

FREESTYLE ThermELUTE™

Fully Automated Sample Preparation for the Mycotoxin Analysis

User Manual



Manufacturer of FREESTYLE™ ThermELUTE™

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www.FREESTYLE-robotic.com!**

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Dear Valued Customer,

With your decision to buy the FREESTYLE™ SPE system in combination with the ThermELUTE™ module you have made an excellent choice. Congratulations!

The new and proven technology follows the nowadays trend of miniaturization and increased work speed for state-of-the-art laboratory equipment.

Having combined three ultimately powerful tools, the FREESTYLE™ SPE, the ThermELUTE™ large volume heat extraction module, and the SMART immunoaffinity columns, a new standard for economy, speed, and sensitivity is set in the field of mycotoxin analysis.

Without leaving the gold standard of immunoaffinity clean-up prior to HPLC measurement, it is now possible to process samples in less than 20 minutes including sample preparation and measurement at a sensitivity that easily allows a direct measurement of samples at baby food levels.

The ThermELUTE™ module was designed for the day-to-day routine operation in analytical laboratories following the general idea of the FREESTYLE™ series.

It is easy and intuitive to use and compatible with all HPLC brands on the market.

So enjoy your new FREESTYLE™ SPE / ThermELUTE™ combination and experience how sample throughput increases in your lab.

Best wishes,

Your LCTech team

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Important Notes



This ThermELUTE™ operation manual is an appendix of the FREESTYLE™ GPC-SPE-EVAporation manual and thus has to be read in combination with the latest version of your FREESTYLE™ manual.

Please read the important notes and the two user manuals carefully. Please follow the instructions given therein.

Make sure that the system is installed and operated on a table with a minimum bearing load of 100 kg for the Basic, GPC, SPE, and ThermELUTE™ system! If the system is upgraded with the EVAporation unit or if numerous solvent bottles are put on the table, make sure the additional load can be carried by the corresponding table.

Any improper operation, application, maintenance or repair may have fatal consequences for health and life of the user as well as of the system!

The system must **not be used** for

- Unstable substances
- Substances that might explode upon mechanical impact or elevated temperature without additional air supply
- Self-igniting substances
- Substances which are ignitable without additional air supply
- Explosives

The system is not certified to be used in a hazardous area.

The system complies with the fundamental requirements regarding design and construction according to the EC guideline which we think is applicable. The standards determine environmental conditions under which the system can be operated safely.

The system can be operated at environmental temperatures of +10 °C to + 30 °C and a relative humidity of 10 % up to a maximum of 90 % non-condensing. The system may be operated at altitudes from sea level up to 2000 m.

The electrical protection class is IP20. Make sure there is sufficient ventilation, in particular in the area of the external power supply: Above and underneath the power supply sufficient space for convection of cooling air must be provided. Do not cover the cooling slots!

Any improper usage, contempt of the safety instructions within this manual, misuse of the system, as well as inappropriate maintenance/repair by non-authorized service personnel and without the use of genuine spare parts and accessories voids any claims against the manufacturer.

The individual components may be connected in the intended way only.

Never put your hand in moving mechanical parts or in gaps intended for mechanical movements!

Keep sufficient distance to the system, in particular when

- the robotic system is initializing
- a sample list is processed
- a rinsing step is processed
- or the system is in a demo or service mode

Please note the **safety area**, which covers

- the left to the right traversing range of the robotic unit
- the front end of the robotic unit (y axis)
- the upper rack area to the upper end of the robotic unit

During operation under the aforementioned conditions no access to the safety area is allowed.

In case you feel unsafe or any uncontrolled conditions may occur, switch off the system with the main switch at the external power supply or push the emergency stop button located right at the system.



Attention: As with all mains-operated systems please follow the following safety advice:

- Protect the system and the external power supply from any dripping or spilling liquids. There is danger of short-circuit or fire (protection class of the power supply is IP20). Do not put them below the racks!
- Do not make any inappropriate alterations of the system.
- Switch off the system in emergency cases (de-energized).
- Operate the system under the given environmental conditions only.
- Operate the system with the rated voltage only: 100-240 Volt AC, 50-60 Hz.
- Replace broken fuses with the same type only.
- Operate the system with a functional ground pin.
- In order to increase your personal safety there should be an earth leakage circuit breaker with a cut-off current of 30 mA or less installed.
- Do not operate the system with broken power cables; a repair is mandatory then.

Never try to move the robotic unit by hand when it is powered, as the system might be damaged or malfunctions might occur at least.

Never take out samples or racks during operation, as there is danger of a collision.

Never put items onto the racks as there is danger of collision.

Cover the needle port upon a prolonged stand-still of the system.

Prior to any transportation of the system (even within the laboratory) secure the robotic arm as well as the injection needle.



Please take note of the following notes about solvents and their handling:

Attention: Use personal protection equipment when handling chemicals.

Collect the solvents in a suitable container only, which is sufficiently vented and has a proper and tight cover lid. The waste tube must be tightly fixed to the upper lid of the waste container; the outlet of the tube should preferably end shortly beneath the lid in order to guarantee a proper function.

Avoid any formation of kinks, loops or rings of the waste tube; otherwise the solvent may not drain properly. The maximum volume of the waste container must be sufficient to collect all solvents during processing.

Alternatively using an LCTech waste level sensor is recommended. If you are using the LCTech waste level sensor please make sure that the waste tube does not come in contact with the floater. Protect the waste tube of being immersed too deep. Empty the waste container prior to any refilling of the solvent supply bottles. Refill any solvents with the appropriate quality only. The solvent must be free of any particulate matter. Make sure the solvent tubes of the reservoir are free of bubbles.

