Lipase Activity

PLU assay
Determination by capillary-GC

C-GC analyses

The injection volume is 0.7µl.
Carrier gas is helium, flow 1.7ml/min (46.2cm/sec), split 30(80°C).

Method

Injector temperature 280°C.
Detector temperature 280°C.

Column temperature program:

0-2	min.	80°C.
2-5.4	min	80-250°C, temperature slope 50°C/min.
5.4-10	min	250°C.

After a series of injections the equipment is cleaned for 3 hours at the following temperatures:

Injector	350°C
Detector	350°C
Column	250°C.

For the C-GC analyses approx. 5µl of the reaction mixture is diluted with 995µl heptane. The C-GC yields all three components (1-propanol, propyllaurate and lauric acid) well seperated. The retention times are approx. 0.9, 4.8, 6.3 minutes, respectively. All analyses are made in duplicate, and mixed standards are analyzed both before and after the samples. It is important, that all flasks, vials etc. are sealed immiediately after use to prevent evaporation of propanol.

The following 6 mixed standards are used:

Table 1

	Concentration (mM)		
Standard no.	**1-Propanol**	**Propyllaurate**	**Lauric acid**
1	0	0	0
2	4	1	4
3	8	2	8
4	12	3	12
5	16	4	16
6	20	5	20

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Calculations

From the analyses made on the standards a standard curve is made for each of the three materials. The slopes are used as response factors.The correlation coefficient is close to 1.

The conversion C of either lauric acid or 1-propanol into propyllaurate is calculated as:

$$C_{LA} = \frac{PL}{PL + LA} = \frac{\frac{\text{area PL}}{\text{RF PL}}}{\frac{\text{area PL}}{\text{RF PL}} + \frac{\text{area LA}}{\text{RF LA}}}$$

$$C_{PR} = \frac{PL}{PL + PR} = \frac{\frac{\text{area PL}}{\text{RF PL}}}{\frac{\text{area PL}}{\text{RF PL}} + \frac{\text{area PR}}{\text{RF PR}}}$$

Where

C_{LA}: Fraction of lauric acid, which is transformed into propyllaurate.
C_{PR}: Fraction of 1-propanol, which is transformed into propyllaurate.
PL: Concentration of propyllaurate (mM).
LA: Concentration of lauric acid (mM).
PR: Concentration of 1-propanol (mM).
Area PL: Area of propyllaurate peak on chromatogram (Counts).
RF PL: Response factor of propyllaurate from standard curve (Counts/mM).

The conversion is then used in the formula below to calculate the activity of the enzyme analyzed in PLU/g.

$$\text{Activity PLU / g} = \frac{M \times C}{W \times t}$$

Where
M: Initial amount of 1-propanol and lauric acid, i.e. 40000 µmole.
C: Fraction ester from the formula above.
W: Amount of dry matter of catalyst in g.
t: Reaction time in min.

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