ANALYTICAL LETTERS, 15(A3), 267-281 (1982)

SPECIATION OF Cr (III) and Cr (VI) VIA REVERSED PHASE HPLC WITH INDUCTIVELY COUPLED PLASMA EMISSION SPECTROSCOPIC DETECTION (HPLC-ICP)[24]

Key Words: HPLC-ICP, high performance liquid chromatography, inductively coupled plasma emission detection, trace inorganic analysis (Cr)

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ABSTRACT:

Chromium ions, viz., chromic (Cr^{+3} = III) and chromate (Cr^{+6} = VI), can be reliably, conveniently, reproducibly, and quickly separated and detected by the use of conventional paired-ion, reversed phase (RP) high performance liquid chromatography (HPLC) together with refractive index (RI) and/or inductively coupled plasma emission spectroscopic (ICP) detection. A number of novel paired-ion approaches have now been developed, using PIC A (tetrabutylammonium hydroxide) or PIC B (sodium n-alkyl sulfonate) separately in the mobile phase. This allows for the retention of each Cr species depending on the particular ion pairing reagent being used, while the remaining Cr ion elutes in the solvent front. Changing the ion pairing reagent reverses the overall situation. The total time for each HPLC analysis is about 10 mins. ICP detection provides for a complete, overall method of speciation for both

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Cr (III) and Cr (VI) via two separate injections, together with quantitation for both species. This method of using paired-ion RP-HPLC can easily be applied to other mixtures of inorganic cations and anions, presumably with equally successful results. Minimum limits of detection are computed for chromate via direct-ICP and HPLC-ICP, using at least two basic methods for such computations. It is suggested that virtually all chromatographic detection limits can be significantly improved by the application of newer, spectroscopic based methods of automated computation of detection limits.

INTRODUCTION[24]

It has long been recognized that chromium can exist in at least two different ionic forms, viz., chromic ion (+3), Cr^{+3}, Cr (III), and chromate ion (+6), Cr^{+6}, Cr (VI), CrO_4^{-2}. It is also well known that each of these forms has very different biological, medical, and toxicological properties, and the problem of chromium toxicity is currently one of considerable interest and concern[1-4]. Trivalent chromium (III) is an essential element in mammalian systems, whereas hexavalent chromium (VI) is considered to be a moderate to severe industrial hazard. Epidemiologic studies have suggested that Cr (VI), chromate anion, is a carcinogen with bronchogenic carcinoma as the principal lesion[1]. Quite obviously, possible health hazards associated with the presence of chromium in foods or beverages for human consumption will/may depend on which particular oxidation state of chromium is/are present. Thus, the safety of drinking water supplies, environmental watersheds, beverages, foods, and industrial environments, can only be determined, at least as regards chromium metal, once a correct determination has been made as to the specific forms or species present. For about the past decade, a large number of toxicological studies have been reported related to chromium and its various ionic species, but almost all of these have omitted a valid analytical determination of the particular and actual species of chromium present in particular samples. The determination of toxic properties for any metal or nonmetal cannot be accurately or reliably made in the absence of appropriate and reliable analytical speciation determinations.

There have been a large number of reports over the past ten years with regard to specific analytical methods for the speciation of chromium ions[5-13]. Most of these methods have used ion exchange resins for pre-concentration and separation prior to atomic absorption or atomic emission spectroscopy, while still others have used ion exchange HPLC separations on-line with non-specific and/or element specific detection. Naranjit et al. have described a method for chromium speciation using anion and cation exchange resins together with atomic absorption spectroscopy[9]. Tande et al. discuss yet

another method which used sodium diethyldithiocarbamate to chelate the chromium ions, and these chelates were then separated and analyzed by reversed phase HPLC with UV detection. Of course, chromic and chromate ions can also be determined using ion chromatography (IC) with either conductivity or electrochemical detection[14, 15]. However, the currently available detectors for IC do not really provide any high degree of specificity for individual ions of interest. Recent developments in electrochemical detection cells and methods suggest that improved speciation may shortly become available.

