

COMPARISON OF L-DOPA CONTENT IN TWO VARIETIES OF BROAD BEANS
(*VICIA FABEA*) BY DIFFERENT EXTRACTION TECHNIQUESRagni N. Vora¹, Ambika N. Joshi*¹ and Nitesh C. Joshi²¹Department of Botany, Jai Hind College, A road, Churchgate, Mumbai-400020, Maharashtra, India.²Department of Botany, Rizvi College of Arts, Science and Commerce, Mumbai-400050, Maharashtra India.

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ABSTRACT

In the present study, attempts are made to develop suitable method(s) for extraction of L-DOPA from the powdered seeds of 2 varieties of *Vicia faba* using different solvents and conditions. The seed powder of both plants was subjected to 6 different extraction methods, where Method 1 was performed with different solvent ratios. All the extracts were analyzed using RP-HPLC and was validated according to The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines. The L-DOPA extraction was best with Acetonitrile and formic acid mixture in cold maceration technique and overall gives good extraction efficiency in both the plants giving 5.063% and 4.422% L-DOPA in *Vicia faba* var major and *Vicia faba* var. minor, respectively. The present investigation was done to study the extraction efficiency of various extraction methods so to compare L-DOPA content in seed extracts of 2 varieties of *Vicia faba*.

KEYWORDS: Comparison, Extraction efficiency, L-DOPA, HPLC, *Vicia faba*.

INTRODUCTION

L-(3,4-dihydroxyphenyl)-L-alanine(L-DOPA) is a precursor to many neurotransmitters like dopamine, norepinephrine (noradrenaline), and epinephrine. L-DOPA crosses the Brain Blood Barrier whereas, dopamine cannot. In the Central Nervous System, L-DOPA converted into dopamine by the enzyme aromatic L-amino acid decarboxylase, also known as DOPA decarboxylase (DDC).^[1]

Vicia faba also known as the broad bean, fava bean, faba bean, field bean, bell bean, ortic bean, is a species of bean family (Fabaceae) native to North Africa, south west and south Asia, and extensively cultivated elsewhere. It is a rigid, erect plant 0.5–1.8 m tall, with stout stems of a square cross-section. Its seeds are rich in L-DOPA, a substance used medically in the treatment of Parkinson's disease. In 1913, Guggenheim identified L-DOPA in the seedlings, pods, and beans of the *Vicia faba*. Since then, improvements after its consumption have been described in patients with Parkinson's disease.^[2] L-DOPA is also a natriuretic agent, which might help in controlling hypertension.^[3] It is a good source of lysine rich protein and good source of levodopa (L-DOPA), a precursor of dopamine, can be potentially used as medicine for the treatment of Parkinson's disease.^[4] Fava Beans contain high amounts of phenolic content which can elevate L-DOPA in blood. L-DOPA is

a neurotransmitter precursor which is considered as an effective remedy for the relief in Parkinson's disease.^[5]

Vicia faba var. *major* (broad beans): It is cultivated mainly for human consumption. Mature seeds are roasted and eaten as snacks in India, or ground to prepare falafel, sauces and various food ingredients such as meat extenders or skim-milk replacers.^[6]

Vicia faba var. *minor* (horse beans, field beans) produces smaller seeds. They are generally used as livestock feed, and are found to have low-tannin, low vicine-convicine and low-trypsin inhibitor contents.^[7] Faba beans have been suggested as an alternative protein source to soy bean for livestock in Europe.^[8-10]

One of the most important steps for getting the bioactive substances from the plant is extraction. Change in the method of extraction, solvents used, different extraction techniques can highly vary the quantity of the bioactive material extracted. Therefore, a suitable extraction method is important for obtaining the extracts with required pharmacological activities. There is a lack of studies done for optimization of extraction technique for L-DOPA despite an extensive work is reported for isolation, identification and pharmacological activities of L-DOPA from various plant sources. Keeping this in view, present investigation was done to study the

extraction efficiency of many methods that could be used to extract L-DOPA from plant samples.

MATERIAL AND METHODS

Collection and preparation of Sample

Vicia faba var major was obtained from Museum Botanical, Department Of Life Sciences, University of Siena, Italy and *Vicia faba var minor* was sent by NBPGR, Delhi for research purpose. The seeds were oven dried at 40°C and powdered using a grinder. It was passed through a sieve to achieve fine powder.

Preparation of Standard

99.9% pure L-DOPA standard was obtained from Pallav Chemicals and 1000 ppm standard stock was prepared.

