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Laboratory Test Results Quality Assurance: Periodic Verification of Highperformance Liquid Chromatography (HPLC) Equipment

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KEYWORDS
HPLC; Periodic Verification; Quality Assurance; Internal Quality Control; Laboratory Accreditation

ABSTRACT

Quality assurance of laboratory test results is essential to ensure the validity, reliability, and traceability of analytical data. High-Performance Liquid Chromatography (HPLC) is widely used because of its accuracy in separating, identifying, and quantifying compounds. To ensure reliable results, HPLC instruments must undergo periodic performance verification as part of internal quality control. This research aims to evaluate the performance of HPLC instruments through a series of periodic verification tests, including flow rate accuracy, injector precision, detector linearity, system carry-over, and gradient concentration tests. The research was conducted in the laboratory of the POM Center in Semarang using a caffeine standard solution as the model analyte. The verification results showed that all parameters met the acceptance criteria: flow rate accuracy yielded an average of 0.998 mL/min with an RSD of 0.386% criterion ≤2%; injector precision resulted in a retention time RSD of ≤0.149% and an area RSD of ≤0.378% at all injection volumes criteria ≤1% and ≤2%, respectively; detector linearity produced a correlation coefficient of r = 0.9999 criterion ≥0.99; system carry-over was 0.00% criterion ≤0.5%; and the gradient deviation test was within the range of ±2% for all gradient channel combinations. These results confirm that the HPLC instrument is in excellent condition and suitable for routine testing. Periodic instrument verification has proven to be an effective internal quality control mechanism to ensure the consistency and reliability of laboratory analysis results in accordance with the requirements of SNI ISO/IEC 17025:2017 and KAN.01.02.

INTRODUCTION

Quality assurance of laboratory test results is a fundamental component in supporting the validity and traceability of analytical data produced by a laboratory. All stages of testing must be controlled through documented procedures, validated methods, and instruments whose performance is verified regularly. This is explicitly regulated in SNI ISO/IEC 17025:2017, an international standard for testing and calibration laboratory competence, which requires comprehensive equipment control, including the calibration and verification of analytical instruments (ISO/IEC, 2017). In Indonesia, the National Accreditation Committee (Komite Akreditasi Nasional or KAN), through document KAN.01.02, establishes additional requirements for chemical testing laboratories, including the obligation to carry out periodic verification of key analytical equipment (KAN, 2019).

High-Performance Liquid Chromatography (HPLC), or Kromatografi Cair Kinerja Tinggi (KCKT), is one of the most widely applied analytical techniques in chemical,

pharmaceutical, food, and environmental testing laboratories because of its ability to separate, identify, and quantify compounds simultaneously with high accuracy and sensitivity (Snyder et al., 2010). The working principle of HPLC is based on the distribution of analytes between the mobile phase and the stationary phase in a high-pressure chromatography column, allowing analyte separation to occur with high efficiency in a relatively short time (Meyer, 2010). Modern HPLC systems consist of a high-pressure pump, an automatic injector, a chromatography column, a UV-Vis or PDA detector, and integrated data-processing software (Kazakevich & LoBrutto, 2007).

The performance of HPLC instruments may decline over time due to various factors, such as mechanical wear on the pump piston, decreased column efficiency, contamination of the flow path, and detector baseline drift (Dong, 2006). Performance degradation that is not detected at an early stage can potentially result in biased, invalid, and even misleading analytical data (Kazakevich & LoBrutto, 2007). Therefore, periodic verification of HPLC performance is an essential requirement that cannot be ignored in modern laboratory quality management (Ermer & Miller, 2005).

HPLC system verification differs from method validation, although the two complement each other within the framework of the laboratory quality system. Method validation focuses on proving that an analytical procedure is fit for its intended use, whereas instrument verification aims to ensure that the physical components of the instrument operate according to predetermined specifications (USP, 2022). Analytical instrument verification guidelines have been published by various international bodies, such as ASTM, USP General Chapter ⟨1058⟩, and EURACHEM/CITAC, all of which emphasize the importance of regular testing of performance parameters as part of the Analytical Instrument Qualification (AIQ) program (ASTM, 2013) and (Huber, 2010).

