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Application Note

Using Natural Products Application Solution with UNIFI for the Identification of Chemical Ingredients of Green Tea Extract

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Abstract

This application note describes how to analyze and identify the chemical ingredients of green tea using the Natural Products Application Solution with UNIFI, which combines UltraPerformance LC (UPLC), orthogonal quadrupole time-of-flight mass spectrometry (QTof MS), and a Traditional Medicine Library.

Benefits

The integration of data acquisition and processing with the Traditional Medicine Library in this solution provides a simple, efficient process to effectively facilitate the identification of chemical ingredients from complex natural product samples. As a result, it greatly improves productivity and reduced the demands for operator's technical expertise level.

Introduction

The practice of using natural products as Traditional Medicines for health benefits and for therapeutic effects is common in many countries worldwide. The efficacy of Traditional Medicines has been affirmed by thousands of years of history as well as by modern clinical practice. However, many challenges still remain for research and development in this area; for example, to reveal the material base and the mechanism of efficacy. The core of these challenges is to gain understanding on all of the chemical ingredients from a Traditional Medicines.

All research for natural products starts from ingredient analysis. However, classical methods used for ingredient analysis are complicated, time-consuming, and inefficient. To summarize, they typically follow one of these approaches:

1. Purchase of standards and comparing them with the components from samples. The cost of this approach is very high and not all compounds have relevant standards available.

2. Using various separation and preparation methods to purify the components. The problem of this approach is its blindness; it is also very time-consuming and prone to a lot of repeated work.

3. Searching for answers from literature. However, the data from previous literature could be dated, and may have been acquired by low-resolution instruments, which will lead to many false positives.

Regardless of the approach, an additional requirement is that the analyst must have a very strong technical background, both in terms of chemistry and natural product knowledge.

The inefficiency in the material base research has always been the bottleneck that limits the modernization of Traditional Medicines. In recent years, the rising popularity of liquid chromatography coupled with mass spectrometry (LC-MS) has helped to improve this situation; yet, no significant breakthrough has been made.

Currently, the mostly commonly used LC-MS solution still includes manually observing the chromatographic peaks one by one, searching possible structural information from various Internet libraries, and checking literature to match the identities of fragments and to rationalize fragmentation pathways for the purpose of determining the chemical structure of the target component. The limitations of such an approach are still the same: time-consuming, inefficient, and high expertise requirement for the operators.

In this application note, the new Waters Natural Products Application Solution with UNIFI is introduced using the analysis of the green tea extract as an example. This solution combines the ACQUITY UPLC I-Class System, the Xevo G2-S QTof MS, and a Traditional Medicine Library that is integrated within the UNIFI Scientific Information System. The application-based solution unites data acquisition and processing within a streamlined workflow that incorporates the Traditional Medicine Library to provide a simple and efficient process for the identification of the chemical ingredients from complex natural product samples. As a result, productivity can be greatly improved and the demand for operator expertise is significantly reduced. The workflow of the ingredient analysis using the Natural Product Application Solution with UNIFI is outlined in Figure 1.

