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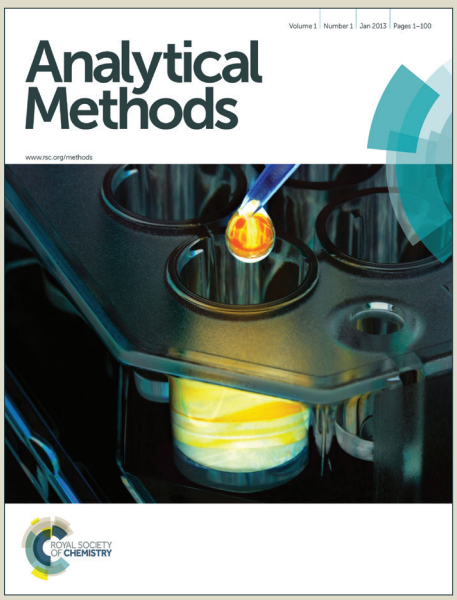

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Nanogram level quantification of molybdenum(VI) by novel hyphenated SDME/DRS-FTIR in human biological fluid

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Abstract:

A novel hyphenated single–drop micro–extraction (SDME) diffuse–reflectance Fourier–transform infrared spectroscopy (DRS–FTIR) were employed for quantification of hexavalent molybdenum, i.e., Mo(VI) in human blood serum and urine. Mo(VI) was extracted as Mo(VI):HDPBA complex in single–drop of dichloroethane solution of N^1–hydroxy–N^1,N^2–diphenylbenzamidine (HDPBA). Mo(VI) formed 1:2 complex in organic phase with HDPBA in acidic medium, which was further analyzed by diffuse–reflectance Fourier–transform infrared spectroscopy (DRS–FTIR). The factors affecting for SDME process, such as extraction solvent, size of the acceptor drop volume, pH, reagent concentration, extraction time and stirring rate were optimized for better extraction efficiency. The extracted micro–drop was analyzed using DRS–FTIR, the most steady and the strongest vibrational IR peak at 911±2 cm^{-1} (υ_1) corresponding to asymmetric stretching mode of MoO_4^{2-} was selected for quantification of Mo(VI). The limit of detection (LOD) and limit of quantification (LOQ) for method were 8.0 ng mL^{-1} and 26.4 ng mL^{-1}, respectively. The absorbance and peak area were determined by SDME/DRS–FTIR method, which showed excellent linearity with correlation coefficient value of 0.99 for the concentration range of 1–100 ng mL^{-1}. The standard deviation (SD) and relative standard deviation (RSD) for 10 replicate measurements were found to be 0.13 ng mL^{-1} and 1.3%, respectively, at a level of 10 ng mL^{-1} Mo(VI) in aqueous phase.

Key words: Single-drop micro-extraction, HDPBA, Mo(VI) determination, quantitative IR peak, DRS-FTIR, blood serum and urine

1. Introduction

Molybdenum is regarded as a vital micronutrient for human as its importance relies on the fact that it forms a significant co–factor complex in various oxotransferases enzymes likes xanthine oxidase, aldehyde oxidase and sulphite oxidase.[1,2] Generally, the form of molybdenum that is taken up by most of the biological systems is molybdate, i.e., MoO_4^{2-}, in the form of Mo(VI), which is a colourless, highly water–soluble dianion in which molybdenum is in its maximum 6^+ oxidation state.[3] However, the high content of Mo(VI) in environment is harmful for humans and animals.[4] Mo(VI) is responsible for drop off in catalase activity that can result in a diseased condition known as podagric syndrome in which there is rise in uric acid concentration. It can also lead to alteration in blood pressure and shrink cholesterol transport.[5] The daily requirement of Mo(VI) in humans is around 25 µg or possibly less while intake of 150 µg/kg body weight may be toxic.[6] The uses of Mo(VI) as an important constituent of metal alloys, lubricants, chemical catalysts, integrated circuits, anti–friction coatings, aircraft parts, missile parts, silicon powder devices etc. in various industries are main sources.[7] Relatively high concentration of Mo(VI) is present in particulate matters emitted from combustion of fossil fuels as well as by weathering and the use of its compounds in agriculture (ammonium molybdate as fertilizer) etc.[8,9]

Determination of very low concentration of Mo(VI) in biological materials (animal and human tissues, blood, and urine) is extremely difficult and many problems need to be solved. Therefore, the development of new methods for the determination of the micro–contents of Mo(VI) and its compounds with low–detection limit, high–sample throughput, applicability to low sample size, etc. particularly in biological samples where sample size is a real challenge to the analytical chemists is matter of vital importance. Mo(VI) has been determined by variety of established methods for instance atomic absorption spectrometry (AAS) has been employed for trace elemental analysis.[10–12] Neutron activation analysis (NAA) have also been reported.[13] For better results some hyphenated techniques have also been employed such as inductively coupled plasma–mass spectrometry (ICP–MS), inductively coupled plasma–atomic emission spectrometry (ICP–AES), flow injection analysis (FIA) and graphite furnace atomic absorption spectrometry (GF–AAS) etc.[14–17]

