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Journal of Chromatography A, 671 (1994) 295-302

JOURNAL OF
CHROMATOGRAPHY A

Progress and problems in organic microanalysis by ion chromatography

J.R. Kreling, J.S. Cowan, F. Block, J. DeZwaan*

Physical and Analytical Chemistry Research, 7255-209-008, The Upjohn Company, Kalamazoo, MI 49001, USA

Abstract

Results generated on a set of known samples, which were submitted as routine samples to the analysts, over a period of many years, are used as a basis for comparing microanalytical results. These comparisons are made for sulfur and chlorine determinations conducted with both the more common titrimetric methods and ion chromatographic methods. The variations observed in the chromatographic methods are studied in greater detail and a means of eliminating nearly all systematic variation is identified. These improved microanalytical procedures result in chromatographic determinations which are not only interference free, but also of significantly greater precision than was obtained using titration based methods. Although a method, similar to that used for sulfur and chlorine, could be developed for bromine, problems in generalizing them to fluorine and phosphorus determinations were encountered.

1. Introduction

Before the routine availability of accurate mass determinations and modern NMR techniques, the determination of elemental composition played a central role in structure elucidation procedures. For this reason a great deal of effort was directed toward developing analytical methods for determining hetero-atom composition (such as sulfur, phosphorous and the halogens) in organic materials [1-3]. These methods generally rely on sample combustion to convert the element of interest to an inorganic form, followed by chemical treatment to convert all of the element of interest to a single species which can then be quantitated by titration. Because most of these titrations are conducted directly on the mixtures produced in a combustion flask, they are subject to both physical and chemical inter-

ferences which may be difficult to control [4,5]. Despite being subject to numerous possible interferences, many of these methods remain in common use.

Microanalytical determinations of hetero-atom content continue to be frequently requested by synthetic and medicinal chemists since they provide an initial indication of sample quality and they are ultimately necessary for a structure proof. The continued need for the rapid and reliable determination of these elements on an expanding number of samples provides the motivation for the development of more efficient analytical methods that are free of interference. It was recognized early [6-9] that ion chromatographic (IC) analysis might provide an advantage over some of the titration procedures in these determinations because the inorganic forms of the hetero-atomic species produced in Schöniger combustions were ionic and could thus be separated and analyzed by IC. Initial indications

* Corresponding author.

were that the IC procedures would be comparable in both accuracy and precision to the classical titrimetric procedures, but would offer the advantage of being subject to fewer interferences than the titrimetric procedures [10].

After several years of routine use for sulfur and chlorine determinations and after improvements to the initial procedures, IC analyses have been shown to not only completely eliminate all interferences but to also be superior to the titration methods in precision for these elements. The chromatographic procedures can also be readily automated and are easier to implement since operators are required to make no subjective judgements regarding either colorimetric endpoints or the possibility of interferences. The direct comparison of precision between assay types, as well as the exact effect of assay modifications on precision can only be made after studying a wide range of well characterized materials over a long time period. Because samples, extracted from the same set of known standard materials, have been consistently and systematically submitted, blindly, as routine samples to the analysts for many years and the deviations from the expected values which were

reported, have been collected and recorded, various procedures and variations of these procedures can be directly compared for the determinations of chlorine and sulfur.

Based on the success of IC for the micro-analysis of sulfur and chlorine, extending this same general procedure to the analysis of bromine, fluorine and phosphorus could result in similar improvements in assay performance while simultaneously diminishing the types of procedures run and the equipment required. The extension of these procedures was readily accomplished for bromine but significant obstacles were encountered in the cases of fluorine and phosphorus. Results on these elements will be presented and discussed.

2. Experimental

IC results were generated using Dionex Models 2010i and 2000i chromatographs using the Dionex columns (Dionex, Sunnyvale, CA, USA) indicated in Table 1. All determinations were made at ambient laboratory temperature. All detection was done using the conductivity

