Phytochemistry Letters 72 (2026) 104136

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Declared vs determined: Analysis of cannabinoids in commercially available products on the Maltese market

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ARTICLE INFO

Keywords:
Cannabidiol
Cannabinoids
HPLC-UV
Labelling accuracy
Quality control

ABSTRACT

The Cannabis market is rapidly expanding, with an increasing variety of formulations creating a complex regulatory environment. Products containing cannabis fall under different legislative frameworks. Medicinal cannabis products are regulated under pharmaceutical legislation, while commercially available formulations, including oils and cosmetics, containing low concentrations of delta-9-tetrahydrocannabinol (Δ^9-THC) fall under a combination of national and European Union (EU) regulations, presenting regulatory and analytical challenges due to fragmented frameworks and the absence of standardised quantification methods. This study aimed to develop and validate a High-Performance Liquid Chromatography with Ultraviolet detection (HPLC-UV) analytical method for the simultaneous quantification of five cannabinoids in commercial cannabis-based oils and cosmetics available in Malta. Validation of the method demonstrated specificity, accuracy, linearity and precision. Analysis of 23 oil and 10 cosmetic products revealed discrepancies between labelled and determined cannabinoid content. THC was detected in 6 samples (0.014–0.165 %), with concentrations below the 0.2 % regulatory threshold, while deviations in cannabidiol (CBD) content exceeded ±10 % label accuracy limit in 19 products. These findings indicate the need for routine quality control and regulatory oversight to ensure consumer safety and product transparency on the Maltase market. Future research should expand this analytical framework to a broader range of cannabis products, including edibles and e-liquid formulations.

1. Introduction

Cannabis products are available in a range of formulations, including plant material, tinctures, oils, edibles, vapes and cosmetics. The expanding market of cannabis products is complex, having fragmented regulations across Europe (Jardim and Delgado-Charro, 2025). Cannabinoid-containing products fall under different legal classifications depending on their authorisation, source and potency, all of which determine their legal status. The complexity of regulations reflects the dual nature of cannabis as both a medicinal and recreational product (Borrego-Ruiz and Borrego, 2025). Historically classified as a controlled substance due to its psychoactive components, cannabis is now increasingly recognised for its therapeutic potential. As a result, the regulatory landscape for cannabis products leads to significant challenges in differentiating legal from illicit cannabis products (Kadkhodaei et al., 2020; Nemeškalová et al., 2020).

The cannabinoid profile determines pharmacological effects, including analgesic, anti-inflammatory, antiemetic and anticonvulsant effects. Cannabinoids modulate the endocannabinoid system with signalling mediated via cannabinoid receptors 1 and 2 (CB1 and CB2). Δ^9-tetrahydrocannabinol (Δ^9-THC) is associated with cognitive and psychomotor impairment and exerts therapeutic effects such as analgesia and antiemesis. Cannabidiol (CBD) is non-intoxicating with limited evidence of dependence relative to THC. CBD preparations have been investigated for their therapeutic effects in epilepsy, neuroinflammation and neurodegeneration, anxiety and schizophrenia (Maurya and Velmurugan, 2018; Canseco-Alba and Rodríguez-Manzo, 2023).

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https://doi.org/10.1016/j.phytol.2026.104136
Received 10 November 2025; Received in revised form 11 February 2026; Accepted 19 February 2026
Available online 20 February 2026

Cannabis-derived consumer products that may claim therapeutic properties but are not authorised as medicines became available in Europe following the 2020 judgment of the Court of Justice of the European Union (Court of Justice of the European Union Fourth Chamber, 2020). This ruling clarified that CBD is not classified as a narcotic drug under the 1961 United Nations (UN) Single Convention on Narcotic Drugs (United Nations, 1961) and is not subject to narcotics control under EU law. Member States may still restrict CBD products on grounds of public health protection. For CBD-containing formulations, a legal threshold of 0.2 % (w/w) Δ⁹-THC is commonly applied to distinguish permitted 'low-THC' products from those considered unauthorised (European Monitoring Centre for Drugs and Drug Addiction, 2020). Compliance with this legal threshold remains challenging due to the lack of standardised monitoring and the variability in formulations and their chemical profiles (Nemeškalová et al., 2020). Products marketed as cannabidiol-rich have been found to contain psychoactive levels of Δ9-THC, exceeding legal limits and posing public health concerns (Nemeškalová et al., 2020).