Due to presence of Mo(VI) in biological samples at low levels, not only its separation from associated elements is necessary, but also the use of a pre–concentration method is usually inevitable. The determination of a metal typically involves an extraction method for the isolation and enrichment of components of interest from a sample matrix. For pre–concentration of metals there are variety of procedures that have been reported, such as solid phase extraction (SPE),[18–20] liquid-phase micro-extraction (LLE),[21] and co–precipitation.[22,23] Novel liquid–liquid extraction (LLE) methods have used liquid membranes and hollow fiber contractors for extraction of Mo(VI).[24] In addition, cloud point extraction (CPE) methods have also been employed.[25–27] But the major drawback in this practice is the increased background and low separation.[28] SPE being a solventless technique might prove to be an efficient method but large amount of eluents and long extraction time make it an unreliable choice. LLE techniques employing ionic liquid suffers with the problem of large volume of ionic liquid requirement. Ionic liquid based dispersive liquid–liquid micro–extraction (IL–DLLME) also employs tedious and expensive process.[7] Liquid–liquid solvent extraction (LLSE) being a

conventional method of extraction uses bulky amount of hazardous solvents.[29] To overcome such types of drawbacks many development in the extraction method have been done such as solid–phase micro–extraction (SPME), liquid–phase micro–extraction and single–drop micro–extraction (SDME).[30,31] In SDME approach, the analyte is generally extracted from aqueous sample to small amount of organic phase. Some of the recently proposed organic reagent for the determination of Mo(VI) include 2–n–octylaminopyridine,[6] sodium diethyl dithiocarbamate,[8] thiocyanate,[16] 4–(2–hydroxyl phenyl ethaminodiol), benzene–1,3–diol,[32] pyrocatechol violet,[33] 4–hydroxybenzaldehydethiosemicabazone,[34] etc. However, most of these methods suffer from number of limitations such as interference, low–metal recovery, and lack of sensitivity, stringent reaction condition and high–blank value. In the present work an attempt has been made to develop a novel single–drop micro–extraction using N^1–hydroxy–N^1,N^2–diphenylbenzamidine (HDPBA) combined with diffuse–reflectance Fourier–transform infrared spectroscopy (DRS–FTIR) first time for the determination of Mo(VI), at 911±2 cm^{-1} after spiking the micro–extract over KBr substrate. The optimization procedure for SDME/DRS–FTIR determination and the merits of the method are discussed.

2. Experimental

2.1 Instrumentation

All spectral scans in the region 4000–400 cm^{-1} were made employing a diffuse–reflectance Fourier–transform infrared spectrometer (DRS–FTIR) equipped with deuterated, L–alanine doped triglycine sulfate (DLaTGS) detector (Model: Nicolet iS10, Thermo Fisher Scientific Instrument, Madison, USA). Sartorius electronic balance with 10 µg precision (Model– CP225D, AG Gottingen, Germany) was used for gravimetric measurement. Micropipette, GalaxoSmithKline Pharmaceuticals Ltd., Finland was used for handling liquid volumes (10–100 µL). A systronics digital pH meter type 335 was employed for the measurement of pH value of solution. Calibrated glass apparatus were used for volumetric measurements. All glassware were cleaned prior use with Ultrasonic cleaning bath, PCI analytics Pvt. Ltd., India, Model 3.5L100H/DIC using mild detergent and after proper washing, rinsed with distilled water. Special care was taken during handling of all glassware to avoid any possible contamination and to maintain the sensitivity of the method. Thermo Fisher Scientific Barnstead Smart2pure, ultra pure water system (conductivity 18.2 Ω) was used to obtain ultra pure water for preparation of aqueous solutions. Homogenous stirring of reaction mixture was performed by 5 MLH magnetic stirrer, Remi Equipment Pvt. Ltd. India.

2.2 Reagents

All reagents and materials used were of analytical grade. A stock solution containing 1000 ng Mo(VI) mL^{-1} was prepared by dissolving appropriate amount of ammonium molybdate (merck KGaA Darmstadt, Germany, 99.9%) in ultra pure water and diluted to 1 L. Appropriately diluted solutions of the above standard Mo(VI) solution were used for further work. Merck, AR grade 1,2–dichloroethane was used for all extraction process. Potassium bromide used in this analysis was of infrared spectrometric grade, Merck KGaA 64271 Darmstadt, Germany. HDPBA was synthesized as according to the reported method[35] and its solution in dichloroethane (0.01%, w/v) were used for extraction and micro–extraction.

2.3 Single–drop micro–extraction (SDME) of Mo(VI)

A 5 mL of 10 ng mL^{-1} Mo(VI) solution was taken in 10 mL vial with a stirring bar placed on a magnetic stirrer. The solution was then acidified using 0.10 mL HCl (0.1N) so that the pH of the solution becomes 3.0. The vial was sealed with polytetrafluoroethylene (PTEE)–coated silicon septum. 10 µL of dichloroethane containing HDPBA (0.01% reagent) is introduced into the acidified solution containing Mo(VI) by the help of 10 µL gas–tight Hamilton manual injection microsyringe (Model 1710, Hamilton, Bonaduz, AG, Switzerland). The needle tip was dipped into the solution and preset about 1 cm lower the surface of the sample. 5 µL of the extractant was squeezed out of the needle and set aside hanging at the needle tip. The solution was stirred at 300 rpm for 5 minutes. After the complete extraction the drop was receded in the microsyringe, and removed from the sample vial. The needle was wiped to remove any probable contamination. The experiments were performed by taking all required precautions.

2.4 DRS–FTIR determination of Mo(VI):HDPBA complex

The FTIR was purged for 30 minutes with >99.99 % analytical grade dry nitrogen gas using iS10 iZ10 external purge kit, Thermo Fisher Scientific, prior to analysis of sample for minimal atmospheric aberration, as water vapor and CO_2 in the sample chamber might lead to additional obscure peaks. Extracted micro-drop containing Mo(VI):HDPBA complex was then delivered over 0.1 g pre–weighed and finely ground KBr for DRS–FTIR spectral scan. For the same, KBr–matrix was dried for one minute at a temperature around 50–65^{o}C. This dried KBr–matrix was then carefully mixed and filled into the sample cup and analyzed by the DRS–FTIR at optimal instrumental condition and software specification as listed in Table 1.

