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Research Article

Extended stability of extemporaneously prepared cefazolin ophthalmic solutions in preservative-free vehicles under frozen storage

Nithima Kharomprat[1], Suporn Charumanee[2], Nantaka Khorana[3], Sudaporn Wongwan[4*]

[1]Department of Pharmacy, Phichit Hospital, Pichit, Thailand
[2]Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand
[3]Faculty of Pharmacy, Payap University, Chiang Mai, Thailand
[4]Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand

ABSTRACT

This study investigated the chemical and physical stability of extemporaneously prepared fortified cefazolin ophthalmic solutions (50 mg/mL) stored in low-density polyethylene containers under freezer conditions (−18 ± 2 °C) for up to 45 days. Formulations were compounded using cefazolin sodium for injection and five preservative-free vehicles: sterile water for injection, normal saline solution, balanced salt solution, and two commercially available lubricant eye drops—Lubric-Eyes® and VISLUBE®. Cefazolin concentrations were quantified using a validated stability-indicating high-performance liquid chromatographic method with photodiode array detection, employing a C18 column and a mobile phase composed of acetonitrile and 0.1% (v/v) acetic acid in water (22:78, v/v). All formulations retained 94.36%–99.89% of their initial cefazolin concentration throughout the 45-day frozen storage, with no significant changes in pH (5.37–6.70), color, odor, or visible particulates. To simulate outpatient post-dispensing conditions, samples stored frozen for 14 days were thawed at 30 ± 2 °C for 1 hr, then stored under refrigeration (5 ± 3 °C) for an additional 14 days. Cefazolin concentrations during this 28-day period remained within 97.53%–100.38% of initial values, although slight discoloration and mild odor changes were observed from Day 21 onward. In conclusion, the present study demonstrates that frozen storage provides superior stability for cefazolin ophthalmic solutions compared to the studies at refrigeration alone that were previously reported in the literature. Importantly, this study provides the first evidence of the compatibility between cefazolin sodium and preservative-free lubricant-based vehicles, highlighting their potential as patient-friendly alternatives in extemporaneous ophthalmic compounding.

Keywords:
Cefazolin; Drug stability; Ophthalmic solutions; Preservative-free vehicles; Frozen storage

1. INTRODUCTION

Bacterial keratitis is a serious corneal infection that can result in permanent vision loss if not promptly diagnosed and treated. It is caused by a broad spectrum of Gram-positive and Gram-negative bacterial pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and coagulase-negative staphylococci[1,2]. In Thailand, common predisposing factors include ocular trauma, contact lens use, ocular surface diseases, and systemic conditions[3]. Early diagnosis and the initiation of appropriate empirical therapy are essential to prevent complications such as corneal scarring and irreversible vision impairment[4].

Fortified topical antibiotics remain the cornerstone of treatment, with cefazolin ophthalmic solution frequently used as first-line therapy for Gram-positive coverage[5]. It is often administered in combination

***Corresponding author:**
* Sudaporn Wongwan Email: sudapornk@nu.ac.th

with gentamicin or tobramycin to ensure broad-spectrum antibacterial activity[4,5]. Due to the lack of commercially available products, cefazolin ophthalmic solutions must be extemporaneously prepared by hospital pharmacists using reconstituted cefazolin sodium powder for injection and appropriate aqueous vehicles. These compounded preparations are generally stored under refrigeration and recommended for use within 5–7 days after reconstitution, or until visible discoloration occurs[6,7]. However, treatment for moderate to severe bacterial keratitis often extends beyond one week[8]. In practical settings, hospital pharmacists require ready-to-use cefazolin ophthalmic solutions to ensure timely and efficient patient care. However, the short shelf life of extemporaneously prepared formulations often necessitates frequent compounding and repeated outpatient visits for refills. This challenge is particularly burdensome in low- and middle-income countries, where such visits may be logistically difficult or financially unsustainable for patients. Therefore, extending the stability of compounded cefazolin ophthalmic solutions is essential to support uninterrupted therapy, reduce the healthcare burden, improve patient adherence, and enhance workflow efficiency in hospital pharmacy settings.

Although strategies such as buffer adjustment or the addition of stabilizer have been proposed to enhance stability, their implementation in hospital pharmacy settings is limited due to formulation complexity and safety concerns[9,10]. In contrast, freezer storage offers a simple, practical, and low-cost approach to prolonging shelf life, and has been successfully applied to other antibiotic eye drops, including vancomycin and ceftazidime[11,12]. Although one study reported that cefazolin ophthalmic solutions remained stable at −80 °C for 42 days, such ultra-low temperatures are not widely accessible in routine clinical practice[13]. Instead, standard household freezer compartments (−18 ± 2 °C) of two-door refrigerators offer a more feasible and widely applicable storage option for use in both hospital and outpatient settings.

In Thailand, cefazolin ophthalmic solutions are typically compounded using either preserved artificial tears or preservative-free aqueous vehicles[6,14]. Evidence from prior studies indicates that long-term use of preserved ophthalmic solutions may lead to ocular surface toxicity, hypersensitivity reactions, and inflammation, thereby contributing to the increasing preference for preservative-free alternatives in clinical practice[15,16]. However, commonly used preservative-free vehicles such as sterile water for injection (SWFI), normal saline solution (NSS), and balanced salt solution (BSS) may cause ocular discomfort, particularly during frequent instillation regimens required in the acute phase of bacterial keratitis[17,18].

Lubricant eye drops such as Lubric-Eyes® and VISLUBE® are preservative-free, hypotonic solutions supplied in sterile single-use containers. Lubric-Eyes® contains hydroxypropyl methylcellulose (HPMC), while VISLUBE® contains sodium hyaluronate—biopolymers recognized for their viscoelastic, mucoadhesive, and corneal epithelial healing properties[19-22]. Given these properties, lubricant-based vehicles have the potential to enhance patient tolerability and adherence while also offering additional therapeutic benefits. However, their application as compounding media for cefazolin ophthalmic solutions has not yet been systematically evaluated.

