

Challenging Reversed Phase Separation of Pharmaceutical Compound Benzodiazepine Achieved by SepaFlash® Phenyl Column (SW-5B22)



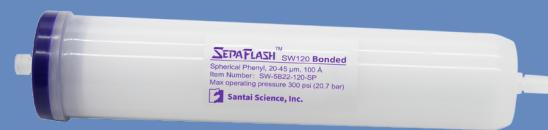
Santai Science Inc.

Chromatography Application Note
ANSS-015

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Benzodiazepine is a potent prescription medication that is most used to treat anxiety and panic disorders. It is also sometimes used to treat seizures and even alcohol withdrawal while in medical detox.

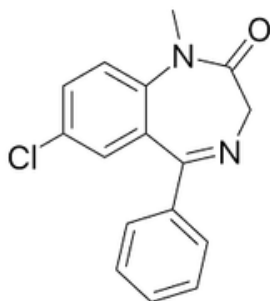
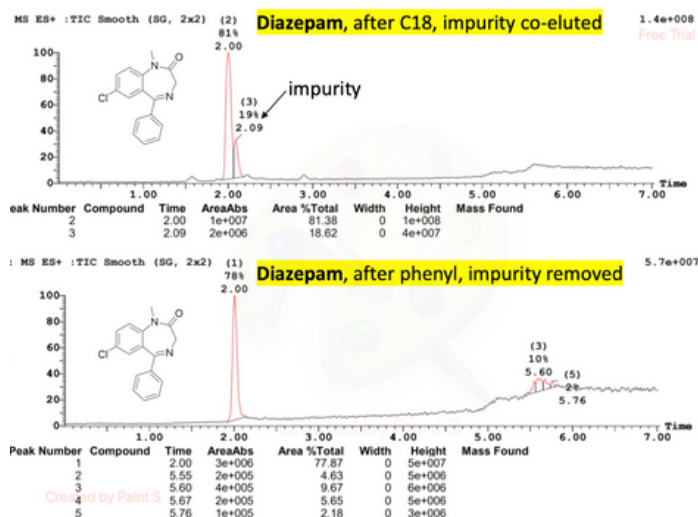


Figure 1 Structure of benzodiazepine

During the synthetic process of the benzodiazepine impurities are often formed, and their removal is critical to the success of the process. Automated Flash chromatography is one of the most effective purification technique on a research scale and it is also translatable to production scale as well.

In the purification of benzodiazepine, the common reversed phase flash chromatography C18 was not able to remove an impurity that co-eluted (2.09 min) with the product (2.00 min) fraction as shown in the LC-MS below.



In order to remove this co-eluting impurity, an alternative reversed phase flash chromatography method using -phenyl column where the C18 groups are replaced by phenyl group was used. This column (Spherical, 20-45 µm, 100Å, SW-5B22-120) resulted in the perfect isolation of the target compound without a trace of the impurity, as shown in the LC-MS, the collected fraction contains only the desired compound that eluted at 2 min.

Instrument	SepaBean™ machine 2
Columns	120 g SepaFlash® Bonded Phenyl column (Spherical, 20-45µm, 100Å, SW-5B22-120)
Wavelength	210 nm 254 nm
Mobile phase	Solvent A: Water Solvent B: Methanol
Flow rate	30 mL/min
Sample loading	2000 mg

Table 1. The experimental setup for flash purification of diazepam

Reversed -phase separation

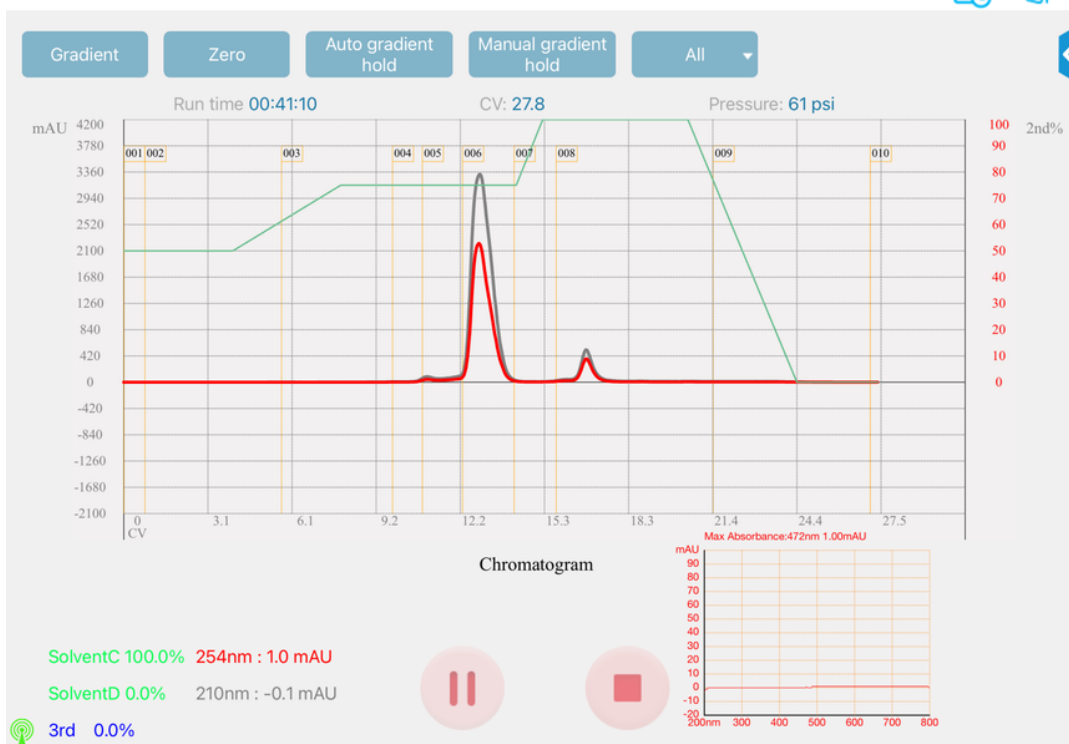


Figure 2. the flash chromatogram of the isolation of pure diazepam from its synthetic isomer impurity by Santai's reversed phase phenyl column (SW-5B222, 20-45 μm , 100 \AA)

As shown in flash chromatogram **Figure 2**, the target pharmaceutical compound benzodiazepam is nicely isolated with a sharp gaussian peak at 12.2 cv, while the impurity eluted around 16 cv. The π - π interaction of the benzodiazepam with the solid phase phenyl groups is a dominating effect. This allows for a different elution rate of the target compound compared to a suspected none planar impurity. Hereby, we are offering an example of an alternative reversed phase purification columns for compounds with aromatic functional group, this could be particularly important for structure isomers while are very common in the pharmaceutical industry.

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