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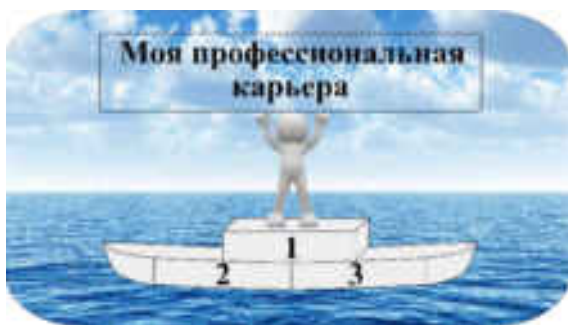


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«ОБРАЗОВАНИЕ И НАУКА В XXI ВЕКЕ»

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**Международный научно-образовательный электронный журнал
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Abstract

Foeniculum vulgare Mill. (fennel, family Apiaceae) is a commercially and medicinally important aromatic plant whose fruits (commonly called seeds) serve as the principal source of pharmaceutical-grade fennel essential oil and a range of secondary metabolites. Among these, coumarins — particularly umbelliferone (7-hydroxycoumarin), bergapten (5-methoxypsoralen), isopimpinellin, and psoralen — represent a pharmacologically significant fraction with documented spasmolytic, anti-inflammatory, photosensitizing, and anticoagulant activities. Despite the dominance of trans-anethole and fenchone in fennel's phytochemical profile, the coumarin fraction has attracted increasing pharmaceutical and cosmetic interest.

This article presents a detailed technical description of the industrial and laboratory-scale process for extracting, isolating, and purifying coumarins from *F. vulgare* fruits: from botanical raw material preparation through solvent extraction, liquid-liquid partitioning, column chromatographic isolation, crystallization, and quality control by HPLC and spectroscopic methods. Process parameters, yield data, and critical technological variables are discussed with reference to current pharmacopoeial and scientific literature.

Keywords: *Foeniculum vulgare*, fennel, coumarin extraction, umbelliferone, bergapten, furocoumarin, solvent extraction, column chromatography, HPLC, phytopharmaceutical technology.

1. Introduction

Foeniculum vulgare Mill. is a perennial or biennial herb native to the Mediterranean basin and widely cultivated across temperate regions of Europe, Asia, and the Americas for culinary, pharmaceutical, and cosmetic purposes. The plant belongs to the family Apiaceae (Umbelliferae), and its mericarp fruits — ovoid, 4-10 mm long, grey-green at maturity — are the pharmacopoeial drug material (*Foeniculi fructus*, Ph.Eur.). Two botanical varieties are recognized: *F. vulgare* var. *dulce* (sweet fennel) with low fenchone content, and *F. vulgare* var. *vulgare* (bitter fennel), the pharmacopoeial standard, characterized by a higher fenchone content and a more complex secondary metabolite profile.

The principal commercially exploited constituents of fennel fruit are the components of the essential oil: trans-anethole (50-80%), fenchone (5-25%), estragole, limonene, and alpha-pinene. However, the non-volatile extractable fraction contains a structurally diverse array of secondary metabolites including: (1) simple coumarins — umbelliferone (7-hydroxycoumarin), herniarin (7-methoxycoumarin), scopoletin; (2) furanocoumarins (linear: psoralen, bergapten, xanthotoxin, isopimpinellin; angular: angelicin); (3) flavonoids (quercetin, kaempferol, rutin, isorhamnetin glycosides); (4) hydroxycinnamic acid derivatives (chlorogenic acid, caffeic acid); and (5) fixed oils (petroselinic acid-rich fatty acids in the non-volatile lipid fraction).

Coumarins are benzopyran-2-one derivatives biosynthesized in the phenylpropanoid pathway from trans-cinnamic acid via ortho-hydroxylation and lactonization. In Apiaceae, the coumarin biosynthetic pathway is particularly active, with prenylation and subsequent cyclization giving rise to the pharmacologically potent furocoumarin subclass. Umbelliferone serves as the central biosynthetic precursor for both linear and angular furanocoumarins in this family.

