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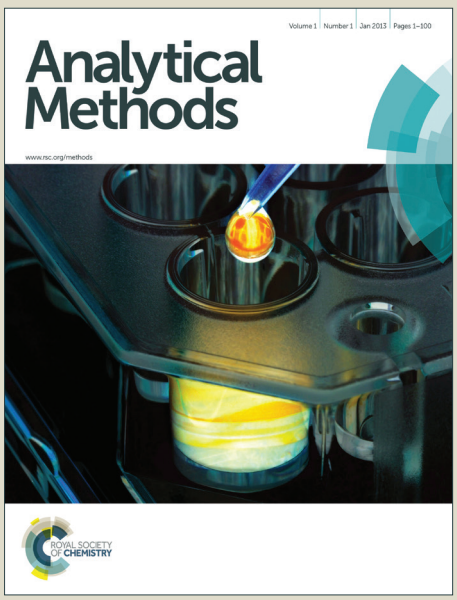

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Title:

Speciation Analysis of Urine Iodine by Ion-pair Reversed-Phase Liquid Chromatography and Inductively Coupled Plasma Mass Spectrometry

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Abstract

This work described the utilization of ion-pair reversed phase liquid chromatography coupled to inductively coupled plasma mass spectrometry (RP-LC-ICP-MS) for iodine speciation analysis in urine. Considering the requirements of green analytical chemistry and the Ar ICP-MS instrument, three aqueous mobile phases were employed for the separation of seven iodine species including iodide, iodate and five iodo amino acids (monoiodothyrosine -MIT, di-iodothyrosine -DIT, tri-iodothyronine -T_3, reversed tri-iodothyronine -rT_3, and thyroxin -T_4). The aqueous mobile phases were composed of an ion-pair reagent (tetrabutylammonium hydroxide -TBAH) and an eluent (ammonium chloride, L-phenylalanine or deoxycholic acid) at low concentrations in ultrapure water. Owing to tremendous difference in retention behavior between these iodinated forms, a gradient elution mode was performed for the rapid separation of IO_3^- and I^-, MIT and DIT, and T_3, rT_3 and T_4, respectively. Iodine species separation was achieved with a 12.5-mm C_{18} guard column in 7 min. The detection limits for IO_3^-, I^-, MIT, DIT, T_3, rT_3 and T_4 were 0.047, 0.046, 0.057, 0.072, 0.093, 0.094 and 0.081 μg L^{-1}, respectively. Application of the proposed method was demonstrated by the speciation analysis of iodine in four real urine samples. The developed method offered satisfactory recoveries in the 93-106% range and good repeatability, showing great potential in routine analysis of iodine speciation in environmental, food and biological samples.

Keywords: iodine speciation; ion-pair reversed-phase liquid chromatography; inductively coupled plasma mass spectrometry; urine.

1. Introduction

Iodine is an essential constituent of the thyroid hormones (3,3',5,5'-tetraiodothyronine -T_4, 3,3',5-triiodothyronine -T_3, 3,3',5'-tri-iodothyronine -rT_3, *etc.*). It plays prominent roles in cellular metabolism, growth, development of body structures, neuronal function and development [1]. The lack of iodine may result in severely adverse effects on the development of various organs (especially the brain) throughout the life cycle [2]. On the other hand, the excessive intake of iodine may lead to hyperthyroidism [3]. The bioavailability and toxicity of iodine are mainly dependent on the chemical forms. For example, the bioavailability of iodo amino acids such as mono-iodotyrosine (MIT) and di-iodotyrosine (DIT) is less than that of mineral iodide [4]. Likewise, inorganic forms of iodine such as iodide and iodate are less toxic than molecular iodine and some organically bound iodine [5]. Most iodine absorbed in the body is excreted in urine, where it is primarily eliminated in the iodide form [6]. Urinary iodine concentration is an excellent marker for assessment of dietary iodine intake and iodine nutrition in human [7]. For instance, the World Health Organization recommends the minimal urinary iodine concentration for iodine sufficiency as 100 μg L^{-1}. Furthermore, the levels of different iodine species in human body fluids (serum, urine, *etc.*) are vital to assess the malfunction of the thyroid gland and also to diagnose other metabolic abnormalities related to thyroid metabolism [2]. Hence, a rapid and highly sensitive method is necessary for routine analysis of iodine speciation in urine.

Several methods have been developed for the analysis of different iodine species in serum and urine based on non-chromatographic techniques (neutron activation analysis, electrochemical detection, X-ray absorption near edge structure, catalytic spectrophotometry, *etc.*) and predominantly the hyphenation between a separation technique and a selective detector [8]. Gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE) are the prevailing separation techniques. The volatile iodinated species can be directly determined by GC while non-volatile iodine species such as inorganic iodine (iodide and iodate) must be derivatized to volatile compounds before GC analysis. Since the derivatization procedure is time-consuming,

