Waters™

Nota applicativa

Exact Mass Measurement of Active Components of Traditional Herbal Medicines by Orthogonal Acceleration Time-of-Flight

Michael McCullagh, Tabisam Khan

Waters Corporation

Abstract

The aim of the study presented is to determine the presence of target Gingkolides in Gingko biloba leaf extract. The data presented shows Gingkolides determined to be present in the extract analyzed. Using negative ion mode electrospray very good mass accuracy is achieved routinely with real time centroid data acquisition and lockmass correction. Acquisition of UV data in parallel is performed. The system used was comprised of a Waters Alliance HT 2795 Separations Module, 2996 PDA Detector, Symmetry C_{18} Column and Waters Micromass LCT Premier oa-Tof Mass Spectrometer.

Introduction

Medical treatment has long been connected with the natural products and actives extracted from plants. This has led to many advances in medicines and has long been the pivotal force in fighting ailments. It has been known and appreciated for many years that certain plants can exhibit healing effects when prepared as part of a mixture, but when taken in isolation can cause detrimental effects. This knowledge is now being used more and more in sophisticated biological and bioanalytical studies of natural products. Despite advances both in utilizing synthetic approaches to drug design, and in sophisticated structure-activity studies, there is still a great need for compounds with a unique mechanism of action. Major breakthroughs have resulted primarily from the study of natural products. Some of the most important drugs have been isolated from plant sources; for example most antibiotics and anticancer drugs. The maidenhair tree, Ginkgo biloba, is an ancient Chinese plant that has been cultivated for its health promoting properties. Ginkgolides, the main active ingredients of Ginkgo biloba, have not only helped to explain the pharmacological basis of several traditional medicines, but have also provided a valuable new class of therapeutic agents. Research on the biochemical effects of Ginkgo biloba extracts is still at a very early stage. Although the terpene fraction of Ginkgo biloba, which contains the ginkgolides, may contribute to the neuroprotective properties of the Ginkgo biloba leaf, it is also likely that the flavonoid fraction, containing free radical scavengers, is important in this respect. The structures of the gingkolides are shown in Figure 4.

The LCT Premier is a newly developed benchtop oa-Tof (orthogonal acceleration time of flight) mass spectrometer. New hardware and software control technology has been incorporated to meet the increased analytical demands in the pharmaceutical, environmental and clinical applications arenas. The high duty cycle of Tof is utilized for qualitative studies, generating full spectra at high mass accuracy (<3 ppm RMS).

The highly specific data generated provides an extra degree of information that aids interpretation of the data. Using real time exact mass centroid data acquisition with the negative ion electrospray, the extract of Gingko biloba leaf has been analyzed using the new LCT Premier oa-Tof. The LCT Premier oa-Tof is presented in Figure 1 and the schematic of the LCT Premier with the analyzer in W geometry is illustrated in Figure 2.

Figure 1. LCT Premier incorporating integral LockSpray.

