

Solid Phase Extraction Application notebook

For Milk analyses



INTRODUCTION

POLYINTELL offers a comprehensive range of sorbents for the challenging fields of sample preparation, sample clean-up, extraction and purification, from conventional SPE products (**AttractSPE™** polymeric sorbents, **SilactSPE** silica based-sorbents) for a crude clean-up to the most innovative SPE for specific application (**AFFINIMIP®SPE**, a Molecularly Imprinted Polymers (MIP) based sorbents).

In addition, the experience of POLYINTELL is continuously enriched with customer interactions and an endless analytical development for new applications. This experience is sometimes communicated through Application notes (available on website and via newsletters).

To make you enjoy of this background and for an wealthy, very instructive and easily-accessed overview of **AFFINIMIP®SPE** and **AttractSPE™** performances, we are pleased to introduce you this Application Notebook which collects a good abstract of POLYINTELL's experience on **AFFINIMIP®SPE** and **AttractSPE™**.

For your convenience, this application notebook will be permanently updated with new protocols and results. Please regularly visit our website www.polyintell.com for the latest version of the Application Notebook.

This Application notebook will be an essential tool to address your technical issues.

TECHNICAL SUPPORT

POLYINTELL has fully integrated technologies platform with specialized teams in organic chemistry, polymer chemistry, analytical and bioanalytical chemistry who are at your disposal to guide you in your challenges.

At POLYINTELL, we are committed to providing the best technical support possible. Our Technical Support Group is a team of highly qualified M.Sc. and PhD Chemists, who are at your disposal to resolve your problem and to answer to your queries. For technical inquiries, feel free to contact us either by email: tech.support@polyintell.com, or by phone at: +33 (0)2 32 09 32 70.

We are also very thankful to customer's feedback about our products, protocols and customer services by email to: contact@polyintell.com

QUALITY POLICY

To develop a long term and durable partnership with its customers, POLYINTELL ensures the best quality of its products and services.

As an ISO9001:2008 certified company, POLYINTELL has implemented Quality management system requirements to show its commitment to quality, customers, and a willingness to work towards improving efficiency.



In addition, to ensure the best quality of its products, the performance is checked by following several QC tests according to each product's quality control procedure. After passing all these tests, the products receive a certificate of analysis which proved the compliance with the defined criterion.

APPLICATIONS available for MILK analyses

Milk is a very complex matrix which requires well suited sample preparation to monitor the presence of mycotoxins or chemical contaminants in order to prevent adulteration or health risks for the consumer.

This specific application notebook contains a wide range of applications for MILK matrices.

ANALYTES	AFFINIMIP® SPE - ANALYTES	MATRICES	REF	PAGE
Bisphenol A	AFFINIMIP® SPE Bisphenol A	Milk (liquid and powdered infant formula) Water Canned food Red and white wines Beer Human urine	FS106	4-5
Tetracyclines (CTC, OTC, TC)	AFFINIMIP® SPE Tetracyclines	Milk	FS112	6
Aflatoxin M1	AFFINIMIP® SPE Aflatoxin M1	Milk	FS116	COMING SOON!
Chloramphenicol	AFFINIMIP® SPE Chloramphenicol	Milk Honey Bovine Urine Shrimp	FS110	7
Sulfonamides	AttractSPE™ SCX	Milk	SCX-25.S.3.60	7
Melamine	AttractSPE™ SCX	Milk	SCX-25.S.6.150	8
Cyanuric acid	AttractSPE™ SAX	Milk	SAX-25.S.6.150	8

DETERMINATION OF BISPHENOL A IN LIQUID INFANT FORMULA

Regulations for Bisphenol A:

Europe (directive 2011/8/EU) : forbidden in infant feeding bottles

PROTOCOL OF PURIFICATION

Sample preparation

Purification with a 3mL/100mg **AFFINIMIP® SPE Bisphenol A** cartridge

Equilibration

- 5mL Methanol -2% Acetic Acid
- 5mL Acetonitrile
- 5mL Water

Loading

Up to 15mL of infant formula

Washing of interferences

- 10mL Water
- 6mL Water/Acetonitrile (60/40)

