Determination of Trace Metals in Oils

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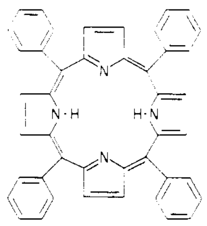
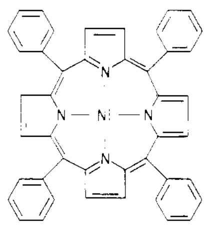
Since some metals in trace quantities have a deleterious effect on certain processes in the petroleum industry, an accurate and rapid procedure is necessary for their determination. Investigators have shown that some of these metals are present as organic complexes which are volatile and that a wet-ash procedure recovers more of the metals present in petroleum fractions than a dry-ash technique. It has never been shown conclusively that a wet-ash procedure recovers quantitatively the metals from volatile porphyrinlike complexes. This paper presents the results of the wet- and dry-ash procedures on synthetic blends of nickel, vanadium, iron, and copper derivatives of porphyrins. Information on the preparation of tetraphenylporphyrin and its metallic derivatives is presented.

THE importance of trace metals in petroleum processing has been reported by several investigators. A review of these reports has been presented by Milner, Glass, Kirchmer, and Yurick (*6*), Karchmer and Gunn (*4*), and Gamble and Jones (*3*). As the deleterious effects of metals such as nickel, vanadium, iron, and copper on catalytic cracking have been more fully realized, attempts have been made to produce cleaner feed stocks for charging to catalytic cracking units. This has been done primarily by the elimination of entrainment in distillation columns. Several years ago, it was not uncommon to encounter overhead distillates containing as high as 25 p.p.m. of metallic contamination. Fractionation efficiency has been so increased that at present it is very rare if the metallic content exceeds a few parts per million. Data have been presented by Woodle and Chandler (*13*), by Wrightson (*14*), and by Gamble and Jones (*3*) to support the postulate that metals appear in the overhead fractions by volatilization during vacuum distillation. It has been noted that porphyrin compounds and their metallic complexes are present in crudes (*1, 2, 6, 11*). It is also known that these compounds are volatile and consequently such metal-containing chelates can be expected to be present in the overhead petroleum fractions.

The procedure for ashing petroleum fractions developed by Karchmer and Gunn (*4*) was shown by these workers to be applicable to the type of stock encountered in the refinery several years ago. However, in the relatively noncontaminated overhead streams produced currently, the metallic impurities are present primarily as soluble, nonfilterable types, presumably volatile metallo-organic complexes. Hence, the conventional dry-ash procedure would not be expected to give quantitative results on these clean overhead fractions and, indeed, has been shown by Milner (*6*) and Gamble and Jones (*3*) to give results which are lower than these obtained by wet-ashing techniques. This is further proof that the metal constituents in these fractions are volatile. However, it has never been shown conclusively that the wet-ash procedure recovers all of the volatile constituents quantitatively. Hence, it was the purpose of this investigation to prepare volatile metallic tetraphenylporphyrin derivatives and to analyze synthetic blends containing these derivatives to determine if the wet-ash procedure gave quantitative results. Samples of tetraphenylporphyrin (TPP), nickel tetraphenylporphyrin (Ni TPP), vanadium oxide tetraphenylporphyrin (VO TPP), iron chloride tetraphenylporphyrin (FeCl TPP), and copper tetraphenylporphyrin (Cu TPP) were synthesized. Tetraphenylporphyrin was chosen because it is relatively easy to synthesize. The structure of tetraphenylporphyrin and its nickel complex may be represented as follows:





VOLATILITY OF METALLIC TETRAPHENYLPORPHYRINS

Evidence of the volatility of metal derivatives of tetraphenylporphyrin was demonstrated by a molecular still distillation of a sample of refined coastal residuum in which 187 p.p.m. of nickel tetraphenylporphyrin was dissolved. Analyses by visible spectroscopy of the 16 fractions obtained from this distillation are presented in Figure 1 and show definitely that this particular metallic porphyrin is volatile, but that no appreciable quantity is distilled below 1150° F. (temperature corrected to atmospheric pressure). The volatilities of nickel, vanadium, and copper tetraphenylporphyrins were proved further by test tube experiments, which showed that these metallic derivatives sublimed under reduced pressures at elevated temperatures without decomposition.