To address these gaps, the present study aimed to evaluate the chemical and physical stability of fortified cefazolin ophthalmic solutions (50 mg/mL) compounded with preservative-free vehicles—SWFI, NSS, BSS, Lubric-Eyes®, and VISLUBE®—when stored at −18 ± 2 °C for up to 45 days. A validated stability-indicating HPLC-PDA method was used to quantify cefazolin concentrations. Physical stability was assessed by monitoring pH and through organoleptic evaluation. To the best of our knowledge, this is the first study to investigate the stability of extemporaneously prepared cefazolin ophthalmic solutions stored under frozen conditions using commercially available, single-use lubricant-based vehicles.

2. MATERIALS AND METHODS

2.1. Materials

Cefazolin sodium reference standard (99.6% purity) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Cefazolin sodium for injection (Batch No. 132125, Registration No. 1A 440/52, Each vial contains Cefazolin Sodium equiv. to Cefazolin base 1 g, Assay: 104.6% LA) was supplied by T.P. Drug Laboratories (1969) Co., Ltd. (Bangkok, Thailand). Sterile water for irrigation (Lot No.099298) and normal saline solution (0.9% w/v NaCl, Lot No. 099791) were purchased from A.N.B. Laboratories Co., Ltd. (Bangkok, Thailand). Balanced salt solution (Lot No. 117JT) was sourced from Alcon Laboratories, Inc. (Texas, USA). Preservative-free lubricant eye drops included Lubric-Eyes® (0.8 mL single-use vials; containing 0.1% w/v dextran 70 and 0.3% w/v hydroxypropyl methylcellulose; Lot No. A22027), obtained from Pharma Innova Co., Ltd. (Pathum Thani, Thailand), and VISLUBE® (0.3 mL single-use vials; containing 0.18% w/v sodium hyaluronate; Lot No. RA0074), supplied by TRB Chemedica (Thailand) Co., Ltd. (Bangkok, Thailand). Chromatographic-grade acetonitrile (LiChrosolv®), glacial acetic acid, and methanol were obtained from Merck KGaA (Darmstadt, Germany). Deionized water and other HPLC-grade

reagents were supplied by RCI Labscan Ltd. (Bangkok, Thailand). Opaque white low-density polyethylene (LDPE) eye dropper bottles (10 mL capacity), sterilized by gamma irradiation, were purchased from PANYAROEK Co., Ltd. (Bangkok, Thailand).

2.2. Preparation of fortified cefazolin ophthalmic solutions

Fortified cefazolin ophthalmic solutions (50 mg/mL) were prepared using cefazolin sodium for injections reconstituted with five preservative-free vehicles: SWFI, NSS, BSS, Lubric-Eyes®, and VISLUBE®. To minimize formulation variability and ensure reproducibility, a standardized compounding procedure was employed, differing slightly from routine hospital pharmacy practice. Specifically, 5.00 g of cefazolin sodium powder, pooled from six randomly selected vials, was accurately weighed and transferred into a 100 mL volumetric flask. The powder was dissolved and brought to volume with sterile water for injection (SWFI) to prepare Formulation 1. The resulting solution was then aliquoted into 5 mL portions and transferred into 10 mL low-density polyethylene (LDPE) eye dropper bottles (n = 18). This procedure was repeated using NSS, BSS, Lubric-Eyes®, and VISLUBE® as diluents to prepare Formulations 2 through 5, respectively. These procedures were conducted under non-sterile conditions, as the primary objective of this study was to evaluate chemical and physical stability.

2.3. HPLC instrumentation and chromatographic conditions

Quantitative analysis of cefazolin was performed using a reverse-phase high-performance liquid chromatography (RP-HPLC) system (Nexera LC-40, Shimadzu, Japan) equipped with a photodiode array (PDA) detector (SPD-M40, Shimadzu, Japan). Chromatographic separation was performed on a Shim-pack GIS C18 column (250 mm × 4.6 mm i.d., 5 µm particle size), maintained at 25 °C. The mobile phase consisted of acetonitrile and deionized water containing 0.1% (v/v) acetic acid in a ratio of 22:78 (v/v), delivered at a flow rate of 1.0 mL/min. The injection volume was 20 µL, and detection was carried out at 270 nm. Each analysis was completed within a 10-min runtime.

2.4. Standard and sample preparation for HPLC analysis

A cefazolin sodium stock solution (1 mg/mL) was prepared by accurately weighing 25 mg of the reference standard and dissolving it in deionized water in a 25-mL volumetric flask. Serial dilutions of the stock solution were performed to obtain working standard solutions at concentrations ranging from 20 to 200 µg/mL, which were subsequently employed for calibration curve construction and preparation of quality control (QC) samples. To ensure analytical reliability, stock solutions were freshly prepared at each scheduled time point throughout the study period.

For sample preparation, each cefazolin formulation was individually sampled by pipetting 100 µL into a separate 50-mL volumetric flask and diluting to volume with deionized water to achieve a final concentration of approximately 100 µg/mL. The resulting solutions were filtered through 0.45 µm-nylon syringe filters and transferred into autosampler vials for HPLC injection.

2.5. Method development and optimization

The HPLC method was adapted from previously published studies and systematically optimized to improve chromatographic performance for cefazolin analysis[6,14]. Critical parameters, including mobile phase composition, type of organic modifier, flow rate, column temperature, and detection wavelength, were evaluated.

Both methanol and acetonitrile were investigated as organic solvents to determine optimal elution conditions for achieving appropriate retention time, peak symmetry, and resolution.