The pharmaceutical relevance of fennel coumarins encompasses: spasmolytic activity of umbelliferone (smooth muscle relaxation via phosphodiesterase inhibition);

photosensitizing activity of bergapten and psoralen (PUVA therapy for psoriasis and vitiligo); anti-inflammatory and antioxidant effects of scopoletin; and potential anticoagulant activity related to structural similarity with 4-hydroxycoumarin anticoagulants. The total coumarin content of *F. vulgare* fruits is typically 0.05-0.4% (w/w) of dry weight, varying by variety, growing region, and harvest time.

2. Botanical Raw Material

2.1 Taxonomic Identification and Varieties

Correct botanical identification is the foundation of any phytochemical extraction process. *F. vulgare* fruits must be distinguished from superficially similar Apiaceae fruits that may occur as adulterants: *Anethum graveolens* (dill), *Carum carvi* (caraway), *Cuminum cyminum* (cumin), and *Pimpinella anisum* (anise). Distinguishing morphological features of fennel fruits include the characteristic five prominent longitudinal ribs, the absence of the winged margin present in anise, and the sweet-anise aroma upon crushing. Microscopy reveals the characteristic vittae (oil canals): six vittae in the commissural face and four vittae on the dorsal face, per pharmacopoeial description.

For coumarin extraction, bitter fennel (*F. vulgare* var. *vulgare*) is preferred over sweet fennel, as it consistently yields higher concentrations of furanocoumarins, particularly bergapten (0.02-0.08% DW in bitter vs. 0.005-0.02% DW in sweet fennel). The coumarin profile also varies by plant organ: roots contain the highest furanocoumarin concentrations, but fruits are the practical industrial source due to their easier harvesting, drying, and storage.

2.2 Harvesting and Primary Processing

Fruits are harvested at full maturity (late summer to early autumn) when they have turned from green to grey-brown and the essential oil content reaches its maximum. Delayed harvesting leads to shattering losses; premature harvesting yields fruits with elevated moisture content and lower secondary metabolite concentrations. Mechanized combine harvesting followed by threshing separates the mericarps from the umbels.

Post-harvest processing includes:

- Cleaning: removal of stems, leaves, soil, and other foreign matter by air classification and screening. Foreign matter must not exceed 2% (w/w) per pharmacopoeial standards.
- Drying: forced-air drying at 40-45 degrees Celsius to a moisture content of not more than 12% (w/w). Temperatures above 50 degrees Celsius cause significant loss of essential oil and risk degradation of heat-labile coumarins, particularly the photosensitive furanocoumarins.
- Storage: dried fruits are stored in sealed, light-proof containers at 15-20 degrees Celsius. Furanocoumarins are susceptible to UV-induced photoisomerization and oxidation; storage in darkness is mandatory.

2.3 Comminution

For solvent extraction of coumarins, the dried fruits are comminuted to increase extraction surface area. Coumarins are located primarily in the pericarp tissues and secretory vittae; grinding ruptures the vittae and disrupts the pericarp, dramatically improving extraction kinetics. A hammer mill or roller mill is used to produce a coarse-to-medium powder (particle size 0.5-2 mm). Over-grinding to fine powder is avoided for two reasons: (1) release of large quantities of essential oil may complicate subsequent liquid-liquid partitioning steps; (2) very fine particles may be difficult to filter from the extract. The milled material is passed through a 1 mm sieve for consistency.

Because furanocoumarins are potent photosensitizers (capable of causing severe photodermatitis upon skin contact followed by UV exposure), all comminution and subsequent processing steps are conducted with appropriate personal protective equipment (gloves, eye protection, dust mask) and in areas protected from direct sunlight or high-intensity UV light sources.

