

# Determination of gold-based antiarthritis drugs and their metabolites in urine by reversed-phase ion-pair chromatography with ICP-MS detection

ZHENG ZHAO, WILLIAM B. JONES, KATHERINE TEPPERMAN, JOHN G. DORSEY\* and R.C. ELDER

*Department of Chemistry and Biological Sciences, Biomedical Chemistry Research Center, University of Cincinnati, Cincinnati, OH 45221-0172, USA*

**Abstract:** A sensitive method for the determination of gold-based drugs auranofin, myochrysin, and their metabolites has been developed. These gold-containing compounds were separated by reversed-phase ion-pair chromatography with tetrabutylammonium chloride as the ion-pairing agent. Gold-specific on-line detection utilized inductively coupled plasma mass spectrometry (ICP-MS). The separation conditions of pH, methanol content, concentration of the ion-pairing agent and ionic strength have been investigated. The detection limit for auranofin, the last peak in the chromatogram, was 0.3 ng. These methods were applied to the analysis of gold-containing species in urine from arthritis patients on auranofin, myochrysin or solganol therapy. The recovery of the total gold-containing species from urine was greater than 90%. Dicyanogold(I) anion,  $[\text{Au}(\text{CN})_2]^-$ , was detected in the urine of several patients.

**Keywords:** Gold-based drugs; gold-containing metabolites; dicyanogold(I) anion; reversed-phase ion-pair chromatography; inductively coupled plasma mass spectrometry.

## Introduction

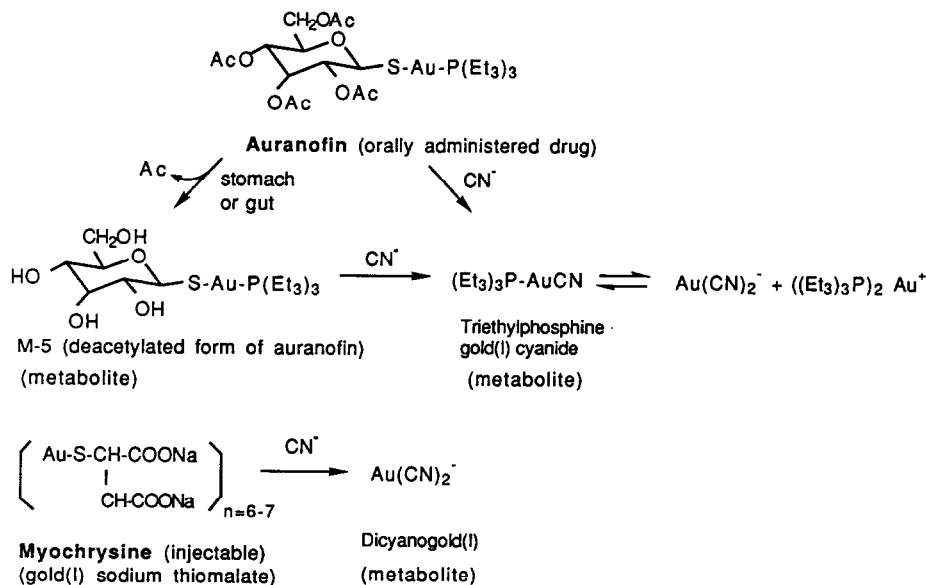
Rheumatoid arthritis (RA) is a progressively debilitating disease with no known cause or cure. Approximately six million Americans are afflicted with this disease. For over 60 years a variety of gold-containing drugs have been used to treat RA, and have proven to be effective antiarthritis drugs. Myochrysin (sodium gold(I) thiomalate), auranofin (triethylphosphinegold(I)tetraacetylthioglucose) and solganol (gold(I) thioglucose) are currently used in the United States. Unfortunately, remission with the use of these gold drugs is obtained for only 50% of the patients [1]. The side-effects, however, are so frequent and severe that treatment must be discontinued in about 25% of cases for the injectable drug myochrysin (gold thiomalate) and 2% for the newer orally administered drug auranofin [2]. The mode of action and cause of the side-effects of the gold drugs are still largely unknown. Clearly, identifying the metabolites of the drugs will enhance the understanding of the pharmacologic properties and actions of these drugs. Development of selective sep-

arations and sensitive detection methods is a necessary prerequisite for progress in this field.

Shaw *et al.* have studied the protein-bound gold distribution in body fluids by gel filtration and quantified gold in collected fractions with atomic absorption spectrophotometry [3]. In this study detection and speciation of the low-molecular weight gold species has been emphasized. The aim was to develop a simple and sensitive method to separate and detect the parent gold drugs, auranofin and myochrysin as well as their low-molecular weight gold-containing metabolites (Fig. 1) in biological samples using liquid chromatography and inductively coupled plasma mass spectrometry.

Previously it has been shown, using an everted intestinal sac with rats and hamsters, that the deacetylated form of auranofin (M-5, triethylphosphinegold(I) thioglucose) is the principal species which passes through the gut wall [4, 5]. Another possible metabolite is triethylphosphinegold(I) cyanide which results from cyanide replacing either the tetraacetylthioglucose ligand of auranofin or the thioglucose ligand of M-5. Cyanide concentrations

\* Author to whom correspondence should be addressed.



