KESANS: International Journal of Health and Science
2808-7178 / 2808-7380
http://kesans.rifainstitute.com/index.php/kesans/index

Cisplatin, Chemotherapy Agent: Production of Reference Standard for Products Determination

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Article Information
Submitted: **23 February 2026**
Accepted: **09 March 2026**
Publish: **15 March 2026**

Keyword: Cisplatin; Cytotoxic; Reference Standard; Assigned Value; Reverse Phase-HPLC;

Abstract
Introduction: *Cisplatin is a cytotoxic drug that is used as cancer therapy. To ensure the success of treatment, the drug products must be examined using reference standards.* ***Objective:*** *Cisplatin has been developed as a reference standard for the quantification of products, both pre-market and post-market.* ***Method:*** *The conducted tests included FTIR assays, water content analysis, impurity assay, and homogeneity evaluation.* ***Result and Discussion****: The RP-HPLC assay results demonstrated the qualified suitability, linearity, and accuracy. The homogeneity test yielded a linearity represented by the equation y = 5.668.152,75x + 411,5 (r =1). The response factor deviation indicated an accuracy of 0,23%, and homogeneity was confirmed with* F_{cal} *= 0,17 (*Ft_{able} *= 3,02).* ***Conclusions:*** *Cisplatin can be used as a reference standard, with an assigned value of 97,15% (U = 1,43%).*

How to Cite **Christy Ambarsari/Cisplatin, Chemotherapy Agent: Production of Reference Standard for Products Determination, Vol. 5, No. 6, 2026**
DOI https://doi.org/10.54543/kesans.v5i6.590
e-ISSN/p-ISSN **2808-7178 / 2808-7380**
Published by **CV Rifainstitut/KESANS: International Journal of Health and Science**

Introduction

In Indonesia, cancer is the third leading cause of mortality (Kementerian Kesehatan, 2022), and the number of people affected grows year after year. In 2050, it is predicted to increase by around 70 percent, with 400 thousand new cases each year and a death rate of up to 240 thousand cases (Kementerian Kesehatan, 2022; Kementerian Kesehatan, 2025). Many patients arrive with severe symptoms, reducing therapy success and increasing treatment costs. Furthermore, delays in early detection of cancer will have an impact on the psychology of patients and their families. (Kementerian Kesehatan, 2025). The most prevalent cancers among women are breast cancer and cervical cancer, whereas men are more likely to develop lung cancer (Kementerian Kesehatan, 2022).

Chemotherapy medications are widely used in cancer treatment and are cytotoxic, meaning they can kill both healthy cells and malignant cells. Facilities that encounter this sort of drug must be serious in their handling, both while delivering drugs by health workers and dealing with biomedical waste, because their mutagenic, teratogenic, or carcinogenic nature is dangerous for officers and the environment (Shamran & Ali, 2022).

Cisplatin is a medicine used to treat head-neck cancer (Mareta Rindang et al., 2021), testicles, ovaries, bladder, cervical, lung cancer, and malignant tumor between the ages of 0-18 years (Mushtaq et al., 2025). It was initially approved in the 1970s, and platinum-based chemotherapy agents (Muscella et al., 2024). When cisplatin is administered to the patient intravenously, the drug enters the cell and undergoes aquaculture, where the chloride component is replaced by water molecules in the conditions of the intracellular environment with low chloride. The transformation activates the molecule and causes it to be attached to the guanine base at the N7 site. The following step predicts that there will be an obstruction to the process of DNA replication and transcription, causing the cell cycle to cease and produce apoptosis, or cancer cell death. Dyspnea (Sinukaban, 2024) and ototoxicity (Saputra et al., 2024) are the possible side effects of cisplatin.

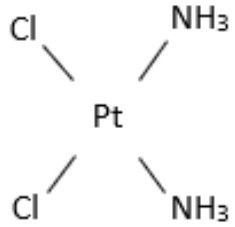


Figure 1. Cisplatin Structure

There are around ten varieties of cisplatin products on post-market that have been registered in Indonesia. The products must be quantitatively examined during the pre- and post-market phase to fulfill cancer therapy goals. Drug regulatory agencies must guarantee that laboratory assays have been carried out by manufacturers and agency laboratories, to prevent patients from consuming substandard products. A reference standard is required for quantitative testing of cisplatin products to ensure that laboratory test results are reliable and unbiased.

