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Rapid Determination of Patulin in Apple Juices by a Graphitized Carbon Black-based SPE Cartridge Coupled with HPLC – PDA and LC – MS/MS

ZHANG Qiao-yan[1*]，ZHANG Xiao-feng[2]，LI Ke[2]，WANG Xia-jun[1]，SHUAI Da-yao[3]，GU Yu-ting[3]

（1. Institute of Agro-product Safety and Nutrition，Zhejiang Academy of Agricultural Sciences，Hangzhou 310021，China；2. Zhejiang Academy of Science and Technology for Inspection and Quarantine，Hangzhou 310016，China；3. Zhejiang Rid Anzheng Testing and Certification Technology Co.，Ltd.，Hangzhou 310023，China）

Abstract：By virtue of the strong adsorption on patulin，a graphitized carbon black-based solid phase extraction（SPE）cartridge（named as GCB cartridge）was fabricated in this paper. Meanwhile，the GCB cartridge was combined with high performance liquid chromatography – photodiode array detector（HPLC – PDA）and liquid chromatography – tandem mass spectrometry（LC – MS/MS）in turn to evaluate the cleanup effects of the home-made GCB cartridge and four commercial SPE cartridges（Retain AX，PAX，MAX and HLB），which were further applied to analysis on the various impurities in the clear apple juice that could affect the accurate quantification of patulin. The significantly improved chromatograms obtained，signal-to-noise ratios（*S/N*s）and matrix effects displayed their capabilities in the decrease of impurity interference. Among them，the GCB cleanup provided an ideal chromatogram in HPLC – PDA analyses，as well as an acceptable matrix effect of −14%，a chromatogram superior to the HLB cleanup and a comparable *S/N* to the Retain AX cleanup in LC – MS/MS analyses. Consequently，a LC – MS/MS method with the stable isotope dilution assay（SIDA – LC – MS/MS）was developed to overcome the impacts of matrix effect and cleanup loss on the accuracy of test results. The performance characteristics of methods based on different SPE cleanups with HPLC – PDA and SIDA – LC – MS/MS，respectively，were compared. The results showed that the GCB cleanup provided a higher sensitivity than the HLB cleanup due to its lower limits of detection and quantitation，while the methods with MAX，HLB and GCB cleanup all showed better recoveries（82% – 102%）and smaller relative standard deviations（≤ 9%）. The developed GCB cleanup only needed two steps（i. e. loading and elution）to carry out by gravity on a SPE manifold within 10 min，which is simpler and faster than other SPE cleanups. The powerful cleanup effects were also reflected in the pigment removal of eight familiar apple juices. Furthermore，some locally moldy apples were processed into the apple juice which was verified to be a positive sample of 34 $\mu g \cdot kg^{-1}$ patulin by the GCB cleanup and the analyses by HPLC – PDA and SIDA – LC – MS/MS，respectively. The better cleanup effects and lower cost of GCB cartridge would be helpful to its wide application in the routine detection of patulin.

Key words：graphitized carbon black；patulin；apple juice；cleanup；HPLC – PDA；LC – MS/MS

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石墨化碳黑净化柱结合HPLC – PDA和LC – MS/MS快速检测苹果汁中展青霉素

张巧艳[1*]，张晓峰[2]，李 可[2]，王夏君[1]，帅大尧[3]，顾宇婷[3]

（1. 浙江省农业科学院 农产品质量安全与营养研究所，浙江 杭州 310021；2. 浙江省检验检疫科学技术研究院，浙江 杭州 310016；3. 浙江锐德安正检测认证技术有限公司，浙江 杭州 310023）

摘 要：基于石墨化碳黑材料制备了一种固相萃取柱（GCB柱），结合高效液相色谱 – 二极管阵列检测法（HPLC – PDA）和液相色谱 – 串联质谱法（LC – MS/MS）评估了GCB柱和4种商业化固相萃取柱（Retain AX、

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*Corresponding author：ZHANG Qiao-yan，associated professor，Research interest：detection technologies and quality control for the nutrition and safety of agricultural products，E-mail：yanyan0014@163.com

PAX、MAX和HLB)的净化效果，并对澄清型苹果汁中影响展青霉素准确定量的杂质进行了分析。结果发现，苹果汁经上述5种净化柱净化后，大大降低了杂质干扰，色谱图、信噪比和基质效应显著改善。其中，GCB净化为HPLC－PDA分析提供了理想的色谱图，为LC－MS/MS分析提供了可接受的基质效应(－14%)、优于HLB净化的色谱图以及与Retain AX净化相当的信噪比。建立的稳定同位素稀释－液相色谱－串联质谱法(SIDA－LC－MS/MS)可以克服基质效应和净化损失对检测结果准确性的影响。采用HPLC－PDA和SIDA－LC－MS/MS分析时，GCB净化比HLB净化的方法更灵敏；MAX、HLB和GCB净化均能得到较好的回收率(82%～102%)和较小的相对标准偏差(≤9%)。所开发的GCB净化方法仅需上样和洗脱2步，且在重力作用下过柱仅需10 min，比其他固相萃取净化更简单快捷，净化能力良好。实际样品经GCB柱净化和HPLC－PDA、SIDA－LC－MS/MS分别分析，展青霉素的平均含量为34 μg·kg^{-1}。GCB柱较好的净化效果和较低的使用成本将有助于其在展青霉素常规检测中的广泛应用。

