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ARTICLE

Multiwalled Carbon Nanotubes as Sorbent for Online Solid-Phase Extraction of Resveratrol in Red Wines Prior to Fused-Core C18-Based Ultrahigh-Performance Liquid Chromatography−Tandem Mass Spectrometry Quantification

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An ultrafast analytical protocol based on online solid-phase extraction (SPE)/high-performance liquid chromatography−tandem mass spectrometry for the determination of resveratrol in red wines has been developed. In the present work, multiwalled carbon nanotubes (MWCNTs) were used as SPE sorbents for the analytes' online extraction and cleanup. The target analytes were separated on a fused-core C18-silica column (Halo, 50 mm × 2.1 mm i.d., 2.7 μm) and quantified by triple-quadrupole linear ion trap mass spectrometry in negative ion multiple-reaction monitoring (MRM) mode. The proposed analytical procedures were carefully optimized and validated. The calibration function is linear from 0.37 to 370 ng mL^{-1} and from 0.13 to 130 ng mL^{-1} for *trans*- and *cis*-resveratrol, respectively. The limits of quantification (LOQs) of *trans*- and *cis*-resveratrol obtained were 0.05 and 0.06 ng mL^{-1}, which means that the proposed method is suitable for trace analysis of resveratrol at low-level concentration. At the three fortified levels (low, medium, and high), recoveries of resvetatrol ranging from 76.9 to 108.3% were obtained. Eight red wine samples from different regions of China were analyzed. The results indicated that the present online SPE-LC-MS/MS system significantly increased sample throughput and decreased solvent consumption, exhibiting great potential to be applied for analyzing resvetatrol in red wines.

KEYWORDS: Multiwalled carbon nanotubes; online solid-phase extraction; fused-core technology; liquid chromatography−tandem mass spectrometry; red wines; resveratrol

INTRODUCTION

In recent years there has been a growing interest in resveratrol (3,5,4′-trihydroxystilbene, $C_{14}H_{12}O_3$) due to its beneficial effects on human health revealed by biological and clinical studies. Resveratrol, a naturally occurring polyphenol, is produced by some fruits such as mulberries and peanuts, whereas grapes and related products such as grape juice, red wine, and other plant extracts are considered the most important dietary sources of these substances (*1*). Two isomeric forms, *trans*-(*E*) and *cis*-(*Z*), exist in wine; *trans*-resveratrol (TRA) has been widely studied, whereas *cis*-resveratrol (CRA) is not a natural constituent of grape. It is likely that CRA is derived from its *trans*-isomer during the winemaking process, storage in the bottle, or analysis (*2*, *3*). Resveratrol has an important role in human health as it presents antibacterial, antifungal, and antioxidant properties (*4*). This compound has also the capacity to protect against global cerebral ischemic injury; inhibit tumor initiation, promotion, and progression (*5*, *6*), and reveal certain chemicopreventive activity against cancer (*7*) as a selective modulator of estrogen receptors (*8*). These effects on human health make resveratrol an important quality indicator of red wine, and it is possible that the TRA concentration can be used for the marketing of red wine. On the basis of these facts, new analytical methodologies for the fast determination of resveratrol in red wines are urgently needed.

Several methods have been developed for the analysis of TRA and CRA in red wines. Sample preparation, such as liquid−liquid extraction and solid-phase extraction (SPE) (*9*, *10*), are most widely utilized prior to the chromatographic separation due to the complex nature of the wine matrix. However, these procedures are laborious and error-prone and require large sample amounts, large volumes of potentially toxic solvents, and multistage operation steps, including evaporation and redissolution before quantification. For analysis and determination, high-performance liquid chromatography (HPLC) is the most commonly used procedure with different detection techniques such as UV diode array detection (DAD) (*11*). The applications of electrochemical detection (*12*), fluorometric detection (*13*), and mass spectrometry (*14*) also have been published. Methods based on gas chromatography−mass spectrometry (*15*) have been proposed for TRA analysis, but this technique generally involves a series of

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inconvenient steps such as extraction, cleanup, or derivatization reaction prior to GC analysis of this substance, and this handling can enhance the TRA to CRA isomerization.

During method development, the use of effective and selective SPE supports is really important to reduce the interference of sample matrix. Carbon nanotubes (CNTs) including single-walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs) have attracted more and more attention in the enrichment of target compounds from a complex matrix. However, SWCNTs easily form big bundles with narrow intertubular gaps, which obstruct the permeation and extraction of the target analytes (*16*, *17*). Compared to SWCNTs, MWCNTs were more competitive with a lower price. On the basis of peculiar structural characteristics, MWCNTs have been exploited in analytical and other fields (*18*, *19*). From the reports by Cai et al. (*20*), the MWCNTs were promising adsorbent materials because of the larger specific area and hydrophobic characteristic of the surface. The adsorption mechanisms involve the establishment of weak interactions, more precisely, $\pi-\pi$ stacking, van der Waals forces, other hydrophobic interactions, and electrostatic forces (*21*). This fact facilitates the adsorption of analytes in a selective and reproducible manner. As a result, MWCNTs have been successfully used as the sorbent for the preconcentration and purification of parabens (*22*), phthalate esters (*23*), etc.

In addition, the development of fused-core particles was considered to be a breakthrough in column technology aimed at reducing analysis times while maintaining column efficiencies and requiring relatively low backpressures (*24*). With a 1.7 μm solid silica inner core surrounded by a 0.5 μm porous silica shell (*25*), the material has a shortened diffusion path, which allows for rapid mass transfer and thus reduced axial dispersion and peak broadening. More importantly, 2.7 μm fused-core particles produce only approximately half the backpressure of the 1.8 μm particles, which makes it possible to use fused-core columns on conventional HPLC systems (*26*). Hsieh et al. (*27*) compared the column efficiency and pressure for fused-core columns and sub-2 μm porous particles. The fused-core particles, bonded with C18 alkyl chains, had a very similar selectivity to the sub-2 μm Zorbax C18 phase, but provided a better shape selectivity. Despite these advantages, fused-core C18−silica column has not been widely used. To reduce analysis time and save solvents, the integration of fused-core columns into online SPE-HPLC-MS/MS systems is generally recommended.

