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Application of FTIR Spectroscopy method for The Quantification of Ascorbic Acid in Bulk Materials and Pharmaceutical Formulation

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Abstract

The present study illustrates the potential using of Fourier Transform Infrared Spectroscopy (FT-IR) in pharmaceutical analysis. A simple, rapid, non-destructive, and green (FT-IR) spectroscopy method for quality control evaluation of Ascorbic acid was developed, using potassium bromide (KBr) as a matrix to quantify the drug in bulk pharmaceutical materials and AUC its dosage forms. The sample preparation was done by mixing and grinding for disk composition and without using organic solvents. Absorbance obtained for the C=C of □□□□unsaturated lactone located at the range (1600-1700) cm-1 was used to develop a calibration curve based on the AUC measurements. Method had been validated according to the International Conference on Harmonization (ICH) guidelines. Linearity, accuracy, precision, robustness, LOD and LOQ were evaluated and showed acceptable results for method validation in the concentration range of (2 - 12.5 mg). The validated method was able to apply for the quality control analysis of Ascorbic Acid raw materials and pharmaceutical dosage forms.

Keywords: Ascorbic acid, Effervescent tablet, Fourier-Transform infrared spectroscopy, Quantitative analysis and Validation

تحليل حمض الأسكوربيك كمياً كمادة أولية وضمن صيغ صيدلانية عبر تقنية الأشعة تحت الحمراء FTIR

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الخلاصة

تظهر الدراسة الآتية إمكانية تطبيق تقنية الأشعة تحت الحمراء FTIR في تحليل الصيغ الصيدلانية، حيث تم تطوير تقنية سريعة ودقيقة غير مخربة وصديقة للبيئة وهي تقنية FTIR لتقييم جودة حمض الأسكوربيك كمادة أولية وضمن الأشكال الصيدلانية وذلك باستخدام بروميد البوتاسيوم كحامل لتشكيل القرص وتحديد كمية المادة الفعالة من خلال حساب المساحة تحت المنحني AUC. تم تحضير العينة من خلال الطحن والمزج لتشكيل القرص دون اللجوء لاستخدام المذيبات العضوية. تم استخدام قيمة امتصاص الرابطة C=C الواقعة ضمن المجال (1600-1700)سم$^{-1}$ لتشكيل منحني المعايرة وذلك بالاعتماد على قياسات المساحة تحت المنحني.

تم التحقق من مصدوقية الطريقة التحليلية وفق توصيات المؤتمر الدولي للموائمة. تم تقييم الخطية، الدقة، المضبوطية، المتانة، الحد الأدنى للكشف والحد الأدنى للكم. كانت نتائج جميع الاختبارات السابقة مقبولة وذلك ضمن مجال التراكيز (2-12.5) ملغ. أظهرت النتائج إمكانية تطبيق تقنية FTIR في تقييم جودة حمض الأسكوربيك كمادة أولية وضمن الأشكال الصيدلانية.

الكلمات المفتاحية : حمض الأسكوربيك، الأقراص الفوارة، الأشعة تحت الحمراء المزودة بمحول فوربيه ، التحليل الكمي واختبارات المصدوقية

Introduction

Vitamins are organic compounds that the body needs in tiny quantities. Since it cannot be synthesized in the human body, it must be supplied externally. One such vitamin is vitamin C[(1)].

Ascorbic acid plays a major role in fighting infection, and wound healing and is a powerful antioxidant that can neutralize damaging free radicals [(2)]. It is necessary to make the protein collagen, a fibrous protein found in connective tissue that is woven into various systems in the body: nerves, immune system, bones, cartilage, blood, etc. Vitamins help make several hormones and chemical messengers used in the brain and nerves [(2)]. Also, Vitamin C is required to stimulate many enzymes that are relating to the neural system, hormones, and detoxification the liver from drugs and toxins [(1)]. Vitamin C rises the absorption of Fe, Ca, and folic acid(B9). It also decreases the allergic reactions, strengthens the immunity, induces the forming of bile in the urinary tract, and eases the secretion of several steroids [(2)]. The drug structure is shown in Figure 1.

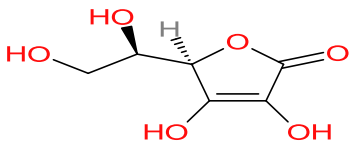

Figure 1 .Ascorbic acid molecule structure

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Several quantitative analytical methods have mentioned the procedure utilized to measure vitamin C in many samples; these include colorimetric[3], titrimetric[4], UV spectrophotometric[5], kinetic[6], electrochemical[7], fluorometric[8], chromatographic[9], and other techniques. However, each method has its own restrictions [10].

For example, the titration method is quick but it needs a large amount of samples and is not suitable for analyzing colored samples. High Performance Liquid Chromatography (HPLC) a chromatographic method, is very accurate but it takes a long time. A rapid, selective and accurate method for quantification of vitamin C concentrations in foods and pharmaceuticals is needed [3].

FTIR spectroscopy is an ordinary, fast, non-destructive method that plays an important role in the rapid determination of many components present in simple and complex matrices[11]. At first FTIR Spectroscopy was viewed as a qualitative tool which used for structural investigations [12,13], identification of compounds [14], polymorphism [15], impurities [16] and for monitoring dissolution of pharmaceutical dosage forms [17], but extensive quantitative research has been published over the past two decades and had proved possibility of using Ftir technique in quantities analysis [11].

