High performance liquid chromatographic determination of resin acids in pulp mill effluent

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ABSTRACT

A high performance liquid chromatographic (HPLC) method was suitable for direct analysis of dehydroabietic acid in pulp mill effluents. Results correlated very well with the dehydroabietic acid and total resin acid contents from a gas chromatographic (GC) method. The HPLC method used to examine resin acid concentration at different stages of effluent purification in three pulp mills gave satisfactory results. The method was simple, fast, and accurate, and it did not involve the use of any toxic chemicals in sample preparation.

Application:

HPLC is a fast method for the determination of resin acids in pulp mill effluent.

RESIN ACIDS ARE COMMONLY recognized toxic materials to aquatic organisms. As early as the 1930s, Ebeling *(1)* found that 5 mg/L of resin acids in pulp mill effluent killed perch in 40 h. Leach and Thakore *(2)* identified the toxic components of the resin acids in pulp mill effluent from digesting 50% Douglas-fir *(Pseudotsuga memzisii)* and 50% western hemlock *(Tsuga heterophyli)* wood chips. They reported that three resin acid soaps in the effluent caused over 80% of the toxicity to juvenile coho salmon—sodium isopimarate, sodium abietate, and sodium dehydroabietate. Chung *et al. (3)* reported that with the common resin acids such as dehydroabietic, pimaric, abietic, etc., the toxicity (96-h LC50) was 0.5–1.0 mg/L with rainbow trout *(Salmo gairdneri)*. As concerns over the environment increase, the problems of toxicity of resin acids to aquatic organisms will extend beyond pulp and paper mills. Other wood processing facilities such as sawmills, log yards, kiln condensate producers, etc., will also need to adopt resin acid reduction.

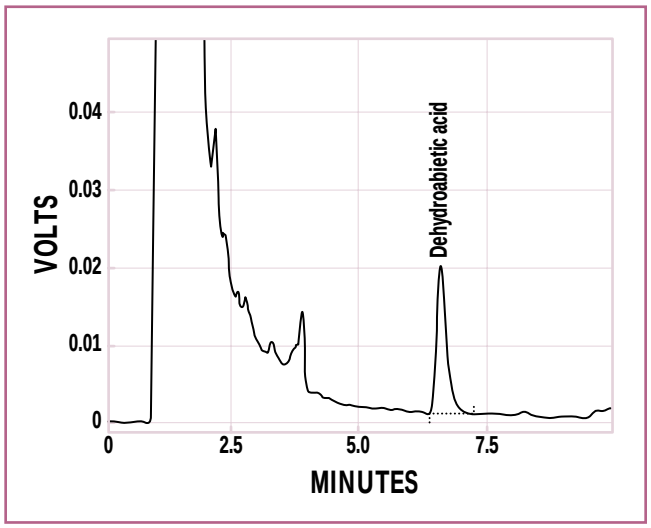
Due to the toxicity of pulp mill effluent, it is necessary to have treatment that reduces the concentration of resin acids before discharge. Under normal conditions of operation, activated sludge treatment can accomplish this. Due to changes of operating conditions or differences in raw material input, however, there is a high probability that the concentration of resin and fatty acids can suddenly increase in the pulp mill effluent. If undetected, this could result in polluting the environment or poisoning the microorganisms in the treatment pond. Upon discovery, changes are possible to alleviate it by allowing longer periods of biodegradation in the aeration treatment system. This would avoid a shutdown or slowdown of pulp mill production.

There are many different methods for resin acid determination. Carpenter *(4)* experimented with a colorimetric method using concentrated sulfuric acid and acetic anhydride to form an observable chromophore. He obtained the resin acid concentration by measuring the absorption at 525-nm wavelength. Pulp mills still use Carpenter's method today for effluent analysis. Because of the instability of the chromophore in the very low concentration range, however, Carpenter's method has some limitations.

