Do we agree with liquid chromatograph operational qualification provided from manufacturers?

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¿Estamos de acuerdo con la cualificación operacional que proporcionan los fabricantes de cromatógrafos de líquidos?

Estem d'acord amb la qualificació operacional que proporcionen els fabricants de cromatògrafs de liquids?

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RESUMEN

Las Buenas Prácticas de Fabricación incluyen el concepto de cualificación del equipo para demostrar, de manera documentada, que el equipo funciona de forma adecuada para el uso previsto y se encuentra en correcto estado de calibración y mantenimiento.
La cualificación del equipo es la base para garantizar la fiabilidad de los resultados y está formado por cuatro fases: DQ, IQ, OQ y PQ. En este artículo, se presenta la forma de llevar a cabo la OQ de un cromatógrafo de líquidos. El test OQ propuesto se basa en un conjunto de pruebas que evalúan el equipo desde un punto de vista modular. También se han discutido y establecido los criterios de aceptación, en base a los criterios utilizados por los fabricantes y al estudio exhaustivo de los resultados históricos del equipo.

Palabras clave: cualificación de equipos, cualificación operacional, cromatografía de líquidos.

SUMMARY

Good manufacturing practices include the concept of equipment qualification which aims to demonstrate in a documented way that equipment operates correctly for its intended use and that it is properly maintained and calibrated.
Equipment qualification is the base to generate quality data and is formed by four phases: DQ, IQ, OQ and PQ. This article presents how to carry out OQ for a liquid chromatograph.
A proposed OQ for a liquid chromatograph is based on a variety of tests that evaluate equipment from a modular point of view. There have also been discussed and set appropriate acceptance criteria whose selection has been based on a search and examination of the criteria used by manufacturers and an exhaustive study of equipment historical results.

Keywords: equipment qualification, operational qualification, analytical instrument qualification, liquid chromatography.

RESUM

Les Bones Pràctiques de Fabricació inclouen el concepte de qualificació de l'equip per demostrar, de manera documentada, que l'equip funciona de manera adequada per a l'ús previst i es troba calibrat i en correcte estat de manteniment.
La qualificació de l'equip és la base per garantir la fiabilitat dels resultats i està format per quatre fases: DQ, IQ, OQ i PQ. En aquest article, es presenta la forma de dur a terme la OQ d'un cromatògraf de líquids.
El test OQ proposat es basa en un conjunt de proves que avaluen l'equip des d'un punt de vista modular. També s'han discutit i establert els criteris d'acceptació, d'acord amb els criteris utilitzats pels fabricants i l'estudi exhaustiu dels resultats històrics de l'equip.

Mots clau: qualificació d'equips, qualificació operacional, cromatografia de líquids.

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INTRODUCTION

The management of laboratory equipment involves conducting a series of activities to ensure their suitability for carrying out measurements. Good manufacturing practices (GMP) include the concept of equipment qualification which aims to demonstrate, in a documented way, that the equipment operates correctly for its intended use and that it is properly maintained and calibrated [1-3].

The following activities are distinguished during the qualification process [2]:
Design Qualification (DQ) defines the functional and operational specifications of the instrument, taking user requirements into account. DQ is usually the responsibility of the seller [4-6].
Installation Qualification (IQ) establishes that the received instrument is the one that was purchased, that it has been installed properly in the selected environment and that this environment is suitable for using the instrument. IQ tends to be an activity shared between seller and owner.
Operational Qualification (OQ) proves that, in the selected environment, the instrument works in accordance with the specifications of the manufacturer. The tests and controls carried out in OQ demonstrate in a modular way that the main working parameters of the equipment are within the established limits. OQ must be carried out after IQ. It must be repeated after an important event (for example, the redeployment of the equipment or maintenance), and periodically in defined intervals (for example, annually) [4, 5, 7].
Performance Qualification (PQ) demonstrates that the instrument works in accordance with the requirements established by the user. PQ is carried out regularly during the customary use of the equipment, demonstrating that the current performance lies within the limits required for its real use. Therefore, PQ can be considered to have two aims: global analysis to prove that the complete instrument works correctly and that it is suitable for its use [3-5, 7, 8].