Perhaps an ideal method for the speciation of Cr (III) and Cr (VI) would involve solely conventional reversed phase (RP) high performance liquid chromatography (HPLC) in combination with element selective detection. This would then avoid the somewhat tedious off-line separations of the two ions before the detection system, which was the basis of a recently described approach[12, 13]. At the same time, such a method would avoid the need for expensive ion exchange columns in the HPLC part, and would permit for the use of the somewhat less expensive RP packing materials. We have recently developed certain paired-ion RP-HPLC conditions for the direct analysis of various metal cations and anions, wherein these approaches have been interfaced with refractive index (RI) and/or inductively coupled plasma (ICP) emission spectroscopic detection[16, 17]. Other workers have also described the use of paired-ion RP-HPLC for the separation of various inorganic cations and anions[18-20]. We have also shown that a PIC B (sodium n-alkyl sulfonate) type counter-ion in the mobile phase permits metal cations to elute as groups of +1, +2, +3, etc. species[16]. In this particular situation, the ICP is then able to speciate for individual monovalent, divalent, etc. cations present within a single eluted peak. In the case of oxyanions of arsenic, we and others have shown that the use of a PIC A type reagent (tetrabutylammonium salt) in the mobile phase allows for the resolution of a number of these species[16, 18]. The ICP is then set on the particular arsenic wavelength of interest during the entire HPLC run, and it then prints out intensities for each arsenic containing species as these elute from the HPLC into the plasma[16].

We have now investigated the use of both PIC A and PIC B type counter-ions in RP-HPLC for the paired-ion separations of Cr (III) and Cr (VI) ions, which is then followed by element selective ICP detection. Since we are dealing with one cation, Cr^{+3}, and one anion, $Cr^{+6} = CrO_4^{-2}$, as the mixture being separated, the use of a PIC A or PIC B type counter ion will only retain one of the two ions, and the other should then appear in the solvent front. However, by first using a PIC A or PIC B type reagent in the mobile phase, and then re-injecting the same mixture with the opposite counter-ion in a new mobile phase, one then has an overall system which has both resolved and retained

both chromium ions. Although this particular approach requires two separate injections and analyses, together with two separate mobile phases for complete confirmation of the speciation results, it is still rapid to perform, simple to operate, and somewhat inexpensive to run routinely. At the same time, it appears to provide a high degree of precision from run to run and day to day, and appears to provide an overall method of true speciation which is not surpassed by any of the currently available alternative methods. Minimum limits of detection are presented for chromate ion via both direct-ICP and HPLC-ICP, and a comparison of these values can then be made. Although the present limits of detection possible with this particular HPLC-ICP system are not directly applicable to real world samples at the ppb levels, they should be adaptable together with appropriate sample pre-concentration.

EXPERIMENTAL

Reagents

Chromic acetate (Cr (III)) was obtained from Matheson, Coleman, and Bell, Inc. (MC&B, Norwood, Ohio), and also from Alfa/Ventron, Inc. (Danvers, Mass.). Chromium chloride (Cr (III)) was obtained from Alfa/Ventron, Inc., and sodium chromate (Cr (VI)) was Mallinckrodt's analytical reagent grade (Mallinckrodt, Inc., Paris, Kentucky) obtained from Alfa/Ventron. Where more than one grade was commercially available, that of the reported highest purity was used. PIC reagents were purchased from Waters Associates, Inc. (Milford, Mass.), and were used directly without further purification. The mobile phase water was purchased from the J.T. Baker Chemical Co. Phillipsburg, N.J.), or used directly from a Corning Mega-Pure still (Corning Corp., Corning, N.Y.)

