

ChiralTek Universal HLE Cartridges Product Manual and Application Notes

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Introduction

The Hydrophilic and Lipophilic Extraction (HLE) cartridges are a new type of universal solid phase extraction (SPE) cartridges manufactured by ChiralTek. The HLE particle was prepared through a specially-designed procedure by bonding multiple chemical function groups, including Diol, C18 (ODS), vinylpyrrolidone and divinylbenzene (HLB) etc., onto a special type of porous composite material. By using a ChiralTek proprietary packing approach, the HLE particles were then packed into kinds of polypropylene tubes with different sizes to produce different model of HLE cartridge products. The following Figure (A) shows a typical photo of model HLE-80 cartridges (specification: 80 mg/3mL, 80-120Å, 50 PCs/pkg).



Figure(A). Typical photo for HLE-80 cartridges

The HLE-80 model cartridges in the Figure (A) are suitable for extraction of small volume samples (0.5 – 50mL).

When worked with automatic SPE instruments, all the HLE cartridges can be used to easily achieve high efficient sample treatments for different types of complicated samples. Another model HLE-300 cartridges (as shown in Figure (B)) are suitable for median volume



Figure(B). Typical photo for HLE-300 cartridges

samples (50 – 500mL, specification: 300 mg/6mL, 80-120Å, 20 PCs/pkg). The HLE-1000 model cartridges (specification: 1000 mg/6mL, 80-120Å, 20 PCs/pkg) are designed for large volume samples (500mL and above).

Unique Characteristics

Since this novel HLE cartridges contains multiple chemical function groups, they can be used under traditional normal and reverse phase elution conditions and modern gradient elution conditions to extract types of hydrophilic and lipophilic compounds from complicated samples. The HLE cartridges can also be used as direct replacement of Waters HLB cartridges due to functioning of vinylpyrrolidone and divinylbenzene groups in the HLE particles.

Multiple elution modes, including gradient elution, can be used to extract and purify different types of small molecular compounds in the samples due to the multiple chemical function groups available in the HLE cartridges. The single HLE cartridge can also be used to fractionate the complicated sample into different groups of compounds according to the polarity, hydrophilic, and lipophilic abilities by using multiple mode elution approaches. It is strongly recommended to use 100% isopropyl alcohol (IPA) as the intermediate solvent when switching between hydrophilic and lipophilic elution conditions.

As the single HLE cartridge contains co-ordinated Diol, C18 (ODS), vinylpyrrolidone and divinylbenzene (HLB) groups, the high-capacity cartridge, e.g., HLE-1000 and HLE-2000 cartridges etc., can be used as multiple functional Flash chromatographic column for separation and purification of complicated samples, such as traditional Chinese medicinal

(TCM) herbs and other nature products, when worked with kinds of automatic SPE instruments. All the HLE cartridges can be used for both manual extraction and automatic extraction.

HLE Cartridges Application and Some Requirements

The finished products of the HLE cartridges were packed into light-tight anti-static bags under nitrogen. The originally-sealed HLE cartridges can be stored for a quite long time (several years) at room temperature. The HLE cartridges could be stored for six months in a cool and ventilated dark place after opening the original sealing package.

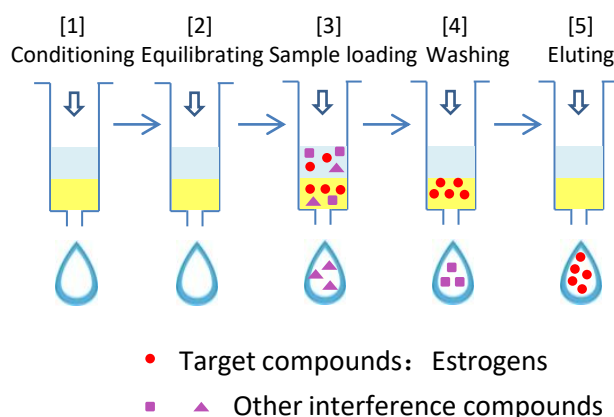
The external packing plastic box for HLE cartridges is strong in compression. The end users are strongly recommended to use the original external packing box from ChiralTek for storing the HLE cartridges and try to avoid any external force extrusion.

