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DIFFERENTIATIVE ANALYSIS AND BIOEQUIVALENCE STUDIES BETWEEN GUMMY VITAMINS AND MULTIVITAMIN TABLETS FOR ADULTS: A SYSTEMATIC REVIEW

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Abstract:

Vitamins are essential substances for human health ,if the intake is not adequate, special dietary supplements are exist like multivitamins. The increased interest in functional materials of natural origin are resulted in high demand for preservative free, clean label or natural ingredient based products. The gummy vitamin supplements are more acceptable to consumers and have fewer limitations compared to other dosage forms. The main object of this review study was to investigate the complete analysis of multivitamin tablets and capsules by HPLC analysis for determination of individual vitamin identifications as well as in gummies also and evaluate bioequivalence between both formulations through the chemical, pharmaceutical and bioequivalences of multivitamin tablets and gummies.

Background:

Article searching:

Medline(pubmed), web of knowledge, Scopus, PsycINFO, upto date, web of science, science direct, online books, and different websites were searched for the Terms performing to multivitamin(vitamin/mineral/multivitamin/dietary supplement/gummy) and the terms relating to bioequivalence, HPLC, pharmaceutical equivalence of the multivitamin etc. forward and backward searching was also performed on trials meeting the inclusion criteria ,to be considered inclusion trials must be randomized and placebo controlled.

***Raw data analysis**: Data regarding bioequivalence studies of the gummies like vitamin D content and vitamin E,B12, folate in gummy and tablet formulations screening laboratory values, Cmax , AUCs and blood concentrations at different time intervals which were in the reference articles directly entered.*

***Considerations**: Inclusion criteria through the previous articles included healthy adults age between 18-45 years old male and females, BMI of 18.5-29.9kg/m2 for bioequivalence studies and exclusion criteria includes woman who are pregnant and breastfeeding, and patients who has the history of gastrointestinal diseases, irritable bowel syndrome, metabolic diseases, kidney stones, smokers, anemia patients etc..*

***Keywords:** Multivitamin, gummies, HPLC, chewable, mobile phase, stationary phase, calibration, linearity, range, selectivity, gelatin, pectin, agar, wavelength, bioequivalence, chemical equivalence, pharmaceutical equivalence, ingredients, monograph, dissolution, assay, clinical trail, AUC, Cmax etc.*

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INTRODUCTION:

Vitamins are essential substances for human health and growth. Gummies are sugar-based candies which are fortified with selected vitamins. Gummy vitamins are designed to be a further palatable volition to regular in the expedients that people will be more inclined to take them. Numerous people prefer gummy vitamins to capsules due to their gooey flavors and delicacy such like taste. These are dissolvable, chewable, greasepaint or sticky vitamins tend to be easier to digest, like capsules, gummies can supply the vitamin, minerals or sauces that might be missing from your diet. Until now no proper method has been proposed for the determination of vitamins in gummies and comparative studies also not been performed yet. In this work we proposed individual analysis of fat soluble as well as water soluble vitamins using HPLC method and bioequivalence studies between multivitamin gummies and tablets by using different methods like chemical equivalence, pharmaceutical equivalence and bioequivalence of vitamins in a single dose multivitamin gummies and tablets.

Vitamins in a small quantity required for normal health and metabolism for humans. In which majorly 13 vitamins are essential for human beings. In which majorly 13 vitamins are essential for human beings including a water soluble vitamins (ascorbic acid, thiamine, riboflavin, niacin, pyridoxin, biotin, folic acid, pantothenic acid & cobalamin) and four fat soluble vitamins (vitamin A,D,E &K). when we discuss individually, niacin (vitamin B3) also describes as nicotinic acid (NA), nicotinamide (NAm),it exists in verity of foodstuffs bioavailability in natural food is low. The daily value of vitamin B3 is 30-100mg. pantothenic acid (vitamin B5), there are generally 3 commercial forms available for vitamin B5 , they are sodium and calcium pantothenate and D-pantothenol. Bioavailability ranged between 40-61% based on urinary extraction. Daily allowance is 20-500mg. Biotin (vitamin B7) recommended daily allowance is 300µg. Folic acid is water soluble B vitamin. Taking 200µg of daily folate could reduce deaths with coronary artery diseases. Recommended daily allowances for other water-soluble vitamins are vitamin B1 (Thiamin) is 30-300mg, vitamin B2 (riboflavin) is 20-200mg, vitamin B6(pyridoxine) is 10-15mg, vitamin B12 (cobalamin) is 5-8mg and vitamin C (ascorbic acid) is 2-12g daily[3].