Apparatus

The HPLC-RI-ICP instrumentation used to obtain the majority of the results described here consisted of a Laboratory Data Control Constametric III pump (Laboratory Data Control, LDC, Riviera Beach, Florida), a Rheodyne Model 7125 syringe injection valve (Rheodyne Corp., Cotati, Calif.), a Micromeritics Model 771 refractive index detector (Micromeritics Corp., Norcross, Ga.), a modified Instrumentation Laboratory Model Plasma-100 inductively coupled plasma emission spectrometer (Instrumentation Laboratory, Inc., Wilmington, Mass.), and a Honeywell Corp. (Minn., Minn.) dual pen recorder. The RI/ICP data were obtained via a dual pen recorder, and/or a separate ICP print-out from the Plasma-100 system. Often, both the recorder ICP chromatogram and the tabular data format from the ICP were obtained at the same time. At other times, the tabular data format could be manually used

to construct a pulsed type or continuous type HPLC-ICP chromatogram. The presentation of both pulsed type chromatograms or continuous type ones, along with simultaneous tabular data presentation, provided additional confirmation of the ICP overall results.

A number of HPLC columns were utilized, but the majority of the results obtained with ICP detection used a 5u commercial Ultrasphere ODS (4.6mm x 15cm) C-18 type column (Altex/Beckman Corp., Irvine, Calif.). The column was maintained at 25°C using a constant temperature water bath, as was the mobile phase.

Methods

In all of this work with paired-ion RP-HPLC, the mobile phase has consisted of the PIC reagent prepared according to the manufacturer's directions, in water, at a final concentration of 0.005M in the counter-ion. In the case of the PIC B-5 (sodium n-pentane sulfonate) reagent, the final pH was about 2.9-3.0, while in the case of the PIC A reagent, this pH was about 7.15. In those experiments using both PIC A & PIC B-5 reagents together in the same mobile phase, this was 0.005M in each. Specific flow rates, effluent split ratios, and more specific HPLC-detector conditions are given below. An approximately 50:50 split of the effluent was used in the dual detector studies, making use of a fixed ratio "T" type splitter after the column (Alltech Assocs., Deerfield, Ill.). Standard solutions of chromic or chromate ions were prepared in the mobile phase used for that particular separation and all HPLC injections were of a 20ul size. Specific amounts of each ion injected or reaching the detector(s) are indicated on the individual chromatograms. The ICP wavelength used throughout this study was 267.71nm for the chromium emission line.

Detection limits were determined in several ways, using standard chromatographic techniques (peak height or peak area) and a signal:noise ratio of 3:1, or using the ICP computer programs designed for this purpose. ICP computed detection limits were done using the standard deviation of the background noise in combination with a single injection of a known amount or concentration of chromate anion. It is of interest that there are almost no literature reports wherein spectroscopic methods of computing detection limits have been applied to any chromatographic systems. The background noise level was determined at the retention time of the species of interest using a blank.

Chromium salts were dissolved in the mobile phase already containing the paired-ion reagent(s), and aliquots of such solutions were injected. Blank injections of the mobile phase alone were performed alongside those samples containing known concentrations of the chromium species.

RESULTS AND DISCUSSION

We have now investigated Cr (III), as chromic acetate and chromic chloride, and Cr (VI), as sodium chromate ($Na_2(CrO_4)$), in three separate paired-ion RP-HPLC systems, wherein the nature of the paired-ion species has been varied. These three different paired-ion mobile phase conditions were: 1) PIC B-5 alone; 2) PIC A alone; and 3) equi-molar amounts of both PIC B-5 and PIC A together. The optimum results for all three systems appears to be that which uses PIC B-5 alone in the mobile phase, and this is illustrated in Figure 1. This separation of Cr (III) and Cr (VI) from each other, as well as an apparent retention of both ions, was obtained using a C_{18} type column with PIC B-5 added to an aqueous mobile phase at a flow rate of 1.0 ml/min. The eluent was split 60:40 between the ICP:RI detectors, with the larger portion going to the ICP. Figure 1 clearly shows the presence of both ions on both the RI and ICP detectors, with the amounts going to each detector indicated. When Cr (III) and Cr (VI) were injected separately under these conditions, their retention times were as found for the mixture of the two. In addition, in the case of the Cr (III) species, the ICP indicated the presence of two other Cr containing species. These are indicated in Figure 1 by question marks (?), because we do not yet know the precise nature of these Cr containing materials. It is possible that they are mixed ligand chelates or hydrated Cr (III) species, possibly containing different ratios of the ion-pairing reagent, but an insufficient amount of information is available to specify their structures. In any event, they appear to be minor components of both Cr (III) salts used, as originally prepared in the mobile phase. The ICP also indicates the presence of at least one additional Cr (III) derived species, which co-elutes along with the Cr (VI) ion under these particular conditions. Under our ICP operating parameters, only Cr containing species eluting from the HPLC into the plasma are being detected and recorded. The total time for this type of an analysis is less than six minutes, with a perfectly useful resolution of the two ions of interest. There is also an apparent retention of the Cr (VI) species, although this is quite small, if real. The Waters PIC B-5 reagent, viz., sodium n-pentanesulfonate anion, should only be ion-pairing with the Cr (III) ion, and there should be no interactions at all with the Cr (VI) ion, since this exists as the negatively charged CrO_4^{-2}.

