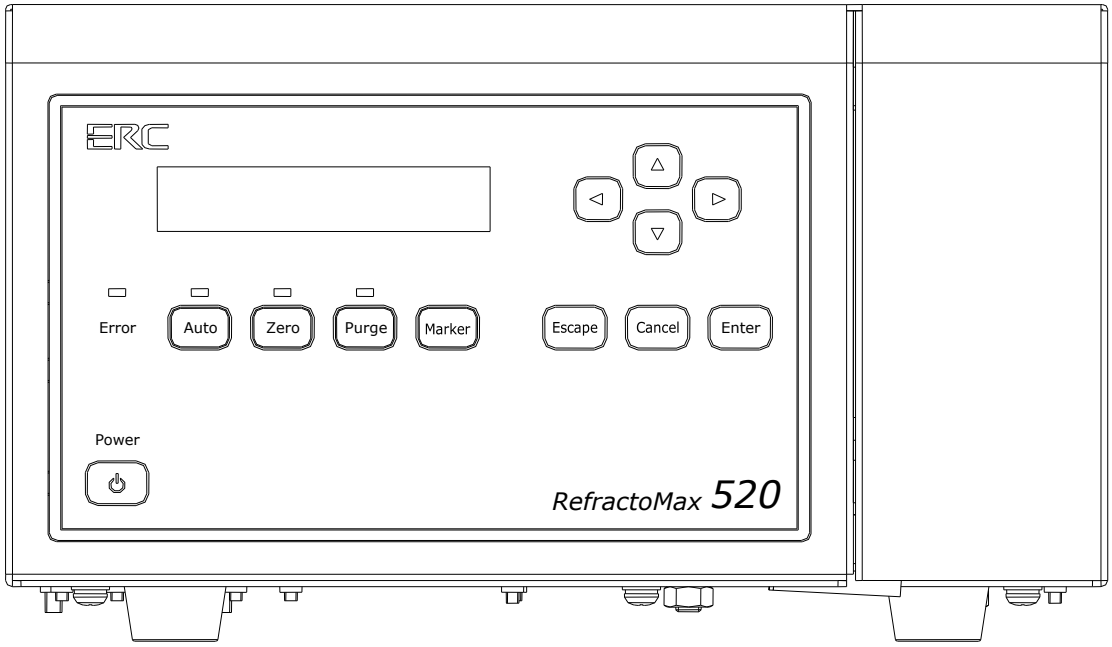
RefractoMax521 RI Detector Operator's Manual

High Sensitive Refractive Index Detector
for High Performance Liquid Chromatography



Publication No. 77-5017-11

Important Notice

The information contained in this manual is subject to change without notice.

ERC Inc. (ERC) assumes no responsibility for any errors that may appear in this manual. This manual is believed to be complete and accurate at the time of publication.

In no event shall ERC be liable for incidental or consequential damages in connection with or arising from the use of this manual.

Spare Parts Availability

It is the policy of ERC to provide maintenance spare parts for a period of seven (7) years after the final production of the instrument. Spare parts may be available after the seven (7) year period but only on an "as available" basis.

The following is a Federal Communication Commission advisory:

WARNING:

This equipment generates, uses, and can radiate radio frequency energy and if not installed and used in accordance with the instruction manual may cause interference to radio communications. It has been tested and found to comply with the limits for Class A computing device pursuant to Subpart J of Part 15 of FCC rules, which are designed to provide reasonable protection against such interference when operated in a commercial environment. Operation of this equipment in a residential area is likely to cause interference in which case the user at his own expense will be required to take whatever measures may be required to correct the interference.

Operating Instructions

This manual is provided to help you establish operating conditions that will make safe and efficient.

Special considerations and precautions are also described in this manual that shall appear in the form of WARNING, CAUTION and NOTE as described below.

It is important that you operate and service equipment in accordance with this manual and any additional information that may be provided by ERC from time to time.

 **WARNING**	*Alerts you to potentially hazardous situations that could result in serious injury, and how to avoid these situation*
 **CAUTION**	*Alerts you to situations that may cause moderate injury and/or equipment damage, and how to avoid these situations.*
	*Information to help you to achieve optimal performance from your equipment.*
	*The following Trademarks and Registered Trademarks are found in this manual.*

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Before Starting:

RefractoMax521 Refractive Index Detector is designed to be an analytical device for pure research purpose and may not be suitable for in vitro diagnostic analysis.

To operate RefractoMax521 Refractive Index Detector properly, you are strongly recommended to go through this Service and Maintenance Manual. Improper use of this detector shall be dangerous and may cause a hazardous result.

Limited Warranty Policy

ERC warrants its products against defects in materials and workmanship for the period of twelve (12) months from the date of shipment out of its factory.
ERC will, at its option, repair or replace products that are proved to be defective.

The aforementioned warranty policy shall not be applied to defects being caused by:

(1) Improper or Inadequate maintenance, adjustment, calibration or operation by the user(s);
(2) User-supplied software, hardware, interfacing or consumable;
(3) Unauthorized modification or misuse;
(4) Operation outside of the environmental and electrical specifications for the product.
(5) Improper site preparation and maintenance; or
(6) User induced contamination or leaks.

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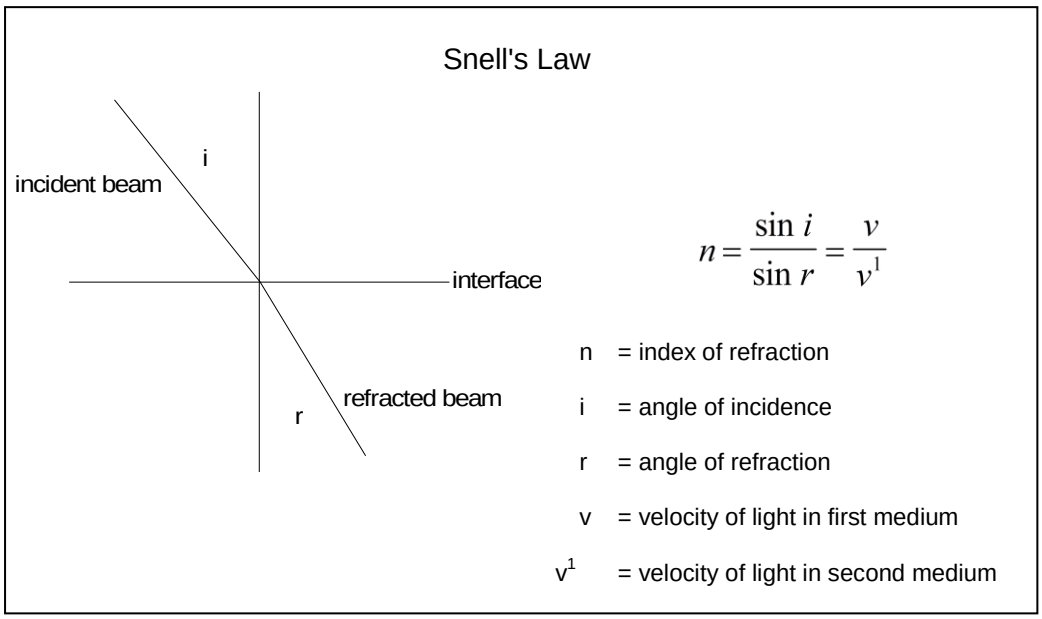
Section 1. Introduction

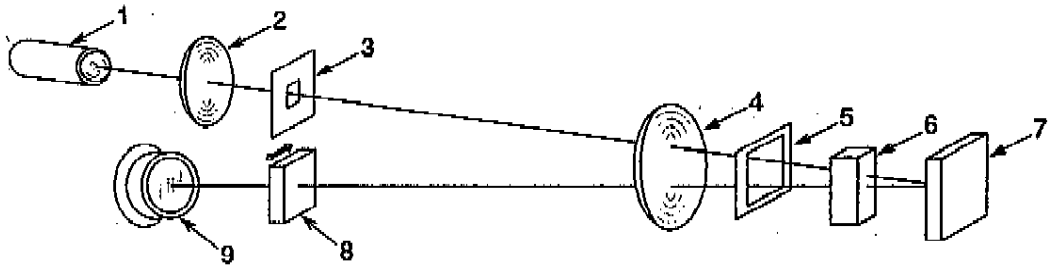
1-1. Principle of refractive index detection

RefractoMax521 Refractive Index Detector (called as "RefractoMax521" hereafter) is a high-performance universal detector designed for analyses requiring the continuous monitoring of the refractive index of a flowing liquid with respect to a reference.

