Report by the Analytical Methods Committee Evaluation of Analytical Instrumentation.

Part IX Instrumentation for High-performance Liquid Chromatography

Analytical Methods Committee
The Royal Society of Chemistry, Burlington House, Piccadilly, London UK W1V 0BN

A method is provided for comparing the features of high-performance liquid chromatography instrumentation.

The Analytical Methods Committee has received and approved the following report from the Instrumental Criteria Sub-Committee.

The following report was compiled by the above Sub-Committee of the AMC, which consisted of Professor S. Greenfield (Chairman), Professor J. N. Miller, Dr. P. J. Potts, Mr. D. C. M. Squirrell, Dr. C. Burgess, Dr. K. E. Jarvis, Professor S. J. Hill and Mr. R. Brown, with Mr. C. A. Watson as Honorary Secretary. The initial input of the features for consideration was undertaken by a working party chaired by Dr. C. Burgess with Mr. G. S. Coppack, Dr. D. G. Jones and Mr. T. Frost, to whom the committee expresses its thanks.

The purchase of analytical instrumentation is an important function of many laboratory managers, who may be called upon to choose between a wide range of competing systems which are not always easily comparable. The objectives of the Instrumental Criteria Sub-Committee are to tabulate a number of features of analytical instruments which should be considered when making a comparison between various systems. As is explained below, it is then possible to score these features in a rational manner, which allows a scientific comparison to be made between instruments.

The over-all object is to assist purchasers in obtaining the best instrument for their analytical requirements. It is also hoped that this evaluation will, to some extent, also help manufacturers to supply the instrument best suited to their customers' needs. It is perhaps pertinent to note that a number of teachers have found the reports to be of use as teaching aids.

No attempt has been made to lay down a specification. In fact, the Committee considers that it would be invidious to do so: rather it has tried to encourage the purchasers to make up their own minds as to the importance of the features that are on offer by the manufacturers.

The ninth report of the Sub-Committee deals with high-performance liquid chromatography.

Notes on the Use of this Document

Column 1. The features of interest.
Column 2. What the feature is and how it can be evaluated.
Column 3. The Sub-Committee has indicated the relative importance of each feature and expects users to decide on a weighting factor according to their own application.
Column 4. Here the Sub-Committee has given reasons for its opinion as to the importance of each feature.
Column 5. Onwards. It is suggested that scores are given for each feature of each instrument and that these scores are modified by a weighting factor and sub-totals obtained. The addition of the sub-totals will give the final score for each instrument.

Notes on scoring

1. (PS) Proportional scoring. It will be assumed, unless otherwise stated that the scoring features will be by proportion, *e.g.*, Worst 0 to Best 100.
2. (WF) Weighting factor. This will depend on individual requirements. An indication of the Sub-Committee's opinion of the relative importance of each feature is indicated as follows: VI (very important), I (important); NVI (not very important). A scale is chosen for the weighting factor which allows the user to discriminate according to needs, *e.g.*, $\times 1$ to $\times 3$, or $\times 1$ to $\times 10$. The factor could amount to total exclusion of an instrument.
3. (ST) Sub-total. This is obtained by multiplying PS by WF.

High-performance liquid chromatography (HPLC) is a well established analytical technique with applications in many areas. An often bewildering range of instrumentation is available from a large number of different manufacturers. Systems range from simple instruments, with a single column and detector, to complex multi-channel systems with auto-samplers and microcomputer-based controllers for continuous operation and sophisticated mass spectrometric or diode-array spectroscopic detectors. Selection of a suitable instrument for purchase is, therefore, not an easy task and the purpose of these notes is to provide some guidance to areas which should be considered, so that the choice is based on a full consideration of the available options. However, the performance of any HPLC method depends primarily on the separation conditions and thus on the nature of the column packing and mobile phase employed. The nature of the analyte and the requirements of sensitivity and selectivity will influence the choice of detector.

The first task in the selection of an instrument is to examine the range of analyses that it will be expected to perform. Care should be taken not to specify these requirements too closely as uses change with time. The analytical scientist should also not try to envisage every potential application or the selection criteria may become too detailed. The choice of the column type and mobile phase are outside the scope of these guidance notes but any specific requirements should be noted, such as special detectors, injectors or accessories.

With these requirements in mind, the user should then evaluate the instruments available on the market while bearing in mind the guidelines and any financial limitations. In many instances it will quickly become clear that a number of different instruments could be satisfactory and non-instrumental criteria may then be important. However, in some specialized cases only one or two instruments will have the ability or necessary features to carry out the assay.

