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84 Patrick Lane, Suite 115, Poughkeepsie, NY 12603 · www.process-nmr.com · Technical White Paper PNA-WP-2026-SAWP-01

¹H NMR Spectroscopy of Saw Palmetto (Serenoa repens) Lipophilic Extracts

Spectral assignment, quantitative proton bookkeeping, metabolomic fingerprinting and authentication against vegetable-oil, lauric-oil and designer-blend adulteration

John C. Edwards, Ph.D.

Process NMR Associates, LLC, 84 Patrick Lane, Suite 115, Poughkeepsie, NY 12603

Version 1.0 · October 1, 2026

Document details

Document code	PNA-WP-2026-SAWP-01
Title	¹H NMR Spectroscopy of Saw Palmetto (Serenoa repens) Lipophilic Extracts: Spectral assignment, quantitative proton bookkeeping, metabolomic fingerprinting and authentication against vegetable-oil, lauric-oil and designer-blend adulteration
Version	1.0 (first issue)
Generation date	October 1, 2026
Filename	PNA-WP-2026-SAWP-01_Saw_Palmetto_1H_NMR_v1.0.docx
Prepared for / by	John C. Edwards, Ph.D., Process NMR Associates, LLC, 84 Patrick Lane, Suite 115, Poughkeepsie, NY 12603
Model identity	claude-opus-5-5 (configured model identifier as reported by the session; the serving model may differ)
Reasoning effort	Not declared by the author for this report (not observable by the model)
Tools engaged	Web search and CrossRef DOI verification (api.crossref.org) for all DOI-bearing references (60 references in total); Python 3.13 (numpy, scipy, pandas, matplotlib, rdata, openpyxl) for the public-dataset analysis, spectral simulation, PCA and workbook; Node.js docx 9.x for document assembly; LibreOffice for rendering checks
AI-assistance statement	AI-drafted material: literature synthesis, document structure, tables, the simulation and analysis code, the figures derived from the public ChemoSpec dataset and from the simulations, and the provisional acceptance windows. Author-supplied material: scope, instrument constraints, standing preferences and the critical framing. No PNA-measured spectra are included in version 1.0; all figures are labeled as public-data or simulated. The author's measured data, calculations and conclusions are to be added in version 1.1.
Status	Working document; version 1.1 reserved for PNA-measured spectra, the reference-set study and measured acceptance windows

Abstract

Saw palmetto (*Serenoa repens* (W. Bartram) Small) fruit extract is the most expensive botanical oil in the dietary-supplement and phytomedicine trade, and its lipid composition, which is dominated by free fatty acids rather than triglycerides, is simple enough that it has become a target for increasingly sophisticated adulteration: dilution with vegetable oils, substitution with lauric-type oils botanically related to the palm, and, since 2018, designer blends of free fatty acids of animal origin formulated to pass the compendial gas-chromatographic ratio tests. In this paper the ¹H NMR spectroscopy of lipophilic saw palmetto extracts is treated from first principles. The chemistry of the hexane, ethanolic and supercritical-CO_2 extracts is summarized, the compendial specifications are tabulated, and a complete ¹H assignment table in $CDCl_3$ is assembled from the saw palmetto literature and the wider lipid-NMR literature, with the α-methylene chemical shift (2.345 ppm for free acids, 2.31 ppm for triglycerides, 2.28–2.30 ppm for alkyl esters) and the glyceryl signals at 4.14, 4.29 and 5.26 ppm identified as the primary speciation markers. A set of proton-bookkeeping relations is derived that returns, from a single spectrum, the fraction of acyl chains bound to glycerol, the oleic, linoleic, linolenic and saturated chain fractions, the mean carbon number of the saturated pool, an iodine-value estimate and semi-quantitative sterol and fatty-alcohol indices. The relations are exercised on sixteen public ≈ 500 MHz spectra of retail saw palmetto products and reference oils (the ChemoSpec SrE.NMR dataset) and on simulated 300 MHz and 60 MHz spectra built for twelve extraction-type and adulteration scenarios. Only two of the fourteen retail products examined carry the free-fatty-acid-dominated profile expected of a compendial extract; most contain 50–75 % glyceride-bound chains and one is a linoleic-rich seed-oil blend. The published chemometric studies (Booker et al. 2014; De Combarieu et al. 2015; Perini et al. 2018) are reviewed, and a preprocessing and modeling workflow (alignment, 0.02-ppm bucketing, total-area normalization, mean-centering, Pareto scaling, PCA, and one-class membership testing in preference to discriminant models) is set out and demonstrated. The limits of the method are stated plainly: hydrolyzed lauric-type oils and designer free-fatty-acid blends are spectroscopically silent in the major ¹H signals, so that stable-isotope ratio analysis and GC sterol and fatty-alcohol profiling remain indispensable for those cases. A PNA acquisition protocol for the 300 MHz and 60 MHz instruments, provisional acceptance windows, and a reference-set plan are proposed for version 1.1, in which the author's own measured spectra will replace the public and simulated data. Sixty references are cited; every journal, report and DOI-bearing entry was verified against the CrossRef record before use, and the compendial, regulatory and data-set entries were verified against the issuing bodies' own pages.

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1. Introduction and scope

Saw palmetto fruit extract occupies an unusual position among botanical ingredients. Its pharmacological interest, which rests on decades of use for lower urinary tract symptoms attributed to benign prostatic hyperplasia, is not tied to any single marker compound; the whole lipid extract is regarded as the active substance, and the compendial monographs define quality in terms of a total fatty-acid content, a pattern of fatty-acid ratios, and small windows for sterols and long-chain alcohols [1–3]. At the same time the plant has a geographically narrow range, confined to the southeastern coastal plain of the United States, the berries are still harvested by hand, and the harvest is subject to hurricanes, rainfall and, since 2018, a Florida permitting regime; the extract has therefore become the most expensive oil in the supplement market, with authentic material quoted at 150–275 US$/kg against 50–135 US$/kg for adulterated material of Asian origin [4,5]. The combination of a simple lipid matrix, a specification expressed almost entirely in fatty-acid terms, and a large price differential has produced exactly the adulteration pattern that such conditions predict: dilution with cheaper vegetable oils, substitution with lauric-type oils whose fatty-acid profile resembles that of the palm, and finally designer blends of free fatty acids, including fatty acids of animal origin, formulated to reproduce the compendial ratios [4–7].

Proton NMR spectroscopy is well suited to this problem for a reason that is rarely made explicit in the saw palmetto literature. The compendial gas-chromatographic method hydrolyzes and methylates the lipid before analysis, so that the information on how the acyl chains are bound, whether as free acids, glycerides or alkyl esters, is destroyed at the first step. Authentic saw palmetto extract is a free-fatty-acid material, with about 85 % of the acyl chains unbound and only a few per cent as triglycerides, whereas every vegetable oil and animal fat is a triglyceride material [8–10]. A ¹H spectrum of the whole extract in $CDCl_3$ reports this speciation directly, through the chemical shift of the α-methylene protons and through the glyceryl signals, without any chemical treatment, and it reports the unsaturation pattern, the chain-length distribution of the saturated pool, and the presence of ethyl and methyl esters, sterols and fatty alcohols in the same five-minute experiment. The published ¹H NMR work on saw palmetto, which consists of three primary studies [3,5,11], the Botanical Adulterants Prevention Program documents [4,6,10], and two pharmacological fractionation papers in which ¹H NMR was used to characterize fractions [12], has concentrated on multivariate fingerprinting. The quantitative, first-principles reading of the spectrum, which is the natural strength of NMR and the basis of the lipid-NMR methods developed for edible oils over the last thirty years [13–18], has not been applied to saw palmetto in the open literature.

The present paper is written as a working document for Process NMR Associates (PNA), 84 Patrick Lane, Suite 115, Poughkeepsie, NY 12603, in preparation for a measured study on the laboratory's Varian Mercury-MVX 300 and its 60 MHz benchtop instruments. Its scope is confined to the lipophilic extracts, that is, the hexane, ethanolic and supercritical-CO_2 extracts and the softgel fills made from them, because these are the materials that are traded, specified and adulterated; the dried berry, its powders and aqueous or hydroalcoholic tinctures are mentioned only where the literature requires it. Section 2 summarizes the botanical and the specifications, Section 3 the extract chemistry, and Section 4 the adulteration problem. Sections 5 and 6 cover sample preparation, acquisition and the complete ¹H assignment, with ¹³C NMR introduced as the speciation complement. Sections 7 and 8 use a public set of sixteen ≈ 500 MHz spectra of retail products and reference oils to illustrate the spectra and to exercise a set of proton-bookkeeping relations; Section 9 uses simulated spectra to explore extraction-type and adulteration scenarios that the public set does not cover; Section 10 treats field strength and the benchtop case. Section 11 reviews and demonstrates the chemometric workflow, Section 12 assembles a decision logic that places NMR among the orthogonal methods, Section 13 proposes the PNA protocol and reference-set plan, and Section 14 is a

critical assessment. No measured PNA spectrum appears in this version; the figures built from public and simulated data are placeholders for the author's own data in version 1.1, and are labeled as such throughout.

2. The botanical, its commercial extracts and the compendial specifications

2.1 Botanical source and harvest economics

Serenoa repens (synonyms *Sabal serrulata* (Michx.) Nutt. ex Schult. & Schult. f. and *Serenoa serrulata* (Michx.) G. Nichols; Arecaceae) is a low, clump-forming fan palm of the sandy soils of the southeastern United States, with Florida supplying most of the commercial harvest. The fruit is a single-seeded drupe that ripens from green through yellow-orange to blue-black between late August and October; it is the partially dried ripe fruit that the United States Pharmacopeia defines as the crude drug, with minimum contents of 2 % (v/w) volatile oil, 7 % lipophilic extract and 9.0 % total fatty acids on the dried basis [1]. Fatty-acid accumulation peaks about three weeks before the fruit turns black, and both the total fatty-acid content (64–127 mg/g whole fruit across Florida locations) and the lauric-to-oleic ratio differ between the green and silver ecotypes, so that the raw material itself carries an appreciable natural variability that is inherited by the extracts [19]. Wild harvesting has in principle been sufficient to meet demand, with normal harvests estimated at 4 000–8 000 metric tons of berries a year, but the poor harvests of 2016–2018 and the July 2018 Florida harvest-permit requirement produced a supply shortfall and a threefold increase in berry prices, which is the immediate economic background to the adulteration episodes described in Section 4 [4,10]. Substitution of the fruit with the Everglades palm, *Acoelorrhaphe wrightii*, has been documented at the raw-material stage, a problem that belongs to DNA barcoding rather than to NMR [4,20].