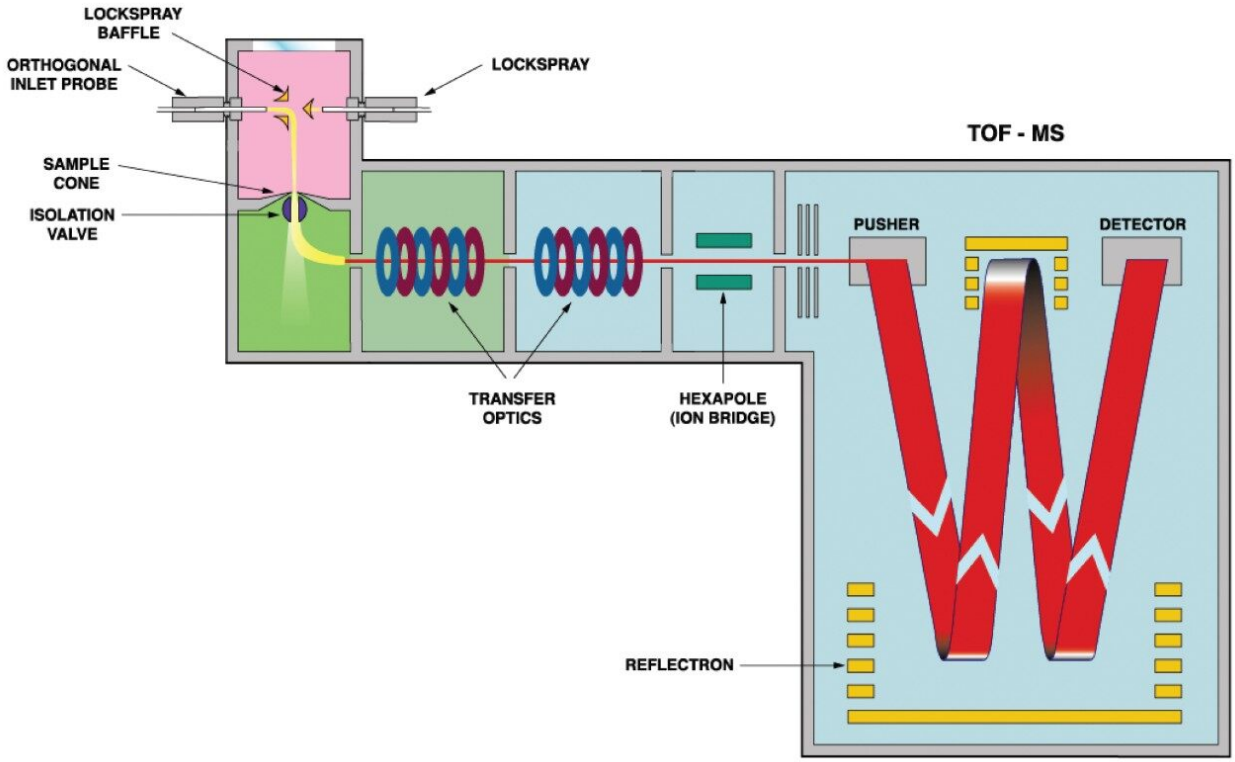

Figure 2. oa-Tof schematic (W mode > 10000 FWHM).

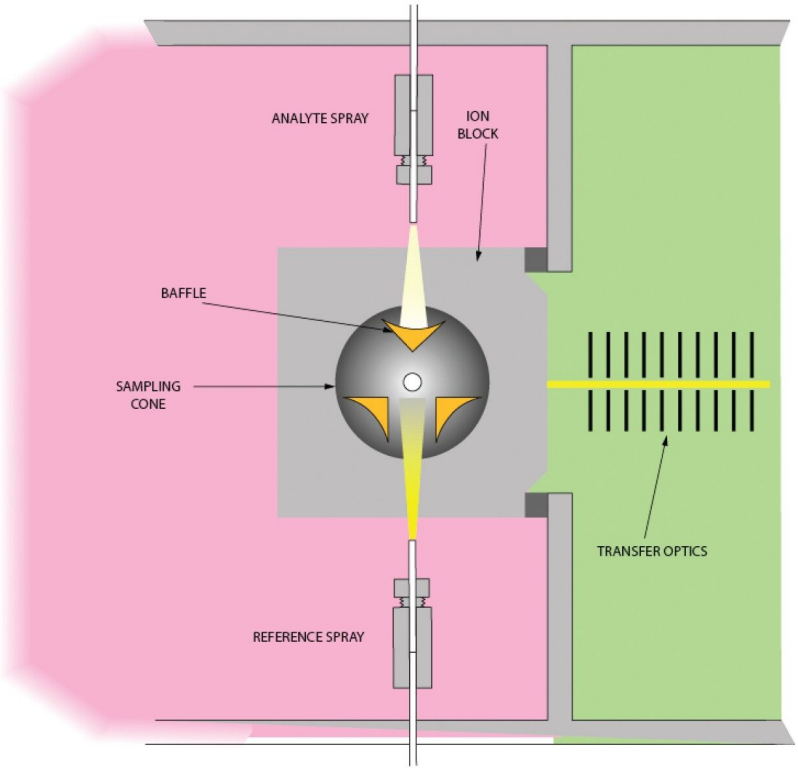

Figure 3. Schematic of the LCT Premier LockSpray source.

