FAM-INFORMATION

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Collaborative Study on the determination of free fatty acids in butter and cheese

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Contents

Abstract

The Swiss Federal Dairy Research Station in Liebefeld (FAM) organized in collaboration with the COST 95 and AIR 3 CT 94 2039 project in Spring 1996 an international collaborative study on the determination of free fatty acids in two butter samples and four cheese samples (processed Emmental, Fontina, Parmigiano-Reggiano and Pecorino Romano).
The following techniques were used: GC separation of the free fatty acids, the methyl-, ethyl- propyl- and butyl esters with flame ionization detection and HPLC separation of the free fatty acids with UV detection.
The total amount of free fatty acids in butter 1 and 2 were 8 and 15 mmol/kg, respectively. In cheese the sum of free fatty acids were in processed Emmental 61 mmol/kg (6 g/kg), in Fontina 27 mmol/kg (3 g/kg), in Parmigiano-Reggiano 49 mmol/kg (8 g/kg) and in Pecorino Romano 41 mmol/kg (7 g/kg)
The precision parameters repeatability r and reproducibility R could only be calculated for the GC separation of the fatty acids without derivatization. The calculation of the precision parameters were performed with the robust method of the Swiss food manual.

The average relative standard deviation of the repeatability (RSD_r) for the butter samples is normally below 2 %. The average RSD_r of the cheese samples is between 3.0 - 5.4 %. The worst RSD_r (> 8 %) were obtained for myristoleic, heptadecanoic and linolenic acid in the cheese samples.
The relative standard deviation of the reproducibility (RSD_R) for the butter samples is normally below 18 %. Only heptadecanoic acid, linoleic acid and arachidoleic acid have RSD_R > 41 %. In processed Emmental, the average RSD_R is 18 %, except for capric and linoleic acid with RSD_R values over 35 %. For Fontina, the average RSD_R is 21 %, except for lauric, myristoleic, pentadecanoic and linolenic acid with RSD_R valuse over 40 %. The average RSD_R in Parmigiano-Reggiano is 13 %, except for acetic, palmitoleic, heptadecanoic and linoleic acid where the RSD_R values are over 28 %. In Pecorino Romano, the average RSD_R is 13 %, except for palmitoleic, heptadecanoic and linoleic acid with RSD_R values over 42 %.

Gas chromatographic separation of fatty acid esters showed for most fatty acids good agreement with the GC separation of free fatty acids. But due to the additional esterification the standard deviation is often higher.
The GC method with TMAH derivatization showed for most fatty acids in butter and cheese samples significant higher values, probably due to hydrolysis of triglycerides during derivatization.

The extraction of the free fatty acids with the aminopropyl cartridges is one of the most critical step in the determination of fatty acids, because artefacts of acetic acid can be present depending of the manufacturer and the batch. Depending of the total amount of free fatty acids, the sample weight have to be adapted to not overload the cartridge.

Introduction

The separation of the free fatty acids with gas chromatographic methods has been performed since the seventies. In the last years, a few authors proposed FFA isolation procedures with solid phase extraction cartridges and GC separation of the free [1, 2] or derivatized fatty acids. Therefore several methods are used today for the quantification of free fatty acids in dairy products. In order to compare these different techniques, it was decided at the 2nd workshop of the COST 95/ AIR 3 CT 94 2039 biochemical group on 1. December 1995 in Vitoria (Spain) to organize a collaborative study on the determination on free fatty acids in butter and cheese.

Experimental

Samples

For this collaborative study, the following samples were chosen. Two butter samples with different levels of free fatty acids and 4 cheese samples (processed Emmental, Fontina, Parmigiano-Reggiano and Pecorino Romano). Table 1 shows the description and origin of the different samples. The samples were distributed on 1. April 1996. Table 2 shows the gross composition of the four cheese samples. The biogenic amines content of the different cheese samples is shown in table 3.

Table 1 Samples

Number	Name	Water content g/kg	Acidity of fat °SH	Description
1	Butter 1	1.15	9.3	butter oil
2	Butter 2	1.12	18.2	butter oil
3	Processed Emmental	559		
4	Fontina	386		3 month
5	Parmigiano Reggiano	312		24 month
6	Pecorino Romano	255		4 month

Table 2 Gross composition of the cheese samples

Number	Name	Fat g/kg	TN mol/kg	NaCl g/kg	Cu mg/kg	WSN mol/kg	NPN mol/kg	Lactic acid L(+)
3	Processed Emmental	216	1.94	8.4	5.8	1.14	0.29	37.4 mmol/kg 49 %
4	Fontina	300	3.02	20.8	0.8	1.57	0.22	57.3 mmol/kg 58 %
5	Parmigiano Reggiano	286	3.72	16.1	8.3	1.21	1.09	167 mmol/kg 57 %
6	Pecorino Romano	364	3.16	59.4	1.1	0.66	0.35	144 mmol/kg 57 %

Legend:
TN Total nitrogen (Protein g/kg = TN x 6.38 x 14.007)
NaCl Salt calculated from chloride
WSN Water soluble nitrogen (5 g cheese in 100 mL water)
NPN Non protein nitrogen (12 % trichloroacetic acid soluble fraction)

Table 3 Biogenic amines in cheese [mg/kg]

Number	Name	Tryptamine	ß-Phenyl-ethylamine	Putrescine	Cadaverine	Histamine	Tyramine
3	Processed Emmental	< 1	< 1	3	18	5	30
4	Fontina	31	16	91	7	75	99
5	Parmigiano Reggiano	< 1	2	< 1	1	8	83
6	Pecorino Romano	< 1	< 1	1	< 1	21	45

Participating laboratories

21 laboratories participated in this collaborative study (table 4). One laboratory sent two different sets of results for statistical evaluation. Therefore 22 sets of results are presented.

Table 4 Participating Laboratories

Name	Company or Institute	Country
Tschager Eduard	Bundesanstalt für Alpenländische Milchwirtschaft, Jenbach	Austria
Nurmela Kari	VALIO Ltd/R&D Centre, Helsinki	Finland
Le Quéré Jean-Luc	INRA Dijon, Laboratoire de Recherche sur les arômes, Dijon Cedex	France
Buscailhon Solange	INRA, Poligny Cedex	France
Grosch Werner	Deut. Forschungsanstalt für Lebensmittelchemie , Garching	Germany
Strohmar	Staatliche Milchwirtschaftliche Lehr- und Forschungsanstalt, Dr. Oskar Farny Institut, Wangen im Allgäu	Germany
Weiss Günther	Deut. Forschungsanstalt für Lebensmittelchemie, Inst. für Chemie und Physik, Freising- Weihenstephan	Germany
Panari Giorgio	Cons. Formaggio Parmigiano Reggiano, Reggio Emilia	Italy
Innocente Nadia	Università di Udine, Dept. of Food Science, Udine	Italy
Battelli Giovanna	Centro Studi Latte, Milano	Italy
Boschelle Ornella	Università di Udine, Dept. of Food Science, Udine	Italy
Piredda Giovanni	Istituto Zootechnico e Caeario per la Sardegna, Olmedo	Italy
Neeter Ronald	NIZO, Institute for Dairy Research, Ede	Netherland
Partidário Ana Maria	INETI Lab. Nacional de engenharia e Tecnologia Industrial, DTIA - Edificio S, Lisboa Codex	Portugal
de Renobales Mertxe	Univ. Pais Vasco /EHU, Dpto. Bioquimica - Fac. Farmacia, Vitoria-Gasteiz	Spain
Alonso Leocadio	CSIC, Instituto de productos lacteos de asturias, Villaviciosa, Asturias	Spain
Saitua Eduardo	AZTI (Instituto Tecnològio Pesquero y Alimentario), Sukarrieta (Bizkaia)	Spain
Juarez Manuela	CSIC FRIO, Ciudad Universitaria Instituto del Frio, Madrid	Spain
Collomb Marius	FAM, Bern	Switzerland
Badertscher René	FAM, Bern	Switzerland
Caperos J.	Kant. Laboratorium, Neuchâtel	Switzerland

Methods

All methods used are shown in table 5. 20 laboratories used a gas chromatographic method with flame ionization detection and 2 laboratories used a liquid chromatographic method with UV detection. The analytical parameters of all laboratories are listed in table 6.

Table 5 Summary of the used methods

Chromatographic method	separation form of fatty acids	Detection	Abbreviation	Number of laboratories
GC	free	FID	G-FFA	10
	methyl ester	FID	G-C1	5
	propyl ester	FID	G-C3	2
	butyl ester	FID	G-C4	1
	TMAH	FID	G-TMA	2
HPLC	free	UV	LC	2

Table 6 Summary of the analytical parameters

Nr	Sample preparation		Sample weight [g]						Reference
Laboratory	Extraction	SPE cartridge	Butter 1	Butter 2	Processed Emmental	Fontina	Parmigiano-Reggiano	Pecorino Romano	
1	Na2SO4 H2SO4/ ether-heptane	Aminopropyl IST Isolute	0.35	0.35	1	1	1	1	de Jong and Badings JHRC (1990)
2	Na2SO4-H2SO4/ ether-heptane		2	2	5	5	5	5	not published
3	Na2SO4-H2SO4/ ether-heptane	Aminopropyl Merck Lichrolut	2	1	1	1	0.2	0.2	de Jong and Badings JHRC (1990)
4	Ether/petroleum spirit				15	15	15	15	Kuzdal-Savoie, Le Lait 461, 9-23 (1967)
5	Na2SO4-H2SO4/ ether-heptane	Aminopropyl Waters WAT020840	1	1	1	1	1	1	de Jong and Badings JHRC (1990)
6	ether-heptane	Aminopropyl WATERS WAT054560	1.5	1.5	3.5	3.5	3.5	3.5	de Jong and Badings JHRC (1990)
7	methyl-butylether		0.2	0.2	0.2	0.2	0.2	0.2	not published
8	ether/heptane 1:1	Aminopropyl Supelco S-7014	1	1	1	1	1	1	de Jong and Badings JHRC (1990)
9	ether-heptane	Aminopropyl IST 470-0050-H			1	1	1	1	de Jong and Badings JHRC (1990)
10	steam distillation		5	5	10	10	10	10	Vandenheuvel F.A. Anal. Chem. 36, 1930 (1964)
20	Na2SO4-H2SO4/ ether-heptane		2.57	2.72	10	15	15	15	not published
21	Ether/H2SO4	Amberlyst A-26 SIGMA A5522	0.5	0.5	1	1	1	1	Needs et al, J. Dairy Res. (1983)
22	Na2SO4-H2SO4/ ether-heptane	Amberlyst A-26	0.5	0.5	1	1	1	1	de Jong and Badings JHRC (1990), FIL Bulletin 265
23	ether		0.13	0.16	0.13	0.18	0.12	0.15	AOAC 991.39
24	CHCl3/HCl/Na2 SO4		3	3	3	3	3	3	Boschelle et al.
40	steam distillation				20	20	20	20	not published
41	ether/hexane	Aminopropyl Amchro CUNAX 20X	1	1	1.5	2.5	2	1.5	de Jong and Badings JHRC (1990)
50	heptane	Amberlyst A-26 Aldrich 21642-9	0.04	0.04	35	50	63	52	
30	ether		0.2	0.2	5	5	5	5	Juarez et al, Chromato. (1992)
31			0.2	0.2	10	10	10	10	Juarez et al Chromato. (1992)
70	NaOH 2 mol/L	Dowex 1-X8 2-, BIO-RAD	4		10	10	10	10	Tschager E. et al Milch. Ber. (1988)
71	steam distillation		2.5	2.5	10	10	10	10	

Nr	Method & deriv.		Equipment and column				Injection			Calibration					Additional parameters		
Laboratory	Separation method	Derivates	Chromatographic system	Stat. phase	Column dim. [m x mm]	Film [µm]	Technique	Volume [µL]	Temperature [°C]	Int. std. added before extraction	Internal standards	Area/Height	Calib. points	Range	Carrier gas	Detector temperature [°C]	Run time [min]
1	GC		CE	DB-FFAP	15 x 0.53	1	on col.	0.7	cold	Y	C5, C13, C17	A	1	C3-C20	H_2	280	40
2	GC		PE Sigma 3B	Supelco CS 80/100, 10% SP 1200/1% H3PO4	3 x 2	pack. col.	Split	2	120	Y	n-C5	A	1	C2-C6.	N_2	200	27
3	GC		HP 6890	J&W DB-FFAP	15 x 0.53	1	on col.	0.5	cold	Y		A	1	C2-C20.	He	240	40
4	GC		Varian	CS AW/DMCS (100-120 mesh) with 10% FFAP	2 x 2.1	pack. col.			190	Y	C5	A	1	C2-C6	N_2	200	29
5	GC		HP 5890	HP-FFAP crosslinked	25 x 0.32	0.52	Split	1	200	Y	C5, C7, C11	A	4	C2-C18:3	He	275	47
6	GC		PE Sigma 3B	HP FFAP	10 x 0.53	1	on col.	1	cold	Y	2-m.-C5, C9, C13, C17	A	1		He	270	50
7	GC		HP 6890	DB-FFAP	30 x 0.32	0.25	PTV	1	cold	N		H	1	C6-C18:1	H_2	300	46
8	GC		HP 5890 II Plus	FFAP-CB, fused silica	25 x 0.32	0.29	on col.	1	cold	N		A	4	C4-C18:2	He	300	38
9	GC		CE 5300	CP-FFAP CB FS Chrompack	25 x 0.53	1	on col.	0.4	65	Y	iC4, C5, C7, C9	A	1		He	250	54
10	GC		Varian 3300	Supelco Nukol	15 x 0.53	0.5	Split	1	150	N	C12-OH	A	1	C2-C6	He	200	38
20	GC	M	CE CE 5160	DB 225	30 x 0.32	0.25	Split	0.4	195	Y	C7	A	1	C2-C20	He	210	0
21	GC	M	CE EL 980	DB Wax	30 x 0.32	0.5	on col.	0.3	40	Y	C5, C11, C13	A	1	C2-C18:3	He	260	65
22	GC	M	Varian 3400 CX	SP 2560	100 x 0.25	0.2	Split	1	260	Y	C5, C9, C13,C17	A	1	C4-C18	He	290	54
23	GC	M	HP 5890 II	SP-2340	60 x 0.25	0.2	Split	1	225	N		A	1	C4-C22.	He	230	50
24	GC	M	CE 5100 Mega	fused silica Capillary Col.	30 x 0.32	0.2	Split	1	210	N	C17	A	1	C4-C20	He	240	24
40	GC	P	HP 5890 /TK 7000	HP Ultra 2	50 x 0.32	0.52	Split	250	140	N		A	1	C1-C6	He	320	20
41	GC	P	PE 3400	Omegawax 250	30 x 0.25	0.25	Split	1	250	N	C5, C7, C13	A	3	C4-C18:2	He	280	53
50	GC	B	HP 5890 Series II	DB-Wax	30 x 0.32	0.5	Split	1	240	N	9-Me-C9	A	1	C6-C18:2	H_2	250	60
30	GC	T	PE Autosystem	WCOT Silar 5CP	50 x 0.22	0.22	Split	0.5	200	Y	C9	A	1	C4-C20.	He	200	40
31	GC	T	PE Autosystem	BPX 70	25 x 0.22	0.25	Split	1	275	Y	C9	A	1	C4-C20.	N_2	275	20
70	LC		Beckman 112	Nucleogel ION 300 OA	600 x 7.8			0		Y	iC4	A	1	C1-C4			90
71	LC		Waters	Biorad HPX 87H	0.3 x 7.8	-		50		N	Succinic acid	H	1	C1-C5			30

Legend

CS	Chromosorb
pack. col.	packed column
M, P, B, T	methyl, propyl, butyl, TMAH esters

Robust Statistics

Robust mean, repeatabilities and reproducibilities were calculated according to the Swiss food manual [3]. With this method there is no outlier elimination procedure to perform. This is a advantage, because the outlier elimination procedure according to the harmonized IUPAC-1987 protocol is sometimes very strict. That means, that ≤ 22.2 % of the data can be eliminated. In real collaborative studies there are sometimes more outliers that should be eliminated. The other restriction was the insufficient number of laboratories for several fatty acids.

Results

Results should have been sent until 31. June 1996. 7 laboratories (33%) sent their results before end of June. Until end of July the results of 19 laboratories (90%) were available. The last corrected results arrived on 5. September 1996.

The calculation of the precision parameters was only possible for the GC separation of free fatty acids without derivatization. Of all other methods, there were not enough results available to calculate the corresponding precision parameters.
The evaluation program for the calculation of the precision parameters according to the Swiss Food Manual was written with Turbo Pascal for Windows Version 1. SYSTAT program modules were used for the descriptive statistics and SYGRAPH program to draw box and category plots [4], which are shown in Appendix G.

The four cheese varieties showed very different levels of free fatty acids (see figure 1).

Processed Emmental showed highest total amount of free fatty acids with 61 mmol/kg, followed by Parmigiano-Reggiano with 49 mmol/kg, Pecorino Romano with 41 mmol/kg and Fontina with 27 mmol/kg.

The processed Emmental sample had highest concentration of formic, acetic, propionic and valeric acid.

Pecorino Romano had highest concentration of butyric, caproic, caprylic, capric, lauric and linolenic acid.

Fontina had highest concentration of isobutyric, isovaleric and isocaproic acid.

The 24 month old Parmigiano-Reggiano cheese sample had highest concentration of the long chain fatty acids myristic, myristoleic, pentadecanoic, palmitic, palmitoleic, heptadecanoic, stearic, oleic and linoleic acid.

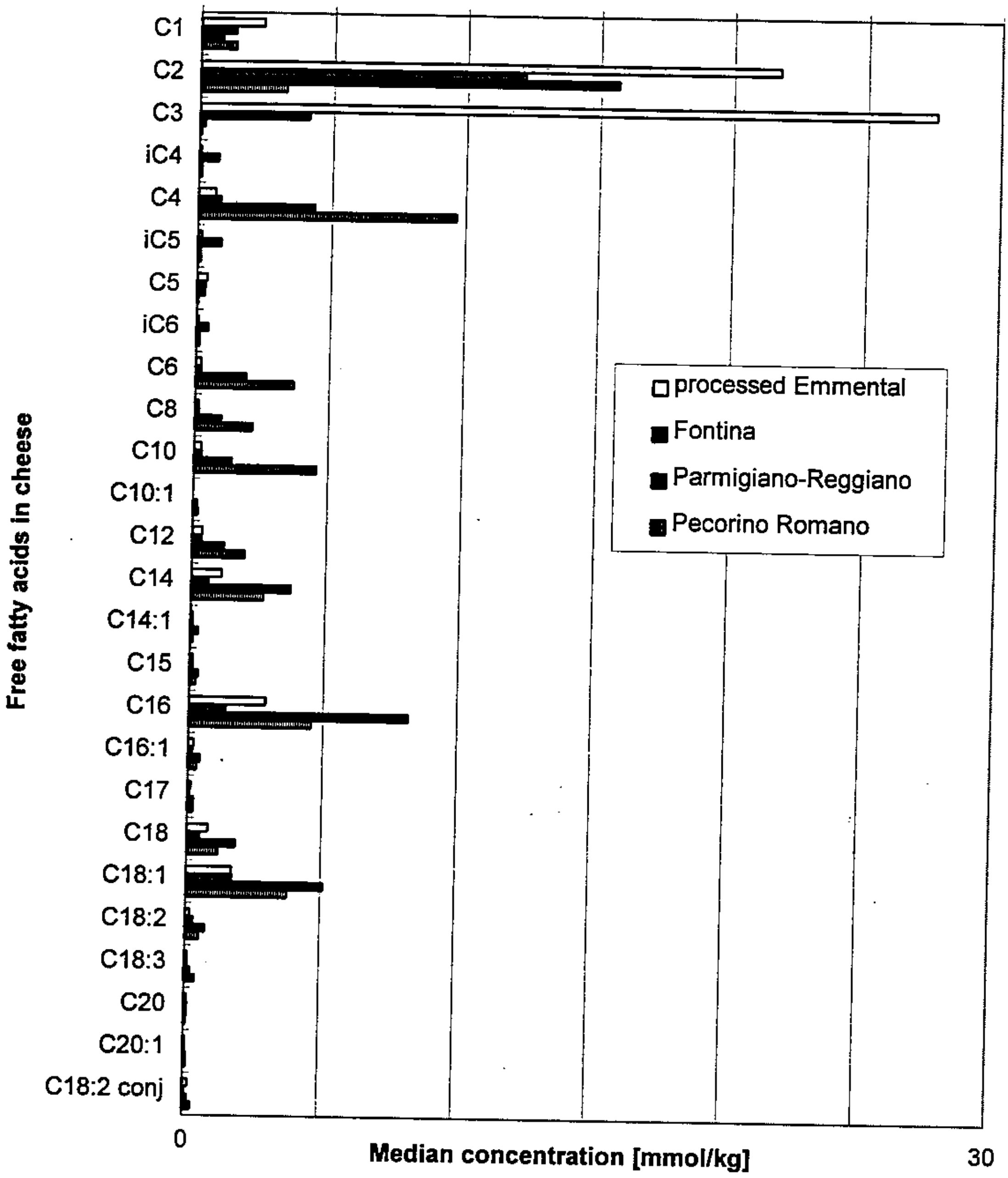

Figure 1 **Distribution of free fatty acids in the four cheese samples**

Precision parameters

In table 7 - 12, the median values of all results, the mean values and the precision parameters of the GC separation of free fatty acids without derivatization are shown.

Table 7 Determination of free fatty acids in butter 1

Fatty acid	all results n	median mg/kg	GC separation of free fatty acids n	mean mg/kg	s_r mg/kg	RSD_r %	s_R mg/kg	RSD_R %
C1	2	25						
C2	5	8	2	6	0.6	8.6	3.0	46
C3	2	22						
C4	12	12	3	4	0.4	9.9	1.0	27
C6	11	10	3	2	0.5	19.9	0.6	23
C8	11	11	3	2	0.1	6.9	0.3	18
C10	14	23	6	16	0.2	1.2	2.4	15
C10:1	3	3						
C12	14	55	6	51	0.7	1.4	5.5	11
C14	14	160	6	147	1.7	1.1	20.4	14
C14:1	8	20	2	17	1.2	7.6	1.3	8
C15	11	25	4	19	0.6	3.3	4.4	23
C16	14	516	6	489	2.1	0.4	64.3	13
C16:1	13	48	5	44	2.2	4.9	9.4	22
C17	9	24	3	24	0.7	2.8	18.7	77
C18	14	165	6	156	3.4	2.2	34.9	22
C18:1	14	485	6	476	2.6	0.6	69.3	15
C18:2	12	46	6	69	1.0	1.5	28.3	41
C18:3	9	21	4	21	1.2	5.6	3.3	16
C20	3	10	2	13	4.4	34.0	4.9	37
C20:1	1	4						
C18:2con.	1	11						

Legend:

n	number of laboratories
median	median value of all obtained results
mean	calculated robust mean value
s_r	standard deviation of repeatability
RSD_r	relative standard deviation of repeatability
s_R	standard deviation of reproducibility
RSD_R	relative standard deviation of reproducibility

Table 8 Determination of free fatty acids in butter 2

Fatty acid	all results		GC separation of free fatty acids					
	n	median	n	mean	s_r	RSD_r	s_R	RSD_R
		mg/kg		mg/kg	mg/kg	%	mg/kg	%
C1	2	38						
C2	5	12	2	9	1.6	17.9	7.1	80
C3	2	0						
C4	12	10	3	4	0.5	12.2	1.3	31
C6	12	9	3	3	0.4	11.4	1.6	47
C8	12	11	3	3	0.3	9.5	0.4	14
C10	15	40	6	32	0.6	1.9	4.0	13
C10:1	4	4						
C12	15	123	6	105	0.7	0.7	7.1	7
C14	14	359	5	332	6.1	1.8	41.4	12
C14:1	9	34	3	43	0.8	1.9	13.0	30
C15	12	55	4	47	1.5	3.1	6.5	14
C16	15	1244	6	1236	26.8	2.2	204.4	17
C16:1	12	81	5	84	1.6	1.9	18.1	22
C17	10	49	3	39	1.0	2.6	18.8	48
C18	14	338	6	334	0.4	0.1	92.2	28
C18:1	15	973	6	1016	8.3	0.8	215.1	21
C18:2	13	74	6	114	1.6	1.4	60.1	53
C18:3	10	43	5	46	1.7	3.7	6.1	13
C20	4	8	2	12	0.2	2.1	8.9	76
C20:1	4	85	2	91	0.5	0.5	126.7	140
C18:2con.	2	38						

Legend see Table 7

Table 9 Determination of free fatty acids in processed Emmental

Fatty acid	all results n	median mg/kg	GC separation of free fatty acids n	mean mg/kg	sr mg/kg	RSDr %	sR mg/kg	RSDR %
C1	3	109						
C2	11	1305	6	1283	98.9	7.7	213.1	17
C3	12	2044	7	2108	139.9	6.6	459.1	22
iC4	2	6						
C4	19	57	8	55	2.7	4.9	4.0	7
iC5	11	14	7	15	1.3	8.8	4.4	29
C5	1	38						
iC6	2	7						
C6	18	24	8	23	1.0	4.3	5.0	21
C8	16	19	7	17	1.3	7.5	7.4	43
C10	16	46	7	39	3.1	7.8	26.2	66
C10:1	5	5	2	23	0.1	0.6	29.2	126
C12	16	75	7	73	3.4	4.6	9.0	12
C14	15	259	6	260	10.3	4.0	47.7	18
C14:1	8	17	2	8	1.1	14.3	7.4	97
C15	12	26	4	26	1.8	6.7	17.5	66
C16	14	732	6	734	24.5	3.3	31.0	4
C16:1	12	49	5	56	1.6	2.8	14.4	26
C17	9	29	3	28	2.4	8.7	26.0	94
C18	15	219	6	216	6.3	2.9	50.3	23
C18:1	15	474	6	541	19.7	3.6	116.8	22
C18:2	14	45	6	50	3.4	6.9	18.0	36
C18:3	8	26	3	29	2.8	9.8	8.2	28
C20	5	31						
C20:1	5	12						
C18:2 con	2	48						

Legend see Table 7

Table 10 Determination of free fatty acids in Fontina

Fatty acid	all results n	all results median mg/kg	GC separation of free fatty acids n	mean mg/kg	s_r mg/kg	RSD_r %	s_R mg/kg	RSD_R %
C1	3	60						
C2	11	732	6	729	38.4	5.3	125.7	17
C3	12	303	7	302	13.1	4.4	41.5	14
iC4	10	64	6	66	1.6	2.5	11.8	18
C4	19	75	8	75	1.0	1.3	9.2	12
iC5	11	89	7	91	3.0	3.2	5.3	6
C5	2	32						
iC6	10	51	7	51	1.9	3.7	10.5	20
C6	19	26	9	27	0.9	3.2	7.5	28
C8	16	21	7	20	0.8	4.2	6.9	35
C10	16	50	7	42	2.7	6.4	12.4	30
C10:1	7	4	2	7	0.1	1.6	5.0	70
C12	16	70	7	72	3.1	4.3	31.0	43
C14	15	148	6	148	4.3	2.9	35.1	24
C14:1	9	21	2	40	5.9	14.7	18.7	47
C15	12	28	4	31	1.3	4.2	12.7	41
C16	15	353	6	360	7.9	2.2	64.9	18
C16:1	13	38	5	41	1.2	2.9	10.1	25
C17	10	20	3	20	2.2	11.4	2.5	13
C18	15	137	6	138	3.9	2.8	38.7	28
C18:1	15	479	6	530	18.0	3.4	167.3	32
C18:2	15	83	6	97	4.5	4.7	26.4	27
C18:3	12	22	5	32	3.2	10.1	14.9	47
C20	5	37						
C20:1	5	21						
C18:2 con	2	16						

Legend see Table 7

Table 11 Determination of free fatty acids in Parmigiano-Reggiano

Fatty acid	all results		GC separation of free fatty acids					
	n	median	n	mean	s_r	RSD_r	s_R	RSD_R
		mg/kg		mg/kg	mg/kg	%	mg/kg	%
C1	3	38						
C2	10	942	6	913	13.8	1.5	261.1	29
C3	9	15	4	19	0.8	4.0	9.3	49
iC4	1	9						
C4	19	384	8	377	3.9	1.0	21.3	6
iC5	2	10						
C5	2	28						
iC6	1	12						
C6	19	220	9	227	2.7	1.2	18.2	8
C8	16	143	7	140	1.5	1.0	15.5	11
C10	15	244	7	242	6.8	2.8	20.1	8
C10:1	7	27	2	28	0.6	2.3	3.6	13
C12	16	239	7	227	7.8	3.4	24.3	11
C14	15	846	6	889	34.2	3.8	163.8	18
C14:1	9	65	2	62	5.5	8.9	6.6	11
C15	12	74	4	81	2.3	2.8	10.8	13
C16	15	2096	6	2190	107.1	4.9	382.1	17
C16:1	12	110	5	123	5.3	4.4	37.8	31
C17	10	59	3	68	3.1	4.5	32.5	48
C18	15	517	6	530	26.3	5.0	73.1	14
C18:1	15	1438	6	1505	31.3	2.1	222.6	15
C18:2	15	207	6	267	12.1	4.5	92.8	35
C18:3	10	56	4	63	1.2	1.9	15.6	25
C20	6	38						
C20:1	6	30						
C18:2 con	2	40						

Legend see Table 7

Table 12 Determination of free fatty acids in Pecorino Romano

Fatty acid	all results n	median mg/kg	GC separation of free fatty acids n	mean mg/kg	sr mg/kg	RSDr %	sR mg/kg	RSDR %
C1	3	61						
C2	10	193	6	180	6.8	3.8	30.8	17
C3	7	5	4	5	0.7	14.5	2.1	42
iC4	1	9						
C4	19	852	8	834	9.0	1.1	97.3	12
iC5	1	10						
C5	2	5						
iC6	1	12						
C6	19	427	9	476	11.7	2.5	37.6	8
C8	16	313	7	304	6.0	2.0	21.0	7
C10	16	788	7	758	17.2	2.3	111.9	15
C10:1	7	30	2	29	0.5	1.9	1.0	4
C12	16	394	7	362	10.4	2.9	35.5	10
C14	15	617	6	630	15.1	2.4	92.8	15
C14:1	9	19	2	22	2.5	11.5	3.5	16
C15	12	51	4	51	1.4	2.7	5.6	11
C16	15	1170	6	1080	28.1	2.6	220.6	20
C16:1	13	80	5	91	3.8	4.2	38.9	43
C17	10	51	3	47	2.7	5.7	21.4	46
C18	15	331	6	343	20.8	6.1	66.9	20
C18:1	15	1061	6	1064	49.9	4.7	115.0	11
C18:2	14	140	5	172	13.7	8.0	91.6	53
C18:3	11	106	4	104	2.9	2.8	23.6	23
C20	7	24	2	10	2.7	27.0	2.7	27
C20:1	6	30	2	14	1.2	8.4	9.1	64
C18:2 con	2	78						

Legend see Table 7

Discussion

Problems with aminopropyl cartridges

The quantification of acetic acid is very important for Swiss type cheese. Unfortunately some aminopropyl cartridges contain very different contents of this acid (several manufacturer uses acetic acid for the rinsing of the resin). A comparison of three SPE is shown in the following table.

Supplier	Name	Part number	Lot. Nr.	Acetic acid concentration
Supelco	Supelclean	57014	SP0906G	high
Varian	Bondelut	611303	112151	medium
Merck	LiChrolut	19697	FE103068	low

Therefore each new SPE batch have to be checked on the content of acetic acid before analysis of free fatty acids. Dependent on the content of free fatty acids and lactic acid the sample weight have to be adapted. Overloaded SPE cartridges have low recoveries. It could be shown, that the maximum capacity of a 500 mg Aminopropyl phase was about 0.1 mmol free fatty acids.

Comparison of the median values of the different methods

The median values of the different methods were compared on the graphical presentations (page G3-G152) according to the instruction on page G1. Table 13 shows the significant differences between the GC method without derivatization and all other methods.

Table 13 Significant differences between the GC method without derivatization and all other methods

Fatty acid	Butter 1	Butter 2	Processed Emmental	Fontina	Parmigiano Reggiano	Pecorino Romano
C2	↑ LC	↑ LC			↑ G-ester	↑ G-ester ↑ LC
C3				↑ LC		↑ G-ester
C4	↑ G-TMA	↑ G-TMA	↑ G-TMA ↑ LC	↑ G-TMA	↑ G-TMA	
iC5			↓ G-ester	↓ G-ester ↑ LC		
C5				↑ LC		↑ LC
C6	↑ G-TMA ↑ G-ester	↑ G-TMA ↑ G-ester	↑ G-TMA	↑ G-TMA		
C8	↑ G-TMA	↑ G-TMA	↑ G-TMA	↑ G-TMA		
C10	↑ G-TMA	↑ G-TMA	↑ G-TMA	↑ G-TMA		↑ G-TMA
C10:1	↑ G-TMA	↑ G-TMA		↑ G-TMA		↑ G-TMA
C12	↑ G-TMA	↑ G-TMA	↑ G-TMA	↑ G-TMA	↑ G-TMA	↑ G-TMA
C14	↑ G-TMA ↑ G-ester	↑ G-TMA	↑ G-TMA	↑ G-TMA	↑ G-TMA	↑ G-TMA
C14:1		↑ G-TMA	↑ G-TMA	↓ G-ester	↑ G-TMA	↑ G-TMA ↓ G-ester
C15			↑ G-TMA	↑ G-TMA		↑ G-TMA
C16	↑ G-TMA	↑ G-TMA	↑ G-TMA	↑ G-TMA	↑ G-TMA	↑ G-TMA
C16:1		↑ G-TMA	↓ G-ester	↑ G-TMA ↓ G-ester		↑ G-TMA
C17	↑ G-TMA			↑ G-TMA		
C18	↑ G-TMA	↑ G-TMA	↑ G-TMA ↓ G-ester	↑ G-TMA	↑ G-TMA ↓ G-ester	↑ G-TMA
C18:1	↑ G-TMA	↑ G-TMA	↑ G-TMA ↓ G-ester	↑ G-TMA	↑ G-TMA	↑ G-TMA
C18:2	↓ G-ester			↓ G-TMA	↓ G-TMA	
C18:3						
C20	↓ G-ester		↑ G-TMA	↑ G-TMA	↑ G-TMA	↑ G-TMA
C20:1			↑ G-TMA		↑ G-TMA	↑ G-TMA

Legend:
The abbreviation of the methods are given in Table 5
↓ method X median value with method X is significant lower than G-FFA ($p < 0.05$)
↑ method X median vaule with method X is significant higher than G-FFA ($p < 0.05$)

Comparison of the precision parameters

The standard deviations of the repeatability s_r and reproducibility s_R could only be calculated for the gas chromatographic separation of fatty acids without derivatization.

Standard deviations of repeatability s_r

Butter samples

The maximum relative standard deviation of the repeatability (RSD_r) for free fatty acids with a concentration over 20 mg/kg is 4.9 %, except for linolenic acid with 5.6 % in butter 1. The average RSD_r for these fatty acids is below 2 %.
The standard deviation of the repeatability s_r for free fatty acids with a concentration below 20 mg/kg is < 1.7 mg/kg, except for arachidic acid with 4.4 mg/kg in butter 1.

Processed Emmental

The maximum relative standard deviation of the repeatability (RSD_r) for free fatty acids with a concentration over 10 mg/kg is 8.8 %, except for linolenic acid with 9.8 %. The average RSD_r for these fatty acids is 5.4 %.

Fontina

The maximum relative standard deviation of the repeatability (RSD_r) for free fatty acids is 6.4 %, except for myristoleic, heptadecanoic and linolenic acid which have RSD_r of 10 - 15 %. The average RSD_r for these fatty acids is 3.5 %.

Parmigiano-Reggiano

The maximum relative standard deviation of the repeatability (RSD_r) for free fatty acids is 5 %, except for myristoleic acid with 8.9 %. The average RSD_r for these fatty acids is 3 %.

Pecorino Romano

The maximum relative standard deviation of the repeatability (RSD_r) for free fatty acids with a concentration over 10 mg/kg is 6.1 %, except for myristoleic and linoleic acid with 11.5 and 8 %, respectively. The average RSD_r for these fatty acids is 3.2 %.

Standard deviations of reproducibility s_R

Butter samples

The maximum relative standard deviation of the reproducibility RSD_R for free fatty acids with a concentration higher than 20 mg/kg is 30 %, except for heptadecanoic acid, linoleic and arachidoleic acid (butter 2) with 41 - 140 %. The average RSD_R for these fatty acids is 18 %.
Capric, lauric, myristic, palmitic and linolenic acid shows lowest RSD_R of 7 - 17%. Stearic and linoleic acid, which are in the same concentration range have much higher values for RSD_R 22 -53 %.

Processed Emmental

The maximum relative standard deviation of the reproducibility RSD_R for free fatty acids with a concentration higher than 30 mg/kg is 26 %, except for capric and linoleic acid with 66 and 36 %, respectively. The average RSD_R for these fatty acids is 18 %.

Fontina
The maximum relative standard deviation of the reproducibility RSD_R for all free fatty acids with a concentration higher than 20 mg/kg is 32 %, except for lauric, myristoleic, pentadecanoic and linolenic acid with 41 - 47 %. The average RSD_R for these fatty acids is 21 %.

Parmigiano-Reggiano
The maximum relative standard deviation of the reproducibility RSD_R for free fatty acids with a concentration higher than 20 mg/kg is 18 %, except for acetic, palmitoleic, heptadecanoic, linoleic and linolenic acid with 25 - 48 %. The average RSD_R for these fatty acids is 12 %.

Pecorino Romano
The maximum relative standard deviation of the reproducibility RSD_R for free fatty acids with a concentration higher than 20 mg/kg is 23 %, except for palmitoleic, heptadecanoic and linoleic acid with 43 - 53 %. The average RSD_R for these fatty acids is 13 %.

Conclusions

The quantification of the free fatty acids with tetramethylammonium hydroxide (TMAH) derivatization gives for most free fatty acids significant higher values than all other methods, probably due to hydrolysis reaction of triglycerides during the derivatization procedure. Only linoleic acid in Parmigiano-Reggiano and Pecorino Romano is significant lower than the G-FFA method.
The determination of free fatty acids with gas chromatography as methyl, propyl and butyl esters or without derivatization gives for most fatty acids comparable results.

Special attention have to be paid on the FFA isolation step with the solid phase extraction cartridges. On the one hand acetic acid artefacts occurs in different concentrations depending on the manufacturer and the batch on the other hand high concentrations of free fatty acids and lactic acid overloads the cartridges and reduces the recoveries of the fatty acids. A mixture of chloroform/2-propanol is used for the elution of the neutral lipids from the aminopropyl cartridge. Because of toxicity and enviromental pollution, chloroform should not be used any longer. The mixture can be replaced by hexane/2-propanol 3+2 [5].

Table 14 gives a summary of the average relative standard deviations of the repeatability s_r and reproducibility s_R of the gas chromatographic separation of fatty acids without derivatization. The unsaturated myristoleic, palmitoleic, linoleic and linolenic acid shows frequently the worst repeatability and reproducibility of all free fatty acids.

Table 14 Summary of precisions parameters

Sample	RSD_r		RSD_R	
	average	outlier	average	outlier
Butter	2.0 %	C18:3	18 %	C17, C18:2, C20:1
Processed Emmental	5.4 %	C18:3	18 %	C10, C18:2
Fontina	3.5 %	C14:1, C17, C18:3	21 %	C12, C14:1, C15, C18:3
Parmigiano-Reggiano	3.0 %	C14:1	13 %	C2, C16:1, C17, C18:2
Pecorino Romano	3.2 %	C14:1, C18:2	13 %	C16:1, C17, C18:2

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1 De Jong C. and Badings H.T. J. Determination of free fatty acids in milk and cheese. Procedures for extraction, clean up and capillary gas chromatorgraphic analysis. High Res. **13** 94-98 (1990)

2 De Jong C., Palma K. and Neeter R. Sample preparation before capillary gas chromatographic estimation of free fatty acids in fermented dairy products. Neth. Milk Dairy J. **48** 151-156 (1994)

3 Anonymous, Swiss food manual, Chapter 60B, Collaborative studies, Berne (1989)

4 SYSTAT. Systat for Windows: Statistics. Version 5.0 Edition, Evanston, IL: SYSTAT Inc. (1992).

5 Badertscher R., Geisinger M.-L. and Gerber P. Ersatz von halogenierten Lösungsmitteln bei der Bestimmung der fettfreien Trockenmasse in Butter. Mitt. Gebiete Lebensm. Hyg. **84**, 509-513 (1993)

Appendix G

Graphical presentations

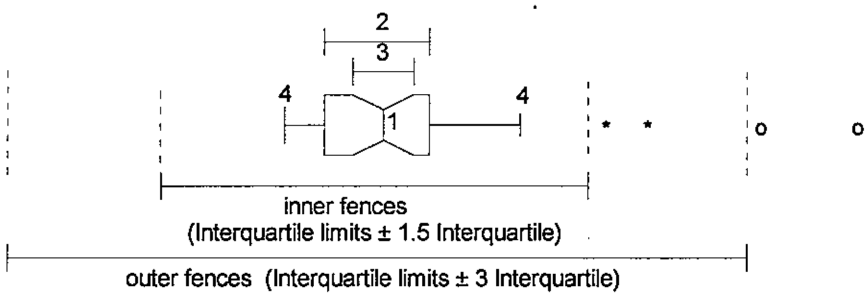
On the following pages the graphical presentation of all free fatty acids with all methods are shown

Sample number	Description	Page
1	Butter 1	G3 - G25
2	Butter 2	G26 - G48
3	Processed Emmental	G49 - G74
4	Fontina	G75 - G100
5	Parmigiano-Reggiano	G101 - G126
6	Pecorino Romano	G127 - G152

Appendix: Graphical presentations

On the following pages all results of the laboratories are shown in category plots. The pooled values are shown for each method group in the notched box plots. The notched box plot is described in figure 2, the category plot in figure 3.

Description of notched box plot



1. Median
2. Interquartile range
3. 95% confidence range (Mc Gill, Tukey and Larsen 1978)
4. Whiskers

* outside value

o far outside value

Figure 2 Description of notched box plot

Description of category plot (example)

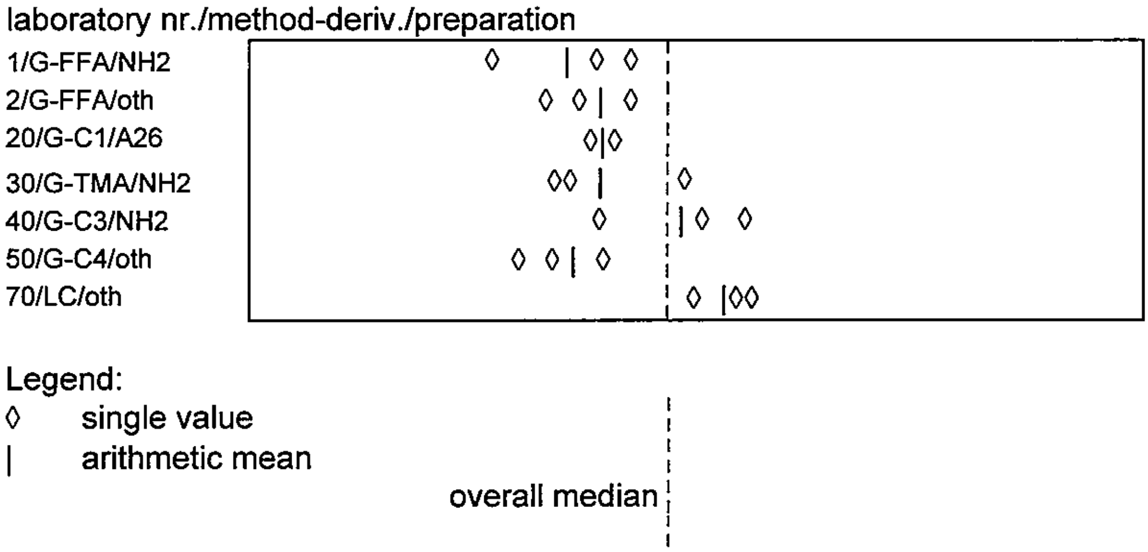
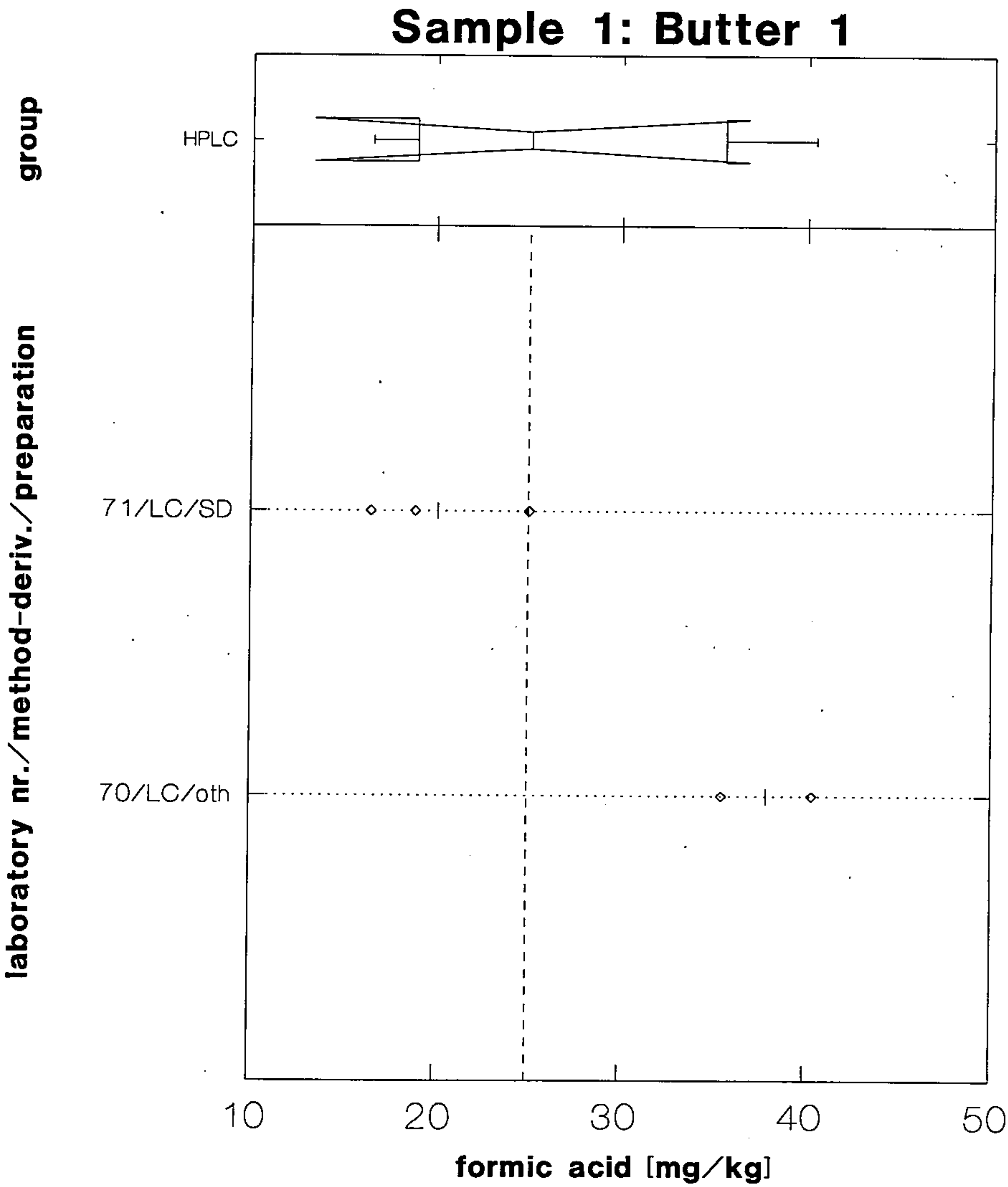


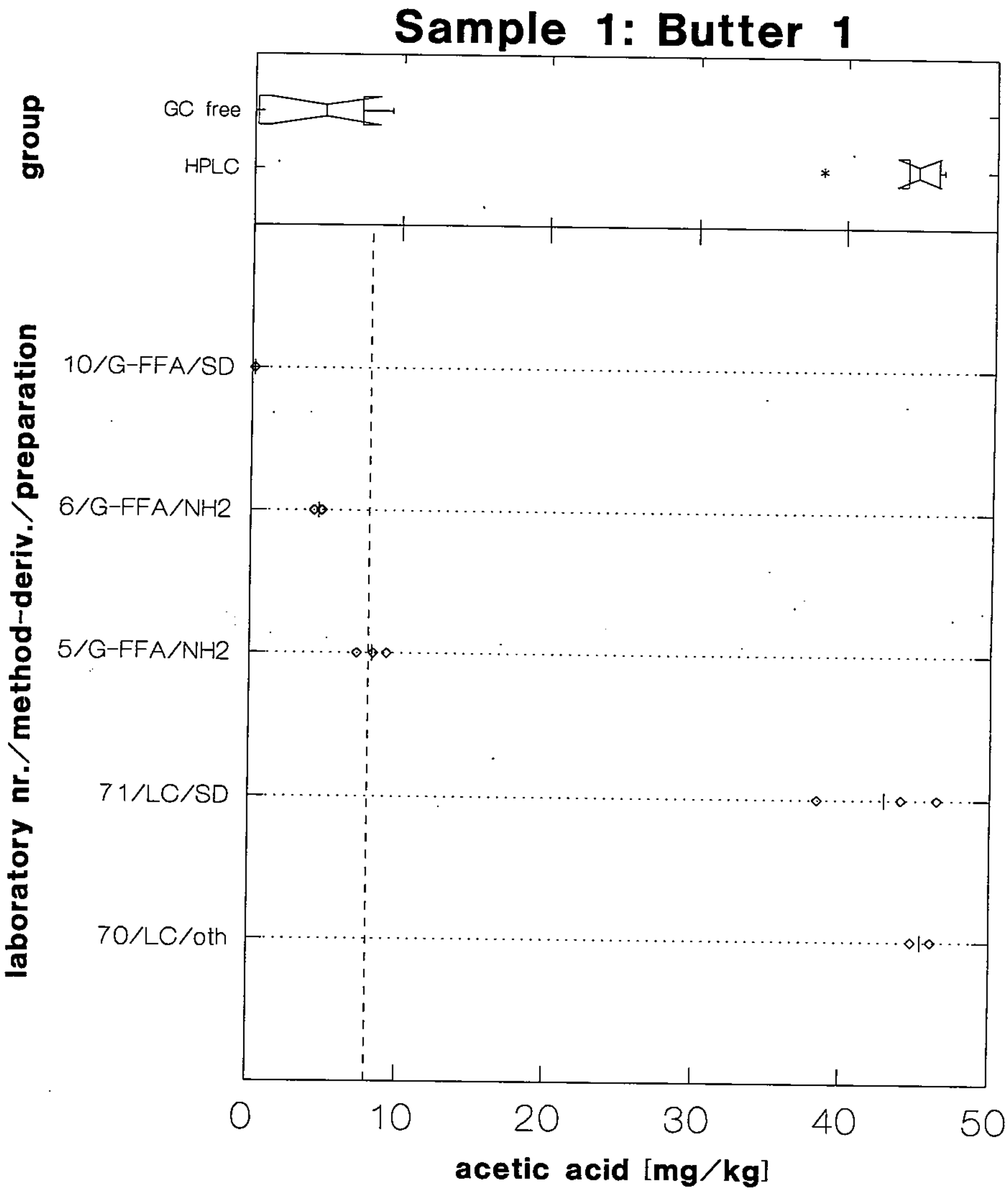
Figure 3 Description of category plot

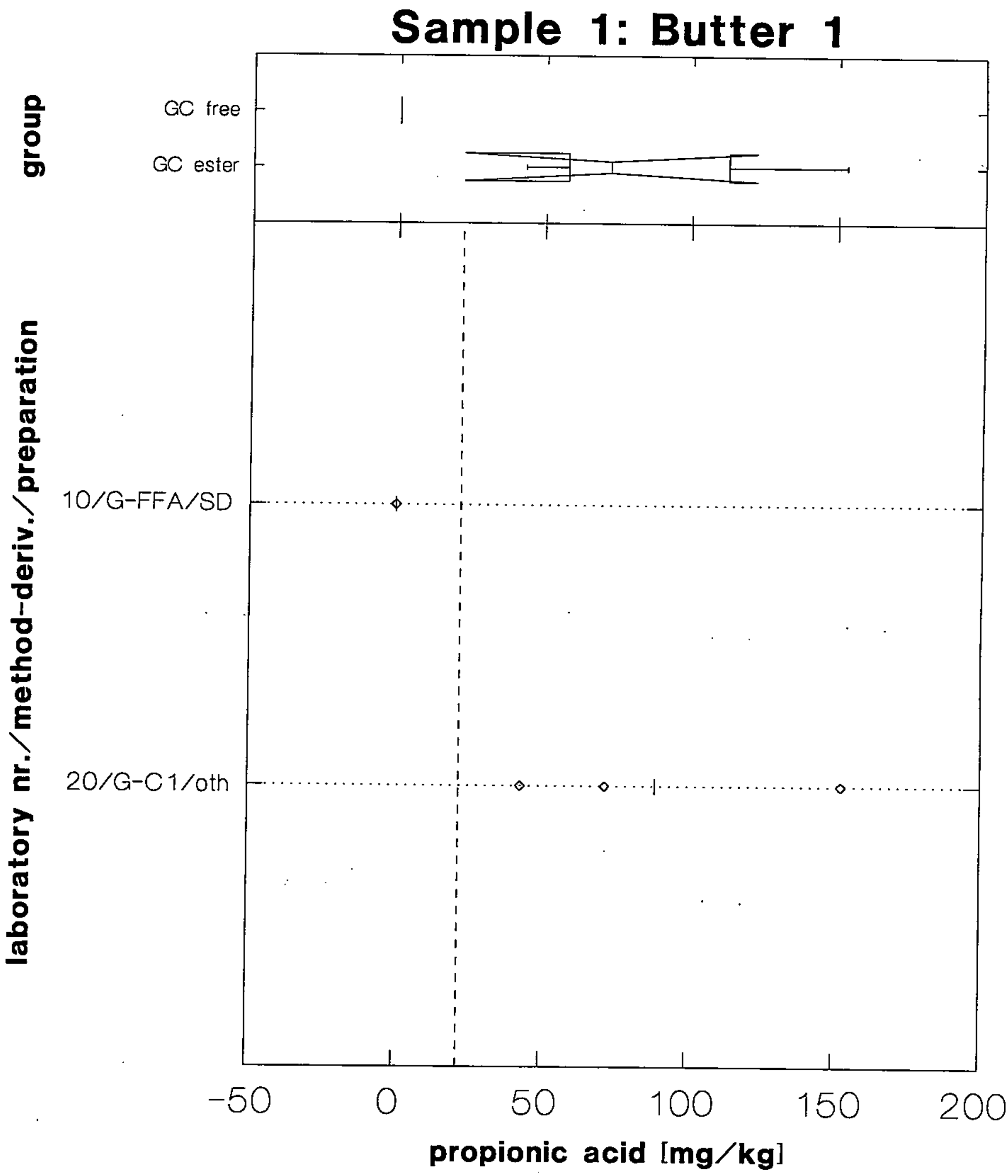
Abbreviations

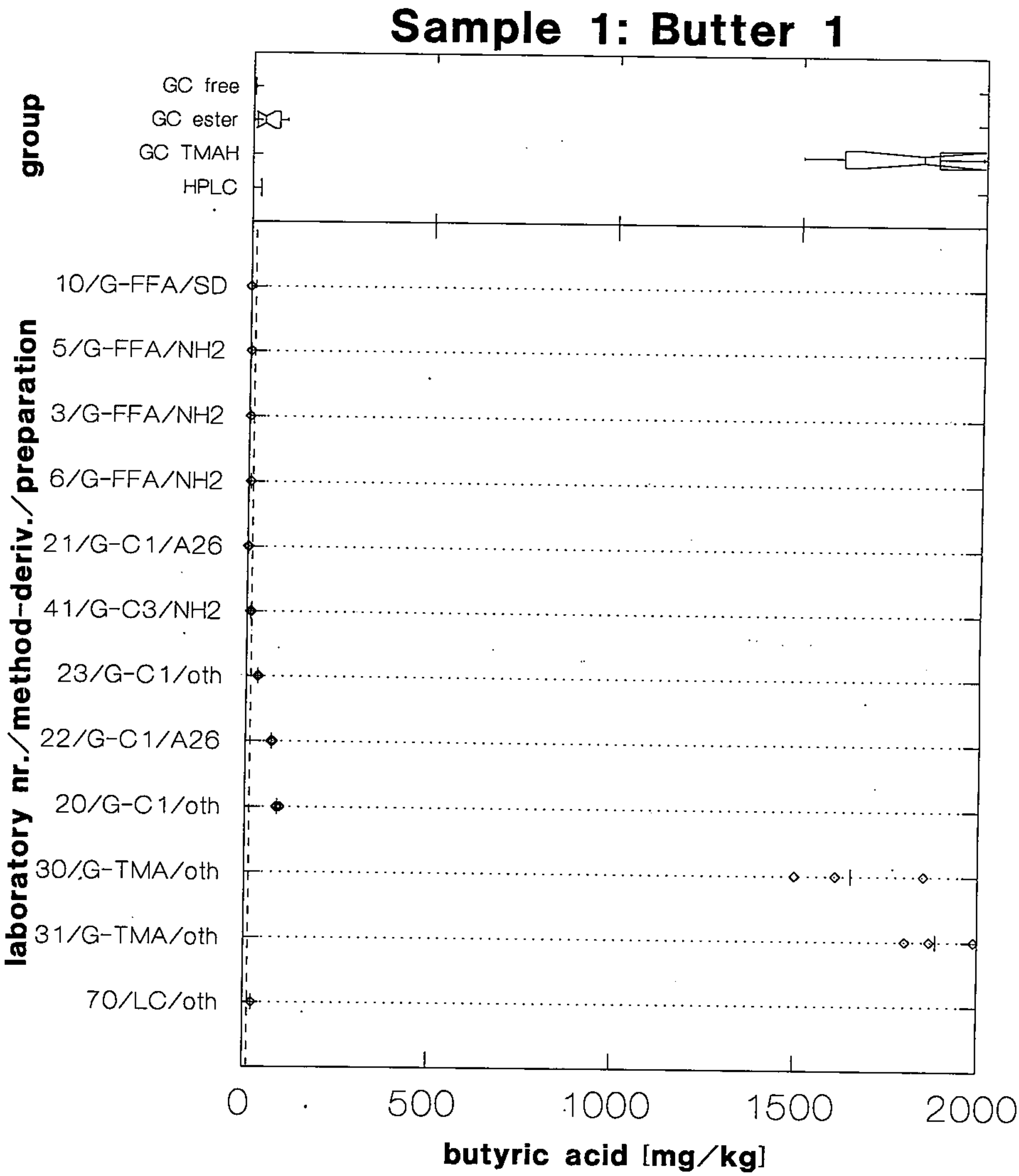
Abb.	Laboratory number	Method used
G-FFA	1-10	GC separation of free fatty acids
G-C1	20-24	GC separation of methyl esters
G-TMA	30-31	GC separation of TMAH derivates
G-C3	40-41	GC separation of propyl esters
G-C4	50	GC separation of butyl esters
LC	70-71	HPLC separation of free fatty acids

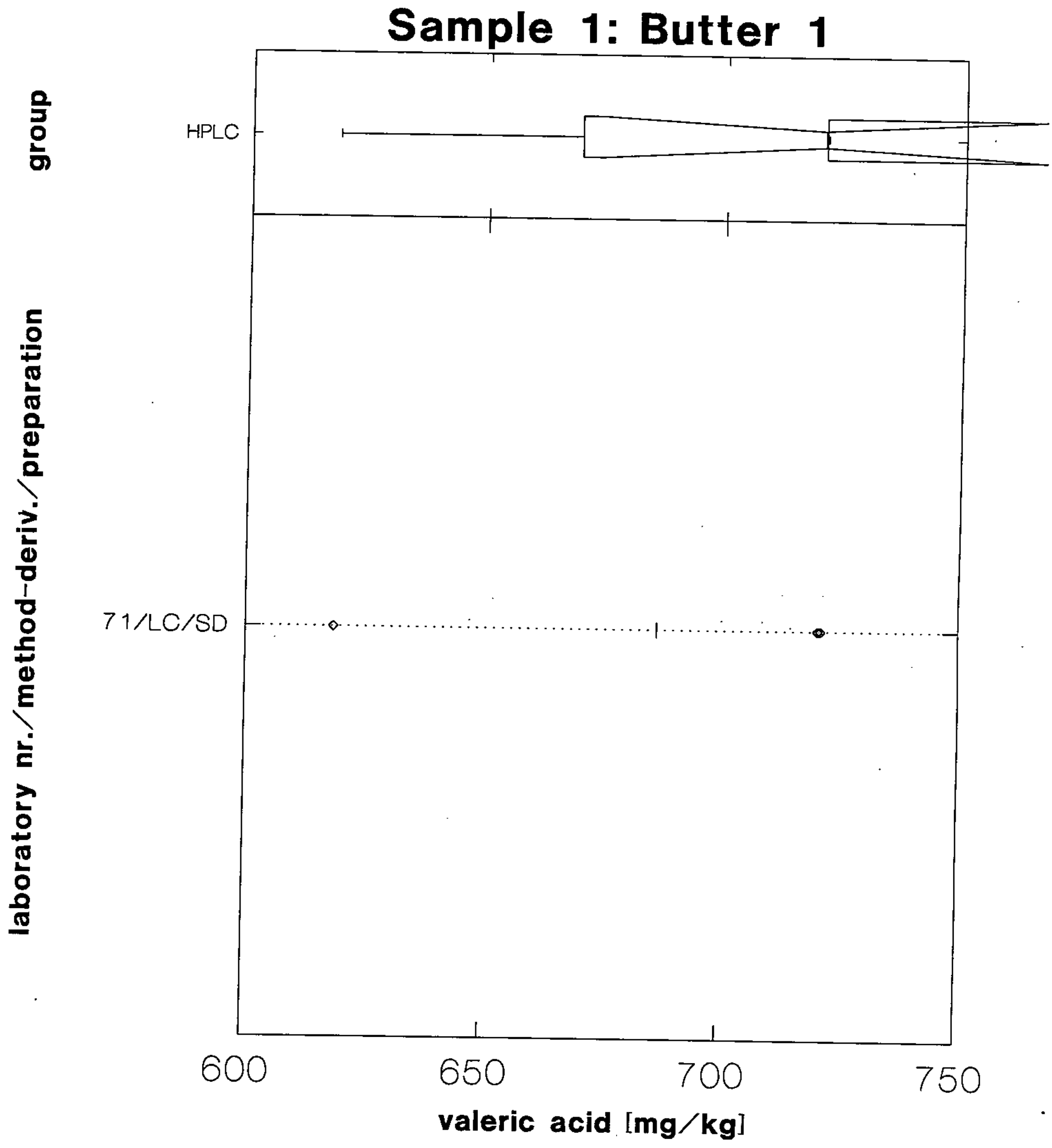
Preparation	Description
NH2	extraction with organic solvents and SPE with Aminopropyl cartridges
A26	extraction with organic solvents and SPE with Abmerlyst A26 cartridges
SD	steam distillation
other	only extraction with organic solvents

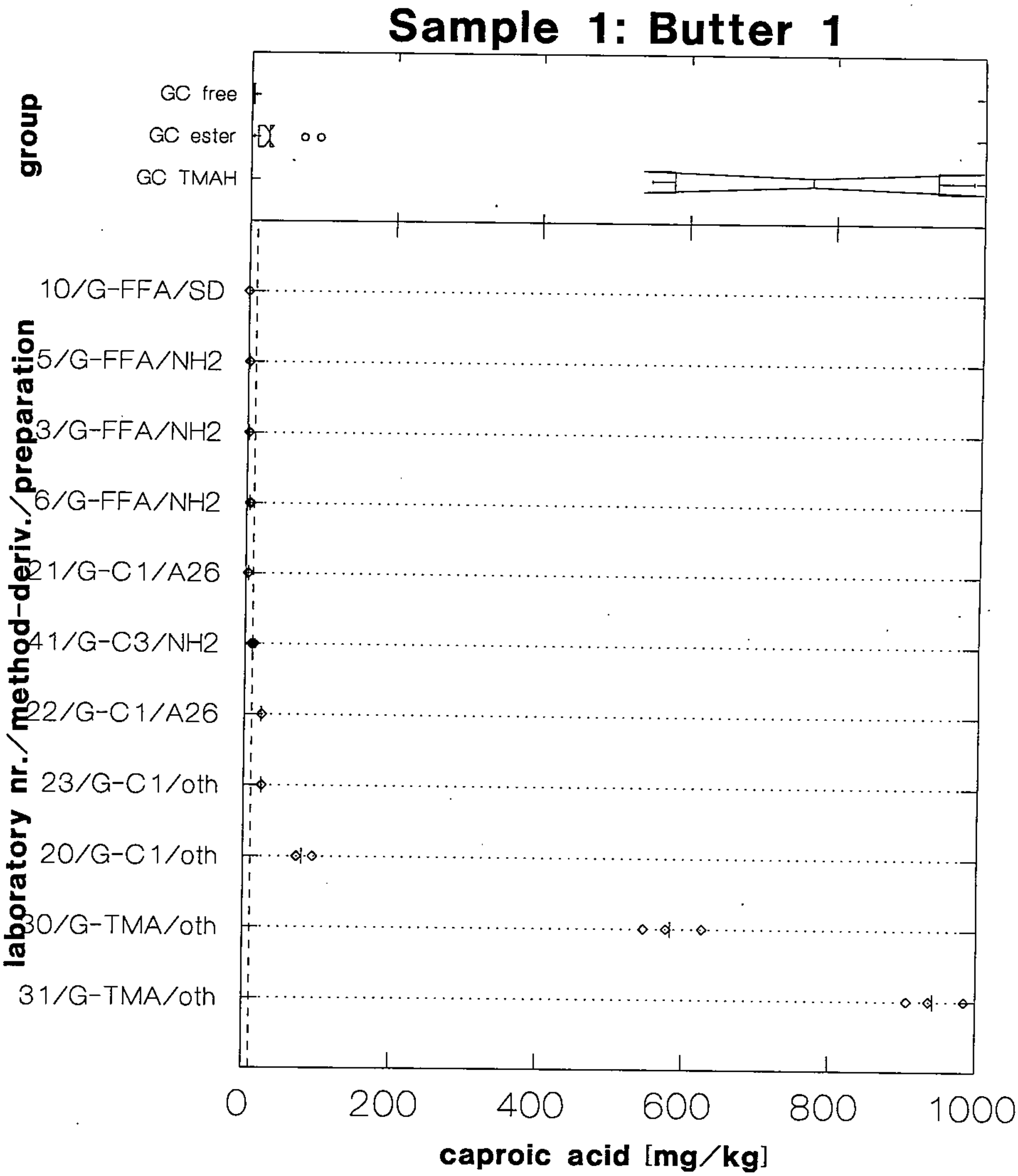

Sample 1: Butter 1
group
HPLC
laboratory nr./method-deriv./preparation
71/LC/SD
70/LC/oth
10
20
30
40
50
formic acid [mg/kg]

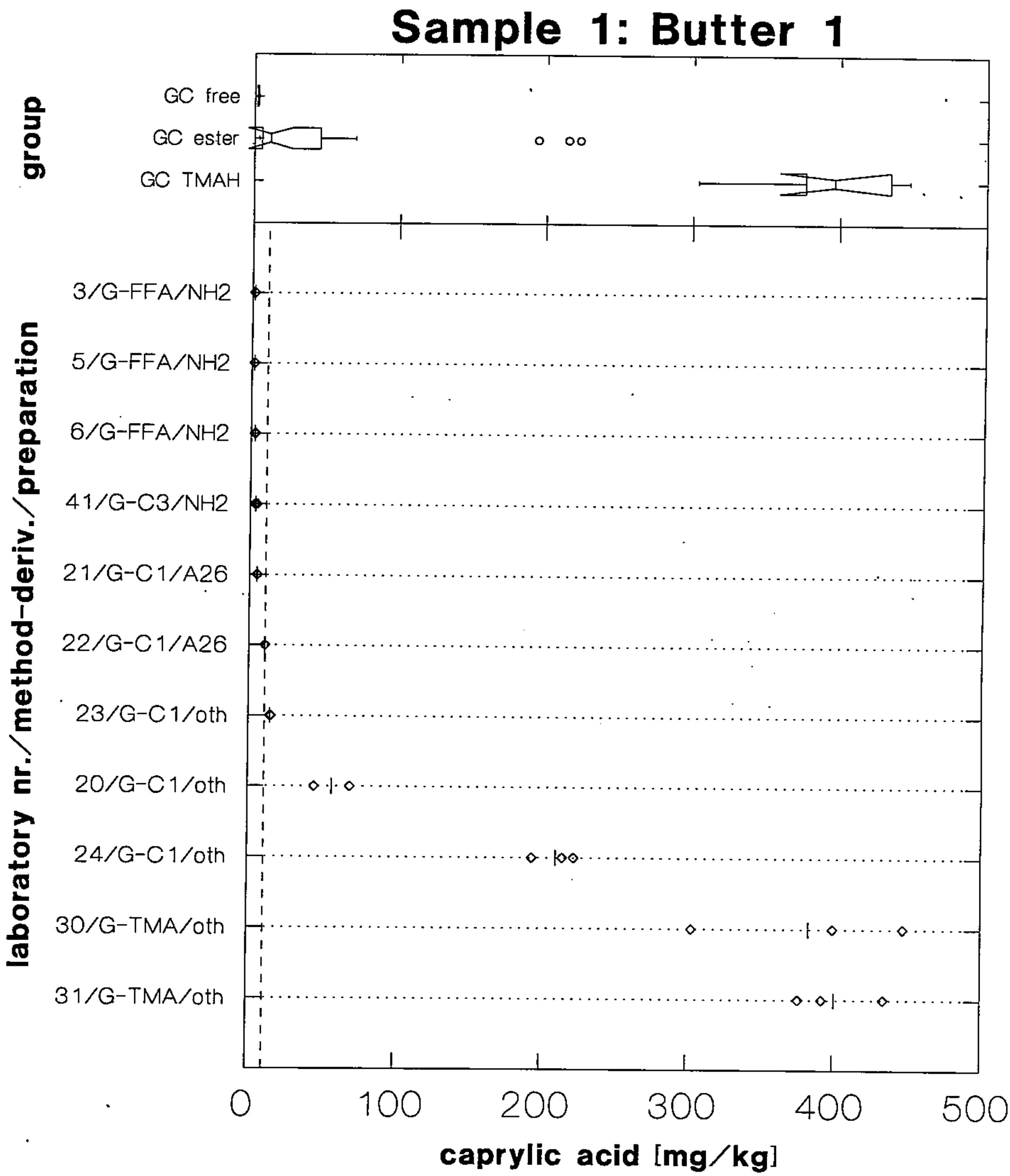

Sample 1: Butter 1
group
GC free
HPLC
laboratory nr./method-deriv./preparation
10/G-FFA/SD
6/G-FFA/NH2
5/G-FFA/NH2
71/LC/SD
70/LC/oth
0
10
20
30
40
50
acetic acid [mg/kg]

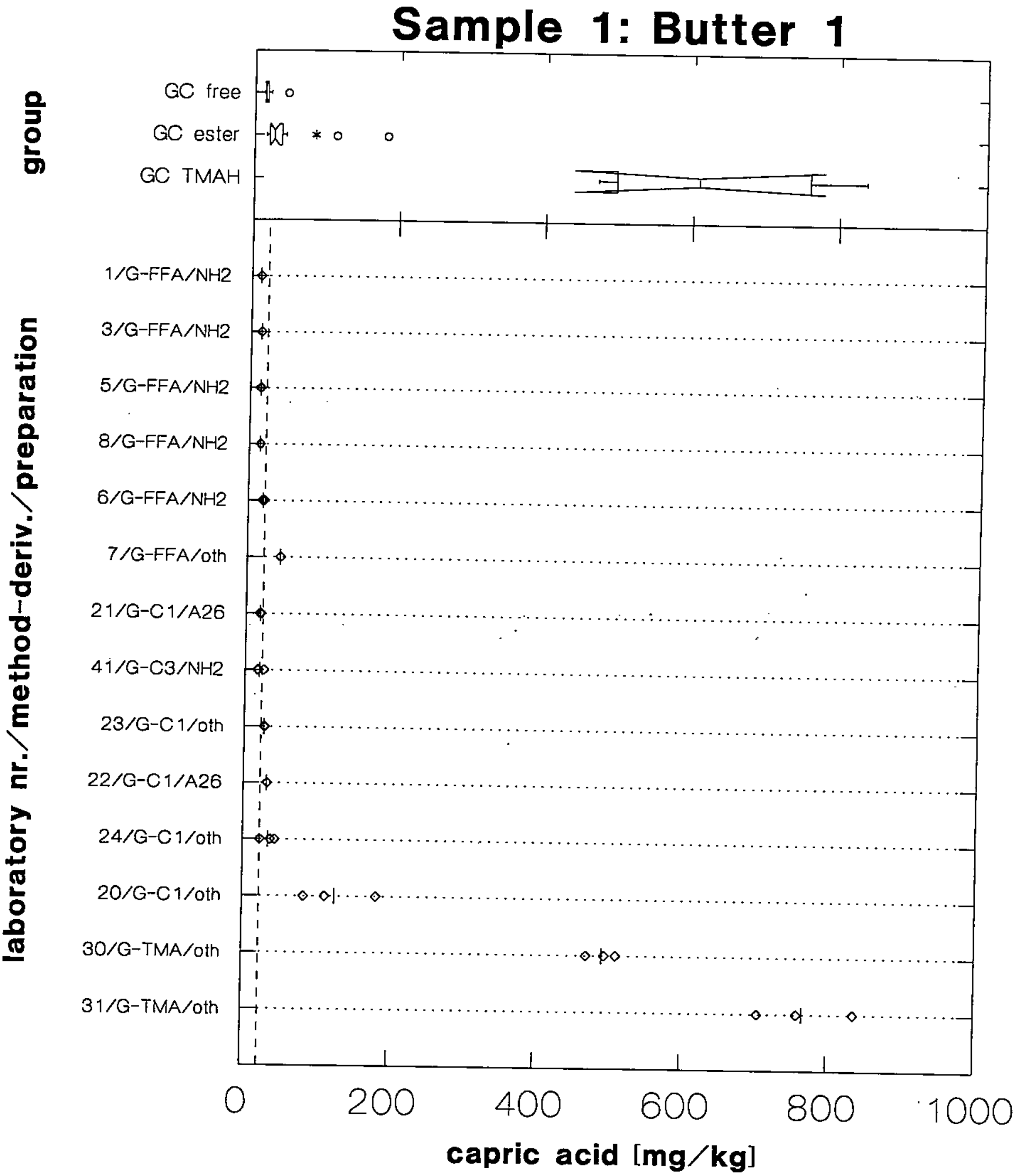

Sample 1: Butter 1
group
GC free
GC ester
laboratory nr./method-deriv./preparation
10/G-FFA/SD
20/G-C1/oth
-50
0
50
100
150
200
propionic acid [mg/kg]


Sample 1: Butter 1
group
GC free
GC ester
GC TMAH
HPLC
laboratory nr./method-deriv./preparation
10/G-FFA/SD
5/G-FFA/NH2
3/G-FFA/NH2
6/G-FFA/NH2
21/G-C1/A26
41/G-C3/NH2
23/G-C1/oth
22/G-C1/A26
20/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
70/LC/oth
0
500
1000
1500
2000
butyric acid [mg/kg]

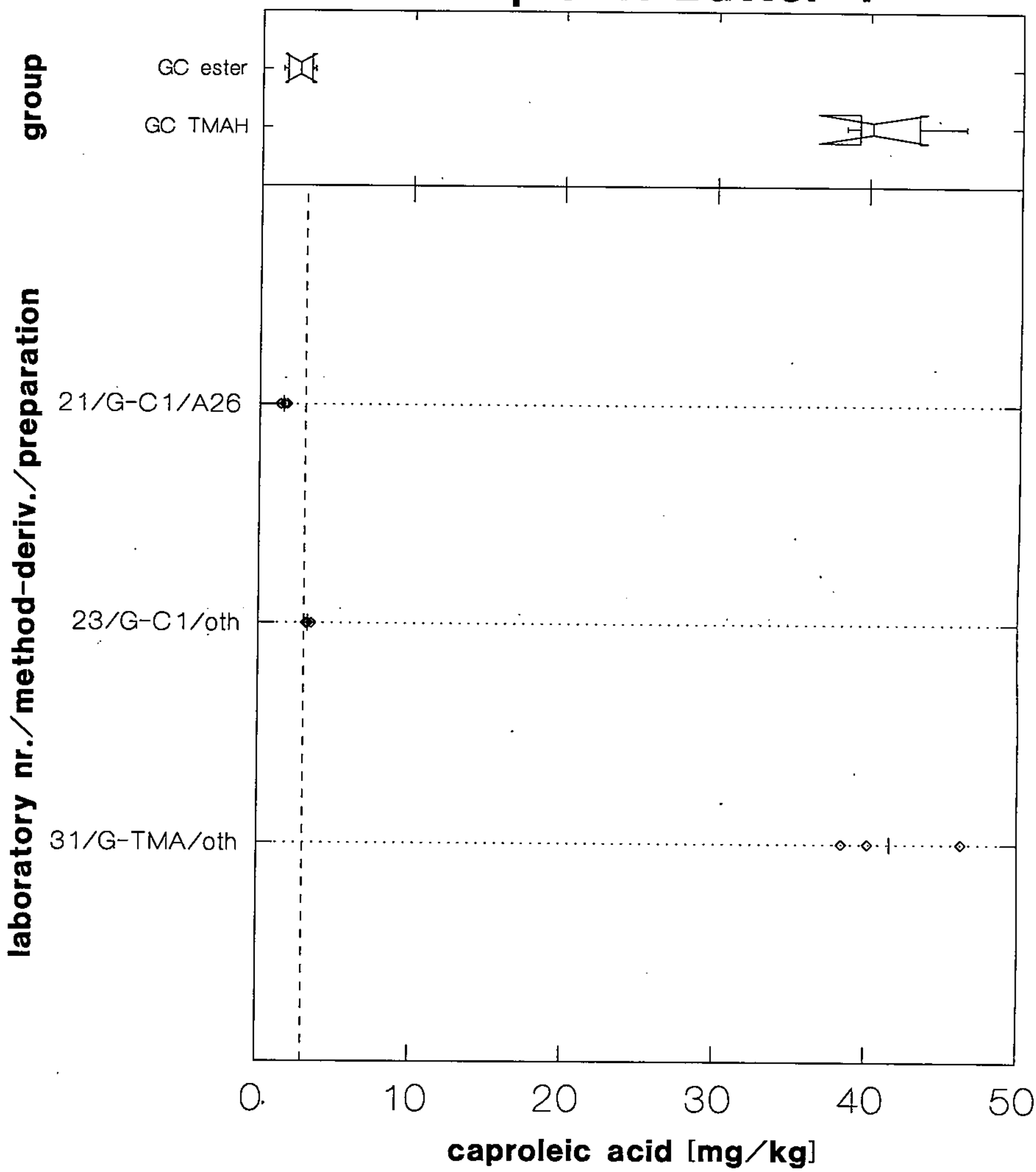

Sample 1: Butter 1
group
HPLC
laboratory nr./method-deriv./preparation
71/LC/SD
600
650
700
750
valeric acid [mg/kg]

