



HAL
open science

Development and cross-validation of simple HPLC-fluorescence and UPLC-MS-UV methods for rapid determination of oleuropein in olive leaves

Morgane Carrara, Mary Kelly, Lauren Griffin, Delphine Margout

► **To cite this version:**

Morgane Carrara, Mary Kelly, Lauren Griffin, Delphine Margout. Development and cross-validation of simple HPLC-fluorescence and UPLC-MS-UV methods for rapid determination of oleuropein in olive leaves. *Phytochemical Analysis*, 2023, 35 (3), pp.476-482. <10.1002/pca.3302>. <hal-04802958>

HAL Id: hal-04802958

<https://hal.science/hal-04802958v1>

Submitted on 25 Nov 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY 4.0 - Attribution - International License

RESEARCH ARTICLE

Development and cross-validation of simple HPLC-fluorescence and UPLC-MS-UV methods for rapid determination of oleuropein in olive leaves

Morgane Carrara¹ | Mary T. Kelly¹  | Lauren Griffin² | Delphine Margout-Jantac¹

¹Qualisud Mixed Research Unit, Faculté de Sciences Pharmaceutiques, Université de Montpellier, Montpellier, France

²School of Pharmacy, Trinity College, University of Dublin, Dublin, Ireland

Correspondence

Mary T. Kelly, Qualisud Mixed Research Unit, Faculté de Sciences Pharmaceutiques, Université de Montpellier, 15 Avenue Charles Fahault, 34093 Montpellier, France.
Email: mary.blondel-kelly@umontpellier.fr

Abstract

Introduction: Olive leaves, abundant by-products of the olive oil industry, are a rich source of oleuropein, an important polyphenol in olive leaves. So far, no published methods have been validated using matrix standards for oleuropein quantification in olive leaves.

Objectives: The study aimed to develop an HPLC method for oleuropein determination in olive leaves using spiked matrix standards prepared from a blank olive leaf matrix, to validate the method with respect to aqueous standards, and cross-validate the HPLC method with UPLC-MS and UPLC-UV techniques.

Methodology: Oleuropein was extracted into methanol and analysed by HPLC with fluorescence detection (FLD; excitation and emission wavelengths 281 and 316 nm, respectively) and by UPLC-MS-UV. For validation, calibration curves of spiked matrix standards (0.4 to 4.8 mg/g) were analysed by the three methods over several days. Oleuropein was then analysed in French olive varieties.

Results: For the HPLC-FLD method, repeatability and intermediate precision were less than 5% RSD and linearity was demonstrated by the Fischer test. Differences in results of the spiked placebos by the three methods were non-significant, as confirmed by ANOVA. Extraction recovery was >90%, and there was a strong linear relationship between authentic and spiked matrix standards. The determination of oleuropein in French olive varieties is reported, including analysis in “Olivière” cultivar for the first time, leaves of which contained twice the amount of oleuropein compared with “Picholine”.

Conclusion: Accurate quantification of oleuropein is possible using aqueous standards. Cross-validation indicates that selective analysis can equally be carried out by HPLC or by UPLC-MS techniques.

KEYWORDS

French olive varieties, HPLC-fluorescence detection, oleuropein, olive leaves, validation

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2023 The Authors. *Phytochemical Analysis* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

Increasing consumer demand for more sustainable production methods combined with stricter environmental regulations are important drivers in developing strategies for the management of by-products. Olive leaves account for 10% of the mass of olive oil produced, and progress in recycling and valorisation means that they have become a promising source of phenolic compounds with high added value.¹

The majority of polyphenols are found in these by-products, and only 0.3% to 1.5% of available phenols occur in olive oil; consequently, extracts of these wastes have enormous potential as nutraceuticals and pharmaceuticals. Oleuropein, the most abundant phenolic compound in olive leaves, is also present in young olives to which it imparts the bitter taste in immature fruit. Different studies have reported varied concentrations of oleuropein in olive leaves; for example, Medina reported that oleuropein showed high variability in a range of 44 to 108 mg/g and that it constituted more than 88%–94% of total phenolic compounds in leaves.² Parameters such as climate and time of harvest determine the concentration of oleuropein and other biophenols.^{3,4} Romani reported differences in oleuropein amounts depending on cultivar, production area, sampling time, and state of leaves (fresh, dried, frozen, or lyophilised).⁵

The antioxidant properties of plant polyphenols form the basis of their health benefits, and as a result, they are increasingly being considered as constituents of pharmaceuticals.⁶ The activities of oleuropein include anti-inflammatory, antioxidant, anti-ageing and antibacterial properties.⁷ It has also been described as having anticancer effects, a topic which has been the subject of reviews.^{8,9} Research has shown that in skin disorders, oleuropein promotes collagen production, inhibits lipid peroxidation post sun exposure, and also reduces swelling of skin, as we have recently reviewed.¹⁰ Its efficacy in the treatment of metabolic disorders is of growing interest,¹¹ and it has yielded promising results as an antiviral agent, including SARS-Cov 2.¹²

