



Troubleshooting

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Columns	Key Words	Question
Flash	AgNO ₃	What is PF-50SIAg?
Flash	Alumine	What are alumine phases ?
Flash	Amine NH ₂	How to use Amine columns?
Flash	Carbon, Activated Carbon	What are PF-AC columns?
Flash	Cyano	How to use Cyano columns?
Flash	DIOL	What is DIOL stationary phase?
Flash	Efficiency	How to compare theoretically Flash columns efficiency?
Flash	Equilibration - Flow rate gradient	Why you should use a flow rate gradient with flash columns during equilibration?



Answer

PF-50SIAG: Silica + AgNO₃ phases.
Impregnated silver nitrate silica 4%.

There are 3 different Alumina phases (Al₂O₃):

- Neutral Alumina (pH=7)
- Basic Alumina (Al-O-Na) (pH=9,7)
- Acid Alumina (Al-O-Cl) (pH=4,5)

Stability: pH 2-11 (more important than silica).

The retention mechanism is based on 3 types:

- Giver of electron (anion and oxygen)
- Giver of proton (hydroxyl group)
- Acceptor of electron (aluminium cation)

Aluminium oxide is prepared from the calcination of the aluminium hydroxide, then cleaned with acidic solution and with methanol.

Then the phase is activated by heating at 800°C.

This phase is an alternative to silica when this one degrades the sample. It is used in normal phase conditions.

Neutral alumina is preferable for purification of non-ionizable products with polar fonction.

A basic treatment of alumina allows a better separation of anionic products.

An acid treatment of alumina allows a better separation of cationic products.

PF-15,30,50NH₂: Phase Amine (100Å, 300m²/g)

This media is a silica end-capped with a primary amine functionality and can be used in normal or reversed phase conditions or HILIC or ions exchange.

In normal phase condition (organic non polar mobile phase), NH₂ media behaves as a relatively high polarity adsorbent and interacts with polar groups of compounds.

Loading of sample is lower than for Normal Phase (< 2%), depending on the resolution on the TLC.

Applications: Purification of polar compounds, Carbohydrates, Carboxylic and Sulfonic Acids, Polar organics compounds, Anions and Organic acids, Removal of TFA, Scavengers for acid chlorides, Basic compounds. (avoid to use a buffer which is difficult to evaporate)

Storage: Under 100% isopropanol immediately after each use.

PF-AC: Activated Carbon

Allows to discolor and take off unwanted products. Pre-packed columns are filled with activated carbon which provides a maximum absorption of impurities.

Application: take off by-products, colorants, impurities, catalysts, degraded reagents.

PF-50CN: Cyano

End-capped phase, with cyano bond, can be used in NP or RP mode.

In RP mode, CN columns are similar to C18 columns, even if the elution order can be different.

Polarity is between NH₂ and C18.

In NP mode (non polar organic mobile phase), CN phase has a different selectivity than NH₂ because of dipole-dipole interaction.

It is a good alternative for silica sensitive products.

Cyano columns are compatible with aqueous and organic solvents.

Applications: Separation of medium to high polar compounds (Anthraquinone, flavonoids,taxol, alcaloids, natural and pigment dyes, amino acids, nucleosides, nucleotides, steroids, vitamins, sulfonamides, benzodiazepines and derivated, nitrosamines, amino,

Storage: Under 100% isopropanol immediately after each use.

DIOL is less polar than NP silica, it is an alternative.

Selectivity is different compared to a virgin silica.

Such columns are compatibles with strong solvents (MeOH, Water), and can be used in NP or RP columns.

It is a good alternative to purify sensitive products as epoxydes which react with silica.

Loading capacity is less than NP (<5%)

Application: For Mid polar compounds, Proteines, peptides, steroids, hormones, Flavonnes, Phenols, amino-acids, nucleotides, vitamins.

Theoretical comparison of 50, 30, 15µm column efficiency

N= Column efficiency

50µm=N

30µm=1,7N => √N=1,3

15µm=3,2N => √N=1,75

Rs=√N

Excessive flow rate during equilibration can generate overpressure.

