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Efficient extraction and sensitive LC-MS quantification of hydroxytyrosol in wine, oil and plasma

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ABSTRACT

Hydroxytyrosol (HT) possesses significant biological activity. However, the methodologies for its quantification always suffered from low sensitivity, intricate treatment and high sample consumption. Here, we presented the very first attempt for specific extraction of HT through cis-diol recognition mechanism. By using easily prepared zirconia as dispersive solid phase extraction medium, HT from small amount of wine (10 μL), oil (20 mg) and plasma (100 μL) was efficiently purified within ten minutes. Coupled with LC-MS/MS analysis, the method limit of detection (LOD) could reach 1 ng/mL in wine, 0.5 μg/kg in oil and 0.1 ng/mL in plasma. Profited by this superior method, HT analysis was successfully performed in diverse wine and oil products as well as human plasma samples after intake of extra virgin olive oil. In addition, we further confirmed the endogenous HT was undetectable from routine human plasma even after upgrading the detection sensitivity through post iso-nicotinoyl chloride derivatization.

1. Introduction

For long, hydroxytyrosol (HT) is regarded as one of the most important nutrient components in olive products and wine. To date, numerous researches have been carried out to demonstrate its potential biological effects as antioxidant, anti-inflammatory and antimicrobial (de las Hazasa, Rubio, Macia, & Motilva, 2018). Besides, the prophylactic and therapeutic functions of HT against cancer, diabetes and cardiovascular diseases have also been increasingly reported (Robles-Almazan et al., 2018). In 2011, the European Food Safety Authority (EFSA) made a positive statement about the health benefits for daily intake of HT and related polyphenols (Efsa Panel on Dietetic Products & Allergies, 2011). Considering the growing scientific interest of HT in the fields of food and clinical science, effective and affordable quantification methodologies has become highly required to investigate its functional mechanisms (Lucci, Saurina, & Núñez, 2017).

Practically, wine, oil and plasma are commonly involved samples in HT related researches. To detect HT from those matrixes, various techniques have been introduced, including gas chromatography (GC), liquid chromatography (LC), electrochemical detection and mass spectrometry (MS) (Lucci et al., 2017). However, the existed methodologies are still compromised by imperfects like low sensitivity, intricate treatment or high sample consumption (as shown in Table 1) (Achaintre, Gicquiau, Li, Rinaldi, & Scalbert, 2018; Bordiga et al., 2016; Caprioli, Boarelli, Ricciutelli, Sagratini, & Fiorini, 2019; Mazzotti et al., 2012; Pastor et al., 2016; Ragusa et al., 2019; Ricciutelli et al., 2017; Rojas et al., 2019). Such predicament could be mainly ascribed to the lack of efficient HT extraction strategy. In most cases, liquid-liquid extraction (LLE) by methanol/water mixture or solid phase extraction (SPE) with C18 cartridges is utilized. Nevertheless, as an amphipathic and highly polar compound, HT could hardly be well recovered by those pretreatment processes. Besides, to prevent the potential adverse solvent effect for chromatographic separation, evaporation of organic solvents or dilution with water was always needed after extraction. These post-treatments would inevitably prolong the operating time and decrease method sensitivity.

Actually, HT is also a cis-diol-containing biomolecule (deriving from its catechol unit). According to our previous researches, nucleosides and glycol-peptides/proteins could be well recognized by metal oxide affinity interaction through cis-diol structure (Wang et al., 2014; Wang, Huang, Lu, Yuan, & Feng, 2013). Inspiring by that, in the present work, we pioneeringly realized selective enrichment of HT from practical samples by zirconia material. Briefly, after diluting with isopropanol/acetonitrile, HT in the matrix was specifically captured through zirconia dispersive SPE (by forming stable cyclic ester between cis-diol group and metal hydroxyl group). Afterward, the captured HT was

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Table 1
Brief comparison of the methods for hydroxytyrosol quantification in wine, oil and plasma samples.

