Inositol – Background and History

Myo-inositol was first discovered in 1850, but interest in the potential nutrition role of inositol did not begin until the 1940's. Inositol was named from the Greek word "inos" meaning "muscle" from which it was first extracted by Scherrer in 1850.

Myo-inositol is considered a quasi-vitamin or conditionally essential nutrient because it satisfies the criteria of vitamin status for only a few species or only under certain conditions.

Myo-inositol was found to be essential for the growth of most cells in culture. Deficiencies in rat and gerbil were shown to produce critical lipodystrophies such as hypolipidemia and fatty liver. Lipid transport now known to be function of VLDL requirement for myo-inositol.

Like other vitamins, inositol acts catalytically, but unlike other vitamins, inositol phosphates are energy-yielding compounds.

Free Inositol Chemical Structures

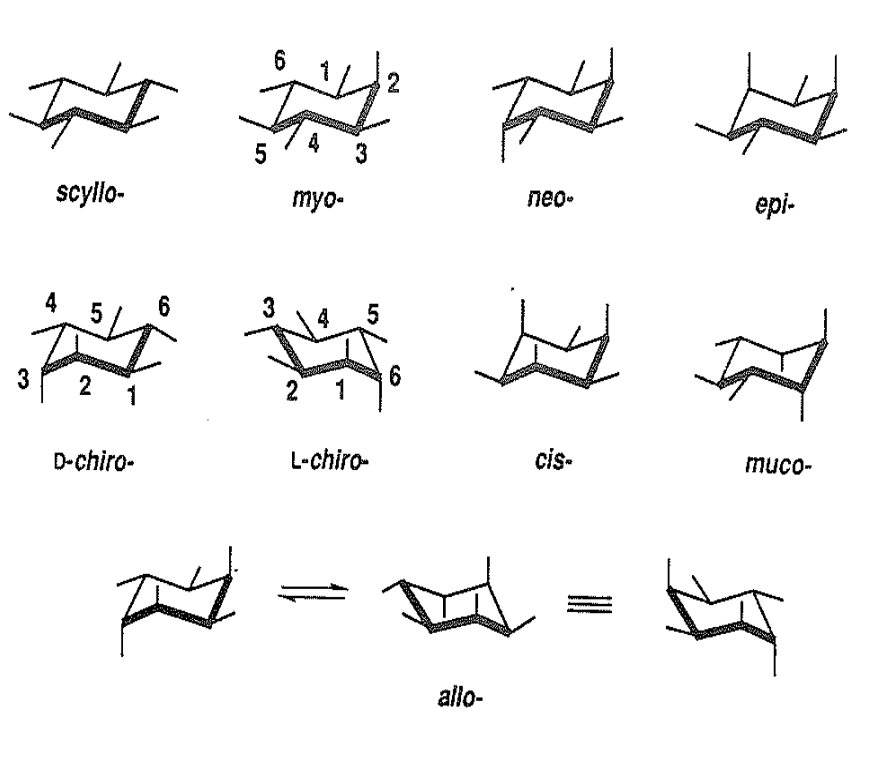


Fig 1. Chair configurations of all nine possible inositol isomers.