3. Results and Discussion

3.1 Single–drop Micro–extraction (SDME) of Mo(VI)

In the present SDME method, the Mo(VI) was extracted by LLSE procedure from solutions containing analyte at nanogram level. Fig. 1 shows the scheme of experimental setup for SDME system (a), and micro-drop formation during the process (b). In this method the organic ligand, i.e., HDPBA combines with Mo(VI), from their nitrogen and oxygen atoms to form a 1:2 Mo(VI):HDPBA complex. The Mo(VI):HDPBA complex was formed in acidic medium (pH, 2–4). The colour change of organic micro-drop from colourless to yellow indicated the formation of the complex. The SDME of Mo(VI) showed relatively a narrow range of HDPBA concentration that is suitable for quantitative analysis. The maximum enrichment of Mo(VI) in complex form in dichloroethane droplet remain intact at an optimum micro-drop volume and reaction time of 5 µL and 7 min, respectively. The concentration of HDPBA for the maximum and steady absorbance intensity of Mo(VI):HDPBA in KBr–matrix was seen when Mo(VI) was extracted with a single–drop of 0.01% HDPBA in dichloroethane.

3.2 Optimization of SDME Parameters

3.2.1 Selection of organic extraction solvent

The Mo(VI):HDPBA complex was extractable into many tested organic polar and non polar solvents, such as methylisobutyl ketone (MIBK), benzene, toluene, xylene, chloroform, carbon tetrachloride and dichloroethane. Dichloroethane was able to extract complex with maximum absorbance efficiency.

3.2.2 Size of acceptor drop of reagent and dilution

The effect of micro–drop volume on the analytical signal of 10 ng mL^{-1} of Mo(VI) reacted with 0.01% HDPBA in dichloroethane at 3.0 pH was determined. A test volume of 5 mL aqueous phase containing 10 ng Mo(VI) was reacted with different drop volume of HDPBA in dichloroethane ranging from 1–10 µL. The results indicated that on increasing the micro–drop volume the extraction efficiency increases. However, drop volumes larger than 7 µL caused accidental dislodgement due to gravity. Therefore, drop volume of 5 µL was used in present SDME experiments (Fig. 2a).

The effect of dilution of aqueous phase during micro-extraction process was also tested. The concentration of molybdenum, i.e., 10 ng mL^{-1} was kept constant while the volume of aqueous phase was varied from 5 mL to 10 mL at the recommended experimental conditions. It was observed that the signal intensity of the Mo(VI):HDPBA complex in organic micro-drop by DRS-FTIR at 911±2 cm^{-1} goes on decreasing steadily with increase in dilution. This could be attributed to the lowering in extraction efficiency of the system due to lesser possibilities of phase interaction up on dilution. However, this loss could be compensated by increasing the reaction time to 15 min. Tests below 5 mL sample volume could not be carried out due to equipment and experimental constraints. Hence, a 5 mL sample volume was chosen for all experiments.

3.2.3 Effect of pH and reagent concentration on SDME

The pH of analyte solution is one of the important factors for formation of metal complexes. Therefore, in order to study the effect of pH on the SDME of Mo(VI) were performed at different pH level ranging from 2.0–9.0 using 0.1N HCl and 0.1N NaOH. The optimum pH for maximum formation and extraction of Mo(VI) in terms of absorbance was found to be in the range 2.0–4.0. Hence, the pH of the analyte was maintained for all the SDME experiment at 3.0. The effect of concentration of HDPBA on the extraction of metal ion was examined by varying the concentration of reagent between 0.01–0.1% (w/v). It was found that the maximum extraction of Mo(VI) occurred at concentration range 0.01-0.05 % (w/v) HDPBA in dichloromethane. However, even at concentrations higher than 0.05% also there was no significant change in the absorbance value of the Mo(VI) ion peak at 911 cm^{-1} but the peak area due to HDPBA becomes dominating with increased concentrations of it which creates difficulties in quantification process. Hence, a concentration of 0.01% (w/v) of HDPBA was used throughout for extraction of Mo(VI).

3.2.4 Effect of extraction time and stirring rate

Extraction is an equilibrium process[36] and it depends on time of extraction. Therefore, extraction time is considered as an important parameter for discussion. The equilibrium point, where the analyte is transferred from aqueous phase to organic phase and extracted maximally is regarded as an ideal time for extraction. A range of 50–400 rpm was taken for optimization of analyte extraction. The results in Fig. 2b indicates that at lower stirring rate, i.e., 50–100 rpm it takes long time to reach the equilibrium with less analyte extraction. Maximum extraction (in terms of peak area) was observed at 300 rpm. At 400 rpm, the drop formation is

unstable due to dislodgement caused by gravity. Hence, a stirring rate of 300 rpm was set for further SDME process. It was also observed that peak area of Mo(VI):HDPBA complex at 911 cm^{-1} increased with extraction time up to 7 minute (Fig.2b). Therefore, 7 minute extraction time was fixed for further SDME experiments.

3.3 DRS–FTIR Determination of Mo(VI)

3.3.1 Characteristic vibrational and qualitative peak of Mo(VI)

The Mo(VI), i. e. MoO_4^{2-} ion, strongly resembles multi–atomic metaloxy anion, its dipole moment changes during vibrations and hence its presence is well identified by DRS–FTIR. The characteristic IR absorption bands of Mo(VI) ion reported 970–740 cm^{-1} (υ_1), 315–268 cm^{-1} (υ_2), 835–750 cm^{-1} (υ_3) and 350–308 cm^{-1} (υ_4) were used for the interpretation of FTIR spectra of Mo(VI) in the present work.[37,38] Peak positions of all the characteristic IR absorption bands of Mo(VI) were checked by employing standards samples. Table 2 shows the comparison of characteristic IR absorption peaks reported earlier to that of FTIR absorption values for the different Mo(VI) compounds found in the present work. In the present work, the spectral study of the solution prepared using ammonium molybdate salt shows strong (broad and sharp) absorption bands at 911±2, 889±2 and 856±2 cm^{-1} (Fig. 3a). A strong and sharp peak at 1400±2 cm^{-1} was also found that corresponded to the presence of NH_4^+. Infrared band found at 856 cm^{-1} is very less intense and hence it is of no analytical interest. A comparably strong and sharp peak with well–defined baseline observed at 911 cm^{-1} was, therefore, selected as the analytical peak for quantification of Mo(VI).

