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Development of vial wall sorptive extraction and its application to determination of progesterone in human serum

Migaku Kawaguchi[a,*], Shin-ichiro Fujii[a], Nobuyasu Itoh[a], Rie Ito[b], Hiroyuki Nakazawa[b], Akiko Takatsu[a]

[a] National Institute of Advanced Industrial Science and Technology (AIST), National Metrology Institute of Japan (NMIJ), 1-1-1 Umezono, Tsukuba, Ibaraki 305-8563, Japan
[b] Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

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ABSTRACT

A novel sample preparation method, vial wall sorptive extraction (VWSE), which uses a vial whose internal wall is coated with polydimethylsiloxane (PDMS), was developed. The method was applied to the determination of progesterone in human serum sample. Human serum sample (0.5 mL) spiked with progesterone-$^{13}C_2$ was pipetted into the VWSE device and vortex mixing was performed for 30 min. Then, the serum sample was removed and the vial rinsed with purified water. Fifty microliter of methanol as liquid desorption (LD) solvent was pipetted into the VWSE device and vortex mixing was performed for 10 min. Then, the extract was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The correlation coefficient (r) of the calibration curve over the concentration range of 0.5–200 ng mL^{-1} was 0.999. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.1 and 0.5 ng mL^{-1}, respectively. The relative recoveries were 97.9% (RSD: 4.4%, n = 6) and 102.8% (RSD: 1.1%, n = 6) for progesterone spiked at 5 and 50 ng mL^{-1}, respectively. This simple, accurate, sensitive, and selective analytical method is applicable to the trace analysis of a minute amount of sample.

1. Introduction

Sorptive extraction is a simple and high-recovery sample preparation technique that enables selective extraction, concentration, and enrichment of trace organic compounds in various matrices. Employing such sorption materials as polydimethylsiloxane (PDMS), it is widely used for the trace analysis of organic compounds in environmental, food or biomedical samples [1–10]. In addition, because sorptive extraction can be performed with a minute volume of solvent or without any solvent at all, it is useful for green analytical chemistry [9,10]. Sorptive extraction also allows miniaturization in sample preparation and meets the demand for shorter analysis time and higher cost effectiveness [11].

Sorptive extraction is by nature an equilibrium technique that is based on solute partitioning between the stationary phase and the aqueous sample, depending on the distribution coefficients of the analyte between the two phases. Solid-phase microextraction (SPME) was developed in 1990 [12] and is now widely used in a variety of analyses [13–16]. However, its sensitivity can be relatively low. The limited enrichment on the SPME fiber is mainly due to the small volume of the PDMS phase (typically 0.5 μL or less). In 1999, stir bar sorptive extraction (SBSE) that uses a stir bar coated with 50–300 μL of PDMS was developed [17]. Increasing the volume of PDMS relative to the volume of the sample can dramatically increase analyte enrichment. SBSE has a wide application range that includes volatile aromatics, halogenated solvents, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, preservatives, odor compounds, endocrine disruptors, and organotin compounds [18,19].

However, SBSE is not suitable for the analysis of a small volume of sample. To subject a sample volume less than 2 mL to SBSE, it was necessary to dilute it to allow stirring [20–27]. In addition, it was reported that recovery was decreasing because the analyte adsorbed to the vial internal wall. Then, this adsorption was avoided by adding some percent of organic solvent [28–32]. Moreover, at least 150–200 μL of solvent was required by the ordinal PDMS stir bar during liquid desorption (LD), which is often used to desorb the analyte from the PDMS stir bar for liquid chromatographic (LC) analysis [33–35]. A large volume of LD solvent leads to a decrease in sensitivity by dilution.

Recently, Wohleb et al. developed the direct vial extraction (DVE) that uses a small PDMS-coated vial which was placed in the cap of large bottle [36]. It is thought that the extraction method using PDMS-coated vial has high utility. However, DVE is not suitable for the analysis of sub-milliliter sample volumes.

* Corresponding author at: Bio-Medical Standard Section, National Metrology Institute of Japan (NMIJ), National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Umezono, Tsukuba, Ibaraki 305-8563, Japan.
Tel.: +81 29 861 4138; fax: +81 29 861 4137.
E-mail address: m-kawaguchi@aist.go.jp (M. Kawaguchi).

