The Identification and Quantification of Gingkolides and Bilobalides in Chinese Herbal Medicines.

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Introduction

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 provided a valuable new class of therapeutic agents. This paper describes the use of a triple quadrupole mass spectrometer for the quantification of the main components of *Gingko biloba* extracts as well as the use of a time of flight instrument for qualitative analyses.

Experimental

The extraction procedure used by the China Pharmaceutical Company was incorporated. A triple quadrupole mass spectrometer was operated in negative ion electrospray mode. An HPLC was used to deliver a short, gradient HPLC method. Mobile phase A was water, mobile phase B was acetonitrile and the column used was a C_{18} 100 □ 2.1mm 3.5□ HPLC column. The instrument response was calibrated between 0.5 pg/uL and 5000 pg/uL for Gingkolide A, Gingkolide B and Bilobalide. A hybrid quadrupole-ToF mass spectrometer fitted with a dual probe source, was used in negative ion electrospray mode. ToF resolution was 20,000 FWHM and accurate-mass data were acquired in centroid mode. The HPLC method described above was used with a more shallow gradient in order to improve chromatographic resolution.

Results

For the quantitative analyses each calibration line showed a good linear response. The samples were then injected in duplicate and the mean amounts of active ingredients, in pg/uL, are shown.

Samples	Gingko_A	Gingko_B	Bilobalide	Gingko_A_isomer
Commercial sample1	1722.1	884.6	889.3	31.5
Commercial sample2	328.0	185.9	172.4	5.8
Nanjing sample 1	1366.2	1122.9	N/A	58.1
Nanjing sample 2.	1323.3	1138.0	N/A	56.2

From the ToF analyses a variety of components can be seen and their accurately determined masses, together with matched elemental compositions are given.

Retention Time/min	Measured Mass	Elemental Composition	ppm deviation	Identity
6.53	455.1202	C20H23O12	2.7	Gingkolide Compound
7.63	325.0921	C15H17O8	-0.6	Isomer of Bilobalide
12.56	325.0918	C15H17O8	-1.8	Isomer of Bilobalide
14.1	325.0918	C15H17O8	-1.6	Bilobalide
15.95	423.129	C20H23O10	-0.2	Isomer of Gingkolide B (Gingkolide J or M?)
16.5	439.1239	C20H23O11	-0.3	Gingkolide C
22.15	423.1296	C20H23O10	1.1	Isomer of Gingkolide B (Gingkolide J or M?)
23.03	423.1295	C20H23O10	0.9	Gingkolide B
23.07	407.1331	C20H23O9	-1.6	Gingkolide A
32.12	405.1201	C20H21O9	3.8	Gingkolide B – H_2O?

Conclusions

Using the tandem mass spectrometer, both commercial formulations and extracted leaf samples may be quantified with a good degree of sensitivity. The data from the hybrid quadrupole-Tof mass spectrometer highlighted the use of accurate mass measurements in identifying the main components of *Gingko Biloba* leaf extract. A complex chromatogram was obtained and many of the peaks could be identified as Gingkolide terpenes or Bilabolides.