MICRO VOLUME INJECTIONS

THE SMARTEST LC-EC APPLICATIONS FOR NEUROSCIENCE ANALYSIS EVER MASTERMINDED

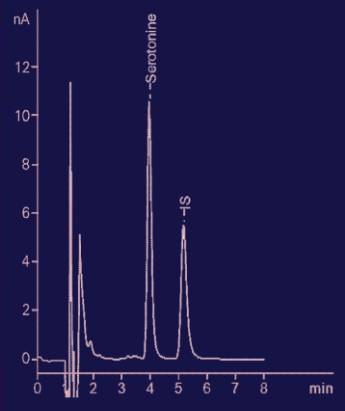
Monoamines and the metabolites
- *Noradrenalin*
- *Dopamine*
- *Serotonin*
- *5-hydroxyindole acetic acid (5-HIAA)*
- *3,4-dihydroxyphenylacetic acid (DOPAC)*
- *homovanillic acid (HVA)*

OPA derivatized amines and amino acids
- *GABA and Glutamate*
- *4-aminobutyrate (GABA)*
- *Glutamate (Glu)*

Choline and Acetylcholine
- *Choline (Ch)*
- *Acetylcholine (ACh)*

Markers for oxidative stress
- *3-nitro-L-Tyrosine*
- *8-OH-DPAT*

Glutathione and other thiols



INTRODUCTION

One of the unique features of the ALEXYS AS autosampler is the option to use an user program for injection.
A user program is used for aspiration and dispensing any volume addressing any vial and timed control of valve position. Particularly in micro LC this provides a powerful tool to minimise sample consumption without compromising reproducibility and peak efficiency.

- Small sample handling
- Inject 3 out of 4 uL
- Minimized sample broadening

Summary

In a user program a well-defined sample volume is aspirated and transported to the sample loop quantitatively with minimum peak broadening. Mobile phase is used as ‘transport liquid’ to push the sample into the loop.

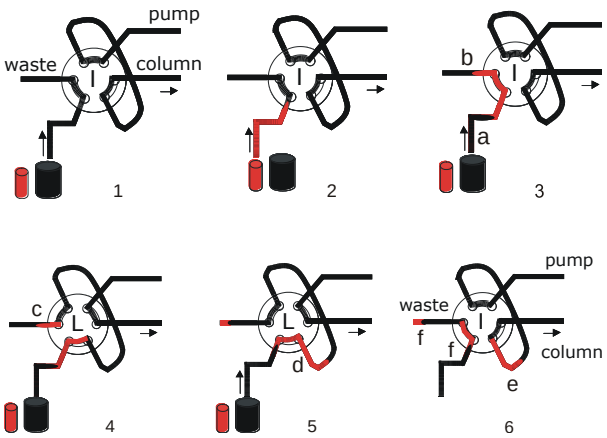


Fig. 1. The sample (red) is aspirated from the vial into the needle tubing (step 1, 2). To minimise sample consumption mobile phase (black) is used to carry the sample into the loop. Diluted front and tail are cut off (step 3-6).

Method

The ALEXYS 100 LC-EC System (cooled, micro) is used with a microbore VT-03 flow cell. The working potential was 350 mV (vs. ISAAC REF) and the DECADE II oven temperature was 35°C. A 100 x 1 mm ID reversed phase column and a flow rate of 0.05 ml/min were applied. The mobile phase pH was 6.0 and contained 50 mM phosphate, 100 mg/L OSA, 8 mM KCl and 0.1 mM EDTA. The individual steps of the injection method have to be translated in programmable steps. An extensive set of autosampler actions is available for programming. The injection performance is evaluated with special attention for reproducibility and column efficiency.

Programming the software

In the ALEXYS data system an injection method is programmed in 'method programming/user program' section of the AS 100 driver. In the AS 100 user manual details are given how to program a sequence of steps (Chapter 4).