In view of its antioxidant properties, oleuropein has considerable potential as a food ingredient in preventing oxidation; however, the production of oleuropein from olive leaves at an industrial scale faces challenges, including its instability in water, decomposing through hydrolysis to form the more apolar aglycone with lower solubility in aqueous systems.¹³ Consequently, reliable analytical determination of oleuropein in olive leaves is a pivotal step to identify varieties that could be considered for large-scale extraction. Furthermore, there is a need to quantify oleuropein in the growing number of nutritional supplements containing olive leaves, particularly given that some products claim to contain concentrations of oleuropein up to 40%. It is significant that Breakspear and Guillaume found that nutritional supplements on the Australian market vary substantially in their concentrations of oleuropein, whereas Medina et al. found that only 17%–26% of polyphenols diffused into olive leaf infusions.^{2,14}

The most widely used method to determine phenolic compounds in olive fruits and leaves is reversed-phase high/ultra-high-performance liquid chromatography (RP-HPLC/UPLC) coupled to UV^{2,14–17} and mass spectrometry (MS).^{18,19} In the majority of cases, no validation results were presented, or validation was carried out

using only authentic standards (ASs),²⁰ one exception being the study by Bertolini et al., where validation results were reported for the determination of oleuropein in an effervescent formulation using spiked placebos.²¹ In some cases, internal standards (ISs) were used such as resorcinol or syringaldehyde, though at what stage the IS's were added is unclear.²

One of the main aims of this study was to develop and validate a rapid and selective method to determine oleuropein in dried olive leaves without internal standardisation. The key elements presented are the production of an oleuropein-free (blank) olive leaf matrix, a validated HPLC method with fluorescence detection (FLD) using spiked placebos (oleuropein-free leaves), and cross-validation of the HPLC-FLD method with UPLV-UV-MS methods on a different instrument.

2 | MATERIALS AND METHODS

2.1 | Reagents and chemicals

All chemicals and reagents were of analytical or HPLC grade or equivalent. Methanol, acetonitrile, acetic acid, dichloromethane, and HPLC-grade water were obtained from Carlo Erba (Val de Reuil, France), and doubly distilled water was used for washing all consumable materials.

2.2 | Instrumentation and operating conditions

2.2.1 | HPLC-FLD

A Shimadzu (Marne-la-Vallée France) Nexera © chromatograph was used consisting of a DGU-20A50 degassing unit, an LC-AD quaternary pump, a SIL20 A autosampler, an SPDM20A photodiode array detector, and a fluorescence detector at λ_{ex} 281 nm and λ_{em} 316 nm. The analytes were separated on an end-capped C₁₈ Nucleodur© column 100 mm × 3 mm internal diameter (i.d.), 3 μm particle size. Column temperature was 40°C, and the injection volume was 2 μL . Low-pressure gradient mobile phase A consisted of water-acetonitrile [95:5] with 0.4% acetic acid; mobile phase B was methanol with 0.4% acetic acid with a flow rate of 0.5 mL/min using a linear gradient programme of: 0 min 25% B - 4 min 35% B. Under these conditions oleuropein elutes at 5.2 min.

2.2.2 | UPLC-UV-MS

Analyses were carried out on a Thermo Ultimate© 3000 instrument with quaternary pump and automatic sampler coupled to an Ultimate 3000 RS type UV spectrophotometer and an ISQ EC mass spectrometer. Data were recorded using Chromeleon 7.2 software (Thermo Scientific). The analytes were separated on a Kinetex© 2.6 μm Biphenyl 100 Å (150 × 2.1 mm) column at 40°C. The mobile phase was methanol acidified with 0.01% formic acid (A) and water acidified with 0.01% formic acid (B) with a flow rate of 0.4 ml/min. The gradient was

0–2 min 75% B, 2–10 min 75%–45% B, 10–13 min 45% B, 13–14 min 45%–0% B, 14–15 min 0% B, 15–16 min 0%–75% B, and 16–20 min 100% B. The UV chromatogram was recorded at 280 and 200 nm. The mass spectrometer was operated in negative ion (ESI) mode with the following source parameters: Sheath Gas Pressure 30 psig; auxiliary Gas Pressure 8 psig; purge gas pressure 0.8 psig, vaporiser temperature 300°C, and transfer tube and ion temperature 350°C. Acquisition was performed in full-scan mode (50–800), and for the assay, the spectrum at mass-to-charge ratio (*m/z*) 539 was extracted.

