ABSTRACT

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Title of the diploma thesis: Development of on-line solid phase extraction using flow methods

The goal of this diploma thesis was to create and validate method using combination of SPE extraction and flow methods (in this case method SIA).

Creatinine was chosen as extracted analyte and urine was chosen as biological material.

At first, the method was optimized (concentration and flow rate of wash reagent and flow rate of sample and standards). Then the limits of the created method were tested. Finally, the trueness and repeatability of results was tested and also stability of diluted samples.

All of these tests (in thesis named experiments) had good results. Final parameters of method are:

- Sorbent – SPH Iontosorb 100
- Wash reagent – 50% acetonitrile, aspirated 0,5 ml
- Elute reagent – 1% water solution of ammonia hydroxide, aspirated 0,5 ml
- Sample diluted by acetic acid with pH 2,4, aspirated 20 µl, or 10 µl according to sample concentration (method alone makes decision about changing aspirated volume, decision algorithm is in the program)
- Method of standard adding is used for measuring creatinine concentration in samples. Used standard have concentration 2mg/l, this standard is aspired at volumes 0, 10, 20 µl. Samples are measured in doublets.

- For detection, absorbance at 235 and 270 nm is measured, for calculations, difference of absorbance at 235 – 270 nm is used.
- Time of one measuring: 130 s

Method using optimized parameters have error rate around 5 %.

Results gained by measuring creatinine in urine samples with created method have good comparison with results gained by using method used at clinical laboratories. Goal of thesis was reached in thanks to follow-up experiments and created method is ready for next enhancing.