

Ultrospec 3000 *pro* UV/Visible Spectrophotometer

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Ultrospec™ 3000 *pro* is a high-specification, stand-alone, Pharmacopoeia-compliant UV/Visible spectrophotometer with integral liquid crystal display and graphical user interface. Built-in applications software includes five modes for nucleic acid quantification and seven modes for protein studies, including Bradford, Lowry, Biuret, and BCA. Ultrospec 3000 *pro* can also be controlled from a PC using SWIFT II applications software modules, which add many detailed functions to the instrument's capabilities, including archive facilities. When used with a PC, data can be downloaded directly to Microsoft™ Excel and stored in a project directory for future manipulation with other results.

Introduction

Ultrospec 3000 *pro* has been designed with consideration for ease of use and elimination of reliance on specialist knowledge. The instrument features a modern, intuitive, menu-driven interface accessed via a keypad and high-resolution liquid crystal display. The user can configure the display and printouts in one of six languages: English, German, French, Spanish, Italian or Russian (Fig 1).

Ultrospec 3000 *pro* functions as a stand-alone instrument, and built-in applications software enables storage of up to 50 customized methods. These methods can be retrieved from any of the five routine operational modes that supplement those already available for nucleic acid quantification and protein determination.

When used with a PC, Ultrospec 3000 *pro* can download data directly into Microsoft Excel, or it can operate in a further range of analytical modes with SWIFT II software modules. There is also a comprehensive range of sample handling accessories and cells, which facilitate measurements of small samples, automatic sample processing, temperature-controlled enzyme kinetics and temperature programmable measurements for determination of nucleic acid melting temperatures.

Ultrospec 3000 *pro* has the performance and incorporated software to comply with the Pharmacopoeia. A bandwidth of 1.8 nm enables high-resolution scanning which is important for assurance of pharmaceutical purity and drug discovery procedures.



Fig 1. Ultrospec 3000 *pro* shown with printer and printer stand. This system combines excellent performance and high specification with minimum bench space.

Important calibration facilities for Good Laboratory Practice

The large number of biopharmaceutical products and their associated complexity has led the European market and the World Trade Organization to enact requirements for acceptance of test results across member states. This resulted in the establishment of acceptable means for determining conformity in test results. Enforcement of legislation is based upon valid calibration and testing. User confidence in test results has to be supported by proper calibrations and proof that instrument measurements are traceable.

The Eurochem Instrumentation Working Group has defined four stages in the process of establishing suitability of an instrument for use:

- design qualification: covers the functional and operational specification
- installation qualification: establishes instrument is received as designed and specified
- operational qualification: demonstrates function according to specification in the lab environment
- performance qualification: demonstrates performance according to the appropriate specification for routine use

For pharmaceutical companies and biotechnology facilities, two important test parameters are spectral resolution, defined by the British Pharmacopoeia, and

stray light, defined by the European Pharmacopoeia. Each Ultrospec 3000 *pro* instrument complies with these tests and is delivered with the appropriate factory final test certificate for verification. An “Instrument Qualification and Performance Verification Logbook” is also included, which details these tests and enables the results to be plotted as a function of time.

Routine instrument calibration is handled on start-up when five key parameters are checked:

- age and % energy of the lamps compared to their values when new
- wavelength accuracy compared to the 656 nm deuterium line
- absorbance values at 220, 340, and 500 nm of a built-in absorbance filter compared to instrument manufacture (or last service by an accredited engineer)
- bandwidth at 656 nm
- instrumental stray light at 220 nm

For laboratories operating under Good Laboratory Practice (GLP) conditions, experimental results can be traced to a specific instrument by entering laboratory, operator, and internal instrument reference names via the keypad. The user is able to obtain an automatic printout of the test results showing also the date and time.

Routine operational modes

As well as basic measurements of absorbance, transmission, and concentration, the user can set and store parameters for up to 50 specific methods.

Much consideration has been given to the routine requirements of life science laboratories and it is in this area that the most comprehensive sets of functions are incorporated.

Nucleic acid measurements

UV absorbance is the most accurate method for nucleic acid quantification. Alternative methods, such as ethidium bromide staining, followed by transmission fluorescence/excitation, are unreliable due to sequence variations that can affect the ratio of bound dye to nucleic acid. Furthermore, UV methods are non-destructive, permitting re-use of samples, provided no other contamination is introduced.