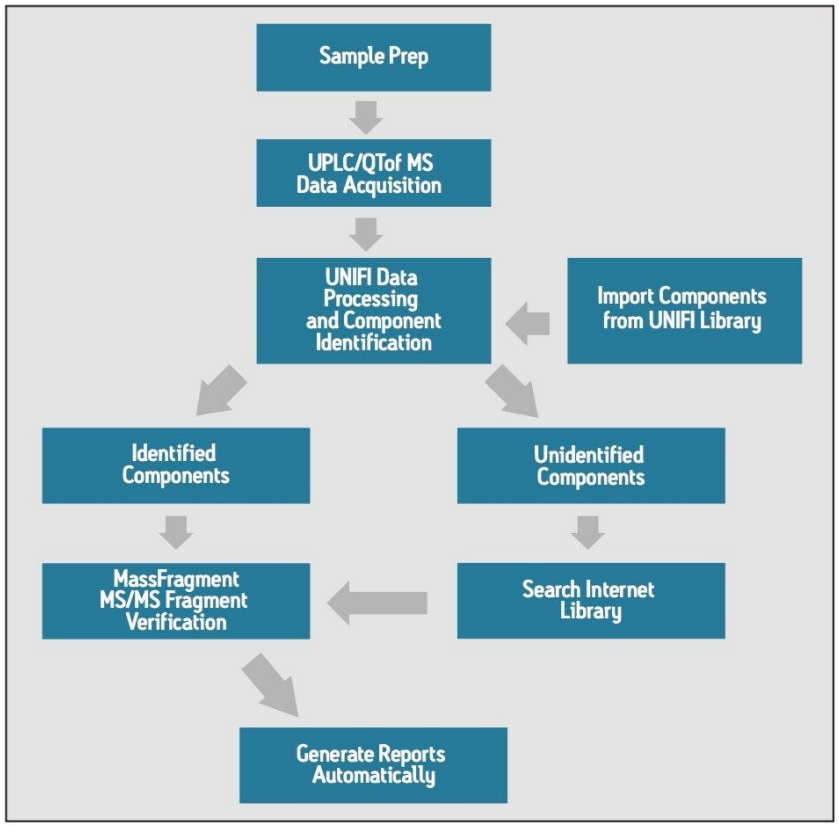


Figure 1. The workflow of ingredient analysis using the Natural Product Application Solution with UNIFI.

Green tea is a natural product drink favored by many. Because it is not fermented, many ingredients found from fresh leaves still remain in their original forms; for example, polyphenols, catechins, pyrocatechins, caffeine, amino acids, vitamins, etc. This provides a good foundation for chemical ingredient analysis, hence it allows us to use green tea as an example to demonstrate use of the Natural Product Application Solution with UNIFI to verify the analytical results with the available standard substances. The entire analytical process, from sample injection to report generation, required merely two hours to complete.

Experimental

Sample preparation

The powder (33 mg) of the green tea extract (Waters, No. 186006962) was dissolved in 2 mL MeOH/H_2O 1/3

solution and then diluted 2X to 8.25 mg/mL final concentration for later use. The catechin standard mixture (Cerriliant No. G-016) was diluted 2X with methanol to 50 µg/mL as final concentration. Injection volume was 1 µL.

LC conditions

LC system:	ACQUITY UPLC I-Class with FTN Sample Manager
Column:	ACQUITY UPLC HSS T3 2.1 x 100 mm, 1.8 µm
Column temp.:	40 °C
Sample temp.:	15 °C
Mobile phase:	A: water (0.1% formic acid); B: acetonitrile

Gradient:

Time	Flow Rate(mL/min)	Solvent A(%)	Solvent B(%)	Curves
0	0.6	99	1	Starting
0.5	0.6	99	1	6
16	0.6	65	35	6
18	0.6	0	100	1
20	0.6	99	1	1

MS conditions

MS system:	Xevo G2-S QTof MS
Acquisition range:	100-1500 Da
Scan time:	0.1 s
Acquisition mode:	ESI+, ESI-; resolution mode; MS^E
Lock mass:	Leucine Enkephalin (LE) 1 ppm (scan for 0.3 s, interval:15 s)
Capillary voltage:	3 KV (ESI+)/2.5 KV (ESI-)
Cone voltage:	100 V
Collision energy:	low CE: 6 eV; high CE: 15-40 eV
Source temp.:	120 °C
Desolvation temp.:	500 °C
Cone gas flow:	30 L/h
Desolvation gas flow:	1000 L/h
Acquisition time:	20 min

Results and Discussion

UPLC and QTof MS with MS^E were used to analyze the chemical ingredients in green tea extract. The Natural Products Application Solution with UNIFI featuring the Traditional Medicine Library was used to process the data. 28 components were initially identified with 16 being confirmed upon verification by MassFragment. The complete analysis took two hours from sample injection to report generation.

The Natural Product Application Solution with UNIFI includes the ACQUITY UPLC I-Class, Xevo G2-S QTof MS, and the UNIFI Scientific Information System containing the Traditional Medicine Library, along with a Waters Green Tea Extract and the Catechin Standard Mixture. This integrated solution also includes 14 preset component and binary analysis workflow templates, and three report templates, which result in a fully automated procedure from data acquisition and processing, to library search and structure verification, to report generation.

