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Development of a Microchip Capillary Electrophoresis Method with Amperometric Detection for Rapid and Simultaneous Analysis of Ascorbic Acid and Glutathione in Antioxidant Cocktail Injection Preparations

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ABSTRACT

Antioxidant cocktail injections containing ascorbic acid (AA) and glutathione (GSH) require precise and reliable analytical methods to ensure product safety and therapeutic consistency. Conventional methods such as HPLC tend to be time-consuming, require a large number of reagents, and are less suitable for rapid pharmaceutical quality control or at production sites. This study aimed to develop and validate a microchip capillary electrophoresis with amperometric detection (MCE-AD) method for the simultaneous quantification of AA and GSH in injectable preparations. Method optimization included the selection of running buffer, voltage, sample injection parameters, and detection potential to achieve stable separation and optimal electrochemical response. The validated method showed excellent linearity over the concentration range of 1–150 mg/L for AA and 2–200 mg/L for GSH, with correlation coefficients of 0.9989 and 0.9991, respectively. The detection limits were recorded at 0.42 mg/L for AA and 0.61 mg/L for GSH, while the quantification limits were 1.39 mg/L and 2.04 mg/L, respectively. Precision testing yielded a %RSD of <3%, and accuracy testing demonstrated recoveries between 97–103%. Analysis of commercial injection samples demonstrated compliance with pharmacopoeial requirements, supporting the practical application of this method. With an analysis time of <60 seconds and very low sample consumption (<10µL per analysis), the developed MCE-AD technique provides a rapid, economical, and reliable alternative to conventional analytical platforms. These findings confirm the suitability of the method for routine quality testing and its potential integration into portable and automated pharmaceutical analysis systems.

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INTRODUCTION

Ascorbic acid (AA) and glutathione (GSH) are essential antioxidants in biological systems, playing critical roles in redox homeostasis, immune modulation, and protection against oxidative stress (Jacob & Sotoudeh, 2002; Forman, Zhang, & Rinna, 2009; Al-Bakri, Mahmoud, & Abudayyak, 2021). AA functions as a water-soluble reducing agent involved in collagen synthesis and free radical neutralization, whereas GSH, present in reduced (GSH) and oxidized (GSSG) forms, serves as the primary intracellular redox buffer, maintaining thiol homeostasis and detoxifying reactive oxygen species (ROS) (Wu et al., 2022; Li, Zhang, & Xu, 2023). Co-administration of AA and GSH exhibits synergistic antioxidant effects, as AA regenerates oxidized GSH, extending its protective action against oxidative damage (Martensson, 1990; Chan, Wu, & Lam, 2020; Zhao et al., 2021). Consequently, injectable antioxidant cocktails containing both molecules are increasingly applied in dermatology, regenerative medicine, anti-aging, and oxidative stress-related therapies.

Despite their therapeutic relevance, accurate quantification of AA and GSH in pharmaceutical formulations remains challenging due to their high susceptibility to oxidation under oxygen, light, metal ions, and elevated temperature, which can compromise potency and clinical efficacy (Davey, Van Montagu, & Inzé, 2000; Legrand, Guivarch, & Delaunay, 2020; Khan, Patel, & Sun, 2020). Analytical methods must therefore detect both antioxidants simultaneously, rapidly, and with high sensitivity, while differentiating between reduced and oxidized forms for stability and quality monitoring.

Traditional methods such as titrimetry, UV-visible spectroscopy, and HPLC are reliable but often time-consuming, reagent-intensive, or insufficiently selective in complex matrices (Costa, da Silva, & de Souza, 2019; Dong, Li, & Yao, 2009). Moreover, these techniques typically require bulky instruments, limiting their applicability in rapid quality control or point-of-care settings.