关键词：石墨化碳黑；展青霉素；苹果汁；净化；高效液相色谱－二极管阵列检测法；液相色谱－串联质谱法

Patulin(4-Hydroxy-4H-furo[3,2-*c*]pyran-2(6H)-one) is a hydrophilic and toxic fungal secondary metabolite which had been classified as a group 3 carcinogen by the International Agency for Research on Cancer due to its impact with hepatotoxicity, nephrotoxicity, genotoxicity, immunotoxicity and neurotoxicity on the mammalian system[1]. Patulin has been found in fruit and vegetable based products, mainly in apples and apple products. In a study, a total of 15 apple juices marketed in Northeast China had been analyzed for patulin content, and the obtained average and maximum contents were 22.8 μg·kg^{-1} and 90.3 μg·kg^{-1}, respectively[2]. In Spain, a previous survey of 100 apple juices from distinct supermarkets had showed that 66% of the samples contained patulin, and 11% exceeded the maximum level of 50 μg·kg^{-1} set by the EU regulation[3]. As a popular and nutrient-rich fruit beverage, the apple juices are needed to have the routine safety inspection on patulin in order to minimize its exposure risk on humans, especially children below the age of 12[4].

The composition of apple juice is complex, including sugar, sorbitol, organic acid, amino acid, phenolic compounds, mineral elements and so on[5]. The cleanup procedures prior to the analyses by high performance liquid chromatography coupled with ultraviolet detector or photodiode array detector (HPLC－UV or HPLC－PDA) and liquid chromatography coupled to tandem mass spectrometry (LC－MS/MS), respectively, are generally essential to reduce the matrix interference and contamination on instrument[6]. Several commercial and lower-cost solid-phase extraction (SPE) cartridges, such as hydrophilic－lipophilic balanced (HLB) cartridge and amine-functionalized anion-exchange cartridges(i.e. MAX, PAX and Retain AX), had been applied in the cleanup of apple juice, and their cleanup effects had been revealed partly by HPLC or LC－MS/MS alone[7-9]. However, those obtained purified liquids still contained certain impurities to cause matrix effects in LC－MS/MS analyses while they might have not achieved perfect baseline separation of patulin and impurities in HPLC chromatograms for the accurate quantitation of patulin.

Due to the remarkable similarity between the structure of some analytes and the surface of graphitized carbon black(GCB), this sorbent had been applied in the determination of coumafuryl, aflatoxin M1 and so on[10]. In our previous work, some common sorbents had been compared and a SPE cartridge based on GCB(named as GCB cartridge) had been designed by virtue of its superior adsorption capability on patulin. In this work, HPLC－PDA analyses were combined with LC－MS/MS analyses to systemically evaluate the cleanup effects of the home-made GCB cartridge and four familiar SPE cartridges(Retain AX, PAX, MAX and HLB) on the clear apple juice in the patulin detection, especially to display the characteristics and alternative of GCB cartridge.

1 Experimental

1.1 Chemicals and materials

Acetonitrile(HPLC grade) was from Merck KGaA(Darmstadt, Germany). Ultrapure water was obtained from a Milli－Q ultrapure water system(Millipore, Beaford, USA). Methanol, acetic acid, ammonium acetate, ethyl acetate and dichloromethane were analytical reagent grade(AR grade), and were supplied by Sinopharm Chemical Reagent(Shanghai, China).

The reference standard of patulin(100 μg·mL^{-1} in acetonitrile) was a national certified reference material

(GBW (E) 100470) from Zhejiang Academy of Agricultural Sciences (Hangzhou, China). The isotope-labeled internal standard of patulin ($^{13}C_7$-patulin, 25 μg·mL^{-1} in acetonitrile) was obtained from Qingdao IniKem BioPharma Tech Co., Ltd (Qingdao, China). Before using, the above stocked standards were respectively diluted into the work standards of proper concentrations by 1% (by volume) acetic acid solution.

The tested SPE cartridges were Oasis HLB (60 mg/3 mL) and MAX (60 mg/3 mL) from Waters (Shanghai, China), Cleanert PAX (150 mg/6 mL) from Agela Technologies (Tianjin, China), HyperSep Retain AX (150 mg/6 mL) from Thermal – Fisher Scientific (Shanghai, China), and the home-made GCB cartridge which was prepared manually by loading 50 mg GCB into an empty SPE cartridge (3 mL) using a lower and an upper polyethylene frits with 10 μm porosity. The sorbent GCB was Cleanert PestiCarb (120 – 400 mesh) from Agela Technologies (Tianjin, China).

