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Journal of Chromatography A, 952 (2002) 289–293

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Evaluation of liquid chromatography–negative ion electrospray mass spectrometry for the determination of selected resin acids in river water

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Received 11 January 2002; received in revised form 25 January 2002; accepted 29 January 2002

Abstract

A liquid chromatography–negative ion electrospray mass spectrometric (LC–ESI-MS) method was evaluated for detection of four prevalent softwood-derived resin acids in natural water. Method detection limits based on a signal-to-noise ratio of 3:1 in river water samples of 0.40, 0.40, 0.30 and 0.25 μg l^{-1} for abietic, dehydroabietic, isopimaric and pimaric acids, respectively, are comparable or lower than reported GC methods. Unlike the majority of GC methods, however, the three structural resin acid isomers (abietic, isopimaric and pimaric acids) do not separate sufficiently under the various LC conditions evaluated in this work. Therefore, LC–ESI-MS may not be suitable for instances where measurement of individual isomeric resin acids is required. However, the method is suitable for trace analysis of resin acids in natural waters where isomeric speciation is not required.

Keywords: Water analysis; Mass spectrometry; Resin acids

1. Introduction

Resin acids are tricyclic, diterpenic carboxylic acids that are predominantly found in the tree bark and wood of conifers. Concentrations can reach as much as one order of magnitude higher in coniferous than deciduous trees. As resin acids form part of the natural antimicrobial defence in trees they can pose a potential health hazard to animal, human and plant life [1]. With 96-h LC_{50} toxicity levels for salmon or rainbow trout falling between 0.4 and 1.7 mg l^{-1}, it is currently believed that resin acids contribute substantially to the overall toxicity of pulp and paper effluents [2–6]. In general, nondissociated resin acid species (low pH conditions) have higher toxicological properties than their dissociated counterparts (high pH conditions) while at high pH their water solubility increases [7,8].

The four predominant resin acids occurring in pulp and paper effluents include abietic (AbA), dehydro-

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)00106-1

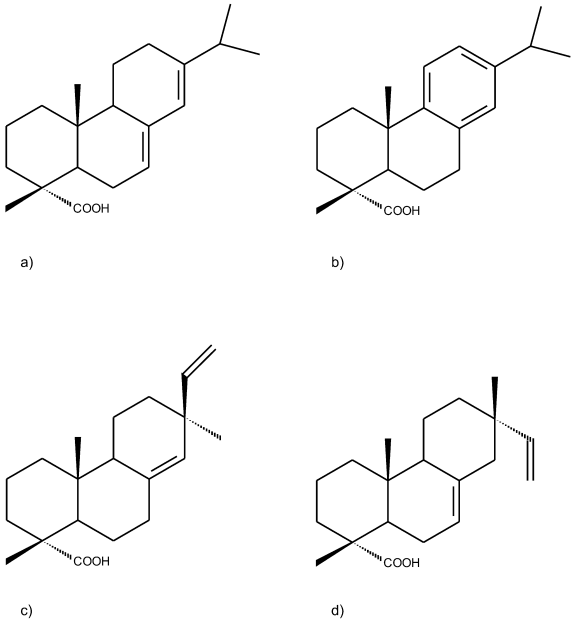

Fig. 1. Chemical structures of the individual resin acids: (a) abietic acid; (b) dehydroabietic acid; (c) isopimaric acid and (d) pimaric acid.

abietic (DhA), isopimaric (IpA), and pimaric (PA) acids (Fig. 1). The former two represent abietane resin acids and the latter two are pimaranes [4,7,9,10]. Unlike pimaranes, abietanes have conjugated double bonds and an isopropyl substituent at C-13. Pimaranes have both methyl and vinyl substituents at C-13 [1,11].

Owing to the prevalence and increasing toxicological evidence related to resin acids in the aquatic environment, there is growing interest in the development of sensitive and robust analytical methods to study the fate and transport of these compounds in aquatic environments. The present study is considered a first step toward a complete solution for fast and sensitive quantification of the four selected resin acids in laboratory and natural water samples using liquid chromatography–negative ion electrospray mass spectrometry (LC–ESI-MS). This method minimises the number of sample preparation steps with no extraction or derivatization requirements with detection limits comparable or lower than reported GC methods [8,12–14]. Unlike the majority of GC methods however, the three structural resin acid isomers (AbA, IpA and PA) do not separate sufficiently using LC under the various conditions evaluated in this work. Therefore, LC–ESI-MS may not be suitable for environmental monitoring in instances where measurement of individual isomeric resin acids is required.