applications of GC analysis to real samples are limited to air, seawater and seaweed until now [8]. Capillary electrophoresis, characterized by high separation efficiency and short separation time, has been utilized to separate various iodine species (iodide, iodate, T_3 and T_4) in human serum and urine [9], foodstuff [10] and table salt [11] with inductively coupled plasma mass spectrometry (ICP-MS) detection. Since the nanoliter sample is dramatically diluted by the sheathing microflow in interfacing CE with ICP-MS, the hybrid method generally suffers from inferior sensitivity. Liquid chromatography is a powerful technique allowing the separation of polar and non-polar compounds, cations and anions, small and big molecules. Adequate resolution, reproducibility, sensitivity, and simplicity of the interface between LC and ICP-MS make their combination one of the most commonly used techniques for speciation analysis. Applications of LC-ICP-MS for iodine speciation analysis are extended to water, aerosol, soil, foodstuff and biological samples [12-25]. However, the use of toxic organic solvents (15-100% v/v methanol or acetonitrile) and high-concentration salts contained in the mobile phases and long separation time are frequently violated by these LC-ICP-MS methods, which go against the principles of green analytical chemistry. Besides, the mobile phases employed in the above LC-ICP-MS methods are detrimental to the sampling interface of the ICP-MS instrument while long separation time is adverse to reducing the operation cost and increasing the throughput of the hyphenated system.

The object of this work is to develop a green method for fast speciation analysis of iodine by coupling LC to ICP-MS using aqueous mobile phases that contain very low concentrations of reagents. In this work, we combined ion-pair reversed phase liquid chromatography with ICP-MS for the speciation analysis of iodine in human urine. Fast separation of seven iodinated forms (iodide, iodate, MIT, DIT, T_3, rT_3 and T_4) was achieved under the gradient elution using three aqueous mobile phases with different elution strengths.

2. Materials and methods

2.1. Chemicals and reagents

Stock standard solutions of 100 mg L^{-1} for iodide and iodate were prepared from potassium iodide

and potassium iodate (≥99.99%, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) with 1 mmol L^{-1} KOH. 3-iodo-L-tyrosine (≥98%, MIT), 3,5-diiodo-L-tyrosine dihydrate (≥98%, DIT), 3,3',5-Triiodo-L-thyronine sodium salt (≥98%, T_3) and 3,3',5'-Triiodo-L-thyronine sodium salt (≥98%, rT_3) were purchased from Suzhou Chemland Pharmaceutical & Technologies Co., Ltd (Suzhou, China) while L-tyrosine (≥98%, T_4) was supplied by Sigma-Aldrich (St. Louis, MO, USA). Standard stock solutions of MIT, DIT, T_3, rT_3 and T_4 (100 mg L^{-1} as iodine) were prepared in 1 mmol L^{-1} KOH and then stored in the dark at 4°C. A series of standard mixture solutions containing 0.4, 1, 4, 10, 50 and 200 μg L^{-1} of the above iodine species (as iodine) were diluted from their stock standard solutions in the ultrapure water. Ammonium chloride (>99.99%), tetrabutylammonium hydroxide (TBAH) (25% v/v in water), L-phenylalanine (Phe, >99%) and deoxycholic acid (DOA, >98%) were purchased from Aladdin Chemistry Co., Ltd (Shanghai, China) to prepare the mobile phases. 10% nitric acid (Jiangyin Chemical Regent, Jiangyin, China) and 10% ammonia (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) were used to adjust pH of the mobile phases. Tetramethylammonium hydroxide (TMAH) (25% v/v in water, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) was used to dilute urine samples and prepare the standard solutions for total iodine measurement. 10 mg L^{-1} tellurium atomic absorption standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). Certified reference materials (CRMs) of iodine in lyophilized human urine (GBW09108i, GBW09109g and GBW09110n with certified values of 67.9 ± 9.0, 142 ± 9.0 and 195 ± 10 μg L^{-1} iodine) were obtained from National Standard Material Center (Beijing, China) to evaluate the accuracy for total iodine determination and iodine speciation in urine. All solutions were filtered through a membrane of 0.45 μm pore size. All used reagents were of analytical or chromatographic grade. Ultrapure water with a resistivity of 18.2 MΩ·cm, obtained from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA) was used throughout the experiment.

2.2. Instrumentation

The liquid chromatography system for iodine speciation was an Agilent 1100 series LC apparatus