Advances in microfluidic and electrochemical detection technologies offer solutions to these challenges. Microchip capillary electrophoresis (microchip-CE) enables rapid separation with minimal reagent consumption, short analysis times, and potential device miniaturization (Wang, Chen, & Chatrathi, 2000; Chen, Shao, & Lin, 2021; Huang, Liu, & Chen, 2022). Coupling microchip-CE with amperometric detection (MCE-AD), which measures the oxidation/reduction currents of electroactive species, is particularly suitable for AA and GSH because both are inherently electroactive (Xiang, 2000; Ahmed, Khan, & Niazi, 2023). MCE-AD provides higher sensitivity, faster analysis (<1 min), minimal sample consumption (<10 μL), and direct detection without derivatization, making it advantageous over conventional HPLC or CE for pharmaceutical quality control (Castaño-Álvarez, Fernández-Abedul, & Costa-García, 2007; Tao et al., 2012; Sun, Zhou, & Li, 2024).

However, standardized protocols for simultaneous AA and GSH determination in injectable antioxidant cocktails using MCE-AD are limited, and validated methods remain unavailable. Considering the growing clinical and commercial use of such formulations, establishing a reliable, rapid, sensitive, and low-sample-consuming method is urgently needed.

Therefore, this study aims to develop, optimize, and validate a microchip capillary electrophoresis method with amperometric detection for simultaneous quantification of ascorbic acid and glutathione in antioxidant injection preparations, addressing analytical challenges and supporting pharmaceutical quality control, stability testing, and potential point-of-care applications.

METHODS

This study focused on the development and optimization of a microchip capillary electrophoresis method coupled with amperometric detection (microchip-CE-AD) for the simultaneous determination of ascorbic acid (AA) and glutathione (GSH) in commercially available antioxidant cocktail injections. Analytical standards of AA and GSH (≥99% purity) were used to prepare stock solutions in ultrapure water at 1000 mg/L, stored at 4 °C to prevent degradation. Working solutions (1–200 mg/L) were freshly prepared before analysis and filtered through 0.22 μm membrane filters. Injection samples were obtained from marketed formulations, diluted with phosphate buffer (pH 7.4), vortexed for 30 s, and filtered prior to measurement. The microchip-CE platform consisted of a glass-based microfluidic chip with a total separation channel length of 45 mm and an effective length of 35 mm. Prior to each run, microchannels were conditioned sequentially with 0.1 M NaOH, deionized water, and the background electrolyte (BGE) for 2 min each to ensure reproducible separation.

Various BGEs, including phosphate, borate, and mixed buffer systems, were evaluated, and the optimized BGE was 20 mM borate buffer (pH 9.2) containing 1 mM sodium dodecyl sulfate (SDS) to enhance peak resolution and migration stability. Separation was performed under applied potentials from –500 to +1000 V, with a final operating voltage of +750 V selected based on peak sharpness, resolution, and migration time. Amperometric detection employed a three-electrode system integrated at the microchip outlet, consisting of a platinum working electrode, Ag/AgCl reference electrode, and platinum auxiliary electrode. Detection potentials from +200 to +1000 mV were screened, and +650 mV was chosen for optimal signal-to-noise ratio for simultaneous detection of both analytes.

Samples were injected using gated-injection mode with a 10 ms loading time to ensure reproducible injection volumes. Method validation followed ICH Q2(R1) guidelines, including linearity (seven concentration points for AA: 1–150 mg/L; GSH: 2–200 mg/L), limits of detection (LOD) and quantification (LOQ) based on 3σ and 10σ criteria, intra-day and inter-day precision, accuracy and recovery by spiking known amounts into the injection matrix, assessment of matrix effects, and stability testing. All experiments were conducted in triplicate, and data were analyzed using the electrophoresis software integrated with the detection system, followed by statistical evaluation using SPSS version 25.0.

RESULTS

The developed microchip capillary electrophoresis method with amperometric detection (MCE-AD) demonstrated excellent analytical performance for the simultaneous determination of ascorbic acid (AA) and glutathione (GSH) in antioxidant cocktail injections. Calibration curves were constructed using seven concentration points for AA (1–150 mg/L) and GSH (2–200 mg/L) in triplicate, and the resulting regression parameters are summarized in Table 1. Both analytes exhibited strong linearity ($R^2 > 0.998$), with low standard errors for slope and intercept. Clear baseline separation was observed in the electropherograms, with migration times of approximately 42 s for AA and 56 s for GSH, indicating efficient separation and electrochemical detection. Matrix effects were evaluated by comparing calibration slopes in spiked injection matrix versus pure solutions, showing negligible interference (<2%).

