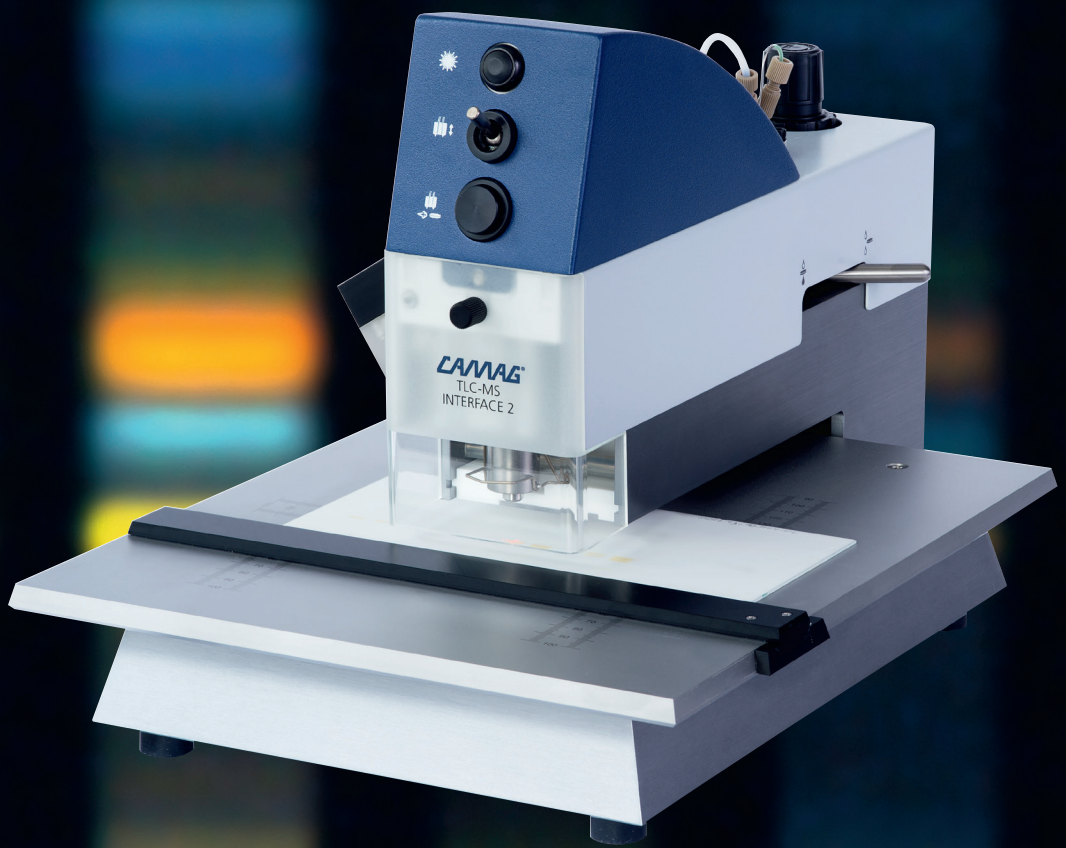
CAMAG®



INSTRUCTION MANUAL
TLC-MS INTERFACE 2

Zertifiziertes Managementsystem SQS ISO 9001

Content

1 Introduction

1.1 Precaution

- Please read this operating manual before starting the installation! This manual contains information and warnings the user has to follow to ensure reliable operation of the instrument
- If the instrument is used in a manner not specified in this manual, the protection provided by the equipment may be impaired
- This sign indicates (on instrument and in this manual) that failure to take note of the accompanying information may result in damage of the instrument
- Attention: For safety reasons the instrument may only be used for the purposes described in the operating manual
- To avoid injury use adequate safety equipment (protective goggles, gloves etc. if applicable) when working with the instrument
- When working with the fluids of the instrument, be sure to take the appropriate caution (protect your eyes from direct contact with liquid)
- The instrument may be used only by properly trained laboratory staff
- Use a damp lint free cloth for cleaning the instrument surface. Do not employ aggressive detergents
- Only authorized personnel may open the instrument. Service and repair is only to be performed by trained specialists. Use spare parts and consumables supplied by CAMAG only. The warranty is voided if parts from other sources are used. Check the service manual before you start service to reduce product-specific risks
- If the instrument is found to be defective, it must be switched off and steps must be taken to ensure that it cannot be switched on by mistake
- If liquids penetrate the inside of the instrument, the power has to be disconnected immediately. Small amounts of liquid can be wiped off and/or dried by means of a hairdryer, with larger amounts of liquid a service technician has to be called. A test of functionality has to be performed in all cases
- Carry out all safety checks and the preventive maintenance as recommended by the manufacturer in order to assure your personal safety and the full functionality of the instrument. Have an authorized service specialist perform any service not described by this manual
- See original manufacturers' manuals for further safety data on third party equipment supplied with the system
- The safety of any system incorporate with the equipment is the responsibility of the assembler of the system

- Lift/move/transport the system with the necessary care and with sufficient manpower (install the transport security devices if applicable, transport it only in the original packaging)

- This symbol indicates that this equipment must not be disposed of as unsorted municipal waste but is to be collected separately as electrical and electronic equipment (WEEE-Directive 2002/96/EC). To properly recycle the instrument or parts of it you are requested to send the equipment back to the distributor, producer or an adequate collection system at the end of its life. This will have potential effects on the environment and human health
- Do not mix batteries -When replacing batteries, replace them at the same time with new batteries of the same type
- Exhausted batteries should immediately be removed from the equipment and disposed of accordingly. When discharged batteries are kept in the equipment for a long time, electrolyte leakage may occur and cause damage to the instrument

- Laser (class 1) inside: Do not look directly into the laser beam

1.2 Parts supplied

Part no	Description
140.0450-1	Pneumatic kit containing:
672.1016	Hose clip Ø 8-16mm
672.0029	Hose PVC
115.0050	Hose fitting
672.0080	Hose black
370.0002	2 Battery 1.5V ,AA size
022.8450	Colum Saver 2µm, SST
B.022.8440E	Instruction Manual
	2 Allen key (for maintenance of 6 port Valve)

Accessories and spare parts

Part no	Description
022.8446	Elution head for circular zones of 4mm diameter
022.8445	Elution head for oval zones of 2mm width
666.0001	10-32 (1/16") RheFlex ® SS Fitting (nut and ferrule)
666.0002	Adapter -1/4-28 x 10-32 PEEK Interface 2
666.0003	Nanotight FEP tubing sleeve 1/16" OD
666.0004	Nanotight peek fitting (fingertight nut and ferrule)
666.4007	F-120 PEEK fitting
666.0017	Flangeless Nut 1/8"
666.0018	Flangeless Ferrule 1/8"
670.0030	Valve dummy connector (for protection)
022.8450	Colum Saver 2µm, SST
022.8420	Waste filter for cleaning sledge
660.0040	O-ring for waste container
	Rotor seal for 6 port valve

2 Unpacking/Installation

2.1 Unpacking

Observe the environmental requirements (2.1 Installation environment) when set-ting up the instrument.

- From the upper compartment of the package carefully unpack all components and accessories listed on the shipping list. Make sure the shipment is complete
- Carefully remove the instrument from the package and place it on a table
- Push the button  (laser cross hair on/off) down and you will see laser crosshairs on the positioning table. If there is no cross please check the batteries

2.2 Installation environment

The place for installation must meet the following requirements:

Connections	5 bar compressed air or N2. (backside of instrument) Short capillaries from HPLC pump to TLC-MS interface 2 and from there to the MS
Bench space	Width 27.5 cm; Depth 45 cm; Height 30 cm
Operating temperature	The temperature should be within a range of 15 to 35 degrees centigrade.
Humidity	Humidity and temperature conditions must not cause condensation.
Additional inline filter	CAMAG strongly recommends using an additional inline filter between the TLC-MS Interface 2 (Valve port 1) and the MS. The porosity of the filter is depending on the usage, CAMAG recommends to use a filter with the porosity of 0.2 µm

2.3 Installation

- Make sure the batteries at the backside of the instrument are correctly inserted
- Connect your LC pump and MS system with the delivered set of 10-32 (1/16”) RheFlex® Two-Piece fittings or with compatible alternatives.
    - Port 2 from the LC pump
    - Port 1 to the MS system
- Connect the black tube to your pressured gas supply system of 4 – 5 bar compressed air or N2.

Your instrument is now ready to use!

