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High-performance liquid chromatography–ultrasonic nebulizer high-power nitrogen microwave-induced plasma mass spectrometry, real-time on-line coupling for selenium speciation analysis☆

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Abstract

The coupling of a high-power nitrogen (N_2) microwave-induced plasma (MIP) mass spectrometry – (MS) (1.3 kW) with high-performance liquid chromatography, connected with concentric nebulizer (CN), ultrasonic nebulizer (USN) and a hydride generation (HG) systems, for the optimization and determination of selenium compounds, has been carried out. The MIP-MS system fulfils the ideal requirement being an on-line real-time chromatographic detector for Se speciation analysis. Interchanging of MIP-MS system fabricated nebulizer (concentric) with an ultrasonic nebulizer increases about 3.4–12 (peak height) and 6.5–10 (peak area) times ion signals for the selenium compounds. The detection limits for selenate, selenite, trimethylselenonium ion (TmSe), selenomethionine (Semet) and selenoethionine (Seet) (in Milli-Q-water) obtained with the optimized HPLC–USN-N_2MIP-MS system are 0.11, 0.14, 0.09, 0.14 and 0.10 $\mu g\,L^{-1}$, respectively, about 12–48 times lower than the HPLC–CN-MIP-MS and 1.5–4.4 (peak height) times lower compared to the HPLC–CN-inductively coupled plasma (ICP)–MS coupling. Considering peak area, the repeatability (R.S.D. for three successive analyses) and intermediate precision (R.S.D. for three successive analyses performed on three different days), achieved for five Se compounds are 0.8–5.6, and 1.1–5.9%, comparable with the HPLC–CN-ICP-MS, HPLC–HG-MIP-MS and HPLC–CN-MIP-MS systems. The combined HPLC–USN-N_2MIP-MS has been adequately applied for the determination of Se compounds in certified National Institute for Environmental studies human urine CRM No. 18. The results reasonably agree with the HPLC–CN-ICP-MS values. This encouraging combination may be an alternative ion source of mass spectrometry for coming generation in regard to the selenium speciation analysis.

Keywords: Microwave-induced plasma mass spectrometry; Mass spectrometry; Nebulizers; Selenium compounds; Organoselenium compounds

1. Introduction

Selenium is an essential element; functions through selenoprotiens [1] but it can be toxic at high concentrations. Toxicity of Se is highly dependent on its compounds and their concentrations [2]. Urinary excretion is the main route of selenium elimination as more than 50% of Se compounds are excreted though urine [3]. Large variations in urinary excretion are found in different parts of the world based on dietary habits and selenium content in diets. In human urine, selenium has been identified as selenite [Se(IV)], selenate, trimethylselenonium (TmSe) and other unknown selenium compounds [4–8].

Several comprehensive reviews of methods concerning Se speciation have been published [9,10]. Ar inductively coupled plasma (ICP)–mass spectrometry (MS) is one of the most widely used and a highly sensitive analytical technique for elemental analysis [1,3,7,11]. The spectroscopic and non-spectroscopic interferences are still serious problems [11] for Se determination. Improvements made in the sampling interface, ion lenses and plasma conditions for

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ArICP-MS [12,13] in recent years reduces the background spectra and some sources of interferences. Argon-related interferences are till problem in ICP-MS. However, collision cells or dynamic reaction cells designed to suppress Ar-related polyatomic species [14–16]. High-resolution ICP-MS separates most of the polyatomic interfering ions from the analyte in the different resolution mode, but the detection limits of analyte decreases with the increasing resolution [17], and the instruments are very expensive. The Ar-associated interference can be avoided by applying other than Ar or mixed gasses with Ar and by using several types of microwave-induced plasma (MIP) sources [18–24]. The Ar, He and nitrogen MIPs [17–23] discharges have been studied as ion sources for plasma MS. HeMIPs are an appealing alternative to ArICP-MS due to high sensitivity, but tuning, difficulties in initiation, and sustainable stable plasma for HeMIP are very difficult and plasma has the low tolerance for wet aerosols [22,23]. The moderate and high-power MIPs (∼300–1300 W) sources, such as plasmas sustained in an Okamoto Cavity offer better analytical performance than those of the ICP discharges at nearly identical power and a more efficient structure than other cavities, including the commonly used Beenakker cavity for a variety of plasmas [17–21]. The major background species were nitrogen related mono/polyatomic ions and background spectral interferences have been not observed above *m*/*z* 45. The primary isotopes of Se and As such as ^{78}Se,$^{+80}Se^{+}$ and $^{75}As^{+}$ can be used for the determination of Se and As. Coupling of LC/HPLC with MIP is rather limited [19,20]. Practically, in all the applications, N_2MIP-MS is mainly devoted for the total elemental analysis. In our publication [21] we describe the feasibility of high-power N_2MIP-MS as an element specific detector for speciation analysis. We have coupled hydride generation (HG) system successfully with N_2MIP-MS and reported the determination of selenite and selenomethionine (Semet) in human urine [21].