PREPARATION OF MATERIALS

Synthesis of Tetraphenylporphyrin. The method employed for the preparation of tetraphenylporphyrin was a modification of the procedure developed by Rothemund and Menotti (*8*). A mixture of 100 ml. of freshly distilled pyrole (Eastman reagent), 200 ml. of benzaldehyde (Eastman reagent), and 200 ml. of pyridine (Baker reagent) was deaerated and placed in a glass cylinder liner from which the oxygen was displaced with Seaford grade nitrogen. The glass liner and its contents were set in a 1-liter bomb (Parr autoclave equipment, Model H.C.-11) from which oxygen had been displaced with Seaford grade nitrogen. One hundred milliliters of pyridine were put in the annular space between the glass liner and the bomb. Any air in the bomb was removed by either evacuation or by pressuring the bomb to 300 pounds per square inch gage with Seaford grade nitrogen and releasing to atmospheric pressure. In case of the latter procedure, the operation was repeated several times.

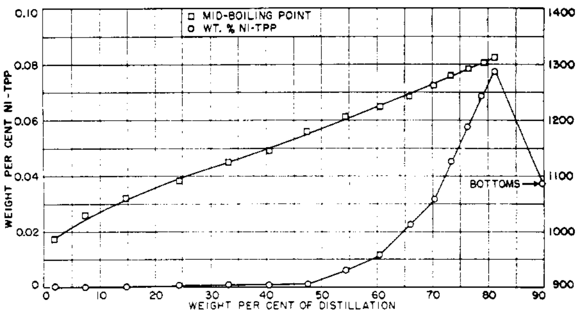


Figure 1. Molecular distillation of nickel tetraphenylporphyrin in refined coastal residum

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The bomb was heated to 360° F. for 48 hours and allowed to cool slowly in the autoclave for 24 hours. The contents in the glass liner were filtered through a glass-fritted disk filter and washed with a mixture of 90 to 10 volume % of ether and acetone until the filtrate was only a light pink color. The crystals were washed with hot methanol and dried in a vacuum oven. The average yield of crude tetraphenylporphyrin was 17.4 grams. These crude crystals were purified by extraction with 200 ml. of benzene in a Soxhlet extractor. The benzene was allowed to cool slowly to about 76° F. (room temperature), and the crystals were removed from the benzene by filtration. These crystals were re-extracted with fresh benzene and recrystallized. The dry crystals from the second extraction were essentially chemically pure tetraphenylporphyrin as shown by the following analysis.

Formula		Mol. Wt.	C	H	N
Tetraphenylporphyrin	Theoretical (calcd.)	614.71	85.97	4.92	9.1
$C_{44}H_{30}N_4$	Found (anal.)	..	85.87	5.15	9.07

Synthesis of Nickel Tetraphenylporphyrin. Nickel tetraphenylporphyrin was prepared by a modification of Method 2 of Rothemund and Menotti (*9*).

Five grams of pure tetraphenylporphyrin were dissolved in 300 ml. of pyridine (Baker reagent), and 5 grams of nickel acetate (Baker reagent) were dissolved in 200 ml. of pyridine. These solutions were mixed and deaerated by applying reduced pressure before charging to an oxygen-free glass liner for a 1-liter Parr autoclave bomb (Model H.C.-11). The glass liner and contents were placed in the bomb from which air had been displaced with Seaford grade nitrogen. One hundred milliliters of pyridine were put in the annular space between the glass liner and the bomb. The bomb was pressured to 300 pounds per square inch gage with Seaford grade nitrogen and then released to atmospheric pressure. This procedure was repeated several times to be sure that the oxygen content of the bomb was negligible. The bomb was repressured to 300 pounds per square inch gage with nitrogen and heated to 400° F. for 24 hours. The bomb and contents were allowed to cool slowly in the autoclave for 24 hours to room temperature. The contents of the glass liner were removed and the liquid volume decreased approximately 50% by distilling under reduced pressure. After cooling to room temperature (about 76° F.), the crystals were filtered from the liquid and purified by extraction with benzene in a Soxhlet extractor. The crystals obtained from the benzene extract were re-extracted with fresh benzene. About 5.2 grams of chemically pure nickel tetraphenylporphyrin were obtained from the second purification step as indicated by the following data.