Drying 30 seconds

Elution (E)

3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

HPLC Method with Fluorescence detection

Column: Hypersil Gold C18 column 150mm x 4.6mm

Mobile phase: gradient profile

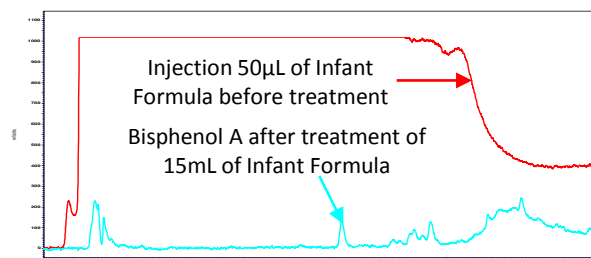
Time (min)	% water	% ACN
0	65	35
2	65	35
12	50	50
20	50	50
20.5	65	35
35	65	35

Flow rate: 1mL/min

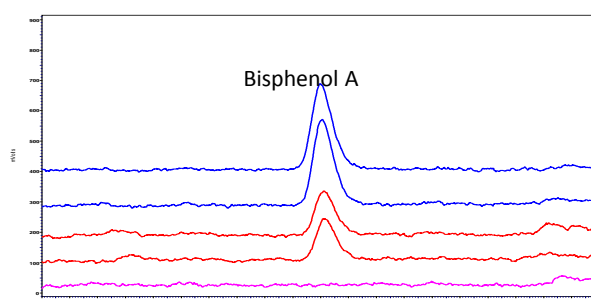
Fluorescence detection: excitation/emission wavelengths: 230 / 315nm

Injection volume: 50µL.

RESULTS



Chromatograms of Infant Formula containing 1µg/L of Bisphenol A before clean-up (Red) and after clean-up (Blue) with **AFFINIMIP® SPE Bisphenol A**.



Chromatograms obtained after clean-up with **AFFINIMIP® SPE Bisphenol A** of 15mL of Infant Formula spiked with Bisphenol A at 2µg/L (tested twice, blue) or at 1µg/L (tested twice, red) or not spiked (pink).

Recovery of Bisphenol A in 15mL of infant formula after **AFFINIMIP® SPE Bisphenol A** clean-up and relative standard deviation calculated from results generated:

- under **repeatability** conditions (n=3, % RSD_r)

C° (µg/L)	Mean (µg/L)	Recoveries %	% RSD _r
1.0	0.9	88.4	1.5
2.0	1.7	85.7	2.7

- under **reproducibility** conditions (% RSD_R).

C° (µg/L)	Mean (µg/L)	Recoveries %	% RSD _R
1.0	0.8	84.4	7.4
2.0	1.7	85.8	5.3

Catalog number:

3mL-100mg sorbent in a PP cartridge

FS106-02 for 25 cartridges

FS106-03 for 50 cartridges

6mL-100mg sorbent in a glass cartridge

FS106-02G for 25 cartridges

FS106-03G for 50 cartridges

[Link to AFFINIMIP® SPE Bisphenol A](#)

DETERMINATION OF BISPHEENOL A IN POWDERED INFANT FORMULA

Regulations for Bisphenol A:

Europe (directive 2011/8/EU) : forbidden in infant feeding bottles

PROTOCOL OF PURIFICATION

Sample preparation

4.4g powdered infant milk was reconstituted in 30 mL of water and warmed up at ~ 50°C during 20 seconds using microwaves. Then 20 mL of acetonitrile were added to 20 mL of warm milk and centrifuged at 4000 rpm during 10 minutes. The supernatant was collected and filtered on filter paper (4-7µm). This extract was diluted 1:1 with water to form the loading solution.