Note the difference between PQ and System Suitability. While PQ is referred to the instrument (as explained before), System Suitability Test is related to an analysis procedure, and it is used to verify that the chromatographic system is appropriate for a specific analysis.

Figure 1 schematically represents the relationship between the different qualification activities that must be applied to the equipment of a chemical analysis laboratory.
In this article, Operational Qualification (OQ) have been designed and applied for a liquid chromatograph working in an accredited laboratory applying validated analytical procedures [9]. Acceptance criteria have been established using the criteria recommended by manufacturers, bibliographic data and our historical results of equipment calibrations.

OPERATIONAL QUALIFICATION (OQ)

1.OQ test

The OQ design of a liquid chromatograph is based on a modular point of view and implies the definition of the parameters to be determined as well as of the corresponding acceptance criteria [5, 8-15].
The parameters that are part of the OQ of a liquid chromatograph (HPLC-UV) are presented below:
Lamp test: Determines if the lamp is in good working condition by evaluating its intensity. This test could be carried out with the equipment's software. This test could be supplemented by the verification of the wavelength accuracy.
Flow accuracy: Tests the accuracy of the flow rate (he selected flow is the one that is actually produced). The flow could be measured using a flowmeter or considering the volume of collected water during an established period of time. The volume of collected water may be measured directly using an appropriate container or by weight (taking into account water density). The deviation from the expected value is determined.
Pump and detector noise: Tests if the residual signal due to these two working modules is suitable. To study this parameter, Milli-Q water is circulated through the chromatographic system with a selected flow and afterwards, the flow is stopped. The obtained noise working without flow corresponds to the detector noise. The pump noise is calculated as the difference between the noise obtained working with a selected flow and the detector noise value obtained previously.
Mobile phase accuracy: Determines if the composition mixtures that are produced between the two channels are accurate. A compound that gives signal on UV detector is selected as a marker (such as acetone). This marker is added to one of the channels and the value selected in the equipment is checked against the experimental one at different percentages of this channel (Figure 2).

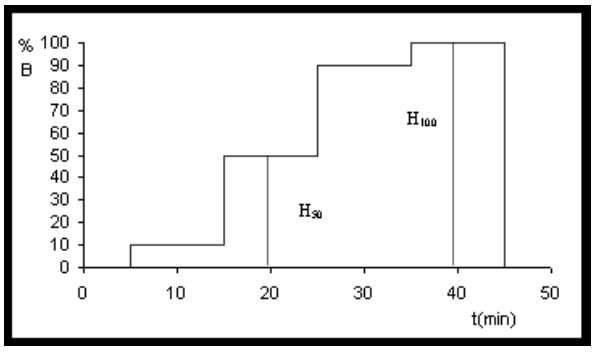

Figure 2: Study of mobile phase accuracy. Channel A: Milli-Q water; Channel B: Milli-Q water + 0.1% acetone (marker) Signal detector to 0, 10, 50, 90 and 100% of channel B.

