International Journal of Chemistry and Applications.
ISSN 0974-3111 Volume 18, Number 1 (2026), pp. 1 - 8

http://www.irphouse.com

Advances in Spectrophotometric and Oxidative Techniques for Medicine Quantification: A Review

Mahammed Talib[1], Mansour Bayati[*2]
[1,2]Department of Chemistry, College of Science,
University of Kufa,
Najaf-54001, Iraq.

Wesam Hassan Ali [3]
[3,] Food Science, Agriculture Faculty,
University of Kufa,
Najaf City-54001. Iraq

***Correspondence**: Mansourk.ali@uokufa.edu.iq

Abstract
For the quantitative measurement of ganciclovir in pharmaceutical formulations, a straightforward, sensitive, and trustworthy spectrophotometric technique was created. Ganciclovir is oxidized with an excess of N-Bromo succinimide acidic media, and the unreacted NBS is then determined using the methylene blue bleaching method at 610 nm. With a good correlation coefficient (R2 = 0.9997), the decline in dye intensity exhibited a linear relationship with GCV content within the range of 1–35 µg/mL. With a Sandell's sensitivity of 0.169 $\mu g \cdot cm^{-2}$ and a molar absorptivity of 3.39×10^3 $L \cdot mol^{-1} \cdot cm^{-1}$, the method demonstrated good sensitivity. Excellent recovery values (99–99.8%) and low %RSD (<1.2%) were confirmed by accuracy precision studies. The measurement was not affected by common excipients. For routine analysis of ganciclovir in pharmaceutical products, the suggested approach proved effective.
Keywords: medication; spectrophotometric method; Oxidative coupling reactions; molar absorption

1.Introduction

The antiviral drug ganciclovir, also called 1,3-dihydroxy-2-propoxymethylguanine, is a viral homolog that has demonstrated efficacy against a variety of viruses, including herpes viruses, varicella-zoster virus, the Epstein-Barr virus, and cytomegalovirus (Abdulzahra et al., 2023) . Ganciclovir is a highly effective antiviral medication that is frequently used to prevent CMV patients who received organ transplants and to treat infections caused by viruses, especially cytomegalovirus retinitis in individuals with compromised immune systems (Ruenroengbun et al., 2021). While Valganciclovir, the drug's precursor, is taken orally and digested to form Ganciclovir after ingestion, Ganciclovir is administered intravenously (Razonable, 2023) . It is noteworthy that the bioavailability of a single dose of the medication valganciclovir is estimated to be around 60% (Balani et al., 2023). GCV assessments are crucial in biological samples due to the drug's high resemblance to endogenous substances (Raglow & Kaul, 2023).

ocular infections , compromised immune systems, and the treatment of AIDS (Bottino et al., 2023). Because of ganciclovir's vital therapeutic uses , accurate and reliable quantification is critical for clinical pharmacokinetics, medicinal product evaluation , and therapeutic drug monitoring (Märtson et al., 2022). Ganciclovir has been quantified using a variety of analytical methods, such as mass spectrometry in conjunction with a liquid chromatography (LC) (Franck et al., 2022), f UV-vis spectroscopic evaluation (Märtson et al., 2021), capillary electrophoresis on gels (Ho et al., 2021), surface enhanced spectroscopy using Raman (Wong, 2021), and spectroscopy technologies (El-Nakib et al., 2025). However, these techniques often have disadvantages, such as expensive equipment, complex specimen processing, comprising time-consuming procedures, that make them inappropriate for general use in laboratory settings with limited resources (Darvishi et al., 2025). Oxidation procedures have attracted a lot of attention lately as a practical technique for pharmaceutical chemistry evaluation (Xu et al., 2024).

