

Fully Automated High Throughput Sample Preparation and Acquisition on an Exploris 240 for Aqueous Samples

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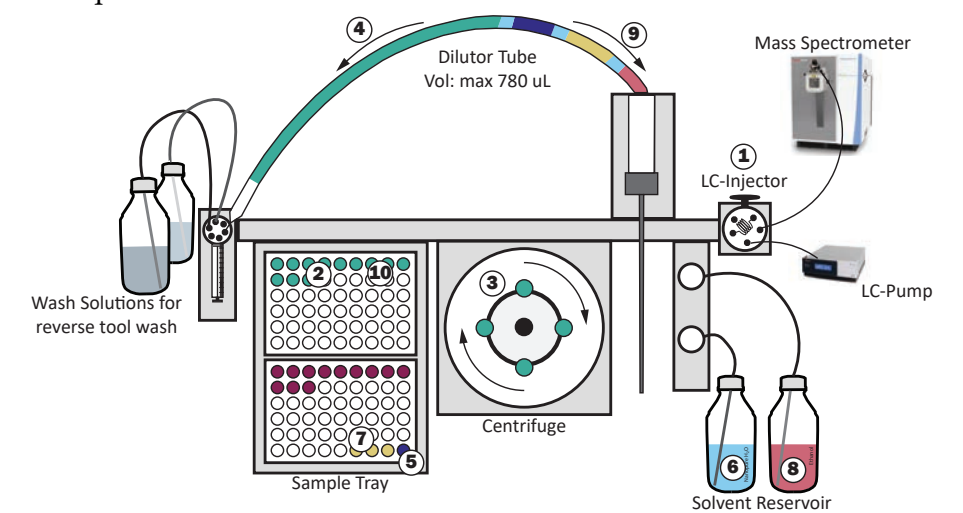
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AIM: DEVELOP A FULLY AUTOMATED SAMPLE PREPARATION

- Sample Preparation needs automation to increase throughput
- As hands off as possible: From native sample to data without interaction
- Automized preparation needs to be reliable and reproducible
- Extracts are desired for reinjection and further experiments
- Requires a user friendly interface so it can be used without programming skills
- Ideal for aqueous environmental samples such as groundwater, surface-water, raw and treated wastewater

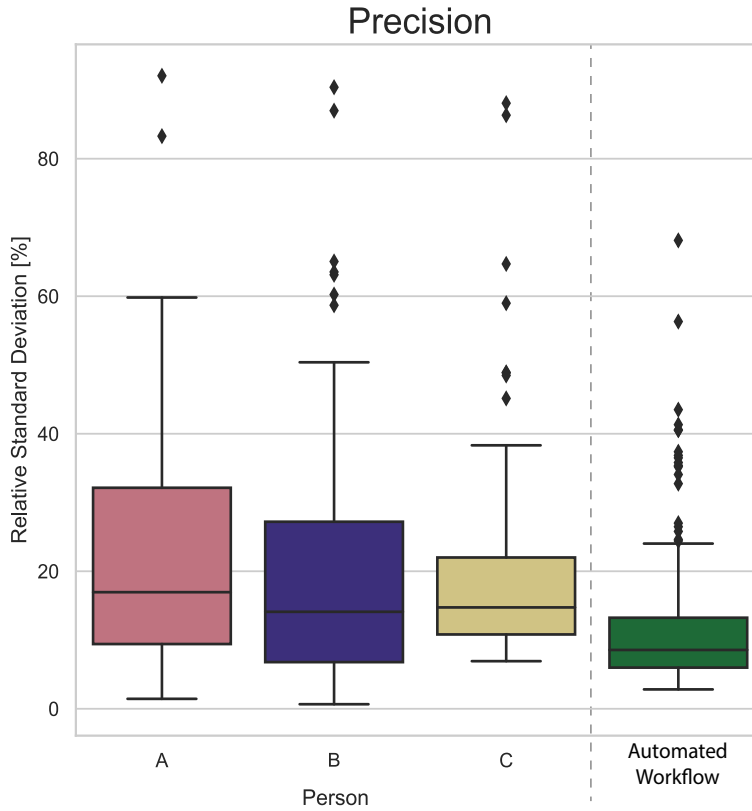
THE DEVELOPED METHOD: (see next page for detailed description)

- Goes from native sample to data without manual interference
- Centrifuges sample and uses a dilutor tool to sequentially add sample, internal standards and standards
- Dispenses this into a new vial thus creating extracts which can be used for reinjections and other experiments.
- Optimizes instrument time through interlacing the preparation and acquisition.