Cannabinoid-containing products fall under specific European Union (EU) legislation depending on the route of administration. CBD-based oils can be classified as food when consumed orally or as a cosmetic when applied topically to the skin. Under Regulation (EC) No 178/2002 on General Food Law and Regulation (EU) 2015/2283 (European Monitoring Centre for Drugs and Drug Addiction, 2023) on novel foods, both plant-derived and synthetic CBD require authorisation before being placed on the market. Hemp seed and its derived products are recognised as traditional food and are not subject to the same authorisation requirements. Cosmetics containing CBD fall under Regulation (EU) No 1223/2009 (Regulation EC No, 1223/2009 of the European Parliament and of the Council of 30 November, 2009 on cosmetic products, 2009). Annex II of this Regulation prohibits substances listed in the 1961 UN Convention, but CBD is not prohibited when derived from cannabis seeds or leaves or produced in a laboratory (European Monitoring Centre for Drugs and Drug Addiction, 2023).

The cosmetic industry shows a growing trend and interest in cannabinoids as reflected in the increasing availability and diversity of topical cannabinoid-containing products such as creams, gels, balms and transdermal patches (Jhawar et al., 2019). CBD is considered a novel ingredient in the food and cosmetic sector and no specific limits on CBD potency have been established by the EU to date (Jardim and Delgado-Charro, 2025)

In response to the European Union Drugs Agency (EUDA) call to improve the monitoring of cannabis products (European Monitoring Centre for Drugs and Drug Addiction, 2023), there is a need for efficient monitoring supported by robust analytical methods for cannabinoid determination to assure product quality, consumer trust and safety. The lack of standardised quality assurance and limited market surveillance of cannabis-containing products raises concerns about potential risks to consumers (Wang et al., 2021). Analytical testing is essential for verifying labelled content, particularly since the concentration and stability of specific cannabinoids can vary across different product types (Nemeškalová et al., 2020).

High-Performance Liquid Chromatography with Ultraviolet detection (HPLC–UV) is commonly used for cannabinoid analysis because it allows the simultaneous quantification of neutral and acidic cannabinoids without derivatisation, unlike Gas Chromatography methods (Kadkhodaei et al., 2020). HPLC-UV is cost-effective and accessible in most analytical laboratories compared to more expensive and complex techniques such as LC-mass spectroscopy, making it a practical solution for routine quality and potency testing (Vella Szijj et al., 2024).

This study describes the validation and application of an HPLC-UV method for the quantification of five cannabinoids in oil and cosmetic matrices. The validated method was applied to commercially available products purchased in Malta, providing insights into product quality assurance and regulatory compliance.

2. Results and discussion

2.1. Method validation

The developed analytical method for quantification of cannabinoids in oil and cosmetic matrices demonstrated specificity and selectivity suitable for this purpose (Fig. 1).

Linearity exceeded R^2 of 0.99 for all tested cannabinoids (CBD, THC, cannabinol (CBN), cannabidiolic acid (CBDA), tetrahydrocannabinolic acid (THCA)) across analysed matrices. Matrix-matched validation was applied for hemp seed oil, olive oil and medium-chain triglyceride (MCT) oil. Similar approaches for oil matrices have been reported by Fries et al., 2024 and Raslan-Jaramillo et al., 2024. For cosmetic products, linearity was established using cannabinoid calibration standards in methanol, where matrix effects were addressed through sample spiking during accuracy evaluation. A similar validation protocol was adopted by Pires et al., 2024, who reported recoveries of 61.37–99.47 % for spiked cosmetic matrices, whereas in this study cosmetic matrices achieved recoveries of 81.11 % - 95.59 %. Other studies (Quiñones et al., 2022; Chaiwangrach et al., 2024) employed blank matrix spiking for cosmetic accuracy testing. Quiñones et al., 2022 demonstrated improved recovery (93–101 %), possibly reflecting focus on simpler water-based formulations, compared with more complex oil-in-water and water-in-oil emulsions tested in this study. Chaiwangrach et al., 2024 reported accuracy of 93.7–104 % in topical ointments using standard addition into blank ointment base, comparable to Quiñones et al., 2022.