In this work, we have successfully coupled the HPLC–ultrasonic nebulizer (USN) with high-power N_2MIP-MS for the separation and determination of Se compounds. Three types of sample introduction mode, i.e. conventional concentric nebulizer, HG system and an USN were used. The USN sample introduction technique was employed to improve the sensitivity and to decrease the solvent load in the MIP. The HPLC–USN-N_2MIP-MS system was optimized and applied to determine of selenium compounds. An assessment of the performance of the USN, concentric nebulizer (CN) and a HG system with HPLC–N_2MIP-MS was carried out. The performance of three sample introduction modes with HPLC–N_2MIP-MS and HPLC–ArICP-MS has been compared in term of analytical figures of merit for five selenium compounds. Finally, selenium compounds in the freeze-dried certified National Institute for Environmental Studies (NIES, Ibaraki, Japan) human urine CRM were determined using HPLC–USN-N_2MIP-MS and HPLC–ICP-MS. The results obtained in this study have been compared with each other and discussed in details.

2. Experimental section

2.1. Reagents and solutions

Certified NIES freeze-dried human urine CRM No. 18 was used as a sample in the present study. The value for the total selenium is 59 ± 9 μg L^{-1} when it is dissolved in 9.57 mL water. The 9.60 mL of pure water was added to the bottle for the reconstitution. All solutions were prepared with Milli-Q (18.3 MΩ cm; Milli-QSP, TOC Water System, Nihon Millipore, Japan) water. The mobile phase for the gel-permeation chromatography was prepared by dissolving 22.8 g tetramethylammonium hydroxide (TMAH; Nacalai Tesque, Kyoto, Japan G.R.; 10% in water) + 2.60 g malonic acid (Nacalai Tesque) in Milli-Q-water, adjusting the pH to 7.9 by adding 2.0 M aqueous ammonia solution (Kanto, Japan, analytical-reagent grade). Finally, the solution was made up to 1000 ml solution with Milli-Q-water (25 mM TMAH + 25 mM malonic acid; LC-1).

All reagents were of analytical grade and were used without further purification. Sodium selenate (selenate) was purchased from Fluka, sodium selenite pentahydrate (selenite) from Merck, Seleno DL-methionine (Semet), seleno-DL-cystine (Secys), seleno-DL-ethionine (Seet), from the Sigma and trimethylselenonium iodide (TmSe), from TRI Chemical Lab., Japan.

2.2. Nitrogen microwave-induced plasma mass spectrometry (N_2MIP-MS)

A P-6000 N_2MIPMS (Hitachi, Japan) was used for this study. The microwave power (2.45 GHz, 1.5 kW max) is produced by a magnetron (H3862: Hitachi) operated by a dc power supply and fed to a cavity known as Okamoto Cavity [19–22] through a rectangular waveguide (WJR-2). Circulating of the cold water from a refrigerator cools the cavity. Operating conditions of N_2MIP-MS are: frequency; 2.45 GHz, forwarded and reflected microwave powers; 1.3 kW and <20 W, plasma gas flow 15 L min^{-1}, carrier gas flow; 1.1 L min^{-1}, peak point 10/mass, dwell time 2.0 ms, number of sweeps 1500 times, nebulizer (Meinhard) concentric, temperature of spray chamber <5 °C, sampling concentration (Pt) 0.8 mm orifice, skimmer concentration (Pt) 0.4 mm orifice, sample uptake rate 1.5 L min^{-1}. Measuring parameters are: $^{76}Se^{+}$, $^{77}Se^{+}$, $^{40}Ar^{37}Cl^{+}$, $^{78}Se^{+}$, $^{80}Se^{+}$, $^{81}Br^{1}H^{+}$, $^{82}Se^{+}$, at *m*/*z* 76, 77, 78, 80, 82; total analysis time 1800 s; quadrupole mass analyzer: QMG420-4 Balzers, Liechtenstein; Mo rods, 200 mm length, 8 mm diameter, radiofrequency of 2.25 MHz, ion detector Channeltron electron multiplier 4831 G, Dalileo, USA, mounted on a quadrupole analyzer with 90° ion deflection and off axis; pulse counter-pulse amplifier C3866 Hamamatsu Photonics, resolution 10 ns, maximum counting rate 10^7 cps.

A HP-4500 ArICP-MS system (Yokogawa Analytical Systems, Japan) was used for this study. The operation conditions of ArICP-MS are: radiofrequency power forwarded

1.2 kW; reflected <10 W, gas argon, plasma gas flow 14.4 L min^{-1}, Auxiliary gas flow 1.04 L min^{-1}, Nebulizer gas flow 1.04 L min^{-1}, Data acquisition mode time resolved analysis, detector mode pulse, nebulizer (Meinhard) concentric, temperature of spray chamber <5 °C, sample uptake rate 1.0 L min^{-1}. Monitored signals $^{76}Se^{+}$, $^{77}Se^{+}$, $^{40}Ar^{37}Cl^{+}$, $^{81}Br^{1}H^{+}$, $^{82}Se^{+}$, at *m*/*z* 76, 77, 78, 82, total analysis time 1800 s.