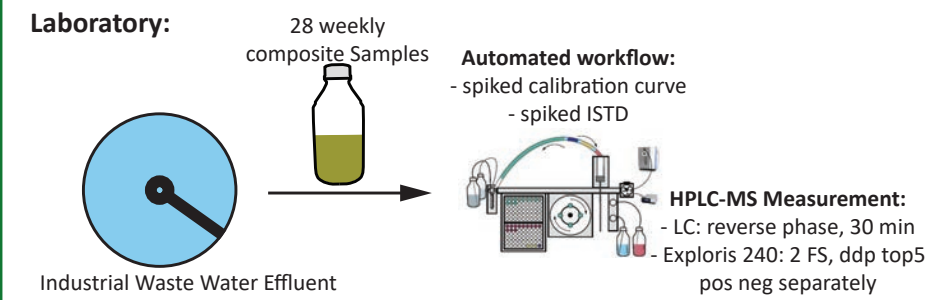


RESULTS:

- Three coworkers were asked to compete against the automated workflow
- 7 samples were prepared each, spiked with standards and internal standards.
- Relative standard deviation of the measured peak area was determined for 150 compounds.
- The automated workflow outperformed the manual performance and required less time in the lab for preparation.
- Reinjection of the same sample produces a relative standard deviation of 5 %.

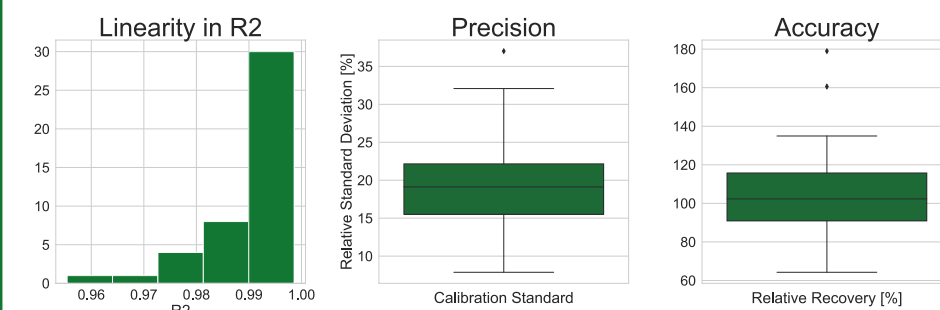


APPLICATION:



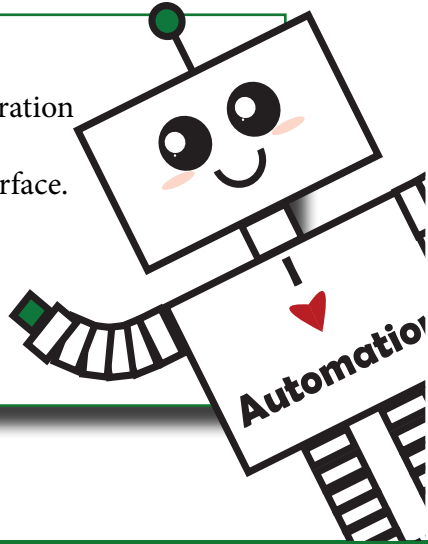
Data Analysis:

Σ 44 Targets and isotope labelled internal Standards



CONCLUSION

- The developed workflow outperforms manual preparation
- Hands off from native sample to data producing, extracts for reinjection all with a userfriendly interface.
- Application of the method to environmental samples showed good measurement parameters and QCs even in heavy matrix.



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METHOD:

- 1 INJECTION:**
FIRST ACTION OF THE WORKFLOW IS AN INJECTION.

Run Time per sample:
- 35 minutes

Liquid chromatography:
- Separated on C18 RP Atlantis T3, 1.5x300 mm
- 100 µL injection Volume
- Linear gradient from 0 % MeOH + 0.1 % FA to 95 % MeOH
- Flow rate: 300 µL/min

Mass Spectrometry:
- Exploris 240 from ThermoFisher
- 1 FullScan at 240k followed by top 5 ddp MS2 at 15k