Figure 2 illustrates another separation of Cr (VI) from Cr (III), wherein we have now made use of the PIC A ion pairing reagent in the mobile phase, at a flow rate of 2.0 ml/min. This separation again utilized a C_{18} type HPLC column, and the split of the eluent was 60:40 to the ICP:RI detectors. The amounts of each Cr ion going to each detector are indicated in Figure 2, and these are somewhat different than the amounts used in Figure 1. Under

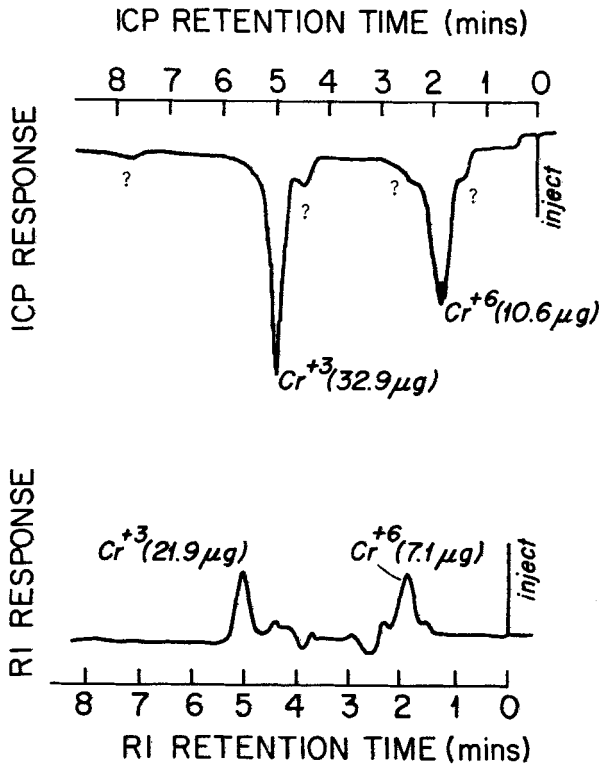

Fig. 1. Paired-ion RP-HPLC-RI-ICP analysis for a mixture of Cr^{+3} (chromic acetate) and Cr^{+6} (sodium chromate, CrO_4^{-2}) using an Altex Ultrasphere ODS (C_{18}) column, 15cm x 5.6mm i.d., with PIC B-5 mobile phase at flow rate 1.0 ml/min, ICP:RI split of 60:40, ICP emission at 267.71nm, amounts indicated at each detector.

the conditions of the PIC A type ion pairing reagent, which is the tetrabutylammonium salt, only the anions present should be retained, and indeed this is now seen to be the situation. Thus, Cr (VI) has an overall retention time of about 8.0 mins, while the Cr (III) species elutes immediately in the solvent front. The total time for this particular analysis is about 9.0 minutes, Figure 2, but this could be shortened somewhat by going to a faster mobile phase flow rate. Both RI and ICP detection methods provide an indication of the presence of the two major Cr ions, but there is an inherently greater degree of specificity via the ICP method of detection[16, 17].

