Mastering nature: targeted metabolomic approach for the phytochemical authentication of *Ophiopogon japonicus*

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Abstract

Ophiopogon japonicus tubers are used in Traditional Chinese Medicine and are currently traded for the treatment of pathological skin conditions. They are morphologically similar to other species including *Liriope spicata*, making them very difficult to discriminate by botanic and organoleptic analyses. The objective of this work was to develop an analytical approach to identify specific markers of *Ophiopogon japonicus* and *Liriope spicata* to enable the differentiation of species entering the trade. A targeted metabolomic analysis by LC-MS based on literature was performed on referent specimens to determine their specific metabolomic signature. Then, a comparative analytical study of various samples of *Ophiopogon japonicus* tubers in powder form, supplied from different regions and suppliers, was assessed to authenticate the natural raw materials. The analytical characterization of certified samples identified a specific molecular signature for these two similar species. The comparative and targeted metabolomic analysis of 4 powdered raw materials from different regions and suppliers allowed us to rigorously identify those from *Ophiopogon japonicus* and to discard non-conforming samples. This study demonstrated the use of metabolomics to differentiate between tubers from two species that are morphologically similar. This is a prerequisite for ensuring that tubers from the correct species are used in cosmetic and dermo-cosmetic products.

Keywords: Targeted metabolomics; molecular signature; authentication; *Ophiopogon japonicus*

Introduction.

Ophiopogon japonicus (*Asparagaceae*) tubers are used in traditional Chinese medicine (TCM) [1] and for many purposes including cosmetics. However, in the practice and trade of TCM, *Ophiopogon japonicus* tubers may be substituted with other plant species in the *Asparagaceae* family, particularly *Liriope spicata*. The cultivated plants of *Ophiopogon japonicus* and *Liriope* species may be botanically discriminated. However, it is difficult to reliably distinguish *Ophiopogon japonicus* tubers from the tubers of *Liriope species* using morphological characteristics [2] (Figure 1). Therefore, other authentication approaches are needed to differentiate *Ophiopogon japonicus* tubers from *Liriope* tubers, such as chemical analysis or molecular biology methods that use genetic profiling. For the analysis of these plant extracts, the genetic profiling is not suitable to authenticate the raw material because of the importance of the chemical profile. Therefore, for this enquiry, high resolution liquid chromatography-mass spectrometry (LC-MS) was selected as the analytical method to chemically characterize plant material labelled as *Ophiopogon japonicus*.

Figure 1. Picture of samples of *Liriope spicata* and *Ophiopogon japonicus* tubers and powders.

The aim of this study was to identify compounds that could be used as markers to differentiate between *Ophiopogon japonicus* and *Liriope spicata*.

Materials and Methods.

For these investigations, certified references were needed. The *Ophiopogon japonicus* sample was analyzed by the Royal Botanic Garden Kew and certified as belonging to this species. The *Liriope spicata* certified reference was kindly supplied by Mrs Fourasté, from the University of Toulouse.

The raw material powder was solubilized at 100g/L in MeOH 80% and stirred for 24 hours. A paper filtration of the solution was followed by a 0.45µm filtration. An additional 0.2µm filtration was made before vial transfer and injection into the UPLC-MS/MS system (Figure 2). The analytical conditions were as follow: (i) instrument and column: WATERS ACQUITY UPLC I-Class with a HPLC Luna C18 (2), 150 x 3 mm; 3 µm column + precolumn; (ii) mobile phase: (A). Ultrapure water / Acetonitrile (90/10 (v/v)) with 0.1% Formic Acid, (B). Methanol / Acetonitrile (90/10 (v/v)) with 0.1% Formic Acid; (iii) flow rate: 0.4 mL min^{-1}; (iv) detector: Waters G2-XS QTof, ESI source; (v) column temperature: 35°C. Mass detection conditions: WATERS Xevo G2-XS QTof; UNIFI software. Polarity mode: +ESI, sensitivity mode.

Figure 2. Picture of the LC-MS/MS system, a WATERS Xevo G2-XS Qtof coupled to an UPLC system WATERS ACQUITY UPLC I-Class used in this study.

Results.

Firstly, a targeted metabolomic analysis was performed on reference specimens of *Ophiopogon japonicus* and *Liriope spicata* to determine their specific molecular signature. For this purpose, markers detailed in the literature and corresponding to *Ophiopogon japonicus* (Figure 3 C and E) and *Liriope spicata* (Figure 3 D and F) were extracted from LC-MS analysis of the reference standards (Figure 3 A and B).