2.4 Connection of the valve

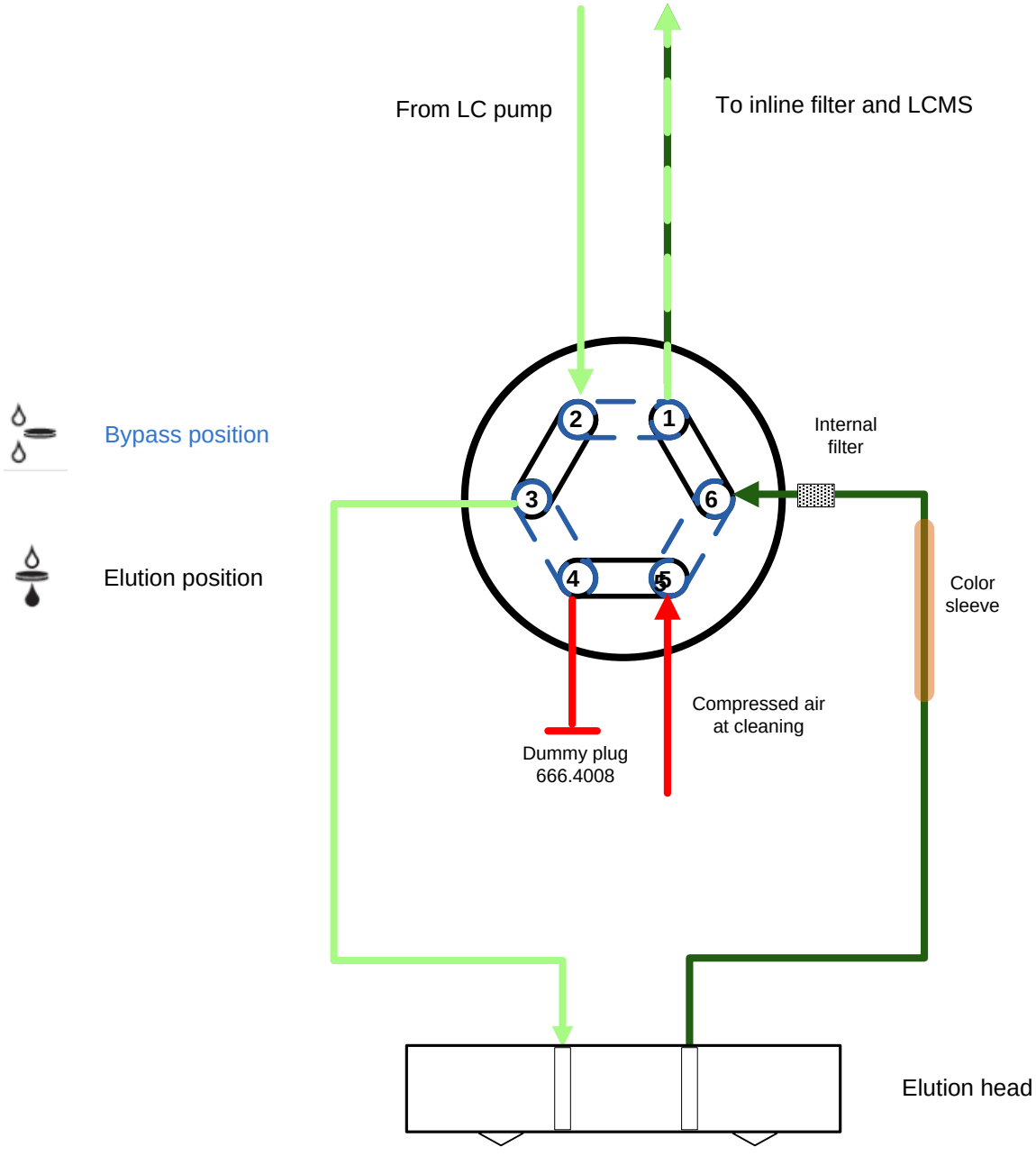


Fig 1

3 Getting started

3.1 Intended use

The TLC-MS Interface 2 is intended for the use described in this manual.

3.2 Instrument

Fig. 2

Control elements:

1	Laser cross hair on/off
2	Piston up/down
3	Cleaning on/off
4	Screw for fixing the security cover
5	Piston with elution head
6	Positioning table
7	Lever in position elution/bypass
8	Air pressure adjustment
9	6-port valve for connection of capillaries from pump and to MS
10	Plate stopper

3.3 Functional principle

The instrument extracts zones (round or oval) from TLC/HPTLC plates or aluminium foils up to 20 x 20 cm. Plates can be positioned accurately and analysed zone by zone. Elutions are possible with appropriate solvents, using the standard flow speed of the HPLC-MS system (e.g. 0.1 ml/min). The system allows a semi-automatic operation involving piston up/down movement, cleaning and backflushing of the elution head, manual positioning and valve switching.

The elution head has two connections on the top side, one inlet and one outlet. On the bottom surface there is a cutting edge seal with a height of about the thickness of the plate layer. When the elution head is pressed onto a foil or glass plate the cutting edge seal cuts into the adsorption layer and creates a leak-free seal.

In bypass position solvent flows directly to the MS-System. With the help of a laser crosshairs the extractor head is easily positioned on a selected zone.

If the position of a zone is not visible, positioning is done with the help of rulers on the instrument table.

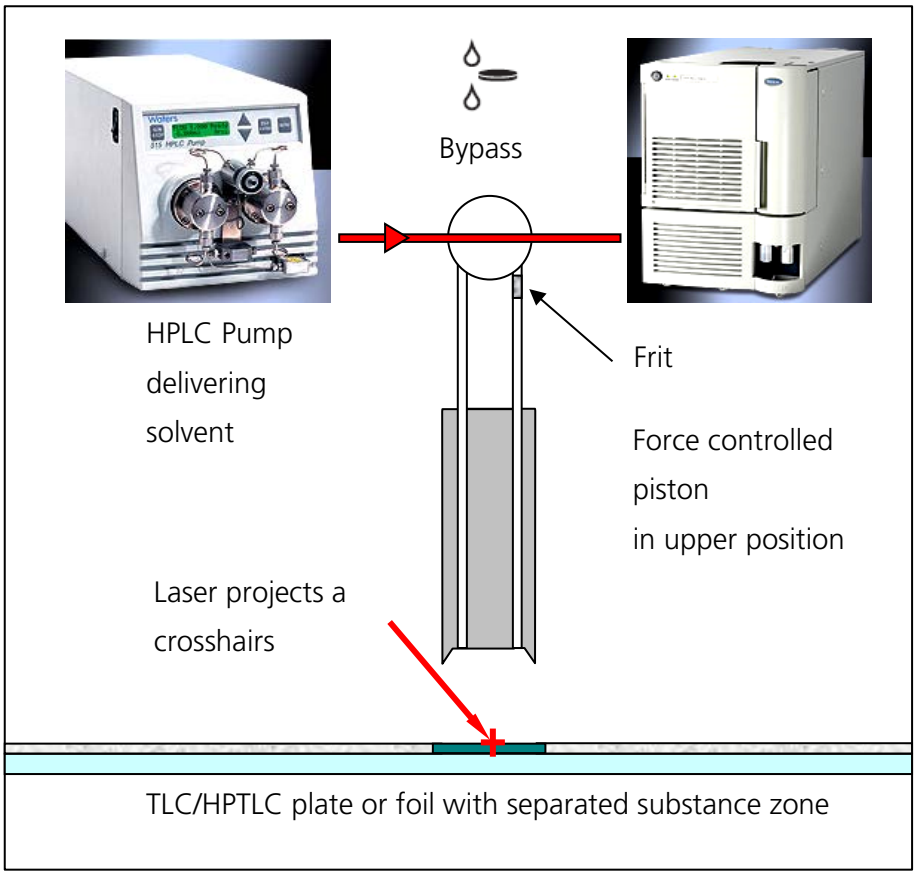


Fig. 3: TLC-MS Interface 2 in bypass position

After lowering the piston, switch the valve to elution position. The solvent passes through the elution head onto the silica gel, elutes the sample and transports it directly to the LC/MS-System.