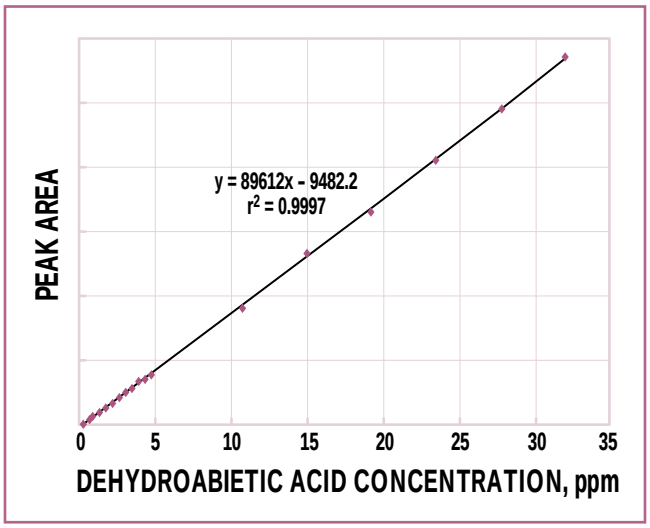
McDonald *(5)* experimented with an improved technique for Carpenter's method and introduced a stable standard, 2-amino-2-methyl-1-propanol salt of levopimaric acid, for the colorimetric analysis. Sithole *(6, 7)* discussed a spectrophotometric method adapted from the analysis of fatty acids in vegetable oil. The resin acids in the extractives formed a complex with cupric ions. The complexes were extracted into a solvent and their concentrations determined colorimetrically at 680 nm. A gas chromatographic (GC) method by Voss and Rapsomatiotis *(8)* is a commonly acknowledged reliable method. It involves the extraction of resin and fatty acids with methyl *tert*-butyl ether followed by derivatization with diazomethane for analysis. Although it takes longer and requires toxic chemicals for sample preparation, its results are highly accurate in the low concentration range. The method also allows the analysis of different components of resin and fatty acids. As a result, it finds common use as a standard method.

Instead of diazomethane derivatization of extracts, triethyloxonium tetrafluoroborate is an effective substitute of diazomethane *(9)*. Lee and Peart *(10, 11)* also reported a GC method that used acetone and methanol in the presence of hydrochloric acid as the solvent for extraction. The detection limit of resin and fatty acids was 0.1 µg/g.

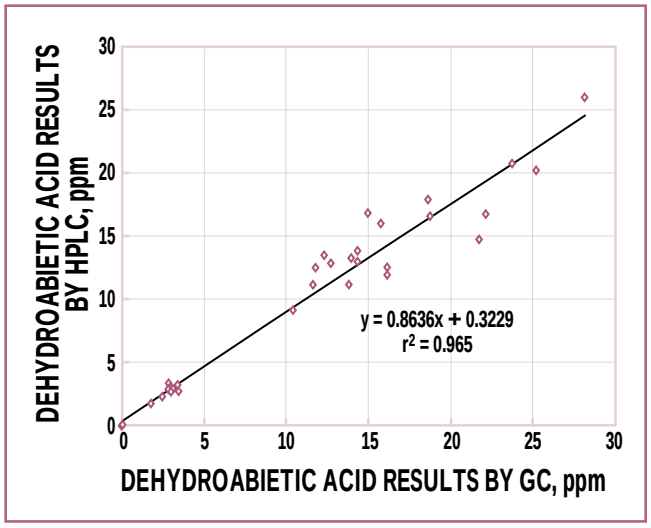
Richardson *et al. (12)* reported the use of the high-performance liquid chromatograph (HPLC) for resin acid analysis in their study of pulp mill effluent with *Pinus radiata*. They used dichloromethane extrac-



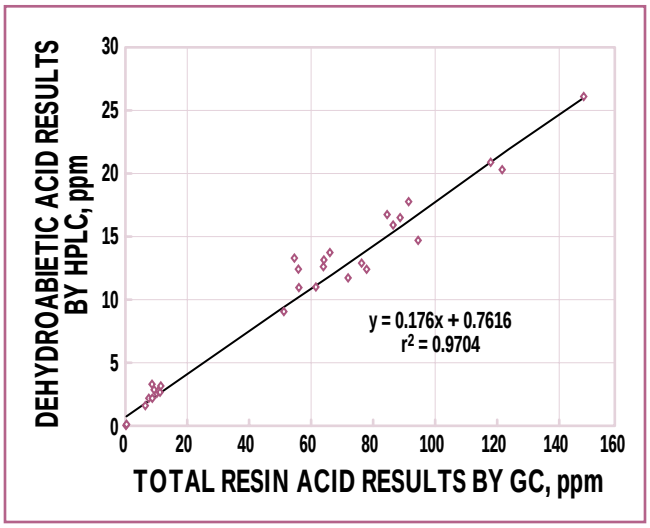
1. Chromatogram of pulp mill effluent containing 2.5 ppm dehydroabietic acid using the UV/VIS detector at 200 nm