Sample	Pretreatment	Detector	Sample consumption	Sensitivity	Reference
Wine	Filtration	HPLC-UV	5 mL	LOQ 240 ng/mL	Ragusa et al., 2019
Wine	Filtration	HPLC-PDA-MS/MS	Not mentioned	LOQ 11 ng/mL	Bordiga et al., 2016
Oil	SPE and evaporation	Voltammetric detection	1 g	LOD 150 ng/mL	Rojas et al., 2019
Oil	LLE and dilution	LC-MS/MS	10 μL	LOQ 710 ng/mL	Mazzotti et al., 2012
Oil	Successive LLE and evaporation	HPLC-DAD-ESI/MS	5 g	LOQ 0.17 mg/kg (about 170 ng/mL)	Ricciutelli et al., 2017
Oil	Successive LLE and evaporation	HPLC-DAD-ESI/MS	0.5 g	LOQ 0.025 mg/kg (about 25 ng/mL)	Caprioli et al., 2019
Plasma	Protein precipitation, benzylamine derivatization, SPE and evaporation	LC-MS/MS	200 μL	LOD 0.3 ng/mL	Pastor et al., 2016
Plasma	Successive LLE, evaporation, dansyl chloride derivatization and evaporation	LC-MS/MS	50 μL	LOQ 11 Nm (about 1.7 ng/mL)	Achaintre et al., 2018
Wine	Dilution and zirconia extraction	LC-MS/MS	10 μL	LOD 1 ng/mL	This work
Oil	Dilution and zirconia extraction	LC-MS/MS	20 mg	LOD 0.0005 mg/kg (about 0.5 ng/mL)	
Plasma	Protein precipitation and zirconia extraction	LC-MS/MS	100 μL	LOD 0.1 ng/mL	

Abbreviation: HPLC-UV, high-performance liquid chromatography-ultraviolet detector; HPLC-PDA-MS/MS, high-performance liquid chromatography-photo-diode array-mass spectrometry; HPLC-DAD-ESI/MS, high-performance liquid chromatography-diode array detection-electrospray ionization/mass spectrometry.

desorbed in formic acid solution (by dissociating the cyclic ester under acidic environment). Benefiting from the excellent purification effect of such affinity extraction, a highly reliable and sensitive LC-MS/MS method was developed for HT quantification from wine, oil and plasma samples.

For wine, four different brands were analyzed (one from France, one from Australia and two from China). The concentration of HT was determined as 0.071–9.2 μg/mL. For oil, peanut oil, corn oil, ordinary olive oil and extra virgin olive oil (EVOO) samples were involved. In this case, HT could be only detected in ordinary olive oil and EVOO with the concentration of 0.4 and 7.3 mg/kg. For plasma, when samples from three healthy volunteers were analyzed, no detectable endogenous HT could be found even after we further upgraded the method sensitivity (to LOD 0.02 ng/mL) through post derivatization treatment. By contrast, after taking extra virgin olive oil, HT could be well detected in plasma from the same group of volunteers. From all the results, the developed method exhibited superior sensitivity and applicability for HT quantification from various sample matrixes so that it might promote future studies in HT related research fields.

2. Hypothesis statement

HT could be specifically purified from sample matrixes by recognition of its cis-diol structure so that the detection performance could be obviously improved.

3. Experimental

3.1. Chemicals and reagents

The reagents of HT (CAS 10597-60-1, H4291-25 mg), zirconium butoxide (CAS 1071-76-7, 333948–100 mL), sodium metabisulfite (CAS 7681-57-4, S9000-500g) and tartaric acid (CAS 147-71-7, T206-100 g) were purchased from Sigma-Aldrich (Beijing, China). HT-d4 (deuterium labeled HT, CAS 1330260-89-3, H977002-2.5 mg) as stable isotope labeled internal standards (SIL-IS) of HT, catechol (CAS 120-80-9, 359694-100 g) and resorcinol (CAS 108-46-3, 140161-50 g) were purchased from J&K Scientific (Beijing, China). Formic acid, methanol, 2-propanol and acetonitrile were of HPLC grade from Fischer Scientific (New Jersey, United States). The water used throughout the study was purified by a Milli-Q apparatus (Millipore, Bedford, MA). The structures of HT, catechol and resorcinol are shown in Supplementary Fig. S1. All the wine and oil products were bought from a local market in Wuhan (China). The product information were obtained from the related brand tags.