Other countries have issued cisplatin reference standard, but the expense is prohibitively exorbitant, raising production, monitoring, medicine, and health insurance expenditures. This study aims to produce cisplatin reference standards according to the Pharmacopoeia monograph and other literature, so that it can be used to determine the value of pre-market dan post-market products. ISO 17034:2016 is also used as the official

standard throughout production process. This study uses reverse phase – high performance liquid chromatography (RP-HPLC) with PDA detector.

Method

Materials

The substances purchased were EPRS cisplatin **batch 7** (Sigma Aldrich, CAS no. 15663-27-1) and cisplatin (TCI, cat. D3371). Other materials included potassium bromide (Merck, cat. 1.04907), sodium 1-octanesulfonate monohydrate (Sigma Aldrich, cat. 74884), tetrabutylammonium hydrogen sulfate (Himedia, cat. GRM7531), potassium dihydrogen phosphate (Supelco, cat. 1.04873), sodium hydroxide (Supelco, cat. 1.06462), 0,9% sodium chloride (Otsuka), ethyl acetat (Supelco, cat. 1.09623), methanol (Smart-Lab, cat. H-1058), N,N-dimethylformamide (Supelco, cat. 8.22275), and water. The materials used are HPLC grade.

Apparatus

The following instruments were used in the study: FTIR spectroscopy (Shimadzu Prestige21), coulometry Karl Fischer (Mettler Toledo C20), high-performance liquid chromatography instrument (Shimadzu/LC 20 AT with autosampler), analytical balance (Mettler Toledo), hotplate and stirrer (Thermo Scientific), pH meter (Mettler Toledo), micro-scale (Mettler Toledo), degasser (Bransonic), micropipettes (Eppendorf). Several stages of work were carried out in a cytotoxic safety cabinet at Biosafety Level 2 Laboratory using specific personal protective equipment.

1. Identification

FTIR

Pellet A was prepared in a vacuum and contains 2 mg of cisplatin and 200 mg of potassium bromide. Pellet B was made in the same procedure, with 2 mg of EPRS cisplatin and 200 mg of potassium bromide. The two pellets were tested alternately using the FTIR instrument, and the wavelength obtained was compared.

Water Content

The moisture content was carried out by weighing about 25 mg of cisplatin each and then measured by coulometric Karl Fischer (KF) 6 times.

Melting Point

First, differential scanning calorimeter (DSC) was verified with tin or stannum (Sn). A total of 2 mg of cisplatin was weighed, pressed into an aluminum pan, and measured. The test was conducted three times.

2. Impurity Test

Mobile Phase

1,08 g of sodium 1-octanesulfonate monohydrate; 1,7 g of tetrabutylammonium hydrogen sulfate; 2,72 g of potassium dihydrogen phosphate was weighed, then added to 1000 mL of water, and the pH was adjusted to 5,9 using 1 N NaOH.

Diluent

0,9% sodium chloride

Validation
The first step was suitability test, which ensured that the system and analysis conditions were appropriate. A total of 5 mg of cisplatin was weighed, transferred to 5-mL volumetric flask, diluted with diluent to volume, and measured five times under the following analysis conditions:

Instrument	: HPLC Shimadzu
Column	: L7, 250 x 4,6 mm i.d.; 3 µm
Column temp	: 35°C
Flow rate	: 0,5 mL/ minute
Detector	: 210 nm PDA
Injection volume	: 10 µL

Linearity was carried out using 5 different cisplatin solutions with concentration ranging from 50% - 150%, each of which was injected into a duplo, and a calibration curve was created. Accuracy was achieved by preparing 1 mg/mL cisplatin solution in 2 separate volumetric flasks and measuring each 5 times. The area acquired was calculated as response factor deviation (dRF) with an acceptable level of no more than 2%.

