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Verification and Maintenance of Analytical Instruments According to ISO/IEC 17025 Standard

EUROPEAN MASTER IN QUALITY IN ANALYTICAL LABORATORIES

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Attest

The current work titled **"Verification and Maintenance of Analytical Instruments According to ISO/IEC 17025 Standard"**

has been conducted by Marta Isabel Zacarias in the Department of Analytical Chemistry at University of Barcelona under our supervision

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Abstract

Equipment verification and preparation of the documents related to this activity were made. The equipment verification was performed on balances and analytical balances, liquid chromatographs and gas chromatographs.

With respect to the balances and analytical balances verification, repeatability, trueness and drift assays were performed. A SOP containing the instructions for the verification, forms to register the primary data obtained from the verification assays, excel sheets to carry out the calculations for the assays, verification notebooks including the form mentioned above and archives to save all the results obtained from such assays were prepared.

The performance verification was carried out for two liquid chromatographs. The following verification assays were performed: injector precision, flow rate precision, injector linearity and carryover, detector linearity, noise and drift, flow rate trueness and gradient accuracy. A SOP containing the instructions for the verification, an excel sheet to carry out the necessary calculations for the verification assays and an archive to save the obtained chromatograms and results were prepared for each instrument. For one of the liquid chromatographs a SOP containing the maintenance instructions was written.

Two gas chromatographs were verified, one with Thermal Conductivity Detector (TCD) and another one with Flame Ionization Detector (FID). The documents related to the verification and mentioned for the liquid chromatographs were also prepared for these equipments. The following verification assays were performed: flow rate precision, detector linearity, noise and drift, oven temperature precision, trueness, linearity and stability. A SOP containing the maintenance instructions was prepared for both gas chromatographs.

The proposed objectives were achieved.

Keywords: Performance Verification, Maintenance, Calibration, Equipment Qualification, Traceability.

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List of Acronyms

VIM – International Vocabulary of Metrology

SOP – Standard Operating Procedure

HPLC – High Performance Liquid Chromatography

GC – Gas Chromatography

DQ – Design Qualification

IQ – Installation Qualification

OP – Operational Qualification

PQ – Performance Qualification

RM – Reference Material

CRM – Certified Reference Material

TCD – Thermal Conductivity Detector

FID - Flame Ionization Detector

1. Objectives

Maintenance, verification and calibration are fundamental activities in order to ensure the suitable performance of the analytical instruments and the reliability of the measurements carried out in analytical laboratories.

The purpose of the present research project is to design processes for the maintenance and verification of some analytical instruments in some laboratories of the Analytical Chemistry Department of the Faculty of Chemistry in the University of Barcelona. The design of such processes includes the following: preparation of material and reagents for verification assays, execution of procedures in order to check if they are suitable or not, and preparation of the documents for the quality management system: (standard operating procedures (SOPS), records, file templates and archives to save the results obtained).

2. Introduction

Good analytical results are essential in order to take reliable decisions. Analytical measurements affect the daily lives of every citizen. Sound, accurate and reliable analytical measurements are fundamental to the functioning of modern society. A wrong result can have an enormous social and economic impact, for instance [1]:

- *"In trade, it could lead to the supply of sub-standard goods and the high cost of replacement with subsequent loss of customers";*
- *"In environmental monitoring, mistakes could lead to hazards being undetected or to the identification of unreal hazards";*
- *"In supply of drinking water, it could lead to harmful contaminants being undetected";*
- *"In healthcare, the incorrect medication or the incorrect content of an active ingredient in a tablet can be catastrophic for the patient".*

In agreement with Sommer et al, the correctness of measurements and measuring instruments is one of the key prerequisites to ensure the quality of products and services, and the accuracy of the instruments must be consistent with their intended use [2].

Calibration and verification are the most important actions to ensure the correct indication of measuring instruments [2]. Taking into account the industrial metrology,

regular calibration of measuring instruments should be carried out in agreement with the implemented quality systems. The industrial metrology ensures the appropriate functioning of measurement instruments used in industry as well in production and testing processes, in order to guarantee the quality of life for citizens and for academic research [3]. The principles of industrial metrology can also be applied to academic research in institutions such as universities, researches centres.

Concerning the legal metrology, periodic verification of instruments should be performed according to legal regulations. Legal metrology is responsible for ensuring the accuracy and reliability of measurement where measured values can affect safety, health or the transparency of financial transactions (e. g. weights and measures) [3].

Table 1 shows an overview of prominent institutions offering guidance on metrology. This table includes metrological institutions and their main purpose.

Institution	Purpose
OIML- International Organisation of Legal Metrology	To promote the global harmonisation of legal metrology procedures [34].
BIPM - Bureau International des Poids et Mesures	To ensure worldwide uniformity of measurements and their traceability to the International System of Units (SI). Deals with scientific metrology [20].
WELMEC – European Cooperation in Legal Metrology	To establish a harmonized and consistent approach to legal metrology [35].
EURAMET - European Association of National Metrology Institutes	To coordinate the cooperation of National Metrology Institutes (NMI) of Europe in fields like research in metrology, traceability of measurements to the SI units, international recognition of national measurement standards and of the Calibration and Measurement Capabilities (CMC) of its members[36].
EURACHEM - European Federation of National Associations of Measurement, Testing and Analytical Laboratories	To establish a system for the international traceability of chemical measurements and promote good quality practices [37].
CITAC - Cooperation on International Traceability in Analytical Chemistry	To foster collaboration among existing organisations to improve the international comparability of chemical measurements. To prepare a directory of international chemical metrology activities [38].

Table 1: Organizations in metrology

The equipment maintenance is also a very important activity. If the maintenance is preventive it will represent a cost-effective method of maintaining equipment, since it prevents failures, damage or malfunctioning. Corrective maintenance is also essential because it allows the reparation of the equipment and accordingly its conservation during more time [15].

Regarding the concept of quality assurance, non-analytical chemical laboratories have become aware that it is important to apply quality criteria to their processes. Some higher education institutions have included theoretical subjects on quality assurance in several curricula, such as pharmacy, engineering and chemistry. Hence, practical teaching becomes also necessary in order to complement the learning of this subject. Moreover, the adoption of a system of readable and comparable degrees throughout Europe has arisen in higher education. Due to this last occurrence and new requirements of the stakeholders, arise the necessity of managing teaching laboratories not only in an adequate and traditional way but also in order to produce an efficient answer to the rapidly occurring changes in the curricula and programmes. The solution for these changes is the implementation of a quality management system in teaching laboratories [11].

In this project the verification of equipment and the preparation of the documents related to this activity like standard operating procedures, forms, file templates and archives were made. The verification was performed on balances and analytical balances, liquid chromatographs and gas chromatographs. Procedures containing the work instructions for the maintenance of laboratory equipment have also been prepared.

The equipment verification, the preparation of the documents above mentioned related with verification were performed for research and teaching laboratories since these laboratories are implementing a Quality Management System (QMS).

The QMS for teaching laboratories aims to improve the technical and economic management as well the performance of these laboratories and the educational quality of the practical work.

2.1. ISO/IEC 17025 and Accreditation

Calibration, verification and maintenance are requirements of ISO/IEC 17025 for the laboratory equipment [21]:

"Equipment used for testing, calibration and sampling shall comply with specifications ..." (5.5.1)

"Before being placed into service, equipment (including that used for sampling) shall be calibrated or checked to establish that it meets the laboratory's specification requirements and complies with relevant standard specifications. It shall be checked and/or calibrated before use". (5.5.2)

"Instructions on use and maintenance of equipment should be readily available..." (5.5.3).

ISO/IEC 17025 is the International Standard which specifies the general requirements for the competence of testing and calibration laboratories. General requirements for the competence of testing and calibration laboratories include management requirements and technical requirements. This International Standard can be applied to all laboratories regardless of the extent of the scope of testing and/or calibration activities or the number of workers. This document should be used by accreditation bodies that recognize the competence of testing as well as calibration laboratories, as a basis for accreditation. This International Standard may also be used by laboratory customers and regulatory authorities in confirming or recognizing the competence of laboratories. This International Standard verifies that testing and calibration laboratories operate a management system, are technically competent, and are able to generate technically valid results [21].

Accreditation is a formal procedure by which an authoritative body confers formal recognition that a body or a person is competent to carry out specific tasks [39].

Laboratory accreditation is a means of determining the technical competence of laboratories to carry out specific activities of testing, measurement and calibration. Laboratory accreditation provides a ready means for customers to identify and choose reliable testing, measurement and calibration services to be able to fulfil their needs. It allows to check if laboratories are performing their work in an adequate way and to suitable standards and thus provides them with a benchmark for keeping that competence [40].

2.2. Calibration, Verification, Maintenance and Equipment qualification

a. Calibration

The balances and analytical balances calibration was not carried out during this research project, although the concept of calibration is discussed briefly since there is a strong relationship between this concept and the equipment verification concept.

ISO/ IEC Guide 99, the International Vocabulary of Metrology (VIM) defines calibration as [4]:

"Operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication".

The main objectives of equipment calibration are: to assess the measurement capacity of the instruments in order to guarantee the comparability of the obtained results with those obtained by other laboratories; to validate the analytical methods and to establish quality control [5].

In agreement with the European Association of National Metrology Institutes (EURAMET), the main reasons for having an instrument calibrated are: to establish and demonstrate traceability; to ensure readings from the instrument are consistent with other measurements; to determine the accuracy of the instrument readings; to establish the reliability of the instrument i.e. that it can be trusted [3].

Generally, calibration is an operation which establishes a relationship between an output quantity and an input quantity for an instrument or a measuring system under specified conditions. Depending on the relationship between the characteristic quantities considered (both from the reference value and from the instrumental signal) and the purpose of calibration two types of calibrations can be identified, direct and indirect calibration [7]. Hence, a measuring instrument can be calibrated directly or indirectly. The type of calibration will depend on its nature, the kind of measurement to be performed and the purpose of the calibration [7].

In direct calibration the value of the standard (known reference value) is expressed in the same magnitude as the response of equipment that carries out the measurement. The analytical balances calibration is a characteristic example of direct calibration. Both the standard mass as the analytical balance indication are expressed in units of mass [8].

In indirect calibration (also known as analytical calibration), the value of the standard (reference value) is expressed in a different magnitude to the equipment answer. The measuring equipment gives a value (namely a signal or instrumental response) which has a different quantity from that characteristic of the standard, and then both are expressed in different units. The indirect calibration procedure consists of obtaining the instrumental response corresponding to a series of standards characterized by a known value of the measurand [5] [8].

The chromatograph calibration and spectrophotometer calibration are examples of indirect calibration. The chromatograph calibration relates the peak height or peak area (instrumental response) with the concentration of the standard solutions. The calibration spectrophotometer establishes the relationship between the concentration of the standard solutions with a magnitude of optical type (absorbance, emission, wavelength, etc.) [5].

b. Verification

The calibration of equipment is not enough to ensure that the operating conditions of a measuring instrument are good and guarantee the comparability of the obtained results with those obtained by other laboratories. This is especially meaningful for analytical instruments in which it is necessary to control numerous operative parameters that affect the sensitivity or selectivity of the instrumental response [5]. For instance, the flow rate of the mobile phase, in the liquid chromatograph, will affect the retention time and the width of the chromatographic peaks [12]. Similarly, it is also necessary to take into account factors which affect the precision of the instrumental response. For instance, the precision of the injector, in the liquid chromatograph, will affect the peak area or peak height [12].

On the other hand, there is laboratory equipment which doesn't perform direct measurements and then doesn't need to be calibrated, however requires the control of the operating conditions which can affect the obtained results. For example, the temperature of a thermostatic bath needs to be controlled [5].

Taking into account what has been mentioned previously, one may conclude that the verification performance is an essential action in order to ensure the correct performance of the measuring equipment in laboratories.

ISO/ IEC Guide 2 defines verification as:

"Confirmation by examination and provision of evidence that specified requirements have been met"[4].

Verification is the confirmation, based on evidences (facts, test results) that some specified requirement has been fulfilled. For instance, the performance of the balance is still in agreement with the calibration certificate [1]. The result from a verification assay will show if the measuring equipment is in agreement with its required specifications, which are generally expressed as tolerances [5]. The verification of measuring instruments includes testing and requires the availability of clear specifications and acceptance/refusal criteria [13].

Verification provides means of checking that the deviations between the values displayed by a measuring instrument and the corresponding known values of measured quantity are under control. For instance, in the balances verification, the balance gives a reading that is close enough or not to the true value of a standard weight to enable the analyst to decide if the equipment should be used [1]. Either of the following is the result of the verification process [5]:

a) The equipment can be in good working conditions and its performance is considered correct. In this case, the date of the next verification is scheduled.
b) The equipment is not in perfect working conditions; however it can be used with restrictions. These restrictions can affect the working ranges.
c) The equipment cannot be used and should be repaired or adjusted.

The verification performance of balances, liquid chromatographs and gas chromatographs was carried out during this research project. Balances play the primary role of weighting the portions to be analysed, so they must be within their specifications (verified). High-Performance Liquid Chromatography (HPLC) and Gas chromatography (GC) are analytical techniques widely used in analytical laboratories. These techniques are used to carry out analysis with numerous applications such as: pharmaceutical, food, chemical, cosmetic, environmental and clinical.

To provide a high level of assurance that the data produced by HPLC and GC analysis are reliable the performance of the equipments should be verified regularly. So the performance verification of the gas chromatographs is an essential activity since it ensures the correct behaviour of these measuring instruments.

c. Calibration and Verification: Differences and Similarities

Taking into account the definitions of calibration and verification mentioned above, one may conclude that they are different activities and, in many cases, are complementary.

Sometimes, in the laboratories, there is misunderstanding of the terms "calibration" and "verification". The concept of equipment verification is referred to as equipment calibration. The misunderstanding of these terms is due to both actions are closely related and are mostly based on the same measuring procedures. Hence, it is essential to clarify both concepts, identifying the differences and similarities (See Figure 1) [5].

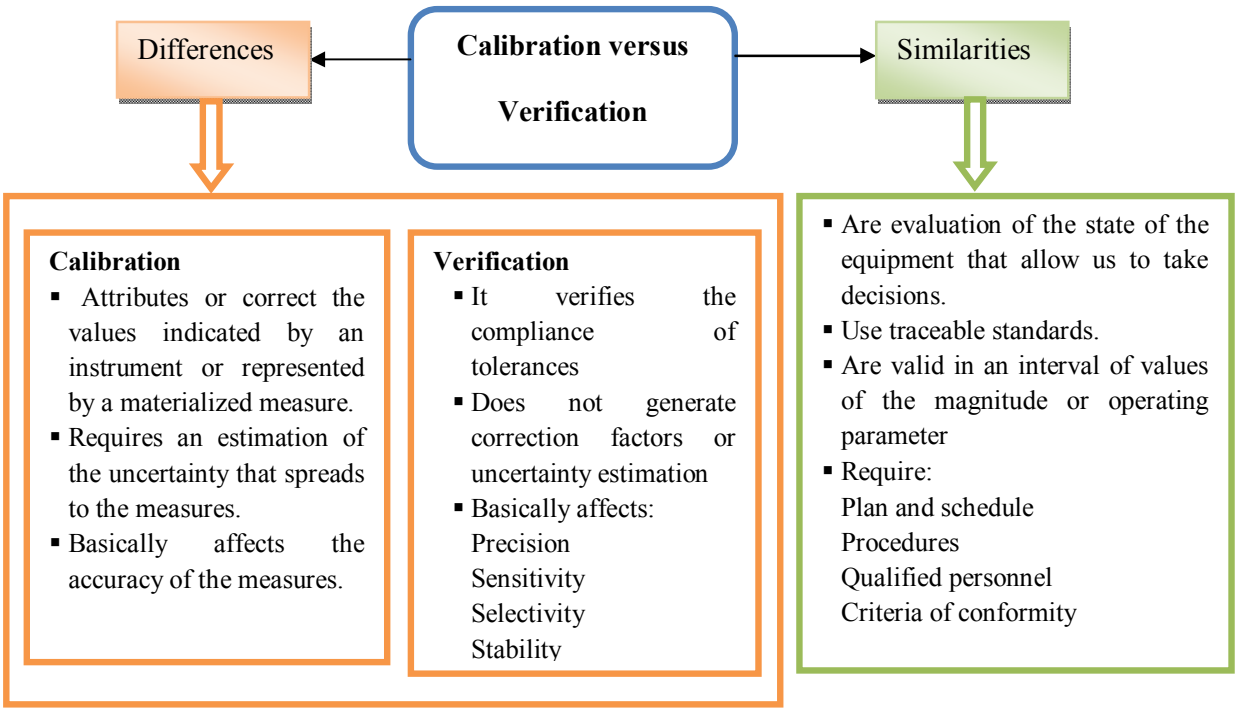


Figure 1: Differences and similarities between calibration and verification [5].

d. Maintenance

There are two types of equipment maintenance: preventive maintenance and corrective maintenance. The preventive maintenance provides a means of ensuring that the instrument is kept in good working conditions and identifying any-long term problems. The preventive maintenance avoids failures, deterioration, damage or malfunctioning of the equipment. Preventive maintenance includes actions such as equipment cleaning, lubricating, reconditioning, adjustment, etc. The laboratory should

have a preventive maintenance plan for equipment containing the activities of maintenance, the periodicity of these actions and responsible people. The laboratory should document the instructions to carry out the activities of preventive maintenance and maintain records of the activities performed. The periodicity for the activities of preventive maintenance will depend upon the features of the equipment, its use (frequency, type of samples processed and personnel qualification) and the environmental conditions of the place where the equipment is located. Anyway, the preventive maintenance intervals established can be modified according to the obtained results from the calibrations or verifications, when it is necessary to analyse compromised samples (with low levels of concentration or abnormal results) or to analyse samples which contaminate the equipment [1] [5].

Some activities of preventive maintenance can be carried out by a qualified personnel who works in the laboratory (for instance the changing of the septum of the injector in the gas chromatograph). Other activities of preventive maintenance should be performed by a technical service provider, for instance, the cleaning of the optical parts of a spectrophotometer [5].

The corrective maintenance aims to repair breakdowns and corrects malfunctioning of the equipment. This type of maintenance is generally performed by a technical service provider and it should be carried out when the equipment broke down and cannot be repaired by the user [5].

e. Equipment Qualification

Calibration, verification and preventive maintenance are the activities included in the process of equipment qualification. In this section, the importance of this process is explained, how it is developed and where the activities of calibration, verification and preventive maintenance can be located within this process.

All equipment (whether it is a refrigerator used to cool samples, volumetric flask or a liquid chromatograph to determine the content of pesticides in drinking water) used in analytical laboratories needs to satisfy a primary requirement that is to be fit for its intended purpose. The GLP (Good Laboratory Practice) and international standards, such as ISO 9001 and ISO/IEC 17025 require that instruments are suitable for their intended use [1].

Equipment Qualification (EQ) is a formal process that provides documented evidence that an instrument is appropriate for its intended use and will be kept in a state of maintenance and calibration consistent with its use. The EQ process includes four stages of "qualification": design qualification (DQ), installation qualification (IQ), operational qualification (OP) and performance qualification (PQ) [14]. (See Figure 2)

Design qualification is the first stage of the EQ process, which defines the operational and functional specifications of the instrument and describes the conscious decisions in the selection of the supplier. The operational specifications should describe the key performance features of the instrument and ranges over which the instrument is required to operate and consistently does. The functional specifications should take into account the overall requirements of the instrument including the operational specifications and other factors relating to its use (for instance, environmental conditions within which, or range over which, the instrument must operate; documentation (protocols for IQ, OP and PQ, model SOPs, etc)). DQ is considered a planning stage and is carried out once, before a new instrument is acquired by the laboratory or before an existing instrument is chosen to be used for a certain task. [1][14].

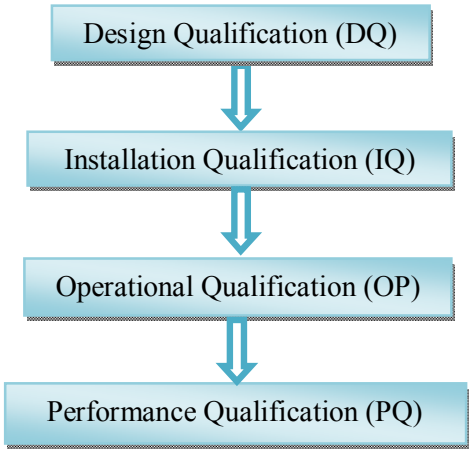


Figure 2: Equipment qualification process

Installation qualification is the stage when checks are carried out in order to confirm that the instrument, its modules and accessories have been received as ordered (according with the specifications agreed between supplier and costumer) and that the instrument is correctly installed in the selected environment. This includes software and hardware. It may be useful to use a check-list to this phase in order to ensure that

everything is checked. IQ stage is generally a one-off check when an instrument is delivered or moved [1][13][14].

Operational qualification stage aims to demonstrate and provide documented evidence that the instrument will operate according to the operational specification in the selected environment. OQ generally is carried out after the IQ of a new instrument or after a significant change to the instrument or a component such as repair. OQ should be performed in agreement with the supplier's procedures and instructions, using suitable materials and protocols [1][14]. OQ phase should be performed regularly but not frequently. The periodicity of future OQ testing depends on factors such as the manufacturer's recommendation, the level of the use of the instrument, the nature of the use of the instrument (for instance the use of aggressive compounds), and "criticality" of the performance of the instrument [1].

Performance qualification serves to demonstrate that the entire instrument functions correctly and to a specification suitable for its routine use. This specification may be the original operational specification or other more adequate for its actual use. PQ involves the testing of the instrument using the specific method or assay to ensure that the method is generating valid results [1][14]. Evidence of continued satisfactory performance (PQ) should be obtained from everyday method-related checks (e.g. system suitability testing, calibration and analytical control).