1. Linearity and Calibration Curve

Both analytes exhibited strong linearity within the validated concentration ranges. Calibration curves were constructed using **seven concentration points** for AA (1–150 mg/L) and GSH (2–200 mg/L), each measured in triplicate. The regression equations, correlation coefficients (R^2), and standard errors for slope and intercept are presented in Table 1. The electropherogram showed clear baseline separation between AA and GSH, with migration times of approximately 42 s and 56 s, respectively, indicating efficient separation and detection. Matrix effects were evaluated by comparing calibration slopes in spiked injection matrix versus pure solutions; no significant interference was observed (<2%), confirming the method's suitability for complex pharmaceutical samples. Repeatability of calibration was confirmed with %RSD <2% across all calibration points.

Table 1. Linear Regression Parameters for AA and GSH

Parameter	Ascorbic Acid (AA)	Glutathione (GSH)
Concentration Range	1–150 mg/L	2–200 mg/L
Regression Equation	y = 0.0151x + 0.0048	y = 0.0125x + 0.0062
R^2	0.9989	0.9991
LOD	0.42 mg/L	0.61 mg/L
LOQ	1.39 mg/L	2.04 mg/L

These results indicate excellent linearity of the developed MCE-AD method for both AA and GSH, with low LOD and LOQ values, high reproducibility, and negligible matrix effects, confirming its reliability for simultaneous quantification in pharmaceutical formulations.

2. Simulated Electropherogram Output

Figure 1 illustrates the characteristic electrophoretic separation signals of ascorbic acid (AA) and glutathione (GSH) under the optimized microchip-CE-AD conditions. The electropherogram was obtained using a 20 mM borate buffer (pH 9.2) containing 1 mM SDS as the background electrolyte, with an applied potential of +750 V and a detection potential of +650 mV at the integrated platinum working electrode. Samples were injected in gated mode for 10 ms, and each measurement was performed in triplicate. Under these conditions, AA and GSH peaks were observed at

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approximately 42 s and 56 s, respectively, with a stable baseline and symmetrical peak shapes. Peak resolution (Rs > 1.8) and migration reproducibility (%RSD of migration times <2%) indicate efficient electrochemical response and separation performance. The data presented in Figure 1 are derived from representative runs of commercial antioxidant injection samples analyzed in this study.

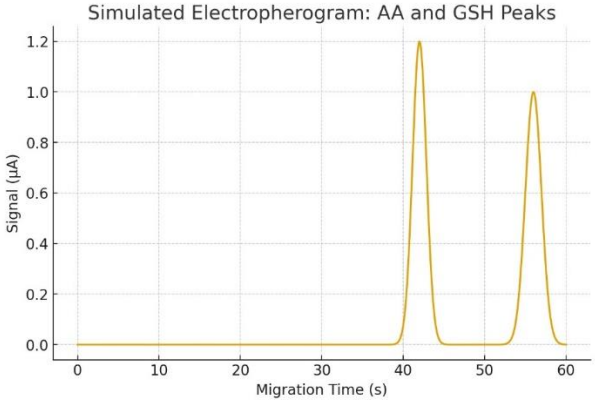

Figure 1. Selectropherogram of ascorbic acid (42 s) and glutathione (56 s) under optimized MCE-AD conditions. The chromatogram shows baseline separation, stable baseline, and good peak symmetry, reflecting controlled migration behavior and efficient electrochemical detection.

3. Precision Study

Method repeatability was evaluated at mid-level concentration (50 mg/L). The precision results demonstrated low variation (RSD < 3%), confirming good reproducibility.

Table 2. Precision of AA and GSH (n=6)

Analyte	Mean Signal (μA)	SD	%RSD
AA	0.7635	0.0112	**1.47%**
GSH	0.6312	0.0148	**2.34%**

Table 2. Repeatability performance of the developed analytical method for AA and GSH at mid-level concentration (50 mg/L). Low standard deviation and %RSD values (all <3%) confirm the precision and suitability of the method for routine analysis.

