Analytical Methods

Cite this: *Anal. Methods*, 2012, **4**, 530

www.rsc.org/methods

PAPER

A HPLC method for the quantification of butyramide and acetamide at ppb levels in hydrogeothermal waters

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Received 12th September 2011, Accepted 14th December 2011
DOI: 10.1039/c2ay05576g

A quantitative analytical method to determine butyramide and acetamide concentrations at the low ppb levels in geothermal waters has been developed. The analytes are concentrated in a preparation step by evaporation and analyzed using HPLC-UV. Chromatographic separation is achieved isocratically with a RP C-18 column using a 30 mM phosphate buffer solution with 5 mM heptane sulfonic acid and methanol (98 : 2 ratio) as the mobile phase. Absorbance is measured at 200 nm. The limit of detection (LOD) for BA and AA were 2.0 μg L^{-1} and 2.5 μg L^{-1}, respectively. The limit of quantification (LOQ) for BA and AA were 5.7 μg L^{-1} and 7.7 μg L^{-1}, respectively, at the detection wavelength of 200 nm. Attaining these levels of quantification better allows these amides to be used as thermally reactive tracers in low-temperature hydrogeothermal systems.

Introduction

Acetamide (AA) and Butyramide (BA) are polar short chain amides that are not easily retained on non-polar reversed-phase (RP) columns commonly used with HPLC with a ultra-violet (UV) detector. Liquid chromatography-mass spectrometry (LC-MS)[1,2] and gas chromatography-mass spectrometry (GC-MS)[3] have been used for low level quantification of amides. AA and BA have been analyzed using gas chromatography-flame ionization detection (GC-FID)[4] at only ppm levels. A high performance liquid chromatography with ultraviolet detection (HPLC-UV) method has also described for acrylamide[5] after a methanol extraction. However, HPLC methods with UV detection for AA and BA in water samples at the ppb levels have not been documented. We developed an analytical technique using HPLC that detects AA and BA at the ppb (μg L^{-1}) level. Amides undergo hydrolysis resulting in the formation of an organic acid and ammonia. At constant hydroxyl ion (OH^-) concentration, the rates of these hydrolysis reactions exhibit pseudo first-order behavior and vary with temperature according to the Arrhenius equation.[6] The rates of hydrolysis of AA and BA make them potentially useful tracers for determining changes in reservoir temperature (thermal depletion) in low-temperature hydrogeothermal reservoirs. However, due to the very large dilution factors that can occur when conducting these tests ($\sim10^6$), detection limits in the ppb range are required.

We conducted two field-scale tracer tests at the Raft River Geothermal area in south-central Idaho in 2010. In the first injection test, BA was used as a reactive tracer and fluorescein disodium salt was used as a non-reactive tracer. In the second tracer test conducted one month later, AA was used as the reactive tracer and naphthalene 2,6-disulfonate was used as a non-reactive tracer. Details of the tracer tests are described by Plummer and others.[7] The expected concentrations of the AA and BA can be calculated from the measured non-reactive tracer concentrations and the known kinetic parameters. The concentrations of AA and BA measured using the method developed are very similar to the expected values based on the known reservoir temperature (Fig. 1). These results suggest that the analytical method described in this paper is capable of measuring AA and BA in the ppb range even in complex matrix of geothermal water.

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Experimental

Chemicals and reagents

Chemicals used in this study include LC-MS grade methanol (EMD Chemical Corporation, Gibbstown, NJ), potassium phosphate, monobasic (KH_2PO_4) (AMERSCO, Solon, OH), phosphoric acid (H_3PO_4) and heptane sulfonic acid (HSA) sodium salt (J. T. Baker, NJ), acetamide (99%) and butyramide (98%) (Alfa Aesar, Ward Hill, MA). In-house nanopure water (>18 mega-ohm) was used in all applications. Blank geothermal waters were obtained from the Raft River Geothermal field in south-central Idaho prior to injection. Since the tracers were injected into this water system, this would represent water with all other ingredients present in it before the tracer injection. Therefore, we used these samples as blank samples for our experiments. Samples with unknown AA and BA concentrations were obtained from wells RRG-1 and RRG-4 at the Raft River Geothermal site for analysis.