3. Extraction of Total Coumarin Fraction

3.1 Physicochemical Properties of Target Coumarins

The selection of an appropriate extraction solvent and process parameters is guided by the physicochemical properties of the target coumarins:

Compound	MW (g/mol)	log P	Solubility	UV Absorbance (nm)
Umbelliferone	162.14	1.37	Slightly sol. H ₂ O; sol. EtOH, EtOAc, CHCl ₃	325 (strong), 254
Herniarin	176.17	2.07	Slightly sol. H ₂ O; freely sol. EtOH, EtOAc	322, 256
Scopoletin	192.17	1.13	Slightly sol. H ₂ O; sol. EtOH, DMSO	340, 297
Bergapten	216.19	2.10	Practically insol. H ₂ O; sol. EtOH, CHCl ₃ , EtOAc	305, 248
Psoralen	186.16	1.54	Slightly sol. H ₂ O; sol. EtOH, EtOAc, CHCl ₃	310, 246

Isopimpinellin	246.22	2.62	Practically insol. H ₂ O; sol. EtOH, CHCl ₃ , hexane	310, 246
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The log P values (octanol-water partition coefficients) of 1.1-2.6 indicate that coumarins are moderately lipophilic compounds, significantly more soluble in organic solvents than in water. Furanocoumarins (bergapten, psoralen, isopimpinellin) are more hydrophobic than the simple hydroxycoumarins (umbelliferone, scopoletin). This polarity difference is exploited in the fractionation stage.

3.2 Solvent Selection and Extraction Strategy

A two-stage extraction strategy is employed for the selective recovery of the coumarin fraction from fennel fruits:

Stage 1 — Defatting: Fennel fruits contain approximately 12-25% (w/w) fixed oil (fatty acid triglycerides, predominantly petroselinic acid). This non-polar lipid fraction must be removed prior to coumarin extraction, as it co-extracts into non-polar organic solvents and severely complicates subsequent purification. Defatting is performed by exhaustive extraction with n-hexane or petroleum ether (40-60 degrees Celsius boiling range) in a Soxhlet apparatus or by cold maceration.

Stage 2 — Coumarin extraction: Following defatting and solvent removal from the marc, the defatted residue is extracted with a medium-polarity solvent. Ethyl acetate (EtOAc) and ethanol (70-96%) are the most widely used, with different selectivity profiles:

Solvent	Coumarins Extracted	Co-extracted Compounds	Assessment
n-Hexane (Stage 1)	Isopimpinellin, some bergapten (from non-defatted drug)	Fixed oils, waxes, essential oil, chlorophylls	Defatting solvent; not primary coumarin extractant
Ethyl acetate	All coumarins (excellent); high furanocoumarin yield	Chlorophylls, some flavonoid aglycones; minimal sugars	PREFERRED for total coumarin fraction; clean extract
Methanol / 96% EtOH	All coumarins including polar glycosides; high yield	Sugars, amino acids, organic acids, polar pigments	High total yield; complex matrix; more purification needed
70% Ethanol	Coumarins + polar phenolics + flavonoid glycosides	Full polar spectrum; highly complex matrix	Suitable for total extract; poor selectivity for coumarins
Dichloromethane	Non-polar coumarins (bergapten, isopimpinellin) selectively	Chlorophylls; essential oil (if not defatted)	Good selectivity; regulated solvent (Class 2)

Ethyl acetate is the recommended extractant for total coumarin fraction isolation at both laboratory and industrial scale. It provides excellent recovery of both simple coumarins (umbelliferone, herniarin, scopoletin) and furanocoumarins (bergapten,

psoralen, isopimpinellin), with minimal co-extraction of highly polar interfering compounds (sugars, amino acids, inorganic salts). EtOAc is also a Class 3 solvent (ICH Q3C) with a permissible daily exposure of 50 mg/day, making it acceptable for pharmaceutical processing with standard residual solvent control.

3.3 Soxhlet Extraction (Laboratory Reference Method)

Soxhlet extraction provides the highest possible coumarin yield from a fixed mass of drug and serves as the reference method for comparative studies and yield determination.