2.2 Extraction solvents and extract types

Three extraction processes are recognized by the pharmacopoeias and by the European Union herbal monograph. The hexane soft extract (drug-to-extract ratio, DER, 7–11:1) is the well-established-use preparation, containing 92 % fatty acids, free or esterified, and 2 % unsaponifiable matter, and is the form on which most of the clinical literature was generated; the ethanolic soft extract (DER 7.5–14.3:1, ethanol 90–96 % m/m) is the traditional-use preparation; and the supercritical-CO_2 extract (DER 6.5–9:1) has been marketed in Belgium since 1996 and in France since 2010 but is not itself a monograph preparation [2,21]. The industrial processes have been described in some detail by De Combarieu and co-workers: ethanolic extraction of the crumbled dried fruit at room temperature with 96 % ethanol, filtration and vacuum concentration at no more than 50 °C; supercritical-CO_2 extraction at 220 bar and 45 °C followed by separation on expansion; and Soxhlet extraction with hexane, in each case with the solvent and water removed under vacuum to an oily extract [3]. The United States Pharmacopeia admits all three routes, hydroalcoholic mixtures, hexane, or supercritical carbon dioxide, with a crude-drug-to-extract ratio of 8.0:1 to 14.3:1, and distinguishes between the lipophilic (hexane, CO_2) and hydroalcoholic extracts only in the window set for long-chain alcohols [1].

The three extract types are not identical in composition, and the differences matter for NMR interpretation. A multiplexed LC-HRMS metabolomic comparison of fifteen laboratory extracts prepared from a single fruit batch and twenty commercial extracts showed that the hexane extracts, and the hexane-derived Permixon product in particular, carried the highest free-fatty-acid content and the lowest content of every glyceride class, whereas the supercritical-CO_2 and ethanolic extracts carried the highest mono-, di- and triglyceride contents; the ethanolic extracts alone contained glycerophospholipids, flavonoids and tyramine [8,12]. The organoleptic differences recorded by the Botanical Adulterants Prevention Program, dark greenish-

brown for ethanolic, yellowish-brown to orange-brown for CO_2 and yellowish-green to orange-yellow for hexane extracts, follow from the same solvent selectivities [4]. Fatty-acid ethyl esters are associated with ethanolic extraction, whether formed during extraction and concentration or carried over from the berry, and were identified by Booker and co-workers in the 4.1–4.2 ppm region of ¹H spectra of commercial products [11,21]. Finished dosage forms add their own complications: softgel fills may be diluted with carrier oils, glycerin or emulsifiers, and the reference hexane extract is presented in capsules as approximately 36 % saw palmetto oil in a polyethylene glycol matrix of molecular weight 10 000 g/mol, which contributes a large singlet at 3.62–3.64 ppm to any $CDCl_3$ spectrum of the capsule contents [11,22].

2.3 Compendial and regulatory specifications

The specifications that an authentication method must be read against are collected in Table 1. The current USP monograph for Saw Palmetto Extract requires not less than 80.0 % fatty acids, not less than 0.2 % sterols and not less than 0.1 % β-sitosterol on the anhydrous basis, together with 0.15–0.35 % long-chain alcohols for the lipophilic extract and 0.01–0.15 % for the hydroalcoholic extract, and it identifies the extract by the ratios of the lauric acid concentration to the concentrations of nine other fatty acids determined by GC-FID after transesterification [1]. The numerical ratio windows are paywalled in the current edition; the freely readable USP 32–NF 27 text of 2009 gives the windows reproduced in Table 1 (for example lauric/oleic 0.60–1.15, lauric/myristic 2.2–2.8, lauric/palmitic 2.8–3.9 and lauric/linoleic 5.0–16), together with a 70.0–95.0 % total fatty-acid window, a 0.2–0.5 % sterol window, an iodine value of 40–50, a saponification value of 210–250 and 1.8–3.5 % unsaponifiable matter [23]. A 2013 Revision Bulletin added a separate ratio set applicable to supercritical-CO_2 extracts, and the windows should therefore be re-checked against the current edition before they are used in a specification [1]. The Capsules monograph requires 22.0–34.0 % lauric acid in the labeled amount of extract, with lauric/caprylic 8.5–17.5 and lauric/myristic 2.2–2.8 [1]. The European Pharmacopoeia fruit monograph requires a minimum of 11 % total fatty acids in the dried material, and the extract monograph sets a minimum lauric acid content, quoted as 23 % in the BAPP bulletin and as 20 % by Booker and co-workers citing the seventh edition, together with an acid-value range that is specific to this material because of its unusually high free-acid content [4,11,21,24]. The National Institute of Standards and Technology maintains a fruit SRM (3250) and an extract SRM (3251) whose certificates carry sterol, carotenoid and tocopherol values, although the fatty-acid values were withdrawn for suspected instability, a cautionary note for anyone proposing to use a stored reference material as an NMR calibrant [25,26].

Table 1. Compendial and regulatory specifications relevant to saw palmetto extract authentication

Document	Material / preparation	Specification (as published)	Notes
USP–NF, Saw Palmetto Extract (current monograph)	Extract of comminuted fruit; hydroalcoholic mixtures, hexane, or supercritical CO_2; crude drug-to-extract ratio 8.0:1 to 14.3:1	NLT 80.0 % fatty acids; NLT 0.2 % sterols; NLT 0.1 % β-sitosterol (anhydrous basis). Long-chain alcohols: lipophilic extract 0.15–0.35 %, hydroalcoholic extract 0.01–0.15 %. Identification by the ratios of lauric acid to nine other fatty acids (GC-FID)	Only the Definition is freely readable; the ratio table and the scCO_2-specific ratio set added by the 2013 Revision Bulletin are paywalled

Document	Material / preparation	Specification (as published)	Notes
USP 32–NF 27 (2009), Saw Palmetto Extract	As above	70.0–95.0 % fatty acids; 0.2–0.5 % sterols; iodine value 40–50; saponification value 210–250; unsaponifiable matter 1.8–3.5 %. Lauric-acid ratio ranges: capric 9.0–16; caproic 8.5–24; caprylic 8.5–17.5; linoleic 5.0–16; linolenic 31.5–55; myristic 2.2–2.8; oleic 0.60–1.15; palmitic 2.8–3.9; stearic 14–26	Freely readable legacy text; used here for the numerical ratio windows, which should be re-checked against the current edition before use in a specification
USP–NF, Saw Palmetto Capsules	Capsules containing Saw Palmetto Extract	Lauric acid 22.0–34.0 % of the labeled extract; lauric/caprylic 8.5–17.5; lauric/myristic 2.2–2.8	Visible portion of the monograph
USP–NF, Saw Palmetto (fruit) and Powdered Saw Palmetto	Partially dried ripe fruit	NLT 2 % (v/w) volatile oil; NLT 7 % lipophilic extract; NLT 9.0 % total fatty acids (dried basis); TLC identification	Raw-material monographs
Ph. Eur., Saw palmetto fruit (monograph 1848) and Sabalis serrulatae extractum	Dried ripe fruit; extract	Fruit: minimum 11 % total fatty acids (as cited by the EMA assessment report). Extract: minimum lauric acid content (23 % per the BAPP bulletin; 20 % per Booker et al. citing the 7th edition) and a specified acid-value range	The EDQM text is not freely readable; the two secondary values for lauric acid disagree and must be resolved against the current edition
EMA/HMPC European Union herbal monograph (EMA/HMPC/280079/2013)	Well-established use: soft extract DER 7–11:1, hexane, containing 92 % fatty acids (free or esterified) and 2 % unsaponifiable matter. Traditional use: soft extract DER 7.5–14.3:1, ethanol 90–96 % m/m	Posology 320 mg daily (hexane extract) or 320 mg daily (ethanol extract)	The $scCO_2$ extract (DER 6.5–9:1) is described in the assessment report market overview but is not a monograph preparation
NIST SRM 3251 (Serenoa repens extract) certificate	Supercritical-CO_2-type extract	Campesterol 0.533 ± 0.031, β-sitosterol 1.666 ± 0.064, stigmasterol 0.247 ± 0.040 mg/g; cycloartenol 0.772 mg/g (reference value); β-carotene 46.8 µg/g; γ-tocopherol 280 µg/g	Fatty-acid values were withdrawn from the certificate because of suspected instability

Two features of these specifications deserve emphasis because they shape the whole authentication problem. First, the fatty-acid ratios are determined after total hydrolysis and methylation; a material containing the right fatty acids in the right proportions passes irrespective of whether those acids were free, glyceride-bound or esterified, and irrespective of their botanical or zoological origin. Second, the sterol and fatty-alcohol windows are the only parts of the specification that look at the minor, non-fatty-acid fraction, and they are the parts least often tested by purchasers. The Botanical Adulterants Prevention Program concluded in 2019, after reviewing thirty-four published methods, that routine GC methods measuring only total fatty acids are not suitable for detecting adulteration and recommended organoleptic inspection together with the acid value and GC profiling of the fatty acids, fatty alcohols and phytosterols [10]. The place of ¹H NMR in that recommendation, as a complementary method rather than a replacement, is examined in Section 12.

