Agela Technologies

Bonna-Agela

BA/BE Analysis Product Guide

Official Website

Best Value
Guaranteed Product Quality
Innovation to Benefit Customers

BA/BE ANALYSIS

LC-MS/MS has become an industrial standardized technique for Bioanalysis (BA) and Bioequivalence testing (BE) including clinical trial from phase I to phase III of new drug development, DMPK, analysis of biomarkers, forensic applications and generic drug testing. However, the complex of biological matrices has to be treated carefully to remove the interferences such as proteins, phospholipids and salts from the samples prior to LC-MS/MS analysis since those interferences may result in wrong conclusions.

Bonna-Agela, superb manufacture in sample preparation, is an excellent partner of you in biological sample treatment. We offer you total solutions from various products to method development. Do you have any problems in bio-sample preparation? Just contact us and our local sales representatives will help you to overcome the problems.

96 Well Plate Series

Cleanert® PPT Protein Precipitation Plates

Cleanert® PPT protein precipitation plates is designed for high throughput biological sample preparation manually and automatically. 96-well format for “in-well” protein precipitation omitting centrifuge and liquid transfer steps in traditional protein precipitation procedure.

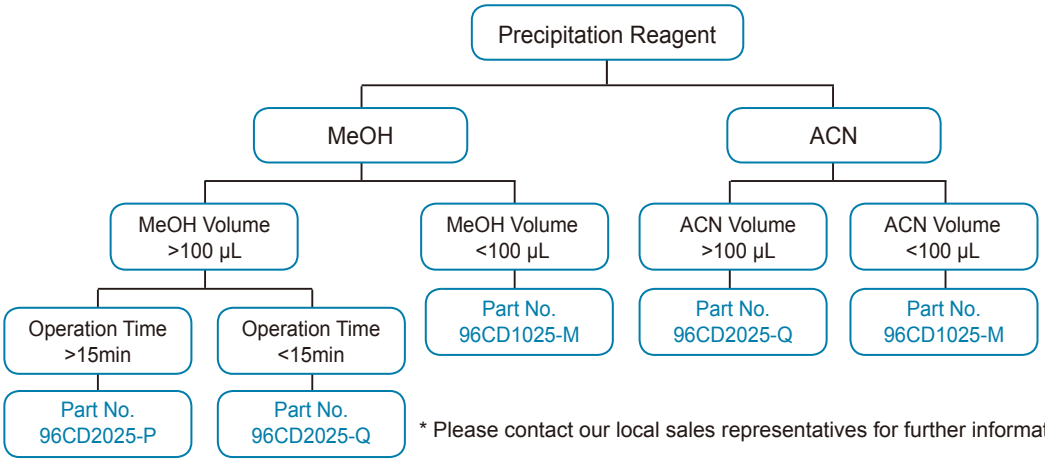
Cleanert® PPT protein precipitation plates provide optimum performance for proteins removal from biological samples effectively. Clean fractions and excellent recoveries can be achieved. It has been utilized in many applications such as dry blood spot (DBS) analysis of amino acids and acylcarnitines.

Cleanert® PPT Plate & Collection Plate

Features

- Rapid and high throughput protein precipitation for bio-samples such as plasma, serum.
- 96-well parallel processing and automation.
- Suitable for both acetonitrile and methanol.
- Applicable for positive pressure or vacuum performance.
- Easy to use for various application.

Selection Guide of Cleanert® PPT Plates

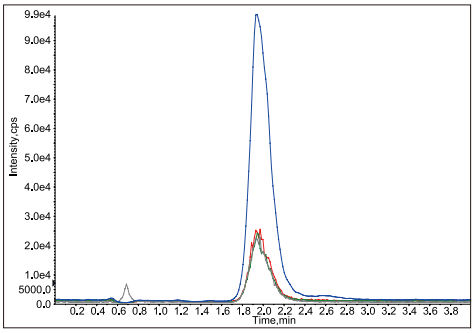


* Please contact our local sales representatives for further information.

