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Diamond Hydride™

# Analysis of Litchi Seed Extracts for Toxic Compounds

## Extended Application Note

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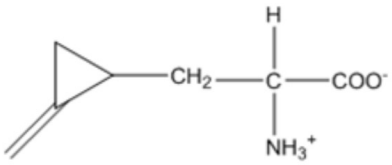
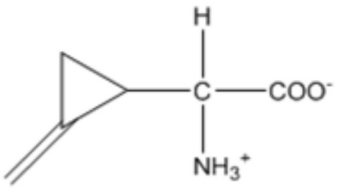



Hypoglycin A




Methylenecyclopropylglycine

**Gradient 1:**

**A:** DI $H_2O$/ 0.1% formic acid
**B:** Acetonitrile/ 0.1% formic acid

| Time (min.) | %B |
|---|---|
| 0 | 95 |
| 6 | 30 |
| 8 | 30 |
| 10 | 95 |

**Gradient 2:**

**A:** DI $H_2O$/ 0.1% formic acid
**B:** Acetonitrile/ 0.1% formic acid

| Time (min.) | %B |
|---|---|
| 0 | 85 |
| 4 | 20 |
| 7 | 20 |
| 10 | 85 |

**Gradient 3:**

**A:** DI $H_2O$
**B:** Acetonitrile/ 0.1% formic acid

| Time (min.) | %B |
|---|---|
| 0 | 95 |
| 6 | 30 |
| 8 | 30 |
| 10 | 95 |

## Introduction

The Litchi (or lychee) tree is indigenous to parts of southern China and has been cultivated elsewhere around the world as well. The tree produces red soapberries with a translucent white pulp and has been a part of Chinese cuisine for thousands of years. In terms of nutrition, it is high in vitamin C and contains low molecular weight polyphenol antioxidants.

Despite its reputed health benefits, Litchi fruit is now believed to be the cause of a serious illness affecting parts of northeast India. The condition, characterized by seizures and other neurological symptoms, has primarily affected undernourished children. Authorities identify it as a type of acute noninflammatory encephalopathy. Clues that the illness was linked to the Litchi fruit were that it coincided with the Litchi harvesting season and that children who had spent time in the orchards were more than twice as likely to be afflicted.

One component found in the Litchi fruit seeds, methylenecyclopropylglycine (MCPG), has been shown to induce hypoglycemia in animals. It is believed to interfere with the metabolic pathyway that synthesizes glucose from fatty acids. Hence, under-nourished children, whose glucose reserves are low, will be more susceptible to the compound's detrimental effects. Another compound also found in the seeds, hypoglycin A, is known to cause Jamaican vomitting sickness and may be a factor in the illnesses as well.

Due to the increased awareness of the role these compounds can play in the consumption of Litchi fruits and the resulting illness, there is a need for analytical methods for their quantitation. LC-MS would be most appropriate for these kinds of analyses due to the complexity of the Litchi extract samples. Use of the Diamond Hydride™ HPLC column was found to be suitable for the analysis.

## Experimental

### Materials

Formic acid LC-MS ultra-grade was from Sigma–Aldrich (St. Louis, MO, USA). Litchi samples and a hypoglycin A/ methylenecyclopropylglycine standard solution were from the USDA. Deionized water (DI $H_2O$) was prepared on a Milli- Q™ purification system from Millipore (Bedford, MA, USA). Acetonitrile (HPLC grade) was obtained from GFS Chemicals, Inc. (Powell, OH, USA).

### *Instrumentation*

An Agilent (Little Falls, DE, USA) 1200SL Series LC system, including degasser, binary pump, temperature-controlled autosampler, and temperature-controlled column compartment was used. The mass spectrometer system was an Agilent (Santa Clara, CA, USA) Model 6210 MSD TOF with a dual sprayer electrospray source (ESI). The first analytical column was a Diamond Hydride™ stationary phase, which had dimensions 2.1 x 150 mm, a particle diameter of 4µm, and a pore size of 100Å. A Diamond Hydride 2.o™ column was also used, which had dimensions 2.1 x 150 mm, a particle diameter of 2.2µm, and a pore size of 120Å. Different gradients were used (see Tables).

