

**EVALUATION OF PHARMACEUTICAL QUALITY PROPERTIES OF SOLID
DOSAGE FORMS USED AS FOOD SUPPLEMENTS**

Master of Science Thesis

Ahmed Fouad Zaidan AL-BAYATI

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Ahmed Fouad Zaidan AL-BAYATI

MASTER THESIS

Department of Pharmaceutical Technology

Supervisor: Assoc.Prof.Dr. Mustafa Sinan KAYNAK

Eskişehir Anadolu University

Graduate School of Health Sciences

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FINAL APPROVAL FOR THESIS

This thesis titled, “Evaluation of pharmaceutical quality properties of solid dosage forms used as food supplements”, has been prepared and submitted by Ahmed Fouad Zaidan AL-BAYATI in partial fulfilment of the requirements in the “Anadolu University Directive on Graduate Education and Examination” for the Degree of Science in Pharmaceutical Technology Department and has been examined and approved on 22/09/2022.

Committee Members

Signature

Member (Supervisor)	Assoc. Prof. Dr. Mustafa SİNAN KAYNAK	
Member	Assoc. Prof. Dr. Ahmet Alper ÖZTÜRK	
Member	Ass.Dr. Esra DEMİRTÜRK	

Prof. Dr. Gülşen AKALIN ÇİFTÇİ

Graduate School Director

ABSTRACT

EVALUATION OF PHARMACEUTICAL QUALITY PROPERTIES OF SOLID DOSAGE FORMS USED AS FOOD SUPPLEMENTS

Ahmed Fouad Zaidan AL-BAYATI

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Anadolu University, Graduate School of Health Sciences,
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In recent years, with the rapid increase in the number of food supplements and vitamin companies and the high demand for them, the difficulty and worry of determining their quality, especially the ease of production and obtaining government approval for the sale of these products, is much easier compared to therapeutic chemical drugs. In this thesis study, quality control tests for USP food supplements were carried out (disintegration, dissolution, and content uniformity), and the remaining physical quality control tests for chemical drugs were all performed. Standards were prepared for these tests, and effective analysis for analysis with the HPLC-dad method has been developed. These tests were performed on tablets because tablets are the most common of the pharmaceutical forms, and vitamin C was chosen because it is common, either alone or in combination with a group of vitamins. According to the results obtained, 3 samples from 5 different samples did not pass the disintegration test. According to the results obtained from HPLC, only 2 out of 5 different samples passed the dissolution test and 3 samples passed the content uniformity test. Many of these products have been found to have quality problems during their manufacture due to insufficient production or excessive pressure.

Keywords: Nutritional Supplement, Tablets quality control, HPLC, Dissolution, Content unformat.

ÖZET

GIDA TAKVİYESİ OLARAK KULLANILAN KATI DOZAJ ŞEKİLLERİNİN FARMASÖTİK KALİTE ÖZELLİKLERİNİN DEĞERLENDİRİLMESİ

Ahmed Fouad Zaidan AL-BAYATI

Farmasötik Teknoloji Anabilim Dalı

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Danışman: Doç. Dr. Mustafa Sinan KAYNAK

Son yıllarda gözlemlenen, gıda takviyesi ve vitamin firmalarının sayısındaki hızlı artış ve bunlara olan yüksek talep, kalitelerini tespit etmenin zor ve endişe verici hale gelmesi, özellikle üretim kolaylığı ve bu ürünlerin satılması için devletten onay alınması tedavi edici kimyasal ilaçlara kıyasla çok daha kolaydır. Bu tez çalışmasında usp gıda takviyeleri için kalite kontrolü testleri istenen testleri yapılmıştır (parçalanma, çözünme ve içerik tekdüzeliği) ve kimyasal ilaçları da istenen diğer kalan fiziksel kalite kontrol testleri tümü yapılmıştır. bu testler için standart hazırlanmıştır ve HPLC-dad ile analiz için etken Bir analiz metodu geliştirilmiştir. Bu testler tabletler üzerinde gerçekleştirilmiştir çünkü tabletler farmasötik formlar arasında en yaygın olanıdır ve C vitamini tek başına veya bir grup vitaminle birlikte yaygın olduğu için seçilmiştir. Elde edilen sonuçlara göre 5 farklı numuneden, parçalanma testinde 3 numune geçemedi, hplc den alan sonuçlara göre çözünme testinden 5 farklı numuneden sadece 2 tane geçmiştir ve içerik tekdüzeliği testinden 3 tane numune geçmiştir. bu ürünlerin birçoğunun üretimleri sırasında yetersiz üretim veya aşırı basınç nedeniyle kalite sorunları yaşadığı tespit edilmiştir.

Anahtar Sözcükler: Gıda takviyeleri, HPLC, Tablet kalite kontrolleri, Çözünme hızı, İçerik tekdüzeliği.

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Ahmed Fouad Zaidan AL-BAYATI

22/09/2022

STATEMENT OF COMPLIANCE WITH ETHICAL PRINCIPLES AND RULES

I hereby declare truthfully that this thesis is my original work; that I have acted in accordance with scientific ethical principles and rules throughout the stages of preparation, data collection, analysis, and presentation of my work; that I have cited the sources of all data and information that could be obtained within the scope of this study and included these sources in the references section; and that this st I also declare that if a case contradictory to my declaration is discovered in my job at any point, I hereby express my approval to all ethical and legal implications that may result.

Ahmed Fouad Zaidan AL-BAYATI

CONTENTS

	<u>Page</u>
COVER PAGE	i
FINAL APPROVAL FOR THESIS	ii
ABSTRACT.....	iii
ACKNOWLEDGEMENTS	v
STATEMENT OF COMPLIANCE WITH ETHICAL PRINCIPLES AND RULES ..	vi
CONTENTS	vii
LIST OF TABLES	xii
LIST OF FIGURES	xii
LIST OF ABBREVIATIONS	xviii
1. INTRODUCTION	1
2. LITRETURE REVIEW	2
2.1. Dietary Supplements.....	2
2.1.1. History of food supplements terms.....	3
2.1.2. Increasing use of dietary upplements.....	3
2.1.3. Types of dietary supplements	5
2.1.3.1. Multivitamin and multimineral supplement	6
2.1.3.2. Single vitamin or mineral supplements.....	7
2.1.3.3. Non vitamin, non mineral supplements	8
2.1.3.4. Herbal supplements	8
2.1.4. General use of vitamin and mineral supplements.....	9
2.1.5. Health associated with dietary supplement	11
2.1.6. Drug Interaction with dietary supplements	13

	<u>Page</u>
2.2. Vitamins	17
2.2.1. Water soluble vitamins.....	19
2.2.2. Fat soluble vitamins	20
2.2.3. Vitamin C.....	20
2.2.3.1. Properties of vitamin C	21
2.2.3.1.1. Physical properties	22
2.2.3.1.2. Chemical properties and stability of vitamin C.....	22
2.2.3.2. Vitamin C benefits.....	24
2.3. Zinc	25
2.4. Dietary Supplements Pharmaceutical Forms.....	27
2.5. Quality Control.....	27
2.5.1. FDA and dietary supplements	29
2.5.1.1. Dissolution test.....	30
2.5.1.2. Tablets disintegration test.....	30
2.5.1.3. Weight variation test	30
2.5.1.4. Hardness.....	31
2.5.1.5. Friability	31
2.5.1.6. Thickness test	31
2.5.1.7. General appearance	32
2.5.1.8. Uniformity content.	32
2.5.2. Good manufacturing practice (GMP).....	32
2.6. Future Public Health Regulation Of Dietary Supplements.....	33
2.6.1. United states and dietary supplements	34

	<u>Page</u>
2.6.2. European union and dietary supplements.....	36
2.6.3. Japan and dietary supplements.....	37
2.6.4. Turkey and dietary supplements.....	38
2.7. HPLC.....	39
2.7.1. Capacity factor (k').....	39
2.7.2. Theoretical number of plates (N)	40
2.7.3. Queuing and peak symmetry factors	40
2.7.4. Selectivity (α) and separation factor (R).....	40
2.7.5. Analytical method validation.....	41
2.7.6. Selectivity (matrix effect)	41
2.7.7. Precision.....	41
2.7.8. Repeatability (system precision).....	42
2.7.9. Reproducibility (method precision)	42
2.7.10. Accuracy	42
2.7.11. Lower limit of measurement (LOD) and lower concentration of determination (LOQ).....	43
2.7.12. Analytical range.	43
2.7.13. Solution stability	44
2.7.14. Robustness analytics	44
3. MATERIALS	45
3.1. Chemicals	46
3.2. Devices.....	47
4. METHODS	49

	<u>Page</u>
4.1. Preparation Of The Main Solutions Used In Thesis Work	49
4.2. Assay Methods	50
4.2.1. Titration	50
4.2.2. UV chromatographic.	52
4.2.3. HPLC-DAD chromatographic.....	61
<i>4.2.3.1. Methods were created for HPLC chromatographic analysis</i>	<i>61</i>
<i>4.2.3.1.1. HPLC method 1.....</i>	<i>61</i>
<i>4.2.3.1.2. HPLC method 2.....</i>	<i>63</i>
<i>4.2.3.1.3. HPLC method 3.....</i>	<i>64</i>
<i>4.2.3.1.4. HPLC method 4.....</i>	<i>65</i>
<i>4.2.3.1.5. HPLC method 5.....</i>	<i>66</i>
<i>4.2.3.1.6. HPLC method 6.....</i>	<i>67</i>
<i>4.2.3.2. Validation of HPLC-DAD chromatographic analyzes</i>	<i>68</i>
4.3. Quality Control According To USP.....	80
4.3.1. Dissolution.	80
4.3.2. Disintegration.	81
4.3.3. Uniformity content.....	82
4.3.4. Weight variation.....	83
4.3.5. Friability.	84
4.3.6. Thickness and hardness.....	85
4.3.7. General appearance	86
5. RESULTS AND DISCUSSION	87
5.1. First Grope (S.tab 1) Quality Control Test Results.	87
5.2. Second Grope (S.tab 2) Quality Control Test Results.	93

	<u>Page</u>
5.3. Third Grope (S.tab 3) Quality Control Test Results.....	99
5.4. Fourth Grope (S.tab4) Quality Tests Results.	105
5.5. Fifth Grope (S.tab 5) Quality Tests Results.....	111
5.6. General Appearance Of Sample Groups.	117
6. CONCLUSION	118
REFERENCES.....	122
CURRICULUM VITAE	

LIST OF TABLES

	<u>Page</u>
Table 2.1. Drug-nutrient interactions.	13
Table 2.2. Drug-nutrient interactions 2.	15
Table 2.3. Drug-nutrient interactions 3.	16
Table 3.1. Vitamin C samples that collected from Turkish marketplace.	45
Table 3.2. Chemicals used in thesis work.	46
Table 3.3. Devices used in thesis work.	47
Table 4.1. Titration Results.	51
Table 4.2. Stander stock for UV chromatographic.	52
Table 4.3. Calibration of Vit C using HCl as dilution solution (1).	54
Table 4.4. Calibration of Vit C using HCl as dilution solution (2).	55
Table 4.5. Calibration of Vit C using HCl as dilution solution (3).	55
Table 4.6. Calibration 1 of Vit C using 98 % KHPO ₄ (pH 3) and 2 % 0.1 N HCl.	59
Table 4.7. Calibration 2 of Vit C using 98 % KHPO ₄ pH 3) and 2 % 0.1 N HCl.	60
Table 4.8. Chromatographic conditions of method 1.	62
Table 4.9. Chromatographic conditions of method 2.	63
Table 4.10. Chromatographic conditions of method 3.	64
Table 4.11. Chromatographic conditions of method 4.	65
Table 4.12. Chromatographic conditions of method 5.	66
Table 4.13. Chromatographic conditions of method 6.	67
Table 4.14. Linear calibration range, LOD and LOQ analysis values of vitamin c.	70
Table 4.15. Data of HPLC calibration.	71

	<u>Page</u>
Table 4.16. HPLC-DAD calibration vitamin C standard day 1.	72
Table 4.17. HPLC-DAD calibration vitamin C standard day 2.	73
Table 4.18. HPLC-DAD calibration vitamin C standard day 3.	74
Table 4.19. Day 1 intraday repeatability	75
Table 4.20. Day 2 intraday repeatability	75
Table 4.21. Day 3 intraday repeatability	76
Table 4.22. Pick time according to pH of mobile phase	77
Table 4.23. Pick time according to temperature of HPLC colon.	78
Table 4.24. Pick time according to flow rate mL/min.	79
Table 5.1. Disintegration test of S.tab 1	87
Table 5.2. Uniformity content of S.tab 1	87
Table 5.3. Dissolution test of S.tab 1 for 60 minutes.	88
Table 5.4. Dissolution test of S.tab 1 in 240 mintes.	90
Table 5.5. Weight variation of S.tab 1	91
Table 5.6. Diameter and hardness tests for S.tab 1.	92
Table 5.7. Friability test of S.tab1.....	92
Table 5.8. Disintegration test of S.tab 2.....	93
Table 5.9. Uniformity content test of S.tab 2.....	94
Table 5.10. Dissolution test of S.tab 2 for 60 minutes.....	95
Table 5.11. Dissolution test of S.tab 2 in 240 minutes.	96
Table 5.12. Weight variation of S.tab 2.	96

	<u>Page</u>
Table 5.13. Diameter and hardness tests for S.tab 2.	98
Table 5.14. Friability test of S.tab 2.	98
Table 5.15. Disintegration test of S.tab3.	99
Table 5.16. Uniformity content test of S.tab3.	100
Table 5.17. Dissolution test of S.tab3 in 60 minutes.	101
Table 5.18. Dissolution test of S.tab 3 in 240 minutes.	102
Table 5.19. Weight variation of S.tab 3.	102
Table 5.20. Diameter and hardness tests of S.tab 3.	104
Table 5.21. Friability test of S.tab3.	104
Table 5.22. Disintegration test of S.tab 4.	105
Table 5.23. Uniformity content test of S.tab4.	106
Table 5.24. Dissolution test of S.tab 4.	107
Table 5.25. Dissolution test of S.tab 4 in 240 mintes.	108
Table 5.26. Weight variation of S.tab 4	109
Table 5.27. Diameter and hardness tests of S.tab 4.	110
Table 5.28. Friability test os of S.tab 4.	110
Table 5.29. Disintegration test for S.tab5.	111
Table 5.30. Uniformity content test for S.tab5.	112
Table 5.31. Dissolution test of S.tab 5.	113
Table 5.32. Dissolution test of S.tab 5 in 240 mintes.	114
Table 5.33. Weight Variation of S.tab 5.	115
Table 5.34. Diameter and hardness tests for S.tab5.	116
Table 5.35. Friability test of S.tab5.	116

	<u>Page</u>
Table 5.36. General appearance of tablest.	117
Table 6.1. Quality control results for all tests.	121

LIST OF FIGURES

	<u>Page</u>
Figure 2.1. Vitamin C structure.	22
Figure 2.2. Vitamin C redox reaction.	23
Figure 4.1. Uv chromatogram of Vit C CONC. 5 µg/mL using HCl as dilution solution. .	53
Figure 4.2. Uv chromatogram of Vit C CONC.45 µg/mL using HCl as dilution solution..	53
Figure 4.3. Calibration graphic of Vit C using HCl as dilution solution (1).	54
Figure 4.4. Calibration graphic of Vit C using HCl as dilution solution (2).	55
Figure 4.5. Calibration graphic of Vit C using HCl as dilution solution (3).	56
Figure 4.6. Uv Chromatogram of Vit C the peak in RFE 265 NM.....	57
Figure 4.7. Uv Chromatogram of Vit C the peak in RFE 242 NM.....	57
Figure 4.8. Calibration 1 graphic of Vit C using 98 % KHPO ₄ pH 3 and 2 % 0.1 N HCl...	59
Figure 4.9. Uv abs. using 98 % KHPO ₄ pH 3 and 2 % 0.1 N HCl.	59
Figure 4.10. Calibration 2 graphic of Vit C using 98 % KHPO ₄ pH 3 and 2 % 0.1 N HCl .	60
Figure 4.11. Chromatogram of method 1.....	62
Figure 4 12. Chromatogram of method 2.....	63
Figure 4.13. Chromatogram of method 3.....	64
Figure 4.14. Chromatogram of method 4.....	65
Figure 4.15. Chromatogram of method 5.....	66
Figure 4.16. Chromatogram of method 6.....	67
Figure 4.17. Chromatogram of placebo.	69
Figure 4.18. Graphic OF day 1 HPLC Calibration.	72
Figure 4.19. Result from HPLC for pick time according to pH of mobile phase.	77
Figure 4.20. Result from HPLC for pick time according to temperature colon.....	78

	<u>Page</u>
Figure 4.21. Result from HPLC for pick time according to pH of mobile phase.	77
Figure 4.22. Result from HPLC for pick time according to temperature colon.....	78
Figure 4.23. Result from HPLC for pick time according to Flow rate mL/min.....	79
Figure 4.24. Tablet disintegration tester.....	82
Figure 4.25. Tablet friability.....	84
Figure 4.26. Thickness and hardness tester.	82
Figure 5.1. Example of HPLC result in a uniformity content test.	88
Figure 5.2. Dissolution 60 minute of s.tab 1.....	89
Figure 5.3. Example of HPLC result in a Dissolution content test in 45 minutes.	89
Figure 5.4. Dissolution 240 minutes of s.tab 1.	90
Figure 5.5. Example of HPLC result in a Uniformity content test for second group sample.	94
Figure 5.6. Dissolution test of S.tab 2 for 60 minutes.	95
Figure 5.7. Dissolution test of S.tab 2 in 240 minutes.....	96
Figure 5.8. Dissolution test of S.tab3 in 60 minutes.	101
Figure 5.9. Dissolution test of S.tab 3 in 240 minutes.....	102
Figure 5.10. Example of HPLC result in a Uniformity content test for Fourth group sample.	106
Figure 5.11. Dissolution test of S.tab 4 in 60 minutes.....	107
Figure 5.12. Dissolution test of S.tab 4 in 240 mintes.....	108
Figure 5.13. Dissolution test of S.tab 5.....	113
Figure 5.14. Dissolution test of S.tab 5 in 240 mintes.....	114

LIST OF ABBREVIATIONS

ACN	: Acetonitrile
DS	: Dietary Supplements
DSHEA	: Dietary Supplement Health and Education Act
EFSA	: The European Food Safety Authority
EU	: The European Union
FDA	: Food and Drug Administration
GMP	: Good Manufacturing Practice
HPLC	: High performance liquid chromatography
LOD	: Limited of detection
LOQ	: Limited of quantitation
MVM	: Multivitamin-mineral
QC	: Quality control
S.Tab	: Group of tablets samples of different companies were tested
SVM	: Single vitamin or mineral
USP	: United states Pharmacopeia
UV	: Ultraviolet light
VIT C	: Vitamin C (Ascorbic Acid)

1. INTRODUCTION

After the Corona phenomenon, the demand for pharmaceutical products has increased especially the nutritional supplements that strengthen the immunity and maintain health. Because of that, the pharmaceutical companies began the race and started producing nutritional supplements and marketing them. These supplements consist of vitamins or minerals in different pharmaceutical forms, such as pills, capsules, and liquid preparations (syrup). These companies did not take the qualitative foundations into consideration among the pharmaceutical preparation of these supplements. They consider that these nutritional supplements are not subject to oversight by the Ministry of Health, where the quality tests required in medicines are not applied on them, although there is a USP General Chapter <2750> Manufacturing Practices for Dietary Supplements and FDA Current Good Manufacturing Practices, that explain the tests that are required, Because of this, pharmaceutical problems have been observed in these pharmaceutical medicines. In this research, we decided to introduce various quality control tests on tablets because they are the most widely used, and because these tests are required by the US Health Administration on therapeutic medicine, and doing all quality control tests (disintegration, dissolution, content uniformity, Weight Variation, Friability, Diameter and hardness tests).

2. LITRETURE REVIEW

2.1. Dietary Supplements

Dietary supplements are products that are taken orally and contain one or more of a substance including vitamins, minerals, botanicals, herbs, enzymes, amino acids, organ tissues, glandular, or metabolites (U.S. Food and Drug Administration, 2017). These supplements appear as a form of a tablet, pill, powder, gel cap, soft gel, liquid, or food item. When the dietary intake is not enough and insufficient, dietary supplements are then added for daily use to meet the micronutrient or macronutrient requirements (U.S. Food and Drug Administration, 2017). Some types of supplements can be classified as multivitamin-mineral (MVM), single vitamin or mineral (SVM), herbal, protein, fatty acid, and weight loss supplements (Bailey et al., 2011).

Regardless of the physical form of dietary supplements, the DSHEA classifies them as "foods," not "drugs," and requires that each supplement must be labeled as such. The DSHEA also defines "dietary ingredient" and "new dietary ingredient" as components of dietary supplements. A person can use these supplements (vitamin, mineral, herb, amino-acid, botanical dietary substance...) to supplement the diet by increasing the amount of total dietary intake. One or any combination of substances is to be considered a dietary ingredient, these substances can be enzymes or tissues from organs or glands, concentrate, metabolite, constituent, or extract. A "new dietary ingredient" is one that fulfills the following criterion and was not available as a dietary supplement in the United States before October 15, 1994 (Binns et al., 2018b).

The European Union (EU) has given the following working definition: "As a complement to a regular diet, food company operators advertise food supplements, which are concentrated sources of nutrients (or other substances) that have a nutritional or physiological effect". These types of dietary supplements appear in a form of pills, tablets, capsules, or liquids in defined doses, and sold as a dosage (Lawal et al., 2021). Directive 2002/46/EC is the most important part of European Union law, which administers dietary supplements containing vitamins and minerals.

The US Food and Drug Administration (FDA) and the Dietary Supplement Health and Education Act (DSHEA) of 1994 defined the dietary supplement as a product that includes an ingredient in the diet used by an individual to supplement his diet and it is taken orally (Seamon & Clauson, 2005).

The European Food Safety Authority (EFSA) says, "Supplements can be used to treat nutritional deficits or to maintain appropriate nutrient consumption. However, excessive vitamin and mineral intake might be detrimental or create undesired side effects in some situations; hence, maximum amounts are required to assure their safe usage in dietary supplements" (Binns et al., 2018b).

Nutritional supplements in the Turkish food codex communiqué on supplementary foods, "vitamins, minerals, proteins, carbohydrates, fibers, fatty acids, capsules, tablets, lozenges, disposable powder packs, liquid ampoules, dropper bottles, and concentrates or extracts of nutrients such as amino acids or other substances of plant, vegetable, and animal origin, bioactive substances, and similar substances that have nutritional or physiological effects, alone or in their mixtures. " "vitamins, minerals, proteins, carbohydrates, fibers, fatty acids, capsules, tablets, lozenges, they are items that can either be in liquid or powder form, and their recommended daily consumption is specified for each product (Atalay & Erge, 2018).

2.1.1 History of food supplements terms

Supplementary foods, or nutraceuticals as we often hear today, constructed by Dr. Stephen DeFelice coined the term by merging the terms "Nutrition" and "Pharmaceutical." This phrase, as defined by the Merriam-Webster Dictionary, refers to foods that give health advantages in addition to their fundamental nutritional attributes (Kalra, 2003).

2.1.2. Increasing use of dietary supplements

Nowadays, the use of dietary supplements is high. Before the turn of the century, the yearly number of dietary supplements that were consumed in the United States was \$12

billion (Burdock, 2000). As of the year 2015, the worldwide market was rated at US \$109 billion, and it is anticipated that this figure would increase to US\$180 billion by the year 2020. The regional shares held by Asia-Pacific, Europe, and North America were, respectively, 31%, 30%, and 25%. (Briend et al., 2015). According to one estimation, the worldwide market would reach \$278 billion by the year 2024 (Hegazy et al., 2020). The variety and make-up of dietary supplements are nearly without limit, and even everyday multivitamins may be tailored to the needs of certain demographics according to factors such as age, gender, physical conditions, and activity level. According to Nowak., typical grocery shop in the United States has more than 500 distinct nutritional supplements, while specialist stores have a substantially greater variety (Nowak., 2010). Although customers are likely to feel overwhelmed by these figures, they only represent a small portion of those that are available. According to the United States Office of Dietary Supplements, "The Dietary Supplement Label Database (DSLDB) is a repository for all the information on the product label (composition, claims, manufacturer contact information, etc.) of dietary supplements." This is one indication of the number of dietary supplements available. The database now has information from over 60,000 different labels, and every month an additional 1,000 are added to it (82, p. 7). According to the findings of the NHANES series, the use of dietary supplements skyrocketed between the years 1980 and 2000, but then leveled out between 1999 and 2012. In 2012, fifty-two percent of those polled acknowledged using supplements on a regular basis (Kantor et al., 2016). There has been a shift over time in the sorts of supplements that people take; the consumption of multivitamin supplements has declined, the consumption of fish oil supplements has grown, and the consumption of vitamin D has increased by a factor of four (to 20 percent)(Kantor et al., 2016). The consumption of supplements has elevated, especially the consumption of multiple supplements, in an indicative sample of elderly Americans (65–84 years) (Qato et al., 2016); 36 percent of responders regularly used five or more supplements or medications, and 15 percent of the combinations showed potentially harmful interactions. Over fifty different dietary supplements were being taken at the same time by one elderly guy(Strewler et al., 2014). Approximately 33 percent of children consume dietary supplements, and pediatric supplements frequently include greater than the recommended daily intake (RDI)(Brasky & Kristal, 2015; Madden et al., 2014). Even though a sizeable section of the population in the

United States utilizes dietary supplements, significantly less than 25 percent of those people do so with their doctor's blessing.

The rising popularity of dietary supplements can be attributed to a number of different causes, including the following:

1. An aging baby boomer population, with responsibilities for wellness, fitness, and increased quality of life.
2. A high consumption of natural substances, while rejecting the substances containing chemicals.
3. Expensive drugs and a suspicion that drug companies have refused "natural" products because their use could not be protected by patents.
4. Hopeful for diseased people to find a cure for a chronic disease (patients with chronic diseases often take more supplementation than those without the disease).

A high consumption of natural substances (Binns et al., 2018a) due to the interest in the "worth" of tradition and the acceptance of GRAS (generally recognized as safe) dietary additives, particularly in developing nations (Burdock, 2000).

In recent decades, there has been a rise in the marketing of various dietary supplements. There are many thousands of goods on the market that are marketed as having positive effects on health, the avoidance of disease, or even an improvement in mental or physical performance.

2.1.3. Types of dietary supplements

Dietary supplements are divided into several categories according to the kinds of components that make up its composition, including as vitamins, minerals, botanicals, amino acids, fatty acids, and enzymes. Formulas for products frequently change according to age, gender, and physiological state, and products are frequently classified according to the demographic that they are designed for (Kirkpatrick, 2011). The dietary supplement's alleged health purpose will often have a function in finding the sort of product as well as the substances that are contained within it. Oral ingestion is required for dietary supplements, which occur in the shape of tablets, capsules, lozenges, gel caps, chewable, powders, or

syrups. Lozenges and gel caps are also acceptable (U.S. Food and Drug Administration, 2015). In the United States, vitamin and mineral supplements make up the largest segment of the market for dietary supplements, with consumers spending around \$30 billion yearly on these products (Homan, 2018). They can be obtained in the form of single nutrients or as mixtures of many different kinds of micronutrients (Dickinson et al., 2014). Single vitamin or mineral (SVM), multivitamin-mineral (MVM), non-vitamin, non-mineral, and herbal supplements are all included in this category. The brands, chemical combinations, and product types available for dietary supplements are quite diverse (Homan, 2018).

2.1.3.1. Multivitamin and multimineral supplement

The term "multi-vitamin multi-mineral" (abbreviated as MVM) refers to a category of dietary supplements that must have at least three different vitamin or mineral components; however, not all products carry this designation (Kirkpatrick, 2011). MVM normally contain all of the necessary micronutrients and provide the recommended daily amount (RDA) that is based on an individual's age as well as their gender. When used on a regular basis, many vitamin and mineral supplements might put a person at risk for an excessive consumption of those nutrients, even though many people already get the necessary vitamins and minerals through their food. There are times when products contain amounts of ingredients that are higher than the RDA, and when multiple daily doses (MVM) are taken in conjunction with other dietary supplements or food products that have been fortified, it may result in daily intakes that are higher than the acceptable upper limit (UL) (Homan, 2018). The UL is the maximum daily intake that is not expected to induce unfavorable complications on health; consequently, exceeding the UL raises the likelihood of an individual having a nutrient toxicity as well as health consequences from excess ingestion of micronutrients (National Institutes of Health Office of Dietary Supplements, 2014). MVM is the dietary supplement that is used the most frequently, with one-third of all Americans indicating that they take it regularly (National Institutes of Health Office of Dietary Supplements, 2016; Rock, 2007). According to the NHANES, 40% of adult males and females report daily consumption of MVM supplements (Bailey et al., 2011). The most frequent reasons for taking a multivitamin, mineral, and herbal supplement are to maintain general health and to fill up nutrient

deficiencies in the diet (Webb, 2006). MVM supplements are a large category of dietary supplements that have tailored formulae for age, gender, and purpose. Some of these purposes include improved performance or energy, menopause, hair development, pregnancy, and aiding persons who suffer from diabetes and other disorders (National Institutes of Health Office of Dietary Supplements, 2016; Yetley, 2007). Tablets, capsules, and gummy products are the most typical forms that products may be found in, and they may contain additional components in addition to vitamins and minerals, such as botanicals, dietary fiber, and macronutrients (Yetley, 2007). In addition to this, compared to other types of dietary supplements, MVM supplements are more likely to be used for much longer periods of time (Homan, 2018; Kirkpatrick, 2011).

2.1.3.2. Single vitamin or mineral supplements

Dietary supplements known as SVMs are those that only contain a single vitamin or mineral (Ashar, 2010). Because of the positive effects on one's health that consuming a sufficient amount of vitamins and minerals via one's food may have, SVM supplements are frequently utilized to treat and prevent chronic diseases like as cancer and cardiovascular disease. For instance, supplementation of vitamins B12, B6, and folic acid has been investigated to see whether there is a lowered risk of cardiovascular illness. Similarly, supplementation of antioxidants A, C, and E has been investigated for a reduced risk of various diseases (Homan, 2018). However, a review of the evidence that looked at twenty-four randomized, controlled studies found no consistent evidence that vitamin and mineral supplementation reduced the incidence of cancer or cardiovascular disease in healthy individuals who did not have nutritional deficiencies (Fortmann et al., 2013). When a person has a nutritional shortage as a result of not enough intakes from the diet or malabsorption difficulties that hinder the body's capacity to absorb nutrients from food, a person will utilize SVM items in order to supplement their diet (U.S. Food and Drug Administration, 2011). When someone has a nutrient shortage, it is possible that taking a large amount of the nutrient as a supplement for a short duration would be helpful in replenishing the vitamin or mineral and reversing the symptoms of the lack (U.S. Pharmacopoeial Convention, 2011). Vitamin and mineral supplements can contain anywhere from ten to one hundred times the daily

recommended intake (DRI), and when used in excess for a prolonged duration, they have the potential to create potentially major adverse effects on one's health (Kirkpatrick, 2011). Because of this, it is essential to speak with a qualified medical practitioner before consuming large quantities of vitamin and mineral supplements. Others who are having iron deficient anemia, breastfed or partially breastfed newborns, strict vegans, menopausal and postmenopausal women, and people who have a limited diet may find that taking an SVM supplement is beneficial (Homan, 2018).