3.3.2 Comparison of spectral characteristics of Mo(VI) ion and Mo(VI):HDPBA complex

The spectral peak obtained for pure Mo(VI), taken in the form of ammonium molybdate, and that obtained for Mo(VI):HDPBA complex in SDME were compared in order to see their spectral characteristics. Fig. 3a-c shows spectra of Mo(VI) before and after extraction with HDPBA, i.e., MoO_4^{2-} in its pure form and in complexed form. There was no appreciable change observed in the position of characteristic spectral peaks at 911 cm^{-1} produced due to both the species, apart from the few those produced due to the presence of some functional groups in the HDPBA. This probably indicates that MoO_4^{2-} does not lose its structure and symmetrical identity even after coordinating with the organic ligand and Mo-O bonds are free to oscillate. Thus, appearance of the characteristic spectral peak positions like in both the forms, i.e., pure as well as complexed, created the criteria for the identification and quantification of Mo(VI) in SDME/DRS–FTIR.

3.4 Calibration and correlation

The reflectance spectrum measured by the diffuse reflectance method is converted into quantitative spectrum using Kubelka Munk conversion formula [$f(R) = (1–R)^2 = k/s$, where k=molecular extinction coefficient; s=scattering coefficient; R=reflectance or power spectrum of sample/power spectrum of dilution material, KBr] that correlates with the concentration of the sample.

The calibration curves for peak height and peak area were prepared by utilizing the respective Kubelka Munk spectrum obtained for the minimum and maximum Mo(VI) concentration range. The software Omnic9 automatically converts the reflectance spectrum into Kubelka Munk spectrum for smoothening of the baseline. The two important parameters, i.e., peak height and peak area data at 911 cm^{-1} obtained on full concentration

range (1–100 ng mL^{-1}) were plotted against the respective absorbance values at 911 cm^{-1}. The calibration curve for peak height and peak area shows excellent linearity with correlation values, 0.999 and 0.998 respectively, the statistical data obtained from calibration curves verifies the ideal rank of calibration as shown in Fig. 4. The relatively high intercept may either be attributed to matrix ions and correlated noise or due to the high blank value. However, there is no significant effect on the results of the present method.[39]

3.5 Selectivity of the method

The selectivity of the present method describing SDME/DRS–FTIR determination of Mo(VI) as Mo(VI):HDPBA complex was investigated for the possible interferences due to presence of various types of ions. Interferences in micro–extraction process due to solvents and interferences in the DRS–FTIR determination of Mo(VI) due to solvent effects, and other ionic interferences were carried out. These can be categorized as internal interference (due to solvent and reagent used in present SDME method) and interference due to foreign species. Dichloroethane is capable of extracting the Mo(VI):HDPBA complex with high % efficiency (>99%) in a single extraction. Boiling point of dichloroethane is 50–65 ^{o}C, and it is completely vaporized on drying KBr–matrix over water bath. Hence we have not found any IR peak for dichloroethane. The HDPBA was used as selective complexing agent for Mo(VI) as it is a strong and co–principal component in the micro–extraction process. So it was very important to study the interferences of the HDPBA, for this we have compared DRS–FTIR spectra of the pure Mo(VI) sample and Mo(VI):HDPBA complex and found the intensity and position of the characteristic peak unaffected in the Mo(VI):HDPBA complex. The IR peak for Mo(VI) could be easily identified in the spectra scanned for ammonium molybdate as well as in the spectra of Mo(VI):HDPBA complex. To study the interference of the foreign species on SDME/DRS–FTIR various types of inorganic and organic chemical species were added prior to SDME process. Firstly, a 1000–fold interference with Mo(VI) (w/w) was tested in the analyte solution (10 ng mL^{-1}). The ratio was then gradually reduced and the effects of interfering species were checked by comparing the quantitative IR peak at 911 cm^{-1}. The presence of Fe(III) reduces the Mo(VI) into Mo(V), which causes difficulty in complexation with HDPBA under normal conditions. Hence to eliminate the interference effect, 0.25 mL of 1.0% (w/v) ammonium fluoride (NH_4F) was employed as a masking agent prior to SDME. A 400-fold excess of Fe(III) could be tolerated using ammonium fluoride as masking agent.[40] HDPBA, being a selective reagent does not form any complex with Fe(II), hence, interference effect from Fe(II) was negligible. The masking process was done to limit Cu(II) interference by addition of small amount of thiourea in the aqueous phase. The effect of Mo(VI) separation in presence of various chemical species is listed in Table 3.

3.6 Analytical features and application to real sample

The analytical features of proposed work compared with other sophisticated techniques have been displayed in Table 4. The standard deviation calculated in the present work was 0.13 ng mL^{-1}. The standard deviation gives an approximation of the average amount each number in a set differs from the main value. The Limit of detection (LOD) in the present work was found to be 8 ng mL^{-1} for Mo(VI). Limit of quantification (LOQ) and relative standard deviation (RSD) in the present method were calculated to be 26.4 ng mL^{-1} and

1.3%, respectively. The limit of detection has been found to be 0.75 μg mL^{-1} in some of the methods like ICP–AES but the major disadvantages in this method are interferences of other ions, high cost and operating expenses etc.[43] The present method has the advantage of non-interference from all the tested ions that are normally found associated with Mo(VI). GF–AAS–DLLME determination of Mo(VI) has been done by Shamsipur and co–workers that accounts for good sensitivity as the LOD observed was 0.007 ng mL^{-1} but the major drawback of the method being the formation of stable molybdenum carbides, so high temperatures are required to run the samples.[8] The present method thus has a considerable advantage over the other preferred methods in terms of sensitivity and less use of toxic solvents. The observed working range in the present work was 1–100 ng mL^{-1}. Thus, these analytical features make SDME/DRS–FTIR suitable technique for analysis of Mo(VI) in complex biological matrices.