**Figure 1**  
Structures of the gold compounds investigated.

can be as high as 1.5  $\mu\text{M}$  in the blood of a smoker [6]. Triethylphosphinegold(I) cyanide, however, is not stable, and will convert to other species such as the dicyanogold(I) anion. Dicyanogold(I) is also a suspected metabolite of any of the gold-containing drugs [7], especially for patients who are smokers. Myochrysine (gold(I) thiomalate, Au-tm) is a polymeric, ionic compound [8].

From the perspective of the liquid chromatography, auranofin and triethylphosphine-gold(I) cyanide are hydrophobic, neutral species, and expected to be well retained on a non-polar (reversed-phase) column. M-5, on the other hand, is polar and very hydrophilic, thus leading to poor retention. The dicyanogold(I) anion is negatively charged, while gold(I) thiomalate (Au-tm) has two ionizable carboxylate groups; thus separation of these compounds by LC should be strongly influenced by both their hydrophilic and ionic properties. In this work, reversed-phase ion-pairing chromatography (RP-IPC) was used to separate these gold compounds. The reason for choosing RP-IPC is to increase the retention of ionic species through modification of the C18 stationary phase, thus overcoming the difficulties arising from mixtures which contain both hydrophobic and ionic species. An inductively coupled plasma mass spectrometer (ICP-MS) was used as the detector. It offers element-specific detection with a low detection

limit such that gold compounds can be determined potentially at physiological levels [9].

## Experimental

### Instrumentation

The LC system included two Waters model 510 HPLC pumps with a model 680 automated gradient controller, a Valco C6W injector with 20, 90 and 200  $\mu\text{l}$  loops, and a UV detector with 214-nm detection (Beckman model 160). A strip chart recorder (Houston Instruments, Austin, TX, USA) was used for data collection.

A Sciex Elan 250 ICP-MS was used for detecting gold-containing complexes in the HPLC effluent. Gold 197 was the mass monitored. The RF power was 1.4 kW, nebulizer Ar gas flow rate was 1 l  $\text{min}^{-1}$ , and the nebulizer spray chamber was cooled at  $-17^\circ\text{C}$  to condense most organic vapour. The column was connected to the nebulizer by means of PTFE capillary tubing (120 cm  $\times$  0.1 mm i.d.). This tubing has a volume of approximately 0.08  $\mu\text{l cm}^{-1}$  and will have a minimal effect on extra-column peak broadening. The data were collected in multielement mode. Separation parameters were optimized with the UV detector.

A VG 30-250 mass spectrometer was used to verify the dicyanogold(I) anion in collected fractions from a patient urine sample. Using

FAB, dicyanogold(I) and its fragments were detected in the selected ion mode.

#### Column and chromatographic conditions

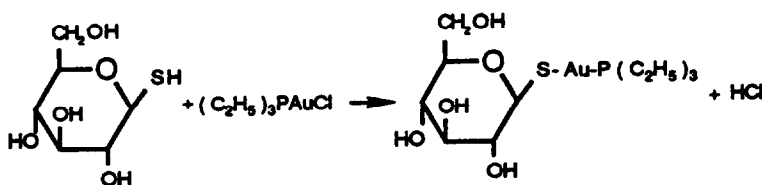
An analytical C18 column (Spherisorb ODS-2) (5  $\mu\text{m}$ , 250  $\times$  4.6 mm) was used for method development. A C18 column (B & J, OD5) (5  $\mu\text{m}$ , 150  $\times$  4.6 mm) was used for the biological sample analyses. A 5 cm guard column was packed in house with 5- $\mu\text{m}$  Spherisorb ODS-2. The mobile phase used in all experiments was comprised of methanol-water, buffer and the ion-pairing reagent. The column was pre-equilibrated with the desired mobile phase for at least 1 h before injection, or overnight when long chain pairing agents were used. All analyses were performed at a flow rate of 1.0 ml min<sup>-1</sup> in an isocratic mode, and the column temperature was maintained at 30°C by a thermostated water bath.

#### Chemicals

All quaternary amines and chemicals used for buffers were obtained from commercial sources, and were of analytical grade. All water used was purified through a Barnstead (Milford, MA, USA) Nanopure system equipped with a 2- $\mu\text{m}$  filter. Prior to use, the mobile phase was filtered through a 0.45- $\mu\text{m}$  Nylon-66 membrane filter.

Auranofin was provided by SmithKline Beecham (Philadelphia, PA, USA). Sodium gold(I) thiomalate was purchased from Aldrich Chemicals (Milwaukee, WI, USA). Potassium dicyanogold(I) was from Sigma Chemical Co. (St Louis, MO, USA). Triethylphosphine-gold(I) cyanide was synthesized by standard procedures [10].

M-5 was prepared by the reaction of (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>PAuCl with thioglucose in 1:1 (v/v) acetone-water at 0°C according to a published procedure [11]:



Stock solutions of the gold compounds were prepared in 70:30 or 50:50 (v/v) methanol-water and were diluted to the desired concentration with the chromatographic mobile phase prior to use.