2.1.3.3. Non vitamin, non mineral supplements

A mix of herbs, probiotics, amino acids, and maybe even fish oil and digestive enzymes can be included in some dietary supplements in addition to vitamins and minerals (Kemper, Amata-Kynvi, Dvorkin, & Whelan, 2003). A little less than forty percent of individuals in the United States have reported using goods outside vitamin and mineral supplements (Kirkpatrick, 2011). Supplements containing fish oil are the most often ingested form of these products in the United States (Xin, Wei, & Li, 2012). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are both kinds of omega-3 fatty acids that may be found in fish oil (Kirkpatrick, 2011). It is well knowledge that dietary sources of EPA and DHA decrease inflammation in the body. These sources have also been connected to other prospective health advantages, including relief from depression and anxiety, as well as protection against cancer; however, none of these claims have been verified. Even though the supplementary version of EPA and DHA does not demonstrate the same health advantages as it does when it is obtained from food, fish oil supplements are the most extensively utilized dietary supplement after vitamins and minerals (Xin et al., 2012).

2.1.3.4. Herbal supplements

Botanical supplements are a subcategory of herbal supplements, and they are made up of a plant or a portion of a plant, including seeds, leaves, stems, bark, roots, or flowers (Botanical Dietary Supplements, 2011). The use of herbal items for the purpose of medical therapy was first documented in China about 2800 B.C., and it has steadily garnered interest

in western culture due to the health advantages that are commonly considered to result from using herbal remedies (Sturluson, 2014). One in six people in the United States use botanical supplements on a daily basis because of the therapeutic or medical benefits they provide (Gershwin et al., 2010).

2.1.4. General use of vitamin and mineral supplements

They are often taken for causes other than nutritional insufficiency, such as for lowering the risk of particular chronic illnesses like as heart disease and cancer, as part of a healthy lifestyle, and for enhancing sleep or physical performance. These are only some of the causes why they are used (Bailey et al., 2019). Dietary supplements are unlike any other product on the market since they combine elements of both diet and medicinal medications (Saldanha, 2005). Food is necessary for the body to function properly, whereas medication is intended to either treat or cure an illness (Gershwin, Borchers, Keen, Hendler, Hagie, & Greenwood, 2010). Items that are under the group of dietary supplements are meant to be consumed in addition to a healthy diet in cases when important nutrients cannot be obtained just through food (U.S. Food and Drug Administration, 2017). They are administered in the same way as pharmaceuticals, most frequently in the form of a pill or capsule and are typically intended to enhance one's health (Saldanha, 2005). Dietary supplements are consumed by clients in the United States primarily for the purpose of improving overall health and lowering the likelihood of developing certain diseases (Bailey et al, 2011). People who do not have any diseases are also interested using these supplements to maintain health and prevent diseases during their lifestyles, such as an unhealthy diet, insufficient physical activity, smoking, and drinking an excessive amount of alcohol (Radimer et al, 2004). Because of this, individuals would look for optimal nutrition from pills and powders more than natural food sources since it is more convenient for them, despite the fact that health care specialists recommend acquiring nutrients from a balanced diet (Homan, 2018). People look for those dietary supplements that are not expensive and easy to be used by consumers according to their lifestyle (self-care health). Another reason people in the United States take dietary supplements is to make up for low amount of vitamins in their regular diet (Bellows et al, 2014). This is more helpful for people who are restricted in their dietary intake or who are at

a certain period in their lives that calls for higher nutritional intake. Those who follow diets that limit their consumption of certain food groups, such as vegetarianism and veganism, as well as those who follow other diets that restrict food group consumption may find that taking dietary supplements of vitamin B12, vitamin D, calcium, iron, and zinc is beneficial (Elorinne et al., 2016). Those who have a decreased calorie intake will have a difficult time getting enough of the nutrients they need from their food and may gain something from taking MVM or a protein supplement (Samadi, Zeinali, Habibi, & GhotbodhinMohammadi, 2016). For instance, those who are facing specific diseases or who have suffered a significant injury may benefit from a protein supplement that contains necessary vitamins and minerals to assist in the battle against infections and to aid in the process of recovering from their condition (Samadi et al., 2016). It is recommended that women of childbearing age who are pregnant or nursing take supplemental folate, iron, or a prenatal vitamin to maintain the high physiological needs of their bodies during pregnancy and breastfeeding (Homan, 2018a). It is suggested that individuals over the age of 50 and women who have gone through menopause take dietary supplements including vitamin B12, calcium, and vitamin D. Last but not least, medical professionals have the ability to recommend a suitable dietary supplement to patients whose health concerns impair digestion, appetite, and the absorption of nutrients (Kirkpatrick, 2011). Many people who use nutritional supplements also utilize items that are designed for the treatment of a variety of different medical ailments (National Institutes of Health Office of Dietary Supplements, 2011). Dietary supplements are an option for those who follow complementary or alternative medical practices, and these individuals may choose to utilize them in conjunction with traditional therapy, or even in place of it (National Center for Complementary and Integrative Health, 2016). Fish oil is regularly used to safeguard the health of the cardiovascular system (Grey et al., 2014). The antioxidant vitamins A, C, and E are connected to immune role and have various reported benefits, including protection against cardiovascular disease and cancer (Bellows et al, 2014). Iron supplements are a typical treatment option for those who suffer from iron deficiency anemia (Kirkpatrick, 2011). Adult women report taking a significant amount of calcium and vitamin D pills on a daily basis in order to strengthen their bones and stave off osteoporosis (Homan, 2018b). Dietary supplements are used by customers to improve their look as well as their physical performance (Calfee & Fadale., 2006). Those who exercise frequently say they use

a variety of supplements to help them recover from their exercises without feeling weak, to build more muscle and strength, and to enhance their overall performance. When it comes to using sports pills to boost their speed and agility, strength, and muscle gain, men are far more likely to do so than women. Protein powders, amino acid mixtures, and many other sport supplements are examples of dietary supplements that can help improve athletic performance (Froiland et al., 2004). When asked about their motivations for using nutritional supplements, women are more likely to cite an enhanced physical appearance brought about by weight reduction, as well as healthier hair, skin, and nails (Bailey et al., 2013). Supplements for weight reduction often include a wide variety of different herbal substances, and specific MVM products that contain B vitamins and collagen are sold as being able to improve the appearance of the user's hair, skin, and nails.

By shielding cells from the effects of oxidative stress, many of the antioxidant substances that may be found in vegetables, fruits, and herbal teas lower the chance of developing chronic illnesses. • Numerous studies have demonstrated that carotenoids in general reduce the risk of developing cancer. Lycopene, for instance, lowers one's chance of developing cancer of the prostate, breast, digestive system, skin, and cervical glands. Because of the glucosinolates that they contain, plant foods like broccoli, cauliflower, and cabbage minimize the likelihood of developing cancer.

2.1.5. Health associated with dietary supplement

Reliability-Usefulness has a long history of being consumed as food because of their low risk of illness. Dietary supplements contain (DS) a number of different phytochemicals that are difficult to evaluate because of variability in the efficacy of the products from one batch to the next. The growing popularity of dietary supplements has given rise to public health concerns over the effectiveness and safety of these products, both in the short term and over the long-term. DS: Benefits and risks are proportional to the dosage that is taken. At very high dosages, there is the potential for adverse consequences. The U.S. Food and Drug Administration estimates that around 50,000 adverse events may place each year that can be linked to the consumption of dietary supplements. This is a

significant understatement, as possibly just 1 percent has been recorded. Most vitamins and minerals have a dose-benefit curve in the shape of U, which indicates that the potential for harm is proportional to the amount consumed. This is a significant understatement, as possibly only one percent has been recorded (Vatistas & Samuels, 2012; Woo, 2007). The DSEA restricted the Food and Drug Administration's capacity to take prompt or preventative action (FDA., 2009; Nowak., 2010).

As the dietary supplements have grown widespread, leading for high usage of them, this increases the reports concerning liver damage. Some of the most common substances that have been implicated are green tea, multi-ingredient nutritional supplements, and anabolic steroids. The immediate liver damage caused by these supplements is comparable to that caused by hepatitis. DS: There is a lack of proof that dietary supplements can prevent deaths from cardiovascular disease or cancer. DS: The Food and Drug Administration of the U.S. has banned products containing ephedrine alkaloids, which are stimulant compounds that can be found in *Ephedra sinica* and a few other plants.

DS databases are used to identify what ingredients do clients use in comparison to those with different diseases using along with or instead of more conventional treatments for disease. There is a role for DS databases in the field of food and nutrition science, provided that the databases do not contain inaccurate data that might cause users to draw the incorrect conclusions. Dietary supplements include nutrients and other bioactive components that may influence health. The safety and usefulness of dietary supplements are not always well established. The information that may be obtained from various media sources, while convenient and affordable, can sometimes be inconsistent and confusing.

This is due to the fact that, unlike a healthy, well-balanced diet, the research that is now available does not establish that dietary supplements may help to prevent illnesses or treat them. Due to the fact that young adults are so dependent on digital technology, it's possible that many of them may turn to media sources as their major source of knowledge on dietary supplements (Scofield & Unruh, 2006).

2.1.6. Drug interaction with dietary supplements

Interactions between drugs and nutrients are difficult to synthesize or collect information about them because there are an infinite number of possible interaction combinations. These combinations are determined by the myriad of elements that are involved in every interaction (Boullata & Hudson, 2012). In addition, the majority of documented interactions are derived from historical case studies, and it is unknown how many of these interactions have therapeutic significance (Ford, 2017). As a general rule, each possible interaction may be placed into one of five categories. These categories are then subdivided according to the effect of a nutrient on a drug or the effect of a drug on a nutrient, as shown in the table below (Boullata & Hudson, 2012; Santos et al., 2016). The information included in Tables 2.1, a good place to begin investigating some of the potential drug-nutrient interactions that are more likely to occur in elderly (Ford, 2017).

Table 2.1. *Drug-nutrient interactions (Ford, 2017).*

Precipitating Factor	Object of Interaction	Potential Consequence
Altered nutrition status	Drug	Treatment failure or drug toxicity
Food or food component	Drug	Treatment failure or drug toxicity
Nutrient or supplement	Drug	Treatment failure or drug toxicity
Drug	Nutritional status	Altered nutritional status
Drug	Specific nutrient	Altered nutritional status

According to the information presented in Table above, drug-nutrient interactions can take place when the precipitating factor is related to nutrients and when drug is the object of interaction. Alternatively, drug-nutrient interactions can take place when the drug is the precipitating factor and overall nutrition status or a specific nutrient is the object of interaction (Ford, 2017). Each one is essential in its own right, but a review of the relevant research reveals that there is a paucity of information on the impact that pharmaceuticals have on the nutritional profile as a whole or on specific nutrients. The impacts that nutrients can have on medications are highlighted in Table 2. 1. and instances of interactions in which pharmaceuticals can have an influence on nutritional status are shown in table above. (Nelms

et al., 2011; Otten et al., 2006; Rogovik et al., 2010; Ross et al., 2011). Tables 2.2. and table 1.3. are not intended to be comprehensive listings of all possible interactions; rather, their purpose is to show frequent drug-nutrient interactions that can take place in people who are bigger. As can be seen in Table 2.3., proton pump inhibitor (PPI) medicines have the ability to interact with a variety of nutrients. Patients suffering from upper gastrointestinal (GI) conditions, such as gastroesophageal reflux disease (GERD), frequently use proton pump inhibitors (PPIs) (Heidelbaugh, 2013). Because of the risk for malabsorption of vitamin C, vitamin B12, calcium, iron, and magnesium that is caused by chronic use of this drug, the high use of these medicines is a cause to worry in terms of nutrition. This is due to the fact that chronic use of this drug causes the drug to be excreted in the urine. When working with elderly who are already at a high risk of malnutrition, it is especially necessary to accept the ethics of understanding the prevalence of these interactions because healthy adults likely have a lower risk of having malabsorption of these nutrients while taking PPIs, whereas elderly and malnourished patients have greater risk of having malabsorption (Ford, 2017). It is essential, both from a nutritional perspective and because there is a dearth of relevant research in this area, to look at the possibility of drug-nutrient interactions in the context of the impairment of nutrients by pharmaceuticals. Even though the list of drug-nutrient interactions that can influence nutritional level that is provided in table above is not exhaustive, it is very comprehensive and can be used to get perception into potential interactions that might be taking place in a population of elderly who live in the community. These kinds of data might be put to use in prospective research that would contribute to filling the informational void that now exists on this therapeutically relevant subject.

Table 2.2. *Drug-nutrient interactions 2 (Ford, 2017).*

Nutrient	Drug	Interaction
Vitamin E	Antiplatelets	May increase risk of bleeding
Vitamin E	Anticoagulants	May increase risk of bleeding
Vitamin E	Iron	May lead to hydroxyl radical production
Vitamin K	Anticoagulants	Antagonist
Folic Acid	Methotrexate	May reduce efficacy in treatment of cancers and can reduce adverse effects when used to treat rheumatoid arthritis and psoriasis.
Folic Acid	Proton Pump Inhibitors	Antagonist
Folic Acid	H2 Receptor Antagonist	Antagonist
Folic Acid	Metformin	Antagonist
Folic Acid	Acetaminophen	May decrease the elimination rate of the drug
Vitamin C	Aluminum	May increase adverse effects (eg. constipation)
Vitamin C	ASA	May increase adverse effects (eg. nausea). Attenuates ASA-induced gastric damage.
Vitamin C	β -blockers	May decrease absorption of the drug.
Vitamin C	Estrogens	May increase plasma estrogen levels up to 55%.
Vitamin C	Iron	May increase absorption of iron
Vitamin C	Anticoagulants	High dosage may reduce response to drug.
Vitamin C	Proton Pump Inhibitors	Antagonist
Vitamin B12	Metformin	Antagonist
Vitamin B12	H2 Receptor Antagonist	Antagonist

Table 2.3. *Drug-nutrient interactions 3 (Ford, 2017).*

Nutrient	Drug	Interaction
Vitamin E	Laxatives	Malabsorption of vitamin E
Vitamin D	Thiazide Diuretics	Hypercalcemia
Vitamin D	Phenobarbital	Decreases metabolism and half life
Vitamin D	Phenytoin	Decreases metabolism and half life
Vitamin K	Laxatives	Malabsorption of vitamin K
Vitamin B6	Isoniazid	Decreases serum vitamin B6
Vitamin B6	Levodopa	Decreases serum vitamin B6
Vitamin B12	Proton Pump Inhibitors	Vitamin B12 malabsorption
Vitamin C	Proton Pump Inhibitors	Vitamin C malabsorption
Calcium	Corticosteroids	Calcium depletion
Calcium	Bisphosphonates	Calcium depletion
Calcium	Antihyperlipidemic	Calcium depletion
Calcium	Loop Diuretics	Calcium depletion
Calcium	H2 Receptor Antagonists	Calcium malabsorption
Calcium	Proton Pump Inhibitors	Calcium malabsorption
Calcium	Laxatives	Calcium malabsorption
Folate	Methotrexate	Folate depletion
Folate	Anticonvulsants	Increased folate metabolism
Folate	Pyrimethamine	Antagonist
Folate	Triamterene	Folate depletion
Folate	Sulfasalazine	Folate depletion
Potassium	Thiazide Diuretics	Potassium depletion
Potassium	Loop Diuretics	Increased potassium excretion
Potassium	Laxatives	Potassium malabsorption

Potassium	NSAIDs	Increased potassium excretion
Magnesium	Laxatives	Magnesium malabsorption
Magnesium	Proton Pump Inhibitors	Magnesium malabsorption
Magnesium	Loop & Thiazide Diuretics	Magnesium depletion
Magnesium	Antihyperlipidemics	Hypermagnesuria
Chromium	Antacids	Chromium malabsorption
Sodium	Corticosteroids	Sodium retention
Sodium	Loop Diuretics	Increased sodium excretion
Iron	H2 Receptor Antagonists	Iron malabsorption
Iron	Proton Pump Inhibitors	Iron malabsorption
Iodine	Sulfonylureas	Impair uptake or release of iodine
Iodine	Lithium	Impair uptake or release of iodine

2.2. Vitamins

Vitamins are organic compounds (non-energy generating) that are required for regular human metabolism and must be consumed in modest amounts. The definition does not include inorganic necessary trace elements, essential amino acids, and essential fatty acids, which are required in considerably greater quantities. The term vitamin comes from the Latin word "Vita," which means "life," and the suffix -amin relates to the word amine (one). The first vitamins were named with letters of the alphabet. The different chemical forms and precursors of vitamin can be called its vitamers. Vitamins, one of the essential dietary factors for life, are compounds that must be taken from the outside through foods for normal growth and continuous of life. The best sources of vitamins are seafood, liver, various meats, cheese, milk, eggs, oilseeds and legumes (Velioglu et al., 2019). Vitamins, which are active elements such as enzymes and hormones, are called biocatalysts in the metabolism of living things (Saldamlı, 2017). Since the human organism cannot produce vitamins except for some vitamins that the body produces far below the amount it needs, vitamins must be taken from outside. In cases where enough vitamins are not taken, deterioration in the functions of cells

and tissues and health problems occur as a result. These vitamins when used as a drug are necessary to prevent deficiency disease and treat it in case it happens. Some vitamins have many medical uses in pharmacological doses. There are many causes of vitamin deficiency such as inadequate intake, malabsorption, increased tissue needs, increased excretion, certain genetic abnormalities, and drug vitamin interaction.

The biochemical effects of vitamins on the body's activity are related to the control of biochemical processes. The impact of vitamins on human health may be classified into three categories:

1. Aiding in growth
2. Aiding in the development of healthy generations
3. Normal nervous and digestive system function, adequate nutrition use, and assistance in body resistance (Cemeroğlu et al.,1992).

So, taking vitamins in very small amounts is sufficient for the body, Vitamin deficiency is not seen in people who have a balanced diet with food. Under normal conditions, there is no benefit in taking more vitamins than in the food, because most of the vitamins are not stored in the body and more than the daily requirement is excreted in the urine (Duconge et al., 2008).

The vitamin family has 13 vitamins divided into two major divisions. The first group of vitamins includes fat-soluble vitamins (A, D, E and K), and the second group includes water-soluble vitamins (B-complex group and vitamin C) including the B-complex group, B1 (thiamine), Vitamins B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine) and B12 (cobalamin), biotin and folic acid. Fat-soluble vitamins need to be taken with a certain amount of fat so that they can be absorbed from the digestive tract (absorption) and used by the body. More than the daily required amount of vitamins in this group is stored in the body. Vitamins A and D are mostly stored in liver tissue. Vitamin E is stored in adipose tissue in the body. Vitamin K can be stored at low levels by the body. When vitamins A and D are taken in excess, they cause toxic effects for the body. Because B-complex vitamins and vitamin C cannot be stored in the body, the needed levels must be obtained through diet on a daily basis. Preforms (pro-vitamin A and D) of some vitamins, which are the main nutritional factors, can be produced in the body. Although nicotinamide does not match the need, the

body produces it through the use of tryptophan (amino acid). Diseases associated with vitamin insufficiency arise when people consume less than the recommended quantity of vitamins for an extended period of time. Vitamin deficiency is more common in people living in poor areas of the world and is often confused with this condition, as it exhibits similar manifestations to protein deficiency. The picture that develops due to the absence of a single vitamin is called "avitaminosis", and the intake of vitamins below the lowest level considered normal is called "hypovitaminosis". Multiple vitamin deficiency is called "polyhypovitaminosis" and excessive vitamin intake is called "hypervitaminosis". In the human organism, fat-soluble vitamins mostly function in cell wall structures, and water-soluble vitamins mostly function as co-factors that regulate the functions of enzymes (Öznure, 2008).

2.2.1. Water soluble vitamins

Water-soluble vitamins are delivered to the tissue by the blood circulation after being absorbed in the gut. Each of them has different degrees of solubility in water (Sauberlich, 1986). This difference also affects their distribution in the body. If these vitamins are taken more than necessary, they are excreted after being stored in the tissues to a certain extent. Vitamin B is inactive in its free form. It must go through a number of chemical steps in order to perform its activities in the body. It performs the function of coenzyme with the addition of other substances or molecular parts (Olson and Bresse, 1990).

Although the physiological functions of vitamin C are well known, the mechanism of metabolism is not clear. Vitamin C is important for the growth of teeth and bones and healing of wounds. It has many other beneficial effects on the body (Öznur, 2012).

2.2.2. Fat soluble vitamins

Vitamins A, K, E, and D are known as fat-soluble vitamins because they are fat-soluble. Although they are necessary for health, each has very important functions in the body. Fat-soluble vitamins circulate with fats from the intestines to the circulation and are absorbed. Any disease or disorder that affects fat absorption causes a deficiency of these vitamins. After circulating, they are transported to the liver tissue. These vitamins are mainly stored in the liver and adipose tissue. Vitamins A, D and K are stored in the liver, while vitamin E is distributed to the body's fat tissues. Because these vitamins can be stored at various rates, short-term deficiencies are less likely to occur than water-soluble ones (Öznur, 2012). Vitamin K is an exception because fat-soluble vitamins are eliminated from the body more slowly than water-soluble ones. Vitamins A and D can accumulate in the body and cause toxicity (Selçuk and Şahna, 2006).

2.2.3. Vitamin C

Vitamin C is a soluble vitamin that dissolves in water (Carr and Maggini, 2017). The recommended daily intake of vitamin C is 500mg-2000mg. The protective effect of high intake of vitamin C against infections has not been scientifically proven. Vitamin C is not found in animal products or grain. It is mostly found in fruits and vegetables (Velioglu et al., 2019). It cannot be synthesized in the human body and should be taken with meal (Megep 2006). Vitamin C deficiency can lead for joint pain, delayed healing of wounds, scurvy, low immunity, Influenza, and gingival bleeding. High levels of vitamin C can lead for diarrhea (Velioglu et al., 2019). When a person is infected, ascorbic acid concentrations decreases in plasma while it increases in urine compared to uninfected individuals living under same conditions. This appears in persons who are vaccinated against smallpox and measles, and even for the common cold (Scrimshaw, 1997). Vitamin C, with its antioxidant feature, cleans free radicals and prevents diseases such as inflammation and URTI by engulfing cancer cells, bacteria, and viruses. Ascorbic acid helps prevent infection by increasing the formation of IgG, IgM, interferon, and PGE1-PGE3 (anti-inflammation agents). In certain investigations, bacteria and viruses are ingested by ascorbic acid in the presence of leukocytes, making the

illness process easier to resist. In case of infection, sugar-free diet is important for glucose, which has structural similarity with vitamin C and competes with it. It has been observed that vitamin C is more effective in viral infections (4). Prof. Dr. Linus Pauling stated that in cases of sudden inflammation, taking 1 g/hour or more of ascorbic acid until the symptoms subside, using antibiotics, histamine and drugs that reduce congestion, vasoconstriction, nasal drops, and foods with high glucose content will worsen the course of the disease and prolong its duration (Yilmaz, 2019). In earlier years, high dosages of ascorbic acid, such as 10 grams or more per day have been used successfully in the treatment of various disorders. Examples of these diseases are polio, pneumonia (viral), and hepatitis (viral) (Scrimshaw, 1997). Overdoses of vitamins, with the exception of vitamin E, do not appear to have a negative effect on the role of the immune system. Humans and a small number of other species require ascorbic acid in their diets. It has consistently been connected with fertility and may have evolutionary relevance, but its specific physiological role in reproduction remains unknown (Yilmaz, 2019).

2.2.3.1. *Properties of vitamin C*

Vitamin C is a significant water-soluble antioxidant. It is a 6-carbon organic acid with a similar structure to glucose. It is a powerful reducing agent, and its active form is generally more active. It is absorbed mostly through the gastrointestinal system and is broadly disseminated extra and intramuscularly. The amount of ascorbic acid ingested each day determines the plasma concentration and total body storage of vitamin C. A typical daily dose of 60mg produces 0.8mg/dl in plasma and 1.5g in the entire body. As the amount consumed increases, so does the amount excreted in the urine. The body can only store up to 2.5g. It partially oxidizes, producing active (dehydroascorbic acid) and inactive (oxalic acid) metabolites (Hancock, 2009).

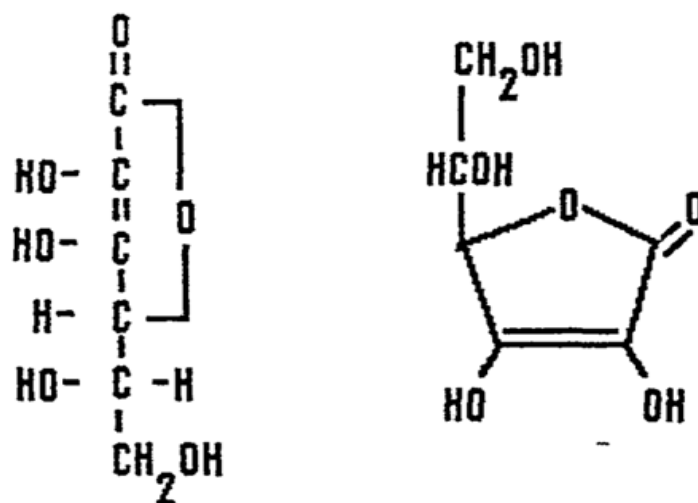


Figure 2.1. Vitamin C structure.

2.2.3.1.1. *Physical properties*

They are colorless, white, oblong crystals. It has a very light specific smell. It has a sour taste and has an acid reaction. Optically active, vitamins in many natural products are oxidized by the effect of air and light; polarized light is shifted to the right. A gram of ascorbic acid dissolves in approximately 3 mL of water, 30 mL of alcohol, 50 mL of pure alcohol, 100 mL of glycerol, and 20 mL of propylene glycol. It can be also dissolved in hot water. It cannot be dissolved in ether, chloroform, benzene, petroleum ether, oils, or oil solvents (Öznur, 2012).

2.2.3.1.2. *Chemical properties and stability of vitamin C*

Its chemical name is 2-oxo-L-threohexono-1,4-lactone-2,3-enediol or L-3-hetothreohexuronic acid lactone. Hydroxyl groups and oxygen atoms give the structure its melting point. It also allows the structure to dissolve in polar molecules such as water (Cemeroglu, 1986). The acid character of Vitamin C comes from en-diol groups and is a monovalent acid. That is, the only hydrogen of the en-diol group is dissociated (Öznur, 2012). Ascorbic acid oxidizes free oxygen in food.

Vitamin C (Ascorbic Acid) is a very sensitive food vitamin. Ascorbic acid is broken down by oxygen, high temperatures, and heavy metals. Even if it is taken in large amounts, the remaining part is excreted in the urine after the tissues meet their needs (E. V. Er, 2019). When the temperature and pH increases, the stability of ascorbic acid decreases (Emese, 2008). This destruction by oxidation is a serious problem, so that during storage, processing and preparation, a considerable quantity of the vitamin C contents in food is lost (Davey, 2000). Ascorbic acid decreases gradually when it is stored at a temperature more than 0°C. Vitamin C is chemically the left-reversing enantiomer of ascorbic acid. Commercial vitamin C is generally composed of ascorbic acid crystals or calcium or sodium salts of ascorbic acid. Ascorbic acid provides protection by capturing free oxygen before oxidizable structures in food Figure 2.2. One of the most important chemical properties of ascorbic acid is its redox potential, causing it to act as a reducing agent and scavenger of free radicals, and by giving an H ion, it inhibits the free radical chain. It is the most acidic in the 3-position hydrogen, the hydrogen atom that is removed in one-electron oxidation reactions (Öznur, 2012).

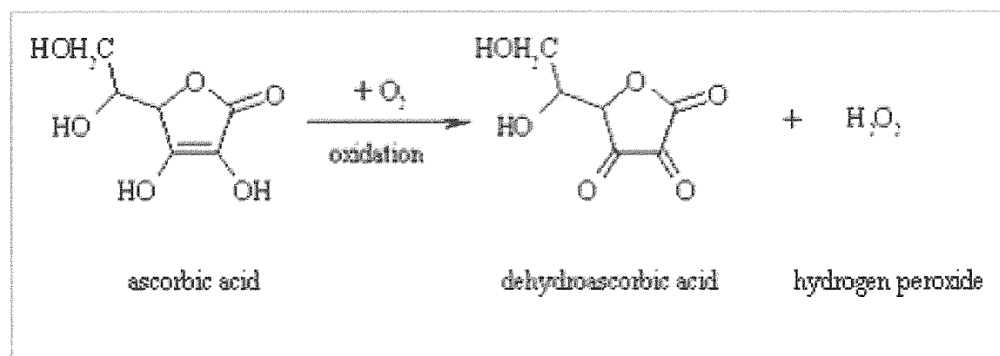


Figure 2.2. *Vitamin C redox Riaction.*

2.2.3.2. Vitamin C benefits

Vitamin C is a water-soluble vitamin (Carr & Maggini, 2017). According to the Brazilian Society for Clinical Nutrition, vitamin C supplementation protects against viral respiratory tract infections (de Faria Coelho-Ravagnani et al., 2021). Because of its antioxidant, antiviral, and antibacterial properties, vitamin C plays a significant role in both adaptive and innate immunity and can control the immune system (Holford et al., 2020). Vitamin C also helps maintain epithelial integrity, increase differentiation and proliferation of T and B cells, produce normal cytokines, and decrease histamine levels. Vitamin C insufficiency impairs immune function and increases susceptibility to infections (Carr and Maggini, 2017). It is necessary for collagen synthesis and wound healing. It is argued that daily consumption of 500-1000 mg of vitamin C accelerates the healing of wounds (Bahador, 2022). Vitamin C works as a cofactor in the hydroxylation of proline and lysine amino acids by proline hydroxylase and lysine hydroxylase enzymes in procollagen, which is required for connective tissue strengthening. In vitamin C deficiency, proline and lysine cannot be hydroxylated, so collagen fibers cannot form crosslinks. The tensile force in the resulting fiber is very low. As a result, scurvy disease occurs. Vitamin C is a non-enzymatic iron reduction agent that converts iron from Fe^{3+} to Fe^{2+} in the stomach and prevents anemia by increasing iron absorption (particularly non-heme iron) (Gürdöl and Ademolu, 2010). Ascorbic acid also prevents megaloblastic anemia by activating the folic acid reductase enzyme to produce tetrahydrofolic acid, the active form of folic acid (Reddy and Lewis, 2009). Vitamin C and zinc are essential for the proper functioning of the central nervous system. Vitamin C has a synergistic effect with vitamin E, which supports the antioxidant resources of the brain, synaptic activity and detoxification (Gromova et al., 2019). Vitamin C is an antioxidant that protects the body from oxidative damage. Vitamin C's antioxidant action protects the health of the heart and, as a result, all bodily tissues. Vitamin C also plays an important function in the development of the lungs, improving the activities of white blood cells and lowering respiratory stress. It protects cholesterol, fatty acids, and lipids on LDL against free radical damage. Ascorbic acid functions as a cofactor in the conversion of tryptophan to the neurotransmitter serotonin, as well as dopamine to norepinephrine. It is also necessary for the production of noradrenaline and adrenaline. It can also prevent bleeding by

strengthening the vessel walls. However, it can decrease the spread of skin malignancies caused by breast cancer and solar radiation. Many experts believe that vitamin C and the cancer process function in the following way: Vitamin C deactivates free radicals before they may harm DNA. As a result, new tumor forms are avoided. Gingival bleeding, weariness, joint discomfort, tooth irregularities, and reluctance can all be symptoms of a deficiency. Vitamin C deficiency in pregnant women has been linked to atherosclerosis, which can lead to preeclampsia (increase in urea with increased blood pressure). It's worth noting that vitamin C has long been proven to lessen the risk of preeclampsia. When taken in excess, it is excreted in the urine, nevertheless, when eaten in excess, it produces allergic skin complaints, diarrhea, and kidney stone development. On average, our daily need for vitamin C is 10 mg (Samur, 2008). Ascorbic acid has a significant anti-cold effect. It cures illnesses like colds. According to Dr. Pauling, ingesting 1-2 g of ascorbic acid each day relieves the common cold (Bahador, 2022).

