ANNEX 4

Analytical data of representative batches of D-tagatose

Analytical data of representative batches of D-tagatose

Analytical data of six representative batches of D-tagatose produced at Nordstemmen (Germany) are presented in Table 1 of this Annex.

Analytical data of six batches of D-tagatose produced at pilot-plant scale are shown in Table 2. HPLC elution profiles of a standard carbohydrate mixture (containing lactose, glucose, galactose, fructose and D-tagatose) and of four representative batches of D-tagatose are shown in Figures 1-5.

Result of an additional analysis of lactose by HPLC and enzymatic test in D-tagatose.

Result of ELISA test of one batch of D-tagatose confirming absence of whey proteins.

Table 1 Analysis of six different batches of D-tagatose from production in Nordstemmen, Germany

Parameter	Batch No.					
	12903 Date: 09.05.03	**13003 Date: 10.05.03**	**13103 Date: 11.05.03**	**16503 Date: 14.06.03**	**16403 Date: 13.06.03**	**16803 Date: 17.06.03**
Loss on drying (%)	<0.01 *	<0.01 *	<0.01 *	<0.01 *	<0.01 *	0.1 *
Assay (%)	99.04	99.06	99.34	99.63	99.69	99.31
D-Galactose (%)	0.65	0.21	0.15	0.37	0.31	0.69
Total ash (%)	<0.01 *	<0.01 *	<0.01 *	<0.01 *	<0.01 *	0.1 *
Arsenic (ppm)[a]	< 0.5 *	< 0.5 *	< 0.5 *	< 0.02 *	< 0.02 *	< 0.02 *
Cadmium (ppm)[a]	< 0.1 *	< 0.1 *	< 0.1 *	< 0.01 *	< 0.01 *	< 0.01 *
Mercury (ppm)[a]	< 0.1 *	< 0.1 *	< 0.1 *	< 0.02 *	< 0.02 *	< 0.02 *
Lead (ppm)[a]	< 0.1 *	< 0.1 *	< 0.1 *	< 0.05 *	< 0.05 *	< 0.05 *
Total (aerobic) plate counts (g^{-1})[c]	< 10 *	< 10 *	< 10 *	< 10 *	< 10 *	< 10 *
Coliforms (g^{-1})[d]	< 10 *	< 10 *	< 10 *	< 10 *	< 10 *	< 10 *
Salmonella[e]	neg.	neg.	neg.	neg.	neg.	neg.

[a] GFAAS

[b] Hy-AAS for 12903, 13003 and 13103. FIMS/SM 3112 for 170661, -662 and -663.

[c] ME198 for 12903, 13003 and 13103. IDF 100B; 1991 for 170661, -662 and -663.

[d] ME168

[e] ME240 and NMKL 71,5 udg.

* Limit of detection. The detection limits are different because different laboratories have made the analysis.

Table 2 Analytical data of six batches of D-tagatose produced at a pilot-plant scale

Parameter [a]	Batch No.					
	B 97 1041	**B 97 1143**	**B 97 1144**	**B 97 1148**	**B 96 1122**	**B 96 1123**
Loss on drying (%)	0.04	0.19	0.12	0.17	0.17	0.18
Assay (%) [b]	99.80	99.53	99.47	99.61	99.60	99.90
D-Galactose (%)	0.20	0.47	0.53	0.39	0.40	0.10
Total ash (%)	< 0.1 *	< 0.1 *	< 0.1 *	< 0.1 *	< 0.1*	< 0.1*
pH	5.80	5.09	5.07	5.02	4.93	4.55
Arsenic (ppm)	< 0.7 *	< 0.7 *	< 0.7 *	< 0.7 *	< 0.7*	< 0.7*
Cadmium (ppm)	< 0.03 *	< 0.03 *	< 0.03 *	< 0.03 *	< 0.03*	< 0.03*
Mercury (ppm)	< 0.01 *	0.05	0.015	0.02	0.19	0.05
Lead (ppm)	< 0.2 *	< 0.2 *	< 0.2 *	< 0.2 *	< 0.2*	< 0.2*
Total (aerobic) plate counts (g^{-1})	600	200	< 100	< 100	< 100	< 100
Coliforms (g^{-1})	< 10	< 10	< 10	< 10	< 10	< 10
Salmonella	neg.	neg.	neg.	neg.	neg.	neg.

[a] except for "Assay" as defined in "Specifications" (see Annex 3)

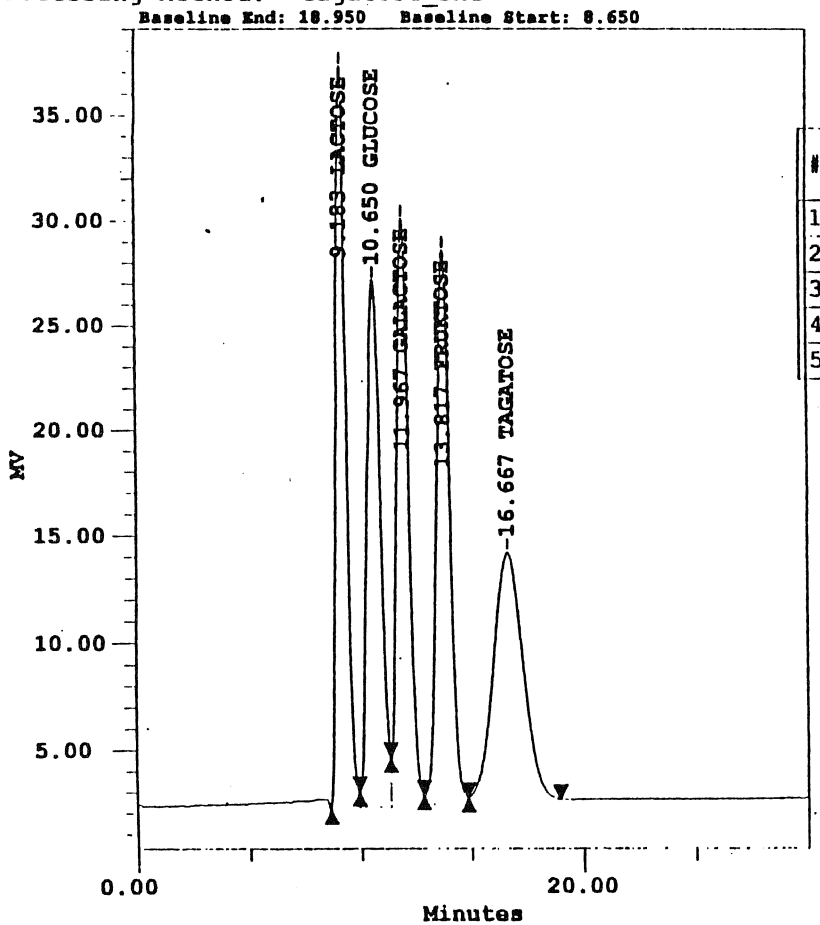
[b] Determined from respective peak area (Sum of all peaks = 100%) (see figs. 2-5 of this annex for illustration)