A Perkin-Elmer FIAS 100 hydride generation system equipped with a gas–liquid separator was used for the HG. The HPLC separated selenium compounds were reacted with the alkaline sodium tetrahydroborate selectively in the HG system [21]. The gaseous products were separated from the liquid in the gas–liquid separator and flushed into the MIPMS nebulizer by a stream of nitrogen gas (126 mL min^{-1}) with 400 mm long PTFE (1.0 mm i.d.) tubing. The operation conditions for HG system are: $NaBH_4$ (0.3% in 0.2% NaOH) flow 4.5 mL min^{-1}, HCl (3.0 M) flow 5.6 mL min^{-1}, sample loop 100 μL, mobile phase flow 1.0 mL min^{-1}, Nitrogen (carrier) gas flow 126 mL min^{-1}. More details about the MIP-MS, ICP-MS and the connections of HG with HPLC have been described previously [19,22–25].

A Meinhard concentric-type nebulizer (Hitachi Electric, Japan, part no. P97M170, 300–8350) and an USN (U5000AT Ultrasonic Nebulizer, CETAC Technologies, NE, USA) with 300 mm × 0.25 mm i.d. PEEK (polyether ether ketone) capillary tubing with a desolvation system were also used for the sample introduction systems. The detailed operation conditions of the USN are: instrument-CETAC, U-5000AT, including desolvation system (heater and cooler systems), Sample uptake rate 1.0 L min^{-1}, desolvation heating 140 °C, cooling 5 °C. The exit of the HPLC column was connected directly to the nebulizers/HG system via the PEEK tubing. A Neslab refrigerating circulator was used to maintain the temperature of the glass spray chamber at 5 °C when the Meinhard concentric-type nebulizer was in use. The up take rate of the normal aqueous sample solution was about ∼0.3 mL min^{-1} when the flow rate of the nitrogen gas (carrier) was 1.1–1.4 L min^{-1}. But with HPLC connection using LC-1 mobile phase at a flow rate of 1.0 mL min^{-1}, the plasma was quite stable.

2.3. Chromatography

The HPLC system consisted of a Shimadzu LC-6A solvent delivery unit (Shimadzu, Japan)/Perkin-Elmer Model Series 410 B10 solvent delivery unit (Perkin-Elmer, Norwalk, CT 06856, USA) and a Rheodyne 9725 six-port injection valve (Cotati, CA, USA) with a 100 μL injection loop. Separations were performed on a gel-permeation column (50 cm × 7.6 mm i.d., 9 μm, polyvinyl alcohol-based resin), connected with a guard cartridge (GF 1G, 7B, Shodex, Showa Denko, Japan). Column was equilibrated by passing at least 100 mL (flow rate 1.0 mL min^{-1}) of the mobile phase through the column before any injection of the selenium compounds. The outlet of the column was connected directly to the nebulizers/HG system with PEEK capillary tubing. The ion signals at *m*/*z* 76, 77, 78, 80 and 82 were recorded with the time-resolved analysis software version of Hitachi, Japan. For quantification, the chromatograms were exported, peak areas and peak heights determined, concentrations were calculated with external calibration curves and with standard addition technique.

3. Results and discussion

3.1. Chromatographic separation of selenium compounds

In the present study, a gel permeation column based on polyvinyl alcohol-based resin, GS-220 was used for the separation of selenium compounds. A gel-permeation chromatographic system (GS-220 column) is found to be especially suitable for the speciation of Se compounds because of its high separation efficiency and durability against the injection of large sample size. The column is stable in high matrix samples like urine as high as 100 μL. The HPLC mobile phase has been optimized for Se compounds by changing the mobile phases with varying pH conditions. The optimized separation conditions for selenous acid, selenic acid, Semet, Seet and TmSe were obtained at pH 7.9, with LC-1 (Fig. 1). Although, GS-220 is made of polyvinyl alcohol, a neutral and hydrophilic compound, it has both negative charge and hydrophobicity character. Negative charge comes from carboxyl groups, which is introduced during polymerization procedure on the resin while hydrophobicity comes from double bonds, added to harden the resin in order to resist mechanical stress during HPLC

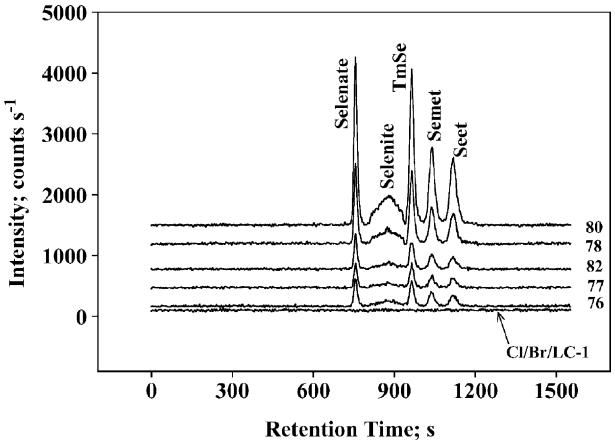

Fig. 1. Chromatogram obtained with a solution of selenate, selenite, trimethylselenonium ion (TmSe), selenomethionine (Semet) and selenoethionine (Seet) (48.5, 48.0, 50, 44.8 and 30.8 μg Se L^{-1}, respectively) in Milli-Q-water, on a GS 220 gel-permeation column with USN-N_2MIP-MS (optimized conditions see Table 1 mobile phase 25 mM TMAH + 25 mM malonic acid at pH 7.95, injection volume 0.100 mL, flow rate 1.0 mL min^{-1}); baseline (B) counts have been enhanced by 100, 400, 1100, 1400 and 700 for *m*/*z* 76, 77, 78, 80 and 82, respectively.