Formula		Mol. Wt.	C	H	N	Ni
Ni tetraphenylporphyrin	Theoretical (calcd.)	671.38	78.71	4.20	8.35	8.74
$C_{44}H_{28}N_4Ni$	Found (anal.)		78.85	4.10	8.16	8.78

Synthesis of Iron Tetraphenylporphyrin. The procedure employed for the preparation of iron tetraphenylporphyrin was Method 1 used by Rothemund and Menotti (*9*). Five grams of pure tetraphenylporphyrin and 1 gram of sodium chloride (Baker reagent) were placed in a 1-liter three-necked flask, equipped with a glass mechanical stirrer, a glass reflux condenser, and a gas inlet tube. Four hundred milliliters of chloroform (Baker reagent) were added and the mixture was heated and stirred under a blanket of Seaford nitrogen until all of the tetraphenylporphyrin was dissolved. In a similar apparatus 2.2 grams of pure iron powder were dissolved in 400 ml. of glacial acetic acid (Baker reagent) under a blanket of Seaford nitrogen at the reflux temperature. The ferrous acetate solution was forced through a glass wool filter into the flask containing the chloroform solution of tetraphenylporphyrin and sodium chloride by means of nitrogen pressure. This mixture was refluxed for 2 hours, and the amount of liquid was reduced to slightly less than 400 ml. by distillation. The contents of the flask were allowed to cool slowly overnight.

The crystals were separated by filtration and were washed consecutively with a 50/50 mixture of glacial acetic acid and water, a small amount of glacial acetic acid, and acetone and then were dried in a vacuum oven. The dried crystals were placed in a Soxhlet extractor and extracted with chloroform. The amount of chloroform was reduced to 50 ml., and 800 ml. of hot methanol were added. This mixture was stirred for about 30 minutes and then allowed to cool slowly. The precipitate was sucked dry and re-extracted with chloroform, then was reprecipitated with methanol as before. About 3.2 grams of iron chloride tetraphenylporphyrin of the following purity were obtained.

Formula		Mol. Wt.	C	H	N	Cl	Fe
FeCl tetraphenylporphyrin	Theoretical (calcd.)	704.00	75.06	4.01	7.96	5.04	7.93
$C_{44}H_{28}N_4FeCl$	Found (anal.)	..	71.28	3.94	7.38	9.54	7.44

Synthesis of Copper Tetraphenylporphyrin. The procedure employed for the preparation of copper tetraphenylporphyrin was Method 1 used by Rothemund and Menotti (*9*). Five grams of pure tetraphenylporphyrin were placed in a 1-liter three-necked flask that was fitted with a glass mechanical stirrer, a glass condenser, and a gas inlet tube. Four hundred milliliters of chloroform (Baker reagent) were added. This mixture was heated by means of a heating mantle and stirred under a Seaford nitrogen blanket until all of the tetraphenylporphyrin was dissolved. In a similar apparatus 2.5 grams of cupric acetate (Baker reagent) were dissolved in 400 ml. of glacial acetic acid (Baker reagent). This solution was then forced through a glass wool filter into the flask containing the chloroform solution of tetraphenylporphyrin by applying nitrogen pressure. This mixture was stirred for 2 hours at the refluxing temperature and then allowed to cool slowly overnight to room temperature (about 76° F.). The crystals were separated from the liquid by filtration, washed with a 50/50 mixture of glacial acetic acid and water, and glacial acetic acid, and then sucked dry. The copper tetraphenylporphyrin was purified by benzene extraction in a Soxhlet apparatus, followed by crystallization, re-extraction with fresh benzene, recrystallization, and drying in a vacuum oven. About 5.5 grams of chemically pure copper tetraphenylporphyrin were obtained from the second purification step

