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Comparison of gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry for the determination of fatty and resin acids in paper mill process waters

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Abstract

A comparative study of the performance of liquid chromatography (LC)–atmospheric pressure chemical ionisation (APCI)–mass spectrometry (MS) and gas chromatography (GC)–mass spectrometry techniques for the determination of resin and fatty acids from paper mill process waters was carried out. These compounds are responsible for the high toxicity of paper mill effluents and little research has been carried out regarding their analysis using mass spectrometric techniques. To prove the usability of GC and LC–MS, 16 treated and untreated water samples of recycle, kraft and pulp paper mills were analysed and good agreement was observed as regards to compounds detected and corresponding concentrations. This paper also reports the limits of detection, recoveries, reproducibility, linearity and precision using the two methods. GC–MS presented better selectivity and lower detection limits (below 0.2 μg/l), but derivatization of the extracts and the short life of derivatives (12–24 h) made the technique tedious and prone to high variations. Although LC–APCI–MS presented coelution of the non-aromatic resin acids, it also showed good sensitivity (limits of detection <3 μg/l) and permitted the detection of resin and fatty acids at μg/l level. In addition, since samples could be directly injected to the chromatographic system, LC–APCI–MS was proven as a powerful technique for quick and unequivocal quality control during papermaking.

Keywords: Paper mill; Water analysis; Fatty acids; Resin acids

1. Introduction

Effluents and closed water circuits from paper recycling industries are complex matrices containing a large number of compounds of extremely high chemical diversity [1,2]. Harmful effects of the effluent are very high [3] but they decrease upon treatment [4]. Toxicity is due to chemical cocktail composed by antifoams, biocides, de-inkers, surfactants, etc. which are released to the whitewater upon production [5,6]. Natural compounds such as fatty and resin acids present in conifers [7] also contribute to the toxic effluents generated by paper mills [8,9]. In addition, fatty and resin acids are mainly respon-

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sible for pitch deposition during paper production which affects the fibre and composition of the paper sheets. Therefore, the survey of fatty and resin acids in whitewater is necessary for (i) production management, in order to have a final paper of outmost quality and (ii) for environmental survey, to diminish toxicity of effluents.

In the literature, most methods used for the determination of fatty and resin acids are based on liquid–liquid extraction using methyl *tert.*-butyl ether [10–13], derivatization to the respective methyl [11,12,14,15], trimethylsilyl [12] or pentafluorobenzyl [10,13] esters, and analysis using gas chromatography (GC) with flame ionisation detector (FID) [12,14,15]. The advantages of such technique is its easy use and high sensitivity, but it often needs of confirmatory analysis for unequivocal identification, either using a column of different polarity or using mass spectrometric (MS) detection. GC–MS with electron ionisation has also been proven as a reliable and selective technique for the detection and quantification of these families of compounds in real effluent waters [10,11,13,16]. The main disadvantage of GC relies in the fact that derivatization is necessary and the life of some derivatives is reduced to 12–24 h [12]. If problems arise during analysis, a new derivatization of the extract is necessary. Recently, liquid chromatography (LC)–MS methods have been optimised for the determination of resin and fatty acids, using either electrospray (ESI) [17] or atmospheric pressure chemical ionisation (APCI) [18]. The advantage of LC–MS is that the water extract can be directly injected in a reverse phase column without the need of a derivatization step and ionisation of the compounds takes place in an interface without the need of any post column addition. Although the technique suffers from some limitations, essentially a poor separation of all resin acid isomers [17–20], it is rapid, highly sensitive and permits a reliable quantification at the low μg/l level. However, in order to proof its final applicability, intercomparison of the traditional methods with newly developed is necessary, using real water samples.

Therefore, this paper is aimed to compare the performance of GC–MS and LC–APCI–MS for the analysis of 10 resin acids and five fatty acids using spiked and real samples collected from different pulp and paper mills, a recycle paper mill and one river effluent. The advantages and limitations of each method are stated, in especial, as regards to their robustness using untreated and treated samples which are characterized by a very high organic content and particle load. The suitability of each method is directed to a faster and cost effective quality control in paper and pulp industries, especially as regards to closed-loop water circuits and for surveying the environmental impact in the case of spills.

2. Experimental

2.1. Chemicals and standards

HPLC grade water, methanol, bis-(trimethylsilyl)-trifluoro-acetamide (BSTFA) and trimethylchlorosilane were purchased from Merck (Darmstadt, Germany). Ammonium acetate was from Sigma (St. Louis, MO, USA) and methyl *tert.*-butylether (MTBE) from Fluka (Buchs, Switzerland).