Recovery performance in oils aligned with literature reports (Silva Sofrás et al., 2023; Raslan-Jaramillo et al., 2024; Fries et al., 2024). Mean recoveries were 104.98 % ± 4.25 % (MCT oil), 101.51 % ± 6.20 % (hemp seed oil) and 103.28 % ± 2.77 % (olive oil). Raslan-Jaramillo et al., 2024 achieved 84.0–103.5 % in sunflower:coconut oil (60:40, v/v). Fries et al., (2024) reported accuracy of 97.0 % - 102.2 % in olive oil and 107.8 % - 110.8 % in sunflower oil, demonstrating the importance of oil matrix composition on method performance. In this study different carrier oils exhibited different recoveries, with higher recoveries in MCT oil compared to hemp seed oil.

Method sensitivity varied by matrix and cannabinoid, with limit of detections (LOD) ranging from 1.42 to 4.47 μg/mL and limit of quantification (LOQ) from 4.33 to 13.5 μg/mL. For oil matrices LOD and LOQ values were comparable with Silva Sofrás et al. (2023) who reported LOD of 1.0–3.66 μg/mL and LOQ of 3.03–11.09 μg/mL for seven cannabinoids using similar HPLC-UV methodology.

Minor variations (±5 %) in chromatographic parameters were evaluated and confirmed robustness. Principal component analysis (PCA) of the robustness data (7 conditions and 15 variables) showed that two principal components (PC1 and PC2) explained 80.6 % of total variance.

The score plot (Fig. 2) demonstrates separation between nominal and modified conditions, with mobile phase composition identified as the most influential parameter. The −5 % organic content condition shows the most extreme deviation, while temperature variations cluster near the nominal method conditions, indicating minimal impact. Mobile phase composition requires strict control. This multivariate evaluation profile aligns with findings from Durante et al., 2022, who used PCA to evaluate chromatographic parameter effects and identified mobile phase control as critical for cannabinoid separation.

Overview of validation results is presented in Table 1 and detailed validation data by matrix are provided in Supplementary Materials S1.

2.2. Method application and labelling accuracy

The validated HPLC-UV method was applied to 33 commercially available cannabis-containing products, obtained from retail stores across Malta. The tested products included 23 oil products with different carrier oils: MCT oil (n = 11), hemp seed oil (n = 10) and olive oil