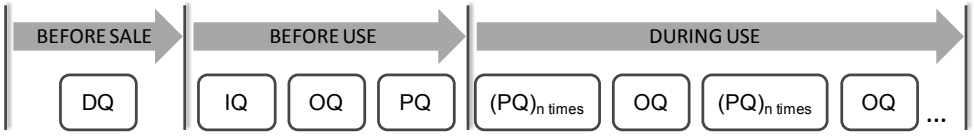

Figure 1: Qualification scheme

Temperature accuracy: Verifies that in the oven, where the column is located, the measured temperature corresponds to the selected one. It requires an external standard probe.
Injection repeatability: Allows to know if the injection of samples and standards are carried out with adequate precision. The repeatability is determined by evaluating the relative standard deviation (RSD) of the areas or heights for a given chromatographic peak obtained by injecting a certain standard several times.
Detector linearity: Assesses whether the detector is linear in the range of study by injecting different certified concentration standards. This test should be done after the injection repeatability test is passed. The injection accuracy is not necessary to be evaluated, because the injected volume remains constant during all the OQ. The resulting values are adjusted to fit a line using the least squares method and verifying the determination coefficient (R^2). Moreover, the relative standard deviation of response factors (RF) is calculated and compared to established criteria. It is important to note that some authors present the calculation of the deviation as a test of linearity, relative to the linear model, of the signal corresponding to absorbance standards of values 1.5 or 2.5 AU.
Carryover: Determines if memory effect exists after injection. Carryover is evaluated by injecting a blank after the injection of a concentrated standard. The percentage value of the residual signal obtained in the blank is compared to the standard's signal with the established acceptance criterion.

Lamp test, flow, pump and detector noise, mobile phase and oven temperature are tested changing test column by a zero dead volume union.

Table 1 summarizes the acceptance criteria of manufacturers and another studies for the parameters selected to carry out the OQ test.

2. Reagents and standards

The reagents and standards used in the OQ test are naphthalene (>99%) from Fluka, anthracene (>99%) from Merck, acetonitrile (gradient for LC) from Merck and Milli-Q water provided by Millipore. The mobile phase (MP) is a mixture of 70% acetonitrile and 30% Milli-Q water. The MP is taken as blank.
Stock solution P1 is prepared weighting 1.30 g of naphthalene and 0.050 g of anthracene and diluting to 100 mL with MP. Stock solution P2 is prepared diluting 5 mL of P1 to 25 mL with MP.
Working solutions of naphthalene and anthracene (D1, D2, D3, D4 and D5) are prepared from the stock solution P2. Working solutions concentrations are:

D1: 260 mg/L naphthalene and 10 mg/L anthracene
D2: 90 mg/L naphthalene and 3 mg/L anthracene
D3: 30 mg/L naphthalene and 1 mg/L anthracene
D4: 10 mg/L naphthalene and 0.3 mg/L anthracene
D5: 3 mg/L naphthalene and 0.1 mg/L anthracene

3. Equipment and chromatographic conditions

The equipment used is a liquid chromatograph Agilent 1100 with a binary pumping system 1100 (G1312A), vacuum degasser HP 1100 (G1322A), autosampler HP 1100 (G1313A), thermostated compartment for columns (G1316A) and diode array detector DAD HP 1100 (G1315A). The chromatographic conditions are detailed in Figure 3.

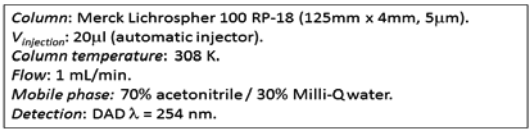

Figure 3: HPLC chromatographic conditions

PARAMETERS	REFERENCES			
	Agilent [14]	Waters [15]	Accred. Qual. Assur. [10]	Accred. Qual. Assur. [5]
Lamp test	5000 counts [range: 221-350 nm]	15 nA [225 nm]	-	-
Flow accuracy	≤ 5%	± 1%	± 2.5 %	≤ 3%
Pump and detector noise	≤ 0.05 mAU	≤ 0.1 mAU detector: ≤ 0.025 mAU	≤ 0.06 mAU	-
Mobile phase accuracy	± 2%	± 0.5%	± 1%	-
Temperature accuracy	± 2 K	± 1 K	± 1 K	± 1 K
Injection repeatability	RSD ≤ 2%	RSD ≤ 0.5%	RSD ≤ 1%	RSD ≤ 1%
Detector linearity	R^2 > 0.999 RSD (RF) ≤ 5%	Deviation% ≤ 5 at 2.5 AU	Deviation% ≤ 5 at 1.5 AU	-
Carryover	Height ≤ 0.4% Area ≤ 0.2%	< 0.1%	< 0.3%	-

Table 1: Acceptance criteria from references

4. OQ Procedure

The OQ test begins with the study of the parameters that do not require working solutions or chromatographic column: lamp test, flow accuracy, pump and detector noise, mobile phase accuracy and temperature. Afterwards, an injection sequence of the working solutions is performed in order to study the injection repeatability, the detector linearity and the carryover (Table 2).