To avoid this, we recommend controlling the flow rate with a flow gradient. Use the equilibration table delivered with the Interchim® flash columns. (Document available in the "technical tips" section of the catalog).



Columns	Key Words	Question
Flash	Flow rate, Optimum Flow rate	How to calculate the optimal flow rate of a flash column?
Flash	Frit	Can you provide details about frits of Flash columns?
Flash	Heat Rise	Why do Flash columns heat during equilibration?
Flash	HILIC-HIA	How to use HILIC-HIA columns?
Flash	Impurities	What types of impurities are present in the silica? And at what concentrations?
Flash	Ion exchange - anion - SAX	How to use Anion Exchange Columns?



Answer

Optimum flow rate calculation = $0.75 \cdot (\text{Diameter}^2/4.6^2)$

$$D_{OPT1} = 0.75 \times \frac{\phi}{4.6^2}$$

Interchim® use a gradient porosity frit technology. For every dimension of silica, a porosity gradient frit is optimized, to have the better diffusion without sample retention.

Causes:

- Solvent displaces air into the pores of silica
- Friction force
- Adsorption of the solvent on the surface of the silica (= heat of adsorption)

The more the solvent is polar, the more the temperature increases.

Consequences: Negative influences on

- The kinetics of mass transfer
- The retention
- The separation of compounds

The amount of heat generated depends on the particles size, flow rate and viscosity of the solvents.

Solution => Equilibration before the injection of the sample.

On rare occasions, the samples can give better separations on a dry cartridge when the compounds are slightly more polar and higher-grade solvent mixtures are used.

The heat factor becomes less important with better retained compounds that sometimes require a little more surface activity of the silica to separate well.

Application: Polar and water soluble compounds.

Cleaning: Rinse with 3-4 column volumes of the most eluting solvent, after every run.

Storage: under isopropanol, after rinsing with 4-5 column volumes.

Aqueous normal phase (ANP) separation of water soluble compounds. Typical mobile phase: water/AcN, MeOH/Water (>50%)

Many compounds are difficult to purify due to their polarity and strong solubility in water. These compounds are insoluble in most of normal phase solvents and are irreversibly retained on silica or alumina. Under reverse phase conditions, the compounds are poorly retained on C18.

The water forms a thin layer at the surface of the silica -> Hydrophilic interaction (almost ionic) with sample

Starting gradient: organic solvent (minimum 20%)

Ending gradient: Mixture containing water

Suitable solvents ANP: Acetone, AcN, IPA, EtOH, MeOH, THF

ANP is an alternative to reverse phase purification for highly polar compounds

Ex: eluent condition: 2CV 100% organic solvent, 10CV : 0% to 50% Water, 3CV 50% Water -> increase the proportion of the water in order to give hydrophilic character, elute polar compounds

Some alternatives to HILIC phase: Diol, Si, NH₂. The user can keep the same solvents used with Hilic to do a purification with Silica, Diol or NH₂. The selectivity can be different.

In some cases, the elution conditions will have to be slightly modified:

Firstly, try to do the purification in HILIC conditions with Diol, NH₂, Silica.

Secondly, if the separation is not optimized, try to modify the % of solvent to increase the resolution.

Silica impurities:

For IR-50SI

Ca: 100 ppm

Na: 100 ppm

Fe: 100 ppm

For PF-15SIHP

Ca: between 1 and 10ppm

Na: 20ppm

Fe: between 1 and 10ppm

Al: between 1 and 10ppm

Zr: 20ppm

Ti: 50ppm

PF-50SAX: Si-(CH₂)₃-N + (CH₃)₃

This phase is a quaternary amine.

The loading capacity is less important than for a normal phase (0.5-5%)

Exemple of solvents used: Hexane/AcOEt

Applications:

- Purification of organic acids (totally retained)

- It is possible to stack a SAX column on a normal phase column to hold acid products, the other compounds will be eluted.

Then, release the acid products by adding 5% of acetic acid in methanol.



Columns	Key Words	Question
Flash	Ion exchange - cation - SCX	How to use Cation Exchange Columns?
Flash	Peptides purification	Are there flash columns dedicated to peptides purification?
Flash	Polyamide	How to use polyamide columns?
Flash	Polymers, PSDVB	How to use PSDVB columns?
Flash	Porosity	How does porosity influence purification?
Flash, Prep	Acid Sites	How to block acid sites of silica?



