Histamine in Fish and Theanine in Tea – Two Cases for Employing Planar Chromatography

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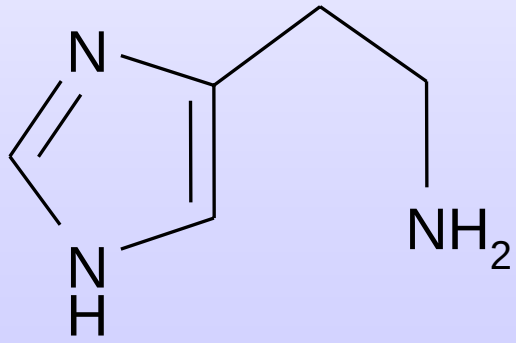
Biogenic amines

Why fish is a problem

- High water content and soft protein
- Enzymes show high activity at low temperatures
- Some fish species obtain high contents of free amino acids



Histamine

Histamine toxication

- Short incubation time

- Decrease of the blood pressure
- Dizziness
- Gastric spasms and headache
- Nausea and vomiting

Histamine in food

< 50	ppm	are safe
50 - 200	ppm	may be toxic
200 – 500	ppm	are toxic
500 – 1000	ppm	are toxic and dangerous

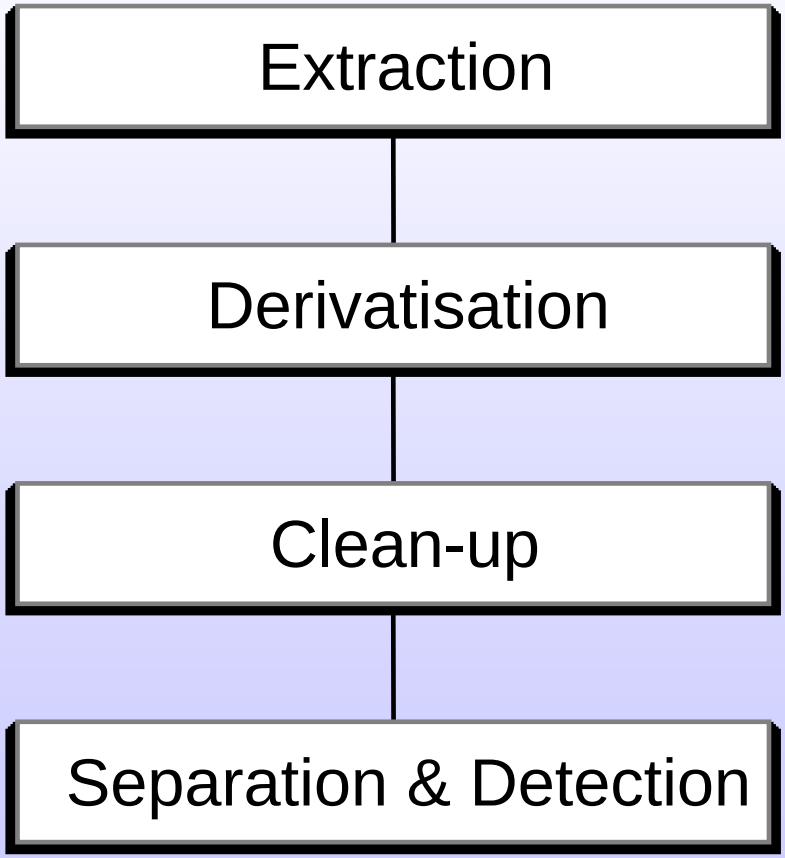
Legal regulations in Germany

- Paragraph 16 FischhygieneVO and guideline 95/71/EU

- Scombridae, Clupeidae, Engraulidae, Coryphaenidae and Istiophoridae
  > 200 mg Histamine/kg » Marketing ban
- Engraulidae in salt brine
  > 400 mg Histamine/kg » Marketing ban

- Appendix 3 of Fischhygiene-VO with guideline 91/493/EU

Analysis of biogenic amines



Development of a TLC - screening-method

CAMAG Automatic TLC Sampler III

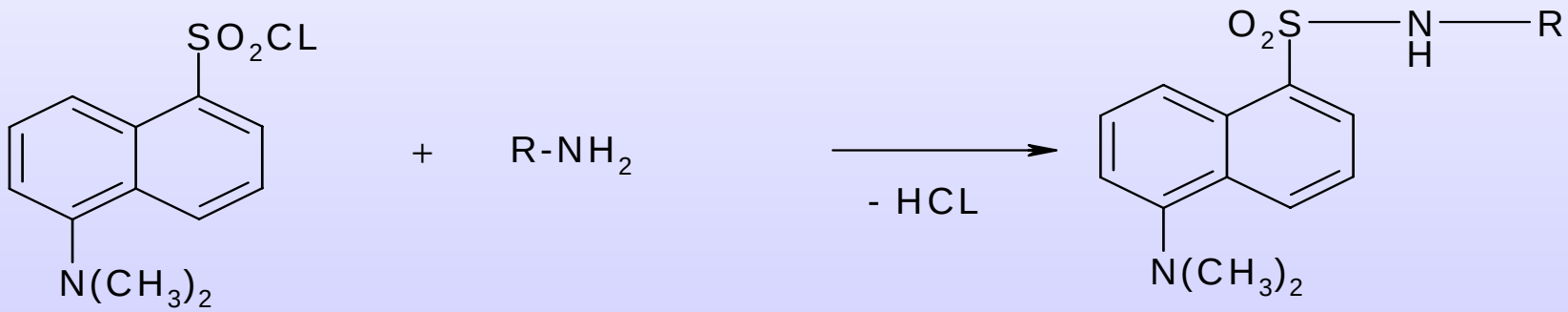
CAMAG TLC Scanner II

Extraction

10 g of the homogenized sample
+ 80 ml TCA (10%)
Ultra-Turrax 90 s
adjust to 100 ml with TCA and filter

Derivatisation

1 ml of the filtrate is adjusted to pH 8
Add 1 ml borate-puffer and 2 ml dansyl chloride solution
Incubate for 1 h
Adjust to 10 ml with water