Extraction of Pregabalin From Plasma

Cleanert® PPT Plates

- 50 µL of plasma diluted 1:1 with of 50% Methanol.
- Add 500 µL Methanol to each well. 100 µL of diluted plasma was added, then shake the plate by vortexer.
- Diluted by 400 µL 0.1% formic acid in Water.
- The recovery of pregabalin is from 93.8% to 100.6% with RSD less than 3.5% at a concentration of 100 ng/mL.



Extraction of amino acids and acylcarnitines from dry blood sports

Cleanert® PPT Plates

Punch the dried blood spot into Cleanert® PPT plate. Add extraction solvent into the plate and vortex for 20min. Employ Cleanert® M96 positive pressure SPE device to filter the sample. Concentrate the filtrate to dryness with Cleanert® V96 nitrogen evaporator. After derivatization and reconstitution the sample was injected in to LC-MS/MS for further analysis.

Cleanert® SLE Solid Supported Liquid Extraction Plates

Solid supported liquid extraction (SLE) can be considered as a micro liquid-liquid extraction with the same principles of liquid-liquid extraction but performed on high quality modified diatomaceous earth. Because of the excellent performance for removing phospholipids, it has been widely used in bio-sample preparation prior to LC-MS/MS analysis such as extraction of 25-OH vitamin D_2/D_3 in plasma.

In SLE, the dry modified diatomaceous earth is packed into a cartridges, or 96-well plate. Load-Wait-Elute, only three steps for SLE: the aqueous-based sample is loaded on to the dry sorbent slowly to saturate diatomaceous earth and wait to form a thin film of aqueous phase on the surface of the porous on the sorbent. A small volume of immiscible organic extraction solvent is added to the top of the SLE device and allowed to percolate by gravity through the supported aqueous phase. The fractions are collected for further treatment or analysis. Cleanert SLE plates have been utilized for the removal of phospholipids and proteins from biosamples.

Cleanert® SLE plates
Left: standard SLE plate;
Right: Assemble SLE plate, up to 600 µL biosample can be load onto the plate.

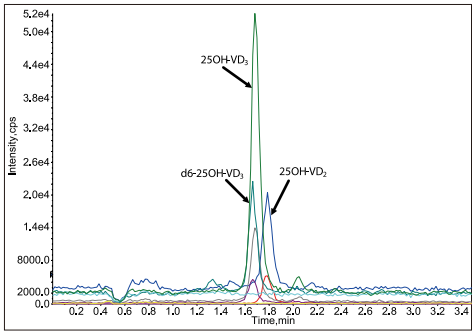
Features

- No vigorous shaking so that emulsions cannot be formed.
- Intimate contact between the aqueous sample and organic phases allows very efficient partitioning. The recoveries and reproducibility of target compounds can be better than LLE.
- SLE process is more technique independent than LLE obtains more reproducible results.
- Reducing the usage of the organic solvent significantly.
- Load-Wait-Elute: Three steps performance.
- Easy to automate with exiting automatic device in Labs.

Extraction of 25OH-VD_2/VD_3 From Plasma

Cleanert® SLE Plate (200 μL/well)

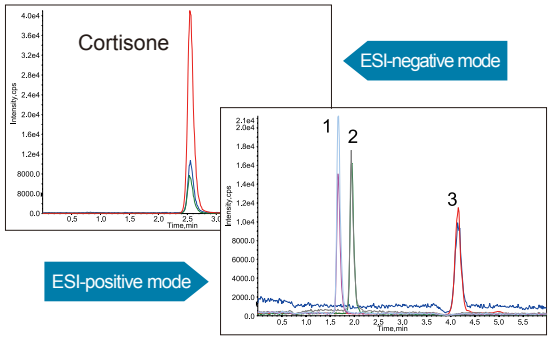
100 μL of plasma was diluted with 100 μL of 50mM NaOH and added to each well of the SLE plate. Wait for 5 min. Elute with 4×500 μL of isooctane under Cleanert® M96 positive pressure device. Collected the elution, concentrated with a Cleanert® V96 Evaporator to dryness and reconstituted with 200 μL of mobile phase for LC-MS/MS analysis.