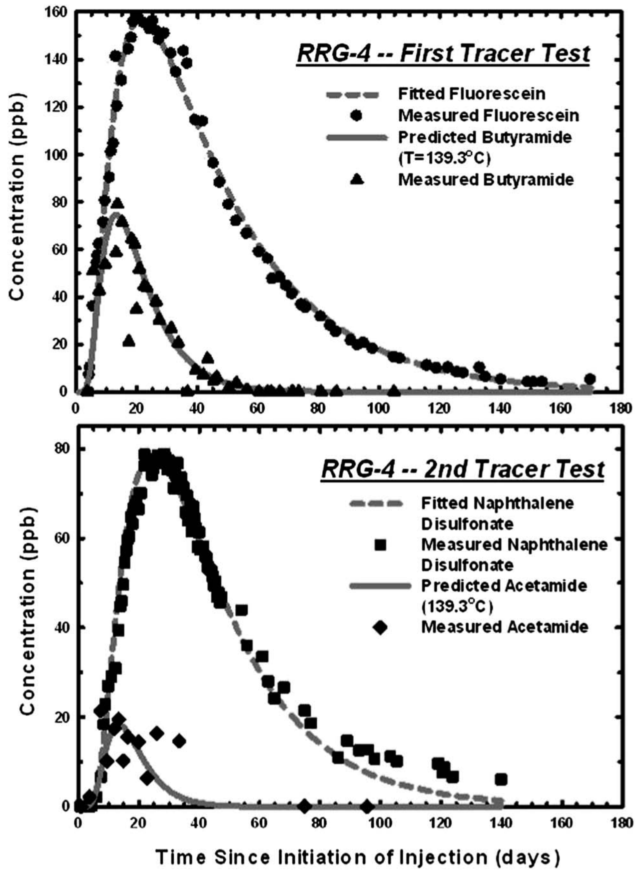


Fig. 1 Breakthrough curves for butyramide and acetamide as well as the corresponding non-reactive tracers at production well RRG-4 for the two tracer injections.

Equipment

Samples were analyzed using a Jasco HPLC system with PU-2089S Plus pumps, CO-2065 Plus Intelligent column oven, AS-2057 Plus Intelligent autosampler, MD-2018 Plus photo-diode array detector, and ChromNAV software.

Chromatographic conditions

Chromatographic separation was achieved with GL Sciences RP C-18 column, Inertsil ODS-SP, 25 cm × 4.6 mm, 5 μm with a guard column, 10 mm × 4 mm with the same particle size. AA and BA were determined at a wavelength of 200 nm. All procedures were carried out isocratically using a mixture of 30 mM phosphate buffer with 5 mM heptane sulfonic acid (HSA), pH ~ 2.6, and methanol (98 : 2 ratio). The column temperature was 35 °C and the flow rate was 0.35 mL min^{-1}. A direct 200 μL injection volume was used for all samples.

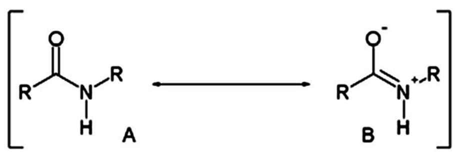
Standard and sample preparation

Standards made up in nanopure water were used initially to test the method. HPLC analyses of these standards showed the retention times of AA and BA. A minimum concentration of 0.10 ppm could be detected with this approach. Since the concentration of these analytes in the Raft River production wells were in the ppb levels, concentration efforts were attempted to detect them at low levels. Initial tests of evaporation of the analytes standards in nanopure water to concentrate AA and BA detected ppb levels of concentration. Therefore, standards of different concentrations of AA and BA were spiked in fifty mL of blank geothermal waters in Erlenmeyer flasks. The effective concentrations of the analytes were in the range from 5–100 ppb in the dilute solutions. The diluted standard solutions were evaporated on hot plates at approximately 80 °C until the volume was reduced to 10 mL. Raft River production well samples, 50 mL, also were evaporated the same manner. These geothermal water samples produced precipitates during evaporation and were filtered using 0.45 μm cellulose acetate membrane filters. The filtered samples were again evaporated to approximately 2–3 mL, cooled to room temperature and filtered a second time into clean 15 mL centrifuge tubes and their volumes were measured. Approximately 1.2 mL of each sample was transferred to 1.5 mL amber vials and analyzed using HPLC with 200 μL injection volume. Calibration curves were built using the evaporated standards and the AA and BA in the unknown samples were quantified using this calibration curve.

Results and discussion

Method development

In amides, the lone pair of electrons on the nitrogen is delocalized into the carbonyl group, thus forming a partial double bond between N and the carbonyl carbon. The resonance structure of amide is shown in A and B[8] (eqn (1)).