## Results and Discussion

Reversed-phase ion-pair chromatography is a technique which employs an ion-pairing agent in the mobile phase to modify the non-polar stationary phase, thus permitting the simultaneous separation of ionic and non-ionic compounds. It is believed that the hydrophobic pairing ion is adsorbed onto the surface of the stationary phase such that the modified stationary phase acts like a dynamic ion-exchanger [12]. Retention of ionic species will be governed by the magnitude of the binding constant and the amount of ion-pairing agent adsorbed on the stationary phase, which depends on the hydrophobicity of the pairing ions and the mobile phase conditions.

#### Selection of ion-pairing agent

The hydrophobic nature of the ion-pairing agents plays an important role in the resulting chromatography. Generally, increasing the length of the alkyl chain of the agent will result in an increase in retention of a given ionic solute. To determine the effects of different ion-pairing agents on the retention of the gold compounds of interest, a mobile phase composition of either 70:30 or 60:40 methanol-water at a pH of 5 was used with an ion-pairing agent (concentration 10 mM). The ion-pairing agents investigated were various quaternary ammonium ions, as their halide salts, with different lengths of carbon chain. These are listed in Table 1. The resulting chromatograms showed that of these pairing ions, tetra-*n*-butylammonium chloride (TBA) was the best at increasing retention for gold(I) thiomalate, while not altering the retention of the neutral molecules.

Since auranofin and triethylphosphine-gold(I) cyanide are neutral molecules, their retention should not be changed by adding an

ion-pairing agent. This expectation holds true when the chain length of the agent is small. However, as the chain length of the ion-pairing agent begins to increase, the retention gradually decreases. There is a dramatic decrease in

**Table 1**  
Ion-pair reagents investigated

Cations:	
Tetraethylammonium chloride (TEA)	$(C_2H_5)_4NCl$
Tetra- <i>n</i> -butylammonium chloride (TBA)	$(C_4H_9)_4NCl$
Tetrapentylammonium bromide (TPA)	$(C_5H_{11})_4NBr$
Decyltrimethylammonium bromide (DTMA)	$C_{10}H_{21}(CH_3)_3NBr$
Hexadecyltrimethylammonium bromide (CTAB)	$C_{16}H_{33}(CH_3)_3NBr$

retention of auranofin when using hexadecyltrimethylammonium bromide (CTAB) compared with using TBA. It is well established that ion-pairing agents adsorb on the surface of hydrophobic stationary phases. Isotherms have been measured for both hydro-organic [13] and purely aqueous [14] mobile phases. Coating the surface alters the apparent chromatographic partition coefficients of neutral molecules, especially for those which interact with residual hydroxyls or metal impurities in the silica base. M-5 is a polar rather than ionic species. Thus, its interaction with a positively-charged ion-pairing agent is expected to be quite weak. In comparison to gold thiomalate, the retention of M-5 is hardly affected by the addition of positively-charged ion-pairing agents.

Since TBA increased the retention times of the ionic species without causing a deterioration in the behaviour of the neutral materials, it was chosen as the ion-pairing agent for all further studies. Also, the TBA cation exhibits an added advantage in that it requires significantly less mobile-stationary phase equilibration time compared to pairing agents with long alkyl chains.

#### *Effect of mobile phase pH on retention*

Since ion pairing is the most important interaction affecting retention, the most extensive retention of weak acids (or weak bases) will be obtained at the pH where they are fully ionized. The gold thiomalate molecule contains two carboxyl groups with the  $pK_1 = 3.5$  and  $pK_2 = 5$  estimated from thiomalic acid. Thus gold(I) thiomalate was expected to be most retained above pH 5. The pH study was carried out at three pH values, pH 5.96, 3.90 and 2.90, adjusted with phosphate buffer. At a mobile phase pH of 5.96, the retention time of gold thiomalate was longest. At pH 3.90, the result was a poorly retained peak which tailed badly. At this pH the acid was partially ionized and exhibited less affinity for TBA. At a pH of 2.90, gold thiomalate was

fully protonated and unionized. Thus the ion-pairing agent was expected to have little, if any, effect on the retention of gold thiomalate. However, as a fully protonated, neutral species gold thiomalate should now be retained by the hydrophobic, reversed-phase interactions and, in fact, its retention time increases over that at pH 3.90. Since, a pH of 2.90 was much less than that of most biological samples expected, pH 5.96 was chosen for further studies.

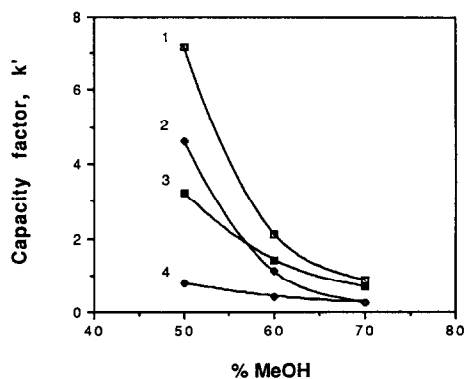
As expected, the neutral molecules auranofin, triethylphosphinegold(I) cyanide and M-5, as well as the anion dicyanogold(I), showed little retention dependence on mobile phase pH.

