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**ClinLab<sup>®</sup>**

Instruction Manual

for the

**Digital**

**Electrochemical Detector**

**EC3000**



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RECIPE

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## 1. COMPONENTS OF THE EC3000 AND ACCESSORY

Order No.	Description	Quantity
EC3000	<b>ClinLab® Digital Amperometric Detector, Model EC3000, complete</b> consisting of: Digital electronic unit, power supply, cell-cable, complete cell, installed in cell housing, ready-to-use, user manual	1 pce.
	<b>Accessory:</b>	
EC4000	<b>ClinLab® ECD-Cell, Model Sputnik®, complete</b> consisting of: cell, tubings, fittings, glassy carbon working electrode, gaskets, reference electrode, wrenches, 3 M KCl, manual	1 pce.
EC4010	<b>ClinLab® Accessory Kit for ECD-Cell, Model Sputnik®</b> consisting of: tubings, fittings, glassy carbon working electrode, gaskets, reference electrode, wrenches, 3 M KCl, in assortment box	1 pce.
EC3101	Power supply	1 pce.
EC3102	Multiple shielded cell-cable for cell EC4000, Model Sputnik®	1 pce.
EC3103	Autozero-cable with universal-terminals	1 pce.
EC3104	Autozero-cable with HITACHI-connector	1 pce.
EC1103	BNC-cable for analog-output with universal-terminals	1 pce.
EC1104	BNC-cable for analog-output with HITACHI-connector	1 pce.
EC1110	BNC-cable for analog-output with adapter for Shimadzu-Integrator	1 pce.
EC3052	User manual	1 pce.

*Pricelist for service and spareparts on request!*

## 2. INTRODUCTION

### 2.1 Unpacking

- Unpack the EC3000 and the accessory from the transport box and
- check the detector and the measurement cell for transport damages. Please contact RECIPE immediately in case you discover any damage.
- Check the contents of the transport box (see section 1).

**Note:** Please retain the original packing materials. These packing materials are intended for the shipping of the unit (e.g. in case of repair). Shipping of the unit in any other packaging may lead to the termination of the warranty.

### 2.2 Warranty

The standard warranty coverage for this unit is in accordance with the conditions of sale. The warranty has a duration of one year from invoice date and covers materials and labour. Please note that the wear-parts cannot be covered by the warranty.

The warranty coverage shall become invalid in any case identified as resulting from inappropriate use, service or the implementation of non-specified spare parts. Similarly the warranty coverage shall be invalidated in the event of inappropriate shipment, packaging or failure to remove aggressive or damaging solvent residues.

### 2.3 Purpose of the unit

The electrochemical detector EC3000 is designed for the use in analytical HPLC systems. Please note that the instrument may only be used in consideration of its technical specifications (see section 7). RECIPE is released of any claim for material or immaterial damages caused by inappropriate use.

### 3. THEORY OF THE ELECTROCHEMICAL DETECTION

#### 3.1 Electrolysis reactions

In contrast to other methods the electrochemical detection changes the chemical nature of the detected substances by oxidation or reduction. After the sample substances are separated in the column they pass the surface of the working electrode (on which the electrochemical reactions will occur). The potential of the working electrode is kept constant in relation to the potential of the electrolyte (measured by the reference electrode).

Thus, the ECD keeps a constant voltage between the working and the reference electrode.

Every chemical reaction needs a certain amount of energy to take place (=activation energy, see figure 1). This energy is supplied by the potential of the ECD.

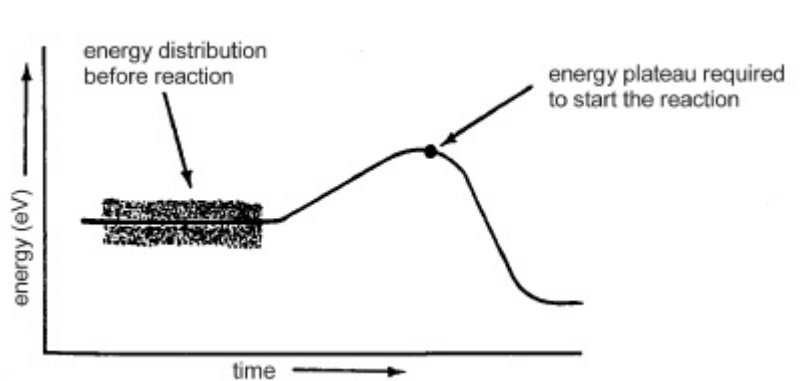


figure 1: kinetic profile of a chemical reaction

In case of a positive potential of the working electrode in relation to that of the electrolyte the molecule loses one or more electrons (oxidation). In case of a negative potential the molecule will be reduced (the electrode gives one or more electrons to the molecule).

All sample molecules contain heat energy (inner energy). The energy distribution in a sample solution makes a typical bell-shaped curve. This is due to the fact that some molecules require more energy than others to reach the plateau necessary to start reaction (see figure 2).

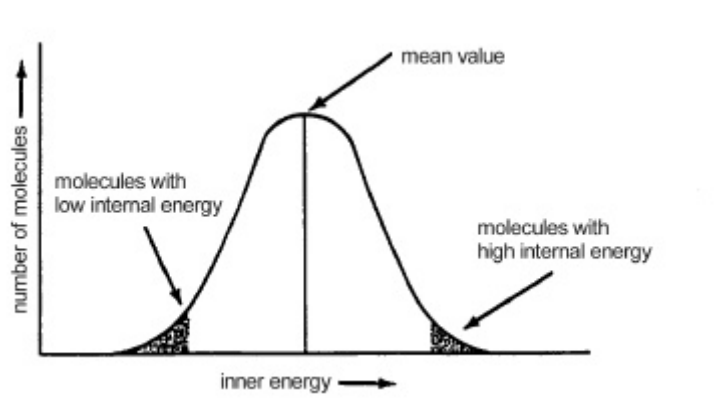


figure 2: energy distribution within a sample of molecules

The following equation shows the energy relation in the electrochemical system:

$$\text{Voltage} = \frac{\text{Total Energy}}{\text{Charge}} \quad \text{or} \quad \text{Total Energie} = \text{Charge} \cdot \text{Voltage},$$

where the voltage is expressed in volts (V) and the charge is Faraday's constant ( $9.65 \cdot 10^4$  C/mol).