Consequently, the aim of this work is to establish a fully automated method for ultrafast determination of resveratrol using online SPE coupled to liquid chromatography, employing MWCNTs as SPE sorbents and fused-core C18 column for compound separation. Online SPE greatly reduces the sample pretreatment steps and decreases the amount of chemical waste and analysis time, which meets the increasing demand for automation and high-throughput analysis. The homemade MWCNT-packed trapping column was especially efficient in absorbing resveratrol and suppressing matrix. The red wine samples could be directly introduced into the SPE column without pretreatment. After concentration and purification, the analytes were eluted into a Halo fused-core column, which delivered hyperfast chromatographic separations without sacrificing reliability. The factors affecting preconcentration and separation of the analytes are discussed in detail. In addition, full validation was performed to assess the linearity, recovery, limit of quantification (LOQ), and precision of the method. The method was successfully applied to the determination of resveratrol in red wines, and the online SPE-HPLC-MS/MS analysis was shown to be a rapid, sensitive, and robust method.

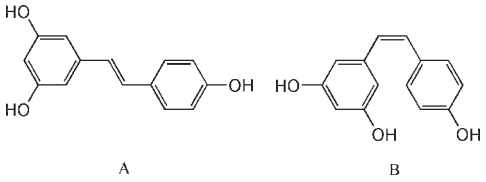

Figure 1. Chemical structures of the *trans*-isomer (**A**) and *cis*-isomer (**B**) of resveratrol.

MATERIALS AND METHODS

Reagents and Materials. Multiwalled carbon nanotubes (purity ≥ 95%) were obtained from Shenzhen Nanotechport Co., Ltd. (Shenzhen, Guangdong, China), with an average diameter of 40−60 nm, length of 5−15 μm, and surface area of 40−300 $m^2\ g^{-1}$. The MWCNTs were already dried for 2 h at 130 °C to remove the adsorbed water beforehand and kept in a desiccator.

The TRA working standard (purity ≥ 98%) was purchased from Shananxi Sciphar Biotechnology Co., Ltd. (Shaanxi, China). A stock solution (3.7 $\mu g\ mL^{-1}$) of TRA was prepared by dissolving the standard in methanol and kept at 4 °C in a dark bottle to avoid exposure to direct light. CRA was obtained after a 4 h exposure of a standard TRA methanolic solution (1.3 $\mu g\ mL^{-1}$) to UV light. After UV irradiation, the signal of TRA remaining was very weak, which meant most TRA was converted into the *cis*-isomer. Then, the mixed standard solution was obtained by combining the TRA and CRA stock solutions. Working standard solutions were freshly prepared by diluting the stock solutions with HPLC mobile phase. **Figure 1** shows the chemical structures of TRA and CRA.

Acetonitrile, methanol, and formic acid were of chromatographic grade and obtained from Merck (Darmstadt, Germany). High-purity water with a resistivity of 18.2 $M\Omega\ cm^{-1}$ was obtained from a Milli-Q water system (Millipore, Bedford, MA). All other organic solvents were of analytical grade and purchased from Huadong Chemicals Co., Ltd. (Hangzhou, Zhejiang, China).

The red wine samples originating from seven regions of China were purchased from a local supermarket (Wumei, Hangzhou, China).

Instrumentation. A schematic diagram for the online SPE-LC-MS/MS system is shown in **Figure 2**. The flow injection system used for delivering loading solvent consisted of a model 515 pump (Waters, Milford, MA) and a Rheodyne 7725i manual injection valve with a 20 μL loop. A precolumn (10 mm × 4.6 mm i.d.) packed with 80 mg of MWCNTs was used for the SPE preconcentration and purification of resveratrol in red wines. All of the PTFE tubes (75 μm i.d.) used for connections were kept at the shortest possible length to minimize the dead volume.

The high-performance liquid chromatograph (HPLC) used was a Waters 2695 system equipped with a binary LC pump, an autosampler and a vacuum degasser. The resveratrol separation was achieved using a Halo fused-core silica column (50 mm × 2.1 mm, i.d., 2.7 μm; Advanced Materials Technology, USA), with a mobile phase flow rate of 0.4 mL min^{-1}. The mobile phase consisted of (A) high-purity water (0.1% formic acid) and (B) acetonitrile. A gradient elution program was applied as follows: 0−4 min, linear increase from 20 to 100% B; 4−5.9 min, hold at 100% B; 5.9−6.0 min, linear decrease from 100 to 20% B. The two compounds were eluted within 2 min. The rest time was used to reestablish equilibrium of the column.

A triple-quadrupole mass spectrometer (4000Q-Trap, Applied Biosystems, Foster City, CA) was coupled to the HPLC system through a turbospray electrospray (ESI) interface operating in negative ionization mode. Instrument control, data acquisition, and processing were performed using the associate Analyst 1.5.1 software. MS/MS data were acquired in the multiple-reaction monitoring (MRM) mode. For each analyte, two transitions between precursor ions and the two most abundant product ions were monitored: the transition 226.8 → 184.8 for quantitative determination and the transition 226.8 → 142.8 for qualitative analysis. To increase sensitivity, the ion source temperature (TEM) was set at 450 °C, and the ion spray voltage (IS) was always set at −4.5 kV. Ion source gas 1 (GS1) and ion source gas 2 (GS2) were used as the drying and nebulizer gases at backpressures of 15 and 18 psi, respectively. Curtain gas (CUR) was 30 psi. Table**1** shows the optimized MS conditions used for the analysis of the target analytes.