2. Calibration curve of dehydroabietic acid using the HPLC



3. Correlation of HPLC and GC results for dehydroabietic acid in pulp mill effluent

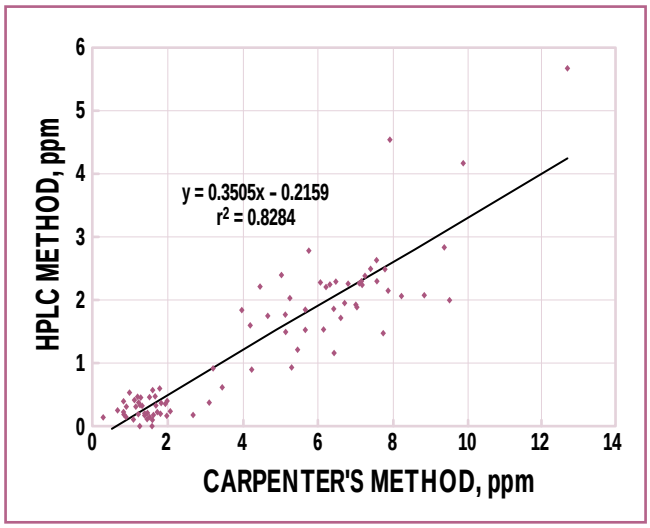


4. Correlation of HPLC dehydroabietic acid content and GC total resin acid content

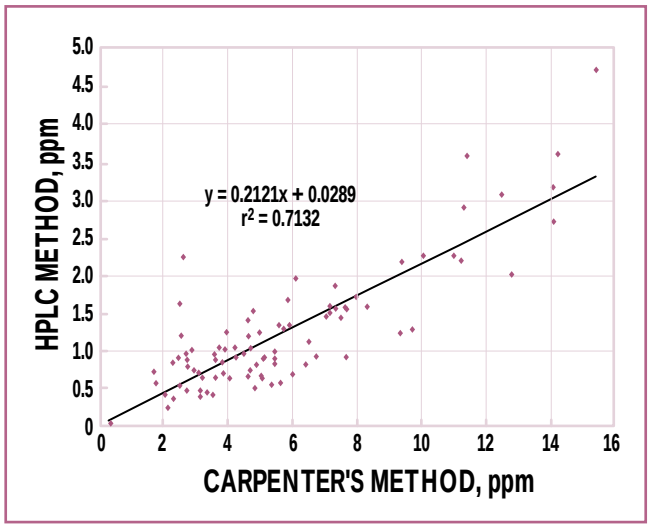
tion of the dehydroabietic acid before the HPLC analysis. Good results followed when using a UV detector and a fluorescence detector. Their note mentioned some work on direct injection of the effluent for analysis. There were no detailed results, however. In later research, Richardson *et al. (13)* reported a method of sample preparation using coumarin derivatives. Working with the same wood species, *Pinus radiata*, Sucking *et al. (14)* reported a method of resin acid analysis with the sample methylated with diazomethane before reverse phase HPLC analysis.

Each method has its strengths and shortcomings. The applicability of any method would depend on the sources of the pulping raw materials, wood species, and the pulping method. A satisfactory method must be fast and accurate to allow ample time to arrest the resin acid problem while it is still in the treatment stage. This will minimize environmental impact and economic loss from a mill shutdown due to a high resin acid surge in the effluent.

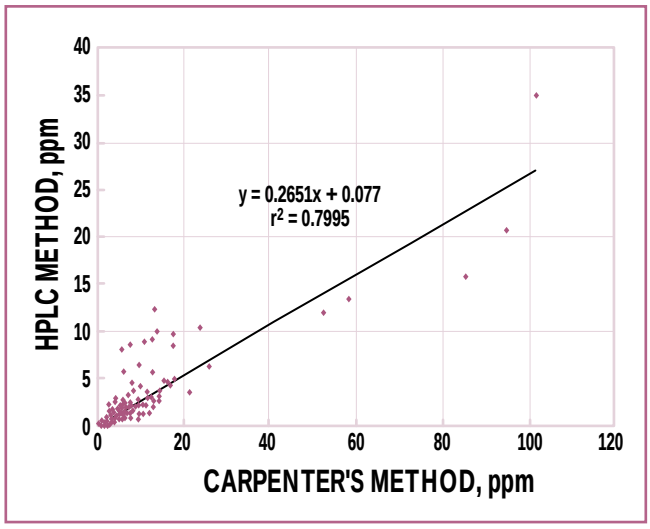
Searching for a fast and reliable method for the determination of resin acids in our pulp mill effluents, we have engaged in some preliminary studies comparing six methods. In this paper, we report the compar-