The current which is produced by the electrochemical reaction is proportional to the concentration of the analyte which is passing the measurement cell at the moment. The following equation shows the correlation between current and concentration:

$$i = N \cdot F \cdot K \cdot D^{2/3} \cdot C$$

$i$   $\equiv$  current produced by the redox reaction  
 $N$   $\equiv$  number of electrons taking part in the reaction (1-8; organic substances typically have 2)  
 $F$   $\equiv$  Faraday's constant  
 $K$   $\equiv$  resistance capacity (cell constant)  
 $D$   $\equiv$  diffusion coefficient of the analyte  
 $C$   $\equiv$  concentration of the analyte in the cell at the moment

Electrochemical reactions will take place within three steps (see figure 3):

1. *Diffusion*: molecules of the analyte diffuse from the solution in the cell to the electrode surface.
2. *Electrolysis*: at the electrode surface the electrons are either added (reduction) or removed (oxidation).
3. *Rediffusion*: the electrolyzed component moves back into the solution.

The speed of the whole reaction is determined by the slowest (diffusion or rediffusion) of the three steps.

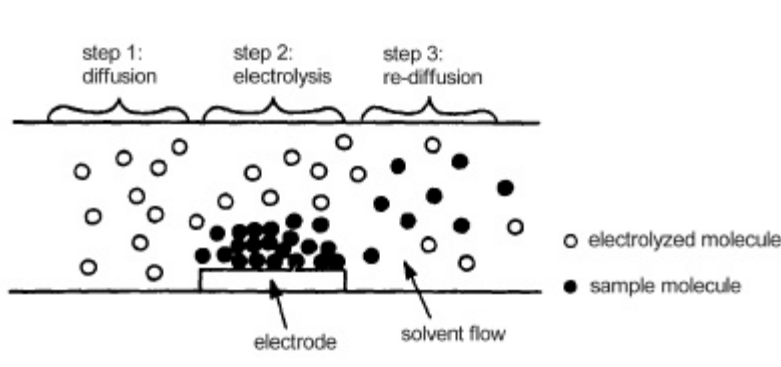


figure 3: steps of an electrochemical reaction

### 3.2 Current-voltage curves

The current of the measurement cell is correlated with the working electrode potential. It is dependent on the ability of the analyte to get oxidized or reduced at different potentials. In case of oxidation a current in positive direction (electrons will move out of the solution into the electrode) will be the result, in case of reduction it will be a negative direction of the current. The relation between potential and resulting current is plotted in a current-voltage diagram, a so called „hydrodynamic voltamogram“.

The electrolysis current is directly proportional to the speed of the reaction at the electrode. If the current is recorded with different potentials the curve describes the speed of the oxidation or reduction in relationship to the potential (see figure 4). Note, that the linear parts of the curve have an inclination to the x-axis (potential). This is caused by electrolysis reactions (transport processes) of interfering substances (e.g. pollution) or by autoprotolysis of mobile phase.

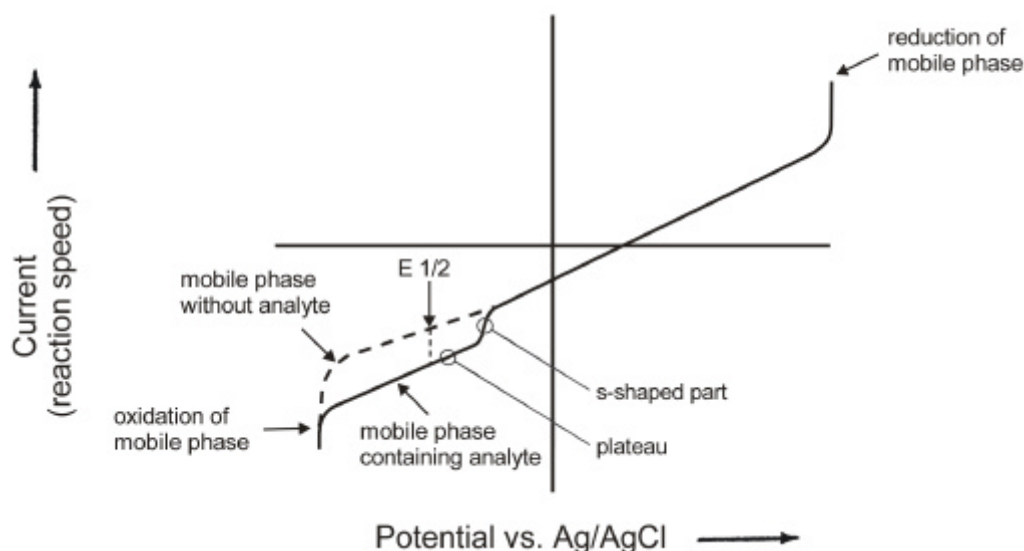


figure 4: current-voltage curve

The s-shaped parts of the curve will show us regions where the potential has a particular strong influence to the reaction speed. In such regions the electronic transfer (oxidation or reduction) is the slowest partial reaction at the electrode (see figure 3). The s-shape results of bell-shaped distribution (see figure 2) of the inner energy of the sample molecules. This means that the s-shaped curve is the integral over the distribution of the inner energy. In the upper region of the s-shaped part (see figure 4) only a few molecules will perform the electrochemical reaction. There are not much molecules which have an inner energy that is high enough, together with the potential, for the reaction to take place. With an increasing (absolute) potential a higher number of molecules will get a sufficient inner energy, so the redox reaction will take place. If the potential is set to a value where approximately all molecules have a sufficient inner energy the curve is declining (see lower part of the s-shaped part in figure 4).

In the so called plateau (note that the curve is not parallel to the potential axis in the plateau) transport processes (diffusion and convection) are the slowest part of the electrolysis reaction. In this part of the curve the potential does not effect the redox reaction. Because of its lower slope the plateau indicates the potential range with the best signal to noise ratio. For a maximum selectivity the working potential should bet set to an (absolute) value just higher as the potential of the s-shaped part of the curve.

At the edges of the curve you see an exponential increase of the current. This results of an electrolysis reaction of the mobile phase itself.

As soon as the working electrode gets older, the slope of the current-voltage-curve will become lower. This is due to changes in the electrode surface which lead to inhibition of the transport processes. An increase of the electrical resistance is the result of this. To compensate this additional resistance a higher potential must be applied. Thus sensitivity will decrease.

### 3.3 Half-wave potentials

The half-wave potential ( $E_{1/2}$ ) is the potential at which the half of the analyte molecules have an inner energy which is higher than the activation energy of the electrochemical reaction. We interpret the "wave" as the s-shaped part in the current-voltage curve in Fig. 4 which is the integral over the energy distribution.

While the height of the wave is dependent to the analyte concentration, the half-wave potential results of the electrochemical characteristics of the analyte. So it is possible to identify the analyte or to suppress interfering substances. For instance, if you perform an analysis with two substances which have different half-wave potentials (at least 150 - 250 mV) you can regulate the applied potential to such an extent that only the substance with the lower half-wave potential will perform the electrochemical reaction. The other substance will pass the measurement cell without being detected.

In general you should use the lowest possible potential set up for your analysis. This will eliminate interfering peaks from the chromatogram and leads to a high sensitivity.

