Enhanced Purification of Capsaicins with the Combi*Flash*® EZ Prep

TELEDYNE ISCO
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Chromatography Application Note AN101

Abstract

In this application note, capsaicins are used as a model for compounds difficult to resolve with flash chromatography. Capsaicin compounds possess very similar structures making them difficult to resolve from each other. Although these compounds can be resolved on flash chromatography columns, such as the Redi*Sep*® Rf Gold C18, preparative high pressure liquid chromatography (prep HPLC) can provide improved resolution due to their smaller particle size and better packing compared to Flash columns.

The Teledyne Isco Combi*Flash*® EZ Prep is a dual function system that is ideal for performing the initial purification of crude mixtures on inexpensive flash columns, with final purification on prep HPLC in a single system requiring lab space similar to a flash-only system. The flash column captures material that would be permanently retained on the HPLC column, and thus enables higher loading of the desired compound(s) on the HPLC column. Undesired materials are removed using the flash column. Detection and identification of the compounds was done with a PurIon mass spectrometer.

Background

Capsaicin compounds are often used as a model for plant extracts. These compounds are of interest for pain relief[1], suppression of tumorigenesis[2] to increase bladder capacity, and reduce nausea[3]. Compounds in the capsaicin family are very similar in structure and polarity making them difficult to resolve.

A high quality flash column such as a Redi*Sep* Rf Gold C18 column, has been shown to resolve a mixture of capsaicins sufficiently well to purify and identify three compounds[4] (Figure 1).

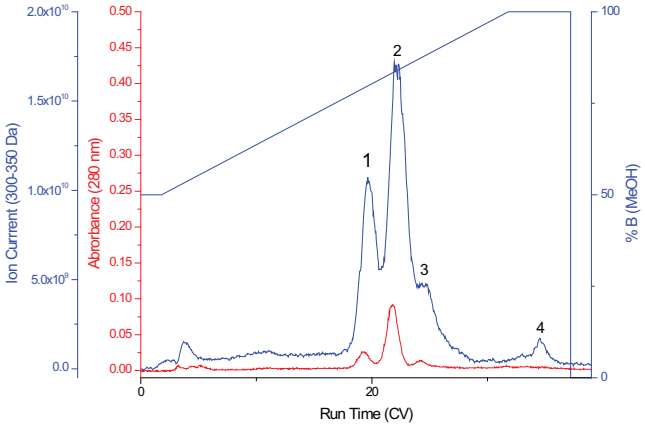
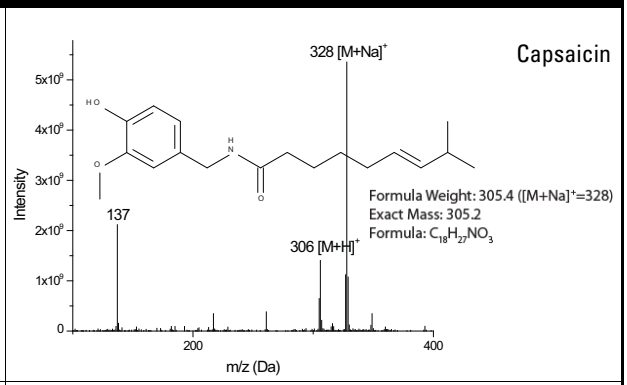
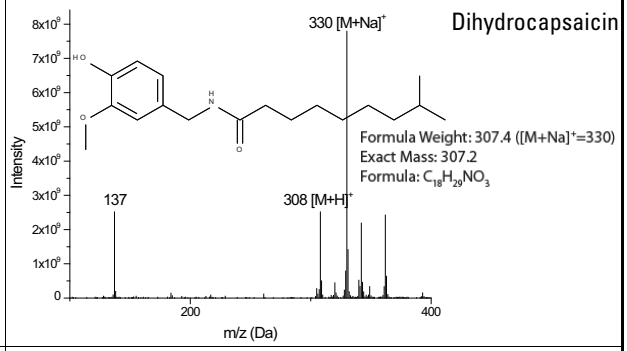
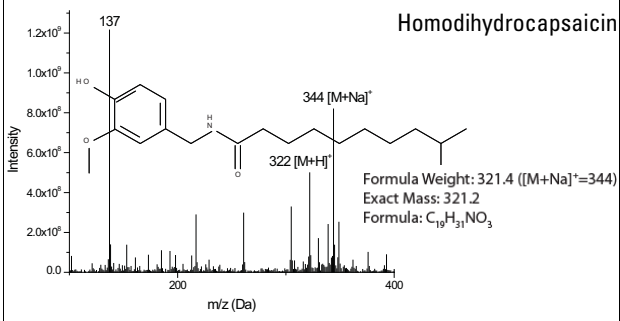
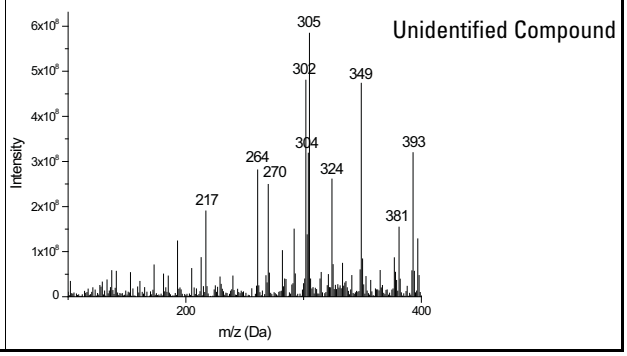