The proposed method was applied to the quantification of Mo(VI) in human blood serum and urine samples. Appropriate volume of the sample pre–treated with 1% (v/v) HCl was pre–concentrated by SDME method under optimized condition and subsequent FTIR analysis was done. 30 samples each for blood serum and urine were analysed to get comprehensible data, the results of the sample having Mo(VI) content have been only shown in the table 5.To test the accuracy of the SDME/DRS–FTIR method, samples were spiked with standard solution of Mo(VI). To validate the present method, the Mo(VI) content in the real samples were also determined by the ICP-MS (PerkinElmer, Elan DRC-e model) technique with flow rate (L min^{-1}) of 0.9, 1.1, and 15.0 for nebulizer, auxiliary and plasma gas 501, respectively at standard operating condition. As can be seen from table 5, the results show good recoveries with spiked samples and were compared with that of standard ICP-MS method.[45] The results indicated good closeness in precision and accuracy with RSD ranging from 1.08-4.44% for standard addition curve and 1.09-4.95% for standard ICP-MS method. The samples were spiked with appropriate amount of analyte at two different concentrations. The results also show considerable recoveries for spiked samples ranging from 87.9-107.4% for present method to 87.4-101.0% for standard ICP-MS method, respectively. The F-test was performed at 95% probability. The calculated values of F (sd_1^2/sd_2^2) were less than the tabulated F-value (table 5) reveals no significant difference from the standard method. Similarly, the t-test was done at 95% confidence level to compare the result of proposed method with earlier reported standard method ICP-MS. In all the cases calculated t-values were less than the tabulated t-value at 95% confidence limit and indicates no statistical difference between both the methods. The certified reference material (CRM) for blood and urine samples was since not available; a low alloy steel sample (No. 223.6, CSIR–National Metallurgical Laboratory) certified value (0.17±0.01 g g^{-1}, n=13) was analyzed instead after acid digestion. The Mo(VI) content in CRM was found to be 0.16±0.22 g g^{-1} (n=6) by SDME/DRS–FTIR method. The Mo(VI) content found by the proposed method agreed well with the value reported in CRM.

4. Conclusions

The newly developed hyphenated technique SDME/DRS–FTIR could be efficiently employed in such case where available sample size is very small. The comparison on characteristics feature of some of the selected techniques used for the determination of Mo(VI) has shown the suitability of the present method. SDME/DRS–

FTIR is very simple, highly sensitive and selective method for the determination of Mo(VI) with a detection limit 8 ng mL^{-1}. The beauty of the present method is the requirement of low amount of toxic consumables and hence also cost effective in comparison with high cost methods. This developed method was successfully employed for the quantification of Mo(VI) in human blood serum and urine.

Conflict of interest

All authors declare that they have no conflict of interest.

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Table 1 Optimum instrumental condition for DRS–FTIR spectral scan of micro–drop

Particulars	Description
Instrument	Fourier transform infrared spectrometer, Nicolet iS10, Thermo fisher Scientific Instrument, Madison, USA
Technique	Diffuse reflectance spectroscopy
Software	Omnic9
Beam Spliter	XT/KBr
Detector	Deuterated, L–alanine doped triglycine sulfate (DLaTGS)
Measurement mode	Absorbance
Resolution	4 cm^{-1}
No. of scanning	32
Sample volume	5 µL
Sample form	Liquid

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Table 2 Infrared absorption bands and different modes of vibrations for some molybdate species

S. No.	Species	Characteristic absorption peak reported[a] (cm^{-1})[37, 38]	Characteristic absorption found in present work (cm^{-1})[b]
1.	Sodium molybdate	930–830	920–850 (910±2)
2.	Potassium molybdate	910–820	910–840 (900±2)
3.	Calcium molybdate	910–810	910–850 (900±2)
4.	Ammonium molybdate	930–840	920–860 (910±2)

[a]Peak value corresponds to asymmetric stretching (Vibrational mode υ_3), [b]values in parentheses are the peak positions obtained in present work with SD of ±2 cm^{-1} for 6 replicate measurements.

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Table 3 Effect of foreign species on separation of Mo(VI) from sample matrix using SDME

Tolerance limit (w/w)	Foreign species
≥ 1000	Cl^-, Br^-, I^-, F^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, formate, acetate, oxalate, succinate, cinnamate
560	CN^-, OH^-, NO_3^-, ClO_3^-, ClO_4^-, BrO_3^-, IO_3^-, IO_4^-, SCN^-, NO_2^-, ClO_2^-,CO_3^{2-},BO_3^{3-}, PO_4^{3-}, HCO_3^-
400	Al^{3+}, Fe^{3+}, Cu^{2+}
100	AsO_4^{3-}, SeO_3^{2-}, AsO_3^{2-}, FeO_4^{2-}, SiO_4^{2-}, MnO_4^-
50	CrO_4^{2-}, $Cr_2O_7^{2-}$
30	VO_4^+

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Table 4 Comparison of characteristic analytical features of some of the selected techniques used for the determination of Mo(VI).