* limit of detection

Figure 1

Millennium Sample Information

Project Name:	tagatose	Username	SYSTEM
Sample Name:	STANDARD Mixture	Set Name	9802102t
Vial:	1	Sample Type:	Standard
Injection:	1	Volume:	20.00 µL
Channel:	410	Run Time:	30.0 min
Date Acquired:	11-02-98 02.02.53	Date Processed:	11-02-98 07.23.07
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	tagatosemetode		
Processing Method:	tagatose_int		



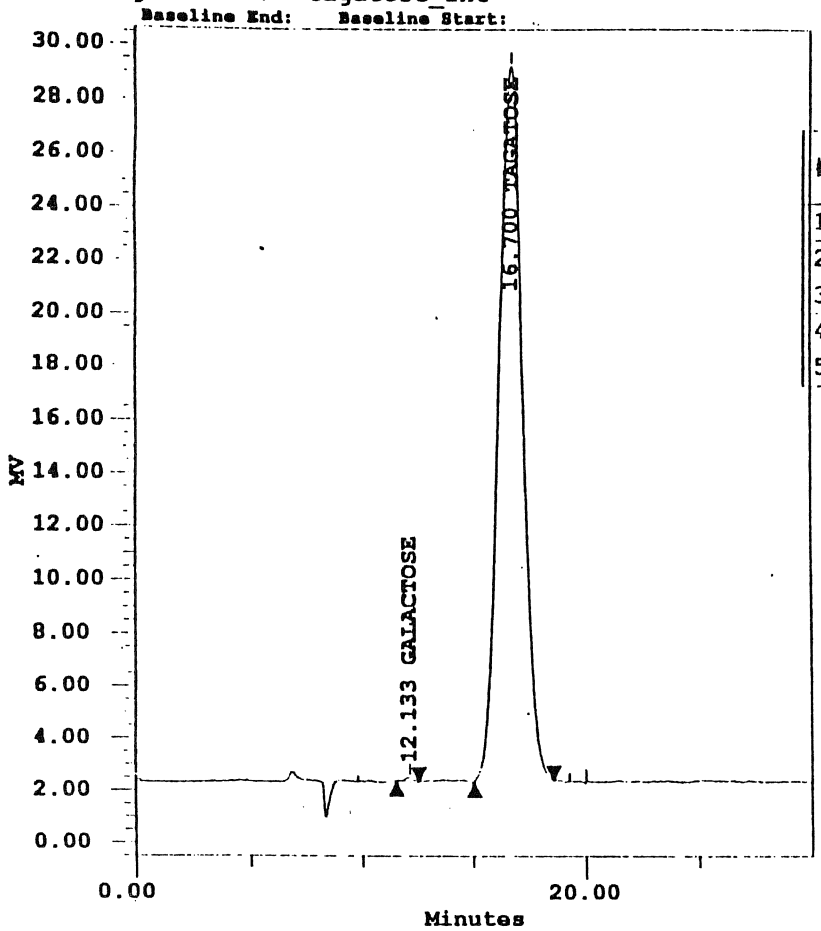
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	% Area	Total Area (uV*sec)
1	LACTOSE	9.183	234960	7493	20.39	1152549
2	GLUCOSE	10.650	227531	5316	19.74	1152549
3	GALACTOSE	11.967	227629	5931	19.75	1152549
4	FRUKTOSE	13.817	232940	5590	20.21	1152549
5	TAGATOSE	16.667	229489	2487	19.91	1152549

Figure 2 - B971041

Millennium Sample Information

Project Name:	Tagatose	Username	SYSTEM
Sample Name:	HAB 7513	Set Name	9802111T
Vial:	2	Sample Type:	Unknown
Injection:	1	Volume:	40.00 µL
Channel:	410	Run Time:	30.0 min
Date Acquired:	11-02-98 08.19.33	Date Processed:	11-02-98 09.01.02
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	tagatosemetode		
Processing Method:	tagatose_int		



