

## Sample Preparation to HPLC Analysis of Confectionery

Like all foodstuff, confectionery is subjected to strict quality controls. Parameters of interest are, for example, nutritional value, moisture or fat content, or the quantification of particular ingredients, such as vitamins or alkaloids. Typically, chromatographic methods like High Performance Liquid Chromatography (HPLC) are used to analyze food samples. Most analytical methods only require a few milligram or gram of sample; the previous size reduction/homogenization process ensures that the small analysis sample is representative of the entire laboratory sample, thus allowing for reproducible results. Moreover, homogenized samples show a much better extraction behavior.

Confectionery occurs in very different textures: it can be hard, sticky, greasy, or moist and is frequently inhomogeneous. For the important step of sample preparation laboratory mills - such as offered by RETSCH in a variety of designs - are indispensable tools. With a suitable mill and the corresponding accessories all types of samples can be easily and reproducibly homogenized. Generally, the sample preparation process needs to be adapted to the sample characteristics as well as to the requirements of the subsequent analysis to avoid falsified results.

When selecting grinding tools and parameters it should be taken into account that the characteristics of the sample must not be altered by the grinding process. Different analysis methods call for different ways of sample preparation. For HPLC analysis a particle size distribution between 0.5 and 0.75 mm is ideal.

The Cologne-based institute IQ.Köln (Institute for Promotion of Quality in the Confectionery Industry) is specialized in the analysis of confectionery. The institute is accredited according to DIN EN ISO 17025 for food testing using methods such as HPLC with UV/VIS, fluorescence and tandem-MS detection and also gas chromatography with flame ionization and MS detection.

### Homogenization of hard candy and toffee

The institute uses RETSCH's knife mill GRINDOMIX GM 200 to homogenize hard candy and toffee. The cutting effect of the sharp knife blades allows for thorough grinding of hard as well as very sticky sweets. The GM 200 produces representative samples within seconds. Features such as variable speed, a

comprehensive selection of grinding containers and lids and the autoclavability of the grinding tools make this mill a versatile tool in the food lab.



Fig. 1: Knife Mill GRINDOMIX GM 200

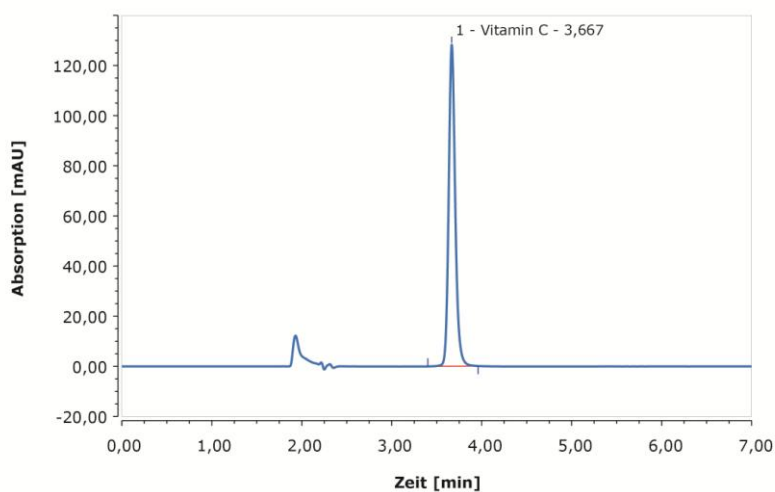
A typical homogenization process involves 100 g of hard candy which is first roughly ground for a few seconds in reverse mode with the blunt side of the knife. The following step involves operation in interval mode for another 15 seconds with 4,000 rpm. Further pulverization to a size below 0.5 mm is achieved by grinding for 6 to 12 seconds at 6,000 rpm. This step by step procedure prevents the sample – which has a high sugar and starch syrup content - from sticking to the knife as is often the case in household mixers. Just like the brittle hard candy the tough-elastic toffees are deep-frozen together with the steel grinding container before they are submitted to size reduction. The lab team of IQ.Köln is highly satisfied with the performance of the GM 200 when grinding these difficult samples. All parts of the mill which come into contact with the sample material (container, lid, and knife) are conveniently cleaned in the dishwasher.

### **Analysis of vitamin C**

Hard candy is often enriched with vitamin C which can be quantified by HPLC. Ascorbic acid is also frequently used as a preservative. The ground candy is

dissolved in water; the vitamin C is stabilized with the help of metaphosphoric acid; after membrane filtration the sample solution is analyzed by reversed-phase HPLC and subsequent UV detection. The standard solvent is phosphate buffer with a flow rate of 1 ml/min; under these conditions the vitamin C elutes after 3.7 minutes.

