



**Bienvenidos
al Seminario**

Automated Preparation of Multi-Compound Calibration Standards

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- ❖ **Standards and working mixture production in pesticide analysis is very complex.**
- ❖ **Automation solutions takes the management and production of standards and mixes to a new level.**

1. Making of Working Standards Solutions
2. SANTE 11312/2021 Requirements
3. Calibration Dilution for Quantitation
4. Standard Management in the Database
5. Automated Standards Preparation Workflow
6. Conclusion



Reference and Calibration Standards

Preparation of multicomponent calibration standards

- essential task in all laboratories
- in qualitative and quantitative analysis



Sample response

- is compared with that of a standard preparation
- quantifies the amount of analyte present

Individual errors and bias in the standard preparation

- => low reproducibility
- => errors in sample results



Image: Restek Multiresidue Pesticide Kit

Making of Working Standards Solutions

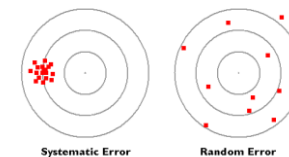
Using certified reference materials (CRM)

- Used as the stock solution (mg/mL range, 10 mL vials)
- Stored until the expiration date
- Errors in stock solution preparation => working standard



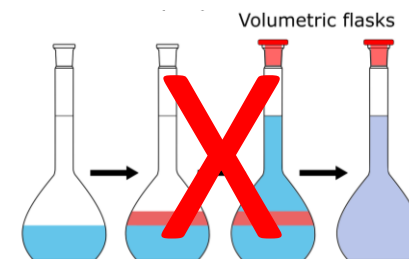
From the stock solution

- the working standard is prepared on the day of analysis,
- usually discarded after the analysis has been completed
- We use typically 2 mL vials, no volumetric flasks
- Errors in working standard preparation
- => Error in sample result



Automation with a PAL System

- Provides high precision and reproducibility
- Minimizes the uncertainty of the analytical procedure



SANTE 11312/2021 Requirements Fulfilled

Method Validation Procedures for Pesticide Residues Analysis in Food and Feed

When preparing stock standards of reference standards with analytes and internal standards [4]:

- Documentation for full traceability.
- Date of preparation, expiry, the identity and mass
- Identity and volume of the solvent (or other diluents)
- Storage at low temperature in the dark, indelibly labelled.
- Moisture must be excluded
- Equilibration to room temperature, remix before use.
- Avoid septum punctures, replace
- Corrected concentrations for the purity.
- Weighing using a 5 decimal place balance.

ANALYTICAL QUALITY CONTROL AND METHOD VALIDATION PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS IN FOOD AND FEED SANTE 11312/2021	
Supersedes Document No. SANTE/2019/12682. Implemented by 01/01/2022	
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Chronect MultiMix Modules and Tools



Cooling + Traceability

- Peltier cooled
- 4°C to 40°C under N₂ flow
- 6 racks of 15 or 54 positions



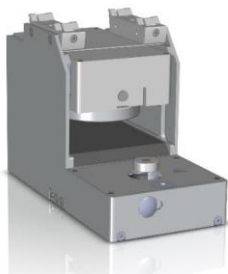
Clear labeling (2D QR-code)