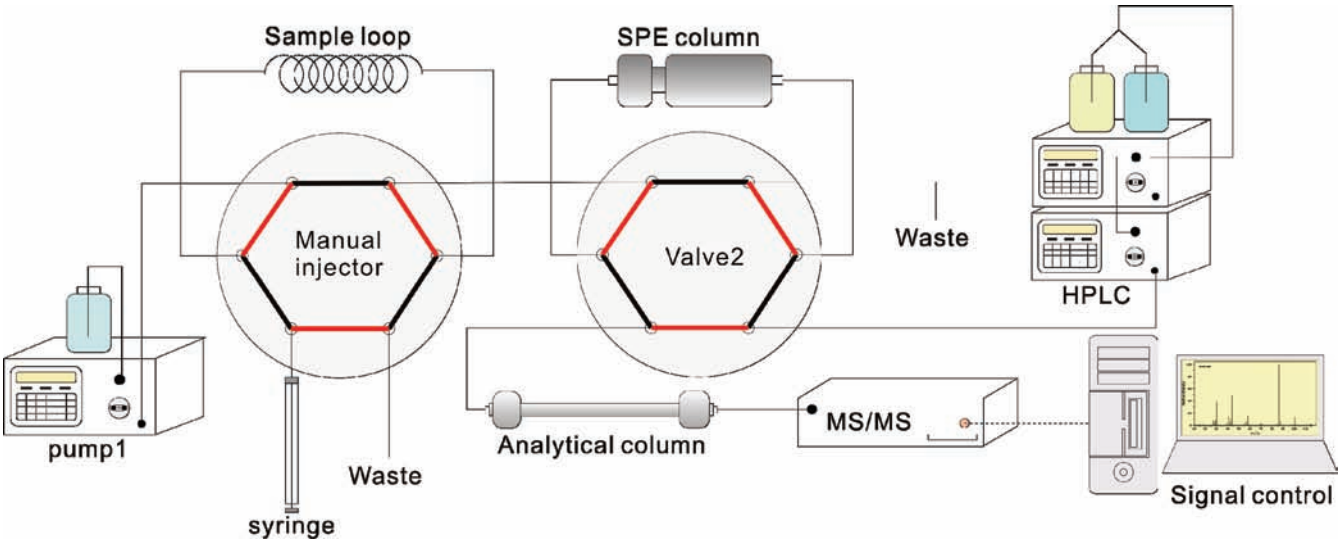

Figure 2. Schematic diagram of the online SPE-LC-MS/MS system. When the manual injector and valve 2 are at position "load" and A, respectively, two ports joined by a black line are connected and two joined by a red line are not when the manual injector and valve 2 are at position "inject" and B.

Table 1. MS Parameters of *trans*- and *cis*-Resveratrol

		MS/MS parameters[a] (V)				
compound	precursor/product ion pairs (*m*/*z*)	DP	EP	CE	CXP	retention time (min)
trans-resveratrol	226.8 → 184.8	−90.0	−10.0	−27.5	−15.0	1.54
	226.8 → 142.8	−90.0	−10.0	−37.0	−15.0	1.54
cis-resveratrol	226.8 → 184.8	−90.0	−10.0	−27.5	−15.0	1.70
	226.8 → 142.8	−90.0	−10.0	−37.0	−15.0	1.70

[a] DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential.

Preparation of MWCNT-Packed Trapping Column. The MWCNT materials were laboratory-packed in a 10 mm × 4.6 mm i.d. (~20 mg sorbent mass) column holder (Shimadzu, Kyoto, Japan) for system evaluation and application. The column was then connected to the six-port valve with PEEK tubing. The stability and potential regeneration of the column were investigated, and the testing results suggested that the SPE cartridges could be properly cleaned and conditioned after each extraction. The column was stable up to at least 50 adsorption−elution cycles without an obvious decrease in the recovery of resveratrol.

Preparation of Samples and Standards. The commercially available red wines were produced in seven regions of China (Jilin, Gansu, Shanghai, Yunnan, Hebei, Shandong, and Henan). All wines were stored in the dark at 4 °C and analyzed immediately after bottle opening, under the same analytical conditions. In a centrifugal tube, 0.1 mL of red wine was diluted with methanol to 5 mL and filtered through a 0.22 μm membrane filter before use. All samples were protected from light to avoid photon-induced isomerization throughout the preparation stages. As for the recovery test, three different concentration levels (low, medium, and high) of resveratrol were added to the red wine prior to analysis. The resulting samples were analyzed as described above. Triplicate experiments were performed at each level.

The standard mixture was weekly prepared by mixing two standard solutions: 0.37 μg mL^{-1} for TRA and 0.13 μg mL^{-1} for CRA. For quantitative analysis, matrix-matched calibration standards were prepared in triplicate at five concentrations range from 0.5 to 500 ng mL^{-1} for total resveratrol. All solutions were stored at 4 °C. The XIC chromatogram of resveratrol standards is shown in **Figure 3**.

Analytical Procedures. The online SPE-HPLC-MS/MS method consisted of four major steps as follows:

Step 1. The six-port switching valve (valve 2) was set at position A (black line, see **Figure 2**), and the flow injection pump (pump 1) delivered loading solvent at a flow rate of 0.4 mL min^{-1}. The red wine sample was injected into the loop with a syringe while the manual injector (valve 1) was at the "load" position (black line).

Step 2. Valve 2 was switched to position B (red line), and the manual injector was switched to "inject" position, so that the red wine sample was introduced into the MWCNT-packed trap column and the resveratrol was extracted. The matrix was constantly washed out by 4 mL of high-purity water at the flow rate of 0.4 mL min^{-1}.

Step 3. Pump 1 was stopped, valve 2 was turned to position A, and the analytes trapped on the SPE column were eluted into the analytical column in the back-flush mode with the chromatographic mobile phase for separation.

Step 4. The manual injector and valve 2 were returned to positions "load" and B, respectively. Data acquisition started simultaneously. Meanwhile, once the first sample had been eluted and HPLC analysis had begun, the automated online SPE system began extraction of the next sample during the previous chromatographic separation. Because sample extraction and analysis can continue to overlap in this manner, the analysis time can be reduced to approximately 6 min per sample.

Peak Identification. TRA and CRA were identified by the comparison of their retention times and MS spectra (MRM mode) with the corresponding standards.

RESULTS AND DISCUSSION

Optimization of Online SPE. By comparison with the performance of C18 SPE reported in the literature (*28*), the recovery values of MWCNT-packed cartridge indicated a similar enrichment and purification ability to resveratrol. However, the lower price made MWCNTs more charming and competitive. Then, a series of experiments were performed to optimize the online SPE conditions to obtain good sensitivity and precision. Various parameters affecting the trapping efficiency (analyte volume, organic modifier ratio, pumping speed, loading, and washing time) were investigated.

