The Speciation of Gold in Mine Wastes and Natural Waters

A thesis submitted for fulfilment of the degree of
Doctor of Philosophy

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2. Method Development

2.1 Introduction

Gold is widely recognised for its use in monetary systems and jewellery, along with uses in medicine, nanotechnology and electronics [4]. The result is that gold mining, exploration and processing are of significant economic importance. The ability to speciate low levels of aqueous gold is required for environmental monitoring of tailings and waters from gold mining sites. The knowledge gained will provide additional information about the mechanisms of gold mobilisation in the environment, guiding the interpretation of regional exploration datasets. In areas with historical mining, speciation studies may help identify contributions from current or previous operations. Knowledge of speciation can also be used to guide the choice and concentration of lixiviant used in gold leaching [143]. In addition, it will be useful for understanding bioremediation processes [144], as well as for the targeted design of recovery strategies for low level aqueous gold [145].

The mobilisation and transport of gold in aqueous solutions has been associated with microbial processes as well as its complexation [10, 18, 56, 146]. Various ligands have been hypothesised to be important for this role. In regions around mining sites, gold is likely to be mobilised as Au(I)-cyanide due to complexation with trace amounts of residual cyanide from mine processing [94]. Other aqueous forms of gold that may be present include $Au(I)OH.H_2O$ [72], Au(I) or Au(III) chloride (and their corresponding mixed halide-hydroxide complexes) [147, 148], Au(I) thiosulfate [149, 150], Au (I) and (III) organic matter complexes [37, 73], and suspended gold nanoparticles [151].

Current techniques for the speciation of Au(I) and Au(III) include liquid-liquid extraction, UV-VIS and Raman spectroscopy [81, 83-85, 96, 97, 121, 152], however, these techniques are unsuitable for speciation of many aqueous solution as their limits of detection are usually in the mg L^{-1} (ppm) range, whereas, the concentration of gold in aqueous samples under standard conditions may be in the ng L^{-1} (ppt) range [102-105, 153, 154]. These techniques are also subject to interferences from the natural water matrix.

HPLC coupled with ICP-MS is a powerful technique for the determination of trace levels of elements [155]. ICP-MS offers detection limits in the low to sub ng L^{-1} (ppt) range for many elements, has a wide linear range and is ideally suited to the detection of trace levels of gold in aqueous solution. A small number of studies have identified aqueous gold species using HPLC-ICP-MS methodologies [132-135], however, none of these studies have focussed on environmental waters. Alternately, they have focussed on the determination of the metabolites of Au(I) containing drugs in blood and urine.

Reversed phase-ion pair chromatography avoids both the quantification difficulties with gradient elution (used with weak anion-exchange chromatography), and the retention of the low molecular weight gold metabolites with size exclusion chromatography [133]. Zhao *et*

al.'s [135] simple isocratic method is an ideal starting point to develop an analytical method capable of differentiation of Au species. Their method was able to detect the dicyanogold(I) anion, $[Au(CN)_2]^-$; an important ligand in the geochemical cycle of gold [56, 135, 156] and had a detection limit of 1.5 µg L^{-1} for the gold drug auranofin.

2.2 Study Scope

The determination of gold in groundwaters is an emerging technique in the field of mineral exploration. The analysis of such waters reduces the heavy reliance on costly drilling programs to identify target areas. The time consuming process of analysing a narrow cylinder of rock that represents a small area, is replaced with the analysis of series of groundwater samples that provide information about a larger geological area. The concentration of gold in groundwater is determined by ICP-MS, and has been used as pathfinder for gold mineralisation [103, 157]. These methods characterise the total gold concentration, and do not give information about the speciation. The ability to speciate gold will provide additional information about the geological conditions under which gold is mobilised. In addition, it will be useful for understanding bioremediation processes and such knowledge could also influence the choice and concentration of lixiviant used in gold leaching.

This chapter presents a HPLC-ICP-MS method for the speciation of Au(I)-thiosulfate, Au(I)-cyanide, Au(III)-chloro-hydroxyl complexes and gold(III)-bromo-hydroxyl complexes.

2.3 Experimental

2.3.1 Reagents and Standards

The chemicals used in this study are shown in Table 2-1, with ~18 MΩcm deionised water (Millipore, Australia) utilised for the preparation of aqueous solutions.

Table 2-1 The chemicals used in this study.

Chemical	Supplier	Grade	Concentration/Purity
Acetonitrile	Ajax Finechem	HPLC	99.7%
Disodium orthophosphate phosphate anhydrous powder	Scharlau	Analytical	>99.0%
Gold Standard in 2% HCl	Choice Analytical	Analytical	1000 mg L^{-1}
Hexadecyltrimethylammonium hydroxide solution (HDTMAOH)	Sigma-Aldrich	Reagent	10 wt. % in H_2O
High Purity HCl	Scharlau	Analytical	37%
Isopropanol	Merck	HPLC	Not stated
Methanol	Merck	HPLC	Not stated
Orthophosphoric acid	Ajax Finechem	Analytical	85%
Potassium gold(I) cyanide	Strem Chemicals	Reagent	99%
Sodium dihydrogen phosphate anhydrous	Chem-Supply	Analytical	>98%
Sodium gold(I) thiosulfate hydrate	Alfa Aesar	Reagent	99.9 % (35.69% Au)
Sodium hydroxide	Ajax Finechem	Analytical	>99%
Sodium tetrabromoaurate	Strem Chemicals	Reagent	99.9+ %
Tetrabutylammonium chloride hydrate (TBAC)	Sigma-Aldrich	Reagent	98%
Tetrabutylammonium hydroxide solution (TBAOH)	Sigma-Aldrich	Reagent	~40% in H_2O (~1.5 M)
Tetraethylammonium hydroxide solution (TEAOH)	Sigma-Aldrich	Reagent	~40% in H_2O
Tetramethylammonium hydroxide solution (TMAOH)	Sigma-Aldrich	Reagent	25 wt. % in H_2O
Tetrapropylammonium hydroxide solution (TPAOH)	Sigma-Aldrich	Reagent	1.0 M in H_2O
Triton™ X-100	Sigma-Aldrich	Analytical	100%

Preparation of the gold standards

Stock solutions (100 mg L^{-1} Au) of sodium tetrabromoaurate, sodium gold(I) thiosulfate, and potassium gold(I) cyanide were prepared gravimetrically in plastic by dissolving the appropriate amount in deionised water (Millipore, Australia) and stored in the dark to prevent the photolytic precipitation of gold [69]. The gold(I) cyanide stock solution was prepared in pH 12 (0.01 M) NaOH solution to prevent the generation of HCN gas. The stock solution of

gold(III) chloride (100 mg L^{-1} Au) was prepared by appropriate dilution of a 1000 mg L^{-1} gold ICP-MS Standard (in 2% HCl, Choice Analytical, Australia) with water. Unless otherwise specified, these gold stock solutions were diluted to the desired concentration (100 µg L^{-1}) with the mobile phase immediately prior use. The Au tune solution (1.0 µg L^{-1}) was diluted from a 1000 mg L^{-1} gold ICP-MS Standard (in 2% HCl, Choice Analytical, Australia) with the mobile phase.

Preparation of the HPLC eluents

All HPLC eluents were prepared from acetonitrile, isopropanol or methanol, sodium dihydrogen phosphate anhydrous, disodium hydrogen phosphate anhydrous powder, and various cationic ion-pairing agents: tetrabutylammonium phosphate and chloride, tetramethyl-, tetraethyl-, tetrapropyl-, tetrabutyl- and hexadecyltrimethylammonium hydroxide. All ion-pairing agents were obtained from Sigma-Aldrich. All were prepared by dissolving the ion-pairing agent in the appropriate amount of solvent, deionised water and buffer. pH values were measured using a pH 211 Microprocessor pH Meter (Hanna Instruments, Italy) and adjusted with either HCl, sodium hydroxide or phosphoric acid. HPLC solvents were filtered through a 0.45 µm nylon membrane (Grace Davison Discovery Sciences, U.S.) prior to use.

2.3.2 HPLC-ICP-MS set up

Chromatographic separations were achieved using an Agilent 1200 Series HPLC coupled with an Agilent 7500cx ICP-MS. During analysis the column was connected to the concentric nebulizer (0.4 mL min^{-1}) by means of PEEK capillary tubing (1 m x 0.1 mm ID) and a tee splitter. Operating conditions for the HPLC-ICP-MS are given in Table 2-2. A range of solvent systems were trialled before arriving at the final mobile phase composition shown in Table 2-2. In all cases, the column was pre-equilibrated with the desired mobile phase for at least half an hour before injection, and the ICP-MS was tuned with a 1.0 µg L^{-1} Au(III)-chloride solution made up in the mobile phase. A reduced bore size torch (1.5 mm ID) was employed to reduce plasma loading and improve plasma stability. Oxygen gas (20% O_2 in Ar) was mixed with the argon nebulizer gas via a T-connection after the spray chamber to sustain the plasma and to prevent carbon deposition on the sampler and skimmer cones of the ICP-MS interface [158].

Table 2-2 Final operational conditions for HPLC-ICP-MS.