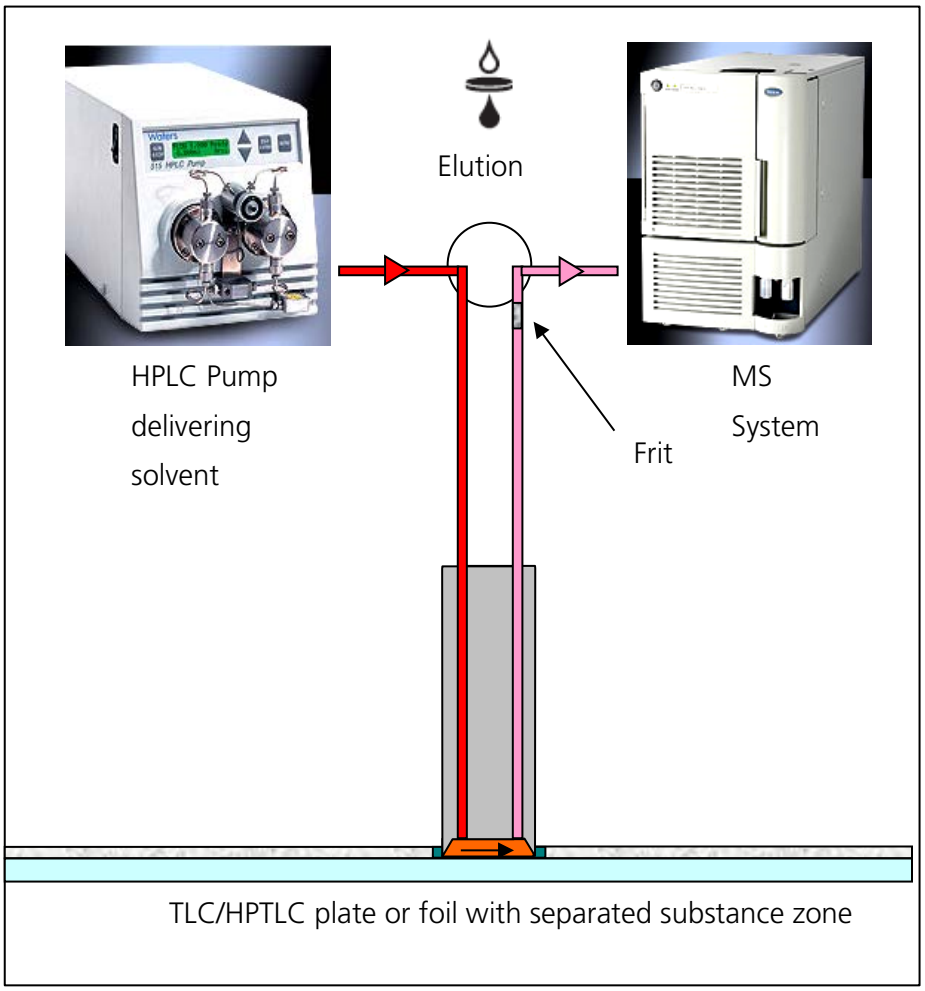


Fig. 4: TLC-MS Interface 2 in elution position

After each elution remove the plate/foil. Make sure the valve is in position "Bypass" and press the cleaning button at least 3 times for 3 seconds. The capillary from the plate to the valve and internal frit are cleaned with the help of compressed air. At the same time the cleaning sledge moves underneath the head to collect residuals of silica powder from the elution head.

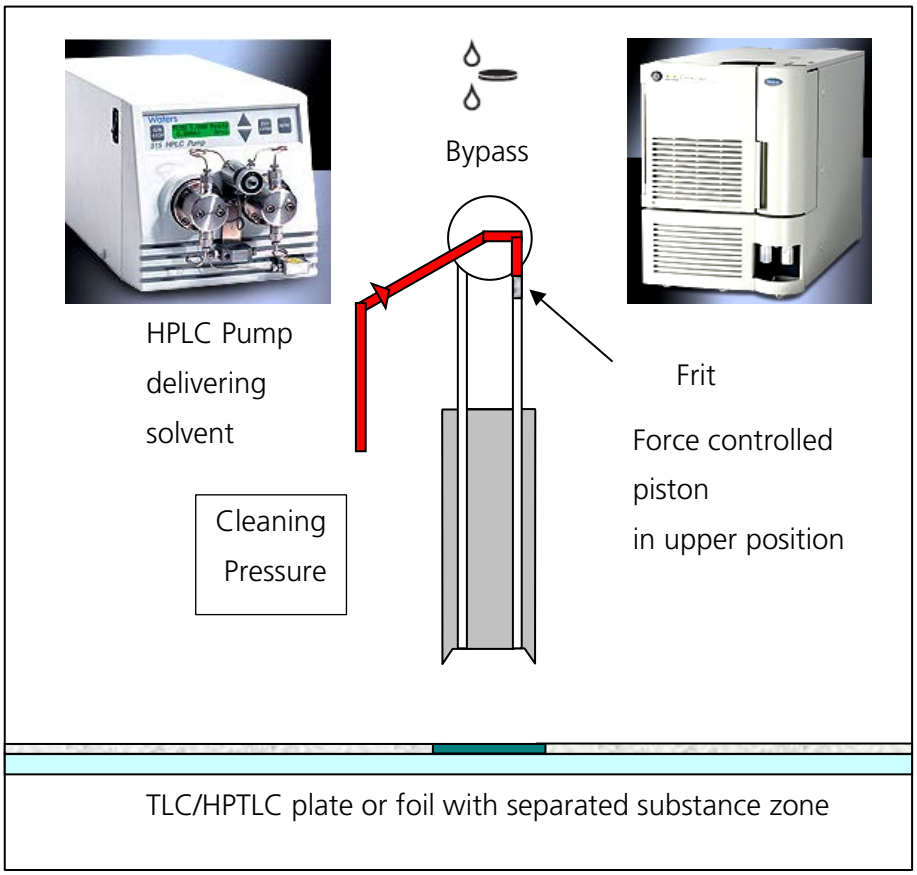


Fig. 5: TLC-MS Interface 2 in bypass position , cleaning active

3.4 Elution process

- Make sure, that the valve is in the rear, Bypass position
- Position your TLC plate/foil on the instrument table
- Press the laser cross hair button and you will see the laser cross hair on the plate. If zones are visible you can easily position the TLC plate with the cross hair
- If zones are not visible you have to evaluate the position with a respective device and position the plate with the help of the plate stopper and ruler on the instrument table accordingly
- Toggle the Piston up/down switch to lower the elution head onto the plate/foil
- Switching the valve to front, Elution position will start the elution. An elution time of about 1 minute should be sufficient (this is strongly depending on the flow rate, solvent type and length of the capillaries)
- Switch the valve back in the rear, Bypass position
- Toggle the Piston up/down switch to bring the elution head into the upper position
- Continuously push the cleaning button at least 3 times for 3 seconds to clean the elution head and capillaries.

3.5 Operation advice

- Elution of a sample–free area on the plate cleans the system from residues of the last elution. In this manner you can also measure the plate background for later correction
- The positioning table must be wiped off before you put a plate or foil on it.
- Capillaries to the detector system should be kept as short as possible
- Increasing the flow rate results in faster elution time but larger elution volume and a rise of pressure
- Caution: It is not recommended to leave the ports of the valve unconnected without protecting them from dust. Please use the included taps or similar parts to protect the ports

Check chapter Maintenance and Service for a trouble-free handling of the instrument.

4 Maintenance and Service

4.1 Cleaning

Cleaning the instrument on a regular basis in an appropriate way assures problem-free functionality over a long period.

A good way to perform a cleaning would be to follow the decontamination instruction outlined below.

4.2 Decontamination

Before transportation or a longer term of not using your system, decontaminate it in an appropriate manner. The decontamination procedure below reflects the minimal requirements, so keep in mind that the procedure for your instrument has to be adapted according the substances used.

Decontamination procedure:

- Disconnect the capillaries to the LC/MS system
- Fill a bottle with 70% Ethanol as cleaning solution and connect it to the pump. Adapt the cleaning solution according to the used substances if necessary
- Place a clean plate underneath the elution head
- Perform 3 elution cycles (each 1 min). After each cycle, press the cleaning button 3 times for 3 sec.
- Place a lint free cloth underneath the elution head
- Move the lever into position "Elution"
- Run the pump for 1 min
- Press the cleaning button 3 times for 3 seconds

- Clean the instrument with a lint free cloth

4.3 User maintenance

Regular check/maintenance (at least every 3 months) by the user is strongly recommended.

User maintenance procedure:

- Check the capillaries
- Check the 6 port valve for signs of leakage
- Clean the instrument with a lint free cloth
- Replace the respective parts as outlined in chapter "Maintenance Data Sheet"

4.4 Maintenance Data Sheet

Purpose	The maintenance data sheet informs about maintenance interval of the respective instrument as well as the proposal for IQ/OQ interval if applicable. In addition, it identifies parts subject to wear with the respective replacement cycle and CAMAG order number.