3.2. Apparatus and parameters

The LC-MS/MS platform consisted of a Shimadzu MS-8050-CL triple quadrupole mass spectrometer (Kyoto, Japan) with an electrospray ionization source (Turbo Ionspray) operating in negative ion mode, a Shimadzu LC-30AD-CL system (Kyoto, Japan), a SIL-30AC-CL autosampler, a CTO-30A thermostat column compartment and a DGU-20A degasser. Data acquisition and processing were performed with Lab Solution 5.53 SP2 software. The targets were monitored by multiple reaction monitoring (MRM) mode. The details are shown in Supplementary Table S1. And the optimal conditions for ionization source were as follows: DL temperature 250 °C, heat block temperature 400 °C, nebulizing gas 2 L/min and drying gas 10 L/min, heating gas 10 L/min and interface temperature 300 °C.

The LC separation was manipulated on a Kinetex 2.6 μm C18 100 Å (100 × 3 mm) column with a flow rate of 0.5 mL/min at 60 °C. Formic acid in water (0.2%, v/v, Solution-A) and formic acid in methanol (0.2%, v/v, Solution-B) were wielded as mobile phases. The gradient was 0–1 min 2% B, 1–3 min 2%-70% B, 3–3.5 min 70%-2% B, 3.5–5 min 2% B. The retention time of HT was 2.8 min. To eliminate

matrix contamination, the fluid from 0 to 0.6 min was discarded to waste by an exchange value.

The transmission electron microscope image was performed on a Tecnai G2 20S-TWIN transmission electron microscope.

3.3. Stock solutions, calibration and quality control samples

Certain amount of HT and HT-d4 standards was weighed and then dissolved in 2-propanol to prepare stock solutions (2 mg/mL). Before use, the synthetic wine, corn oil and blank plasma samples were all analyzed by the established LC-MS/MS protocol to exclude HT contamination. All the solutions were stored at −80 °C before use.

3.3.1. Wine samples

Further dilution of HT stock solution for calibration curve and quality control (QC) samples was operated by using synthetic wine, which was a mixture of tartaric acid (4 g/L) and ethanol (12%, v/v) as a wine-like medium (Bordiga et al., 2016). The concentration range of calibrations was 0.01–50 μg/mL. The spiked concentrations in high-, medium-, low- and LLOQ-level of QC samples were 50, 2, 0.05 and 0.01 μg/mL. HT-d4 solution was prepared in water (1 μg/mL).

3.3.2. Oil samples

Further dilution for calibration curve and quality control (QC) samples was operated by using corn oil. The concentration range of calibrations was 0.01–50 mg/kg. The spiked concentrations in high-, medium-, low- and LLOQ-level of QC samples were 50, 2, 0.05 and 0.01 mg/kg. HT-d4 solution was prepared in 2-propanol (1 μg/mL).

3.3.3. Plasma samples

Further dilution of HT stock solution for calibration curve and quality control (QC) samples was operated by using blank plasma, which was prepared by continuously exposing plasma samples to light for 48 h at room temperature (to ensure the complete degradation of potential HT contamination) (van de Merbel et al., 2011; Yuan, Huang, Gao, Wang, & Li, 2018). The concentration range of calibrations was 0.5–20 ng/mL. The spiked concentrations in high-, medium-, low- and LLOQ-level of QC samples were 20, 5, 1 and 0.5 ng/mL. HT-d4 solution was prepared in water (2 ng/mL).

All the plasma samples were recruited from healthy volunteers in heparinized tubes and spiked with sodium metabisulfite (0.5 mg/mL) immediately. After centrifugation (3000 rpm, 3 min at 4 °C), the separated plasma was stored under −80 °C until use. The whole study was supervised under the Ethics Committee of Renmin Hospital of Wuhan University. The consent procedure was based on the standard procedure. All the plasma samples were obtained with permission.

3.4. Preparation of zirconia suspension

Water (1 mL) was mixed in 2-propanol (30 mL) in a beaker (100 mL). After cooling down to 0 °C, zirconium butoxide solution (10% v/v in 2-propanol, 10 mL) was added drop-by-drop under gender magnetic stirring. Then the mixture was kept with the lid closed at room temperature for 1 h to complete the reaction. In the end, the precipitation was rinsed with ethanol and water (50 mL) successively and re-dispersed in 2-propanol/water (80%, v/v, 5 mL) obtaining the zirconia suspension. Before pipetting for d-SPE, the turbid solution was sufficiently re-mixed.