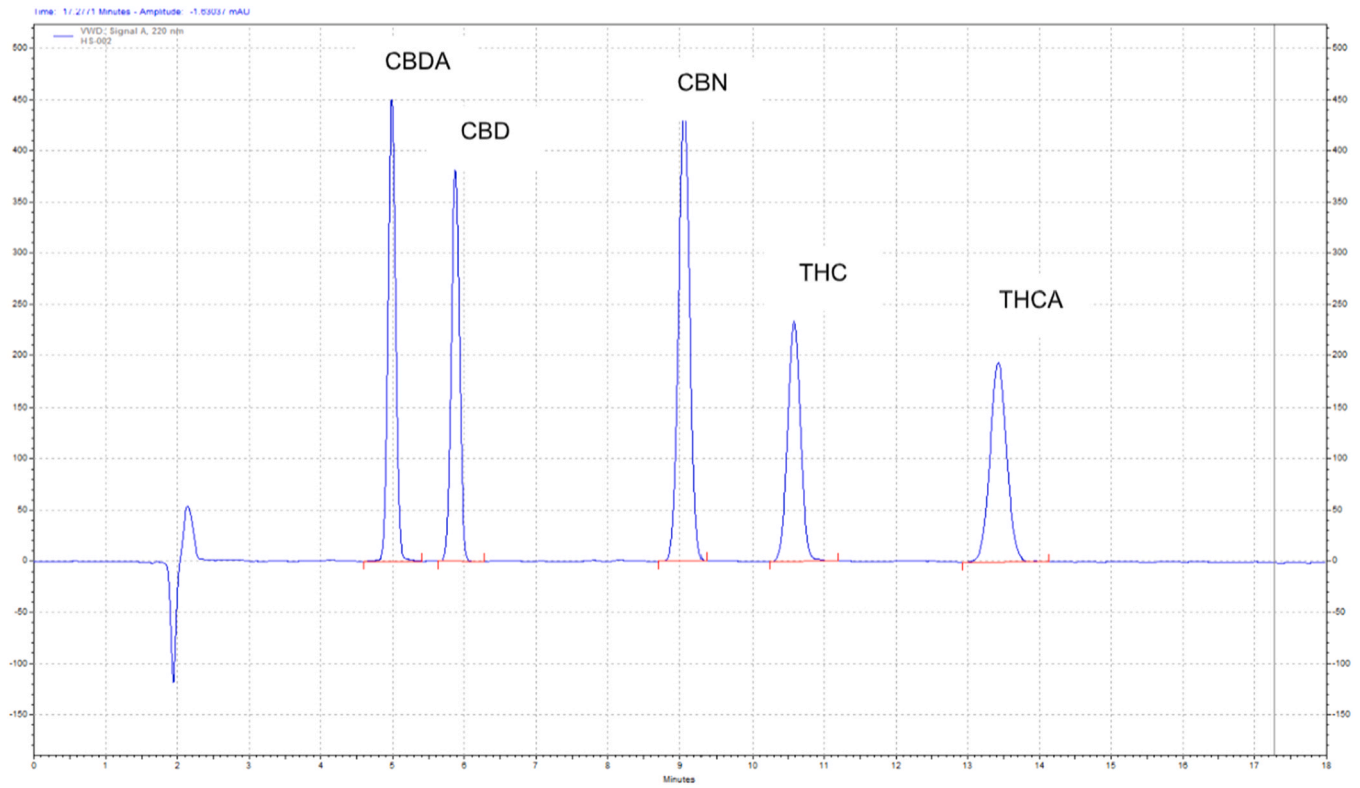

Fig. 1. HPLC chromatogram of cannabinoids in hemp seed oil detected at 220 nm.

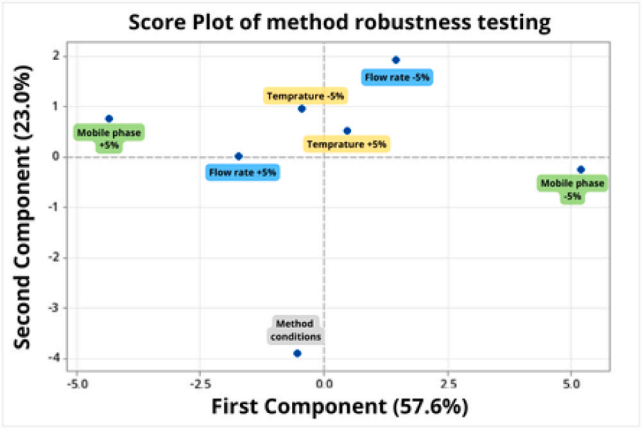

Fig. 2. PCA score plot showing distribution of experimental conditions and nominal method. Each point represents chromatographic performance under specific modifications: method conditions (grey), mobile phase (green), temperature (yellow) and flow rate (blue).

(n = 2), as well as 10 cosmetic formulations: creams (n = 3), balms (n = 4) and others.

CBD was detected in all oil products, with concentrations measured ranging from 2.25 % to 19.7 % (v/v). These analysed concentrations were compared to the labelled CBD content, which varied from 3 % to 25 % (v/v) (Fig. 3). Out of 23 analysed oil products, 10 accurately matched the labelled CBD concentration within the defined ±10 % acceptance range. Two oil products did not provide any information about cannabinoid concentrations, indicating concerns about product safety and transparency on the cannabis market.

THC was detected in six oil-based samples at concentrations between 0.014 % and 0.165 %, all below the regulatory threshold of 0.2 % confirming that these products adhere to legal standards (Fig. 4). No THC was detected in cosmetic samples.

Among tested oil matrices, MCT-based oil formulations demonstrated more accurate label claim information (7 out of 11 within ±10 %) compared to hemp seed oils (2 out of 10 within ±10 %).

Cannabis-based oils formulated with MCT oil as a carrier had higher accuracy of labelling compared to products formulated with hemp seed oil. The reasons for this observation could not be fully explained within the scope of this study. Possible contributing factors may include differences in product labelling and storage practices or cannabinoid

Table 1
Validation results overview by matrix.

Matrix	Linearity	Accuracy	Intraday Precision(RSD%)	Interday precision	LOD (µg/mL)	LOQ (µg/mL)
Hemp seed oil	$R^2 > 0.99$	101.51 %±:6.20 %	2.70 ± 3.05 (n = 25)	4.60 ± 2.39 (n = 25)	1.74–3.17	5.28–6.25
MCT oil	$R^2 > 0.99$	104.98 %± 4.25 %	5.74 ± 4.73 (n = 15)	6.13 ± 3.74 (n = 15)	1.63–4.47	4.94–13.5
Olive oil	$R^2 > 0.99$	103.28 %± 2.77 %	1.97 ± 2.09 (n = 25)	4.24 ± 2.33 (n = 25)	1.42–1.56	4.33–4.72
Cosmetics	$R^2 > 0.99$	87.05 % ±4.85 %	0.32 ± 0.27 (n = 9)	0.60 ± 0.29 (n = 9)	1.82–3.90	5.52–11.80