1.2 Sample pretreatment

A commercial clear apple juice from a local supermarket (Hangzhou, China) was free of patulin which was used to evaluate the cleanup effects of five cartridges. The related cleanup procedures were derived from the previous reports[9,11] and our optimized modifications. All cleanup processes were carried out by gravity on a SPE manifold without controlling the flow rate.

In the HLB, MAX, PAX and Retain AX cleanup, respectively, the SPE cartridge was firstly preconditioned with 3 mL methanol and 3 mL water. Then, 2 mL of the clear apple juice was loaded into the preconditioned cartridge. The analyte-containing HLB cartridge was washed with 3 mL water and the analyte was eluted with 4 mL ethyl acetate[11], while the Retain AX cartridge was washed with 0.1% acetic acid solution and water in sequence (3 mL), and eluted with 4 mL methanol[9]. Both MAX and PAX are the mixed-mode anion-exchange cartridges. According to a Chinese national standard (GB 5009.185 – 2016), the analyte-containing MAX or PAX cartridge was washed with 5 mmol·L^{-1} ammonium acetate solution and water in sequence (3 mL), and the analyte was also eluted with 4 mL methanol.

The cleanup by GCB cartridge was simpler than others by virtue of abandoning the precondition and washing steps. Firstly, 2 mL of the apple juice was directly loaded into the GCB cartridge and the analyte was absorbed by GCB. After the residual liquid in the cartridge was discarded by pressurized air, 4 mL dichloromethane was thoroughly passed through the cartridge to obtain the eluent.

All purified samples were further processed as follows: 20 μL acetic acid was added into the collected eluent in order to inhibit the degradation of patulin. Then the sample was evaporated to near dryness under a gentle stream of nitrogen at 40 ℃. The residue was redissolved in 1.0 mL of 1% acetic acid solution and mixed with a vortex mixer for 30 s. The concentrated purified liquid was filtered into a glass injection vial for the HPLC – PDA and LC – MS/MS analyses.

1.3 HPLC – PDA analysis

A Waters 2695 high performance liquid chromatograph (Waters Corporation, Milford, USA) equipped with autosampler, quaternary pump with vacuum degasser and photodiode array detector (Waters 996) was used in this study. Instrument control and data analysis were carried out using the Waters Empower 3 software (Version 7.20.00.00).

The chromatographic separation was performed on a Waters XSelect HSS T3 column (250 mm × 4.6 mm, 5 μm) (Waters Corporation, Milford, USA). The flow rate was maintained at 1.0 mL·min^{-1} with the mobile phase consisted of water (A) and acetonitrile (B). The gradient elution program was as follows: 0 – 11.0 min, 5% B; 11.0 – 11.2 min, 5% B to 50% B; 11.2 – 12.0 min, 50% B; 12.0 – 12.2 min, 50% B to 5% B; 12.2 – 17.0 min, 5% B. The column temperature was set at 40 ℃, and the injection volume was 100 μL. Chromatograms were recorded at 276 nm with spectra (190 – 400 nm) taken continuously throughout the elution for confirmation.

1.4 LC – MS/MS analysis

The analysis was performed on a Shimadzu LCMS – 8045 triple quadrupole mass spectrometer with LC – 30AD liquid chromatography system (Shimadzu Corporation, Kyoto, Japan). The data were acquired and

processed by the Shimadzu LabSolutions software(Version 5. 109).

The chromatographic separation was carried out on a Shim-pack GIS C_{18} column (75 mm × 2. 1 mm, 2 μm) (Shimadzu Corporation, Kyoto, Japan). The flow rate was maintained at 0. 3 mL·min^{-1} with the mobile phase consisted of water (A) and acetonitrile(B). The gradient elution program was as follows: 0 – 5. 0 min, 5% B; 5. 0 – 5. 2 min, 5% B to 100% B; 5. 2 – 6. 0 min, 100% B; 6. 0 – 6. 2 min, 100% B to 5% B; 6. 2 – 8. 0 min, 5% B. The column temperature was set at 40 ℃, and the injection volume was 5 μL.

The mass spectrometry system utilized an electrospray ion(ESI) source operated in the negative ion mode. The optimal ESI source parameters were as follows: interface temperature, 300 ℃; interface voltage, −3 kV; nebulizing gas flow, 3 L·min^{-1}; heating gas flow, 10 L·min^{-1}; drying gas flow, 10 L·min^{-1}; desolvation line (DL) temperature and heat block temperature were maintained 250 ℃ and 400 ℃, respectively. High purity nitrogen was employed as the nebulizing and drying gas, and argon(99. 998% purity) as the collision gas. The multiple reaction monitoring(MRM) mode was used for quantitation. For patulin, a channel at *m/z* 153 > *m/z* 109 (CE: 11 eV) was selected for quantitation and a channel at *m/z* 153 > *m/z* 81 (CE: 13 eV) for qualification, while for $^{13}C_7$-patulin, a channel at *m/z* 160 > *m/z* 115(CE: 11 eV) was chosen for quantitation and a channel at *m/z* 160 > *m/z* 86(CE: 12 eV) for qualification.