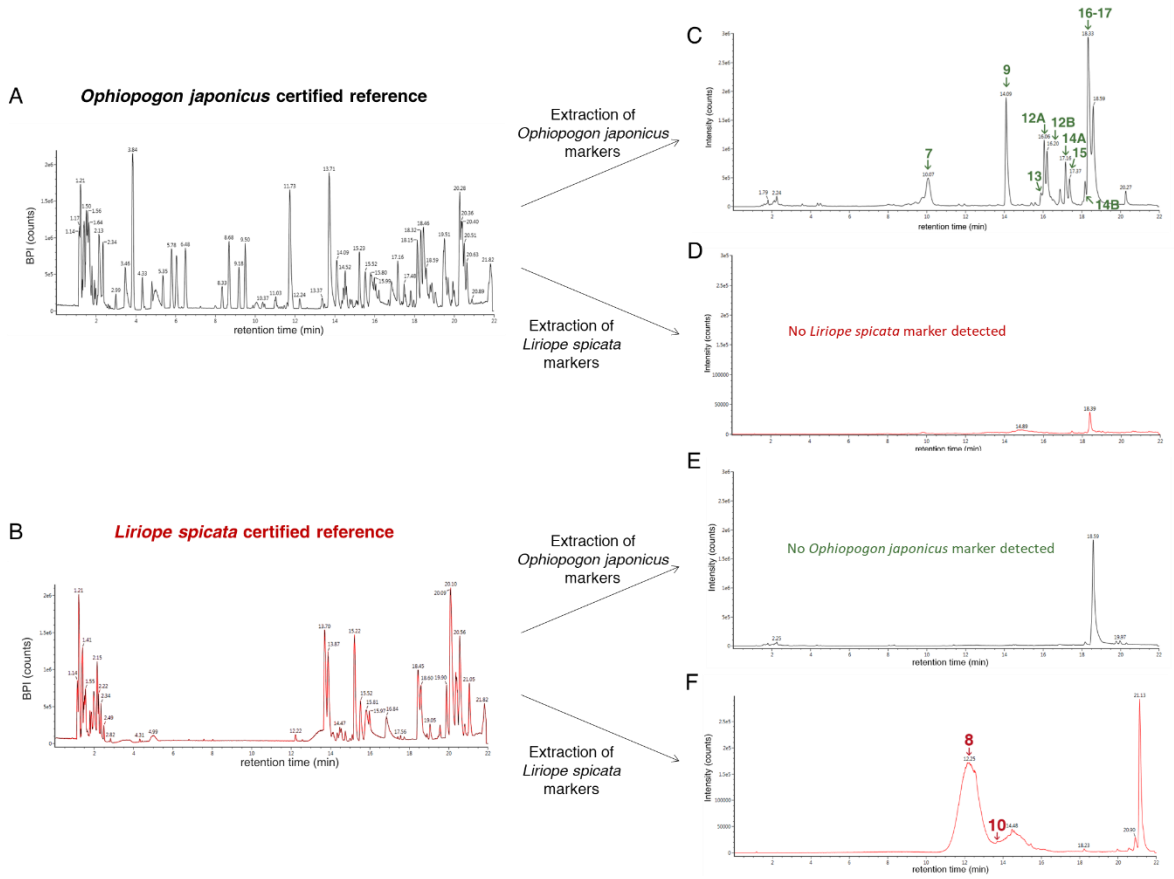

Figure 3. LC-MS chromatograms (positive ESI) of the extracts prepared from the *Ophiopogon japonicus* (A) and *Liriope spicata* (B) certified references. Extraction of *Ophiopogon japonicus* markers (C and E) and *Liriope spicata* (D and F) markers.