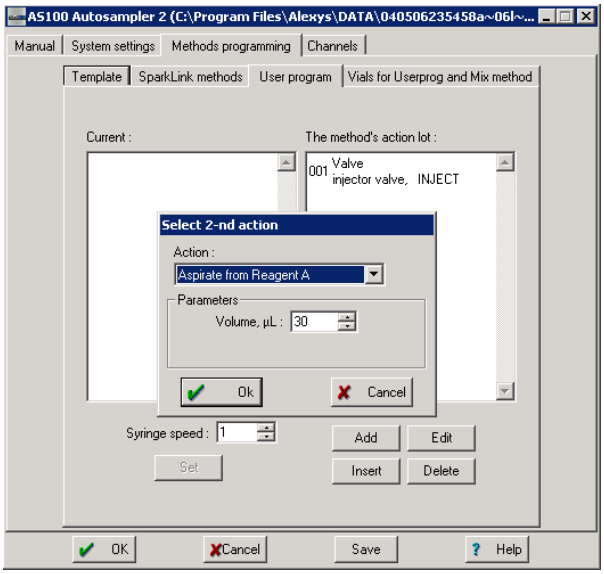


Fig. 2. Click Add, Delete, Insert or Edit to create a user program. Click 'Set' to add the programmed steps to the system method.

Injection steps are:

Program step	Description
001 Valve injector valve, INJECT	switch valve
002 Aspirate from Reagent A Volume - 0030 µL	fill valve with mobile phase
003 Aspirate from Sample Volume - 0004 µL	sample intake (X)
004 Aspirate from Reagent A Volume - 0002 µL	fill non-relevant remainder of needle (Y)
005 Valve injector valve, LOAD	switch valve
006 Aspirate from Reagent A Volume - 0002 µL	take up another 2 µL transport liquid (Z) to push 2 µL sample into the loop
007 Valve injector valve, INJECT	switch valve
008 Aspirate from Wash Volume - 0200 µL	needle wash
009 End	

See appendix for relevant AS 100 settings, and a 'UP514' example.

Method optimisation

Although the principle is straightforward, optimisation is important to minimise sample consumption, without compromising peak reproducibility and plate numbers.
It is important that the sample is aspirated until the diluted front has passed the entrance of the valve, because only then a non-diluted sample front is transported into the sample loop. All cases in *Table 1* meet that requirement. In case of '432' some of the diluted tail is aspirated in the loop resulting in smaller peak height. From *Table 1* it appears that aspirating 5 µL sample followed by 2 µL transport liquid resulted in the largest peak area. If sample volume is really an issue the '422' method applies as well and, as indicated in *Fig. 3* provides excellent reproducibility. Peak efficiencies are similar in all cases.
The precision of the '422' method was investigated by running a sequence of 10 replicate injections of 0.1 µmol/L DA and 5-HT. The RSD in peak area was better than 0.5 and 0.7% for DA and 5-HT respectively. RSD in retention times were better than 0.2% in all cases. These results do not differ significantly from partial loop fill injections of 5-HT.

CONCLUSION

To minimise sample consumption the applicability of injection programming with the AS 100 has been demonstrated for micro HPLC. Injection volumes of only 2 µL can be programmed out of 4 µL aspirated sample. Under optimised conditions RSD of peak areas was better than 0.7 % and RSD of retention times was better than 0.2%. Plate numbers and RSD of peak areas and retention times were similar to a partial loop injection procedure.

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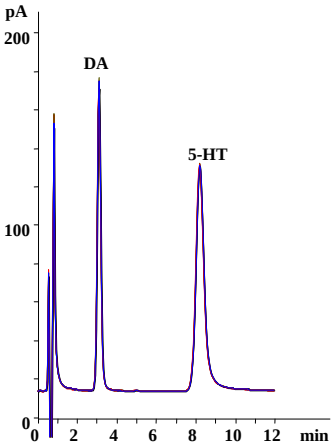


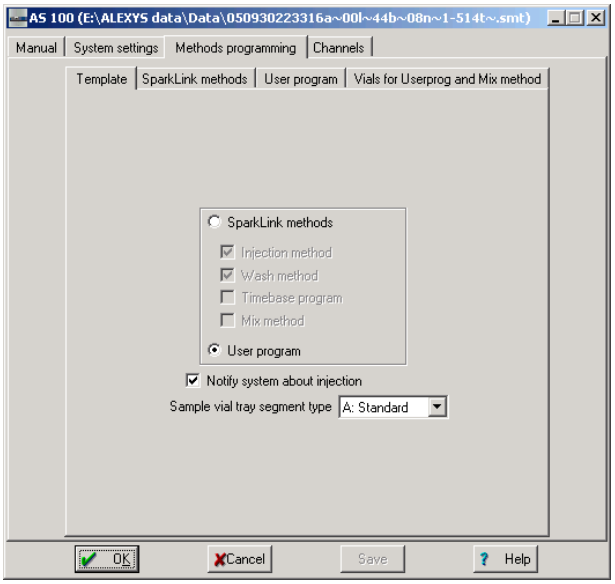
Fig. 3. Overlay of 10 replicates of 0.1 µM DA and 5-HT with injection programming using the '422' method.