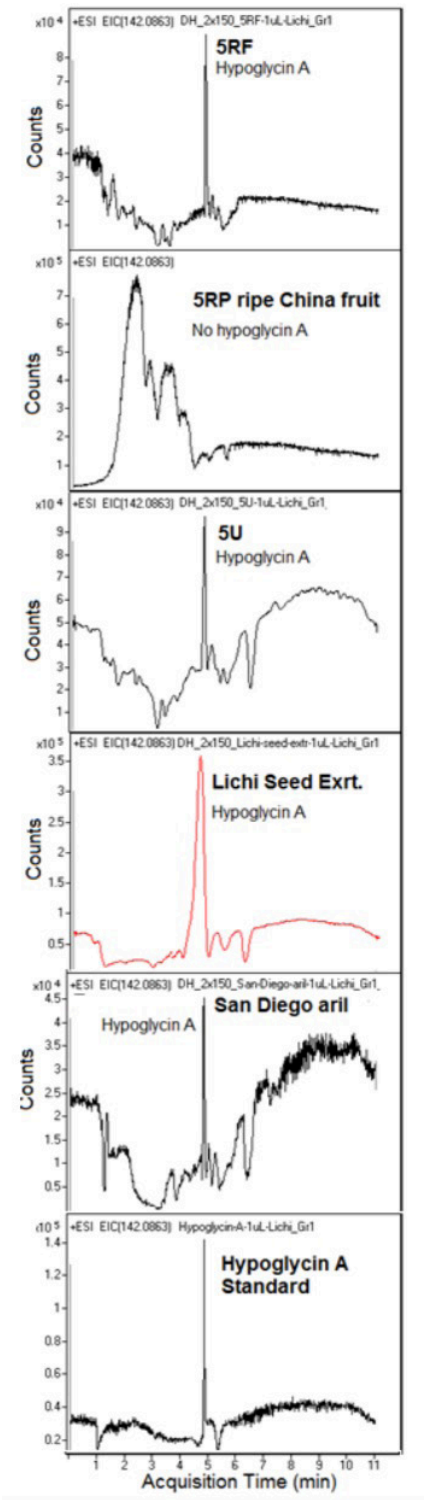



Figure 1

The 4µm column was used with Gradients 1 and 2 and the 2.o™ column was used in Gradient 3. In all cases, the injection volume was 1µL. The flow rate was 0.4 mL/min for Gradients 1 and 2 and was 0.3 mL/min for Gradient 3.

### Samples Preparation

- **Hypoglycin A standard** (100 pmoles/mL) in water with 0.1% formic acid, 85% purity; also contains methylenecyclopropylglycine (MCPG).
- **Litchi seed extract** – ground Litchi seeds were extracted with 85% ethanol and the extract was concentrated. The composition was unknown but was predicted to contain both hypoglycin A and MCPG.
- **5U** - This is an immature Chinese variety Litchi sample from India. The fruit pulp was centrifuged and filtered to yield this sample. It was diluted 4X for the analysis.
- **5RF** – This is a ripe Chinese variety Litchi sample from India. The fruit pulp was centrifuged and filtered to yield this sample. It was diluted 4X for the analysis.
- **5RP** – This is an overripe Chinese variety Litchi sample from India. The fruit pulp was centrifuged and filtered to yield this sample. It was diluted 4X for the analysis.
- **San Diego aril**

## Results and Discussion

Analysis of the samples using Gradient 1 showed that hypoglycin A was observed as a well-retained peak in four out of five samples. **Figure 1** shows the EICs for the hypoglycin $[M+H]^+$ ion in each sample. The bottom figure shows the data for the reference standard for verification by retention time. In the overripe 5RP Litchi sample, it was not detected.

The data suggested that whether the fruit was overripe was a significant factor in predicting whether hypoglycin would be present in the sample. Hence, growers could use this information to select proper harvesting times for the Litchi fruits to ensure consumer safety.

The second hazardous compound found in the fruit extract was methylenecyclopropylglycine (MCPG). It also can be observed as the $[M+H]^+$ ion in the EICs:

| Name | Formula | m/z $[M+H]^+$ |
|---|---|---|
| Hypoglycin A | $C_7H_{11}NO_2$ | 142.0863 |
| Methylenecyclopropylglycine (MCPG) | $C_6H_9NO_2$ | 128.0706 |

MCPG was observed in four of the five samples. Here, the pattern was the same as with hypoglycin; only the overripe fruit did not have a detectable level of MCPG. The data for each sample is shown in **Figure 2**. The retention times for MCPG and hypoglycin A were about the same using this gradient but specificity can be obtained from the EICs. Nevertheless, it was desirable to obtain chromatographic separation as well.

