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Research Article

Solvent-driven stability: UV spectroscopy study of phenolic substances

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Abstract: In contemporary industry, the analysis of various substances often requires handling samples with appropriate solvents, relying on stock solutions for research purposes. This study investigates the stability of gallic acid, ellagic acid, and quercetin naturally occurring polyphenolic compounds with potential pharmaceutical applications. The study explored the stability of these materials in different solvent environments and at varied temperatures, highlighting the critical role of solvent choice and temperature in preserving compound integrity. Gallic acid, quercetin, and ellagic acid were each dissolved at a concentration of 5 µg/mL in different solvents. UV spectroscopic analysis was conducted periodically over one month, with samples stored in controlled environments. Stability was assessed by examining UV absorption spectra, and data were analyzed using statistical methods. The results indicated that the choice of solvent significantly impacted compound stability. Gallic acid showed the highest stability in ethanol (100%) and DMSO (10%) at both room and refrigerated temperatures. Ellagic acid demonstrated optimal stability in DMSO (10%), with variability in other solvents. Quercetin exhibited the highest stability in DMSO (10%), while ethanol showed significant variability. Refrigeration enhanced stability across all solvents. These findings underscore the importance of selecting appropriate solvents and storage conditions to preserve the quality of active pharmaceutical ingredients (API). The results offer valuable insights for improving the stability of stock solutions in pharmaceutical development and quality control, providing crucial information for enhancing the preservation of APIs.

1. INTRODUCTION

Stability study of standard substances used in pharmaceutical analysis in various solvents is crucial for determining their integrity and degradation. There are several methods used for determining stability study. For example, forced degradation, chromatographic assays, and UV/Vis spectrophotometric methods are used to develop the stability indicating methods that involve subjecting the drug substance to conditions that are more extreme than normal manufacturing, storage, and by producing representative samples for method development and validation (Ambhore *et al*., 2021; Bhaskar *et al*., 2020; Blessy *et al*., 2014; Jakkam *et al*., 2021; Robnik *et al*., 2019; Singh & Singh, 2018; Srivastava 2017; Verma *et al*., 2022). The International Council for Harmonisation (ICH) of technical requirements for pharmaceuticals

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for human use guidelines formally codify them as a regulatory requirement (Bhaskar *et al*., 2020). For pharmaceutical products to be safe, effective, and of high quality, it is essential to evaluate the stability of the drugs and the degradation products they produce (Bhaskar *et al*., 2020; Jakkam *et al*., 2021; Verma *et al*., 2022).

Stock solutions are prepared by dissolving a known mass of a substance in a solvent to obtain a specific concentration. The cost of the APIs used in preparing stock solutions is a crucial consideration, as it directly impacts the overall cost of the solution. Additionally, the stability of stock solutions is essential to ensure that the concentration of the drug substance remains constant over time, which is vital for accurate and reliable experimental results. Various factors, such as pH, temperature, and light exposure, can influence the stability of stock solutions. Since the same stock solutions are used many times for the analytical methods until their completion. Therefore, proper storage conditions and handling procedures are necessary to maintain the stability of stock solutions (Karinen *et al*., 2011).

Pharmaceutical analysis involves evaluating medicinal ingredients and formulations to ensure their effectiveness, quality, and safety. Gallic acid is widely used for its versatility in these analyses, particularly as a reference antioxidant for measuring phenolic content in plant extracts and other analytes in gallic acid equivalents (Babich *et al*., 2011; Badhani *et al*., 2015a). It plays a significant role in synthesizing compounds like propyl gallate and trimethoprim, which are important in the food and pharmaceutical industries. Gallic acid is also recognized for its therapeutic properties, including antifungal, antiviral, and antioxidant activities, making it useful in treating conditions such as albuminuria and diabetes. Additionally, it and its esters serve as antioxidants in food and pharmaceuticals, protecting against oxidative damage from reactive oxygen species (Badhani *et al*., 2015b). Ellagic acid, another polyphenol, has been studied for its antioxidant benefits and is often extracted using solvents like methanol. Research indicates that ellagic acid's solubility issues in various solvents can affect its stability and efficacy in medicinal applications (Daniel *et al*., 1989; Polaka *et al*., 2021). Quercetin, a flavonoid with strong antioxidant properties, is also extensively studied for its stability and solubility in stock solutions, emphasizing the importance of these factors in pharmaceutical formulations (Alliangana, 1996; Görög, 2018).