Taking into account the description of the equipment qualification process, it can be concluded that the activities of calibration, verification and preventive maintenance are included in performance qualification stage.

2.3. Traceability and Standards

The comparability is a key property of good results. It is important to compare results obtained in different laboratories or in the same laboratory at different times with confidence. This is possible if all laboratories are using the same measurement scale, or the same "reference points". In many cases, the comparability of results is supported by chain of calibrations which establishes a relationship with primary national or international standards and ideally with SI units of measurement. The calibration of analytical balances is a familiar example. Each balance is calibrated using reference masses which are themselves checked against the national standards, the international standards and the primary reference kilogram. This unbroken chain of comparisons

reaching a known reference value "provides" traceability to a common reference point ensuring that different laboratories are using the same units of measurement [17][18][19].

Traceability is defined by the International Vocabulary of Metrology (VIM) as: *"The property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty"* [9]. The word "unbroken" means that there is no loss of information considering the different stages in an analytical procedure that allows getting a measurement result [16]. Each stage needs to be related to either a reference method, a reference material or an SI unit. The comparability of measurements is achieved through the traceability chain, the unbroken chain of comparisons, all having stated uncertainties. Traceability provides the linkage that ensures that measurements performed in different laboratories or at different times are comparable [17]. This chain ensures that a measurement result or the value of a standard is traceable to references at higher levels, leading to the primary standard [3]. (See Figure 3)

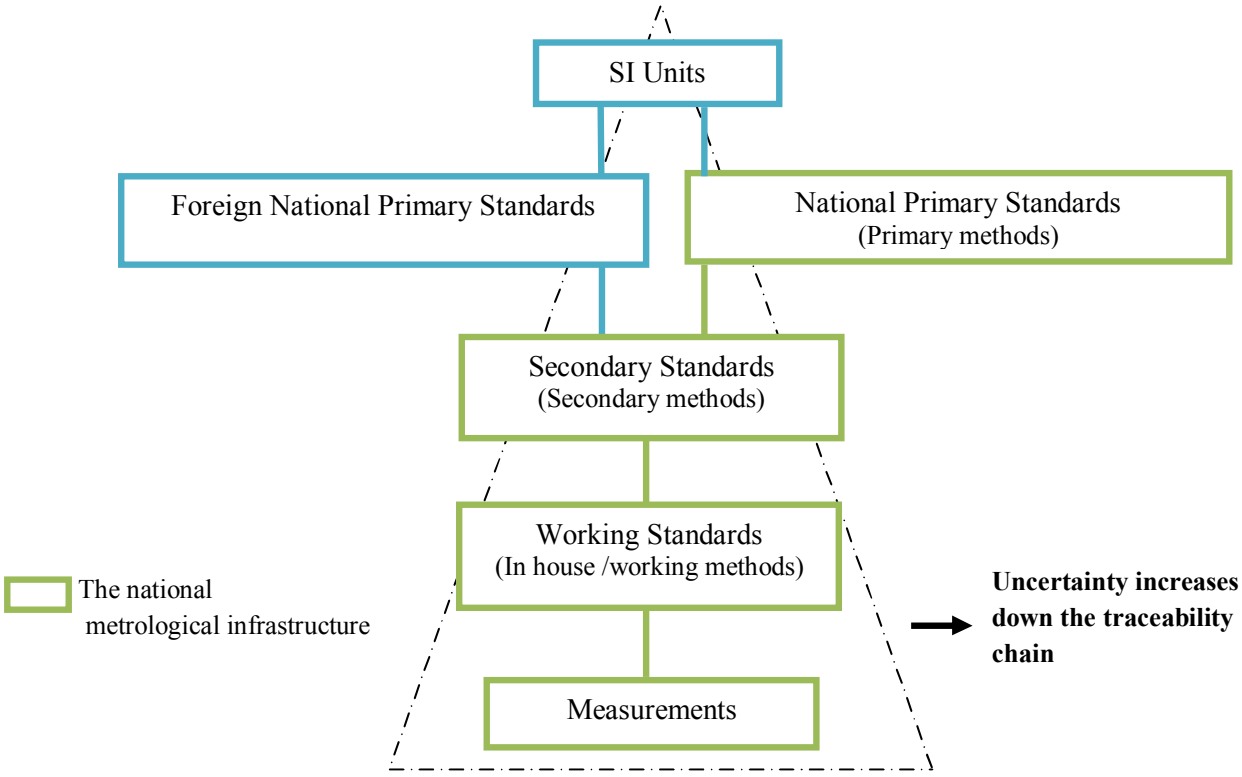


Figure 3: The traceability chain [3].

A primary standard is the standard which has the highest metrological qualities and whose value is accepted without reference to the other standards of the same quantity. Primary standards are used to calibrate secondary standards [20].

Secondary standards are defined as standards whose value arises from the comparison with a primary standard of the same quantity. Secondary standards are used to calibrate working standards [20].

Working standards are standards used routinely to calibrate or check measuring instruments, material measures or reference materials. This type of standards may be used to ensure that the routine measurements are performed in a correct way - a check standard [1][20].

Traceability of a measurement result is related with traceability of a method, which in turn is related with traceability of the equipment used and traceability of the standards [16].

In accordance with EURACHEM/CITAC the traceability of a measurement result should be established through the combination of the following procedures [19]:

1. Use of traceable standards to carry out the calibration of the measuring equipment;
2. By using a primary method or establishing a comparison to the results from this method.
3. By using a pure substance Reference Material (RM);
4. By using an adequate matrix Certified Reference Material (CRM);
5. By using an accepted, closely defined procedure.

The result obtained from a primary method is generally traceable directly to the SI. A primary method of measurement is defined as [19]: "... *a method having the highest metrological qualities, whose operation is completely described and understood in terms of SI units and whose results are accepted without reference to a standard of the same quantity*."

The traceability of results obtained from a primary method is achieved by comparing directly the measurement results of the primary method and the test or calibration method [19].

Traceability can be confirmed by measuring a sample composed of, or including, a known quantity of a pure substance Reference Material (RM). A RM is defined by VIM as [19]: "*Material, sufficiently homogeneous and stable with reference to specified*

properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties".

The substance RM can be incorporated in the sample by spiking or by standard additions. As a consequence, the difference between in the response of the measurement system to the standard used and the sample tested must be evaluated due to the fact of the correction for the difference in response and its uncertainty may be large. If the uncertainty result is unacceptably large or unquantifiable thus the traceability has not been established [19].

Traceability can be also achieved by comparing the measurement results on a certified matrix CRM with certified value(s). In this procedure there is an adequate matrix CRM available, so it can reduce the uncertainty comparing with the use of a pure substance RM. However, if there is not a good match between the composition of the sample and the reference material, the uncertainty result can be unacceptably large or unquantifiable. Accordingly, if the uncertainty is not unquantifiable thus traceability has not been established [1][19].

The use of a closely defined and generally accepted procedure is very often the only factor which provides the suitable comparability of measurement results. When it is expected that results of a method or alternative procedure are comparable with an accepted procedure, traceability to the accepted values is established trough the comparison of the results obtained by the alternative and accepted procedures [19].

Traceability among standards is the most relevant basis for traceability of results. Traceability of equipment is defined as "*the detailed, timely, and customised recording of installation, periodic calibrations and corrections (if necessary), malfunctioning and repairs, hours of use, samples processed, standards used, etc., in such way that all questions (what?, how?, who?, etc) should have a detailed answer in the pertinent documents*"[16].

The calibration of measuring equipment used must be always traceable to adequate standards. Generally, the quantification stage of the analytical procedure is calibrated through the use of a pure substance reference material whose value is traceable to the SI. However this activity doesn't provide traceability of results to SI for the complete analytical procedure. So it is also necessary to establish traceability for the results of operations carried out before the quantification stage, such as extraction and clean up of the sample, using the corresponding procedures [5][19].

3. Experimental Part

3.1. Performance Verification of Balances and Analytical Balances

A Standard Operating Procedure (SOP) containing the instructions to carry out the verification of balances and analytical balances was written. This SOP is named as "Instructions for verification of balances and analytical balances", coded as **PNT/QA-D/EQP/053/01** and can be found in Annex I.

The verification of balances and analytical balances was carried out weekly. Four analytical balances and one balance were verified. The balances and analytical balances verification included the following: cleaning and level adjustment activities, and trueness, repeatability and drift assays. The cleaning and level adjustment activities were performed before carrying out the verification assays in order to guarantee that each balance would operate in suitable conditions. The cleaning of each balance was firstly done using an adequate brush to remove solids in the weighing chamber. The pan, pan support and anti draft-ring were cleaned with tissue wet with ethanol. After cleaning the level adjustment of each balance was done. After performing all the activities mentioned above, the verification assays were carried out.

The trueness, repeatability and drift assays were performed using a standard mass of 100 g with the following specifications: code number E7249, tolerance: +/- 0.50 mg, uncertainty: +/- 0.15 mg. The calibration certificate of the standard mass is included in Annex I.

The balance was warmed up for 15 minutes in order to stabilize. During this time, the standard mass was placed close to the balance in order to reach the room temperature. While wearing gloves, the standard mass was held with tweezers. The trueness and repeatability assays were carried out by weighing the standard mass until five replicates were obtained. The masses were registered in the form named as "Verification Form of Balances" (see Figure 4) which can be found in Annex I in the SOP coded as **PNT/QA-D/EQP/053/01**. Between measurements, the standard mass was removed from the pan and the balance was adjusted to zero. From the values obtained (mass) in the trueness assay, the average value of the replicate measurements, the absolute error and the relative error were determined.

The trueness of each balance was expressed through the relative error.

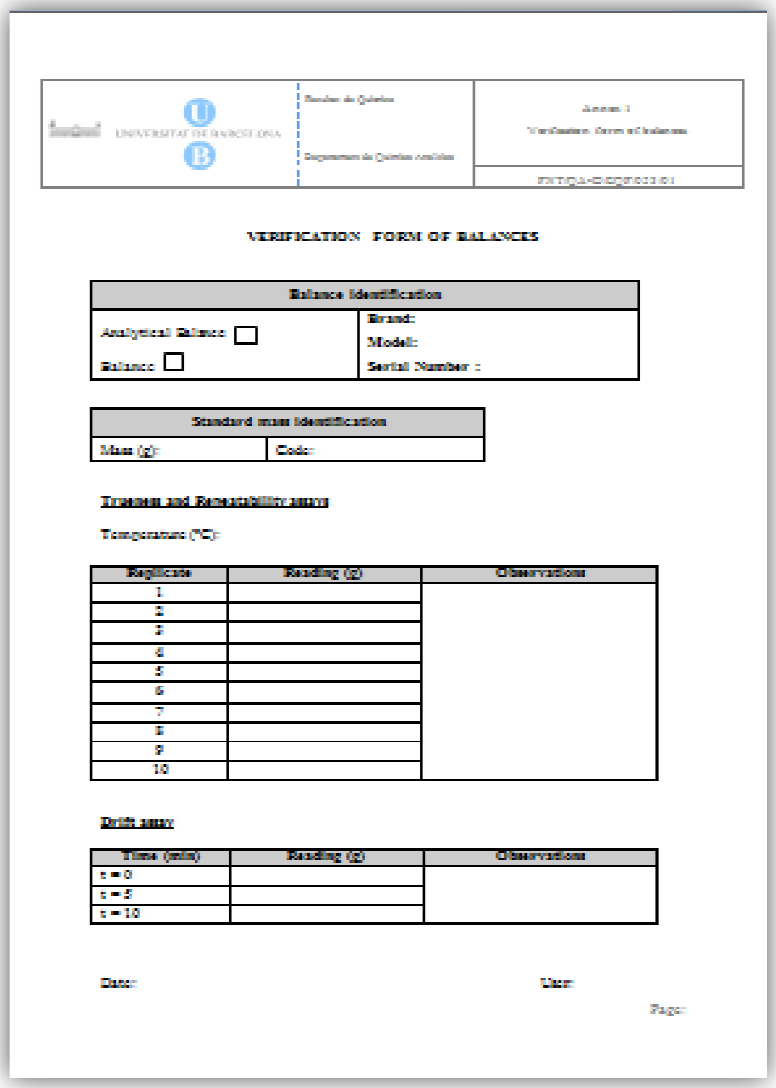
From the values of the measurements carried out in the repeatability assay, the standard deviation, the relative standard deviation and the coefficient of variation were

calculated. The repeatability of each balance was expressed through the coefficient of variation.

The drift assay was performed following the procedure below:

1. The balance was adjusted to zero and then the standard mass was weighed. The result of the measurement was registered in the form "Verification Form of the Balances" (see Figure 4).
2. The mass was left onto the weighing pan and the value of the measurement was read after 5 and 10 minutes.

In the drift assay, the drift value was calculated by subtracting the minimum value from the maximum value of the masses obtained.



VERIFICATION FORM OF BALANCES

Balance Identification	
Analytical Balance ☐ Balance ☐	Brand: Model: Serial Number :

Standard mass identification	
Mass (g):	Code:

Temperature (ºC):

Replicate	Reading (g)	Observations
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Time (min)	Reading (g)	Observations
t = 0		
t = 5		
t = 10		

Date: User:

Figure 4: Verification Form of Balances

All the calculations necessary for the verification assays of balances and analytical balances were encoded in an excel sheet prepared for this purpose and saved in a file

template called "Balance verification form". Figure 5 shows an example of an excel sheet prepared for an analytical balance. This excel sheet includes the identification of the balance (brand, model, serial number and the type of the balance), the standard mass identification (code, number of the certificate, value of the mass, uncertainty and tolerance) and the acceptance /refusal criteria to compare against the obtained results and accordingly to decide if the equipment is or not suitable for use.

The excel sheets containing the calculations for the verification assays carried out for each balance can be found in Annex I, in the SOP coded as **PNT/QA-D/EQP/053/01**, as the example given in Figure 5.

Annex 2: Balance verification form

Procedure: PNT/QA-D/EQP/053/01: Instructions for verification of balances and analytical balances

Balance Identification	
Brand:	Mettler Toledo
Model:	AG245
Serial Number:	1117512803
Analytical Balance:	X
Balance:	.

Standard Mass Identification	
Code:	E7249
Certified nº:	MS60716
Value: [illegible]	99,9951
Uncertainty:	+/- 0,15 mg
Tolerance:	+/- 0,05 mg

Acceptance / Refusal Criteria	
Trueness (RE), %	0,01
Repeatability (CV),	0,0001
Drift (D), g	0,001

Trueness and repeatability assays

Date:	17-09-2009	21-09-2009	29-09-2009	05-10-2009	13-10-2009	19-10-2009	27-10-2009
Replicate	Reading (g)	Reading (g)	Reading (g)	Reading (g)	Reading (g)	Reading (g)	Reading (g)
1	100,0000	99,9998	100,0000	99,9998	100,0000	99,9991	99,9999
2	99,9998	99,9997	100,0000	99,9999	100,0000	99,9991	99,9999
3	99,9999	99,9997	99,9999	99,9999	99,9999	99,9991	99,9999
4	99,9998	99,9998	99,9999	100,0000	99,9999	99,9991	99,9999
5	99,9998	99,9998	99,9999	100,0000	99,9999	99,9991	99,9999
Average (g)	**99,9999**	**99,9998**	**99,9999**	**99,9999**	**99,9999**	**99,9991**	**99,9999**
Absolute Error (g)	0,0048	0,0047	0,0048	0,0048	0,0048	0,0040	0,0048
Relative Error (%)	0,0048	0,0047	0,0048	0,0048	0,0048	0,0040	0,0048
Accepted/ Not accepted trueness	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted
SD (g)	8,944E-05	5,477E-05	5,477E-05	8,367E-05	5,477E-05	0,0000	0,0000
CV(%)	8,944E-05	5,477E-05	5,477E-05	8,367E-05	5,477E-05	0,000E+00	0,000E+00
Accepted/ Not accepted repeatability	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted
Tolerance (g)	1,111E-04	6,801E-05	6,801E-05	1,039E-04	6,801E-05	0,0000	0,0000
Confidence interval (g)	99,9999 ± 0,0002	99,9998 ± 0,0001	99,9999 ±0,0001	99,9999 ± 0,0002	99,9999 ± 0,0001	99,9991 ± 0,0000	99,9999 ± 0,0000

Drift assay

Date:	17-09-2009	21-09-2009	29-09-2009	05-10-2009	13-10-2009	19-10-2009	27-10-2009
Time (min)	Reading (g)	Reading (g)	Reading (g)	Reading (g)	Reading (g)	Reading (g)	Reading (g)
t = 0	99,9998	99,9998	99,9999	100,0001	99,9999	99,9991	99,9999
t =5	99,9998	99,9997	99,9999	100,0003	99,9999	99,9992	99,9998
t = 10	99,9998	99,9997	99,99999	100,0005	99,9999	99,9993	99,9998
Drift (g)	0,0000	0,0001	0,0001	0,0004	0,0000	0,0002	0,0001
Accepted/ Not accepted drift	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted

Figure 5: Excel sheet for the calculations in the verification assays of an analytical balance

Verification notebooks for balances and analytical balances were prepared in order to register the values obtained in the verification assays. Figure 6 shows the cover page of the verification notebook for an analytical balance. This page includes the identification of the balance (brand, model and serial number), the number of the notebook, the beginning date (the date of the first register) and the final date (the date of the last register). All the verification notebooks prepared have this format page. These notebooks include the form "Verification Form of Balances" (see Figure 4).

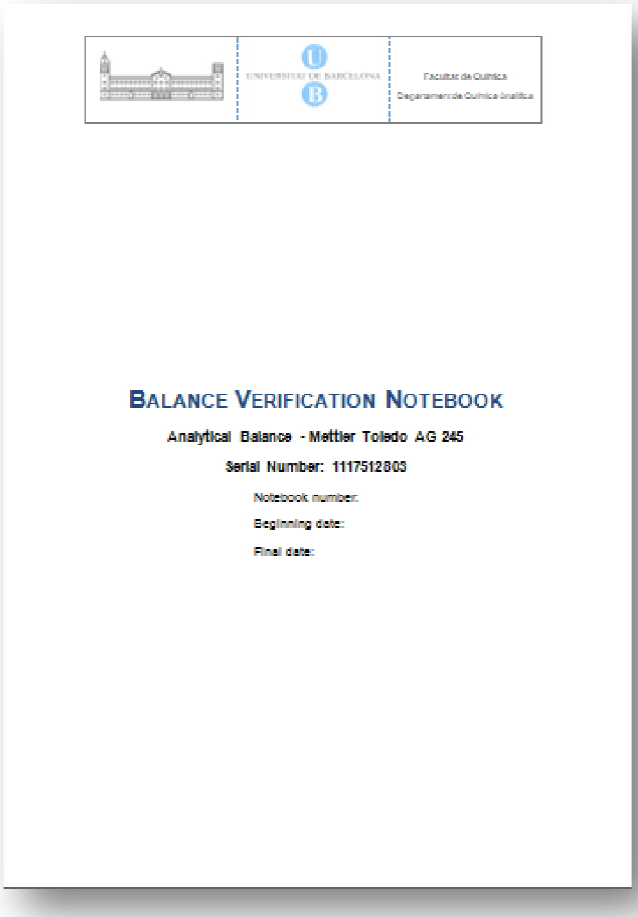


Figure 6: Verification notebook for an analytical balance

3.2. Performance Verification of High-Performance Liquid Chromatography (HPLC)

The performance of two liquid chromatographs was verified. One of the chromatographs is used at the teaching laboratory by students. This equipment is an HP 1050 with UV/VIS detector. The other liquid chromatograph is used for research. The latter equipment is an Agilent 1100 with UV/VIS detector.

The performance of the Liquid Chromatograph was evaluated by examining the key functions of the various modules that are included in the system: pump, injector and UV/VIS detector.

Performance Verification of the Liquid Chromatograph HP 1050 with UV/VIS detector

A SOP containing instructions for the verification of the liquid chromatograph HP 1050 with UV/VIS detector was written. This document also includes the periodicities for the verification assays. This SOP was named as “Instructions for verification of the

liquid chromatograph with UV/VIS detector (HP 1050)", coded as **PNT/QA-D/EQP/055/01** and can be found in Annex II.

The verification of this equipment included the following assays: injector and flow rate precision, flow rate trueness, detector linearity, noise and drift, and gradient accuracy.

The injector precision and flow rate precision were evaluated using the same assay. In order to evaluate the ability of the injector to draw the same amount of sample in replicate injections (injector precision) and the ability of the pump to deliver a constant flow rate (flow rate precision), five replicate injections of 8.0 mg/L anthracene standard solution with an injection volume of 100 µL were performed. The average value, the standard deviation, the relative standard deviation and the coefficient of variation were calculated from the values obtained (peak area in the chromatograms) in the injector precision assay. The precision of the injector was evaluated through the coefficient of variation.

The calculations mentioned in the injector precision were also performed to evaluate the precision of the mobile phase flow rate but now using the retention times obtained in the chromatograms instead of peak area. The flow rate precision was also evaluated through the coefficient of variation of the retention time for the five replicate injections.

The flow rate trueness assay was performed by setting the flow rate at 1.0 mL/min and using a stopwatch to measure the time that it takes to collect 10 mL of mobile phase from the pump into a 10 mL volumetric flask. The flow rate was calculated by taking the ratio of the volume of the mobile phase collected and the corresponding time. The trueness of the mobile phase flow rate was determined by calculating the relative error of the obtained volumetric flow rate.