Working solution	Number of injections	Test parameter
Blank	1	Detector linearity
D5	3	Detector linearity
D4	3	Detector linearity
D3	6	Injector repeatability and detector linearity
D2	3	Detector linearity
D1	3	Detector linearity
Blank	1	Carryover

Table 2: OQ injection sequence

RESULTS

The results corresponding to the application of the OQ test in the year 2010, and the minimum and maximum values obtained from 2001 to 2010 appear in Table 3.
For each of the OQ test parameters is presented control charts, bibliographic references and Institut Químic de Sarrià (IQS) acceptance criteria. To establish this IQS acceptance criteria is considered bibliographic data and results of calibrations and verifications carried out since 2001.

Lamp test:
The results for the lamp test in 2009 and 2010 are shown in Table 4. For this test there are no previous records.

	OQ 2009	OQ 2010
Lamp test (221-350 nm)	7444 counts	15889 counts

Table 4: Results of the OQ test between 2009 and 2010 (Agilent 1100 chromatograph)

This test is carried out frequently during routine analysis and the lamp replacement is almost annually. So, note that values obtained in 2009 and 2010 are really different, because lamps are different too.
The acceptance criterion is provided by the manufacturer. In this work, it is accepted being greater than 5000 counts in the range of interest (221-350 nm). The result is the average of the counts obtained in the wavelength range selected.
This test controls that the lamp intensity is appropriate and when it does not comply with the acceptance criterion, the lamp should be replaced by a new one.

Flow accuracy:
The results of the flow accuracy obtained between 2001 and 2010 are presented in Table 5 indicating the percentage of deviation obtained over selected flow values of 1 and 2 mL/min. The bibliographic acceptance criteria are detailed in Table 6.

	Flow accuracy	
	1 mL/min	2 mL/min
OQ 2001	0.94%	0.49%
OQ 2002	0.48%	0.18%
OQ 2003	0.02%	0.31%
OQ 2005	0.44%	0.15%
OQ 2006	1.68%	2.70%
OQ 2008	1.20%	0.80%
OQ 2009	0.62%	0.65%
OQ 2010	1.10%	0.85%

Table 5: Results of flow accuracy (expressed as % deviation).

PARAMETERS		RESULTS		
		OQ 2010	Minimum result (2001-2010)	Maximum result (2001-2010)
Lamp test (221-350 nm)		15889 counts	7444 counts (2009)	15889 counts (2010)
Flow accuracy	1 mL/min	1.1%	0.02% (2003)	1.7% (2006)
	2 mL/min	0.85%	0.15% (2005)	2.7% (2006)
Noise	Pump	0.014 mAU	0.010 mAU (2001)	0.050 mAU(2005)
	Detector	0.023 mAU	0.009 mAU (2006)	0.060 mAU (2005)
Mobile phase accuracy	10%	0.1%	<0.1% (2009)	0.4% (2006)
	50%	0.2%	<0.1% (2003)	0.8% (2009)
	90%	0.3%	0.1% (2005)	0.8% (2009)
Temperature accuracy		1.0 K	0.5 K (2009)	1.0 K (2010)
Injection repeatability	RSD	0.08%	0.04% (2009)	1.2% (2006)
Detector linearity	R^2	0.9999	0.9997	0.9999
	RSD FR	1.2%	1.2% (2010)	4.6% (2009)
Carryover	Naphthalene	0.01%	-	-
	Anthracene	0.02%	-	-

Table 3: OQ test results

	Flow accuracy
Agilent14	≤ 5%
Waters15	± 1%
Quality Assur. and Accred.10	± 2.5%
Acred. Qual. Assur. (Bedson)5	≤ 3%

Table 6: Flow accuracy bibliographic acceptance criteria.

