Appendix No. 1 to the Test certificate ML 2717/22

Records documenting sample analysis using U-HPLC-HRMS/MS

Testing strategy

For the purposes of analysis of sample ML 2717/22 and to find out in what quantity the substance protodioscin occur in the sample, the analysis was carried out using ultra-high-performance liquid chromatography coupled to tandem high-resolution mass spectrometry (KM15, system (C): U-HPLC-HRMS/MS) technique).

Testing conditions

Sample ML 2717/22 was extracted with 70% aqueous ethanol. The extract was then separated using ultra-high performance liquid chromatography with a reverse phase column. Target analytes detection was performed using a quadrupole HRMS/time-of-flight analyzer (TripleTOF 6600, SCIEX). Quantification of protodioscin was performed by using the calibration curve, for its construction analytical standard of these compounds was employed. PeakView 2.0 and MultiQuant 3.0 software were used for data processing.

Test results

In the records of sample ML 2717/22, the analyte protodioscin was monitored using target analysis. The presence of analyte in the sample was assessed based on the exact m/z value of the [M+Na]+ adduct, isotopic profile, retention time and characteristic fragmentation ions (MS/MS spectrum). **Figure 1** compares the extracted ion chromatograms of the 50 ng/ml (5 µg/g) standard and 10 000x diluted sample ML 2717/22.

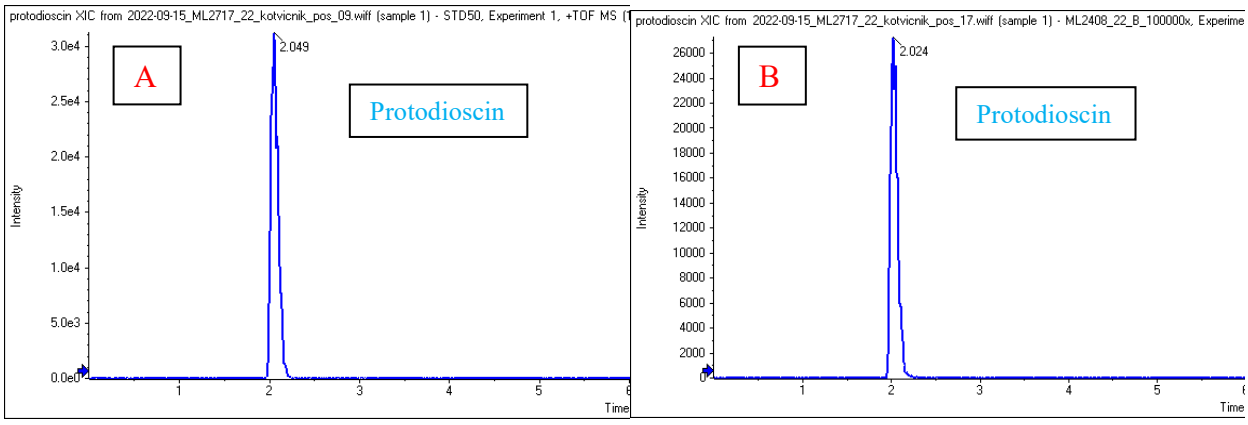

Figure 1: Extracted ion chromatogram A) solvent standard - concentration of 50 ng/ml (5 µg/g), B) 10 000x diluted sample ML 2717/22

Interpretation of test results:

Sample ML 2717/22 was investigated for the amount of protodioscin by target analysis using instrumental technique, U-HPLC-HRMS/MS. Analysis of generated data based on the exact value of m/z adducts, retention

time, isotopic profile and characteristic fragments confirmed the presence of protodioscin in concentration 439 ± 66 mg/g (43,9 ± 6,6 % wt). It can be concluded that the sample contains declared amount 40 % of protodioscin.

End of the appendix

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