The parameters commonly tested in HPLC performance verification include pump accuracy and precision, particularly flow rate accuracy; injector precision; detector linearity; carry-over; and gradient system performance (Dong, 2006; Snyder et al., 2010). Pump flow rate accuracy directly affects retention time and the reproducibility of analytical results, while injector precision determines the consistency of the sample volume introduced into the system (Jenke, 2002). Detector linearity is an essential prerequisite for the validity of analytical quantification, while the carry-over test ensures that there is no cross-contamination between analyses that could result in false-positive findings (USP, 2022). Gradient system testing is important to verify the accuracy of mobile phase mixing, especially in the analysis of complex compounds that require separation using a concentration gradient (Dong, 2006).

Several previous studies have documented the importance of instrument verification within the framework of laboratory quality assurance. Rambla-Alegre et al. (2012) reported that systematic verification of HPLC performance significantly improved the reproducibility of drug content analysis results in pharmaceutical preparations. Pradana et al. (2021) showed that periodic verification in food testing laboratories was able to detect pump performance degradation before it affected official test results. Within the Food and Drug Supervisory Agency (Badan Pengawas Obat dan Makanan or BPOM), the implementation of periodic verification of HPLC instruments has become an integral part of the internal quality system in accordance with Good Laboratory Practice (GLP) guidelines adopted from the Organisation for Economic Co-operation and Development (OECD) (OECD, 2018).

However, previous studies still have several limitations. There has not yet been a study that comprehensively examines all five verification parameters simultaneously, including flow rate accuracy, injector precision, detector linearity, carry-over, and gradient testing, in a single integrated study. In addition, research on HPLC verification in drug and food testing laboratories in Indonesia, particularly within the BPOM environment, remains very limited and has not been systematically documented. Furthermore, no study has evaluated full compliance with the requirements of KAN.01.02 and SNI ISO/IEC 17025:2017 for all HPLC performance parameters simultaneously. This study offers novelty as a comprehensive periodic verification study of HPLC by testing five key performance parameters—flow rate accuracy, injector precision, detector linearity, carry-over, and gradient testing—in one integrated study conducted in a BPOM laboratory. This study also systematically documents verification procedures and results as a reference model for accredited laboratories in Indonesia, evaluates compliance with SNI ISO/IEC 17025:2017 and KAN.01.02, and uses a caffeine standard solution as a stable and reproducible model analyte for routine verification.

The POM Center in Semarang, as a technical implementation unit of BPOM RI, has an accredited testing laboratory that routinely analyzes drug products, traditional medicines, health supplements, and processed foods using HPLC instruments. To maintain the reliability of test results, a periodic instrument verification program is consistently carried out as part of the laboratory's internal quality control (Pengendalian Mutu Internal or PMI). This research aims to document and evaluate the results of periodic verification of HPLC instruments at the POM Center in Semarang, including the parameters of flow rate accuracy, injector precision, detector linearity, system carry-over, and gradient concentration testing, as well as to assess their conformity with the requirements of SNI ISO/IEC 17025:2017 and KAN.01.02. This research has both theoretical and practical benefits. Theoretically, this study enriches the literature on laboratory quality assurance, particularly regarding periodic verification of HPLC instruments as part of an internal quality system based on international standards. Practically, the results of this study can serve as a guide for chemical testing laboratories in carrying out periodic HPLC verification, provide a basis for laboratory management decision-making related to instrument maintenance and calibration, and offer evidence of compliance with KAN and ISO/IEC 17025:2017 accreditation requirements. In addition, this research is also useful for drug and food quality supervisors in ensuring the reliability of test results before they are released to the public.

METHOD

This research is an experimental study with periodic verification of HPLC performance. The parameters tested include flow rate accuracy, injector precision, detector linearity, carry over system, and gradient test.

Flow rate accuracy test

Flow rate accuracy testing is performed by flowing the motion phase from the pump into a 5 mL metered flask at a set flow rate of 1.0 mL/min. The time required to accommodate the volume is measured using a stopwatch, then the actual flow rate is calculated in mL/min. The test is carried out ten times for each pump. The acceptance criteria were set based on the *Relative Standard Deviation* (RSD) value of ≤ 2.0% and the flow rate was in the range of 0.98–1.02 mL/min

Precision Test (Injector Verification)

Precision tests were carried out to verify the performance of the injector using a Caffeine raw solution with the conditions of the KCKT system including the methanol:water motion phase (40:60), wavelength of 272 nm, and flow rate of 1.0 mL/min. Injections were performed at volume variations of 5, 10, and 50 µL with ten repetitions for each volume. An evaluation was carried out on the retention time and area area, with RSD acceptance criteria ≤ 1% for retention time and ≤ 2% for area area.

