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RESEARCH ARTICLE

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A validated LC–MS/MS method for quantitative determination of L-dopa in Fagioli di Sarconi beans (*Phaseolus vulgaris* L.)

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Abstract

An analytical method based on ultrasound assisted extraction (UAE) and liquid chromatography coupled to electrospray tandem mass spectrometry (LC–ESI/MS/MS) was validated and applied for determining L-dopa in four ecotypes of Fagioli di Sarconi beans (*Phaseolus vulgaris* L.), marked with the European label PGI (Protected Geographical Indication). The selectivity of the proposed method was ensured by the specific fragmentation of the analyte. Simple isocratic chromatographic conditions and mass spectrometric detection in multiple reaction monitoring (MRM) acquisition mode were used for sensitive quantification. The LC–ESI/MS/MS method was validated within a linear range of 0.001–5.000 μg/mL. Values of 0.4 and 1.1 ng/mL were obtained for the limits of detection and quantification, respectively. The repeatability, inter-day precision, and recovery values ranges were 0.6%–4.5%, 5.4%–9.9%, and 83%–93%, respectively. Fresh and dried beans, as well as pods, cultivated exclusively with organic methods avoiding any synthetic fertilizers and pesticides were analyzed showing an L-dopa content ranging from 0.020 ± 0.005 to 2.34 ± 0.05 μg/g dry weight.

KEYWORDS
beans, LC–MS/MS, L-dopa, quantitative analysis, validation

1 | INTRODUCTION

L-dopa or levodopa (LD) (Figure 1) belongs to the class of the catecholamine compounds, and it is an amino acid analogue. It acts in the areas of brain that are related to emotional and psychomotor functions, like neurotransmitters.[1] LD is nowadays considered as a gold standard for the pharmacological treatment of Parkinson's disease motor symptoms.[2] The symptoms of Parkinson's disease (PD) are linked to dopamine depletion; however, dopamine is not an effective treatment for PD as it is too polar to easily pass through the blood–brain barrier while LD, the natural precursor, can cross the blood–brain barrier and be converted to dopamine in the brain. The effectiveness of the drug decreases after a long time intake and severe side effects could happen after a $t_{1/2}$ half-life of 50–90 min, including on–off oscillations, that is, motor fluctuations, hallucinations, dyskinesia and orthostatic hypotension.[2–5] Accordingly, extended-release L-dopa formulations integrated with other drugs have been developed in order to enhance the bioavailability and half-life and minimize side effects. In addition, the administration of a food diet based on specific vegetables belonging to the Leguminosae family as natural adjuvants is strongly recommended.[1,6] Indeed, Leguminosae have been exploited as an alternative source for LD isolation. Legumes belonging to genus *Mucuna*, commonly known as velvet beans, were found to contain the maximum level of levodopa, and

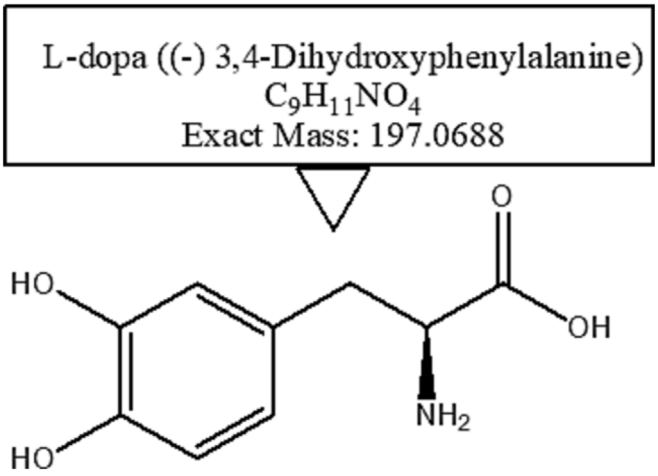

FIGURE 1 Chemical structure of L-dopa and its exact mass value.