Figure 2 shows the UPLC-QTof MS base peak ion (BPI) chromatogram of the green tea extract. The advantages of using UPLC for the analysis of complex samples are fully demonstrated here. Not only is the run time shortened (effective separation time of 15 min), but also it enhanced the separation efficiency and peak capacity. In the meantime, MS information with accurate mass measurements was also provided by the QTof MS. Additionally, with the MS^E data acquisition strategy, chemists can obtain the molecular weight information of the compounds (from low-collision-energy MS scan) and the respective fragmentation ion information (from high-collision-energy MS scan) from a single injection. All provides a solid foundation for in-depth ingredient analysis and structure identification in future steps.

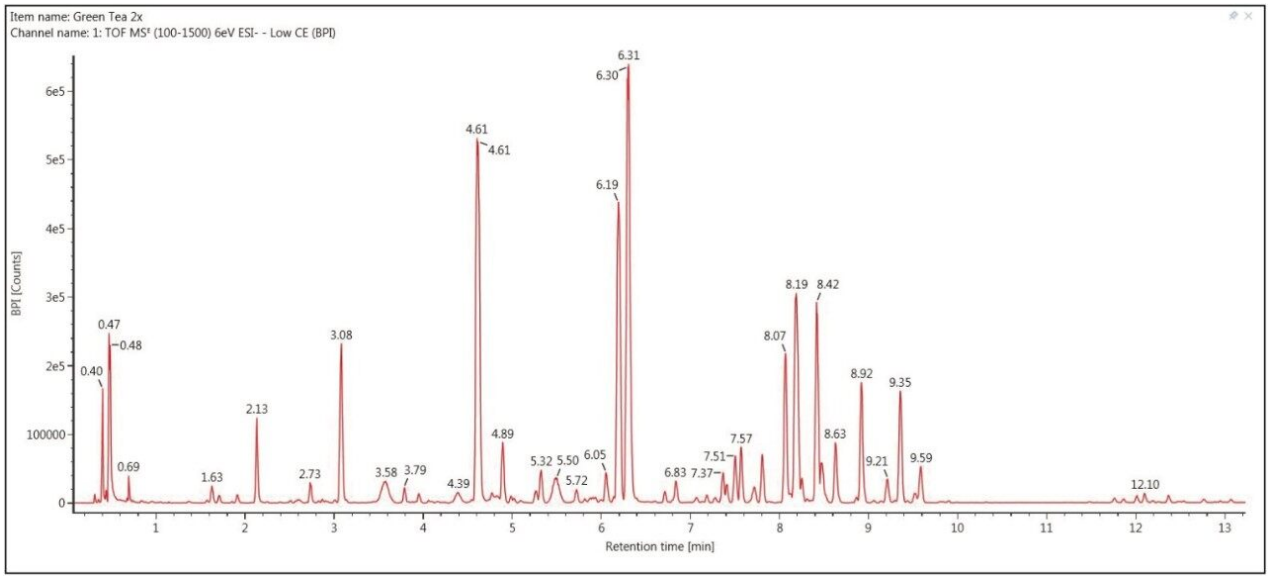


Figure 2. The UPLC-QTof MS base peak ion (BPI) chromatogram of green tea extract.

Another critical matter that contributes to bottlenecks in natural products research is that, although there are a large number of libraries and databases available on the Internet, there is no commercially available library that has been systematically incorporated with instrumental analysis for the purpose of facilitating natural product ingredient analysis research.

For the first time, the Waters Natural Product Application Solution with UNIFI has filled this gap. The new Traditional Medicine library follows the 2010 edition of Chinese Pharmacopoeia and lists all the herbs included in this pharmacopoeia. Figure 3 shows the basic infrastructure of this library and the information it contains. This includes compound name (both in Mandarin and English), chemical structure, molecular formula, average molecular mass and mono-isotopic molecular mass of each compound in accurate mass, as well as the plant origins. For each listed herb, the library provides its plant name (including Mandarin, Mandarin Pinyin Phonetic, and Latin) and the major compounds reported from literatures. In addition, the library also labels each listed compound according to chemical classes, which provides a foundation for fragment analysis.

