Determination of perfluorinated compounds in *Eichhornia crassipes* (Mart.) Solms using LC-MS/MS in combination with sample preparation by ultrasound-assisted extraction and solid-phase extraction

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Abstract

Fifteen perfluorinated compounds, including perfluorocarboxylic acids and perfluoroalkyl sulfonate salts (with 4 to 12 carbon atoms in their structure) in *Eichhornia crassipes* (Mart.) Solms samples underwent analysis using Liquid Chromatography- tandem Mass Spectrometry (LC-MS/MS). This analysis was conducted alongside sample preparation employing ultrasound-assisted extraction and solid-phase extraction. The optimization of solvent extraction involved the use of 5 ml of methyl tert-butyl ether (MTBE), 10 minutes of sonication, and three repeated extraction cycles. The weak anion exchange (WAX) cartridge was selected after evaluating and comparing the solid-phase extraction efficiencies of both WAX and C18 cartridges. The evaluated method demonstrated successful analysis of all 15 PFCs in plant samples, achieving favorable recoveries ranging from 71 to 116% (with a coefficient of variation of 1.2-6.6%). The quantification limits for these 15 PFCs in *Eichhornia crassipes* (Mart.) Solms samples ranged between 0.30 to 0.54 ng/g.

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1 Introduction

Perfluorinated compounds (PFCs) are organic substances where all the carbon-hydrogen (C-H) bonds in their molecular structure are replaced by carbon-fluorine (C-F) bonds. These chemicals stem from industrial applications, specifically anionic and neutral surfactants that are widely used across textile manufacturing, electroplating, mining, petrochemical industries and serve multiple purposes such as coatings, fire suppressants, hydraulic fluids, and insect repellents since the 1950s [1]. Due to their resistance to degradation, high propensity for biological accumulation, and long-term usage, PFCs have been found extensively in soil, water, air, wildlife, and even in human beings [2-4]. As a result, compounds like perfluorooctane sulfonate (PFOS), perfluorooctane sulfonyl fluoride (PFOSF), and related substances were classified as Persistent Organic Pollutants (POPs) in Annex B in 2009. Perfluorooctanoic acid (PFOA) and its salts were later added to Annex A in 2019, followed by perfluorohexane sulfonate (PFHxS) and related substances being included in Annex A under the Stockholm Convention in 2022 [5].

Currently, interest in research has focused on managing persistent organic pollutants (POPs), which include PFCs. Techniques involving physicochemical treatments, notably advanced oxidation processes, or the use of adsorbents such as activated carbon, ion exchange resins, biological materials, and molecularly imprinted polymers, demonstrate a high treatment efficiency (> 90%) for these compounds. However, these methods come with limitations, often requiring substantial amounts of chemicals during the treatment process,

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potentially leading to secondary pollution. From an environmental perspective, these technologies have not yet proven to be sustainably effective.

Consequently, the remediation process through the utilization of plants and/or microorganisms to treat PFC compounds in the environment is currently gaining attention. Specifically, the employment of plants (i.e. phytoremediation) represents an eco-friendly technology, leveraging plant mechanisms to transform, relocate, isolate, extract, and/or detoxify pollutants present in sediments, soils, groundwater, and surface water. Greger et al. (2021) used *Carex rostrata* to remediate PFOS and PFOA, witnessing a respective decrease of 63% and 42% in these compounds' concentrations in water after 12 days of treatment using these plants [6]. Zhang et al. (2019) employed *Juncus effusus* to evaluate the distribution of PFC compounds within plant-soil-water systems and microbial ecosystems [7].

This article focuses on optimizing and validating the analysis process of 17 PFC compounds which belong to the perfluorocarboxylic acid and perfluoroalkyl sulfonate groups in *Eichhornia crassipes* (Mart.) Solms samples. It involves utilizing liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS), along with sample preparation techniques like ultrasound-assisted extraction and solid-phase extraction. The findings of this study are the scientific platform for further research on phytoremediation solutions to remove PFCs from contaminated water.