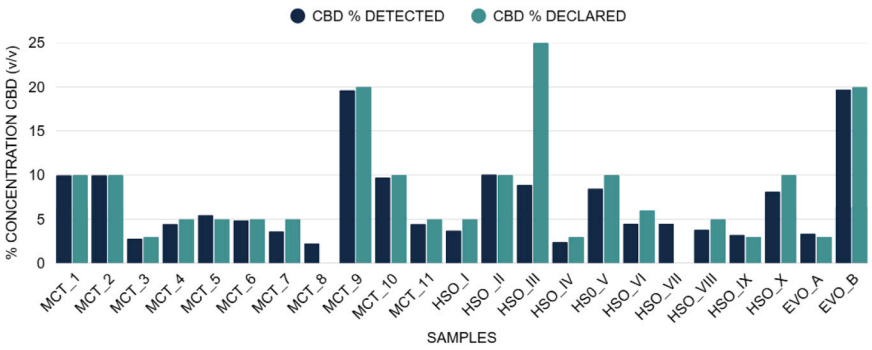

Fig. 3. Detected vs declared CBD content in oil samples (MCT = Medium Chain Triglyceride; HSO = Hemp seed oil; EVO = Extra Virgin Olive oil).

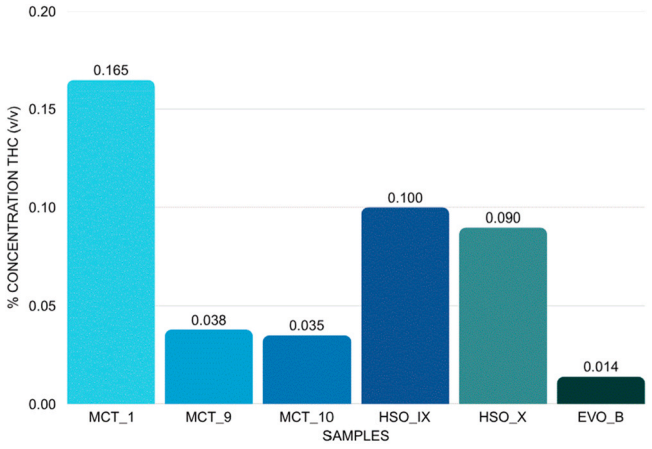

Fig. 4. Concentrations of THC across some of the analysed samples (MCT = Medium Chain Triglyceride; HSO = Hemp seed oil; EVO = Extra Virgin Olive oil).

stability in a given matrix, as it has been suggested that CBD exhibits greater stability when formulated in MCT oil (Tonoyan et al., 2022). The composition of cannabis-derived ingredients and processing may also influence the cannabinoid profiles. A key limitation is that cannabis-derived ingredients represent variable active materials and may be influenced by processing. Detailed information on ingredient type, origin and processing was not consistently available. The findings should be interpreted as market surveillance of finish product and observed discrepancies cannot be attributed to specific cannabis composition or manufacturing practices.

The prevalence of label inaccuracies observed in this study aligns closely with findings reported in European and US markets. According to Miller et al., 2022 4 of 11 (36 %) hemp seed oils tested in the US market were labelled accurately to their measured cannabinoid content. Pavlovic et al., 2018 evaluated 14 CBD oils available in Europe, finding 5 accurately labelled (36 %) and detected levels of THC, with one exceeding the legal limit of 0.2 %.

In cosmetic products, measured CBD concentrations ranged from 0.13 % to 4.58 % and one product did not contain any detectable CBD. Concentrations on the label reported values between 0.2 % and 5 % with one product without a label claim (Fig. 5).

Two of the ten tested cosmetic products met the ±10 % acceptance range for CBD content for label and measured accuracy. These findings are consistent with previous studies reporting poor labelling compliance in cannabinoid-containing cosmetics: Spindle et al., 2022 found that 24 % of cosmetic product samples were accurately labelled, while Oleinik (2022) reported that 22.5 % cosmetic products analysed met label accuracy criteria.