The detector linearity assay was carried out by injecting standard solutions of anthracene in methanol with different concentrations (2.0 mg/L, 4.0 mg/L, 8.0 mg/L, 10.0 mg/L). The injected volume of each solution was 100 µL. The response of each injection (peak-area) was plotted against the corresponding concentration. The regression coefficient of the plot was used to assess the linearity of the detector.

A mobile phase of methanol and water (80:20) with a flow rate of 2 ml/min was used and a detection wavelength of 254 nm was selected to perform the verification assays described above.

It is fundamental to measure the noise of the detector since excessive noise can reduce the sensitivity of the detector affecting the quantification of low-level analytes

afterwards. Detector drift may affect the determination of the baseline and integration of peaks, so it is necessary to measure the drift of the detector. The measurements of the detector noise and drift were performed following a procedure indicated in the operating manual of the equipment. The noise and drift assays were carried out with water flowing as mobile phase through the system at a rate of 1 ml/min. The wavelength of detection selected was 254 nm. In order to print the chromatogram that allows performing the noise and drift measurements, it is necessary to select adequate attenuation values in the integrator. The attenuation values to carry out both the noise measurement and drift measurement have been chosen in according to the suggestion given in the operating manual of the instrument. An attenuation value of -3 was chosen for noise measurement while an attenuation value of -1 was chosen for drift measurement. Noise data were acquired during 15 minutes on the other hand drift data were acquired during 20 minutes. The determination of the detector noise was performed using the mathematical expression (1) since the obtained scale in the integrator is in milivolts (mV), the noise measurement is obtained in millimeters (mm) and the detector noise level is expressed in absorbance units (AU).

$$\text{Noise}(\text{AU}) = \frac{\text{A}}{\text{B}} \times \frac{2\text{AU}}{1000} \times 2^{\text{C}} \qquad (1)$$

Where:

A = noise measurement in millimeters (mm)

B = complete voltage scale measured in millimeters (mm). For Integrators 3394/6 the complete scale is 150 mm.

C= attenuation (-3)

The term **A**, in the expression, was obtained by dividing the chromatographic baseline into 15 segments, each of 1 min- interval. The biggest vertical distance (Y-value) peak-to-peak, in each segment, was measured using a graduated ruler. This distance was obtained by drawing parallel lines between the highest and lowest data point (see figure 7, as example of this measurement**)**. The average value was calculated from the Y-values and is termed as A. The noise value is expressed in absorbance units (AU).

Figure 7: Noise measurement

The determination of the detector drift was determined using the following mathematical expression:

$$\text{Drift (AU/ 20 min)} = \frac{A}{B} \times \frac{2AU}{1000} \times 2^{C} \qquad (2)$$

Where:

A = drift measurement in millimeters (mm)

B = complete voltage scale measured in millimeters (mm). For Integrators 3394/6 the complete scale is 150 mm.

C= attenuation (-1)

The term **A** was obtained by measuring the difference in the response between the end and the beginning of the chromatogram using a graduated ruler. This difference was measured by drawing a straight line (see Figure 8, as example of this measurement). The slope of the straight line gives the drift of the detector. The drift value calculated from the mathematical expression (2) was converted into absorbance units per hour (AU/h)

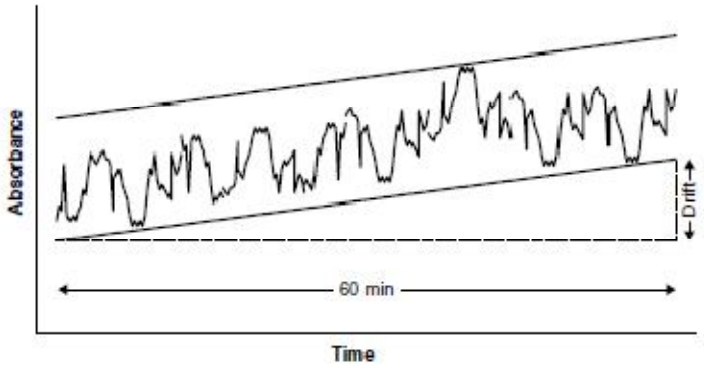


Figure 8: Drift measurement

For gradient accuracy, the ability of the pump to deliver the mobile phase at different solvent strengths over the time by varying the composition of the mobile phase accurately is fundamental to get the adequate chromatographic separation and reproducibility. The gradient accuracy assay was performed using two different mobile phases (a mobile phase of methanol and water (80:20) and a mobile phase containing 0.5 % of acetone in methanol/water). The channel A contained the mobile phase of acetone in methanol/water and the channel B contained the mobile phase of methanol and water. The mobile phase of acetone in methanol /water was used as UV tracer. A gradient profile was set, in the chromatograph, in order to change (in a stepwise way)

the composition of the mixture from 100% B to 100 % A and then changed back to 100 % B. The following was done to carry out to determine the gradient accuracy:

The absorbance change from 100 % B (baseline) to 100 A % was measured and expressed as height, *H*, in the plot of absorbance versus time. The height, *H*, was measured (using a graduated ruler, in centimeters) from the baseline until the maximum value of the heights in the obtained chromatogram (see Figure 9).

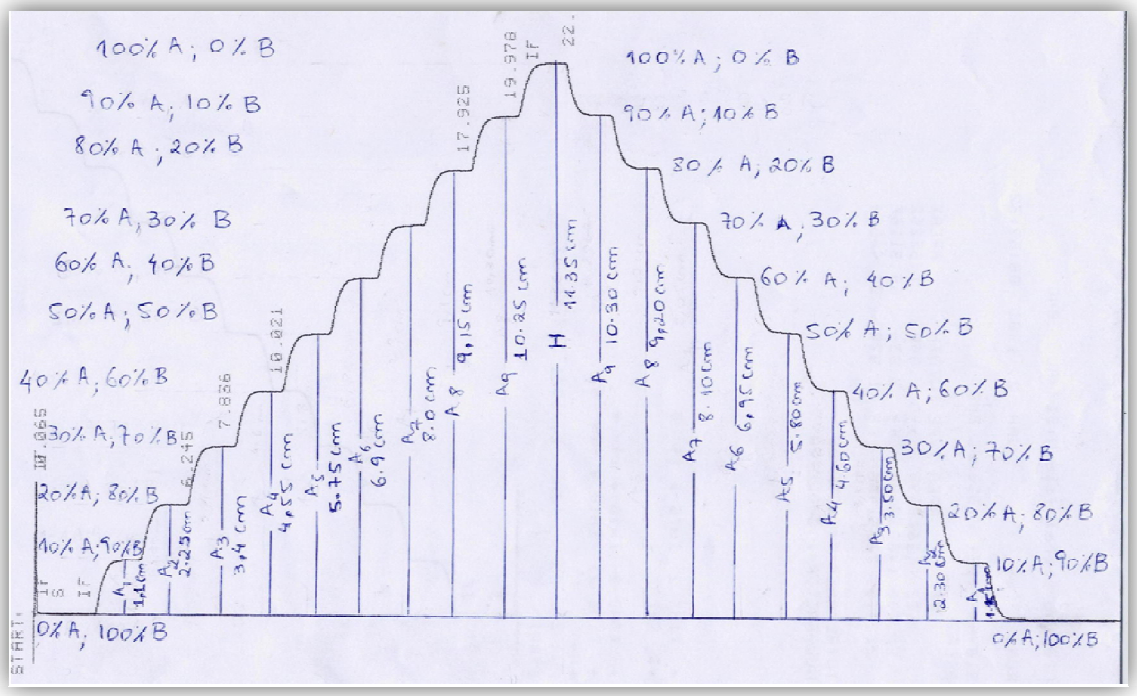


Figure 9: Gradient accuracy measurement performed for the HP 1050

Afterwards, the height of the gradient step in which the mobile phase composition was 90% A and 10% B was measured and the gradient composition was calculated using the following expression:

$$\text{Gradient composition (\%)} = \frac{\text{height of the gradient step (cm)}}{\text{H (cm)}} \times 100$$

Where:

H is the height when the composition of the mixture has 100% A and 0 % B.

The absolute error and the relative error were calculated and then the gradient accuracy was expressed as the relative error.

The gradient composition, the absolute error and relative error were determined for the remaining gradient steps in order to arrive at the corresponding gradient accuracy.

All the calculations necessary for the verification assays of the Liquid Chromatograph 1050 were processed using an excel sheet prepared for this purpose, a file template named as "Liquid Chromatograph HP 1050 Verification Form", which can be found in Annex II, in the SOP coded as **PNT/QA-D/EQP/055/01**, as it can be seen in Figure 10.

Annex 1: Liquid chromatograph HP 1050 verification form

Procedure:	PNT/QA-D/EQP/055/01: Instructions for verification of the liquid chromatograph HP 1050
Assay:	Injector precision
Date:	29-09-2009

Replicate	Area
1	12536856
2	12522272
3	12508896
4	12513328
5	12502840
Average value	12516838
Standard deviation, *s*	13244
Relative standard deviation , s_r	0,001
Coefficient of variation, *CV* (%)	0,1
Accepted/Not Accepted precision	**Accepted**

Acceptance/Refusal Criteria	
CV (%) ≤	1

Figure 10: Excel sheet for the calculations in the verification assays of the HP 1050

Performance Verification of the Liquid Chromatograph Agilent 1100 with UV/VIS detector

An SOP containing instructions for the verification of the liquid chromatograph Agilent 1100 with UV/VIS detector was written. This document also includes the periodicities for the verification assays. This SOP was named as "Instructions for the verification of the liquid chromatograph with UV/VIS detector (Agilent 1100)", coded as **PNT 0351000 APR/172 ED. 1** and it is included in Annex II.

The verification of this liquid chromatograph included the following assays: injector precision, flow rate precision, injector linearity and carryover, detector linearity, noise and drift, flow rate trueness and gradient accuracy.

In the verification of this instrument the same methodology as used for the liquid chromatograph HP 1050 was utilized for the injector precision, flow rate precision, detector linearity and flow rate trueness assays except that a flow rate of 1.0 ml/min instead of 2.0 ml/min was employed.

This equipment has an automatic injector which has the ability of varying the injection volume. Different volumes of sample are drawn into a sample injection loop

by a syringe. The uniformity of the sample loop and the ability of the syringe to draw different amounts of sample in the right proportion influence the linearity of the injection volume. The injector linearity is important when performing a method in which the use of variable injection volume is a requirement. The linearity of the injector was tested by making injections of different volumes, 10, 30, 50, 80, and 100 µL of an anthracene standard solution with 8.0 mg/L of concentration. The response of each injection (peak-area) was plotted against the corresponding injection volume and the regression coefficient of the plot was used to assess the injector linearity.

Small amounts of analyte may get carried over from the sample injected before and lead to the contamination of the next sample to be injected. The carryover will affect the accuracy of the quantification of the next sample. The carryover assay was carried out by injecting a blank (methanol) after an anthracene standard solution (solution of anthracene dissolved in methanol) with a concentration of 8 mg/L. The level of carryover was determined by calculating the ratio between the responses (peaks-area) of the anthracene found in the methanol sample and the anthracene standard solution.

The detector noise and drift assays were done by making a run with the mobile phase of methanol and water flowing through the system at 1.0 mL/min during 20 minutes. After getting the noise registered, a short-term noise measurement was performed. For this measurement, the chromatographic baseline was divided into 20 segments, each of 1 minute interval. Parallel lines were drawn for each segment to enclose the peak-to-peak variation in signals (see Figure 11, as example of this measurement). The vertical distance (Y-value) between the parallel lines in each segment was measured. The Y-values was divided by the number of segments in the measurement. The obtained value is the noise level of the detector and was expressed in absorbance units (AU).

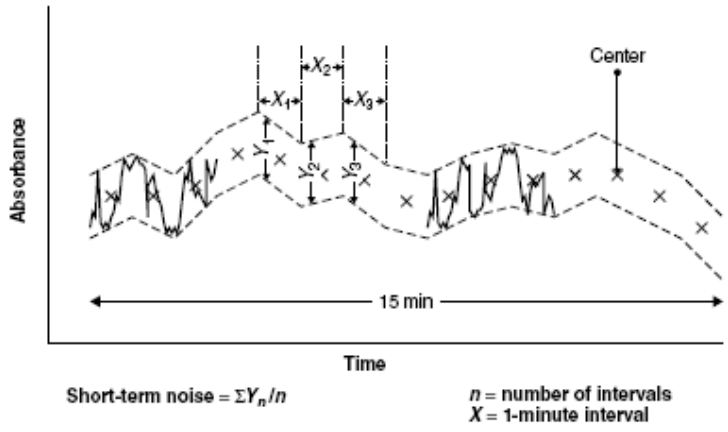


Figure 11: Noise measurement for the Agilent 1100

The drift (the trend of the signal) measurement was performed by measuring the difference in the response between the end and the beginning of the chromatogram, using a graduated ruler. This difference was measured by drawing a straight line (see Figure 8). The slope of the straight line gives the drift of the detector. This obtained slope is in centimetres and then was converted to AU/h using the obtained scale in units of absorbance.

The gradient accuracy assay was performed using the methodology applied to the gradient accuracy assay in the Liquid Chromatograph HP 1050, although a different measurement method was used to determine it. Half of the chromatogram ("half of the pyramid") was integrated in order to obtain the area of halves of the chromatogram (see Figure 12).

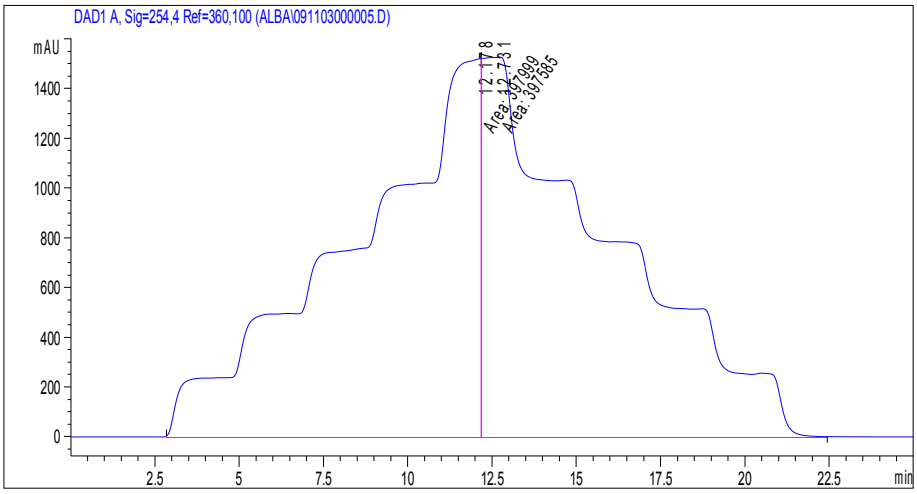


Figure 12: Gradient accuracy measurement for the Agilent 1100

The gradient accuracy was calculated by taking the quotient of the difference of the two areas of the pyramid by the average of their areas. The gradient accuracy value was expressed in percentage.

All the calculations necessary for the verification assays of this liquid chromatograph were carried out using an excel sheet prepared for this purpose and saved in a file template called "Liquid chromatograph Agilent 1100 verification form", (see Figure 13) which can be found in Annex II in the SOP coded as **PNT 0351000 APR/172 ED.**

Annex 1: Liquid chromatograph Agilent 1100 verification form

Procedure:	PNT 0351000 APR/172 ED. 1: Instructions for verification of liquid chromatograph with UV/VIS detector (Agilent 1100)
Assay:	Injector Precision
Date:	30-10-2009

Replicate	Peak Area
1	6704,33
2	6732,08
3	6752,91
4	6726,20
5	6758,04
Average value	6734,71
Standard deviation, *s*	21,67
Relative standard deviation , s_r	0,003
Coefficient of variation, *CV* (%)	0,3
Accepted/Not Accepted precision	Accepted

Acceptance/Refusal Criteria	
CV (%)≤	1

Figure 13: Excel sheet for the calculations in the verification assays of the Agilent 1100

3.3. Liquid Chromatography Maintenance

A SOP containing the instructions for the cleaning and maintenance of the Liquid Chromatograph HP 1050 was written. This document was named as "Cleaning and Maintenance Instructions for the Liquid Chromatograph with UV/VIS Detector (HP1050) and coded as **PNT/QA-D/EQP/054/01**. This document was prepared based from the reference manual of the equipment. The maintenance and cleaning activities for this equipment include the following: cleaning of the tubes of the system, cleaning of the solvent inlet filters and helium sparging frits, changing the PTFE frit and the cleaning of the lower part pieces of the purge valve (ball and seat), cleaning and changing of the different parts of the pump, cleaning of the needle port manual injection valve, reforming of the needle seal of the manual injection valve, changing of the deuterium lamp and cleaning of the detector cell. All the procedures to carry out the maintenance activities mentioned above are described in the SOP coded as **PNT/QA-D/EQP/054/01** which is included in Annex II. The periodicities of maintenance are included in this document.

3.4. Performance Verification of Gas Chromatography

A gas chromatograph with thermal conductivity detector (TCD) and another one with flame ionization detector (FID) were verified. For each gas chromatograph was written a SOP containing the instructions for its verification. The SOP for the gas chromatograph with TCD was named as "Instructions for Verification of the Gas Chromatograph with Thermal Conductivity Detector (TCD) – HP 5890 Series II",

coded as **PNT/QA-D/EQP/057/01** and can be found in Annex III. The SOP for the gas chromatograph with FID was named as "Instructions for Verification of the Gas Chromatograph with Flame Ionization Detector (FID) – HP 5890 Series II", coded as **PNT/QA-D/EQP/058/01** and can be found in Annex III.

The verification of the gas chromatographs was carried out by checking the main functions of the modules which make part of the system. For both instruments the following verification assays were performed: flow rate precision, detector linearity, noise and drift, oven temperature precision, trueness, linearity and stability.

The flow rate precision assay was carried out by performing five replicate injections of an aqueous ethanol solution with a concentration of 31.8 mg/L. The injected volume was 0.2 µL.

The detector noise and drift assays were carried out by making a run without injecting any substance. The noise and drift data were acquired during 20 minutes. An attenuation value of -3 was chosen, in the integrator, since it allows to print the chromatogram with a scale which is suitable to perform the noise and drift measurements. For the gas chromatograph with TCD, the determination of the detector noise was performed using the mathematical expression (3) since the noise measurement is done in millimeters, the scale of integrator is obtained in milivolts and the detector noise level is expressed in volts (V)

$$\text{Noise}(\text{V}) = \frac{\text{A}}{\text{B}} \times \frac{1\,\text{V}}{1000} \times 2^{\text{C}} \qquad (3)$$

Where:

A = noise measurement in millimeters (mm)

B = complete voltage scale measured in millimeters (mm). For Integrators 3394/6 the complete scale is 150 mm.

C= attenuation (-3)

The term A, in the expression, was obtained by dividing the chromatographic baseline into 20 segments (each of 1 min- interval) since the measurement time was 20 minutes. The methodology used to carry out the noise measurement is described above in the noise measurement for the Liquid Chromatograph HP 1050 (see Figure 7, as example of this measurement).

The drift calculation was done by using the mathematical expression (3) applied to the noise calculation. Here the term A is the drift measurement in millimeters. This term was obtained by measuring the difference between the beginning and the end of the

chromatogram (see Figure 8, as example of this measurement). The drift level was expressed in volts per hour (V/h).

For the gas chromatograph with FID, the determination of the detector noise was performed using the mathematical expression (4) since the noise measurement is done in millimeters, the scale of integrator is obtained in milivolts and the detector noise level is expressed in amperes (A). Considering the internal resistance of the instrument, 1 mV corresponds to an electric current of 1×10^{-12} A.

$$\text{Noise(A)} = \frac{\text{A}}{\text{B}} \times 1 \times 10^{-12}\,\text{A} \times 2^{\text{C}} \qquad (4)$$

Where:

A = noise measurement in millimeters (mm)

B = complete voltage scale measured in millimeters (mm). For Integrators 3394/6 the complete scale is 150 mm.

C= attenuation (-3)

The drift determination was done by using the mathematical expression (4) but now term A is the drift measurement. This term was obtained by measuring the difference between the beginning and the end of the chromatogram (see Figure 8, as example of this measurement). The drift level was expressed in amperes per hour (A/h).