Formula		Mol. Wt.	C	H	N	Cu
Cu tetraphenylporphyrin	Theoretical (calcd.)	676.26	78.12	4.18	8.29	9.41
$C_{44}H_{28}N_4Cu$	Found (anal.)	..	78.28	4.20	8.23	9.39

Synthesis of Vanadium Tetraphenylporphyrin. The method used for the preparation of vanadyl tetraphenylporphyrin is as follows:

Ten grams of c. p. vanadium pentoxide were placed in a 500-ml. three-necked flask fitted with a glass Trubore stirrer, reflux condenser, and gas inlet tube; 50 ml. of concentrated hydrochloric acid were added and the mixture was warmed and stirred gently. After 15 minutes the oxide had usually dissolved. If not, additional acid was added and heat applied until a clear green solution resulted. Two milliliters of ethyl alcohol (*7*) were added, and the solution was refluxed until a clear blue solution was obtained; this generally required 20 minutes. The heating mantle was removed and the stirred solution was neutralized to pH 5 to 7 by the addition of a saturated aqueous solution of sodium carbonate. The light cocoa-colored precipitate was filtered on a sintered-glass filter under vacuum, and washed with two 50-ml. portions of distilled water and two 25-ml. portions of absolute ethyl alcohol. The precipitate was sucked dry and immediately scraped out of the funnel into a 200-ml. round-bottomed flask containing 5 grams of tetraphenylporphyrin (twice extracted with benzene) and 30 grams of c. p. naphthalene.

Immediate transfer of the precipitate was found to be very important, because prolonged exposure of the solid to air resulted in darkening of its color and failure of the subsequent reaction. The flask was fitted with a long air condenser and was heated with a free flame. Water and naphthalene boiled out the top of the condenser until all the water was gone. More naphthalene was added at this point if necessary to maintain a convenient liquid volume. The mixture was then refluxed for 2 additional hours, or until a bright red melt was obtained. The contents of the flask were poured into water, and the lumps were broken up and placed in a Soxhlet extractor. Naphthalene was extracted with either acetone or alcohol; the extraction flask was changed and the porphyrin was extracted with 300 ml. of chloroform.

The extraction required about a week because the sintered-glass extraction thimble became clogged with very finely divided black material, probably unreacted vanadium oxides. This material was so finely divided that it passed through the sintered-glass thimble (it also passed through conventional paper thimbles) and contaminated the solid porphyrin in the flask. The black material was not volatile whereas the porphyrin was; thus, vacuum sublimation was chosen as the method of purification.

Filtration of the chloroform extract gave about 3 grams of material which were dried in vacuo at 110° C. and purified by sublimation at 450° C. and 1 to 5 microns of pressure. A nonvolatile, black residue remained behind. The dark blue-violet, crystalline sublimate was triturated to a dark red-violet powder prior to analysis. The powdered sublimate was found to be essentially chemically pure.

Formula		Mol. Wt.	C	H	N	O	V
VO tetraphenylporphyrin	Theoretical (calcd.)	679.65	77.75	4.15	8.24	2.36	7.50
$C_{44}H_{28}N_4VO$	Found (anal.)	..	77.40	4.25	8.45	2.12	7.68

Preparation of Synthetic Blends. Synthetic metallic tetraphenylporphyrin blends in gas oil were prepared for subsequent analytical studies. The gas oil used in these blends had a boiling range between 600° and 1100° F. and contained 0.32 p.p.m. of nickel, 0.04 p.p.m. of vanadium, 1.0 p.p.m. of iron, and no copper as determined by several analyses by the wet-ash procedure. Prior to the preparation of the blends the gas oil was heated to approximately 300° F. under a nitrogen blanket and filtered through a medium porosity glass-fritted disk filter. To individual-weighed samples of this filtered gas oil accurately weighed quantities of nickel tetraphenylporphyrin, vanadium tetraphenylporphyrin, iron tetraphenylporphyrin, and copper tetraphenylporphyrin of known purities were added. These mixtures were agitated mechanically in glass equipment under a Seaford nitrogen blanket at approximately 300° F. for 48 to 72 hours, followed by filtration through a medium porosity glass-fritted disk filter by means of reduced pressure. The residue on the fritted disk was dissolved by sulfuric acid digestion and analyzed to determine the amount of the particular metal that did not dissolve in the oil. This quantity of metal was subtracted from the amount originally added to obtain the actual metal content of the filter gas oil.

