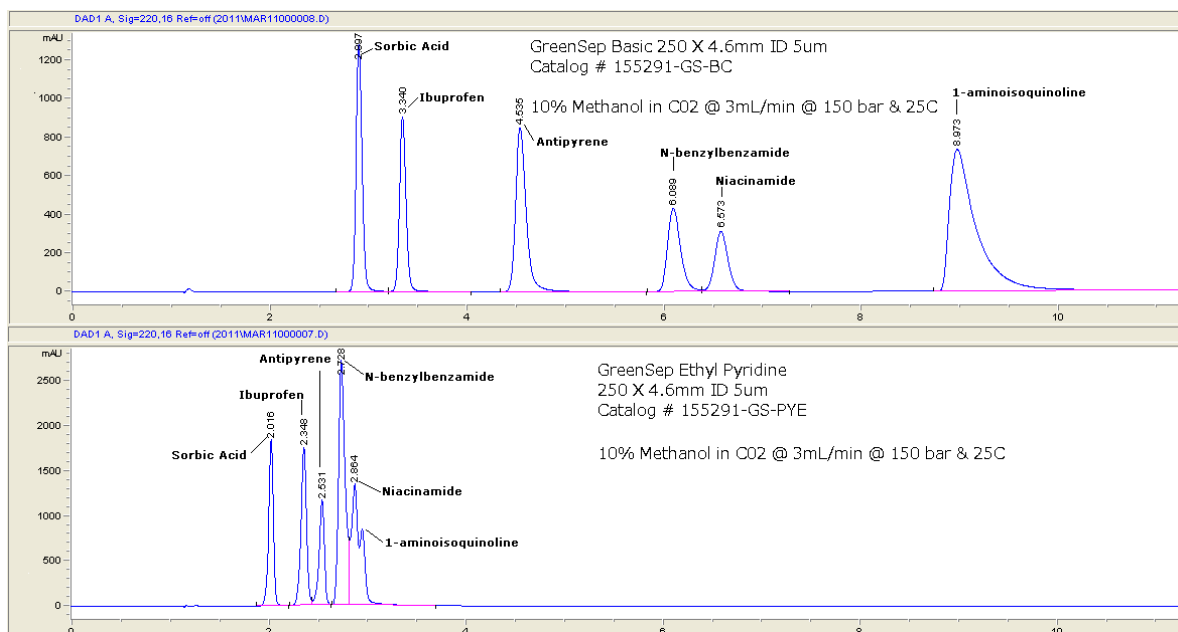


# APPLICATION NEWS

## GreenSep™ Basic SFC Columns

GreenSep™ *Basic* – Supercritical fluid chromatography (SFC) is a powerful chromatographic technique for the separation of complex mixtures. It has been useful in the areas of preparative chromatography and rapid analysis chromatography. Many SFC separations have been forced to utilize older types of stationary phases from “normal phase” HPLC such as unmodified silica, diol, amino and cyano. These phases are poorly adapted to SFC and present a number of limitations for SFC separations. Limitations include: low capacity, poor selectivity, and poor peak shape for SFC separations.

At ES Industries we have developed a new line stationary phases specifically engineered for SFC separations, one of these phases is GreenSep Basic. This stationary phase has proven superior to conventional stationary phases (such as diol, cyano etc...) in the areas of separation selectivity, peak shape and loading capacity. GreenSep Basic is based on imidazole chemistry providing a highly basic character for this stationary phase. The chromatogram shown below is a prime example of the superior peak shape performance and separation capacity obtainable with the GreenSep Basic column with SFC. This chromatogram also compares GreenSep Basic with GreenSep Ethyl Pyridine. The chromatogram contains chemicals that are functionalized with amine groups and acidic groups, demonstrates the retention capability that GreenSep Basic can deliver to the SFC chromatographer. This chromatogram also shows the superior retention on GreenSep Basic when compared to GreenSep Ethyl Pyridine. GreenSep Basic offers the chromatographer greater flexibility in developing separation. GreenSep Basic is the SFC column ideally suited for the retention and rapid separation of chemicals containing amine groups. GreenSep Basic can easily replace conventional stationary phases used in SFC and deliver superior performance.