1. 5 Data manipulation

The blank apple juice was spiked with patulin at 50 μg·L^{-1} prior to cleanup. The work standard of patulin (100 μg·L^{-1}) was prepared daily as the calibration solution in order to obtain the measured concentrations of the spiked apple juice after different cleanups. Recovery was expressed in terms of the percentage of measured concentration to fortified concentration.

After the purified liquid from the blank apple juice was evaporated to near dryness, the residue of each cleanup was redissolved in 1. 0 mL of the work standard of patulin (100 μg·L^{-1}) to prepare the corresponding matrix-matched work standard for LC – MS/MS analyses. In order to evaluate the matrix effect on analyte ionization, the peak area of patulin of the matrix-matched work standard(a) was compared with that of the work standard(b) at 100 μg·L^{-1}. The percentage, $(a-b)/b \times 100\%$, was used to describe the matrix effect, where the positive and negative values reflected the matrix-induced enhancement and suppression effect, respectively. The matrix effect with the value ranging between −20% and 20% was generally considered insignificant[12].

All the experiments were performed with six replicates, and the results were expressed as mean value.

2 Results and discussion

2. 1 Online fingerprinting of HPLC – PDA

Of particular importance in the HPLC analyses of processed apple juices is the separation of patulin from intrinsic phenolic compounds in general and 5-hydroxymethylfurfural (HMF) in particular[13]. HMF is an intermediate product of the well-known Maillard reaction, an aldehyde that is formed as a result of dehydration of ketopentoses. Most processed apple juices had been exposed to high temperatures for enzyme denaturalization and pasteurization which made some intrinsic sugars into HMF[14]. HMF had exhibited the similar chromatographic retention behavior with patulin[15]. Here, using our optimized instrument conditions, the patulin peak (11. 6 min) could be well separated from the impurity I peak (9. 5 min) (resolution > 2) (Fig. 1B – Fig. 1H). The impurity I had been identified as HMF based on its characteristic PDA spectra and retention time in line with that of the reference standard of HMF(GBW(E) 100251). Nevertheless, a mass of impurities in the unpurified clear apple juices brought about severe baseline drift of chromatographic signals and interference to target peak (Fig. 1G and Fig. 1H), which affected the accuracy and sensitivity of patulin detection. Those impurities among and beside the patulin peak may be phenolic compounds and should be removed by SPE cleanup prior to HPLC analyses.

Four available cartridges (Retain AX, PAX, MAX and HLB) and our GCB cartridge were used and their cleanup effects on the spiked apple juices at 50 μg·L^{-1} of patulin were reflected in the HPLC chromatograms. The results showed that all of the tested SPE cartridges had certain cleanup capability on the test apple juice because they provided a flatter baseline around the patulin peak (Fig. 1B – Fig. 1F), compared with that of the

unpurified spiked apple juices (Fig. 1H). In a previous report, Retain AX cartridge had exhibited the best cleanup performance for the patulin detection by LC – MS/MS[9]. In our HPLC analyses, it was found that there was still a small interference peak (impurity II at 11.9 min around) appearing in the patulin peak after the Retain AX cleanup (Fig. 1B), similar to the cleanup result of PAX cartridge (Fig. 1C). What's more, after cleanup by MAX, HLB and GCB cartridge, respectively, the ideal chromatographic peak with symmertrical profile was obtained for the patulin detection (Fig. 1D – Fig. 1F), while none of peaks appeared at the target retention time (about 11.6 min) in their purified blank apple juices (data not shown). In particular, the home-made GCB cartridge was revealed its better cleanup capability on the test apple juice for the accurate quantification of patulin by HPLC, since there was no impurity peak at all beside and among the target peak (between 10 min and 14 min) in its chromatogram (Fig. 1F).