This work is aimed to improve FTIR as a faster, simpler, and inexpensive method for measuring ascorbic acid in effervescent tablets. The FTIR spectrum of ascorbic acid will be corrected and then measuring its area under the curve (AUC). This technique was accomplished, in the intent of sharpening the peaks and make the information more facilitate to calculate [18].In this study, there was no sample treatment such as derivation or purification.

Comparing this method with HPLC, the two methods had high sensitivity, but HPLC was time consumimg [25].comparing it with Titration method, The titration method required many steps to prepare the sample. And when compared FTIR with UV method, it was solvent free so it's Eco-friendly, in addition to its precision and selectivity due to it's identifications feature [12].

Table 1. Ascorbic acid content detrmination by different methods

Methods	Equipment	Solution and materials needed	Analyzed durtion	LOD,LOQ mg
Iodin Titaration[22]	- burette and stand - 100 mL or 200 mL volumetric flask - 20 mL pipette -10 mL and 100 mL measuring cylinders - 250 mL conical flasks	-Iodine solution -Starch indicator solution -H_2SO_4 solution -Distilled water	45-50 minutes	1.00, 3.00
UV- Visible[5]	UV-Spectrophotometer of Shimadzu	-Methanol -Distilled water	15-20 minutes	0.00096, 0.00291
HPLC[25]	HPLC of Shimadzu	- sodium phosphate buffer	90 minutes	0.0036, 0.012
FTIR	spectrophotometer Interspec 200-X	Potassium bromide (KBr)	15-20 minutes	0.003417, 0.0103

Materials and Methods

Chemical and Equipment

The ascorbic acid standard used for this study was provided by RAJKOT INDIA, potassium bromide (KBr) was provided by a PIKE TECHNOLOGIES, UNITED STATES. FTIR (Fourier transforms infrared) spectrophotometer Interspec 200-X is used to acquire infrared spectra. FTIR spectra had been recorded in the wavenumber range 4000–400 cm-1 using 16 scans for every sample with a resolution of 4 cm-1. Deuterated Lanthanum α Alanine triglycine sulphate (DLATGS) was utilized as the FTIR detector. Data were collected and analyzed using Version 1.2.15 of OPUS Spectragryph.

Samples

Three different batches of two ascorbic acid effervescent tablets products (A, B) were purchased from local Syrian pharmacies. Classical KBr discs were KBr discs have been made for both pure ascorbic acid and samples. Samples were prepared by weighing the chemicals and KBr accurately on an analytical balance. With an agate mortar and pestle, ascorbic acid and KBr were grinded and mixed. The resulting mixture was pressed into a mold using an PKIE TECHNOLOGIES manual hydraulic press. The discs had been fabricated with a 7 mm diameter under constant pressure of 12 tons for 5 minutes to obtain a transparent disk [20].

Calibration curve:

Using six different ascorbic acid stock concentrations ranging from 0.26 to 1.62 mg a calibration curve was represented. To obtain each concentration, potassium bromide used as a dilute, was added to a particular amount of ascorbic acid, and to ensure sample homogeneity, Ascorbic acid and KBr were grounded together very well. The area under the curve (AUC) for each calibration standard was measured at wavenumbers ranging from 1600 to 1700 cm^{-1} corresponding to the C=C of α–β unsaturated lactone stretch. Each calibration standard was analyzed in triplicate and the average of the triplicate measurements was used to construct the calibration curve [18].

Validation method

The developed method had been validated by the following parameters: linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantification (LOQ) tests according to the recommendations of the International Conference on Harmonization (ICH) [19,21].

Linearity

Linearity was evaluated using the same process to represent a calibration curve, preparing standard ascorbic acid concentrations between 0.26 and 1.62 w/w at wavenumber rang 1600-1700 cm-1 table1. Each quantification was analyzed by FTIR in triplicate. Linearity was assessed by calculating the correlation coefficient (r) [20] .

Precision

The precision was evaluated by repeatability. Repeatability was assessed by measuring three concentrations of the ascorbic acid each concentration was analyzed in 3 replicates [20]. The AUC value of the chosen wave number range was calculated, then the contents were determinate through the calibration curve. The value of the relative standard deviation "RSD" is to be accepted when it's less than 2%.

Accuracy

Recovery experiments were conducted at a concentration range of 50%, 100% and 150% to validate the accuracy of the test method. The analysis was performed in triplicate. Accuracy was determined utilizing the mean percentage recovery, and the percentage relative standard deviation (%RSD) [20].

LOD and LOQ

The limit of detection (LOD) of an analytical method is known as the minimum concentration at which the presence of an analytic can be concluded with reasonable statistical confidence. LOD and LOQ were measured by standard calibration. LOD and LOQ were evaluated at 3.3σ/S and 10σ/S. where σ is the standard deviation of the blank and S is the slope of the y-intercept of the regression equation [20] .

Robustness

Robustness aims to make certain that the analytical method unaffected by slight modifications in analytical parameters.

The robustness of the proposed method was assessed by analyzing small changes in the compression time of disks by ±2 min and evaluating it as the relative standard deviation [20].

Determination of ascorbic acid content in effervescent tablets using FTIR method:

Two different brands of ascorbic acid effervescent tablets were used to determine their amount of the active substance. 20 tablets from each batch were accurately weighed, averaged, and grind. A certain amount of each effervescent tablet powder was weighed. For FTIR: a suitable dilution with KBr was made to obtain 0.359 mg w/w ascorbic acid. Samples were thoroughly mixed by finely grinding. The analysis was done in three replicates.