Purification with a 3mL/100mg AFFINIMIP® SPE Bisphenol A cartridge

Equilibration

- 5mL Methanol -2% Acetic Acid
- 5mL Acetonitrile
- 5mL Water

Loading

Up to 40mL of infant formula

Washing of interferences

- 10mL Water
- 6mL Water/Acetonitrile (60/40)

Drying 30 seconds

Elution (E)

3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

HPLC Method with Fluorescence detection

Column: Hypersil Gold C18 column 150mm x 4.6mm

Mobile phase: gradient profile

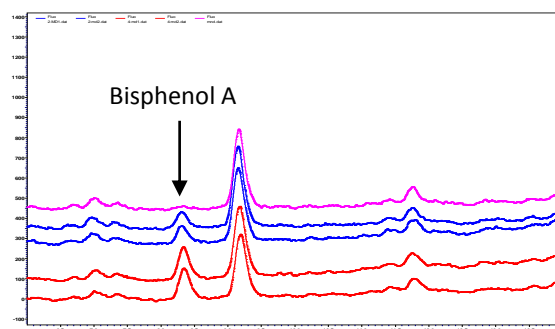
Time (min)	% water	% ACN
0	65	35
2	65	35
12	50	50
20	50	50
20.5	65	35
35	65	35

Flow rate: 1mL/min

Fluorescence detection: excitation/emission wavelengths: 230 / 315nm

Injection volume: 50µL.

RESULTS



Chromatograms obtained after clean-up with **AFFINIMIP® SPE Bisphenol A** of equivalent at 10mL of Infant Formula spiked with Bisphenol A at 4.3µg/L (tested twice, red) or at 2.1µg/L (tested twice, blue) or not spiked (pink).

Recovery of Bisphenol A spiked at different concentrations after **3mL/100mg AFFINIMIP® SPE Bisphenol A** clean-up of 40mL of loading solution (equivalent to 10mL of reconstituted Infant milk) and relative standard deviation calculated from results generated under **repeatability conditions**

Concentration of BPA in reconstituted milk (µg/L)	Mean concentration (µg/L)	Recoveries %	RSD _r %
2.1	2.3 (n=5)	108	8.7
4.3	4.0 (n=4)	95	3.7

Catalog number:

3mL-100mg sorbent in a PP cartridge

FS106-02 for 25 cartridges

FS106-03 for 50 cartridges

6mL-100mg sorbent in a PP cartridge

FS106-02B for 25 cartridges

FS106-03B for 50 cartridges

6mL-100mg sorbent in a glass cartridge

FS106-02G for 25 cartridges

FS106-03G for 50 cartridges

[Link to AFFINIMIP® SPE Bisphenol A](#)

DETERMINATION OF TETRACYCLINES IN MILK

Regulations for Tetracyclines:

Codex Alimentarius: 100µg/L sheep or cattle milk (26th CAC session, 2003)

PROTOCOL OF PURIFICATION

Sample preparation

1.5mL of Milk were mixed with 6mL of EDTA/Mc Ilvaine's Buffer and the mixture was centrifuged at 4000rpm for 10 minutes at a temperature below 15°C. The supernatant was collected and 750µL of a 1N NaOH solution was added and the solution was then adjusted to pH 10 with a NaOH solution (this mixture was the loading solution).

Purification with a 1mL/10mg AFFINIMIP® SPE Tetracyclines cartridge

Equilibration

- 1mL Acetonitrile
- 1mL Water

Loading

Load the loading solution (7.5mL)

Washing of interferences

- 2mL Water/Acetonitrile (60/40)

Drying 3 minutes

Elution (E)

2mL Methanol with 2% Formic acid

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

HPLC Method with UV detection

Column: Hypersil Gold C18 column 150mm x 2.1mm, 3µm

Mobile phase: gradient profile

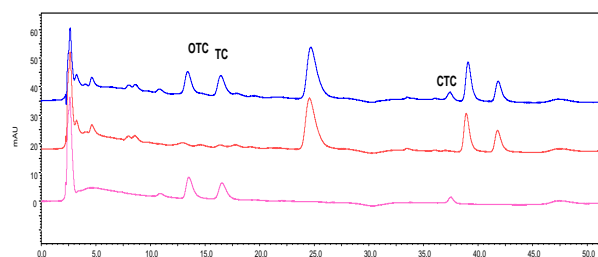
Time (min)	% 10mM Oxalic Acid Water	% 10mM Oxalic Acid ACN	% MeOH
0	90	5	5
20	90	5	5
21	80	10	10
40	80	10	10
41	90	5	5

Flow rate: 0.2mL/min

UV detection: 355nm

Injection volume: 100µL.

