Simple method for determination of food additives E953 and E965

Hydrogenated compounds produced from starch and sucrose

Authors: Sylvain Morel[1], Pranathi Perati[2], Julie Moriceau[1], Jeff Rohrer[2], Jean-François Garnier[1]

[1]Thermo Fisher Scientific Customer Solution Center, Villebon / Yvette FRANCE
[2]Thermo Fisher Scientific, Sunnyvale, CA, USA

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Goal

Develop an easy method for determining hydrogenated compounds in foods using eluent generation, HPIC, and HPAE-PAD technologies

Introduction

Polyols are commonly used in food formulations as sweeteners, emulsifiers, stabilizers, and texturizers, and their use in food applications is regulated. In Europe, a list of approved food additives and their conditions of use has been established by Regulation (EU) 1129/2011.[1,2] The pharmaceutical industry also uses polyols as excipients. Many publications report that polyols like sorbitol and xylitol are found naturally in fruits and vegetables. However, most polyols used for products are “synthetic” polyols based on starch or sucrose transformations. For example, isomalt (E953) is prepared from sucrose via enzymatic and chemical processes. Sucrose is converted to isomaltulose and then hydrogenated to isomalt. Isomalt synthesis results in a mixture of two diastereomeric disaccharides, -α-D-glucopyranosyl-1,6-mannitol and -α-D-glucopyranosyl-1,6-sorbitol. Maltitol is derived from a different route. Its synthesis is based on maltose hydrogenation. Maltitol (E965) is also named 4-O-α-glucopyranosyl-sorbitol. Maltitol and isomalt are structurally very similar, making their determination in products containing both a challenge.

Polyols have several health advantages due to their lower caloric value in comparison with common sugars and the fact that they do not promote tooth decay. These advantages have resulted in their global use as sugar substitutes (sugar substitutes = high- and low-intensity sweeteners and fructose syrups). The market for polyols is predicted to increase by 2026, and most of the increase will be in the food and beverage (F&B) market.[3] Demand for polyols in oral care products will also increase but remain significantly lower than F&B. Based on this information, analytical demand for robust analytical methods will increase. To improve analytical sample throughput, we developed a simple method for polyols analysis based on ion chromatography. The first pillar of our development was "just add water" in our system, ensuring high quality eluent production and a safe environment for your scientists. The second pillar is a high-performance chromatography system to ensure a good ratio between run time and analytical quality. This application brief describes a simple method for determination of isomalt and maltitol additives in food samples using the recently introduced Thermo Scientific™ Dionex™ CarboPac™ PA300-4µm column and electrolytic eluent generation.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-6000™ HPIC™ system:
  - Dionex ICS-6000 DP Dual Pump: Analytical Gradient—Analytical Isocratic with Degas (P/N 21181.60009)
  - Dionex ICS-6000 EG module (P/N 22181-60019), with 1 – Degas Unit for EG (SB/MB) (P/N 075522)
  - Dionex ICS-6000 DC with Dual Temperature Zones, Two Injection Valves, Microbore (P/N 22181-60049)
  - Thermo Scientific™ Dionex™ Electrochemical Detector (P/N 072042)
  - Valve pod 0.4 µL (P/N 074699)
  - Thermo Scientific™ Dionex™ AS-AP Autosampler and sample tray cooling, 250 µL sample syringe (P/N 074925)
  - Dionex ICS-6000 ED Electrochemical Detector cell (P/N 072044)
  - Dionex ED electrode, Au, with gasket and polishing kit (P/N 079850)
  - Dionex ED reference electrode pH, Ag/AgCl (P/N 061879)
  - Diverter Valve Assembly, includes one 2 way 6-port valve and mounting hardware (P/N 074123)
  - Thermo Scientific™ Dionex™ EO Eluent Organizer Tray with two 2-liter bottles (P/N 072057)
  - Thermo Scientific™ Dionex™ IC PEEK Viper™ Fitting Kit for Dionex ICS-6000 with ED (P/N 088804)
  - Degas unit for EG (P/N 075522)
- Thermo Scientific™ LP Vortex Mixer (P/N 15298834)
- Fisherbrand™ Analytical Balance (Model FAS224)
- Thermo Scientific™ Barnstead™ Smart2Pure™ Pro water purification (Model Smart2pure Pro UV/UF 16LPH)
- Thermo Scientific™ F1-ClipTip™ Variable Volume Single Channel Pipette 2–20 µL (P/N 4641180N)
- Thermo Scientific™ F1-ClipTip™ Variable Volume Single Channel Pipette 20–200 µL (P/N 4641210N)
- Thermo Scientific™ F1-ClipTip™ Variable Volume Single Channel Pipette 100–1,000 µL (P/N 4641230N)