HPLC	**Agilent 1200 Series**
Analytical Column	Agilent Zorbax Eclipse XDB (4.6 x 150 mm, 5 µm)
Mobile Phase	6: 17.5: 76.5 v/v/v isopropanol: acetonitrile: water, 1 mM TBAC, 5 mM NaH_2PO_4/Na_2HPO_4. (adjusted to pH 7.03 with H_3PO_4,)
Flow rate	1.0 mL min^{-1}
Injection volume	25 µL
Column Temperature	30 ºC
ICP-MS	**Agilent 7500cx**
Forward Power	1600 W
Plasma gas flow	Ar, 15 L min^{-1}
Auxillary gas flow	Ar, 0.9 L min^{-1}
Nebulizer gas flow	Ar, 0.8 L min^{-1}
Blend gas	20% O_2 in Ar, 12%
Torch sampling depth	10 mm
Mass monitored	Au^{197}
Acquisition mode	Time resolved analysis
Detection	Electron multiplier (pulse mode voltage 1070 V)
Integration time/mass	0.1 s
Peripump rate	0.24 rps
Spray chamber	- 5 º C

2.3.3 HPLC-ICP-MS procedure

After lighting the ICP-MS plasma and allowing it to stabilise for half an hour, the ICP-MS was tuned for gold. This was done by introducing the 1 µg L^{-1} Au tune solution into the ICP-MS through tygon peristaltic pump tubing (ID 1.02 mm, Ismatec, Switzerland) at a rate of 0.24 rps (0.8 ml $min^{-1)}$. The ICP-MS was tuned for gold counts (~6000 counts, <3 %RSD), by adjusting the torch horizontal and vertical sampling position. The flow rates of the carrier gas and make-up gas and the percentage of blend gas were set so that the optimal signal to noise was obtained (0.7 L min^{-1}, 0.3 L min^{-1}, and 12% respectively). To return the background counts back to baseline levels, the instrument was washed with 2% HCl in 0.1% Triton™ X-100 rinse solution, and then water. The HPLC column and ICP-MS nebuliser were then connected with a PEEK tubing (1 m x 0.1 mm i.d.), and the two instruments were also connected with a remote start cable. The mobile phase was pumped through the ICP-MS for at least half an hour before commencing analysis. Raw data was initially recorded using the

Agilent MassHunter Workstation Software (with MassHunter Chromotographic software), then extracted into Origin Pro v. 7.5 for analysis and presentation purposes. The data is presented qualitatively in y-offset chromatograms and reported as counts at peak maximum.

2.3.4 Modelling Studies

The Geochemist's Workbench software (RockWare®,USA) [125] was used to construct log-log activity gold speciation diagrams. Thermodynamic properties were taken from the Lawrence Livermore National Laboratory database (version R9), with properties for Au complexes from Usher *et al.* [84].

2.4 Results and Discussion

2.4.1 Preliminary work

Initial HPLC conditions were based on those of Zhao *et al.* [135] who separated gold-based drugs auranofin, myochrysine and their Au(I) metabolites using a C18 column and a methanol: ammonium formate mobile phase containing tetrabutylammonium chloride (TBAC) as an ion-pairing agent. Our initial studies employed a methanol: buffer mobile phase. The buffer comprised of 10 mM TBAC and 10 mM $NH_4H_2PO_4$ prepared in 50:50 v/v methanol: water. The first gold complexes analysed were individual standards (~100 µg L^{-1}) of Au(III)-chloride, Au(I)-thiosulfate and Au(I)-cyanide, however, the high methanol concentration in the mobile phase reduced the plasma stability, often extinguishing the plasma. Consequently, the organic solvent was changed to acetonitrile. This solvent has a stronger eluting power than methanol and thus, could be used at lower concentrations whilst retaining separation [159]. Incorporation of a 30 % v/v acetonitrile mobile phase resulted in a stable plasma.

The ion-pairing agent, buffer composition, buffer concentration and organic modifier were systematically examined in order to develop the separation conditions for mine water samples. Unless otherwise stated, the optimisation was undertaken by evaluating individual solutions made from each of the Au(III)-bromide, Au(III)-chloride, Au(I)-thiosulfate and Au(I)-cyanide standards ([Au] = 100 µg L^{-1}). This was done to avoid interactions that may occur between the different species, which would affect the interpretation of the overall results.

2.4.2 Choice of ion-pairing agent

The hydrophobicity of an ion-pairing agent is related to the length of its alkyl chain, and influences the retention of the ionic compounds [135, 160]. As such, a range of alkyl ammonium ion-pairing agents of varying alkyl chain lengths (listed in Table 2-3) were investigated for their effect on the retention times of the various Au(III)-chloro-hydroxl, Au(III)-bromo-hydroxyl, Au(I)-thiosulfate and Au(I)-cyanide species. To keep the counter-ion consistent for this study, alkyl ammonium hydroxide ion-pairing agents were chosen due to their availability in varying sizes. This study was undertaken in the absence of buffer using 30: 70 v/v acetonitrile: water mobile phase with 1 mM ion-pairing agent. The resulting mobile phases had a high pH due to the hydroxide counter-ion from the ion-pairing agents, so the pH was adjusted to 7 with HCl. We chose not to have a buffer in an effort to reduce the complexity of the mobile phase.

Table 2-3 The tetra-ammonium-hydroxide ion-pairing agents investigated.

Ion-pairing agent name	Formula
Tetramethylammonium hydroxide (TMAOH)	$(CH_3)_4NOH$
Tetraethylammonium hydroxide (TEAOH)	$(C_2H_5)_4NOH$
Tetrapropylammonium hydroxide (TPAOH)	$(C_3H_7)_4NOH$
Tetrabutylammonium hydroxide (TBAOH)	$(C_4H_9)_4NOH$
Hexadecyltrimethylammonium hydroxide (HDTMAOH)	$C_{16}H_{33}(CH_3)_3NOH$

As can be seen in Figure 2-1, the use of the smaller ion-pairing agents (TMAOH, TEAOH and TPAOH), resulted in poor retention of the gold complexes (all elute within 2 minutes), whilst TBAOH gave good separation of the Au(III)-chloride, Au(III)-bromide and Au(I)-cyanide complexes. Au(I)-thiosulfate did not elute within 15 minutes. The superior performance of TBAOH is likely to be due to a change in the separation mechanism from ion-pairing formation in the mobile phase to dynamic ion-exchange, i.e. solute ions binding to ion-pairing agents already bound to the stationary phase [160]. This change in mechanism results from the ion-pairing agent's increased hydrophobicity and increased attraction of the ion-pairing agent to the stationary phase along with decreased binding between the tetrabutylammonium salt and the solute ion. In addition to being able to separate the various complexes, the use of TBAOH was found to result in five peaks for the Au(III) halide complexes. These are ascribed to the expected mixed Au(III) halide-hydroxide complexes, $[AuX_{(4-n)}(OH)_n]^-$ (where X = Cl, Br and n=0–4). Figure 2-2 shows the effect of increasing the ion-pair alkyl chain length on the retention of the gold cyanide complex, $[AuCN_2]^-$. The retention time of $Au(CN)_2^-$ with the 50:50 TPAOH:TBAOH mobile phase confirmed Horvath *et al.*'s [160] finding that a mixture of different ion-pair agents has the same capacity factor as an ion-pair agent with the same mean chain length.

Given that TBAOH is a strong base and unsuitable for HPLC columns without significant adjustment to the pH of the mobile phase, it was decided to investigate the suitability of other tetrabutylammonium ion-pairing agents as a replacement. Similar separations were achieved with TBAP and TBAC, however, TBAP is extremely hygroscopic and this affected the reproducibility of mass weighed out and therefore, the separations. Hence, TBAC was chosen as it was cheap, readily available and avoided the difficulties with working with TBAP. Although 1 mM Cl^- ion from the TBAC may affect the speciation of the Au(III)-halide complexes in samples with lower chloride concentrations, typical samples of interest will contain an excess of chloride. According to Gray [161], salt solutions with soluble gold (Au > 10^{-6} M) contain at least 10 mM Cl^- or 100 mM Cl^- (at pH 0 – 3 and pH 0 – 5.5 respectively). Moreover, typical Australian brines contain ~2400 to 5300 mM of chloride [162] (The Cl^- concentrations in the water samples are shown in Section 3.4.4). At these high concentrations of chloride, the speciation of gold will be unaffected by the use of TBAC.

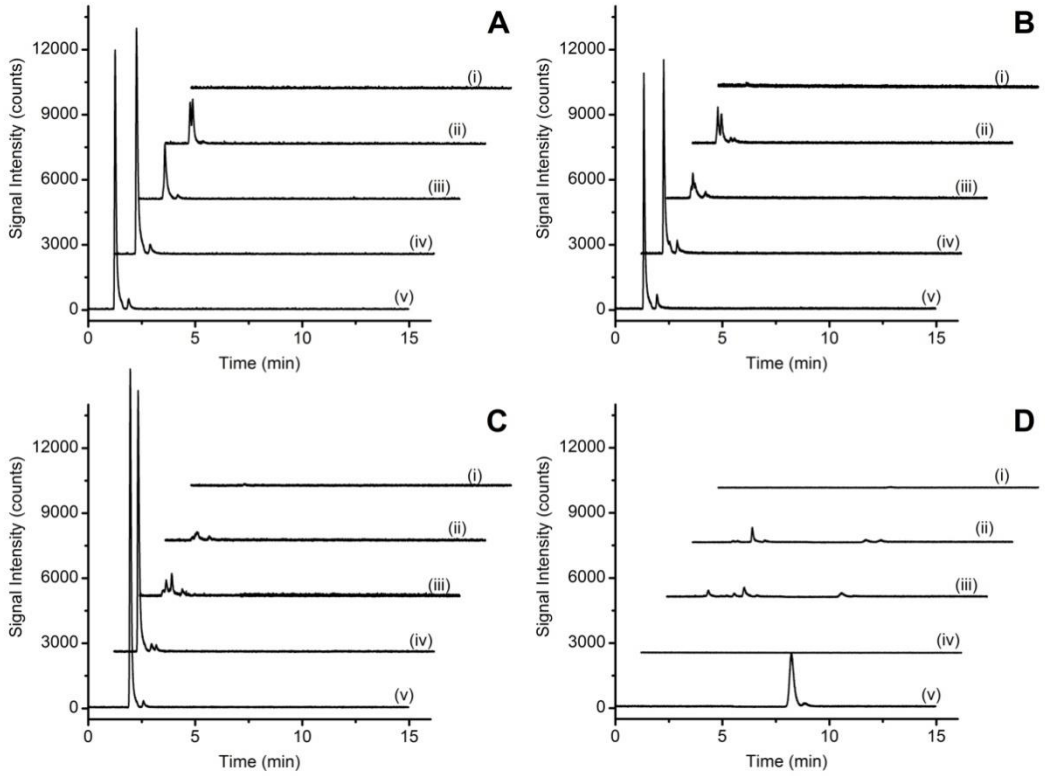

Figure 2-1 **Effect of ion-pairing agent's alkyl chain length on gold signal and separation.**

Chromatograms show (i) Blank (ii) Au(III)-chloride, (iii) Au(III)-bromide, (iv) Au(I)-thiosulfate and (v) Au(I)-cyanide; Mobile phase: 30:70 v/v acetonitrile:water, pH 7 (adjusted with HCl), 1 mM ion-pairing agent (A) TMAOH, (B) TEAOH, (C) TPAOH, (D) TBAOH.

