UCT

PHARMACEUTICAL METHODS

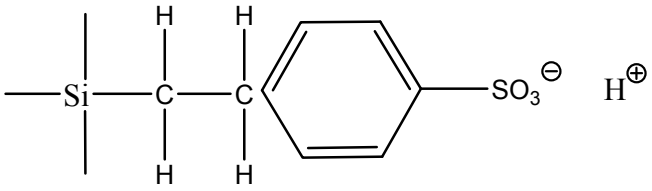
PURIFICATION OF SMALL MOLECULE LIBRARIES BY PHARMASIL® ION EXCHANGE SPE

February 3, 2009

Principle: The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. Ion exchange chromatography has been demonstrated to expedite work up and purification of organic molec**ules** synthesized in solution, and in the automated construction of small molecule libraries. The advantage of ion exchange chromatography over more traditional small molecule purification modes such as flash chromatography or HPLC is that one can reliably predict the elution characteristics of a broad range of molecules soley by the presence or absence of an ionizable site on the molecule.

Application: This application details the use of Pharmasil® BCX1HL, a highly loaded strong cation exchange sorbent, for the purification of amine compounds from organic synthesis mixtures. In combinatorial chemistry and organic synthesis, reactions are often carried out in solvents such as DMSO or DMF in $MeCl_2$. Once the reaction is complete, it is usually necessary to separate the products of the reaction from excess reagents and by-products. This can be done using a highly loaded. Strong cation exchanger to selectivelyretain the basic compounds from the reaction mixture. The sorbent can also be used as scavengers in the synthesis of ureas.

Chemistry of Pharmasil® BCX1HL Sorbent



Advantages of Pharmasil® Based Sorbents

- Clean background
- High recoveries
- High levels of purification of anaytes
- Applicable to a broad range of compounds
- Simple easy to develop methods

Purification Profile

This profile is based on the use of a Pharmasil® BCX1HL 500 mg column (columns are available with varying volumes). This column is capable of purification of up to 50 mg of basic product with a molecular weight of < 300amu. The method can be scaled up as necessary by using columns of higher bed mass of sorbent and increasing the solvent volumes proportionately**. The following profile is meant to be a guideline for these types of purifications. Each drug class has its own specific requirements based on solubility, stability, and pKa and may require slight adjustments in methodology. Therefore think of the following profile as a beginning rather than a final method.**

Sample Pre-treatment

Samples may or may not require pretreatment before addition. The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. This may require the addition of an acid or buffer. Ion exchange can be done out of organic solvents such as methanol or ethyl acetate as long as the compound of interest is ionized.

Column Conditioning

Condition the column with the appropriate solvents. (ethylacetate/hexane, methanol/ethylacetate, methanol, often times the elution solvent makes an excellent conditioning solvent).

Column Equilibration

Equilibrate the column with the same solvent you pretreat the sample with.(buffer, ethylacetate/hexane, etc.)

Sample Application

Apply the sample to the column under gravity. Positive pressure or vacuum can also be used just be certain the application rate does not exceed 1-2 mL per min. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the compound of interest exceeds the capacity of the sorbent you will not get the highest recovery of your compound. If you think this is a problem use a larger bed mass.

Product Purification

Elute neutral and polar reagents and byproducts with ethyl acetate, 25% methanol/ethylacetate, or buffers**. (Caution: when using buffer washes be sure the pH of the buffer remains 2 pH units below the pKa of the compounds of interest you want to retain on the column)**

Product Elution

Elute compound of interest with ethylacetate/ammonium hydroxide, ethylacetate/triethylamine, or ethylacetate/methanol/ammonium hydroxide.(the important factor is to be sure the pH of the elution solvent is 2 pH units above the pKa of your compound of interest. These solutions can be easily dried down to remove unwanted solvents before analysis.