2 Experiments

2.1 Reagents

The PFC standard mixture (PFAC-MXB 2 ppm) from Wellington Lab consisting of 11 perfluorocarboxylic acid compounds (ranging from C4-C14) and 4 perfluoroalkyl sulfonate compounds (C4, C6, C8, and C10); and an mass-labeled PFC mixture (MPFAC-MXA 2ppm) from Wellington Lab comprising 13C perfluorocarboxylic acids (C4, C6, C8, C9, C10, C11, and C12) and 18O, 13C perfluoroalkyl sulfonates (C6 and C8). In this study, mass-labeled PFCs were used as surrogates (SR). As SRs, mass-labeled PFASs were added to each sample immediately before the sample treatment process to control the recoveries of each sample preparation process. The solid-phase extraction cartridges used were WAX (weak anion exchange type) and C18 from Oasis, Water, USA. The chemicals and solvents used, including sodium hydroxide, tetrabutyl ammonium hydrogen sulfate (TBA), sodium carbonate, methyl tert-butyl ether (MTBE), ammonia, and methanol, are all pure analytical grade supplied by Merck, Sigma.

2.2 Sample preparation

Eichhornia crassipes (Mart.) Solms sample was first homogenized using a standard blender. A total of 2 g of the homogenized sample was weighed into a 50 mL polypropylene (PP) tube, to which 25 µL of a 100 ng/mL surrogate (SR) mixture was added. This was followed by the addition of 8 mL of 0.4 M NaOH, with subsequent shaking. The sample was stored overnight in a refrigerator to ensure uniform distribution of the SR within the sample. Then, 2 mL of 0.5 M TBA and 4 mL of 0.25 M Na_2CO_3 were added and shakened well. After that, MTBE was added and shakened vigorously using a Vortex mixer for 1 minute, and conducted ultrasonic extraction. Then, the sample was centrifuged at 5000 rpm for 5 minutes. The upper MTBE extract was transferred to a fresh PP tube and repeated the MTBE extraction process thrice. Combine the MTBE extracts and evaporate them to dryness using N_2 gas. Sequentially, add 2 mL of methanol and 18 mL of deionized water to obtain the solid-phase extraction (SPE) sample. Activate the SPE cartridge with 4 mL of 0.1% w/w ammonia/methanol, 4 mL of methanol, and 4 mL of deionized water. Load the sample through the activated cartridge at a rate not exceeding 2 drops/second. Rinse the cartridge with 4 mL of 0.025 M acetate buffer to eliminate impurities. Elute the PFCs by using 4 mL of methanol, followed by 4 mL of 0.1% ammonia/methanol. Concentrate the eluent to 1 mL of methanol. Filter the resultant solution through a 0.2 µm nylon membrane, transfer it to a 1.5 mL sample vial, and store it at 4°C until analysis.

2.3 LC-MS/MS condition

The analysis of PFCs was performed using a Shimadzu LC-MS/MS 8040 system, equipped with a Shim-pack FC-ODS C18-ACF3 analytical column (100 mm×2.2 µm) and an ACE-C18 guard column (2.1 mm×2.2 µm). The solvent program employed a mixture of mobile phase A: 2 mmol/L ammonium acetate/methanol (9:1/v:v) and mobile phase B: methanol. Detailed MS/MS parameters used for the analysis of PFCs are outlined in Table 1.