3.3.1 Defatting Stage

1. Place 50 g of powdered fennel fruit (sieve No. 1, particle size 0.5-1 mm) in a cellulose extraction thimble.
2. Extract with 250 mL n-hexane in a Soxhlet apparatus. Maintain solvent reflux at 60-70 degrees Celsius (heating mantle setting approximately 120 degrees Celsius). Run for 4-6 hours (approximately 15-20 siphoning cycles). The hexane siphonate becomes nearly colorless when defatting is complete.
3. Remove the thimble from the Soxhlet and allow the marc to air-dry in a fume hood overnight to remove residual hexane completely. Residual hexane in the marc inhibits subsequent EtOAc extraction by occupying surface pores.
4. Record the mass of the defatted marc. Expected fixed oil content of fennel: 12-20% (w/w) of dry drug.

3.3.2 Coumarin Extraction Stage

5. Transfer the air-dried defatted marc to a fresh Soxhlet thimble.
6. Extract with 300 mL ethyl acetate at reflux temperature (78-80 degrees Celsius) for 6-8 hours (approximately 20-25 siphoning cycles). The EtOAc siphonate is initially deep yellow-green (chlorophylls and carotenoids); it becomes pale yellow when extraction approaches completion.
7. Combine all siphonate fractions. Evaporate solvent under reduced pressure (40 degrees Celsius, rotary evaporator). The crude residue is a dark green-brown

gum or resinous solid containing coumarins, chlorophylls, carotenoids, and residual fixed oil.

8. Typical crude EtOAc extract yield: 3-7% (w/w) relative to dried defatted drug.
Coumarin content of crude extract: 2-8% (w/w) depending on variety and growing conditions.

3.4 Percolation (Industrial Method)

For large-scale production, Soxhlet extraction is replaced by multi-stage countercurrent percolation or continuous countercurrent extraction, as described in Section 3 of the absyntol article. The industrial process for fennel coumarin extraction follows a two-stage sequence analogous to the laboratory method:

Defatting percolation: The milled drug is loaded into stainless steel percolators. Hexane is passed through the drug bed in a countercurrent arrangement (3-4 percolators in series) at a controlled flow rate of 2-3 mL/min per 100 g drug. The defatted marc is dried under nitrogen sweep.

Coumarin extraction percolation: The defatted marc is loaded into fresh percolators and extracted with ethyl acetate (or acetone for higher furanocoumarin selectivity) in a multi-stage countercurrent system. Total solvent:drug ratio is 8:1 to 12:1 (v/w) to ensure extraction efficiency above 90% for all coumarin components. The combined EtOAc percolates are concentrated in a falling-film evaporator under vacuum (40 degrees Celsius, 80-100 mbar).

3.5 Ultrasound-Assisted Extraction (UAE)

UAE significantly accelerates coumarin extraction from fennel fruits through acoustic cavitation-induced cell wall disruption and enhanced mass transfer. Optimized conditions for *F. vulgare* coumarin UAE reported in the literature: 40 kHz probe sonicator; 70% ethanol as solvent; solid:liquid ratio 1:20 (w/v); temperature 40 degrees Celsius; extraction time 30 minutes. Under these conditions, total coumarin yield (expressed as umbelliferone equivalents) is comparable to 6-hour Soxhlet extraction.

UAE is particularly suited to selective bergapten extraction when ethyl acetate replaces ethanol.

4. Purification and Isolation of Individual Coumarins

4.1 Liquid-Liquid Partitioning

The crude EtOAc extract contains coumarins as the principal pharmacologically active compounds, but also chlorophylls, carotenoids, fatty acid residues, and wax components. A systematic liquid-liquid partitioning procedure is used to reduce complexity before chromatographic purification:

9. Dissolve crude EtOAc extract in 90% methanol:water (9:1 v/v), 200 mL per 10 g of extract.
10. Extract three times with an equal volume of n-hexane (3 x 200 mL). The hexane layer removes residual fixed oil, waxes, highly non-polar carotenoids, and essential oil residues. The aqueous methanol layer retains coumarins. Combine hexane layers (discard or reserve for separate analysis).
11. Concentrate the aqueous methanol layer to remove methanol by rotary evaporation at 40 degrees Celsius. Dilute the residue with water to achieve approximately 30% aqueous solution.
12. Extract the aqueous solution three times with ethyl acetate (3 x 100 mL). Coumarins partition strongly into the EtOAc phase; polar pigment residues, organic acids, and residual sugars remain in the aqueous phase.
13. Wash the combined EtOAc extract with saturated NaCl solution (brine, 1 x 50 mL) to remove emulsified water. Dry over anhydrous Na₂SO₄, filter, and evaporate solvent at 40 degrees Celsius.