RESULTS



UV Chromatograms (355nm) obtained after clean-up with AFFINIMIP® SPE Tetracyclines of 1.5mL of Milk spiked with Tetracyclines at 50µg/L (blue) or not spiked (red) or of 1.5mL of water spiked with Tetracyclines at 50µg/L (pink)

Recovery of Tetracyclines spiked at 50µg/L after AFFINIMIP® SPE Tetracyclines clean-up of 1.5mL of Milk and relative standard deviation calculated from results generated under **repeatability conditions**.

Tetracyclines	C° (µg/L)	Mean (µg/L)	Recoveries %	% RSD _r (n=3)
Tetracycline	50.8	48.9	96.3	5.1
Oxytetracycline	50.7	47.3	93.3	2.7
Chlortetracycline	51.0	34.8	70	13.8

Recovery of Tetracyclines spiked at 50µg/L after AFFINIMIP® SPE Tetracyclines clean-up of 1.5mL of Milk and relative standard deviation calculated from results generated under **reproducibility conditions**.

Tetracyclines	C° (µg/L)	Mean (µg/L)	Recoveries %	% RSD _r (n=3)
Tetracycline	50	44.3	88.7	9.5
Oxytetracycline	50	53.7	107.3	10.7
Chlortetracycline	50	40.7	81.3	9.6

Catalog number:

1mL-10mg sorbent

FS112-02A for 25 cartridges 1mL

FS112-03A for 50 cartridges 1mL

kit of 12 reservoirs 15ml and adapters for use with 1, 3 & 6 mL columns

ACC-AR2

[Link to AFFINIMIP® SPE Tetracyclines](#)

DETERMINATION OF CHLORAMPHENICOL IN MILK

PROTOCOL OF PURIFICATION

Sample preparation

Whole milk is centrifuge during 15min at 5000 rpm. Loading solution is the intermediate layer or is the skimmed milk.

Purification with a 1mL/50mg AFFINIMIP® SPE Chloramphenicol cartridge

Equilibration

- 2mL Acetonitrile
- 2mL Water

Loading

1mL of loading solution for 15µg/kg (or 10mL for 0.3µg/Kg)

Washing of interferences (W1)

- 1mL Water
- 1mL (Water - 0.5% AA)/ACN (95/5)
- 2mL of Ammonia (1%) in Water
- 2mL (Water-1% Ammonia)/ACN (80/20)

Drying 1 min

Washing of interferences (W2)

0.25mL Diethyl ether

Elution (E)

2mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

Regulations for Chloramphenicol:

Europe (2003/181/EC): prohibited with a minimum required performance limits of 0.3µg/Kg

Catalog number:

1mL-50mg sorbent

FS110-02A for 25 cartridges
1mL

FS110-03A for 50 cartridges
1mL

[Link to AFFINIMIP® SPE Chloramphenicol](#)

AttractSPE™ SCX

DETERMINATION OF SULFONAMIDES IN MILK

PROTOCOL OF PURIFICATION

Purification with a 3mL/60mg AttractSPE™ SCX cartridge

Equilibration

- 2mL Methanol
- 2mL Water

Loading

5mL Milk

Washing of interferences

- 2mL Methanol/Water (5/95)
- 1mL 0.5M HCl
- 2mL Methanol/Water (20/80)

Elution (E)

2.5mL Ammonium bicarbonate/Methanol (10/90)

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

Analyse LC/MS-MS

Regulations for Sulfonamides:
Sulfadimethoxine 0.01ppm in milk (U.S. FDA 21 CFR 556.640)
Sulfaethoxyypyridazine 0 in milk (U.S. FDA 21 CFR 556.650)

Catalog number:

6mL-150mg sorbent

SCX-25.S.3.60 for 25 cartridges
SCX-50.S.3.60 for 50 cartridges

[Link to AttractSPE™ SCX](#)

DETERMINATION OF MELAMINE IN MILK

PROTOCOL OF PURIFICATION

Sample preparation

Add 4mL water to 5g liquid infant formula or 1g dry infant formula. Shake during 10-20min with 20mL 50/50 ACN/Water and centrifuge for 10minutes at 3400 rpm.