among the various species, *M. holtonii*, *M. pruriens* and *M. monosperma* showed high L-dopa amounts in their seeds, which are promising.[7,8] Other best plant sources of L-dopa are seed sprouts, pods, and broad beans from fava bean (*Vicia faba* L.), which is employed both as weld beans or broad beans for animal or human food consumption.[9] Although high amount of dopamine precursor have been detected in velvet bean, its employment as a source of L-dopa is quite limited because of its restricted distribution to the tropical regions of Africa and Asia.[10] On the contrary, the wider geographical distribution of fava makes this plant a potentially more appealing source of LD than the velvet bean, and simultaneously, other leguminous matrices are being investigated for these purposes, such as *Phaseolus* beans.[11] Although no studies concerning the use of *Phaseolus vulgaris* in patients affected by Parkinson's disease are available, it is being viewed as a potential therapeutic means in PD, and several efforts are being done towards the identification of the best cultivating conditions under which the amount of L-dopa in *Phaseolus* could be enhanced.[12]

The analytical determination of L-dopa is challenging because of the chemical instability of this compound, which requires a careful and reproducible matrix extraction and analytical determination.[13–15] Among the analytical methods for LD determination in food matrices, liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) offers some advantages as the characteristic fragmentation pathway of the analyte provides structural information necessary for LD characterization. Moreover, LC–MS/MS remains the most sensitive analytical technique for quantitative analysis, with limit of detection (LOD) and limit of quantification (LOQ) values lower than those from other analytical techniques reported in the literature.[1]

A literature screening revealed the presence of few methods for LD determination in plants different from *M. pruriens* and *V. faba*. LD determination in *M. pruriens* and *V. faba* matrices has been mainly conducted by high performance liquid chromatography coupled to diode array detection set at 280 nm and chromatographic column with C-18 stationary phase with acidified aqueous solutions mixed with methanol or acetonitrile as mobile phases.[16–18] On the other hand, for other food matrices including sunflower, sesame and pumpkin seeds, or sweet potatoes, electrochemical methods based on the use of modified electrodes have been proposed, mainly because of the ease of LD to oxidize.[19,20] To the best of our knowledge, no detailed LC–MS/MS assay for LD determination in *P. vulgaris* L. beans has been described so far. Therefore, the main goal of this present study was to develop and validate an efficient and sensible LC–ESI/MS/MS method for LD determination and quantification in Fagioli di Sarconi (*P. vulgaris* L.) beans and pods, cultivated in Basilicata (southern Italy) and marked with the PGI (Protected Geographical Indication) label, in order to promote their application as potential pharmacological adjuvants for Parkinson's disease treatment. According to validation guidelines, a validation study is required when a matrix sample is changed to confirm the performance characteristics, and because of the potential application of foodstuff as natural adjuvants for the treatment of Parkinson's disease, the validation of a sensitive and selective method for LD quantitative determination in common beans is of utmost importance for the whole scientific community.

2 | EXPERIMENTAL

2.1 | Chemicals and reagents

The analytical standards of LD (3,4-dihydroxy-L-phenylalanine) and LD-(phenyl-d_3) (3-(3,4-dihydroxyphenyl-2,5,6-d3)-L-alanine) were purchased from Sigma Aldrich (Milano, Italy). The hydrochloric acid (HCl, 37%), which was used for standards dissolution and beans extraction, and methanol (HPLC grade), which was used for chromatographic separation, were obtained from Sigma Aldrich (Milano, Italy). Formic acid (98%), which was used as an additive of aqueous mobile phase was purchased from Carlo Erba Srl (Milano, Italy). Deionized water was prepared with a Milli-Q (Millipore, Darmstadt, Germania) water purification system.

2.2 | Apparatus and equipment

Besides the usual laboratory tools, the following equipments were used: ultrasonic bath sonicator (Sonorex Super RK 100/H; Bandelin electronic, Berlin, Germany); centrifuge (Kontron A8.24 rotor, Augsburg, Germany); 0.20 µm polytetrafluoroethylene (PTFE) syringe filter (Whatman, Maidstone, UK); Nexera-e liquid chromatograph system (Shimadzu, Kyoto, Japan) equipped with a CBM-20A controller; four LC-30AD dual-plunger parallel-flow pumps; a CTO-20AC column oven; a $DGU20A_{SR}$ degasser; and a SIL-30AC autosampler connected to a SCIEX QTRAP 6500 MS (SCIEX, Foster City, CA, USA), interfaced through an electrospray ionization (ESI) source. A Kinetex C_{18} reversed-phase column (dimension 100 × 2.1 mm; stationary phase with particle size 2.6 µm) (Phenomenex, Aschaffenburg, Germany) was used for chromatographic separation.