The HLE column bed will be changed to light yellow colour after conditioning with isopropylalcohol or methanol. This light yellow colour is normal for the composite particles. The HLE column wall is made from polypropylene. The two frits are made from polyethylene. Most of the common normal phase and reversed phase elution solvents and solutions are compatible with the polypropylene and polyethylene materials. If non-standard solvents are needed for elution, please contact ChiralTek for technical support.

Since the strong alkalic compounds (e.g., NaOH) can cause damages to the HLE column bed, they cannot be used as elution solution additives or sample solution additives. The HLE cartridges have many different models with different specifications and column capacities. Please choose proper model according to the sample volume and total concentration of all possible compounds in the sample.

Easy Method Development by Using Universal HLE Cartridges

Since this novel HLE cartridges can be used under multiple elution conditions, including traditional normal and reverse phase elution conditions and modern gradient elution conditions, it is very easy to establish proper extraction and purification methods for types of samples. For example, a typical reversed phase method using HLE-80 cartridges to extract Estrogens in 1~3mL female blood sample is shown in the following Figure (C).



Figure(C). Typical reversed phase extraction method using HLE-80 cartridges to extract Estrogens in blood samples

The procedure of a typical reversed phase extraction includes following five steps: conditioning column, equilibrating column, sample loading, washing out interference, and eluting target compounds. The detailed operation is described as follows:

[1] *Conditioning column*: the HLE-80 cartridge was conditioned by 3 mL \times 2 methanol at a flow rate of 3 mL/min.

[2] *Equilibrating column*: the HLE-80 cartridge was equilibrated by 3 mL physiological saline solution (or pure water) at a flow rate of 3 mL/min.

[3] *Sample loading*: 1~3 mL of blood samples (whole blood, serum, or plasma) was passed through the HLE-80 cartridge at a flow rate of 1.5 mL/min.

[4] *Washing column*: 3 mL D.I. water (or pure water) was used to wash the HLE-80 cartridge to remove interference compounds

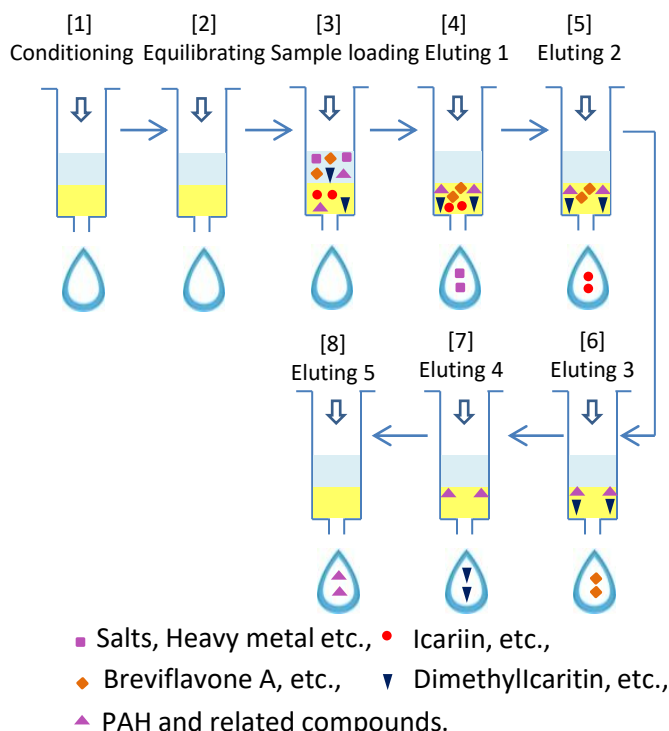
at a flow rate of 3 mL/min. Then the HLE cartridge was dried by nitrogen gas by using automatic SPE instrument or centrifuging under vacuum via manual extraction.