- Barcode Printing/Reading
- Rotating



Re-mixing

- Vortex mixer
- Up to 2000 rpm



No septum exchange

- Vial decapping and re-capping



Low risk of carryover

- Pipetting tools
- Disposable pipettes
- 200 and 1000 µL

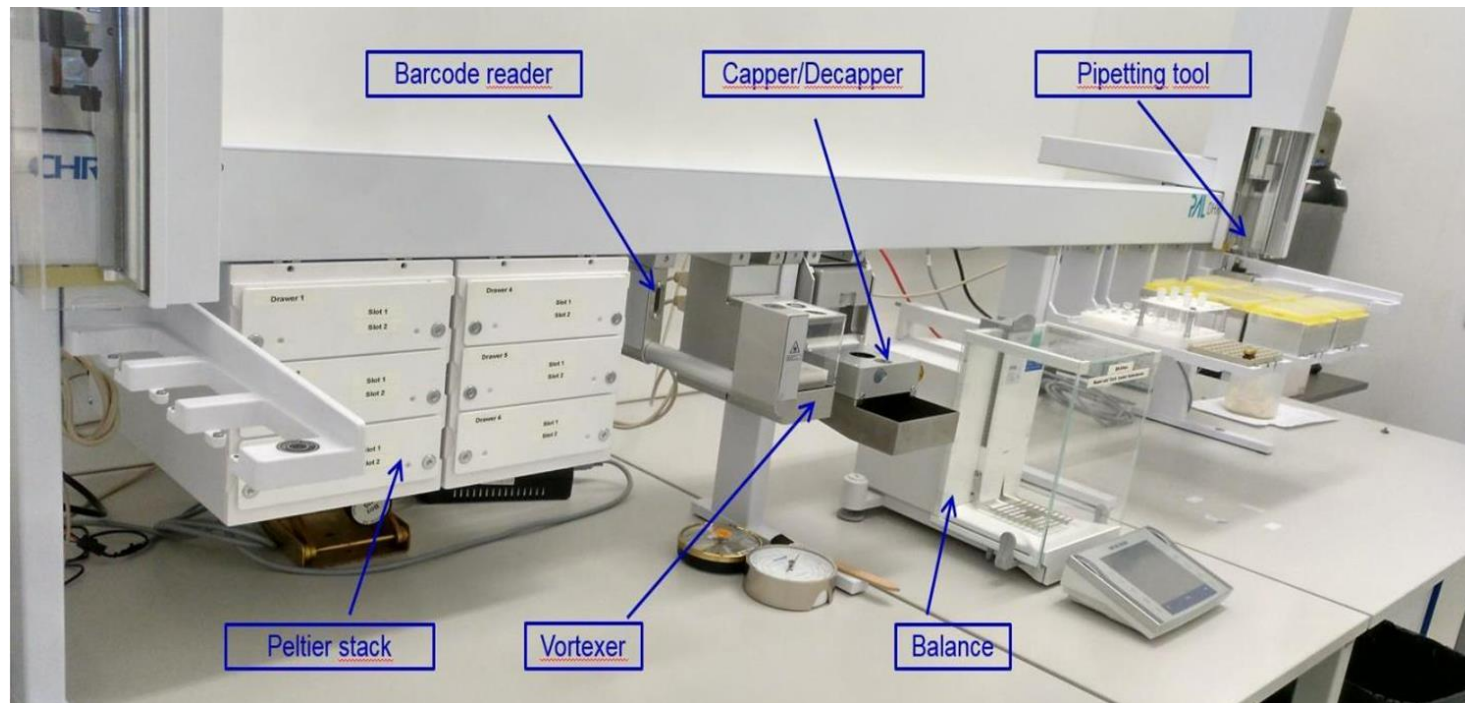


Gravimetric control + Traceability

- 5 digit analytical balance
- Integrated by CHRONOS

Automated Preparation of Multicomponent Calibration Standards

The AxelSemrau CHRONECT MultiMix Workstation



How Accurate Are Your Dilutions?

Analytical procedures rely on the accuracy of the preparation

- of the initial stock solution
- of the working standard solutions

The serial dilution scheme can play a significant role

- Use serial dilutions with larger volumes [2]

PAL System Automation: Gravimetric specifications [3]:

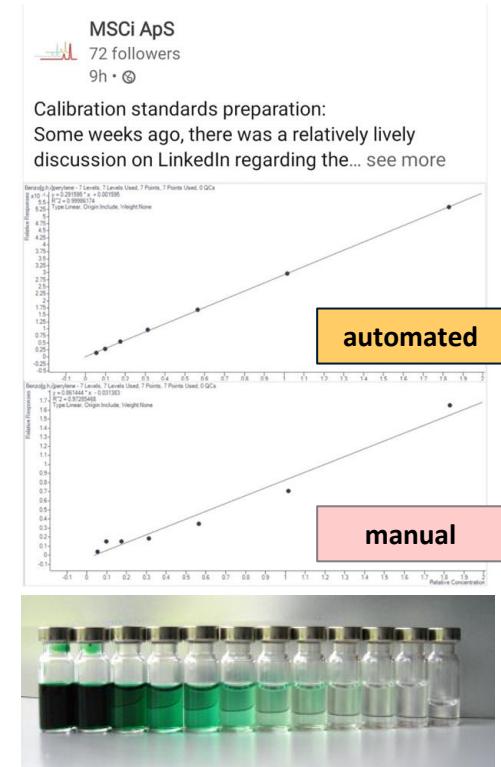
- Syringe RSDs < 0.1 %
 - (100 μ L, 10-100% transfer volume, single dispense)
- Pipette RSDs \leq 0.5 %
 - (1000 μ L, 10-100% transfer volume, single dispense)
- Dilutor RSDs \leq 0.1 %
 - (1000 μ L, 10-100% transfer volume, single dispense)



Calibration Dilution for Quantitation

General requirements of SANTE 11312/2021 [4]:

- Multi-level calibration with three or more concentrations is preferred.
- Bracketing calibration must be used.
- Matrix-matched calibration with extracts of a blank matrix, preferably of the same type as the sample.
- Analyte protectants are added to sample extracts and the calibration standard solutions.
- Inject the calibration standards at the start and end of a sample sequence.
- Sample standard addition can compensate for matrix effects and losses.



Standard Management in the Database

Upon receipt of a new reference standard all relevant data and the certificate are transferred to the database:


The screenshot shows a software interface for standard management. Key elements include:

- Standard name:** Metalaxyl
- Location:** Metalaxyl
- Batch, CAS-Number, Amount:** 776236, 57837-19-1, 1 x 100 mg
- Expiration date:** 01.04.2020

A table at the bottom shows currently available solutions:

Art	Konzentration	Menge	Lösemittel	Lagerbedingungen	Herstellung	MHD	Entsorgung	Kürzel
Standard	10,58 mg/l	10 ml	Acetonitril	KS	30.11.2018	30.11.2019		
Stamm	1,058 mg/ml	10 ml	Acetonitril	TKS	15.08.2018	15.08.2019		WR
Standard	10,58 mg/l	10 ml	Acetonitril	KS	15.08.2018	15.08.2019		

currently available solutions (incl concentration, location, expiration date, solvent, etc.)



Standard Management in the Database

- All solutions prepared from neat reference materials were registered.
- Stock solutions: Usually 10 mL with a concentration of approx. 1 mg/mL

The screenshot shows a software interface for managing standards. The main form is titled 'Eintrag ändern/aktualisieren'. It contains several fields and buttons:

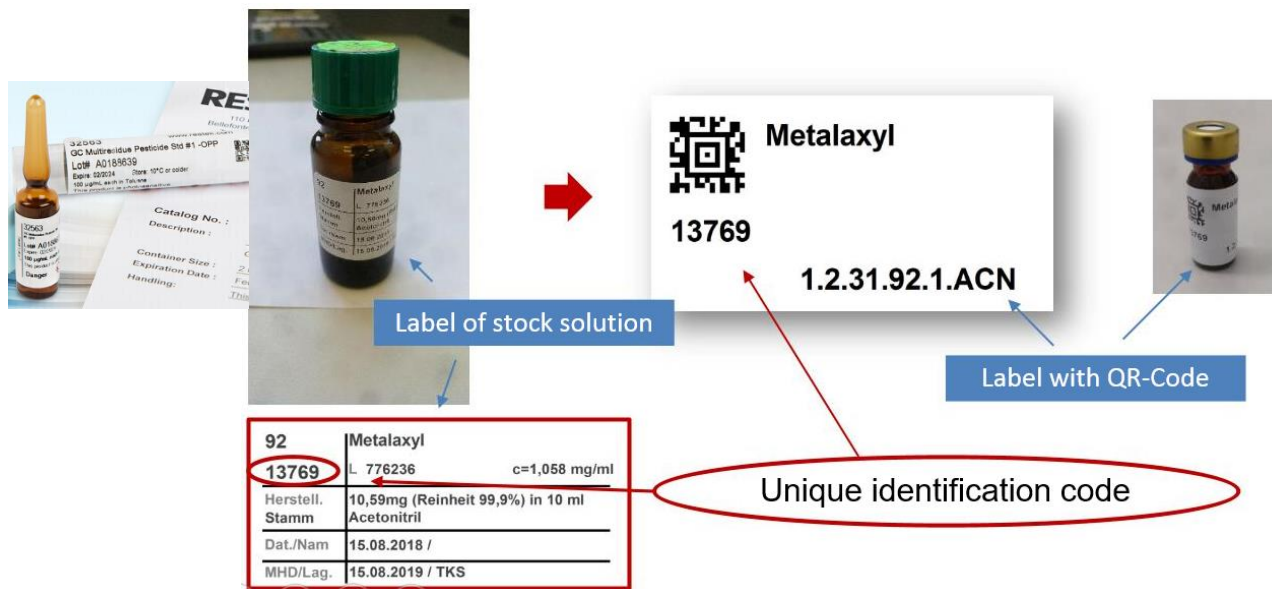
- Type of solution:** 'Art des Standards auswählen' dropdown menu, currently set to 'Stamm'.
- Solvent:** 'Lösungsmittel' dropdown menu, currently set to 'Acetonitril'.
- Concentration:** 'Konzentration' input field with '1,058' and 'mg/ml' unit.
- Expiration date:** 'MHD' (Maximum Hold Date) input field with '15.08.2019'.
- Preparation date:** 'Herstellung' (Manufacture) input field with '15.08.2018'.
- Other fields:** 'hergestellte Menge' (10 ml), 'Entsorgung' (Disposal), and 'Lagerhinweis (optional)'.
- Buttons:** 'ändern' (change) and 'abbrechen' (cancel).

Annotations in blue boxes point to these specific fields. The background shows a search results table and a list of standards.

Lagerbedingungen	Herstellung	MHD	Entsorgung	Kürzel
Standard Stamm	10,58 mg/l	10 ml	Acetonitril	KS
Standard	1,058 mg/ml	10 ml	Acetonitril	TKS
Standard	10,58 mg/l	10 ml	Acetonitril	KS

Standard Management in the Database

- Automatic generation of codes and labels.
- The aliquots for „Working standards“ were made in 2 mL screw cap vials.



Standard Management in the Database

- Permanent volume control of the stock solutions for the identification of solvent losses.
- Using the actual mass of stock solution the immersion depth into the bottle is calculated.

Aliquots for „Working standard preparation system“

Total amount of stock solution

PSM-Nr
92

Metalaxyl

Synonym(e)
englisch metalaxyl

Merkmale
CAS-Nummer
Verbindungsklasse
Wirkstoffart
MKS-Fraktion

Lösungen

Art	korr Konz	Ar
Standard	10.00000 mg/l	10
Standard	780 mg/ml	1.0
Standard	363 mg/ml	1.0
Standard	10.58000 mg/l	10
Standard	10.58000 mg/l	10
Standard	10.58000 mg/l	10


Historie anzeigen

aktuelle Menge [µL] Gesamtmenge [mL] Abf.