Table 1. Comparison between different volumes selected. X is the aspirated sample volume, Y is the following transport liquid. Z is the aspirated transport volume after switching to Load being in all cases 2 µL of a 0.1 µM DA and 5-HT mixture in 0.01M PCA.

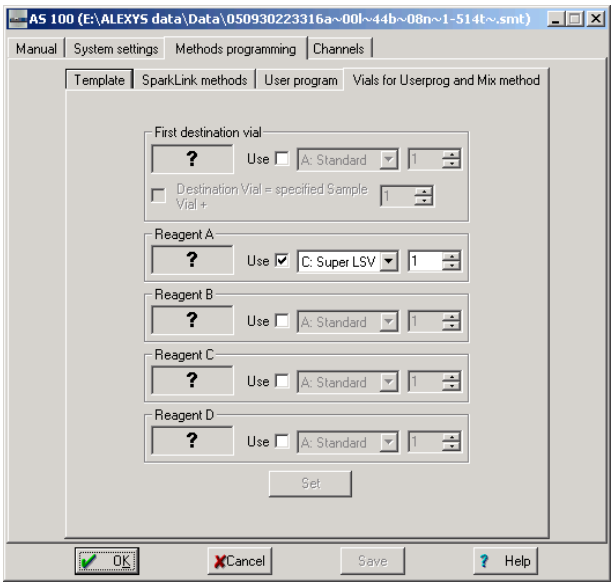
	DA		5-HT	
injection method XYZ	peak area (pA*sec)	Plate number plates/m	peak area (pA*sec)	Plate number plates/m
422	1705	28497	2570	34963
432	1563	28010	2346	34956
512	1739	28273	2620	34864
522	1830	28647	2772	34518

Appendix 1. AS 100 settings

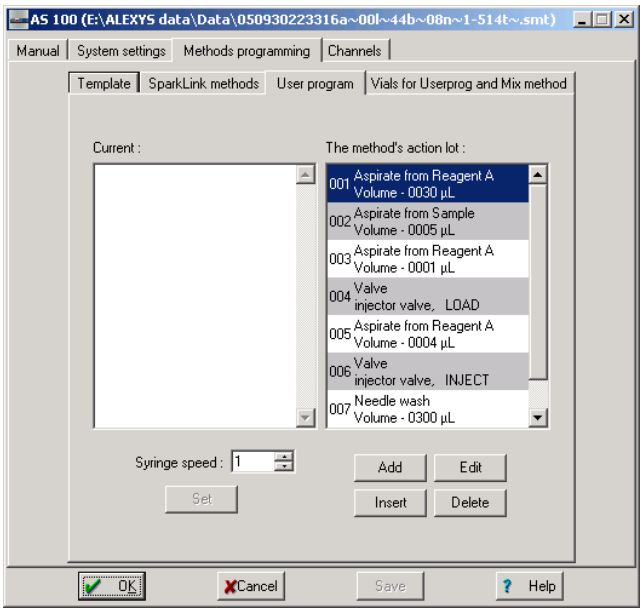
The AS 100 driver must be configured in a way that the user program is selected for injection. This is done in the ‘template’ tab. In the ‘Vials’ tab the vial containing transport liquid (mobile phase) must be assigned. In this example a large sample vial (LSV) is used, but also an A-type vial can be used.



Select ‘User program’.



Reagent A vial must contain mobile phase. With one vial containing 1 mL mobile phase, about 20 injections can be done.



In this example a UP514 is used. Click the ‘User program’ tab and add the following lines:

Line	
001	Aspirate from Reagent A Volume - 30 µL
002	Aspirate from Sample Volume - 5 µL
003	Aspirate from Reagent A Volume - 1 µL
004	Valve injector valve, LOAD
005	Aspirate from Reagent A Volume - 4 µL
006	Valve injector valve, INJECT
007	Needle wash Volume - 300 µL
008	End