2.3 | Calibration curve and blank samples

Stock solutions of LD and LD-(phenyl-d_3) at a concentration of 1000 μg/mL were prepared in 0.1 mol/L HCl. The same acidic solution was used to prepare the working standard solutions from the stock solutions employed to build the calibration curve for analyte quantification by using the internal standard (IS) method approach. To build the calibration curve, nine working standard solutions were prepared from the stock solutions at concentrations of 0.001, 0.025, 0.050, 0.100, 0.250, 0.50, 1.0, 2.0, and 5.0 μg/mL. Commercial dried chickpea extracts were used as blank samples to spike LD external standard solution and LD-(phenyl-d_3) IS solution. Blank samples were spiked at low, medium, and upper levels of concentration (LL, ML, UL) of LD, that is, 0.001, 0.500 and 5.000 μg/mL, respectively. Each sample and working standard solutions were spiked with LD-(phenyl-d_3) at 0.5 μg/mL as IS solution.

2.4 | Fagioli di Sarconi (*P. vulgaris* L.) beans extraction

Fresh and dried samples of PGI Fagioli di Sarconi (*P. vulgaris* L.) beans and pods were provided by a local producer in Paterno (Basilicata, Italy). Four ecotypes were analyzed, that is, Cannellino rosso, Munachedda nera, San Michele rosso, and Risi. Extraction conditions proposed by Tesoro et al[21] were used for the LD extraction from fresh and dried beans and from pod samples. Briefly, beans and pods were reduced to a fine powder in a commercial coffee mill. HCl (0.1 mol/L) was used as the extracting solution by employing an extraction ratio of 1:10 weight dry/volume. All powdered samples were sonicated for 20 min in an ice bath (4°C) and then centrifuged for 10 min at 6000*g*. The extraction was repeated twice, after which the collected supernatants were filtered on PTFE 0.2 μm filters and stored at 4°C in the dark until the LC–MS/MS analyses. LD quantification on bean extracts was performed by the IS method.

2.5 | Liquid chromatography and mass spectrometric conditions

Chromatographic separation was carried out on a Shimadzu Nexera chromatograph with a Kinetex C_{18} analytical column. A mobile phase consisting of 99% A (formic acid 0.2% v/v) and 1% B (methanol) was eluted with an isocratic flow rate of 200 μL/min. Injection volume was set at 10 μL, and the total run time for each sample analysis was 10 min. Mass spectra were obtained using an AB SCIEX QTRAP 6500 system equipped with electrospray ionization source. Positive ion mode was chosen for the electrospray ionization. The optimized source-dependent parameters were: capillary voltage, 5 kV; sheath gas pressure, 25 psi; curtain gas (CUR), 40 psi; collision gas (CAD), medium; ion spray voltage, (IS) 4500; and source temperature 349°C. Quadrupoles 1 and 3 were both maintained at unit resolution and set at a scan speed value of 10 Da/s. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode, and collision induced dissociation (CID) was used as a fragmentation technique. The selected mass transitions were *m/z* 198.0 → 181.0 and *m/z* 198.0 → 152.0 for LD and *m/z* 201.0 → 184.0 and *m/z* 201.0 → 155.0 for LD-(phenyl-d_3). Collision energies of 20 eV were used for both LD and LD-(phenyl-d_3). Both the transitions were needed for LD identification in beans extracts, but only the *m/z* 198.0 → 152.0 transition was considered for quantification. The CID coupled to tandem mass spectrometry (CID–MS/MS) spectrum obtained for the analytical standard of LD and the proposed fragmentation pathway are shown in Figure 2.[22,23] In order to acquire and analyze data, Analyst 1.6.2 software was used. For the quantification of data, the target ion peak area ratios of LD to that of the IS were