UV spectroscopy is a widely used instrument that plays a vital role in pharmaceutical analysis for ensuring medication stability, purity, and adherence to regulatory guidelines. It assesses chemical and physical stability under various conditions, providing crucial insights (Blessy *et al*., 2014). This technique, endorsed by organizations like WHO and ICH, evaluates stability in solvents and complex compounds (Görög, 2018). UV spectroscopy's versatility extends to biomolecular and nanocomposite stability studies, emphasizing its role in innovative drug formulations. Monitoring UV absorption spectra aids in detecting medication deterioration, guiding researchers in assessing stability patterns across different solvents over time (Baek & Patra, 2015). The research goal was to use UV spectroscopy to study the stability of diverse pharmacological compounds dissolved in different solvents over a month at different temperature conditions. The study also determined the stability profiles of API and solvent effects on APA stability by analyzing UV absorption spectra over time. This research helps to analyse, create and formulate pharmaceutical products and understand solvent characteristics and drug substance stability.

2. MATERIAL and METHODS

Phenolic compounds, namely Gallic acid, Ellagic acid, and Quercetin, were employed in the study. Solvents, including methanol, ethanol, and Dimethyl sulfoxide, were procured from Sigma-Aldrich in the year 2021. Borosilicate transparent test tubes with screwed caps were utilized for the meticulous preparation of stock solutions. Accurate sample weighing was conducted using the Acculab ALC-210.4 Analytical Balance, with a precision of 210 g x 0.1 mg. Spectroscopical analysis was performed using the SHIMATZU UV Spectrophotometer 1700 at Sri Adichunchanagiri College of Pharmacy. Additionally, a sonicator was used to ensure complete dissolution of the drug substances in the selected solvents.

2.1. Preparation of Stock Solutions and Experimental Setup

Stock solutions were prepared by weighing the phenolic compounds and dissolving in measured volume of respective solvents like methanol (100%), ethanol (100%), hydroalcohol (70%), and Dimethyl sulfoxide (10%) to attain a concentration of 5µg/mL. These solutions were then sonicated for 15 minutes to dissolve the drugs in the suitable solvents. The solutions were subsequently subjected to UV spectroscopy measurements. The experimental setup involved recording the UV absorption spectra of the solutions at specific intervals: 1 Day 1, Week 1, 2Weeks, 3Weeks, and 4 Weeks at room temperature (25°C) and refrigerator condition (4°C). Two maximum wavelengths were selected for each drug substance, and absorbance was measured consistently at these wavelengths over time.

2.2. Storage Conditions

The stock solutions were stored in transparent borosilicate test tubes with screwed caps to accurately replicate pharmaceutical storage conditions. Two storage conditions were used for comparison: the refrigerator condition, where samples were kept at 4°C, mimicking common pharmaceutical storage practices for temperature-sensitive compounds, and the room temperature condition, where samples were stored at 25°C to simulate typical storage in a pharmaceutical manufacturing facility. Over the course of one month, any changes in the UV absorption spectra, such as shifts in peak intensity or position, were meticulously monitored for each drug substance-solvent combination under both conditions. These changes could indicate the degradation or instability of the drugs in the selected solvents under the above conditions.

2.3. UV Spectroscopy Study

The UV spectroscopy study was conducted using a UV-Vis Spectrophotometer 1700 with a measurement range of 200-800 nm and a wavelength resolution of 1 nm. A 1 cm quartz cuvette was used for all measurements, and the scan speed was set at 200 nm/min. Each sample required a minimum volume of 2 mL, and absorbance was recorded at two maximum wavelengths for each drug substance, as indicated in the data section. Measurements were taken at regular intervals to monitor stability over time. A baseline calibration was performed using the solvent as the blank before each measurement, ensuring accurate results. These technical specifications provided a consistent and reliable method for assessing the stability of the drug substances (Chakraborty *et al*., 2018).

2.4. Statistical Analysis

Statistical analysis was performed to evaluate the stability of the phenolic compounds (Gallic acid, Ellagic acid, and Quercetin) in various solvents (methanol, ethanol 100%, ethanol 70%, and Dimethyl sulfoxide (DMSO 10%)) under two storage conditions: room temperature (25°C) and refrigerated (4°C). Descriptive statistics, including mean absorbance and standard deviation, were calculated for each compound-solvent combination at five time points (1 Day, 1 Week, 2 Weeks, 3 Weeks, and 4 Weeks), providing insights into the consistency of the compounds' stability over time. To determine whether significant differences existed between solvents, ANOVA was used to compare the mean absorbance values across groups, while the non-parametric Kruskal-Wallis test was applied to compare the medians, accounting for non-normal data distributions. These tests revealed significant differences in the stability of the compounds, particularly for Gallic acid, Quercitin and Ellagic acid, in different solvents at temperature conditions, helping to identify which solvent systems maintained the compounds' stability more effectively.