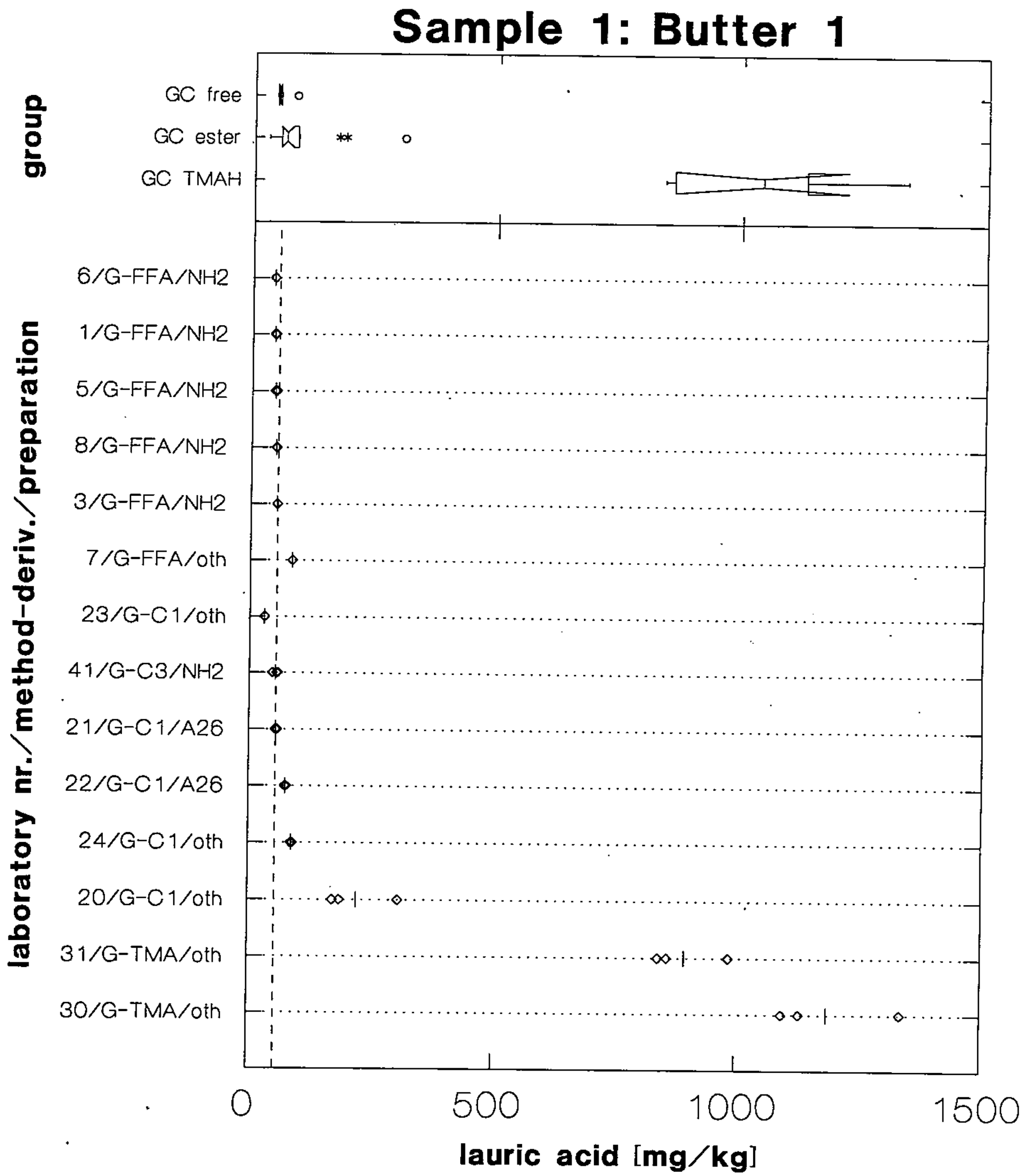

Sample 1: Butter 1
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
10/G-FFA/SD
5/G-FFA/NH2
3/G-FFA/NH2
6/G-FFA/NH2
21/G-C1/A26
41/G-C3/NH2
22/G-C1/A26
23/G-C1/oth
20/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
200
400
600
800
1000
caproic acid [mg/kg]

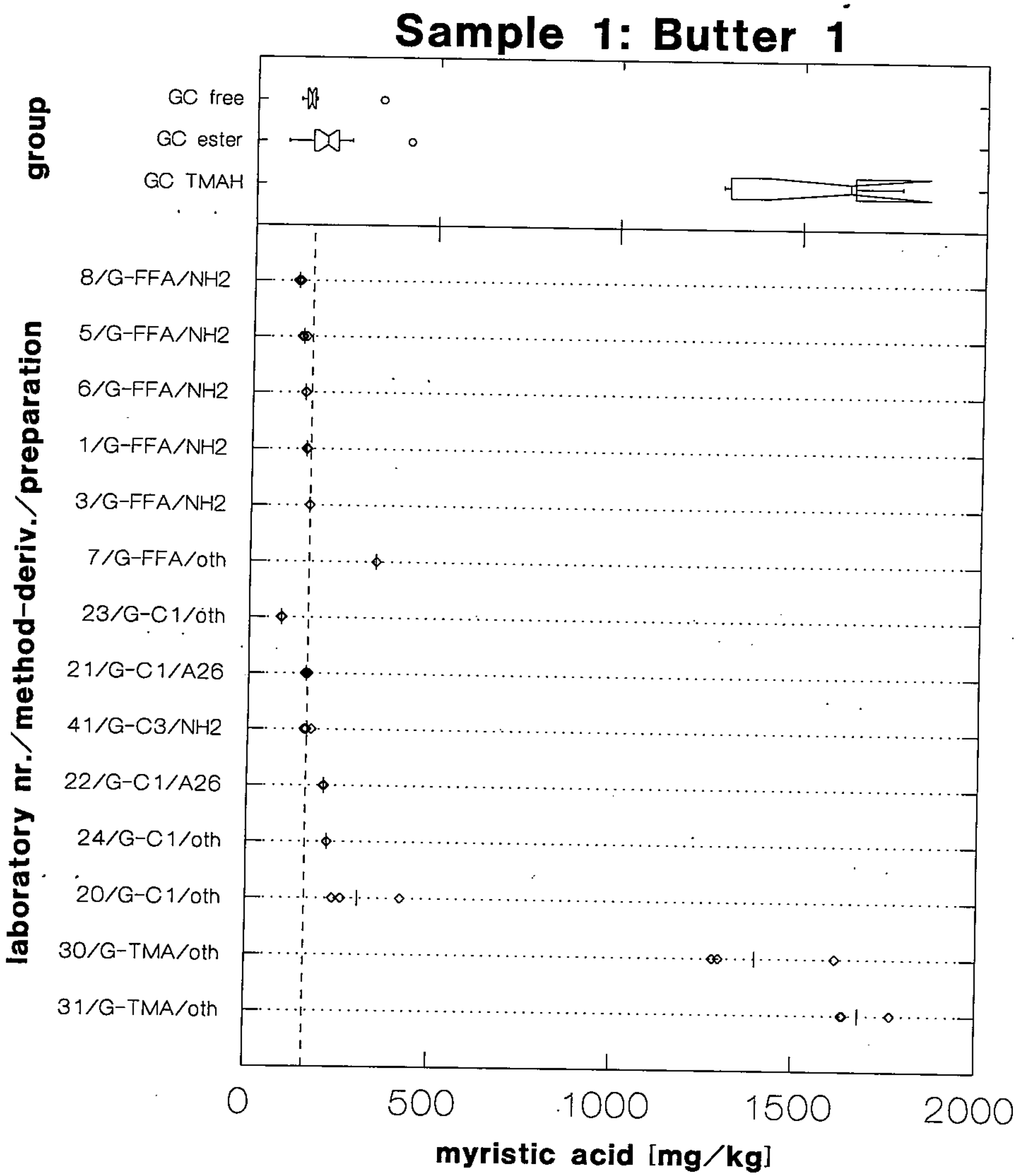

Sample 1: Butter 1
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
3/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
41/G-C3/NH2
21/G-C1/A26
22/G-C1/A26
23/G-C1/oth
20/G-C1/oth
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
100
200
300
400
500
caprylic acid [mg/kg]

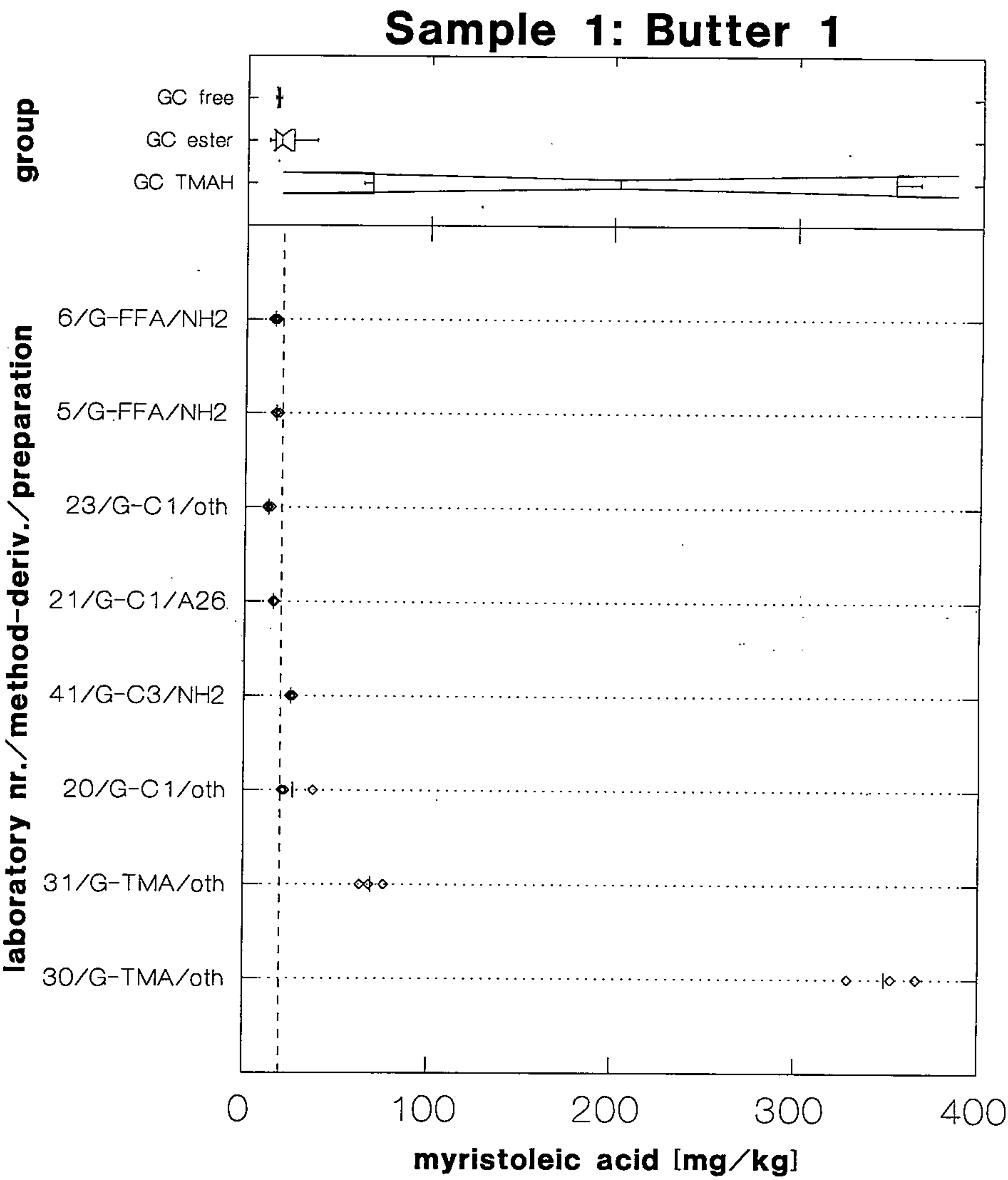

Sample 1: Butter 1
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
1/G-FFA/NH2
3/G-FFA/NH2
5/G-FFA/NH2
8/G-FFA/NH2
6/G-FFA/NH2
7/G-FFA/oth
21/G-C1/A26
41/G-C3/NH2
23/G-C1/oth
22/G-C1/A26
24/G-C1/oth
20/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
200
400
600
800
1000
capric acid [mg/kg]

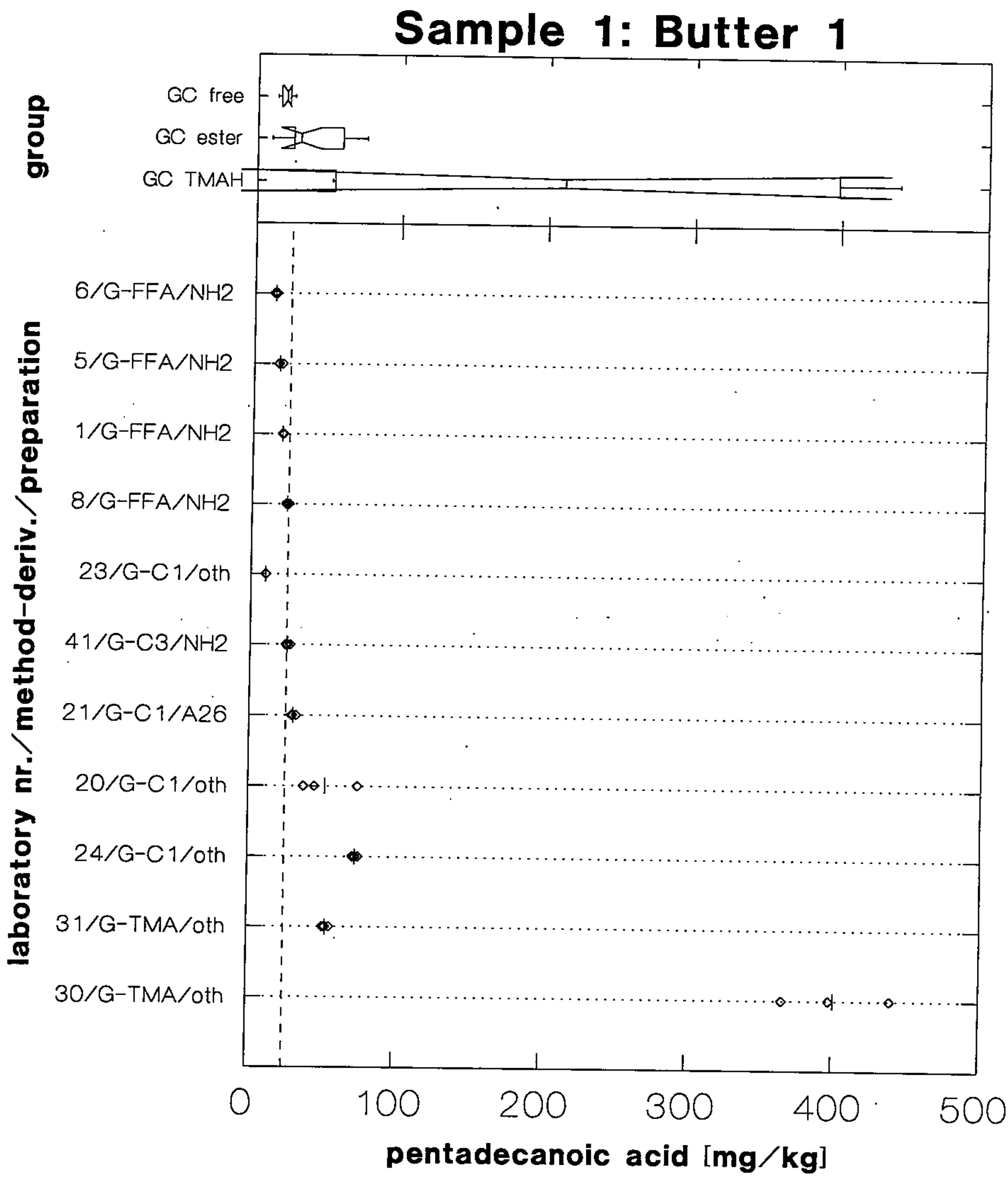
Sample 1: Butter 1

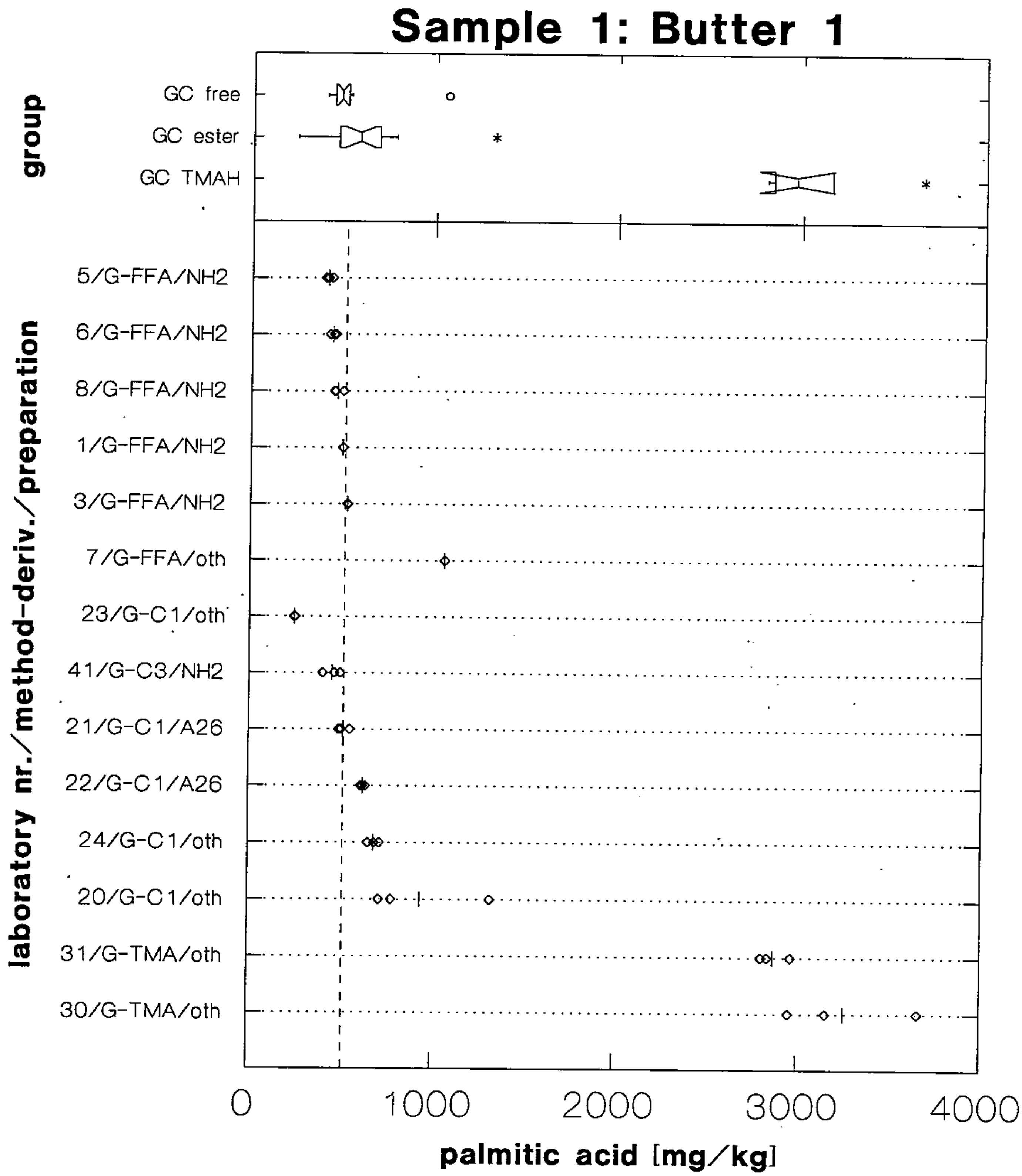



Sample 1: Butter 1
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
6/G-FFA/NH2
1/G-FFA/NH2
5/G-FFA/NH2
8/G-FFA/NH2
3/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
22/G-C1/A26
24/G-C1/oth
20/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
500
1000
1500
lauric acid [mg/kg]

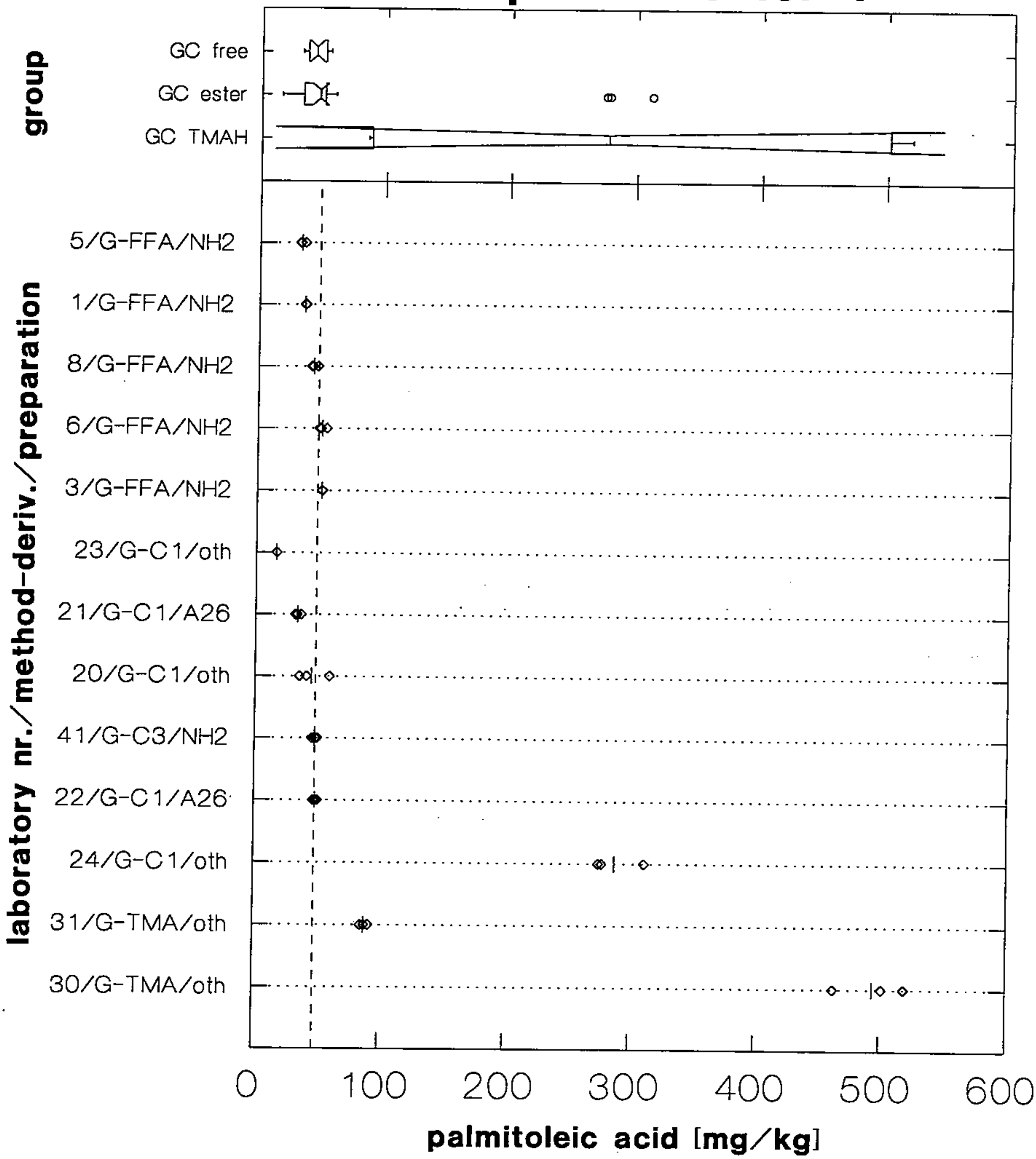

Sample 1: Butter 1
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
21/G-C1/A26
41/G-C3/NH2
22/G-C1/A26
24/G-C1/oth
20/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
500
1000
1500
2000
myristic acid [mg/kg]


Sample 1: Butter 1
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
6/G-FFA/NH2
5/G-FFA/NH2
23/G-C1/oth
21/G-C1/A26
41/G-C3/NH2
20/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
100
200
300
400
myristoleic acid [mg/kg]

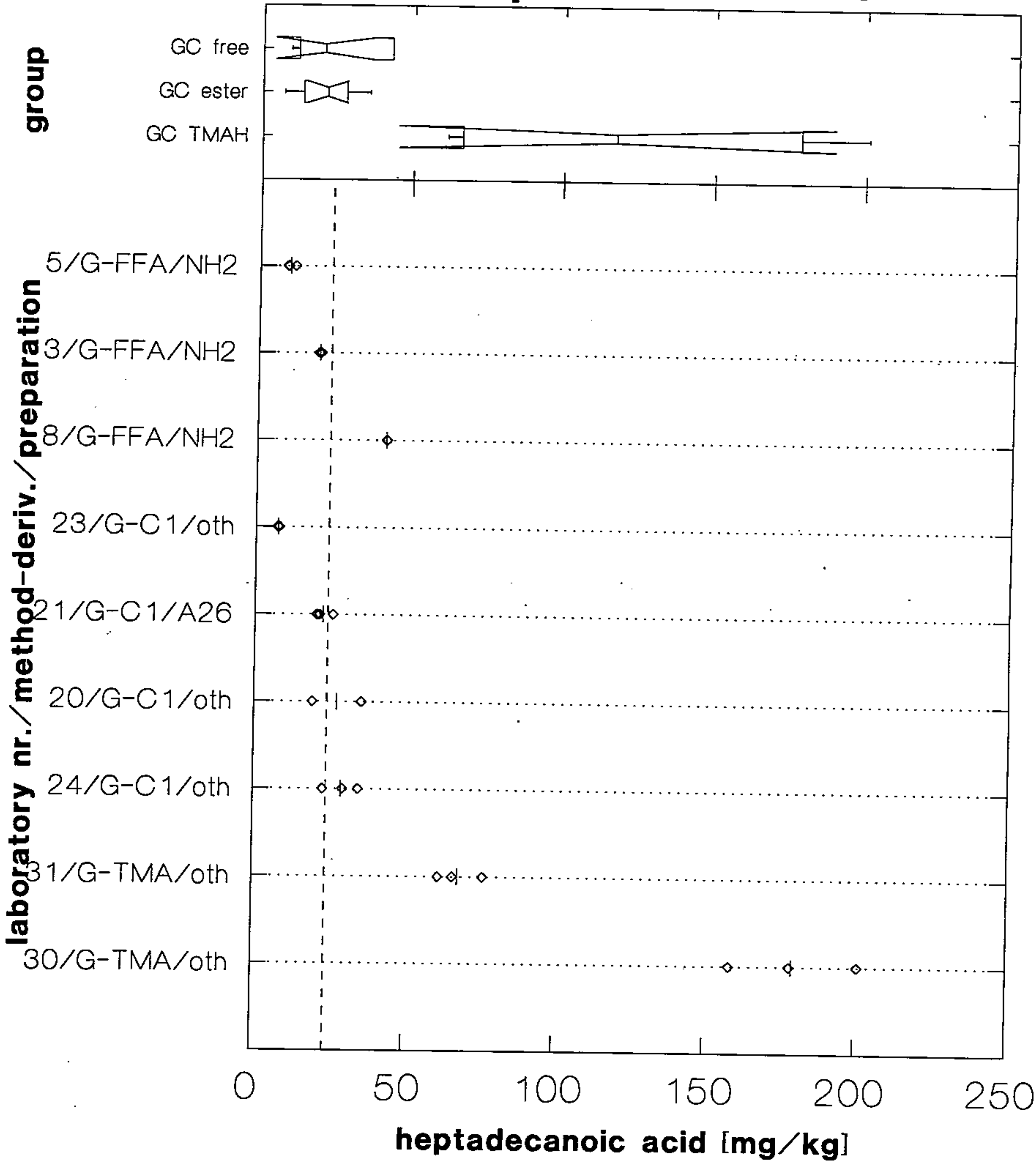

Sample 1: Butter 1
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
6/G-FFA/NH2
5/G-FFA/NH2
1/G-FFA/NH2
8/G-FFA/NH2
23/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
20/G-C1/oth
24/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
100
200
300
400
500
pentadecanoic acid [mg/kg]

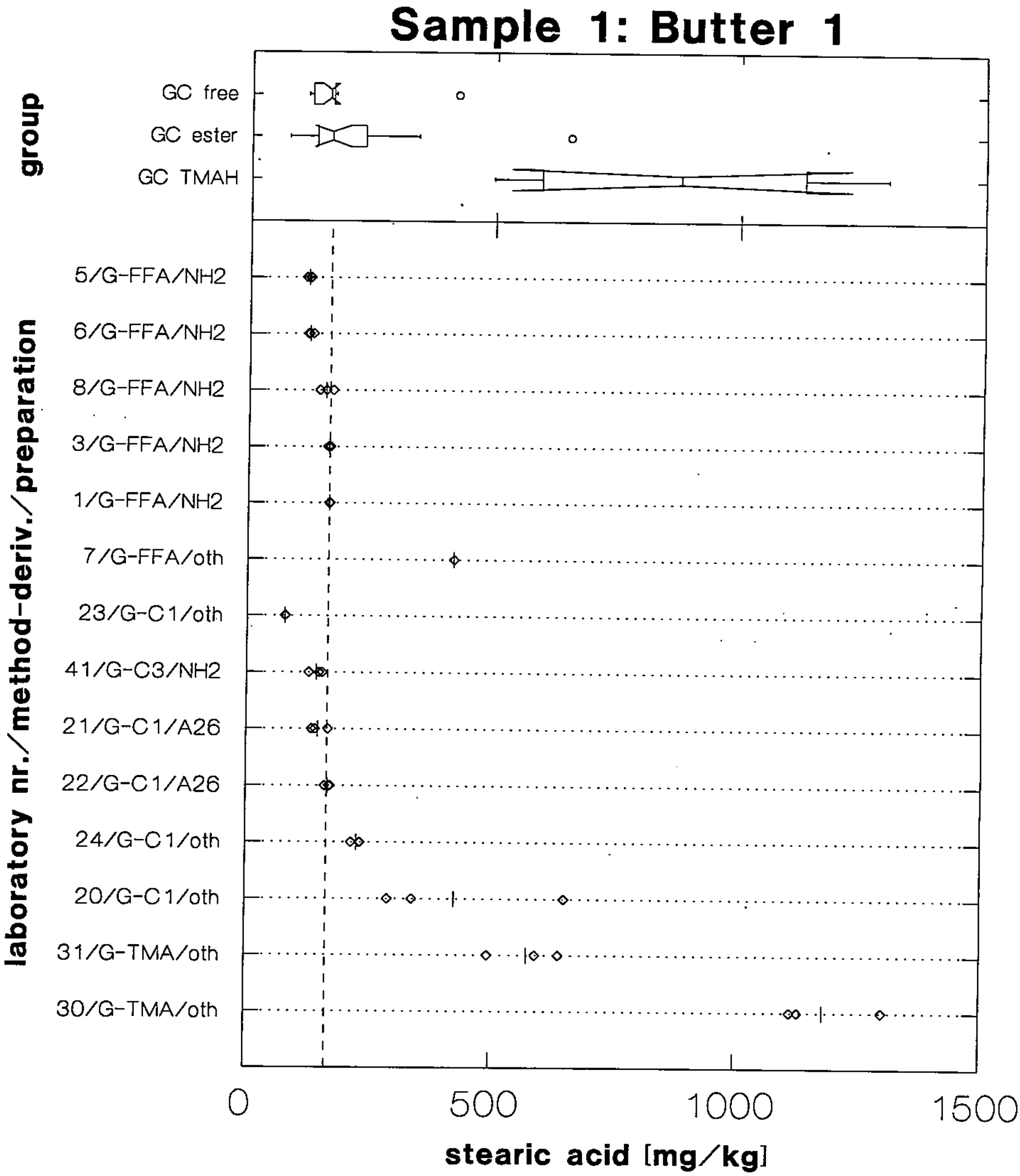

Sample 1: Butter 1
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
6/G-FFA/NH2
8/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
22/G-C1/A26
24/G-C1/oth
20/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
1000
2000
3000
4000
palmitic acid [mg/kg]

Sample 1: Butter 1

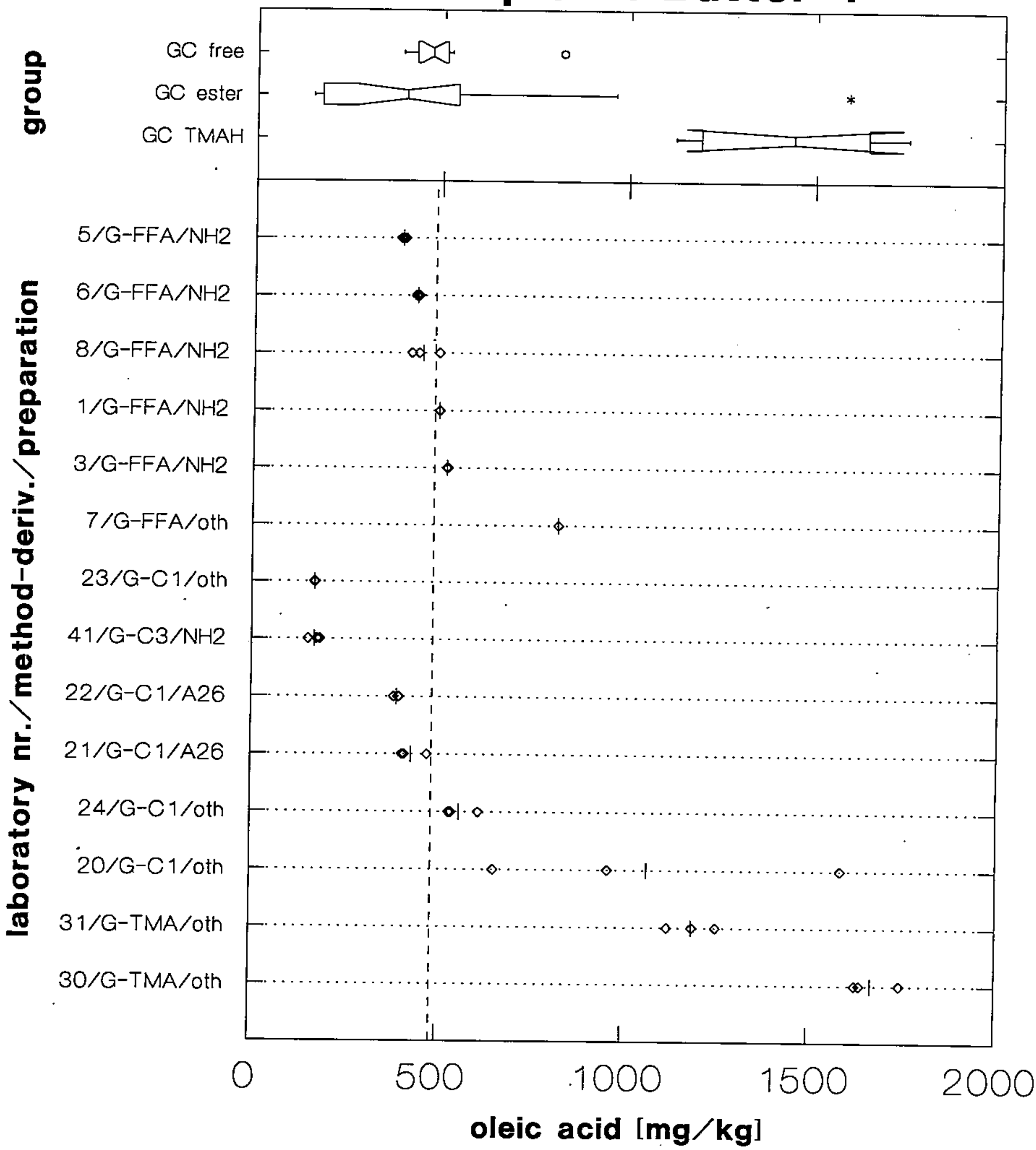


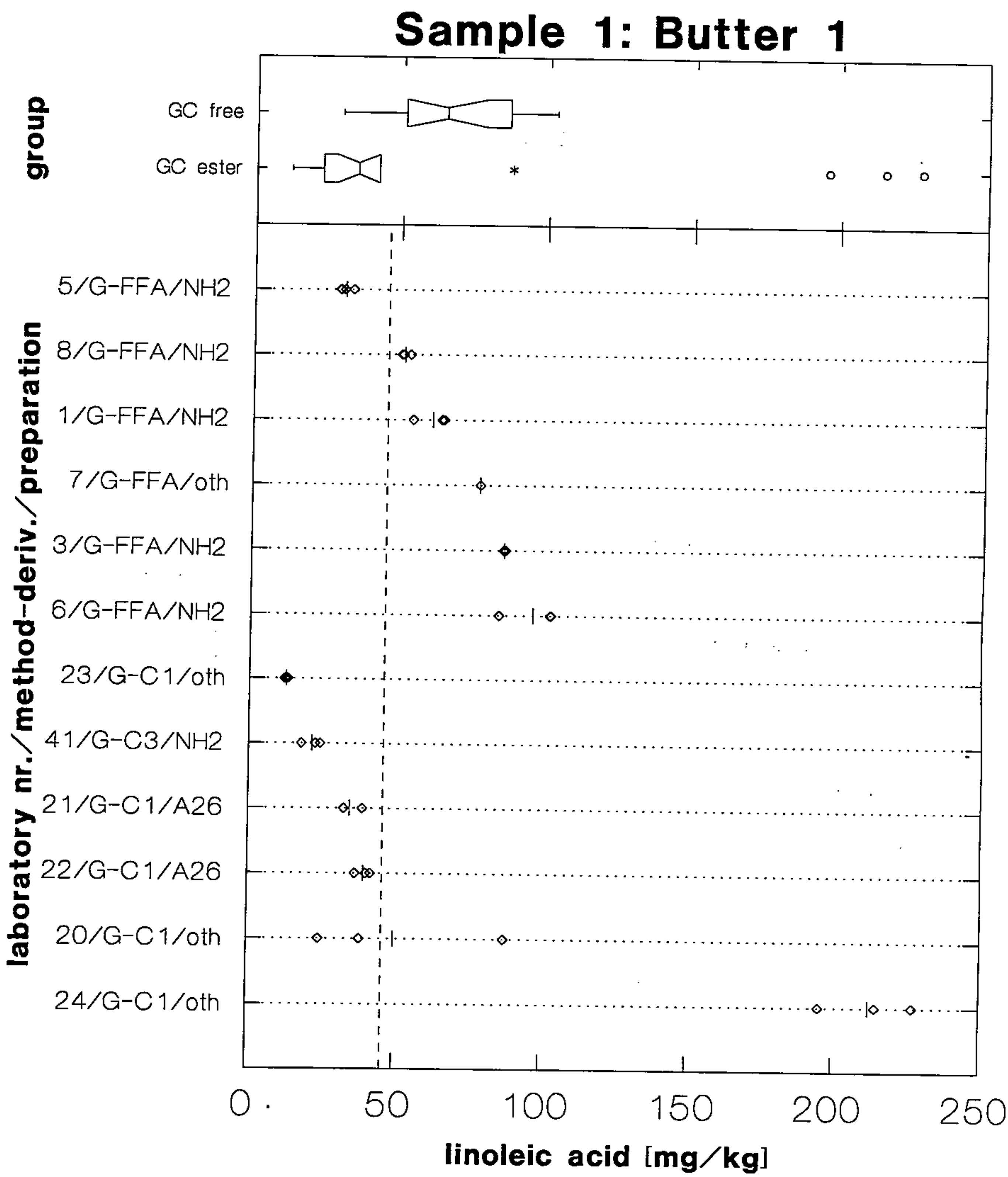
Sample 1: Butter 1

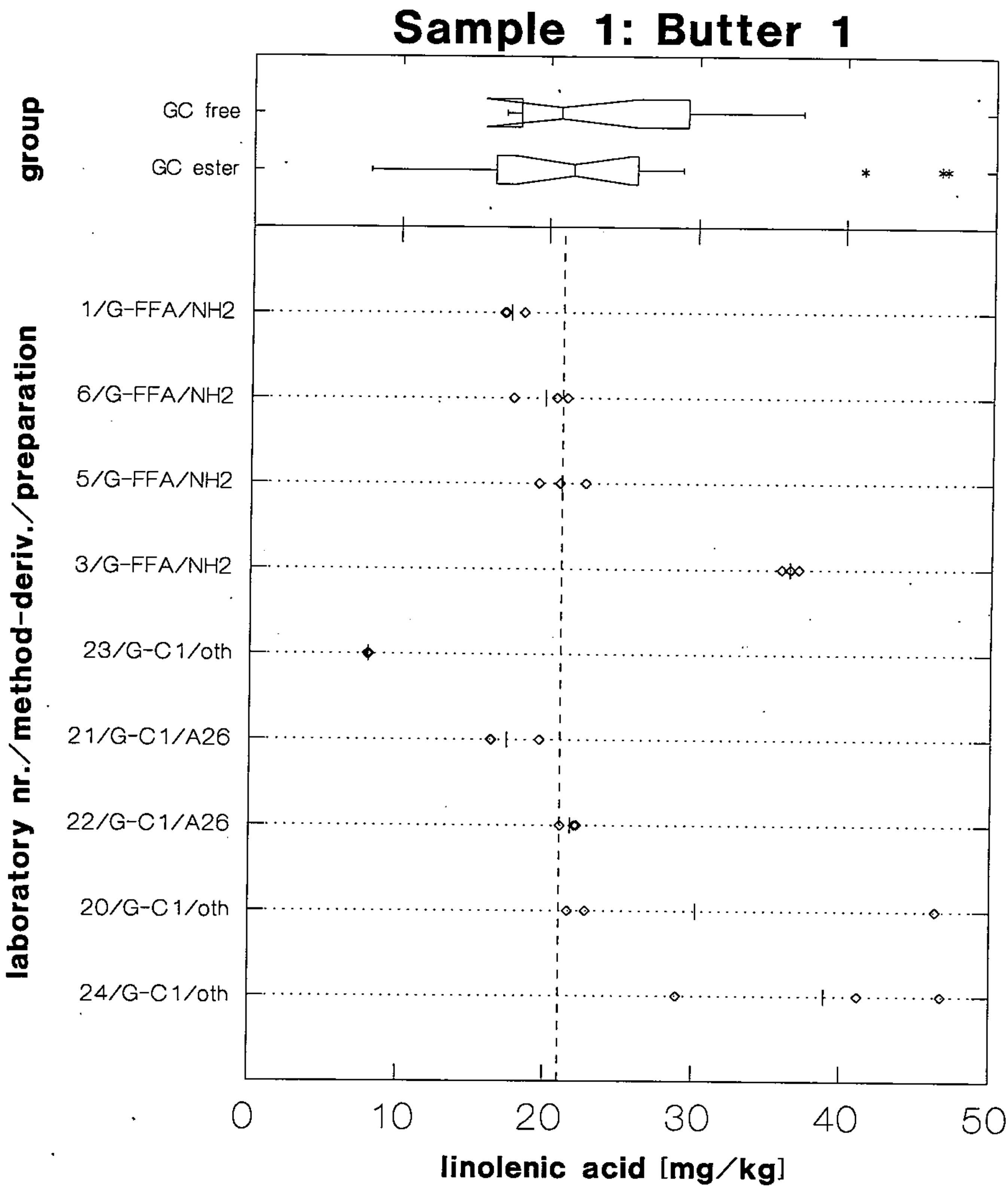



Sample 1: Butter 1
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
6/G-FFA/NH2
8/G-FFA/NH2
3/G-FFA/NH2
1/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
22/G-C1/A26
24/G-C1/oth
20/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
500
1000
1500
stearic acid [mg/kg]

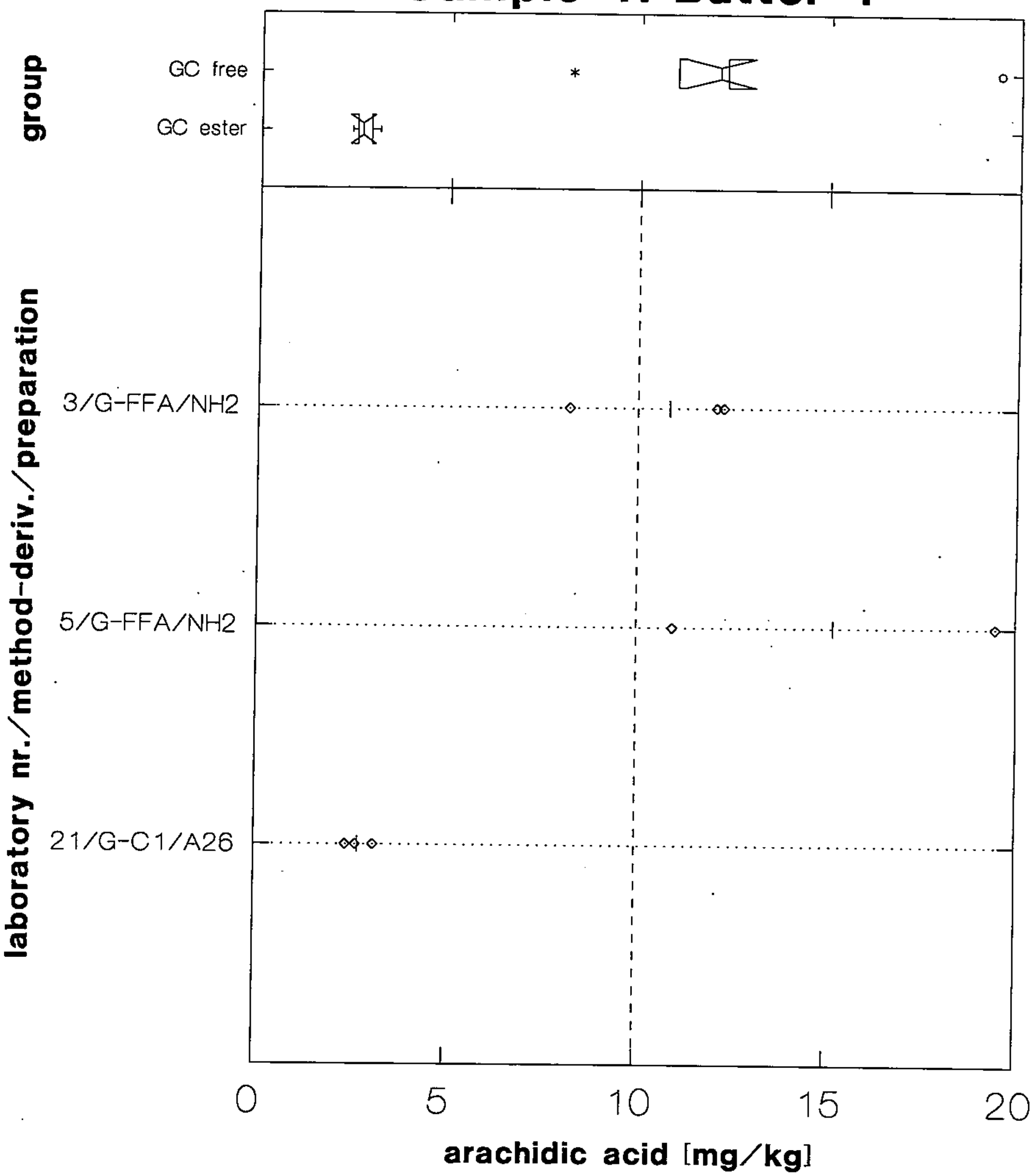
Sample 1: Butter 1

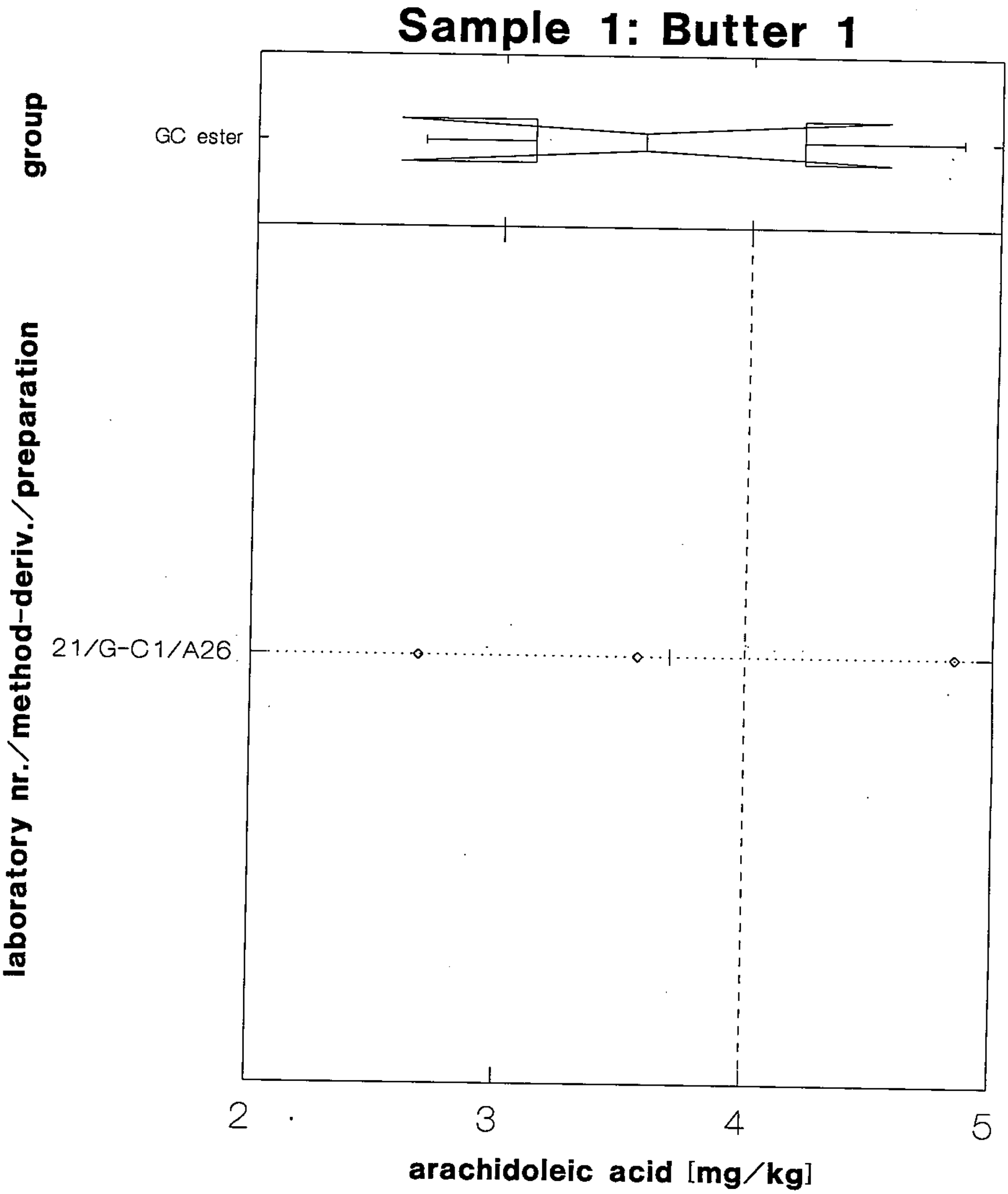


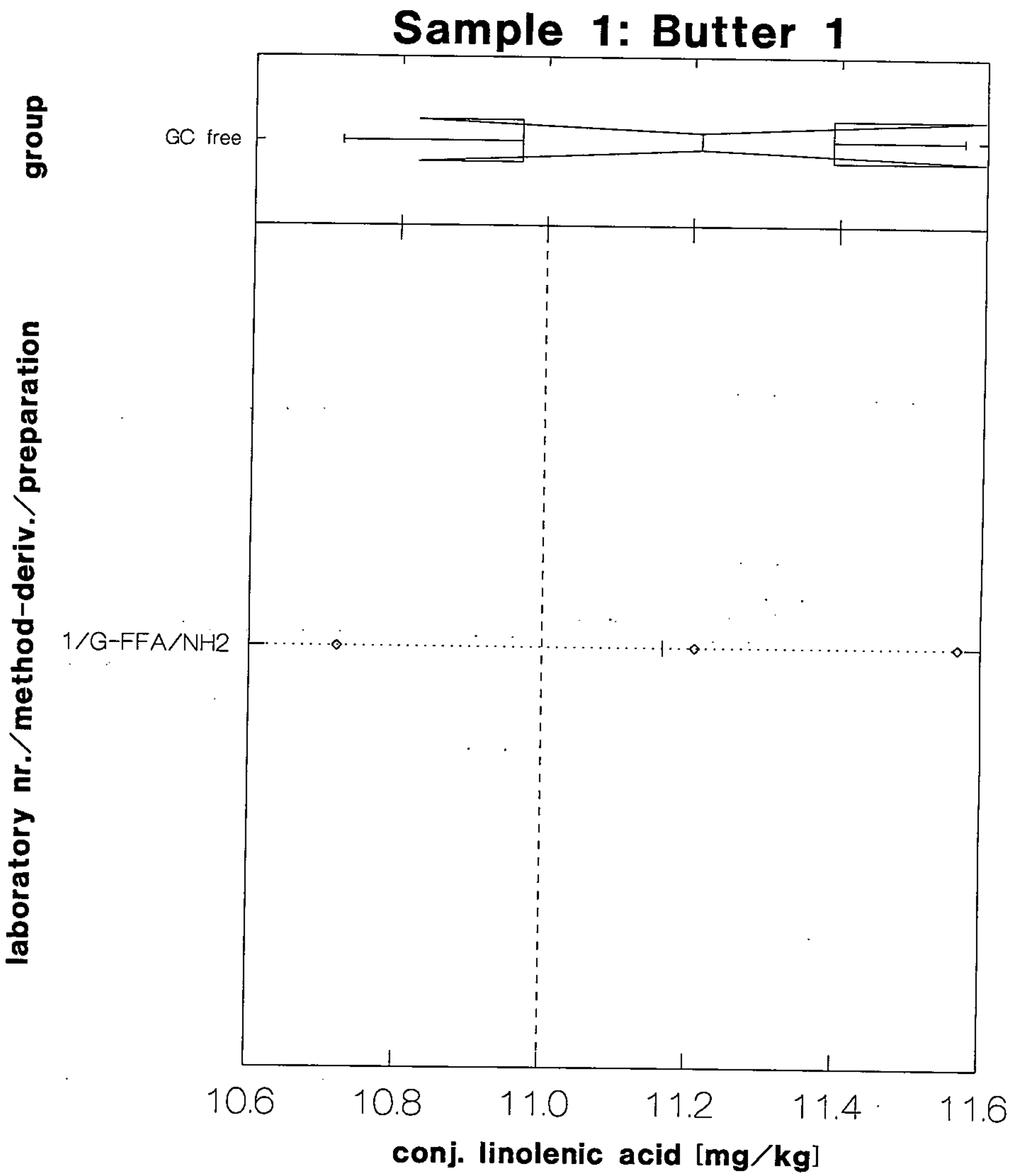

Sample 1: Butter 1
group
GC free
GC ester
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
8/G-FFA/NH2
1/G-FFA/NH2
7/G-FFA/oth
3/G-FFA/NH2
6/G-FFA/NH2
23/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
22/G-C1/A26
20/G-C1/oth
24/G-C1/oth
0
50
100
150
200
250
linoleic acid [mg/kg]

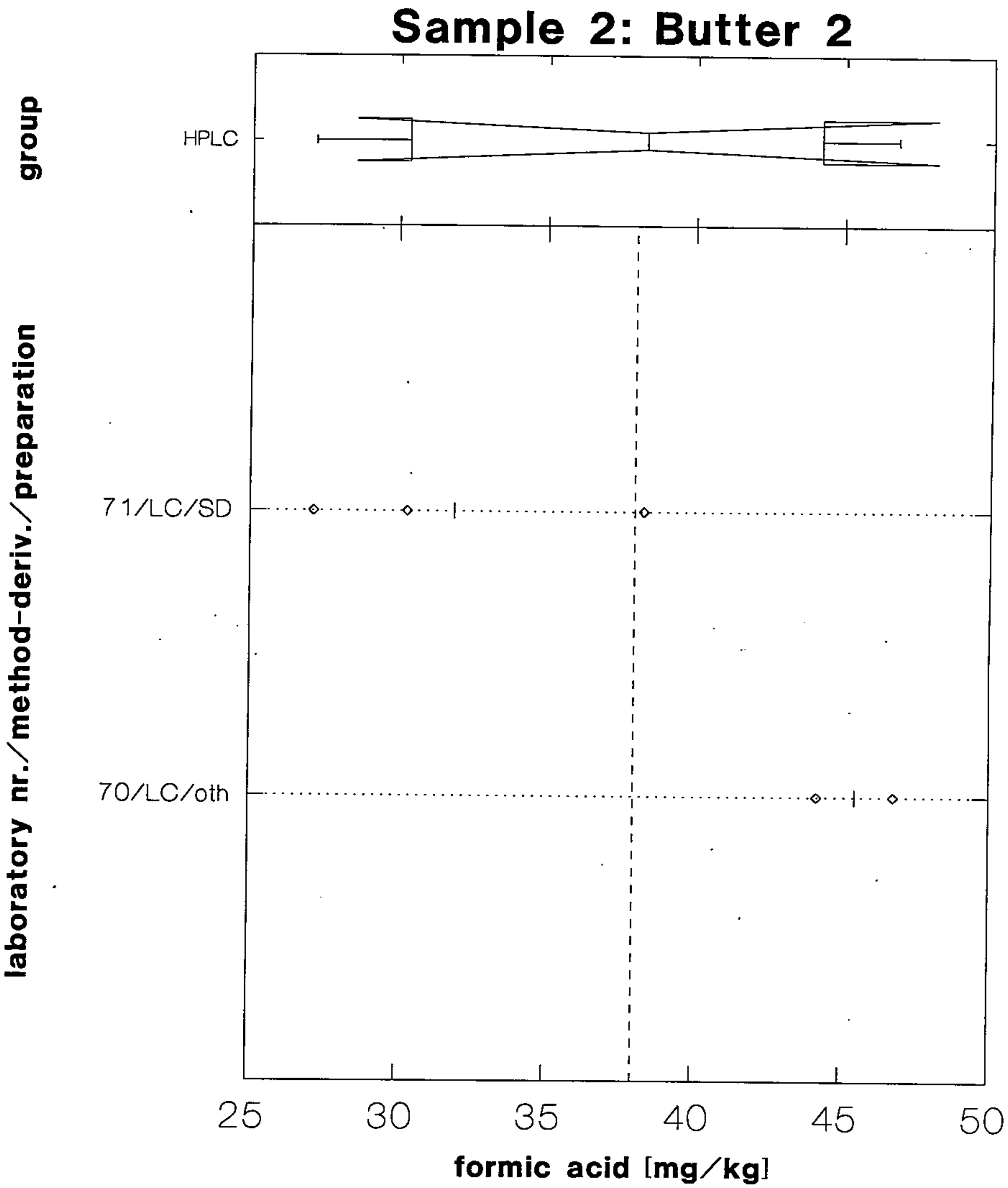

Sample 1: Butter 1
group
GC free
GC ester
laboratory nr./method-deriv./preparation
1/G-FFA/NH2
6/G-FFA/NH2
5/G-FFA/NH2
3/G-FFA/NH2
23/G-C1/oth
21/G-C1/A26
22/G-C1/A26
20/G-C1/oth
24/G-C1/oth
0
10
20
30
40
50
linolenic acid [mg/kg]

Sample 1: Butter 1

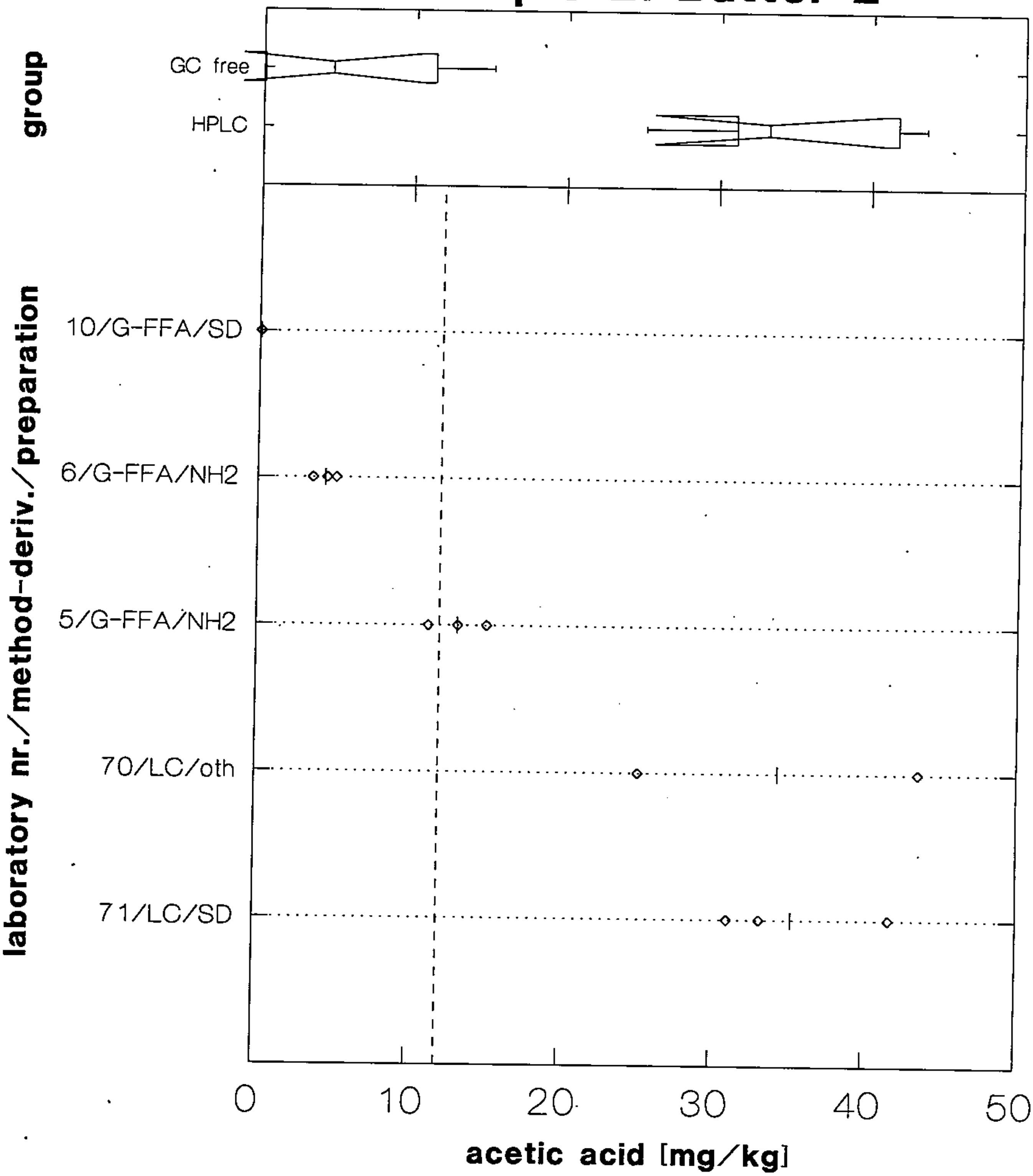



Sample 1: Butter 1
group
GC ester
laboratory nr./method-deriv./preparation
21/G-C1/A26
2
3
4
5
arachidoleic acid [mg/kg]

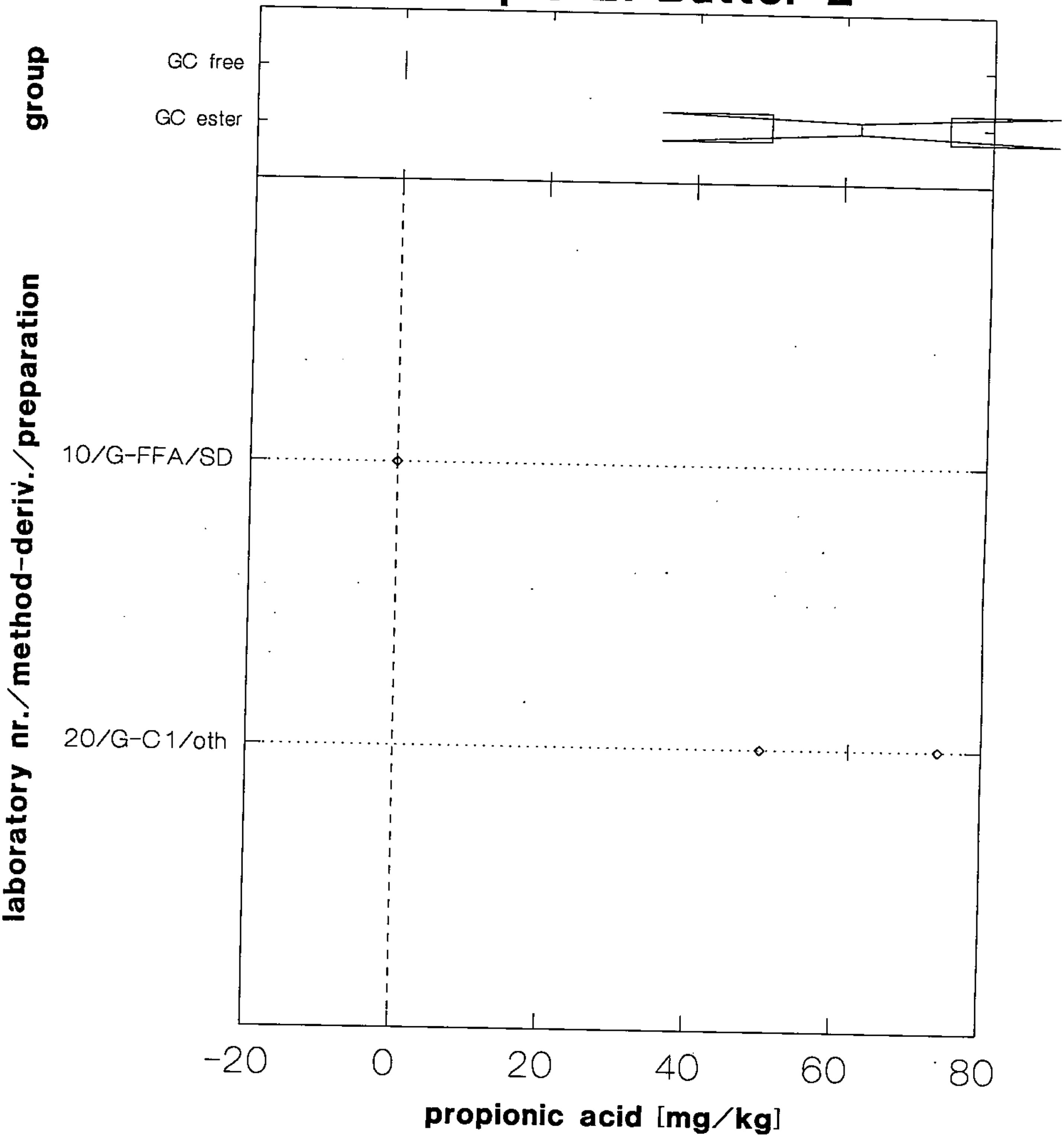

Sample 1: Butter 1
group
GC free
laboratory nr./method-deriv./preparation
1/G-FFA/NH2
10.6
10.8
11.0
11.2
11.4
11.6
conj. linolenic acid [mg/kg]

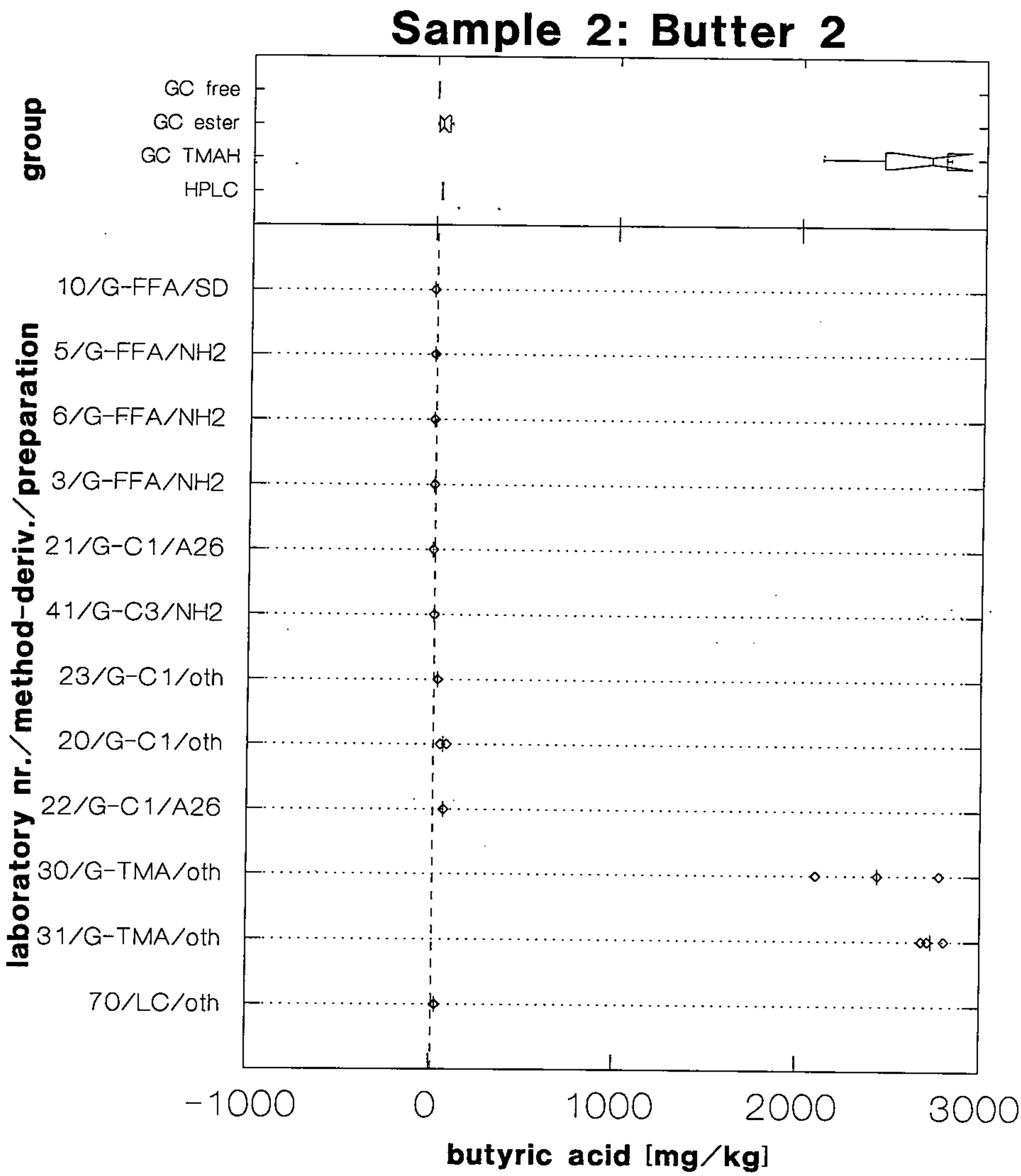

Sample 2: Butter 2
group
HPLC
laboratory nr./method-deriv./preparation
71/LC/SD
70/LC/oth
25
30
35
40
45
50
formic acid [mg/kg]

Sample 2: Butter 2

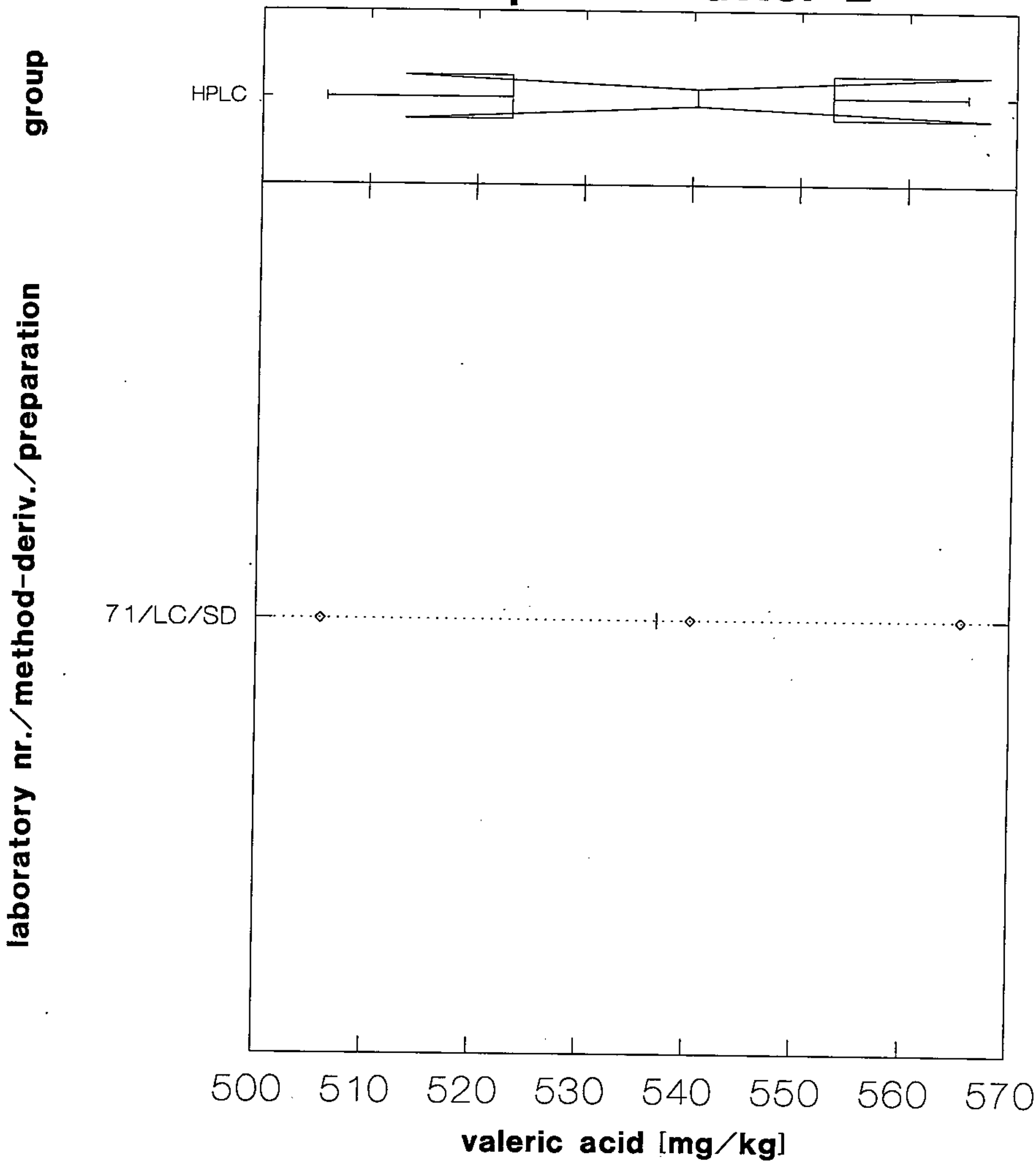


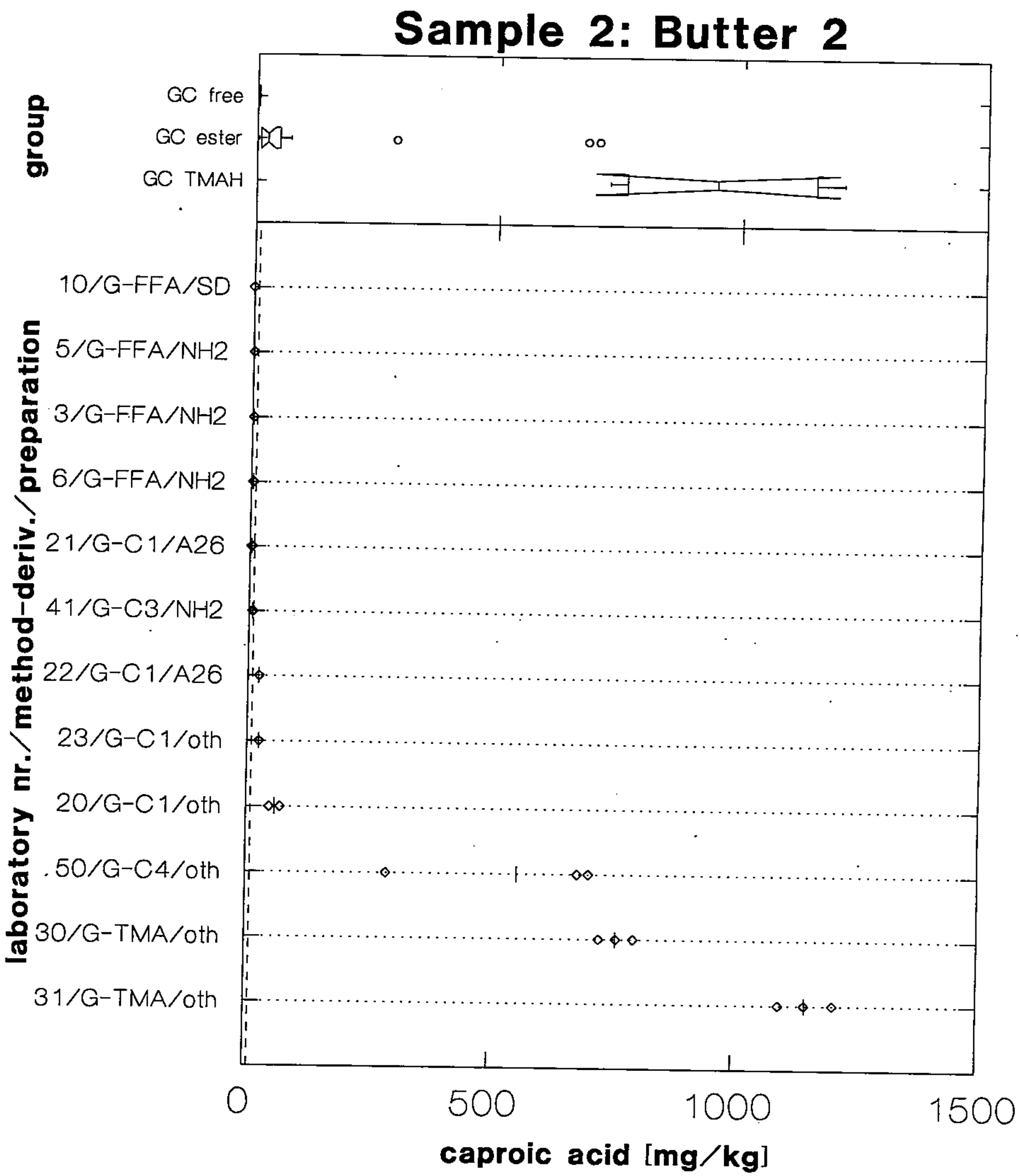
Sample 2: Butter 2

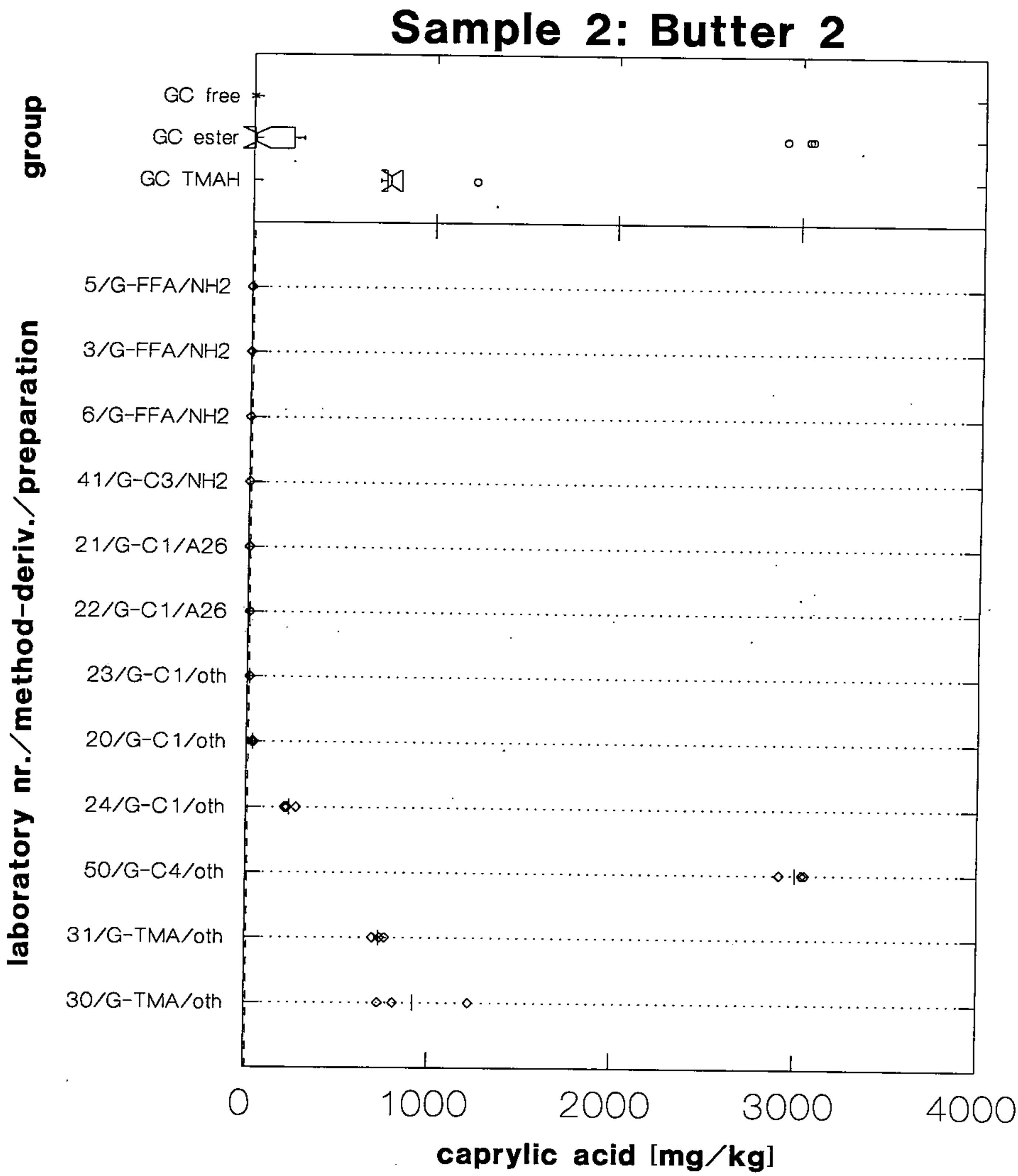


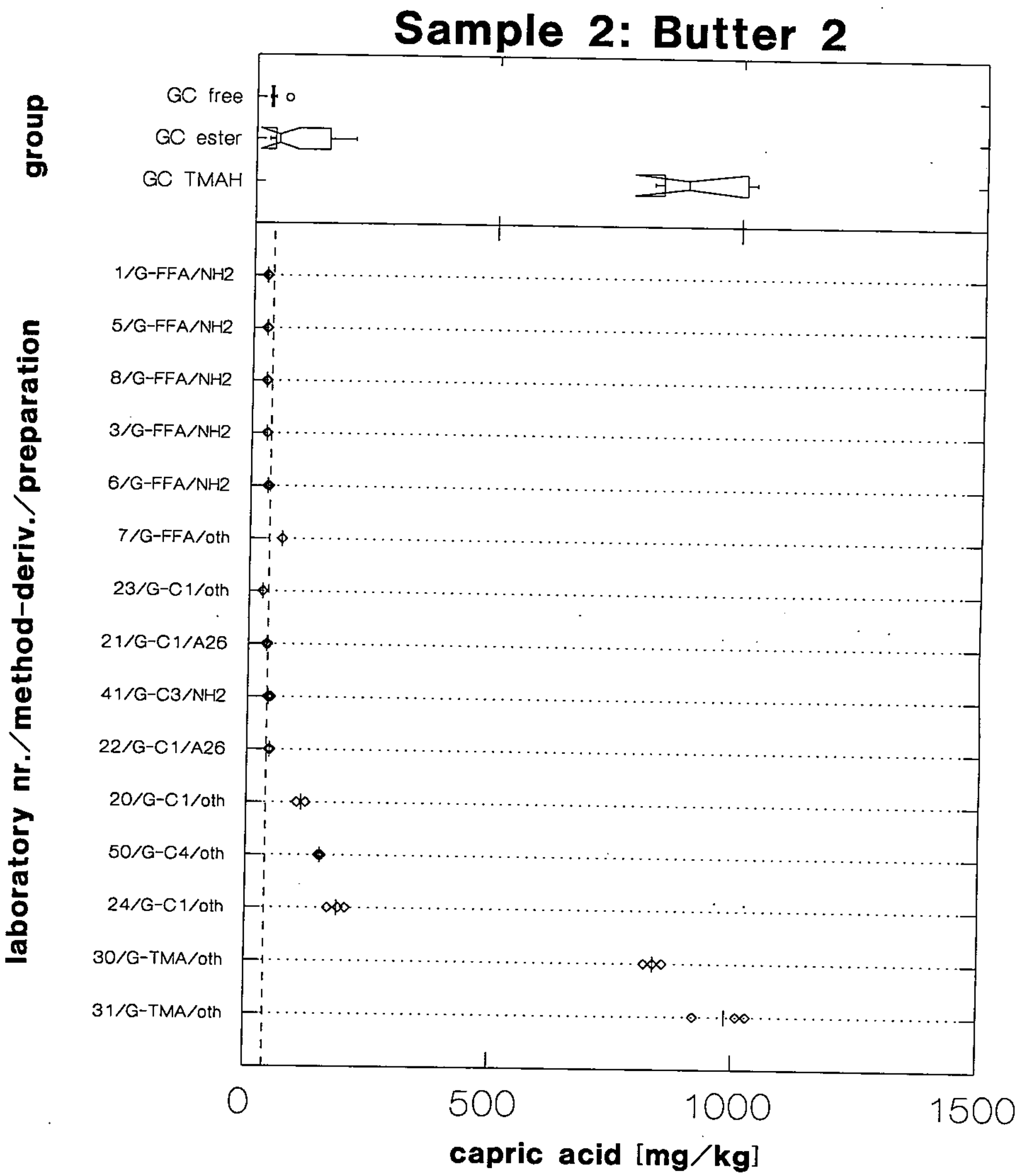

Sample 2: Butter 2
group
GC free
GC ester
GC TMAH
HPLC
laboratory nr./method-deriv./preparation
10/G-FFA/SD
5/G-FFA/NH2
6/G-FFA/NH2
3/G-FFA/NH2
21/G-C1/A26
41/G-C3/NH2
23/G-C1/oth
20/G-C1/oth
22/G-C1/A26
30/G-TMA/oth
31/G-TMA/oth
70/LC/oth
-1000
0
1000
2000
3000
butyric acid [mg/kg]