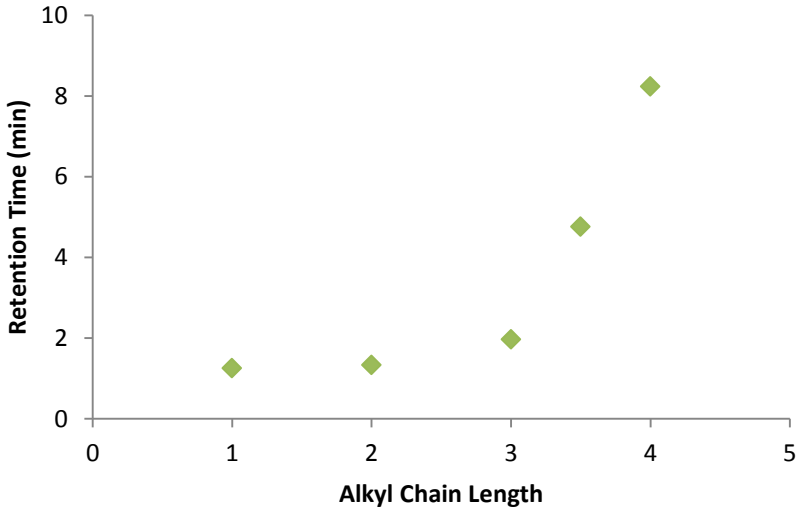

Figure 2-2 **Effect of ion-pairing agent alkyl chain length on $[Au(CN)_2]^-$ retention time.**

Mobile phase: 30:70 v/v acetonitrile:water, pH 7 (adjusted with HCl), 1 mM ion-pairing agent (TMAOH, TEAOH, TPAOH, TBAOH). The "3.5" carbon ion-pairing agent is a 50:50 v/v mixture of TPAOH and TBAOH.

2.4.3 Choice of buffer

As demonstrated by Usher *et al.* [84], Au(III)-halides are generally expected to exist in aqueous solution as a mixture of Au(III)-halide-hydroxide complexes $[AuX_{(4-n)}(OH)_n]^-$, (where X = Cl or Br and n = 0–4) with the exact speciation changing with pH and halide ion concentration, however, this mixture of complexes was not observed when a $NH_4H_2PO_4$ buffer was used in the mobile phase. Figure 2-3 shows chromatograms of Au(III)-chloride and Au(III)-bromide from early work trialling a methanol organic modifier, TBAP ion pairing agent and $NH_4H_2PO_4$ buffer. The broad, overlapping peaks in Figure 2-3, suggests the formation of a single dominant complex. Such complex could be the $Au(NH_3)_4^{3+}$ ion (arising from the $NH_4H_2PO_4$ buffer), which is known to be very stable [163]. To improve the separation of the Au(III)-halides, the suitability of the $NH_4H_2PO_4$ buffer used by Zhao *et al.* [135] was accessed by modelling the speciation of Au(III)-complexes with Geochemist's Workbench (GWB).

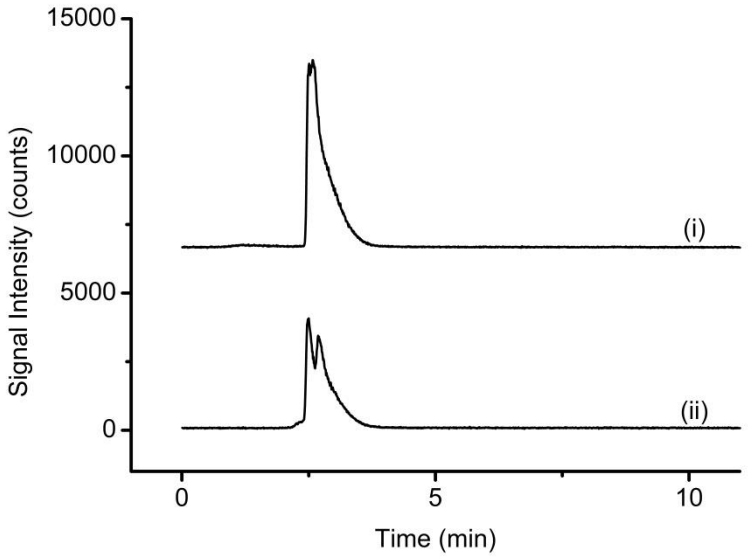

Figure 2-3 **A comparison of the Au(III)-halides in an ammonium phosphate buffer.**
HPLC-ICP-MS chromatograms show (i) Au(III)-chloride, (i) Au(III)-bromide in mobile phase: 35: 65 v/v methanol: water, 1 mM TBAP, 5 mM $NH_4H_2PO_4$.

GWB was used to calculate the log-log activity-activity diagram describing the speciation of Au(III) in the presence of Cl^- and NH_3 ligands at pH 7 (Figure 2-4 (A)). At [Au] = 10^{-9} M, (the concentration of gold as it elutes from the column) and at the pH and buffer concentrations (NH_3 activity >> 10^{-6}) used throughout this study, chloride and hydroxide ligands are readily exchanged with amine ligands to produce $Au(NH_3)_4^{3+}$ as the dominant species in solution. This effect was similar for bromide, as shown in Figure 2-4 (B). Given that both Au(III) salts form $Au(NH_3)_4^{3+}$, when mixed with the ammonium phosphate buffer, it was impossible to identify which species (chloride or bromide) was originally in solution. Therefore, an

alternative buffer system without the amine ligand was required. There is no reported evidence for Au(III) inorganic phosphate complexation; as such phosphate was chosen as an alternative buffer. In Figure 2-4 (C) and (D), Au(III) is modelled with $H_2PO_4^-$ ions to simulate the speciation of Au-chloride and bromide in a sodium dihydrogen phosphate buffer. As can be seen, four Au(III) halide species ($[Au(OH)_4]^-$, $[Au(OH)_3X]^-$, $[Au(OH)_2X_2]^-$ and $[Au(OH)X_3]^-$ (where X= Cl^- or Br^-)) are expected across the sodium dihydrogen phosphate concentrations modelled. The distribution of the various gold halide hydroxyl species is different for chloride and bromide and suggests that it will be possible to determine which species (chloride or bromide) was originally in solution. Au(III) and halide ions were also modelled in water (Figure 2-4 (E) and (F)) to confirm that the Au(III)-halide-hydroxyl species resulting from the sodium dihydrogen phosphate buffers are typical of the Au(III) species expected to exist in water. As this was the case, the ammonium phosphate buffer used by Zhao *et al.* [135] was replaced with a sodium dihydrogen phosphate buffer to avoid the formation of $Au(NH_3)_4^{3+}$ ion.

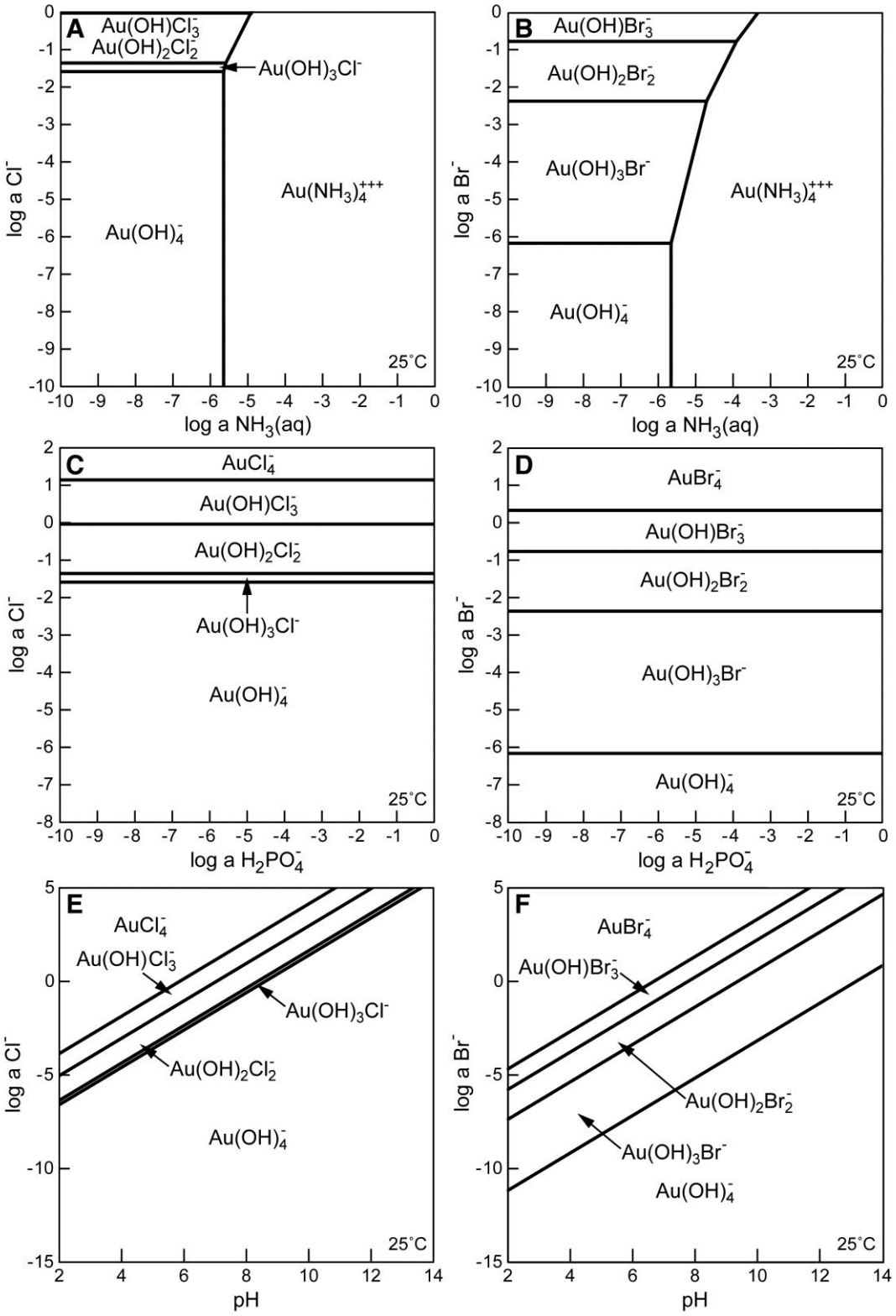

Figure 2-4 **Log-log activity-activity speciation diagram of Au(III)-halide species.**