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In this study, we propose a novel sample preparation method called "vial wall sorptive extraction (VWSE)." In the VWSE device, the vial internal wall was coated with PDMS. In addition, miniaturization of VWSE for application to an autosampler was performed by using a conventional vial.

In this study, the VWSE was applied for the determination of progesterone in human serum sample. Progesterone is one of the steroid hormones that are involved in regulating the female reproductive process. Progesterone participates in the regulation of the menstrual cycle and is especially important in preparing the uterus for implantation of the blastocyst and in the maintenance of pregnancy. Serum progesterone levels in adult females normally range from ~0.15 to ~25 ng mL^{-1} but can rise to ~230 ng mL^{-1} during pregnancy [37]. Serum progesterone levels are often determined to assess corpus luteum function and to detect luteal phase defects. A single progesterone determination may be as valuable as repeated human chorionic gonadotropin (hCG) measurements to estimate the risk of early pregnancy complications [38] and has greater predictive value for pregnancy outcome than a single hCG determination [39].

In the present paper, optimization of the conditions for VWSE is described. A comparison of VWSE and SBSE for progesterone analysis was also performed and the usefulness of the VWSE method was discussed.

2. Experimental

2.1. Materials and reagents

Progesterone material and frozen human serum were obtained by Wako Chemical Inc. (Osaka, Japan). European Reference Materials (ERM)-DA 347, lyophilized human serum reference material for progesterone (unspiked), was obtained from Institute for Reference Materials and Measurement (IRMM, Geel, Belgium). Progesterone-$^{13}C_2$ as surrogate standard was purchased from Euriso-Top (Saint-Aubin Cedex, France). SILPOT 184 W/C for PDMS polymer syntheses was purchased by Dow Corning Toray Co., Ltd. (Tokyo, Japan). The water purification system used was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA). Other reagents such as methanol, ethanol, acetonitrile and acetic acid were purchased from Wako Pure Chemical Inc. The 2 mL screw vials and 1.5 mL high-recovery screw vials were purchased from Agilent Technologies (Palo Alto, CA, USA). For SBSE, 10 mL headspace vial and 250 μL vial insert were purchased from Agilent Technologies. PDMS (24 μL) stir bar was purchased from Gerstel (Mülheim an der Ruhr, Germany).

2.2. Standard solutions

Standard solutions of progesterone were prepared in ethanol. For calibration standard, the progesterone standard solution was diluted by ethanol and a fixed concentration of progesterone-$^{13}C_2$ was added.

The human serum sample spiked with standard solution (100 ng mL^{-1}) was used for optimization of VWSE or SBSE condition.

2.3. Preparation of VWSE device

The main reagent of SILPOT 184 and the cross-linking agent of SILPOT 184 were mixed in a 10:1 ratio. Then, the mixture was degassed under reduced pressure. Two hundred microliter of the mixture was added to a 2 mL screw vials and 1.5 mL high-recovery screw vials. PDMS polymerization was performed for 15 min at 95 °C. Prior to use, conditioning was performed with 1 mL of methanol.

2.4. Instrumentation and LC-MS/MS measurement

A high performance liquid chromatographic separation was performed using Agilent 1100 LC (Agilent Technologies). A tandem mass spectrometry (MS/MS) was performed using a Thermo TSQ Quantum Discovery (Thermo Fisher Scientific K.K., Tokyo, Japan) equipped with an electrospray ionization (ESI) source with positive ion mode. The injection volume was set to 5.0 μL. An XDB (150 mm × 2.1 mm, 5 μm) analytical column (Agilent Technologies) was used for separation at 40 °C. The 0.05% acetic acid in water/methanol (35/65, v/v) was used as the mobile phase. The flow rate was set to 0.2 mL min^{-1} for 20 min. Then, methanol content was increased stepwise to 100% and held at this concentration for 10 min to clean the column. Finally, the mobile phase was returned to the initial composition in a stepwise manner and the column was equilibrated with the initial mobile phase composition for 10 min. The working conditions for electrospray MS/MS were as follows: ion transfer tube temperature was set at 300 °C; spray voltage was set at 4000 V; nitrogen sheath gas and auxiliary gas were set at 40 and 5 A.U., respectively; collision gas pressure was set at 4.0 mTorr; collision energy was set at 25 V; and skimmer offset was set at 8 V. When working in the selected reaction monitoring (SRM) mode, m/z 315/97 and 317/99 were assigned to the precursor/product ion of progesterone and progesterone-$^{13}C_2$, respectively.

