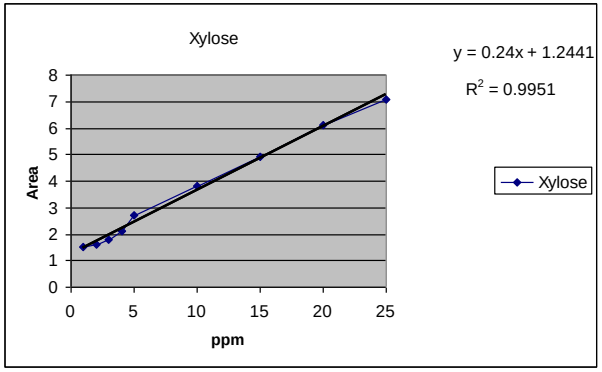

Glucose
y = 0.4226x + 1.0867
R2 = 0.9977
Glucose
Area
ppm

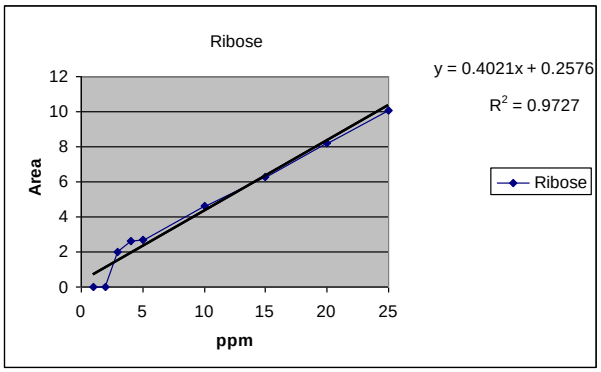

Rhamnose
y = 0.2612x + 0.9772
R2 = 0.9936
Rhamnose
Area
ppm


Mannose
y = 0.3403x + 1.3969
R2 = 0.9938
Mannose
Area
ppm


Arabinose
y = 0.3735x + 0.695
R2 = 0.9779
Arabinose
AREA
ppm


Fructose
y = 0.2859x + 1.4113
R2 = 0.9904
Fructose
AREA
ppm


Xylose
y = 0.24x + 1.2441
R2 = 0.9951
Xylose
Area
ppm


Ribose
y = 0.4021x + 0.2576
R2 = 0.9727
Ribose
Area
ppm

25 ppm A group					
#	Time	Area	Height	Width	Symmetry
EOF	10.59	13.40	1.20	0.19	1.21
Xylitol	11.14	10.50	0.75	0.23	0.72
Raffinose	11.67	12.90	1.00	0.21	0.84
Mannitol	12.18	5.90	0.77	0.13	0.00
Sucrose	12.30	6.40	0.97	0.11	1.24
Lactose	13.53	5.80	0.75	0.13	1.23
Galactose	13.85	11.70	1.40	0.14	1.01
Glucose	14.30	11.80	1.40	0.14	1.05
Rhamnose	14.59	7.60	0.94	0.14	0.64
Mannose	15.04	10.00	1.20	0.13	1.14
Xylose	15.25	7.10	0.86	0.14	0.87
Ribose	15.73	10.10	0.72	0.23	0.21

20 ppm A group					
#	Time	Area	Height	Width	Symmetry
EOF	10.65	13.50	1.20	0.18	0.90
Xylitol	11.14	10.70	0.78	0.23	0.75
Raffinose	11.68	10.30	0.72	0.24	0.61
Mannitol	12.21	5.20	0.68	0.13	0.95
Sucrose	12.34	5.20	0.80	0.11	1.66
Lactose	13.56	4.90	0.65	0.12	1.09
Galactose	13.87	9.50	1.10	0.15	0.91
Glucose	14.36	9.50	1.10	0.15	0.97
Rhamnose	14.63	6.00	0.76	0.13	0.91
Mannose	15.07	7.90	1.00	0.13	0.00
Xylose	15.29	6.10	0.69	0.15	0.07
Ribose	15.80	8.20	0.61	0.22	0.61

15 ppm A group					
#	Time	Area	Height	Width	Symmetry
EOF	10.65	14.00	1.20	0.19	1.99
Xylitol	11.19	8.30	0.58	0.24	1.21
Raffinose	11.69	7.50	0.49	0.25	0.48
Mannitol	12.20	3.90	0.55	0.12	0.84
Sucrose	12.35	3.80	0.60	0.11	0.13
Lactose	13.59	4.30	0.48	0.15	1.17
Galactose	13.90	7.40	0.86	0.14	0.50
Glucose	14.38	7.20	0.89	0.14	1010.26
Rhamnose	14.66	4.90	0.62	0.13	0.89
Mannose	15.11	6.70	0.79	0.14	0.00
Xylose	15.33	4.90	0.56	0.13	0.37
Ribose	15.89	7.70	0.51	0.25	0.75

10 ppm A group					
#	Time	Area	Height	Width	Symmetry
EOF	10.75	13.80	1.20	0.20	2.84
Xylitol	11.29	5.80	0.38	0.24	0.56
Raffinose	11.80	5.40	0.39	0.23	0.07
Mannitol	12.32	3.10	0.39	0.13	1.40
Sucrose	12.46	3.10	0.42	0.12	0.00
Lactose	13.74	3.00	0.36	0.14	0.00
Galactose	14.05	5.80	0.59	0.16	0.80
Glucose	14.52	5.30	0.56	0.16	1.13
Rhamnose	14.83	3.90	0.46	0.14	0.05
Mannose	15.26	4.90	0.61	0.13	0.24
Xylose	15.49	3.80	0.42	0.15	0.64
Ribose	15.99	4.60	0.32	0.24	0.26

5 ppm A group					
#	Time	Area	Height	Width	Symmetry
EOF	10.63	13.70	1.20	0.19	1.41
Xylitol	11.22	3.90	0.25	0.26	0.96
Raffinose	11.83	3.70	0.21	0.30	0.49
Mannitol	12.20	2.40	0.26	0.15	0.97
Sucrose	12.35	2.00	0.35	0.09	1.06
Lactose	13.57	2.40	0.33	0.13	1.03
Galactose	13.87	3.50	0.41	0.14	0.86
Glucose	14.36	3.50	0.40	0.14	0.96
Rhamnose	14.64	2.20	0.37	0.10	0.62
Mannose	15.08	3.40	0.38	0.15	0.40
Xylose	15.31	2.70	0.36	0.13	0.00
Ribose	15.80	2.70	0.22	0.20	0.34

4 ppm A group					
#	Time	Area	Height	Width	Symmetry
EOF	10.68	14.40	1.20	0.19	3.49
Xylitol	11.27	3.20	0.20	0.27	0.39
Raffinose	11.79	2.70	0.21	0.21	0.06
Mannitol	12.21	1.90	0.23	0.15	0.68
Sucrose	12.34	1.40	0.23	0.10	0.23
Lactose	13.60	2.10	0.26	0.16	3.13
Galactose	13.92	2.90	0.32	0.15	0.44
Glucose	14.37	2.60	0.33	0.13	1.01
Rhamnose	14.66	1.80	0.25	0.12	0.38
Mannose	15.11	2.50	0.33	0.12	2482.78
Xylose	15.35	2.10	0.29	0.12	0.88
Ribose	15.81	2.60	0.23	0.15	0.23

3 ppm A group					
#	Time	Area	Height	Width	Symmetry
EOF	10.66	13.90	1.20	0.20	0.45
Xylitol	11.16	2.90	0.19	0.23	1.78
Raffinose	11.71	2.60	0.20	0.20	9.81
Mannitol	12.26	1.70	0.22	0.15	0.93
Sucrose	12.36	1.20	0.19	0.10	0.31
Lactose	13.63	1.90	0.23	0.13	1.69
Galactose	13.93	2.20	0.24	0.15	0.44
Glucose	14.43	2.30	0.30	0.13	1.29
Rhamnose	14.73	1.60	0.24	0.11	1.09
Mannose	15.15	2.10	0.25	0.14	0.34
Xylose	15.39	1.80	0.25	0.12	0.64
Ribose	15.94	2.00	0.18	0.25	0.71

2 ppm A group					
#	Time	Area	Height	Width	Symmetry
EOF	10.71	13.80	1.20	0.19	1.29
Xylitol	11.23	2.80	0.18	0.20	0.54
Raffinose	11.65	2.50	0.19	0.19	0.01
Mannitol	12.29	1.50	0.28	0.09	1.02
Sucrose	12.41	1.10	0.19	0.10	0.00
Lactose	13.70	1.70	0.21	0.12	0.34
Galactose	14.04	2.10	0.26	0.14	0.24
Glucose	14.48	1.80	0.21	0.14	0.42
Rhamnose	14.79	1.60	0.19	0.14	0.01
Mannose	15.29	2.30	0.22	0.16	2.88
Xylose	15.51	1.60	0.21	0.13	0.00
Ribose	nd	nd	nd	nd	nd

1 ppm A group					
#	Time	Area	Height	Width	Symmetry
EOF	10.74	14.20	1.20	0.19	2.22
Xylitol	11.22	1.00	0.13	0.13	0.12
Raffinose	11.70	1.20	0.16	0.16	0.01
Mannitol	12.22	1.30	0.15	0.13	2.82
Sucrose	12.34	1.00	0.23	0.07	0.00
Lactose	13.62	1.30	0.17	0.11	0.20
Galactose	14.05	2.00	0.19	0.17	0.04
Glucose	14.53	1.70	0.17	0.17	0.28
Rhamnose	14.84	1.40	0.19	0.17	0.14
Mannose	15.25	1.90	0.22	0.19	1.44
Xylose	15.55	1.50	0.18	0.15	1.11
Ribose	nd	nd	nd	nd	nd

Peak Area			
	stdev	Mean	RSD (%)
Xylitol	3.57	5.46	65.48
Raffinose	4.01	5.42	73.98
Mannitol	1.68	2.99	56.07
Sucrose	1.97	2.80	70.42
Lactose	1.58	3.04	52.04
Galactose	3.59	5.23	68.55
Glucose	3.67	5.08	72.29
Rhamnose	2.27	3.44	66.01
Mannose	2.94	4.63	63.49
Xylose	2.09	3.51	59.46
Ribose	3.23	5.41	59.63