stage. These two help to improve separation of various compounds. In other words, GS-220 separates compounds based on not only the size of the molecule but also the charge-state and hydrophobicity. Selenate at pH 7.9 carries two negative charges. So, it is repelled by the negatively carboxylic group of the resin and elutes in the front of the chromatogram (Fig. 1). Increasing pH, repulsion between carboxylic group and selenate may increase. Hence, this anionic repulsion may be responsible for decreasing the retention time of selenate with increasing pH values. Selenous acid is about 25% mono-negatively and about 75% di-negatively charged at pH 7.9. The lower anionic repulsion with stationary phase compare to selenate may be accountable for the weak retention of this compound on the column elutes slowly after the selenate, and that generates broad peak. A more details about the separation methods were described previously [7,8]. The LC-1 mobile phase provides the organic compounds (TMAH and malonic acid) and is matching well with the limited loading of the MIP source. The normal aqueous sample solution up take rate in this N_2MIP is very low and about 0.3 mL min^{-1} when the flow rate of nitrogen gas (carrier) is in the range of 1.1–1.4 L min^{-1}. But with the HPLC connection using LC-1 mobile phase at a flow rate of 1.0 mL min^{-1}, the plasma is quite stable. The high-power N_2MIP produces the annular (doughnut) shaped plasma that has a superior operational stability and a higher tolerance for the injection of high aqueous aerosol samples [20]. This plasma is robust and not extinguished even if an air sample is injected. So, the high mobile phase flow rate (1.0 mL min^{-1}) has adjusted adequately with the MIP system. The relative intensities of ions and signals for each selenium compound (ions signals) are different (Table 1). Ion intensity is highest for the Seet and lowest for the Semet (peak area). But, the ion signals for selenate and selenite are highest and lowest, respectively, considering the peak height measurement. The variability of the ion signals for Se compounds may be based on different analytical residence time of the analytes in the plasma. The different analytical residence time of the analytes in the plasma reaches to the variable decomposition rate of the Se compounds, and that ultimately increases/decreases the central analyte rich region. Coupling of the HPLC to the MIP-MS is straightforward; the HPLC column is simple connected to the nebulizers. The USN and MIPMS were adjusted well. Hence, the

Table 1
Total counts[a] of different selenium species with two different nebulizing systems

Selenium species (100 μg Se L^{-1})	HPLC–CN-MIP-MS, area	HPLC–USN-MIP-MS, area
Selenate	5,230 ± 250	34,100 ± 540 (6.5)
Selenite	4,680 ± 175	37,700 ± 960 (8)
TmSe	5,430 ± 156	39,500 ± 630 (7.3)
Semet	2,780 ± 120	28,500 ± 410 (10)
Seet	3,950 ± 165	40,500 ± 420 (10)

x: fold increased value compared to HPLC–CN-MIP-MS.
[a] Average of three determinations.

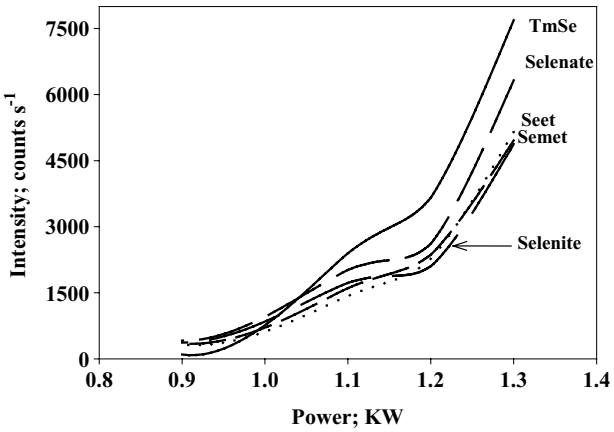

Fig. 2. Dependence of ion signals of the five selenium compounds (selenate, selenite TmSe, Semet and Seet) on the microwave power using HPLC–USN-N_2MIP-MS (conditions same as in Fig. 1).

MIP-MS has been performing well as an on-line real-time chromatographic detector for Se compounds analysis.

3.2. Optimization of experimental parameters

In an attempt to obtain the highest sensitivity for five selenium compounds, the influence of various operation parameters were studied on the analytical performance of the HPLC–USN-MIP-MS system and optimized individually using the mixture of five selenium compounds. Increasing the microwave power, ion signals (peak area and peak height) for the selenium compounds have increased sharply up to 1.3 kW (Fig. 2). The manufacturer does not allow a further increase of power. A 1.3 kW microwave power was used during the measurements. It has been found that the background counts (peak areas and peak heights) increase with increasing the MW power for the selenium isotopes (*m*/*z* 76, 77, 78, 80 and 82). Fig. 3 (inset) shows the increase