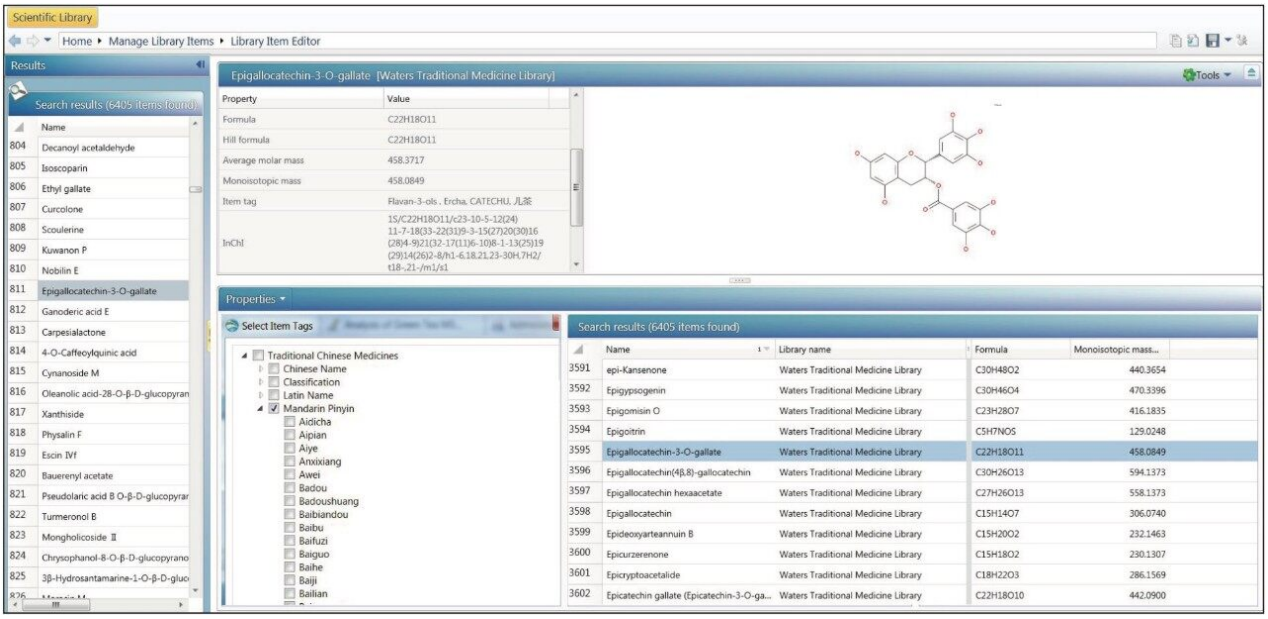


Figure 3. The basic infrastructure of the Traditional Medicine Library.

The Natural Products Application Solution with UNIFI enables the automatic incorporation of the Traditional Medicine Library into the component and binary analysis workflows. Figure 4 shows an example of sample analysis by utilizing the UNIFI Component Analysis Workflow.

Figure 4A shows the complete list of these 14 preset analysis workflow templates. By clicking on any item in this list, the corresponding results are shown in windows (4B, 4C, and 4D) on the right side of the Figure 4. For example, if the “Component – Good Match” is clicked, the information of all identified components with response values greater than 2000 counts and exact mass error less than 5 mDa will be displayed.

Figure 4B shows the component table that lists all components that found a match from the Traditional

Medicine Library, and they fit the criteria described above. Each listed chemical component contains the following information: compound name, molecular formula, exact mass mono-isotopic molecular weight, response intensity, retention time, exact mass error in mDa, ionization mode, adduct ions and identification status. Figure 4C is the corresponding chromatogram for the component being clicked from the table, (caffeine, in this case). Figure 4D is the MS^E spectra for the component shown in Figure 4C, including the low-energy full scan spectrum and the high-energy fragmentation spectrum.

