PhytoReport #4

Ginkgo Adulteration & Identification

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Introduction

Ginkgo biloba is the oldest living tree species on earth, dating back to the Paleozoic period, over 225 million years ago. The medicinal use of ginkgo leaf is first mentioned in Chinese medicine in the Ming dynasty, in 1436 [1].

In the present day, standardized Ginkgo biloba extract (GBE) has been one of the most popular and respected herbal remedies on both sides of the Atlantic for over 25 years. Ginkgo is also one of the most widely tested phytomedicines, and has been the subject of over 120 clinical studies, for cerebral insufficiency, dementia, and peripheral vascular disease.

Therapeutic grade ginkgo extracts, as used in these clinical trials, are highly concentrated. It takes (on average) 50 kilos of dried ginkgo leaf to make one kilo of extract, which is also required to contain guaranteed levels of several key compounds, including flavonol glycosides and terpene lactones.

This ginkgo extract is expensive and complex to manufacture, but scientific literature gives little or no support for the clinical benefits of other dosage forms, or low concentration extracts made from the leaf [2]. The detailed specifications for ginkgo extracts were set initially to standardize and ensure overall potency/effectiveness of preparations made with the extract. However, due to the high cost of production, and high demand, diluted or adulterated material has become commonplace in the supplement ingredient market.

This **PhytoReport #4** reviews current trends in this area. It includes an overview of the well-established adulteration method using added rutin/quercitin, but the principle focus is on a newer method, which uses Fructus saphorae as an adulterant.

Root of Ginkgo Adulteration

The record of adulteration of ginkgo products in the U.S. is well documented with reliable data:

ConsumerLabs (1999):	25% of tested products failed
ConsumerLabs (2003):	75% of tested products failed
ENI/AHP/Eurofins Lab (2006):	47% of tested products failed
ConsumerLabs (2007):	41% of tested products failed
ConsumerLabs (2008):	62% of tested products failed

The bulk of adulteration in these samples was found in the flavonol glycosides, not the terpene lactone component of the extracts. This is because ginkgo leaves

naturally contain a proportion of terpene lactones that make it easier to reach the required 6%, than to reach the 24% content of flavonoids.

How much money can actually be saved by adulterating ginkgo extracts with added flavonoids? Some manufacturers calculate that from 10%, to a maximum of 50% of the extract cost can be saved; however the higher the level of adulteration used, the easier it is to detect.

The key here is that in a market where large contracts are awarded based upon differences of only a few percentage points in cost, even a small saving can result in a company that uses adulteration gaining domination in a market segment, over a company that does not.

Adulteration of ginkgo creates a number of problems. For consumers, the resulting diluted extract does not conform to the concentrated standard used in clinical trials. Additionally, the U.S. Dietary Supplement Health and Education Act requires that a finished dietary supplement product be labeled truthfully relative to its contents. The addition of flavonoids from an outside source is illegal both from this point of view, and that the extract no longer corresponds to the product upon which the structure function claims were made [3].

Adulteration Methods & Identifications

Rutin Adulteration

The most common method of adulterating ginkgo is to add an inexpensive flavonoid source that artificially inflates the total flavonoid level in the extract to meet the required 24%. Rutin, a flavonoid source extracted from buckwheat, and costing about $10/kg, has been the most common additive used for this purpose. After hydrolization for analysis, rutin tests out at 90% quercitin.

As rutin consists primarily of quercetin (Q), rutin does not raise kaempferol (K) or isorhamnetin (I) when added to a ginkgo extract. The quercetin level in a typical rutin-adulterated ginkgo product is usually disproportionately higher (Figure 1). For this reason, adulteration with rutin is now not as effective as it once was, because it is quite easy to identify in the finished product [4].

Accordingly, a ratio of Q/K, K/Q, or Q/K/I has been commonly used to identify rutin adulteration. For example, a ratio of Q/K 1.25 – 1.65 was originally set up by Dr. Willmar Schwabe Pharmaceuticals in Germany. It was based upon the testing data of its trademarked ginkgo product ‘EGb761’, the first GBE used extensively in clinical trials and approved by the German Commission E Monograph.