### 3.4 Electrochemical measurement cell

Three electrodes are included within the measurement cell: reference, working and auxiliary electrode. Figures 5 and 6 show the electrochemical measurement cell EC4000, model Sputnik® integrated within the cell housing and the cell electrode components.



figure 5: electrochemical measurement cell EC4000, model Sputnik®

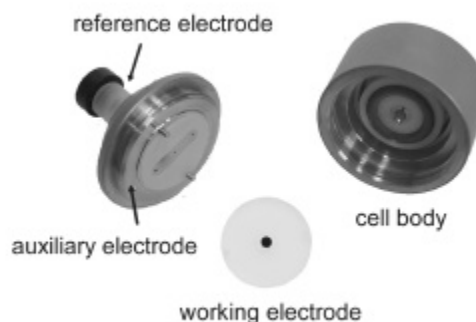


figure 6: cell components reference electrode, auxiliary electrode and working electrode

The difference of the potential of working and reference electrode will be kept constant by detector electronics. This difference is equal to the value given by the pre-set potential (see section 6.2.5).

During the analyte flows through the measurement cell it will be electrolyzed by the applied potential. An electrical current through the working electrode is the result of this electron transfer. In the detector electronic the current is converted to a voltage signal which is boosted by a buffer amplifier and then passed to a noise filter. The recorder/integrator outputs provide this current-proportional voltage signal (see figure 7).

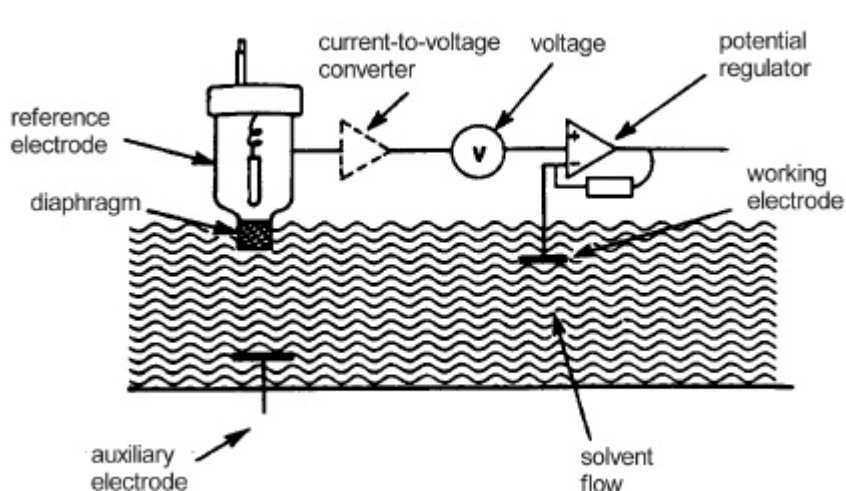


figure 7: measurement cell – electrical principle

## 4. ELEMENTS AND CONNECTIONS ON FRONT AND REAR PANEL

### 4.1 Front panel



- 1: On [ I ] / Off [ 0 ] keys, 2: [ Enter ] key, 3: [ Esc ] key, 4: Function keys [ F1 ] - [ F4 ],  
 5: Function keys [ F5 ] - [ F9 ], 6: Cursor keys [ Cursor left, right, up, down, center ],  
 7: Gamma control keys [ + ] / [ - ], 8: Switching keys [ + ] / [ - ], 9: Info key [ ? ]

figure 8: front panel of the EC3000

### 4.2 Rear panel



- 10: "Power supply" socket, 11: "Serial connector" socket, 12: "Control" socket,  
 13: "Analogue out" BNC-socket, 14: "Active cell" socket, 15: "Passive cell" socket

figure 9: rear panel of the EC3000

## 5. INSTALLATION

### 5.1 Electrochemical detector EC3000

#### 5.1.1 Location and environment

The location of the instrument should conform to the following:

- Free of large temperature variation
- Away from direct sunlight
- Well ventilated and away from aggressive gases
- Away from strong electric or magnetic fields
- Free of vibrations

**Please note:** The detector, and the measurement cell in particular, must not be exposed to direct sunlight or draught. Interference of baseline stability or the reproducibility of analytical results may be the consequences.

The housing of the EC3000 allows the stacking of other HPLC components.

**Please note:** For the stacking of HPLC components please be aware that the electrochemical measurement cell is sensitive to vibrations.

#### 5.1.2 Mains connection

- The current connection is performed via the "power supply" connector (10) (see figure 9) with the power supply order no. EC3101 included in delivery.
- The power supply EC3101 allows an input voltage range of 100 - 240 V at an AC frequency range of 47 - 63 Hz.
- The mains connector of the EC3101 can be detached from the transformer socket and be replaced by a connector complying with the local requirements (see figure 10).



*figure 10: the power supply EC3101*

## 5.2 Electrochemical measurement cell EC4000, model Sputnik®

### 5.2.1 Assembly of the cell

At delivery, the mechanical components of the Sputnik® are already mounted (see figure 11). The disassembly and re-assembly of the cell for the purpose of maintenance is described in section 7.2.

### 5.2.2 Assembly of fittings and capillaries

The cell is connected with PEEK tubings (inlet tubing I.D. = 0.25 mm, outlet tubing I.D. = 0.50 mm) and PEEK fittings. To avoid deathvolume (noise) the tubings have to be installed carefully. At this, please pay attention that the capillaries are cut right-angled at their end. Slide the capillaries into the fittings and screw the fittings into the drill wholes ("IN" and "OUT" respectively) of the cell top.

### 5.2.3 Filling of the reference electrode

Unscrew the cap of the reference electrode housing. Fill the KCl solution (order no. EC2900) up to the marking (=indentation, see dashed line in figure 12). Take care that no air bubbles remain on the diaphragm. Screw the cap again.

### 5.2.4 Ventilation of the measurement cell

Start pumping. Unscrew the reference electrode slightly and wait a few seconds until some mobile phase is pressed through the gap. For removing air bubbles underneath the reference electrode screw ( $\frac{1}{4}$  rotation) in and out the housing repeatedly (see figure 12). Finally screw the reference electrode tightly. Dab the overflown buffer.