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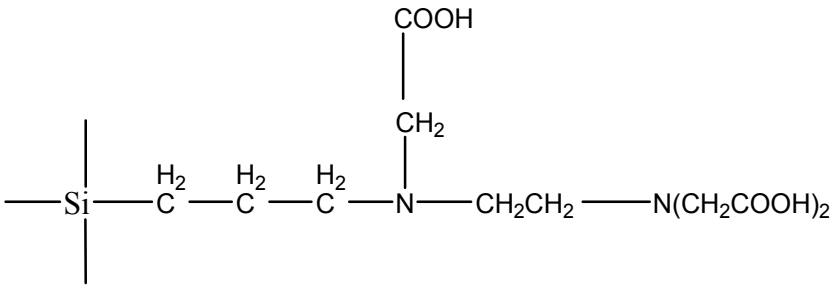
PURIFICATION OF SMALL MOLECULE LIBRARIES
TIN (Sn) REMOVAL BY PHARMASIL® ION EXCHANGE SPE

February 3, 2009

Principle: The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. Ion exchange chromatography has been demonstrated to expedite work up and purification of organic molecules synthesized in solution, and in the automated construction of small molecule libraries. The advantage of ion exchange chromatography over more traditional small molecule purification modes such as flash chromatography or HPLC is that one can reliably predict the elution characteristics of a broad range of molecules solely by the presence or absence of an ionizable site on the molecule.

Application: This application details the use of Pharmasil® TAX, a highly loaded weak cation exchange sorbent, for the removal of tin catalysts from organic synthesis mixtures. In combinatorial chemistry and organic synthesis tin compounds are common catalysts. Once the reaction is complete, it is usually necessary to separate the products of the reaction from the catalysts. If the catalyst is not removed it can interfere with further testing as well as ruin expensive analytical equipment. This can be done using a highly loaded weak cation exchanger to selectively remove the tin catalyst from the reaction mixture.

Chemistry of Pharmasil® TAX Sorbent



Advantages of Pharmasil® Based Sorbents

- Complete removal of tin catalyst
- Clean background
- High recoveries
- High levels of purification of anaytes
- Applicable to a broad range of compounds
- Simple easy to develop methods

Purification Profile

This profile is based on the use of a Pharmasil® TAX 500 mg column (columns are available with varying volumes). This column is capable of removal of up to50mg of tin. The method can be scaled up as necessary by using columns of higher bed mass of sorbent and increasing the solvent volumes proportionately

The following profile is meant to be a guideline for these types of purifications. Each drug class has its own specific requirements based on solubility, stability, and pKa and may require slight adjustments in methodology. Therefore think of the following profile as a beginning rather than a final method.

Sample Pre-treatment

Samples may or may not require pretreatment before addition. The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. This may require the addition of an acid or buffer. Ion exchange can be done out of organic solvents such as methanol or ethyl acetate as long as the compound of interest is ionized**...** Tin catalysts are strong cations and are charged across the complete pH range.

Column Conditioning

Condition the column 1 mL of Methanol followed by 1 mL of water.

Column Equilibration

Condition the column with buffer: If sample is a base, you want the pH at 7-8.

If sample is an acid, you want the pH at 3-4.

Sample Application

Apply the sample to the column under gravity. The tin will stick to the column. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the tin of exceeds the capacity of the sorbent you will not get the highest removal of tin. If you think this is a problem use a larger bed mass.

Product Purification

Wash the column with 1 mL of buffer used in column equilibration.

Product Elution

Elute compound of interest with 1 mL of methanol.

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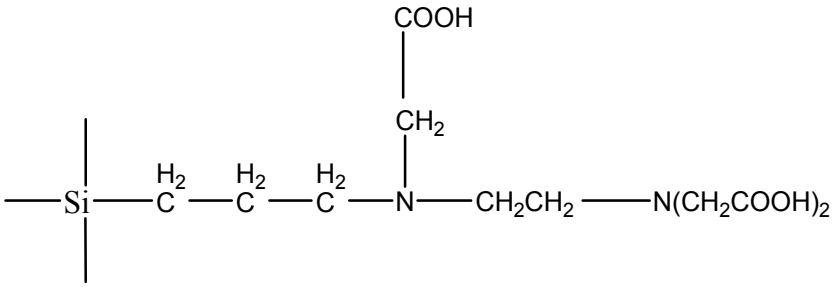
PURIFICATION OF SMALL MOLECULE LIBRARIES
Palladium (Pd) REMOVAL BY PHARMASIL® ION EXCHANGE SPE

February 3, 2009

Principle: The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. Ion exchange chromatography has been demonstrated to expedite work up and purification of organic molecules synthesized in solution, and in the automated construction of small molecule libraries. The advantage of ion exchange chromatography over more traditional small molecule purification modes such as flash chromatography or HPLC is that one can reliably predict the elution characteristics of a broad range of molecules solely by the presence or absence of an ionizable site on the molecule.