Recently, USP35-NF30 has set an even tighter K/Q > 0.7 (or Q/K < 1.42) and I//Q (> 0.1) to more closely reflect the naturally occurring ratios of these flavonoids in fresh Ginkgo biloba leaf. This approach and similar ratios are recommended by AHP (American Herbal Pharmacopeia), AHPA and others in the industry.

Because these ratios are now well established, and testing for individual flavonoids is a rational step in laboratory analysis of ginkgo extracts, companies who wish to commit the resources have a way to protect themselves and their customers from ginkgo adulteration by added rutin. Additional protection is gained from dealing with manufacturers or suppliers who themselves have clear commitments and programs to prevent this problem from occurring in their production chain.

Fructus Sophorae and Rutin Adulteration

In contrast to the easily detected 'rutin method' described above, there is a new trend in ginkgo adulteration that involves the addition of *Fructus sophorae* (FS) into the extract manufacturing process. It is interesting to note at this point also that the Australian TGA has also recognized the problem of FS adulteration and is working to develop a program (along the lines below), to detect the presence of FS in Ginkgo extracts.

Fructus saphorae, the fruit of the Japanese Pagoda tree, has some traditional uses in Chinese and Japanese herbal medicine, primarily for calming the digestion. However, its use as a ginkgo adulterant is for a different reason altogether, and relates to its unique content ratio of flavonoids: FS contains high levels of kaempferol (up to 10 times more than ginkgo leaf), and lower levels of quercitin (Figure 1: Typical HPLC chromatogram of Ginkgo and FS extract). This makes it an ideal material to use for adulterating ginkgo extract.

Because of this flavonoid ratio, the addition of highly concentrated FS extract is able to make poor quality ginkgo look more potent by altering the K/Q (or Q/K) ratio. Due to the relatively high cost of highly concentrated FS extract however, adulteration with FS only is not common: adulteration with both FS and Rutin have become the main trend in recent years. This is because adding FS is an effective way to bring a rutin-added ginkgo extract into specification by raising the K/Q ratio into the range of a pure ginkgo leaf product.

While these additions considerably cut down the cost of manufacturing a 'Ginkgo' extract, an FS and rutin-adulterated GBE usually passes most sophisticated HPLC tests, even when a K/Q (or Q/K) ratio is set under a well-established specification. This adulteration has recently become a persistent quality issue in our industry. As mentioned above, a cost change of only a few % points often affects the outcome of large and small annual contracts.

At the early stage, the detection of FS and Rutin-adulterated ginkgo presented a noteworthy challenge. One reason is that highly concentrated FS extract contains mainly flavonoids (K, Q, I) that are very similar to those found in Ginkgo, while other compounds that may be used for adulteration screening when only present in very small percentages were not identified in FS.

Facing this challenge, ENI, working with some other responsible manufacturers and labs, continued its analytical work by thoroughly studying the HPLC chromatogram of the flavonoids in real ginkgo extract, pure FS extract, as well as FS and rutin adulterated extracts. It was found that careful comparison of the HPLC chromatogram fingerprint could be an effective way to determine if total and/or

individual flavonol glycosides are exclusively from ginkgo, or have been added from FS extracts.

Though FS has a similar flavonol glycosides profile to that of ginkgo, it shows a clearly noticeable peak, lying between the quercetin and kaempferol peaks. This peak consistently appears at a relatively larger size in FS and rutin-adulterated extract than in real ginkgo extract. The peak was later identified as 'genistein'. However, this peak is not as obvious in the HPLC chromatogram when it is run under regular Ginkgo flavonoids HPLC conditions (e.g. wavelength at 360~370 nm), as this wavelength was set to be optimal for K and Q analysis but not genistein. Furthermore, it was found that natural Ginkgo extract also contains a small amount of genistein.