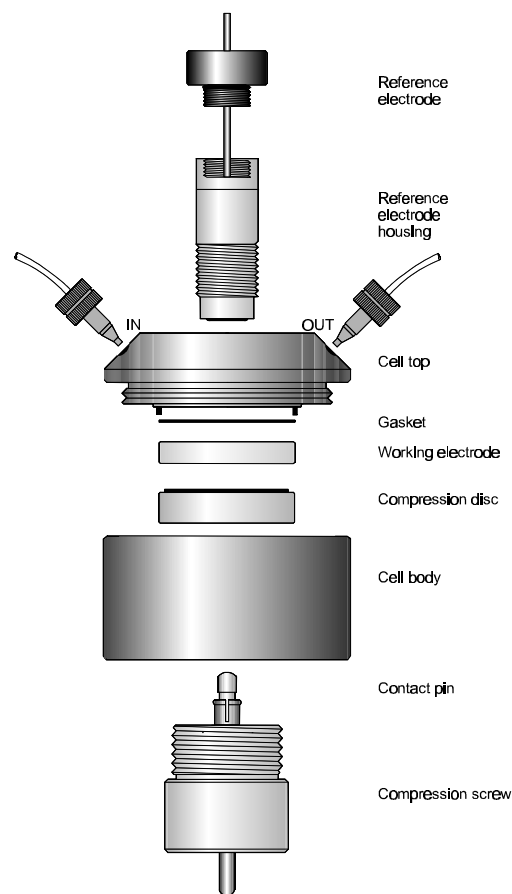


figure 11: components of the Sputnik®

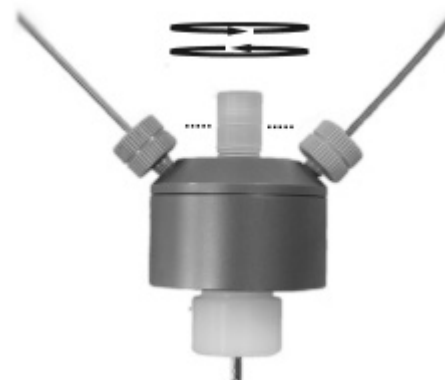


figure 12: ventilation of the measurement cell

### 5.2.5 Electrical installation of the cell

Install the measurement cell within the mounting device of the cell housing (order no. EC4603) and fix it with the fixing screw (**16**). Afterwards connect the cell-cable (order no. EC3102) with housing and measurement cell in the following order (see figure 13 and figure 14):

- Plug the serial connector (**21**) of the cell cable (see figure 13) in the "passive cell" socket (**15**) (see figure 9) of the detector.
- Put the shielding plug (**17**) (= red, 4 mm) into the socket of the cell housing.
- Put the grounding plug (**18**) (= red, 2 mm) into the socket of the cell head.
- Clamp the reference electrode (**19**) (black coupling plug)
- Finally clamp the working electrode (**20**) (black crocodile clip)

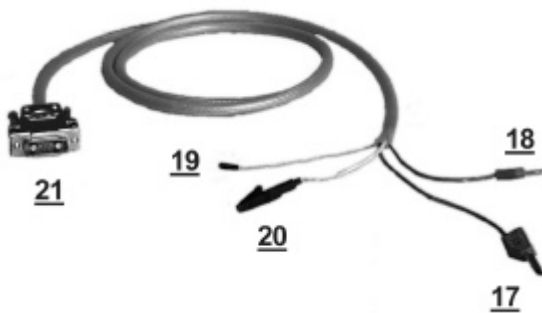


figure 13: connecting plugs of the cell-cable

figure 14: measurement cell installed in the mounting device of the cell housing

### 5.3 Signal connection

For connecting the EC3000 with the electrochemical measurement cell EC4000, model Sputnik use the cell cable order no. EC3102 (see section 5.2.5).

Depending on the evaluation unit (integrator or PC interface) being used, RECIPE offers several autozero- and analog output cables:

Autozero-cable

- with universal-terminals (order no. EC3103)
- with HITACHI-connector (order no. EC3104)

BNC-cable for analog output

- with universal terminals (order no. EC1103)
- with HITACHI-connector (order no. EC1104)
- with adapter for Shimadzu-Integrator (order no. EC1110)