Maintenance interval	
Maintenance	12 month
IQ/OQ	12 month

Consumable parts		
Part No.	Description	Replacement cycle
370.0002	2 Batteries 1.5V AA size	36 months/100h
022.8450	In-line frit	6 months/depends on usage
115.8448	Elution head, round 4mm h= 0.25mm	36 months/if leakage is detected
115.8446	Elution head, oval 4x2 mm h=0.25mm	36 months/if leakage is detected
941.0040	Waste filter for cleaning sledge	36 months
660.0040	O-ring for waste container	36 months
	Rotor seal for 6 port valve	36 months/if leakage is detected or the pressure in the system is too high

4.5 Replacing of elution head

- Remove the security cover
- Remove the blue top cover
- Remove the elution head capillaries to port 3 and port 6 (the filter) of the valve
- Make sure the filter to port 6 of the valve is changed if the head is replaced
- Unfasten the elution head clip
- Remove the elution head carefully through the top of the instrument
- Insert the new head through the top of the instrument
- Fasten the elution head clip
- Mount the capillaries (color sleeve to the filter on port 6 of valve)
- Mount the blue top cover
- Mount the security cover
- Make an fake extraction to check if the elution head is properly positioned and no leaks are visible

5 Technical data

General data	
Plate types	TLC/HPTLC glass plates and aluminium foils of sizes up to 20 x 20 cm and layer thickness up to 300µm
Standard flow rate	50 - 300 µL/min
Down force	400 N max
Gas supply pressure	4 – 6 bar compressed air or N2
Electrical data	
Laser type	5 mW, laser class 1
Electrical power	Two batteries 1.5 V, AA/LR6 size
Battery duration	Up to 100 hours
Mechanical data	
Dimensions	275 x 425 x 275 mm (w x d x h)
Weight	14.5 kg
Chemical data	
Elution head capillaries	Stainless steel passivated and rinsed with reagent-grade methanol
Frit	2µm stainless steel
Elution head	Stainless steel

6 Application hints

6.1 Practical procedure for elution

Installation

The TLC-MS Interface 2 can be connected to any brand of MS.

The type of MS is mainly defined by the tasks of your laboratory and by the availability. For simple tasks, like confirmation of a structure or mass, an easy to use single quadrupol MS is adequate. For more complex MS/MS tasks (or if it is the only available instrument) a triple quadrupol MS, or an ion trap MS may be suitable. For identification of unknowns even a time of flight (TOF), Q/TOF or an Orbitrap MS can be used.

Eluent delivery

For the delivery of the eluent, a HPLC pump is needed. You may use the internal pump of the HPLC-MS. Remove the HPLC column if installed and connect the respective capillary from the TLC-MS Interface 2 to the solvent delivery capillary of the HPLC (as described in this manual).

You may alternatively use a stand-alone HPLC pump.

Connecting the TLC-MS Interface 2 to the MS

Connect the capillary from the HPLC pump to port 2 of the valve and port 1 directly to the MS (it is recommended to bypass the UV detector cell, because it is sensitive to back pressure from the TLC-MS Interface 2).

It is highly recommended to use an inlet filter before the MS. The inlet filter is a standard feature of most MS. For more information check chapter 2.2 Installation environment.

Setting up the MS

Select the ionization mode according to substance requirements and availability: ESI, APCI or APPI ionization, select appropriate mode (positive or negative).

Tune the MS according to the MS manufacturer's recommendations.

Optimizing MS parameters

Ideally the substance to be investigated is available in pure form. If not, another substance with similar properties can be used to verify the ionization conditions.

Eluents are often mixtures of acetonitrile or methanol with water. Mixtures of 80 % organic phase with 20 % of water have been successfully used. Depending on the nature of the substances to be investigated, protonation can be enforced by adding 0.1 % of formic or acetic acid. Deprotonation can be enforced by adding 0.1 % of ammonia.

A flow of 0.2 mL/min is suitable for the elution.

Caution: Do not use any nonvolatile buffer salts (e.g. phosphates) in the eluent and use only MS grade solvents!

A general rule of thumb for HPLC-MS: Dilute the solutions you typically use for UV-detection (approx. 1 mg/mL in e.g. methanol) by a factor of 1000 or 100, resulting in concentrations of 1 to 10 µg/mL. Use flow injection analysis (FIA) of 0.1 µL and watch the MS signal. If the MS signal is too small, inject 1 µL or more, until you get a clean mass spectrum.

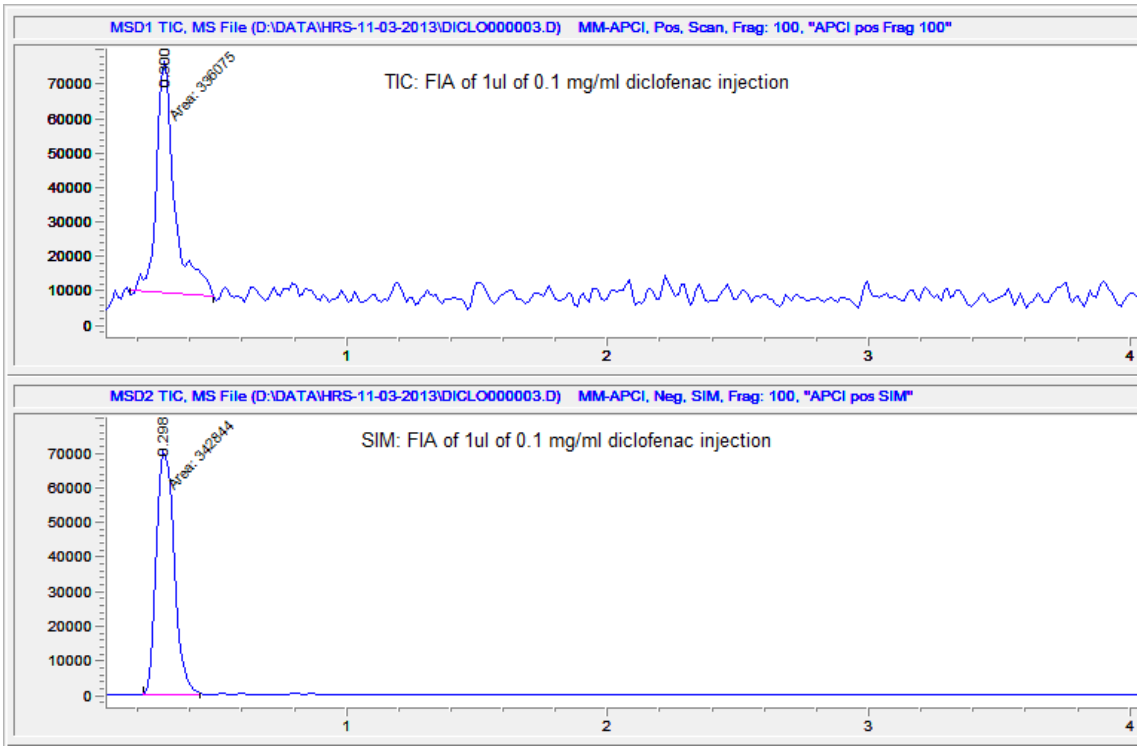


Fig. 6: Flow injection analysis FIA of 1 µL of 0.113 mg/mL diclofenac in methanol, upper curve total ion current (TIC), lower curve single ion monitoring (SIM) of the masses 296.00, 297.00 and 298.00 dalton.

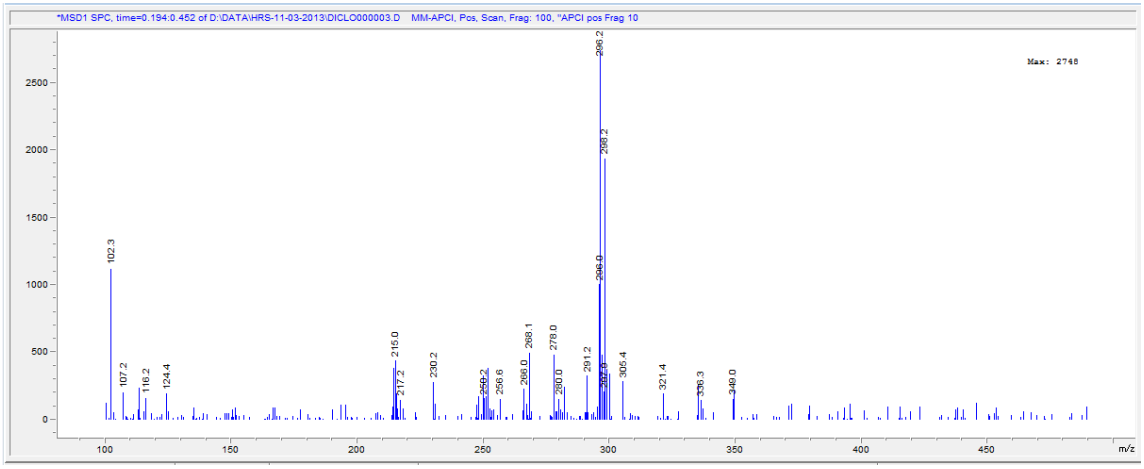


Fig. 7: Flow injection analysis FIA of 1 µL of 0.113 mg/mL diclofenac in methanol; spectrum of diclofenac.