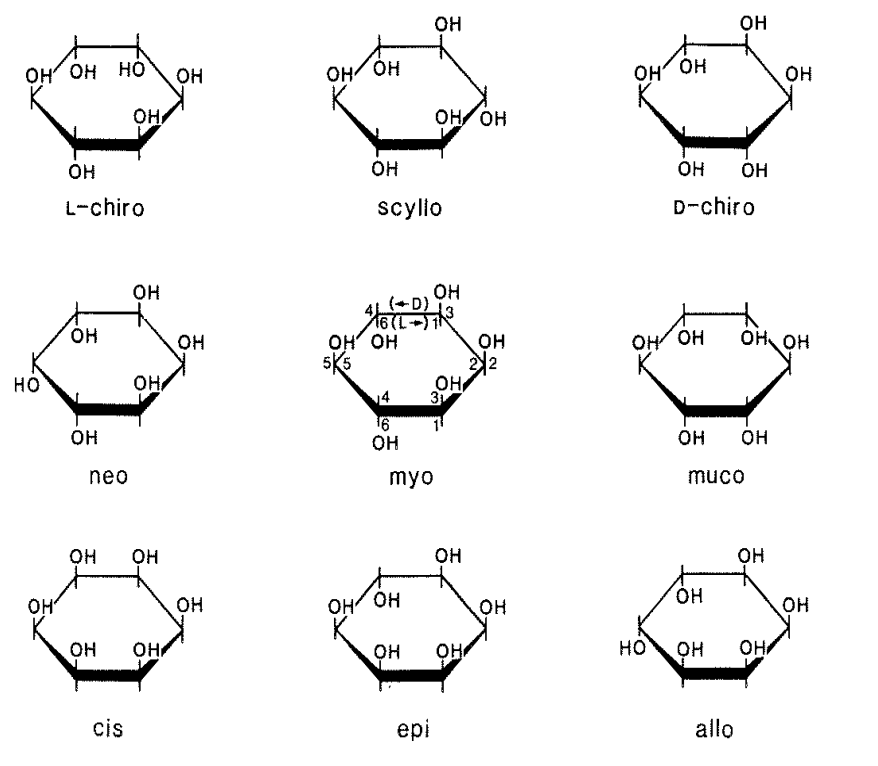


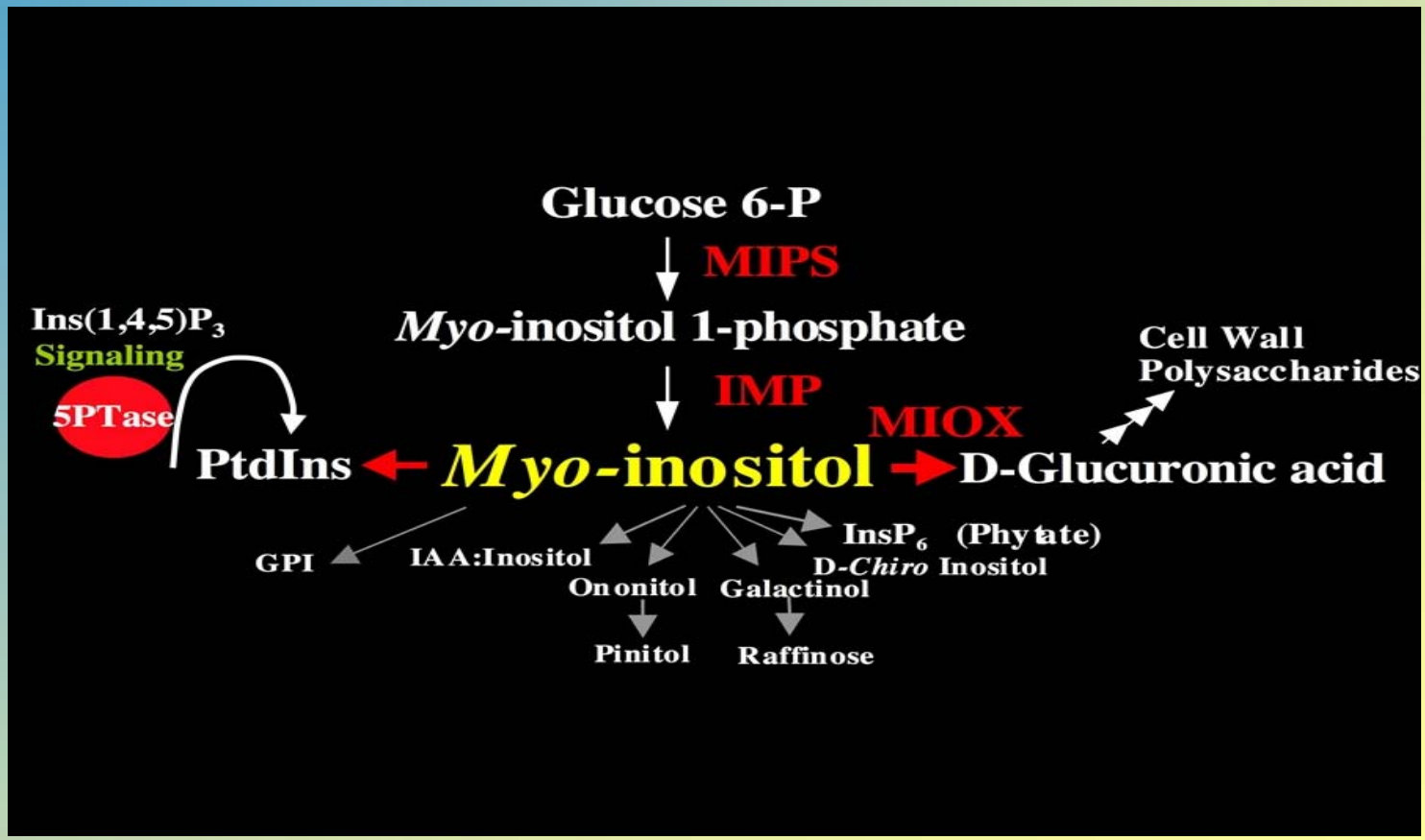
Fig 2. Chemical structures of all nine possible inositol isomers.

