Authors should write taking account of the following corrections.

Acacia Nilotica: *Acacia nilotica*

Mobile phase: (10:90 v/v)

mg/ml: mg/mL

µl: µL

ml: mL

Title: ~~An optimized method for the~~ The simultaneous determination of some water-soluble vitamins in gum of *Acacia* ~~*Nilotica*~~ *nilotica* by high performance liquid chromatography

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Abstract: A rapid, simple and precise method by ~~enzymatic hydrolysis~~ HPLC (high performance liquid chromatography) has been developed for simultaneous determination of water-soluble vitamins as~~:~~ thiamine (B_1), nicotinamide (B_3), panthotenic(B5) , pyridoxine (B_6) and biotin (B_8) in gum of *Acacia* ~~*Nilotica*~~ *nilotica* using ~~HPLC~~enzymatic hydrolysis. The method uses a C18column ~~(4.6 x 150 mm, 5 µm) type ZORBAX Eclipse XDB~~ -. Mobile phase such as methanol-0.1M sodium dihydrogen phosphate (pH = 2.5~~; (0.1M)~~), 10:90 (v/v) is found most suitable for rapid separation and identification of ~~all~~ this water–soluble vitamins. Good linearity was observed between the concentration of analytes and peak area (r = 0.9999). Each vitamin was quantitatively determined at its maximum wavelength. Recovery percentages ranged from 97% to 99%.

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Keywords: Water – soluble vitamins; Gum of *Acacia* ~~*Nilotica*~~*nilotica*; HPLC.

Introduction: Acacia gums have a complex and branched structure, which makes them have good adhesive and cohesive properties. These properties are useful in pharmaceutical preparations. They are used as dental and other adhesives and as bulk laxatives. These hydrophilic polymers are useful as tablet binders, emulsifiers, suspending agents, gelling agents, stabilizers, thickeners, protective colloids and suspending agents keeping tablets [1].They can also be used as tablet disintegrants [2]. Their adhesive property could be used in the apparatus of colostomies and also in fixing dental prosthesis [3].

For internal use, they help in the preparation of medicines to soothe coughs, diarrhea, dysentery and hemorrhages; for external use, they calm inflammations, so the presence of vitamins in acacia gums is very important since vitamins are essential for human health.

Comment [P1]: Reference should be given.

As far as we know, other researchers have not reported the presence or absence of vitamins B1, B3, B5, B6, B8 in Acacia gum, particularly that of *Acacia* ~~*Nilotica*~~*nilotica*.

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These vitamins are very important for the production of energy (B_1), normal growth and development (B_3), the regulation of neurotransmitters (messengers of nerve impulses) (B_5), balance and regulation of blood sugar (B_6) and the processing of several products such as glucose and fatty acids (B_8).

~~The determination of water-soluble vitamins has always been a particular problem due to the instability of these compounds and complex matrices in which they exist.~~

Because of the importance of these nutritional vitamins, microbiological assay and several analytical methods have been developed for the determination of these substances in food, pharmaceutical supplements andbiological fluids [4-~~7~~8]. There are many analytical methods for performing the assay of vitamins in food, pharmaceutical and physiological specimens such as spectrophotometry [5], [8-10] spectrophotoflurorimetry [5], voltammetry [6], [11], the gas chromatography [12-14] and high performance liquid chromatography [15-25]. Normally, it is necessary to determine more than one vitamin; the analytical method must be able to determine multiple components in complex samples, which can lead to interference in chemical analysis. ~~To avoid possible interferences with soluble possible interferences with soluble vitamin determination, we are developed an enzymatic hydrolysis in gum Acacia Nilotica.~~

2. Materials and Methods

3. Results and Discussion

Table1.~~Program change of~~ ~~wavelengths~~ Wavelengths in the elution time for the determination of vitamins in gum *Acacia* ~~*Nilotica*~~*nilotica*.

All calculations prove that vitamin B is in the order of 12,000 ppm. Apart from vitamin B_8, there are vitamins B_1 and B_3 but with low levels. We can conclude that the method gives a good resolution of vitamin B_8.

