# Analyzing wood extractives from process streams by using alkaline reversed-phase extraction followed by *in situ* ion pairing

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**ABSTRACT** *A new and convenient analytical procedure for determining fatty acids, resin acids, and triterpenoic components in pulp mill process waters is described. The extractives are isolated from alkaline aqueous samples using disposable reversed-phase columns. The cations of the adsorbed acid salts are exchanged, in situ, for quaternary ammonium ions. The acids are methylated by thermal decomposition of the quaternary salts in the injector of the gas chromatograph. The rapid isolation, cleanup, and derivatization procedures are the major advantages of this method.*

**KEYWORDS**
Analysis
Black liquors
Extraction
Fatty acids
Methylation
Resins

Wood extractives often cause problems in pulping and papermaking. They may not only be responsible for the formation of pitch deposits in the process system or in the pulp, but they may also affect the quality of the product. Moreover, the extractives contribute to the environmental stress. Rapid, accurate, and sensitive analytical procedures are therefore needed to classify and quantify the resin components. These methods must be applicable to various kinds of sample liquids such as diluted paper mill effluents and black liquors.

Our aim was to develop a rapid method for selectively isolating extractives in pulp and paper mill process waters using reversed-phase methods and to determine the content using a harmless derivatization reagent and a rapid separation technique. We chose to develop the method primarily for kraft black liquors, since these liquors are among the most difficult sample matrices to handle.

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## Experimental methods

We found that fatty acids, resin acids, and triterpenoic wood extractives were efficiently retained on a hydrocarbon-substituted solid phase, even at high pH—in other words, when the acids are ionized (**Fig. 1**). The pH of the sample was kept constant or raised to a pH≥ 12, and some of the problems associated with the isolation of extractives from lignin-containing liquors were thereby avoided. The undesired substances were washed through the column with aqueous alkali containing methanol.

Gas chromatography was used to determine the isolated extractives. To be able to separate resin, fatty acids, and triterpenoics on the same gas chromatography column with sufficient resolution, the acids had to be derivatized. The commonly used methyl esters were prepared through pyrolysis methylation, by addition of a harmless methylating agent already in the extraction procedure. The counterions to the immobilized fatty and resin carboxylates were thereby exchanged for a quaternary ammonium salt (Q-salt). This salt was obtained by rinsing with an alkaline phenyl trimethylammonium solution, leaving an amount of the methylation agent equivalent to the number of functional groups to be derivatized. The lipophilic resin and fatty acid ion pairs were then eluted from the column, together with the neutrals. The pyrolysis methylation was accomplished in the gas chromatography's injector, according to the reaction scheme described in **Fig. 2**. A rapid gas chromatographic method was developed for quantifying the extractives. We used splitless injections, a common methyl silicon column, and a flame ionization detector. When only resin and fatty acids were being determined, we used a column with butanediol succinate (BDS) as stationary phase.

### Procedure

Our procedure, as outlined in **Fig. 3**, was as follows:

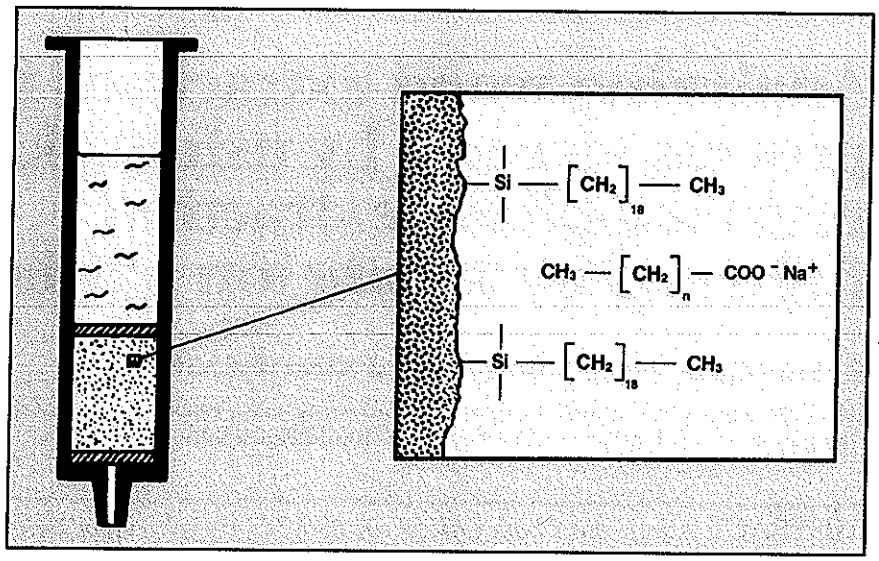
1. The solid-phase column was activated by rinsing with methanol (1 mL) followed by water (2 mL).