Answer

PF-50SCX

This phase is a bounded silica with tosic acid (strong acid)

The loading capacity is lower than NP (0,5-5%)

Example of solvents used: DCM/MeOH, Hexane/AcOEt

Applications:

Allows to purify basic compounds.

It is possible to isolate basic compounds (totally caught on the phase). Meanwhile, neutral and acidic compounds are eluted.

Then, release the basic compounds.

It is possible to stack a SCX column on Si columns. SCX column will catch basic products, the other compounds will be eluted.

Then basic products will be released by adding 5% of NH_4OH .

Interchim® propose Flash columns dedicated to peptides:

PT-15C18T

PT-15C18N

PFB-15C18T

PFB-15C18N

PT-15C18

Typical Loading is 2% (but depends on purification)

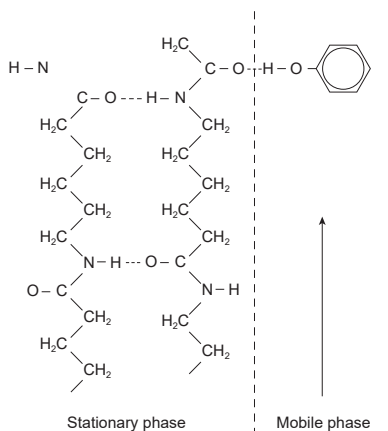
PF-100P6: Purification of natural substances with phenol groups, polyphenols and nitro.

Solvents used: AcOEt, Dichloromethane, Alcohol, Water.

Applications: Phenols, aromatic compounds, nitro and amino compounds, chalcones, flavonoids, DNP-amino acids

Storage: 2-3 CV of organic solvent 70-90% (Acetonitrile, MeOH,...)

Polyamide 6 as stationary phase for the separation of phenols



PF-X: PSDVB Polymers

Atoll™ X, 40 μm - 800 m^2/g

Solvents used: Acetonitrile/water (7/1), water/MeOH. Acetonitrile or THF can be added to the mobile phase to increase performance.

Can be used with Dichloromethane, Cyclohexane, Chloroform.

Applications: extraction and preconcentration of non or slightly polar compounds, analysis in reverse phase.

Storage: 2-3 CV of 70-90% organic solvent (Acetonitrile, MeOH,...)

Do not clean with 100% water, leave a minimum 1% of organic solvent.

Advantages:

- Stability of pH: 1<pH<14 (ideal for basic compound)
- Less selective
- More important loading
- Specific surface more important than for C18 phase

If the porosity decreases, the surface area increases.

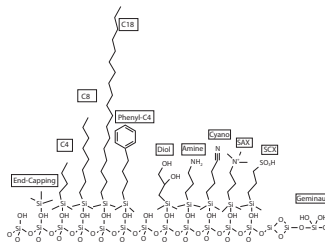
For a specific application, it is advisable to use large pore silica, especially with biomolecules.

To block an acid site of virgin silica, use Triethylamine (0,1 to 0,5%) mixed to a solvent.



Columns	Key Words	Question
Flash, Prep	Bonded Silica	What are the different bonded silicas?
Flash, Prep	Dry-Load, Celite	How to realize a dry-load?
Flash, Prep	HILIC	How HILIC mode works?
Flash, Prep	Methanol	Can I use 100% methanol with silica?
Flash, Prep	Plates, theoretical plates, EHTP	Equivalent height of theoretical plate EHTP: How to compare?
Flash, Prep	Polarity	What is the evolution of the polarity of stationary phases, according to the bonds?
Flash, Prep	Retention	Retention mechanism in purification

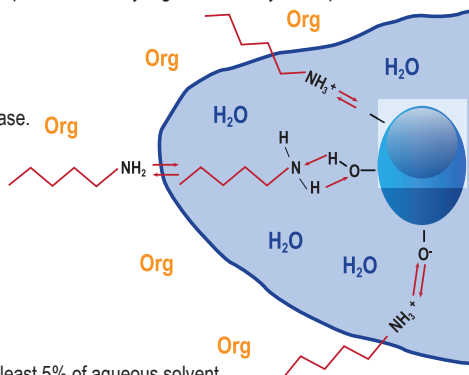
Representation of graft stationary phases



Dilute the product in a solvent, add the celite (1:1 or 1:2), dry to adsorb the product, then introduce in the dry-load, and complete with celite or sand (to reduce the dead volume).