RefractoMax521 is a deflection or Snell type refractive index detector. Snell's law states that a parallel light beam, when passing through a dielectric interface separating two media of different refractive index at an angle of incidence greater than zero, will be refracted as a function of the magnitude of difference of the refractive indices of the two media.



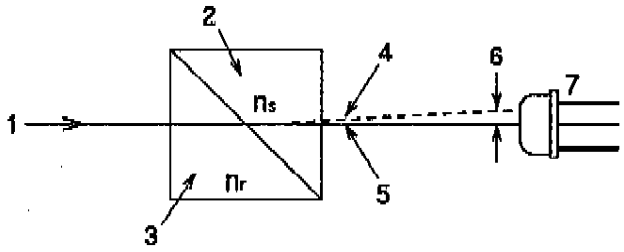
Optical System of RefractoMax521



[1] Tungsten lamp [2] condense lens [3] First Slit [4] Collimator Lens
[5] Second Slit [6] Flow Cell [7] Mirror [8] Null Glass [9] Photodiode

Light from a low-power, long-lifetime tungsten lamp is collimated by a lens and slit and passed through reference and sample cells, reflected off a mirror, passed back through the optical cells, and focused by lenses onto a pair of photo sensing diodes (photo sensor).

During operation, RefractoMax521's reference and sample cells are filled with mobile phase. The reference cell is then isolated from the flow path and mobile phase flows through the sample cell only. As long as no difference exists between the refractive indices of the media of the two cells, there is no refraction of the light passing through them.



[1] Light Beam [2] Sample Cell [3] Reference Cell [4] Light Axis (Ns>Nr)

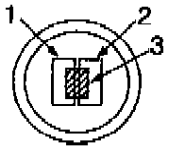
[5] Light Axis (Ns=Nr) [6] Distance between …[4]&[5] at the photo sensor

[7] Photo sensor

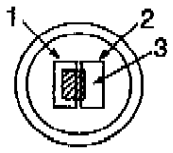
Ns: Refractive Index of mobile phase in Sample Cell

Nr: Refractive Index of mobile phase in Reference Cell

The light shines on a pair of photodiodes, each of which gives an electrical signal; these signals are amplified and the difference between the two signals is measured. Zero refraction should generate a zero-volt difference in these signals. An electrically controlled mechanical linkage allows the user to optimize the photodiodes' outputs for zero deflection via a refractive lens in the optical path. Additional circuitry enables the user to easily correct the signal output to electronic zero.



Ns = Nr



Ns > Nr

[1] photo sensor A [2] photo sensor B [3] light beam

When a change occurs in the refractive index of the mobile phase, the light passing through the interface between the sample and reference cells is refracted, causing the light intensity on one photodiode to increase and on the other to decrease. This difference gives a signal having both amplitude and polarity; the signal is amplified to drive a chart recorder or integrator.

1-2. Specifications

● Construction	Deflection type
● Refractive Index Range	1.00 ~ 1.75
● Range	1/4 ~ 512 μRIU
● Linearity	600 μRIU
● Noise	≤2.5 nRIU(Response : 3 seconds)
● Response Time	0.1, 0.25, 0.5, 1.0, 1.5, 2, 3, 6 sec.
● Polarity	Positive/Negative
● Auto Zero	Optical & Electrical Auto-Zero
● Auto Zero Range	All Refractive Index Range
● Auto Zero Resolution	≤1 (@8mV/μRIU) / 4 (@2mV/μRIU) nRIU
● Offset Range	0 to 500mV (same with Integrator output sensitivity)
● Offset Resolution	10mV (same with Integrator output sensitivity)
● Integrator Output	0 to 1V/FS (Sensitivity: 2mV/μRIU, 8mV/μRIU)
● Recorder Output	0 to 10mV/FS
● Event Marker	Marker out: ≥5% of FS
● Temperature Control	OFF, 30 to 55°C (1°C increment): 80°C Thermal Protector
● Operator Support	Span Check/Validation Display
● External Communication	RS232C
● Cell Volume	8μl
● Maximum Flow Rate	10 ml/min (mobile phase: pure water)
● Pressure Rating	50 kPa (0.5 kgf/cm^2)
● Internal Volume	Inlet Port / Flow Cell: approx. 60μl Flow Cell / Outlet Port: approx. 480μl Total: approx. 540μl
● Wetted Material	SST316, Teflon, Quarts Glass
● Power Requirement	AC100 ~ 240V +/- 10%: 50-60Hz
● Power Consumption	120VA maximum
● Dimensions	H150 x W260 x D450 (mm)
● Weight	12 kgs (26 lbs)
● EMC Standards	EN61326-1,EN61326-2-1
● Safety Standards	EN61010-1

Conditions to secure safety (EN61010-1)

Indoor use
Altitude up to 2000m
Temperature 5°C to 40°C
Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C
Transient over voltages according to installation category Ⅱ
Pollution Degree 2 in accordance with IEC 60664

Section 2. Unpacking and Installation

2-1. Installation Site Requirements

To install RefractoMax521, please make sure that any of followings shall be kept away from it to prevent interference on your analysis. Refractive Index Detector, in general, is very sensitive to the change in ambient temperature and airflow that results a drift of baseline.

- Air fan (air-conditioner-cooler, heating equipment and ventilation)
- Open doors or windows
- Direct sunlight
- Dart or dusts
- Vibration source
- Electro-Magnetic Wave or High Frequency

2-2. Power Requirements

RefractoMax521 requires 50-60 Hz single-phase power source capable of providing AC 100/240V +/- 10%.

CAUTION

Running RefractoMax521 on a voltage other than the correct single-phase supply will void the warranty.

CAUTION

RefractoMax521 is designed to operate with single-phase (phase-neutral) power ONLY.
If your facility provides only phase-phase (i.e., three-phase) power consult ERC.

2-3. Unpacking and Inspection

This section describes in details how to install RefractoMax521. This detector is designed to be operator installed.

CAUTION

Each steps of the installation site preparation must meet local safety, electrical, and building codes. These codes take precedence over any recommendations in these instructions, and compliance to them is the responsibility of the customer.

CAUTION

RefractoMax521 weighs 12 kgs (26 lbs). Use proper lifting techniques to avoid potential injuries.
To remove the detector from the box, hold the bottom of the cabinet. Never attempt to lift by front panel or terminals.
RefractoMax521 is packed with a number of accessories. Do not discard the packing material until all parts are accounted for.

CAUTION

Check the detector carefully for evidence of shipping damage.

If there is any evidence of damage, any item(s) missing in the carton or discrepancies, please notify that to the carrier immediately and to ERC.

2-4. Standard Accessories

Image	Description	Part Number	Thermo Scientific™ Sales P/N	Quantity
	RS-232 Ext. Cord, Modem Cable, 5 m	8914.0143	8914.0143	1
	RS-232<>USB Interface Cable	8073.2000	6073.2000	1
	Outlet Tubing, PTFE, 2.5 × 1.5 × 1500 mm, with nut and ferrule	8809170	6060.1239	1
	Fuse, 3.15 A	2401120	Included in 6810.9011	2
	Operator´s Manual	n.a.	n.a.	1

Note: The detector is equipped with a power cord that is appropriate to your power source.

2-5. Loosing the locking screws

To prevent damage may occur during the shipment, the optical block is fixed by two (2) locking screws (5mm Allen bolts). As you are installing RefractoMax521, please make sure to loosen these screws.

1. Move RefractoMax521 to the side of a bench to make the locking screws accessible while supporting it to prevent it from falling.
2. Loosen the locking screws, but do not remove them, using the 5mm hexagonal key.
3. Move the detector securely back onto the bench.

Once the locking screws were loosened, the rubber insulator equipped with the optical block becomes functional and absorb an external shock or vibration.

CAUTION

Do not remove the screws. Do not run them out so far that the detector's weight rests on the screw heads instead of its feet.

note

Detector will not stabilize if locking screws are not loosened

2-6. Making a connection

2-6-1. Power Line

The power switch is located on the lower left of front panel as viewed in 3-1. The power should be OFF before connecting the power cord to the detector. The receptacle for the power cord is located on the rear of the detector. Refer to 3-2.
Connect the end of grounding cable to the ground terminals on the back panel and to a known ground.