Results and Discussion

The method is anchored on the measurement of the AUC of the C=C band stretch of unsaturated lactone which is in the range 1600 – 1700 cm-1 [23] Figure. 3. This is because this absorption functional group does not appear in pharmaceutical excipients[23]. Figue.4. illustrate the response of the C=C band which had showed the most linear response relative to several Concentrations of the ascorbic acid standards in KBr ranging from 0.26 to 1.62 mg by FTIR spectroscopy.

The proposed method has been validated according to ICH guidelines. Calibration curve is represented by the formula y = bx+a. where x is ascorbic acid concentration and y is AUC Figure.2. The linear regression equation was y = 48.371x+5.939 with a calibration correlation coefficient of 0.9944, which was within the acceptance criteria Table 1. The data of LOD and LOQ were obtained at 0.003417 and 0.0103 w/w. This indicates that FTIR sensitivity is particularly good [20].

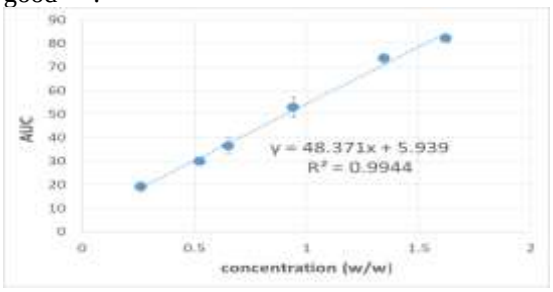

Figure 2 Linearity of ascorbic acid at 1600-1700 cm-1 (n=3 for each measurement)

Table 2. Linearity studies results

S.NO	Concentration of standard ascorbic acid in KBr pellet,mg	AUC
1	0.262948	19.17267
2	0.52381	29.95667
3	0.65153	36.47
4	0.941038	52.87333
5	1.344268	73.63
6	1.621951	82.1
Regression equation: y = 48.371x + 5.939		
The correlation coefficient R^2 = 0.994		

