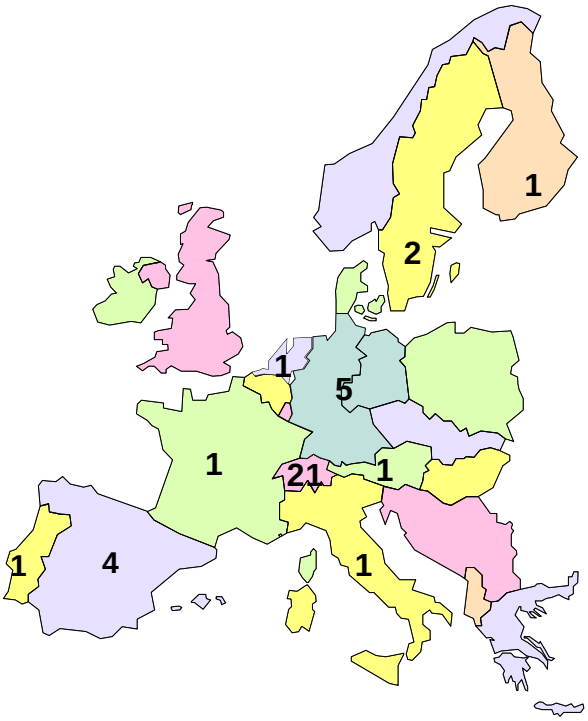
First FAM Collaborative Study on the Determination of Biogenic Amines in Standard Solution, Wine, Cheese and Feed

Calculation of the precision parameters for the HPLC dansyl method according to the IUPAC-1987 protocol and the Swiss food manual



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Contents

Abstract

The Federal Dairy Research Institute (FAM) organized an international collaborative study on the determination of biogenic amines in standard solution, wine, spiked wine, feed and two cheese samples in summer 1994. The total amount of biogenic amines in the samples was 57 mg/L for wine, 5'300 mg/kg for feed, 3'200 mg/kg for defatted and lyophilized cheese 1 and 1'800 mg/kg for low fat cheese powder 2. 38 laboratories of 10 European countries participated in this study. The following methods were used: HPLC separation of free amines, dansyl-, OPA- and dabsyl-derivates and ion exchange separation of free amines with ninhydrin and OPA postcolumn derivatization. The precision parameters repeatability r and reproducibility R could only be calculated for the HPLC separation of dansyl derivates with UV and fluorescence detection. The calculation of the precision parameters was performed with the classical analysis of variance, including outlier elimination procedure (IUPAC-1987 protocol) and the robust statistic (Swiss food manual).

The mean values with HPLC of dansyl derivates and UV or fluorescence detection were for most amines in the different samples quite close together. The median values obtained using ion exchange chromatography with ninhydrin or OPA postcolumn reaction were not significantly different. The other HPLC methods gave for some amines significantly different results. The recoveries of the biogenic amines in the spiked wine samples were better than 80 % for all amines, except for ß-phenylethylamine with a recovery of < 75 %. Interference with the internal standard 1,7-diaminoheptane for the feed sample and UV detection of dansyl derivates could be observed. This results were therefore slightly lower than fluorescence results.

The determination of histamine with fluorescence detection showed poor repeatability and reproducibility. This determination is therefore not recommended.

The median relative standard deviation of repeatability and reproducibility in the liquid samples (standard solution, wine and spiked wine) was ≤ 1.4 and ≤ 5 mg/L, respectively. The median relative standard deviation of repeatability and reproducibility in the solid samples (feed, cheese 1 and cheese 2) was ≤ 26 and ≤ 97 mg/kg, respectively. Especially high values for reproducibility were obtained for putrescine with UV detection $s_R \leq 260$ mg/kg ($RSD_R \leq 28$ %) and cadaverine $s_R \leq 300$ mg/kg ($RSD_R \leq 16$ %) in feed and tyramine with UV detection in cheese 1 $s_R \leq 290$ mg/kg ($RSD_R \leq 24$ %).

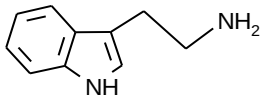
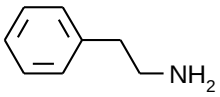
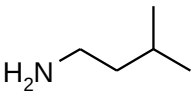
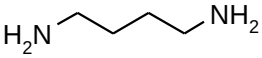
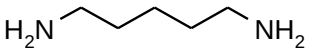
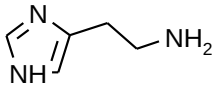
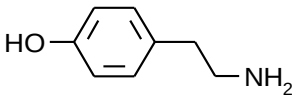
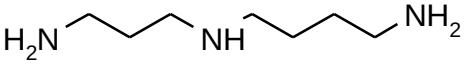
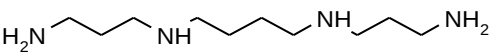
Introduction

The separation of biogenic amines on ion exchange resins is known since the sixties. But only laboratories equipped with sophisticated amino acid analyzers, which are quite expensive, were able to quantify the biogenic amines. In the 1970's HPLC methods with different precolumn and postcolumn derivatization procedures became very popular. The last collaborative study in Switzerland on the determination of biogenic amines in cheese and fish was performed in Spring 1988 with the method of the Cantonal laboratory of Zurich. This precolumn derivatization method with dansylchloride became then the standard method for the Swiss food manual [1]. In the last years precolumn derivatization with OPA became popular. In order to compare the currently used methods and to determine the precision parameters of the Swiss food manual method with UV and fluorescence detection, a new collaborative study was organized.

Experimental

Table 1 shows the list of biogenic amines used in this collaborative study.

Table 1 Biogenic amines tested

Biogenic amine	Abbreviation	Structure
Tryptamine	TRA	
ß-Phenylethylamine	PHA	
Isopentylamine	ISA	
Putrescine	PUT	
Cadaverine	CAD	
Histamine	HIA	
Tyramine	TYA	
Spermidine	SPD	
Spermine	SPM	

Samples

For this collaborative study, the following samples were chosen: Red wine sample Rioja 1990, fish meal of herring, defatted and lyophilized Appenzeller cheese and a low fat cheese powder. Table 2 shows the description and origin of the different samples.

Table 2 Samples

Number	Name	Description
1	Standard solution	40 - 60 mg/L of each biogenic amine in 0.01 mol/L H_2SO_4
2	Wine	Rioja 1990, Embotellado por Federico Paternina, S.A. Haro-España
3	Wine spiked	The same wine after addition of 8 - 25 mg/L of each amine
4	Feed	Herring fish meal: Protein 705 g/kg, fat 95 g/kg, moisture 77 g/kg and ash 133 g/kg.
5	Cheese 1	An extra old Appenzeller cheese (6 month) with the following composition: Protein 257 g/kg, fat 317 g/kg and moisture 385 g/kg. The cheese was grated and extracted with 10 x 3 L heptane. The residue was lyophilized (3 days) and then grinded and packed in Minigrip and welded. The dry matter of this hygroscopic sample was 964 g/kg at packing time.
6	Cheese 2	A cheese powder specialty (spice) with a very low fat content (< 2 %) and a dry matter content of 770 g/kg.

Participating laboratories

38 laboratories participated in this collaborative study (Table 3). One laboratory sent two differents sets of results for statistical evaluation. Therefore 39 set of results are presented.