After putting in more work and trials, a modified method was developed, which is specific and optimal for Genistein (e.g. wavelength at 260 nm). As a result, this HPLC chromatogram shows significant differences in genistein peak levels between the pure Ginkgo extract, pure FS, as well as FS and Rutin adulterated Ginkgo extract (Figure 3).

Figure 3 (A & B) illustrates a comparison of the HPLC fingerprints of an NIST certified ginkgo extract with that of a tested and verified commercial lot of pure ginkgo extract (source, ENI). Both HPLC chromatograms show a very small Genistein peak (0.03% and 0.1% respectively). By contrast, in a typical pure FS extract, and in an FS and rutin-adulterated ginkgo extract, genistein was found to be over 8% and 3% respectively [Figure 3 (C & D)].

Based on the testing data under the method specific for Genistein, it is observed that if genistein content in a claimed 'ginkgo extract' is over 0.5%, the extract is most likely adulterated with FS and/or Rutin. For some severe cases, where genistein content is reported at over 3%, the extract is most likely adulterated with a significant amount of FS and Rutin. These criteria may be used to objectively determine if there is, and the severity of, an FS adulteration.

Reference:

[1] Foster, S. (1996). Ginkgo-Ginkgo biloba. Botanical booklet No.304. Austin (TX): American Botanical Council.

[2] Blumenthal, M., et al (1998). *The Complete German Commission E Monographs - Therapeutic Guide to Herbal Medicines.* Austin (TX): American Botanical Council; Boston (MA): Integrative Medicine Communication. 136-8.

[3] Myers, S. (2008). *Adulteration Stifles the Ginkgo Biloba Market. Natural Products Insider Magazine*. Retrieved on 11/07/12 from the following link http://www.naturalproductsinsider.com/articles/2008/10/adulteration-stifles-the-ginkgo-biloba-market.aspx

[4] Chen, P., et al. (2007). Chromatographic fingerprint analysis for evaluation of Ginkgo biloba products. *Anal Bioanal Chem.* 389, 251-261.

Fig. 1 (A)

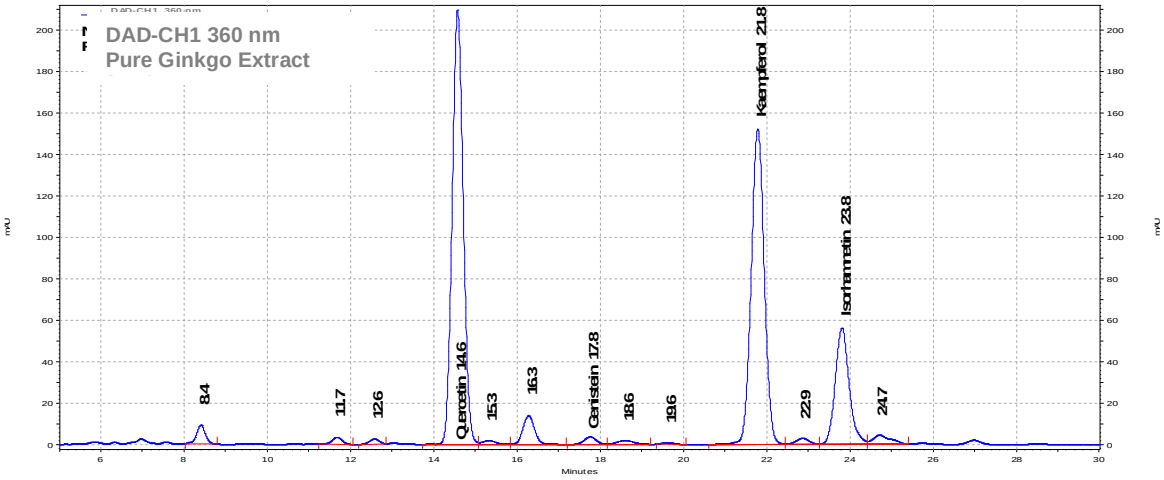

Fig. 1 (B)