The supernatant is the loading solution.

Purification with a 6mL/150mg AttractSPE™ SCX cartridge

Equilibration

- 5mL 0.1M NaOH in Acetonitrile
- 5mL 0.1M HCl in Acetonitrile
- 5mL Acetonitrile
- 5mL 4% Formic acid in Water

Loading

3mL 4% Formic acid in Water

2mL of loading solution

Washing of interferents

- 5mL Acetonitrile
- 5mL 0.2% diethylamine in Acetonitrile

Elution (E)

4mL 2% diethylamine in Acetonitrile

The elution fraction was filtered and then evaporated under nitrogen and dissolved in the mobile phase before HPLC analysis.

Analyse LC-MS/MS

Regulations for Melamine:
Codex alimentarius 35th CAC session (july 2012):
Maximum limit 0.15mg/kg for liquid infant milk

Catalog number:**6mL-150mg sorbent**

SCX-25.S.6.150 for 25 cartridges

SCX-50.S.6.150 for 50 cartridges

[Link to AttractSPE™ SCX](#)

DETERMINATION OF CYANURIC ACID IN MILK

PROTOCOL OF PURIFICATION

Sample preparation

Add 4mL water to 5g liquid infant formula or 1g dry infant formula. Shake during 10-20min with 20mL 50/50 ACN/Water and centrifuge for 10minutes at 3400 rpm.

The supernatant is the loading solution

Purification with a 6mL/150mg AttractSPE™ SAX cartridge

Equilibration

- 5mL 0.1M HCl in Acetonitrile
- 5mL 0.1M NaOH in Acetonitrile
- 5mL Acetonitrile
- 5mL 5% NH₄OH in Water

Loading

3mL 5% NH₄OH in Water

2mL of loading solution

Washing of interferents

- 5mL Acetonitrile

Elution (E)

2mL 4% Formic acid in Acetonitrile

The elution fraction was filtered and then evaporated under nitrogen and dissolved in the mobile phase before HPLC analysis.

Analyse LC-MS/MS

Catalog number:**6mL-150mg sorbent**

SAX-25.S.6.150 for 25 cartridges

SAX-50.S.6.150 for 50 cartridges

[Link to AttractSPE™ SAX](#)

SPE ACCESSORIES

POLYINTELL proposes the complete set of equipments required to carry out SPE experiments:

Manifold



ACC-MAN1 Like all chromatography techniques, Use of SPE cartridges needs a precise control of flow rate for maintaining reproducible extractions. Solid Phase extraction Vacuum Manifold allows you to control the flow and to process up to 12 (12-port version) or 24 (24-port version) AFFINIMIP® SPE samples simultaneously, to gain significantly time during sample preparation steps.

SPE Adapter & Reservoir kit



ACC-AR1 Tube adapters serve to pile one SPE tube on top of another to provide different selectivities. A larger empty syringe barrel can be stacked on top of a smaller SPE tube to act as a larger load reservoir. Or, they can serve as an adapter for positive pressure methods (e.g. from a syringe or air/ N2 line).

Mini-Vap



ACC-VAP1 The 6-Port Mini-Vap concentrator/evaporator processes six vials at one time. The Mini-Vap includes a needle valve for fine metering of air or nitrogen drying gas.

Mini PUMP

ACC-PUMP Mini diaphragm vacuum pump for solid phase extraction experiments
 Portable
 ➤ 5.5L/min
 ➤ ~120 torr vacuum
 ➤ Oil-free
 ➤ portable

Vacuum pump trap

ACC-TRAP SPE Vacuum pump trap kit
 Installed between the manifold and the vacuum pump, it collects all liquids that are aspirated preventing contamination of the vacuum pump with a capacity of 1L.