Clean-up

Add 5 ml of diethyl ether

Shake vigorously and centrifuge

Remove the organic layer and shake the water residue twice more

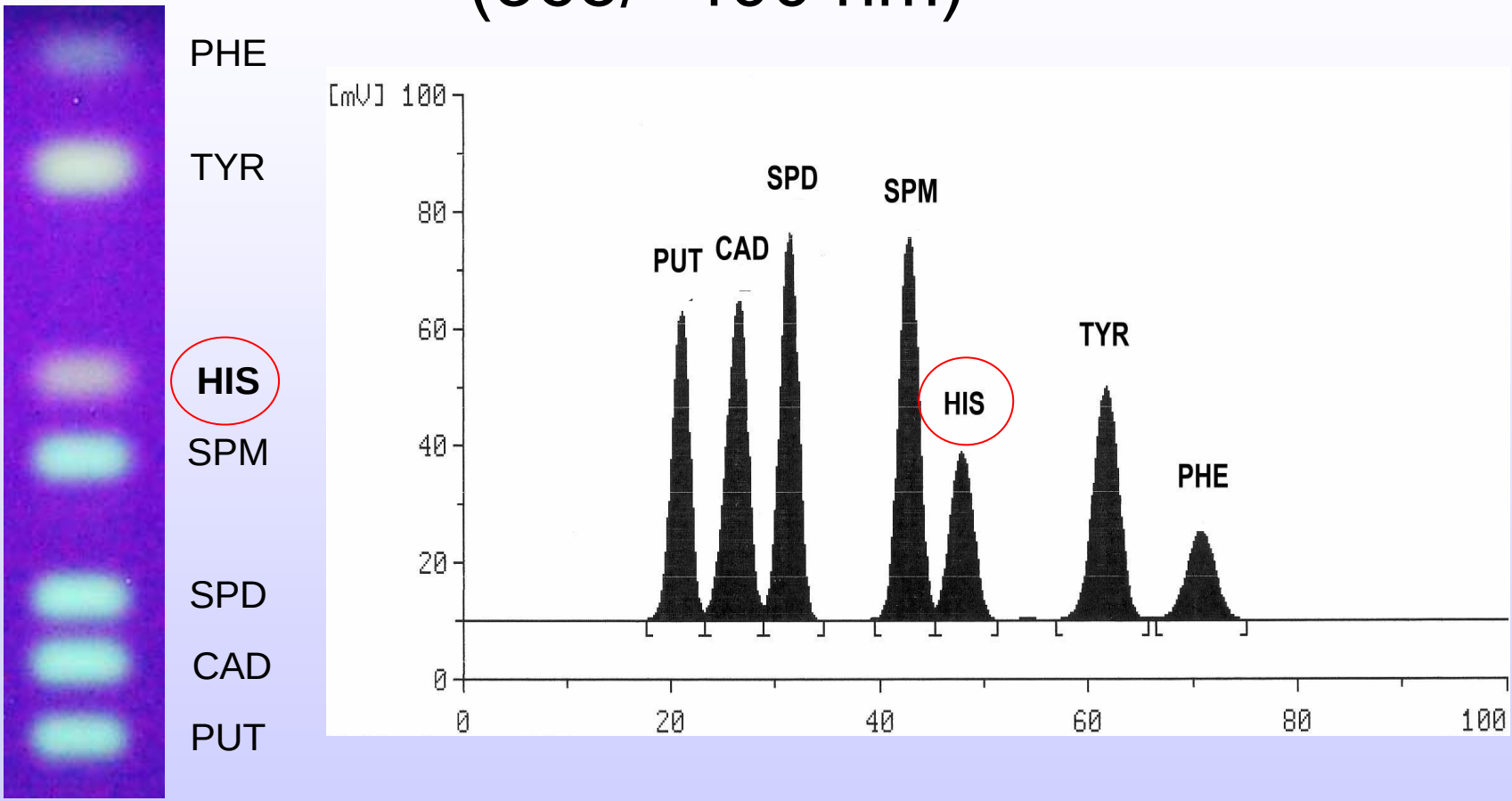
Reduce the combined extracts to dryness and dissolve the residue in 5 ml acetonitrile

Separation

TLC plates silica gel 60 (Merck) (20 x 10 cm, layer thick-ness 0.25 mm, pre-washed with developing solvent)

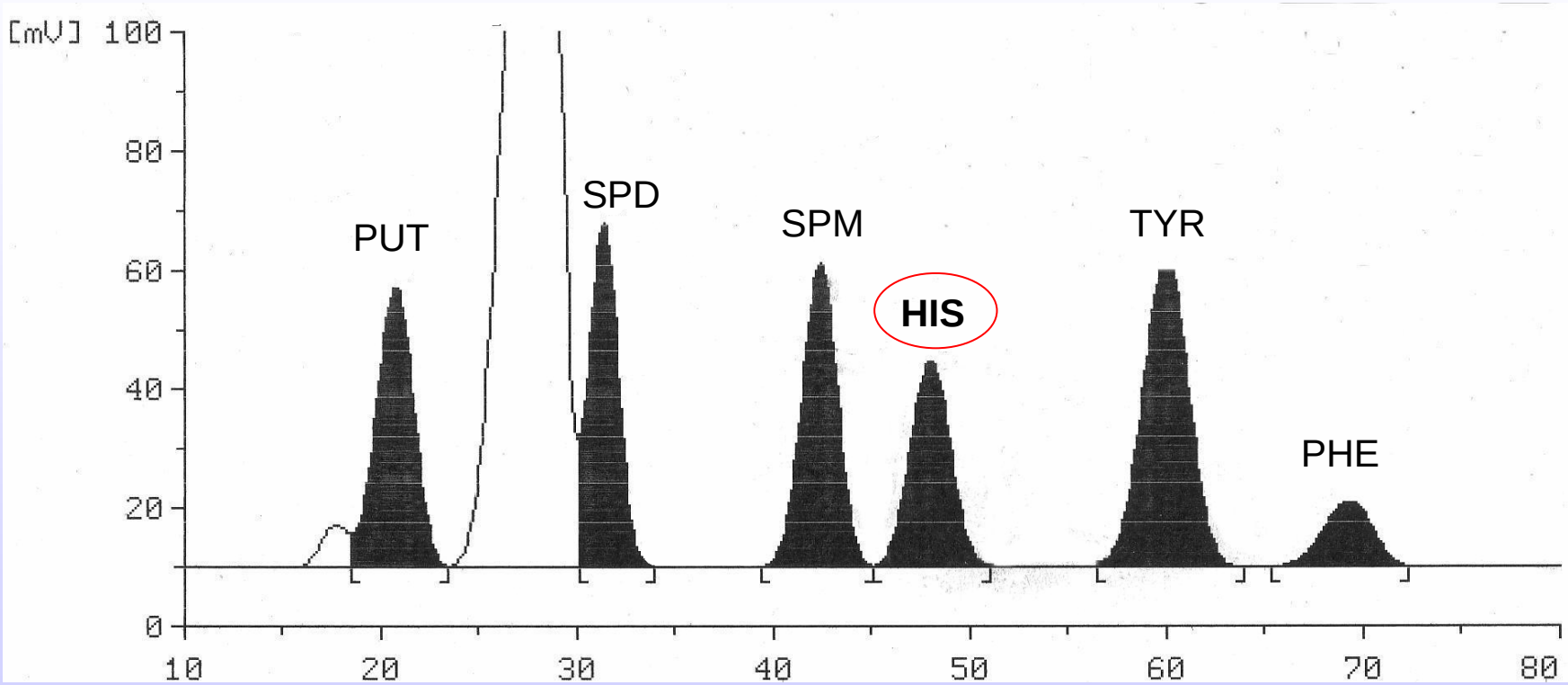
Separation was performed in a horizontal development chamber

Densitogram of a standard solution (365/>400 nm)



benzene : chloroform : triethylamine (10:6:7)