Leergewicht [g] Gesamtgewicht [g] Art

Felder zur Korrektur freigeben

Datum	Art	Leer	gesGew	Aktion	VolVerl...	KonzALT	Konz_NEU	Abfüllung
11.02.2019	Start	3,27989	4,62285		µL	-	-	13769 k1
20.02.2019	Entnahme	3,27989	4,60209		26,5µL	-	-	13769 k1
23.02.2019	Kontrolle	3,27989	4,60169	KEINE	0,5µL	1,058	1,058	13769 k1
01.03.2019	Kontrolle	3,27989	4,6013	Konzentration...	0,5µL	1,058	1,05832	13769 k1
06.03.2019	Kontrolle	3,27989	4,60092	Konzentration...	0,5µL	1,05832	1,05863	13769 k1
15.03.2019	Entnahme	3,27989	4,57732		30,2µL	-	-	13769 k1
16.03.2019	Kontrolle	3,27989	4,57723	KEINE	0,1µL	1,05863	1,0587	13769 k1
28.03.2019	Kontrolle	3,27989	4,57691	KEINE	0,4µL	1,05863	1,05889	13769 k1
04.04.2019	Kontrolle	3,27989	4,57644	KEINE	0,6µL	1,05863	1,05901	13769 k1
04.04.2019	Entnahme	3,27989	4,55303		29,9µL	-	-	13769 k1
04.04.2019	Kontrolle	3,27989	4,55244		0,8µL	1,05863	1,05912	13769 k1
08.04.2019	Entnahme	3,27989	4,51698		45,3µL	-	-	13769 k1
08.04.2019	Entnahme	3,27989	4,51694		0,1µL	-	-	13769 k1