Table 1 MS/MS parameters for analysis of PFCs

No.	Compounds	Acronym	Precursor ion (m/z)	Production (m/z)	Voltage potential Q1 (V)	Collision Energy (V)	Voltage potential Q3 (V)
Target PFCs							
1	Perfluoro-n-butanoic acid	PFBA	212.85	169.05;18.90	22;22	10;42	28;16
2	Perfluoro-n-pentanoic acid	PFPeA	262.85	219;19.2	27;27	8;45	19;18
3	Perfluoro-n-hexanoic acid	PFHxA	312.8	269;118.95	22;22	9;21	25;18
4	Perfluoro-n-heptanoic acid	PFHpA	362.8	319;169.15	25;25	9;18	30;29
5	Perfluoro-n-octanoic acid	PFOA	412.8	368.95;169.05	20;20	10;19	22;28
6	Perfluoro-n-nonanoic acid	PFNA	462.8	418.95;219.05	22;22	10;17	26;20
7	Perfluoro-n-decanoic acid	PFDA	512.85	469.2;219.1	24;24	11;19	30;12
8	Perfluoro-n-undecanoic acid	PFUdA	562.8	518.95;269.1	40;40	12;17	34;26
9	Perfluoro-n-dodecanoic acid	PFDoA	612.8	568.95;318.75	22;22	12;20	38.29
10	Perfluoro-n-tridecanoic acid	PFTrDA	662.8	618.95; 169.25	32;32	13;31	40;29
11	Perfluoro-n-tetradecanoic acid	PFTeDA	712.8	669; 169.3	34;34	13;36	30;26
12	Potassium perfluoro-1-butanesulfonate	PFBS	298.85	80.05;99.05	20;20	40;35	29;15
13	Sodium perfluoro-1-hexanesulfonate	PFHxS	398.8	79.95;98.95	27;27	46;35	28;16
14	Sodium perfluoro-1-octanesulfonate	PFOS	498.85	80.15;99.05	24;24	50;43	28;15
15	Sodium perfluoro-1-decanesulfonate	PFDS	598.85	79.9; 98.85	30;30	50;51	29;13
Mass- labeled PFCs							
1	Sodium perfluoro-1-hexane($^{18}O_2$)sulfonate	MPFHxS	403	73.9;102.9	19;19	49;39	28;15
2	Sodium perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	MPFOS	503	79.9;99.1	24;24	55;48	29;15
3	Perfluoro-n-($^{13}C_4$)butanoic acid	MPFBA	217	172.05	22	8	29
4	Perfluoro-n-(1,2-$^{13}C_2$) hexanoic acid	MPFHxA	314.95	270.15;119.15	15;15	8;20	25;20
5	Perfluoro-n-(1,2,3,4-$^{13}C_4$) octanoic acid	MPFOA	416.95	372.05;172.2	20;20	10;19	23;30
6	Perfluoro-n-(1,2,3,4,5-$^{13}C_5$) nonanoic acid	MPFNA	467.95	423.1;219.15	22;22	10;16	26;20
7	Perfluoro-n-(1,2-$^{13}C_2$) decanoic acid	MPFDA	514.9	469.95;219.10	24;24	11;19	30;19
8	Perfluoro-n-(1,2-$^{13}C_2$) undecanoic acid	MPFUdA	564.9	519.95;169.1	28;28	11;26	34;28
9	Perfluoro-n-(1,2-$^{13}C_2$) dodecanoic acid	MPFDoA	614.9	569.9;169.1	30;30	12;30	36;28

2.4 Sample preparation optimization

The sample processing method used in this study was based on the approach outlined by Zhang et al. (2019) [7]. It was further refined by investigating various factors such as the choice of extraction solvents, ultrasonication duration, cartridge types, as elaborated below. During the optimization process, *E. crassipes* sample (considered as the matrix sample) was spiked with SR mixture of 9 mass-labeled PFCs. These SR compounds exhibit similar chemical characteristics to PFCs but are not naturally found. The recovery efficiency of these labeled compounds was evaluated under different sample processing conditions to serve as a proxy for assessing the efficacy of the sample preparation when analyzing PFCs.

Solvent extraction volume and ultrasonication time

MTBE serves as the solvent for extracting PFC compounds after alkaline digestion. It is notably crucial to optimize both the solvent volume and ultrasonication time to attain maximum sample recovery efficiency. MTBE is incorporated into each of the three extractions with volumes of 5 mL, 10 mL, and 15 mL, then undergoing ultrasonication for 5 minutes, 10 minutes, and 15 minutes, respectively. The comparison of SR recovery efficiency under different extraction conditions aids in selecting the ideal solvent volume and ultrasonication time.