The result is a semi-purified coumarin-enriched fraction with a coumarin content typically 20-45% (w/w) of the residue. This fraction is suitable for TLC screening, bioactivity assays, and as the feedstock for column chromatographic isolation.

4.2 Column Chromatographic Isolation

4.2.1 Silica Gel Column Chromatography

Silica gel (SiO₂) column chromatography is the standard method for separation of individual fennel coumarins. Stationary phase: silica gel 60 (particle size 63-200 micrometers for gravity columns; 40-63 micrometers for medium-pressure liquid chromatography, MPLC). Column dimensions (laboratory scale): 4 cm internal diameter x 40-50 cm height for 2-5 g of crude extract load. Solvent systems are developed by TLC optimization:

- n-Hexane:EtOAc gradient (100:0 to 30:70 v/v): provides good separation of furanocoumarins (eluted at higher EtOAc concentrations) from residual non-polar lipids (eluted first with pure hexane).
- n-Hexane:acetone gradient (95:5 to 50:50 v/v): particularly effective for separation of bergapten from isopimpinellin and for isolating umbelliferone in the more polar fractions.
- DCM:MeOH gradient (100:0 to 90:10 v/v): provides excellent resolution of all coumarin classes, including the most polar scopoletin and umbelliferone glucoside.

Procedure: The semi-purified extract is dissolved in a minimum volume of the starting mobile phase and carefully loaded onto the pre-equilibrated column as a narrow band. Mobile phase is eluted under gravity or at controlled pressure (MPLC: 2-5 bar). Fractions of 10-20 mL are collected and monitored by TLC (silica gel 60 F254 plates, UV visualization at 254 and 365 nm; anisaldehyde-H₂SO₄ spray for universal detection). Coumarins exhibit characteristic fluorescence under UV 365 nm: umbelliferone (bright blue fluorescence, R_f 0.35 in hexane:EtOAc 1:1), bergapten (weak blue fluorescence, R_f 0.55), isopimpinellin (greenish, R_f 0.65), herniarin (blue, R_f 0.45).

Fractions containing the same coumarin (confirmed by identical TLC profile) are pooled and evaporated. Representative isolated yields from 5 g of semi-purified extract:

- Bergapten: 15-30 mg (0.3-0.6% w/w of crude extract)
- Umbelliferone: 8-20 mg (0.16-0.4% w/w)
- Isopimpinellin: 5-15 mg (0.1-0.3% w/w)
- Herniarin: 5-12 mg (0.1-0.24% w/w)
- Psoralen: 2-8 mg (0.04-0.16% w/w)

4.2.2 Sephadex LH-20 Gel Filtration

Sephadex LH-20 (hydroxypropylated dextran gel, particle size 25-100 micrometers) provides size-exclusion separation combined with reversed-phase partition in organic solvents. It is particularly useful for removing residual polymeric pigments (chlorophyll degradation products, melanoid pigments) that co-elute with coumarins on silica gel. Elution with methanol or methanol:dichloromethane (1:1 v/v) resolves coumarins from high-molecular-weight interferences in a single pass. Sephadex LH-20 is frequently used as a second-stage purification following silica gel chromatography.