There are five methods available, which are easily selected, to give detailed measurements for nucleic

acid samples. Nucleic acids can be characterized by concentration measurements, corrections for turbidity of the sample matrix, and 260/280 ratio for DNA, RNA, and oligonucleotides. Additionally a quick check on purity can be performed using a scan from 200–350 nm. The scan can quickly determine if the sample is contaminated with buffer salts, synthetic oligonucleotide protecting groups, or protein. For routine quality control, the rapid scan enables identification checks within a second. Because the sample is unaltered, the solution can be used for further analyses. Small sample analyses are conveniently handled using the ultra-microvolume single-cell holder for sample volumes down to 7 μ l.

The theoretical melting temperature (T_m) for an oligonucleotide is the temperature at which the oligonucleotide is half denatured. If the base sequence is known, T_m can be calculated theoretically using known thermodynamic parameters. Ultrospec 3000 *pro* has a base sequence input facility that calculates theoretical T_m for synthetic oligonucleotides and primers if the nucleotide concentration and total molarity of buffer salts are also entered. Molecular weight, conversion factor (μ g/ml), and concentration in pmol/ μ l are calculated as well. Ultrospec 3000 *pro* can also display useful information for selecting the appropriate cell to use, formulae for mass to moles conversion and the codon dictionary.

Protein measurements

Protein analyses are commonly required in parallel with nucleic acid investigations. Parameters for four common chemical methods for protein assays are included:

- Bradford—quantification based upon binding with Coomassie™ Blue and measurement at 595 nm
- Lowry—reaction of peptide bonds with alkaline copper tartrate solution and Folin reagent followed by measurement at 750 nm
- Biuret—reaction of peptide bonds with cupric ions and measurement at 546 nm
- BCA—reaction of peptide bonds with cupric ions, oxidation to cuprous ions and reaction with bicinchononic acid with measurement at 562 nm

Additionally protein can be analysed by three direct, non-destructive UV methods derived from empirical factors:

- 260, 280 nm method, which measures protein in the presence of nucleic acids

- 280, 205 nm, based on amino acid residue and aromatic side chain absorption
- 215, 225 nm, based on peptide bond absorption

Standard curve data is displayed for each assay, showing slope, intercept and correlation coefficient.

Micro assays, where low volumes of sample are used, can be accommodated by increasing the integration (measurement) time; this increases the precision of the measurement.

Useful information for factors relating absorbance to concentration for some common proteins and look-up tables for amino acids can be displayed as required.

Spectral scanning

Ultrospec 3000 *pro* has all the facilities necessary to cover characterization of biological molecules. The wavelength range of 190–1100 nm can be scanned at 0.1 nm intervals at scan speeds up to 6200 nm/min, with a resolution of < 1.8 nm bandwidth. The 1st, 2nd and 4th derivatives, which are the most important derivative spectroscopy functions for analysis, are easily selected and displayed on screen. This is useful to enhance spectral differences for comparison of anti-sense oligonucleotides. For routine analysis using the standard eight-cell changer, seven samples and a reference are scanned in 25 sec.

Analysis of sample mixtures using multi-wavelength equation entry

Some official analytical methods for drug preparations employ multi-wavelength methods (e.g. the analysis of thiazide antibacterial drugs). For these types of analyses, Ultrospec 3000 *pro* has been designed to take the user conveniently through the set-up procedure for up to two equations of nine wavelengths and factors. Once stored, the same analyses can be repeated by simply pressing the “Run” key.

Routine enzyme kinetics

Ultrospec 3000 *pro* provides analysis functions for reaction rate. Absorbance and correlation coefficients are calculated and displayed (Fig 2). There is a choice of serial and parallel modes. The latter enables several assays to be measured simultaneously. The following parameters are available:

- user defined time interval for up to 600 data points
- display best-fit line through data points
- statistical fit of line

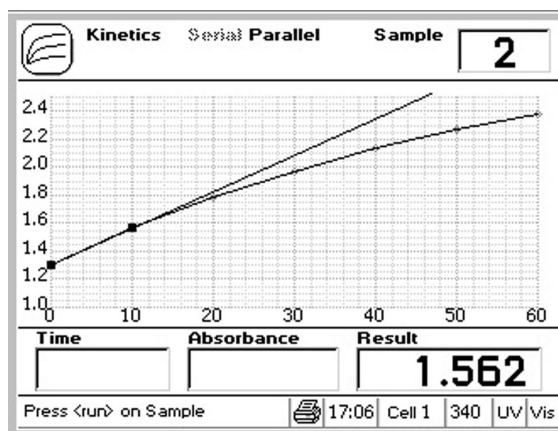


Fig 2. A typical Ultrospec 3000 *pro* screen display for reaction kinetics studies.