Table 1
Chromatographic conditions

	Assay		
	Sulfur/chlorine	Bromine	Fluorine
Column	Dionex AS3 separator column and AG3 guard column	Dionex AS9-SC separator column and AG9-SC guard column	Dionex HPICE AS1 separator column
Mobile phase	30 mM NaHCO ₃ and 2.4 mM Na ₂ CO ₃ in water	0.75 mM NaHCO ₃ and 2.0 mM Na ₂ CO ₃ in water	0.5 mM HCl in water
Flow-rate	2 ml/min	1 ml/min	1 ml/min
Temperature	Ambient	Ambient	Ambient
Injection volume	50 μl	50 μl	50 μl
Combustion solution	0.6% H ₂ O ₂	0.6% H ₂ O ₂	H ₂ O
Detection	Suppressed conductivity at 100 μS range using a Dionex Anion Micro-Membrane Suppressor (AMMS) with 0.0125 M H ₂ SO ₄ as regenerant (regenerant flow-rate = 3 to 4 ml/min)	Suppressed conductivity at 100 μS range using a Dionex Anion Micro-Membrane Suppressor (AMMS) with 0.0125 M H ₂ SO ₄ as regenerant (regenerant flow-rate = 3 to 4 ml/min)	Suppressed conductivity at 30 μS range using a Dionex AFS-2 Fiber Suppressor with 5 mM tetrabutyl-ammonium hydroxide as regenerant (regenerant flow-rate = 2 ml/min)

detectors incorporated into the Dionex systems. The suppression techniques and the chromatographic conditions used are given in Table 1.

All samples were prepared for chromatographic analysis using Schöniger oxygen flask combustion [11,12]. Sample sizes for combustion were established by targeting for 0.6 mg of sulfur, chlorine or fluorine and 1 mg of bromine and phosphorus, based on the estimated percentage by mass of these particular elements in the sample. The accurately weighed samples were wrapped in ashless combustion paper (A.H. Thomas). Combustion flasks of 1 l (A.H. Thomas, No. 66970-G20) were charged with oxygen and exactly 20 ml of an absorbent solution (see Table 1). These relatively large sample sizes were targeted to minimize the effects of low levels of the analyte elements which can be present in the combustion papers themselves (by far the largest blank, *ca* 5 μg , is observed for chlorine). Following combustion the samples were mechanically shaken for at least 20 min before transfer and analysis.

The instrument response was calibrated using standard solutions. These were prepared from chemically stable non-hygroscopic organic salts. Specifically, a standard solution was prepared by dissolving 936 mg of ephedrine sulfate and 909 mg of lincomycin hydrochloride in two liters of deionized (18 M Ω filtered) water. This solution and a 7:10 dilution of this solution were used as calibration standards. The measured area response was found to be linear over this range and well beyond (both lower and higher concentrations) for both sulfate and chloride.

The instrumental responses obtained were digitized and stored on a Harris computer. All integrations, standard curves, and calculations were completed on the computer using standard software.

The classical sulfur determinations reported were done by quantitatively transferring the contents of the combustion flask to a beaker and titrating with a 0.005 M Ba(ClO₄)₂ solution. The endpoint was determined by observing the color change produced in a mixture of thiorin and methylene blue indicators. The classical results of chlorine reported were obtained by a coulometric titration [13] with Ag⁺ after acidify-

ing the contents of the combustion flask with nitric acid. A generating current of 20 mA was used and the endpoint was established by observing the change in potential between a silver indicating electrode and a saturated calomel electrode. Schöniger combustions for these assays were conducted as described above except that a 6% H₂O₂ absorbing solution was used for the sulfur determination and a 0.1 M KOH absorbing solution was used for the chlorine determination.

3. Discussion

One means of assessing the performance of a microanalytical procedure is to submit stable, non-hygroscopic samples which are indistinguishable from routine samples and to then tabulate the differences between the reported values and the known values. If the average of these differences is calculated a result of zero should be obtained if the assay is unbiased, while the standard deviation of these differences will give a measure of the precision of the assay. A program of this type has been in place for the evaluation of these assays at Upjohn for many years and the results obtained for sulfur and chlorine are presented for certain time periods. Since the same basic set of reference materials have been used over this entire time frame, it is possible to compare the various assay performances directly with between 30 and 40 blind knowns being submitted for each assay each year.

Reported in Table 2 are the standard deviations, calculated for the differences between the measured and expected values of the sulfur and chlorine blind knowns, as determined by the procedures which were in routine use for various time periods. It should be noted that the average difference from theory was well under 0.1% for all assays in all periods which demonstrates the overall accuracy of each of the methods. The data in Table 2 demonstrate that the precision of the sulfur and chlorine assays during 1961 and over the 1981–1985 period were basically identical. During these time periods, the same titrimetric procedures were being used, with the only significant difference being the person conduct-

Table 2

Standard deviations (with the number of submissions, *n*, in parentheses) observed for the differences between the expected and measured values for sulfur and chlorine using a set of knowns which were systematically submitted blindly over different time periods

