



Australian Government

Department of Education, Employment and Workplace Relations

MSL977007A Apply specialised knowledge of liquid chromatography techniques to analysis

Release: 1

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Modification History

Not applicable.

Unit Descriptor

Unit descriptor	This unit of competency covers the ability to analyse samples using advanced liquid chromatography (LC/HPLC) instruments including (LC-MS). The unit includes establishing client needs for routine and non-routine samples, optimising enterprise procedures and instruments for specific samples, obtaining valid and reliable data and reporting test results. Personnel are required to recognise atypical test data/results and troubleshoot common analytical instrument and procedure problems and perform routine instrument maintenance.
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Application of the Unit

Application of the unit	This unit of competency is applicable to experienced laboratory technical officers/technicians, laboratory supervisors and technical specialists who conduct instrumental analysis in laboratories providing consultancy, research and development and quality assurance services. These services may be provided for a wide range of industry sectors, such as forensic science (e.g. toxicology, dyes and inks), environmental monitoring (e.g. herbicides and pesticides), chemical, biomedical (e.g. structural analysis of complex molecules and protein identification), food (e.g. pesticide residues and nutrition supplements) and pharmaceuticals testing (e.g. pharmacokinetics). Industry representatives have provided case studies to illustrate the practical application of this unit of competency and to show its relevance in a workplace setting, at the end of this unit of competency under the section 'This competency in practice'.
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Licensing/Regulatory Information

Not applicable.

Pre-Requisites

Prerequisite units		

Employability Skills Information

Employability skills	This unit contains employability skills.
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Elements and Performance Criteria Pre-Content

Elements describe the essential outcomes of a unit of competency.	Performance criteria describe the performance needed to demonstrate achievement of the element. Where bold italicised text is used, further information is detailed in the required skills and knowledge section and the range statement. Assessment of performance is to be consistent with the evidence guide.
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Elements and Performance Criteria

ELEMENT	PERFORMANCE CRITERIA
1. Determine sample characteristics and appropriate analytical methods	1.1. Interpret client request and/or perform presumptive tests to identify sample characteristics that may affect analysis 1.2. Liaise with client or sample provider to review client needs, testing requirements and sample history, if necessary 1.3. Identify analytical standards, reference materials, test methods and enterprise procedures that may be applicable 1.4. Select the most appropriate standard test method that is consistent with testing requirements and instrument availability 1.5. If no standard method exists, adapt or modify a test method to suit the sample characteristics 1.6. If necessary, seek advice from supervisor about any proposed variations and document all approved changes to test methods 1.7. Schedule analysis using enterprise procedures
2. Prepare samples, standards and mobile phase	2.1. Log sample into instrument software 2.2. Obtain a representative analytical portion of the laboratory sample 2.3. Prepare sample in accordance with selected test method 2.4. Prepare mobile phase in accordance with selected test method using buffers and other additives to enhance separation and ionisation, concentrate analytes and prevent build up of salts, as necessary 2.5. Filter and degas mobile phase 2.6. Prepare validation checks and/or calibration standards for analytical portions 2.7. Use specialised procedures for ultra trace sample and standard preparation as required
3. Set up instrument and perform trial analysis	3.1. Configure the injector, column, solvent delivery and detector sub-systems according to the selected test method 3.2. Prime the pump, flush the system and monitor pressures and baseline to ensure the system is fully equilibrated 3.3. Perform other pre-use, calibration and safety checks using enterprise procedures 3.4. Set instrumental parameters in accordance with those specified in selected test method 3.5. Check and optimise each instrument sub-system

ELEMENT	PERFORMANCE CRITERIA
	3.6. Conduct performance tests using standards and samples 3.7. Assess instrument performance in terms of response, resolution and run-time

ELEMENT	PERFORMANCE CRITERIA
4. Optimise instrument performance	4.1. Apply an understanding of analyte and column chemistry, temperature control and varying mobile phase composition to determine strategies for enhancing separation and detection of required species 4.2. Adjust instrumental parameters in a logical and efficient sequence to optimise performance 4.3. When optimum separation is achieved, check that the detector and system software can correctly identify and quantify the required species
5. Perform analysis	5.1. Measure analyte response for standards, validation checks and samples using optimised instrument settings 5.2. Conduct sufficient measurements to obtain reliable data 5.3. Use system software to produce calibration graphs, chromatographs and/or mass spectra, confirm data quality and calculate uncertainties 5.4. Check that results are consistent with estimations and expectations 5.5. Analyse trends in data and/or results and report out of specification or atypical results promptly to appropriate personnel 5.6. Return instrument to standby or shutdown condition in accordance with enterprise procedures 5.7. Report results with the appropriate accuracy, precision, uncertainty and units
6. Perform routine maintenance and troubleshoot instruments	6.1. Flush and store the column with the recommended solvent to protect the stationary phase during downtime 6.2. Regularly check that the pump, lines, valves, injector, column and detector sub-systems are clean/undamaged and replace consumable items as necessary 6.3. Change columns and detectors in accordance with manufacturer's instructions and ensure that the system is free of leaks and properly conditioned before re-use 6.4. Investigate possible causes for the absence of peaks and presence of ghost peaks, split/distorted peaks, band broadening and apply recommended remedial actions 6.5. Investigate possible causes for baseline instability and non-reproducible retention times and apply recommended remedial actions