5mg/ml H2O

Peak Results

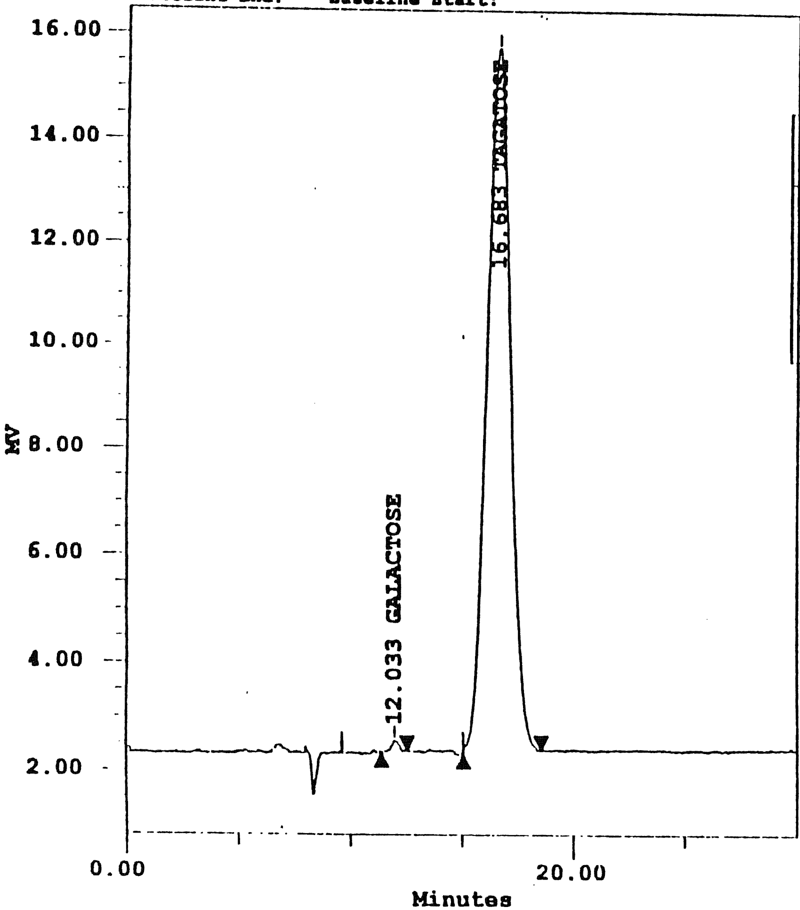
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	% Area	Total Area (uV*sec)
1	LACTOSE	9.350				426450
2	GLUCOSE	10.830				426450
3	GALACTOSE	12.133	861	33	0.20	426450
4	FRUKTOSE	14.100				426450
5	TAGATOSE	16.700	425589	5747	99.80	426450

Millennium Sample Information

Project Name:	tagatose
Sample Name:	HAB 75114
Vial:	3
Injection:	1
Channel:	410
Date Acquired:	11-02-98 08.50.48
SampleWeight:	1.00000
Acq Meth Set:	tagatosemetode
Processing Method:	tagatose_int

Username SYSTEM

Set Name	98021117
Sample Type:	Unknown
Volume:	20.00 μL
Run Time:	30.0 min
Date Processed:	11-02-98 09.59.34
Dilution:	1.00000



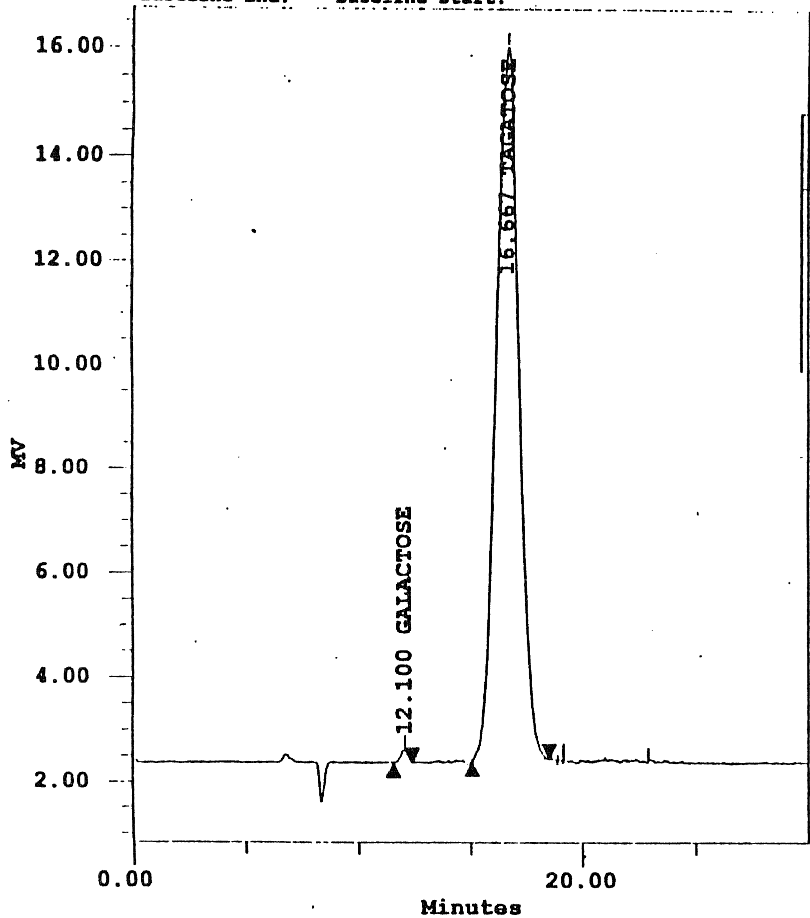
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	% Area	Total Area (uV*sec)
1	LACTOSE	9.350				209708
2	GLUCOSE	10.830				209708
3	GALACTOSE	12.033	996	43	0.47	209708
4	FRUKTOSE	14.100				209708
5	TAGATOSE	16.683	208712	2862	99.53	209708

Millennium Sample Information

Project Name:	tagatose	Username SYSTEM	
Sample Name:	HAB 75115	Set Name 9802112T	
Vial:	4	Sample Type:	Unknown
Injection:	1	Volume:	20.00 μL
Channel:	410	Run Time:	30.0 min
Date Acquired:	11-02-98 09.48.10	Date Processed:	11-02-98 11.14.35
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	tagatosemetode		
Processing Method:	tagatose_int		