Impurity Assay
Amount of 1 mg/mL cisplatin were generated in up to 3 volumetric flasks and then injected each with triplo. All peaks, including primary peak and impurities, had their retention times recorded. The percentage of impurities were calculated using the following formula:

$$\%impurity = \frac{impurity\ area}{main\ area + \sum impurity\ area}$$

3. Homogeneity Test

Mobile phase
Ethyl acetate: Methanol: Dimethylformamide: Water (25: 16: 5 :5).

Diluent
Dimethylformamide (DMF)

Validation
Suitability and linearity assay were performed using 1 mg/mL cisplatin solution in DMF according to the impurity method. The conditions for the analysis were as follows:

Instrument	: HPLC Shimadzu
Column	: L8, 250 x 4,6 mm i.d.; 5 µm
Flow rate	: 2,0 mL/ minute
Detector	: 310 nm PDA
Injection volume	: 40 µL

Accuracy was conducted by preparing 1 mg/mL EPRS cisplatin solution in DMF in up to 4 volumetric flasks and measuring them 5 times each. Accuracy data is utilized as a control standard to quantify cisplatin in the homogeneity assay.

Homogeneity Assay

A total of 200 vials containing 50 mg of cisplatin were produced, with 10 vials selected at random. Each of the selected vials was prepared of 1 mg/mL in DMF and then measured in duplo. The solution was generated during 2 sessions. The area acquired was used to calculate the cisplatin concentration, which was then statistically evaluated using a single factor ANOVA.

Value Determination

The value of cisplatin as a reference standard is calculated in assigned value (AV) by considering the moisture content and organic impurities using the below formula.

$$AV = (100\% - water\ content)x\ \frac{(100\% - impurity)}{100\%}$$

Results and Discussion

1. Identification

FTIR

IR spectroscopy is also known as vibration spectroscopy or molecular spectroscopy due to its capability to analyze specific functional groups in substances. Some FTIR instruments evaluate organic compounds between 4000 – 400 cm^{-1} (Fadlelmoula et al, 2022; Hong et al, 2021) as well as 2000 – 400 cm^{-1} for fingerprint region. However, FTIRs with various manufacturers measures at a frequency of 3600–1200 cm^{-1} with fingerprint area of 1500–500 cm^{-1} (Mukai et al, 2022) . The wavelength measured in this study are shown in Table 1. FTIR is widely employed in analytical chemistry, both qualitatively (Hong et al., 2021) and quantitatively (Fadlelmoula et al., 2022), because it is non-destructive, non-invasive, requires only a little sample, and can be applied to wide range of materials, including solids, liquids, powders, etc (Johnson, Walsh, Naiker, & Ameer, 2023). In this experiment, cisplatin contains at least six wavelengths that are identical to EPRS cisplatin. The difference in wavelength between the two materials is less than 4 cm^{-1}.

Table 1
FTIR Wavelength Results

EPRS Cisplatin (cm^{-1})	Cisplatin (cm^{-1})
502.48	503.44
738.77	737.80
798.56	798.56
868.97	868.97
996.28	997.24
1102.37	1101.40
1298.15	1299.11
1312.62	1311.65
1405.20	1405.20
1539.26	1538.30