All standards were of the highest purity commercially available. Resin acids were purchased from Helix Biotechnologies (Vancouver, Canada) and were used without further purification. Dehydroabietic, neoabietic and isopimaric acids were above 99% purity. Levopimaric, 12,14-dichlorodehydroabietic and sandaracopimaric acids were above 95% purity, the latter containing isopimaric acid. Abietic, palustric and chlorodehydroabietic acids (mixture of 12- and 14-isomers) were between 90 and 95% purity, and pimaric acid was between 85 and 90% containing 10–15% of sandaracopimaric acid. Fatty acids were supplied by Fluka with a purity >99%.

Stock individual standard solutions (1 mg/ml) were prepared by dissolving accurate amounts of pure standards in methanol and were stored at −20 °C. Working standard solutions were obtained by further dilution of stock solutions with methanol. For GC–MS analysis, standard solutions were evaporated to dryness and derivatized as explained below for the extracts of real water samples.

2.2. Sample collection

Sixteen water samples corresponding to different production processes were analysed:

(1) A river effluent from a paper mill that used eucalyptus wood as raw material (E1).

(2) Whitewaters collected at six different locations of a recycle paper mill:

- Well water used by the industry (R1)
- Water at four points of the close-water circuit (R2, R3, R4 and R5)
- Water from a small river close to the industry (R6)

(3) Untreated and biologically treated whitewaters from various pulp and paper mills:

- Kraft pulp mill that used pine wood as raw material ($S1_{untreated}$ and $S1_{treated}$)
- Printing paper mill ($S2_{untreated}$, $S2_{treated\ 1}$ and $S2_{treated\ 2}$)
- Packaging board mill that worked with an open system ($S3_{untreated}$ and $S3_{treated}$)
- Packaging board mill that worked with a closed system ($S4_{untreated}$ and $S4_{treated}$)

All samples were collected with amber glass bottles and kept at 4 °C in the dark. These samples were yellowish and showed a very high total organic carbon (up to 5000 mg/l) and presence of particulate material coming from the paper paste and suspended microfibres from the paper sheets. In all cases, analyses were performed within 2 weeks.

2.3. Sample preparation

Water samples were filtered through 0.45-μm filters to remove particulate matter. No pH adjustment was performed, since pH of whitewater was between 6.2 and 6.5, well water was at pH 7.5 and river water at a pH of 8.2. pHs between 7 and 10 are suitable values for an efficient extraction of fatty and resin acids with minimum isomerization and increased recoveries since there is a lower adsorption onto lignins, which are hydrophilic [10,13,21]. Two different sample preparation protocols were used for GC–MS and LC–APCI–MS analysis: liquid–liquid extraction plus derivatization and direct sample introduction, respectively.

2.3.1. Liquid–liquid extraction and derivatization

For GC–MS analysis, an aliquot of 4 ml of water sample was measured in a screw-capped test tube for liquid–liquid extraction. A 2-ml volume of MTBE containing margaric acid ($C_{17}H_{34}O_2$) was added in the first extraction. Margaric acid was used as internal standard (I.S.), since it did not coelute with the other species and is very unusual to find it in water samples [22]. The tube was vigorously shaken by hand for 2 min and centrifuged at 300 *g* for 5 min. The clear MTBE layer was carefully pipetted off and the extraction was repeated twice with 2-ml volumes of MTBE (free of I.S.). The combined MTBE extracts were evaporated with a Reacti-Vap3 (Pierce) operating under a gentle stream of nitrogen to dryness.

Afterwards, the extracted resin acids were derivatized to the respective trimethylsilyl (TMS) esters. For derivatization, 80 μl of BSTFA and 40 μl of trimethylchlorosilane were added to the residue of evaporation. The solution was kept in an oven at 70 °C for 20 min and was thereafter ready for analysis.

2.3.2. Direct sample introduction

For LC–APCI–MS analysis, the <0.45-μm water fraction was diluted with methanol (0.8 water sample:0.2 methanol) and directly introduced into the chromatographic system. Since no sample preparation was performed, no internal standard was used, although margaric acid could also be used as internal standard for LC.