(Agilent Technologies, USA), which consisted of a G1311A four-channel gradient pump, a G1322A vacuum degasser, a G1316A thermostated column compartment and a 7725i Rheodyne manual injector with a 5 μL sample loop (Rheodyne, LP, Rohnert Park, CA, USA). A Thermofishier X Series^{II} argon ICP-MS (Thermo Fisher Scientific Inc., USA) was utilized to detect the iodine-containing analytes eluted out of the chromatographic column. Separation of iodine species was performed on a Zorbax Eclipse Plus C_{18} guard column (5 μm, 12.5 mm × 4.6 mm i.d., Agilent Technologies, USA). A PEEK tubing (30 cm long, 0.25 mm i.d.) was used to connect the column directly to the concentric nebulizer (TR-30-A1, Meinhard Glass Products, USA) of the ICP-MS instrument. A conical spray chamber (cooled to 3 ºC) of the X Series^{II} ICP-MS instrument was used to remove the coarsest aerosol droplets. Measurements of pH were made with a HI 98128 pH-meter (Hanna Instrument, Italia). The temperature of thermostated column compartment was fixed at 25°C. 10 μg L^{-1} Te (^{125}Te) was used as the internal standard for total iodine determination and speciation analysis of iodine [18, 19], and was added into the mobile phases and the samples before measurement. Three types of mobile phases were employed in the gradient elution process. The mobile phases A and B consisted of 4 mmol L^{-1} TBAH at pH 8.5 containing 0.5 mmol L^{-1} ammonium chloride and 0.5 mmol L^{-1} L-phenylalanine, respectively. The mobile phase C was composed of 4 mmol L^{-1} TBAH and 20 mmol L^{-1} deoxycholic acid (pH 8.5). The mobile phase A was used for the elution of iodide and iodate while the mobile phase B was employed for the elution of MIT and DIT. Usage of the mobile phase C was to achieve fast elution of T_3, rT_3 and T_4. The gradient process with all steps linear was employed as the following: 0.0-0.5 min: 100% A; 0.5-1.3 min: 100% B; 1.3-1.5 min: 100% B → 80% B and 20% C; 1.5-2.4 min: 80% B and 20% C; 2.4-2.6 min: 80% B and 20% C →50% B and 50% C; 2.6-3.8 min: 50% B and 50% C; 3.8-4.0 min: 50% B and 50% C → 100%C; 4.0-5.5 min: 100% C; 5.5-6.0 min: 100% C → 100% A; 6.0-7.0 min: 100% A. The flow rate of the mobile phases was constant at 1.5 mL min^{-1}. The ICP-MS instrument system was daily optimized using a multi-element solution containing 10 μg L^{-1} of ^{7}Li, ^{59}Co, ^{115}In, ^{208}Pb and ^{238}U in 1% HNO_3. The optimized ICP-MS operating conditions were summarized in Table 1.

2.3. Sample collection and pretreatment

All experiments on human subjects were performed in compliance with the relevant laws and institutional guidelines approved by Hangzhou Normal University, Hangzhou, China. The required consent was obtained for any experimentation with human subjects. The urine samples of two male and female volunteers (MU1 and MU2, FU1 and FU2) were obtained from the middle stream and stored in the dark at 4°C until analysis [23]. They were analyzed by the proposed method within 24 h since their collection. Considering the ultra-low concentrations of organic iodine species in urine and the complex matrix of urine, these samples were diluted 2-fold with ultrapure water containing 10 μg L^{-1} tellurium and then filtered through 0.45 μm filters. For total urine iodine quantification by ICP-MS, the urine samples were diluted 10-fold with 0.5% (v/v) TMAH containing 10 μg L^{-1} tellurium as an internal standard. The certified reference materials of iodine in lyophilized human urine (GBW09108i, GBW09109g and GBW09110n) were pretreated with the same procedure as described above.

3. Results and discussion

3.1 Optimization of the ion pair RP-LC parameters

To achieve baseline separation of iodine species in the shortest time, the chromatographic parameters including the type of the eluent and ion-pairing reagent and their concentrations, the pH value and flow rate of the mobile phases, and the column temperature were investigated. It was reported in our previous work that effective separation of iodide, iodate, MIT and DIT was all achieved by individual usage of TMAH, TBAH and tetraethylammonium hydroxide (TEAH) as the ion-pairing reagent [22]. When TMAH, TEAH and TBAH was separately added into the mobile phases (4.0 mmol L^{-1}), baseline separation of all iodine species could only be achieved with TBAH as the ion-pairing reagent, whereas the co-elution of iodide and iodate, MIT and DIT co-elution, and the co-elution of rT_3 and T_3 were observed using TMAH and TEAH. Therefore, TBAH was selected as the ion-pairing reagent. The target iodine species were divided into three groups according to the chemical structure (Fig. 1). The first group consisted of iodide and iodate and the

second group included MIT and DIT. The remaining three organic iodine forms (rT_3, T3 and T_4) belonged to the third group. Considering that the iodine species have disparate retention behaviors due to their significant difference in the chemical sturture (shown in Fig. 1), various reagents were separately utilized to elute the analytes in group from the chromatographic column. Firstly, inorganic reagents such as sodium thiosulfate, ammonium chloride and ammonium nitrate were employed as the eluents (0.5 mmol L^{-1}) for the separation of iodide and iodate. The best separation of iodide and iodate was achieved with ammonium chloride added in the mobile phase while peak overlapping between iodide and iodate was observed by using sodium thiosulfate. As a result, ammonium chloride was selected as the eluent for the separation of iodide and iodate. Secondly, three aromatic compounds including 5-sulfosalicylic acid dihydrate, L-tyrosine and L-phenylalanine (0.5 mmol L^{-1}) were separately tested to elute organic iodinated amino acids (MIT and DIT). Satisfactory resolution between MIT and DIT was all achieved whereas L-phenylalanine offered the fastest elution speed (about 1.0 min). Therefore, L-phenylalanine was chosen as the eluent to achieve rapid separation of MIT and DIT. Since rT3, T3 and T4 were not eluted from the column even when using 100 mmol L^{-1} L-phenylalanine within 60 min, some other organic reagents with stronger elution strengths including sodium 2-naphthalenesulfonate, berberine hydrochloride and deoxycholic acid were examined to elute rT_3, T_3 and T_4 from the column. The experimental results indicated that rapid baseline separation of rT_3 and T_3 within 7.0 min was obtained when using 4 mmol L^{-1} deoxycholic acid and T_4 could be eluted within 1.5 min from the column by 20 mmol L^{-1} deoxycholic acid. However, when 4 mmol L^{-1} sodium 2-naphthalenesulfonate or berberine hydrochloride were used as the eluents, the separation time of rT_3 and T_3 was in excess of 26 min. T_4 could not be eluted from the column within 60 min even when using 100 mmol L^{-1} sodium 2-naphthalenesulfonate and berberine hydrochloride. Hence, deoxycholic acid was finally employed as the eluent for the separation of rT_3, T_3 and T_4.