ASHING PROCEDURES

The procedure of Karchmer and Gunn (*4*) for the determination of ash in petroleum was used for the dry-ashing technique. Normally, 1 quart of material was burned. The wet-ashing procedure was a modification of the method reported by Milner (*6*). The modified procedure is simple, rapid, and adaptable to routine operation. It has been in routine use in this laboratory for over a year.

Modified Wet-Ash Procedure. An appropriate amount of oil was weighed into a 700-ml. Vycor beaker and 1.0 ml. of concentrated sulfuric acid was added for each gram of sample. (The size of sample for analysis depends on the expected concentration of metals, but usually 100 grams are used when analyzing for trace amounts. No more than 100 grams should be treated with sulfuric acid at one time. If more sample is required, additional 100-gram batches should be treated and the subsequent solutions combined.) The beaker containing the sample and acid was placed on a high temperature hot plate (about 800° F.) and heated as rapidly as possible. When boiling or frothing started the mixture was stirred vigorously with a Vycor stirring rod and, by use of large beaker tongs, the container was removed alternately off and on the hot plate to control foaming. How hot and when to stir definitely affects the sludging characteristics of most oil samples. In most cases a few minutes were required before a dry coke was obtained and all danger of spattering and foaming was passed.

When the sludge began to thicken, the beaker was heated on the hot plate at a high temperature and was stirred intermittently. At first, heavy fumes of sulfur oxides were emitted; however, the organic sludge soon began to crack and the ignitable hydrocarbon vapors were ignited and allowed to burn until a completely dry coke remained. The burning could be aided considerably by stirring periodically. The beaker containing dry coke was removed to a muffle furnace and reduced to an inorganic ash at 1000° F. aided by a slow flow of air or oxygen. This work, up to placing in the muffle furnace, must be done under a well constructed hood. By elimination of the sulfur oxide fumes and burning the hydrocarbon vapors, the final ashing step in the muffle furnace may be done in the open laboratory.

After the sample had been reduced to an inorganic ash it was removed very carefully from the muffle furnace and, after cooling, 20 ml. of 1 to 1 hydrochloric acid were added, and the sample was boiled to effect solution. Then it was transferred quantitatively to a 250-ml. beaker, reduced to 3 to 4 ml., filtered, and made to a convenient volume for aliquoting. Transferring from the 700-ml. beaker to the 250-ml. beaker is done because of the danger of forming dry spots in the large beaker. If a large amount of acid insoluble material is present it may be necessary to fuse the sample to effect solution. However, this procedure was not necessary in the samples used in this study.

Baker's c. p. hydrochloric acid and Du Pont c. p. sulfuric acid were used. The over-all reagent blank was approximately 0.025 p.p.m. of iron, 0.035 p.p.m. of nickel, and no vanadium. Of these amounts the sulfuric acid contained 0.020 p.p.m. of iron and 0.014 p.p.m. of nickel. Blanks on copper were high and hence copper in low concentrations was not determined.

The dry and wet ashes were then analyzed chemically for the desired metals. Nickel was determined by dimethylglyoxime (*6*), iron by thiocyanate (*10, 12*), vanadium by phosphotungstate (*6*), and copper by polarography (*5*). All reagent blanks were carefully determined and subtracted from the readings obtained on samples.

Accuracy of Wet-Ash Analyses. During the work on the synthesis of the metallic porphyrin compounds, preliminary results indicated that nickel was one of the more volatile metal derivatives of tetraphenylporphyrin. Therefore, the ability of the wet-ash procedure to determine accurately the amount of volatile metallo-organic complexes introduced into an oil was first tested on blends of nickel tetraphenylporphyrin. Blends 1 and 2 were prepared in white oil containing no detectable amount of extraneous nickel; blend 3 was prepared in a gas oil. The data in Table I present the results obtained.