2. Experimental

2.1. Sample collection

Saale River water samples were collected twice weekly approximately 5 km from the confluence of the Saale and Elbe Rivers and analysed over a 5-month period between April and August, 2001. Analysis was completed to evaluate the ruggedness of the LC–ESI-MS method and to determine the extent of resin acid contamination from local pulp and paper mills situated upstream. Spiked water samples of both Saale River and Milli-Q laboratory water were also analysed. Aliquots of these water samples were spiked with each individual resin acid and combinations thereof.

2.2. Chemicals

Neat standards of the four resin acids were purchased from Helix Biotech (Vancouver, Canada). Dehydroabietic and isopimaric acids were above 99% purity. Abietic acid was between 90 and 95% purity; pimaric acid was between 85 and 90%, containing traces of sandopimaric acid. Ammonium acetate (NH_4Ac) of minimum 98% purity was purchased from Riedel-de Haen (Germany). Glacial acetic acid and HPLC grade acetonitrile were purchased from Fisher Scientific (Edmonton, Canada).

2.3. Standard preparation

Resin acid standards were prepared using a 10 mg ml^{-1} stock solution prepared in methanol and stored at 4 °C for no longer than 2 weeks. Calibration was established with four to six standards prepared from the stock solution using appropriate dilutions with a 50:50 mixture of eluent A and eluent B (see below).

2.4. Instrumental

HPLC analysis was conducted using a Waters 2690 (Milford, MA, USA) separation module. The HPLC pumps were primed with fresh eluents on a weekly basis or sooner as required. The selected method employed a 250 mm×2.0 mm, 5 μm particle size, Luna C_8 reversed-phase analytical column (Phenomenex, Torrance, CA, USA.) maintained at 30±1 °C. The mobile phase consisted of 10 mmol ammonium acetate in water (eluent A) and of 10 mmol ammonium acetate in acetonitrile (eluent B) at a flow-rate of 200 μl min^{-1}. Volumes of 10 μl sample and calibration standard were injected using a Waters 2690 autosampler.

Mass spectrometric analysis was conducted using a Quattro Ultima mass spectrometer (Micromass, UK) equipped with an electrospray interface operating in the negative ion mode. MS conditions were as follows: source temperature 90 °C, desolvation temperature 220 °C, cone voltage setting 90 V, capillary voltage setting 2.74 V, cone gas N_2 81 l h^{-1}, desolvation gas N_2 265 l h^{-1}. Low and high mass resolution was set at 14.1 and 14.3, respectively and ion energy was 1.4. Entrance voltage was 36 V, collision energy 16 eV, and exit voltage 66 V. The multiplier was set at 650 V. At these conditions mass resolution was approximately 1 Da. Quantitative analysis was performed using selected ion monitoring of m/z 299.3 for DhA and 301.3 for AbA, IpA and PA. MASSLYNX version 3.4 software was utilised for all instrumental control and data acquisition.

3. Results and discussion

Several mobile and stationary phase conditions were evaluated to attain optimum method sensitivity and analysis time while maximising separation of AbA, IpA and PA. Both C_8 and ABZ (Supelco, Bellefonte, PA, USA; similar to C_{18} column chemistry) analytical columns were evaluated for retention and separation of the resin acids. Although the retention times were greatly increased using the ABZ stationary phase (>20 min) compared to that of the Luna C_8, separation was not improved.

Neither gradient nor acidic elution significantly improved the separation of the resin acid isomers. Further, a preliminary gradient program consisting of a linear gradient ending with 100% acetonitrile greatly reduced method sensitivity to approximately 10% of the isocratic method; optimal ionisation requires an aqueous eluent. The conditions for optimum analysis time, separation and method sensitivity of these resin acids were with the selected method in which a C_8 stationary phase and 10 mmol NH_4Ac (as an ion-pairing agent) in water–acetonitrile (20:80, v/v) isocratic mobile phase was employed. The four resin acids eluted between 8 and 13 min under isocratic conditions of 20:80 eluent A–B (Fig. 2). Although good sensitivity and analysis time were achieved, these conditions still did not adequately separate the three isomers for reliable individual quantification.