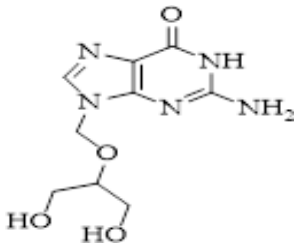

Figure 1: Chemical structure of Ganciclovir (Di Carmine et al., 2021)

Spectroscopic qualitative and quantitative analysis of nature frequently uses colorimetric reagents (Dadi & Yasir, 2022) . N-bromosuccinimide (NBS) , a bronzing reagent with exceptional specificity for both free-radical replacements and electrophilic insertion into unsaturated compounds due to its ability to liberate minute amounts of bromine (Van Kerrebroeck et al., 2022), is one of the colorimetric reagents. N-bromosuccinimide and luminol react quickly in an alkaline media to produce uric acid chemiluminescence in saliva samples. N-bromosuccinimide (NBS) is a notable oxidant that is frequently utilized in chemical processes because of its advantageous properties, especially its exceptional selectivity for aromatic and nucleophile chemicals (Nimbhal & Singh, 2024). Consequently, NBS becomes an excellent option for the oxidative degradation of drugs such as Ganciclovir (GCV) (Ganin et al., 2025). The choice of NBS is based on its ability to accurately identify the functional groups present in ganciclovir, hence encouraging the generation of an oxidation byproduct that is easily quantifiable and characterized by analytical techniques (Abdulzahra et al., 2023). Combinations of organic processes known as oxidative reactions are highly significant and have numerous applications, particularly in analytical chemistry (Wang et al., 2023). When an oxidizing agent is present, two organic molecules are linked under specific reaction conditions in these reactions. When these substances oxidize, a range of compounds are created that interact to create a colored result. This vibrant material can be measured using spectroscopic techniques.

2.Methods

To prepare the stock standard solution, one gram of ganciclovir was carefully dissolved in a thousand milliliters of distilled water (D.W.). To create different concentrations, a suitable volume of the initial solution was diluted with the same solvent.

3.Discussion

To employ an oxidation process, an excess of N-Bromo succinimide was introduced. The unreacted N-Bromo succinimide was used to bleach the methylene blue hue. The absorbance,

measured at 610 nm, increased linearly with Ganciclovir concentration. To determine the ideal conditions for Ganciclovir identification in pharmaceutical preparations, the effects of several parameters on color formation were investigated. One of the considerations was the volume of the dye. The results are displayed in Table 1. Experiments were conducted to identify the optimal and effective quantities of dyes (methylene blue, malachite green, and crystal violet) that may be evaluated using spectrophotometric methods.

Table 2: Influence of volume oxidant on the absorbance of product (Abdulzahra et al.)

sequence	Volume	Abs.
1	0.5	0.196
2	1	0.2
3	1.5	0.202
4	2	0.302
5	2.5	0.291
6	3	0.285

Table 3: Benefit of various volume acids on the absorbance of the product (Abdulzahra et al.)

sequence	Volume	Abs.
1	0.25	0.196
2	0.5	0.299
3	0.75	0.269
4	1	0.209
5	1.25	0.2
6	1.5	0.197

Impact Of Oxidant Volume

The color of methylene blue dye was compared to various concentrations of 0.001 M N-bromosuccinimide (0.5–3 mL). At the time of these findings, ganciclovir was not present. The results are summarized in Table 2. It was discovered that only two milliliters of the N-bromosuccinimide solution were needed to bleach the methylene blue dye pigment as effectively as possible. This volume was therefore chosen to be used in subsequent tests.

The Impact of Acid

The impact of acid on the oxidation of ganciclovir was examined. The study found that the product's absorption was enhanced by the acid. All of the acids under investigation, including HCl, CH3COOH, H2SO4, and HNO3, were found to be able to produce the color result. Conversely, HCl provided the best absorption and the highest color stability. The subsequent investigations utilized 0.5 mL of 1M HCl. An overview of the investigation's results is given in Table 3.

Effect of Temperature

The effect of temperature on the color intensity of methylene blue was investigated. It was discovered that the hue produced at 20°C produced the most absorption. Color loss was observed at both high and low temperatures. For any subsequent research, it is therefore recommended to maintain the temperature at 20°C.

Sequence of Additions(Abdulzahra et al.)
The oxidant reagent (NBS) should be added in the sequence that the analytical procedure specifies in order to obtain the best results. Deviating from the recommended sequence resulted in decreased stability and a drop in color intensity. The results of the experiment are summarized in Table 4.