- Analysis of very polar compounds
- Analysis of basic ionizable drugs, not separated in reverse phase.
- Development of more robust methods compared to grafted phases.
- Better sensibility on MS thanks to the high organic percentage of the mobile phase.
- Easier preparation of sample (solubility).

- Polarity of analytes
- Degree of solvation
- Eventual ion exchange



For the utilisation on LC/MS, prefer volatile buffers.

It is possible to use 100% Methanol, the silica is not dissolved.

- calculation method (4sigma, 0,606h, Dorsey-Follet,...)
- instrument used (dead volumes)
- mobile phase
- sample

Diagram illustrating the increasing polarity of stationary phases:

- Silica: —Si—OH
- Amine: $\text{—Si—O—CH}_2\text{—CH}_2\text{—NH}_2$
- Diol: $\text{—Si—O—CH}_2\text{—CH}_2\text{—O—CH}_2\text{—CH}_2\text{—OH}$
- Cyan: $\text{—Si—O—CH}_2\text{—CH}_2\text{—CN}$
- C-18: $\text{—Si—O—CH}_2\text{—CH}_2\text{—(CH}_2\text{)}_{17}\text{CH}_3$

Increasing polarity is indicated by an upward arrow.

- Silanol free
- Silanol link
- Silanol gemine

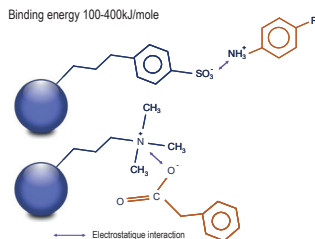


Columns	Key Words	Question
Flash, Prep	Retention mechanisms, Ion exchange	How do SCX or SAX interact with compounds?
Flash, Prep	Retention mechanisms, Normal phase	How do polar interactions work?
Flash, Prep	Transposition C18 TLC - Flash C18	Non-reproducible separation between TLC C18 and Flash C18. What to do?
Prep	Capacity - Loading capacity	What is the Loading capacity for a prep column?
Prep	Diameters	What are the different diameters available for prep HPLC columns?

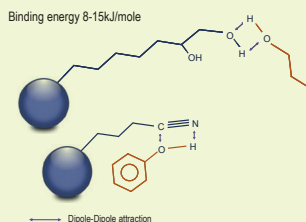


Answer

Ion interaction SCX,SAX
Electrostatic attraction



Polar Interactions
stationary polar phase, apolar mobile phase



A) The behavior of the grafted phases can be very different between TLC and Flash purification:

The proportion of water in the mobile phase has a very important influence on the velocity of the mobile phase in TLC. The more the apolar graft is dense (short chains), the fewer are the remaining silanols on the surface, the lower is the speed. The hydrophobic repulsion of the grafting becomes more important than the capillary forces - In purification, this phenomenon does not exist, or is very limited, because of the use of a pump.

B) With C18 silica it is difficult to elute with a solvent which contains water.

Only a transposition from an HPLC column filled with the same Flash stationary phase avoids these problems.

The loading capacity is an important element of preparative HPLC.

Several factors can affect the loading capacity: the dimensions of the column and the specific surface of the stationary phase used. Another factor affecting this capacity is the way the sample is introduced on the top of the column.

The sample enters the column into a spot which has the dimension of the internal diameter of the capillary 1/16 connection. If the sample is not evenly dispersed across the head of the live bed, overload regions are created when others are underloaded.

For example, if using a 50mm column i.d. and a connecting capillary of 500µm internal diameter without a distributor, then the sample will be focused on only 0.01% of the surface of the column head. This is obviously a huge loss of capacity. Not to mention that the column head clogs prematurely in the area of arrival of the sample, which will reduce the life time of the column.

