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COUPLED GAS CHROMATOGRAPHY–ATOMIC ABSORPTION SPECTROMETRY

WAYNE R. WOLF

Nutrient Composition Laboratory, Nutrition Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md. 20705 (U.S.A.)

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SUMMARY

The specific, precise detection of volatile metal chelates has been obtained by coupling the effluent from a gas chromatograph directly to the burner head of a commerical atomic absorption spectrometer (AAS). Quantitation of chromium in the nanogram range has been accomplished with a detection limit of 1.0 ng. The chelation-extraction–gas chromatographic separation procedure coupled with the selective detection by AAS gives a relatively interference-free system that has been used to quantitatively analyse for chromium in standard biological materials NBS SRM 1571 Orchard Leaves and SRM 1569 Brewers Yeast. Metal chelates of iron, copper and cobalt have also been detected by this system.

INTRODUCTION

The analysis of trace amounts of metals by formation, extraction and gas chromatographic (GC) separation of volatile metal chelates has been demonstrated and used for a wide variety of metals and matrices[1,2]. In extending these procedures to trace metal analysis of very complex matrices such as biological materials, the specificity of the detection system becomes of extreme importance. If one is interested in the metal content of the GC effluent then the ideal detection system must be sensitive to the metal and offer positive identification of its presence and quantity. The majority of detection systems used in GC are not selective for metals. For example, the electron capture detector is very sensitive to the fluorinated ligands commonly used to form suitable volatile chelates but insensitive to the metal. Therefore, confirmation is not positive that the metal chelate is eluted with the peak of detector response and not decomposed on the column.

Sensitive and precise detection of metals in GC effluents by identification of chelates or chelate fragments in a coupled mass spectrometer has been demonstrated[3] and is a very powerful tool in this type of analysis. However, the high cost, limited availability, and necessary modifications of most existing equipment often restricts its use on a routine basis.

The above stated requirements for specificity and sensitivity for detection of metals are in general well met by atomic spectroscopic methods. There have been

several reports of diverting GC effluent into various spectroscopic detectors for the determination of metal chelates[4–6]. The application of the most widely used atomic spectroscopic method of metal analysis, flame atomic absorption spectrometry (AAS), to analysis of metals in the gas phase has been almost exclusively limited to the specific cases of hydride generation of difficult to analyse metals such as arsenic and selenium and diversion of the hydrides into the flame[7,8]. A recent report has used GC for the separation of organo-selenium compounds and detection in a furnace atomization device[9].

A preliminary report on the coupling of a gas chromatograph directly to the burner head of a conventional, commercially available atomic absorption spectrometer has recently been published[10]. That report demonstrated the separation and detection of five different chromium chelates and that fluorination of the chelate is not a prerequisite for detection. It also showed the preliminary use of this coupled GC–AAS system for quantitative trace chromium analysis for inorganic standards and NBS SRM 1571 Orchard Leaves. Since one of the major tasks of this laboratory is to quantitate chromium in all types of biological samples, especially those with the biologically active form of chromium, I would like to report the further use and refinements of the GC–AAS determination of chromium in a sample of brewers yeast which is presently being prepared as a reference material for chromium in biological material[11]. I would also like to report on the extension of GC–AAS detection to several other metals and to comment on the potential applications, advantages and disadvantages of this technique for trace element analysis.

EXPERIMENTAL*

The GC–AAS set-up and interface are similar to that described previously[10] except that the home-made gas chromatograph has been replaced by an inexpensive, commercially available system (Model 69550; Gow-Mac, Madison, N.J., U.S.A.) with better temperature and flow control.

The procedure for sample digestion of biological samples for chromium analysis consists of wet ashing with concentrated sulfuric acid (subboiling distilled, National Bureau of Standards, Gaithersburg, Md., U.S.A.) and hydrogen peroxide (50%, stabilized; Fisher Scientific, Pittsburgh, Pa., U.S.A.). This procedure has been developed to ensure quantitative retention of the chromium during the digestion procedure. The ashing takes place in a reflux system, utilizing an analytical distillation condenser (Cat. No. 9244), distillation trap (Cat. No. 9086) and 25-ml round-bottom flask (Ace Glass, Vineland, N.J., U.S.A.). The sample is placed in the flask, 1.0 ml H_2SO_4 and 1.0 ml water added, the sample allowed to stand for 2 h, 1.0 ml H_2O_2 added and the sample allowed to stand overnight at room temperature. The sample is then heated gently under reflux until bubbling stops. Successive 0.5-ml aliquots of H_2O_2 are added until the solution clears. Excessive water is then distilled off of the sample allowing the conc. H_2SO_4 to further char the sample. Repeated cycles of addition of H_2O_2 and distillation of excess water are carried out until no more charring

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occurs. The sample is then neutralized with a saturated solution of NaOH (Bromcresol Green indicator added) and buffered with 10 ml of sodium acetate–acetic acid buffer (pH 4.7).