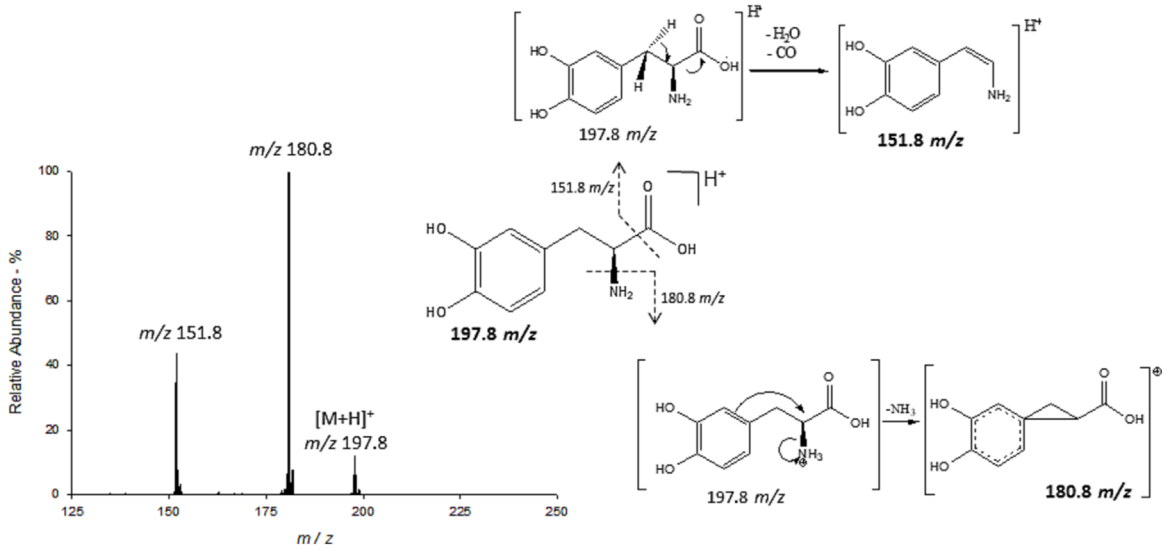

FIGURE 2 Collision induced dissociation coupled to tandem mass spectrometry (CID–MS/MS) spectrum of L-dopa acquired in positive ion mode and proposed fragmentation pathway of the protonated molecule of L-dopa $[M + H]^+$ at *m/z* 197.8.[22,23]

compared with the calibration curve, where ratios of the peak area of the calibration standards were plotted against their concentrations.

2.6 | LC–MS/MS method validation

A comprehensive validation of the proposed LC–MS/MS method for the determination of LD in *P. vulgaris* samples was performed, following the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines.[24] The method has been validated for specificity, linearity, LOD, LOQ, precision and trueness.

2.6.1 | Specificity and linearity

The specificity of the method was measured by its inability to be interfered by other components of the sample at the established retention times (RTs) for the analytes and the IS. As regard to the identification requirements for MS detection, an analysis of the correspondence of the RTs of the analytes in the bean extracts with that of the average of the calibration standards measured in the same sequence with a tolerance of ±0.2 min was done. The ion ratio (IR) was calculated as the ratio between the response of the signal with the lower area and that of the peak with the higher area. It was found to be within ±30% of the IR calculated from the average of the calibration standards from the same sequence. A calibration curve was obtained by plotting the area of the ratio of the external and internal standards against the known concentration of each compound. Linearity was evaluated at nine concentration levels ($k = 9$) and analyzed in three independent replicates ($n = 3$) over the range 0.001–5 μg/mL. Linearity was evaluated by the residual distribution analysis and the determination coefficient (R^2) of calibration curve and was also supported by statistic *t*-test for R^2.

2.6.2 | LOD and LOQ

The LOD, equivalent to the lowest detectable analyte concentration, and the LOQ, equivalent to the lowest quantifiable analyte concentration, were calculated according to the method proposed by ICH based on the standard deviation of the blank response (σ) and the slope (S) of the calibration curve, as: $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$.[24–26] Afterwards, the calculated LOQ values were validated by analyzing spiked blank samples at the level of the interest.