2.3. Zinc

Zinc protects several respiratory systems from viral infections by protecting respiratory epithelium, enhancing mucociliary clearance, lowering viral replication, reducing local inflammation, and avoiding secondary respiratory infections (Wessels et al., 2020). New evidence recommends that zinc may have a function in the emergence of COVID-19 symptoms. Both zinc deficiency and COVID-19 infection are associated with loss of taste and smell. The use of zinc supplements can shorten the recovery period of the sense of taste and smell (Abdelmaksoud et al., 2021). It has been stated that zinc supplementation at doses above 75 mg/day may cause antiviral effects against cold viruses, including influenza viruses, and it shortens the reported duration of colds (Hemilä, 2017). It is a metalloenzyme component that promotes cell growth and proliferation, sexual competence, and fertility. It improves the immune system and taste. It plays a significant function in the antioxidant equilibrium of the body since it is a cofactor of the superoxide dismutase enzyme, which is one of the intracellular antioxidant enzymes (Karabulut and Gülay, 2016). Adult men require 15 mg of zinc per day, while women require 12 mg (Samur, 2008). Infections reduce both serum copper and zinc. Castillo-Dunan et al. performed careful metabolic experiments to

demonstrate the influence of diarrhea on zinc and copper levels. During infection, the hepatic ceruloplasmin production is stimulated leading to an increase in copper concentrations, so that they cannot be estimated from serum concentrations. In this study, it was observed that serum copper concentrations were remarkably less in patients with diarrhea compared to the control group. Conversely, during an acute infection, the metal is redistributed to the liver leading to a decrease in zinc concentrations found in plasma. Because of that, the reduced retention of zinc during diarrhea thus interferes with the redistributive effect of the infection (Bahador, 2022).

12 Zinc is the Q10 (coenzyme) of over 300 enzymes, and its deficiency causes weakening of the immune system. Zn (zinc) supplementation improves immunity and protects against illness in healthy people. At the same time, it has been noted that it decreases the duration of diarrhea in children by improving the intensity of the diarrhea and the course of the disease. In influenza infections, Zinc has shown its helpful effect. Zinc is necessary for the activity of many enzymes as a cofactor, and its deficiency decreases the activity of several cells in the immune system. Zn deficiency, often a part of protein energy malnutrition (PCM), is clearly related with an immunodeficiency condition. These are lymphopenia, thymic atrophy, and altered T-cell subsets and cytokine response. Clinical studies showed an increased sensitivity to infections, skin rashes, and diarrhea. Zn supplementation has been found to improve immune functions, including cytokine production, and decreases the occurrences of diarrhea and pneumonia in both adults and children. However, excessive zinc consumption should be avoided since it might change the immunological response. In the deficiency of vitamin E and selenium, such as PCM, the antioxidant impairs immunity, accelerates the rapid progression of viral and other infections and the development of cardiomyopathy. Zinc and other vitamins are necessary for appropriate growth and development, as well as for the senses of taste and smell. The recommended dietary dose for zinc increases from 3 mg/d for ages 7 months to 3 years to a maximum of 11 mg/d for men 14-18 years old. Zinc is mostly found in fish, eggs, poultry, meat (particularly red meat), cheese, milk, legumes, and whole grains (Yilmaz, 2019). In 2004, greater than 37% of the zinc in the American diet came from meat, fish, and poultry. This is with addition to 25% of cereal products and 16% of dairy products. Zinc deficiency can make the immune system weak, increase the risk of infection, and decrease infection-fighting ability. Signs and

symptoms may include poor growth, loss of appetite, altered immune function, hair loss, skin and eye lesions, and delayed puberty (Deshpande, 2013).

2.4. Dietary Supplements Pharmaceutical Forms

There are many forms of dietary supplements such as tablets, capsules, soft gels, gel caps, liquids, and powders (Dişli & Yeşilada, 2019). They can also be made into other products, as a snack bar or toothpaste, to give vegans with vitamin B12 (Siebert et al., 2017). However, the most prevalent kind of oral route administration that comes to mind is tablets, which hold the most significant and prominent position among all pharmaceutical forms. In the pharmaceutical industry, oral dose forms are more important. They are pharmaceutical manufacturers who work on both large and small scales. The size, shape, and thickness of the tablets are more significant in the packing process than their organoleptic features, which include their color and odor (Sowjanya et al., 2015). Various pharmacopoeia tablet quality control tests: Tablets are tested for active ingredient, Disintegration number, Content Consistency and Labeling is number four according to the British Pharmacopoeia.

The API determines the different sorts of tablets. There are many varieties such as coated tablets, uncoated tablets, effervescent tablets, gastro resistant tablets, modified release tablets, and dispersible tablets, soluble tablets, enteric coated tablets, controlled release tablets, and sustained release tablets. The quality control tests for these tablets are determined by the tablet's nature. These tests eliminate any damaged or broken tablets, as well as any pills that are not blinded to the substance (Sowjanya et al., 2015).

2.5. Quality Control

Quality assurance is a broad expression referring to all elements that affect the quality of a product, either one by one or all together. The total planned arrangements made with the aim of making sure that pharmaceutical products are of the necessary quality for their intentional use is known as quality assurance. As a result, it covers GMP as well as other quality considerations. The need of defect testing and reporting to management is emphasized by quality. The choice to investigate or refuse the release is made by

management. There have been several complaints about the quality of nutritional supplements. Research of multivitamin and mineral supplements discovered significant variations between the quantities reported on the labels and those found in the pills themselves (Andrews,2017). According to the International Organization for Standardization (ISO), quality control is the operational processes and actions used to meet quality criteria. In process, quality control allows manufacturers to track any changes that occur during the use of technical processes. The manufacturer meets all standards due to in-process quality control for the whole product. One of the most important requirements is that the product be safe (Chavan et al., 2018).

In-process quality controls are used to monitor and, if required, alter the production process to meet the specifications. This might also include equipment and environmental control. In-process materials must be examined for physical characteristics and quality features, with the outcomes of the production process determining whether they are authorized or rejected by the quality control department. Rejected in-process material should be noticed and monitored using a quarantine system to avoid its usage in production. Standard operating procedures for in-process controls and testing should be created and followed.

Finished Product Controls (FPC) are performed after the production process. These tests are used to investigate qualitative and quantitative qualities, as well as test procedures and acceptance limits, with which the completed product must conform for the duration of its shelf life. The completed product specifications should take into account the quality features connected with the production process. An adequate specification for each area of the quality considered should be developed throughout the development and validation of the manufacturing process. The goal of specs is to identify the features that are regarded significant and should be tested on a regular basis. Marketing permission establishes the completed product specification limitations at the time of batch release, ensuring that the specifications presented at the end of shelf life are assured, and is based on a critical comprehensive examination of the data collected from the batches assessed.

Pharmacopoeias vary by country, such as the British Pharmacopoeia (BP), the United States Pharmacopoeia (USP), the European Pharmacopoeia (PhEur), the International Pharmacopoeia (PhInt), and the Japanese Pharmacopoeia (JPh) (JP). This research was done

to illustrate the quality characteristics for pharmaceutical tablets according to pharmacopoeias, which are used in quality control tests on in-process and final goods (Sarma et al., 2016).

2.5.1. FDA and dietary supplements

The United States Pharmacopeia has tools to check the quality of dietary supplements. The Dietary Supplements Health and Education Act (DSHEA) asked the U.S. Food and Drug Administration (FDA) to make the current good manufacturing practice (GMP) regulations for dietary supplements (DS). The goal was to protect public health by making sure the quality of dietary supplements. The GMP regulations say that manufacturers must set their own quality standards for identity, purity, strength, composition, and the absence of contaminants (Sarma et al., 2016). During many GMP inspections by the FDA, it was found that the private standards set by these manufacturers are often not enough to make sure that the quality of dietary ingredients and DS is good enough. With GMP compliance and more use of the public standards made by the United States Pharmacopeial Convention (USP), DS can have the same quality and consistency as medicines.

Strengthening the GMP rules so that they require conformance with relevant United States Pharmacopeia–National Formulary (USP–NF) standards, or other public compendial standards if there are no USP standards, could help protect public health. Another big worry is that some products sold as DS contain synthetic drugs and similar drugs. Using the new USP General Chapter, "Adulteration of Dietary Supplements with Drugs and Drug Analogs," may protect consumers from dangerous drugs that are put in DS to look like they are safe (Trust, 2018).

Foods with function claims are a new group of foods that came out in April 2015 and are labeled with their nutritional or health benefits, so that clients can make better decisions (Sarma et al., 2016).

2.5.1.1. Dissolution test

Dissolution is a formal test that is used to check the percentage release from the dosage structure. Evaluation tests are done before the tablets are prepared in which they will determine the correct granule testing procedure to compress the tablets. Granules are the API formulations which are compressed to form the tablets. API is combined with excipients and binders for granules preparation. This dough is used to prepare granules which are dried and then undergo evaluation test.

1. Particle Size and Shape determination: The size and shape depends upon the processing requirements and during granulation. The methods for determining the shape are: Sieving, Sedimentation rate, Microscopy, Light screening.
2. Surface area of granules: It is usually used for drug substances, but not for the granules.
3. Density: It is the compressibility, porosity and dissolution of tablet. Methods for density are: Granular density (Sowjanya et al., 2015).

2.5.1.2. Tablets disintegration test

Disintegration is the formal test which identifies the duration taken for the tablet to break down into fine particles when placed in the solution. It helps to know API solubility in the gastric fluids of the digestive system. Controlled and sustained release tablets are performed by this test, but it is ideal for other tablets. Temperatures are maintained according to each tablet. It is calculated by the duration for the tablet to dissolve in liquid and then it is compared with the standard duration in the pharmacopoeia (Abdul Althaf et al., 2012; Zhang & Zhang, 2014).

2.5.1.3. Weight variation test

It is done to make sure the tablet is the same uniformity (Abdul Althaf et al., 2012).

2.5.1.4. Hardness

It is done to see how steady the tablet is. It is the amount of force needed to break the tablet when it is set on its edge. The test is done to see if the pressure on the tableting machine needs to be changed. The hardness affects how the test works. If the tablet is too hard, it might not break up in the needed duration. And if the tablet is too soft, it won't be able to handle the next steps, like being coated or put in a box. If a tablet is too hard, we first check to see if it breaks apart, and then the batch is thrown out. If the disintegration is within the allowed range, batch will be accepted.

The following factors may affect tablet hardness: Tablet compression and compressive force, Binder quantity (More binder a more hardness). The granulation process used in tablet preparation wet method gives more hardness than direct method, Slugging method gives the best hardness (Abdul Althaf et al., 2012).

2.5.1.5. Friability

The Roche Friabillator is used to test whether the tablets are easy to break or not. Before putting the tablets in the machine, the initial weight of each tablet is checked, and batches are separated, so that each can be checked separately. The tablet's starting and ending weights are checked. If there are clearly cracked, cleaved, or fractured tablets in the tablet sample after tumbling, the sample fails the test. For most products, a maximum mean weight loss from samples of no more than 1.0 percent is considered acceptable (Pharmacopia U.S, 2016).

2.5.1.6. Thickness test

The thickness test is not a formal test. It is only done on a small number of tablets. A Vernier caliper or screw gauge is used to measure the thickness of a tablet (Sowjanya et al., 2015).

2.5.1.7. General appearance

For consumers to accept a tablet, the way it looks, how it stands out, and how elegant it is as a whole are all crucial important. This is done to control the uniformity from lot to lot and from tablet to another. Measurements of size, shape, color, smell, taste, etc. are used to control how something looks in general. The size and shape of the tablet may differ from one to another (Chatsiricharoenkul et al., 2011; Lokesh et al., 2012).

2.5.1.8. Uniformity content.

The potency (drug content) of a tablet is represented in terms of gm, mg, or mcg (for certain powerful medications) of drug per tablet and is stated on the product's label.

characteristics that might directly contribute to tablet content uniformity issues:

- Non-uniform active ingredients dispersion throughout the powder combination or granulation.
- During the different production procedures, powder mixtures or granulations are separated (demixed).
- Tablet weight variation.

2.5.2. Good manufacturing practice (GMP)

Good Manufacturing Practice makes sure that pharmaceutical products are manufactured and administered in compliance with the quality standards and conscious use, and the specific characteristics of the product or the information that is essential to the registration are deemed necessary, and a part of Good Manufacturing Practice of Quality Assurance is related to both production and quality control, and must be demonstrated that they can be produced in accordance with specifications (Tan, 2008) .

1. Critical steps of production procedures and significant changes in procedures are confirmed.
2. All necessary skills for GMP should be provided, including:

- a. Appropriate trained and qualified personnel
 - b. Appropriate facilities and space
 - c. Appropriate instruments and assistances
 - d. Right material, labels and containers
 - e. Acceptable processes and directions
 - f. Proper storage and handling
3. Directions and processes should be written in a clear and understandable language as a direction form and especially applicable according to the possibilities at hand.
 4. Operators should be trained to perform the procedures correctly (Kaya, 2015).
 5. During production, manual or other records should be kept to show that all the operations are actually done according to the procedures and instructions, and that the product is of the desired quality and quantity. Any significant deviation should be fully recorded and investigated.
 6. The production record of the entire history of the series to be examined, including its distribution, should be kept in understandable and easily accessible forms.
 7. Distribution (wholesale) of pharmaceutical products should be done in a way that minimizes any risk that may affect their quality.
 8. A system should be in place to allow the withdrawal of any amount, after sale or distribution.

Complaints regarding advertised products should be investigated, the causes of quality faults should be investigated, suitable measures should be taken regarding faulty products and recurrence of the situation should be prevented (Velioglu, 2019).

2.6. Future Public Health Regulation Of Dietary Supplements

The majority of countries care more for market approach and industrial interest, while ignoring public health. In comparison to other countries or international groups, the EU and Japan have stronger regulations (Binns et al., 2018b). Any system would be overwhelmed by the hurdles of assessing and controlling maybe 100,000 goods in any scenario. With the potential of problems and side effects, the US\$180 billion industry demands stronger

adjustment and oversight of product labeling, side effect warnings (prohibiting if considerable), and effectiveness (if health professions are made). The dietary supplements that are taken on medical orders and as part of clinical practice guidelines for certain life stages should be controlled independently. In pregnancy and newborns, folate, iodine, iron, vitamin D, and vitamin K are examples. The National Health and Medical Research Council of Australia make these suggestions, which are included in clinical guidelines. Between food and drugs, dietary supplements exist in a gray area. Pharmaceutical-style rules for dietary supplements would simply be impossible to execute. It's difficult to imagine carrying out or analyzing 100,000 randomized controlled studies. Direct regulation, market-based incentive mechanisms, post market surveillance, and legal liability incentives (Brewster, 2007) are all used in the food business, while enforcement levels differ by state. Certain food ingredients are usually regulated hardly, and food cannot be marketed as such unless they meet high compositional and morality criteria, such as those set by the Australian Food Standards Authority (Aust, 2016). Nowak submits that the US Congress proceed more proactive laws customized after the EU's Food Supplements Directive (Nowak.,2010).

2.6.1. United states and dietary supplements

Food Supplements Health and Education Act (DSHEA), which went into force in the United States in 1994, said that the term "Food Supplements" refers to vitamins, minerals, herbal remedies, amino acids, enzymes, organ tissues, glands, and metabolites that are added to food and ingested by mouth. Extracts and concentrations are also included in this definition. There are different forms such as tablets, capsules, soft gels, gelatin capsules, liquids, and powders. In any case, the label must declare that the product is not conventional and contains complimentary compounds. According to the DSHEA, these items are classified as "food" rather than "medicine" (Kasabali., 2007). The DSHEA classifies dietary supplements as foods rather than food additives. Unlike food additives, these products are not normally regarded as safe if they were introduced prior to 1994 (Hughes, 2001). Firms do not require FDA clearance to produce and distribute food supplements in the United States. Because the FDA does not have a specific rule on the subject, the company must set its own production techniques and is responsible for the safety of the food supplements it

creates. The composition of the product must be written on the label (Dietary Supplements Center for Food Safety and Applied Nutrition, 2007). This rule includes herbal remedies that have been proven to be effective to prevent chronic diseases in the scope of food assistance. These plants can now be sold as foods, thanks to the definitions of tablets, capsules, and liquid dosage forms. Although medical claims for these products are prohibited, claims that herbal remedies change the structure and function of the human body may be made in advertising or on labeling (Kasabali., 2007). On October 6, 2000, the FDA adopted a regulation regarding structural and function claims for food supplements. Many "minor ailments" that are accessible over-the-counter (OTC) are now covered by food aid thanks to this rule. Examples include antacids, carminatives, antiemetics, sexual stimulants, sedatives, digestive aids, laxatives, sleeping pills, stimulants, and attenuators (Israelsen, 200). Since these 27 laws were passed, the food supplement business has risen tremendously. Food supplements accounted for more than 80% of the herbal products market in the US and 15-20% in Europe. Despite the fact that legality varies from nation to country, the market for herbal products is developing in mature countries (Dietary Supplements Center for Food Safety and Applied Nutrition, 2007). In the domain of food supplements, new terminologies have emerged, particularly in the United States. Functional foods include nutraceuticals, designer foods, pharmaceuticals, and phytochemicals. These expressions refer to "one or more herbal substances, in single or mixed form, entering the composition of a food product, exhibiting a protective, preventive, and possibly therapeutic effect in the pathogenesis of cancer and the development of other chronic diseases, with a supportive or synergistic effect in the pathogenesis of cancer and other chronic diseases, with a supportive or synergistic effect Animal and isolated organ research, as well as epidemiological and clinical studies¹⁵, have all demonstrated the efficacy of these products. Nutraceuticals and functional foods are terms used in the United States to describe foods or food components that provide health benefits in addition to basic nutrition. Nutraceutical refers to both traditional and new food and food constituents (tablets, capsules, etc.), whereas functional food refers to traditional food forms. In other words, whereas nutraceutical refers to any food extract with scientifically proven health benefits in the treatment or prevention of diseases, non-toxic, functional food refers to nutrients that reduce disease risk and promote health. However, in reality, the terms nutraceutical and functional food are usually used interchangeably

(Dillard,2000). There is now no formal regulation governing nutraceuticals or functional foods in the United States. The FDA believes that present food regulation is acceptable and that firms should determine the intended use of the product (food or food supplement) early in the research phase. If a structural and functional claim is made, the label must indicate, "This claim has not been reviewed by the FDA." This product is not intended to be used for disease diagnosis, treatment, or prevention." (Soller,2000). A breakthrough that will astound the scientific community is also taking place in the United States. The FDA is also chastised in this 1994 act (Dietary Supplement Health and Education Act). According to an urgent distribution letter sent to all food support producing companies on June 22, 2007, valid good manufacturing practices (cGMP, current Good Manufacturing Practices - Current Good Manufacturing Practices) will be adopted from now on, which will be based on quality production and safe production in terms of pollution and infection. Because cGMP rules will be followed, certification in the areas of production, packaging, labeling, and storage will be required; good production conditions will be planned in accordance with quality control rules; laboratory analyses for finished products will be requested; and consumer records control will be required. The FDA has given the manufacturers standards for determining the compounds in the food supplements and herbal preparations they make, as well as a document stating that they are free of natural toxins, bacteria, pesticides, silicon, lead, and other heavy metals, as well as they are not well packaged and labeled. Manufacturers with more than 500 workers must comply with these GMP criteria until June 2008, food supplement providers with fewer than 500 workers must conform until June 2009, and manufacturers with less than 20 workers must comply by June 2010¹²⁷ (FDA Issues Dietary Supplements Final Rule, 2007).

2.6.2. European union and dietary supplements

The rules from the European Commission are meant to make sure that food supplements are good, safe and correctly labeled. In the EU, food supplements are controlled like foods, and most of the rules are about the vitamins and minerals that are used to make them (Binns et al., 2018). There are EU-wide high and low levels set for each vitamin and mineral added to supplements. Companies wish to advertise a substance that is not included

in the permitted list needed for submitting an application to the European Commission (Zell-Kanter and Leikin, 2015). In the EU, many botanical products that are considered dietary supplements in other places are considered foods. Most of the time, these items are labeled as "natural foods," and maintains about their health benefits are often made. The same things can be called food or medicine depending on how they are used and where they are sold. The EFSA knows that this method has some problems: "While most of these products have a long history of use in Europe, some concerns exist about their safety and quality. These include the risk of chemical or microbiological contamination and the need to ensure that concentrations of bioactive agents are within safe limits" (Tanaka, 2004). Some botanicals are thought of as traditional herbal medicines, and they are used in both medicines and dietary supplements. The European Medicines Agency is in charge of figuring out if herbal medicines are safe and effective when they are used as medicine (Binns et al., 2018b).

2.6.3. Japan and dietary supplements

In 2015, nutritional and dietary supplements brought in 1.19 trillion yen, which is about \$11 billion (Representative, 2007). The Ministry of Health, Labor, and Welfare (MHLW) divides foods into two groups: General Foods and Food with Health Claims (FHC). The Consumer Affairs Agency is in charge of FHC, which includes dietary supplements (Binns et al., 2018b). FHC can also be broken down into three subgroups:

- Foods with Nutrient Function Claims (FNFC) all foods that are labeled with the claims about how nutrients work that the MHLW says are allowed. For 17 ingredients, standards and specifications have been set to show what their nutritional function is (12 vitamins and 5 minerals). These foods can be made and sold without getting agreement from the government as long as they meet the standards and requirements (self-certification).
- Foods for Specified Health Uses (FOSHU) are foods that contain dietary ingredients that help the body's physiological functions to keep people healthy and improve certain health conditions. These need to be approved by each person and come in both traditional food forms and capsules and tablets (Tanaka, 2004).
- Foods with Function Claims: A new group of foods that came out in April 2015 and are labeled with their nutritional or health benefits so that clients can make better decisions.

2.6.4. Turkey and dietary supplements

In our country, there are two ministries used as intervention areas for the status of food supplements nutraceutical or nutritional supplements. These two ministries are the Ministry of Health and the Ministry of Agriculture and Rural Affairs. Each has their own responsibilities and controls:

1. Regulatory Department under the Ministry of Health is responsible for new drugs, Intermediate Products and Medical Equipment Branch Office.
2. Department of Economic Studies is responsible for General Directorate of Pharmaceuticals and Industrial Studies Branch.
3. Quality Control Department is responsible for Control Planning and Follow-up Branch.
4. Branch Directorates is responsible for Pharmaceuticals Security Monitoring and Evaluation (Üretimi, 2006).
5. In the Ministry of Agriculture and Rural Affairs, Food Codex, Domestic Food Control and Nutrition, Food Foreign Trade and Control Branch Offices affiliated to the Food Control Services Department, which works under the General Directorate of Protection Control, and in addition to these, Adana, Ankara, Antalya, Artvin, Balıkesir, Bursa , Çanakkale, Edirne, Eskişehir, Gaziantep, Giresun, Hatay, Mersin, İstanbul, İzmir, Kocaeli, Konya, Samsun, Tekirdağ, Trabzon Provincial Directorates of Agriculture are authorized to issue domestic production and import permits of nutraceuticals (Aşan, 2006) It is under the control of the Ministry of Agriculture.

In European Union countries, food supplements such as herbal products and vitamins are also sold in markets. However, even a very small portion of food products in Europe can get permission from the Ministry of Agriculture, which is not as easy as in our country (kasabalı., 2007).

2.7. HPLC

The pump, sampler, separation column, detector, and recording system are all components of the solvent distribution section of an HPLC device. According to the formation of the mobile phase in HPLC technique, there are two types of systems: isocratic and graduated elution. Isocratic elution is the elution of a mobile phase in stationary components using a single pump. If the k' values of the components to be separated are close, k' can be kept at the desired value by using a solvent or solvent mixture in the stationary component as the mobile phase. Graded elution is an elution with mobile phases with different polarities using more than one pump. It is impossible to find a single solvent for multicomponent samples with very different k' values. In this case, graduated elution is used. During the separation, solvents of different strengths are successively pumped into the column, from a predetermined and weakly eluting solvent to a strongly eluting solvent. Graded elution is usually achieved using solvent mixtures. Apart from this, some properties of the mobile phase can be changed, and gradual elution can be made. Some of these are changing the ionic strength, pH, taking advantage of the hydrogen bonding feature, changing the dielectric constant (Kmetec, 1992).

2.7.1. Capacity factor (k')

The capacity factor is a measure of a compound's residence time in the column $2,1056 \pm 0,0198$. Each component must be calculated separately. The retention time of the component not retained in the girth is the t_0 value used to determine the capacity factors of the standards. The capacity factor value of each standard in a good chromatography method should be in the range of $2 < k < 10$ (Dolan, 2017).

$$k' = \frac{t_r - t_0}{t_0} \quad (2.1)$$

t_r = Compound retention time.

t_0 = Retention time of the component not retained in the column.

2.7.2. Theoretical number of plates (N)

Theoretical plate number is a theoretical concept that determines column efficiency and is thought to consist of many plates adjacent to each other with enough areas to create complete equilibrium between the liquid and solid phases. A good chromatographic system should have more than 2000 theoretical plates that determine column performance and efficiency.

$$N = 16 \left(\frac{tr}{w} \right)^2 \quad (2.2)$$

2.7.3. Queuing and peak symmetry factors

A symmetrical peak has a tailing factor of the one. The tailing factor should be in the $0.5 \leq T \leq 2$ range, and the peak symmetry factor should be in the $0.5 \leq T \leq 1.5$ range (Dolan, 2017).

2.7.4. Selectivity (α) and separation factor (R)

The selectivity factor (α) is a measure of a chromatography system's ability to separate structurally related compounds (compounds A and B). The selectivity factor in a good chromatography method should be greater than one.

$$\alpha = \frac{trB - t_0}{trA - t_0} \quad (2.3)$$

The separation factor is a numerical value that represents the chromatographic separation of two compounds. The separation factor is calculated using the theoretical plate number, capacity factor, and selectivity factors. When the separation factor is 1.5, the two compounds are completely separated (Dolan, 2017).

$$R = \frac{\sqrt{N}}{4} \left(\frac{trB - t_0}{1 + K_b} \right) \left(\frac{\alpha - 1}{\alpha} \right) \quad (2.4)$$

2.7.5. Analytical method validation

Analytical method validation is the process of demonstrating that a method meets all of the requirements of the intended analytical application through laboratory studies.

2.7.6. Selectivity (matrix effect)

The separation of the substance to be separated from other substances or impurities in the analyzed mixture is referred to as selectivity. The signal of the substance to be analyzed in the working mixture solution determines the selectivity of an analytical method; it is determined by checking whether it interferes with the impurity, auxiliary, and solution signals. It is the most important validation parameter, and the first test to be performed while developing the method (Eurachem, 1998; FDA, 1987; USP, 2009).

2.7.7. Precision

The analytical method's precision is the parameter that indicates the similarity of the results of repeated samples collected from the same sample and prepared under the same conditions. Precision is a measure of random error that is calculated by calculating the standard deviation, variance, and relative standard deviation of repeat analytical findings from the same sample. Two types of research are used to determine precision: repeatability (system precision) and reproducibility (method precision) (Eurachem, 1998; FDA, 1987; USP, 2009).

2.7.8. Repeatability (system precision)

The repeatability parameter measures the performance of the equipment used. It is the calculation of the standard deviation to assess the similarity of measurement results obtained on the same day by the same researcher under the same measurement conditions (same devices and equipment, same laboratory conditions). It should be done at different concentrations (at least three) in the measuring range. At each concentration level, at least six repetitions should be performed. The standard deviation and relative standard deviation are then computed for each concentration.

2.7.9. Reproducibility (method precision)

It is the evaluation of a method's standard deviation in order to evaluate the similarity of measurement data obtained on different days across a vast time interval under diverse measurement conditions (different devices, equipment and chemicals and different working conditions). Trials are conducted at various concentrations within the measurement range.

2.7.10. Accuracy

The analytical method's accuracy is defined as the similarity of the analysis results tested with this method to the actual results. Accuracy is also known as recovery. The analytical procedure has been demonstrated to be correct over the given range. It is important to determine how much of the analyte determined in the sample medium is recovered in comparison to the pure analyte solution. The matrix is treated with standard solutions of three known concentrations.

It is recovered using the separation procedure and quantified by comparing it to a known amount (Eurachem, 1998; FDA, 1987; USP, 2009).

2.7.11. Lower limit of measurement (LOD) and lower concentration of determination (LOQ).

The analytical method's lower detection limit, the detection of the examined item, It is the smallest concentration at which it can be detected and quantified. Although there are several methods for calculating the LOD value, the signal-to-noise (S/N) ratio of 3 is commonly used for active substance analysis. It is calculated by diluting each active substance separately from the working standards, analyzing with the current method, and determining the concentrations where the signal/noise ratio is 3. The acceptance of the tested substances is the lower limit of concentration (LOQ) of an analytical method.

It is the smallest concentration that can be numerically measured with acceptable accuracy and precision. The approach that the S/N ratio should be 10 is commonly used to determine the LOQ. It is calculated by diluting each active substance separately from the working standards, analyzing with the current method, and determining the concentrations where the signal/noise ratio is 10 (Eurachem, 1998; FDA, 1987; USP, 2009).