Effect of the TBAH concentration in the range of 0.5-10.0 mmol L^{-1} on the separation of iodide and iodate was first investigated with other conditions constant (pH at 8.5, column temperature at

25°C and the mobile phase flow rate as 1.5 mL min^{-1}). The retention times of iodide and iodate increased with the TBAH concentration. Meanwhile the resolution was improved. Baseline separation of two inorganic iodinated ions could be achieved only if the TBAH concentration was higher than 4.0 mmol L^{-1}. Thereby, 4.0 mmol L^{-1} TBAH was added into the mobile phase. Then the effect of the concentration of ammonium chloride in the range of 0.1-5.0 mmol L^{-1} on the separation of iodide and iodate was investigated. The separation time was reduced with the concentration of ammonium chloride while the resolution was gradually impaired. 0.5 mmol L^{-1} ammonium chloride was a good compromise between the analytical speed and resolution. Fig. 2a shows the separation of iodide and iodate in 10 μg L^{-1} standard solution with 4 mmol L^{-1} TBAH + 0.5 mmol L^{-1} ammonium chloride at pH 8.5 as the mobile phase. Followingly, the TBAH and Phe concentrations were optimized in the ranges of 0.5-10.0 mmol L^{-1} and 0.1-5.0 mmol L^{-1}, respectively, for obtaining the fastest separation of MIT and DIT. Similarly, the retention times of MIT and DIT increased with the TBAH concentration but reduced with the Phe concentration. Meanwhile the resolution was improved on increasing the TBAH concentration but decreasing the Phe concentration. 4.0 mmol L^{-1} TBAH and 0.5 mmol L^{-1} Phe were selected considering short analysis time and good resolution of MIT and DIT, which is demonstrated by Fig. 2b. The concentration of TBAH (0.5-10.0 mmol L^{-1}) on the separation of rT_3, T_3 and T_4 was investigated. A similar influence of the TBAH concentration in the mobile phase on the separation of the three organic iodine species was also observed. Then the DOA concentration was evaluated in the range of 0.5-20.0 mmol L^{-1}. The retention times of the three analytes were, to a large extent, compressed with the DOA concentration. Baseline separation was always achieved with the DOA concentration below 4.0 mmol L^{-1}. Further increasing the DOA concentration to 10 mmol L^{-1} resulted in the co-elution of rT_3 and T_3. When the DOA concentration was above 10 mmol L^{-1}, it might lead to the co-elution of rT_3, T_3 and T_4. A completely overlapping peak with a width of 1.4 min was obtained with 20 mmol L^{-1} DOA. To achieve the rapid separation, a gradient elution procedure on varying the DOA concentration was employed, which consisted of 4 mmol L^{-1} DOA for 1.2 min, a ramp from 4 to 10 mmol L^{-1} DOA within 0.2 min, elution at 10 mmol

L^{-1} DOA for 1.8 min, another ramp from 10 to 20 mmol L^{-1} DOA within 0.1 min, and final elution at 20 mmol L^{-1} DOA for 1.7 min. A typical chromatogram in Fig. 2c shows the separation of rT_3, T_3 and T_4 using the above gradient elution procedure.

Generally, the introduction of high-content carbon into the plasma may lead to spectral and non-spectral interferences in the elemental analysis by ICP-MS. Although enhanced signals for As, Se, Te, Au and Hg in the presence of carbon are generally observed, contradictory statements can be found for iodine [26, 27]. The utilized mobile phases 100% A, 100% B, 80% B + 20% C, 50% B + 50% C and 100% C are corresponding to 0.77, 0.82, 1.96, 3.68 and 6.53 g L^{-1} carbon. The effect of carbon content from the mobile phases on iodine intensities was investigated by comparing the intensities of each iodine species (10 μg L^{-1}) individually prepared in the above mobile phases and ultrapure water. The ^{127}I intensities of 10 μg L^{-1} single iodine species in the mobile phases were about 95.3-103.6% of those prepared in ultrapure water and the iodine intensities were independent of chemical form of iodine. We also monitored the baseline on performing the gradient elution and a very steady baseline was obtained throughout. These results indicated no significant influence on iodine intensity in the presence of organic carbon from the mobile phases, which was in accordance with the observation by Allain et al. [27].

