Appendix D: Quality Control / Product Selection

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Overview

Research papers:

Cheema-Dhadli, Surinder, Mitchell L. Halperin and Clifford C. Ieznoff (1973). Inhibition of enzymes which interact with citrate by (-)-hydroxycitrate and 1,2,3,-tricarboxybenzine. European Journal of Biochemistry 38 (1973) 98-102.

Lowenstein, John M. and henri Brunengraber (1981). Hydroxycitrate. In John M. Lowenstein, ed., volume 72 in Methods in Enzymology, Lipids (New York: Academic Press, 1981) 486-497.

Glusker, Jenny P. (1992). Structural aspects of Citrate Biochemistry. In Earl R. Stadtman and P. Boon Chock, eds., Current Topics in Cellular Regulation, volume 33, From Metabolite, to metabolism, to Metabolon (New York: Academic Press Inc., 1992) 169-184.

Hoffman-La Roche Information Services and Administration, Preclinical R&D, Information on Hydroxycitric Acid.

Branchaud, Bruce, Associate Professor of Chemistry, University of Oregon, Letter to Interhealth (February 23, 1994).

Quality Control/Product Selection

Overview

The dried fruit rind of *G. cambogia* and its relatives contains HCA *only* in the form of the lactone. Further, the highest natural yield of the rind, a concentration rarely seen, is about 30% lactone by weight. This means that an unextracted source will never contain greater than 30% HCA as lactone, and more commonly the range will be from 4-6% with the species *G. indica* to about 15% with *G. cambogia*. Products which look very dark and gritty probably are no more than the ground rind and should be avoided.

HCA can be made available as a free acid dissolved in liquid, as an HCA lactone and as a salt of HCA. Free HCA is extremely hygroscopic and cannot be supplied in dry form. Theoretically, liquid free HCA would be the preferred form, but in fact it turns out to be next to impossible to bring this item to the market without its having already turned into its lactone, a chemically altered derivative of HCA that does not inhibit ATP-citrate lyase. Alkaline metal salts of HCA (HCA complexed with sodium, calcium, etc.), however, are quite stable, resist lactone formation and confer potent biological activity. In fact, HCA salts have been used almost entirely in research studies after it was determined that the lactone was significantly less effective than the salt form of HCA. Then there is the further difficulty of accurately measuring the concentration of HCA being provided in the liquid and salt forms. Many products which claim to be 50% HCA are really only 50% acids, which means a mixture of HCA along with whatever other acids are present. Although HCA is the primary acid in the rind of *G. cambogia,* other organic acids such as citric, glutamic and acetic acids are present as well.

Various analytic methods can be used to determine the HCA levels of products. In principle, the HCA content of a material can be analyzed by simply calculating the degree of acidity of the pertinent fraction of the product. This is called a "titration." Different acids have slighly different degrees of acidity, i.e., the acetic acid in vinegar is more acid than the malic acid found in apples but less acid than the hydrochloric acid found in our stomachs. Unfortunately, what is simple in theory is often impossible in practice. A mixture of acids with closely related pH values, meaning very close degrees of acidity, when plotted on a graph will create an overlapping and difficult to interpret picture. Any manufacturer using such an approach will have a poor notion of the true quality of the product and will overstate the HCA content by a wide margin because this method is more useful for determining the total acid content than for determining any particular element within a group of similar acids. If any other acids are used in the extraction process, the testing procedure becomes even more difficult.

Two other methods which are far more reliable than titration are the HPLC (high-performance liquid chromatography, sometimes also called high-pressure liquid chromatography) method and the GCMS (gas chromatography mass spectroscopy) method. Each of these methods can be calibrated very finely to reveal both the presence of specific acids and the amount of each acid present. In the opinion of many experts in the field of chemistry, HPLC is the method preferred for the testing of (–)-hydroxycitric acid products. For instance, this is the judgment of Dr. Robert

Rosen, Director of the Mass Spectrometry Laboratory at the Center for Advanced Food Technology, Department of Food Science, Rutgers University and likewise of Dr. Bruce Branchaud, Associate Professor of Chemistry at the University of Oregon. Notwithstanding, even HPLC results can be affected by variations in analytical techniques, apparatuses, operators and the quality of the reference standard, which is generally not available as a stock item in chemical supply houses. For example, recently, laboratories employing HPLC methodolgies using *ion exchange* reversed phase columns reported higher HCA values than laboratories using *simple* reversed phase columns. The exact reason for the differences in these values is not yet known, and new techniques may reveal even more accurate methods of analysis.

CitriMax is a standardized calcium salt of HCA containing ~50% (–)hydroxycitric acid as routinely certified by independent laboratory HPLC analysis and confirmed by the Mass Spectrometry Laboratory at the Center for Advanced Food Technology, Rutgers University.

Clouatre; Dallas and Michael E. Rosenbaum (1994). **The Diet and Health Benefits of HCA (Hydroxycitric Acid)** (New Canaan, CT: Keats Publishing, Inc., 1994).

<u>The Lactone Occurs Naturally in Selected *Garcinia* Species</u>

Observational Study:

"The acid is present to the extent of 20-30% in the dried fruit rinds of *Garcinia cambogia, Garcinia atrovirdis*, and *Garcinia indica*, which are used for culinary purposes and are available commercially in India. The acid can be isolated from these materials in the form of its lactone...."