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GreenSep™ Basic 3u 120A				
	<u>4.6mm (ID)</u>	<u>4.0mm (ID)</u>	<u>3.2mm (ID)</u>	<u>2.1mm (ID)</u>
5cm	115191-GS-BC	114191-GS-BC	11d191-GS-BC	112191-GS-BC
10cm	125191-GS-BC	124191-GS-BC	12d191-GS-BC	122191-GS-BC
15cm	135191-GS-BC	134191-GS-BC	13d191-GS-BC	132191-GS-BC
25cm	155191-GS-BC	154191-GS-BC	15d191-GS-BC	152191-GS-BC

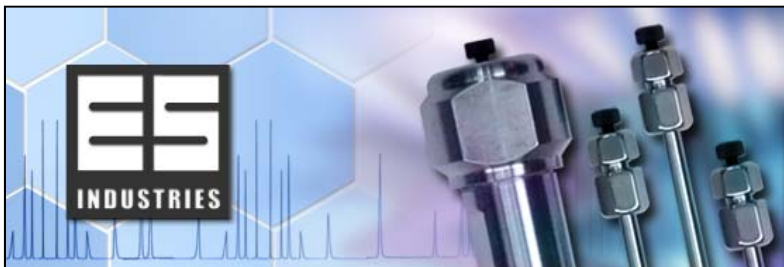
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GreenSep™ Basic 5u 120A				
	<u>4.6mm (ID)</u>	<u>4.0mm (ID)</u>	<u>3.2mm (ID)</u>	<u>2.1mm (ID)</u>
5cm	115291-GS-BC	114291-GS-BC	11d291-GS-BC	112291-GS-BC
10cm	125291-GS-BC	124291-GS-BC	12d291-GS-BC	122291-GS-BC
15cm	135291-GS-BC	134291-GS-BC	13d291-GS-BC	132291-GS-BC
25cm	155291-GS-BC	154291-GS-BC	15d291-GS-BC	152291-GS-BC

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GreenSep™ Basic 10u 120A				
	<u>4.6mm (ID)</u>	<u>4.0mm (ID)</u>	<u>3.2mm (ID)</u>	<u>2.1mm (ID)</u>
10cm	125391-GS-BC	124391-GS-BC	12d391-GS-BC	122391-GS-BC
15cm	135391-GS-BC	134391-GS-BC	13d391-GS-BC	132391-GS-BC
25cm	155391-GS-BC	154391-GS-BC	15d391-GS-BC	152391-GS-BC
30cm	165391-GS-BC	164391-GS-BC	16d391-GS-BC	162391-GS-BC

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# APPLICATION NEWS

## GreenSep™ Basic for the Optimized SFC Separation of Caffeine and Caffeine Related Compounds

Caffeine and caffeine related compounds such as theobromine, paraxanthine and theophylline are found in natural products including cacao (chocolate), tea, kola and coffee. The chemical structures of caffeine, theobromine, paraxanthine and theophylline are all very similar (Figure 1) and differ by methyl group substitutions on the heterocyclic nitrogen contained in the xanthine base structure. These compounds can be separated by HPLC. However, the SFC separation of these caffeine related compounds has several advantages over HPLC including increased speed of analysis and environmental protection (a “Green” technique).

We tested several stationary phases for the SFC separation of caffeine, theobromine, paraxanthine and theophylline including GreenSep Silica (Figure 2) and GreenSep Ethyl Pyridine (Figure 3) both of which failed to provide complete separation of the mixture. The stationary phase that is able to baseline separate the mix of caffeine, theobromine, paraxanthine and theophylline is GreenSep Basic (Figure 4). GreenSep Basic is the ES Industries imidazole based stationary phase.