Make sure the open ends of the solvent tubes are at the bottom of the solvent supply bottle and not directed upwards. Using sinter filters is recommended.

Make sure any solvent supply bottles and waste containers are vented properly in order to prevent any vacuum formation and to ensure a correct solvent delivery. Where appropriate connect any filter or waste air tubing. Make sure the tubing to the injection valve of the ThermELUTE™ is connected properly to the HPLC and check for leakage.

Please take note of the detailed explanations given in the user manuals in the individual chapters.

This introductory chapters are intended to point out particular circumstances solely and do not contain the full procedures recommended by LCTech.

1. Intended Purpose

The FREESTYLE™ SPE with ThermELUTE™ module is a new technological approach for the sample preparation/clean-up of mycotoxins with on-line injection into an HPLC system.

Using the system, crude extracts originating from standard extraction protocols according to LCTech's SMART column manuals can be cleaned-up in order to remove unwanted matrix components prior to analyzing the analytes.

Due to the fast and sensitive process, respectively, the samples mustn't be overloaded and filtered properly to avoid detector overload or column clogging.

The ThermELUTE™ module is operating in combination with LCTech's SMART columns (AflaCLEAN™ SMART and OtaCLEAN™ SMART) for aflatoxin and ochratoxin A analysis; further applications will be implemented.



Figure 1: Different types of nuts

The unique technology allows the processing of SMART immunoaffinity columns on the FREESTYLE™ system directly connected to any HPLC brand. Due to its parallel sample clean-up and HPLC measurement and the low sample volumes applied to the SMART columns, a process time of about 20 minutes or less is achieved. As a consequence the sample throughput in analytical laboratories is significantly increased while any manual handling is reduced to a minimum.

Using the water-based large volume injection methodology, the use of toxic organic solvents (e. g. methanol) is dramatically reduced as are the amount of sample and the solid waste of the used columns.

Simultaneously the analytical sensitivity is increased significantly so that samples regulated at baby food levels can easily be measured without any additional manual intervention.

As the system is designed for a 24/7 operation any overpressure failures will be detected and documented; the corresponding sample is skipped, the instrument cleaned and the subsequent sample processed. This ensures a high throughput during a 24 h operation.

The ThermELUTE™ module and racks were designed to cover the requirements for a robust day-to day routine operation under standard laboratory conditions.

2. Environmental Conditions / Technical Data

2.1. Physical Data

Environmental temperature admissible during

Storage:	0 °C + 50 °C
Operation:	+10 °C + 30 °C
Max. relative humidity at operation (non-condensing):	10 % - 90 %

Measures W x H x D, weight:

ThermELUTE™ module	107 x 310 x 420 mm, 7 kg
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Max. rack capacity; ThermELUTE™ module mounted permanently

Type of vial	Max. number of vials	Max. number of racks	Number of vials total
16 mL	30	4	120
SMART column rack	60	2	120

Performance parameters ThermELUTE™ module

Temperature range column heating	up to 100 °C
Maximum pressure injection valve	up to 410 bar / 6,000 psi

2.2. Electrical Data

Operating voltage:	24 V DC
Maximum rating:	50 W

2.3. Process Engineering SPE

The FREESTYLE™ SPE SMART column gripper was designed for a serial processing of SMART-type immunoaffinity columns to which a single-use injection needle is attached.

In order to operate the ThermELUTE™ module, a FREESTYLE™ SPE module is mandatory.

Each sample is processed individually. The processing is done by positive pressure produced by the tight connection between SPE gripper, SMART column gripper, and the column in combination with the solution forced through the cartridge. The higher the flow rate the higher will be the resulting pressure in the system.

In contrast to SPE methods which can be freely parameterized via the SPE method editor by the customer, the ThermELUTE™ is operated with dedicated methods for the individual SMART column types; this was necessary to obtain process times as short as possible without compromising the maximum performance of the columns.

Therefore, only a limited set of parameters can be modified by the customer.

A cross-contamination in a subsequent sample does not occur, this is ensured by integral rinsing processes and corresponding rinsing ports.

Nevertheless, it should be decided whether running baby food samples after animal feed samples that may be highly contaminated, is performed without an intermediate blank sample.

Sample loop:	Stainless steel, 700 µL
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Wetted parts:	Stainless steel, PTFE
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3. Explanation of the ThermELUTE™ Components

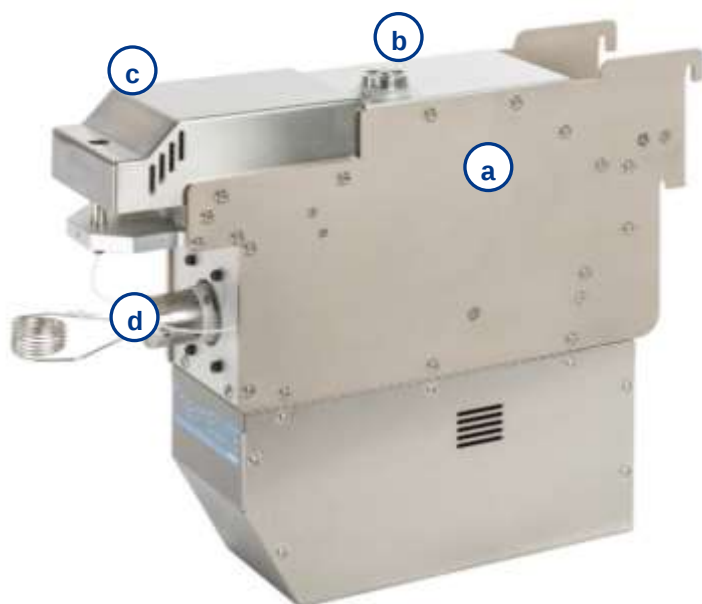


Figure 2: ThermELUTE™ module (side view)

- a** ThermELUTE™ module housing
- b** SMART column gripper in its rest position
- c** Injection port with column heater bars (hidden)
- d** High pressure injection valve with 700 μ L stainless steel sample loop



Figure 3: ThermELUTE™ module (front view)

4. Rack Types, Needles, and Clamping Tool

Besides the ThermELUTE™ module itself that is permanently fixed at the first position of the system's platform, two types of racks are needed in general.