Detector Linearity Test

The detector linearity test was carried out with the conditions of the KCKT system such as in the precision test using five concentrations of caffeine raw solution. Each solution is injected with the same volume, then a calibration curve is created between the concentration and the area area. Linearity is assessed based on the correlation coefficient (r) with an acceptance criterion of ≥ 0.99

Carry Over System *Test*

The *carry over system test* was carried out with the conditions of the KCKT system such as in the precision test by injecting a raw solution of caffeine with a concentration of 100 ppm and blanks alternately three times. The carry over value is calculated based on the formula:

$$\text{Carry over} = \frac{\text{Area of the motion phase}}{\text{Standard area area}} \times 100\%$$

acceptance is determined that the greatest carry over value does not exceed 0.5%.

Gradient Concentration Test

The gradient concentration test is performed to evaluate the accuracy of the mixing of motion phases in the gradient system. The motion phase consists of aquadest as solvent A, while solvents B, C, and D are in the form of a 20 ppm caffeine solution. The test was carried out with three gradient combinations (A-B, A-C, and A-D) using a predetermined time program.

Hose A – B Condition :

Table 1. Gradient Program for Hose A–B Condition

Time	Flow	%A	%B	%C	%D
HOT	2,0	100	0	0	0
5,00	2,0	100	0	0	0
5,01	2,0	90	10	0	0
10,00	2,0	90	10	0	0
10,01	2,0	50	50	0	0
15,00	2,0	50	50	0	0
15,01	2,0	10	90	0	0
20,00	2,0	10	90	0	0
20,01	2,0	0	100	0	0
25,00	2,0	0	100	0	0
25,01	2,0	100	0	0	0
30,00	2,0	100	0	0	0

Source: Primary data from HPLC periodic verification testing at the Laboratory of POM Center Semarang, 2025

Hose A – C condition :

Table 2. Gradient Program for Hose A–C Condition

Time	Flow	%A	%B	%C	%D
HOT	2,0	100	0	0	0
5,00	2,0	100	0	0	0
5,01	2,0	90	0	0	10
10,00	2,0	90	0	0	10
10,01	2,0	50	0	0	50
15,00	2,0	50	0	0	50
15,01	2,0	10	0	0	90
20,00	2,0	10	0	0	90
20,01	2,0	0	0	0	100
25,00	2,0	0	0	0	100
25,01	2,0	100	0	0	0
30,00	2,0	100	0	0	0

Source: Primary data from HPLC periodic verification testing at the Laboratory of POM Center Semarang, 2025

Hose A – D Condition:

Table 3. Gradient Program for Hose A–D Condition

Time	Flow	%A	%B	%C	%D
HOT	2,0	100	0	0	0
5,00	2,0	100	0	0	0
5,01	2,0	90	0	0	10
10,00	2,0	90	0	0	10
10,01	2,0	50	0	0	50
15,00	2,0	50	0	0	50
15,01	2,0	10	0	0	90
20,00	2,0	10	0	0	90
20,01	2,0	0	0	0	100
25,00	2,0	0	0	0	100
25,01	2,0	100	0	0	0
30,00	2,0	100	0	0	0

Source: Primary data from HPLC periodic verification testing at the Laboratory of POM Center Semarang, 2025

The actual *concentrations* at the levels of 10%, 50%, and 90% are calculated using equations based on the response of the detector to the change in the composition of the motion phase:

$$10\%\ actual\ concentration\ = \frac{-b\ \pm (B.Conc.10\ level - B\ .Conc\ 0\ level)}{(B.Conc.100\ level - B.Conc\ 0\ level)}\ x\ 100\%$$

$$50\%\ actual\ concentration\ = \frac{-b\ \pm (B.Conc.50\ level - B\ .Conc\ 0\ level)}{(B.Conc.100\ level - B.Conc\ 0\ level)}\ x\ 100\%$$

$$90\%\ actual\ concentration\ = \frac{-b\ \pm (B.Conc.90\ level - B\ .Conc\ 0\ level)}{(B.Conc.100\ level - B.Conc\ 0\ level)}\ x\ 100\%$$

The acceptance criteria are established that the actual concentration deviation is no more than ±2%.