Pipetting using:
Liquid classes
Level control

Pipetting process

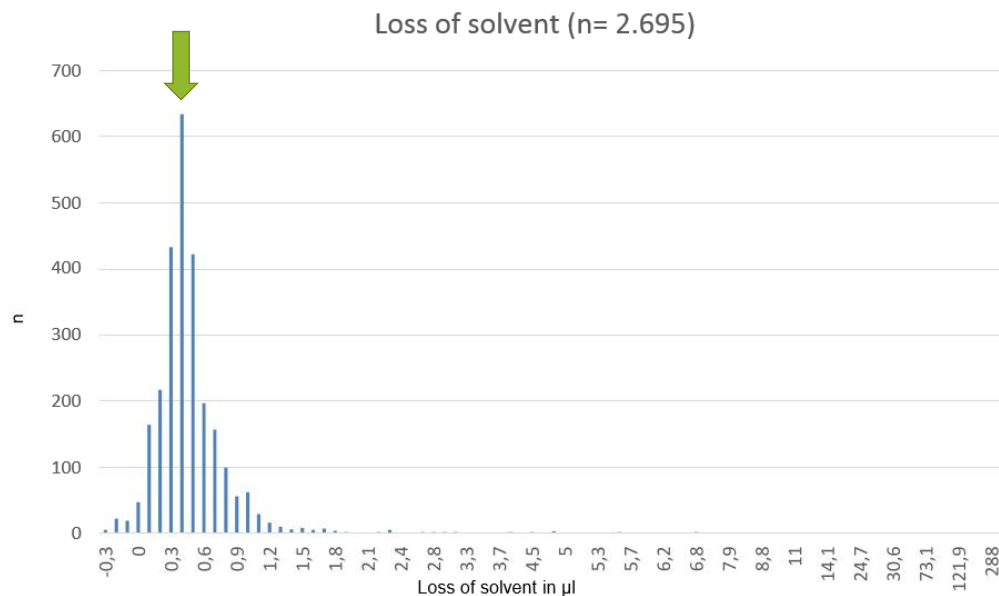
Corrected concentration

Solvent loss control

Solvent loss

Standard Management in the Database

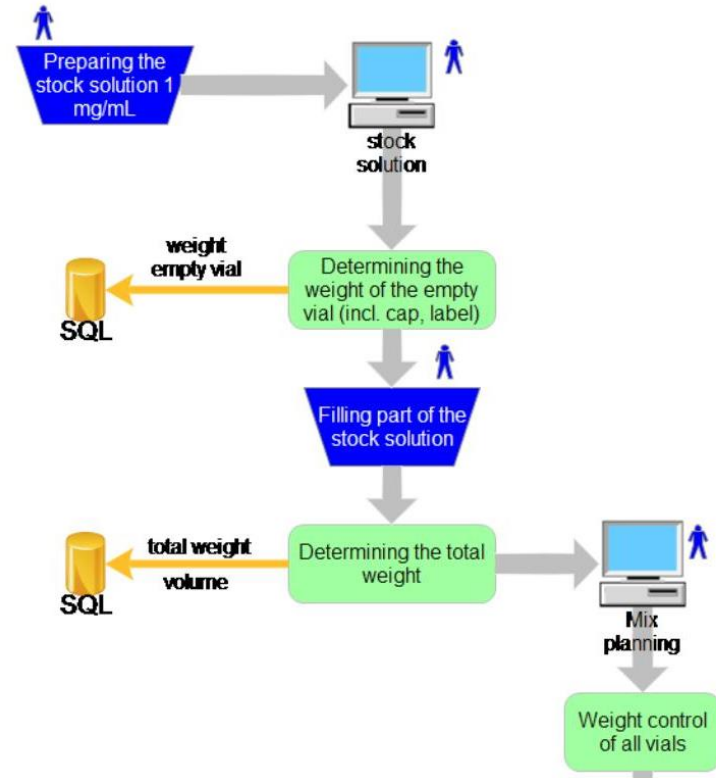
- Volume losses are recorded, usually low, typically 0,4 μL per week
 - Resulting concentrations get adjusted accordingly
 - Leaks may be detected by larger deviations, then exchange of the vial



Standards Preparation Workflow (1)

(1) Load the stock solutions

- Locate into cooled trays
- Update database
- Automatically entered to the database
 - Empty vial weight
 - Filled vial weight
 - Weight control all vials
- Capacity 30 vials/tray
 - Multiple trays
- Concentration range of ca. 1 mg/L



MultiMix Standard Mix Preparation

- Standard Mixes are then created automatically.
- Volumes to be pipetted are automatically calculated using the concentration of stock solutions and target concentration in the mixture.

Bezeichnung: LC Mix 27_

Lösungsmittel: Acetonitril

Mixart: Kilobrier-Mix

Auffüllvolumen: 10

Faktor: 1

Validieren

Der Mix wurde am 10.04.2019 16:16:03 von SRO erstellt.

Konz	EndKonz	µl Entnahme	Abweichung	Anzahl Wirkstoffe/N
1.010 mg/ml	1.000 µg/mL	10	- %	
1.023 mg/ml	2.000 µg/mL	19,6	- %	
1.061 mg/ml	1.000 µg/mL	9,4	- %	
1.002 mg/ml	2.000 µg/mL	20	- %	
1.019 mg/ml	2.000 µg/mL	19,6	- %	
1.062 mg/ml	2.000 µg/mL	18,8	- %	144831K1
1.083 mg/ml	1.000 µg/mL	9,2	- %	144091K1
1.046 mg/ml	1.000 µg/mL	9,6	- %	140301K1
1.021 mg/ml	2.000 µg/mL	19,6	- %	144881K1
1.054 mg/ml	1.000 µg/mL	9,4	- %	
1.111 mg/ml	2.000 µg/mL	18	- %	
1.005 mg/ml	2.000 µg/mL	19,8	- %	
1.059 mg/ml	1.000 µg/mL	9,4	- %	
1.041 mg/ml	2.000 µg/mL	19,2	- %	
1.020 mg/ml	1.000 µg/mL	9,8	- %	
1.030 mg/ml	2.000 µg/mL	19,4	- %	145631K1
1.015 mg/ml	2.000 µg/mL	19,8	- %	145651K1
1.090 mg/ml	2.000 µg/mL	18,4	- %	145861K1
1.015 mg/ml	2.000 µg/mL	19,8	- %	145931K1
1.052 mg/ml	1.000 µg/mL	9,6	- %	136901K1
1.006 mg/ml	2.000 µg/mL	19,8	- %	145951K1
0.970 mg/ml	1.000 µg/mL	10,4	- %	145851K1
1.033 mg/ml	2.000 µg/mL	19,4	- %	145271K1
0.989 mg/ml	1.000 µg/mL	10,2	- %	137451K1
1.040 mg/ml	2.000 µg/mL	19,2	- %	128421K1
1.037 mg/ml	2.000 µg/mL	19,2	- %	128431K1
1.027 mg/ml	2.000 µg/mL	19,4	- %	126771K1
1.109 mg/ml	1.000 µg/mL	9	- %	138861K1
0.996 mg/ml	2.000 µg/mL	20	- %	145451K1
0.988 mg/ml	2.000 µg/mL	20,2	- %	109981K1
...	146511K1
...	128861K1
...	130491K1

