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Application of silica-based monolith as solid phase extraction cartridge for extracting polar compounds from urine

T. Nema, E.C.Y. Chan, P.C. Ho*

Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore

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ABSTRACT

The silica monolith with ionizable silanol groups and large surface area was found able to function as an offline cation exchange solid phase extraction (SPE) cartridge for extracting polar analytes. The prepared cartridge was housed in a 2-mL syringe fixed over a SPE vacuum manifold. The unique property of this silica monolithic cartridge was demonstrated by extracting epinephrine, normetanephrine and metanephrine from urine samples. These analytes were chosen as model compounds for testing because of their high hydrophilicity, and being candidates monitored for clinical diagnosis. The extracted analytes, after concentration and reconstitution were then quantitated by high-performance liquid chromatography coupled to mass spectrometer (HPLC/ESI/MS). Multiple reactions monitoring was carried out with transitions: 184 → 107, 184 → 134 and 198 → 148 for analyzing epinephrine, normetanephrine and metanephrine, respectively. The limit of detection was 3 ng/mL for metanephrine and 5 ng/mL for normetanephrine and epinephrine. The relative standard deviations of measurements ranged from 2 to 10%. The sorbent offered good linearity with coefficient of determination (r^2) > 0.99, over a concentration range of 20–200 ng/mL. The relative recoveries ranged from 60 to 67%, 55 to 59% and 99 to 105% for epinephrine, normetanephrine and metanephrine, respectively. The prepared cartridge had shown potential and was found robust in extracting the polar analytes repeatedly without any significant loss in efficiency.

1. Introduction

Sample pretreatment prior to analysis is essential in analytical process especially when the sample matrices are complex. Samples free from interfering matrices not only simplify analysis but also maximize the sensitivity of detection by concentrating analyte within the detection limit as well as eliminating interfering contaminants. Liquid–liquid (LLE) and solid phase extraction (SPE) are the two major methods used widely for this purpose. While both methods are applied for sample clean-up, SPE has the advantage of ease in operation and environmental friendliness. In SPE, the use of toxic organic solvents is minimized. Therefore based on these considerations, SPE was found useful to extract analytes from complex mixtures like environmental [1–3] and biological samples [4]. The basic principle underlying the retention of analytes on SPE sorbent is based on reversible hydrophobic, polar and ionic interactions. Such interactions depend on the chemistry and nature of the sorbent and analyte. The availability of numerous solid phase chemistries is the major advantage that SPE has over other extraction techniques. Sorbents used in SPE can be broadly classified into organic and inorganic sorbents. Organic sorbents are polymer-based whereas inorganic sorbents are silica-based. The physicochemical properties like, polarity, acidity and basicity of the sorbent and the analytes have great impact on the extraction efficiency. Of special significance is the extraction of polar analytes from the sample matrices. Polar analytes are difficult to isolate and preconcentrate as it is difficult to retain them selectively using SPE for accurate quantitation. For the sorbent to retain polar compounds efficiently, it should have a large and highly specific surface area to provide large number of hydrophilic interaction sites to retain the polar analytes. The polarity of the sorbent has to be competitive to the sample matrix in order to have high affinity for the polar analytes. Numerous sorbents for polar analyte extraction, either as offline and online solid phase extractor, have been extensively reviewed [5,6]. One of the techniques is solid phase microextraction (SPME) which has shown great potential in extracting polar analytes from liquid samples [7–9]. Nonetheless, SPME suffers from limited sites for interaction due to low volume of the stationary phase as well as the limited number of coating fibers.

In recent years, polymer-based sorbents have found increasing attention in the extraction of polar compounds and various modifications in the preparation of polymer-based sorbents have been

* Corresponding author. Tel.: +65 6516 2651; fax: +65 6779 1554.
E-mail address: phahocl@nus.edu.sg (P.C. Ho).