2.3 | Samples and standards

2.3.1 | Olive leaves

Olive leaves of Picholine and Olivière varieties (used for the production of olive oil) were collected in March 2022, in the Hérault department of the Occitanie region of France (latitude 43.5912356, longitude 3.2583626). The leaves were immediately removed and stored protected from light and humidity until analysis in June 2022. Leaves of four other, mainly ornamental varieties, were collected locally and analysed in order to provide additional data for the validation study. These varieties could not be identified, but insofar as could be established, they were Italian or Greek hybrids; in the interest of simplicity, these varieties were named UNK 1, UNK 2, etc.

2.3.2 | Preparation of oleuropein-free (blank) olive leaves

After milling and sieving, leaf powder was dried to constant weight with a final water content of 4% to 5%. Approximately 50 mL of methanol was added to 2 g of leaf powder, and oleuropein was removed by extraction in an ultrasonic bath at approximately 55°C for 45 min. The supernatant was discarded and the procedure repeated. The leaves were then dried for 2 h at 105°C and maintained in a low-humidity environment before use. The quantity of oleuropein remaining in the blank after double extraction acted as the first point on the calibration curve.

2.3.3 | Standards and samples

A stock solution of 250 mg/L oleuropein was prepared in methanol. This solution is stable for 6 months at –20°. ASs at five calibration points (5, 10, 20, 40 and 60 mg/L) were prepared daily in methanol–water [1:1] by serial dilution of the stock solution. Spiked standards (dried-leaf placebos) containing 0.4, 0.8, 1.6, 3.2, and 4.8 mg/g were prepared by adding the appropriate volume of the stock solution to 50 mg of blank leaves in an haemolysis tube and the total volume made up to 2 mL with methanol in 5-mL haemolysis tubes. The samples were vortex-mixed for 30 s and then dried under a stream of

nitrogen at 30°C. The samples were ground to a fine powder and prepared in the same manner as the spiked standards.

2.3.4 | Extraction

Two millilitres of methanol were added to the spiked standards (or samples), mixed on a rotary mixer for 30 min, and centrifuged at 5000 rpm for 5 min. The supernatants were removed and the above process was repeated with another 2 mL of methanol. The 2 × 2 mL supernatants were combined, diluted 100-fold with methanol–water (50/50), and filtered through a 0.45 µm filter before injection.

2.3.5 | Validation

Calibration curves of the spiked standards were obtained from unweighted least-squares linear regression analysis of the data. The slope and intercept of the calibration graphs were determined through linear regression of the peak areas versus concentration plot. Individual peak areas were then interpolated on the calibration graphs to determine found (back-calculated) concentrations. The quality of fit was determined using back-calculated-to-nominal concentrations, and linearity was confirmed by the Fischer test. Within-day precision (repeatability) and between-day (intermediate precision) variability and accuracy were determined by carrying out replicate analyses of the spiked leaf calibration standards. Repeatability was determined by preparing and analysing the spiked leaf standards five times within a single day, and the intermediate precision was determined by carrying out the same operations for 5 days over a 10-day period. The precision was given by mean relative standard deviation (RSD) of the back-calculated concentrations; recovery was determined by comparing back-calculated and nominal concentrations and expressed as $100 \times (\text{mean found concentration} / \text{nominal concentration})$. The relationship between detector responses of ASs (in methanol–water) and spiked placebos was measured by analysing four calibration standards and a blank of each set. The concentrations of oleuropein were 0.4, 0.8, 1.6, and 3.2 mg/g for the spiked placebos, which, after extraction (4 mL methanol) and dilution (100-fold in 50% aqueous methanol), give injection solutions of 5, 10, 20, and 40 mg/L oleuropein, respectively (for 100% recovery). These solutions were compared with AS solutions at the same concentrations. Five replicates of each concentration were prepared on the same day by the same operator under the same operating conditions.

3 | RESULTS AND DISCUSSION

3.1 | Method development and extraction

The first objective was to develop an analytical method with a short analysis time using the minimum amount of organic solvents. A 3.5-mm i.d. column allowed a low flowrate to be used (0.5 mL/min),

and increasing column temperature to 40°C reduced run time and back-pressure in the system. Under these conditions, oleuropein eluted at 5.2 (\pm 0.1) min with a total run-time including column re-equilibration of 13 min. The excitation and emission wavelengths were optimised for oleuropein at 281 and 316 nm, respectively, and it is worth noting that under these conditions, extremely strong responses are obtained for hydroxytyrosol and tyrosol, which may also be determined at the parts per billion (ppb) level by this method.

