Method optimisation and sugar analyses by liquid chromatography with refractive index detection

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Introduction

IFPEN took part in a big project in sustainable development. This project had to develop an ethanol production process with biomass. One of the most important directives was biomass conversion research into fuel and some other chemical intermediaries. It is therefore important to develop processes, technologies and products to manufacture the second generation bio ethanol from vegetables or green waste. IFPEN's teams are involved in the process development, pre-treatment, prototype conception and enzymatic hydrolyze optimisation. The sugar analysis formed part on the latter point.
Those samples contained monomeric sugars (glucose, xylose, galactose, arabinose and manose) and one glucose oligomere (cellobiose). These were the different sugars to be quantified with an internal calibration, although different compounds in samples could interfere with the sugar signal. Therefore specific samples had to be prepared.

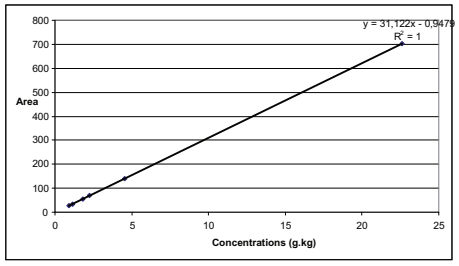
Experimental methods

The samples were prepared by a 1/10 mass dilution and a filtration with a C18 cartridge (silicon matrix with some alkyl functions C18). This filtration was used to eliminate degradation products due to previous treatments.
Injections were automatic, and the samples were kept in a refrigerated room at 4°C. When the temperature increased, sugars degraded themselves.

To analyse the sugars, two Biorad deashing cartridges holder were used to protect the column from different salts. The HPLC was equipped with a BIORAD Aminex carbohydrate Analysis Column. Stationary phase was some 9µm polystyrene divinylbenzene balls. Ultra-pure water was the only mobile phase used in this case, because of this column. The rate of flow was 0.6mL.min^{-1} with a temperature of 90°C of the column. To detect signals, a refractive index detector heat at 50°C was coupled to the HPLC. It measured the difference between the sugars refractive index with ultra-pure water (as a reference because it was the mobile phase).

To quantify the sugars in each sample, an external calibration technique was used with 19 calibration samples. Those samples were composed by a known concentration of each sugar, from low concentration to high concentration, to observe a linear increase of the calibration curve (area in function of the sugars concentration). There was one curve for each sugar. We determined the concentration of sugars in the samples with the calibration curve equation.

External calibration of glucose



External calibration curve of glucose

Conclusion

Concentration of each sugar of each sample was determined with the external calibration. The results were logged and processed with internal software. IFPEN has a strict quality policy, for example, 95% results have to be given on time, and 100% had to be given on time with 20% more. These criteria were measured as part of the client satisfaction survey. Research is continuously carried out to improve sugar separation methods and to reduce the actual analysis time.