2. The pH of an alkaline kraft black liquor sample was raised to pH 12, if necessary, and methanol was added to a final concentration of 15% methanol in the aqueous sample.
3. The sample was allowed to penetrate the reversed-phase bed.
4. The column was rinsed with 5 mL of 0.01 *M* NaOH (containing 15% methanol), followed by 2 mL of an aqueous quaternary ammonium salt solution (phenyl trimethyl ammonium hydroxide 0.2 *M*, adjusted to pH 11 with $CO_2$).
5. The liquid was displaced by passing nitrogen through the column.
6. The extractives were eluted using a sequence of ethanol (1 mL) and dichloromethane (4 mL).
7. Cholesterol dissolved in dichloromethane was added as an external standard, and sodium sulfate was added to remove residual water before the gas chromatographic injection.

A Pye-Unicam autoinjector (PU 4700) was used together with a Hewlett Packard gas chromatography unit (HP 5790A). The extractives were separated on a 15-m × 0.54-mm, fused silica column, with a 1.5-$\mu$m film of DB-1, obtained from J&W Scientific, Calif. A retention gap (1 m × 0.54 mm) was inserted before the DB-1 column and a fused silica capillary column (0.5 m × 0.32 mm) was used as flow restrictor and transfer line to the detector. Helium was used as the carrier gas. The temperature of the injector was 300°C, and the flame ionization detector (FID) was set to 310°C. We used a temperature program of 180°C for 2 min, raised to 300°C at a rate of 8°C/min, and finally maintained at 300°C for 13 min. The peak integrations, standard calculations, and grouping of the extractives in three categories were performed using a laboratory data system (Trivector 2000). Peak identities were confirmed by mass spectrometry (Finnigan INCOS 50).

Gravimetric determinations were performed via adsorption of resin and fatty acid mixtures from alkaline solutions (pH≥ 12). The columns were rinsed with 0.01*M* NaOH and eluted with a sequence of ethanol (1 mL) and dichloromethane (4 mL). Samples from a black liquor were also isolated as described above but acidified with acetic acid before elution. The eluates were thoroughly evaporated and weighed on a 5-decimal electronic balance. The determinations were reproducible with a relative standard deviation of 1% for the alkaline and 3% for the acidified samples.