WARNING:
SHOCK HAZARD

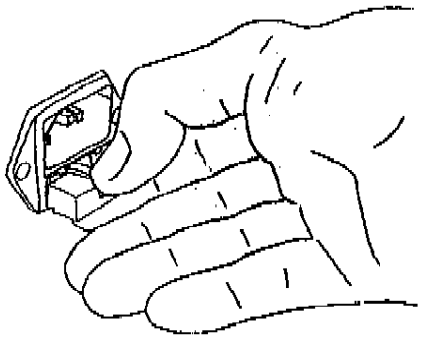
Electrically conducting spills can occur when conductive HPLC solvents are spilled on or in the instrument.
Ground the instrument properly to protect the operator from electrical shock. Proper grounding also protects the instrument from power line noise.
Verify that the instrument is properly grounded through the power line ground terminal. Do not remove or otherwise disable the power cord's ground prong.

2-6-2. Replacing Fuse

WARNING:
FIRE HAZARD

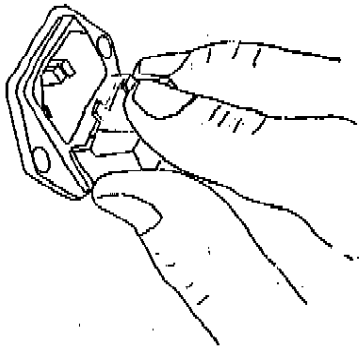
Replace only with same type and rating of fuse.
Fuse for RefractMax521: 3.15A(T3.15AL/250V)

To remove fuse holder



Turn off the power of detector.
Remove the power cord.
Push the lever located just above of Fuse holder cover. Pull the holder out as you hear clicking sound.

To re-install fuse holder



Replacing fuse.
Push the fuse holder in until clicking Sound is heard.
Make sure the holder is locked.

2-6-3. Tubing Connections

- Connection to the Inlet

CAUTION

RefractoMax521 must be the last component in your LC system. No detector or backpressure regulator can follow it. Do not use narrow-bore tubing for the outlet.

Hook up the narrow-bore stainless steel tubing of the Standard (0.25 mm ID, 1M long) with the inlet (IN) port of front panel.

- Connection on the Outlet Side
  Hook up Teflon® tubing (1.5 mm ID, 1.5 m long) of the Standard with the outlet (OUT) port of front panel. Lead the opposite end of tubing into drain bottle.

CAUTION

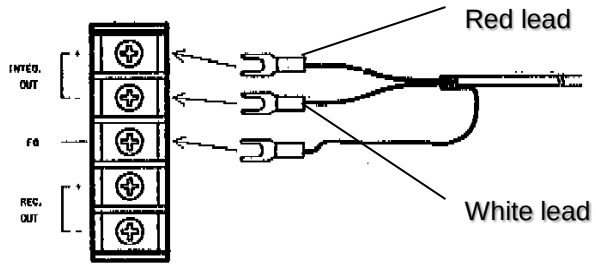
Do not expose the purge valve against backpressures greater than 50 kPa (7 psi). Also, never subject the reference and sample cells to back pressures greater than 700 kPa (100 psi). High backpressures can break the cells.

2-6-4. Cable Connections

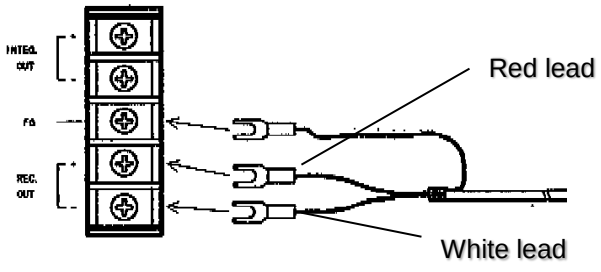
CAUTION

The GROUND terminals, on the lower right side of the rear panel (see), are not the same as the recorder ground or Integrator ground terminals. Do not inter change these connections.

- Integrator Connection
  Using the output signal cable supplied in the Standard, connect one end to integrator, white lead to integrator negative (–) and red lead to integrator positive (+).
  Connect the third lead to the GROUND (FG).



- Recorder connection
  Using the output signal cable supplied in the Standard, connect one end to the chart recorder, white lead to recorder negative (–) terminal and red lead to recorder positive (+).
  Connect the opposite end to the recorder's 10 mV terminal.
  Connect the third lead to the GROUND (FG).



2-7. Solvent Recommendations

Grade of Solvents

HPLC grade solvents are recommended for better instrument performance and chromatographic data.

Solvent Filtering

All solvents, including deionized water, should be filtered by in-line solvent filter.

Solvent Degassing

Solvents should be properly degassed prior to use. Solvents that are not properly degassed may cause bubble formation in pump heads and in the detector cells potentially causing pressure flow problems and a noisy chromatographic baseline. Degassing is especially critical when the column temperature is elevated above room temperature, or with solvents that have a high gas solubility, e.g., methanol. Analysis times that run greater than 5 to 6 hours will also require degassing of solvents.

On-line (continuous) solvent degassing is most ideal to have a constant state of mobile phase solvent over time, as the degassed solvent would start reintroducing the air.

Additional Precautions

Some solvents may corrode the wetted surfaces of the detector, if they are left in the detector after operation. The quartz cell window is easily etched by strong bases. It is recommended that some solvents be rinsed from the detector for overnight and weekend storage.

The solvents used with the detector are limited by the materials used for the wetted parts they come in contact with (e.g., quartz glass, Teflon® and 316 stainless steel). These limitations should be considered as you are choosing mobile phase solvents.

When an organic solvent that contains halogens, such as chloroform and methylene chloride, is used, flush out entire flow path with a solvent compatible with your chromatographic condition. (for example, hexane or another hydrocarbon)

For isocratic work with a normal phase column, the alcohols methanol or 2-propanol, a non-carcinogenic aromatic such as the xylenes, acetone, or an ether that is non-volatile and not a ready producer of peroxides can be used. DO NOT use ethyl ether.

If a solvent listed below is in use, flush out all the flow path sufficiently with an inert solvent that is compatible with your chromatographic system. Buffers, acids, and other highly ionic aqueous solutions should be flushed out with large amounts of water (5-10 times the volume of liquid from pump head to detector outlet If you neglect this flushing, the pump, injector, and column may become corroded and badly damaged.

Sulfuric Acid, Boric Acid, Citric Acid, Acetic Acid, Lactic Acid,

Acetic Anhydride, KOH, NaOH, Hydrazine, Sodium formate

Ammonium salts: -formate, -perchlorate, - nitrate, -citrate, -oxalate, -sulfate, -H_2PO_4, $_2CO_3$

K, Na salts: -bicarbonate, -chlorate, -nitrite

Following solvents should be avoided.

Hydrohalogenic, Metal Halides >2M, KCl, Ammonium Halides,

Ammonium Formate, All hypochlorites, Tetrachloromethane

Acids: HCl, HF, etc.

CAUTION

Fluorocarbon solvents will alter Teflon® over long exposure. Flush with pentane or another light hydrocarbon.

note

If you want to replace one solvent with an immiscible one, flush out the existing mobile phase with an intermediary solvent inter-miscible with your initial and final solvents. For example, you want to replace water in your HPLC with chloroform; water and chloroform are immiscible. Replace the water in your system with 2-propanol, which is freely miscible with water and chloroform. When you are certain all water is removed, replace the 2-propanol with chloroform. See the Miscibility Chart in Appendix.