Under the selected negative ion electrospray conditions the four resin acids studied produced intense $[M-H]^-$ ions with no fragment ions being observed. MS–MS of the $[M-H]^-$ ions was performed so that product ion spectra could be used to differentiate the three isomers in lieu of chromatographic separation. However, $[M-H]^-$ precursor ions did not dissociate using argon as the collision gas at various collision cell energies, thus useful product-ion scans could not be obtained.

3.1. Detection, recovery and reproducibility

Based on a five-point calibration, a linear response was observed from 0.01 to 3.5 ng of resin acid on column using 10 μl injections. Method detection limits based on a signal-to-noise ratio of 3:1 in river water samples were 0.40, 0.40, 0.30 and 0.25 μg l^{-1} for abietic, dehydroabietic, isopimaric and pimaric acids, respectively. In comparison, typical GC–MS detection limits for unconcentrated effluent and water samples are generally in the range of 5 μg l^{-1} [8,12,13]. In light of this, LC–MS was deemed a sensitive quantification method for the four individual resin acids while minimising sample preparation with no extraction or derivatization requirements compared to reported GC methods. The key limitation of the LC–MS method, then, is the inability to separate the three isomer resin acids as may be achieved with the more complex GC methods.

The reproducibility of the method was ≥97% based on analysis of laboratory standards and dupli-

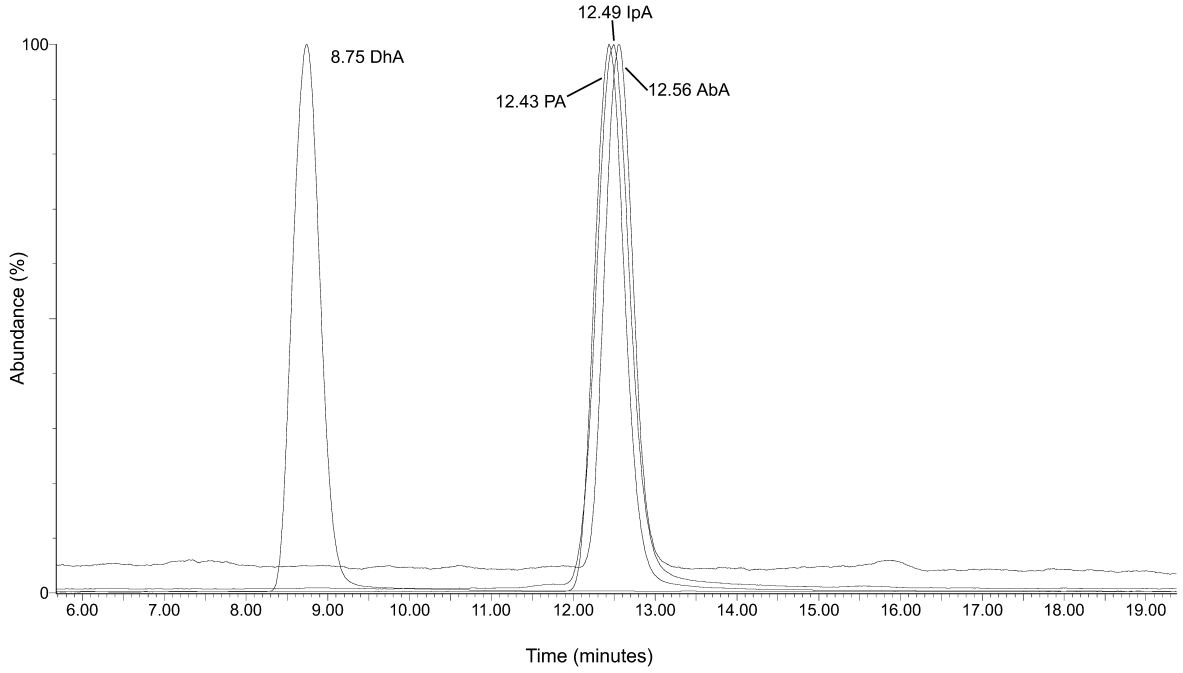

Fig. 2. LC ion chromatogram of AbA, DhA, IpA and PA using isocratic acetonitrile–water (80:20) with ammonium acetate.

cate Saale River samples taken throughout a 5-month period, in which more than 1000 determinations of resin acids were performed. There was little or no matrix interference based on the observed recovery of matrix spikes (Table 1). Environmental samples indicate the presence of DhA and the isomeric resin acids in the Saale river (Fig. 3). For those samples in which resin acids were detected, the overall mean and 95% confidence interval values were 0.177 ± 0.022 and 0.150 ± 0.020 mg l^{-1} for DhA and resin acids isomers, respectively. Resin acid concentrations in natural water samples were below the detection limits for some 50% of the natural water samples investigated.