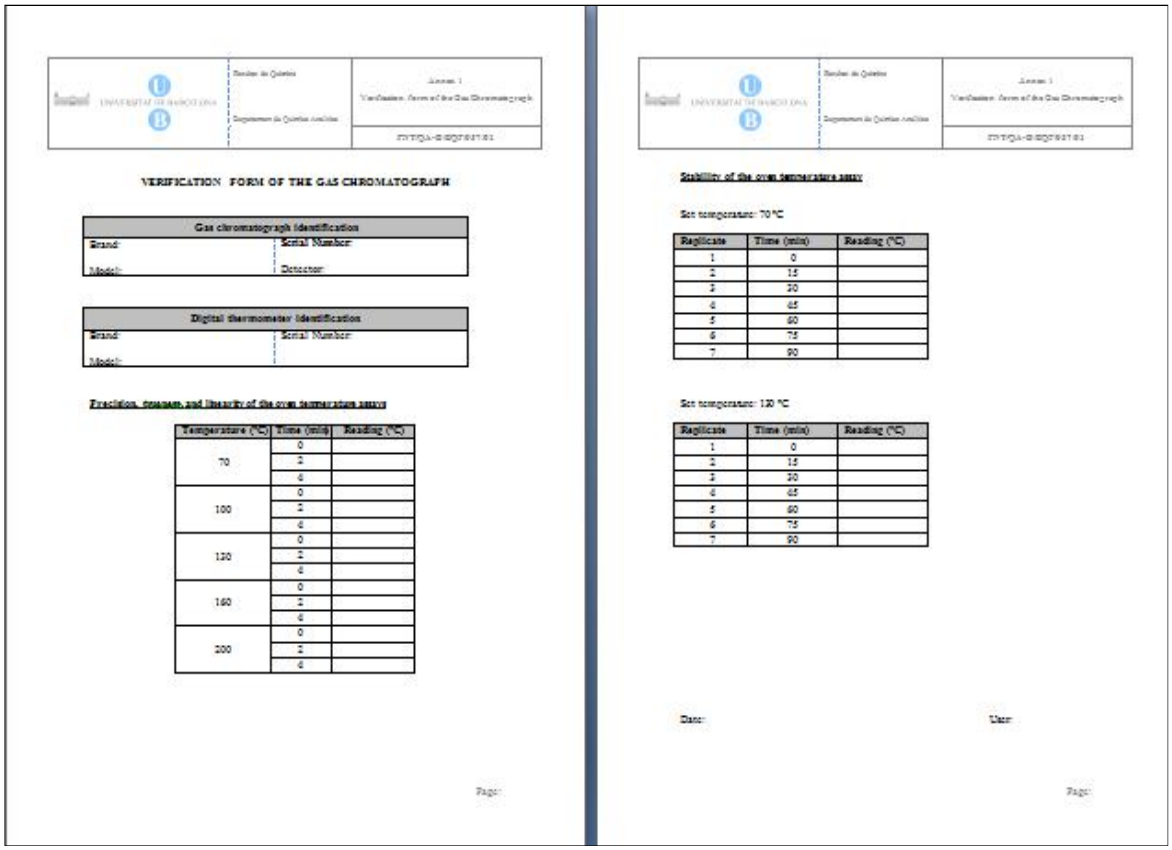
The detector linearity assay was performed by injecting standards solutions of butanol in ethanol with different concentrations (18.2 mg/L, 36.4 mg/L, 45.0 mg/L, 53.4 mg/L and 61.6 mg/L). The injected volume was 1µL.

The precision, trueness and linearity of the oven temperature were evaluated by placing a calibrated digital thermometer (Brand: TESTO, Model: 945, identification 00543843/105(168543)) in the column compartment to measure the temperature. This thermometer has been calibrated between the temperatures of 0ºC and 200ºC in agreement with the internal procedure ITC-306 (the calibration certificate of the thermometer can be found in Annex III). Working temperatures were preset and three readings of each temperature were done. The first reading was performed when the temperature value got stable in the digital thermometer and the remaining readings were done every 2 minutes. The precision of the oven temperature was determined by calculating the average value, the relative standard deviation and the coefficient of variation and was expressed through the coefficient of variation. The trueness of the oven temperature was determined by calculating the average value and the absolute error and it was assessed through the absolute error.

With respect to the linearity of the oven temperature, this parameter has been assessed by plotting the experimental temperature against the set temperature. The regression coefficient of the plot allows concluding if the change of the oven temperature is linear when temperature changes are preset.

The stability of the oven temperature was evaluated by placing the calibrated thermometer in the column compartment and programming the thermometer in order to perform automatically 6 readings every 15 minutes. The preset temperatures were 70º C and 130ºC which are the minimum and maximum working temperatures for these instruments, respectively. The stability of the oven temperature was evaluated by calculating the difference between the maximum and the minimum temperature during the measurement time.

The obtained results from trueness, precision, linearity and stability of the oven temperature assays were registered in the form named as "Verification form of the gas chromatograph" (see Figure 14) which can be found in Annex III in SOPs coded as **PNT/QA-D/EQP/057/01** and **PNT/QA-D/EQP/058/01**.



VERIFICATION FORM OF THE GAS CHROMATOGRAPH

Gas chromatograph identification	
Brand:	Serial Number:
Model:	Detector:

Digital thermometer identification	
Brand:	Serial Number:
Model:	

Precision, exactness and linearity of the oven temperature assays

Temperature (ºC)	Time (min)	Reading (ºC)
70	0	
	2	
	4	
100	0	
	2	
	4	
130	0	
	2	
	4	
160	0	
	2	
	4	
200	0	
	2	
	4	

Page:

Stability of the oven temperature assay

Set temperature: 70 ºC

Replicate	Time (min)	Reading (ºC)
1	0	
2	15	
3	30	
4	45	
5	60	
6	75	
7	90	

Set temperature: 130 ºC

Replicate	Time (min)	Reading (ºC)
1	0	
2	15	
3	30	
4	45	
5	60	
6	75	
7	90	

Date: User:

Page:

Figure 14: Verification form for the gas chromatographs

The calculations necessary for the verification assays of the gas chromatographs were carried out in excel sheets prepared for this purpose. The excel sheet for the gas chromatograph with TCD was named as "Verification form of the gas chromatograph with TCD" (see Figure 15) and can be found in Annex III in the SOP coded as **PNT/QA-D/EQP/057/01**. The excel sheet for the gas chromatograph with FID was named as "Verification form of the gas chromatograph with FID" (see Figure 16) and can be found in Annex III in the SOP coded as **PNT/QA-D/EQP/058/01**.

Annex 2: Verification form of the gas chromatograph with TCD

Procedure:	PNT/QA-D/EQP/057/01: Instructions for verification of the gas chromatograph with thermal conductivity detector (TCD) - HP 5890 SERIES II
Assay:	Flow rate precision
Date:	06-11-2009

Replicate	Retention time (min)
1	2,120
2	2,130
3	2,129
4	2,123
5	2,122
Average value (min)	2,125
***s* (min)**	0,004
s_r	0,002
CV (%)	0,2
Accepted/Not accepted	**Accepted**

Acceptance / Refusal Criteria	
CV(%) ≤	1

Figure 15: Excel sheet for the calculations in the verification assays of the GC with TCD

Annex 2: Verification form of the gas chromatograph with FID

Procedure:	PNT/QA-D/EQP/058/01: Instructions for verification of the gas chromatograph with flame ionization detector (FID) - HP 5890 Series II
Assay:	Flow rate precision
Date:	16-11-2009

Replicate	Retention time (min)
1	1,492
2	1,492
3	1,495
4	1,495
5	1,485
Average value (min)	1,492
***s* (min)**	0,004
s_r	0,003
***CV* (%)**	0,3
Accepted/Not accepted	**Accepted**

Acceptance / Refusal Criteria	
CV (%) ≤	1

Figure 16: Excel sheet for calculations in the verification assays of the GC with FID

3.5. Gas Chromatography Maintenance

A SOP containing the instructions for the cleaning and maintenance of the Gas Chromatographs 5890 Series II either with FID or TCD was written. This SOP was named as "Cleaning and Maintenance Instructions for the Gas Chromatograph HP

5890", coded as **PNT/QA-D/EQP/056/01** and can be found in Annex III. This document was prepared based from the reference manual of the equipment.

The maintenance and cleaning of these gas chromatographs includes the injector and the detector modules and the chromatographic column.

The maintenance of the injector module includes the changing of the septum, the checking of the leaks and the liner and/ or insert care for packed column inlet and split/splitless capillary inlets.

The maintenance of the chromatographic column includes the conditioning of the column. The detector maintenance includes the changing of ON/OFF and needle valves for the gas chromatograph with TCD and FID. Besides the maintenance operations described previously, the chromatograph with FID also can need the jet exchange/replacement.

The SOP for the maintenance of the gas chromatographs includes the frequency that the assays should be performed.

4. Results and Discussion

4.1. Performance Verification of Balances and Analytical Balances

Acceptance/Refusal criteria for trueness, repeatability and drift were adopted in order to evaluate the condition of the balances and analytical balances verified. Tables 2 and 3 show the acceptance/refusal criteria for the analytical balances and balances, respectively. These criteria were obtained from the certificates of calibration of the analytical balances and balances.

Acceptance / Refusal Criteria for Analytical Balances	
Trueness (*RE*), %	0.01
Repeatability (*CV*), %	0.0001
Drift (*D*), g	0.001

Table 2: Acceptance/ refusal criteria for analytical balances

Acceptance / Refusal Criteria for Balances	
Trueness (*RE*), %	0.2
Repeatability (*CV),* %	0.01
Drift (*D*), g	0.02

Table 3: Acceptance/ refusal criteria for balances

Table 4 and 5 show the primary data registered in the form "Verification form of the balances" for the trueness, repeatability and drift assays on 17/09/09, as example, for an analytical balance (Mettler Toledo AB204) as mentioned in section 3.1 of the Experimental Part.

Replicate	**Reading (g)**
1	100.0000
2	99.9998
3	99.9999
4	99.9998
5	99.9998

Table 4: Primary data obtained from the trueness and repeatability assays on 17/09/09 for an analytical balance.

Time (min)	**Reading (g)**
t = 0	99.9998
t = 5	99.9998
t = 10	99.9998

Table 5: Primary data obtained from the drift assay on 17/09/09 for an analytical balance.

Tables 6 and 7 provide the obtained results shown in tables 4 and 5 but now registered in the excel sheet and the results obtained from the calculations carried out in the trueness, repeatability and drift assays for an analytical balance.

Date:	**17-09-2009**
Replicate	Reading (g)
1	100.0000
2	99.9998
3	99.9999
4	99.9998
5	99.9998
Average (g)	99.9999
Absolute Error, *AE* (g)	0.0048
Relative Error, *RE* (%)	0.0048
Accepted/ Not accepted trueness	Accepted
Standard Deviation, *s* (g)	8.944E-05
Coefficient of Variation, *CV* (%)	8.944E-05
Accepted/ Not accepted repeatability	Accepted
Tolerance (g)	1.111E-04
Confidence interval (g)	99.9999 ± 0.0002

Table 6: Obtained results and calculations registered in the excel sheet for the trueness and repeatability assays of an analytical balance.

Date:	**17-09-2009**
Time (min)	Reading (g)
t = 0	99.9998
t =5	99.9998
t = 10	99.9998
Drift (g)	0.0000
Accepted/ Not accepted drift	Accepted

Table 7: Obtained results and calculations registered in the excel sheet for the drift assay of an analytical balance.

Table 8 shows the final results obtained from the calculations carried out in the trueness (relative error, in second column), repeatability (coefficient of variation, in fourth column) and drift (drift value, in the sixth column) assays for the analytical balance AB204. This table also shows the assessment result (accepted/not accepted) for each verification assay. Column 3, 5 and 7 reveals the assessment result for the trueness, repeatability and drift assays, respectively. Similarly, Tables 9, 10, 11 and 12 show the final results obtained from the verification assays of the other analytical balances and one balance.

a) Analytical Balance Mettler Toledo AB204

Date	Relative Error (%)	Accepted/Not Accepted Trueness	Coefficient of Variation (%)	Accepted/ Not Accepted Repeatability	Drift (g)	Accepted/Not Accepted Drift
17/09/2009	0.0052	Accepted	7.071E-05	Accepted	0.0000	Accepted
21/09/2009	0.0050	Accepted	4.472E-05	Accepted	0.0006	Accepted
29/09/2009	0.0059	Accepted	4.472E-05	Accepted	0.0002	Accepted
05/10/2009	0.0053	Accepted	1.225E-04	Accepted	0.0006	Accepted
13/10/2009	0.0050	Accepted	8.944E-05	Accepted	0.0003	Accepted
19/10/2009	0.0115	Accepted	3.768E-04	Not Accepted	0.0003	Accepted
22/10/2009	0.0095	Accepted	5.477E-05	Accepted	0.0000	Accepted
27/10/2009	0.0068	Accepted	5.477E-05	Accepted	0.0000	Accepted
02/11/2009	0.0059	Accepted	0.0000	Accepted	0.0002	Accepted
09/11/2009	0.0118	Accepted	8.366E-05	Accepted	0.0003	Accepted
16/11/2009	0.0093	Accepted	4.472E-05	Accepted	0.0002	Accepted
23/11/2009	0.0091	Accepted	4.472E-05	Accepted	0.0001	Accepted
30/11/2009	0.0113	Accepted	4.472E-05	Accepted	0.0000	Accepted
09/12/2009	0.0109	Accepted	4.472E-05	Accepted	0.0002	Accepted
14/12/2009	0.0128	Accepted	4.472E-05	Accepted	0.0001	Accepted
18/01/2010	0.0105	Accepted	8.367E-05	Accepted	0.0001	Accepted
25/01/2010	0.0104	Accepted	8.367E-05	Accepted	0.0000	Accepted

Table 8: Final results obtained from the verification assays of the analytical balance AB204.

Analysing the results presented in Table 8 for the trueness of the balance analytical balance AB204, it can be observed all the obtained results are acceptable in agreement with the acceptance criteria. This means that this balance will generate accurate results when used since values of mass in the vicinity of the true value were obtained.

With respect to the precision (repeatability) of this balance, it can be observed that this performance parameter was unacceptable for one measurement (19/10/2009) out of 16 measurements made. However, analysing the overall results it can be concluded that this balance can give precise results when successive measurements of the same substance under the same condition is done.

When it comes to the drift parameter which values are shown in table 8 (column 6), it can be observed that this performance attribute was always accepted for any verification. This analytical balance provides mass indications, for a sample or any object, which did not change significantly during the course of the weighing activity. It means that the weight of the sample, even if it was left onto the pan of analytical balance for some period of time, did not change continuously.

Analysing the global condition of the analytical balance AB204, it can be concluded that its working condition is good and accordingly this equipment suitable for its use.

b) Analytical Balance Mettler Toledo AG245

Date	Relative Error (%)	Accepted/Not Accepted Trueness	Coefficient of Variation (%)	Accepted/ Not Accepted Repeatability	Drift (g)	Accepted/Not Accepted Drift
17/09/2009	0.0048	Accepted	8.944E-05	Accepted	0.0000	Accepted
21/09/2009	0.0047	Accepted	5.477E-05	Accepted	0.0001	Accepted
29/09/2009	0.0048	Accepted	5.477E-05	Accepted	0.0001	Accepted
05/10/2009	0.0048	Accepted	8.367E-05	Accepted	0.0004	Accepted
13/10/2009	0.0048	Accepted	5.477E-05	Accepted	0.0000	Accepted
19/10/2009	0.0040	Accepted	0.000E+00	Accepted	0.0002	Accepted
27/10/2009	0.0048	Accepted	0.000E+00	Accepted	0.0001	Accepted
02/11/2009	0.0048	Accepted	5.477E-05	Accepted	0.0000	Accepted
09/11/2009	0.0047	Accepted	0.000E+00	Accepted	0.0001	Accepted
16/11/2009	0.0047	Accepted	0.000E+00	Accepted	0.0001	Accepted
23/11/2009	0.0047	Accepted	5.477E-05	Accepted	0.0001	Accepted
30/11/2009	0.0048	Accepted	5.477E-05	Accepted	0.0000	Accepted
09/12/2009	0.0047	Accepted	5.477E-05	Accepted	0.0000	Accepted
14/12/2009	0.0047	Accepted	5.477E-05	Accepted	0.0000	Accepted
18/01/2010	0.0047	Accepted	5,477E-05	Accepted	0.0001	Accepted
25/01/2010	0.0048	Accepted	8,944E-05	Accepted	0.0001	Accepted

Table 9: Final results obtained from the verification assays of the analytical balance AG245.

Analysing the results shown in Table 9 obtained from the trueness assay (column 2) of the analytical balance AG245, it can be observed that all results are acceptable considering the acceptance criteria. It means that this balance provides trustful indications of the mass of an object and thus the user can be sure that the obtained measurement results are true. Considering the results for the repeatability (column 4), it can be noted that the variation between the replicate measurements is not meaningful in according with the acceptance criteria. This means that there is an agreement among the measurements when a user carries out weighing of multiple replicates of a sample. Observing the drift values obtained (column 6), it can be said that all values are also acceptable with regards to the acceptance criteria. There are even some days that this analytical balance provided a null drift value. Analysing the overall state of this analytical balance, it can be said that this equipment is in good working conditions and can be used with confidence.

c) Analytical Balance Scaltec SBA 32

Date	Relative Error (%)	Accepted/Not Accepted Trueness	Coefficient of Variation (%)	Accepted/ Not Accepted Repeatability	Drift (g)	Accepted/Not Accepted Drift
17/09/2009	0.0040	Accepted	2.793E-04	Not Accepted	0.0004	Accepted
21/09/2009	0.0037	Accepted	8.944E-05	Accepted	0.0003	Accepted
29/09/2009	0.0036	Accepted	7.981E-04	Not Accepted	0.0005	Accepted
05/10/2009	0.0046	Accepted	7.071E-05	Accepted	0.0011	Accepted
13/10/2009	0.0045	Accepted	6.301E-04	Not Accepted	0.0005	Accepted
19/10/2009	0.0032	Accepted	2.588E-04	Not Accepted	0.0010	Accepted
27/10/2009	0.0040	Accepted	1.095E-04	Accepted	0.0005	Accepted

Table 10: Final results obtained from the verification assays of the analytical balance SBA 32

Analysing the data in Table 10 obtained from the verification of the analytical balance SBA 32, it can be observed that the amount of measurement results is reduced. This is because the equipment broke down consequently not so many observations were made. On November 3 this equipment stopped working and then it was not possible to continue carrying out its verification. Afterwards, the responsible person by this equipment was contacted and technical service to repair the analytical balance was requested.

Analysing the results obtained for the trueness assay (columns 2 and 3), it can be observed that there were no problems since the relative error is always acceptable considering the acceptance criteria. In respect to the precision (columns 4 and 5), this analytical balance was not in good working condition since only three results were accepted. Regarding the drift values (columns 6 and 7), it is observed that all the obtained results were accepted.

Taking into consideration all the performance attributes verified, it can concluded that the analytical balance SBA 32 was not in good working conditions and therefore was not suitable for operation.

d) Analytical Balance AND GR200

Date	Relative Error (%)	Accepted/Not Accepted Trueness	Coefficient of Variation (%)	Accepted/ Not Accepted Repeatability	Drift (g)	Accepted/Not Accepted Drift
17/09/2009	0.0042	Accepted	5.477E-05	Accepted	0.0000	Accepted
21/09/2009	0.0041	Accepted	1.140E-04	Accepted	0.0001	Accepted
29/09/2009	0.0041	Accepted	8.367E-05	Accepted	0.0004	Accepted
05/10/2009	0.0044	Accepted	8.944E-05	Accepted	0.0002	Accepted
13/10/2009	0.0043	Accepted	3.647E-04	Not Accepted	0.0006	Accepted
19/10/2009	0.0037	Accepted	7.071E-05	Accepted	0.0006	Accepted
27/10/2009	0.0043	Accepted	7.071E-05	Accepted	0.0001	Accepted
03/11/2009	0.0041	Accepted	5.477E-05	Accepted	0.0001	Accepted
09/11/2009	0.0042	Accepted	8.367E-05	Accepted	0.0004	Accepted
16/11/2009	0.0041	Accepted	4.472E-05	Accepted	0.0001	Accepted
23/11/2009	0.0043	Accepted	5.477E-05	Accepted	0.0001	Accepted
30/11/2009	0.0043	Accepted	8.944E-05	Accepted	0.0002	Accepted
09/12/2009	0.0043	Accepted	7.071E-05	Accepted	0.0001	Accepted
14/12/2009	0.0046	Accepted	5.477E-05	Accepted	0.0001	Accepted
18/01/2010	0.0043	Accepted	4.472E-05	Accepted	0.0000	Accepted
25/01/2010	0.0044	Accepted	5.477E-05	Accepted	0.0001	Accepted

Table 11: Final results obtained from the verification of the analytical balance GR200

Looking at the results for the trueness assay (columns 2 and 3) in Table 11, it can be seen that the analytical balance GR200 generates measurement results which show a level of agreement with the true value of standard mass weighed taking into account the acceptance criteria adopted for this performance attribute. With respect to the results of the repeatability assay (columns 4 and 5), it can be observed that the result obtained on October 13 was rejected. Following this date, all the results are acceptable considering the acceptance criteria. Since October 13 all the coefficients of variation are acceptable so it cannot be said that the balance will not give precise measurements knowing that October 13 gave the opposite. Concerning the drift values (columns 6 and 7), it can be observed that this analytical balance doesn't show relevant drift since all the drift values are accepted considering the acceptance criteria. In global terms, the working condition of the analytical balance GR 200 is satisfactory and accordingly this equipment is suitable for use.

e) Balance Sartorius LP 2200P

Date	Relative Error (%)	Accepted/Not Accepted Trueness	Coefficient of Variation (%)	Accepted/ Not Accepted Repeatability	Drift (g)	Accepted/Not Accepted Drift
19/10/2009	0.007	Accepted	4.472E-03	Accepted	0.01	Accepted
27/10/2009	0.005	Accepted	0.000E+00	Accepted	0.00	Accepted
02/11/2009	0.009	Accepted	5.477E-03	Accepted	0.01	Accepted
09/11/2009	0.005	Accepted	0.000E+00	Accepted	0.01	Accepted
16/11/2009	0.005	Accepted	0.000E+00	Accepted	0.00	Accepted
23/11/2009	0.005	Accepted	0.000E+00	Accepted	0.00	Accepted
30/11/2009	0.007	Accepted	4.472E-03	Accepted	0.00	Accepted
09/12/2009	0.005	Accepted	0.000E+00	Accepted	0.00	Accepted
14/12/2009	0.005	Accepted	0.000E+00	Accepted	0.00	Accepted
18/01/2010	0.005	Accepted	0.000E+00	Accepted	0.00	Accepted
25/01/2010	0.007	Accepted	4.472E-03	Accepted	0.01	Accepted

Table 12: Results obtained in the verification of the balance LP 2200

With respect to the trueness, the user can carry out measurements with confidence using this balance because the mass of the standard mass weighed here was close to the true value in agreement with the acceptance/ refusal criteria. Considering the precision of the balance (columns 4 and 5), it can be observed that this performance attribute is almost perfect since some measurements resulted to no variation among replicates as can be seen in table 12. In the same manner it can be observed that the obtained drift values (columns 6 and 7) are also acceptable being majority of them null. Analysing the global state of the balance LP 2200P, it can be concluded that its working conditions are good and consequently this equipment is suitable for operation.