Lewis, Y. S. (1969). Isolation and properties of hydroxycitric acid. In John M. Lowenstein, ed., volume 13 in Methods in Enzymology, **Citric Acid Cycle** (New York: Academic Press, 1969) 613-619.

Review Article:

"Hydroxycitric acid lactone is the major constituent of the extract [of *Garcinia cambogia* as prepared here]...."

Lowenstein, John M. and Henri Brunengraber (1981). Hydroxcitrate. In John M. Lowenstein, ed., volume 72 in Methods in Enzymology, **Lipids** (New York: Academic Press, 1981) 486-497.

HCA Lactone is Less Effective Than the Salt with Oral Administration

In Vivo Study:

"Additional experiments indicate that the effective dose of orally administered (–)-hydroxycitrate required to produce maximal inhibition can be decreased by giving the sodium salt rather than the lactone.

The experimental data reported here suggest that the hydrolysis product of (–)-hydroxycitrate lactone, i.e., (–)-hydroxycitrate is the active inhibitor of lipogenesis."

Sullivan, Ann C., James G. Hamilton, O. Neal Miller, and Victor R. Wheatley (1972). Inhibition of lipogenesis in rat liver by (–)-hydroxycitrate. **Archives of Biochemistry and Biophysics** 150 (1972) 183-190.

HCA Lactone Does Not Inhibit ATP-Citrate Lyase

Review Article:

"The lactone does not inhibit ATP:citrate lyase. It is hydrolyzed to hydroxycitrate by heating a 1 *M* solution with 3.1 equivalents of sodium hydroxide at 90° for 1 hr."

[Note: a similar procedure is possible with other alkaline metals and earth metals.]

Lowenstein, John M. and Henri Brunengraber (1981). Hydroxcitrate. In John M. Lowenstein, ed., volume 72 in Methods in Enzymology, **Lipids** (New York: Academic Press, 1981) 486-497.

Free HCA In Solution Rapidly Converts to Lactone

Chemical Analysis:

"In solution, compound [(–)-hydroxycitric acid] slowly lactonizes to RO 20-1569 (ca. 10% in 7 days at 20° [Centigrade])."

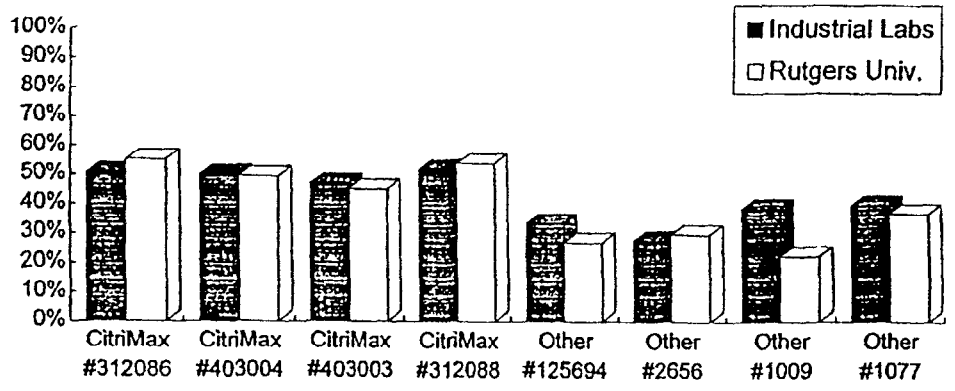
Hoffmann-La Roche, Inc. Information Services

Independent Laboratories Confirm Potency/Label Claim of CitriMax

Test Results:

Using HPLC and pharmaceutical-grade HCA reference standard acquired from the research and development laboratoroies at Hoffmann-La Roche, Industrial Laboratories, Denver, CO and the Mass Spectrometry Laboratory at the Center for Advanced Food Technology, Rutgers University have independently confirmed the potency and label claim of CitriMax. All samples of CitriMax tested met label claim, while all samples of another leading brand tested were below label claim.

Product Control No.	CitriMax #312086	CitriMax #403004	CitriMax #403003	CitriMax #312088	Other #125694	Other #2656	Other #1009	Other #1077
Label Claim (-)Hydroxycitrate)	50%±5%	50%±5%	50%±5%	50%±5%	50%±2%	50%±2%	50%±2%	50%±2%
Industrial Laboratories Results	50.7%	50.1%	47.0%	51.9%	33.5%	27.6%	38.6%	39.6%
Rutgers University Results	55.1%	49.3%	45.0%	53.8%	26.9%	29.74%	22.2%	36.7%



Articles

Cheema-Dhadli, Surinder, Mitchell L. Halperin and Clifford C. Leznoff (1973). Inhibition of enzymes which interact with citrate by (–)-hydroxycitrate and 1,2,3,-tricarboxybenzene. **European Journal of Biochemistry** 38 (1973) 98-102.

Lowenstein, John M. and Henri Brunengraber (1981). Hydroxcitrate. In John M. Lowenstein, ed., volume 72 in Methods in Enzymology, **Lipids** (New York: Academic Press, 1981) 486-497.

Glusker, Jenny P. (1992). Structural Aspects of Citrate Biochemistry. In Earl R. Stadtman and P. Boon Chock, eds., Current Topics in Cellular Regulation, volume 33, **From Metabolite, to Metabolism, to Metabolon** (New York: Academic Press, Inc., 1992) 169-184.

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