2.4. Gas chromatography–mass spectrometry

A Trace GC–MS instrument (Thermoquest) equipped with a HP-5MS column (30 m×0.25 mm I.D. with 0.25 μm film thickness) containing 5% phenyl methyl siloxane (model HP 19091S-433) was used. The oven temperature was held at 120 °C for 2 min and programmed to 300 °C at a rate of 4 °C/min. The final temperature was held for another 5 min. The inlet, ion source, and GC interface temperatures were 260, 200, and 270 °C, respectively. The carrier gas was helium at 10 p.s.i. The mass spectrometer was operated in the electron impact ionisation mode with an ionising energy of 70 eV and an emission current of 150 μA. Full scan data were obtained by scanning from m/z 45 to 600 at a rate of 1.5 scans/s.

Quantitative analysis was performed in selected ion monitoring (SIM) mode using margaric acid as internal standard (I.S.). Series of injected standards

in the range from 0.01 to 20 μg/ml containing 7 μg/ml of internal standard were generated daily to obtain the calibration curves. All injections were done in the splitless mode and 1 μl of the sample was injected.

2.5. Liquid chromatography–mass spectrometry

A HP 1100 autosampler equipped with a 100-μl loop and an HP 1090A LC pump, both from Hewlett-Packard (Palo Alto, CA, USA) were used as optimised in a previous work [18]. Chromatographic separation was done using a C_{18} reversed-phase column Lichrospher 100 RP-18 (5 μm, 250×4 mm I.D.) with a guard column (5 μm, 4×4 mm I.D.) of the same packaging material from Merck (Darmstadt, Germany). An elution gradient with two solvents was used: (A) methanol and (B) water with 25 m*M* CH_3COONH_4 (pH 7.0). The gradient started with 70% A and linearly increased to 100% A in 30 min, condition that was kept isocratic for 5 min. The flow-rate and the column temperature were set at 0.8 ml/min and 40 °C, respectively.

Detection was carried out using a MSD HP 1100 mass-selective detector, equipped with an atmospheric-pressure chemical ionisation (APCI) interface operating in negative mode. Operating conditions of the MS system were as follows: drying gas (N_2) at a flow of 6 l/min and a temperature of 325 °C, nebulizer pressure of 40 p.s.i., vaporizer temperature of 500 °C, corona current of 8 μA, and capillary and cone voltages of 3500 and 80 V, respectively.

Quantitative analysis was performed in selected ion monitoring (SIM) mode using external standard calibration. Calibration curves were generated daily at the range from 1 to 200 ng injected of the target compounds. The injection volume was of 100 μl.

3. Results and discussion

3.1. Mass spectra

Resin acids are tricyclic diterpenoids with a carboxylic moiety. Two main groups may be distinguished: aromatic and non-aromatic resin acids. The aromatics are basically dehydroabietic ($C_{19}H_{27}COOH$), 12- and 14-chlorodehydroabietic ($C_{19}H_{26}ClCOOH$) and 12,14-dichlorodehydroabietic ($C_{19}H_{25}Cl_2COOH$) acids. The formation of the two later is induced by the chlorobleaching process of wood pulps. Among the non-aromatic resin acids, some have an isopropyl substituent on the C-13 and present two conjugated double bonds (palustric, levopimaric, abietic, and neoabietic acids), whereas the other present a vinyl and a methyl group in this position and two non-conjugated double bonds (pimaric, sandaracopimaric and isopimaric acids), but all of them respond to the same molecular formula ($C_{19}H_{29}COOH$). On the other hand, fatty acids are open long chain carboxylic acids, which can be saturated (palmitic, $C_{16:0}$; margaric, $C_{17:0}$; and stearic acids, $C_{18:0}$) or unsaturated (oleic, $C_{18:1}$; and linoleic acids, $C_{18:2}$).

The similar structure of resin acids, and particularly of the non-aromatic that have the same molecular mass (Table 1), was a main problem when dealing with their identification by APCI–MS with negative ionisation. Moreover, these compounds were highly resistant to fragmentation even at high cone voltage and corona current, since the cyclic structure is a stabilizing factor. Working at the optimal conditions only the $[M–H]^-$ ion was obtained for the fatty and non-chlorinated resin acids, and the chlorinated resin acids also showed the ions corresponding to the two chlorine isotopes. Thus, dehydroabietic (DHA), chloroDHA, dichloroDHA and fatty acids can be identified by APCI–MS since they show different $[M–H]^-$ ions, whereas the seven non-aromatic resin acids present a common $[M–H]^-$ ion at m/z 301.