Forms of Inositol Found in Foods

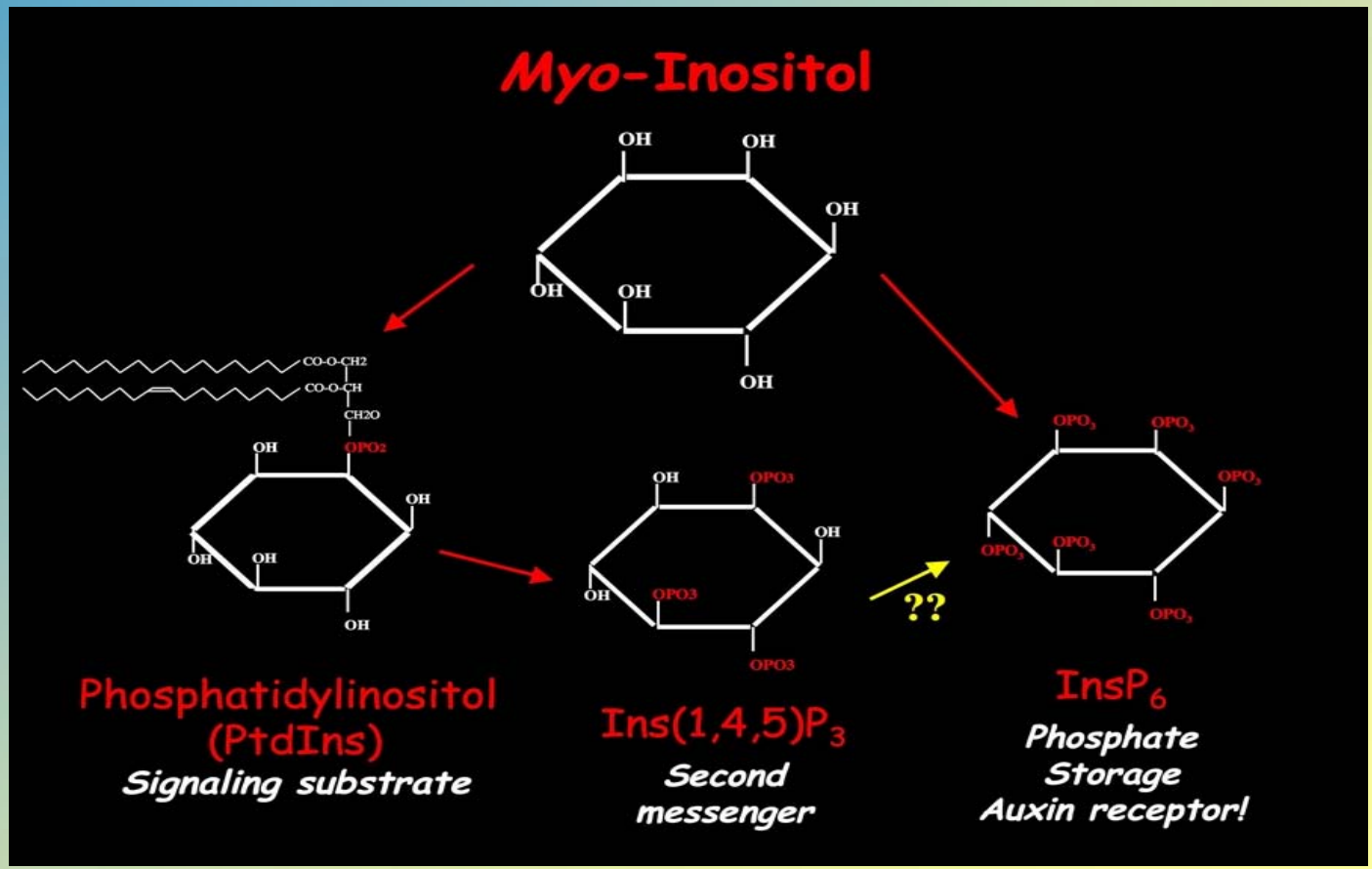
Myo-inositol occurs in foods as

1. free myo-inositol
2. phytic acid and inositol phosphates
3. inositol-phospholipids

Inositol Biosynthesis and Metabolism



Myo-inositol Biosynthesis



Free Inositols

Myo-inositol (optically inactive), is the only form of the nine cyclohexanehexols with known biological importance.