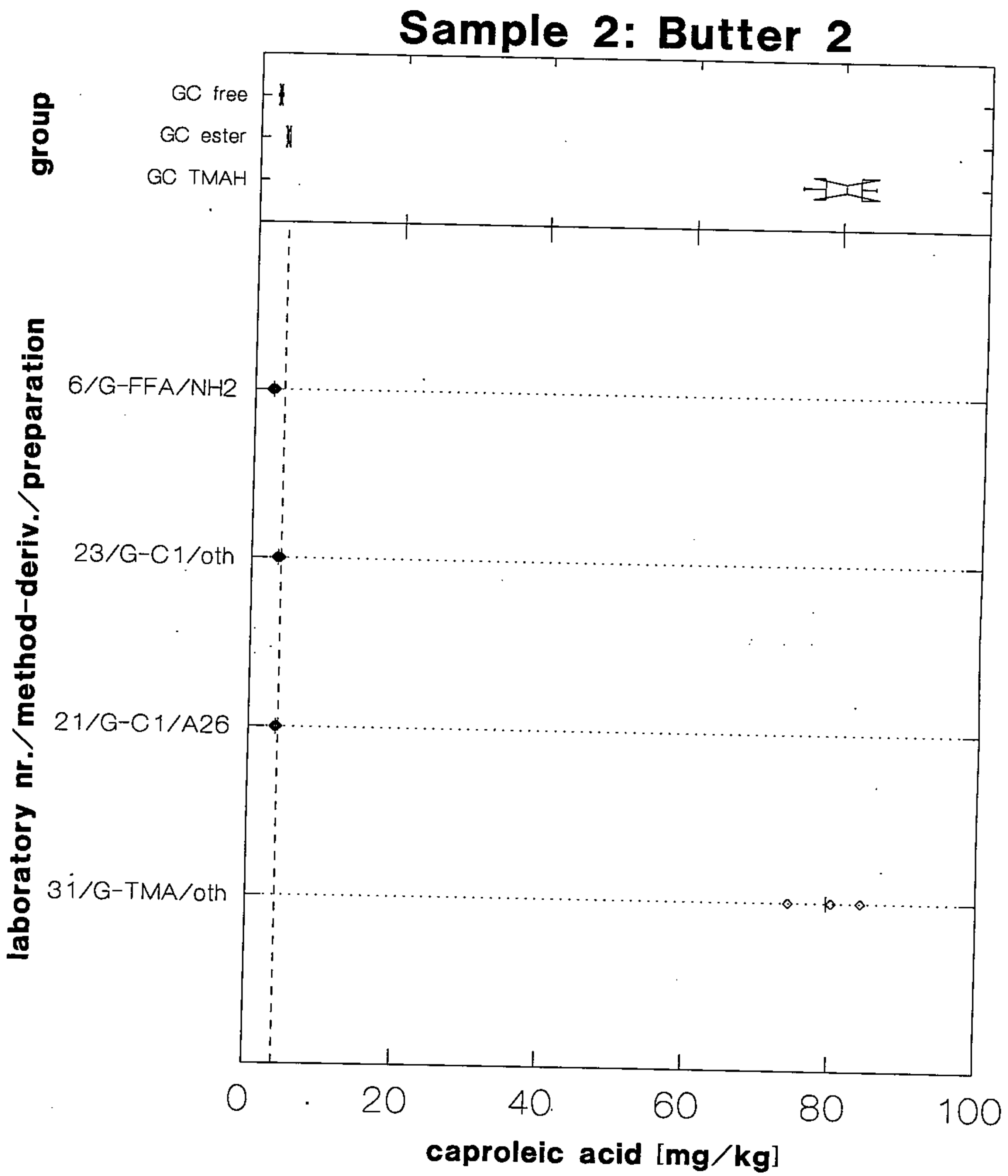
Sample 2: Butter 2

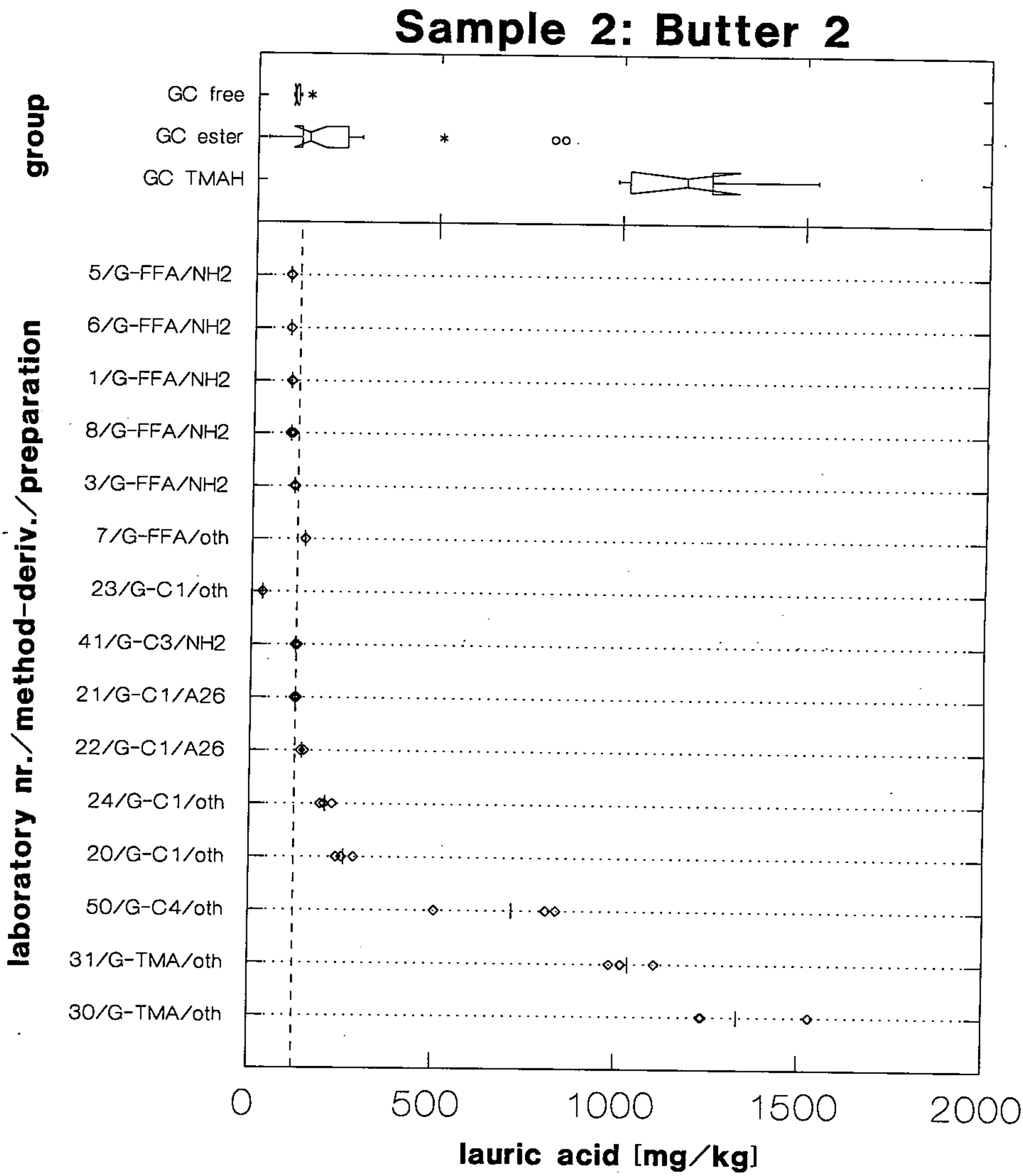


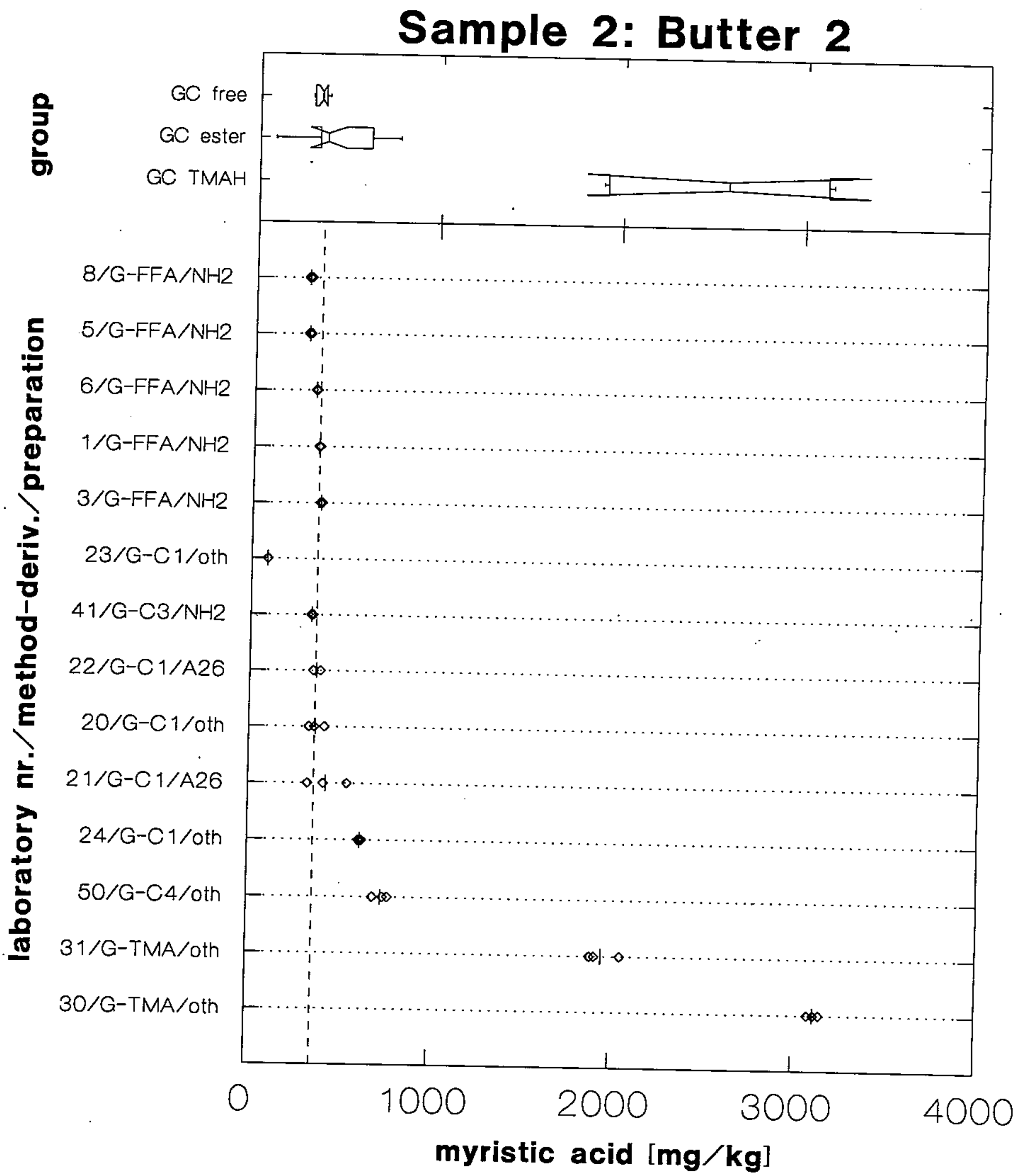

Sample 2: Butter 2
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
10/G-FFA/SD
5/G-FFA/NH2
3/G-FFA/NH2
6/G-FFA/NH2
21/G-C1/A26
41/G-C3/NH2
22/G-C1/A26
23/G-C1/oth
20/G-C1/oth
50/G-C4/oth
30/G-TMA/oth
31/G-TMA/oth
0
500
1000
1500
caproic acid [mg/kg]


Sample 2: Butter 2
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
3/G-FFA/NH2
6/G-FFA/NH2
41/G-C3/NH2
21/G-C1/A26
22/G-C1/A26
23/G-C1/oth
20/G-C1/oth
24/G-C1/oth
50/G-C4/oth
31/G-TMA/oth
30/G-TMA/oth
0
1000
2000
3000
4000
caprylic acid [mg/kg]

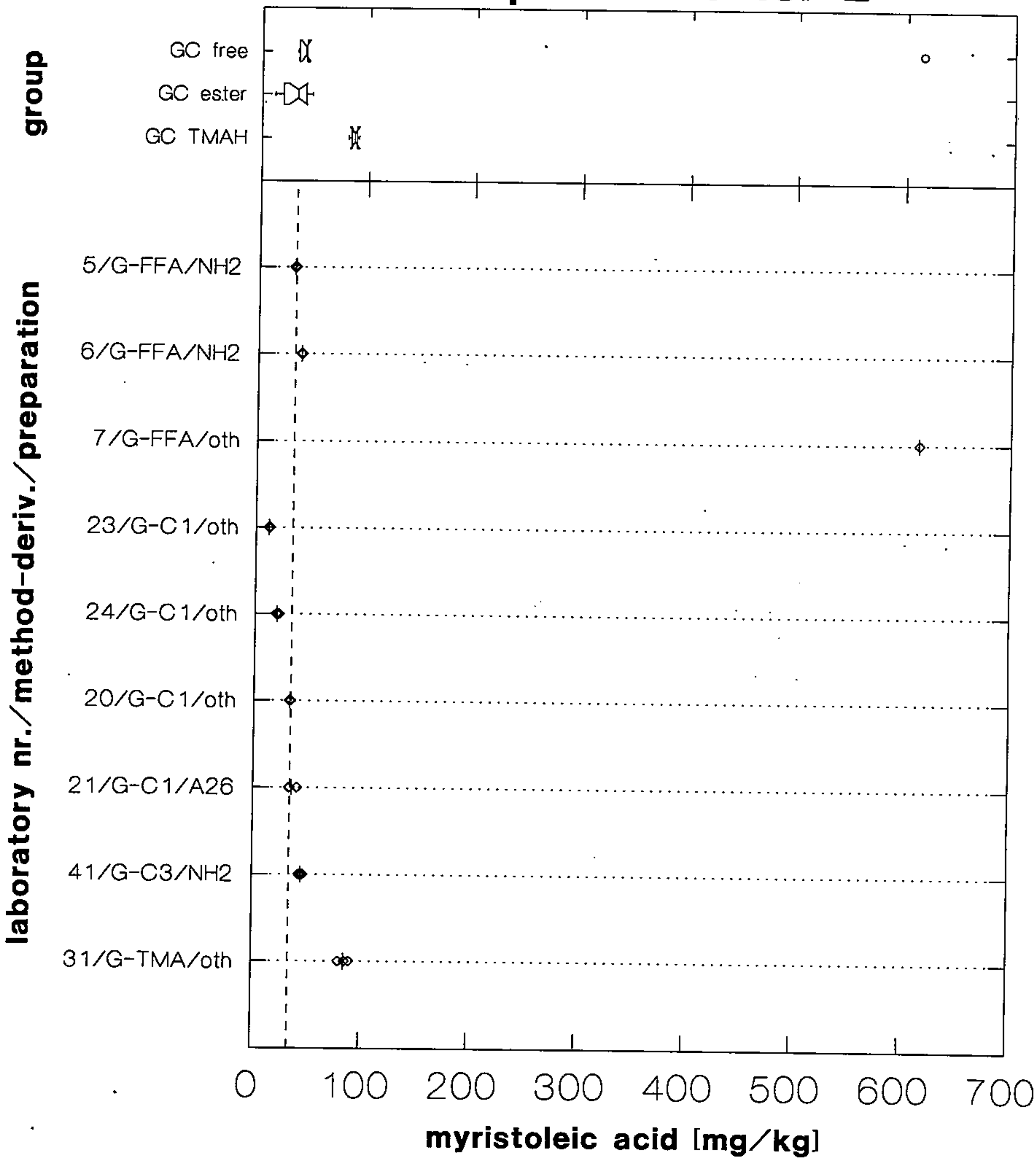

Sample 2: Butter 2
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
1/G-FFA/NH2
5/G-FFA/NH2
8/G-FFA/NH2
3/G-FFA/NH2
6/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
21/G-C1/A26
41/G-C3/NH2
22/G-C1/A26
20/G-C1/oth
50/G-C4/oth
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
500
1000
1500
capric acid [mg/kg]

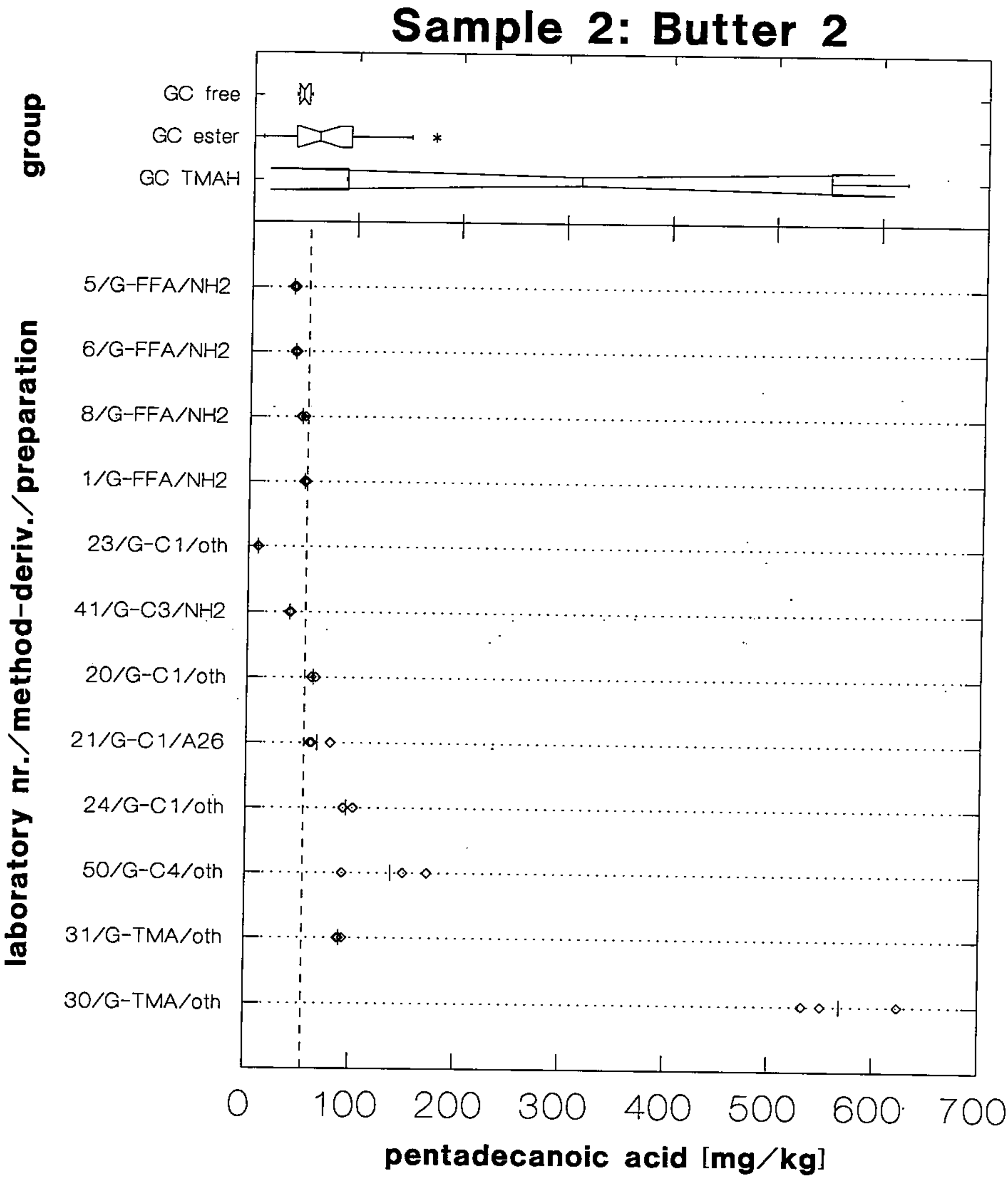

Sample 2: Butter 2
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
6/G-FFA/NH2
23/G-C1/oth
21/G-C1/A26
31/G-TMA/oth
0
20
40
60
80
100
caproleic acid [mg/kg]

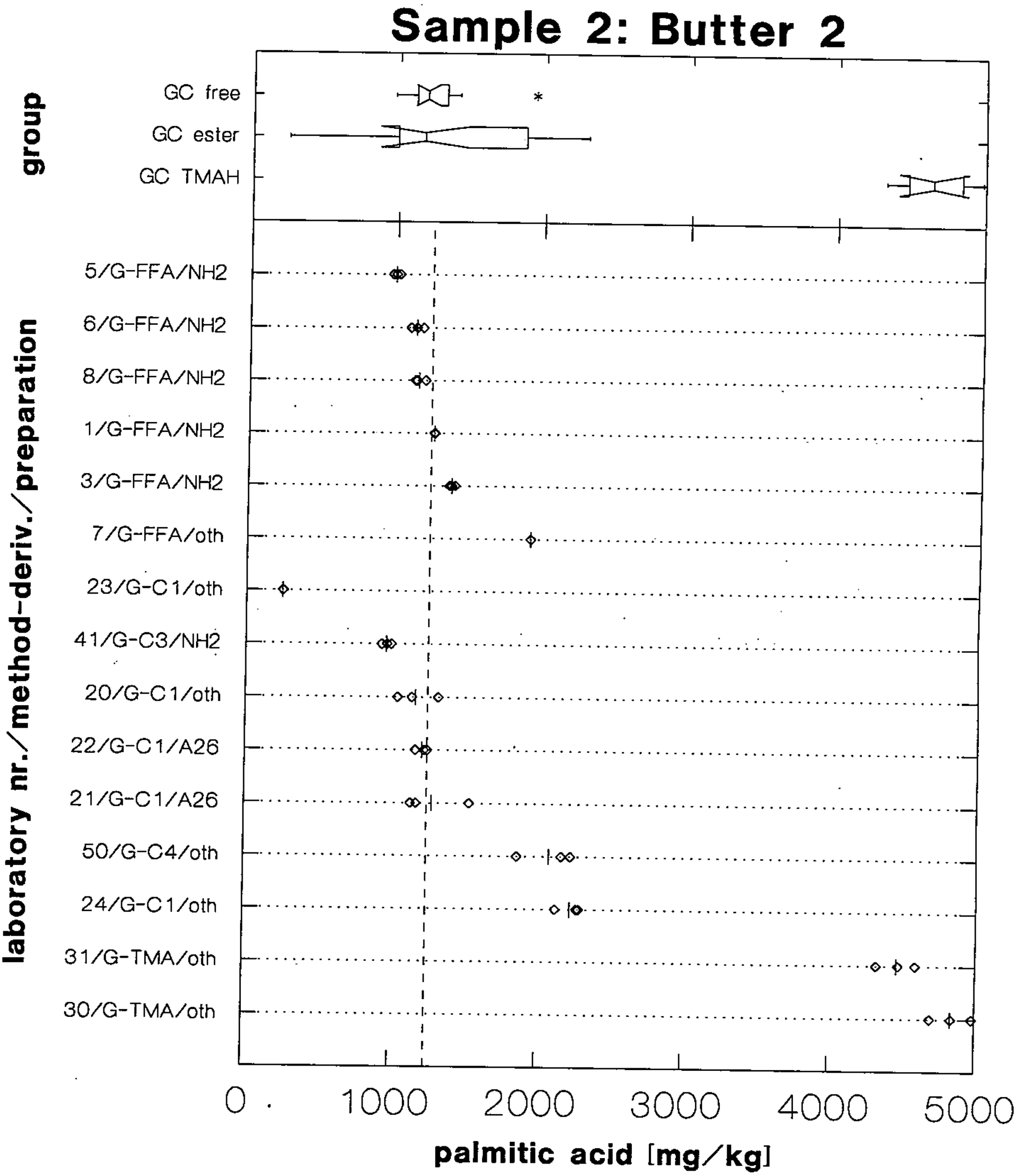

Sample 2: Butter 2
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
6/G-FFA/NH2
1/G-FFA/NH2
8/G-FFA/NH2
3/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
22/G-C1/A26
24/G-C1/oth
20/G-C1/oth
50/G-C4/oth
31/G-TMA/oth
30/G-TMA/oth
0
500
1000
1500
2000
lauric acid [mg/kg]


Sample 2: Butter 2
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
23/G-C1/oth
41/G-C3/NH2
22/G-C1/A26
20/G-C1/oth
21/G-C1/A26
24/G-C1/oth
50/G-C4/oth
31/G-TMA/oth
30/G-TMA/oth
0
1000
2000
3000
4000
myristic acid [mg/kg]

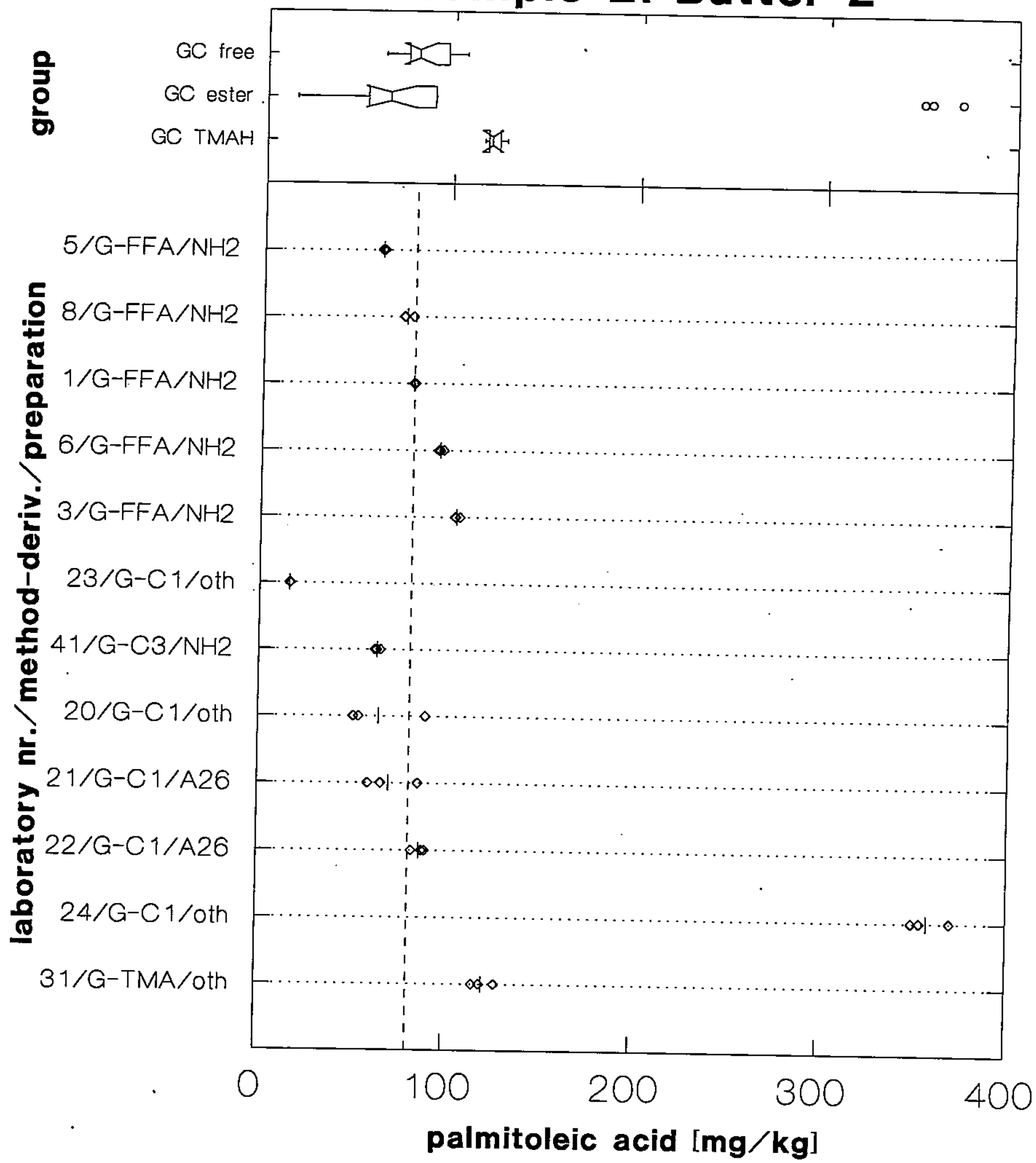
Sample 2: Butter 2



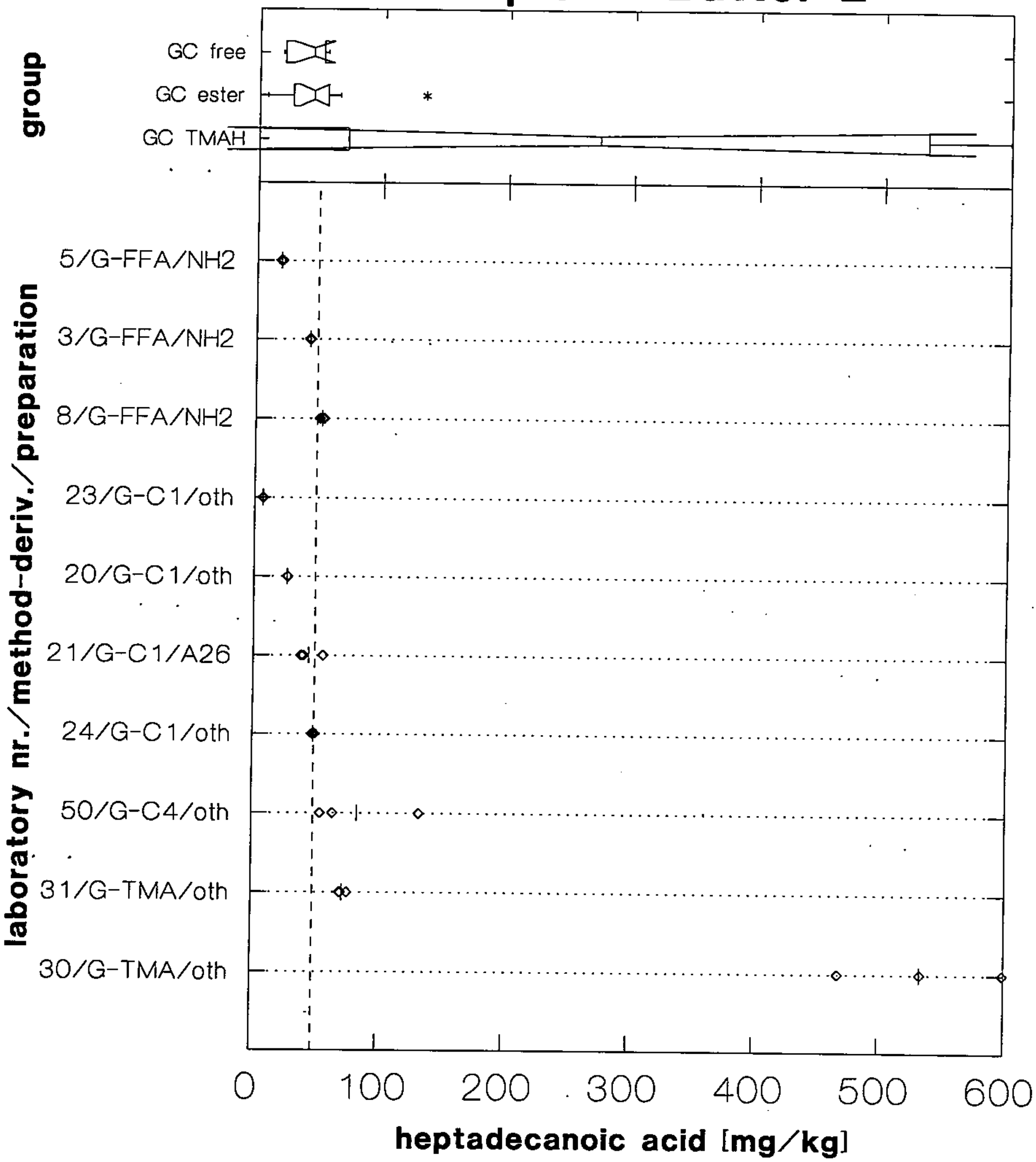

Sample 2: Butter 2
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
6/G-FFA/NH2
8/G-FFA/NH2
1/G-FFA/NH2
23/G-C1/oth
41/G-C3/NH2
20/G-C1/oth
21/G-C1/A26
24/G-C1/oth
50/G-C4/oth
31/G-TMA/oth
30/G-TMA/oth
0
100
200
300
400
500
600
700
pentadecanoic acid [mg/kg]

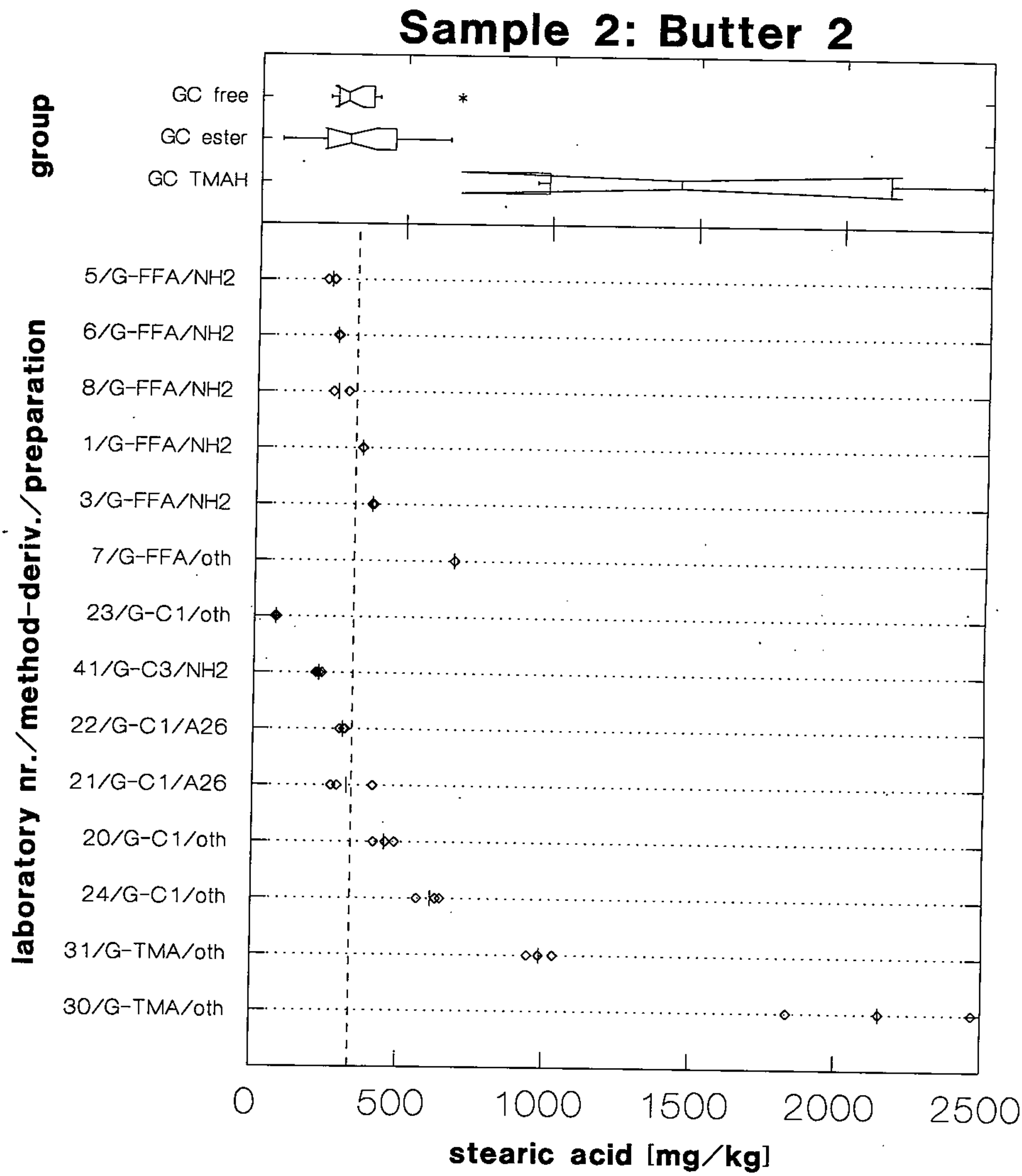

Sample 2: Butter 2
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
6/G-FFA/NH2
8/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
41/G-C3/NH2
20/G-C1/oth
22/G-C1/A26
21/G-C1/A26
50/G-C4/oth
24/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
1000
2000
3000
4000
5000
palmitic acid [mg/kg]

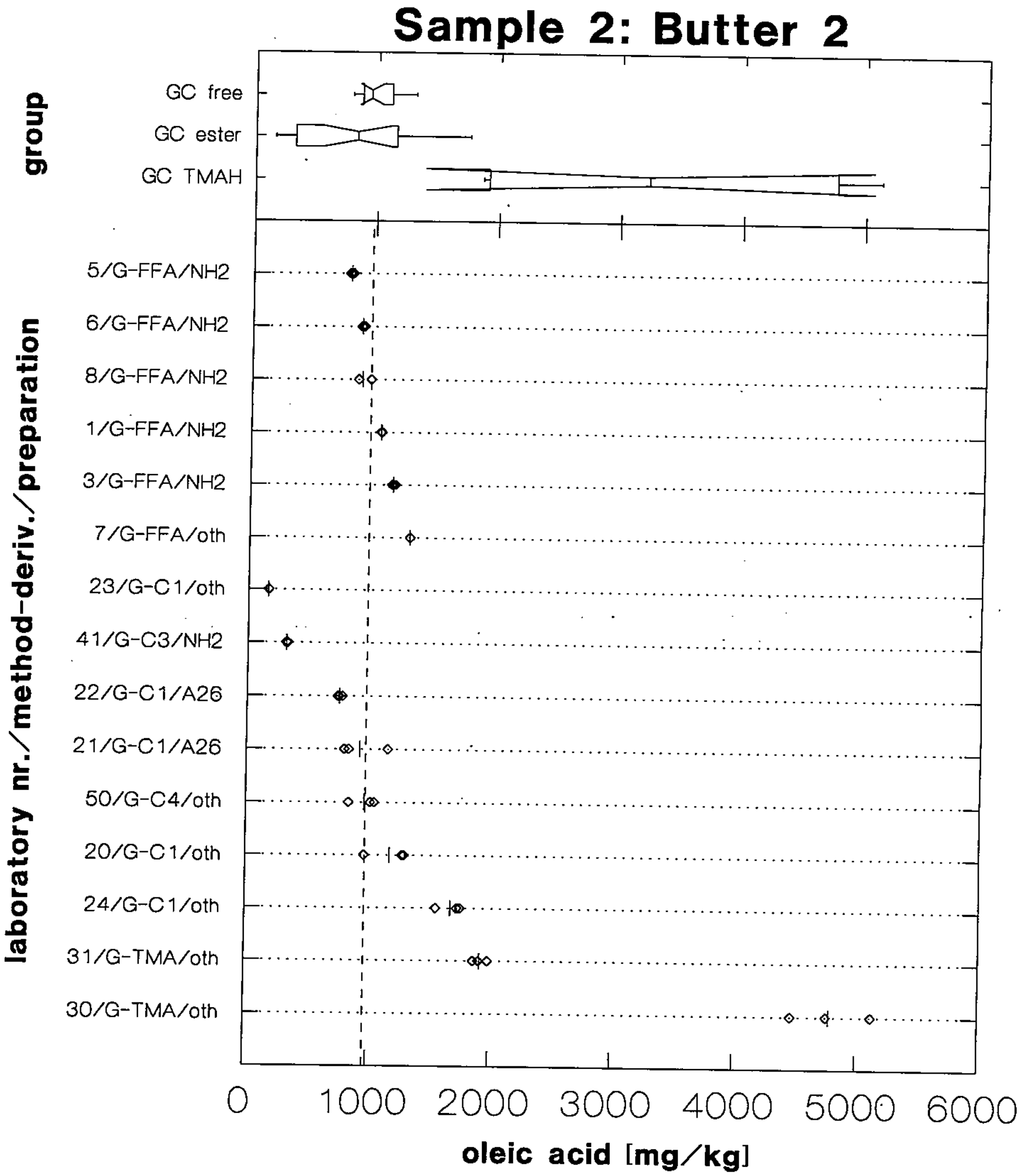
Sample 2: Butter 2

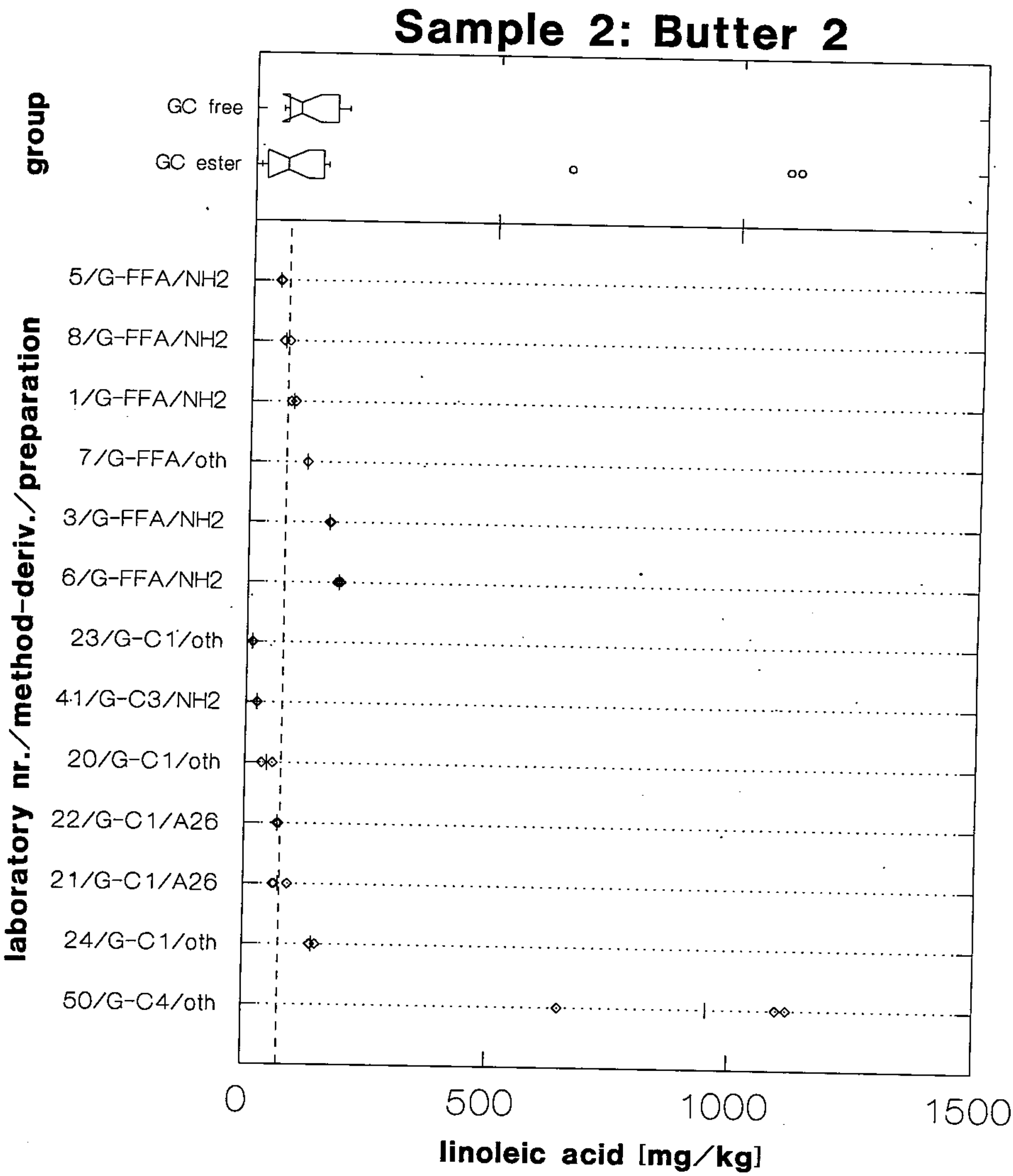


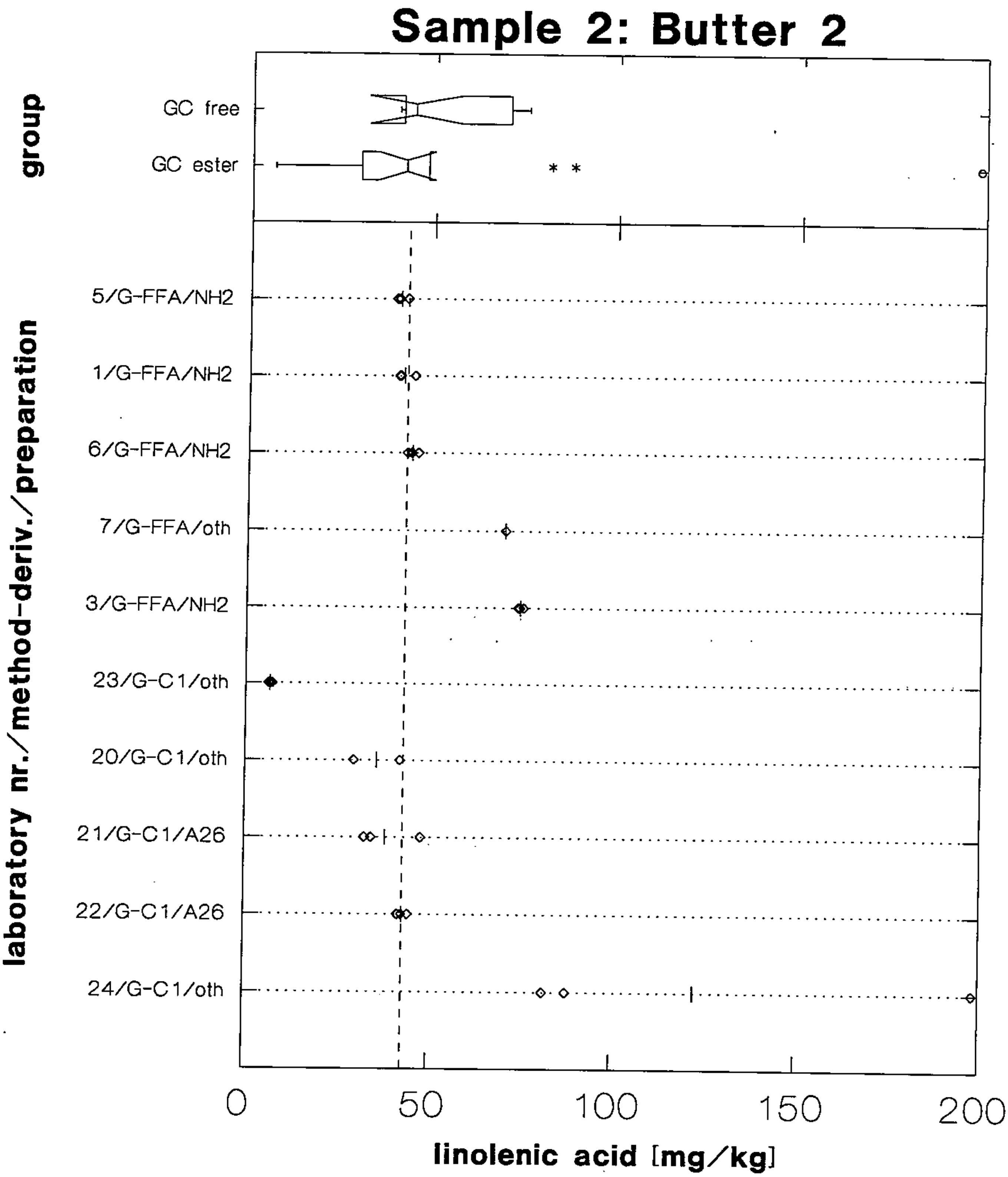
Sample 2: Butter 2



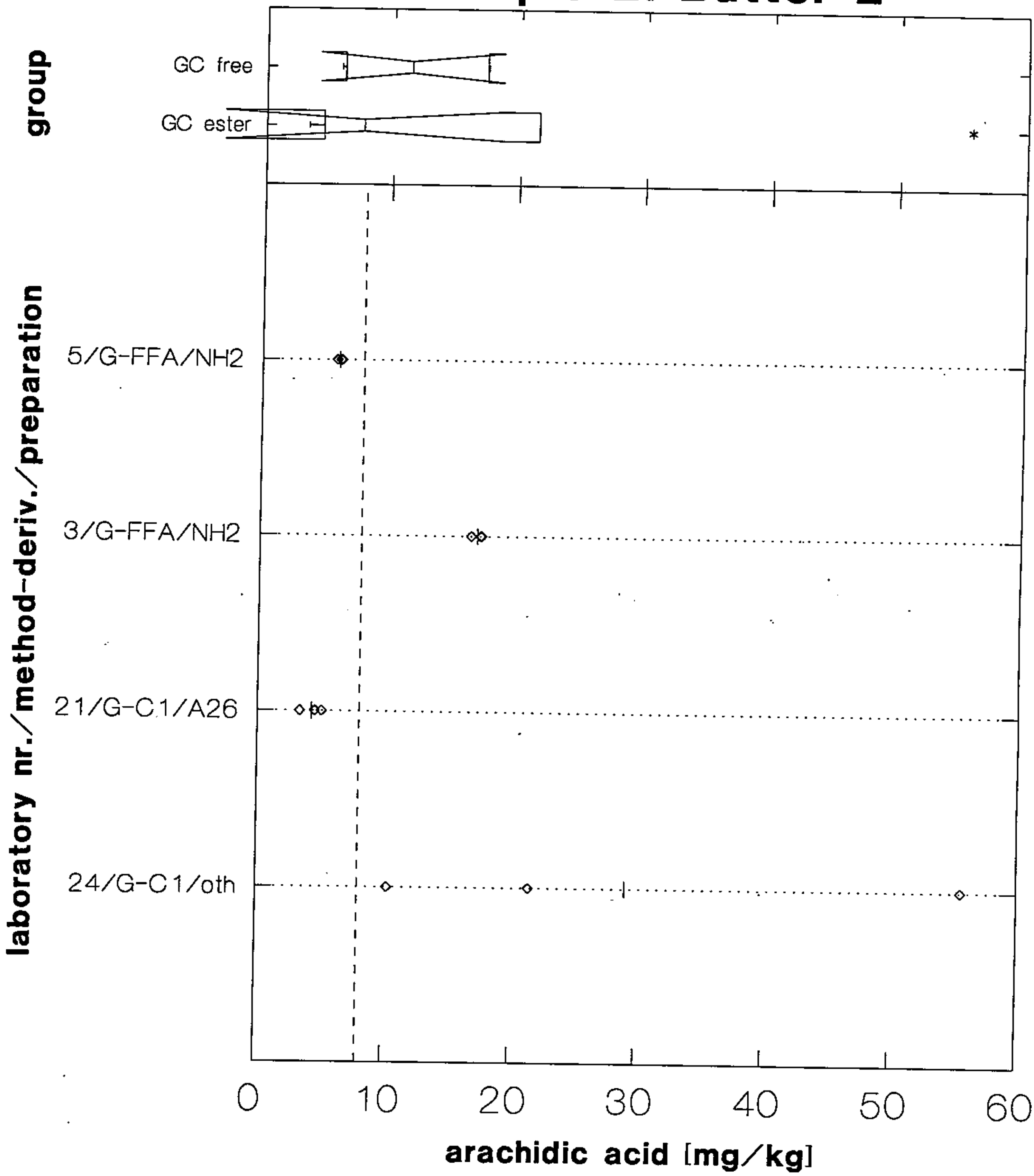

Sample 2: Butter 2
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
6/G-FFA/NH2
8/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
41/G-C3/NH2
22/G-C1/A26
21/G-C1/A26
20/G-C1/oth
24/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
500
1000
1500
2000
2500
stearic acid [mg/kg]


Sample 2: Butter 2
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
6/G-FFA/NH2
8/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
41/G-C3/NH2
22/G-C1/A26
21/G-C1/A26
50/G-C4/oth
20/G-C1/oth
24/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
1000
2000
3000
4000
5000
6000
oleic acid [mg/kg]

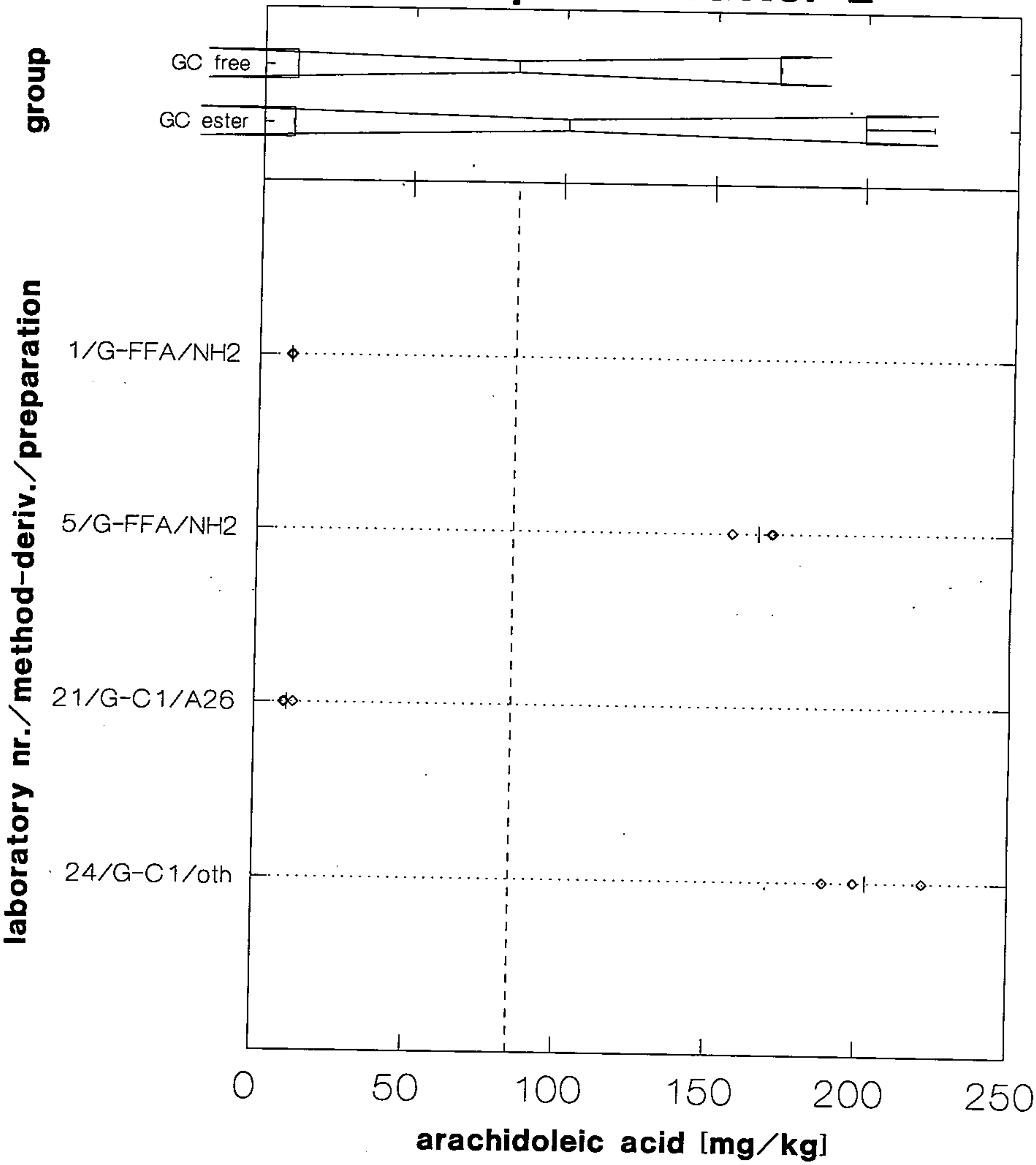

Sample 2: Butter 2
group
GC free
GC ester
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
8/G-FFA/NH2
1/G-FFA/NH2
7/G-FFA/oth
3/G-FFA/NH2
6/G-FFA/NH2
23/G-C1/oth
41/G-C3/NH2
20/G-C1/oth
22/G-C1/A26
21/G-C1/A26
24/G-C1/oth
50/G-C4/oth
0
500
1000
1500
linoleic acid [mg/kg]

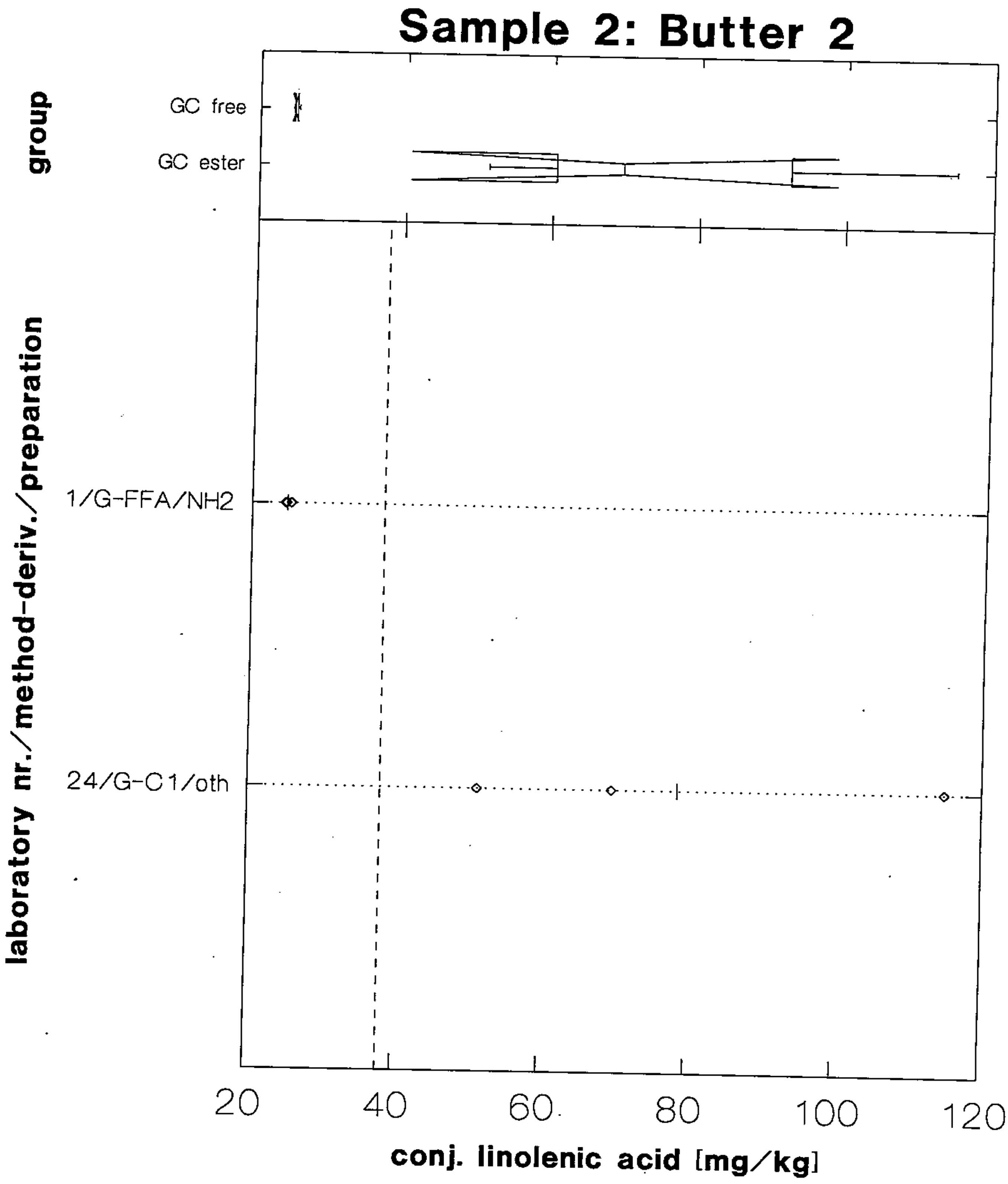

Sample 2: Butter 2
group
GC free
GC ester
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
1/G-FFA/NH2
6/G-FFA/NH2
7/G-FFA/oth
3/G-FFA/NH2
23/G-C1/oth
20/G-C1/oth
21/G-C1/A26
22/G-C1/A26
24/G-C1/oth
0
50
100
150
200
linolenic acid [mg/kg]

Sample 2: Butter 2

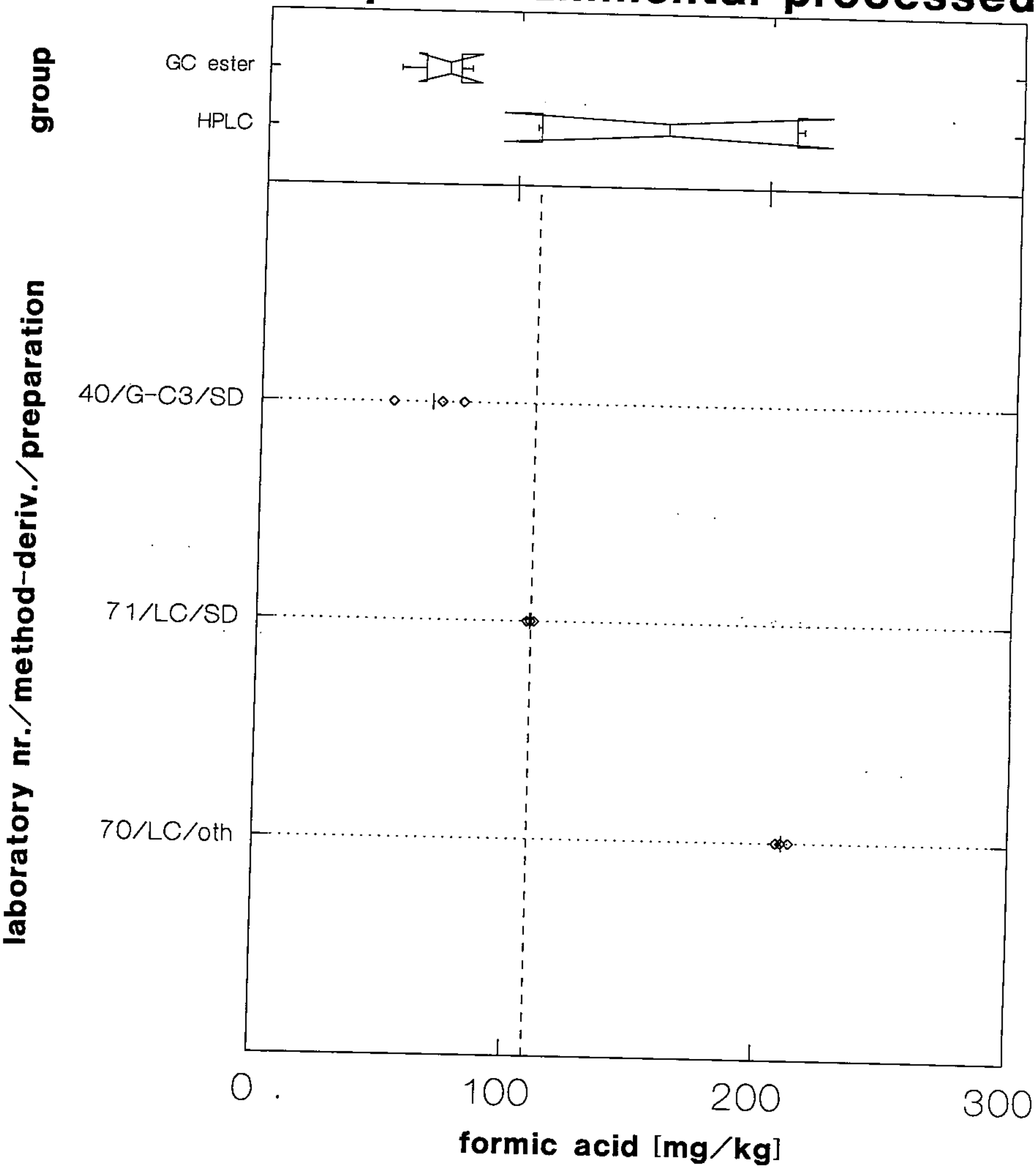


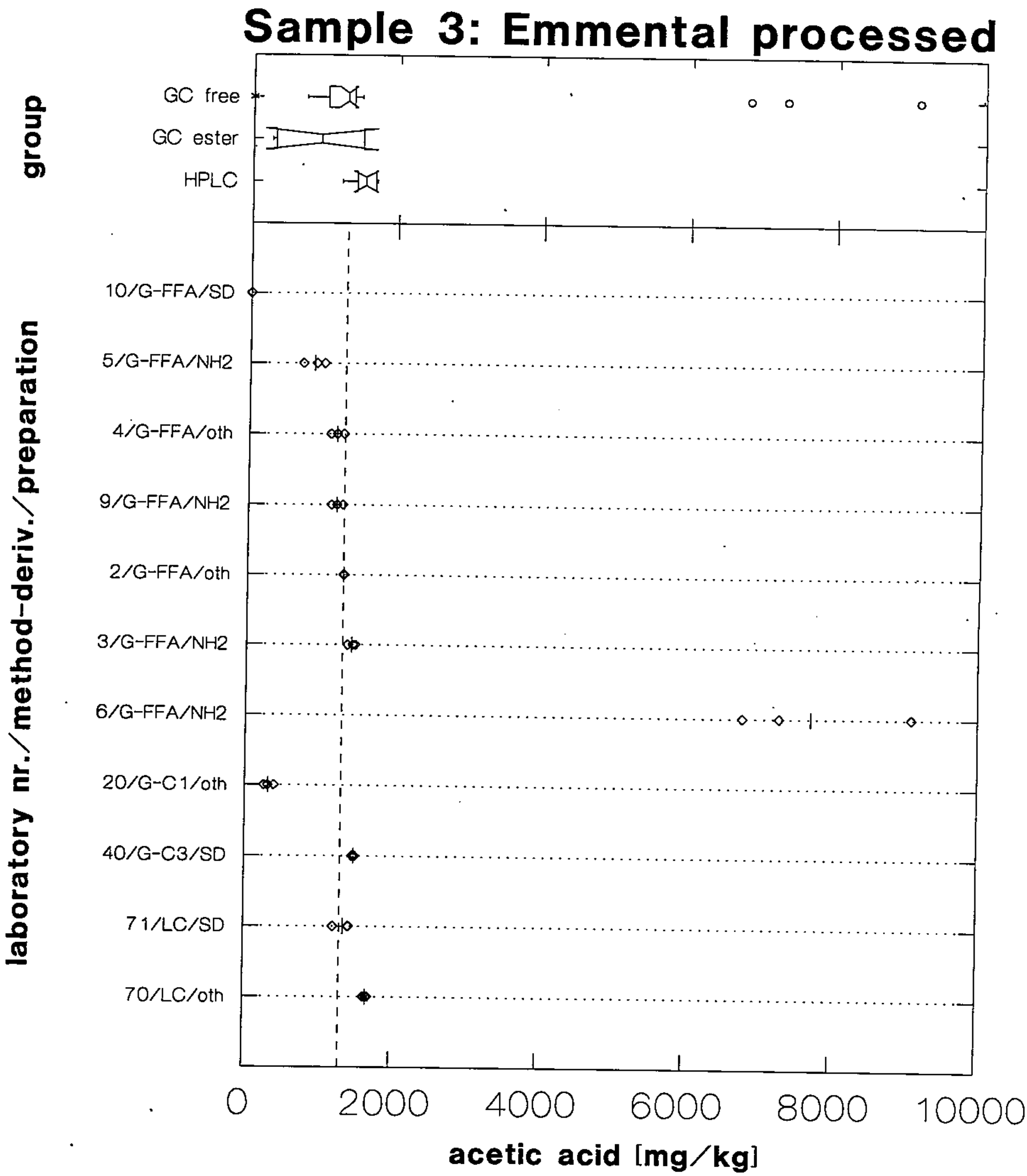
Sample 2: Butter 2

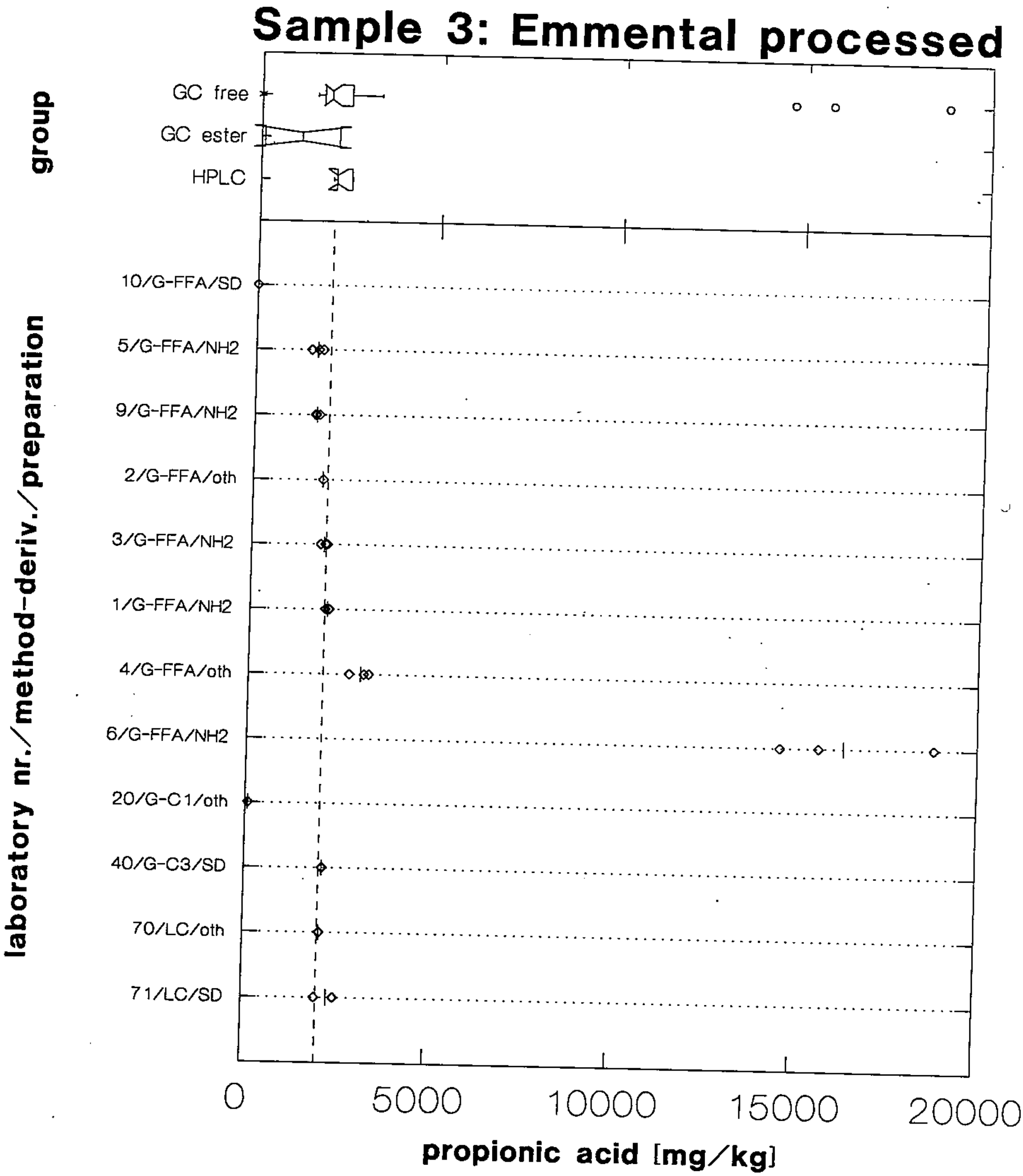


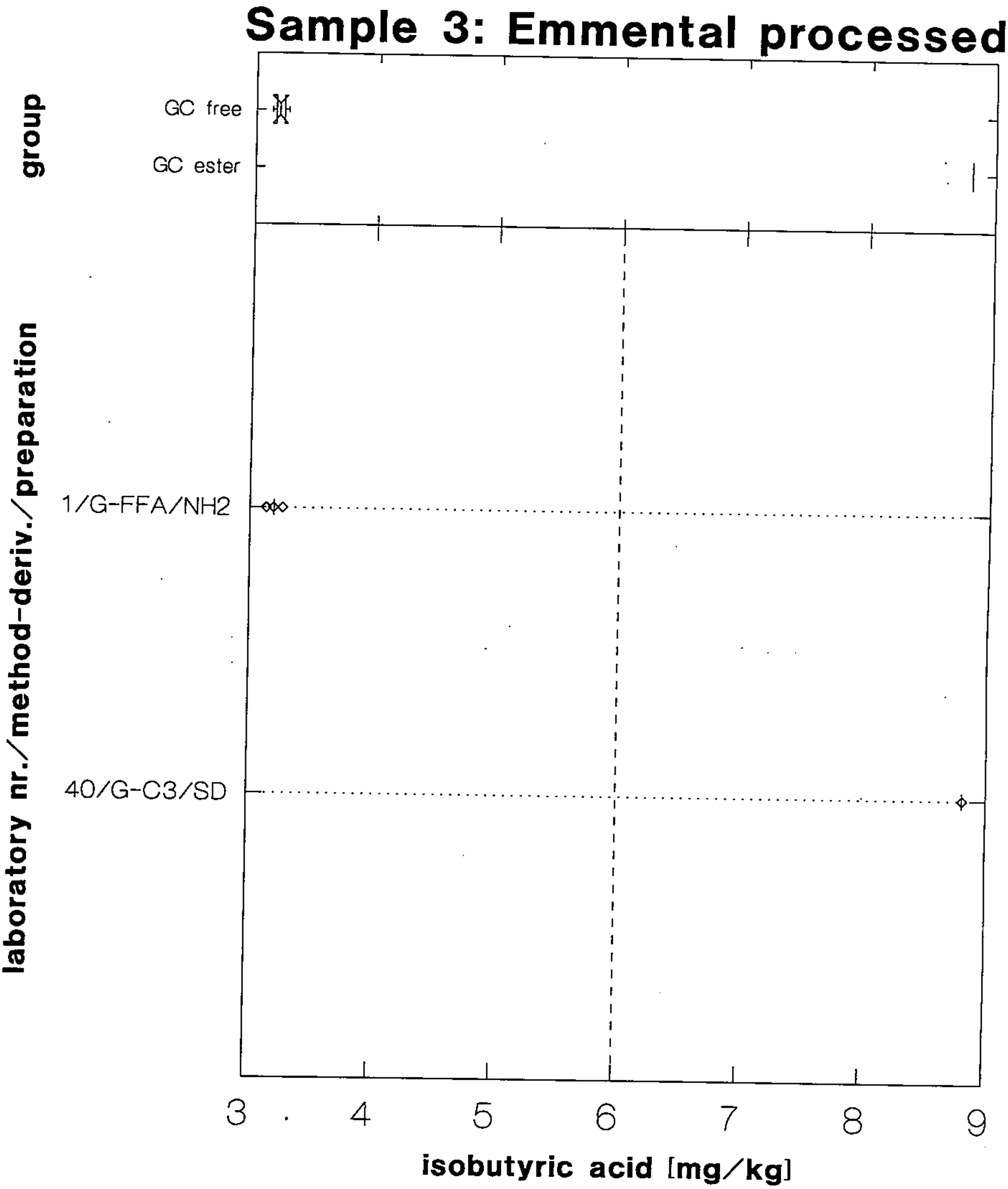

Sample 2: Butter 2
group
GC free
GC ester
laboratory nr./method-deriv./preparation
1/G-FFA/NH2
24/G-C1/oth
20
40
60
80
100
120
conj. linolenic acid [mg/kg]