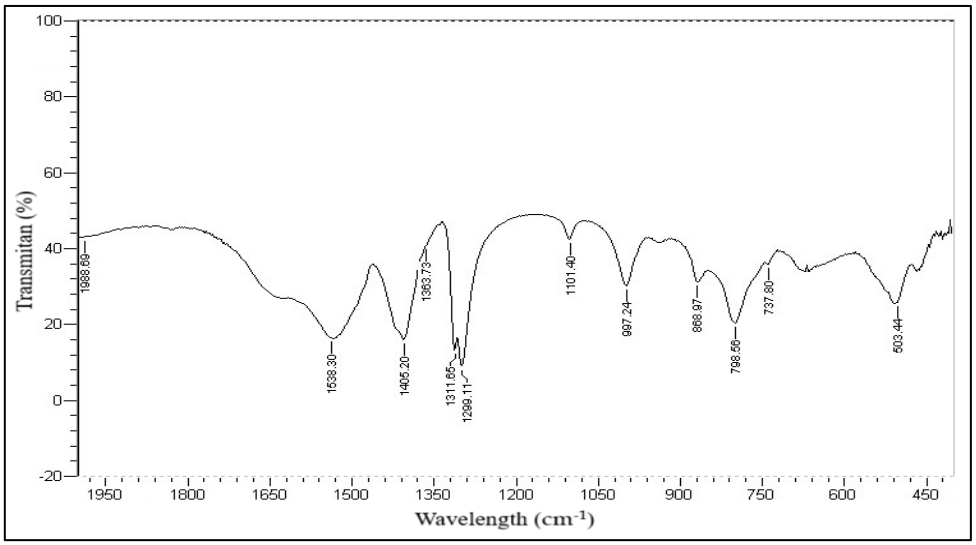


Figure 2. Cisplatin FTIR spectrum

Water Content

The KF coulometry method is generally used for compounds that are susceptible to high temperature heating (Olszewska-Pastuszak, Suchorab, Tabiś, & Pluta, 2025). In contrast, the water content of heat-resistant substances can be determined using the loss on drying approach. If a non-heat-resistant substance is applied to the loss on drying method, measurement inaccuracies will occur because certain components are loss during heating. The measurement results using Coulometry KF are provided below. The acceptance requirement for water content in cisplatin is no more than 1,0%, hence the cisplatin employed in this investigation fits the requirements.

Table 2
Cisplatin Water Content Results

No.	Weight (Gram)	Result (%)
1	0.0248	0.1638
2	0.0251	0.1906
3	0.0251	0.1625
4	0.0245	0.2169
5	0.0262	0.1958
6	0.0272	0.1967
Mean		0.19

Melting Point

Tin's melting point ranges from 229,9 to 233,9°C. In the study, the verification measurement data were achieved at 232,29°C, allowing the DSC to be used for testing. According to the measurement results, cisplatin has a melting point more than 220°C, hence this study is still included in the criterion. (https://www.usbio.net/biochemicals/236910/Cisplatin)

Table 3
Cisplatin Melting Point Results

No.	Weight (Gram)	Result (°C)
1	1.880	300.43
2	2.253	314.36
3	2.227	312.44
Mean		309.08

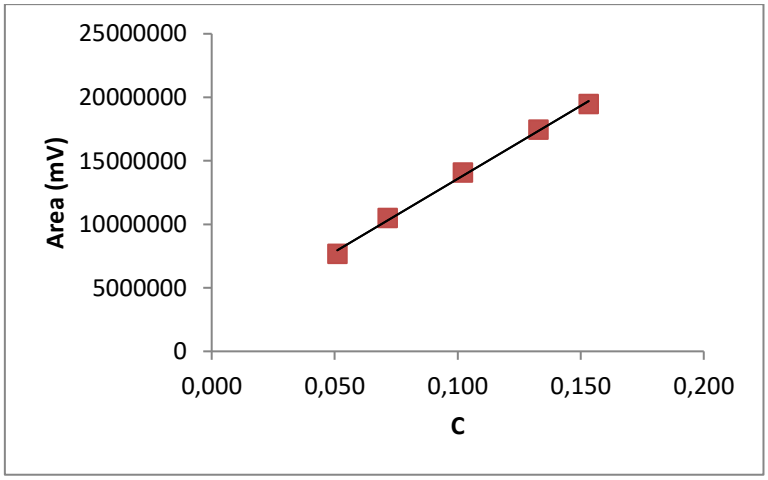
2. Impurity Test

Validation

Suitability, linearity, and accuracy testing are carried out in accordance with International Council of Harmonization (ICH) guidelines. With the analysis conditions applied, the cisplatin retention time (RT) was 7,97 minutes after injection. The RSD area = 0,09% (n=5).

Table 4
Suitability System of Impurity Testing

No.	RT	Area	Tailing Factor	Theoritical Plate
1	7.971	13537837	1.343	12.605
2	7.966	13518169	1.342	12.640
3	7.966	13508740	1.343	12.680
4	7.968	13512317	1.347	12.726
5	7.970	13513300	1.344	12.705
Mean	**7.97**	**13518073**	**1.34**	**12.671**



C = concentration (%)

Figure 3. Cisplatin Calibration Curve

Meanwhile, the linearity of 5 solutions with concentrations of 0,051; 0,071; 0,102; 0,133; and 0,153% was produced a calibration curve with the equation y = 114.948.729,54x + 2.086.869,86 with r = 0,999 (Figure 3). Requirement of r ≥ 0,995.