In contrast, under electron impact (EI) conditions when using GC–MS, the mass spectra of the fatty and resin acid TMS esters were quite complicated. Most works using GC–MS deals with methyl ester derivatives [14] or pentafluoronbenzyl ester derivatives [13] and these spectra are already described in the literature. This is not the case for TMS esters that have been used for GC–FID [12]. Table 1 shows the characteristic ions supporting the identification of the target compounds. A very important feature was that most compounds showed a clearly recognizable molecular ion. Fatty acids presented highly intense peaks mostly related with the trimethylsilylated (TMS) carboxylic group ($[CH_2–CH_2–COOTMS]^+$, $[CH_2–COOTMS]^+$, $[COOTMS]^+$ and $[TMS]^+$),

Table 1
Fragmentation patterns of the fatty and resin acids: m/z with relative abundances (%) in parenthesis and the quantification ion in bold

Compound	M_W	$[M]^+$	$[M–CH_3]^+$	$[CH_2–CH_2–COOTMS]$	$[CH_2–COOTMS]$	[COOTMS]	[TMS]	Other ions	RF[a]
Fatty acids									
Palmitic	256	328 (49)	**313 (68)**	145 (36)	132 (56)	117 (100)	73 (95)	–	1.1
Margaric (I.S)	270	342 (6)	**327 (71)**	145 (38)	132 (58)	117 (100)	73 (95)	–	–
Linoleic	280	352 (3)	**337 (42)**	–	129 (34)	–	73 (100)	262 (24)	0.3
Oleic	282	354 (4)	**339 (52)**	145 (37)	129 (65)	117 (81)	73 (100)	–	0.4
Stearic	284	356 (77)	**341 (70)**	145 (42)	132 (59)	117 (100)	73 (97)	–	1.0
Resin acids				$[M–TMS–CO_2]^+$ [b]	$[M–TMS–CO_2–CH_3]^+$ [b]				
Pimaric	302	374 (10)	359 (17)	**257 (28)**	238 (18)		73 (100)	121 (87)	0.3
Sandaracopimaric	302	374 (12)	**359 (21)**	257 (26)	241 (21)		73 (100)	121 (84)	0.7
Isopimaric	302	374 (3)	359 (17)	256 (75)	**241 (100)**		73 (83)	–	1.2
Palustric	302	374 (2)	359 (42)	257 (9)	**241 (100)**		73 (69)	148 (31)	1.3
Levopimaric	302	374 (2)	359 (14)	256 (13)	241 (15)		73(100)	**159 (52)**,121 (51)	0.6
DHA	300	372 (1)	**357 (8)**	255 (8)	239 (100)		73 (18)	173 (9)	0.3
Abietic	302	374 (1)	359 (1)	**256 (100)**	241 (58)		73 (35)	185 (31)	1.6
Neoabietic	302	374 (20)	359 (68)	256 (5)	239 (10)		73 (41)	**148 (30)**,135 (100),121 (35)	0.7
ChlorDHA	334.5	406 (8)	391 (10)	**273 (100)**	–		73 (50)	207 (28)	1.6
DichlorDHA	369	440 (9)	425 (8)	**307 (53)**	–		73 (100)	241 (31)	0.9

[a] RF: Response factor of the quantification ion of the target compound with respect to the quantification ion of the I.S.
[b] Loss of CO_2, HCO_2 or HCO_2H.

except for linoleic acid that showed a slightly different fragmentation with a peak at m/z 262 corresponding to the loss of a TMS and a methyl group. For these compounds $[M]^+$ and $[M–CH_3]^+$ were the less intense but the most selective peaks, the second being used for quantitative purposes. With respect to resin acids, common fragments were observed but with different relative abundances that allowed to obtain different fragmentation patterns for most of them. Thus, all the esters presented the fragment corresponding to the TMS at high abundance. Moreover, the carboxyl moiety was eliminated as CO_2, HCO_2 or HCO_2H, and always together with the TMS group. The loss of a methyl radical is also observed. Criteria of selectivity and sensitivity were followed to choose the most suitable ions for quantification. Table 1 also includes, in bold, the ion of each target compound used for quantification and their respective response factors with respect to the internal standard. Although some peaks presented low response factors (0.3), they were still suitable for quantification.

3.2. Method performance and quality parameters

The performance of LC–APCI–MS and GC–MS was contrasted by comparing, first, the chromatographic separation obtained by the two techniques and, second, the quality parameters of the respective methods.