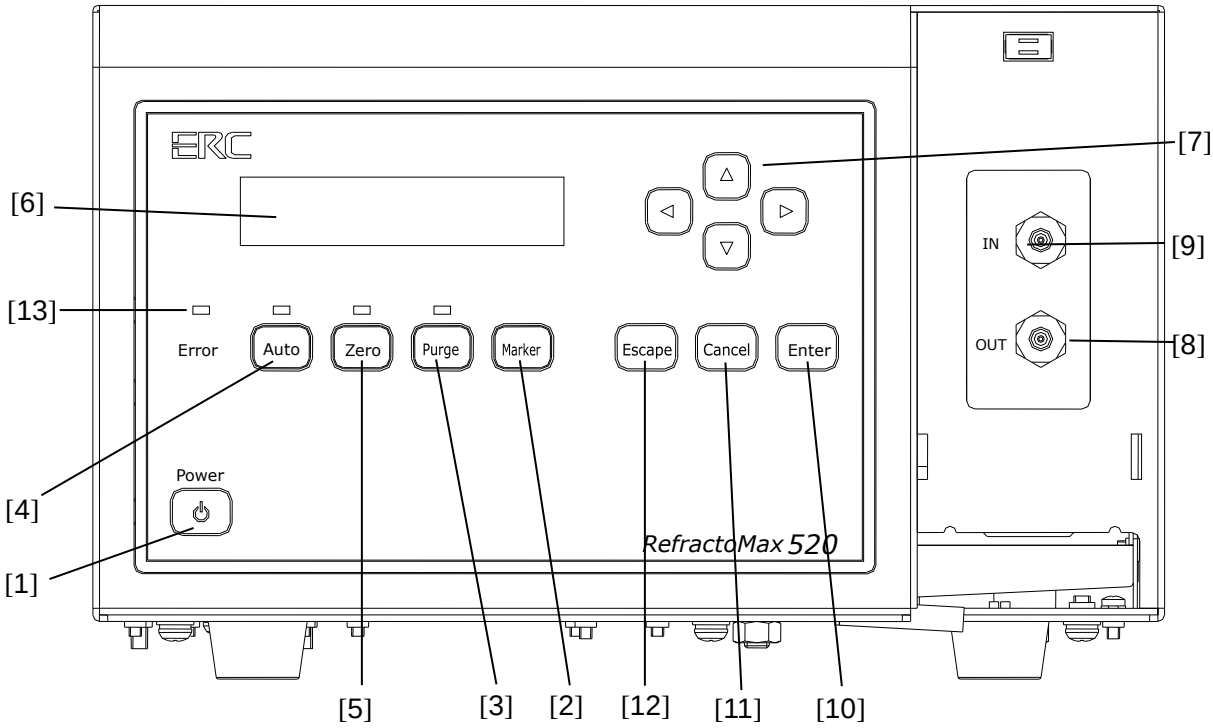
2-8. Characteristics of commonly used mobile phase

(Those in bold should not be employed)

	Polarity $E^2(Al_2O_3)$	Viscosity $_CP20\ ^oC$	Refractive Index	UV Cut-off (nm)	Flash Point (^oC)	Fire Point (^oC)	Upper	Lower	Vapor Density	Boiling Point (^oC)	Gravity
Fluoroalkanes	-0.25		1.25								
n-Pentane	0.00	0.23	1.358	210	<-40	308.9	1.5	7.8	2.5	36.1	0.6
Hexane	0.00		1.375	210	-21.7	233.9	1.2	7.5	3.0	68.9	0.7
Isooctane	0.01		1.404	210							
Petroleum ether	0.01	0.3		210							
n-Decan	0.04	0.92	1.412		46.1	207.8	0.8	5.4	4.9	173.9	0.7
Cyclohexane	0.04	1.00	1.427	210	-20	260	1.3	8	2.9	81.7	0.8
Cyclopentane	0.05	0.47	1.406	210							
Diisobutylene	0.06		1.411	210							
i-Pentene	0.08		1.371		-17.8	272.8	1.5	8.7	2.4	30	0.7
Carbon disulfide	0.15	0.37	1.626	380	-30	100	1.3	44	2.6	46.1	1.3
Carbon tetrachloride	0.18	0.97	1.466	265							
Amyl chloride	0.26	0.43	1.413	225	12.8	343.3	1.6	8.6	3.7	106.1	0.9
Busy chloride	0.26		1.436	220	-9.4	460	1.8	10.1	3.2		
					o-17.2	463.9	1.6	6.0		144.4	
Xylene	0.26	0.62-0.8 to 1.50		290	m-25	527.8	1.1	7.0	3.78	138.9	0.9
					p-25	528.9	1.1	7.0		138.3	
i-Propyl ether	0.28	0.37	1.368	220	-27.8	443.3	1.4	21	3.5	68.9	0.7
i-Propyl chloride	0.29	0.33	1.378	225	-32.2	593.3	2.8	1037	2.7	35	0.9
Tolune	0.29	0.59	1.496	285	4.4	536.1	1.4	6.7	3.1	110.6	0.9
n-Propyl-chloride	0.30	0.35	1.389	225	<-17.8		2.6	11.1	2.7	46.1	0.9
Chlorobenzene	0.30	0.80	1.525		32.2	637.8	1.3	7.1	3.9	132.2	1.1
Benzene	0.32	0.65	1.501	280	-11.1	562.2	1.4	7.1	2.8	80	0.9
Ethyl bromide	0.37		1.424			511.1	6.7	11.3	3.8	37.8	1.4
Ethyl ether	0.38	0.23	1.353	220	-45	180	1.9	48	2.6	35	0.7
Ethyl sulfide	0.38	0.45	1.442	290							
Chloroform	0.40	0.57	1.443	245							
Methylene-chlolride	0.42	0.44	1.424	245	-50	518.9	3.8	15.4	2.2	38.5	0.9
Methyl I-butyl ketone	0.43		1.394	330							
Tetrahydrofurane	0.45		1.408	220	-14.4	321.1	2	11.8	2.5	66.1	0.9
Ethylen dichloride	0.49	0.79	1.445	230	13.3	412.3	6.2	16	3.4	83.9	1.3
Methyl ethyl ketone	0.51		1.381	330	-6.1	515.6	1.8	10	2.5	80	0.8
i-Nitropropane	0.53		1.400	380	48.9	420.6	2.6		3.14	131.1	1.0
Acetone	0.56	0.32	1.359	220	-17.8	537.8	2.6	12.8	2.0	56.7	0.8
Dioxane	0.56	1.54	1.422	260	12.2	180	2.0	22	3.0	101.1	1.0
Ethyl acetate	0.58	0.45	1.370	260	4.4	460	1.8	8	3.5	90	0.9
Methyl acetate	0.60	0.37	1.362	210	-10	501.7	3.1	16	2.6	60	0.9
Amyl alcohol	0.61	4.1	1.410		32.8	300	1.2	10.0	3.0	137.8	0.8
Dimethyl sulfoxide	0.62	2.24									
Aniline	0.62	4.4	1.586		70	617.2	1.3		3.2	184.4	1.0
Dimethyl amine	0.63	0.38	1.387	275	<-17.8	312.2	1.8	10.1	2.5	56.7	0.7
Nitromethane	0.64	0.67	1.394	380	35	418.3	7.3		2.1	101.1	1.1
Acetonitrile	0.65	0.37	1.344	210	5.6				1.4	81.7	0.8
Pyridine	0.71	0.94	1.510	305	20		1.8	12.4	2.7	115	1.0
Butyl cellosolve	0.74			220							
i-Propanol n-Propanol	0.82	2.3	1.38	210	11.7	398.9	2.0	12	2.1	82.8	0.8
Ethanol	0.88	1.20	1.361	210	12.8	422.8	4.3	19	1.6	78.3	0.8
Methanol	0.95	0.60	1.329	210	11.1	463.9	7.3	36	1.1	63.9	0.8
Ethylene glycol	1.11	19.9	1.427	210	111.1	412.8	3.2			197.2	1.1
Acetic acid	large	1.26	1.372								