#### 5.3.1 Connection to a HPLC system with integrator

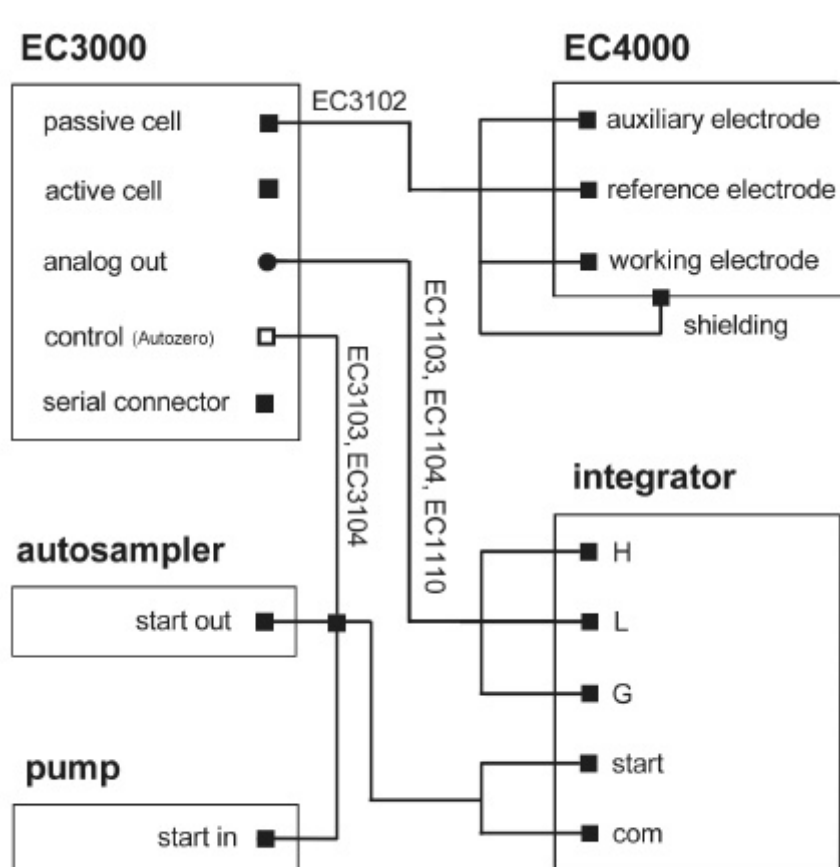


figure 15: connection of the EC3000 to a HPLC system with integrator

### 5.3.2 Connection to a HPLC system with PC interface

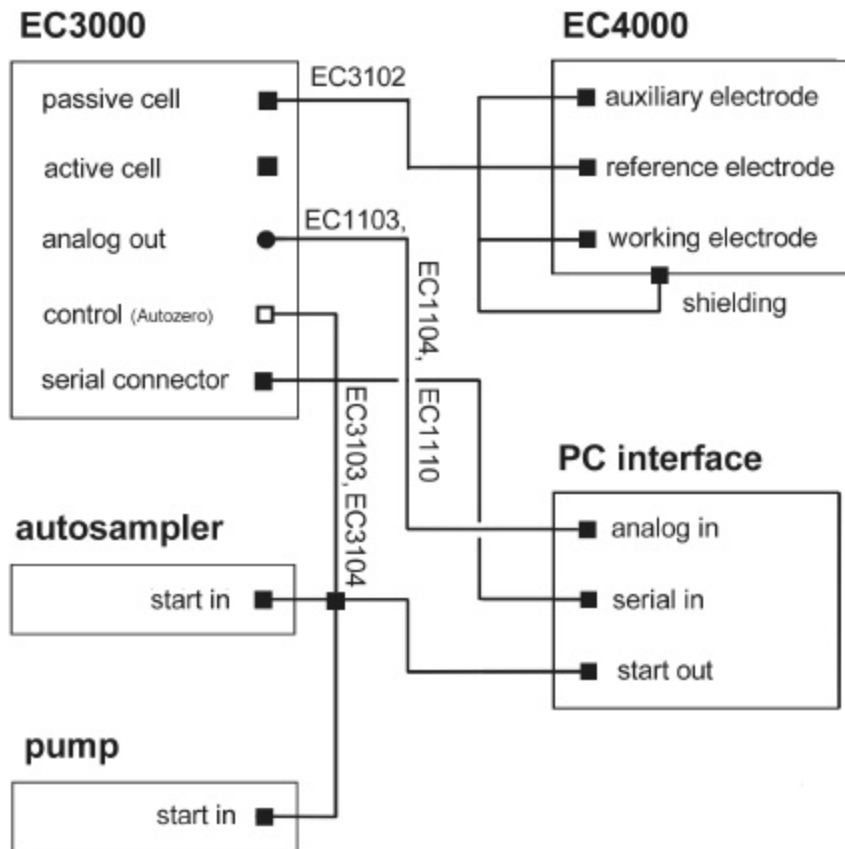


figure 16: connection of the EC3000 to a HPLC system with PC interface

## 6. OPERATION

### 6.1 Power-Up

After connecting the power supply (order no. EC3101) the detector is already supplied with operating voltage. The display yet is still dark and without indication.

The instrument is switched on and off with the "on/off" keys (1) (see figure 8). The EC3000 is switched on with [ I ] and is switched off with [ 0 ].

After switching on with [ I ] the display is illuminated and three acoustic signals for confirmation are given after about one second.

### 6.2 Operation-Menu

#### 6.2.1 General

The whole operation of the EC3000 is carried out with the foil key-pad (see figure 8). For external operation via PC interface (remote control) see section 6.2.3.

- Between the menus skip forward with [ ENTER ] (2) and backward with [ ESC ] (3).
- Within the menu the setting of values or numbers is carried out by the cursor keys (6). That is: [ cursor up ] steps up the value, [ cursor down ] steps down the value.

The stepwise adjustment of values can be fastened with factor 10 or 100 by the "cursor fast" function. The "cursor-fast" function is activated by pressing the [ cursor-centre ] -key and the [ cursor up ] or [ cursor down ] -key accordingly.

In case of misentry an acoustic signal is given.

#### 6.2.2 Start-Display

On the start-display diverse information, e.g. the detector-CPU-ID-code number, software version and the available operation modes are shown. The instrument carries out a system self-test. For the failure free system the result "successful finished" is indicated. In case of malfunction a status indication is given and the further command execution within the menus is blocked.

**Please note :** In case no indication is visible at the display, the gamma control (gamma control keys (7), see figure 8) has to be adjusted. In general, the surface temperature of illuminated displays may slightly change in operation. For this, it might be necessary to re-adjust the gamma control in the warming-up period. The display is illuminated by LEDs (LED=Light Emitting Diode).

### 6.2.3 Language selection and operation mode

The function keys [ F1 ] ... [ F5 ] (function keys (4), see figure 8) are allocated with the language selection. With the basic equipment of the EC3000 "English" and "German" is selectable.

[ F1 ]	"German"	available
[ F2 ]	"English"	available
[ F3 ]	"Francais"	not installed
[ F4 ]	"Italian"	not installed

The function keys [ F7 ] ... [ F9 ] (function keys (5), see figure 8) are assigned for the selection of the operation mode. The basic equipment does not include the operation modes "Remote" and "Service". For this, "manual operation mode via keyboard" is pre-adjusted with the basic equipment.

[ F7 ]	"Manual"	pre-adjusted
[ F8 ]	"Remote"	not installed
[ F9 ]	"Service"	not installed

### 6.2.4 Measurement modes and detector selection

The function keys [ F1 ] ... [ F5 ] (function keys (4), see figure 8) are intended for the selection of the measurement modes. With the basic equipment only the measurement mode "DC-amperometry" is available. For this, this mode is already pre-adjusted.

[ F1 ]	"DC-amperometry"	pre-adjusted
[ F2 ]	"Pulsed amperometry"	not installed
[ F3 ]	"Cyclic voltametry"	not installed
[ F4 ]	"Calibration of gradient"	not installed

The function keys [ F7 ] ... [ F9 ] (function keys (5), see figure 8) are allocated with the detector selection.

[ F7 ]	"test"	is an integrated replacement cell, which serves the testing of the electronics.
[ F8 ]	"passive"	is the usual measurement cell, e.g. EC4000 model Sputnik <sup>®</sup> . The cell is plugged to the "passive cell" socket (15) at the rear panel.
[ F9 ]	"active"	is a measurement cell with integrated pre-amplifier. It is plugged in the "active cell" socket (14) at the rear panel.

## 6.2.5 Menu setting for DC-amperometry

The function keys [ F1 ] .... [ F9 ] (function keys (4, 5), see figure 8) are allocated with the menu setting for DC-amperometry.

[ F1 ]	Potential -2,00V.....+2,00V	Setting with cursor keys (6) by steps of 10mV, by steps of 100mV with "cursor-fast" function, -2,00V.....+2,00V polarity switching with [ + ] and [ - ] (8) accordingly.
[ F2 ]	Measuring range +/-10pA....20µA	Setting with cursor keys (6), by step sequence 10 – 20 – 50 – 100 etc. The measuring range basically is referred to an output voltage of 1,00V at the analogue output (BNC-socket). The measuring range may be selected independently from the effective basic current, because the basic current is compensated by the setting of an offset or by auto-zero.
[ F3 ]	Filter 5Hz.....0,02 Hz	Setting with cursor keys (6), no filter – 5 – 2 – 1 – 0,5 – 0,2 – 0,1 – 0,05 – 0,02 Hz
[ F4 ]	Offset up to +/- 50 µA	Compensation of the basic current, setting with cursor keys in 0,02nA - steps, by 2,00nA- steps with "cursor-fast" function, polarity switching with [ + ] and [ - ] (8) accordingly.
[ F5 ]	auto-zero "on"	If the auto-zero function is activated, the entry of "offset" is blocked. A value being possibly adjusted before with [ F4 ] is <b>not</b> used for the basic current compensation. Auto-zero is enabled and will be started by pressing the key [ F5 ]. With auto-zero being enabled, the next menu is "cell cleaning" (see below).
[ F6 ]	auto-zero "off"	The auto-zero function is blocked in the following menu. A compensation of the basic current may be carried out with offset [ F4 ].
[ F7 ]	programme no.	Programme number 0...99, selection with cursor keys, by steps of 10 with "cursor-fast" function
[ F8 ]	save programme	For the storing of the adjustments being defined with [ F1 ] .... [ F5 ] at the programme number previously selected with [ F7 ]. At this, already existing programmes are overwritten without warning! The programmes remain stored after switching off the detector.
[ F9 ]	load programme	For the loading of a programme being selected with [ F7 ]. If still no programme exists at the selected programme number, it is indicated on the display.