Application: This application details the use of Pharmasil® TAX, a highly loaded weak cation exchange sorbent, for the removal of palladium catalysts from organic synthesis mixtures. In combinatorial chemistry and organic synthesis palladium compounds are common catalysts. Once the reaction is complete, it is usually necessary to separate the products of the reaction from the catalysts. If the catalyst is not removed it can interfere with further testing as well as ruin expensive analytical equipment. This can be done using a highly loaded weak cation exchanger to selectively remove the tin catalyst from the reaction mixture.

Chemistry of Pharmasil® TAX Sorbent



Advantages of Pharmasil® Based Sorbents

- Complete removal of palladium catalyst
- Clean background
- High recoveries
- High levels of purification of anaytes
- Applicable to a broad range of compounds
- Simple easy to develop methods

Purification Profile

This profile is based on the use of a Pharmasil® TAX 500 mg column (columns are available with varying volumes). This column is capable of removal of up to50mg of palladium. The method can be scaled up as necessary by using columns of higher bed mass of sorbent and increasing the solvent volumes proportionately

The following profile is meant to be a guideline for these types of purifications. Each drug class has its own specific requirements based on solubility, stability, and pKa and may require slight adjustments in methodology. Therefore think of the following profile as a beginning rather than a final method.

Sample Pre-treatment

Samples may or may not require pretreatment before addition. The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. This may require the addition of an acid or buffer. Ion exchange can be done out of organic solvents such as methanol or ethyl acetate as long as the compound of interest is ionized... Palladium catalysts are strong cations and are charged across the complete pH range. Adjust the sample to pH 9 with buffer or ammonium hydroxide.

Column Conditioning

Condition the column 1 ml of Methanol followed by 1 ml of water.

Column Equilibration

Condition the column with buffer of pH 9.

Sample Application

Apply the sample to the column under gravity. The palladium will stick to the column. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the palladium exceeds the capacity of the sorbent you will not get the highest removal of palladium. If you think this is a problem use a larger bed mass.

Product Purification

Wash the column with 1ml of buffer used in column equilibration.

Product Elution

Elute compound of interest with 1ml of methanol.

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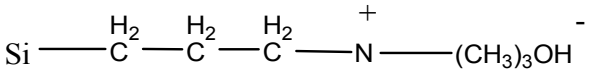
PURIFICATION OF SMALL MOLECULE LIBRARIES
TFAA REMOVAL BY PHARMASIL® ION EXCHANGE SPE

February 3, 2009

Principle: The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. Ion exchange chromatography has been demonstrated to expedite work up and purification of organic molecules synthesized in solution, and in the automated construction of small molecule libraries. The advantage of ion exchange chromatography over more traditional small molecule purification modes such as flash chromatography or HPLC is that one can reliably predict the elution characteristics of a broad range of molecules solely by the presence or absence of an ionizable site on the molecule.

Application: This application details the use of Pharmasil®™ CHQAX, a highly loaded quaternary amine exchange sorbent, for the removal of acid catalysts from organic synthesis mixtures. In combinatorial chemistry and organic synthesis TFAA is a common catalyst. Once the reaction is complete, it is usually necessary to separate the products of the reaction from the catalyst. If the catalyst is not removed it can interfere with further testing as well as ruin expensive analytical equipment. This can be done using a highly loaded quaternary amine exchanger to selectively remove the acid catalyst from the reaction mixture.

Chemistry of Pharmasil® CHQAX Sorbent



Advantages of Pharmasil® Based Sorbents

- Complete removal of acid catalyst
- Clean background
- High recoveries
- High levels of purification of anaytes
- Applicable to a broad range of compounds
- Simple easy to develop methods

Purification Profile

This profile is based on the use of a Pharmasil® CHQAX 500 mg column (columns are available with varying volumes). This column is capable of removal of up to 50mg of TFAA. The method can be scaled up as necessary by using columns of higher bed mass of sorbent and increasing the solvent volumes proportionately.

The following profile is meant to be a guideline for these types of purifications. Each drug class has its own specific requirements based on solubility, stability, and pKa and may require slight adjustments in methodology. Therefore think of the following profile as a beginning rather than a final method.

Sample Pre-treatment

Samples may or may not require pretreatment before addition. The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. This may require the addition of a pH 7 buffer. Ion exchange can be done out of organic solvents such as methanol or ethyl acetate as long as the compound of interest is ionized... acid catalysts are strong anions and are charged across the complete pH range.