Table 4: Sequence of addition effect

sequence	Order of addition	Abs.
1	Drug+Acid+NBS+Dye	0.301
2	Drug+NBS+Acid+Dye	0.268
3	NBS+Drug+Acid+Dye	0.273
4	Acid+NBS+Drug+Dye	0.209
5	Acid+Drug+NBS+Dye	0.211

Table 5: The impact of time for oxidation Drug

sequence	Time(min)	Abs.
1	5	0.259
2	10	0.3
3	15	0.263
4	20	0.255
5	25	0.249
6	30	0.242

The Impact of Time on Oxidation and Bleaching of The Dye
We examined the effects of time on the oxidation of N-bromosuccinimide Ganciclovir and the maximum bleaching time of methylene blue. The results are displayed in Tables 5 and 6. It was found that methylene blue dyes needed fifteen minutes to bleach, whereas ganciclovir needed ten minutes to completely oxidize.

Influence of Interference
The impact of specific excipients that were labeled in pharmaceutical preparations was evaluated by measuring GANCICLOVIR in the presence of various excipients, including glucose, potato starch, tween 80, sucrose, benzoic acid, aspartate, lactose, PVP, and cellulose that is microcrystalline. The experimental results demonstrated that these excipients did not impede the experimental process, as shown in Table 7.

Absorption Spectra
After adding 2 mL of methylene blue and incubating for 15 minutes, a 1 mL diluted aqueous solution of ganciclovir, 0.5 mL of HCl, and 2 mL of N-bromosuccinimide spontaneously produces a rich blue color. The maximum absorption is shown at 610 nm in Figure 2.

Calibration Graph(Abdulzahra et al.)
A linear relationship between absorbance and the active ingredient g concentration was found within the range of 1-35 μg/mL−1. The determination coefficient value ($R2 = 0.9997$) indicates a strong connection. The calibration graph was obtained by following the fundamental procedure, as shown in Figure 3. The test's objective was to determine how concentration

impacted the color product's absorption. The Sandal sensitivity was determined to be 0.169 g/cm−2. The high molar absorptivity of the color product was found to be 3.39 × 103 L.mol−1.cm−1. For the limit of detection (LOD) and the limit of quantitation (LOQ), the following formulas were verified.

Table 6: The impact of time for Dye bleaching

sequence	Time(min)	Abs.
1	5	0.271
2	10	0.289
3	15	0.302
4	20	0.268
5	25	0.254
6	30	0.251

Table 7: Influence of various interferences

0.1 ml interfering	%Error	%Recovery
Benzoic acid	0.901	100.901
PVP	1.040	101.040
Sucrose	1.412	101.412
Lactose	1.252	101.252
Microcrystalline cellulose	-0.119	99.881
Glucose	-0.781	99.219
Starch	1.120	101.120
Aspartate	1.101	101.101
Tween 80	-0.021	99.979

where "s" denotes the standard deviation of the intercept and the slope of the intensity line was 0.0059. All quantitative optical properties are listed in Table 8.

Precision and accuracy

The accuracy and precision of the methods were evaluated using five replication tests of the drug's unique solution at two distinct Ganciclovir concentrations. The results, which demonstrate the degree of accuracy and precision attained, are shown in Table 9.

Applying The Method(Abdulzahra et al.)

Ganciclovir found in the pharmaceutical product was satisfactorily estimated using NBS in the investigated analysis procedure. The calculated values of %RSD, %Error, and Recovery are shown in Table 10. Additionally, a T-test was run, but no discernible difference was seen. This suggests that the method is appropriate for determining ganciclovir.

Figure 2: A): the maximum absorption for drug B): the maximum absorption for GANCICLOVIR-NBS

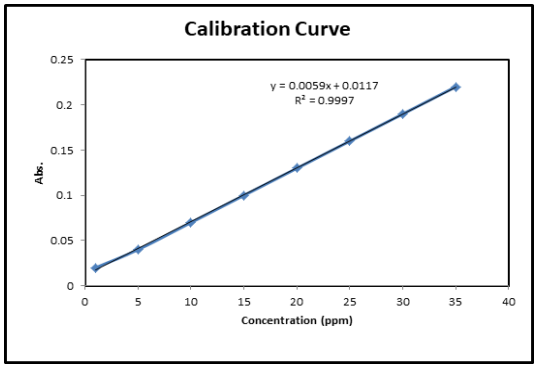

Figure 3: Calibration graph for the color product

Table 8: Analytical optical characteristics as reliability for the procedure tested