4.2. Performance Verification of HPLC

Taking into account section 3.2 of the Experimental Part, the obtained results on a date for the verification assays of Liquid Chromatography will be presented, as example.

4.2.1. Liquid Chromatograph HP 1050 with UV/VIS detector

a) Injector precision assay

Table 13 shows the obtained results from the injector precision assay on 29/09/2009 and the acceptance/refusal criteria.

Replicate	Area
1	12536856
2	12522272
3	12508896
4	12513328
5	12502840
Average value	12516838
Standard deviation ,*s*	13244
Relative standard deviation, s_r	0.001
Coefficient of variation, *CV* (%)	0.1
Accepted/Not Accepted precision	Accepted

Acceptance/Refusal Criteria
$CV\ (\%) \leq 1$

Table 13: Results obtained from the injector precision assay on 29/09/2009 and the acceptance/ refusal criteria (HP 1050).

Considering Table 13 and comparing the obtained coefficient of variation and the corresponding value of the acceptance criterion, it can be observed that the precision of the injector is right. This means that the injector draws very closely the same amount of sample in replicate injections which is crucial to the precision and accuracy for peak-height or peak-area comparison of the standards and samples. The acceptance criteria for the precision of the injector should be stringent due to the importance of this performance attribute and as a result a value of equal or less than 1% was assigned taking into account the suggested specifications for the precision of the injectors in HPLC.

b) Flow rate precision assay

Table 14 shows the obtained results from the flow rate precision assay on 29 September 2009 and the acceptance/refusal criteria.

Replicate	Retention time (min)
1	4.745
2	4.740
3	4.732
4	4.727
5	4.720
Average value (min)	4.733
Standard deviation ,s (min)	0.010
Relative standard deviation, s_r	0.002
Coefficient of variation, CV (%)	0.2
Accepted/Not Accepted precision	Accepted

Acceptance/Refusal Criteria
CV (%) ≤ 1

Table 14: Results obtained from the flow rate precision assay on 29/09/09 and the acceptance/ refusal criteria (HP 1050).

An ideal pump is the one which is able to provide a wide range of flow rates while maintaining an adequate level of precision. A poor flow rate precision will affect the retention time by causing variation in the retention time of early eluting peaks. Considering Table 14, it is observed that the retention times obtained for replicate injections of the standard solution used are similar having a coefficient of variation of 0.2 %. This means that the precision of the flow rate is in good condition in agreement with the acceptance/refusal criteria defined. The value assigned for an acceptable coefficient of variation must be very small ($CV \leq 1\%$). This is necessary to maintain a consistent flow rate.

c) Flow rate trueness assay

Table 15 shows the obtained results from the flow rate trueness assay on 29 September 2009 and the acceptance/refusal criteria. The acceptance criteria values for the flow rate trueness are based on the values suggested by Herman Lam in the chapter "Performance Verification of the HPLC" of his book [12].

Replicate	Time (min)
1	9.97
2	9.82
3	9.82
4	9.87
Average value (min)	9.87
Volume collected (ml)	10.00
Obtained flow rate (ml/min)	1.014
Theoretical flow rate (mL/min)	1.000
Accepted /Not Accepted trueness	Accepted
Absolute error	0.0135
Relative error, *RE* (%)	1.35
Accepted /Not Accepted trueness	Accepted

Acceptance/Refusal Criteria
Obtained flow rate ± 2 % of the set flow rate
$RE\ (\%) \leq 2$

Table 15: Results obtained from the flow rate trueness assay on 29/09/09 and the acceptance/refusal criteria (HP 1050).

The flow rate trueness can be defined as the level of agreement between the set flow rate in the equipment and the flow rate obtained experimentally. The trueness of the flow rate is considered one of the premier requirements for the liquid chromatography. The pump should maintain an accurate flow rate in order to provide stable interactions between analytes and the stationary phase [12]. If the analysis of a certain compound is to be carried out using a certain flow rate, it is fundamental that the system provides that flow rate in order to obtain right and reliable results. Analysing Table 15, it is observed that the obtained flow rate is acceptable although the relative error is slightly high. If we take into account the time necessary to collect the predetermined volume (the volume of the volumetric flask) of the mobile phase, it can be observed that the first value is higher than the rest of the values. During the first collection the volumetric flask used was dry. This same volumetric flask was used in the subsequent collection. This means that the previous is relatively drier and so the latter will take shorter time to be fill up to the predetermined volume. Such difference of values demonstrates that it is necessary to use dry volumetric flasks to carry out this assay in the same and right conditions and accordingly to improve the obtained results.

d) Detector linearity assay

Table 16 shows the obtained results from linearity of the detector assay on 29 September 2009 and the acceptance/refusal criteria. The acceptance criteria for the linearity of the detector were defined taking into account that perfect linearity is a

hypothetical concept and equipments only can approach to the ideal linearity. This can be justified considering that practical detectors have always imperfections in mechanical and electrical devices [24].

Concentration (mg/l)	Peak area
2.05	3172766
4.10	6417162
8.20	12537928
10.25	14742608
Regression coefficient, r	0.998
Accepted/ Not Accepted linearity	Accepted

Acceptance/Refusal Criteria
$r \geq 0.99$

Table 16: Results obtained from the detector linearity assay on 29/09/2009 and the acceptance/refusal criteria (HP 1050).

The detector linearity is very important when the purpose of work is to carry out quantitative analysis. If the concentration of the analyte in the analysed samples changes the detector must produce a linear response to concentration variation within a reasonable range. The linearity of the detector is important to the accuracy for the peak area and peak height comparison between standards and samples and accordingly to the determination of analyte (s) in these samples.

Figure 17 shows the obtained graph of the peak area versus concentration of the standard solutions and the corresponding regression coefficient of the calibration curve which allows to assess the linearity of the detector.

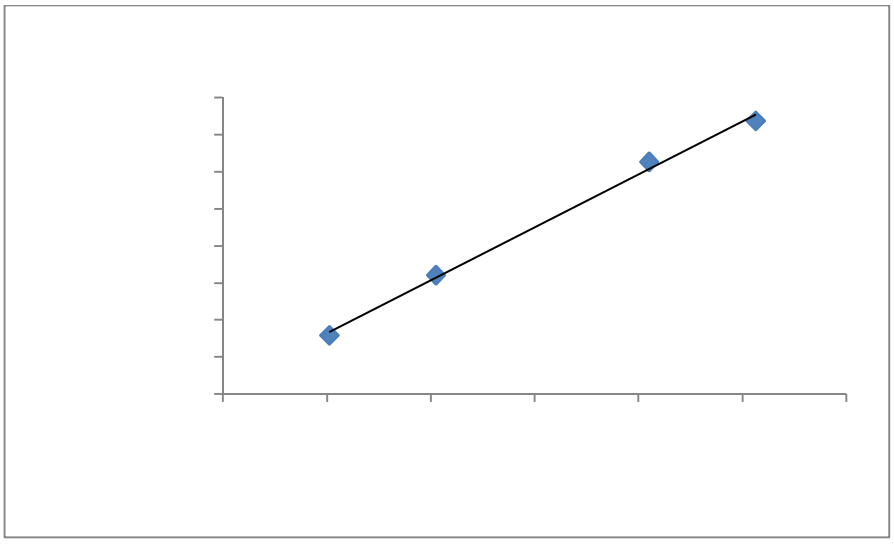


Figure 17: Detector linearity (HP 1050)

Analysing the obtained graph (Figure 17) for the detector linearity it can be observed that there is linear relationship between the instrumental response (peak area) and the concentration variation. Moreover, considering the obtained regression coefficient (r = 0.998) it can be concluded that response of the detector is linear since this value is higher than 0.99 (the minimum limit for the acceptance criteria).

As suggestion, the detector of this liquid chromatograph should be left turn on during 1 one hour before carrying out the evaluation of the detector linearity in order to ensure that the detector has warmed up and is really stable.

e) Detector noise and drift assays

For the detector noise assay is fundamental to identify the noise in the obtained chromatographic baseline and to decide how the measurement of noise should be performed taking into account the baseline. Noise can be defined as random fluctuations of the baseline over a few seconds. The analysts generally perform "peak-to-peak" noise measurement, which is the difference between the highest and the lowest data point. This method for noise measurement should be carried out by constructing parallel lines embracing the maximum excursions of the recorder trace over the measurement interval (see Figure 18) [22][25].

Figure 18: Noise measurement proposed by Hinshaw

In agreement with Dolan, the noise measurement should be performed by drawing lines roughly barely touch the extremes of the most of the noise and then by measuring vertically the distance between the lines (See Figure 19) [29].

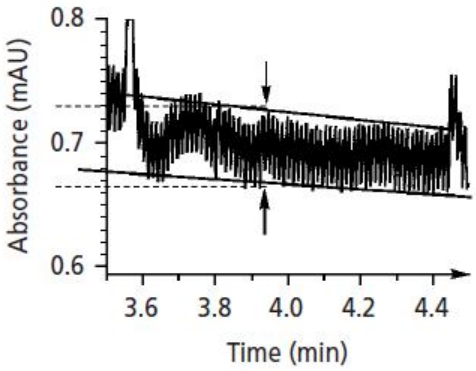


Figure 19: Noise measurement proposed by Dolan [29].

The first mentioned method, the peak-to-peak noise measurement, was used to carry out the measurement of the noise of the Liquid Chromatograph HP 1050 since this method takes into account all the fluctuations of the baseline over few seconds. Considering the chromatographic baseline and its scale, it has been decided to carry out a short- term noise measurement. Using the short-term noise measurement a bigger number of values are obtained than by using the long-time noise measurement therefore the first is more accurate. For this type of measurement, the chromatographic baseline is divided into segments of 1-min interval, since this measurement was done in 15 minutes, 15 segments were made.

Figure 20 shows the obtained chromatographic baseline from the detector noise assay and the noise measurement carried out.

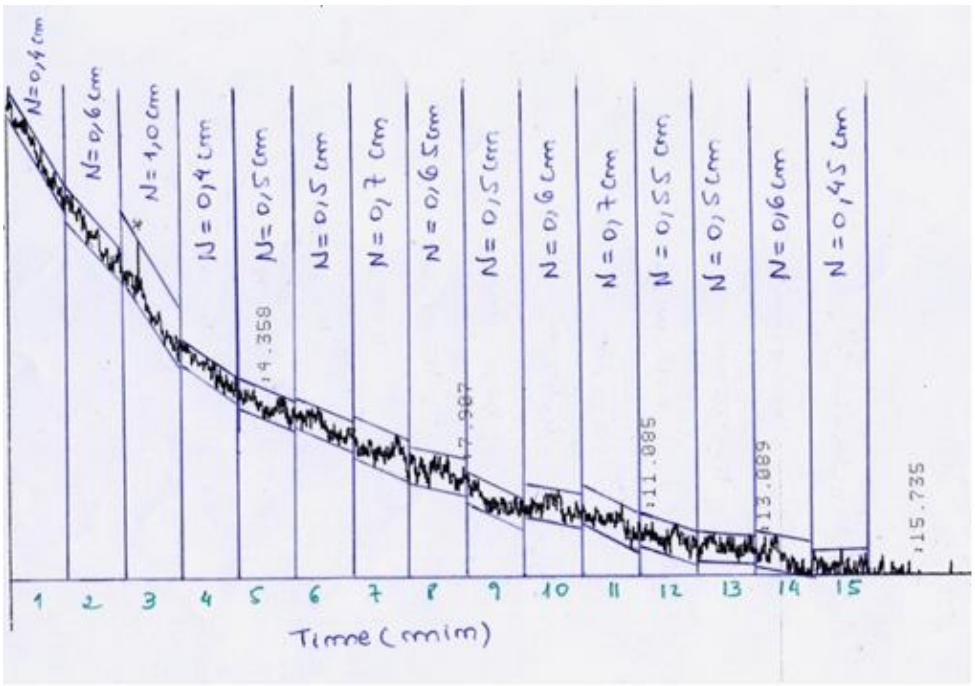


Figure 20: Noise measurement (HP 1050)

The obtained chromatographic baseline was printed in an amplified way in order to ease the analysis and then the noise measurement.

Analysing the obtained chromatographic baseline (Figure 20), it can be observed that baseline becomes more stable over time. In order to get a more stable baseline it would be good to leave the detector turn on more time (it has been left turned on during one hour) before performing the noise assay. The zero value (key, in the integrator, which sets the position of the baseline on the chart) should be increased because the baseline cannot be completely drawn longer than 15 minutes as it can be seen in Figure 20.

Table 17 shows the obtained results from the detector noise assay on 30 September 2009 and the acceptance/refusal criteria. The acceptance criteria for the evaluation of the detector noise were defined taking into account the noise level specification for liquid chromatograph HP 1050, included in the manual of this equipment, and the use given to the equipment.

Noise calculation	
A (mm)	5.8
B (mm)	150
C	-3
Noise (AU)	9.6 E-06
Accepted/Not accepted noise	Accepted

Acceptance/Refusal Criteria
Noise (AU) $\leq$ 1.5 E-5

Table 17: Results obtained in the detector noise assay on 30/09/2009 and the acceptance/ refusal criteria (HP 1050).

Analysing the obtained result for the noise level of the detector (9.6×10^{-6} AU) it can be observed that this result is acceptable considering the acceptance criteria. The chromatographic baseline and noise value evidence that the detector lamp is in good condition. When the detector lamp fails abnormal peaks are observed, which are mostly square-topped [27].

The obtained noise level can result from electronic and pump noise which is generated over the time with the age of the equipment. In addition flow cell might not be well-cleaned contributing to the noise level.

Generally the detector noise is caused by the following factors: electronic, pump and photometric noise; poor lamp intensity and a dirty flow cell. So before starting the noise assays it is strictly necessary to check the intensity and hours of use of the lamps and to make sure that the flow cell is clean and free of gas bubbles [12][28][29].

In the detector drift assay, the analysis of the obtained baseline and the measurement of the drift are the fundamental steps to estimate the drift of the detector. To analyse the obtained baseline it is essential to know what drift is. Drift can be defined as baseline fluctuations with a frequency significantly larger than that of the eluted peak [22].

Figure 21 shows the obtained baseline and the drift measurement.

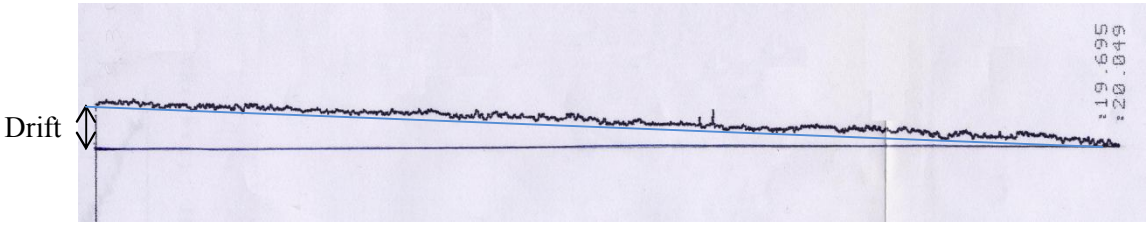


Figure 21: Drift measurement (HP 1050).

The obtained chromatographic baseline (See Figure 21) for the detector drift assay shows a downward trend of the signal over the time, which corresponds to the drift of the detector. The best way of estimating the drift is to draw a straight line following this downward trend and determine its slope. The obtained slope was 7.0 mm that is an estimation of the detector drift. The slope can only be measured using the units of length (centimetres) hence it is necessary to use a mathematical expression in order to convert these units in absorbance units per hour. This mathematical expression has been presented above, in the section 3.2 of the experimental part in the drift assay.

Table 18 shows the obtained results from the detector drift assay on 30 September 2009 and the acceptance/refusal criteria. These criteria were defined taking into account the drift specification for this liquid chromatograph, included in the manual of the equipment, and the use given to the equipment.

Drift calculation	
A (mm)	7.0
B (mm)	150
C	-1
Drift (AU/20 min)	4.67E-05
Drift (AU/min)	2.33E-06
Drift (AU/ h)	1.40E-04
Accepted/Not accepted drift	Accepted

Acceptance/Refusal Criteria
Drift (AU/h) $\leq$ 5.0 E-4

Table 18: Results obtained from the detector drift assay on 30/09/2009 and the acceptance/refusal criteria (HP 1050).

The obtained value for the drift was accepted which means that the equipment is suitable for use, although the drift value is close to the refusal criterion value. This value of drift can be caused by a not well cleaned flow cell; electronic, pump and photometric noise. The age of this liquid chromatograph is another factor which contributes to the drift in the detector since the performance of the equipment deteriorates over the time. Changes in ambient temperature, in solvent composition, or in flow rate are almost every time the responsible factors by the drift [12].

f) Gradient accuracy assay

Some analyses which are carried out, in this equipment, require the use of a gradient of mobile phases. The majority of analyses generally require only two solvents but up to four solvents can be used. In order to be sure that the results from these analyses are accurate it is fundamental to evaluate the performance of the pump to deliver mobile phase with different percentages of different solvents by changing its composition.

The chosen method for measuring the gradient accuracy is not the best accurate method since it is performed using a graduated ruler and can add also systematic human errors (due to the human eye or the way that the operator carries out the measurement). The measuring method was adopted taking into account the provided conditions.

For the gradient accuracy assay, some authors suggest to integrate half of the chromatogram ("half of the pyramid"), using the equipment software, in order to get the area of the halves of the chromatogram. Afterwards the gradient accuracy determination is done by calculating the ratio between the difference of the two halves of the pyramid area and the average value. The latest method was not applied because it was not possible to get the areas of the halves of the pyramid using the equipment software.

Table 19 shows the obtained results from the gradient accuracy assay on 21 October 2009 and the acceptance/ refusal criteria. These criteria were defined taking into account the measuring method chosen and its features (the use of a ruler to carry out the measurements) and the criteria generally suggested in recommended procedures (±2 % of the step gradient composition).

Analysing the obtained results, presented in Table 19, while increasing the percentage of mobile phase containing 0.5 % of acetone in methanol/ water, it is observed that the gradient accuracy for all gradient steps was accepted. It can be noticed that for the mixture having 90 % of B and 10 % of A the maximum limit for the

acceptance criteria was obtained. This can be probably due to the existence of air bubbles in the pump. So, before starting this assay it is extremely important to degas the mobile phases and then purge the channels in which the solvents will flow in order to avoid air bubbles in the pump system.

In relation to the second part of Table 19, while decreasing the percentage of mobile phase in the channel A, it can be observed that the composition of all gradient steps meets the acceptance criteria.

As conclusion it can be said that the pump is in good working condition with respect to the gradient accuracy, since it delivers the mobile phase at various solvent strengths over the time by varying the composition of the mobile phase accurately.

Increasing the percentage of mobile phase containing 0.5 % of acetone in methanol/ water								
Heights	**%A**	**%B**	**Height of the step gradient (cm)**	**Theoretical values of the height of the step gradient (cm)**	**Gradient composition (%)**	**Theoretical Gradient composition (%)**	**Gradient accuracy (%)**	**Accepted / Not accepted accuracy**
H	100	0	11.35	11.35	100.00	100.00	0.00	-
A9 = 90 H	90	10	10.25	10.22	90.31	90.00	0.30	Accepted
A8 = 80 H	80	20	9.15	9.08	80.62	80.00	0.80	Accepted
A7 = 70 H	70	30	8.00	7.95	70.48	70.00	0.70	Accepted
A6 = 60 H	60	40	6.9	6.81	60.79	60.00	1.32	Accepted
A5 = 50 H	50	50	5.75	5.68	50.66	50.00	1.30	Accepted
A4 = 40 H	40	60	4.55	4.54	40.09	40.00	0.20	Accepted
A3 = 30 H	30	70	3.40	3.40	29.96	30.00	0.10	Accepted
A2 = 20 H	20	80	2.25	2.27	19.82	20.00	0.90	Accepted
A1 = 10 H	10	90	1.10	1.14	9.69	10.00	3.08	Accepted
Decreasing the percentage of mobile phase containing 0.5 % of acetone in methanol/ water								
H	100	0	11.35	11.35	100.00	100.00	0.00	-
A9 = 90 H	90	10	10.30	10.22	90.75	90.00	0.80	Accepted
A8 = 80 H	80	20	9.20	9.08	81.06	80.00	1.30	Accepted
A7 = 70 H	70	30	8.10	7.95	71.37	70.00	2.00	Accepted
A6 = 60 H	60	40	6.95	6.81	61.23	60.00	2.06	Accepted
A5 = 50 H	50	50	5.80	5.68	51.10	50.00	2.20	Accepted
A4 = 40 H	40	60	4.60	4.54	40.53	40.00	1.30	Accepted
A3 = 30 H	30	70	3.50	3.40	30.84	30.00	2.80	Accepted
A2 = 20 H	20	80	2.30	2.27	20.26	20.00	1.30	Accepted
A1 = 10 H	10	90	1.15	1.14	10.13	10.00	1.32	Accepted

Acceptance/ Refusal Criteria
Gradient accuracy ± 3 % of the step gradient composition

Table 19: Results obtained from the gradient accuracy assay on 21/10/2009 and the acceptance/refusal criteria (HP 1050).