Experimental

HPLC

HPLC:	Waters Alliance HT 2795 Separations Module
Column:	Waters Symmetry C_{18} (250 mm x 4.6 mm, 5 µm) with Guard Column (20 mm x 3.9 mm, 5 µm)
Column temperature:	35 °C
Flow:	1 mL/min - split 1:4

Mobile phase:	A: H_20 (0.2% HCOOH), B: MeCN
Gradient:	0–10 min: 15% B; 10–40 min: 15-30% B; 40–50 min: 30–15% B

MS

Mass spectrometer:	Micromass LCT Premier oa-Tof
Ionization mode:	ESI Voltage -ve = 2.5kV
Sample cone voltage:	100V
Reference mass:	Leucine enkephalin, [M-H]- =554.2615
Acquisition parameters:	100–800 Da 1spectrum/second 10500 FWHM 0.1 second inter scan delay

Extraction Procedure

The extraction procedure used by the China Pharmaceutical Company is described as follows;

- 50 g Ginkgo Biloba leaf powder was refluxed with 200 mL 60% acetone for 1hr, then filtered.
- Extraction solvent was recovered and remaining residue was extracted with ethyl acetate.
- Ethyl acetate was recovered and remaining residue was extracted with water.
- Water was extracted with diethyl ether.
- Acetone, ethyl acetate and diethyl ether fractions were combined and evaporated.
- Samples were reconstituted in 60% acetone.

Results and Discussion

The extract of Gingko biloba was analyzed using the LCT Premier in negative ion mode. Presented in Figure 4 are the structures of the Gingkolides of interest. The negative mode total ion chromatogram obtained from the extract of Nanjing Gingko Biloba leaf is shown in Figure 5, where Gingkolides A, B and C determined to be present are shown. From the chromatogram it can be seen that five major components were present in the purified extract. Under the chromatographic conditions used it was not possible to achieve complete separation of the Gingkolides.

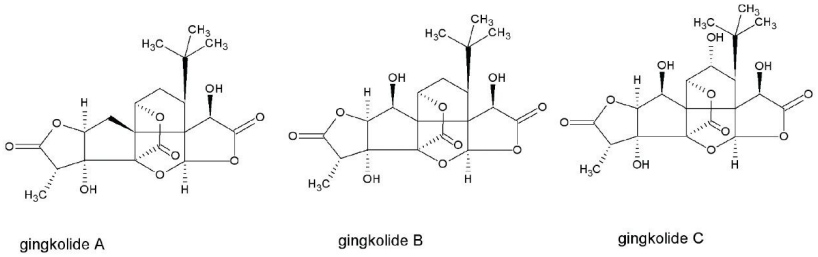

Figure 4. Chemical Structure of Gingkolides.