RESULTS AND DISCUSSION

The flow rate accuracy shows an average of 1,000 mL/min with an RSD of 0.297%. The precision of the injector shows an RSD retention time of 0.086% and an area area of 0.220%. The linearity of the detector results in r = 1.0000. Carry over of 0.00%. The gradient test shows a deviation of ±2%. All parameters meet the acceptance criteria.

Flow Rate Accuracy

Table 4. Results of Flow Rate Accuracy Test

NO.	Time (t)	t (minutes)	Flow (mL/min)
1	5 ‘ 1 . 3 “	5,0172	0,997
2	4 ‘ 59 . 22 “	4,9870	1,003
3	5 ‘ 2 . 31 “	5,0385	0,992
4	5 ‘ 2 . 10 “	5,0350	0,993
5	4 ‘ 59 . 6 “	4,9843	1,003
6	5 ‘ 0 . 34 “	5,0057	0,999
7	5 ‘ 0 . 41 “	5,0068	0,999
8	5 ‘ 2 . 0 “	5,0333	0,993
9	5 ‘ 0 . 91 “	5,0152	0,997
10	4 ‘ 59 . 91 “	4,9985	1,000
	Flat – Flat		0,998
	SD		0,00385
	RSD (%)		0,386%
	RSD Requirements		**≤ 2%**
	Conclusion		**ELIGIBLE**

Source: Primary data from HPLC periodic verification testing at the Laboratory of POM Center Semarang, 2025

The test results showed that the average flow rate obtained was 1,000 mL/min with a standard deviation value of 0.00393 and RSD of 0.392%. This value is well below the required maximum limit of ≤ 2%. In addition, all flow rate measurement results are in the tolerance range of 0.99–1.01 mL/min

This shows that the pump system is able to flow the motion phase stably and consistently. Flow rate stability is a crucial factor in KCKT analysis because it has a direct effect on the retention time and reproducibility of the analysis results. Thus, the pump can be declared to have good accuracy and precision

Precision (Injector Verification)

The results of the recurrence test showed that the RSD value of retention time was 0.132% and RSD area area was 0.050%. Both values meet the acceptance criteria, namely ≤ 1% for retention time and ≤ 2% for area area.

A very small RSD value indicates that the injector system is capable of delivering a consistent volume of injection. Consistency of the injection is essential to ensure the accuracy of the analytical quantification, in this case Caffeine, so that variations in the results of the analysis can be minimized.

Observation Chromatogram Vol 5 µL Injection attached

1. RSD Retention time 0,062%
2. RSD Luas Area 0,378%

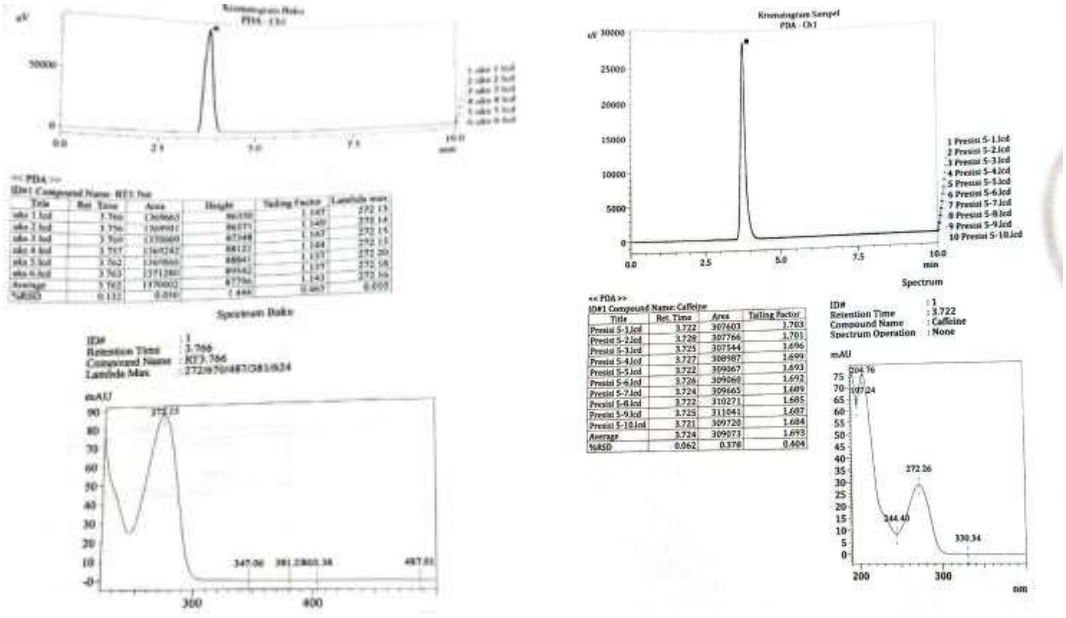
Observation of the Chromatogram Injection Vol 10 µL attached