The guidelines are intended to be used as a check list of features to be considered, mostly of the instrument itself, but some also of its service requirements and of the relationship of the user with the manufacturer. Their relative importance will depend on the installation requirements of the instrument as well as the uses to which it will be put. Therefore, to some extent, the selection process will inevitably be subjective, but if all the points have been considered, it should be an informed choice.

In addition, because a separation depends so much on the column, mobile phase and operating conditions, it may sometimes be difficult to assess the actual operating performance of a particular feature from the manufacturer's specifications. For some applications it may be necessary to evaluate the performance of the instrument under consideration using the system suitability test mixture chosen for a particular application. The purpose of this is to demonstrate the system's ability to perform a critical separation. HPLC instruments are often sold as complete systems, so that compromises between features may have to be accepted, but it will still be important to distinguish between critical features and those which are optional.

The Committee consider that, in general, HPLC equipment is safe in normal use, but care should be taken to allow sufficient cooling time when changing columns and to take suitable precautions when handling flammable solvents. In addition, eye protection should be worn when aligning or changing UV lamps. It is recommended that a suitable leak detector should be fitted in the column oven.

Finally, as many laboratories are now working to quality standards such as GMP/GLP/NAMAS/ISO Guide 25, some consideration should be given to third party recognition of the manufacturer to standards such as ISO 9001. Such accreditation should extend to the service organisation, which is particularly important when working to NAMAS or GLP criteria.

Previous Reports in this Series from the Analytical Methods Committee

Evaluation of Analytical Instrumentation

Part 1. Atomic-absorption Spectrophotometers, Primarily for Use with Flames, *Anal. Proc.*, 1984, **21**, 45.

Part 2. Atomic-absorption Spectrophotometers, Primarily for use with Electrothermal Atomizers, *Anal. Proc.*, 1985, **22**, 128.

Part 3. Polychromators for Use in Emission Spectrometry with ICP Sources, *Anal. Proc.*, 1986, **23**, 109

Part 4. Monochromators for Use in Emission Spectrometry with ICP Sources, *Anal. Proc.*, 1987, **24**, 3.

Part 5. Inductively Coupled Plasma Sources for Use in Emission Spectrometry, *Anal. Proc.*, 1987, **24**, 266.

Part 6. Wavelength Dispersive X-ray Spectrometers, *Anal. Proc.*, 1990, **27**, 324.

Part 7. Energy Dispersive X-ray Spectrometers, *Anal. Proc.*, 1991, **28**, 312

Part 8. Instrumentation for Gas–Liquid Chromatography, *Anal. Proc.*, 1993, **30**, 296.

Instrument Evaluation Form

Type of Instrument: High Performance Liquid Chromatograph								
Manufacturer:								
Model No.:								
Feature	Definition and/or test procedures and guidance for assessment	Importance	Reason	Score				
Non-instrumental criteria *Selection of manufacturer*	Laboratories in possession of other HPLC systems should score highest for the manufacturer with the best past record based on the following sub-features:							
(*a*) Previous instruments								
(*i*) Innovation	Company's record for developing instruments with innovative features.	I	The manufacturer should be alert to developments in technology and chromatography.	PS WF ST				
(*ii*) Reliability record	Company's record for instrument reliability.	I	Indicates history of sound design/ manufacturing concepts.	PS WF ST				
(*iii*) Similarity of operation, layout and design to existing instruments in the laboratory	For routine purposes this may be important. However, this may be less important for research applications.	I	Similarity of layout means that operators can draw on in-house expertise, resulting in reduced training costs and time. It may also maximise the use of spares and fittings.	PS WF ST				
(*iv*) Confidence in the supplier	Confidence gained from past personal experience.	I/NVI	Good working relationship already in place.	PS WF ST				
(*b*) Servicing	Score according to manufacturer's claims and past record, judged by the sub-features (*i*) to (*v*) below:							
(*i*) Service contract	Availability of suitable service contracts from the supplier, agent or third party contractor.	VI	Suggests long-term commitment to user. Often ensures preferential service and guarantees a specific response time to call-outs.	PS WF ST				
(*ii*) Availability and delivery of spares	Range of stock carried by, or quickly available to, the manufacturer/agent/ contractor.		Rapid delivery of spares reduces downtime.	PS WF ST				
(*iii*) Call-out time	Availability of adequate service such as the time for the engineer to reach the laboratory following a call.	I(VI)	Keeps laboratory in operation by reducing down time [see also (*i*)].	PS WF ST				

