



AUTOSAMPLER **ASAM L40**



Instruction manual

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0. Introduction

Autosampler ASAM L40 is destined for multiple injection of liquid samples into HPLC chromatograph CHROM L10 made by Labio a.s., Prague, Czech Republic. It can be used in connection with other manufacturer chromatographs too. It allows injection upto 40 samples and (in connection for example with Dataapex Clarity chromatostation) sequential record and evaluation of chromatograms.

0.1 Technical parameters:

| | |
|--------------------------------------|-------------------------|
| Sample quantity: | 1 - 40 |
| Sample volume: | 0,8 – 2 ml |
| Injected volume: | 5 μ l – 100 μ l |
| Minimal interval between injections: | 3 min |
| Vials: | Chromacol, 2-SVWST-CP |
| Voltage, input: | 230 V, 65 W |
| Weight: | 10 kg |

1 Description of ASAM L40 function:

Autosampler consists of electronic block in single case with two line alphanumeric display and a keyboard staying besides the chromatograph, from carousel part placed usually on the upper panel of a chromatograph and an injection block with motorized loop injector with electronic part and power supply situated either inside or outside of the chromatograph unit in a separate box.



Fig. 1: Back side of the unit

Electronic block of ASAM L40 is connected (see back side of el. unit on Fig. 1) via 5 pin type connector to Chrom L10 chromatograph unit in order to allow to start a analysis cycle. It is connected by a multiwire cable with 25 pin PC connector to a carousel unit and optionally by a two wires cable to an electronic board of loop injector unit (in case loop injector is installed in Chrom L10, this cable is not necessary). Net socket is on the left side of the case together with main switch and a fuse.

Sample injection into a loop is made by standard syringe Hamilton type 8123 with total volume 500 μ l, which is situated on vertically moved arm of carousel tower. The arm movement is provided by the step motor. Next step motor is used to provide the syringe piston movement using a toothed rod for. Vials with samples are located in plastic made carousel which is moved by third step motors. The magazine (see Fig. 2) is equipped with 40 positions for vials and 2 positions for (i) solvent for washing reservoir and (ii)

waste liquid reservoirs. Reservoirs are made of polypropylene block and equipped with PTFE folie covers to avoid liquid evaporation. A free hole in carousel is used to move the syringe down to an injection port.



Fig. 2. Carousel part on the chromatograph

The injection port is made of stainless steel in combination with PTFE sealing (see Fig 3) and connected via an O.D. 1,6 mm PEEK capillary to the loop injector. Syringe is located just above the injection port. The syringe can be removed when piston fixing tool and two fixing bolts with top part of plastic sheet are removed (see Fig. 4).



Fig. 3 Injection port under the carousel

All vials for the autosampler are standard style, they are equipped with a cap and under the cap is inserted a silicon rubber/PTFE membrane. The plastic carousel with vials is covered by a ring made of stainless steel with the same diameter as the carousel. The ring has holes with pressed metal cones on the same place vials are in the magazine. It is to conduct injection needle inside the vial cap properly.

The function of auto-sampler starts with magazine movement to the position of washing reservoir with solvent under the syringe position. The syringe is moved down to permeate the needle to the reservoir through a PTFE membrane. Solvent is sucked to the full syringe volume and syringe is moved up. The magazine rotates to the waste position, syringe is going down again and the solvent is flushed to the waste reservoir.

Attention: the carousel part movement starts already in a moment when ASAM G40 is switched on in order to make an autotest of the system.

When injecting, the sampler rotates the magazine to the position of first vial and syringe is moved down to deep the needle inside the vial.



Fig. 4 Syringe connection

Full syringe volume is then sucked and flushed 2 times. Then the prescribed volume is filled into the syringe and it is moved up. The magazine is then rotated to bring the through hole under the syringe.