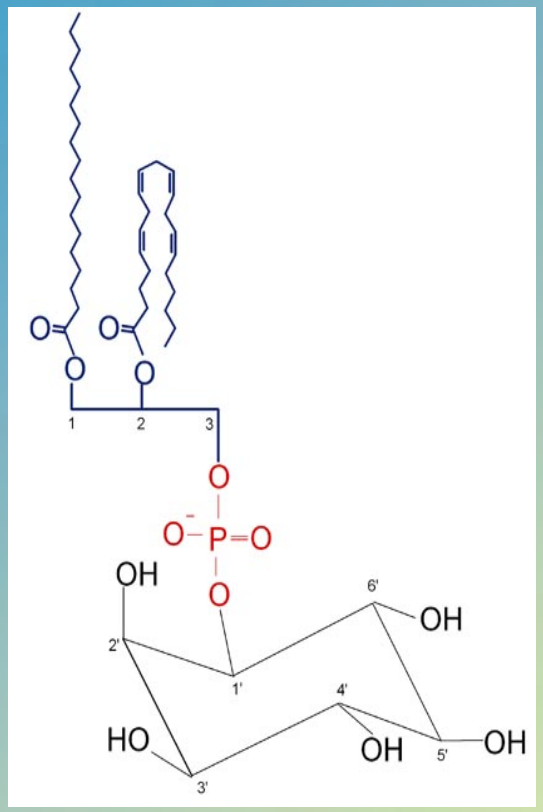
Although other free inositols such as chiro-, muco-, epi-, and scyllo have been isolated from natural sources, their roles are uknown.

Free myo-inositol is produced in all eukaryotic cells and so is present in all animal tissues.

Most if not all mammals can synthesize myo-inositol from glucose.

Free myo-inositol levels of 12-48 mg/100mL and 4-11 mg/100mL have been reported in human and cow milk respectively.

Phosphatidylinositol Chemical Structure and Description



Phosphatidylinositol is the primary myo-inositol containing phospholipid in animal products.

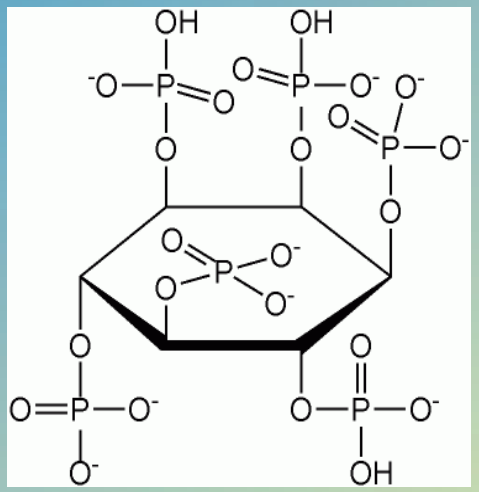
Phosphatidylinositols are predominately stearic acid at the 1-position and arachidonic acid at the 2-position.

Little information is available about the absorption mechanism for phospholipid myo-inositol, but it may be similar to that of phosphatidylcholine. In tissues myo-inositol is found as the free form, as phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-biphosphate (PIP2)

American diets typically provide ~ 900 mg myo-inositol per day with half of the myo-inositol in the phospholipid form.

Phosphatidylinositol levels of 0.22±0.09 mg/100mL and 0.36 ±0.10 mg/100mL have been reported in human and cow milk respectively.

Phytic Acid Chemical Structure and Description



Most of the inositol in plant material such as seeds and grains is present predominately as phytic acid and its salts (phytate).

Plants store phosphorus as phytic acid (myo-inositol hexaphosphate) until it is needed for seed germination.

Phytic acid forms insoluble salts which reduce the absorption of minerals from animals and humans.

Because most mammals have little or no intestinal phytase activity, phytic acid is poorly utilized as a source of myo-inositol. The theory that phytic acid might be completely hydrolyzed by the gut, absorbed as myo-inositol, and rephosphorylated intracellularly has not been proven.