2.7.12. Analytical range.

It is the work done to find the lower and upper concentration ranges where the analytical test method produces accurate, precise, and linear results. Linearity studies determine an analytical method's operational range. Linearity experiments should be carried out with at least five distinct concentrations, including upper and lower limits. Each concentration should be evaluated at least three times with the system. Linearity tests should be done on three different days (Eurachem, 1998; FDA, 1987; USP, 2009).

2.7.13. Solution stability

The conditions and period during which the standard and sample solutions created in the analytical procedure are stable are determined in the solution stability study. Standard and sample solutions are evaluated at various time intervals by storing them at room temperature and in the refrigerator, and the results are compared to the starting values. The time it takes for standard and sample solutions to be stable is measured in hours or days. The dwell time to choose is determined by the analysis. If the active component deteriorates throughout this period and storage circumstances, the method limits and specifies these parameters (Eurachem, 1998; FDA, 1987; USP, 2009).

2.7.14. Robustness analytics

An analytical method's robustness is a measure of method capacity that tries to assure method dependability throughout typical use with deliberate and minor modifications to method parameters. The establishment of the system fitness parameter as a result of evaluating the durability parameter ensures the validity of the analytical approach whenever it is applied. The outcomes are compared to the baseline values (before to the change), and the system fitness characteristics must be provided. For the robustness investigation, the following parameters might be adjusted (Eurachem, 1998; FDA, 1987; USP, 2009).

- Effect of changes in the pH of the mobile phase (± 0.2 pH)
- Effect of change in mobile phase buffer concentration ($\pm 10\%$)
- Mobile phase composition (percentage of organic solvent)
- Different columns (different lot and/or manufacturer)
- Column temperature ($\pm 10\%$) - Flow rate ($\pm 50\%$)

3. MATERIALS

In this thesis, the objective is to control the quality control of pharmaceutical tablets using a high-pressure liquid chromatography (HPLC) device, UV chromatography and Titration to develop appropriate sample preparation steps for analysis and to control the quality of vitamin C, the sample tablets are collected in the various content concentrations either alone or in compositions with other vitamins in the Turkish market. to the quality control tests required by USP-NS.

Table 3.1. *Vitamin C samples that collected from Turkish marketplace.*

Food supplements	Amount of Vitamin C (mg)		Ex.
S.tab 1.	1000	Water soluble	12.2024
S.tab 2.	1000	Water soluble & oil soluble with mineral	10.2023
S.tab 3.	30	Water soluble with mineral	12.2024
S.tab 4.	70	Water soluble & oil soluble with mineral	01.2025
S.tab 5.	500	Water soluble with mineral	08.2024

3.1. Chemicals

Table 3.2. *Chemicals used in thesis work.*

<u>Substance</u>	<u>Company</u>
Acetonitrile	CARLO ERBA Reagents, France
HCl	Sigma-Aldrich, Germany
Dimethyl sulphoxide (<i>cell culture grade</i>)	Sigma-Aldrich, Germany
Ethanol	Sigma-Aldrich, Germany
Iodine	Prolabo, Belgium
Methanol	Merck, USA
Monobasic potassium phosphate	Sigma-Aldrich, Germany
Ortho-phosphoric acid	Merck, USA
Phosphoric acid	Sigma-Aldrich, Germany
Potassium iodide	Sigma-Aldrich, Germany
Iodine 0.1 N	CARLO ERBA Reagents, france
Di-potassium hydrogen phosphate	Merck, USA
Disodium hydrogen phosphate	Sigma-Aldrich, Germany

3.2. Devices

Table 3.3. *Devices used in thesis work.*

<u>Device</u>	<u>Company</u>
Micropipette	Eppendorf, Germany
Pure Water Device	Millipore, Milli-Q Synthesis A10 Ultra- Pure, France
pH meter	Mettler Toledo SevenCompact S210-U, switzerland
Ultrasonic bath	Wisd Wuc A03H, South Korea
Vortex	Jeiotech VM-96B, Korea
Micropipette tip	Eppendorf, Germany
Vortex	Scilogex MX-S, Germany
Horizontal Shaker	MLW, Shuttler, Thys 2, Germany
Centrifuge	Eppendorf, Centrifuge 5417r, Germany
Carbon dioxide incubator	Hera Cell 240i, USA
Autoclave	Hirayama, Japan

Oven	Nüve FN 500, Turkey
High Performance Liquid Chromatography (HPLC)	SHIMADZU – 20 A, Japan
Column	Zorbax SB=C18 3.5 µm 4.6 * 100mm
UV – Spectrophotometer	Shimadzu UV 160A, Japonya
precision scale	Mettler Toledo XS 105, Switzerland
4 °C refrigerator	Arçelik No Frost 2002, Turkey
Magnetic Stirrer	IKA RT 15, Germany
Dissolution tester	Pharma test PTWS 820D, Germany
Disintegration tester	Sotax DT2, switzerland
Filtration membranes	Nylon 25 mm 0.22 µm
Syringe filter	25mm, PTFE 0.45 µm

4. METHODS

4.1. Preparation Of The Main Solutions Used In Thesis Work

- Prepared HCl 0.1 N solution: add 8.212 of HCl to 250 ml D.W. using measuring cylinder and made volume up to 1000 ml by D.W.
- Prepared 30 mM pH 3 monobasic potassium phosphate (KH_2PO_4) solution: weighed 4.083 g of monobasic potassium phosphate and dissolved in 1000 ml D.W. into the glass beaker. After mixing and degassing, adjusted the pH to 3 using phosphoric acid.
- Prepared pH 3.3 di-potassium hydrogen phosphate (K_2HPO_4) solution: Weighed 4.083 g of di-potassium hydrogen phosphate and solution in 1000 ml D.W. into the glass beaker. After mixing and degassing adjusted the pH to 3.3 using phosphoric acid.
- Prepared pH 3.3 disodium hydrogen phosphate 0.05 mol/L (Na_2HPO_4) solution: Weighed 7.1 g of di-potassium hydrogen phosphate and solution in 1000 ml D.W. into the glass beaker, after mixing, degassing, and filtering adjusted the pH to 3.3 using phosphoric acid.

4.2. Assay Methods

4.2.1. Titration

Titration is one of the oldest examination methods that depend on the chemical properties of materials, And USP refers to use iodometric titration for determination the amount of ascorbic acid (Davydova, 2010).

Preparing Solutions:

- Iodine Solution (standard solution): In 100 ml beaker placed a 2 g of potassium iodide and 100ml 0.1N iodine, made volume to 1000 ml by distilled water.
- Starch Indicator Solution: Weighed 0.25 g of starch, dissolved it in 50ml distilled water close to boiling point and completed the solution to 100 ml by DW, waited until it cools down before using it.
- Vitamin C Standard Solution: Weighed 1000 mg of standard Vit C, dissolved in 900 ml 0.1 N HCl
- Tablet sample solution: 1000 mg tab, dissolved in 900 ml of 0.1 HCl, and well mixed.
- Vit C standard and tablet sample was mixed with a few of indicator solution placed in 250 ml conical flasks to Titration the sample.

This method was worked on, and results were obtained, but for the lack of accuracy of the system due to practical errors that could occur, other analytical methods were relied upon. Where it worked as follows:

Table 4.1. *Titration Results.*

Stock	mL from stock	mL from starch	completed to by H₂O	Consumer of titrant	Color
s.t.	2	2	25	2,80	blue-black
tab	2	2	25	2,6	blue-black
s.t.	2	1	25	2,8	blue-black
tab	2	1	25	2,8	blue-black
s.t.	1	2	25	1,5	blue-black
tab	1	2	25	1,4	blue-black
s.t.	1	1	25	1,5	blue-black
tab	1	1	25	1,4	blue-black
s.t.	0.5	2	25	0,9	blue-black
tab	0.5	2	25	0.6	blue-black
s.t.	0.5	1	25	1	blue-black
tab	0.5	1	25	0.8	blue-black
s.t.	0.25	2	25	0.4	blue-black
tab	0.25	2	25	0,4	blue-black
s.t.	0.25	1	25	1	blue, yellow
tab	0.25	1	25	0.8	blue, yellow

This method of analysis was not relied upon due to the difficulty of analyzing samples with low concentration, their inaccuracy, the possibility of practical errors during the analysis and the lack of accurate color discrimination. It was noted that the blue color did not appear when a small amount of starch was added to the solution because the iodine ions did not find

starch to form the blue color complex and the color changes with changing the concentration of vitamins and starch.

4.2.2. UV chromatographic

Table 4.2. *Stander stock for UV chromatographic.*

	Concentration		weighing	Pipette	dilution	Stock to be used
	µg/mL	mg/mL	mg	mL	mL	
Stock I	1000	1	100	-	100	Weighing will be done
Stock II	100	0,1	-	10	100	Stock I

A. Analysis of Vitamin C using hydrochloric acid as a dilute solution.

- Stock 1: weighted 100 mg of stander Vit C and dissolved it in 100 ml of HCl 0.1 N mixing and degassing the solution in 100 mL Volumetric flask.
- Stock 2: take 10 ml of stock 1 and complete it to 100 ml with 0.1 N HCl mixing and degassing the solution in 100 volumetric flask (100 µg/ml).
- dilution solution for preparing the standard was 0.1 N HCl.
- REF.: was 265 NM

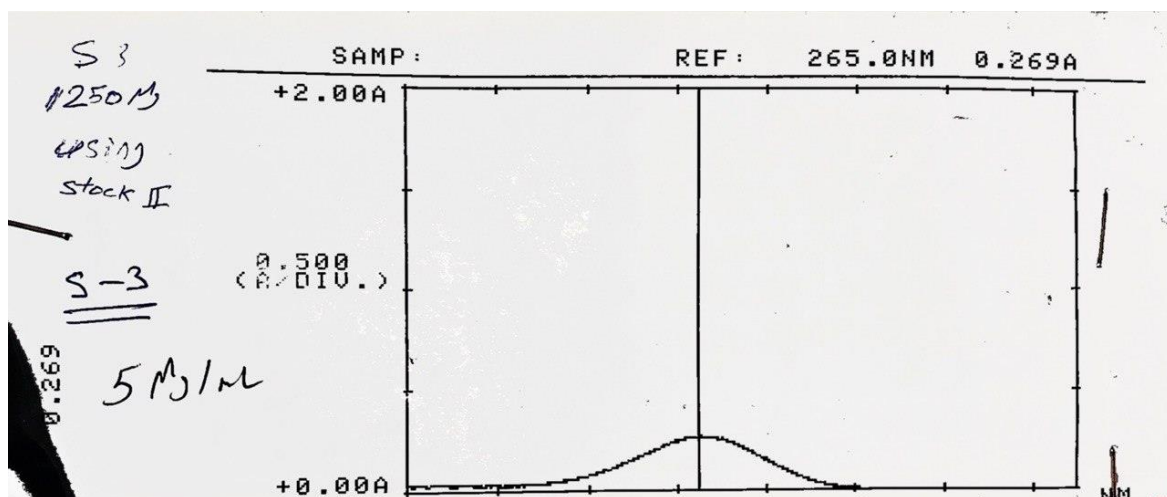


Figure 4.1. Uv Chromatogram of Vit C CONC. 5 µg/mL using HCl as dilution solution.

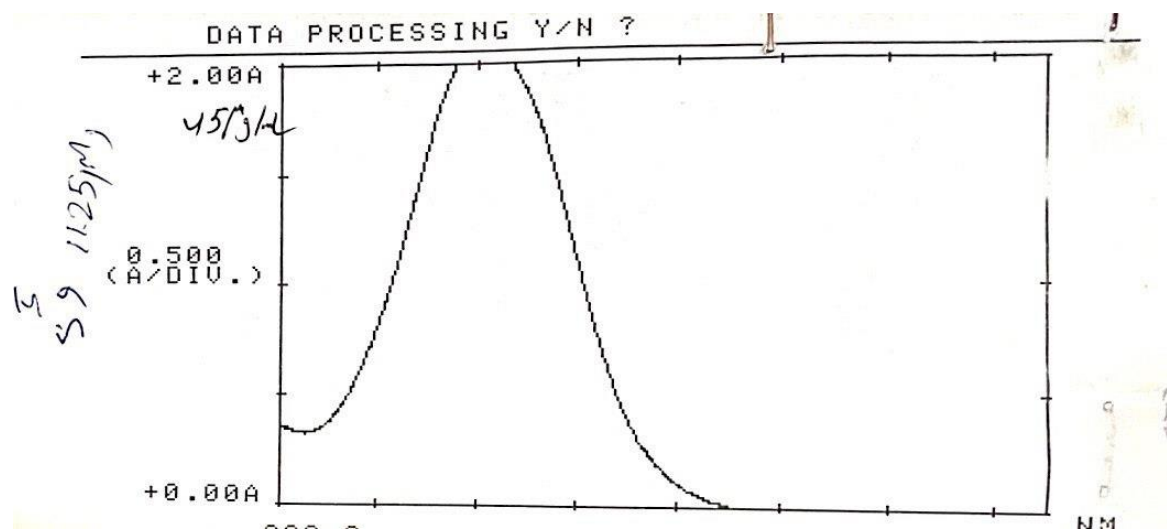


Figure 4.2. Uv Chromatogram of Vit C CONC.45 µg/mL using HCl as dilution solution.

➤ **Calibration test of Vit C using HCl as dilution solution 1.**

Table 4.1. Calibration of Vit C using HCl as dilution solution (1).

CONC.	2,5	5	7,5	10	15	20	25	30
Stock N.	2	2	2	2	2	2	2	2
μL stock	100	200	300	400	600	800	1000	1200
μL HCl 0.1N	3900	3800	3700	3600	3400	3200	3000	2800
Sample 1	0,112	0,224	0,354	0,482	0,741	0,999	1,217	1,372
Sample 2	0,124	0,250	0,349	0,471	0,738	1,002	1,234	1,431
Sample 3	0,114	0,305	0,348	0,469	0,732	1,031	1,216	1,449
Sample 4	0,108	0,251	0,335	0,464	0,732	1,164	1,206	1,407
Sample 5	0,142	0,243	0,355	0,469	0,723	1,006	1,252	1,418
Sample 6	0,144	0,238	0,401	0,457	0,755	0,958	1,181	1,461
AV.	0,124	0,252	0,357	0,469	0,737	1,027	1,218	1,423

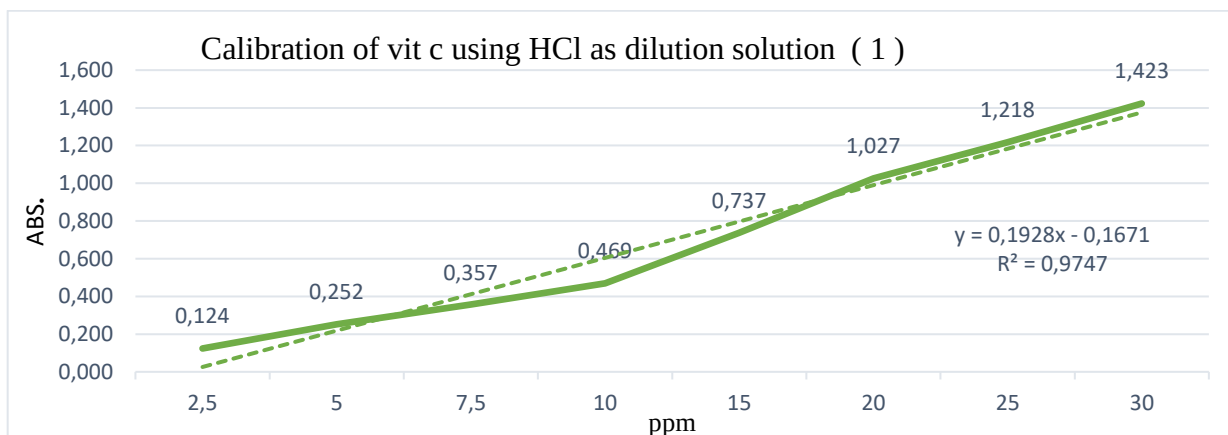


Figure 4.3. Calibration graphic of Vit C using HCl as dilution solution (1).

➤ **Calibration test of Vit C using HCl as dilution solution 2.**

Table 4.2. Calibration of Vit C using HCl as dilution solution (2).

CONC.	2,5	5	7,5	10	15	20	25	30
stock N.	2	2	2	2	2	2	2	2
μL stock	100	200	300	400	600	800	1000	1200
μL HCl 0.1N	3900	3800	3700	3600	3400	3200	3000	2800
sample 1	0,124	0,320	0,408	0,541	0,794	1,054	1,314	1,590
sample 2	0,129	0,278	0,401	0,536	0,809	1,068	1,319	1,596
sample 3	0,128	0,263	0,399	0,520	0,829	1,075	1,313	1,598
sample 4	0,133	0,262	0,397	0,526	0,802	1,073	1,320	1,573
sample 5	0,135	0,260	0,427	0,514	0,812	1,069	1,331	1,575
sample 6	0,150	0,260	0,386	0,520	0,801	1,068	1,327	1,447
AV.	0,133	0,274	0,403	0,526	0,808	1,068	1,321	1,563

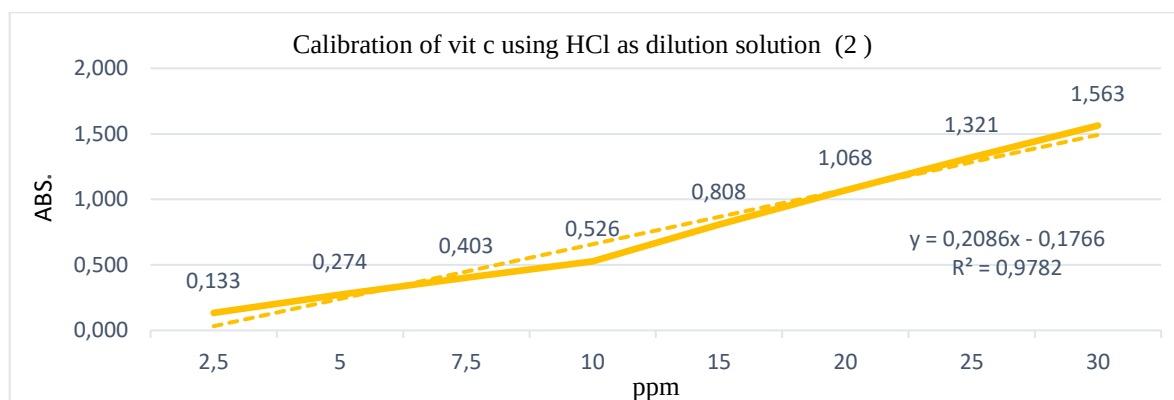


Figure 4.4. Calibration graphic of Vit C using HCl as dilution solution (2).

➤ **Calibration test of Vit C using HCl as dilution solution 3.**

Table 4. 3. Calibration of Vit C using HCl as dilution solution (3).

CONC.	2,5	5	7,5	10	15	20	25	30
Stock N.	2	2	2	2	2	2	2	2
μL stock	100	200	300	400	600	800	1000	1200
μL HCl 0.1N	3900	3800	3700	3600	3400	3200	3000	2800
Sample 1	0,157	0,292	0,411	0,556	0,821	1,082	1,350	1,595
Sample 2	0,147	0,297	0,418	0,559	0,821	1,098	1,354	1,602
Sample 3	0,187	0,293	0,420	0,555	0,824	1,090	1,358	1,603
Sample 4	0,157	0,293	0,438	0,552	0,833	1,097	1,343	1,619
Sample 5	0,158	0,295	0,436	0,549	0,827	1,099	1,357	1,576
Sample 6	0,157	0,294	0,439	0,543	0,833	1,096	1,359	1,588
AV.	0,161	0,294	0,427	0,552	0,827	1,094	1,354	1,597

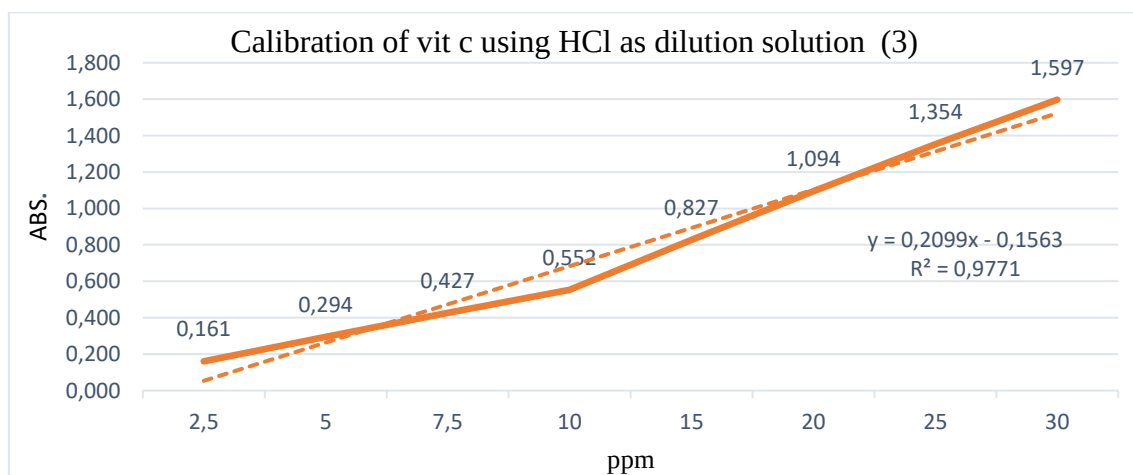


Figure 4.5. Calibration graphic of Vit C using HCl as dilution solution (3).

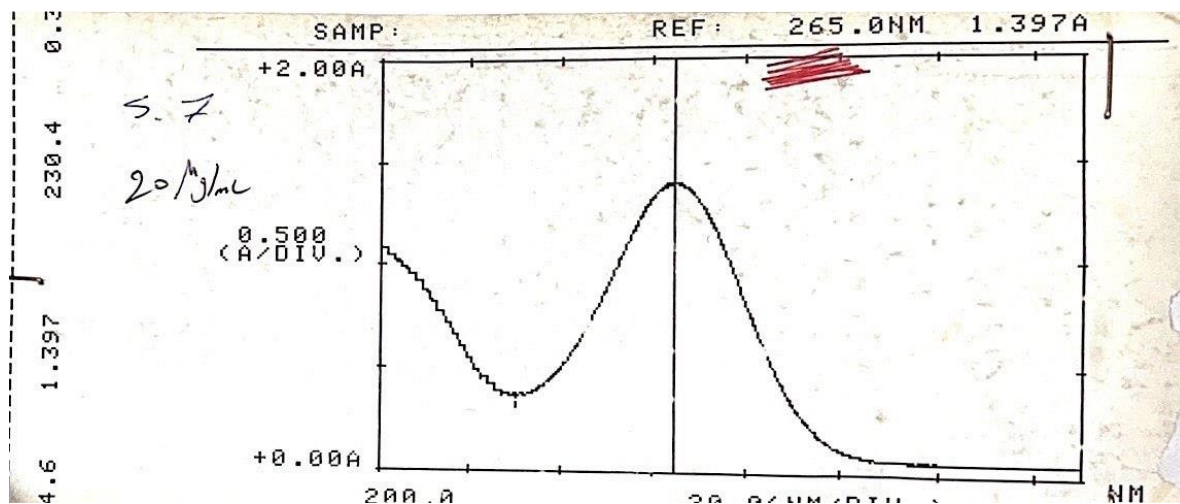


Figure 4.6. Uv Chromatogram of Vit C the peak in RFE 265 NM.

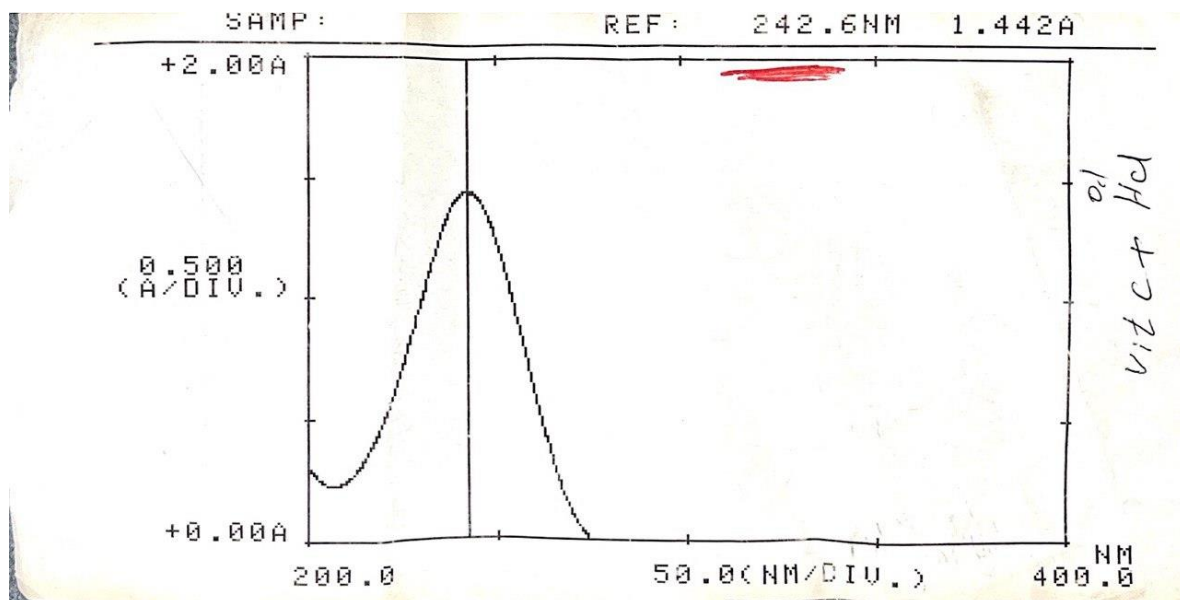


Figure 4.7. Uv Chromatogram of Vit C the peak in RFE 242 NM.

In the first attempts, HCl solution was used to prepare the samples from stock 1 & 2 to ensure that calibration of the method and find the work area, but we found when the concentration of vitamin C in the sample increased the peak change from 265 NM to 244 NM, as in seen in figure (4.7. & 4.8.), the UV absorption in 10 ppm concentration decreasing in every test, that's maybe happen because changing the acidity of the sample with increasing the vitamin C concentration, Therefore, the dilution solution was changed to K_2HPO_4 at the (pH 3) and test the calibration of the method with K_2HPO_4 dilution solution.

B. Vitamin C UV analyse using KH_2PO_4 pH 3 (98% and 2%) of HCl 0.1 N as dilute solution.

- Stock 1: weighted 100 mg of stander Vit C and dissolved it in 20 ml of HCl 0.1 N and 80 mL of KH_2PO_4 pH 3 mixing and degassing the solution in 100 mL Volumetric flask.
- Stock 2: took 10 ml of stock 1 and completed it to 100 ml with 98% of KH_2PO_4 at pH 3 and 2 % of 0.1 N HCl mixing and degassing the solution in Volumetric flask (100 $\mu\text{g}/\text{ml}$).
- dilution solution for preparing the standard was KH_2PO_4 pH 3 98% and 2% of HCl 0.1 N, the solution is prepared as follows mix 98 mL KH_2PO_4 at pH 3 with 2 mL of HCl 0.1 N.
- REF.: was 243 NM

- Calibration of Vit C using 98 % KHPO₄ (pH 3) and 2 % 0.1 N HCl dilution solution

Table 4.6. Calibration 1 of Vit C using 98 % KHPO₄ (pH 3) and 2 % 0.1 N HCl.

conc.	2,5	5	7,5	10	12,5	15
stock NO.	2	2	2	2	2	2
dissolution (μL)	625	1250	1875	2500	3125	3750
Total Volume (mL)	25	25	25	25	25	25
stander no.	S1	S2	S3	S4	S5	S6
abs.	0,136	0,275	0,406	0,541	0,675	0,812

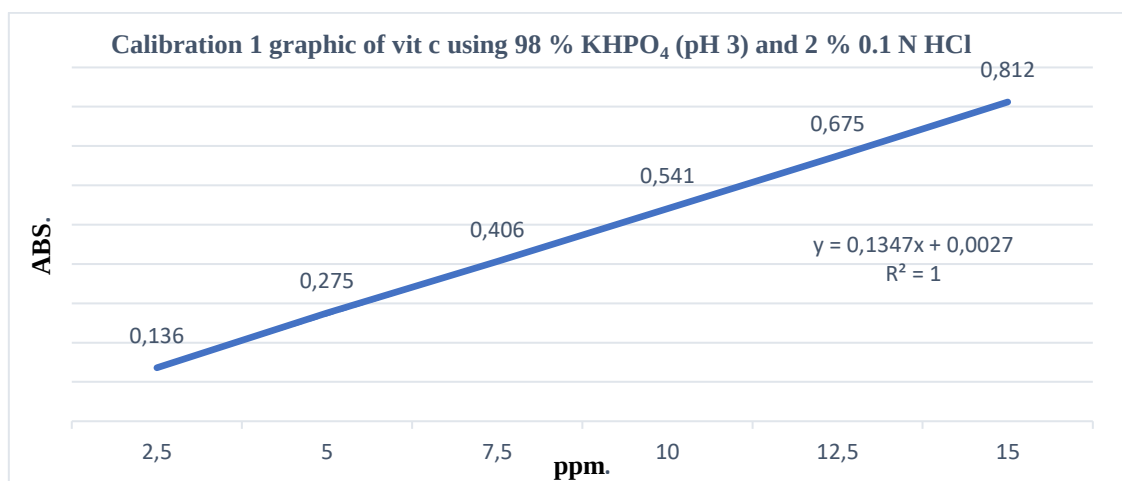


Figure 4.8. Calibration 1 graphic of Vit C using 98 % KHPO₄ (pH 3) and 2 % 0.1 N HCl.

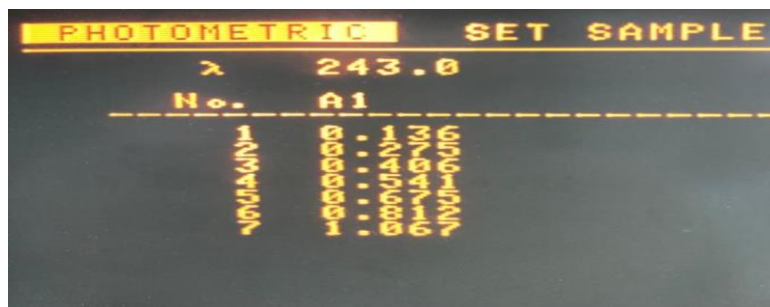


Figure 4.9. Uv abs. using 98 % KHPO₄ (pH 3) and 2 % 0.1 N HCl.