3. RESULTS

3.1. Gallic Acid Stability

3.1.1. *Room temperature (25°C)*

The stability of gallic acid was assessed across different solvents over four weeks. In DMSO 10%, the mean absorbance ranged from 0.1292 in Week 1 to 0.1619 in Week 4, with an average standard deviation of 0.082, indicating good stability. Ethanol 100% exhibited relatively high

and stable absorbance values, peaking at 0.335 in Week 2, and had an average standard deviation of 0.028, suggesting excellent stability. Ethanol 70% maintained a mean absorbance around 0.215, with moderate variability (average standard deviation of 0.052). Methanol displayed moderate stability, with absorbance ranging from 0.206 in Week 1 to 0.275 in Week 4, and an average standard deviation of 0.088, indicating higher variability. ANOVA and Kruskal-Wallis tests revealed significant differences in absorbance means (F-statistic: 12.58, $p < 0.001$) and medians (H-statistic: 21.96, $p < 0.001$) across solvents, highlighting variability in gallic acid stability (Figure 1 and Table 1).

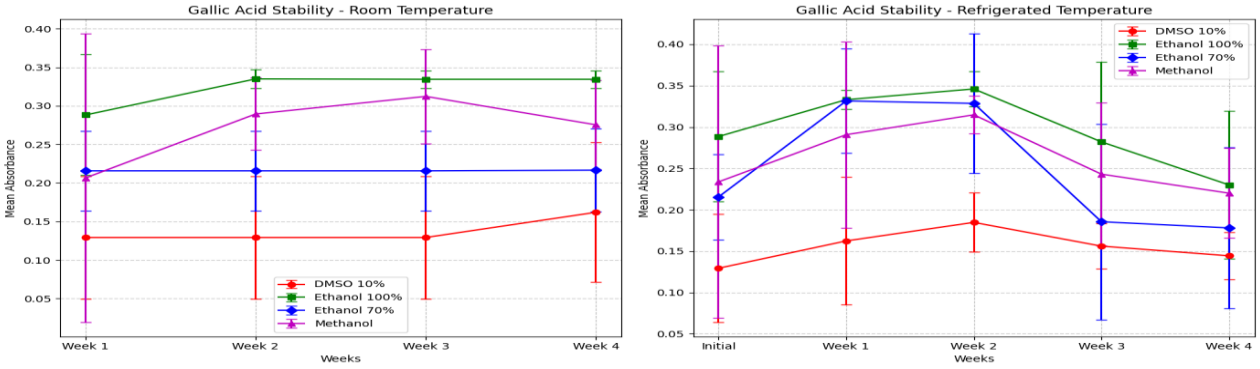

Figure 1. Stability of gallic acid in different solvents over time. The absorbance of gallic acid was monitored in DMSO 10%, ethanol 100%, ethanol 70%, and methanol over four weeks. The results indicate variations in stability, with methanol and ethanol 70% showing higher absorbance values, while DMSO 10% exhibited the lowest absorbance, suggesting differences in solvent effects on gallic acid stability.

Table 1. Statistical summary of gallic acid stability in different solvents in room temperature condition.

Solvent	Week	Mean Absorbance	Std Dev Absorbance
DMSO 10%	Week 1 Absorbance	0.1292	0.0794
	Week 2 Absorbance	0.1292	0.0794
	Week 3 Absorbance	0.1292	0.0794
	Week 4 Absorbance	0.1619	0.0905
Ethanol 100%	Week 1 Absorbance	0.2882	0.0783
	Week 2 Absorbance	0.3350	0.0122
	Week 3 Absorbance	0.3345	0.0115
	Week 4 Absorbance	0.3345	0.0115
Ethanol 70%	Week 1 Absorbance	0.2157	0.0519
	Week 2 Absorbance	0.2157	0.0519
	Week 3 Absorbance	0.2157	0.0519
	Week 4 Absorbance	0.2166	0.0540
Methanol	Week 1 Absorbance	0.2065	0.1871
	Week 2 Absorbance	0.2895	0.0469
	Week 3 Absorbance	0.3123	0.0612
	Week 4 Absorbance	0.2754	0.0582