Methods	Linear range (ng mL^{-1})	Detection limit (ng mL^{-1})	Analysis time (min)	Sample volume (µL)	Interferences	Reference
IL–DLLME/FO–LADS[a]	5.0–100	1430	7	10000	Fe^{3+}, Bi^{2+}, Pb^{2+}, Cu^{2+}, V^{5+}	7
GF–AAS–DLLME[b]	0.04–0.8	7	5	15000	Pb^{2+}, Hg^{2+}, Sn^{2+}, Fe^{2+}, Cr^{2+}, Ag^{1+}, Cl^{-}, Br^{-}, NO_3^{2-}	8
Kinetic–Catalytic Method	4.0–40	1.2	10	5000	Fe^{2+}, Fe^{3+}, S^{2-},	41
HNAAQ[c]	0–30	0.083	15	10000	Al^{3+}, Ga^{3+}, In^{3+}, Ti^{3+}, Cu^{2+}	42
VA–SFODME–FAAS[d]	2.0–4000	4.9	10	120	–	43
ICP–AES[e]	1.0–50	0.75	15	10000	Fe^{3+}	44
SDME/DRS–FTIR	1.0–100	8.00	7	5	Fe^{3+} interferences but could be effectively masked with ammonium fluoride	Present Work

Note: [a]Ionic liquid–based dispersive liquid–liquid microextraction fiber optic–linear array detection spectrophotometry, [b]Graphite furnace–atomic absorption spectrometry–Diespersive liquid–liquid microextraction, [c]Hydroxy–napthaldehydene aminoquinolene, [d]Vortex–assisted Solidified floating organic drop microextraction–Flame atomic absorption spectrometry, [e]Inductively coupled plasma–atomic emission spectrometry.

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Table 5 Determination of Mo(VI) in biological samples using SDME/DRS–FTIR and recovery test

Samples[a]	No. of Samples	Present SDME/DRS-FTIR method				ICP-MS			[#]*F*-test	[*]Student's *t*-test
		Spiked (ng/mL)	Found[b] (ng/mL)	Recovery (%)	RSD (%)	Found[c] (ng/mL)	Recovery (%)	RSD (%)		
Blood serum	1.	-	8.26 ± 0.09		3.84	8.34 ± 0.07		2.75	1.65	1.46
		10.00	18.24 ± 0.21	99.7	2.80	18.43 ± 0.11	101.0	1.53		
		15.00	23.21 ± 0.20	99.3	1.58	23.14 ± 0.20	97.6	1.65		
	2.	-	8.33 ± 0.08		4.44	8.55 ± 0.07		3.78	1.31	1.96
		10.00	18.73 ± 0.24	104.8	3.57	18.54 ± 0.14	99.8	2.12		
		15.00	23.95 ± 0.22	107.4	1.84	23.52 ± 0.22	99.6	1.85		
	3.	-	8.99 ± 0.04		4.04	8.96 ± 0.03		2.97	1.78	2.01
		10.00	18.95 ± 0.23	99.5	3.93	18.87 ± 0.23	98.9	3.92		
		15.00	23.96 ± 0.12	99.6	1.08	23.92 ± 0.12	99.5	1.09		
Urine	1.	-	9.58 ± 0.16		3.49	9.42 ± 0.14		3.32	1.31	1.08
		10.00	19.29 ± 0.30	96.9	2.92	18.40 ± 0.30	89.1	2.95		
		15.00	23.43 ± 0.21	87.9	1.42	23.32 ± 0.21	88.3	1.48		
	2.	-	10.08 ± 0.14		3.60	9.96 ± 0.12		3.37	1.36	1.65
		10.00	19.93 ± 0.31	98.5	3.68	19.72 ± 0.21	97.5	2.52		
		15.00	23.99 ± 0.44	89.1	3.21	23.71 ± 0.44	87.4	3.21		
	3.	-	9.62 ± 0.21		3.73	9.64 ± 0.20		3.55	1.10	1.66
		10.00	19.53 ± 0.23	99.0	2.23	19.42 ± 0.23	97.7	2.21		
		15.00	24.51 ± 0.35	98.8	2.21	23.87 ± 0.35	92.0	2.21		

[a] Samples obtained from the hospitalized patients from Pt. Jawaharlal Nehru Memorial Medical College Hospital, Raipur, C.G., India

[b] The average and standard deviation of six replicates measurements (*N*: 6) obtained by the proposed method

[c] The average and standard deviation of six replicates measurements (*N*: 5) obtained after detection with ICP-MS as comparative method

[#] The tabulated $F_{5,4}$-values at 95% confidence level is 6.26 for nine degrees of freedom at probability level of 0.05

* The tabulated *t*-value at 95% confidence level is 2.571 for $N_1 = 6$ (i.e., for $\upsilon_1 = N_1 - 1 = 5$, $\upsilon_2 = N_2 - 1 = 4$)

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Figures Caption:

Fig.1 Schematic of experimental Setup for the SDME extraction (a), micro-drop formed at needle tip in present work (b)

Fig.2 Effect of micro-drop volume (a), and extraction time at different rate (b) on extraction efficiency

Fig.3 DRS-FTIR spectra of molybdate as ammonium molybdate in pure form (a), HDPBA (b), Mo(VI):HDPBA complex formed in SDME (c), and quantitative behavior of characteristic vibrational peak a 911 cm^{-1} (d).

Fig.4 Calibration curve for concentration vs relative peak height (a), and relative peak area at 911 cm^{-1}.

