ABSTRACT

In pharmacy, sweeteners are used as excipients, which are important ingredients of various pharmaceutical forms. Excipient is every ingredient that is not an active substance and is present in the pharmaceutical product or is used for manufacturing of pharmaceutical products. The excipient can in some cases actively affect the physiological functions or may in certain doses cause (unwanted) side effects. That is why correct selection and use of excipients is extremely important and it contributes to safety, efficiency and quality of the pharmaceutical product. In terms of analytics, this means that qualitative tests should prove the absence of unauthorized additives and the quantitative analyses should ensure that the concentration of used additives does not exceed the allowed limit.

At the determination of the excipients concentration in semi-products and finished pharmaceutical products, which is mainly done with chromatography, the main problem is the absence of chromophores in the structure of excipients. In this master's thesis I researched the most suitable way of detecting excipients with different HPLC detectors (RI, CAD and ELSD, MS) and made a comparison between them with reference to precision, sensitivity (limit of detection and limit of quantitation), linearity range and robustness of the detector. For comparison of validation parameters between different detectors, I analysed a standard mixture of sugars (sucrose, glucose, and fructose).

At the determination of the sugars concentration I focused more on CAD (Charged aerosol detector) and ELSD (Evaporative light scattering detector) detectors, which are the new generation of detectors for High Performance Liquid Chromatography (HPLC). They are considered universal instruments, which are able to provide the response for each analyte that is less volatile than the mobile phase.

For all the HPLC detectors (CAD, ELSD, MS, RI detector), which I compared in this master's thesis, I was able to use the same chromatographic conditions. I only changed the specific settings of each individual detector (for instance: temperature of detectors, temperature of columns or samples, gas flow rate, nebulizer temperatures and evaporation temperatures). I used isocratic elution with water as the mobile phase. The elution of sugars was performed on Rezex RCM-monosaccharide Ca 2+ column with dimensions 300 mm x 7.8 mm, 8 μm particles and column temperetature 65 ºC, with flow 0.6 ml/min and injection volume 20 μL.

As the most suitable detector with good repeatability and sensitivity for determination of individual components without UV chromophores was proven CAD detector. It is also distinguished by simple and reliable operation. Although we have shown that MS detector is approximately 200 times more sensitive, its disadvantage is the high price of MS instruments, which limits its use for routine analyses. The good characteristic of the mass spectrometer is definitely its ability to obtain the structural information. The choice of optimization parameters for the ELSD detection is very demanding compared to CAD detector which has significantly less settings and is therefore more user-friendly.

Keywords: method development, excipients, sweeteners, liquid chromatography, detectors, *DAD, RI, CAD, ELSD, LC-MS*