Test	Statistic	*p*-value	Conclusion
ANOVA (Absorbance Mean Comparison)	F = 12.58	8.87×10^{-6}	Significant differences between solvents
Kruskal-Wallis (Absorbance Median Comparison)	H = 21.96	6.63×10^{-5}	Significant differences between solvents

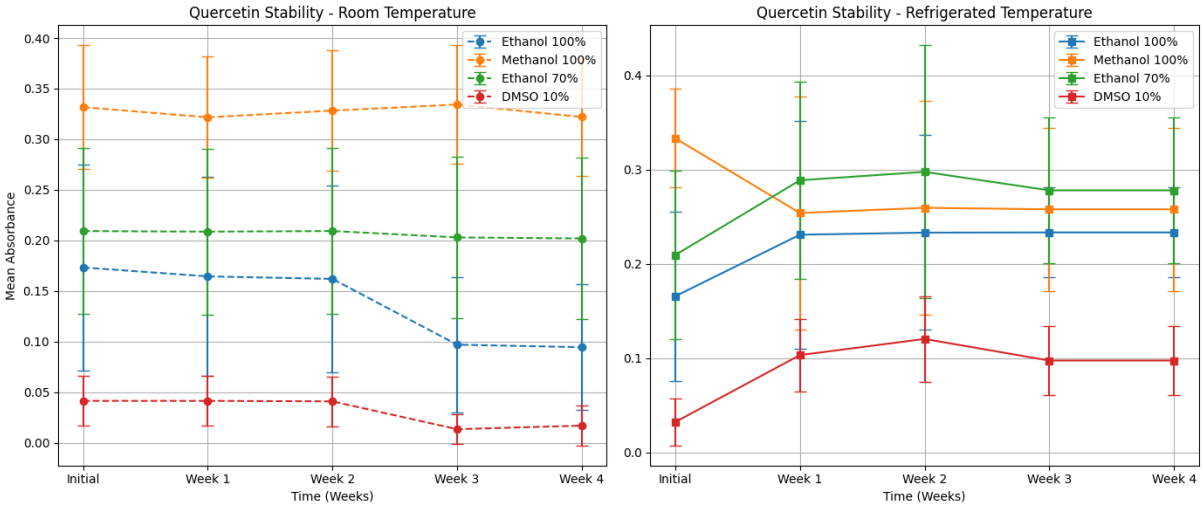

Figure 2. Stability of quercitin across different solvents. Absorbance measurements of quercetin in DMSO 10%, ethanol 100%, ethanol 70%, and methanol over four weeks revealed significant differences in stability. DMSO 10% demonstrated excellent stability with minimal fluctuations, while ethanol 70% and methanol exhibited moderate to high absorbance values, indicating varying solvent interactions with ellagic acid.

Table 2. Statistical summary of gallic acid stability in different solvents in refrigerator.

Solvent	Week	Mean Absorbance	Std Dev Absorbance
DMSO 10%	Initial Absorbance	0.1293	0.0651
	Week 1 Absorbance	0.1623	0.0770
	Week 2 Absorbance	0.1847	0.0358
	Week 3 Absorbance	0.1560	0.0274
	Week 4 Absorbance	0.0717	0.0482
Ethanol 100%	Initial Absorbance	0.2885	0.0788
	Week 1 Absorbance	0.3330	0.0113
	Week 2 Absorbance	0.3460	0.0212
	Week 3 Absorbance	0.2820	0.0967
	Week 4 Absorbance	0.2235	0.1263
Ethanol 70%	Initial Absorbance	0.2155	0.0516
	Week 1 Absorbance	0.3315	0.0629
	Week 2 Absorbance	0.3285	0.0842
	Week 3 Absorbance	0.1855	0.1182
	Week 4 Absorbance	0.1370	0.0454
Methanol 100%	Initial Absorbance	0.2337	0.1645
	Week 1 Absorbance	0.2907	0.1126
	Week 2 Absorbance	0.3147	0.0229
	Week 3 Absorbance	0.2430	0.0863
	Week 4 Absorbance	0.1263	0.1187

Statistical Test	Test Statistic	*p*-value	Conclusion
ANOVA (Absorbance)	38.86	<0.001	Significant differences in absorbance means across solvents
Kruskal-Wallis Test	38.99	<0.001	Significant differences in absorbance medians across solvents

3.1.2. *Refrigerated condition (4°C)*

Under refrigerated conditions, gallic acid in DMSO 10% showed consistent absorbance with minimal fluctuation, ranging from 0.1293 to 0.0717, and an average standard deviation of 0.019, indicating excellent stability. Ethanol 100% had absorbance values between 0.173 and 0.092, with an average standard deviation of 0.088, suggesting moderate stability. Ethanol 70% displayed slightly higher variability, with absorbance ranging from 0.209 to 0.2020, and an average standard deviation of 0.094. Methanol demonstrated relatively stable absorbance, ranging from 0.331 to 0.322, with an average standard deviation of 0.066. ANOVA and Kruskal-Wallis tests indicated significant differences in absorbance means (F-statistic: 38.86, $p < 0.001$) and medians (H-statistic: 38.99, $p < 0.001$) across solvents (Figure 1 and Table 2).