Verification of the Liquid Chromatograph Agilent 1100

a) Injector precision assay

Table 20 shows the obtained results from injector precision assay on 30 October 2009 and the acceptance/refusal criteria. These criteria were defined taking into account that the injector must be very precise during injections thus it was decided that the variation of peak areas among replicate injections must be equal or lower than 1%.

Replicate	Peak Area
1	6704.33
2	6732.08
3	6752.91
4	6726.20
5	6758.04
Average value	6734.71
Standard deviation ,s	21.67
Relative standard deviation , s_r	0.003
Coefficient of variation, CV (%)	0.3
Accepted/Not Accepted precision	Accepted

Acceptance/Refusal Criteria
$CV\,(\%) \leq 1$

Table 20: Results obtained from the injector precision assay on 30/10/2009 and the acceptance/refusal criteria (Agilent 1100).

Analysing the obtained results in Table 20 for peak area, from five replicate injections of anthracene standard solution in methanol, it can be observed that there is a small and not meaningful variation among the results taking into account the acceptance criteria. From these results one may conclude that the autosampler draws almost the same amount of standards and/or samples in replicate injections, so it is suitable for establishing comparisons between the responses of standards and samples which is an activity carried out routinely in this equipment.

b) Injector linearity assay

Table 21 shows the obtained results from injector linearity assay on 30 October 2009 and the acceptance/refusal criteria. The acceptance criteria for the linearity of the injector must be defined in a way that ensures this linearity, it means that the assigned value must be very close to 1, the perfect linearity. Hence, the assigned value for this matter is 0.99.

Injected volume (µL)	Peak area
10	1418.45
30	4129.24
50	6748.68
80	10740
100	13308.9
Regression coefficient, *r*	0.9997
Accepted/ Not Accepted linearity	Accepted

Acceptance/Refusal Criteria
$r \geq 0.99$

Table 21: Results obtained from the injector linearity assay on 30/10/2009 and the acceptance/refusal criteria (Agilent 1100).

Figure 22 shows the graph of the peak area against the injected volume and the regression coefficient which allows to evaluate the injector linearity.

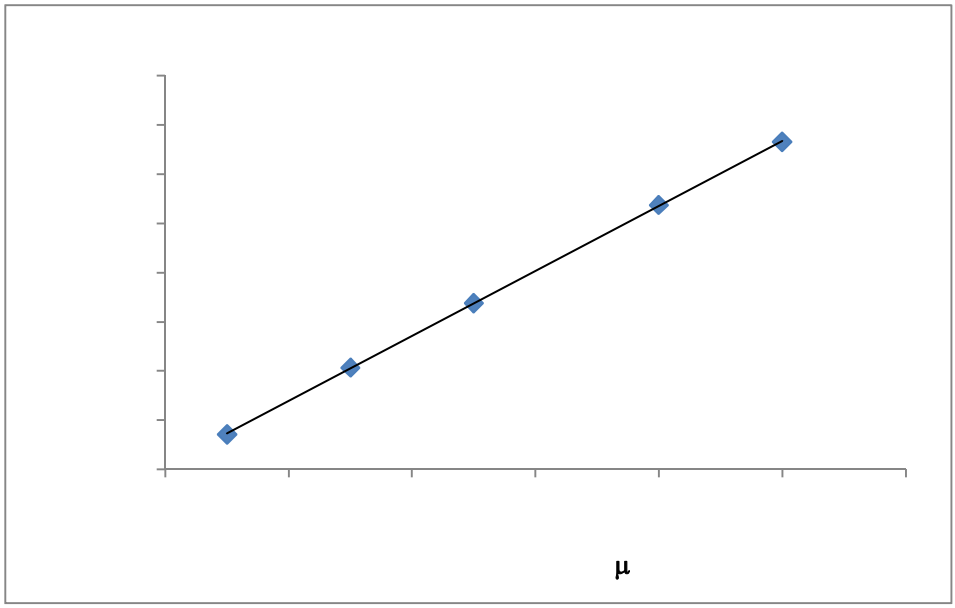


Figure 22: Injector linearity (Agilent 1100)

Looking at the graph of the response of injection (peak area) versus the injected volume, it can be observed that the relationship between the two variables is directly proportional. The correlation coefficient, the factor used in the evaluation of the injector linearity, is very close to 1. This means that injector (the autosampler) works almost perfectly when different volumes of sample are injected, producing a proportional response.

Analysing the regression coefficient taking into account the acceptance criteria defined, it can be said that the autosampler has a very good linearity of response.

c) Injector carryover assay

There is sample carryover if the peak that corresponds to the analyte (anthracene) appears in the chromatogram of a blank (methanol) injection.

It is necessary to define acceptance criteria for the autosampler carryover tests, in order to ensure that the level of carryover detected will not affect the quantification of subsequent samples more concentrated than the previous injected. The maximum allowable carryover was set to be 1%. This decision was made taking into consideration the accuracy required for the analyses using this equipment, an example of which is the identification of drugs.

Table 22 shows the obtained results from injector carryover assay on 29 October 2009 and the acceptance/refusal criteria.

Solution	Peak area
Anthracene (8 mg/l)	13446.9
Analyte in methanol	0
Carryover (%)	0
Accepted/ Not Accepted carryover	Accepted

Acceptance/Refusal Criteria
Carryover (%) ≤ 1

Table 22: Results obtained from the injector carryover assay on 29/10/2009 and the acceptance/refusal criteria (Agilent 1100).

Analysing the obtained results for the peak areas (Table 22), it can be observed that there is no carryover, since the anthracene peak did not appear in the chromatogram obtained for the blank injection. It can therefore be concluded that the autosampler is not contaminated by the last injected sample, consequently it will not contaminate the diluted samples injected after concentrated samples.

If there were some level of carryover it would be necessary to take corrective actions. Most carryover problems can be solved by adjusting tubing and fittings, choosing the best wash solvent and possibly by adding extra wash cycles [23].

Sample residue left in the autosampler is the most common source of carryover. In order to avoid sample carryover, all the components of the injector that come in contact with the sample (the injection needle, the needle seat, the injection loop) should be cleaned after the injection [23].

d) Flow rate precision assay

Table 23 shows the obtained results from flow rate precision assay on 30 October 2009 and the acceptance/refusal criteria. The values assigned to the acceptance criteria for the precision of the flow rate must be very small in order to have a consistent flow rate of the mobile phase. Due to this fact, it was agreed that the flow rate precision value must be equal or less than 1% to be acceptable.

Replicate	Retention time (min)
1	4.597
2	4.577
3	4.590
4	4.579
5	4.585
Average value (min)	4.586
Standard deviation ,*s* (min)	0.01
Relative standard deviation, s_r	0.002
Coefficient of variation, *CV* (%)	0.2
Accepted/Not Accepted precision	Accepted

Acceptance/Refusal Criteria
$CV\,(\%) \leq 1$

Table 23: Results obtained from the flow rate precision assay and the acceptance/refusal criteria (Agilent 1100).

Analysing Table 23 it can be observed that the obtained retention times by injecting five replicates of the anthracene standard solution are very similar having only a coefficient of variation of 0.2 %. The coefficient of variation value denotes that the pump provides a given flow rate and maintains this flow rate accurately while performing replicate injections which are preset to have the same flow rate. This means that the pump, in respect to this performance attribute, is in very good condition.

e) Flow rate trueness assay

Table 24 shows the obtained results from flow rate trueness assay on 02 November 2009 and the acceptance/refusal criteria to evaluate the flow rate trueness of the mobile phase. The acceptance criteria values for the flow rate trueness were defined in agreement with the values suggested by Herman Lam in the chapter "Performance Verification of the HPLC" of his book [12].

Replicate	Time (min)
1	9.82
2	9.85
3	9.78
Average value (min)	9.82
Volume collected (ml)	10
Obtained flow rate (ml/min)	1.019
Theoretical flow rate (mL/min)	1.00
Accepted /Not Accepted flow rate	Accepted
Absolute error (mL/min)	0.02
Relative error, *RE* (%)	1.9
Accepted /Not Accepted trueness	Accepted

Acceptance/Refusal Criteria
Obtained flow rate ± 2 % of the set flow rate
RE (%) ≤ 2

Table 24: Results obtained from the flow rate trueness assay on 02/11/2009 and the acceptance/refusal criteria (Agilent 1100)

Analysing the obtained results for the flow rate trueness assay (Table 24), it can be verified that the trueness of the flow rate of the mobile phase is acceptable in accordance with the acceptance criteria, although it is close to the value defined as not acceptable. The closeness between the obtained flow rate and the unacceptable value is due to the collection of mobile phase into wet volumetric flask. The use of wet volumetric flasks reduced the time necessary to collect the predetermined volume (the volume of the volumetric flask) and accordingly affected the volumetric flow rate. As a conclusion, the volumetric flasks used in this assay must be dry. Considering that the volumetric flow rate trueness is accepted, it can be concluded that the pump of this equipment is operating in suitable condition. This means that the pump is generating accurate flow rates, the flow rate value defined in the software is really generated by the pump.

f) Detector linearity assay

Quantitative analysis is routinely carried out in this measuring equipment. Taking into consideration that the analyses of samples of different concentrations are performed in this equipment, it is therefore necessary to check if the detector produces a linear response when the samples are injected.

Table 25 shows the obtained results from detector linearity assay on 29 October 2009 and the acceptance/refusal criteria to evaluate the linearity of the detector to the variation of the concentration. The acceptance criterion was defined to be equal or lower than 0.99. This value will ensure that the detector response is linear within a reasonable

range. A linear relationship between the instrumental response (peak-area) and the concentration of samples must exist in order to produce reliable results.

Concentration (mg/l)	Peak area
2.05	1703.06
4.10	3442.97
8.20	6793.11
10.25	7891.20
Regression coefficient, *r*	0.997
Accepted/ Not Accepted linearity	Accepted

Acceptance/Refusal Criteria
$r \geq 0.99$

Table 25: Results obtained from the detector linearity assay on 29/10/2009 and the acceptance/refusal criteria (Agilent 1100).

Figure 23 shows the graphic drawn of the peak area versus concentration of the standard solutions and the regression coefficient to assess the linearity of the detector.

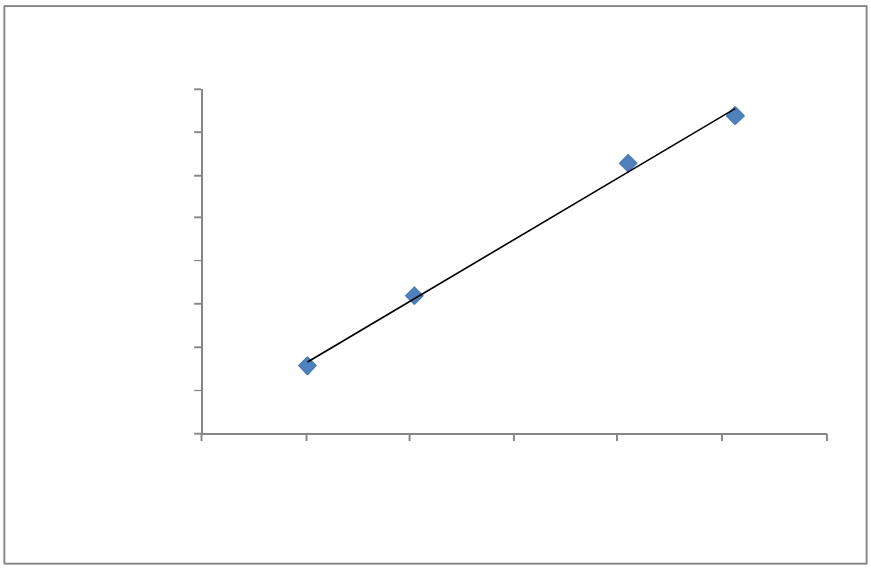


Figure 23: Detector linearity (Agilent 1100)

Analysing the obtained graph for the detector linearity (Figure 23), it can be observed that there is a linear relationship between the instrumental response and the variation of concentration. The regression coefficient of the plot allows quantifying this linearity, which is 0.997. It can be concluded that the detector produces a linear response when the concentration of samples changes, so it is suitable for its intended use.

g) Detector noise and drift assays

Figure 24 shows the obtained chromatographic baseline for the noise assay and the noise measurement performed.

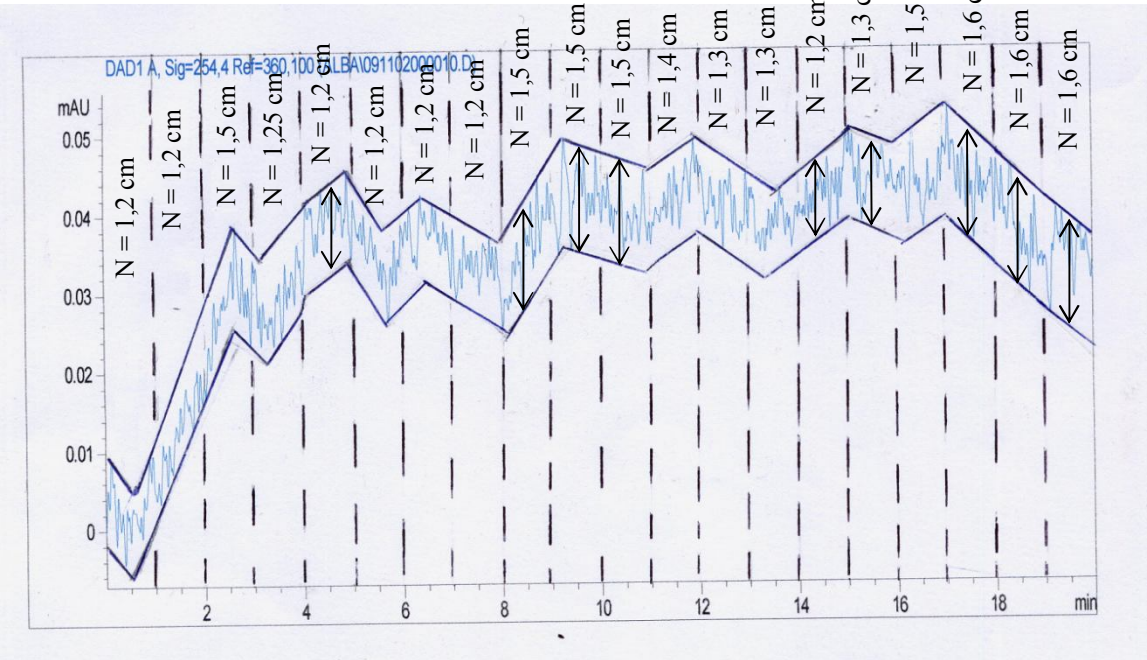


Figure 24: Noise measurement (Agilent 1100)

Analysing the obtained baseline for the noise assay (Figure 24), it can be observed that until the first 2 minutes the baseline was not stable since there is a marked upward trend. In order to get a baseline more stable it would be good to leave the detector turn on more time (it has been left turned on during one hour in order to stabilize) before to perform the noise assay.

Table 26 shows the obtained results from detector noise assay on 02 November 2009 and the acceptance/refusal criteria. The manufacturer's specification for the noise level and the use given to the equipment are the factors considered in setting the acceptance/refusal criteria.

Noise measurement	
Noise measurement (cm)	1.4
Noise measurement (mAU)	1.2 E-02
Noise measurement (AU)	1.2 E-05
Accepted/Not Accepted noise	Accepted

Acceptance/Refusal Criteria
Noise (AU) $\leq$ 1.5 E-5

Table 26: Results obtained from the detector noise assay on 02/11/2009 and the acceptance/refusal criteria (Agilent 1100).

Analysing the obtained result for the level of noise of the detector (1.2 E-05 AU), it can be said that this noise level in not significant considering the acceptance criteria adopted.

Taking into account the obtained baseline (Figure 24) and the noise value, it can be concluded that the detector lamp is in good conditions, since normal peaks were observed. Abnormal peaks, generally square-topped, are observed if the detector lamp fails. The obtained noise level can result from electronic and pump noise which is generated over the time with the age of the equipment. Other factor that may have contributed to the obtained noise level is that the flow cell is not well-cleaned.

The poor lamp intensity also can cause a relevant noise level. Lamps that have been in use a long time generally have low intensity which can cause a high noise level. Taking into account the noise level obtained it can be said that the detector lamp is in good condition.

Generally the detector noise is caused by the following factors: electronic, pump and photometric noise; poor lamp intensity and a dirty flow cell. So before starting the noise assays it is strictly necessary to check the intensity and hours of use of the lamps and to make sure that the flow cell is clean and free of gas bubbles [12][28][29].

The Figure 25 shows the obtained chromatographic baseline and the drift measurement.

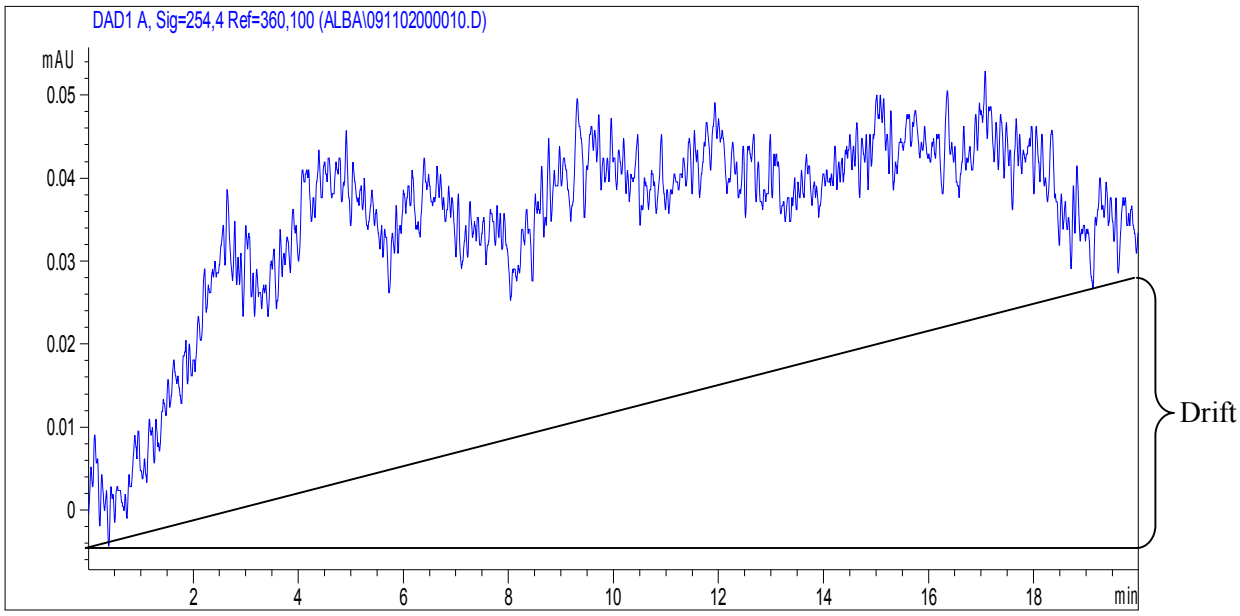


Figure 25: Drift measurement (Agilent 1100)

The obtained baseline for the detector drift assay (Figure 25) shows an upward trend of the signal over the time (during 20 minutes), which corresponds to the drift of the detector. The best way of determining the drift is to draw a straight line following this upward trend and calculate the corresponding slope. Table 27 includes the obtained slope value which is the measurement of the drift (in centimetres), its conversion into AU/h and the acceptance/ refusal criteria. These criteria were defined taking into account the manufacturer's specification for the drift level and the use given to the equipment.

Drift measurement	
Drift measurement (cm)	3.6
Drift measurement (mAU/20 min)	3.273E-02
Drift measurement (mAU/ min)	1.636E-03
Drift measurement (AU/ h)	9.8E-05
Accepted/Not Accepted drift	Accepted

Acceptance/Refusal Criteria
Drift (AU/h) $\leq$ 5.0 E-4

Table 27: Results obtained from the detector drift assay on 02/11/2009 and the acceptance/refusal criteria (Agilent 1100).

Analysing the obtained value for the drift (9.8E-05 AU/h), it can be said that the level of drift is not meaningful taking into account the acceptance criteria defined. This level of drift can be caused by the following factors: a flow cell not well cleaned, pump, electronic and photometric noise.

The age of this liquid chromatograph is also a factor which contributes to the drift in the detector since the performance of the equipment deteriorates over the time. Changes in ambient temperature, in solvent composition, or in flow rate are almost every time the responsible factors by the drift.

h) Gradient accuracy

This equipment has a quaternary pump which is able to deliver a mobile phase containing different percentages of various solvents over time. Generally they are used to carry out analyses of compounds using methods that require binary, ternary or quaternary solvent gradients. So it is a requirement that the ability of the pump to deliver a mobile phase at different solvent strengths over time by changing the composition of the mobile phase be tested in order to check if these changes are accurate.

Table 28 shows the obtained results from gradient accuracy assay on 03 November 2009 and the acceptance/refusal criteria. The acceptance criteria for the gradient accuracy have been defined following the suggested values by Herman Lam in the chapter "Performance Verification of the HPLC" of his book [12].

Gradient accuracy measurement	
1 st half of chromatogram area	397999
2 nd half of chromatogram area	397585
Difference between areas	414
Average area	397792
Gradient accuracy (%)	0.10
Accepted/Not Accepted accuracy	Accepted

Acceptance/Refusal Criteria
Gradient accuracy (%) ≤ 1

Table 28: Results obtained from the gradient accuracy assay on 03/11/2009 and the acceptance/ refusal criteria (Agilent 1100).

Analysing the obtained result for the gradient accuracy (0.10 %) shown in Table 28, it can be verified that the gradient accuracy is right in agreement with the acceptance/refusal criteria adopted. This means that the pump is able to change the composition of the mobile phase accurately.