Many laboratories seek advanced, sensitive workflows with optimized analysis efficiency and productivity while reducing the manual burden placed on their operators. For these reasons, we used an instrument setup based on a Thermo Scientific™ Dionex™ ICS-6000™ HPIC™ system (Figure 1). This modular platform equipped with an eluent generator (EG) is the perfect tool for meeting the needs of these laboratories. Using this configuration, the analyst just needs to add water to ensure accurate and reproducible eluent preparation and consumables regeneration. In addition, electrolytic eluent generation reduces pump maintenance because the pump seals and pistons come in contact only with deionized water. Additionally, this technology enables the generation of gradients without a proportioning valve. The high-pressure pump allows the use of 4 µm columns to produce high-resolution separations that enable reductions in analysis time. Pulsed amperometric detection was chosen to ensure high analyte sensitivity and selectivity when working with complex samples.

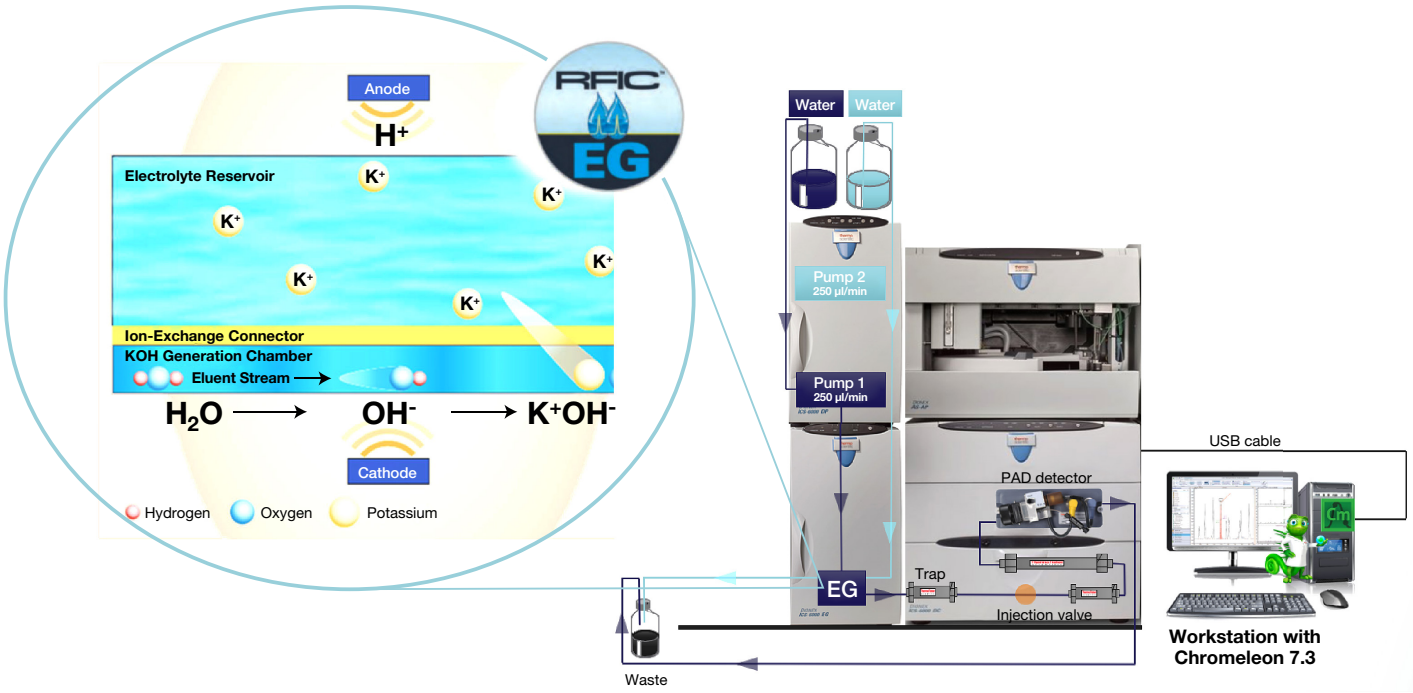

Figure 1. Schematic principle of Reagent-Free™ Ion Chromatography Eluent Generation (RFIC™-EG) and Dionex ICS-6000 fluidic pathway