3.2. Quercetin Stability

3.2.1. *Room temperature (25℃)*

Quercetin exhibited varying stability across different solvents. In DMSO 10%, absorbance remained low, ranging from 0.0324 in Week 1 to 0.0975 in Week 4, with an average standard deviation of 0.017, indicating excellent stability. Ethanol 100% showed the highest absorbance values, peaking at 0.2333 in Week 4, but demonstrated significant variability, with an average standard deviation of 0.119. In Ethanol 70%, absorbance fluctuated between 0.2094 and 0.2779, with moderate variability (average standard deviation: 0.077). Methanol exhibited moderate stability, with absorbance decreasing from 0.3331 in Week 1 to 0.2578 in Week 4 and an average standard deviation of 0.072.

Statistical analysis revealed significant differences between solvents. ANOVA results (F-statistic: 8.43, *p*-value: 4.23×10^{-4} and Kruskal-Wallis test results (H-statistic: 18.92, *p*-value: 0.0023) confirmed variability in quercetin stability across solvents (Figure 2, and Table 3).

Table 3. Statistical summary of quercetin stability in different solvents in room temperature.

Solvent	Week	Mean Absorbance	Std Dev Absorbance
Ethanol 100%	Week 1 Absorbance	0.1657	0.0899
	Week 2 Absorbance	0.2309	0.1207
	Week 3 Absorbance	0.2331	0.1032
	Week 4 Absorbance	0.2333	0.0475
Methanol	Week 1 Absorbance	0.3331	0.0523
	Week 2 Absorbance	0.2539	0.1237
	Week 3 Absorbance	0.2594	0.1129
	Week 4 Absorbance	0.2578	0.0867
Ethanol 70%	Week 1 Absorbance	0.2094	0.0894
	Week 2 Absorbance	0.2886	0.1049
	Week 3 Absorbance	0.2975	0.1342
	Week 4 Absorbance	0.2779	0.0771
DMSO 10%	Week 1 Absorbance	0.0324	0.0249
	Week 2 Absorbance	0.1033	0.0385
	Week 3 Absorbance	0.1204	0.0452
	Week 4 Absorbance	0.0975	0.0361

Test	Statistic	*p*-value	Conclusion
ANOVA (Absorbance Mean Comparison)	F = 8.43	4.23×10^{-4}	Significant differences between solvents
Kruskal-Wallis (Absorbance Median Comparison)	H = 18.92	0.0023	Significant differences between solvents

3.2.2. *Refrigerated condition (4°C)*

Quercetin stability varied across solvents under refrigerated conditions. In DMSO 10%, absorbance remained consistently low, starting at 0.0415 and decreasing to 0.0170 by Week 4, with an average standard deviation of 0.021, indicating strong stability. Ethanol 100% showed a decline in absorbance from 0.1733 at the initial measurement to 0.0945 by Week 4, with moderate variability (average standard deviation: 0.0842). Ethanol 70% exhibited relatively stable absorbance, fluctuating between 0.2093 and 0.2020, with low variability (average standard deviation: 0.0810). Methanol maintained the highest absorbance values, starting at 0.3317 and remaining relatively stable at 0.3220 by Week 4, with minimal variation (average standard deviation: 0.0595). Statistical analysis confirmed significant differences among solvents. ANOVA results (F-statistic: 27.42, *p*-value: <0.001) and Kruskal-Wallis test results (H-statistic: 26.98, *p*-value: <0.001) indicated strong variability in quercetin stability across different solvents (Figure 2, and Table 4).

Table 4. Statistical summary of quercetin stability in different solvents in refrigerated temperature.