Whereas LC using a C_{18} column usually achieves chromatographic separation of fatty acids and aromatic resin acids, the non-aromatic resin acids easily coelute due to their similar structure, which together with their identical mass spectra leads to the impossibility of LC–MS to quantify non-aromatic resin acids individually. In a previous work [18], the use of a C_8 column allowed a certain resolution of these non-aromatic resin acids, although global quantification of these compounds had to be done. Moreover, with the C_8 column lower sensitivity was observed for fatty acids, and the C_{18} column was suggested for further quantification studies. As shown in Fig. 1, the LC–APCI–MS method used in this work allows the single quantification of fatty and aromatic resin acids

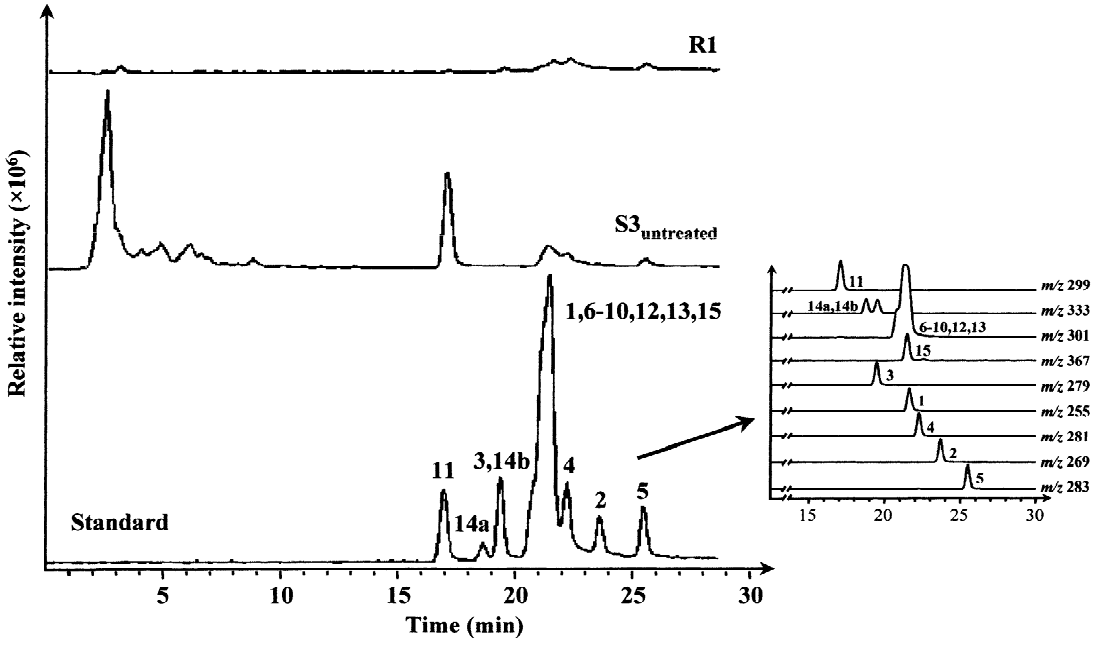

Fig. 1. Total ion current (TIC) LC–APCI–MS chromatograms (SIM mode) of a well water (R1), an untreated process water coming from a packaging board mill that worked with an open system ($S3_{untreated}$), and a mixture of standards of the five fatty acids and the 10 resin acids (16 ng injected). See Section 2 for LC–APCI–MS conditions. Acids: 1=palmitic; 2=margaric; 3=linoleic; 4=oleic; 5=stearic; 6=pimaric; 7=sandaracopimaric; 8=isopimaric; 9=palustric; 10=levopimaric; 11=dehydroabietic (DHA); 12=abietic; 13=neoabietic; 14a,14b=12- and 14-chlorDHA; 15=12,14-dichlorDHA.