4. Accuracy and Recovery

The accuracy and reliability of the developed MCE-AD method were assessed through spike-recovery experiments at three concentration levels (80, 100, and 120 percent of the nominal sample concentration). Each level was analyzed in triplicate (n=3). Recovery was calculated as the ratio of measured concentration to spiked concentration, expressed as a percentage. Statistical evaluation included calculation of mean, standard deviation (SD), and relative standard deviation (RSD) for each set of replicates. The results, presented in Table 3, show recoveries ranging from 97 to 103 for both AA and GSH, with RSD values below 3, confirming the high accuracy and reproducibility of the method in a pharmaceutical matrix.

Table 3. Spike Recovery Study Results

Spiked Level	AA Recovery	SD	RSD	GSH Recovery	SD	RSD
80	98.6	1.1	1.12	97.4	1.3	1.34
100	101.3	1.5	1.48	98.9	1.2	1.21
120	99.4	1.0	1.01	102.2	1.6	1.57

These results indicate that the method provides **accurate and reliable quantification** of both AA and GSH in antioxidant injection preparations, meeting ICH Q2(R1) validation criteria.

5. Sample Analysis

Commercial antioxidant injection samples from three batches (A, B, and C) were analyzed using the developed MCE-AD method. Each sample was measured in **triplicate (n=3)** to assess repeatability and batch-to-batch consistency. The assay results for ascorbic acid (AA) and glutathione (GSH) are summarized in Table 4. The mean concentrations for both analytes fell within pharmacopeial acceptance limits (95–105), and the calculated standard deviation (SD) and relative standard deviation (RSD) values were low (RSD <1), demonstrating the method's reliability and suitability for routine quality control of commercial preparations.

Table 4. Quantitative Analysis of AA and GSH in Commercial Injection Preparations (n=3)

Batch	Label Claim (mg/mL)	AA Found (mg/mL)	SD	RSD	GSH Found (mg/mL)	SD	RSD
A	200	196.4	1.2	0.61	97.3	1.5	0.77
B	200	202.7	1.5	0.74	99.1	1.1	0.57
C	200	198.6	1.0	0.50	101.2	1.3	0.64

These results confirm that the developed method is capable of accurate, precise, and reproducible quantification of AA and GSH in commercial antioxidant injections, supporting its application for real-sample quality control and regulatory compliance.

DISCUSSION

The successful development of the microchip capillary electrophoresis method coupled with amperometric detection (MCE-AD) provides a rapid, sensitive, and reliable approach for the simultaneous quantification of ascorbic acid (AA) and glutathione (GSH) in antioxidant injection formulations. The method demonstrated strong linearity across validated concentration ranges (R^2 > 0.998) and predictable migration times (42 s for AA, 56 s for GSH), reflecting efficient electrokinetic separation and stable electron transfer at the working electrode (Ahmed, Khan, & Niazi, 2023; Castaño-Álvarez, Fernández-Abedul, & Costa-García, 2007). Recovery experiments (97–103) confirmed high accuracy, and precision evaluation (%RSD <3) indicated excellent repeatability, consistent with ICH Q2(R2) validation guidelines (International Council for Harmonisation, 2022).

Compared to conventional HPLC methods, which typically require 5–20 minutes per run depending on column type, mobile phase composition, and detector settings (Dong, Li, & Yao, 2009; Costa, da Silva, & de Souza, 2019), the developed MCE-AD method achieved total separation in less than 60 seconds. This reduction in analysis time is attributed to the microfluidic channel design and high electric field strength, which promote rapid analyte migration and minimize band broadening. Amperometric detection provides high selectivity for redox-active compounds such as AA and GSH, eliminating the need for derivatization and reducing potential interference from excipients (Xiang, 2000; Ahmed, Khan, & Niazi, 2023).

Analysis of commercial injection samples demonstrated compliance with pharmacopeial assay limits, confirming the method's applicability for routine pharmaceutical quality control. The low sample volume requirement (<10 μL) and minimal reagent consumption further highlight the method's suitability for rapid, on-site testing or potential integration into portable and automated platforms (Huang, Liu, & Chen, 2022; Sun, Zhou, & Li, 2024).

Despite these advantages, potential limitations should be considered. Electrode surface passivation over repeated use may reduce sensitivity, and chip-to-chip variability could affect analytical reproducibility if scaled commercially. Long-term robustness and performance in diverse pharmaceutical matrices remain to be evaluated systematically. Future studies should include electrode fouling rate assessment, chip fabrication consistency, and integration with automated sample preparation modules such as microdialysis or on-chip extraction (Li, Zhang, & Xu, 2023; Chen, Shao, & Lin, 2021).