3.5. Sample pretreatment

3.5.1. Wine samples

Calibration, quality control or practical samples (10 μL) were mixed with HT-d4 solution (1 μg/mL, 10 μL). Then acetonitrile (1000 μL) was added following with vortex (1 min) and centrifugation (15,000 rpm, 2 min at 4 °C) for precipitation. After transferring supernatant (950 μL) into a new tube, the prepared zirconia aqueous dispersion (50 μL) was added and vortexed (1 min) for extraction. The supernatant was then discarded. After washing with water (1 mL), formic acid solution (5% v/v in water, 50 μL) was used to elute the adsorbed targets on zirconia material by vortexing (1 min) and centrifuging (15,000 rpm, 2 min at 4 °C). The supernatant (20 μL) was injected for LC-MS/MS analysis.

3.5.2. Oil samples

Calibration, quality control or practical samples (20 mg) were mixed with HT-d4 solution (1 μg/mL, 20 μL) and 2-propanol (1000 μL) following with vortex (1 min) for completely dissolving the oil samples. Then the prepared zirconia aqueous dispersion (50 μL) was added and vortexed (1 min) for extraction. Afterward, the material was separated by centrifugation and washed with 2-propanol/water (50%, v/v, 1 mL) and water (1 mL) successively. In the end, formic acid solution (5% v/v in water, 50 μL) was used to elute the adsorbed targets from zirconia material by vortexing (1 min) and centrifuging (15,000 rpm, 2 min at 4 °C). The supernatant (20 μL) was injected for LC-MS/MS analysis.

3.5.3. Plasma samples

Calibration, quality control or practical samples (100 μL) were mixed with HT-d4 solution (2 ng/mL, 50 μL). Then acetonitrile (2000 μL, 4 °C) was added following with vortex (0.5 min) and centrifugation (15,000 rpm, 2 min at 4 °C) for protein precipitation. After transferring supernatant (2000 μL) into a new tube, the prepared zirconia aqueous dispersion (50 μL) was added and vortexed (1 min) for extraction. The supernatant was then discarded. After washing with water (1 mL), formic acid solution (5% v/v in water, 50 μL) was used to elute the adsorbed targets on zirconia material by vortexing (1 min) and centrifuging (15,000 rpm, 2 min at 4 °C). The supernatant (20 μL) was injected for LC-MS/MS analysis.

3.6. Method validation

The calibration curves were determined using the peak ratios of HT to HT-d4 versus the nominal spiked concentrations by a linear least squares regression model. The lowest concentration of the calibration curve was accepted as lower limit of quantitation (LLOQ). The ratio of signal to noise (S/N) was higher than ten at limit of quantification (LOQ) levels. The details are listed in Table 2. The carry-over effects were evaluated by analyzing the blank matrix after three successive injections of high-level of QC samples. The residues should be less than 15% of LLOQ.

The matrix effect was evaluated by comparing the results of two experimental groups. For Group-I, HT and HT-d4 were spiked into the pretreated blank matrixes just before LC-MS/MS analysis. For Group-II, HT and HT-d4 were spiked into formic acid solution (5%, v/v) and analyzed. Both groups were spiked at medium-level of HT. The matrix effect was evaluated by the ratio of the results from Group-I to Group-II, which should be within 85–115%.

Accuracy was calculated by dividing the measured concentration by the nominal spiked value in QC samples, which could also be regarded as the method recovery. And imprecision was evaluated as coefficient of variation (CV) of the measurements. Both accuracy and imprecision were investigated for four levels (high-, medium-, low- and LLOQ-

Table 2
The calibration curves and sensitivity for hydroxytyrosol detection in wine, oil and plasma.

Samples	Linear range	Calibration curves			LOD
		Slope	Intercept	R^2	
Wine	0.01–50 μg/mL	0.947	−0.0017	0.999	1 ng/mL
Oil	0.01–50 mg/kg	0.938	0.0009	0.999	0.5 μg/kg
Plasma	0.5–20 ng/mL	0.944	0.0607	0.999	0.1 ng/mL