The syringe is moved down to the deepest position and the volume is injected into injection port of the sampler. The syringe is kept in the position and loop injector changes its position to insert a loop into a mobile phase path. The syringe is stepping up and the magazine movement to the position of washing starts again. The syringe is filled with solvent again. The magazine rotates to the waste position and the solvent is flushed to the waste vial. In the meantime loop is switched to filling position.

The autosampler is ready to start next injection process.

2. Description of installation on CHROM L10

1. Installation of ASAM L40 to the CHROM L10 chromatograph is easy. Injection valve is already installed in the chromatograph. The carousel is put on the top panel of the instrument (see Fig. 2). The connection of a line between chromatograph and the electronic box has to be done then, following by the connection of electronic box to the carousel unit. All instruments have to be switched off during this procedure.

2. The standard input capillary of the injector is not to be removed. Capillary from an injecting port of autosampler is equipped by a needle on its end. The needle is connected to an injection port of chromatograph which is regularly used for manual sampling (see Fig 5). Both injection ports have the same design with sealing PTFE insert.

3. Central bolt on the carousel is released and plastic magazine with covering lid is removed and filled with vials. Solvent reservoir is filled. Magazine is assembled back to the position.

4. The side covering sheet on the carousel tower is removed and a syringe is then inserted in. The grip of the piston is inserted into the hole in the piston holder on the endpart of vertical connecting rod. The holder bolt is tightened such way, to leave the grip a bit free. The syringe is then moved up against the piston to be totally empty. It is fixed in this position using two bolts and plastic plate (see Fig. 4). Mentioned bolts have to be tightened properly to preserve the syringe movement!

5. The chromatograph is then switched on.

Taking care to give out all objects which can prevent the autosampler movement, the autosampler is switched on too. The system use the start routine to check all positions and movements of the unit and washing syringe. Then stops in the start position.

6. The ASAM L40 is programmed according manual (see Section 3). Time of analysis is selected to be longer than time of gradient program and time of wavelengths program programmed on the chromatograph.

7. The chromatograph is checked to be preprogrammed rightly and in „Pump running“ state. The system is started by the START button on the autosampler. Operator has to follow all operations of autosampler during first run to be ready to switch it off when not expected event occurs.

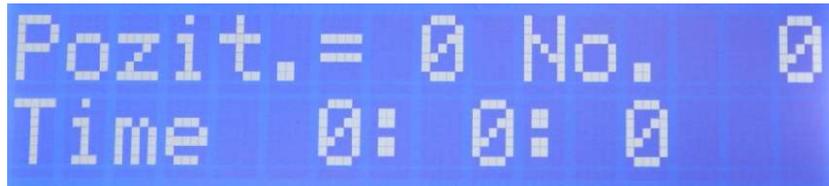


Fig. 5 Chromatograph injection port

3 Description of autosampler programming:

ASAM L40 is programmed in order to set washing parameters, injecting volume (to a loop) and to select either sequential or random approach to vials. Finally of course a time of particular analysis is selected. Programming is done by a twoline alphanumeric display and eight buttons keyboard. There are horizontal and vertical arrows on the keyboard used either to list in menu (vertical) or changed values (horizontal). There is an ENTER button confirming each programmed value.

When switched on the following message is shown on the ASAM L40 display:



Pozit. = 0 No. 0
Time 0: 0: 0

Listing in the menu next screen appears, where position time is selected. This is in fact time of analysis increased for small reserve:



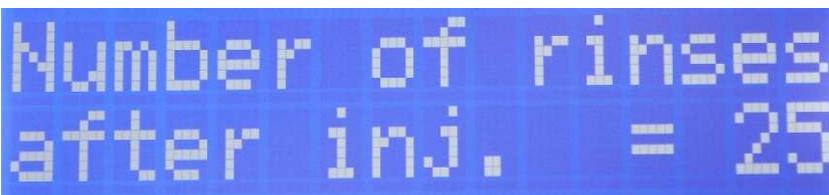
Position time =
9:59:30

Next screen defines number of rinses with solvent before injection. Usually 2-3 rinses is enough.