Feature	Definition and/or test procedures and guidance for assessment	Importance	Reason	Score				
(*iv*) Effectiveness of service engineers	The ability of the service engineers, as judged from previous experience and reports of others, including the carrying of adequate spares.	I	Ability to repair on-site avoids return visit or removal of equipment for off-site repair, so reduces down time and may reduce service cost.	PS WF ST				
(*v*) Cost of call-out and spares	It *may* be inappropriate to score this feature if in-house servicing is contemplated.	I	The proximity of the service centre may be a factor in travel costs.	PS WF ST				
(*c*) Technical support	As in (*b*) score in consideration of sub-features (*i*)–(*iii*) below.	VI for new user						
(*i*) Advice from applications department	The advice and training available from the manufacturer's applications department.		This helps in-house staff with new applications problems.	PS WF ST				
(*ii*) Technical literature	The range and quality of technical literature, including the operating manual.		Guidance on optimum use of instrument suggests manufacturer's awareness of applications.	PS WF ST				
(*iii*) Telephone assistance	Willingness of the manufacturer/supplier/contractor to give effective advice over the telephone. This can normally only be evaluated by reference to existing users.		Rapidly available technical help reduces the number of call outs and enhances productivity.	PS WF ST				
Instrumental Criteria								
1. *General features*								
(*a*) Facilities required for:								
(*i*) Access and location of connections and controls on instrument	Score according to convenient access taking into account the proposed location of the instrument.	I	Depending on bench position and layout, connections and controls may limit accessibility for servicing and installation, particularly at the rear of the instrument.	PS WF ST				
(*ii*) Power requirements	Many systems require multiple power inputs. Score maximum for instruments with the minimum of separate power leads.	NVI	Excessive numbers of power cables when combined with other services create hazards and make servicing more difficult.	PS WF ST				
(*iii*) Power failure effects	Score highest for systems that allow recovery from power failure with minimal data/control loss.	I(VI)	Down time is increased if power failure necessitates manual re-priming of pumps or resetting of instrument control parameters. This is critical for unattended operation with long runs.	PS WF ST				
(*iv*) Size of equipment	Score according to convenience of installation, taking into account the proposed location of the instrument.	I(VI)	Dimensions may be critical if space is limited.	PS WF ST				
2. *Solvent reservoirs*								
(*a*) Capacity	Score highest for reservoirs having the largest practical capacity.	I	Large capacity reservoirs reduce down time and make long runs possible.	PS WF ST				
(*b*) De-gassing/filtration	Score highest for systems which allow *in-situ* de-gassing/filtration.	VI	Solvents need to be free from dissolved gases and particulates to prevent bubble formation in the detector and to prevent back-pressure problems caused by column blockage.	PS WF ST				
(*c*) Ease of cleaning and handling	Score highest for reservoirs which are easily removed and cleaned.	I	Rapid solvent changeover reduces down time.	PS WF ST				
(*d*) Spillage containment	Score highest for systems which have integral trays to contain spillage of solvent.	I	Minimises safety hazards.	PS WF ST				
3. *Pumping systems*								
(*a*) Instrument control	Score highest for instruments which allow adequate software control of all key operational functions.	VI	Software control facilitates method compliance.	PS WF ST				
(*b*) Flow rate	Score highest for best accuracy and reproducibility commensurate with the application. Flow rates should be independently checked at constant temperature by the use of standard flow meters or gravimetric procedures.	VI	Poor control leads to poor method reproducibility.	PS WF ST				