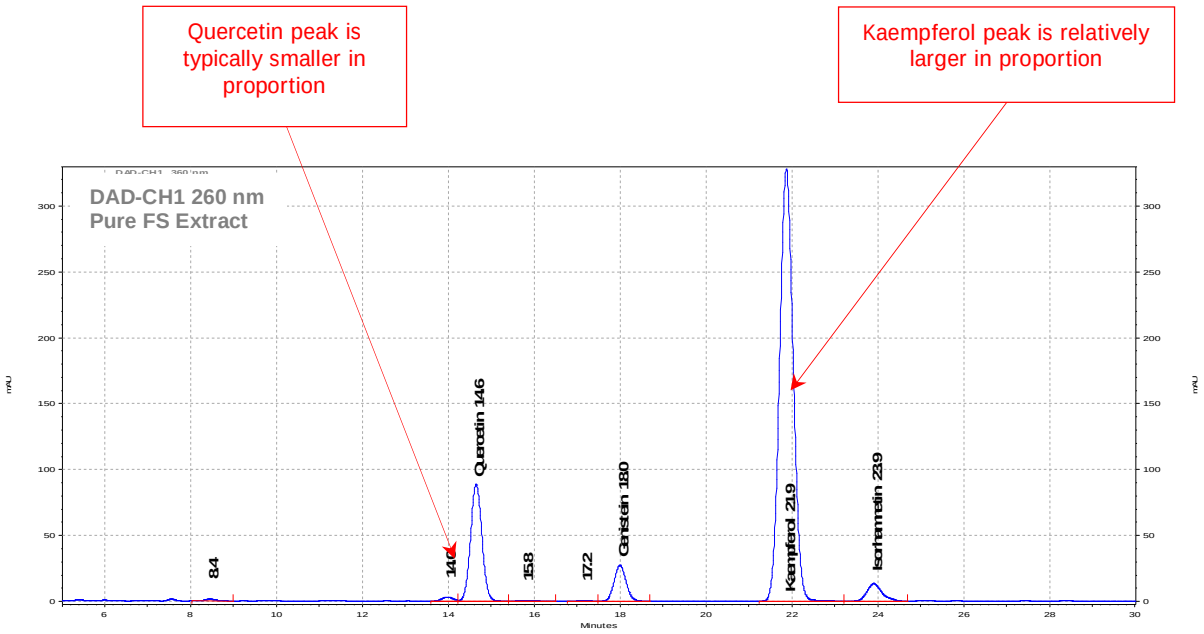

Figure 1: Comparison of HPLC chromatogram under USP Ginkgo Flavonoids test condition: (A) A typical tested and verified commercial Ginkgo biloba extract (source, ENI); (B) A typical lot of pure Fructus sophorae (FS) extract

Fig. 2 (A)