Table 3 Participating Laboratories

Name	Company or Institute	Country
Ginzinger W.	Bundesanstalt für Alpenländ. Milchwirt., Jenbach	Austria
Eklund E.	Finnish Customs Laboratory, Espoo	Finland
Nicolas M.	Labororatoire central d'hygiene alimentaire, Paris	France
Krause I.	FML Weihenstephan, Institut Chemie und Physik, Freising-Weihenstephan	Germany
Herrel D.	MILUPA AG , Friedrichsdorf/Ts.	Germany
Bauer Ch.	MUVA , Kempten	Germany
Friedhart G.	Staatliche Milchwirt. Lehr- und Forschungsanstalt, Dr. Oskar Farny Institut, Wangen im Allgäu	Germany
Petridis K.	Uni Hamburg, Abt. Lebensmittelchemie, Hamburg	Germany
Moret S.	Università degli studi di Udine, Dipartimento di scienze degli alimenti, Udine	Italy
Haaksman I.	Hoofdgroep TNO Voeding Afd. BFC , AJ Zeist	Netherland
Alves A.	Faculdade de Engenharia da Universidade do Porto, Dep. de Engenharia Quimica, Porto	Portugal
Pozo R.	AZTI (Instituto Tecnològio Pesquero y Alimentario), Sukarrieta (Bizkaia)	Spain
De Llano D. G.	CSIC, Instituto de Productos Lacteos de Asturias, Villaviciosa	Spain
Hitos P.	Ministerio de Agricultura, Pesca y Alimentacion Laboratorio Arbitral (M.A.P.A.), Madrid	Spain
Vidal-Carou C.	Universidad de Barcelona, Nutricion y Bromatologia fac. Farmacia, Barcelona	Spain
Eriksson S.	AnalyCen , Lidköping Vänern	Sweden
Thim A.M.	National Food Administration , Uppsala	Sweden
Grüter A.	COOP Zentrallabor, Basel	Switzerland
Schneider J.	FAG , Posieux	Switzerland
Bilic N.	FAM , Liebefeld-Bern	Switzerland
Fuchs D.	FAM , Liebefeld-Bern	Switzerland
Bill R.	FAW, Wädenswil	Switzerland
Bussmann W.	Kant. Laboratorium, Solothurn	Switzerland
Caperos J.	Kant. Laboratorium, Neuchâtel	Switzerland
Etter R.	Kant. Laboratorium, Zürich	Switzerland
Huber D.	Kant. Laboratorium, St. Gallen	Switzerland
Känzig A.	Kant. Laboratorium, Aarau	Switzerland
Kaufmann T.	Kant. Laboratorium, Luzern	Switzerland
Ramseier C.	Kant. Laboratorium, Basel	Switzerland
Rutschmann M.	Kant. Laboratorium, Steinhausen	Switzerland
Seiler K.	Kant. Laboratorium, Schaffhausen	Switzerland
Noser J.	Kant. Laboratorium, Füllinsdorf	Switzerland
Meier P.	Laboratoire cantonal, Epalinges	Switzerland
Walker H.	Laboratoire cantonal, Fribourg	Switzerland
Weinhold D.	Laboratoire cantonal, Genève	Switzerland
De Rossa M.	Laboratorio cantonale, Lugano	Switzerland
Schneller R.	Migros-Genossenschafts-Bund, Zentrallabor, Zürich	Switzerland
Spycher E.	VSF, Zollikofen	Switzerland

Methods

All methods used are shown in Table 4. 34 laboratories used an HPLC method: 25 laboratories used the Swiss food manual method with dansylchloride precolumn derivatization, 7 precolumn derivatization with OPA (ortho-phtalaldehyde), 1 postcolumn derivatization with OPA, 1 precolumn derivatization with dabsylchloride and 1 HPLC separation of free amines. 12 of the laboratories which applied the Swiss food manual method applied both detection methods. The sum of laboratories with UV and fluorescence detection is therefore > 25. 4 laboratories used an ion exchange method with postcolumn ninhydrin reaction, except one laboratory which used OPA postcolumn derivatization. Table 5 gives a compilation of the analytical and chromatographic parameters used in this collaborative study. Laboratory 1 - 25 used precolumn derivatization with dansylchloride and HPLC separation with UV and/or fluorescence detection. Non harmonized OPA precolumn derivatization (ethanediol, mercaptoethanol, mercaptosulfonic acid sodium salt and without thiol component) followed by HPLC separation and fluorescence detection was used of the laboratories 30 - 36. Laboratory 40 used HPLC with OPA-mercaptoethanol postcolumn derivatization and fluorescence detection. Precolumn derivatization with dabsylchloride and HPLC separation with detection at 436 nm was applied by laboratory 50. Laboratory 60 used ionpair HPLC for the determination of histamine and tyramine with UV detection at 215 nm. Ion exchange chromatography was applied of laboratory 70 with OPA postcolumn derivatization and of the laboratories 80 - 82 with ninhydrin postcolumn derivatization.

Table 4 Summary of the used methods

Separation method	Derivatization reagent	Derivatization type	Detection	Abbreviation	Number of laboratories
HPLC	dansylchloride	precolumn	UV / FL	LC-Dan	25
	OPA	precolumn	FL	LC-OPA	7
	OPA	postcolumn	FL	LC-OPP	1
	dabsylchloride	precolumn	UV	LC-Dab	1
	-	without	UV	LC	1
IEC	OPA	postcolumn	FL	IC-OPA	1
	ninhydrin	postcolumn	UV	IC-Nin	3

Legend:
UV ultraviolet or visible light
FL fluorescence

Short description of the Swiss food manual method

This method [2] uses an extraction mixture of 25 mL acetonitrile and 25 mL perchloric acid 0.2 mol/L. After homogenization and filtration, 200 µL are derivatized with dansylchloride. The excess of the reagent is destroyed by sodium glutamate. The derivates are extracted with ethyl acetate. The organic layer is evaporated and the residue is diluted in 200 µL of acetonitrile. The HPLC separation is performed on a C_{18} column at 35°C with a binary gradient which consist of a pH 8.0 buffer solution, deionised water, ethanol and acetonitrile. The detection is possible in the UV at 254 nm and with fluorescence measurement at excitation 254 nm / emission 485 nm.

Table 5 Summary of the analytical parameters

Nr	Experience		Equipment	Column		Chromatographic parameters			Detection parameters nm			Calibration parameters			Remarks
Laboratory	Years	Determinations/year		Stationary phase	Length x I.D. mm	Flow mL/min	Injection volume µL	Run time min	UV	Excitation	Emission	Int. or Ext. standard	Area or Height	Number of cal. points	IS = Internal standard
1	>3	<50	HP 1050	Phenomenex C18	250x 3.2	0.5	10	35	254			I	A	4	Feed: Interference of internal standard in UV
2	>3	<200	HP 1090	Hypersil ODS	250x 4	1.4	5	30	254	254	485	I	A	1	
3	<1	<50	Kontron MT II	Hypersil ODS	250x 4	1.4	5	30	254	254	485	I	A	1	
4	>3	<50	Merck LC 6200, F1050	Lichropher	250x 4	1.5	5	35		360	490	I	A	2	ISA/PUT: Bad chromatographic resolution IS/TYA: Bad chromatographic resolution
5	>3	<200	Waters	Hypersil ODS	250x 3	1	520	36	254	328	470	I	A	1	PHA: Calibration plot not linear. Fish samples: Always higher values for all amines with UV detection, compared to fluorescence detection
6	>3	<50	PE Integral 4000	Nucleosil 100-7C18	250x 4	1.5	20	35	250	359	445	I	A	1	Feed: Unknown Interference with TRA and fluorescence detection
7	<1	<200	Merck FLD 1050	Nucleosil 5, C18	250x 4	1	5	35	254	360	490	I	A	1	PHA/ISA: Bad chromatographic resolution. Feed: Interference of internal standard in UV
8	>3	<50	HP 1050/1046	LiChrospher	250x 4	0.7	15	46	254	254	485	I	A	4	ISA/PUT: Bad chromatographic resolution
9	>3	>200	Kontron	LiChrochart	125x 4	1	60	42	254	328	470	I	H	3	
10	>3	<50	Varian	Spherisorb ODS 2	250x 4.6	1.3	20	34	254	328	470	I	A	5	PHA/ISA/PUT: Bad chromatographic resolution
11	>3	<50	HP 1050	Nucleosil 100-7 C18	250x 4	1	20	15	254			I	A	1	
12	<3	>200	Waters	RP 18 Millipore	300x 3.9	1	10	55	254	254	485	E	A	2	PHA/ISA: Bad chromatographic resolution
13	<3	<200	Varian LC 5000	Spherisorb ODS-2	250x 4.6	0.8	20	52	254	254	485	I	A	1	ISA/PUT: Bad chromatographic resolution
14	>3	<50	Varian	LiChrospher 100 RP18	125x 4	0.7	5	30	254			I	A	4	Feed: Interference of internal standard in UV
15	>3	>200	Varian 9010	Phase Sep. C18	150x 4.6"	0.8	10	13	254			I	A	1	ISA/PUT: Bad chromatographic resolution
16	<1	<50	Beckman	Ultrasphere ODS	250x 4.6	1.4	50	40		254	485	I	A	1	ISA/PUT: Bad chromatographic resolution
17	>3	>200	Waters	Spherisorb S5 ODS 2	250x 4.6	1.3	10	36	254	254	485	E	A	5	TYA: Quantification problems for cheese 1 with different dilutions
18	<1	<50	HP 1050	EGT RP18 endcapped	125x 4	0.6	5	42	210	360	490	I	A	1	ISA/PUT: Bad chromatographic resolution. Feed: Unknown Interference with TRA and UV detection