Studies on phenol extraction using water at high temperatures demonstrated that it is an efficient method for the recovery of high-value natural bioactive compounds.¹⁷ This high temperature effect could be attributed to both the nature of the matrix and structure of the bioactive compounds to be extracted.²² Microwave-assisted extraction for oleuropein from olive leaves was first described by Japón-Luján et al. in 2006,²³ and Da Rosa described microwave extraction coupled to HPLC-DAD for the determination of oleuropein in olive leaves. The procedure required strict control of extraction conditions, as the extraction time was 2–3 min and it was necessary to rapidly cool the samples in an ice bath.²⁴ However, since this approach has grown in popularity for the extraction of oleuropein from olive leaves,^{25,26} the starting point for this study was based on a method described by Martínez-Navarro, where olive leaves were extracted in water using a domestic microwave at 800 W for 30 s.¹⁶ Approximately 50 mg of powdered oleuropein leaves was placed in a test tube with 4 mL of water and microwaved using the conditions described above. Microwaving was effective in extracting oleuropein

from the leaves, but it caused the contents of the test tubes to rise rapidly, probably due to uneven heating in the domestic microwave. Even by reducing duration and power setting, it was impossible to control the temperature within the test tubes, and clearly sample volume ratios and test tube/bottle size would need to be optimised and tailored to each experimental situation.

The next step was to investigate extraction in an ultra-sonic bath, as this technique has been described by several authors, though in some cases long extraction times are reported.^{25,27} Samples (50 mg) were weighed into 10-mL test tubes with 4 mL water or 70% aqueous methanol and placed in the ultrasonic bath at the lowest setting for 30 min. Again, it proved difficult to ensure uniform heating; adding ice to the ultrasonic bath failed to maintain an even temperature, and variation in heating among samples resulted in poor repeatability of extraction. Furthermore, in some cases, the rapid rise in temperature caused the methanol to evaporate resulting in sample loss. Given this difficulty, the more conventional rotary mixing was compared with ultrasonic extraction. Samples were mixed for 15 min on a rotary mixer, and then either a further mixing for 15 min on the rotary mixer or 15 min of sonication followed. There was little difference between the two extraction methods, so rotary mixing was adopted, which has the added advantage of enabling several samples to be extracted simultaneously. Extraction with 100% methanol with rotary mixing gave greater recovery than with 50% methanol (Figure S1). Regardless of the volume used, only ~80% of added oleuropein was recovered in a single extraction (Figure 1); most of the remainder was removed in

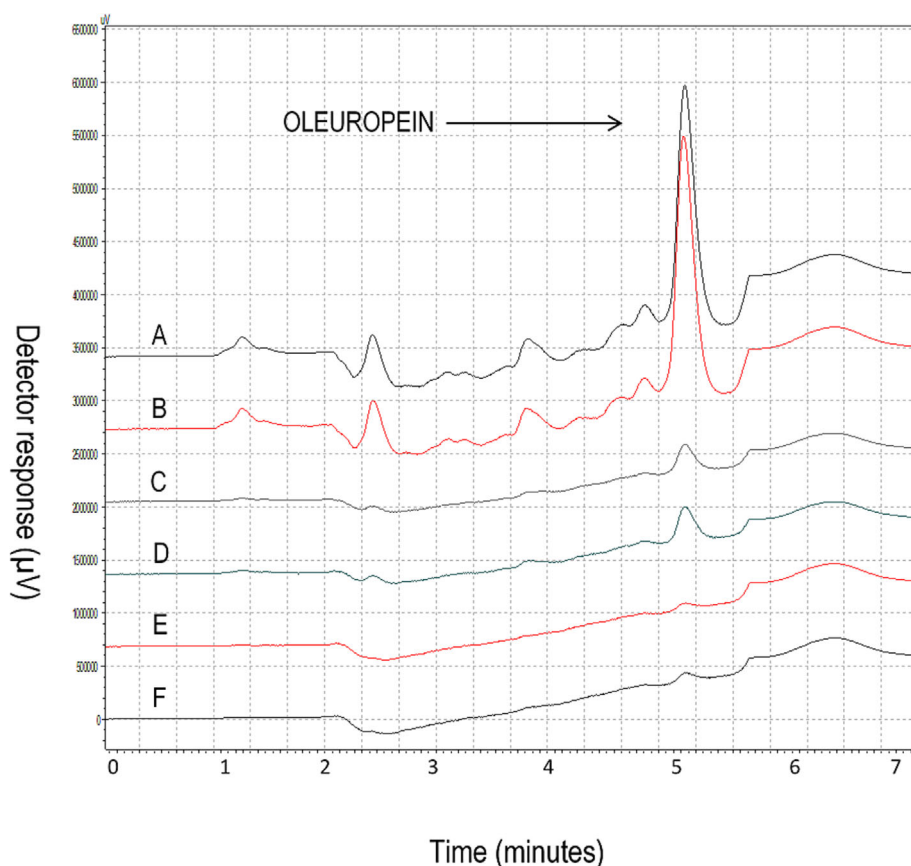


FIGURE 1 Comparison of ultrasound (US) and rotary extraction (Ext) using 50 mg of sample extracted into 10 mL methanol. HPLC method, chromatographic conditions described in the text. A – rotary 1st Ext; b – US 1st ex. t.; c – rotary 2nd Ext; d – US 2nd Ext; e – rotary 3rd Ext; f – rotary 3rd Ext.