The above results now provide us with an overall approach to the reliable speciation of Cr (III) and Cr (VI) via two separate injections of the same

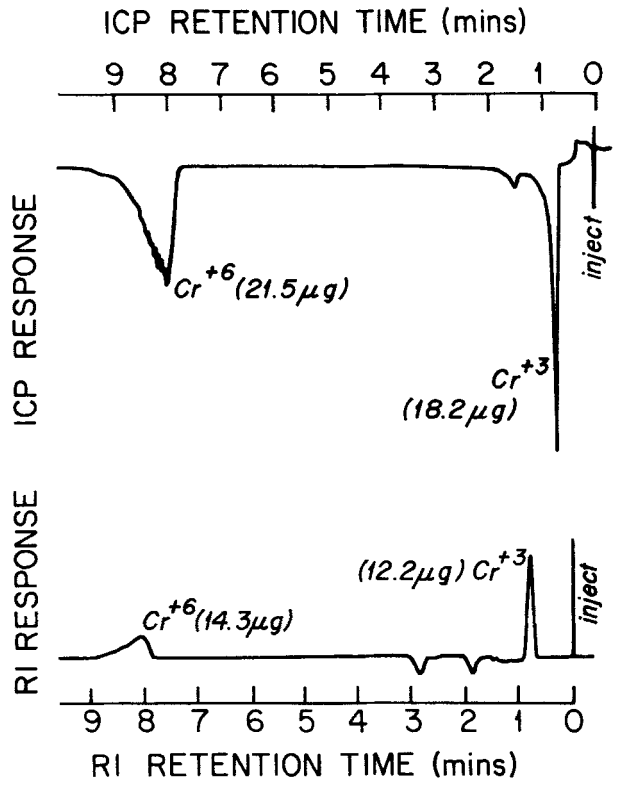

Fig. 2. Paired-ion RP-HPLC-RI-ICP analysis for a mixture of Cr^{+3} (chromic acetate) and Cr^{+6} (sodium chromate, CrO_4^{-2}), using an Altex Ultrasphere ODS (C_{18}), 15cm x 4.6mm i.d., with PIC A mobile phase at flow rate of 2.0ml/min, ICP:RI split of 60:40, ICP emission at 267.71nm, amounts indicated at each detector.

sample under two very different paired-ion RP-HPLC conditions. It is clear that each separate HPLC-ICP analysis for Cr ions requires less than 10 mins total time, outside of the time initially required to set-up and equilibrate the HPLC-ICP instrumentation. Because we are now using two oppositely charged ion pairing reagents, the order of the elution for the two Cr ions is reversed, and therefore both the qualitative and quantitative results should agree under very different HPLC elution times and peak shapes, Figures 1 and 2. The overall speciation for both Cr ions is therefore improved by the use of these two different HPLC mobile phases, above and beyond that provided by the HPLC retention times and ICP element specificity for chromium. It would be difficult to imagine a situation wherein other Cr ions would elute coincidentally with

the Cr (III) and Cr (VI) ions under both sets of paired-ion conditions. We suggest that this combined approach for Cr ion speciation provides an unusual and unique degree of element and ion specificity and selectivity. One other approach was of interest, and that involved the possible use of a mixed paired-ion mobile phase, containing both the PIC B-5 and PIC A together.

Strangely enough, there is very little in the literature which discusses the possible uses of a mixed paired-ion mobile phase in RP-HPLC, either for organic or inorganic type ions. When equi-molar (0.005M) amounts of PIC A and PIC B-5 are added in the same flask, there is no formation of any insoluble precipitate, which suggested that some free PIC A and PIC B-5 reagents remained uncoordinated to each other. However, when this mobile phase was used for the analysis of the Cr ions, now injected separately, it became readily apparent, Figure 3, that the actual amounts of free reagents were considerably less than when either of these had been used alone. Thus, Figure 3 is a summary of separate injections of first Cr (III) (left) and then Cr (VI) (right), with the amounts going to the ICP alone indicated. No RI detection was used in this study. The overall results indicate that in the case of Cr (III) there is virtually no retention at all. In the case of Cr (VI), there appears to be some retention, but much less than was seen when PIC A was used alone, Figure 2. Also, the shape of the Cr (VI) peak is very different than that seen in Figure 2, it is much broader and less well defined. These initial results suggest that it is not feasible at present to utilize a mixed paired-ion type mobile phase for the separation of these Cr ions. This may be due to the fact that organic paired-ion reagents tend to coordinate (ion-pair) with each other in preference to their separate coordination (ion-pairing) with the metal ions present in solution.