Time period	Standard deviations (μg)	
	Sulfur	Chlorine
1961	12.9 (43)	10.4 (46)
1981–1985	12.4 (245)	9.2 (260)
1986–1987	9.9 (81)	10.0 (78)
1987–1992	6.4 (237)	5.6 (234)

Sample sizes targeted the presence of 600 μg of the element of interest.

ing the assay. When the routine method of determination was changed to IC in 1986 for both sulfur and chlorine, the assay precision observed was very similar to the classical assays. Although there were no significant improvements in either accuracy or precision, as estimated by results on selected (interference free) materials of known composition, substantial practical benefits were realized from the change in procedure. The most important of these benefits was the complete elimination of chemical or physical interferences which had adversely affected up to 20% of these determinations using the titrimetric procedures.

Although the precision obtained using the chromatographic analysis was at least as good as had been obtained using the titrimetric procedures, the relative deviations on the order of 1.5%, which were observed for the blind knowns, was significantly greater than the 0.6% relative standard deviation obtained for peak areas upon reanalysis of samples. This difference in variability indicates that the assays are not performing at the limit of the chromatographic capabilities of the equipment and that improvements in sample preparation or assay calibration might result in improved results.

Unlike the titrimetric procedures, which relied on quantitative transfers, the chromatographic

analysis determines concentration and thus relies on absolute volumetric accuracy. Of particular importance is the ratio of the volume of adsorbent solution delivered to the combustion flask and the volume of the glassware used to prepare the standard solutions which are used daily for calibration. Any actual difference in the ratio of these two volumes would produce a systematic bias in the results, while any variability in this ratio (temperature effects, etc.) would appear as assay variability. In an effort to reduce uncertainty related to volumetric variability, an automatic burette (Metrohm Herisau Model E 415), instead of a pipette, is used to deliver the adsorbent solution to the Schöniger flask.

The incorporation of standards, combusted using the same procedure as the samples, among the routine, service samples, provides an additional means of evaluating assay performance. It should be noted that these standards are not the materials which are systematically submitted to the analyst blindly but are in fact knowingly prepared by the analyst. Monitoring assay behavior, both within day (a single series of determinations) and between days, using these combusted standards, could be useful in characterizing the nature of assay variability observed and thus improving overall assay performance. An assay monitoring program of this type was implemented for sulfur–chlorine determinations by routinely inserting combusted samples of lincomycin hydrochloride (S = 6.95%; Cl = 7.69%) every fifth sample during the determinations of sulfur and chlorine (sulfur and chlorine determinations are always made during a single chromatographic series). Dividing the theoretical value of sulfur or chlorine by the corresponding experimental result for the standard sample, using the calibration obtained from stock solutions of dissolved standards, yields a ratio (*R*) whose magnitude is related to assay accuracy and precision. The variation observed in *R* is directly influenced by sources of variation in the assays and therefore related to assay performance.

In an effort to separate sample preparation (handling) variations from chromatographic (in-

strumental) variations, variations in R were determined both within and between standard samples during a single chromatographic analysis period by preparing multiple combusted standard samples and running them more than once during the period (usually once early and once late in the run). The results obtained for the within day variations in R for the January through June (1993) time period are given in Table 3, and indicate essentially the same variability for the within-sample and between-sample variation over this time period. Because these variations are essentially the same, the sample weighing and combustion process do not appear to add significant variability to the assay.

The between-day variability of the assays is established by determining the standard deviations of the daily mean values of $R(R^*)$. R^* values were calculated by averaging all values of R determined during the course of running a single sample set, including both the determinations on separate combusted standards (typically 5 per day) and repeats of these standards (typically 3 per day). The variations found in these values are given in Table 3 for both sulfur and chlorine. The magnitude of these deviations correspond to relative standard deviations of 0.44% for sulfur and 0.54% for chlorine. The average values of the R^* values over the January through June time period was 0.96 for sulfur and 0.97 for chlorine. Both the within-day variability (R) and the between-day variability (R^*) will contribute to the overall variability of the assay as reflected by the data in Table 2.

Since both sulfate and chloride were present in both the stock standard solutions used and in the

material (lincomycin hydrochloride) used as a combusted standard, it is possible to directly compare the variations observed in R^* values determined for each element. One means of making this comparison would be to plot the difference between the R^* of sulfur on a given day with the value of R^* on the preceding day versus the corresponding differences observed in the chlorine R^* values. This comparison is made in Fig. 1. Based on the manner in which these data scatter around a line of unit slope, passing through the origin, a correlation between changes in the sulfur ratio and chlorine ratios must exist. A systematic bias is, therefore, present and the variability reflected in R^* is not completely random. Although the exact nature of this systematic bias has not been explicitly defined, and may be complex, it is clear that, if the (daily) assay bias which is represented by the correlation of the variations in Fig. 1 could be eliminated, overall assay performance should be significantly improved.