SPE cartridge selection

Once the optimal solvent volume and ultrasonication time for the samples were determined, these settings remained consistent. The subsequent factor requiring optimization is the choice of cartridge during the sample processing stage. Due to the dual hydrophilic and hydrophobic nature of PFC compounds, various types of solid-phase extraction (SPE) cartridges like HLB, C18, and WAX can be employed for their separation. The selection of a suitable cartridge depends on laboratory conditions and the sample recovery efficiency. Notably, C18 and WAX

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cartridges are commonly utilized for cleaning and enriching samples for PFC compound analysis in plants. The C18 column, which are silica-based and non-polar, interacts predominantly with non-polar compounds (fluorine-containing), while the WAX column interacts with polar groups (carboxylate or sulfonate groups). In this study, we examined the effectiveness of two types of cartridges, C18 and WAX, comparing the recovery efficiency of the SR compound using these cartridges during the sample cleanup and enrichment via SPE to determine the most suitable cartridge type.

3 Results and Discussion

3.1 Sample preparation

Solvent extraction volume and ultrasonication time

The study employed MTBE as the extraction solvent subsequent to alkaline digestion. MTBE was introduced in three extraction cycles at varying volumes: 5 mL, 10 mL, and 15 mL, followed by ultrasonication for durations of 5 minutes, 10 minutes, and 15 minutes correspondingly. Table 2 shows the recovery efficiency range of surrogate compounds (mass-labeled PFCs) introduced into the *E. crassipes* (Mart.) Solms sample matrix before processing, at a concentration of 1.25 ng/g. The findings indicate the MTBE's effective extraction capacity for PFC compoundsof over 65%. Analysis from Table 2 reveals that in order to conserve solvent volume within shorter ultrasonication periods, 5 mL of MTBE per extraction was utilized in 10 minutes of ultrasonication, resulting in a recovery efficiency from 84% to 105% for the surrogate compounds.

Table 2 Recoveries of the surrogate for optimization of solvent volume and ultrasonic time

	Recovery ranges of the surrogate (%)		
Extraction time	**Volume of MTBE/each time**		
	5 mL	10 mL	15 mL
5 minutes	65 % - 93 %	87 % - 102 %	89 % - 107 %
10 minutes	84 % - 105 %	88 % - 110 %	93 % - 113 %
15 minutes	86 % - 103 %	92 % - 108 %	95 - 115 %

Cartridge selection

Two solid-phase extraction cartridges that were optimized in this study are C18 and WAX cartridges, utilizing identical solvent activation and elution solvent ratios. Findings indicate that the recovery efficiencies of the surrogate compounds using the WAX cartridge ranged from 85% to 119%, whereas for the C18, despite an acceptable range (>70%), exhibited lower efficiency, varying between 72% and 113%. Consequently, the WAX cartridge was selected for the cleanup and enrichment process during SPE extraction.

Table 3 Average recoveries of surrogates for using WAX and C18 cartridges

No	**Compound**	**Average recoveries of the surrogate (%) (n = 5)**	
		Cartridge C18	**Cartridge WAX**
1	MPFBA	89 ± 6	102 ± 15
2	MPFHxA	93 ± 10	98 ± 8
3	MPFOA	100 ± 8	94 ± 6
4	MPFNA	105 ± 13	119 ± 19
5	MPFDA	96 ± 10	111 ± 4
6	MPFUdA	72 ± 9	85 ± 5
7	MPFDoA	83 ± 12	93 ± 11
8	MPFHxS	76 ± 4	112 ± 9
9	MPFOS	113 ± 11	91 ± 3

3.2 Calibration curve

This research conducted quantitative analysis on 15 PFC compounds, comprising 11 perfluorocarboxylic acids ranging from C4 to C14 and 4 perfluoroalkyl sulfonate salts: C4, C6, C8, and C10. Nine mass-labeled compounds served two roles: as surrogates (added to the sample before processing to control the processing efficiency) and internal standards (used in preparing standard solutions and determining concentrations using the internal standard method). Internal calibration curves with six data points were established for each PFC concentration, ranging from 0.5 ng/mL to 25 ng/mL, and each internal standard had a concentration of 1 ng/mL. The linear correlation coefficients (R2) for all calibration curves were greater than 0.99. Figure 1 illustrates the quantitative calibration curves for PFOA and PFOS compounds.