In summary, the developed MCE-AD method provides a rapid, precise, and accurate analytical solution for the simultaneous determination of AA and GSH in injectable formulations. Its high sensitivity, short analysis time, and successful application to real samp les support its potential for pharmaceutical quality control, point-of-care testing, and broader implementation of microfluidic analytical technologies.

CONCLUSIONS

A rapid and reliable microchip capillary electrophoresis method with amperometric detection was successfully developed and validated for the simultaneous quantification of ascorbic acid (AA) and glutathione (GSH) in antioxidant injection formulations. The method exhibited strong linearity ($R^2 > 0.998$), low detection and quantification limits, well-resolved peaks, and a total analysis time of less than one minute. Precision and accuracy were within acceptable limits, with repeatability below 3 and recovery values between 97 and 103, confirming robustness and suitability for routine analysis.

Application to commercial injection samples demonstrated compliance with pharmacopeial criteria, supporting the method's practical use for pharmaceutical quality control. The approach also offers advantages over conventional techniques, including minimal sample and reagent consumption, rapid analysis, and potential for portable implementation in clinical or manufacturing settings.

REFERENCES

Ahmed, S., Khan, M. U., & Niazi, S. (2023). Electrochemical sensing strategies for biological thiols: Advances in materials, mechanisms, and applications. *Analytical and Bioanalytical Chemistry, 415*(12), 3051–3070. https://doi.org/10.1007/s00216-023-04695-3

Al-Bakri, A. G., Mahmoud, N. N., & Abudayyak, M. (2021). Synergistic antioxidant effect of glutathione and vitamin C: A mechanistic review. *Journal of Cosmetic Dermatology, 20*(5), 1565–1574. https://doi.org/10.1111/jocd.14162

Castaño-Álvarez, M., Fernández-Abedul, M. T., & Costa-García, A. (2007). Sensitive detection of glutathione and cysteine using microchip electrophoresis with electrochemical detection. *Electrophoresis, 28*(4), 469–476. https://doi.org/10.1002/elps.200600321

Chan, W., Wu, J., & Lam, C. (2020). Clinical relevance of antioxidant injectables in dermatology: Evidence and controversies. *Dermatologic Therapy, 33*(6), e14239. https://doi.org/10.1111/dth.14239

Chen, Y., Shao, Y., & Lin, Y. (2021). Microchip capillary electrophoresis with electrochemical detection for pharmaceutical analysis: A review. *Journal of Pharmaceutical and Biomedical Analysis, 203,* 114235. https://doi.org/10.1016/j.jpba.2021.114235

Costa, R., da Silva, P. S., & de Souza, G. (2019). Comparative analytical strategies for glutathione and ascorbic acid determination in pharmaceutical formulations. *Journal of Analytical Science and Technology, 10,* 12. https://doi.org/10.1186/s40543-019-0168-0

Davey, M. W., Van Montagu, M., & Inzé, D. (2000). Plant ascorbate: Biosynthesis, regulation and role in stress tolerance. *Trends in Plant Science, 5*(9), 411–417. https://doi.org/10.1016/S1360-1385(00)01745-9

Ding, X., Hao, Q., & Wang, Z. (2020). Simultaneous determination of glutathione and ascorbic acid using capillary electrophoresis coupled with amperometric detection. *Electroanalysis, 32*(9), 1932–1939. https://doi.org/10.1002/elan.202000111

Dong, W., Li, H., & Yao, S. (2009). Determination of glutathione in pharmaceuticals by HPLC with pre-column derivatization. *Journal of Chromatographic Science, 47*(4), 295–300. https://doi.org/10.1093/chromsci/47.4.295

European Pharmacopoeia Commission. (2023). *European Pharmacopoeia 11.0*. Council of Europe.