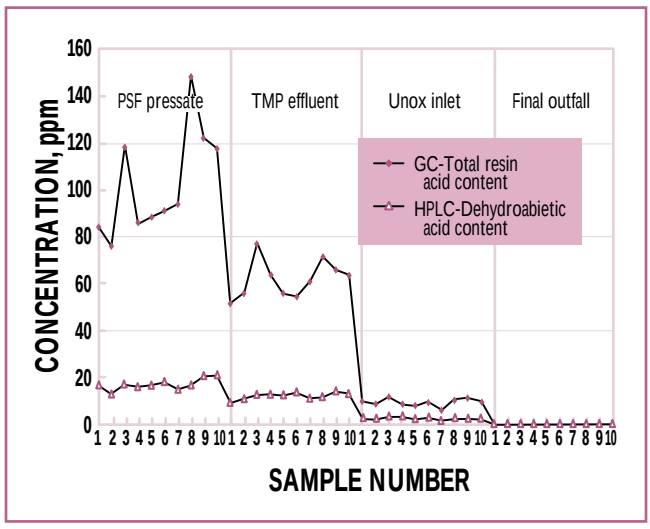
5. Relationship between HPLC and Carpenter's method with combined results from the clarifiers



6. Relationship between HPLC and Carpenter's method with combined results from the biological treatment basin



7. Relationship between HPLC and Carpenter's method with all data from the different stages of effluent treatment



8. Reduction of resin acid in pulp mill effluents using western hemlock

ative results of three selected methods including a modified method for direct injection of pulp effluent for HPLC analysis and applications.

EXPERIMENTAL METHODS

Carpenter's method

Effluent samples of 400 mL are acidified and extracted with 100 mL of petroleum ether and 100 mL of water:acetone:methanol mixture (1:2:1). Extracts are transferred to a dish and evaporated to dryness on a water bath. This then is redissolved in chloroform and extracted again using 65% sulfuric acid with acetic anhydride. The resultant colored layer is transferred into a 50-mL volumetric flask and made up to the mark with 65% sulfuric acid. Absorbance is read at 525 nm using a Novaspec spectrometer. Total resin acid concentration is quantified using external abietic acid standards.

Gas chromatography method

The samples were prepared essentially according to the method of Voss and Rapsomatiotis *(8)* with the exception that diethyl ether was used as solvent for extraction instead of methyl *tert*-butyl ether. A 500-mL water sample is acidified using 1 mL of 36 *N* H_2SO_4 and extracted three times with 100 mL of diethyl ether. The extract is filtered through sodium sulfate and glass wool before

being evaporated to 5 mL and derivatized with diazomethane to produce the corresponding methyl ester derivatives. The extracts are then purified on a Florisil column followed by concentration to 1 mL. Derivatives are analyzed by gas chromatography with a flame ionization detector.

Modified direct injection meth-od—HPLC

To investigate the Richardson *et al.* HPLC analytical methods, three different extraction techniques were tested: solid phase extraction, dichloromethane extraction as outlined in Richardson's paper, and direct injection. We also tested two different detectors for the analysis—fluorescence and UV/visible (VIS)—to determine which would be best for this analysis. The solid phase extraction used Chromosep C-18, 200 mg/3.0 mL cartridges. The resin acids were washed off the cartridge using acetone, dichloromethane, and methanol.

Concentration range, ppm	Correlation coefficient (r^2)
0 – 3.5*	0.98
0 – 12**	0.96
10 – 30*	0.79
40 – 160**	0.86
0 – 30*	0.97
0 – 160**	0.97

* Dehydroabietic acid vs. GC dehydroabietic acid
** HPLC dehydroabietic acid vs. GC total resin acid

I. Accuracy of HPLC direct injection method at different concentration ranges

Sources	Concentration range, ppm	r^2
Combined final outflow	0 – 3	0.001
Combined aeration lagoon	2 – 7	0.055
Clarifiers	0 – 14	0.828
Biological treatment basin	0 – 16	0.713
Chemical containment basin	0 – 100	0.794
Total	0 – 100	0.800

II. r^2 values for sampling areas

MATERIALS

For the comparison with the GC method, we obtained 40 samples from a coastal pulp mill—Howe Sound Pulp and Paper Ltd.—mainly using western hemlock for pulping. The samples came from four locations at different stages of treatment. Samples were plug screw feed pressate, thermal mechanical pulp effluent, effluent from inlet to aeration lagoon (unox inlet), and from the final treated effluent. At each location, we took a 2-L sample each day for 10 days. These samples were divided into two equal portions—one for the standard GC analysis and the other for the modified HPLC analysis. The analyses were made separately in two different labs and the results correlated.