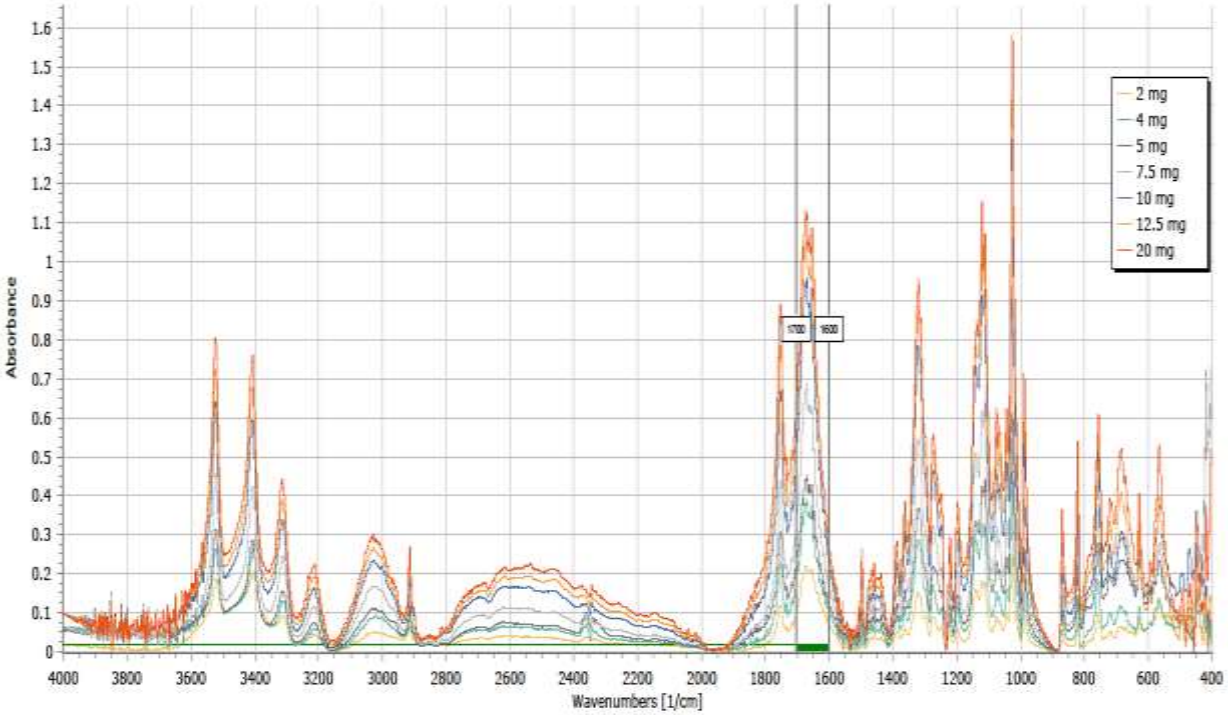

Figure 3 FTIR spectrum of standard Ascorbic acid

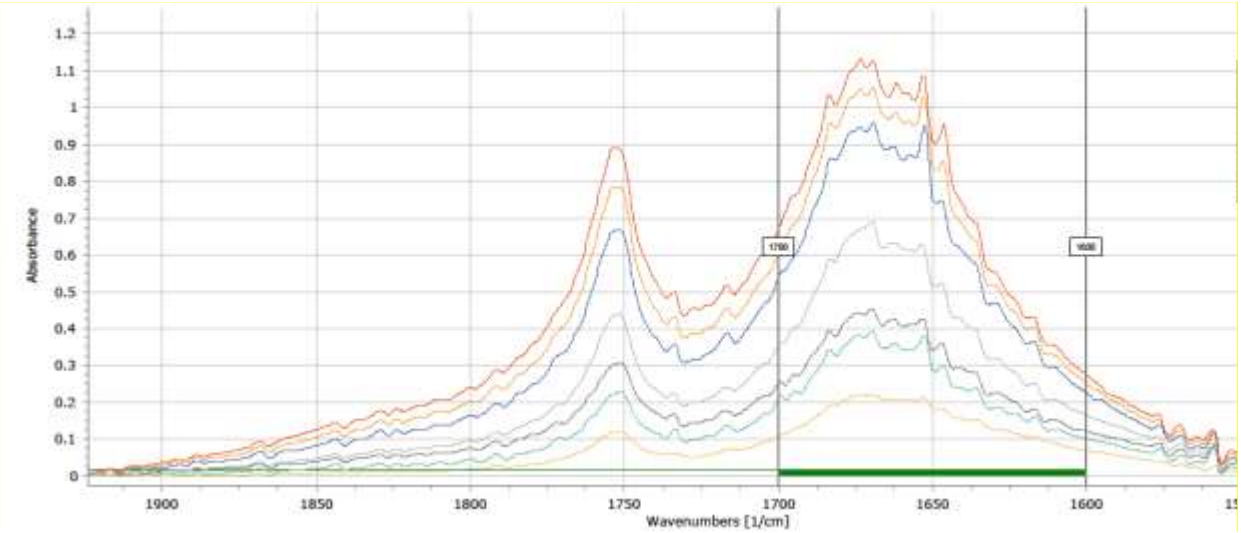

Figure 4 Response of the C=C band area (1600-1700 cm-1) to various concentrations of the ascorbic acid working standards by FTIR spectroscopy

Table 2 illustrates the repeatability of ascorbic acid standards as a result of parameter validation analysis. On the other hand, Table 3 illustrates the accuracy results by FTIR.

Table 3 Repeatability precision data (n=3)

Concentration w/w	Average AUC Measured	SD	RSD%
0.523	29.95	0.0043	0.821%
1.344	73.63	0.017	1.274%
1.621	82.1	0.008	0.494%

Mean RSD%=0.863%

Precision was demonstrated by the relative standard deviation (RSD) and Evaluated by repeatability [20,26]. The RSD value for the repeatability assay was 0.863%. This result was within an acceptable range. The FTIR method was high precision According to the result of precision studies [14] table 2. The accuracy of the FTIR method was estimated by the standard addition method at 3 variable levels (50, 100, and 150%) of the working concentration of the method in triplicate, and the recovery results are mentioned in Table 3 [19]. Well recoveries of ascorbic acid were acquired in the range of 99.19% to 99.92%.

Table 4 Accuracy of the FTIR spectroscopy method.

Standard Concentration	Accuracy Level	Amount of ST added	Average Recovery%	SD%	RSD%
0.52 mg w/w	50%	0.26 w/w	99.92	0.823	0.823
	100%	0.52 w/w	99.19	1.294	1.304
	150%	0.78 w/w	99.58	0.541	0.543
Average			99.56	0.886	0.89

Robustness results were evaluated by RSD of the absorbance measured for each variation. The RSD% values were less than 5%, demonstrating the robustness of the analytical method for ascorbic acid determination by FTIR spectroscopy. Figure. 5. shows the results as a chart.

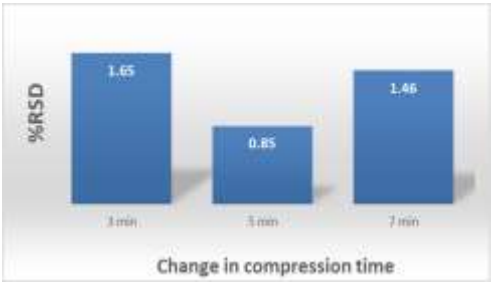

Figure 5. Robustness test results

According to the results of the validation studies, this method can be utilized to quantify the active ingredient content in pharmaceutical formulations [20].In this study, we implemented the proposed and examined the method by validating to determinate ascorbic acid content in effervescent tablet dosage to quantify ascorbic acid content in effervescent tablet dosage forms. Three different batches of two brands of ascorbic acid effervescent tablets have been quantitatively analyzed as test samples using the developed method and the results are summed up in Table 4. Table 4 illustrates the AUC measurement results for effervescent tablet A with a claimed dose of 1000 mg. Table 5 shows the AUC measurement results for effervescent tablet A with a claimed dose of 1000 mg.

Table5.Ascorbic acid effervescent tablets of A product assay results (n=3).

Batch No.	Active ingredient (mg)	Average of AUC	Amount recovered(mg)	Recovery%
1	1000	23.61667	1029.66	102.966
2	1000	24.09667	1058.9	105.89
3	1000	23.75667	1057.091	105.709

Table 6. Ascorbic acid effervescent tablets of B product assay results (n=3).

Batch No.	Active ingredient (mg)	Average of AUC	Amount recovered(mg)	Recovery%
1	1000	24.0333	1039.109	103.9109
2	1000	24.2566	1052.43	105.243
3	1000	22.73333	938.5736	93.8573

The figures below show the infrared spectrum of A and B effervescent tablets determinate by FTIR. Each batch of A and B products was measured in triplicate.

