

Tentamen T1 Chemische Analysemethoden  
1 maart 2013

Naam:

Student nummer:

**Geef uw antwoord op dit papier. U mag uw tekstboek, aantekeningen, liniaal en een rekenmachine gebruiken.**

(1) (10 punten) De specifieke activiteit van een enzym (in arbitraire eenheden) werd bepaald met behulp van twee onafhankelijke methoden in zes verschillende monsters. Elk monster werd één keer met Methode 1 gemeten en één keer met Methode 2. De resultaten van de metingen zijn samengevat in Tabel 1.

**Tabel 1**

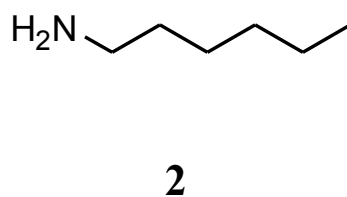
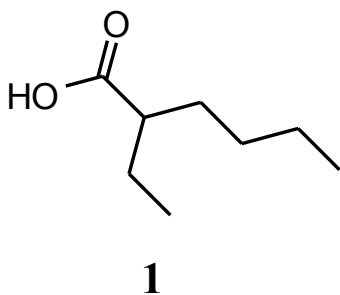
Methode	Enzymatische activiteit (zes monsters)					
	Monster 1	Monster 2	Monster 3	Monster 4	Monster 5	Monster 6
1	139	147	160	158	135	141
2	148	159	156	164	149	134

Bepaal of deze twee methoden significant verschillende uitkomsten geven op 95% betrouwbaarheidsniveau. (*Laat alle tussenberekeningen zien en licht je antwoord toe*).

(2) (5 punten) (a) Which method in Question 1 provided a more precise measurements? Why?

(2) (5 punten) (b) Is it possible to determine what method is more accurate? Why or why not?

3) (20 punten)



a. A mixture of compound **1** and **2** is loaded on a bare silica column at pH 10. The column is eluted with a mixture of MeOH en H<sub>2</sub>O. What is the elution order? Justify your answer (5 *punten*)

b. Would the elution order change at pH 4? Explain your answer (5 *punten*)

**c.** Give two properties of the column that you would change to improve the resolution (5 *punten*)

**d.** Suppose your mixture of compounds **1** and **2** is buffered at pH 10. Would you use a gradient that increases or decreases the pH to efficiently remove compound **2** from the column? Explain your answer (5 *punten*)

$$1) \bar{x}_1 = \frac{\sum x_i}{n} = \frac{146.6}{9} = 16.3$$

$$s_1 = \sqrt{\frac{\sum (x_i - \bar{x}_1)^2}{n-1}} = 10.3$$

$$\bar{x}_2 = \frac{\sum x_i}{n} = \frac{151.6}{9} = 16.8$$

$$s_2 = \sqrt{\frac{\sum (x_i - \bar{x}_2)^2}{n-1}} = 10.6$$

$$s_{pooled} = \sqrt{\frac{s_1^2(n_1-1) + s_2^2(n_2-1)}{n_1+n_2-2}} = \sqrt{\frac{(10.3)^2(8) + (10.6)^2(8)}{102}}$$

$$= 10.5$$

$$t_{calc} = \frac{|\bar{x}_1 - \bar{x}_2|}{s_{pooled}} \sqrt{\frac{8(8)}{122}} = 0.825 < t_{table} = 2.228$$

⇒ difference is not significant.

2(a) The standard deviations are very similar, so both methods have a similar precision, but method 1 has a slightly smaller standard deviation, so it could be considered very slightly more precise.

(b) No, we can't say which is more accurate since we don't know the true value.

3 (a) Bare silica is negatively charged as is 1, so 1 would elute first, then 2.

(b) at pH 4, 1 will be neutral, but 2 will be positively charged, thus 1 would elute first.

3(c) - increase column length  
- decrease particle size (in HPLC)

3(d) To remove compound 2 more efficiently, you would like it to lose its positive charge and thus the pH should increase.