Chromatographic separation of iodine species was carried out in a pH range of 6.5-9.5 for the mobile phases containing ammonium chloride or L-phenylalanine when keeping other conditions constant. As deoxycholic acid was of low solubility under acidic conditions, the influence of the pH value on the separation was examined in the range of 7.5-9.5 for the mobile phase containing DOA. The retention times of all target analytes decreased with the pH value and the resolutions became worse. Resolutions better than 1.2 between the seven iodine species were only achieved at pH values below 8.5. Therefore, pH 8.5 was chosen as the optimum pH value. The effect of the mobile phase flow rate in a range of 0.75-2.0 mL min^{-1} on iodine speciation was studied. As the flow rate increased, the retention times of all iodine species reduced significantly. Fast separation within only 5 min or less was obtained when the mobile phase flow rate was higher than 1.5 mL min^{-1}.

However, higher flow rates of the mobile phase induced the descending sensitivity because the nebulising efficency of the Meinhard concentric nebulizer decreased with the sample uptake rate especially at sample uptake rates more than 1.0 mL min^{-1}. Therefore, the mobile phase flow rate was selected as 1.5 mL min^{-1} as a compromise between rapid analysis and high sensitivity. The effect of the column temperature from 20°C to 50°C on the separation was also tested. According to the experimental results, the column temperature shifted the retention times of organic iodine species considerably, whereas the elution of iodide and iodate was hardly influenced by the column temperature. When a high column temperature (> 25°C) was used, peak overlapping of organic iodine species was encountered. The separation was therefore performed at 25°C.

3.2. Analytical figures of merit of the ion pair RP-LC-ICP-MS system

Under the optimized conditions, a series of standard solutions containing 0.4, 1.0, 4.0, 10, 50 and 200 μg L^{-1} of each iodine species were measured (10 times for 1.0 and 10 μg L^{-1} I-mixture standard solution and 3 times for the other standard solutions). Fig. 3a shows the typical chromatogram of 10 μg L^{-1} iodine mixture standard solution under the gradient elution. Detection limits (*S/N*=3) and linearities were obtained from linear calibration curves by peak areas of these standard solutions. The retention times, repeatabilities of peak heights and peak areas were calculated on the basis of ten chromatograms of 10 μg L^{-1} iodine-mixture standard solution. All these results are listed in Table 2. The method limit of quantification (LOQ) for each analyte in urine was calculated from 10 times of the standard deviations for the blank signals considering the dilute factor (2-fold). Method LOQs for I^-, IO_3^-, MIT, DIT, rT_3, T_3 and T_4 were 0.31, 0.31, 0.38, 0.48, 0.62, 0.63 and 0.54 μg L^{-1}, each. The relative standard deviations of peak heights and peak areas for the seven iodine species (10 μg L^{-1} for each iodine species) were in the ranges of 0.7-2.5% and 0.9-3.1%, respectively. The repeatability of the migration times of the iodine species ranged from 0.2% to 1.4% while the repeatability of the peak width varied from 0.6% to 2.1%. The repeatabilities of retention time, and peak width, height and area were variable in the ranges of 0.8-1.9%, 1.1-2.7%, 2.8-5.4% and 3.6-6.5% for 1.0 μg L^{-1} iodine species, respectively. These results proved satisfactory precisions of

the present hybrid method. In comparison with the other LC-ICP-MS methods [11-24], the proposed method offered a significant time advantage. Up to seven iodine forms were separated within 5.0 min in one run for our method while more than 20 min was consumed for fewer analytes. It indicated an observable decrease of the operation cost of the Ar ICP-MS instrument and significant enhancement of the analytical throughput. Besides, organic solvents such as methanol and acetonitrile were eliminated from the mobile phases in our method. The aqueous mobile phases containing only inorganic and organic salts below 20 mmol L^{-1} were quite suitable to the ICP-MS instrument.

3.3. Iodine speciation in urine

As there is no commercially available certified reference material for iodine speciation, spike recovery test of certified reference materials of human urine (GBW09108i, GBW09109g and GBW09110n) for total iodine was performed to evaluate the accuracy of the proposed method. The results are shown in Table 3. The fairly good recoveries (94-106%) validated its accuracy. Besides, the sum concentrations of all iodine species were 63.1 ± 1.7, 135.0 ± 3.2 and 186.8 ± 3.6 μg L^{-1} in GBW09108i, GBW09109g and GBW09110n, respectively, which accounted for 96.6-97.5% of total iodine. They were in the certified value ranges, indirectly confirming the accuracy of our method.

The stability of iodine-contained analytes in urine during the storage was examined with the spiked urine MU1 (spiked with 10 μg L^{-1} iodine species except iodide). After storage over a 24-hour period in the dark at 4°C, iodide concentration in urine decreased by about 2.6% while the other iodine species of less than 1% were lost, showing good short-term stability of iodine species in the urine matrix. However, more than 10% of iodide was transformed after 7-day storage whereas the other six iodine forms were still stable (< 3.6%). Therefore, urine samples were analyzed within 24 h since their collection. Besides, the matrix effect was also investigated by comparing the intensities of iodine mixture standard solutions (4, 10, 50 and 200 μg L^{-1}) prepared in the urine matrix (MU1) and ultrapure water. It was observed that peak areas of iodate in the urine matrix were about

94-98% of those in ultrapure water while the ratios of peak areas of the other six iodine species (subtracting peak areas of background iodinated forms in urine) in both matrices varied from 97-102%. The experimental results indicated no significant matrix effect from the two-fold diluted urine, which could be ascribed from the dilution of 5-μL diluted urine by the high flowing rate mobile phases during chromatographic separation.

