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Review Article

A Review On Analytical Method Development And Validation For Broccoli

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ABSTRACT

A simple accurate and selective methods are developed and validation for determination of phytoconstituents in broccoli by products. Broccoli is a fresh green vegetable in the cabbage family and it contains main phytoconstituent is sulforaphane which is found in isothiocyanate group of organosulfur compounds. The sulforaphane is mainly used to prevent cancer. In this review, the methods were developed and it also includes validation parameters for the determination of phytoconstituents in broccoli. Results are consistent with expected ranges. The broccoli UV range results in three bands UVA 315-400 nm, UVB 280-315 nm, UVC 100-280 nm. The detection limits and quantitative limits of these compounds were in the range of 0.15-0.46 and 0.42-2.47 µg/ml respectively.

INTRODUCTION

Broccoli is a cruciferous green plant in the cabbage family. It is derived from Latin word “Branchium” which means branch ‘Wild cabbage or Wild related to cabbage, cauliflower, kale and mustard. The biological source of broccoli is (Brassica olearaceae var. italica). The genus is Brassica and the species is Brassica olearaceae. It belongs to the family Brassicaceae. They are native to the Eastern Mediterranean. It is leading protein-rich cole crop. The cole crop is derived from Latin word meaning stem or stalk of a plant. For optimal growth, it requires firm, fertile soil with good drainage and

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partial to full sun. The leaves, stems and flowering heads of wild mustard or edible but bitter. It has a high-quality vegetable for fresh use and it is frozen vegetable. This is good source for vitamin C, calcium, zinc, potassium. It will prevent the cancer, diabetes and cardiovascular disease. It plays a major role for a particular product against cancer in humans and prevent the heart disease and includes anticancer, anti-diabetic, anti-microbial, anti -inflammatory, anti -oxidant, cardio protective activities.

PLANT PROFILE:

STEM:

Broccoli forms an upright branching thick green stem with leathery, oblong blue grey or green basal leaves. The consumable portion of broccoli comprises the inflorescence and adjacent stems.

Figure 1: Stem of broccoli

LEAVES:

Broccoli has a large, flat and wide leaves that grow on the broccoli plant. They are grey –blue to green in colour and can be 15-18cm long and 10-13cm wide.

Figure 2: Leaves of broccoli

ROOT:

Broccoli, it has a fibrous root system. It is rather shallow reaching only 45 to 60 cm into the soil but it can spread laterally in a 45 cm radius. Therefore, the optimal row spacing for broccoli is 90cm.

Figure 3: Roots of broccoli

FLOWER:

Broccoli is green flowering head. It has large flower heads, usually green in colour, arrangementin a treelike structure on branches sprouting from a thick edible stalk. The are dense green edible clusters of flower buds (flowers) which is not harvested, bloom four-petaled yellow flowers and produced silky.

Fig.4 Flower of broccoli

SEED:

Broccoli seeds are tiny, green and brown. They are naturally made from the crown of the broccoli and then dried. They have a mild taste and can be used to add colour of salads, coleslaw dressing, dips and fresh vegetable salads.

Fig.5 Seed of broccoli

PHYTOCHEMISTRY:

Broccoli contains sulforaphane, glucosinolates, flavonoids, hydroxycinnamic acid, and other minor compounds. Glucosinolates are hydrolysed and give bioactive compounds they are isothiocyanates, thiocyanates, nitriles, epithionitriles.

ANALYTICAL METHODS:

Analytical method development and validation play an important role in the drug discovery development and manufacture of pharmaceuticals. The resulting official test methods are employed by quality control labs to assuring drug product quality by evaluating identity, purity, potency and performance. The methods are involved in this is UV, HPLC, GC, IR, and validation parameters were explained and the results were within the limits respectively.

REVIEW OF LITERATURE:

1. Magdalena Tuszynska et al., Development and validated HPLC method for determination of Flavonoids in Broccoli. The separation was achieved on a reveresed-phase C18 column using a mobile phase composed of methanol/water (60/40) and phosphoric acid 0.2 % at a flow rate of 1.0ml min-1and detected by a DAD detector at 370nm.It has a linearity, With R2>0.99. The recovery is within 98.07-102.15 % and 97.92 -101.83 % for quercetin ana kaemferol which is important for flavonoids.
2. Ali Ali Redha et al., Development of Liquid chromatography-Mass spectrometry (LC-MS) Method for Quantification of broccoli sulforaphane. The method was demonstrated a highly reproducible retention time (72.04 ± 0.008min), producing a sharp, symmetrical, and well–defined peak in standard and test samples. The sulforaphane in the pure a96nd test samples 178m/z ([M+H]). The standard curve demonstrated expectional correlation, exceeding R2=0.9663. The LOD is 1.3ng/mL and LOQ is 3.9ng/mL, indicating high sensitivity. LC-MS analysis yielded reliable results for SFN identification and quantification.
3. Kyung Ho Row et al., A simple solid-phase extraction (SPE) method for the determination of sulforaphane in broccoli has been developed by using HPLC method. The absorption wavelength was determined at 205nm.The elution mobile phase was ACN/H2O (20:80, v/v). The sulforaphane content in broccoli extracts was determined to be 0.513mg/g. The linearity was obtained from 0.05 to 200µg/mL(r=0.998). The SPE method were provide a higher yield of sulforaphane compared with other extraction methods.
4. Chiang Mai J et al., Determined the HPLC method for simultaneous estimation of phenolic compounds in Broccoli seed samples. The octadecyl silyl column were used. The elution system of acetonitrile - acetic acid solution (PH 3.0)-methanol as tashe mobile phase with flow rate of 1.0mL/min. The catechin, epicatechin, epigallocatechin gallate, gallic acid, quercetin and rutin were obtained in the range of 96-103 %, respectively.
5. Violetta Constataniou - Kokotou et al., Development method UPLC-HRMS-based method for simultaneous detection of sulforaphane and indole-3-carbinol in broccoli. The correlation coefficient and LOD and LOQ were 0.993, 0.77 mg/L and 2.35mg/L for sulforaphane and 0.997,0.42mg/L,1.29mg/L for indole -3-carbinol, respectively. The variation is been is 72 ± 9-304 ± 2 mg and 77 ± 1-117 ± 3mg per 100 g of fresh florets, respectively.
6. Steven J. Schwatrz et al., Compared between HPLC and Electrospray detection and Tandem Mass spectroscopy method with selected reaction monitoring detection for glucosinolates. The following detection limits were observed: glucoriberin,1.75pmol; sinigrin,1.38pmol; progoitrin,1.36pmol; glucoerucin,0.60pmol; and 0.63pmol. In comparison the HPLC, the LC-ESI/MS/MS analysis using SRM detection allows direct