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.3 or above

Reagents and consumables

- Dionex CarboPac PA300-4µm analytical column, 2 × 250 mm (P/N 303346)
- Dionex CarboPac PA300-4µm guard column, 2 × 50 mm (P/N 303347)
- Thermo Scientific™ Dionex™ BorateTrap™ inline trap column (P/N 047078)
- Gold working electrode (1 mm) with 1 mil gaskets (P/N 061875, 1 mil = 25.4 µm)
- Reference electrode pH, Ag/AgCl (P/N 061879)
- Thermo Scientific™ Titan3™ syringe filter, 17 mm PVDF membrane (0.45 µm, P/N 44513-PV)
- FisherBrand™ 1 mL plastic syringe PP (Fisher Scientific Cat No. 14955456)
- Thermo Scientific™ Dionex™ AS-AP Autosampler Vial Kits, 1.5 mL glass with caps and septa, 100 Each (P/N 055427)
- Thermo Scientific™ Dionex™ CR-ATC 600 (P/N 088662)
- Thermo Scientific™ Dionex™ EGC 500 KOH cartridge (P/N 075778)
- D-Sorbitol, 97%, ACROS Organics™ (Fisher Scientific Cat No. 132730050)
- Xylitol, 99+%, ACROS Organics™ (Fisher Scientific Cat No. 225985000)
- D-Mannitol, ACS reagent ACROS Organics™ (Fisher Scientific Cat No. 423922500)
- Maltitol, 95%, ACROS Organics™ (Fisher Scientific Cat No. 295800250)
- Isomalt, Sigma-Aldrich™ (PHR1769-1G)
- Myo-inositol, 99+%, Sigma-Aldrich™ (I5125-50G)

Instrument method

Table 1. IC conditions

Parameter	Value
Column	Dionex CarboPac PA300-4µm, 250 × 2 mm with guard column
Eluent	KOH gradient generated by Dionex ICS-6000 EG Eluent Generator For details, see Table 2
Eluent source	Dionex EGC 500 KOH cartridge (P/N 075778), Dionex CR-ATC 600 trap column (P/N 088662) externally regenerated with DI water at 200 µL/min flow rate, high-pressure degasser module A Dionex BorateTrap column was installed between the eluent generator and the injection valve.
Flow rate (eluent)	250 µL/min
Injection volume	0.4 µL
Column temperature	30 °C
Detection	Pulsed amperometric detection at 20 °C Waveform—Table 3

Table 2. KOH eluent program generated by the Dionex ICS-6000 EG module

Time (min)	Concentration (mM)
0	50
11	50
11	90
16	90
16	50
31	50

Table 3. PAD waveform (vs. Ag/AgCl)

Time (ms)	Voltage (V)	Integration
0	0.100	Off
200	0.100	On
400	0.100	Off
410	-2.000	Off
420	-2.000	Off
430	0.600	Off
440	-0.100	Off
500	-0.100	Off

Sample preparation

Diluent preparation: Dissolve 100 mg of sodium azide in DI water in a 1 L volumetric flask.

Polyols stock solution (100 ppm): Weigh 100 mg of each compound in a separate 100 mL volumetric flask; dissolve powders in diluent solution using a magnetic stirrer at room temperature. Dilute 1 to 10 with fresh diluent. The ready-to-use stock solution was stored at 4 °C.

Sample preparation: If necessary, homogenize the solid sample to a fine powder with liquid nitrogen. Add 1 g of homogenized sample to 96 mL of methanol/aqueous solution (10/90 vol/vol). Ultrasonicate the suspension for 20 min and stir for 20 min using a magnetic stirrer. Add 2 mL each of Carrez I solution and Carrez II solution (for Carrez solutions preparation, refer to AN248[4]) to the sample solution and stir for 5 min. Allow particles to settle overnight and dilute supernatant 1:100 using the diluent (the dilution step can be adjusted based on your sample concentration). Filter (0.45 µm) the diluted sample into an injection vial.