Ascorbic acid effervescent tablets A and B contained ascorbic acid 102.96-105.7; 93.85-105.243% respectively. The amount recovered from the active ascorbic acid compound was fully matched with the labeled content. It is within the acceptable range mentioned in the USP35 Pharmacopoeia of 90.0% - 110.0% [22].

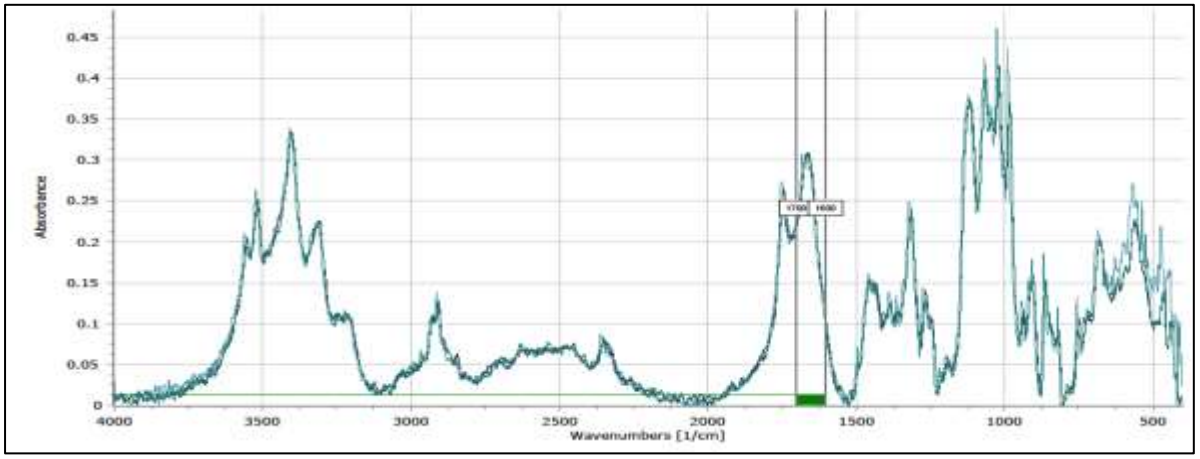

Figure 6. FTIR spectrum of A product batch NO.1

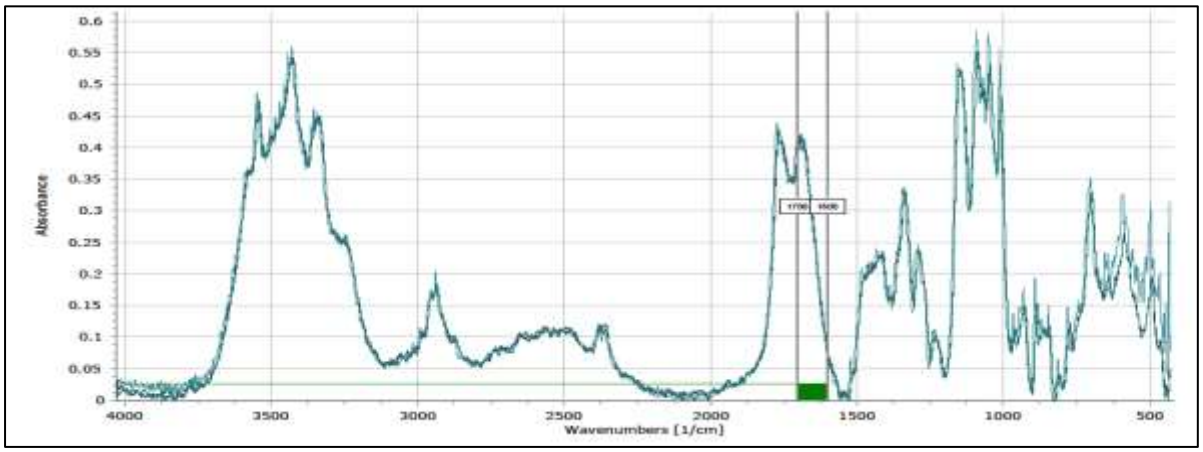

Figure 7. FTIR spectrum of A product batch NO.2

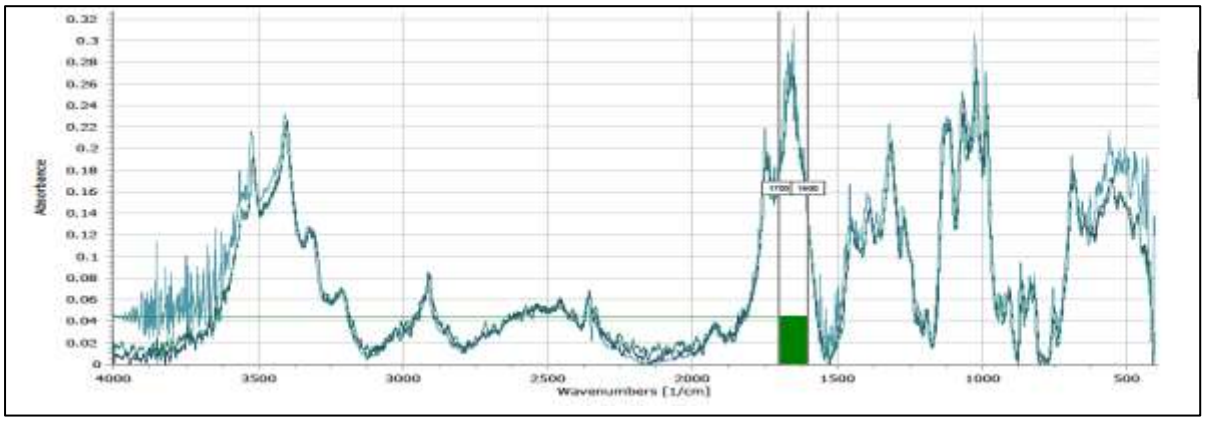

Figure 8. FTIR spectrum of A product batch NO.3

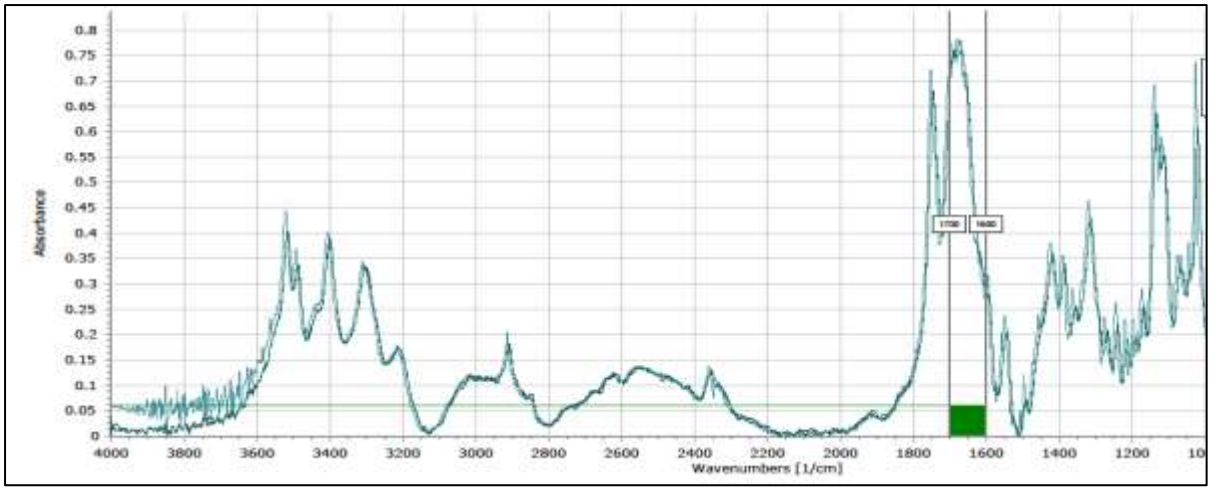

Figure 9. FTIR spectrum of B product batch NO.1

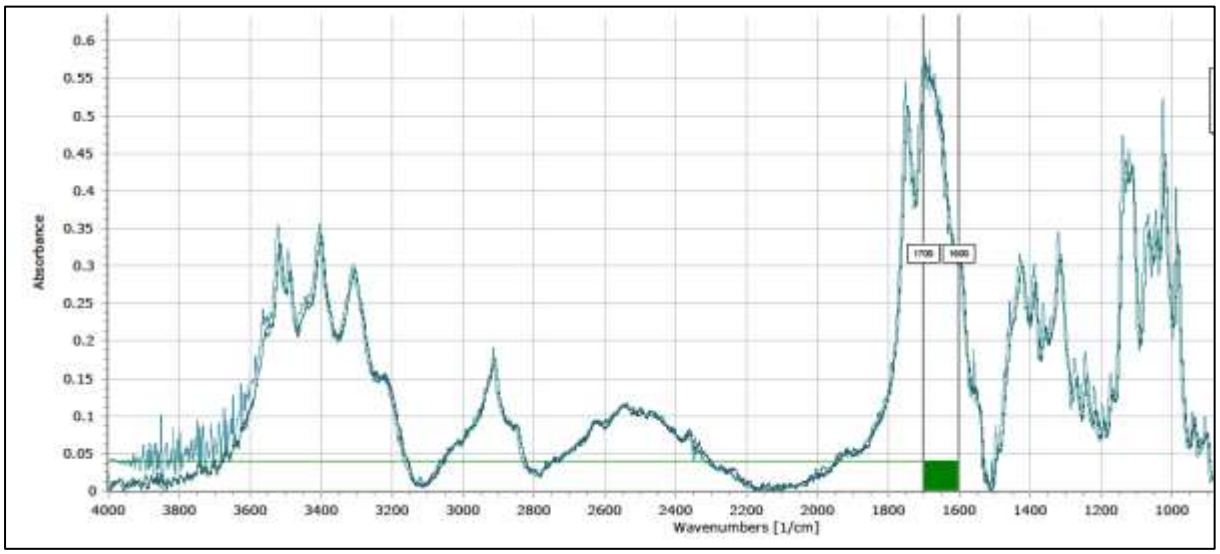

Figure 10. FTIR spectrum of B product batch NO.2

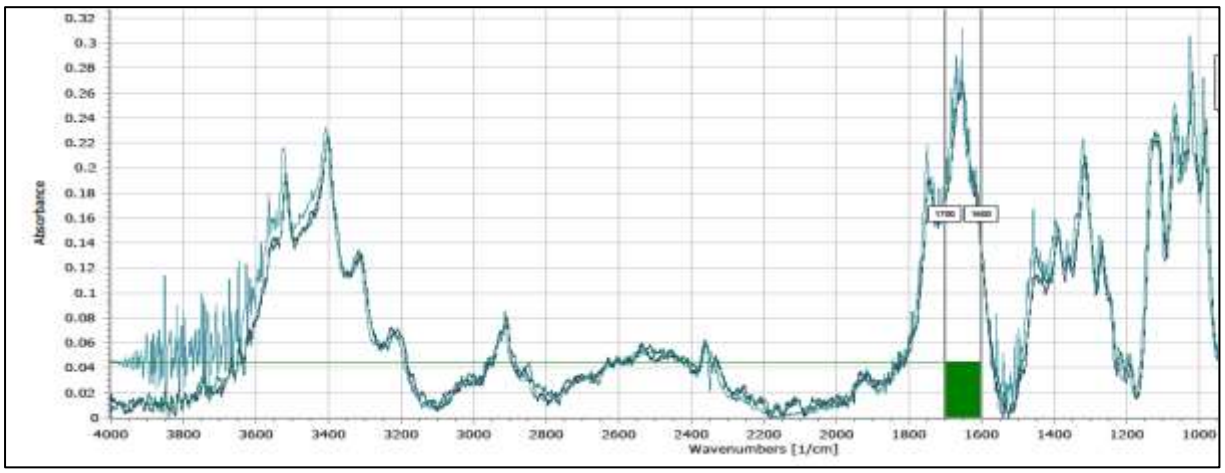

Figure 11. FTIR spectrum of B product batch NO.3

Figure. 12. shows the results of comparisons between the iodine titration traditional method and the FTIR spectroscopy method.

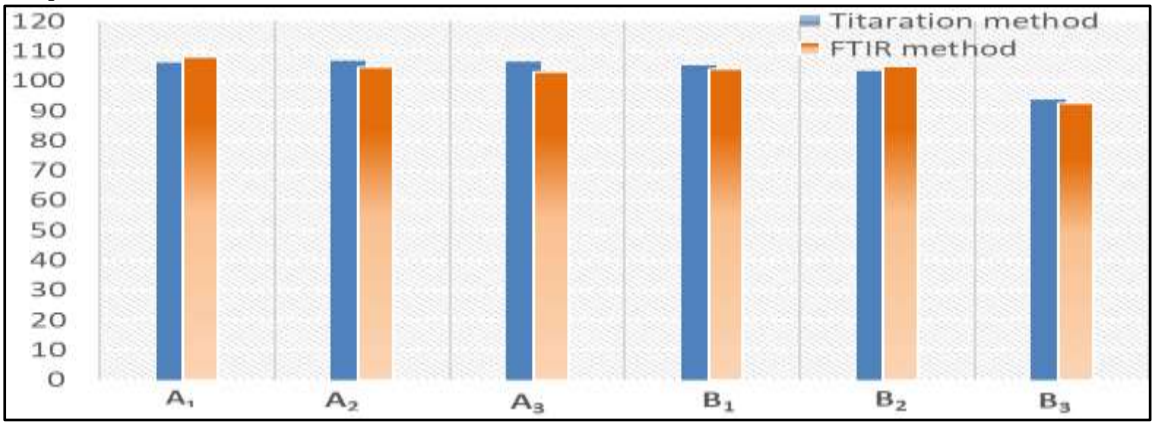

Figure 12. comparison of Ascorbic acid determination content in effervescent tablet by Titration method and Ftir *method*