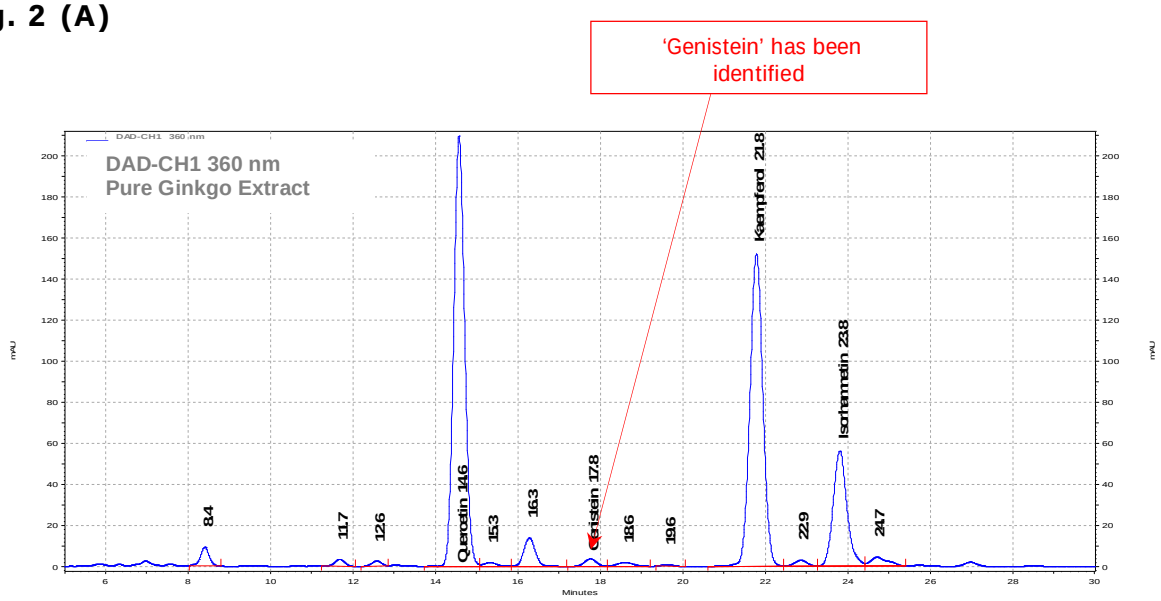

Fig. 2 (B)

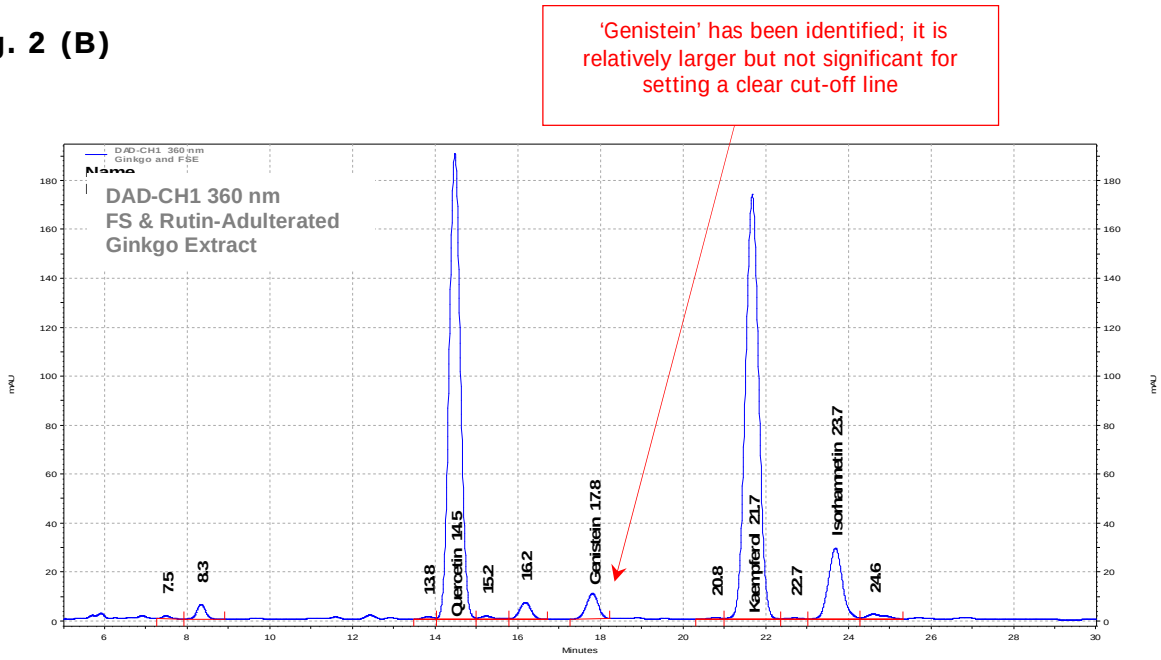

Figure 2: Comparison of HPLC chromatogram under USP ginkgo flavonoids test condition: (A) A typical tested and verified commercial Ginkgo biloba extract (source, ENI) (B) A typical lot of Fructus sophorae adulterated Ginkgo extract. There is relative difference but not significant to draw a cut-off line to differentiate pure Ginkgo extract from FS and Rutin adulterated Ginkgo extract.