2.6.3 | Precision

The precision of the proposed method was studied as "repeatability" and "intermediate precision", expressed as percentage relative standard deviations (RSD%). These parameters were calculated for three replicates ($n = 3$) at three different concentration levels, that is, 0.001, 0.5, and 5 μg/mL, corresponding to LL (low level) as well as the LOQ, ML (medium level), UL (upper level). The RSD% for three replicates ($n = 3$) of three levels ($k = 3$) over the linear range in the same day ($p = 1$) was referred to as repeatability, which is the precision under the same operating conditions over a short time interval. Intermediate precision, that is, the within-laboratories variations (different days, different analysts, different equipment, etc.) was calculated as the RSD% within several days ($p = 3$) for three replicates ($n = 3$) of three levels ($k = 3$) over the linear range.[24,27]

2.6.4 | Recovery

The recovery test was performed to evaluate the trueness of the analytical procedure. As a certified reference material is not commercially available, recoveries were estimated by spiking chickpea extract samples (i.e., samples considered as blank) with known amounts of LD at least at three concentration levels ($k = 3$) over the linear range for three replicates ($n = 3$) at each level over 3 days ($p = 3$). The fortified concentration levels were: 0.001 μg/mL, 0.5 μg/mL and 5 μg/mL, corresponding to LL as well as the LOQ, ML, and UL, respectively. The recovery was calculated based on the difference between the amount of analyte measured in the spiked sample and the amount of analyte determined in the blank, divided by the amount of the spiked analytical standard.

2.6.5 | Matrix effect

To determine the degree of ion suppression or enhancement, matrix effect was quantitatively assessed by the post-extraction spike method. In detail, matrix effect was calculated as the ratio of the response of LD in neat solution to the response of LD spiked into the blank matrix sample that was carried through the sample preparation process.[28] Two concentration levels ($k = 2$) over the linear range for three replicates ($n = 3$) at each level were used, that is, 0.001 μg/mL (Low Quality Control, LQC) and 5 μg/mL (High Quality Control, HQC).

2.7 | Application of the method

The validated method was successfully used to determine LD concentrations in the four ecotypes of PGI Fagioli di Sarconi beans (*P. vulgaris* L.), that is, Cannellino rosso, Munachedda nera, San Michele rosso and Risi. Moreover, commercial black bean samples were also analyzed for comparison. Beans and pods of selected ecotypes were used for LD quantification, both dried and fresh. Ten microliter of each undiluted extract were injected into the LC–MS system. Quantitative analyzes were replicated three times ($n = 3$) for each sample extract, in order to express the data as mean ± standard deviation.

3 | RESULTS AND DISCUSSION

3.1 | Method development

Method development involved several steps in order to optimize chromatographic separation and mass spectrometric detection parameters. As far as extraction is concerned, the same parameters already optimized by the authors for LD extraction from *V. faba* beans have been employed.[21] LD shows poor stability in aqueous solutions, naturally degrading over time. Therefore, HCl 0.1 M has been employed as solvent for ultrasound assisted extraction ensuring a reproducibility with RSD% lower than 4.30%.[21]

3.1.1 | Chromatographic separation

The chromatographic conditions were chosen on the basis of a previously conducted study for the development and validation of a liquid chromatography–ultraviolet (LC–UV) method, aiming at LD determination in *V. faba* extracts. A careful investigation of the separative chromatographic conditions was carried out by Tesoro et al.[21] Briefly, different mobile phases, containing an acidic aqueous solution of acetic acid or formic acid at a concentration of 0.2% and methanol at a percentage varying from 1% to 5%, were tested. A percentage not higher than 5% of the methanolic organic phase was suggested to limit the stationary phase collapse. A clear improvement in terms of resolution of the LD chromatographic peak was observed with the increase in percentage of the aqueous phase, while the employment of formic acid ensured a longer RT for LD compared with acetic acid, as formic acid is known to be an ion-pairing reagent, whereas acetic acid is not.[21,29] Based on these results, formic acid 0.2% v/v containing 1% of methanol resulted in the best mobile phase for LD separation and was repurposed here for LD separation with an LC-ESI/MS/MS method. Two short-length columns, that is, Discovery C_{18} column, 150 × 2.1 mm, 5 µm and Kinetex Phenomenex C_{18} 100 × 2.1 mm, 2.6 µm column, were tested for method development. As the Kinetex gave satisfactory peak shapes for LD and a good signal/noise response compared with the other one, it was chosen for further analyzes.