This method has shown the ease of sample handling, selectivity and time-saving as compared to different methods which include volumetric calibration, HPLC and visible/UV spectroscopy. Another benefit is that it does not use organic solvents, so it is a cost-effective and eco-friendly method.

Conclusion

FTIR spectrophotometry has been demonstrated as a technique advanced for the quantitative evaluation of ascorbic acid in effervescent tablets. This technique can be applied to assay ascorbic acid content. All the results we obtained through this study indicate that FTIR can be presented as a replacement analytical method for ascorbic acid effervescent tablets.

Conflicts of Interest

The authors declare that they have no conflicts of interest related to this work.

Ethics Statements

The study does not require ethical approval from an ethics committee.

References

1. Journal BS. Optimum conditions for ascorbic acid determination in three Iraqi citrus using HPLC technique. Baghdad Sci.J. 2014; 11(3): 1233 –42.
2. Aruoah MK, Al-Jowari SA-K. The effects of zinc and vitamin C supplementation on the glycemic profile in type 2 diabetic patients. Iraqi Sci.J. 2022;:70–6. Doi: 10.24996/ ijs. 2022. 63.1.8
3. Hashmi MH, Shahid MA, Akhtar MA, Chughtai NA. Colorimetric determination of ascorbic acid. Mikrochimica Acta. 1973;61(6):901–6.DOI: BN 10. 1007/ B F012 18 181