1. Solid-phase extraction cartridge with a fatty acid salt adsorbed in the $C_{18}$ phase




### Materials

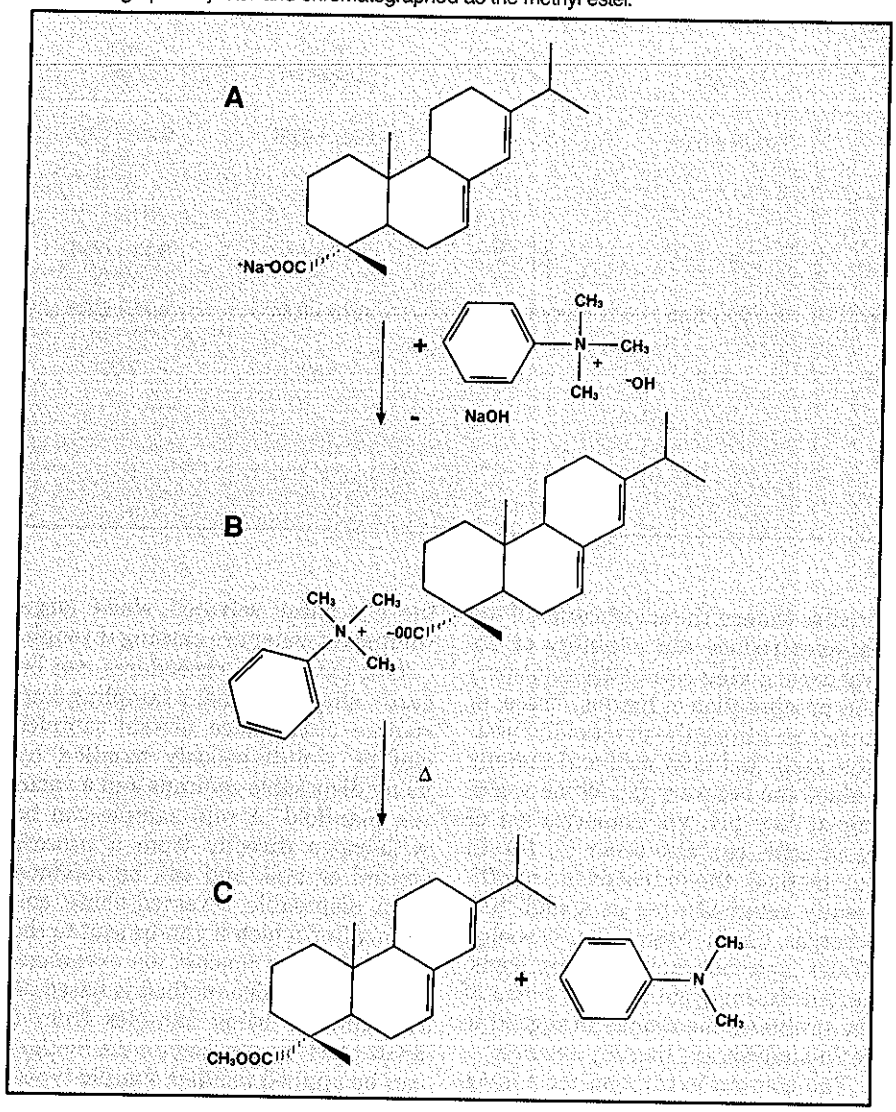
Abietic, dehydroabietic, isopimaric, levopimaric, neoabietic, and palustric acids were purchased from Helix Biotech Ltd, B.C., Canada. Commercial tall oil fractions of resin acids (Beviros 95), fatty acids (Bevacid 2), and pimaric acid were received as gifts from Bergvik Kemi, Söderhamn, Sweden. Phenyl trimethylammonium hydroxide, cholesterol and *n*-tricosanoic acid were obtained from Fluka AG, Switzerland. Authentic samples from different kraft pulp process waters were obtained from MoDo, Ornskoldsvik, Sweden. As extraction media, we used disposable syringe containers obtained from Analytichem Inc, U.S., or J. T. Baker, U.S. These were filled with 200 mg or 500 mg silica gel (40-$\mu$m particle size, 60 A porosity), chemically bonded to $C_{18}$ alkyl chains and end capped with trimethylsilyl chloride.

## Results

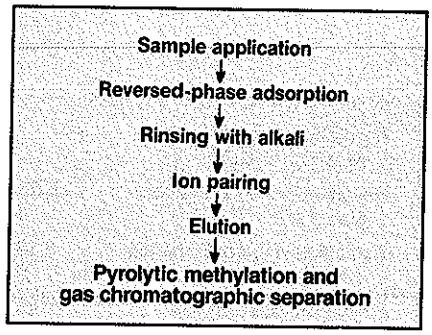
Resin and fatty acid mixtures of known concentration were determined by gas chromatography directly from the solution and via an alkaline or acidic reversed-phase extraction without any difference in recoveries. Thus, the resin and fatty acid were efficiently adsorbed and eluted from the $C_{18}$ phase, even as salts. The effect of different reversed-phase chain lengths ($C_2$–$C_{18}$) was studied. The highest yields of extractives were achieved when an octadecyl phase, $C_{18}$, was used. The accuracy of the method showed no difference between dilute resin and fatty acid mixtures and black liquor samples with a high lignin and extractives content. Black liquor samples were added both in concentrated (0.5 mL) and diluted form (100 ml) before addition, without any deficiency in recoveries. The sample volumes added to the extraction column were portioned so as not to exceed the column capacity, stated to be approximately 5% of the weight of the solid-phase (*1*).