Sample 3: Emmental processed

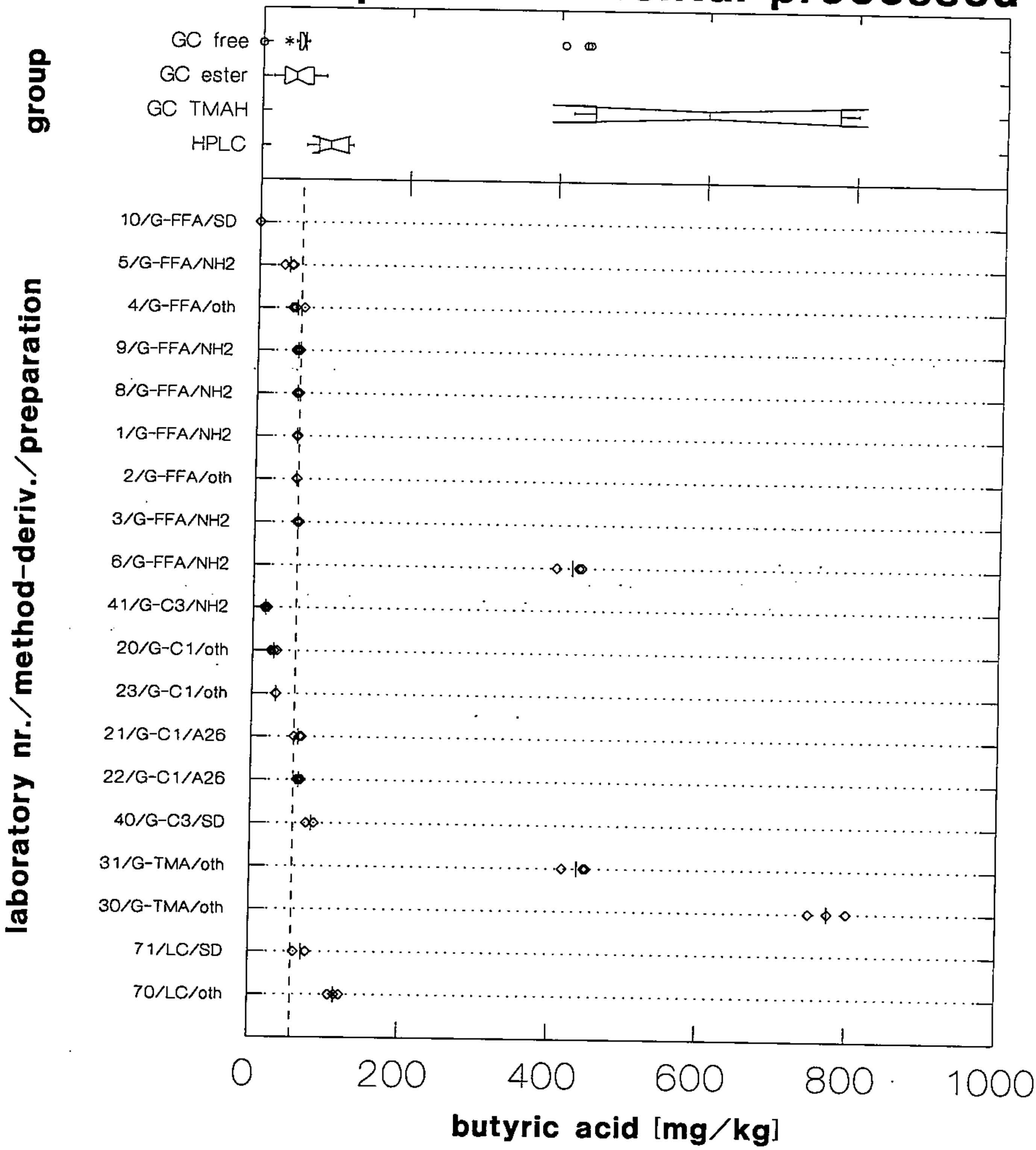


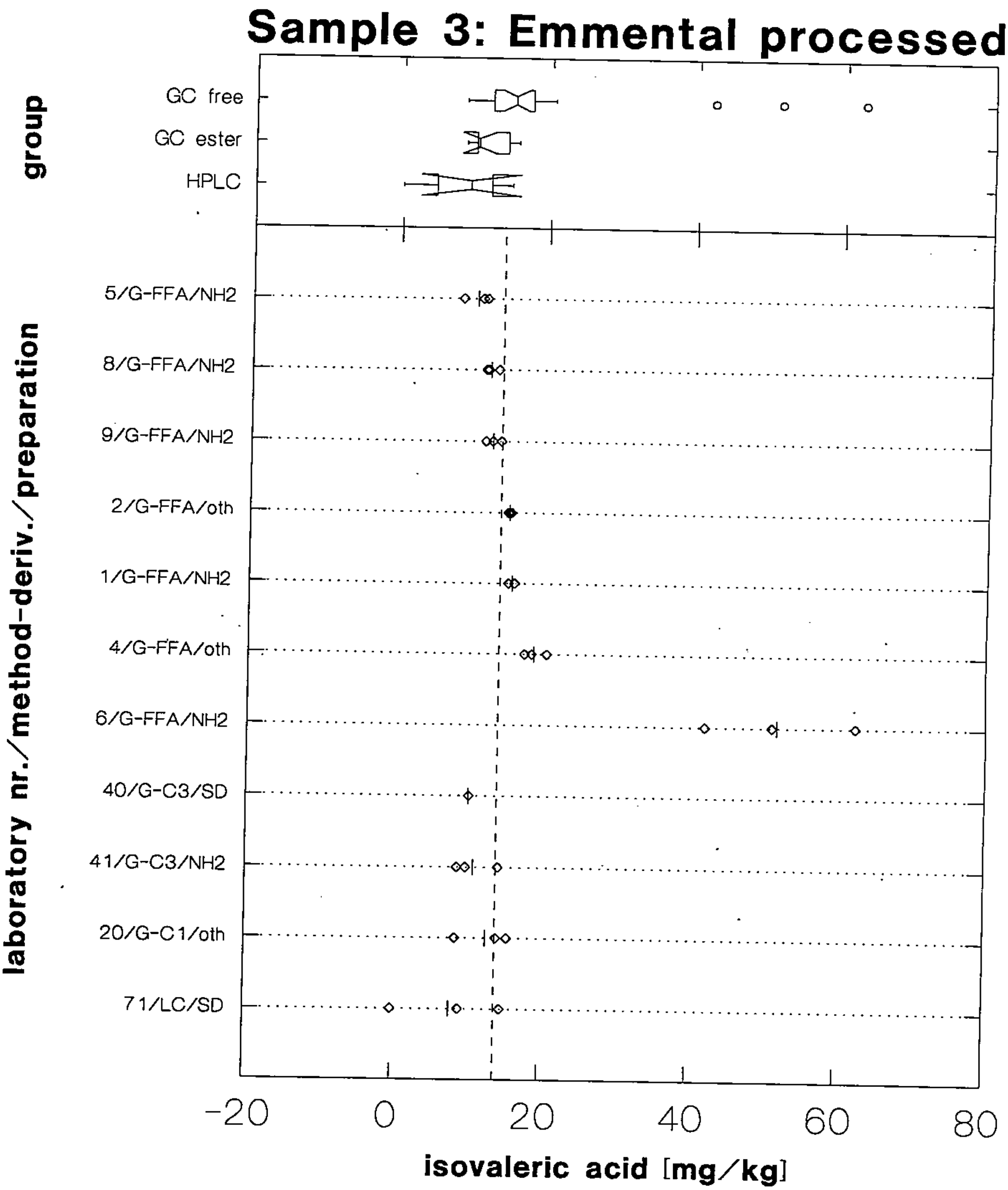

Sample 3: Emmental processed
group
GC free
GC ester
HPLC
laboratory nr./method-deriv./preparation
10/G-FFA/SD
5/G-FFA/NH2
4/G-FFA/oth
9/G-FFA/NH2
2/G-FFA/oth
3/G-FFA/NH2
6/G-FFA/NH2
20/G-C1/oth
40/G-C3/SD
71/LC/SD
70/LC/oth
0
2000
4000
6000
8000
10000
acetic acid [mg/kg]


Sample 3: Emmental processed
group
GC free
GC ester
HPLC
laboratory nr./method-deriv./preparation
10/G-FFA/SD
5/G-FFA/NH2
9/G-FFA/NH2
2/G-FFA/oth
3/G-FFA/NH2
1/G-FFA/NH2
4/G-FFA/oth
6/G-FFA/NH2
20/G-C1/oth
40/G-C3/SD
70/LC/oth
71/LC/SD
0
5000
10000
15000
20000
propionic acid [mg/kg]

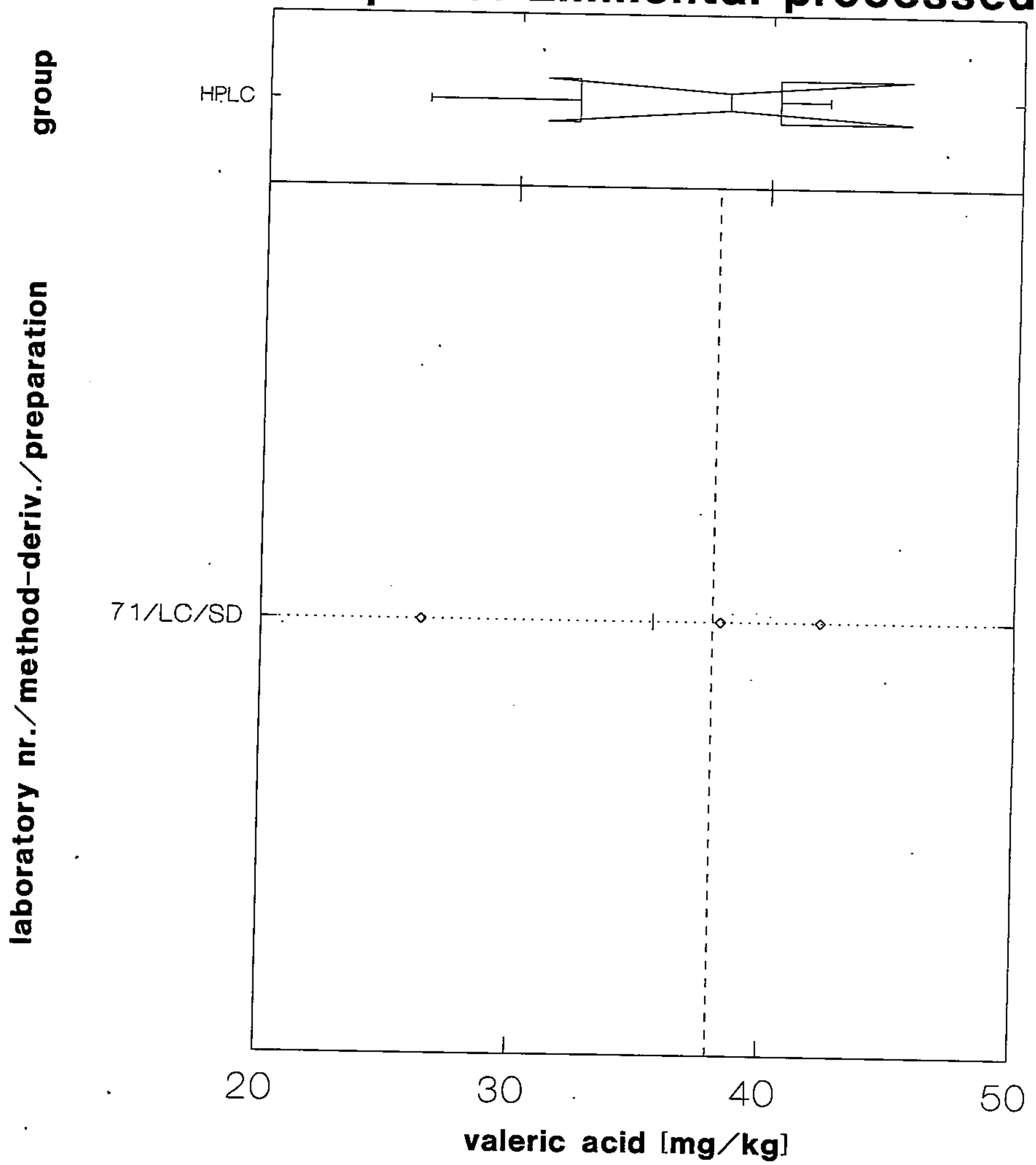

Sample 3: Emmental processed
group
GC free
GC ester
laboratory nr./method-deriv./preparation
1/G-FFA/NH2
40/G-C3/SD
3
4
5
6
7
8
9
isobutyric acid [mg/kg]

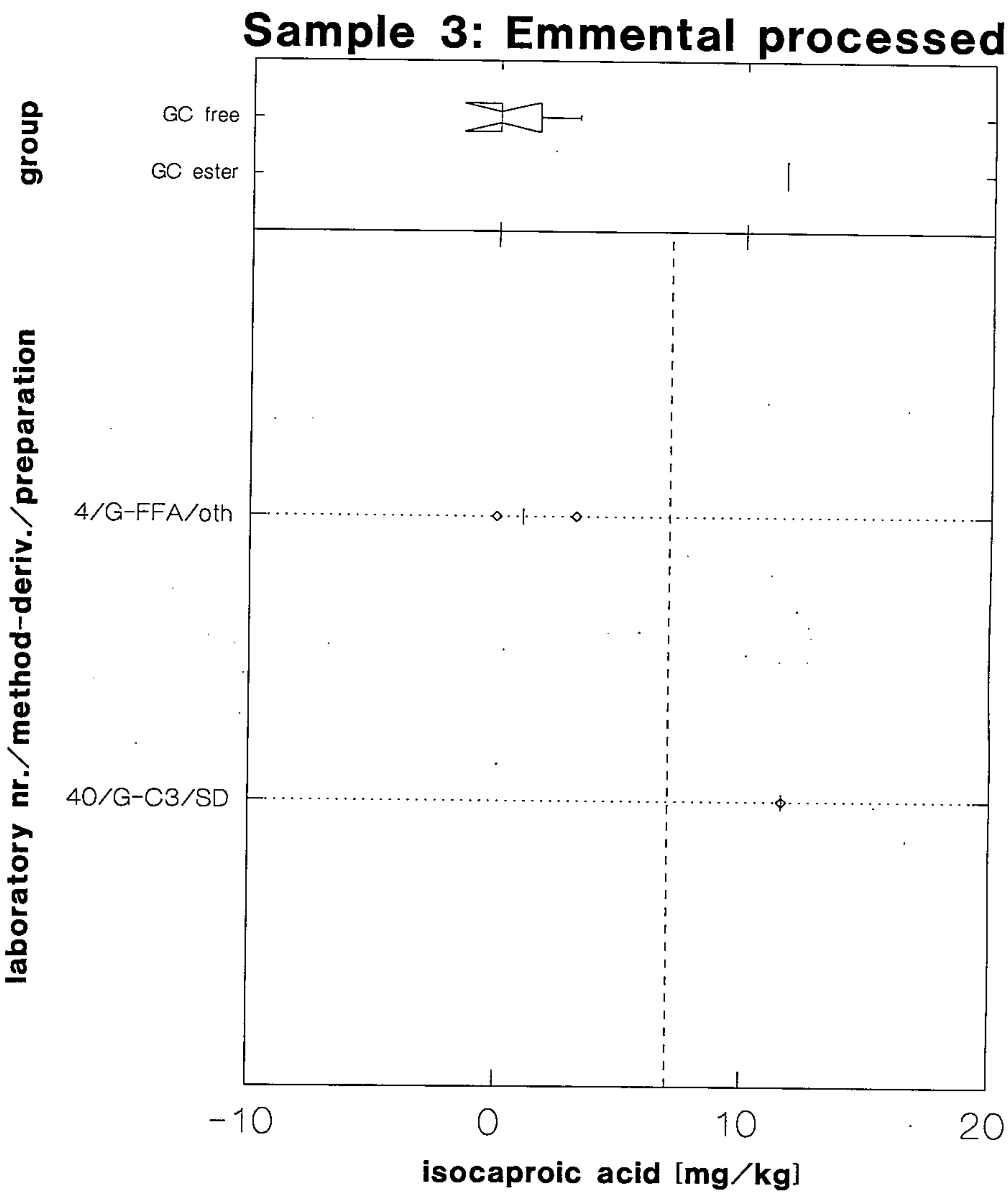
Sample 3: Emmental processed



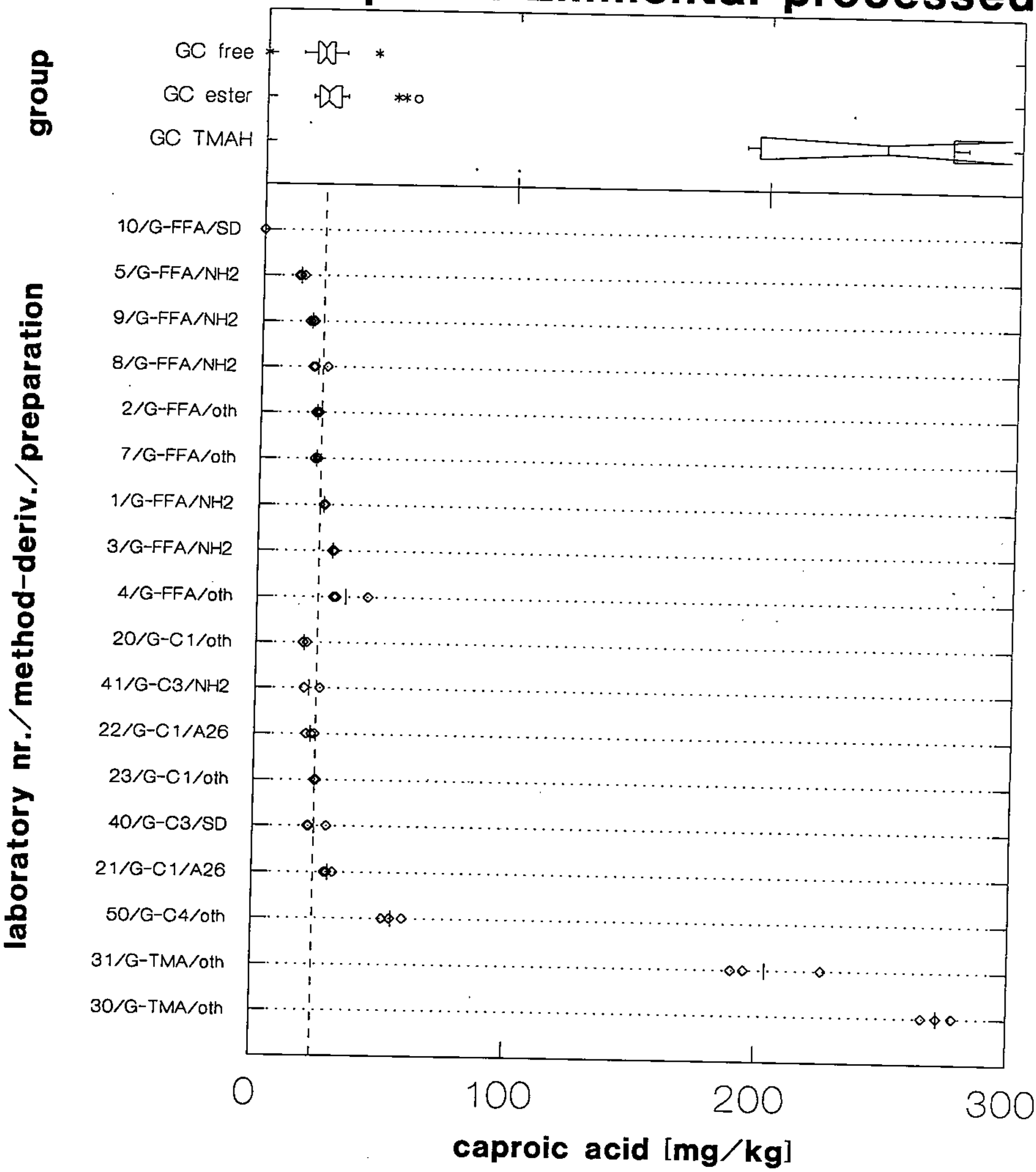

Sample 3: Emmental processed
group
GC free
GC ester
HPLC
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
8/G-FFA/NH2
9/G-FFA/NH2
2/G-FFA/oth
1/G-FFA/NH2
4/G-FFA/oth
6/G-FFA/NH2
40/G-C3/SD
41/G-C3/NH2
20/G-C1/oth
71/LC/SD
-20
0
20
40
60
80
isovaleric acid [mg/kg]

Sample 3: Emmental processed

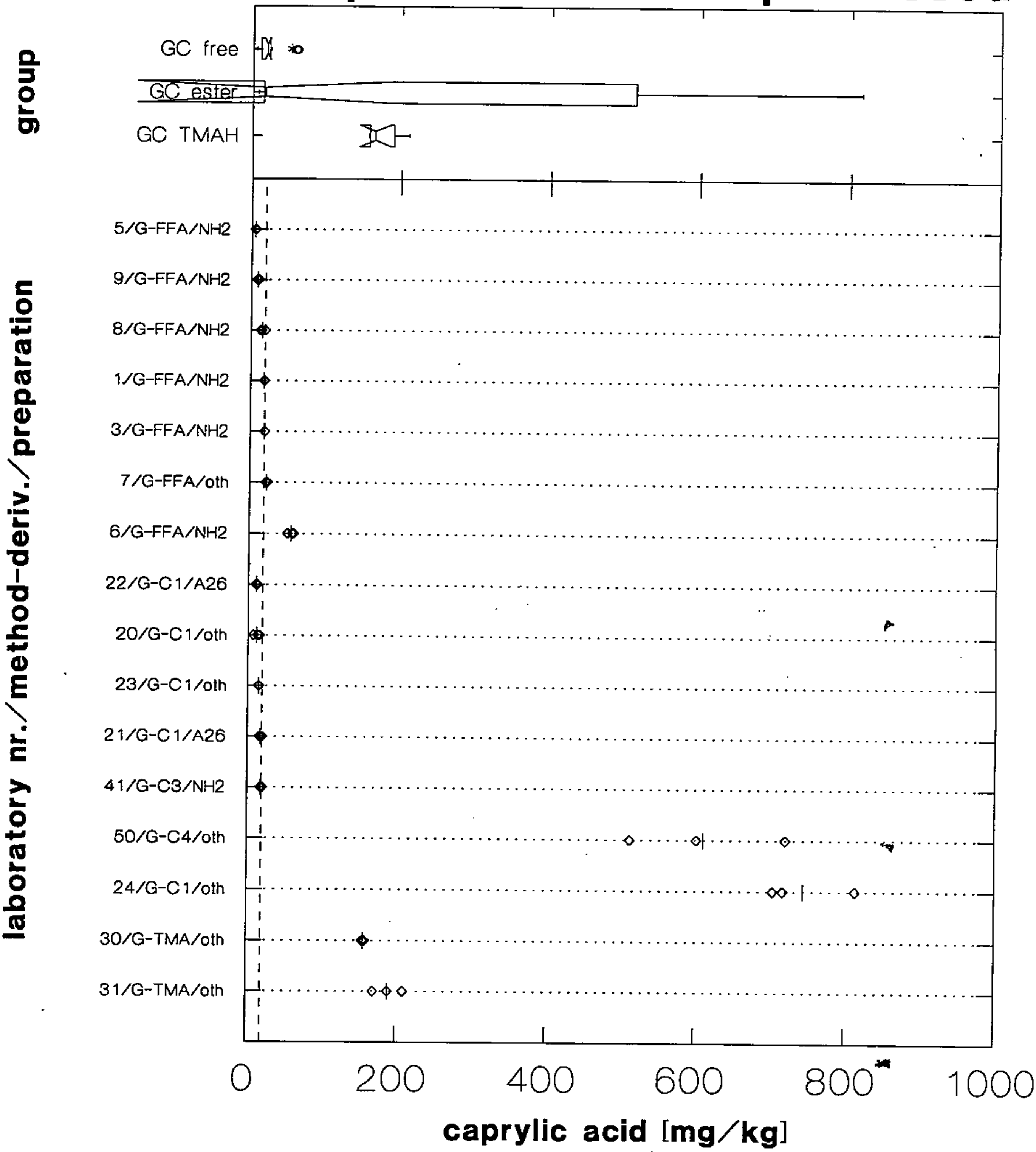



Sample 3: Emmental processed
group
GC free
GC ester
laboratory nr./method-deriv./preparation
4/G-FFA/oth
40/G-C3/SD
-10
0
10
20
isocaproic acid [mg/kg]

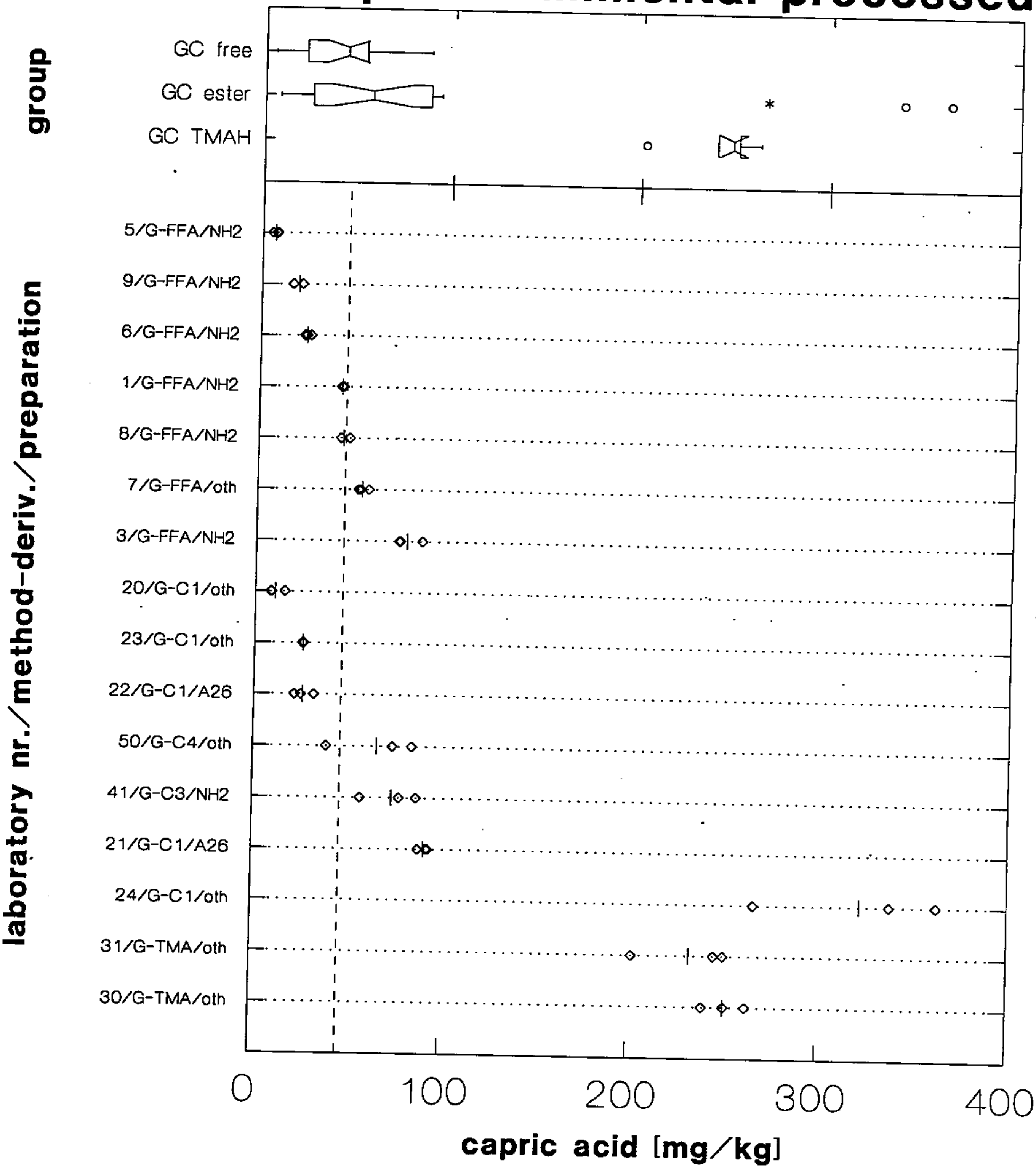
Sample 3: Emmental processed



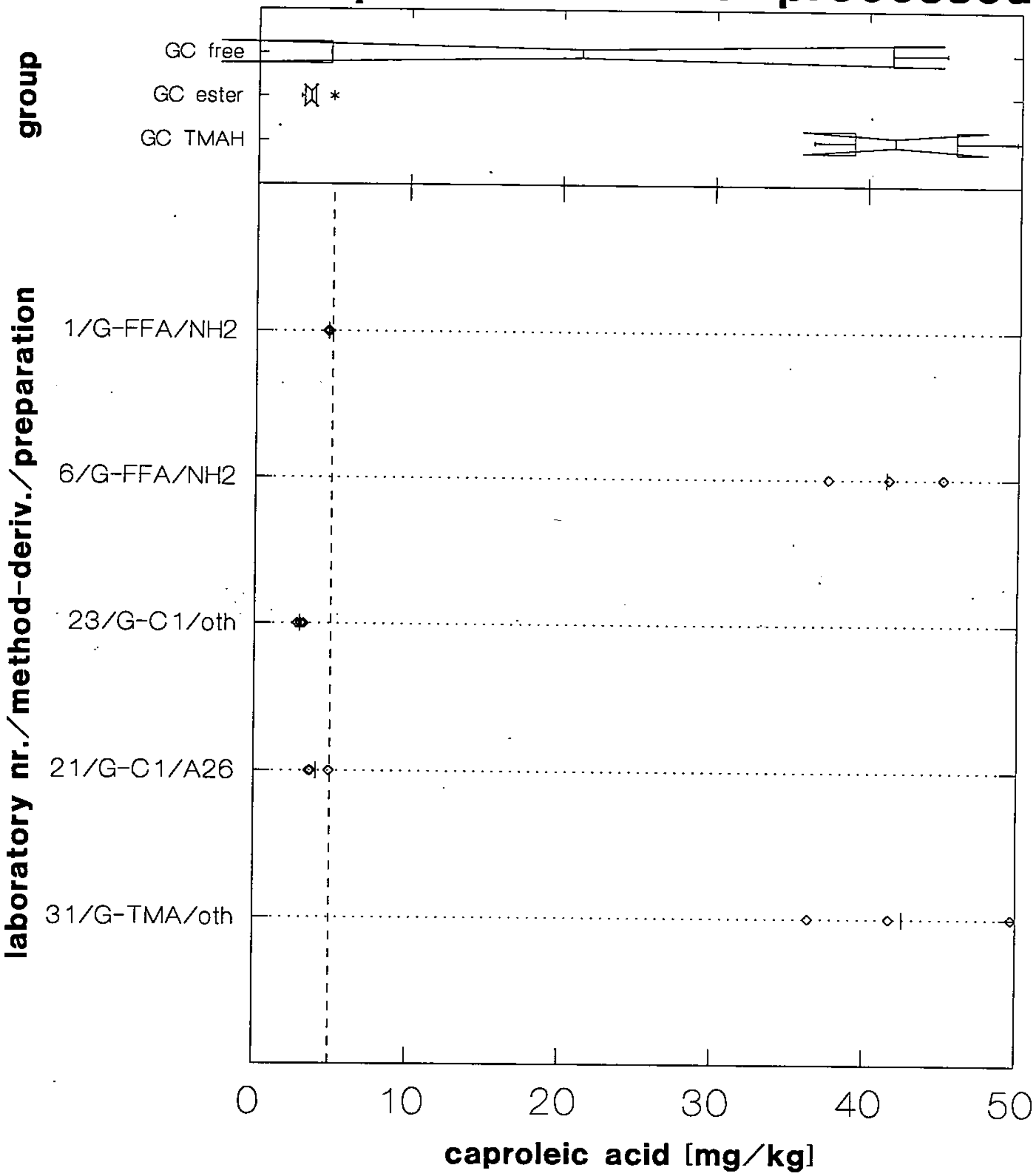
Sample 3: Emmental processed

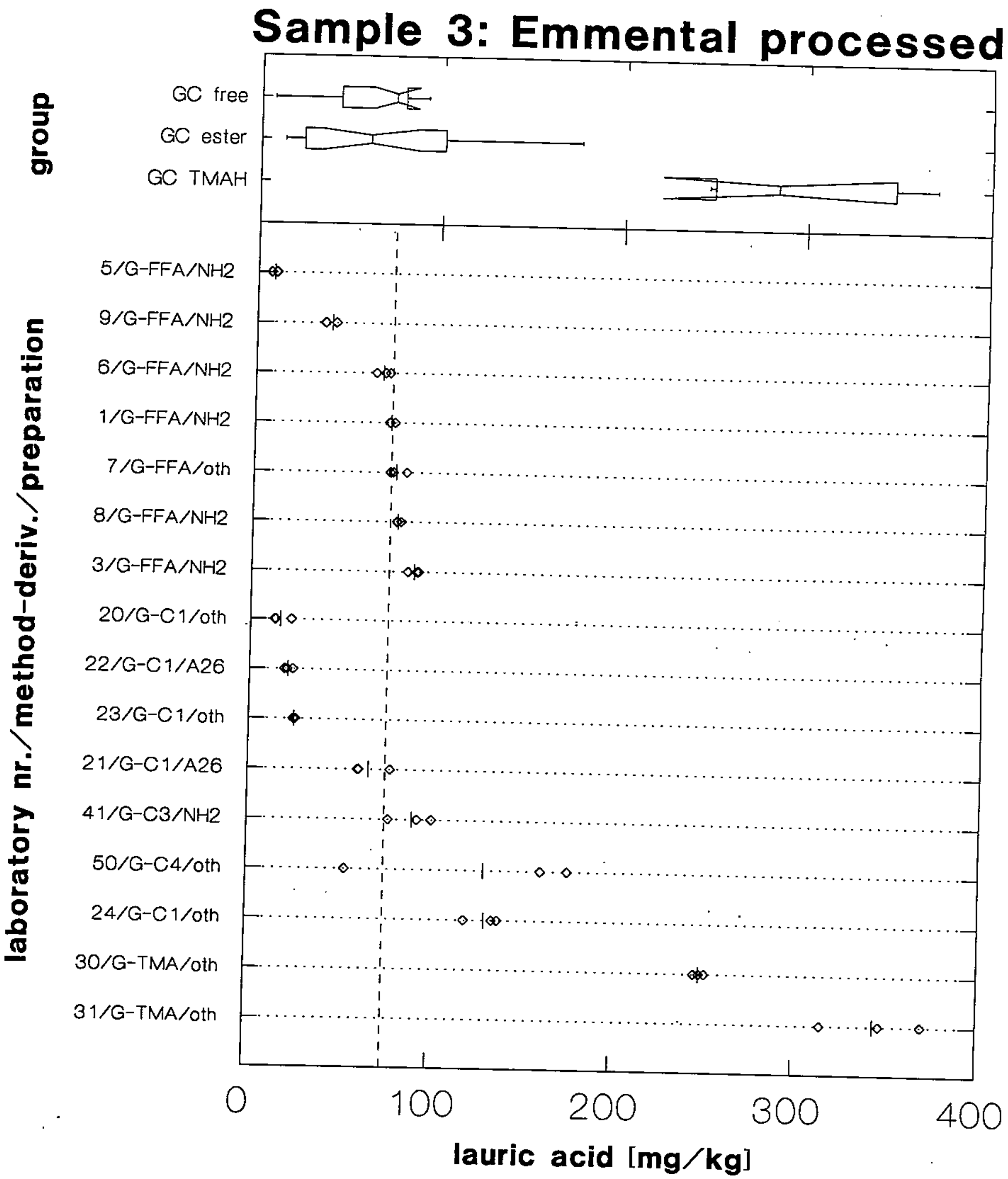


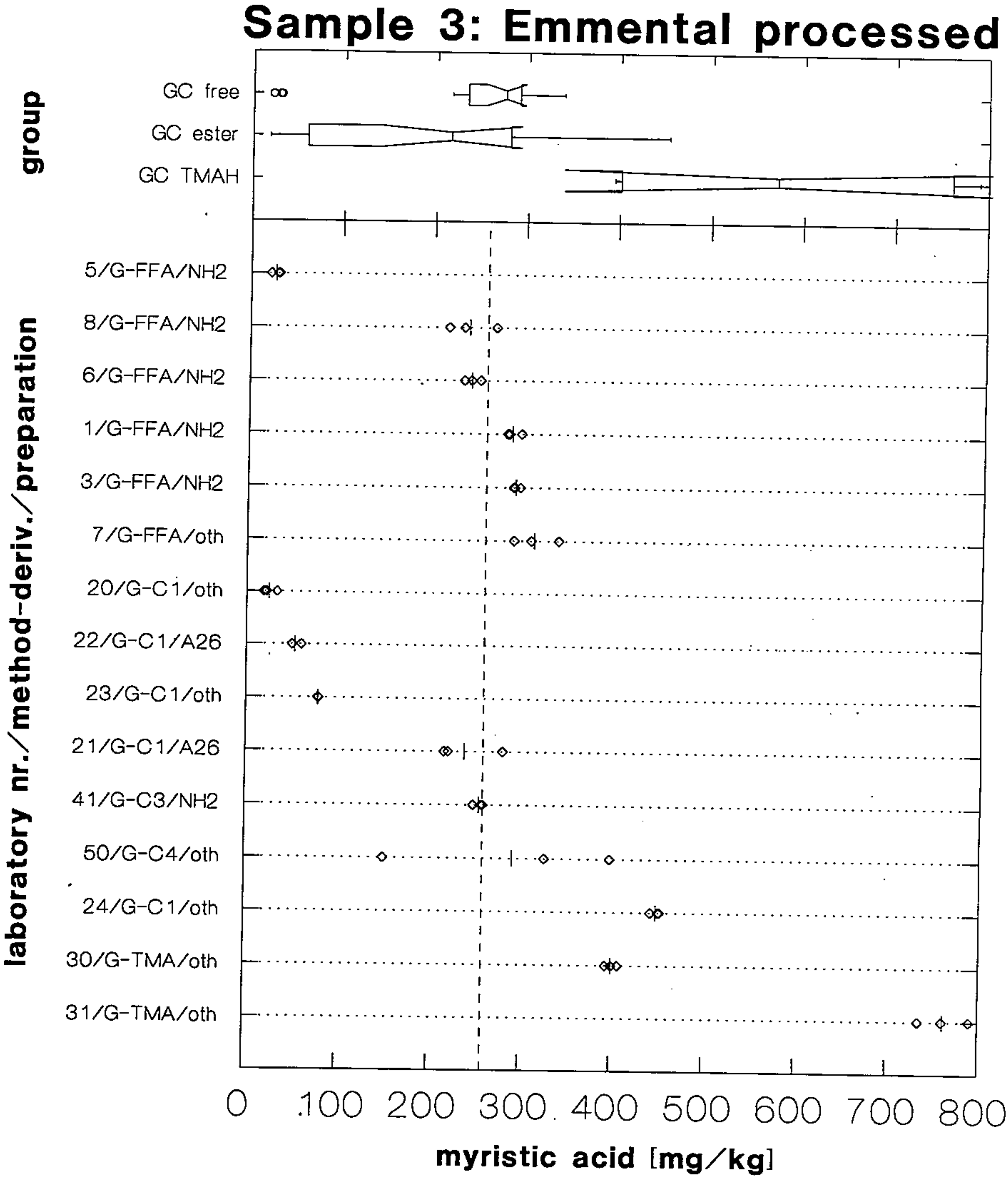
Sample 3: Emmental processed



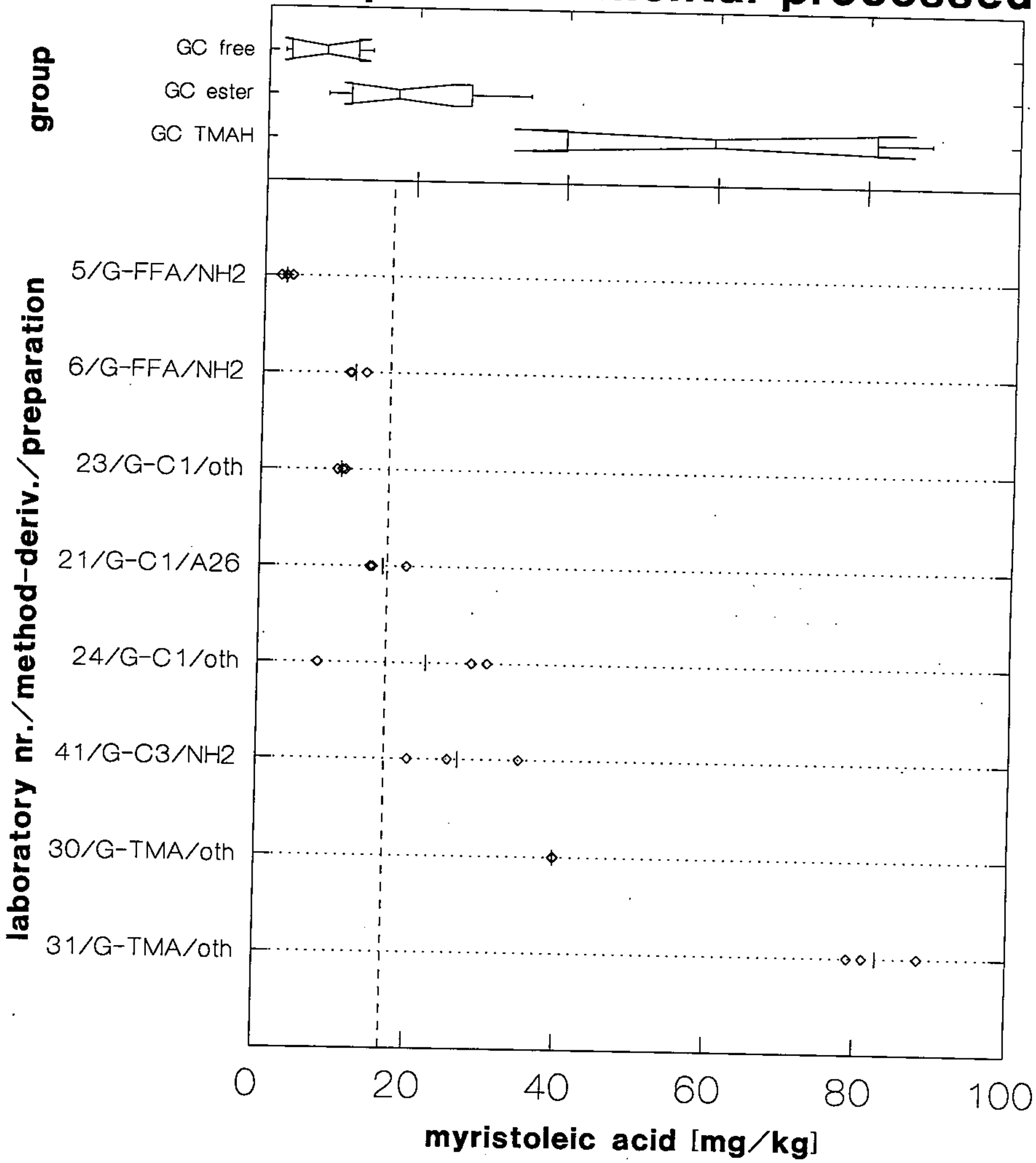
Sample 3: Emmental processed



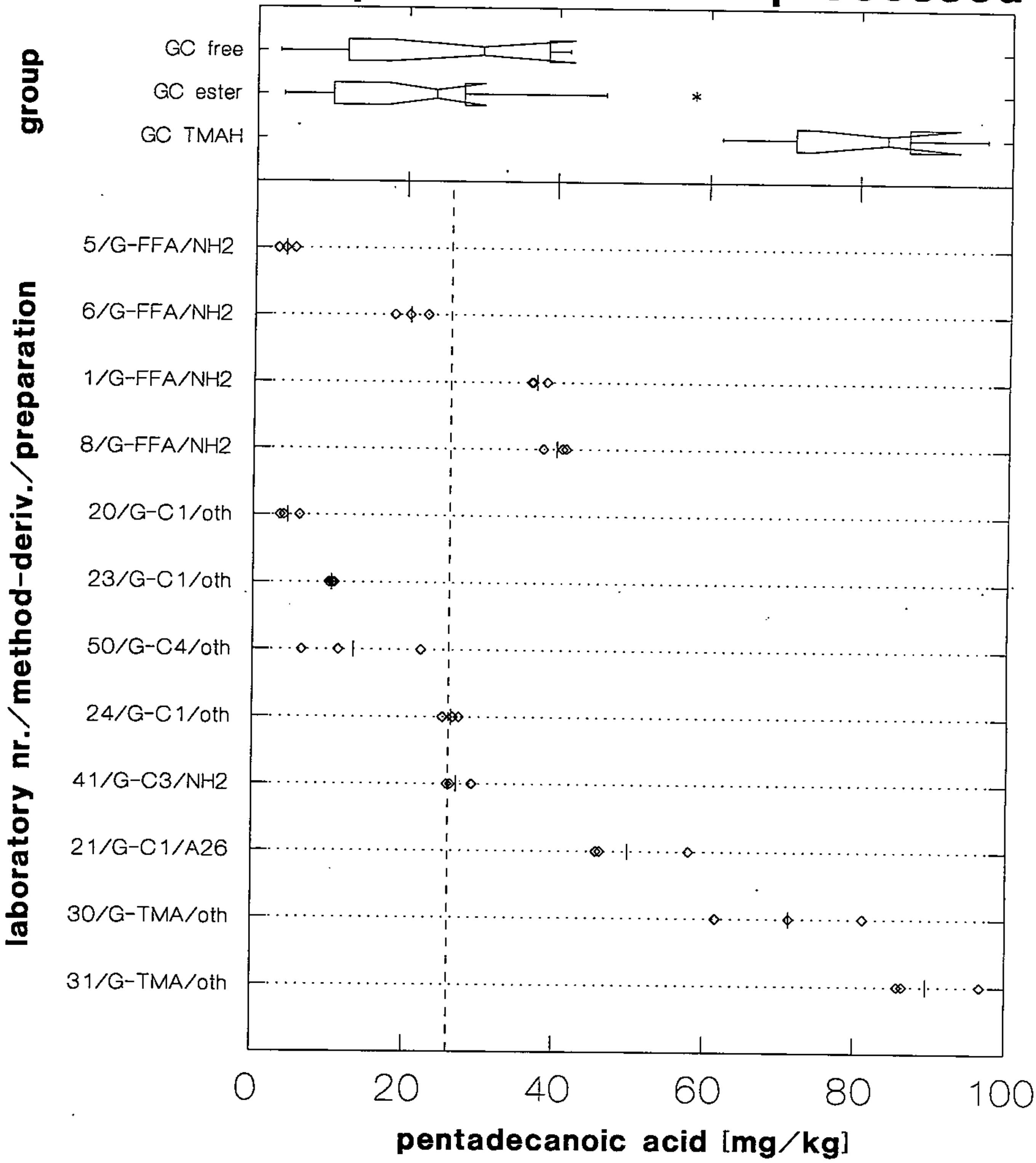

Sample 3: Emmental processed
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
9/G-FFA/NH2
6/G-FFA/NH2
1/G-FFA/NH2
7/G-FFA/oth
8/G-FFA/NH2
3/G-FFA/NH2
20/G-C1/oth
22/G-C1/A26
23/G-C1/oth
21/G-C1/A26
41/G-C3/NH2
50/G-C4/oth
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
100
200
300
400
lauric acid [mg/kg]


Sample 3: Emmental processed
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
8/G-FFA/NH2
6/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
7/G-FFA/oth
20/G-C1/oth
22/G-C1/A26
23/G-C1/oth
21/G-C1/A26
41/G-C3/NH2
50/G-C4/oth
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
100
200
300
400
500
600
700
800
myristic acid [mg/kg]

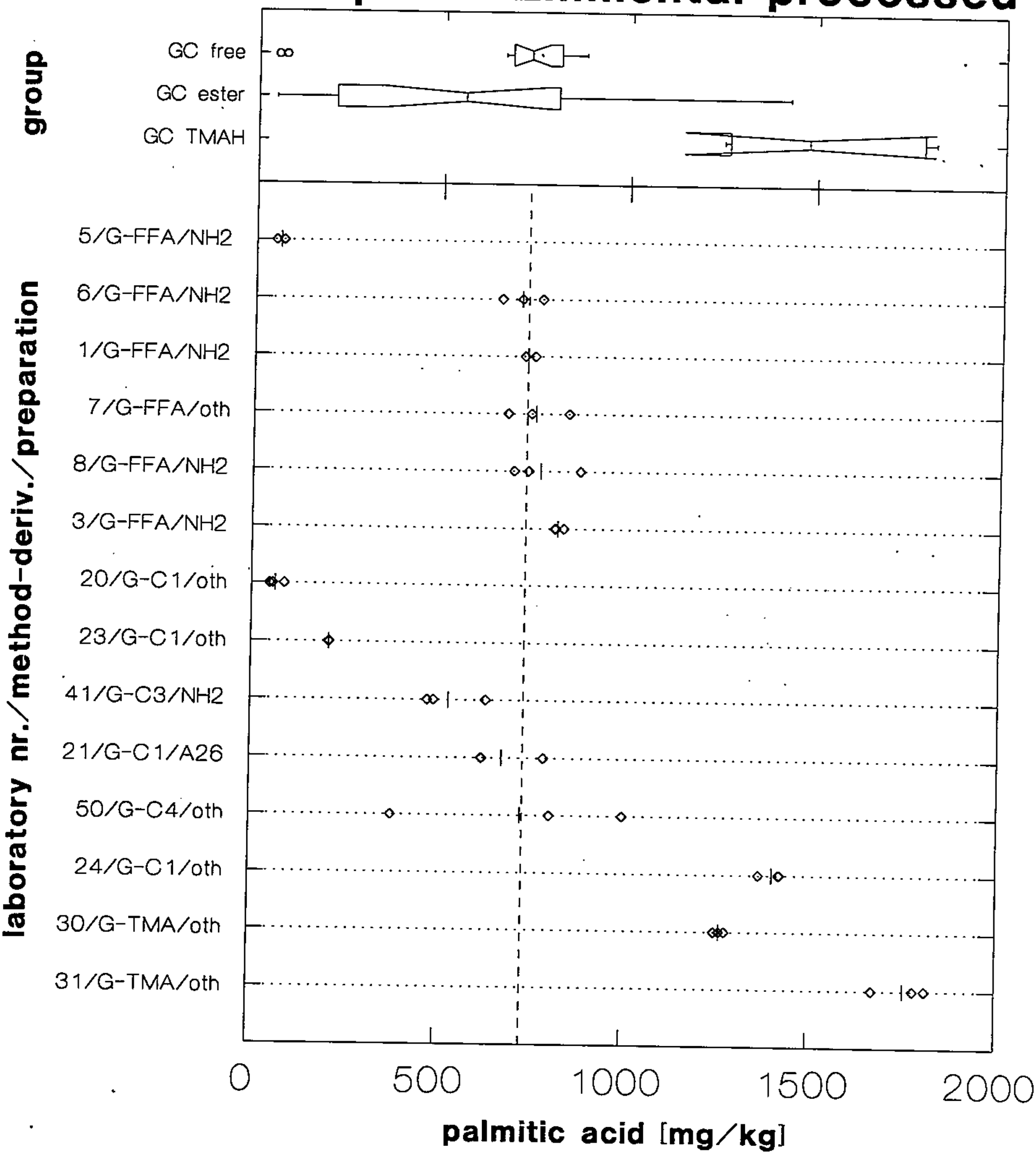
Sample 3: Emmental processed

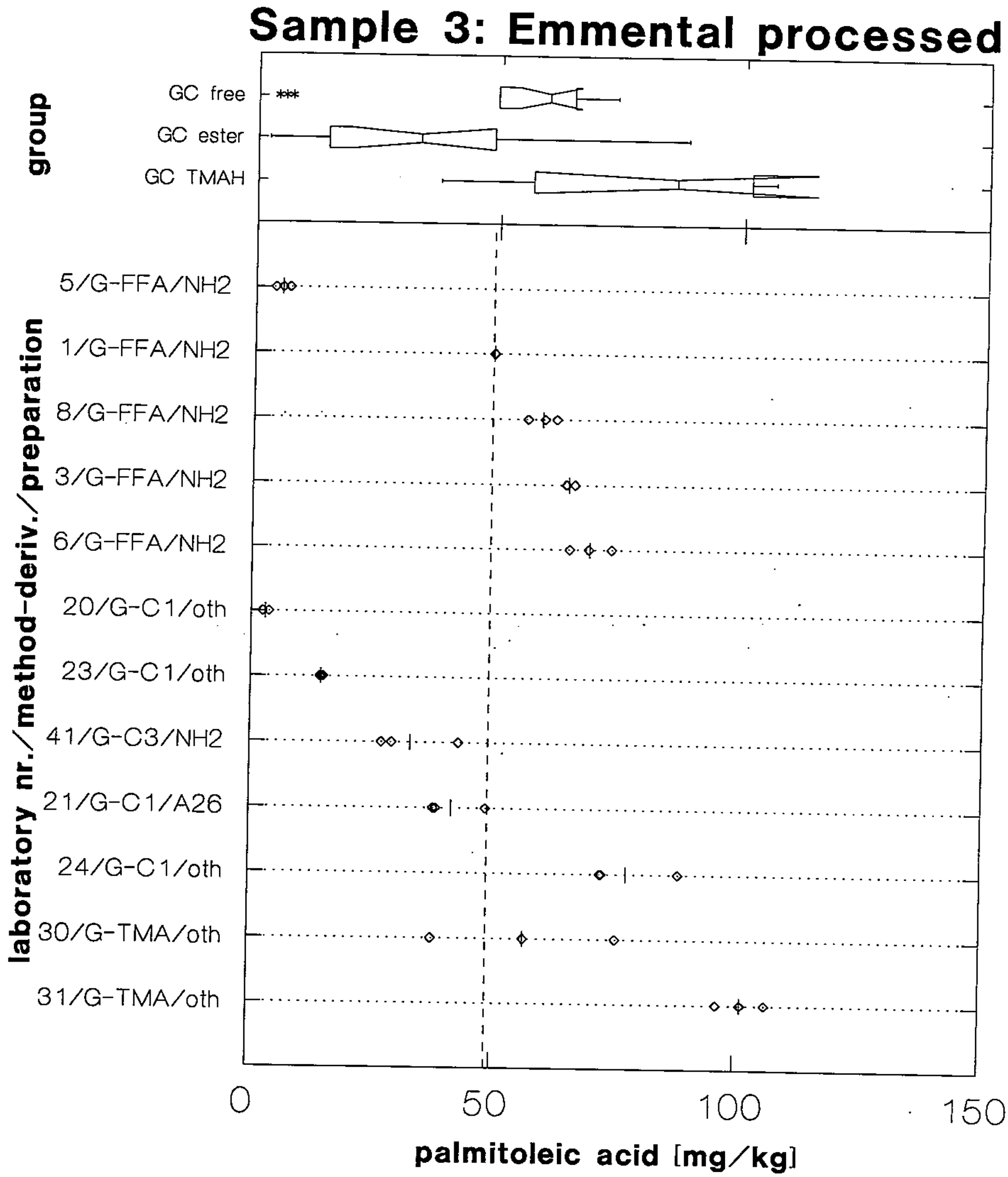


Sample 3: Emmental processed

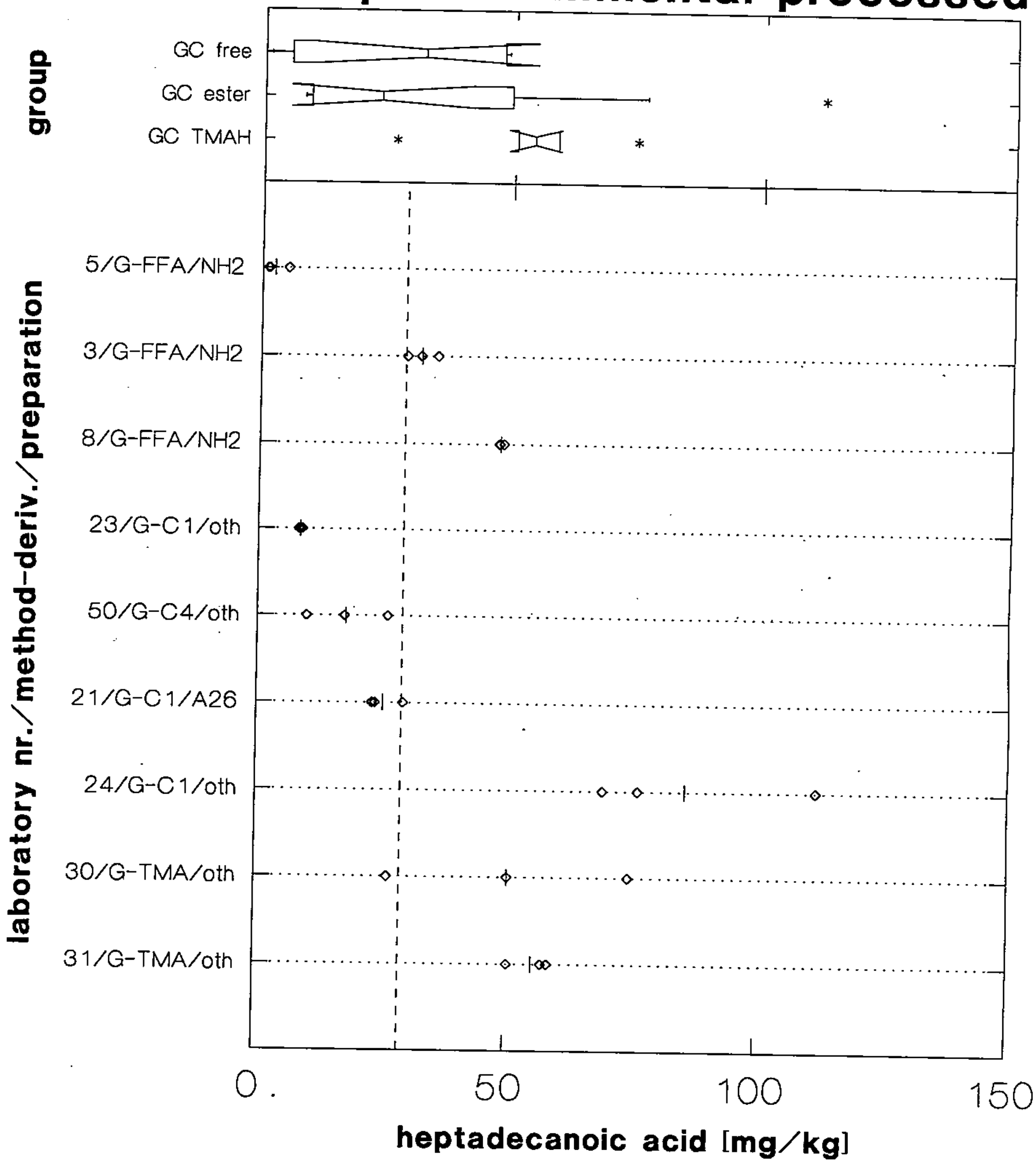


Sample 3: Emmental processed

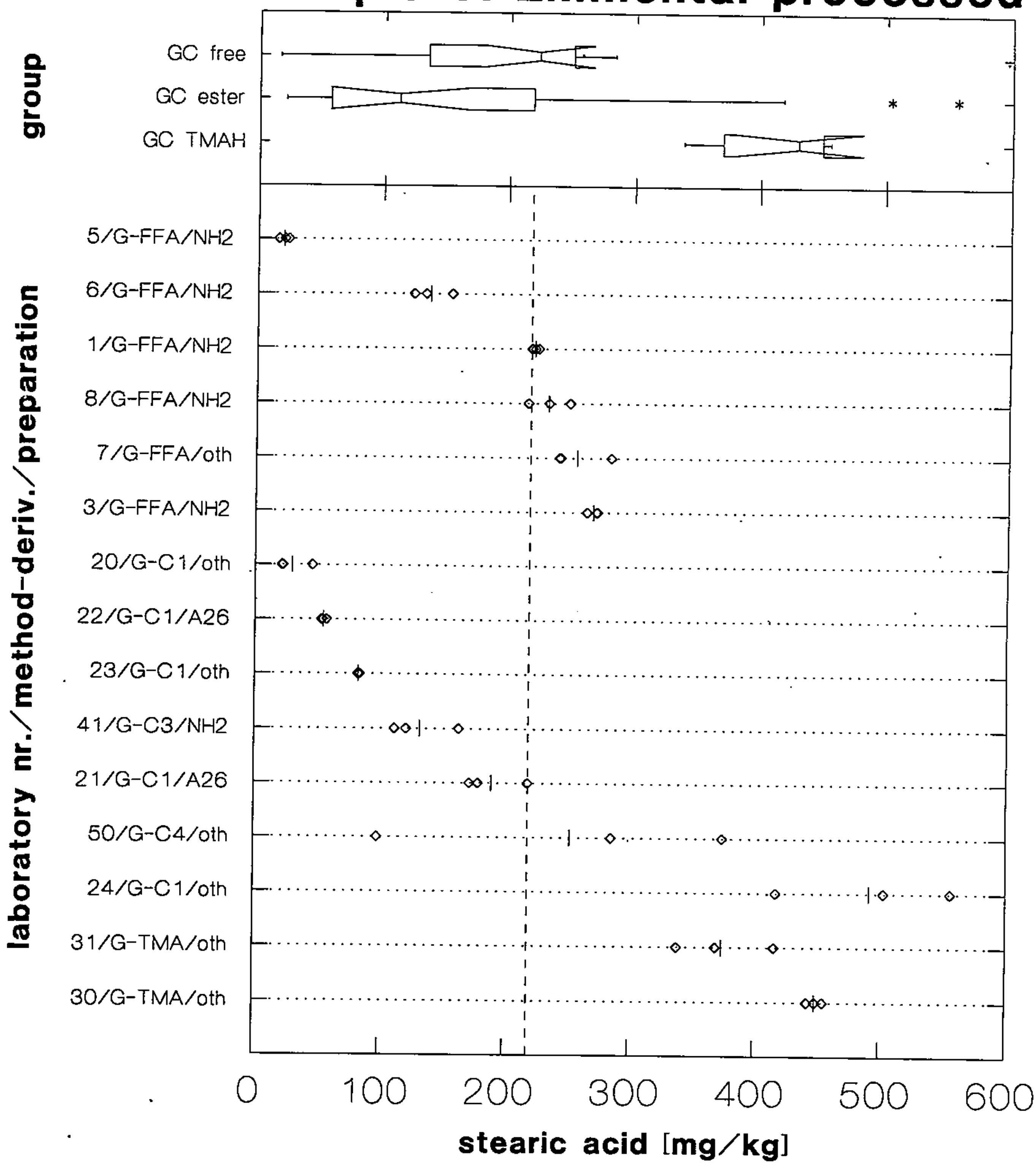



Sample 3: Emmental processed
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
1/G-FFA/NH2
8/G-FFA/NH2
3/G-FFA/NH2
6/G-FFA/NH2
20/G-C1/oth
23/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
50
100
150
palmitoleic acid [mg/kg]

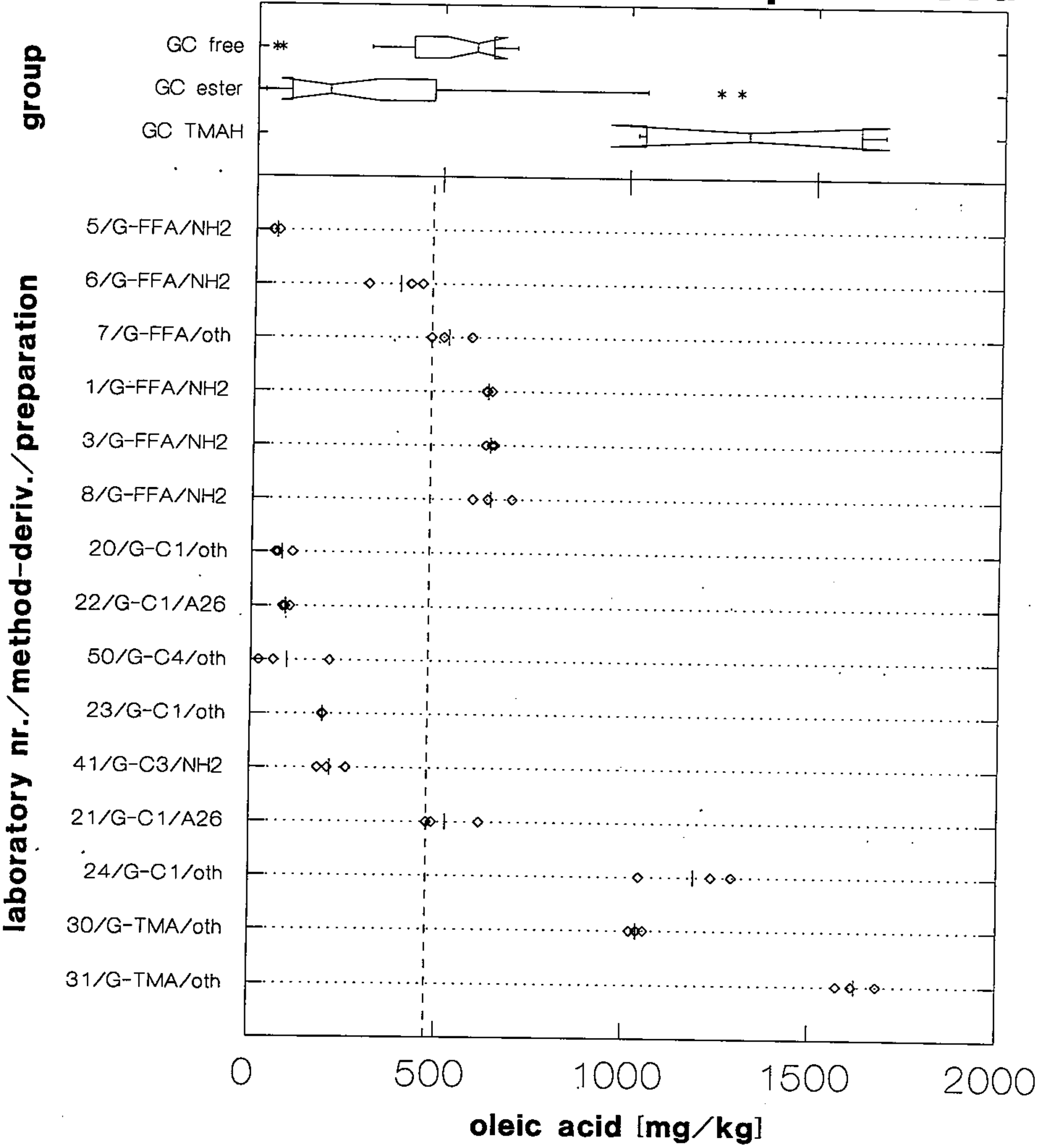
Sample 3: Emmental processed

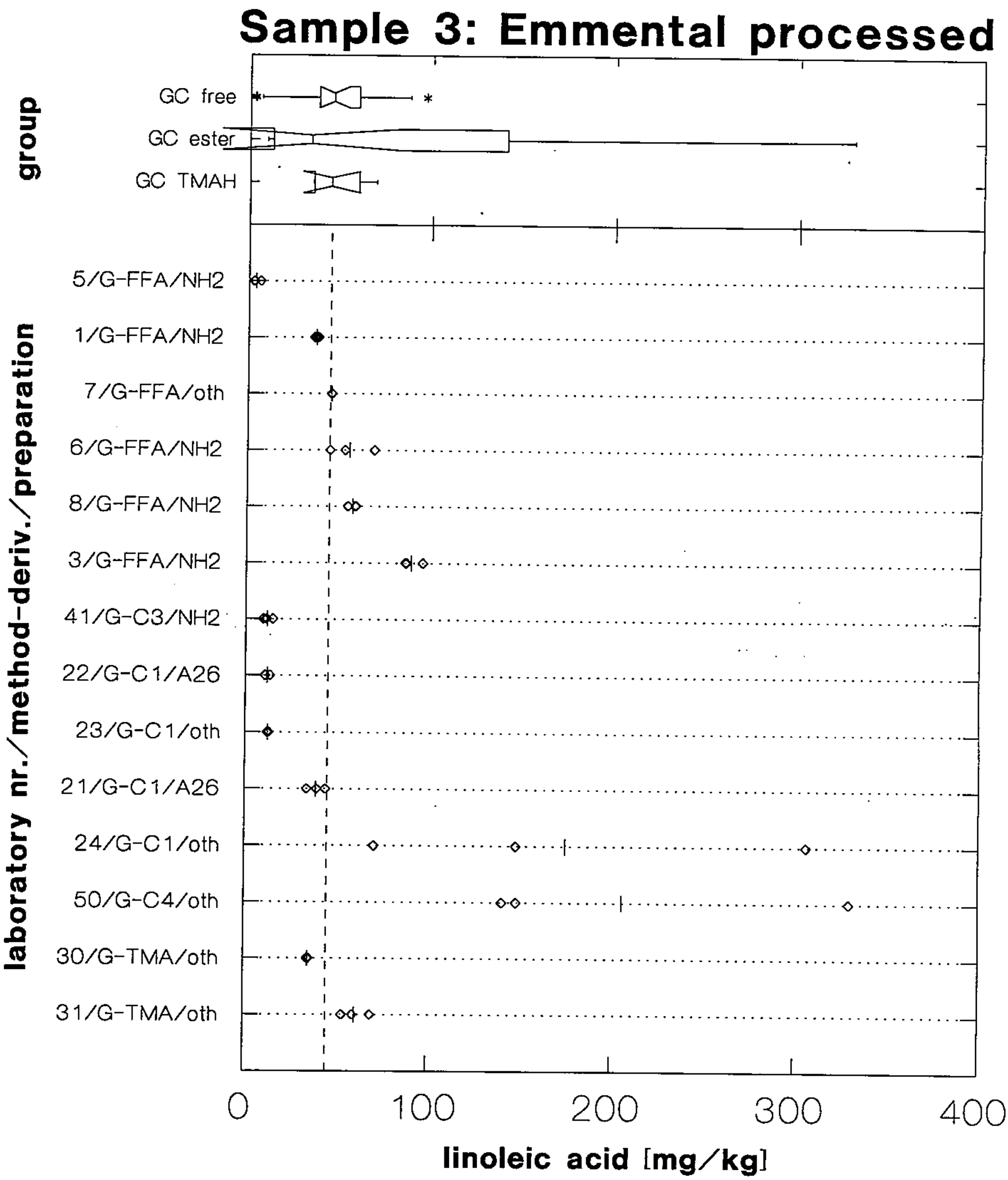


Sample 3: Emmental processed

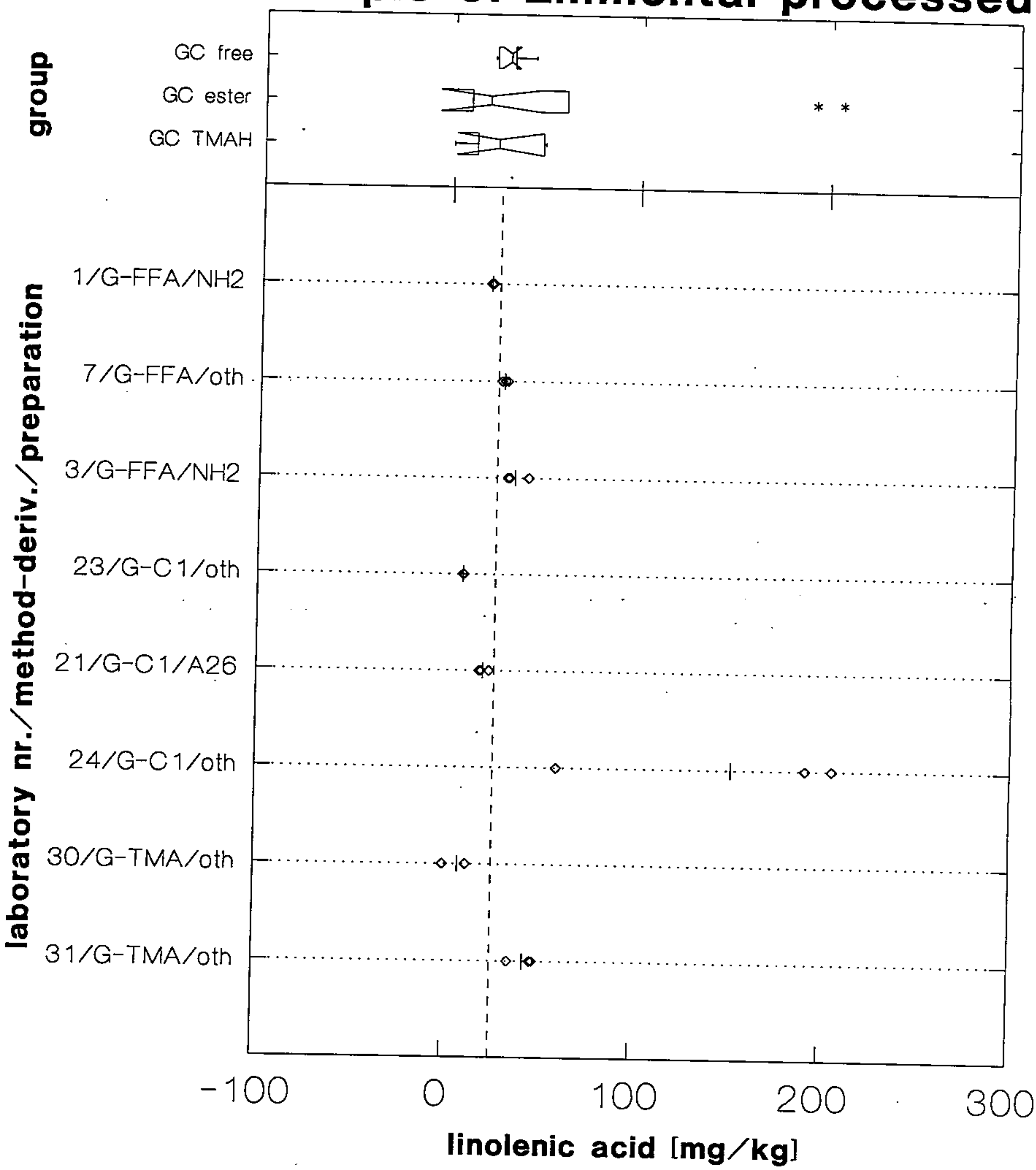


Sample 3: Emmental processed

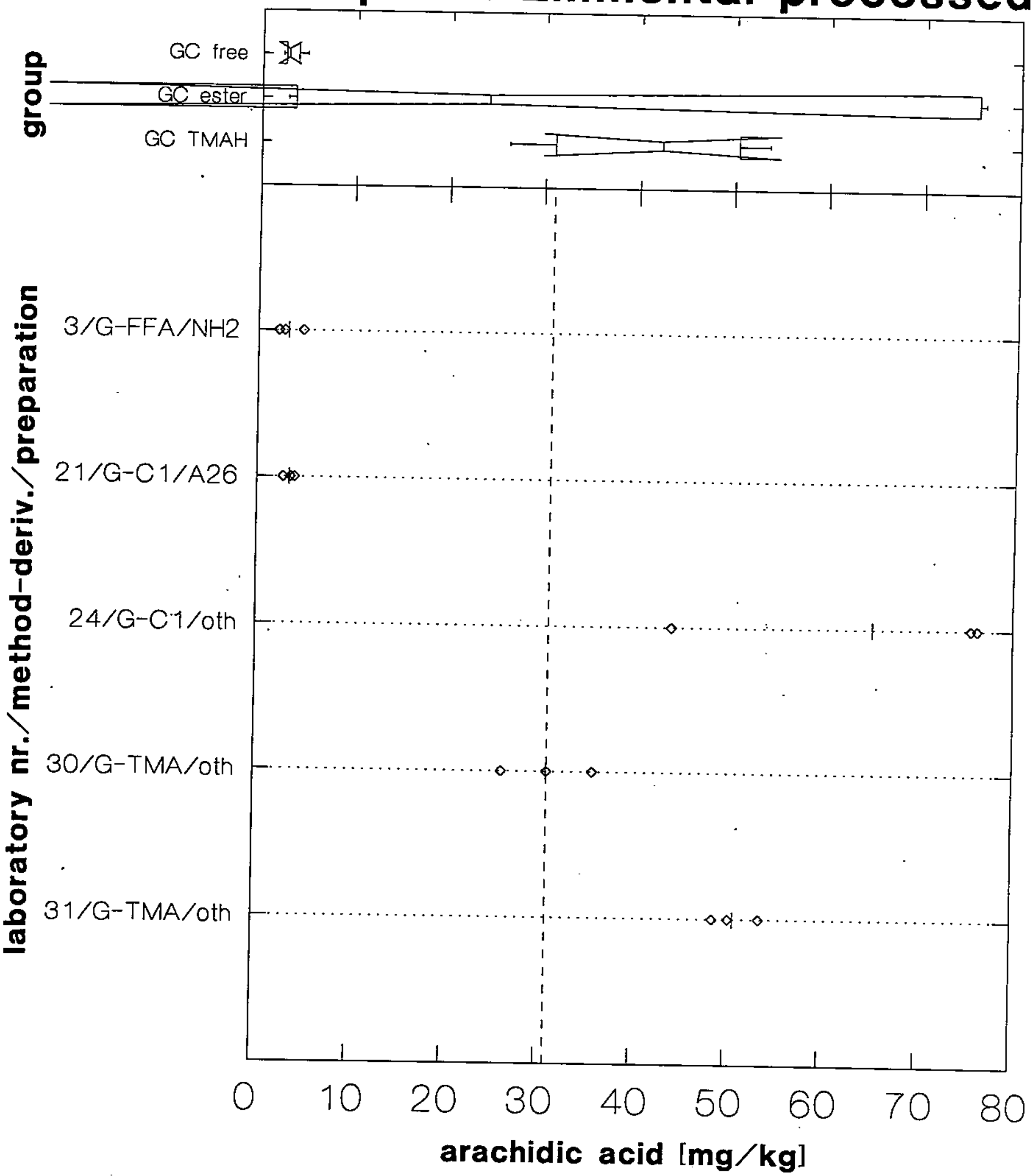



Sample 3: Emmental processed
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
1/G-FFA/NH2
7/G-FFA/oth
6/G-FFA/NH2
8/G-FFA/NH2
3/G-FFA/NH2
41/G-C3/NH2
22/G-C1/A26
23/G-C1/oth
21/G-C1/A26
24/G-C1/oth
50/G-C4/oth
30/G-TMA/oth
31/G-TMA/oth
0
100
200
300
400
linoleic acid [mg/kg]