[5] *Eluting target compounds*: 3 mL methanol was used to elute the Estrogens from the HLE cartridge at flow rate of 1.5 mL/min. The final elute was directly collected and concentrated into 0.5 mL sample vial by using automatic SPE instrument or dried under nitrogen and reconstituted into 0.5 mL vial through manual approach. Finally the extract was injected into LC-MS or GC-MS for analysis and detection.

The above extraction method was developed basing on endogenous level of Estrogens in normal female blood. Some parameters, e.g., final eluting volume of methanol etc., need to be further optimized if the exogenous Estrogens and their metabolites are to be extracted and analyzed.

By using a series of fractionations via multiple mode elution approaches, the high-capacity cartridge, e.g., HLE-1000 and HLE-2000 cartridges etc., can be used as multiple functional Flash chromatographic column for fractionation and purification of complicated samples, such as traditional Chinese medicinal herbs or other natural products. For example, the 50% ethanolic crude extract of *Epimedium* herb was fractionated and purified by HLE-1000 cartridge as shown in the Figure (D).

The procedure of a typical multiple mode elution procedure includes following steps: conditional column, equilibrating column, sample loading, washing out interference, followed by several eluting steps, e.g., eluting Fraction 1, eluting Fraction 1, eluting Fraction 2, eluting Fraction 3, eluting Fraction 4, and eluting Fraction 5. The detailed operation of fractionation of crude extract of *Epimedium* herb, by using HLE-1000 cartridge as a Flash chromatographic column, is described as follows.



Figure(D). Typical multiple mode elution method using HLE-1000 cartridge to fractionate crude extract of *Epimedium*

[1] *Conditioning column*: the HLE-1000 cartridge was conditioned by 6 mL × 3 IPA at a flow rate of 6 mL/min.

[2] *Equilibrating column*: the HLE-1000 cartridge was equilibrated by 6 mL × 2 mL 50% ethanol at a flow rate of 6 mL/min.

[3] *Sample loading*: 500 mL of 50% ethanolic crude extract of *Epimedium* herb was passed through the HLE-1000 cartridge at a flow rate of 3 mL/min. The total sample loading onto the HLE-1000 cartridge should be no more than 0.2 gram of dry weight of the crude extract powder.

[4] *Eluting Fraction 1*: 6 mL × 2 pure water was used to elute the ionic compounds, e.g., inorganic salts and heavy metals etc., from the HLE-1000 cartridge at flow rate of 3 mL/min. The elute was directly collected and concentrated into 0.5 mL sample vial by using automatic SPE instrument or dried under nitrogen and reconstituted into 0.5 mL vial through manual approach. The obtained Fraction 1 was then injected into ICP-MS for analysis and detection.

[5] *Eluting Fraction 2*: 6 mL × 3 pure methanol was used to elute polar compounds, e.g., Icariin, Epimedin A, Epimedin B, and

Epimedin C, etc., from the HLE cartridge at flow rate of 3 mL/min. After concentrated, the obtained Fraction 2 was then injected into HPLC or LC-MS for analysis.

[6] *Eluting Fraction 3*: 6 mL×3 IPA was used to elute median polar compounds, e.g., Breviflavone A and Breviflavone B etc., from the HLE-1000 cartridge at flow rate of 3 mL/min. After concentrated, the obtained Fraction 3 was then injected into HPLC or LC-MS for analysis and detection.

[7] *Eluting Fraction 4*: 6 mL×2 ethyl acetate was used to elute weak polar compounds, e.g., Icaritin and Dimethyl Icaritin etc., from the HLE-1000 cartridge at flow rate of 3 mL/min. After concentrated, the obtained Fraction 4 was then injected into HPLC or GC-MS for analysis.

[8] *Eluting Fraction 5*: 6 mL×2 n-hexane was used to elute non-polar compounds, e.g., PAH and related compounds etc., from the HLE-1000 cartridge at flow rate of 3 mL/min. After concentrated, the obtained Fraction 5 was then injected into HPLC (normal phase) or GC-MS for analysis and detection.