(1)

Amides are very weak bases compared to amines because of the presence of the carbonyl group, and do not have as clearly noticeable acid–base properties in water. This lack of basicity is explained by the electron-withdrawing nature of the carbonyl group where the lone pair of electrons on the nitrogen is delocalized by resonance. On the other hand, amides are much stronger bases than carboxylic acids, esters, aldehydes, and ketones. The presence of the C=O group makes the acid derivatives polar compounds.[9] Amides have high boiling points because of their capability for strong intermolecular hydrogen bonding.[9]

The polarity of the low carbon chain amides makes the detection of these compounds difficult using a non-polar column like C-18 on the HPLC. Therefore, a column with a low carbon load was used for the experiments. However, controlling the pH of the mobile phase[10] was necessary to retain the amides in the protonated form by RP-HPLC. Phosphate buffer (30 mM, pH 2.6) (95%) and methanol (5%) were initially selected as solvents for the HPLC analysis to obtain a reasonable retention time of 10 min for BA at a flow rate of 1.0 mL min^{-1} and 35 °C. The solvent system selected worked well for the separation of BA. However, this solvent condition was not enough to resolve AA from the

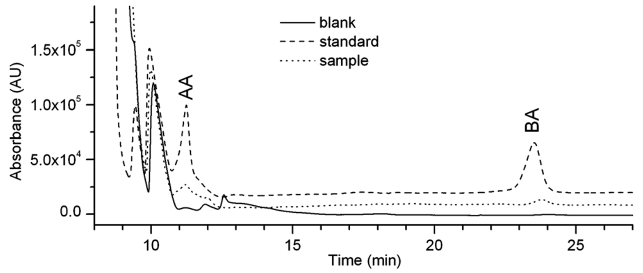


Fig. 2 Evaporated blank ground water, standards (200 ppb) of acetamide (AA) and butyramide (BA) concentrated to 4000 ppb in ground water, and ground water sample with BA (13.74 ppb) and AA (21.44 ppb) concentrated to 360 ppb and 540 ppb.

solvent peak. An ion-pairing agent (5 mM heptane sulfonic acid) was added to the phosphate buffer to improve the retention of this polar, low molecular weight compound.[11] A resolved peak of AA was obtained with 98% of the buffer solution with HSA and 2% methanol at a flow rate of 0.35 mL min^{-1} at 35 °C. This buffer-to-methanol ratio increased the capacity factor (k') of AA and resulted in retention times of 11.3 and 23.8 min for AA and BA, respectively (Fig. 2). The limit of detection (LOD) of BA was also increased with the addition of HSA, due to the effect of the ion-pairing agent on k' and LOD.[12,13] A peak in the blank geothermal water near the AA peak of the standard (Fig. 2) may produce some interference.

A detection wavelength of 200 nm was selected for quantitation of the analytes based on their UV spectra (Fig. 3). The detection limit of the analytes in this condition was only 100 µg L^{-1} (100 ppb); however, the concentration of the amides in the geothermal reservoir water was expected to be only in the low ppb levels. Therefore, it was necessary to concentrate the samples prior to introducing them on the HPLC. Efforts to concentrate the sample were made using a solid phase extraction (SPE) cartridge provided by Phenomenex, Strata-X. According to its specifications, it is a reversed phase functionalized polymeric sorbent that gives strong retention of neutral, acidic, or basic compounds. These cartridges were useful for volumes up to 5 mL for BA, but, higher volumes were needed for the lower detection limits required for the samples. Hence, evaporation was attempted with confidence because of the high boiling points[9] (>200 °C) for AA and BA. Sample volumes of 50–100 mL were evaporated in volumetric flasks at 80–85 °C on hot plates and reduced to 2–3 mL. Care was taken not to evaporate the liquid completely to avoid analyte loss.

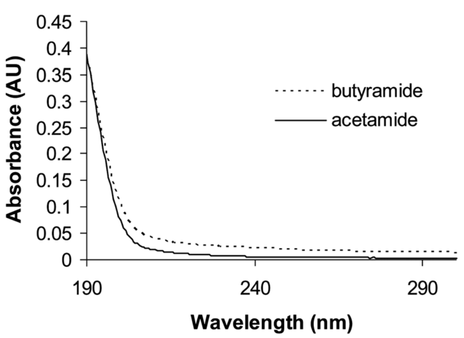


Fig. 3 UV spectra of acetamide and butyramide.

HPLC-UV analysis

Satisfactory detection of BA and AA was achieved by HPLC with a photodiode array (PDA) detector with an isocratic analysis in 35 min using a RP-C_{18} column with low carbon load. The mobile phase was a mixture of 30 mM phosphate buffer with 5 mM HSA, pH 2.6, and methanol in 98 : 2 ratio. With this set up, retention times of 11.3 min and 23.8 min were obtained for AA and BA respectively. Absorption at 200 nm wavelength, near the lower end of the near UV range where the absorption coefficient is greatest (Fig. 3), was used to determine peak area for quantitation. Peak identification was possible by comparison of the retention time to the standard.