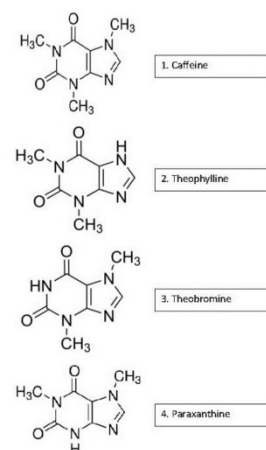


Figure 1

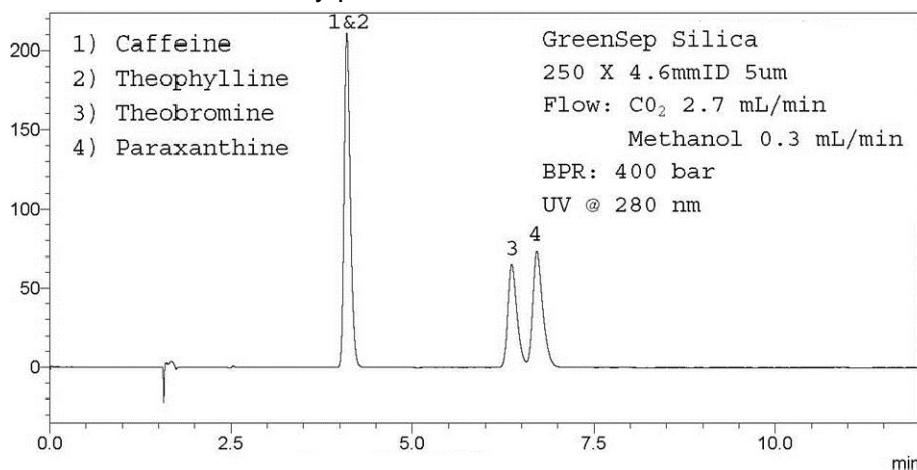


Figure 2

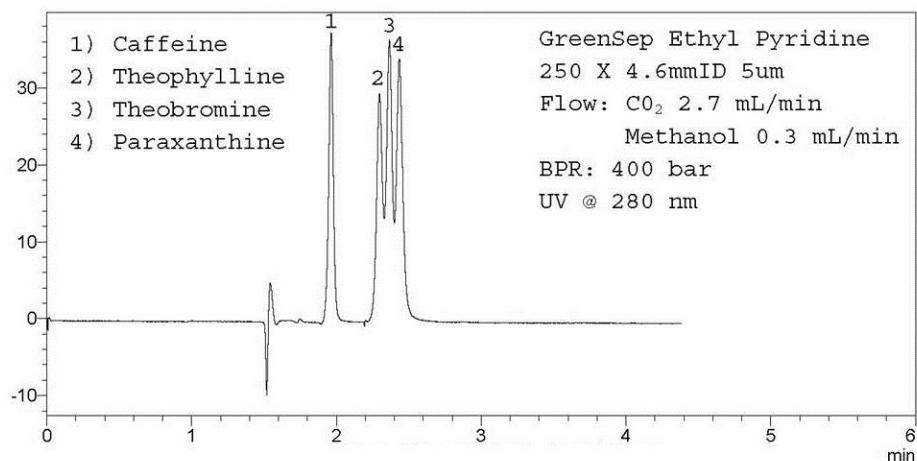


Figure 3

Back pressure regulator (BPR) for the Figure 4 chromatogram was set at 400 bar. At a 400 bar BPR setting the separation was completed in less than 4 minutes, by comparison the same separation, which took over 5 minutes to complete was performed at a 100 bar BPR setting (Figure 5). The difference in separation time, between 400 and 100 bar, can be attributed to the density difference for the carbon dioxide mobile phase.