The methanol content of the process water samples was studied, and we found that at least 15% by volume was required to obtain good recoveries. The yield of extractives, particularly resin acids, was lowered when the methanol concentration was too high. We obtained a better recovery of triterpenoics with increasing methanol concentration in the range studied (0–40%). A high methanol concentration was found to favor the elution of lignin fragments during the alkaline rinsing.

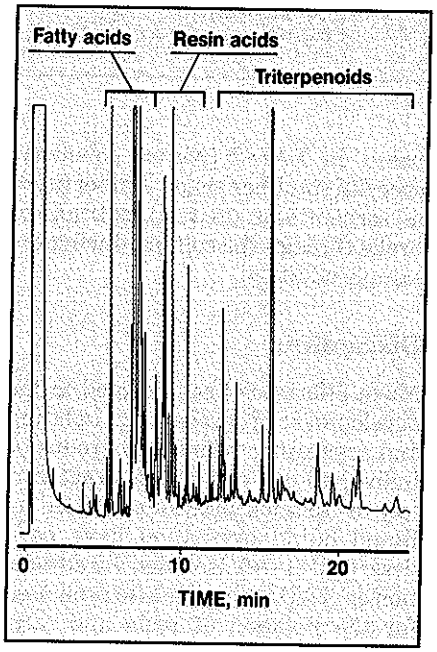
**2.** A resin acid (A) is trapped in the reversed-phase gel as its sodium salt. It is ion paired (B) with a phenyl trimethylammonium ion and eluted as such. It is derivatized (C) in the gas chromatographic injector and chromatographed as the methyl ester.




**3.** Analysis scheme for determining extractives




**4.** Gas chromatogram of extractives isolated from a kraft black liquor.




The solid-phase extraction method required no sample filtration, since fibers and other insoluble material in the samples were retained on the upper polyethylene frit of the disposable extraction column and were extracted in the final elution step by the organic solvents.

In this work, we prepared the methyl esters of the resin and fatty acids via pyrolysis methylation using a harmless methylating agent, phenyl trimethylammonium hydroxide. Halogen-containing solvents such as chloroform and carbon tetrachloride may inhibit pyrolysis methylation (*2*). We found indeed that lower yields were obtained when $CHCl_3$ was used as solvent. However, the solvent mixture used (dichloromethane:ethanol, 4:1) did not suffer from this limitation.

The completeness in derivatization with phenyl trimethylammonium hydroxide was compared with the results from methylation with diazomethane as reagent (*3*). Five different extractions were made and examined by gas chromatography, using double injections, with no significant difference between methylation with diazomethane or via the pyrolysis methylation of either the fatty acids or the resin acids (Student's *t*-test for paired data, $P > 0.7$).

The method depends on a complete ion exchange of the acids counterions for the derivatizing Q-salt. The addition of small amounts of calcium ions to the samples gave lower yields of resin and fatty acids. The recoveries could be restored by adding sodium oxalate to the samples.

We chose gas chromatography since it gives a rapid analysis with good resolution power. We considered a group separation to be sufficient, and the results are therefore presented in three categories—fatty acids, resin acids, and triterpenoics (**Fig. 4**). The overlaps between the groups were less than 10% and could be reduced by an elaborate grouping of the individual peaks before presentation. Using a wide-bore column and splitless injections, we minimized the discrimination pertaining to the components boiling at higher temperatures.