4. Quantitative determination of vitamin C concentration of common edible food sources by redox titration using Iodine Solution. Lett. Appl. NanoBioScience. 2020;10(3):2361–9.
5. Pancham YP, Sanjay SS. UV-Spectrophotometric method for quantification of ascorbic acid in bulk powder. J. Pharm. Innov. 2020; 9(5):05-08
6. Karayannis MI. Kinetic determination of ascorbic acid by the 2,6-dichlorophenolindophenol reaction with a stopped-flow technique. Anal. Chim. Acta. 1975;76(1):121–30. https://doi.org/10.1016/S0003-2670(01)81993-0
7. Pisoschi AM, Pop A, Serban AI, Fafaneata C. Electrochemical methods for ascorbic acid determination. Electrochim. Acta. 2014;121:443–60. https://doi.org/10.1016/j.electacta.2013.12.127
8. Dilgin Y, Nişli G. Fluorimetric determination of ascorbic acid in vitamin C tablets using methylene blue. Chem. Pharm. Bull. 2005;53(10):1251–4. DOI:10. 1248/ cpb.53. 1251
9. Ahmida, M.H.S. Determination of ascorbic acid in vitamin C (tablets) by high-performance liquid chromatography. Asian J. Chem. 2009;21: 6463–6467.
10. Fahelelbom KM, Saleh A, Al-Tabakha MM, Ashames AA. Recent applications of quantitative analytical FTIR spectroscopy in pharmaceutical, biomedical, and clinical fields: A brief review. Rev Anal Chem. 2022;41(1):21–33. Doi:10.1515/revac-2022-0030 .