2.6. Method validation

The analytical method was validated in accordance with the International Council for Harmonisation (ICH) Guideline Q2(R2)[23]. The following parameters were assessed: specificity, linearity, accuracy, precision, robustness, and the limits of detection (LOD) and quantitation (LOQ).

2.6.1. Specificity

Specificity was evaluated by comparing chromatograms of blank vehicle solutions and degraded cefazolin samples with that of a cefazolin sodium standard solution. Degraded samples were prepared by dissolving cefazolin sodium for injection in deionized water and storing the solution at room temperature without light protection for a minimum of seven days. During this period, progressive visual discoloration from yellow to dark brown was observed, attributed to cefazolin degradation, primarily associated with β-lactam ring hydrolysis, including substitution and oxidative reactions[14].

Chromatographic specificity was evaluated using PDA detection. The purity index values of

cefazolin peaks in all analyzed samples exceeded 0.999, indicating the absence of co-eluting interference from degradation products or formulation excipients.

2.6.2. The limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined based on the signal-to-noise (S/N) ratio method. Serial dilutions of the cefazolin sodium reference standard were prepared and analyzed under the optimized HPLC conditions.

The LOD was defined as the lowest concentration that produced an S/N ratio of approximately 3:1, while the LOQ corresponded to the lowest concentration yielding an S/N ratio of approximately 10:1.

2.6.3. Linearity

Linearity was evaluated over the concentration range of 20–200 µg/mL using six calibration levels, with each concentration analyzed in triplicate. Calibration curves were constructed by plotting peak areas versus concentration, and linear regression analysis was performed. A correlation coefficient (r) of ≥ 0.999 was considered indicative of acceptable linearity.

2.6.4. Accuracy

Accuracy was assessed using the standard addition method at three selected concentration levels: 80, 100, and 120 µg/mL. A cefazolin sodium standard stock solution (1 mg/mL) was used to spike 0.8, 1.0, and 1.2 mL into separate 10 mL volumetric flasks, followed by dilution to volume with deionized water. Each concentration level was prepared and analyzed in triplicate. Percentage recovery was calculated by comparing the measured concentrations with their corresponding theoretical values. A recovery range of 98–102% across all selected concentration levels was considered acceptable, indicating that the method was accurate for quantifying cefazolin sodium.

2.6.5. Precision

Precision was evaluated in terms of repeatability (intra-day precision) and intermediate precision, including inter-day and between-analyst variability. Six replicates of a 100 µg/mL cefazolin sodium standard solution were analyzed.

Repeatability was assessed by performing all six measurements on the same day under identical conditions. Intermediate precision was evaluated by analyzing six replicates over three consecutive days (inter-day precision) and by two independent analysts (between-analyst precision). A percent relative standard deviation (%RSD) of less than 2% was considered acceptable.

2.6.6. Robustness

Robustness was assessed by introducing deliberate, minor variations to key chromatographic parameters of the optimized HPLC method. These variations included changes in flow rate (±0.1 mL/min), column temperature (±5 °C), acetonitrile content in the mobile phase (±2%, v/v), and detection wavelength (±5 nm). The method was considered robust if the %RSD values remained below 5%, indicating that such variations had a minimal impact on method performance.

2.6.7. Forced degradation study

A forced degradation study was undertaken to demonstrate the specificity and to confirm the stability-indicating capability of the developed HPLC method. Cefazolin sodium standard solutions were subjected to forced degradation under four stress conditions: acidic hydrolysis (0.1 M HCl), alkaline hydrolysis (0.1 M NaOH), oxidative degradation (6% H_2O_2), and thermal degradation (60 °C). All samples were exposed to these conditions for 1.5 hr at ambient temperature, except for the thermal degradation condition, which was conducted in a water bath maintained at 60 °C. These conditions were selected to induce sufficient degradation and evaluate the method's ability to selectively quantify cefazolin in the presence of its degradation products. A control sample was prepared by dissolving the cefazolin sodium reference standard in deionized water without applying any stress conditions.

All samples were analyzed using the optimized HPLC method. Peak purity was assessed using PDA detection, and values exceeding 0.999 confirmed the absence of co-eluting degradation products under the cefazolin peak.

2.7. Stability Study

All cefazolin ophthalmic formulations (Formulations 1–5), including the control, were stored in the freezer compartment of a standard two-door refrigerator at −18 ± 2 °C and were evaluated for chemical and physical stability on days 0, 7, 14, 28, 35, and 45.

To simulate outpatient post-dispensing conditions, a subset of samples (Formulations 1–5) previously stored at −18 ± 2 °C for 14 days was thawed at room temperature (30±2 °C) for 1 hr and subsequently stored under refrigerated conditions (5 ± 3 °C) for an additional 14 days. These post-thaw

samples were analyzed on refrigerated storage Days 0, 3, 7, 10, and 14, corresponding to Days 14, 17, 21, 24, and 28 of the overall study periods.

2.7.1. Chemical Stability

Cefazolin concentrations at each predefined time point were quantified using the validated stability-indicating HPLC-PDA method. Chemical stability was assessed in accordance with United States Pharmacopeia (USP) guidelines, which define acceptable stability as the retention of 90%–110% of the initial drug concentration[24,25]. Results were expressed as the percentage of cefazolin remaining relative to the baseline concentrations (Day 0).

2.7.2. Physical Stability

Physical stability was evaluated by measuring the pH using a calibrated pH meter (SevenCompact pH meter S220, METTLER TOLEDO™, Switzerland) and by conducting organoleptic assessments of color, odor, and clarity in triplicate (n = 3). Visual inspection for particulate matter was conducted against both black and white backgrounds to ensure detection of any visible particles.