2.5. Sample preparation using VWSE

Human serum sample (0.5 mL) spiked with progesterone-$^{13}C_2$ was pipetted into a VWSE device containing 200 μL PDMS. Then, the sample was mixed with a vortex mixer (DeltaMixer, TAITEC Co., Ltd., Saitama, Japan) and extraction was performed for 30 min. After extraction, the serum was removed and the device was washed with 1 mL of purified water 3 times. Then, 50 μL of methanol was added and LD was performed for 10 min with the vortex mixer. The device was set to the autosampler and the extract was directly injected to LC-MS/MS.

2.6. Sample preparation using SBSE

Human serum sample (0.5 mL) spiked with progesterone-$^{13}C_2$ was diluted to 2 mL by adding purified water and placed in a headspace vial. A PDMS stir bar (24 μL of PDMS) was put inside the vial. SBSE was performed at room temperature for 60 min while stirring at 500 rpm. Then, the stir bar was placed inside a 2 mL standard vial with a 250 μL insert vial that was filled with 150 μL of methanol. Analyte desorption was performed with an ultrasonic device for 15 min. After LD, the stir bar was removed with a magnetic rod and the extract was subjected to LC-MS/MS.

2.7. Evaluation of absolute recovery

Standard solution (100 ng mL^{-1}) was used for the evaluation of absolute recovery. The sample was applied to VWSE or SBSE method. After extraction, the remaining sample was analyzed by LC-MS/MS without performing liquid desorption. The absolute recovery was calculated below the equation.

$$\text{Absolute recovery}(\%) = \frac{\text{initial concentration} - \text{quantitative value}}{\text{initial concentration}} \times 100$$

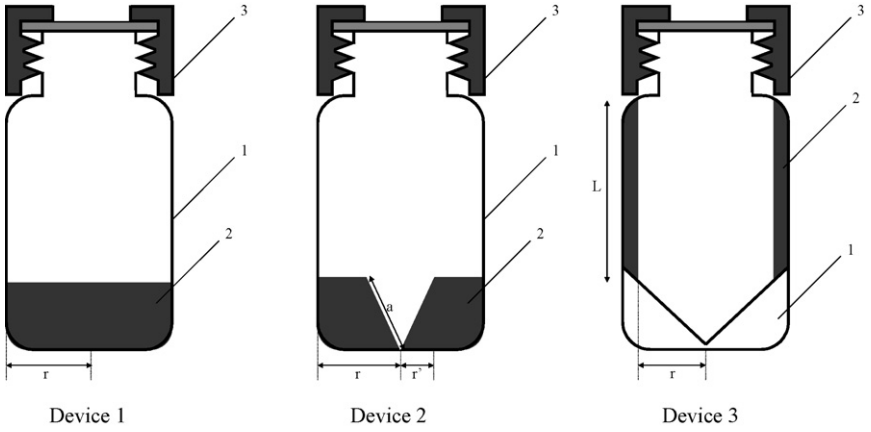

Fig. 1. Schematic of VWSE devices. Device 1: 2 mL screw vial with PDMS column. $r = 0.5$ cm, $\pi \approx 3.14$. Contact area = $\pi r^2 \approx 0.785$ cm^2. Device 2: 2 mL screw vial with PDMS column and blank inverted cone. $r = 0.5$ cm, $r' = 0.28$ cm, $a = 0.41$ cm, $\pi \approx 3.14$. Contact area = $\pi r^2 - \pi r'^2 + \pi a r' \approx 0.899$ cm^2. Device 3: 1.5 mL high-recovery screw vial with PDMS film coating internal wall surface. $r = 0.46$ cm, $L = 1.8$ cm, $\pi \approx 3.14$, film thickness ≈ 400 μm. Contact area = $2\pi r \times L \approx 5.20$ cm^2. 1: Vial, 2: PDMS, 3: Septum cap.