To prevent this problem, our preparative columns are equipped with splitters whose design maximizes the dispersion of the volume and mass of the sample injected over the entire surface of the column head.

Prep columns are available as standard in internal diameters 10.0 - 21.2 -30.0 and 50.0mm. The lengths are 50 and 250mm (purification range from mg to g).



Columns	Key Words	Question
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Prep

Dry-load HP

How to install a Dry-Load HP (High Pressure)?

Prep	Filters, In-line filters, columns saving	How to prevent the degradation of a prep column?
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Prep

Inox tubes - Quality - Ra

What is the Ra?

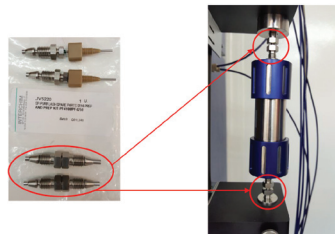
Prep	Life time improvement	How to improve the life time of a prep column?
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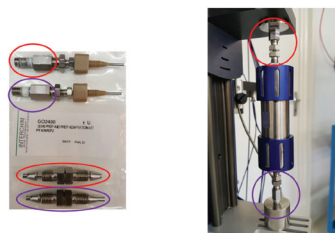
Answer

HP Dry-loads have Valco threads 5/16"-24 for 1/8" OD tubes.

Installation on a puriFlash® 4250 or 4125 system (on the column support) requires the connectors contained in our JV5220 kit:



Installation on a puriFlash® 4125 system (on the pre-column support) as well as on the puriFlash® 215, 430 and 450 systems requires the connectors contained in our GO2400 kit:



To prevent the degradation of a prep column, the installation of an in-line filter in preparative column inlet with diameter 10 to 50mm is recommended.

Holder 21.2mm + filter 2µm reference CE4600.

Sintered replacement reference CE4620.

Use a 1/16"-1/16" PEEK (T78560) or Inox (DT2800) coupler between the in-line filter and the column inlet for 10 to 30mm diameter preparative columns.

Use a 1/16"-1/8" coupler (included in our JV5220 kit) between the in-line filter and the column inlet for 50mm diameter preparative columns.

The Ra, value of tube's surfacing, is very important in Preparative Chromatography. Measured electronically or optically, it represents the difference between peaks and the valleys of the tube surface. The more the Ra is low, the more the surface is smooth. The friction against the tube surface leads the mobile phase to move in laminary regime.

The molecules in the center of the flow move more quickly than those closer to the edges of the tube. It can induce an enlargement of peaks and a bad efficiency. To minimize this edges effect, the finished process of prep column fabrication is a key to their very high quality.

It makes it possible to obtain a surface of tube extremely smooth (The Ra is 8µinch), significantly reducing the edges effects and so increasing the efficiency of Modulocart Prep.

In order to improve the life time of the columns, we recommend to gradually increase the flow as well as the variation of the composition of the mobile phase during commissioning.

Example: with a reversed phase column 50.0mm diameter under ACN / H₂O 70/30:

Composition of the desired mobile phase: ACN / H₂O 10/90; Desired flow rate: 120mL/min

Start gradually (5 minutes at 30mL/min ACN / H₂O 10/90, then 5 minutes at 60mL/min then 5 minutes at 90mL/min to finish at the desired flow).

To perform a gradient change during or at the end of the cycle, we recommend avoiding composition variations that are too abrupt.

Example: do not go from 120mL/min in ACN / H₂O- (90/10) to 120mL/min in ACN / H₂O- (10/90) in a few minutes). A duration of 15-20 minutes for this change of mobile phase composition is recommended.



Columns	Key Words	Question
Prep	Loading capacity	How to overload?
Prep	Seal	Advice for seal
Prep	Transposition HPLC-Prep - Injected quantity	How to transpose the mass loaded, from an analytical method to a preparative column?
Prep	Transposition HPLC-Prep - Resolution	How to calculate the resolution, from a analytical mode to a preparative mode?



Answer

If concentration overload is generally preferable (within the solubility limits of the analytes in the chosen solvent) to volume overload, the latter remains simpler to implement.