Baseline End: **Baseline Start:**



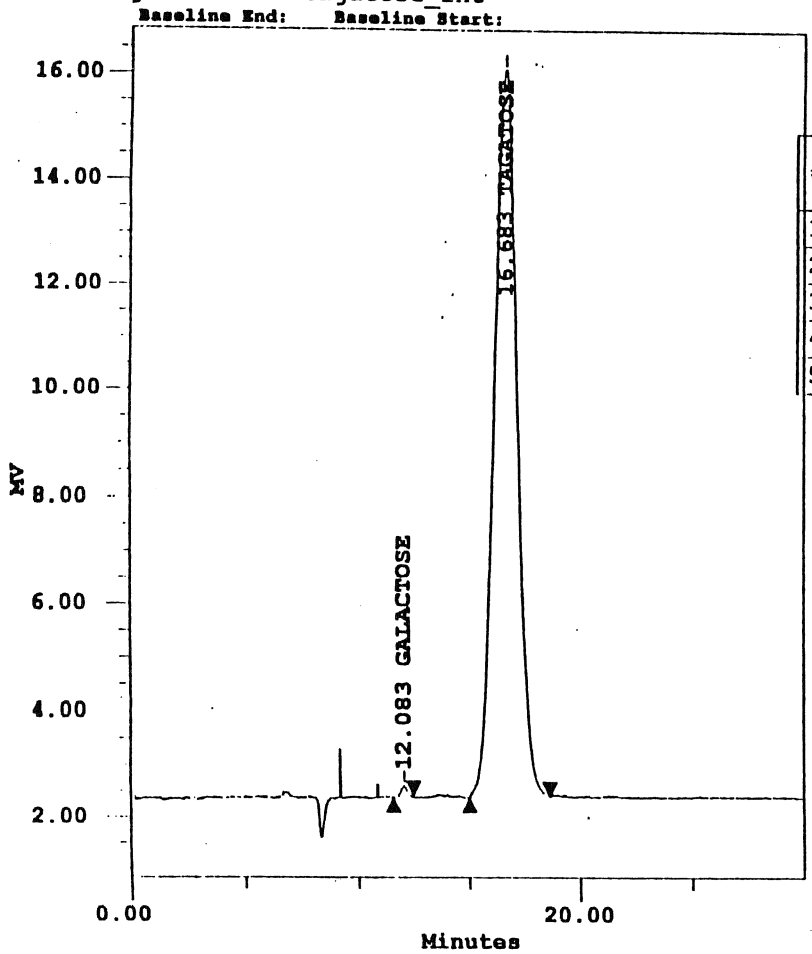
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	% Area	Total Area (uV*sec)
1	LACTOSE	9.350				208588
2	GLUCOSE	10.830				208588
3	GALACTOSE	12.100	1114	52	0.53	208588
4	FRUKTOSE	14.100				208588
5	TAGATOSE	16.667	207474	2909	99.47	208588

Millennium Sample Information

Project Name:	tagatose
Sample Name:	HAB 75116
Vial:	5
Injection:	1
Channel:	410
Date Acquired:	11-02-98 10.19.26
SampleWeight:	1.00000
Acq Meth Set:	tagatosemetode
Processing Method:	tagatose_int

Username	SYSTEM
Set Name	9802112T
Sample Type:	Unknown
Volume:	20.00 µL
Run Time:	30.0 min
Date Processed:	11-02-98 11.15.13
Dilution:	1.00000



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	% Area	Total Area (uV*sec)
1	LACTOSE	9.350				209036
2	GLUCOSE	10.830				209036
3	GALACTOSE	12.083	824	46	0.39	209036
4	FRUKTOSE	14.100				209036
5	TAGATOSE	16.683	208212	2932	99.61	209036

Eurofins Danmark A/S

ARLA FOODS AMBA
TEKNOLOGICENTER
SØNDERRUPVEJ 22

6920 VIDEBÆK

Att.: Driftlaboratoriet

Reference No. 98737.3
Sample B203080036

Kolding 03.04.2002

CERTIFICATE OF ANALYSIS

Sample	Food
Date of receipt	08.03.2002
Date of analys.	08.03.2002
Sample identity:	**D-Tagatose, Batch 11207**
Requisitions no.	**22458 LeBH**

Analysis	Result		C_{95} [1]
Lactose (HPLC)	**< 0.0100**	**g/100g**	
BI metode (dionex)			
Lactose (enz.)	**< 25.0**	**mg/kg**	
Boehringer Mannheim			

[1] C_{95} = 95% Interval of confidence

Jens Søndermark

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www.eurofins.dk

ELISA test of D-tgatose for the presence of alpha-lactalbumin

Regarding ELISA a-Lacprodan 80 analytical results on D-Tagatose lot 11207.

PURPOSE.
D-Tagatose lot11207 has been analysed in AFI ELISA a-Lacprodan 80 (AFI-DB K055) with the purpose of tracing impurties from milk proteins.

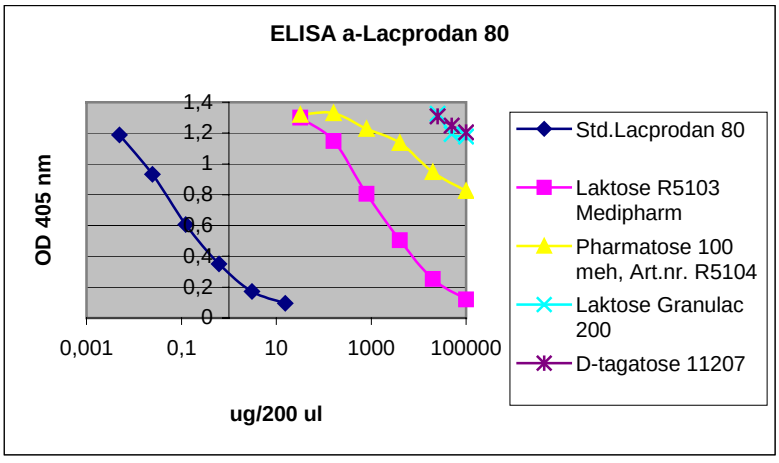
METHOD:
ELISA a-Lacprodan 80 is an extremely sensitive immuno chemical method based on antigen-antibody-reactions, which makes it possible to detect concentrations of milkproteins down to nano- and picogram range.