➤ Calibration (2) of Vit C using 98 % KHPO₄ (pH 3) and 2 % 0.1 N HCl dilution solution

Table 4.7. Calibration 2 of Vit C using 98 % KHPO₄ pH 3) and 2 % 0.1 N HCl.

conc.	2,5	5	7,5	10	12,5	15
stock	2	2	2	2	2	2
dissolution (μL)	625	1250	1875	2500	3125	3750
Total Volume (mL)	25	25	25	25	25	25
stander no.	S1	S2	S3	S4	S5	S6
abs.	0,178	0,296	0,446	0,577	0,711	0,851

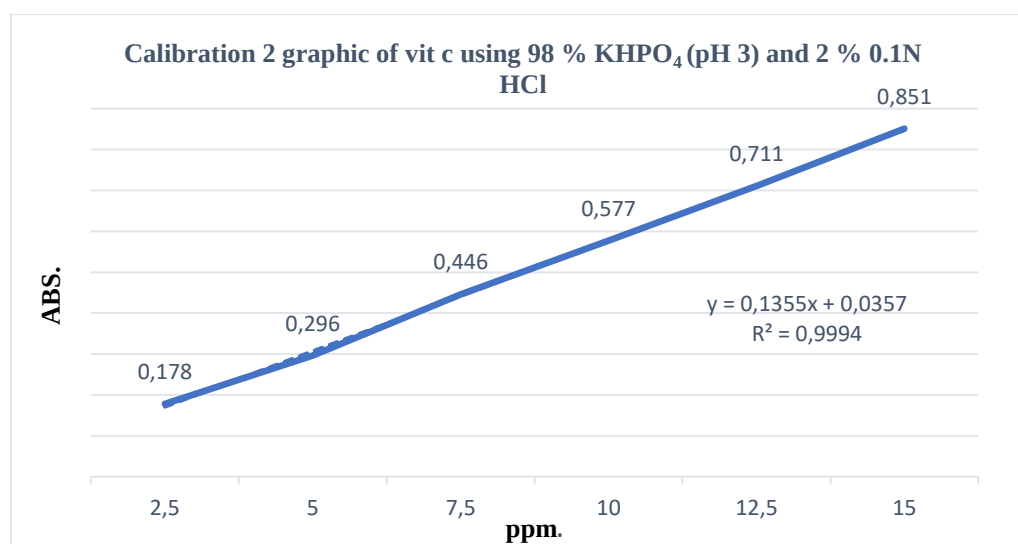


Figure 4.10. Calibration 2 graphic of Vit C using 98 % KHPO₄ (ph 3) and 2 % 0.1 N HCl.

4.2.3. HPLC-DAD chromatographic

4.2.3.1. *Methods were created for HPLC chromatographic analysis*

Before proceeding to sample analysis, chromatographic separation conditions for standard solutions were tried to be determined. a method was created for chromatographic analysis by applying different gradient programs. The separation methods applied for chromatographic analyzes are given below by numbering. After these applications, optimum chromatographic conditions were determined and applied to the samples

4.2.3.1.1. *HPLC method 1*

- Main Standard of Vit C: weighted 25 mg of stander Vit C and dissolved it in 100 ml of mobile phase mixing and degassing the solution in Volumetric flask.
- Ende Standard 2 of Vit C: Taken 2 ml of stander (1) to the Volumetric flask and completed it to 25 ml By HCl (0.1 N), The sample was mixed, degassed, and filtered before analysis.
- Blank: Taken 2 ml of mobile phase solution completed the volume to 25 ml with HCl (0.1 N) mixing and degassing the solution.

- **Chromatographic conditions of method 1.**

Mobile Phase A:	K ₂ HPO ₄ (pH:3.3)
Mobile Phase B:	Acetonitrile (100%)
Column:	Zorbax SB=C18 3.5 µm 4.6 * 100mm
Injection volume:	50 µl
Wavelength:	210 nm
Temperature:	20°C

Table 4.8. *Chromatographic conditions of method 1.*

Injection time, min.	Mobile Phase A, %	Mobile Phase B, %	Flow rate mL/min
5	80	20	1

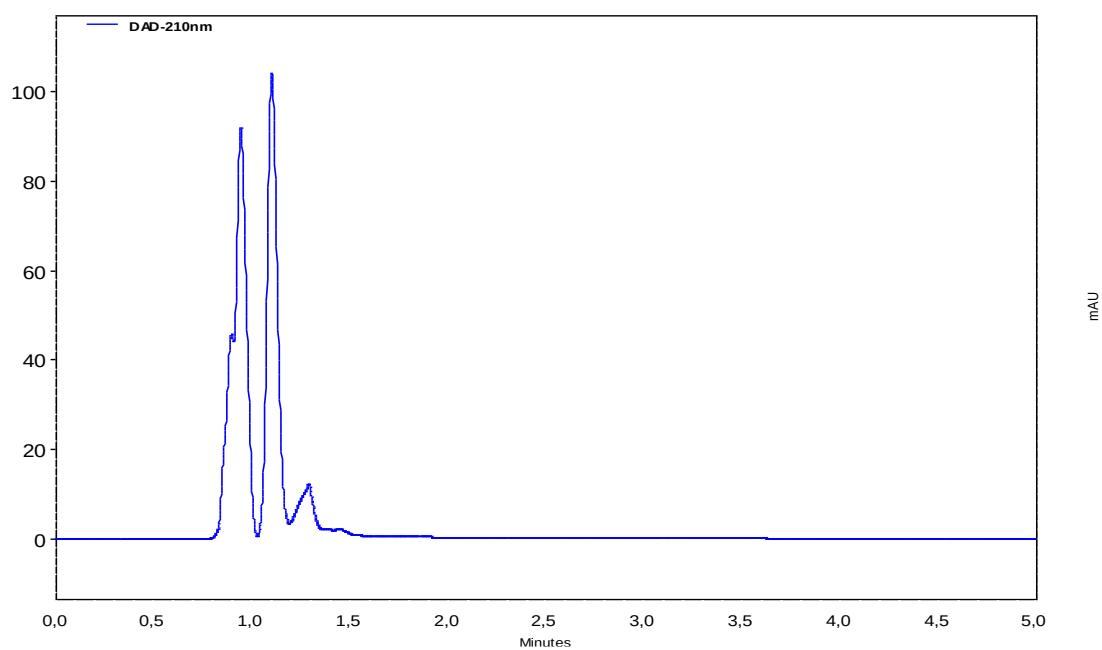


Figure 4. 11. *Chromatogram of method 1.*

4.2.3.1.2. HPLC method 2.

- Main Standard of Vit C: weighted 25 mg of stander Vit C and dissolved it in 100 ml of mobile phase mixing and degassing the solution in Volumetric flask.
- Ende Standard of Vit C: Taken 2 ml of stander (1) to the Volumetric flask and completed it to 25 ml By HCl (0.1 N), The sample was mixed, degassed, and filtered before analysis.
- Blank: Taken 2 ml of mobile phase solution completed the volume to 25 ml with HCl (0.1 N) mixing and degassing the solution.

- **Chromatographic conditions of method 2.**

Mobile Phase A:	K ₂ HPO ₄ (pH:3.3)
Mobile Phase B:	Acetonitrile (100%)
Column:	Zorbax SB=C18 3.5 µm 4.6 * 100mm
Injection volume:	50 µl
Wavelength:	210 nm
Temperature:	20°C

Table 4.9. *Chromatographic conditions of method 2.*

Injection time, min.	Mobile Phase A, %	Mobile Phase B, %	Flow rate mL/min
5	95	5	1

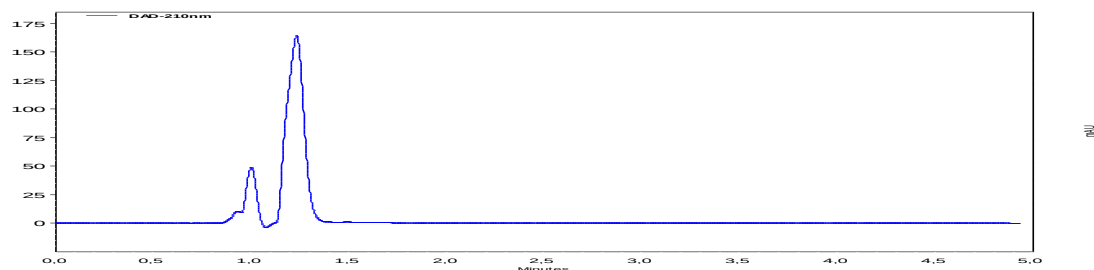


Figure 4 12. *Chromatogram of method 2.*

4.2.3.1.3. HPLC method 3

- Main Standard of Vit C: weighted 25 mg of stander Vit C and dissolved it in 100 ml of HCl 0.1 N mixing and degassing the solution in Volumetric flask.
- Ende Standard of Vit C: Taken 2 ml of stander (1) to the Volumetric flask and completed it to 25 ml by mobile phase, The sample was mixed, degassed and filtered before analysis.
- Blank: Taken 2 ml of 0.1 HCl solution completed the volume to 25 ml with Mobile phase mixing and degassing the solution.

Chromatographic conditions of Method 3.

Mobile Phase A:	K ₂ HPO ₄ (pH:3.3)
Mobile Phase B:	Acetonitrile (100%)
Column:	Zorbax SB=C18 3.5 µm 4.6 * 100mm
Injection volume:	50 µl
Wavelength:	210 nm
Temperature:	20°C

Table 4.10. *Chromatographic conditions of method 3.*

Injection time, min.	Mobile Phase A, %	Mobile Phase B, %	Flow rate mL/min
5	99	1	0.8

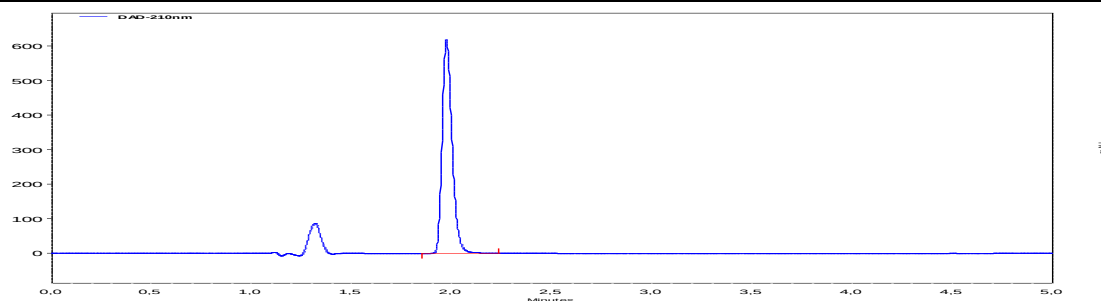


Figure 4.13. *Chromatogram of method 3.*

4.2.3.1.4. HPLC method 4

- Main Standard of Vit C: weighted 25 mg of stander Vit C and dissolved it in 100 ml of HCl 0.1 N mixing and degassing the solution in Volumetric flask.
- Ende Standard of Vit C: Taken 2 ml of stander (1) to the Volumetric flask and completed it to 25 ml by mobile phase, The sample was mixed, degassed and filtered before analysis.
- Blank: Taken 2 ml of 0.1 HCl solution completed the volume to 25 ml with Mobile phase mixing and degassing the solution.

- **Chromatographic conditions of Method 4.**

Mobile Phase A:	Na ₂ HPO ₄ (pH:3.3)
Mobile Phase B:	Acetonitrile (100%)
Column:	Zorbax SB=C18 3.5 µm 4.6 * 100mm
Injection volume:	50 µl
Wavelength:	210 nm
Temperature:	20°C

Table 4.11. *Chromatographic conditions of method 4.*

Injection time, min.	Mobile Phase A, %	Mobile Phase B, %	Flow rate mL/min
5	95	5	0.8

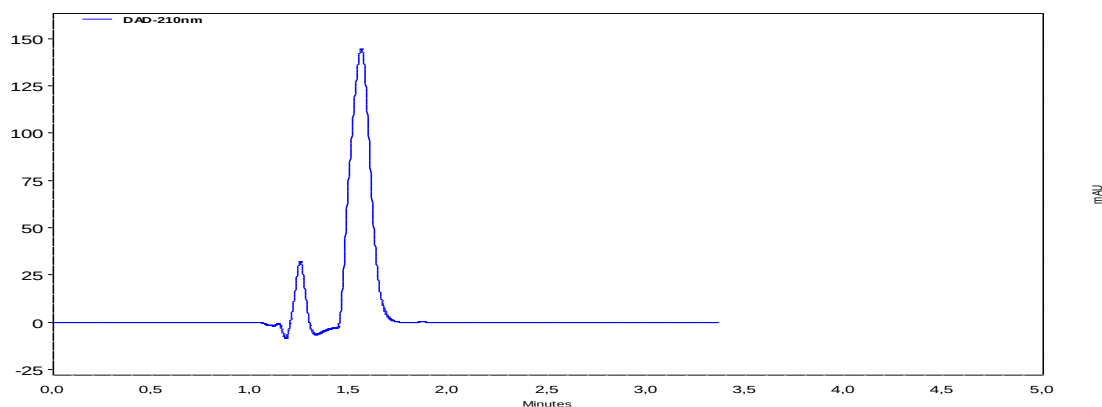


Figure 4.14. *Chromatogram of method 4.*

4.2.3.1.5. HPLC method 5

- Standard 1 of Vit C: weighted 25 mg of stander Vit C and dissolved it in 100 ml of HCl 0.1 N mixing and degassing the solution in Volumetric flask.
- Standard 2 of Vit C: Taken 2 ml of stander (1) to the Volumetric flask and completed it to 25 ml by mobile phase, The sample was mixed, degassed and filtered before analysis.
- Blank: Taken 2 ml of 0.1 HCl solution completed the volume to 25 ml with Mobile phase mixing and degassing the solution.

- **Chromatographic conditions of Method 5**

Mobile Phase A:	Na ₂ HPO ₄ (pH:3.3)
Mobile Phase B:	Acetonitrile (100%)
Column:	Zorbax SB=C18 3.5 µm 4.6 * 100mm
Injection volume:	50 µl
Wavelength:	210 nm
Temperature:	20°C

Table 4.12. *Chromatographic conditions of method 5.*

Injection time, min.	Mobile Phase A, %	Mobile Phase B, %	Flow rate mL/min
6	95	5	0.5

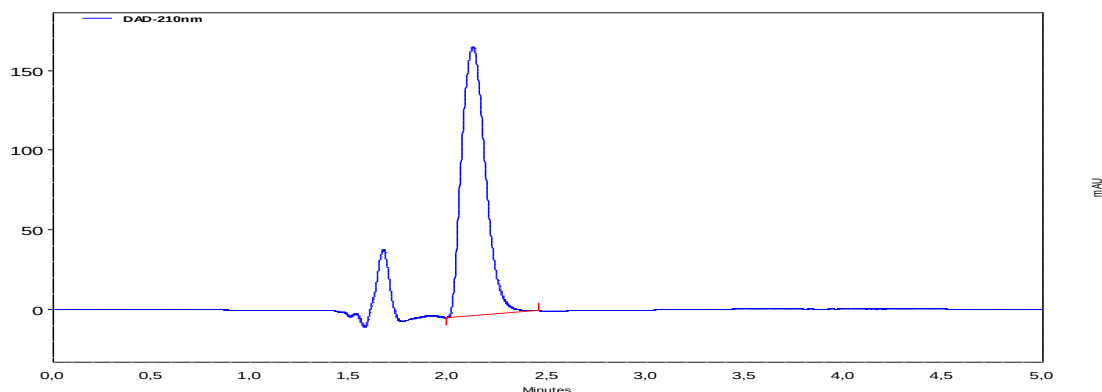


Figure 4.15. *Chromatogram of method 5.*

4.2.3.1.6. HPLC method 6

- Standard 1 of Vit C: weighted 25 mg of stander Vit C and dissolved it in 100 ml of HCl 0.1 N mixing and degassing the solution in Volumetric flask.
- Standard 2 of Vit C: Taken 2 ml of stander (1) to the Volumetric flask and completed it to 25 ml by mobile phase, The sample was mixed, degassed and filtered before analysis.
- Blank: Taken 2 ml of 0.1 HCl solution completed the volume to 25 ml with Mobile phase mixing and degassing the solution.

- **Chromatographic conditions of Method 6**

Mobile Phase A:	Na ₂ HPO ₄ (pH:3.3)
Mobile Phase B:	Acetonitrile (100%)
Column:	Zorbax SB=C18 3.5 µm 4.6 * 100mm
Injection volume:	50 µl
Wavelength:	210 nm
Temperature:	20°C

Table 4.13. *Chromatographic conditions of method 6.*

Injection time, min.	Mobile Phase A, %	Mobile Phase B, %	Flow rate mL/min
6	99	1	0.5

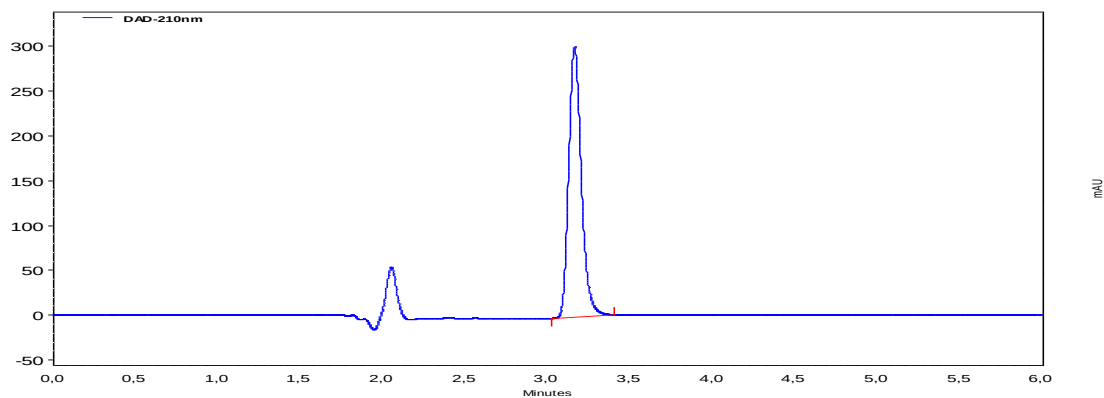


Figure 4.16. *Chromatogram of method 6.*

Optimum conditions for chromatographic separation by HPLC were determined by Method 4 and applied to the samples. Therefore, in the findings of the analysis of the samples, Method 4 was named as the "valid method".

4.2.3.2. Validation of HPLC-DAD chromatographic analyzes

➤ Validation of Chromatographic Analyzes

by applying the standard, valid method from the intermediate stock solutions (0.02 mg/mL), the preparation of which is described in Section 4.2.3.1.6. The validation results obtained are given below.

➤ Selectivity (α) and Separation Factor

A separation factor means that the two compounds are completely separated. In a good chromatography method, the selectivity factor should be $\alpha > 1$, which is provided by the applied gradient program. As we noted in Figure 4.17.

➤ Parameters of the Applied Analysis Method placebo (solvent) effect

used as HCl and Mobile Phase solvent, which will be analyzed for vitamin c. These components can affect the recovery efficiency in analysis and chromatographic separations in HPLC analysis. For this reason, the solvent effect must be taken into account in the analysis. In this study, to observe the solvent effect, HCl and Mobile Phase chromatographic analyzes without vitamin C were performed.

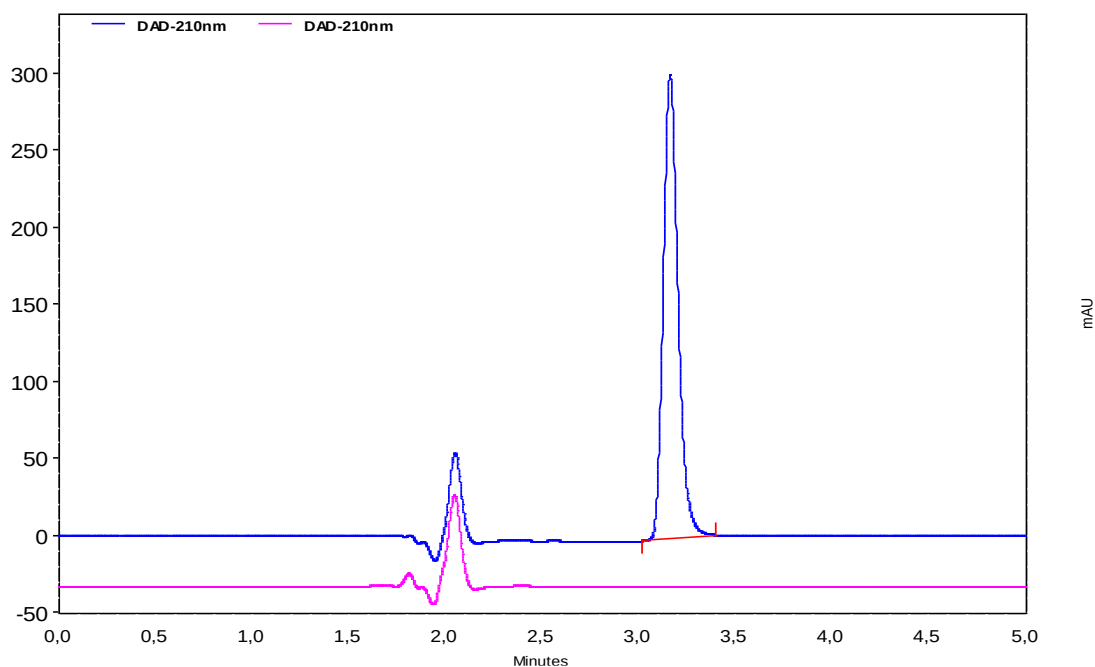


Figure 4. 17. *Chromatogram of placebo.*

As seen in figure 4.17. HCl and Mobile Phase solvent (purple segment) effects were not observed at the retention time or relative retention times in the chromatographic analyzes of standard substances (the blue segment).

➤ **The limit of detection (LOD):**

The lower limit of detection of an analytical method is the lowest concentration at which the analyzed substance can be detected and at which quantitative determination becomes difficult. In order to determine the LOD value of the HPLC analysis method, which was developed for the analysis of active substances, the approach that the signal to noise (S/N) ratio should be 3 was used. By making separate dilutions of each active ingredient from the 20 ppm, vitamin c working standards, HPLC analyzes were performed with the current method and the concentrations with a signal/noise ratio of 3 were determined. The determined LOD values are shown in table 4.14.

➤ **The limit of quantification (LOQ):**

The lower limit of quantification (LOQ) of an analytical method is the lowest concentration of the substances tested that can be measured numerically with acceptable accuracy and precision. For the analysis of active substances

The developed HPLC quantification method used the approach that the signal-to-noise (S/N) ratio should be 10 to determine the LOQ value. By making separate dilutions of each active ingredient from the working standards of 20 ppm, HPLC analyzes were performed with the current method, and the concentrations with a signal/noise ratio of 10 were determined and given in table 4.14.

Table 4.14. *Linear calibration range, LOD and LOQ analysis values of vitamin c.*

Xort LOD	Xort LOQ
0.051	0.015

- **Determining the working range with the current method:**

The linear operating range for HPLC analysis of the standard mixture solutions consisting of standard solutions of vitamin c standard at certain concentrations, with the current method, is given in table 4.13. In addition, standard solutions were prepared separately in the concentration range of the studied vitamin c (1, 5, 10, 20, 25, 30 ppm) according to the optimized separation conditions for HPLC analysis, and the calibration curves were created by considering the peak areas given by each concentration. Standard solutions were injected into the HPLC system 3 times and the linearity study was repeated for 3 days. The results obtained with the calibration curves of the standards are given in table 4.15. and figures 4.18, 4.19, and figure 4.20.

➤ **Determination of the Accuracy of the Analysis Method Applied:**

Data of calibration charts of vitamin C standard.

Table 4.15. *Data of HPLC calibration.*

DAY NO.	Calibration Equation ($y=mx+n$)	R2	% CV
Day 1	$y = 55178x - 5815,4$	0,9997	0,212
Day 2	$y = 55684x - 6642,1$	0,9996	0,192
Day 3	$y = 55667x - 12930$	0,9993	0,469

➤ **HPLC-DAD calibration Vitamin C standard day 1.**

Table 4.4. *HPLC-DAD calibration Vitamin C standard day 1.*

conc. (ppm)	Rep. 1.	Rep. 2.	Rep. 3.	AV.
1	63148	61295	62889	62444
5	254560	254565	256149	255091.3
10	539956	540433	540147	540178.7
20	1113342	1115104	1107319	1111922
25	1366968	1371956	1363444	1367456
30	1653761	1651181	1642674	1649205

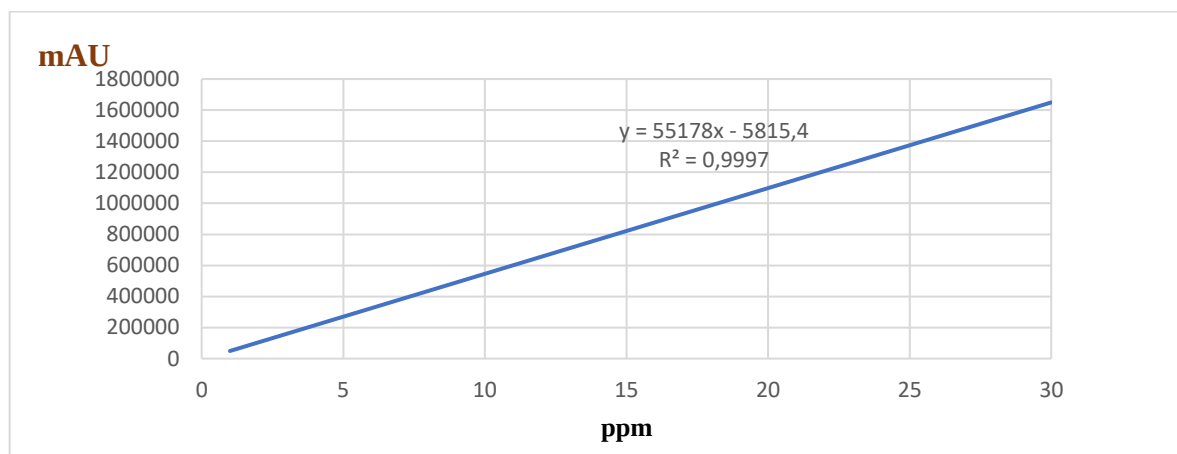


Figure 4.18. *Graphic OF day 1 HPLC Calibration.*

➤ **HPLC-DAD calibration Vitamin C standard day 2.**

Table 4.17. *HPLC-DAD calibration Vitamin C standard day 2.*

conc. (ppm)	Rep. 1.	Rep. 2.	Rep. 3.	AV.
1	45619	45363	45180	45387.33
5	273850	272759	272759	272757.7
10	565977	563695	561803	563825
20	1099838	1094824	1096524	1097062
25	1371105	1367907	1362392	1367135
30	1682329	1681115	1680101	1681182

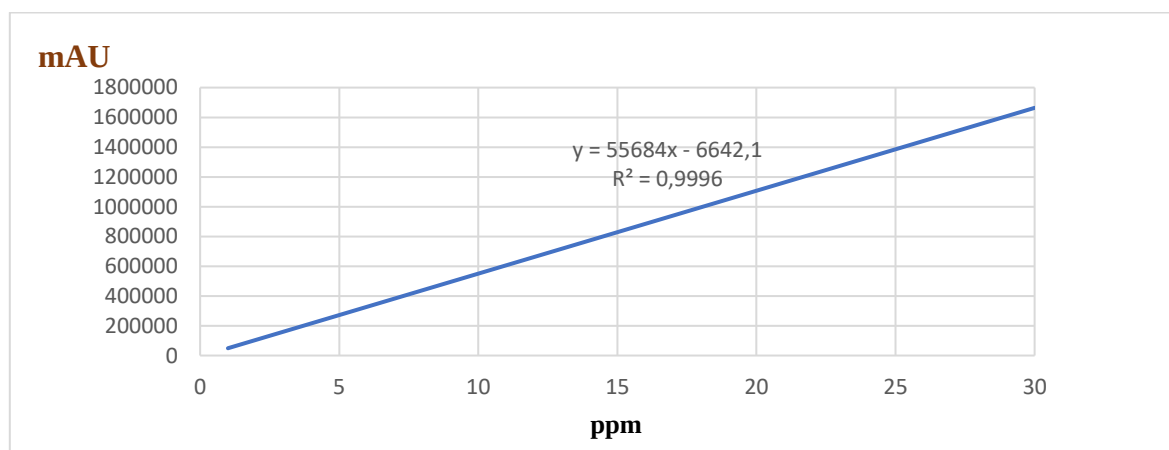


Figure 4.19. *Graphic of day 2 HPLC calibration.*

➤ **HPLC-DAD calibration Vitamin C standard day 3.**

Table 4.5. *HPLC-DAD calibration Vitamin C standard day 3.*

conc. (ppm)	Rep. 1.	Rep. 2.	Rep. 3.	AV.
1	45138	45827	44111	45025.33
5	262555	262074	260200	261609.7
10	553874	553604	551639	553039
20	1100922	1099224	1095871	1098672
25	1353467	1349672	1348891	1350677
30	1682652	1677383	1677139	1679058

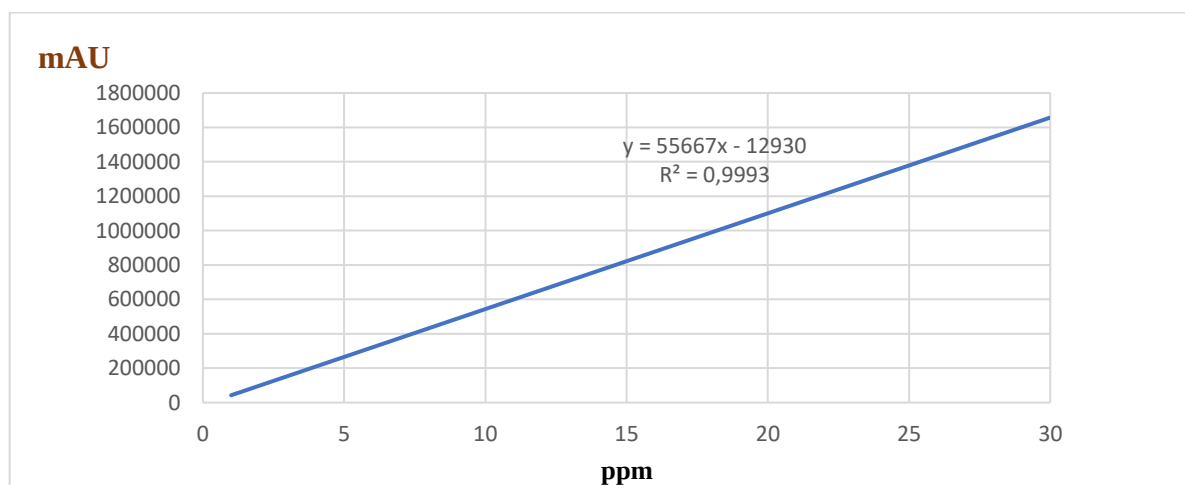


Figure 4.20. *Graphic Of day 3 HPLC calibration.*

➤ **Day 1 Intraday Repeatability**

Table 4.6. *Day 1 intraday repeatability.*

Rep. N	Rep. 1	Rep.2	Rep.3	Rep.4	Rep.5	Rep.6
Std. 20	1136173	1140962	1142088	1143223	1141203	1140161
ppm						
Av.	1140635					
Sd.	2423.742					
Rsd.	0.212491					

➤ **Day 2 Intraday Repeatability**

Table 4.20. *Day 2 intraday repeatability*

Rep. N	Rep. 1	Rep.2	Rep.3	Rep.4	Rep.5	Rep.6
Std. 20	1097832	1100824	1096524	1096229	1100973	1097161
ppm						
Av.	1098257					
Sd.	2119.625					
Rsd.	0.192999					

➤ Day 3 Intraday Repeatability

Table 4.21. Day 3 intraday repeatability

Rep. N	Rep. 1	Rep.2	Rep.3	Rep.4	Rep.5	Rep.6
Std. 20 ppm	1100032	1099928	1097798	1096811	1100673	1098975
Av.	1099036					
Sd.	1480.165					
Rsd.	0.134678					

➤ Robustness

It is a test done to determine how much the results are affected when changes are made on the method parameters intentionally.

