Cambridge Isotope Laboratories, Inc.
www.isotope.com

Preparation and Analysis of

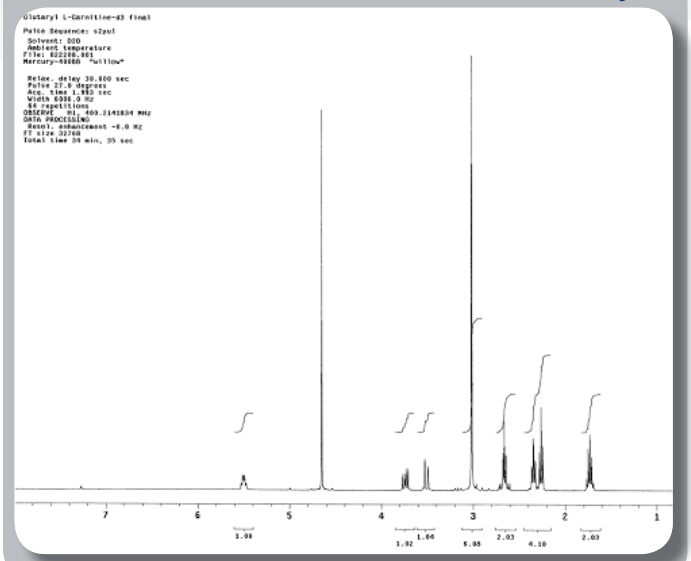
Acyl Carnitines

from Cambridge Isotope Laboratories

Cambridge Isotope Laboratories provides O-acyl carnitines of high chemical purity as individual components and kits. As part of this program, CIL offers:

- Straight chain O-acyl carnitines from C0 to C26 in high chemical purity with D_3, D_6, or D_9 labeling.
- Branched chain and other substituted O-acyl carnitines, including glutaryl, isovaleryl, 3-hydroxyisovaleryl, and 2-decenoyl carnitines, also with D_3, D_6, or D_9 labeling.
- High purity unlabeled reference standards corresponding to all labeled analogs.
- Kits prepared under batch record control, analyzed against certified standards with excellent reproducibility and quality assurance.

^{1}H NMR spectrum of O-glutaryl-L-carnitine (N-methyl-D_3)



Reference Materials

Before isotopically labeled carnitine standard solutions can be formulated and tested, corresponding unlabeled ("native") reference materials must be purified and characterized. We have observed that unlabeled materials available from other manufacturers are often of insufficient purity to use as reference standards. At CIL, we independently synthesize and purify each of these reference materials. The identity and purity of native carnitines are established using quantitative nuclear magnetic resonance (NMR) spectroscopy, high performance liquid chromatography (HPLC), and melting point determinations. Quantitative NMR is the primary analytical technique, using a common reference material for all the carnitines analyzed.

With pure, well-characterized reference materials in hand, we take similar steps to synthesize, purify, and analyze labeled carnitines. Enrichment, the amount of stable isotope incorporation, is measured relative to native analogs by NMR or liquid chromatography – mass spectrometry (LC/MS) techniques. The ^{1}H NMR spectrum of O-glutaryl-L-carnitine (N-methyl-D_3) is shown.

Unlabeled Certified Standard Solutions

Solutions of the unlabeled carnitines are formulated in triplicate following a formulation batch record. The gravimetry is traceable to U.S. National Institute of Standards and Technology (NIST) standards. The weights and balances are calibrated on a regular schedule. Class A volumetric glassware is used. These rigorous procedures allow us to control and calculate the uncertainty for concentrations of the unlabeled certified standard solutions, according to EURACHEM/CITAC guidelines.

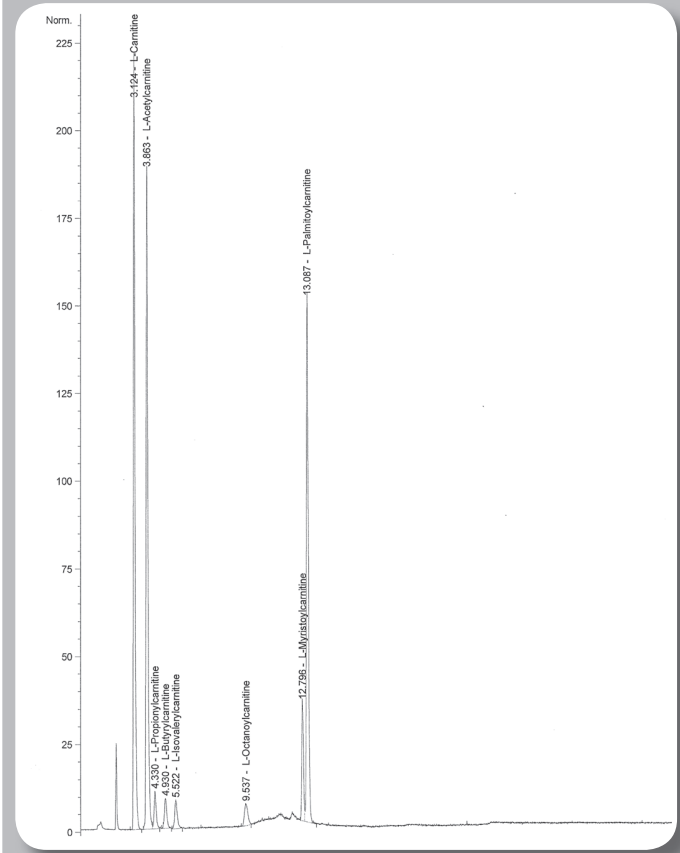
NSK-B Formulation and Dispensing

Labeled carnitine standard solutions are formulated using similar procedures. Once the concentration of the labeled carnitine solution has been verified against the unlabeled standard (described in detail, below), the solution is metered into vials using a calibrated pipette. The mass of solution added to each vial (and hence the amount of labeled standard) is individually verified. The transfer process is organized into discrete blocks, referred to as "dispenses", to enhance traceability. The solutions in the individual vials are evaporated under vacuum in a carefully controlled environment.