Fig. 3 (A)

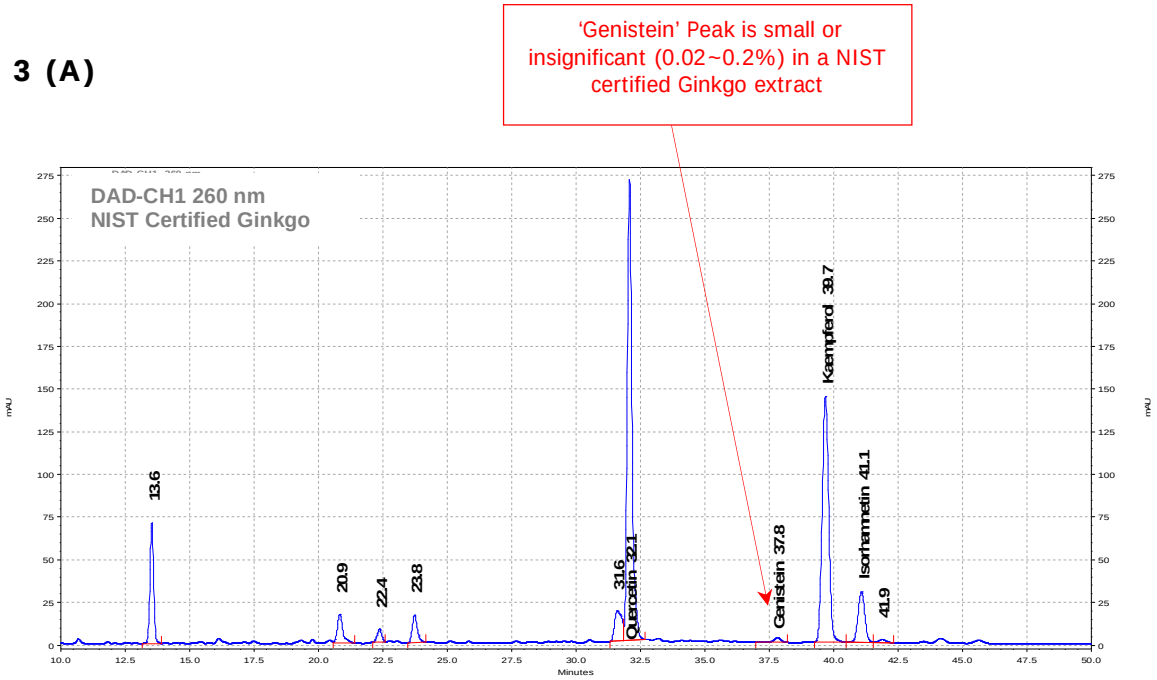

Fig. 3 (B)

