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Application Note

DART-QDa for the Rapid Differentiation of *Curcuma longa* and *Curcuma xanthorrhiza* in Adulterated Spices using LiveID

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Abstract

This application note describes the use of Waters DART QDa System with Live ID to successfully differentiate between seemingly identical samples of turmeric spice based on differing levels of high value antioxidant anti-inflammatory chemoprotective active ingredients in the samples. The application will be of use to food safety scientists interested in food adulteration and authenticity studies, and quality control scientists testing cosmetic formulations.

Benefits

- Real-time identification of *C. longa* and *C. xanthorrhiza* with little sample preparation and no chromatographic separation required
- Possibility to detect food adulteration and differentiate cosmetic ingredients within seconds
- Intuitive software, accessible to the non-expert user, to develop and validate robust models for various authenticity, integrity, and quality control challenges

Introduction

Curcuma longa (*C. longa*) and *Curcuma xanthorrhiza* (*C. xanthorrhiza*) are plants belonging to the ginger family (Zingiberaceae), which are native to tropical South Asia but are widely cultivated in the tropical regions of the world. The dried rhizome of those plants, known as turmeric, is considered a traditional spice, dye and medicine, particularly in Ayurveda or traditional Chinese medicine.[1] Currently turmeric is getting much attention since studies on its constituents have proposed many pharmacological properties (e.g. anti-oxidative, anti-inflammatory or anti-mutagenic properties,)[2] although some of those constituents have recently been declared a PAIN (pan assay interference compound.)[3] Extracts of *C. longa* and *C. xanthorrhiza* are also being increasingly used in cosmetic formulations as natural replacements for petrochemically derived ingredients offering antioxidant, anti-inflammatory, chemoprotective, and cosmetic coloring properties.[4]

The proposed pharmacological effects of turmeric are mainly attributed to so called curcuminoids, more precisely to the substances curcumin, demethoxycurcumin, and bisdemethoxycurcumin. While *C. longa* contains all three of those curcuminoids, *C. xanthorrhiza* lacks bisdemethoxycurcumin, and is therefore considered less valuable, which makes it cheaper on the international market. For this reason, commercially available powder of *C. longa* is often adulterated with *C. xanthorrhiza*, which causes financial damage for customers and food industry. This application note presents a fast and easy opportunity for the detection of such spice adulteration using DART-QDa analysis coupled with LiveID Software.

Experimental

Sample Preparation Extraction Procedure

150 mg of dried and ground sample were weighed into 2 mL reaction tubes and mixed with 1 mL ACN:water 75:25 (v:v). After sonication for 15 min at 25 °C, the samples were centrifuged at 16800 rcf for 3 min. The supernatant was isolated and filtered through H-PTFE filters into 1.5 mL reaction tubes. The extracts were diluted 1:99 (v:v) with extraction solvent. Finally, each extract was spotted onto a QuickStrip card and analyzed using DART QDa.

The mass spectra of authentic samples of *C. longa* (n=10) and *C. xanthorrhiza* (n=10) were used to train a chemometric model. The parameters of the model can be seen in Table 1.

PCA components	10
LDA components	1
Outlier by	5 standard deviations
Binning resolution	1
Mass range	200–400 *m/z*

Table 1. Parameters of the LiveID Authenticity Model.

MS Conditions

MS system:	ACQUITY QDa
MS source:	DART SVP
Ionization gas:	He
Ionization mode:	DART +ve
Gas temperature:	450 °C
Cone voltage:	10 V
Sample speed:	1.00 mm/s
Sampling frequency:	10 Hz
Acquisition mode:	Full Scan
Acquisition range:	100–600 *m/z*

Data Management

Chromatography Software

MS software:	MassLynx
Informatics:	Live ID sample recognition software

Results and Discussion

DART-QDa Authenticity Model

The combined mass spectra were clustered in two groups using a Principle Component Analysis (PCA) combined with a Linear Discriminant Analysis (LDA). The resulting clusters can be seen in Figure 1.

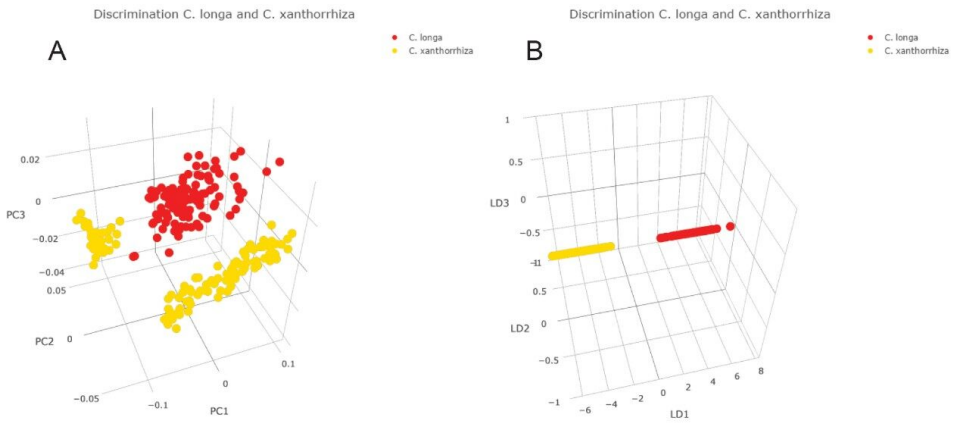

Figure 1A. PCA and 1B. PCA/LDA plot generated in LiveID for the DART-QDa authenticity model.