Nr	Experience		Equipment	Column		Chromatographic parameters			Detection parameters nm			Calibration parameters			Remarks
Laboratory	Years	Determinations/year		Stationary phase	Length x I.D. mm	Flow mL/min	Injection volume µL	Run time min	UV	Excitation	Emission	Int. or Ext. standard	Area or Height	Number of cal. points	IS = Internal standard
19	>3	<200	Gynkotek	LiChrospher 100 RP18	125x 4	0.7	5	42	254	360	490	I	H	1	PHA in feed and cheese 1: 5 to 10 times higher values with UV detection Cheese: Interference for SPD and UV detection
20	<3	<50	Kontron	?	?	0.8	20	35	254	254	485	I	A	4	
21	?	?	?	?	?	?	?	?	?	?	?	?	?	?	HIA: Bad deriv. stability
22	>3	<50	Varian	Nucleosil 120 C18	125x 4	0.5	10	55	254			I	A	1	Wine: Unknown Interference with TRA and UV detection
23	>3	<50	SYKAM	LiChrosorb RP18	250 x 4	1	20	50	254	337	520	E	A	1	Only height evaluation for UV detection possible
24	>3	<50	Perkin Elmer 3B	LiChrospher 100 RP18	250x 4	1.5	20	50	255			I	H	4	
25	<3	<200	Varian 9010	Merck C18		1	50	30	254	360	490	I	A	1	Feed: Unknwon interference with TRA and with IS ISA/PUT: Bad chromatographic resolution
30	>3	>200	Waters	Spherisorb ODS 2	250x 4.6	0.8	20	66		340	420	I	A	2	mercaptoethanol Bad repeatability of retention times
31	<3	>200	Jasco, HP 1046	Knauer	125x 4.6	0.8	10	95		330	450	I	A	10	mercaptoethanolsulfonic sodium salt. PHA: Interference with unknown peak
32	>3	>200	HP 1090	Lichrosorb RP-8	200x 4.6	1	20	10		358	447	E	A	3	no thiol. Only determination of HIA
33	<3	>200	Beckman/ Merck	Superspher RP-18 100	125x 2	0.2	1	18		340	450	I	A	1	ethanediol, Isocratic separation. TYA/HIA: Bad chromatographic resolution. HIA: Unknown interference peak
34	<3	<200	Merck	WATERS Resolve C18	100 x 8	1	20	74		345	445	E	H	1	mercaptoethanol CAD: Interference with unknown peak
35	>3	>200	LKB	Spherisorb ODS II	125 x 4	1.1	20	64		345	440	I	A	2	thiol ? IS diamino-heptane: interference with unknown peak. Feed, cheese: A lot of unknown and interfering peaks
36	<1	>200	Merck	Superspher 100 RP-18	125x 4	0.6	20	40		338	450	E	A	5	mercaptoethanol
40	>3	>200	Waters	Novapack C18	150x 3.9	1	20	67		340	445	E	A	5	postcolumn mercaptoethanol
50	<1	<200	Waters	Spherisorb OSD 2 3µm	150x 4.6"	1	20	65	436			E	A	1	SPD: tailing
60	<3	<50	LDC	Spheri-5 ODS	220x 4.6	1	20	25	215			E	A	3	Method only HIA and TYA
70	<3	<50	Biotronik LC 5001	BTC 2710	100x 3.2	0.35	50	103		390	460	E	A	1	
80	>3	>200	Biotronik LC 6001	BTC 2710	75x 4	0.28	50	155	440			I	A	1	
81	<3	<50	Beckman 119CL	Beckman W3 11µm	70x 4.6	0.37	50	33	570			E	A	1	Method only for PUT, HIA and CAD
82	>3	<50	LKB	?	?	?	40		570			E	A	1	HIA: Bad resolution in some runs

Results

The samples were distributed in September 1994. Results should be sent until end of January 1995. Figure 1 shows the arrival of the data in Liebefeld. This figure can help to organize other collaborative studies.

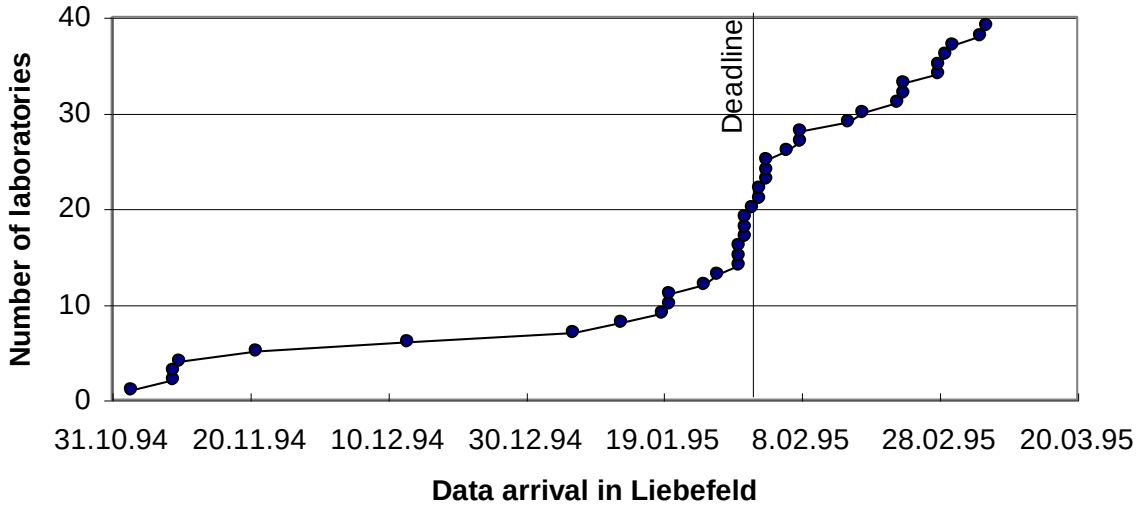


Figure 1 Results arrived in Liebefeld

The calculation of the precision parameters was only possible for the precolumn derivatization procedure with dansyl chloride and HPLC separation. Of all other methods, there were not enough results available to calculate the corresponding precision parameters.
The evaluation programs for the calculation of the precision parameters according to the harmonized IUPAC-1987 protocol and the Swiss food manual method were written with Turbo Pascal for Windows Version 1. The detailed statistical results of the IUPAC evaluation is given in Appendix FL and UV.
SYSTAT program modules were used for the descriptive statistics and SYGRAPH program to draw box and category plots [3], which are shown in Appendix G.