One rack type is used for the crude samples to be cleaned-up; the second one is to hold the SMART columns.



Figure 4: SMART column rack (frame P/N 11915 and tray P/N 13497) for 60 SMART columns with needles.

As a typical rack for the crude samples LCTech recommends to use a 16 mL tray (P/N 11933 with frame P/N 11915).

For a full setup that allows processing of up to 120 samples, two SMART columns and four 16 mL racks are needed. Please note that this number of racks can only be used if there is no EVAporation chamber mounted.

For operation of the SMART columns, in particular for the injection into the injection valve, the SMART columns must be equipped with a special needle (P/N 13544).



Figure 5: SMART column with needle attached taken by the SMART column gripper.

As the gaps between column cap and column body as well as needle and column body need to be adjusted properly in order to ensure an error-free processing of the columns, using a special clamping tool (P/N 13678) is mandatory.

5. Using the Software – General Aspects

The figures and instructions are based on the software version October 2013.

The FREESTYLE™ system software is the main control element of the LCTech systems and has to be used in order to control the ThermELUTE™ module and racks, respectively.

The design was programmed to ensure a simple as well efficient sample processing in analytical laboratories.

5.1. Main Screen

Operating the ThermELUTE™ module with racks, the main screen of the FREESTYLE™ software is used as normal.

Attention! Upon stopping the instrument during a run with “Stop all” or by pushing the emergency stop button and after subsequent re-initialization, the SPE column gripper will open and the SMART column gripper will fall down! Please take measures that the gripper and needle, respectively, will not be damaged by falling down.

5.2. Menu Functions

For the menu functions please refer to the FREESTYLE™ manual.

5.3. Method Editing

5.3.1. Method Editing – General Introduction

Depending on the process required in the lab, an individual method has to be parameterized for any ThermELUTE™, GPC, SPE, EVA or FLEX process.

In order to do so open the *Module Selector* (refer to chapter 6.1 in the FREESTYLE™ manual) must be opened and select from the pull-down menu. After this clicking to the “Methods” button, the corresponding screen for setting of the parameters will appear.

5.3.2. Method Editing – ThermELUTE™



Figure 6: Method definition dialogue

Selecting and activating/deactivating of individual methods is performed as described in the FREESTYLE™ main manual.

As it can be seen the ThermELUTE™ methods are straight forward, meaning only a limited number of parameters can be changed by the customer. **And even within these parameters LCTech recommends not to change anything as the values are already determined for best performance.** The only exception here is the sample volume, but even there we recommend working with 10 mL in general.

After the method was given a new name and the appropriate SMART column process (AflaCLEAN™ SMART or OtaCLEAN™ SMART, respectively) was selected from the menu list, the first thing to be set is the size of the vial for the crude sample. This is done by selecting the appropriate size from the “Bottle types” scroll-down selection; in general this is a 16 mL vial with screw cap and seal.

The next settings to be parameterized are the sample volume to be loaded onto the SMART column, the flow rate of the sample, and the initial system cleaning by drag bars and the port selection.

The next settings to be parameterized are the sample volume to be loaded onto the SMART column, the flow rate of the sample, and the initial system cleaning by drag bars and the port selection.



Figure 7: Settings for sample volumes, flow rate, initial system cleaning and the port selection

It needs to be mentioned that the 10 mL of sample may appear as a comparatively low volume; please note that the entire sample is injected onto the HPLC column! Therefore, except from baby food samples, all other samples need to be diluted appropriately as a contamination of 10 mL of a 10 ppb sample, such a column eluate will dramatically overload any standard HPLC fluorescence detector.

The initial cleaning should be performed with water in order to avoid any organic solvent being present and disturbing the SMART column performance significantly!

The next procedural step is the cleaning of the system, in particular the sample needle and needle loop.

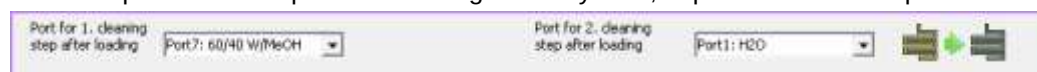


Figure 8: Cleaning step after loading

There are two cleaning cycles implemented; the first one using an organic solvent/water mixture to remove matrix components, the second to wash out the organic phase and residual water soluble matrix compounds.

The next step is the washing step of the SMART column, which is set-up as the loading step.



Figure 9: Washing step

The next parameter field handles the elution process. Here a proper setting is crucial for a good result. If there is no reasonable cause for a change, the setting shouldn't be changed by the user.