These results indicate that inaccurate label information regarding cannabinoid content remains a concern across both oil and cosmetic products. Similar studies conducted in other European countries and the US have reported comparable findings. There is a need for continuous monitoring and evaluation of cannabis products (European Monitoring Centre for Drugs and Drug Addiction., 2023). Robust analytical determination of cannabinoids is crucial to distinguish between legal and illegal products, supporting evidence-based policymaking in the cannabis sector.

3. Materials and methods

3.1. Equipment and chemicals

Analysis was performed using an Agilent 1260 Infinity Series liquid chromatography system equipped with an autosampler (G7129A) and quaternary pump (G7111B) coupled to a Variable Wavelength Detector (G7114A). Chromatographic separation was achieved using Avantor ACE 5 C18-AR (250 × 4.6 mm; 5 µm) HPLC column. Data acquisition

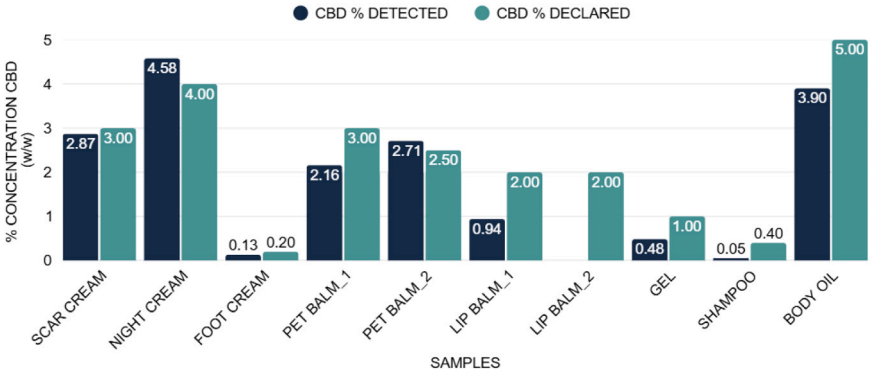

Fig. 5. Detected vs declared CBD content in cosmetic samples.

and processing were carried out using Open-Lab CDS ChemStation Software. Calculations were performed using Excel and Minitab Statistical Software (Minitab LLC, State College, PA, USA). HPLC-grade acetonitrile (ACN), methanol (MeOH), and acetic acid were purchased from J.T. Baker (Phillipsburg, NJ, USA).

3.2. Cannabis samples

Cannabis-containing consumer products available on the Maltese market were purchased between January and April 2025 from cannabis stores, vape shops and general retailers. Cannabis products sold through non-pharmacy retail outlets in Malta do not undergo medicinal product registration or marketing authorisation. Manufacturer and batch number were recorded for all analysed products. Oils samples were categorised by carrier oil and cosmetic samples were classified based on type of formulation as water-in-oil (w/o), oil-in-water (o/w) or aqueous preparations.

Thirty three commercial samples were analysed, comprising 23 oil formulations (11 in MCT oil, 10 hemp seed oil and 2 olive oil) and 10 cosmetic products (including 3 creams, 4 balms and other product types). For purpose of this study, label accuracy was defined as measured cannabinoid content within ±10 % of the manufacturer's stated label claim (Pavlovic et al., 2018; Miller et al., 2022; Spindle et al., 2022).

3.3. Standards and reagents

Certified reference standard solutions of CBD and CBN (1 mg/mL in methanol), CBDA and THCA (1 mg/mL in acetonitrile), and Δ9-THC (4.98 mg/mL in methanol) were obtained from LGC (Wesel, Germany) stored at −20 °C.

A working range of mixed cannabinoid standards was prepared by creating a serial dilution of standards in methanol over the concentration ranges from 12.5 μg/mL to 200 μg/mL. The calibration curves were constructed for oil-based matrices by matrix-matched standards preparation. This involved combining mixed cannabinoid standards with blank, cannabinoid-free oil solvents using a ratio of 1:1 (v/v) to account for matrix effects. Due to the lack of representative cosmetics blanks free of cannabinoids, spiking was performed on commercially available samples. Cosmetics specificity was evaluated using standard addition at known concentrations to assess potential matrix-related bias in the absence of an identical blank matrix.

3.4. Sample preparation

Commercial cannabis-based oil formulations were prepared by diluting each sample 1:100 (v/v) in methanol, followed by sonication for 15 min. Samples were vortexed before sonication and before injection to HPLC to ensure homogeneity.