Shown are Au(III) ions in the presence of NH_3 and (A) Cl^- ligands, (B) Br^- ligands; Au(III) in the presence of $H_2PO_4^-$ and (C) Cl^- ligands, (D) Br^- ligands at pH = 7; Au(III) in water and (E) Cl^- ligands; (F) Br^- ligands; Log a[Au+++] = 10^{-9}, T = 25 º C, P = 1.013 bars, a[H_2O] = 1.

2.4.4 pH effect

The effect of the pH of the buffer was investigated by varying the buffer pH between 6.5 and 7.5 whilst maintaining the acetonitrile content at 30% and the tetrabutylammonium ion pairing agent at 1 mM. The buffer concentration was maintained at 2.5 mM throughout. Overall, the effect of increasing the pH of the mobile phase from pH 6.5 to pH 7.5 resulted in decreased retention times (especially for the Au(I)-complexes Figure 2-5 (A), (B) and (C)) and increased peak intensities for the Au(III)-complexes. Figure 2-5 (A) and (C) shows the retention time of Au-thiosulfate decreased from 7.2 minutes to 3.4 minutes, whilst the Au-cyanide decreased from 8.5 minutes to 6.0 minutes. At the same time, the peak intensity of the Au(III) peaks increased with the increase of pH. From pH 6.5 – 7.5, the dominant Au-chloride and Au-bromide peaks increased from 730 counts and 189 counts to 2330 counts and 1475 counts respectively. The number of Au-chloride peaks also change with the variation in mobile phase pH. The two broad Au-chloride peaks at pH 6.5 increased to four peaks at pH 7, as the co-eluting compounds were separated. On increasing the pH from 7 to 7.5, there is a decrease in the number of Au-chloride species from four peaks to three peaks, due to the decreased retention times and co-elution (this is consistent with changes in H^+ affecting the Au-chloro-hydroxyl speciation as shown in Figure 2-4). Therefore, a pH of 7 was chosen for the remainder of the optimisation as a compromise between increased peak intensity and the number of resolved Au-chloride peaks.

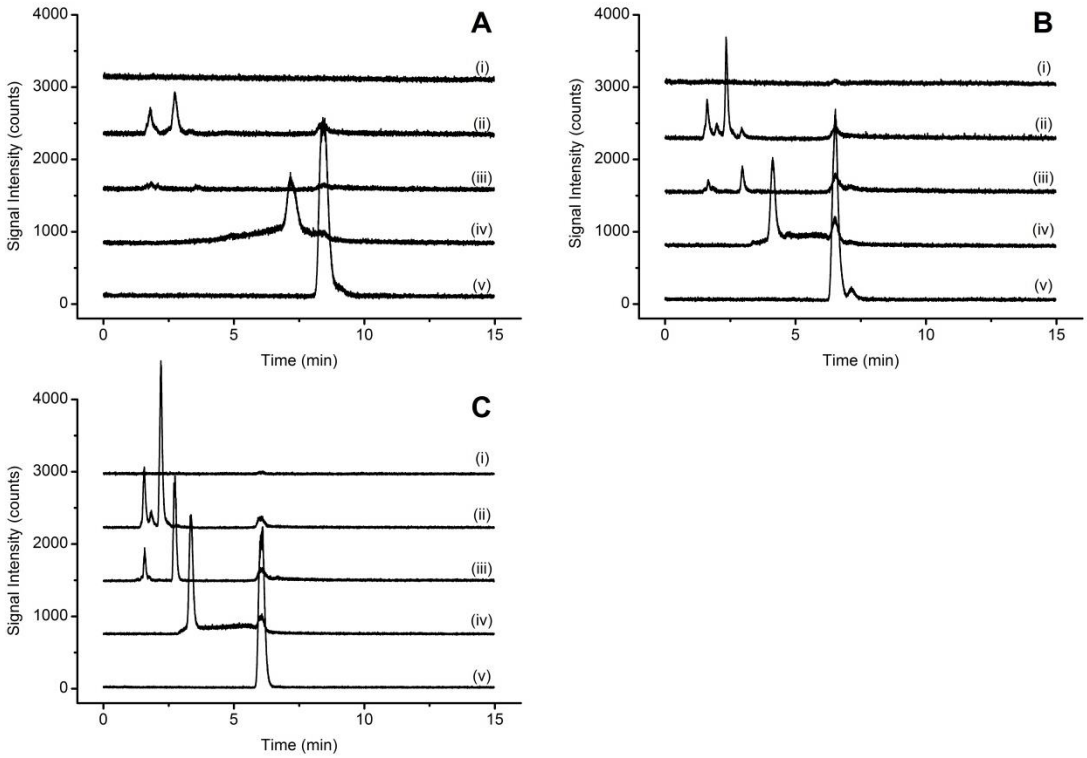

Figure 2-5 **Effect of pH on the signal and retention times on the gold species.**
Chromatograms show (i) Blank (ii) Au(III)-chloride, (iii) Au(III)-bromide, (iv) Au(I)-thiosulfate and (v) Au(I)-cyanide; Mobile phase: 30:70 v/v acetonitrile:water, 1 mM TBAP, 2.5 mM NaH_2PO_4/Na_2HPO_4 (A) pH 6.5; (B) pH 7; (C) pH 7.5.

2.4.5 Phosphate buffer effect

The effect of the phosphate concentration in the buffer was investigated by varying its concentration whilst maintaining the acetonitrile content at 30% and the tetrabutyl ammonium ion pair agent at 1 mM and the pH at 7. When compared to the chromatogram obtained in the absence of phosphate (Figure 2-6 (A)), the incorporation of a 2 mM phosphate buffer resulted in a broad peak for Au(I)-thiosulfate at approximately 9 minutes, as well as decreasing the retention time for the Au(III)-halide and Au(I)-cyanide complexes (Figure 2-6 (B)). Increasing the buffer concentration to 5 mM and 10 mM further decreased the retention time for all four Au species, with some loss of resolution between Au(III)-bromide and Au(I)-thiosulfate at 10 mM phosphate concentrations. Consequently, 5 mM phosphate buffer was chosen for all further work.

Given the dramatic change in retention time for Au(I)-thiosulfate, in the presence of phosphate buffer, it was decided to model Au(I) speciation in the presence of thiosulfate and dihydrogen phosphate ions (as shown in Figure 2-7 (A)). As can be seen, increasing the phosphate concentration (going from left to right) results in no change in Au(I)-thiosulfate speciation. This suggests the dihydrogen phosphate ions are acting as a competing ion. At low phosphate concentrations the Au(I)-thiosulfate with its high 3^- charge is tightly bound to the column. Increasing the concentration of competing ions shifts the equilibrium to elute the Au(I)-thiosulfate anion in an ion-exchange mechanism.

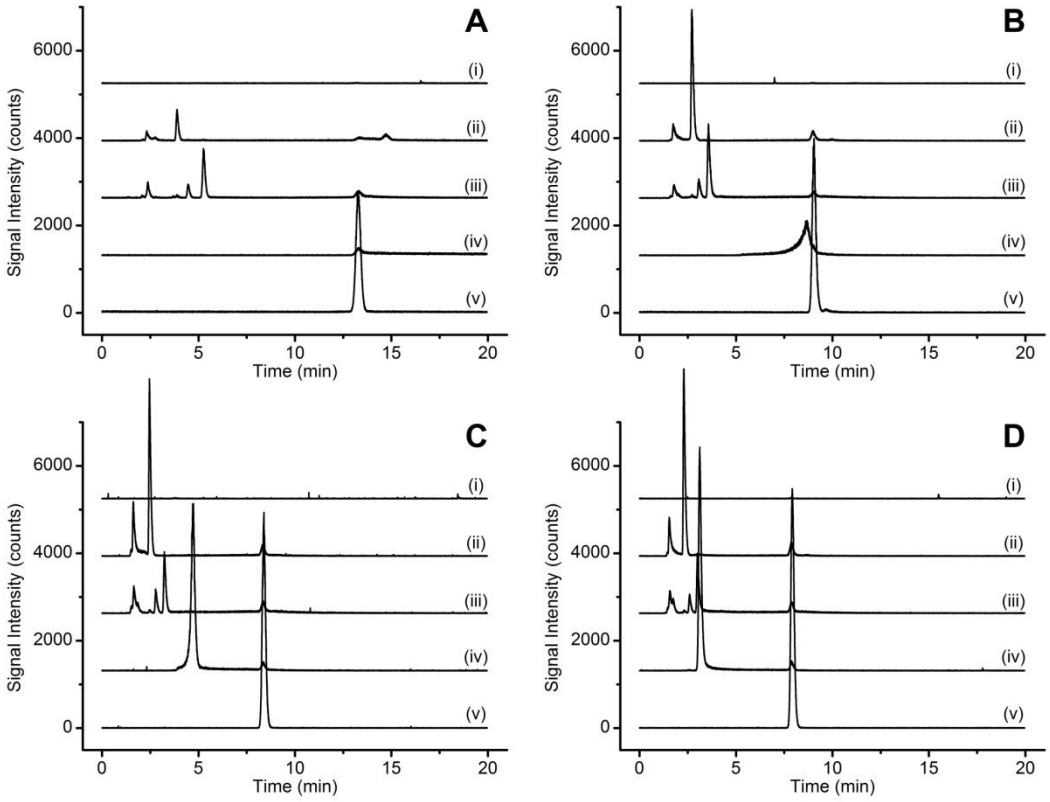

Figure 2-6 **Effect of phosphate buffer concentration on gold signal and separation.**

Chromatograms show (i) Blank (ii) Au(III)-chloride, (iii) Au(III)-bromide, (iv) Au(I)-thiosulfate and (v) Au(I)-cyanide; Mobile phase: 30:70 v/v acetonitrile:water, 1 mM TBAC, pH 7 (A) adjusted with NaOH; (B) 2 mM NaH_2PO_4/Na_2HPO_4, (C) 5 mM NaH_2PO_4/Na_2HPO_4; (D) 10 mM NaH_2PO_4/Na_2HPO_4, (pH adjusted with H_3PO_4).