3. Chemistry of the lipophilic extract

3.1 Fatty-acid profile and its natural variability

The fatty-acid profile of the extract is dominated by two acids, lauric (C12:0) and oleic (C18:1), each at roughly 30 % of the total, followed by myristic (C14:0) and palmitic (C16:0) at about 10 % each and linoleic (C18:2) at about 5 %; caproic, caprylic, capric, stearic and linolenic acids complete the profile at 0.5–3 % each [8,11,22]. Measured lauric-acid ratios for a hexane extract and an ultrahigh-pressure supercritical-CO_2 extract, each compared with its multi-lot typical values, were reported by Cartwright and co-workers as, for example, lauric/oleic 1.0–1.2, lauric/myristic 2.6–2.8, lauric/palmitic 3.5–3.7, lauric/linoleic 5.9–6.9 and lauric/linolenic 35–51, with total fatty-acid contents of 87.9–91.5 % in the lots examined [22]. Table 2 sets a typical literature profile beside the USP ratio windows and beside the Codex Alimentarius ranges for the vegetable oils that have been documented or suspected as adulterants [27,28]. The table makes two points that recur through this paper. The lauric-type oils, coconut, palm kernel and babassu, share the lauric and myristic dominance of saw palmetto, differing mainly in their much lower oleic and linoleic contents and higher caprylic and capric contents, and babassu and coconut are the oils that Perini and co-workers identified as the adulterants in two of nine commercial extracts [5]. The long-chain oils, olive, canola, sunflower and palm, differ from saw palmetto in every column, and their presence is betrayed as readily by the fatty-acid profile as by any other measurement; it is only when they are blended with lauric-type materials in proportions chosen to reproduce the saw palmetto profile that the fatty-acid test fails, which is precisely the strategy described in the industry commentary collected by the Botanical Adulterants Prevention Program [6,10].

Table 2. Fatty-acid profiles (% of total fatty acids) of authentic saw palmetto extract and of the oils documented or suspected as adulterants

Fatty acid	Saw palmetto extract (typical, literature)	USP lauric-acid ratio window (USP 32–NF 27)	Coconut oil (Codex)	Palm kernel oil (Codex)	Babassu oil (Codex)	Palm oil (Codex)	Olive oil (Codex CXS 33)	Low-erucic rapeseed/canola (Codex)	Sunflower oil (Codex)
C6:0 caproic	≈1	8.5–24 (lauric/caproic)	ND–0.7	ND–0.8	ND	ND	ND	ND	ND
C8:0 caprylic	1.5–2	8.5–17.5	4.6–10.0	2.4–6.2	2.6–7.3	ND	ND	ND	ND
C10:0 capric	2–3	9.0–16	5.0–8.0	2.6–5.0	1.2–7.6	ND	ND	ND	ND
C12:0 lauric	27–33	— (reference acid; ≥ 20–23 % in Ph. Eur.)	45.1–53.2	45.0–55.0	40.0–55.0	ND–0.5	ND	ND	ND–0.1
C14:0 myristic	10–12	2.2–2.8	16.8–21.0	14.0–18.0	11.0–27.0	0.5–2.0	≤ 0.05	ND–0.2	ND–0.2
C16:0 palmitic	8–10	2.8–3.9	7.5–10.2	6.5–10.0	5.2–11.0	39.3–47.5	7.5–20.0	2.5–7.0	5.0–7.6
C16:1 palmitoleic	< 0.5	not specified	ND	ND–0.2	ND	ND–0.6	0.3–3.5	ND–0.6	ND–0.3
C18:0 stearic	1.5–2.5	14–26	2.0–4.0	1.0–3.0	1.8–7.4	3.5–6.0	0.5–5.0	0.8–3.0	2.7–6.5
C18:1 oleic	28–36	0.60–1.15	5.0–10.0	12.0–19.0	9.0–20.0	36.0–44.0	55.0–83.0	51.0–70.0	14.0–43.0
C18:2 linoleic	3–6	5.0–16	1.0–2.5	1.0–3.5	1.4–6.6	9.0–12.0	3.5–21.0	15.0–30.0	45.4–74.0
C18:3 linolenic	0.5–1	31.5–55	ND–0.2	ND–0.2	ND	ND–0.5	≤ 1.0	5.0–14.0	ND–0.3
Saturated chains, total	≈ 58–64	—	≈ 90	≈ 80	≈ 80	≈ 50	≈ 14–20	≈ 6–8	≈ 10–12
Mean carbon number of the saturated pool, $\bar{n}_s$	≈ 12.8–13.0	—	≈ 12.4	≈ 12.6	≈ 12.6	≈ 16.1	≈ 16.3	≈ 16.5	≈ 16.8

The natural variability of the profile is not negligible and must be built into any acceptance window. The 2025 study of fruit from diverse Florida locations found oleic acid at 35–60 % and lauric acid at 16–20 % of

the fruit fatty acids in mature fruit, with the lauric-to-oleic ratio differing between ecotypes and with the stage of ripeness, so that extracts prepared from unripe or mixed-ripeness berries, a practice listed among the adulteration routes, will show a shifted oleic/lauric balance without any foreign material being present [4,19]. Among finished products, the twenty commercial supplements analyzed by Penugonda and Lindshield showed oleate at 27.6–62.4 % and laurate at 8.9–29.4 % of the total fatty acids in the liquid products, with total fatty acids of 833–941 mg/g in liquids but only 69–327 mg/g in powders and 0.1–97 mg/g in tinctures [29]. Booker and co-workers found a 177-fold range of fatty-acid content per dose among monopreparations, and four of their monopreparations failed the European lauric-acid criterion, three because of elevated oleic and linoleic acid and one because of a linolenic acid content that pointed to another plant oil [11]. A 2023 survey of twenty-eight United States products by GC found only one that met the USP criteria [30]. Variability of this magnitude is the reason why a single-ratio test cannot carry an authentication decision and why the multivariate treatment of Section 11 is needed.

3.2 Speciation of the acyl chains: free acids, glycerides and alkyl esters

The feature of saw palmetto extract that distinguishes it from every oil and fat it might be adulterated with is that its fatty acids are predominantly free. Marti and co-workers summarized the composition of the extracts as approximately 85 % free fatty acids, about 2 % methyl and ethyl esters and 5–6 % triglycerides, with triterpenes at about 1 % and fatty alcohols at 0.8–1.1 % [8]; Booker and co-workers quote 70–95 % free fatty acids together with their ethyl esters and glycerides [11]; and the European assessment report, drawing on the fourteen-brand comparison of Habib and Wyllie, records a free-acid content varying from 41 to 81 % of the total lipid and a glyceride content varying from 7 to 52 %, a range so wide that it must be understood as describing the commercial market rather than the authentic extract [9,21]. Table 3 collects these class-level data together with the ¹H signals through which each class is observed. The origin of the free-fatty-acid dominance is not addressed by the sources reviewed here; lipolysis in the ripening and drying fruit is the obvious hypothesis and could be tested directly by ¹H NMR of fresh against dried berries. Its practical consequence is not in doubt: the extract has an acid value far higher than that of any vegetable oil, high enough that the European Pharmacopoeia specifies an acid-value range rather than an upper limit [4]. Partial glycerides, 1-monolaurin and 1-monomyristin among them, and the sn-1,2 and sn-1,3 diglycerides are present as minor constituents, enriched in the CO_2 and ethanolic extracts, and De Combarieu and co-workers assigned their glyceryl signals in the extract spectra against purchased reference compounds [3,8].

Table 3. Constituent classes of lipophilic saw palmetto extracts: literature values and their ¹H NMR visibility

Class	Reported content (lipophilic extracts)	Source(s)	Diagnostic ¹H signal(s) in $CDCl_3$
Free fatty acids	≈ 85 % (hexane, $scCO_2$ types); 41–81 % of total lipid across 14 brands; 70–95 % quoted for extracts in general	Marti 2019; Habib & Wyllie 2004 (as quoted by EMA); Booker 2014	α-CH_2 triplet at 2.34–2.35 ppm; broad COOH at 10–12 ppm
Triglycerides	5–6 % (hexane/$scCO_2$); glycerides 7–52 % of total lipid across brands; highest in $scCO_2$ and ethanolic extracts, lowest in Permixon-type hexane extracts	Marti 2019; Habib & Wyllie 2004; EMA assessment report	glyceryl CH_2 dd at 4.14 and 4.29 ppm; CH at 5.26 ppm; α-CH_2 at 2.31 ppm
Mono- and diglycerides	minor; enriched in $scCO_2$ and ethanolic extracts; 1-monolaurin and 1-monomyristin isolated from the ethanolic extract	Marti 2019; De Combarieu 2015 (assignments)	1,2-DG: 5.08, 4.32/4.23, 3.73 ppm; 1,3-DG: 4.17/4.14, 4.07 ppm; 1-MG: 4.19/4.14, 3.93, 3.70/3.60 ppm
Methyl and ethyl esters	≈ 2 % methyl + ethyl esters; ethyl esters associated with ethanolic extraction	Marti 2019; Booker 2014; EMA assessment report	OCH_3 singlet 3.67 ppm; OCH_2 quartet 4.12 ppm and CH_3 triplet 1.25 ppm; α-CH_2 at 2.28–2.30 ppm