Chelation of the chromium is carried out as follows: 1.0-ml aliquots of the buffered solution are transferred to reaction tubes fitted with PTFE plugs (10 × 100 mm hydrolysis tubes, No. 29560; Pierce, Rockford, Ill., U.S.A.); 0.20-ml of distilled ligand (trifluoroacetylacetone; Pierce) is added to each tube; the tube sealed and placed in the oven at 100° for 2 h. Reacted samples are taken from the oven allowed to cool and extracted with 4 0.250-ml aliquots of hexane. The first aliquot of hexane contains an internal standard of tris-heptafluorooctanedionate, Cr(III) [$Cr(fod)_3$] used to correct for volumetric changes in the extraction solvent. Combined extracts are concentrated under a stream of nitrogen gas to approx. 0.200 ml and aliquots injected into the GC–AAS instrument for analysis.

For the brewers yeast samples, it was necessary to destroy excess H_2O_2 by adding 1.0 ml of a saturated NaOH solution after digestion and refluxing for an additional 2 h before buffering the solution. It was also necessary to analyse the brewers yeast digest by the method of additions, adding successive amounts of chromium to separate 1.0-ml aliquots of the digest. These two steps were added routinely to the procedures for analysing diet composites and are necessary to accurately quantitate chromium in a variety of matrices.

Chelates of other metals prepared in previous investigations[1,12] and stored in closed vials for several years were used as opened. The analytical GC conditions were: column, 18 in. × 3 mm I.D. PTFE tubing, packed with 5% SE-30 on Chromosorb P AW DMCS, 80–100 mesh (Hewlett-Packard, Avondale, Pa., U.S.A.); nitrogen carrier gas flow-rate, 120 ml/min; column temperature, 160°; injection port temperature, 150°. Atomic absorption conditions: air acetylene flame; 2-in. single slot burner; standard conditions[13], Model 303 Atomic Absorption Spectrometer (Perkin-Elmer, Norwalk, Conn., U.S.A.).

RESULTS

Using the internal standard $Cr(fod)_3$ in the extraction step gives a chromatogram containing three peaks per injection as shown in Fig. 1. This figure shows a succession of five 30-μl injections of standard solutions into the GC–AAS and a 30-μl injection of the extract from a diet composite sample. Each injection gives a small peak for the solvent followed by a varying peak for the $Cr(tfa)_3$ (trifluoroacetylacetonate). By taking a ratio of the peak heights of the $Cr(tfa)_3$ peak and the $Cr(fod)_3$ peak a calibration curve is generated as shown in Fig. 2. The first injection in Fig. 1 contains 7.7 ng of chromium in the $Cr(tfa)_3$ peak and 54 ng of chromium in the $Cr(fod)_3$ peak. The addition of the internal standard allows quantitative extraction with multiple solvent addition. It also allows concentration of the extract as shown in the last injection. The ratio in this sample is approximately the same as the lowest standard but is about twice as concentrated to give a good sized peak for quantitation. This allows the maximum use of the extract and the quantitation of samples which contain very low amounts of chromium, such as most food samples where the chromium content is often 100 ng/g or less.

Table I shows the recovery of solutions of known amounts of chromium (92.1%

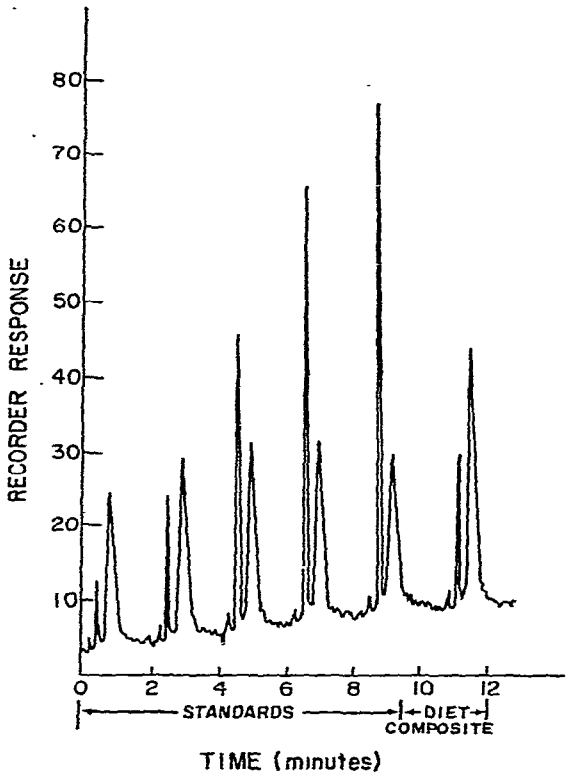

Fig. 1. Chromatograms of multiple injections of chromium chelates into GC–AAS. Each group of three peaks is a single injection. The three peaks are solvent, $Cr(tfa)_3$ and $Cr(fod)_3$, respectively, in order of elution.