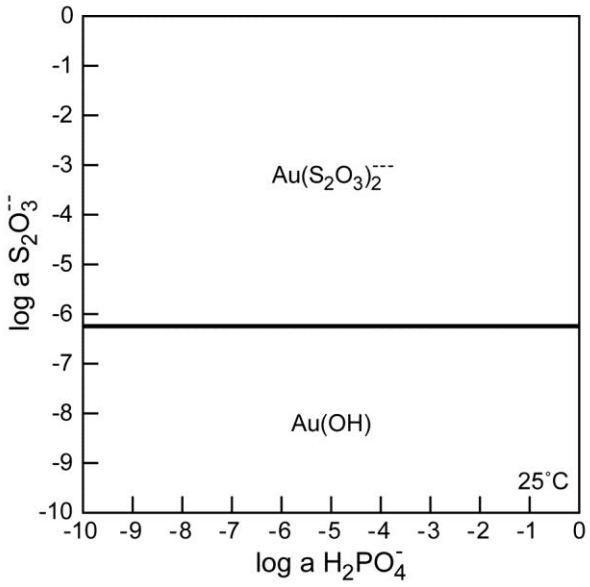

Figure 2-7 **Log-log activity-activity speciation diagram of Au(I) with $H_2PO_4^-$ and $S_2O_3^{2-}$ ions.**

Log a[Au+] = 10^{-9}, T = 25 ºC, P = 1.013 bars, a[H_2O] = 1, pH = 7.

2.4.6 Choice of organic modifier

Although the Au species appear to be well separated with the 5 mM phosphate buffer, it was observed that some of the gold appears to react with the acetonitrile in the mobile phase to form a small peak due to a complex that elutes at the same time as Au(I)-cyanide as shown in Figure 2-8. It is thought that this is likely to be due to the reaction of the gold species with cyanide impurities that can be found in acetonitrile [164]. Throughout our studies, it was observed that the purity of the acetonitrile significantly affected the formation of this peak, with some sources resulting in much larger intensities of this peak. Consequently, it was decided to investigate the use of isopropanol as an alternate organic modifier. Unlike the dipolar acetonitrile, isopropanol is considered a more acidic solvent [165] and possesses a greater elutropic strength [159], however, separations obtained when substituting various concentrations of isopropanol for acetonitrile were poor. Whilst the separations were poorer, it was observed that the resulting ICP-MS signal increased with the reduced organic content of the mobile phase. In addition, when using isopropanol as the organic modifier, the extra peak due to the reaction of gold complexes with cyanide impurities was not observed. Therefore, it was decided to investigate the use of a mixed isopropanol-acetonitrile organic modifier.

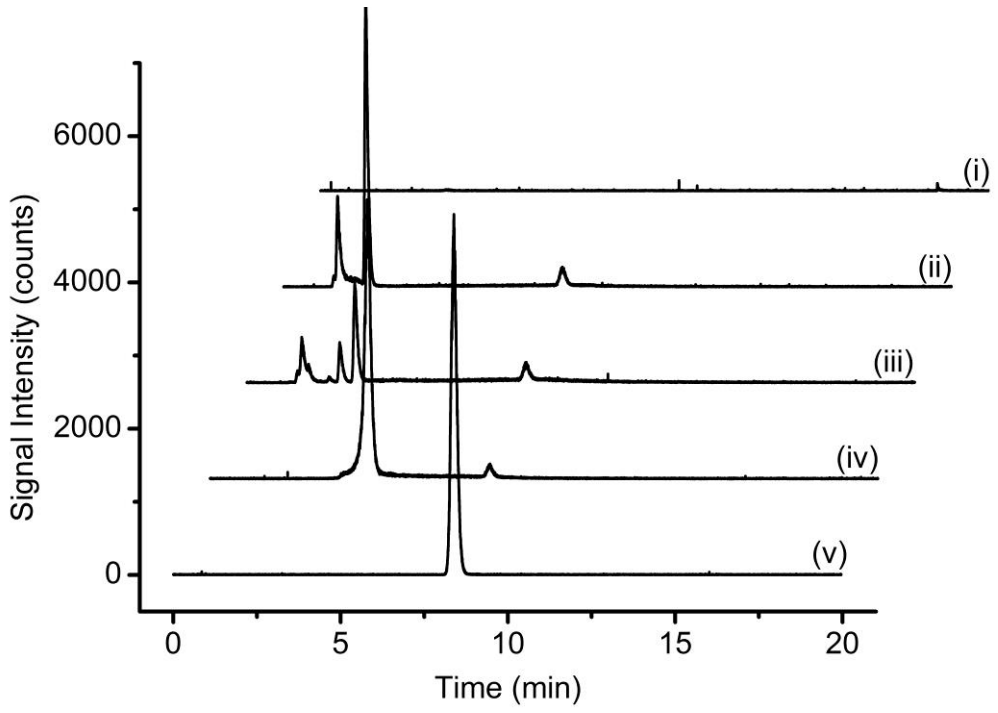

Figure 2-8 **Effects of cyanide impurities in acetonitrile on the gold species.**
Chromatograms of (i) Blank (ii) Au(III)-chloride, (iii) Au(III)-bromide, (iv) Au(I)-thiosulfate and (v) Au(I)-cyanide; Mobile phase: 30:70 v/v acetonitrile: water, 1 mM TBAC, 5 mM NaH_2PO_4/Na_2HPO_4; pH 7. Peak at 8 minutes is an Au-cyanide complex from acetonitrile.

2.4.7 Isopropanol-acetonitrile system

Use of a 10: 10: 80 v/v isopropanol: acetonitrile: buffer system (Figure 2-9 (B)) resulted in an immediate improvement to the peak shape and intensity when compared with using just isopropanol (Figure 2-9 (A)), however this solvent system was too strong with the Au(III)-halides and Au(I)-thiosulfate all eluting before 3 minutes. Lowering the solvent content to 5: 20: 75 v/v isopropanol: acetonitrile: buffer improved the separation, however, it decreased the peak intensities of the gold chloride complexes with the gold bromide peaks disappearing completely, as shown in Figure 2-9 (D) (most likely due to peak broadening). Consequently, a mobile phase composition of 6: 17.5: 76.5 v/v isopropanol: acetonitrile: buffer (Figure 2-9 (C)) was chosen as the optimal. Under these conditions Au(III)-halide-hydroxide complexes are resolved, and the production of $[Au(CN_2)]^-$ from impurities in the acetonitrile is reduced. Furthermore, the reduced organic content of this mobile phase resulted in a greater signal-to-noise ratio for the final isopropanol-acetonitrile solvent system when compared to the previous acetonitrile mobile phase (Figure 2-8).

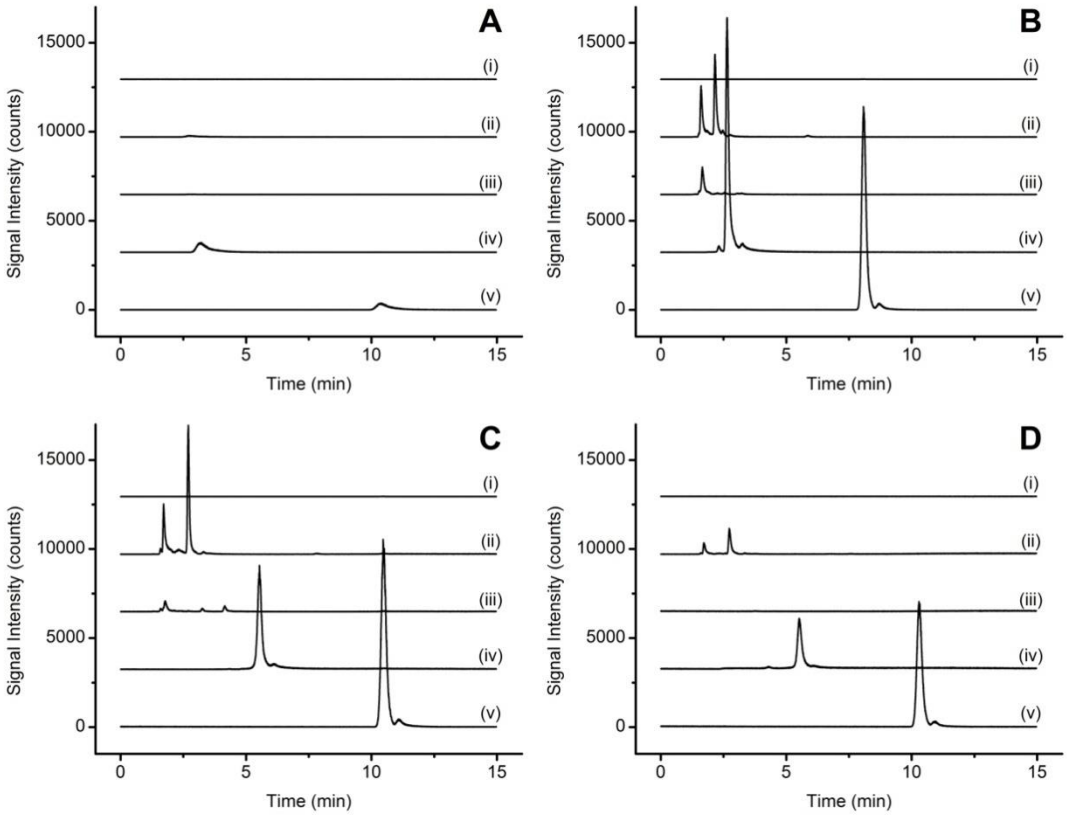

Figure 2-9 **Effect of isopropanol/acetonitrile on the gold signal and separation.**