First, analyte breakthrough volume was measured by introducing 20 μL red wine samples with different dilution ratios into the SPE column at a flow rate of 0.4 mL min^{-1}. The column effluent was directly monitored by the MS detector. Because of the high sensitivity and low detection limit, a sample diluted 50-fold was chosen for subsequent experiments, resulting the minimum contamination of the SPE column brought by the sample matrix.

Then, the effect of the loading solvent flow rate was investigated over the range from 0.2 to 1 mL min^{-1}. Higher flow rate could cause slight sample leakage and a decrease of the lifetime of

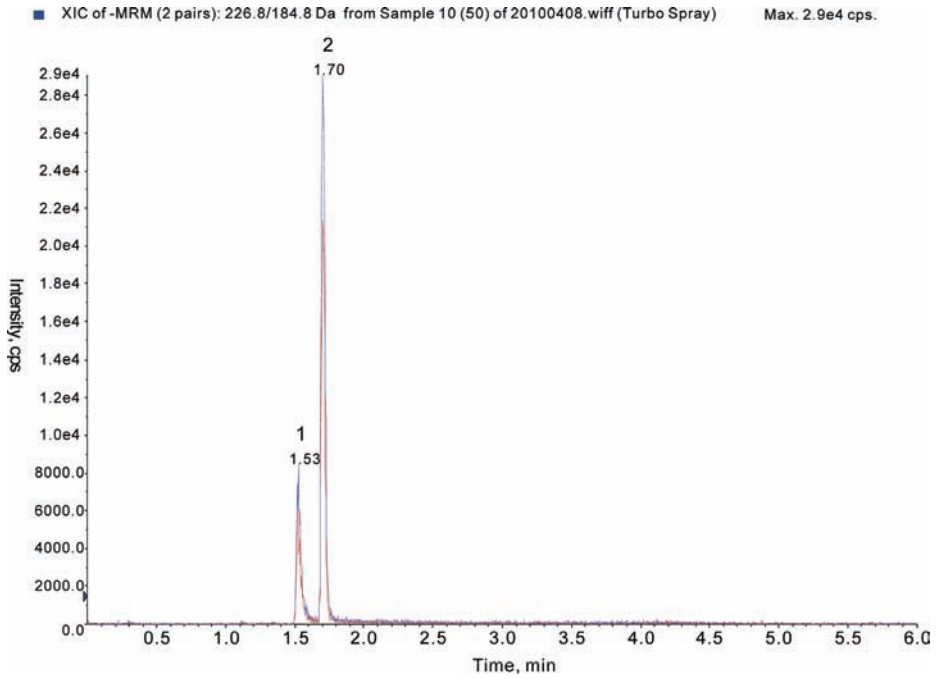

Figure 3. XIC chromatograms of TRA and CRA standards. Peaks: 1, TRA; 2, CRA.

the MWCNT-packed trapping column owing to the high backpressure. A flow rate of 0.4 mL min^{-1} was an optimal value for introducing resveratrol into the SPE column.

The ionization efficiency of samples in electrospray source may be affected by matrix interference. Given that red wine matrix is quite complex, the percentage of organic modifier for cleanup of the sample matrix was studied. No desorption was detected when the acetonitrile concentration was ≤10%. However, more coextracted matter was retained on the SPE column when high-purity water was used as solvent. When acetonitrile concentration was 20%, the recovery of resveratrol decreased. Consequently, 10% acetonitrile was adopted as the optimum loading solvent.

Moreover, the pH value plays an important role for the quantitative recoveries of TRA and CRA during the SPE procedure. The pH of the loading solvent was adjusted to pH 2−3 with $HCOO^-$/HCOOH buffers, to pH 4−6 with CH_3COO^-/CH_3-COOH buffers, and to pH 7 with CH_3COONH_4 buffer solution. According to the results, the retention of TRA and CRA on MWCNTs was high in the acidic solution. The highest retention for resveratrol was obtained at pH 2, and the signals remained almost constant from 2 to a pH value of 3. The adsorption decreased considerably with the increase of solution pH to 7. At higher pH, a less marked reduction of the signal was observed. Therefore, pH 3 was selected as the optimum value.

Finally, the total duration for loading sample and washing matrix was optimized in the range from 30 to 180 s, at a sample flow rate of 0.4 mL min^{-1}. When longer loading times were used, the recoveries of resveratrol decreased, because resveratrol was washed out by the excess washing solvent. When the time was < 30 s, more coextracted matter was retained on the SPE column. Finally, 60 s was adopted for the proposed method.

Optimization of Chromatographic Conditions. It is important to optimize the chromatographic conditions before mass spectrometric detection is attempted, including the types of column and composition of mobile phase, because impurities in the samples can greatly decrease the sensitivity of determination by affecting the ionization of the compounds of interest. To achieving good resolution and symmetric peak shapes of analyte in a shorter run time, the selection of analytical columns with high separation efficiency is a prerequisite. The chromatographic performance of Halo fused-core C18−silica column (50 mm × 2.1 mm i.d., 2.7 μm) was compared with that of conventional fully porous particle packed columns (1) Agilent Zorbax SB-C18 250 mm × 4.6 mm i.d., 5 μm; (2) Waters Atlantis T3-C18 150 mm × 2.1 mm i.d., 3 μm; and (3) Phenomenex Synergi Fusion-RP 80A 50 × 2.0 mm i.d., 4 μm, for online SPE-HPLC-MS/MS analysis. It was found that the separation efficiency and sensitivity of the Halo column was obviously performed much better due to its special structure (a 1.7 μm solid core particle fused with a 0.5 μm porous shell). This novel fused-core column packing technique greatly decreased the diffusion path, allowed rapid mass transfer, and thus reduced axial dispersion and peak broadening. Compared to the sub-2 μm particles, similar efficiency separations could be achieved on conventional HPLC systems using these fused-core columns, saving the expensive cost of ultrahigh-pressure instrumentation. As a result, the Halo C18 column was finally chosen in this work.