A simple means of approximating this correction would be to use the daily average, R^* , as a correction factor for the elemental composition, $\%X$, determined using the standard solution calibrations as shown in Eq. 1.

$$\%X_{\text{corrected}} = R^* \cdot \%X_{\text{measured}} \quad (1)$$

When the effects of daily biases are removed from the experimental values of elemental composition, using Eq. 1, a substantial improvement in assay performance can be demonstrated as shown in Table 2 for the 1987–1992 time period. The variability of the assay results,

Table 3
Standard deviations determined based on a sample size of 600 μg with n , the number of determinations, in parentheses

Element	Standard deviation (μg)		
	R value within-day		Between-day R^*
	Within sample	Between samples	
Sulfur	5.4 (111)	4.9 (213)	2.8 (37)
Chlorine	3.1 (111)	4.4 (213)	3.1 (37)
Bromine	5.5 (42)	5.6 (86)	

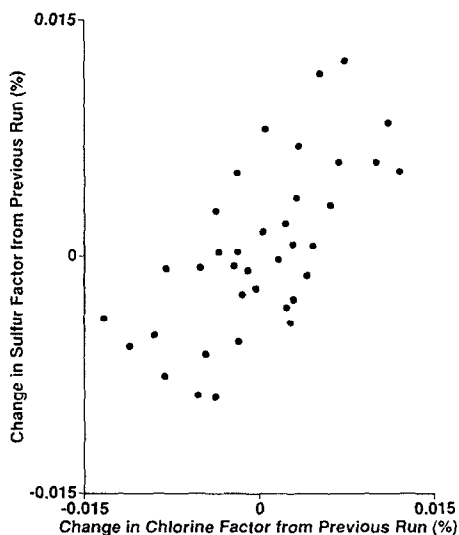


Fig. 1. Change from the previous value observed in R^* determined for sulfur plotted versus the corresponding change observed for chlorine.

determined using a wide range of sample types and over a long time period, are now more nearly comparable to the chromatographic precision available from our instrumentation.

Because of the excellent microanalytical results produced by the IC technique for sulfur and chlorine, extending this procedure to other hetero-atomic species such as bromine, fluorine and phosphorus would be desirable. This could potentially lead to improvements in the accuracy and precision of these determinations, as well as require the maintenance and support of only one overall assay procedure for all of these elements. This generalization of the procedure was first attempted for bromine determinations. The chromatographic conditions used for the bromine assay are given in Table 1. An example of the chromatogram obtained for a sample containing chlorine, nitrogen, bromine and sulfur is shown in Fig. 2. Unlike the more common titration assays, it is clear from Fig. 2 that no significant inferences from chlorine in the sample should be expected. The major problem encountered with this assay, after several years of routine use, is degradation in column performance leading to incomplete resolution of the bromide and nitrate peaks. This is a significant

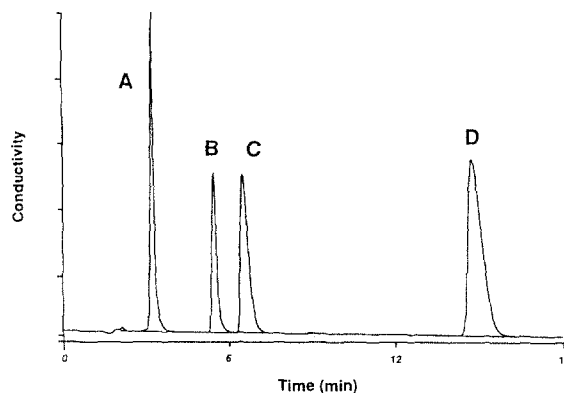


Fig. 2. Chromatogram of a combustion solution containing chloride (A), bromide (B), nitrate (C) and sulfate (D) ions which was obtained using the chromatographic system developed for bromine analysis.

problem because nitrate will be present in all samples analyzed and, at the level of accuracy and precision expected from this determination, anything less than complete baseline resolution of these peaks degrades assay performance. Column lifetimes are limited to around a thousand samples because of this problem.