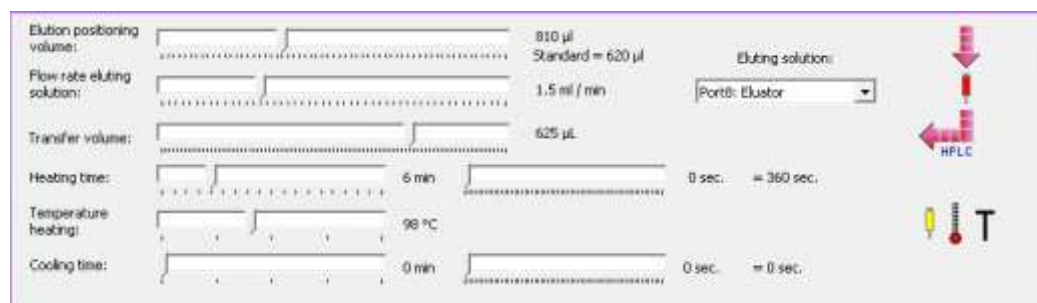


Figure 10: Elution process

This will get clearer by explaining the function of the individual settings.

“Elution positioning volume” will set the eluent volume in such a way that the SMART column is optimally flushed and the ends of the eluent inside the tubing have an optimal position. As the elution and transfer into the sample loop is an ongoing integral process without any intermediate opening of tubing, it needs to be set as precise as possible.

The elution flow rate is in general lower as any other flow rate during the process and has been optimized for the individual SMART column types.

The appropriate eluent has to be selected by a scroll-down selection. Please note that for the elution of aflatoxins a special eluent is needed!

The “Transfer volume” determines the value that is loaded into the sample loop, taking into account any thermal dilatation as well as the dead volumes; it is thus crucial for the result and shouldn't be changed at any time!

For an optimal result the heating time and temperature need to be set; typically they are 98 °C for a duration of 6 min.

A cleaning cycle with an organic solvent/water mixture is performed to remove residual traces of matrix compounds or analytes. The system is now ready to process the next sample depending on the signal status the FREESTYLE™ has received from the HPLC software.

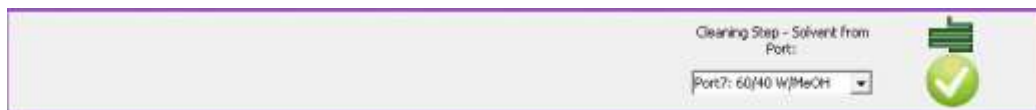


Figure 11: Cleaning step

Finally, the value at which an overpressure should be detected can be set. This is a digital value from 50 to 231; in general a value of 60 is ideal for processing ThermELUTE™ samples correctly. In case of detecting an overpressure failure, the system stops the processing of the corresponding sample, puts the column back to the SMART column rack, and rinses all contaminated parts.

Additionally, a timer must be set from the start of the sample until the injection for the HPLC. This is important to keep up the correct order of samples in the HPLC sample list in case of an overpressure failure.

A maximum count of triggered samples in series can also be set, where the system stops all the following samples in case of a mechanical problem (e.g. blockage of the column).

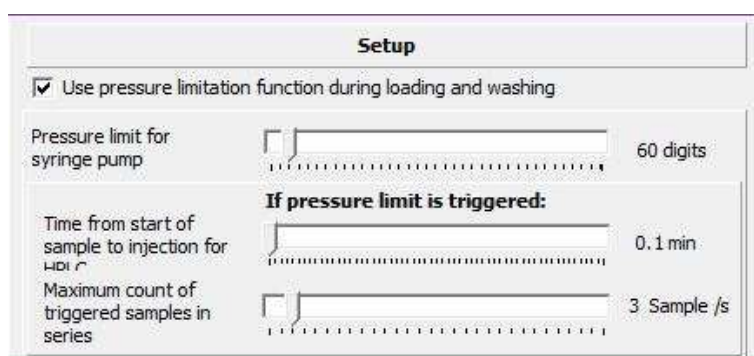


Figure 12: Setup

In order to ensure a safe and proper timing of processing and measurement and to avoid injections of the ThermELUTE™ into a running HPLC run, waiting timers must be set. They ensure that the HPLC measurement has definitely finished and the HPLC is ready for the next injection. The minimum of the timer has to be set to the duration of the actual ThermELUTE™ method. If the corresponding chromatogram lasts longer than the ThermELUTE™ method itself, the timer has to be set higher until both times match.

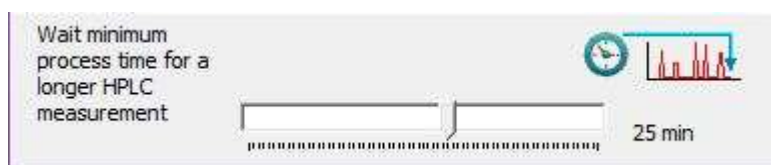


Figure 13: Timer if HPLC run takes longer than ThermELUTE™ process.