METHODS:

Multivitamin tablet analysis by HPLC

For water soluble vitamins HPLC conditions:

According to some previous articles mobile phase was 15:85(v/v) methanol-10mM hexane sulfonate, 1% acetic acid and 0.13% triethyl amine in water. the flow rate was 1.2ml/min at 36oC at a pressure of 340 kpa(2400psi).we performed UV-absorbance detection at 275nm (295nm from0.09 min for samples containing high concentrations of vitamin C).

For fat soluble vitamins HPLC conditions:

And they used a mobile phase of 92:8 methanol-water. The flow rate was 1.5mL/min at 35 oC at pressure of 260Kpa(1800psi). We performed UV-absorbance detection at 265nm for 8.0min and 285 nm thereafter.

Preparation of calibration standards:

Folic acid solution (1.00mg/ml) was prepared in 0.1Msodium bicarbonate and adjusted to PH 7.0 with phosphoric acid. We prepared riboflavin (0.100mg/mL) and all other water-soluble vitamins(1.00mg/mL) in water. Fresh standard solutions for each water soluble vitamin were prepared weekly and kept refrigerated until used. We prepared mixed water-soluble vitamin standard solutions. The mixed water-soluble vitamin calibration solution was transferred into capped. Amber auto sampler vials and used immediately. Under these conditions most water soluble vitamins stable except ascorbic acid, remaining discernible degradation in several hours. We prepared fat soluble vitamin standards in acetonitrile and kept them stable in capped amber vials under refrigeration.

Sample preparation:

For water soluble vitamins in multivitamin tablets or feed premixes, we followed a four-step procedure. First, we ground a one multivitamin tablet or 1 g of freed premix with a mortar and pestle and transferred to ground powder into a 125-mL Erlenmeyer flask. second, we poured exactly 90ml of 2%acetic acid in water to the mixture and stirred with magnetic stir bar for 1 min. third ultrasonicated the mixture at approximately 40oC for 5 min. Fourth we filtered the extract through a 0.45-µl membrane filter into amber jars while it was still warm. after this four-part procedure we analysed samples immediately by injecting 5-10µl of the sample solution into the high-performance liquid chromatogram.

Table 1:Summery of the parameters used in the analysis performance for the multivitamin tablets as well capsules.

Summary of analytical method performance parameters for vitamin analysis		
Performance parameters	**Water-soluble vitamins**	**Fat-soluble vitamins**
Retention time precision (%)	<0.3	<0.15
Peak area precision (%)	<1.0*	<1.1
Table assay precision (%)	<3	<3
Spike recovery (%)	90-100*	98(vitamin A) 87(vitamin E)
Limit of detection (ng, S/N=3)	0.6-1.2	<1(vitamins A and E) <10(vitamin E)
Selectivity	PI<1.1 R>3 n>6000 (Confirmation via spectral annotations of λ_{max} and purity index)	PI<1.1 R>5 n>8000
Range(ng)	1->1000	1->10,000(vitamin A) 5->20,000(vitamin E)
Linearity (r)	>0.9995	>0.999
Mobile-phase preparation ruggedness (%)	±5	±3
Column life time (injections)	>1000	>1000
Compatibility with other columns	fair	Excellent
	sensitive	20-45° C
***except for vitamin C , which is unstable in a water solution. 10% for vitamin C,**		

By considering the previously published different analytical approaches they reflects determination of water soluble vitamins by using HPLC method like vitamin C, niacin, niacinamide , pyridoxine , thiamine, folic acid, riboflavin recovered approximately 95.9% and fat soluble vitamins like vitamin A, vitamin E, vitamin K recovered average of 90.7% as per label claim of multivitamin tablets, and in capsules fat and water soluble vitamins are recovered average of above 95% as they included in the label claim . This method is commonly used by most of researchers because it has high degree of accuracy and precision.