Loading plots (Figures 2 and 3) show the significant ions contributing to the differentiation of classes. The ion at 369 *m/z* (curcumin) seems to be the major feature in PC1, accountable for approximately 70% of the variance. The ions at 217 (ar-turmerone) and 235 *m/z* (procurcumenol, isoprocurcumenol, curcumenol, curcumenone) are the main features in PC2 and responsible for approximately 16% of the variance. The corresponding structures are annotated in the spectra shown in Figure 4.

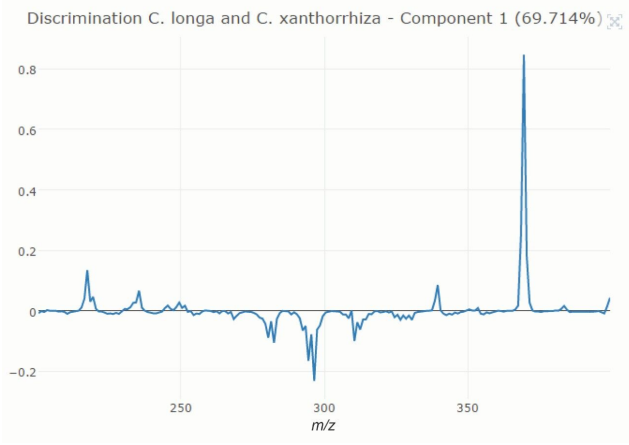

Figure 2. Loading plot for PC1 created in LiveID.

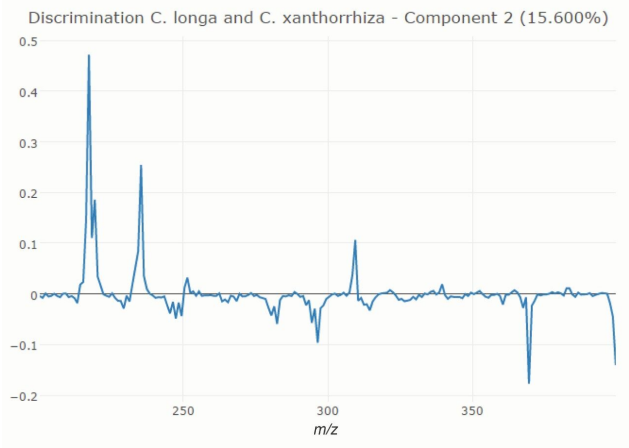

Figure 3. Loading plot for PC2 created in LiveID.

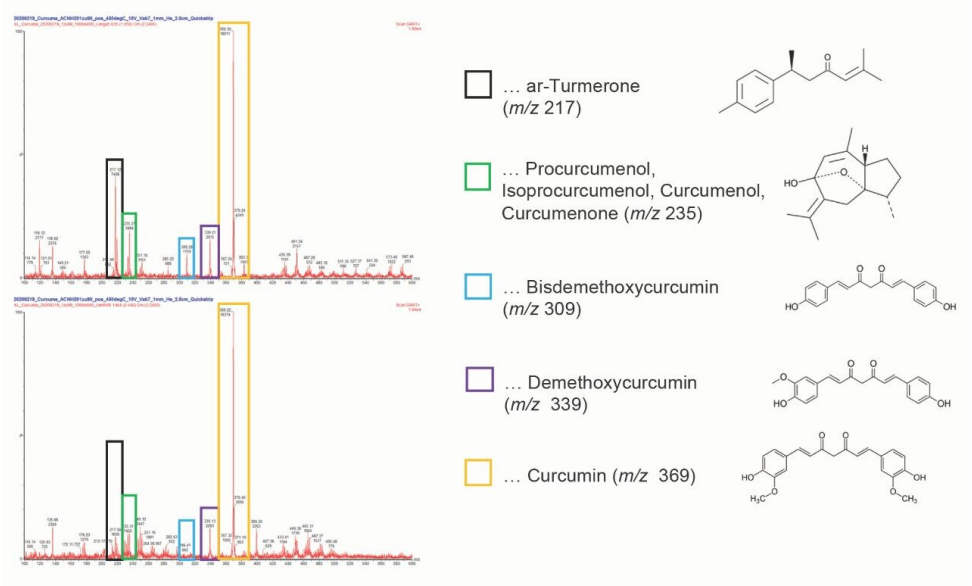

Figure 4. Mass Spectra of C. longa (top) and C. xanthorrhiza (bottom) with differing components highlighted.

Model Validation

The created authenticity model was cross-validated using the "leave 1 file out" option provided by LiveID. The associated validation report can be seen in Figure 5. Out of 240 spectra, obtained from two different turmeric species, all could be associated with the correct species. This results in a correctness score of 100%. Additionally, the model was validated using the "leave 20% out" option provided by LiveID, which also resulted in a correctness score of 100%.