Chromatograms show (i) Blank (ii) Au(III)-chloride, (iii) Au(III)-bromide, (iv) Au(I)-thiosulfate and (v) Au(I)-cyanide; Mobile phase: 1 mM TBAC, 5 mM NaH_2PO_4/Na_2HPO_4, pH 7 (pH adjusted with H_3PO_4), and (A) 10% isopropanol, (B) 10:10:80 v/v/v isopropanol: acetonitrile: water, (C) 6: 17.5: 76.5 v/v isopropanol: acetonitrile: water, (D) 5: 20: 75 v/v/v isopropanol: acetonitrile: water.

The exception to this was the Au(III)-bromide complex which had reduced signal-to-noise in the mixed solvent system. The differences in signal magnitude between the four gold complexes were investigated by analysing the complexes with and without the LC column. The resultant peak areas in Table 2-4 show that the Au(III)-bromide signal is consistently lower than the other complexes regardless of whether or not a column is used. This suggests that the reduced signal-to-noise is a result of the incomplete ionisation of Au(III)-bromide in the plasma with the isopropanol-acetonitrile solvent system. Furthermore, it is clear that under these conditions, Au(I)-bromide and Au(III)-thiosulfate are not entirely recovered with mass balances of approximately 70%.

Table 2-4 Mass balance recovery of gold standards (n=1).

	Peak Area (counts.min)	**Peak Area without column (counts.min)**	**Recovery**
Au-chloride	1137	1281	89 %
Au-bromide	604.8	880.8	69 %
Au-thiosulfate	1145	1674	68 %
Au-cyanide	1852	1919	98 %

2.4.8 Application of method to model solutions

It is well documented that gold complexes are labile in solution, with the overall speciation being influenced by the solution pH and ionic composition of the aqueous environment. For example Au(III)-chloride and Au(III)–bromide can be readily hydrolysed to form mixed halide complexes [84]. Previous spectroscopy studies have monitored the hydrolysis of Au(III)-chloride [81, 83, 166] and the exchange of chloride and bromide ligands in Au(III)-complexes [84]. The gold concentrations used in UV-VIS studies ranged from ~10^{-4} M to ~10^{-3} M [83, 84, 166], and ~10^{-3} M to ~10^{-2} M for the Raman studies [81, 83]. In this section, the developed HPLC-ICP-MS method was used to monitor the stability of Au(III)-chloride, Au(III)-bromide, Au(I)-thiosulfate and Au(I)-cyanide over time and observe any hydrolysis effects. The different gold complexes were also mixed together to observe any ligand exchange that may occur between the Au-complexes. The gold concentrations in this study were at lower and more environmentally relevant concentrations at 100 μg L^{-1} (~10^{-7} M).

Application to monitoring gold hydrolysis

During method development, all of the gold standards were diluted from their stock solutions in the half hour before beginning the HPLC-ICP-MS analysis, however, when the gold standards were analysed a day later, there was a noticeable change in the peak ratios in the Au-chloride chromatogram. This aging effect on the complexation of a Au-chloride standard was also observed by Lee and Gavriilidis [88], who monitored the hydrolysis of $[AuCl_4]^-$ with UV-VIS over 720 minutes. At pH 5, 7, 9 and 11, the authors suggested the initial dominant complexes were $AuCl_2(H_2O)OH$, $[AuCl_2(OH)_2]^-$, $[AuCl(OH)_3]^-$ and $[Au(OH)_4]^-$ respectively, while the dominant complexes at 720 minutes could be $[AuCl_2(OH)_2]^-$, $[AuCl(OH)_3]^-$, $[Au(OH)_4]^-$ at pH 9 and $[Au(OH)_4]^-$ at pH 11 [88].

To assess any possible changes in speciation over time, freshly prepared standards of Au(III)-chloride, Au(III)-bromide, Au(I)-thiosulfate and Au(I)-cyanide (diluted in water to ~100 mg L^{-1}) were monitored over time with HPLC-ICP-MS. The Au(I)-thiosulfate and Au(I)-cyanide standards were sampled every 60 minutes over 480 minutes. The resulting chromatograms show that the speciation of Au(I)-thiosulfate and Au(I)-cyanide remain unchanged over time, and the peak height plots do not vary significantly (Appendix A). However, a significant change in speciation was observed with the Au(III)-chloride standard (Figure 2-10), where, over time, the number of Au(III)-chloride peaks reduced from five peaks (t = 0 minutes) to two peaks (t = 480 minutes).

This process can be explained by the dilution of the 1000 mg L^{-1} Au stock in 2% HCl, where there is a low pH (approx. pH 0) and sufficient Cl^- ligands (5.5×10^{-1} M) from the 2% HCl to stabilise the $[AuCl_4]^-$ complex, as shown in Figure 2-4 (E). Upon dilution of the original stock to form the 100 mg L^{-1} standard, the concentration of the chloride ligands decreases and allows the formation of the other $[AuCl_{(4-n)}(OH)_n]^-$ complexes, where n = 0–4, as shown in the first injection in Figure 2-10. The rate of change in speciation is quite rapid: initially there were five peaks where the largest peak eluted at 1.7 minutes. An hour after the first injection, the $[AuCl_4]^-$ peak at 2.7 minutes was significantly reduced. Two hours after the first injection the dominant species is no longer the $[AuCl(OH)_3]^-$ complex (large peak at 1.7 minutes), instead, the dominant complex is represented by the peak at 1.6 minutes corresponding to $[Au(OH)_4]^-$.

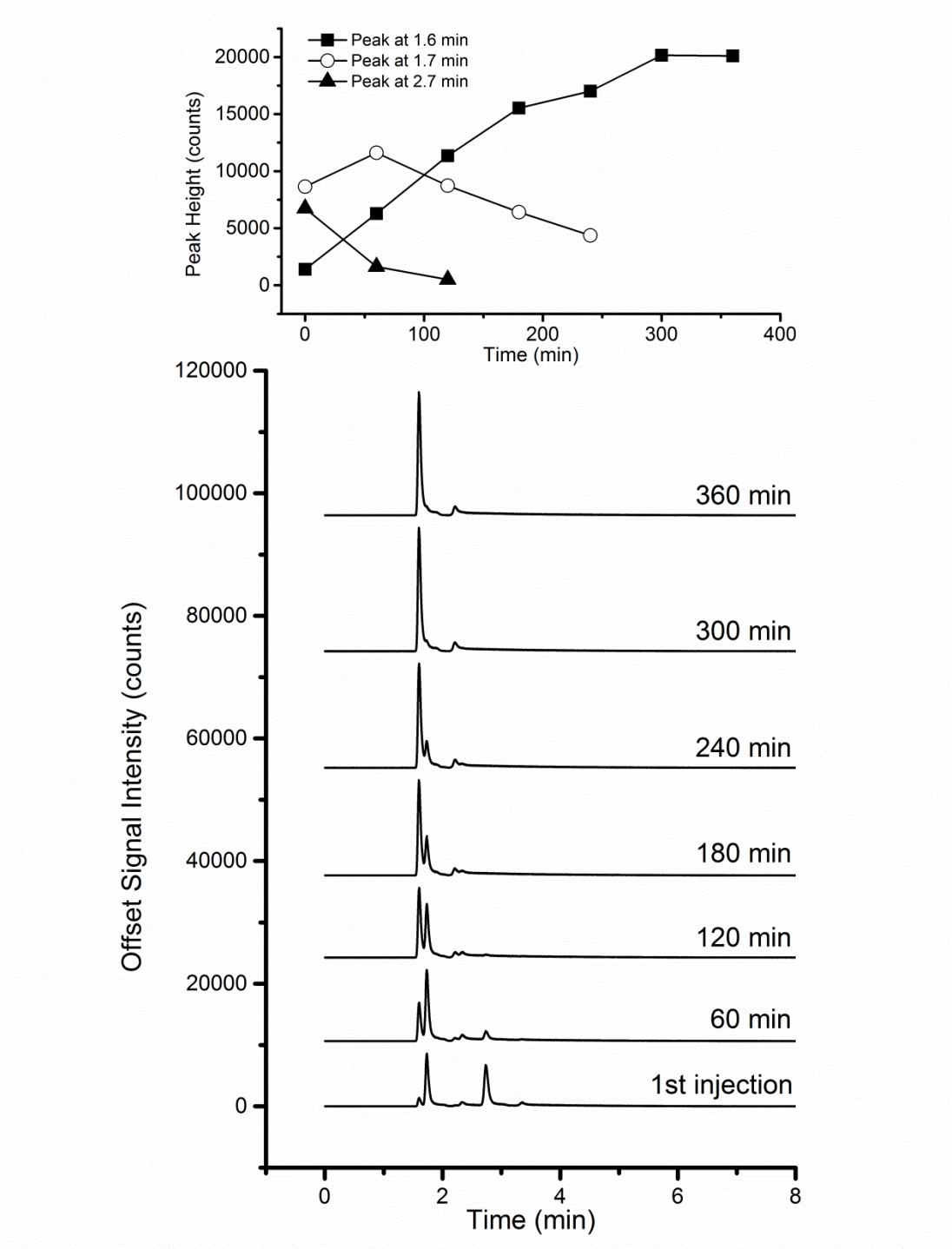

Figure 2-10 **Effect of dilution and aging time on Au(III)-chloride stability in water.**

Chromatograms show injections of the standard spaced 60 minutes apart with an inserted plot of peak heights over time. The chromatograms are y-offset at increasing intervals beginning at ~10600 counts. Mobile phase: 6: 17.5 v/v isopropanol: acetonitrile, 1 mM TBAC, 5 mM NaH_2PO_4/Na_2HPO_4, pH 7.