The analytical sensitivities in condition of samples eluted with methanol/water, acetonitrile/water, and acetonitrile/water (0.1% formic acid) were compared. The results indicated that methanol gave rise to better selectivity, whereas acetontrile gave rise to better elution strength and shorter retention time. Furthermore, acetontrile generated lower backpressure than methanol, which made the Halo column especially suitable for conventional LC equipment. The 0.1% formic acid in water solution played an important role in improving peak shape. Results of multiple injections indicated that under such a situation nice peak shape and high sensitivity of resveratrol could be achieved. The retention time of the last peak in our test was < 2 min with a flow rate of 0.4 mL min^{-1}.

Optimization of MS-MS Conditions. Acquisition parameters of the mass spectrometer were optimized by direct continuous pump infusion of standard working solutions of the analytes (100 ng mL^{-1}) at a flow rate of 10 μL min^{-1} in the mass spectrometer. Full-scan spectra were acquired over the m/z range of 50−500 amu with a dwell time of 1.0 s and a step size of 0.1 amu for identification of the precursor ions, and the deprotonated molecular ion $(M - H)^-$ at m/z 226.8 was selected as precursor

Table 2. Calibration Data of Resveratrol Standards

analyte	slope	intercept	R^a	LOD[b]	LOQ[c]	linearity
trans-resveratrol	1194.3	146.28	0.9996	0.02	0.05	0.37−370
cis-resveratrol	1465.6	83.61	0.9997	0.02	0.06	0.13−130

[a] Regression coefficient. [b] Limit of detection (3 S/N, ng mL^{-1}). [c] Limit of quantitation (10 S/N, ng mL^{-1}).

ion for TRA and CRA. The declustering potential (DP) was optimized for achieving the highest signal response of $(M - H)^-$. Further identification of the most abundant fragment ions and selection of the optimum collision energies (CE) for TRA and CRA were carried out in the product ion scan mode. The fragmentations of m/z 184.8 and 142.8 were comparatively high intensity. On this point, the MRM transition (226.8 → 184.8) was used as a quantifier, whereas the mass transition (226.8 → 142.8) was used as a qualifier for TRA and CRA. The optimal parameters are given in **Table 1**. In view of that TRA and CRA are isomers of each other with the same ionization behavior, the retention time is the parameter used to identify isomers.

To achieve maximum MRM transition sensitivity, a flow injection analysis (FIA) methodology was adopted by introducing a 5 μL standard solution of resveratrol (1 μg mL^{-1}) into the mass spectrometer using the initial chromatographic conditions. The optimum conditions including curtain gas (CUR), ion source gas 1 (GS1), ion source gas 2 (GS2), temperature (TEM), and collision-activated dissociation (CAD) were summarized under Instrumentation.

Method Validation. Method performance was investigated with respect to various parameters such as linearity, selectivity, limit of detection (LOD), limit of quantification (LOQ), recovery, and precision (*29*).

The calibration curves in the linear range of 0.37−370 ng mL^{-1} for TRA and 0.13−130 ng mL^{-1} for CRA were produced by fortified blank samples with working standard solutions of resveratrol at six concentration levels. All measurements were done in duplicate. Then the calibration curves were constructed by plotting the peak area versus the concentration of each analyte. **Table 2** shows the results of the standard calibration curves of integrated peak area (n = 3) and linearity (R^2). The high correlation coefficient values (R^2 > 0.9996) indicated good linearity between their peak areas (y), investigated compound concentration (x, ng mL^{-1}) in relatively wide concentration ranges, and excellent sensitivity of the analytical method.

Selectivity was conducted in the MRM mode. For each analyte, two transitions between precursor ions and the two most abundant product ions were monitored. The more abundant one was used for quantification and the other one for confirmation. By comparing the relevant retention time and the MS/MS signals of the analytes in the matrix with those of standard solutions, the target analytes could be easily distinguished and no interference peaks were observed. The results showed that the retention time agreement of *trans*- and *cis*-resveratrol was within ±2.5%, and the relative abundance of the two selected ion transitions was within a margin of ±25%, which indicated a good selectivity of the analytical method.

The LOD and LOQ were considered as the analyte minimum concentrations that can be confidently identified and quantified by the method. The LOD was determined by analyzing blank sample at levels that provided signals at 3 times above the background noises. In a similar way, the LOQ was identified at signal-to-noise ratios equal to 10. **Table 2** summarizes LOD and LOQ values of individual compounds and clearly indicates a lower limit determinable analytical range.

Table 3. Intraday Recovery and Precision (RSD) Data from Spiked Red Wine

analyte	added amount (mg)	average recovery (%)	RSD (%)
trans-resveratrol	0.104 ± 0.003	76.9	12.77
	0.911 ± 0.003	95.0	5.48
	14.635 ± 0.471	102.7	3.41
cis-resveratrol	1.020 ± 0.008	108.3	6.67
	10.745 ± 0.157	104.2	4.09
	102.522 ± 1.030	99.1	1.56

Table 4. Intraday and Interday Precisions[a]

	precision			
analyte	intraday (ng mL^{-1})	RSD (%)	interday (ng mL^{-1})	RSD (%)
trans-resveratrol	0.061 ± 0.005	6.81	0.040 ± 0.003	7.48
	1.477 ± 0.031	2.12	1.319 ± 0.101	7.69
	15.550 ± 0.428	2.75	15.524 ± 0.696	4.48
	163.840 ± 7.816	4.77	161.135 ± 7.804	4.84
cis-resveratrol	0.440 ± 0.024	5.45	0.387 ± 0.026	6.80
	3.736 ± 0.102	2.73	3.681 ± 0.112	3.05
	37.397 ± 0.979	2.62	37.362 ± 1.659	4.44
	382.180 ± 17.478	4.57	375.901 ± 17.903	4.76

[a] The concentration data of analytes were shown as means ± SD (n = 6).

Recoveries were performed by employing the method of standard addition and obtained by subtracting the quantities of resveratrol in unspiked ones from spiked ones. At all fortification levels, recoveries were in the range of 95.0−108.3%, whereas relative standard deviation (RSD) values ranged from 1.56 to 6.67% except at low levels of TRA with a recovery 76.9% and an RSD 12.77%. The results obtained are shown in **Table 3**.