Fig. 2: Chromatogram of vitamin C analysis with reversed-phase HPLC



### Fatty, granular food samples such as cocoa beans

The best combination of size reduction mechanisms for medium-hard, granular food stuff like grain or cocoa beans is impact and shearing. The cocoa bean bursts open through impact, and the smaller parts are then further reduced in size by shearing. The **Ultra Centrifugal Mill ZM 200** is ideally suited for this job; this rotor mill grinds the sample between rotor and fixed ring sieve by impact and shearing. The material is fed to a hopper, falls onto the rotor and is thrown outward by centrifugal acceleration. When the sample hits the wedge-shaped rotor teeth running at high speed it is precrushed and then pulverized between rotor and ring sieve. The sample only remains in the grinding chamber for a very short interval so that the characteristic properties are not altered.

The lab team at IQ.Köln uses the ZM 200 to grind 100 g of cocoa beans with a 1.5 mm distance sieve at 18,000 rpm for 10 seconds and obtains a mean particle size below 0.75 mm. With a distance sieve there is more space between rotor and sieve which helps to reduce frictional heat and prevents the apertures from

being blocked by fatty particles. The pulverized sample is then collected in a cassette. This is part of a patented principle where the cassette containing the sample is removed together with the ring sieve, thus ensuring sample recovery without loss and prevention of cross contaminations. For small amounts up to 20 ml RETSCH offers a mini cassette with matching rotor and ring sieves.



*Fig. 3: Cocoa beans before and after grinding in the ZM 200*

### **Alkaloid analysis**

The alkaloids contained in cocoa beans and cocoa nibs – which are mainly theobromine and caffeine – are also quantified by HPLC. Cocoa beans contain up to 0.2% caffeine and 1 to 2.5% theobromine. The invigorating effect of theobromine on the human organism is much weaker than that of caffeine; however, it has a positive effect on the mood.

The ground beans are first cooked in water and then clarified. After centrifugation and membrane filtration the supernatant is analyzed by reversed-phase HPLC and subsequent UV detection. A water/ethanol mixture (80%/20%) is used as standard solvent at a flow rate of 1 ml/min. Under these conditions theobromine elutes after 3.5 minutes and caffeine after 9.1 minutes.

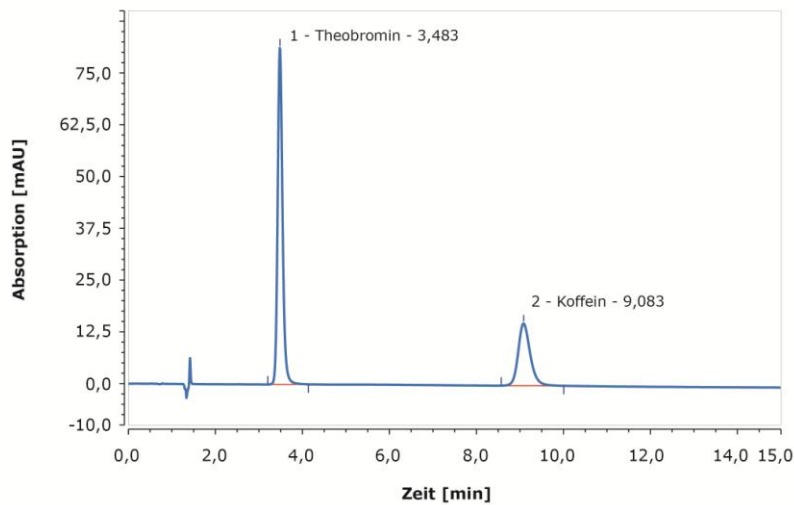


Fig. 4: Chromatogram of alkaloid analysis by reversed-phase HPLC

## Conclusion

RETSCH offers rotor and knife mills which are perfectly suited to grind all types of food quickly, reproducibly and without interfering with subsequent analysis. A wide selection of accessories allows for flexible adjustment to material properties and sample volumes. Tough-elastic samples or those containing volatile components are best processed by cryogenic grinding, which means embrittling the sample material with dry ice or liquid nitrogen. The use of RETSCH mills and grinders greatly improves the efficiency of a food laboratory, particularly if a variety of sample types needs to be processed on a daily basis.

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