4. Conclusions

The LC–ESI-MS method proved to be a rapid, highly sensitive procedure for the quantitative analy-

Table 1
Recovery of the four individual resin acids using the chosen LC–MS method with spiked natural river water samples

Resin acid	Spike concentration (mg l^{-1})	Recovered concentration (mg l^{-1})
Abietic acid	3.33	3.35 ± 0.01
	0.05	0.053 ± 0.003
Dehydroabietic acid	3.33	3.41 ± 0.03
	0.05	0.048 ± 0.004
Isopimaric acid	3.33	3.45 ± 0.01
	0.05	0.066 ± 0.006
Pimaric acid	3.33	3.39 ± 0.06
	0.05	0.075 ± 0.003

$n = 20$; error values are based on 95% confidence limits.

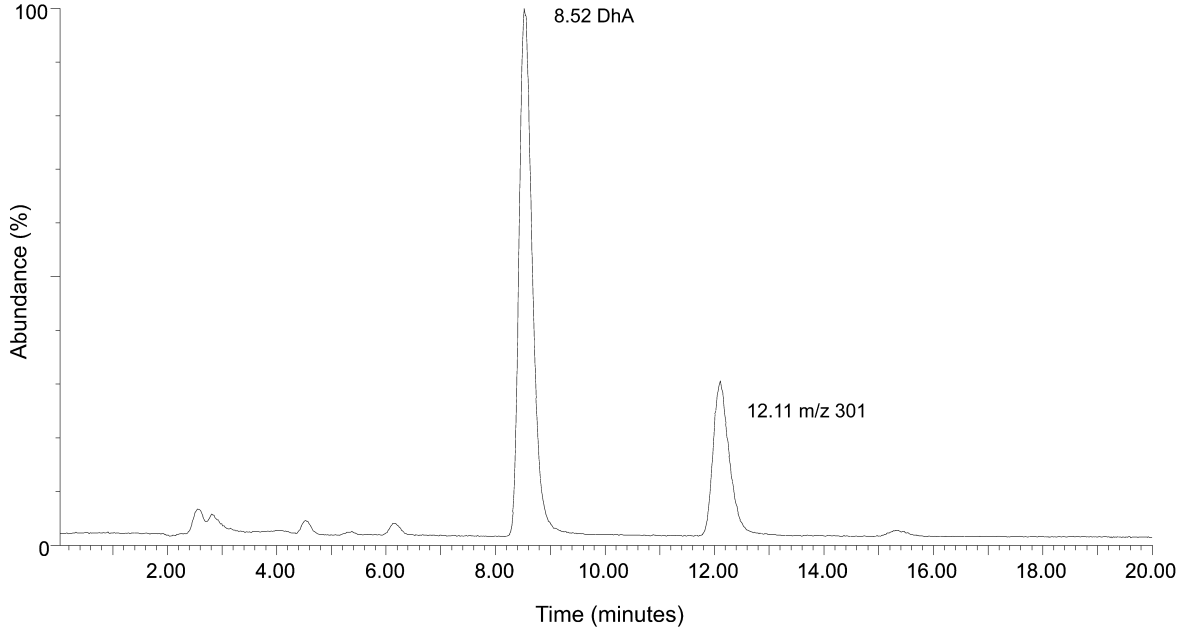

Fig. 3. Chromatogram of DhA and the three resin acid isomers in a natural water sample from the Saale river.

sis of abietic, dehydroabietic, isopimaric, and pimaric resin acids in spiked natural waters at detection limits of 0.40, 0.40, 0.30 and 0.25 $\mu g\ l^{-1}$, respectively. However, the three structural resin acid isomers (AbA, IpA and PA) do not separate sufficiently under the various LC conditions evaluated. Therefore, LC–ESI-MS is not recommended for environmental monitoring in instances where measurement of individual isomeric resin acids is required. Application of LC–ESI-MS is, however, well-suited for resin acids analysis of environmental water samples in which isomeric speciation is not required.

Acknowledgements

This work was conducted in support of the Canada–Germany Bilateral Agreement. The authors thank Hajo Dahlke of the UFZ Environmental Research Centre in Magdeburg, Germany, for collection of Saale river water samples and Wolf von Tuempling of UFZ Environmental Research Centre in Magdeburg, Germany, for technical support.

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