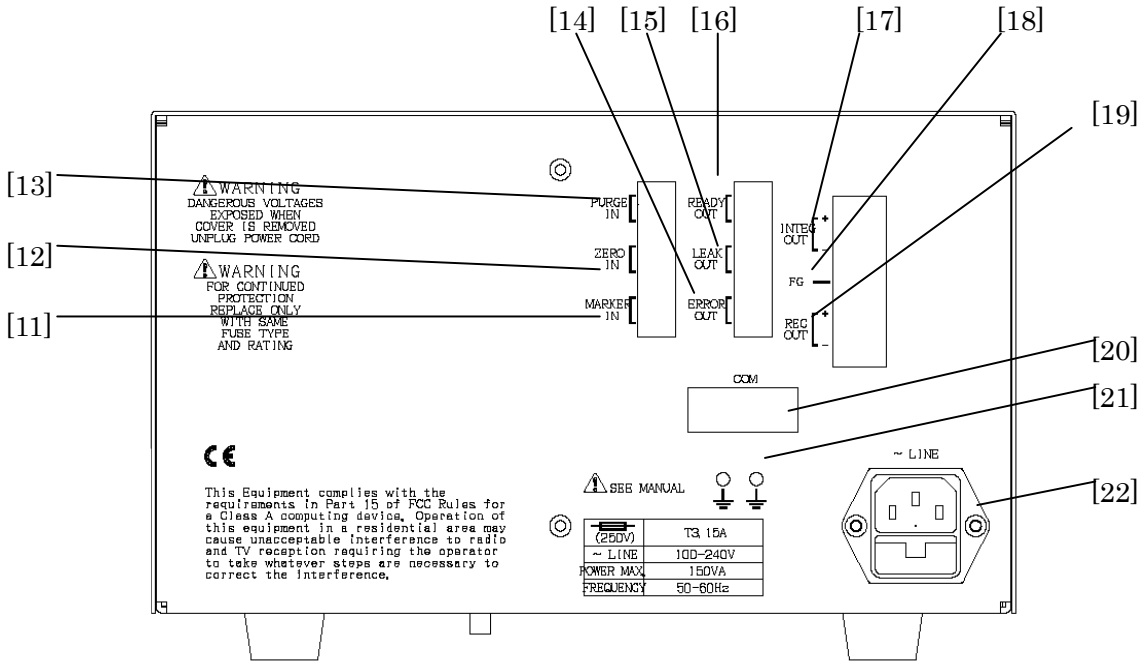
Section 3. Function Keys and Displays

3-1. Front Panel



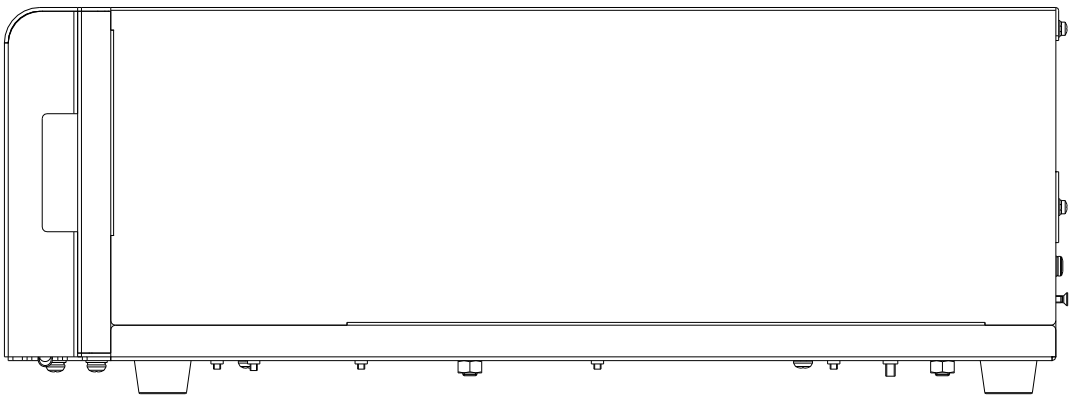
[1]	Power Switch:	Press this key once to turn on or off the unit.
[2]	Event Marker Key:	Press to generate a marker signal. (5% of Full Scale)
[3]	Purge Key:	Press to turn purge valve on or off to change flow path. When the valve is on, LED above the key illuminates and “PURGE” appears on the lower line of LCD display. Solvent flows through reference side of flow cell instead of sample side.
[4]	Auto Set Up Key:	Press this key to do Auto Set Up. As “Auto Set Up” is going on, LED above the key illuminates and “AUTO” appears at the lower line of LCD display.
[5]	Auto-Zero Key:	Press this key to do Auto-Zero. As Auto-Zero is going on, LED above is illuminated and “AUTO ZERO” appears on the lower line of LCD display.
[6]	LCD Display:	Liquid Crystal Display
[7]	Arrow Keys:	Press to change screen, to move cursor or to edit values.
[8]	Outlet Port:	
[9]	Inlet Port:	
[10]	Enter Key:	Press to save the edited data or to confirm the command.
[11]	Cancel Key:	Press this key to scrap the edited data.
[12]	Escape Key:	Press this key once to return to Monitor Screen.
[13]	Error LED:	Error message

3-2. Rear Panel



[11] MAKER IN: External Signal In (Event Marker)
[12] ZERO IN: External Signal In (Auto Zero)
[13] PURGE IN: External Signal In (Purge On)
[14] ERROR OUT: Signal Out (Error)
[15] LEAK OUT: Signal Out (Solvent Leak)
[16] READY OUT: Signal Out (Ready)
[17] INTEG. OUT: Integrator Out
[18] FG: Ground for signal cable
[19] REC. OUT: Recorder Out
[20] COM: RS-232C Port
[21] Ground Terminal
[22] Power Inlet

3-3. Side View



3-4. LCD Display (User Screens)

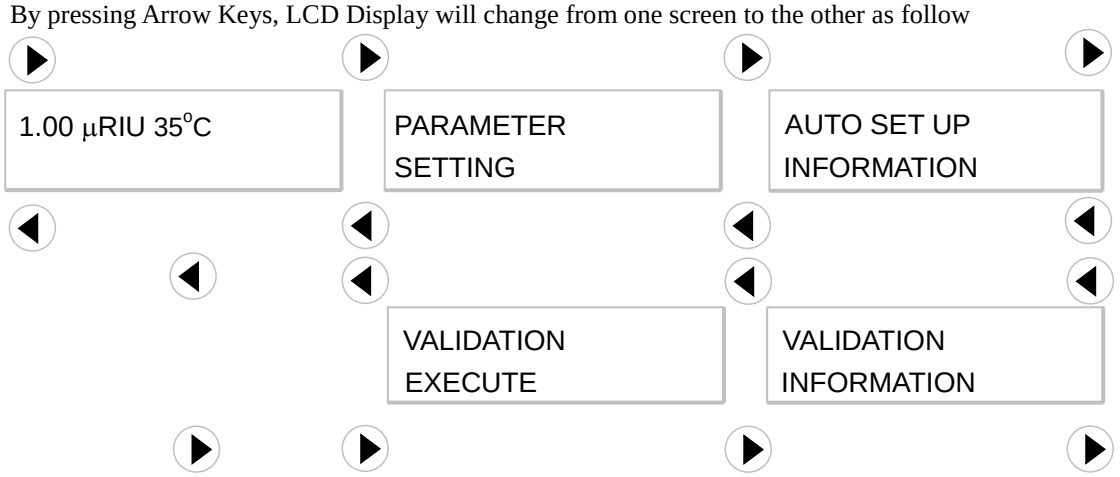
RefractoMax521 has a LCD display on the front panel as shown in 3-1. Front View here above. The display consists of following four screens.

3-4-1. Default Screens (Start Up Screens)

Refractoamax 521 Ver.RA21E01	Model name and Program version number show up as the power switch is on.
INITIALIZING	"INITIALIZING" appears during the initialization.
0.0 μRIU 35°C TEMP.UNSTABILIZED	Monitor screen shows up after the initialization. "TEMP.UNSTABILIZED" indicates the unit has not reached to the default temperature (35°C) yet.
0.0 μRIU 35°C RD	As the unit reaches the default temperature (35°C) "TEMP.UNSTABILIZED" goes off and "RD" shows up on the upper line (Ready).
note	*When RefractoMax521 finished the initialization, LCD Display will automatically shows "Monitor Screen". Should the screen have a message "TEMP. UNSTABILIZED" originally, you may go ahead reviewing and/or editing those parameters by using Arrow Keys and other functional keys.*

3-4-2. Screen Sequence

By pressing Arrow Keys, LCD Display will change from one screen to the other as follow



3-4-3. Parameter Setting Screen

PARAMETER
SETTING

Parameter Setting Screen Home

PARAMETER
REC. RANGE 512uRIU

Recorder Range Setting
By pressing Enter key, a cursor appears to indicate the range is selectable.
Select 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 or 0.25 by pressing Up or Down Arrow keys. ▲ ▼
Press Enter key to confirm the selection.

PARAMETER
INTEG. RANGE 500uRIU

Integrator Range Setting
By pressing Enter key, a cursor appears to indicate the range is selectable.
Select 500 or 125 by Arrow keys. ▲ ▼
Press Enter key to confirm the selection.

PARAMETER
TEMPERATURE 35°C

Temperature Setting
By pressing Enter key, a cursor appears to indicate the temperature setting is editable.
Select temperature between 30 and 55°C or OFF by Arrow keys. ▲ ▼
Press Enter key to confirm the selection.