ELEMENT	PERFORMANCE CRITERIA
	6.6. Identify the need for repairs or servicing and determine whether local repair/maintenance is technically possible and economic 6.7. Arrange for repair or servicing from an accredited agent or other appropriate personnel in accordance with enterprise procedures
7. Maintain a safe work environment	7.1. Identify risks, hazards, safety equipment and control measures associated with sample handling/preparation and test method 7.2. Use personal protective equipment and safety procedures specified for test method and materials to be tested 7.3. Minimise the generation of wastes and environmental impacts 7.4. Ensure the safe collection/disposal of mobile phase and other laboratory wastes 7.5. Clean, care for and store equipment and consumables in accordance with enterprise procedures
8. Maintain laboratory records	8.1. Enter approved data and results into laboratory information management system (LIMS) 8.2. Maintain logs of instrument calibration checks, use and maintenance in accordance with enterprise procedures 8.3. Maintain security, integrity and traceability of samples, results and documentation 8.4. Communicate results to appropriate personnel in accordance with enterprise procedures

Required Skills and Knowledge

REQUIRED SKILLS AND KNOWLEDGE

This section describes the skills and knowledge required for this unit.

Required skills

Required skills include:

- establishing client needs for routine and non-routine samples
- interpreting client requests, test methods and procedures accurately
- selecting, adapting and modifying standard test methods for unknown samples (including consideration of suitable polar/non-polar stationary and mobile phases, solvent composition, compatibility of sample solvent with mobile phase, buffer, sample loop volume, temperature, flow rate, column type, column length and detection)
- preparing samples and standards, optimising procedures and equipment to suit sample/test requirements
- setting up, starting up and shutting down equipment
- checking the calibration/qualification status of equipment
- selecting, configuring, checking and optimising instrument sub-systems
- performing routine instrument maintenance and replacement of consumables
- obtaining valid and reliable data
- calculating analyte concentrations with appropriate accuracy, precision, uncertainty and units
- recognising atypical data/results and troubleshooting common analytical procedure and equipment problems
- recording and reporting data/results using enterprise procedures
- maintaining security, integrity and traceability of samples and documentation
- assessing risks, applying specified control measures and working safely
- minimising waste, ensuring safe collection and disposal
- applying relevant principles of good laboratory (GLP) procedures
- maintaining technical knowledge by accessing journals, technical updates, suppliers' product notes and test methods

Required knowledge

Required knowledge includes:

- sample preparation procedures including specialised techniques such as:
 - handling unstable/hazardous chemicals and samples, fragile/labile biological material
 - liquid-liquid extraction, solid-phase micro extraction, derivatisation, filtering and dilution/concentration
- principles for separation of analytes such as:
 - chemical composition of stationary and mobile phases and their types of

REQUIRED SKILLS AND KNOWLEDGE

- interaction
 - selection of solvents based on polarity, viscosity, ultraviolet (UV) cut-off
 - requirements for solvent purity and pre-treatment including filtration, degassing, buffering and modifying solvents
 - isocratic and gradient elution flow programming (linear, concave and convex gradients and step)
 - recovery and recycling of solvents
- separation by polarity:
 - normal phase systems and hydrophilic (interaction chromatography)
 - reverse phase systems and hydrophobic (interaction chromatography)
 - order of elution in normal and reverse phase systems
- separation by charge:
 - ion exchange chromatography(IEC) and ion chromatography (IC)
 - ion suppression and ion pairing techniques
 - non-suppressed systems
- separation based on molecular size:
 - size exclusion chromatography (SEC)
 - gel-permeation chromatography (GPC)
 - relationship between retention time and molecular mass
- bioaffinity chromatography principles
- chromatography concepts and calculations involving:
 - retention times, peak widths, peak asymmetry, capacity factor k' and resolution
 - column selectivity, column efficiency (plates/m), optimum flow rate, minimum theoretical plate height, Van Deemter and related equations
 - limit of detection, limit of quantitation and their application to quality control procedures
- operation, construction, selectivity, typical applications, troubleshooting and routine maintenance of LC columns including:
 - (semi) preparation columns, packed columns and capillary columns
 - column oven (role of temperature in achieving close separations)
 - checking for leaks, changing of columns, lines and valves
 - system flushing and conditioning and storage of columns
- operation, construction, selectivity, typical applications, troubleshooting and routine maintenance of LC sample introduction systems including:
 - manual and auto-injection, injector valves, solvent reservoirs, selector valves, gradient programmer, mixing manifolds and column switching
 - pump designs such as reciprocating piston or diaphragm, pressures, flow rates
- operation, construction, selectivity, sensitivity, linear range, typical applications, troubleshooting and routine maintenance of LC detectors including:

REQUIRED SKILLS AND KNOWLEDGE

- UV fixed wavelength and dispersion/diode array multi-wavelength detectors
- conductivity detector (ECD)
- electrochemical detector (ECD)
- fluorescence detectors
- refractive index (RI)
- evaporative light scattering (ELSD)
- mass spectrometry (LC-MS) (LC-MS-MS) using full scan or selective ion monitoring (SIM)
- flow splitting, tandem detectors (e.g. UV and MS)
- routine quality control procedures such as use of manual/computer calibration charts and/or standards
- computer control software for operating and optimising instrument (peak detection and integration, drift parameters, baseline correction and instrument/integrator zero)
- procedures for optimising instrument performance such as:
 - optimising separation by changing solvent composition including use of mobile phase gradient computer control programs
 - investigation of elution order in normal and reverse phase systems
 - effects on instrumental outputs and analytical results by fine tuning injection, mobile phase flow rate, column pressures and changing column type or detector
 - aligning MS interface cones
- steps in identifying and quantifying analytes including relative retention data, peak area normalisation and response factors
- calculation steps to give results in appropriate units and precision
- troubleshooting and maintenance procedures recommended by instrument manufacturer
- enterprise and/or legal traceability requirements
- relevant health, safety and environment requirements

Evidence Guide

EVIDENCE GUIDE

The Evidence Guide provides advice on assessment and must be read in conjunction with the performance criteria, required skills and knowledge, range statement and the Assessment Guidelines for the Training Package.

Overview of assessment

Critical aspects for assessment and evidence required to demonstrate competency in this unit

Assessors should ensure that candidates can:

- interpret client requests, test methods and procedures accurately
- select, operate and maintain a variety of LC sample valves, solvent delivery components, pumps, columns and detectors
- install injectors, solvent delivery systems and columns
- safely set up, start up and shut down instrument using enterprise procedures
- prepare samples and calibration standards in accordance with test method
- check calibration/qualification status of equipment
- optimise instrument sub-systems and procedures and equipment to suit sample/test requirements
- operate equipment to obtain valid and reliable data
- use software to identify analytes and calculate concentrations with appropriate accuracy, precision and units
- recognise atypical data/results
- troubleshoot common analytical procedure and equipment problems
- record and report data/results using enterprise procedures
- maintain security, integrity and traceability of samples and documentation
- follow OHS procedures and principles of GLP.

Context of and specific resources for assessment

This unit of competency is to be assessed in the workplace or simulated workplace environment. This unit of competency may be assessed with:

- *MSL976003A Evaluate and select appropriate test methods and procedures*
- *MSL977003A Contribute to the validation of test methods*
- *MSL977004A Develop or adapt analyses and*

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	<i>procedures.</i> Resources may include: - laboratory with specialised analytical instruments - laboratory reagents and equipment - SOPs and test methods.
Method of assessment	The following assessment methods are suggested: - review of test data/results/calibration graphs obtained by the candidate over time to ensure accuracy, validity, precision and timeliness of results - inspection of results and technical records (e.g. maintenance schedules and quality control logbooks) completed by the candidate - observation of candidate using LC instruments to measure analytes - feedback from clients, peers and supervisors - oral or written questioning of relevant LC concepts, chemical principles underpinning sample preparation and separation of species, instrument design and optimisation, analytical techniques and enterprise procedures. In all cases, practical assessment should be supported by questions to assess underpinning knowledge and those aspects of competency which are difficult to assess directly. Where applicable, reasonable adjustment must be made to work environments and training situations to accommodate ethnicity, age, gender, demographics and disability. Access must be provided to appropriate learning and/or assessment support when required. The language, literacy and numeracy demands of assessment should not be greater than those required to undertake the unit of competency in a work like environment.
This competency in practice	Industry representatives have provided the case studies below to illustrate the practical application of this unit of competency and to show its relevance in a workplace setting. Food and beverage processing A technician is measuring phosphate levels in samples of a popular cola drink by ion chromatography. Using a standard method, he/she notes that there is a long