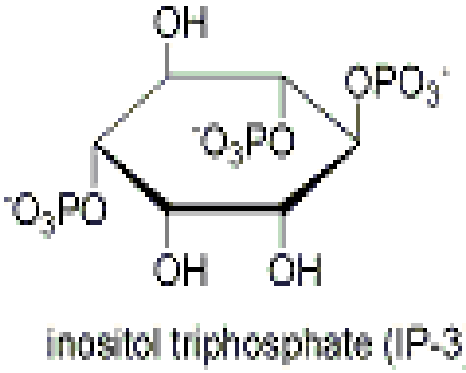
Inositol Phosphates

There are 66 possible myo-inositol phosphate isomers.

Approximately half of the possible isomers are present in mammalian cells.

The most widely recognized inositol phosphate is inositol 1,4,5-triphosphate (IP3), which serves as a second messenger to activate the release of Ca ions from intracellular stores.

Physiological function of each IP is determined by specific position of phosphate groups on inositol ring.



inositol triphosphate (IP-3)

Inositol Function

- Insulin signal transduction
- Cytoskeleton assembly
- Intracellular Ca^{2+} concentration control
- Maintenance of cell membrane potential
- Cell osmolite
- Serotonin activity modulation
- Lipid transport and catabolism
- Gene expression

Myo-inositol Metabolism in Mammalian Kidneys

In mammalian kidneys, myo-inositol oxygenase catalyzes the oxidation of myo-inositol to D-glucuronic acid (Fig 1)

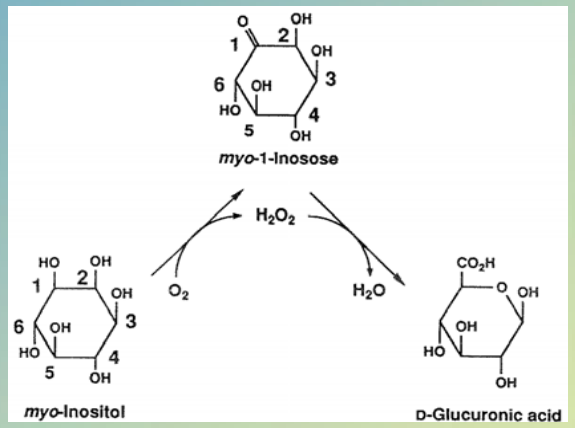


Fig 1. Mechanism of action of myo-inositol oxygenase.

The kidneys clear myo-inositol from plasma, convert it to glucose, and metabolize glucose to CO_2 by way of the pentose phosphate cycle. (Fig 2 and Fig 3)

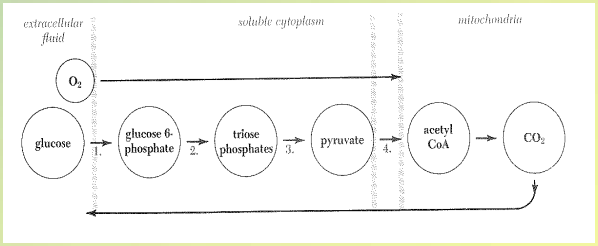


Fig 2. Oxidation of Glucose

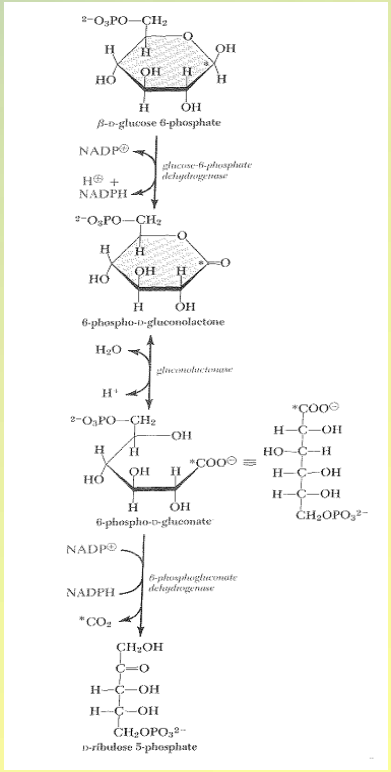


Fig 3. Pentose Phosphate Pathway

Recommended Minimum Daily Amounts

No reported deficiencies of inositol in humans.

No recommended daily intake of inositol, but the average diet supplies 300-1000 mg/day. Endogenous synthesis ~2-4g/day.

Myo-inositol addition to IF based on higher levels in human vs bovine milk.

ANZ, IFA, Codex, EU and GB minimum fortification of inositol in infant formula is 27 mg/L or 4 mg/100 kcal.

There are no maximum IFA or Codex inositol fortifications for infant formulas, but Codex has a guidance upper limit and ANZ, EU ,and GB have a maximum limit of 270 mg/L or 40 mg/100 kcal.