Class	Reported content (lipophilic extracts)	Source(s)	Diagnostic ¹H signal(s) in $CDCl_3$
Phytosterols (β-sitosterol, campesterol, stigmasterol, Δ⁵-avenasterol, cycloartenol and others; free, esterified and glycosylated)	0.2–0.5 % (USP window); SRM 3251: β-sitosterol 1.67 mg/g, campesterol 0.53 mg/g, stigmasterol 0.25 mg/g, cycloartenol 0.77 mg/g	USP–NF; NIST SRM 3251; Hiermann 1989; Perini 2018	H-18 singlet 0.68 ppm; H-3 multiplet 3.52 ppm; H-6 at 5.35 ppm (hidden under olefinic signals)
Long-chain fatty alcohols (C26, C28, C30)	0.15–0.35 % (USP, lipophilic extract); 0.8–1.1 % quoted by Marti et al.	USP–NF; Marti 2019	CH_2OH triplet 3.64 ppm; CH_2CH_2OH at 1.56 ppm
Polyprenols, hydrocarbons, carotenoids, tocopherols, volatiles	≈ 1 %; β-carotene 46.8 µg/g and γ-tocopherol 280 µg/g in SRM 3251	Marti 2019; NIST SRM 3251	Not observable at the concentrations present in a 1D ¹H spectrum of the whole extract
Polar co-extractives of ethanolic extracts (glycerophospholipids, flavonoids, tyramine)	present only in ethanolic extracts	Marti 2019; Chua 2014 (fraction NMR)	Largely insoluble in $CDCl_3$; phospholipids would be seen by ³¹P NMR

3.3 Unsaponifiable minor constituents

The unsaponifiable fraction, 1.8–3.5 % by the legacy USP window, carries the constituents on which the sterol and fatty-alcohol specifications rest and on which the detection of designer blends ultimately depends. β-Sitosterol is the dominant sterol, followed by campesterol and stigmasterol, with analytically significant amounts of Δ⁵-avenasterol, 24-methylenecholesterol and clerosterol reported by Perini and co-workers and cycloartenol certified in the NIST extract SRM at 0.77 mg/g [5,26]. Hiermann isolated from the fruit the palmitate, myristate and laurate esters of β-sitosterol, its 6-O-acyl glucosides and diglucoside, and the free sterol, together with the flavonoids rutin, rhoifolin, isoquercitrin and kaempferol-3-O-glucoside, so that the sterol pool of an extract is distributed among free, esterified and glycosylated forms in proportions that depend on the extraction solvent [31]. The collaborative AOAC method for campesterol, stigmasterol and β-sitosterol, which saponifies the sample in ethanolic potassium hydroxide, extracts the unsaponifiables into toluene, silylates and quantifies by GC-FID, remains the reference procedure and reports total sterols irrespective of their original form [32]. Long-chain fatty alcohols, chiefly hexacosanol, octacosanol and triacontanol, are present at 0.15–0.35 % in the lipophilic extract and at an order of magnitude less in hydroalcoholic extracts, which is why the USP sets different windows and why a hydroalcoholic extract sold as a lipophilic one, or a designer blend containing no plant wax alcohols at all, fails on this parameter before it fails on any other [1,10]. Carotenoids and tocopherols are present at the tens to hundreds of micrograms per gram and are responsible for the color of the extract, a point of some forensic interest because color adjustment with added carotenoids has been reported by industry sources as a means of making blends look authentic [6,26]. None of these minor constituents except the sterols and fatty alcohols produces a signal that can be seen in a one-dimensional ¹H spectrum of the whole extract, and even those two appear at the 0.1–0.3 % level relative to the major signals, which fixes the signal-to-noise requirement of the NMR method discussed in Section 8.

3.4 Excipients and dosage-form matrices

A sample received as a softgel or capsule is a formulated product, and the formulation contributes to the spectrum. Polyethylene glycol, used as the matrix of the reference hexane-extract capsules, gives an intense singlet at 3.62–3.64 ppm that was the dominant feature of one product in the Booker survey and that obliterates the fatty-alcohol CH_2OH triplet at 3.64 ppm [11,22]. Glycerin, lecithin, beeswax, medium-chain triglycerides and carrier oils such as olive, soybean or pumpkin seed oil appear in combination products and

in softgel fills, and in the public dataset examined in Section 7 the products labeled as containing added oil are, by the dataset documentation, typically diluted with olive oil [11,33]. A triglyceride carrier declared on the label and an undeclared triglyceride adulterant are indistinguishable by NMR; what NMR can do is to quantify the triglyceride fraction so that it can be compared with the declaration. Gelatin and vegetable-capsule shells are insoluble in $CDCl_3$ and are simply excluded, but softgel fills should be weighed after the shell has been opened and drained rather than dissolved whole.

4. The adulteration problem and the limits of fatty-acid profiling

4.1 Economic drivers and documented adulterants

The Botanical Adulterants Prevention Program bulletin, first issued in February 2017 and revised in October 2018, documents dilution or substitution of saw palmetto extract with canola, coconut, olive, palm, peanut and sunflower oils, dilution with specially formulated blends of lower-cost oils that emulate the fatty-acid profile, and, in the revised version, blends of fatty acids obtained from animal fats mixed in the same ratios that the fatty acids occur in authentic extract [4]. At the raw-material stage, substitution of the fruit with *Acoelorrhaphe wrightii*, dilution with exhaustively extracted berry powder, and the use of unripe berries are listed. The economic figures recorded in the same document, extract prices of 150–235 US$/kg in 2016 and 190–275 US$/kg in 2017 against 50–135 US$/kg for material from Asia, a 2016 extract production of about 520 metric tons valued at 105 million US$, and United States retail sales of 34.7 million US$ in 2018, explain both the incentive and the scale [4,10]. No statistics on the frequency of adulteration exist, but the available surveys are not reassuring: a 2017 industry test of six competitor extracts found four engineered and two outright imitations, DNA barcoding of thirty-seven supplements found two containing related species that cannot legally be sold, Booker and co-workers identified added olive and soybean oil among fifty-seven products, and the 2023 GC survey found one compliant product in twenty-eight [4,11,20,30].

4.2 Designer blends and the failure mode of GC profiling

The decisive demonstration that the compendial test can be passed by fraudulent material was provided by Perini and co-workers in 2018. Nine commercial extracts labeled as originating outside the European Union or the United States were compared with thirty authenticated extracts produced between 2009 and 2017. By the USP fatty-acid method only three of the nine failed; the other six passed the lauric-acid ratios. When the minor constituents, triglycerides and phytosterols, were examined by GC and HPLC, and when the extracts were examined by ¹H NMR, all nine commercial samples fell outside the authentic cluster. The bulk $\delta^{18}O$ values of the authentic extracts (+27.2 to +40.7 ‰) were far above the +9 to +31 ‰ range of plant oils, and the $\delta^{18}O/\delta^{2}H$ pattern of the commercial samples placed them among animal fats, consistent with a combination of pig, chicken and lamb fat; the authors concluded that the six ratio-compliant samples were designer blends of animal-origin fatty acids and the three non-compliant samples were coconut, babassu and an unidentified vegetable-oil blend [5]. The follow-up isotopic study established the reference ranges on twenty authentic extracts, bulk $\delta^{13}C$ −31.0 to −29.7 ‰, $\delta^{2}H$ −176 to −165 ‰ and $\delta^{18}O$ +27.2 to +40.7 ‰, with compound-specific $\delta^{13}C$ of −37.4 to −30.5 ‰ and $\delta^{2}H$ of −187 to −136 ‰ for the capric, caprylic, lauric, myristic, palmitic and oleic acids, and found all nine commercial extracts outside these ranges and closer to meat fat and to pure fatty acids [7]. The BAPP review of 2023 adds that the oil mixtures used in low-harvest years often have a fatty-acid profile indistinguishable from saw palmetto oil while failing on color, odor, fatty alcohols or sterols, and quotes the program's director to the effect that sunflower, coconut and canola oils mixed at the right ratios give something that looks like the saw palmetto fatty-acid profile [6].

A chemical point that the literature leaves implicit should be made explicit here, because it bears directly on what NMR can and cannot see. Animal fats contain almost no lauric or myristic acid, so a blend of animal-origin fatty acids that passes the lauric-acid ratios cannot be made from animal fat alone; the lauric, myristic, caprylic and capric acids must be sourced from the oleochemical industry, where they are produced in quantity by hydrolysis and fractional distillation of coconut and palm-kernel oils, and the oleic, palmitic and stearic acids may then be taken from rendered fat. The result is a free-fatty-acid material, not a triglyceride material, and the glyceride-based ¹H markers that detect ordinary vegetable-oil adulteration so easily are therefore silent. The same is true of any hydrolyzed lauric oil used on its own. This is the central limitation of ¹H NMR for saw palmetto authentication, and it is treated quantitatively in Section 9 rather than being left as a qualification.

4.3 What a lipid-speciation method must deliver

The requirements that follow from Sections 2–4 can be stated in a few lines. A method intended to complement the compendial GC test should report the speciation of the acyl chains, since that is the one property in which every oil and fat differs from the authentic extract; it should report the unsaturation pattern and the chain-length distribution independently, since designer blends reproduce the fatty-acid ratios by construction but are unlikely to reproduce the full distribution of positional and minor isomers; it should look at the minor constituents, sterols and fatty alcohols, that designer blends lack; it should do so on the whole extract without derivatization, so that no information is destroyed before the measurement; and it should produce a multivariate fingerprint that can be compared with an authentic reference set by a membership test rather than a two-class discrimination, since the adulterant population is open-ended. ¹H NMR meets the first, fourth and fifth requirements fully, the second partially, and the third only at the margin of its sensitivity, which is the honest summary of the method that the remainder of this paper substantiates.