The control chart is shown in Figure 4. In this control chart, you can distinguish two marks: NOTICE, in which the equipment is still fine but the module requires some revision and ALARM, in which the equipment is not available for use. On the basis of historical results and bibliographic acceptance criteria the IQS notice and alarm criteria have been established.

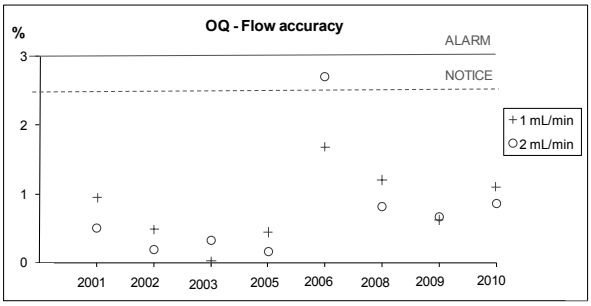

Figure 4: Control chart for flow accuracy

It was decided to choose as IQS acceptance criterion a percentage of deviation of ≤ 3%. According to historical results, this seems the most appropriate value, discarding criteria may be considered too broad. The control chart could serve to detect trends that may help to anticipate problems in the equipment.

Pump and detector noise:

The historical results from the noise of the pump and the detector are presented in Table 7. The consulted acceptance criteria are in Table 8. The noise control chart of the pump and the detector is shown in Figure 5.

	Pump noise (mAU)	Detector noise (mAU)
OQ 2001	0.010	0.013
OQ 2002	0.016	0.012
OQ 2003	0.022	0.025
OQ 2005	0.050	0.060
OQ 2006	0.020	0.009
OQ 2008	0.015	0.019
OQ 2009	0.012	0.025
OQ 2010	0.014	0.023

Table 7: Results of the pump and the detector noise.

	Noise of pump/detector
Agilent[14]	≤ 0.05 mAU
Waters[15]	≤ 0.1 mAU
Quality Assur. and Accred.[10]	≤ 0.06 mAU
Acred. Qual. Assur. (Bedson)[5]	-

Table 8: Pump and the detector bibliographic acceptance criteria.

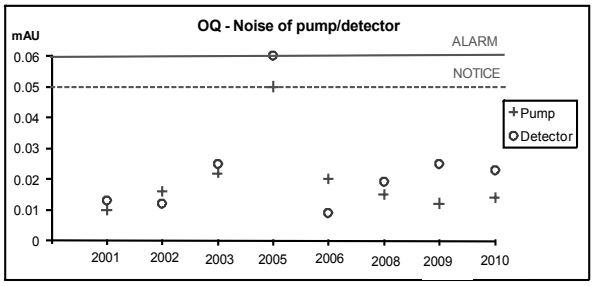

Figure 5: Control chart for the pump and the detector.

As shown in the results, the pump and the detector noise tend to behave similarly. The anomalous result obtained in 2005 was corrected shortly after the manufacturer maintenance service. The IQS acceptance criterion value has been established in 0.06 mAU. The majority of sources consulted reinforce this approach as well as historical results available.

Mobile phase composition accuracy:

The results of the mobile phase composition (MP) for selected values of 10, 50 and 90% of channel B are presented in Table 9. The bibliographic acceptance criteria are shown in Table 10. For instance, the control chart for 50% is presented in Figure 6.

	Mobile phase composition accuracy		
	10%	50%	90%
OQ 2001	9.8%	50.0%	89.5%
OQ 2002	10.1%	50.2%	90.1%
OQ 2003	9.9%	50.0%	90.1%
OQ 2005	10.1%	50.1%	90.1%
OQ 2006	9.6%	49.8%	89.7%
OQ 2008	10.2%	50.2%	90.2%
OQ 2009	10.0%	49.2%	89.2%
OQ 2010	9.9%	50.2%	89.7%

Table 9: Results of the mobile phase composition accuracy

	Mobile phase composition accuracy
Agilent[14]	± 2%
Waters[15]	± 0.5%
Quality Assur. and Accred.[10]	± 1%
Acred. Qual. Assur. (Bedson)[5]	-