Statistics

To perform statistical evaluation of collaborative studies, results of at least 8 laboratories are necessary. In this collaborative study only the Swiss food manual method with precolumn dansylchloride derivatization and HPLC separation was applied of enough laboratories (number 1 - 25) to calculate the precision parameters.
The calculation of the precision parameters mean, repeatability and reproducibility were performed according to the harmonized IUPAC-1987 protocol with Cochran and Grubb's outlier elimination procedure [4] and with the robust statistical method of the Swiss food manual [5].
For the evaluation of repeatability and reproducibility all "zero values" (below detection limits) were eliminated. This avoids problems of the evaluation because of different detection limits.

Statistical analysis with harmonized IUPAC-1987 protocol

Means, repeatabilities and reproducibilities were calculated according to the harmonized IUPAC-1987 protocol [4].

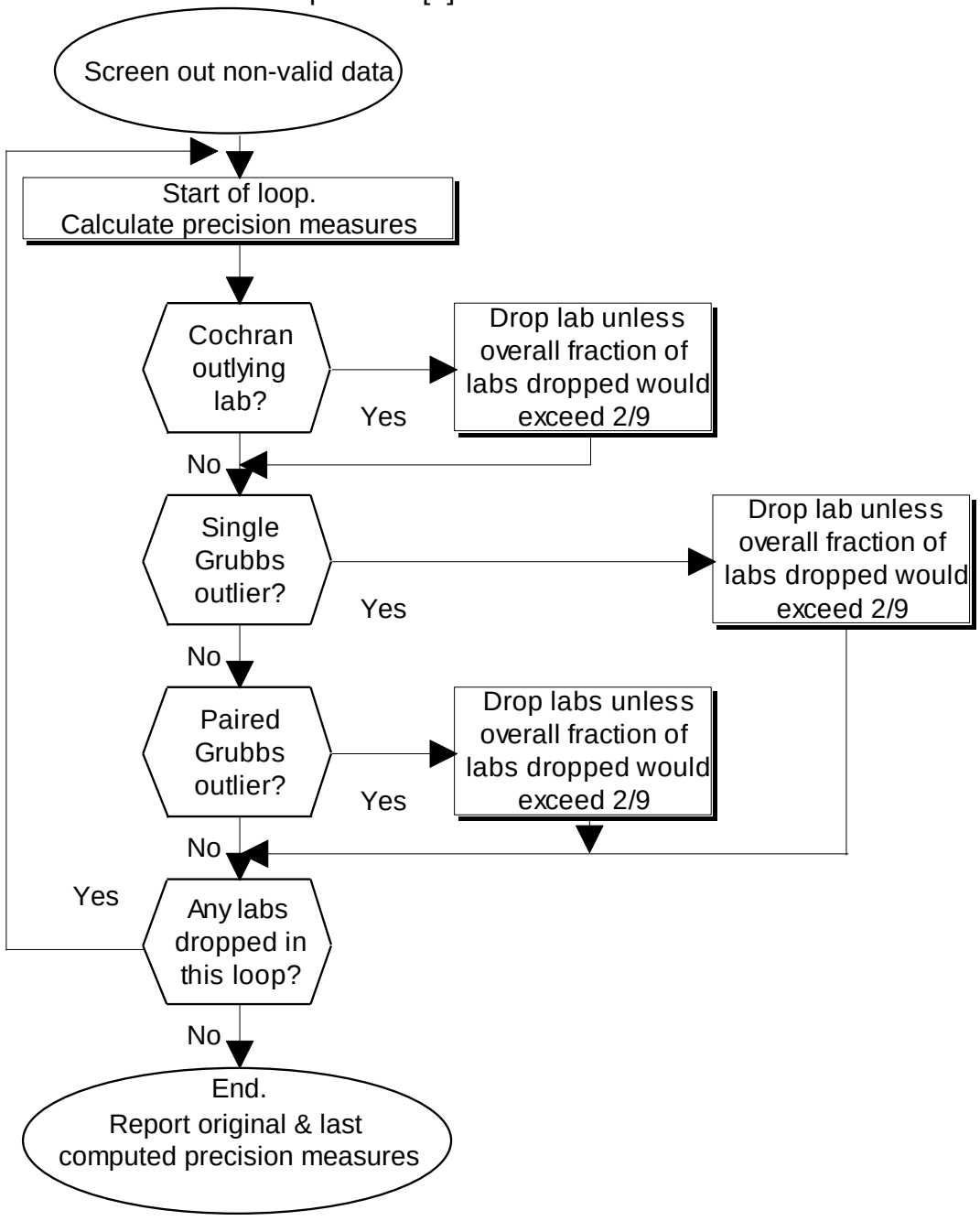


Figure 2 Flow chart according to the harmonized IUPAC-1987 protocol.

Robust statistical analysis according to Swiss food manual

Robust mean, repeatabilities and reproducibilities were calculated according to the Swiss food manual [5]. 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.

Outliers elimination according to the harmonized IUPAC-1987 protocol

The outlier elimination procedure was performed for the results of the Swiss food manual method with fluorescence detection (Table 6) and UV detection (Table 7). The laboratory numbers of the outliers are shown in the following two tables.

Table 6 Outliers with HPLC of dansyl derivates and fluorescence detection

Amine	Standard solution			Wine			Wine spiked		
	Cochran (variance)	Grubb (mean)		Cochran	Grubb (mean)		Cochran	Grubb (mean)	
	too high	too low	too high	too high	too low	too high	too high	too low	too high
TRA		16*					6		
PHA	9,3	16*			12			12*,16*	
ISA		16*							2,6
PUT				18,9	12*,13'		6,9	12	
CAD		16*	2*				6,9	12	
HIA		16*,12							
TYA		16		6	16*	12*	6,10		
SPD		16							
SPM	9								

Amine	Feed			Cheese 1			Cheese 2		
	Cochran (variance)	Grubb (mean)		Cochran	Grubb (mean)		Cochran	Grubb (mean)	
	too high	too low	too high	too high	too low	too high	too high	too low	too high
TRA	3								
PHA	18,12	16',25'	3**	23,3,7			8,23		
ISA	18	16'	3						
PUT		10	16,12			16*	23'	12*,10	16*,3'
CAD	25				12	3		12*	
HIA	12			10			12,3		16'
TYA	9	16,25'	3,12'	3	16,25			16*	4,3
SPD	6,2		16'						
SPM			16						

Legend:

1 to 25	Number of the outlying laboratory ($p < 0.01$)
*	Straggler ($p < 0.05$) in Grubb Test
**	Straggler ($p < 0.05$) in Dixon Test
'	Outliers exceeding 2/9

Table 7 Outliers with HPLC of dansyl derivates and UV detection

Amine	Standard solution			Wine			Wine spiked		
	Cochran (variance)	Grubb (mean)		Cochran	Grubb (mean)		Cochran	Grubb (mean)	
	too high	too low	too high	too high	too low	too high	too high	too low	too high
TRA	3		2*						
PHA	10,9	15*		3,10,1	20	15*',22*'			
ISA	19,9	22*'	2*	18,22,1'					
PUT				9,5	13		6	15	
CAD		15*	2,10	14,1	5'	13'			
HIA	10,3		2*			20*	9,6		14*,20*,2*'
TYA	10			3	21		6	15,9*	
SPD				1					
SPM	9,18	25*,10*'							

Amine	Feed			Cheese 1			Cheese 2		
	Cochran (variance)	Grubb (mean)		Cochran	Grubb (mean)		Cochran	Grubb (mean)	
	too high	too low	too high	too high	too low	too high	too high	too low	too high
TRA	22		3				10	25*,23*'	3*
PHA				22,23,3			10,22		5*
ISA			3*						
PUT						10	2,18		
CAD		20*,21*		22			18		
HIA		20*,5*	21*,3*		11*	3*			
TYA	10,9,25	20*',5*'	3*	10,3			18	25*,20*	3*
SPD			19	20					
SPM	14,15,3',18'								

Legend see Table 6

Precision parameters calculated with IUPAC-1987 protocol

In the following tables 8 - 13, the results of the statistical evaluation according to the harmonized IUPAC-1987 protocol are given. For each amine and sample the precision parameters are shown for the two different detection method (fluorescence and UV). In Table 14 the recoveries of the added biogenic amines to the wine sample are given.