The proposed method was applied for iodine speciation analysis in four urine samples from two male and two female volunteers after 2-fold dilution (Table 3). The results of the recovery tests (spiked with 50 μg L^{-1} for iodide and 10 μg L^{-1} for the other iodine forms) and total iodine are also provided in Table 3. Fig. 3b demonstrates a typical chromatogram of the urine sample MU1. Iodide was observed the dominant species, showing 96.6-98.4% of total iodine in urine samples. Besides, low concentrations of rT_3, T_3 and T_4 were also found in human urine samples. These results were similar to those from Michalke *et al.* [9, 24].

4. Conclusion

In this work, ion-pair reversed-phase liquid chromatography was utilized for iodine speciation analysis in urine combined with inductively coupled plasma mass spectrometry detection. By using 4 mmol L^{-1} TBAH as the ion-pair reagent and 0.5 mmol L^{-1} ammonium chloride and L-phenylalanine and 20 mmol L^{-1} deoxycholic acid as the elution reagents, fast separation of seven iodine compounds (IO_3^-, I^-, MIT, DIT, T_3, rT_3 and T_4) was achieved within 7 min via a 12.5-mm C_{18} guard column under the gradient elution. The aqueous mobile phases were quite suitable to the ICP-MS instrument. The detection limits for various iodine species were in the range of 0.046-0.094 μg L^{-1}. Iodide was found the dominate iodine species in urine. The proposed method was of high efficiency, low argon consumption and powerful detection capacity, showing a potential for routine analysis of iodine speciation in environmental, food and biological fields. In combination with preconcentration techniques, the detection limits of the present method are anticipated to be decreased in the near future, which will be applicable for routine analysis of trace organic iodine species.

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Figure captions

Fig. 1. The chemical structures of monoiodothyrosine (MIT), di-iodothyrosine (DIT), L-phenylalanine (Phe), tri-iodothyronine (T_3), reversed tri-iodothyronine (rT_3), thyroxin (T_4) and deoxycholic acid (DOA).

Fig. 2. Chromatograms showing the separation of iodide and iodate with the mobile phase A (a), and MIT and DIT with the mobile phase B (b), and rT_3, T_3 and T_4 with the mobile phases B and C under a gradient elution (c). The gradient elution was as the following: 0-1.2 min: 80% B and 20% C; 1.2-1.4 min: 80% B and 20% C → 50% B and 50% C; 1.4-3.2 min: 50% B and 50% C; 3.2-3.3 min: 50% B and 50% C → 100%C; 3.3-5.0 min: 100% C. The concentration of each iodine species was 10 μg L^{-1} (as I).

Fig. 3. Typical chromatograms of iodine species in 10 μg L^{-1} standard mixture solution (a) and the diluted urine MU1 (b).

Tables

Table 1 Operating conditions of the ICP-MS system

Parameters	Value
RF power, W	1200
Sampler cone (orifice diameter, mm)	1.1
Skimmer cone (orifice diameter, mm)	0.9
Cooling gas, L min^{-1}	13.02
Auxiliary gas, L min^{-1}	0.75
Nebulizer gas, L min^{-1}	0.85
Isotope monitored	^{127}I, ^{125}Te
Dwell time, ms	100
Acquisition time, s	300

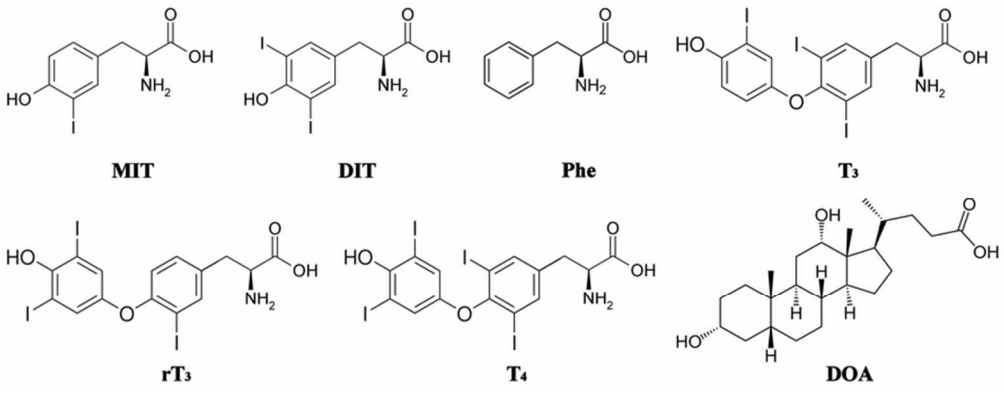
Table 2 Retention time, linearity, detection limit and repeatability of iodine species

Analyte	Retention time ($t_R \pm SD$, s)	Linear range (μg L^{-1})	R	Detection limit (μg L^{-1})	Repeatability (RSD %，n=10)	
					Peak height	Peak area
IO_3^-	7.2 ± 0.1	0.4-200	0.9995	0.047	0.7	0.9
I^-	22.0 ± 0.2	0.4-200	0.9994	0.046	0.9	1.2
MIT	40.5 ± 0.3	0.4-200	0.9991	0.057	1.6	2.0
DIT	60.7 ± 0.3	0.4-200	0.9992	0.072	1.4	1.8
rT_3	101.6 ± 0.6	0.4-200	0.9988	0.093	2.3	2.7
T_3	182.4 ± 0.5	0.4-200	0.9990	0.094	2.5	3.1
T_4	253.0 ± 0.5	0.4-200	0.9994	0.081	1.8	2.2