Table 10: Mobile phase composition accuracy acceptance criteria

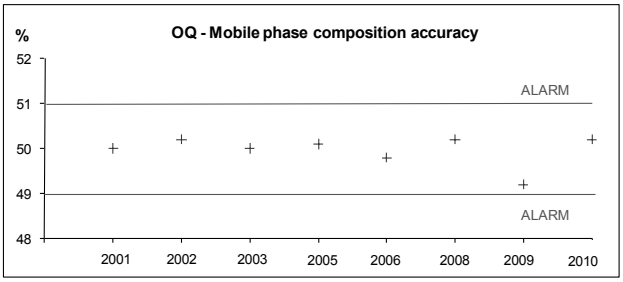

Figure 6: Control chart for mobile phase composition accuracy (50%).

As shown in Table 9, none of the deviations is greater than 1%. Thus, an acceptance criterion of 1% is considered

appropriate because larger variations in the composition cause substantial changes in retention times.

Temperature:

The study of this parameter began in 2005 and Table 11 presents the results for the oven´s temperature. Bibliographic acceptance criteria can be seen in Table 12.

	Temperature (K)	
OQ 2005	T(313 K) = 312.1 K	T(353 K) = 349.0 K
OQ 2006	T(313 K) = 312.3 K	T(353 K) = 349.3 K
OQ 2008	-	
OQ 2009	T(308 K) = 307.5 K	
OQ 2010	T(308 K) = 307.0 K	

Table 11: Results of the temperature of the oven.

	Temperature
Agilent[14]	≤ 2 K (T=313 K) / ≤ 3 K (T=353 K)
Waters[15]	± 1 K
Quality Assur. and Accred.[10]	± 1 K
Acred. Qual. Assur. (Bedson)[5]	± 1 K

Table 12: Oven temperature bibliographic acceptance criteria.

The acceptance criterion of 1K is considered too demanding because the uncertainty of the certified temperature standard is around 1K. It is established a maximum deviation of the temperature of 2 K working at 313 K.

Injection repeatability:

The results of the injection repeatability, estimated as RSD% area, are shown in Table 13 and Figure 7 (control chart). Bibliographic acceptance criteria for injection repeatability are presented in Table 14.

	Injection repeatability (RSD% area)
OQ 2001	0.08
OQ 2002	0.07
OQ 2003	0.07
OQ 2005	0.07
OQ 2006	1.22
OQ 2008	0.08
OQ 2009	0.04
OQ 2010	0.08

Table 13: Results of the injection repeatability

	Injection repeatability
Agilent[14]	RSD% ≤ 2
Waters[15]	RSD% ≤ 0.5
Quality Assur. and Accred.[10]	RSD% ≤ 1
Acred. Qual. Assur. (Bedson)[5]	RSD% ≤ 1

Table 14: Injection repeatability bibliographic acceptance criteria

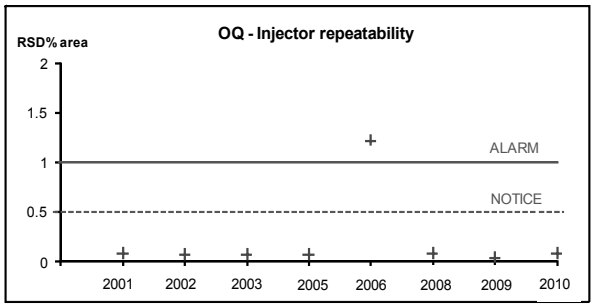

Figure 7: Control chart for injector repeatability

The 2006 result is the only value that does not comply with the IQS acceptance criteria (1%). The maintenance activities allowed solving the problem improving the injector repeatability.

Detector linearity:

The linearity results obtained are shown in Table 15. It details the results of coefficient of determination (R^2) and coefficient of variation of the response factors (CV% RF). In Table 16 the bibliographic acceptance criteria are presented. The control chart of the detector linearity which shows the response factors can be seen in Figure 8.