Quantitative optical characteristics	**Proposed method**
Molar absorptive (L. mol-1.cm $^{-1}$)	3.39×103
Regression equation (y = bx + a)	y = 0.0059x + 0.0117
Beer's Law limits (μg/mL) (x)	1-35
Sandell's sensitivity μg/cm2	0.169 g.cm−2
Slope (b)	0.0059
Intercept (a)	0.0117
Correlation coefficients (r)	0.9997
λmax (nm)	610
RSD%	0.01
Recovery %	99
(LOQ) quantification Limit (μg.mL^{-1})	0.365
(LOD) detection Limit (μg.mL^{-1})	1.219

4.Conclusion

Numerous analytical benefits have been shown by the suggested spectrophotometric approach for measuring ganciclovir utilizing N-bromosuccinimide as an oxidizing agent (Abdulzahra et al., 2023). Because the process is quick, easy, and doesn't require sophisticated equipment, it can be used in labs with little funding (Maqsood et al., 2024). The technique provides strong connection between absorbance and drug concentration, great sensitivity, and good linearity over a broad concentration range (Farahani et al., 2025). Its dependability for regular quality control analysis was shown by the satisfactory accuracy and precision results. Additionally, the technique demonstrated good selectivity with no influence from widely used pharmaceutical excipients (Akash & Rehman, 2025). All things considered, the oxidation-bleaching method is a reliable and efficient analytical technique for determining the amount of ganciclovir in pharmaceutical dosage forms(Dispas et al., 2022).

Table 9: Precision and accuracy of the methodology evaluated

Ganciclovir Present (ppm)	Ganciclovir found (ppm)	%Error	%Recovery	%RSD
5.5	5.00	0.4	99.6	1.2
16	14.0	0.2	99.8	1.03
26	24.8	0.04	99.96	1.1

Table 10: Values of %RSD, %Error, and %Recovery

Sample	T test	Conc. Present mg. L-1 after dilution	Conc. Found. mg. L^{-1}	%RSD	%Error	%Recovery
Ganciclovir 500mgcapsules/Medindia-India	0.598406	100	99.6	0.022	0.4	99.6
Natclovir 250mg capsules/Wellona Pharma-India	0.76626	75	74.8	0.10799	0.26	99.74

5.References

[1] Abdulzahra, M. A., Abed, A. M., & Ali, A. M. Influence of Leptin and Adiponectin on Polycystic Ovary Syndrome in Obese Iraqi Patients.

[2] Abdulzahra, M. A., Ali, A. M., Awad, A. A.-H., & Hassib, T. F. a. (2023). Estimation of ganciclovir drug by oxidative coupling reactions with NBS as an Oxidant. Azerbaijan Pharmaceutical and Pharmacotherapy Journal, 22, 5-9.

[3] Akash, M. S. H., & Rehman, K. (2025). Comprehensive Insights into Pharmaceutical Analysis. In Essentials of Pharmaceutical Analysis (pp. 1-62). Springer.

[4] Balani, S. S., Sadiq, S., Jensen, C. J., & Kizilbash, S. J. (2023). Prevention and management of CMV infection in pediatric solid organ transplant recipients. Frontiers in Pediatrics, 11, 1098434.

[5] Bottino, P., Pastrone, L., Curtoni, A., Bondi, A., Sidoti, F., Zanotto, E., Cavallo, R., Solidoro, P., & Costa, C. (2023). Antiviral approach to cytomegalovirus infection: an overview of conventional and novel strategies. Microorganisms, 11(10), 2372.

[6] Dadi, M., & Yasir, M. (2022). Spectroscopy and Spectrophotometry: Principles and Applications for Colorimetric. Colorimetry, 81.

[7] Darvishi, M., Heidari, M. M., Kheradmand, R., Niaki, N. M., Tabean, M., & Mobed, A. (2025). Nanomaterial‐Enhanced Electrochemical Sensors for Clinical Monitoring of Acyclovir: Integration Into Molecular Diagnostics. Journal of Clinical Laboratory Analysis, 39(20), e70104.

[8] Di Carmine, G., Abbott, A. P., & D'agostino, C. (2021). Deep eutectic solvents: alternative reaction media for organic oxidation reactions. Reaction Chemistry & Engineering, 6(4), 582-598.

[9] Dispas, A., Sacré, P.-Y., Ziemons, E., & Hubert, P. (2022). Emerging analytical techniques for pharmaceutical quality control: Where are we in 2022? Journal of pharmaceutical and biomedical analysis, 221, 115071.