(3) kein Gesamtgewicht hinterlegt

(4) Volumenprüfung (Lösung in VStation)

(5) Volumenprüfung (Entnahmemenge)

schließen

Abschluss

MultiMix Standard Mix Preparation

- Exact concentrations in the prepared mixture are determined by using a balance.
- and can be exported to quantification software (e.g. Masshunter, Multiquant, etc).

PSMNr	Wirkstoff	Konz	EndKonz	µl Entnahme	Abweichung	PSMStdID_A...
6	Methodathion	1.006 mg/ml	1.002 µg/mL	10	1%	14334ik1
7	Dimethoat	1.036 mg/ml	2.049 µg/mL	19,8	2%	12654ik1
27	Trichlorfon	1.027 mg/ml	1.073 µg/mL	10,5	8%	12674ik1
48	Pirimiphos-methyl (F)	1.002 mg/ml	2.076 µg/mL	20,7	4%	13491ik1
50	Fenthion	1.400 mg/ml	2.036 µg/mL	14,5	2%	4455ik1
52	Parathion (F)	1.030 mg/ml	2.048 µg/mL	19,9	2%	12720ik1
53	Malathion	1.002 mg/ml	1.131 µg/mL	11,3	13%	12721ik1
56	Phosphamidon	1.046 mg/ml	1.085 µg/mL	10,4	8%	12724ik1
57	Disulfoton-sulfon	1.050 mg/ml	2.096 µg/mL	20	5%	12725ik1
58	Disulfoton-sulfoxid	1.054 mg/ml	1.049 µg/mL	10	5%	13529ik1
66	Dichlorvos	1.174 mg/ml	2.139 µg/mL	18,2	7%	12727ik1
91	Mevinphos (Summe der E- und Z-Isome...	1.005 mg/ml	2.112 µg/mL	21	6%	14140ik1
92	Metalaxyl	1.058 mg/ml	1.130 µg/mL	10,7	12%	13769ik1
106	Cyanazin	1.008 mg/ml	2.012 µg/mL	20	1%	14000ik1
107	Triadimefon	1.040 mg/ml	2.124 µg/mL	20,4	6%	13716ik1
108	Pirimicarb	1.020 mg/ml	1.140 µg/mL	11,2	14%	13717ik1
111	Azinphosmethyl (F)	1.014 mg/ml	2.154 µg/mL	21,2	8%	11521ik1
114	Triadimenol	1.000 mg/ml	2.116 µg/mL	21,2	6%	11522ik1
134	Chloridazon	1.032 mg/ml	2.074 µg/mL	20,1	4%	12819ik1
151	Propham	1.031 mg/ml	1.975 µg/mL	19,2	-1%	11430ik1
155	Triazophos (F)	1.052 mg/ml	1.101 µg/mL	10,5	10%	13690ik1
156	Methamidophos	1.012 mg/ml	2.019 µg/mL	20	1%	11524ik1
157	Piperonylbutoxid	0.972 mg/ml	0.802 µg/mL	8,3	-20%	12824ik1
159	Imazail	1.043 mg/ml	1.903 µg/mL	18,3	-5%	12826ik1

Concentration of stock solution

Calculated concentration in mixture (by balance)

Calculated volume pipetted in µl (by balance)

Deviation from target value in %

MultiMix Standard Mix Preparation (2)