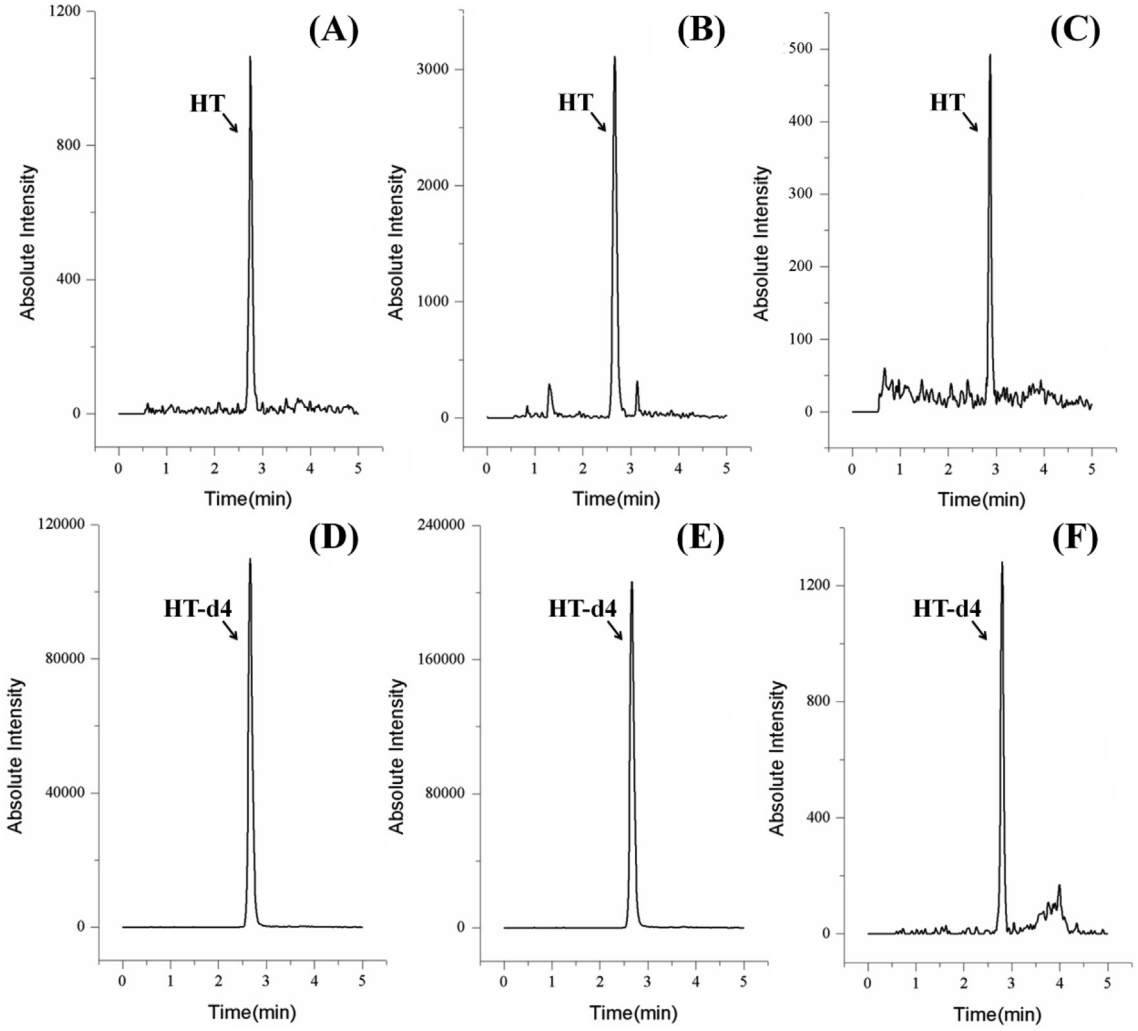

Fig. 1. The typical LC-MS/MS chromatograms of hydroxytyrosol (HT) and hydroxytyrosol-d4 (HT-d4) in quality control samples at LLOQ-level: (A) and (D) for wine; (B) and (E) for oil; (C) and (F) for plasma.

level). The measurements were performed in one day (intraday) and ten consecutive days (interday). The values of accuracy should be 85–115% (80–120% for LLOQ-level). And imprecision should not be higher than 15% (20% for LLOQ-level).

The repeatability of the methodology was evaluated through extracting QC samples (medium-level) by zirconia from one synthesis batch or five different batches. The intra-batch and inter-batch recovery should be 85–115% with CV $\leq$ 15%.

The stability of HT in sample matrix before pretreatment and in desorption solution after pretreatment was studied by using medium-level QC samples. The storage was performed at −80 °C, 4 °C and room temperature (25 °C) for 1, 6, 24 h. Freeze-thaw stability was tested after three cycles of freezing (-80 °C) and thawing (25 °C). During stability test, no specific light-protection treatment was carried out.