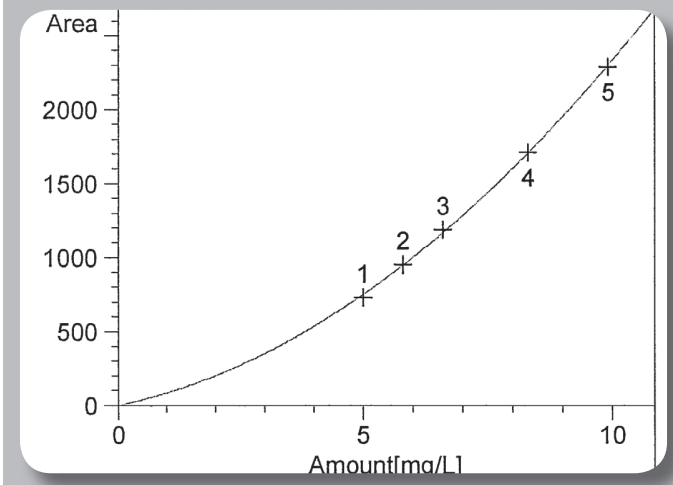
Sampling and Analysis

Samples of the finished product are taken to verify the reconstituted concentrations of the carnitines. Quality control samples are drawn according to American National Standards Institute / American Society for Quality Control (ANSI/ASQC) sampling guidelines.

Certified carnitine standards are formulated at five concentrations, bracketing the target concentrations for the product (0.750x, 0.875x, 1.000x, 1.250x, 1.500x). The carnitines are analyzed by HPLC, using an evaporative light scattering detector (LSD), which is sensitive to a wide range of materials, including carnitines, at low concentrations. Other typical HPLC detectors (e.g., ultraviolet, UV, RI) are not sensitive enough to analyze carnitines at the required concentrations. As with many analytical detectors, the response is non-linear. Quadratic or cubic equations are fitted to the calibration curves, with typical correlation coefficients ranging from 0.99995 to 0.99911. Calibration standards are run, interspersed among the product samples with typically 5 standard concentrations before each set of 5 (or 6) samples.

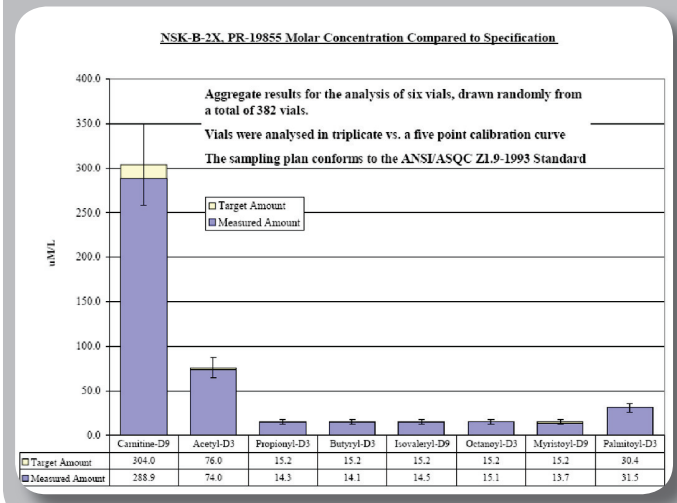


Quadratic Calibration Curve for L-Palmitoylcarnitine



Area = 9.992 x (Amount)^2 + 27.31 x (Amount) - 4.76 e^{-1} Correlation: 0.99984

NSK-B-2X, PR-19855 Molar Concentration Compared to Specification



	Carnitine-D9	Acetyl-D3	Propionyl-D3	Butyryl-D3	Isovaleryl-D9	Octanoyl-D3	Myristoyl-D9	Palmitoyl-D3
Target Amount	304.0	76.0	15.2	15.2	15.2	15.2	15.2	30.4
Measured Amount	288.9	74.0	14.3	14.1	14.5	15.1	13.7	31.5

Calculations and Results

The ELSD measures concentrations by weight (mg/L). To compare these values to the specification, the concentrations are converted to micro-moles per liter (µM/L). The measured molar concentrations compare well to the corresponding targets. The upper and lower bounds represent the target concentration +/- 15%.

Available from CIL:

NSK-B Labeled Carnitine Standards
Set B, 10 vials
NSK-B-1 Labeled Carnitine Standards
Set B, 1 vial
NSK B-G Supplemental Labeled Carnitine Standards
Set B-G, 10 vials
NSK-B-G-1 Supplemental Labeled Carnitine Standards
Set B-G, 1 vial

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