4.2.3 Preparative HPLC

For analytical-grade reference standards and high-purity isolates (purity > 98%), preparative reversed-phase HPLC is the method of choice. Column: C18 preparative column (21.2 mm x 250 mm, 5 or 10 micrometer particle size). Mobile phase: acetonitrile:0.1% aqueous formic acid gradient (20:80 to 70:30 v/v over 30 min). UV detection: 310 nm (selective for furanocoumarins) and 325 nm (selective for umbelliferone). Flow rate: 15-20 mL/min. Injection volume: 500-2000 microliters of semi-purified fraction dissolved in methanol at 5-10 mg/mL.

Preparative HPLC achieves baseline separation of all major fennel coumarins in a single 30-minute run with purities of 95-99.5%. Fractions are collected by peak

triggering, pooled, and freeze-dried or evaporated under nitrogen to yield white-to-pale yellow crystalline solids.

4.3 Crystallization

For compounds obtained in sufficient quantity by column chromatography, recrystallization provides an economical final purification step that is especially effective for bergapten and umbelliferone:

4.3.1 Bergapten Crystallization

Bergapten (5-methoxypsoralen) crystallizes readily as colourless needles. Dissolve the column-purified fraction in a minimum volume of hot ethanol (96%, 60-70 degrees Celsius). Add hot distilled water dropwise until the solution becomes faintly turbid. Allow to cool slowly to room temperature, then to 4 degrees Celsius overnight. Filter crystals under vacuum, wash with cold 30% aqueous ethanol, and dry at 50 degrees Celsius under vacuum. Purity by HPLC: 97-99%. Melting point of pure bergapten: 188-190 degrees Celsius (literature value: 188 degrees Celsius). Yield: 60-80% recovery from column-purified fraction.

4.3.2 Umbelliferone Crystallization

Umbelliferone crystallizes as white needles from hot water or aqueous ethanol. Dissolve the column-purified fraction in a minimum volume of hot water (80-90 degrees Celsius). Allow to cool slowly to room temperature, then to 4 degrees Celsius. Filter under vacuum, wash with cold water, and dry at 50-60 degrees Celsius. Purity: 95-98%. Melting point: 228-230 degrees Celsius (literature: 230 degrees Celsius). Umbelliferone exhibits intense blue fluorescence under UV 365 nm, which serves as a convenient purity indicator.

5. Quality Control and Standardization

5.1 Identity Tests

TLC Identity Test (Ph.Eur. method for *Foeniculi fructus*): Stationary phase: silica gel 60 F254. Mobile phase: ethyl acetate:toluene:formic acid:water (20:65:10:10 v/v). Detection: (a) UV 254 nm — quenching zones; (b) UV 365 nm — fluorescence zones (coumarins fluoresce blue or green); (c) anisaldehyde-H₂SO₄ spray followed by heating to 100 degrees Celsius for universal chromogenic detection. Reference solutions of bergapten and umbelliferone at 1 mg/mL in methanol are run alongside the sample. The extract must show zones at R_f values corresponding to the reference standards.

HPLC Fingerprinting: A characteristic HPLC-DAD (diode array detection) fingerprint at 310 nm is established for each batch. Retention times and UV absorption spectra (200-400 nm, full scan) of all peaks are compared to reference standards and to an approved reference chromatogram. Peaks deviating by more than 5% in retention time or displaying non-matching UV spectra are flagged for investigation.

5.2 Quantitative Assay of Total Coumarins

Spectrophotometric method (screening): Total coumarin content may be estimated by spectrophotometry after reaction with Emerson's reagent (4-aminoantipyrine) or by direct UV absorbance at 325 nm, expressed as umbelliferone equivalents. This method is rapid and suitable for routine quality monitoring of crude extracts but lacks specificity.

HPLC-UV Quantitative Method (official assay): This is the pharmacopoeial reference method for individual coumarin quantification. Conditions: C18 analytical column (4.6 mm x 150 mm, 5 micrometer); mobile phase A: 0.1% formic acid in water; mobile phase B: acetonitrile; gradient from 10% to 70% B over 25 minutes; flow rate 1.0 mL/min; UV detection at 310 nm; column temperature 30 degrees Celsius. External calibration with certified reference standards of bergapten, umbelliferone, psoralen,

and isopimpinellin. Linearity range: 0.5-100 micrograms/mL for each coumarin. LOD: 0.05-0.1 micrograms/mL; LOQ: 0.2-0.5 micrograms/mL.