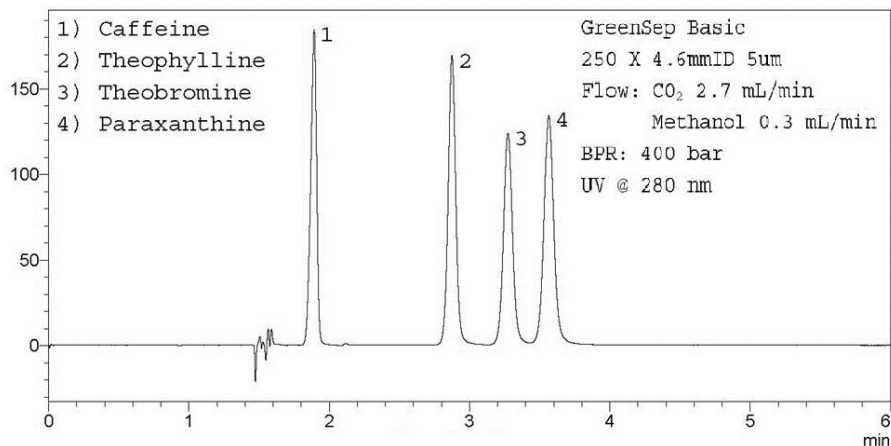


Figure 4

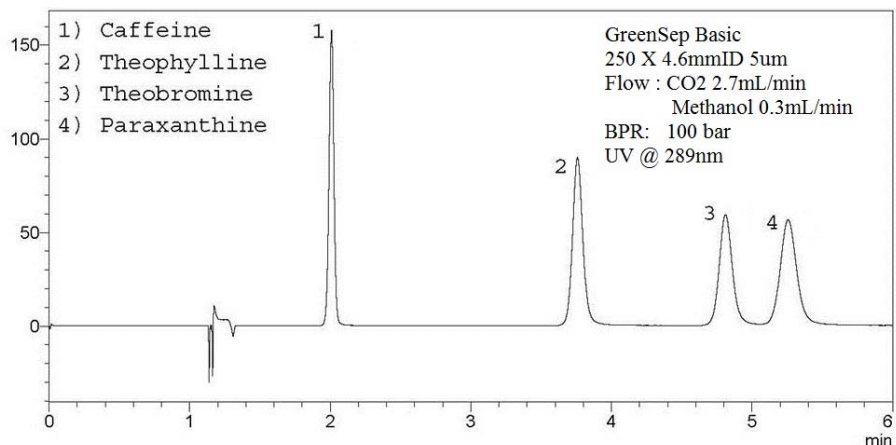


Figure 5

An example chromatogram from a methanol extract of Mayan Cacao powder, purchased at a grocery store, is shown in Figure 6. The chromatogram identifies both caffeine and theobromine. Theobromine is considered to more gently stimulate than caffeine and is believed to be one of the more healthful chemicals found in chocolate.

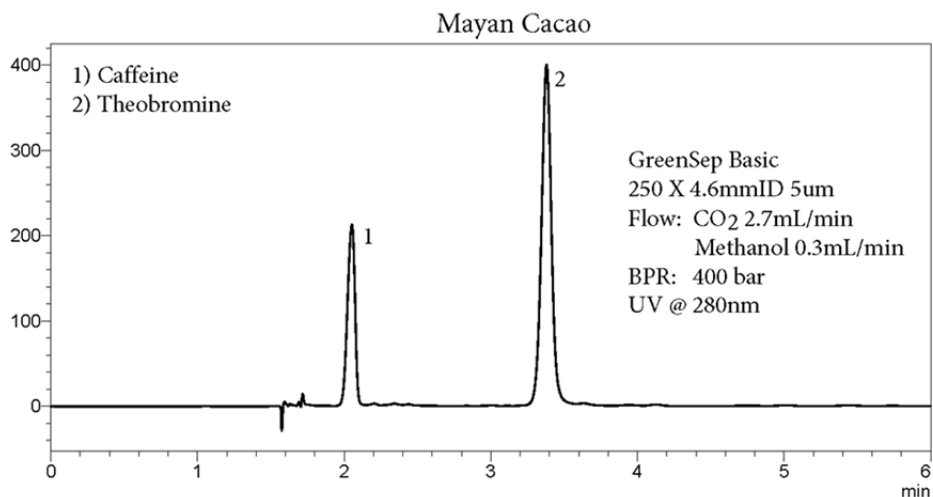
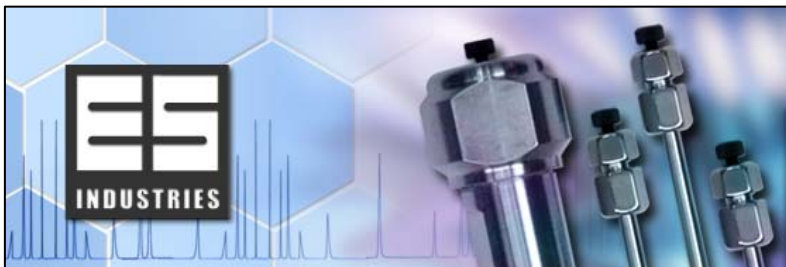