Solvent	Week	Mean Absorbance	Std Dev Absorbance
Ethanol 100%	Initial Absorbance	0.1733	0.1015
	Week 1 Absorbance	0.1645	0.0985
	Week 2 Absorbance	0.1620	0.0922
	Week 3 Absorbance	0.0970	0.0668
	Week 4 Absorbance	0.0945	0.0621
Methanol 100%	Initial Absorbance	0.3317	0.0611
	Week 1 Absorbance	0.3217	0.0601
	Week 2 Absorbance	0.3283	0.0596
	Week 3 Absorbance	0.3343	0.0586
	Week 4 Absorbance	0.3220	0.0580
Ethanol 70%	Initial Absorbance	0.2093	0.0817
	Week 1 Absorbance	0.2087	0.0817
	Week 2 Absorbance	0.2093	0.0817
	Week 3 Absorbance	0.2030	0.0801
	Week 4 Absorbance	0.2020	0.0801
DMSO 10%	Initial Absorbance	0.0415	0.0247
	Week 1 Absorbance	0.0415	0.0247
	Week 2 Absorbance	0.0410	0.0244
	Week 3 Absorbance	0.0135	0.0149
	Week 4 Absorbance	0.0170	0.0196

Statistical Test	Test Statistic	*p*-value	Conclusion
ANOVA (Absorbance)	27.42	<0.001	Significant differences in absorbance means across solvents
Kruskal-Wallis Test	26.98	<0.001	Significant differences in absorbance medians across solvents

3.3. Ellagic Acid Stability

3.3.1. *Room temperature (25°C)*

Ellagic acid stability varied across solvents, with notable differences in absorbance trends. In DMSO 10%, absorbance remained relatively stable but fluctuated between 0.08075 in Week 1 and 0.03120 in Week 5, with an average standard deviation of 0.0237, indicating minimal degradation. Ethanol 100% exhibited moderate stability, with absorbance ranging from 0.22943 in Week 1 to 0.20500 in Week 5, though it showed considerable variability (average standard deviation: 0.1576). Ethanol 70% initially maintained absorbance levels around 0.16165 in Week 1 but significantly declined to 0.03540 by Week 5, with an average standard deviation of

0.0848, suggesting instability over time. Methanol displayed relatively stable absorbance values, ranging from 0.23370 in Week 1 to 0.20683 in Week 5, with an average standard deviation of 0.1467, indicating a more consistent profile.

Table 5. Statistical summary of ellagic acid in different solvents in room temperature.

Solvent	Week	Mean Absorbance	Std Dev Absorbance
DMSO 10%	Week 1	0.08075	0.02709
DMSO 10%	Week 2	0.03775	0.01163
DMSO 10%	Week 3	0.07655	0.02518
DMSO 10%	Week 4	0.04415	0.03077
DMSO 10%	Week 5	0.03120	0.02168
Ethanol 100%	Week 1	0.22943	0.15736
Ethanol 100%	Week 2	0.19175	0.15186
Ethanol 100%	Week 3	0.20643	0.15919
Ethanol 100%	Week 4	0.21610	0.16126
Ethanol 100%	Week 5	0.20500	0.15956
Ethanol 70%	Week 1	0.16165	0.12697
Ethanol 70%	Week 2	0.15420	0.12648
Ethanol 70%	Week 3	0.15455	0.13427
Ethanol 70%	Week 4	0.03685	0.01705
Ethanol 70%	Week 5	0.03540	0.01928
Methanol	Week 1	0.23370	0.14385
Methanol	Week 2	0.20733	0.14373
Methanol	Week 3	0.20957	0.14319
Methanol	Week 4	0.21860	0.14259
Methanol	Week 5	0.20683	0.15994

Test	F/H Statistic	*p*-value	Conclusion
ANOVA	38.182	0.01092	Significant differences between solvents
Kruskal-Wallis	120.892	0.00705	Significant differences between solvents

Statistical analysis revealed significant differences between solvents. The ANOVA results (F-statistic: 3.8182, *p*-value: 0.01092) and Kruskal-Wallis test results (H-statistic: 12.0892, *p*-value: 0.00705) confirmed variability in ellagic acid stability across different solvents, suggesting that solvent choice significantly affects its stability over time (Figure 3, Table 5).