Densitogram of a spiked fish extract



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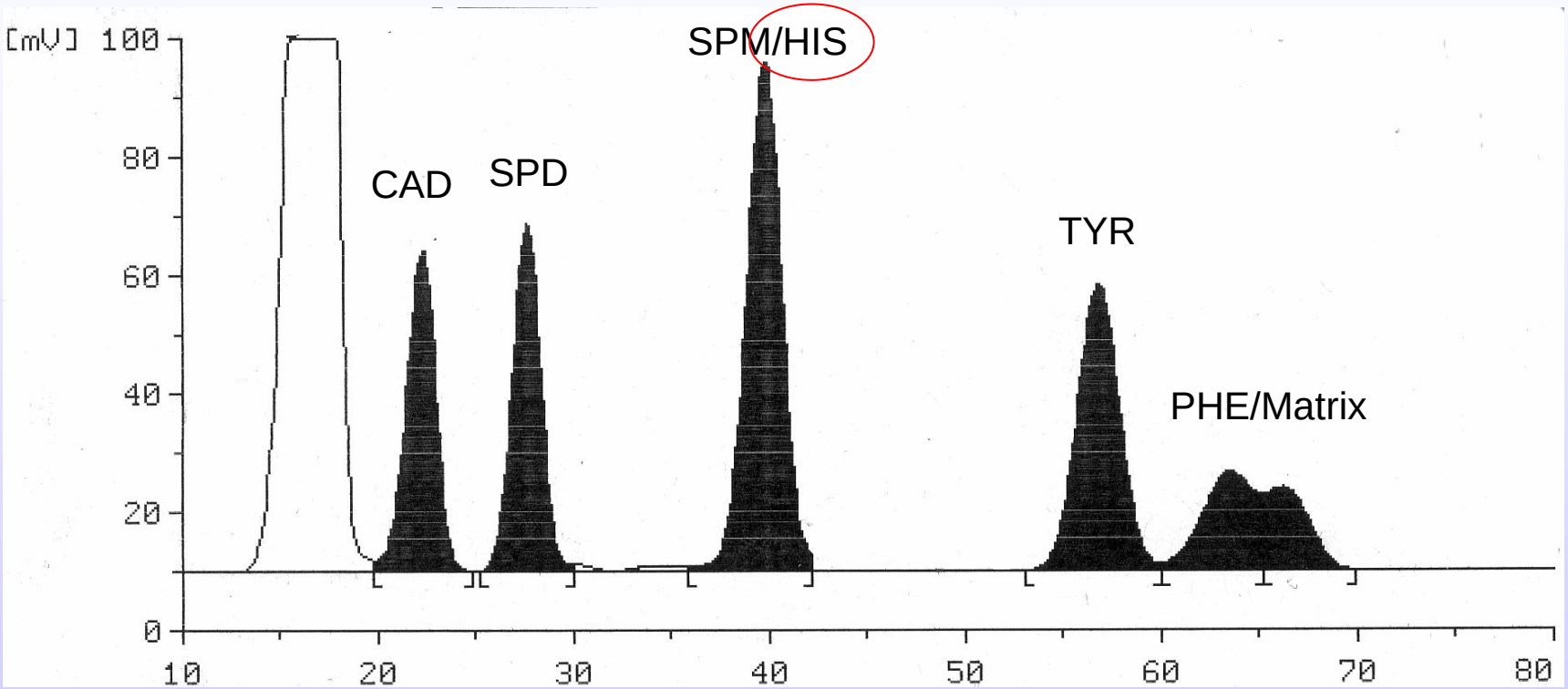
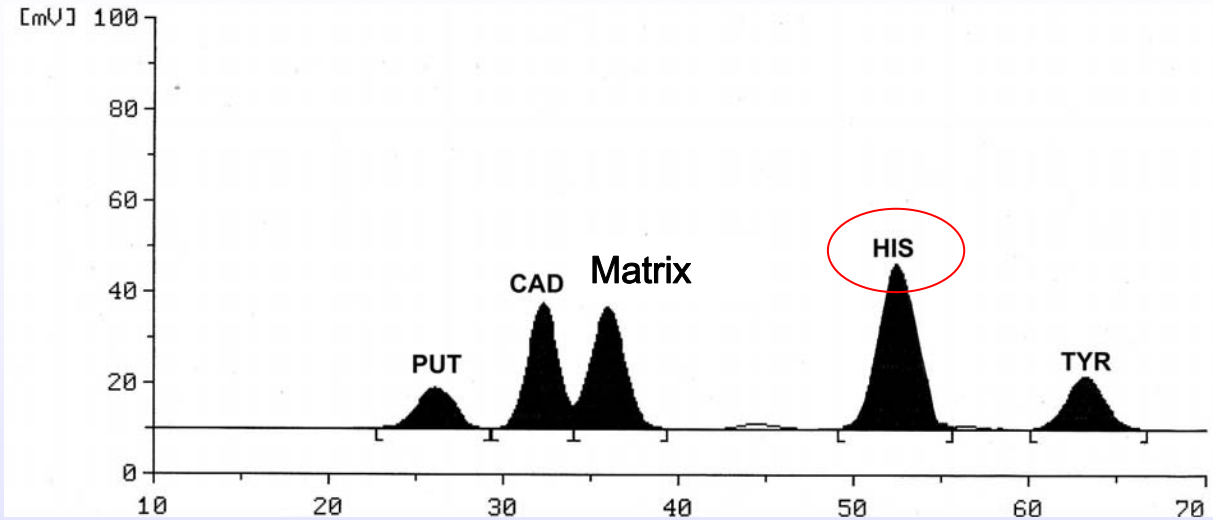


benzene : chloroform : triethylamine (10:6:2)

Validation data for histamine

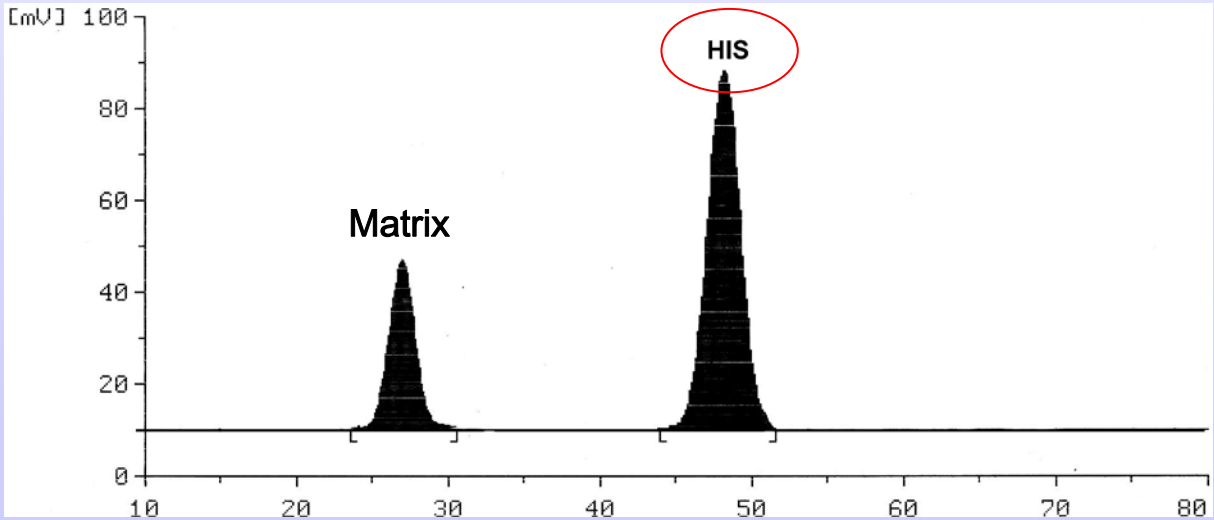
Linearity	50 – 250 mg/kg
Recovery (200 mg/kg)	108 %
Confidence interval (n = 5, P = 0,95)	± 9.9 mg
$LOD_{DIN\ 32645}$	17.5 mg/kg
$LOQ_{DIN\ 32645}$	56 mg/kg