The table below gives a very general idea of the quantities that can be reached in preparative chromatography. These indications are however not exhaustive. Depending on the compounds (their solubilities), the column resolution and the operating conditions, the indicated charges may be lower or greater.

ID Column	Standard flow rate	Loading for selectivity > 1.5	Loading for selectivity close to 1.2
4.6mm	1mL/min	3 to 20mg	0.5 to 3mg
10.0mm	5mL/min	20 to 100mg	5 to 20mg
21.2mm	20mL/min	100 to 400 mg	30 to 100mg
30.0mm	40mL/min	150 to 1000mg	50 to 150mg
50.0mm	12 mL/min	500 to 2500mg	100 to 500mg

The 1J8300 basic seal is made of FKM fluorocarbon (limited compatibility with DMSO).

For applications using this solvent we can offer a replacement seal Hifluor FKM, reference 1K0630.

$$m2 = m1 \cdot (r2 / r1) \cdot (L2 / L1)$$

With:

Ray of analytica column: r1

Length of analytica column: L1

Injected analytica mass: m1

Ray prepared of column: r2

length prepared of column: L2

Injected mass prepared: m2

The goal is to purify the maximum amount of product in a minimum of injections. We will actually seek to increase the mass m2 by loading the column to the limit of resolution of impurities and the product of interest.

$$Rs_{prep} = 1 / \sqrt{Dp} \cdot L / U \cdot Rs_{ana}$$

Analytic resolution: Rsana

Diameter of analytic paticules: dpana

Length of analytic columns: lana

Lineary speed of analytic column: uana

Injected analytic mass: m1

Resolution preparative: Rsprep

Diameter of preparative particule: dpprep

Length of preparative columns: lprep

Lineary speed of preparative column: uprep

Injected preparative mass: m2

with

$$L : l_{prep} / l_{ana} , U : u_{prep} / u_{ana} , Dp : d_{pprep} / d_{pana}$$



Troubleshooting Instruments

Back to
SUMMARY

Module	Key Words	Question
Autosampler	CE	Is the Autosampler CE marked?
Autosampler	Connection	Can we connect the autosampler on a puriFlash® system already installed?
Autosampler	Contamination	Can the needle contaminate the sample?
Autosampler	Electrical power	What is the electrical power of the autosampler?
Autosampler	Injection Mode	What are the different ways to inject the sample with the autosampler?
Autosampler	Injection Volume	Which volume can we inject with AS?
Autosampler	Needles	Is the AS supplied with different needles?
Autosampler	Racks	Can we use Interchim® Racks with the autosampler?
Autosampler	Rinsing	What is the rinsing procedure with AS?
CarouXel	Columns	Which columns can be used on a CarouXel?
CarouXel	Connection	Can we connect a CarouXel on a puriFlash® already installed?
ELSD (External)	Pressure, Flow rate	What pressure and what gas flow rate can we use with an ELSD?
ELSD (External)	Rinsing	Should I rinse my ELSD detector after each run?
ELSD (Integrated)	Solvent Carrier	Why do we have to use a solvent carrier with an ELSD detector?
General	Fluorinated solvents	Can I use fluorinated solvents with a puriFlash® system?
General	Leak Detectors	Are the puriFlash® systems equipped with leak detectors?
General	Nitric acids, acids	Is nitric acid compatible with puriFlash® systems?
General	Rinsing	Is it necessary to rinse the puriFlash® system?
General	Soda	Can I use soda with a puriFlash® system?
General	Solvent filters, strainer	Can I use solvent filters?



Answer

Yes, the autosampler is CE marked.

Yes.

Theoretically not: Before and after every picking-up, the needle is automatically rinsed.

Max power of the Autosampler is 68W.
The Power of the syringe motor is 48W.

There are 2 different modes:

- Standard Mode: the sample can be surrounded by two air bubbles, to avoid side effects with the mobile phase
- Bracketing mode: used to add upstream and downstream of this sequence "bubble - sample - bubble" a solvent segment (DMSO) different from the carrier solvent or the rinsing solvent. At the ends of these two segments of solvents, is added an air bubble.