the second extraction, so the final protocol retained was 2×2 mL extraction of 50 mg powdered leaf, which gave total recovery in excess of 90%. Chromatograms of ASs and spiked standards (at the same concentrations after extraction and dilution) are presented in the supporting information (Figure S2).

3.2 | Validation

Validation results for the HPLC-FLD method are presented in Table 1; those for the UPLC-MS and UPLC-UV methods are presented in Tables S1 and S2, respectively. In the interest of clarity, only back-calculated concentrations are presented rather than detector responses. For HPLC-FLD, repeatability, expressed as the intraday RSD, was generally less than 5%, and accuracy was 95% to 105%. In addition, the following data were obtained: The mean slope of the detector response versus concentration curve was 3,301,401 ($\pm 81,242$); RSD 2.46%; mean coefficient of determination (R^2) 0.9994 (± 0.0004). The equation of the curve "mean back-calculated concentration versus nominal concentration" was $y = 0.9966x + 0.0565$ ($R^2 = 1$). For intermediate precision, the RSD was less than 6%, and accuracy was generally between 96% and 102%. The mean slope of the detector response versus concentration curve was 3,250,083 ($\pm 121,928$; RSD, 3.75% with a coefficient of determination (R^2) of 0.9986 (± 0.0016). The equation of the mean back-calculated versus nominal concentration was $y = 0.9941x + 0.2519$ ($R^2 = 0.9996$). The equivalent values for the UPLC-MS method were mean overall RSD 3.96% (repeatability) and 2.69% (intermediate precision), and for the

UPLC-UV method, 2.88% and 2.15%, respectively (see Tables S1 and S2, respectively). Linearity was determined by the Fischer test based on residual values, that is the differences between nominal and found (back-calculated) concentrations. No significant differences were observed: the function f (nominal C - found C) exhibits significant linearity (Fisher, $p < 0.001$, $R^2 = 0.999$) with an intercept of about 0 (intraday = -0.080 and interday = -0.106) and a slope of about 1 (intraday = 1.001 and interday = 1.002). The AS detector responses versus spiked placebo detector responses were highly correlated ($R^2 = 0.9996$), and as Figure 2 shows, there was also a strong correlation between spiked placebo back-calculated concentrations

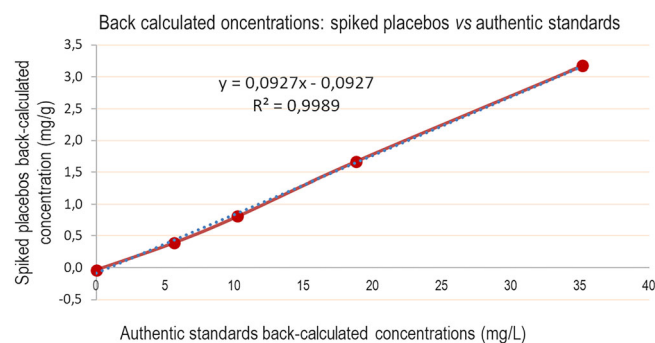


FIGURE 2 Correlation between detector responses for authentic standards and spiked placebo standards. HPLC-fluorescence method. Concentration range: 5–60 mg/L authentic standards and spiked standards (after extraction and dilution).

TABLE 1 Validation data for the HPLC-FLD method.

Nominal concentration	0.4	0.8	1.6	3.2	4.8	0.4	0.8	1.6	3.2	4.8
Found concentration (AF mg/g)	Repeatability					Intermediate precision (interday)				
AF	0.40	0.79	1.58	3.08	4.81	0.35	0.81	1.80	3.27	4.69
Recovery %	100	99	99	96	100	87	101	112	102	98
AF	0.41	0.80	1.60	3.17	4.82	0.41	0.82	1.66	3.09	4.85
Recovery %	101	100	100	99	100	102	102	104	97	101
AF	0.40	0.81	1.68	3.12	4.83	0.40	0.81	1.68	3.12	4.83
Recovery %	100	101	105	98	101	100	101	105	98	101
AF	0.36	0.77	1.52	3.22	4.74	0.40	0.78	1.61	3.28	4.75
Recovery %	91	96	95	100	99	100	98	100	102	99
AF	0.42	0.85	1.66	3.34	4.76	0.41	0.81	1.60	3.12	4.85
Recovery %	104	106	104	105	99	102	102	100	97	101
Mean AF	0.40	0.81	1.61	3.19	4.79	0.39	0.81	1.67	3.17	4.79
Mean recovery %	99	101	100	100	100	102	101	104	99	100
SD AF	0.02	0.03	0.06	0.10	0.04	0.02	0.02	0.08	0.09	0.07
SD recovery %	0.05	0.04	0.04	0.02	0.01	0.06	0.02	0.05	0.03	0.01
RSD %	5.11	3.73	4.01	3.21	0.78	6.23	1.91	4.70	2.89	1.50

