UNIVERSITY OF OREGON

February 23, 1994

Guy Woodman
InterHealth Company
1320 Galaxy Way
Concord, CA 94520

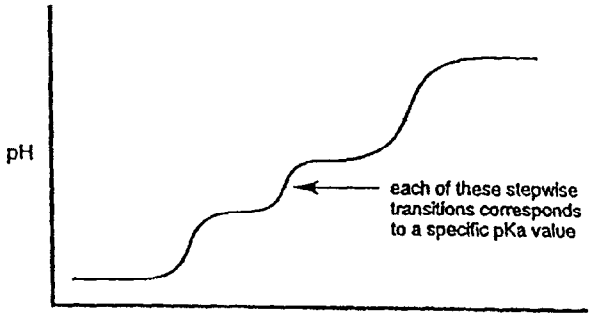
Dear Mr. Woodman,

I have carefully examined the two assay procedures you sent me, including Sabinsa's method for the detection and quantitation of (–)hydroxycitric acid (HCA) in Citrin® and InterHealth's method for testing its HCA product, CitriMax™. Following are my comments.

SABINSA METHOD

The Sabinsa method, as described, is intrinsically non-specific since it really only measures total acid content in a simple titration. In principle, it might be possible to determine (–)HCA by careful titration using a pH meter rather than the simple colorimetric method employed by Sabinsa; since different acids have somewhat different acidities (pKa values) they will titrate at different pH values. A plot of NaOH used vs. pH could, *in principle*, measure individual acids and look like the graph below. I emphasize "in principle" here because the actual case would tend to be much more complicated and unreliable since many pKa values are very similar. The similarity in pKa values would cause overlapping "stepwise transitions" making the data ambiguous and extremely difficult to interpret.

Hypothetical Titration Of A Mixture Of Acids



Furthermore, if the extraction process used in the manufacture of Citrin® involves the use of acids, this could dramatically change the observed analytical results using the Sabinsa method. This is not a problem with the InterHealth HPLC method (see below). In summary, the Sabinsa procedure

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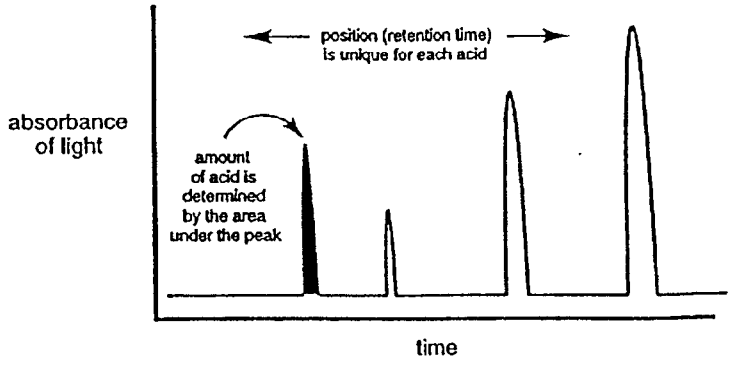
[redacted]

is not an accurate method of analysis for (–)HCA but essentially a method of determining total acid content in a sample.

INTERHEALTH METHOD

In principle, the InterHealth HPLC method, properly executed and calibrated, is highly specific for both the detection of a given acid (based on the retention time or how long it takes for the sample to travel through the column) and for the quantitative determination of how much is present (based on the area under the curve in each chromatogram).

Hypothetical HPLC Of A Mixture Of Acids



The HPLC column specified in the InterHealth method is a state-of-the-art column specifically designed for the analysis of organic acids. In addition, use of an HPLC calibration curve, as specified in the InterHealth method, is the only true way to get reliable HPLC quantitative data. "Coelution" or overlapping, of two different acids at exactly the same position is possible with the HPLC method, but "spiking" the sample with a reference standard, as InterHealth has done, eliminates this unlikely possibility, giving further validation of this method.

Furthermore, unlike the Sabinsa method, the use of phosphoric acid, or any other acid, in the extraction process in the InterHealth method does not perturb the assay since the procedure does not rely on the sample's acidity to determine the presence and amount of compounds such as HCA in the sample. In summary, the InterHealth HPLC assay is a far superior and highly reliable method of assaying for (–)hydroxycitric acid.

I hope that these comments are helpful. If you have any other questions, please don't hesitate to call.

Sincerely,

Bruce Branchaud

Bruce Branchaud
Associate Professor of Chemistry

industrial
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Summary [–]Hydroxycitric Acid Analytical Procedure

Scope:
The method is appropriate for purified [–]HCA extracts and source materials.

Method Principle:
The finely ground sample is extracted with a buffered aqueous solution. The extraction solution is filtered and diluted, prior to analysis by high performance liquid chromatography.

Precision:
2% relative standard deviation

Accuracy:
Recovery ranges from 95 to 105%

References:
Kucera, Paul; Moros, Stephan A.; and Mlodozeniec, Arthur R.; "Differential Frontal Analysis of Carboxylic Acids.," *Journal of Chromatography*, 210 (1981) 373-388.

Quality Control:
Each sample set includes:
1. Three point standard curve
2. Replicate sample analysis
3. Spiked sample analysis
4. Blank sample
5. Repeat standard at end of run

Apparatus:
1. High Performance Liquid Chromatograph
2. Volumetric Glassware
3. Electronic Balance
4. Vials
5. Filters

Reagents:
1. Pure water
2. Buffer

Reference Standard:
Pharmaceutical grade [–]Hydroxycitric Acid—100% pure

Procedure:
1. Grind sample.
2. Weigh appropriate amount of sample into blender and blend with solvent.
3. Heat solution.
4. Cool sample and adjust to known volume.
5. Dilute sample solution to appropriate volume.
6. Filter and prepare for HPLC analysis.
7. Analyze by HPLC utilizing proper standards.

Calculations:
Calculation of concentration is based upon the linear calibration curve produced by the standard from the HPLC analysis.

$$\%HCA = \frac{[(Sl \times Area) - Int]}{SW_{/250mL} \times DF} \times 100\%$$

Where:

Sl	=	Slope of calibration curve.
Area	=	Area of HCA peak from sample chromatogram.
Int	=	Intercept from the calibration curve.
SW	=	Sample weight in grams.
DF	=	Dilution factor used to bring the solution into the proper concentration range.

[redacted]

SABINSA CORPORATION
PRODUCT INFORMATION

Method of Analysis for (-) Hydroxy Citric Acid in Citrin

Weigh about 2 gms of Calcium HCA extract and suspend in 100 ml water. Warm till all the materials go into solution.

Pass the solution through the Dowex 50 resin, well washed and packed in a column, and collect the eluate in a flask. Wash the resin with Carbon dioxide free water and collect the eluate, till the eluate is neutral pH. Combine all the eluate and titrate with 1N Sodium Hydroxide using Phenolphthalein as indicator.

After the end point, the same may be warmed to 80°C and if any color changes, the titration may be continued to get the end point.

Calculation: Each ml of 1N NaOH = 0.06904 gm of (-)HCA.