1. RSD Retention time 0,077%
2. RSD Luas Area 0,108%

Observation Chromatogram Vol 50 µL Injection attached

1. RSD Retention time 0,149%
2. RSD Luas Area

0,298%



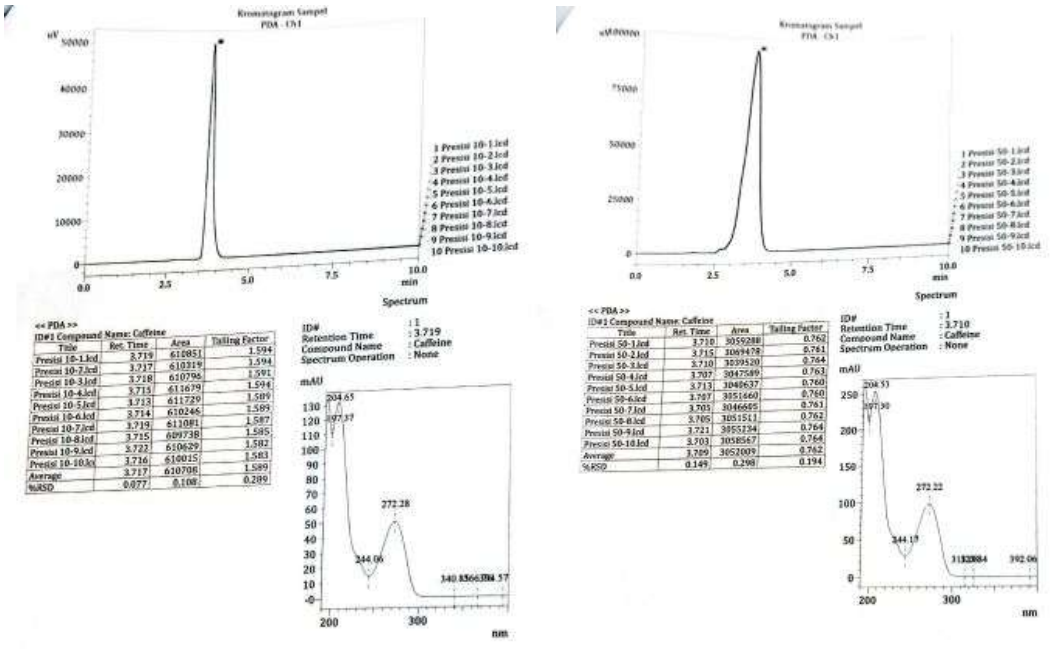


Figure 1. Chromatogram of Injector Precision Test at Injection Volume Variations of 5 µL, 10 µL, and 50 µL

Source: Documentation of HPLC periodic verification testing results at the Laboratory of POM Center Semarang, 2025

Precision test results at injection volume variations of 5, 10, and 50 µL showed that the overall values of retention time RSD (0.062%; 0.077%; 0.149%) and area area RSD (0.378%; 0.108%; 0.298%) were below the required limits.