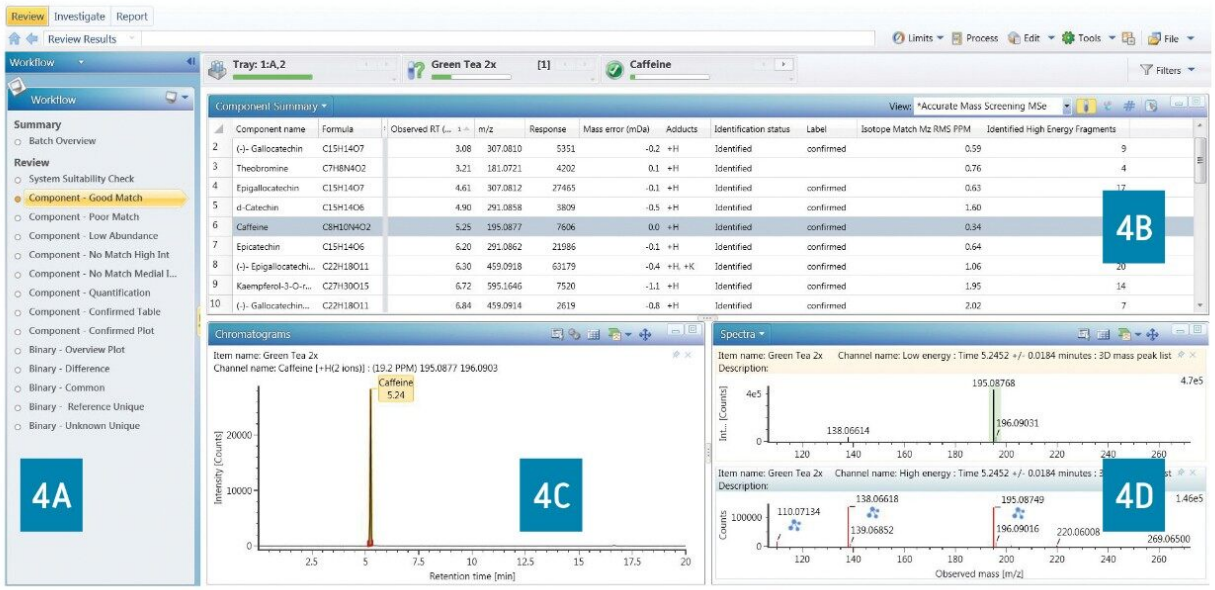


Figure 4. The list of identified components from green tea after processing by UNIFI.

All ingredient analysis solutions offered before UNIFI Software required researchers to manually extract each individual chromatographic peak, check its corresponding mass spectrum, and deduce the possible molecular formula based on the exact mass. Afterwards, researchers need to search online libraries based on molecular formula, deduce fragmentation pathways based on the fragment ions, then finally determine the chemical structure of the target component. Now with the Natural Products Application Solution with UNIFI, after all relevant components are imported from the Traditional Medicine Library, data processing and library search are performed automatically as a single step. The identification result is presented directly (Figure 5), while the rationality of the structure is automatically verified based on fragment ions via MassFragment.