Chromatographic conditions and instrumentation

Chromatographic separation was performed with AGILENT HPLC (Model no. 1220 Infinity) equipped with quaternary pump and auto injector (20µl). OpenLab CDS Version A.04.06 chromatographic software was used for data acquisition. Kromasil100–5–C18 (250mm × 4.6mm × 5µ); Part / Serial No: M05CLA25/E117509 column was used for analysis. Mobile Phase used was Water / Methanol / AcetoNitrile (100:60:40) (v/v) containing 0.2% Triethylamine, pH = 3.3 was filtered through 0.45 micron membrane filter (Millipore) and degassed by sonication; flow rate of 1 ml / min was maintained throughout the run. Column effluent was monitored at 280 nm with variable wavelength UV detector.^[11]

Method Validation

Validation of the HPLC method was carried out as per ICH guidelines.^[12] Parameters such as Linearity, Accuracy, Precision, LOD and LOQ were taken up as tests for analytical method validation and the values are listed in table 1.

Table 1: Method Validation.

Parameters	Values
Linearity	100-700 ppm
Correlation coefficient	0.992
Accuracy (Standard Addition Method).	98.83% recovery
Precision	
Intraday	0.189
Interday	0.754
Limit of Detection (LOD)	2 ppm
Limit of Quantitation (LOQ)	5 ppm
Stability	Assay not decreased below 8%

Preparation of the Plant extracts

Extracts were made using various techniques as listed below:

Method 1:^[13]

This method was proposed by Takashi *et. al.*, 2011. The preparation of the sample remained the same and only

the solvents used for the extraction have been changed so as to check the extraction efficiency of the same procedure with different concentration. The various Solvent systems used were:

Method 1.1: acetonitrile:water:formic acid (80:20:1)

Method 1.2: acetonitrile:water (50:50),

Method 1.3: acetonitrile:water:formic acid (50:50:1),

Method 1.4: acetonitrile:formic acid (100:1),

Method 1.5: acetonitrile:water (80:20)

Method 2:^[14]

1. The seed powder was defatted with acetone and then suspended in water: ethanol (1:1) with 0.1 % ascorbic acid for 3 overnights. This was performed with regular change of solvents.
2. It was diluted 1:100 by using water: ethanol (1:1) with 0.1% ascorbic acid for HPLC analysis.

Method 4:^[15]

1. The seed powder was suspended in Water: Methanol (50:50) (v/v) and let it stand for 2 hrs unlike the original method.

Method 5:^[16]

1. In this method, heat reflux was done for the seed powder using 0.1N HCl solution.

Method 6:^[17]

1. The seed powder was treated with water: ethanol 30:70, kept in tightly closed container for 7 days.
2. The supernatant was separated.

RESULTS AND DISCUSSIONS

The HPLC method discussed in the present work provides a convenient and accurate way for analysis of L-DOPA in *Vicia faba*. The retention time of standard L-DOPA is 2.363 mins as shown in fig. 1. As shown in fig. 2 and 3, respectively, *Vicia faba var. major* shows retention time of 2.283 mins and *Vicia faba var. minor* shows retention time of 2.293 mins; it confirms the presence of L-DOPA in both the varieties of this plant samples.

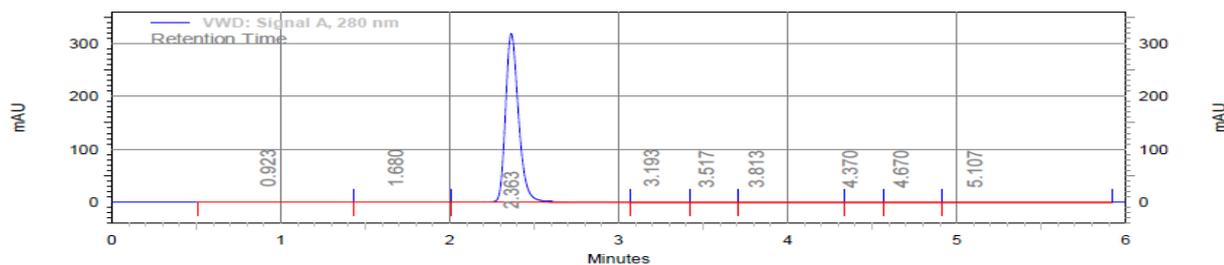


Figure 1: Chromatogram of the Standard.