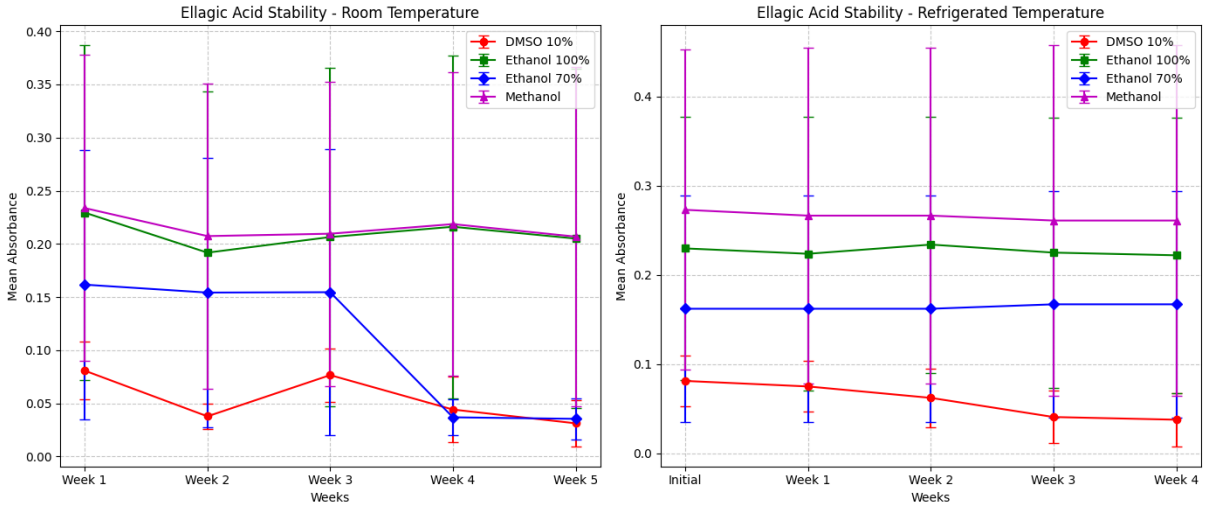

Figure 3. Stability of ellagic acid in various solvents over time. Ellagic acid absorbance was measured in DMSO 10%, ethanol 100%, ethanol 70%, and methanol over four weeks. The results show high variability in ethanol 100%, moderate stability in methanol, and excellent stability in DMSO 10%, highlighting solvent-dependent effects on quercetin stability.

3.3.2. *Refrigerated condition (4°C)*

Under refrigerated conditions, ellagic acid stability varied across solvents. In DMSO 10%, absorbance remained relatively stable, decreasing from 0.0810 initially to 0.0375 by Week 4, with an average standard deviation of 0.0298, indicating excellent stability. Ethanol 100% showed moderate stability, with absorbance ranging from 0.2297 initially to 0.2220 in Week 4, and an average standard deviation of 0.1502, suggesting some variability. Ethanol 70% maintained consistent absorbance values between 0.1620 and 0.1670 across all weeks, with an average standard deviation of 0.1273, reflecting moderate stability. Methanol 100% exhibited a gradual decline from 0.2730 initially to 0.2610 by Week 4, with an average standard deviation of 0.1907, suggesting relatively stable behavior despite slight fluctuations. Statistical analysis confirmed significant differences in ellagic acid stability across solvents.

The ANOVA results (F-statistic: 12.79, *p*-value: 2.38×10^{-6}) and Kruskal-Wallis test results (H-statistic: 32.03, *p*-value: 5.17×10^{-7}) demonstrated significant variations, indicating that solvent choice strongly influences ellagic acid stability under refrigerated conditions (Figure 3, Table 6).

Table 6. Statistical summary of ellagic acid in different solvents in room temperature.

Solvent	Week	Mean Absorbance	Standard Deviation (SD)
DMSO 10%	Initial Absorbance	0.0810	0.0280
	Week 1 Absorbance	0.0748	0.0283
	Week 2 Absorbance	0.0620	0.0332
	Week 3 Absorbance	0.0405	0.0295
	Week 4 Absorbance	0.0375	0.0299
Ethanol 100%	Initial Absorbance	0.2297	0.1481
	Week 1 Absorbance	0.2237	0.1532
	Week 2 Absorbance	0.2340	0.1438
	Week 3 Absorbance	0.2250	0.1516
	Week 4 Absorbance	0.2220	0.1544
Ethanol 70%	Initial Absorbance	0.1620	0.1273
	Week 1 Absorbance	0.1620	0.1273
	Week 2 Absorbance	0.1620	0.1273
	Week 3 Absorbance	0.1670	0.1273
	Week 4 Absorbance	0.1670	0.1273
Methanol 100%	Initial Absorbance	0.2730	0.1796
	Week 1 Absorbance	0.2665	0.1888
	Week 2 Absorbance	0.2665	0.1888
	Week 3 Absorbance	0.2610	0.1966
	Week 4 Absorbance	0.2610	0.1966