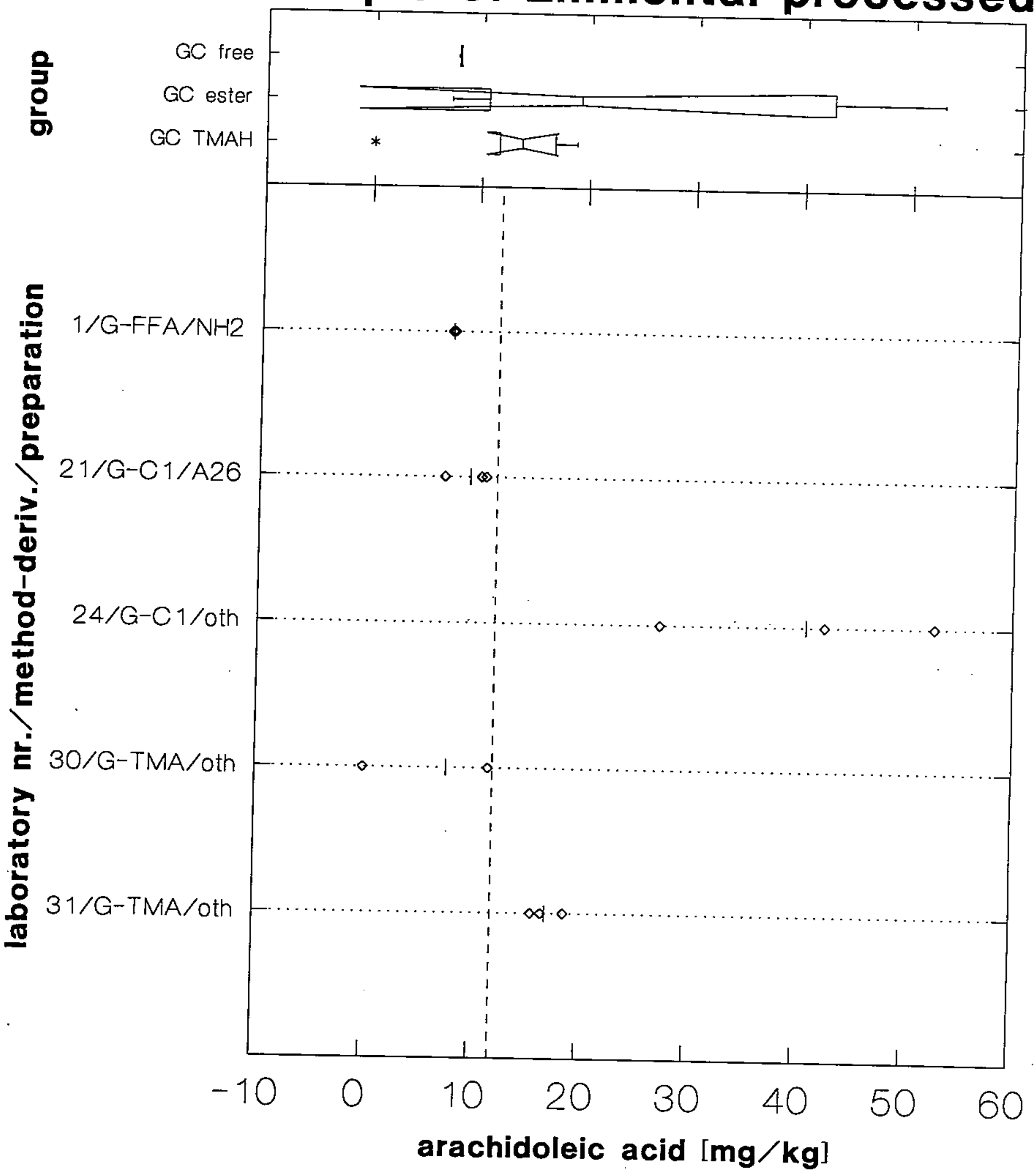
Sample 3: Emmental processed



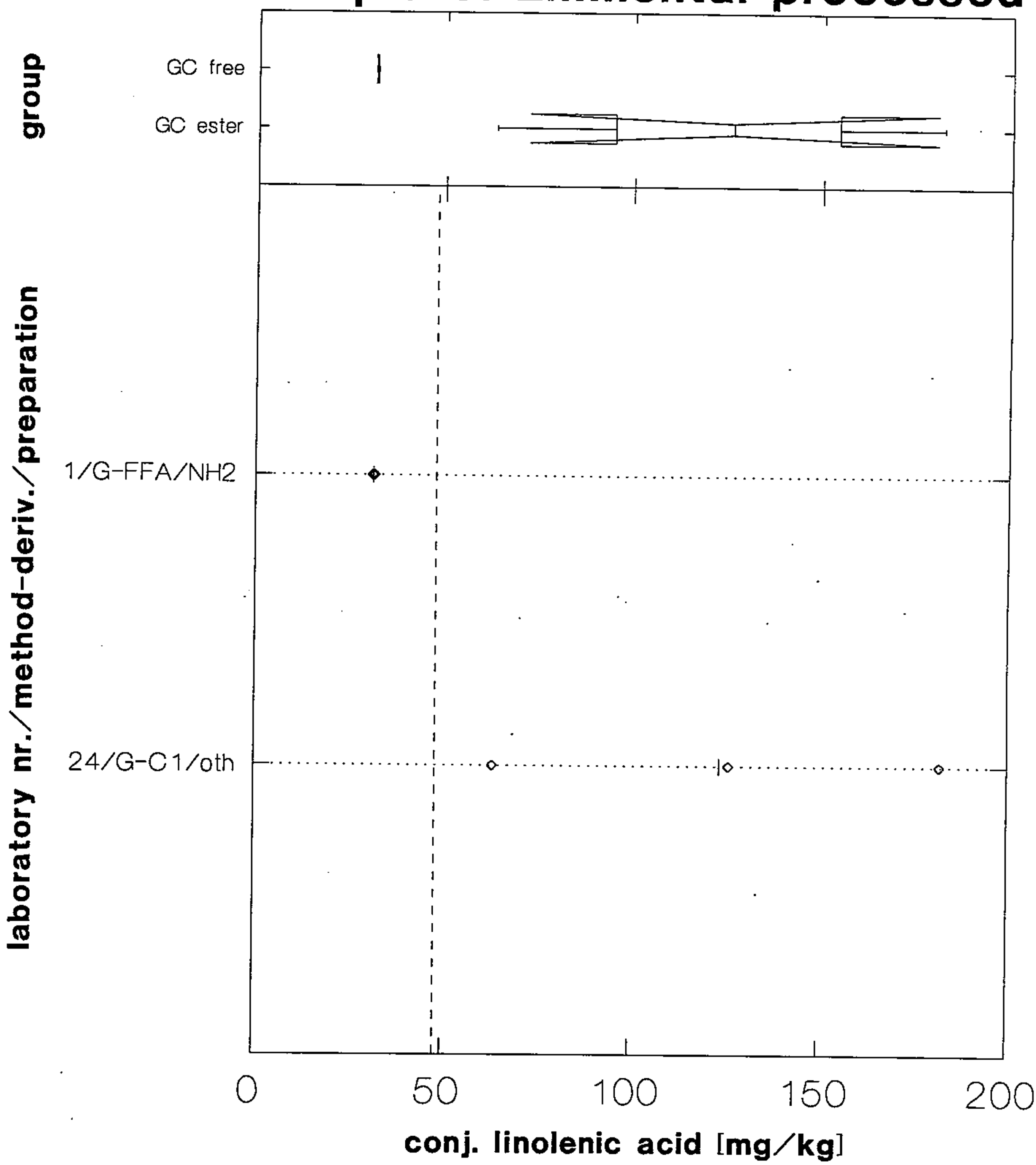
Sample 3: Emmental processed



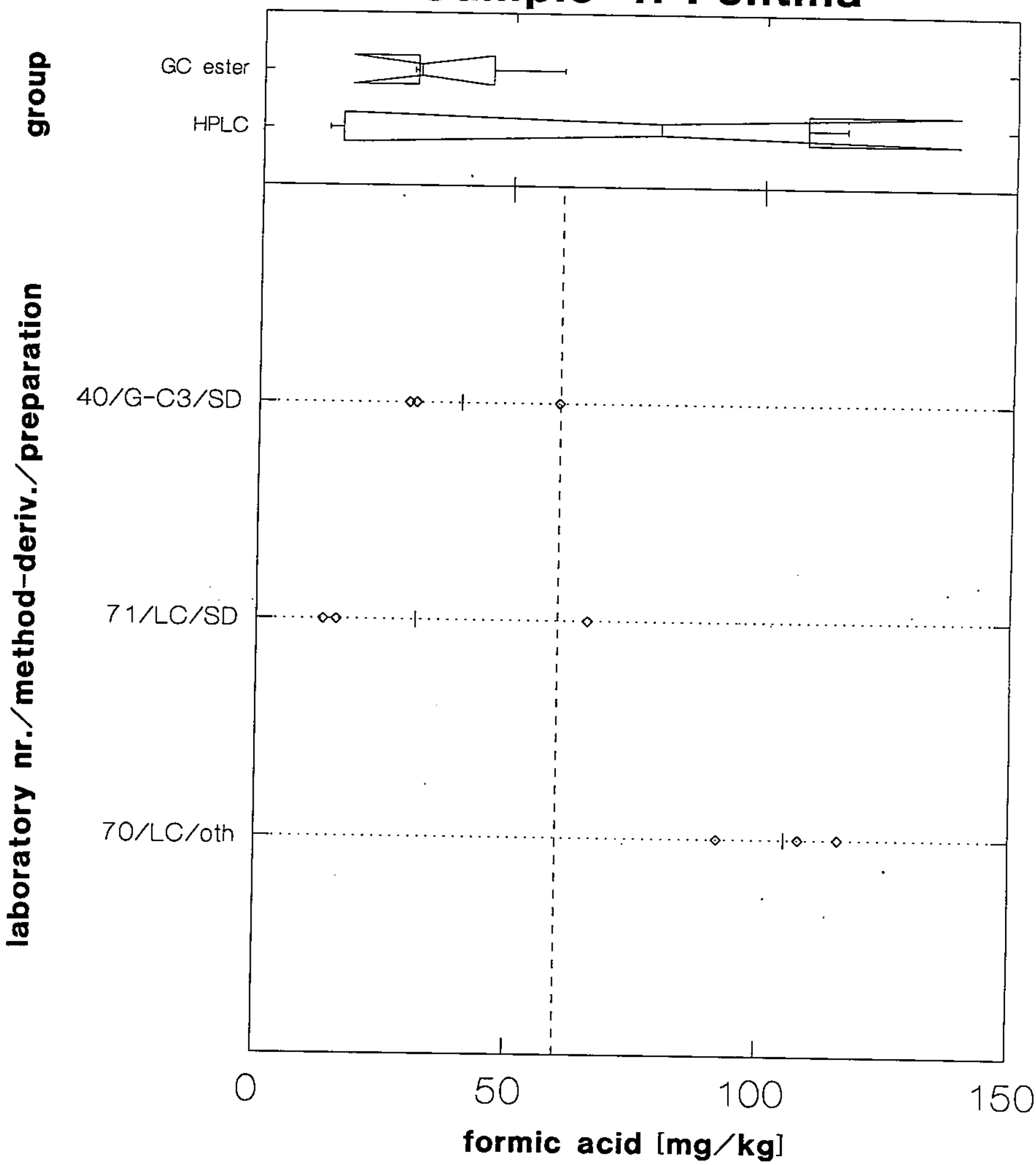
Sample 3: Emmental processed

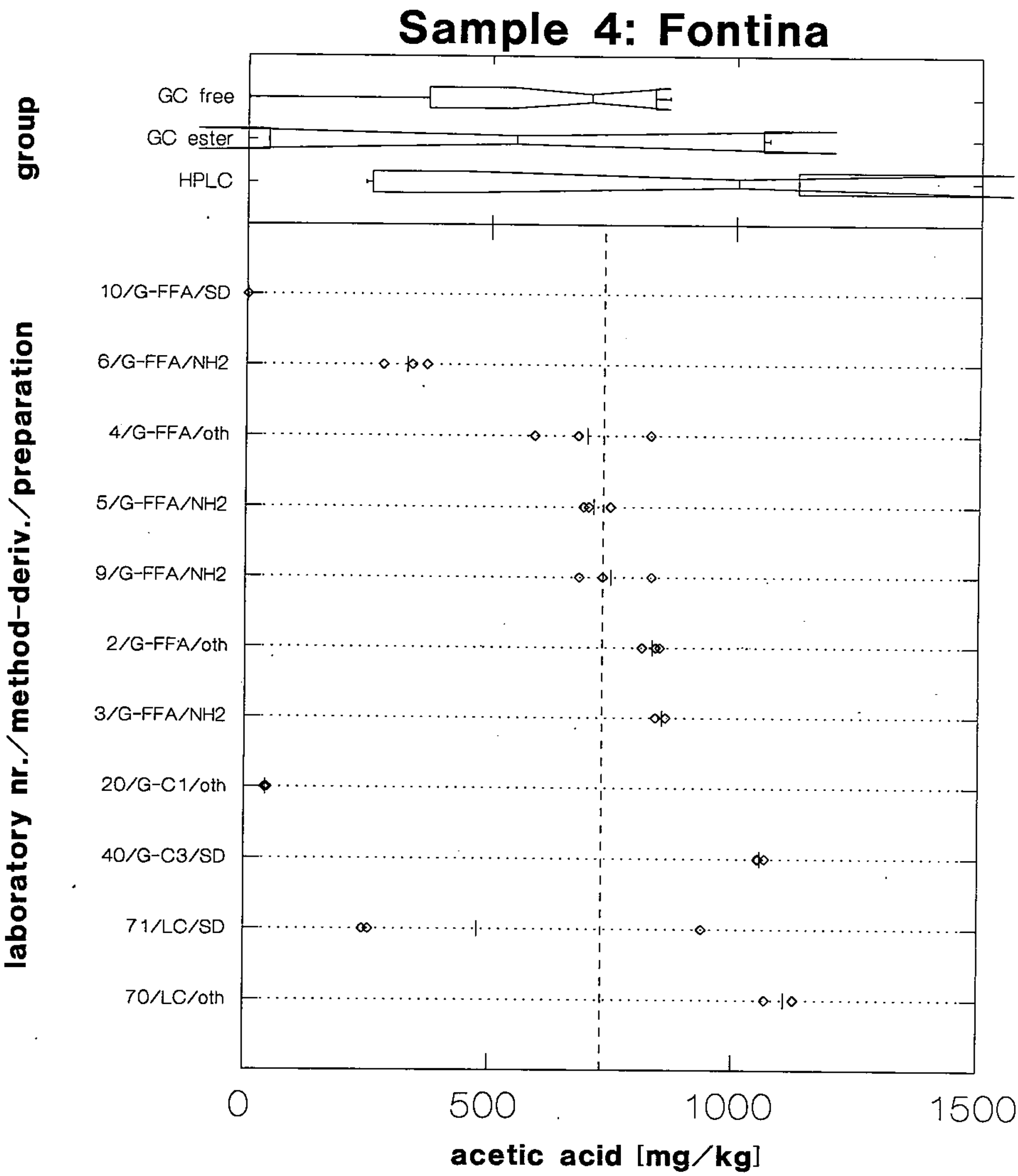


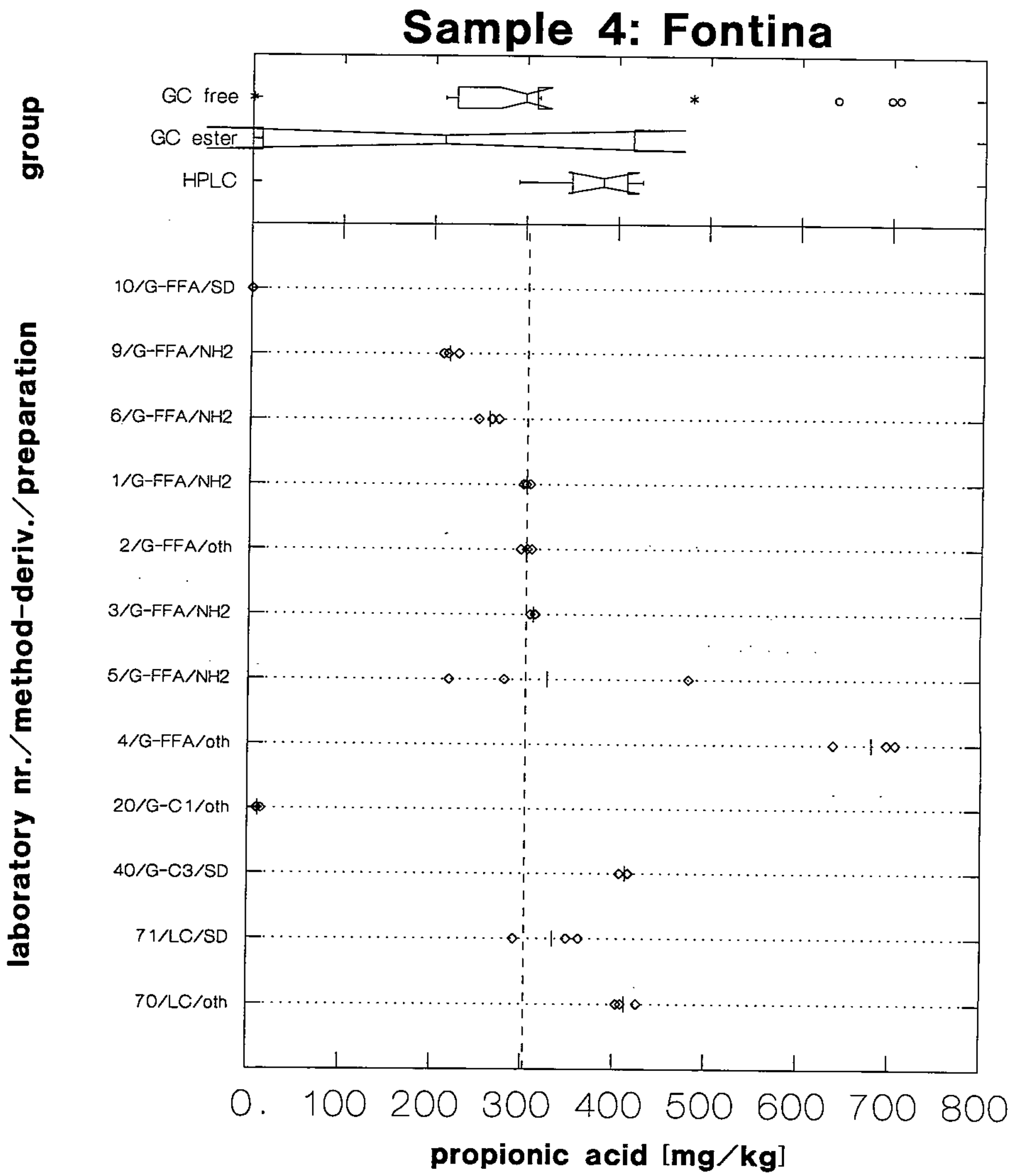
Sample 3: Emmental processed

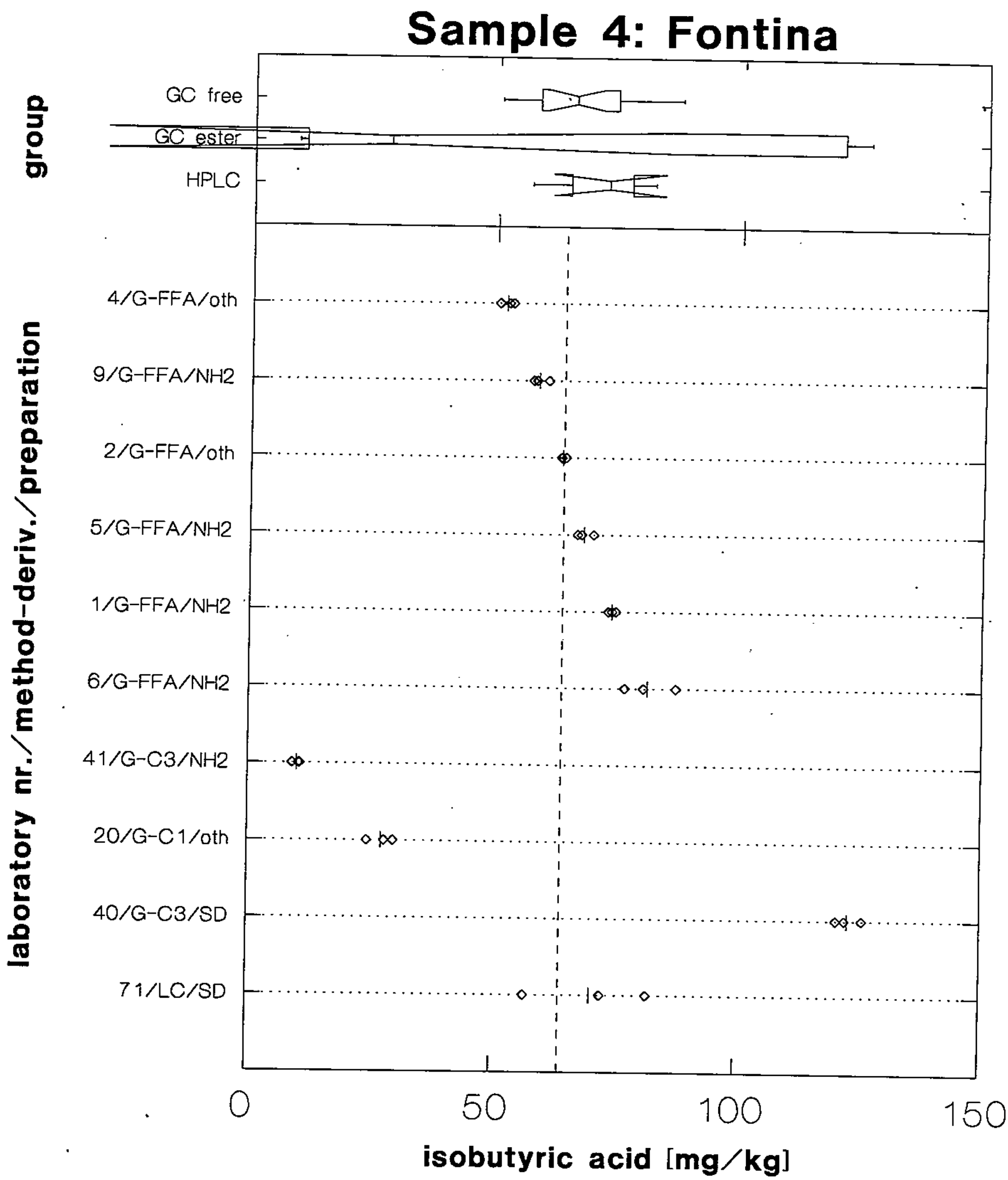


Sample 4: Fontina

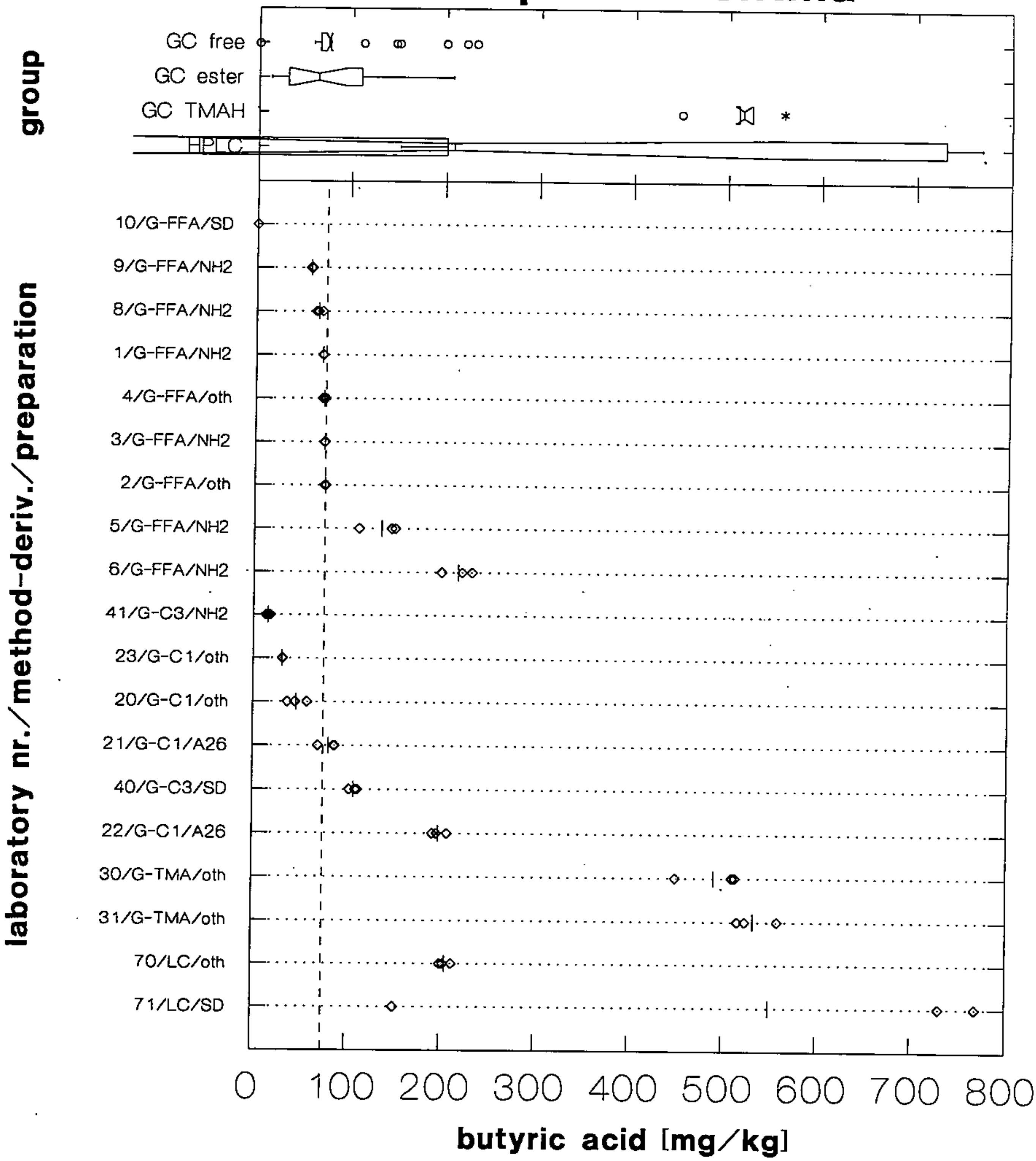



Sample 4: Fontina
group
GC free
GC ester
HPLC
laboratory nr./method-deriv./preparation
10/G-FFA/SD
6/G-FFA/NH2
4/G-FFA/oth
5/G-FFA/NH2
9/G-FFA/NH2
2/G-FFA/oth
3/G-FFA/NH2
20/G-C1/oth
40/G-C3/SD
71/LC/SD
70/LC/oth
0
500
1000
1500
acetic acid [mg/kg]

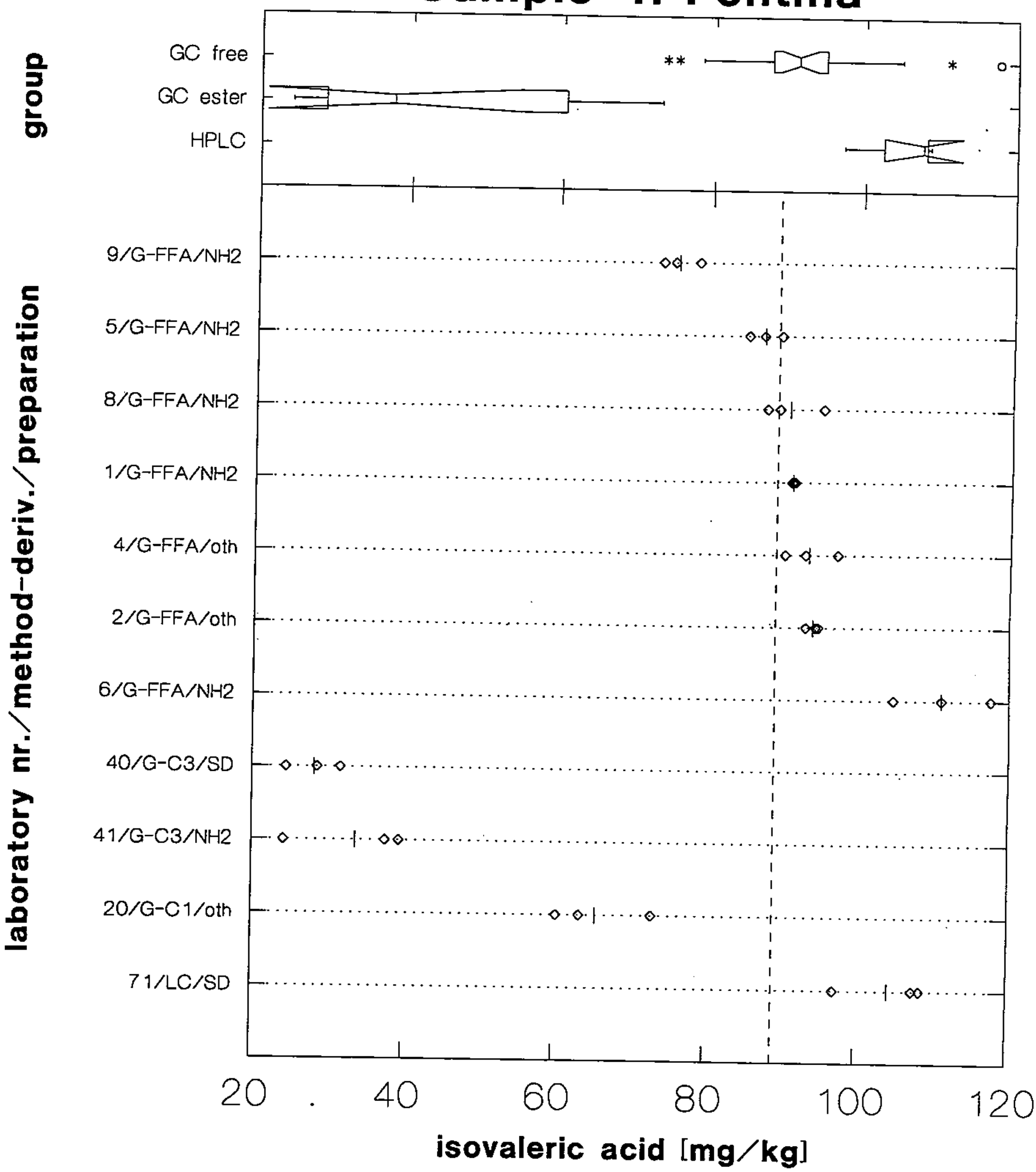

Sample 4: Fontina
group
GC free
GC ester
HPLC
laboratory nr./method-deriv./preparation
10/G-FFA/SD
9/G-FFA/NH2
6/G-FFA/NH2
1/G-FFA/NH2
2/G-FFA/oth
3/G-FFA/NH2
5/G-FFA/NH2
4/G-FFA/oth
20/G-C1/oth
40/G-C3/SD
71/LC/SD
70/LC/oth
0. 100 200 300 400 500 600 700 800
propionic acid [mg/kg]


Sample 4: Fontina
group
GC free
GC ester
HPLC
laboratory nr./method-deriv./preparation
4/G-FFA/oth
9/G-FFA/NH2
2/G-FFA/oth
5/G-FFA/NH2
1/G-FFA/NH2
6/G-FFA/NH2
41/G-C3/NH2
20/G-C1/oth
40/G-C3/SD
71/LC/SD
0
50
100
150
isobutyric acid [mg/kg]

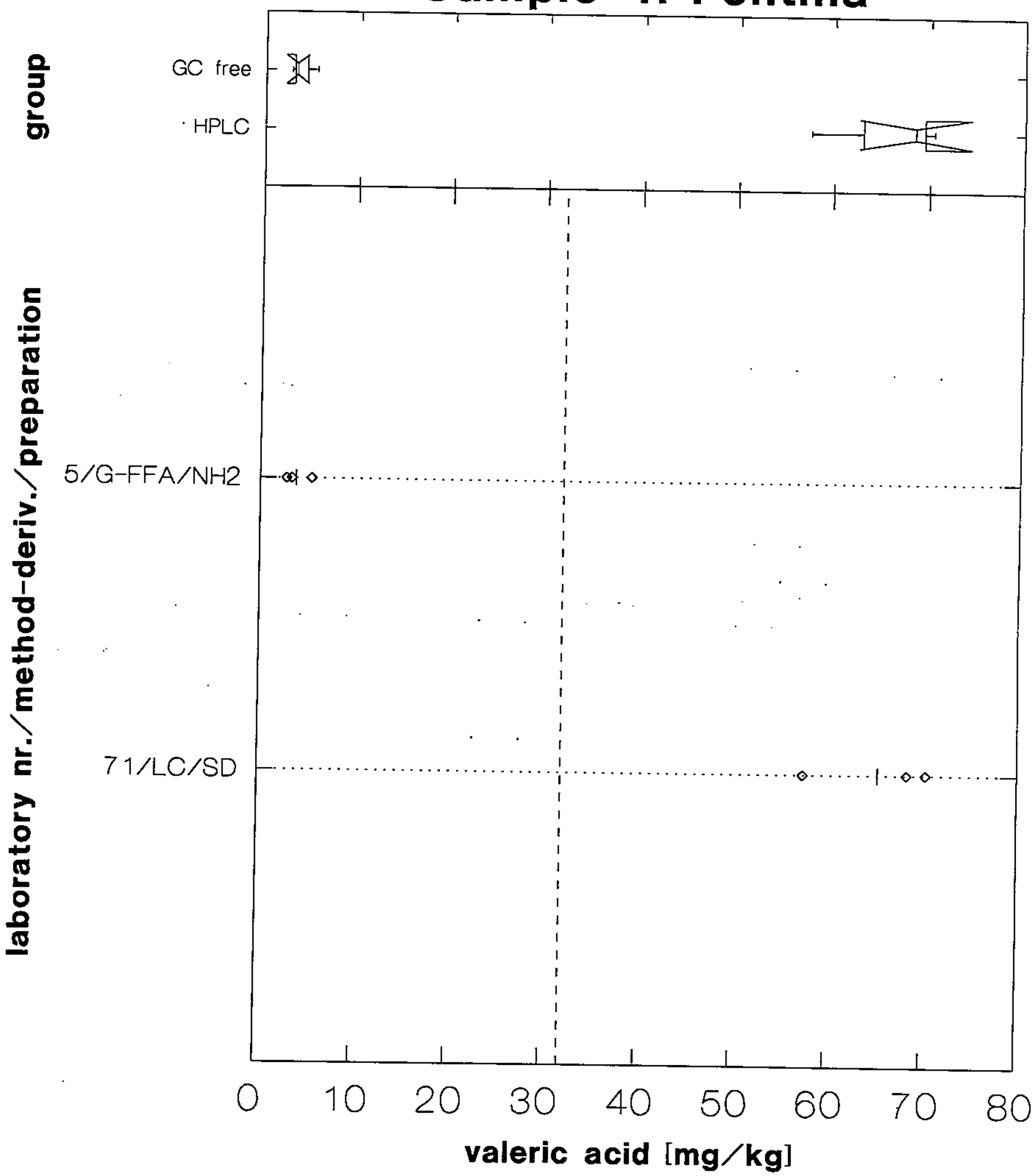
Sample 4: Fontina

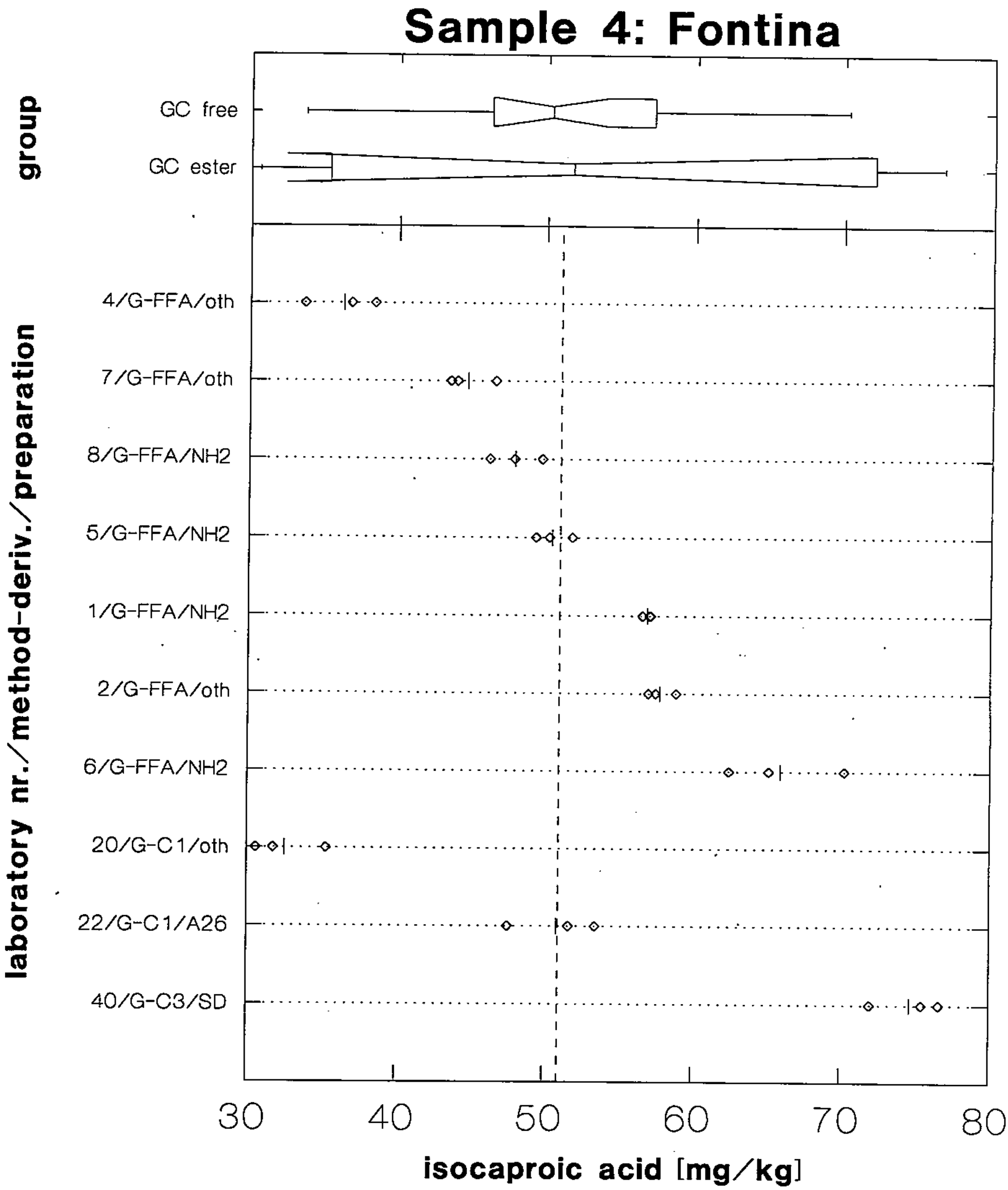


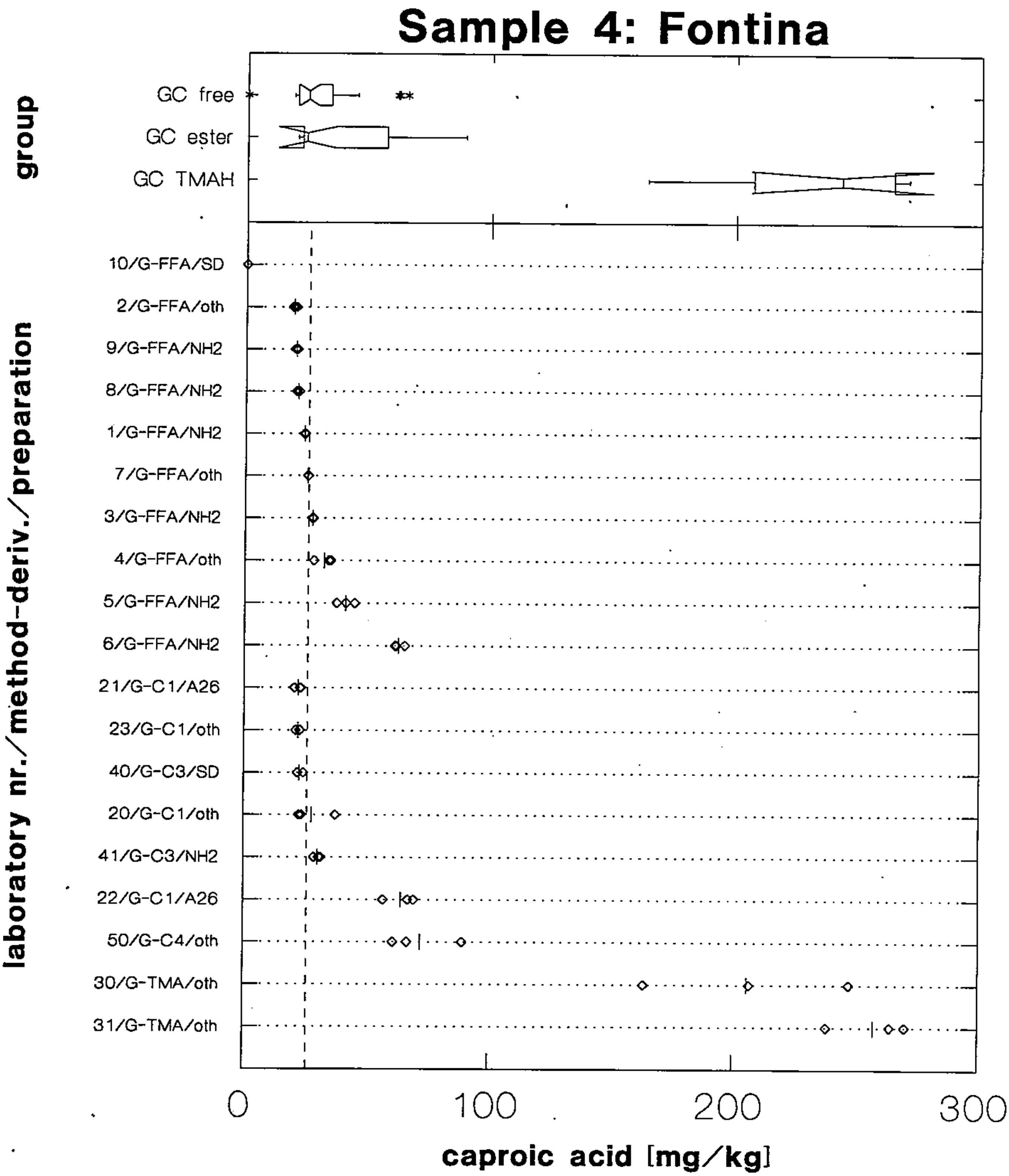
Sample 4: Fontina

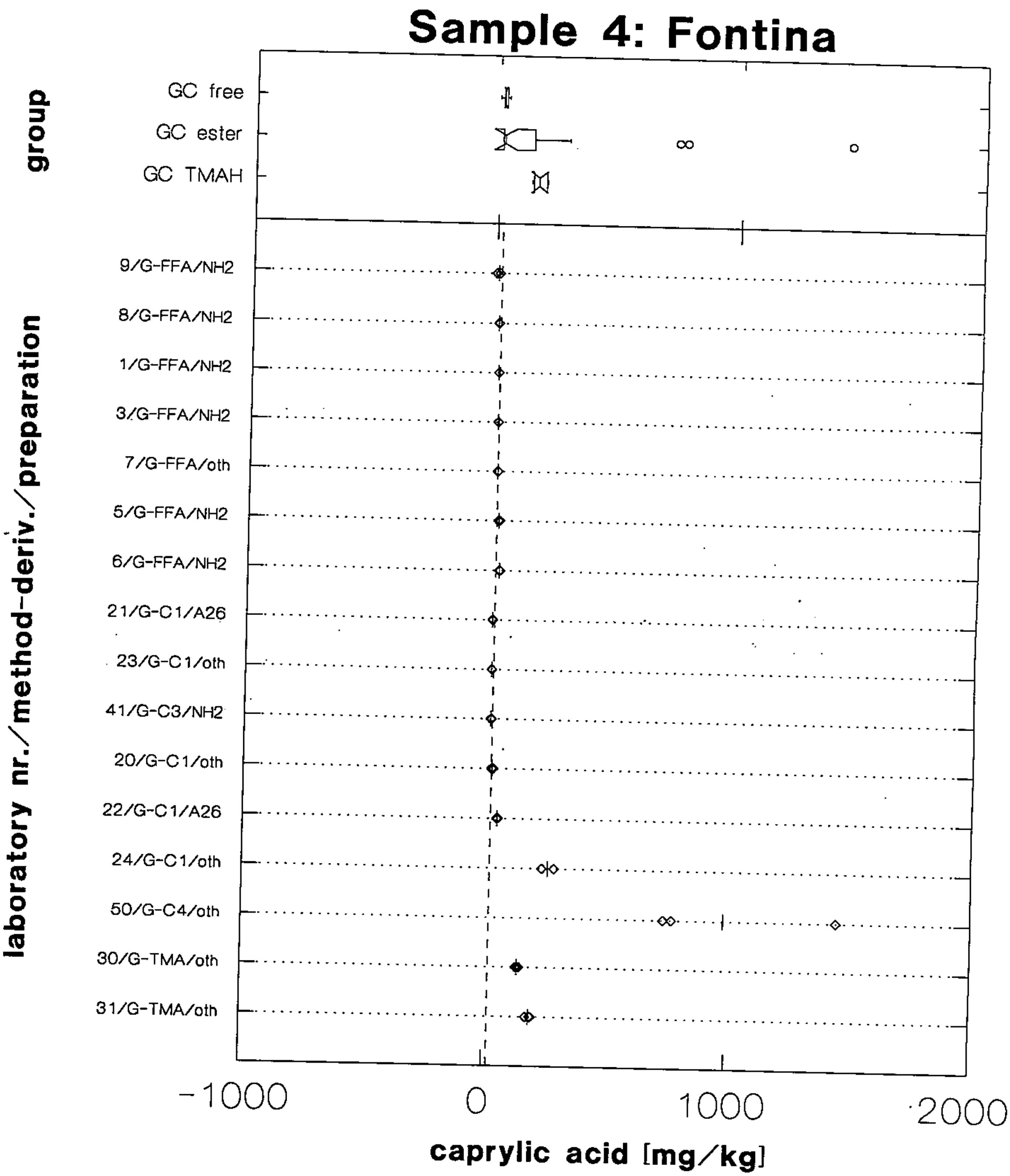


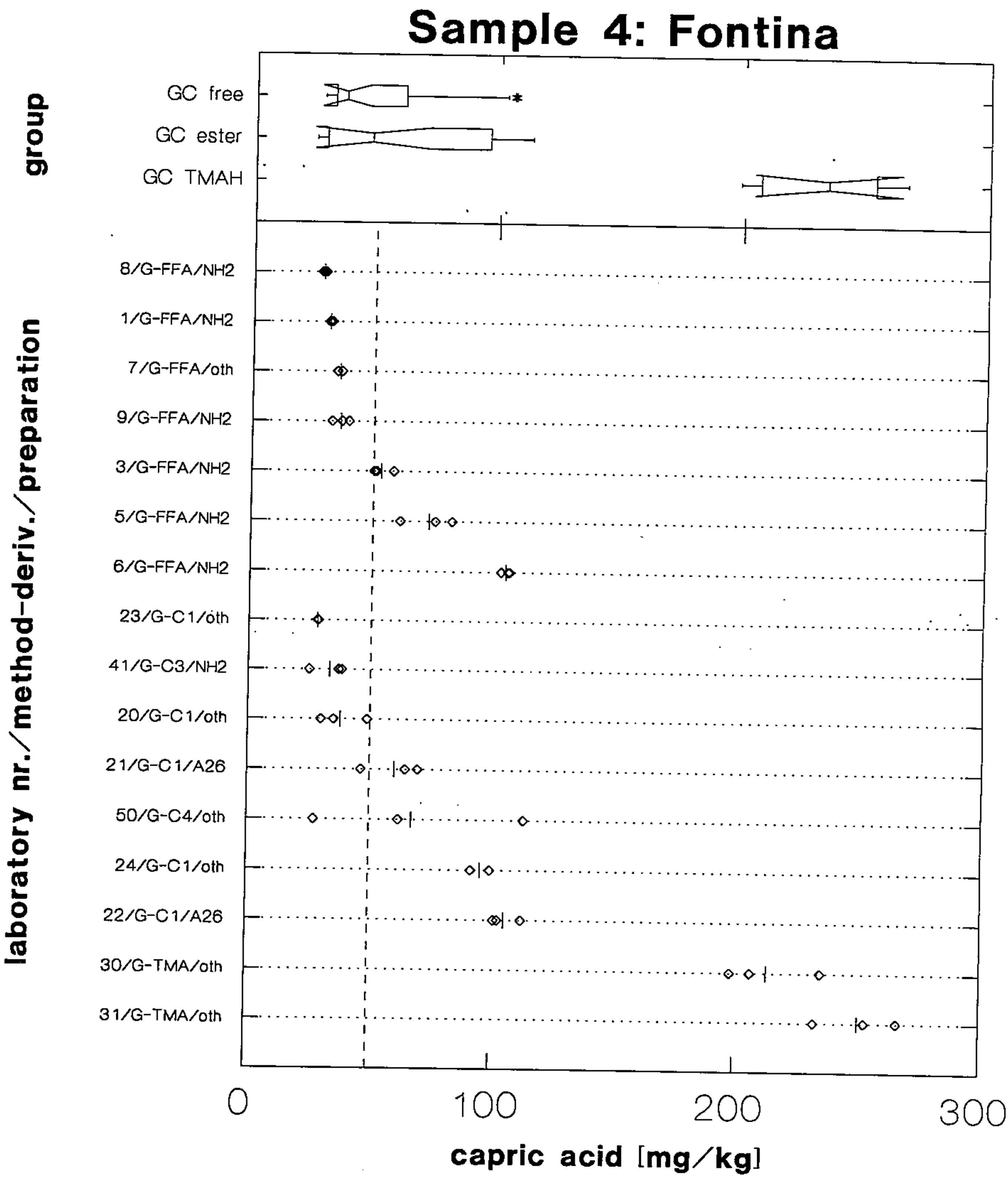
Sample 4: Fontina



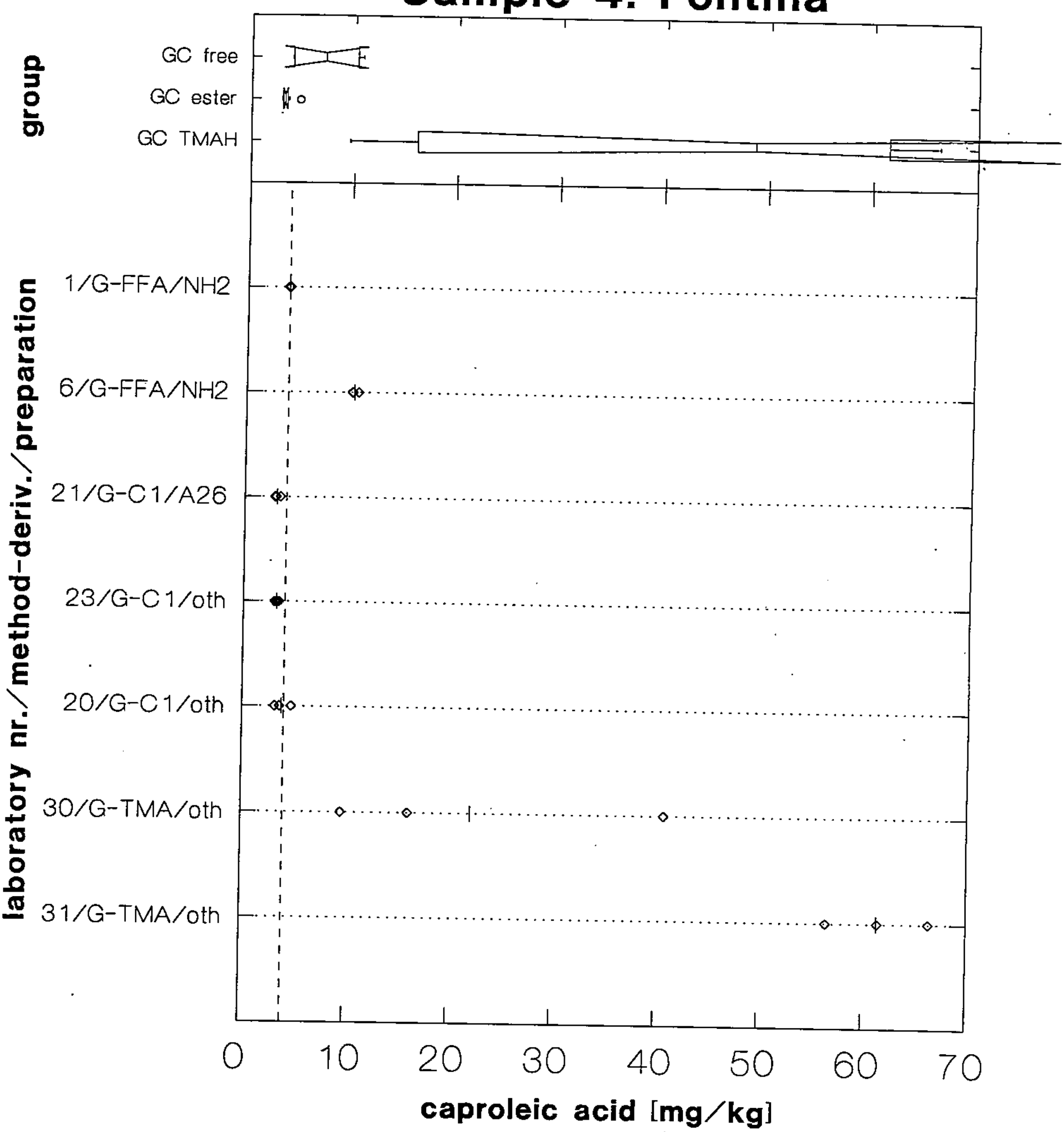

Sample 4: Fontina
group
GC free
GC ester
laboratory nr./method-deriv./preparation
4/G-FFA/oth
7/G-FFA/oth
8/G-FFA/NH2
5/G-FFA/NH2
1/G-FFA/NH2
2/G-FFA/oth
6/G-FFA/NH2
20/G-C1/oth
22/G-C1/A26
40/G-C3/SD
30
40
50
60
70
80
isocaproic acid [mg/kg]


Sample 4: Fontina
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
10/G-FFA/SD
2/G-FFA/oth
9/G-FFA/NH2
8/G-FFA/NH2
1/G-FFA/NH2
7/G-FFA/oth
3/G-FFA/NH2
4/G-FFA/oth
5/G-FFA/NH2
6/G-FFA/NH2
21/G-C1/A26
23/G-C1/oth
40/G-C3/SD
20/G-C1/oth
41/G-C3/NH2
22/G-C1/A26
50/G-C4/oth
30/G-TMA/oth
31/G-TMA/oth
0
100
200
300
caproic acid [mg/kg]

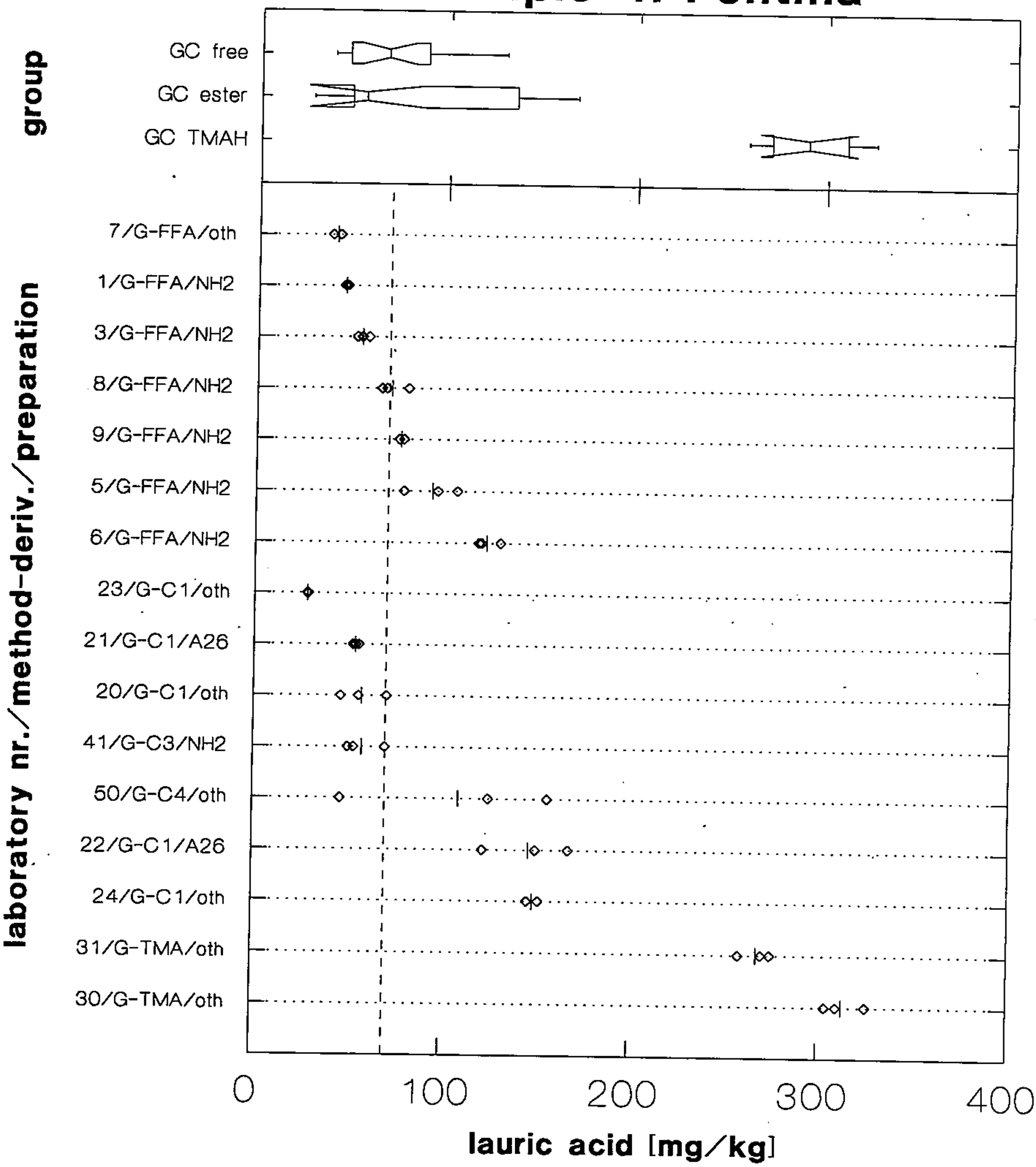

Sample 4: Fontina
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
9/G-FFA/NH2
8/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
7/G-FFA/oth
5/G-FFA/NH2
6/G-FFA/NH2
21/G-C1/A26
23/G-C1/oth
41/G-C3/NH2
20/G-C1/oth
22/G-C1/A26
24/G-C1/oth
50/G-C4/oth
30/G-TMA/oth
31/G-TMA/oth
-1000
0
1000
2000
caprylic acid [mg/kg]

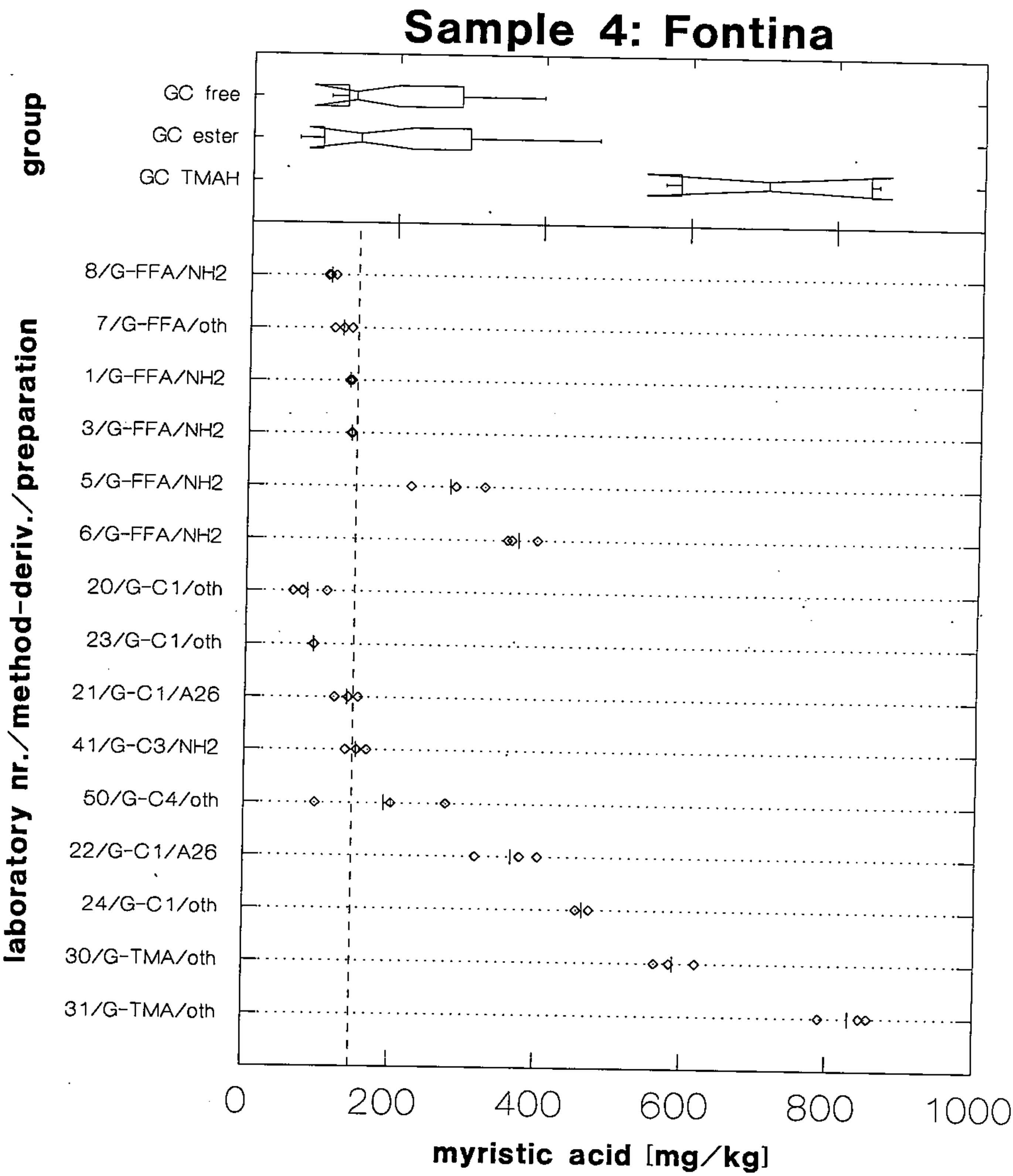

Sample 4: Fontina
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
1/G-FFA/NH2
7/G-FFA/oth
9/G-FFA/NH2
3/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
23/G-C1/oth
41/G-C3/NH2
20/G-C1/oth
21/G-C1/A26
50/G-C4/oth
24/G-C1/oth
22/G-C1/A26
30/G-TMA/oth
31/G-TMA/oth
0
100
200
300
capric acid [mg/kg]

Sample 4: Fontina

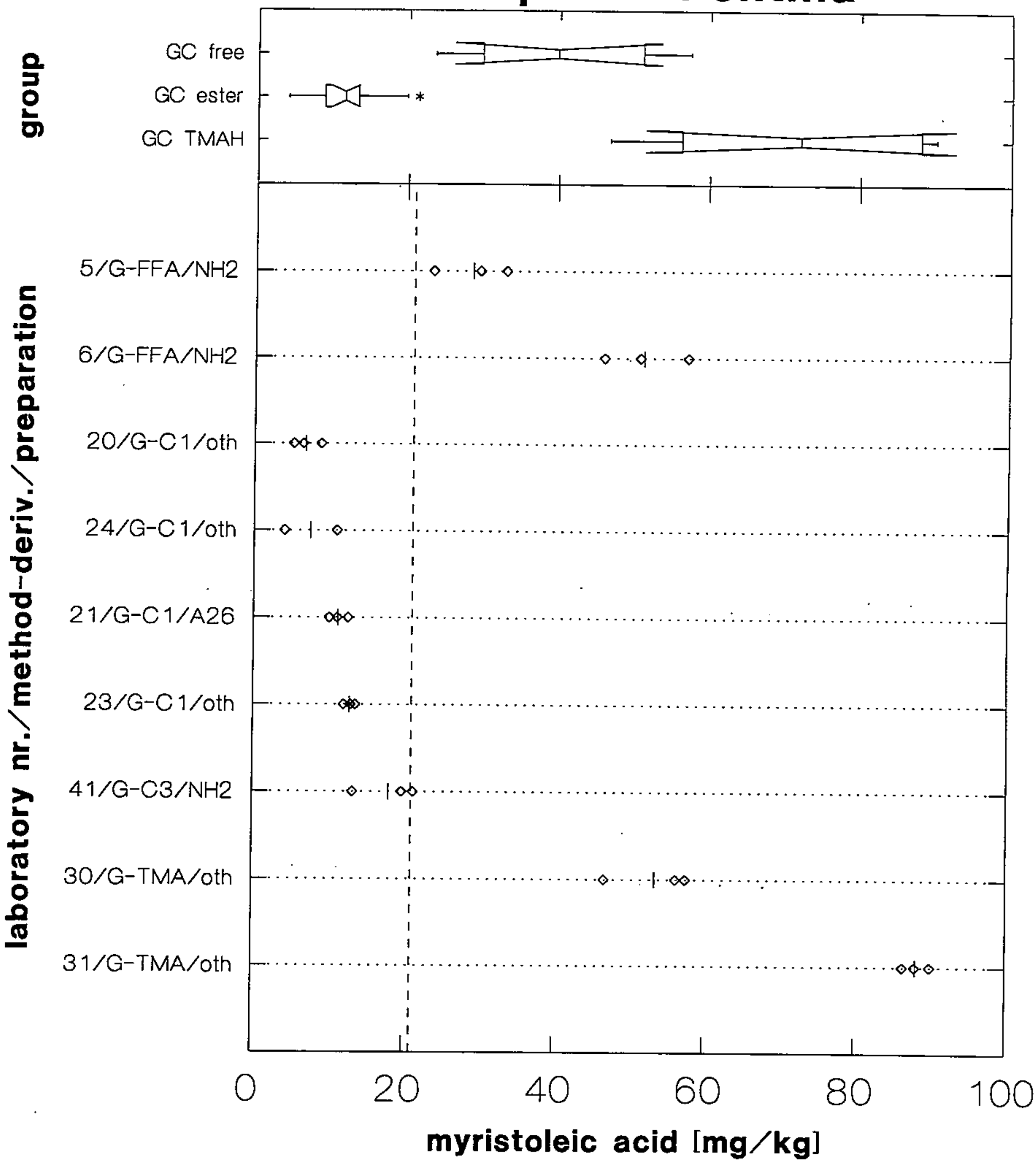


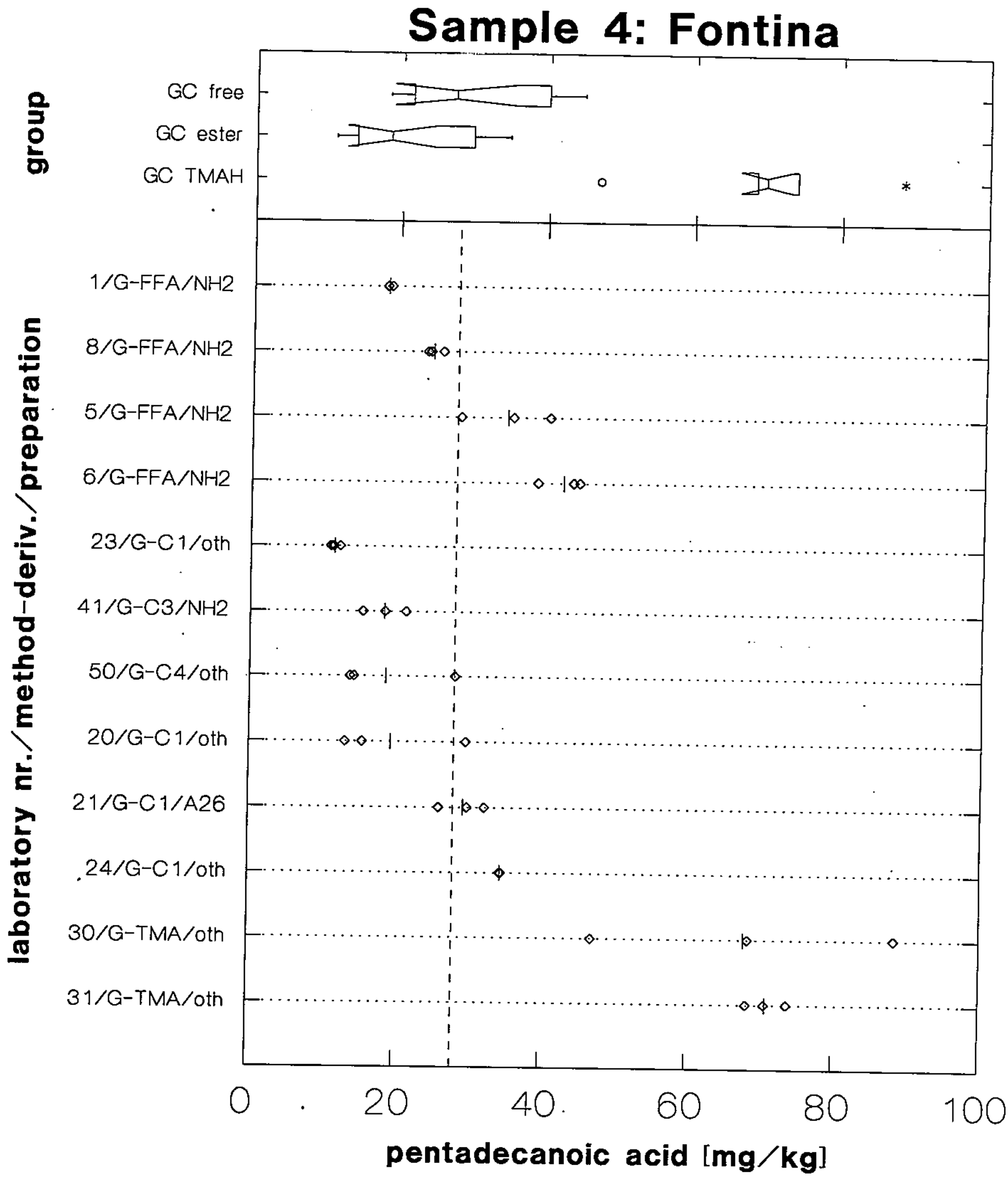
Sample 4: Fontina

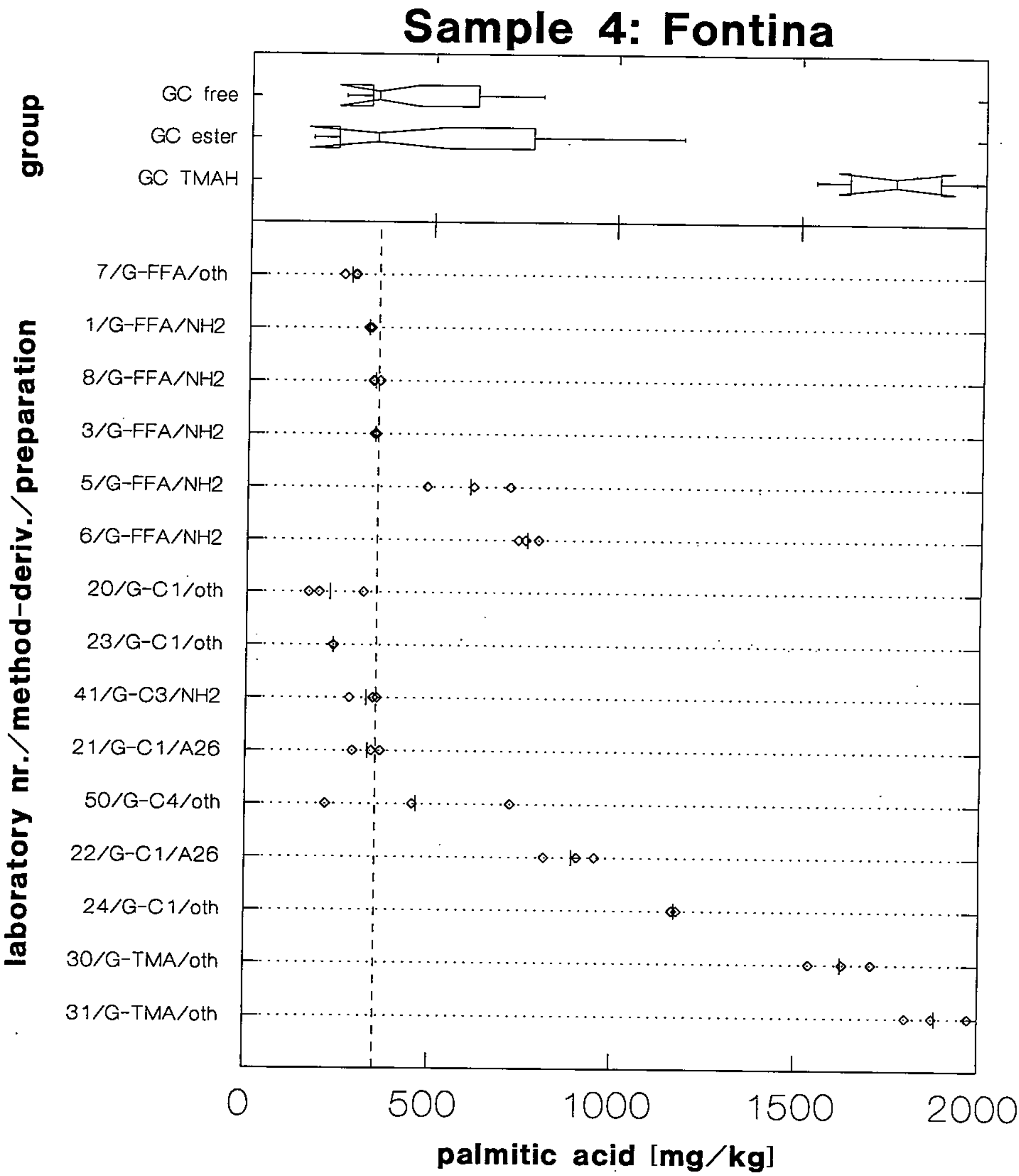



Sample 4: Fontina
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
7/G-FFA/oth
1/G-FFA/NH2
3/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
20/G-C1/oth
23/G-C1/oth
21/G-C1/A26
41/G-C3/NH2
50/G-C4/oth
22/G-C1/A26
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
200
400
600
800
1000
myristic acid [mg/kg]