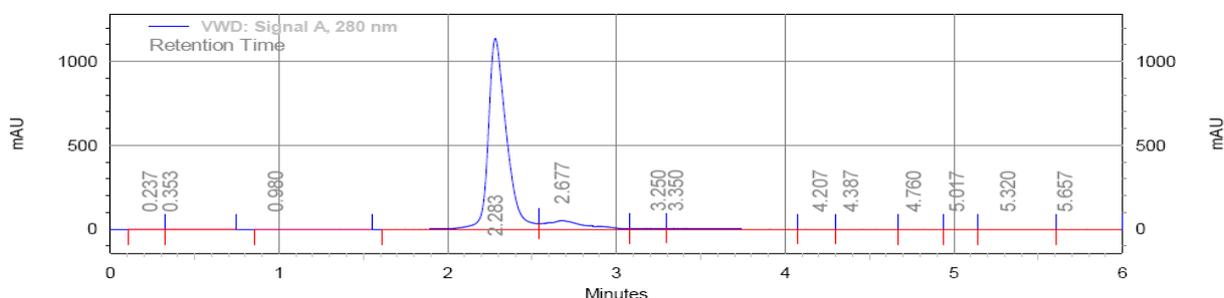


Figure 2: Chromatogram of Method 1.3 for *Vicia faba var. major*.

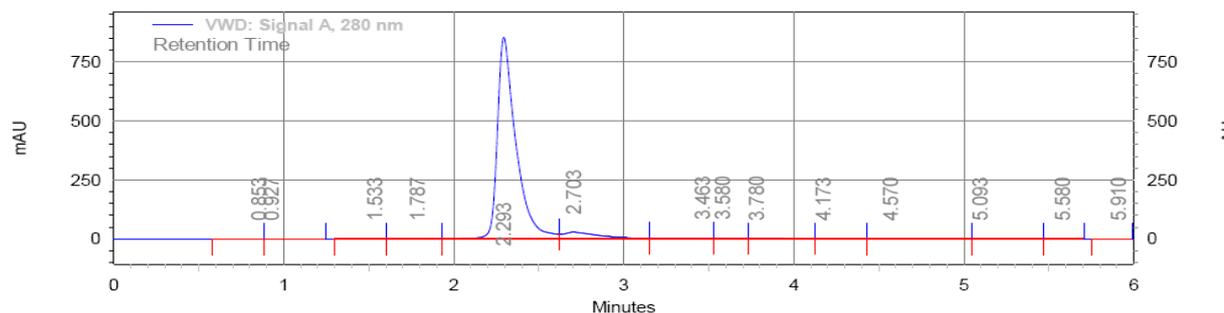


Figure 3: Chromatogram of Method 1.3 for *Vicia faba var. minor*.

For quantitation purposes all the plant extract were made in triplicates and tested by HPLC. The Area under the Curve/ peak area was considered and used for calculations. The Formulae used were as follows:

$$\text{Response factor} = \frac{\text{Peak Area}}{\text{Standard Amount}}$$

$$\text{Amount of Unknown in the sample} = \frac{\text{Peak Area}}{\text{Response Factor}}$$

$$\% \text{ Content} = \frac{C \times V \times D}{10000 \times W}$$

Where,

C = conc in mg/L

D = dilution factor

V = final total volume

W = Weight of the sample taken in g

In proposed method, Linearity was observed in the concentration range of 100–700 ppm. The mean values of L-DOPA content in the seed powder extracted by each of these methods are compiled in the Figure 4.

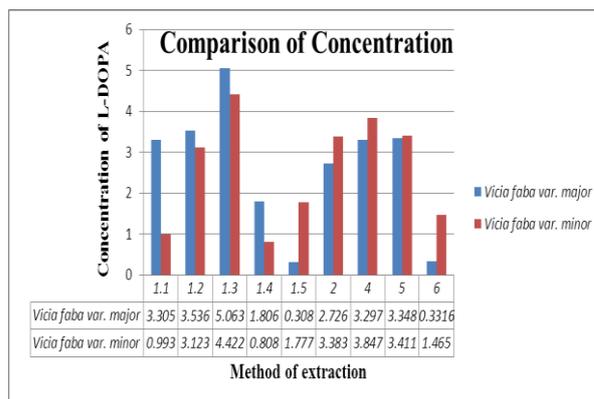


Figure 4: Comparison of extraction efficiency of L-DOPA from both the varieties of *V. Faba*.

Method 1 gave a good separation and comparing the various ratios of Acetonitrile, Formic acid and water used; acetonitrile:water:formic acid (50:50:1) gave the best extraction efficiency of 5.063% and 4.422% in *Vicia faba var. major* and *Vicia faba var. minor*, respectively. Method 1.5 showed the least concentration of L-DOPA in *V.faba var. major*; whereas Method 1.4 showed the least concentration for *Vicia faba var. minor*. Overall if considered, in some methods extraction of L-DOPA is

higher in major variety while in others it is high in the minor variety, this may be due to the reaction of other phytochemicals with the solvents used for extraction.