Extraction of Steroid Hormones From Serum

Cleanert® SLE Plate (200 μL/well)

Add 10 μL methanol to 200 μL serum. Vortex for 30s. Load the sample onto Cleanert® SLE plate and stand for 5 min. Dispense 2×600 μL MTBE (2 aliquots) to elute by gravity. Concentrated elution and reconstitute by mobile phase. The target compounds contain progesterone, testosterone, boldenone and cortisone. The recovery data range from 86% to 107% at concentration of 5 ng/mL.



1 Boldenone; 2 Testosteron; 3 Progesterone

Solid Phase Extraction Plates

Series Plates*	Functional Groups	Target Compounds
Cleanert® PEP	Balanced hydrophobic & hydrophilic property	Nonpolar to polar compounds
Cleanert® PAX	Strong anion exchanger	Week acidic compounds
Cleanert® PCX	Strong cation exchanger	Week basic compounds
Cleanert® PWAX	Week anion exchanger	Strong acidic compounds
Cleanert® PWCX	Week cation exchanger	Strong basic compounds

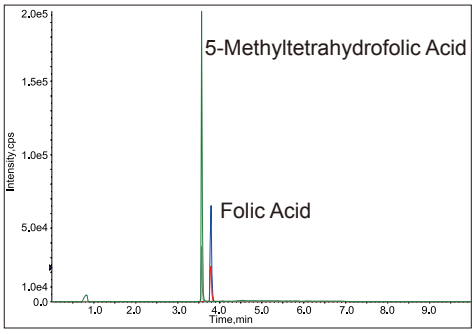
* Average particle diameter: 40 - 60 μm, 30 μm; Average pore size: 70 Å; Specific surface area: 600-800 m^2/g.

Another SPE plate is Cleanert® MAS-M, which is designed for the enrichment of phospholipids from plasma. For further information please contact our local sales representatives.

Folic Acid and 5-Methyltetrahydrofolic Acid in Serum

Cleanert® PAX Plate (30 mg/well)

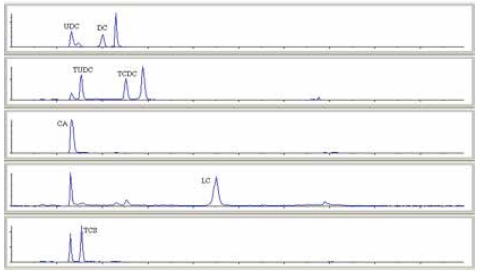
Serum sample was mixed with ascorbic acid and 2-mercaptoethanol (antioxidant). 400 μL of serum was 1:1 diluted with antioxidant and load on to Cleanert® PAX plate which was preconditioned with MeOH, H_2O & antioxidant solution. After wash with H_2O contained 2% of formic acid, followed by MeOH, The plate was eluted with 500 μL MeOH containing 2% formic acid. After drying and reconstituting the sample was analyzed by LC-MS/MS.



Extraction of Bile Acids in Serum

Cleanert® MAS-MAW Mixed Mode SPE Plate

100 μL of serum diluted 1:1 with 1% formic acid.
The plate was conditioned with 2 mL of acetonitrile and 2 mL of 3% formic acid. 200 μL of diluted serum was load on to each well and wash the plate with MeOH/H_2O(1:1). The plate was eluted with 2 mL of MeOH and 2 mL of triethylamine/H_2O/MeOH(2:10:88) subsequently. LC-MS/MS analysis shows the recoveries of 8 bile acids range from 95.2% to 116.4% with RSD less than 7.5% at a concentration level of 50 ng/mL.