4. Results and discussion

4.1. Synthesis and characterization of zirconia material

For synthesis, zirconium butoxide was simply hydrolyzed by water. Relatively low temperature was utilized to control the reaction rate so that zirconia could precipitate gradually and homogeneously. According to transmission electron microscope image, the particle diameter of the prepared zirconia was about 100 nm (Supplementary Fig. S2). Such morphotype ensured large specific surface area, which would benefit the extraction performance. By weighing the dried suspension, the dosage of zirconia for every extraction could be calculated as less than 1 mg. Taking the simple material preparation and low material consumption together, the present extraction strategy was cost-effective and highly applicable for practical usages.

4.2. Method development

The recognition of prepared zirconia toward cis-diol structure was investigated in the first place. Catechol and resorcine were chosen as model compounds. The experimental details are shown in Supplementary Fig. S3. From the results, cis-diol containing catechol could be specifically isolated from resorcine (the non-cis-diol analogue), which demonstrated excellent selectivity of zirconia extraction strategy. Benefiting from such feature, specific purification of HT from wine, oil and plasma matrixes was achieved for the very first time.

During method development, several parameters were optimized including sampling condition, material dosage and eluting condition. (I) Sampling condition. Previously, we found non-aqueous or basic environment was important to realize zirconia extraction toward cis-diols (Wang et al., 2014; Wang et al., 2013). As HT was unstable in basic solution (Wani et al., 2018), we chose to load the samples under neutral condition with high content of organic solvents (over 95%). And to diminish hydrophilic interference, pure water was used in the following washing step. (II) Material dosage. The dosing amount of

zirconia suspension was tested from 20 to 200 μL. Profited by the nanoscale particle diameter (large surface area, scilicet), we found only 50 μL of the wet-gel zirconia was adequate for HT extraction from all the tested sample matrixes. (III) Eluting condition. Acidic concentration (1%–10%) and organic content (methanol and acetonitrile) for desorption was investigated. Unlike the commonly used hydrophobic extraction of HT, the present strategy was based on cis-diol-directed affinity mechanism. In consequence, we found water diluted formic acid (5%) without any other organic solvent could achieve good eluting efficiency. Such feature made the supernatant after desorption could be immediately injected for LC separation without causing any adverse solvent effect, so that the unfavorable solvent evaporation process was prevented.

4.3. Method validation

For method validation, linearity, sensitivity, carry-over effect, matrix effect, accuracy, imprecision and stability were investigated according to Food and Drug Administration (FDA) guideline for bioanalytical method validation and Clinical and Laboratory Standards Institute (CLSI) document C62-A.

The linear ranges of HT in wine, oil and plasma matrixes were investigated within 0.01–50 μg/mL, 0.01–50 mg/kg and 1–20 ng/mL respectively. The lowest concentrations of the calibration curves were accepted as LLOQ, while the LOD was determined as 1, 0.5 and 0.1 ng/mL for wine, oil and plasma respectively. Such sensitivity presented a general upgrade comparing with the existed methodologies. The typical LC-MS/MS chromatograms for blank matrixes and QC samples at LLOQ-level are shown in Supplementary Fig. S4 and Fig. 1. In addition, when blank matrix was analyzed after three successive injections of high-level of QC samples, little quantitative residues could be observed (below LOD) so that the carry-over effect was negligible.

As for matrix effect, the ratio of Group-I to Group-II was calculated as 96.6–99.4% for all the tests. This result indicated after zirconia purification and HT-d4 normalization, all the matrixes would not cause adverse effect on quantification.

During intraday and interday analysis, the recovery was observed as 90.4–103.2% for high-, medium- and low-level tests and 82.7–93.3% for LLOQ-level tests (Supplementary Table S2). And the corresponding imprecision was lower than 12.1% for high-, medium- and low-level tests and 17.4% for LLOQ-level tests. These results indicated the developed method was reliable for HT detection from wine, oil and plasma matrixes.

For method repeatability validation, the recovery of intra-batch tests was obtained as 95.1–99.8% with CV less than 7.7%. And the recovery of inter-batch tests was obtained as 96.3–104.2% with CV less than 8.4%. In consequence, benefiting from the simple material preparation strategy, the extracting performance of zirconia revealed excellent reproducibility.