#### *Organic modifier content*

After selecting the ion-pairing agent (TBA) of appropriate concentration (10 mM), and a suitable mobile phase pH (5.96), the effect of methanol concentration on retention was investigated. In RP-IPC, the ionic solute retention depends largely on the surface concentration of the adsorbed ion-pairing agent which in turn is strongly affected by the concentration of the organic modifier [15]. Adsorption isotherms of ion-pairing agents show that an increase in the concentration of methanol causes a rapid decrease in the concentration of the ion-pairing agents on the surface of the stationary phase [15]. This retention dependence was observed here as shown in Fig. 2, the retention of gold(I) thiomalate increased dramatically as the methanol content decreased. The phenomenon was probably caused by an increase of the surface concentration of the pairing ion, TBA, as the methanol concentration in mobile phase decreases.

M-5, on the other hand, has only a weak attraction to TBA, and its polar character is the dominant factor in its retention. Thus, its retention increased only very slowly with a decrease in methanol content.

The capacity factors,  $k'$ , of auranofin and triethylphosphinegold(I) cyanide showed a

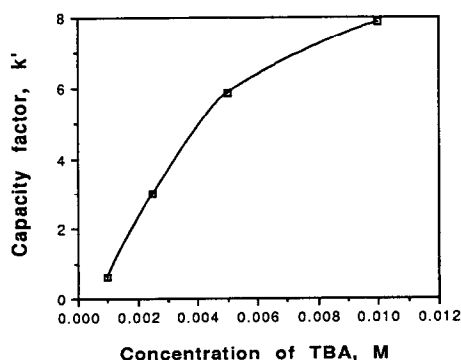


**Figure 2**  
Effect of the organic modifier on retention. Pairing ion: tetrabutylammonium, 10 mM; pH 5.96 with 25 mM phosphate buffer. 1, Auranofin; 2, gold(I) thiomalate; 3, triethylphosphine gold cyanide; 4, M-5.  $k' = (\text{retention volume} - \text{void volume})/\text{void volume}$ .

strong dependence on the content of modifier. These two compounds are neutral, hydrophobic species, and the retention increases as the percentage of methanol decreases. The peak shape for auranofin deteriorated below 50% methanol, so the methanol concentration could not be reduced further to increase the  $k'$  of M-5 greater than 1. Figure 2 demonstrates that these gold compounds were well separated with reasonable  $k'$  values at methanol–water (50:50, v/v).

#### Concentration of ion-pairing agent

The effect of TBA concentration on the retention of Au-tm is shown in Fig. 3. When the concentration of TBA was increased from 1 to 10 mM, the retention of the ionic analyte, gold(I) thiomalate, increased rapidly from 1 to 5 mM TBA, then slowly increased thereafter. This behaviour is expected from the adsorption isotherm of the ion-pairing agent on

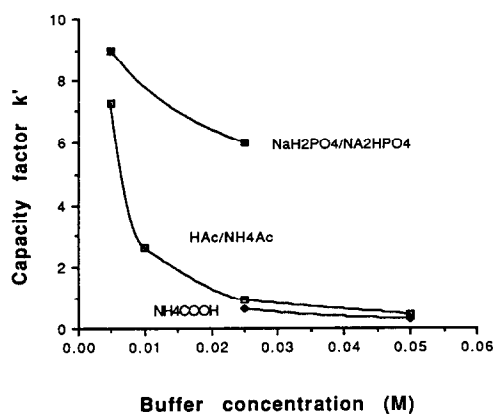


**Figure 3**  
Effect of TBA-Cl concentration on the retention of gold(I) thiomalate.

the hydrophobic surface. More TBA will be absorbed [12] on the stationary phase surface as the concentration increases, resulting in greater retention of gold(I) thiomalate. The retention of gold(I) thiomalate will eventually level off when the surface of the stationary phase is saturated with ion-pairing agent. The retention behaviour of dicyanogold(I) should be similar to this.

#### Ionic strength effect on retention of gold(I) thiomalate

Considering that ICP-MS was chosen to monitor gold-containing species, all components of the mobile phase must be compatible with this instrument. In the pH study, phosphate buffer was used. However, phosphate buffer is not volatile and may block the sampler and skimmer on the ICP-MS after a few hours [16]; therefore a more volatile buffer was needed for long-term stability. Ammonium acetate and ammonium formate were tested and their effects on retention of Au-tm are shown in Fig. 4. For all the buffers, an increase in buffer concentration was expected to decrease the retention of gold thiomalate since increasing the ionic strength decreases the ion-pairing agent concentration on the surface of the stationary phase. Compared to phosphate buffer, however, the drop in retention of gold thiomalate was greater for the other two salts, implying that another factor is contributing to the shift. One possible explanation is a competition between buffer and ion-pairing agent for the analyte due to the similarity in functional groups. Doubling the



**Figure 4**  
Effect of buffer solutions on the retention of gold(I) thiomalate. Mobile phase: methanol–water (50:50, v/v) containing 10 mM TBA-Cl (pH 6). Flow rate: 1.0 ml  $\text{min}^{-1}$ ; detector: 214 nm.

concentration of TBA in the mobile phase (20 mM) has a small positive effect on the retention of Au-tm. However, increased total dissolved solids in the mobile phase decrease the long-term stability of the ICP-MS. Therefore, increasing the TBA concentration was not a useful way to increase the retention of Au-tm.