The performance of the chromatographic assay, on a set of standard materials is presented in Table 4. Also presented in Table 4 are the results reported on these samples by well known external laboratories. In the case of the chlorine containing samples, only one lab indicated the ability to analyze bromine in the presence of chlorine. The sample sent to the laboratory, which indicated the ability to determine bromine in the presence of chlorine, was clearly marked as containing chlorine when submitted. Although the results reported in Table 4 represent a one time event to compare laboratories, they are representative of our experience with outside determinations of bromine.

Fluorine is another example of an element for which microanalytical results from outside sources are often less than satisfactory. The IC analysis of fluoride ion was also found to give good precision for both dissolved standards (R.S.D. = 0.95%) and combusted standards (R.S.D. = 1.5%). The chromatographic system used for fluoride determination is given in Table 1 and is based on an ion-exclusion column for

Table 4
Results obtained by various labs on bromine standards

Expected result (%)	IC result (%)	Result (%)		
		Laboratory 1	Laboratory 2	Laboratory 3
37.33	37.36	37.79	34.38	37.99
21.39 ^a	21.01	43.49	NR ^b	NR
18.11	18.05	18.33	17.58	17.67
20.06	20.04	NR	20.52	20.44
20.56 ^a	20.48	39.65	NR	NR

^a Sample contained chlorine and was labelled as such.

^b NR = Not run.

weak acids, since insufficient retention of fluoride ion is obtained on typical anion columns.

Although the assay precision obtained for fluoride ion appeared good, the data obtained on combusted materials indicated the presence of a significant bias in the assay. The results in Table 5 were generated by using dissolved fluorine standards for calibration. The negative bias was always observed when samples were combusted and indicates that there is a significant recovery problem associated with the Schöniger combustion. The recovery problem for fluoride following sample combustion in a Schöniger flask has been reported earlier [14]. The elimination of borosilicate glass in the combustion flask through the use of plastic combustion flasks did not eliminate the recovery problems as was indicated

Table 5
Results obtained on combusted fluorine standards using dissolved standards to quantify the ion chromatographic response

Sample	F (μg)		
	Expected	Found	Difference
1	596	549	-47
2	621	584	-37
3	583	538	-45
4	662	615	-47
5	614	581	-33
1	596	555	-41
2	621	576	-45
3	583	537	-46

in some publications [15,16]. Because the source and nature of this assay bias remains unexplained, the chromatographic determination of fluorine has not been implemented as a routine assay.

Another element for which sample combustion problems complicate the analysis is phosphorus. In this case it is well known that many different phosphorus species are formed. For the purpose of analysis by IC, the most desirable form would be orthophosphate since this species could be analyzed using the same chromatographic system used for bromine. In addition to orthophosphate, however, substantial levels of pyrophosphate, tripolyphosphate and, sometimes, tetrapolyphosphate are formed. Fig. 3 illustrates the

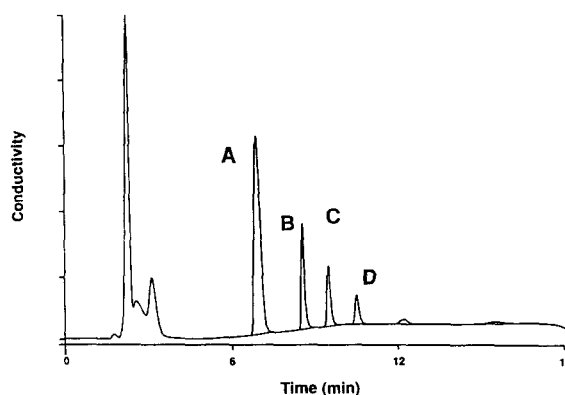


Fig. 3. Gradient chromatogram showing the presence of orthophosphate (A), pyrophosphate (B), tripolyphosphate (C), tetrapolyphosphate (D), etc. in the absorbent solution from a Schöniger combustion of a phosphorus-containing sample.

various phosphorus-containing species produced during the combustion of a phosphorous containing sample. The gradient conditions described in ref. 17 were used to produce the chromatogram in Fig. 3. Although chemical procedures for converting all forms of phosphorus to orthophosphate have been developed and widely applied, they involve refluxing the sample after addition of strong acid. In addition to adding a major interference to the anion chromatogram, procedures of this type are labor intensive and are not ideal for concentration determinations. Recently enzymatic procedures, that require no refluxing, have been developed in our laboratory which will completely convert all phosphorus species, generated in a Schöniger combustion, to orthophosphate. Since the cost of the enzymes is negligible (cents per sample) and since they can be readily obtained, this procedure appears to form the basis for a future IC micro-determination for phosphorous.

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