SAMPLES:
Though lactose products from different suppliers based on standard chemical analysis seem to be very comparable, we have from previous projects observed a very different quality of even pharmaceutical grade products due to small contents of milkproteins.

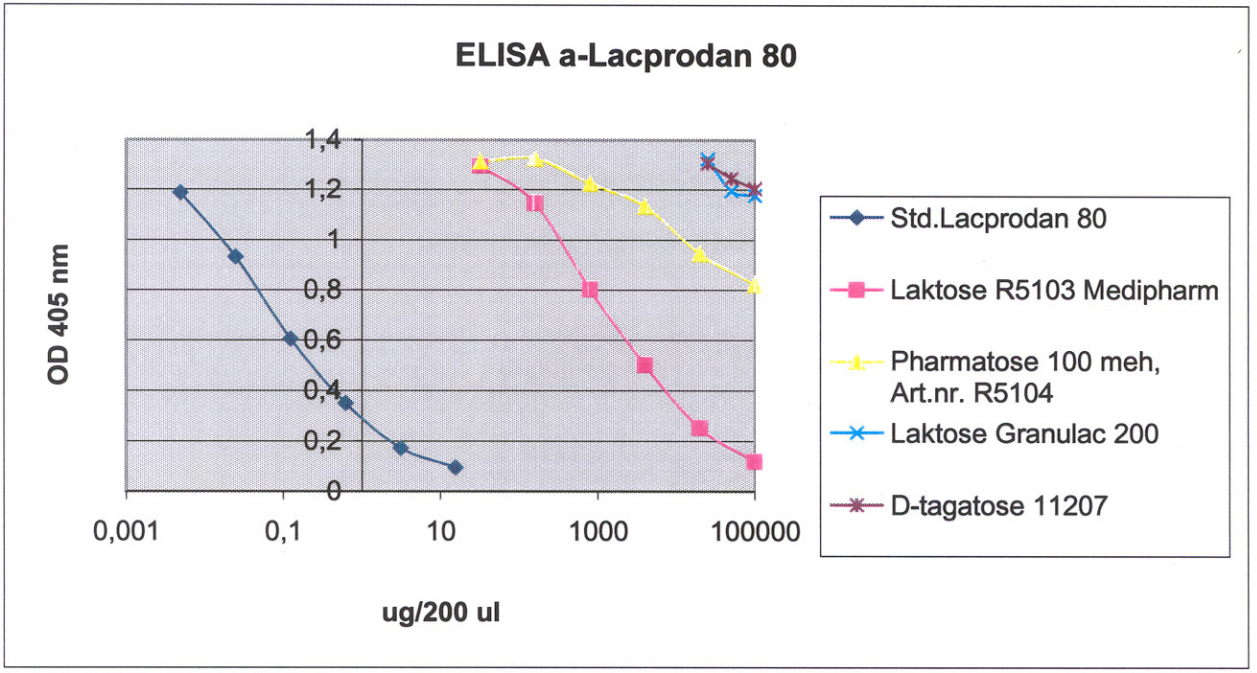
In some applications, even very small contents can be of decisive role, for instance in hypoallergenic infant formulas for allergic babies.

4 lactose samples were chosen to demonstrate the very different content of milkproteins. The results are presented as concentrations of ug protein equivalents/g protein and as a graphic presentation.

Lactose Granulac 200 would be considered suitable for hypoallergenic infant formulas, whereas Lactose R5103 Medipharm and Pharmatose would be discarded as suitable. Tagatose is in the same range as Lactose Granula 200 and could be classified as suitable for hypoallergenic infant formulas.



The graphs demonstrate a standard curve and a curve for each of the 4 samples.
x-axis shows the content of powder in a given sample dilution, whereas y-axis shows optical density of a given sample dilution. The higher the concentration of powder and the higher the optical density, the lower the content of immunoactive components. The Lactose Granulac 200 and D-Tagatose 11207 graphs demonstrate no content of milkproteins.



Regnr.	Sample ID	Startkonc.	Solubility	ug proteinequi/g powder
12165	D-Tagatose 11207	100 mg/ml	fully soluble	<< 10
12117	Lactose R5103 Medipharm	100 mg/ml	fully soluble	227+/-14
12118	Lactose Granulac 200	100 mg/ml	fully soluble	<< 10
12167	Pharmatose 100 mesh, Art.nr. R5104	100 mg/ml	fully soluble	< 10 (approx 4)

ANNEX 5

Determination of D-tagatose in foods

Analysis of selected foods for D-Tagatose

MD FOODS INGREDIENTS AMBA

Author:

Hans Jørgen S. Christensen
(R&D Department)

Report Date:

June 18, 1998

Analysis of selected foods for D-Tagatose

Objective.

The objective of this work is to describe a methodology for analysis of D-Tagatose in some common foods. Methodology and data is used to provide support for D-Tagatose as a Generally Recognized As Safe (GRAS) material.

Introduction.

A specific, precise and accurate method has to be available to quantify the levels of D-Tagatose in relevant foods. In this work HPLC is used as the general analytical procedure for the determination of D-Tagatose.

A standard curve is made from different solutions of D-Tagatose. Concentration of added D-Tagatose is plotted against measured peak area, and detection limits is determed.

D-Tagatose has been tested in several foods. Selected test foods containing D-Tagatose are analysed to quantitate the amount of D-Tagatose. The stated amounts of used D-Tagatose are taken from production recipes, and is therefore not exact from an analytical point of view.

The method is validated by spiking/recovery studies, where a certain amount of D-Tagatose is added to blanks without D-Tagatose and afterwards extracted according to the standard procedure. The recovery is calculated. Blanks without added tagatose are analysed to determine possible background signals that could interfere with the signal from D-Tagatose.

The following foods is analysed - chocolate, cereals, soft drinks and ice-creme.

Materials and Methods.