Representative chromatograms with other chromatographic parameters are shown in Figure 1,

Comment [P3]: Reference should be given.

Comment [P4]: **8.** Kaynar P.Quantitative determination of vitamin B6 in dietary foods for special medical purposes by microbiological assay method. Afr J Microbiol Res., 2013; 7 (27): 3489-93.

Comment [P5]: References should be controlled and again adjusted.

Comment [P6]: The objective of this research should be written

Comment [P7]: **Materials and Methods:** The date of the study, institution that performed the study, and materials and methods should be clearly presented. (HPLC conditions, analytical column, mobile phase, preparation stock standard and working standard solutions, exctraction of vitamins from sample). Statistical methods should be clearly stated.

Comment [P8]: This section should be written. The results should be stated clearly and only include the current research. In Discussion, the study findings should be compared with the findings of the current researchers. Authors should mention their comments in this section.

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Comment [P9]: ?

2 and Table 2.

Comment [WU10]: To be written.

Figure 1 and 2; Typical chromatograms of ~~water soluble vitamins~~vitamin B_8, vitanin B_3 and vitamin B_1.……

……..sept solutions between 1 and 30 mg / L for B8……..

Comment [WU11]: Not understood.

The reasons of the lack of B_6 and B_8 detection limit and quantification limit values should be written.

Table 5. Study of determining recovery rate by the addition of 500µl of ~~each vitamin~~vitamin B_1, B_3 and B_8.

139 …(Sigma~~-Aldrich~~)…….. Standars of ~~Thiamine~~thiamine, ~~Nicotinamide~~nicotinamide, ~~Pantothenic~~pantothenic, ~~Pyridoxine~~ pyridoxine and ~~Biotin~~ biotin were purchased from Sigma 141 ~~Aldrich.~~

Taka~~-Diastase~~ diastase enzyme from *Aspergillus oryzae* powder, slightly beige was obtained from Sigma~~-Aldrich~~. All chemicals and reagents used are of HPLC and were used without further purification. All solutions must be filtered through a 0.45 ~~mm~~ µm membrane (Millipore), protected from light and stored at 4°C.……

Comment [WU12]: Materials and Method is written

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150 The HPLC system (~~technology~~ Aagilent) was equipped with a pump type technology agilent 1200 series, a vacuum degassing unit model G1322A, a UV-VIS spectrometer to 8 wavelengths, a fluorescence detector (G1321 Agilent 1200 Series), an analytical column (Agilent)~~ZORBAX Eclipse XBD type~~ C18 (6 * 250mm, 5µm), During the analysis the column was equilibrated at 30°C and a manual injector uses an injection valve sample seven lane Rheodyne 7725i. The chromatographic peaks were recorded and elaborated automatically by employing a computerized program 'Agilent ChemStation'.

Comment [WU13]: It shpuld be controlled.

Comment [WU14]:

157 The analyzes were performed by gradient elution of wavelength at room temperature, at a flow rate of 1 mL / min. The total execution time required is less than 20 min.

161 The vitamin stock solution: 100 mg / L were prepared by dissolving 10 mg of each ~~reagent~~ standard ~~(Thiamine: Vitamin B1, Nicotinamide: Vitamin B3, Pantothenic: Vitamin~~

Comment [WU15]: Trademark is written.

~~B5, Pyridoxine: Vitamin B6, Biotin: Vitamin B8~~ in 100 mLl of methanol in dark volumetric flasks.

Comment [WU16]: Trademark

Biotin B8 1.0, 2.0 ,5.0 ,10.0 ,15.0, 20.0, 30.0

Comment [WU17]: It should be written

170 Accurately weigh 1 g of gum *Acacia* ~~*Nilotica*~~ *nilotica* which is a fine powder in a 250 ~~ml~~ mL ~~Erlenmeyer~~ erlenmeyer flask, a….

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This article should be written according to writing rules of the journal.

Current references should be given.