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S10. Optimized chromatographic analysis of ergosterol in wild and cultivated mushrooms

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Abstract

Sterols belong to the unsaponifiable fraction of several matrices, where they can be found as free or conjugated structures. In the latter, the 3β-hydroxyl group is esterified with a fatty acid or a hydroxycinnamic acid, or glycosylated with a hexose (usually glucose) or a 6-fatty acyl hexose [1]. Ergosterol (an important vitamin D_2 precursor), is clearly the main sterol in mushrooms. Typically, the analysis of individual sterols includes the extraction of lipids, saponification (which might include a previous acid hydrolysis), extraction of unsaponifiable matter and separation/partial purification of sterols. The subsequent separation step might be performed by high performance liquid chromatography (HPLC), which is faster than gas chromatography analysis and operates under milder column temperatures and non-destructive detection conditions [2]. Herein, an analytical method for ergosterol determination in cultivated and wild mushrooms was developed using HPLC coupled to ultraviolet detection. The chromatographic separation was achieved in a Inertsil 100A ODS-3 reverse phase column using an isocratic elution with acetonitrile:methanol (70:30, *v:v*) at a flow rate of 1 mL/min. Different extraction methodologies were tested, using *n*-hexane, methanol:dichloromethane (75:25, *v:v*) or chloroform:methanol (20:10, *v:v*).

After studying the linearity (11 levels) for ergosterol (t_R = 13.0±0.1 min; coefficient of variation, CV = 0.65%), a seven-level calibration curve (y = 0.6566$\varkappa$ + 0.01098; R^2 = 0.9996) was made using the peak/area ratio versus concentration of the standard (in μg/mL). The average of triplicate determinations for each level was used. The limit of detection (LOD), calculated as the concentration corresponding to 3.3 times the standard error of the calibration curve divided by the slope, was 0.3498 μg/mL; the limit of quantification (LOQ), calculated using the concentration corresponding to ten times the standard error of the calibration curve divided by the slope, was 1.060 μg/mL. The precision of the equipment was evaluated injecting seven consecutive times the sterols extract of the same mushroom. The optimized method proved to be precise (CV = 1.25%). Repeatability was assessed by applying the extraction procedure three times to the same dried mushroom powder. The method proved also to be repeatable (CV = 1.88%). The method accuracy was evaluated by the standard addition procedure (percentage of recovery). The standard mixture was added to the samples in three concentration levels (25, 50 and 100 % of the peak/area concentration, each one in triplicate) before the extraction. The method showed good recovery (above 90%).

Overall, a reproducible and accurate HPLC-UV technique was fully optimized. Chromatographic signals presented good resolution, indicating the suitability of this methodology to assess sterol profiles in future studies. Moreover, the chosen extraction and saponification steps do not require complex conditions, being suitable for routine analysis.

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