On the identification and quantification of ergothioneine and lovastatin in mushroom species: A comparison between different analytical approaches

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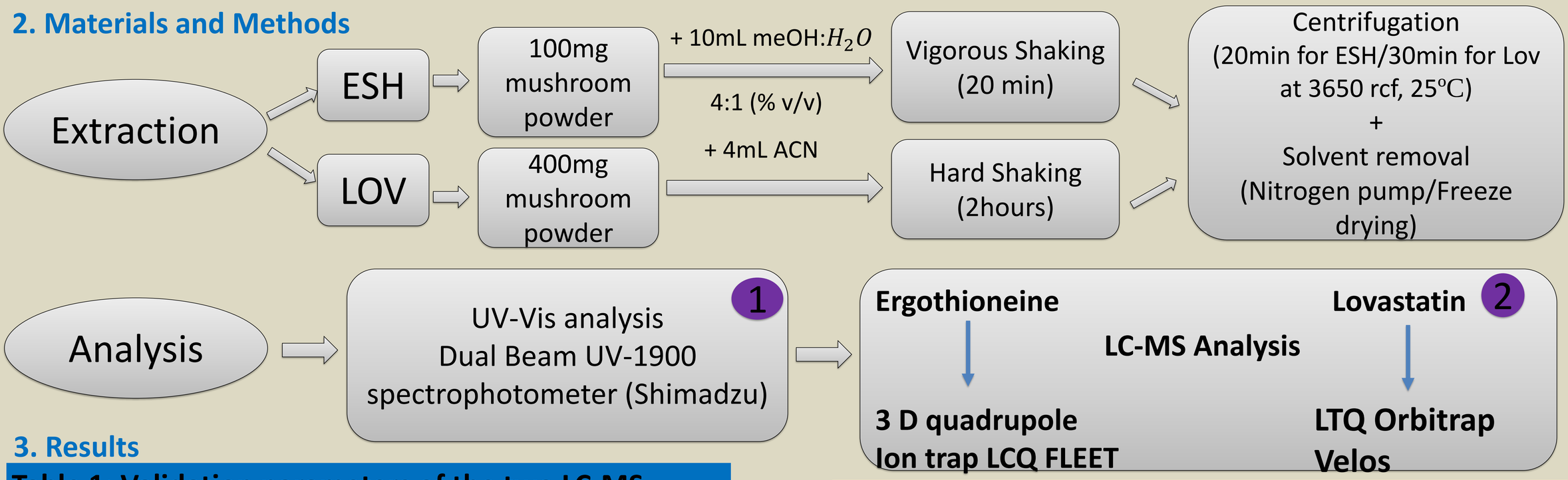
1. Introduction

Mushrooms are considered to be one of the main sources of health promoting bioactive compounds, such as ergothioneine (ESH) and lovastatin (LOV). In the present project we aim to evaluate the content of ergothioneine and lovastatin in different types of mushrooms (A. *Bisporus, P. Ostreatus, P. Citrinopileatus)* as well as that of *Pleurotus Citrinopileatus* cultivated in substrates from winery (Grape Marc, GM) and olive oil (OL) by – products using liquid chromatography mass spectrometry (LC-MS) and Ultraviolent- Visible Spectroscopy (UV-Vis).

2. Materials and Methods



3. Results

Table 1: Validation parameters of the two LC-MS methods

Analytical figures of merit	ESH	LOV
Concentration range (μg mL^{-1})	0.05 – 45	0.001 – 1
Slope (a) (±sa)	0.0307 (±0.00023)	35.47 (±0.18)
Intercept (b) (±sb)	0.0012 (±0.0051)	0.090 (±0.065)
R^2	0.9993	0.9998
LOD (μg mL^{-1})	0.02	0.00039
LOQ (μg mL^{-1})	0.06	0.0012
Accuracy (%)	102,95	105.17
Intra-Day Precision (n=3, % RSD)	2.0	4.91
Inter-Day Precision (N=3)	1.9	3.21
Extraction Recoveries (%)	80%	75%

[1] N: The number of consecutive days required for inter – day precision determination; [2] n: the number of QC replicates

Table 2: ESH-LOV content in conventional cultivated mushrooms (Wheat Straw, Control) using UV-Vis and LC-MS

Method	Ergothioneine Content (mg/ kg dry sample)[a] (n=3)		
	Agaricus	*Ostreatus*	*P. Citrinopileatus* – *WS*
UV – Vis	7100 (±300)B	9200 (± 800)A	8300 (±1100)A
LC – MS	521.2 (±14.7)C	607.3 (±11.2)B	822.1 (±20.6)A
Method	**Lovastatin Content (mg/ g dry sample)[a] (n=3)**		
UV – VIS	1050 (±80)A	930 (±100)A	840 (±250)A
LC-MS	1.39 (±0.014)A	1.11 (±0.042)B	0.158 (±0.05)C

[a] Each value is expressed as mean ± standard error (n=3). Means with different letters within a line are significantly different (P < 0.05)