[10] El-Nakib, H. E., Abdel Aziz, S. E., Schaletzky, J., & Ahmed, N. S. (2025). Analytical Methodologies for Anti-Infective Orphan Drugs: A Comprehensive Review of FDA Approvals (2013–2023) Part 2. Critical Reviews in Analytical Chemistry, 1-25.

[11] Farahani, F. Z., Sohrabi, M. R., & Tadayon, F. (2025). Spectral resolution techniques for the simultaneous spectrophotometric determination of anti-Parkinson drugs in their combined pharmaceutical dosage form and biological sample based on multivariate calibration and absorbance subtraction methods. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 327, 125399.

[12] Franck, B., Autmizguine, J., Marquet, P., Ovetchkine, P., & Woillard, J. B. (2022). Pharmacokinetics, pharmacodynamics, and therapeutic drug monitoring of valganciclovir and ganciclovir in transplantation. Clinical Pharmacology & Therapeutics, 112(2), 233-276.

[13] Ganin, A. S., Sobyanina, M. M., & Moskalik, M. Y. (2025). Synthesis of N-Heterocycles and Linear Adducts from Allyl-Containing Substrates and Sulfonamides. Current Organic Chemistry, 29(15), 1145-1158.

[14] Ho, S. A., Slavin, M., Roberts, J. A., & Yong, M. (2021). Optimization of Ganciclovir use in allogeneic hematopoietic cell transplant recipients–the role of therapeutic drug monitoring. Expert Review of Anti-infective Therapy, 19(6), 707-718.

[15] Maqsood, A., Chen, C., & Jacobsson, T. J. (2024). The future of material scientists in an age of artificial intelligence. Advanced Science, 11(19), 2401401.

[16] Märtson, A.-G., Edwina, A. E., Burgerhof, J. G., Berger, S. P., de Joode, A., Damman, K., Verschuuren, E. A., Blokzijl, H., Bakker, M., & Span, L. F. (2021). Ganciclovir therapeutic drug monitoring in transplant recipients. Journal of Antimicrobial Chemotherapy, 76(9), 2356-2363.

[17] Märtson, A.-G., Edwina, A. E., Kim, H. Y., Knoester, M., Touw, D. J., Sturkenboom, M. G., & Alffenaar, J.-W. C. (2022). Therapeutic drug monitoring of ganciclovir: where are we? Therapeutic Drug Monitoring, 44(1), 138-147.

[18] Nimbhal, A., & Singh, R. (2024). N-Chlorosuccinimide: A versatile reagent in organic synthesis. Current Organic Chemistry.

[19] Raglow, Z., & Kaul, D. R. (2023). A new antiviral option for cytomegalovirus prevention after kidney transplant. JAMA, 330(1), 27-29.

[20] Razonable, R. R. (2023). Oral antiviral drugs for treatment of cytomegalovirus in transplant recipients. Clinical Microbiology and Infection, 29(9), 1144-1149.

[21] Ruenroengbun, N., Numthavaj, P., Sapankaew, T., Chaiyakittisopon, K., Ingsathit, A., Mckay, G. J., Attia, J., & Thakkinstian, A. (2021). Efficacy and safety of conventional antiviral agents in preventive strategies for cytomegalovirus infection after kidney transplantation: a systematic review and network meta‐analysis. Transplant International, 34(12), 2720-2734.

[22] Van Kerrebroeck, R., Horsten, T., & Stevens, C. V. (2022). Bromide oxidation: A safe strategy for electrophilic brominations. European Journal of Organic Chemistry, 2022(35), e202200310.

[23] Wang, S., Zhao, Y., Chan, A. W., Yao, M., Chen, Z., & Abbatt, J. P. (2023). Organic peroxides in aerosol: key reactive intermediates for multiphase processes in the atmosphere. Chemical reviews, 123(4), 1635-1679.

[24] Wong, D. (2021). Clinical pharmacokinetic monitoring and resistance testing for the optimisation of antiviral therapy targeting human cytomegalovirus University of New South Wales (Australia)].

[25] Xu, W.-X., Qu, Q., Teng, X.-Q., Zhuang, H.-H., Liu, S.-F., Wang, Y., & Qu, J. (2024). Personalized application of antimicrobial drugs in pediatric patients with augmented renal clearance: a review of literature. European Journal of Pediatrics, 183(1), 51-60.