5.4. Rack Arrangement

Clicking „Arrange Rack“ the dialogue box shown below is displayed:

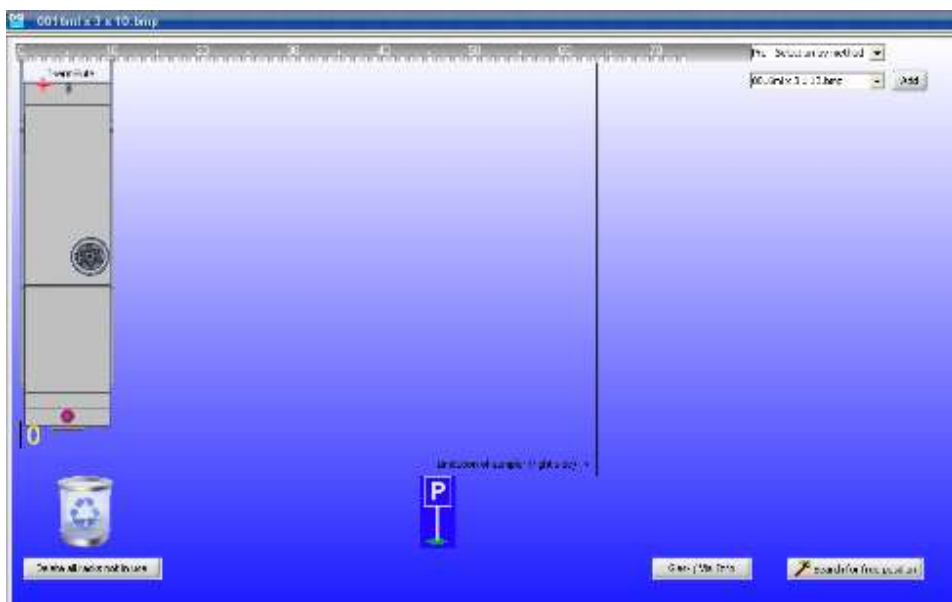


Figure 14: Dialogue box „Arrange Rack“

The ThermELUTE™ module will always be displayed as it is fixed firmly to the robotic area. For arrangement of the racks please follow the instructions given in the FREESTYLE™ main manual.

At least one special SMART column rack (frame P/N 11915 and tray P/N 13497) and a rack for the crude samples must be selected. In the figure shown below the rack arrangement for a processing of 60 samples is displayed.

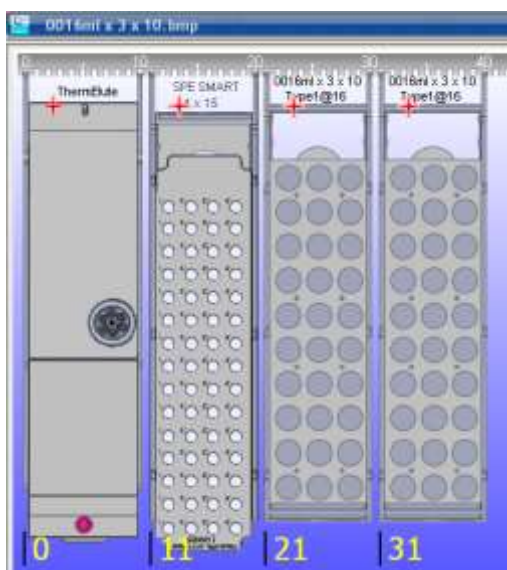


Figure 15: Rack arrangement for a processing of 60 samples

5.5. Report

Via the button



a method report for any ThermELUTE™ method can be generated.



Figure 16: Report generation

The selection of methods to be reported follows the main FREESTYLE™ manual.

An exemplary report is shown below.

		
LCTech FreeStyle - Report on Methods: ThermElute		Date: 16.10.2013 Time: 11:44:23
Name:	thermi.tel	
Column:	AttaCLEAN_SMART.col	
Initial System Cleaning Port:	1	H2O
Sample Volume:	10 ml	Bottle type: Type1@4 ml
Flow rate sample:	3 ml / min.	
Port for 1. cleaning step after loading	7	60/40 W/MeOH
Port for 2. cleaning step after loading	1	H2O
Volume washing solution:	2 ml	
Flow rate washing solution:	3,1 ml / min.	
Port for 2. Washing solution:	1	H2O
Elution positioning volume:	810 µl	
	Standard = 620 µl	
Flow rate eluting solution:	1,5 ml / min.	
Eluting solution:	8	Eluator
Temperature heating:	98 °C	
Heating time:	360 sec.	
Cooling time:	0 sec.	
Cleaning Step - Solvent from Port:	7	60/40 W/MeOH

Figure 17: Exemplary report

6. Advice on Sample Preparation

The FREESTYLE™ SPE equipped with a ThermELUTE™ module is a system combination that enables analytical laboratories to significantly increase their sample throughput. The key words here are miniaturization and process optimization.

In order to ensure an error-free processing of the samples in a sample sequence, some important advice is given by LCTech.

First the samples should be extracted according to the SMART column manuals. The sample extraction and dilution should be performed as described in the extraction and clean-up procedure of the corresponding column type. In case it is not clear which extraction process will give an optimal result, it is recommended to use a defatting procedure in combination with the Tween 20 protocol, where applicable, as described in the corresponding column manuals.

The second step is to filter any turbid samples or samples where a later precipitation is expected as any particulate matter may have an adverse effect on the sample processing.

As a general rule it can be said that the more an extract is diluted, the better it will be for the processing. Due to the fact that the whole sample applied onto the SMART column is also injected into the HPLC, the sensitivity is normally sufficient. Therefore, it is advisable to dilute samples from an origin that is known to have high contaminations in order to avoid any overloading of the fluorescence detector (see figure below). As a recommendation all samples should have a volume of 10 mL, meaning 10 mL of baby food can be taken as is, whereas e. g. an animal feed sample is further diluted (e. g. 2.5 mL sample + 7.5 mL buffer).

Note: In order to aspirate a precise volume of 10 mL, it is mandatory to at least fill in 12 mL of crude sample.

In case of materials that may have high contamination levels, as mainly found during the analysis of spices, nuts or cereals, it is recommended to run a blank after in case that baby food should be measured subsequently.