The current Codex standard (CODEX STAN 72-1981) does not define the forms (free and/or bound) of myo-inositol to be declared.

Why is Inositol Testing an Issue

No clear definition of what should be reported as inositol. Should inositol only include free myo-inositol or should inositol phosphates, and/or inositol containing phospholipids be included?

Limited or no inositol regulations for infant, infant follow-on, and adult nutritional products.

No official methods for measuring inositol.

Analytical Methods

1. Microbiological
2. Enzymatic
3. Gas Chromatography
4. High Performance Liquid Chromatography

Microbiological Inositol Method

Can be used to measure free or total myo-inositol (myo-inositol + inositol phosphates including phytate + inositol containing phospholipids) depending on how the sample is prepared. For total inositol analyses, samples are mixed with strong acid and heated for several hours at high temperatures.

Many microbiological methods are based on an adaptation of the procedure of Atkin et al. for the assay of vitamin B6.

Myo-inositol is extracted from samples with acid and filtered. A portion of the filtrate is inocculated with a microorganism and incubated for 16-20 hours. The myo-inositol concentration is calculated from the turbidity of the incubated sample compared to that of standards.

Microbiological method is a 2 day assay because of a 16-20 hour incubation.

In general microbiological methods are more prone to failure because of under or over growth of the microorganism.

In general microbiological methods are more imprecise than chromatographic methods.

Using a 10 gram sample size, the lowest confidence level of the microbiological method for free myo-inositol has been reported to be 3 mg/100 g with a coefficient of variation of 6%.

Enzymatic Methods

- Several Enzymatic methods for the quantitation of myo-inositol have been reported.
- These assays are based on the oxidation of myo-inositol and the reduction of NAD+ by myo-inositol dehydrogenase (MIDH) and use of either a coupled NADH-chromophoric end-point or a coupled NADH-malate fluorescence end-point.
- The sample preparation procedure for this assay can become complicated if samples contain glucose or NADH which must be destroyed with a base and an acid respectively before the analysis of myo-inositol can begin.
- This type of assay is relatively specific for myo-inositol, but several compounds such as ribitol, fructose, mannose, myo-inositol 2-monophosphate can interfere with the assay.
- This type of method demonstrates good sensitivity. One enzymatic inositol method reported a detection limit of 1 mg/L and an RSD of 9.5%.

Gas-Liquid Chromatography

Can be used to measure free or total myo-inositol (myo-inositol + inositol phosphates including phytate + inositol containing phospholipids) depending on how the sample is prepared. For total inositol analyses, samples are mixed with strong acid and heated for several hours at high temperatures.

Myo-inositol in prepared sample filtrates is converted to a hexa-O-trimethylsilyl ether. Prepared samples are chromatographed on conventional packed or capillary columns and detected by FID.

Analytical run times vary from 5-50 minutes. Most methods have not been developed for use with infant formula. Chromatograms can be complicated because of other carbohydrates present in infant and adult nutritional samples.

Myo-inositol has limited solubility in the reaction mixture used for the preparation of the TMS derivative.

Sample preparation is more labor intensive than LC methods because myo-inositol, which is insoluble in organic solvents, has to be converted to a compound that is soluble in organic solvent and can be volatized.

One total inositol method reported a detection limit of 1 mg/100g and an RSD of 15-35% using either acid or alkaline/enzyme digestion.

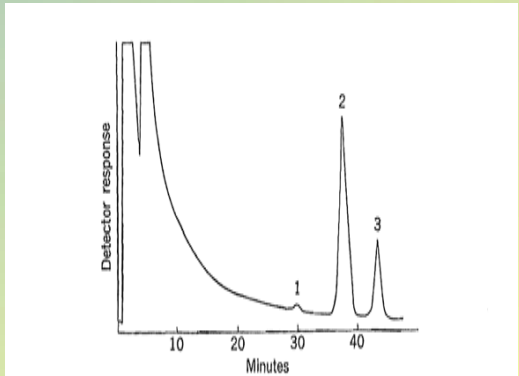


Fig. 1. A gaschromatogram of inositol extracted from human milk and triphenylmethane. 1, Scylloinositol; 2, myoinositol; 3, triphenylmethane.