Marker	Chemical formula	Assigned coumpound (or isomer)	Retention time (min)	Theoretical Ion [M+H]+	Detected ion / attribution	*Ophiopogon japonicus* certified reference Area	*Liriope spicata* certified reference Area
1	$C_6H_{14}N_4O_2$	Arginine	1.22	175.1195	175.1188 / +H	13 452 602	17 277 774
2	$C_{11}H_{12}N_2O_2$	Tryptophan	2.34	205.0977	205.0965 / +H	431 883	268 973
3	$C_{16}H_{18}O_8$	*p*-Coumaroyl-quinic acid	4.8	339.108	Not detected	Not detected	Not detected
4	$C_{22}H_{35}NO_5$	Alkaloid	4.39	394.2593	394.2584 / +H	Not detected	Not detected
5 A	$C_{22}H_{33}NO_4$	Alkaloid	3.34	376.2488	376.2478 / +H	Not detected	Not detected
5 B		Alkaloid	3.70			Not detected	Not detected
6 A	$C_{23}H_{37}NO_5$	Alkaloid	4.31	408.275	408.2740 / +H	Not detected	Not detected
6 B		Alkaloid	4.70			Not detected	Not detected
7	$C_{51}H_{82}O_{23}$	Ophiopogonin I*	10.05	1063.5325	1063.5313 / +H	6 926 644	Not detected
8	$C_{44}H_{70}O_{17}$	Spirost-5-ene diol tri-glycoside	12.20	871.4691	871.4679 / +H	Not detected	8 920 510
9	$C_{20}H_{22}O_7$	7-Hydroxy-3-(2-hydroxy-4-methoxybenzyl)-5-8-dimethoxy-6-methyl-4-chromanone*	14.08	375.1444	397.1254 / +Na	12 598 135	Not detected
10	$C_{56}H_{90}O_{25}$	Steroidal	13.80	1163.5849	Not detected	Not detected	Traces
11 A	$C_{45}H_{72}O_{17}$	Spirost-5-ene diol (or) Spirost-5-en-3-ol tri-glycoside derivative	13.60	885.4848	885.4833 / +H	Not detected	38 158 269
11 B			15.43		907.4650 / +Na	Not detected	715 827
12 A	$C_{19}H_{20}O_7$	5,7-Dihydroxy-3-(2-hydroxy-4-methobenzyl)-8-methoxy-6-methyl-4-chromanone*	16.04	361.1287	361.1283 / +H	4 371 853	Not detected
12 B		5,7-Dihydroxy-3-(2-hydroxy-4-methobenzyl)-8-methoxy-6-methyl-4-chromanone*	16.18		361.1281 / +H	4 657 081	Not detected
13	$C_{19}H_{18}O_7$	Ophiopogonanone H*	15.88	359.1131	359.1094 / +H	1 729 334	Not detected
14 A	$C_{20}H_{22}O_5$	O^5,8-Dimethyl-ophiopogonanone B*	17.16	343.1545	365.1350 / +Na	10 188 793	Not detected
14 B			18.03		365.1362 / +Na	735 843	Not detected
15	$C_{18}H_{16}O_6$	Ophiopogonanone A*	17.36	329.1025	329.1015 / +H	1 864 654	Not detected
16	$C_{19}H_{20}O_5$	8-Methylophiopogonanone B*	18.31	329.1389	329.1359 / +H	16 204 603	Not detected
17	$C_{44}H_{70}O_{17}$	Spicatoside A	18.38	871.4691	893.4498 / +Na	7 719 809	Not detected
18*	$C_{44}H_{70}O_{16}$	Ophiopogonine D injected reference	20.28	855.4742	877.4558 / +Na	30 862 695	3 793 099

Table 1. LC-MS analysis results summary for *Ophiopogon japonicus* and *Liriope spicata* certified references. Identification of *Liriope spicata* markers (in red) and *Ophiopogon japonicus* markers (in green). *: reported to occur in *Ophiopogon* species [2-4].

Hence, chromatograms obtained from referent samples revealed 10 specific markers of *Ophiopogon japonicus* and 2 specific markers of *Liriope spicata (Table 1)*. In particular, the range of homoisoflavonoids detected in the *Ophiopogon species*, is in accordance with published data that the abundance (diversity and amounts) of homoisoflavonoids in *Liriope* species is lower than in *Ophiopogon japonicus* [2-5].

Then, a comparative analytical study of various samples supplied as *Ophiopogon japonicus* tubers in powder form, was assessed to authenticate the natural raw materials. The analysis of 4 different samples of powdered raw material is presented in Figure 4. Results revealed that sample #1 contained the markers present in *Liriope spicata* but none of the markers of *Ophiopogon japonicus* , revealing that this sample was falsified. The sample #2 contains no *Ophiopogon japonicus* markers and alkaloids not present in the *Asparagaceae* family (*i.e.* the family that both *Ophiopogon japonicus* and *Liriope spicata* belong to), thus also suggesting falsification. The chromatograms obtained for the two other samples (#3 and #4), from different regions and suppliers, displayed the specific markers of authentic *Ophiopogon japonicus*.