Densitograms of two fish samples



"Mackerel smoked"

178 mg/kg histamine



"Tuna in oil"

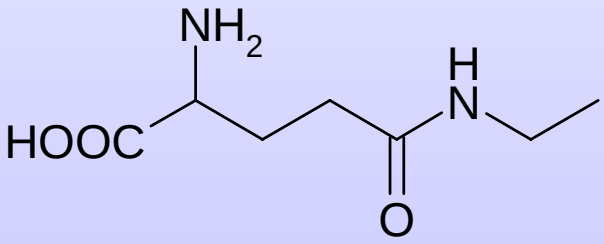
2804 mg/kg histamine

Histamine contents using different methods

	TLC	HPLC	ELISA	Fluorimetry
Mackerel, smoked	178	174	251	-
Salmon, smoked	n.d.	<5	<1	1
Tuna in oil	2804	2541	2893	-
Tuna (Interlaboratory tests)	39	-	-	37

Contents of histamine in mg/kg

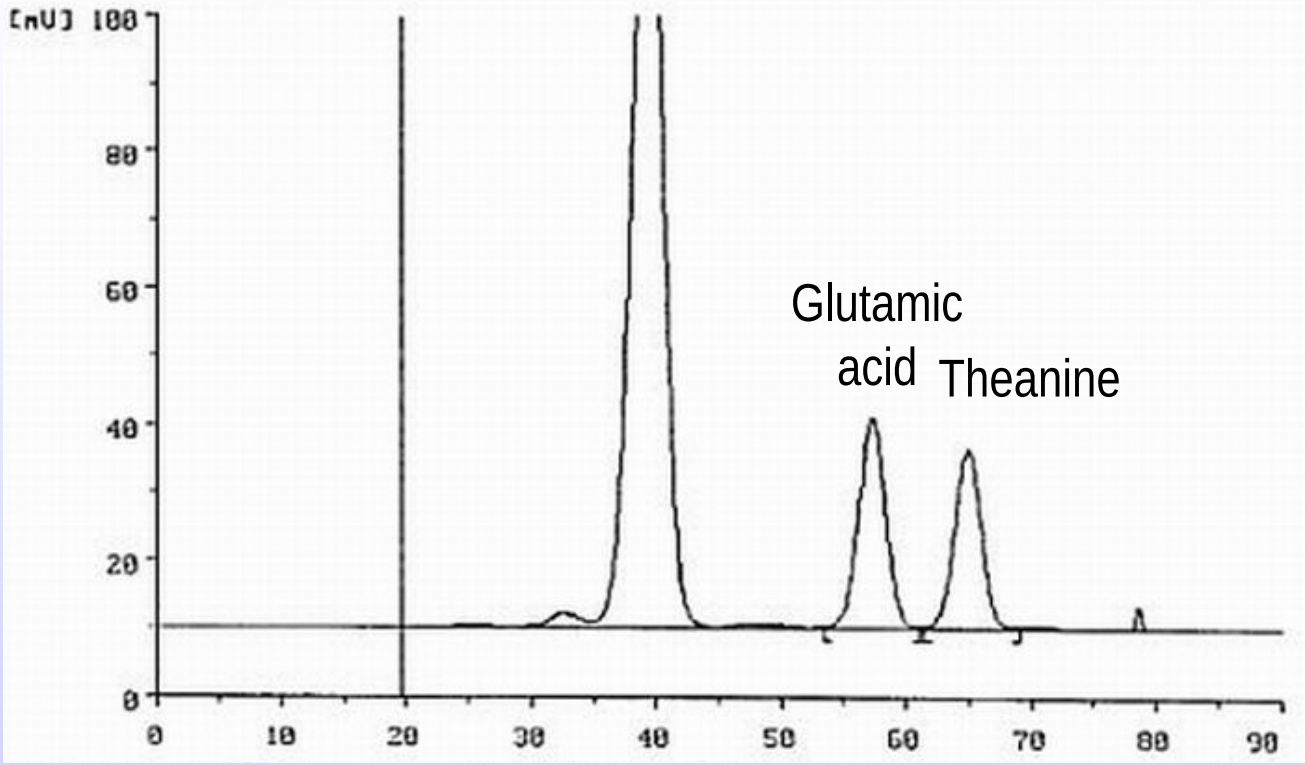
Theanine



- charakteristic constituent of tea
- 50 % of the total free amino acids
- theanine content in tea leaves

  c. 0.2 - 2 %
- sweeter taste
- pleasant tranquilising or gentle freshening effect

Densitogram of a standard mixture



chloroform:methanol:glacial acetic acid (75:20:5)

Detection

- Excitation wavelength of dansylated amino acids

  (Literature data): 250 - 370 nm;

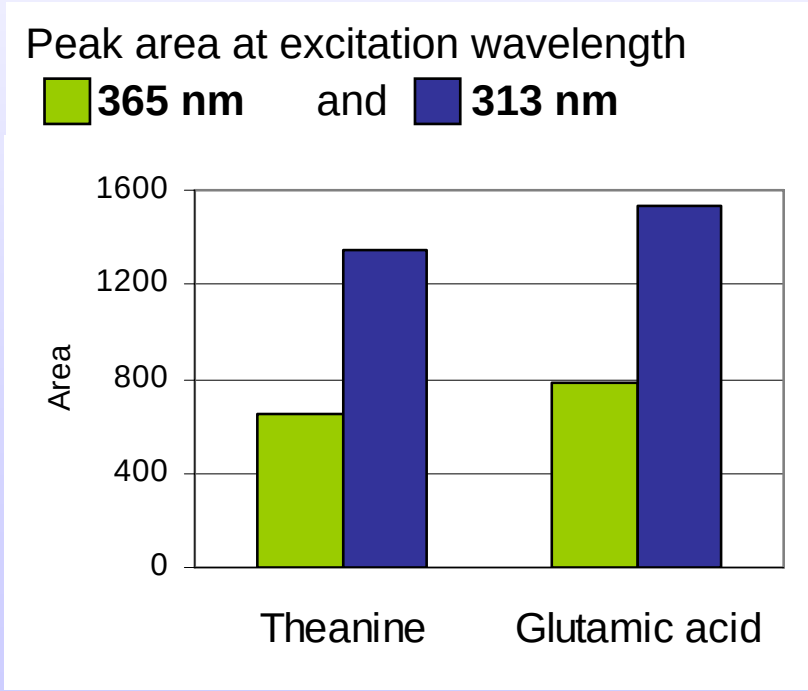
  CAMAG: 313 nm

- Kretzschmar et al.:

  365 nm for histamine

- **Detection**

  > 400 nm



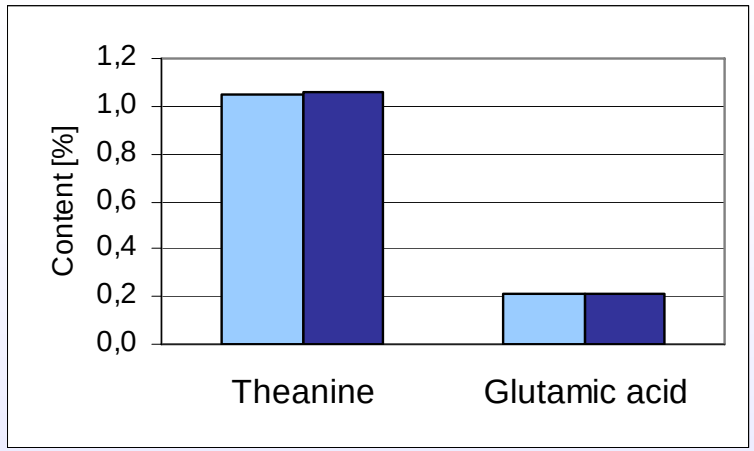
Sample preparation:

0.2 g of ground tea

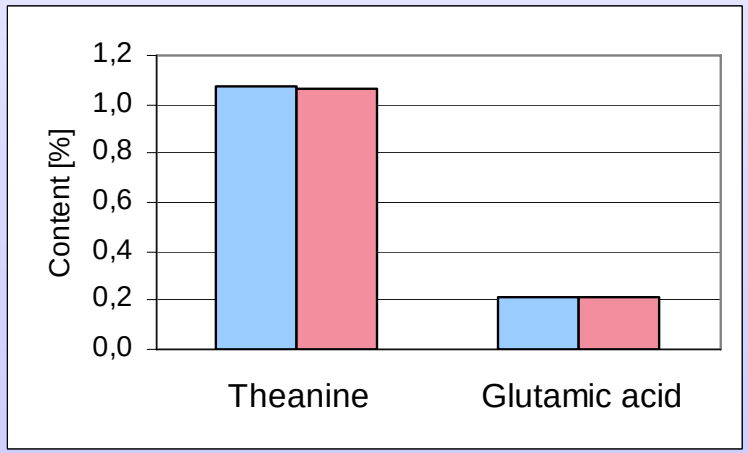
are extracted with cold

water (15 ml)

(10 min in an ultrasonic bath)