The above method was developed basing on one subspecies, *Epemidium Brevicornum*, of the *Epemidium* herbs. Since composition and concentration of ethanolic crude extract of different *Epemidium* subspecies are different, the multiple mode elution methods for different subspecies samples may need to be further optimized.

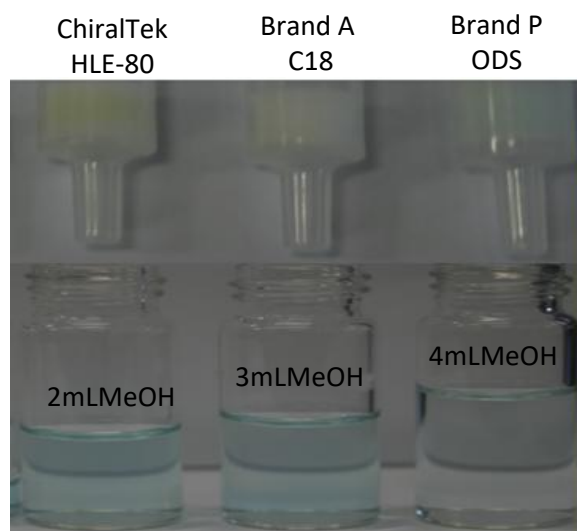
Compared to SPE Cartridges from Other Manufacturer

The ChiralTek HLE cartridges exhibited better recovery when compared to SPE cartridges from other manufacturers. The following experiment was illustrated by the example of extraction of 1 ppm Methyl Blue in 3 mL water. After conditioned by 3 mL ×2 methanol following by loading same volume of 3 mL of Methyl Blue solution, the color of the column beds of ChiralTek HLE, Brand A, and Brand P cartridges with similar specifications is shown in Figure (E).



Figure(E). Comparison of column bed colour of three manufacturers' cartridges after loading same amount of Methyl Blue.

After eluting ChiralTek, Brand A, and Brand P cartridges with 2 mL, 3mL, and 4mL of methanol respectively, the column beds and collected solutions are as shown in Figure (F).



Figure(F). Comparison of colour of column beds and collected solutions of three manufacturers' cartridges after eluting.

The ChiralTek HLE cartridge was completely eluted by using the smallest volume of methanol (the blank HLE cartridge is in light yellow colour). However, although the largest volume of methanol, double volume for eluting HLE cartridge, could not completely elute the Brand P cartridge since the column bed of the Brand P cartridge was still in light blue colour after the eluting procedure. This demonstrated that the ChiralTek universal HLE cartridge exhibited much better recovery and extraction yield when compared with the SPE cartridges from other suppliers.

HLE Cartridges Product List

Product List of ChiralTek HLE Cartridges			
Product No.	Model	Specification	Remarks
880-HLE-80	HLE-80	80mg/3mL, 80-120Å, 50 PCs/box	For small volume samples (0.5 - 50mL)
880-HLE-150	HLE-150	150mg/3mL, 80-120Å, 50 PCs/box	For small volume samples (0.5 - 50mL)
880-HLE-300	HLE-300	300mg/6mL, 80-120Å, 20 PCs/box	For median volume samples (50 - 500mL)
880-HLE-1000	HLE-1000	1000mg/6mL, 80-120Å, 20 PCs/box	For large volume samples (500-1000mL)
880-HLE-2000	HLE-2000	2000mg/12mL, 80-120Å, 10 PCs/box	For very large volume samples (1000mL and above)

This manual may not be updated on time, please visit English website <http://chiraltek-column.com/Downloads.php> or Chinese website <http://cbook.antpedia.com/6755> for downloading the latest version of full product manual and application notes for the HLE cartridges.

Please call an international phone number (+65)-93656129 to directly contact ChiralTek technical support team in Singapore. You also can call a special local phone number (+86)-95040358310 in the mainland of China to directly contact ChiralTek support team in Singapore.

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