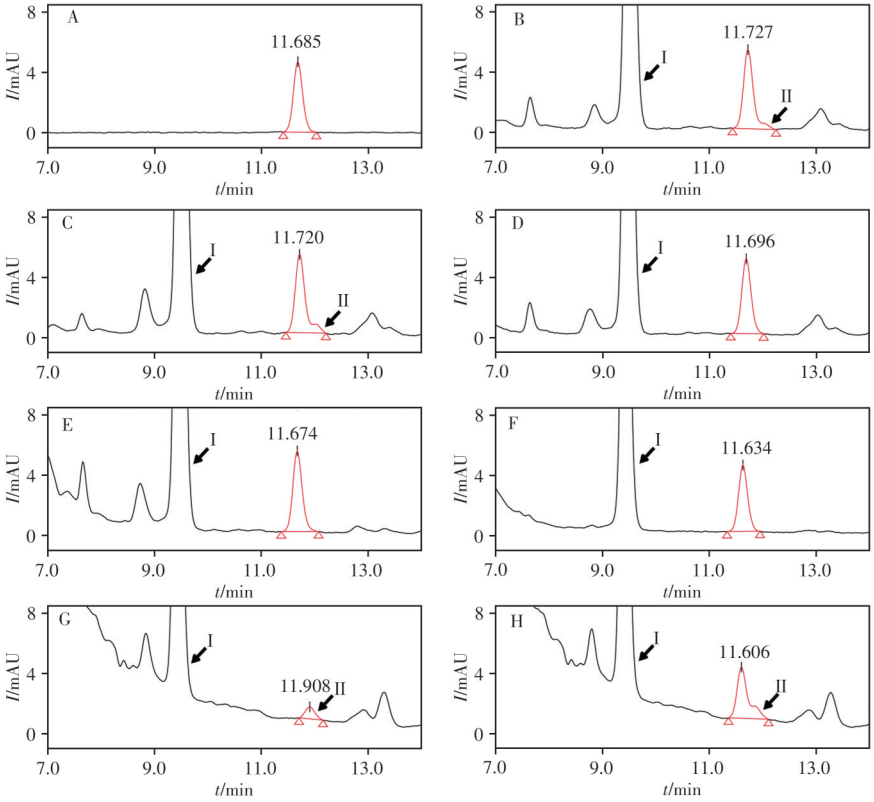

Fig. 1 HPLC – PDA chromatograms of the tested apple juices before and after cleanups

A: the work standard of patulin at 100 μg·L^{-1}; B – F: the purified samples of the spiked apple juice at 50 μg·L^{-1} of patulin by Retain AX(B), PAX(C), MAX(D), HLB(E) and GCB(F) cartridges, respectively; G: the unpurified blank apple juice; H: the unpurified spiked apple juice at 50 μg·L^{-1} of patulin. I and II were different impurities of concern

2.2 Online fingerprinting of LC – MS/MS

Patulin is an electrophilic, low-molecular-weight α, β-unsaturated γ-lactone (M_W: 154)[16]. One of the most important aspects when analyzing low-molecular-weight compounds using LC – MS/MS, is minimizing the interference ions that increase the background noise from the tested samples[17]. In our LC – MS/MS analyses, the unpurified clear apple juice spiked with patulin at 100 μg·L^{-1} strived to provide a chromatographic peak of the extracted ion (*m/z* 153 > *m/z* 109) at 3.3 min by the optimized instrument conditions, but it could be considered undetectable because of its signal-to-noise ratio (*S/N*) less than 3 (Fig. 2H). The larger response difference of target peaks in Fig. 2A and Fig. 2H showed that the sensitivity of patulin detection was heavily influenced by the intrinsic matrixes of apple juice. In a previous report on the unpurified apple juice by LC – MS/MS, no signal response had been obtained for patulin, but the almost normal responses were observed for bisphenol A, aflatoxin B1 and ochratoxin A at 30 μg·L^{-1}[8]. As we know, fructose, sucrose and glucose account for nearly 85% of the soluble solid in apple juice[5]. The background noise derived from these polar low-molecular-weight sugars could produce the selective and forceful sign suppression effect on patulin. In addition, followed by the

unpurified samples, the signal response of patulin work standard was found to be decreased greatly, which could be returned to normal only after repeated flushing with the mobile phase for at least two hours. It was speculated that the saccharides in apple juice might adhere to the orifice of mass spectrometer to prevent the following patulin molecular from being ionized in ESI source. Thus, it was indispensable to reduce those soluble impurities like sugars for the effective determination of patulin in clear apple juice by LC – MS/MS.