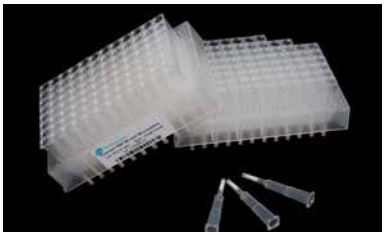
As a total solution supplier, Bonna-Agela also offers you state-of-art equipments for high throughput sample preparation in both clinical and DMPK applications.

Cleanert® PEP Plate Series

Cleanert® PEP (Polar Enhanced Polymer) plates are 96-well format packed with polystyrene/divinylbenzene (PS) materials with various functional. The plates are suitable for extraction of target compounds from bio matrix with pH range from 1 to 14. Furthermore, the capacity of polymer packing material is 3 to 10 times then traditional bonded silica. Cleanert® PEP plate series have a balanced hydrophilic and hydrophobic property, which can be used to extract a variety of polar and non-polar compounds such as screening of antibiotics.

Cleanert® Micro Plate

Cleanert® Micro SPE Plate is designed for extraction of target compounds from small volume of bio-samples. The plates are integrated with individual cartridges packed with polymer materials in the amount of 5 mg or 10 mg in each well that allows low volume elution that may omit further evaporation and reconstitution steps. The Cleanert® Micro SPE plates are available with the sorbents of PEP, PCX, PAX, PWCX, and PWAX. Cleanert® PEP neo is packed with modified PEP material, which was first introduced in 2010. Cleanert® neo omits condition and equilibration steps for fast biosample preparation.



Cleanert® Micro Plate

Features

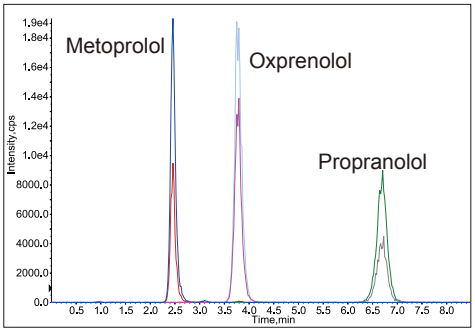
- Small volume solvent for elution;
- Various cartridges can be assembled on the same plate for special application;
- Ideal cleanup and extraction for small sample.

Determination of β-blockers in Plasma

Cleanert® PEP 96 Well Microplate (5 mg/mL)

Condition: 200 μL Methanol, 200 μL Water;
Load: 200 μL plasma;
Wash: 200 μL 5% Methanol in Water;
Elution: 50 μL 2% Formic acid in Acetonitrile: Isopropyl alcohol(4:6);
Reconstitute: Add 150 μL Water into elution.
Result:

Compounds	Concentration (ng/mL)	Recoveries Range (n=6)	RSD (n=6)
Metoprolol	50	91.6% ~ 103.1%	< 4.0%
Oxprenolol	50	99.9% ~ 109.9%	< 4.0%
Propranolol	50	98.1% ~ 107.7%	< 4.0%



Instrument and Apparatus

Cleanert® M96 Positive Pressure Device

Cleanert® M96 is a efficient automatic device with 96-well format special designed for R&D laboratories in pharmaceutical industry and biosample analysis laboratories. The device can handle 96-well plates such as solid phase extraction (SPE) plates, solid supported liquid extraction (SLE) plates, protein precipitation (PPT) plates as well as filtration plates from various brands.

Since the positive pressure is applied to drive liquid pass through the plates smoothly. It eliminates the differential of the flow-rate between each well that encountered frequently on vacuum SPE devices. Therefore, Cleanert® M96 provides highly uniform performance which improves the reproducibility of the recoveries. Cleanert® M96 allows for gas pressure up to 0.4 MPa, supplying greater motive force for viscous sample flow.