PARAMETER
TIME CONSTANT 3 SEC

Time Constant Setting
By pressing Enter key, a cursor appears to indicate the time constant is selectable.
Select 6, 3, 2, 1.5, 1, 0.5, 0.25 or 0.1 by Arrow keys. ▲ ▼
Press Enter key to confirm the selection.

PARAMETER
POLARITY +

Polarity Selection
By pressing Enter key, a cursor appears to indicate the polarity is selectable. Select + or - by Arrow keys. ▲ ▼
Press Enter key to confirm the selection.

PARAMETER
BASELINE SHIFT 0

Baseline Shift Setting
By pressing Enter key, a cursor appears to indicate the baseline shift setting is editable.
Select baseline shift between 0 and 50 by Arrow keys. ▲ ▼
Press Enter key to confirm the selection.

PARAMETER
SELECT DEFAULT

Default Setting
If you want to reset parameter setting and bring it back to default setting, press Enter key. A cursor and “NO” show up. Select “YES” by Arrow keys. ▲ ▼
Press Enter key to confirm the selection.

ERASE
CURRENT SETTING? NO

Default Setting (2)
After pressing Enter key above, this message shows up to reconfirm if you do want to bring it back to default setting. Select “YES” by Arrow keys. ▲ ▼
Press Enter key.
Parameter is changed back to default setting and the unit will beep.
The LCD display goes back to Monitor Screen.

PARAMETER
LCD CONTRAST 4

LCD Contrast Setting
By pressing Enter key, a cursor appears to indicate that you can change LCD contrast.
Select contrast between 1 and 7 by Arrow keys. ▲ ▼
Press Enter key to confirm the selection.

PARAMETER
AUTO MODE STANDARD

Auto Set Up mode Setting
By pressing Enter key, A setup changes a judgment standard by three kinds, FINE, STANDRD, and COARSE.
Select by Arrow keys. ▲ ▼
Press Enter key to confirm the selection

Auto Set Up mode

	Fine	Standard (Default)	Coarse
Purge Cycle	30 sec.		
Number of Cycle	3		
Time to Auto Zero	240 sec.		
Equilibration Time	80 min.	60 min.	40 min.
Measuring Time	80 min.	60 min.	40 min.
Drift	100 nRIU/h	500 nRIU/h	2500 nRIU/h
Noise	50 nRIU		

Parameter Default Setting

Parameter	Setting value	Unit	Default
REC. RANGE	0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 (12 steps)	uRIU/10mV	512
INTEG. RANGE	125 or 500	uRIU/1V	500
TEMPERATURE	OFF, 30 to 55 (increment: 1^{o}C)	Celsius	35^{o}C
TIME CONSTANT	0.1, 0.25, 0.5, 1, 1.5, 2, 3, 6 (8 steps)	Second	3
POLARITY	+ or -	N/A	+
BASELINE SHIFT	0 to 50 (increment: 10mV)	10mV	0
SELECT DEFAULT	YES or NO	N/A	NO
LCD CONTRAST	1 to 7 (increment: 1)	N/A	4

3-4-4. Auto Set Up Information Screen

AUTO SET UP
INFORMATION

Auto Set Up Information Screen Home
It moves to each setting screen by Up or Down Arrow keys.

LAST S.SEQ
DATE **.**.**

Date of the latest Auto Set Up of this unit

DRIFT
100.0 nRIU

Drift result of the latest Auto Set Up of this unit

NOISE
10.0 nRIU

Noise result of the latest Auto Set Up of this unit

3-4-5. Validation Information Screen

VALIDATION INFORMATION	**Validation Information Screen Home**
LAST VALIDATION DATE **.**.**	Date of the latest Validation of this unit
SPAN 512.5μRIU	Result (Span) of the latest Validation of this unit
LAMP VOLTAGE 3.75V	Current Lamp Voltage of this unit

3-4-6. Validation Execute Screen

Validation Execute Screen Home

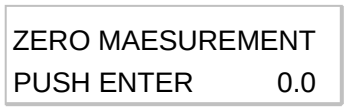
Press Enter key to get into following screen sequence.

Enter

Validation Date Entry Screen

Refer to 5-2-2. Span Validation about the detail.

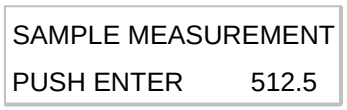
Enter



Zero Measurement Screen

Refer to 5-2-2. Span Validation about the detail.

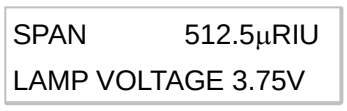
Enter



Sample Measurement Screen

Refer to 5-2-2. Span Validation about the detail.

Enter



Span Validation Result Screen

Refer to 5-2-2. Span Validation about the detail.

3-4-7. Error Messages

To alert you about the situation that is hazardous or may cause a deterioration of your analysis, RefractoMax521 flashes following five different error messages.

100.0 μRIU 35°C
LEAKAGE ERROR

Solvent Leak Error

This indicates there is a solvent leakage inside of cabinet.

CAUTION

When this error message comes on, power off the detector at once.

Please double-check if there is any solvent leak. In case you cannot fix problems, please contact our local representatives in your area.

600.0 μRIU 35°C
HOME POSITION ERROR

Null Glass Home Position Error

This indicates the null glass doesn't come back to its home position.
If you are commanding Auto-Zero via external control devise, refer to the procedure for "Optical Balance Error.
If you have this error as you are turning on RefractoMax521, please contact our local representatives in your area.

100.0 μRIU 70°C
OVERHEAT ERROR

Overheating Error
This indicates overheating of optical block.
Please contact our local representatives in your area.

100.0 μRIU 70°C
OPT.BALANCE ERROR

Optical Balance Error
This indicates that Auto-Zero wasn't successful.
Insufficient solvent exchange of reference flow path might cause this.

Try purging procedure to fill reference flow path with fresh solvent at once.

100.0 μRIU 35°C
PARAMETER ERROR

Parameter Error

This indicates the units failed to store data.

3-4-8. Other Messages

There are four other messages as described below. Some of them are more like a warning message.

0.0 μRIU 35°C
INTENSITY

Low Light Intensity

This indicates inadequate light intensity. There are several possible causes for this error as follow.

1. Different state of solvents between sample-side flow cell and reference-side flow cell. Purge reference-side flow path with fresh solvent.
2. Air bubble in flow cells. Repeat purge on and off with flow to remove the air bubble.
3. Optical axis is off from the center. Press Zero Key to do “Auto-Zero”.
4. The flow cell is empty.

0.0 μRIU 35°C
TEMP.UNSTABILIZED

Unstable Temperature

This indicates temperature of optical system hasn’t met the preset temperature.

0.0 μRIU 35°C
PURGE

Purge

This indicates the purge valve is on. Solvent flows through both of reference cell and sample cell.

0.0 μRIU 35°C
AUTO ZERO

Auto-Zero

This indicates the Auto-Zero adjustment function is working.

Section 4. Getting Started

4-1. Just in Case !

Prior to operation, check the following one more time.

.

- Locking screws are loosened.
- Mobile phase solvent is freshly made and degassed well.
- All wetted parts are chemically compatible to the mobile phase solvent.
- Mobile phase flushed through the entire flow path; all incompatible or immiscible solvents have been flushed out.
- Cable connections are properly made to chart recorder, integrator, data system, or other external equipment.
- All tubing connections are properly made and checked for leaks.
- Power cord is plugged into appropriate power receptacle.
- Proper fuse is installed.
- Drain tube is installed.
- Power switch is ON.

CAUTION

Before activating the purge valve (LED light OFF), pump about 10 ml of liquid through the cell.
This will flush possible dust or particulate matter and reduce the possibility of damaging the valve seals.

4-2. Start Up

(1) Start pumping mobile phase solvent at flow rate 1 ml/min to reference cell (Purge On).
(2) Press Purge key in every 10 seconds to on/off the purge valve for few minutes.
(3) Keep pumping mobile phase solvent to reference cell for about 20 minutes from the above step (2).
(4) Press Purge key to turn off the valve. Mobile phase solvent flows to sample-side flow cell.
(5) Wait until the baseline is stabilized.
(6) Press Zero Key to do Auto Zero.

WARNING:
CHEMICAL HAZARD

Wearing protective gloves and goggles is advised.

4-3. Parameter Setting

Set the parameter on Parameter-Setting Screen (Refer to 3-4-3).