Gummies HPLC analysis:

There are three types of composition of gummies gelatin gummies (dissolve at room temperature), pectin gummies, carrageenan gummies. Generally, gummies major ingredients Gelatin or Pectin or Agar (5-8 %), water (15-20%), sucrose (28-50%), and corn syrup solids (40-55 %). The formulations of gummy vitamin products pose additional challenges with respect to a tablet or capsule preparation due to vitamins stability issue in a gummy delivery system. Due to stability issue, manufacturers add an excessive amount of the nutrients during manufacture to compensate for loss during storage and achieve the declared shelf-life

Determination of water- and fat-soluble vitamins in gummies:

Determination of six water-soluble vitamins [calcium pantothenate (B5), pyridoxine (B6), biotin (B8), folic acid (B9), cyanocobalamin (B12) and ascorbic acid (C)] and three fat-soluble vitamins [retinyl palmitate (A), cholecalciferol (D3) and alpha-toco pheryl acetate (E)] in gummies was studied by RP-LC with UV detector. Sample was prepared as follows: • Gummy was dissolved in the mixture of water/methanol (v/v=1:1) • Ethanol was used to precipitate the gelatin • Nitrogen gas was used to evaporate the solvent • The final sample was injected onto a C-8 column

Table:2 Elution program for the RP-HPLC determination of Water- and fat- soluble vitamins in Gummies

Time (min)	Solvent A (0.025 M phosphate buffer, P^H =3.5) %	Solvent B (acetonitrile) %
0.0	100.0	0.0
3.5	100.0	0.0
4.0	87.0	13.0
12.0	70.0	30.0
12.5	0.0	100.0
53.0	0.0	100.0
54.0	100.0	0.0

Table. 3 Detection wavelengths for water- and fat-soluble vitamins

Vitamin	Detection wave length (nm)
B5, B8	210
D3	265
C, B6, B9, E	293
A	324
B12	361

Vitamins were detected at different wavelengths. Table No. 3 Detection wavelengths for water- and fat-soluble vitamins. Separation and determination of the vitamins was achieved by RP-LC. For most vitamins, quantitative results close to the label claim are determined. The stability of the vitamin extracts and potential interference by coextracted sugar are being investigated to improve these results prior to validation of the final method. wavelengths for water- and fat-soluble vitamins in gummies are detected as respectively 210nm for vitamin B5 and vitamin B8., 265nm for vitamin D.,292nm for vitamin C, Vitamin B6, vitamin B9, vitamin E., 324nm wave length for vitamin A and 361nm wavelength for vitamin B12. Separation and determination of the vitamins was achieved by RP-HPLC. For most vitamins, quantitative results close to the label claim are determined. In Table No :4 we have all collected data of individual vitamin analysis in different pharmaceutical dosage forms.

RESULTS:

Gummies and multivitamin tablets, capsules are chemically analyzed for vitamin and mineral content with verified reference materials and recommended daily allowances (RDAs). For each ingredient predicted mean percentage differences between analytical and labelled amounts were calculated. For 10 for 12 nutrients, most products had labeled amounts at or equivalence to RDAs. The mean percentage measured content of all ingredients (except vitamin B6, vitamin B12 , folic acid in gummies) were same as labelled content. And for some ingredients like vitamin C in MV tablets and capsules, and niacinamide, vitamin B6, B1,vitamin A were exceeded labelled amounts. (Table 4).

Table No :4 we have all collected data of individual vitamin analysis in different pharmaceutical dosage forms.