Press the [ Enter ] key (2) to start the measurement or skip to the next menu:

[ Enter ]	start of measurement	In case the auto-zero function is disabled, the present measurement values are indicated (see section 6.2.7).
	skip to the next menu	With auto-zero being activated, the next menu (see section 6.2.6) is displayed.

## 6.2.6 Menu setting for cell cleaning

The function keys [ F1 ] ... [ F9 ] (function keys (4, 5), see figure 8) are allocated with the menu setting for the automatic cell cleaning.

**Please note: Any combination of measuring- (section 6.2.5) and cell cleaning programmes (see section 6.2.6) may be loaded.**

[ F1 ]	Potential -2,00V.....+2,00V	Setting of the cleaning potential with cursor keys (6) by steps of 10 mV, by steps of 100 mV with the "cursor fast"- function, polarity switching with [ + ] and [ - ] (8) accordingly.
[ F2 ]	Starting delay 10....900 sec.	Starting delay of cell cleaning programme from auto-zero-start, setting of the delay with the cursor keys (6) by steps of 10 sec., with the "cursor-fast" function by steps of 100 sec.
[ F3 ]	Interval 1....100 sec.	Interval of cell cleaning process, interval setting with cursor keys (6) by steps of 1 sec., with the "cursor-fast" function by steps of 10 sec.
[ F4 ]	Cycle 1....10	It defines the number of analysis-intervals being performed, before cycle the cell cleaning-interval is started. Setting with the cursor keys (6) from 1 .... 10.

[ F5 ]	Enabling of cell cleaning	The cell cleaning is enabled and is started with the conditions enabling defined in [ F1 ] ...[ F4 ] by the first auto-zero.
[ F6 ]	Disabling of cell cleaning	The cell cleaning is disabled within the following menu.
[ F7 ]	programme no.	Programme number 0...99, selection with cursor keys, by steps of 10 with "cursor-fast" function
[ F8 ]	save programme	For saving the adjustments being defined with [ F1 ] .... [ F5 ] at the programme number being selected with [ F7 ]. At this, already existing programmes are overwritten without warning! The programmes remain stored after switching off the detector.
[ F9 ]	load programme	For the loading of a programme being selected with [ F7 ]. If still no programme exists at the selected programme number, it is indicated on the display.

Press the [ Enter ] key (2) to start the measurement:

[ Enter ]	start of measurement	The present measurement values are indicated (see section 6.2.7)
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## 6.2.7 The present measurement display

Within this menu, The present measurement values are indicated. If auto-zero is activated, the sequence of an enabled cell cleaning programme is started not until the auto-zero is finished. Within this menu, the auto-zero may be re-started with the function key [ F5 ]:

[ F5 ]	auto-zero now	Starts the auto-zero, starting may be performed externally. The auto-zero function calculates the optimal compensation value. It may require some seconds until the indication "present current" is at a value near "0"
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Any other keys are out of function, with exception of: [ ESC ] (3), Gamma keys [ + ] / [ - ] (7) and the [ I ] / [ 0 ] keys (1) (see figure 8).

In the upper part of the display the measured values are indicated in the corresponding unit of measurement. In the lower part the selected menu-settings are reported (see figure 17).

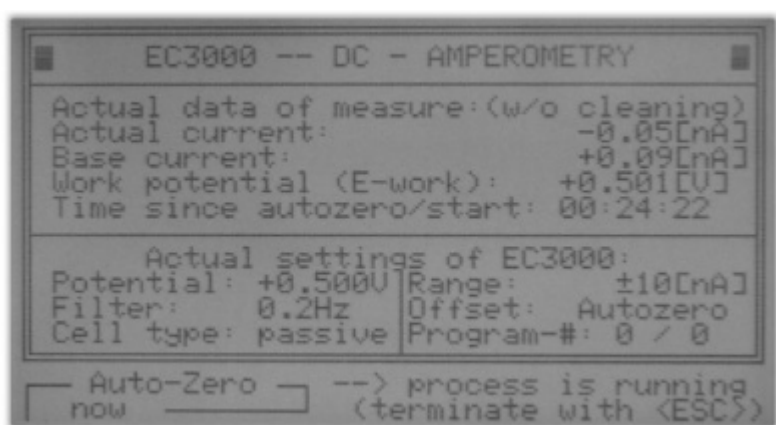


figure 17: sections of the measurement display

During measurement the access to the selected settings is only possible by stepping back in the corresponding sub-menu:

[ ESC ]	skip to the sub-menu	The present measurement values remain, the corresponding sub-menu is indicated on the display.
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**Please note:** In both upper corners of the display, two animated rectangles are stating the proper running of the programme sequence. The stopping of these rectangles indicates the hang-up of the programme. If so, re-boot the EC3000 by detaching it completely (switching off is not sufficient!) from the power supply.

## 7. MAINTENANCE

### 7.1 Passivation of the HPLC-system

#### 7.1.1 Reason for passivation

HPLC systems basically have to be passivated before using them with electrochemical detection systems. The reason is that metal parts with an unprotected metal surface (steel capillaries, mechanical components of the pump etc.) may emit metal ions to the mobile phase. These ions undergo oxidation and reduction processes within the electrochemical measurement cell and therefore may cause heavy disturbances of the detection process (e.g. high base current, noisy base line etc.).

It is advisable, to repeat the passivation of the HPLC system from time to time (dependent on usage, normally every 2 to 3 months), especially in case of malfunctions which can not be attributed to a single component of the system.

#### 7.1.2 Performing the passivation

It is important that all fluidic components of the HPLC system are passivated (pre-oxidized), **with exception of the analytical column and the detector cell**. For passivating the system follow the instructions below:

- Connect pump, injection system, column heater, detector and all capillaries **with exception of the column and the detector cell**.
- Put the outlet-capillary into a safe waste container.
- Flush the system for 15 min at a flow of 1 - 2 ml/min with HPLC water.
- Flush then for 10 min with 2-propanol.
- Afterwards flush for 15 min with HPLC water.
- Flush the system for 30 min with half concentrated nitric acid (1 volume each of concentrated nitric acid (65 %) and HPLC water) at a flow rate of 1 ml/min.
- Afterwards purge the system with HPLC water (1 - 2 ml/min) until the pH of the waste solution is neutral. Change the water in the eluent container several times to be sure that the nitric acid will be washed out the frit.
- Finally flush the system for about 15 min with appropriate mobile phase.
- Connect the column and the detector cell.