Table 8 Determination of biogenic amines in standard solution

Amine	Det	n	add.	mean	s_r	RSD_r	r	s_R	RSD_R	R	mean/add.
			mg/L	mg/L	mg/L	%	mg/L	mg/L	%	mg/L	%
TRA	FL	11	52.3	44.8	1.3	3.0	3.8	8	18	23	86
	UV	13	52.3	39.6	0.7	1.8	2.0	5	13	15	76
PHA	FL	11	57.8	49.9	1.0	1.9	2.7	7	13	19	86
	UV	15	57.8	49.0	1.6	3.2	4.5	6	13	17	85
ISA	FL	11	45.0	40.8	2.3	5.7	6.5	5	13	15	91
	UV	10	45.0	41.1	1.2	2.9	3.4	2	6	7	91
PUT	FL	15	48.4	46.9	1.4	2.9	3.9	4	9	12	97
	UV	19	48.4	47.7	1.4	2.9	4.0	4	7	10	99
CAD	FL	13	44.9	46.3	1.6	3.5	4.6	2	5	6	103
	UV	15	44.9	46.0	1.3	2.9	3.7	2	4	5	102
HIA	FL	10	48.3	43.8	3.6	8.2	10.2	5	11	13	91
	UV	17	48.3	44.0	1.2	2.7	3.3	4	8	10	91
TYA	FL	14	52.2	50.0	2.0	3.9	5.5	5	9	13	96
	UV	17	52.2	49.6	1.4	2.7	3.8	3	6	9	95
SPD	FL	10	50.4	48.4	1.6	3.4	4.6	6	12	17	96
	UV	15	50.4	46.6	1.2	2.6	3.4	6	12	16	93
SPM	FL	10	64.2	54.8	1.4	2.5	3.9	18	33	52	85
	UV	11	64.2	54.6	1.6	2.9	4.6	7	13	20	85
Minimum					0.7	1.8	2.0	2	4	5	76
Maximum					3.6	8.2	10.2	18	33	52	103
Median					1.4	2.9	3.9	5	11	14	91

Legend:

Det	detection with
UV	UV
FL	fluorescence
n	number of laboratories after outlier elimination
add.	addition (real concentration)
mean	calculated mean value (without outliers)
s_r	standard deviation of repeatability
RSD_r	relative standard deviation of repeatability
r	repeatability
s_R	standard deviation of reproducibility
RSD_R	relative standard deviation of reproducibility
R	reproducibility
mean/add.	calculated mean/addition * 100 %

Table 9 Determination of biogenic amines in wine

Amine	Det	n	mean	s_r	RSD_r	r	s_R	RSD_R	R
			mg/L	mg/L	%	mg/L	mg/L	%	mg/L
PHA	FL	11	2.2	0.2	9.3	0.6	0.4	19	1
	UV	9	2.4	0.2	9.0	0.6	0.5	21	1
ISA	FL	10	3.9	0.4	10.0	1.1	0.6	14	2
	UV	9	3.7	0.3	7.9	0.8	0.8	21	2
PUT	FL	11	31.5	0.5	1.7	1.5	3.7	12	10
	UV	16	30.2	1.2	3.8	3.3	3.8	13	11
CAD	FL	7	0.8	0.1	13.9	0.3	0.4	53	1
	UV	6	1.6	0.1	8.9	0.4	0.5	34	2
HIA	FL	7	11.0	0.5	4.3	1.3	2.2	20	6
	UV	18	9.4	0.6	6.5	1.7	1.2	13	4
TYA	FL	12	7.0	0.5	7.8	1.5	0.9	12	2
	UV	16	7.6	0.5	6.7	1.4	1.4	19	4
Minimum				0.1	1.7	0.3	0.4	11.7	1.2
Maximum				1.2	13.9	3.3	3.8	52.9	10.8
Median				0.4	7.9	1.2	0.8	19.2	2.3

Legend see Table 8

Table 10 Determination of biogenic amines in spiked wine

Amine	Det	n	mean	s_r	RSD_r	r	s_R	RSD_R	R
			mg/L	mg/L	%	mg/L	mg/L	%	mg/L
TRA	FL	11	17.1	0.6	3.8	1.8	7.7	45	22
	UV	14	18.2	1.1	6.3	3.2	7.4	40	21
PHA	FL	13	23.5	1.0	4.1	2.7	3.1	13	9
	UV	18	22.4	1.7	7.7	4.9	3.0	13	8
ISA	FL	10	11.2	0.5	4.5	1.4	1.3	11	4
	UV	14	12.0	0.8	6.4	2.2	1.7	14	5
PUT	FL	12	50.1	1.6	3.1	4.4	5.8	12	16
	UV	17	49.1	1.8	3.6	5.0	4.7	10	13
CAD	FL	12	27.5	0.8	2.9	2.3	2.5	9	7
	UV	18	28.7	1.7	5.9	4.8	3.1	11	9
HIA	FL	10	31.9	2.3	7.2	6.5	5.6	18	16
	UV	15	31.3	0.9	2.9	2.6	2.2	7	6
TYA	FL	13	31.7	1.3	4.0	3.6	5.5	17	16
	UV	15	33.2	1.3	4.0	3.8	2.3	7	7
Minimum				0.5	2.9	1.4	1.3	7	4
Maximum				2.3	7.7	6.5	7.7	45	22
Median				1.2	4.0	3.4	3.1	12	9

Legend see Table 8

Table 11 Determination of biogenic amines in feed

Amine	Det	n	mean	s_r	RSD_r	r	s_R	RSD_R	R
			mg/kg	mg/kg	%	mg/kg	mg/kg	%	mg/kg
TRA	FL	9	112	14	13	41	84	75	236
	UV	11	79	8	10	22	35	44	98
PHA	FL	8	129	5	4	14	10	8	28
	UV	14	141	19	14	54	61	44	174
ISA	FL	8	64	4	6	11	9	14	25
	UV	11	56	8	15	24	18	33	52
PUT	FL	11	1076	32	3	92	69	6	195
	UV	19	905	44	5	125	253	28	717
CAD	FL	11	1967	43	2	122	254	13	719
	UV	16	1845	54	3	153	261	14	740
HIA	FL	10	1144	123	11	349	328	29	928
	UV	16	975	32	3	92	99	10	280
TYA	FL	9	914	19	2	55	49	5	140
	UV	12	927	17	2	49	81	9	231
SPD	FL	7	48	1	3	4	21	44	60
	UV	12	51	6	11	16	23	46	66
SPM	FL	7	35	6	17	17	10	29	29
	UV	8	29	2	7	5	17	59	49
Minimum				1	2	4	9	5	25
Maximum				123	17	349	328	75	928
Median				16	6	45	55	28	157