The following modifications can be made in the liquid chromatography method:

- pH of mobile phase.
- temperature.
- Flow rate mL/min.

Table 4.22. *Pick time according to pH of mobile phase.*

pick time according to pH of mobile phase	
pH 3,1	3,03
PH 3,1	3,04
PH 3,1	3,04
PH 3,3	2,82
PH 3,3	2,81
PH 3,3	2,81
PH 3,5	2,89
PH 3,5	2,88
PH 3,5	2,89

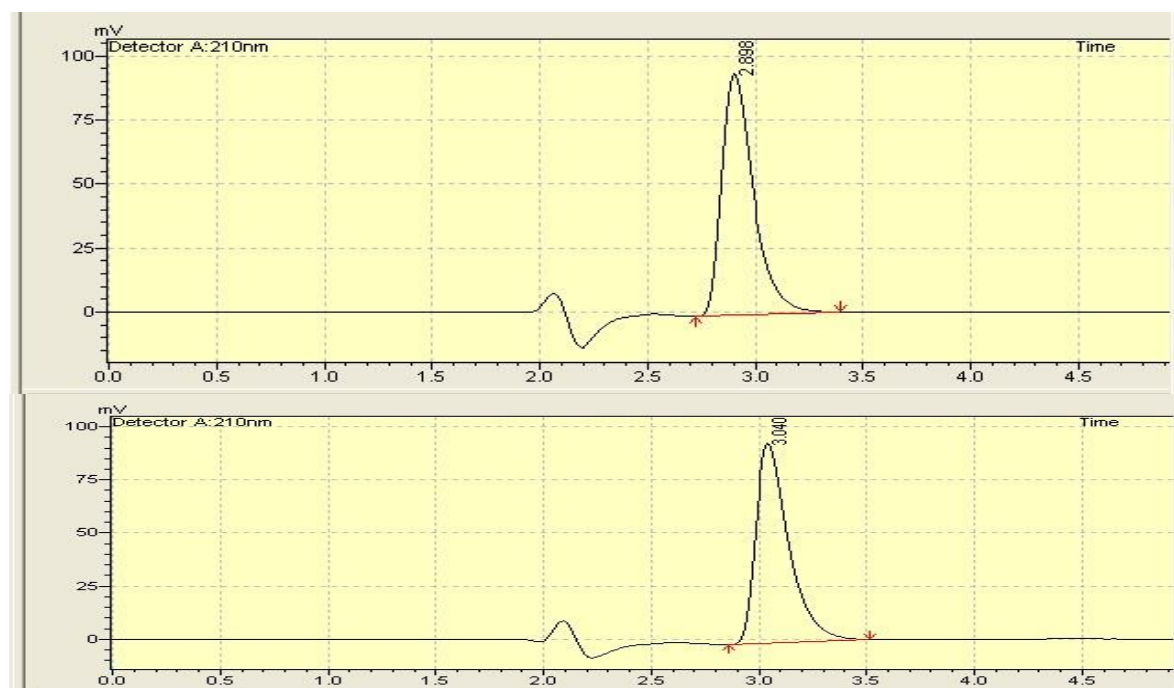


Figure 4. 21. *Result from HPLC for pick time according to pH of mobile phase.*

Table 4. 23. *Pick time according to temperature of HPLC colon.*

pick time according to temperature of HPLC colon	
15°C	3,23
15°C	3,23
15°C	3,23
25°C	2,84
25°C	2,82
25°C	2,87
40°C	2,78
40°C	2,78
40°C	2,78

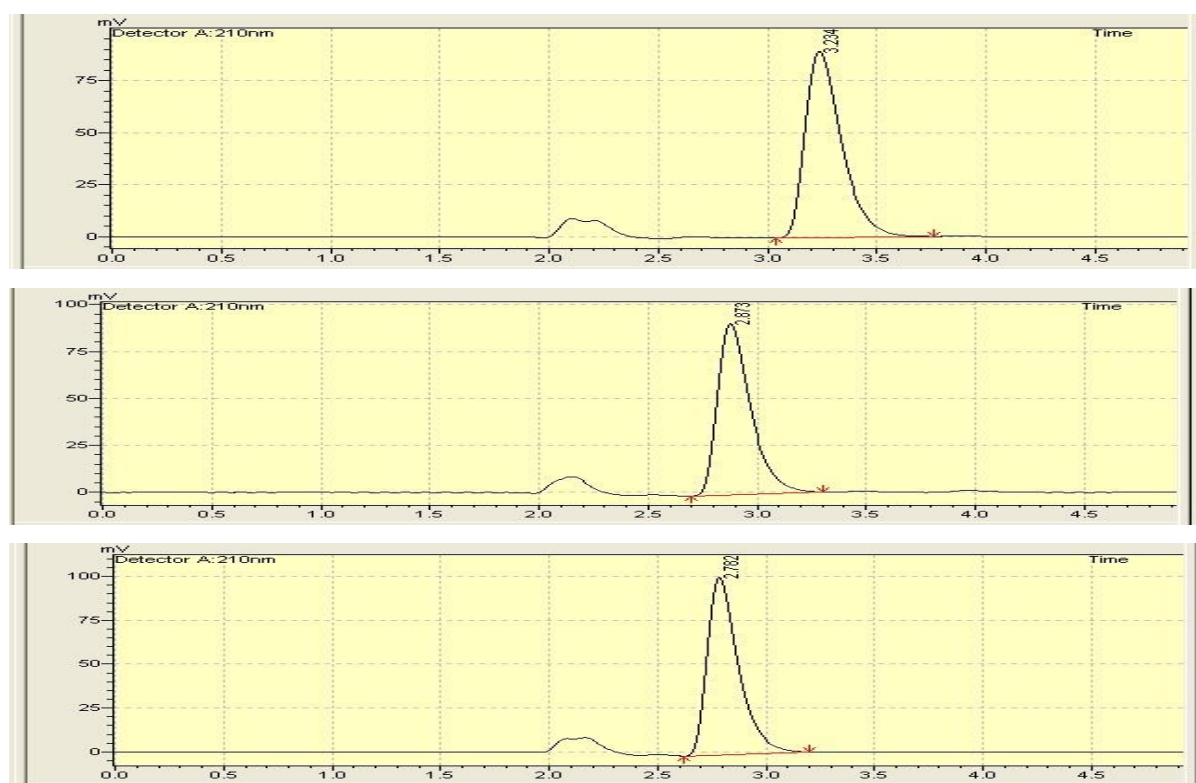


Figure 4. 22. *Result from HPLC for pick time according to temperature colon.*

Table 4. 24. *Pick time according to Flow rate mL/min.*

pick time according to Flow rate mL/min.	
0,4 mL/min	3,82
0,4 mL/min	3,81
0,4 mL/min	3,81
0,5 mL/min	2,82
0,5 mL/min	2,81
0,5 mL/min	2,81
0,6 mL/min	2,54
0,6 mL/min	2,54
0,6 mL/min	2,54

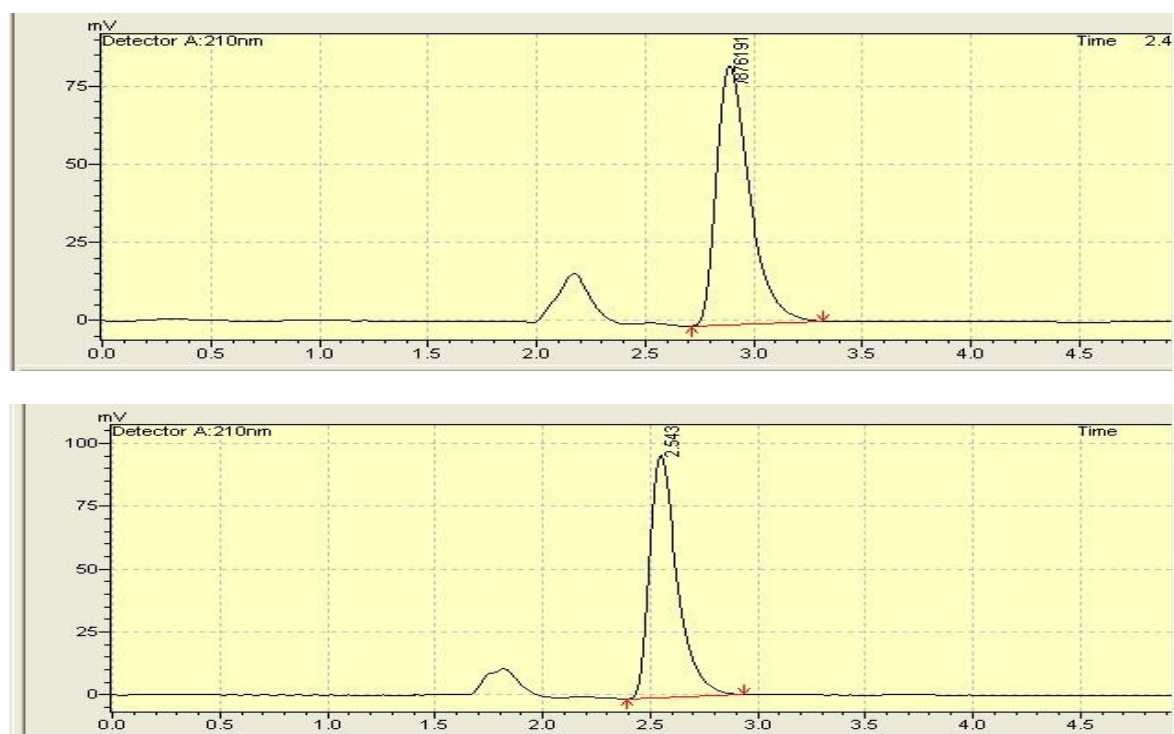


Figure 4. 23. *Result from HPLC for pick time according to Flow rate mL/min.*

4.3. Quality Control According To USP

4.3.1. Dissolution.

Before moving on to sample analysis, the method for analysis was created. After these applications, optimum chromatographic conditions were determined and applied to the samples.

Dissolution conditions:

- Medium: 0.1 N HCl; 900 mL.
- Time: 1 hour.
- Apparatus 2: 75 rpm.

Place 1 tab of the apparatus in 900 ml of 0.1N HCl at pH 1.2 medium, taking care to remove air bubbles from the surface of the dosage unit, and immediately operate the apparatus at rates of 75 rpm and (37°, 5°). Withdraw 5 ml of a specimen from a zone midway within 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 45 minutes, and 60 minutes, and replace the aliquots extracted for analysis with equivalent quantities of new Dissolution Medium at 37°.

These conditions are all according to USP, but we did an experiment for a longer time, up to 4 hours, because some of the brands did not dissolve perfectly.

Dissolution for 240 minutes: Samples were taken under the same conditions and samples were taken at the following time: 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 45 minutes, 60 minutes, 75 minutes, 90 minutes, 120 minutes, 150 minutes, 180 minutes, and 240 minutes.

Prepare the specimen that taken from dissolution media:

- first taking 5 ml from media and filtering the specimen, and replace the aliquots extracted for analysis with equivalent quantities of new Dissolution Medium at 37°.
- All sample taken to become the expected concentration at 0.02 µg/ml.
- For tablet that contain 1000 mg Vit C: take 0.09 ml from 5 mL specimen by micropipette and complete the solution with mobile phase to 5 ml, mixing and degassing, the end sample.
- For tablet that contain 500 mg vit C: take 0.18 ml from from 5 mL specimen by micropipette and complete the solution with mobile phase to 5 mL, mixing and degassing the end sample.
- For tablet that contain 70 mg Vit C: take 1.29 ml from from 5 mL specimen by micropipette and complete the solution with mobile phase to 5 mL, mixing and degassing the end sample.
- For tablet that contain 30 mg Vit C: For 30 mg tab, take 3 mL from from 5 mL specimen by micropipette and complete the solution with mobile phase to 5 mL, mixing and degassing the end sample.
- Samples were analyzed in HPLC with Chromatographic conditions that was mentioned.

4.3.2. Disintegration.

Place one tablet in each of the basket's six tubes (tablets are selected randomly), Place the basket rack in a 1-L beaker of 0.1N HCl at pH 1.2, Start the device (move the basket assembly carrying the tablets) and time how long it takes for all six tablets to disintegrate into particles and travel through the disintegration medium. The tablets should dissolve within 30 minutes (uncoated tablets). Disintegration time standards as low as 5 minutes, if one pill fails to dissolve within 30 minutes, the disintegration test is repeated on 12 additional tablets. Within 30 minutes, at least 16 of the 18 pills tested fully dissolve.

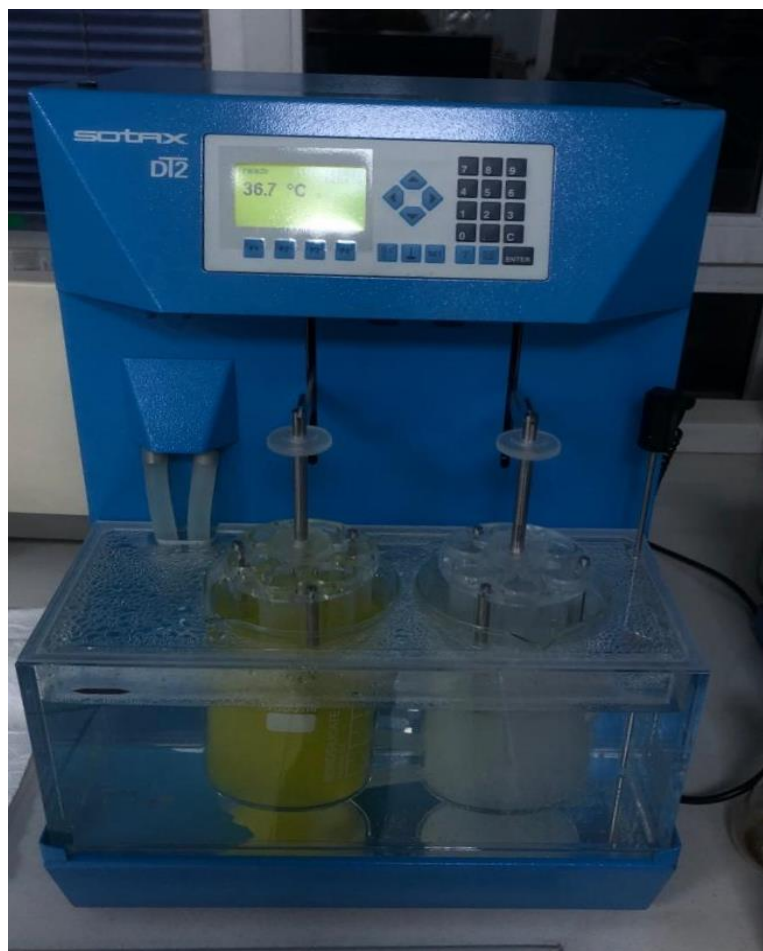


Figure 4.24. *Tablet disintegration tester.*

4.3.3. Uniformity content.

Official potency analysis procedures need taking a composite sample of pills, milling them up, mixing them, and analyzing them to get an average potency result. The official potency range for high dose medications in tablet form is not less than 95 percent and not more than 105 percent of the listed amount.

➤ **The Preparing of tablet to assay the content uniformity:**

- All sample taken to become the expected concentration at 0.02 µg/ml.
- For 1000 mg tab, dissolved and mix the tab within 500 ml of 0.1 HCl then take 0.5 ml from this solution by micropipette and complete the solution with mobile phase to 50 ml and mixing, degassing, and filtering the end solution.
- For 500 mg tab: For 500 mg tab, dissolved and mix the tab within 500 ml of 0.1N HCl then take 0.5 ml from this solution by micropipette and complete the solution with mobile phase to 25 ml and mixing, degassing, and filtering the end solution
- For 70 mg tab: For 70 mg tab, dissolved and mix the tab within 500 ml of 0.1N HCl then take 1.43 ml from this solution by micropipette and complete the solution with mobile phase to 10 ml and mixing, degassing, and filtering the end solution
- For 30 mg tab: For 30 mg tab, dissolved and mix the tab within 500 ml of 0.1N HCl then take 3.4 ml from this solution by micropipette and complete the solution with mobile phase to 10 ml and mixing, degassing, and filtering the end solution
- Samples were analyzed in HPLC with Chromatographic conditions that was mentioned.

4.3.4. Weight variation.

Each brand's tablets were chosen at random and individually weighed using an analytical balance, computing the average weight, and comparing the individual tablet weights to the average. If no more than two tablets are outside the percent restriction and no tablet differs by more than double that percentage limit, the tablets pass the USP test. The following formula was used to calculate the percentage of weight changes:

$$\% \text{ Of weight variations} = (\text{individual weight} - \text{Average weight} / \text{Average weight}) * 100$$

If the Average weight of tablets (mg) 130 or less the Maximum % difference allowed is ± 10 for 130 – 324 is ± 7.5 and for More than 324 Maximum % difference allowed is ± 5 .

4.3.5. Friability.

Conducted by individually measuring 10 tablets if it weighs more than 650mg if less weight the tablets until you reach 650mg, Place the tablets in the Roche friabilator drum and turn on the machine, adjusting the rotation to 100. and a speed of 25 rpm. At the conclusion of this procedure, take the tablets from the friabilator, dedust, and reweigh them (W). (Any broken tablet should be rejected before reweighing.) Friability is expressed as a percentage weight loss. The batch is acceptable when the value of friability is less than or equal to 1%.

$$\% \text{ Friability} = (\text{weight before test} - \text{weight after test} / \text{weight before test}) * 100.$$



Figure 4.25. Tablet friability.

4.3.6. Thickness and hardness.

Hardness: Using an Erweka hardness tester, test the batch of tablets provided. Determine the average crushing strength of ten pills (taken randomly). For uncoated tablets, a crushing strength of 4-8 Kg (40-80 N) is appropriate.

Thickness The thickness of each tablet can be measured with a micrometer. The thickness should be controlled within 5% of a defined value.



Figure 4.26. Thickness and hardness tester.

4.3.7. General appearance

Color: 1. Many pharmaceutical tablets rely on color for quick identification and customer acceptability.

2. The color of a product must be uniform inside a single tablet (this is known as 'mottling').

3. Nonuniformity of coloration not only lacks visual appeal, but it may also be connected with poor product quality.

Odor: The presence of odor in a batch of pills could suggest a problem with stability. The presence of an odor may be due to the medicine (vitamins), added substances (flavoring agents), or the dosing form (film-coated tablets).

5. RESULTS AND DISCUSSION

5.1. First Grope (S.tab 1) Quality Control Test Results.

Table 5.1. *Disintegration test S. Tab 1.*

Disintegration test	
Tablet No.	Disintegration Time
1.	In the range between 5-30 mint
2.	In the range between 5-30 mint
3.	In the range between 5-30 mint
4.	In the range between 5-30 mint
5.	In the range between 5-30 mint
6.	In the range between 5-30 mint

Table 5. 2. *Uniformity content for S. Tab 1*

Uniformity content test			
Table label amount		1000 mg	
Tablet No.	% Actual amount Compared with label amount	Tablet No.	% Actual amount Compared with label amount
1.	104,77	6.	103,74
2.	102,27	7.	106,88
3.	99,25	8.	100,06
4.	107,05	9.	107,66
5.	101,06	10.	102,32
AV.		103,5	
RSD (%)		2,91	

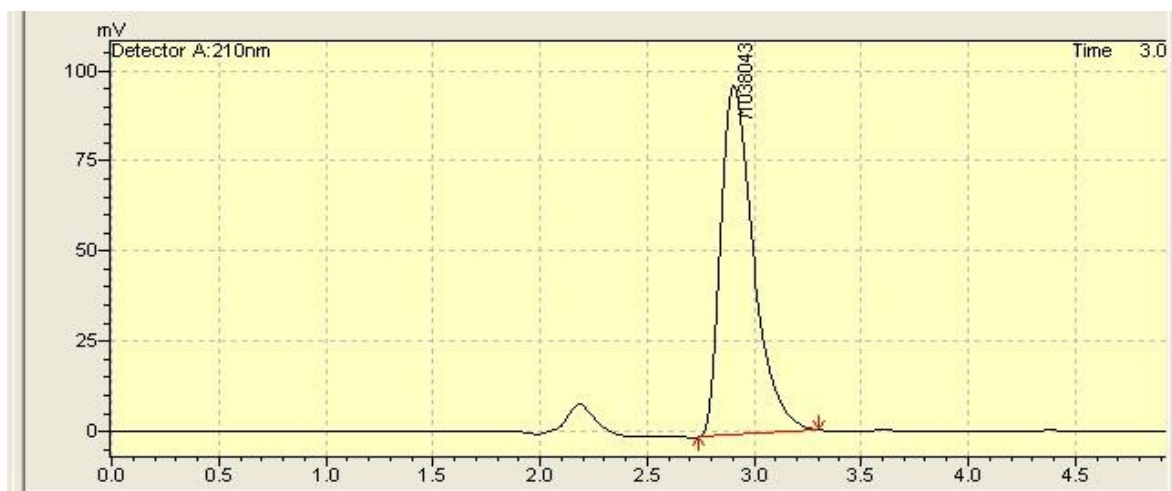


Figure 5. 1. Example of HPLC result in a uniformity content test.

Table 5.3. Dissolution test of S.tab 1 for 60 minutes.

Dissolution test		
Time (minute)	Average of dissolved according to libel amount (%)	RSD (%)
5	7,29	1,90
10	16,56	1,82
15	24,98	1,78
20	38,42	1,68
30	53,99	1,89
45	69,35	1,64
60	93,94	1,35

- The test was carried out on 6 randomly selected tablets, the average of the results was taken.

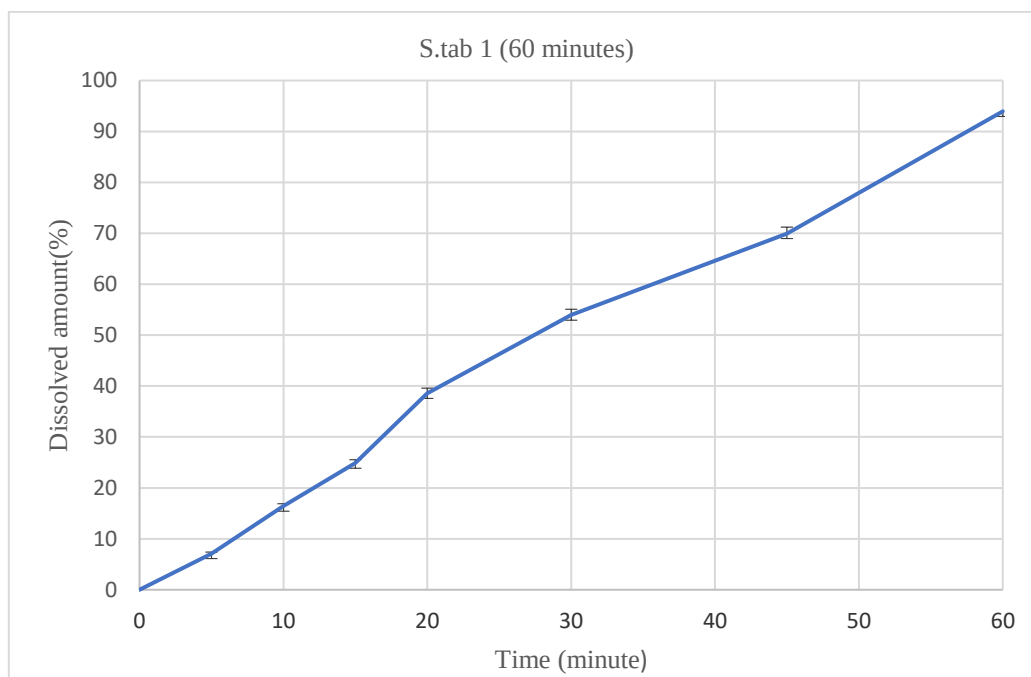


Figure 5. 2. *Dissolution 60 minute of s.tab 1.*

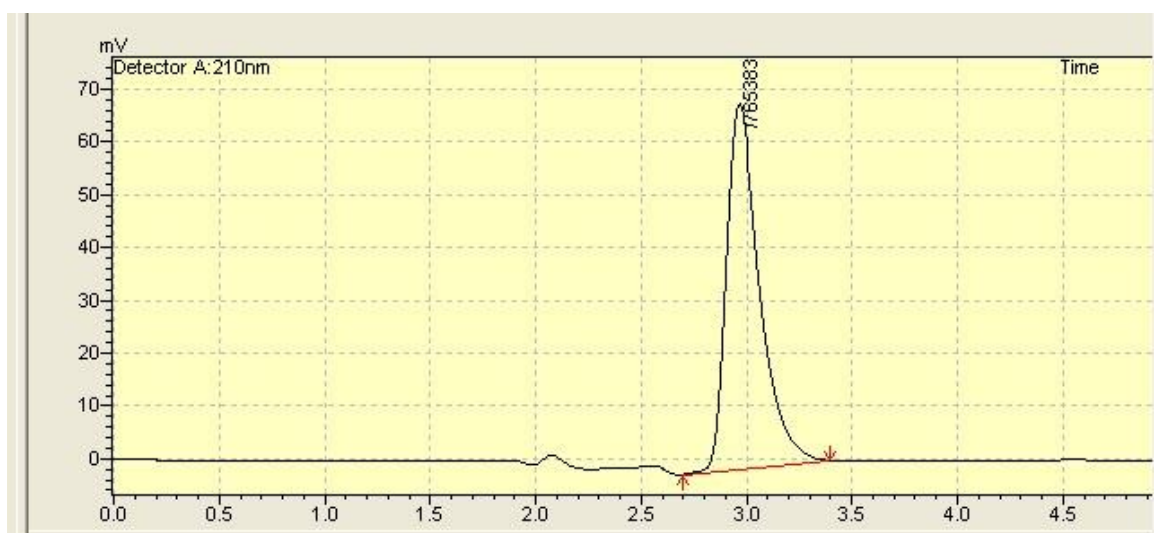


Figure 5. 3. *Example of HPLC result in a Dissolution content test in 45 minutes.*

Table 5. 4. *Dissolution test of S.tab 1 in 240 mintes.*

Dissolution test of S.tab 1 in 240 mintes		
Time (minute)	Average of dissolved according to libel amount (%)	RSD (%)
5	7,40	1,14
10	17,19	1,30
15	24,97	1,54
20	36,71	1,26
30	50,10	1,22
45	71,95	1,65
60	94,21	1,15
75	100,07	1,71
90	93,91	1,30
120	87,06	1,12
150	80,95	1,28
180	79,41	1,23
240	78,13	1,11

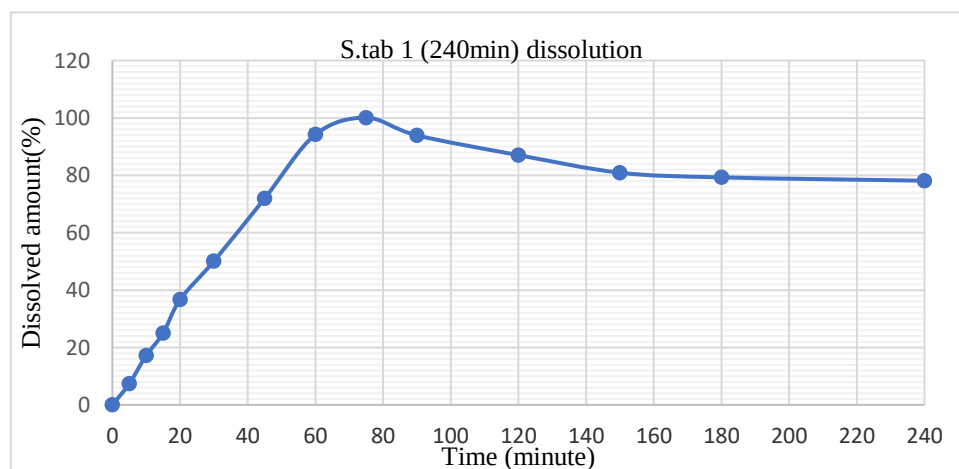


Figure 5. 4. *Dissolution 240 minutes of s.tab 1.*

- The test was carried out on 6 randomly selected tablets, the average of the results was taken

Table 5.5. *S.tab 1 Weight Variation.*

Weight Variation					
Tablet No	Weight (mg)	%	Tablet No	Weight (mg)	%
1.	1794,40	-0,23	11.	1781,84	-0,93
2.	1796,41	-0,12	12.	1793,15	-0,30
3.	1795,70	-0,16	13.	1790,88	-0,43
4.	1814,51	0,88	14.	1811,35	0,71
5.	1785,50	-0,73	15.	1787,62	-0,61
6.	1799,31	0,04	16.	1797,69	-0,05
7.	1830,53	1,77	17.	1799,67	0,06
8.	1790,91	-0,43	18.	1798,77	0,01
9.	1800,31	0,09	19.	1797,49	-0,06
10.	1810,40	0,66	20.	1795,80	-0,16
A.V.			1798,61		
SD			11,101		
RSD (%)			0,617		

Table 5. 6. *Diameter and hardness tests for S.Tab 1.*

Diameter and hardness tests for S.Tab 1							
Tablet No	width(mm)	%	Depth(mm)	%	length(mm)	%	Hardness
1.	23,16	-0,39	10,32	-0,58	7,77	-1,77	253,2
2.	23,30	0,22	10,38	0,00	7,98	0,88	>
3.	23,27	0,09	10,32	-0,58	7,89	-0,25	299,3
4.	23,26	0,04	10,42	0,39	7,91	0,00	292,2
5.	23,25	0,00	10,37	-0,10	7,87	-0,51	255,6
6.	23,24	-0,04	10,40	0,19	8,07	2,02	>
7.	23,22	-0,13	10,41	0,29	7,90	-0,13	>
8.	23,24	-0,04	10,32	-0,58	7,98	0,88	236,7
9.	23,30	0,22	10,42	0,39	7,85	-0,76	269,2
10.	23,25	0,00	10,41	0,29	7,92	0,01	>
AV.	23.25		10.38		7.91		
SD	0,040		0,042		0,082		
RSD (%)	1,174		0,409		1,037		

Table 5. 7. *Friability test of S.tab1.*

Friability test of S.tab1	
weight before test	18023,74
weight after test	18011,1
% Friability	0,07

5.2. Second Grope (S.tab 2) Quality Control Test Results.

Table 5. 8. *Disintegration test of S.tab 2.*

Disintegration test			
Tablet No.	Disintegration Time	Tablet No.	Disintegration Time
1.	More Thean 2 hr.	13.	More Thean 2 hr.
2.	More Thean 2 hr.	14.	More Thean 2 hr.
3.	More Thean 2 hr.	15.	More Thean 2 hr.
4.	More Thean 2 hr.	16.	More Thean 2 hr.
5.	More Thean 2 hr.	17.	More Thean 2 hr.
6.	More Thean 2 hr.	18.	More Thean 2 hr.
7.	More Thean 2 hr.	19.	More Thean 2 hr.
8.	More Thean 2 hr.	20.	More Thean 2 hr.
9.	More Thean 2 hr.	21.	More Thean 2 hr.
10.	More Thean 2 hr.	22.	More Thean 2 hr.
11.	More Thean 2 hr.	23.	More Thean 2 hr.
12.	More Thean 2 hr.	24.	More Thean 2 hr.