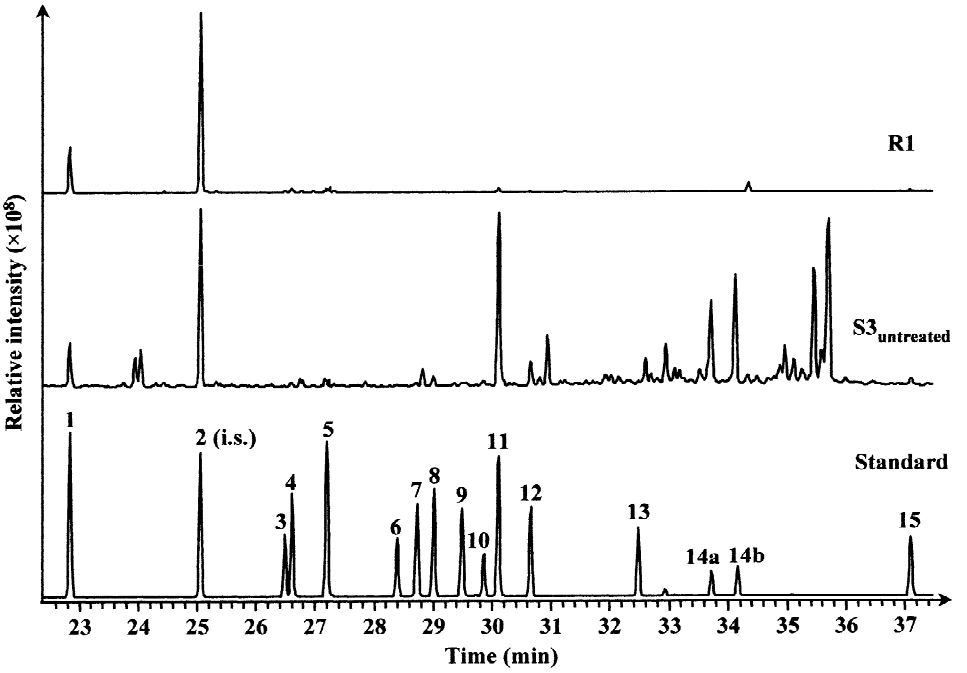

Fig. 2. Total ion current (TIC) GC–MS chromatograms (SIM mode) of a well water (R1), an untreated process water coming from a packaging board mill that worked with an open system ($S3_{untreated}$), and a mixture of standards of the five fatty acids and the 10 resin acids (7 ng injected). See Section 2 for GC–MS conditions and Fig. 1 for peak identification.

and a global determination of non-aromatic resin acids in less than 30 min. In contrast, the non-polar GC column provides very good separation of the TMS esters of fatty and resins acids, but 40 min are required for the elution of all compounds (Fig. 2).

Linearity ranges, repeatability, reproducibility, sensitivity and recovery rates of all target compounds were determined by the two techniques and are shown in Table 2. Linearity ranges were longer for GC–MS (from 0.01 to 20 ng injected) than for

Table 2
Quality parameters of LC–APCI–MS and GC–MS methods

Parameter	Resin acids		Fatty acids	
	GC–MS	LC–APCI–MS	GC–MS	LC–APCI–MS
Linearity range (ng injected)	0.01–20	1–200	0.01–20	1–200
R^2	>0.999	>0.99	>0.999	>0.99
Repeatability (%RSD, $n=5$)	4–5	2–4	2–8	3–7
Reproducibility (%RSD, $n=5$)	6–13	6–7	2–11	6–9
$LD_{instrumental}$ (pg injected)	0.1–3	75–250	0.7–6	40–150
LD_{method} (μg/l water)[a]	0.004–0.1	0.9–3	0.03–0.2	0.5–2
% Recovery ($n=5$)[b]	81–94	75–87	92–106	76–86

[a] Estimated from $LD_{instrumental}$: sample volume = 4 ml, concentration factor = 25 and injection volume = 1 μl for GC–MS; dilution factor = 1.25 and injection volume = 100 μl for LC–APCI–MS.

[b] Determined at 5 and 15 ng injected volume for GC–MS and LC–APCI–MS, respectively.

LC–APCI–MS (from 10 to 200 ng injected) and, in both cases, good correlations were obtained. In order to evaluate repeatability and reproducibility, five consecutive injections and six injections in different days were performed, respectively. Relative standard deviation (RSD) percentages were lower than 10% for the two techniques and for most compounds, and they were slightly better for the LC–APCI–MS method. Instrumental limits of detection ($LD_{instrumental}$), defined as the minimum amount of analyte which produced a peak with a signal-to-noise ratio of 3, was also measured for each compound. Limits of detection of the method (LD_{method}) were also estimated from the $LD_{instrumental}$ by considering the corresponding sample volume, concentration or dilution factor and injection volume. LDs were one order of magnitude lower with GC–MS, but those obtained with LC–APCI–MS were still below the levels of fatty and resin acids encountered in process waters and effluents that are of various μg/l.