5. ¹H NMR of saw palmetto extracts: sample preparation and acquisition

5.1 Solvent, concentration and referencing

Deuterochloroform is the solvent of every published saw palmetto extract spectrum, and there is no reason to depart from it: the free acids, glycerides, esters, sterols and fatty alcohols are all freely soluble, the solvent signal at 7.26 ppm lies in an empty region, and the chemical-shift literature for lipids in $CDCl_3$ is extensive [3,11,34]. De Combarieu and co-workers dissolved approximately 10 mg of extract in 0.75 mL by ultrasonication and referenced to $CHCl_3$ at 7.26 ppm; Booker and co-workers used $CDCl_3$ containing 0.05 % tetramethylsilane for softgel fills and switched to CD_3OD for crude powders and dry extracts, with diffusion-edited acquisition to suppress ethanol and water in tinctures [3,11]. For the purposes of this paper a higher concentration is preferable. The sterol H-18 singlet and the fatty-alcohol triplet appear at roughly 0.1–0.3 % of the methyl integral, and a 300 MHz instrument needs a methyl signal-to-noise ratio above about 1 000 before those signals can be integrated with 10 % precision; 40–60 mg of extract in 0.7 mL gives that in 32–64 scans without the line-broadening that accompanies the viscosity of a more concentrated solution. Tetramethylsilane referencing is preferred over the residual $CHCl_3$ signal because the $CHCl_3$ shift moves slightly with the concentration of acidic solute. Softgel fills are expressed from the opened shell, weighed, and dissolved; powders and dry extracts, which contain the polar co-extractives of ethanolic extraction, may leave an insoluble residue that should be removed by centrifugation rather than filtration through cellulose, which retains lipid.

Two solvent-related artifacts deserve mention. Water in $CDCl_3$ gives a singlet at about 1.56 ppm, directly under the fatty-alcohol CH_2CH_2OH signal and at the edge of the β-methylene multiplet, and the carboxyl proton of the free acids exchanges with it, so that the broad COOH signal at 10–12 ppm moves and broadens with the water content, the concentration and the temperature [18]. The solvent should therefore be stored over molecular sieves; basic drying agents must not be used, since they would deprotonate the acids and alter the α-methylene shifts that carry the speciation information. Acid traces in aged $CDCl_3$ are of no consequence for a material that is itself 85 % carboxylic acid.

5.2 Quantitative acquisition conditions

The published fingerprinting studies were acquired under conditions chosen for throughput rather than for quantitation: a 45° pulse with a 1 s relaxation delay and 128 transients at 400 MHz, or a 30° pulse with a 1 s delay and 256 transients at 500 MHz [3,11]. Such conditions are acceptable for a fingerprint compared with a reference set acquired identically, but they are not acceptable for the proton-bookkeeping relations of Section 8, which assume that every integral is proportional to the number of protons. The protons of a lipid have spin-lattice relaxation times that differ by a factor of three to five across the spectrum: the terminal methyl and the olefinic protons relax slowly, the mid-chain methylene envelope and the glyceryl protons relax quickly, and the α-methylene protons lie between. A short delay therefore depresses the methyl and olefinic integrals relative to the envelope and the glyceryl signals, which biases the chain count downward and the glyceride-bound fraction upward, and the public spectra examined in Section 8 show exactly this signature. The remedy is conventional: the longest T_1 in the sample is measured once by inversion recovery, the relaxation delay plus acquisition time is set to at least five times that value for 90° pulses (or the Ernst-angle compromise is used with a correspondingly shorter delay, as treated in the PNA relaxation white paper PNA-WP-2026-RELAX-01), and the condition is verified by confirming that the terminal-methyl integral divided by three equals the α-methylene integral divided by two within 2 %. The quantitative mode of the protocol in Section 13 is built on this check. Zailer and co-workers have shown, in a seventeen-laboratory comparison of ¹H NMR edible-oil analysis, that fifteen signal integrals including the free-fatty-acid, double-bond and linoleic/linolenic parameters can be reproduced across laboratories with a 90 % z-score pass rate once the acquisition conditions are harmonized, which is the standard that an interlaboratory saw palmetto method should be held to [35].

5.3 Processing

For quantitation the free induction decay is zero-filled once, apodized with 0.3 Hz exponential broadening, phased, and baseline-corrected with a low-order polynomial fitted through signal-free windows (for example −1.5 to −0.3, 3.0–3.4, 3.85–4.0, 4.6–5.0, 5.6–7.0 and 7.5–7.9 ppm); automatic phasing must be checked by eye at the α-methylene and glyceryl regions, where dispersive tails from the envelope distort the small signals, and the integration limits of Table 6 are then applied without local baseline manipulation, which was found in the course of this work to bias the methyl integral by up to 10 % when a local baseline is anchored on the tail of the envelope. For chemometrics the spectra are aligned (Section 11.2), bucketed and normalized, and the solvent and reference regions are excluded. The processing of the public dataset followed these rules and is reproduced in the companion workbook.

6. Spectral assignment

Table 4 assembles the ¹H assignments for the lipophilic extract and for the constituents that adulteration or formulation may introduce. The extract-specific assignments are those of De Combarieu and co-workers,

who built a reference database by recording, under the conditions of the extract spectra, the spectra of purchased free fatty acids, fatty alcohols, monoglycerides, diglycerides and triglycerides, and who tabulated twenty-two signals for the whole extract [3]; the chemical shifts of the alkyl esters, the sterols and the oxidation products are taken from the edible-oil and lipid-NMR literature [13,15,17,34,36–41]. The assignments are discussed below by region, with attention to the signals that carry authentication information and to the overlaps that limit them.

Table 4. ¹H NMR assignments for lipophilic saw palmetto extracts and their adulterants in $CDCl_3$ (TMS = 0 ppm)

δ (ppm)	Multiplicity, J (Hz)	Protons	Species	Source / comment
0.68	s	H-18 (C-18 methyl)	β-sitosterol, campesterol, stigmasterol (also cholesterol)	Hatzakis 2010; Alexandri 2017. Present in the public spectra at 0.1–0.2 % of the terminal-CH_3 integral
0.81–0.86	d, t	H-26, H-27, H-29 side-chain methyls	Phytosterols	Overlapped by the terminal CH_3 triplet
0.88	t, 7.0	ω-CH_3	All saturated and ω-9 chains; fatty alcohols	De Combarieu 2015 (0.89); Guillén & Ruiz 2003
0.89–0.90	t, 7.0	ω-CH_3	Linoleic (ω-6) chains	Slight downfield displacement; Knothe & Kenar 2004
0.97	t, 7.5	ω-CH_3	Linolenic (ω-3) chains	De Combarieu 2015; Guillén & Ruiz 2003; semi-quantitative below ≈ 1 % because of envelope tails
1.01	s	H-19	Phytosterols	Alexandri 2017
1.22–1.38	m (envelope)	$(CH_2)_n$	All acyl chains and fatty alcohols	De Combarieu 2015 (1.26); intensity 2(n − 4) H per saturated chain, 20 H per oleic, 14 H per linoleic chain
1.25	t, 7.1	OCH_2CH_3	Fatty-acid ethyl esters	Buried under the envelope; Rosset 2011
1.56	m	CH_2CH_2OH	Fatty alcohols	De Combarieu 2015; coincides with the H_2O singlet in $CDCl_3$
1.61–1.64	m	β-CH_2 (C-3)	All acyl chains (1.64 free acids; 1.61 glycerides)	De Combarieu 2015; Guillén & Ruiz 2003
1.97	br	OH	Monoglycerides and 1,3-diglycerides (exchangeable)	De Combarieu 2015
2.00–2.06	m	allylic CH_2	All unsaturated chains (4 H per chain)	De Combarieu 2015 (2.06); Guillén & Ruiz 2003
2.28	t, 7.5	α-CH_2 (C-2)	Ethyl esters	Alkyl ester shift; Rosset 2011
2.30	t, 7.5	α-CH_2 (C-2)	Methyl esters	Gelbard 1995; Knothe & Kenar 2004
2.31 (sn-1,3), 2.33 (sn-2)	t, 7.5	α-CH_2 (C-2)	Triglycerides	Lie Ken Jie & Lam 1995; Sacchi 1997
2.32–2.35	t, 7.5	α-CH_2 (C-2)	1,2- and 1,3-diglycerides, monoglycerides	Nieva-Echevarría 2014
2.34–2.35	t, 7.5	α-CH_2 (C-2)	Free fatty acids	De Combarieu 2015 (2.35); Skiera 2014; Nieva-Echevarría 2014. The free-acid/glyceride α-CH_2 pair is the primary speciation marker
2.44	br d	OH	Monoglycerides and 1,2-diglycerides (exchangeable)	De Combarieu 2015
2.77	t, 6.5	bis-allylic CH_2	Linoleic chains (2 H); linolenic chains (4 H at 2.80)	De Combarieu 2015 (2.81); Knothe & Kenar 2004
3.52	m	H-3 (CHOH)	Free phytosterols	Hatzakis 2010
3.60, 3.70	dd	CH_2OH (α′)	1-Monoglycerides	De Combarieu 2015
3.62–3.64	s	OCH_2CH_2O	Polyethylene glycol excipient	Booker 2014 (sample SP12, a hexane extract in PEG); interferes with the fatty-alcohol triplet
3.64	t, 6.6	CH_2OH	Long-chain fatty alcohols	De Combarieu 2015
3.67	s	OCH_3	Methyl esters	Gelbard 1995

δ (ppm)	Multiplicity, J (Hz)	Protons	Species	Source / comment
3.73	m	CH_2OH (α′)	sn-1,2-Diglycerides	De Combarieu 2015
3.93	m	CHOH (β)	Monoglycerides	De Combarieu 2015
4.07	m	CHOH (β)	sn-1,3-Diglycerides	De Combarieu 2015
4.12	q, 7.1	OCH_2	Fatty-acid ethyl esters	Booker 2014 (4.1–4.2 ppm, caproic acid ethyl ester identified from a reference standard); Rosset 2011
4.14, 4.29	dd, 11.9/6.0 and 11.9/4.3	glyceryl CH_2 (sn-1,3)	Triglycerides	De Combarieu 2015 (4.15/4.30, all acylglycerols); Lie Ken Jie & Lam 1995
4.14–4.20	dd	CH_2OCOR	1,3-Diglycerides and 1-monoglycerides	Nieva-Echevarría 2014
4.23, 4.32	dd	CH_2OCOR	sn-1,2-Diglycerides	Nieva-Echevarría 2014
5.08	m	CHOCOR (β)	sn-1,2-Diglycerides	De Combarieu 2015
5.26	m	CHOCOR (β)	Triglycerides	De Combarieu 2015 (5.27); Gafner 2023 (ca. 5.25 ppm adulteration marker)
5.30–5.42	m	–CH=CH–	All unsaturated chains (2 H per double bond); sterol H-6 at 5.35	De Combarieu 2015 (5.36)
7.26	s	$CHCl_3$	Solvent	Reference region excluded from chemometrics
9.5–9.8	d, t	CHO	Aldehydes from lipid oxidation	Booker 2014 (singlet at 9.54 ppm in one product); Guillén & Uriarte-type assignments
10–12	br	COOH	Free fatty acids (exchangeable; position and width depend on water, concentration and temperature)	Skiera 2014