Number of rinses
before inj. = 25

The same situation is with after injection rinses:



Number of rinses
after inj. = 25

Injection volume on the autosampler ASAM L40 screen is in fact not an injection volume. It is the volume which is injected into loop injector loop. It has to be relatively high in order to flush previous sample from connecting capillary. 80 % (400 ul) is usually enough.

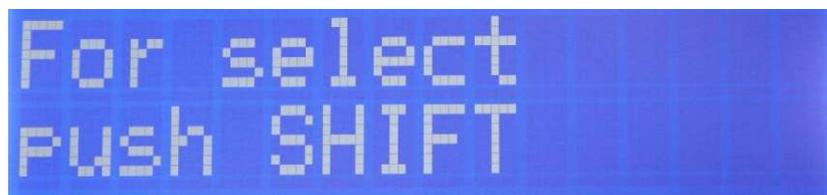


Inject. volume =
84 %

On ASAM L40 you can select either random, or sequential access to vials.



Random acces

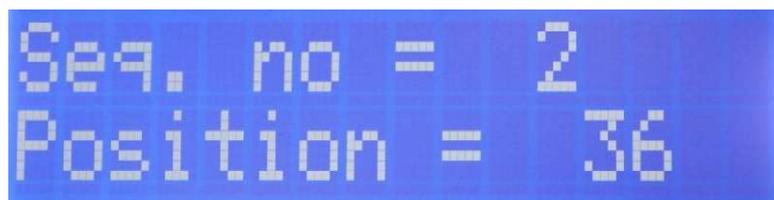


For select
Push SHIFT



Sequent. acces

In case of random access is necessary to define all positions which will be used in a row:



Seq. no = 2
Position = 36

In case of sequential access only last position has to be selected:



Last pos.
10

When all programming work is done and everything is ready with autosampler and datastation, injection sequence can start using a START button on the keyboard. In case chromatograph itself is not ready for injection next screen appears:

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Pozit.= 0 No. 0
Wait for READY

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If chromatograph is ready to start its program, following screen is appeared:

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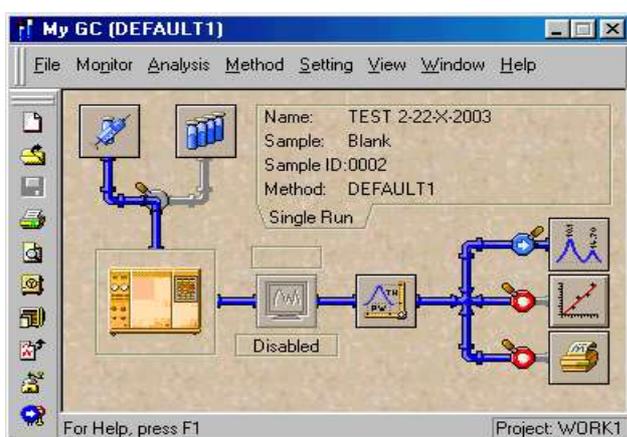
Akt. Pozit. = 1
Time 0: 1:17

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4 Use of Clarity chromatostation with ASAM L40 autosampler:

Chromatostation Clarity, can (in SEQUENCE mode) automatically evaluated analyses made with autosampler. The cooperation with ASAM L40 autosampler run with Clarity in the mode of „passive sequetion“, when integrator run is started by GC unit. For such purposes chromatostation has to be connected to the chromatograph by the starting cable (jack on the left back side of the chromatograph). It is necessary to specify evaluating method and to define calibrating file and fill sequence table before start to work. There are specified codes which are given to the sequence of chromatograms.

4.1. Analyses of samples using autosampler ASAM L40 and chromatostation



It is necessary to develop calibration file for the type of analysis which will be run. The method of the preparing of calibrating file is the same as for single analysis (see Clarity manual).