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explored for the purpose. Many of these sorbents also showed great potential with regards to their reusability but for limited extent. Polymer-based sorbents for extraction of polar compounds have been extensively reviewed by Fontanals et al. [6]. Their advantage of being used over extended pH range makes them more popular in SPE. In spite of all the potential advantages, the major drawback of the polymer-based sorbent is its tendency to swell in organic solvents which leads to undesirable changes in its pore structure and makes them mechanically unstable. This instability can lead to their collapse or may result into run to run variability after multiple usages [10]. It is because of these limitations, polymer-based sorbent cannot be used repeatedly and has to be disposed after single or few usages. The silica-based sorbent on the other hand provides good organic solvent resistance and mechanical stability. However, the limited pH working range (2–8) of silica renders it less amenable to certain applications when compared to polymer-based sorbents. Nonetheless, if the pH values of the reagents used during SPE are compatible with the working range of silica, silica-based cartridge may be explored for repeated polar compound isolation. The existing SPE cartridge based on silica are mostly particle packed with a particle size in the range of 50–60 μm and requires the filling of particles in the holder (syringe barrel) between porous frits. The efficiency of extraction depends on the quality of packing, i.e., more uniform packing will give less variation in the recoveries of samples. In addition, particle size also has significant effect in the quality of packing and performance of the cartridge. It is known that smaller the particle greater is the surface area that will favor rapid mass transfer. Although, reduction in particle size can facilitate the efficiency of the sorbent material for extraction of analyte(s), it is limited by the increase in back pressure. According to Darcy' Law, back pressure is inversely proportional to the square of particle size [11]. Because of that, small particles will create high back pressure that will be detrimental to the vacuum pump used for suction during the SPE process. Nonetheless, if the small particle is desired then the bed length has to be compromised in order to counteract the increased back pressure. This in turn limits the number of interaction sites on the sorbent surface, and therefore the capacity of the sorbent [12]. Particle packed SPE sorbents could also be packed as hard cakes after multiple usages as the particles are progressively compressed under the applied pressure. This will further increase back pressure, causing undue stress on the vacuum pump. In addition to these, particle based SPE cartridges form channels after few usages. This led to rapid movement of the analytes thorough the channel before they get sufficient equilibration time to interact with the stationary phase. Henceforth, the repeatability of the cartridge for multiple usages was limited and result into its rejection. These limitations urge the need for more reliable and easy SPE material which can provide high surface area and efficiency in performance without any undue problems. This led to the exploration of the monolith for SPE application. In comparison to packed particles, monolith offers high porosity and permeability, rendering them an attractive alternative for SPE. Furthermore, monolith does not require end frits like particle packed SPE to retain particles within the vicinity of the holder.

Monolith as stationary phase was first introduced in the field of separation science in early 1990s [13,14]. Subsequently, monolith was leveraged in diverse separation science applications involving high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrochromatography (CEC) [15–21]. The details of the diverse applications are reviewed in a series published by Svec [22–24]. While monolith is a popular material for column fabrication and it appears intuitive that it may play a role in SPE and protein/enzyme immobilization, it is surprising that the latter applications are broadly overlooked. Recently, monolith began to gain recognition as a sample preparation tool which is evident in a number of publications [5,6]. The ease of modification of the surfaces of monolith for desired extraction renders it an amenable tool for offline and online sample preparation.

It is well known that secondary interactions with underivatized silica provide hindrances for the effective elution of selected analytes especially basic compounds. Silanol groups which have the tendency to exist as $Si–O^-$ above pH 4 are the potential sites for such secondary interactions. As a result, they contribute to cation exchange secondary interaction [25]. Therefore, an extra effort has to be put in to successfully elute the analytes. On the other hand for sorbent to be effective as solid phase extractor, it should have high surface area which can provide high rates of mass transfer with a consistent flow rate at low applied pressure. These requirements comply with monolithic stationary phase as they are considered to be a single particle which does not contain inter-particle voids. Therefore, solvent can flow through the stationary phase via the interwoven network structure of the monolith. In light of these observations and attributes of monolith, we hypothesize that the silica silanol groups of monolith can be explored as weak cation exchangers for the selective retention of polar basic analytes. The aim of this study is to explore the application of silica monolith as offline solid phase extractor of polar basic analytes (epinephrine, normetanephrine and metanephrine). Moreover the sample cleaning efficiency was also evaluated to render the analytes free from the matrix interferences. This study presents a simple but robust method for the extraction of polar analytes from urine using high surface area silica monolith acting as a weak cation exchange cartridge.

2. Experimental

2.1. Chemicals

Tetramethoxysilane and polyethylene glycol (PEG) were purchased from Sigma–Aldrich (Milwaukee, WI). Epinephrine, normetanephrine and metanephrine were purchased from Sigma (St. Louis, MO, USA). Formic acid was from Fluka (Buchs, Switzerland). Acetonitrile (ACN) was of HPLC-grade and all other chemicals used were of analytical grade. Milli-Q water (Millipore Bedford, MA, USA) was used throughout the experiment. Standard stock solutions of normetanephrine, metanephrine and epinephrine were prepared in 0.1 N HCl to give a final concentration of 1 mg/mL. The working standard solutions were prepared by appropriate dilution of stock. The prepared samples were stored in the dark at −20 °C.