Over time the $[Au(OH)_4]^-$ peak continues to increase in intensity as the equilibrium between the Au(III)-chloride complexes shifts towards the formation of the hydroxide complex (Figure 2-10). Although Lee and Gavriilidis [88] prepared their standards differently (higher gold concentration, 2.5×10^{-3} M $HAuCl_4$, and pH adjusted with 0.1 M Na_2CO_3), they also observed an equilibrium shift towards the formation of the hydroxide complexes at pH 5 – 11. Similarly to their study, the equilibrium shift toward $[AuOH]^-$ dominance occurs over hours. As expected, the Au(III)-bromide standard also underwent similar changes in speciation towards the formation of the hydroxide complexes (Figure 2-11).

The hydrolysis of Au(III)-chloride was found to occur at a slower rate when the standard is prepared in mobile phase instead of water. A comparison of the peak intensities in Figure 2-10 and Figure 2-12 shows a slower decrease in intensity, in the latter, for the peaks at 1.7 minutes and 2.7 minutes. This slower rate of hydrolysis is due to a stabilising effect from the phosphate buffer in the mobile phase. Also the presence of the TBAC ion-pairing agent stabilises the $[AuCl_4]^-$ complex, by providing additional chloride ligands to the matrix. Therefore, instead of $[AuCl(OH)_3]^-$ being the initial dominant complex (when the standard was prepared in water), in a mobile phase matrix, the initial dominant complex was the $[AuCl_3OH]^-$ complex (the peak at 2.7 minutes).

This study showed that changes to the speciation of gold halide complex may occur from dilution, and that groundwater samples should be analysed without dilution where possible. In terms of preparing standards for quantification, differences between a gold standard matrix and the sample matrix may result in differing gold species. Therefore, matrix matching via multiple standard additions is recommended for quantifying gold species. Additionally, the samples should be left overnight after spiking with gold, to allow the speciation of gold to equilibrate.

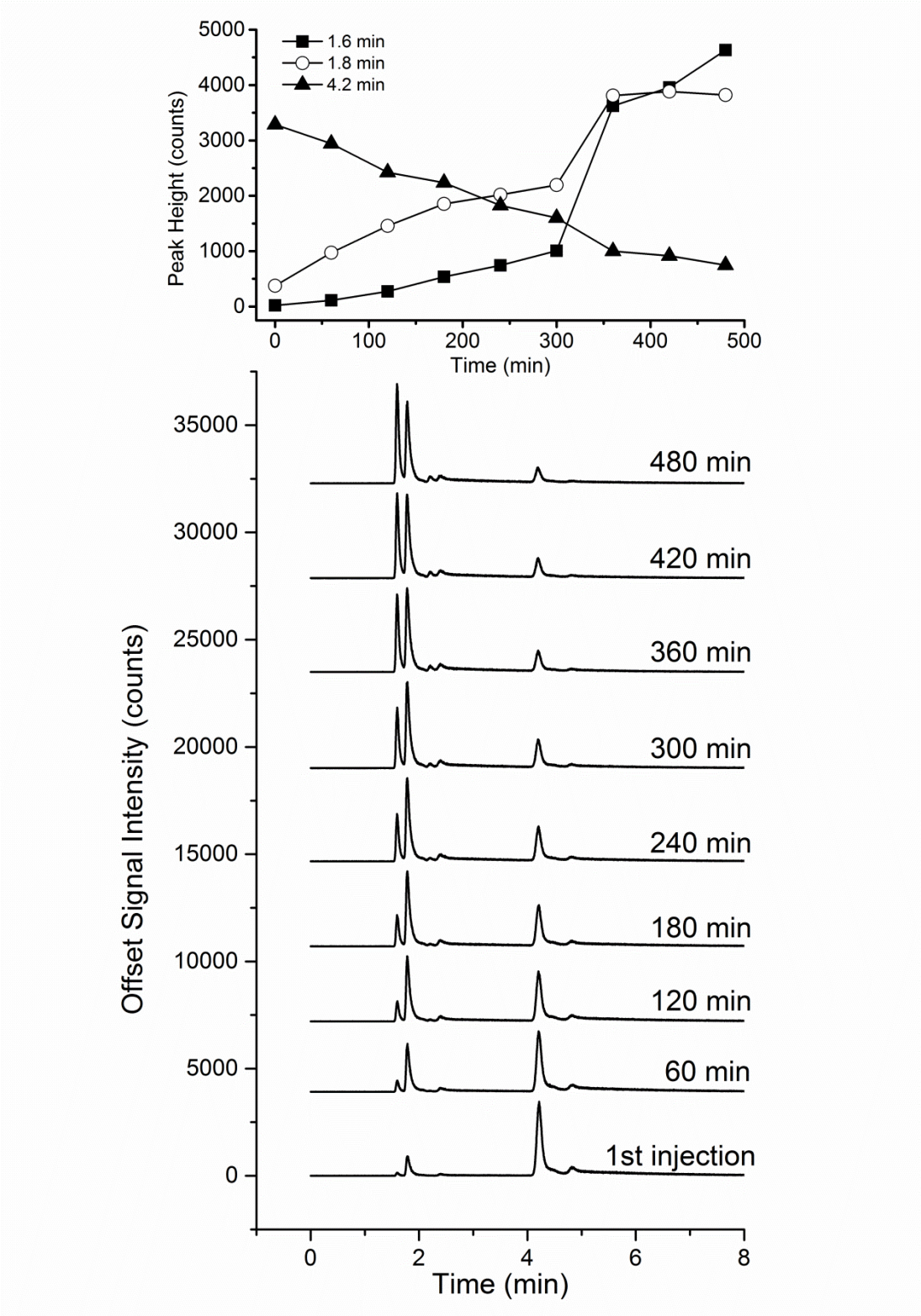

Figure 2-11 **Effect of dilution and aging time on Au(III)-bromide stability in water.**

Chromatograms show injections of the standard spaced 60 minutes apart; Chromatograms are y-offset at intervals of ~3500 counts; Mobile phase: 6: 17.5 v/v isopropanol: acetonitrile, 1 mM TBAC, 5 mM NaH_2PO_4/Na_2HPO_4, pH 7.

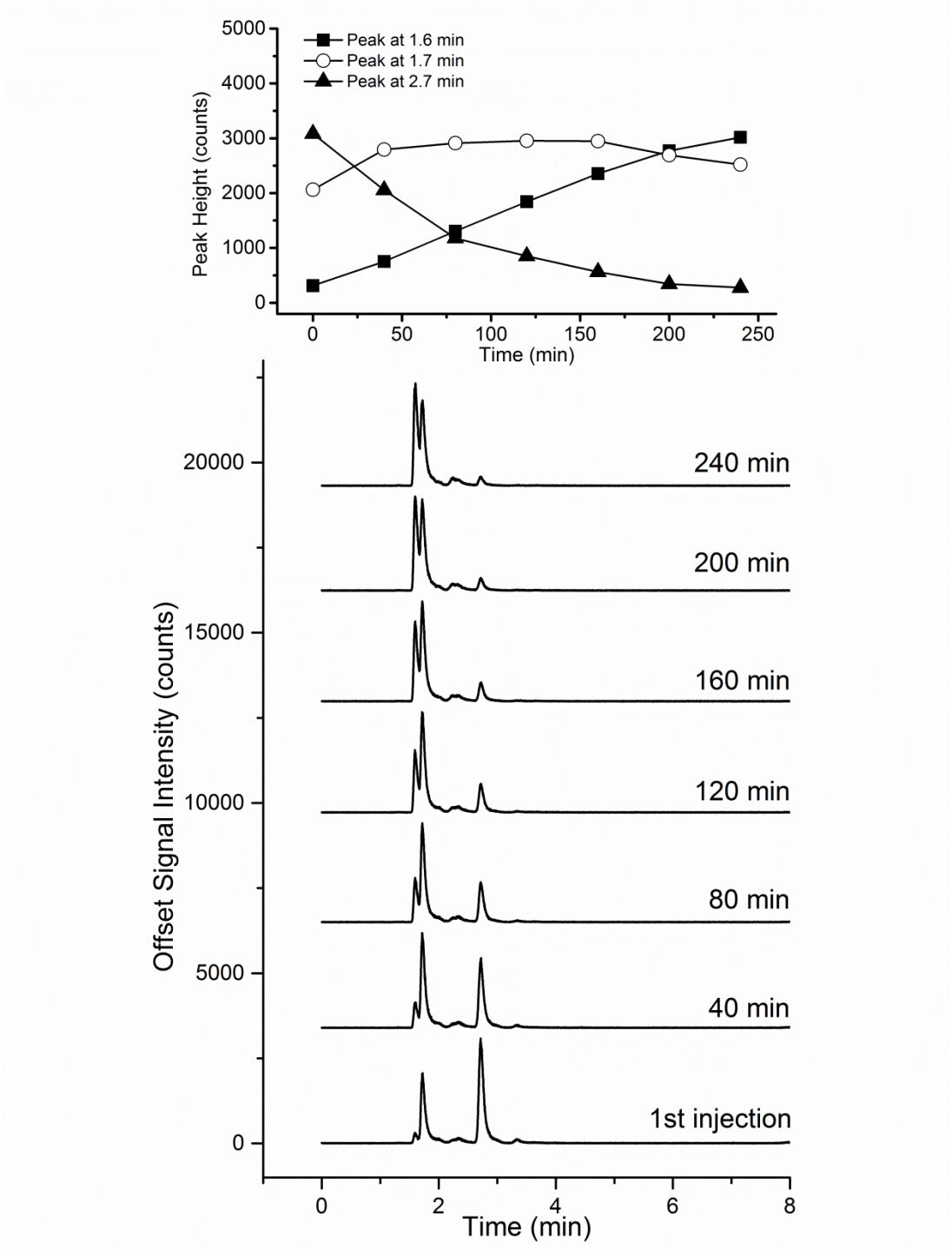

Figure 2-12 **Effect of dilution and aging time on Au(III)-chloride stability in mobile phase.** *Chromatograms show injections of the standard spaced 40 minutes apart with an inserted plot of peaks heights over time. The chromatograms are y-offset at intervals of ~3200 counts. Mobile phase: 6/ 17.5 v/v isopropanol: acetonitrile, 1 mM TBAC, 5 mM NaH_2PO_4/Na_2HPO_4, pH 7.*