Figure 1: Purification of capsaicin compounds on a Redi*Sep* Rf Gold C18 column

Table 1: Mass Spectra and Assigned Structures for Purification in Figure 1

1		Capsaicin 328 [M+Na]+ 137 306 [M+H]+ Formula Weight: 305.4 ([M+Na]+=328) Exact Mass: 305.2 Formula: $C_{18}H_{27}NO_3$
2		Dihydrocapsaicin 330 [M+Na]+ 137 308 [M+H]+ Formula Weight: 307.4 ([M+Na]+=330) Exact Mass: 307.2 Formula: $C_{18}H_{29}NO_3$
3		Homodihydrocapsaicin 137 344 [M+Na]+ 322 [M+H]+ Formula Weight: 321.4 ([M+Na]+=344) Exact Mass: 321.2 Formula: $C_{19}H_{31}NO_3$
4		Unidentified Compound 305 302 349 264 270 304 324 393 217 381

A fragment common to the capsaicin compounds is the peak at m/z=137 created from the phenolic portion of the molecule (Figure 2)[5]. This fragment allows easy identification of those peaks which are capsaicinoids from those peaks not containing this compound family. For example, peak 4 in Figure 1 and Table 1 does not exhibit this fragment and is therefore presumed not to be a capsaicinoid.

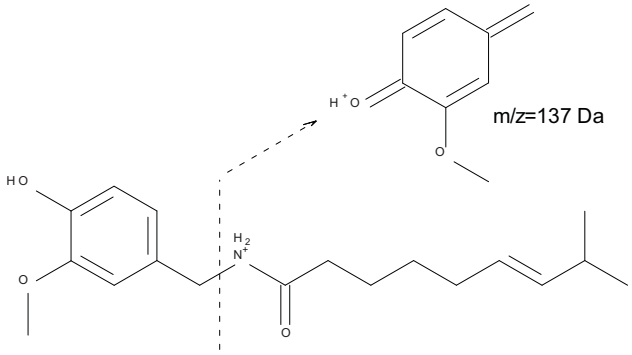


Figure 2: Schematic representation of the daughter ion produced from capsaicin at m/z = 137 Da

Experimental and Results

All experiments were run on a Combi*Flash* EZ Prep purification system (PN 68-5230-025) and a Teledyne Isco Combi*Flash* PurIon L system (PN 68-5237-084) both controlled by PeakTrak®. Pure chemicals were obtained from Sigma-Aldrich (St. Louis, MO) and solvents were ACS grade from VWR Scientific (Radnor, PA). The carrier solvent (used to dilute and deliver the sample from the Combi*Flash* system to the mass spectrometer) was 0.1% formic acid in methanol.

Thai green peppers (*Capsicum annuum*, 2.25 kg) purchased at a local grocery store were ground to a paste with a blender and extracted with toluene[6]; the extract was dried to yield 30.8g extract.