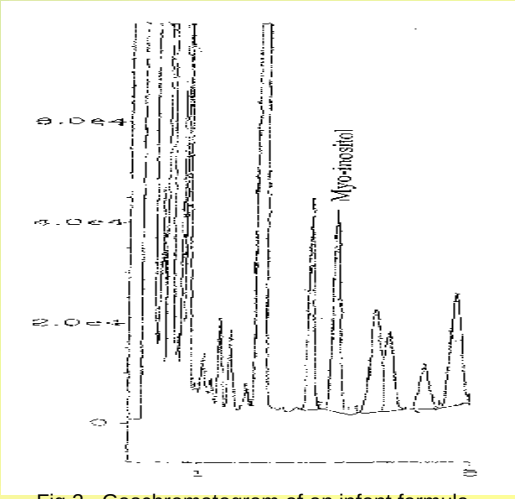


Fig 2. Gaschromatogram of an infant formula

Liquid Chromatography – RP and NP Methods

Several RP and NP methods with UV or fluorescence detection reported.

Methods based on pre-column derivatization of sample extracts with phenylisocyanate, *p*-nitrobenzoyl chloride, or isatoic anhydride; RP chromatographic separation with C_{18} or amino columns, or NP separation on silica columns. Sample extracts are prepared by precipitating proteins with dilute acid and lyophilizing a portion of sample filtrate.

UV detection demonstrate moderate sensitivity, fluorescence detection has higher sensitivity.

Typical derivatization times are ~ 60 min. Analytical run times vary from ~20-50 min. Most of these methods developed for use with biological samples. Chromatograms can be complex due to other carbohydrates present in milk, infant and adult nutritionals.

Methods can be used to measure free or total myo-inositol (myo-inositol + inositol phosphates including phytate + inositol containing phospholipids) depending on how the sample is prepared.

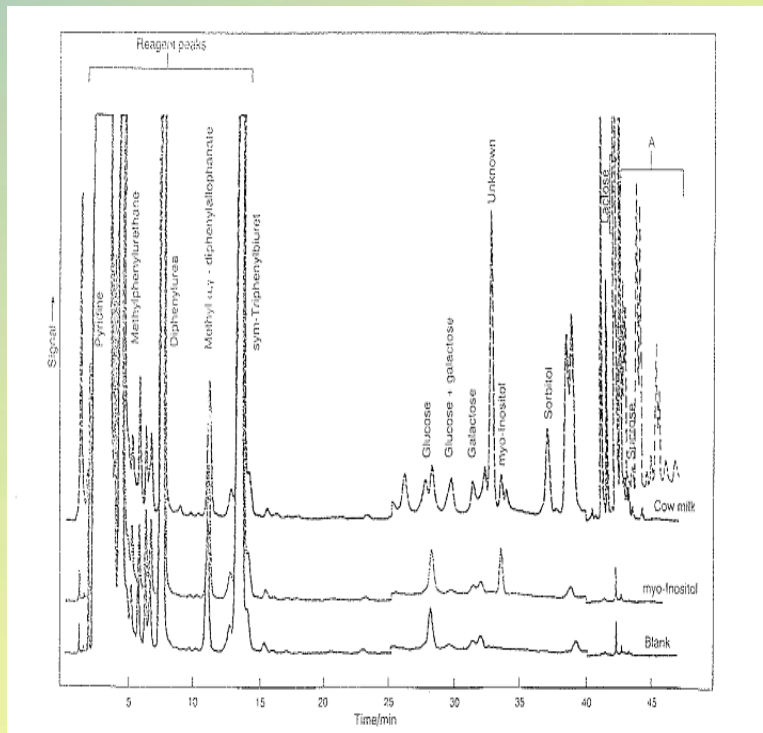


Fig. 1 Overlay chromatograms of phenylurethane derivative extracts of lyophilizates. Column: 5 µm Resolve C_{18}; gradient elution as described in Table 1. Detection: 240 nm; 0.16 a.u.f.s. (25–40 min). Injection volume, 5 µl. A, components unique to human milk

Liquid Chromatography – Metal Ligand

Methods using metal ligand chromatography with refractive index (RI), evaporative light scattering (ELSD), and pulsed amperometry (PAD) detection reported.