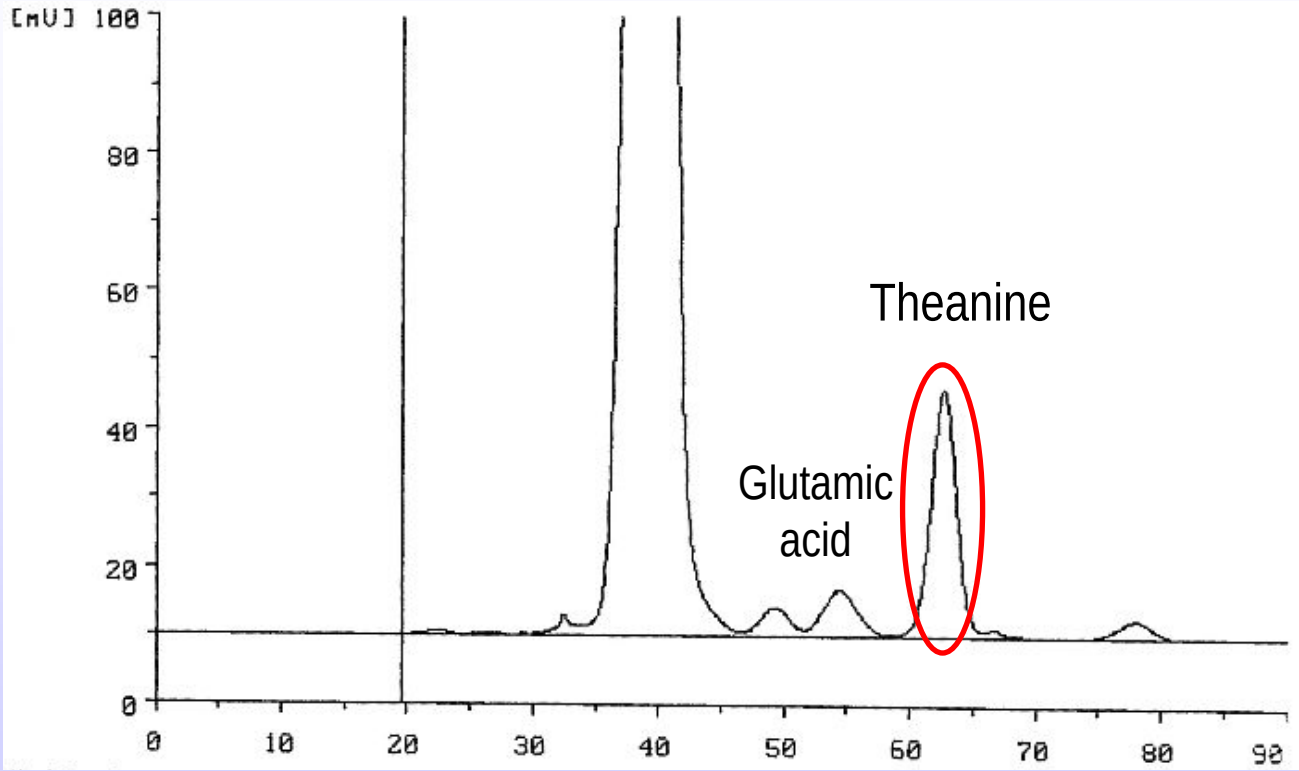


single extraction
twofold extraction



cold water extraction
hot extraction

Densitogram: black tea, Nepal

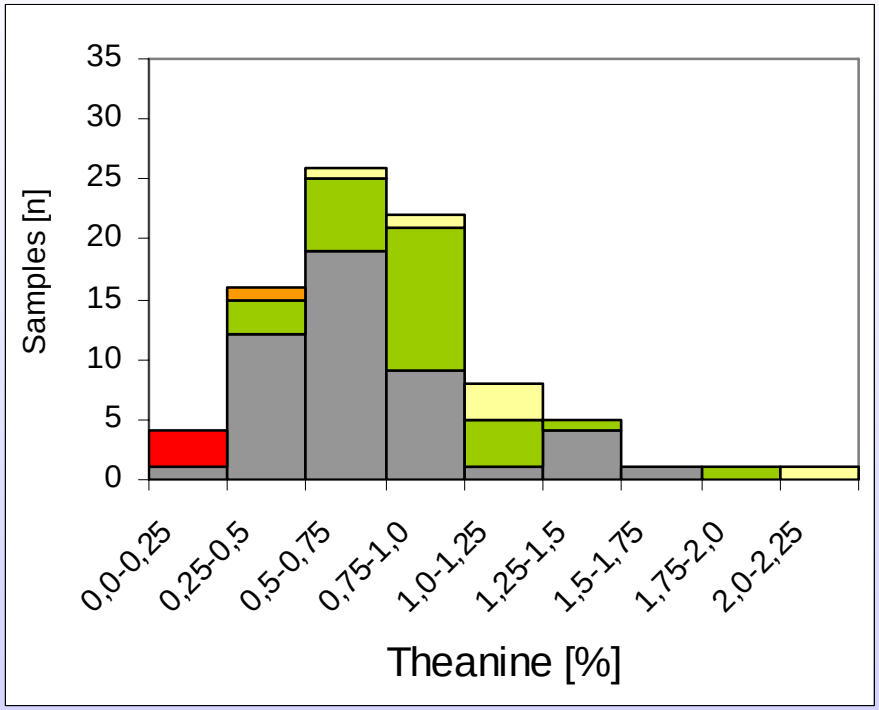


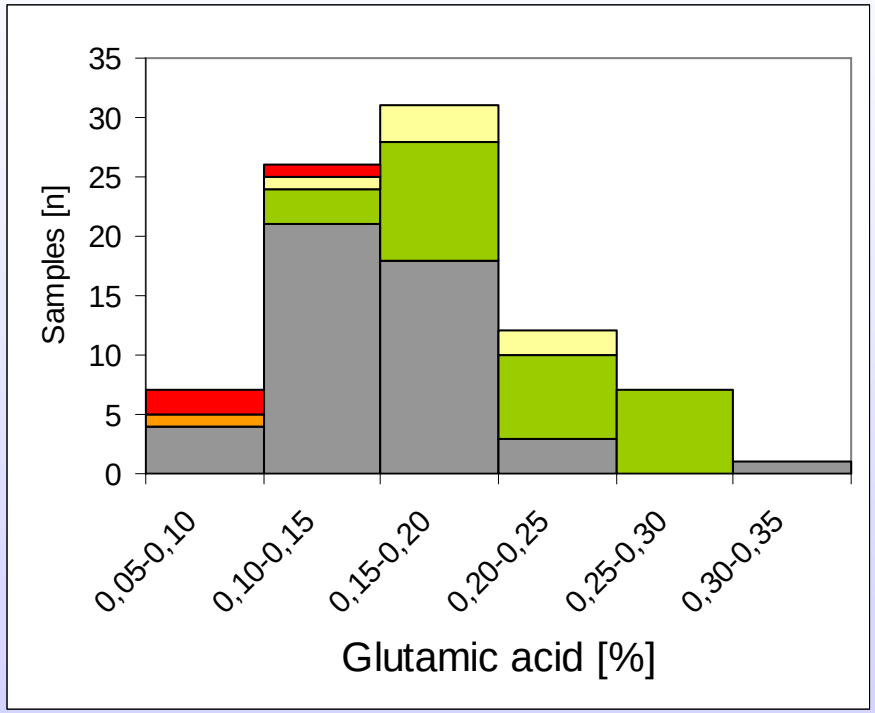
Validation data

	Theanine	**Glutamic acid**
Linearity	0.045 – 3.75 %	
Recovery	94 %	99 %
Confidence interval (n = 5, P = 0,95)	± 0.02 %	± 0.01 %
LOD*	0.10 %	0.08 %
LOQ*	0.35 %	0.28 %

* DIN 32645

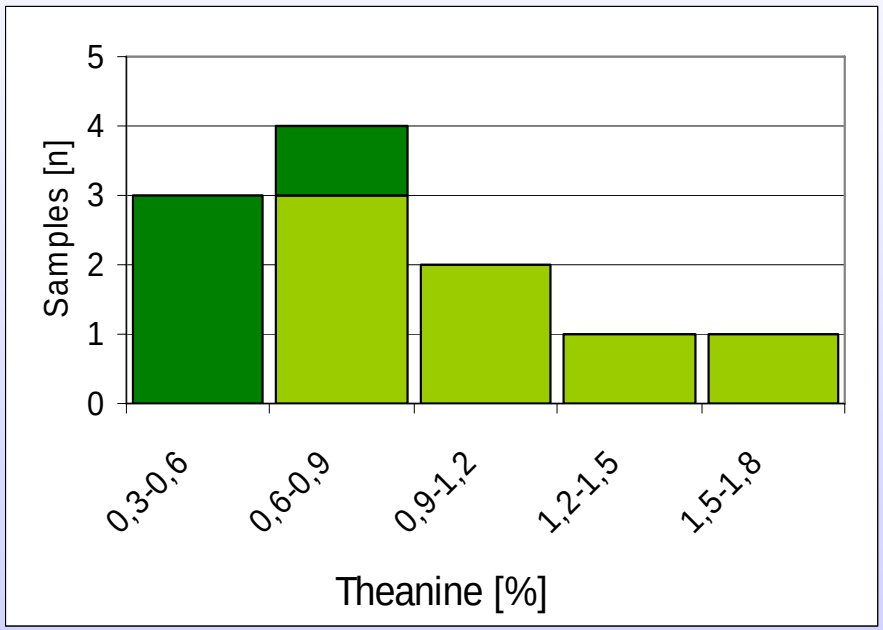
Contents of theanine and glutamic acid in different teas



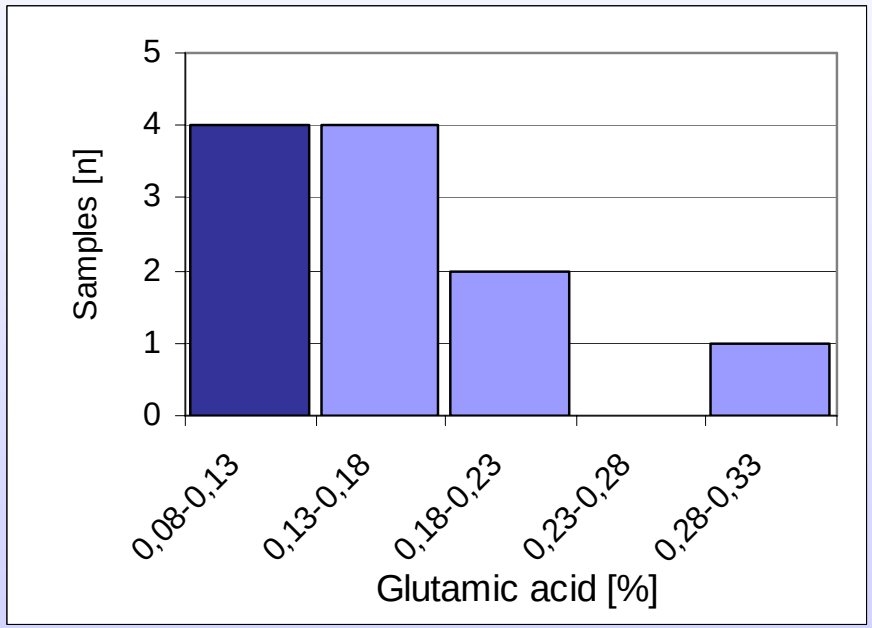


Pu-Erh Tea (n = 3)
Oolong Tea (n = 1)
White Tea (n = 6)
Green Tea (n = 27)
Black Tea (n = 47)