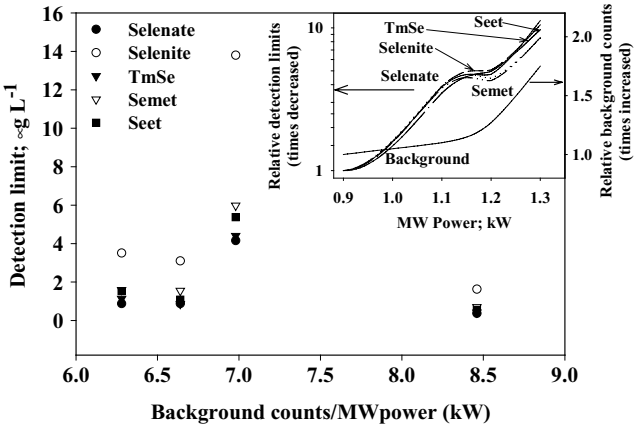

Fig. 3. Dependence of detection limits of selenium compounds with background counts/microwave power (kW) using HPLC–USN-N_2MIP-MS (conditions same as in Fig. 1). Inset: variation of relative detection limits of Se compounds and relative background counts for *m*/*z* 80 with microwave power (kW).

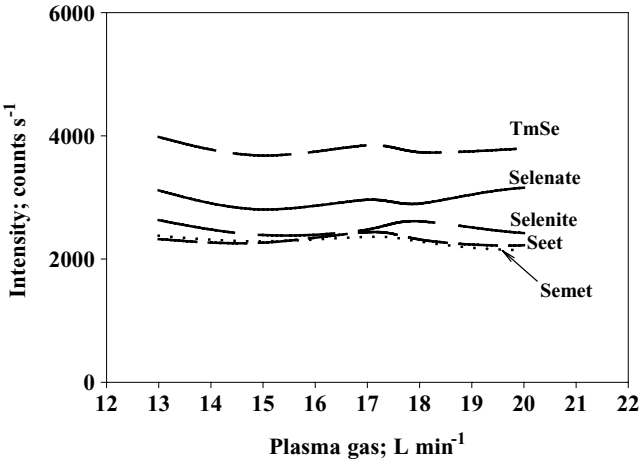

Fig. 4. Dependence of ion signals of the five selenium compounds on the plasma gas using HPLC–USN-N_2MIP-MS (conditions same as in Fig. 1).

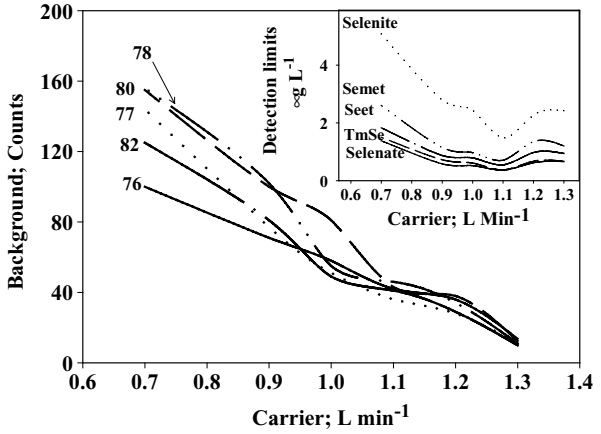

Fig. 6. Variation of background ion signals of *m*/*z* 76, 77, 78, 80 and 82 with carrier gas flow using HPLC–USN-N_2MIP-MS (conditions same as in Fig. 1). Inset: variation of detection limits for five selenium compounds (selenate, selenite TmSe, Semet and Seet) on the carrier gas flow rate.

of relative background counts against MW power for *m*/*z* 80. It was observed that the relative background ion signals for *m*/*z* 76, 77, 78, 80 and 82 increases linearly with increasing MW power. But, the ion signals for selenium compounds increase about 10 times. So, ionization and retention of Se compounds in the plasma was increased (one-fold) with increasing MW power. Plasma gas flow rate (13–20 L min^{-1}) does not alter the ion signal of Se compounds significantly (at 1.3 kW, Fig. 4). The minimum variation of ion signals with plasma gas up to 20 L min^{-1} is due to the stability of the plasma region. The increase of the carrier gas flow rate first gradually increases the ion signals of the Se compounds then decreases (maxima at 1.0 L min^{-1} for selenite; 1.1 L min^{-1} for selenate, TmSe, Semet and Seet; Fig. 5). This effect probably results from an increase in the amount of aerosol carried into the plasma per unit time and decrease in the residence time of the analyte species in the plasma. The 1.0–1.1 L min^{-1} carrier flow rate (0.7–1.3 L min^{-1}) executes optimize signals for Se species when both peak area and

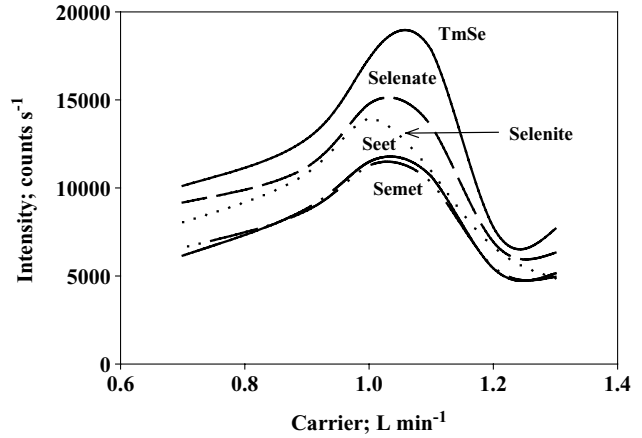

Fig. 5. Dependence of ion signals of the five selenium compounds (selenate, selenite TmSe, Semet and Seet) on the carrier gas using HPLC–USN-N_2MIP-MS (conditions same as in Fig. 1).