Note: Intraday precision (repeatability), interday variability (intermediate precision), and recovery % based on found concentrations (AF mg/g) obtained by back-calculation of the signal on the individual calibration curves. For intraday, the equation was $y = 0.9966x + 0.0565$ ($R^2 = 1$); for interday the equation was $y = 0.9941x + 0.0201$, ($R^2 = 0.9996$).

Abbreviations: FLD, fluorescence detection; RSD, relative standard deviation (coefficient of variation); SD, standard deviation.

TABLE 2 Oleuropein concentration in olive leaves (mg/g dry weight).

	Picholine	Olivière	UNK 1 ^a	UNK 2	UNK 3	UNK 4	UNK 4h ^b	Olivière 2020 ^c
	14.44	41.32	32.5	16.5	5.96	21.68	21.9	3.85
	15.54	37.92	33.36	19.08	6.06	19	21.46	4.29
	16.22	38.7	36.42	185.6	5.62	21	21.58	3.99
	16.44	39.3	33.3	16.62	-	21.44	21.72	4.36
Mean	15.66	39.31	33.89	59.45	5.88	20.78	21.66	4.12
SD ^d	0.90	1.45	1.73	84.11	0.23	1.22	0.19	0.24
RSD ^e %	5.74	3.70	5.10	141.48	3.92	5.87	0.87	5.92

Abbreviations: RSD, relative standard deviation (coefficient of variation); SD, standard deviation.

^aUnknown variety.

^bUnknown variety extracted for 4 h.

^cOlivière leaves stored for 12 months under non-controlled conditions.

^dStandard deviation.

^eRelative standard deviation (coefficient of variation).

and AS back-calculated concentrations ($R^2 = 0.9989$). Similar results were obtained for the UPLC-MS and UPLC-UV methods (data not shown).

3.3 | Application to the analysis of French olive varieties

Savournin et al. reported concentrations of oleuropein in four French varieties (Cailletier, Lucques, Tanche, and Verdale—Picholine hybrid) with Lucques having the highest concentration (12.56%–14.16%).²⁸ These values were higher than usually reported and were attributed to the microwave drying method rather than the extraction procedure. Other authors found between 9% and 14.3% oleuropein in leaves of olive trees depending on the harvest time and agronomic conditions.²⁹ Notable differences in the concentration of oleuropein in the leaves of different olive cultivars were found among Mediterranean countries; in a major review article, Talhaoui et al. reported values varying between $24.7\text{--}143.2 \times 10^3 \mu\text{g/g}$ dry weight (DW) depending on the provenance and time of year when the samples were collected.¹⁵ However, in another review, Özcan et al. reported relatively minor differences (31 to 53 mg/g DW) in oleuropein concentrations among 11 olive cultivars originating in France, Spain, Italy, and Tunisia.³⁰

There are relatively few publications on oleuropein concentrations in cultivars particular to the South of France, where the most common varieties include Picholine, Lucques, Verdale, and Negrette. There are no publications on Olivière, another variety widely found in the Languedoc Region of France. In addition to cultivars used for the production of olive oil, (Picholine, Olivière, Lucques) analysed in this study, three unidentified cultivars, mainly used as decorative trees, of probably Greek (Bleu Grec) and Spanish (UNK 4) origin were also analysed. The values (mg/g) in Table 2 show some variation among the cultivars, ranging from a mean concentration of 5.88 mg/g for UNK 3 to 39.31 mg/g for Olivière 2022. These values are not inconsistent with concentrations of oleuropein described above, but are considerably lower than some given elsewhere, as cited in Section 1

(Introduction). Such vast differences in reported oleuropein concentrations are probably due to different cultivars (often not specified), harvest times, storage conditions (water content of the leaves is rarely specified), milling/grinding procedures, and extraction methods.

Table 2 also shows that no benefit is gained from increasing extraction time (Bleu Grec 4 h) and that the concentration of oleuropein in Olivière variety decreases by almost 90% in dried leaves stored for 12 months. This observation contrasts with that of Martinez-Navarro et al., who found that oleuropein was stable for at least 6 months when leaf powder was stored at any temperature with a relative humidity of 57%.³¹ However, it should be pointed out that the 2021 leaf samples in this study were not stored under controlled conditions. Nonetheless, the promising results obtained here for the widely planted Olivière cultivar paves the way forward for the exploration of this variety as a valuable and abundant source of naturally occurring oleuropein.