Validation of HPLC-UV method

Performance of the method was evaluated with regard to calibration, linearity, repeatability, limit of detection (LOD), limit of quantification (LOQ) and recovery. Standards made in nanopure water without evaporation in the range of 0.1–5 ppm produced linear curves for BA with $r^2 > 0.99$. Evaporated ground water samples spiked with BA and AA standards also produced linear curves with $r^2 > 0.98$–0.99 where the evaporated standard concentration ranged from 0.1–4.5 ppm and the concentration before evaporation was in the range of 5–100 ppb as shown in Fig. 4. The calibration curves were obtained by plotting concentration against peak area at 200 nm wavelength.

Each batch of samples was analyzed randomly with standards made up in the same matrix (geothermal ground water) to verify the validity of the calibration curve. In addition, calibration standards were analyzed when any change in analytical conditions was made. The LOD and LOQ was determined as LOD = 3.3 (SD/S) and LOQ = 10 (SD/S) where S is the slope of the calibration curve at levels approximating the LOD and SD is the standard deviation of y-intercepts of regression line. Based on this calculation, the LOD for BA and AA were 2 µg L^{-1} and 2.5 µg L^{-1}, respectively and LOQ for BA and AA were 5.7µg L^{-1} and 7.7 µg L^{-1}, respectively, at the detection wavelength of 200 nm. The precision and accuracy of the method was verified by analyzing 50 mL of groundwater spiked with 40 ppb and 100 ppb

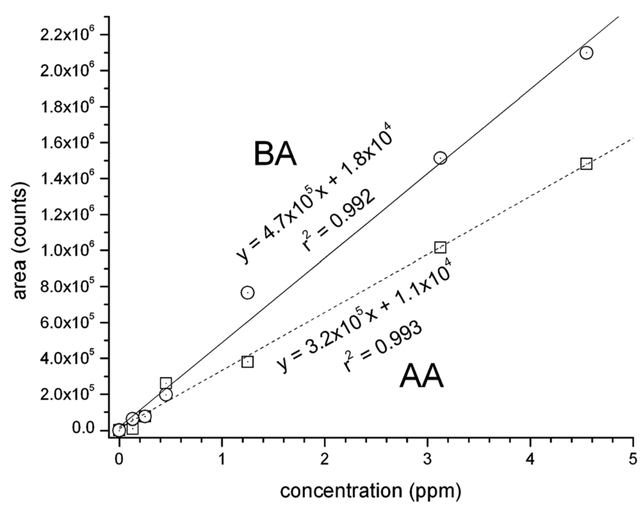


Fig. 4 Standard curves for AA and BA from evaporated ground water.

Table 1 Accuracy and Precision of butyramide and acetamide in geothermal waters[a]

Analytes	Spike Level (μg L^{-1})	Detected (μg L^{-1})	sd[b]	Recovery (%) (mean)	Relative percent difference
BA	40	36.8	3.4	92.1	8.2
BA	100	98.6	2.1	98.6	1.4
AA	40	31.1	7.5	77.8	25.0
AA	100	93.5	5.5	93.5	6.7

[a] n = 3. [b] standard deviation of three detected concentration.

of the analytes. The recovery of BA and AA was determined by the triplicate analyses of these two samples after evaporation as described in the text. The mean percentage recovery of BA exceeded 90% in both spike levels. The recovery of AA is 77% in the 40 ppb spike level and 93% in the 100 ppb spike. The relative percent difference of both BA and AA, the standard deviation of the triplicate analyses are all listed in Table 1.

Process error was determined by evaporating four blank geothermal water samples (50 mL) spiked with the same amount (40 ppb) of AA and BA standards. The relative errors for BA was 7.2% and for AA was 30.8%. The magnitude of these errors could be attributed to the compounding of errors at each step in the procedure such as measuring and transferring large volumes of samples and multiple filtering. The higher error for AA could be partially the result of its elution close to a signal given from the groundwater itself where the AA peak was not symmetrical. There is a possibility that AA is hydrolyzed partially during the evaporation.

Conclusions

The quantitative analytical method for the determination of butyramide and acetamide described in this paper is capable of achieving LODs as low as 2 μg L^{-1} for butyramide and 2.5 μg L^{-1} for acetamide. LOQs of 5.7 μg L^{-1} for butyramide and 7.7μg L^{-1} for acetamide were attained. The higher LOD and LOQ for acetamide compared to butyramide are partially attributed to interference from a naturally occurring peak in the geothermal water. These levels of quantification better allow these amides to be used as thermally reactive tracers in low-temperature hydrogeothermal systems.

Acknowledgements

This work was supported by the U.S. Department of Energy, Office of Geothermal Technologies, under DOE Idaho Operations Office Contract DE-AC07-05ID14517.

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