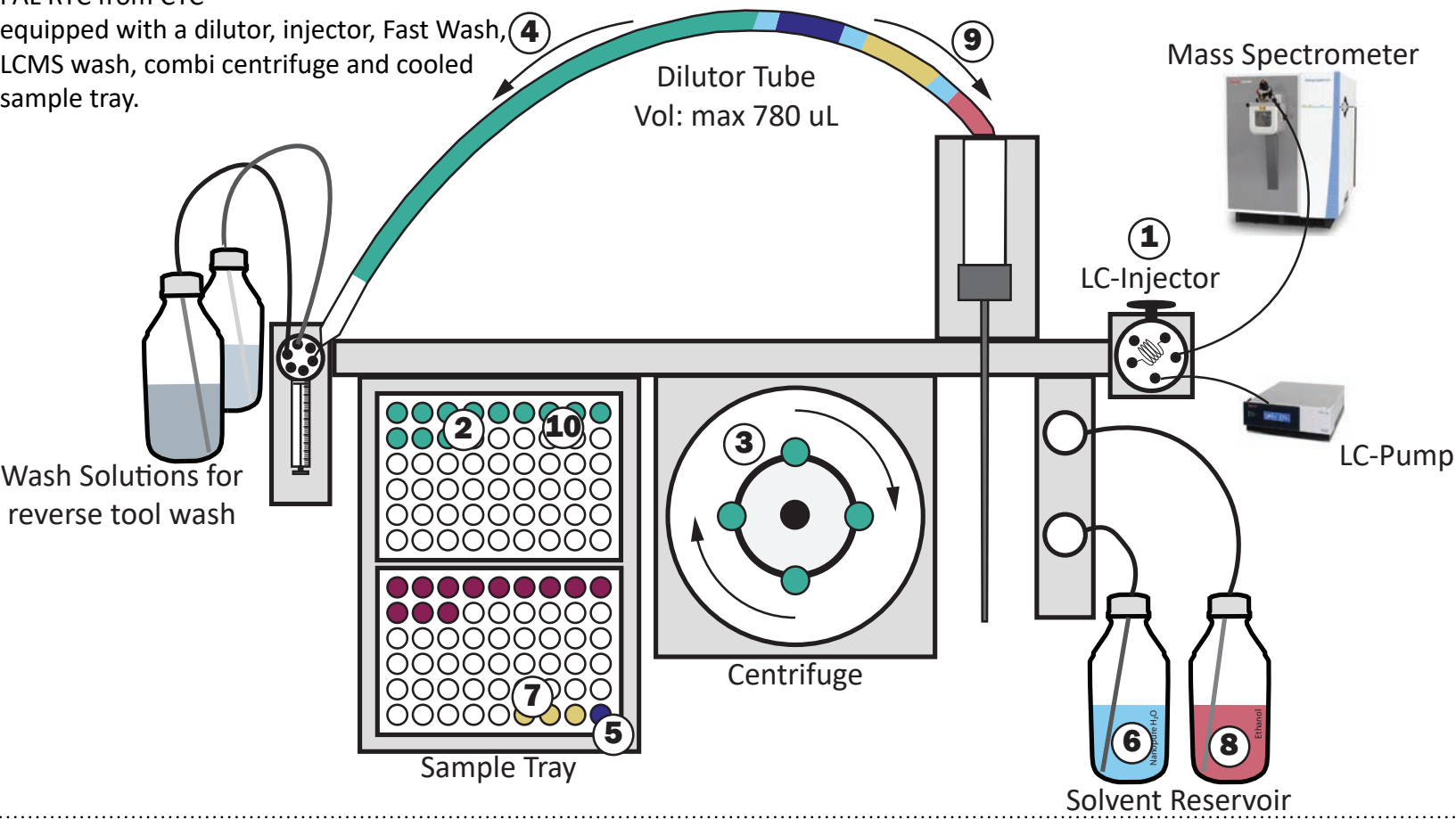
- 2 TRAYHOLDER:**
Two racks are used for this method. The native samples (1.5 ml) are placed in the back. The front rack holds empty vials. Now each native sample has a corresponding aligned empty vial.
- 3 CENTRIFUGATION:**
4 Samples are moved to the centrifuge with a magnetic tip on the dilutor tool. Samples are centrifuged at 2000g for 3-5 minutes depending on the matrix. Afterwards the centrifuged samples are moved back to the sample rack.

- 4 ASPIRATION OF SAMPLE:**
A dilutor tool equipped with a 780 µL tube and a 1 ml syringe was used. At first, the native sample is aspirated, normally between 500 to 650 µL.
- 5 SEPARATION AND CLEAN WITH NANOPURE**
A small volume (10 µL) of Nanpure is aspirated from the solvent reservoir to clean the outside of the needle and separate the sample from standards.
- 6 ADDITION OF ISOTOPE LABELED INTERNAL STANDARD (ISTD)**
To account for instrument fluctuations, degradation and other fluctuations, ISTD are added to each sample. A small volume of the mix (normally 10 µL) is also aspirated into the dilutor tube.
- 5 SEPARATION AND CLEAN WITH NANOPURE**
Step 5 is repeated in order to clean the outside of the needle.
- 7 ADDITION OF STANDARD AND EQUALIZE VOLUME**
If applicable (Calibration points, standard addition) a specific volume of an organic standard stock solution is added through aspiration of the dilutor. Volumes range from 10 to 50 µL.
- 8**
In order to always have the same sample amount and organic content the workflow aspirates an equalizer volume of organic solvent depending on the added standard volume.