Table 5. 9. *Uniformity content test S.Tab 2.*

Uniformity content test	
Table label amount	1000 mg
Tablet No.	% Actual amount Compared with label amount
1.	108,27
2.	106,88
3.	107,05
4.	106,98
5.	105,92
6.	108,12
7.	107,41
8.	105,85
9.	106,94
10.	108,02
AV.	107,06
RSD (%)	0,786

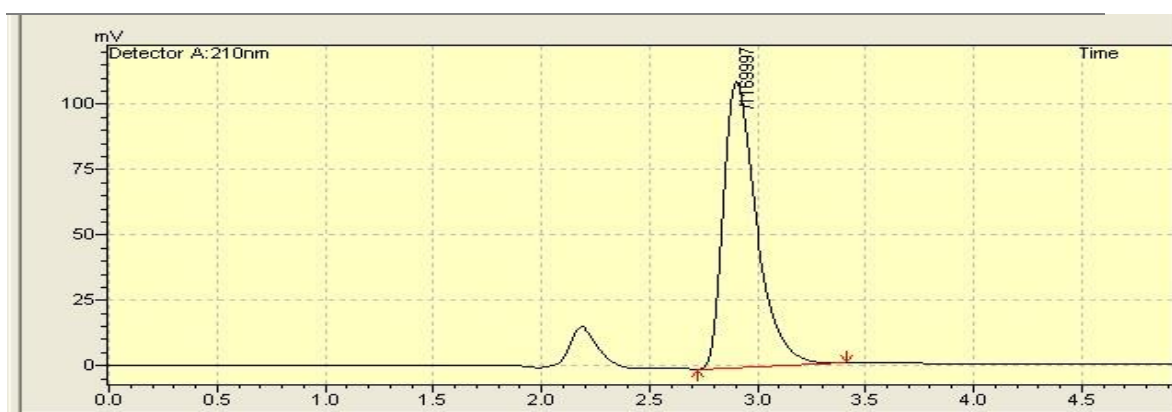


Figure 5. 5. *Example of HPLC result in a Uniformity content test for second group sample.*

Table 5.10. *Dissolution test of S.tab 2 for 60 minutes.*

Dissolution test of S.tab 2		
Time (minute)	Average of dissolved according to libel amount (%)	RSD (%)
5	6,87	2,00
10	9,43	2,32
15	13,99	4,84
20	17,22	4,38
30	25,99	5,43
45	38,85	5,74
60	44,37	3,68

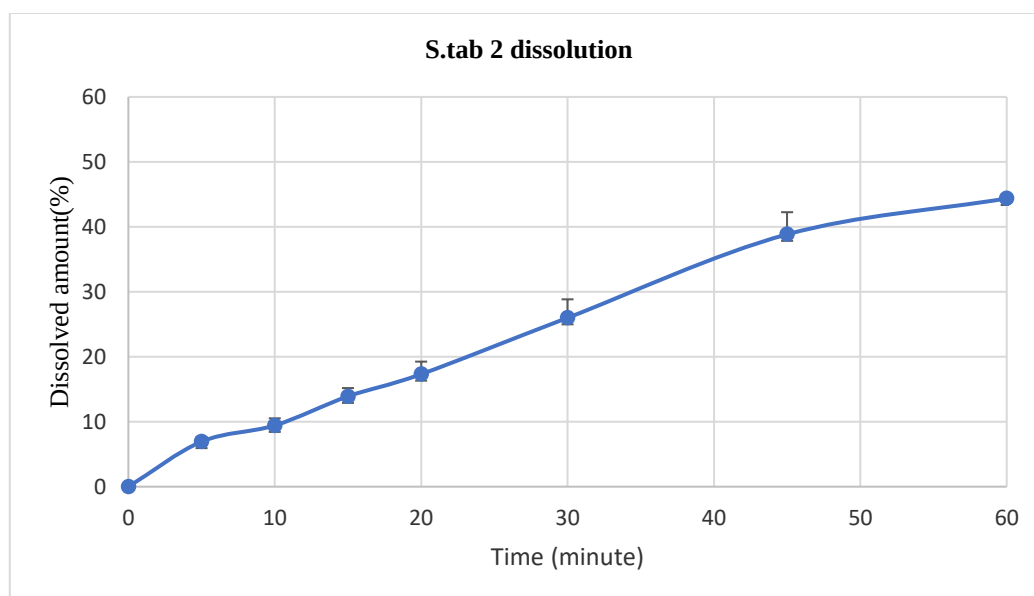


Figure 5. 6. *Dissolution test of S.tab 2 for 60 minutes.*

- The test was carried out on 12 randomly selected tablets, the average of the results was taken

Table 5. 11. *Dissolution test of S.tab 2 in 240 minutes.*

Dissolution test of S.tab 2 in 240 mintes		
Time (minute)	Average of dissolved according to libel amount (%)	RSD (%)
5	7,34	4,74
10	13,61	4,46
15	18,15	3,97
20	22,03	6,32
30	27,64	6,62
45	36,86	6,08
60	43,56	6,62
75	48,78	6,81
90	54,03	6,88
120	59,83	6,75
150	68,12	7,16
180	74,52	5,74
240	86,58	4,66

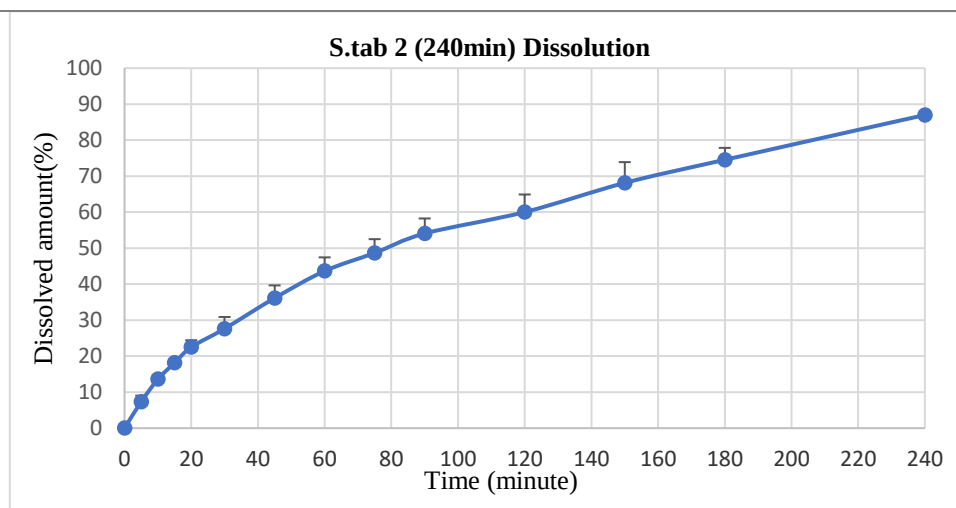


Figure 5. 7. *Dissolution test of S.tab 2 in 240 minutes.*

- The test was carried out on 6 randomly selected tablets, the average of the results was taken

Table 5. 12. *Weight Variation of S.tab 2.*

Weight Variation					
Tablet No	Weight (mg)	%	Tablet No	Weight (mg)	%
1.	1528,66	-0,23	11.	1516,18	-1,04
2.	1534,87	0,18	12.	1532,45	0,02
3.	1495,79	-2,37	13.	1516,05	-1,05
4.	1548,39	1,06	14.	1529,51	-0,17
5.	1539,40	0,47	15.	1534,69	0,17
6.	1529,73	-0,16	16.	1549,57	1,14
7.	1561,84	1,94	17.	1558,44	1,72
8.	1472,74	-3,88	18.	1511,88	-1,32
9.	1559,60	1,79	19.	1551,40	1,26
10.	1542,82	0,70	20.	1528,61	-0,23
A.V.			1532,13		
SD			22,075		
RSD (%)			1,441		

Table 5. 13. *Diameter and hardness tests for S.Tab 2.*

Diameter and hardness tests							
Tablet No	width(mm)	%	Depth(mm)	%	length(mm)	%	Hardness
1.	20,14	-0,35	11,58	-0,26	6,93	-1,98	100,3
2.	20,26	0,25	11,56	-0,43	7,07	0,00	125,1
3.	20,22	0,05	11,62	0,09	7,15	1,13	158,7
4.	20,22	0,05	11,67	0,52	7,11	0,57	106,4
5.	20,21	0,00	11,69	0,69	7,17	1,41	135,6
6.	20,22	0,05	11,59	-0,17	6,97	-1,41	153,7
7.	20,18	-0,15	11,56	-0,43	7,16	1,27	104,2
8.	20,24	0,15	11,64	0,26	7,09	0,28	134,2
9.	20,21	0,00	11,62	0,09	6,98	-1,27	152,6
10.	20,14	-0,35	11,58	-0,26	6,93	-1,98	100,3
AV.	20,21		11.61		7.07		
SD	0,039		0,046		0,090		
RSD (%)	0,195		0,399		1,267		

Table 5. 14. *Friability test of S.tab 2.*

Friability test	
weight before test	15336,82
weight after test	15193,75
% Friability	0,932

5.3. Third Grope (S.tab 3) Quality Control Test Results.

Table 5.15. *Disintegration test of S.tab3.*

Disintegration test			
Tablet No.	Disintegration Time	Tablet No.	Disintegration Time
1.	In 7 mints	13.	In 6,5 mints
2.	In 6 mints	14.	In 7 mints
3.	In 4 mints	15.	In 5 mints
4.	In 6.5 mints	16.	In 4.7 mints
5.	In 4 mints	17.	In 6 mints
6.	In 6 mints	18.	In 5 mints
7.	In 6 mints	19.	In 6 mints
8.	In 5 mints	20.	In 5,5 mints
9.	In 7 mints	21.	In 6 mints
10.	In 6.5 mints	22.	In 6 mints
11.	In 7 mints	23.	In 7,5 mints
12.	In 6 mints	24.	In 5 mints

Table 5. 16. *Uniformity content test of S.tab3.*

Uniformity content test	
Table label amount	30 mg
Tablet No.	% Actual amount Compared with label amount
1.	96,14
2.	103,02
3.	97,73
4.	101,27
5.	99,07
6.	99,729
7.	99,729
8.	99,729
9.	99,729
10.	99,729
AV.	99,729
RSD (%)	1,84

Table 5.17. *Dissolution test of S.tab3 in 60 minutes.*

Dissolution test of S.tab 3		
Time (minute)	Average of dissolved according to libel amount (%)	RSD (%)
5	41,73	2,04
10	87,25	2,08
15	97,03	2,15
20	102,63	1,30
30	100,80	1,93
45	100,42	1,76
60	99,64	1,57

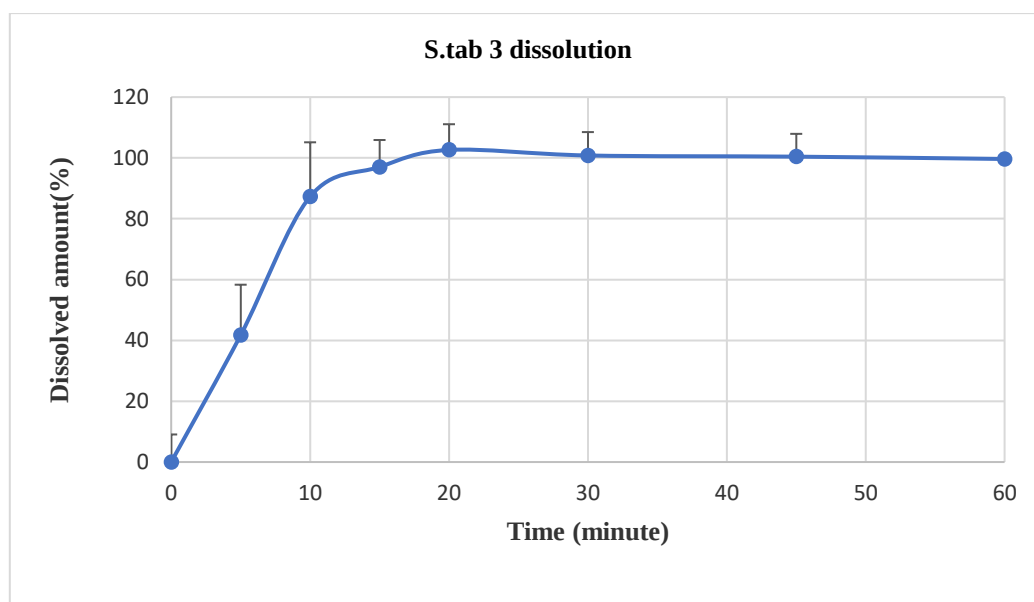


Figure 5. 8. *Dissolution test of S.tab3 in 60 minutes.*

- The test was carried out on 6 randomly selected tablets, the average of the results was taken

Table 5. 18. *Dissolution test of S.tab 3 in 240 minutes.*

Dissolution test of S.tab 3 in 240 mintes		
Time (minute)	Average of dissolved according to libel amount (%)	RSD (%)
5	33,98	1,24
10	61,22	1,98
15	74,95	1,97
20	84,26	1,32
30	99,47	1,49
45	96,81	2,09
60	93,53	1,12
75	90,88	1,17
90	84,81	1,08
120	80,26	1,21
150	76,73	1,16
180	71,30	1,94
240	64,49	1,26

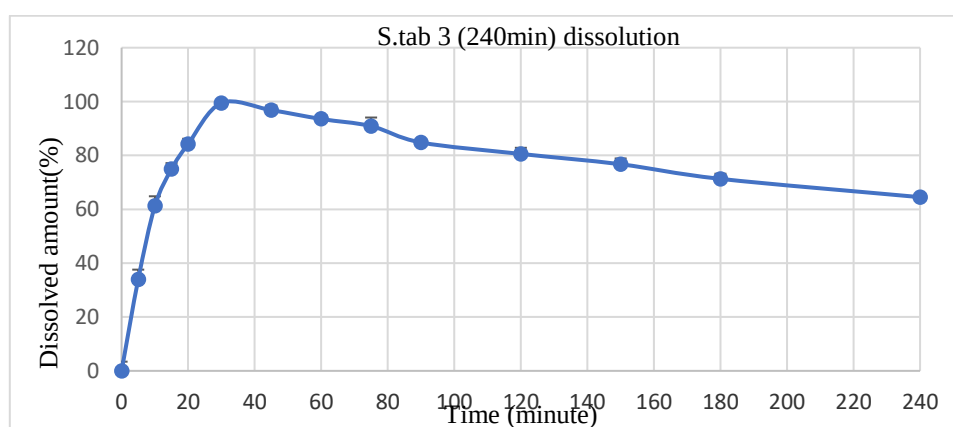


Figure 5. 9. *Dissolution test of S.tab 3 in 240 minutes.*

- The test was carried out on 6 randomly selected tablets, the average of the results was taken

Table 5. 19. *Weight variation of S.tab 3.*

Weight Variation					
Tablet No	Weight (mg)	%	Tablet No	Weight (mg)	%
1.	369,85	-0,03	11.	366,29	-0,99
2.	374,81	1,31	12.	367,53	-0,66
3.	371,40	0,39	13.	375,54	1,51
4.	371,75	0,48	14.	366,31	-0,99
5.	365,20	-1,29	15.	370,96	0,27
6.	370,05	0,02	16.	367,37	-0,70
7.	371,05	0,29	17.	369,14	-0,22
8.	371,87	0,51	18.	372,12	0,58
9.	369,04	-0,25	19.	370,94	0,26
10.	368,28	-0,46	20.	369,95	-0,01
A.V.			369,97		
SD			2,693		
RSD (%)			0,728		

Table 5. 20. *Diameter and hardness tests of S.tab 3.*

Diameter and hardness tests					
Tablet No	Width (mm)	%	Length (mm)	%	Hardness
1.	10,19	0,00	4,24	2,91	>300
2.	10,14	-0,49	4,09	-0,73	>300
3.	10,16	-0,29	4,09	-0,73	>300
4.	10,20	0,10	4,11	-0,24	>300
5.	10,23	0,39	4,16	0,97	>300
6.	10,21	0,20	4,05	-1,70	>300
7.	10,19	0,00	4,13	0,24	>300
8.	10,20	0,10	4,09	-0,73	>300
9.	10,18	-0,10	4,14	0,49	>300
10.	10,21	0,20	4,09	-0,73	>301
AV.	10,19		4,12		
SD	0,026		0,053		
RSD (%)	0,255		1,282		

Table 5. 21. *Friability test of S.tab3.*

Friability test	
Weight before test	6665,38
Weight after test	6663,94
% Friability	0,022

5.4. Fourth Grope (S.tab4) Quality Tests Results.

Table 5.22. *Disintegration test of S.tab 4.*

Disintegration test			
Tablet No.	Disintegration Time	Tablet No.	Disintegration Time
1.	1 mint	13.	1.5 mint
2.	2 mints	14.	1 mint
3.	1 mint	15.	2 mints
4.	1.5 mint	16.	1 mint
5.	1 mint	17.	1.5 mint
6.	2 mints	18.	1,8 mints
7.	1 mint	19.	2 mints
8.	2 mints	20.	1 mint
9.	2 mints	21.	1.5 mint
10.	1 mint	22.	2 mints
11.	1.5 mint	23.	2 mints
12.	1 mint	24.	1.5 mint

Table 5.23. *Uniformity content test of S.tab4.*

Uniformity content test	
Table label amount	70 mg
Tablet No.	% Actual amount Compared with label amount
1.	75,52
2.	78,76
3.	84,21
4.	76,06
5.	73,86
6.	72,35
7.	76,52
8.	75,81
9.	81,70
10.	73,34
AV.	76,81
RSD (%)	4,879

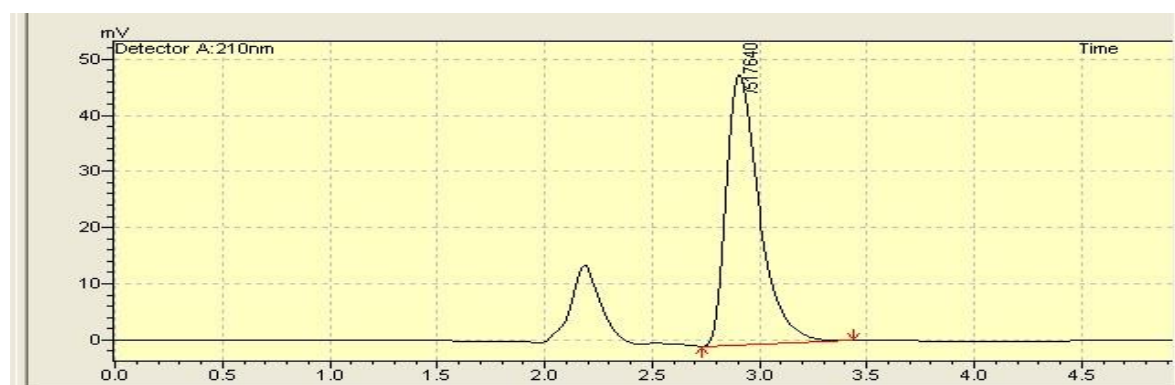


Figure 5. 10. *Example of HPLC result in a Uniformity content test for Fourth group sample.*

Table 5. 24. *Dissolution test of S.tab 4.*

Dissolution test of S.tab 4		
Time (minute)	Average of dissolved according to libel amount (%)	RSD (%)
5	73,76	7,24
10	75,18	7,39
15	70,97	7,37
20	68,95	7,22
30	67,15	7,64
45	65,19	7,22
60	64,47	7,18

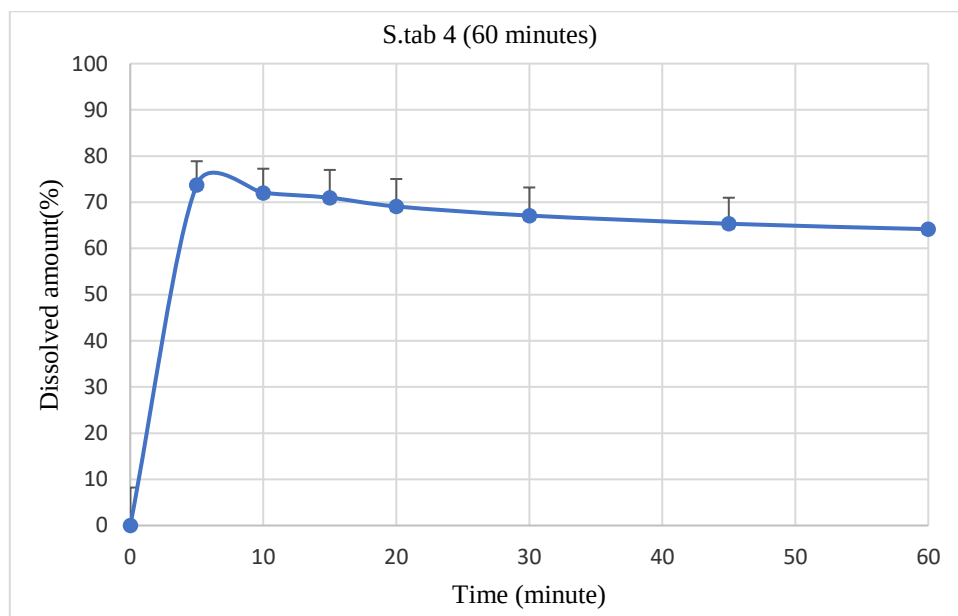


Figure 5. 11. *Dissolution test of S.tab 4 in 60 minutes.*

- The test was carried out on 12 randomly selected tablets, the average of the results was taken

Table 5. 25. *Dissolution test of S.tab 4 in 240 mintes.*

Dissolution test of S.tab 4 in 240 mintes		
Time (minute)	Average of dissolved according to libel amount (%)	RSD (%)
5	71,56	7,74
10	77,94	7,56
15	75,77	4,39
20	73,03	4,42
30	71,34	6,74
45	67,52	4,02
60	66,57	5,13
75	59,71	7,74
90	55,02	7,98
120	52,80	6,11
150	51,16	6,65
180	48,25	7,79
240	44,38	7,06

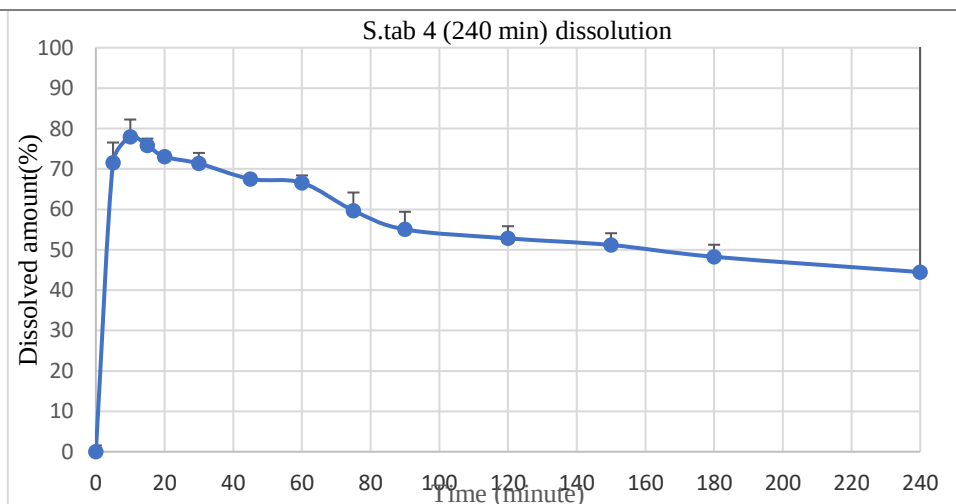


Figure 5. 12. *Dissolution test of S.tab 4 in 240 mintes.*

- The test was carried out on 6 randomly selected tablets, the average of the results was taken

Table 5. 26. *Weight variation of S.tab 4.*

Weight Variation					
Tablet No	Weight (mg)	%	Tablet No	Weight (mg)	%
1.	760,04	3,38	11.	713,59	-2,94
2.	659,63	-10,28	12.	788,96	7,31
3.	663,14	-9,80	13.	708,15	-3,68
4.	747,88	1,72	14.	770,56	4,81
5.	723,15	-1,64	15.	798,41	8,60
6.	774,81	5,39	16.	646,79	-12,03
7.	777,18	5,71	17.	655,76	-10,81
8.	751,87	2,27	18.	774,95	5,41
9.	695,01	-5,47	19.	787,62	7,13
10.	739,23	0,55	20.	767,29	4,36
A.V.			735,20		
SD			46,143		
RSD (%)			6,684		

Table 5. 27. *Diameter and hardness tests of S.tab 4.*

Diameter and hardness tests							
Tablet No	width(mm)	%	Depth(mm)	%	Length(mm)	%	Hardness
1.	16,96	-0,01	9,31	0,22	6,27	4,45	>300
2.	16,8	-0,96	9,31	0,22	6,01	0,12	164,4
3.	16,99	0,17	9,29	0,00	5,87	-2,22	97,3
4.	16,99	0,17	9,28	-0,11	5,71	-4,88	156,6
5.	16,99	0,17	9,28	-0,11	5,83	-2,88	87,3
6.	16,96	-0,01	9,26	-0,32	6,26	4,28	>300
7.	16,98	0,11	9,29	0,00	5,86	-2,38	138,4
8.	16,97	0,05	9,28	-0,11	5,99	-0,22	205
9.	16,99	0,17	9,27	-0,22	6,36	5,95	164,4
10.	16,99	0,17	9,30	0,11	5,88	-2,05	185,2
AV.	16,96		9,29		6,004		
SD	0,058		0,016		0,220		
RSD (%)	0,344		0,176		3,658		

Table 5. 28. *Friability test os S.tab 4.*

Friability test	
Weight before test	7906,92
Weight after test	7888,03
% Friability	0,239

5.5. Fifth Grope (S.tab 5) Quality Tests Results.

Table 5. 29. *Disintegration test for S.tab5.*

Disintegration test			
Tablet No.	Disintegration Time	Tablet No.	Disintegration Time
1.	In 1 mint	13.	In 1 mint
2.	In 1 mint	14.	In 2 mints
3.	In 1 mint	15.	In 1 mint
4.	In 1 mint	16.	In 1 mint
5.	In 1 mint	17.	In 1 mint
6.	In 1 mint	18.	In 1 mint
7.	In 1 mint	19.	In 1 mint
8.	In 2 mints	20.	In 2 mints
9.	In 1 mint	21.	In 1 mint
10.	In 1 mint	22.	In 2 mints
11.	In 1 mint	23.	In 1 mint
12.	In 1 mint	24.	In 1 mint

Table 5. 30. *Uniformity content test for S.tab5.*

Uniformity content test	
Table label amount	500 mg
Tablet No.	% Actual amount Compared with label amount
1.	42,82
2.	43,39
3.	44,51
4.	46,73
5.	49,66
6.	46,73
7.	42,23
8.	46,52
9.	44,12
10.	46,73
AV.	45,344
RSD (%)	5,085

Table 5. 31. *Dissolution test of S.tab 5.*

Dissolution test of S.tab 5		
Time (minute)	Average of dissolved according to libel amount (%)	RSD (%)
5	44,12	5,87
10	43,28	5,77
15	42,24	6,72
20	41,03	5,98
30	38,05	4,21
45	36,32	6,86
60	35,76	5,25

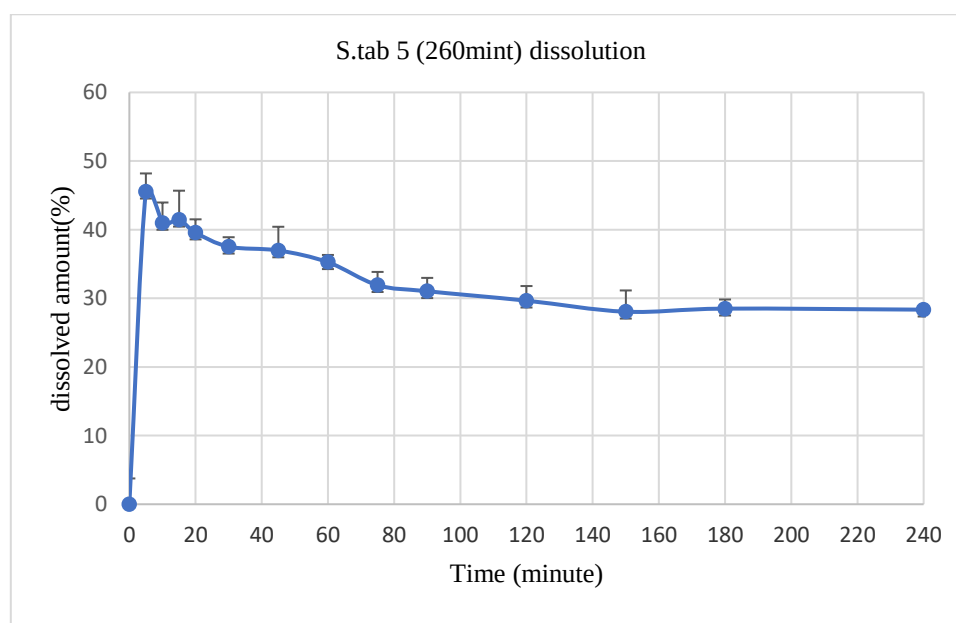


Figure 5. 13. *Dissolution test of S.tab 5*

- The test was carried out on 12 randomly selected tablets, the average of the results was taken

Table 5. 32. *Dissolution test of S.tab 5 in 240 mintes.*

Dissolution test of S.tab 5 in 240 mintes		
Time (minute)	Average of dissolved according to libel amount (%)	RSD (%)
5	45,54	5,34
10	40,98	5,28
15	41,45	5,56
20	39,58	5,87
30	37,52	5,87
45	36,97	7,07
60	35,30	6,79
75	32,61	5,93
90	31,12	6,11
120	29,66	5,87
150	28,05	7,91
180	28,54	5,96
240	28,35	5,94

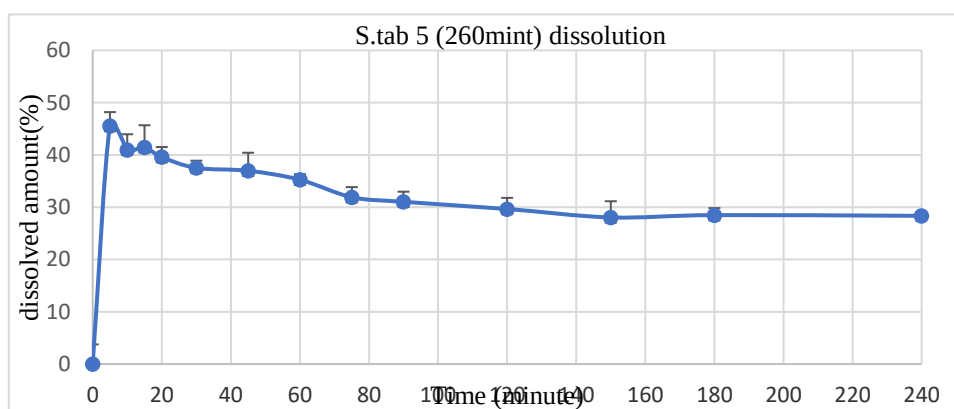


Figure 5. 14. *Dissolution test of S.tab 5 in 240 mintes.*

- The test was carried out on 6 randomly selected tablets, the average of the results was taken

Table 5. 33. *Weight Variation of s.tab 5.*

Weight Variation					
Tablet No	Weight (mg)	%	Tablet No	Weight (mg)	%
1.	912,51	8,71	11.	878,33	4,64
2.	832,67	-0,80	12.	733,14	-12,66
3.	845,67	0,74	13.	931,28	10,94
4.	777,21	-7,41	14.	876,28	4,39
5.	865,98	3,16	15.	780,63	-7,00
6.	806,45	-3,93	16.	907,23	8,08
7.	798,59	-4,86	17.	699,45	-16,67
8.	866,05	3,17	18.	936,68	11,59
9.	820,21	-2,29	19.	773,04	-7,91
10.	794,8	-5,32	20.	952,22	13,44
A.V.			839,421		
SD			69,540		
RSD (%)			8,320		

Table 5. 34. *Diameter and hardness tests for S.tab5.*

Diameter and hardness tests							
Tablet No	Width(mm)	%	Depth(mm)	%	length(mm)	%	Hardness
1.	17,03	-0,53	9,41	0,43	6,66	5,05	75,10
2.	17,11	-0,06	9,42	0,53	6,39	0,79	29,40
3.	17,15	0,17	9,40	0,32	6,68	5,36	88,50
4.	17,08	-0,24	9,28	-0,96	5,86	-7,57	11,30
5.	17,05	-0,41	9,30	-0,75	6,68	5,36	89,40
6.	17,19	0,40	9,30	-0,75	6,37	0,47	61,80
7.	17,16	0,23	9,39	0,21	6,22	-1,89	22,60
8.	17,15	0,17	9,41	0,43	6,02	-5,05	21,30
9.	17,11	-0,06	9,41	0,43	6,40	0,95	47,10
10.	17,18	0,34	9,33	-0,43	6,14	-3,15	21,60
AV.	17.121		9.37		6.34		
SD	0,054		0,056		0,284		
RSD (%)	0,318		0,594		4,484		

Table 5. 35. *Friability test of S.tab5.*

Friability test	
Weight before test	8291,06
Weight after test	7867,12
% Friability	5,113

5.6. General Appearance Of Sample Groups.

Table 5. 36. *General appearance of tablest.*

General Appearance			
S.tab NO:	Color	Odor	Tablet level of flaws
1	white	No odour	Consistent, no flaws
2	white	No odour	Consistent, no flaws
3	pink	No odour	Consistent, no flaws
4	white	Little unacceptable smell	presence of black dots and spots
5	white	No odour	Gloss differin

6. CONCLUSION

Uniformity of content

From the results of the accurate analysis of ten pills from each group, the following shows: The first group (s.tab1), second (s.tab2), third (s.tab3) They pass the test but the fourth (s.tab4) and fifth (s.tab5) groups were not able to pass the test, as it was found that there was a decrease in the content of the fifth group by 23%, and in the fifth group it was found that there was a decrease by 55% of the content with label amount.