2.8. Efficiency of sorptive extraction

The mass of an analyte extracted into PDMS at equilibrium (expected recovery) is calculated as follows:

$$K_{\mathrm{PDMS/S}} = \frac{C_{\mathrm{PDMS}}}{C_{\mathrm{S}}} = \left(\frac{m_{\mathrm{PDMS}}}{m_{\mathrm{S}}}\right)\left(\frac{V_{\mathrm{S}}}{V_{\mathrm{PDMS}}}\right) \quad (1)$$

where C_{PDMS} is the analyte concentration in PDMS; C_{S} is the analyte concentration in sample; m_{PDMS} is the mass of analyte in PDMS; m_{S} is the mass of analyte in sample; V_{PDMS} is the volume of PDMS; and V_{S} is the volume of sample. Using $V_{\mathrm{S}}/V_{\mathrm{PDMS}} = \beta$, the phase ratio of the sample-PDMS system is expressed as:

$$\frac{K_{\mathrm{PDMS/S}}}{\beta} = \frac{m_{\mathrm{PDMS}}}{m_{\mathrm{S}}} = \frac{m_{\mathrm{PDMS}}}{m_0 - m_{\mathrm{PDMS}}} \quad (2)$$

$$\mathrm{Recovery} = \frac{m_{\mathrm{PDMS}}}{m_0} = \frac{K_{\mathrm{PDMS/S}}/\beta}{1 + K_{\mathrm{PDMS/S}}/\beta} = \frac{K_{\mathrm{PDMS/S}}}{\beta + K_{\mathrm{PDMS/S}}} \quad (3)$$

where m_0 is the total amount of analyte originally present in the sample.

In the VWSE method, 0.5 mL of sample volume and 200 μL of PDMS were used for calculation. In the SBSE method, 2 mL of sample volume and 24 μL of PDMS were used.

3. Results and discussion

3.1. VWSE device design and optimization of extraction time

Three VWSE devices were designed and their recoveries and extraction times examined (Fig. 1). The internal walls of the devices were coated with 200 μL of PDMS. The device 1 was the result of just putting PDMS solution in the vial and curing. The device 2 was obtained by inserting a V-shaped object before curing, and the device 3 was obtained by curing while horizontally rotating the vial. Moreover, device 3 did not contain PDMS at the bottom, because thin PDMS film was damaged by organic solution such as methanol or acetonitrile for long immersed time. The extraction time profiles (0–75 min) of progesterone are shown in Fig. 2. The extraction of progesterone reached equilibrium after approximately 30 min with device 3. In contrast, equilibrium was not observed even up to 75 min with devices 1 and 2. The extraction time with device 3 was shorter than those with devices 1 and 2. In addition, the absolute recovery of progesterone (100 ng mL^{-1}) using device 3 was 94%, which was higher than those using the other two devices. On the basis of these results, device 3 was selected as the VWSE device in this study and the extraction time was set at 30 min.

Contact areas of PDMS and sample were calculated for each device. Contact areas for devices 1–3 were approximately 0.785, 0.899, and 5.20 cm^2, respectively. Device 3 had a large contact area compared with the other two devices. Therefore, it was thought that the contact area is an important parameter in the VWSE method.

3.2. Optimization of LD condition

Important parameters for LD in VWSE are the selection of solvent, the volume of solvent, and the desorption time. First, we evaluated methanol and acetonitrile as LD solvent but found no difference between them (data not shown). Because methanol was used as the mobile phase of LC, it was selected as LD solvent. Second, the optimum volume of methanol (50, 100, and 150 μL) was evaluated. The largest peak area was obtained with 50 μL of methanol. As the minimum sample volume for direct injection by the autosampler was 50 μL, the volume of methanol was set at 50 μL. Then, the optimum desorption time using 50 μL of methanol was investigated from 1 to 20 min. The LD time profile of progesterone using a VWSE device is shown in Fig. 3. The largest peak area was obtained after approximately 10 min. Therefore, this condition was used for the determination of progesterone.