Results and discussion

Figure 2 shows a chromatogram obtained from a low volume injection (0.4 µL) of the polyols standard solution. Six polyols were separated in less than 10 min. All peaks were baseline resolved. The total run time, including column rinse and equilibration, was 31 min. The rinsing step removes stronger retained anionic compounds that are present in some food samples. Isomalt and maltitol calibration curves up to 100 µg/mL were fit with linear models (Figure 3). The chromatograms in Figure 4 illustrate results of representative samples obtained injecting 1:100 diluted samples. In the mint sweet, small quantities of sorbitol could be detected in the presence of large quantities of isomalt and maltitol.

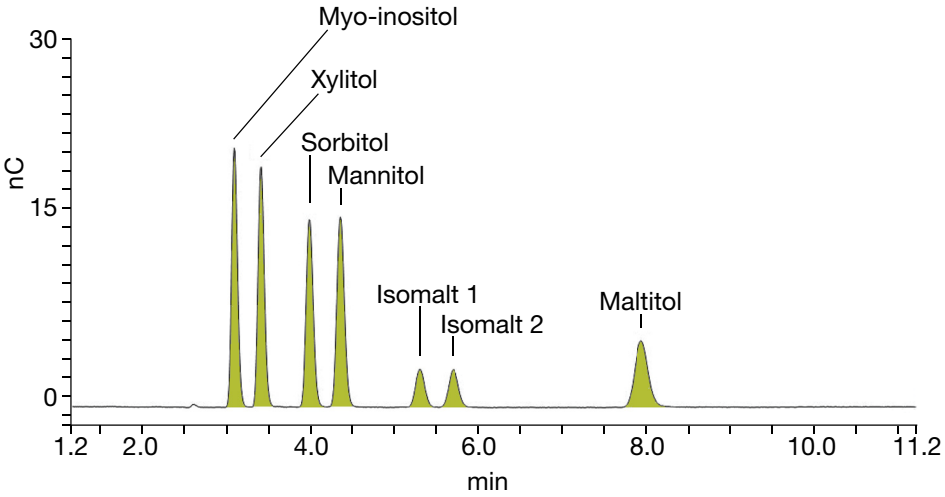

Figure 2. Chromatogram showing the separation of a 6.25 µg/mL polyols standard solution using a Dionex CarboPac PA300-4µm column on a Dionex ICS-6000 system