The pepper extract (1.0 g) was dissolved in a mixture of methanol and water and dried onto 5 g neutral alumina. This was run on a 24 g Redi*Sep* Rf neutral alumina column (Teledyne Isco PN 69-2203-441) with a hexane/ethyl acetate gradient with mass-directed fractionation using the range from 300-400 Da. This column and solvent system were chosen on the basis of column screening as described in Reference 4.

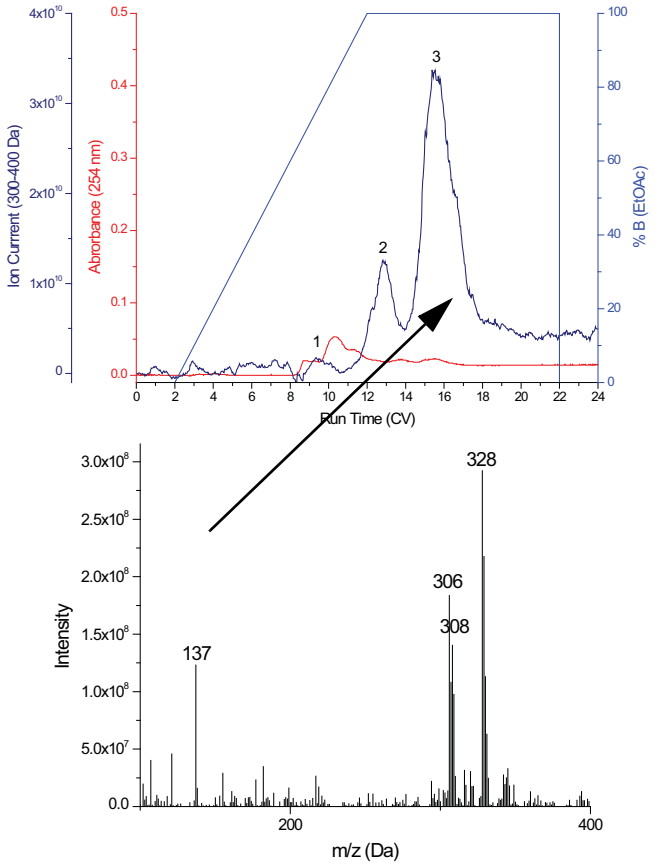


Figure 3: Initial purification of crude capsaicin extract with neutral alumina

Table 2: Fractions from Alumina Purification

Fraction	Elution Range (CV)	Mass Recovered (g)
1	8.4-10.7	0.1630 g
2	10.7-14.4	0.0312 g
3	14.4-18.2	0.0180 g

Fraction 3 (0.0180 g) was then run on the Combi*Flash* EZ Prep with a Redi*Sep* Prep C18 20x150 mm, 5 µm column (PN 69-2203-810) equipped with a 20x30 mm guard column (PN 69-2203-804) using the gradient depicted in Figure 4. The gradient is similar to that used for the flash C18 column (Figure 1). Mass directed fractionation used a range of 250-400 Da. Capsaicins were identified by the fragment at 137 Da, their mass ($[M+H]^+$ and $[M+Na]^+$) and comparison with authentic purchased samples.