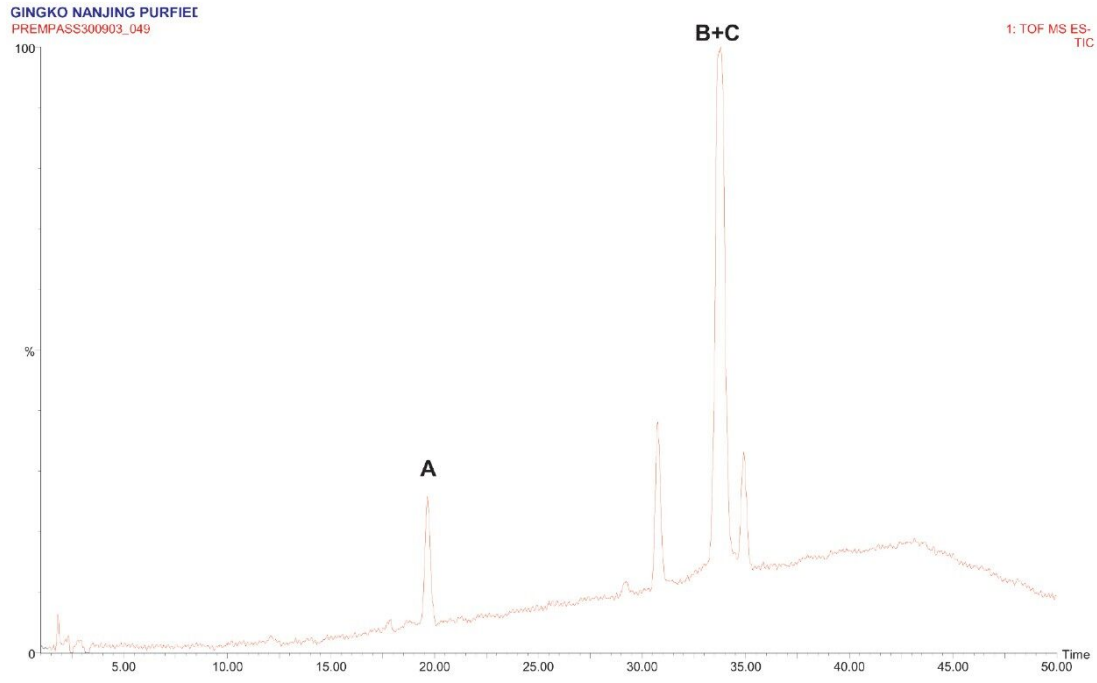

Figure 5. Negative mode total ion chromatogram obtained for the extract of Nanjing Gingkolide Biloba.

The reference compound Leucine enkephalin ionizes in negative mode to produce ([M-H]-=554.2615), and is sampled independently from the analyte spray to provide a lockmass. As shown in Figures 6 and 7 it is not seen in the analyte mass spectrum. Lockmass correction takes place automatically in real time, the independent sampling enhances the mass accuracy obtained. The exact mass spectrum obtained for Gingkolide C is presented in Figure 6. Gingkolide C was mass measured within less than 1ppm error. For Gingkolides A and B the exact mass spectrum is shown in Figure 7, where mass measurement has been obtained within 3ppm error. For the Gingkolides identified, in each case a formate adduct has been observed.