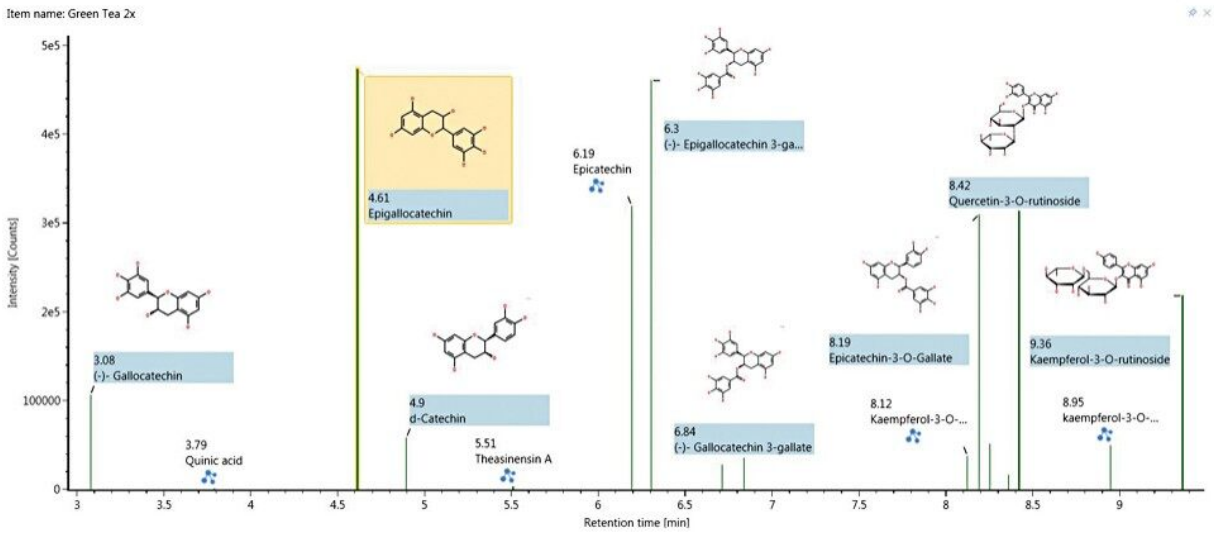
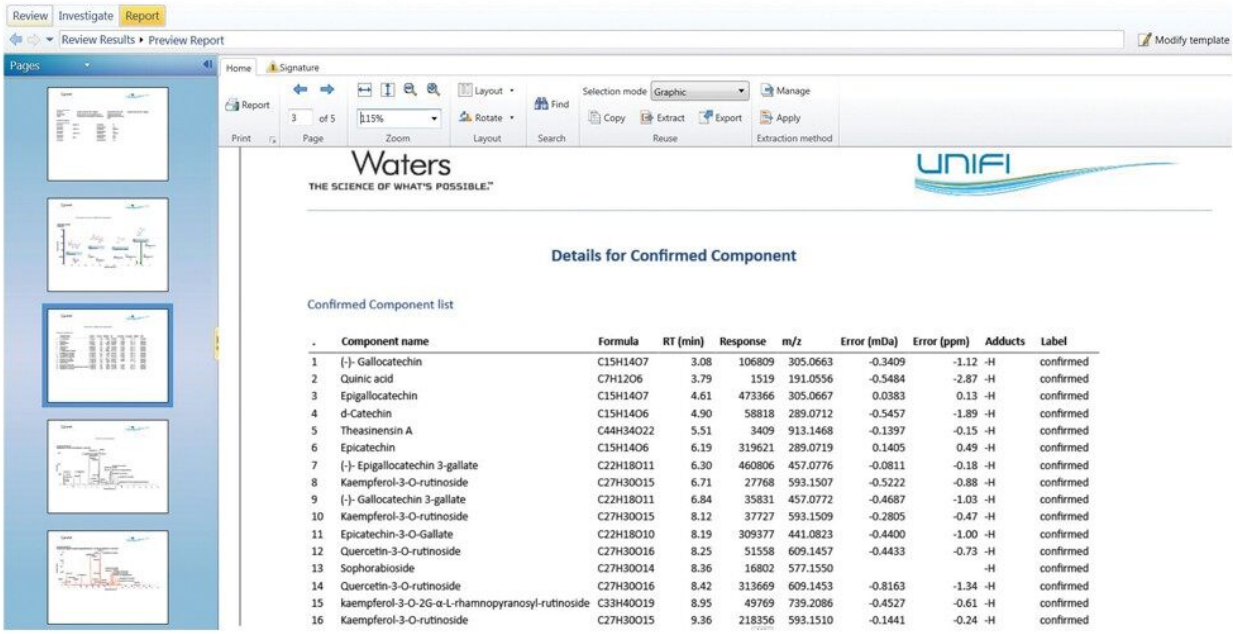


Figure 5. Summary Plot of the identified components of green tea extract after processing by UNIFI

As shown in Figure 4D, each fragment ion that corresponds to the compound precursor ion can be examined by simply clicking on the blue icon . Researchers only need to determine if the verified fragment structure is sensible. If yes, then it can be potentially concluded that the proposed compound structure is correct, and this component can be defined as confirmed. If a false positive is suspected, further identification and structural elucidation can be carried out by using the UNIFI Structural Elucidation Tool to search more online libraries and again utilizing MassFragment. By the same token, all unidentified components can be investigated in this manner. Final results of all confirmed components can be shown as a table by clicking the “Component – Confirmed Table” (Figure 4A), or as a plot by clicking “Component - Confirmed Plot” (Figure 5), or as a list that can be generated using a report template (Figure 6).