Precision was evaluated by calculating intra- and interday RSDs of three replicate runs of the procedure of online SPE-HPLC-MS/MS to ensure instrument and method performance. Three different fortified concentrations (low, medium, and high) were analyzed in 1 day for intraday precision and over 6 days for interday precision, respectively. The results obtained are shown in **Table 4**.

All of these data revealed that the described method had an accepted degree of precision.

Application of the Method. The proposed method was applied to analyzing eight kinds of red wines produced from seven regions of China. The chromatograms of the eight red wine samples are given in **Figure 4**. **Table 5** shows the ranges and the average concentrations of resveratrol found in the red wine samples. Reported RSDs were for six replicate samples from the same bottle of wine. The results demonstrated a successful application of this online SPE-HPLC-MS/MS assay for the quantification of resveratrol. Differences could be observed in the content of TRA and CRA. The concentration of TRA ranged from 41.85 to 1634.06 ng mL^{-1} and that of CRA from 167.15 to 1938.62 ng mL^{-1}. The total resveratrol content ranged from 290.33 to 2730.64 ng mL^{-1}. According to **Figure 5**, the highest average level of total resveratrol was found in wines from Gansu, whereas wines from Shanghai had the second highest level of total resveratrol. The lowest average level of total resveratrol was found in wines from Jilin province. This fact was under the influence of various elements such as climate, geographical area of cultivation, growing conditions, winemaking techniques, and storage conditions. Red wines made from the grapes that grow in northern China had comparatively lower resveratrol concentrations than the wines made from the grapes grown in more

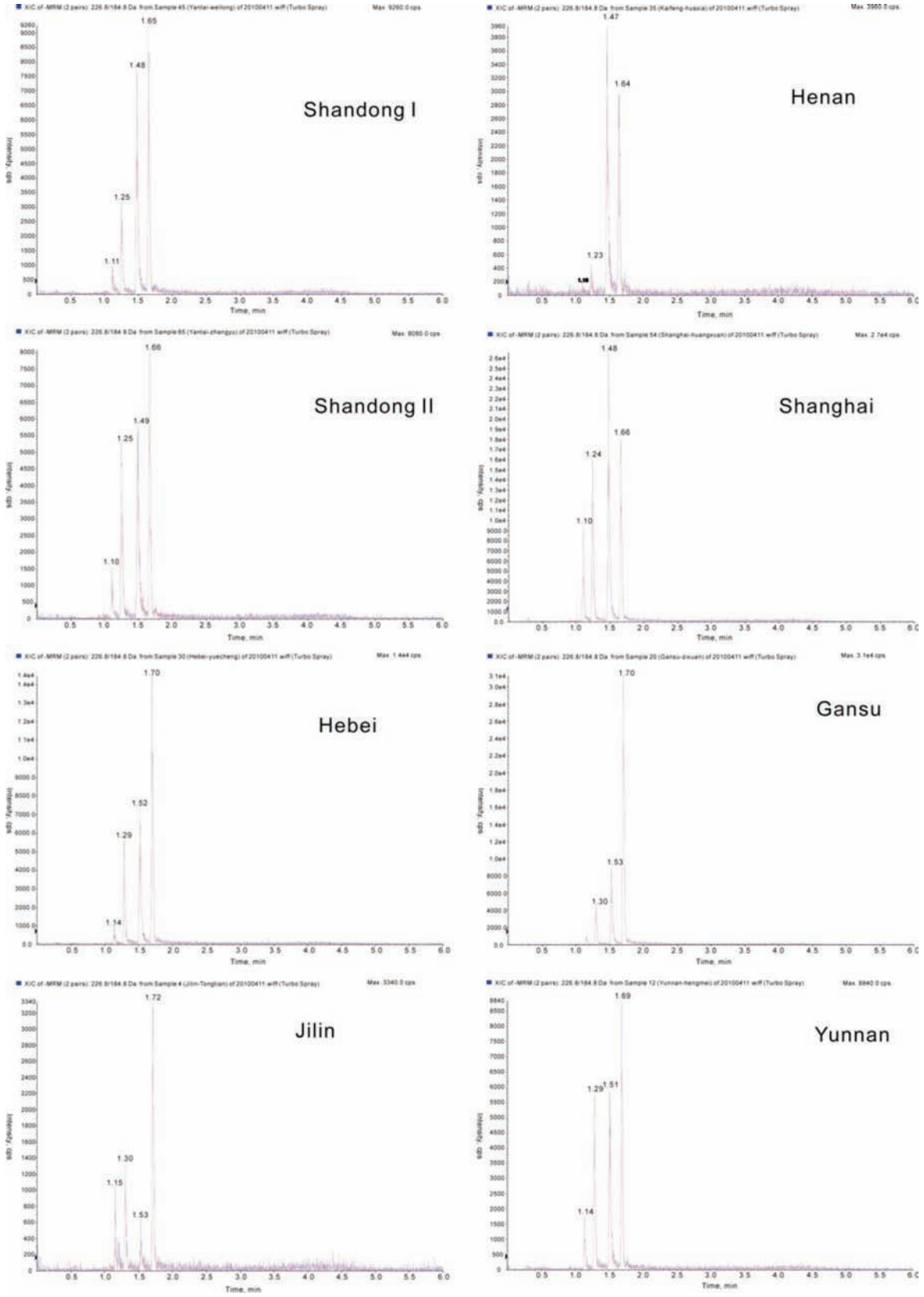

Figure 4. XIC chromatograms of the eight red wine samples.

hospitable climates of sunnier southern locations. The ratios of TRA to CRA from the selected red wines were also investigated in this study. According to the data, the *trans*-isomer content was greater than that of *cis*-isomer in the wines from Henan, Shanghai, and Shandong (I and II) province, for which the ratios of TRA/CRA were 1.76, 1.52, 1.02, and 1.12, respectively. The TRA content was equal to CRA in wines from Shandong and Yunnan. For the rest, CRA was obviously more than TRA, which may be due to the exposure of wine to light during the winemaking process or possibly from the light exposure of wine bottles during storage.