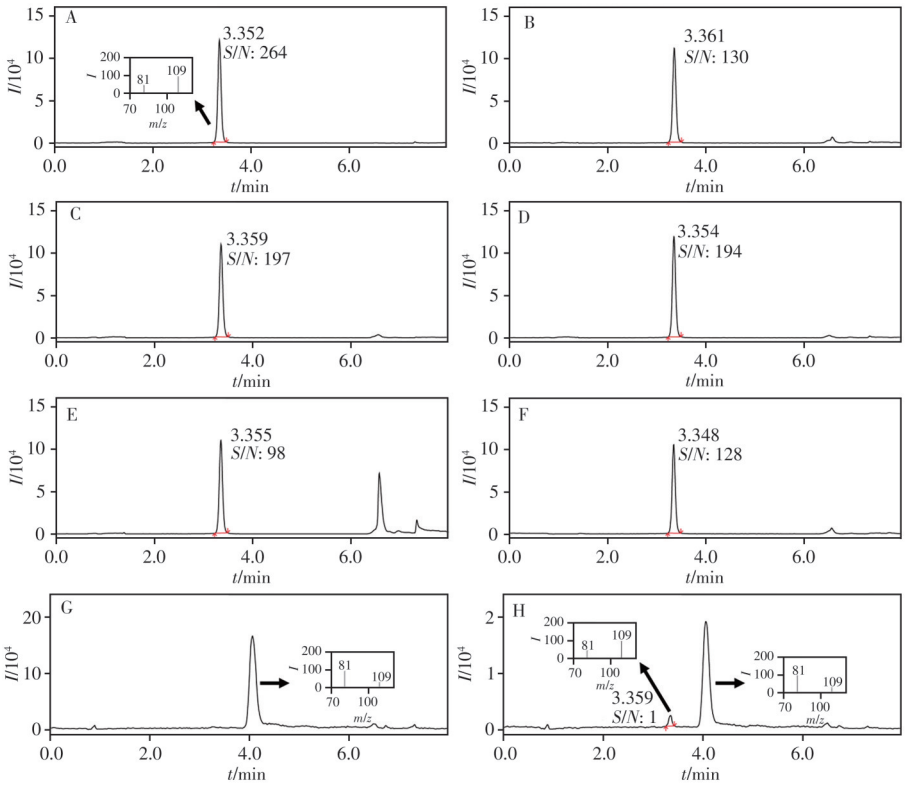

Fig. 2 LC – MS/MS chromatograms of the quantitative ion of patulin extracted from various work standards

A: the work standard of patulin at 100 μg·L^{-1}; B – F: the matrix-matched work standards of patulin at 100 μg·L^{-1} related with the Retain AX (B), PAX(C), MAX(D), HLB(E) and GCB(F) cleanup, respectively; G: the unpurified blank apple juice; H: the unpurified spiked apple juice at 100 μg·L^{-1} of patulin. The distinguishable centroids from the target analyte and the impurity were inserted into A, G and H

In this work, the blank apple juice was purified by Retain AX, PAX, MAX, HLB and GCB cartridges, respectively, and finally made into their related matrix-matched work standards at 100 μg/L of patulin to reflect the cleanup effects by LC – MS/MS analyses. The results in Fig. 2 showed that the above five SPE cleanups had better performances in the removal of interfering substances from the apple juice, depending on the significantly higher *S/N*s of their target peaks (Fig. 2B – Fig. 2F) than that of the unpurified apple juice containing patulin (Fig. 2H), with the high-to-low order as follows: PAX > MAX > Retain AX> GCB > HLB. These cleanups could not only decrease the background noise to make the target responses increased greatly, but also eliminate the larger impurity at 4. 1 min as shown in Fig. 2G and Fig. 2H. Although the matrix-induced suppression effects were still found in the MRM mode after cleanups, they had been remarkably weakened into the acceptable level (−20% – 20%), with the weak-to-strong order as follows: MAX < Retain AX < PAX < HLB < GCB. The enhancement of suppression effects was not exactly proportional to the lowering of *S/N*s, probably due to the complex interactions among the target analyte, the residual polar low-molecular-weight sugars and other impurities. Here, the GCB cleanup (Fig. 2F) provided the insignificant matrix effect of −14% as well as the superior chromatogram to the HLB cleanup (Fig. 2E) and the comparable *S/N* to the Retain AX cleanup (Fig. 2B), which indicated that our home-made GCB cartridge was potential for the cleanup of apple juice in the patulin detection by LC – MS/MS.

Further, the impacts of matrix effect and cleanup loss on the accuracy of test results can be compensated by using the stable isotope dilution assay (SIDA) method[18]. The selected isotope-labeled internal standard ($^{13}C_7$-patulin) had been verified its conformity to the target analyte (patulin) in the recovery test and the matrix

effect. The above SPE cleanups coupled with SIDA – LC – MS/MS methods had been respectively established to allow omission of matrix-matched calibration.