Multivitamin tablets										
	Vit C	**Vit B_3**	**NAm (B_3)**	**Vit B_6**	**Vit B_1**	**Vit B_9**	**Vit B_2**	**Vit A**	**Vit D**	**Vit E**
Label	60	2	20	2.0	1.5	0.40	1.7	-	0.01	30.0
Avg	68.8	0	18.6	2.48	1.47	0.418	1.66	4.48	0.014	32.2
% Recovery	101%	96%	93%	98%	95%	95%	92%	91%	94%	87%
Multivitamin capsules										
Label	150	-	25	2.0	10	1.0	5.0	2.75	-	-
Found	176	-	26	2.23	11.3	1.06	5.38	3.95	-	-
% Recovery	117%	-	104%	111.5%	113%	94.3%	92.94%	143.64%	-	-
	Gummies									
	Vit C	**Vit B_5**	**Vit B_7**	**Vit B_6**	**Vit B_{12}**	**Folic Acid**	**-**	**Vit A**	**Vit D**	**Vit E**
Label	4595.588	1194.9	13.79	459.56	1.38	59.743	-	144.761	2.298	2527.574
Found	3919.469	760.29	24.8	41.1	0.12	24.04	-	251.286	2.391	145.0034
% recovery	85.29%	63.6%	179%	8.8%	8.7%	40.24%	-	73.6%	104%	57.4%

Bioequivalence studies of gummies and multivitamin tablets:

Bioavailability is referred to as the extent and rate to which the active drug ingredient or active moiety from the drug product is absorbed and becomes available at the site of drug action[1]. The objective of this investigation was to compare bioavailability between single oral dose multivitamin gummies vs. multivitamin tablets in healthy adults. An initial crossover, randomized clinical trial involving healthy adults who are taking multivitamin gummies (study 1) and healthy adults who were taking multivitamin tablets (study 2), comparison with specific characteristics or functions or their standards.

1.Chemical equivalence:

Our main goal was to determine relations between analytically measured and labelled ingredient content and to compare adult multivitamin tablets and gummies composition with recommended dietary allowances (RDAs) and tolerable upper intake levels. Adults' multivitamins and gummies were purchased while following the plan for HPLC analysis and chemically analyzed for vitamin and mineral content with certified reference materials for each ingredient. Not all multivitamin supplements are same, unfortunately most multivitamins contains synthetic vitamins or semi synthetic nutrients with some using mega doses that can actually do more harm than good. If we study the cross over study between the chemical ingredients most of the ingredients like vitamin C, niacin, niacinamide, pyridoxine, thiamine, folic acid, riboflavin and fat solubles like vitamin A, D,E are same for both gummy and tablets. but gummy vitamin are fewer vitamins and minerals than tablets. Because they are in liquid form made from gelatin, starch, water, sugars. Gummies sometimes include selected nutrients. (eg. Vitamin D and calcium) and often contain 3-5g of sugars (sucrose or corn syrup) in each gummy vitamin approximately they contain 20% of our daily sugar. Generally gummies coated for prevention of sticky nature(eg. Thin corn starch coating). The basic gummy formulation is comprised of sweeteners, gelling agents, acidulants, colors and flavors. Gelatin used for their bouncy and rubbery nature.

2.pharmaceutical equivalence:

Definition: pharmaceutical equivalence means the drug products in identical dosage forms and route of administration that contain identical amounts of the identical ingredients, it implies that two drug products when they are identical in strength, purity content uniformity, disintegration, and dissolution characteristics[1].

Dissolution: this testing is used on the product's end use and strength on average it takes a multivitamin approximately 30 minutes to dissolve in stomach. Instead of there are many types of other bioassays, microbial and chemical assays are used for analysis of vitamins[1].