Disintegration test:

From the results of Disintegration test each group, the following shows: The first group (s.tab1) and third (s.tab3) They pass the test, the other remaining groups did not pass the test, Although the samples tested were raise to 24 tab. As all samples of the second group took more than two hours to disintegrate and it is possible that the cause was excessive pressure and an increase in the percentage of the effective content, as there is in the sample 1000 mg of vitamin C added to other vitamins. While the opposite was observed, where all the samples of the fourth and fifth groups took less than five minutes to disintegrate, and it is possible that the reason was low compression or Misuse of binder or not adjusting the humidity in manufacturing.

Dissolution test:

According to what is stipulated in Pharmacopeia, the tablet must be dissolve completely within an hour inside the Dissolution tester that contains acid like the stomach, and according to the results analyzed by using the HPLC. It turned out that the first (s.tab1) and third (s.tab3) group had succeeded in the test, while the second group (s.tab2) had failed and its dissolved was delayed, and this is due to its weak disintegration, while in the last two groups (S.tab4 & S.tab 5) it was dissolved in the first minutes because it was easy to disintegrate.

The test was based on the rate of dissolve of 12 tablets from each group at the times that were mentioned in the US pharmacopeia.

Dissolution test to 240 minutes:

Some groups were late in Dissolution, so an additional 6 tablets from each group were tested, In the second group (s.tab2), it was observed that they continued to dissolve past the 240 minutes.

Weight Variation test:

In the difference in weight, (20) tablets were randomly weighed from each group in the sensitive scale and the percentage of difference in the weight of the tablets was calculated. It was found that the first, second and third group differed by less than 5%, while the fourth group showed that there were (11) tablets that differed from the average by more than 5% degrees, while the fifth group There are also (11) tablets that differ from the average by more than 5% degrees, and the difference in these tablets reaches 17%. This discrepancy in weight within one group may be attributed to poor processing, fragility of tablets, errors in pressure and flow.

Hardness test:

The hardness of the tablets was tested with sensitive devices and on 10 tablets from each group tested. The results were as follows: The first group was that the force required to break the tablets was between 230 and 300 Newtons. In the second group, the required force was more than 100 Newtons. The third group was more than 300 Newtons, and the fourth group The required force between 97 and 300 Newtons, while the fifth group was the required force between 11 and 88 Newtons, where 5 of the tablets are less than 40 Newtons and 2 are tablets more than 80 Newtons, while the force to break the grains must be between 40-80 Newtons according to pharmacopeia.

These problems in the hardness of the tablets can occur due to the weakness or strength of the pressure that occurs during the pressing of the tablets. Or because of the moisture content, and for this we must rely on the test that is carried out within 5 days of manufacturing the tablets, and for this reason we cannot rely on these results to cross the 5 days on the manufacture of these tablets.

Friability:

After using the rotating tumbling apparatus and measuring by the sensitive scale for friability test, we found that all groups had a change in their weight of less than 1% of the starting weight of the test, except for the fifth group (s.tab5), which was more than 5%, while the USA pharmacopeia states that the change should not be more than 1%. The reason for the failure of the fifth group in the test is due to a defect in production, which causes a defect in the hardness of the tablets, and this leads to the occurrence of the tendency of tablets to powder, which might impair the elegant look, customer approval of the tablet, and also contribute to weight fluctuation or content homogeneity issues.

Thickness test:

The thickness of the tablets was tested with sensitive devices, and 10 tablets from each group were tested. The percentage difference between the tablets for all groups was less than 5% of the average thickness of the tablets, except for the fifth group, where it became clear that there are 5 tablets that differ in length by more than 5% of the average thickness of this group, and this difference is due to friability.

Table 6. 2. *Quality control results for all tests.*

	Disintegration	Dissolution	Uniformity Content	Weight Variation	Hardness	Thickness	friability
S. Tab1	pass	pass	pass	pass	fail	pass	pass
S. Tab 2	fail	fail	pass	pass	fail	pass	pass
S. Tab 3	pass	pass	pass	pass	fail	pass	pass
S. Tab 4	fail	fail	fail	fail	fail	pass	pass
S. Tab 5	fail	fail	fail	fail	fail	fail	fail

REFERENCES

- Abdul Althaf, S., Sailaja, P., & Ashwin Kumar, M. (2012). Formulation, evaluation and mathematical modelling of clopidogrel bisulphate & aspirin immediate release bilayer tablets. *Pharm Anal Acta*, 3, 194.
- Abdelmaksoud AA, Ghweil AA, Hassan MH, Rashad A, Khodeary A, Arefzf, Sayedf MAA, Elsamman MK ve Bazeed SE (2021). Olfactory disturbances as presenting manifestation among egyptian patients with COVID-19: possible role of zinc. *Biological Trace Element Research*: 1-8.
- Andrews, K. W., Roseland, J. M., Gusev, P. A., Palachuvattil, J., Dang, P. T., Savarala, S., ... & Bailey, R. L. (2017). Analytical ingredient content and variability of adult multivitamin/mineral products: national estimates for the Dietary Supplement Ingredient Database. *The American journal of clinical nutrition*, 105(2), 526-539.
- Aşan, M. (2006). 27.05. Examination of the Decree-Law on the Production, Consumption and Inspection of Foods dated 2004 and numbered 5179 (Doctoral dissertation, Bursa Uludag University (Turkey)).
- Ashar, B. H. (2010). The dietary supplement health and education act: time for a reassessment: comment on “acute selenium toxicity associated with a dietary supplement”. *Archives of internal medicine*, 170(3), 261-263.
- Bailey, R. L., Gahche, J. J., Lentino, C. V, Dwyer, J. T., Engel, J. S., Thomas, P. R., Betz, J. M., Sempos, C. T., & Picciano, M. F. (2011). Dietary supplement use in the United States, 2003–2006. *The Journal of Nutrition*, 141(2), 261–266.
- Bailey, R. L., Gahche, J. J., Miller, P. E., Thomas, P. R., & Dwyer, J. T. (2013). Why US adults use dietary supplements. *JAMA Internal Medicine*, 173(5), 355–361. <https://doi.org/10.1001/jamainternmed.2013.2299>.
- Bailey, R. L., Pac, S. G., Fulgoni, V. L., Reidy, K. C., & Catalano, P. M. (2019). Estimation of total usual dietary intakes of pregnant women in the United States. *JAMA Network Open*, 2(6), e195967–e195967.

- Bailey, R.L., Gahche, J.J., Lentino, C.V., Dwyer, J.T., Engel, J.S., Thomas, P.R., ... & Betz, J.M. (2011). Dietary supplement use in the United States, 2003-2006. *J Nutr*, 141:261-266.
- Binns, C. W., Lee, M. K., & Lee, A. H. (2018). Problems and prospects: public health regulation of dietary supplements. *Annual Review of Public Health*, 39, 403–420.
- Blendon, R. J., DesRoches, C. M., Benson, J. M., Brodie, M., & Altman, D. E. (2001). Americans' views on the use and regulation of dietary supplements. *Archives of Internal Medicine*, 161(6), 805-810.
- Botanical Dietary Supplements. (2011). National Institutes of Health Office of Dietary Supplements. Retrieved from [https://ods.od.nih.gov/factsheets/Botanical Background -HealthProfessional](https://ods.od.nih.gov/factsheets/BotanicalBackground-HealthProfessional).
- Boullata, J. I., & Hudson, L. M. (2012). Drug–nutrient interactions: a broad view with implications for practice. *Journal of the Academy of Nutrition and Dietetics*, 112(4), 506–517.
- Brasky, T. M., & Kristal, A. R. (2015). Learning from history in micronutrient research. In *Journal of the National Cancer Institute* (Vol. 107, Issue 1, p. dju375). Oxford University Press US.
- Brewster, N. A. T., & Goldsmith, P. D. (2007). Legal systems, institutional environment, and food safety. *Agricultural Economics*, 36(1), 23-38.
- Burdock, G. A. (2000). Dietary supplements and lessons to be learned from GRAS. *Regulatory Toxicology and Pharmacology*, 31(1), 68–76.
- Burns, R. D., Schiller, M. R., Merrick, M. A., & Wolf, K. N. (2004). Intercollegiate student athlete use of nutritional supplements and the role of athletic trainers and dietitians in nutrition counseling. *Journal of the American Dietetic Association*, 104(2), 246-249.
- Calfee, R., & Fadale, P. (2006). Popular ergogenic drugs and supplements in young athletes. *Pediatrics*, 117(3), e577-e589.
- Carr, A. C., & Maggini, S. (2017). Vitamin C and immune function. In *Nutrients* (Vol. 9, Issue 11). MDPI AG. <https://doi.org/10.3390/nu9111211>.

- Cemeroğlu, B., "Basic Analysis Methods in Fruit and Vegetable Processing Industry", (1992).
- Cemeroglu, B., & Acar, J. (1986). Fruit and Vegetable Processing Technology,—Food Technology Association.
- Chatsiricharoenkul, S., Niyomnaitham, S., Pongnarin, P., Sathirakul, K., & Kongpatanakul, S. (2011). Bioequivalence study of 10 mg olanzapine tablets in healthy Thai volunteers. *J Bioequiv Availab*, 3, 82–85.
- Davydova, N. (2010). Disintegration and dissolution of dietary supplements. *Pharmacopeial Forum*, 36(2), 567.
- DE Faria Coelho-ravagnani C, Corgosinho FC, Sanches Flfz, Prado CMM, Laviano A ve Mota JF (2021). Dietary recommendations during the COVID-19 pandemic. *Nutrition Reviews* 79(4): 382-393.
- Denny, A. (2008). An overview of the role of diet during the ageing process. *British Journal of Community Nursing*, 13(2), 58.
- Deshpande, J. D., Joshi, M. M., & Giri, P. A. (2013). Zinc: The trace element of major importance in human nutrition and health. *Int J Med Sci Public Health*, 2(1), 1-6.
- Dickinson, A., Blatman, J., El-Dash, N., & Franco, J. C. (2014). Consumer usage and reasons for using dietary supplements: report of a series of surveys. *Journal of the American College of Nutrition*, 33(2), 176–182.
- Dillard, C. J., & German, J. B. (2000). Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture*, 80(12), 1744-1756.
- Dolan, J. (2017). Method adjustment the USP way.
- Duconge, J., Miranda-Massari, J. R., Gonzalez, M. J., Jackson, J. A., Warnock, W., & Riordan, N. H. (2008). Pharmacokinetics of vitamin C: insights into the oral and intravenous administration of ascorbate. *Puerto Rico Health Sciences Journal*, 27(1).

- Bahador,T. (2022). tip fakültesi öğrencilerinin covid 19 döneminde gıda takviyesi kullanımı , beslenme alışkanlıkları , fiziksel aktivite düzeyi ve tip fakültesi öğrencilerinin covid 19 alışkanlıkları , fiziksel aktivite düzeyi ve depresyon- anksiyete - stres.
- Eurachem, G. (1998). A laboratory guide to method validation and related topics. *LGC Ltd, Teddington, UK*.
- FDA, U. (1987). General principles of validation, Rockville, MD. *Center for Drug Evaluation and Research (CDER)*.
- FDA, U. S. (2009). *Warning on Hydroxycut products*. Updated May.
- FDA Issues Dietary Supplements Final Rule. 2007 [cited 2007 August 25], Available from: URL: <http://www.fda.gov/bbs/topics/NEWS/2007/NEW01657.htm>.
- Food and Nutrition Board Institute of Medicine. (1998). Dietary Reference Intakes: A Risk Assessment Model for Establishing Upper Intake Levels for Nutrients. Washington, D.C.: National Academy Press.
- Food Stand. Aust. N. Z. (2016). Food standards code. Food Stand. Aust. N. Z., Canberra. <http://www.foodstandards.gov.au/code/Pages/default.aspx>.
- Ford, K. L. (2017). Vitamin and Mineral Supplement Use by Older Adults with Complex Medication Needs; Potential for Adverse Drug-Nutrient Interactions (Doctoral dissertation, University of Saskatchewan).
- Fortmann, S. P., Burda, B. U., Senger, C. A., Lin, J. S., & Whitlock, E. P. (2013). Vitamin and mineral supplements in the primary prevention of cardiovascular disease and cancer: an updated systematic evidence review for the US Preventive Services Task Force. *Annals of internal medicine*, 159(12), 824-834.
- Froiland, K., Koszewski, W., Hingst, J., & Kopecky, L. (2004). Nutritional supplement use among college athletes and their sources of information. *International journal of sport nutrition and exercise metabolism*, 14(1), 104-120.
- Gahche, J., Bailey, R., Burt, V., Hughes, J., Yetley, E., Dwyer, J., ... & Sempos, C. (2011).

- Dietary supplement use among US adults has increased since NHANES III (1988-1994). NCHS data brief, (61), 1-8.
- Gershwin, M. E., Borchers, A. T., Keen, C. L., Hendler, S., Hagie, F., & Greenwood, M. R.C. (2010). Public safety and dietary supplementation. *Annals of the New York Academy of Sciences*, 1190(1), 104-117.
- Gershwin, M. E., Borchers, A. T., Keen, C. L., Hendler, S., Hagie, F., & Greenwood, M. R. C. (2010). Public safety and dietary supplementation. *Annals of the New York Academy of Sciences*, 1190(1), 104-117.
- Grey, A., & Bolland, M. (2014). Clinical trial evidence and use of fish oil supplements. *JAMA internal medicine*, 174(3), 460-462.
- Grey, A., & Bolland, M. (2014). Clinical trial evidence and use of fish oil supplements. *JAMA internal medicine*, 174(3), 460-462.
- GROMOVA O, TORSHIN IY, PRONIN A ve KILCHEVSKY M (2019). Synergistic application of zinc and vitamin c to support memory and attention and to decrease the risk of developing nervous system diseases. *Neuroscience and Behavioral Physiology*: 1-8.
- Gürdöl F ve Ademoğlu E (2010). *Biyokimya*. İkinci baskı. Nobel Tıp Kitapevleri Ltd. Şti.
- Hancock, R. D. (2009). Recent patents on vitamin C: opportunities for crop improvement and single-step biological manufacture. *Recent patents on food, nutrition & agriculture*, 1(1), 39-49.
- Hegazy, N., Sayed, H. A., Hasan, A. A., & Salem, M. R. (2020). Popularity of the consumption of dietary supplements and its associated factors among students in an Egyptian university: a cross-sectional study. *Open Access Macedonian Journal of Medical Sciences*, 8(E), 566–573.
- Heidelbaugh, J. J. (2013). Proton pump inhibitors and risks of mineral deficiency: evidence and clinical implications. *Therapeutic Advances in Drug Safety*, <https://doi.org/10.1177/2042098613482484>

- Hemila H (2017). Zinc lozenges and the common cold: a meta-analysis comparing zinc acetate and zinc gluconate, and the role of zinc dosage. *JRSM open* 8(5): 2054270417694291.
- Holford P, Carr AC, Jovic TH, Ali SR, Whitaker IS, Marik PE ve Smith AD (2020). Vitamin C—An adjunctive therapy for respiratory infection, sepsis and COVID-19. *Nutrients* 12(12): 3760.
- Homan, E. M. (2018). *Dietary Supplements and College Students: Use, Knowledge, and Perception*. Kent State University.
- Israelsen, L. D., & Blumenthal, M. (2000). FDA issues final rules for structure/function claims for dietary supplements under DSHEA. *HerbalGram*, 48, 32-38.
- J. Emese and P.F. Nagymate, The Stability of Vitamin C in Different Beverages. *British Food Journal*. Vol 110, Issue 3, 2008, pp 296-309 (2008).
- Kalra, E. K. (2003). Nutraceutical-definition and introduction. *Aaps Pharmsci*, 5(3), 27–28.
- Karabulut H ve Gülay MŞ (2016). Antioksidanlar. *Veterinary Journal of Mehmet Akif Ersoy*.
- Kasabalı, k., " RESEARCH ON PRODUCTS USED AS FOOD SUPPORT", (2007).
- Kantor, E. D., Rehm, C. D., Du, M., White, E., & Giovannucci, E. L. (2016). Trends in dietary supplement use among US adults from 1999-2012. *Jama*, 316(14), 1464–1474.
- Kaya, K. (2015). Gıda sanayi işletmelerinin kalite güvence sistemlerini önemseme düzeyleri üzerine bir araştırma: Şanlıurfa örneği.
- Kemper, K. J., Amata-Kynvi, A., Dvorkin, L., & Whelan, J. S. (2003). Herbs and other dietary supplements: healthcare professionals' knowledge, attitudes, and practices. *Alternative therapies in health and medicine*, 9(3), 42.
- Kirkpatrick, S. I., & Tarasuk, V. (2008). Food insecurity is associated with nutrient inadequacies among Canadian adults and adolescents. *The Journal of Nutrition*, 138(3), 604.

- Kirkpatrick, H. (2011). Thesis written by. *Sciences-New York, May*.
https://etd.ohiolink.edu/apexprod/rws_etd/send_file/send?accession=kent1619198478121933&disposition=inline.
- Lokesh, P. N. V, Abdul, A. S., & Sailaja, P. B. (2012). Design, development and formulation of orodispersible tablets of a model drug using response surface methodology. *Pharmaceut Anal Acta*, 3(9), 1–15.
- Madden, M. M., DeBias, D., & Cook, G. E. (2014). Market analysis of vitamin supplementation in infants and children: evidence from the dietary supplement label database. *JAMA Pediatrics*, 168(3), 291–292.
- Megep Food Technology Vitamins and Minerals. MEB, 89s, Ankara (2006).
- M.W. Davey, M. Van, D. Montagn, M. Inze, A. Sanmartin, A Kanallis and N. Smirnoff (2000), Plant L-ascorbic acid: Chemistry Function Metabolism, Bioavailability and Effects of Processing. *J. Sci. Food Agric.* 80, 2000, 825-860.
- National Institutes of Health Office of Dietary Supplements. (2016). Multivitamin/mineral Supplements. Retrieved from [https://ods.od.nih.gov / factsheets /MVMS-HealthProfessional](https://ods.od.nih.gov/factsheets/MVMS-HealthProfessional).
- Nelms, M., Sucher, K. P., Lacey, K., & Roth, S. L. (2011). *Nutrition Therapy and Pathophysiology* (2nd ed.). Belmont, CA: Brooks/Cole, Cengage Learning.
- Nowak, R. E. (2010). DSHEA's failure: why a proactive approach to dietary supplement regulation is needed to effectively protect consumers. *U. Ill. L. Rev.*, 1045.
- Office of Nutrition Policy and Promotion Health Products and Food Branch. (2006).
- Canadian 82 Community Health Survey, Cycle 2.2, Nutrition (2004)- A Guide to Accessing and Interpreting the Data. Ottawa: Ministry of Health, Health Canada.
- Olson, R.E., Munson, P.L., Mueller, R.A. and Bresse (1990), G.R., "Water-soluble vitamins In: *Principles of Pharmacology*", (Chapman and Hall, New York), Ch 59.

- Otten, J. J., Pitz Hellwig, J., & Meyers, L. D. (Eds.). (2006). *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*. Washington, DC.: The National Academic Press.
- Otten, J. J., Pitz Hellwig, J., & Meyers, L. D. (Eds.). (2006). *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*. Washington, DC.: The National Academic Press. Overview of Dietary Supplements. Center for Food Safety and Applied Nutrition, 2001 [cited 2007 August 25], Available from: URL: <http://www.cfscan.fda.gov/~dms/supplnt.html>
- Öznur, D (2012). " Determination of ascorbic acid (vitamin c) in colored materials by spectrophotometric method".
- Pharmacopia, U. S. (2016). *Briefing 1216 Tablet Friability*,. 31(6), 5–7.
- Qato, D. M., Wilder, J., Schumm, L. P., Gillet, V., & Alexander, G. C. (2016). Changes in prescription and over-the-counter medication and dietary supplement use among older adults in the United States, 2005 vs 2011. *JAMA Internal Medicine*, 176(4), 473–482.
- Radimer, K., Bindewald, B., Hughes, J., Ervin, B., Swanson, C., & Picciano, M. F. (2004). Dietary supplement use by US adults: data from the National Health and Nutrition Examination Survey, (1999–2000). *American journal of epidemiology*, 160(4), 339-349.
- Representative, U. T. (2007). National trade estimate report on foreign trade barriers. *Washington, DC*.
- Richardson, M. A., Sanders, T., Palmer, J. L., Greisinger, A., & Singletary, S. E. (2000). Complementary/alternative medicine use in a comprehensive cancer center and the implications for oncology. *Journal of Clinical Oncology*, 18(13), 2505-2514.
- Rock, C. L. (2007). Multivitamin-multimineral supplements: who uses them? *The American journal of clinical nutrition*, 85(1), 277S-279S.
- Rogovik, A., Vohra, S., & Goldman, R. D. (2010). Safety considerations and potential interactions of vitamins: Should vitamins be considered drugs? *Annals of Pharmacotherapy*, 44, 311.

- Ross, A. C., Taylor, C. L., Yaktine, A. L., & Del Valle, H. B. (Eds.). (2011). Dietary Reference Intakes: Calcium and Vitamin D. Washington, DC: The National Academic Press.
- Saldamlı İ Food Chemistry. Hacettepe University Publications, 682s, Ankara. (2017).
- Saldanha, L.G. (2005). Dietary Supplements in the USA: Who's Using What and Why. National Institutes of Health Office of Dietary Supplements. Retrieved from https://ods.od.nih.gov/News/Dietary_Supplement_Use_in_the_USA_FNCE_2005.aspx.
- Samadi, M., Zeinali, F., Habibi, N., & Ghotbodini-Mohammadi, S. (2016). Intake of dietary supplements and malnutrition in patients in intensive care unit. *International journal of preventive medicine*, 7.
- Samur F (2008). Vitaminler mineraller ve sağlığımız.
- Santos, D. A., Lima, K. P., Março, P. H., & Valderrama, P. (2016). Vitamin C determination by ultraviolet spectroscopy and multiproduct calibration. *Journal of the Brazilian Chemical Society*, 27(10), 1912–1917. <https://doi.org/10.5935/0103-5053.20160071>.
- Santos, C. A., & Boullata, J. I. (2005). An Approach to Evaluating Drug-Nutrient Interactions. *Pharmacotherapy*, 25(12), 1789–1800.
- Sauberlich, HE, "Bioavailability of vitamins", *Prog. Food Nutr. science* 9:1-33 (1985).
- Scofield, D. E., & Unruh, S. (2006). Dietary supplement use among adolescent athletes in central Nebraska and their sources of information. *Journal of strength and conditioning research*, 20(2), 452.
- Scrimshaw N.S., SanGiovanni J.P., *The American Journal of Clinical Nutrition*, Volume 66, Issue 2, August 1997, p. 464S–477S.
- Selçuk, İ. Oktar, S. ve Şahna, E "Farmakoloji Anabilim Dalı", Fırat Üniversitesi Tıp Fakültesi, Elazığ ., (2006).
- Soller, R. W. (2000). Regulation in the herb market: the myth of the, 'unregulated industry'. *HerbalGram*, 49, 64-67.

- Sorice A, Guerriero E, Capone F, Colonna G, Castello G ve Costantini S (2014). Ascorbic acid: its role in immune system and chronic inflammation diseases. *Mini reviews in medicinal chemistry* 14(5): 444-452.
- Sowjanya, P., Subashini, D., & Rakesh, S. (2015). *Research and Reviews : Journal of Pharmacy and Pharmaceutical Sciences A Review on Method development and validation by HPLC*. 4(1), 42–50.
- Strewler, A., Conroy, R., & Kao, H. (2014). Approach to overuse of herbal and dietary supplements: a teachable moment. *JAMA Internal Medicine*, 174(7), 1033–1034.
- Sturluson, T. (2014). History of Herbal Medicine. Herbal Resource. Retrieved from <https://www.herbal-supplement-resource.com/history-of-herbal-medicine/>.
- Tan, T. (2008). *İlaç sektöründe kalite iyileştirme teknikleri ve bir uygulama* (Doctoral dissertation, Marmara Üniversitesi (Turkey)).
- Tanaka, H., Kaneda, F., Suguro, R., & Baba, H. (2004). Current system for regulation of health foods in Japan. *Japan Medical Association Journal*, 47(9), 436-440.
- U.S. Food and Drug Administration. (2011). Dietary Supplements Background Information. Retrieved from [https://ods.od.nih.gov/factsheets/Dietary Supplements-Health Professional](https://ods.od.nih.gov/factsheets/Dietary%20Supplements-Health%20Professional).
- U.S. Food and Drug Administration. (2017). Avoiding Drug Interactions. Retrieved from <https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm096386.htm>.
- Üretimi, G. (2004). Tüketimi ve Denetlenmesine Dair Kanun Hükmünde Kararnamenin Değiştirilerek Kabulü Hakkında Kanun. *Kabul Tarihi*, 27.
- Vatistas, T. J., & Samuels, J. G. (2012). The regulation of dietary supplements in the United States: advocating for a reasonable approach, protecting patient safety, and the role of nursing. *Policy, Politics, & Nursing Practice*, 13(2), 113–116.
- Velioglu Er, E. (2019). A study on the determination of the use of food supplements: The case of Thrace (Master's thesis, Institute of Science and Technology).

- Webb, G. P. (2006). An Overview of Dietary Supplements and Functional Food. Dietary Supplements and Functional Foods, 1-35.
- Wessels I, Rolles B ve Rink L (2020). The potential impact of zinc supplementation on COVID-19 pathogenesis. *Front Immunol* 2020; 11: 1712.
- Woo, J. J. Y. (2007). Adverse event monitoring and multivitamin-multimineral dietary supplements. *The American Journal of Clinical Nutrition*, 85(1), 323S-324S.
- Xin, W., Wei, W., & Li, X. (2012). Effect of fish oil supplementation on fasting vascular endothelial function in humans: a meta-analysis of randomized controlled trials. *PloSone*, 7(9), e46028.
- Yetley, E. A. (2007). Multivitamin and multimineral dietary supplements: Definitions, characterization, bioavailability, and drug interactions. *American Journal of Clinical Nutrition*, 85(1). <https://doi.org/10.1093/ajcn/85.1.269s>.
- Yilmaz, A. H. (2019). *Balık yağı içeren takviye ile multivitamin şurup kullanan çocuk hastalarda hastalık sıklığının karşılaştırılması*.
- Zell-Kanter, M., Quigley, M. A., & Leikin, J. B. (2015). Reduction in ephedra poisonings after FDA ban. *New England journal of medicine*, 372(22), 2172-2174.
- Zhang, X., & Zhang, S. (2014). Bioequivalence Study of Two 30 Mg Tolvaptan Tablets Formulations in Healthy Chinese under Fed Condition. *J Bioequiv Availab*, 6, 181–185.