6.1 The aliphatic region (0.6–1.7 ppm)

The terminal methyl triplet at 0.88 ppm (J ≈ 7.0 Hz) counts every acyl chain and every fatty alcohol molecule with three protons, and is the usual chain-count reference in oil analysis [14,16]. Linoleic chains place their methyl slightly downfield, at 0.89–0.90 ppm, a displacement that is visible as asymmetry of the triplet at 500 MHz and that the public evening-primrose-oil spectrum shows clearly; linolenic chains place theirs at 0.97 ppm, well separated, which is the classical route to ω-3 content [13,15]. In saw palmetto extract the 0.97 ppm triplet is small, since linolenic acid is below 1 % of the chains, and it is contaminated by the Lorentzian tails of the methylene envelope, so that the linolenic estimate of Section 8 is semi-quantitative. The sterol H-18 singlet at 0.68 ppm and the H-19 singlet at 1.01 ppm are the only sterol signals free of overlap; the side-chain methyl doublets at 0.81–0.86 ppm lie under the terminal methyl triplet [40,41]. The methylene envelope at 1.22–1.38 ppm carries 2(n − 4) protons per saturated chain of carbon number n, 20 protons per oleic chain and 14 per linoleic chain, which is the basis of the chain-length index derived in Section 8; its shape also differs between lauric-type and long-chain pools, the lauric-rich envelope being narrower and centered at 1.26 ppm whereas unsaturated long chains add intensity at 1.30–1.37 ppm from the methylenes adjacent to the allylic positions, a difference that appears in the second principal component of the public data (Section 11.3). The β-methylene multiplet at 1.61–1.64 ppm is at 1.64 ppm for free acids and about 1.61 ppm for glycerides, a small displacement that is not resolved at 300 MHz but that contributes to the loadings at higher fields; the fatty-alcohol CH_2CH_2OH multiplet at 1.56 ppm and the water singlet share the low-field shoulder of this multiplet [3]. The ethyl-ester methyl triplet at 1.25 ppm (J = 7.1 Hz) is buried in the envelope and cannot be used.

6.2 The allylic, α-methylene and bis-allylic region (1.9–2.9 ppm): speciation by the α-methylene shift

The allylic multiplet at 2.00–2.06 ppm counts four protons for every unsaturated chain and the bis-allylic triplet at 2.77 ppm (J ≈ 6.5 Hz) two protons for every linoleic chain and four for every linolenic chain, so that the two integrals together with the methyl integral give the saturated, monoenoic and dienoic chain fractions by the relations that Guillén and Ruiz, Knothe and Kenar, and Barison and co-workers established for oils [13–16]. The α-methylene region between 2.20 and 2.45 ppm is the most informative region of the saw palmetto spectrum and the one in which the extract differs qualitatively from every oil. The chemical shift of the protons on C-2 depends on the group to which the carboxyl is bound: 2.34–2.35 ppm for the free acid, 2.31 ppm for the sn-1,3 chains and 2.33 ppm for the sn-2 chain of a triglyceride, 2.32–2.35 ppm for partial glycerides, 2.30 ppm for a methyl ester and 2.28 ppm for an ethyl ester [17,18,36,38]. In an authentic extract the region is dominated by a single triplet at 2.345 ppm with a weak glyceride triplet 0.035 ppm upfield; in a vegetable oil the free-acid triplet is absent; and in a blend the two triplets interleave, the relative intensity of the two patterns giving the glyceride-bound fraction directly. At 500 MHz the two triplets are separated by 17 Hz, which is more than twice the coupling constant and allows the two patterns to be seen as such (Figure 2); at 300 MHz the separation is 10.5 Hz and the patterns overlap but are still assignable; at 60 MHz they are not separated, and the region must be integrated as a block (Section 10). The free-acid α-methylene triplet is also the signal that Skiera and co-workers used for the ¹H NMR acid value of pharmaceutical lipids, validated against the European Pharmacopoeia titration on 305 oils and fats, and Satyarthi and co-workers showed that it reports the free fatty acids exclusively, whereas titration returns a gross acidity including other acidic impurities [18,42]. The hydroxyl protons of partial glycerides appear as broad signals at 1.97 and 2.44 ppm and exchange with water; they are not used quantitatively [3].

6.3 The oxygenated region (3.5–4.4 ppm): glycerides, alkyl esters, fatty alcohols, sterol H-3 and polyethylene glycol

Everything between 3.5 and 4.4 ppm belongs to carbon bearing oxygen, and the region is empty in a pure free-fatty-acid material. Triglycerides contribute the two doublets of doublets of the sn-1,3 glyceryl methylenes at 4.14 and 4.29 ppm (J ≈ 11.9 Hz geminal, 6.0 and 4.3 Hz vicinal), four protons per molecule, which is the signal that the Botanical Adulterants Prevention Program documents single out as the visual adulteration marker because it is much weaker in saw palmetto extract than in vegetable oils [6,10,36]. The partial glycerides are assigned, following De Combarieu and co-workers, to 4.07 ppm (methine of sn-1,3-diglycerides, with their methylenes at 4.14–4.17 ppm overlapping the triglyceride signal), 3.73 ppm (hydroxymethyl of sn-1,2-diglycerides, whose acyloxymethylenes appear at 4.23 and 4.32 ppm) and 3.93, 3.70 and 3.60 ppm (methine and hydroxymethyl of 1-monoglycerides, whose acyloxymethylene lines lie at 4.14–4.19 ppm) [3,17]. Nieva-Echevarría and co-workers derived equations that return the molar percentages of triglycerides, 1,2- and 1,3-diglycerides, 1- and 2-monoglycerides and free fatty acids from these signals on standard mixtures, and the equations transfer to saw palmetto with the caveat that the 4.14–4.20 ppm block is a sum over three glyceride classes and the ethyl ester [17]. The ethyl-ester OCH_2 quartet at 4.12 ppm (J = 7.1 Hz) is the signal in which Booker and co-workers identified caproic acid ethyl ester against a reference standard and which drove part of their principal-component loadings; its four lines interleave with the lower doublet of doublets of the triglyceride, so that at 300–500 MHz the ethyl ester cannot be integrated separately and must be fitted or taken from ¹³C NMR [11,39]. The methyl-ester OCH_3 singlet at 3.67 ppm, the signal of the classical Gelbard transesterification assay, is well resolved and three protons per ester [38]; the fatty-alcohol CH_2OH triplet at 3.64 ppm (J ≈ 6.6 Hz) lies 9 Hz from it at 300 MHz and is resolved there, but is obliterated by the polyethylene glycol singlet at 3.62–3.64 ppm in capsule contents formulated in that

matrix [3,11]. The free-sterol H-3 methine at 3.52 ppm is the signal through which Hatzakis and co-workers quantified free sterols in olive oil, in combination with ³¹P NMR of the phosphitylated hydroxyl; at the 0.2–0.5 % sterol level of saw palmetto extract it is at the limit of a 300 MHz experiment [40].

6.4 The olefinic region and the triglyceride methine (5.0–5.5 ppm)

The olefinic multiplet at 5.30–5.42 ppm counts two protons per double bond, and the triglyceride glyceryl methine quintet-like multiplet at 5.26 ppm counts one proton per triglyceride; the two are resolved by 24 Hz at 300 MHz and by 40 Hz at 500 MHz but merge at 60 MHz [3,14]. The olefinic integral is used in Section 8 only as a consistency check on the unsaturation derived from the allylic and bis-allylic signals, because the sterol H-6 proton at 5.35 ppm and the slow relaxation of olefinic protons make it a less reliable primary measurement. The sn-1,2-diglyceride methine at 5.08 ppm is well separated and is a useful indicator of partial glyceride content and, in oils, of hydrolytic history [17]. Trans double bonds, present at a few per cent in ruminant fats and at trace levels in refined oils, shift the olefinic protons to about 5.38 ppm and the allylic protons to about 1.97 ppm; at 500 MHz and above these displacements are resolvable and are, in principle, one of the few ¹H leads toward an animal-origin designer blend, although the lauric-type acids in such a blend would be of plant oleochemical origin and would dilute the trans signature [41].

6.5 Exchangeable and low-field signals

The carboxyl proton of the free acids gives a broad signal between 10 and 12 ppm whose position and width depend on water content, concentration and temperature, and which is therefore recorded but not integrated [18]. Aldehyde protons at 9.5–9.8 ppm, from alkanals, 2-alkenals and 2,4-alkadienals formed by lipid oxidation, were observed as a singlet at 9.54 ppm in one product by Booker and co-workers and are a useful indicator of storage history; in the public spectra of Section 7 weak unassigned signals near 9.3 ppm occur in several products [11]. Carotenoid olefinic protons between 6.1 and 6.7 ppm and tocopherol aromatic methyls near 2.1 ppm are below the detection limit of a one-dimensional spectrum of the whole extract at the concentrations certified for the NIST extract SRM [26].