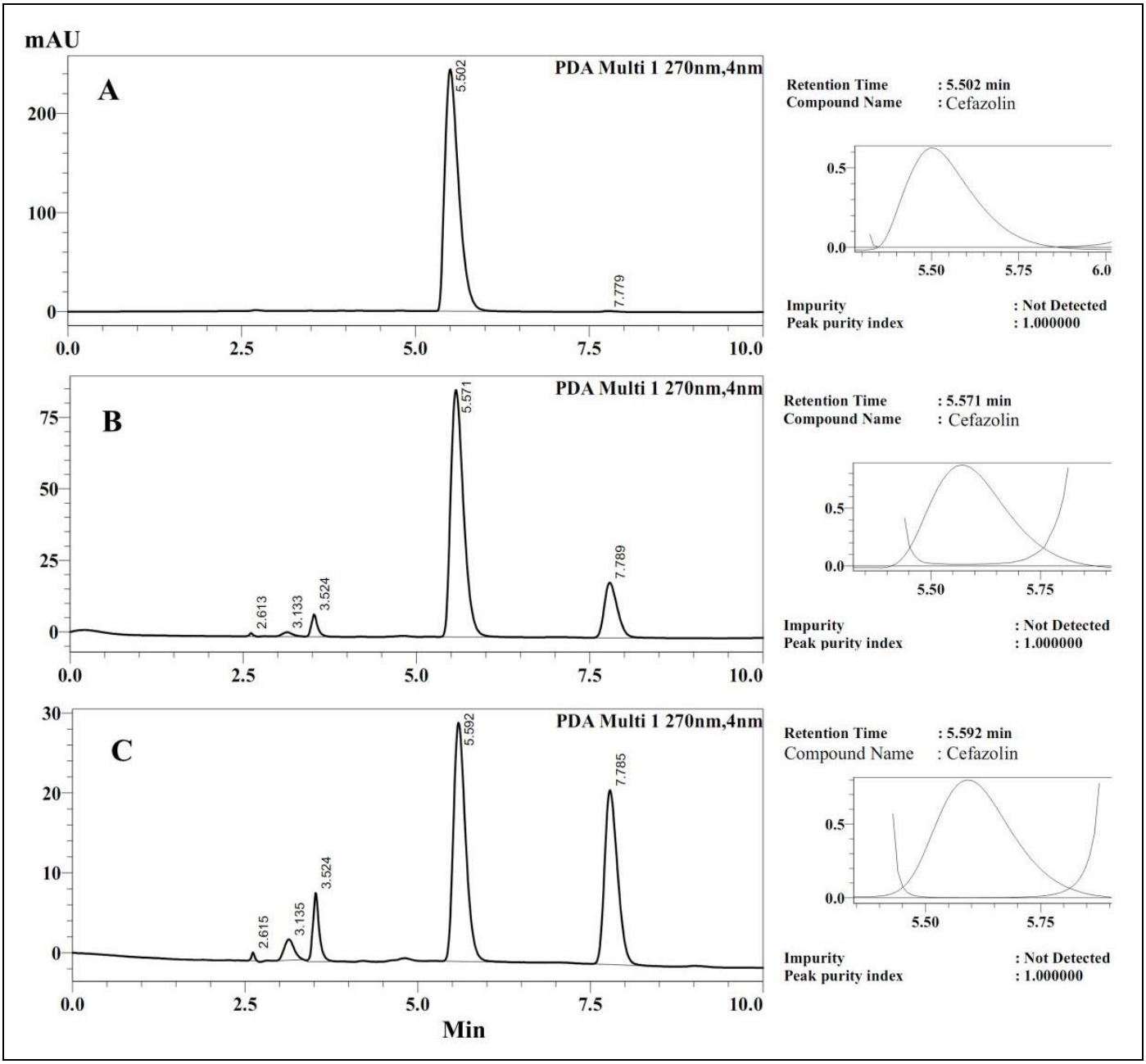

Figure 1. Representative HPLC chromatograms and peak purity indices of cefazolin sodium solutions (100 µg/mL in deionized water): (A) freshly prepared standard solution; (B) sample degraded after 7 days at ambient temperature, exhibiting yellow discoloration; (C) sample degraded after more than 7 days at ambient temperature, showing dark brown discoloration.

Table 1. Summary of validation parameters for the HPLC-PDA method used for the quantification of cefazolin sodium.

Parameter	Result
Linear range (µg/ml)	20 – 200
Correlation coefficient (r)	0.9999 – 1.0000
Regression equation	Y= 29022X – 80151
Accuracy (mean recovery, n = 9)	99.29% – 100.64%
Intra-day precision (%RSD, n = 6)	0.33
Intermediate precision	
– Inter-day precision (%RSD, n = 6)	1.28
– Between analysts (%RSD, n = 6)	1.40
Limit of detection (LOD, ng/mL)	15
Limit of quantitation (LOQ, ng/mL)	90

3. RESULTS AND DISCUSSION

3.1. Method development and validation

A reliable reverse-phase HPLC-PDA method was developed and optimized for the quantification of cefazolin sodium in ophthalmic formulations. Initial trials employing methanol and 0.1% acetic acid in water (20:80, v/v) as the mobile phase resulted in prolonged retention time (t_R = 22.79 min) and a broadening peak (Tailing factor = 2.75). To address these issues, alternative organic modifiers with greater eluotropic strength than methanol, such as acetonitrile, were explored. Substituting methanol with acetonitrile at the same volume ratio significantly improved peak symmetry (Tailing factor = 1.69) and reduced the retention time (t_R = 6.07 min).