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retention time and obtains a broad asymmetric peak because the recommended flow rate could not be obtained with the laboratory's instrument. He/she then decides that the simplest way to speed up the elution time is to modify the mobile phase by increasing the % of methanol. The technician was pleased to obtain a well resolved analyte peak despite the faster eluting peaks now being unresolved. However, this was of no consequence to the analysis as the client only required the phosphate concentration.

Education and training

An experienced technician sets up and runs some pre-use checks for an LC instrument that is used by several laboratory personnel with periods of downtime in between. To his/her dismay, the initial standard produces one broad peak instead of three pronounced UV absorbance peaks as expected. He/she checks that the column has not been changed and that the mobile phase composition and flow rates are correct. He/she then checks each sub-system carefully and then waits for the system to equilibrate again. After re-running the standard, the result is the same and so he/she then suspects something is wrong with the stationary phase. The technician notices that when the instrument was last used, the mobile phase had a high percentage of water. He/she now suspects that there is bacterial growth in the column because it has been stored incorrectly. After trying to regenerate the column unsuccessfully, the technician installs a new one and then flushes and conditions the instrument in accordance with the laboratory procedures. He/she then adds the following information to the instrument logbook:

- Store columns in 50/50 methanol/water without any acid.
- If you are using a salt, unlikely in LC-MS, wash the entire system, solvent bottles, LC, solvent lines, and column, in a non-salt containing solvent. (Otherwise salt may precipitate out and plug your LC or column or may cause corrosion.)
- Flush with pure water first and then leave the system in 50/50 methanol/water mixture. (Some salts may precipitate out in high organics so an initial water wash is advised. The 50/50 methanol/water solution helps to stop bacterial growth which can muck up the

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	system!

Range Statement

RANGE STATEMENT

The range statement relates to the unit of competency as a whole. It allows for different work environments and situations that may affect performance. Bold italicised wording, if used in the performance criteria, is detailed below. Essential operating conditions that may be present with training and assessment (depending on the work situation, needs of the candidate, accessibility of the item, and local industry and regional contexts) may also be included.

Codes of practice

Where reference is made to industry codes of practice, and/or Australian/international standards, it is expected the latest version will be used

Standards, codes, procedures and/or enterprise requirements

Standards, codes, procedures and/or enterprise requirements may include:

- Australian and international standards, such as:
 - AS ISO 17025-2005 General requirements for the competence of testing and calibration laboratories
 - AS/NZS 2243 Set:2006 Safety in laboratories set
 - AS/NZS ISO 9000 Set:2008 Quality management systems set
 - AS 2830.1 Good laboratory practice - Chemical analysis
 - AS 3741 Recommended practice for chemical analysis by ion chromatography
 - BS EN 12916 Petroleum products - Determination of aromatic hydrocarbon types in middle distillates - HPLC method with refractive index detection.
 - DIN EN 12630 Fruit and vegetable juices - Determination of glucose, fructose, sorbitol and sucrose - Method by HPLC.
 - ISO 10304 set Water quality - Determination of dissolved anions by LC of ions
 - ISO 20481 Coffee and coffee products - Determination of caffeine content using HPLC
 - ISO/IEC Guide 98-3:2008 Uncertainty of measurement - Part 3 Guide to the expression of uncertainty in measurement