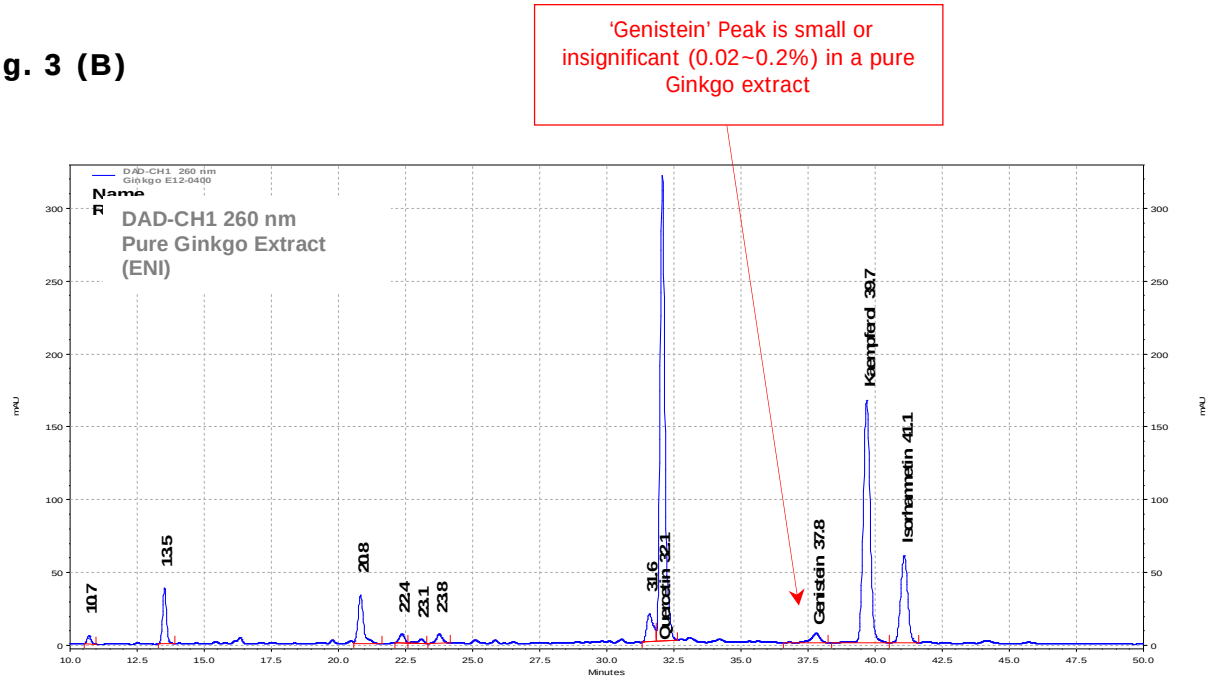

Figure 3: Comparison of HPLC chromatogram under specific condition optimal for Genistein: (A) A typical lot of NIST Certified Ginkgo biloba extract; (B) A typical tested and verified commercial Ginkgo biloba extract (source, ENI);

Fig. 3 (C)