D-Tagatose (crystalline, > 99% purity, > 99.9 dm)

Cereals: (Frosties, Crunchy, Bran Flakes and Corn Flakes) from Kellog®

Soft drinks: (Cola) from Coca-Cola® and test sample from MD Foods Ingredients

Chocolate: Milk chocolate from Toms® and dark chocolate from MD Foods Ingredients

Ice: Ice creme and sherbet ice from Frisko® and ice creme from MD Foods Ingredients

Standard curve.

Solutions of D-Tagatose in the range of 2, 4, 6, 8, 10, 20, 40, 60, 80, 100, 200 and 300 mg/ml was analysed on HPLC, and a standard curve in the range 0-10 mg/ml was plotted with concentration against measured area.

Standard Procedure

Treatment of sample in principle according to Nordic Committee on Food Analysis. No. 155, 1996. (UDC 547.45:577.15:543.9), point 5.1.1 (copy enclosed).
The food is homogenized with a mixer and 5.0 g is dissolved into a 200 ml volumetric flask. Add about 140 ml of water and incubate for 15 minutes at 70 °C in a water bath. The sample is stirred continuously. Allow the sample to cool to room temperature and dilute to 200 ml with water. Place the flask in a refrigerator for 20 minutes to separate fat. The sample is hereafter filtered. Discharge the first few millilitres of the filtrate. The filtered sample is analysed by HPLC.

Soft drinks are analysed directly by HPLC

In the spiking experiments D-Tagatose is added simultaneously with the food sample.

Determination of D-Tagatose by HPLC

Instrumentation

HPLC equipped with a refractive index detector.

column:	Biorad Aminex carbohydrate HPX-87C column (300 mm x 7.8 mm, 9 µm) heated to 85 °C.
mobile phase:	Deionized water with 50 ppm calcium acetate
flow rate:	0.6 ml/min
detector:	Refractive index

Retention time for D-Tagatose under these conditions is app. 16.8 min.

Validation.

The validation studies consists of spiking/recovery studies.

Spiking. Samples of foods are added D-Tagatose at levels of one-half of, equal to and twice the typical use level of D-Tagatose in the actual food.

Recovery. The percent recovery is calculated as $(\frac{a-b}{c}) * 100$

where

"a" is the level of spiked sample analytically determined in the spiked sample

"b" is the background level

"c" is the amount of D-Tagatose added to the sample

Results and Comments.

Standard curve.
Different solutions of D-Tagatose ranging from 0-300 mg/ml was analysed by HPLC. Measured area was plotted against concentration of D-Tagatose. Calibration curve for 0-10 mg/ml is shown in enclosure page no. 6.

R Squared was calculated to be 0.999 showing linearity between 0.2 and 300 mg/ml D-Tagatose. Expected doses of D-Tagatose in foods are inside the detection limits.

Table 1 is showing the results of the spiking experiments and detection of background signals from different foods without D-Tagatose. Only Cola-light gave a minor background signal with app. the same retention time as D-Tagatose.

The spike recovery is calculated as the percentage re-found D-Tagatose in relation to the added amount correlated for background signals. All experiments are done in triple and the standard deviation is calculated.

Table 1. Determination of background signals and spiking recovery.
Samples of food without D-Tagatose are treated according to the standard procedure and analysed by HPLC. Signals with elution time equal to D-Tagatose is called background signal.

Food spiked with D-Tagatose (w/w%)	Background signal "b"	Spike Recovery (%)	SD
Cereals:			
Corn Flakes			
0.0	ntd		
2.5		99.3	7.6
10.0		96.3	4.7
20.0		99.2	1.4
Soft drink:			
Cola			
0.0	ntd		
Cola (light)			

0.0	< 1.5%		
0.1		102.6	7.6
1.0		100.1	4.9
2.0		97.9	6.7
Chocolate:			
Milky (Toms)			
0.0	ntd		
5.0		98.7	3.2
25.0		99.0	2.6
50.0		104.0	3.6
Ice:			
Creme (regnbue)			
0.0	ntd		
5.0		98.1	2.7
10.0		98.0	8.5
20.0		97.6	5.3
Sherbet (filur)			
0.0	ntd		
5.0		85.4	3.1
10.0		87.0	0.8
20.0		87.1	2.2

ntd: no trace detectable
SD: standard deviation

Table 2 is showing the comparison of the added amount of D-Tagatose with the detected amount. The added amounts of D-Tagatose according to the recipes are not exact from an analytical point of view, and minor deviation can be expected.

Examples of HPLC chromatograms for respectively background signals and products with D-Tagatose are shown at page no. 7 and 8.