peak height measurement are considered. The background counts were dramatically reduced when the gas flow rate was increased from 0.7 to 1.3 L min^{-1} as shown in Fig. 6. Background counts most likely depend on by the photon emission from the plasma. When the flow rate is below 1.0 L min^{-1}, the gas would not pass through the plasma by forming a central channel, causing a stronger photon emission in the sampling area. Therefore, the Se compounds would spread throughout the plasma, rather than be concentrically located along the channel as occurs under normal ICP or MIP operating conditions, and this dispersion will lead to a low signal intensity and high background level. Obviously, a higher gas flow rate has lowered the background counts because photons are not deflected. The photon noise are also reduced with increasing carrier flow, but limitations on the gas flow rate due to the lowering of the plasma temperature and the dilution effect on the Se compounds have to be considered and a compromise has to be made. The detection limits for Se compounds were found to be decreased with increasing carrier flow up to 1.1 L min^{-1} (Fig. 6, inset). However, it is true that over 1.1 L min^{-1}, the DL does not increases significantly. Thus, we have used a carrier flow rate of 1.1 L min^{-1} for the experiment.

3.3. Effect of sample introduction on the plasma characteristic

The ionization efficiency of Se (IP 9.75 eV) using a CN is reported to be 31% for ArICP, and much lower value for MIP sources [25]. It has been found that the replacement of the conventional nebulizer with the USN, the ion signals for Se compounds were increased at least 6.5–10 (considering peak area) and 3.4–12 (considering peak height) times (Table 1). CN produces considerable numbers of big particles; those are introduced into the plasma. The poor ionization efficiency for Se compounds with bigger particles, and

different ionization regions for the bigger and the smaller particles result in significant dilution of the analyte ion. The USN produces aerosols of higher number density and more uniform and smaller particle size distribution than the CN [36]. The smaller particles are more rapidly desolvated; easily transported; and ionized efficiently in the MIP compared to the aerosol generated from the CN. These facts clearly illustrate the USN causes better efficiencies for the Se compounds. Thus, the USN is recommended and used for the measurements.

3.4. Evaluation of detection limits, repeatability and intermediate precision for selenium compounds between the HPLC–USN-MIP-MS, HPLC–ICP-MS, HPLC–MIP-MS and HPLC–HGMIP-MS systems

The detection limits (3σ of the blank; $n = 5$; Table 2) found in HPLC–USN-MIP-MS for the five selenium compounds are about 12–48 (peak area); 6–20 (peak height) times better than those of the HPLC–CN-MIP-MS, and 1.5–4.4 (peak area) times better than HPLC–CN-ICP-MS (*m/z* 82). The detection limits for Semet (6 times) and Seet (10 times) are also improved compare to HPLC–HG-MIP-MS (Table 2). The values indicate that the detection limits of selenium species improve excellently with using online HPLC–USN-MIP-MS. Additionally, the detection limits obtained with the HPLC–CN-ICP-MS are about 8–30 (peak height) and about 6–31 (peak area) times lower compare to the detection limits obtained using the HPLC–CN-MIP-MS (Table 2). The detection limit of selenium is about 1–2 orders of magnitude lower than those reported for other CN-N_2MIP systems [18–20]. The main reasons for lowering the detection limit may be a difference in the microwave power (500 W/1300 W) that has been used and a different in the shape (annular) of the plasma formed. But due to the high ionization potential of selenium the detection limit in the CN-N_2MIP is higher than that for ArICP-MS (Table 2). The higher detection limit of selenium in N_2MIP-MS is because of the interference of the high concentration of $^{30}NO^+$ in the nitrogen plasma [18–20]. The detection limits of elements with high ionization energy of more than 8.0 eV is about one order of magnitude worse than those for ArICP-MS as the degree of ionization of these elements are in the range 0.3–25% in the N_2MIP and 15–85% in the ArICP. Another reason for the difference in the detection limits between N_2MIP and the ArICP is attributed due to the difference in the plasma temperature, which is higher for the ArICP. The repeatability (R.S.D.s %) of the investigated selenium compounds using HPLC–USN-MIP-MS (Table 3) are in the range of 0.8–5.6%, which are comparable with the HPLC–CN-MIP-MS (3.4–8.2%), HPLC–CN-ICP-MS (2.4–5.1%) and HPLC–HG-MIP-MS (3.1–5.4%; Table 3). The intermediate precision using the HPLC–USN-MIP-MS (1.1–5.9%; Table 3) is better/comparable than that of the HPLC–CN-ICP-MS (1.5–7.6%; Table 3), HPLC–CN-MIP-MS (2.5–8.7%; Table 3) and the HPLC–HG-MIP-MS (4.9–9.9%). Thus, the fact that the USN connected to the HPLC–MIP-MS was found to be superior to the normal HPLC–CN-MIP-MS and also to the HPLC–CN-ICP-MS in terms of lower detection limits is an important result of this work.