3.3. Validation of VWSE method and VWSE for the determination

The calculated limit of detection (LOD) of progesterone in human serum sample was 0.1 ng mL^{-1} by VWSE in combination with LC-MS/MS, when the ratio of the compound's signal to the background signal (S/N) was 3. In addition, the calculated limit of quantification

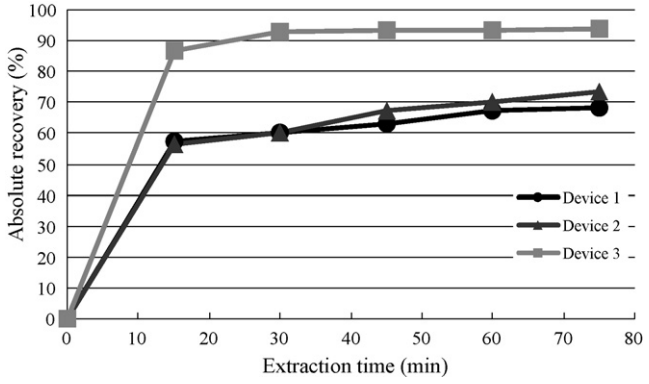

Fig. 2. Extraction time profiles of progesterone using VWSE.

Table 1
Comparison of VWSE method and SBSE method.

Method	Conditions					Optimized conditions and method validations				
	Sample volume[a] (mL)	Volume of PDMS (μL)	Phase ratio (β)	LD solvent (μL)	Contact area (cm^2)	Extraction time (min)	LD time (min)	Absolute recovery (%)	LOD ($ng\,mL^{-1}$)	LOQ ($ng\,mL^{-1}$)
VWSE	0.5	200	2.5	50	5.2	30	10	94	0.1	0.5
SBSE	2	24	83.3	150	0.942	60	15	70	0.5	2.0

[a] In SBSE, the human serum sample (0.5 mL) was diluted to 2 mL by adding purified water.

(LOQ) when S/N > 10 was 0.5 $ng\,mL^{-1}$ for progesterone in human serum sample. The VWSE method shows linearity over the calibration range (0.5–200 $ng\,mL^{-1}$) and the correlation coefficient (r) is higher than 0.999 for progesterone standard solution.

The relative recovery and precision of the method were assessed by replicate analysis ($n = 6$) of human serum samples spiked at 5 and 50 $ng\,mL^{-1}$ levels. Non-spiked and spiked samples were subjected to VWSE in combination with LC-MS/MS. Relative recovery was calculated by subtracting the results for the non-spiked samples from those for the spiked samples. The results were obtained by using isotope dilution mass spectrometry. The relative recoveries of spiked progesterone were 97.9% (RSD: 4.4%, $n = 6$) at 5 $ng\,mL^{-1}$ level and 102.8% (RSD: 1.1%, $n = 6$) at 50 $ng\,mL^{-1}$ level. Therefore, the method is applicable to the determination of progesterone in human serum samples.

Measurement accuracy was evaluated by analyzing progesterone in lyophilized human serum reference material for progesterone [European Reference Material (ERM)-DA 347] as Certified Reference Material (CRM). The ERM-DA 347 sample was subjected to VWSE and the results were compared to the certified value. Typical chromatograms are shown in Fig. 4. The concentration of progesterone in ERM-DA 347 measured by the present method was 3.2 ± 0.1 $ng\,mL^{-1}$, in good agreement with the certified value (3.19 ± 0.07 $ng\,mL^{-1}$).

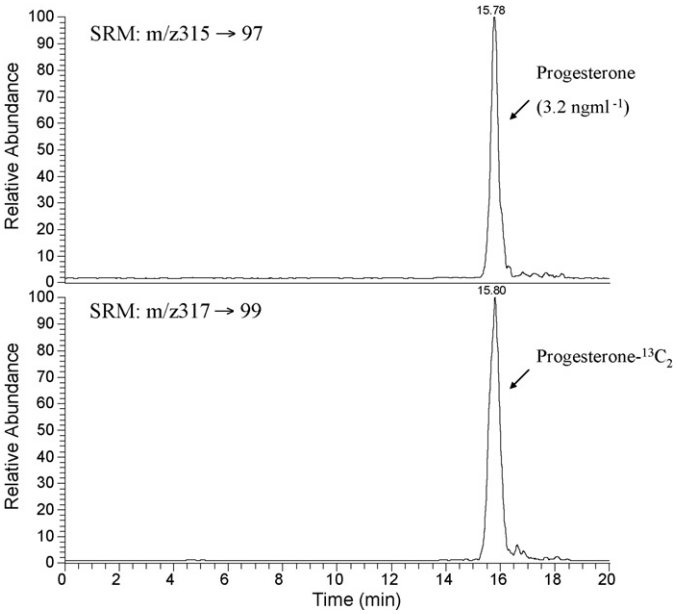

Fig. 4. Typical chromatograms of progesterone and progesterone-$^{13}C_2$ in ERM-DA 347 sample.