Column Conditioning

Condition the column with 1 mL of methanol followed by 1 mL of DI water.

Column Equilibration

Condition the column with pH 7 buffer.

Application

Apply the sample to the column under gravity. The TFAA will stick to the column. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the TFAA exceeds the capacity of the sorbent you will not get the highest removal of TFAA. If you think this is a problem use a larger bed mass.

Product Purification

Wash the column with 1ml of buffer used in column equilibration.

Product Elution

Elute compound of interest with 1ml of methanol.

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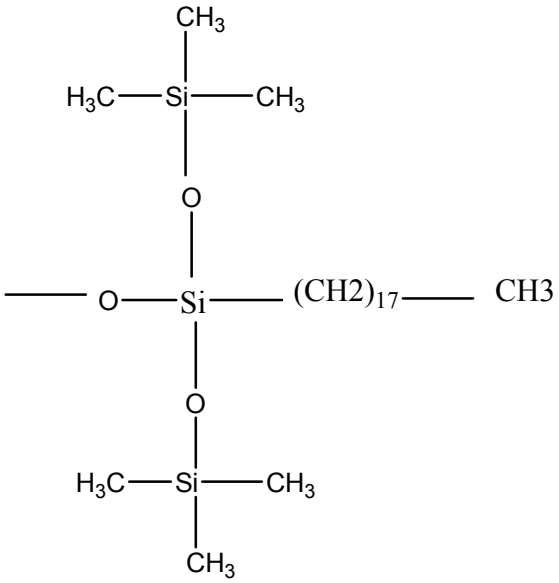
PURIFICATION OF SMALL MOLECULE LIBRARIES
DESALTING SAMPLES USING PHARMASIL® REVERSE PHASE SPE

February 3, 2009

Principle: The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. SPE has been demonstrated to expedite work up and purification of organic molecules synthesized in solution, and in the automated construction of small molecule libraries. Samples that have been synthesized in aqueous salt, buffer solutions, or low polarity organic solvents containing salts may require the removal of those salts prior to analysis. Pharmasil® Reverse Phase SPE can be used to desalt these libraries.

Application: This application details the use of Pharmasil® CEC18, a highly loaded reverse phase sorbent, for desalting synthetic mixtures. In combinatorial chemistry and organic synthesis salts are sometimes present in the reaction mixtures. Once the reaction is complete, it is usually necessary to separate the products of the reaction from the salts. If the salt is not removed it can interfere with further testing as well as ruin expensive analytical equipment. This can be done using a highly loaded reverse phase SPE column to selectively remove the salt from the reaction mixture.

Chemistry of Pharmasil® CEC18 Sorbent



Advantages of Pharmasil® Based Sorbents

- Complete removal of salts
- Clean background
- High recoveries
- High levels of purification of anaytes
- Applicable to a broad range of compounds
- Simple easy to develop methods

Purification Profile

This profile is based on the use of a Pharmasil® CEC18 500 mg column (columns are available with varying volumes). This column is capable of removal of salts. The method can be scaled up as necessary by using columns of higher bed mass of sorbent and increasing the solvent volumes proportionately

The following profile is meant to be a guideline for these types of purifications. Each drug class has its own specific requirements based on solubility, stability, and pKa and may require slight adjustments in methodology. Therefore think of the following profile as a beginning rather than a final method.

Sample Pre-treatment

Samples may or may not require pretreatment before addition. The primary concern using desalting columns is to adjust the pH of the compound of interest so that it is totally molecular. This may require the addition of an acid or base. Desalting can be done out of low polarity organic solvents such as hexane or methylene chloride as long as the compound of interest is protonated.

Column Conditioning

Condition the column 1 ml of Methanol followed by 1 ml of water.

Column Equilibration

Condition the column with buffer: If sample is a base, you want the pH to be >9

If sample is an acid, you want the pH to be<2.5

Apply the sample to the column under gravity. The salts will flow through the column and the sample will stick to the column. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the compound exceeds the capacity of the sorbent you will not get the highest recovery. If you think this is a problem use a larger bed mass.

Product Purification

Wash the column with 1ml of DI water or hexane. .

Product Elution

Elute compound of interest with 1ml of methanol, ethyl acetate, or the organic solvent of your choice.

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