5.3 Quality Specifications

Parameter	Specification	Method
Total coumarin content (as umbelliferone equivalents)	$\geq 0.05\%$ (w/w) in raw drug	HPLC-UV 325 nm or spectrophotometric
Bergapten content	$\geq 0.01\%$ (w/w) in drug; $\geq 10\%$ (w/w) in isolated fraction	HPLC-UV 310 nm, external standard
Purity of isolated bergapten	$\geq 97.0\%$ (w/w)	HPLC-UV area normalization
Purity of isolated umbelliferone	$\geq 95.0\%$ (w/w)	HPLC-UV area normalization
Residual solvents: EtOAc	$\leq 5,000$ ppm (ICH Q3C Class 3)	GC headspace, FID detector
Residual solvents: n-hexane	≤ 290 ppm (ICH Q3C Class 2)	GC headspace, FID detector
Water content (isolated compounds)	$\leq 1.0\%$ (w/w)	Karl Fischer titration
Heavy metals (drug)	Pb ≤ 5 ppm; Cd ≤ 0.5 ppm; As $\leq$	ICP-MS

	3 ppm; Hg $\leq$ 0.1 ppm	
Microbial quality (drug)	TAMC $\leq 10^5$ CFU/g; TYMC $\leq 10^3$ CFU/g; E. coli absent	Ph.Eur. 5.1.4
Aflatoxins B1+B2+G1+G2	B1 ≤ 2 ppb; total ≤ 4 ppb	HPLC-FLD post-column derivatization
Melting point (bergapten)	188-190 degrees Celsius	Capillary melting point apparatus
Melting point (umbelliferone)	228-232 degrees Celsius	Capillary melting point apparatus

5.4 Structure Confirmation of Isolated Compounds

For each new batch of isolated coumarins, structural identity is confirmed by a combination of spectroscopic methods:

- UV-Vis spectroscopy: Characteristic absorption maxima in methanol. Bergapten: 248, 305 nm (log epsilon 4.24, 4.06). Umbelliferone: 254, 325 nm (log epsilon 3.97, 4.20). The bathochromic shift upon addition of 5% NaOH solution confirms phenolic hydroxyl groups (umbelliferone shifts from 325 to 390 nm, confirming the 7-hydroxy group).
- Infrared spectroscopy (ATR-FTIR): Characteristic absorption bands for the coumarin lactone carbonyl stretch at 1715-1730 cm^{-1} ($\text{C}=\text{O}$), aromatic C-H at 3050-3100 cm^{-1} , and C-O-C stretching at 1200-1250 cm^{-1} (furan ring oxygen in furanocoumarins).
- ^1H -NMR and ^{13}C -NMR (if required for reference standard qualification): Full assignment of proton and carbon signals confirms the substitution pattern. Bergapten is characterized by two furan ring protons (H-2' at delta 7.79 ppm,

H-3' at delta 6.96 ppm, $J = 2.2$ Hz), the H-8 psoralen proton (delta 7.70 ppm), and the methoxy singlet (H-OMe at delta 3.98 ppm).

- Mass spectrometry (HPLC-ESI-MS/MS): Molecular ion confirmation and fragmentation pattern. Bergapten: $[M+H]^+$ $m/z = 217.05$; key fragment at $m/z = 189$ (loss of CO from lactone). Umbelliferone: $[M+H]^+$ $m/z = 163.04$; key fragment at $m/z = 107$ (loss of $CO_2 + CO$).