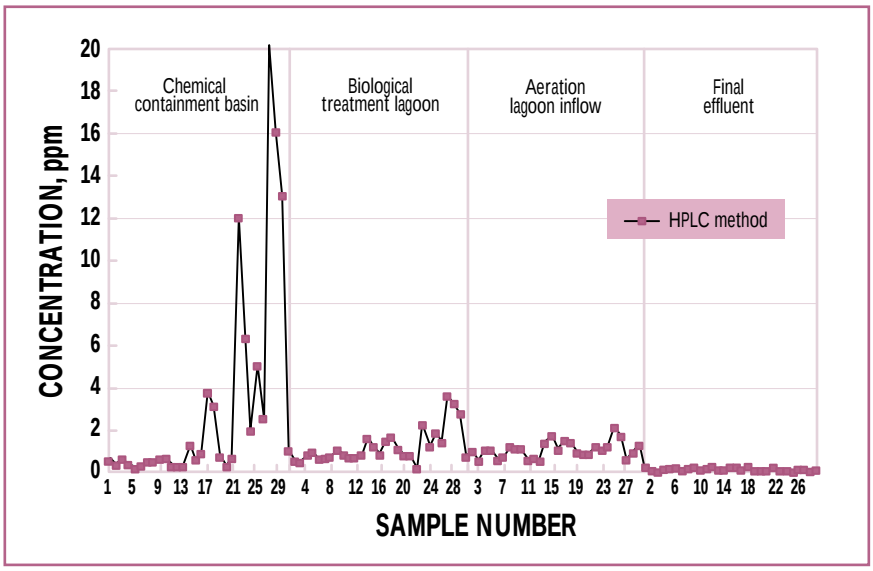
In the same manner, the correlation between the modified HPLC analysis with Carpenter's colorimetric analysis was performed with effluent samples from two pulp mills in northern British Columbia. These mills specialized in a combination of spruce, pine, and fir species *(Piceas glauca, Pinus contorta, Abies balsamea)*. The effluent samples came from eight locations from both pulp mills each day for 29 days. At the Canfor Intercontinental Pulp Mill, the samples came from the clarifier inflow, biological treatment basin inflow, and the chemical containment basin. At the Canfor Prince George Pulp and Paper Mill, the samples came from the clarifier outflow, biobasin inflow, and the chemical containment basin. The two mills combine their secondary treatment in one aeration lagoon. Two additional samples came from there—one from the combined aeration lagoon inflow and one for the combined outflow. Our study used 232 samples. Each sample was divided into two portions—one for HPLC analysis and one for Carpenter's method. The chemistry laboratory at Intercontinental Pulp Mill performed both methods for subsequent correlation of results.

INSTRUMENTATION

For Carpenter's method, the work used a Novaspec spectrophotometer. The sample was analyzed at 525 nm.

For the GC analysis, a Varian Model 3700 gas chromatograph was used equipped with a flame ionization detector using the following conditions: 30 meters of 0.25 mm ID SPB-5 fused silica capillary column, temperature from 80–280°C at 4°C/min hold 5, injector temperature at 240°C, detector temperature of 280°C, and helium as carrier gas. The GC analysis was performed by Econotech Ltd., an independent industrial laboratory, using the standard procedure for testing resin acids *(8)*.

For the direct injection HPLC analysis, a Shimadzu SCL-10A HPLC was used with LC-10AD pump and a SPD-10AV variable wavelength UV/VIS detector at 200 nm, a 20-µL



9. Reduction of resin acid in effluents from pulp mill using spruce, pine, and fir

injection port, a Whatman Universal RP Guard Column, and a Zorbax Rx-C8 4.6 mm x 250 mm column with a mobile phase of 65% acetonitrile and 35% 0.1% acetic acid at 2 mL/min.