#### Chosen separation conditions

The selected conditions were: ion-pairing agent TBA; methanol–ammonium formate (25 mM; pH 6) (50:50, v/v). A typical chromatogram of standards detected by UV is shown in Fig. 5. The first peak in Fig. 5(a) is an impurity presumably resulting from the synthesis of M-5. The myochrysin peak (Fig. 5b) tails, possibly due to the polymeric character of this material.

#### LC-ICP-MS

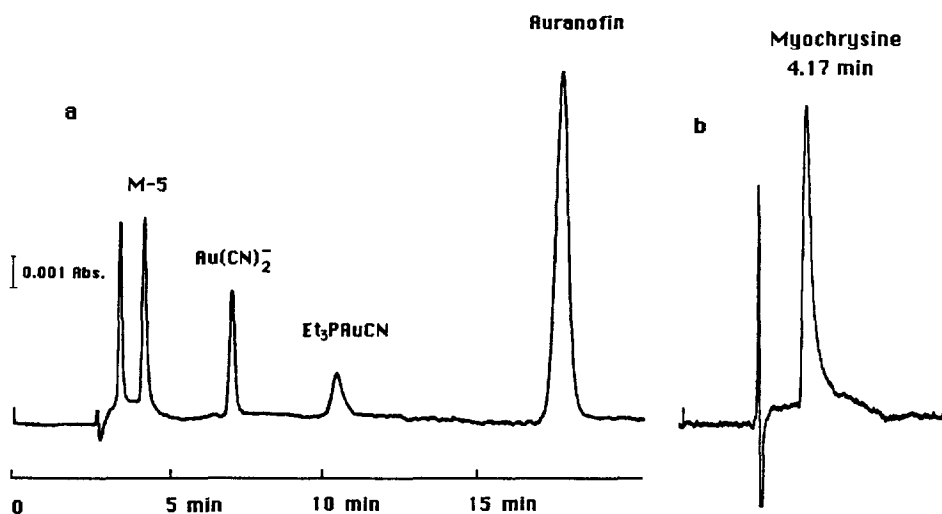
The coupling of the LC column to the ICP-MS was accomplished by directly connecting the column to the nebulizer using PTFE capillary tubing. The response, i.e. the signal intensity of the analyte is a complex function of several operational variables. Generally, the common factors that are open for the operator to adjust are radio frequency power, nebulizer Ar flow rate, and interface position. The RF power and nebulizer flow rate appear to operate as a paired set of variables in that if one is changed, the other must also be changed

to remaximize the signal intensity. These two variables were optimized with a  $100 \text{ ng ml}^{-1}$  gold standard in the mobile phase which is used in the chromatographic separation, as shown in Figs 6 and 7. Generally, organic solvents require a higher forward power and a reduced nebulizer argon flow rate to optimize the signal. The chromatogram of the standards with the ICP-MS detector is shown in Fig. 8. There are two small peaks that appear before the myochrysin peak (Fig. 8b), which can be attributed to impurities in the standards.

Chromatography inherently dilutes the analyte due both to convective and diffusional band broadening in the void volume of the column and any extra-column tubing. Karger *et al.* [17] have shown that

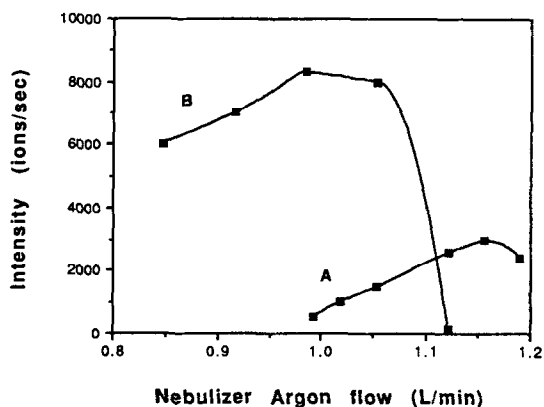
$$h = q_{\text{inj}} \sqrt{\frac{N}{2\pi}} / V_{\text{R}}^2,$$

where  $h$  is peak height,  $q_{\text{inj}}$  is the amount injected,  $N$  is the column efficiency (theoretical plates) and  $V_{\text{R}}$  is the retention volume of the compound of interest. Because of the variable of the injection volume it is only correct to report a limit of detection in terms of the amount injected [18]. Furthermore, as the limit of detection increases (worsens) with increasing retention volume, the last peak in the chromatogram will have the highest (poorest) limit of detection. A typical calibration curve for aqueous standards of auranofin with