Test	Statistic	Value	*p*-value
ANOVA	F-statistic	12.79	2.38×10^{-6}
Kruskal-Wallis	H-statistic	32.03	5.17×10^{-7}

4. DISCUSSION

The study aimed to evaluate the stability of gallic acid, ellagic acid, and quercetin in various solvents over a one-month period under room and refrigerated conditions. Using UV spectroscopy, we observed significant variability in stability depending on the solvent and storage temperature, providing insights into solvent-solute interactions and the effects of environmental conditions on phenolic compounds. At room temperature for gallic acid, ethanol (both 100% and 70%) demonstrated superior stability compared to other solvents. In ethanol

100%, absorbance values were consistently high and stable, reflecting minimal degradation. This suggests that ethanol is highly effective in preserving gallic acid's stability for a period of up to one month.. Conversely, methanol introduced variability, with absorbance fluctuating over time, indicating potential structural changes or instability of gallic acid in this solvent. Dimethyl sulfoxide (DMSO 10%) also provided relatively stable results, though less pronounced than ethanol, suggesting moderate effectiveness. Quercetin displayed high stability in DMSO 10% at both room and refrigerated conditions, with low variability in absorbance values. Ethanol 100% exhibited higher absorbance values but with notable variability, which may be attributed to quercetin's potential susceptibility to degradation in ethanol. Methanol showed moderate stability, with absorbance values decreasing over time, indicating some instability. The inconclusive statistical results for quercetin may highlight issues with the data or suggest a need for more precise measurement techniques. Ellagic acid stability was notably high in DMSO 10%, with consistent absorbance values, indicating excellent stability. Ethanol 100% and 70% exhibited considerable variability, which could be attributed to the solvent's impact on the chemical structure or interaction of ellagic acid. Methanol also showed relatively high stability but with some fluctuations, similar to gallic acid.

The refrigerated conditions generally enhanced the stability of all three phenolic compounds compared to room temperature. This aligns with the common understanding that lower temperatures slow down degradation processes (Karinen *et al*., 2011; Salazar-Orbea *et al*., 2023). For instance, gallic acid in DMSO and quercetin in DMSO showed exceptional stability under refrigerated conditions, suggesting that lower temperatures can significantly improve the preservation of these compounds. Conversely, ethanol and methanol solutions also showed improvements under refrigeration but were not as effective as DMSO.

The findings highlight the importance of solvent selection in pharmaceutical formulations. Ethanol, particularly at 100%, proved to be the most reliable solvent for maintaining the stability of gallic acid and quercetin. DMSO was also effective, especially under refrigerated conditions. These results suggest that careful consideration of solvent properties and storage conditions is crucial for developing stable and effective pharmaceutical formulations. This is the first study to report the importance of API in different solvents at different conditions. This study helps researchers to either employ the preserved stock solutions of expensive API for various analytical purposes or dispose of the stock solutions. Further studies need to be conducted using HPLC, NMR, LCMS and other advanced instruments to find out the stability of many more expensive APIs that were routinely used in pharmaceutical analysis.

5. CONCLUSION

This study evaluated the stability of gallic acid, ellagic acid, and quercetin in various solvents like methanol, ethanol (100%), ethanol (70%), and Dimethyl sulfoxide (10%)—under room (25°C) and refrigerated (4°C) temperatures using UV spectroscopy. The results highlighted that solvent choice significantly influences the stability of these phenolic compounds. Gallic acid and quercetin showed excellent stability in ethanol (100%) and ethanol (70%), with consistent UV absorbance and minimal fluctuations. Dimethyl sulfoxide (10%) also maintained high stability for all compounds, though some variability was observed in the results. Methanol exhibited greater variability, possibly due to solvent-induced structural changes. Ethanol (70%) and Dimethyl sulfoxide (10%) emerged as particularly favorable solvents for maintaining the stability of these phenolic compounds. This study emphasizes the significance of choosing suitable solvents for preserving active pharmaceutical ingredients (APIs), aiding drug development and quality control. It presents a simple, cost-effective approach for evaluating solvent-driven stability. Further research is needed to assess the stability of other expensive APIs commonly used in pharmaceutical analysis.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Manoj Madanahalli Ramesh: Investigation, resources, visualization, methodology, software, formal analysis, and writing original draft. **Annegowda Hardur Venkatappa**: Methodology, supervision, and validation. **Richard Lobo**: Enriched the manuscript's scientific quality and clarity.

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