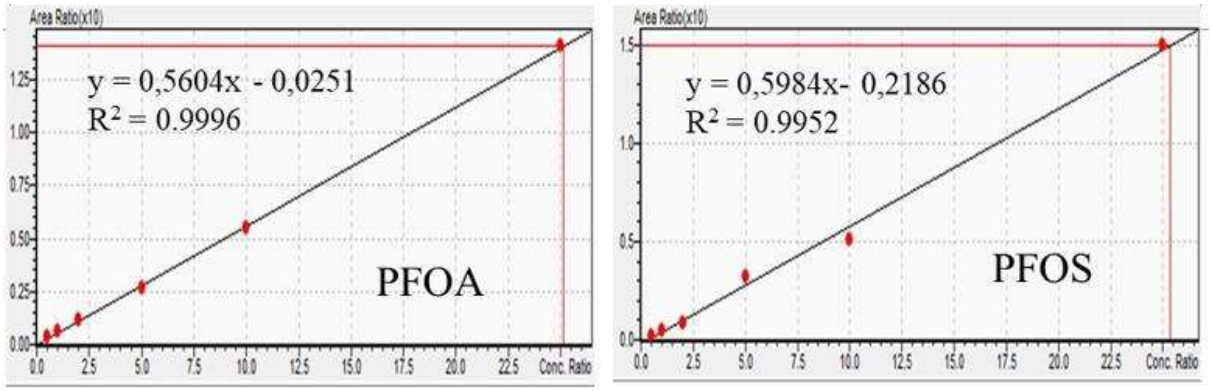

Figure 1 Calibration curves for quantitative analysis of PFOA and PFOS

3.3. Method evaluation

Method Detection Limit and Method Quantification Limit

The Instrument Detection Limit (IDL) and the Instrument Quantification Limit (IQL) for the 15 PFCs were established by performing five repeated injection of the mixture standard solution with PFCs concentration at 0.5 ng/mL and based on the standard deviation (SD) values derived from this injection. The LOD and LOQ values were determined as 3 and 10 times the SD, respectively. The Method Detection Limit (MDL) for each PFC was computed based on the LOD value and the enrichment factor during the sample processing. For the Eichhornia crassipes (Mart.) Solms sample, the MDL values for each PFC ranged from 0.09 ng/g to 0.16 ng/g, and the Method Quantification Limit (MQL) values ranged from 0.30 ng/g to 0.54 ng/g. These findings are similar to those found in the study of Zhang et al. (2019), wherein the MDL values fluctuated between 0.08 ng/g and 0.18 ng/g, and the MQL values ranged from 0.27 ng/g to 0.39 ng/g [7].

Table 4 Limits of detection and limits of quantification of the method for analysis of PFCs in Eichhornia crassipes (Mart.) Solms sample

No.	Compound	SD (ng/mL)	IDL (ng/mL)	MDL (ng/g)	IQL (ng/mL)	MQL (ng/g)
1	PFBA	0.08	0.24	0.12	0.80	0.38
2	PFPeA	0.08	0.24	0.11	0.80	0.37
3	PFHxA	0.09	0.27	0.13	0.90	0.43
4	PFHpA	0.10	0.30	0.14	1.00	0.48
5	PFOA	0.08	0.24	0.11	0.80	0.37
6	PFNA	0.08	0.24	0.11	0.80	0.36
7	PFDA	0.08	0.24	0.11	0.80	0.37
8	PFUdA	0.10	0.30	0.14	1.00	0.46
9	PFDoA	0.10	0.30	0.14	1.00	0.45
10	PFTrDA	0.10	0.30	0.14	1.00	0.48
11	PFTeDA	0.10	0.30	0.14	1.00	0.48
12	PFBS	0.11	0.33	0.15	1.10	0.49
13	PFHxS	0.06	0.18	0.09	0.60	0.30
14	PFOS	0.08	0.24	0.11	0.80	0.36
15	PFDS	0.12	0.36	0.16	1.20	0.54

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Recovery and precision

The evaluation of recovery efficiency in analyzing PFCs in *E. crassipes* samples was performed using the actual matrix of *E. crassipes*. Five samples of *E. crassipes* were spiked with PFC standards at 5 ng/g, and another set of five samples were spiked at 20 ng/g. Processing and analysis followed the outlined procedure for both spiked and background *E. crassipes* samples. Recovery efficiency of the analyzed compounds within the background *E. crassipes* samples was determined based on the contents of PFCs in spiked samples, background samples, and the quantity of spiked standards. The chromatogram of the real sample spiked with standards at concentration of 20 ng/g is shown in Figure 2.