The storage stability of HT was acceptable in wine and oil matrixes under all the tested circumstances (recovery 91.8–96.6%). While in the case of plasma samples, obvious degradation was observed after 6 h (recovery 41.1%) and 24 h (recovery 15.3%) storage at room temperature and 24 h storage at 4 °C (recovery 44.2%). During freezing and thawing experiments, HT was also less stable in plasma matrix (recovery 72.1%) than wine (recovery 90.4%) and oil (recovery 89.7%) matrixes.

5. Real sample analysis

5.1. Wine sample

The present method was successfully applied to HT detection in four different brands of red wine purchased randomly from a local market. As shown in Table 3, concentrations of HT were determined as 0.071–9.2 μg/mL in these samples. For comparison, we also analyzed the wine sample with the lowest HT concentration after direct filtration treatment (Fig. 2). In this case, the signal of HT was seriously overwhelmed by interferences with the ratio of signal to noise (S/N) lower than ten. Moreover, we also performed the recovery experiments by spiking HT (5 μg/mL) to this wine sample. In consequence, the recovery was obtained as 94.7%. All these results clearly demonstrated the excellent purification capacity of zirconia extraction for HT from wine matrixes.

5.2. Oil sample

One peanut oil, one corn oil, one ordinary olive oil and one EVOO were analyzed in this part. As expected, no detectable HT could be observed in peanut and corn oil samples, whereas considerable content of HT was presented in both ordinary olive oil and EVOO samples. The concentrations were 0.4 and 7.3 mg/kg respectively. Such difference was also reported in previous researches. In addition, HT was spiked in these two oils (25 mg/kg) to investigate the method recovery for real samples. The results showed 98% for ordinary olive oil and 91% for EVOO, which again indicated favorable applicability of the developed methodologies for HT detection in oil matrixes.

5.3. Plasma sample

For long, quantification of HT in plasma remains quite a challenge. Even in the latest methods, multiple elaborate pretreatments were forced to integrate. For example, Torre's group innovatively combined protein precipitation, benzylamine derivatization, hydrophobic SPE and nitrogen evaporation to detect HT from 200 μL plasma (LOQ 0.3 ng/mL) (Pastor et al., 2016). And the comprehensive method from Achaintre's group realized detection of HT from 50 μL plasma (LOQ 1.7 ng/mL) through successive LLE, dansyl chloride derivatization and two cycles of solvent evaporation (Achaintre et al., 2018). For improvement, in this work, we pioneerringly simplified the pretreatment steps and achieved higher sensitivity (LOD 0.1 ng/mL) by using only protein precipitation and novel zirconia extraction for 100 μL plasma. With this superior method, we firstly investigated whether endogenous free HT was widely existed in plasma, which is still a controversial issue so far. Three healthy volunteers (without any supplements of olive or wine products) were analyzed. In consequence, no HT could be identified in all the samples. Considering HT was believed to present in dopaminergic pathways as 3,4-dihydroxyphenylethanol (Domínguez-Perles, Auñón, Ferreres, & Gil-Izquierdo, 2017), we further upgraded the method sensitivity to LOD 0.02 ng/mL through post derivatization treatment with isonicotinoyl chloride (as shown in Supplementary Fig. S5) (Le et al., 2019). However, even under such circumstance, negative results were obtained. In contrast, free HT with concentration of 0.8–4.9 ng/mL were well detected in plasma from the same group of volunteers after taking EVOO (10 g) for 1 h (as shown in Table 3), indicating the satisfying applicability of the proposed method for plasma samples.

5.4. Other samples

For further evaluation, olive leaves and olive pomace samples were introduced for HT quantification. The experimental details are shown as "Pretreatment of olive leave and olive pomace samples" in Supplementary material. After zirconia extraction, HT could be well detected in both samples. The amounts presented in olive leaves and olive pomace were calculated as 33.2 and 1.1 mg/kg respectively. Such results provided a proof-of-concept for expanding the usage of the methodology for other food/clinical sample matrixes.

5.5. Hypothesis response

HT from various sample matrixes could be efficiently extracted by

Table 3
Detail information for hydroxytyrosol detection in real samples.