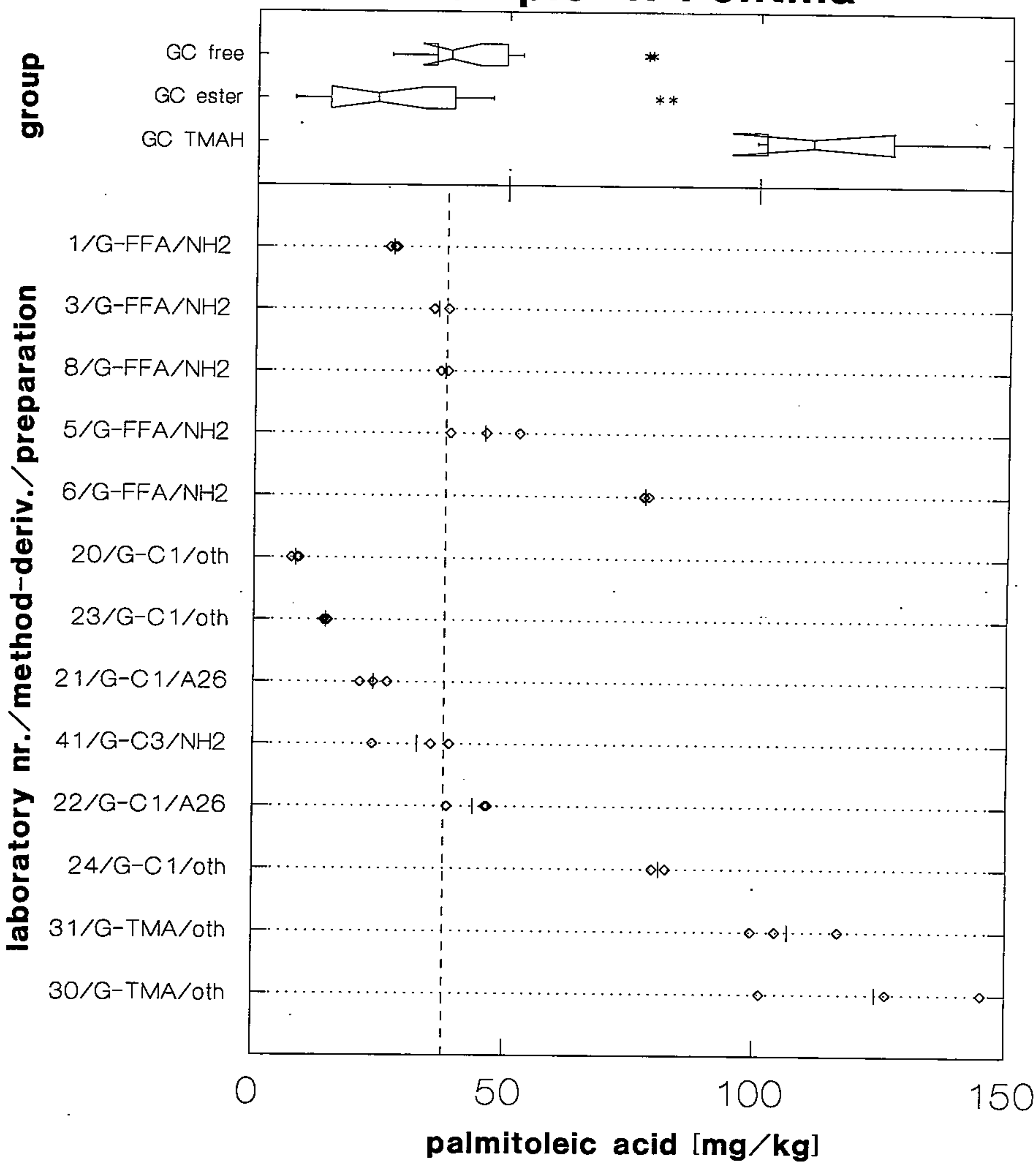
Sample 4: Fontina



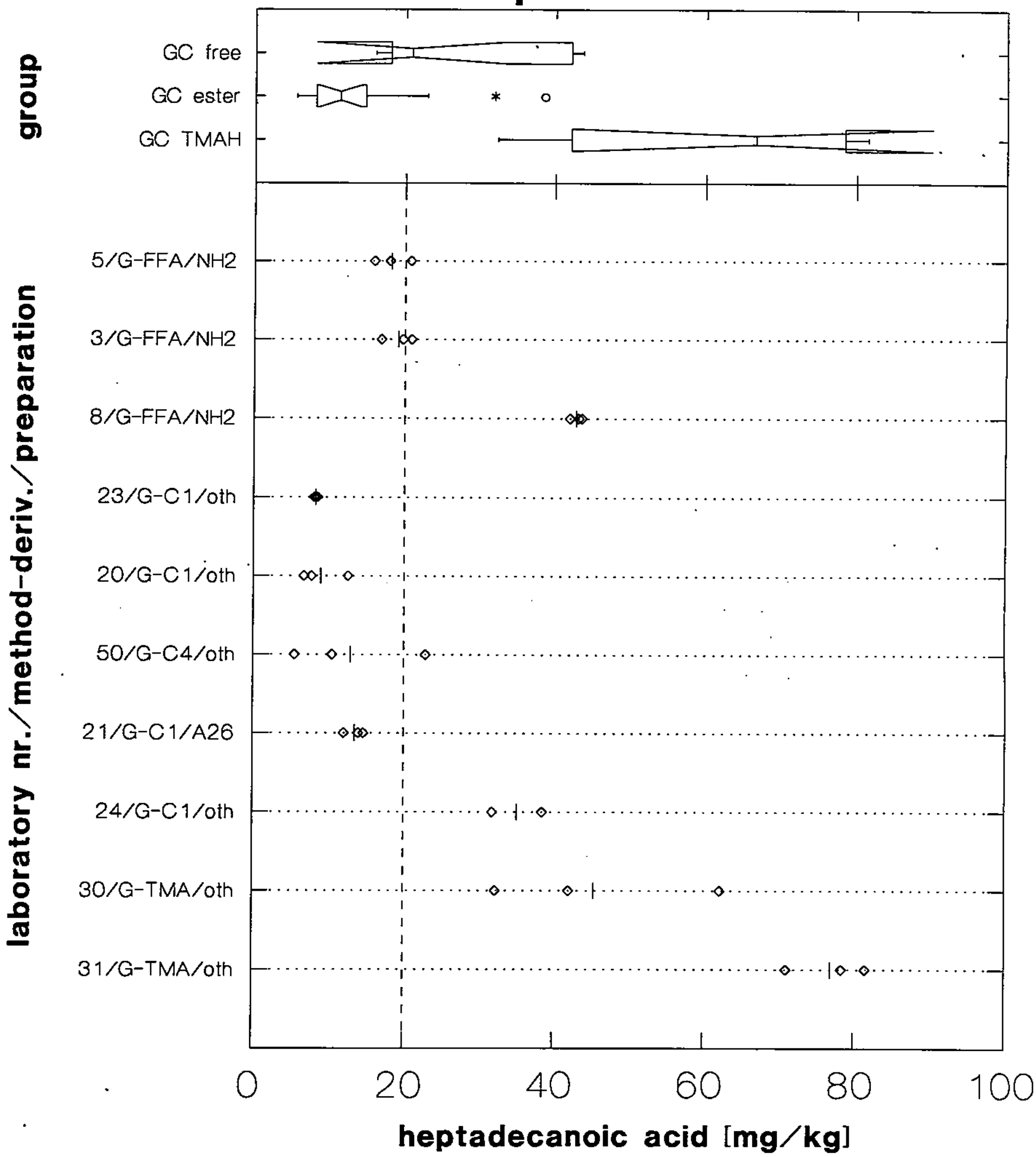

Sample 4: Fontina
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
1/G-FFA/NH2
8/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
23/G-C1/oth
41/G-C3/NH2
50/G-C4/oth
20/G-C1/oth
21/G-C1/A26
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
20
40
60
80
100
pentadecanoic acid [mg/kg]


Sample 4: Fontina
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
7/G-FFA/oth
1/G-FFA/NH2
8/G-FFA/NH2
3/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
20/G-C1/oth
23/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
50/G-C4/oth
22/G-C1/A26
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
500
1000
1500
2000
palmitic acid [mg/kg]

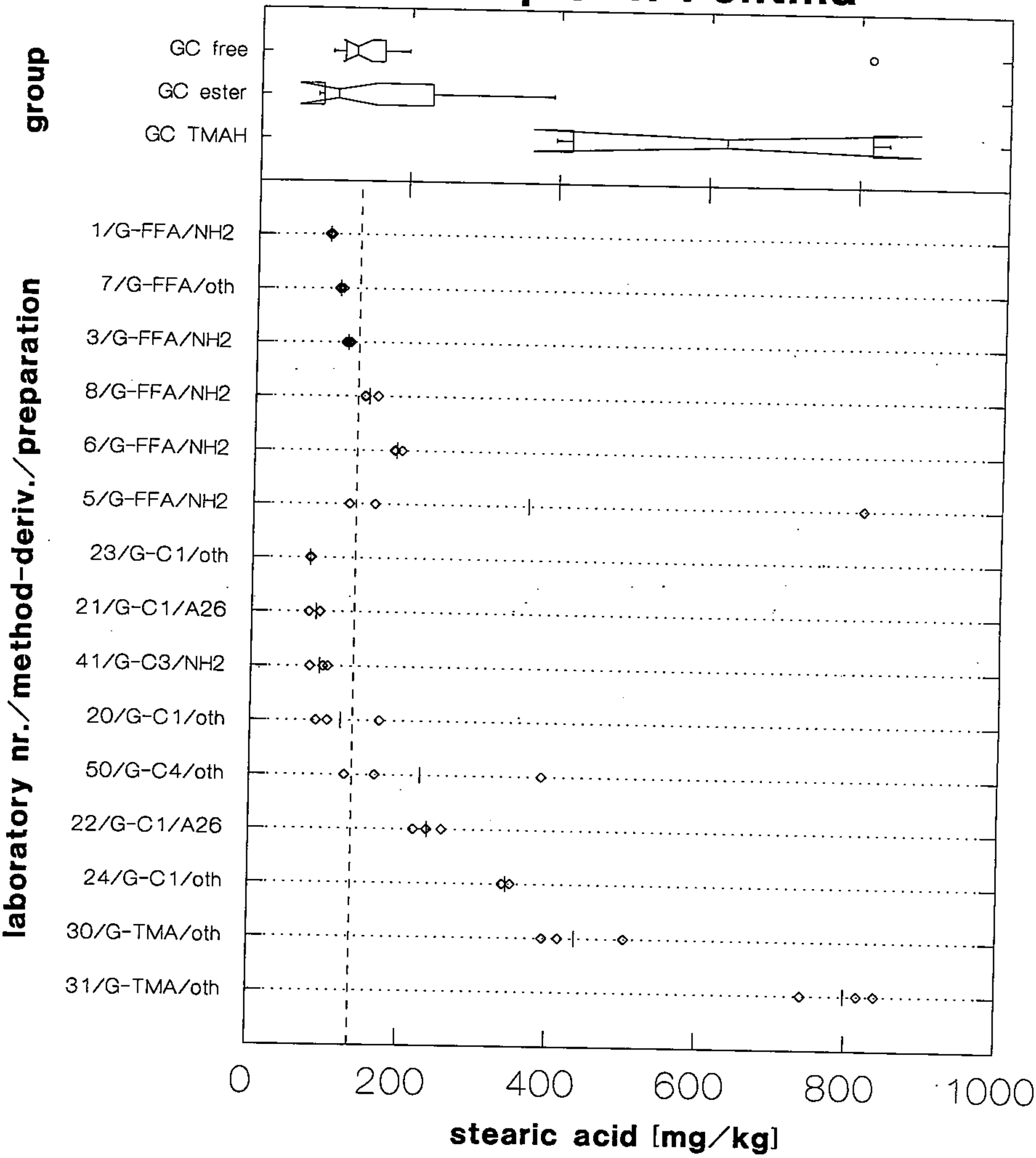
Sample 4: Fontina



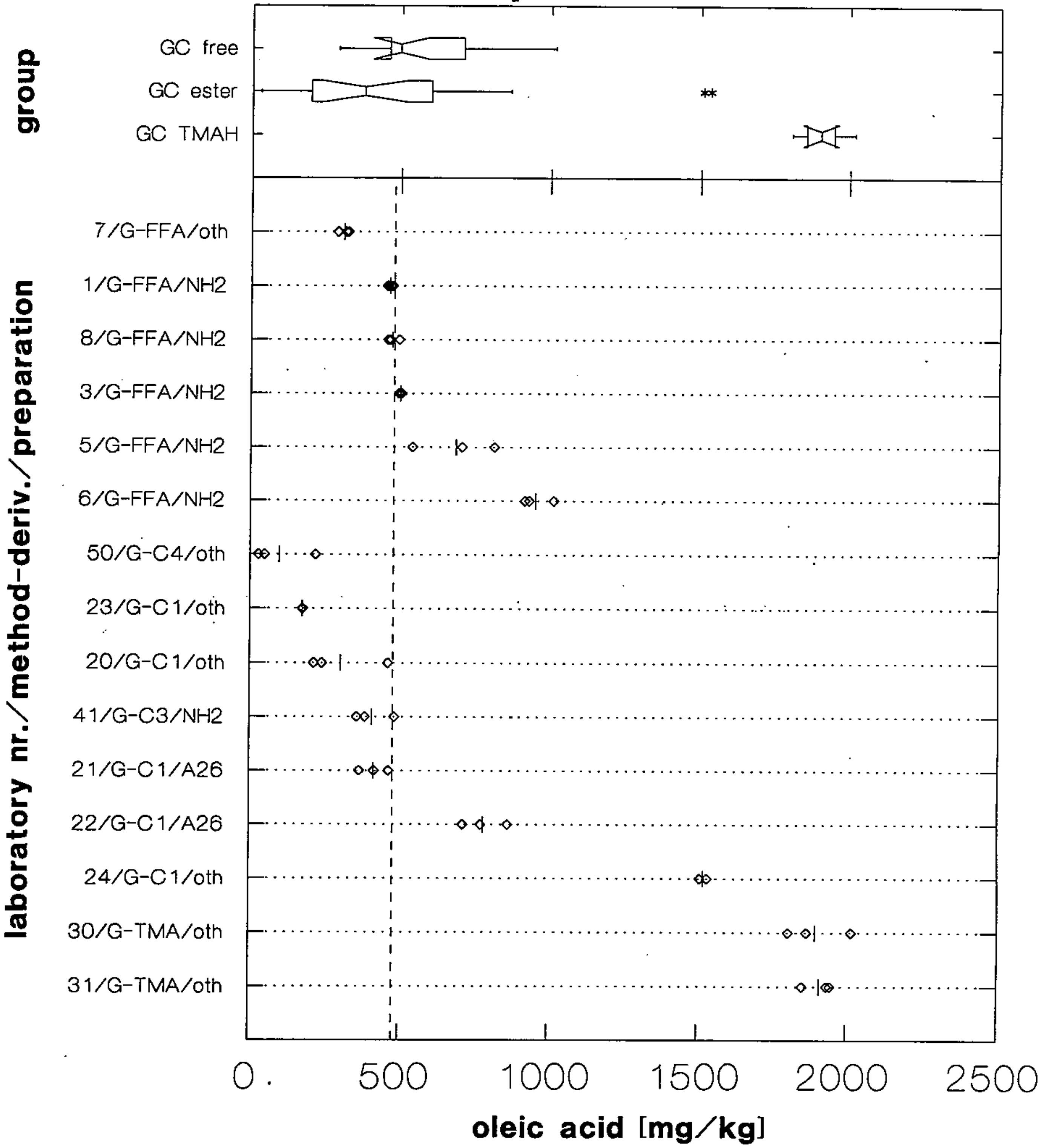
Sample 4: Fontina

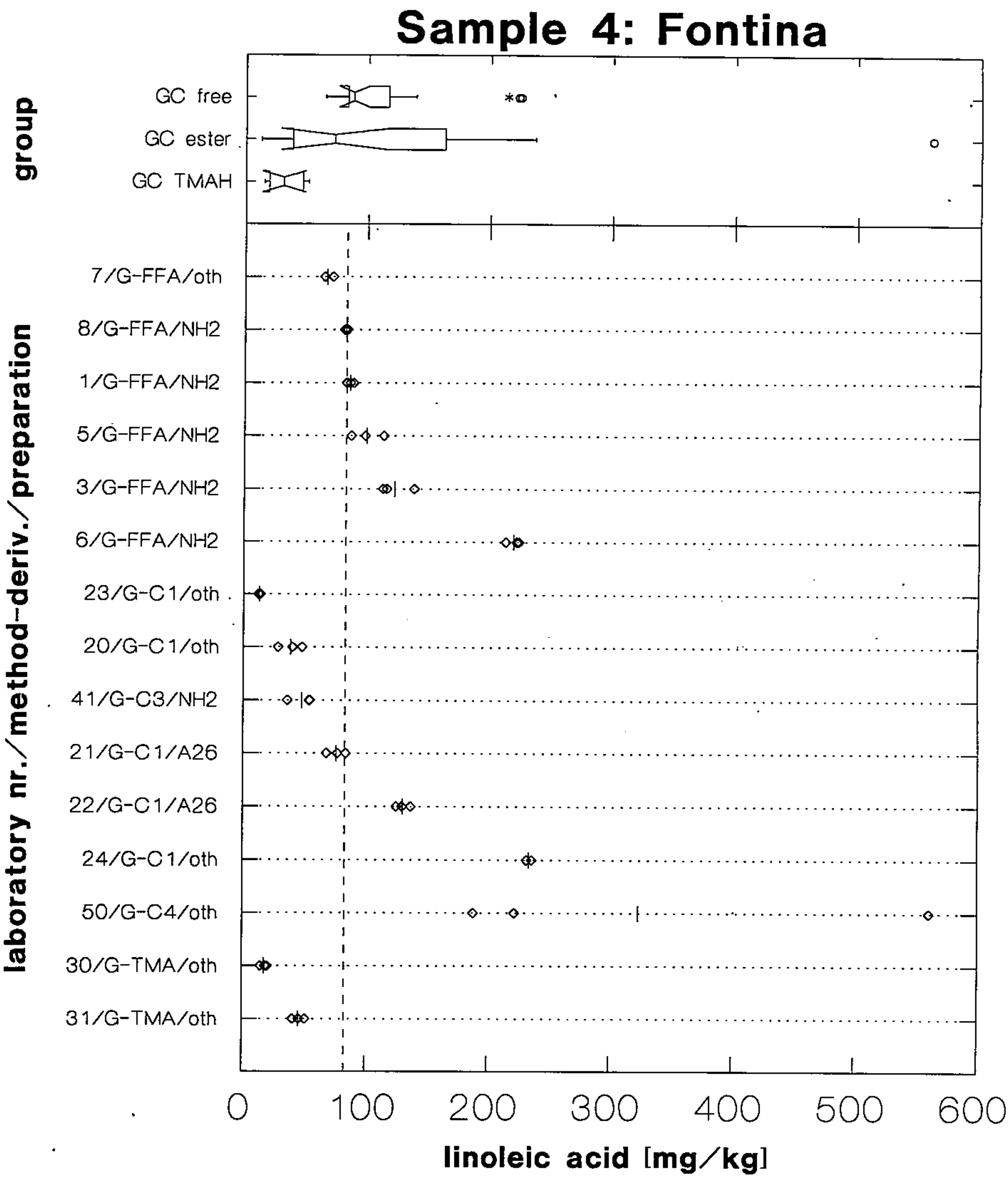


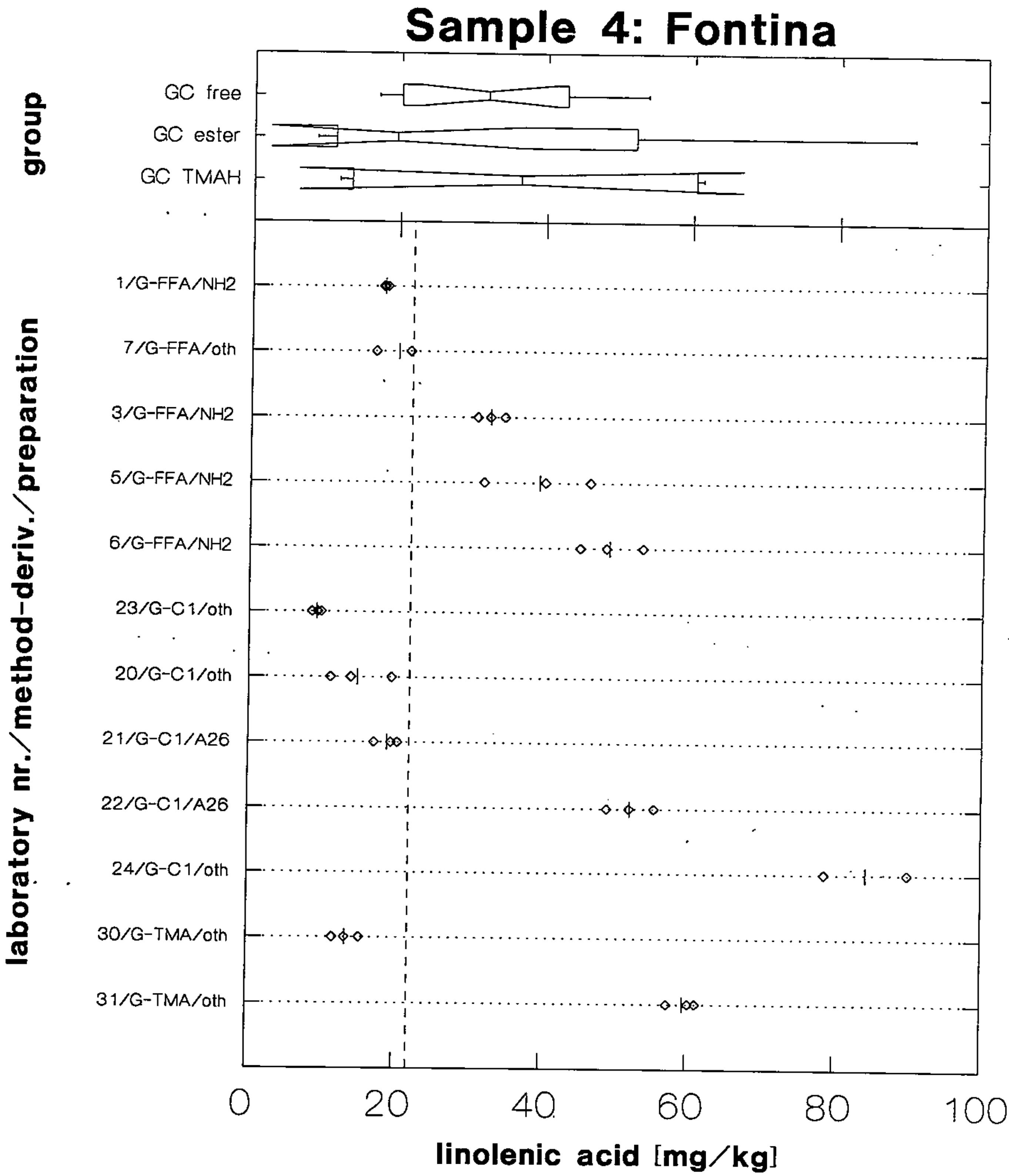
Sample 4: Fontina

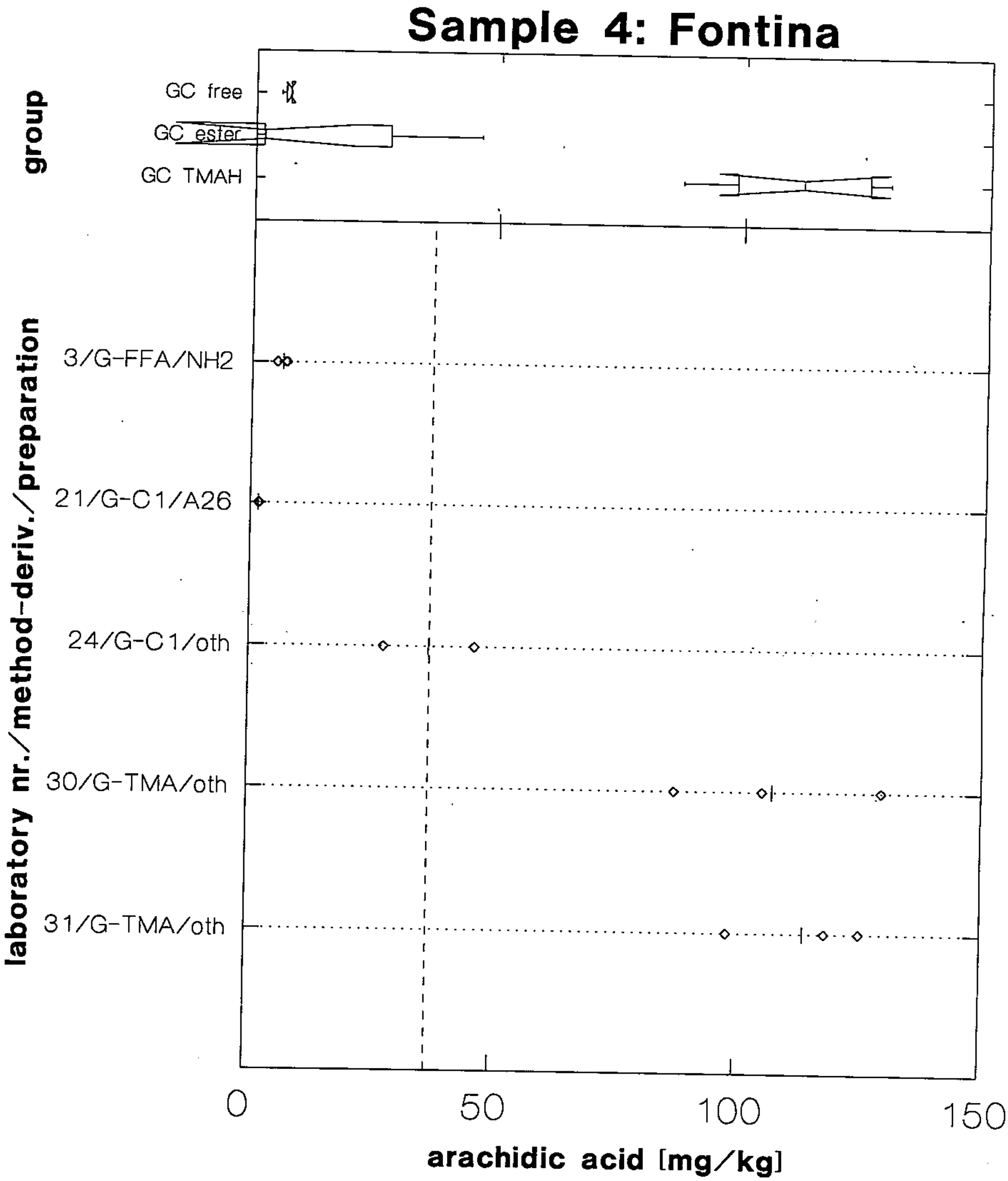


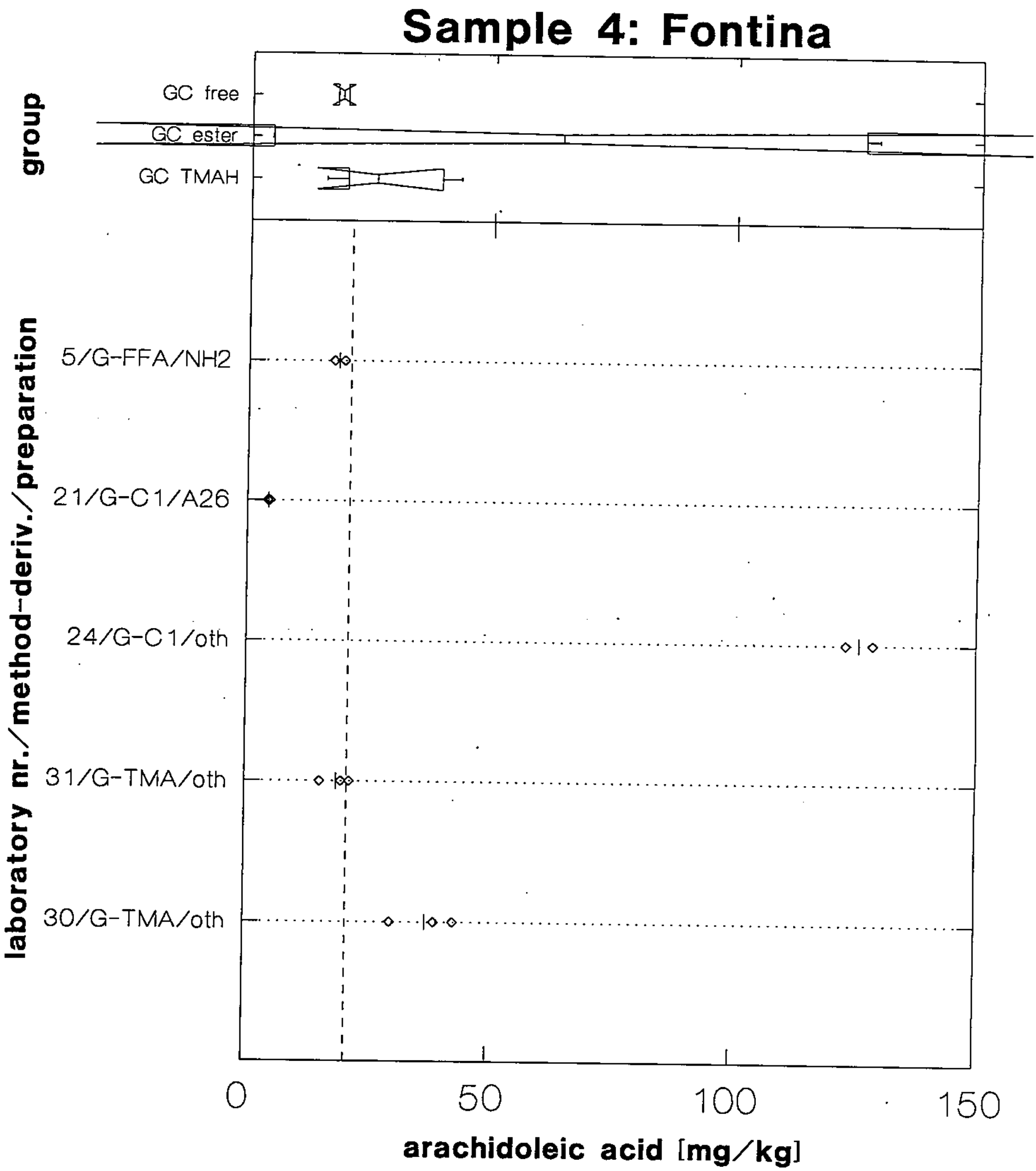
Sample 4: Fontina



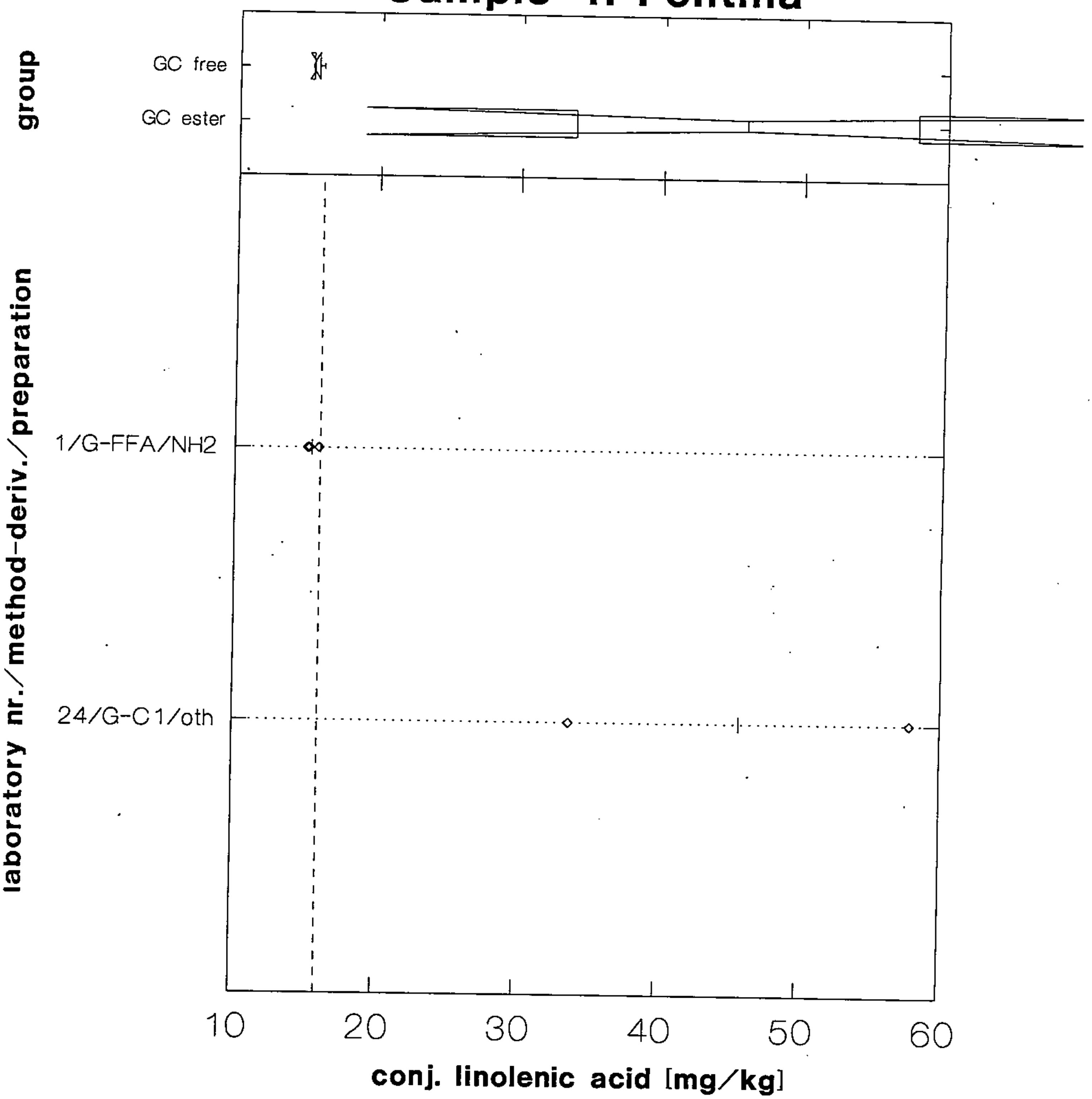

Sample 4: Fontina
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
7/G-FFA/oth
8/G-FFA/NH2
1/G-FFA/NH2
5/G-FFA/NH2
3/G-FFA/NH2
6/G-FFA/NH2
23/G-C1/oth
20/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
22/G-C1/A26
24/G-C1/oth
50/G-C4/oth
30/G-TMA/oth
31/G-TMA/oth
0
100
200
300
400
500
600
linoleic acid [mg/kg]

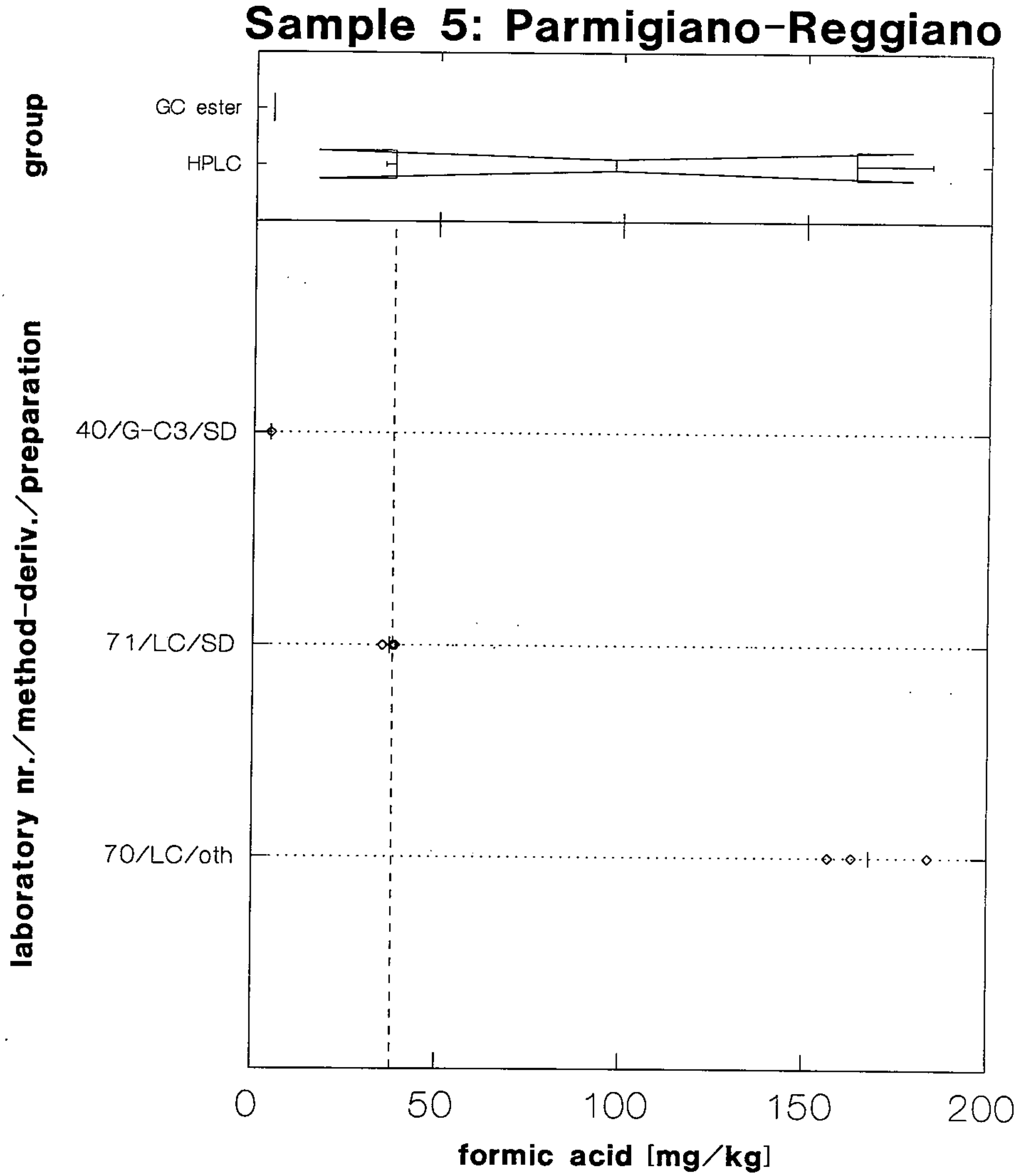

Sample 4: Fontina
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
1/G-FFA/NH2
7/G-FFA/oth
3/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
23/G-C1/oth
20/G-C1/oth
21/G-C1/A26
22/G-C1/A26
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
20
40
60
80
100
linolenic acid [mg/kg]

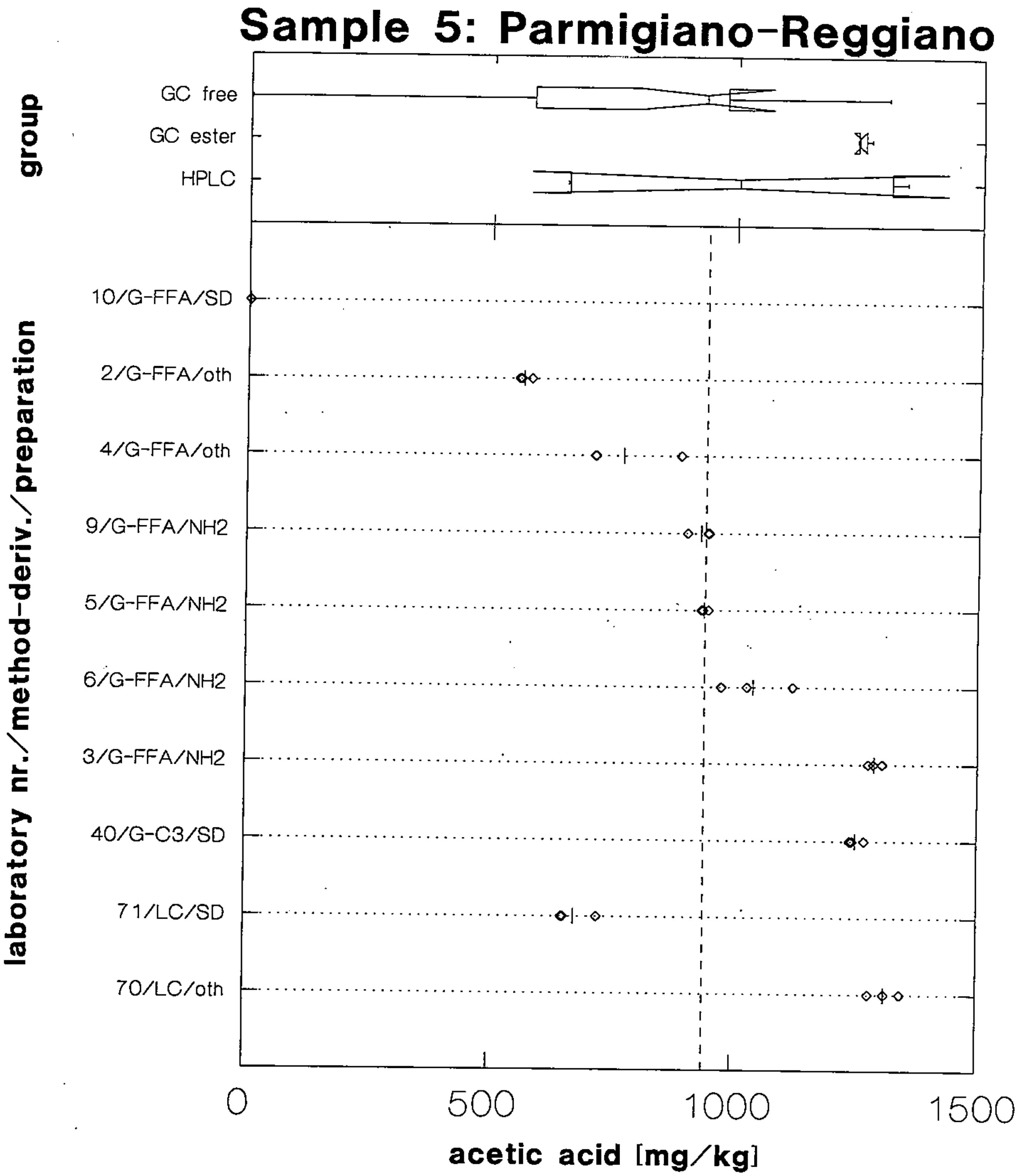

Sample 4: Fontina
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
3/G-FFA/NH2
21/G-C1/A26
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
50
100
150
arachidic acid [mg/kg]

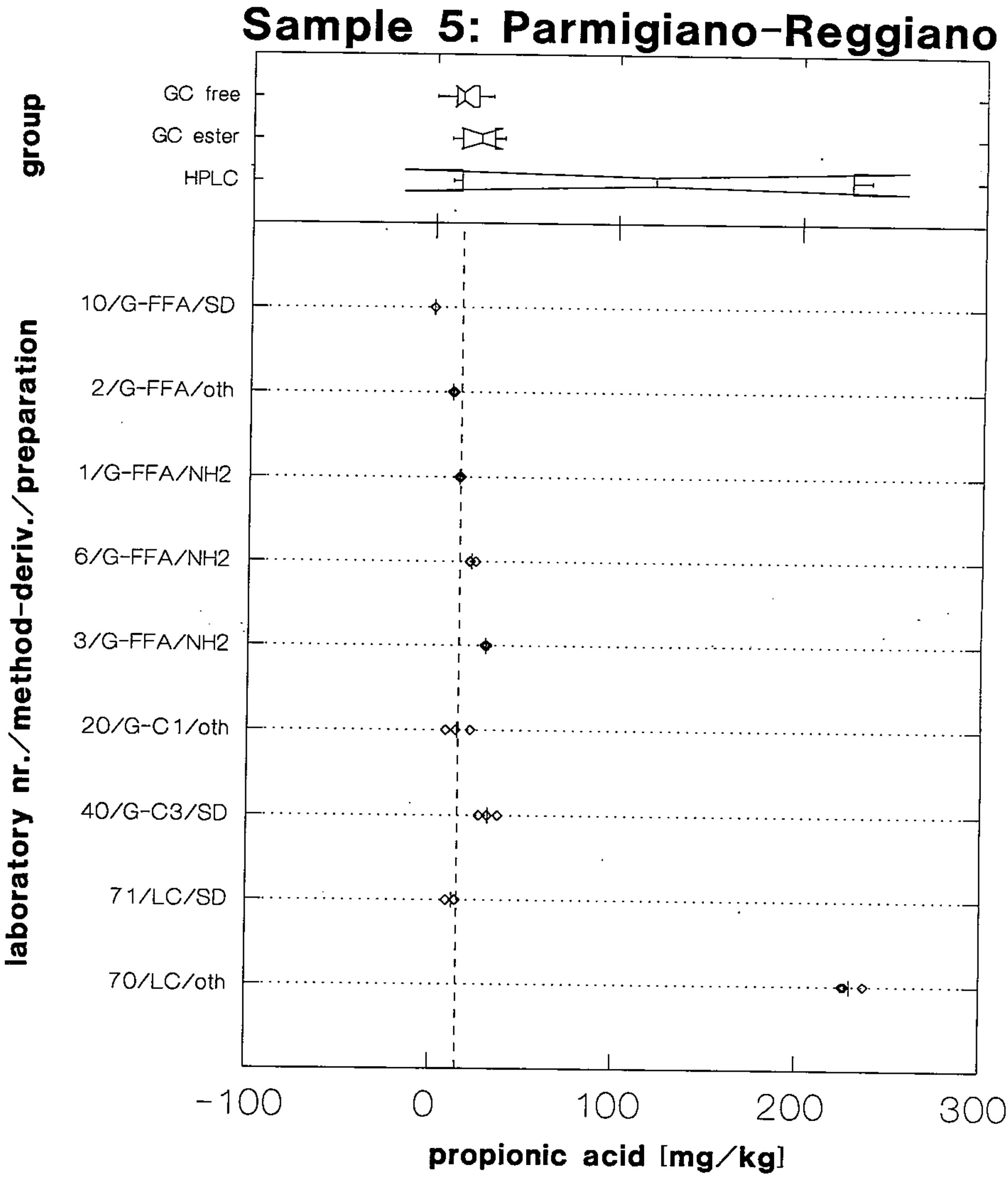

Sample 4: Fontina
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
21/G-C1/A26
24/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
50
100
150
arachidoleic acid [mg/kg]

Sample 4: Fontina

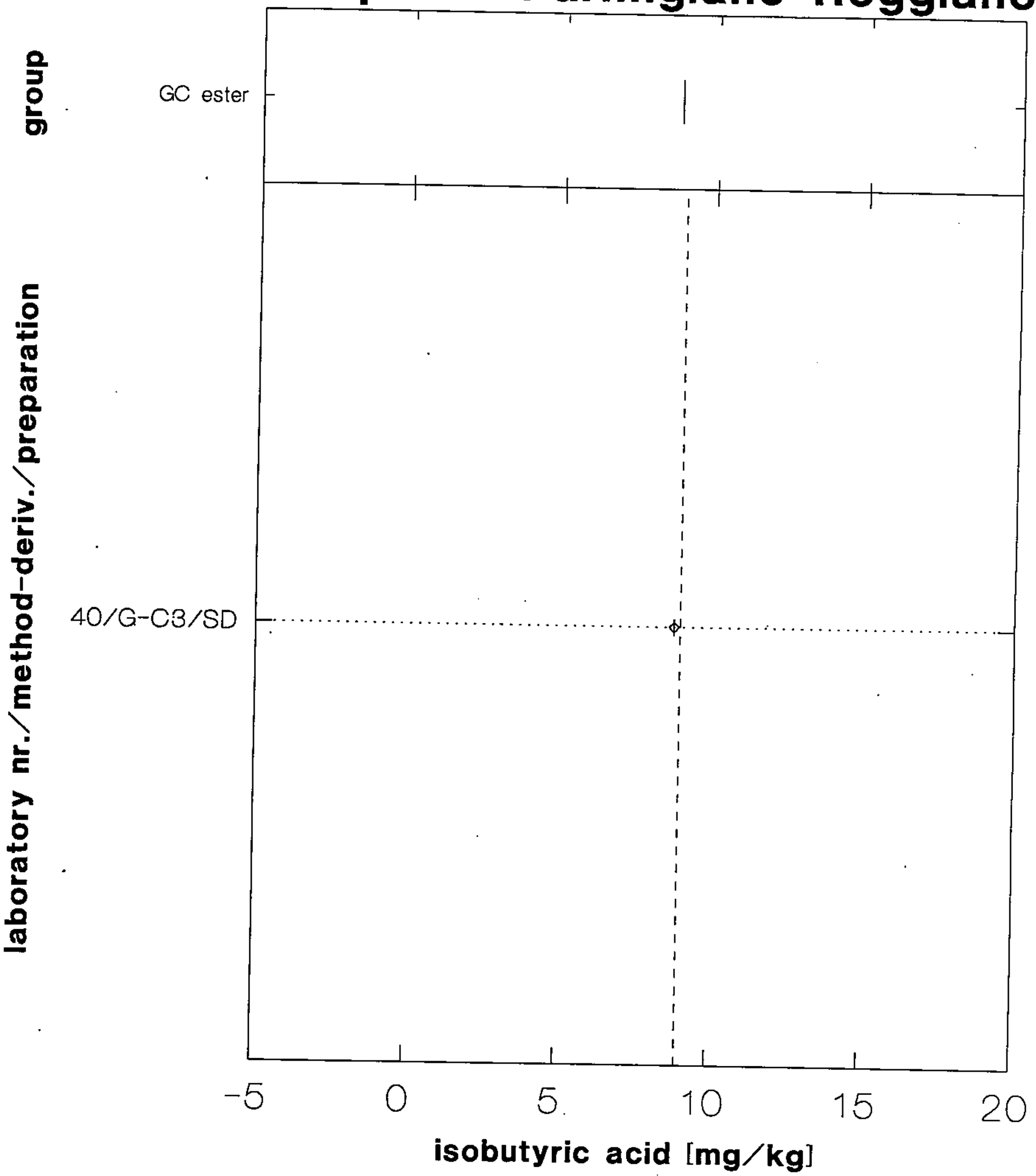



Sample 5: Parmigiano-Reggiano
group
GC ester
HPLC
laboratory nr./method-deriv./preparation
40/G-C3/SD
71/LC/SD
70/LC/oth
0
50
100
150
200
formic acid [mg/kg]

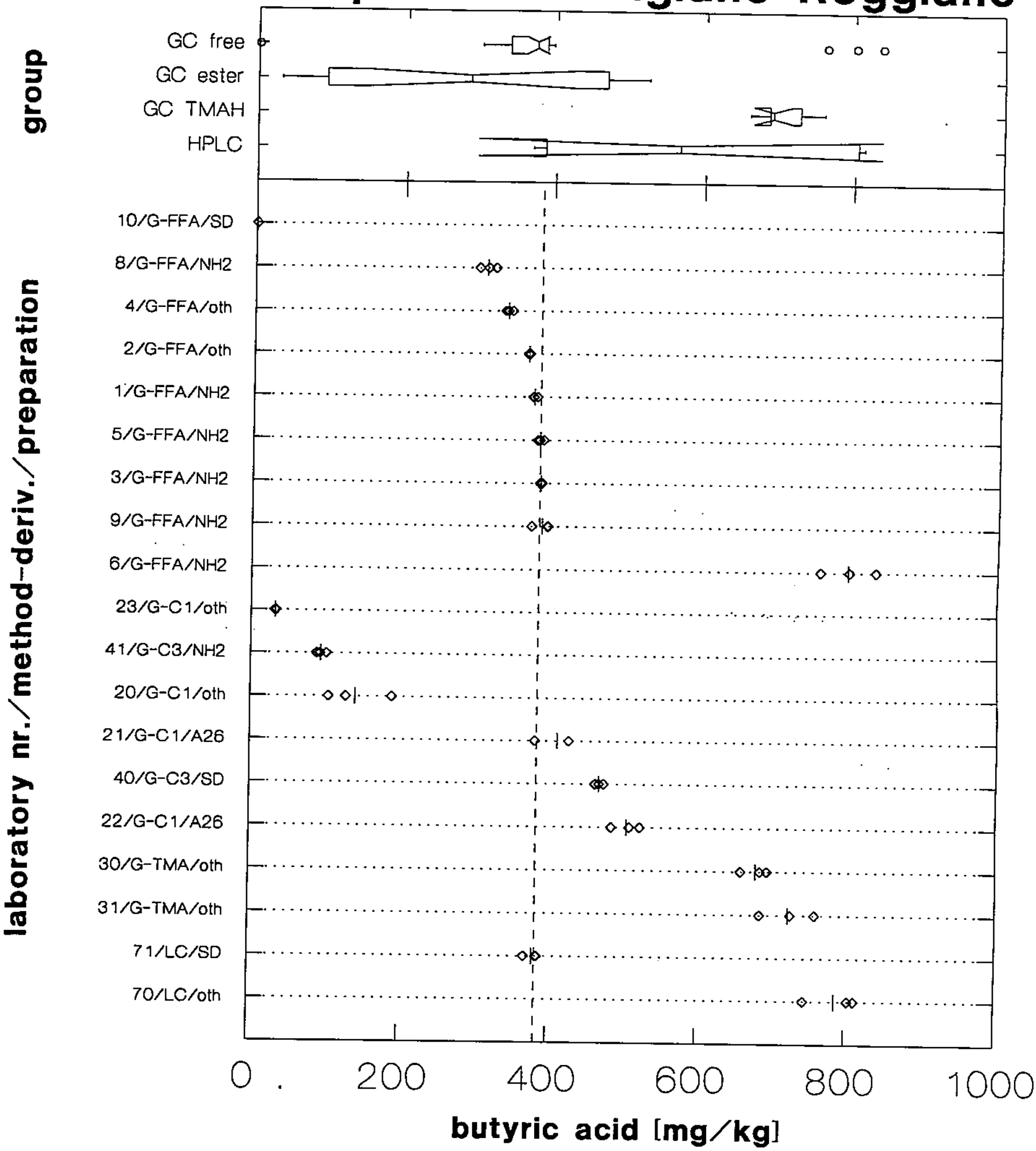

Sample 5: Parmigiano-Reggiano
group
GC free
GC ester
HPLC
laboratory nr./method-deriv./preparation
10/G-FFA/SD
2/G-FFA/oth
4/G-FFA/oth
9/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
3/G-FFA/NH2
40/G-C3/SD
71/LC/SD
70/LC/oth
0
500
1000
1500
acetic acid [mg/kg]

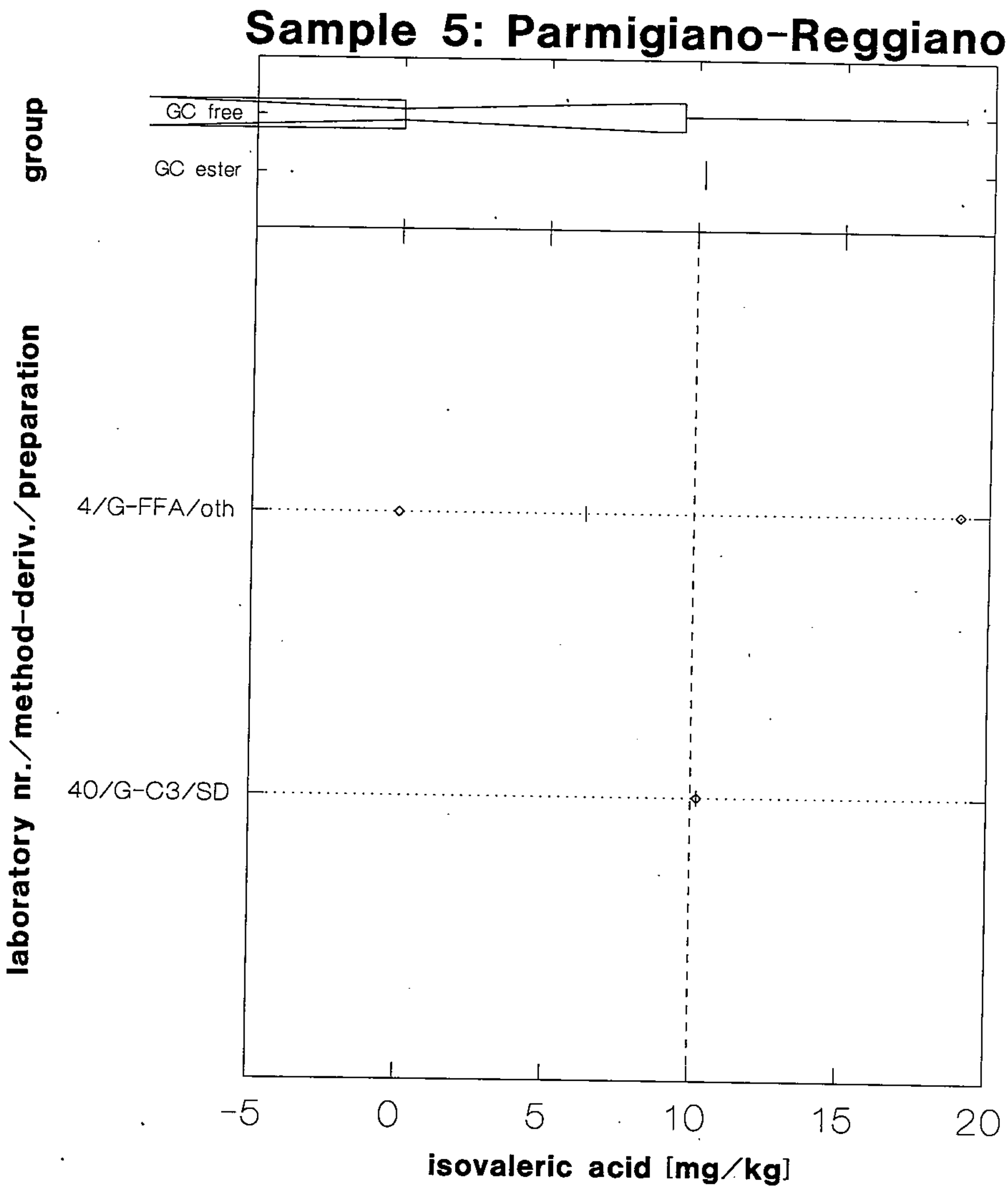

Sample 5: Parmigiano-Reggiano
group
GC free
GC ester
HPLC
laboratory nr./method-deriv./preparation
10/G-FFA/SD
2/G-FFA/oth
1/G-FFA/NH2
6/G-FFA/NH2
3/G-FFA/NH2
20/G-C1/oth
40/G-C3/SD
71/LC/SD
70/LC/oth
-100
0
100
200
300
propionic acid [mg/kg]

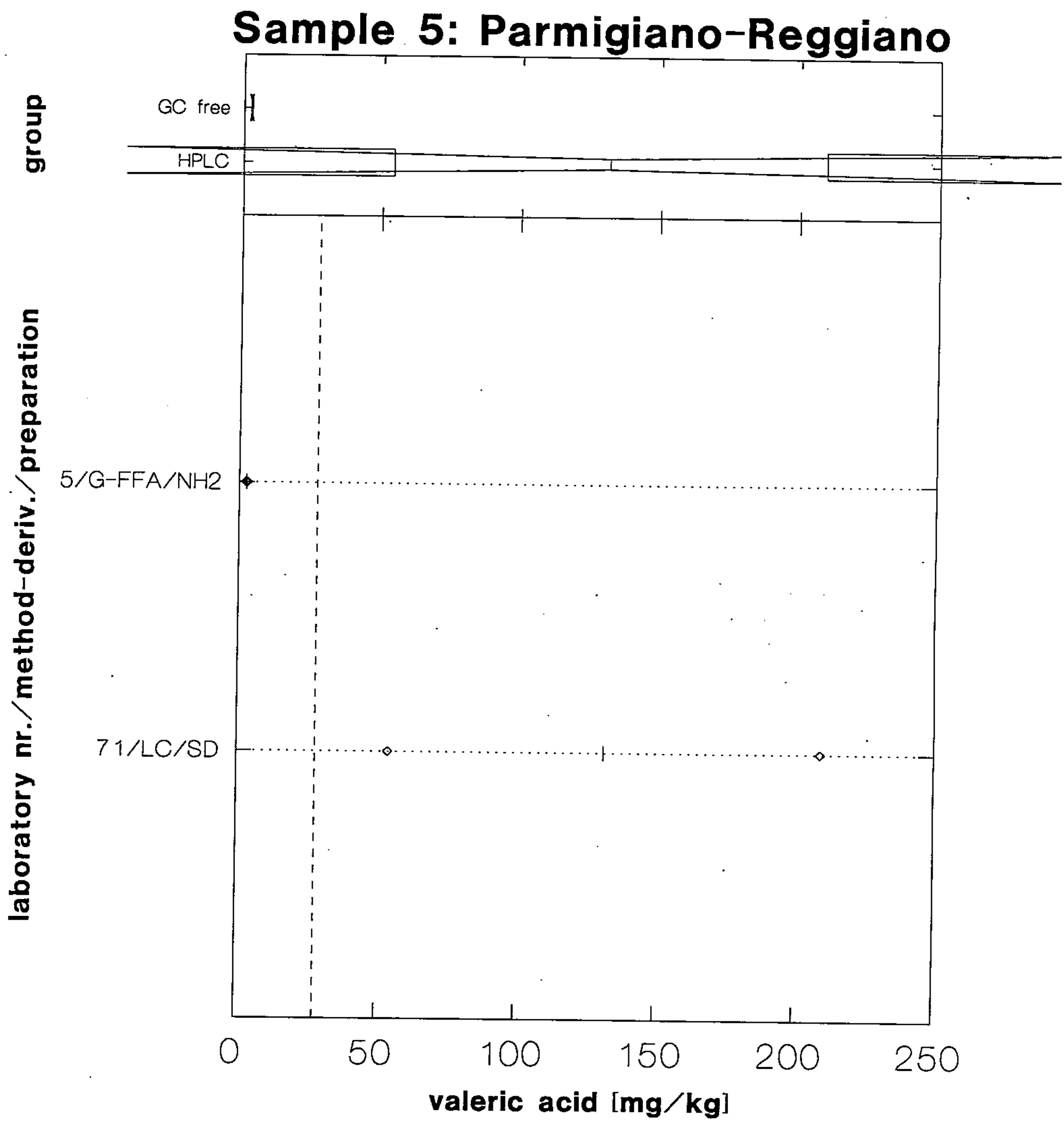
Sample 5: Parmigiano-Reggiano



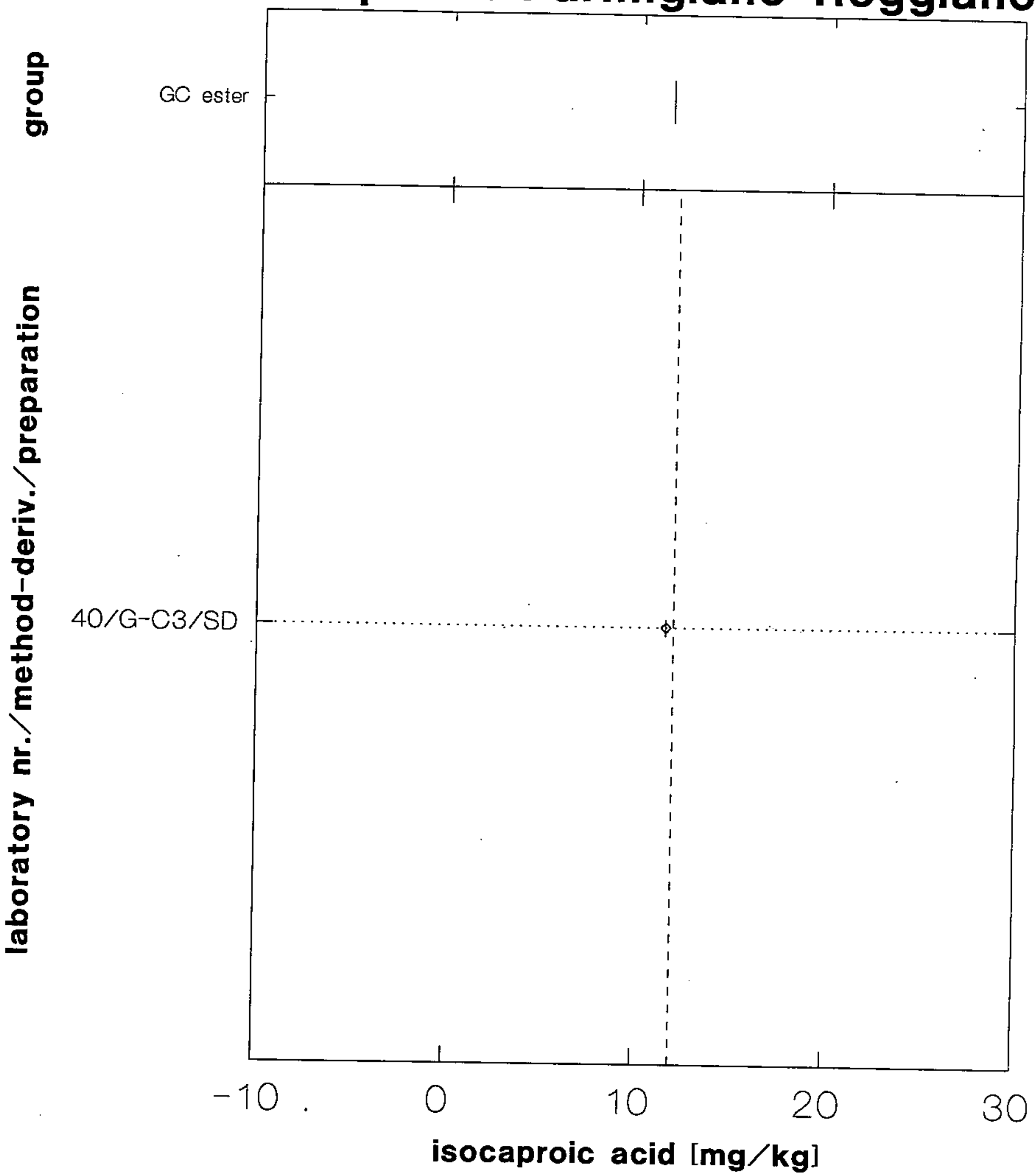
Sample 5: Parmigiano-Reggiano



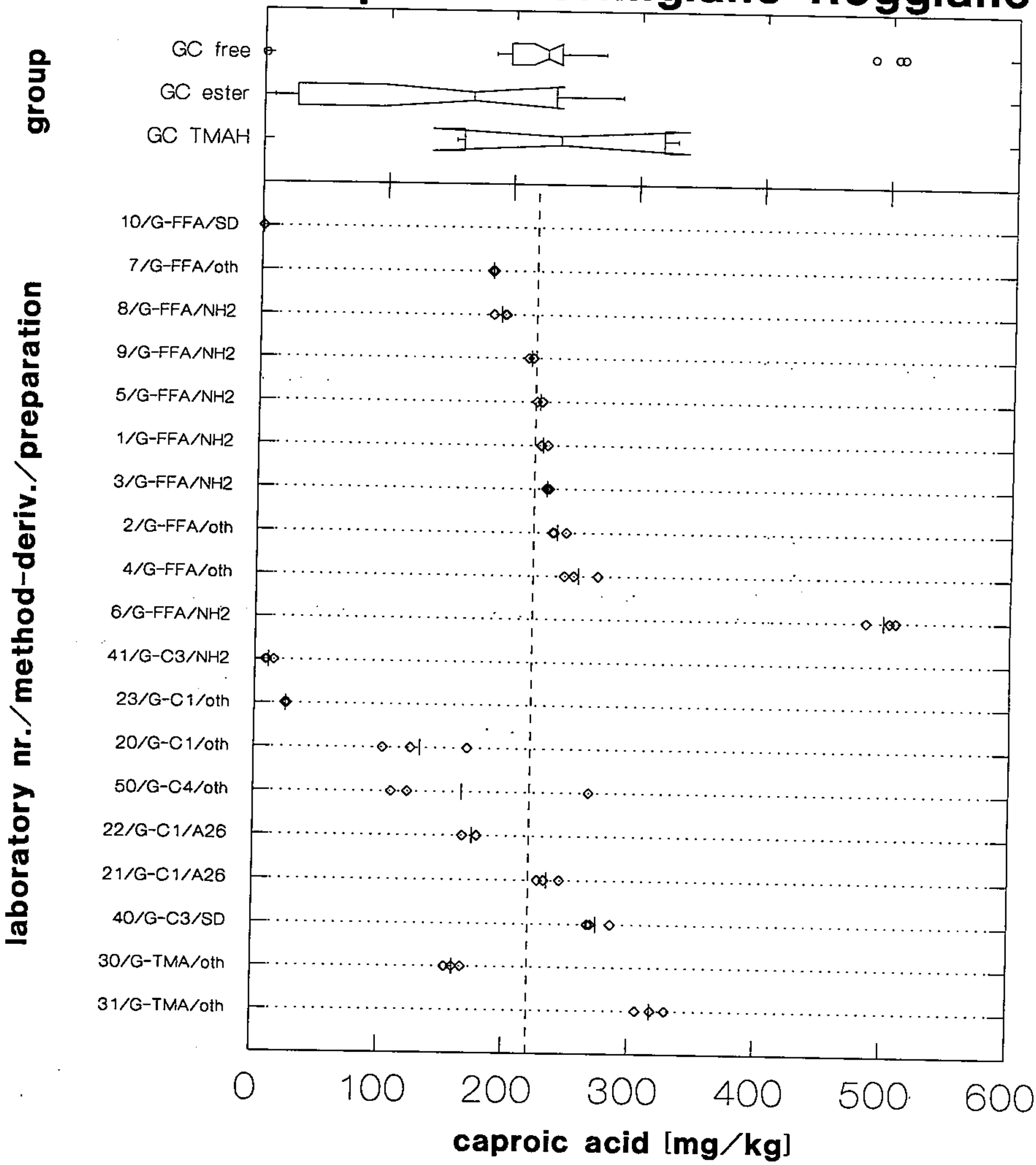

Sample 5: Parmigiano-Reggiano
group
GC free
GC ester
laboratory nr./method-deriv./preparation
4/G-FFA/oth
40/G-C3/SD
-5
0
5
10
15
20
isovaleric acid [mg/kg]

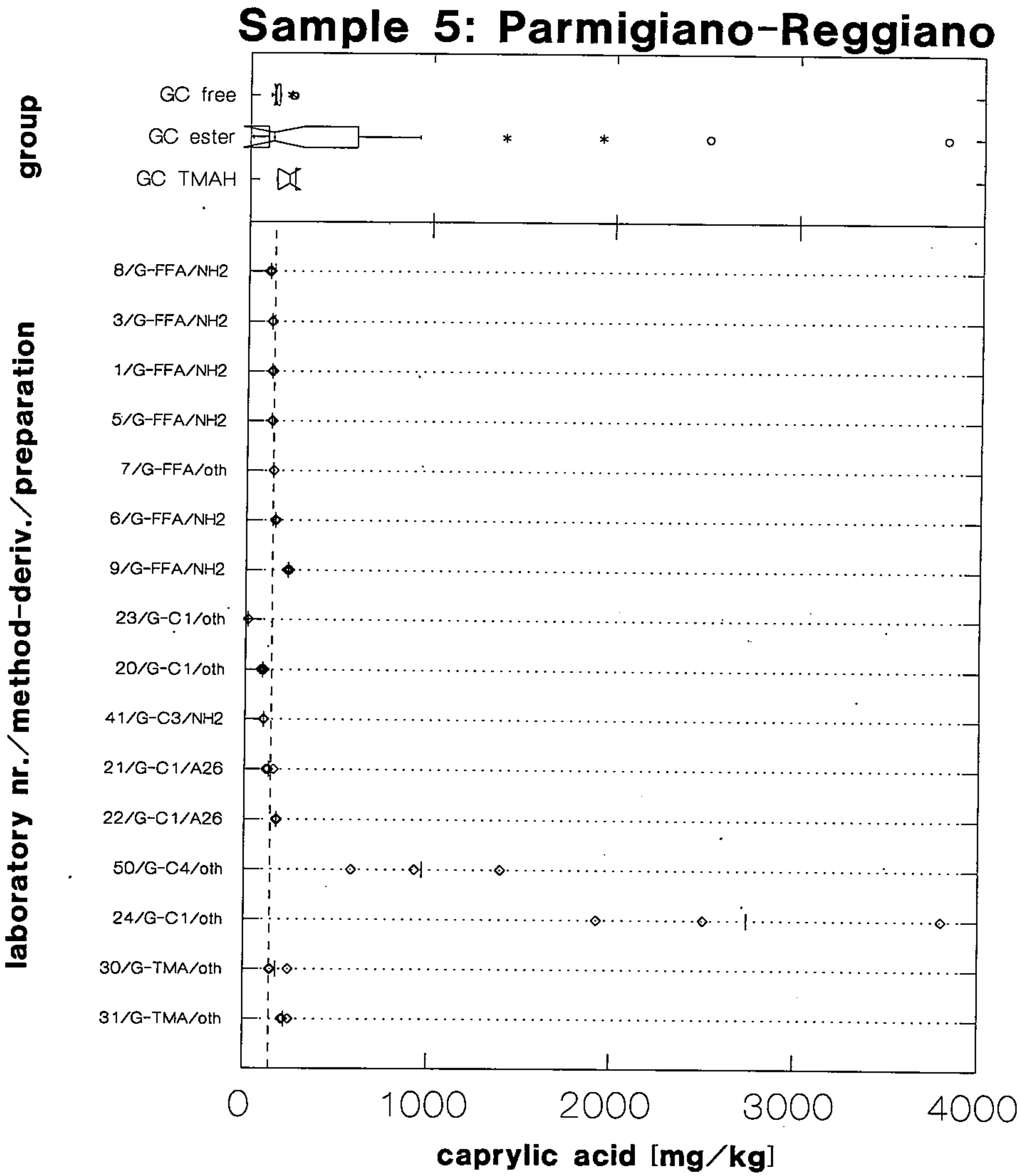

Sample 5: Parmigiano-Reggiano
group
GC free
HPLC
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
71/LC/SD
0
50
100
150
200
250
valeric acid [mg/kg]