Validation report

Download as .csv

Description	: 10 PC, Outlier 5 SD, Binning 1, One Out
Created	: 4/2/2020, 5:05:38 PM

Model details

Name	: Discrimination C. longa and C. xanthorrhiza
Type	: PCA-LDA
Outlier threshold	: 5 (standard deviations)
PCA components	: 10
LDA discriminants	: 1
Mass range	: 200 - 400 (*m/z*)
Bin size	: 1 (*m/z*)

Validation parameters

Validation type	: Leave 1 file out

Results summary

	Spectra	Passes	Failures	Outliers	Correctness score
Total	240	240	0	0	100.00%

Confusion matrix

	C. longa	C. xanthorrhiza	Outlier	Total
C. longa	120	0	0	120
C. xanthorrhiza	0	120	0	120

Figure 5. Cross validation report for the DART-QDa authenticity model.

To further test the robustness of the model, two samples of turmeric powder were purchased from local supermarkets and extracted using the described method. The extracts were analyzed using the created LiveID authenticity model. Both samples could be recognized as *C. longa* and *C. xanthorrhiza*, respectively, with a correctness score of 100% (Figure 6).

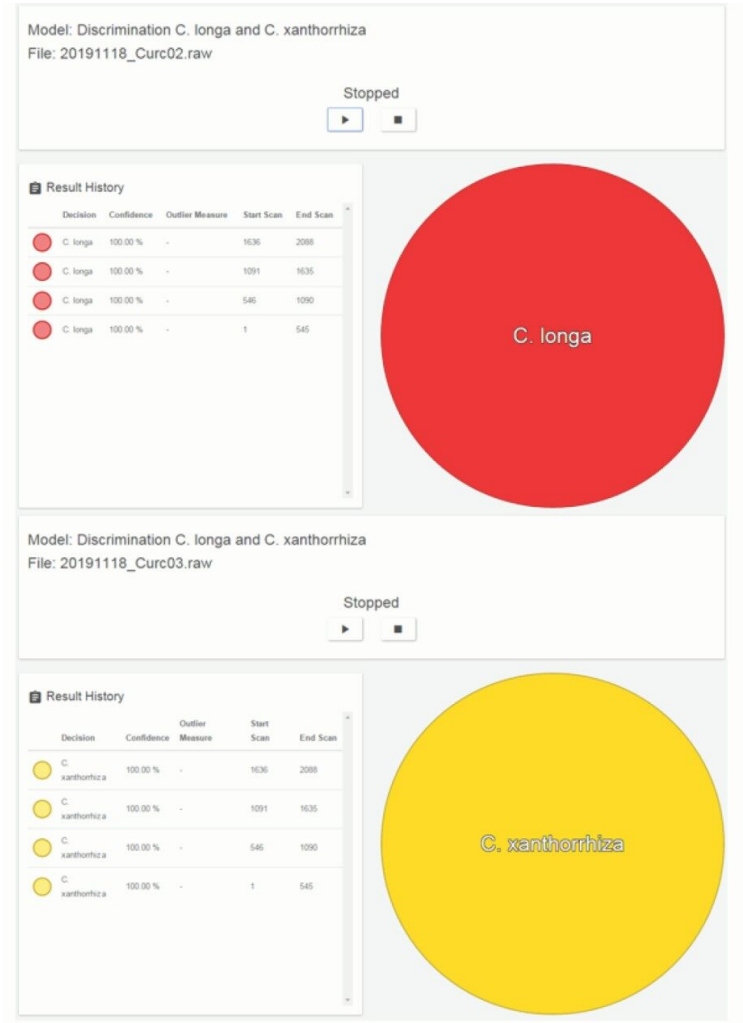

Figure 6. Recognition results for unknown samples of turmeric.

Conclusion

- DART QDa System with LiveID is an appropriate technique for the rapid discrimination of related botanical species
- DART QDa detected the key ions present in extracts of two turmeric species
- DART QDa represents a time saving alternative to LC-MS in the discrimination of botanical species
- It can be expected that the model can be updated to differ mixtures of *C. longa* and *C. xanthorrhiza*

References

1. Li S.; Chemical Composition and Product Quality Control of Turmeric (*Curcuma longa L.*). *TOPHARMCJ*. 2011, 5(1): 28–54.
2. Jayaprakasha G.K.; Jagan M.R.; Sakariah K.K. Improved HPLC Method for the Determination of Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin. *J Agric Food Chem*. 2002, 50(13): 3668–72.
3. Baell J; Walters M.A. Chemistry: Chemical Con Artists Foil Drug Discovery. *Nature*. 2014 , 513(7519):481–3.
4. Mieloch M.; Witulska M. Evaluation of Skin Colouring Properties of *Curcuma Longa* Extract. *Indian J. Pharm Sci.* 2014, 76 (4): 374–378.

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Featured Products

ACQUITY QDa Mass Detector

DART QDa System with LiveID

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