4.3. Performance Verification of Gas Chromatography (GC)

Taking into account section 3.4 of the Experimental Part, the obtained results on a date for the verification assays of Gas Chromatography will be presented, as example.

4.3.1. Gas Chromatograph with Thermal Conductivity Detector (TCD)

a) Flow rate precision assay

Table 29 shows the obtained results from the flow rate precision assay on 06 November 2009 and the acceptance/ refusal criteria. The acceptance/ refusal criteria for the flow rate precision were defined taking into account that there must be a very small variation of flow rates in order to get a consistent flow. Due to this reason, the precision of the flow rate must be equal or less than 1% to be acceptable.

Replicate	Retention time (min)
1	2.120
2	2.130
3	2.129
4	2.123
5	2.122
Average value (min)	2.125
Standard deviation, s (min)	0.004
Relative standard deviation, s_r	0.002
Coefficient of variation, CV (%)	0.2
Accepted/Not accepted precision	Accepted

Acceptance/Refusal Criteria
CV (%) ≤ 1

Table 29: Results obtained from the flow rate precision assay on 06/11/2009 and the acceptance/refusal criteria (GC with TCD).

The column efficiency and consequently the retention time of a compound eluted are affected by carrier gas flow rate. It is fundamental to check the ability of the flow controller to maintain a consistent flow of the carrier gas. If there is a poor flow rate precision there will be a significant variation in the retention time of an eluted peak among replicate injections of a sample.

Analysing the obtained result for the precision of the flow (Table 29), it can be said that the flow controller is operating in good conditions since the obtained retention times for replicate injections of a standard solution injected are similar having a coefficient of variation of 0.2 %. This means that the flow rate of the carrier gas is precise in agreement with the acceptance/refusal criteria defined.

b) Detector linearity assay

Table 30 shows the obtained results from detector linearity assay on 06 November 2009 and the acceptance/ refusal criteria. The acceptance criterion was defined to be equal or lower than 0.99. This value will ensure that the detector response is linear within a reasonable range.

Concentration (mg/L)	Peak-area
18.7	3964320
36.7	7507107
45.1	9202106
53.3	10701477
Regression coefficient, r	0.9999
Accepted/ Not Accepted	Accepted

Acceptance/Refusal Criteria
$r \geq 0.99$

Table 30: Results obtained from the detector linearity assay on 06/11/2009 and the acceptance/refusal criteria (GC with TCD).

This equipment includes a TCD which is able to detect any compound that has a different thermal conductivity to the carrier gas.

Samples of different concentrations are analysed using this equipment, so it is important to check if the detector produces a linear response to variations in the concentration within a reasonable range.

Figure 26 shows the obtained graph for peak-area versus concentration of the standard solutions and the regression coefficient which allows to evaluate the linearity of the detector.

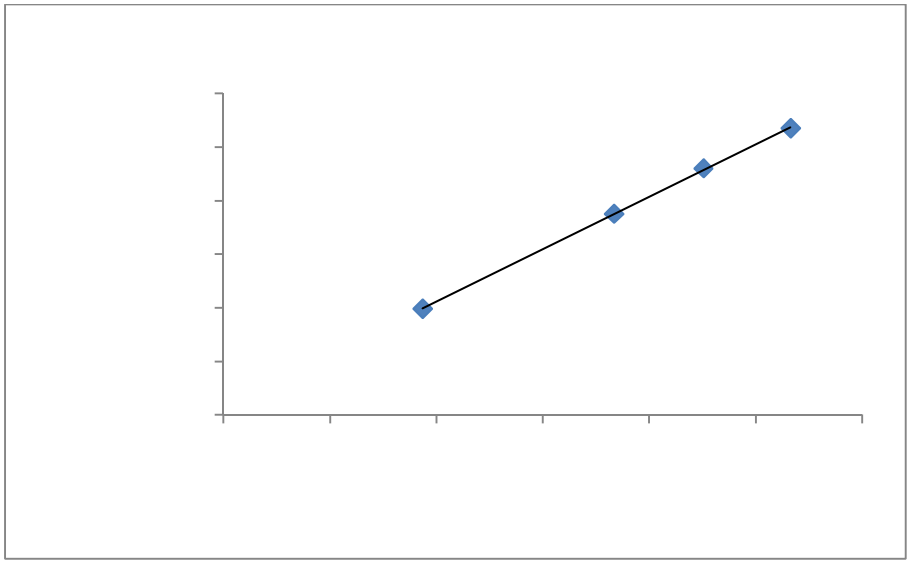


Figure 26: Detector linearity (GC with TCD).

Analysing the obtained graph for the detector linearity (Figure 26) it can be observed that the instrumental response is directly proportional to the variations in the concentration. Considering the regression coefficient (r = 0.9999) of the plot peak-area versus concentration and taking into consideration the acceptance criteria for the detector linearity, it can be confirmed that this detector shows a linear response to the variation of the concentration.

c) Detector noise and drift assays

The Figure 27 shows the obtained baseline and the corresponding noise measurement for the detector noise assay.

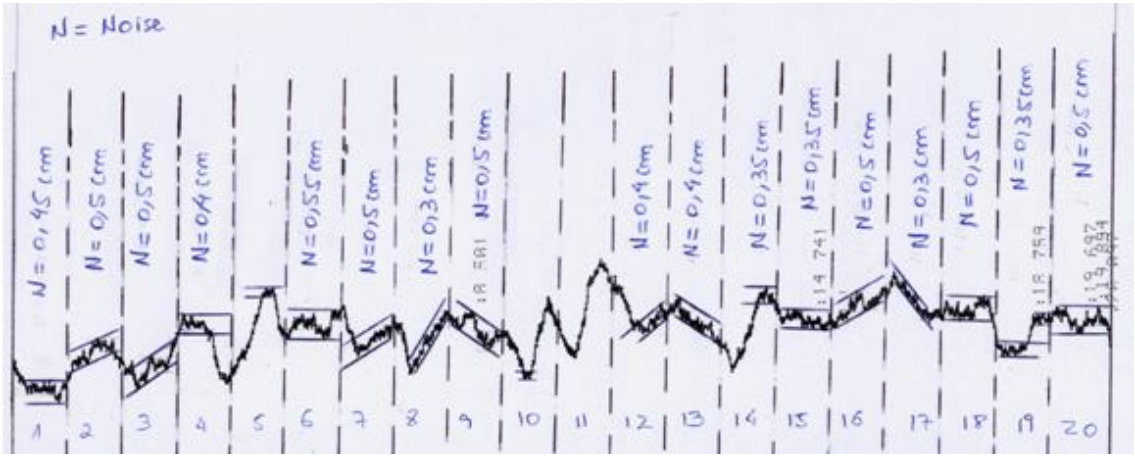


Figure 27: Noise measurement (GC with TCD)

The obtained chromatographic baseline shows wander and noise. Generally noise is called "short-term noise" since wander is also noise. Wander is a type of detector noise which is random in direction but at a lower frequency than the short-term noise (See Figure 28) [33].

Figure 28: Wander and noise [33]

Looking at the obtained chromatogram (Figure 27), it can be observed that wander is mainly present in segments 5, 10, 11 and 14. The noise (short-term noise) was not measured in segments 5, 10 and 11 since it was not possible to indentify peak-to-peak variation.

Table 31 shows the obtained results from detector noise assay on 17 December 2009 and the acceptance/refusal criteria. These criteria were defined taking into account suggested manufacturers' specifications for maximum level of noise in TCDs (10 µV) and the use given to the equipment.

Noise calculation	
A (mm)	4.1
B (mm)	150
C	-3
Noise (V)	3.60E-06
Accepted/Not accepted noise	Accepted

Acceptance/Refusal Criteria
Noise (V) ≤ 1.0 E-5

Table 31: Results obtained from the noise assay on 17/12/2009 and the acceptance/refusal criteria (GC with TCD).

Comparing the obtained noise level (3.60×10^{-6} V) with the maximum value that can be acceptable for level of noise, it can be concluded that the detector shows a noise level which is not significant. This level of noise will not significantly contribute uncertainty to peak areas or heights and therefore the determination of analytes in samples will not be affected.

Electronic noise from the instrument or from external sources and chemical noise from the influx of the response-provoking molecules in the background are factors that contribute to the detector noise level. Other factors such as leaking fittings, column bleed or contamination in the pneumatics, inlet, column or detector can increase the level of noise making it meaningful [25].

Figure 29 shows the obtained baseline in the drift assay and the drift measurement done.

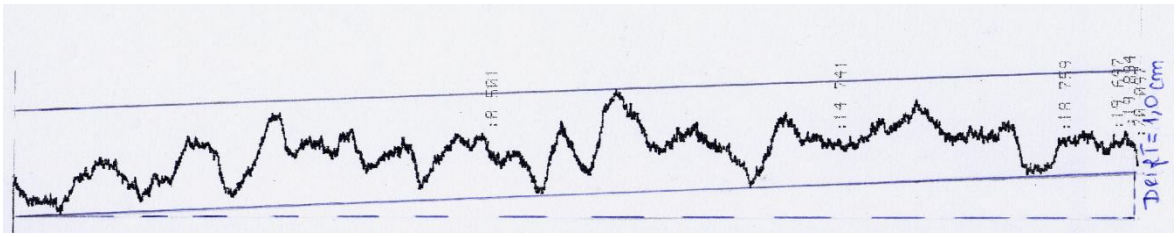


Figure 29: Drift measurement (GC with TCD)

Analysing the obtained baseline (Figure 29) an upward trend over the measurement time (20 minutes) which corresponds to the drift of the detector can be observed. The best strategy to carry out the drift measurement is therefore to draw a straight line following this upward trend and to determine the slope of this line.

Table 32 shows the obtained results from detector drift assay on 17 December 2009 and the acceptance/refusal criteria. These criteria were defined taking into account suggested manufacturers' specifications for maximum level of drift in TCDs (1.0×10^{-4} V/h) and the use given to the equipment.

Drift calculation	
A (mm)	10
B (mm)	150
C	-3
Drift (V/20 min)	8.3E-06
Drift (V/min)	4.2E-07
Drift (V/ h)	2.50E-05
Accepted/Not accepted drift	Accepted

Acceptance/Refusal Criteria
Drift (V /h) ≤ 1.0 E-4

Table 32: Results obtained from the detector drift assay on 17/12/2009 and the acceptance/ refusal criteria (GC with TCD).

Analysing the obtained drift value (2.50×10^{-5} V/h), one can say that the detector shows a drift level that will not affect significantly the analyses performed in this equipment taking into account the acceptance/refusal criteria defined. The obtained value for drift can result from the detector has not been allowed to fully stabilize.

d) Linearity of the oven temperature assay

Temperature programming is required to analyse highly complex mixtures. Programmed temperature gas chromatography (PTGC) is a process in which the column temperature is increased during the run. This method is very effective for optimizing an analysis and is frequently used for screening new samples. Due to the use of this method, in this gas chromatograph, it is necessary to assess the linearity oven temperature in order to check if the equipment produces a linear response to the temperature variation.

Table 33 shows the obtained results from linearity of the oven temperature assay on 01 December 2009 and the acceptance/refusal criteria. These criteria were defined in order to ensure that the oven produces a linear response to changes of temperature within a reasonable range.

Set temperature (^{0}C)	**Reading (^{0}C)**	**Correction factor (^{0}C)**	**Experimental temperature (^{0}C)**
70	70.5	- 0.3	70.2
100	101.2	- 0.6	100.6
130	131.6	- 0.6	131.0
160	161.8	- 0.6	161.2
200	201.9	- 0.6	201.3
Regression coefficient, *r*		1.0	
Accepted/ Not Accepted		Accepted	

Acceptance/Refusal Criteria
$r \geq 0.99$

Table 33: Results obtained from the linearity of the oven temperature assay on 01/12/2009 and the acceptance/ refusal criteria (GC with TCD).

The presented values for the experimental temperature in Table 33 include the correction factors obtained from the calibration certificate of the digital thermometer.

Figure 30 shows the graph of the experimental temperature against the set temperature.

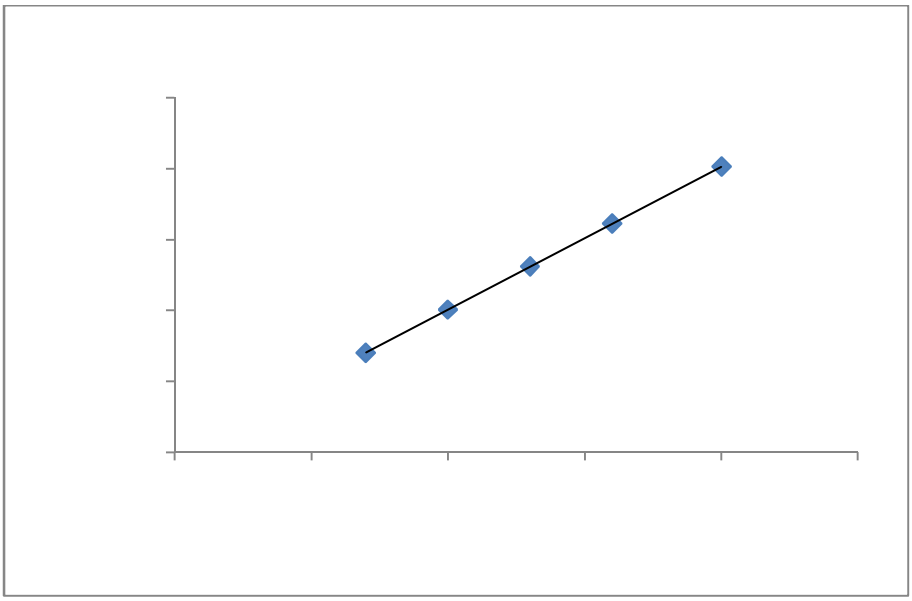


Figure 30: Linearity of the oven temperature (GC with TCD)

Analysing the graph of the experimental temperature versus the set temperature (see Figure 30), it can be observed that the instrumental response (experimental temperature that was read in the calibrated thermometer) is directly proportional to the variation of the set temperature (this temperature was defined in the equipment). Regarding the regression coefficient of the plot (r =1) it can be concluded that there is a true linear relationship between the instrumental response and the variation of the temperature, taking into account the acceptance/refusal criteria defined. A temperature programming can be used, in this equipment, with confidence considering that the temperature of the oven changes in a linear way.

e) Precision *and trueness of the oven temperature assays*

The efficiency of a GC column changes with the temperature of the column. Generally, the retention factor, k', decreases with the increase of the temperature, and thus the retention of the analysis also decreases with the temperature. The partition of solutes between the carrier gas and the stationary phase is highly dependent on the temperature of the chromatographic system. If this partition is a desirable attribute, the conditions for a particular analysis can be tailored but the control of the temperature must be taken into account if a repeatable separation is to be achieved. The chromatographic column must be heated in a uniform way and should match the set

temperature at all times. Therefore is essential to assess the ability of the oven to maintain an accurate and consistent temperature.

Tables 34 and 35 show the acceptance/ refusal criteria for the precision and trueness of the temperature of the oven, respectively. These criteria were defined taking into account the accuracy and the precision required by the analyses performed in this gas chromatograph.

Acceptance / Refusal Criteria	
CV (%) ≤	1

Table 34: Acceptance/ refusal criteria for the precision of the oven temperature assay (GC with TCD)

Acceptance / Refusal Criteria
Obtained temperature value ± 3 ^{0}C of the set temperature

Table 35: Acceptance/ refusal criteria for the trueness of the oven temperature assay (GC with TCD)

Table 36 shows the obtained results from the trueness and precision of the oven temperature assays on 1 December 2009. The values obtained experimentally (replicates) include the correction factors obtained from the calibration certificate of the digital thermometer.

	Set temperature (^{o}C)				
	70.0	**100.0**	**130.0**	**160.0**	**200.0**
Replicate 1 (^{o}C)	70.2	101.0	131.3	161.5	201.7
Replicate 2 (^{o}C)	70.1	100.4	130.9	161.1	201.2
Replicate 3 (^{o}C)	70.2	100.3	130.8	161.0	201.1
Average value (^{o}C)	70.2	100.6	131.0	161.2	201.3
Standard deviation, s (^{o}C)	0.058	0.379	0.265	0.265	0.321
Relative Standard deviation, s_r	0.001	0.004	0.002	0.002	0.002
Coefficient of variation, CV (%)	0.08	0.4	0.2	0.2	0.2
Accepted/ Not Accepted precision	Accepted	Accepted	Accepted	Accepted	Accepted
Absolute error, AE (^{o}C)	0.2	0.6	1.0	1.2	1.3
Accepted/ Not Accepted trueness	Accepted	Accepted	Accepted	Accepted	Accepted

Table 36: Results obtained from the trueness and precision of the oven temperature assay on 01/12/2009 and the acceptance/ refusal criteria (GC with TCD).

Analysing the obtained results for the precision shown in Table 36 (the coefficient of the variation for replicate readings of the temperature) of the oven temperature, it can observed that the variation among the temperatures is not significant taking into account the acceptance/refusal criteria defined. This means that the oven is able to maintain consistent temperatures during GC runs since there is closeness of agreement among

independent test results (replicates) obtained under the same conditions of measurement.

Looking at Table 36, the obtained results for the trueness of the oven temperature show that the variation between the temperatures preset in the equipment and the temperatures obtained in the digital thermometer is not significant based on the acceptance/refusal criteria. It can be also observed that the difference between the preset temperature value and the obtained temperature value increases with the increasing temperature. A conclusion can be drawn that the oven provides accurate temperatures since and absolute error is lower than 3°C.

f) Stability of the oven temperature assay

Some analyses performed in this gas chromatograph require isothermal conditions (the same temperature all the time). The chromatographic oven must maintain a steady column temperature in order to prevent peak retention time shifts due to temperature variation that happens due to the conditioning and heating/cooling cycles that occur sometimes. Therefore is essential to check if the oven is able to maintain the same temperature over a period of time while performing the analysis.

Table 37 shows the obtained results from the stability of the oven temperature assay for the minimum and the maximum working temperatures used in this equipment and the acceptance/refusal criteria. These results were obtained on 1 December 2009.

Set temperature: 70^0 C

Time (min)	Reading (^{0}C)
0	69.9
15	70.1
30	70.1
45	70.1
60	70.3
75	70.2
Difference of temperature (^{0}C)	0.4

Set temperature: 130^0 C

Time (min)	Reading (^{0}C)
0	131.6
15	130.9
30	130.8
45	130.8
60	130.9
75	130.7
Difference of temperature (^{0}C)	0.9

Acceptance / Refusal Criteria	
Difference between maximum and minimum temperatures (ºC) ≤	2

Table 37: Results obtained from the stability of the oven temperature assay for the set temperatures at 70 and 130^0 C and the acceptance/ refusal criteria (GC with TCD)

Looking at Table 37 and analysing the obtained temperature values when the set temperature was 70 ºC, it can be observed that difference between the maximum temperature and the minimum temperature during 75 minutes is very small (0.4 ºC). Considering the acceptance/ refusal criteria defined for the stability of the oven temperature, it can be said that the oven maintained the preset temperature (70 ºC) during the measurement time.

Considering the obtained temperature values when the set temperature was 130 ºC (see Table 37), it can be verified that the difference between the maximum temperature value and the minimum temperature value (0.9 ºC) during 75 minutes is not significant taking into account the acceptance/ refusal criteria defined.

Comparing the obtained temperature difference when the preset temperature was 70 ºC and obtained temperature difference when the preset temperature was 130 ºC, it can be observed that the temperature difference was bigger when the preset temperature was higher. Therefore, it can be said that the chromatographic oven is less efficient in the temperature control for higher temperatures.

It can therefore be concluded that the chromatographic oven is able to maintain a steady working temperature (70 ºC or 130 ºC) during the time necessary to perform the analyses in this equipment. Thus the column temperature will be constant and accordingly there will be no peak retention time shift due to the temperature variation.

4.3.2. Gas Chromatograph with Flame Ionization Detector (FID)

a) Flow rate precision assay

Table 38 shows the obtained results from the flow rate precision assay in the gas chromatograph with FID on 16 November 2009 and the acceptance/ refusal criteria.

The acceptance/ refusal criteria for the precision of the flow rate has been defined taking into consideration that the variation of the flow rate of the carrier gas must be very small in order to obtain precise measurement results. Hence, a value of 1% or values lower than 1% were assigned to the acceptance criteria in order to guarantee precise measurement results.

Replicate	Retention time (min)
1	1.492
2	1.492
3	1.495
4	1.495
5	1.485
Average value (min)	1.492
Standard deviation (min)	0.004
Relative Standard deviation, s_r	0.003
Coefficient of variation, CV (%)	0.3
Accepted/Not accepted precision	Accepted

Acceptance/Refusal Criteria
CV (%) ≤ 1

Table 38: Results obtained from the flow rate precision assay on 16/11/2009 and the acceptance/ refusal criteria (GC with FID)

The control of the carrier gas is essential for the column efficiency and consequently for the qualitative analysis. For qualitative analysis it is essential to have a constant flow rate in order to obtain retention times in replicate measurements with a degree of agreement among them [31]. Therefore is fundamental to assess the ability of the flow controllers to maintain the flow of the carrier gas.

Analysing the obtained results, showed in Table 38 for the retention time of replicate measurements, it can be observed that there is a very small variation among them, having a coefficient of variation of 0.3 % which is not meaningful taking into account the acceptance/refusal criteria defined. This means that there is a good flow rate control and as consequence the results obtained from the identification of a compound in replicate measurements will be precise using this equipment.

b) Detector linearity assay

This equipment has a FID. In general terms, this type of detector is known by its very stable response and sensitivity to most organic compounds [30].