Other than the already reported use of HPLC-GFAA, in an off-line manner, there are no reported practical approaches, on-line, for Cr speciation of real world samples. The recently reported use of a pre-concentratin, selective extraction of one Cr species in the presence of the other, followed by separate ICP analyses, is usable, but it is an indirect, off-line method[12, 13]. The problem in using an on-line HPLC-ICP approach to solve this trace analysis Cr speciation problem resides, at the present time, in obtaining usable minimum detection limits (MDLs). The published MDL for chromium via the Instrumentation Laboratory Plasma 100 instrument is about 3 ppb. We have independently determined that with our non-routine operating plasma in the direct mode, Cr has an MDL of about 15ppb. This method of determining detection limits utilizes a computer determined standard deviation of the background noise level. It then computes the amount of Cr necessary to produce a signal/noise peak of 2.0-3.0, utilizing a single injection of a known amount of Cr.

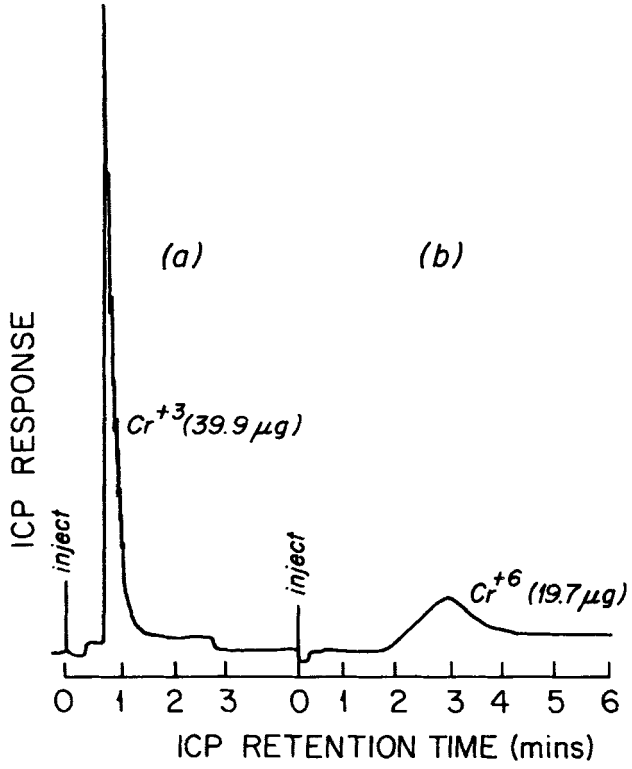

Fig. 3. Paired-ion RP-HPLC-ICP analysis of Cr^{+3} (chromic acetate) and Cr^{+6} (sodium chromate, CrO_4^{-2} using an Altex Ultrasphere ODS (C_{18}) column, 15cm x 4.6mm i.d., with a mixture of both PIC A and PIC B-5 (0.005m) in mobile phase at flow rate of 2.0ml/min, ICP emission at 267.71nm, amounts indicated to ICP alone.

For some strange reason, this method of determining MDLs is rarely, if at all, used in any form of chromatography-detection system. The same approach can be used to determine the MDL for Cr ions (as chromate) via HPLC-ICP, in combination with a separate computer program, different from that used for the direct-ICP calculations. We have now used this method, via the HPLC conditions of Figure 1, to determine the MDL for Cr as chromate ion. The value we obtain is about 10-20ppm via HPLC-ICP, but this obviously depends on the particular HPLC conditions employed. With longer retention times for chromate, one would expect worse MDLs using the peak height method. We have also determined the MDL for both Cr (III) and Cr (VI) using both PIC A and PIC B-5 HPLC conditions, Figures 1 and 2, using measured peak heights for