SPE ACCESSORIES – Product list

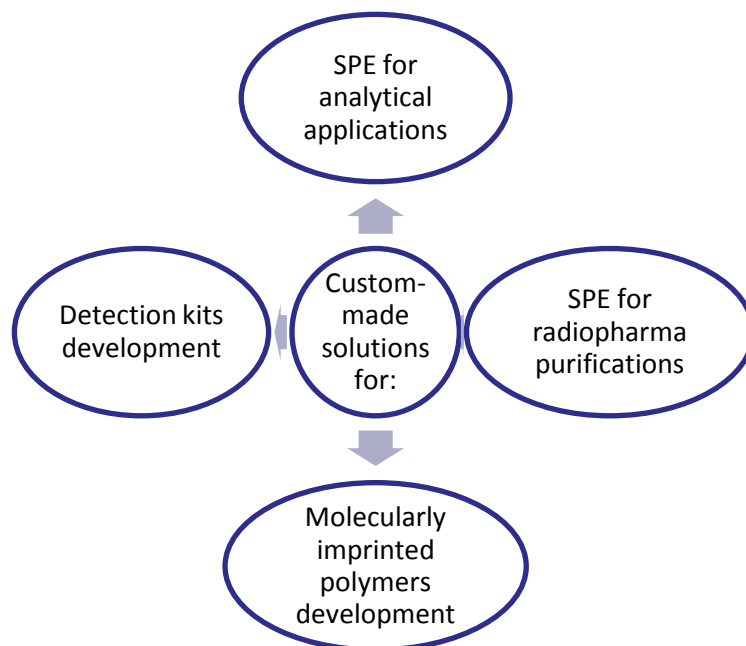
SPE Accessories	Designation	Definition	Reference
Manifold	SPE Vacuum Manifold	12-port model	ACC-MAN1
SPE Adapter & Reservoir kit	SPE Adapter & Reservoir kit	Kit of 12 reservoirs 60ml and adapters for use with 1,3 & 6 mL cartridges	ACC-AR1
Mini-Vap	Mini Evaporator/Concentrator	6 port Mini-Vap Evaporator/Concentrator for use with 1 to 250mL containers	ACC-VAP1
Mini PUMP	Mini vacuum pump	Laboport diaphragm vacuum mini pump, 5.5L/min	ACC-PUMP
Vacuum pump trap	SPE Vacuum pump trap kit	1L trap kit	ACC-TRAP

CUSTOM-MADE PRODUCTS & SERVICES

POLYINTELL offers full services for the design and synthesis of polymers complying with your specifications.

With fully integrated technology platforms in organic, polymer and analytical chemistry, POLYINTELL has been partner in more than 40 projects and helps its customers by innovative solutions for their complex challenges.

POLYINTELL has developed a library of monomers giving a family of selective stationary phases based on its proprietary technology, which have shown a strong potential for the extraction, purification and detection of various compounds.



Did you know ?

Molecularly imprinted polymer (MIP) is a polymer with a «memory» of the shape and the functional groups of a target molecule. This material is designed in order to recognize selectively this molecule, even in the presence of compounds with structure and functionality similar to those of this molecule. High molecular recognition properties can be achieved with these MIPs for a variety of molecules and can be useful for its extraction, purification and detection.

Why trusting POLYINTELL?

POLYINTELL supplies

- The most comprehensive bank of sorbents
 - From Silica to Polymers
 - From conventional to very selective
 - Reversed-phase, ionic exchange, MIPs etc...
- Our Expertise on MIP, sample preparation, SPE protocol and detection kit development
- Quick and efficient development
- Reactivity

PROJECT DEVELOPMENT

No matter if we run a short term project (2-3 days) or long term project (4 to 6 months) we always follow a well-established procedure. The following scheme describes an example of a procedure for the development of a custom-made product based on our customer's requirements.