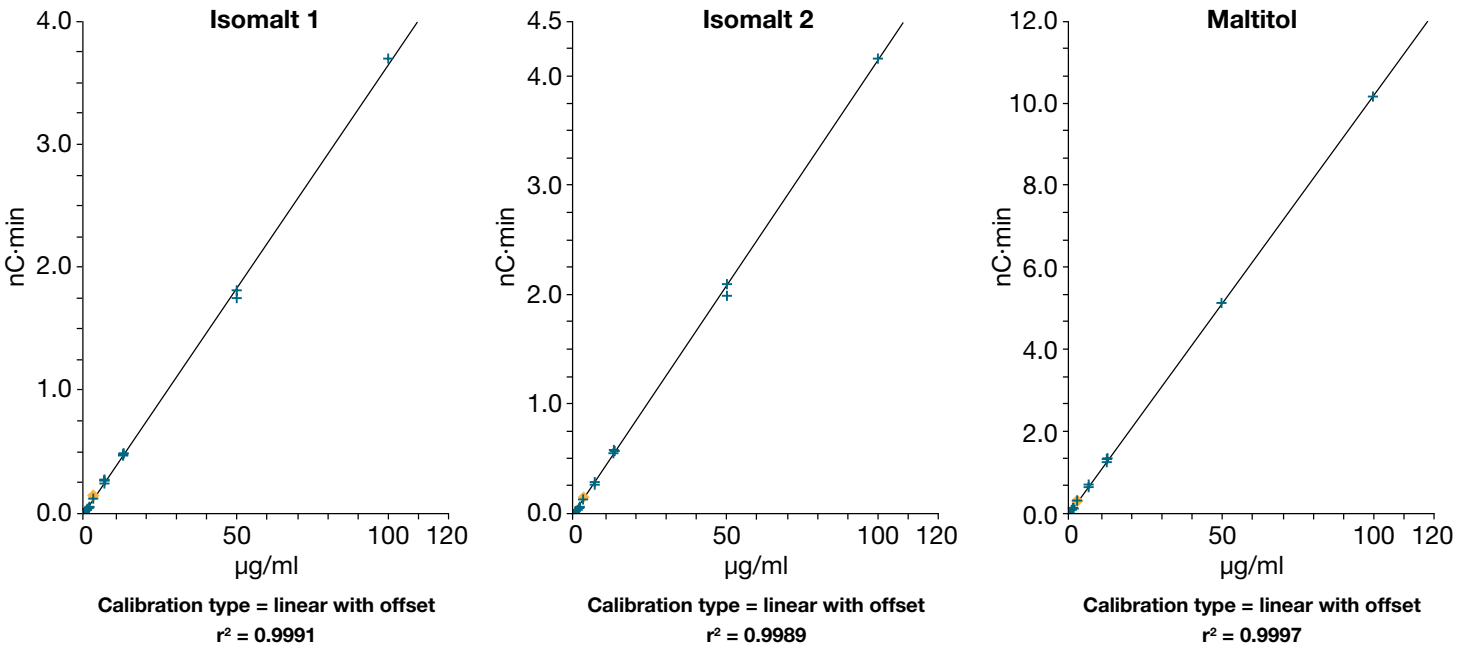

Figure 3. Calibration curves for isomalt 1 & 2 and maltitol

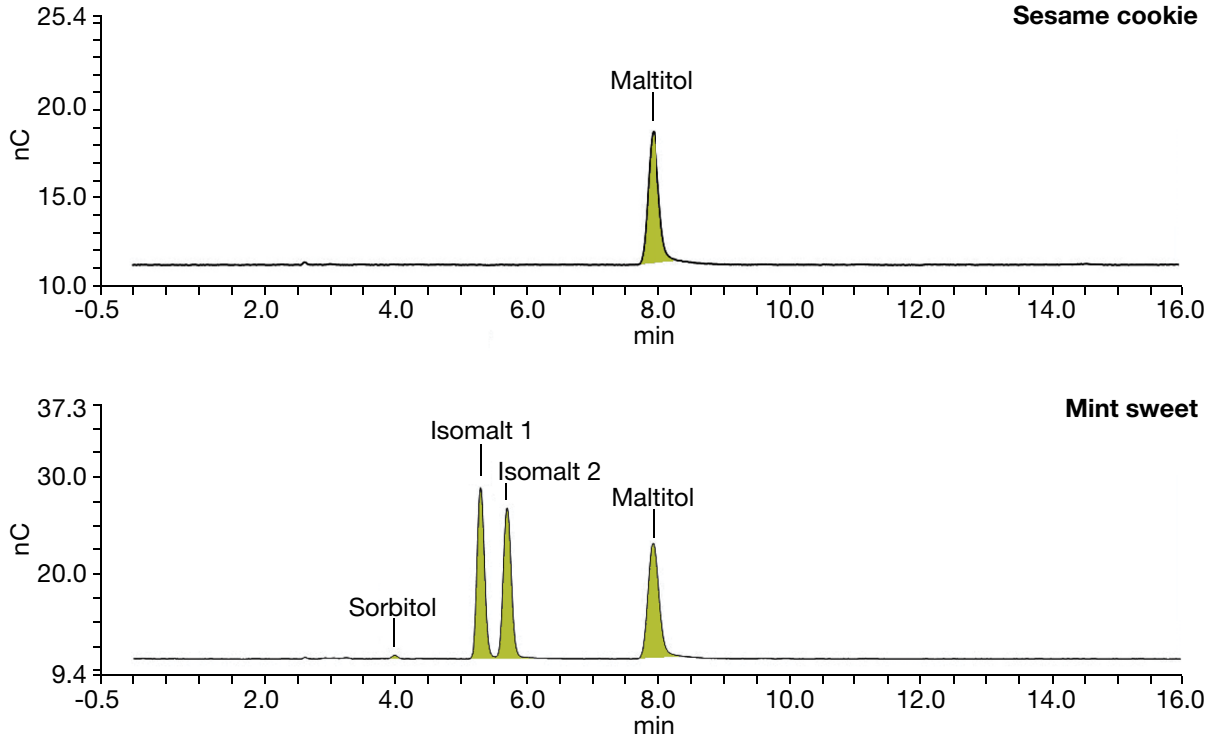

Figure 4. Chromatograms obtained after injection of 1:100 diluted extract of food samples (upper trace: sesame cookie and lower trace: mint sweet)