Legend see Table 8

Table 12 Determination of biogenic amines in cheese 1

Amine	Det	n	mean	s_r	RSD_r	r	s_R	RSD_R	R
			mg/kg	mg/kg	%	mg/kg	mg/kg	%	mg/kg
PHA	FL	12	345	13	4	38	97	28	273
	UV	15	359	13	4	36	80	22	227
PUT	FL	14	68	7	10	20	22	32	61
	UV	17	69	6	9	18	13	19	37
CAD	FL	13	597	22	4	63	49	8	140
	UV	18	563	29	5	82	98	17	276
HIA	FL	11	808	68	8	193	147	18	415
	UV	19	811	30	4	85	109	13	308
TYA	FL	11	1319	58	4	163	145	11	410
	UV	16	1228	34	3	96	290	24	821
Minimum				6	3	18	13	8	37
Maximum				68	10	193	290	32	821
Median				26	4	72	97	19	275

Legend see Table 8

Table 13 Determination of biogenic amines in cheese 2

Amine	Det	n	mean	s_r	RSD_r	r	s_R	RSD_R	R
			mg/kg	mg/kg	%	mg/kg	mg/kg	%	mg/kg
TRA	FL	12	57	5	8	14	29	52	83
	UV	10	63	8	13	22	10	16	29
PHA	FL	11	33	2	6	5	13	38	36
	UV	11	31	3	11	10	10	31	27
PUT	FL	10	390	7	2	19	31	8	89
	UV	18	367	16	4	46	58	16	164
CAD	FL	12	693	23	3	66	111	16	313
	UV	18	629	23	4	66	94	15	266
HIA	FL	8	239	25	10	71	54	23	154
	UV	21	242	17	7	49	38	16	109
TYA	FL	12	387	17	4	49	37	10	104
	UV	15	414	16	4	45	35	8	99
Minimum				2	2	5	10	8	27
Maximum				25	13	71	111	52	313
Median				16	5	45	36	16	102

Legend see Table 8

Table 14 Recovery of added amines to the spiked wine sample

Amine	Det	add.	wine spiked - wine	recovery
		mg/L	mg/L	%
TRA	FL	19.7	17.1	87
	UV	19.7	18.2	93
PHA	FL	29	21.4	74
	UV	29	20.0	69
ISA	FL	9.3	7.3	79
	UV	9.3	8.3	90
PUT	FL	20.9	18.6	89
	UV	20.9	18.9	90
CAD	FL	29.5	26.7	91
	UV	29.5	27.1	92
HIA	FL	22.7	20.9	92
	UV	22.7	21.9	97
TYA	FL	25.8	24.7	96
	UV	25.8	25.7	100

Legend see Table 8

Robust estimation of precision parameters

In the following tables 15 - 20, the results of the statistical evaluation according to the Swiss food manual are given. For each amine and sample the robust precision parameters are shown for the two different detection method (fluorescence and UV). In Table 21 the recoveries of the added biogenic amines to the wine sample are given.

Table 15 Determination of biogenic amines in standard solution

Amine	Det	n	add.	mean	s_r	RSD_r	r	s_R	RSD_R	R	mean/add.
			mg/L	mg/L	mg/L	%	mg/L	mg/L	%	mg/L	%
TRA	FL	12	52.3	42.9	1.2	2.8	3.4	9	21	26	82
	UV	15	52.3	41.2	0.4	0.9	1.1	6	15	18	79
PHA	FL	14	57.8	48.6	1.2	2.5	3.5	8	16	22	84
	UV	18	57.8	48.2	1.0	2.1	2.8	8	16	22	83
ISA	FL	12	45.0	40.4	1.2	3.1	3.5	4	9	11	90
	UV	14	45.0	40.6	0.8	2.0	2.3	3	7	8	90
PUT	FL	15	48.4	46.7	0.7	1.5	1.9	4	8	11	96
	UV	19	48.4	47.5	1.2	2.6	3.5	3	6	8	98
CAD	FL	15	44.9	46.5	0.6	1.4	1.8	2	5	6	104
	UV	18	44.9	46.1	0.8	1.6	2.1	2	4	5	103
HIA	FL	12	48.3	42.6	0.7	1.6	1.9	6	14	16	88
	UV	20	48.3	44.5	1.5	3.3	4.1	4	8	10	92
TYA	FL	15	52.2	49.8	0.9	1.8	2.6	5	10	14	95
	UV	18	52.2	50.2	1.0	2.0	2.9	2	4	5	96
SPD	FL	11	50.4	49.8	0.8	1.6	2.3	5	10	14	99
	UV	15	50.4	46.5	1.3	2.7	3.6	4	8	11	92
SPM	FL	11	64.2	57.1	1.2	2.0	3.3	9	15	25	89
	UV	15	64.2	53.1	2.6	4.9	7.3	10	18	27	83
Minimum					0.4	0.9	1.1	2	4	5	79
Maximum					2.6	4.9	7.3	10	21	27	104
Median					1.0	2.0	2.8	4	10	13	91

Legend see Table 8

Table 16 Determination of biogenic amines in wine

Amine	Det	n	mean	s_r	RSD_r	r	s_R	RSD_R	R
			mg/L	mg/L	%	mg/L	mg/L	%	mg/L
PHA	FL	12	2.1	0.1	3.8	0.2	0.4	20	1.2
	UV	15	2.6	0.2	9.1	0.7	0.7	25	1.9
ISA	FL	10	3.8	0.3	7.5	0.8	0.5	12	1.3
	UV	12	4.0	0.2	5.8	0.6	1.1	27	3.1
PUT	FL	15	29.3	0.4	1.5	1.2	4.3	15	12.3
	UV	19	29.0	1.1	4.0	3.3	3.9	13	11.0
CAD	FL	7	0.8	0.1	11.6	0.3	0.3	45	1.0
	UV	10	2.0	0.2	10.6	0.6	0.8	41	2.3
HIA	FL	9	9.7	0.5	4.6	1.3	2.2	22	6.1
	UV	19	9.3	0.3	3.2	0.8	0.9	10	2.6
TYA	FL	15	7.1	0.5	7.0	1.4	1.2	17	3.4
	UV	18	7.4	0.3	4.3	0.9	1.5	20	4.2
Minimum				0.1	1.5	0.2	0.3	10.0	1.0
Maximum				1.1	11.6	3.3	4.3	45.4	12.3
Median				0.3	5.2	0.8	1.0	20.3	2.9

Legend see Table 8

Table 17 Determination of biogenic amines in spiked wine

Amine	Det	n	mean	s_r	RSD_r	r	s_R	RSD_R	R
			mg/L	mg/L	%	mg/L	mg/L	%	mg/L
TRA	FL	12	18.1	0.7	3.8	1.9	4.6	26	13
	UV	14	17.8	0.6	3.4	1.7	6.6	37	19
PHA	FL	15	22.6	0.8	3.5	2.2	3.7	16	10
	UV	18	22.1	0.8	3.7	2.3	2.7	12	8
ISA	FL	12	11.8	0.3	2.6	0.9	1.4	12	4
	UV	14	12.0	0.5	3.8	1.3	1.8	15	5
PUT	FL	15	49.7	0.8	1.6	2.3	4.4	9	12
	UV	19	48.8	1.2	2.6	3.5	5.3	11	15
CAD	FL	15	27.3	0.7	2.5	1.9	2.3	9	7
	UV	18	28.6	0.9	3.0	2.5	2.7	10	8
HIA	FL	10	32.6	1.6	4.9	4.6	4.6	14	13
	UV	20	32.1	0.8	2.4	2.2	3.3	10	9
TYA	FL	15	32.9	1.3	4.1	3.8	2.5	8	7
	UV	18	32.9	0.7	2.2	2.1	2.5	7	7
Minimum				0.3	1.6	0.9	1.4	7	4
Maximum				1.6	4.9	4.6	6.6	37	19
Median				0.8	3.2	2.2	3.0	11	9