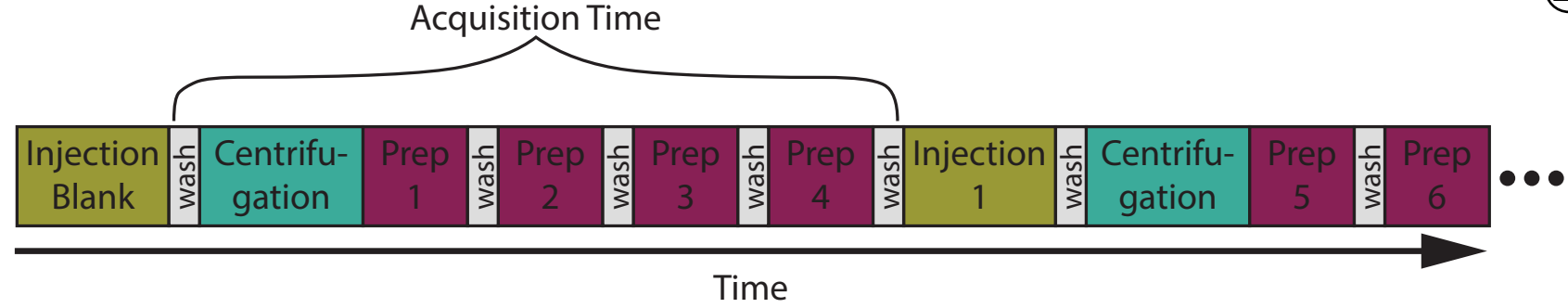
- 9 DISPENSE AND MIX SAMPLE**
The entire sample is located in the dilutor tube at this point. The dilutor head moves to the tray containing the empty vials and dispenses the entire content into the vial. The content is aspirated into the tube and dispensed again a few times for complete mixing.
- 10 PREPARE SAMPLES 2,3 AND 4**
The Steps 4 to 9 are repeated for the remaining centrifuged samples. Afterwards the autosampler waits for the acquisition to end and will then continue with the next injection and preparation of samples 5 to 8. At one point all samples are prepared and the autosampler will continue with injections until the sequence is done.

SETUP:

PAL RTC from CTC
equipped with a dilutor, injector, Fast Wash,
LCMS wash, combi centrifuge and cooled
sample tray.



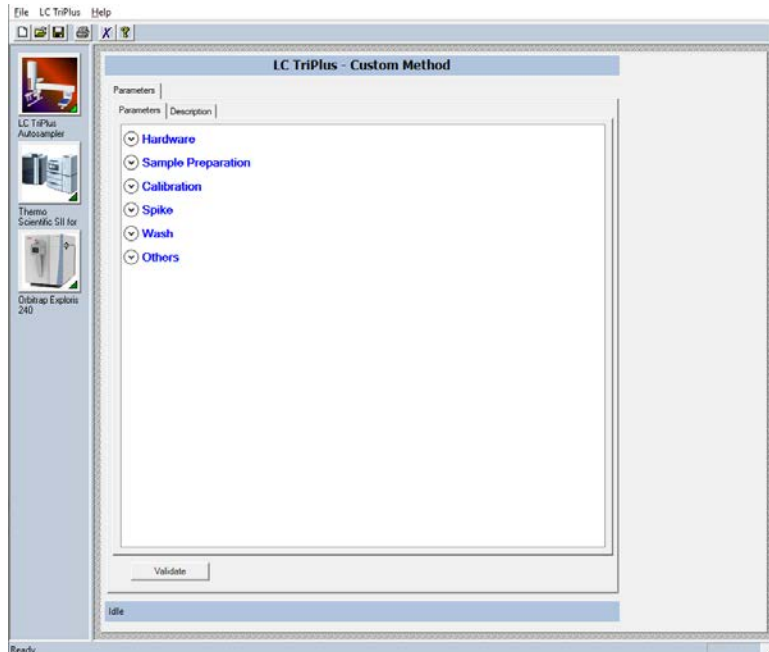
TIME LINE:



SETTINGS THROUGH GUI

The workflow was scripted using PALScriptEditor and its object based programming language. The Script was exported as xml file and can be imported through Xcalibur Method editor.

ONE METHOD FOR ALL:
All settings are set in one method, from defining calibration points, to sample volume, centrifugation speed or disabling sample preparation completely.



KEY POINTS:

- OPTIMIZED TIME USAGE THROUGH INTERLACED ACQUISITION AND PREPARATION
- CREATES EXTRACTS, AVAILABLE FOR REINJECTIONS
- CAN GENERATE CALIBRATION CURVES AND SPIKE SAMPLES
- FULLY HANDS OFF FROM SAMPLE TO DATA
- CONTROLLED AND ADJUSTED THROUGH A GUI IN THE XCALIBUR METHOD EDITOR
- DEVELOPED AND IDEAL FOR AQUEOUS ENVIRONMENTAL SAMPLES (GROUND WATER, SURFACE WATER, RAW AND TREATED WASTE WATER)