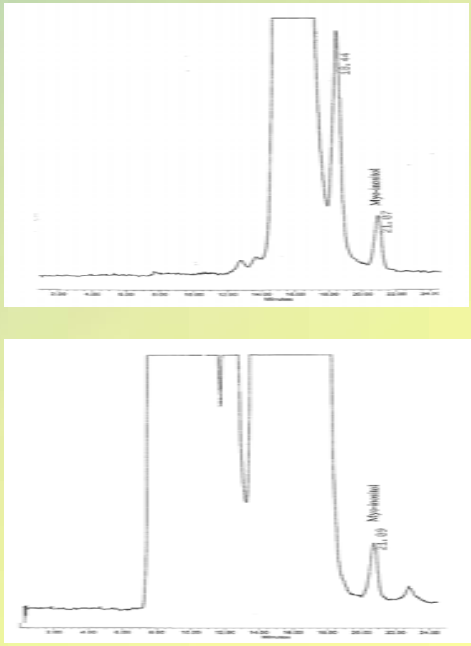
Myo-inositol is extracted from samples with dilute acid and filtered. Filtrate neutralized and separated with a water mobile phase on Ag, Ca, or Pb metal-ligand columns. Column eluant analyzed directly by RI or ELSD, or mixed with a high pH solution (eg NaOH) and detected by PAD. For total inositol analyses, samples are mixed with strong acid and heated for several hours at high temp.

RI and ELSD detection systems have moderate sensitivity. PAD has good sensitivity, but requires use of corrosion resistant pumps and system tubing. With PAD some sensitivtiy is lost because of the dilution of the column eluant. Metal ions that leach from the metal-ligand columns can interfere with the PAD detector.

Chromatography is complicated because of other carbohydrates present in infant and adult nutritional samples. RI and ELSD are not specific for myo-inositol while PAD is specific for carbohydrates.

Method can be used to measure free or total myo-inositol (myo-inositol + inositol phosphates including phytate + inositol containing phospholipids) depending on how the sample is prepared.

One metal-ligand method reported a quantitation limit of 60 mcg/L in prepared samples and an RSD of 2.6%.



Separation of myo-inositol in infant formulas with a silver metal-ligand column and PAD detection

Liquid Chromatography – Ion Exchange

Methods using ion chromatography with pulsed amperometry detection (PAD) have been reported.

Methods use dilute acid to precipitate proteins and extract myo-inositol. For total inositol analyses, samples are mixed with strong acid and heated for several hours at high temperatures. Sample filtrates are analyzed using a hydroxide mobile phase with anion exchange chromatography and PAD.

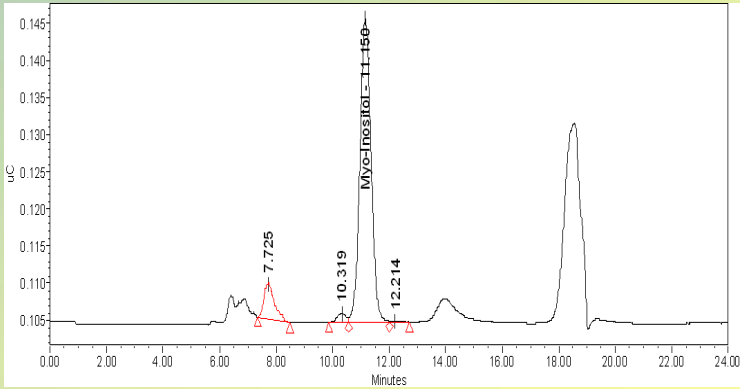
Anion exchange with PAD detection has good sensitivity.

Depending on system configuration, run times can be ~ 25 min or several hrs. For infant nutritionals, run times can be shortened from 2-3 hr to ~ 25 min by column switching.

Anion exchange with a hydroxide mobile phase requires a corrosion resistant system.

Ion exchange methods can be used to measure free or total myo-inositol (myo-inositol + inositol phosphates including phytate + inositol containing phospholipids) depending on how the sample is prepared.

One ion exchange method reported a quantitation limit of 13 mcg/L in prepared samples and an RSD of 2.5%.



Analysis of an infant formula using anion exchange chromatography with a sodium hydroxide mobile phase and PAD

Analytical Challenges

Challenges will depend on forms of myo-inositol to be measured.

Fewer challenges if only free myo-inositol is to be measured.

If specific phosphorylated forms of myo-inositol are to be included in a myo-inositol measurement, developing a sample preparation procedure to selectively release bound forms will be challanging.

Soy-based formulas will provide additional problems due to phytate.

Other challenges include eliminating carbohydrate interferences without significantly increasing sample preparation time or analysis time.

Matrices

Milk and milk-based infant and adult nutritional formulas

Whey-based infant and adult nutritional formulas

Soy-based infant and adult nutritional formulas

Rice-based infant and adult nutritional formulas

Hydrolyzed protein-based infant and adult nutritional formulas

Amino acid-based (with and without intact protein), infant and adult nutritional formulas