Legend see Table 8

Table 18 Determination of biogenic amines in feed

Amine	Det	n	mean	s_r	RSD_r	r	s_R	RSD_R	R
			mg/kg	mg/kg	%	mg/kg	mg/kg	%	mg/kg
TRA	FL	10	114	20	17	55	81	71	228
	UV	13	90	10	11	28	41	45	115
PHA	FL	13	126	2	1	4	19	15	55
	UV	14	129	4	3	10	44	34	124
ISA	FL	11	65	3	4	8	13	21	38
	UV	12	59	5	8	13	17	29	49
PUT	FL	14	1060	18	2	52	90	8	254
	UV	19	950	24	3	69	188	20	531
CAD	FL	14	2016	36	2	102	232	12	657
	UV	18	1805	28	2	78	294	16	831
HIA	FL	11	1190	134	11	378	389	33	1101
	UV	20	975	21	2	59	129	13	365
TYA	FL	14	904	29	3	82	106	12	300
	UV	18	882	13	2	38	133	15	376
SPD	FL	10	50	3	5	7	18	36	51
	UV	13	50	3	5	7	20	40	56
SPM	FL	8	36	4	11	11	9	24	25
	UV	12	36	5	13	13	20	56	58
Minimum				2	1	4	9	8	25
Maximum				134	17	378	389	71	1101
Median				12	4	33	62	22	176

Legend see Table 8

Table 19 Determination of biogenic amines in cheese 1

Amine	Det	n	mean	s_r	RSD_r	r	s_R	RSD_R	R
			mg/kg	mg/kg	%	mg/kg	mg/kg	%	mg/kg
PHA	FL	15	363	7	2	20	74	20	209
	UV	18	374	17	5	49	104	28	293
PUT	FL	15	73	4	5	10	17	23	48
	UV	18	69	6	9	17	11	17	32
CAD	FL	15	600	23	4	64	53	9	150
	UV	19	588	20	3	56	68	12	192
HIA	FL	12	834	31	4	86	129	15	365
	UV	21	831	27	3	76	88	11	248
TYA	FL	14	1302	28	2	78	188	14	533
	UV	18	1303	38	3	106	244	19	690
Minimum				4	2	10	11	9	32
Maximum				38	9	106	244	28	690
Median				21	4	60	81	16	229

Legend see Table 8

Table 20 Determination of biogenic amines in cheese 2

Amine	Det	n	mean	s_r	RSD_r	r	s_R	RSD_R	R
			mg/kg	mg/kg	%	mg/kg	mg/kg	%	mg/kg
TRA	FL	12	56	4	7	12	26	46	72
	UV	14	63	3	5	8	13	21	37
PHA	FL	13	32	1	4	3	13	41	38
	UV	14	37	4	11	12	16	44	46
PUT	FL	15	388	10	3	28	44	11	125
	UV	20	370	8	2	24	54	15	154
CAD	FL	15	680	18	3	51	88	13	249
	UV	19	628	20	3	58	79	13	224
HIA	FL	11	288	22	8	62	101	35	286
	UV	21	240	8	3	21	23	10	65
TYA	FL	15	394	14	4	41	52	13	148
	UV	19	413	15	4	42	37	9	106
Minimum				1	2	3	13	9	37
Maximum				22	11	62	101	46	286
Median				9	4	26	41	14	115

Legend see Table 8

Table 21 Recovery of added amines to the wine spiked sample

Amine	Det	add.	wine spiked - wine	recovery
		mg/L	mg/L	%
TRA	FL	19.7	18.1	92
	UV	19.7	17.8	90
PHA	FL	29	20.6	71
	UV	29	19.5	67
ISA	FL	9.3	8.0	86
	UV	9.3	8.1	87
PUT	FL	20.9	20.4	97
	UV	20.9	19.8	95
CAD	FL	29.5	26.5	90
	UV	29.5	26.6	90
HIA	FL	22.7	22.8	100
	UV	22.7	22.8	101
TYA	FL	25.8	25.8	100
	UV	25.8	25.5	99

Legend see Table 8

Discussion

Comparison of the median values of the different methods

The median values of the different methods were compared on the graphical presentations (page G2-G55) according to the instruction on page G1. Table 22 shows the significant differences.

Table 22 Significant differences between the median values of the methods

Amine	Standard solution	Wine	Wine spiked	Feed	Cheese 1	Cheese 2
TRA	↑ LC-OPP ↓ LC-Dab					↑ LC-OPP
PHA	↓ LC-Dab					
ISA	↑ LC-OPA ↑ LC-Dab		↑ LC-OPA	↑ LC-OPP ↑ LC-Dab		
PUT		↑ LC-OPP ↓ LC-Dab	↓ LC-OPP	↑ LC-OPP ↑LC-OPA	↑ LC-Dab	↑ LC-Dab
CAD	↓ LC-OPP				↓ LC-Dab	↓ LC-Dab
HIA	↑ LC-OPP ↓ IC-OPA	↑ LC-OPP	↓ LC	↓ LC-Dab ↓ LC	↓ LC-Dab	↑ LC-Dab
TYA				↓ LC-Dab ↓ LC	↓ LC-Dab	↓ LC-Dab
SPD	↑ LC-Dab			↑ LC-Dab		
SPM	↓ LC-Dab			↑ LC-Dab		

Legend:
The abbreviation of the methods are given in Table 4
↓ X results with method X are significant lower ($p < 0.05$)
↑ X results with method X are significant higher ($p < 0.05$)

Comparison of the precision parameters calculated with IUPAC and robust statistics

The precision parameters could only be calculated for the Swiss food manual method (precolumn derivatization with dansylchloride and HPLC separation). The two detection methods fluorescence and UV detection were separately calculated.

Mean values (Table 8 - Table 21)

The detection with fluorescence is about 5 - 10 times more sensitive than with UV. No difference in the detection limits for tyramine. The sensitivity for histamine with fluorescence is about 4 - 5 times lower compared to the UV detection. Higher values for the repeatability for histamine with fluorescence detection can be explained by this phenomena (see * chapter standard deviation of repeatability and reproducibility).

Standard solution
The mean values calculated with the two statistical methods gave the same results 100 ± 4 %. The yield of all biogenic amines were better than 80 %, except for tryptamine with UV detection (76 - 79 %). In some chromatograms there was a bad resolved artifact peak just after tryptamine present. Only the yield of cadaverine were slightly over 100 % (102 - 104 %).

Wine
The mean value of ß-phenylethylamine with fluorescence detection is about 10 % lower than with UV detection. The same effect can be observed with cadaverine, where the fluorescence results were only half of the UV results. The mean value of histamine with fluorescence is 15 % , but not significantly higher than with UV detection.

Wine spiked
The recoveries were better than 80 % for most amines. ß-Phenylethylamine and isopentylamine had recoveries of 67-74 and 79-90 %, respectively.

Feed
The mean values of fluorescence results were for most amines slightly higher than for UV results. This effect can again be explained by an UV active interference peaks eluting together with the internal standard 1,7-diaminoheptane.

Cheese 1
In this cheese sample only five amines could be detected: ß-phenylethylamine, histamine, tyramine, putrescine and cadaverine. The mean results calculated with the two statistical methods were all in the range from 93 to 107 %. No difference between the fluorescence and the UV detection could be observed.

Cheese 2
In this cheese samples only isopentylamine, spermidine and spermine could not be detected. The mean results calculated with the two statistical methods were all in the range from 85 to 110 %, except for histamine with fluorescence detection, where the mean value calculated with the robust method is 20 % higher. The reason is the non symmetrical distribution of the data.

Standard deviations of repeatability s_r (Table 8 - Table 21)

Standard solution

All standard deviations of repeatability were ≤ 2.6 mg/L, except for histamine with fluorescence detection s_r ≤ 3.6 mg/L*.
The median of all repeatabilities was ≤ 1.4 mg/L.