2.3 Method performance characteristics

The methods based on different SPE cleanups with HPLC – PDA and SIDA – LC – MS/MS, respectively, were compared their performance characteristics, except for those by the Retain AX and PAX cartridge due to the interference of impurity Ⅱ to patulin in their HPLC chromatograms (Fig. 1B and Fig. 1C). The spiked level of the analyte giving the *S/N* of 3 times is generally regarded as the limit of detection (LOD) of the method as well as that of the *S/N* of 10 times as the limit of quantitation (LOQ)[19]. The results in Table 1 showed that the GCB cleanup could provide the higher sensitivity for confirmation and quantitation of patulin in apple juice since it owned lower LOD and LOQ than that of the HLB cleanup in both HPLC – PDA and SIDA – LC – MS/MS analyses. Various levels of the spiked apple juices were used for the recovery tests with the relative standard deviations (RSDs) for the measured concentrations (n = 6). The methods by the MAX, HLB and GCB cleanup all showed better recoveries (82% – 102%) and smaller RSDs (≤ 9%) (Table 1), which ensured their accuracy and precision meeting the method performance acceptability criteria (recovery: 70% – 120%, RSD: ≤ 15%)[19]. In addition, the GCB cleanup could provide the linear calibration curves in the range of 0.01 – 10 mg·kg^{-1} for HPLC – PDA method and 0.004 – 10 mg·kg^{-1} for SIDA – LC – MS/MS method, and their correlation coefficients of linearity were above 0.999. In our previous tests, the frequently-used organic solvents (i.e. methanol, acetonitrile, ethyl acetate and dichloromethane) had been tried to elute the patulin molecules adsorbed in the GCB cartridge, and the baseline separation between patulin and impurities could been achieved in the HPLC chromatogram only when using dichloromethane as the elution solvent. In future, the mixed solvent dominated by dichloromethane and its elution volume would be further optimized to hunt for the higher recovery of the GCB cleanup.

As far as the silica gel cartridge such as Retain AX, PAX and MAX as well as the macroporous polymer cartridge like HLB is concerned, the precondition with solvents to promote the stretching of sorbent is usually necessary for them to catch target compound. In contrast, the GCB sorbent was free of the precondition might due to its inorganic hydrophobic nature and the similarity between the patulin molecules and the GCB surface[10] that may facilitate their mutual interaction. However, it was observed that the yellow pigment in apple juice was also absorbed by the GCB cartridge. If the loading-sample GCB cartridge was washed with water or aqueous solution (5 mmol·L^{-1} ammonium acetate solution or 0.1% acetic acid solution) in order to remove more impurities, some patulin molecules would be washed away from the GCB cartridge before elution, leading to a large decrease in the recovery. Moreover, the residual pigment would be eluted by dichloromethane into the yellowish eluent which affected the resolution of patulin. Once the washing step was left out, the acceptable recoveries could be obtained (Table 1), and the yellow pigment would be firmly attached to the GCB cartridge to ensure the colorless eluent and the favorable HPLC chromatogram for the patulin detection (Fig. 1F). In a word, our GCB cleanup only needed two steps (i.e. loading and elution) to carry out by gravity on a SPE manifold within 10 min, simpler and faster than other SPE cleanups (for 20 – 40 min). The microstructure of GCB material was observed by a Regulus 8100 field emission scanning electron microscope (SEM) (Hitachi Ltd., Tokyo, Japan). The obtained SEM image (Fig. 3) exhibited the extensive micropore structure and the huge specific surface area of this material which should be helpful for the liquids in freely passing through the GCB cartridge to rapidly complete the absorption and desorption on patulin.

Table 1 Method performances of the patulin detection based on various SPE cleanups coupled with HPLC – PDA and SIDA – LC – MS/MS

SPE mode	HPLC – PDA					SIDA – LC – MS/MS				
	LOD/ (μg·kg^{-1})	LOQ/ (μg·kg^{-1})	Recovery (RSD)/%			LOD/ (μg·kg^{-1})	LOQ/ (μg·kg^{-1})	Recovery (RSD)/%		
			15*	50*	100*			5*	50*	100*
MAX	4	15	89(8)	93(6)	87(6)	1	3	98(5)	98(4)	99(4)
HLB	5	15	85(9)	90(7)	87(7)	2	5	102(6)	100(5)	99(4)
GCB	3	10	86(7)	82(7)	83(6)	1	4	99(5)	99(5)	100(4)

* referred to the spiked level with the unit "μg·kg^{-1}"

2.4 Application of GCB cartridge

Eight familiar brands of clear apple juices with different color from pale-yellow to yellow-brown were sampled from our local supermarkets (Hangzhou, China). They could freely pass through the GCB cartridge without vacuum, and the cleanup times were all less than 10 min. The powerful cleanup effects were clearly reflected in the colorless appearances of eight purified samples. The analyses by HPLC – PDA and LC – MS/MS indicated that patulin was absent in these apple juices. Further, a series of higher concentrations of patulin were spiked into the yellow-brown apple juice to evaluate the capacity of GCB cartridge. 20 μg patulin could be completely absorbed by 50 mg GCB sorbent, and the recovery based on the cleanup by the GCB cartridge and the analysis by HPLC – PDA was above 75%.