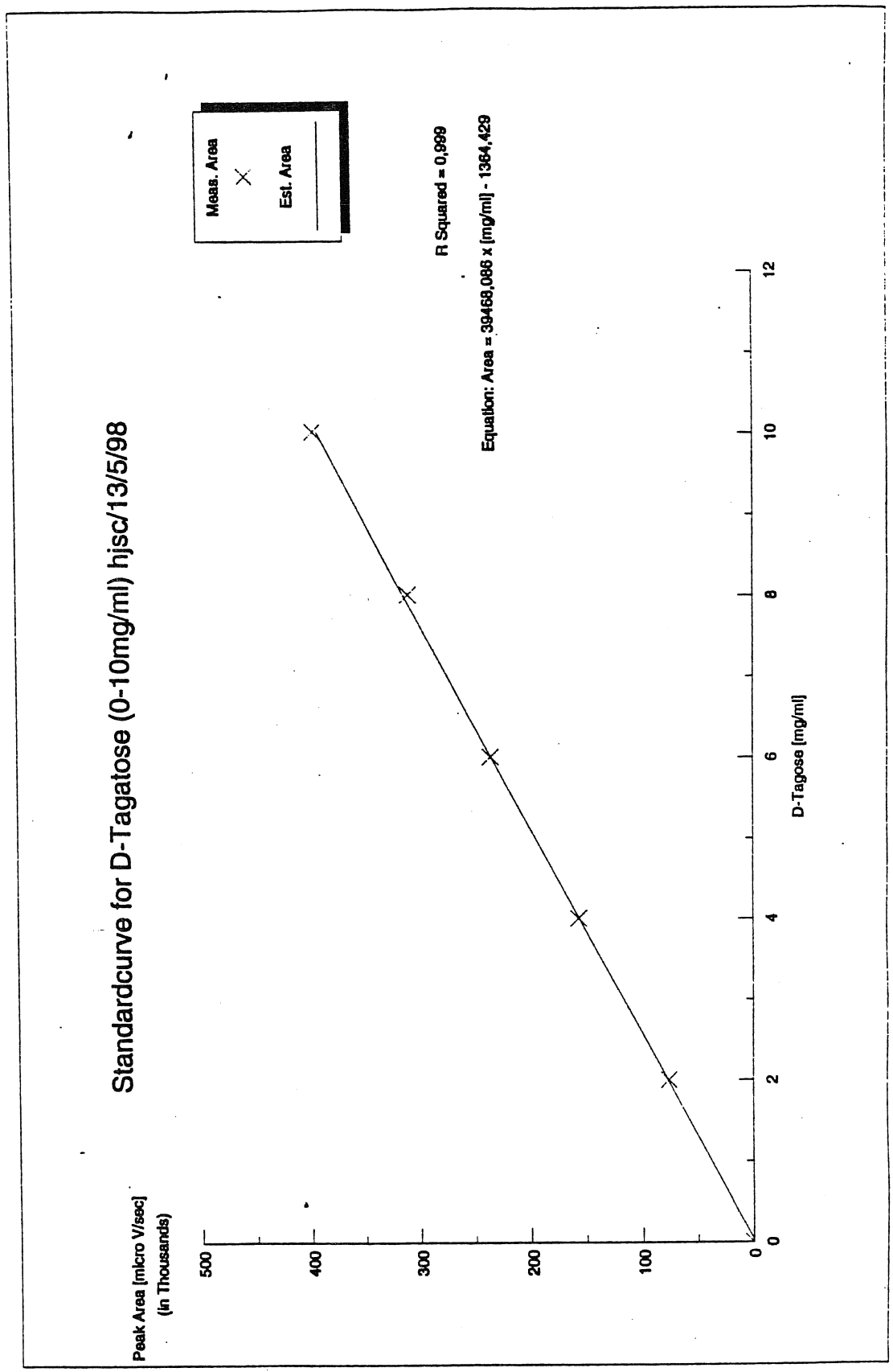
Table 2. Analysis of D-Tagatose in selected foods.
Samples of foods produced with D-Tagatose are treated according to the standard procedure and analysed by HPLC.

Food	Recipe D-Tagatose (w/w %)	Found D-Tagatose (w/w %)	SD
Cereals:			
Bran flakes	17-19	19.4	0.6
Frosties	36-38	36.1	0.8
Crunchy Nut	25-26	27.9	0.3

Chocolate:			
Dark	25	25.5	0.7
Soft drink:			
Orange	6.7	6.7	0.1
Ice creme:			
Soft Ice	6	6.1	0.1

Enclosure page

Nordic Committee on Food Analysis


Standardcurve for D-Tagatose (0-10mg/ml) hjsc/13/5/98
Meas. Area
Est. Area
R Squared = 0,999
Equation: Area = 39468,086 x [mg/ml] - 1364,429
Peak Area [micro V/sec]
(in Thousands)
500
400
300
200
100
0
0
2
4
6
8
10
12
D-Tagose [mg/ml]

Cereals

Corn flakes®
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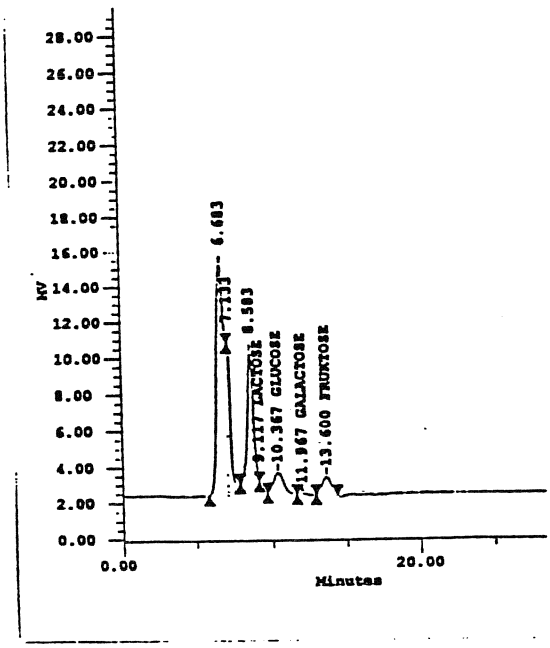
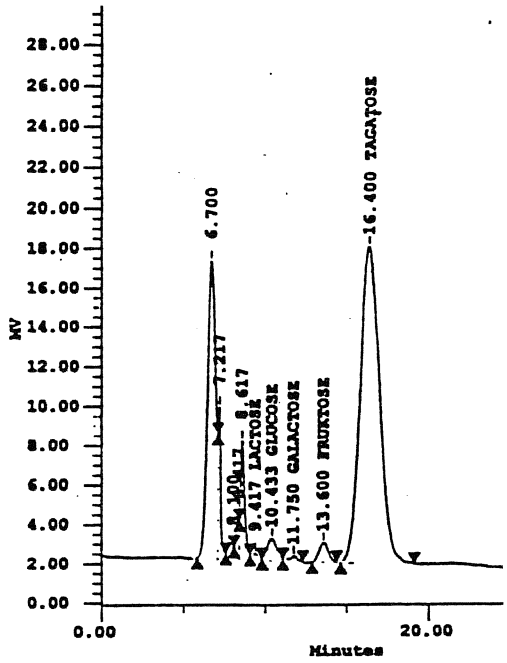