Table I. Accuracy of Wet-Ash Procedure on Synthetic Blends Containing Nickel Tetraphenylporphyrin

Sample No.	1	2	3
Composition, p.p.m. Ni			
By synthesis	0.22	1.07	10.42
By wet-ash	0.23	1.05	10.15
	0.24	1.10	10.43
	0.23	1.05	10.41
	0.23	1.04	10.41
	0.22	1.03	
	0.20		
Av.	0.225	1.054	10.35
Recovery, % of synthetic value	102	99	99

It is seen from these data that the nickel was accurately and totally determined in all concentrations studied, to within the reproducibility of the test.

Comparison of Dry- and Wet-Ash Results. In order to test further the accuracy of the wet-ash procedure and to compare the results with the dry-ash technique, blends containing nickel, iron, vanadium, and copper in gas oil were prepared. The results of both types of analyses are compared in Table II.

Table II. Comparison of Wet-Ash and Dry-Ash Results at High Concentrations of Metals

Sample No.	4		5		6		7	
Metal added	Ni		V		Fe		Cu	
	Compn. by Synthesis, P.P.M.							
	15.62		12.44		15.20		16.20	
	Compn. by Analysis, P.P.M.							
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
	16.1	8.7	11.5	11.0	15.5	10.4	16.7	7.7
	16.4	8.5	11.2	11.0	15.6	9.9	16.3	7.2
		8.2	11.7	11.0	15.4	10.3	16.0	6.7
			11.8	10.8	15.8	10.4	16.4	
			12.3			9.8		
						9.7		
Av.	16.25	8.47	11.70	10.95	15.55	10.09	16.35	7.20
Recovery, % of synthetic value	104	54	95	88	102	72	101	45

The data show that all the metals studied were quantitatively recovered by the wet-ash procedure but not by the dry-ash technique. In the case of vanadium, very little was lost by dry-ashing; however, the difference in the dry- and wet-ash value is statistically significant.

The comparison of wet- and dry-ash results was extended to cover low concentration of nickel, iron, and vanadium. Copper was not studied in low concentration because of reagent blank difficulties. The samples for this study were prepared by dilution of samples 3, 5, and 6 with a catalytic cycle stock containing 0.02 p.p.m. of iron, 0.03 p.p.m. of nickel, and no detectable amount of vanadium. The three blends were then mixed to make sample 8. Table III shows the results obtained on this sample.

Table III. Comparison of Wet-Ash and Dry-Ash Results at Low Concentrations

(Sample 8)

Metal added	Ni		V		Fe	
	Compn. by Synthesis, P.P.M.					
	0.53		0.31		0.52	
	Compn. by Analysis, P.P.M.					
	Wet	Dry	Wet	Dry	Wet	Dry
	0.52	0.41	0.29	0.31	0.51	0.46
	0.50	0.44	0.33	0.29	0.51	0.44
	0.54	0.41	0.33	0.29	0.53	0.43
	0.52	0.39	0.33	0.30	0.53	0.41
Av.	0.52	0.41	0.32	0.30	0.52	0.44
Std. dev.	0.0163	0.0206	0.0200	0.0096	0.0115	0.0208
Recovery, % of synthetic value	98	76	103	97	100	85

These data show that significant losses of nickel and iron were obtained by the dry-ash method, whereas the loss of vanadium was not statistically significant. By comparing the dry-ash results on nickel in Tables II and III, it can be seen that 76% recovery of nickel was obtained on the 0.5-p.p.m. samples, whereas only 54% recovery was obtained on the 15-p.p.m. samples. Similarly, the recoveries of iron and vanadium were greater at the low concentration level (see Tables II and III). The higher recoveries of nickel, iron, and vanadium by the dry-ash procedure at the 0.5-p.p.m. level as compared to the 15-p.p.m. level cannot be fully explained at this time. One explanation may be that 100-gram samples of the 0.5 p.p.m. blends were dry-ashed, whereas 1-quart (approximately 800 grams) samples were used in the work at the 15-p.p.m. level. The initial dry ashing results on the concentrated blends were obtained at an early stage of the experimental work by the regular routine procedure which specifies 1 quart of sample. However, as the work progressed it was necessary to reduce the amount of sample used to 100 grams, because of sample availability. In order to test this possibility, a series of six dry-ash determinations were made using 100-gram portions of sample 4 which contained 15.62 p.p.m. of nickel. The amount determined by dry ash was 11.8 p.p.m. (average of 12.2, 12.1, 10.5, 11.6, 11.9, 12.5). This represents a recovery of 76%, which checks exactly with the value of 76% obtained on sample 8 when using 100-gram portions during dry-ashing.