Use FIA to optimize MS parameters: Nebulizer pressure, drying gas temperature and drying gas flow, capillary voltage and fragmentor voltage (consult the manufacturer's recommendations for your individual brand of HPLC-MS).

Optimizing TLC conditions

If available use HPTLC plates. Prior to use, prewash plates by developing once to the upper edge with neat methanol. Dry the plates for 10 min at 60°C in a vacuum drying oven or by heating for 20 min at 120°C in a clean (!) convection oven.
The preferred sample application technique is the spray-on technique using the Automatic TLC Sampler 4 or the Linomat 5. The spray-on technique allows application of relatively large volumes in the form of narrow bands, resulting in a good resolution of the sample zones after chromatography.
Use the sample solution with the concentration optimized by FIA experiments (usually 1 to 10 µg/mL). Apply a series of bands with increasing volume, e.g. 1, 2, 4, 8, 16 µL. The selected band length depends on the type and the number of samples. For many different samples on the same plate, a short band of 4 mm may be adequate. For fewer samples which are to be analyzed more than once, a band length of 10 to 20 mm is preferred.

Alternatively samples can be applied with fixed volume capillaries of 0.5 to 5 µL. In this case select a fixed volume and apply a series of individual dilutions.

Develop the plate in an appropriate device, e.g. the Automatic Developing Chamber ADC2 or a Twin Trough Chamber, which allows for control of chamber saturation.

6.2 Fittings

In order to connect your tubing together and to connect it to the equipment in your HPLC system; nuts, ferrules, and unions must be used. A ferrule is used to form the seal between components. Utilizing the nut and ferrule combination, tubing is held in place by a compression action.

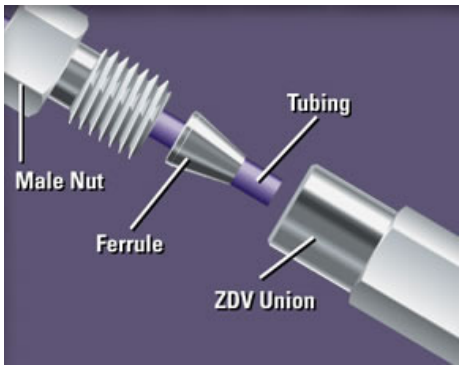


Fig. 8: Fittings

Installing high pressure fittings

Before connecting fittings, ensure the end of the tubing has a clean, square, burr-free cut. This is very important, as the square cut makes a flat surface for the tubing to contact the

bottom of the receiving port. Improper cutting of the tubing could lead to dead volume, resulting in peak distortion such as fronting, tailing or broadening.

Procedure with stainless steel nuts and ferrules:
Slide the fitting at least 5 mm from the end of the clean cutting tube.

- Insert the assembly into the receiving port, pushing the tubing into the port until it bottoms out.
- Using the delivered wrench, tighten an additional ½ to 1 turn past finger-tight. Remove the fitting to confirm the ferrule is swaged onto the tube.
- Because the ferrule is permanently attached to the tubing we highly recommended that the fitting only be used in the receiving port into which it was initially swaged. Failure to this may result in dead volume and/or leaks.

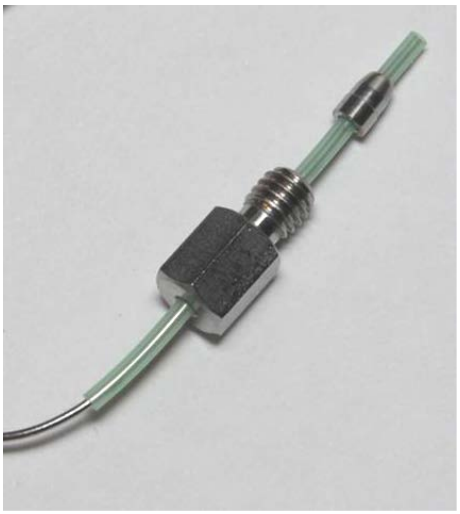


Fig. 9: Tubing sleeve

Making capillary tubing connections to 1/32" capillaries

Smaller tubing sizes like 1/32" OD are being used with increasing frequency in chromatography and related disciplines, and using these tubing sizes requires some special skills normally unnecessary with 1/16" OD tubing. This is especially evident when using the smaller tubing in receiving ports normally designed for the larger, 1/16" OD tubing. Generally, the high pressure receiving ports in most of the equipment on the market have an internal geometry supporting 1/16" OD tubing. This means that the port has an internal pocket into which the 1/16" OD tubing extends. As long as tubing with the same diameter
is used, potential dead volume resulting from the connection is kept to a minimum. However, when the tubing is smaller than that which the receiving port was designed for, it's easy for dead volume to be introduced into the connection.
Numerous manufacturers have developed ways to adapt smaller, capillary tubing into the receiving ports meant for larger OD tubing. Of the options available, two stand out as the most popular. The first option involves customized ferrules which look similar to those used for the larger tubing, but which feature smaller holes drilled through them to better accommodate the capillary tubing.
Fortunately, the second option - the use of a special tubing sleeve - overcomes both of the disadvantages exhibited by the customized ferrules. Tubing sleeves generally have a controlled outer diameter (usually 1/16") which allows them to fit into standard threaded ports. And, since the sleeve will slide through a ferrule until it bottoms out in a receiving

port, the tubing pocket beyond the ferrule will be completely filled to help avoid dead volume in the connection.

Tubing sleeves also offer several side benefits. One is the structural support sleeves provide to the outside of the capillary tubing, helping prevent damage to the tubing as it leaves the fitting. Furthermore, the extrusion process generally allows a more concentric connection than precision drilling and machining can provide, allowing for more accurate tubing-to-thru-hole alignment and decreasing inline turbulence and mixing. For all these reasons, using a tubing sleeve to connect capillary tubing into a receiving port is the method of preference - unless the receiving port is specifically designed for use with the size of tubing you are using!

6.3 Dead volumes

One of the concerns you will have as a chromatographer is about the dead volume existing in your system. Dead volume is described as a small space within your HPLC system where remixing of the sample may occur or where your sample can be diluted with mobile phase. Dead volume can cause critical problems in your analysis of chemical compounds, as it may bring about band broadening or split peaks in the end result, making it difficult to obtain good data on your samples. You must keep this in mind when making connections throughout your system.

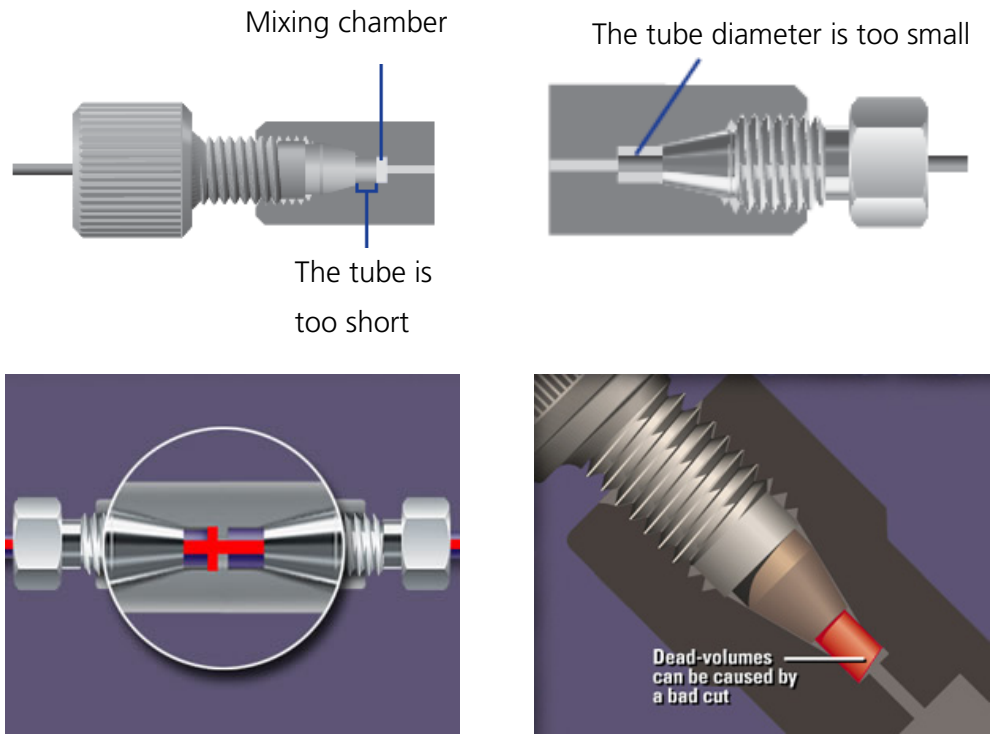


Fig. 10: Potential dead volumes in capillary-based applications

6.4 System leaks

Telltale signs of system leaks

Before you even see your first drip from a fitting, your system can tell you that the problem exists. The most common signs of system leaks are as follows:

1. No flow or pressure
2. Pump pressures up, but no flow
3. Noisy baseline
4. Baseline drift

While all of these symptoms could also indicate problems completely unrelated to leaking fittings, it is always easiest to start there. Not only are fitting leaks usually easy to repair, they are also the least expensive part of the system that can cause problems.

Check to make sure your tubing is seated properly

When using finger tight fittings, the tubing must bottom out in the receiving port before the nut and ferrule are tightened. If a gentle tug disengages your tubing after the fittings have been tightened, loosen the fitting, push the tubing to the bottom of the receiving port, and re-tighten the fitting.

The fitting may not be tightened enough

Stainless steel nuts and ferrules require a wrench to tighten them, even after repeated use. Finger tight fittings also require a good turn; however, don't use a wrench unless instructed to do so, or you may damage the fitting.

Notice: Over tightening can cause leakage too!

You may be using incompatible fittings

Make sure you are using a nut and ferrule that are compatible with each other and with the components of your system

Check the condition of the nut and ferrule

After repeated use, nuts (and especially ferrules) will gradually become de-formed to the point of being incapable of creating the seal they were de-signed to make. Always keep an extra supply of all the nuts and ferrules you are using so that you can replace them quickly and avoid unnecessary down time.

Check the receiving port for damage

Sometimes a leaking connection has nothing at all to do with the nut and ferrule, but with the receiving port. Ports that have had stainless steel fittings swaged into them are especially susceptible to damage. Check the receiving port for visible burrs or scratches and replace if necessary.

Evaluate chemical compatibility

Using fittings made of material that is incompatible with your mobile phase is a sure way of creating leaks.

6.5 Application example

Sample: diclofenac in methanol at a concentration of 0.113 mg/mL

Layer: 20x10 cm HPTLC plate Si60 F254, prewashed with methanol; UV 254 nm

Application: 1, 2, 4, and 10 µL as 8 mm bands

Mobile phase: toluene – ethyl acetate – acetic acid 50 : 40 : 1 (v/v/v)

Development: ADC2, saturated chamber, 70 mm from lower edge of plate

Under UV 254nm mark zones with a soft pencil.

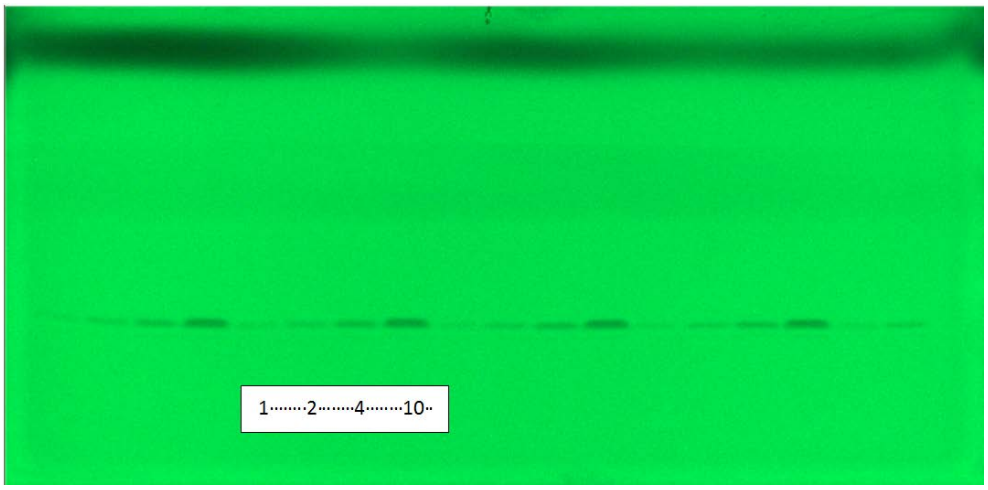


Fig. 11: Different volumes of 0.113 mg/mL diclofenac in methanol ; 8 mm bands

MS measurement

Start the HPLC pump.

The eluent used in the presented example was 95% methanol – 5% water and 0.02% formic acid, with a flow rate of 0.2 ml/min.

Start the MS for data acquisition only (no injection).

The following MS settings were used:

Source	APCI
Mode	positive
Capillary Voltage	4'000 V
Charging V	2'000 V
Corona Voltage	4'000 nA
Drying Gas Temp.	350°C
Vaporizer Temp	250°C
Nebulizer	5 l/min
Nebulizer Pressure	20 psig
Fragmentor	100 V
Mass Range	100 – 500 m/z
Scan	20 min

Place the elution head over the first sample zone, lower the elution head and switch the valve to elution position. Make a note of the time, when the elution started. After approx. 1 to 1.5 minutes, the elution is finished. Switch the valve to rear position to stop the elution. Lift the elution head and press the cleaning button 2-3 times. Place the elution head over the next sample zone and repeat the process.

This procedure allows you to register a series of samples into one MS file.

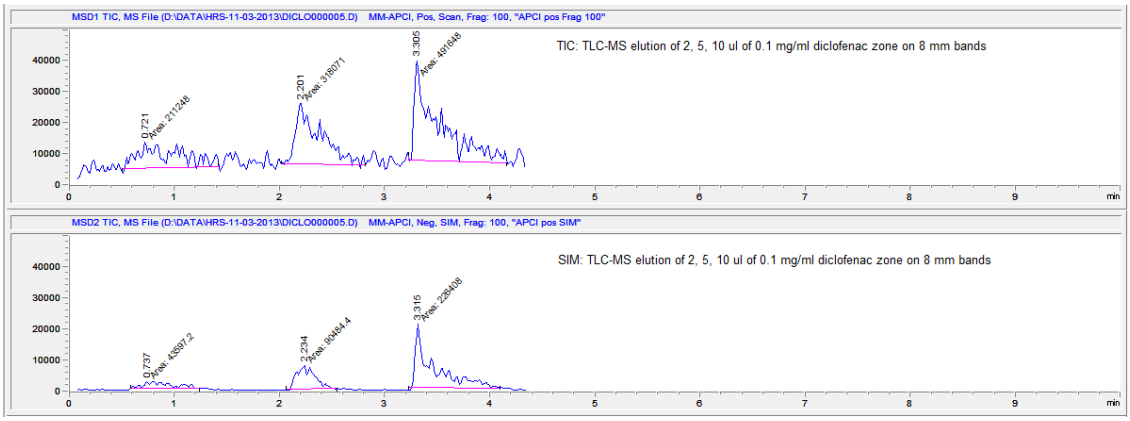


Fig. 12: Elution of three consecutive sample zones of diclofenac; 2, 4 and 10 µL per 8 mm band. Upper curve TIC, lower curve SIM.

Alternatively you can start the MS for each individual elution, which provides an individual MS file for each sample.