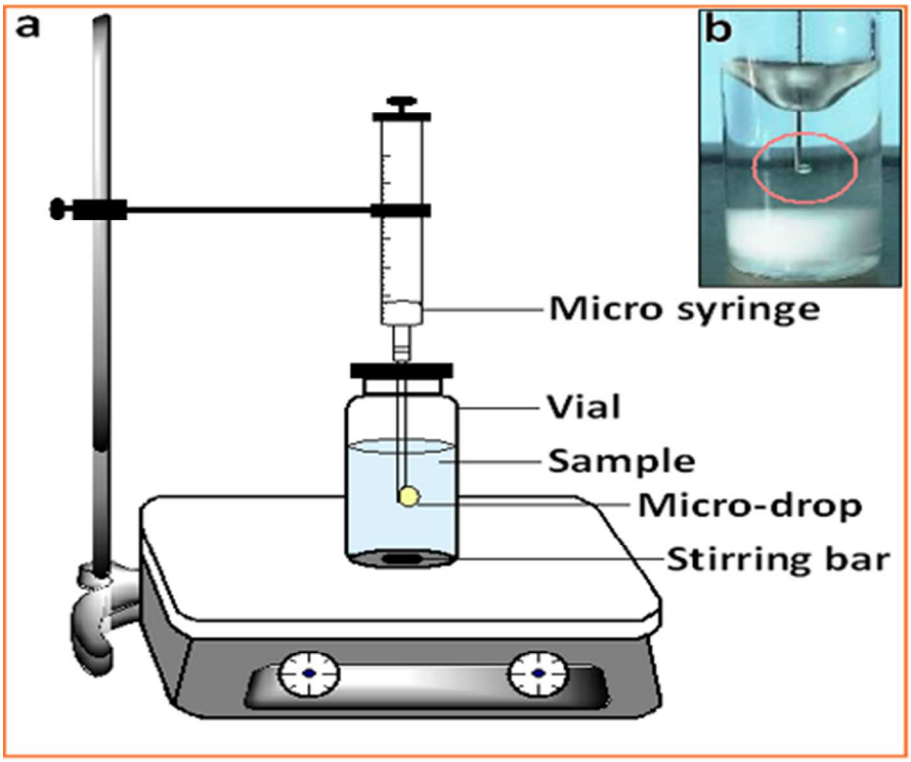

Fig 1

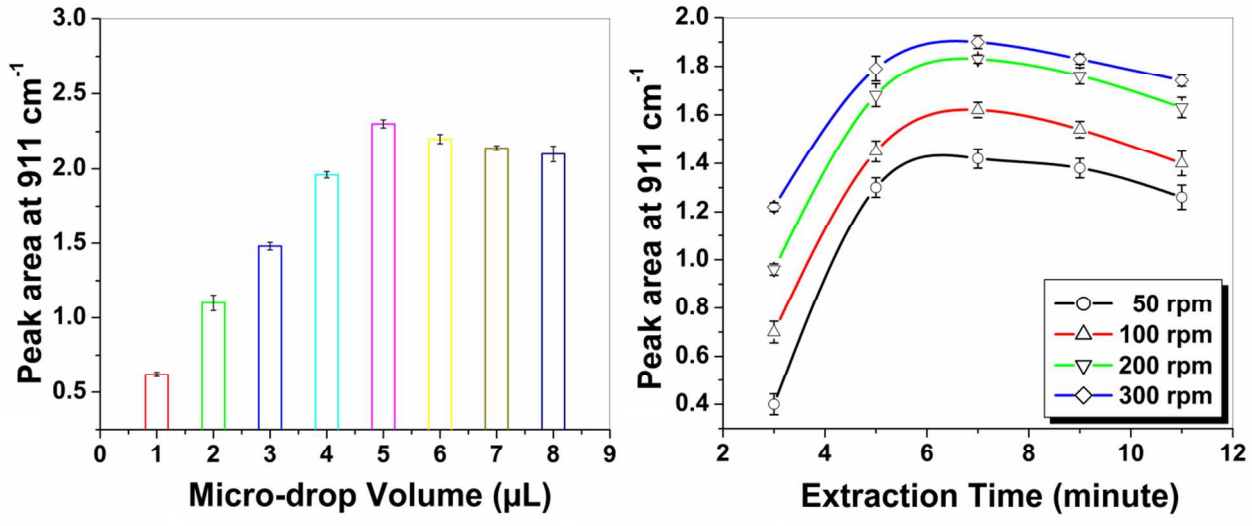

Fig 2

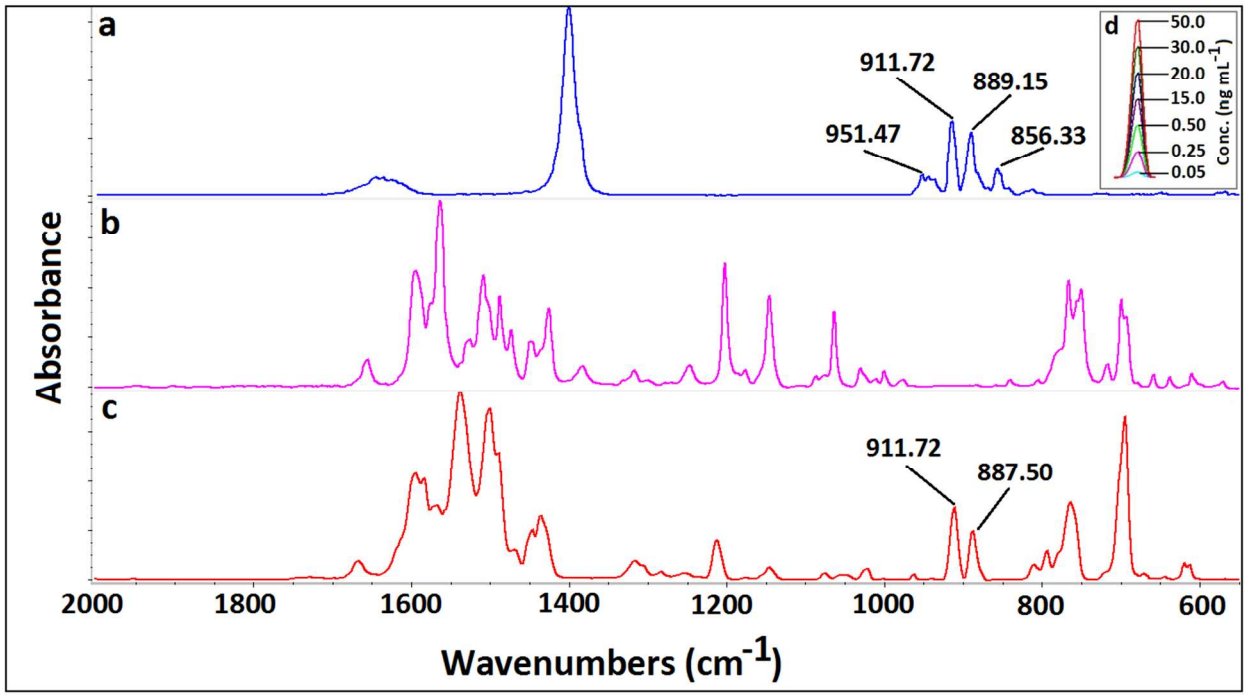

Fig 3

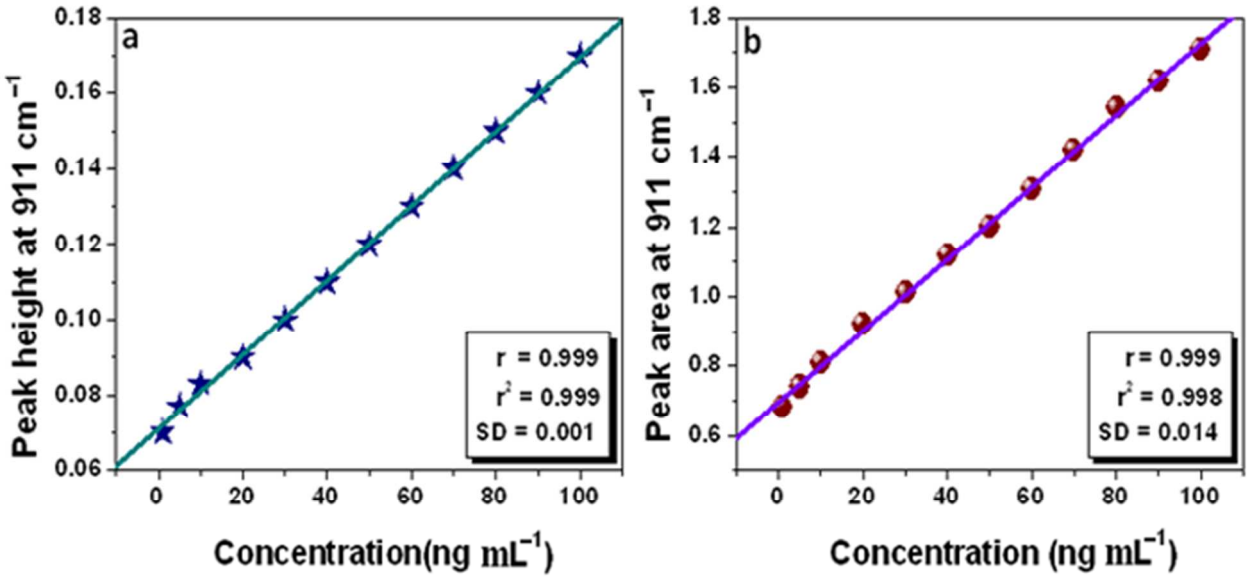

Fig 4