TABLE 1

CHROMATOGRAPHICALLY DETERMINED MINIMUM DETECTION LIMITS (MDLS) FOR CHROMIUM

CHROMIUM ION	PIC A HPLC[a]	PIC B-5 HPLC[b]
Cr (III)	0.22ug/ul[c]	1.75ug/ul[c]
Cr (VI)	0.45ug/ul[c]	0.4ug/ul[c]

a. calculated using a peak height ratio of 3:1 on the ICP chromatogram, together with HPLC conditions as indicated in Figure 2.
b. calculated using a peak height ratio of 3:1 on the ICP chromatogram, together with HPLC conditions as indicated in Figure 1.
c. ug/ul = ppt (parts per thousand), 0.22ug/ul = 220ppm, 1.75ug/ul = 1.75ppt, etc.

each ion on the ICP chromatograms. This was done manually, using the same method that chromatographers have long used to crudely calculate MDLs *via* any detection system. Our results using this older approach are indicated in Table 1. These manual determinations of MDLs for both chromic and chromate ions were done using a signal:noise ratio of 3:1, and therefore the observed MDLs are considerably greater (larger) than those determined by computer program, as above. Clearly, the computer controlled, automated method of determining MDLs for any HPLC peak is to be preferred over the older, somewhat cruder manual calculations. The spectroscopic method is just as valid and reliable, if not more so, than our somewhat older, manual methods.

Clearly, a MDL of even 10-23ppm for chromate is not satisfactory, and would not serve usefully as a general HPLC-ICP approach for trace metal analysis-speciation. Pre-concentration methods for Cr from aqueous samples might suffice to provide the HPLC-ICP method with final concentrations usable with this MDL. However, to be truly useful and applicable to real world samples, some improvements are required in a final MDL for chromium and other metal species *via* HPLC-ICP speciation methods. We have initially determined relative sensitivities, not true detection limits, for chromate *via* HPLC-ICP and direct-ICP methods, Table 2. This table lists the ICP peak heights and peak areas for a known amount (mass) of Cr (VI) injected onto the HPLC in 20ul, and for the same absolute amount of Cr (VI) going to the ICP *via* the direct-ICP approach[16]. That is, we are here injecting 1.8ug/20ul of Cr (VI) onto the HPLC-ICP, the ICP peak heights and areas for this amount of ion are then compared with an identical amount and concentration of the same ion reaching the ICP *via* direct nebulization. The only variables in this type of a comparison are the band spreading (broadening) effects due to the

TABLE 2

COMPARISON OF SENSITIVITIES FOR DIRECT-ICP AND HPLC-ICP WITH Cr (VI) ALONE[a]

METHOD OF COMPARISON	DIRECT-ICP[b]	HPLC-ICP[c]	DIRECT-ICP/ HPLC-ICP
ICP PEAK HEIGHTS (cm)	19.5 ± 0.7[d]	2.13 ± 0.09[e]	9.2
ICP PEAK HEIGHTS (ICP units)	777,287 ± 24,104	58,614 ± 2,654	13.3
ICP PEAK AREAS (ICP units)	855,105 ± 26,515	589,879 ± 31,185	1.5

a. analysis for Cr (VI) performed with sodium chromate in PIC B-5 in water.
b. determined by direct nebulization into ICP of a solution of sodium chromate at a concentration of 1.8ug/20ul, ICP emission at 267.71nm.
c. determined using C-18 type column, 15cm x 4.6mm i.d., with PIC B-5 mobile phase at 1.0 ml/min flow rate, ICP emission at 267.71nm.
d. numbers represent the average ± standard deviations for five separate runs on the same day (n=5).
e. numbers represent the average ± standard deviations for three separate runs on the same day (n=3).

HPLC interface in the HPLC-ICP arrangement. The ICP operational parameters are identical in both situations. The final results, Table 2, indicate that the relative differences in responses for chromate are greater when peak heights are used rather than peak areas. Thus, the ratios for direct-ICP/ HPLC-ICP in using peak height (cm) measurements is about 9.2, while using peak heights in terms of ICP arbitrary units, this ratio is about 13.3. Ideally, as a referee has indicated, these two ratios should be identical. In practice, because the methods of measuring these two ratios are different, viz., a manual method with a ruler and an electronic computer program, the final ratios are somewhat different. In the manual method, the errors in measuring the peak heights(cm) for direct-ICP and HPLC-ICP may be different, and therefore this ratio will be different from that obtained by using the computer print-out of ICP peak heights (ICP units), Table 2. When peak areas are used for the same comparison, then the ratio is only about 1.5. These overall results suggest quite strongly that the ICP is acting as a mass sensitive detector in the HPLC mode, and that the use of peak areas should provide better HPLC-ICP detection limits than peak height measurements. Even for the worst-case situation, Table 2, there is still only about one order of magnitude difference between the two modes (13.3 vs. 1.5).