Application of method to studying ligand exchange in standard solutions

The previous work was undertaken by evaluating individual solutions ([Au] = 100 µg L^{-1}) made from each of the Au(III)-chloride, Au(III)-bromide, Au(I)-thiosulfate and Au(I)-cyanide standards, however, the simultaneous analysis of the four complexes was trialled to determine if a mixed gold standard could be used for quantification. Figure 2-13 shows a series of chromatograms containing a mix of Au(III)-chloride, Au(III)-bromide, Au(I)-thiosulfate and Au(I)-cyanide (20 µg L^{-1} of each), followed by chromatograms of mixes containing only three of the four gold complexes of interest. This systematic removal of one of the complexes was done in order to confirm the identity the gold peaks.

As expected from the individual standards, the Au(III)-chloro-hydroxyl and Au(III)-bromo-hydroxyl peaks co-eluted. Furthermore, it is likely that upon mixing, some of the chloride ligands are exchanged for the bromide ligands (and vice versa) to form mixed chloride–bromide complexes, as described by Usher *et al.* [84]. The numerous possible complexes $[AuX_{(4-n)}(OH)_n]^-$ (where X = Cl or Br, n = 0 – 4), results in more overlapped peaks, and broadening of the resultant trace. Most notably, an interaction was observed between Au(I)-thiosulfate and Au(I)-cyanide. A comparison of Figure 2-13 (i), (iv) and (v) shows three Au(I)-thiosulfate peaks in the presence of the gold(I) cyanide, and only two peaks from Au(I)-thiosulfate when Au(I)-cyanide is not present. The extra peak present seen with Au(I)-cyanide could correspond to a Au(I)-thiocyanate complex, although, more work is required in order to fully understand the influence of one gold complex on another and to identify the additional peaks. It is clear, however, that individual standards must be used to avoid interactions that may occur between the different species, which would affect the interpretation of the overall results.

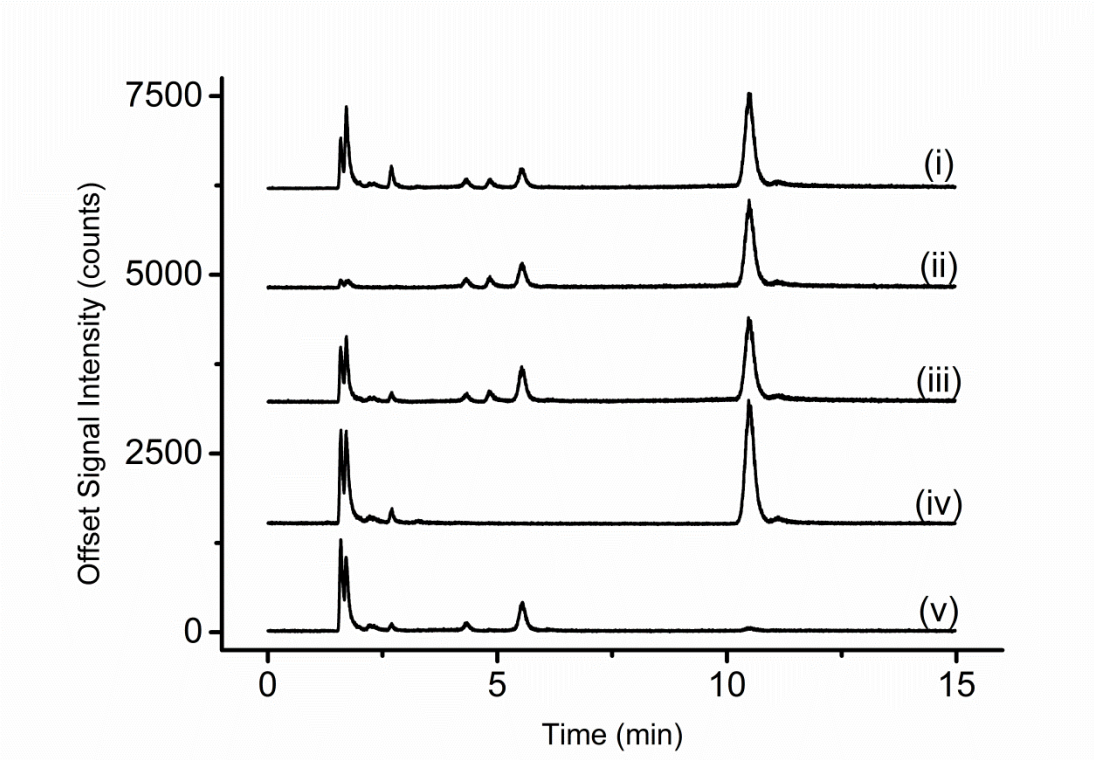

Figure 2-13 **The interaction effect from mixing gold standards on the speciation of gold.** *Chromatograms of (i) a mix of the four gold standards (20 µg L^{-1} each); (ii) mix without Au(III)-chloride; (iii) mix without Au(III)-bromide; (iv) mix without Au(I)-thiosulfate; (v) mix without Au(I)-cyanide. The chromatograms are y-offset at intervals of ~1500 counts. Mobile phase: 6/ 17.5 v/v isopropanol: acetonitrile, 1 mM TBAC, 5 mM NaH_2PO_4/Na_2HPO_4, pH 7.*

2.4.9 Analytical figures of merit

As clearly shown above, the quantitation of gold cannot be achieved with a mixed gold standard. Instead, the samples must first be screened (to determine the gold species) and then the suitable standards for standard addition must be made. The limits of detection (LOD) and quantitation (LOQ) were determined from individual standards (not mixed) from 0.25 µg L^{-1} to 50 µg L^{-1}. Due to the hydrolysis effects observed in freshly prepared Au(III)-halide standards, all standards were left overnight (to form the hydrolysed complex) before filtering and analysis with HPLC-ICP-MS. As shown in Table 2-5, the detection limits for the gold complexes are in the ppt (ng L^{-1}) level. The LOD and LOQ values are for the Au(I)-thiosulfate complex (0.58 µg L^{-1} and 1.4 µg L^{-1} respectively), where in standards 1 µg L^{-1} and below, a small Au(I)-cyanide peak is also visible in the chromatogram. At these very low concentrations some of the Au(I) thiosulfate complex is converted to Au(I)-cyanide by the cyanide impurities from the acetonitrile.

Table 2-5 Analytical figures of merit.

$LOD = (s_B + 3.3\sigma_B)/S$, where s_B= signal of the blank, σ_B= standard deviation of the blank and S= slope; $LOQ = (s_B + 10\sigma_B)/S$.

	Slope (counts min^{-1})	R^2 value	LOD (μg L^{-1})	LOQ (μg L^{-1})
Total Au(III)-chloro-hydroxyl complexes	102.9 ± 0.7	0.9997	0.1	0.2
Total Au(III)-bromo-hydroxyl complexes	48.0 ± 0.2	0.9999	0.2	0.4
Au(I)-thiosulfate	16.9 ± 0.4	0.9980	0.6	1
Au(I)-cyanide	40.1 ± 0.2	0.9999	0.2	0.5

2.5 Conclusions

A HPLC-ICP-MS method has been developed to distinguish between Au(III)-chloro-hydroxyl complexes, Au(III)-bromo-hydroxyl complexes, Au(I)-thiosulfate and Au(I)-cyanide. The detection limits were 0.081 μg L^{-1}, 0.17 μg L^{-1}, 0.58 μg L^{-1} and 0.21 μg L^{-1} respectively. The optimised HPLC conditions comprised of a buffer of 1 mM tetrabutylammonium chloride and 5 mM sodium dihydrogen phosphate/disodium hydrogen orthophosphate prepared in 6: 17.5: 76.5 v/v/v isopropanol: acetonitrile: water. Equilibrium thermodynamic modelling indicates that the mobile phase composition will not influence the overall speciation of environmental samples. As part of the method development, different processes used to prepare gold standards were also studied. Changes to the speciation of gold were observed when: different gold standards were mixed together (due to interactions between different species); and when the Au-halide standards were monitored over time (due to hydrolysis upon dilution). The hydrolysis of the Au-halide standards to a $[Au(OH)_4]^-$ complex occurred over hours. With these observations in mind, groundwater samples should be analysed without dilution or concentration where possible. Where samples are manipulated, the samples should be left overnight to allow the speciation of gold to equilibrate.

Due to changing distributions of the Au(III)-halide-hydroxides in the Au(III) standards (from pH/equilibrium effects), the low intensities of certain peaks and the fact that some of these peaks are shoulder peaks and not completely resolved, identifying various Au-halide-hydroxides for quantification will be difficult. However this method is able to differentiate between Au(III)-chloride and Au(III)-bromide by their different retention times. The retention time of the gold peaks also indicates whether it belongs to a Au(I) or Au(III) complex. Therefore this method is able to observe changes to the distribution of gold species, allowing future application to monitoring gold oxidation reactions. The application of the developed method to environmental water samples including spike-and-recovery and detection limits are explored in the next chapter.