Migration Time			
	stdev	Mean	RSD (%)
Xylitol	0.05	11.21	0.49
Raffinose	0.07	11.72	0.56
Mannitol	0.05	12.23	0.38
Sucrose	0.05	12.36	0.39
Lactose	0.07	13.62	0.51
Galactose	0.08	13.94	0.60
Glucose	0.08	14.42	0.56
Rhamnose	0.09	14.71	0.63
Mannose	0.09	15.15	0.61
Xylose	0.11	15.39	0.70
Ribose	0.09	15.85	0.58

[nd] not detect

25 ppm B group					
#	Time	Area	Height	Width	Symmetry
EOF	10.60	13.70	1.20	0.19	2.18
Sorbitol	11.39	14.80	0.68	0.36	0.22
Cellobiose	13.74	6.60	0.74	0.15	0.53
Maltose	13.94	5.50	0.72	0.13	0.00
Arabinose	14.30	10.00	1.00	0.16	1.19
Fructose	15.05	8.60	0.70	0.21	0.19

20 ppm B group					
#	Time	Area	Height	Width	Symmetry
EOF	10.54	14.40	1.20	0.20	1.47
Sorbitol	11.36	11.40	0.51	0.37	0.35
Cellobiose	13.71	5.40	0.61	0.15	1.64
Maltose	13.91	4.70	0.58	0.13	0.35
Arabinose	14.26	8.30	0.81	0.17	0.75
Fructose	15.11	7.30	0.57	0.21	0.51

15 ppm B group					
#	Time	Area	Height	Width	Symmetry
EOF	10.56	13.90	1.20	0.19	1.42
Sorbitol	11.36	8.90	0.39	0.34	0.13
Cellobiose	13.73	4.40	0.53	0.14	1.69
Maltose	13.93	3.60	0.44	0.13	0.46
Arabinose	14.29	5.70	0.63	0.15	0.56
Fructose	15.17	5.30	0.46	0.18	0.69

10 ppm B solution					
#	Time	Area	Height	Width	Symmetry
EOF	10.57	14.10	1.20	0.19	0.50
Sorbitol	11.37	5.50	0.29	0.32	0.36
Cellobiose	13.73	3.30	0.38	0.15	0.68
Maltose	13.96	2.80	0.34	0.14	0.00
Arabinose	14.32	4.60	0.48	0.17	0.58
Fructose	15.20	4.30	0.32	0.20	0.00

5 ppm B solution					
#	Time	Area	Height	Width	Symmetry
EOF	9.46	12.70	1.30	0.17	2.10
Sorbitol	10.18	3.90	0.18	0.34	0.00
Cellobiose	12.12	2.00	0.24	0.13	4.11
Maltose	12.30	1.70	0.23	0.11	0.58
Arabinose	12.59	3.00	0.32	0.16	0.00
Fructose	13.37	2.60	0.20	0.22	0.55

4 ppm B solution					
#	Time	Area	Height	Width	Symmetry
EOF	9.34	12.30	1.20	0.17	1.56
Sorbitol	10.07	2.90	0.19	0.25	0.58
Cellobiose	11.95	1.90	0.21	0.16	1.72
Maltose	12.11	1.90	0.20	0.15	0.00
Arabinose	12.40	2.60	0.26	0.16	0.34
Fructose	13.21	2.70	0.18	0.25	0.44

3 ppm B solution					
#	Time	Area	Height	Width	Symmetry
EOF	10.67	12.40	1.20	0.17	2.31
Sorbitol	11.40	2.00	0.17	0.26	0.06
Cellobiose	13.99	1.50	0.22	0.12	0.91
Maltose	14.14	1.90	0.19	0.17	0.26
Arabinose	14.55	2.00	0.23	0.14	0.01
Fructose	15.52	2.30	0.18	0.21	0.01

2 ppm B solution					
#	Time	Area	Height	Width	Symmetry
EOF	10.70	13.30	1.20	0.18	2.08
Sorbitol	nd	nd	nd	nd	nd
Cellobiose	14.04	1.30	0.21	0.11	2.87
Maltose	14.23	1.40	0.21	0.12	0.69
Arabinose	14.64	1.70	0.22	0.13	0.07
Fructose	15.61	2.30	0.19	0.18	0.31

1 ppm B solution					
#	Time	Area	Height	Width	Symmetry
EOF	10.69	12.70	1.20	0.18	2.02
Sorbitol	Nd	nd	nd	nd	nd
Cellobiose	nd	nd	nd	nd	nd
Maltose	14.28	1.10	0.15	0.12	0.02
Arabinose	nd	nd	nd	nd	nd
Fructose	15.64	1.30	0.17	0.13	0.00

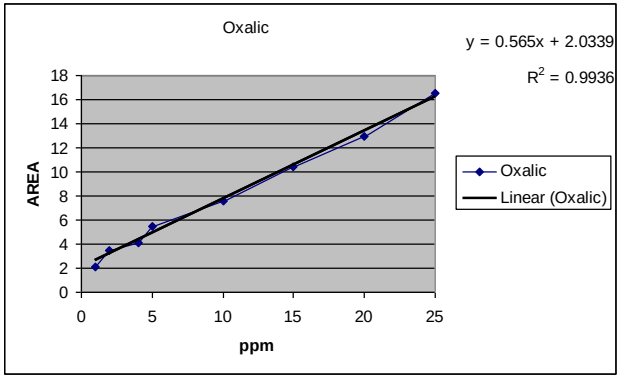
[nd] not detect

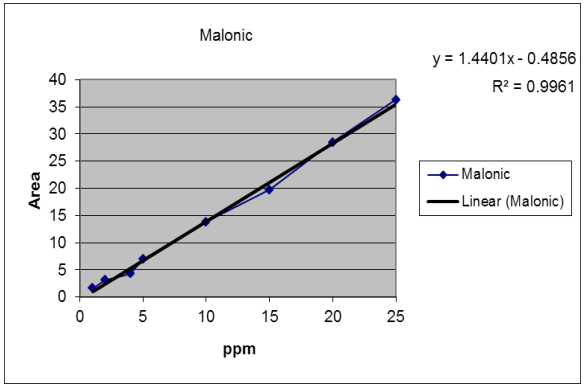
Area peak			
	stdev	mean	RSD(%)
Sorbitol	4.79	7.06	67.82
Cellobiose	1.98	3.30	59.95
Maltose	1.55	2.73	56.65
Arabinose	3.06	4.74	64.64
Fructose	2.51	4.08	61.61

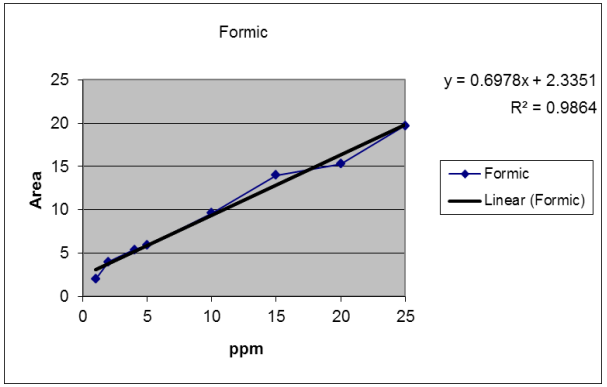
Migration time			
	stdev	mean	RSD(%)
Sorbitol	0.02	11.38	0.17
Cellobiose	0.16	13.92	1.18
Maltose	0.14	14.15	1.00
Arabinose	0.17	14.51	1.14
Fructose	0.20	15.49	1.29

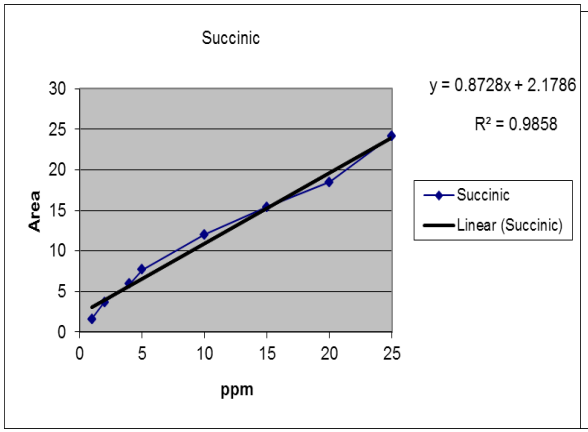
stdev Standard deviation

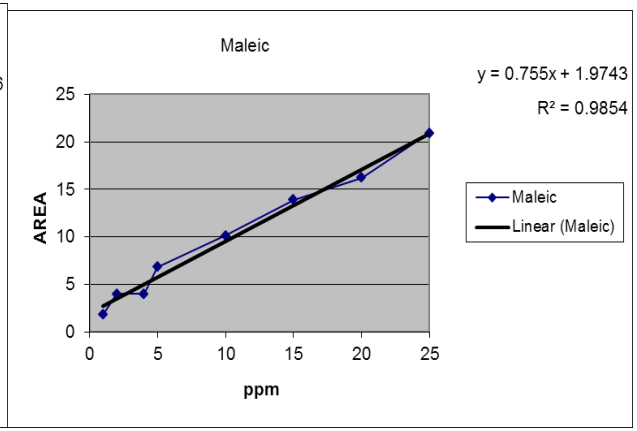
Calibration curve with area regression equation and co-efficient of each organic acids Appendix VII, 1