Further optimization involved varying the acetonitrile proportion (20%–25% v/v), adjusting the flow rate (1.0–1.5 mL/min), evaluating column temperatures (25–35 °C), and screening detection wavelengths (254–305 nm). The final optimized conditions consisted of a mobile phase comprising acetonitrile and 0.1% (v/v) acetic acid in deionized water (22:78, v/v), a flow rate of 1.0 mL/min, a column temperature of 25 °C, and a detection wavelength of 270 nm. Cefazolin was eluted at approximately 5.5 min under the optimized conditions (Figure 1A), yielding well-resolved peaks with tailing factors <2.0, theoretical plate numbers >3,000, and %RSD values <2.0% across six replicate injections. In Figure 1A, a minor impurity peak at 7.7 min, accounting for 0.37% of the total peak area by normalization, was consistently observed but did not interfere with the quantification of cefazolin.

The results of method validation were consistent with the ICH Q2(R2) guidelines. Specificity was confirmed by the absence of interference from formulation excipients or degradation products in the cefazolin peak. Cefazolin solutions degraded after seven days of post-reconstitution, exhibiting a yellow discoloration and a distinct degradation peak at approximately 7.7 min (Figure 1B). Prolonged storage resulted in dark brown discoloration of the solutions, a pronounced decrease in the cefazolin peak, and the emergence of a major degradation peak at 7.7 min, along with several minor impurity peaks observed between 2 and 4 min (Figure 1C). The progressive darkening of degraded cefazolin solutions appeared to correlate with an increased intensity of the degradation peak at 7.7 min. Despite these changes, peak purity indices for cefazolin sodium remained consistently above 0.999 across all samples as shown in Figure 1A–1C, confirming the specificity of the analytical method for quantifying cefazolin in the presence of its degradation products.

Table 2. Robustness testing results of the optimized HPLC method under minor variations in analytical conditions (n = 3).

Parameters	Variations	Peak areas	%RSD
Percent of acetonitrile in the optimized mobile phase (v/v)	20	3018107, 3011826, 3021151	0.26
	22	3027153, 3019859, 3012156	
	24	3021151, 3015470, 3006689	
Column temperature (°C)	20	3019982, 3013045, 3002922	1.36
	25	2945316, 2934018, 2928689	
	30	3027153, 3019859, 3012156	
Detection wavelength (nm)	265	2887884, 2876947, 2871118	1.98
	270	3019982, 3013045, 3002922	
	275	2945316, 2934018, 2928689	
Flow rate (mL/min)	0.9	3264823, 3265102, 3263712	9.30
	1.0	2957921, 2955959, 2957720	
	1.1	2632499, 2631000, 2628495	

Table 3. Forced degradation profile of cefazolin sodium under various stress conditions.

Stress Condition	Degradation (%)	Retention Time (s) of Degradation Product (s) (min)
Control	0.21	7.1
0.1 M HCl	13.90	3.2, 4.5, 7.1
0.1 M NaOH	99.19	2.1, 2.5, 3.5, 4.9, 7.1
6% H_2O_2	9.75	3.2, 7.1
Thermal (60°C)	4.56	3.1, 4.5, 7.1

The developed method demonstrated excellent linearity over the concentration range of 20–200 µg/mL, with the correlation coefficient (r) > 0.999. LOD and LOQ were 15 ng/mL and 90 ng/mL, respectively. Accuracy yielded recovery values ranging from 99.29% to 100.64% across all concentrations. Intra-day and inter-day precision studies showed %RSD values below 2%. Additionally, intermediate precision assessed by two independent analysts resulted in a %RSD of 1.40%, confirming the reproducibility of the method. A summary of the validation parameters is presented in Table 1.

Robustness testing demonstrated consistent method performance under minor variations in column temperature, mobile phase composition, and detection wavelength, as detailed in Table 2. However, changes in flow rate significantly affected reproducibility, highlighting the need to maintain a constant flow rate of 1.0 mL/min for reliable results.

Forced degradation studies revealed that cefazolin was most susceptible to alkaline hydrolysis, followed by acidic, oxidative, and thermal degradation. These findings are consistent with previous reports indicating that alkaline conditions accelerate cefazolin degradation more rapidly than acidic environments[26]. A summary of the degradation profiles is presented in Table 3. Forced degradation samples exhibited additional chromatographic peaks, including a consistent degradation product eluting at approximately 7.1 min, which is presumed to be the same as the major degradation peak previously observed in Figure 1. Chromatograms of cefazolin sodium standard solution obtained from the forced degradation study are shown in Figure 3. These degradation products are likely attributed to hydrolytic cleavage of the β-lactam ring under strongly acidic (pH < 2) and basic (pH > 10) conditions, as well as potential oxidative degradation involving the thioether moiety of cefazolin sodium[14,27]. Despite the presence of these degradation products, the cefazolin peak maintained a purity index above 0.999 across all stress conditions, confirming the specificity and stability-indicating capability of the developed analytical method.

Additionally, the stability of cefazolin sodium stock solutions was evaluated, revealing that the drug gradually degraded in aqueous media over time. This degradation resulted in a reduction in the actual concentration of the standard solution, while the nominal concentration remained unchanged. Consequently, calibration curves constructed using degraded standards led to overestimation of cefazolin concentrations in the ophthalmic formulations due to disproportionately higher sample peak areas. Therefore, to ensure analytical accuracy and reliability, cefazolin sodium stock solutions should be freshly prepared or used within three days of preparation.

3.2. Stability of cefazolin ophthalmic solutions

The chemical stability of fortified cefazolin ophthalmic solutions compounded with five preservative-free vehicles—SWFI, NSS, BSS, Lubric-Eyes®, and VISLUBE®—was evaluated over a 45-day period under frozen storage at −18 ± 2 °C. Cefazolin content remained within 94.36%–99.89% of the initial concentration throughout the study period, indicating acceptable chemical stability across all formulations (Table 4). Figure 2A illustrates a gradual decline in cefazolin content across all formulations during the 45-day frozen storage period. Notably, Formulation 4, containing Lubric-Eyes®, exhibited a more pronounced reduction in drug concentration after Day 28 compared to the control. A two-way ANOVA was conducted to compare the percentage of cefazolin remaining between the control and Formulation 4 throughout the frozen storage period. The analysis demonstrated that Formulation 4 exhibited a statistically significant difference in cefazolin concentration at multiple times compared to the control ($p < 0.05$). These results indicate that cefazolin reconstituted in Lubric-Eyes® was more prone to degradation under frozen storage conditions than when reconstituted in deionized water.