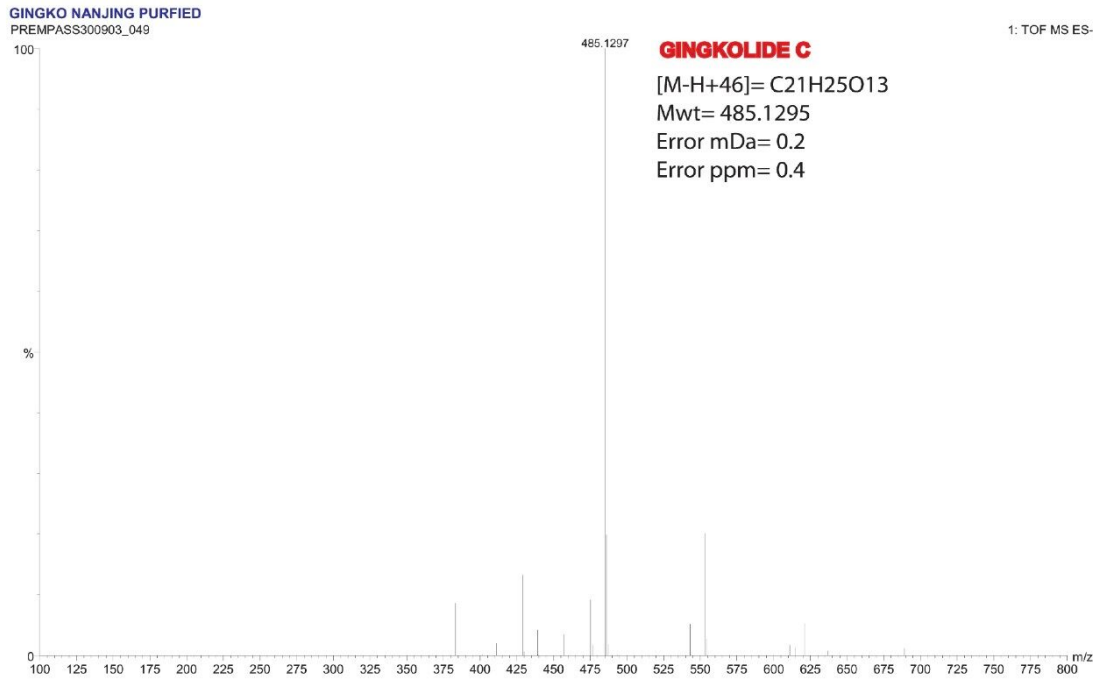

Figure 6. Exact mass spectrum obtained for Gingkolide A.

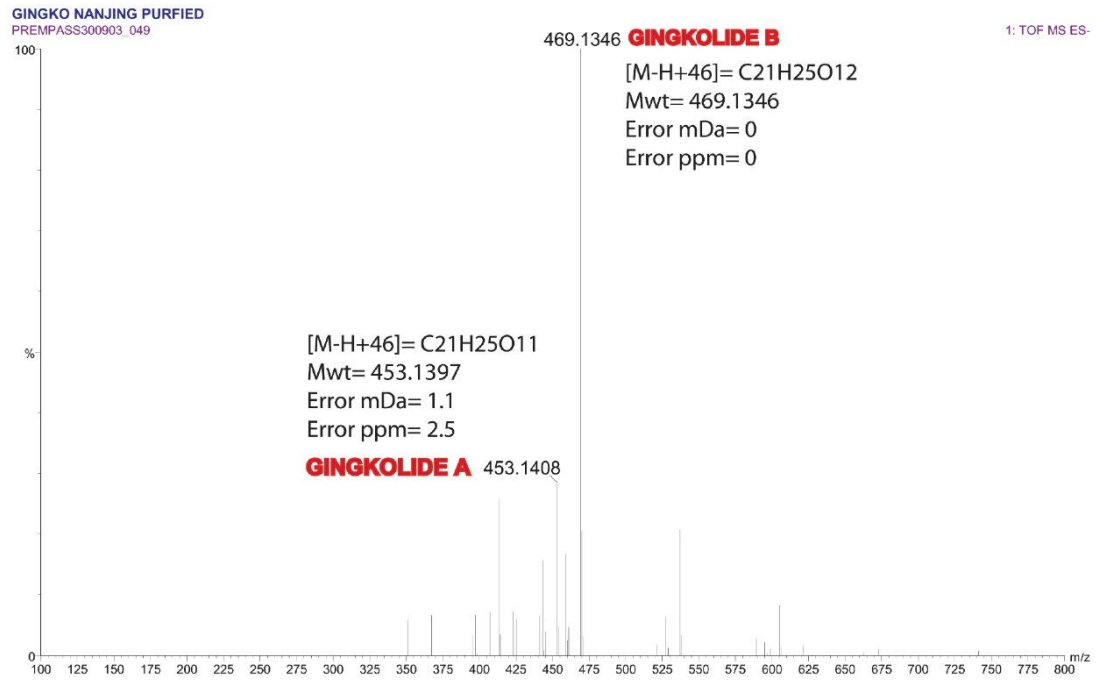

Figure 7. Exact mass spectrum obtained for Gingkolide B and C.

Conclusion

- Extracts of Gingko Biloba samples have been successfully analyzed allowing the specific Gingkolide profile to be obtained.
- Analysis has been performed using centroid acquisition mode and exact mass measurement performed in real time.
- 0.4 ppm error was obtained for negative mode acquisition of Gingkolide A.
- 0.0 ppm error was obtained for negative mode acquisition of Gingkolide B.
- 2.5 ppm error was obtained for negative mode acquisition of Gingkolide C.
- The LCT Premier oa-Tof with integral LockSpray and independent reference mass acquisition enables the routine rapid acquisition of highly specific data.
- Analyte confirmation is achieved using exact mass measurement and enabled complete confidence in the identification of the Gingkolides.

Featured Products

Alliance HPLC System <https://www.waters.com/534293>

720000980, November 2004