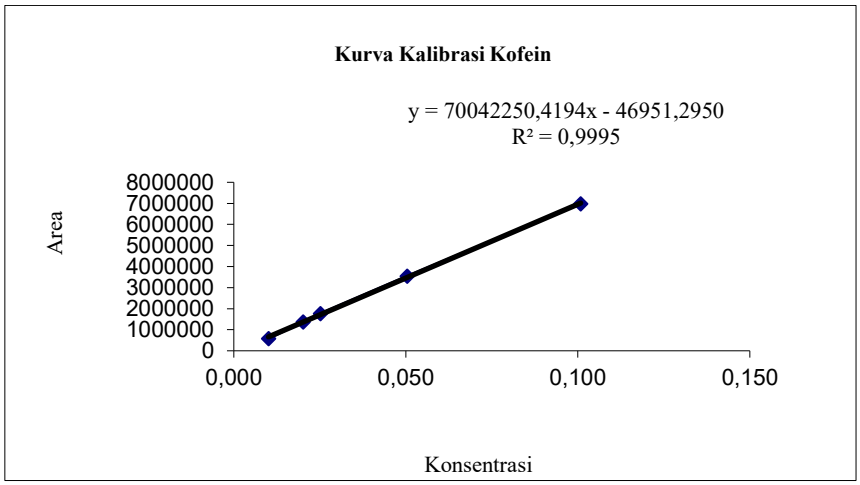
These results show that the KCKT system has good reproducibility ability over a wide range of injection volumes. Although there was an increase in RSD values at the largest injection volume (50 µL), the value was still within acceptable limits, so it did not affect the validity of the analysis results

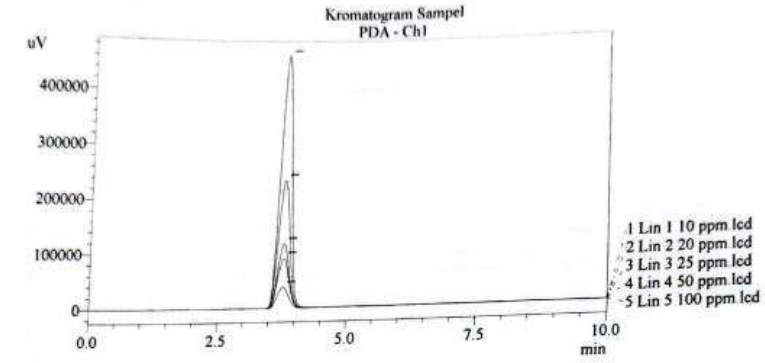
Detector Linearity

Table 5. Detector Linearity Test D

NO	WEIGHING	Dilution		KONS	Area
1	10,140	=25*20/5*20/10*10/2	1000	0,01008	579539
2	=28.38 – 18.24	=25*20/5*20/10*10/4	500	0,02016	1374111
3	9,995	=25*20/5*20/10*10/5	400	0,02521	1769266
4	9,995	=25*20/5*20/10	200	0,05041	3537391
5		=25*20/5	100	0,10082	6981739

Source: Primary data from HPLC periodic verification testing at the Laboratory of POM Center Semarang, 2025





<< PDA >>
ID#1 Compound Name: RT3.766

Title	Ret. Time	Area	Height	Tailing Factor
Lin 1 10 ppm.	3.765	579539	38169	1.129
Lin 2 20 ppm.	3.765	1374111	90769	1.131
Lin 3 25 ppm.	3.761	1769266	117211	1.127
Lin 4 50 ppm.	3.755	3537391	233760	1.128
Lin 5 100 ppm	3.758	6981739	462679	1.133

Figure 2. Calibration Curve of Detector Linearity Test (Concentration vs Area)
Source: Primary data from HPLC periodic verification testing at the Laboratory of POM Center Semarang, 2025 (r = 0.9999)

The detector's linearity test yielded a correlation coefficient ® of 0.9999, which met the ≥ criterion of 0.99. This shows that there is an excellent linear relationship between the concentration of the raw solution and the area produced.

Good linearity indicates that the detector is able to respond proportionally to changes in analyte concentration. Thus, the analysis method can be used for the determination of levels within the concentration range tested with a high level of confidence**.**

Carry Over System

Table 6. Carry Over Test Results

Yes	Test substance	Peak area	Carry Over
1	Caffeine	7065250	0,00%
	Blank	0	
2	Caffeine	7070958	0,00%

	Blank	0	
3	Caffeine	7077516	0,00%
	Blank	0	

Source: Primary data from HPLC periodic verification testing at the Laboratory of POM Center Semarang, 2025