2.2. Cartridge preparation and characterization

Monolithic cartridge was prepared by sol–gel technology [26]. Briefly, 2 mL of tetramethoxysilane was mixed with a solution of 0.44 g polyethylene glycol (Mw 10,000) and 0.45 g urea in 5 mL 0.01 M acetic acid. The mixture was stirred for 45 min at 0 °C. The solution was kept at 40 °C where it gelled within 2 h and subsequently aged for 18 h at the same temperature. The aged gel was treated at 120 °C for 3 h which led to formation of mesopores by the hydrolysis of urea to ammonia [17]. After cooling it down to room temperature (25 °C), the cartridge was washed with 50:50 water:methanol for 12 h. Finally the washed cartridge was treated at 550 °C for 8 h. The prepared cartridge was cut in 2 cm length and fixed in a 2-mL syringe and used on Varian 24 position vacuum manifold (Fig. 1). The prepared cartridge was characterized with regards to its surface area and macroporous and mesoporous structures. Surface characteristics were analyzed based on nitrogen sorption performed on a gas adsorption analyzer (TriStar 3000, Australia). Surface morphology was photographed using scanning electron microscope (SEM, JEOL JSM-6701F, Japan). Infrared (IR) analysis of the monolith was recorded on a FTIR spectrometer (Spectrum 100 FTIR, PerkinElmer, USA).

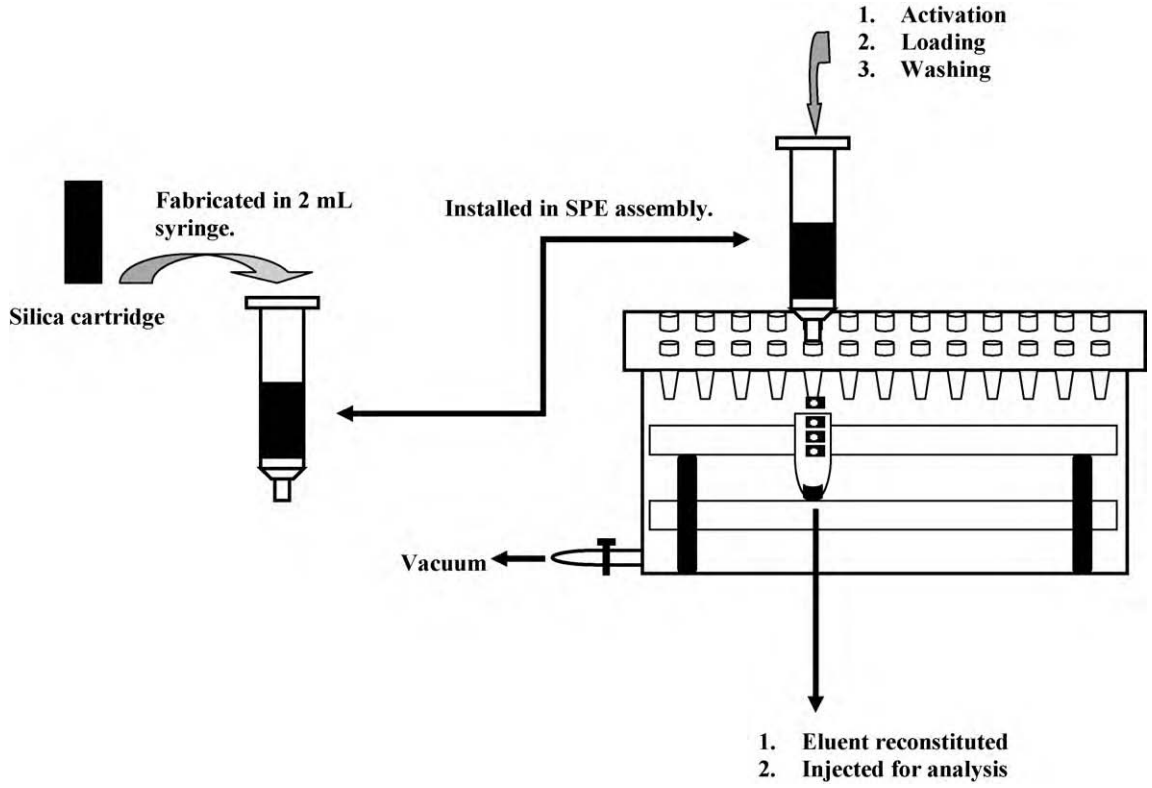


Fig. 1. Extraction steps using the prepared underivatized silica cartridge on SPE manifold.