Table 3
Repeatability[a], intermediate precision[b] and correlation coefficients[c] (r^2) for selenium compounds in HPLC–USN-MIP-MS^{X1}; HPLC–CN-ICP-MS^{X2}; HPLC–CN-MIP-MS^{X3} and HPLC–HG-MIP-MS^{X4}

Analyte	Repeatability				Intermediate precision				r^2
	X1	X2	X3	X4	X1	X2	X3	X4	
Selenate	0.8	3.5	4.6	–	1.1	1.5	2.5	–	0.998
Selenite	2.4	5.1	8.2	3.1	2.1	7.6	8.3	4.9	0.997
TmSe	3.9	2.5	5.7	–	4.0	3.4	6.2	–	0.999
Semet	5.5	3.4	3.4	5.4	5.6	5.3	8.7	9.9	0.998
Seet	5.6	2.4	3.9	4.8	5.9	7.0	6.9	7.9	0.999

[a] Repeatability is determined from peak areas by calculating the relative standard deviation (R.S.D. of three successive analyses. Concentration of each analyte was 50.0 μg Se L^{-1}).
[b] Intermediate precision is determined for peak areas by calculating the RSD of three analyses performed on three different days; concentration for each analyte was 100 μg Se L^{-1}.
[c] Concentrations range is 0.99–50.0 μg Se L^{-1} for selenium compounds.

3.5. Analytical performance

The performance of the HPLC–USN-N_2MIP-MS has been characterized by the linearity of the standard curves, repeatability, intermediate precision and limits of detection

Table 2
Detection limits[a] for selenium compounds (using peak area)

Analyte	Detection limit (μg Se L^{-1})				
	HPLC–USN-MIP-MS	HPLC–CN-ICP-MS	HPLC–CN-MIP-MS	HPLC–USN-MIP-MS in urine matrix	HPLC–HG-MIP-MS
Selenate	0.11	0.17	5.34	0.28	–
Selenite	0.14	0.26	6.27	0.34	0.12
TmSe	0.09	0.18	1.05	0.22	–
Semet	0.14	0.42	2.42	0.34	0.84
Seet	0.10	0.44	2.27	0.23	0.95

[a] Detection limits are determined as the elemental concentrations giving a signal three times the standard deviation ($n = 5$) of the blank (Milli-Q-water injected with LC-1 mobile phase).

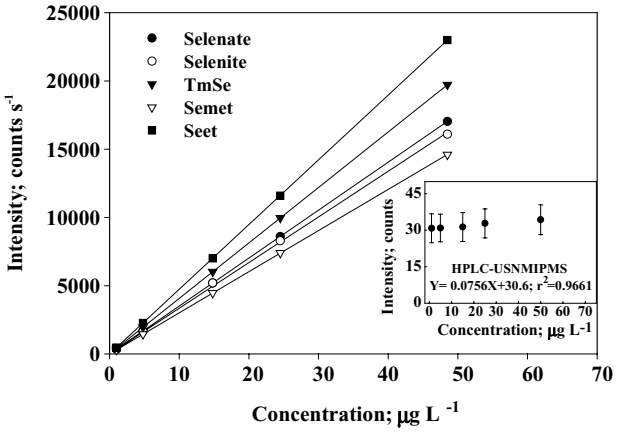

Fig. 7. Analytical curves and variation of the ion signals with concentrations for five selenium compounds (selenate, selenite TmSe, Semet and Seet) using HPLC–USN-N_2MIP-MS (conditions same as in Fig. 1). Inset: dependence of background ion signals on the concentration of selenium in HPLC–USN-N_2MIP-MS (conditions same as in Fig. 1 and details in Table 1).

(based on 3σ of the reagent blanks) within a series are calculated on three replicate measurements and are listed in the Tables 2 and 3 (Fig. 7). The intensities for the HPLC–N_2MIP-MS signals (on-line injection of the mixture of five selenium compounds) given, indicate that a linear range of at least 2 order of magnitude (Fig. 7). Repeatability is good for all species with respect to peak areas is better than 5.6% (Table 3). The intermediate precision for selenium compounds is better than 5.9% for peak areas. The detection limits, for selenium compounds which are spiked in the NIES human urine (using HPLC–USN-MIP-MS), are about 2.3–2.5 times higher than those calculated in the Milli-Q-water (Table 2). The repeatability (2.3–6.7%) and the correlation coefficient (0.995–0.9999) obtained for the Se compounds, are adequately comparable with those of the HPLC–MIP-MS and HPLC–ICP-MS.

3.6. Interference and background study

During our urine analysis by ICP-MS, monitoring ^{77}Se and ^{82}Se modes (*m*/*z* 77 and 82), interfering peaks from $^{40}Ar^{37}Cl^{+}$ and $^{81}Br^{1}H^{+}$ at *m*/*z* 77 and 82 have been appeared on the chromatogram at the retention times of 871 and 1162 s, respectively. The additional $^{40}Ar^{37}Cl^{+}$ peak is overlapped with TmSe peak at *m*/*z* 77 modes. The $^{81}Br^{1}H^{+}$ peak was not found simultaneously in the *m*/*z* 77 mode at the retention time 1162 s. So, Br forms an additional peak in the chromatogram at *m*/*z* 82 mode by forming $^{81}Br^{1}H^{+}$ in Ar-ICPMS.