recovery with a coefficient of variation of 9.4%). Table II shows the analysis of NBS Standard Reference Material 1571 Orchard Leaves which is certified by NBS for chromium content. These samples were quantitated by comparing the peak height ratios of extracts of individual samples to the ratio of standard solutions as shown in Figs. 1 and 2.

In attempting to extend this procedure to the brewers yeast sample, initial results were much lower than observed by independent methods[11]. It was subsequently discovered that in contrast to the results observed with the Orchard Leaves the chelation-extraction was not quantitative for the chromium in the matrix of the brewers yeast digest. This observation has been noted in other analytical methods comparing chromium in various materials[14,15]. Adding the step of boiling the digest in basic solution increased the amount of chromium observed in the brewers yeast sample. Table III shows the effect of adding varying amounts of NaOH to the digest of 1 g of brewers yeast. The chromium seen by comparison to external standards is seen to increase to 1.0 ml of NaOH added. The chromium seen by internal standards or methods of additions is constant at levels of NaOH above 0.25 ml. The percent recovery of the samples is shown in the next to last column. This value was calculated from the slope of the additions line obtained by plotting chromium added *versus* chromium

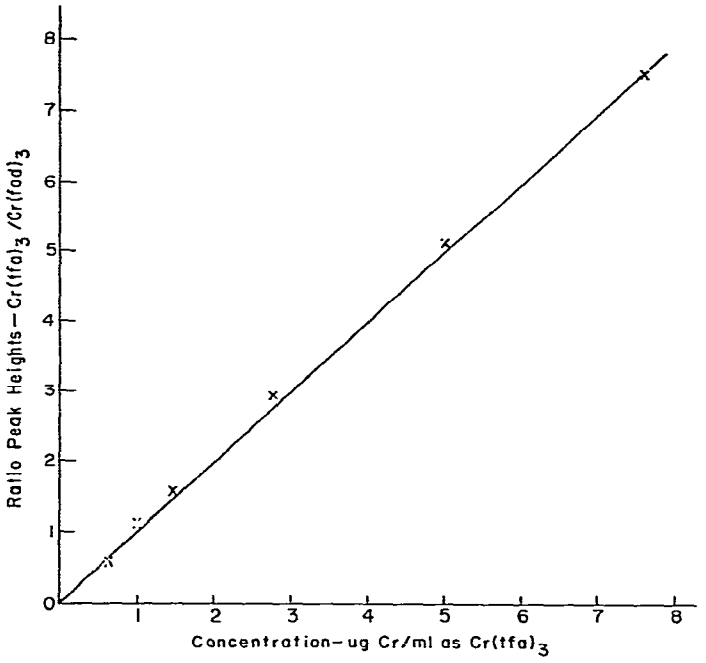

Fig. 2. Calibration curve for chromium by GC–AAS using an internal standard.

observed. The last column in Table III shows the precision and linearity of the standard additions line.

Extending this procedure to a series of diet composites also yields recoveries in the 60–70% range with highly linear addition lines. Thus the procedure appears to be applicable to a wide variety of biological samples for chromium analysis.

Since previous workers have reported the GC of a wide number of metals as volatile chelates, the feasibility of this GC–AAS system for the detection of other met-

TABLE I

RECOVERY OF INORGANIC CHROMIUM

S.E.M. = standard error of the mean.

Cr added (ng)	*Cr observed (ng)*	*Recovery (%)*
250	214	85.6
250	219	87.6
250	235	94.0
210	200	95.2
210	193	91.9
371	352	94.9
371	306	98.7
88.2	75.2	85.3
88.2	70.7	80.2
92.8	76.0	81.9
92.8	91.0	98.1
92.8	104.0	112.1
	Mean	92.1% ± 2.5 (S.E.M.)