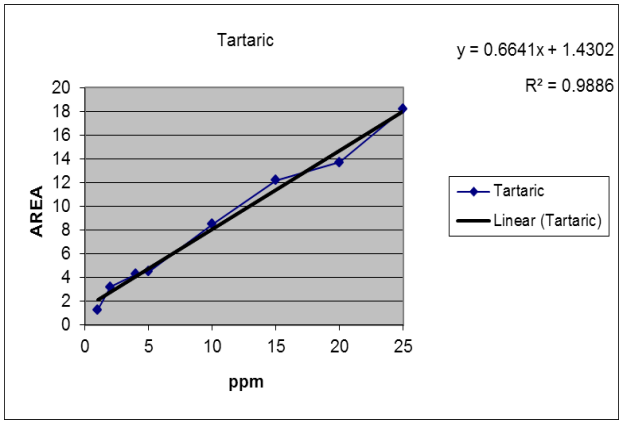


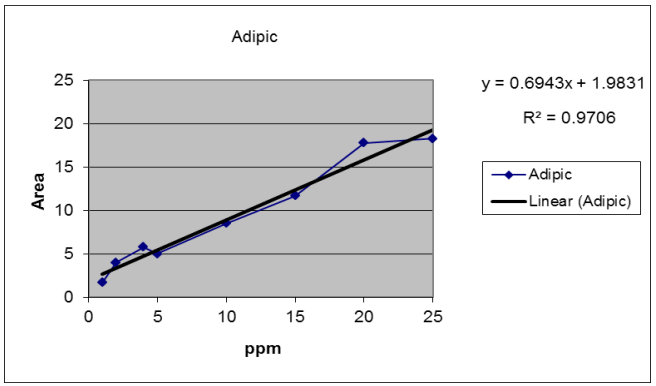


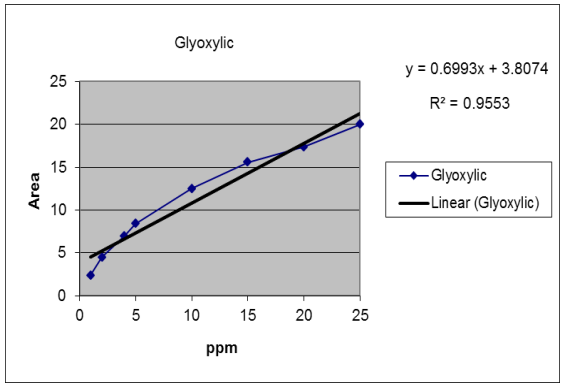


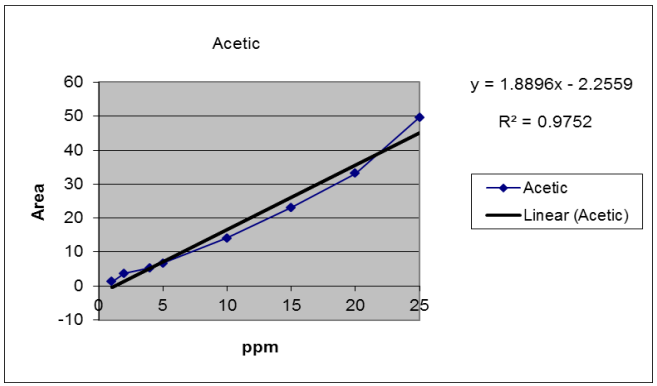


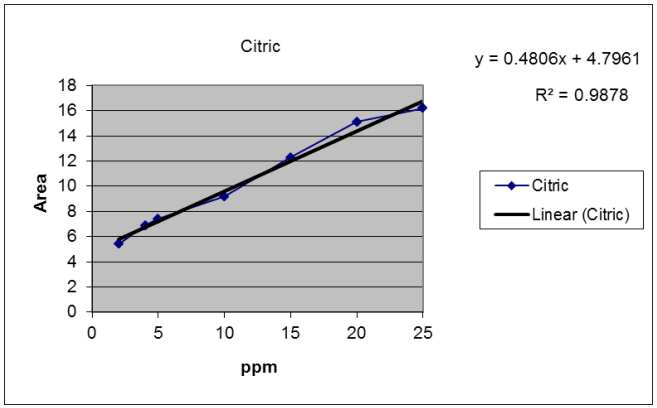


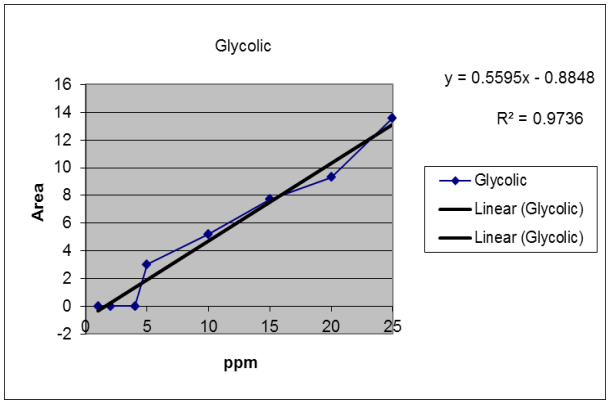


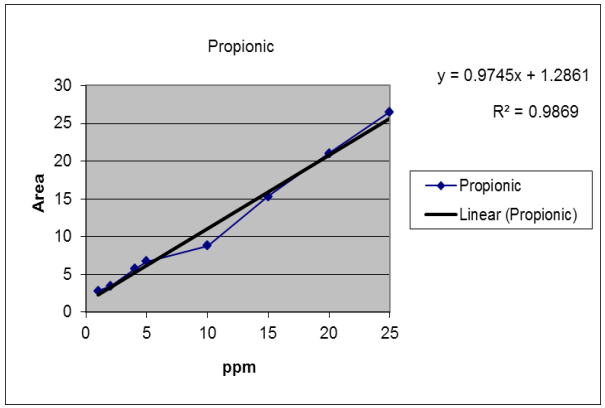

Adipic
y = 0.6943x + 1.9831
R² = 0.9706
Adipic
Linear (Adipic)
Area
ppm

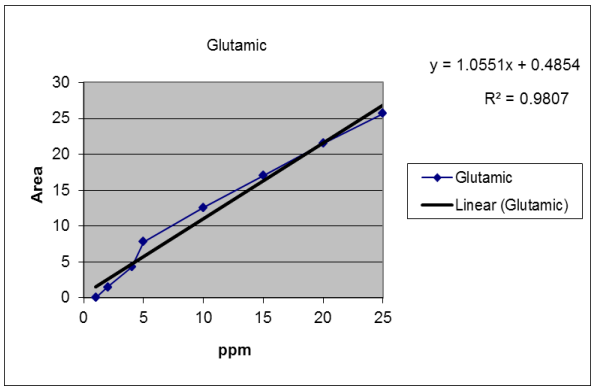

Glyoxylic
y = 0.6993x + 3.8074
R² = 0.9553
Glyoxylic
Linear (Glyoxylic)
Area
ppm

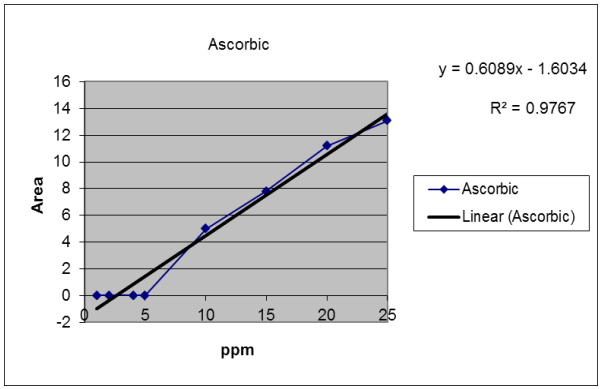

Acetic
y = 1.8896x - 2.2559
R² = 0.9752
Acetic
Linear (Acetic)
Area
ppm

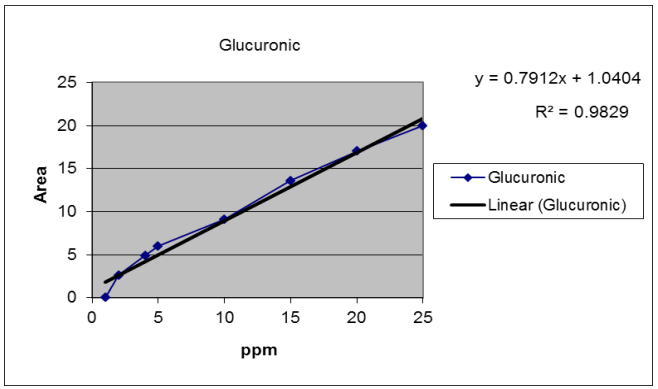

Citric
y = 0.4806x + 4.7961
R² = 0.9878
Citric
Linear (Citric)
Area
ppm

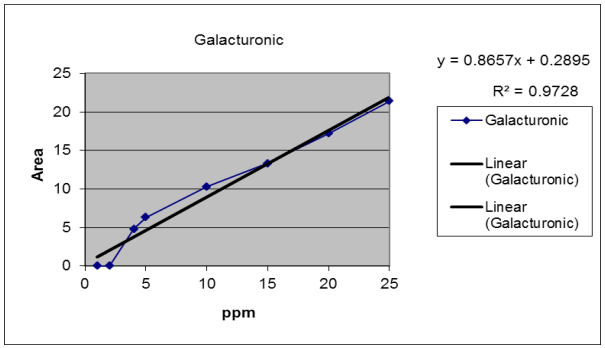

Glycolic
y = 0.5595x - 0.8848
R² = 0.9736
Glycolic
Linear (Glycolic)
Linear (Glycolic)
Area
ppm


Propionic
y = 0.9745x + 1.2861
R² = 0.9869
Propionic
Linear (Propionic)
Area
ppm


Glutamic
y = 1.0551x + 0.4854
R² = 0.9807
Glutamic
Linear (Glutamic)
Area
ppm


Ascorbic
y = 0.6089x - 1.6034
R² = 0.9767
Ascorbic
Linear (Ascorbic)
Area
ppm


Glucuronic
y = 0.7912x + 1.0404
R² = 0.9829
Glucuronic
Linear (Glucuronic)
Area
ppm


Galacturonic
y = 0.8657x + 0.2895
R² = 0.9728
Galacturonic
Linear (Galacturonic)
Linear (Galacturonic)
Area
ppm

25 ppm Organic acids					
#	Time	Area	Height	Width	Symmetry
Oxalic	5.28	16.50	6.70	0.04	5.08
Malonic	5.72	36.30	9.70	0.06	5.13
Formic	5.76	19.70	12.30	0.03	2.74
Maleic	5.97	20.90	9.40	0.04	5.38
Tartaric	6.06	18.20	9.30	0.03	3.56
Succinic	6.14	24.20	12.00	0.03	4.32
Adipic	6.79	18.30	9.30	0.03	0.57
Glyoxylic	7.14	20.00	9.70	0.03	0.53
Acetic	7.21	49.60	13.40	0.06	0.99
Citric	7.26	16.20	3.40	0.08	0.00
Glycolic	7.89	13.60	4.50	0.05	1.31
Propionic	7.93	26.50	8.30	0.05	0.19
Glutamic	9.22	25.70	5.30	0.08	0.22
Ascorbic	9.99	13.10	2.80	0.08	0.43
Glucuronic	10.26	19.80	4.00	0.08	0.37
Galacturonic	10.46	21.40	3.00	0.11	0.53