The analysis of samples with different concentration is performed using this gas chromatograph, thus it is fundamental to check the ability of the detector to produce a linear relationship between the instrumental response and the variation of the concentration withtin a reasonable range.

Table 39 shows the obtained results from the detector linearity on 17 November 2009 and the acceptance/refusal criteria. These criteria have been defined in the same way that the criteria for the linearity of the detectors mentioned above.

Concentration (mg/L)	Peak-area
18.7	164217200
36.7	326917280
45.1	405326560
53.3	444601600
61.5	485037600
Regression coefficient, *r*	0.99
Accepted/ Not Accepted	Accepted

Acceptance/Refusal Criteria
$r \geq 0.99$

Table 39: Results obtained from the detector linearity assay on 17/11/2009 and the acceptance/refusal criteria (GC with FID)

Figure 31 shows the plot of peak are versus concentration of standard solutions and the regression coefficient which allows to evaluate the linearity of the detector.

Analysing the plot of peak area versus concentration (Figure 31) drawn in order to evaluate the linearity of the detector, it can be observed that there is a linear relationship between the intrumental response (peak area) and the concentration variation. Considering the regression coefficient value, it can be said that the detector shows a linear response taking into account the acceptance/refusal criteria defined. Considering the regression coefficient value ($r = 0.99$) , it follows that this detector shows the maximum allowable value to be considered to have a linear response. As conclusion it can be said that the linearity of the detector is acceptable in accordance with the acceptance criteria.This means that the detector is able to generate a linear response when the concentration of standards or samples changes.

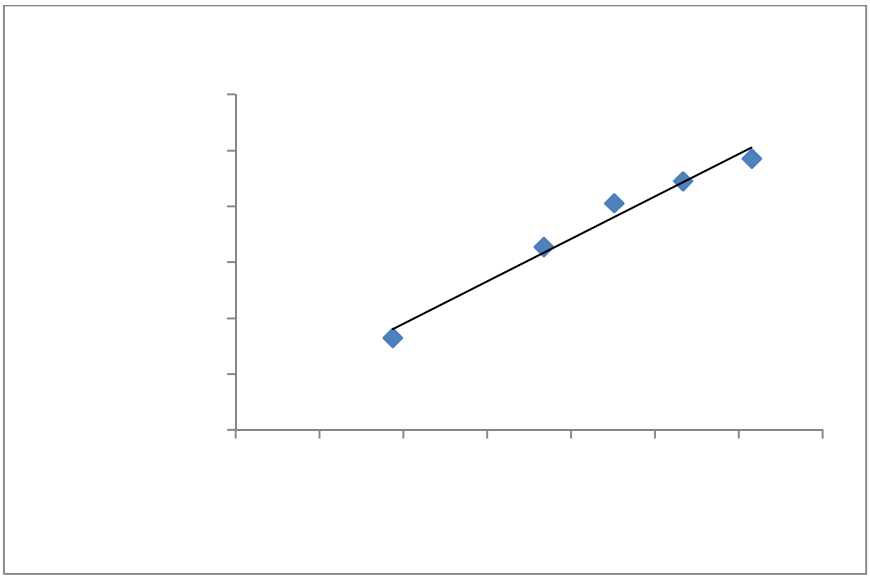


Figure 31: Detector linearity assay (GC with FID)

c) Detector noise and drift assays

The Figure 32 shows obtained baseline from the detector noise assay and the noise measurement performed.

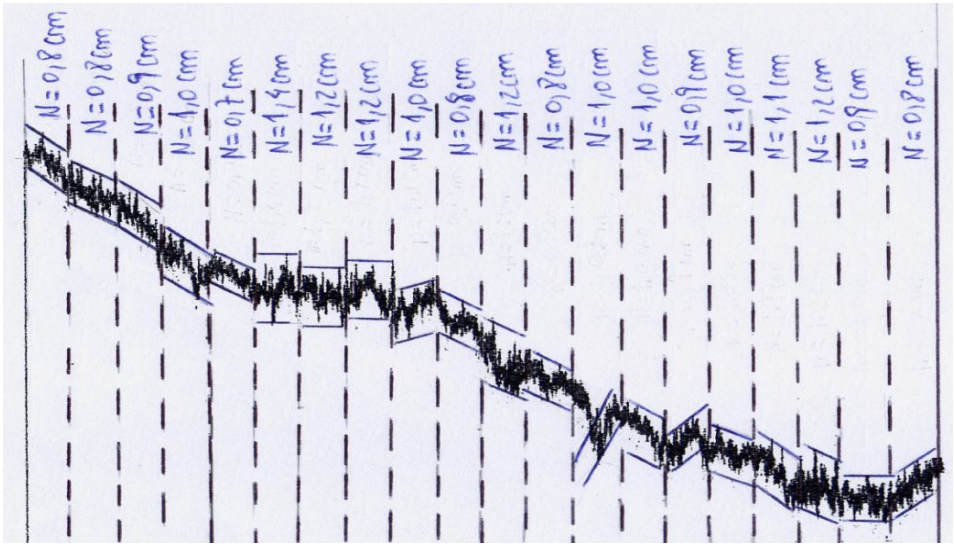


Figure 32: Noise measurement (GC with FID)

Table 40 shows the obtained results from detector noise assay on 17 November 2009 and the acceptance/refusal criteria. These criteria were defined taking into account suggested manufacturers' specifications for maximum level of noise (1×10^{-14} A) in FIDs and the use given to the equipment.

Noise calculation	
A (mm)	9.85
B (mm)	150
C	-3
Noise (A)	8.21E-15
Accepted/Not accepted noise	Accepted

Acceptance/Refusal Criteria
Noise (A) ≤ 6.0 E-14

Table 40: Results obtained from the detector noise assay on 17/11/2009 and acceptance/refusal criteria (GC with FID)

Considering the noise value shown in Table 40, it can be said that the noise level is not significant taking into consideration the acceptance criteria defined. The noise level obtained will not affect significantly the sensitivity of the detector and hence the quantitation of low-level analytes.

Figure 33 shows the obtained chromatographic baseline from the detector drift assay and the drift measurement performed.

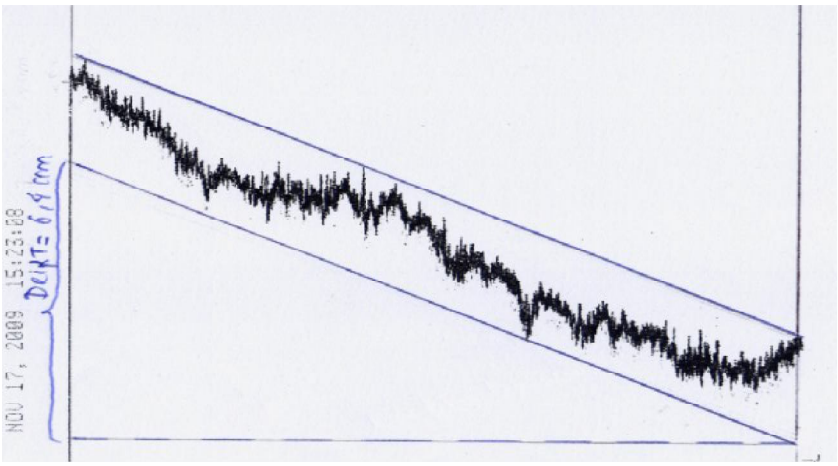


Figure 33: Drift measurement (GC with FID)

Analysing the obtained baseline, it can be observed that there is a downward trend over the measurement time (20 minutes). This trend represents the detector drift.

Table 41 shows the obtained results from detector drift assay on 17 November 2009 and the acceptance/refusal criteria. These criteria were defined taking into account suggested manufacturers' specifications for maximum level of drift in FIDs (1×10^{-13} A/h) and the use given to the equipment.

Drift calculation	
A (mm)	64
B (mm)	150
C	-3
Drift (A/20 min)	5.33E-14
Drift (A/min)	2.67E-15
Drift (A/ h)	1.60E-13
Accepted/Not accepted drift	Accepted

Acceptance/Refusal Criteria
Drift (A/h) ≤ 6.0 E-13

Table 41: Results obtained from the detector drift assay (GC with FID)

Analysing the obtained drift level that can be seen in Table 41, it can be noticed that this level for this detector is not significant taking into account the acceptance/ refusal criteria defined. Therefore this drift level will not affect significantly the analysis of compounds carried out in this equipment.

d) Linearity of the oven temperature assay

Complex mixtures, analysed in this equipment, require programmed temperature gas chromatography.Therefore this requires an evaluation of the linearity of the oven temperature in order to check if there is a linear relationship between the instrumental response (the experimental temperature) and the temperature variation.

Table 42 shows the obtained results from linearity of the oven temperature assay on 30 November 2009 and the acceptance/refusal criteria. These criteria were defined in order to ensure that the oven shows linearity to changes of temperature within a reasonable range.

The presented values for the experimental temperature include the correction factors obtained from the calibration certificate of the digital thermometer.

Set temperature (^{0}C)	Reading (^{0}C)	Correction factor (^{0}C)	Experimental temperature (^{0}C)
70	70,5	- 0.3	70.2
100	101.1	- 0.6	100.5
130	131.7	- 0.6	131.1
160	162.0	- 0.6	161.4
200	202.3	- 0.6	201.7
Regression coefficient, *r*		1.0	
Accepted/ Not Accepted		Accepted	

Acceptance/Refusal Criteria
$r \geq 0.99$

Table 42: Results obtained from the linearity of the oven temperature assay and the acceptance/ refusal criteria (GC with FID)

Figure 34 shows the graph of the experimental temperature versus set temperature and the corresponding regression coefficient.

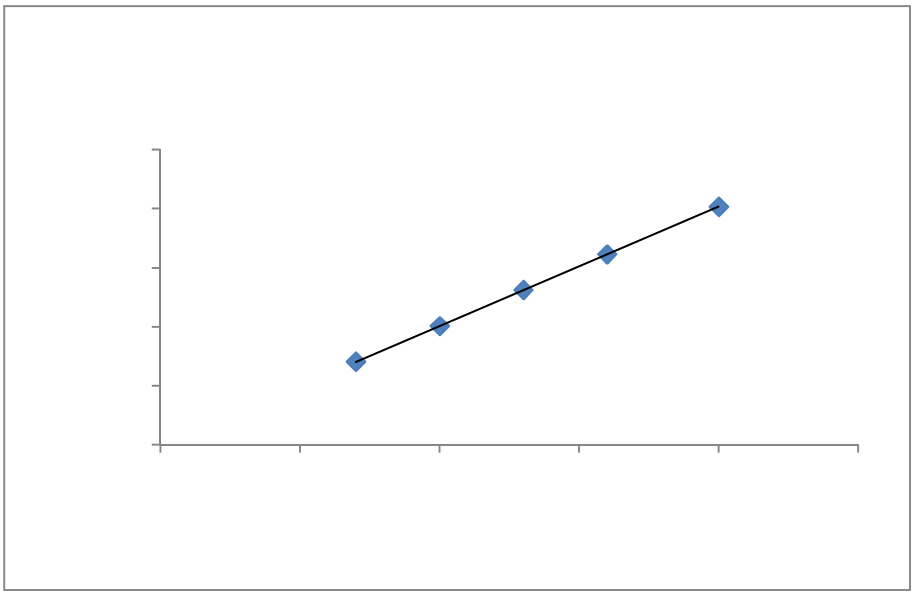


Figure 34: Linearity of the oven temperature (GC with FID)

Looking at the graph of the experimental temperature against the set temperature (See Figure 34), it can be observed that there is a linear relationship between the experimental temperature read in the digital thermometer and the variation of the set temperature in the equipment. Moroever, considering the regression coefficient of the plot ($r = 1$), it can be concluded that the temperature of the column oven shows a linear response to the variation of the temperature taking into account the acceptance/ refusal criteria defined. As consequence, the programmed temperature mode can be used with confidence, in this equipment, since the temperature of the oven shows a linear response to the increase of the temperature.

e) Precision and trueness of the oven temperature assays

The efficiency of the GC column is affected by changes in the temperature column. Usually the retention factor, *k'*, decreases as temperature increases, and thus the retention time of a compound also decreases with the temperature. If a desired retention time should be achieved, the chromatographic column must be heated uniformly, the column temperature must be accurate (the displayed temperature in the chromatograh screen should be the set temperature) and must be maintained. Due to these performance attributes (accuracy and precision) that the column temperature must

show, it is fundamental to assess the ability of the oven temperature to maintain an accurate and consistent temperature.

Tables 43 and 44 show the acceptance/refusal criteria for the precision and trueness of the oven temperature, respectively. These criteria were defined taking into account the accuracy and the precision required by the analyses performed in this gas chromatograph.

Acceptance / Refusal Criteria	
CV (%) ≤	1

Table 43: Acceptance/ refusal criteria for the precision of the oven temperature assay (GC with FID)

Acceptance / Refusal Criteria
Obtained temperature value ± 3 ^{0}C of the set temperature

Table 44: Acceptance/ refusal criteria for the trueness of the oven temperature assay (GC with FID)

Table 45 shows the obtained results from the trueness and precision of the oven temperature assays on 30 November 2009.

Set temperature (^{0}C)	70.0	100.0	130.0	160.0	200.0
Replicate 1	70.3	100.5	131.2	161.4	201.9
Replicate 2	70.1	100.5	131.1	161.4	201.7
Replicate 3	70.1	100.4	131.0	161.3	201.6
Average value	70.2	100.5	131.1	161.4	201.7
Standard deviation, s (^{0}C)	0.115	0.058	0.100	0.058	0.153
Relative standard deviation, s_r	0.002	0.001	0.001	0.000	0.001
Coefficient of Variation, CV (%)	0.165	0.057	0.076	0.036	0.076
Accepted/ Not Accepted precision	Accepted	Accepted	Accepted	Accepted	Accepted
Absolute error, AE (^{o}C)	0.2	0.5	1.1	1.4	1.7
Accepted/ Not Accepted trueness	Accepted	Accepted	Accepted	Accepted	Accepted

Table 45: Results obtained from the trueness and precision of the oven temperature assays on 30/11/2009 (GC with FID).

Analysing Table 45, it can be observed that the variation of the temperature among replicate measurements is very small, being the coefficient of variation for all temperatures less than 1%. Therefore it can be said that the oven is able to provide almost the same column temperature in replicate measurements since the temperature variation between replicate measurements is not significant taking into account the acceptance criteria.

Analysing Table 45 in respect to the trueness of the oven temperature, it can be observed that the variation between the obtained temperature average value and the

temperature true value is not meaningful for all the preset temperature values with regards to the acceptance criteria. This means that the temperature provided by the oven is accurate. It can also be observed that the difference between the preset temperature value and the obtained temperature value increases with the increasing of the temperature.

f) Stability of the oven temperature assay

The majority of the analyses carried out in this equipment require the same temperature during the measurement time. Therefore the chromatographic oven must maintain a steady column temperature during the measurement times in order to avoid peak retention time shifts due to temperature changes. Hence, there is a need to check if the oven has the ability to maintain the same temperature during the period of time that the compounds analysis are performed.

Table 46 shows the obtained results on 30 November 2009 from the stability of the oven temperature assay for the minimum and the maximum working temperatures used in this equipment and the acceptance/refusal criteria. These criteria were defined taking into account the required stability for the temperature by the analysis performed in this equipment.

Set temperature: 70^0 C

Time (min)	Reading (^{0}C)
0	65.1
15	68.7
30	69.0
45	69.1
60	69.2
75	69.2
Difference of temperature (^{0}C)	4.1[1]
Difference of temperature (^{0}C)	0.5[3]

Set temperature: 130^0 C

Time (min)	Reading (^{0}C)
0	122.8
15	127.9
30	128.4
45	128.7
60	128.7
75	128.8
Difference of temperature (^{0}C)	6.0[2]
Difference of temperature (^{0}C)	0.9[4]

Acceptance / Refusal Criteria	
Difference between maximum and minimum temperatures (ºC) ≤	2

Table 46: Results obtained from the stability of the oven temperature assay for the set temperatures at 70 and 130^0 C and the acceptance/ refusal criteria (GC with FID)

Looking at Table 46, it can be observed that for the two preset temperatures (70^{0}C and 130^{0}C) the gas chromatograph shows these temperatures at a certain time although they were not yet reached since the digital thermometer showed lower temperatures.

Considering this fact, it can be said that during the first 15 minutes the oven temperature is not accurate. If the stability of the oven temperature is to be assessed taking into account the first 15 minutes, it is verified that the oven doesn't show stability over the time taking into account the acceptance criteria defined. This is so since the difference between the maximum and the minimum temperatures exceed the maximum allowable value (2ºC). Taking into consideration the observations and the conclusions mentioned above, the stability of the oven has been assessed without considering the first value of temperature.

Considering the temperature values shown in Table 46 for the two working temperatures (70^{0}C and 130^{0}C) after 15 minutes, it can be said that the oven maintain a

[1] The difference between the maximum and the minimum temperatures was calculated considering the temperature at zero minute.
[2] The difference between the maximum and the minimum temperatures was calculated considering the temperature at zero minute.
[3] The difference between the maximum and the minimum temperatures was calculated without considering the temperature at zero minute.
[4] The difference between the maximum and the minimum temperatures was calculated without considering the temperature at zero minute.

steady temperature during the measurement time, since the variation between the maximum and the minimum temperatures is lower than 2ºC.

The stability of the temperature of the oven assay should be started when the preset temperature in the chromatograph is attained and when the temperature read out in the digital thermometer does not show big oscillations. As a conclusion the assay should begin 15 minutes after the temperature value preset in the chromatograph occurs, since it was observed that the temperature reading became stable after this time.

With respect to the execution of analyses in which the temperature programming is a requirement, the operator should wait also at least 15 minutes after the preset temperature occurs in the chromatograph in order to get accurate and trustful results.

5. Conclusions

1. ISO/IEC 17025 is the standard which specifies the requirements for technical competence of testing and calibration laboratories. Procedures for verification and maintenance of some instruments have been designed and implemented. The corresponding documents have been written.

2. Taking into account the high number of students using the equipment at teaching laboratories, it has been decided to carry out equipment maintenance and verification in order to control the performance and to ensure the maximum yield and the maximum duration of the equipment utilized.

3. The SOPs for maintenance and verification of equipment were prepared to ensure that such activities are performed in a suitable way, always using the same procedure, in a traceable way. Moreover these SOPs were done with a future perspective of being used by students to carry out such activities and making part of their formation.

4. A SOP containing the instructions for verification of balances and analytical balances, a form to register the raw data obtained from the verification assays, excel sheets to carry out the calculations for the verification assays, verification notebooks to save and organize the verification registers and an archive to save the final results obtained from the verification assays were prepared.

   The performance of four analytical balances and one balance were verified. From the results obtained in the verification assays, it can be concluded that three analytical balances and the balance are in good working conditions with respect to the trueness, precision and drift and accordingly are appropriate for its use. One analytical balance (Scaltec SBA 32) was not in good conditions with respect to the precision. This analytical balance broke down. The technical service was requested.

5. Two SOPs were prepared for the the liquid chromatograph (HP 1050), one containing the instructions for the maintenance and the other containing the instructions for verification. With respect to the verification of this equipment, an excel sheet to carry out the necessary calculations for the verification assays and an archive to save the data obtained from these assays were prepared.

The verification of the Liquid Chromatograph HP 1050 was performed. Taking into consideration that all the obtained results from the verification assays were accepted, it can be said that the equipment is in good working conditions and it is suitable for use.

6. A SOP containing the verification instructions for the liquid chromatograph Agilent 1100 was prepared as well an excel sheet to perform the calculations of the verification assays and an archive to save the obtained chromatograms and the final results obtained.
The performance verification of the main modules of this equipment was done and it can be concluded that the equipment is in good working conditions since all the obtained results were accepted in agreement with the acceptance/ refusal criteria defined.

7. Two SOPs containing the verification instructions for the gas chromatographs HP 5890 SERIES II were prepared, one for the the gas chromatograph with TCD and another for the gas chromatograph with FID. Futhermore, an excel sheet to perform the calculations of the verification assays and an archive to save the final results of the verification assays and chromatograms were also done for each gas chromatograph.
The two gas chromatographs above mentioned were verified. From the obtained results in verification assays for the gas chromatograph with TCD, it can be said that this equipment is in good work conditions and accordingly it is suitable for its purpose. With respect to the gas chromatograph with FID all results obtained from the verification assays were accepted, so it can be said that this instrument is in good working conditions.
One SOP containing the maintenance instructions for these chromatographs was prepared.

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[33] "Noisy Detector, Including Wander, Drift, and Baseline Spikes" http://www.chem.agilent.com/en - US/Support/FAQs/GC/Hardware/7890/Pages/KB005106.aspx

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[35] WELMEC – European Cooperation in Legal Metrology. http://www.welmec.org/

[36] EURAMET - European Association of National Metrology Institutes. http://www.euramet.org/

[37] EURACHEM - European Federation of National Associations of Measurement, Testing and Analytical Laboratories. http://www.eurachem.org/

[38] CITAC – Cooperation on International Traceability in Analytical Chemistry. http://www.citac.cc/

[39] ISO – International Organization for Standardization: http://www.iso.org/iso/resources/conformity_assessment/mechanisms_for_performing_conformity_assessment.htm~

[40] ILAC - International Laboratory Accreditation Cooperation; "Why become an accredited laboratory?. http://www.ilac.org/index.php?id=300

7. Annexes

Annex I

Balances and Analytical Balances

Annex II

High Performance Liquid Chromatography

Annex III

Gas Chromatography