2.3. Solid phase extraction

The extraction (loading, washing and elution) was carried out after installing the prepared cartridge in a 2-mL syringe. The extraction was initiated with the activation of cartridge using 1.5 mL methanol followed by 1.5 mL of milli-Q water. A 1-mL aliquot of sample matrix (water or urine) was directly transferred over the preactivated cartridge and allowed to flow through under applied vacuum (40–45 kPa). No prior treatment of sample matrix was done before loading on to the cartridge. The cartridge was washed with 2 mL of water and drained completely before adding the elution solvent. Finally, the adsorbed analytes were eluted with 5 mL of 0.1% formic acid. The eluted analytes were collected and lyophilized. The residue was reconstituted with 500 μL of 0.1% formic acid. 5 μL of the reconstituted sample was injected into HPLC coupled to a triple quadrupole/linear ion trap mass spectrometer. For consecutive use the cartridge was washed with 1 mL of 0.1% formic acid and finally with 5 mL of water before loading the subsequent sample. In order to test the efficiency and carryover effect of the cartridge, 50 ng/mL of each of the three analytes was spiked in a urine sample that was subsequently divided into five portions. Each urine portion was extracted consecutively on the same cartridge. Extraction of each spiked urine sample was followed by extraction of an unspiked urine sample. After each extraction, the cartridge was washed again as described above.

Table 1
Optimized parameters for LC/MS quantitation.

Analytes	Transitions	DP (V)	EP (V)	CE (V)	CXP (V)
Epinephrine	184 → 107	15	4	25	3
Normetanephrine	184 → 134	15	4	25	3
Metanephrine	198 → 148	15	4	25	3

DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision exit potential.

The sample cleaning efficiency was evaluated based on the osmolalities of the samples measured using the Vapro Osmometer 5520 (Wescor, Inc., USA) to verify that the analytes were free from interfering ions. For this, three spot urine samples collected in the morning, afternoon and at night were cleaned using the silica cartridge. The osmolalities of the urine samples before SPE and their corresponding extracts were recorded to measure the cleaning efficiency. The results were analyzed statistically by two-way ANOVA to verify the level of significance in the difference between the osmolalities of extracts before and after SPE.

2.4. Chromatographic conditions

The Agilent 1200 series HPLC system comprised of a binary pump (G1312A), an online degasser (G1379B), an autosampler (G1329A) and a system controller. The data were analyzed using Analyst software. The separation was performed in the isocratic condition using a C_{18} column (150 mm × 2 mm ID × 5 μm particles) (Phenomenex, Torrance, USA) protected by a guard column (C_{18} ODS, 3.0 mm ID × 4 mm) (Phenomenex). The mobile phase consisted of 10% ACN in 0.012% formic acid and flow rate was set at 0.25 mL/min. The system was operated at ambient temperature. The column outlet was directly connected to the ESI probe with PEEK tubing.

2.5. Mass spectrometry conditions

LC was coupled to 3200 QTRAP mass spectrometer (Applied Biosystem, USA). The mass spectrometer and LC parameter were controlled using Analyst software 1.4.2 (Applied Biosystem, USA). The analytes were quantitated using multiple reaction monitoring (MRM) to study the transition of the parent to the product ions (Table 1). The MS was operated in positive ion spray mode with the parameters settings as follow: curtain gas = 10; collision

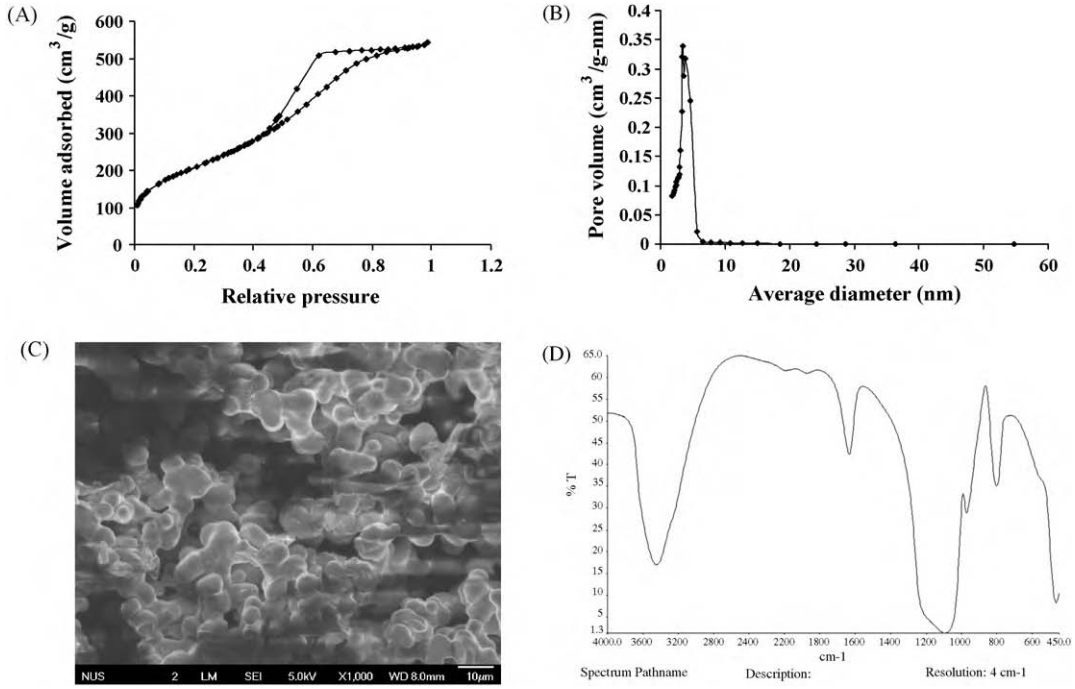


Fig. 2. Physical and chemical characteristics based on Nitrogen adsorption and desorption isotherms (A); pore size distribution (B); SEM photograph (C); and FTIR spectrum analysis (D) of the prepared silica cartridge.