Table 3: ESH-LOV content in alternative cultivated mushrooms (Grape Marcs, GM and olive oil by-products, OL) using UV-Vis and LC-MS

Method	Ergothioneine Content (mg/ kg dry sample)[a] (n=3)		
	WS	GM	OL
UV – Vis	8300 (±1100)A	11800 (±1400)A	6700 (±1100)A
LC – MS	822.1 (±20.6)A	637.2 (±24.5)B	884.5 (±20.0)A
Method	**Lovastatin Content (mg/ g dry sample)[a] (n=3)**		
UV – Vis	840 (±250)A	860 (±180)A	904 (±0.241)A
LC-MS	0.158 (±0.05)B	0.218 (±0.014)A	0.161 (±0.009)B

[a] Each value is expressed as mean ± standard error (n=3). Means with different letters within a line are significantly different (P < 0.05)

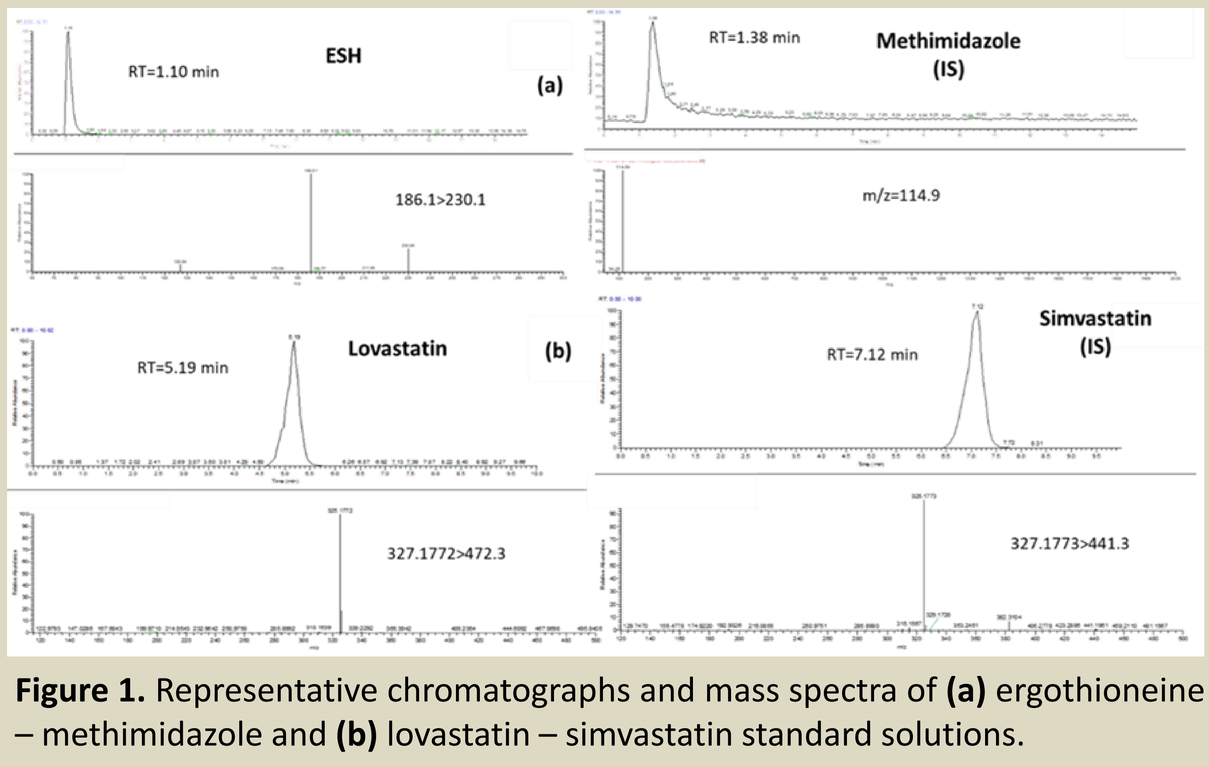


Figure 1. Representative chromatographs and mass spectra of **(a)** ergothioneine – methimidazole and **(b)** lovastatin – simvastatin standard solutions.

4. Conclusion

- **The use of UV – Vis method was hindered due to co-absorbance of different constituents.**
- **LC – MS/MS methodologies were developed, optimized and validated having (a) shorter analysis time and (b) higher resolution**
- ***Pleurotus* genus and especially, *P. Citrinopileatus* contained higher amounts of ergothioneine than *A. Bisporus.***
- ***Agaricus Bisporus* contained higher amounts of lovastatin than P. Ostreatus and especially from P. Citrinopileatus.**
- **Olive oil (OL) and grape marcs (GM) contained the highest amount ergothioneine and lovastatin respectively.**
- **Since by products can affect ergothioneine and lovastatin biosynthetic pathways, a colleration between their bioactive compounds would be an area of investigation.**

5. Acknowledgement

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