The injection system (manual injection valve or autosampler) requires supplementary passivation:

##### *a.) Manual injection valve*

For the manual injection valve the passivation procedure as indicated above has to be performed in the same manner and simultaneously with the remaining HPLC system. In this way inject 2-propanol, HPLC-water and half concentrated nitric acid several times. Finally flush the injection valve with an appropriate amount of water to remove the residues of nitric acid completely.

### ***b.) Autosampler***

For the autosampler the passivation procedure as indicated above has to be performed in the same manner and simultaneously with the remaining HPLC system. In this way inject 2-propanol, HPLC-water and half concentrated nitric acid several times and with the largest possible volume. Finally flush the injection valve with an appropriate amount of water to remove the residues of nitric acid completely.

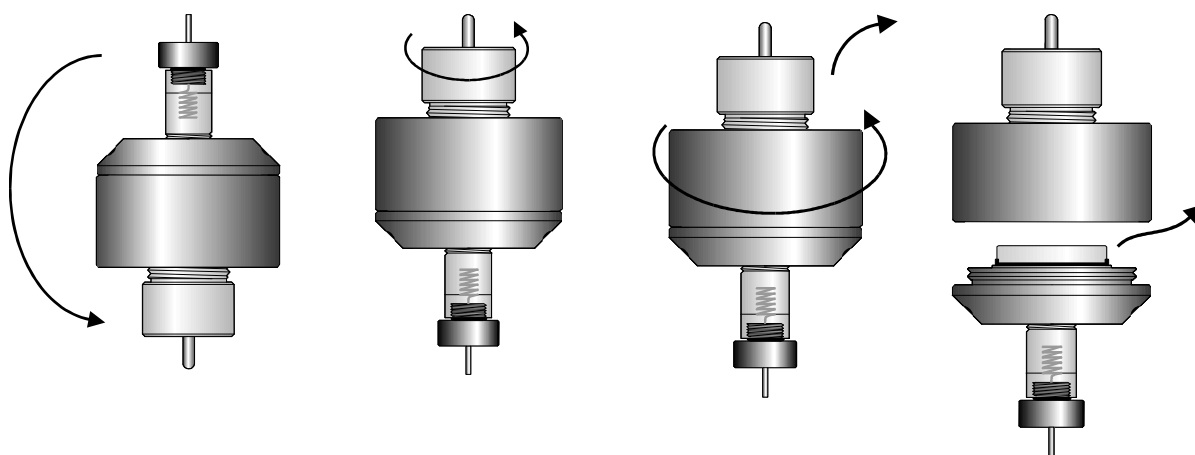
## **7.2 Maintenance of the electrochemical measurement cell EC4000**

For the purpose of maintenance, any cell components can be disassembled and re-assembled without using tools. This ensures a most easy handling of the measurement cell.

### **7.2.1 Disassembly of the measurement cell**

For the disassembly of the cell follow the instructions below:

- Switch off the EC3000 ([ 0 ] key **(1)**).
- Switch the pump to STAND BY or off.
- Disconnect the serial connector (**21**) and the plugs (**17 - 20**) in the reverse order as shown in section 5.2.5.
- Remove the cell from the mounting device of the cell housing.
- Disconnect the capillaries.
- Turn the bottom side of the cell up (see figure 18a).
- Loosen the compression screw (approx. 1 rotation counterclockwise) with the supplied wrench (see figure 18b).
- Hold the cell top in one hand and unscrew the cell body counterclockwise with the other (see figure 18c).
- Remove the working electrode (see figure 18d).



*figure 18a-d: disassembly of measurement cell EC4000, model Sputnik®*

### 7.2.2 Cleaning and activation the working electrode

The sensitivity of the measurement cell slowly decreases over the time, depending on the application and on the number of analyses being performed. In order to re-establish the sensitivity of the measurement cell the surface of the working electrode has to be cleaned and activated from time to time. The procedure of cleaning and activation depends on the material of the working electrode (glassy carbon, gold, copper etc.). Please contact RECIPE for cleaning instructions.

**Please note:** The working electrode can be used from its both sides.

### 7.2.3 Maintenance of the reference electrode

In order to avoid an increase of noise and an enhancement of the work potential (E-work), regularly check the KCl liquid level.

For the maintenance of the reference electrode please follow the instructions given in sections 5.2.3 and 5.2.4.

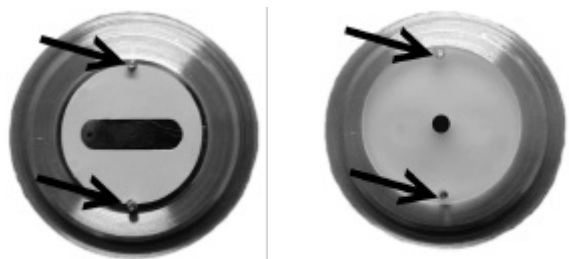
### 7.2.4 Cleaning and passivation of the auxiliary electrode

Contact RECIPE for cleaning and passivation of the auxiliary electrode surface!

### 7.2.5 Re-assembly of the measurement cell

For the re-assembly of the cell follow the instructions below:

- Assemble the gasket (=spacer) and the working electrode. Take care that the groove of the gasket and the working electrode fits in the pin! (see figure 19a-b and figure 20a).
- Screw the cell body tightly (clockwise). Do not turnabout the cell until the compression screw is tightned! (see figure 20b).
- Fix the compression screw by hand at first and tighten it afterwards with the supplied wrench (see figure 20c).
- Turn the cell in the upright position (see figure 20d).