gas (CAD) = medium; ion spray voltage = 5000 V; source temperature (TEM) = 300 °C; ion source gas 1 (GS1) = 45 psi; ion source gas (GS2) = 35 psi; and interface heater = on. Quadrupole 1 (Q1) and quadrupole 3 (Q3) were maintained at unit resolution and dwell time was set at 200 ms.

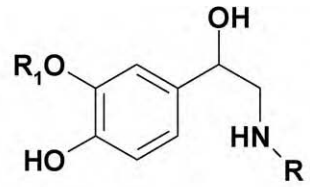
3. Results and discussion

3.1. Monolithic cartridge characteristics

Fig. 2 represents the characteristic features of the prepared monolithic silica cartridge based on their characterization parameters. Bimodal pore structure is the characteristic feature of the monoliths which is characterized by the presence of mesopores and macropores. These pores have their own significance in the process of extraction. Mesopores provide the desired surface area for the interaction with the analyte whereas macropores are responsible for the permeability of the solvents with low back pressure. The high surface area mesopores facilitate the mass transfer between the phases in efficient and short duration. The nitrogen adsorption/desorption isotherms of the synthesized silica cartridge is shown in Fig. 2A. The isotherm exhibits type IV curve which had a characteristic hysteresis loop of H_2 type particular for mesoporous sorbents. This agrees well with the previously reported characteristics of mesoporous materials [27,28]. Fig. 2B shows the derived mesopore size distribution based on Barrett–Joyner–Halenda (BJH) method which exhibited a mean pore diameter of 4.5 nm. The surface area and pore volume was found to be 745.14 m^2/g and 0.84 cm^3/g, respectively, using Brunauer–Emmett–Teller (BET) method. SEM photograph (Fig. 2C) shows the macropores present in the silica cartridge. IR analysis (Fig. 2D) confirmed the distribution of hydroxyl groups on the heat treated cartridge. The presence of band in the range between 3800 and 3000 cm^{-1} was attributed to Si–OH stretching bands. Strong band in the region of 1100–1250 cm^{-1} corresponded to Si–O–Si stretching bands [29] and the peak at 1650 cm^{-1} was characteristic of SiO_2. These results suffice the applicability of the prepared cartridge for SPE.

3.2. Cartridge SPE conditions

Fundamental prerequisite for the extraction of polar compounds from the biological matrices demands highly polar stationary phase with high surface area. This will provide higher affinity for the analytes in competition to sample matrix. With this principle in mind, the performance of the prepared cartridge was evaluated based on its extraction efficiency of the model polar analytes. The underivatized silica cartridge contains siloxanes and silanols as the major functional groups. Silanols are responsible for the ion exchange whereas siloxanes provide the weak reversed phase binding characteristic [30]. The basic mechanism underlying the adsorption of analytes is based on polar ionic interactions. The schematic of the process is demonstrated in Fig. 3. The underivatized silica materials contains hydroxyl group that is bonded to silica backbone. The silanol group (Si–OH) on the surface of cartridge is acidic and exist as $Si–O^-$ at pH above 4 and will retain cations if the selected pH maintains both entities in charged state. Thus, the cartridge acts as weak cation exchanger. Electrostatic interaction between the positively charged model analytes (quaternary amines) and negatively charged silica cartridge possessing number of hydroxyl group and high surface is the probable mode of adsorption. A routine SPE condition was applied based on previous literatures [31,32]. The retention based on ion exchange mechanism depends heavily on pH of the environment. The pH should be selected at which the analyte and the sorbent functional groups remain in charged state. Therefore, based on the nature of the sorbent and the analytes, pH was adjusted to 6.5 before loading the sample on to the cartridge. The cartridge was washed with twice the volume of the loaded matrix to eliminate unwanted impurities. Biological samples are known to possess polar ionic endogenous compounds which can be retained strongly on underivatized cartridge because of their affinity towards silanols. In order to remove these endogenous compounds, the polarity of the washing solvent has to be such that these interfering compounds find higher affinity towards them for their successful removal. Since the presence of organic solvent could have altered the polarity of water, the cartridge was washed



Compound	R	R_1
Epinephrine	$-CH_3$	-H
Normetanephrine	-H	$-CH_3$
Metanephrine	$-CH_3$	$-CH_3$