In a preliminary experiment on the selection of detector, we compared both UV/VIS and fluorescence. A calibration curve was plotted using data from each detector with standard dehydroabietic acid at a concentration range of 0.1–2.5 ppm at 0.1–0.2 ppm intervals. The correlation coefficients, r^2, for the detectors were 0.995 and 0.999 for the fluorescence and UV/VIS detectors, respectively. Peak height in the HPLC chromatogram indicated that the fluorescence detector showed greater sensitivity. The noise from the fluorescence detector was far higher than the UV/VIS, however, especially at low concentrations. Our UV/VIS data also gave more consistent results than that of a fluorescence detector compared with the results from GC analysis. Note that Richardson *et al. (12)* preferred a fluorescence detector in their experiment.

The dehydroabietic acid standard of over 99% purity came from Helix Biotech in Vancouver, BC. Its peak appeared at approximately 6.75 min and was analyzed using external standards. Since dehydroabietic acid toxicity to fish reportedly occurs above 0.5–1.2 ppm concentration in the effluent *(3)*, additional samples were prepared to extend the calibration curve below 0.01 ppm concentration. The concentration samples below 0.1 ppm were also used for deriving the method detection limit (MDL) noted in Glasser *et al. (15)* at pH 9.

We also observed that effluent samples with high resin acid and high fiber content such as the effluents from thermal mechanical pulp (TMP) gave extremely low results by the direct injection to HPLC. In a sequence of tests, we found that some resin acids in acidic and neutral conditions precipitated and adhered to the fibers. As the pH of samples was adjusted over 8, the solubility of resin acids increased rapidly and reached a plateau at pH 9–11. For this reason, the pH values of samples from TMP were adjusted to approximately 10 with 10% NaOH solution before injection. By raising the pH, the resin acids became more soluble than the fibers. Note that our tests with pH adjustment used normal fiber contents of effluents from TMP operations of all three mills. There was no specific experiment to examine the effect of increasing fiber content above normal operating conditions. Other work *(9)* has shown that the adjustment of pH to approximately 10 also stabilizes resin acids in solutions.

Figure 1 shows the HPLC chromatogram of a unox inlet effluent sample with dehydroabietic acid concentration of 2.5 ppm. The dehydroabietic acid peak is well resolved without interference from other components. Because of this independent nature in the HPLC chromatogram, we considered this suitable for analytical purposes. We used it later to develop an in-line, direct resin acid detection method in the pulp mill.

The spiking method for chemical analysis *(9)* was tested with standard 0.5 ppm dehydroabietic acid solutions. *O*-heptadecanoic acid and *o*-methyl podocarpic acid (4 ppm) were respectively added to the resin acid solutions. They were analyzed with HPLC. *O*-heptadecanoic acid peak was not shown in the chromatogram, and the *o*-methyl podocarpic acid gave a peak at 3.45 min. The dehydroabietic acid peak appeared at 6.75 min. This spiking method with *o*-methyl podocarpic acid has some merit for future application research. Our objective is the development of a simple in-line, high-speed determination of resin acid in mill production conditions while taking advantage of the single and independent nature of the dehydroabietic acid peak in HPLC chromatogram. Our method development experiments therefore were done only with direct injection of mill effluents without the addition of spiking chemical.

KEYWORDS
Abietic acids, accuracy, chemical analysis, colorimetry, effluents, effluent treatment, gas chromatography, liquid chromatography, pollution control, pulp mills, resin acids, spectrometers, test methods, toxicity.

RESULTS AND DISCUSSION

To conform with the objective of finding a fast and easy method for in-mill application, the experimental design was such that the results from the GC method were used as standard. They were correlated to that of the HPLC method. In mill results later, the HPLC and Carpenter's methods were compared. Carpenter's method had been used in our mill quality control until the middle of 1995. It provided some needed information. With the increasingly stringent environmental and pollution regulations, however, a better method was necessary.

Correlation with GC method

Figure 2 shows the calibration curve derived from the direct injection HPLC analysis of dehydroabietic acid. It demonstrates the excellent linearity that extends below the 1-ppm range with a correlation coefficient of 0.99. The MDL for dehydroabietic acid with the direct injection HPLC method was 0.012 ppm.

Because the HPLC method detected only dehydroabietic acid while the GC method detects many individual acids, we correlated the HPLC dehydroabietic acid results with both the total resin acid content and the dehydroabietic acid alone obtained from the GC method. Using results from mill samples, Fig. 3 shows the relationship between the HPLC dehydroabietic acid and the GC dehydroabietic acid content. The range of the acid content was 0–28 ppm. The correlation coefficient is 0.97 indicating a good fit between the two methods.