Section 5. Maintenance and Validation

5-1. Cell Cleaning

In many cases, performance degradation in sensitive instruments equipped with flow-through cells is caused by cell contamination. The use of filtered solvents with in-line solvent filters will protect the cell from contamination and reduce the amount of cleaning required. However contamination from trapped particulates or bubbles, from precipitates, or from thin films of residues can still occur.

5-1-1. Preparations

To introduce cleaning solution into RefractoMax521 by solvent delivery pump, connect a tubing line directly from the pump to RefractoMax521 inlet port bypassing the column. Some cleaning solutions should be injected, however, directly into the flow cells by syringe due to their high corrosiveness or safety concern.

CAUTION

Remember that flow cells can stand with only up to 70 kPa (100 psi). So, gently flush the cells under all conditions. If you encounter a high backpressure in RefractoMax521, use extreme caution to proceed. You will be risking flow cell rupture, and flow cell assembly replacement is not a recommended customer procedure.

note

Clean all internal lines of RefractoMax521 by injecting cleaning solution with PURGE OFF, and inject cleaning solution again with PURGE ON.

Particulate matter can be removed by forcing liquid through the cell using the syringe. Sometimes it helps to reverse flow and inject in the outlet port.

5-1-2. Cleaning

Depends on the solvents in use, the cleaning procedure is varied. Following is a procedure for typical.

(1) Inject cleaning solution (acetone) by syringe from the inlet port (5 ml).
(2) Inject de-ionized water by syringe from the inlet port (5 ml).
(3) Inject nitric acid solution (15%) by syringe from the inlet port (5 ml).
(4) Expel nitric acid solution completely by flowing de-ionized water adequately.
(5) Exchange de-ionized water with the mobile phase solvent.

If buffers or solutions of high salt content have been in use, the cells may be contaminated by precipitated salt. Pumping a large amount of distilled, de-ionized water, such as 1 ml/min, for up to several hours, is the simplest clean-up procedure.

An elevated cell temperature will speed dissolution. The water wash can be acidified, if the precipitated salt is more soluble in acidic solutions. However, do not use strongly basic (pH 10 or higher) solutions, as these will etch the refractive index cells.

If contamination is suspected when a non-aqueous solvent is in use, flush the cells with a solvent that is (1) miscible with your mobile phase, (2) a good solvent for the predicted contaminant, and (3) generally of greater polarity than your mobile phase.

WARNING:
EXPLOSION HAZARD

Do not allow nitric acid to contact methanol. An explosion could result. Completely rinse the flow cell with water following cleaning with nitric acid.

WARNING:
CHEMICAL HAZARD

Corrosive acids are used. Use extreme caution to avoid spillage on skin, clothing, or the instrument. Protective gloves are advised.

CAUTION

Never put hydrochloric acid in the cell. This acid in any concentration will corrode the cell.
Diluted (10-20%) or concentrated nitric acid is a good cleaning solution.
The sample and reference cells should be filled with water or air (blown dry) before proceeding.

Filled the syringe with cleaning solution and connect to the inlet port of the RefractoMax521. Carefully inject the cleaning solution. For safety, make sure that outlet tubing is led to drain bottle.

Flush acid cleaning solutions from the cells with large amounts of water, such as 1 ml/min. Flush for 15 to 30 minutes.

note

For shutdown and storage, please review Section 6. Shutdown Procedure.

5-2. Validation

From time to time, you are recommended to validate your HPLC system to keep an accuracy and credibility of your analysis.

5-2-1. Validation Information

VALIDATION INFORMATION	Validation Information Screen Home
LAST VALIDATION DATE **.**.**	Date of the last Validation of this unit
SPAN 512.5 μRIU	Result (Span) of the last Validation of this unit
LAMP VOLTAGE 3.75V	**Lamp Voltage reading at the last Validation of this unit** This indicates an applied voltage to the lamp. As it exceeds 4.5V, purge the reference side flow path with fresh solvent at once. (Applied voltage to the lamp is automatically raised as light intensity from the flow cell drops. In many cases the reduction of intensity is due to dirt in the reference flow cell.) If the voltage stays high, you may want to replace the light source.

5-2-2. Span Validation

To do Span Validation, follow the below procedure.

(1) Preparation of standard sucrose solution:
Weigh out 350 mg sucrose and transfer quantitatively to a volumetric flask.
Dissolve in the 100 ml de-ionized, filtered, degassed water and dilute to flask's mark.

note ***Use freshly made sucrose solution always.***

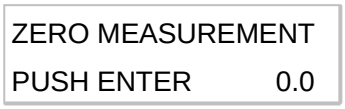
(2) Equilibrate RefractoMax521 by pumping de-ionized water through both reference and sample cells. Use the same de-ionized water as that used to prepare the sucrose standard solution. Start pumping at flow rate to 1 ml/min.
(3) Make sure that the baseline is stabilized and the drift is equal or less than 500 nRIU/h by monitoring recorder chart.

Enter

Press Enter key to get into following screen sequence.

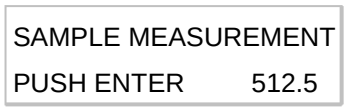
Validation Date Entry
After pressing Enter key above, this screen shows up for you to enter the date (YY.MM.DD).
A cursor moves from left to right by pressing Enter key.
At each digit, you can change figure by Arrow keys.
Upon completing date entry, press Enter key for next step.

Enter



Zero Measurement Screen
Press Auto-Zero key to do Auto Zero.

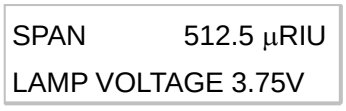
Enter



Sample Measurement Screen

(4) Disconnect and remove tubing from the inlet port of RefractoMax521.
(5) Make sure that the purge valve is off.
(6) Filled the syringe with standard sucrose solution and gently inject from the inlet port.
(7) Validation screen will come on with the measuring result.
(8) The result should be within 487 to 537 (512 uRIU +/-5%)
(9) Press Enter key to approve the above sample measurement result.

Enter



Enter

By pressing Enter key, the above validation results will be stored. The screen will go back to Validation Execute Home Screen

note ***Make sure that you have put the tube back to inlet port.***

5-2-3. Auto Set Up Execute

Monitor Screen

AUTO

Auto Set Up Date Entry
After pressing Enter key above, this screen shows up for you to enter the date (YY.MM.DD). A cursor moves from left to right by pressing Enter key. At each digit, you can change figure by Arrow keys.
Upon completing date entry, press Enter key for next step.

Enter

Auto Set Up Screen

note *Auto Set Up cannot be performed other than Monitor Screen.*

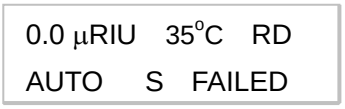
▼

Case1:

When stability is obtained within regulation time.

▼

Case2:

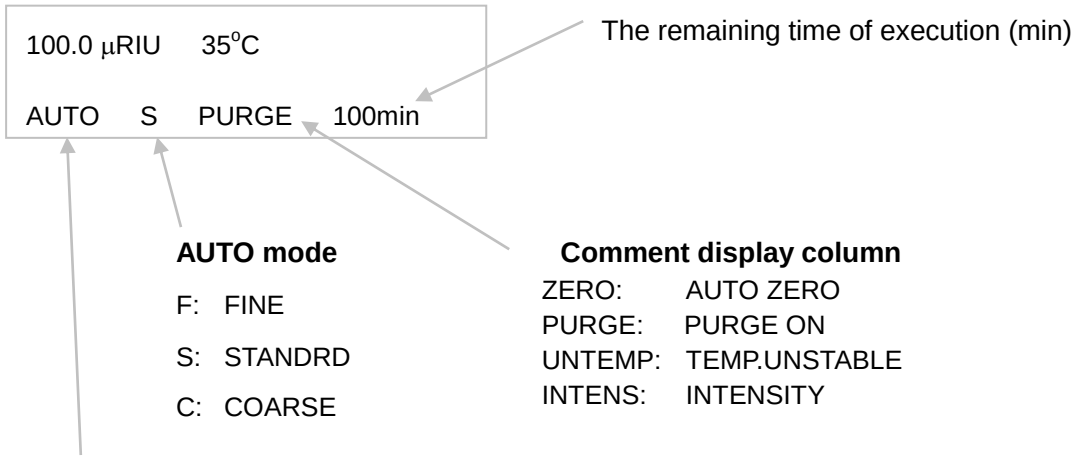


When stability is not obtained within regulation time.
When a drift and a noise do not enter in a judgment standard within regulation time, it judges that it will be in the state where still sufficient stability is obtained, and is displayed as "FAILED" on a screen.