CBD-based cosmetic samples were prepared by accurately weighing 0.1 g of each product. The weighed samples were gently heated on a hot plate at 40°C and dissolved in 10 mL of 96 % ethanol. Ultrasound-assisted extraction was employed for 20 min at room temperature. To ensure the absence of interfering excipients, all samples were centrifuged for 10 min at 10,000 rpm.

All prepared samples were filtered through a 0.45 μm regenerated cellulose syringe filters and diluted tenfold for quantification of higher concentration cannabinoids before chromatographic analysis.

3.5. Analytical parameters

An HPLC-UV method was validated for the simultaneous quantification of CBD, CBN, THC, THCA, and CBDA. Chromatographic separation was achieved within a run-time of 15 min. The mobile phase consisted of acetonitrile and 0.5 % acetic acid (75:25, v/v), at a flow rate of 1.5 mL/min. Chromatographic separation was performed on an ACE C18-AR (250 ×4.6 mm; particle size 5 μm) column maintained at a temperature of 30°C. Detection was carried out at 220 nm using ultraviolet absorbance. The injection volume was 10 μL.

3.6. Validation

The developed analytical method was validated following International Council on Harmonisation (ICH) guidelines (International Council for Harmonisation ICH, 2022)

Specificity was tested by analysing blank cannabinoid-free oils and solvents to confirm the absence of interfering peaks at the retention times of the target analytes. Selectivity was confirmed by the injection of cannabinoid reference standards to identify retention times and optimal resolution. The specificity and selectivity of the method was verified through analysis of mixed standards in addition to representative samples.

For cosmetic matrices, matched cannabinoid-free blanks were not available. Selectivity was assessed by comparing chromatograms of unspiked cosmetic extracts and cosmetic extracts spiked with known concentrations of cannabinoids for each representative formulation group (balm, cream and gel), confirming that cannabinoid peaks increased proportionally with the amount added. This standard addition was conducted during method validation and before each cosmetic sample was analysed.

Linearity was evaluated by analysing mixed cannabinoid standards and matrix-matched standards for oils ranging from 12.5 μg/mL to 200 μg/mL for cosmetics standards and 6.25 μg/mL to 100 μg/mL for MCT, hemp seed and olive oil formulations. Calibration curve linearity was assessed across five concentration levels and the square of the correlation coefficient (R^2) was determined, with an acceptance of $R^2 > 0.99$.

Accuracy was evaluated by spiking samples with known concentrations of analytes (low, medium and high levels) and calculating their recovery percentage. Acceptance criteria for recovery varied based on the complexity of matrix: 85–115 % for oil formulations and 80–120 % for cosmetics.

Precision was determined by both intraday and interday precision testing. Intraday precision was determined by analysing spiked samples at known concentration levels in triplicate on the same day. Interday precision was calculated by analysis of spiked samples on three different days. For both precision assessments, the percentage relative standard deviation (RSD%) was calculated, with acceptable values below 15 %. Limit of detection (LOD) and limit of quantification (LOQ) were determined using the standard deviation of the regression line of the analyte (σ) and the slope (S). The values were calculated as follows: $LOD = (3.3\sigma)/S$ and $LOQ = (10\sigma)/S$.

Robustness of the method was assessed by intentionally varying key analytical parameters by ±5 % from their original settings, including mobile phase composition ratio, flow rate and column temperature to ensure reliability under minor fluctuations.

4. Conclusion

A validated, robust HPLC-UV method for quantification of cannabinoids was successfully applied to commercial cannabis-based oil and cosmetic products in Malta. The discrepancies observed between labelled and measured cannabinoid content highlight the need for routine quality control to enhance product safety, ensure labelling accuracy, and strengthen regulatory oversight and market monitoring of cannabidiol-containing formulations. This work demonstrates the practical applicability of chromatographic surveillance for consumer products and provides important baseline data for the Maltese market.

Future studies will extend this analytical framework to include additional formulations, such as edibles and vaping products, incorporating improved traceability and batch-level sampling to enable a more comprehensive evaluation of formulation characteristics, processing

variables and label compliance across products and batches.

CRediT authorship contribution statement

Szyrner Karolina Delfina: Writing – original draft, Validation, Investigation, Formal analysis. **Paul I Buhagiar:** Validation. **Anthony Serracino-Inglott:** Supervision, Conceptualization. **Janis Vella Szijj:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Professor LaFerla, Dean of the Faculty of Medicine and Surgery at the University of Malta, for his support in granting the internal funding that made this research possible.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phytol.2026.104136.

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