The injection volume depends on both the volume of the tube in the sampler rack and the injection loop on the injection valve (we can not put more sample volume than the loop can contain)
If you want to make a large injection, you can play on the volume of the syringe, (syringe 10mL by default - 25mL syringe optional). It is also possible to do several filling cycles with the syringe.

By default, the autosampler has a beveled needle, but a flat-bottomed needle can be installed as an option.

Yes, the autosampler is designed to use all puriFlash® racks.

Different rinsing procedures:

- Independent rinsing station
- External bottle for rinsing solvent
- Additional port for bracketing solvent

It is possible to use columns from F0004 to F0330 format

Yes.

2 to 2.3L/min with a pressure of 2 bar.

Yes, after each purification, cleaning the DEDL is essential. The DEDL must be purged with isopropanol by using the predefined method "ELSD Nebulizer cleaning" (lasts 4 minutes). When the signal on the DEDL is stable, you can stop the purge.

ELSD is a destructive detection mode. Interchim® therefore uses a solution on its systems that consists in dividing the stream (Solvent + Sample) in order to reduce as much as possible the quantity of product lost.
In addition, the sample is pushed to the nebulizer with isopropanol which at the same time allows the system to be cleaned. This prevents the products from crystallizing.

The puriFlash® systems are compatible with fluorinated solvents (methyl nonafluorobutyl ether or methyl nonafluoroisobutyl ether). However, rinsing the system with isopropanol is essential.

Yes, leak detectors are available by default on all the modules of the new generation 5 of the puriFlash® systems: pump, fraction collector, detector.

The puriFlash® system can work up to 25% nitric acid. A weak acid like ethanoic acid does not cause any problem.

Yes, after each purification, a cleaning with the most eluting solvent of the purification is essential to eliminate any product remaining in the system. Then, a conditioning of the apparatus with isopropanol is recommended in order to avoid miscibility problems between the next and the previous purification.

To use soda is possible, but it is recommended to rinse the pistons of the pump with water, and do not let the soda stagnate on the pistons.
You can create a last step in your method to rinse the system.

It is not advised to use solvent filters. Cavitation phenomena may appear, thus disturbing flow accuracy and gradient accuracy.



Module	Key Words	Question
General	THF	Is THF compatible with puriFlash® systems?
Mass	Calibration peaks	There are still some calibrant peaks. How to remove them?
Mass	Cleaning, Source	How to clean the source?
Mass	Gain	Can we adjust Mass gain during autotune?
Mass	Injection	When we inject on the mass with FIA, which sample concentration must we use?
Mass	Pressure, Flow rate	What pressure and which gas flow rate should be used for the mass spectrometer?
Mass	Sensitivity	How can I increase the sensitivity of my CMS?
Pump	Accuracy, Precision	What does accuracy and precision mean?
Pump	Acetic acid, Ammonia	Can we use acetic acid or Ammonia with a puriFlash® system?
Pump	Belt	What material the pump belt is made of?
Pump	Buffers	Can we use salt buffers with the puriFlash® systems?
Pump	Flow rate	Why is my flow rate higher than expected?
Pump	Mixing Chamber	What material the mixing chamber seal is made of?
Pump	Pistons	What material the pistons are made of?
Pump	Seals	What material the pump head seals are made of?
RI Iota 2	Cleaning	Do we have to clean the cell of the RI?
RI Iota 2	Equilibration	Is there a particular way to equilibrate the column with a RI?
RI Iota 2	Maintenance	Does the optical block need maintenance?
RI Iota 2	Mix of solvents	How to proceed with the RI when we use a mix of solvents?
RI Iota 2	Position	How to position the RI if we use other detectors?
RI Iota 2	Procedure	What are the good operating procedures for a RI detector?
RI Iota 2	Procedure	What to do, if we want to use the RI after a long unused time?
RI Iota 2	Pulsations	How to avoid pulsations of the RI signal?



Answer

PuriFlash® systems are compatible with THF. But we do not recommend to store the device under THF. You must rinse the device with isopropanol.

It is necessary to rinse 2h with acetonitrile and / or MeOH to eliminate all the calibrant. If there are still traces, try to put the tube back in the calibrant bottle so that it is no longer in contact with the liquid. If the problem persists, it means that there is calibrant stuck in the system. It is probably necessary to change a check valve or contact the After Sales Service.