Enter or Cancel

Monitor Screen

LCD display explanation：



Indicates that RefractoMax521 is under Auto Set Up execution.

note:

1) *When an error occurs during Auto Set Up execution, execution is stopped and the contents of an error are displayed.*
2) *Communication (RS232C) with the exterior cannot be performed during Auto Set Up execution.*
3) *During Auto Set Up execution, Escape key is invalid.*

Section 6. Shutdown Procedure

6-1. Corrosive Solvents

Some solvents may corrode the detector, if they are left in the detector and should be thoroughly flushed from the entire system, including the reference and sample flow cell.

The quartz flow cell window, in particular, is easily etched by strong bases. Do not turn power to RefractoMax521 off without rinsing these solvents from the detector.

Some solvents can be left in the cells at the end of an operation. For example, water, acetonitrile, 2-propanol, the xylenes, and paraffinic hydrocarbons are quite innocuous. They may be left in RefractoMax521 overnight or over a weekend.

6-2. No Flow Shutdown Versus Reduced Flow Shutdown

A continuous slow flow through RefractoMax521 is the preferable shutdown procedure if the situation permits. (Especially if buffers, tetrahydrofuran and organohalocarbons are in use.)

Reduced flow may be 0.5 ml/min to 0.01 ml/min but the HPLC pump must be able to stay primed at reduced flow.

Buffers:

Even if the buffer is non-corrosive, it is better to keep the solvent flowing at a reduced rate to eliminate the possibility of salt precipitation in the flow cells and tubing.

Tetrahydrofuran:

Because THF does oxidize, you may find that, if you keep solvent flowing at a reduced rate, the chromatographic system takes less time to re-stabilize upon start-up. Generally a reduced-flow shutdown procedure will minimize re-stabilization time; the time saved is noticeable with THF as the solvent.

Organohalocarbons, such as Methylene chloride and Chloroform:

Keep a small amount of flow to keep down the amount of corrosive chloride impurities in the cell.

6-3. Long-Term Storage

If RefractoMax521 will not be used for a week or more, the sample and reference cells should be blown dried.

If the detector is to be exposed to sub-freezing temperatures, an antifreeze flush, such as methanol must be used. Ideally, you are suggested to expel residual solution out of RefractoMax521 and to blow and dry.

Section 7. Troubleshooting

7-1. Introduction

Malfunctions within RefractoMax521 can arise from three general sources:

- RefractoMax521 itself can be dirty, or operating non-optimally.
- The HPLC system can have a broken, dirty, or non-optimally operating component, but the problem is manifesting itself in RefractoMax521.
- A mobile phase and/or column problem, which by its very nature is spread throughout the HPLC system but appears as a malfunction of RefractoMax521.

To troubleshoot, you must be able to isolate the performance of RefractoMax521 within the HPLC system from its performance outside the HPLC system. Therefore, this section begins with guidelines for testing RefractoMax521 as a stand-alone.

Following is the Troubleshooting Table that lists the observed phenomenon with the possible cause and the suggested solution.

7-2. Make sure how the detector itself is working.

You must know how well the detector performs by itself before you can troubleshoot it in an HPLC system.

To perform the tests, disconnect all cables from the detector except one signal cable from recorder output to a calibrated, functioning recorder. The recorder should match input to the output of RefractoMax521 (10 mV).

Before proceeding, verify also that the locking screws on the optics module have been loosened; that the correct voltage is supplied and the correct fuse installed; and that degassed water is in the reference cell, the sample cell, and the entire flow path.

7-3. To isolate the cause of problem

As you go through the Troubleshooting Table, you will have occasional instructions for HPLC systems. Generally, however, the rule of thumb is to add one component at a time back into the HPLC system so that, should the condition arise again, the component causing the problem is indicated.

You will begin by adding the pump to RefractoMax521 first and you will add the column last.

If another type of detector is available, it is a good idea to use it before RefractoMax521 to aid in troubleshooting as reference. Let's assume that you have a UV detector before RefractoMax521 and only RefractoMax521 has a noisy baseline.

One possible implication is that the noise arises from pressure fluctuation, to which the refractive index detector is more sensitive. On the other hand, if both detectors are showing noise, a power line current may be indicated.

If both detectors show anomalous baseline performance, such as huge peaks that continue indefinitely, a bleed-off problem (material from the column or immiscible solvents trapped in the system) is more likely.

7-4. Troubleshooting (Diagnosis Chart)

Problem	Possible Cause	Solution
Noise	Bubble in the pump.	Purge pump heads. Use only premixed/degassed mobile phase.
	Bubble in the detector.	Elevate drain reservoir above the level of the flow cells to create slight backpressure. Premix and degas mobile phase well.
	Dirty flow cell(s).	Clean flow cells. Refer to Cell Cleaning.
	Weak lamp.	Check validation screen.
	Ambient temperature fluctuations.	Move detector to a more stable environment.
	Vapor pressure of the mobile phase is too high for the detector.	Reduce or turn temperature control off. Modify method to exclude or decrease concentration of troublesome solvent.
	Electrical transients from power line or radio frequency source.	Isolate the detector power source from other heavy equipment, motors, etc. Ground the detector to earth.
Noise appears in 8-10 hours of operation.	Formation of gases in the mobile phase reservoir.	Consider using on-line solvent degasser(R).
Cyclic Noise	Ambient temperature fluctuation.	Move the detector to a more stable environment. Place a cover over the detector
	Bubbles in reference cell.	Flush detector with Purge on/off
Cyclic Noise matching pump stroke frequency	Drain tube is too small (narrow bore).	Verify that correct exit line is installed. Check outlet tubing for crimps
Drift	Flow cell(s) dirty.	Clean reference and sample cells. Refer to Cell cleaning procedure.
	Flow cell(s) damaged.	Check for liquid in rear drain tube indicating a broken cell..
	Contamination from HPLC System.	Flush HPLC system with a solvent stronger than the mobile phase (less polar for reverse phase, more polar for normal phase, etc.) until contaminant disappears.

Problem	Possible Cause	Solution
Drift (continued)	Contaminated or non-HPLC grade solvents.	Prepare fresh mobile phase (premixed/degassed)
	Vapor pressure of mobile phase is too high for operating temperature causing bubble formation in reference cell.	Reduce or turn temperature control off. Modify method to exclude or decrease concentration of troublesome solvent.
	Tetrahydrofuran (THF) in the mobile phase will oxidize in the reference cell.	Add an antioxidant to stabilize the THF, if compatible with other chromatographic requirements. Allow >2 hours stabilization time for oxidation in reference cell to reach a steady state condition.
Baseline drift occurs in few hours of start.	Reference cell solvent has aged and deteriorated.	Flush reference cell with mobile phase.
Baseline will not zero	Sample and reference cells do not contain identical solutions.	Flush sample and reference cells with mobile phase.
	Reference cell contains air bubbles.	Flush sample and reference cells with mobile phase.
	Flow cell(s) dirty.	Clean flow cell. Refer to Cell Cleaning procedure.
	Flow cell(s) damaged.	Check for liquid in drain tube indicating a broken cell.
	Deteriorating lamp or lamp out of adjustment.	Check validation screen.
Intensity Alarm	Flow cell(s) is dirty.	Clean flow cell. Refer to Cell cleaning procedure.
	Lamp is burned out.	
	Flow cell(s) is empty.	Fill with the eluent.

Appendix. Miscibility Chart of Solvents

	Acetic Acid	Acetonitrile	Chloroform	Cyclohexane	Methylene Chloride	Dimethyl Formamide	Dioxane	Ethyl Ether	Hexane	Methanol	Methyl t-Butyl Ether	Trimethylpentane	Pentane	Propanol-2	Tetrahydrofuran	Water
Acetic Acid																
Acetonitrile																
Chloroform																
Cyclohexane		X														
Methylene Chloride																
Dimethyl Formamide				X												
Dioxane																
Ethyl Ether																
Hexane		X				X										
Methanol				X					X							
Methyl t-Butyl Ether																
Trimethylpentane		X				X				X						
Pentane		X				X				X						
Propanol-2																
Tetrahydrofuran																
Water			X	X	X			X	X		X	X	X			

X = Immiscible

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