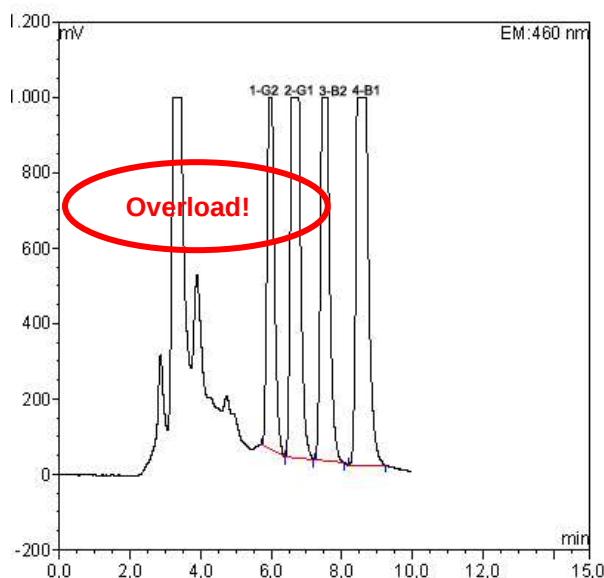


Figure 18: Exemplary hazel nut sample spiked with 10ppb total aflatoxin

(1 ng G2; 4 ng G1; 1 ng B2; 4 ng B1) extracted according to LCTech extraction and clean-up procedure, 10 mL processed using AflaCLEAN™ SMART and ThermELUTE™.

Chromatogram represents 0.28 g matrix equivalents.

7. System Calibration

For determination of the toxin concentration in an individual sample, the full set-up, FREESTYLE™ SPE, ThermELUTE™, and HPLC need to be calibrated with standards.

Depending on the HPLC system's and software's capabilities, respectively, there are two ways how the calibration can be performed.

The first, and recommended way, is to perform the calibration by using a sequence of standards and run them with SMART columns over the system. The advantage is that any systematic errors are suppressed, leaving the extraction efficiency and the matrix interference as the only two unknowns.

The second way is to use the autosampler of the HPLC system to produce a calibration curve. Here normally the standards are diluted in an organic solvent and water mixture. The injection volumes in general are low, typically between 10 and 100 µL. Although this procedure is quite different to the aqueous large volume injection in the ThermELUTE™ module, the results of both injection types can be compared and thus can be used for calibration.

As this approach needs HPLC software that is capable of handling two different autosamplers in one sequence, it has to be checked by the user whether the software can be set in such a way that it fulfills this specific requirement.

This topic will be asked by the service installation prior to the instrument's installation.

Due to the accurate operation of the system, excellent calibration curves can be expected (see figure below). Low toxin level in lower ppt range (ng/kg) could be analysed and quantified by using post column derivatisation device UVE™ for aflatoxins B/G without further sample concentration or evaporation processes.

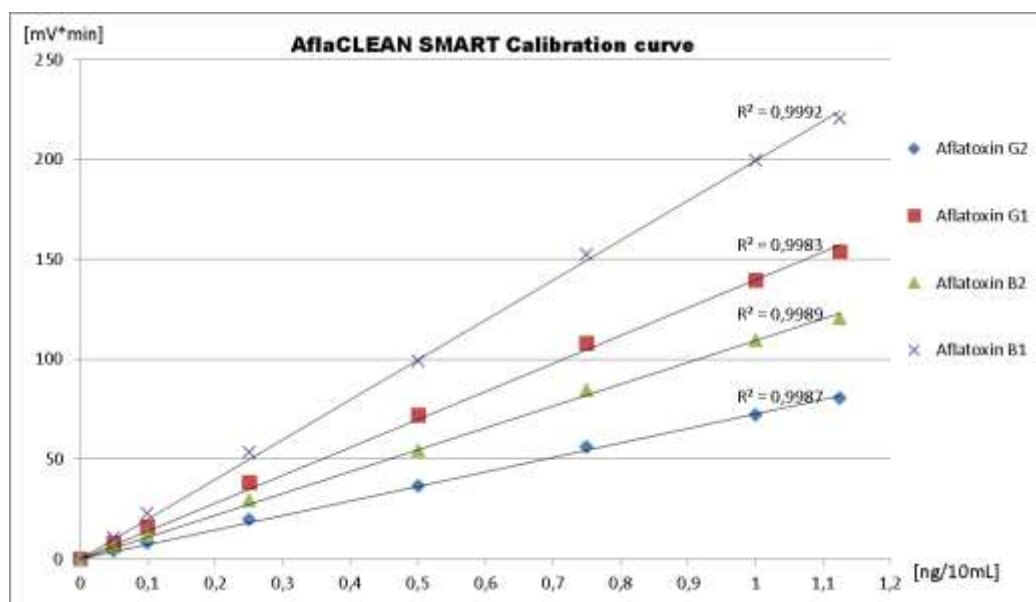


Figure 19: 8-point calibration from 0 – 1.125 ng/10 mL ($n = 3$ for each data point).

8. Troubleshooting / FAQ (frequently asked questions)

This chapter gives you valuable information on questions and problems that might occur during operation of the FREESTYLE™ SPE with ThermELUTE™ module:

For the elimination of most of the errors there are simple rules to follow:

- Most of the errors have an obvious logical cause.
- It is quite unlikely two errors occur at the same time.
- Errors can be found by logically locating the symptoms.
- For most of the errors there is a defined starting point. Try to precisely determine the time when the error occurred the first time. Then the following questions are important: What was before, during, and after the error occurred? The LCTech service engineer needs this information to locate the problem quickly.