Features

- Highly uniform flow for each well on the plate.
- Improvement of reproducibility, accuracy and performance efficiency.
- Parallel processing up to 96 samples for increased sample throughput.
- Dual pressure regulators allow the user to set different pressure for extraction and plate drying.
- High pressure (up to 0.4 MPa) supplying greater motive force for viscous samples.
- Adapt to different height of 96-well.

Cleanert® M96 positive pressure device

Cleanert® M48 Positive Pressure Device

Cleanert® M48 is a positive pressure device specially designed for high-throughput sample preparation in research and analytical laboratories. It can simultaneously process up to 48 SPE cartridges by applying evenly gas pressure on individual cartridges to press liquid through SPE cartridges smoothly. It reduces largely the variation on the flow-rate between each cartridge, compared to vacuum SPE devices, thus improving sample-to-sample consistency. For some viscous sample, vacuum may not provide enough flow. However, Cleanert® M48 can provide up to 0.4 MPa positive pressure to help the sample pass through SPE cartridges smoothly.

Features

- Low variation on the flow-rates between the cartridges; improved extraction consistency.
- High-throughput by processing up to 48 samples simultaneously.
- Good flexibility: can control on/off for gas by each 12 to save gas; can run any number of samples between 1-48.
- Dual-pressure control: separated coarse and fine tuning to get precise pressure control and quick tuning.
- Easy set-up and operation: ready to run; needs only purged gas source.
- The SPE stage indicator: manually switch the indicator position to remember the current stage of your operation.
- The whole SPE process can be completed in an inert (N_2) environment.

Cleanert® M48 positive pressure device with patented technology

Cleanert® V96 N_2 Evaporator

Bonna-Agela Technologies Extends Innovation in Sample Preparation Instruments Range By Introducing Cleanert® V96 Nitrogen Evaporator with unique Gas Heating System.

Cleanert® V96 N_2 Evaporator purpose is to middle out the sample clean up process between manual and high scale liquid handling system. This is ideal product combination for the customers looking out for semi-automation sample clean up procedure for their lab.

Features

- Gas and electric system are well separated in the Instrument to provide safety to the user during the operation;
- Unique design directs N_2 gas evenly and directly into each well of the plate;
- To avoid waste of nitrogen gas it is allowed to entered only in heated mode;
- Interchangeable Needle Heads helps in cleaning the needle comfortably;
- N_2 Evaporator is advised to be operated in fume hood cupboard;
- Two individual evaporating head for two 96 well plates used simultaneously helps in high throughput analysis.

Cleanert® V96 vaporator for 96-well collection plate

*** For more specification product information, please contact our local sales representative and customer service specialist.**

HPLC columns for BA/BE Analysis

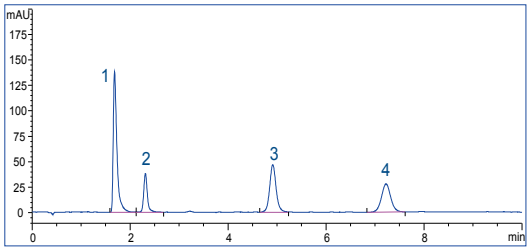
Bonshell C18 Plus

Bonshell (Core-shell) is a solid 1.7 μm core covered with 0.5 μm of porous layer. The total particle size is 2.7 μm. Because of the core supporting structure, Bonshell could be operated under high pressure. The particles result in less band broadening and thus deliver extremely high efficiencies. It has similar performance as 1.8 μm HPLC columns with only 1/2 of pressure. Bonshell is the best choice for the HPLC & UHPLC application.

Bonshell C18 Plus columns are designed for enhance peak shape for various sample matrix, especially useful for the separation of acidic, basic, and other neutral compounds by reverse-phase liquid chromatography.

Silica Pure: >99.999%; Particle Size: 2.7 μm; Pore Size: 70 Å; Specific Surface Area: 150 m^2/g;
C%: 8%; pH Range: 1.5-9.0.