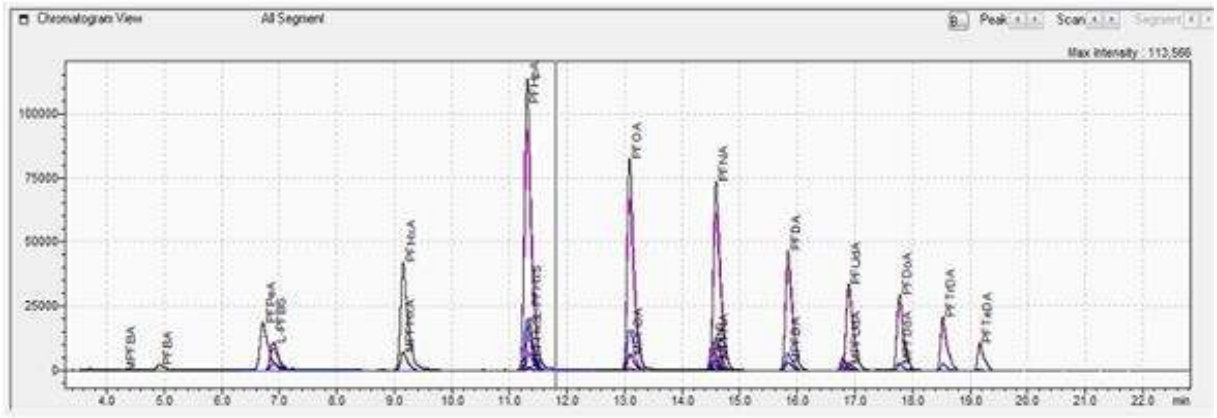

Figure 2 Chromatogram of the real sample spiked with standards at concentration of 20 ng/g

4 Conclusion

The research method was assessed for analyzing 15 PFC compounds—perfluorocarboxylic acids and perfluoroalkyl sulfonate salts—in *E. crassipes* samples, representing a plant species which are capable of absorbing contaminants from water. In the sample handling process, PFCs were extracted, cleanup and concentrated from *E. crassipes* using MTBE solvent assisted by ultrasound, followed by solid-phase extraction via a weak anion exchange column. Subsequently, the quantification of PFCs was conducted using LC-MS/MS. The method evaluation, encompassing recovery efficiency, repeatability concerning actual sample matrix, and detection limits, indicates the method's suitability for assessing PFC presence in *E. crassipes*, enabling research into plant-based solutions for tackling PFC-contaminated water.

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Phân tích nhóm hợp chất peflo hóa trong mẫu bèo tây bằng phương pháp sắc ký lỏng khối phổ hai lần kết hợp với xử lí mẫu bằng chiết dung môi hỗ trợ bởi siêu âm và chiết pha rắn

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Tóm tắt 15 hợp chất peflo hóa thuộc nhóm axit peflocacboxylic và muối pefloankyl sunfonat (chứa 4 đến 12 nguyên tử C trong phân tử) trong mẫu bèo tây đã được phân tích bằng sắc ký lỏng ghép nối khối phổ 2 lần (LC-MS/MS) kết hợp với xử lí mẫu bằng chiết dung môi hỗ trợ bởi siêu âm và chiết pha rắn. Điều kiện chiết dung môi đã được tối ưu hóa là sử dụng 5mL MTBE, siêu âm 10 phút, chiết 3 lần lặp lại. Cột chiết pha rắn WAX đã đư c lựa chọn sau khi khảo sát và so sánh hiệu quả chiết pha rắn sử dụng hai loại cột WAX và C18. Kết quả thẩm định phương pháp đã tối ưu hóa cho thấy, đối với mẫu thực vật, cả 15 PFCs được phân tích cho hiệu suất thu hồi tốt từ 71 tới 116% (1.2-6.6% CV). Giới hạn định lượng đối với 15 PFCs trong bèo tây dao động từ 0,30 đến 0,54 ng/g.

Từ khóa PFCs, LC-MS/MS, MTBE, WAX, bèo tây