RANGE STATEMENT

	(GUM) • Eurachem/CITAC Guide CG4 Quantifying uncertainty in analytical measurement • NATA supplementary requirements for the field of testing • Australian code of good manufacturing practice (GMP) • principles of good laboratory practice (GLP) • material safety data sheets (MSDS) • national measurement regulations and guidelines • enterprise procedures, standard operating procedures (SOPs) and operating manuals • quality manuals, equipment and procedure manuals • equipment startup, operation and shutdown procedures • calibration and maintenance schedules • cleaning, hygiene and personal hygiene requirements • data quality procedures • enterprise recording and reporting procedures • material, production and product specifications • production and laboratory schedules • quality system and continued improvement processes • safety requirements for equipment, materials or products • sampling procedures (labelling, preparation, storage, transport and disposal) • schematics, work flows and laboratory layouts • statutory and enterprise occupational health and safety (OHS) requirements • stock records and inventory • test procedures (validated and authorised) • waste minimisation, containment, processing and disposal procedures
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RANGE STATEMENT	
LC instruments and techniques	LC instruments and techniques may include: • analytical and (semi) preparative columns • sample and solvent introduction systems such as: • sample valves, sub μL, μL and mL • sample loop design • solvent selector valves, mixing manifolds, binary/quaternary pumps and control program • LC detectors such as: • UV absorbance • conductivity detector (ECD) • electrochemical detector (ECD) • fluorescence • refractive index (RI) • evaporative light scattering (ELSD) • 'hyphenated' mass spectrometry (LC-MS) (LC-MS-MS) • fraction collectors • replaceable items, such as sample injection valves, (high pressure) tubing and fittings, column connectors and lamps • data systems such as recorders, electronic integrators, and software packages for peak detection and integration
Testing that uses LC instruments	Testing that uses liquid chromatography instruments may include: • determination of the molecular weight distribution for large molecules such as proteins and polymers • therapeutic drug analysis • diagnostic pathology tests • forensic testing of samples (e.g. toxicology, dyes and inks) • environmental monitoring of pollution in air, water or soil (e.g. herbicides, pesticides, nitrates and phosphates) • control of starting materials, in-process materials and final products in the food, beverage and pharmaceutical industry sectors

RANGE STATEMENT	
Presumptive tests	Presumptive tests may include: • pH • sample solubility in water/methanol mix (free of precipitates and miscible in one phase) • colour test • compatibility of sample solvent and mobile phase
Sample preparation	Sample preparation may include: • identification of any hazards associated with the samples and/or analytical chemicals • derivatisation • grinding, dissolving, extraction, filtration, refluxing, centrifuging, evaporation, washing and drying • solid-phase micro-extraction • determination of, and if appropriate, removal of any contaminants or impurities or interfering substances • ultra-trace procedures requiring high purity solvents, clean rooms, ultra clean glassware and specialised glassware • on column concentration
Instrumental parameters	Instrumental parameters may include • LC parameters: • manual/auto sample; injector volume, pre- and post-sample washes • fixed or variable solvent ratios • solvent flow rate • column temperature • detector/source parameters, single/split system • MS parameters: • vacuum pressures and gas flows • nebuliser gas flow • ionisation control • interface cone alignment • ion lens voltage • mass analyser control • solvent delay • scan, mass start/end, scan time and inter-

RANGE STATEMENT	
	- scan delay - selective ion monitoring (SIM)

RANGE STATEMENT	
Common analytical procedure and equipment problems	Common analytical procedure and equipment problems may include: • system leaks • efficiency of roughing pump (oil and bearing wear) and turbo/molecular pumps • irregular sample or solvent delivery • contamination of sample, solvents, lines or other system elements • dead volume • bubbles in system • build up of salts/dissolved solids in sample valves, injectors, MS spray chamber and/or cones • lack of suitable reference standards • poor separation due to inappropriate selection of column or operating parameters solvent mix and flow • poor sensitivity • absence of peaks and presence of ghost peaks, split peaks or distorted peak shapes and broad solvent peaks • baseline instability and non-reproducible retention times
Hazards	Hazards may include: • electric shock • biohazards, such as microbiological organisms and agents associated with soil, air, water, blood and blood products, human or animal tissue and fluids • corrosive chemicals • sharps and broken glassware • hot surfaces • flammable liquids and gases • fluids under pressure and sources of ignition • disturbance or interruption of services
Addressing hazards	Addressing hazards may include: • use of MSDS • accurate labelling of samples, reagents, aliquoted samples and hazardous materials • personal protective equipment such as gloves,

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	safety glasses, coveralls • use of fumehoods, direct extraction of vapours, gases • use of appropriate equipment such as biohazard containers, laminar flow cabinets, Class I, II and III biohazard cabinets • handling and storage of all hazardous materials and equipment in accordance with labelling, MSDS and manufacturer's instructions
Occupational health and safety (OHS) and environmental management requirements	OHS and environmental management requirements: • all operations must comply with enterprise OHS and environmental management requirements, which may be imposed through state/territory or federal legislation - these requirements must not be compromised at any time • all operations assume the potentially hazardous nature of samples and require standard precautions to be applied • where relevant, users should access and apply current industry understanding of infection control issued by the National Health and Medical Research Council (NHMRC) and State and Territory Departments of Health

Unit Sector(s)

Unit sector	Testing
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Competency field

Competency field	
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Co-requisite units

Co-requisite units		