To evaluate this method, we challenged the system robustness by repeated injections of food samples. The standard mixture (QC solution) was injected twice before and after each block of sample injections (10 injections). Figure 5 illustrates maltitol peak area stability in samples and the QC solution. Table 4 summarizes analytical results for all detected compounds in this food sample: sorbitol, isomalt 1&2, and maltitol. Data collected from the 85 injections in Figure 5 show that the peaks areas remain stable. Relative standard deviations remain very stable, ranging from 0.61% for sorbitol to 0.37% for isomalt.

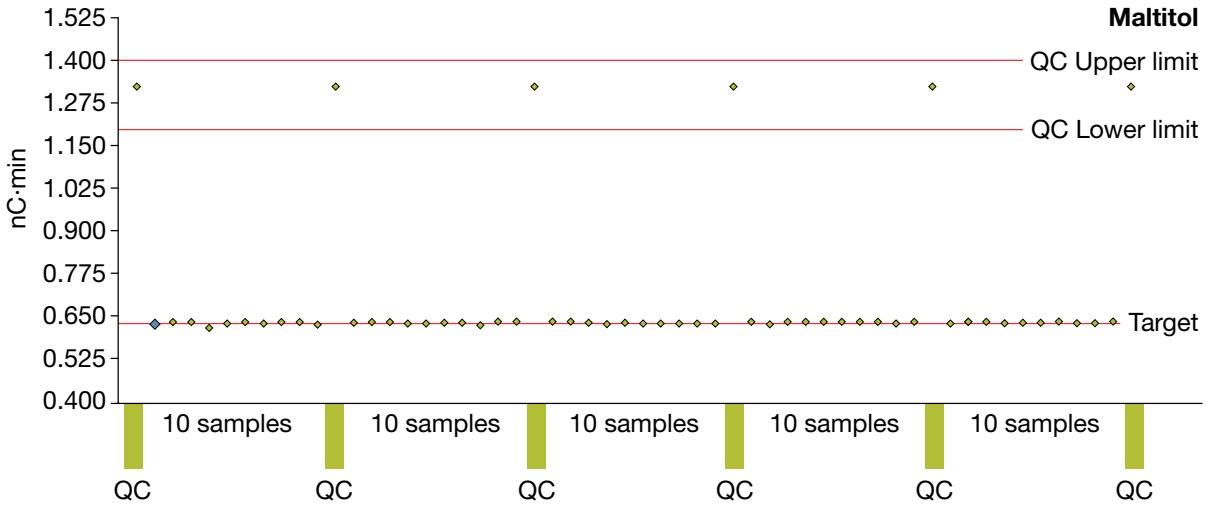

Figure 5. Maltitol peak area trending using the automated control chart feature in Chromeleon CDS

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Table 4. Analytical parameters: Mean and relative standard deviation. Calculations are based on the 85 food sample injections.

Area (nC·min)	Sorbitol	Isomalt 1	Isomalt 2	Maltitol
Mean	1.32	2.04	2.06	0.64
RSD	0.61	0.42	0.37	0.55

Conclusion

- This HPAE-PAD method is reliable and allows separation and quantification of polyols in complex samples.
- The eluent generator improves operator safety and workflow accuracy due to minimal need for manual eluent generation. Automated electrolytic eluent generation ensures the generation of high-purity and accurate eluent concentrations, delivering consistent and reproducible retention time and baseline stability. These benefits translate to increased uptime and throughput. Reagent-free, electrolytic eluent generation eliminates the variation inherent to manual eluent preparation and related CO_2 intrusion. Automatic electrolytic eluent generation not only takes the manual labor out of ion chromatography, but it is also easy to adopt.
- Automated data reprocessing with Chromeleon CDS workstation software enables fast method implementation in labs engaged in high-throughput work.

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