20 ppm Organic acids					
#	Time	Area	Height	Width	Symmetry
Oxalic	5.31	12.90	5.90	0.04	5.40
Malonic	5.75	28.50	7.80	0.06	3.67
Formic	5.78	15.30	10.50	0.02	2.24
Maleic	6.00	16.20	7.80	0.03	4.87
Tartaric	6.09	13.70	7.90	0.03	3.53
Succinic	6.17	18.50	10.20	0.03	4.14
Adipic	6.83	17.80	8.80	0.03	0.55
Glyoxylic	7.20	17.40	9.60	0.03	0.54
Acetic	7.25	33.10	12.20	0.05	0.61
Citric	7.32	15.10	2.60	0.07	0.06
Glycolic	7.93	9.30	4.70	0.03	1.33
Propionic	7.97	21.00	6.60	0.05	0.36
Glutamic	9.29	21.50	5.30	0.07	0.26
Ascorbic	10.07	11.20	2.90	0.06	0.42
Glucuronic	10.31	17.00	3.50	0.08	0.53
Galacturonic	10.54	17.20	2.30	0.13	0.57

15 ppm Organic acids					
#	Time	Area	Height	Width	Symmetry
Oxalic	5.35	10.40	5.30	0.03	4.65
Malonic	5.78	19.70	6.40	0.05	3.27
Formic	5.82	14.00	9.00	0.03	3.01
Maleic	6.04	13.90	6.70	0.03	3.05
Tartaric	6.14	12.20	6.60	0.03	1.97
Succinic	6.22	15.40	8.30	0.03	2.78
Adipic	6.89	11.70	7.80	0.02	0.89
Glyoxylic	7.27	15.60	8.40	0.03	0.66
Acetic	7.31	23.10	10.30	0.04	0.43
Citric	7.41	12.30	3.30	0.06	0.04
Glycolic	8.01	7.70	4.40	0.03	1.13
Propionic	8.05	15.30	5.20	0.05	0.42
Glutamic	9.40	17.00	4.20	0.07	0.58
Ascorbic	10.18	7.80	2.00	0.07	0.44
Glucuronic	10.45	13.60	2.90	0.08	0.67
Galacturonic	10.66	13.30	1.80	0.13	0.56

10 ppm Organic acids					
#	Time	Area	Height	Width	Symmetry
Oxalic	5.39	7.60	3.90	0.03	4.32
Malonic	5.82	13.80	4.20	0.06	0.00
Formic	5.86	9.60	7.20	0.02	3.32
Maleic	6.09	10.10	5.20	0.03	2.62
Tartaric	6.19	8.50	6.10	0.02	3.02
Succinic	6.26	12.00	6.30	0.03	2.02
Adipic	6.96	8.50	6.90	0.02	0.93
Glyoxylic	7.34	12.50	6.90	0.03	0.63
Acetic	7.40	14.10	5.70	0.04	0.54
Citric	7.50	9.20	1.80	0.08	0.16
Glycolic	8.10	5.20	2.90	0.03	0.00
Propionic	8.12	8.80	3.40	0.04	0.00
Glutamic	9.52	12.60	3.30	0.06	0.49
Ascorbic	10.31	5.00	1.60	0.05	0.76
Glucuronic	10.59	9.10	2.00	0.08	1.04
Galacturonic	10.78	10.30	1.60	0.11	0.13

5 ppm Organic acids					
#	Time	Area	Height	Width	Symmetry
Oxalic	5.43	5.50	3.00	0.03	0.41
Malonic	5.85	6.90	2.90	0.04	1.05
Formic	5.90	7.60	4.90	0.03	2.79
Maleic	6.14	6.80	3.30	0.03	1.52
Tartaric	6.24	4.50	2.90	0.03	1.04
Succinic	6.31	7.70	4.40	0.03	1.14
Adipic	7.03	5.00	3.20	0.03	0.85
Glyoxylic	7.43	8.40	4.70	0.03	0.63
Acetic	7.49	6.70	3.10	0.04	0.75
Citric	7.63	7.40	0.98	0.13	0.23
Glycolic	8.19	3.00	2.10	0.02	0.00
Propionic	8.21	6.70	3.30	0.03	0.68
Glutamic	9.64	7.80	2.00	0.06	0.18
Ascorbic	n.d	n.d	n.d	n.d	n.d
Glucuronic	10.73	6.00	1.50	0.06	0.00
Galacturonic	10.94	6.30	1.10	0.07	0.00

4 ppm Organic acids					
#	Time	Area	Height	Width	Symmetry
Oxalic	5.46	4.10	2.00	0.03	1.22
Malonic	5.88	4.30	2.20	0.03	1.54
Formic	5.93	5.40	3.70	0.02	2.45
Maleic	6.17	4.00	2.80	0.02	2.70
Tartaric	6.27	4.30	2.50	0.03	1.25
Succinic	6.35	5.90	3.30	0.03	1.33
Adipic	7.08	5.80	3.10	0.03	0.94
Glyoxylic	7.49	7.00	3.70	0.03	1.33
Acetic	7.54	5.30	2.60	0.03	0.48
Citric	7.73	6.90	1.20	0.10	0.21
Glycolic	n.d	n.d	n.d	n.d	n.d
Propionic	8.25	5.70	2.60	0.04	0.65
Glutamic	9.74	4.30	1.90	0.04	0.42
Ascorbic	n.d	n.d	n.d	n.d	n.d
Glucuronic	10.82	4.90	1.00	0.08	0.52
Galacturonic	11.06	4.80	1.10	0.07	0.37

$^{n.d}$ not detect

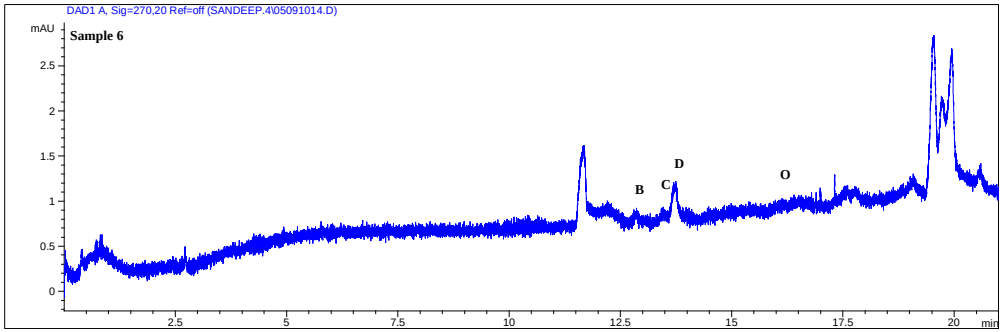
2 ppm Organic acids					
#	Time	Area	Height	Width	Symmetry
Oxalic	5.49	3.50	2.00	0.04	1.67
Malonic	5.90	3.10	1.90	0.03	1.13
Formic	5.95	4.40	2.40	0.03	0.82
Maleic	6.20	4.00	2.40	0.03	0.19
Tartaric	6.30	3.20	1.40	0.04	0.86
Succinic	6.38	3.70	1.80	0.03	0.84
Adipic	7.11	4.00	1.90	0.04	0.40
Glyoxylic	7.53	4.50	2.20	0.03	0.62
Acetic	7.58	3.70	1.60	0.04	0.36
Citric	7.75	5.40	1.20	0.08	0.11
Glycolic	n.d	n.d	n.d	n.d	n.d
Propionic	8.31	3.40	1.50	0.04	0.62
Glutamic	9.81	1.50	1.10	0.02	0.20
Ascorbic	n.d	n.d	n.d	n.d	n.d
Glucuronic	10.94	2.60	0.80	0.05	1.42
Galacturonic	n.d	n.d	n.d	n.d	n.d

1 ppm Organic acids					
#	Time	Area	Height	Width	Symmetry
Oxalic	5.45	2.10	0.95	0.04	0.96
Malonic	5.87	1.60	1.20	0.03	7.25
Formic	5.91	2.00	1.30	0.03	1.05
Maleic	6.16	1.80	0.88	0.04	0.00
Tartaric	6.28	1.30	0.66	0.03	0.54
Succinic	6.34	1.60	1.20	0.02	1.32
Adipic	7.10	1.70	0.83	0.03	0.28
Glyoxylic	7.52	2.40	1.10	0.04	0.81
Acetic	7.59	1.30	0.63	0.04	2.20
Citric	n.d	n.d	n.d	n.d	n.d
Glycolic	n.d	n.d	n.d	n.d	n.d
Propionic	8.30	2.80	1.20	0.03	2.20
Glutamic	n.d	n.d	n.d	n.d	n.d
Ascorbic	n.d	n.d	n.d	n.d	n.d
Glucuronic	n.d	n.d	n.d	n.d	n.d
Galacturonic	n.d	n.d	n.d	n.d	n.d

n.d not detect

Migration time			
	stdev	mean	rsd%
Oxalic	0.07	5.39	1.38
Malonic	0.07	5.82	1.14
Formic	0.07	5.86	1.23
Maleic	0.08	6.10	1.39
Tartaric	0.09	6.19	1.47
Succini	0.09	6.27	1.41
Adipic	0.13	6.97	1.81
Glyoxy	0.15	7.36	2.03
Acetic	0.15	7.42	2.05
Citric	0.20	7.51	2.62
Glycoli	0.12	8.03	1.52
Propior	0.15	8.14	1.82
Glutam	0.23	9.52	2.36
Ascorb	0.14	10.14	1.36
Glucuro	0.26	10.59	2.44
Galactu	0.23	10.74	2.17