These data are in agreement with the results of Gamble and Jones (*3*), who have shown that the recovery of metals by dry-ashing is dependent on the amount burned at one time, the size of the dish in which the sample is burned, and the rate of burning. Inasmuch as the modified wet-ash procedure described in this work determines volatile metals quantitatively and in some cases is as fast or actually faster than the dry-ashing technique, further studies on improving the recovery by dry-ashing were of limited interest and hence not pursued.

Recovery of Metals from Natural Petroleum Stocks. Numerous experiments on petroleum fractions produced in the refinery show losses of nickel and iron by the dry-ashing techniques similar to the losses reported in Tables II and III on the synthetic blends. However, losses of vanadium on some natural petroleum fractions were higher than those experienced with the synthetic blends. These data are presented in Table IV.

Table IV. Recovery of Vanadium from Natural Petroleum Fractions

Sample No.	11		12		13	
Type of stock	Gas Oil		Deasphalted Residua			
Ashing method	Wet	Dry	Wet	Dry	Wet	Dry
Vanadium, p.p.m.	0.37	0.32	8.48	7.60	19.2	17.2
	0.39	0.34	8.48	7.50	19.6	16.2
	0.35	0.30	8.58	7.20	19.4	17.1
	0.38	0.30	8.60	7.40	19.2	16.6
Av.	0.372	0.315	8.535	7.425	19.35	16.77
Std. dev.	0.0171	0.0191	0.064	0.171	0.195	0.465
Loss by dry-ashing, % of wet ash value	15		13		13	

The dry-ash determinations reported in Table IV were done on 100-gram portions and hence should be compared to the vanadium results in Table III. By this comparison it is seen that significant losses on vanadium were realized from the natural stocks, whereas no significant loss was obtained on the synthetic sample. Likewise, the losses of nickel and iron, although similar, are not the same for all types of refinery fractions. Hence, it is not feasible to use a single dry-ash to wet-ash conversion factor for all stocks.

Effect of Ignition after Initial Sulfonation. After the initial sulfonation step in the wet-ash procedure, the unreacted organic vapors must be driven off before the sample can be put into the muffle furnace for reduction to ash. This operation usually requires several hours. In an effort to speed up the procedure, ignition of the vapors after sulfonation and prior to introduction into the muffle furnace was studied. The results are presented in Table V.

Table V. Effect of Ignition after Sulfonation

(Sample 8)

Metal added	Ni		V		Fe	
	Compn. by Synthesis, P.P.M.					
	0.53		0.31		0.52	
	Compn. by Analysis, P.P.M.					
	Ign.[a]	Non-ign.[b]	Ign.	Non-ign.	Ign.	Non-ign.
	0.52	0.52	0.29	0.29	0.51	0.47
	0.50	0.50	0.33	0.33	0.51	0.51
	0.54	0.52	0.33	0.26	0.53	0.53
	0.52	0.50	0.30	0.29	0.53	0.45
Av.	0.52	0.51	0.31	0.29	0.52	0.49
Std. dev.	0.0115	0.0115	0.0206	0.0282	0.0115	0.045

[a] Ignition.
[b] Nonignition.

These data show that there is no significant difference in the values obtained when either igniting or not igniting the vapors. Hence, the procedure can be greatly speeded up by igniting the oil remaining after the initial sulfonation.

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Paper Chromatographic Method for Determining Alkaloids in Tobacco

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A method of determination of the kinds of alkaloids and their approximate amounts in tobacco and related species of *Nicotiana* is described. This method is designed for the analysis of the large number of samples required in connection with a plant-breeding program. Sufficient accuracy for this purpose is obtained by visual comparison with known spots after separation by paper chromatography.