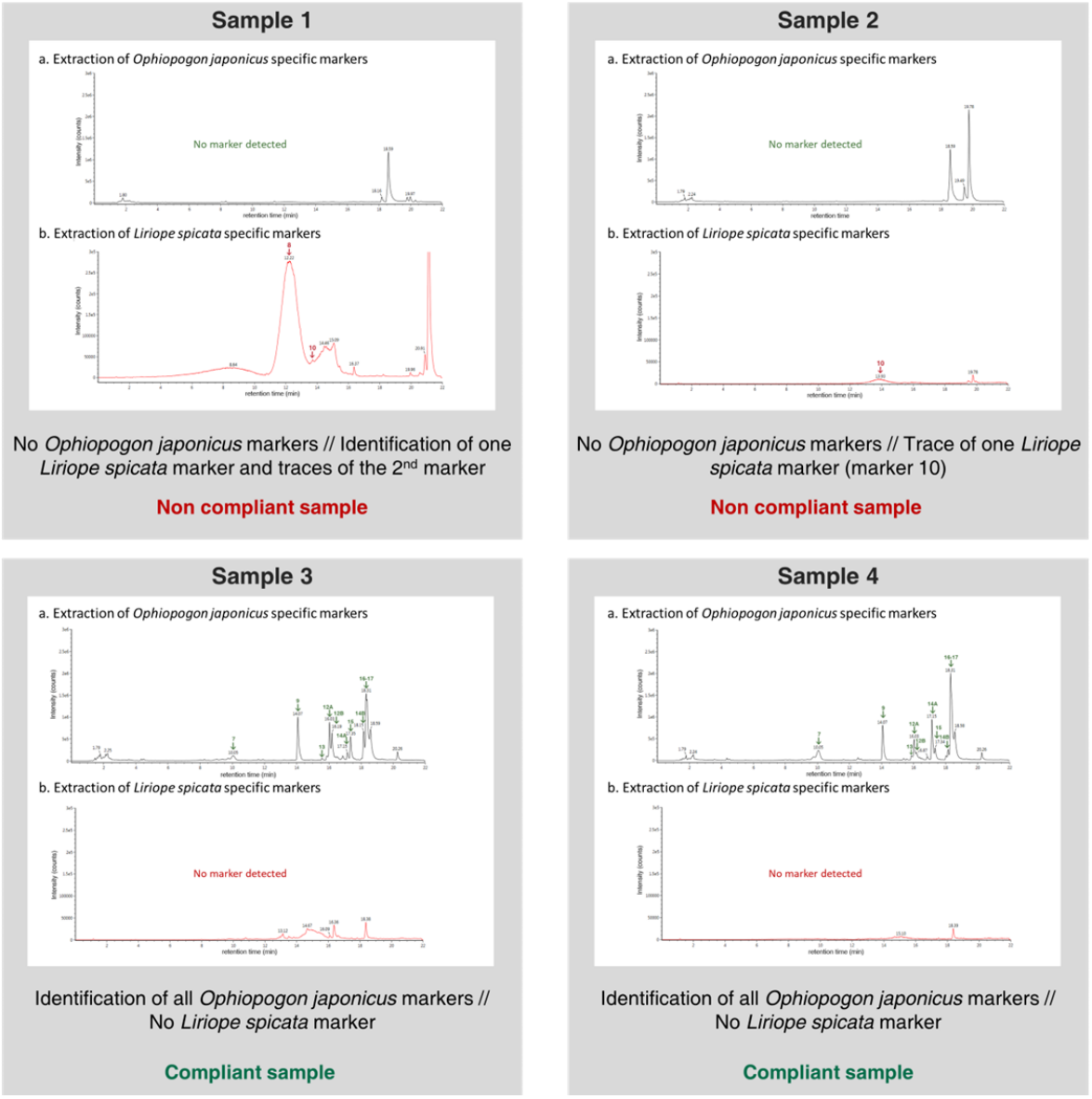

Figure 4. Extracted LC-MS chromatograms of 10 markers of *Ophiopogon Japonicus* (chromatograms a. for each sample) and 2 markers of *Liriope spicata* (chromatograms b. for each sample).

Discussion.

The analytical characterization of certified samples of tubers of *Ophiopogon japonicus* and *Liriope spicata* identified a specific molecular signature for these two species whose tubers are morphologically similar. The comparative and targeted metabolomic analysis of four powdered raw materials from different regions and suppliers allowed us to rigorously identify those from *Ophiopogon japonicus* and to discard non-conforming samples. This approach makes it possible to go beyond the organoleptic, microscopic and macroscopic analyses to discriminate non-differentiable powders. It allows the authentication of raw materials that cannot be discriminated using classical botanically and organoleptically techniques. This reliable methodology will permit a strict quality control of our future supplies of this starting raw material.

Conclusion.

This study demonstrated the use of metabolomics to establish the molecular signature of raw materials that are difficult to discriminate botanically and organoleptically and thus to authenticate them rigorously. This is a prerequisite for the development of cosmetic and dermo-cosmetic products.

Acknowledgments.

We are grateful to Mrs Fourasté for her expertise on *Liriope spicata.*

Conflict of Interest Statement.

NONE.

References.

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