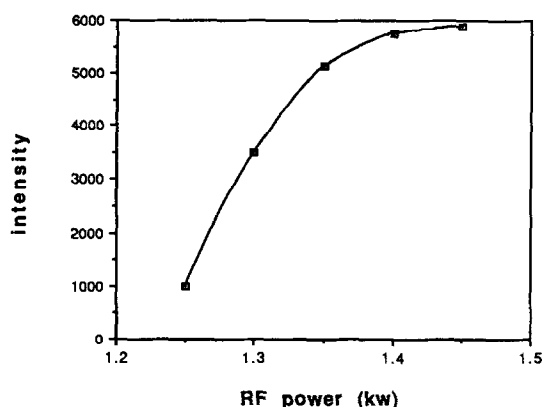


**Figure 5**

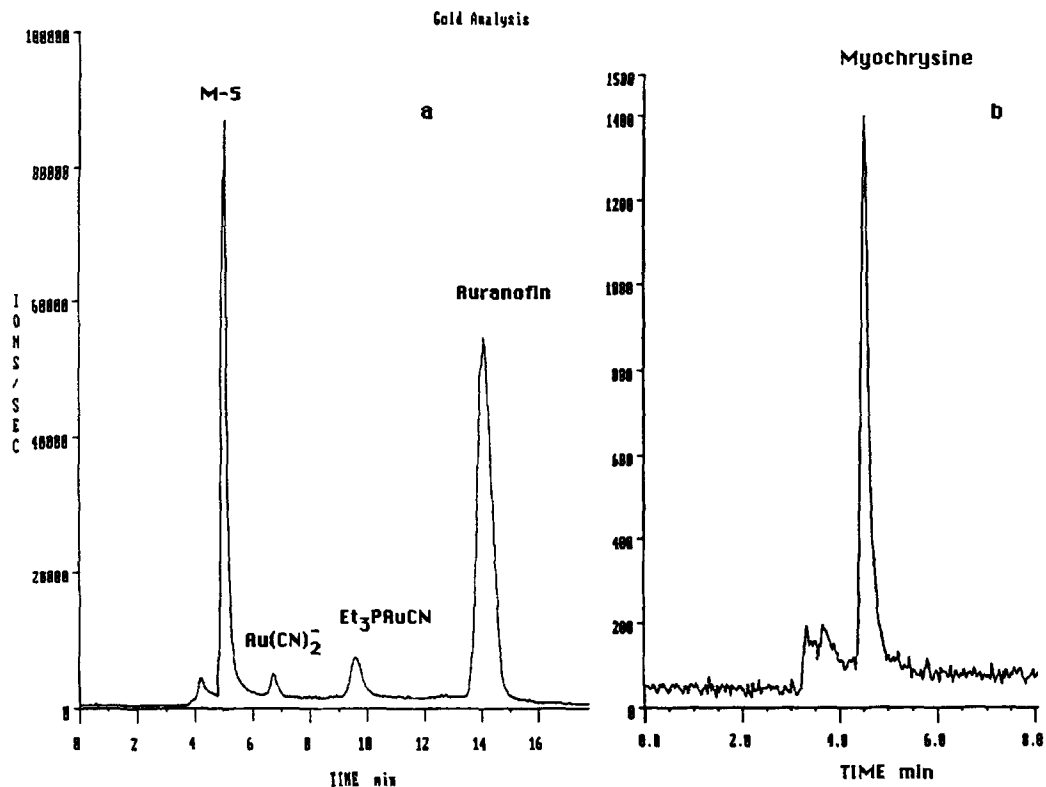
(a) Chromatogram of auranofin and its metabolites. (b) Chromatogram of gold(I) thiomalate. Column: Spherisorb/C18 ( $250 \times 4.6 \text{ mm}$ ,  $5 \mu\text{m}$ ); mobile phase: methanol–water (50:50, v/v) containing 10 mM TBA-Cl and 25 mM  $\text{NH}_4\text{COOH}$  (pH 6.3). Flow rate:  $1.0 \text{ ml min}^{-1}$ ; column temperature:  $30^\circ\text{C}$ ; detection: UV detector at 227 nm.



**Figure 6**  
Effect of the nebulizer gas flow rate on the signal intensity. (A) 100 ppb Au in water. (B) 100 ppb Au in the mobile phase containing 50% MeOH. RF power, 12.5 kW. Plasma gas flow, 12.5 l min<sup>-1</sup>. Auxiliary gas flow, 1.45 l min<sup>-1</sup>.



**Figure 7**  
Effect of the RF power on the signal intensity. Plasma gas flow: 12.5 l min<sup>-1</sup>; auxiliary gas flow: 1.45 l min<sup>-1</sup>; nebulizer gas flow: 0.916 l min<sup>-1</sup>. Sample: 10 ppb Au in the mobile phase containing 50% MeOH.