ACKNOWLEDGEMENT

The authors wish to thank the olive oil mill “Oli d’Oc” at Clermont l’Herault in the Occitanie region of France for kindly supplying the olive leaves used in this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Mary T. Kelly  <https://orcid.org/0000-0003-1415-0096>

REFERENCES

- Şahin S, Bilgin M. Olive tree (*Olea europaea* L.) leaf as a waste by-product of table olive and olive oil industry: a review. *J Sci Food Agric*. 2018;98(4):1271–1279. doi:10.1002/jsfa.8619
- Medina E, Romero C, García P, Brenes M. Characterization of bioactive compounds in commercial olive leaf extracts, and olive leaves and their infusions. *Food Funct*. 2019;10(8):4716–4724. doi:10.1039/C9FO00698B

3. Lukić I, Pasković I, Žurga P, et al. Determination of the variability of biophenols and mineral nutrients in olive leaves with respect to cultivar, collection period and geographical location for their targeted and well-timed exploitation. *Plan Theory*. 2020;9(12):1667. doi:10.3390/plants9121667
4. Kabbash EM, Ayoub IM, Gad HA, Abdel-Shakour ZT, El-Ahmady SH. Quality assessment of leaf extracts of 12 olive cultivars and impact of seasonal variation based on UV spectroscopy and phytochemical content using multivariate analyses. *Phytochem Anal*. 2021;32(6):932-941. doi:10.1002/pca.3036
5. Romani A, Mulas S, Heimler D. Polyphenols and secoiridoids in raw material (*Olea europaea* L. leaves) and commercial food supplements. *Eur Food Res Technol*. 2016;3(243):429-435.
6. Scott KA, Cox PB, Njardarson JT. Phenols in pharmaceuticals: analysis of a recurring motif. *J Med Chem*. 2022;65(10):7044-7072. doi:10.1021/acs.jmedchem.2c00223
7. Barbaro B, Toietta G, Maggio R, et al. Effects of the olive-derived polyphenol oleuropein on human health. *Int J Mol Sci*. 2014;15(10):18508-18524. doi:10.3390/ijms151018508
8. Rishmawi S, Haddad F, Dokmak G, Karaman R. A comprehensive review on the anti-cancer effects of oleuropein. *Life (Basel)*. 2022;12(8):1140. doi:10.3390/life12081140
9. Zheng Y, Liu Z, Yang X, Liu L, Ahn KS. An updated review on the potential antineoplastic actions of oleuropein. *Phytother Res*. 2022;36(1):365-379. doi:10.1002/ptr.7325
10. Carrara M, Kelly MT, Roso F, Larroque M, Margout D. Potential of olive oil mill wastewater as a source of polyphenols for the treatment of skin disorders: a review. *J Agric Food Chem*. 2021;69(26):7268-7284. doi:10.1021/acs.jafc.1c00296
11. Ahamad J, Toufeeq I, Khan MA, et al. Oleuropein: a natural antioxidant molecule in the treatment of metabolic syndrome. *Phytother Res*. 2019;33(12):3112-3128. doi:10.1002/ptr.6511
12. Abdelgawad SM, Hassab MAE, Abourehab MAS, Elkaeed EB, Eldehna WM. Olive leaves as a potential phytotherapy in the treatment of COVID-19 disease. A Mini-Review *Front Pharmacol*. 2022;13:879118. doi:10.3389/fphar.2022.879118
13. Monteiro M, Silva AFR, Resende D, et al. Strategies to broaden the applications of olive biophenols oleuropein and hydroxytyrosol in food products. *Antioxidants*. 2021;10(3):444. doi:10.3390/antiox10030444
14. Breakspear I, Guillaume C. A quantitative phytochemical comparison of olive leaf extracts on the Australian market. *Molecules*. 2020;25(18):4099. doi:10.3390/molecules25184099
15. Talhaoui N, Taamalli A, Gómez-Caravaca AM, Fernández-Gutiérrez A, Segura-Carretero A. Phenolic compounds in olive leaves: analytical determination, biotic and abiotic influence, and health benefits. *Food Res Int*. 2015;77:92-108. doi:10.1016/j.foodres.2015.09.011
16. Martínez-Navarro EM, Cebrián-Tarancón C, Moratalla-López N, Lorenzo C, Alonso GL, Salinas RM. Development and validation of an HPLC-DAD method for determination of oleuropein and other bioactive compounds in olive leaf by-products. *J Sci Food Agric*. 2021;101(4):1447-1453. doi:10.1002/jsfa.10758