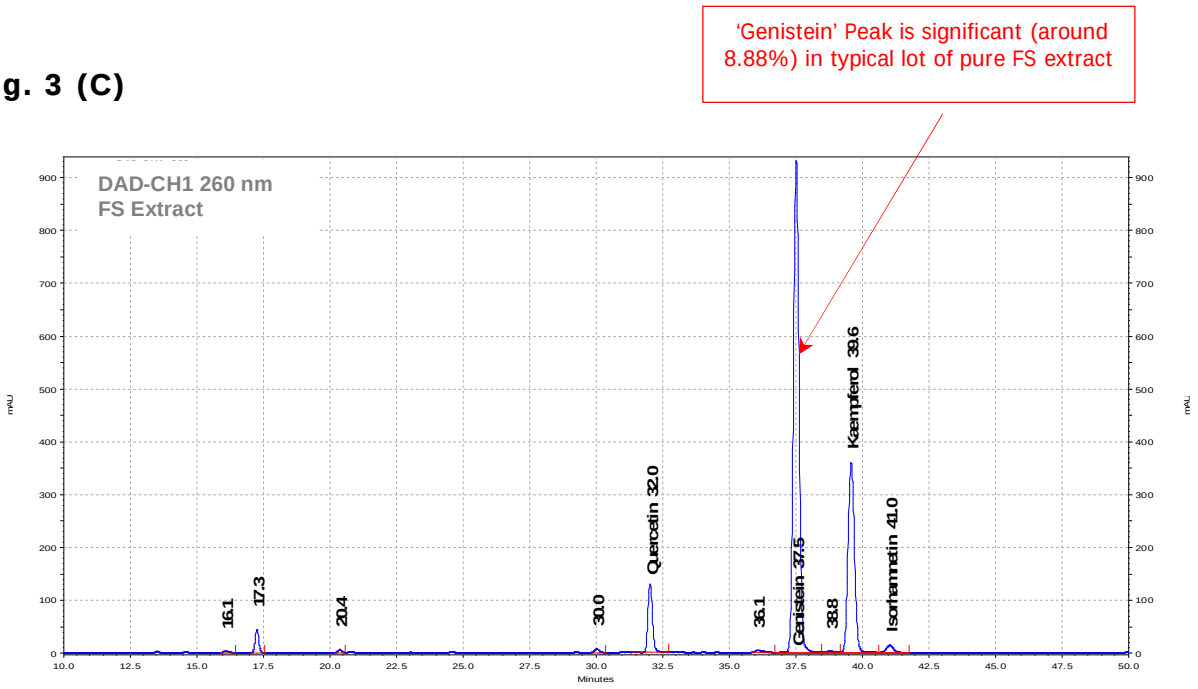

Fig. 3 (D)

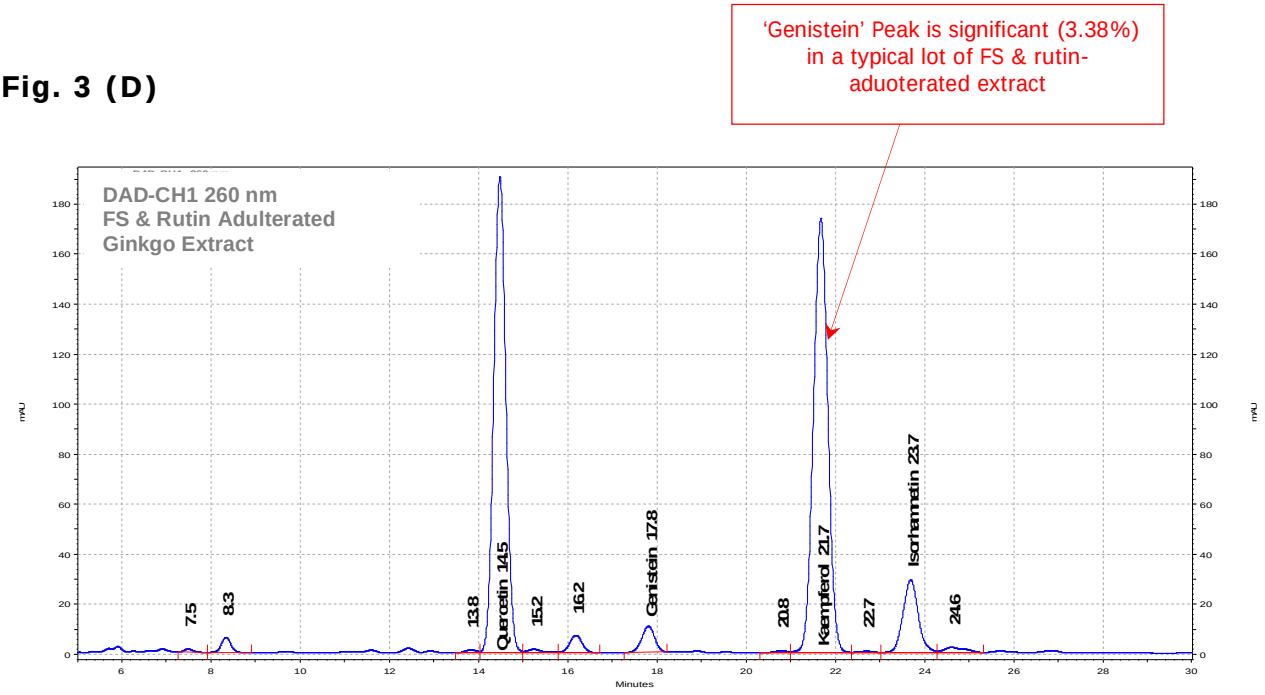

Figure 3: Comparison of HPLC chromatogram under specific condition optimal for Genistein: (C) A typical lot of pure Fructus sophorae (FS) extract (D) A typical lot of Fructus sophorae adulterated Ginkgo extract. Under this method, Genistein peak stands out clearly in the FS and Rutin adulterated Ginkgo extract.

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