3.1.2 | Mass spectrometric detection

The Q-Trap instrument parameters were optimized to achieve signal sensitivity and stability by direct injection of the LD standard solution into the electrospray ion source operated at both polarities at a flow rate of 10 µL/min. As LD contains a basic functionality $>NH_2$, the signal intensity was higher in positive ion mode. The predominant peak in the primary ESI mass spectra of the LD standard corresponds to the protonated molecule $[M + H]^+$ at *m/z* 197.8. Product ions scanned after collision with nitrogen showed signals at *m/z* of 180.8 and 151.8 (Figure 2), due to the loss of NH_3 and H_2O and CO, respectively.[1] The study of the CID–MS/MS mass spectrum allowed to select the specific transitions of LD to be set in the Q-TRAP mass spectrometer. MRM acquisition mode allowed to achieve a specific and selective detection of LD.

3.2 | Method validation

The specificity of a method is defined as the ability to unequivocally assess the analyte in the presence of compounds that may be expected to be present (i.e., impurities, degradants, matrix, etc.).[24] Here, the specificity of the proposed LC-ESI/MS/MS method was evaluated by verifying the absence of interfering peaks at the established RTs of LD in bean extracts. The ion ratio met the required identification criteria as it was within ±30% to that obtained from the average of the calibration standards from the same sequence. It was possible to confirm that no other peaks interfered with the analyte of interest in MRM detection mode.[24,30] The extracted ion

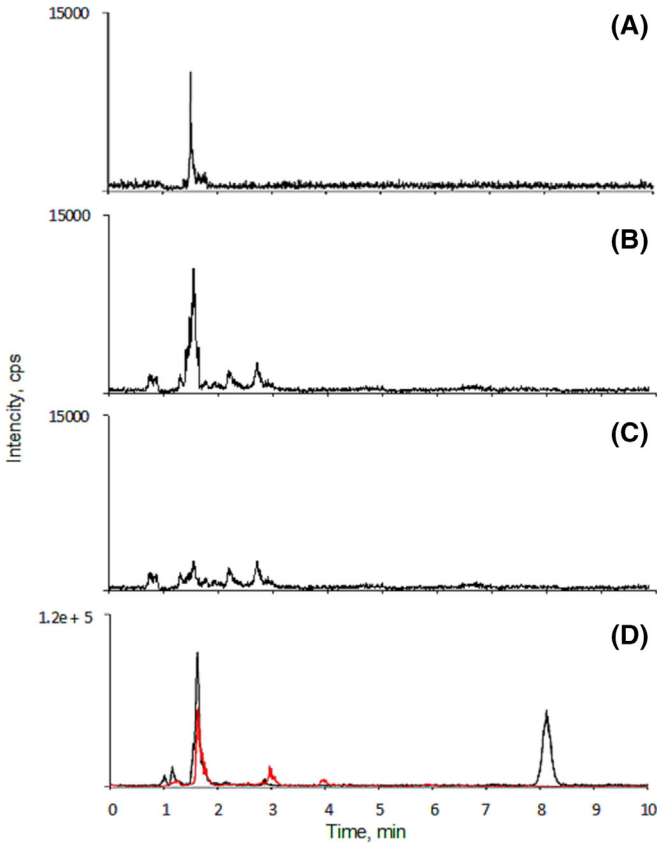

FIGURE 3 Representative extracted ion chromatograms (XICs) of a standard solution of L-dopa at 0.001 µg/mL (A) A blank sample spiked with L-dopa at 0.001 µg/mL. (B) Unspiked blank sample. (C) Extract of Fagioli di Sarconi (*Phaseolus vulgaris* L.) beans, Risi ecotype. (D) Red line is used for XIC corresponding to *m/z* 198.0 → 181.0 transition, while black line is used for XICs corresponding to *m/z* 198.0 → 152.0 transition. In bean extract samples (D), the presence of an isobaric compound at a longer RT than L-dopa responding to *m/z* 198.0 → 152.0 transition is detectable.

TABLE 1 Validation data for L-dopa in *Phaseolus vulgaris* L. samples, by using LC–MS/MS method.