Figure 4 shows the relationship between the total resin acid content as determined by GC analysis and dehydroabietic acid content as determined by the direct injection HPLC method. Range of total resin acid content was 0.05–148 ppm. Values correlated well with a correlation coefficient of 0.97. This relationship demonstrates that knowledge of the dehydroabietic acid content will give the total resin acid content.

Table I shows the correlation coefficients of the HPLC determination at different concentration ranges. These correlation coefficients for the HPLC dehydroabietic acid vs. GC dehydroabietic acid and the HPLC dehydroabietic acid vs. GC total resin acid substantiated the linear fit of the two methods. The high correlation coefficient (0.96–0.98) in the low concentration range is especially impressive.

These results confirm that it is possible to use the fast HPLC method to determine dehydroabietic acid content as an "indicator" for assessing total resin acid concentration in pulp and paper mill effluent. This will enable mill personnel to obtain resin acid data quickly and accurately at the mill site.

As we reviewed our past data gathered from both northern pulp mills, we found that these spruce, pine, and fir mills had dehydroabietic acid as the resin acid of the highest concentration. It was usually responsible for the toxicity of the effluents. Similar results occurred with the southern pulp mill that uses mainly hemlock. Here dehydroabietic acid was highest in the effluent inflow. The relative percentage of each resin acid of the total resin acid content remained constant throughout the treatment process. The calculation with the GC results supported this observation. These showed that the dehydroabietic acid accounted for approximately 20–30% of the total resin acids in the western hemlock pulping effluents. They were consistent among the sources of samples (n = 40). The percentage of dehydroabietic acid in the total resin acids of spruce, pine, and fir effluent was similarly approximately 20–25% (n = 180) with a calculation assuming that the resin acid content obtained with Carpenter's method represents the total resin acid content. Quantification of dehydroabietic acid can therefore indirectly provide the total resin acid content and the toxicity of the effluent.

Correlation with Carpenter's method

Similar to Fig. 4, Figs. 5–7 show the correlation between the results of the direct injection HPLC and Carpenter's method. The correlation was for each source of the effluent: chemical containment basin, biological treatment basin inflow, aeration inflow, and combined final effluents. There were separate correlations because the concentration ranges were different at each source. This provided an opportunity for examination of fitness of Carpenter's method at low and high concentrations. As Table II shows, the correlation coefficient of the two methods changes with the concentration range.

Our results indicate that correlation between Carpenter's method and the HPLC method is poor at a resin acid concentration below 10 ppm. This is different from the correlation between the GC and HPLC results. Those results showed excellent linear fit with high and low resin acid content. The present Carpenter's results are consistent with the work of McDonald showing that Carpenter's method has limitation in the low concentration range.

The modified HPLC method is an accurate method. Because it is fast

and simple and requires no toxic chemicals, one could use it under mill conditions.

Application of the modified HPLC method in pulp mills

The concentrations of resin acids in pulp mill effluents as they pass through various treatment systems were monitored using the HPLC method. Figure 8 shows the plot of resin acid concentration in the effluent of a pulp mill specializing in western hemlock pulping.

The pine, spruce, fir (PSF) pressate is a mechanical refining effluent involving no chemical treatment. Total resin content at this sampling spot can be as high as 140 ppm as measured by GC. This effluent is then combined with the rest of the TMP effluent that is also sampled. From here, the mechanical pulp effluent combines with the kraft effluent of lower concentration and feeds into the unox. This was also sampled. The final outflow was also monitored. Both HPLC and GC methods confirmed the reduction of resin acids in the pulp effluent.

The northern spruce, pine, and fir mills were also monitored for their reduction of resin acids in the effluent. Figure 9 shows results for a kraft pulp mill. These tests again demonstrated the usefulness and effectiveness of the HPLC method for the fast resin acid determination in the effluent. The results showed the effective reduction of the resin acids through various stages of the effluent treatment.

CONCLUSION

The direct injection HPLC analysis is suitable for the fast detection of resin acids in pulp mill effluent with reasonable accuracy. The method is most desirable for high-speed pulp production systems where the toxicity of the effluent requires detection in the shortest possible time. It will provide information for pollution abatement avoiding the shutdown of production due to high resin acid content. TJ

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Received for review Nov. 14, 1995.
Accepted May 14, 1996.

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