Physical stability assessments revealed no visible changes in color, odor, or clarity in any formulation during frozen storage. No particulate matter was detected upon visual inspection. However, given the limitations of visual assessment in detecting subvisible particles, future studies should incorporate techniques such as light obscuration or microscopic particle counting, particularly for ophthalmic preparations.

The pH values of all formulations remained stable throughout the 45-day freezer storage period, suggesting minimal acid–base degradation. The observed pH ranges were as follows: Formulation 1

(pH 5.42–5.67), Formulation 2 (pH 5.37–5.40), Formulation 3 (pH 5.97–6.02), Formulation 4 (pH 5.60–5.78), and Formulation 5 (pH 6.55–6.70). These values remained within the pH range of 4.5–8.5, which is considered optimal for maintaining cefazolin stability[28,29]. Accordingly, all tested vehicles were suitable for compounding cefazolin ophthalmic solutions, as they maintained formulation pH within a stability-favorable range throughout the study period.

These findings confirm that freezer storage at −18 ± 2 °C effectively extends the chemical and physical stability of preservative-free cefazolin ophthalmic solutions, surpassing previous reports of cefazolin stability in preserved artificial tear-based vehicles, which demonstrated limited stability (≤28 days) under refrigerated conditions[6,14].

Additionally, lubricant-based formulations demonstrated chemical stability comparable to conventional aqueous formulations, despite containing excipients such as HPMC and sodium hyaluronate, which could potentially affect drug solubility and stability. This study not only confirms the stability of HPMC-based formulations under frozen storage but also provides the first evidence supporting the use of sodium hyaluronate-based vehicles as suitable alternatives for cefazolin ophthalmic compounding. Beyond their physicochemical stability, these lubricant eye drops may offer additional clinical benefits. Their hypotonicity could help reduce ocular irritation and enhance patient comfort during intensive dosing regimens. Moreover, both HPMC and sodium hyaluronate possess mucoadhesive properties that may enhance precorneal retention time. Notably, sodium