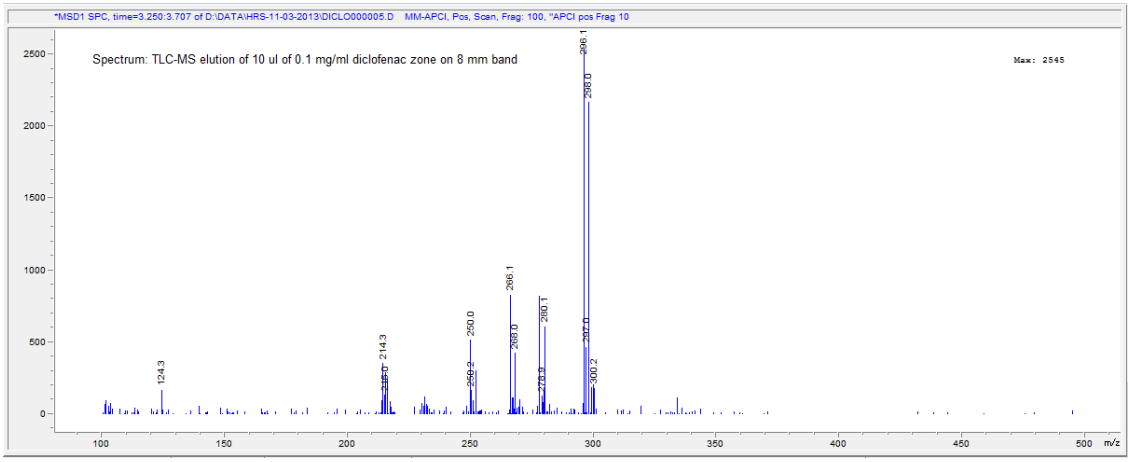


Fig. 13: Spectrum of a diclofenac zone with 10 µL per 8 mm band

Separation of Caffeine, Paracetamol, Acetylsalicylic Acid

Sample solutions

A stock solution containing 3 mg/ml caffeine, paracetamol, acetylsalicylic acid in ethyl acetate was prepared and diluted to

1	1'500	µg/ml
2	750	µg/ml
3	300	µg/ml
4	100	µg/ml
5	50	µg/ml

Sample Application

HPTLC-Plates Si 60 F254 20x10 cm, Merck (Darmstadt), were pre-cleaned with methanol in a CAMAG Twin-Trough Chamber and dried at 60°C for 20 minutes. Sample solutions were applied with a CAMAG Automatic TLC Sam-pler ATS4, 5 µl band wise, 4 mm wide. Distance from the lower edge 10 mm.

Solvent

Toluene: Ethyl acetate: Formic Acid 50:45:5

Chromatography

CAMAG ADC automatic development chamber with the following parame-ters: Saturation 10 minutes; migration distance 60 mm; drying time 5 minutes.

Documentation

CAMAG Visualizer (or DigiStore 2) Documentation System at 254nm.

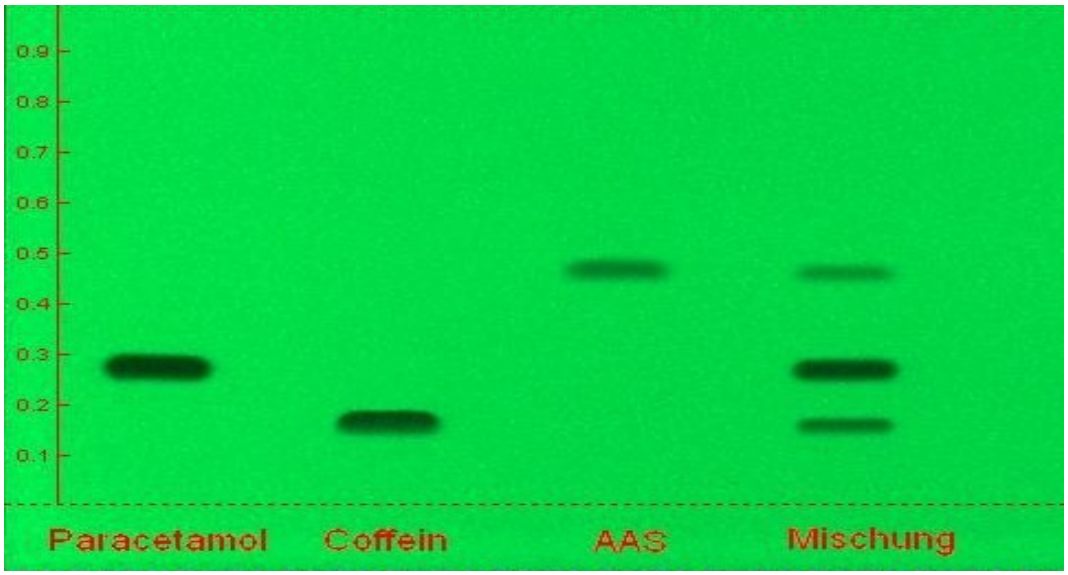


Fig. 14: Separation of Paracetamol, Caffeine, Acetylsalicylic Acid, mixture (from left to right), 25µg substance per spot.

HPLC Conditions

Column:	none	
Elution Solvent:	Eluent A: 95%	acetonitrile with 0.1% formic acid
	Eluent B: 5%	H_2O with 0.1% formic acid
Flow:	0.2 ml/min.	
Run time:	6 minutes	

The TLC-MS Interface 2 was directly connected to the HPLC pump (at the position of the 6-port valve of the HPLC normally connected to the column). The exit of the Interface was directly coupled to the MS inlet valve.

MS conditions Agilent MSD Trap XCT plus

Source	APCI	
Mode	positive	and negative
Corona	4'000nA	in negative mode 20'000nA

Dry Temp.	325°C	
Vaporizer Temp	400°C	
Nebulizer	60 psi	
Drying gas	5 l/min	
HV Capillary	3'500 V	
Scan	80-1800 m/z	
ICC Target	20'000	
Max. accu time	200'000 µs	
Averages	6	
Target mass	800m/z	
Compound Stability	100%	in negative mode 50%
Run Time	6min	
1. Segment	0-4min	to MS
2. Segment	4-6min	to waste

All MS runs were started manually.

CAMAG TLC-MS Interface 2

The selected sample zone is positioned below the elution head (either by means of the laser crosshairs, or by means of the x-y-scale). The elution head is lowered onto the HPTLC plate.

The total time for the elution was 6 minutes:

1.5 min	bypass
0.5 min	elution
4 min	bypass

Results

Separation of Caffeine, Paracetamol and Acetylsalicylic Acid

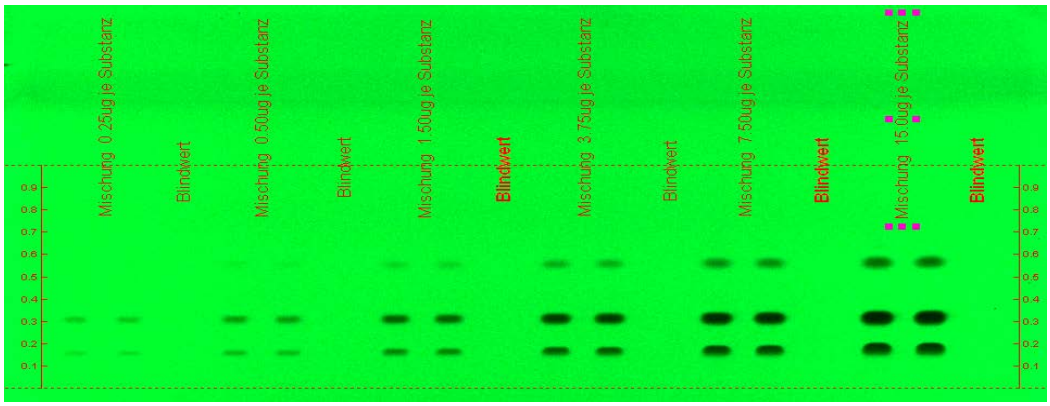


Fig. 15: Example chromatogram with band length 4 mm. Substance concentrations from left to right 0.25, 0.5, 1.5, 3.75, 7.5 and 15 µg/spot. Rf values: Caffeine <Paracetamol <Acetylsalicylic acid.

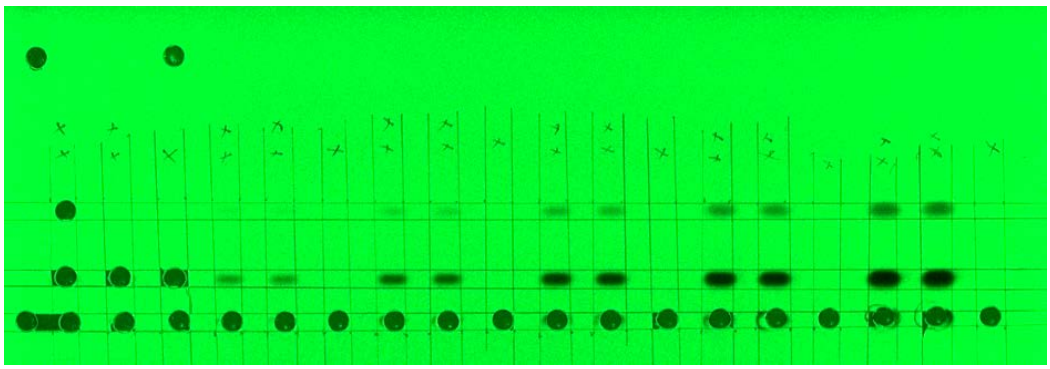


Fig. 16: Same plate as in Fig. 15, partially extracted with CAMAG TLC-MS Interface 2.