6.6 ¹³C NMR as the speciation complement

The overlaps that limit ¹H speciation in the 2.2–2.4 and 4.0–4.4 ppm regions disappear in the ¹³C spectrum, where the carbonyl carbon alone separates the free acid (179.5–180.5 ppm) from the triglyceride (173.2 and 172.8 ppm for the sn-1,3 and sn-2 positions) and from the methyl and ethyl esters (174.0–174.5 ppm) by margins of 1–7 ppm, and where the ethyl-ester OCH_2 carbon (60.1 ppm), the methyl-ester OCH_3 carbon (51.4 ppm), the fatty-alcohol CH_2OH carbon (63.1 ppm) and the glycerol carbons of the tri-, di- and monoglycerides (62–72 ppm) are all resolved [37,41,43]. Table 5 lists approximate shifts; the partial-glyceride values are marked as such and should be confirmed against the reference compounds on the MVX300 before they are used in a report. The price of the ¹³C experiment is sensitivity and relaxation: the carbonyl carbons have no attached protons, long T_1 values and a small nuclear Overhauser enhancement, so that quantitative ¹³C requires inverse-gated decoupling, a relaxation agent or long delays, and acquisition times of an hour or more at 75 MHz on a 50 mg sample. Gunstone's early review of high-resolution ¹³C for lipid structure and composition remains the clearest statement of what the carbonyl, olefinic and glycerol regions can deliver, and Sacchi, Addeo and Paolillo showed on virgin olive oil how the regiospecific information of the sn-2 carbonyl complements the ¹H data [37,43]. For saw palmetto the ¹³C carbonyl region is proposed in Section 13 as the confirmatory speciation measurement to be applied to any sample whose ¹H glyceride fraction or ethyl-ester content is in doubt. A ³¹P alternative, in which the hydroxyl-bearing constituents (free acids, partial glycerides, sterols, fatty alcohols) are phosphitylated with 2-chloro-4,4,5,5-

tetramethyldioxaphospholane and quantified in a single ^{31}P spectrum, has been developed for olive oil by Spyros, Dais and co-workers and used for seed-oil adulteration and for sterol speciation; it would deliver the fatty-alcohol and free-sterol contents of saw palmetto extract, the two parameters the ^{1}H spectrum sees least well, in one experiment, at the cost of a derivatization step [40,44–46]. A multinuclear ^{1}H, ^{13}C and ^{31}P scheme of this kind has been assembled for the quality control of krill oil, where composition and the detection of foreign oils are handled in a single workflow, and it is the closest published analogue of what is proposed here for saw palmetto [47].

Table 5. Approximate ^{13}C chemical shifts ($CDCl_3$) that separate the acyl-chain speciation of saw palmetto extracts

δ (ppm)	Carbon	Species	Comment
179.5–180.5	C-1 (COOH)	Free fatty acids	Resolved by 6–7 ppm from every ester carbonyl; the cleanest speciation signal in the whole spectrum
174.0–174.5	C-1 ($COOCH_3$ / $COOCH_2CH_3$)	Methyl and ethyl esters	Separate from triglyceride carbonyls by ≈ 1 ppm
173.2 and 172.8	C-1, sn-1,3 and sn-2	Triglycerides	Regiospecific pair; the sn-2 carbonyl of saturated versus unsaturated chains is further split by ≈ 0.04 ppm
129.7–130.2 / 127.9–128.1	olefinic CH	Oleic / linoleic chains	Internal (linoleic) olefinic carbons appear upfield of the oleic pair
71.8, 140.8, 121.7	C-3, C-5, C-6	Phytosterols	Weak at 0.2–0.5 % w/w; long acquisitions required
68.9 and 62.1	glycerol CH and CH_2	Triglycerides	Isolated region
72.1, 62.6, 61.5	CH, CH_2OCO, CH_2OH	sn-1,2-Diglycerides	Approximate; to be confirmed against reference standards
68.3, 65.0	CHOH, CH_2OCO	sn-1,3-Diglycerides	Approximate
70.3, 65.1, 63.3	CHOH, CH_2OCO, CH_2OH	1-Monoglycerides	Approximate
63.1	CH_2OH	Fatty alcohols	Isolated
60.1 and 14.3	OCH_2, OCH_2CH_3	Ethyl esters	The OCH_2 carbon is 2 ppm from the triglyceride CH_2 carbons, removing the ^{1}H overlap problem
51.4	OCH_3	Methyl esters	Isolated
34.1 / 34.0 and 34.2	C-2	Free acids / glycerides (sn-1,3 and sn-2)	Small but systematic differences
14.1	ω-CH_3	All chains	Chain-count reference

7. Illustration with public spectra: the ChemoSpec SrE.NMR dataset

7.1 Provenance and what is and is not known about the acquisition

No measured PNA spectra of saw palmetto exist at the time of writing, and the spectra in the published papers are available only as figures. A public dataset was therefore used. The ChemoSpec package for R, maintained by Bryan A. Hanson of DePauw University, distributes a dataset named SrE.NMR consisting of sixteen ^{1}H spectra: fourteen retail saw palmetto products, divided by their label descriptions into four "pure extract" (pSrE) and ten "adulterated extract" (adSrE) samples, the latter described in the documentation as typically containing added olive oil, together with one evening primrose oil and one olive oil as triglyceride references. The documentation states that the NMR data were collected at Purdue University with the assistance of Professor Daniel Raftery and Mr Tao Ye, that the saw palmetto samples are "largely fatty

acids" whereas the reference oils are "mixtures of triglycerides", and that the spectra of the two groups are "subtly different" [33]. The dataset was extracted from the CRAN source archive (version 6.3.1) and read into Python; it comprises 32 768 intensity points per spectrum over −4.18 to 16.48 ppm, referenced to tetramethylsilane at 0.000 ppm with the residual $CHCl_3$ signal at 7.267 ppm, so that the solvent was $CDCl_3$. The field strength is not documented but can be inferred: the terminal-methyl triplet lines fall at 0.8660, 0.8799 and 0.8931 ppm, a spacing of 0.01355 ppm which corresponds to 7.0 Hz at 517 MHz, and the bis-allylic triplet spacing of 0.01325 ppm corresponds to 6.5 Hz at 491 MHz; the instrument was therefore a 500 MHz spectrometer. The pulse angle, relaxation delay, number of scans, sample concentration and date of acquisition are unknown, and the retailer codes embedded in the sample names (CVS, GNC, Nature's Way, Solaray and others) identify the retail source but not the formulation. These spectra are used here as illustrations of real spectral behavior and as a test bed for the bookkeeping relations; they are not used as a reference set, and their group labels, which derive from product labels rather than from analysis, are treated as hypotheses to be tested rather than as ground truth.

7.2 Qualitative observations

Figure 1 shows five of the sixteen spectra, a labeled-pure product, two labeled-adulterated products, and the two reference oils, each scaled to its methylene-envelope maximum, with the 2.5–6.0 ppm region expanded twelvefold in the right-hand panel. Figure 2 overlays the diagnostic regions of the labeled-pure product, one labeled-adulterated product and olive oil after normalization of each spectrum to its terminal-methyl integral, so that the three traces represent equal numbers of chains. The qualitative content of the two figures is the whole argument of this paper in compressed form. In the α-methylene region the pure-labeled product shows the free-acid triplet centered at 2.341 ppm (lines at 2.356, 2.341 and 2.327 ppm) as its strongest feature, with the glyceride triplet (2.325, 2.310 and 2.295 ppm) interleaved at lower intensity; olive oil shows only the glyceride pattern, with the sn-2 chain contributing the additional lines at slightly lower field; and the adulterated-labeled product shows both patterns at comparable intensity. In the glyceryl region the olive oil shows the two doublets of doublets at 4.14 and 4.29 ppm at a level four to five times higher per chain than the pure-labeled product, which nevertheless shows them clearly, together with additional lines at 4.16–4.20 and 4.07–4.10 ppm from 1,3-diglycerides and 1-monoglycerides and a cluster of partial-glyceride signals between 3.60 and 3.95 ppm that is more prominent in the pure-labeled product than in olive oil. The triglyceride methine at 5.26 ppm and the olefinic multiplet are resolved by 40 Hz, and the terminal-methyl region shows the near-identity of the methyl triplets after normalization, with the linolenic triplet at 0.976 ppm visible in all three as a trace. The sterol H-18 singlet at 0.680 ppm is present in every saw palmetto spectrum at 0.1–0.2 % of the methyl intensity and in the evening primrose oil at 1 %, and no methyl-ester singlet at 3.67 ppm is visible in any spectrum. In none of the fourteen products is an ethyl-ester quartet evident at 4.12 ppm above the triglyceride lines, which is consistent with United States retail products being predominantly CO_2 or hexane extracts rather than ethanolic extracts, but which cannot be asserted from the ¹H spectra alone for the reason given in Section 6.3. Several of the adulterated-labeled products show weak signals near 9.31 ppm that are not assigned, and one pure-labeled product shows a weak broad signal at 10.08 ppm that is probably the carboxyl proton. These observations are converted into numbers in the next section.