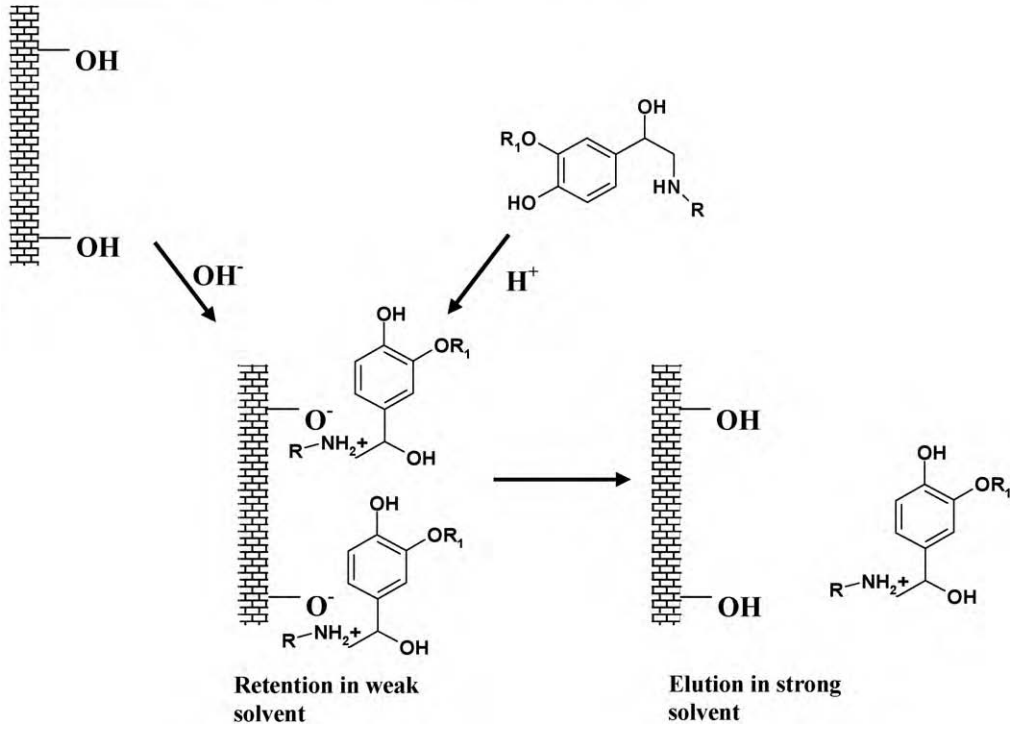


Fig. 3. Mechanism of adsorption and desorption of analytes in SPE.

with milli-Q water to eliminate the accumulation of the endogenous compounds. The analytes were then eluted out of the cartridge by regeneration of the free silanol groups (Si–OH) on the cartridge surface with mild acidified aqueous solution. In this study, the elution solvent was optimized by varying the concentration of formic acid in the elution solvent. It was found that higher the percentage of acid used will increase the efficiency of elution. However, it will also require higher volume of washing solvent required to neutralize the cartridge for the subsequent uses. Therefore, 0.1% formic acid was selected for the elution of analytes from the sorbent after washing. The pH (found to be 3 with 0.1% formic acid) provided good recoveries without any deleterious effect on the sorbent. After rinsing the sorbent, the cartridge was reused again for SPE. The repeatability and the efficiency of adsorption are discussed in later section.

3.3. Adsorption

The adsorption capacity of the cartridge was evaluated based on the concentration of analytes before and after loading the cartridge. Water was mimicked as the sample matrix and was spiked with the three analytes simultaneously, in increasing concentration. The volume of 1 mL was always kept constant. The concentration of 100, 200 and 500 ng/mL of the three analytes was prepared in water and the mixed analytes were allowed to pass through the cartridge. As the matrix was water, the eluted solution was directly injected into HPLC/ESI/MS system to estimate the adsorption capacity. On analyzing the eluents, it was found that the three analytes were absent at all three concentrations. The result confirmed the fast and high adsorption capability of the cartridge. Further increase in concentration could be explored but the high sensitivity of the instrument limited the concentration to 500 ng/mL; furthermore, concentrations of biogenic amines higher than 500 ng/mL might not reflect the endogenous concentrations that could be encountered in clinical practice.