Table No :5 USP classification with respective dissolution test requirements

Class		Dissolution
I.	Fat soluble vitamins	Not required
II.	Water soluble vitamins	One index vitamin, folic acid if present
III.	Water soluble vitamins with minerals	One index vitamin and one index element, folic acid if present
IV.	Fat and water soluble vitamins	One index water soluble vitamin folic acid if present
V.	Fat and water soluble vitamins with minerals	One index water soluble vitamin and one index element folic acid if present.
VI.	Minerals	One index element

The general dissolution conditions for index vitamins and index minerals are medium -0.1 N hydrochloric acid, 900 mL, Apparatus 1 - 100 rpm for capsules, Apparatus 2 - 75 rpm for tablets, time - 1 hr.

Monograph compounds for strength:
Sample preparation: immerse 25-30 chewable gels in liquid nitrogen in a cryogenic vessel for 10min. cool a blender jar by swirling liquid nitrogen for about 1 min and discard the contents. Transfer the powder normally equivalent.

Dissolution test for gummies:
Major ingredients included in the formulation of gummies are gelatin or pectin or agar(5-8%), water (15-20%),sucrose(28-50%) and corn syrup solids (40-50%).due to the stability and gummy delivery system these formulations are challengeable , for the stability issue manufactures add an excess amount of nutrients for the achievement of shelf life and to reduce the loss during storage.Some vitamins are quite unstable in the chewable gel matrix (high water content and low PH). majority of vitamin products contain stabilized forms of vitamins with protective coating which could effect the release of vitamins. Gelatin aging impair the release of nutrients from matrix[4]. Table:4By following some previous articles here we compare the dissolution and assay results of gummies

Table No:6 Here the results of comparison of dissolution samples with the assay results of gummies :

Product	Label claim (µg)	Dissolution (% release)	Assay (%label claim)
A	687.5 (1250 IU)	153	153.04
B	385 (700 IU)	76	140.07
C	687.5(1250 IU)	54	96.07

Report: Mineral chewable gels should meet dissolution requirements for folic acid,vitamin A, index water soluble vitamins and index minerals similar to tablets and capsules to ensure vitamins are released from matrix. acceptance criteria for the PH is highly dependent on the formulation, recommended value is NMT 3.7, water activity criteria used to control the product microbial contamination. When compare the dissolution results with assay results of the multivitamin gummies for different products most of the times (in case of B and C) getting the half percentage of assay with dissolution release. And in case of multivitamin tablets dissolution is not applicable.

3.Bioavailibility
The concept of vitamin bioavailability for dietary supplements lacks the standard scientific and regulatory definitions. commonly used definitions include concepts of absorption and availability for use and storage (eg. utilization). bioequivalence closely related to the bioavailability bioavailability and bioequivalence factors basis for adjustments for some nutrient values. Systematic information on the bioavailability and bioequivalence of vitamins and minerals in marketed products is scare.

TableNo:7 Examples of considerations of bioavailability and bioequivalency of the Dietary Reference Intakes (DRIs)

Nutrient	Bioavailability and bioequivalence
Vitamin A	DRI expressed as µg retinol activity equivalents (RAE): 1 µg RAE = 1 µg *all-trans*-retinol = 2 µg supplemental *all-trans*-β-carotene = 12 µg dietary *all-trans*-β-carotene = 24 µg other dietary provitamin A carotenoids
Iron	Algorithm for estimating dietary iron bioavailability: 18% bioavailability from a total diet based on differences in absorption from heme- and nonheme-iron sources in a mixed diet
Niacin	No adjustment is made for bioavailability, but the requirement is expressed in niacin equivalents (NEs), which allows for some conversion of the amino acid tryptophan to niacin
Vitamin B-6	Bioavailability of 75% is assumed from a mixed diet
Folate	DRI expressed as dietary folate equivalents (DFEs): 1 µg DFE = 0.6 µg folic acid from fortified food or as a supplement taken with meals = 1 µg food folate = 0.5 µg supplement taken on an empty stomach
Vitamin B-12	An assumed absorption from foods of 50% is included in the DRI, advise adults aged ≥51 y that foods fortified with vitamin B-12 or supplements containing vitamin B-12 be used to meet the DRI because of reduced absorption of food forms