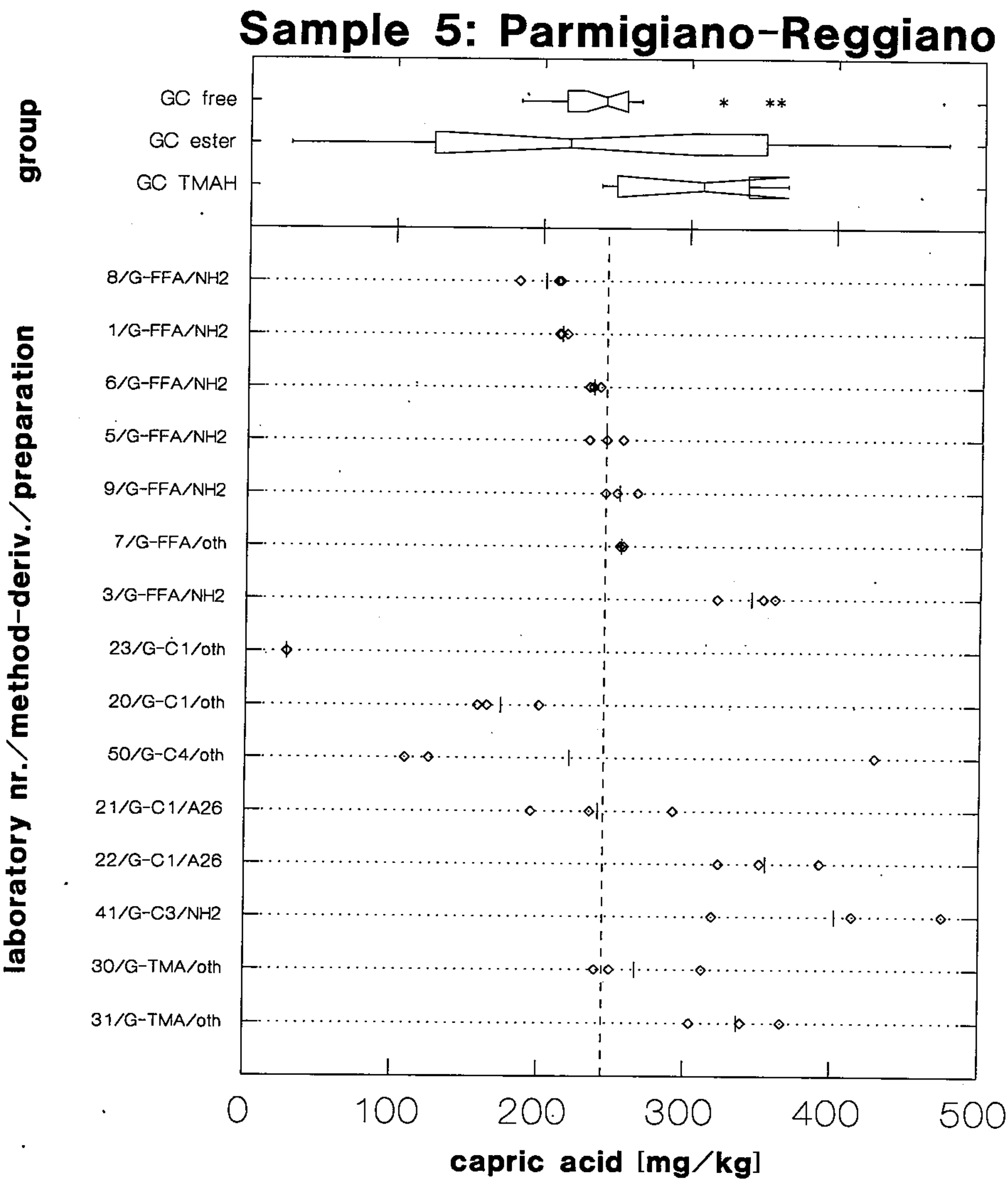
Sample 5: Parmigiano-Reggiano

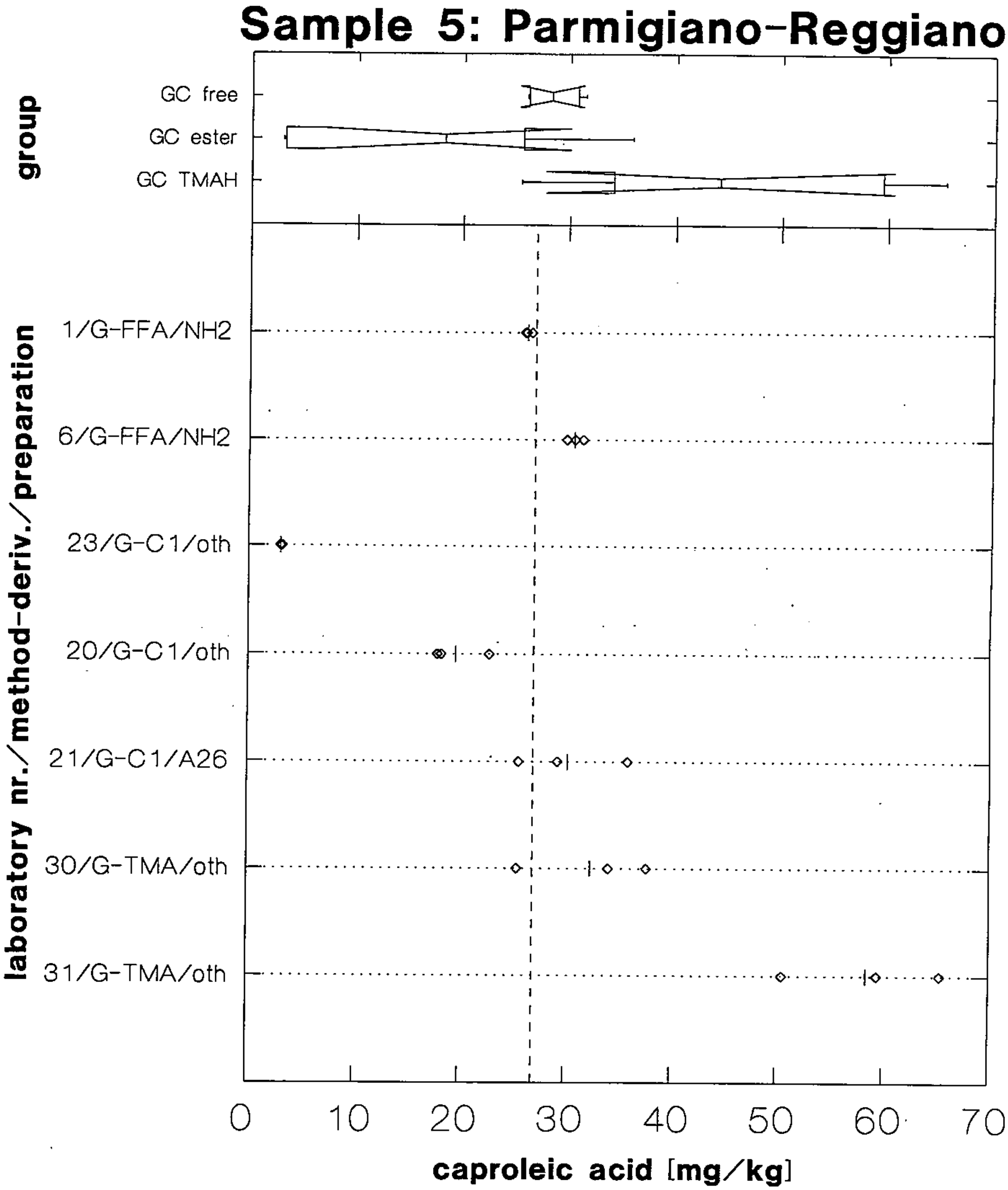


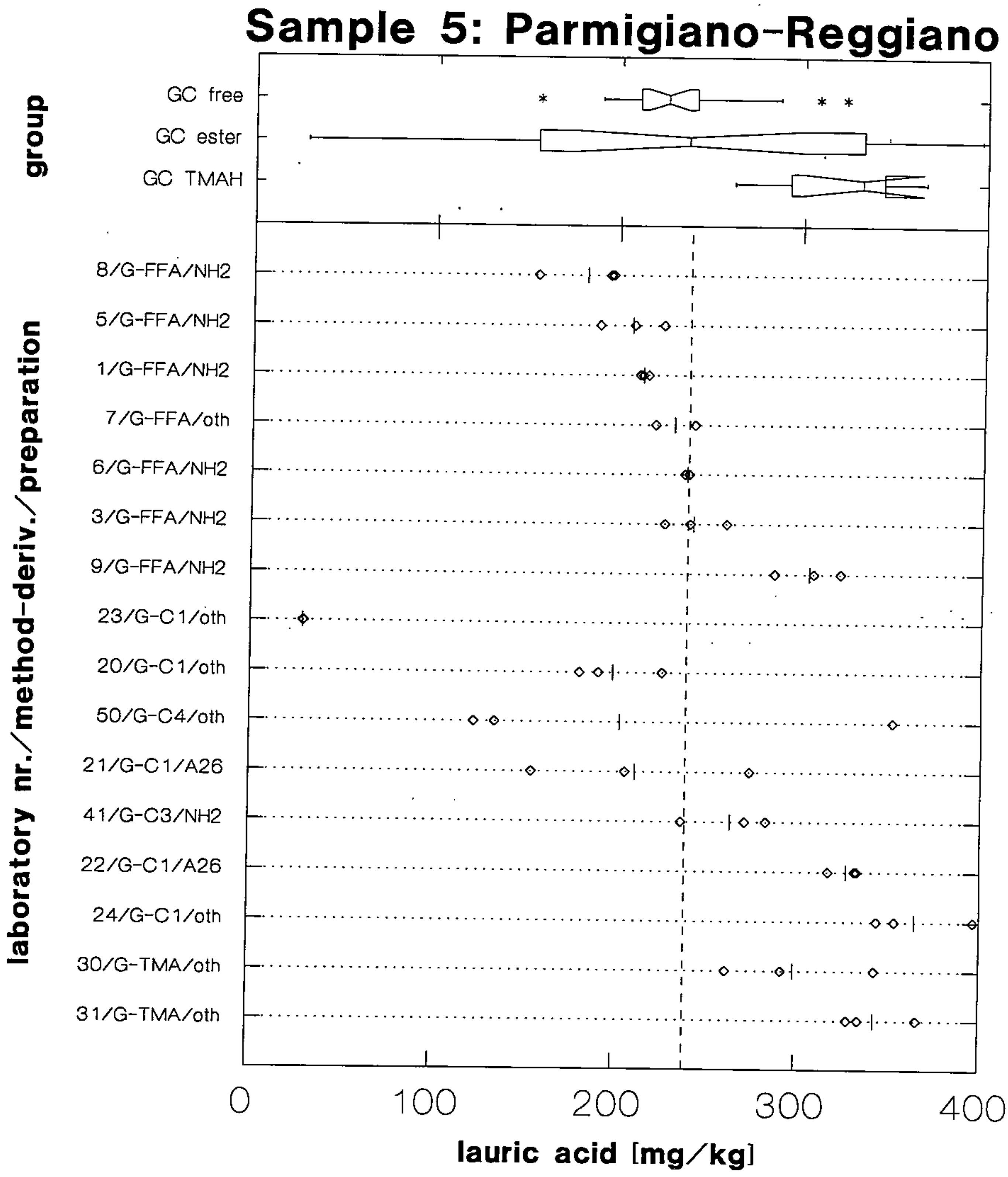
Sample 5: Parmigiano-Reggiano

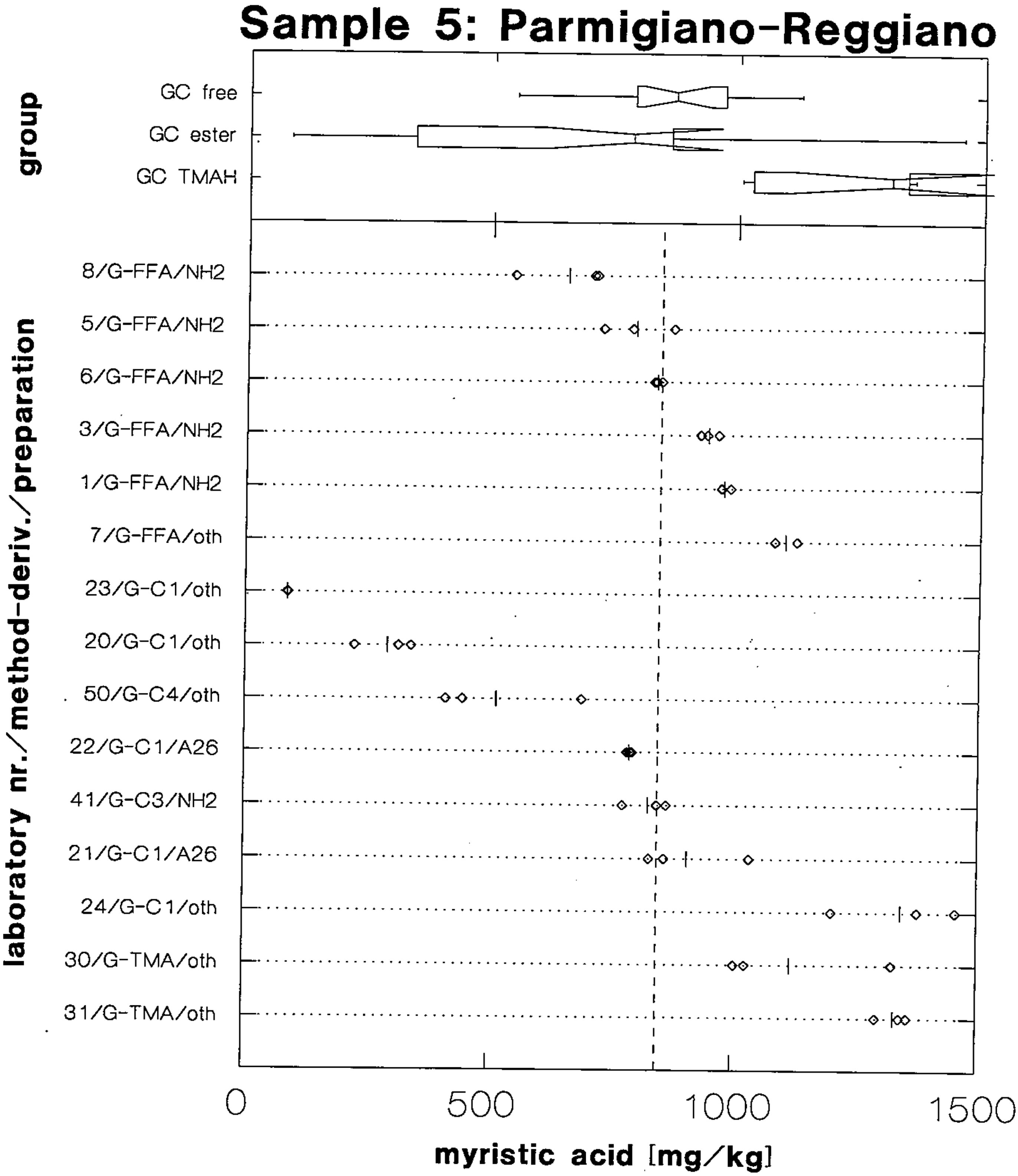



Sample 5: Parmigiano-Reggiano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
3/G-FFA/NH2
1/G-FFA/NH2
5/G-FFA/NH2
7/G-FFA/oth
6/G-FFA/NH2
9/G-FFA/NH2
23/G-C1/oth
20/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
22/G-C1/A26
50/G-C4/oth
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
1000
2000
3000
4000
caprylic acid [mg/kg]

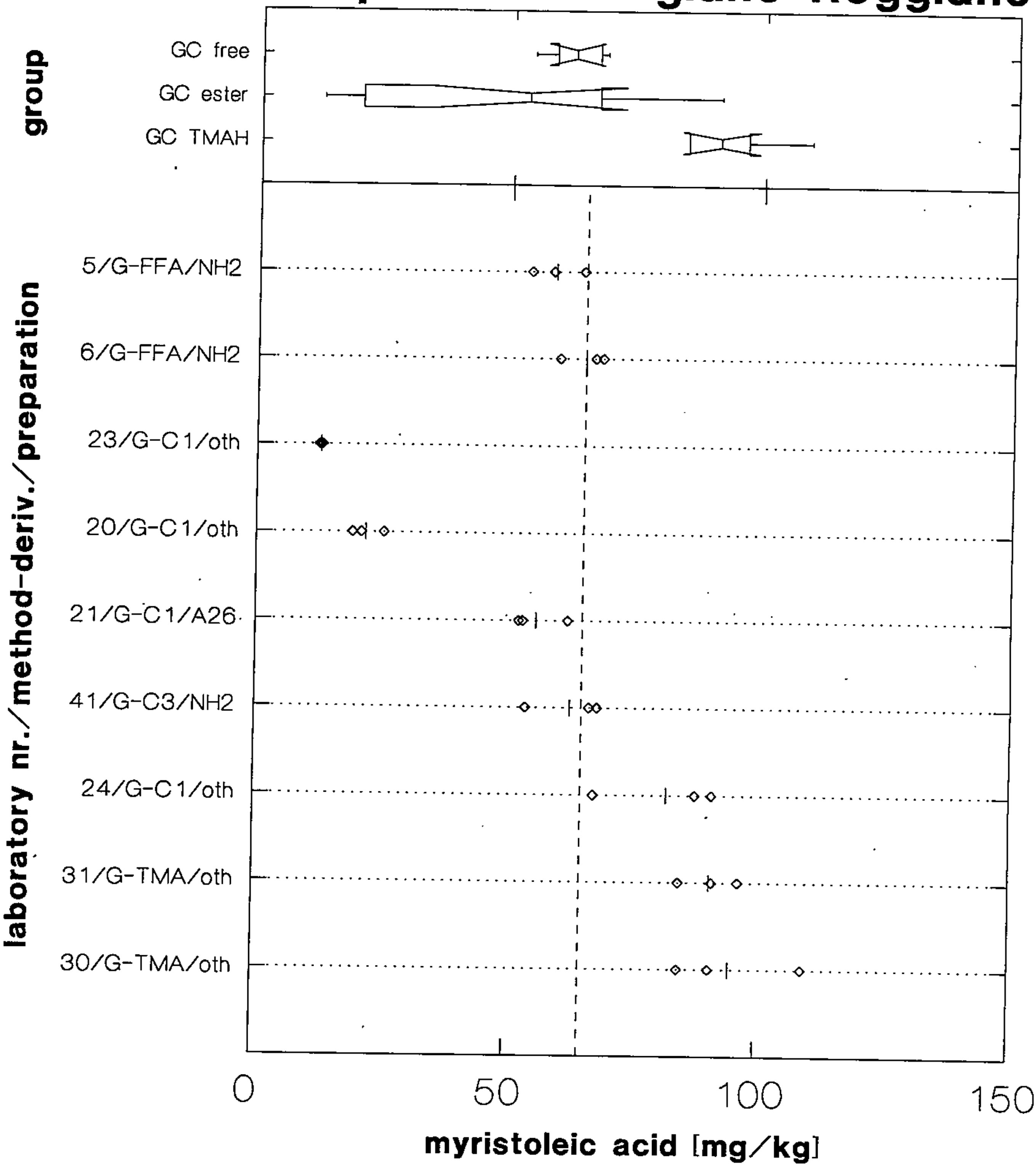

Sample 5: Parmigiano-Reggiano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
1/G-FFA/NH2
6/G-FFA/NH2
5/G-FFA/NH2
9/G-FFA/NH2
7/G-FFA/oth
3/G-FFA/NH2
23/G-C1/oth
20/G-C1/oth
50/G-C4/oth
21/G-C1/A26
22/G-C1/A26
41/G-C3/NH2
30/G-TMA/oth
31/G-TMA/oth
0
100
200
300
400
500
capric acid [mg/kg]

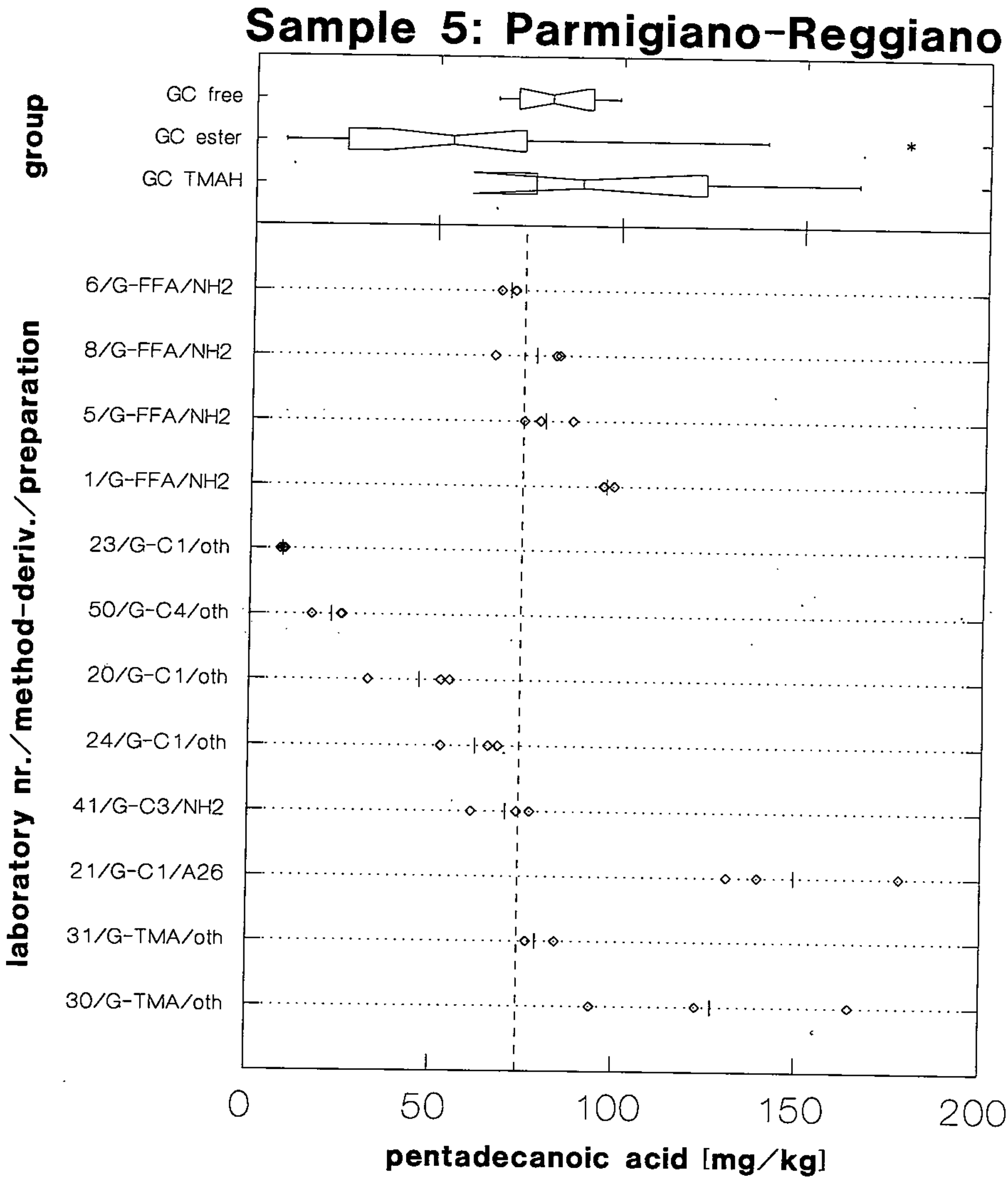

Sample 5: Parmigiano-Reggiano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
1/G-FFA/NH2
6/G-FFA/NH2
23/G-C1/oth
20/G-C1/oth
21/G-C1/A26
30/G-TMA/oth
31/G-TMA/oth
0
10
20
30
40
50
60
70
caproleic acid [mg/kg]


Sample 5: Parmigiano-Reggiano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
5/G-FFA/NH2
1/G-FFA/NH2
7/G-FFA/oth
6/G-FFA/NH2
3/G-FFA/NH2
9/G-FFA/NH2
23/G-C1/oth
20/G-C1/oth
50/G-C4/oth
21/G-C1/A26
41/G-C3/NH2
22/G-C1/A26
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
100
200
300
400
lauric acid [mg/kg]

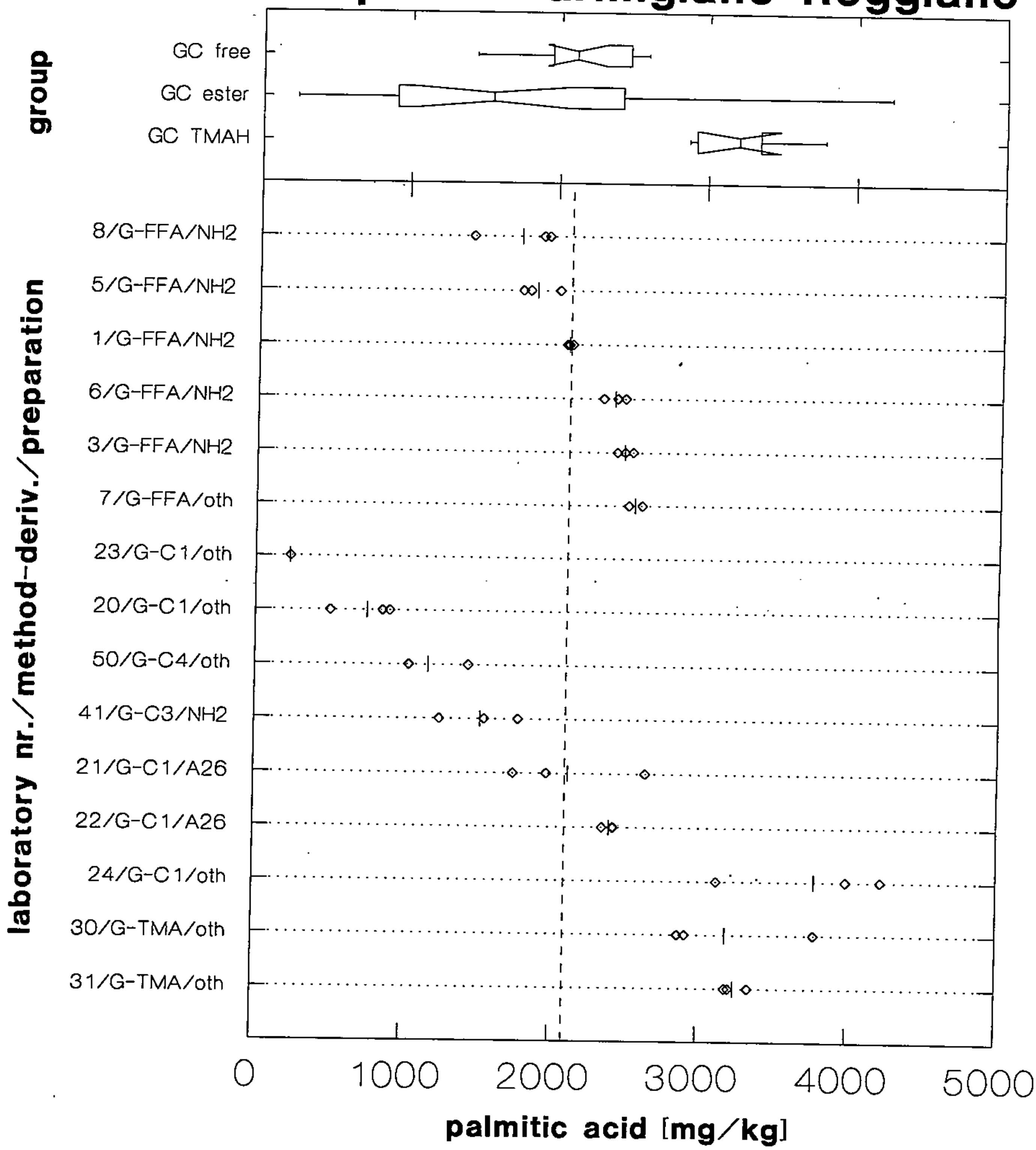

Sample 5: Parmigiano-Reggiano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
3/G-FFA/NH2
1/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
20/G-C1/oth
50/G-C4/oth
22/G-C1/A26
41/G-C3/NH2
21/G-C1/A26
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
500
1000
1500
myristic acid [mg/kg]

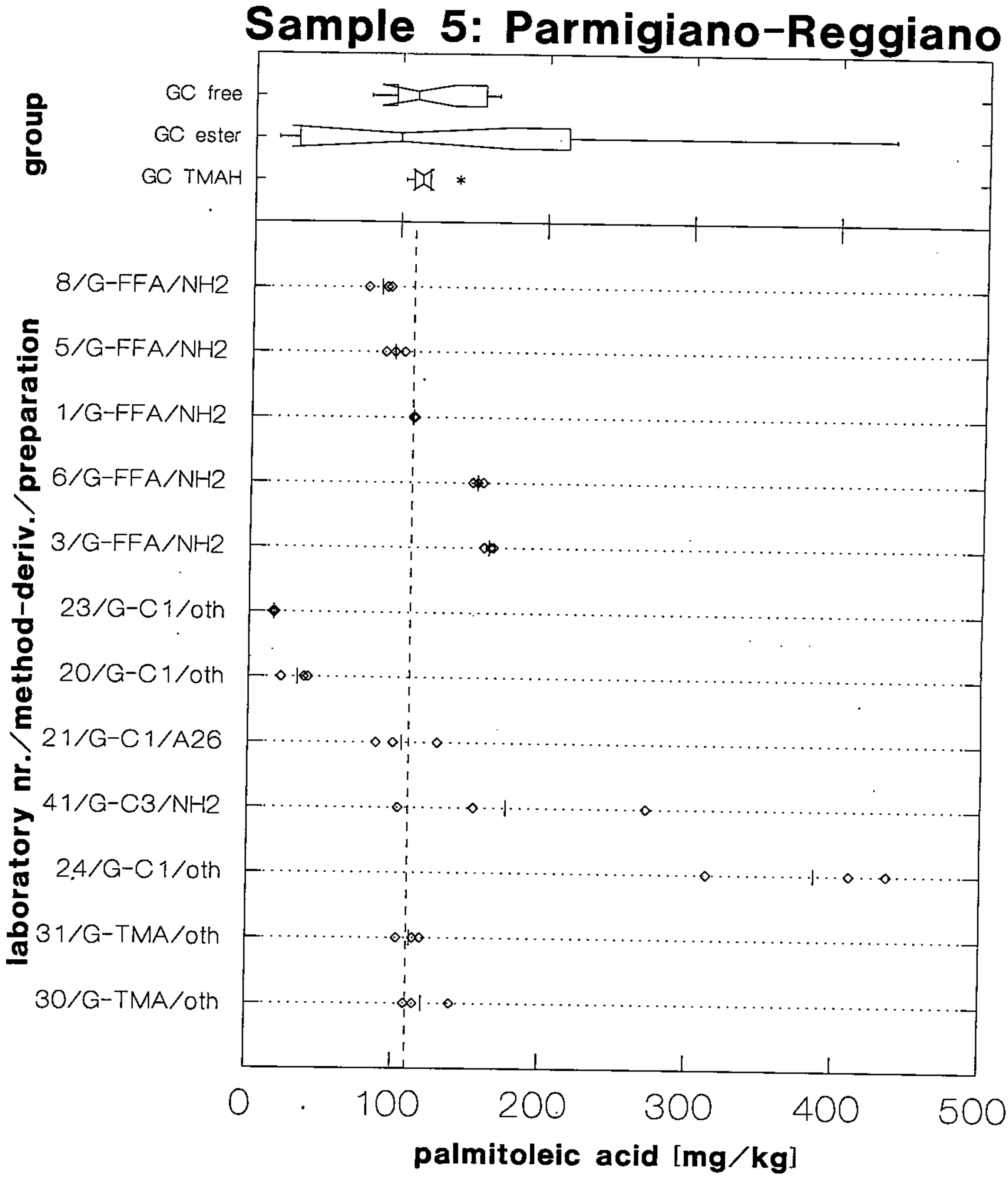
Sample 5: Parmigiano-Reggiano



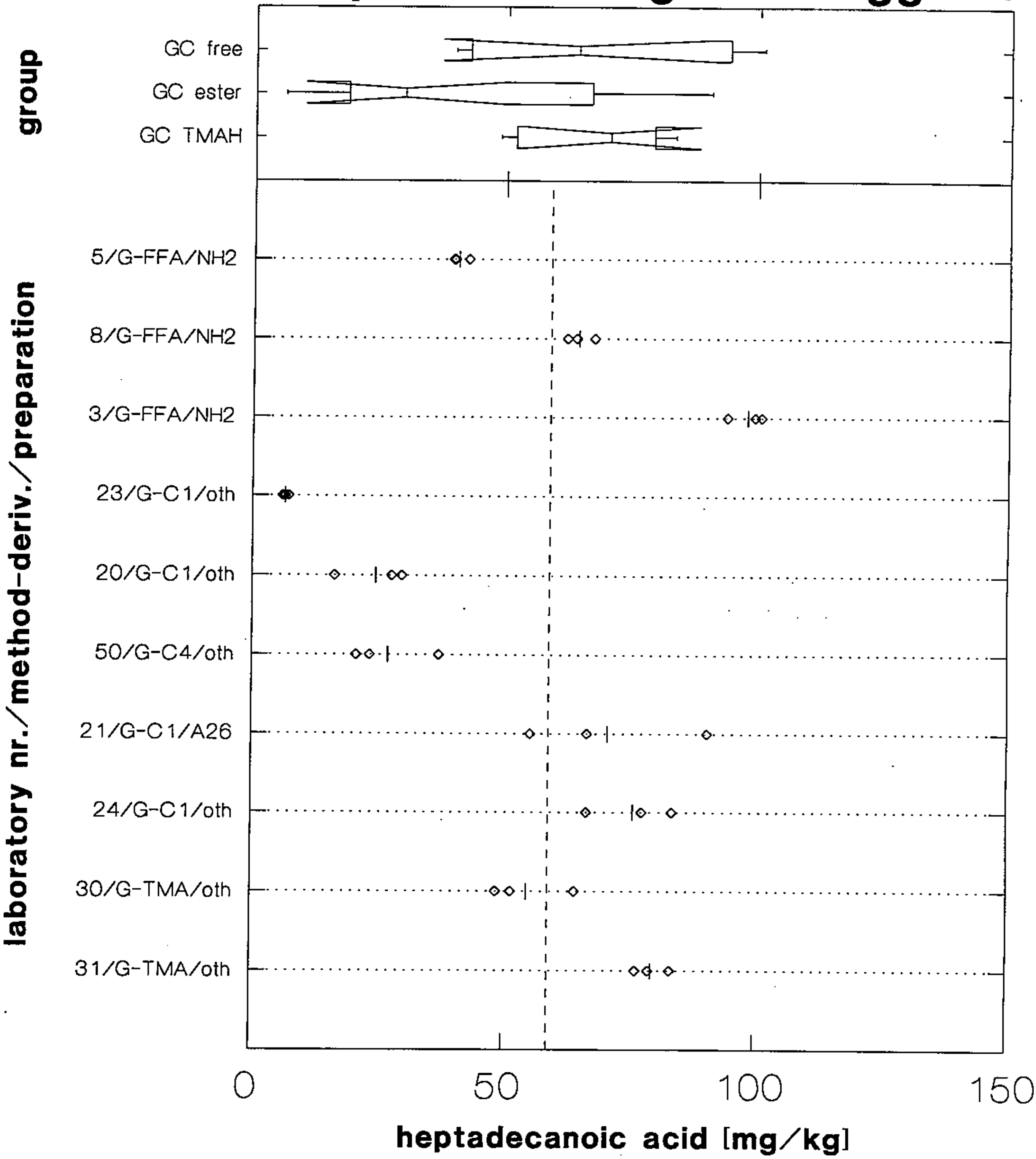

Sample 5: Parmigiano-Reggiano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
6/G-FFA/NH2
8/G-FFA/NH2
5/G-FFA/NH2
1/G-FFA/NH2
23/G-C1/oth
50/G-C4/oth
20/G-C1/oth
24/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
31/G-TMA/oth
30/G-TMA/oth
0
50
100
150
200
pentadecanoic acid [mg/kg]

Sample 5: Parmigiano-Reggiano

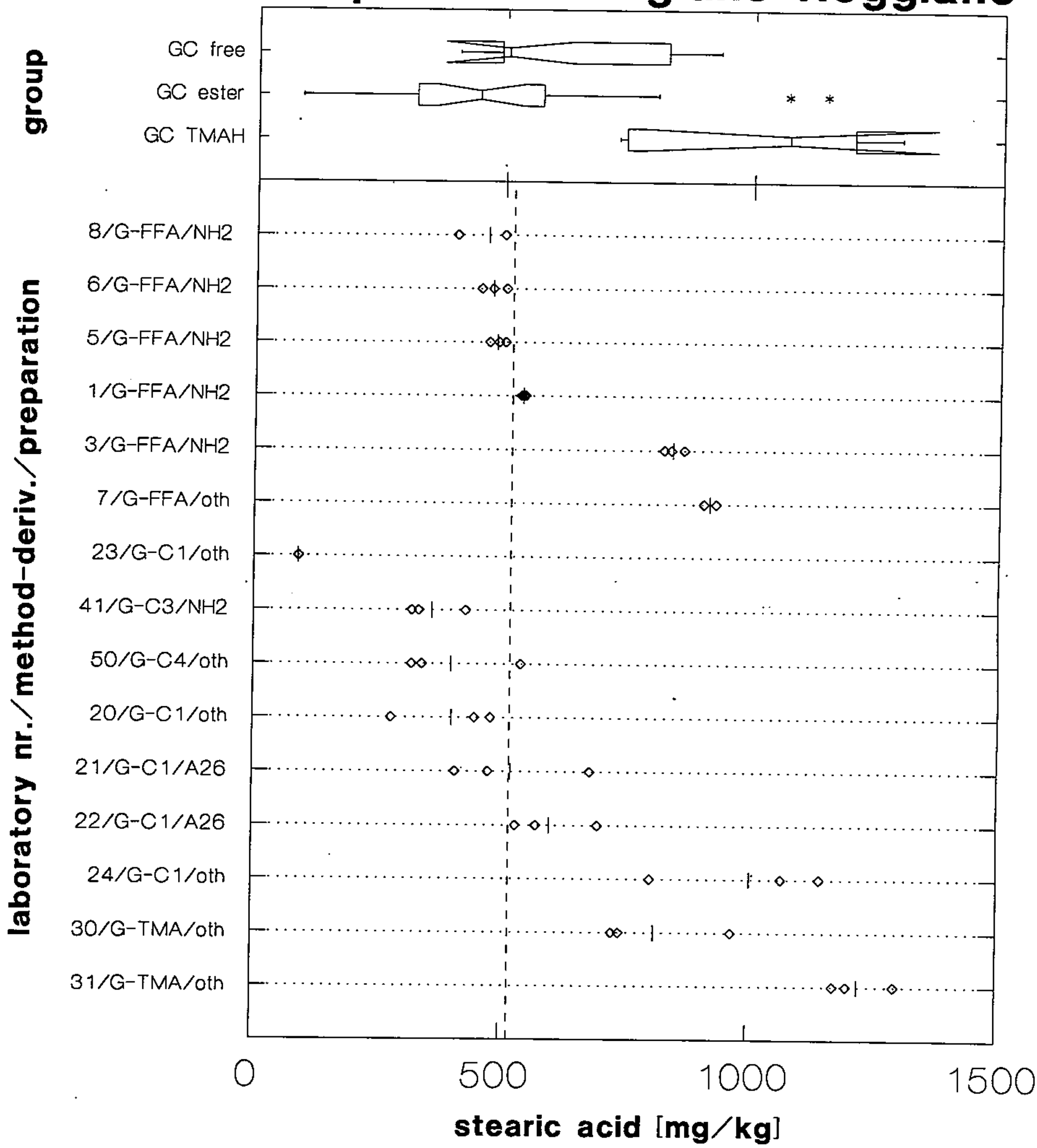



Sample 5: Parmigiano-Reggiano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
5/G-FFA/NH2
1/G-FFA/NH2
6/G-FFA/NH2
3/G-FFA/NH2
23/G-C1/oth
20/G-C1/oth
21/G-C1/A26
41/G-C3/NH2
24/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
100
200
300
400
500
palmitoleic acid [mg/kg]

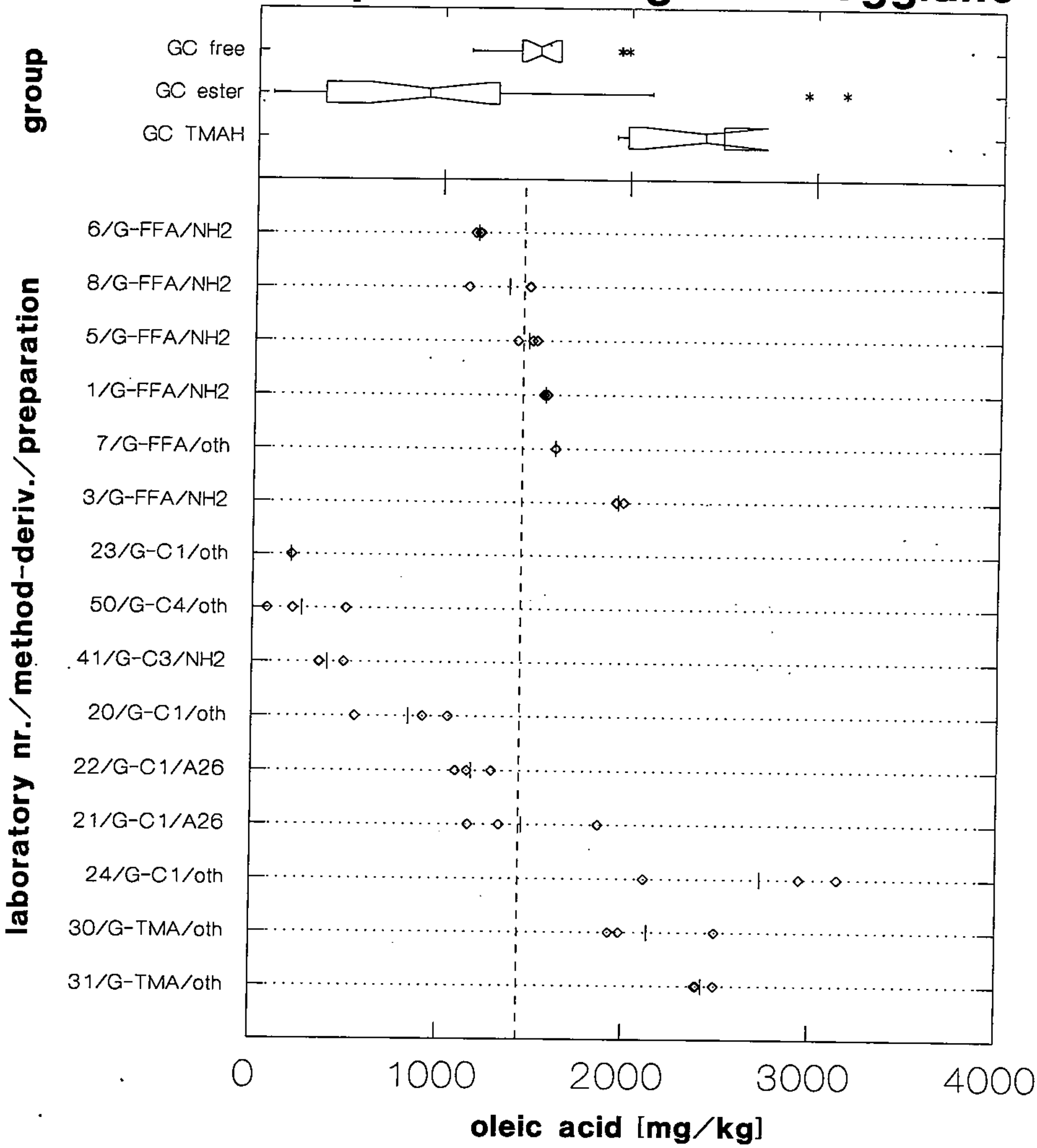
Sample 5: Parmigiano-Reggiano



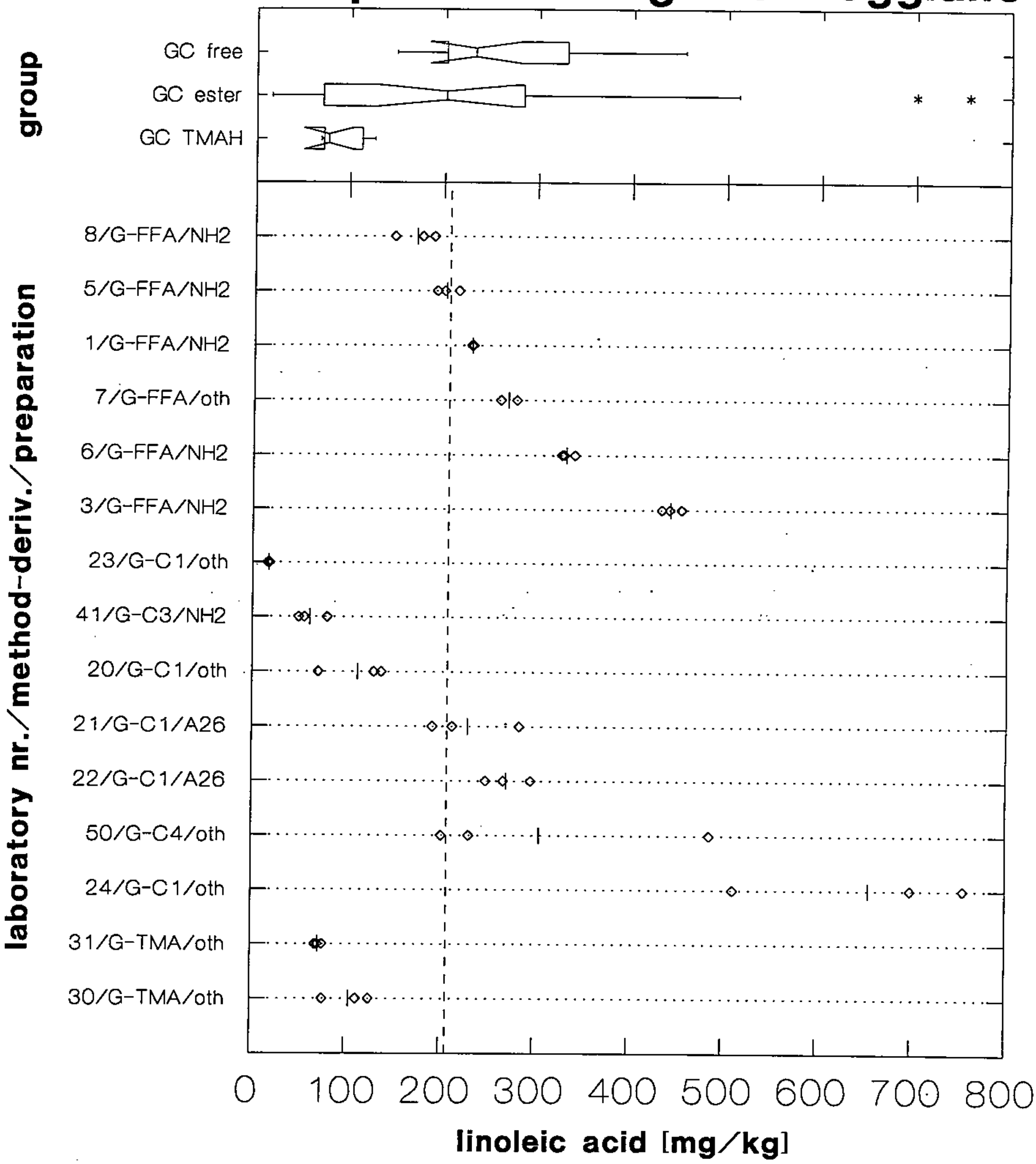
Sample 5: Parmigiano-Reggiano

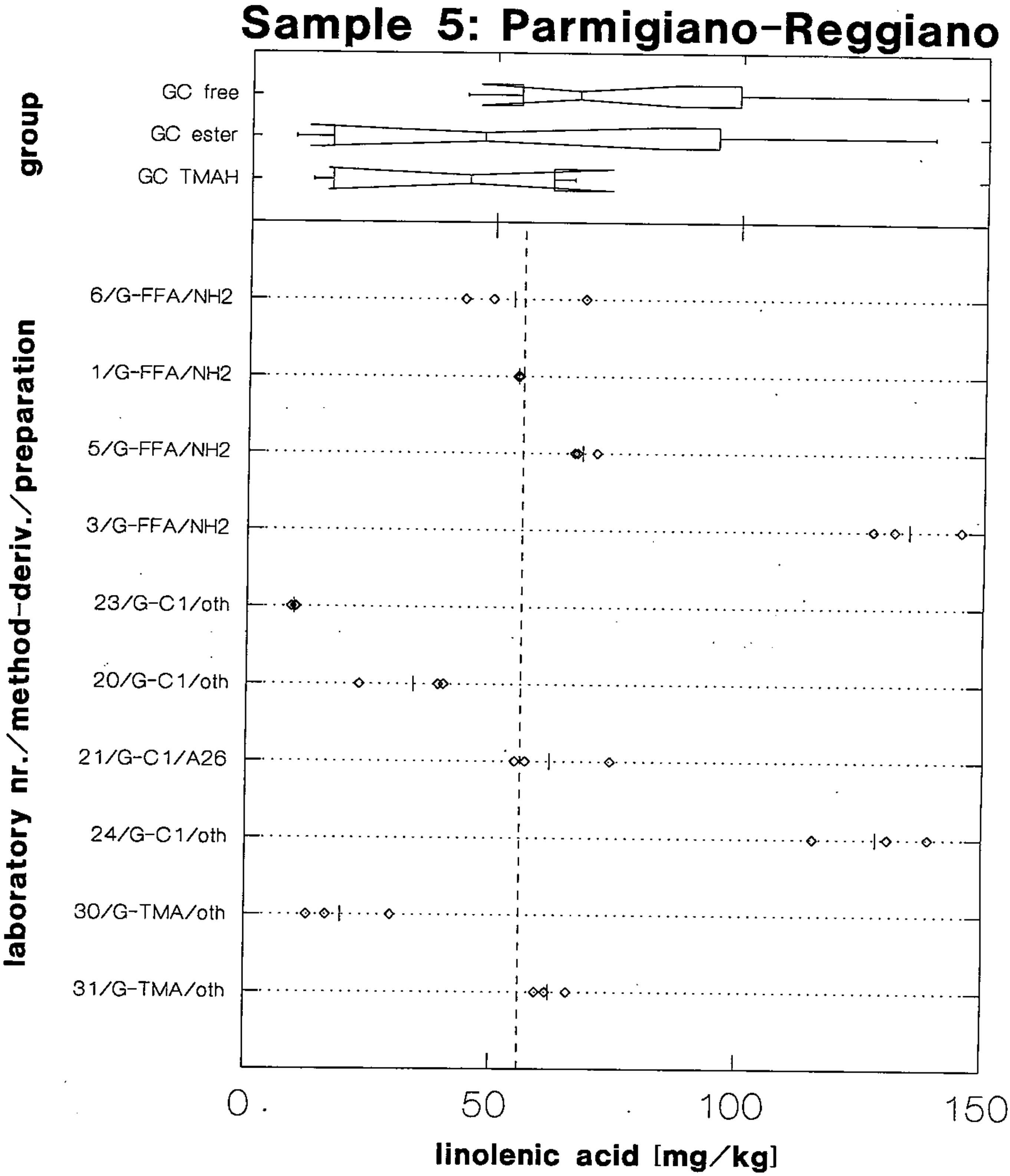


Sample 5: Parmigiano-Reggiano

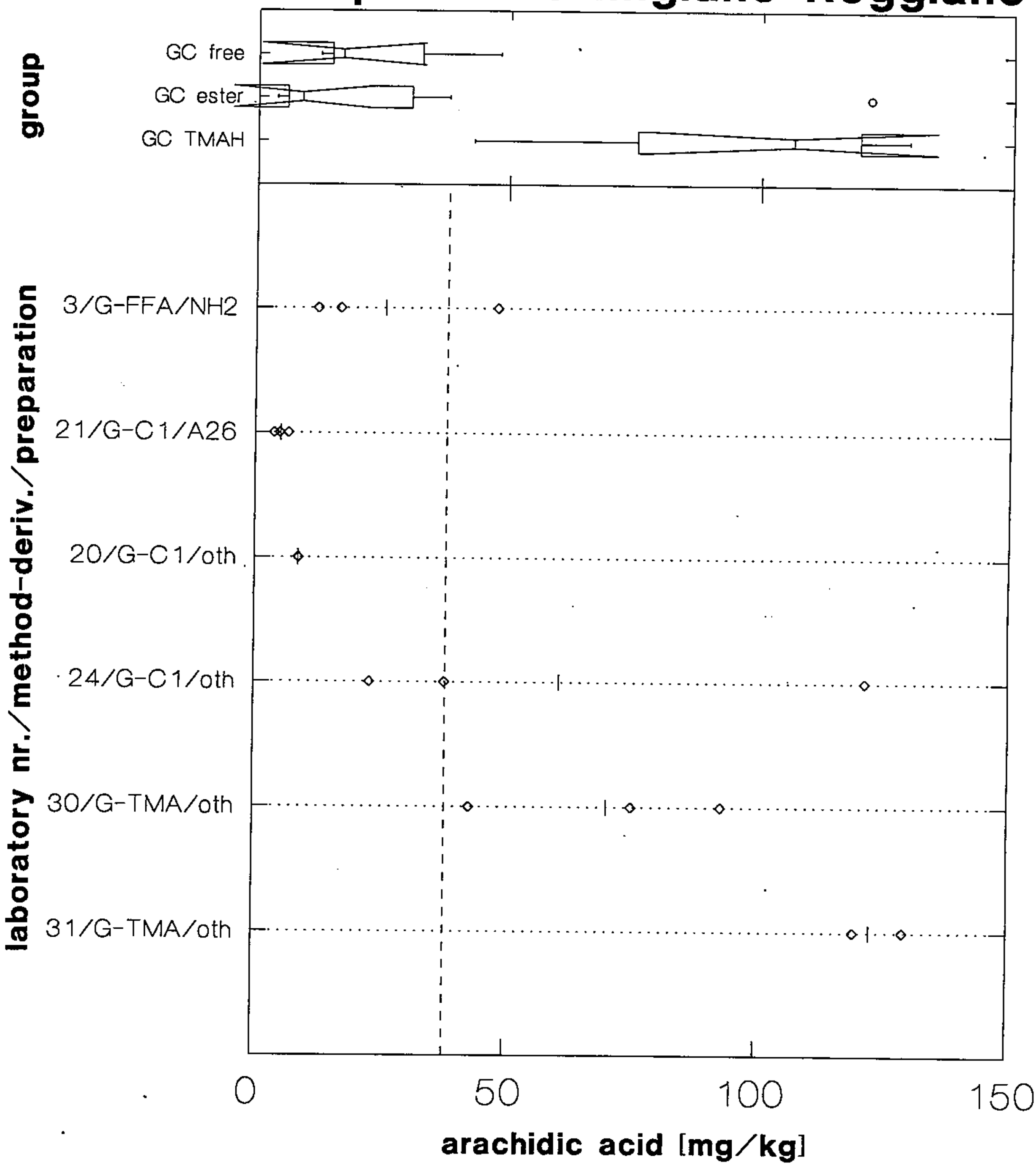


Sample 5: Parmigiano-Reggiano

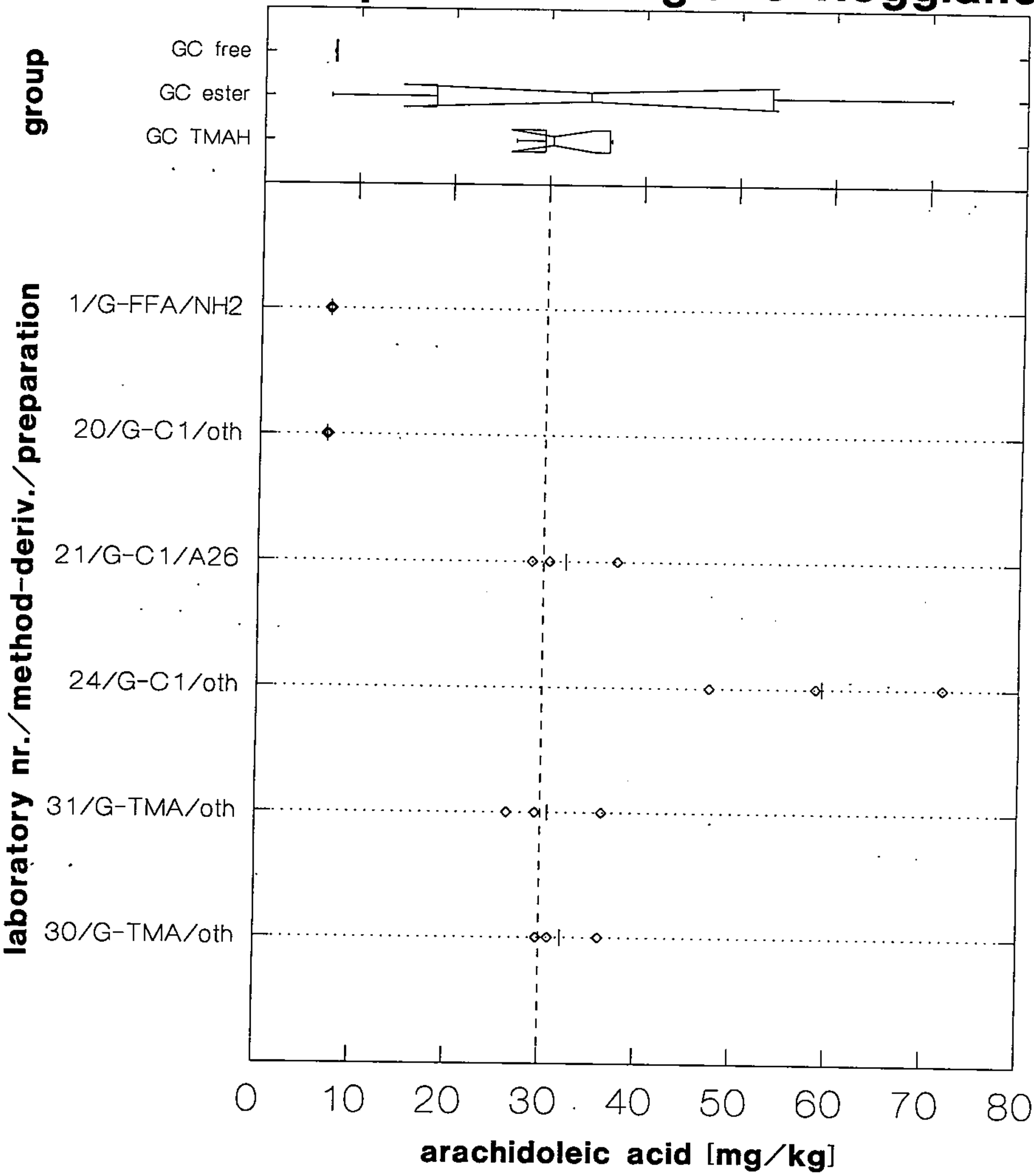



Sample 5: Parmigiano-Reggiano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
6/G-FFA/NH2
1/G-FFA/NH2
5/G-FFA/NH2
3/G-FFA/NH2
23/G-C1/oth
20/G-C1/oth
21/G-C1/A26
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
50
100
150
linolenic acid [mg/kg]

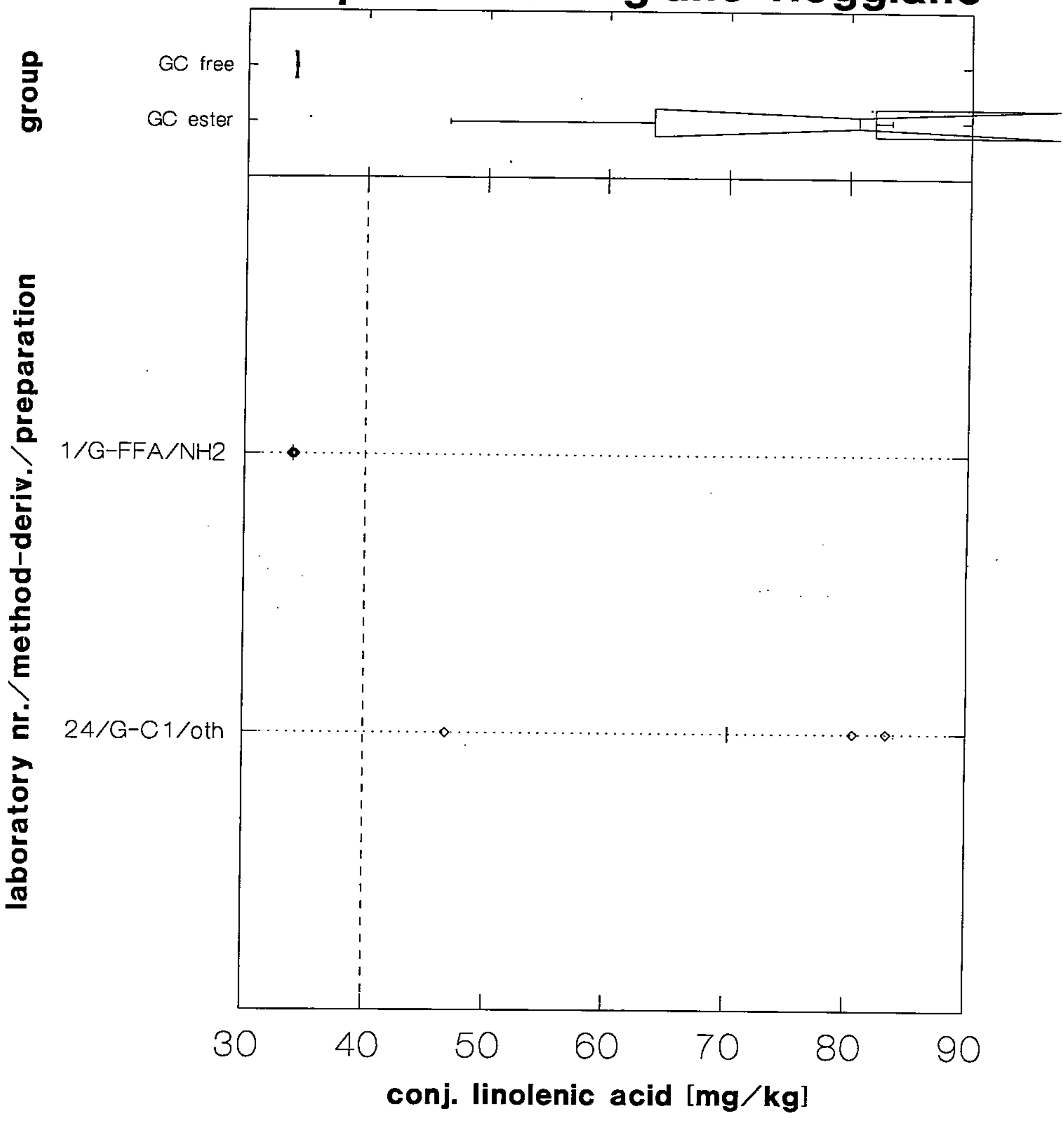
Sample 5: Parmigiano-Reggiano

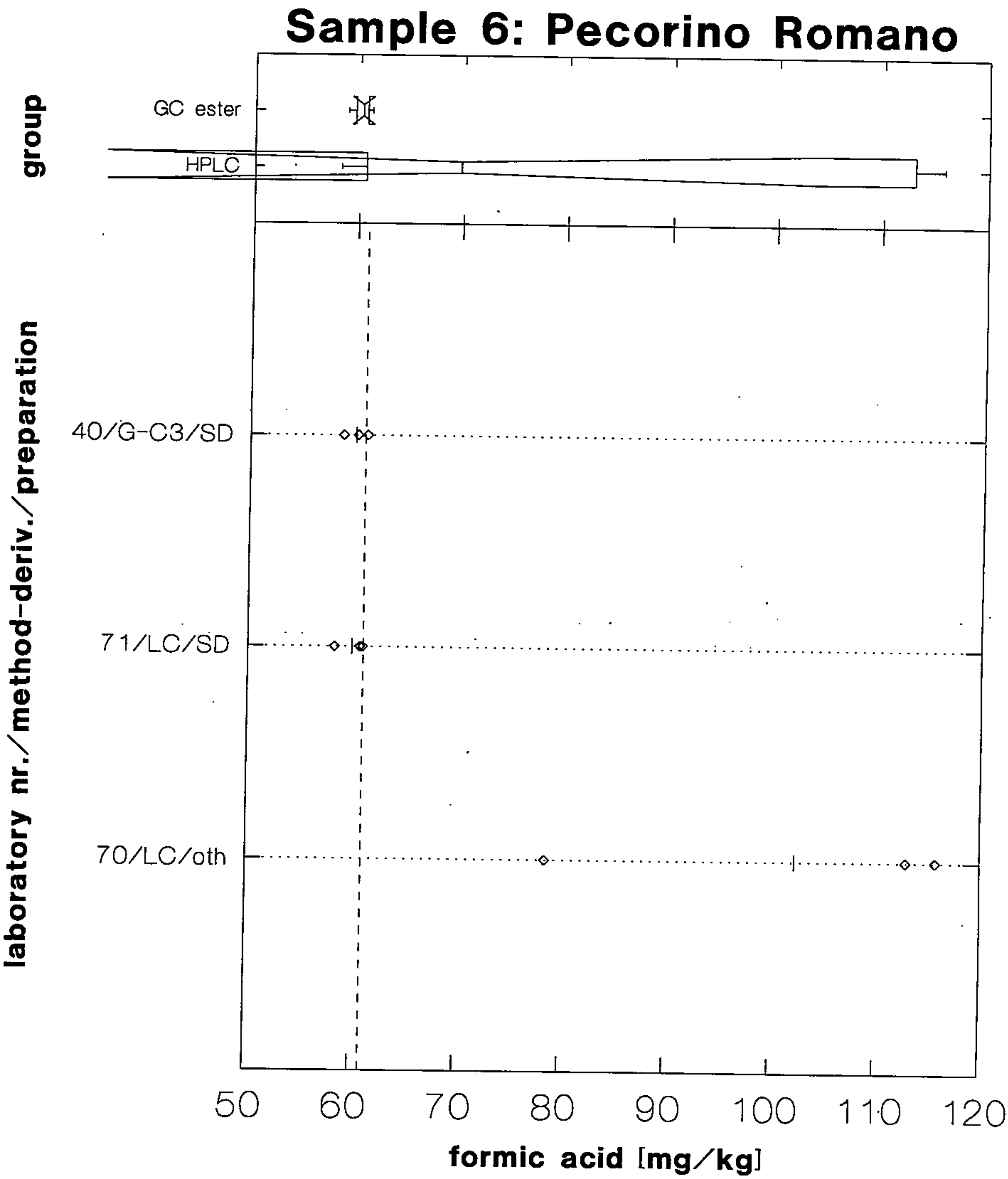


Sample 5: Parmigiano-Reggiano

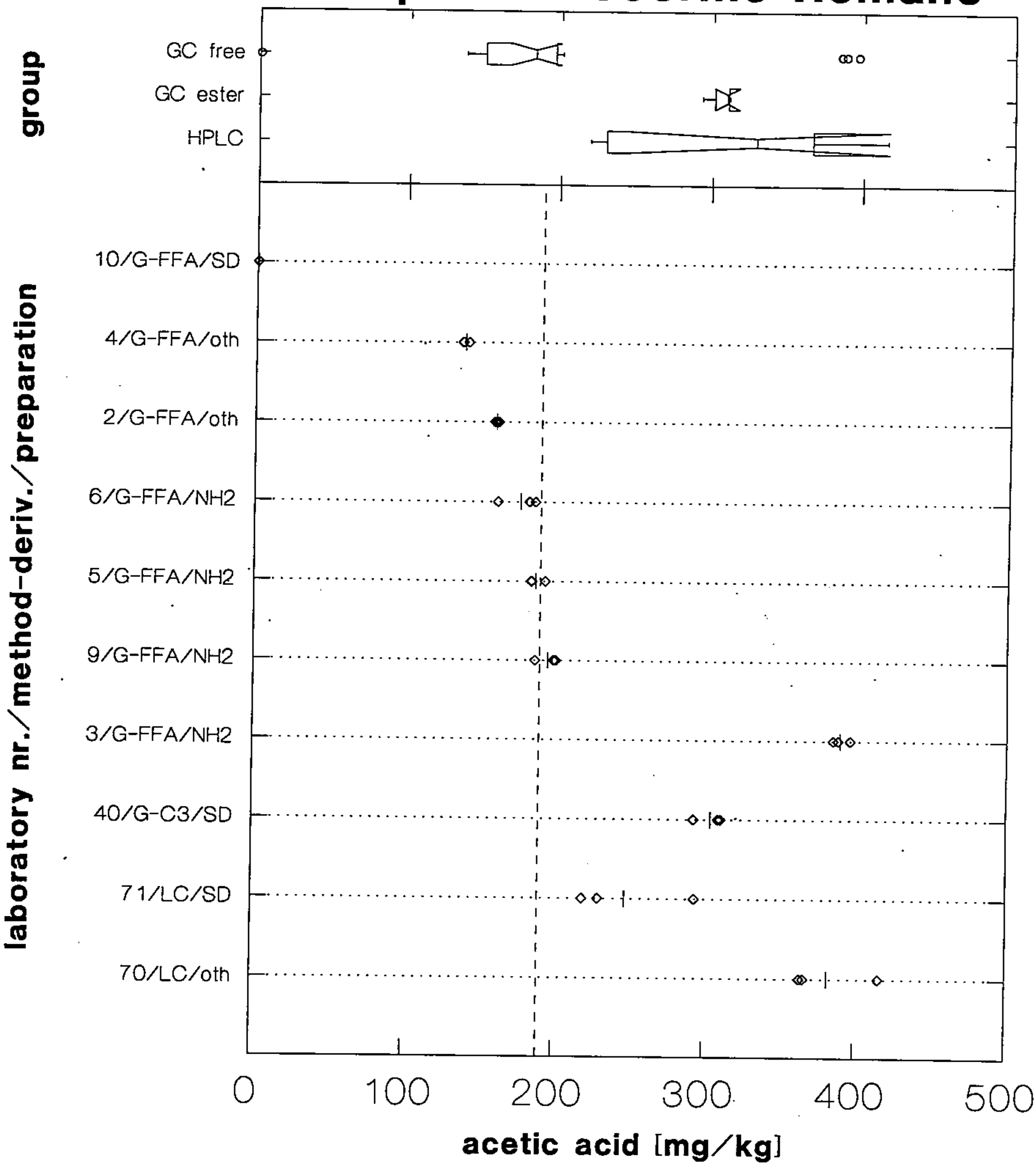


Sample 5: Parmigiano-Reggiano

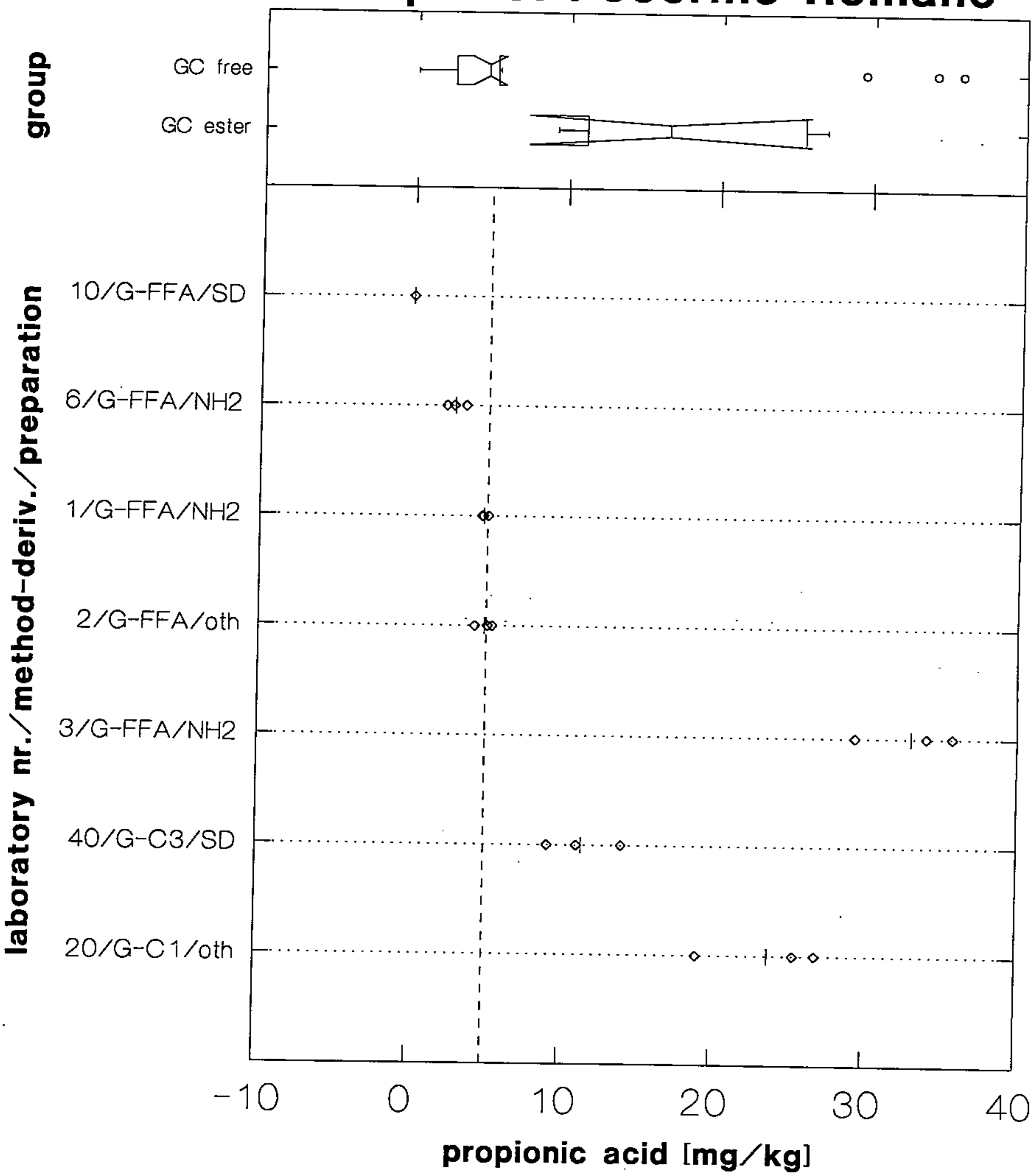



Sample 6: Pecorino Romano
group
GC ester
HPLC
laboratory nr./method-deriv./preparation
40/G-C3/SD
71/LC/SD
70/LC/oth
50
60
70
80
90
100
110
120
formic acid [mg/kg]