3.4. Osmolality

Molality is the number of particles dissolved in a mass weight of fluid (mmol/kg). Osmolality is a measure of the total number of osmotically active particles in a solution and is equal to the sum of the molalities of all the solutes present in that solution. The osmolality of physiological fluids tends to be dominated by small molecules which are present in high concentrations. For instance in urine, sodium, potassium, chloride, bicarbonate and urea are the components present at high concentration which individually

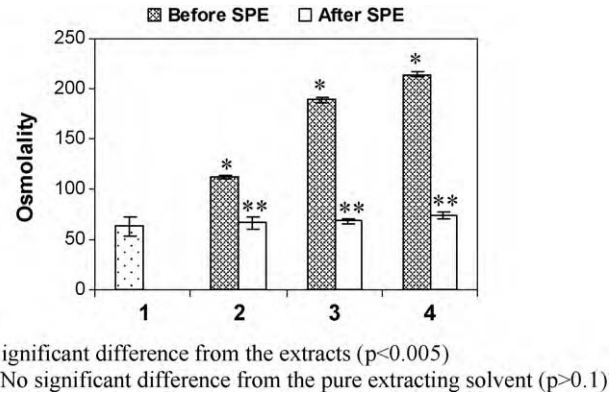


Fig. 4. Comparison of the osmolality of the urine samples ($n = 3$) before and after SPE: (1) osmolality of the pure extracting solvent; (2), (3) and (4) are the osmolalities of the blank urine samples collected at different time point before and their extracts after SPE.

affects osmolality. The urine osmolality is the best measure of urine concentration. In this study, osmolality was measured using an osmometer. It works on the principle of measuring the vapor pressure of the solution (altered in proportion to the number of solute particles) with respect to pure solvent.

The purpose of SPE is to prepare samples free from the interfering endogenous molecules and ions. The presence of endogenous small molecules and ions in the sample can compete with the analyte ions in the detector, leading to ion suppression. Measurement of the osmolality of urine samples collected at different times before and their respective extracts after SPE can be applied to determine the sample cleaning efficiency of the cartridge. Despite the various initial osmolality levels of the urine samples, it was found that the osmolality of the extracts after sample clean-up decreased significantly and was comparable to the that of the original eluting solvent ($p < 0.001$, but no significant differences among the extracts from the respective urine samples, as tested by two-way ANOVA) (Fig. 4). The finding indicated the high efficiency of the prepared cartridges in cleaning up the endogenous ions in urine samples.

3.5. Method validation

Extracts were recovered via SPE method using an in-house weak cation exchange silica cartridge. The reconstituted samples after lyophilization were directly injected into HPLC/ESI/MS for analysis. Calibration curve in the linearity range of 20–200 ng/mL was constructed from triplicate measurements of each concentration level of the calibration samples in spiked water, following all steps of SPE. The linearity of the calibration curve was checked using least squared regression analysis taking peak area versus concentration (x) as the regression parameters with $1/x$ weighting factor. The coefficients of determination, r^2 were found to be more than 0.99 for all analytes as shown in Table 2. The intra- and inter-day precision was expressed as the % relative standard deviation (%RSD) of the triplicate measurement at low (50 ng/mL), medium (100 ng/mL) and high (500 ng/mL) concentrations for each analyte. The %RSDs for intra- and inter-day variation at all concentrations were thereafter found less than 10% for the respective analytes (Table 3). The method sensitivity was established by examining the limits of detection (LOD) and limits of quantitation (LOQ). LOD was defined as the lowest detectable concentration with a signal to noise ratio of at least 3 and the LOQ was defined as the lowest quantifiable concentration with a signal to noise ratio of at least 10. The LODs and LOQs for the analytes under these conditions are presented in Table 2.

Table 3
The precision of the assay in intra- and inter-day variation of the analysis in aqueous samples.

Analytes	Precisions (%RSD)					
	Intraday ($n = 3$)			Interday ($n = 3$)		
	Low	Medium	High	Low	Medium	High
Epinephrine	3.4	2.8	3.8	4.1	9.7	4.6
Normetanephrine	5.0	5.9	2.3	5.8	7.5	3.8
Metanephrine	4.7	4.0	5.5	3.4	5.2	7.2

Table 4
The precision of the assay in intra- and inter-day variation of the analysis in urine samples.

Analytes	Precisions (%RSD)					
	Intraday ($n = 3$)			Interday ($n = 3$)		
	Low	Medium	High	Low	Medium	High
Epinephrine	7.4	5.6	9.8	7.8	6.3	8.9
Normetanephrine	7.2	6.4	3.6	5.4	7.2	5.3
Metanephrine	4.6	6.4	5.4	4.3	5.2	6.8

3.6. Analysis of spiked urine

Epinephrine, normetanephrine and metanephrine are present in trace amounts in healthy urine samples [32]. Urine samples were spiked with analytes to the desired concentrations (20, 50, 100, 200 ng/mL) before they were subjected to SPE extraction. The SPE steps were identical to that applied for the water spiked samples. The relative standard deviations for intra- and inter-day variation at all concentrations with spiked urine were within 10% (Table 4). The result of percentage relative recoveries (ratio of the peak areas of the spiked urine extracts to spiked water extracts) for urine samples is shown in Table 5. The results indicated that recoveries of normetanephrine and epinephrine were lower than that of metanephrine. Although 100% analytes were adsorbed onto cartridge but the interaction between epinephrine and normetanephrine with sorbent as compared to metanephrine was not sufficiently strong. This observation was possibly related to the differences in the competency of the three analytes with the sorbent in presence of many polar endogenous ions present in urine

Table 5
Relative recoveries of analytes from urine samples after SPE ($n = 3$).