NUMEROUS methods have been devised for the determination of nicotine in tobacco and insecticides, and many of them give satisfactory results when nicotine is the predominant alkaloid present (*5*). If other related compounds are present the numerical value obtained is usually intermediate between the true nicotine content and the total alkaloid value. The most frequently used methods provide no indication of which samples contain nicotine and which may contain principally related alkaloids. It has been known for some time that certain strains of commercial tobacco contain principally nornicotine (*8*), and that the predominant alkaloid in most of the species of the genus *Nicotiana* investigated is not nicotine. In most cases nornicotine is predominant, in some nicotine, and in a few anabasine (*10*).

Plant breeders have found that certain of these wild species offer better sources of parental material from which to obtain resistance to many of the important tobacco diseases than does commercial tobacco, *Nicotiana tabacum*, and have made interspecific crosses with commercial tobacco in the development of new varieties which are being released for commercial use. If either parent produces predominantly nornicotine, the first generation of the crosses which have been studied contain predominantly the non-nicotine alkaloid, but the inheritance of the factors controlling alkaloid type in later generations requires further study. Information concerning the physiological effects on the smoker and the effects on smoking quality of these alkaloids, other than nicotine, is limited and selection of varieties similar to present commercial varieties in proportion of different alkaloids cannot be conducted without improved analytical methods.

Several methods have been devised which differentiate among pyridine alkaloids. Some have utilized reactions differentiating secondary and tertiary amines (*1*, *2*) which divide the tobacco alkaloids into two classes usually referred to as nicotine and nornicotine. The determination of nicotine by these methods appears to be satisfactory, but several secondary amines are often present and nornicotine is not necessarily the one present in greatest amount. Others have used various column (*4*) or paper chromatography (*3*, *7*, *9*, *11*) methods. The latter type of method is capable of distinguishing at least 20 to 40 compounds present in some tobacco samples, which are probably related to nicotine (*6*). There is no evidence at the present time that significant quantities of most of these constituents are present in any tobacco, but it is advantageous to use a method capable of detecting and distinguishing between various pyridine compounds should they appear in quantity in future breeding material. Observations in this laboratory indicate that the types of alkaloid formed by a tobacco strain are characteristic of the strain and that the amount present is dependent on enviromental or cultural conditions. In many instances the same plants must be used for analysis and for seed. Thus topping is impossible, resulting in a much lower total alkaloid content than would be attained in farm practice, and consequently precision is less important than simplicity, reliability, and qualitative selectivity. On the basis of 3 years of experience with various paper chromatographic methods and modifications, the following methods seem best adapted to this use.

APPARATUS

PAPER CHROMATOGRAPHIC CHAMBER, either ascending or descending type.

CYANOGEN BROMIDE TANK, cylindrical jar with ground edge and plate glass cover, at least 9 inches in diameter and 19 inches high, if full-sized sheets are used.

TRACING BOX, or other means of observing papers by uniform transmitted light.

PIPETS, of 10-µl. delivery.

WARING BLENDOR, or equal (if green leaf samples are used).

REAGENTS AND SUPPLIES

CHROMATOGRAPHIC FILTER PAPER, Whatman No. 1, full-sized sheets.

ACETONE, according to ACS specifications.

ACETONE SOLUTION, 50%.

PAPER PRETREATING SOLUTION, equal parts of 0.1*M* pH 6.5, phosphate buffer and methanol.

DEVELOPING SOLUTION, 100 ml. of *tert*-amyl alcohol, 20 ml. of water, and 0.3 gram of ethyl *p*-aminobenzoate.

CHAMBER SATURATING SOLUTION, 100 ml. of water and 12.5 ml. of *tert*-amyl alcohol.

SODIUM ACETATE BUFFER, 0.2*M*, pH 5.6.

CYANOGEN BROMIDE CRYSTALS.

STOCK SOLUTIONS OF NICOTINE, NORNICOTINE, AND ANABASINE, each containing 1 mg. of alkaloid per ml. in 50% acetone. From these, four standards, also in 50% acetone, are made up containing the following numbers of micrograms of alkaloid per 10 µl. (or mg. per 10 ml.).

γ/10 µl.	
Nicotine	Nornicotine
8	1
4	4
2	2
	Anabasine
1	0.2