Before starting analyses with Clarity and autosampler, the chromatostation instructions has to be modified. The modification starts with the basic screen opening sequence window when clicking on the icon in left top corner of the screen.

Sequential file is opened in dialog window **Open Sequence**. The sequential table is appeared. The table has to be filled. **Sv** is

the number of starting position (=1), **Ev** is number of the last position. **IV** specifies number of injections from one vial (=1). Then the row **Sample ID** and **Sample is filled** (specification of samples which is used in the protocol). Next are **Sample Amount**, **ISTD Amount** (internal standard amount), **Sample Dilut(ion)**, **Inj. Volume** and finally **File Name**. File name presents the name of individual analysis (one row). The symbol % can be used to introduce serial number starting with 1.

The row **Std** is defined as **No** (sequence without calibration change). Row **Lvl** is empty. **Method Name** means the method file name which will be used. **Report Style** means the file name where the printing details are saved.

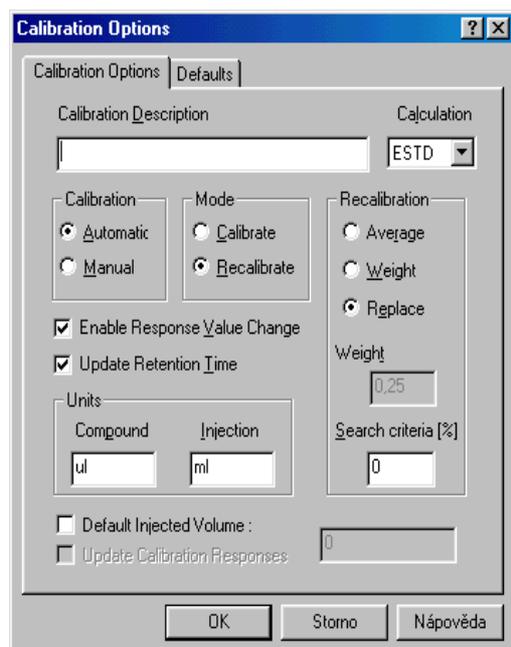
Caution : In case the row **Std** is filled like „Yes“ and in row **Lvl** in given some number, the station automatically makes the recalibration and calibration data are replaced. Results have no sense then.

It is necessary to click on **Sequence icon**, to start sequential analyses and in the sequential window to activate the button **Run**. In the row **State** in bottom part of the window appears the message **Waiting**. It means that computer is waiting on the start of the chromatograph with autosampler.

Starting analyses (pressing **START** button in working mode), the fully automatic process begins. System takes samples from vials, gives the file name for the analysis, the separation and its monitoring proceeds, primarily file is named, the curve is recorded and integrated and concentrations are calculated using calibration file. Results are sent to the printer.

In the row **Vial No**: in the bottom part of sequential window is displayed actual position number during the process.

Sequential analyses can proceed with continual recalibration in CSW. On first positions in the magazine must be inserted vials with standards when recalibration is accepted. The same quantity of standards with the same concentrations and the same order as in original calibration has to be used.



Sequential table (opening the window before) is to be filled. In rows **Std** is used „Yes“ and in rows **Lvl** a number if calibration level is written.

The windows **Calibration** is then open and prepared calibration file is opened. In **Calibration** window a window **Calibration Options** is then opened .

The option **Calibration Automatic** is selected. Next option is **Enable Response Value Change**. **Recalibrate** is selected as the mode. Type of **Recalibration** is specified. The option **Replace** is selected when old values are to be replaced. **Average** makes the old and new values average and **Weight** means that new values are added to old ones in the ratio which has to be specified.

Then **Calibration** window is closed, and **Sequence** opened. RUN button is activated and the sequence of analyses started by the GC unit.

Standards are first evaluated now and new results are used to evaluate concentrations in analysed samples.

3. Manufacture and maintenance service

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