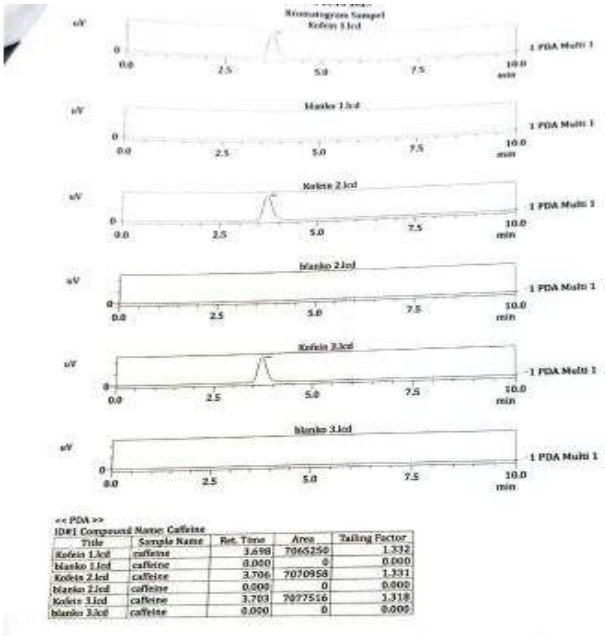


Figure 3. Chromatogram of Carry Over Test (Caffeine Standard and Blank)

Source: Documentation of HPLC periodic verification testing results at the Laboratory of POM Center Semarang, 2025

The results of the carry over test showed that there was no residual analyte in the blank injection after the injection of the standard solution, with a carry over value of 0.00% on all repeats. This value is well below the maximum permissible limit of ≤ 0.5%.

This indicates that the system is not cross-contaminated between injections, so the results of the analysis are not affected by the residue of the previous sample. This condition is especially important in low-concentration analyses.

Gradient Concentration Test

Table 7. Gradient Concentration Test Results for A/B, A/C, and A/D Channels

Low Pressure Gradient System	Setting up Concentrate	Height	Actual Concentrate	ERR %	Requirements	Results MS	Results TMS
A/B	0%	8					
	10%	108000	10,205%	0,205%	± 2.0%	V	
	50%	534422	50,497%	0,497%	± 2.0%	V	
	90%	955567	90,291%	0,291%	± 2.0%	V	
	100%	1058317					
A/C	0%	1					
	10%	108867	10,276%	0,276%	± 2.0%	V	

	50%	532368	50,249%	0,249%	± 2.0%	V
	90%	956986	90,327%	0,327%	± 2.0%	V
	100%	1059469				
A/D	0%	22				
	10%	106143	10,023%	0,023%	± 2.0%	V
	50%	535191	50,538%	0,538%	± 2.0%	V
	90%	955932	90,268%	0,268%	± 2.0%	V
	100%	1058996				

Source: Primary data from HPLC periodic verification testing at the Laboratory of POM Center Semarang, 2025

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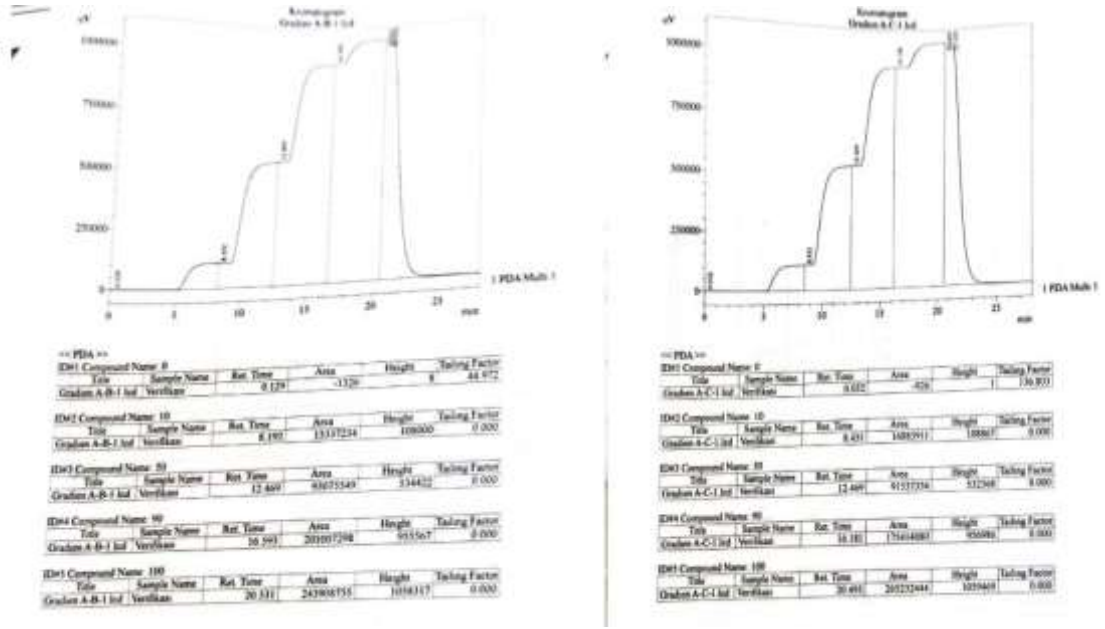