Area peak			
	stdev	mean	rsd %
Oxalic	5.05	7.83	64.53
Malonic	12.85	14.28	90.04
Formic	6.10	9.75	62.52
Maleic	6.77	9.71	69.75
Tartaric	5.95	8.24	72.23
Succinic	7.83	11.13	70.39
Adipic	6.28	9.10	68.99
Glyoxylic	6.37	10.98	58.07
Acetic	17.05	17.11	99.61
Citric	4.22	10.36	40.78
Glycolic	4.05	7.76	52.22
Propionic	8.74	11.28	77.51
Glutamic	8.99	12.91	69.59
Ascorbic	3.60	9.28	38.77
Glucuronic	6.51	10.43	62.43
Galacturon	6.39	12.22	52.29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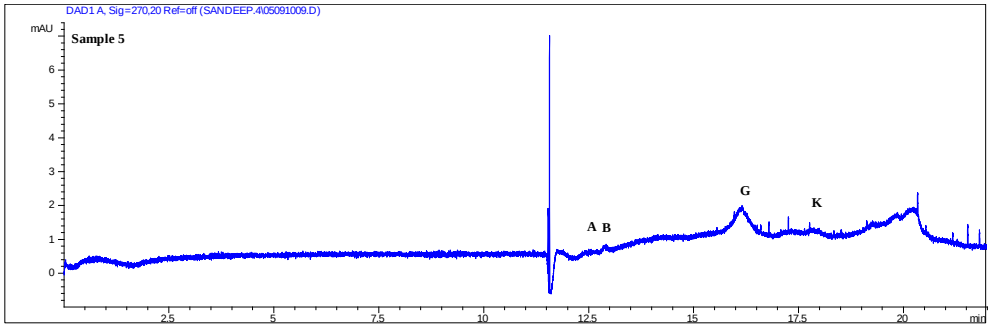
Sample 6
mAU
B
C
D
O
min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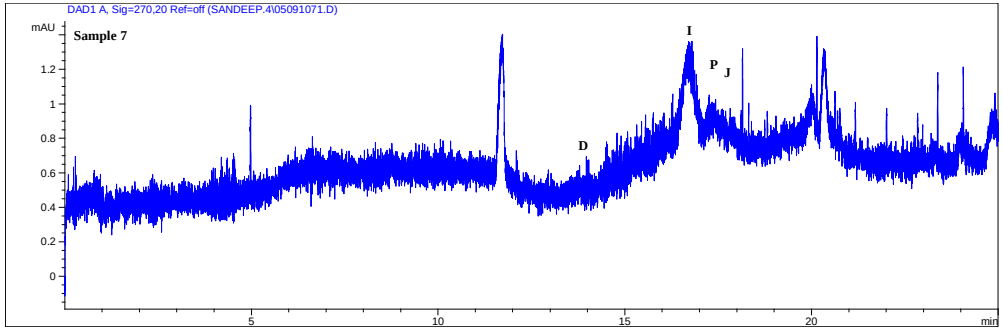
Sample 5
mAU
A
B
G
K
min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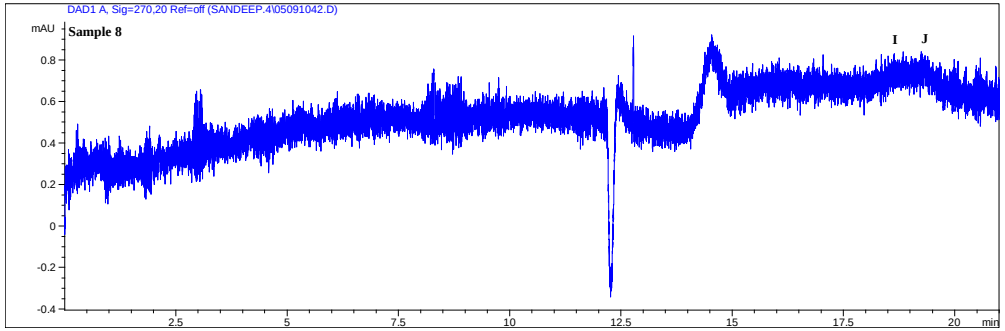
Sample 7
mAU
D
I
P
J
min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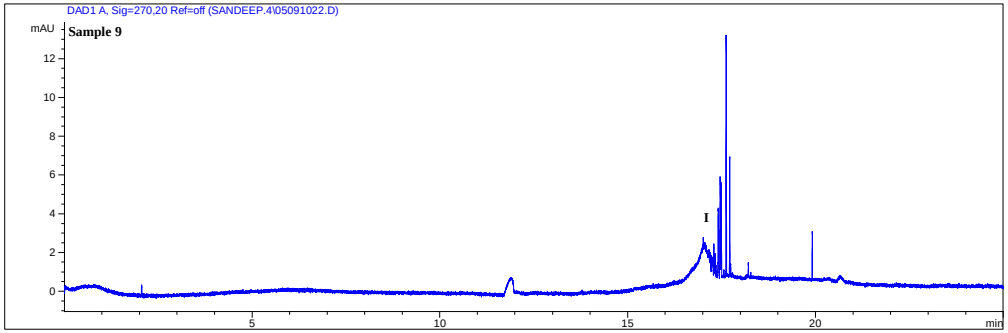
Sample 8
mAU
I
J
min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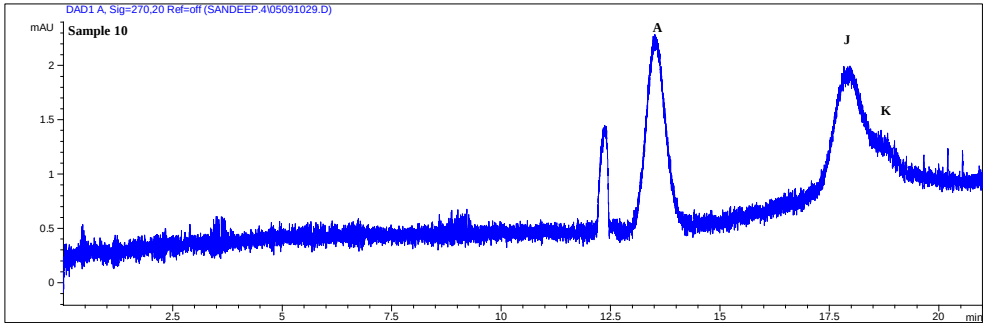
Sample 9
mAU
I
min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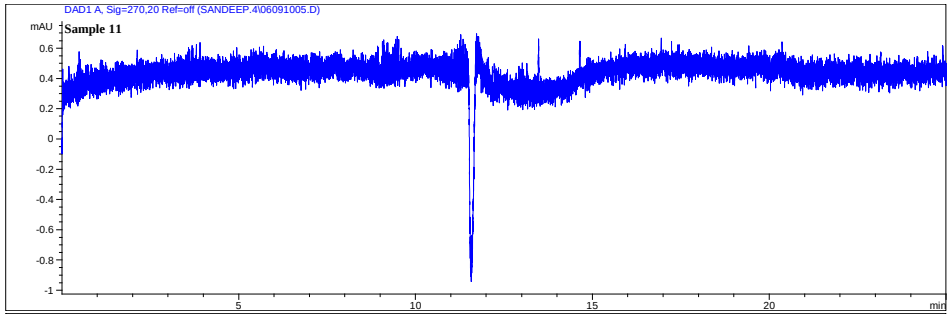
Sample 10
mAU
A
J
K
min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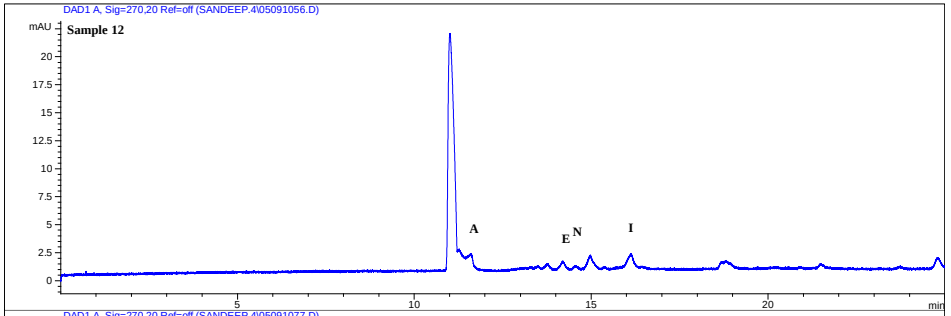
Sample 11
mAU
min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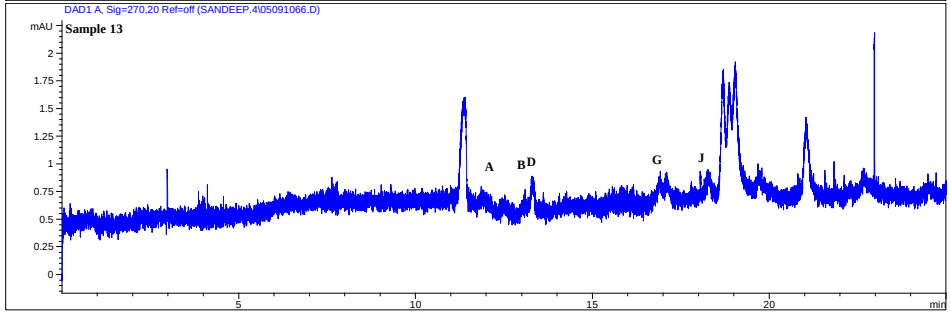
Sample 12
mAU
A
E
N
I
min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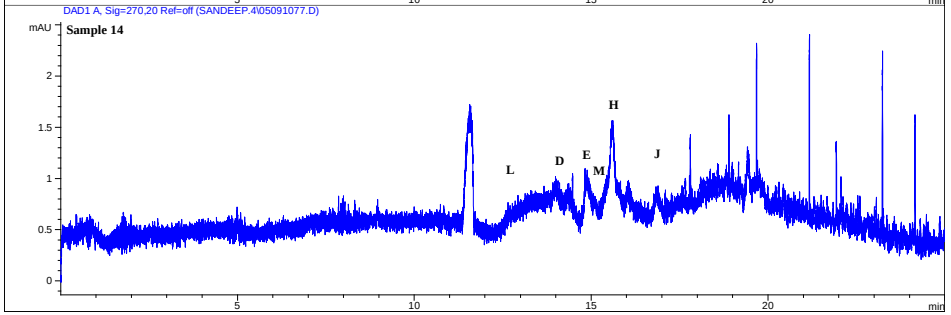
Sample 13
mAU
A
B
D
G
J
min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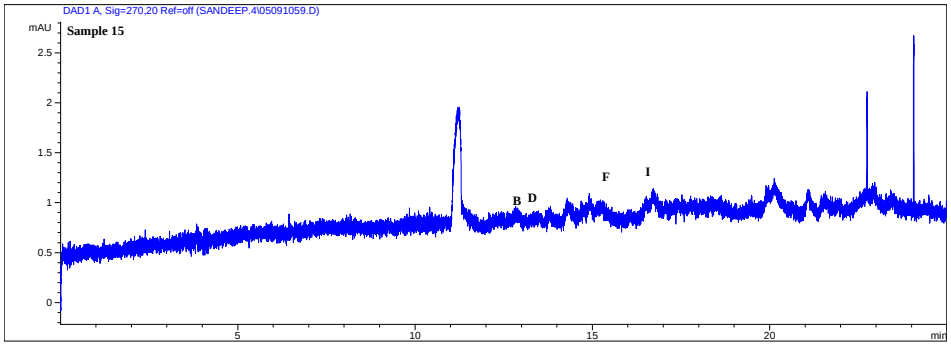
Sample 14
mAU
L
D
E
M
H
J
min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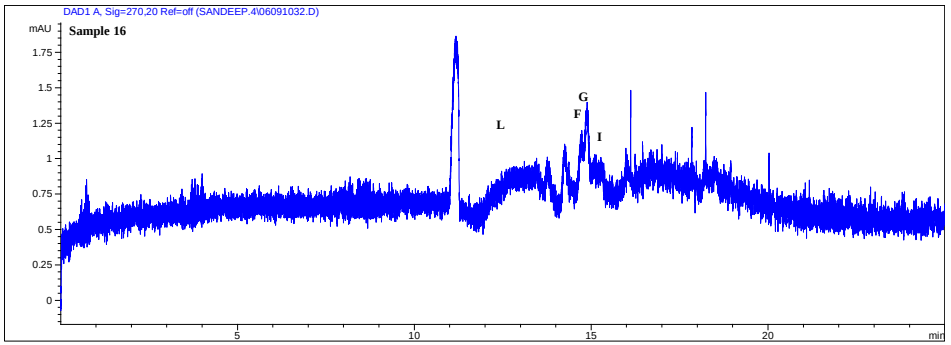
Sample 15
mAU
B
D
F
I
min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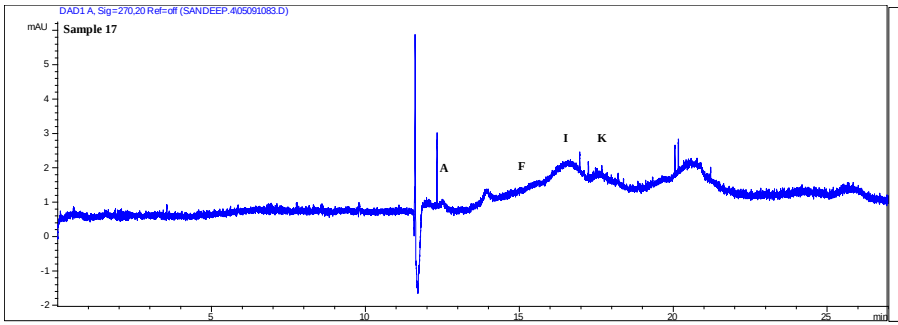
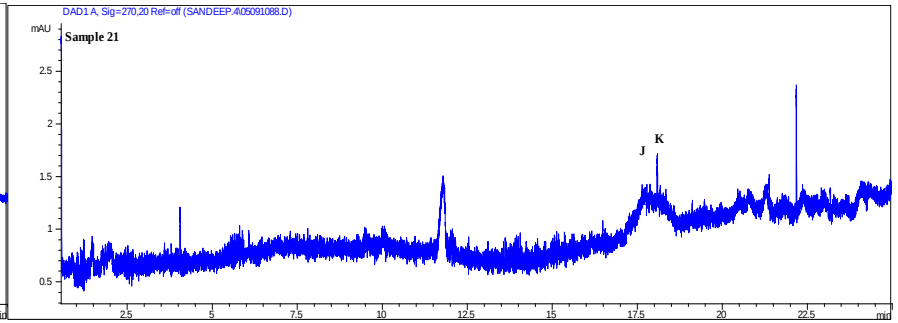
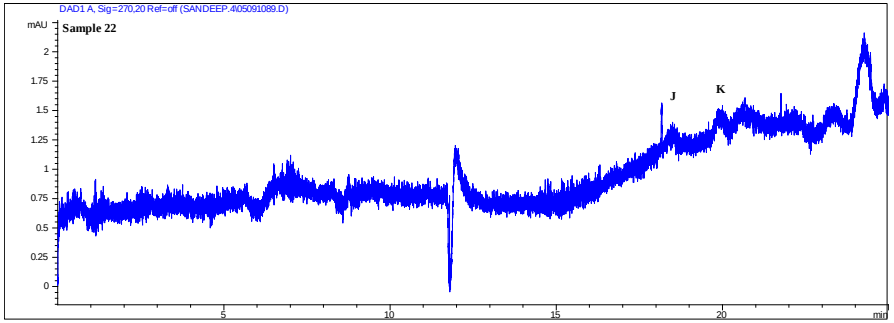
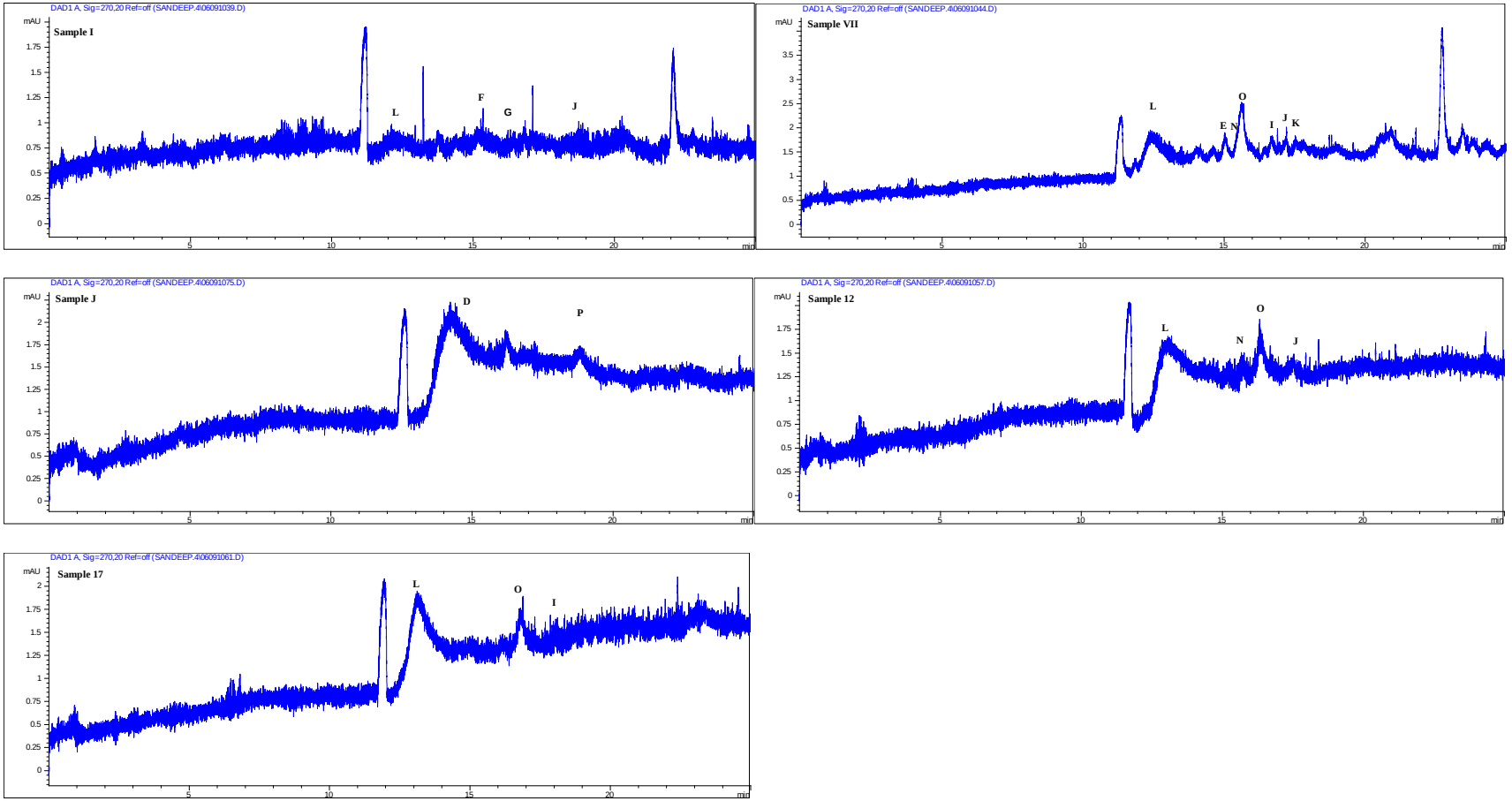
Sample 16
mAU
L
G
F
I
min