**Figure 8**  
Chromatogram of standards detected by ICP-MS. (a) Auranofin and its metabolites: M-5, Et<sub>3</sub>PAuCN and Au(CN)<sub>2</sub><sup>-</sup>, (b) gold(I) thiomalate. Mobile phase: 50% MeOH, 10 mM TBA-Cl and 25 mM NH<sub>4</sub>COOH; flow rate: 1 ml min<sup>-1</sup>.

a column of 11,000 theoretical plates satisfied the relationship  $y = -5.67 + 266x$  ( $n = 4, r^2 = 0.999$ ) over a range 0.3–200 ng of Au injected ( $x$ ). The RSD of the peak area counts ( $y$ ) over the calibration curve concentration range was 0.8 to 5.9% (for  $n = 3$ ). The

biological gold levels were well within this range. The detection limit for auranofin, based on a signal equal to three times the standard deviation of the noise divided by the sensitivity, was found to be 0.3 ng. As the ICP-MS detector response is essentially independent of

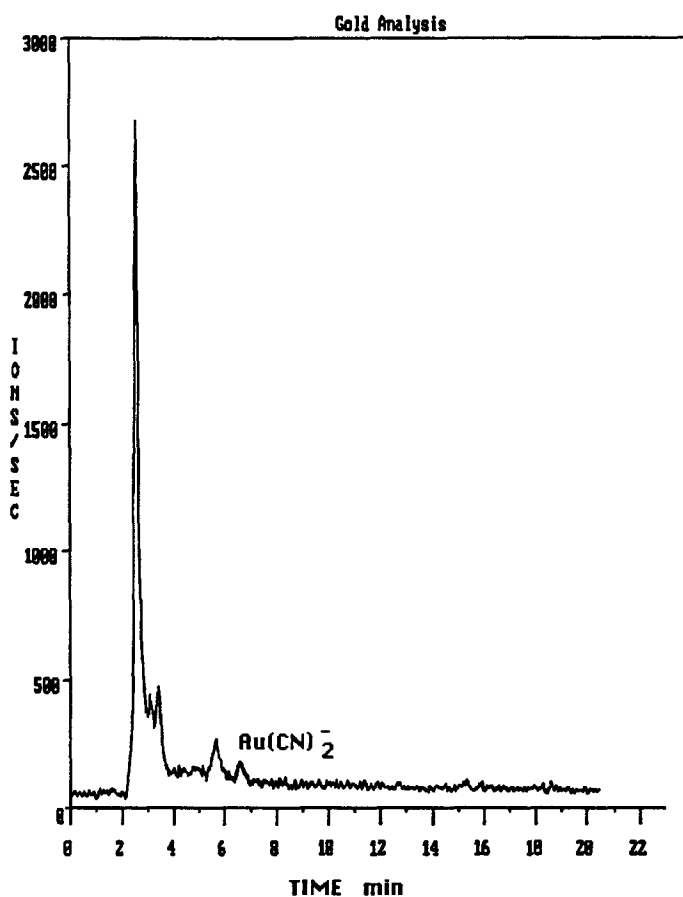
the molecular form of the gold(I), the detection limits for the other gold compounds will be about the same or lower since auranofin has the longest retention time. Given a typical injection volume of 200  $\mu\text{l}$  and a typical total gold concentration of 500  $\text{ng ml}^{-1}$ , the detection limit for auranofin suggests that components present in amounts as small as 0.3% of the total will be detectable. The RSD for 10 replicate injections of a 800  $\text{ng ml}^{-1}$  aqueous Au standard was 4.2%. The RSD for 10 replicate injections of urine samples containing 200  $\text{ng ml}^{-1}$  Au was 3.2%.

The absolute recovery of Au for urine samples was estimated to be greater than 90%. This percentage was established by comparing total peak area in the chromatogram with peak area obtained when flow injection analysis was performed on the same urine sample with the mobile phase as the carrier stream.

#### *Analysis of clinical samples*

The developed method was applied to the analysis of urine samples taken from two arthritic patients immediately before the next administration of gold drug. One patient was being given auranofin, while another was given myochrysin. The patient given myochrysin was a smoker. Urine samples were filtered through a 0.45  $\mu\text{m}$  membrane upon collection from the hospital, and then refrigerated before analysis. A 200  $\mu\text{l}$  sample loop was used for urine analysis.

The chromatogram (Fig. 9) of one auranofin patient's urine shows several gold-containing species. The resolution of the first peak is poor and a significant amount of gold is eluted from the column unretained. There are two small peaks eluted at 5.6 and 6.6 min, respectively. The 6.6 min peak corresponds to the retention time for  $[\text{Au}(\text{CN})_2]^-$ . Comparison with the



**Figure 9**

Chromatogram of a urine sample from an arthritis patient on auranofin therapy. Analytical column: B & J OD5 Octadecyl, 15  $\times$  0.46 cm, 5  $\mu\text{m}$ ; guard column: Spherisorb ODS2, 5  $\times$  0.46 cm, 5  $\mu\text{m}$ ; mobile phase: methanol-water (50:50, v/v) containing 0.01 M TBA-Cl and 25 mM  $\text{NH}_4\text{COOH}$ , pH 6; flow rate: 1  $\text{ml min}^{-1}$ ; column temperature: 30°C; detection: ICP-MS with  $m/z$  at 197.

