

**Flash and prep-HPLC purification of chemically related pyrazines – all in one**
*PrepChrom C-700*
**1. Introduction**

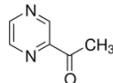
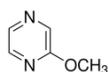
Pyrazines – a class of heterocyclic aromatic and nitrogen-containing organic compounds – play a significant role in a wide range of areas, leading from pharmaceutical to food and fragrance industry.

Pyrazines are not only important components of many fruits and vegetables, such as grapes, peppers, peas and tobacco, but are also responsible for the typical flavor of toasted and roasted food [1, 2]. Furthermore, they are found in natural medicines and are nowadays still of large biological interest and thus used as anti-cancer, anti-inflammatory and anti-trombotic compounds [2, 3, 4].

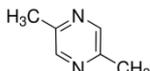
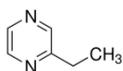
Hence, the efficient chromatography purification of pyrazines, providing good resolution and yielding high purity, remains essential and the efforts for optimizing their chromatographic separation are ongoing [5, 6].

**2. Experimental**
**Mixture components**

- ① 2-methoxy pyrazine      ② 2-acetyl pyrazine



- ③ 2-ethyl pyrazine      ④ 2,5-dimethyl pyrazine



The sample was prepared by dissolving 50 mg of each compound in 1 mL acetone, thus resulting into a total sample concentration of 200 mg/mL.

**Equipment and method parameters**

System	PrepChrom C-700	
BUCHI Flash Cartridge	Interchim Prep LC-column	
Material: Silica (80 g)	Material: PuriFlash 60 Å	
Particle size (µm): 40-63	Particle size (µm): 10	
Length (mm): 194	Length (mm): 150	
I.D. (mm): 31	I.D. (mm): 30	
Flow rate	40 mL/min	
Solvents	A: Hexane; B: Ethyl acetate	
Gradient	15% B for 1 min 15% to 100% B in 20 min 100% B for 1 min	
Detection	254 nm	
Threshold	50 mAU	
Sample load	0.5 g	

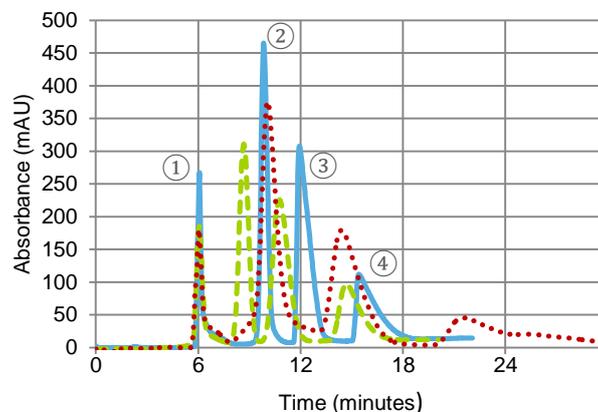
**3. Results and Discussion**


Figure 1. Prep-HPLC purification (blue full line), flash purification (normal run, light green dashed line) and flash purification (AGO, red dotted line).

Figure 1 shows the chromatographic separation of the four chemically related pyrazines mentioned above using a flash cartridge (green and red lines) and a prep-HPLC column (blue line) on the same system. As expected, peak widths and separation are better using the prep-HPLC column. The difference in resolution is clearly due to the difference in efficiency, *i.e.*, to particle size and shape.

Special software features as the “*automatic gradient optimization*” option (AGO, red dotted line in Figure 1), allowing to hold current gradient parameters when a peak appears on the chromatogram during the run, improves the flash separation of a difficult solution and thus the collection of the different mixture constituents.

**4. Conclusion**

For the purification of complex systems, the PrepChrom C-700 system not only allows the use of two different types of columns on one single system, namely flash and prep-HPLC columns, but also includes several software features which enable efficient purification and guarantee a well defined separation of the different mixture constituents.

**5. References**

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- [5] K. Zaitso, *Chromatography* **2008**, 29 (1)
- [6] H.-D. Belitz, W. Grosch and P. Schieberle, *Food Chemistry*, Springer **2009**