11. Harshou N, Trefi S, Bitar Y. Fourier transform infrared spectroscopy for quantitative determination of valsartan in bulk materials and in pharmaceutical dosage forms. Bulletin of Pharmaceutical Sciences Assiut. 2022Dec;45(2):747–60.

12. Umar Y, Abu-Thabit N, Jerabek P, Ramasami P. Experimental ftir and theoretical investigation of the molecular structure and vibrational spectra of acetanilide using DFT and dispersion correction to DFT. J Theo Comput Chem. 2019;18(02):1950009. Doi: 10.1142/s0219633619500093

13. Ouhaddouch H, Cheikh A, Idrissi MO, Draoui M, Bouatia M. FT-IR spectroscopy applied for identification of a mineral drug substance in drug products: Application to bentonite. J Spectrosc. 2019;2019:1–6.

14. Siozou E, Sakkas V, Kourkoumelis N. Quantification and classification of diclofenac sodium content in dispersed commercially available tablets by attenuated total reflection infrared spectroscopy and multivariate data analysis. Pharmaceuticals. 2021;14(5):440. Doi: 10.3390/ph14050440

15. Perveen N. Comparative purity study of UV spectrophotometric and Fourier-transform infrared spectroscopic (FTIR) techniques for the determination of ciprofloxacin hydrochloride tablets. Biomed J Sci Tech Res. 2020;32(3). Doi: 10.26717/ BJSTR.2020. .005246.

16. Grabska J, Beć KB, Huck CW. Current and future applications of IR and NIR spectroscopy in ecology, Environmental Studies, wildlife and plant investigations. Compr. Anal. Chem. 2022;:45–76.

17. Nugrahani I, Mussadah M. development and validation analysis of acyclovir tablet content Ddetermination method using FTIR. Int. J. Appl. Pharm. 2013;8(3). Doi: 10.22159/ ijap. 2016v8i3. 12946

18. Balusani R. FT-IR spectroscopic approach for the quantitative analysis of few commercial drugs in bulk and pharmaceutical formulations. J Adv Sci Res. 2022;13(05):95–104. Doi : 10. 55218/ JASR.202213511

19. Borman P, Elder D. Q2(R1) validation of Analytical Procedures. ICH Quality Guidelines. 2017;:127–66.

20. Nugrahani I, Manosa EY, Chintya L. FTIR-derivative as a green method for simultaneous content determination of caffeine, paracetamol, and acetosal in a tablet compared to HPLC. Vib Spectrosc. 2019;104:102941. Doi: 10.1016/j.vibspec.2019.102941

21. Singh Atamjit, Baghel Us, Sinha Manish, Ashawat Ms. Quantitative analysis of rosuvastatin calcium in bulk and solid pharmaceutical dosage forms using green and rapid fourier-transform infrared spectroscopic method. Indian J Pharm Sci. 2020;82(4). Doi:10.36468/pharmaceutical-sciences.689

22. USP35, NF 30: 2012: 2 supplement: U.s.pharmacopoeia and National Formulary. Rockville: USP Convention; 2012.

23. Bunaciu AA, Bacalum E, Aboul-Enein HY, Elena Udristioiu G, Fleschin Ș. FT-IR spectrophotometric analysis of ascorbic acid and biotin and their pharmaceutical formulations. Anal Lett. 2009;42(10):1321–7. Doi: 10.1080/00032710902954490.

24. Nerdy N, Margata L, Surbakti CI, Lux Putra ED, Nasution MA, Bakri TK. Development and validation of FTIR spectrophotometry to identify and determine chloramphenicol in marketed capsules. Rasayan J Chem. 2021;14(03). Doi: 10.31788/RJC. 2021. 1436369

25. Abe-Matsumoto LT, Sampaio GR, Bastos DH. Is titration as accurate as HPLC for determination of Vitamin C in supplements? —titration versus HPLC for vitamin C analysis. American J. Anal. Chem. 2020;11(07):269–79 Doi: https:// doi.org / 10.4236/ ajac. 2020. 117021

26. P. Nithila, Raghavendrababu N, PadmavathiI Y, Neena G, Sushma K, Poojitha A. New FTIR method development and validation for quantitative analysis of favipiravir in bulk and pharmaceutical dosage forms. Int. J. Curr. Pharm. Res. 2022;:25–9. Doi: 10. 22159/ ijcpr. 2022v14i5.2022