Table 3 Iodine speciation analysis in CRMs and real urine samples (n=3) [a]

Sample	IO_3^-		I^-		MIT		DIT		rT_3		T_3		T_4		Total iodine	
	Conc. [b]	R [c]	Conc.	R	Conc.	R	Conc.	R	Conc.	R	Conc.	R	Conc.	R	Determined	Certified
GBW09108i	ND [d]	98	61.4 ± 1.6	97	ND	96	ND	97	ND	96	0.9 ± 0.0	94	0.8 ± 0.0	101	65.3 ± 0.8	67.9 ± 9.0
GBW09109g	ND	102	132.3 ± 3.0	99	ND	99	ND	100	ND	101	1.1 ± 0.1	95	1.6 ± 0.1	103	139.2 ± 2.0	142 ± 9.0
GBW09110n	ND	106	181.5 ± 3.3	99	ND	104	ND	103	0.7 ± 0.0	104	1.9 ± 0.1	95	2.7 ± 0.1	105	191.5 ± 2.7	195 ± 10
MU1	ND	103	133.2 ± 6.1	98	ND	102	ND	101	ND	102	1.0 ± 0.1	97	1.5 ± 0.1	105	237.1 ± 3.2	-
MU2	ND	101	177.8 ± 5.7	96	ND	101	ND	102	ND	99	1.6 ± 0.1	93	2.3 ± 0.2	99	184.0 ± 2.5	-
FU1	ND	97	324.4 ± 8.2	96	ND	98	ND	96	0.8 ± 0.0	95	2.4 ± 0.2	93	4.8 ± 0.3	99	335.2 ± 3.9	-
FU2	ND	102	279.6 ± 7.9	97	ND	101	ND	99	ND	98	2.1 ± 0.2	94	4.4 ± 0.2	101	288.4 ± 3.7	-

[a] in μg L^{-1}. [b] Concentration. [c] Recovery (%). [d] Not detected.



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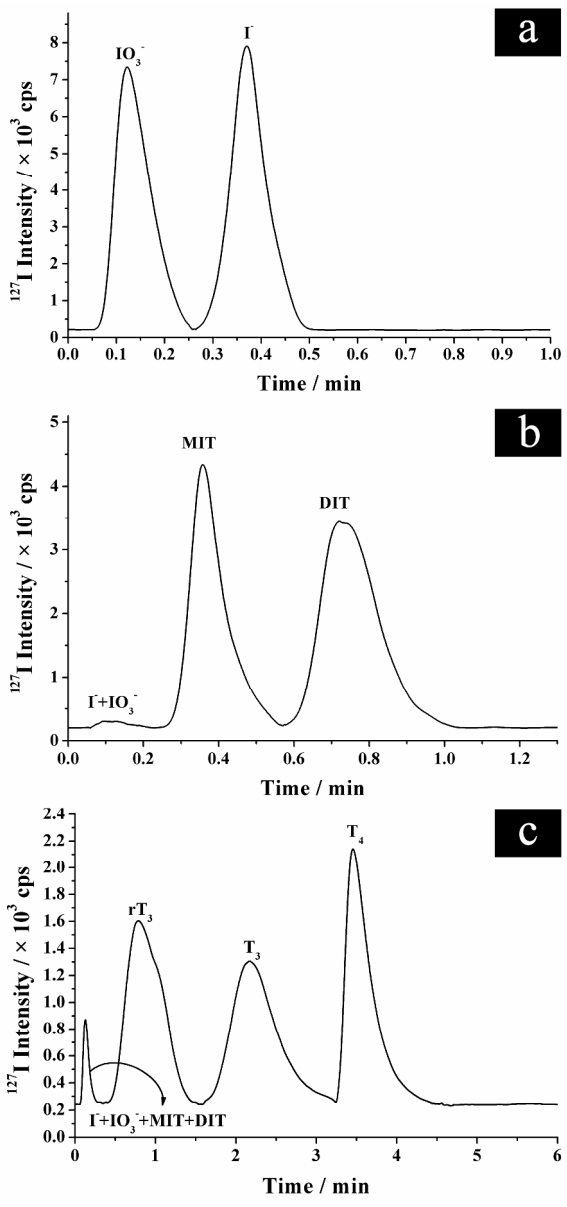