TABLE II

CHROMIUM CONTENT OF NBS SRM 1571 ORCHARD LEAVES

Sample	*μg Cr per g of sample*
1a	2.87
1b	2.42
1c	2.36
2a	2.56
2b	2.16
2c	2.08
Mean	2.41 ± 0.12 (S.E.M.) or 92.7% of NBS value of 2.6

TABLE III

INFLUENCE OF ADDING NaOH DURING DIGESTION ON RECOVERY OF CHROMIUM FROM BREWERS YEAST (NBS SRM 1569)

Sample	*NaOH added (ml)*	*μg Cr per g yeast*		*% Recovery (addition methods)*	r^2
		External standards	*Addition methods*		
1	0	0.46	1.65	39.2	0.979
2	0.25	0.88	1.81	62.0	0.997
3	0.50	0.83	2.35	45.7	0.990
4	0.75	1.01	2.07	46.2	0.995
5	1.00	1.65	2.45	71.2	0.990
6	1.25	1.32	2.40	60.7	0.991
		Mean	2.12* ± 0.14 (S.E.M.)		

* NBS value is 2.12 ± 0.05.

als was briefly explored. Chelates of several metals were available from previous studies[1,12]. Small amounts of these materials were dissolved in hexane and aliquots injected into the GC–AAS. The chelates which gave a response in the GC–AAS are listed in Table IV. The iron and copper chelates showed broadened peaks and evidence of decomposition. This is probably due to the fact that these compounds had been prepared several years previously and were not repurified. Also the metal transfer line from the GC to the AAS might cause decomposition of these metal chelates as previous workers have noticed[1]. The detection limits in Table IV are approximations

TABLE IV

DETECTION OF VARIOUS METALS BY GC–AAS

Chelates: fod = heptafluorooctanedionate; tfa = trifluoroacetylacetonate; ofhd = octafluoroheptanedionate.

Chelate	*GC–AAS approximate detection limit (ng)*
$Co(fod)_3$	80
$Fe(fod)_3$	300*
$Fe(tfa)_3$	800*
$Cu(ofhd)_3$	500*

* These samples showed broadened, skewed peaks and evidence of decomposition.

based upon the weight of sample and the assumption of pure samples, and should be somewhat lower with fresh, purified samples. The data in Table IV do show that different metals can be detected with this system and there is a good potential for trace analysis of a variety of metals.

DISCUSSION

The use of AAS as a detector for GC analysis of metal chelates or other inorganic compounds which can be handled in the gas phase has some very significant advantages. It is a detection system which a wide number of laboratories and investigators are familiar with and does not require the highly sophisticated technical support of several other detection systems which have been used. The selectivity and separation during extraction-chromatography gives a detection system that is relatively interference-free. The ability to use a wide volume range per injection and to concentrate extracts greatly compensates for the better sensitivity of other less selective detection systems. One disadvantage is the extended sample preparation and chemistry involved in the chelation and extraction. However, I feel that these types of problems and chemistries are better understood and more susceptible to solution at the present time than the problems involved with the very complex, poorly understood physical and chemical phenomena which occur in many of the newer atomization devices for atomic spectroscopy. This system has the potential for a very workable, useful system in answering the many analytical questions of the content of the essential trace element chromium in our food supply. It also has obvious potential for other metals and other matrices.

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REFERENCES

1 R. W. Moshier and R. E. Sievers, *Gas Chromatography of Metal Chelates*, Pergamon Press, New York, 1965.
2 G. Guiochon and C. Pommier, *Gas Chromatography in Inorganic and Organo-metallics*, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1973.
3 W. R. Wolf, M. L. Taylor, B. M. Hughes, T. C. Tieman and R. F. Sievers, *Anal. Chem.*, 44 (1972) 616.
4 H. Kawaguchi, T. Sakamoto and A. Mizuike, *Talanta*, 20 (1973) 321.
5 R. M. Dagnell, T. S. West and P. Whitehead, *Analyst (London)*, 98 (1973) 647.
6 F. A. Servallo and T. H. Risby, *J. Chromatogr. Sci.*, 12 (1974) 585.
7 F. J. Fernandez, *At. Absorption Newslett.*, 12 (1973) 93.
8 H. C. Freeman and J. F. Uthe, *At. Absorption Newslett.*, 13 (1974) 75.
9 Y. K. Chau, P. T. S. Wong and D. P. Goulden, *Anal. Chem.*, 47 (1975) 2279.
10 W. R. Wolf, *Anal. Chem.*, 48 (1976) 1717.
11 R. Alvarez, *Standard Reference Materials, SRM 1569*, National Bureau of Standards, Gaithersburg, Md., to be published.
12 R. E. Sievers, personal communications.
13 *Analytical Methods for Atomic Absorption Spectroscopy*, Perkin-Elmer, Norwalk, Conn., 1971.
14 W. R. Wolf, W. Mertz and R. Masironi, *J. Agr. Food Chem.*, 19 (1974) 1037.
15 W. R. Wolf, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 34 (1975) 927.