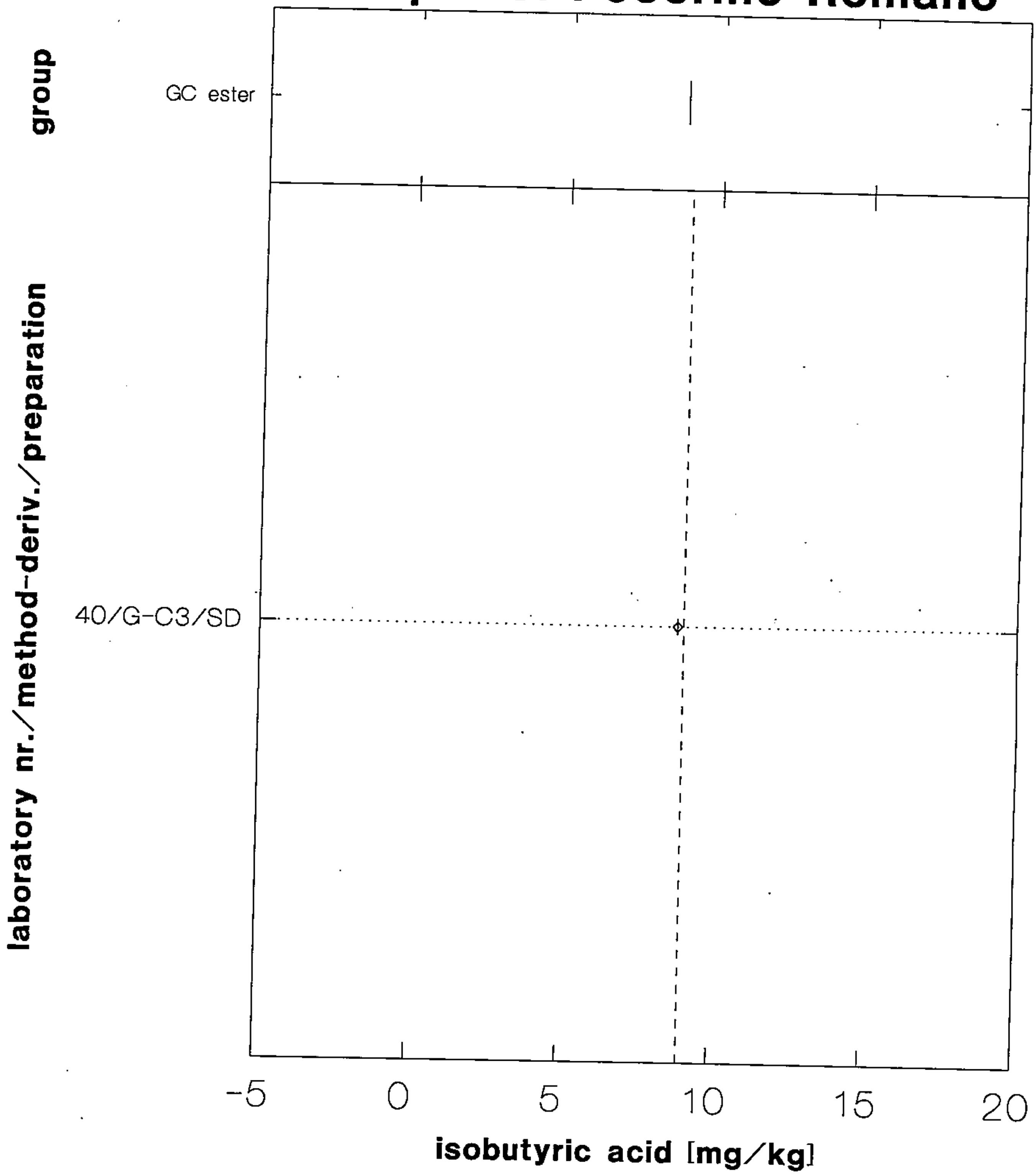
Sample 6: Pecorino Romano

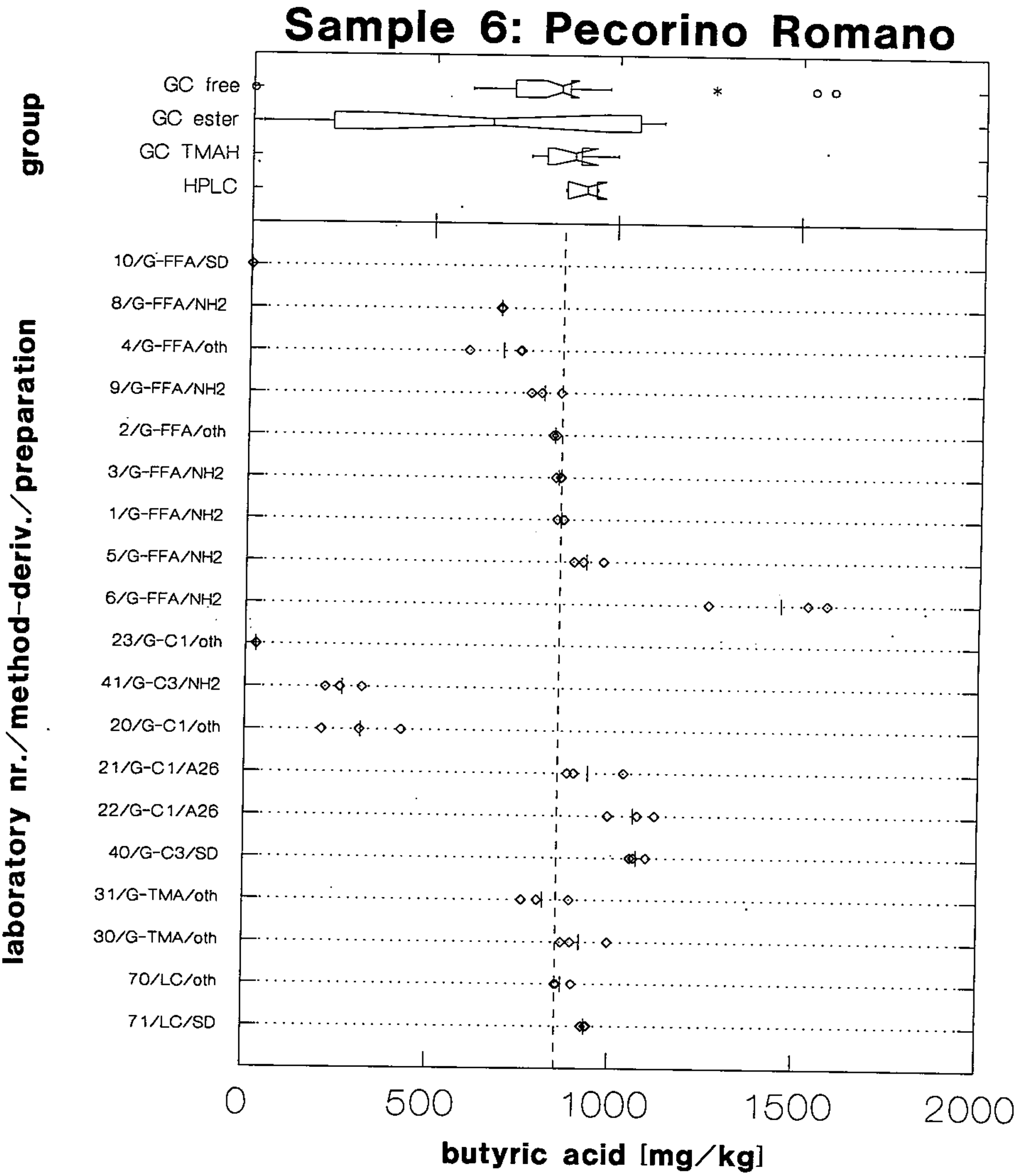


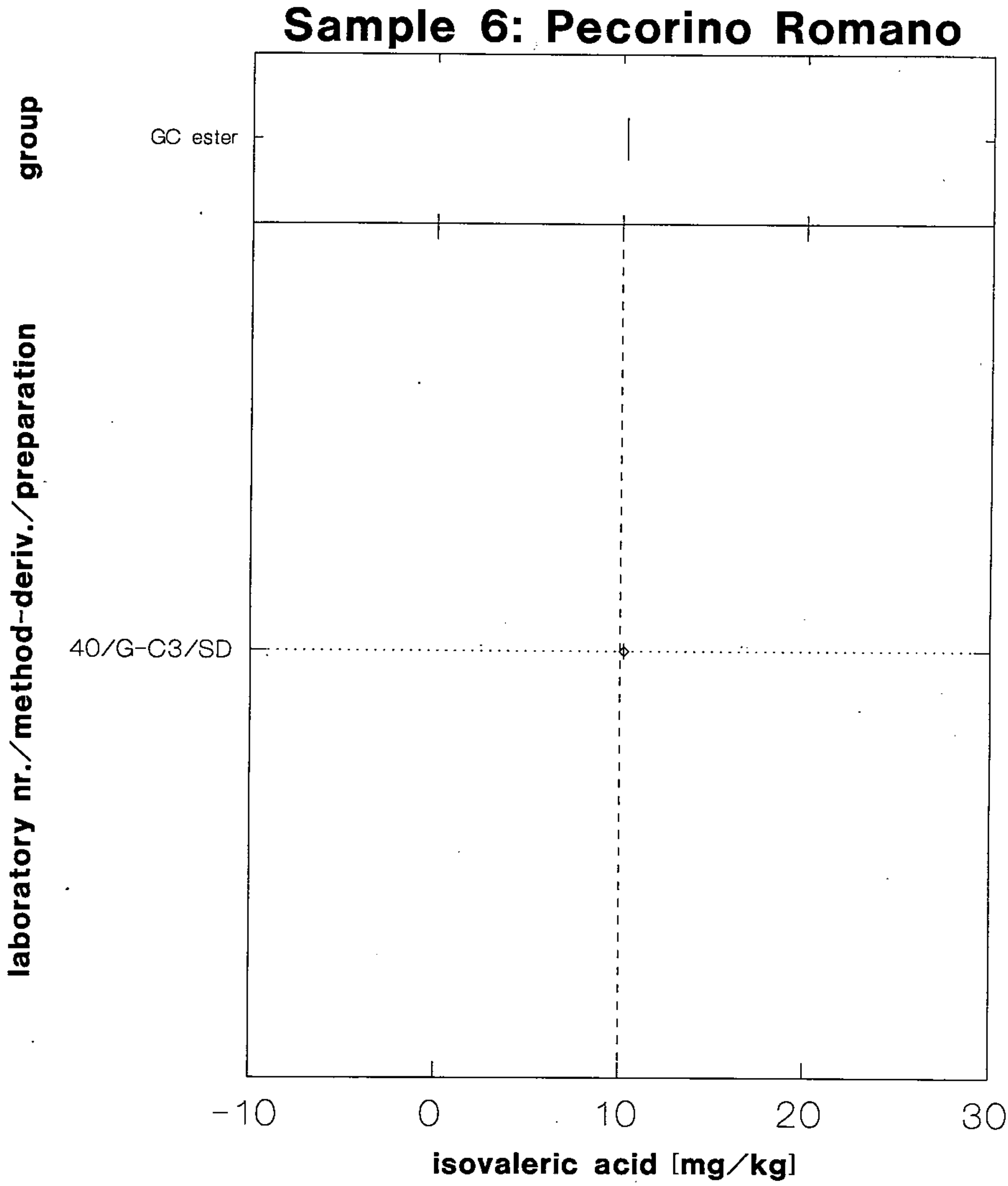
Sample 6: Pecorino Romano



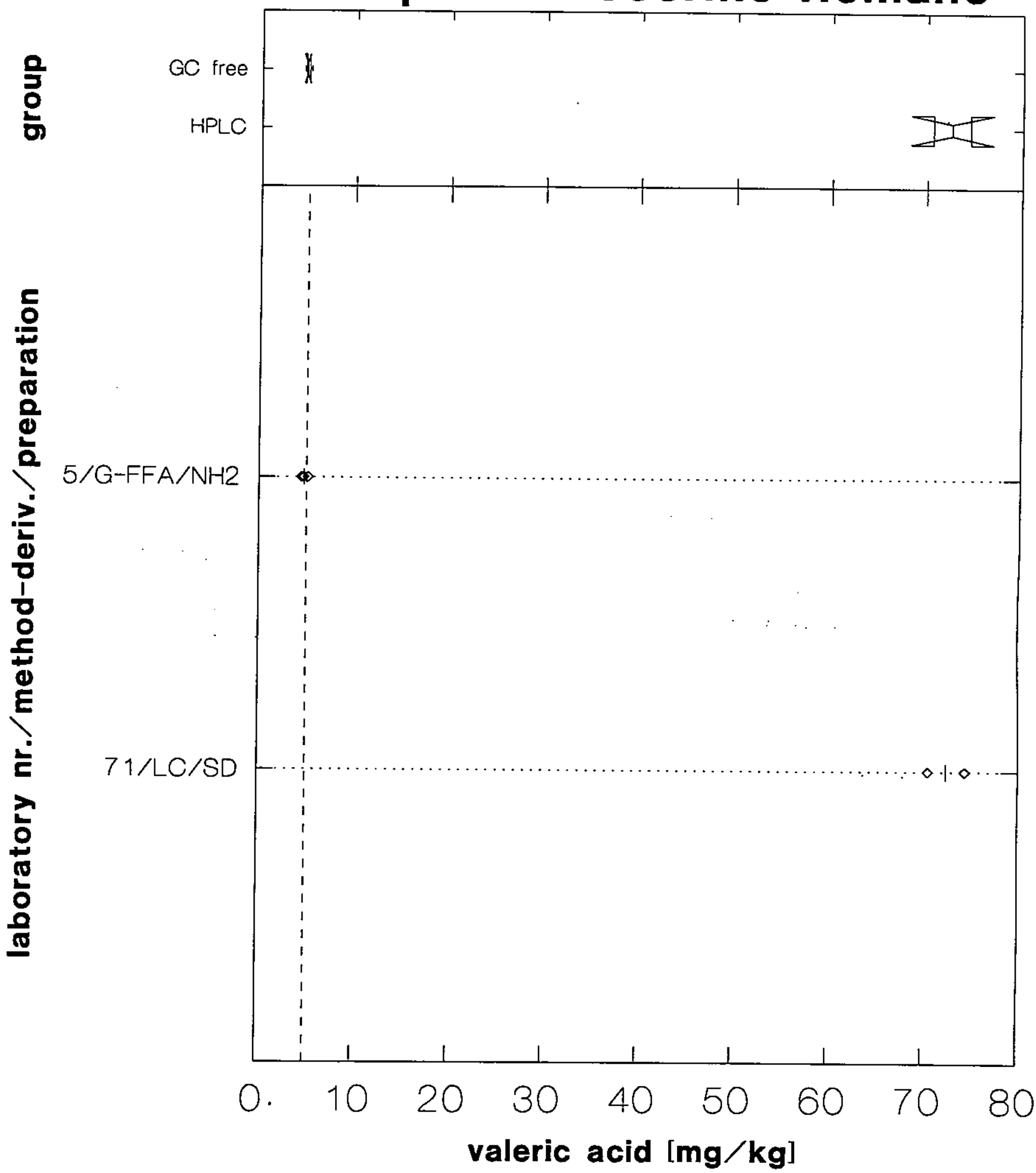
Sample 6: Pecorino Romano

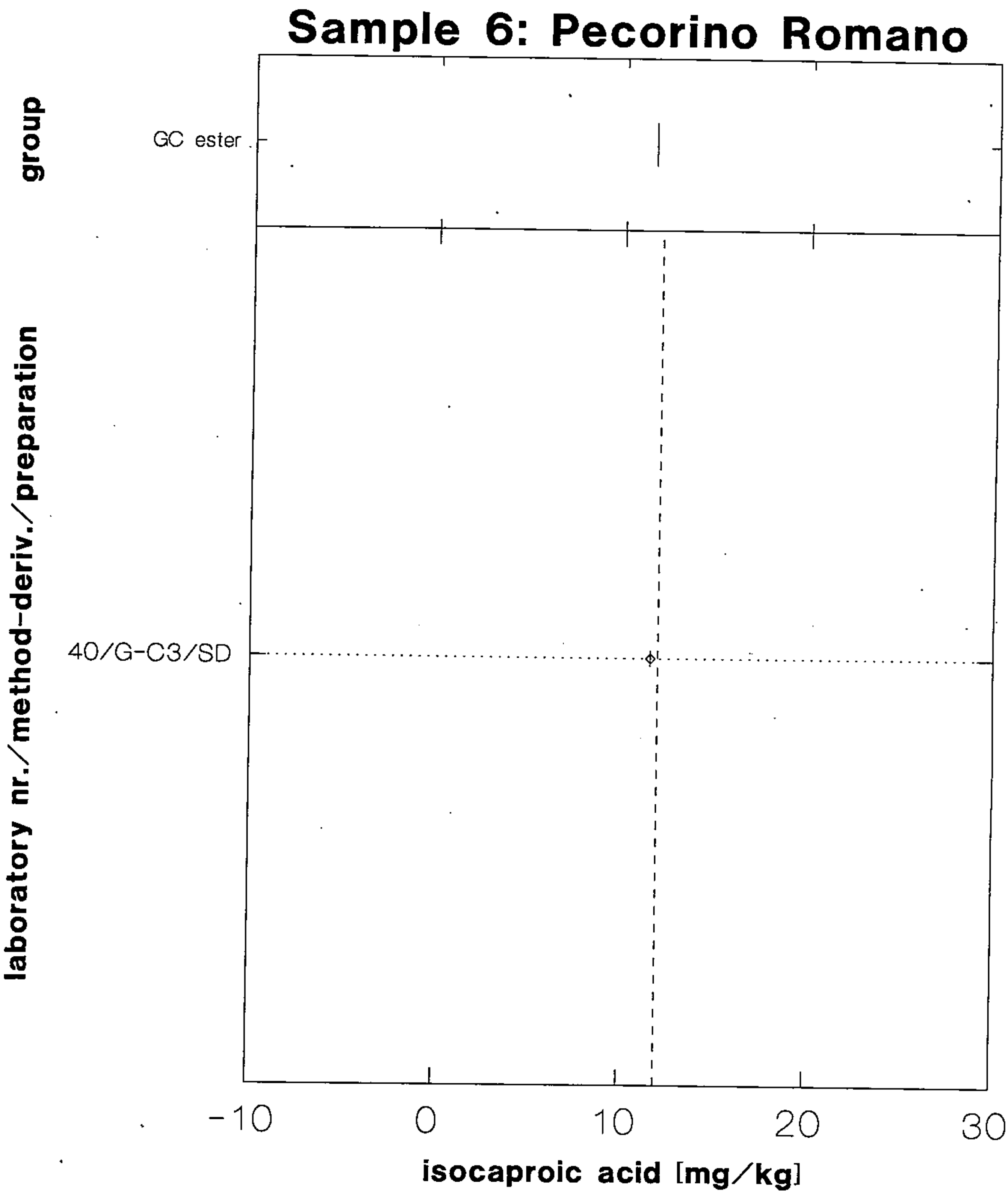


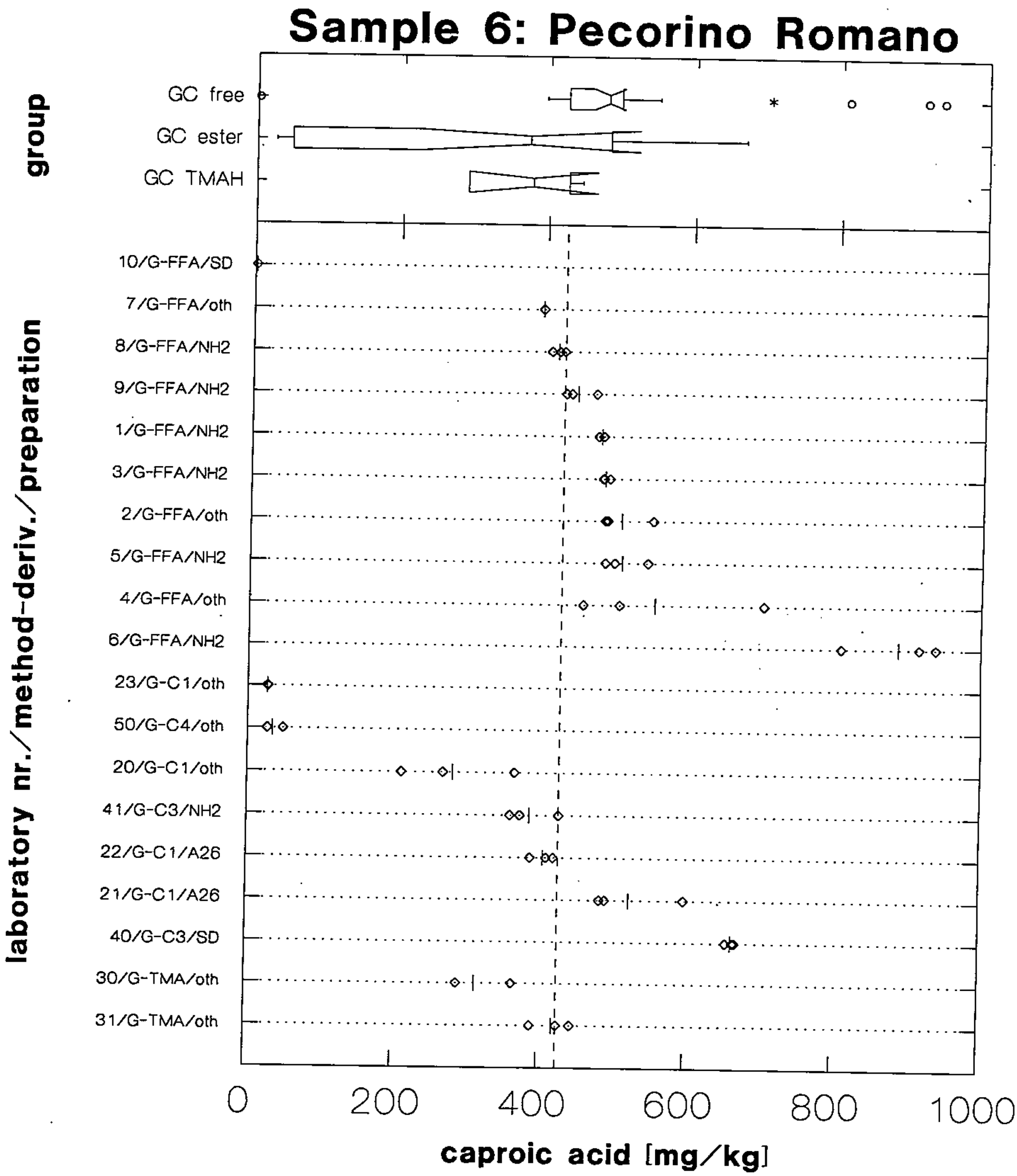

Sample 6: Pecorino Romano
group
GC free
GC ester
GC TMAH
HPLC
laboratory nr./method-deriv./preparation
10/G-FFA/SD
8/G-FFA/NH2
4/G-FFA/oth
9/G-FFA/NH2
2/G-FFA/oth
3/G-FFA/NH2
1/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
23/G-C1/oth
41/G-C3/NH2
20/G-C1/oth
21/G-C1/A26
22/G-C1/A26
40/G-C3/SD
31/G-TMA/oth
30/G-TMA/oth
70/LC/oth
71/LC/SD
0
500
1000
1500
2000
butyric acid [mg/kg]

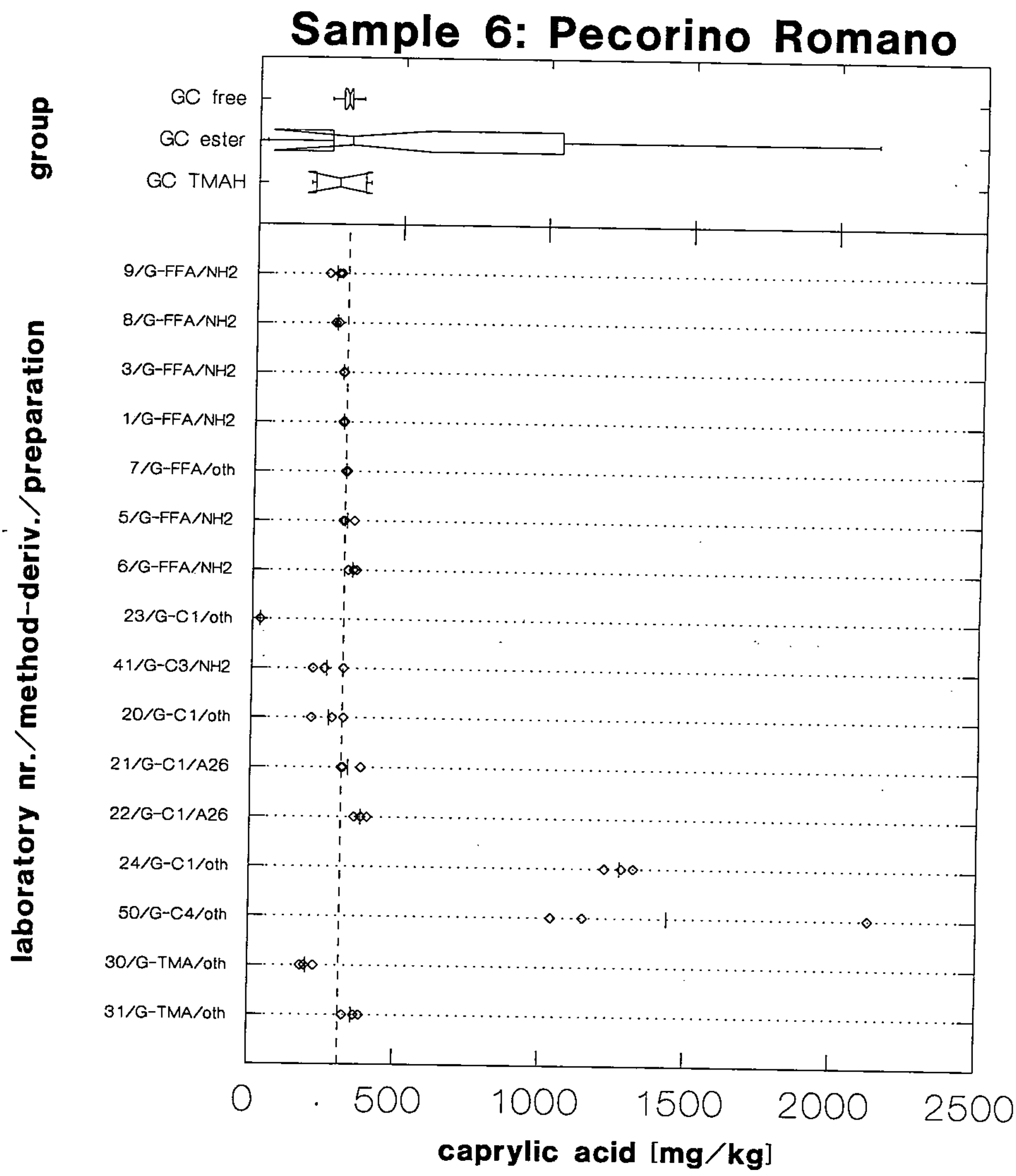

Sample 6: Pecorino Romano
group
GC ester
laboratory nr./method-deriv./preparation
40/G-C3/SD
-10
0
10
20
30
isovaleric acid [mg/kg]

Sample 6: Pecorino Romano

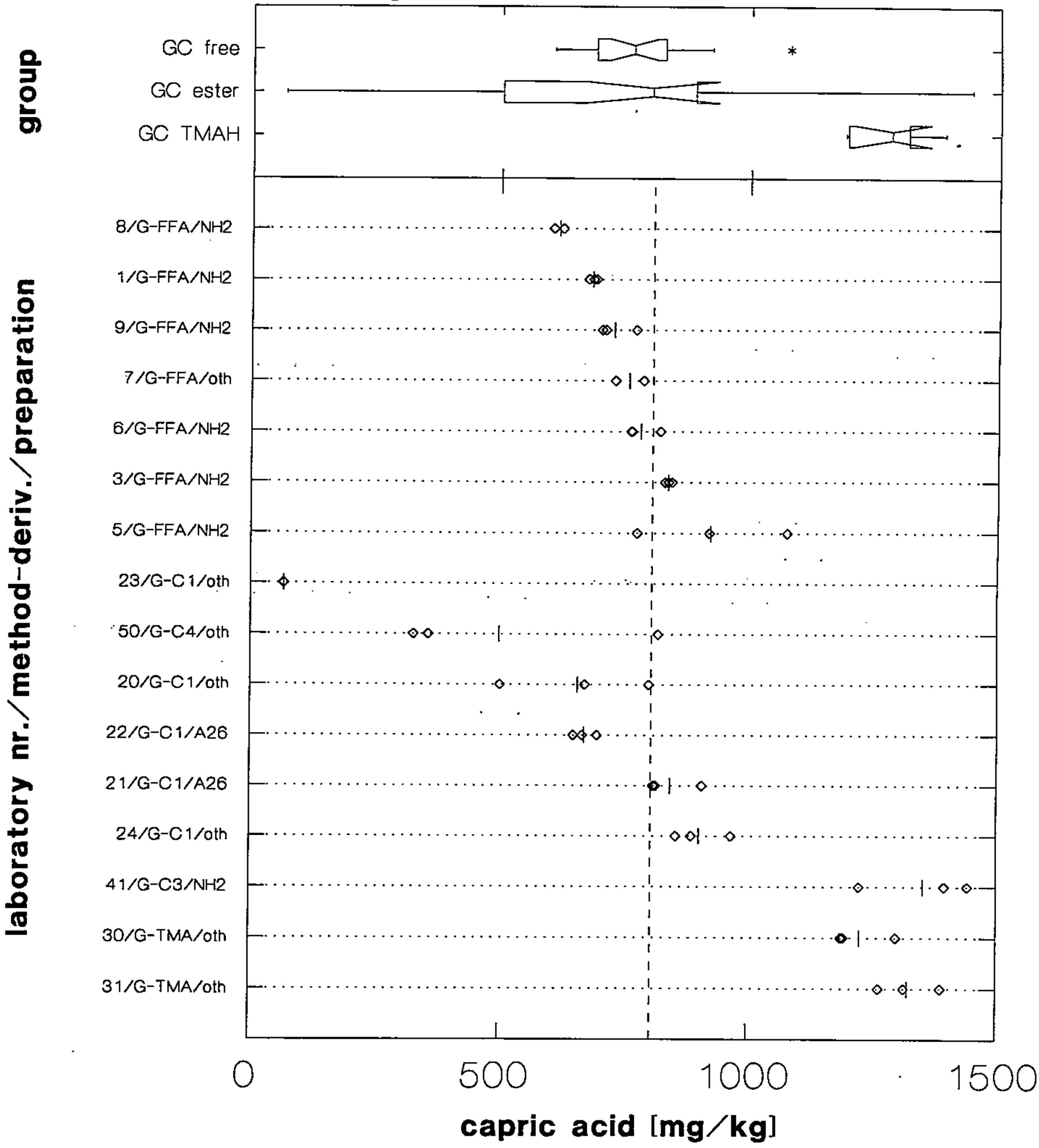


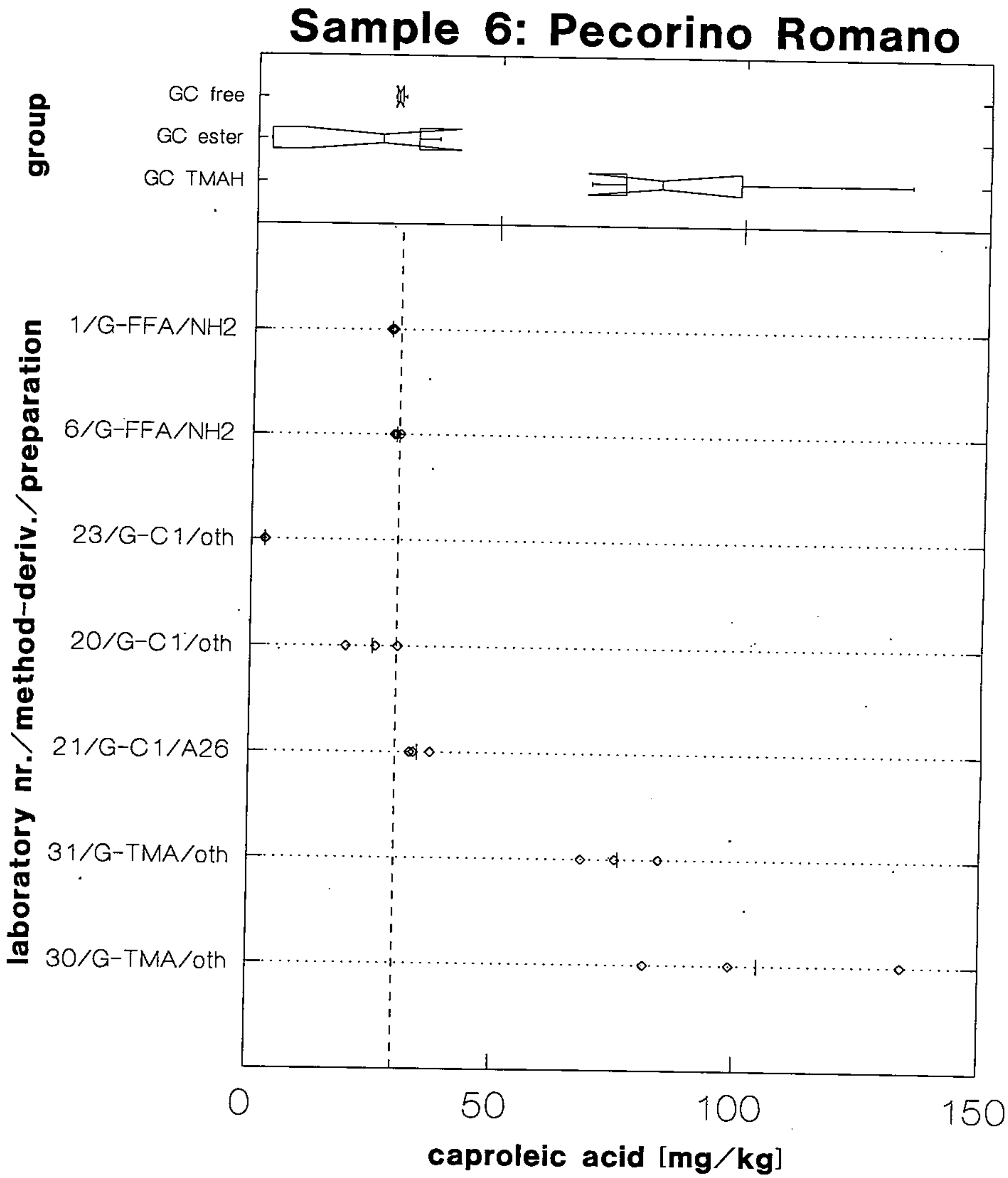

Sample 6: Pecorino Romano
group
GC ester
laboratory nr./method-deriv./preparation
40/G-C3/SD
-10
0
10
20
30
isocaproic acid [mg/kg]


Sample 6: Pecorino Romano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
10/G-FFA/SD
7/G-FFA/oth
8/G-FFA/NH2
9/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
2/G-FFA/oth
5/G-FFA/NH2
4/G-FFA/oth
6/G-FFA/NH2
23/G-C1/oth
50/G-C4/oth
20/G-C1/oth
41/G-C3/NH2
22/G-C1/A26
21/G-C1/A26
40/G-C3/SD
30/G-TMA/oth
31/G-TMA/oth
0
200
400
600
800
1000
caproic acid [mg/kg]

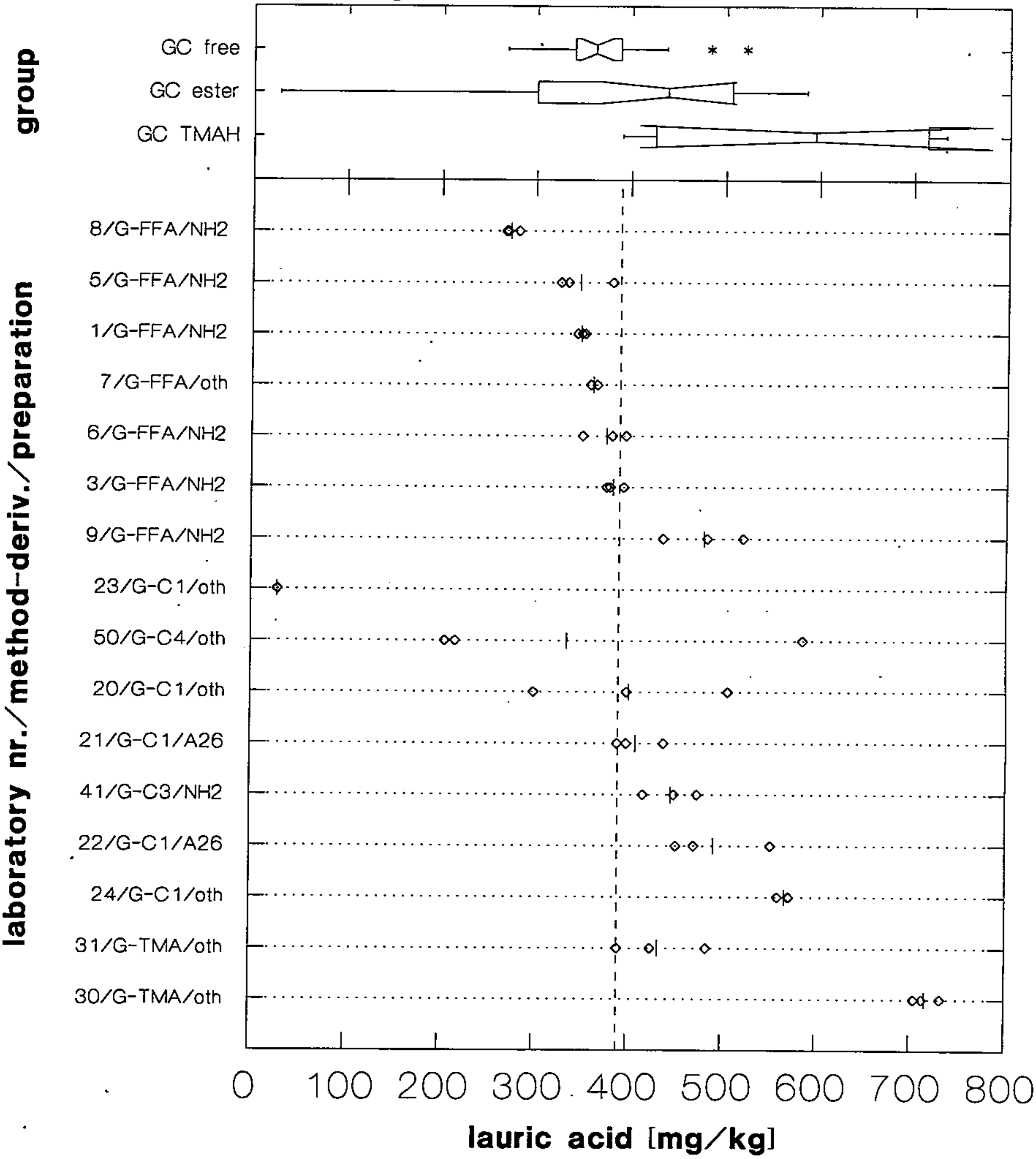

Sample 6: Pecorino Romano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
9/G-FFA/NH2
8/G-FFA/NH2
3/G-FFA/NH2
1/G-FFA/NH2
7/G-FFA/oth
5/G-FFA/NH2
6/G-FFA/NH2
23/G-C1/oth
41/G-C3/NH2
20/G-C1/oth
21/G-C1/A26
22/G-C1/A26
24/G-C1/oth
50/G-C4/oth
30/G-TMA/oth
31/G-TMA/oth
0
500
1000
1500
2000
2500
caprylic acid [mg/kg]

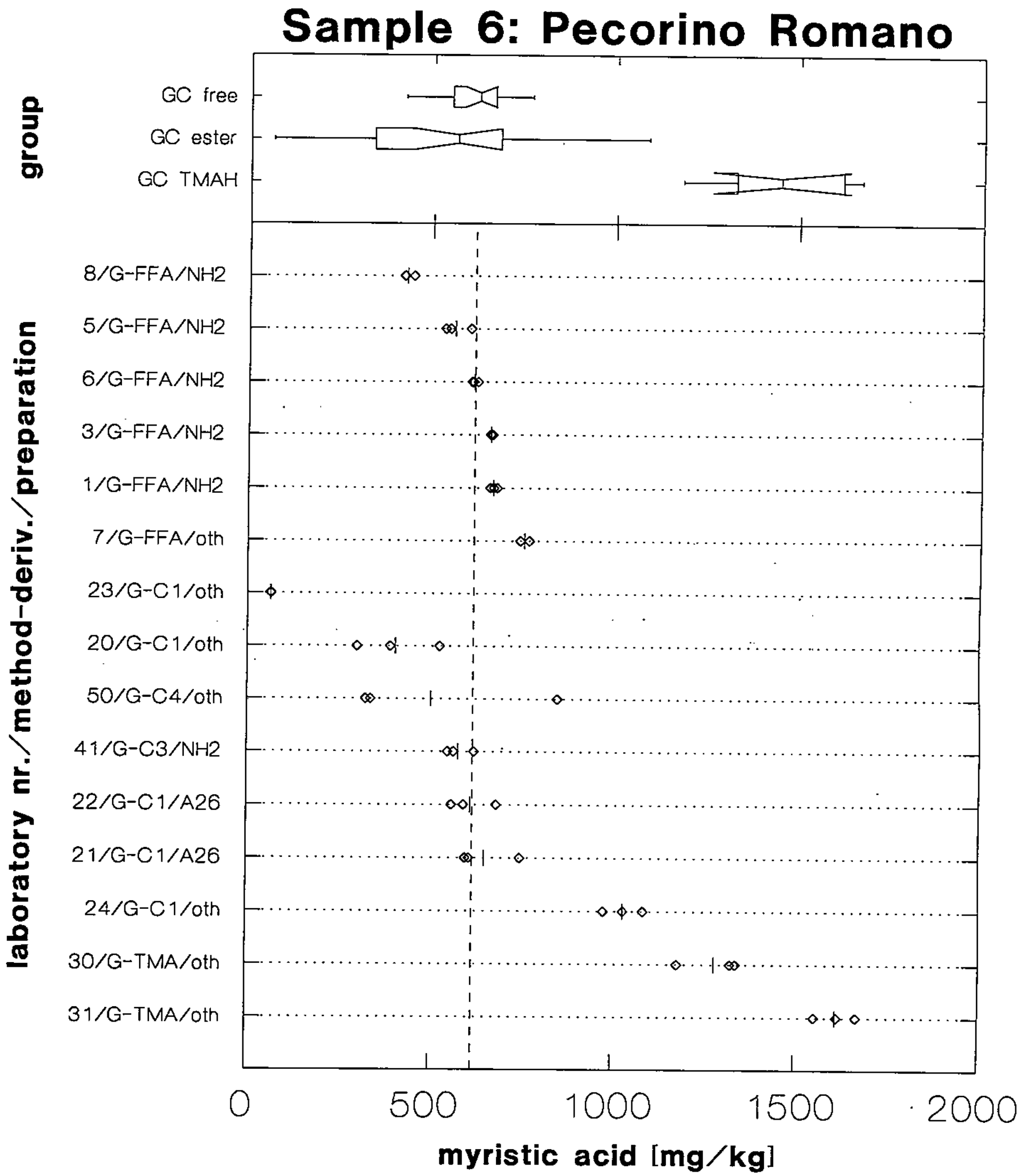
Sample 6: Pecorino Romano



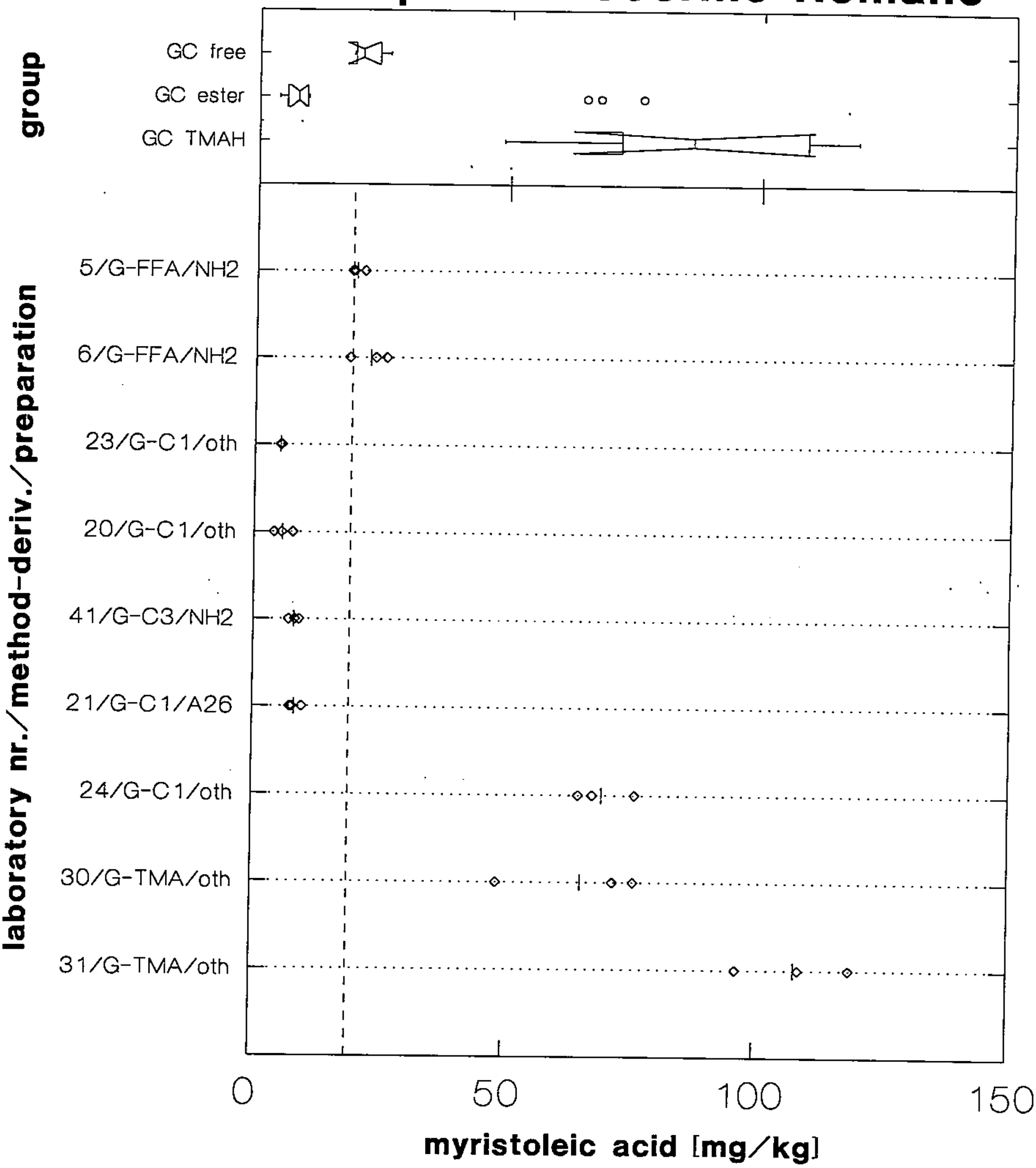

Sample 6: Pecorino Romano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
1/G-FFA/NH2
6/G-FFA/NH2
23/G-C1/oth
20/G-C1/oth
21/G-C1/A26
31/G-TMA/oth
30/G-TMA/oth
0
50
100
150
caproleic acid [mg/kg]

Sample 6: Pecorino Romano

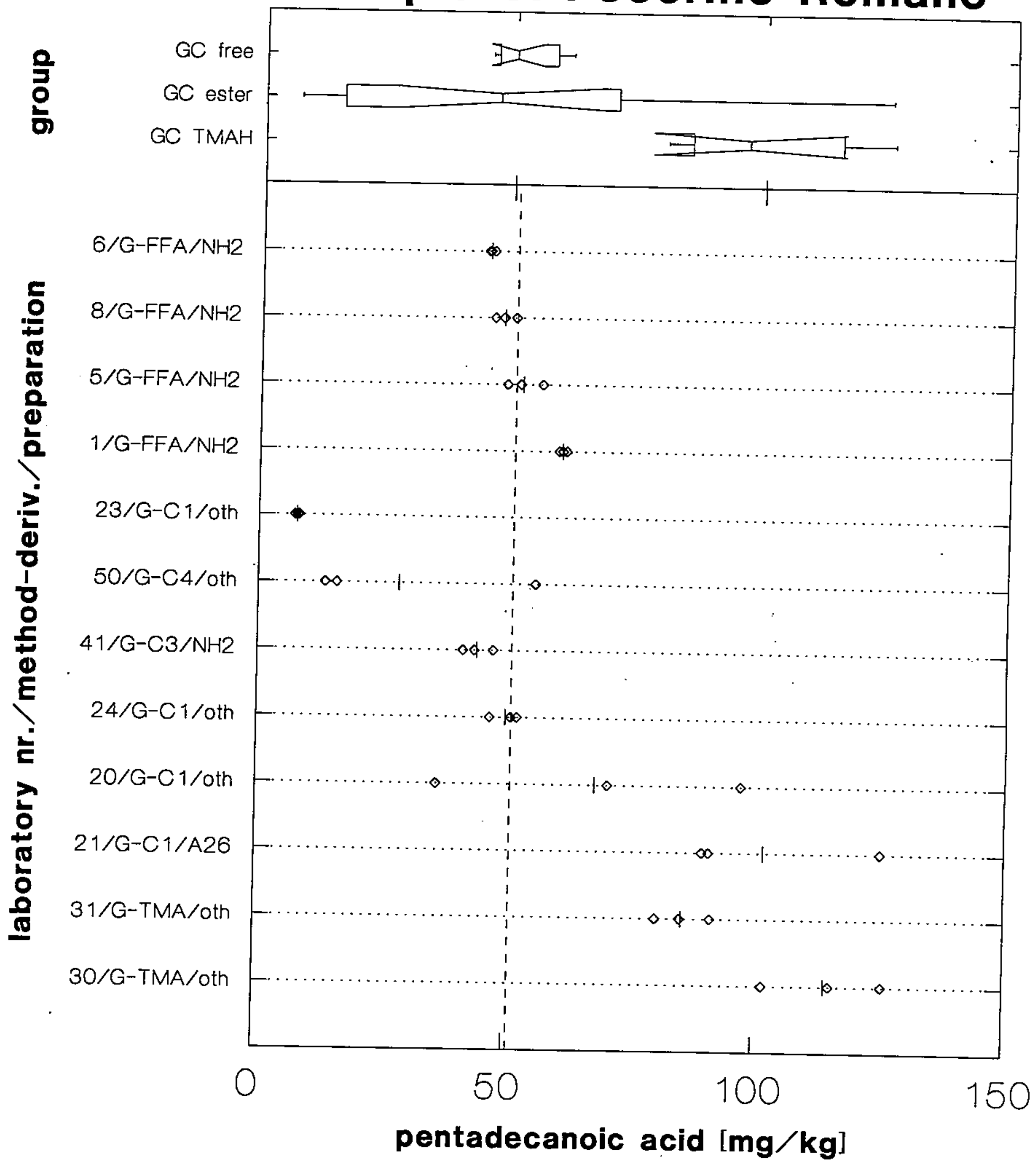



Sample 6: Pecorino Romano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
3/G-FFA/NH2
1/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
20/G-C1/oth
50/G-C4/oth
41/G-C3/NH2
22/G-C1/A26
21/G-C1/A26
24/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
500
1000
1500
2000
myristic acid [mg/kg]

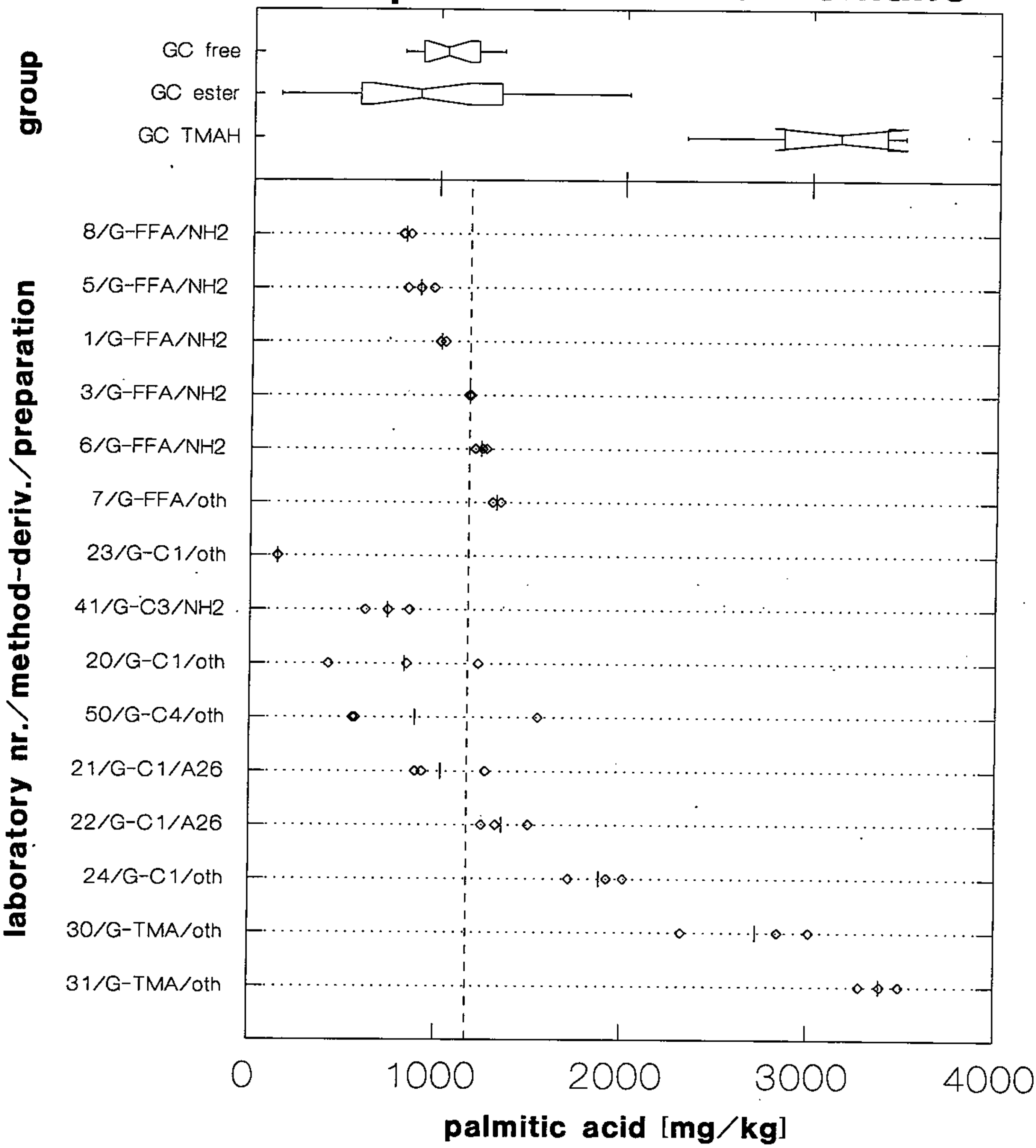
Sample 6: Pecorino Romano



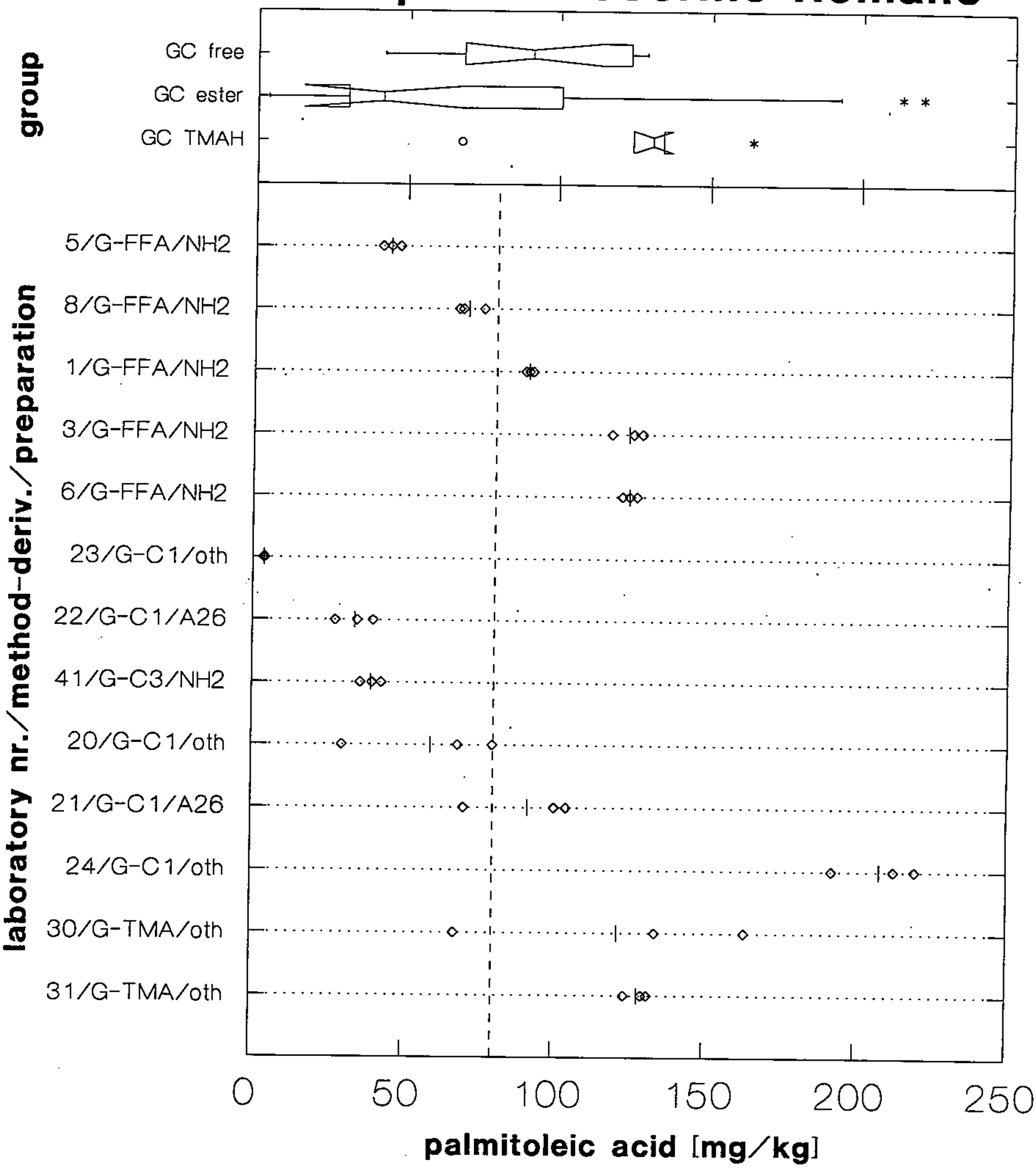
Sample 6: Pecorino Romano

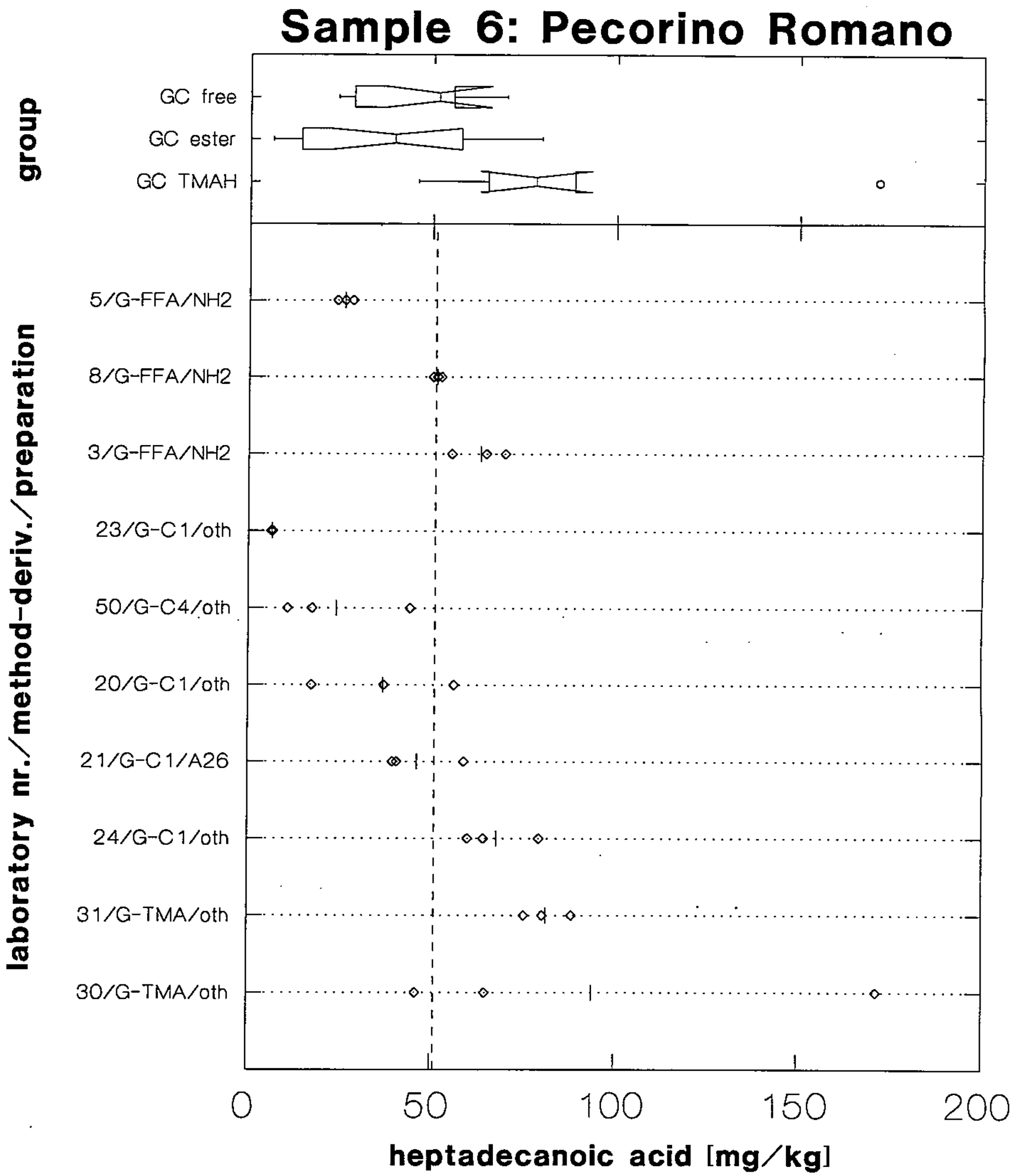


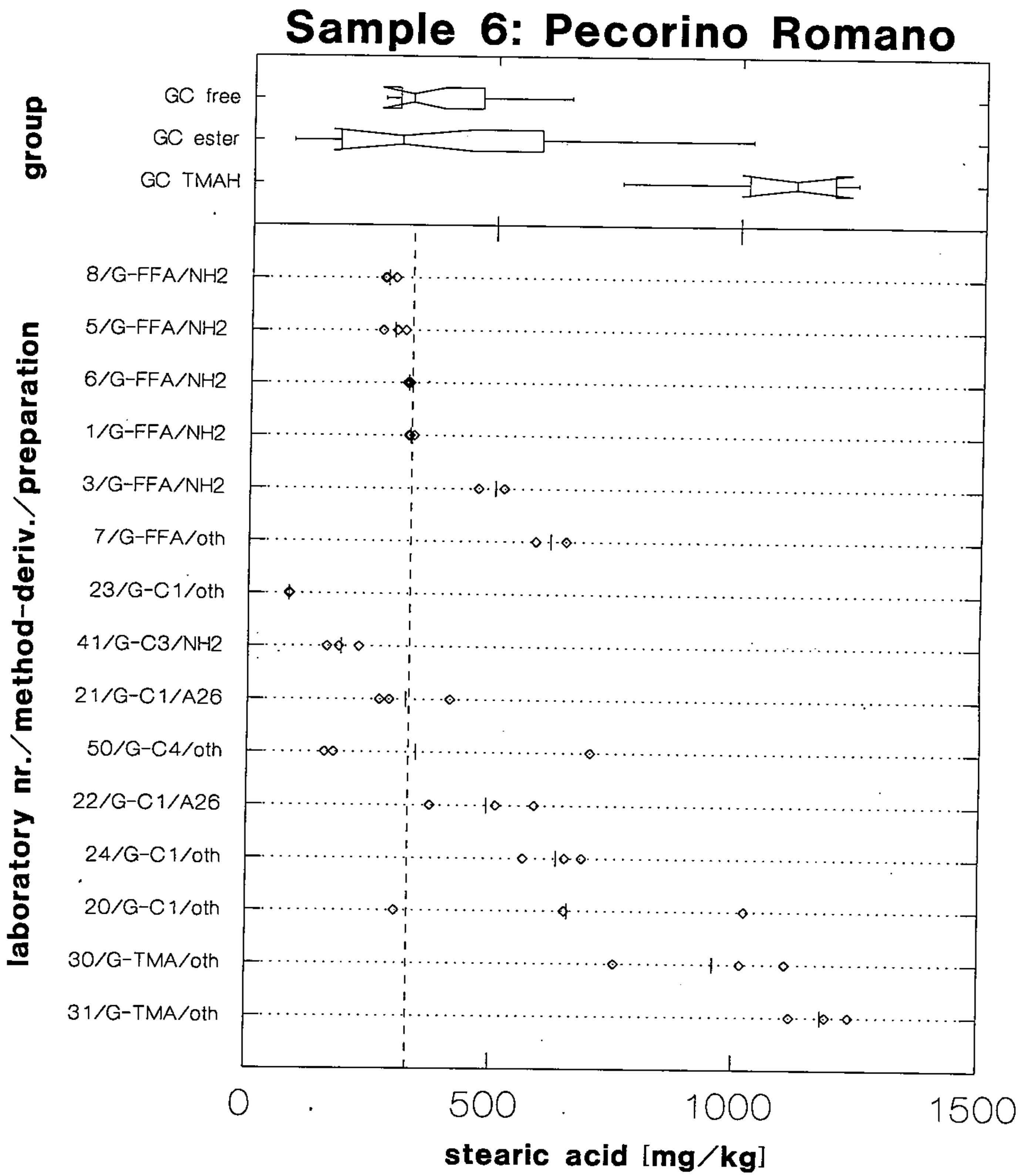
Sample 6: Pecorino Romano

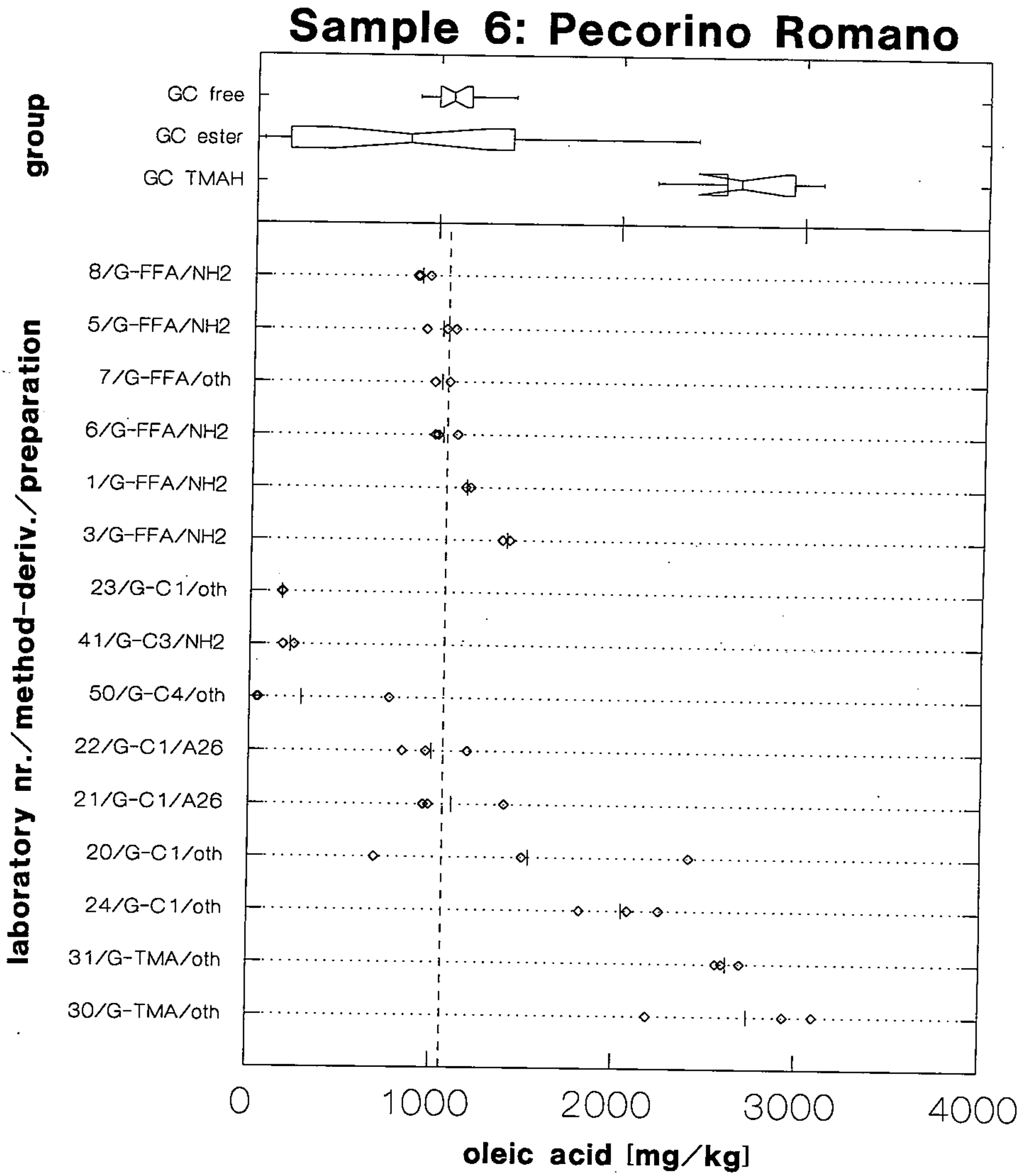


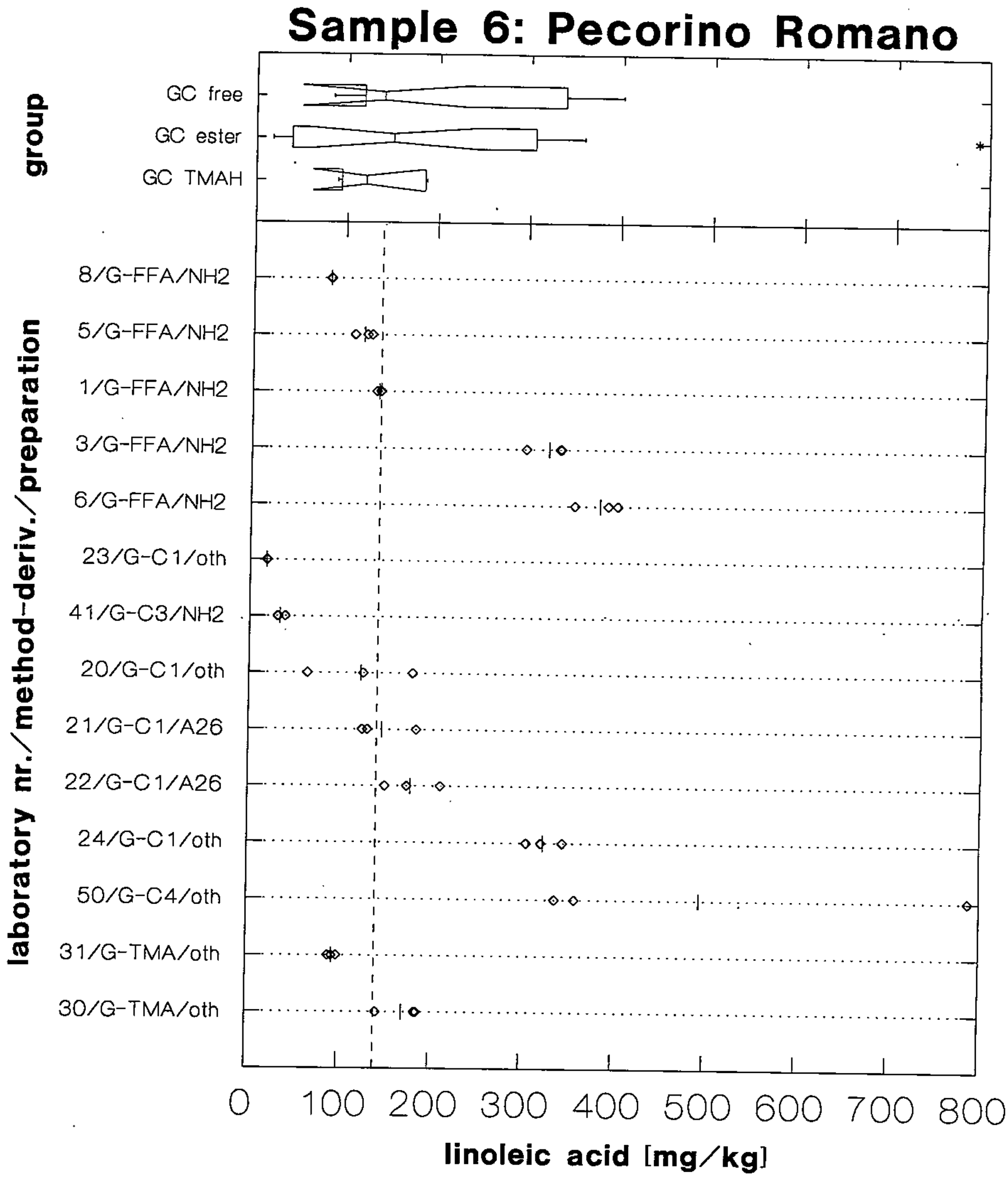
Sample 6: Pecorino Romano



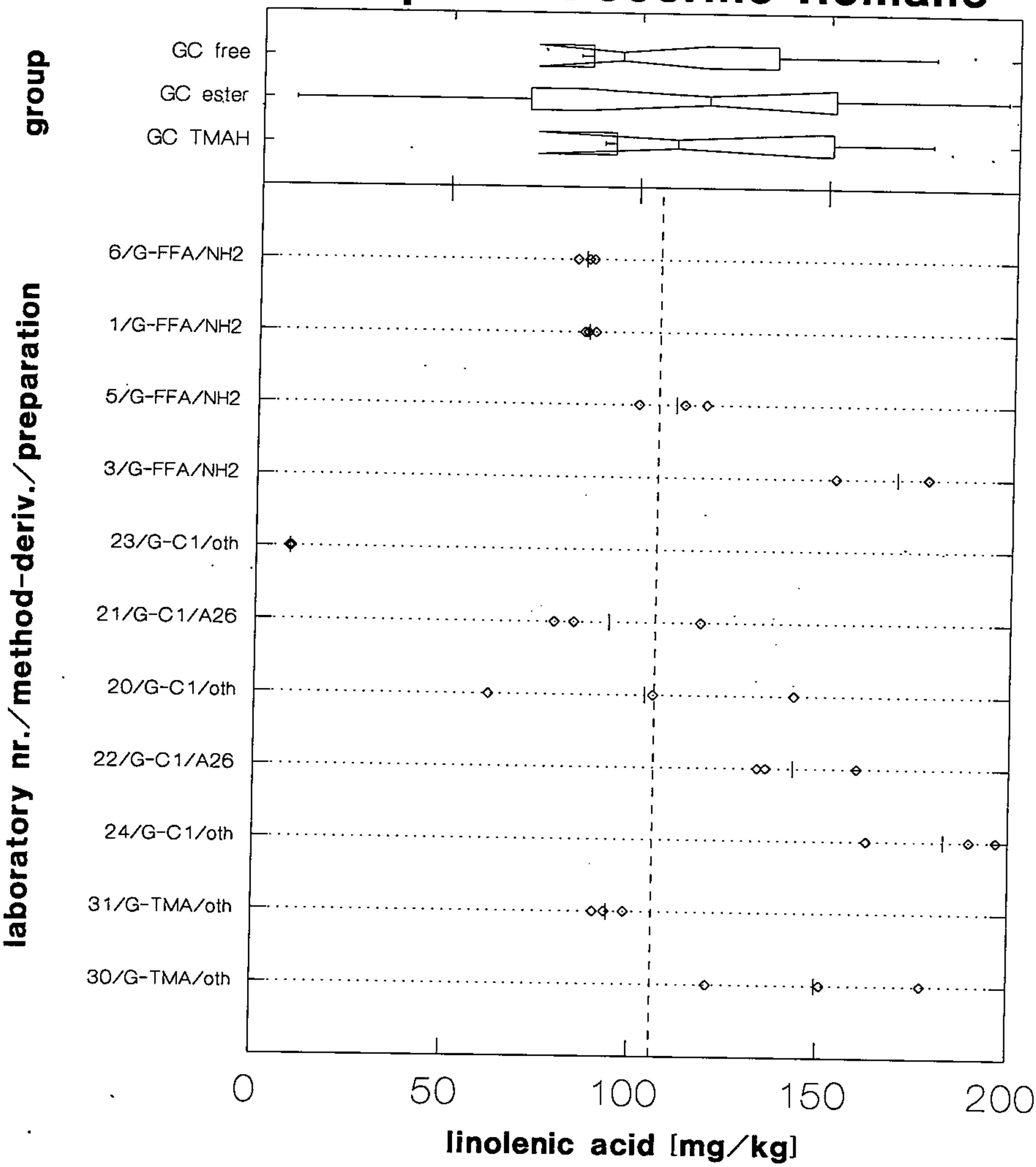

Sample 6: Pecorino Romano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
5/G-FFA/NH2
8/G-FFA/NH2
3/G-FFA/NH2
23/G-C1/oth
50/G-C4/oth
20/G-C1/oth
21/G-C1/A26
24/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
50
100
150
200
heptadecanoic acid [mg/kg]


Sample 6: Pecorino Romano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
5/G-FFA/NH2
6/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
7/G-FFA/oth
23/G-C1/oth
41/G-C3/NH2
21/G-C1/A26
50/G-C4/oth
22/G-C1/A26
24/G-C1/oth
20/G-C1/oth
30/G-TMA/oth
31/G-TMA/oth
0
500
1000
1500
stearic acid [mg/kg]

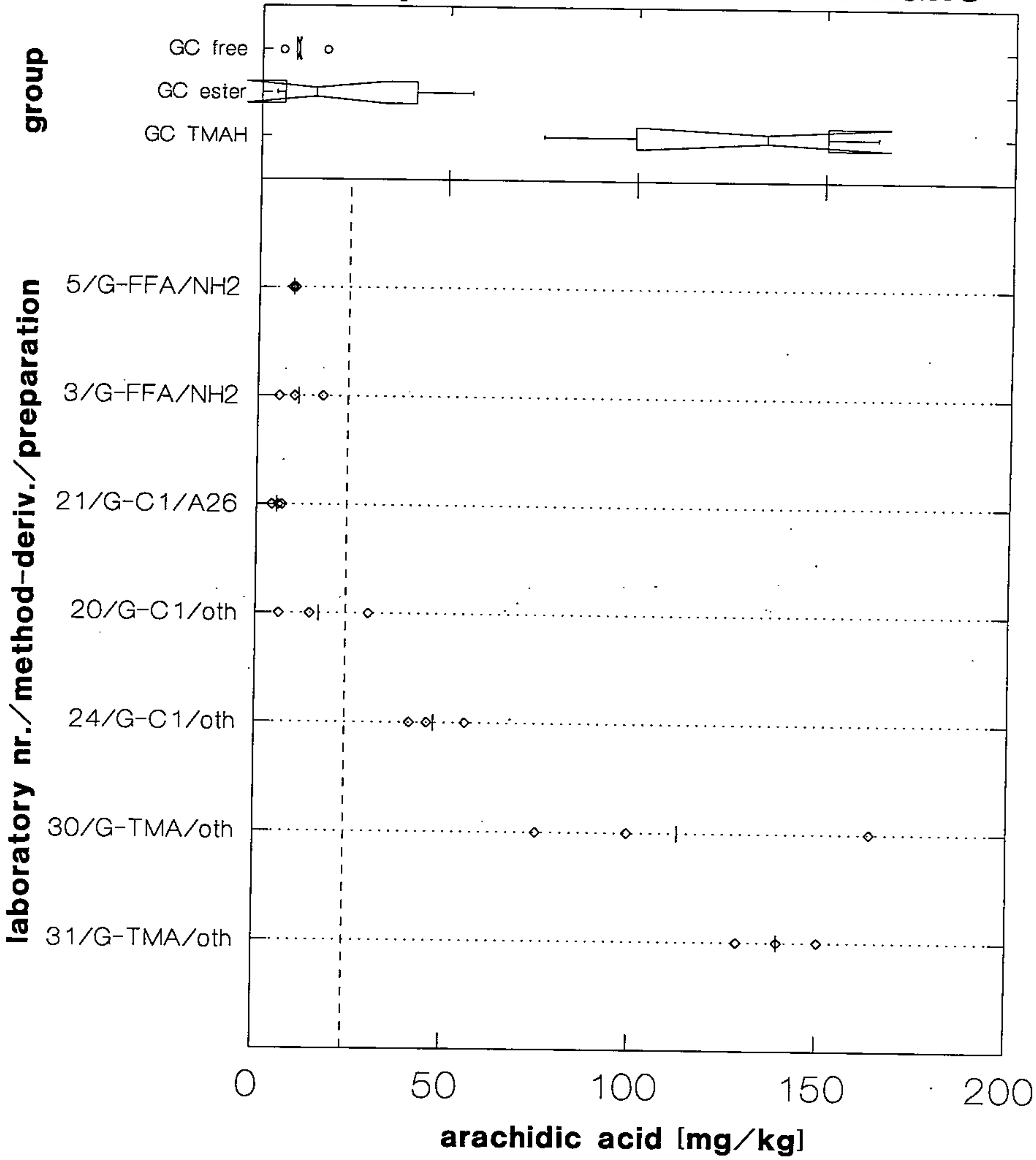

Sample 6: Pecorino Romano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
5/G-FFA/NH2
7/G-FFA/oth
6/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
23/G-C1/oth
41/G-C3/NH2
50/G-C4/oth
22/G-C1/A26
21/G-C1/A26
20/G-C1/oth
24/G-C1/oth
31/G-TMA/oth
30/G-TMA/oth
0
1000
2000
3000
4000
oleic acid [mg/kg]


Sample 6: Pecorino Romano
group
GC free
GC ester
GC TMAH
laboratory nr./method-deriv./preparation
8/G-FFA/NH2
5/G-FFA/NH2
1/G-FFA/NH2
3/G-FFA/NH2
6/G-FFA/NH2
23/G-C1/oth
41/G-C3/NH2
20/G-C1/oth
21/G-C1/A26
22/G-C1/A26
24/G-C1/oth
50/G-C4/oth
31/G-TMA/oth
30/G-TMA/oth
0
100
200
300
400
500
600
700
800
linoleic acid [mg/kg]

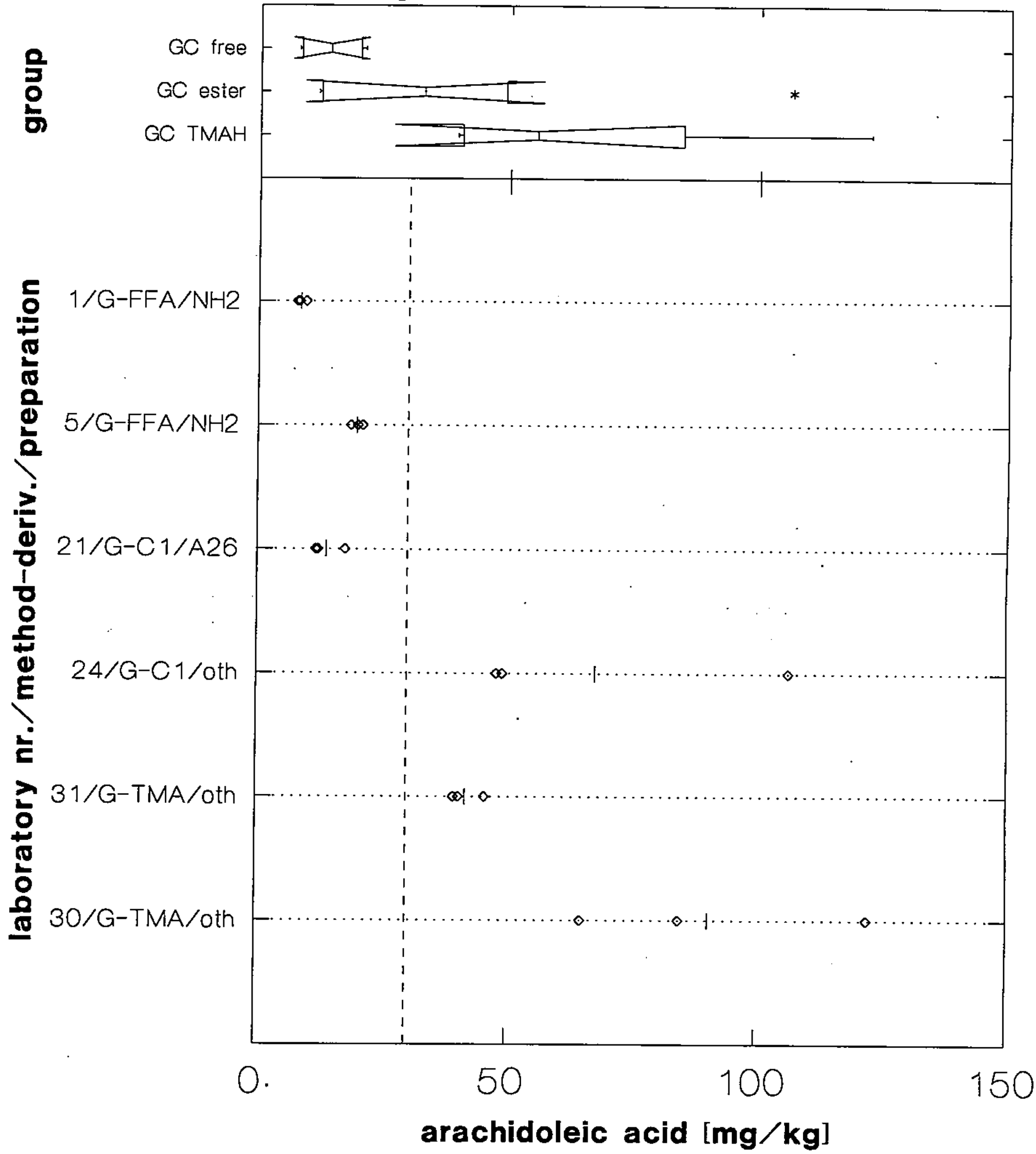
Sample 6: Pecorino Romano

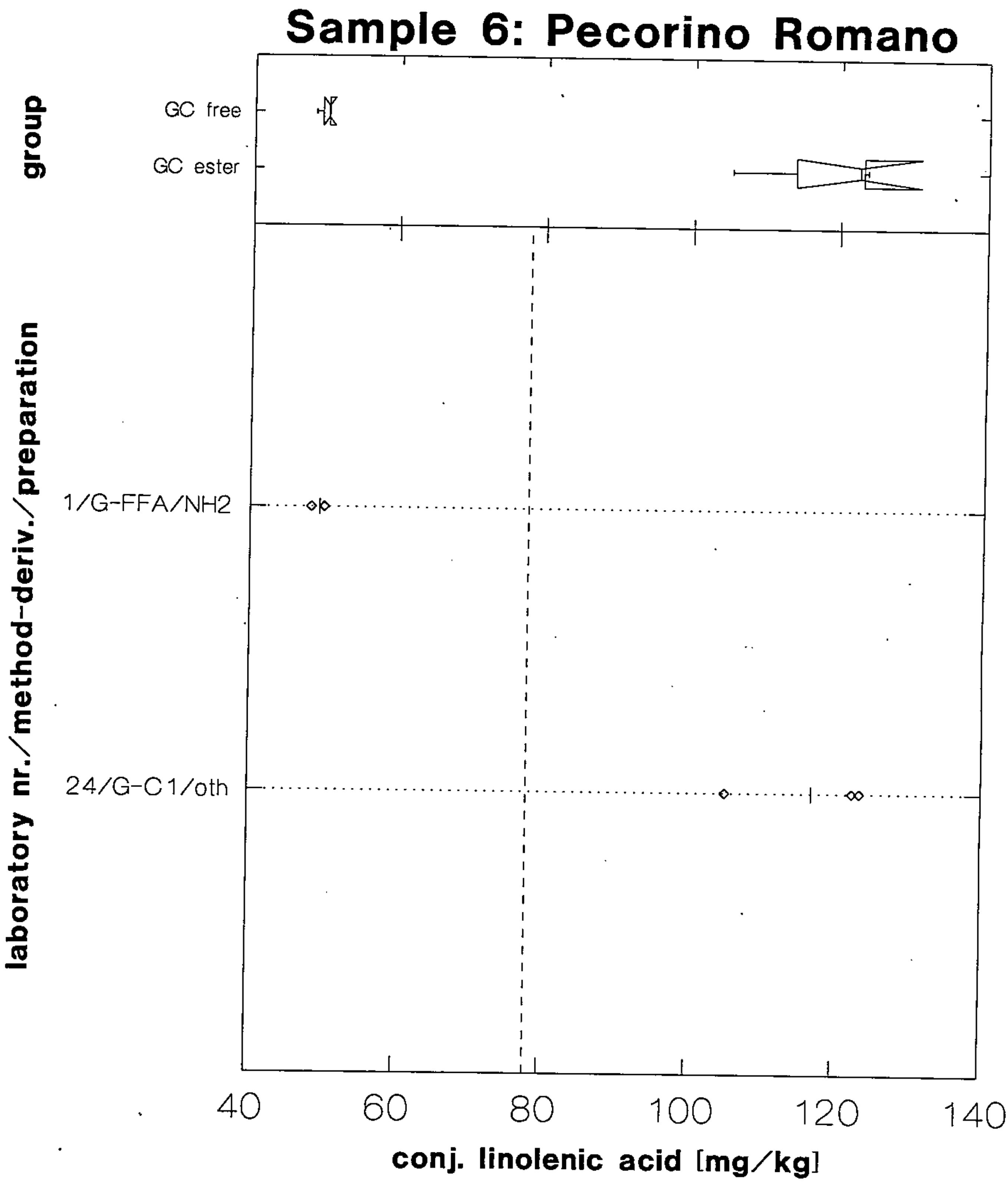


Sample 6: Pecorino Romano



Sample 6: Pecorino Romano




Sample 6: Pecorino Romano
group
GC free
GC ester
laboratory nr./method-deriv./preparation
1/G-FFA/NH2
24/G-C1/oth
40
60
80
100
120
140
conj. linolenic acid [mg/kg]