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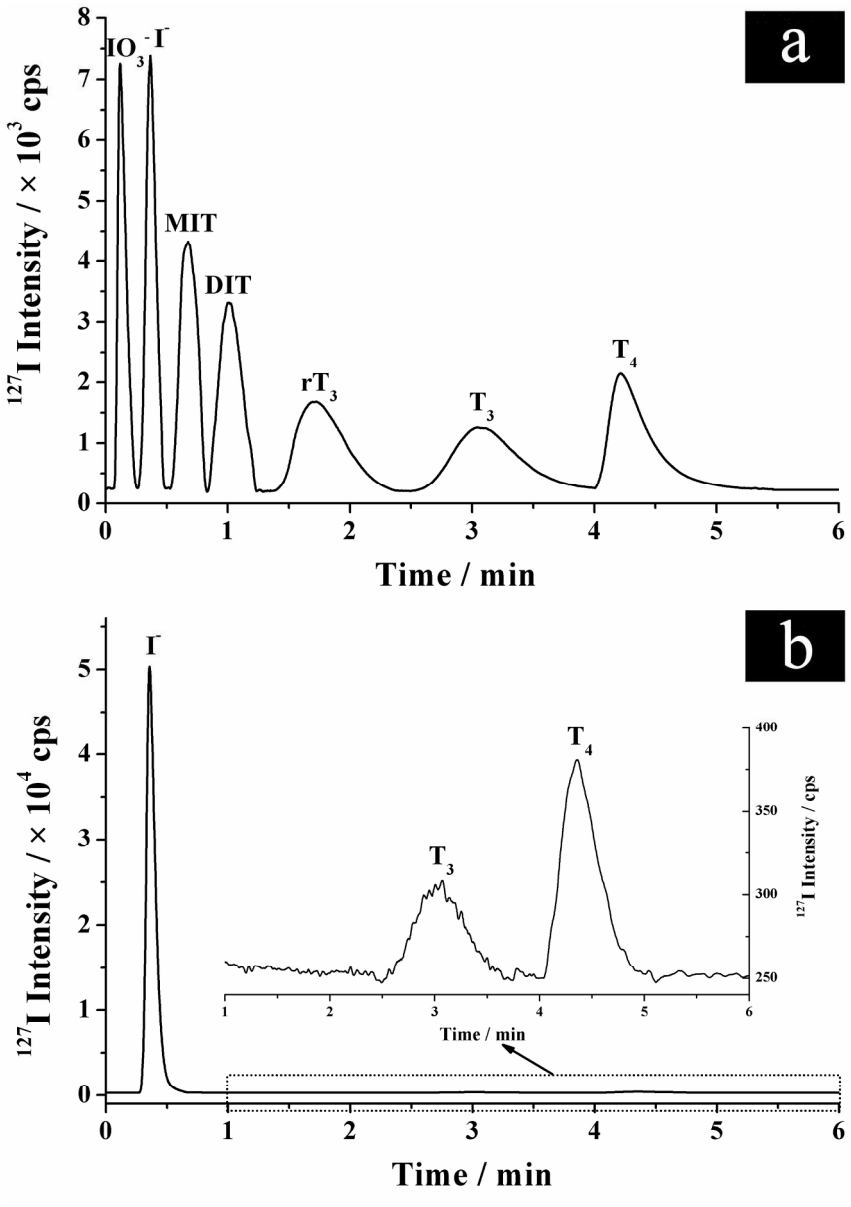

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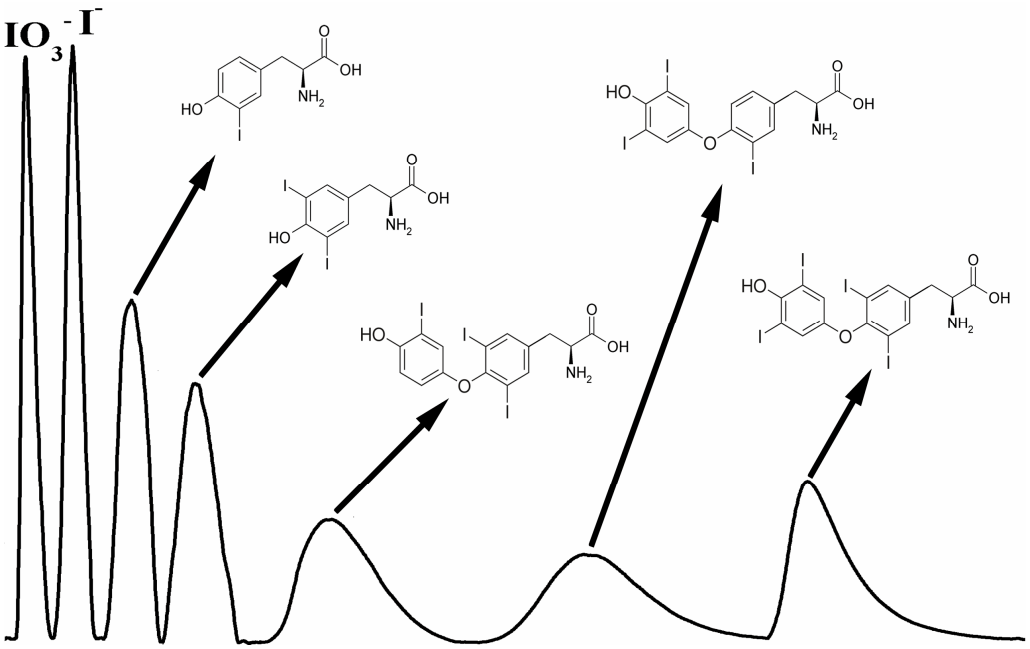
Graphic Abstract



This work proposed a green method for fast separation of seven iodinated forms within 7 min under the gradient elution using three aqueous mobile phases, which was highly efficient, environment-friendly and ICP-MS-compatible.