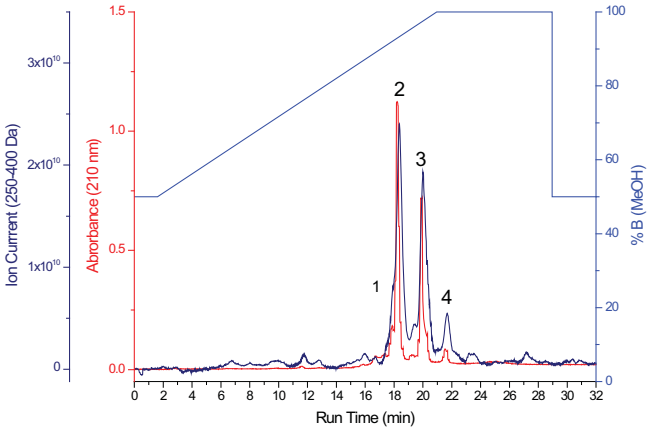
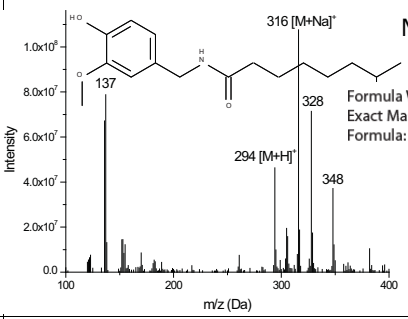
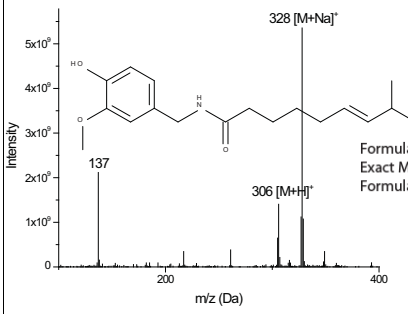
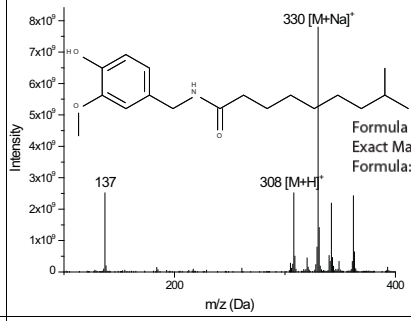
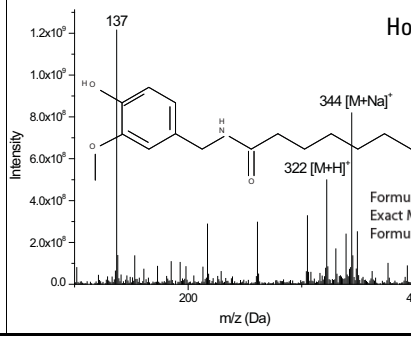


Figure 4: Final capsaicin purification on prep HPLC column

Table 3: Fractions Collected from RediSep Prep HPLC Column

Fraction	Elution Range (Minutes)	Mass Recovered (g)
1	0-16.6	0.0015
2	16.6-17.9	0.0014
3	17.9-19.0	0.0056
4	19.0-19.3	0.0003
5	19.3-19.6	0.0004
6	19.6-22.0	0.0044
7	22.0-23.7	0.0002
8	23.7-32.2	0.0018
	Total Recover: 0.0156 (87% Recovery)	

Table 4: Mass Spectra and Assigned Structures for Purification *Figure 4*

1		Nordihydrocapsaicin Formula Weight: 293.4 ([M+Na]⁺=316) Exact Mass: 293.2 Formula: $C_{17}H_{27}NO_3$
2		Capsaicin Formula Weight: 305.4 ([M+Na]⁺=328) Exact Mass: 305.2 Formula: $C_{18}H_{27}NO_3$
3		Dihydrocapsaicin Formula Weight: 307.4 ([M+Na]⁺=330) Exact Mass: 307.2 Formula: $C_{18}H_{29}NO_3$
4		Homodihydrocapsaicin Formula Weight: 321.4 ([M+Na]⁺=344) Exact Mass: 321.2 Formula: $C_{19}H_{31}NO_3$

The chromatogram in Figure 4 demonstrated the improved resolution of the prep HPLC column on the EZ Prep. The original three compounds detected using the flash C18 column exhibit baseline resolution on the HPLC column run on the EZ Prep. In addition, compound #1 is detected as a shoulder on the peak corresponding to compound 2 in Figure 4; compound 1 was not detected in the flash C18 run. The use of the PurIon mass spectrometer allowed identification of the shoulder as a member of the capsaicin family.

Conclusion

The Combi*Flash* EZ Prep system combines the features and benefits of both flash and prep HPLC in a single space-saving unit. Flash chromatography can be run quickly on samples with well-resolved peaks. It can serve to remove impurities and increase the sample load on the prep HPLC columns since solutions loaded on the prep HPLC column has a higher concentration of the desired compound. The prep HPLC portion of the system allows improved resolution of related compounds and closely eluting impurities.

References

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