Contents of theanine and glutamic acid in first flush and second flush teas

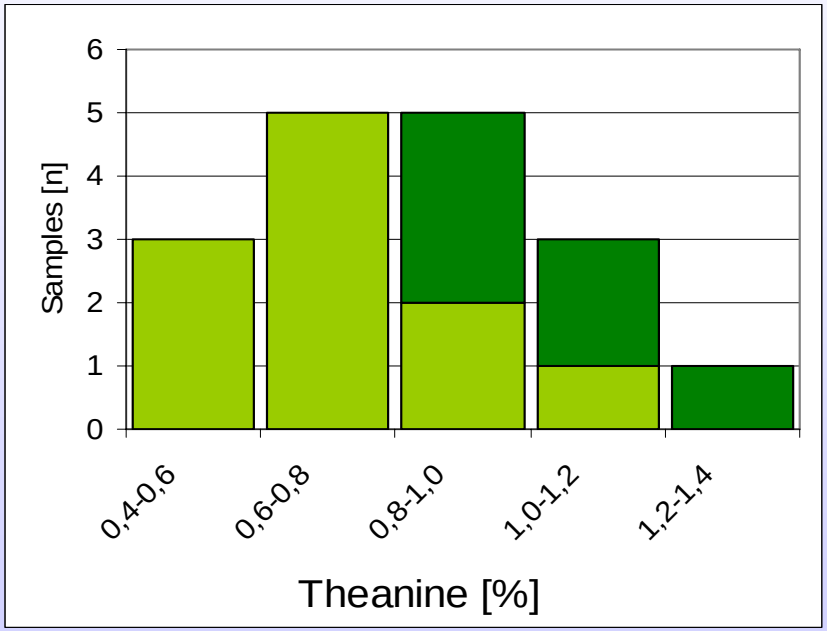


First Flush (n=7)
Second Flush (n=4)

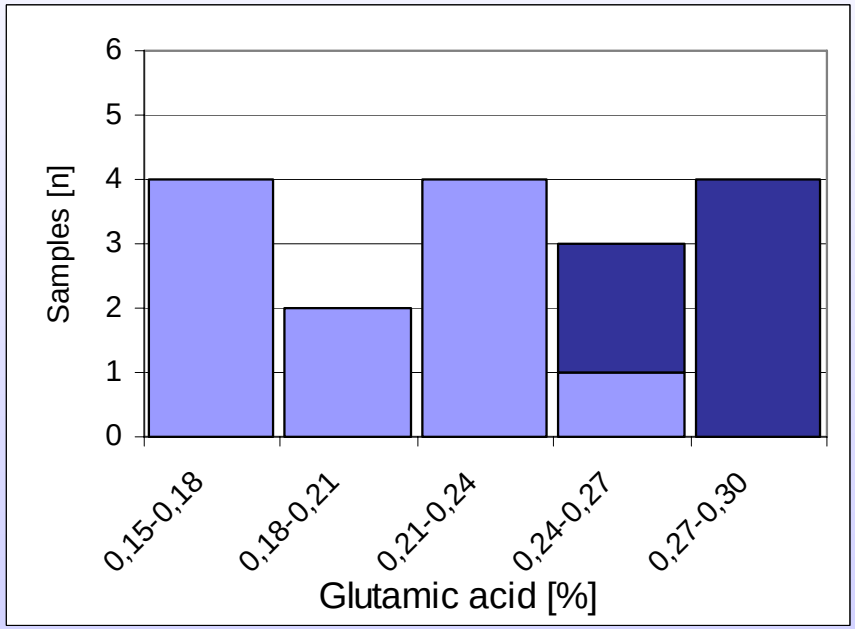


First Flush (n=7)
Second Flush (n=4)

Contents of theanine and glutamic acid in Japanese teas and Chinese teas

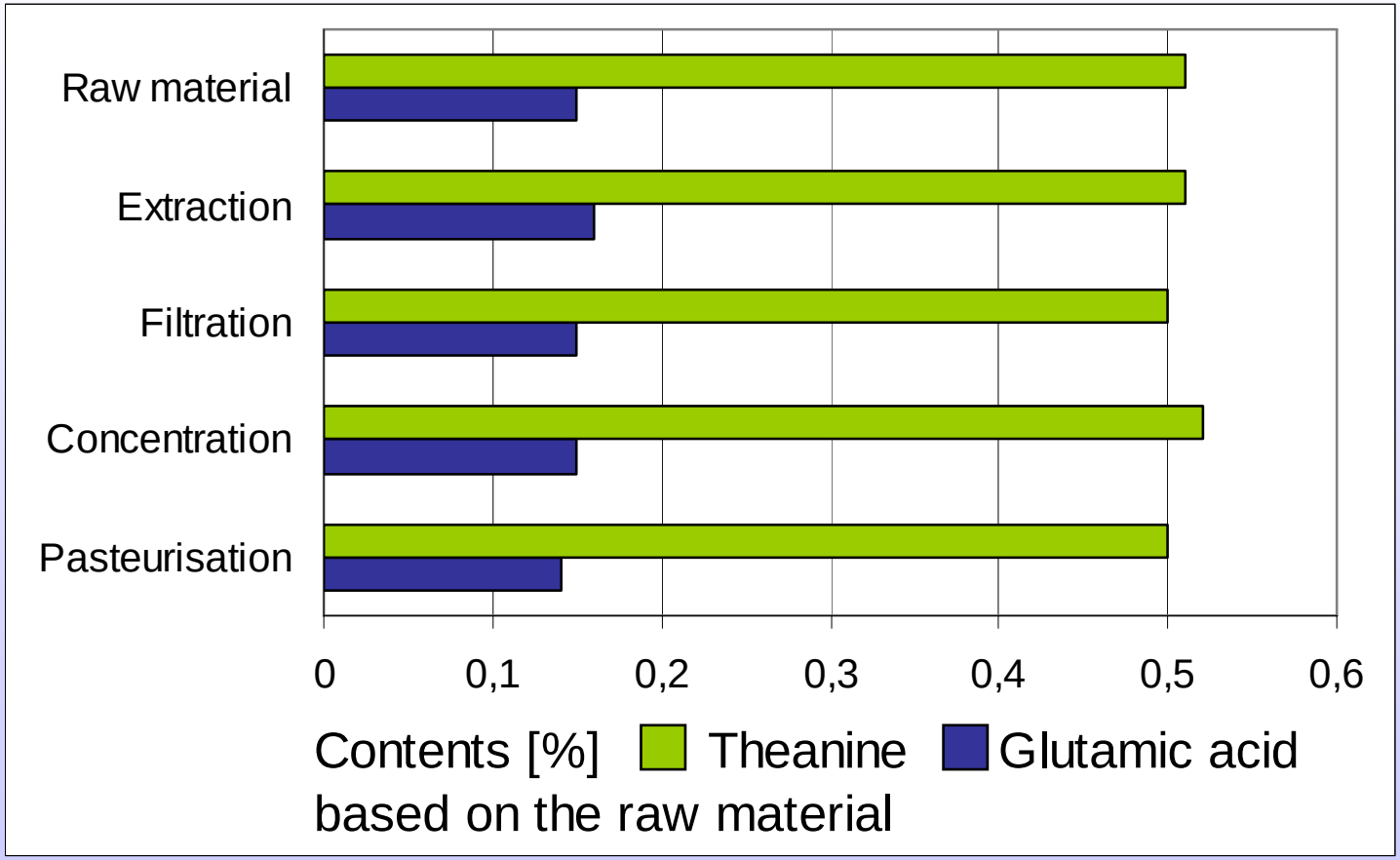


China (n = 11)
Japan (n = 6)