Repeated analysis of the resin content in 1 mL of an authentic black liquor gave a relative standard deviation of ±4% for the whole procedure. Our method was compared with other procedures (**Table I**). When a method

I. Comparisons of various methods

| | Total extract by gravimetric determination, g/L | Gas chromatographic determination,* g/L | | | |
|---|---|---|---|---|---|
| | | Fatty acids | Resin acids | Triterpenoids | Total yield |
| Reversed-phase extraction, eluted as Q-salts | ... | 4.32 + 0.09 | 1.04 + 0.04 | 0.93 + 0.06 | 6.29 + 0.13 (n = 7) |
| Reversed-phase extraction, eluted at pH 2 | 6.65 + 0.23 (n = 5) | ... | ... | ... | ... |
| Extraction with *t*-butylmethyl ether at pH 9 (*9*) | 8.36 + 0.53 (n = 5) | 4.26 + 0.56 | 1.08 + 0.10 | 1.11 + 0.2 | 6.48 + 0.66 (n = 6) |
| Extraction with dichloromethane at pH 2 | 5.45 + 0.92 (n = 5) | 2.72 + 0.49 | 0.70 + 0.11 | 0.75 + 0.14 | 4.17 + 0.60 (n = 5) |
| Precipitation on pulp and extraction with dichloromethane (*15*) | 8.88 + 0.06 (n = 5) | 4.32 | 1.00 | 1.88 | 7.20 |

n = number of samples
* = mean of double injections.

based on XAD-2 (porous polymeric styryldivinyl-benzene, Amberlite®) as sorbent was used (*4*), extractives broke through the column during the sample addition.

## Discussion

Much effort has been devoted to the development of methods for isolating and separating resins into groups or individual components. A great variety of isolation procedures based on liquid–liquid extraction have been described (*4–10*). Isolation via adsorption on XAD resins has also been used for the isolation of resin and fatty acids (*8, 11–14*). A different approach for isolating extractives is based on the precipitation of the extractives onto pre-extracted pulp, which is then filtered, washed, and re-extracted (*15, 16*). Methods based on liquid–liquid extraction are in general both laborious and time-consuming, while the extraction efficiency for adsorption on XAD resin is reported to be poor (*17–19*).

The methods of isolation may give incomplete and/or incorrect recoveries of the resin components, especially when acidified samples are analyzed. Pitch in pulp mill process systems often contains both lipophilic and amphiphilic extractives. The isolation of extractives on a lipophilic solid-phase would normally require the acids to be in their protonated, more lipophilic form. However, when starting from alkaline lignin-containing process waters, the acidification of the samples reduce the solubility of extractives, giving rise to the emulsions and precipitation of lignins. These, in turn, can adsorb the practically insoluble resin and fatty acid and thereby reduce the recovery (*9*). When working at low pH, an isomerization of resin acids can also occur (*8, 10*). In our method, the extractives are efficiently retained even at a high pH, whereas lignin fragments, carbohydrates, and inorganic salts are transported through the column. Some of the problems mentioned in regard to resin analysis are thereby avoided.

The column extraction techniques (*1, 20–22*) have the advantages of low solvent consumption, no problems with phase separation, and minimal need for glassware and sample transfers. In general, modern reserved-phase isolation techniques are simple and rapid, and the recoveries can be made quantitative (*21*). The solid-phase extractions eliminate disturbances from possible emulsions (*22, 23*), and they can also be useful for enriching dilute samples (*24*). The sorbent is stable under weakly alkaline conditions and for short periods of time at a pH as high as 13. The addition of methanol to aqueous samples containing lipophilic compounds has previously been suggested (*1*). Methanol increases the solubility of the lipophilic compounds and diminishes the tendency for foam to form. The presence of methanol in the aqueous phase also assists in keeping the $C_{18}$ phase solvated when large volumes of water are passing through.

This extraction procedures can be easily adapted to field sampling and routine work, since several samples can be simultaneously handled by using disposable columns and a vacuum manifold. A mill sample can be withdrawn directly from a process stream or tank and can be retained on a disposable reversed-phase column, after which it can be sent to the laboratory for rinsing, ion exchange, and analysis. The method is based on experiments using samples from a kraft black liquor. Before the method can be applied to other sample types, minor adjustments may be necessary, such as adjusting the concentration of the organic modifier (methanol) in the sample and washing solutions.