The Natural Products Application Solution with UNIFI contains three report templates. The report templates related to ingredient analysis are: NP Component Summary Template and NP Component Details Template. For example, a summary report of the status of the components identification can be easily obtained by importing the NP Component Summary Template (Figure 6). This report contains sample information, data acquisition and processing methods, as well as related data information.



.	Component name	Formula	RT (min)	Response	m/z	Error (mDa)	Error (ppm)	Adducts	Label
1	(-)- Gallocatechin	C15H14O7	3.08	106809	305.0663	-0.3409	-1.12	-H	confirmed
2	Quinic acid	C7H12O6	3.79	1519	191.0556	-0.5484	-2.87	-H	confirmed
3	Epigallocatechin	C15H14O7	4.61	473366	305.0667	0.0383	0.13	-H	confirmed
4	d-Catechin	C15H14O6	4.90	58818	289.0712	-0.5457	-1.89	-H	confirmed
5	Theasinensin A	C44H34O22	5.51	3409	913.1468	-0.1397	-0.15	-H	confirmed
6	Epicatechin	C15H14O6	6.19	319621	289.0719	0.1405	0.49	-H	confirmed
7	(-)- Epigallocatechin 3-gallate	C22H18O11	6.30	460806	457.0776	-0.0811	-0.18	-H	confirmed
8	Kaempferol-3-O-rutinoside	C27H30O15	6.71	27768	593.1507	-0.5222	-0.88	-H	confirmed
9	(-)- Gallocatechin 3-gallate	C22H18O11	6.84	35831	457.0772	-0.4687	-1.03	-H	confirmed
10	Kaempferol-3-O-rutinoside	C27H30O15	8.12	37727	593.1509	-0.2805	-0.47	-H	confirmed
11	Epicatechin-3-O-Gallate	C22H18O10	8.19	309377	441.0823	-0.4400	-1.00	-H	confirmed
12	Quercetin-3-O-rutinoside	C27H30O16	8.25	51558	609.1457	-0.4433	-0.73	-H	confirmed
13	Sophorabioside	C27H30O14	8.36	16802	577.1550			-H	confirmed
14	Quercetin-3-O-rutinoside	C27H30O16	8.42	313669	609.1453	-0.8163	-1.34	-H	confirmed
15	kaempferol-3-O-2G-α-L-rhamnopyranosyl-rutinoside	C33H40O19	8.95	49769	739.2086	-0.4527	-0.61	-H	confirmed
16	Kaempferol-3-O-rutinoside	C27H30O15	9.36	218356	593.1510	-0.1441	-0.24	-H	confirmed

Figure 6. Summary report for the status of component identification, which is easily obtained by importing the NP Component Summary Template.

Conclusion

Green tea ingredient analysis has been used as an example to introduce the Natural Products Application Solution with UNIFI. UPLC overcomes the shortcomings of traditional HPLC separations, such as long separation time, limited resolution, and low peak capacity. QTof MS provides molecular weight and fragmentation information in exact mass, and sufficient dynamic range, laying a solid foundation for natural product component identification and quantification.

The Natural Products Application Solution with UNIFI is accompanied by the Traditional Medicine Library, with the ability to automatically identify component structures. It is a new solution for ingredient analysis of complex natural product samples. The UNIFI informatics platform enables this entire process to be completed all at once, from injecting sample to processing data to printing report. The entire green tea ingredient analysis was completed in only two hours. This solution also contains preset workflow templates and various report templates, provide further time saving for method re-editing.

In effect, we provide a simple and efficient process for the identification of chemical ingredients in complex natural product samples. Productivity is greatly improved and demands for an operator's technical expertise is greatly reduced, so that these analyses can be performed much more routinely, and experienced operators can now apply their knowledge to more advanced laboratory challenges. As a result, this provides a breakthrough for alleviating the bottleneck of natural product research.

Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Natural Products Application Solution with UNIFI <https://www.waters.com/134777097>

UNIFI Scientific Information System <https://www.waters.com/134801359>

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