Table 5
Accuracy Results of Cisplatin

Data	**I**	**II**
C	**0.0988**	**0.1021**
No	**Area**	
1	13536300	13875577
2	13537837	13871687
3	13518169	13858511
4	13508740	13862169
5	13512317	13860910
Mean	13522673	13865771
RSD	0.10	0.05
RF	136869156	135752602

The accuracy test results are displayed in Table 5. The average area divided by concentration yields the response factor (RF). The RF deviation (dRF) between two data points and the absolute value indicates accuracy. Accuracy is calculated by the following formula. The formula used to calculate accuracy is as follows. The accuracy value of cisplatin was 0,82%, meeting the criterion.

$$\% \text{ dRF} = \left(\frac{(RF\ 1 - RF\ 2)}{RF\ 1}\right) x100$$

Impurity Assay

Cisplatin weighed 4,94; 5,107; and 5,134 g, when added to 5-mL of solvent, respectively. According to the measurement results, cisplatin retention time was 7,9 minutes, impurity a at 8,5 minutes, and impurity b at 11,6 minutes. The chromatogram is shown in Figure 4. The resolution between cisplatin and impurity a is not less than 1,5. Meanwhile, the impurity assay's resultant area was determined using Table 6. Cisplatin contains 2,66% total impurities.

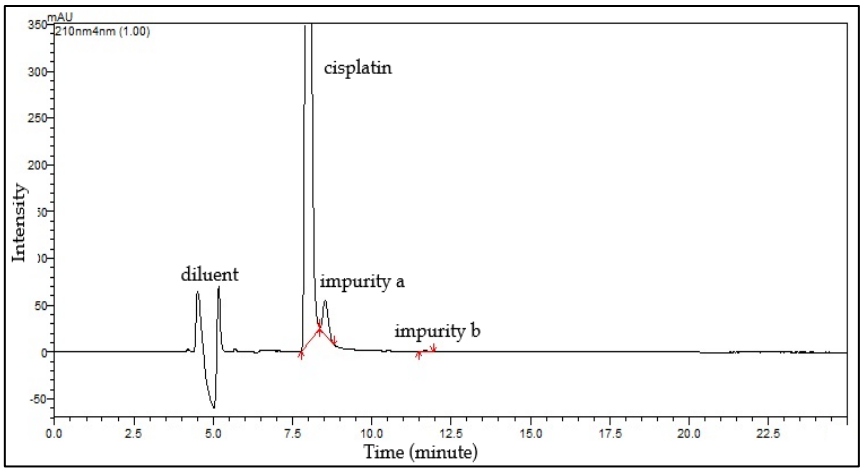


Figure 4. Chromatogram of Impurity Assay

Table 6
Impurity Assay Results

Peak	1		2		3	
	Area	**R**	**Area**	**R**	**Area**	**R**
Cisplatin	13536300		13537837		13518169	
Impurity a	436129	3.119	438402	3.134	437677	3.134
Impurity b	12102	0.087	11321	0.081	11192	0.080
Sum	13984531	3.205	13987560	3.215	13967038	3.214
Cisplatin	13862169		13860910		13854488	
Impurity a	393803	2.760	397691	2.787	405318	2.840
Impurity b	10256	0.072	9886	0.069	10439	0.073
Sum	14266228	2.832	14268487	2.856	14270245	2.913
Cisplatin	14076236		14080091		14099454	
Impurity a	262948	1.833	268029	1.867	266543	1.854
Impurity b	9735	0.068	8333	0.058	9368	0.065
Sum	14348919	1.900	14356453	1.925	14375365	1.919
	Mean of impurity				2.66	
	RSD				0.22	

R = % of impurity

3. Homogeneity Test

Validation

The homogeneity test was validated since the analysis conditions differed from those for the impurity test. The suitability test results revealed that the employment of the mobile phase of ethyl acetate: methanol: dimethylformamide: water and DMF solvent retained cisplatin at 5,5 minutes, with an RSD area of 0,06% (Table 7). Thus, the analysis conditions for the homogeneity test can be applied. The linearity solution measurement yielded the findings shown in Table 8. By plotting the concentration as the X-axis and the average area as the Y-axis, the equation y = 5.668.152,75x + 411,5 (r = 1) (Table 8.).