quantification of glucosinolates with improved sensitivity and selectivity.

7. Gunnar B. Bengtsson et al., Determination of flavonoids in broccoli by HPLC method with Chlorophyll fluorescence (ChlF). It includes red light (685nm), green light (530nm), and UV (382nm), blue light (450nm) by excitation of ChlF, relative epidermal absorbance of blue light was correlated with flavonoid Content ($r=0.6$; $p<0.001$). For quercetin alone the correlation with flavonoid Content ($r=0.77$, $p<0.001$).and for green light had weaker correlation with flavonoid Content ($r\leq0.40$, $p<0.05$). whether postharvest irradiation treatment for commercial purpose.
8. J. Lopez -cervantes et al., Determination and validation of sulforaphane in broccoli by products using RP-HPLC method. RP-HPLC which follows: column C18 using a mobile phase, a 30:70(v/v) mixture of acetonitrile: water; flow rate, 0.6mL/min with UV photodiode detector. The analytical method exhibited optimal linearity (R2=1) at 202nm, with recovery efficiencies of 97.5% and 98.1% for fresh and lyophilized florets, respectively.
9. Yumei Liu et al., Development method for sulforaphane in broccoli and cabbage by RP-HPLC method. The solvent is ethyl acetate. The result is been the linear equation was Y=5.907.07X + 4556.71, R2= 0.9996 and repeatable HPLC condition the recovery of 96.3 % (n=6, RSD=1.3 %). The ideal conditions for extracting sulforaphane from broccoli and cabbage were established as:pH7.0, 5-20:1 buffer-to-material ratio and 1-2 hours reaction time at room temperature.
10. William C.K. Chiang et al., Determination of sulforaphane and sulforaphane nitrile in broccoli by using Gas chromatography/Mass spectrometry. It is the thermal degradation were reduced by use of appropriate injector linear and control of the Carrier gas flow rates. They linear concentration range is 13-266 µg /mL and mean RSD is 87.5 % ± 2.4 % and 81.4 ± 5.8 % for sulforaphane and sulforaphane nitrile, respectively. This technique facilitates rapid and reliable analysis of plant samples.
11. D. Gonzalez-Gomez et al., Determination and validation of chlorophyll A and B in Broccoli and cabbage plants by UV Chemometric method. The Chlorophyll contain limit of detection is 0.174 and 0.0324 µg / mL -1 and selectivity is 0.946 and 0.942 and also sensitivity is 0.0324 and 0. 0183 absorbance mL µg -1 were calculated to establish the robustness of the proposed methodology. They accuracy results were compared with chromatography.
12. Samira Sahamishirazi et al., Determination of glucosinolates, in broccoli by using NIRS spectroscopy. Derived calibrations for total –GSLs, aliphatic-GSLs, glucoraphanin, 4-methoxyglucobrassicin in quantitative have RPD =1.36, 1.65, 1.63, 1.11While for indole–GSLsglucosinigrin,1-methoxyglucobrassicin in qualitative have RPD=0.95,0.62,0.67,0.81,0.56. The result shows that NIRS have good potential for determination of glucosinolates respectively.
13. R. Font et al., Determination of glucosinolates in Broccoli eaves by using NIRS spectroscopy. This method is cost effective the calibration results in coefficient of determination and standard deviation to standard error of cross validation ratio of 0.83 and 2.38 for t-GSLs, 0.70 and 1.85 for GNA, 0.62 and 1.63 for GNAST,0.60 and 1.58 for NGBS. This shows an NIRS is high potential analytical method for individual glucosinolates routine analysis in cabbage, respectively.

14. Yasunori Hamauzu et al., Determination of flavonoids in cooked broccoli by using HPLC-MS method. The result in the apparent retention factor range is 35.6 % -147.5 % and true retention factor range is 30.4 % -174.1 %, depending on the cooking method and chemical structure of flavonoids. respectively.
15. Helle Olsen et al., Determination of flavonoids and hydroxycinnamic acid in curly kale by HPLC-DAD-ESI-MS method. The flavonoid profile of curly kale revealed total flavanol and hydroxycinnamic acid contents of 646 mg of RE/100g and 204 mg RE/100g with main compounds and the total content are 44 and 58 mg/100g respectively.

CONCLUSION:

- The review was summarized the simple, rapid and accurate analytical method for Broccoli. It consists of the main phytoconstituent was sulforaphane and is widely used to prevent cancer, diabetics, inflammation, heart diseases.
- The newly developed analytical validation method like UV, IR, HPLC, MECC, MS, extraction could be determined simultaneously and the validation parameter also be concluded.
- The validation parameter is linearity, accuracy, precision, specificity, LOD and LOQ respectively.
- The validation parameter shows the regression coefficient was R2 > 0.99 and parameter shows within the limit. The analytical method development for Broccoli could be concluded.

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