Feature	Definition and/or test procedures and guidance for assessment	Importance	Reason	Score				
(*c*) Flow range	Score highest for wide range of flow rates if microbore, analytical and preparative columns are to be used.	I	Flow rates required vary from below 1 ml min^{-1} to over 30 ml $min.^{-1}$	PS WF ST				
(*d*) Pulse monitoring	Score highest for systems with built-in accurate pulse monitoring and for lowest pulsation.	I	Pulsation in the flow can give rise to noise problems particularly with electrochemical detectors and hence should be checked.	PS WF ST				
(*e*) Gradient formation; accuracy and reproducibility	Score highest for best accuracy and reproducibility of the eluent mixture commensurate with the application. It may be inappropriate to score this feature as not all applications require gradient elution.	VI	Poor control leads to poor method reproducibility.	PS WF ST				
(*f*) Recycling of mobile phases	Score highest for systems possessing this feature. It may be inappropriate to score this feature.	I	Recycling allows the re-use of mobile phase if costs or runtime are critical.	PS WF ST				
(*g*) Materials of construction	Score highest for durability, as judged from the quality of construction.	I	Poor quality or inappropriate materials can lead to contamination of mobile phases and corrosion of casings and connectors.	PS WF ST				
(*h*) Ease of maintenance	Score highest for systems which have clear 'built in' diagnostics of pump functionality and easy removal of check valves, pistons and heads.	VI	Well maintained pumps are essential to ensure that flow rates are accurate and reproducible.	PS WF ST				
(*i*) Eluent switching	Score highest for systems which allow eluents to be exchanged during analysis.	I	Allows several sets of samples to be analysed in conjunction with column switching [see 5(*c*)].	PS WF ST				
4. *Sample introduction*								
(*a*) Sample loop injection (manual)	For simple instruments, manual injection is usually adequate. Score highest for systems which have the ability to accept fixed or variable loops which: are easy to change; have minimal carry over; have sample loop/ valve preheat; are inert to the solvent system; have the highest reproducibility; have appropriate volumes.	VI	Consistent sample introduction onto the column is a critcal factor in obtaining reproducible peak shapes, areas and retention times. Consistency may mean determining reproducibility of partial as well as complete filling of the sample loop.	PS WF ST				
(*b*) Sample loop injection (automatic)	In addition, for more complex systems, score highest for systems which have these additional features: have thermostating of the sample tray; have full software control of injection numbers and sequence; are able to perform liquid transfers or dilutions.	VI	As above.	PS WF ST				
5. *Columns and fittings*	(Column materials and stationary phases are outside the scope of this evaluation.)							
(*a*) Pre-columns	If applications require one, score highest for systems which: are easy to fit; have low dead volume; are robust; are low cost.	I	Pre-columns can prolong the life of the main column but must not significantly reduce the overall efficiency of the system.	PS WF ST				
(*b*) Cartridge columns	Score highest for systems which allow a full range of column configurations and connections.	I	Cartridge columns can make column changing easier and quicker.	PS WF ST				
(*c*) Column switching	Score highest for systems which allow full control of valves and pneumatic systems.	I	Allows several sets of samples and/or columns with different eluents to be run consecutively. This may be important for method development.	PS WF ST				
(*d*) Connectivity (compatibility of components)	Score highest for fittings with standard thread sizes and uniform external dimensions.	I	Allows ease of interchangeability of components between systems and reduces spares requirements.	PS WF ST				
6. *Column ovens*								
(*a*) Oven design; size, shape and special features	Oven design must allow easy accommodation of user selected columns and have an adequate thermal capacity. These are special requirements for multi-column, post-column reactor or preparative work. Score accordingly.	I	Usually only one column is employed but for some applications pre-columns or guard columns are required. Sufficient space is required for ease of installation or replacement.	PS WF ST				