Resulting detection limits

Detection limits in MS strongly depend on the ability of substances to be ionized, as well as on the selected ionization source (mainly ESI, APCI or APPI).

If unknown substances have to be identified, the MS has to be run in "scan" mode, and the TIC (total ion current) is registered.

Known substances can be measured with a much higher sensitivity, if only the ion of interest (e.g. the ion with a m/z of 195.1 Da in the case of caffeine). In this case, the extracted substance can be measured in the SIM mode (single ion monitoring), or in the SRM mode (single reaction monitoring) in the case of MS/MS experiments.

Identification of substance (caffeine) by means of MS

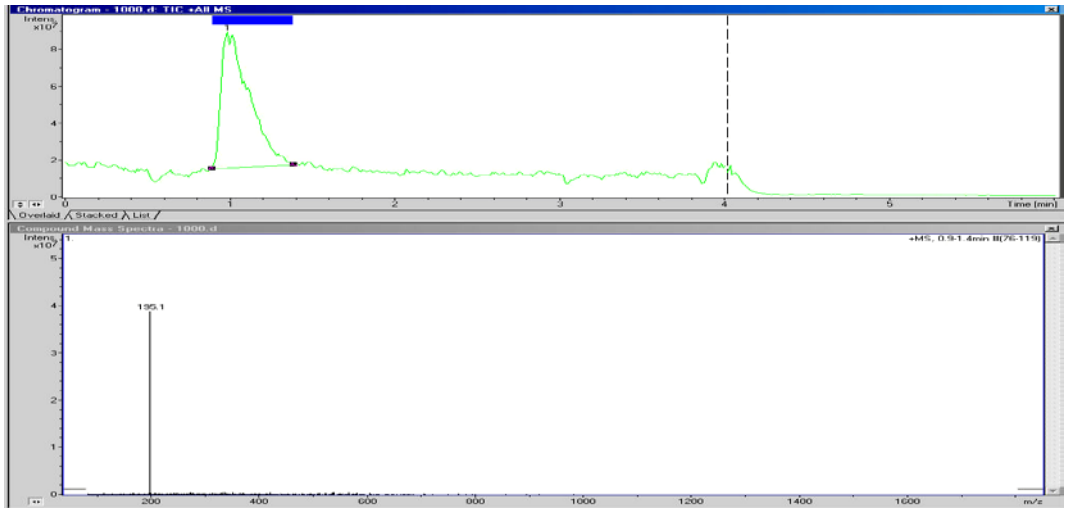


Fig. 17: ***Upper part****: Elution of 500 ng caffeine per spot, TIC has been registered between 80 and 1800 Da.*

Lower part*: MS of caffeine with APCI ionization; very low background noise.*

Measurement near the limit of quantity

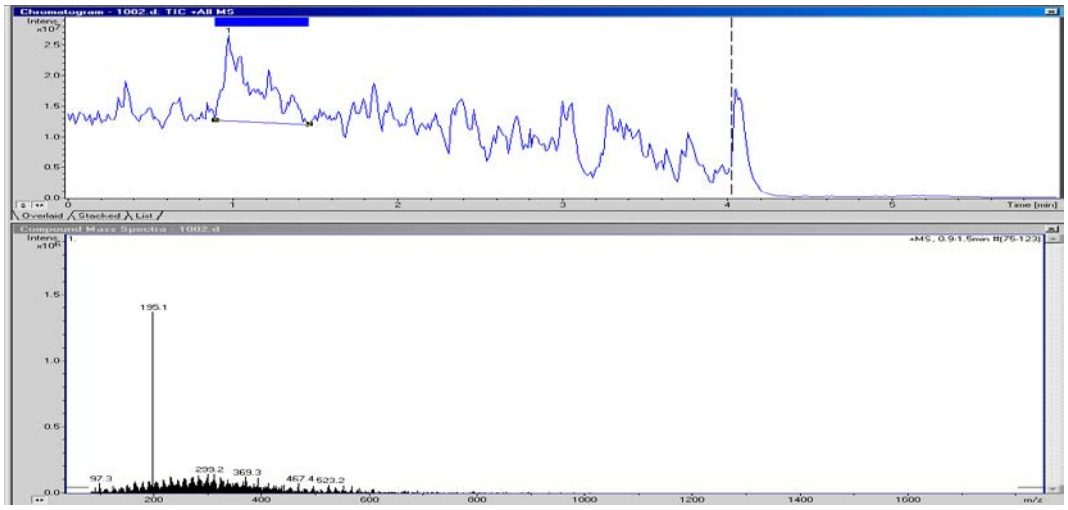


Fig. 18: Upper part: Elution of 25 ng caffeine per spot, registration of the TIC (total ion cur-rent), baseline shows relative high noise.

Lower part: MS of caffeine with APCI ionization, [M+H]+ ion (195.1 Da) is still clearly visible, background noise from the plate reaches approx. 10%.

Measurement below the limit of detection

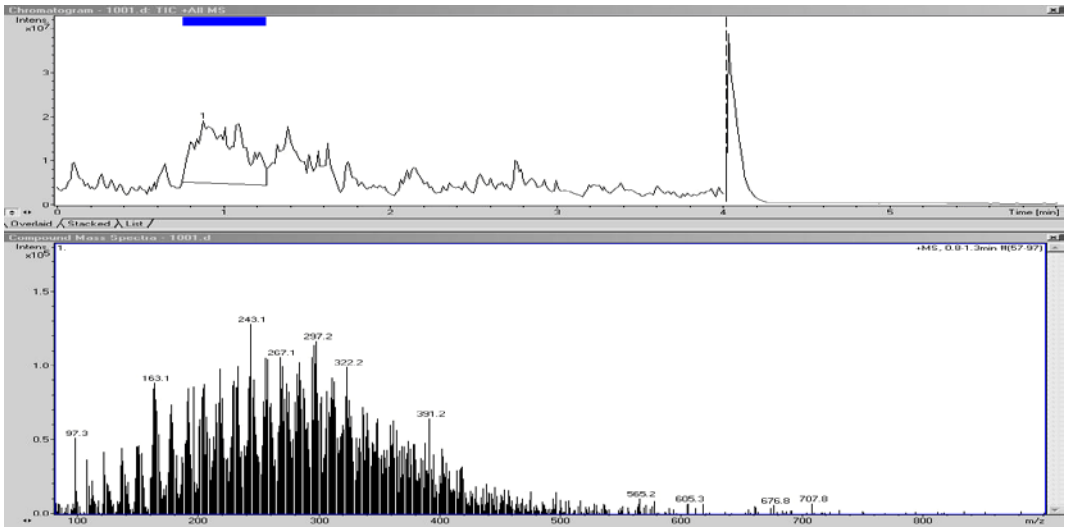


Fig. 19: 5ng Caffeine extracted from the plate. Background noise is larger than mass signal 195.1 Da.

CAMAG (Switzerland) · Sonnenmattstrasse 11 · CH-4132 Muttenz 1
Telephone +41 61 467 34 34 · Fax +41 61 461 07 02 · E-Mail: info@camag.com

CAMAG (Germany) · Bismarckstraße 27-29 · D-12169 Berlin
Telephone +49 30 516 55 50 · Fax +49 30 795 70 73 · E-Mail: infoberlin@camag.com

CAMAG Scientific Inc. (USA) · 515 Cornelius Harnett Drive · Wilmington, NC 28401
Telephone 800 334 3909 · Fax 910 343 1834 · E-Mail: tlc@camag.com

www.camag.com

DECLARATION OF CONFORMITY

EC – Declaration of Conformity

We, CAMAG Chemie-Erzeugnisse und Adsorptionstechnik AG
Sonnenmattstrasse 11
4132 Muttenz
Switzerland

declare under our sole responsibility that the product

CAMAG® TLC-MS Interface 2

Product name

022.8440/ 022.8441

Article number(s)

to which this declaration relates is in conformity with the following provisions of directive(s):

- 2006/95/EC
- 2004/108/EC

Following standard(s) or other normative document(s):

- EN61010-1: 2010
- EN61326-1: 2013

Year of the CE characteristic assignment: 2015

Muttenz, 09 June 2015

Walter Rahm, Head of Quality Management

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