Table 5. Concentrations of *trans*- and *cis*-Resveratrol in Eight Red Wine Samples from Seven Regions of China

	analyte concentration[a] (ng mL^{-1})					
red wine region	*trans*-resveratrol	RSD (%)	*cis*-resveratrol	RSD (%)	total resveratrol	TRA/CRA ratio
Jilin (JL)	41.85 ± 2.68	6.40	248.48 ± 24.45	9.84	290.33	0.17
Gansu (GS)	792.02 ± 51.72	6.53	1938.62 ± 189.65	9.78	2730.64	0.41
Shanghai (SH)	1634.06 ± 225.52	13.80	1075.23 ± 93.79	8.72	2709.29	1.52
Yunnan (YN)	544.63 ± 43.35	7.96	579.79 ± 54.50	9.4	1124.42	0.94
Hebei (HB)	658.10 ± 76.99	11.70	1191.85 ± 142.79	11.98	1849.95	0.55
Shandong I (SD I)	636.54 ± 56.65	8.90	621.35 ± 75.98	12.23	1257.89	1.02
Shandong II (SD II)	580.20 ± 26.64	4.59	516.78 ± 53.61	10.37	1096.98	1.12
Henan (HN)	293.61 ± 23.89	8.14	167.15 ± 6.12	3.66	460.75	1.76

[a] The concentration data of analytes are shown as content = means ± SD (n = 6).

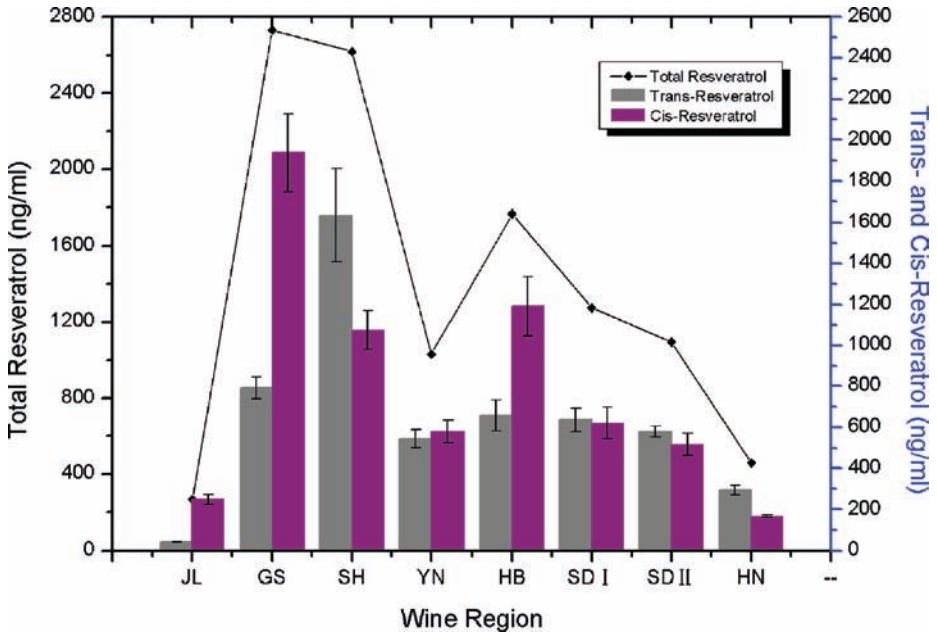

Figure 5. Resveratrol concentrations of eight red wine samples from seven regions of China. (The former two peaks in each chromatogram might be the glycosides of resveratrol.)

In summary, a fully automated methodology, based on online SPE-HPLC-MS/MS analysis, has been developed for rapid determination of TRA and CRA in red wines. A multiwalled carbon nanotube-packed trapping column was used for extraction and cleanup of the sample, and a fused-core C18 column was used for target analyte separation. Compared to the existing methods, the proposed approach affords high-throughput (6 min per sample), improved accuracy (because aqueous calibration standards are processed in the same way as samples), high sensitivity (LOQ values usually < 0.06 ng L^{-1}), and high selectivity. Moreover, no laborious and time-consuming sample pretreatment steps were needed in this assay. The risk of analyte loss was decreased as well, because the analysis and sample extraction took place in a closed, automated system. Additionally, red wines from seven regions of China were analyzed. The results showed the concentration of resveratrol appeared to be mostly influenced by the place of production. Wines from Gansu presented the highest total resveratrol concentration. The TRA/CRA ratios were < 1.0 except for red wines from Henan, Shanghai, and Shandong (I and II) province, which could be due to the different winemaking processes that contribute to *cis*-isomer formation. These facts demonstrate that the whole system was of great potential to be applied for the analysis of TRA and CRA in red wines. This proposed method could also be utilized for resveratrol analyses in grape juice, jams, and jellies and other related products.

ABBREVIATIONS USED

LC-MS/MS, liquid chromatography−tandem mass spectrometry; TRA, *trans*-resveratrol; CRA, *cis*-resveratrol; MRM, multiple-reaction monitoring; ESI, electrospray ionization.

LITERATURE CITED

(1) Daniel, O.; Meier, M. S.; Schlatter, J.; Frischknecht, P. Selected phenolic compounds in cultivated plants: ecologic functions, health implications, and modulation by pesticides. *Environ. Health Perspect.* **1999**, *107*, 109−114.

(2) Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P.; Trollat, P. Resveratrol content of wines of different ages: relationship with fungal disease pressure in the vineyard. *Am. J. Enol. Vitic.* **1995**, *46*, 1−4.

(3) Romero-Pérez, A. I.; Lamuela-Raventós, R. M.; Waterhouse, A. L.; de la Torre-Boronat, M. C. Levels of *cis*- and *trans*- resveratrol and their glucosides in white and rose *Vitis vinifera* wines from Spain. *J. Agric. Food Chem.* **1996**, *44*, 2124−2128.

(4) Kolouchová-Hanzlíková, I.; Melzoch, K.; Filip, V.; Smidrkal, J. Rapid method for resveratrol determination by HPLC with electrochemical and UV detections in wines. *Food Chem.* **2004**, *87*, 151−158.