Figure 6



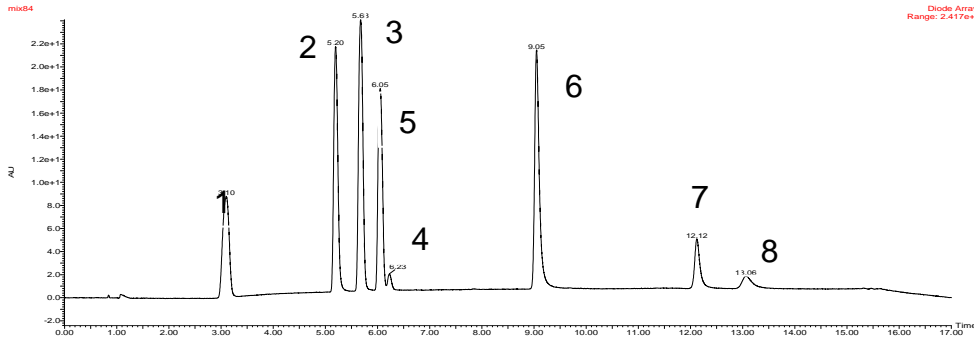
## APPLICATION NEWS

### High Resolution Separation of Purines using GreenSep™ Basic

Supercritical fluid chromatography (SFC) is a powerful chromatographic technique for the separation of complex mixtures. It has been useful in the areas of preparative chromatography and rapid analysis chromatography. Many SFC separations have been forced to utilize older types of stationary phases from “normal phase” HPLC such as unmodified silica, diol, amino and cyano. These phases are poorly adapted to SFC and present a number of limitations for SFC separations. Limitations include: low capacity, poor selectivity, and poor peak shape for SFC separations.

At ES Industries we have developed the GreenSep line stationary phases specifically engineered for SFC separations. One of the phases in the GreenSep line is GreenSep Basic. This stationary phase has proven superior to conventional stationary phases (such as diol, cyano etc...) in the areas of separation selectivity, peak shape and loading capacity. GreenSep Basic is based on imidazole chemistry providing a highly basic character for this stationary phase. The chromatogram shown below is a prime example of the superior peak shape performance and separation capacity obtainable with the GreenSep Basic column with SFC for a high resolution separation of a mixture of purines. This chromatogram also compares GreenSep Basic with a standard unbounded silica column using mobile phase containing no additives with methanol as the modifier solvent. The chromatogram contains purines that are structurally similar and clearly demonstrates the retention capability that GreenSep Basic can deliver to the SFC chromatographer. This chromatogram also shows the superior retention and peak shape on GreenSep Basic when compared to unbounded silica. GreenSep Basic offers the chromatographer greater flexibility in developing separation. GreenSep Basic is the SFC column is ideally suited for the retention and rapid separation of chemicals various functional groups. GreenSep Basic can easily replace conventional stationary phases used in SFC and deliver superior performance.

**Injection of Eight Compound Mixture  
GreenSep Basic 250 x 4.6 mm, 5µm  
Catalog # 155291-GS-BC**



**SFC Conditions**

Gradient: Methanol

Start(%)	End(%)	Time(min)
5	5	1
5	23	8
23	35	10
35	35	14
35	5	14.5
5	5	17.5

- (1) Caffeine
- (2) 6-dimethylaminopurine
- (3) Theophylline
- (4) Theobromine
- (5) - Hydroxyethyltheophylline
- (6) 2-aminopurine
- (7) Hypoxanthine
- (8) Xanthine