In view of the above results, Table 2, we would expect that, at worst, the MDL for Cr (VI) using HPLC-ICP should be about 15ppb x 13.3 = 200 ppb (parts-per-billion). Clearly, it is not going to be possible, using peak

height measurements, to obtain limits of detection via HPLC-ICP that are identical with direct-ICP methods. There have been several recent literature reports which indicate that HPLC-ICP detection limits for certain metals can be equal to or better than the direct-ICP results, at least for certain arsenic species[21, 22]. Such reports are of interest, because on theoretical principles alone, it would be difficult to imagine obtaining these MDLs via HPLC-ICP. Clearly, the nature of the interface and nebulizer arrangement in any HPLC-ICP arrangement can affect the final MDLs, this being in addition to the band broadening effects already discussed. We suspect that the approximately three orders of magnitude difference between our direct-ICP and HPLC-ICP MDLs for chromate (15ppb vs 10ppm) is due to these parameters. We can account, at present, for about a 10-15 fold difference on the basis of the band broadening and extra-column effects inherent in the HPLC operation, Table 2. However, the remaining almost two orders of magnitude difference cannot be due to factors inherent in the HPLC operations, and must therefore be ascribed to the nature of the interface-nebulizer arrangement. There is a distinct possibility that conventional cross-flow or concentric tube type nebulizers for direct-ICP operations are just not operationally compatible, in terms of MDLs, for HPLC-ICP operations. Other more suitable arrangements will have to be developed in this regard, and our efforts, as well as those of others, are now being directed towards solving this important problem.

At the same time, we recognize the importance of applying any final HPLC-ICP methods for chromium speciation to real world samples, including: foods, beverages, water supplies, environmental matrices, and related items. We also recognize that pre-concentration of real world samples, as currently used in ion chromatography methods, would provide us with an alternative approach for realizing improved minimum detection limits via HPLC-ICP[23]. Pre-concentration for trace inorganic analysis has its own set of problems, and if this approach is avoided, it might provide a more fool-proof method of speciation via direct sample injection onto HPLC-ICP.

ACKNOWLEDGEMENTS

The authors wish to thank B.L. Karger, B. Bidlingmeyer, and P. Rahn for their interest and assistance in the HPLC areas, as well as G. Davies for his assistance in the inorganic chemistry area. We appreciate the sincere interest of K. Panaro of the Boston District Food and Drug Administration, and for his indicating to us the importance and need for a rapid method of performing chromium speciation and analysis.

This work was supported, in part, by a research and development contract from the Analytical Instrument Division of Instrumentation Laboratory, Inc.

Additional funding was provided, in part, by a grant from the NIH Biomedical Research Support Grant to Northeastern University, No. RR07143. We wish to indicate our sincere appreciation for these sources of financial assistance.

This is contribution number 103 from the Institute of Chemical Analysis, Applications, and Forensic Science at Northeastern University.

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24. Abbreviations used: HPLC = high performance liquid chromatography; ICP = inductively coupled plasma emission spectroscopy; RI = refractive index; Cr = chromium; PIC A = tetrabutylammonium hydroxide; PIC B-5 = sodium n-pentane sulfonate; RP = reversed phase; IC = ion chromatography; MDL = minimum detection limits; ODS = octadecyisilane (C-18); Cr (+3) = Cr (III) = chromic cation; Cr (+6) = Cr (VI) = CrO_4^{-2} = chromate anion; ppb = parts-per-billion; ppm = parts-per-million; ppt = parts-per-thousand.

Received November 23, 1981
Accepted December 17, 1981