Frosties®
(36-38% w/w D-Tagatose)



Chocolate

Toms®
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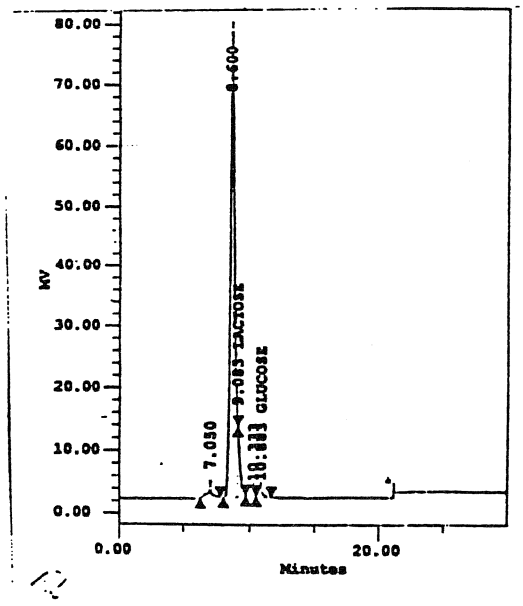
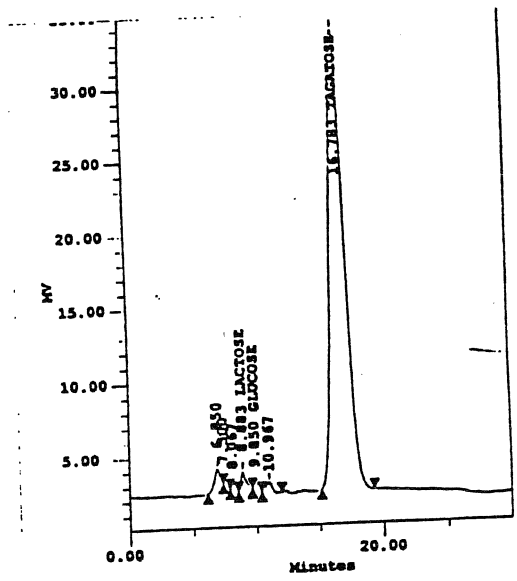


MD Foods
(50% w/w D-Tagatose)



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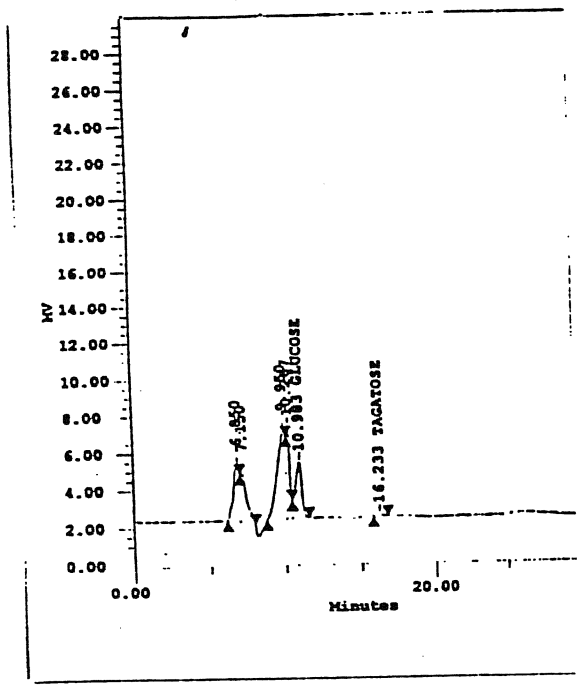
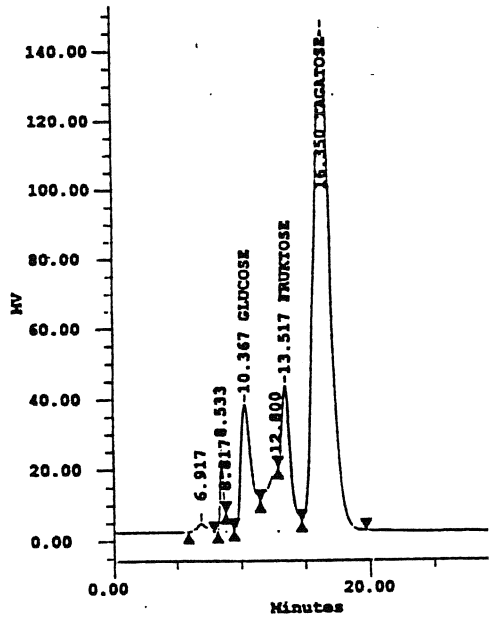
Soft drinks

Cola-light® (blank)

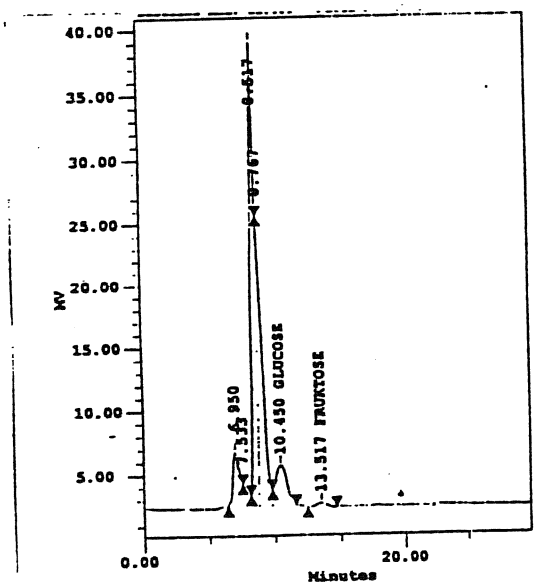


Orange (6.7% w/w D-Tagatose)



Ice Creme

Frisko® (blank)



MD Foods (12% w/w D-Tagatose)