(5) Sánchez, J. B. J.; Corral, E. C.; Delgado, M. J. S.; Orea, J. M.; Ureña, A. G. Analysis of *trans*- resveratrol by laser ionization mass spectrometry and HPLC with fluorescence detection: comparison between both techniques. *J. Chromatogr., A* **2005**, *1074*, 133−138.
(6) Moreno-Labanda, J. F.; Mallavia, R.; Perez-Fons, L.; Lizama, V.; Saura, D.; Micol, V. Determination of piceid and resveratrol in Spanish wines deriving from Monastrell (*Vitis vinifera* L.) grape variety. *J. Agric. Food Chem.* **2004**, *52*, 5396−5403.
(7) Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W. W.; Fong, H. H. S.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **1997**, *275*, 218−220.
(8) Levenson, A. S.; Gehm, B. D.; Pearce, S. T.; Horiguchi, J.; Simons, L. A.; Ward, J. E., III; Jameson, J. L.; Jordan, V. C. Resveratrol acts as an estrogen receptor (ER) agonist in breast cancer cells stably transfected with ER α. *Int. J. Cancer* **2003**, *104*, 587−596.
(9) Goldberg, D. M.; Yan, J.; Ng, E.; Diamandis, E. P.; Karumanchiri, A.; Soleas, G. J.; Waterhouse, A. L. A global survey of *trans*-resveratrol concentrations in commercial wines. *Am. J. Enol. Vitic.* **1995**, *46*, 159−165.
(10) Mattivi, F. Solid phase extraction of *trans*-resveratrol from wines for HPLC analysis. *Z. Lebensm. Unters. Forsch* **1993**, *196*, 522−525.
(11) Ali, A.; Strommer, J. A simple extraction and chromatographic system for the simultaneous analysis of anthocyanins and stilbenes of *Vitis* species. *J. Agric. Food Chem.* **2003**, *51*, 7246−7251.
(12) McMurtrey, K. D.; Mynn, J.; Pobanz, K.; Schultz, T. P. Analysis of wines for resveratrol using direct injection high-pressure LC with electrochemical detection. *J. Agric. Food Chem.* **1994**, *42*, 2077−2080.
(13) Pezet, R.; Pont, V.; Cuenat, P. Method to determine resveratrol and pterostilbene in grape berries and wines using HPLC and highly sensitive fluorimetric detection. *J. Chromatogr., A* **1994**, *663*, 191−197.
(14) Domínguez, C.; Guillén, D. A.; Barroso, C. G. Automated solid-phase extraction for sample preparation followed by HPLC with DAD and MS for the analysis of resveratrol derivatives in wine. *J. Chromatogr., A* **2001**, *918*, 303−331.
(15) Careri, M.; Carradini, C.; Elviri, L.; Nicoletti, I.; Zagnoni, I. Direct HPLC analysis of quercetin and trans-resveratrol in red wine, grape, and winemaking byproducts. *J. Agric. Food Chem.* **2003**, *51*, 5226−5231.
(16) Bekyarova, E.; Murata, K.; Yudasaka, M.; Kasuya, D.; Iijima, S.; Tanaka, H.; Kahoh, H.; Kaneko, K. Single-wall nanostructured carbon for methane storage. *J. Phys. Chem. B* **2003**, *107*, 4681−4684.
(17) Banks, C. E.; Crossley, A.; Salter, C.; Wilkins, S. J.; Compton, R. G. Carbon nanotubes contain metal impurities which are responsible for the "electrocatalysis" seen at some nanotube-modified electrodes. *Angew. Chem., Int. Ed.* **2006**, *45*, 2533−2537.
(18) Trojanowicz, M. Analytical applications of carbon nanotubes: a review. *Trends Anal. Chem.* **2006**, *25*, 480−489.
(19) Wang, S.; Zhao, P.; Min, G.; Fang, G. Z. Multi-residue determination of pesticides in water using multi-walled carbon nanotubes solid-phase extraction and GC-MS. *J. Chromatogr., A* **2007**, *1165*, 166−171.
(20) Cai, Y.; Jiang, G.; Liu, J.; Zhou, Q. Multi-walled carbon nanotubes as a solid-phase extraction adsorbent for the determination of bisphenol A, 4-*n*-nonylphenol, and 4-*tert*-octylphenol. *Anal. Chem.* **2003**, *75*, 2517−2521.
(21) Valcárcel, M.; Cárdenas, S.; Simonet, B. M.; Moliner-Martinez, Y.; Lucena, R. Carbon nanostructures as sorbent materials in analytical processes. *Trends Anal. Chem.* **2008**, *27*, 34−43.
(22) Márquez-Sillero, I.; Aguilera-Herrador, E.; Cárdenas, S.; Valcárcel, M. Determination of parabens in cosmetic products using multi-walled carbon nanotubes as solid phase extraction sorbent and corona-charged aerosol detection system. *J. Chromatogr., A* **2010**, *1217*, 1−6.
(23) Xiao, S. F.; Wang, Z. H.; Luo, G. A. The progress in functionalization of carbon nanotube. *Chin. J. Anal. Chem.* **2005**, *2*, 261−266.
(24) Unger, K. K.; Skudas, R.; Schulte, M. M. Particle packed columns and monolithic columns in HPLC-comparison and critical appraisal. *J. Chromatogr., A* **2008**, *1184*, 393−415.
(25) Kirkland, J. J.; Langlois, T. J.; DeStefano, J. J. Fused core particles for HPLC columns. *Am. Lab.* **2007**, *39*, 18−21.
(26) Abrahim, A.; Al-Sayah, M.; Skrdla, P.; Bereznitski, Y.; Chen, Y.; Wu, N. Practical comparison of 2.7 μm fused-core silica particles and porous sub-2 μm particles for fast separations in pharmaceutical process development. *J. Pharm. Biomed. Anal.* **2010**, *51*, 131−137.
(27) Hsieh, Y.; Duncan, C. J. G.; Brisson, J. M. Fused-core silica column HPLC/MS determination of rimonabant in mouse plasma. *Anal. Chem.* **2007**, *79*, 5668−5673.
(28) Arce, L.; Tena, M. T.; Rios, A.; Valcárcel, M. Determination of trans-resveratrol and other polyphenols in wines by a continuous flow sample clean-up system followed by capillary electrophoresis separation. *Anal. Chim. Acta* **1998**, *359*, 27−38.
(29) International Conference on Harmonization (ICH), Q2 (R1). Validation of analytical rocedures: text and methodology, 2005.

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