We have monitored the other major selenium isotopes for five selenium compounds (Fig. 1). LC-1 mobile phase the background signal was increased than the Milli-Q-water (directly injected to the plasma through CN). However, with the USN the background counts under optimize conditions were decreased rapidly 8–47, for *m*/*z* 76, 77, 78, 80 and 82. It is also observed that the DLs for selenium compounds were decreased logarithmically with increasing MW power (Fig. 3, inset). So, it is expected that with further increasing the MW power, the DL for selenium compounds will be improved. Fig. 3, inset, shows the relation between the DL with BC/MW power. With USN the background counts decreased to about 10–40, as desolvation system was used (140 °C) in the USN. The desolvation system helps to remove the solvent vapor (TMAH) due to the evaporation at 140 °C. Consequently, the organic load and the matrix, which are originated from the mobile phase, are decreased in the MIP. The more uniform desolvated aerosols are distributed rather central analyte zone of the plasma. The low dispersion of the analyte leads to a low background level. Hence, the background counts were decreased with using the USN compared to CN. It is observed that with increasing the selenium compounds concentrations the background counts does not changes significantly (Fig. 7, inset) in the HPLC–USN-MIP-MS.

3.7. Selenium compounds in NIES human urine

To authenticate the developed HPLC–USN-MIP-MS method, the NIES human urine CRM No. 18 is analyzed for the determination of selenium compounds (Fig. 8). The peaks are identified according to the retention times (Fig. 1) with those of the authentic standards and confirmed also by spiking with the standard selenium compounds (Fig. 8). Selenate, TmSe and two unknowns Se compounds are detected and reconfirmed by HPLC–ICP-MS using various columns with different mobile phases described previously [8]. TmSe, confirmed by spiking with standard TmSe in the NIES human urine (Fig. 8), is estimated using standard addition technique. The experimental value is 3.04 ± 0.5 µg L^{-1}, agreeable with the HPLC–ICP-MS value (3.42 ± 0.2 µg L^{-1}) and the HPLC–MIP-MS (3.0 ± 0.8 µg L^{-1}).

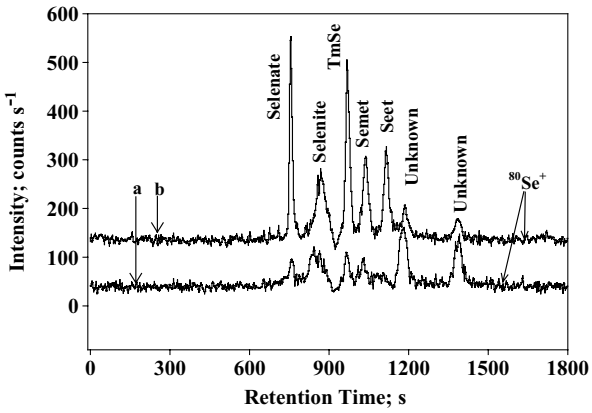

Fig. 8. Chromatogram obtained (a) for the reconstituted NIES human urine CRM No. 18, and (b) urine (1:2 with Milli-Q-water) spiked with 8.5, 8.5, 8.2, 6.6 and 6.9 µg Se L^{-1} of selenate, selenite, TmSe, Semet and Seet, respectively, on GS-220 gel-permeation column; 100 µL injected (conditions same as in Fig. 1).

The unknown selenium compounds are present as a major selenium species in the urine followed by selenite, selenate and TmSe (Fig. 8). The value of TmSe, thus, found will give an idea about the presence of TmSe in NIES Urine and the concentration found should be rechecked with different procedures. With the direct injection of the 100 μL urine, the separation of selenium compounds in GS-220 column is adequate (Fig. 8). The percentage recoveries of spiked selenium compounds in the urine are in the range of 95–103%. So, urine matrix has minimum influence to suppress the ion signals of selenium compounds.

4. Conclusions

High-power doughnut-shaped nitrogen MIP-MS coupled with HPLC is a powerful selenium speciation analysis technique due to the high sensitivity and low susceptibility of interference. The USN, an optimized sample introduction system, has enhanced the sensitivity. With USN, the N_2MIP is much more efficient for ionization of Se compounds, than the CN technique. Therefore, HPLC–USN-N_2MIP-MS enables to determine the Se compounds in ultratrace levels. The detection limits, repeatability, intermediate precision of the system are adequate for the determination of selenium compounds.

The limited information has been available for elemental speciation using HPLC–USN/HG/CN-N_2MIP-MS. N_2MIP-MS eliminates the argon related polyatomic interference. The major isotopes of ^{78}Se and ^{80}Se can be used for the analysis. Interference arising from the urine matrix in ICP-MS system has been overcome. Hence, N_2MIP-MS will be very useful detector for Se compounds estimation in samples with high matrix. Se compounds can be determined using all Se-isotopes (*m*/*z* 74, 76, 77, 78, 80 and 82; natural abundance 0.87, 9.00, 7.60, 23.5, 49.8 and 9.2%). The HPLC–USN-MIP-MS coupling is promising, reliable, and successfully applied for the analysis Se compounds in NIES candidate human urine. Accurate and precise determination of trace levels of Se compounds is very important. The results, however, clearly indicate that MIP-MS system now has enough capability to act as an on-line real-time chromatographic detector for the determination of Se species in human urine.

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