Resin and fatty acids and neutrals can be determined by different chromatographic methods. Thin layer chromatography (TLC) is extensively used (*25*), but capillary gas chromatography (*26*) and high-performance liquid chromatography (HPLC) (*27, 28*) offer the best separation power.

Resin and fatty acids are separated on a gas chromatography capillary column coated with a BDS phase (*29*). The use of HPLC (high-performance liquid chromatography) with direct injection of pulp mill effluents has been examined (*30*). However, the samples had to be filtered before injection, and this resulted in low yields, since particles coated with adsorbed extractives were also re-

moved. There is also a problem in finding suitable detectors for HPLC. However, if the acids are derivatized with a strong chromophore absorbing at high wavelengths, an HPLC separation and UV detection could be a selective way to determine resin and fatty acid (*31*), with response factors of 1.0 between the different acids.

During the development of this method, gas chromatography was chosen for determining the extractives. To be able to analyze both resin and fatty acid and triterpenoics in the same gas chromatography run, we had to derivatize the acids. The derivatization procedures commonly used for resin and fatty acid (*26*) are often tedious, and some include the use of toxic reagents. Harmless tetramethylammonium hydroxide can replace diazomethane in the methylation of resin acids (*32*). The use of an aqueous phenyl trimethylammonium hydroxide solution as methylating reagent instead of tetramethylammonium hydroxide (*33*) for the ion exchange was preferred, because of its greater lipophilicity and the lower temperature required for the pyrolysis methylation (*34*). The methylating reagent was introduced in the extraction procedure. In this way, the total time for analysis can be reduced, and the excess of methylating reagent can be avoided.

Generally, side reactions can occur if an excess of the derivatization reagent is used. To overcome these problems, the reagent has to be added in a calculated amount, or the samples have to be titrated with the reagent. In this method, only the required amount of the reagent was trapped by ion pairing, while the excess was washed away. Margarinic acid, $C_{17:0}$, and *n*-tricosanoic acid, $C_{23:0}$, are commonly used as quantitative standards and can also be used in this method, preferably as their quarternary ammonium salts. However, the results can be misleading when these acids are used as standards, since they can already be present in the samples, especially when samples originating from birch are analyzed. This potential error could be corrected for by using a standard addition method.

This method is compared with some commonly used methods in Table I. In spite of the limited number of analyses, some generalization can be made. The total yield determined by gas chromatography revealed a good agreement between our method and the method using *t*-butylmethylether (*9*). In both methods, the extractions were performed at a high pH. Extraction with dichloromethane at low pH gave lower yields, partly because of the difficulties experienced in the phase separation. The method based on precipitation of pulp followed by extraction with dichloromethane (*15*) gave the highest total yield, as a result of good recovery of triterpenoics. In the reversed-phase extraction method, we found the best agreement between the gravimetrically determined total extracts and the sum of the grouped extractives determined via gas chromatography.

Gravimetric determination is usually not the preferred method in assaying industrial process waters, since the determination is not selective, and interference from the matrix cannot be fully excluded. A gravimetric determination might, however, be of value for comparative determinations of total extracts. The reliability of a simple gravimetric determination is improved upon in our solid-phase isolation of extractives by the permanent retention of fibers, fines, and other solids, combined with the possibility of washing out inorganic salts, low-molecular-weight acids, carbohydrates, lignin fragments, etc.

## Conclusions

The method is a rapid analysis of fatty and resin acids from complex liquids such as kraft black liquors. The lipophilic extractives were efficiently retained on a disposable reversed-phase extraction column. The interfering substances were washed away with alkaline water, and an equivalent amount of a harmless derivatization agent was introduced already in the isolation sequence. A group separation on a wide-bore gas chromatography column gave reliable determinations. The whole procedure, including isolation, rinsing, derivatization, and determination of wood resin components, was accomplished in less than 1 h.□

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