CONCLUSION

Use of suitable extraction methods will increase versatile utilization of *Vicia faba* seeds with high levels of bioactive compounds for the management of chronic diseases like Parkinson's. The present investigation suggests Acetonitrile to remain the best solvent from all the solvents used for maximum extraction of L-DOPA for both *Vicia faba var. major* and *Vicia faba var. minor*. This will further help standardize procedures for extraction of L-DOPA from broad beans and make a natural medicine against the symptoms of various diseases. However, for industrial application purposes, further investigations are required to develop mathematical model to control and predict the optimization parameters of the extraction process.

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REFERENCES

1. Simuni T, Hurtig H. Levodopa: a pharmacologic miracle four decades later. In: Factor SA, Weiner WJ (eds). Parkinson's Disease-Diagnosis and Clinical Management. New York; Demos, 2008; 471–490.
2. Rabey JM, Vered Y, Shabtai H, Graff E, Harsat A, Korczyn AD. Broad bean (*Vicia faba*) consumption and Parkinson's disease. *Adv Neurol*, 1993; 60: 681–4.
3. Vered Y, Grosskopf I, Palevitch D, Harsat A, Charach G, Weintraub MS, Graff E. (The influence of *Vicia faba* (broad bean) seedlings on urinary sodium excretion). *Planta Med*, 1997; 63(3): 237–40.
4. Singh AK, Bhatt BP, Sundaram PK, Gupta AK, Singh D. Planting geometry to optimize growth and productivity in faba bean (*Vicia faba* L.) and soil fertility. *J Environ Biol*, 2013; 34(1): 117–22.
5. Etemadi F, Hashemi M, Xing B, Mashayekhi H. Accumulation Trend of L-Dopa in Different Plant Parts of Fava Beans Varieties. 2014 Stockbridge School of Agriculture, University of Massachusetts Amherst, MA. 01003.
6. Muehlbauer F, Tullu A. *Vicia faba* L. Purdue Univ., Cent. New Crops Plants Prod., New Crop Factsheet, 1997.
7. McVicar, R, Panchuk D, Brenzil C, Hartley S, Pearse P, Vandenberg A. Faba bean. Gov. Saskatchewan, Agriculture, Crops, 2013.
8. Smith LA, Houdijk JGM, Homer D, Kyriazakis I. Effects of dietary inclusion of pea and faba bean as a replacement for soybean meal on grower and finisher pig performance and carcass quality. *J Anim Sci.*, 2014; 91(8): 3733–41.
9. Blair R. Nutrition and feeding of organic pigs. Wallingford, UK; Cabi Series, CABI: 2007.
10. Jezierny D, Mosenthin R, Bauer E. The use of grain legumes as a protein source in pig nutrition: a review. *Animal Feed Sci Technol*, 2010; 157(3–4): 111–28.
11. Rathod BG, Patel NM. Development of validated RP-HPLC method for the estimation of L-Dopa from *Mucuna pruriens*, its extracts and in Aphrodisiac formulation. *Int J Pharm Sci Res.*, 2014; 5: 508–13.
12. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf.
13. Takashi H, Takahashi I, SaijoMasaaki K, Tomohide F, Tomoko N, Yuji M. Quantitative Determination of L-DOPA in Dietary Supplements Containing *Mucuna pruriens* by High Performance Liquid Chromatography. *Chiba Prefecture Wei Yan Annual Report No.*, 60: 2011; 53–6.
14. Misra L, Wagner H. Extraction of bioactive principles from *Mucuna pruriens* seeds. *Indian J Biochem Biophys*, 2014; 44: 56–60.
15. Vachhani UD, Trivedi M, Bajaj A, Shah CP. A HPTLC method for quantitative estimation of L-dopa from *MucunaPruriens* in polyherbal aphrodisiac formulation. *Res J Pharm Biol Chem Sci*, 2011; 2(2): 389.
16. Raina AP, Khatri R. Quantitative Determination of L-DOPA in Seeds of *Mucuna Pruriens* Germplasm by High Performance Thin Layer Chromatography. *Indian J Pharm Sci*, 2011; 73(4): 459–62.
17. Kasture V, Sonar V, Patil P, Musmade D. Quantitative Estimation of L-Dopa from Polyhebal Formulation by using RP-HPLC. *Am J Pharmtech Res.*, 2014; 4(3): 408–414.