6. Yield Summary and Process Optimization

The following table summarizes typical yields at each processing stage, calculated from 1 kg of dried *F. vulgare* bitter fennel fruits (moisture content 10%, bergapten content of raw drug approximately 0.05% DW):

Processing Stage	Mass Obtained	Notes
Dried fennel fruit (starting material)	1,000 g	Moisture $\leq$ 12%; bergapten $\sim$ 0.05% DW
Defatted marc (after hexane Soxhlet)	820-850 g	150-180 g fixed oil removed (15-18%)
Crude EtOAc extract (after Soxhlet, evaporation)	35-55 g	Dark green resinous solid; 3.5-5.5% yield
Semi-purified coumarin fraction (after LLE)	12-20 g	Coumarin content $\sim$ 25-40% w/w of fraction
Bergapten (after silica gel + crystallization)	350-500 mg	Purity $\geq$ 97%; $\sim$ 70% recovery from semi-purified fraction
Umbelliferone (after silica gel + crystallization)	200-350 mg	Purity $\geq$ 95%
Isopimpinellin (after preparative HPLC)	100-200 mg	Purity $\geq$ 95%; smallest fraction
Total coumarin yield (all compounds combined)	700-1,100 mg	$\sim$ 0.07-0.11% yield relative to starting drug

Process optimization focuses on three key variables: (1) Particle size of milled drug — reducing from 2 mm to 0.5 mm increases total coumarin yield by approximately 18% due to improved cell disruption; (2) Solvent polarity in Stage 2 extraction — replacing

EtOAc with acetone increases total coumarin yield by 10-15% but increases co-extraction of interfering polar compounds; (3) Temperature during evaporation — maintaining below 45 degrees Celsius prevents degradation of bergapten (which undergoes slow thermal decarboxylation above 60 degrees Celsius at low pH).

7. Safety Considerations

The extraction and isolation of fennel coumarins, particularly furanocoumarins, requires stringent safety measures:

- Photodermatitis hazard: Bergapten, psoralen, and isopimpinellin are potent photosensitizers. Skin contact followed by UV light exposure (sunlight or artificial UV sources) causes severe photodermatitis, hyperpigmentation, and potentially blistering. All personnel must wear nitrile gloves, long-sleeved laboratory coats, and eye protection. Processing areas must be shielded from direct sunlight and UV light sources. Warning signs must be posted.
- Solvent safety: n-Hexane is a neurotoxic solvent (N-hexane metabolite 2,5-hexanedione is responsible for peripheral neuropathy with chronic exposure). Handling must be in a properly ventilated fume hood; personal exposure limit (PEL) must be respected. Ethyl acetate is less hazardous (PEL 400 ppm) but is flammable (flash point -4 degrees Celsius); all operations with EtOAc must exclude ignition sources.
- Regulatory status: Bergapten and other furanocoumarins are regulated in cosmetic products (EU Cosmetics Regulation EC 1223/2009 restricts bergapten to 1 ppm in sunscreen products intended for use in sunlight, and bans it entirely in products for use in UV booths). Pharmaceutical-grade isolated furanocoumarins are controlled substances in some jurisdictions and require appropriate documentation for purchase, use, and disposal.

8. Conclusion

The technological process for obtaining coumarins from *Foeniculum vulgare* is a multi-stage procedure requiring careful control of each step to maximize yield, selectivity, and purity of the target compounds. The two-stage extraction strategy — defatting with

n-hexane followed by ethyl acetate extraction of the coumarin fraction — provides an effective and scalable approach that removes the major interfering lipid matrix while achieving high recovery of all coumarin classes.

Liquid-liquid partitioning reduces extract complexity prior to chromatographic isolation, significantly improving column performance and reducing solvent consumption. Silica gel column chromatography with optimized solvent gradients, complemented by crystallization or preparative HPLC, yields individual coumarins of pharmaceutical purity. Comprehensive quality control by HPLC, spectroscopy, and physicochemical testing ensures conformity with regulatory standards.

The photochemical sensitivity and dermatotoxic potential of furanocoumarins necessitate rigorous safety protocols at every stage of the process. As demand for natural furanocoumarin reference standards, botanical supplements, and pharmaceutical preparations continues to grow, optimized, validated extraction and purification processes for *F. vulgare* coumarins will remain of significant industrial and scientific importance.

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