China (n = 11)
Japan (n = 6)

Behaviour of theanine and glutamic acid during the production of tea extract



Summary

Histamine in fish

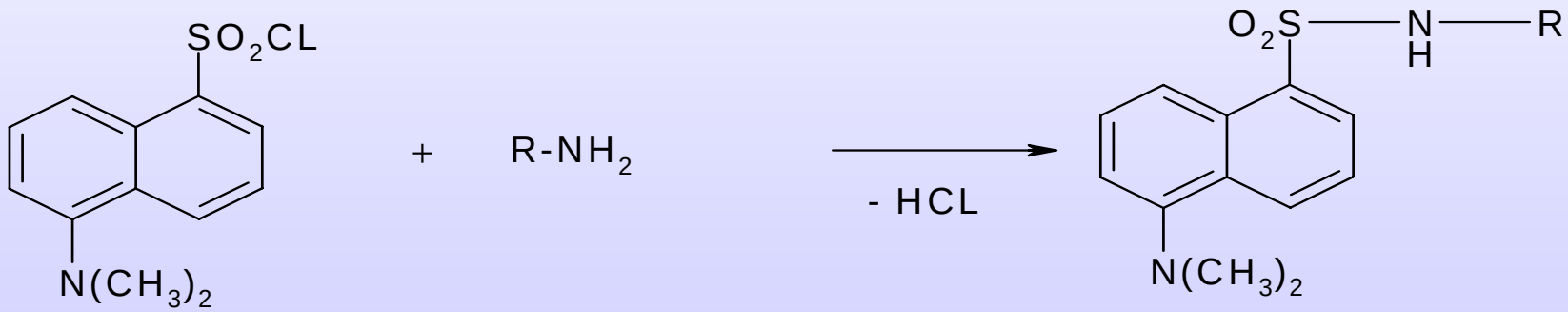
12 samples can be analyzed within 6 hours

Theanine in tea

24 samples can be analyzed within 8 hours

Derivatisation

1 ml of the filtrate is adjusted to pH 8
Add 1 ml borate-puffer and 2 ml dansyl chloride solution
Incubate for 1 h
Adjust to 10 ml with water

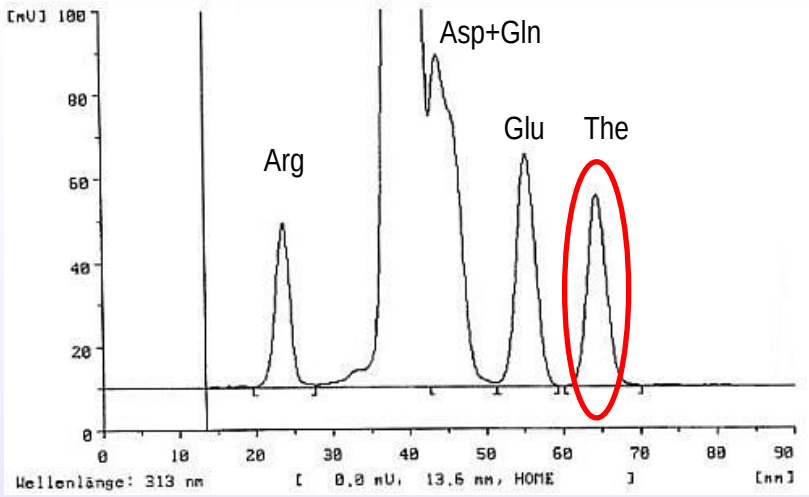


Borate puffer
Mix 67.3 ml of a 0.25 M borax solution with 32.7 ml of a 1 M boric acid,
for preparing 100 ml you need 6.225 g boric acid and 9.525 g sodium borate

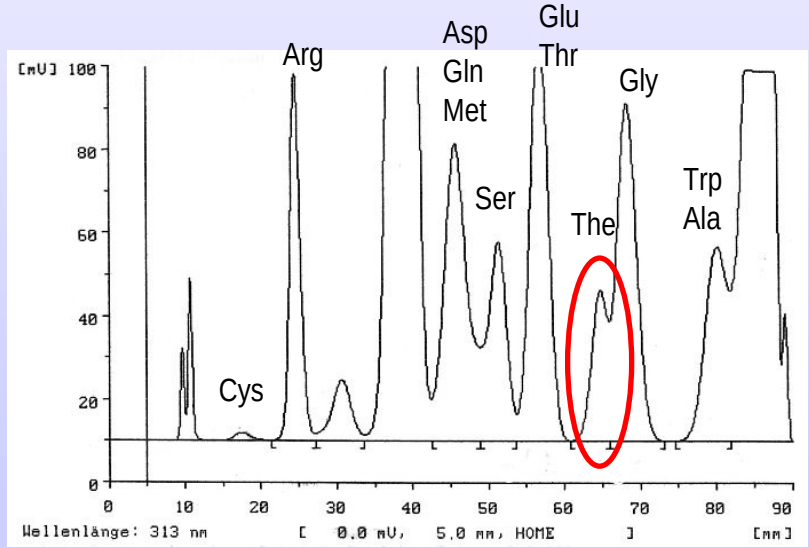
- Trennung der wichtigen AS

  The, Arg, Asp, Glu und Gln

- weitere Aminosäuren:
  - Elution in ausreichendem Abstand zu Theanine (außer Gly)
  - Coelution von Glu und Thr



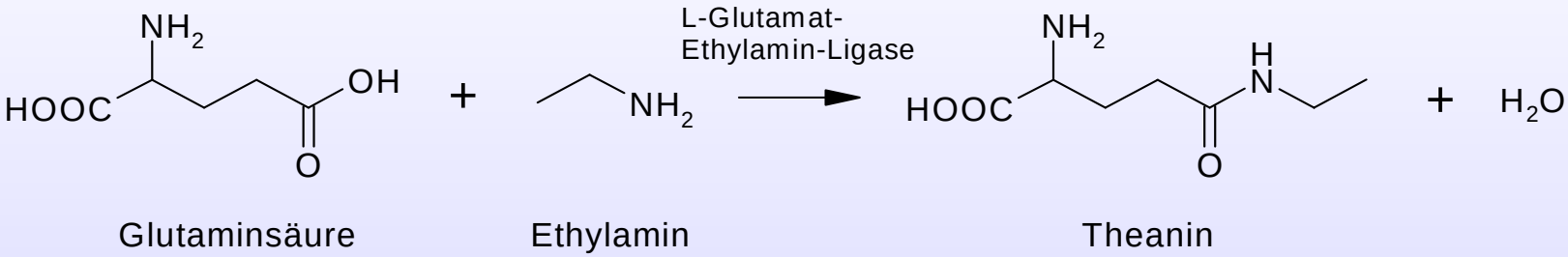
Trennung der Aminosäuren Arg, Asp, Glu, Gln und The



Trennung eines Aminosäuregemisches

$$BAI = \frac{c_{\text{Histamin}} + c_{\text{Putrescin}} + c_{\text{Cadaverin}}}{1 + c_{\text{Spermin}} + c_{\text{Spermidin}}}$$

Theanine biosynthesis



- in den jungen Wurzeln der Teepflanze
- danach Transport in die jungen Blätter (Wickremasinghe et al. 1972)