Feature	Definition and/or test procedures and guidance for assessment	Importance	Reason	Score				
(*b*) Temperature control	Score highest for ovens with the ability to maintain precise temperature control over the range (ambient + 5) °C to 80 °C.	VI	Accuracy and precision of temperature control are important, particularly in separations using buffers or ion pairing, *etc*., if reproducible chromatography is to be obtained.	PS WF ST				
(*c*) Temperature uniformity	Score highest for least temperature gradient effects and additional for adequate control of eluent temperature at the column inlet and exit so that it is unaffected by the flow rates and solvent compositions used.	I	The oven's thermal capacity and control of temperature distribution must be adequate to prevent temperature effects distorting the chromatography.	PS WF ST				
(*d*) Solvent inert construction	Oven should be able to handle leaks of organic solvents without corrosion or other damage.	I	Solvent resistant materials are necessary for long-term durability.	PS WF ST				
(*e*) Resistance to solvents	Score highest for systems which have built-in leak detection.	I	Minimises risk of fire and operator exposure to solvent vapours.	PS WF ST				
7. *Detectors* (*general*)								
(*a*) Detector types	In most instances detector selection is dictated by the analyte and/or matrix and the selectivity and sensitivity required.							
(*b*) Availability	If appropriate to the applications, score maximum for the widest range of detectors which can be fitted to the standard instrument.	I	Enables a wide range of applications to be carried out and increases versatility.	PS WF ST				
(*c*) General features								
(*i*) Linear dynamic range	Score maximum for widest linear dynamic range in the spectral region of interest or relevant operational settings.	VI	Enables accurate quantification to be made over largest possible concentration range.	PS WF ST				
(*ii*) High signal to noise ratio	Score highest for highest signal to noise ratio.	VI	A high signal to noise ratio facilitates lower detection limits.	PS WF ST				
(*iii*) Sensitivity to back pressure	Whilst this is largely detector dependant, score highest for least effect.	I	Sensitivity to back pressure causes problems, *e.g.*, noise and leaks.	PS WF ST				
(*iv*) Solvent/ solute limitations	Whilst this is largely detector dependant, score highest for the widest range of solvents and solutes which can be accommodated.	I	The larger the range of solvents and solutes which can be accommodated, the greater is the utility of the detector.	PS WF ST				
(*v*) Ability to connect detectors in series with minimal dead volume increases	Where detectors are to be connected in series, score highest for designs which minimise dead volume.	VI	Dead volume will cause band broadening and loss of chromatographic efficiency.	PS WF ST				
(*vi*) Flow cells	Score highest for systems which have flow cells that: are easy to remove and clean; have low dead volume; have ease of introduction of liquid standards; are adequately thermostatted.		Flow cells may become blocked and require cleaning. Adequate thermostatting is essential to prevent signal drift. Low dead volume minimises band broadening.	PS WF ST				
8. *UV detectors*	UV detection is the most widely employed as many solutes of interest contain chromophores. The complexity of the detector required depends upon the application.							
(*a*) Filter detectors	For the simplest type, score highest for detectors which have: a wide range of filters; narrowest bandpass; ease of lamp changing.	VI	Such detectors have wide applicability and high sensitivity, as well as ease of maintenance, but are limited by the wavelengths selectable.	PS WF ST				
(*b*) Continuously variable wavelength	Score highest for detectors which have: wide wavelength range; narrowest bandpass; best accuracy and precision of wavelength selection.	VI	Such detectors have wide applicability and high sensitivity where wavelength selection is critical.	PS WF ST				

Feature	Definition and/or test procedures and guidance for assessment	Importance	Reason	Score				
(*c*) Diode array	Score highest for detectors which have: a wide wavelength range; the best resolution; the highest sensitivity; a flexible readout capability.	VI	Such detectors have wide applicability and high sensitivity where complete spectral information is required.	PS WF ST				
9. *Fluorescence detectors*	Some chromophores will emit light as fluorescence when irradiated with UV–visible radiation. For some applications a simple filter based detector will be adequate.							
(*a*) Fixed wavelength	Score highest for detectors which have: a wide range of filters for excitation and emission; the narrowest bandpass; ease of lamp changing.	VI	Such detectors have high sensitivity and selectivity as well as ease of maintenance.	PS WF ST				
(*b*) Variable wavelength	Score highest for detectors which have: a wide wavelength range; the narrowest bandpass; best accuracy and precision of wavelength selection.	VI	Such detectors have very high sensitivity and selectivity when optimised excitation and emission wavelengths are necessary.	PS WF ST				
10. *Other detectors*	If appropriate, score highest for systems which have available specialist detectors including: refractive index; reaction detectors; mass detectors; electrochemical.	I(VI)	A specific application may require a particular detection method since the solute may not contain a chromophore or additional sensitivity or specificity is needed.	PS WF ST				
11. *Data handling*	The selection of a data handling system is outside the scope of this study as many software packages are available for data handling which are interchangeable between personal computers. Providing the instrument can output the data to a suitable computer, it should not affect the choice of the instrument, so scoring is inappropriate. However, the following features should be taken into consideration.							
(*a*) Integrated systems	Facility to carry out instrument control, data capture, collation and integration and extensive reporting capabilities.	VI	The ability to carry out these tasks in a controlled manner is essential for data and information integrity.	PS WF ST				
(*b*) Interfacing requirements for non-integrated systems								
(*i*) Connectivity and control links	Score highest for systems employing adequately documented industry standard interfaces and protocols.	VI	Provision of such hardware and software is essential to secure data communication to other systems.	PS WF ST				
				Sum of Sub-totals				
12. *Value for money* (Points per £)	Sum of the previous sub-totals divided by the purchase price of the instrument. Subject to proportional scoring and weighting factors, including ST in grand total.	I	'Simple' instruments are often good value for money, whereas those with unnecessary refinements are often more costly.	PS WF ST				
				Grand Total				

Paper 7/01013C