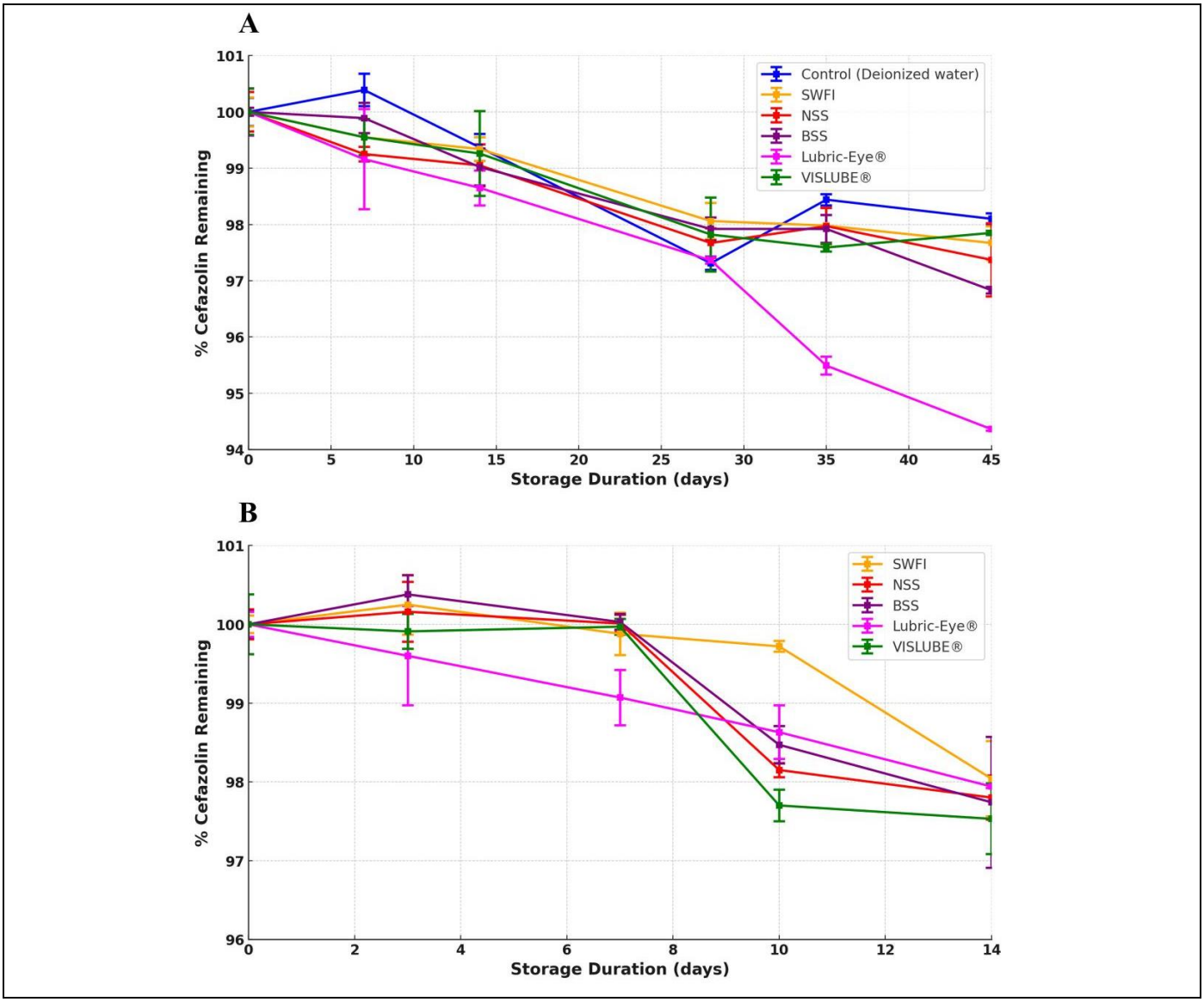

Figure 2. (A) Chemical stability of fortified cefazolin ophthalmic solutions (50 mg/mL) prepared with five preservative-free vehicles—sterile water for injection (SWFI), normal saline solution (NSS), balanced salt solution (BSS), Lubric-Eyes®, and VISLUBE®—during storage at −18 ± 2 °C for 45 days. (B) Chemical stability of the same formulations after 14 days of frozen storage followed by an additional 14 days of storage at 4 ± 2 °C, simulating a 28-day outpatient use period.

Table 4. Chemical stability of fortified cefazolin ophthalmic solutions stored at −18 ± 2 °C over a 45-day period (n = 3).

Formulation	Type of Vehicle used in Formulations	Initial Drug Content (mg/mL)* at Day 0	% Drug Concentration Remaining During Frozen Storage *				
			Day 7	Day 14	Day 28	Day 35	Day 45
Control	Deionized water	55.77 ± 0.25	100.39 ± 0.29	99.37 ± 0.24	97.31 ± 0.12	98.44 ± 0.10	98.10 ± 0.10
1	SWFI	51.24 ± 0.26	99.55 ± 0.32	99.34 ± 0.21	98.06 ± 0.32	97.98 ± 0.31	97.67 ± 0.30
2	NSS	50.94 ± 0.35	99.25 ± 0.13	99.05 ± 0.37	97.67 ± 0.26	97.97 ± 0.32	97.37 ± 0.65
3	BSS	51.14 ± 0.07	99.89 ± 0.27	99.02 ± 0.33	97.92 ± 0.20	97.92 ± 0.25	96.83 ± 0.06
4	Lubric-Eye®	52.66 ± 0.42	99.16 ± 0.89	98.65 ± 0.31	97.36 ± 0.06	95.49 ± 0.16	94.36 ± 0.03
5	VISLUBE®	51.19 ± 0.41	99.55 ± 0.32	99.26 ± 0.75	97.82 ± 0.66	97.59 ± 0.07	97.85 ± 0.04

*means ± SD, and %RSD <2%
SWFI—sterile water for injection, NSS—normal saline solution, and BSS—balanced salt solution

hyaluronate has also been shown to promote corneal epithelial healing, which could further support therapeutic outcomes in the management of bacterial keratitis. Although the present study primarily focused on chemical and physical stability, the inclusion of these excipients suggests potential added therapeutic value. Further investigations are warranted to assess their sterility, clinical efficacy, and patient acceptability to fully establish their suitability for routine use in hospital pharmacy compounding.

Notably, VISLUBE® in single-use lubricant eye drop containers were utilized in this study, as they were readily available and routinely used at Phichit Hospital, Thailand, during the study period. However, the recent availability of preservative-free multi-dose packaging systems may provide a more practical and scalable alternative for ophthalmic compounding in hospital pharmacy settings. These advanced systems are designed to maintain sterility throughout repeated use without the need for preservatives, utilizing innovative mechanisms such as Tip-Seal technology.

To simulate outpatient post-dispensing conditions and typical at-home use, a secondary study was conducted in which previously frozen formulations were thawed and stored under refrigerated conditions (5 ± 3 °C) for an additional 14 days (Days 15–28). This simulation reflects real-world scenarios in which patients store cefazolin ophthalmic solutions at home after receiving them from the pharmacy. During the 14-day post-thaw period, all formulations maintained chemical stability within the acceptable range (97.53%–100.38%) (Figure 2B, Table 5). The pH of all formulations remained stable within the range of 5 to 6, and no visible particulate matter was observed throughout the study period. While minor discoloration and faint odor changes were observed after Day 21, these did not compromise chemical integrity; however, they may affect patient acceptability.

4. CONCLUSIONS

This study demonstrates that extemporaneously prepared fortified cefazolin ophthalmic solutions (50 mg/mL), when compounded with five preservative-free vehicles and stored at −18 ± 2 °C, remain chemically and physically stable for up to 45 days. Among these, lubricant-based vehicles—Lubric-Eyes® (HPMC-based) and VISLUBE® (sodium hyaluronate-based)—exhibited stability profiles comparable to those of conventional aqueous diluents. Notably, this is the first study to provide evidence supporting the compatibility and suitability of sodium hyaluronate-based vehicles for cefazolin ophthalmic compounding.

For outpatient use, preservative-free cefazolin ophthalmic solutions dispensed in frozen form should be thawed at room temperature (30 ± 2 °C) for approximately 1 hr prior to use. Following thawing, the cefazolin eye drops should be stored under refrigerated

Table 5. Chemical stability of post-thaw cefazolin ophthalmic solutions during 14 days of refrigerated storage (5 ± 3 °C) following prior frozen storage at −18 ± 2 °C (n = 3).