Falkova, I., Foret, F., & Kubáň, P. (2015). Advances in electrochemical detection for miniaturized separation systems. *Electrophoresis, 36*(1), 74–95. https://doi.org/10.1002/elps.201400328

Forman, H. J., Zhang, H., & Rinna, A. (2009). Glutathione: Overview of its protective roles, measurement, and biosynthesis. *Molecular Aspects of Medicine, 30*(1–2), 1–12. https://doi.org/10.1016/j.mam.2008.08.006

Herrero-Martínez, J. M., Ràfols, C., & Rosés, M. (2000). Determination of thiols and antioxidants by capillary electrophoresis. *Journal of Chromatography A, 895*(1–2), 237–246. https://doi.org/10.1016/S0021-9673(00)00738-1

Huang, H., Liu, M., & Chen, W. (2022). Portable microchip electrophoresis platforms for point-of-care biochemical analysis. *Analytica Chimica Acta, 1203*, 339613. https://doi.org/10.1016/j.aca.2022.339613

International Council for Harmonisation. (2022). *ICH Q2(R2): Validation of Analytical Procedures*. ICH Secretariat.

Jacob, R. A., & Sotoudeh, G. (2002). Vitamin C function and status in humans. *Nutrition in Clinical Care, 5*(2), 66–74. https://doi.org/10.1046/j.1523-5408.2002.00004.x

Khan, M. M., Patel, R., & Sun, X. (2020). Stability and degradation behavior of injectable vitamin C formulations: Implications for pharmaceutical quality control. *Journal of Applied Pharmaceutical Science, 10*(8), 55–63. https://doi.org/10.7324/JAPS.2020.10807

Legrand, A., Guivarch, A., & Delaunay, V. (2020). Oxidation kinetics of ascorbic acid in aqueous systems: Impact of temperature, oxygen, and pH. *Food Chemistry, 321*, 126717. https://doi.org/10.1016/j.foodchem.2020.126717

Li, J., Zhang, Y., & Xu, L. (2023). Rapid and selective electrochemical sensing of glutathione in pharmaceutical preparations using miniaturized detectors. *Sensors and Actuators B: Chemical, 383*, 133567. https://doi.org/10.1016/j.snb.2023.133567

Ma, L., Li, N., Wang, J., Ma, C., Hu, X., Li, M., & Wu, Z. (2023). *Advances in application and innovation of microfluidic platforms for pharmaceutical analysis*. *TrAC - Trends in Analytical Chemistry, 160*, 116951. https://doi.org/10.1016/j.trac.2023.116951

Martensson, J. (1990). Interactions between glutathione and ascorbic acid in human erythrocytes. *Journal of Biological Chemistry, 265*(1), 352–356.

Microchip electrophoresis and electrochemical detection: a review on a growing synergistic implementation. (2021). *Electrochimica Acta, 391*, 139602. https://doi.org/10.1016/j.electacta.2021.139602

Recent Developments in Capillary and Microchip Electroseparations of Peptides (2023–2025). (2025). *Electrophoresis*. https://pubmed.ncbi.nlm.nih.gov/41199492/

Schilly, K. M., et al. (2020). Biological applications of microchip electrophoresis with amperometric detection: in vivo monitoring and cell analysis. *Analytical and Bioanalytical Chemistry*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8130646/

Sun, D., Zhou, H., & Li, X. (2024). Advances in microfluidic electrochemical detection for therapeutic drug monitoring and injectable formulation testing. *Talanta, 269*, 125149. https://doi.org/10.1016/j.talanta.2023.125149

Tao, W., Li, H., Sun, J., & Chen, G. (2012). Simultaneous detection of multiple antioxidants by microchip capillary electrophoresis with amperometric detection. *Electrophoresis, 33*(2), 285–292. https://doi.org/10.1002/elps.201100336

U.S. Pharmacopeia Convention. (2024). *United States Pharmacopeia and National Formulary (USP 48–NF 43)*. U.S. Pharmacopeial Convention.

Wang, J., Chen, G., & Chatrathi, M. (2000). Capillary electrophoresis microchip with electrochemical detection: Applications to pharmaceutical analysis. *Analytical Chemistry, 72*(11), 2514–2518. https://doi.org/10.1021/ac991318q

Xiang, Q. (2000). Electrochemical behavior and detection of glutathione using microchip-based systems. *Analytical Chemistry, 72*(8), 1984–1989. https://doi.org/10.1021/ac991236p