Column: Bonshell C18 Plus, 2.7 μm, 2.1×50 mm
Sample: (1) Terbinafine hydrochloride; (2) Ibuprofen;
(3) Lovastatin; (4) Simvastatin;
Mobile Phase: ACN:H_2O:0.1mol/LCH_5NO_2(pH 3.75)
=50:37.5:12.5(v/v/v)
Detector: UV233 nm Flow Rate: 0.3 mL/min
Injection:1 μL Temperature: 30°C



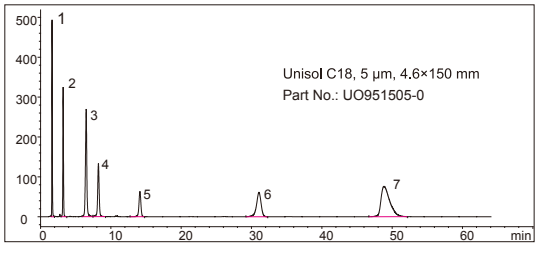
Unisol C18

The columns have good peak shape with basic compounds and 100% aqueous compatibility. Unisol C18 has excellent separation performance with a wide range of properties from hydrophilic to hydrophobic, especially for polar compounds. It shows robust and reproducible performance for LC-MS/MS.

Silica pure: > 99.999%; Particle Size: 3 μm; Pore Size: 100 Å; Specific Surface Area: 410 m^2/g;
C%: 17%; pH: 1.5-9.0

Good Peak Shape for Acid, Basic and Neutral Compounds

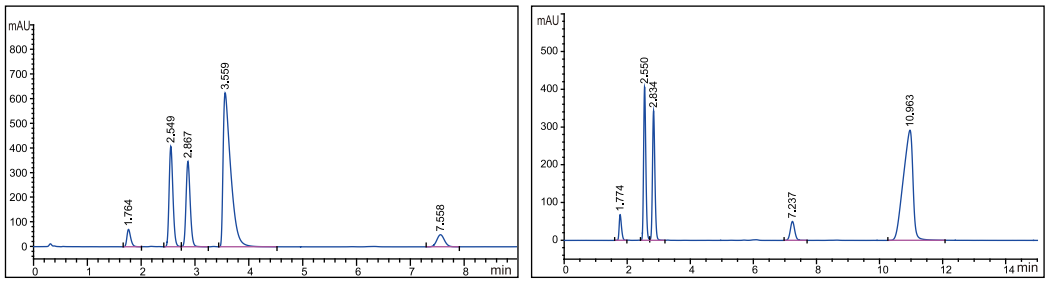
Mobile Phase: 35% 20 mM KH_2PO_4 pH=7.0,
65% Methanol
Temperature: 23°C; Flow Rate: 1 mL/min
Detector: UV 254 nm
Sample:
(1) Uracil ; (2) Propranolol hydrochloridel ; (3) Butyl Phthalatel ;
(4) Dimethyl Phthalatel ; (5) Naphthalenel ; (6) Acenaphthenel ;
(7) Amitriptyline



Unisol C18(2)

Unisol C18(2) columns were made by unique bonding process and polar end-capped. It has good peak shape for basic compounds as well as 100% aqueous compatibility. The columns have excellent separation performance for samples with a wide range of properties from hydrophilic to hydrophobic, especially for polar compounds.

Silica pure: >99.999%; Particle Size: 3 μm; Pore Size: 110 Å; Specific Surface Area: 340 m^2/g;
C%: 14%; pH Range: 1.5-9.0.



When modified the pH from 2.6 to 5.8,
the retention time of Amoxicillin changed from 3.559 min to 10.963 min.

*** Please contact our local sales representative and customer service specialist to obtain more information about HPLC column.**

Accessories and Supplies

- Clarinert™ Syringe Filters
- Vials, Caps and Septa
- 96 Well Collection Plate
- 96 Well Silica Mat
- Disposable Syringe

- DA Series GC Columns
- Standard Substance
- Other Consumables
(Injection Needle, Disposable Gloves ,etc.)

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