Modifier: Methanol / Additive: None

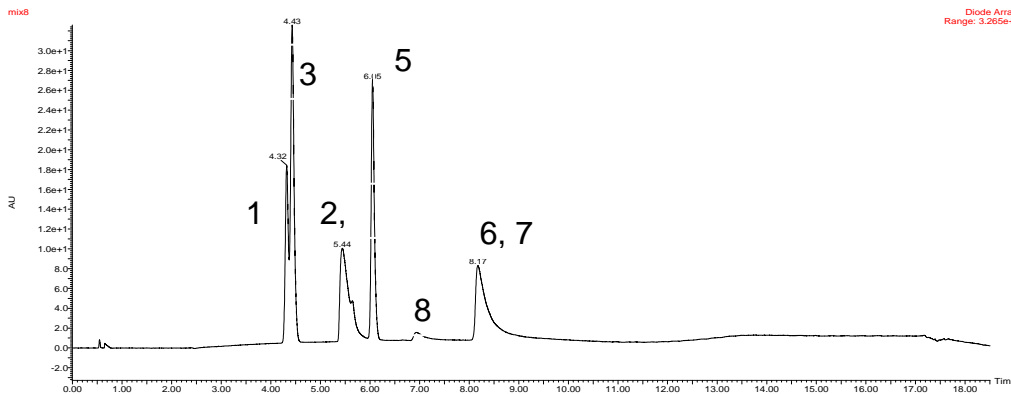
System Back Pressure: 140 bar

Column Oven Temperature: 60<sup>0</sup> C

Detection: UV @ 254nm

Flow: 4mL/min

**Unbonded Silica Column 150 x 4.6 mm, 5µm**



**SFC Conditions**

Gradient: Methanol

Start(%)	End(%)	Time(min)
5	5	1
5	23	8
23	60	12
60	60	16
60	5	16.5
5	5	19.5

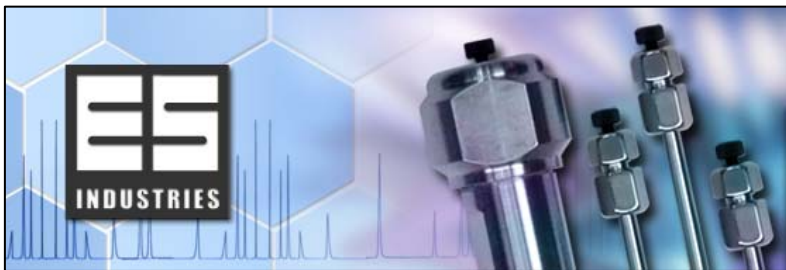
Modifier: Methanol / Additive: None

System Back Pressure: 140 bar

Column Oven Temperature: 60<sup>0</sup> C

Detection: UV @ 254nm

Flow: 4mL/min

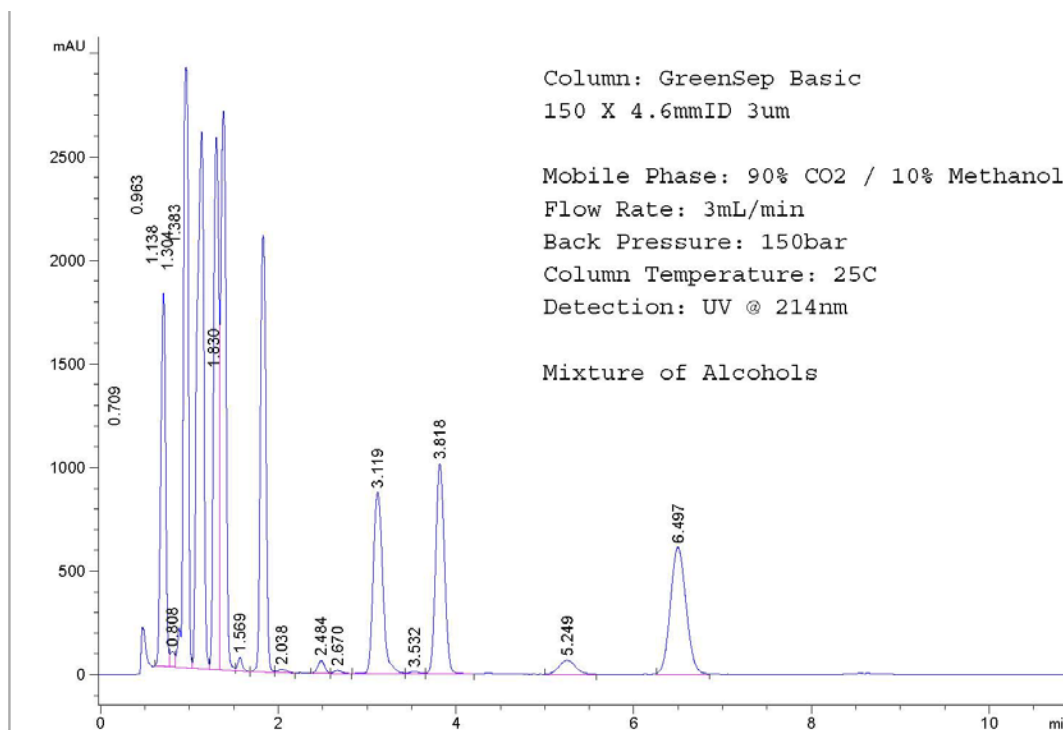


## APPLICATION NEWS

### GreenSep™ Basic 3µm SFC Columns

GreenSep™ *Basic* - Supercritical fluid chromatography (SFC) is a powerful chromatographic technique for the separation of complex mixtures. It has been useful in the areas of preparative chromatography and rapid analysis chromatography. Many SFC separations have been forced to utilize older types of stationary phases from “normal phase” HPLC such as unmodified silica, diol, amino and cyano. These phases are poorly adapted to SFC and present a number of limitations for SFC separations. Limitations include: low capacity, poor selectivity, and poor peak shape for SFC separations.

At ES Industries we have developed a new line stationary phases specifically engineered for SFC separations, one of these phases is GreenSep Basic 3µm. This stationary phase has proven superior to conventional stationary phases (such as diol, cyano etc...) in the areas of separation selectivity, peak shape and loading capacity. GreenSep Basic 3µm is based on imidazole chemistry providing a highly basic character for this stationary phase. The chromatogram shown below for a mixture of alcohols is a prime example of