Calibration curve					Precision		
Linear range (t calc.)[a]	Linear equation[b], R^2	LOD (ng/mL)	LOQ (ng/mL)	Level (μg/mL)	Repeatability[c] RSD%	Interm. Precision RSD %	Recovery %
0.001–5 μg/mL (45.98)	$y = (2.03 \pm 0.04)x + (0.24 \pm 0.16)$ $R^2 = 0.997$	0.4 ± 0.1	1.1 ± 0.1	0.001 (LOQ)	4.5%	9.9%	83%
				0.5 (ML)	3.9%	5.4%	91%
				5 (UL)	0.6%	7.5%	93%

Abbreviations: LC–MS/MS, liquid chromatography coupled to tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; ML, medium level; RSD%, percentage relative standard deviations; UL, upper level.

[a]Calculated t-value was compared to tabulated value $t_{0.01,4} = 3.499$ ($k = 7$).

[b]Calibration fitting: $y = x(m \pm s_m) + q \pm s_q$.

[c]The repeatability was estimated for three replicates ($n = 3$) of three levels ($k = 3$) over the linear range in the same day ($p = 1$). The intermediate precision was calculated within 3 days ($p = 3$) over the linear range. P-value for correlation coefficients is $p < 0.01$, and the residuals follow a random distribution.

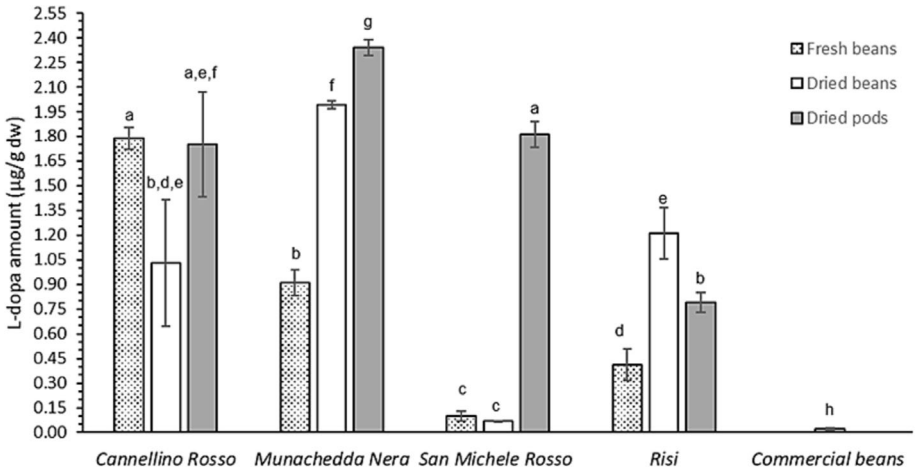

FIGURE 4 Histogram about quantitative results for L-dopa in fresh, dried, and pods extracts of PGI Fagioli di Sarconi (*Phaseolus vulgaris* L.) beans. The results for a commercial dried black beans extract are also reported. The amounts of L-dopa are reported as μg/g dry weight ± standard deviation. Values marked by the same letter are not significantly different ($p < 0.05$).

chromatograms (XICs) acquired in MRM mode of an LD standard solution at 0.001 μg/mL, a blank sample spiked at 0.001 μg/mL, an unspiked blank sample and an extract of Fagioli di Sarconi beans (Risi ecotype), are reported in Figure 3A–D. In Figure 3D the XICs corresponding to m/z 198.0 → 181.0 (red line) and m/z 197.8 → 152.0 (black line) transitions are reported, respectively. It is worth noting that the RT and both the selected transitions were important to identify LD in beans extracts because of the occurrence of an isobaric compound that responded to m/z 198.0 → 152.0 transition.