Finally, recoveries of all acids were compared using the two techniques by analysis of fortified water samples at 200 μg/l, level close to those of real process water samples. All recoveries were between 76 and 106% with a precision (% RSD) lower than 8 and 10% using LC–APCI–MS and GC–MS, respectively. Moreover, recoveries were slightly higher for GC–MS that used the liquid–liquid extracted sample than for LC–APCI–MS that used direct sample introduction. This might be attributed to the amount of sample injected that for GC–MS was in the middle of the calibration curve, whereas for LC–APCI–MS was at the low limit of the curve. In addition, filtration was the solely sample treatment and since there was no sample clean-up, matrix interferences might cause poor recoveries due to ionisation suppression of target analytes. Although in LC–APCI–MS experiments margaric acid was a test compound, given the response of this compound, a way to improve recoveries and precision with this technique would be the use of such compound as internal standard as done in the GC–MS determinations

3.3. Quantification of water samples

The two analytical procedures compared in this work were also applied to the analysis of process waters and effluents corresponding to different production processes of paper industries. Figs. 1 and 2 show, as an example, the total ion current (TIC) chromatograms of a dirty water ($S3_{untreated}$) and a clean water (R1) obtained by LC–APCI–MS and GC–MS, respectively. The two techniques allowed the identification and quantification of palmitic, dehydroabietic and non-aromatic resin acids. The rest of fatty acids, except for margaric, were observed in some samples, but at the limit of detection. Moreover, whereas, a global concentration of non-aromatic resin acids was obtained by LC–APCI–MS, GC–MS permitted the identification and quantification of the individual species.

Fatty and resin acid concentrations found in the analysed waters are given in Table 3. The effluent, the well water and the river water, that were considered clean waters presented only low concentrations of palmitic acid, resin acids not being detected. In the rest of samples, dehydroabietic acid was the most abundant resin acid, followed by abietic and isopimaric acids. This agree with previous works stating that resin acids with conjugated double bonds can easily undergo isomerization forming thermodynamically more stable isomers with dehydroabietic and abietic being the favoured final products [13,20,21]. The chlorinated dehydroabietic acids, that are less usual compounds, were not found in any sample. Process waters of the recycling factory (R2–R5) contained considerable amounts of palmitic and most resin acids, with very similar concentrations between the different sampling points. This revealed the low efficiency of physical treatment for the removal of this type of compounds. Palustric and neoabietic acids were not detected in these waters, probably due to their tendency to isomerise to DHA and abietic acids. With respect to the process waters of pulp and paper mills, untreated and treated waters from S1 and S2, were relatively clean, only containing moderated concentrations of palmitic acid and low concentrations of DHA acid. In contrast, untreated samples from S3 and S4 were considerably charged containing the highest concentrations of DHA acid and significant amounts of the rest of resin acids. Comparing untreated and treated waters, it should be pointed out that biological treatment seems quite efficient for the removal of resin acids. However, this was not the case for palmitic acid that

Table 3
Concentration of fatty and resin acids (μg/l) in water samples from paper mills

Sample	Palmitic		DHA		Pimaric	Sandaracopimaric	Isopimaric	Palustric	Levopimaric	Abietic	Neoabietic	Non-aromatic RA	
	GC–MS	LC–MS	GC–MS	LC–MS	GC–MS							GC–MS[a]	LC–MS
E1	49	46	–	–	–	–	–	–	–	–	–	–	–
R1	50	60	–	–	–	–	–	–	–	–	–	–	–
R2	166	151	68	54	4.3	1.7	4.5	–	3.6	16.2	–	30	27
R3	63	82	66	67	5.4	2.0	5.3	–	2.9	18.3	–	34	46
R4	102	118	58	61	3.2	1.1	3.5	–	3.2	12.8	–	24	25
R5	81	82	53	51	2.6	0.8	2.8	–	3.7	11.0	–	21	23
R6	40	60	–	–	–	–	–	–	–	–	–	–	–
$S1_{untreated}$	106	90	4.0	–	–	–	–	–	–	–	–	–	–
$S1_{treated}$	202	203	5.0	8.1	–	–	–	–	–	–	–	–	–
$S2_{untreated}$	51	46	12.0	10.0	–	–	–	–	–	–	–	–	–
$S2_{treated\ 1}$	275	300	2.5	–	–	–	–	–	–	–	–	–	–
$S2_{treated\ 2}$	253	267	8.0	–	–	–	–	–	–	–	–	–	–
$S3_{untreated}$	53	50	400	403	6.5	8.3	30.7	4.8	6.3	64.2	–	121	127
$S3_{treated}$	63	67	202	218	2.9	1.7	4.2	–	4.3	23.8	–	37	45
$S4_{untreated}$	84	56	73	75	–	9.6	64.6	7.0	2.4	87.2	59.1	230	256
$S4_{treated}$	132	127	5.0	–	–	–	–	–	–	–	–	–	–
Slope	1.04±0.11		1.02±0.05									1.09±0.10	
R^2	0.968		0.997									0.994	