- automatic endpoint selection
- user- or instrument-defined reaction rate

Ultrospec 3000 *pro* can be used in conjunction with the water-jacketed, temperature-controlled single cell holder, six-position Peltier-heated multi-cell changer, and eight-position water-jacketed, temperature-controlled multi-cell changer. Calculations can be performed on whole data files, or selected segments. Results can be viewed and plotted.

Quantification of samples using standard curve assays

Ultrospec 3000 *pro* can accommodate standard curves set up with known concentrations at up to three replicates. Calibration can be by one of four different methods: regression, spline, interpolation, or factor. Each sample is analysed on pressing the “Run” key.

Quantification of samples using test kits

Many types of test kits used in food and clinical analyses are based on enzyme-substrate reactions in which rates of reaction are measured. Ultrospec 3000 *pro* calculates the reaction point over the defined linear part of the assay and results are directly converted to concentrations with output of the mean and standard errors of replicates.

Interface with other devices

Printer output is available to all standard types of printers including thermal, dot matrix, ink jet, and laser. All the appropriate drivers are incorporated as standard.

The system can be turned into a powerful automatic analyser via connection to an autosampler, which can also be run with the SWIFT II software Quantification module.

Operation with a PC—SWIFT II software

Operation of Ultrospec 3000 *pro* is further enhanced by interfacing the instrument with a PC running SWIFT II software modules, which add many detailed functions to the instrument's capabilities, including archiving facilities (Fig 3).

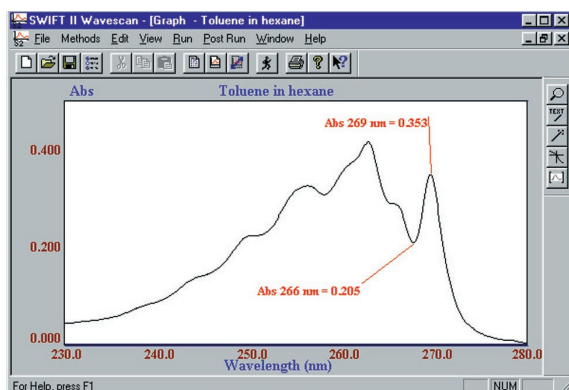


Fig 3. Spectrum of toluene in hexane (0.02% v/v solution) obtained using SWIFT II software. The ratio of the absorbance at the maximum (269 nm) and minimum (266 nm) should be at least 1.5 to fulfil the Pharmacopoeia; the results shown give a ratio of 1.722, demonstrating that Ultrospec 3000 *pro* passes the test.

In addition to the Instrument control mode, the software is based on six modules:

- Wavescan
- Reaction kinetics
- Time drive
- Quantification
- Multi wavelength
- Fraction analysis

Operation with a PC—download of data to spreadsheets

Ultrospec 3000 *pro* will download results directly to Microsoft Excel when the PC has Biochrom Data Capture software installed and the instrument is connected to a PC by a serial cable. The Data Capture program and serial cable are both supplied with Ultrospec 3000 *pro*.

Absorbance-wavelength data generated from a scan and absorbance-time data generated from a reaction kinetics experiment are stored as columns of numbers, which may be subsequently converted to a more conventional graph using the spreadsheet. Results can be formatted or manipulated as appropriate prior to inclusion in reports or archiving. All application modes may be processed this way. When the Data Capture Utility has been installed, the user activates the Communications Module by pressing “Go Run > Start”.

An Excel template is supplied with the Data Capture Utility that contains three worksheets: the data capture sheet, a short instruction sheet and the macro itself, which is locked to avoid accidental misuse.

The option to save data onto a new sheet is presented to re-run an experiment, followed by the Set Up dialogue box and Communications Module.

Conclusion

Ultrospec 3000 *pro* provides a wide range of functions that are essential for operation under GLP and compliance with the Pharmacopoeia requirements. The instrument's ease of use is enhanced by built-in software, which is designed to guide and inform the life scientist about the optimum solutions for their investigations. Convenient archiving of data is ensured by communication facilities suitable for any PC.

ORDERING INFORMATION

Ultrospec 3000 <i>pro</i> UV/Visible Spectrophotometer (includes Biochrom Data Capture software, serial cable, and qualification logbook)	1	80-2111-30
SWIFT II Method Applications Software	1	80-2108-31
Seiko DPU-414 printer	1	Inquire
Printer support plinth	1	80-2106-60
Six-position Peltier-heated cell changer (requires Temperature Control Unit)	1	80-2106-04
T _m Peltier-heated cell holder (requires Temperature Control Unit)	1	80-2106-14
Temperature Control Unit	1	80-2105-49