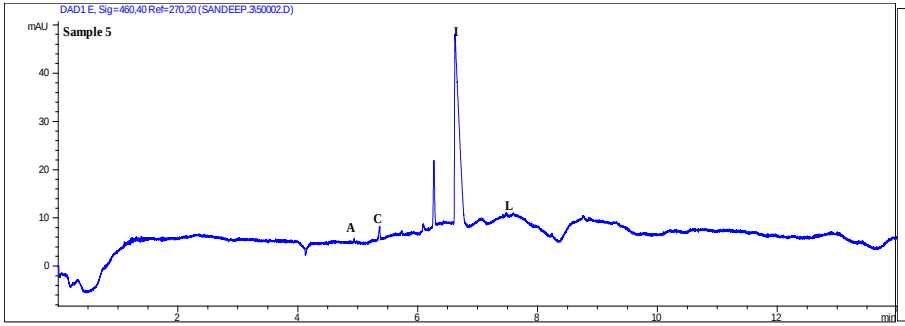




Peak identification: A, xylitol; B, raffinose; C, mannitol; D, sucrose; E, lactose; F, galactose; G, glucose; H, rhamnose; I, mannose; J, xylose; K, ribose; L, sorbitol; M, cellobiose; N, maltose; O, arabinose; P, fructose.