After checking for linearity (by means of the residual distribution analysis and the F-test), the calibration curve was considered acceptable with the coefficient of determination (R^2) above 0.997 within concentration range (0.001–5) μg/L for LD. In addition, a statistical t-test was performed to evaluate the significance of the R^2 correlation coefficient and the significance (p-value) of this correlation was also provided ($p < 0.01$). As the calculated t value resulted higher than the reference value ($t_{0.01,4} = 3.499$ [$k = 7$]), a linear relationship for the experimental data set was confirmed. LOD and LOQ were 0.4 ± 0.1 ng/mL and 1.1 ± 0.1 ng/mL (validated value with spiking experiments), respectively (Table 1). The obtained values were lower than LOD reported in other analytical methods based on LC–MS/MS; therefore, the high sensitivity of this method could be ascertained.[31,32] The RSD% ranged from 0.6% to 4.5% for repeatability and from 5.4% to 9.9% for intermediate precision (Table 1). The accuracy of the method was determined by recovery tests, analyzing chickpea extract samples spiked with standard solutions of LD at 0.001, 0.5 and 5 μg/mL. The obtained results were always higher than 83%, in accordance with the recovery values reported in other works.[33] Evaluation of matrix effect forms an important and integral part of validation for quantitative LC–MS/MS methods and it is generally evaluated to assess the degree of ion suppression or, more rarely, ion enhancement which are both unpredictable effects for atmospheric pressure ionization sources. Here, the observed results were well within acceptable limits as the RSD% of the area ratios of the post-spiked recovery samples at the LQC and HQC levels of 10.1 and 3.6, respectively. Therefore, the minor suppression of analyte signal due to endogenous matrix interferences did not affect analyte quantification.[21,25–27,34]

3.3 | Application of the validated method to bean extract samples

The validated method was successfully used to determine LD concentrations in fresh, dried, and pods of Fagioli di Sarconi beans (*P. vulgaris* L.) protected with the PGI mark. Four ecotypes of Fagioli di Sarconi

PGI beans were selected, namely, Cannellino Rosso, Munachedda Nera, Risi, and San Michele Rosso. Moreover, commercial dried bean extracts were analyzed as well for comparison. LD quantification was performed through the IS method. Figure 4 shows the amounts in μg/g (dry weight) of LD quantified in each sample extract. The two-tailed *t*-test of significance was performed to evaluate significant differences between the obtained values. The results showed a significant difference in the LD content between the various ecotypes of fresh Fagioli di Sarconi beans, with Cannellino Rosso ecotype presenting the highest amount of analyte (1.79 ± 0.07 μg/g dry weight). Anyway, the amount of LD detected in bean matrices was lower than *V. faba*; as for this matrix, the reported content is in the order of mg/g dw.[21] For dried beans, LD content ranged between 0.070 ± 0.001 and 1.99 ± 0.03 μg/g dry weight, obtained for San Michele Rosso and Munachedda Nera, respectively. Significant differences ($p < 0.05$) were also found between the fresh and the respective dried ecotypes, with the exception of San Michele Rosso. Cannellino Rosso and Munachedda Nera ecotypes generally showed the highest LD content in fresh and dried samples and pods, compared with the other analyzed beans. Moreover, both PGI Fagioli di Sarconi beans and pod extracts exhibited a more significant amount of LD than commercial black bean samples purchased at a local store (packed shelf-stable beans). This result would highlight the importance of organic cultivation of the local product and its added value compared with products derived from long intensive cultivation processes.[35] Apart from Risi, significantly higher quantities of LD were found for pods than fresh and dried beans; while for Cannellino Rosso, the analyte content was around 1.80 μg/g dry weight for both dried pods and fresh beans. Among all the ecotypes, Munachedda Nera pods presented the highest analyte content. Currently, the interest in the search of food matrices exploitable as source of bioactive compounds with proved health benefits for the treatment and prevention of many disease is increasing,[36,37] as evidenced by the data about pods allowed to repurpose PGI Fagioli di Sarconi pods as potential pharmacological adjuvants for Parkinson's disease. In this way, a waste product would be valorized for pharmacological purposes.

4 | CONCLUSIONS

This study reports for the first time an ultrasound-assisted extraction method of LD from *P. vulgaris* L. bean and pod matrices and its quantification by using LC–ESI/MS/MS. The LC–MS/MS method developed for LD determination is simple, specific, rapid, selective, sensitive, and suitable for routine measurement of Leguminosae samples with a composition comparable to those of beans and low levels of LD. The validated method has been successfully applied to local sample extracts of Fagioli di Sarconi beans in order to evaluate their potential use as food adjuvants for the treatment of Parkinson's disease. As there are no public domain articles concerning the quantification of LD in *P. vulgaris* L. by LC-ESI/MS/MS, this current research becomes unique for the quantification of the target analyte by liquid chromatographic separation coupled to mass spectrometry methodology. The present method provided excellent specificity and linearity with a LOQ of 0.001 μg/mL for LD. Moreover, a 10-min chromatographic run time allows the analysis of several plant samples per day.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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