Formulation	Type of Vehicle used in Formulations	Initial Drug Content (mg/mL)* on Day 0 of Refrigerated Storage (following 14 days of frozen storage)	%Drug Concentration Remaining During Refrigerated Storage*			
			Day 3	Day 7	Day 10	Day14
1	SWFI	50.90 ± 0.11	100.25 ± 0.38	99.88 ± 0.27	99.72 ± 0.07	98.04 ± 0.48
2	NSS	50.46 ± 0.19	100.16 ± 0.38	100.01 ± 0.11	98.15 ± 0.09	97.80 ± 0.28
3	BSS	50.64 ± 0.17	100.38 ± 0.24	100.03 ± 0.10	98.47 ± 0.24	97.74 ± 0.83
4	Lubric-Eye®	51.95 ± 0.16	99.60 ± 0.63	99.07 ± 0.35	98.63 ± 0.34	97.94 ± 0.02
5	VISLUBE®	50.81 ± 0.38	99.91 ± 0.22	99.97 ± 0.10	97.70 ± 0.20	97.53 ± 0.45

*means ± SD, and %RSD <2%
SWFI—sterile water for injection, NSS—normal saline solution, and BSS—balanced salt solution

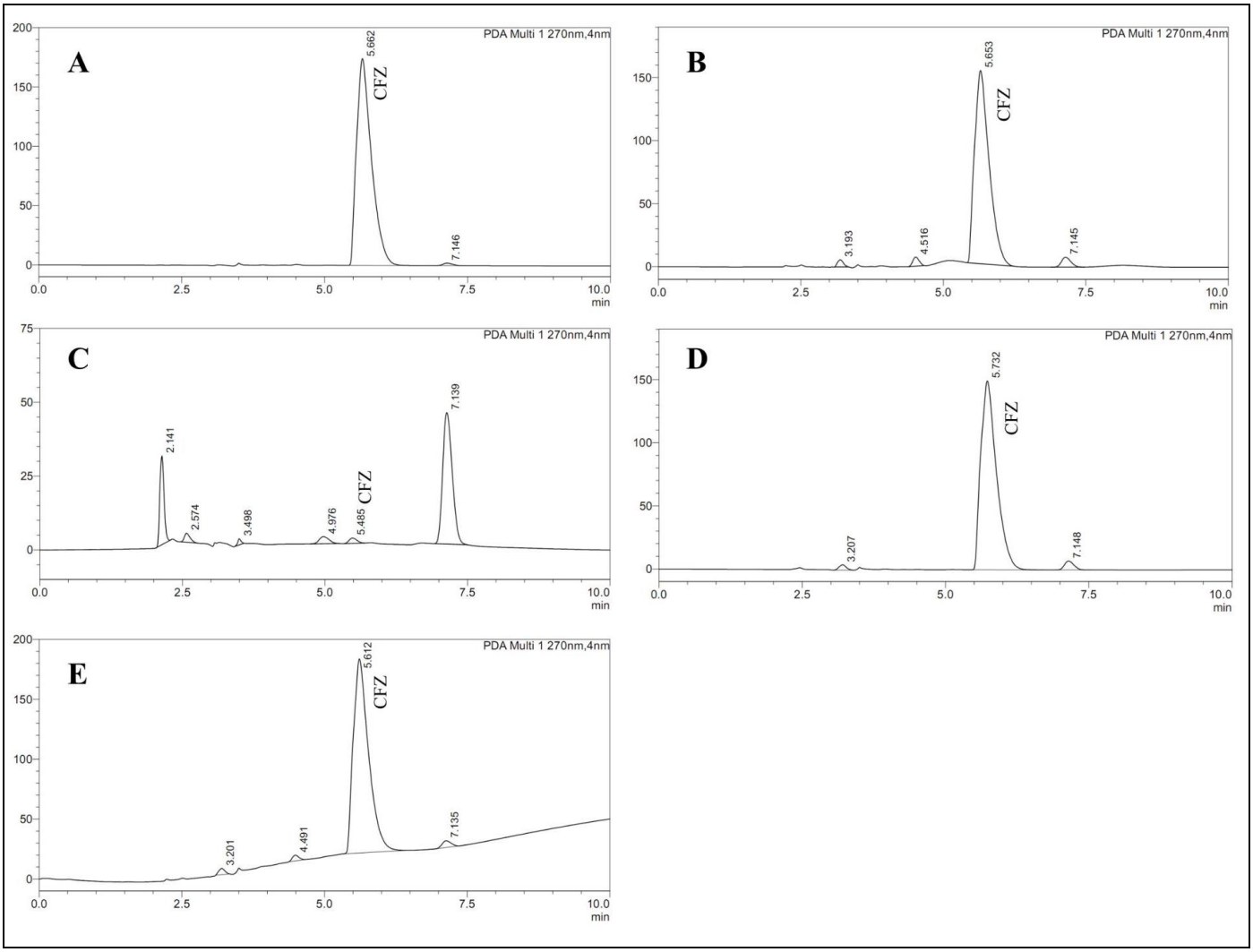

Figure 3. Representative HPLC chromatograms of cefazolin (CFZ) sodium standard solutions (100 µg/mL) under various conditions: (A) unstressed (control); and stressed conditions including (B) acidic degradation, (C) alkaline degradation, (D) oxidative degradation, and (E) thermal degradation.

conditions (5 ± 3 °C) and used within 14 days. Re-freezing of the thawed product is not recommended, as the stability of the formulations under repeated freeze–thaw cycles has not been evaluated or established.

These findings confirm that frozen storage effectively extends the shelf life of preservative-free cefazolin ophthalmic solutions, offering a practical alternative to conventional refrigerated storage. Maintaining frozen, ready-to-use formulations may enhance workflow efficiency and facilitate prompt dispensing in emergency situations within hospital pharmacy settings. Furthermore, this extended usability has the potential to improve treatment continuity, reduce the frequency of pharmacy visits, and promote better adherence in outpatient care. Overall, this study provides a foundation for advancing extemporaneous ophthalmic compounding practices within hospital pharmacies, contributing to improved formulation stability and enhanced patient outcomes.

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Author contribution

NIK: Conceptualization, Preliminary Study, Investigation, Formal analysis, Resources, Writing – Review and Editing.

SC: Validation, Formal analysis, Writing – Review and Editing.

NAK: Validation, Formal analysis, Writing – Review and Editing.

SW: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Visualization, Resources,

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