Therefore, there is no need to disassemble the system just because the emergency stop was pressed.

Attention:

- Never try to solve problems by unauthorized repair!
- Do not open the system!
- Do not remove any covers or safety devices!
- Checking and repairing the electrical components of the system has to be done by LCTech personnel only. Any attempt by third-party personnel, which was not authorized by LCTech voids any claims against LCTech and the guarantee!
- Contact:

LCTech GmbH
Daimlerstraße 4
84419 Obertaufkirchen
Germany
Fax: +49 8082 2717-0
E-mail: service@LCTech.de

8.1. Questions and Answers

- Q:** Can I use one extraction protocol for all matrices?
A: This is not recommended as the extraction protocols given in the SMART column manual should strictly be kept. This ensures an ideal extraction and avoids problems with chromatographic difficulties using the ThermELUTE™.
- Q:** What do I do if my matrix is not described in the SMART column manual and I'm not sure about the right extraction protocol?
A: Use the protocol with n-hexane defatting and TWEEN 20 application in order to minimize matrix interferences.
- Q:** Are there any matrices I cannot process?
A: No, all food and animal feed matrices can be processed on the system.

- Q:** Do I have to filter the samples?
A: All samples should be filtered, to avoid precipitation which contaminate the FREESTYLE™ tubing.
- Q:** Do I have to use the clamping tool?
A: Yes, using this tool is essential to ensure a proper processing of the SMART columns with needle attached. The clamping tool sets the overall length of the column/needle assembly and adjusts the gaps between needle and column body as well as column body and cap.
- Q:** Can I use 1 or 2 mL of sample instead of 10 mL?
A: Principally this can be done, but the recommendation is to fill up to 10 mL. This improves the precision and the sample is more diluted resulting in a better SMART column performance.
- Q:** In my chromatograms I see an overload of the fluorescence detector. What to do?
A: Dilute your sample; in many cases a 10 mL sample representing as little as 0.07 g of matrix is sufficient. Check linearity of detector to ensure proper quantification of the signal in the detector.
- Q:** Does the different length of the flow paths when using the HPLC autosampler and the ThermELUTE™, respectively, have an effect on my toxin calculation?
A: No, the differences can normally be neglected. A slight spreading of peaks could occur if the standards are injected using an autosampler instead using the injection path of the ThermELUTE™ device.
- Q:** Can I use UPLC?
A: No, as the injection mode is a large volume injection with water, so it is not recommended for typical UPLC columns. Furthermore, it is best when the time for sample clean-up and HPLC measurement are more or less the same. In combination with photochemical derivatization UPLC cannot be used.
- Q:** In my lab I want to analyze baby food samples. Do I need to concentrate?
A: No, the sensitivity is high enough to get results that comply with the corresponding directives without using any intermediate concentration step of the SMART column eluate.
- Q:** Do I have to use the special eluent for the AflaCLEAN™ SMART columns?
A: Yes, without the eluent the recovery of some aflatoxins will be low.
- Q:** In my sequence I had several SMART columns where an error message for the processing was displayed. What can I do?
A: Optimize your extraction and crude sample preparation for these samples, meaning use lower matrix weight in and avoid matrix interference in chromatography by using the defatting and Tween 20 dilution procedure. The sample should be filtered to remove turbidity or precipitates from the sample.
- Q:** Do I have to use post-column derivatization?
A: Depending on the sensitivity required, a post-column derivatization is recommended for the aflatoxins B1, G1, and ochratoxin A.
- Q:** Column not processed due to overpressure, what can I do?
A: Check that clamping tool is used to ensure cap sits properly on column, check the column gripper for any possible clogging. Contact local service technician for support.
- Q:** Leaking injection port, what can I do?
A: Contact local service technician to adjust injection port.

9. Delivery and Unpacking

9.1. Delivery and Receipt of Goods

The ThermELUTE™ module is shipped in a single piece cardboard box. Depending on the size of order, there may be additional boxes. Compare the number of received boxes with the number on the delivery note. Inspect the packaging carefully. Claim any visible damage or signs of improper treatment immediately to the haulier (important!).

Please send information about your claim to LCTech via:

Fax: +49 8081 9368-10 or

E-mail: service@LCTech.de

9.2. Unpacking

Carefully remove the outer packing material and transport the ThermELUTE™ module to its working place by help of appropriate transportation equipment. Inspect the ThermELUTE™ module and accessories for any hidden damages, e. g. broken parts inside the package, and claim them immediately to the haulier (important!).

Please send information about your claim to LCTech via:

Fax: +49 8081 9368-10 or

E-Mail: service@LCTech.de

10. Installation and Connection to HPLC

The installation of the ThermELUTE™ module has to be done by an authorized service technician only, as a proper adjustment is mandatory for a trouble-free operation and several internal settings of the instrument need to be done.

The ThermELUTE™ module cannot be removed easily by the user. The connection to the AUX-socket must be kept; otherwise the instrument will display an error message during booting of the software.

The ThermELUTE™ module needs to be connected to the HPLC software in order to ensure a correct timing for the injection of a SMART column eluate and an optimized parallelization of the column processing as well as the HPLC measurement.

A standard connection is shown in the figure below.

Typically the FREESTYLE™ software is waiting for an HPLC ready signal. The ThermELUTE™ then answers with an injection signal where the sample loop is loaded onto the HPLC column and the HPLC starts the measurement.

Depending on the HPLC system/software used the logic of the sent signals may be invers, meaning in some cases the signal “high”, in others the signal “low” is the trigger for the injection and thus has to be set accordingly in the FREESTYLE™ basic settings of the software; see explanation below.