Spiked concentration (ng/mL)	%Relative recoveries (%RSD)		
	Epinephrine	Normetanephrine	Metanephrine
20	60 (8.8)	55 (6.8)	101 (6.7)
50	62 (7.5)	56 (5.5)	105 (6.2)
100	65 (6.9)	57 (4.9)	99 (5.7)
200	67 (6.5)	59 (3.1)	103 (4.9)

Table 2
LOD, LOQ, linearity range, and the coefficient of determination, r^2 for the calibration curve of the respective analyte in aqueous samples.

Analytes	LOD (ng/mL)	LOQ (ng/mL)	Linearity range (ng/mL)	Linearity	r^2
Epinephrine	5	16.7	20–200	$y = 161.59x - 2413.4$	0.9952
Normetanephrine	5	16.7	20–200	$y = 266.09x - 536.33$	0.9999
Metanephrine	3	10.0	20–200	$Y = 145.19x + 435.04$	0.9920

Table 6
Relative recoveries of the analytes on a single cartridge after multiple extractions.

No. of extractions	50 ng/mL concentration		
	Epinephrine	Normetanephrine	Metanephrine
1st	62	55	101
2nd	64	57	99
3rd	61	52	104
4th	67	59	102
5th	65	54	106
%RSD	3.7	4.8	2.6

samples. The decreasing order of hydrophilicity of the three analytes is as follow: epinephrine > normetanephrine > metanephrine (the order is predicted based on the elution of the analytes from the column). Since the polarity of epinephrine and normetanephrine was on the higher side as compared to metanephrine therefore, their competitiveness to the binding sites on the sorbent with other ions in urine was higher. Thus, this reduces the chance of the two analytes (epinephrine and normetanephrine) to interact with the sorbent and accounts for their lower recovery.

3.7. Extraction efficiency and cartridge carry over

The extraction efficiency of the cartridge for repeated use was evaluated based on the recoveries after multiple extractions on the same cartridge. A defined concentration (50 ng/mL) of the analytes was spiked in a urine sample and was extracted on the same cartridge. Extraction of each spiked urine sample was followed by one extraction of an unspiked urine sample to monitor the carryover effect. After each extraction, the cartridge was washed extensively and the last portion of the washed eluent from the cartridge was also analyzed to monitor the carryover. 5 mL of water was selected for washing based on its ability to neutralize the cartridge pH before proceeding to the next step of sample extraction. The uniform pH condition was utilized for each extraction to ensure reproducible recoveries. Although extraction of five replicate samples was carried out to demonstrate the robustness of the prepared cartridge, it had been reused for many more times with minimal compromise on its reproducibility. Table 6 represents the relative recoveries of simultaneous extraction of three analytes with multiple extractions on the same cartridge.

4. Conclusions

Our attempt to utilize the principle of underivatized silica monolith as SPE sorbent for extraction of polar analytes from urine was proven to be effective. Although the recoveries of epinephrine and normetanephrine from the silica monolithic cartridges were approximately 60%, the optimal recovery of metanephrine (100%) proved to be promising. In the urine samples, there are endogenous ions competing with the polar compounds for the binding sites in the cartridge; therefore, the recovery appears to be lower for the polar compounds (e.g., epinephrine and normetanephrine) than for the comparatively less polar compound (e.g., metanephrine). Nevertheless, the % of recoveries for epinephrine and normetanephrine are still consistently around 60%. Our results are encouraging, as the cartridges were effective in extracting epinephrine, normetanephrine and metanephrine simultaneously, from urine samples with high levels of reproducibility. Furthermore, the sample matrices were directly loaded on to the cartridge without any prior treatment which was the added advantage of the prepared cartridge. With the application of the prepared cartridges, the developed assay was validated suitable for selectively extracting polar basic analytes even in the presence of complex matrix like urine. The finding of the highly reproducible recoveries after consecutive multiple extractions on the same cartridge is particularly interesting. This indicates that it will be economical to use these cartridges as they can be reused for many times without significant carryover effects and loss of efficiency. This unique property is different from that of the conventional SPE system which has limited number of reusability [33]. The observed robustness of the developed underivatized silica monolithic cartridge is accounted by its unique approach in sample extraction via weak rather than strong ion exchange interaction.

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