Clean the source with 1-3% formic acid (soak in a beaker and then in an ultrasound bath for 30 minutes).
Clean the cone with MeOH and specific cloth.

During an autotune, the gain of the detector is not regulated. It is good to check the intensity of a control compound during installation and to read the detector gain value. When we see the sensitivity decreasing (on average after one year) it is necessary to manually increase this gain and re-record the tune.

The concentration must be 5-20 µg/mL. Too much concentration can interfere with ionization.

5.5 bar and 10L/min Nitrogen.

- Reduce the range of TIC.
- Decrease the split ratio, decrease the make-up or dilution flow rate.
- Decrease the capillary temperature.

In the fields of science, engineering, industry, and statistics, the accuracy of a measurement system is the degree of closeness of measurements of a quantity to that quantity's actual (true) value.

The precision of a measurement system, related to reproducibility and repeatability, is the degree to which repeated measurements under unchanged conditions show the same results.

Yes, we can use acetic acid or Ammonia with a puriFlash® system.

The pump belt is made of Polyurethane and nylon.

Yes, but it is essential to use the washing discs supplied with the system to avoid crystallization at the back of the pistons which would damage the seals.

With low flow rates (<1.5mL/min) without any column (backpressure), it is possible that the solvent is siphoned, disrupting the measurements. If a column (or more generally a back pressure) is installed, the flow becomes correct again.

The Mixing chamber seal is made of PTFE.

Pistons are made of ceramic.

Pump head seals are made of PTFE charged with graphite carbon, thus extremely resistant to solvents.

The cleaning of the cell is to be done very rarely, unless there is a significant decrease in sensitivity.
The materials used for the block are stainless steel, teflon and quartz.

We recommend to equilibrate the column at least one hour before the IOTA is started. Indeed, the RI is dependent on the temperature, and during equilibration the column heats up.

No. The optical unit needs no maintenance or adjustment.

If you use a mix of solvents, it is recommended to mix them in a bottle before starting the run.

Never put another detector in serie behind a refractometer. If multiple detectors are used, the refractometer must always be put in last position.

Before using RI detector, let it a while in the room, in order to stabilize the temperature of the detection part (minimum half a day).

In case of prolonged shutdown of the device it is necessary to rinse carefully the measurement and reference circuits.

The check valves of your pump must be clean, otherwise you will see pulsations on the chromatogram.



Troubleshooting Instruments

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SUMMARY

Module	Key Words	Question
Unit Control	Remote Control	Is it possible to remote control the system?
Unit Control	Touchscreen	What is the technology of the puriFlash® touchscreen?
UV Detector	Baseline	What can be the reason for a non-constant baseline?
UV Detector	BPR Back Pressure Regulator	Where should the BPR (Back Pressure regulator) be placed?
UV Detector	Flow Cell	Is it possible to change the UV flow cell?
UV Detector	Flow Cell, Cleaning	How to clean the UV flow cell?
UV Detector	Flow cell, specifications	What are the specifications of the UV Flow cell?
UV Detector	SCAN	What is the entire scale for the SCAN function?
Valves	Loops	What are the different loops available with puriFlash® systems?



Answer

Networking and remote control of puriFlash® systems is possible.
It is the customer's responsibility to network the puriFlash®.

It is a capacitive touchscreen.

A non-constant baseline can be induced by solvents with a high rate of dry residue.
Solvents with a high residual content after evaporation, or with non-volatile stabilizers, can cause disturbances along the entire baseline (not only on peaks). Thus, they increase both the height of the baseline and the background noise.

The BPR is inside the device, and is placed between the UV cell outlet and the fraction collector inlet.

Yes 3 different flow cells are available:

0.3mm 45µL

1.3mm 55µL

2.4mm 70µL

It is possible to clean the UV cell with formic acid, then rinse with water to remove acid, and use Isopropanol to stock it.

Max flow rate 500mL/min.

Max pressure 20 bar.

The SCAN function is now available for all wavelengths from 200nm up to 800nm.

Injectons are possible from 500µL to 50mL. 2 kits are available for standard injections, and large injections.