(2) Mixing steps

- Weight control all vials in sequence

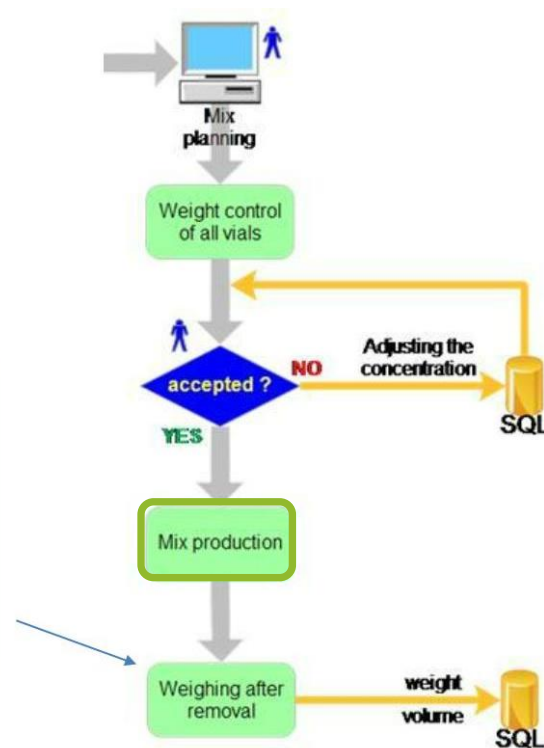
Automatically entered to the database

- Vial weights
- Concentration adjustment in case of deviation

Quality control + Traceability

- Weighing of all stock solutions
- Update database

To complete the process, the stock solutions are weighed again and the new weight is used to determine future solvent losses



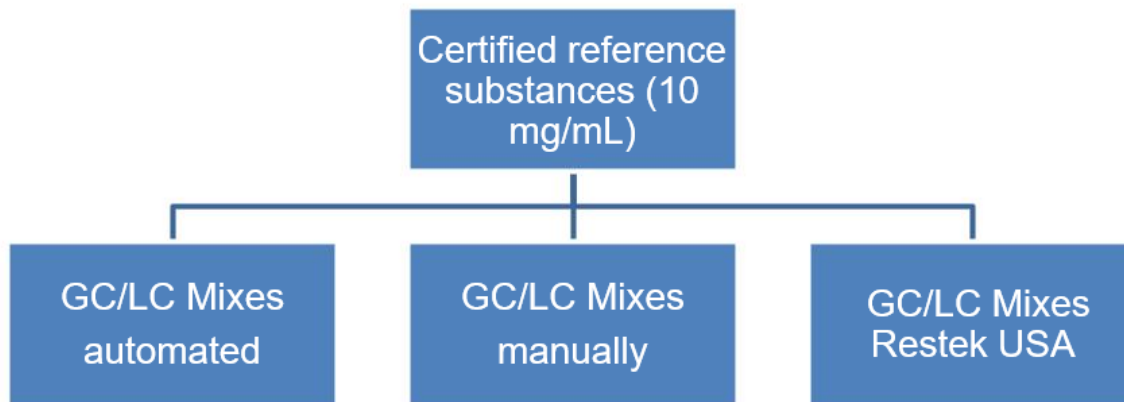
Validation

In order to validate the system and prove its accuracy

- 10 mixes each were prepared manually (volumetrically) and automatically from certified standard solutions
- The precision of the automated preparation system was better than manual pipetting.

Confirmation of accuracy

- The automatically prepared mixes were measured by Restek against their own certified mixes.



Conclusion

See our poster
A 17

Comments by collaboration partner on automated mixture preparation:

- The system is in routine use for more than 4 years now.
- About 650 substances can currently be added to a mix
 - Extension possible with additional cooling stacks
- Adaptation of the analytical spectrum to new regulations is much easier now
- New working standards are produced faster
- Improved quality - Used mixes are replaced more often without additional cost and effort
- Easy export to GC-MS and LC-MS quantification software

Comparison between manual and automated mix preparation

- Example of a mix of 500 substances:
- **Manual:** 32 working hours pipetting and documentation = 4 working days!
- **Automated:** 5 min planning and 20 h automated mix preparation including documentation



Thank You for Attending

... and our collaboration partner
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