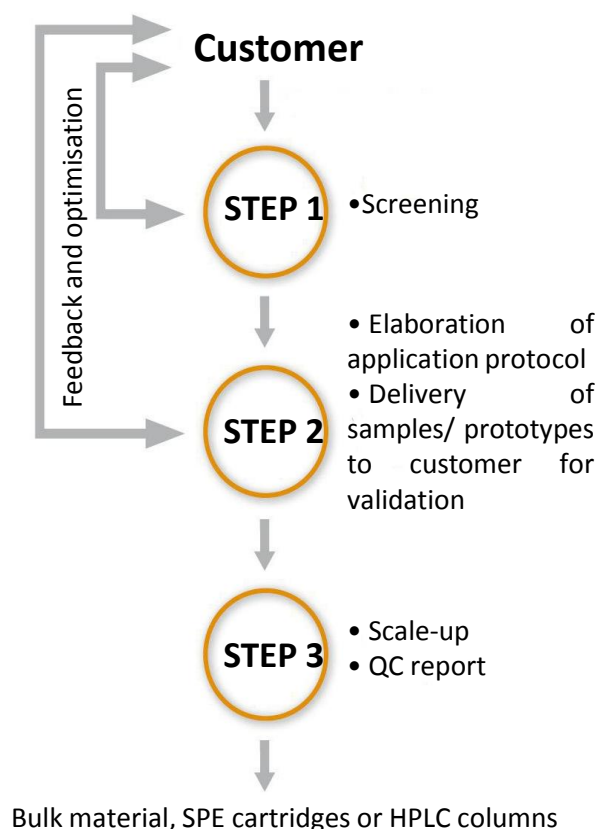
After signing of Confidential Disclosure Agreements (CDA), key data are exchanged. A quotation based on our experience in separation science including a process sheet is developed. During the whole procedure, a permanent feedback is established with you.

Step 1: We offer you a screening of our library which consists of several hundreds POLYINTELL phases to find the suitable one for your separation problem. The knowledge of the structure of the target substance, its functional groups and the solubility data give us valuable hints for the choice of the screening phases.

Step 2: For the selected phase, a protocol is implemented for your application. Then samples and / or prototypes are delivered to you for testing, evaluation and validation.

Step 3: When the selected phase suits your application and has been validated, a scale-up is planned. A QC report is delivered with the product. The format of the product is correlated to your application and can be bulk material, SPE cartridges, HPLC columns etc...

Procedure for custom-made polymer phase



If you need the development of new polymer for your application, please send us an email to contact@polyintell.com or describe your needs using <http://www.polyintell.com/services/request-service-online/>. You can describe your application and our scientists will shortly evaluate your queries before contacting you as soon as possible.

About POLYINTELL

Founded in 2004, **POLYINTELL is a worldwide leader in the design and the development of intelligent polymers.** Pioneer in Molecularly Imprinted Polymers (MIP) and expert in polymer chemistry, POLYINTELL develops, manufactures and markets innovative solutions for sample preparation, selective extraction, purification and detection of specific target analytes.

Our mission is to develop and market innovative products of high value to customers by a practical contribution to their work. **By offering you a most comprehensive range of solid phase extraction products, AFFINIMIP® SPE products based on molecularly imprinted polymers, AttractSPE™ a range of polymeric phases and Silica based products, associated reagents and small equipment, the analytical chemists can find any solution for sample preparation, selective extraction, sample clean-up and purification needs in various sectors: food safety, pharmaceutical industry, clinical diagnosis, environment and doping. we are your partner of your choice.**

Furthermore, by exploiting our library of innovative polymers and our know-how in chromatography and solid phase extraction, we have a strong capacity to adapt these polymers to meet any specific requirements and to solve unsatisfied purification and extraction needs.

ORDERING INFORMATION

For any order, please, choose one of the following ways:

❖ By mail:

POLYINTELL
Pharma Parc II
Voie de l'innovation
27100 Val de Reuil, FRANCE

❖ By phone: +33 (0)2 32 09 32 70

❖ By fax: +33 (0)2 32 59 61 01

❖ By email: contact@polyintell.com

❖ On-line by **E-commerce**: To save your time and a quicker delivery, order via our web site www.polyintell.com



Information about new products or new matrices:

❖ **Newsletters**

Stay up-to-date with the news on POLYINTELL's latest innovations by subscribing free of charge to our newsletter, delivering new information straight to your inbox.



<http://www.twitter.com/POLYINTELL>