	Detector linearity	
	R2	RSD% RF
OQ 2001	0.9999	1.4
OQ 2002	0.9999	1.3
OQ 2003	0.9999	1.4
OQ 2005	0.9999	2.4
OQ 2006	0.9999	1.9
OQ 2008	0.9998	1.4
OQ 2009	0.9998	4.6
OQ 2010	0.9999	1.2

Table 15: Results of the linearity of the detector.

	Detector linearity
Agilent[14]	$R^2 > 0.999$ / RSD% RF ≤ 5
Waters[15]	Deviation% ≤ 5 at 2.5 AU
Quality Assur. and Accred.[10]	Deviation% ≤ 5 at 1.5 AU
Acred. Qual. Assur. (Bedson)[5]	-

Table 16: Detector linearity bibliographic acceptance criteria.

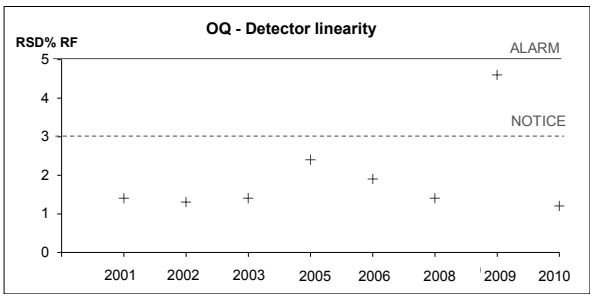

Figure 8: Control chart for the detector linearity.

The IQS acceptance criterion established for the Detector linearity are $R^2 > 0.999$ and RSD% RF ≤ 5%. The RSD% RF provides much more relevant information than R^2, because as noted, there are not significant differences in the values of R^2.

Carryover

The result for the carryover (% height) by 2010 is presented in Table 17. There are not historical records of this parameter. The bibliographic acceptance criteria are presented in Table 18. The IQS acceptance criterion value has been established in 0.2%.

	Naphthalene	Anthracene
D1 (mAU)	1057	714
Blank (mAU)	*	0.023
Blank/D1(%)	*	0.003
* Below detection limit		

Table 17: Carryover results

	Carryover
Agilent[14]	Height ≤ 0.4% / Area ≤ 0.2%
Waters[15]	< 0.1%
Quality Assur. and Accred.[10]	< 0.3%
Acred. Qual. Assur. (Bedson)[5]	-

Table 18: Carryover bibliographic acceptance criteria.

The IQS acceptance criteria for the OQ test parameters are summarized in Table 19.

	Acceptance criteria
Lamp test (221-350 nm)	> 5000 counts
Flow accuracy	≤ 3%
Noise of pump/detector	≤ 0.06 mAU
Mobile phase accuracy	≤ 1%
Temperature	≤ 2 K
Injection repeatability	RSD% area ≤ 0.5
Detector linearity	RSD% RF ≤ 5
Carry Over	≤ 0.2%

Table 19: IQS acceptance criteria

OQ test should be carried out annually. The established planning could be modified by the laboratory responsible if necessary (for instance, after troubleshooting, location or module changes, etc.). Note that the lamp test is performed independently with a higher frequency before routine analysis.

CONCLUSIONS

The OQ proposed for a liquid chromatograph is based on a series of tests that can assess each of its modules (pump, injector, oven and detector). To establish acceptance criteria suitable to the needs of the laboratory, bibliographic and values recommended by the manufacturers are used, being adapted to the historical results of equipment calibrations.

The acceptance criteria adopted for Lamp test and Detector linearity parameters coincide exactly with those provided by manufacturers. For Flow accuracy, Pump and detector noise, Temperature accuracy, Injection repeatability and Carryover parameters, the acceptance criteria established are in the middle of the values provided by the manufacturers. And finally, Mobile phase composition accuracy acceptance criteria has been established as the lowest value provided by manufacturers.

OQ designed ensure the smooth operation of the liquid chromatograph according to requirements specified by the manufacturer and the laboratory. Proper planning of these activities provides confidence in the tests carried out with the equipment. This proposal will be advantageous, even from an economic perspective, since it will prevent possible equipment failures and avoid erroneous results.

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