3.1. Bioequivalence studies:

Objectives:

I. Bioequivalence of vitamin E,B12,folate in a single dose multivitamin gummies vs tablets
II. Bioequivalence studies of vitamin D in gummies and tablets in healthy adults

Method I: In this cross over study phase I clinical trial that involved healthy adults who were taking either gummy or tablet multi vitamin consists vit E,B12 and folate as a single dose serial blood samples were collected at times intervals of 0, 0.5, 1-, 2-, 4-, 6-, 8-, 9-, 10-, 24-, hours followed by 2 weeks wash out period. In phase II participants to crossover taken the multivitamin which form previously not given, and blood samples were collected at same time points. Time course of absorption of gummy and tables were compared. C_{max} and area under the curve (AUC) calculated for individual vitamins at specific time based on $T_{1/2}$. and final data is analysed.

Results :

only healthy subjects are considered for this study. For Vit E there were no difference in AUCgeometric mean ratios, log AUC GMR ,C_{max} compared as GMR and for T_{max} GMR in both gummy and tablets. For Vit B12 also no statistical differences on any of metrics with C_{max} , and T_{max} GMR are similar between gummy and tablets. For folate there was a significant difference in absorption . Folate absorption peaked earlier in gummy group that is T_{max} is 1.59hrs in gummy and 4.08 hrs in tablets. Shifting mean points in gummy groups at earlier time is higher mean, at mid points in tablet group. Shorter T_{max} in gummies suggested a difference in bioavailability compare to tablet. For folate geometric ,logAUC GMR and C_{max} ratios showed no statistical difference in gummy and tablets.

Conclusion :

After completion of overall study, both gummy and tablets showed similar absorption for vitamin E and B12. The time course of absorption of folate is differed significantly between gummy and tablets means more rapid absorption in gummies. And C_{max} for both gummy and tablets were same for 3 vitamins.

Method II:

Bioequivalence Studies of Vitamin D Gummies and Tablets in Healthy Adults as shown in the consolidated standards of reporting trials previous article there were 15 participants who consented to participate in this bioequivalence trial: 8 females and 7 males. All pregnancy tests of the female participants were negative at the time of enrollment and study visits. Nine participants completed the study. Study 1 (n = 9). Mean ± SD changes from baseline vitamin D3 blood concentrations (ng/mL) at each time point measured.

Results : Blood concentrations of participants who were taken the gummy and vitamins for vitamin D study at different time intervals. Table No:8

Table 8:

Vitamin D 3		
Hour	Gummy mean ± SD	Tablet mean ± SD
3	8.0±6.7	7.8±4.7
6	25.0±14.4	18.2±8.3
10	33.8±11.2	27.0±7.0
24	24.1±6.3	19.9±6.0
48	12.7±3.0	12.0±3.2

DISCUSSION:

Most of people taking multivitamin gummies over tablets, now a days and there is several reasons for that when consider the benefits of taking gummies are many.one included that they are more easily absorbed by body than tables because they are in liquid form and body can easily digest them. other reasons that they are enjoyable to take , variety of flavors are available, so one can always choose right one (eg:for vegan or sugar free ones). At the same time tablets can be difficult to swallow, but gummies are chewy this makes them great option for people who have trouble swallowing tablets, they come in variety of flavors(like orange, apple, strawberry etc.,) which making them more appealing to children and adults ,in another way they are more portable than the tablets just because they do not require any water or special storage conditions like tablets, they can be taken with you on the go making them perfect for busy people, they are absorbed better in gummy format, and easier on stomach moreover these are a good idea for stay healthy.

CONCLUSION:

Based on the obtained research and review articles regarding the data were presenting the individual vitamin analysis in multivitamin tablets and gimmies concluded that there are differences in labelled and obtained mean percentages in HPLC method and except the folate all vitamins have the same AUC geometric mean, Tmax, Cmax in gummies as well as multivitamin tablets.

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