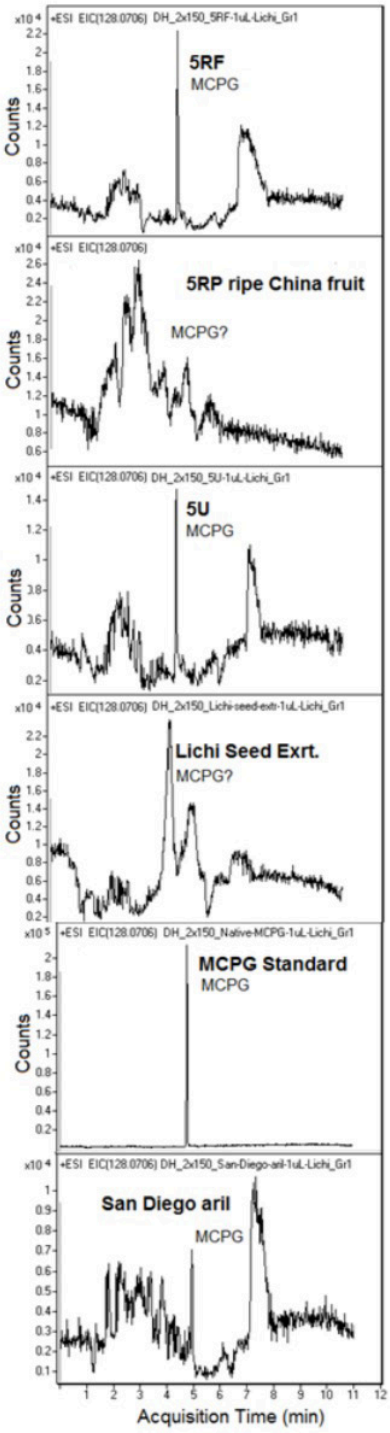



Figure 2

For this reason, Gradient 2 was tried using the Litchi seed extract sample. Here, an interesting phenomenon was observed. The EIC for hypoglycin A showed two other peaks present, while the EIC for the reference standard showed only one peak (refer to **Figure 3**).

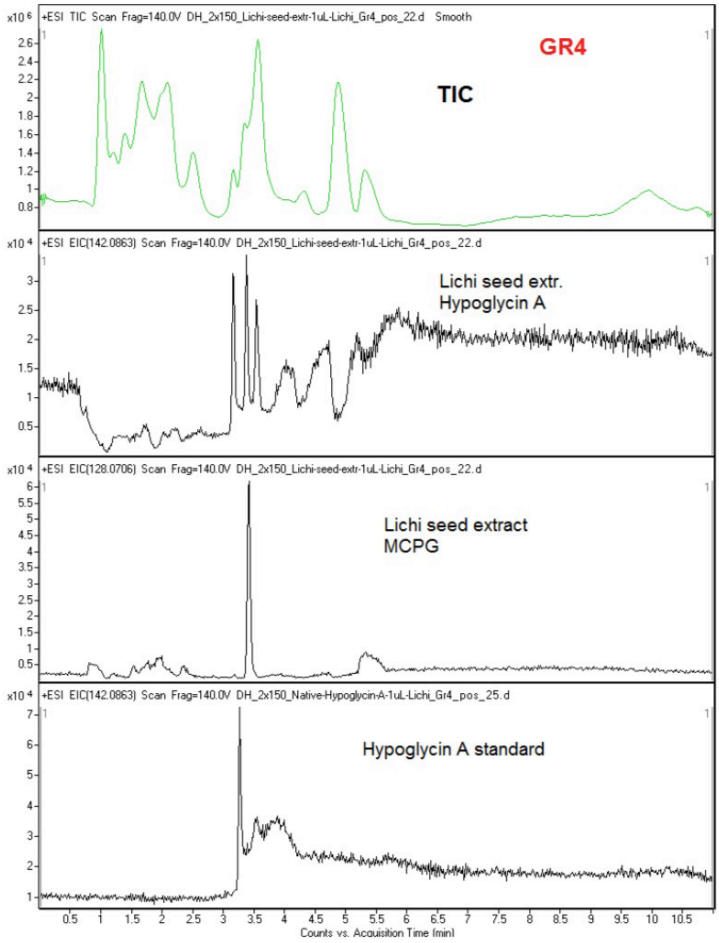



Figure 3

Because they appear at the same m/z as hypoglycin A, the extra peaks are likely to be isomers. As for the separation, slightly better selectivity was observed between MCPG and hyopglycin A in the standards than using the previous gradient.

A Diamond Hydride 2.o™ column was used next. This stationary phase material has a smaller particle size and therefore produces higher efficiency. This may lead to better chromatographic resolution of hyopglycin A and MCPG if the peaks are sharper.

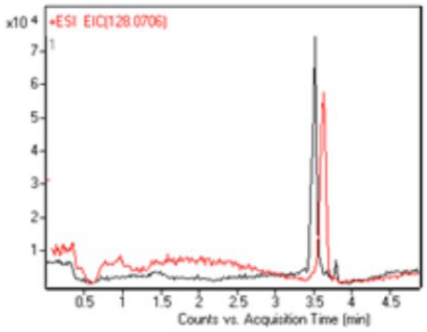



Figure 4

In **Figure 4**, the use of the Diamond Hydride 2.o™ column was able to produce near baseline resolution of MCPG and hyopglycin A. Gradient 3 was used in this case. With this method, chromatographic specificity can be obtained as well as mass spectral specificity. This may be useful for laboratories that don't have access to more sophisticated detection methods such as MS.

## Conclusion

The Diamond Hydride™ column has been shown to be highly suitable for the analysis of hyopglycin A and MCPG in Litchi fruit extracts. The methodology can be applied to testing of these compounds in food products sold to the consumer, which may present serious health risks for malnourished individuals. Peaks were observed in the EICs for both compounds as well as two isomers of hypoglycin A. Use of a near-UHPLC particle size column and a different gradient were able to produce better chromatographic separation of the two hazardous compounds.