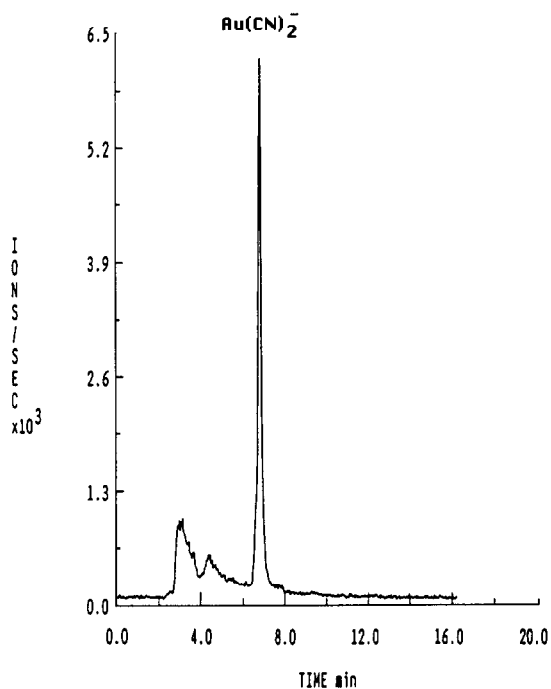


standard chromatogram shows the parent drug auranofin has been converted to other gold-containing species. Based on the detection limit, the maximum amount of auranofin which could be present in this sample is less than 0.3% of total gold, which is 99.6 ng determined by flow injection analysis.

The chromatogram of a urine sample from a patient on myochrysin therapy is shown in Fig. 10. Besides several poorly retained gold-containing compounds, there is a major peak eluted at 6.6 min, at the same retention time for the standard gold compound  $K[Au(CN)_2]$ . This component also had the same retention as a standard sample of  $[Au(CN)_2]^-$  when chromatographed on a polystyrene-divinylbenzene (PRP-1) column under the same mobile phase conditions. Since this component has the same retention characteristics on two different columns as does a known sample of  $K[Au(CN)_2]$ , it is very likely that the component is in fact  $[Au(CN)_2]^-$ . To further check this component a fraction was collected and the parent anion,  $[Au(CN)_2]^-$ , was detected by negative ion, quadrupole mass spectrometry.

## Conclusions

A powerful analytical tool for the sensitive



**Figure 10**  
Chromatogram of a urine sample from an arthritis patient on myochrysin therapy. Conditions same as in Fig. 9.

and element-specific analysis of gold compounds in body fluids has been developed and applied to clinical samples. The detection limit is 0.3 ng gold. The parent drugs, auranofin and myochrysin, were not found in urine samples.  $[Au(CN)_2]^-$  was detected in one cigarette smoker. This is the first time that  $[Au(CN)_2]^-$  has been detected *in vivo*. This finding confirms the hypothesis that  $CN^-$  forms a complex with the gold drug *in vivo* as previously found in *in vitro* studies by Graham *et al.* [19].

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## References

- [1] R. Srinivasan, B. Miller and H. Paulus, *Arthritis Rheum.* **22**, 105–110 (1979).
- [2] B. Tumiati, *J. Rheumatol.* **15**, 177 (1988).
- [3] C.F. Shaw III, N.S. Memmel and D. Krawczak, *J. Inorg. Biochem.* **26**, 185–195 (1986).
- [4] K. Tepperman, R. Finer, S. Donovan, R.C. Elder, J. Doi, D. Ratliff and K. Ng, *Science* **225**, 430–432 (1984).
- [5] R.C. Elder and K. Tepperman, in *Proceedings of the First International Conference on Gold and Silver in Medicine*, pp. 135–154 (1987).
- [6] A.R. Pettigrew and G.S. Fell, *Clin. Chem.* **19**, 466–471 (1973).
- [7] G.G. Graham and M.M. Dale, *Biochem. Pharmacol.* **39**, 1697–1702 (1990).
- [8] R.C. Elder and M.K. Eidsness, *Chem. Rev.* **87**, 1027–1046 (1987).
- [9] S.G. Matz, R.C. Elder and K. Tepperman, *J. Anal. At. Spectrom.* **4**, 767–771 (1989).
- [10] A.L. Hormann, C.F. Shaw III, D.W. Bennett and W.M. Reiff, *Inorg. Chem.* **25**, 3953–3957 (1986).
- [11] B.M. Sutton, E. McGusty, D.T. Walz and J. DiMartino, *J. Med. Chem.* **15**, 1095–1098 (1972).
- [12] M.T. Gilbert, *High Performance Liquid Chromatography*, p. 235. Wright, Bristol (1987).
- [13] C.T. Hung and R.B. Taylor, *J. Chromatogr.* **202**, 333–345 (1980).
- [14] A. Berthod, I. Girard and C. Gonnet, *Anal. Chem.* **58**, 1356–1358 (1986).
- [15] A. Bartha, G. Vigh and Z. Varga-Puchony, *J. Chromatogr.* **260**, 337–345 (1983).
- [16] D.T. Heitkemper, Ph.D. Dissertation, University of Cincinnati, OH, USA, p. 18 (1989).
- [17] B.L. Karger, M. Martin and G. Guiochon, *Anal. Chem.* **46**, 1640–1647 (1974).
- [18] J.P. Foley and J.G. Dorsey, *Chromatographia* **18**, 503–511 (1984).
- [19] G.G. Graham, T.M. Haavisto, H.M. Jones and G.D. Champion, *Biochem. Pharmacol.* **33**, 1257–1262 (1984).

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