Sample	Information	Measured hydroxytyrosol concentration
Wine-1	Origin: France; Price: 270 Yuan/750 mL	9.2 μg/mL
Wine-2	Origin: Australia; Price: 170 Yuan/750 mL	5.4 μg/mL
Wine-3	Origin: China; Price: 150 Yuan/750 mL	5.6 μg/mL
Wine-4	Origin: China; Price: 30 Yuan/750 mL	0.071 μg/mL
Peanut oil	Origin: China; Price: 50 Yuan/200 mL	Below LOD
Corn oil	Origin: China; Price: 20 Yuan/150 mL	Below LOD
Ordinary olive oil	Origin: China; Price: 60 Yuan/500 mL	0.4 mg/kg
Extra virgin olive oil	Origin: Spain; Price: 180 Yuan/500 mL	7.3 mg/kg
Plasma-1	Plasma from volunteer-I	Below LOD
Plasma-2	Plasma from volunteer-II	Below LOD
Plasma-3	Plasma from volunteer-III	Below LOD
Plasma-4	Plasma from volunteer-I (1 h after ingestion of EVOO)	4.9 ng/mL
Plasma-5	Plasma from volunteer-II (1 h after ingestion of EVOO)	1.4 ng/mL
Plasma-6	Plasma from volunteer-III (1 h after ingestion of EVOO)	0.8 ng/mL

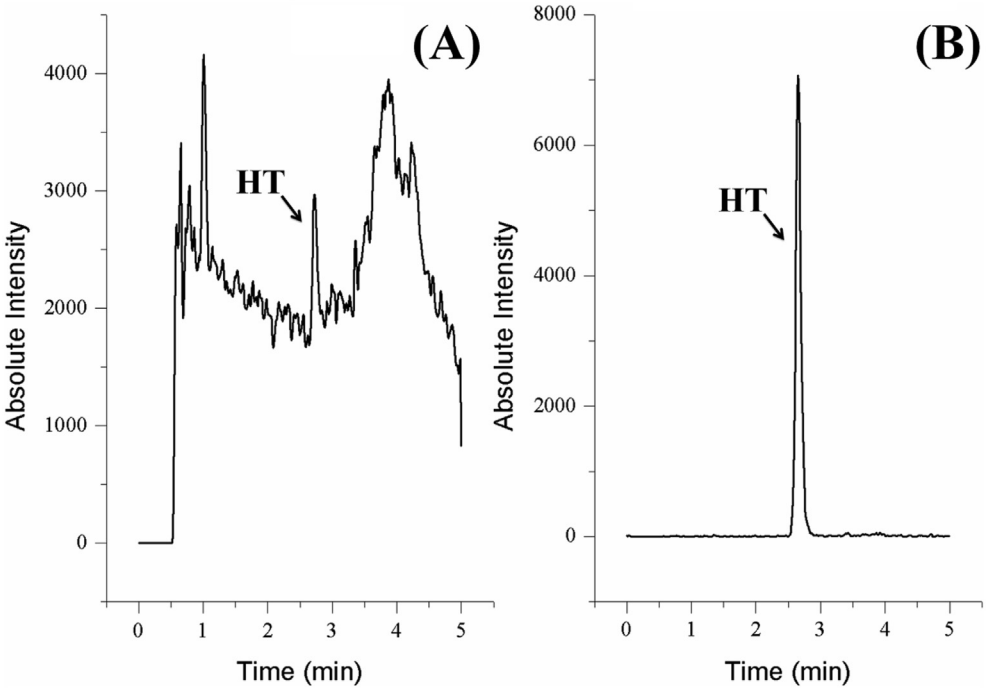

Fig. 2. Comparison of LC-MS/MS analysis of hydroxytyrosol (HT) in Wine-4: (A) analysis after filtration and (B) analysis of wine after zirconia purification.

using zirconia material, which obviously benefitted its quantification with LC-MS/MS.

6. Conclusions

In conclusion, this work put forward the very first strategy for selective extraction of HT through cis-diol recognition technology. By using the simply prepared zirconia material, HT could be efficiently captured from wine, oil and plasma matrixes. Coupled with LC-MS/MS analysis, the whole methodology exhibited excellent applicability (easy-handling, high-throughput and proper sample consumption) and sensitivity (LOD 1, 0.5 and 0.1 ng/mL for wine, oil and plasma respectively). We expected the present method could facilitate further development of HT-related researches.

CRediT authorship contribution statement

Shao-Ting Wang: Conceptualization, Methodology, Writing - original draft. **Juan Le:** Validation, Writing - review & editing. **Rui Peng:** Investigation. **Yan Li:** Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.126803.

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