Table 7
Suitability System of Homogeneity Test

No.	RT	Area	Tailing Factor	Theoritical Plate
1	5.515	474071	1.210	7598
2	5.527	474608	1.211	7606
3	5.547	474568	1.212	7611
4	5.556	474732	1.214	7612
5	5.565	474677	1.215	7594
Mean	**5.542**	**474531.2**	**1.212**	**7604**

Table 8
Linearity Results of Homogeneity Test

C	Area	Mean Area	SD	RSD
0.045	255501 255205	255353	209	0.08
0.068	383867 383449	383658	296	0.08
0.090	510792 511331	511062	381	0.07
0.113	639360 638660	639010	495	0.08
0.135	766583 765239	765911	950	0.12

C = concentration (%)

Homogeneity Assay

Four accuracy solutions were measured, as well as 20 solutions from vials. The accuracy of solutions A, B and C, D was calculated using average dRF = 0,23% (requirement ≤ 2%). The average rates for series 1 and 2 are **99,81% and 99,76**%, respectively (Table 9). The average combined rate of series 1 and 2 was then calculated to be **99,79**% (RSD = 0,41%). Statistical calculations using ANOVA single factor on ten vials yielded F_{cal} = 0,17, which is less than F_{table} = 3,02. This finding demonstrated that the 200 vials that had contained cisplatin were homogeneous.

Table 9
Homogeneity Test Calculation

Result	Accuracy		Vial									
	A	B	1	2	3	4	5	6	7	8	9	10
Series 1												
Mean	5675 64	5652 83	5961 15	5674 82	5556 47	5826 13	5600 01	5943 01	5670 72	5912 03	5615 91	5409 14
RF	5692 720	5696 119	5670 800	5682 771	5686 108	5740 025	5704 982	5681 649	5693 494	5660 690	5697 956	5693 832
dRF	0.06											
Q	99.9	99.9	99.4 6	99.6 7	99.7 2	100. 67	100. 06	99.6 5	99.8 5	99.2 8	99.9 3	99.8 6
Q Mean			99.81									
Series 2												
Mean	5857 65	5627 90	6138 54	5707 02	5496 47	6275 68	5848 62	5356 49	5580 62	5739 55	5814 46	5707 92
RF	5715 894	5739 237	5712 395	5739 154	5694 644	5648 672	5686 000	5721 518	5734 294	5717 822	5715 014	5710 199
dRF	0.41											
Q	99.9	99.9	99.8 4	100. 31	99.5 3	98.7 3	99.3 8	100. 00	100. 22	99.9 3	99.8 8	99.8 0
Q mean			99.76									

Q = quantification (%)

Value Determination

A total of 200 vials were quantified, taking into consideration the uncertainty caused by organic impurities, moisture or water content, homogeneity and stability assay. The components that comprise the calculation include the uncertainty of the analytical or micro-balance, the uncertainty and volume of flask, the weighing of the tiniest materials, and so on. Based on the calculation findings, cisplatin value in vials estimated using the AV formula yielded a result of 97,15% (Uncertainty = 1,43%, k = 2).

4. Management of Cytotoxic Drug Waste

In this study investigation, cytotoxic drugs may be exposed during both the preparation and laboratory testing phases. Officers who work must first be trained and adhere to set protocols. The personal protective equipment must safeguard personnel from exposure. If there is a spill in the laboratory, neutralize it right away using 12% sodium hypochlorite. To safeguard the laboratory from any risk, the remaining cytotoxic drug in the glassware must be treated with a hypochlorite solution.

Conclusion

Cisplatin purchased from TCI can be used as a reference standard to calculate the value of cisplatin products pre-market and post-market with assigned value of 97,15% (Uncertainty = 1,43%, k = 2). This is part of the quality control conducted by the manufacturer and the authorized supervisory institution.

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