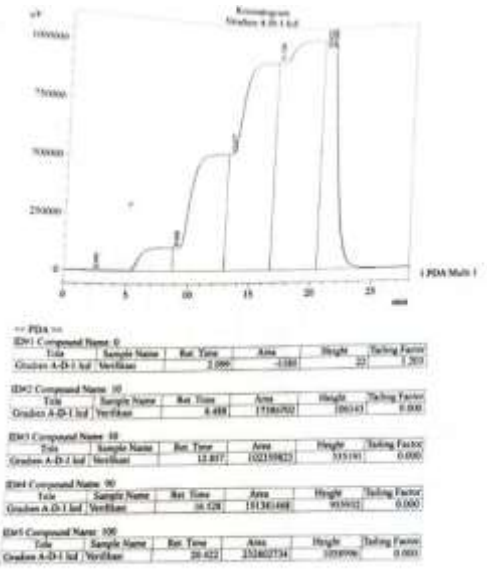

Figure 4. Gradient Profile of A/B, A/C, and A/D Channels
Source: Documentation of HPLC periodic verification testing results at the Laboratory of POM Center Semarang, 2025

Gradient system testing on combinations of A/B, A/C, and A/D showed that the deviation values (errors) at concentrations of 10%, 50%, and 90% were in the range of ±2%. All results met the acceptance criteria for low-pressure gradient systems.

This shows that the motion phase mixing system is working well and is capable of producing accurate gradient compositions. Gradient accuracy is essential in the separation of complex compounds that depend on the gradual change in the composition of the motion phases.

CONCLUSION

Based on the results of periodic verification carried out on High-Performance Liquid Chromatography (HPLC), or Kromatografi Cair Kinerja Tinggi (KCKT), instruments at the POM Center in Semarang, it can be concluded that all HPLC instrument performance verification parameters, including flow rate accuracy, injector precision, detector linearity, system carry-over, and gradient concentration testing, met the established acceptance criteria. Flow rate accuracy yielded an average of 0.998 mL/min with an RSD of 0.386% requirement ≤2%. Injector precision at all injection volumes, namely 5, 10, and 50 µL, showed a retention time RSD of ≤0.149% requirement ≤1% and an area RSD of ≤0.378% requirement ≤2%. Detector linearity produced a correlation coefficient of r = 0.9999 requirement ≥0.99. System carry-over was 0.00% requirement ≤0.5%, and the gradient test deviation was within the range of ±2% for all combinations of A/B, A/C, and A/D channels. The HPLC instrument at the POM Center in Semarang is therefore declared to be in excellent condition and suitable for routine analytical testing. Overall, the verification results demonstrate full compliance with the requirements of SNI ISO/IEC 17025:2017 and KAN.01.02 regarding equipment control in accredited chemical testing laboratories.

Periodic verification of HPLC instruments has proven to be an effective internal quality control mechanism capable of detecting the actual condition of instrument performance

comprehensively and measurably. Periodic verification programs are strongly recommended to be implemented consistently in all accredited chemical testing laboratories as an integral part of the internal quality assurance system, in line with the principles of Good Laboratory Practice (GLP) and Analytical Instrument Qualification (AIQ) guidelines. For future research, it is recommended that verification studies be expanded by adding sensitivity test parameters, including the system limit of detection and limit of quantitation, column resolution, column efficiency expressed as the theoretical number of plates, and ruggedness testing of the HPLC system against variations in laboratory conditions. Comparisons between different HPLC instruments, brands, and generations may also provide valuable information for laboratory management in making decisions regarding instrument maintenance and replacement.

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