Wine

All standard deviations of repeatability were ≤ 0.6 mg/L, except for putrescine with UV detection s_r ≤ 1.2 mg/L. The origin of this bad repeatability is probably the interference with isopentylamine or the excess of dansylchloride, not destroyed by the addition of sodium glutaminate, gives interference with the UV signal of putrescine. The median of all repeatabilities was = 0.3 mg/L.

Wine spiked

All standard deviations of repeatability were ≤ 1.8 mg/L, except for histamine with fluorescence detection s_r = 2.3 mg/L*.
The median of all repeatabilities was ≤ 1.2 mg/L.

Feed

All standard deviations of repeatability were ≤ 54 mg/kg, except for histamine with fluorescence detection s_r ≤ 134 mg/kg*.
The median of all repeatabilities was ≤ 16 mg/kg.

Cheese 1

All standard deviations of repeatability were ≤ 38 mg/kg, except for histamine and tyramine with fluorescence detection s_r ≤ 68 mg/kg*, calculated with IUPAC method.
The median of all repeatabilities was ≤ 26 mg/kg.

Cheese 2

All standard deviations of repeatability were ≤ 23 mg/kg, except for histamine with fluorescence detection s_r ≤ 25 mg/kg*.
The median of all repeatabilities was ≤ 16 mg/kg.

Standard deviations of reproducibility s_R (Table 8 - Table 21)

Standard solution

All standard deviations of reproducibility were ≤ 10 mg/L, except for spermine with fluorescence detection s_R = 18 mg/L, calculated with the IUPAC method. This is a classical demonstration of the big outlier influence. Robust value for the same data is only s_R = 9 mg/L and comparable with the results of UV detection.
The median of all reproducibilities was ≤ 5 mg/L.

Wine

All standard deviations of reproducibility were ≤ 1.5 mg/L, except for putrescine s_R ≤ 4.3 mg/L and histamine with fluorescence detection s_R = 2.2 mg/L*.
The median of all reproducibilities was ≤ 1 mg/L.

Wine spiked

All standard deviations of reproducibility were ≤ 5.8 mg/L, except for tryptamine s_R ≤ 8 mg/L.
The median of all reproducibilities was ≤ 3.1 mg/L.

Feed

All standard deviations of reproducibility were ≤ 130 mg/kg, except for cadaverine s_R ≤ 294 mg/kg, putrescine with UV detection s_R ≤ 253 mg/kg and histamine with fluorescence detection s_R ≤ 390 mg/kg*.
The median of all reproducibilities was ≤ 62 mg/kg.

Cheese 1

All standard deviations of reproducibility were ≤ 104 mg/kg, except for tyramine s_R ≤ 290 mg/kg and histamine with fluorescence detection s_R ≤ 147 mg/kg.
The median of all reproducibilities was ≤ 97 mg/kg.

Cheese 2

All standard deviations of reproducibility were ≤ 58 mg/kg, except for cadaverine s_R ≤ 111 mg/kg and histamine with fluorescence detection s_R ≤ 101 mg/kg*.
The median of all reproducibilities was ≤ 41 mg/kg.

Conclusions

Some of the HPLC dansyl chromatograms showed not baseline separation of all biogenic amines, especially for ß-phenylethylamine, isopentylamine and putrescine. In order to improve chromatography and to reduce interference's, change of stationary phase or gradient is very easy and cheap (see Figure 3). Multi point calibration is recommended to improve the accuracy and to check the linearity. Due to the bad repeatability and reproducibility the fluorescence detection of the histamine derivate can not be recommended. A combination of UV (for histamine) and fluorescence detection will give the most precise results.

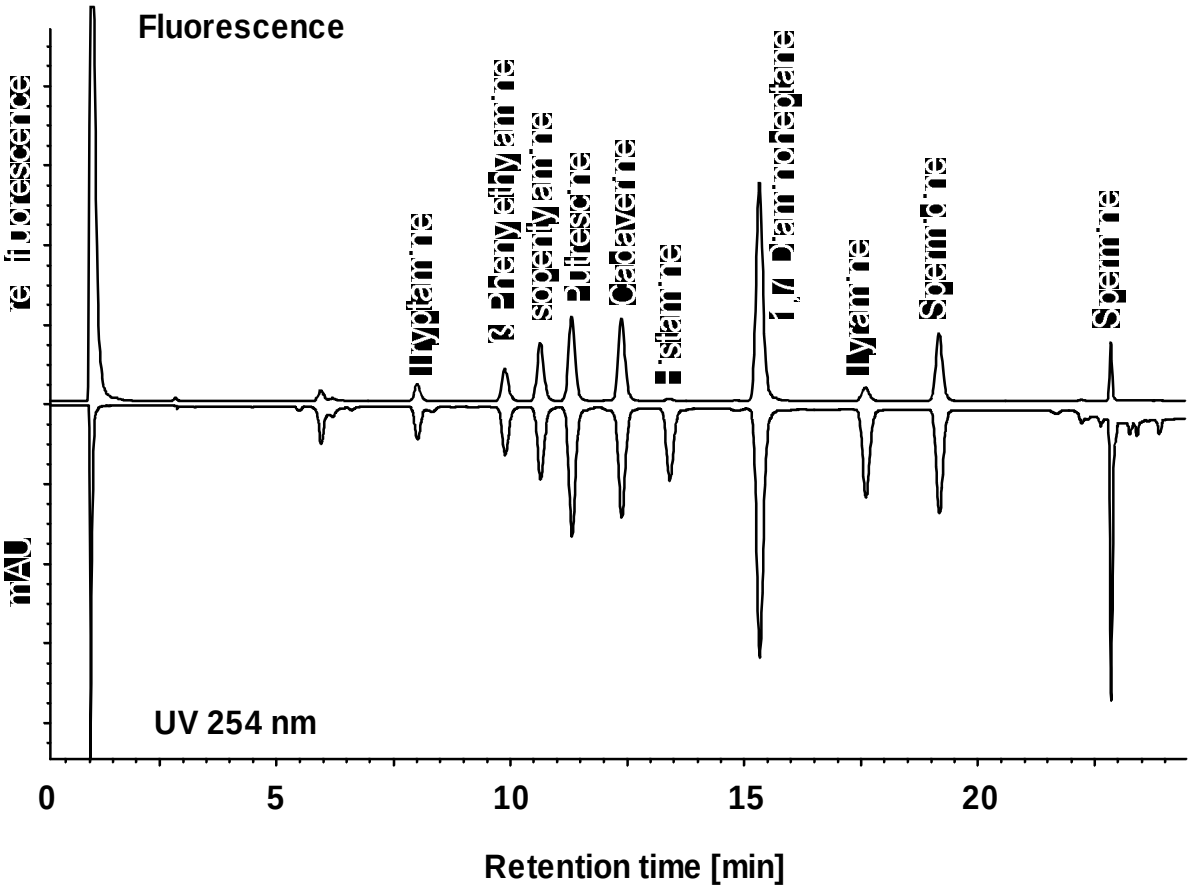


Figure 3 HPLC separation of dansyl derivates on 5 µm Hypersil ODS 250 x 4 mm at 35°C with a stepwise linear gradient: 0 - 20 min, 5 - 63 % solvent B, 20 - 21 min, 63 - 100 % solvent B. UV detection at 254 nm, fluorescence detection Ex: 254 nm, Em: 485 nm [6].

HPLC of OPA derivates can be very important for the future. Automatization of this precolumn derivatization technique is very easy to perform on modern programmable autosamplers. After harmonization of the different methods and thiols used, a new collaborative study should be organized and compared with the Swiss food manual method.

One laboratory used the German food manual fluorescence method (§ 35) for the determination of histamine in the two cheese samples. The histamine content found in the two cheese samples with this method was 519 and 155 mg/kg, respectively. Compared with the HPLC dansyl method with UV detection, the German food manual method gave recoveries of 61 and 63 %.

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