Peak identification: A, xylitol; B, raffinose; C, mannitol; D, sucrose; E, lactose; F, galactose; G, glucose; H, rhamnose; I, mannose; J, xylose; K, ribose; L, sorbitol; M, cellobiose; N, maltose; O, arabinose; P, fructose.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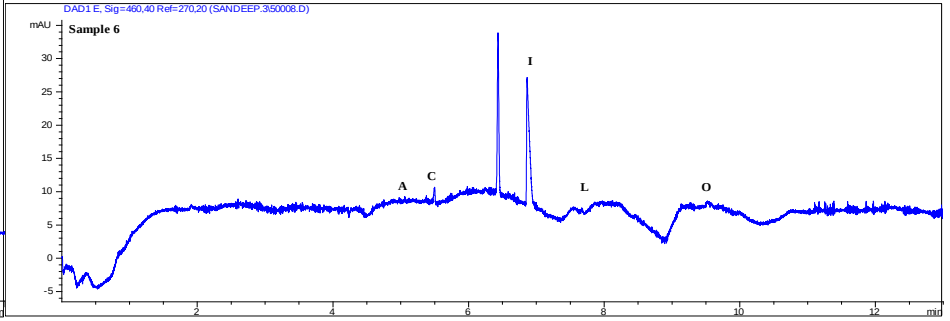
Sample 5
mAU
A
C
I
L
min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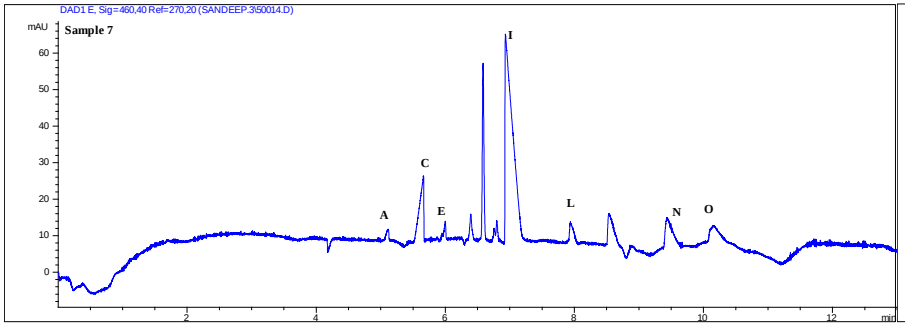
Sample 6
mAU
A
C
I
L
O
min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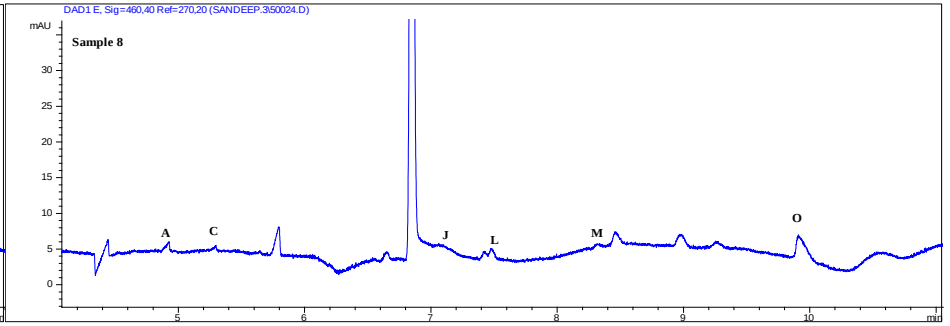
Sample 7
mAU
A
C
E
I
L
N
O
min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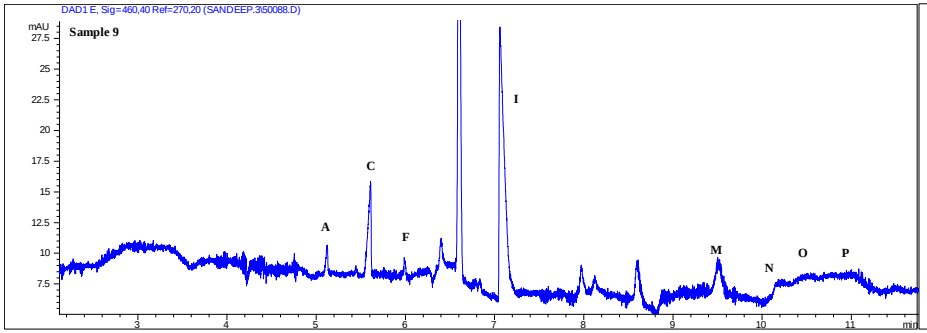
Sample 8
mAU
A
C
J
L
M
O
min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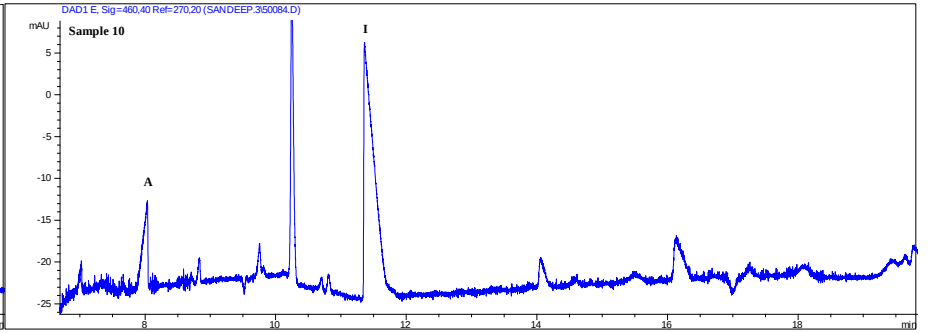
Sample 9
mAU
A
C
F
I
M
N
O
P
min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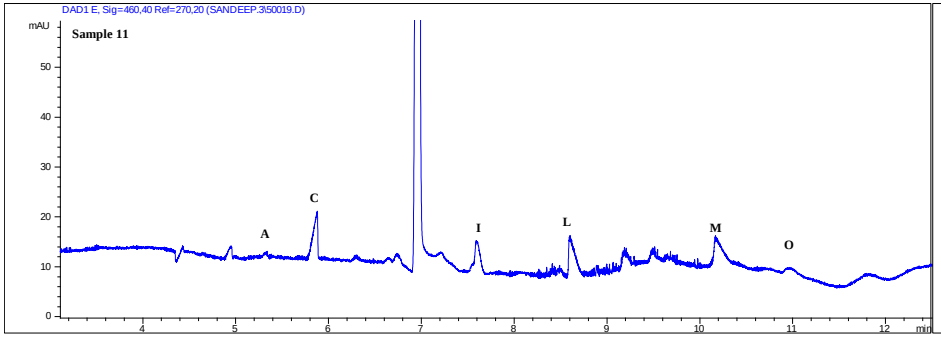
Sample 10
mAU
A
I
min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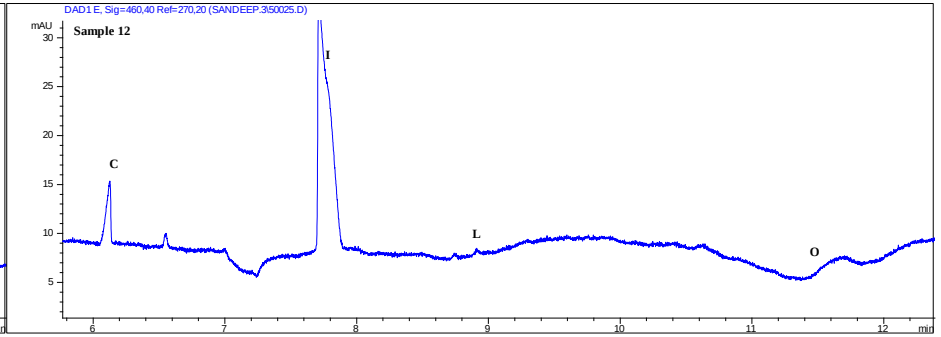
Sample 11
mAU
A
C
I
L
M
O
min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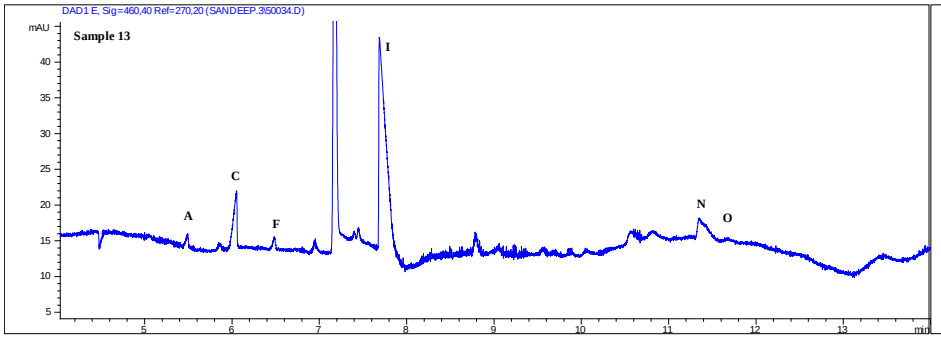
Sample 12
mAU
C
I
L
O
min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Sample 13
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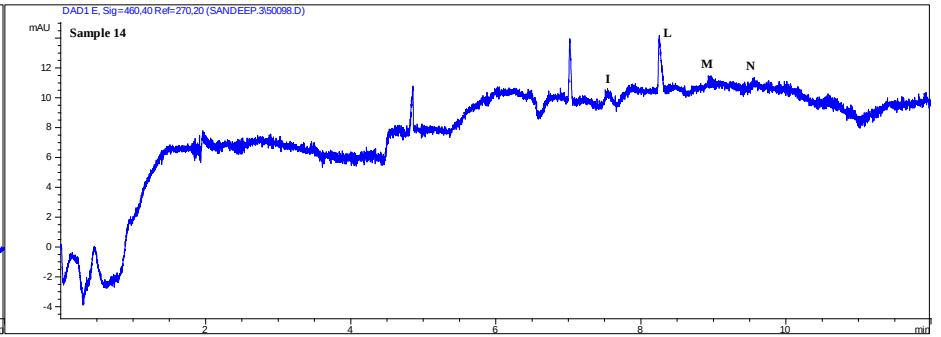