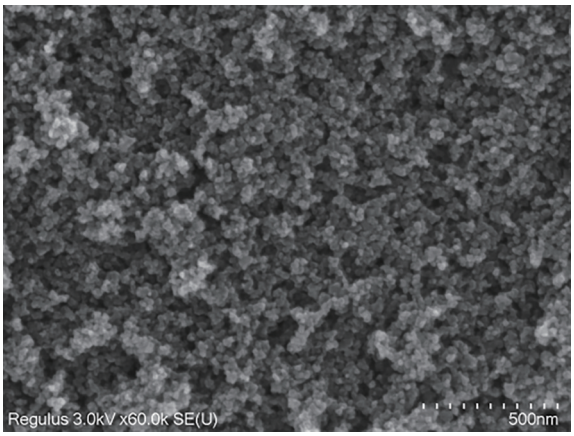

Fig. 3 Scanning electron microscope(SEM) image for the graphitized carbon black material(Cleanert PestiCarb) made into the GCB cartridge

the tested material was mounted on a bronze stub and sputter-coated with gold. A field emission scanning electron microscope was used to observe at an accelerating voltage of 3.0 kV, and under the magnification of 60 000

In addition, some locally moldy apples were collected from a local market(Hangzhou, China). After the rotten areas were removed entirely, they were made into a clear apple juice according to the reported processing technology[20]. The 34 μg·kg^{-1} patulin was found in this apple juice after the GCB cleanup, and its characteristic chromatograms by HPLC – PDA and SIDA – LC – MS/MS were shown in Fig. 4. Obviously, patulin could present in those seemingly healthy areas of moldy apples. In our life, many people think that the partially rotten apple is still safe to eat by cutting off the deteriorated part. In fact, this kind of apple could have become a "poisonous apple".

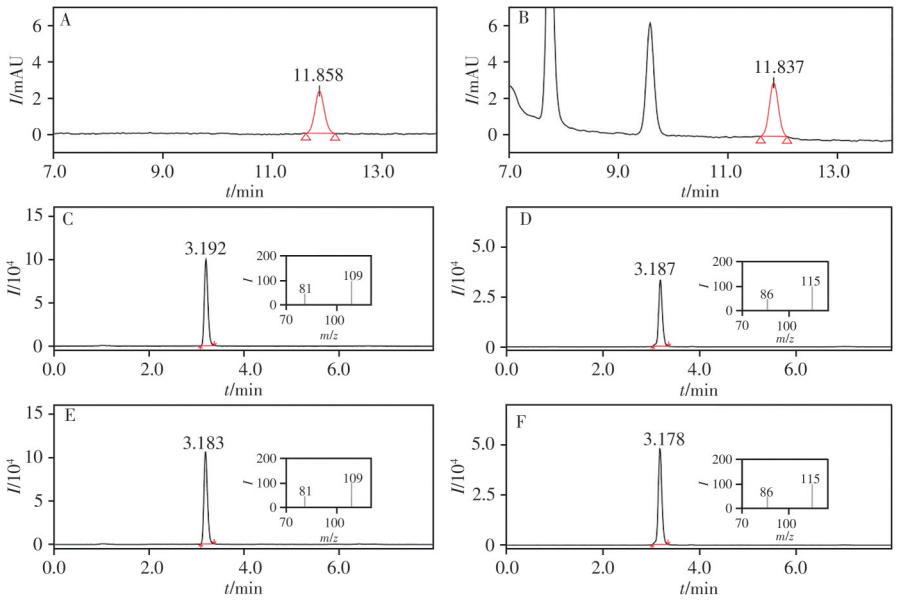

Fig. 4 Characteristic chromatograms of the home-made apple juice containing patulin

A and B: HPLC – PDA chromatograms of the work standard of patulin at 50 μg·L^{-1}(A) and the purified positive apple juice(B). C – F: LC – MS/MS chromatograms of the extracted quantitative ions of patulin at 50 μg·L^{-1}(C) and $^{13}C_7$-patulin at 25 μg·L^{-1}(D) from the mixed work standard as well as the extracted quantitative ions of patulin(E) and $^{13}C_7$-patulin(F) from the purified positive apple juice which was spiked with $^{13}C_7$-patulin at 25 μg·L^{-1} before cleanup. Their characteristic centroids were inserted in C – F

3 Conclusions

In the patulin detection, the impurities in clear apple juice could bring severe interference to target peak in HPLC – PDA and strong suppression effect on signal response in LC – MS/MS. The online fingerprinting of HPLC – PDA and LC – MS/MS exhibited the remarkable cleanup effects of the Retain AX, PAX, MAX, HLB

and GCB cartridge on the clear apple juice reflected in their chromatograms, *S/N*s and matrix effects. Our home-made GCB cartridge was cost-effective and easy to process. After the GCB cleanup, the ideal chromatograms, better *S/N* and acceptable matrix effect were obtained, and the comparable sensitivity, accuracy and precision of the method were achieved by HPLC – PDA and SIDA – LC – MS/MS, respectively. Based on its simpler and faster cleanup steps as well as the significant cleanup effects in the removal of the pigment and other impurities from apple juice, it was concluded that the GCB cartridge was promising in the routine determination of patulin by HPLC – PDA or SIDA – LC – MS/MS. Besides, the low cost(around 1/4 or 1/6 price of other four SPE cartridges) and easy processing of GCB cartridge were also beneficial properties for its wide application.

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