In any case the HPLC brand, the used software with version and the control logic has to be clarified in advance.

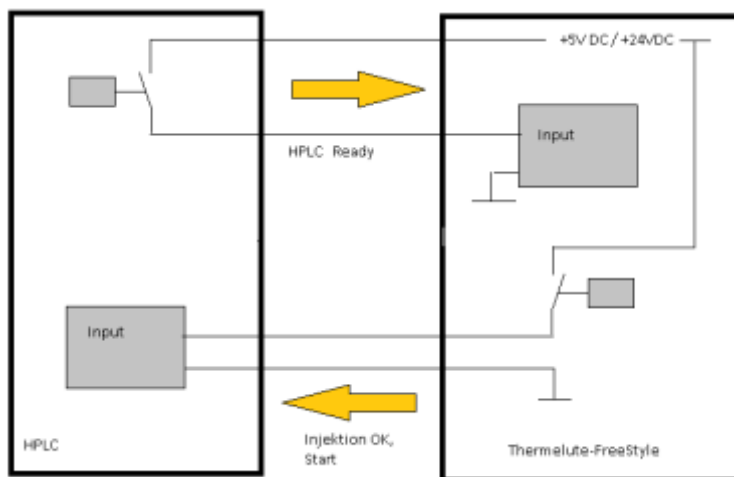


Figure 20: Standard connection

On the rear side of the ThermELUTE™ is a 7 pole round connector (see figures below).



Figure 21: 7 pole round connector

The FREESTYLE™ ThermELUTE™ and the HPLC are connected by means of an LCTech adapter box with attached cables and connectors. Currently there are two models available:

- Adapter box for Dionex / Thermo UCI50-box with, Mini-DIN connector;
P/N 13678
- Adapter box for Agilent 1260 Series, 9-pole Sub-D connector;
P/N 13679



Figure 22: P/N 13678



Figure 23: P/N 13679

The 7-pole round connector needs to be attached to the backside of the ThermELUTE™ module:

- **HPLC connection for Dionex / Thermo:** The Mini-DIN connector goes to Analog Inputs section, Plug 8 of the UCI-50 unit.

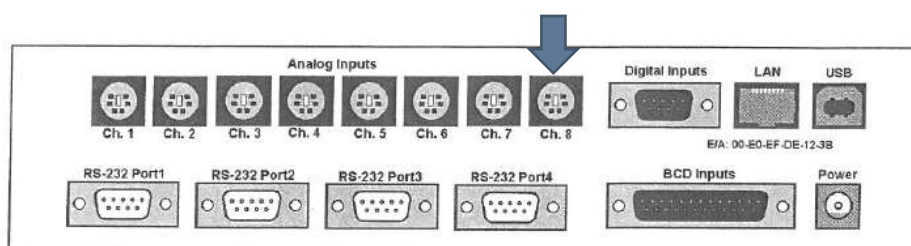


Figure 24: UCI-50 Unit

Software configuration Thermo/Dionex ULTIMATE 30000:

NOTE: It is recommended to ask for support by trained Thermo/Dionex service technicians:

1. Copy the file CMServer.cfg from folder C:\Chromel\bin of your actual PC system where Chromeleon is installed
 2. Send this file to your local Thermo/Dionex support together with the information which version you have (Help-Button, then click About-Button)
 3. The server file needs to be reconfigured so that Channel8 of your UCI50 is connected to a variable in your Chromeleon software
 4. After you have received the file CMServer.cfg back, then Stop Chromeleon software server, make a safety copy of your actual CMServer.cfg to e.g. CMServer.cf, and copy the newly received file into this directory C:\Chromel\bin
 5. Start Chromeleon Server again
 6. In Chromeleon you can generate a new device-method by help of the Wizard. If you are asked for the Inject-device, you select „Inject-Valve“.
 7. Set Relay Start_ext to „ON“ time to -0.1 and duration to 2secs, and store this method.
- **HPLC connection for Agilent 1260 Series:** Plug the 9-pole Sub-D into the connector with description „REMOTE“ on detector box.

Software configuration of Agilent HPLC:

1. Open the configuration settings of Chemstation Software
2. Remove the sampler as input source
3. Select Configuration „Manual Injection“ and save this option.

When the method starts it will wait with recording until the signal is given through „REMOTE“ connector from LCTech FREESTYLE™ adapter.

NOTE: It is recommended to ask for support by trained Agilent service technicians, especially if other models than the 1260 Series are to be connected.

NOTE: Depending on model and software configuration you can set either signal LOW as „READY for operation“ or signal HIGH. FREESTYLE™ will wait before injection cycle until the „READY“ signal is given by HPLC. If FREESTYLE™ ThermELUTE™ has injected the sample, it will give signal to the HPLC-unit as a start signal for measurement.

The maximum measurement cycle time of the HPLC must be configured shorter than the FREESTYLE™ ThermELUTE™ cycle that is about 25 min! Otherwise it may happen that ThermELUTE™ injects into a running measurement cycle.

11. Spare Parts

In case spare parts need to be replaced, use LCTech original parts only. All parts have been carefully selected and tested and ensure a proper function. Please do not change even simple things such as tubing as it is relevant for a proper liquid handling and is calibrated!

Below you will find spare parts for the corresponding systems; for spare parts of the FREESTYLE™ Basic and SPE module please refer to the FREESTYLE™ main manual.

13499	SMART column gripper
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13768	Clamping Tool
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13593	Sample loop, 700 µL, stainless steel
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