the superior peak shape performance and separation capacity obtainable with the GreenSep Basic column with SFC. GreenSep Basic 3µm is ideally suited to high performance/high speed SFC preparative chromatography applications. GreenSep Basic offers the chromatographer greater flexibility in developing separation. GreenSep Basic is the SFC column is ideally suited for the retention and rapid separation of chemicals containing amine groups. GreenSep Basic can easily replace conventional stationary phases used in SFC and deliver superior performance.



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GreenSep™ Basic 3u 120A				
	<u>4.6mm (ID)</u>	<u>4.0mm (ID)</u>	<u>3.2mm (ID)</u>	<u>2.1mm (ID)</u>
5cm	115191-GS-BC	114191-GS-BC	11d191-GS-BC	112191-GS-BC
10cm	125191-GS-BC	124191-GS-BC	12d191-GS-BC	122191-GS-BC
15cm	135191-GS-BC	134191-GS-BC	13d191-GS-BC	132191-GS-BC
25cm	155191-GS-BC	154191-GS-BC	15d191-GS-BC	152191-GS-BC

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GreenSep™ Basic 5u 120A				
	<u>4.6mm (ID)</u>	<u>4.0mm (ID)</u>	<u>3.2mm (ID)</u>	<u>2.1mm (ID)</u>
5cm	115291-GS-BC	114291-GS-BC	11d291-GS-BC	112291-GS-BC
10cm	125291-GS-BC	124291-GS-BC	12d291-GS-BC	122291-GS-BC
15cm	135291-GS-BC	134291-GS-BC	13d291-GS-BC	132291-GS-BC
25cm	155291-GS-BC	154291-GS-BC	15d291-GS-BC	152291-GS-BC

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GreenSep™ Basic 10u 120A				
	<u>4.6mm (ID)</u>	<u>4.0mm (ID)</u>	<u>3.2mm (ID)</u>	<u>2.1mm (ID)</u>
10cm	125391-GS-BC	124391-GS-BC	12d391-GS-BC	122391-GS-BC
15cm	135391-GS-BC	134391-GS-BC	13d391-GS-BC	132391-GS-BC
25cm	155391-GS-BC	154391-GS-BC	15d391-GS-BC	152391-GS-BC
30cm	165391-GS-BC	164391-GS-BC	16d391-GS-BC	162391-GS-BC

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