Nanogram level quantification of molybdenum(VI) by Novel Hyphenated SDME/DRS-FTIR in human biological fluid

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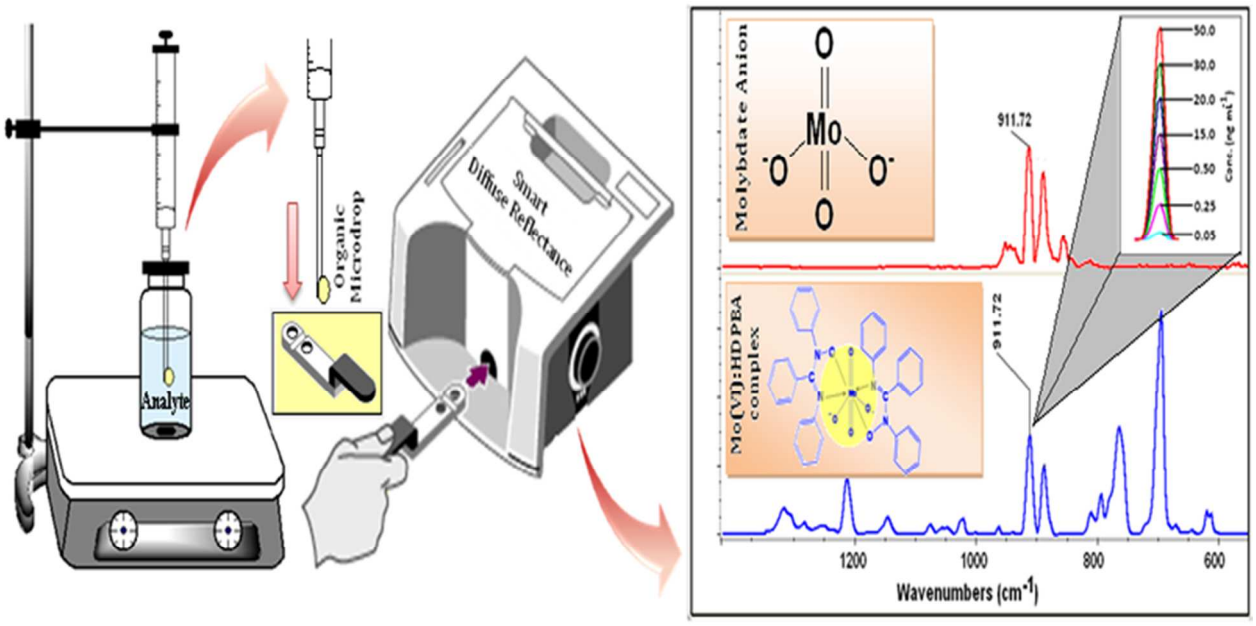

Graphical Abstract