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Sample 14
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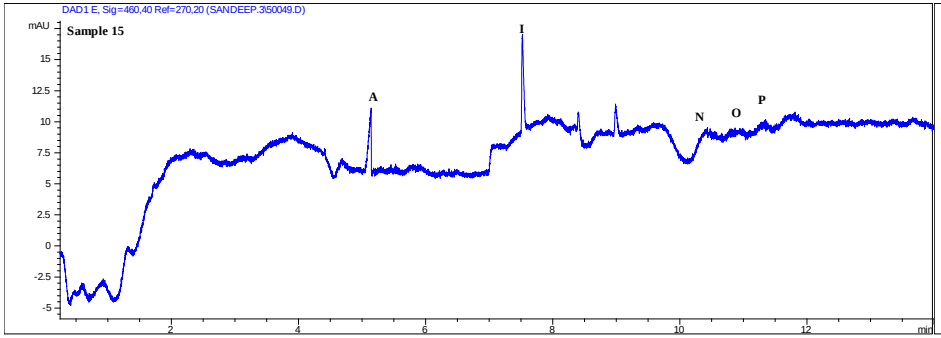
M
N
min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Sample 15
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N
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P
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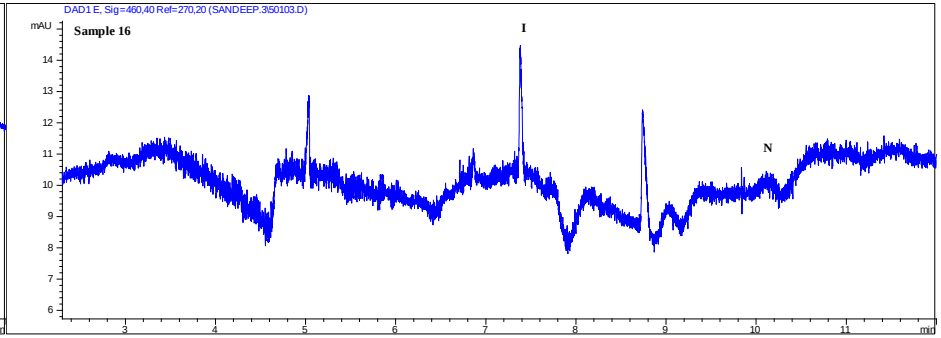
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Sample 16
mAU
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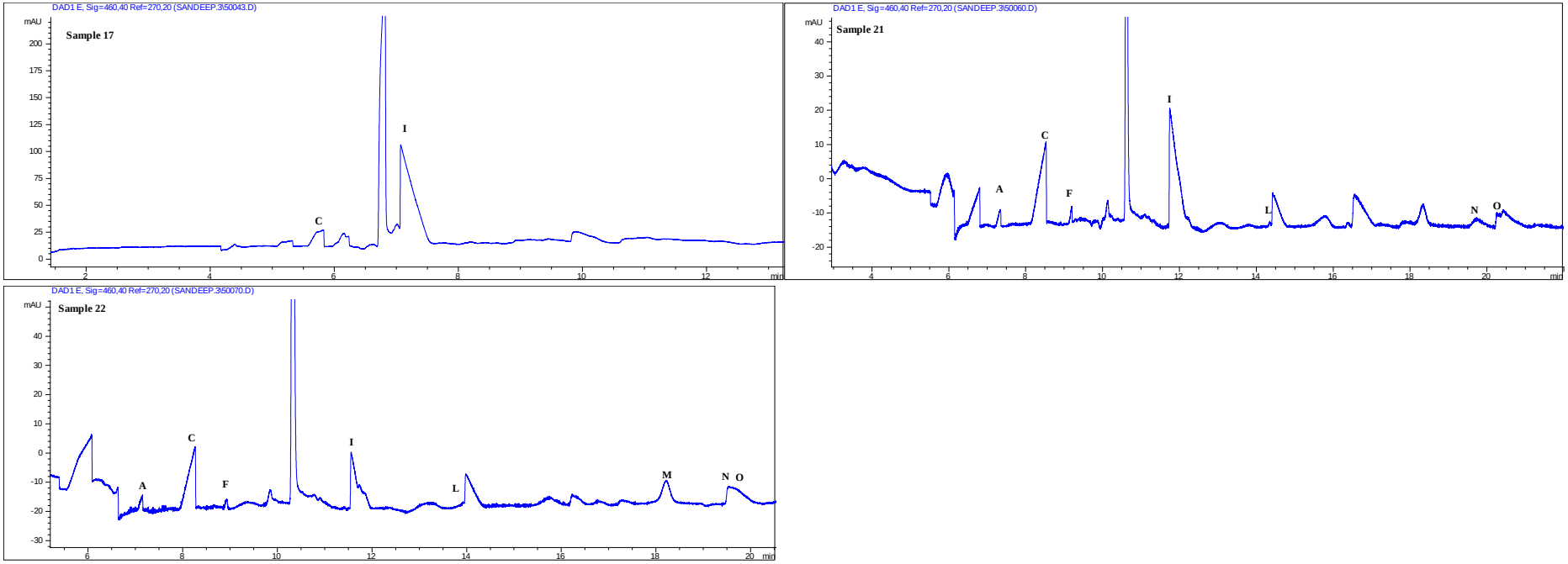
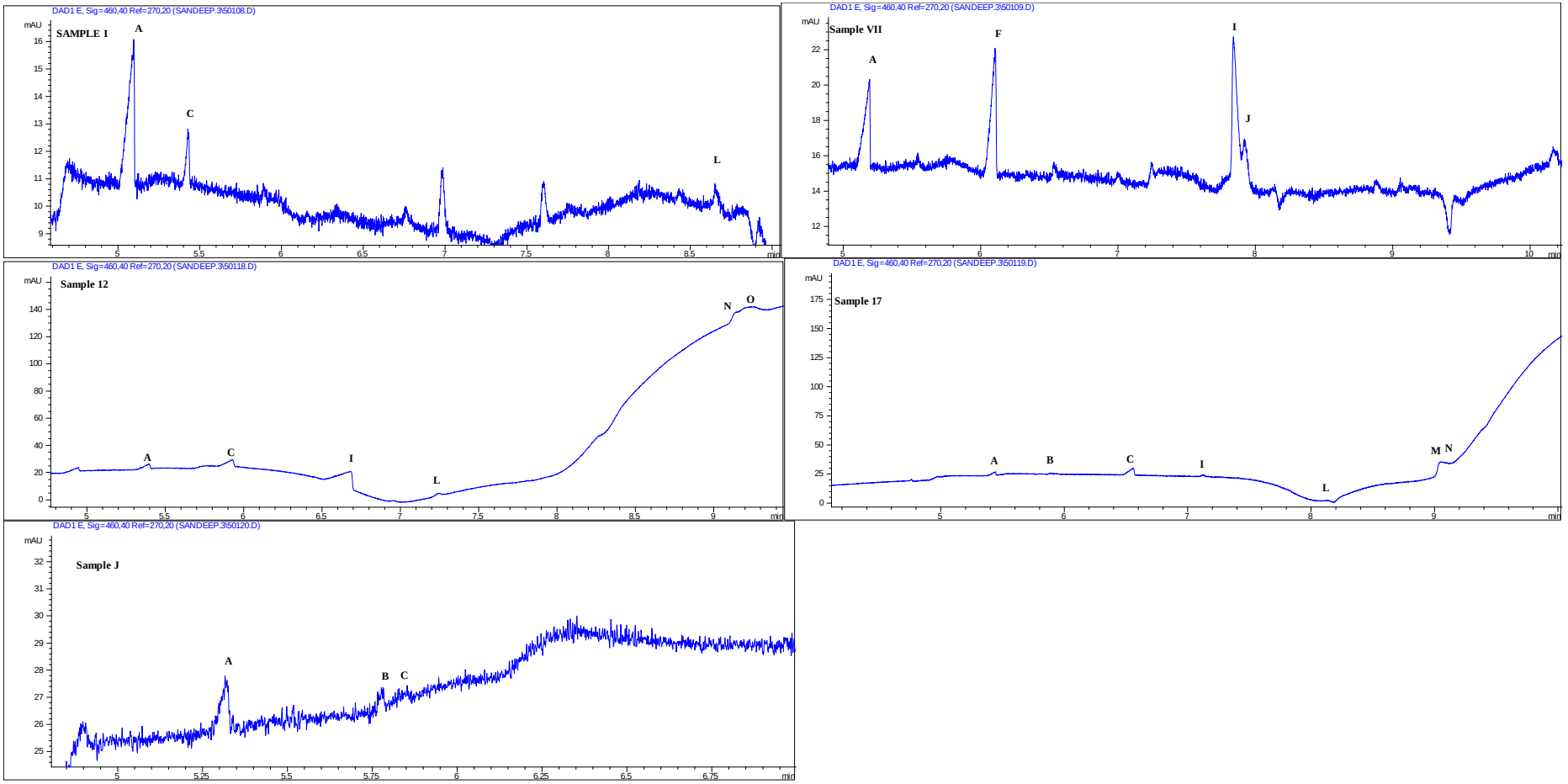
N
min



Peak identification: A, oxalic acid; B, malonic acid; C, formic acid; D, maleic acid; E, tartaric acid; F, succinic acid; G, adipic acid; H, glyoxylic acid; I, acetic acid; J, citric acid; K, glycolic acid; L, propionic acid; M, glutamic acid; N, ascorbic acid; O, glucuronic acid and P, galacturonic acid.



Peak identification: A, oxalic acid; B, malonic acid; C, formic acid; D, maleic acid; E, tartaric acid; F, succinic acid; G, adipic acid; H, glyoxylic acid; I, acetic acid; J, citric acid; K, glycolic acid; L, propionic acid; M, glutamic acid; N, ascorbic acid; O, glucuronic acid and P, galacturonic acid.