[a] Sum of the individual non-aromatic resin acids (pimaric, sandaracopimaric, isopimaric, palustric, levopimaric, abietic and neoabietic); (–) non detected.

increased its concentration after biological treatment. This increase might be related with the bacteria used for the treatment (production of acid by bacteria or extraction of acid present in their structure).

In relation to the comparison of the results obtained by LC–APCI–MS and GC–MS, the two techniques showed an excellent agreement. Thus, for palmitic and DHA acids good correlations were observed between concentrations obtained by the two methods, with slopes close to 1 and intercepts non-significantly different from zero. For the non-aromatic resin acids a good regression coefficient was also achieved between the sum of the concentrations obtained by GC–MS and the global value quantified by LC–APCI–MS. In this case, a slope close to the unit and an intercept non-significantly different from zero were also attained. Moreover, only at very low concentrations of DHA acid, GC–MS showed a higher capacity of detection than LC–APCI–MS. These results demonstrate that LC–MS is perfectly suitable to determine fatty and resin acids at the levels found in process waters.

3.4. Advantages and disadvantages of LC–APCI–MS and GC–MS

A list of advantages and disadvantages of the application of LC–APCI–MS and GC–MS for the determination of fatty and resin acids in water samples was performed from all the information obtained in this work (Table 4).

GC–MS presented slightly better sensitivity, selectivity and linearity than LC–APCI–MS. Recoveries were also higher using GC–MS, but LC–APCI–MS was more reproducible and precise. In spite of these differences, quality parameters are acceptable for the two methods, and suitable for quantification of fatty and resin acids in waters of pulp and paper mills. Moreover, the two techniques permit the use of an internal standard to improve the analysis of these compounds, margaric acid being the most appropriate candidate. This fatty acid is not detected in process waters of paper industries in any significant amount, does not coelute with the rest of compounds, present intermediate elution times and show similar response factors with respect to the other fatty and resin acids.

Finally, some advantages of LC–APCI–MS versus GC–MS can be remarked. LC allows a direct introduction of the sample contributing to more rapid results and less sample manipulation, although this approach might not be suitable for very charged samples. In contrast, GC analysis requires derivatization of fatty and resin acids. This step represents an additional source of error because of the sample handling and the low stability of trimethylsilyl ester derivatives that hydrolyses very easily. In general, it can be stated that LC–APCI–MS is more robust than GC–MS for the analysis of these compounds.

Table 4
Advantages and disadvantages of GC–MS and LC–APCI–MS for the analysis of fatty and resin acids from treated and untreated effluent samples

Parameter	GC–MS	LC–APCI–MS
Sensitivity	+ + +	+ +
Selectivity	+ + +	+
Linearity	+ + +	+ +
Reproducibility	+ +	+ + +
Recovery	+ + +	+ +
Precision	+ +	+ + +
Internal standard quantification	+ + +	+ + +
Direct analysis	–	+ + +
Robustness	+ +	+ + +

4. Conclusions

GC–MS and LC–APCI–MS methods for the analysis of 10 resin acids and five fatty acids in water samples of paper industries have been developed, applied and compared. The two methods show good linearity and precision, and limits of detection suitable for the analysis of fatty and resin acids found in process waters of pulp and paper mills. Excellent agreement is observed between the concentrations of the target compounds obtained with the two techniques, with dehydroabietic, abietic and isopimaric acids being the most abundant resin acids and palmitic the solely fatty acid present at quantifiable levels.

The GC–MS provides a more exhaustive analysis of the water composition in terms of the single resin acids, but is more time consuming, requires a higher sample manipulation, an the limited stability of the derivatives can be an obstacle for the analysis of fatty and resin acids. In contrast, the LC–APCI–MS

method provides less information about the exact non-aromatic resin acid composition of water samples, but is an ideal technique for a rapid routine monitoring of fatty acids, DHA acid and the global concentration of non-aromatic resin acids, with minimum sample manipulation.

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