3.4. Comparison of VWSE and SBSE

VWSE and SBSE were compared and the results are shown in Table 1. In the extraction time profile (0–90 min) of progesterone using SBSE, progesterone extraction reached equilibrium after approximately 60 min. The extraction time of VWSE (30 min) is shorter than that of SBSE. Two reasons are considered. One is the difference in contact area. The contact area of the PDMS stir bar (24 μL) used in SBSE was approximately 0.942 cm^2 while that of VWSE was 5.20 cm^2 (device 3). The other is the difference in phase ratio (β). Bicchi reported that β in SBSE is related to the extraction time to equilibrium [40]. A smaller phase ratio leads to a shorter extraction time to equilibrium. In this study, the phase ratios of VWSE and SBSE were 2.5 and 83.3, respectively. Clearly, the phase ratio of VWSE is smaller than that of SBSE.

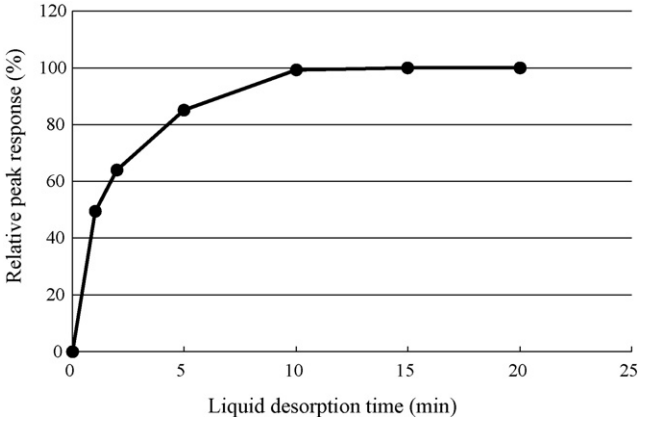

Fig. 3. LD time profile of progesterone using VWSE.

VWSE efficiency and SBSE efficiency were compared. In sorptive extraction, theoretical recovery is calculated using Eq. (3). Therefore, a smaller phase ratio also leads to higher extraction efficiency. The absolute recoveries of progesterone (100 $ng\,mL^{-1}$) by VWSE and SBSE were 94% and 70%, respectively. The fact that the recovery by VWSE is higher than that by SBSE proves that VWSE efficiency is superior to SBSE efficiency.

LD conditions in SBSE were evaluated. Methanol was selected as LD solvent. The 150 μL needed to enable complete immersion of the stir bar. LD of the analyte from the PDMS stir bar was performed with an ultrasonic device for 15 min. The volume of LD solvent for VWSE and SBSE was 50 and 150 μL, respectively. Because the concentration factor of VWSE was higher than that of SBSE, high-sensitivity analysis was expected using VWSE.

Finally, we compared the figures of merit of VWSE and SBSE. LOD and LOQ of SBSE-LD-LC-MS/MS method were 0.5 and 2 $ng\,mL^{-1}$, respectively. The VWSE method is superior to the SBSE method because it exhibited approximately 4–5 times higher sensitivity. The extraction time of VWSE is shorter than that of SBSE. Therefore, it was thought that the VWSE method is applicable to high-throughput analysis.

4. Conclusions

A novel sample preparation method that uses a PDMS-coated conventional high-recovery (1.5 mL) vial, the so-called VWSE

method, was developed. Comparison of VWSE and SBSE methods indicated that VWSE is simpler, faster, and more sensitive. In addition, the VWSE method could be applied to small-volume samples. The VWSE device could be set to the autosampler and the extract could be directly injected into the analytical instrument. Therefore, the VWSE method is suitable for automated high-throughput analysis.

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