*figure 19a-b: Fixing of gasket and working electrode by fixing pins*

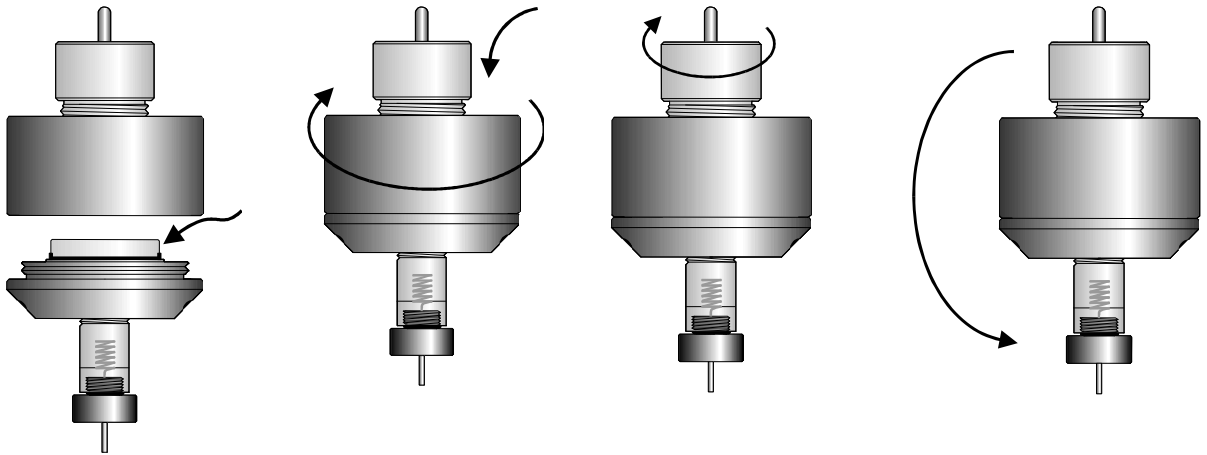


figure 20a-d: re-assembly of the measurement cell EC4000, Model Sputnik<sup>®</sup>

- Ventilate the measurement cell according to the instructions given in section 5.2.4.
- Assemble the cell in the mounting device of the cell housing and
- connect the capillaries (see section 5.2.2).
- Switch on the pump.
- Connect the plugs (17-20) and the serial connector (21) according to the instructions given in section 5.2.5.

## 8. TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTION
Pump error (pressure fluctuation)	Air in the pump	Open purge valve, suck off mobile phase and pump through system at maximum flow rate
	Defect pump head valve	Replace
	Flow rate not constant	- Check pump for constant pressure and flow rate - Leak or air in the pump ?
Spikes on the baseline	Air bubbles in the detector cell	Increase the pressure in the detector cell by briefly closing off the outlet tube (Caution: consider maximum cell-pressure) or disconnect the column and flush the cell with mobile phase
	Air bubbles in the mobile phase	Degass the mobile phase
	Air bubbles at diaphragma of reference electrode	Remove air bubbles, remove KCl and replace with fresh KCl, filling without air bubbles
	Interference from mains	Relocate instrument to interference-free place or install interference filter
Baseline drift	System not yet in equilibrium	Pump mobile phase through the system for a longer period of time
	Leak in the cell	Check cell and connections for leaks
	Temperature drift	Check column heater
	Mobile phase contaminated	Replace mobile phase
Noisy baseline		Check reference electrode insert, working electrode, cell connections
High baseline noise	Multiple signal grounding	Remove grounding clip at the rear side of the detector
Peaksplitting	Column packing defect	Replace column
	Defect injection valve	- Disassemble and clean injection system or call service - If Rheodyne system used, replace Vespel rotorseal with Tefzel rotor-seal

<b>PROBLEM</b>	<b>POSSIBLE CAUSE</b>	<b>SUGGESTED SOLUTION</b>
Broad peaks, tailing	Column at end of useful life	Replace column
	Dead volume	Check system
Poor recovery	Internal standard partially degraded	Compare internal standard with standard solution
	Washing steps carried out incorrectly	Check washing solutions; replace if necessary
	Pipettes out of adjustment or defect	Check pipettes
	Injection volume too low	Check injection system
	Sample preparation columns sucked off too fast	Ensure columns sucked off slowly
	Incorrect sample preparation	Follow sample preparation instruction strictly
Interfering peaks in chromatogram	Injection system contaminated	- Rinse injection system with water followed by 2-propanol
		- In case of automatic injectors: clean needle
		- check external rinsing
	Peaks originate from degradation products of old samples or standards	Use only fresh or properly stored samples
	Rheodyne injection system	Replace Vespel rotorseal with Tefzel rotorseal
High backpressure	Accumulation of particles in the column	Replace column
Retention times changed	Column temperature fluctuates	Check column heater
	Leak in system	Locate and eliminate

<b>PROBLEM</b>	<b>POSSIBLE CAUSE</b>	<b>SUGGESTED SOLUTION</b>
Significant decrease of detector sensitivity	Contamination or reaction products	Clean working electrode
	Potential of reference electrode is drifting	Check reference electrode using another one
Base current too high	Reference electrode defect	- Check potential of reference electrode using another one - If difference is more than $\pm 20$ mV, refill electrode with 3 M KCl
	Mobile phase contaminated	Replace mobile phase
	Column contaminated	Replace column
	Surface of working electrode contaminated	Clean working electrode with chrome-sulphuric acid, rinse well with distilled water and reinstall

## 9. TECHNICAL DATA

### 9.1 Technical data EC3000

<b>Principle:</b>	amperometric detector with thin-layer cell three-electrode wiring
<b>Measurement cell:</b>	see section 9.2
<b>Electronics:</b>	
Working potential:	0 to $\pm 2.00$ V
Input current range:	to $\pm 50$ $\mu$ A
Measuring range:	$\pm 10$ pA to 20 $\mu$ A
Auto-zero-range:	to $\pm 50$ $\mu$ A
Manual offset range:	to $\pm 50$ $\mu$ A
LCD-display:	simultaneous indication of all relevant measurement data
Filter:	5 Hz to 0.02 Hz ( 0.2 to 50 sec.)
Detector noise level:	< 0.3 pA
Cleaning potential:	0 to $\pm 2.00$ V
Cleaning duration:	1 to 100 sec
Delay time before applying cleaning potential:	10 to 1500 sec
Cleaning cycle:	every 1 <sup>st</sup> to 10 <sup>th</sup> cycle
Storage capacity for measurement programmes:	0 to 99
Storage capacity for cell-cleaning programmes:	0 to 99
Analogue output:	$\pm 1$ V per measurement range
Auto-zero interface:	active low, switching contact, active high, current- / voltage input,
Power supply:	12 V DC 2 A
Power pack EC3101:	115 / 230 V AC
Dimensions:	510 mm x 260 mm x 160 mm ( w x d x h )
Weight:	8.1 kg

### 9.2 Technical data EC4000, Model Sputnik<sup>®</sup>

Working electrode:	glassy carbon in zirconium oxide; optional Ag, Au, Cu, Ni, Pt in Kel-F
Auxiliary electrode:	stainless steel
Reference electrode:	silver/silverchloride reference electrode, refillable
Diaphragm:	base-stable zirconium oxide diaphragm
Cell volume:	1.5 $\mu$ l with 30 $\mu$ m spacer; optional 0.75 $\mu$ l with 15 $\mu$ m spacer or 2.5 $\mu$ l with 50 $\mu$ m spacer
Materials:	stainless steel, PTFE, PEEK, zirconium oxide, glassy carbon, Kel-F