17. Monteleone JI, Sperlinga E, Siracusa L, et al. Water as a solvent of election for obtaining oleuropein-rich extracts from olive (*Olea europaea*) leaves. *Agronomy*. 2021;11(3):465. doi:10.3390/agronomy11030465
18. Kishikawa A, Ashour A, Zhu Q, Yasuda M, Ishikawa H, Shimizu K. Multiple biological effects of olive oil by-products such as leaves, stems, flowers, olive milled waste, fruit pulp, and seeds of the Olive Plant on skin. *Phytother Res*. 2015;29(6):877-886. doi:10.1002/ptr.5326
19. Kritikou E, Kalogiouri NP, Kostakis M, et al. Geographical characterization of olive oils from the North Aegean region based on the analysis of biophenols with UHPLC-QTOF-MS. *Foods*. 2021;10(9):2102. doi:10.3390/foods10092102
20. Khemakhem I, Gargouri OD, Dhoubi A, Ayadi MA, Bouaziz M. Oleuropein rich extract from olive leaves by combining microfiltration, ultrafiltration and nanofiltration. *Sep Purif Technol*. 2017;172:310-317. doi:10.1016/j.seppur.2016.08.003
21. Bertolini T, Vicentini L, Boschetti S, Andreatta P, Gatti R. A novel liquid chromatography method using diode-array detector for the determination of oleuropein in dietary supplements. *J Pharm Biomed Anal*. 2016;129:198-202. doi:10.1016/j.jpba.2016.07.001
22. Olmo-García L, Bajoub A, Benlamaalam S, et al. Establishing the phenolic composition of *Olea europaea* L. leaves from cultivars grown in Morocco as a crucial step towards their subsequent exploitation. *Molecules*. 2018;23(10):2524. doi:10.3390/molecules23102524
23. Japón-Luján R, Luque-Rodríguez JM, Luque de Castro MD. Multivariate optimisation of the microwave-assisted extraction of oleuropein and related biophenols from olive leaves. *Anal Bioanal Chem*. 2006;385(4):753-759. doi:10.1007/s00216-006-0419-0
24. da Rosa GS, Vanga SK, Garipey Y, Raghavan V. Comparison of microwave, ultrasonic and conventional techniques for extraction of bioactive compounds from olive leaves (*Olea europaea* L.). *Innov Food Sci Emerg Technol*. 2019;58:102234. doi:10.1016/j.ifset.2019.102234
25. Otero DM, Oliveira FM, Lorini A, Antunes BD, Oliveira RM, Zambiasi RC. Oleuropein: methods for extraction, purifying and applying. *Rev Ceres*. 2020;67(4):315-329. doi:10.1590/0034-737x202067040009
26. da Rosa GS, Martiny TR, Dotto GL, et al. Eco-friendly extraction for the recovery of bioactive compounds from Brazilian olive leaves. *Sustain Mater Technol*. 2021;28:e00276. doi:10.1016/j.susmat.2021.e00276
27. Contreras MDM, Lama-Muñoz A, Espínola F, Moya M, Romero I, Castro E. Valorization of olive mill leaves through ultrasound-assisted extraction. *Food Chem*. 2020;314:126218. doi:10.1016/j.foodchem.2020.126218
28. Savournin C, Baghdikian B, Elias R, Dargouth-Kesraoui F, Boukef K, Balansard G. Rapid high-performance liquid chromatography analysis for the quantitative determination of oleuropein in *Olea europaea* leaves. *J Agric Food Chem*. 2001;49(2):618-621. doi:10.1021/jf000596+
29. Martínez-Navarro ME, Cebrián-Tarancón C, Salinas MR, Alonso GL. Evolution of oleuropein and other bioactive compounds in Arbequina olive leaves under different agronomic conditions. *Horticulturae*. 2022;8(6):530. doi:10.3390/horticulturae8060530
30. Özcan MM, Matthäus B. A review: benefit and bioactive properties of olive (*Olea europaea* L.) leaves. *Eur Food Res Technol*. 2017;243(1):89-99. doi:10.1007/s00217-016-2726-9
31. Martínez-Navarro ME, Cebrián-Tarancón C, Oliva J, Salinas MR, Alonso GL. Oleuropein degradation kinetics in olive leaf and its aqueous extracts. *Antioxidants (Basel)*. 2021;10(12):1963. doi:10.3390/antiox10121963

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Carrara M, Kelly MT, Griffin L, Margout-Jantac D. Development and cross-validation of simple HPLC-fluorescence and UPLC-MS-UV methods for rapid determination of oleuropein in olive leaves. *Phytochemical Analysis*. 2024;35(3):476-482. doi:10.1002/pca.3302