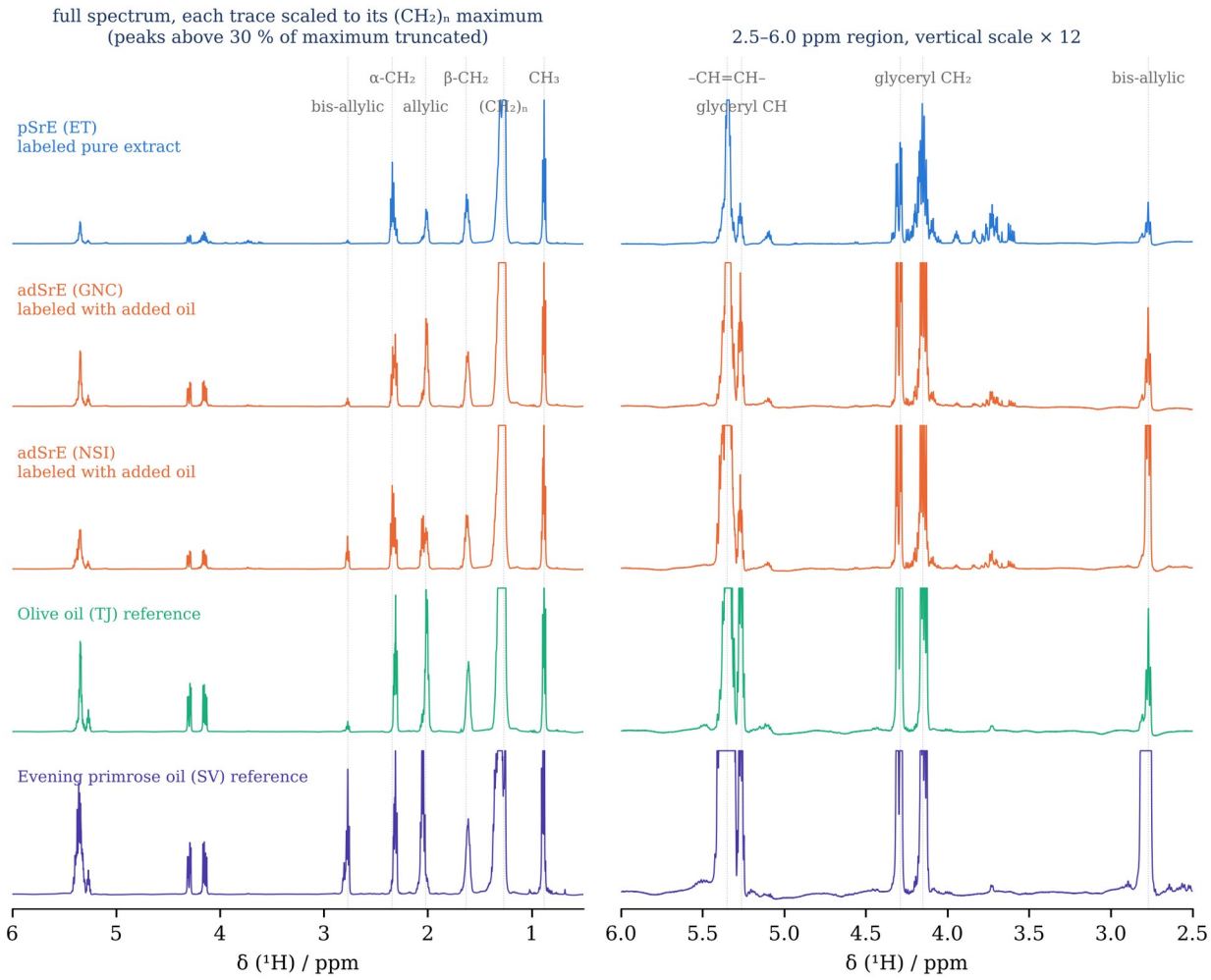

Figure 1. Public ¹H NMR spectra (≈ 500 MHz, $CDCl_3$, TMS = 0) of retail saw palmetto products and reference oils from the ChemoSpec SrE.NMR dataset [33]. Left: 0.5–6.0 ppm, each trace scaled to its methylene-envelope maximum with intensities above 30 % of that maximum truncated. Right: the 2.5–6.0 ppm region at twelve times the vertical scale. From top: ET (labeled pure extract), GNC and NSI (labeled as containing added oil), olive oil (TJ), evening primrose oil (SV). The dataset labels pSrE and adSrE derive from product descriptions, not from analysis. These are not PNA spectra; they will be replaced by MVX300 spectra of the reference set in version 1.1.

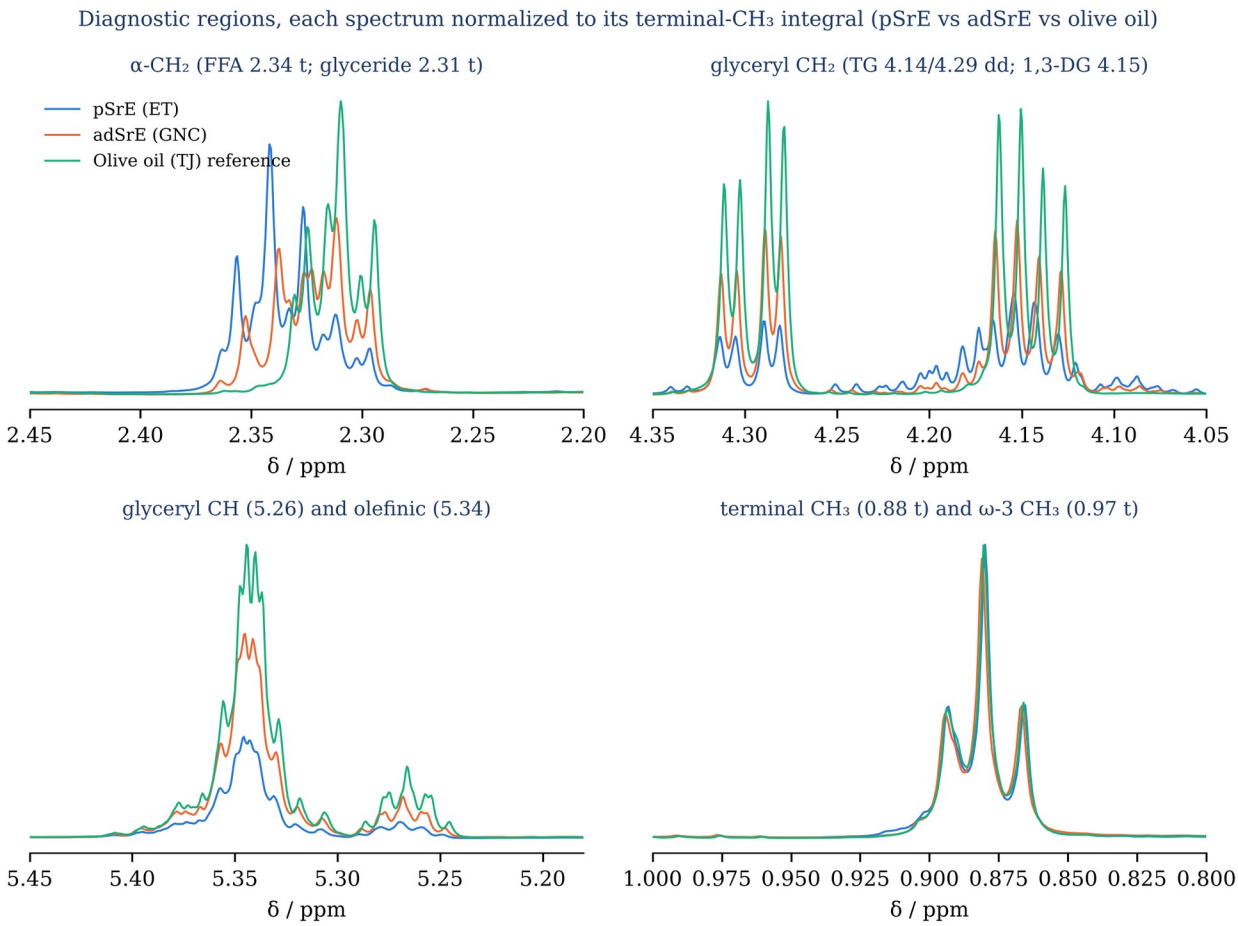

Figure 2. Diagnostic regions of three public spectra after normalization of each spectrum to its terminal-methyl integral (equal numbers of chains): ET (labeled pure extract, blue), GNC (labeled with added oil, orange) and olive oil (green). Upper left: the α-methylene region, with the free-acid triplet centered at 2.341 ppm and the glyceride triplet at 2.310 ppm. Upper right: the glyceryl methylene region, with the triglyceride doublets of doublets at 4.14 and 4.29 ppm and partial-glyceride lines at 4.07–4.20 ppm. Lower left: the triglyceride methine at 5.26 ppm and the olefinic multiplet. Lower right: the terminal-methyl triplet and the trace linolenic triplet at 0.976 ppm. Data from the ChemoSpec SrE.NMR dataset [33].

8. Quantitative proton bookkeeping

8.1 Equations and assumptions

The relations collected in Table 6 are the standard lipid-NMR bookkeeping of Guillén and Ruiz, Knothe and Kenar, Barison and co-workers and Nieva-Echevarría and co-workers, re-based on the α-methylene integral rather than the glyceryl integral as the chain-count reference, because in a free-fatty-acid material the glyceryl signal counts only the minority of chains [13–17]. The α-methylene integral between 2.20 and 2.45 ppm counts two protons for every acyl chain whatever its head group, and is isolated from the allylic multiplet on one side and from the bis-allylic triplet on the other; it is therefore taken as the definition of the chain count N. The terminal-methyl integral divided by three is retained as a check: in a fully relaxed spectrum of an extract containing no fatty alcohols the two counts agree to within the integration error, and a methyl count below the α-methylene count by more than a few per cent is diagnostic of differential saturation. The

unsaturated chain count follows from the allylic integral, the linoleic count from the bis-allylic integral after correction for the linolenic contribution, and the saturated count by difference; the glyceride-bound fraction of the chains follows from the ratio of the glyceryl methylene integral (4.03–4.37 ppm) to the α-methylene integral, with the factor 1.5 arising from the four glyceryl methylene protons shared by three chains in a triglyceride. The relation is exact for triglycerides and is inflated by partial glycerides, which carry two or four glyceryl protons per one or two chains, and by the ethyl-ester OCH_2 quartet, so that the quantity returned is best described as the fraction of acyl chains bound to glycerol or to ethanol, an upper bound on the triglyceride-bound fraction. The mean carbon number of the saturated pool is obtained from the methylene envelope after subtraction of the contributions of the unsaturated chains, whose envelope proton counts are fixed by their structures (20 for oleic, 14 for linoleic, 8 for linolenic); the relation is a weighted average over the saturated chains and is sensitive to error when the saturated fraction is small, which is why it is reported only when saturated chains exceed 15 % and should be trusted only above about 40 %. Double bonds per chain and an iodine value on the free-acid basis follow from the chain fractions, and the sterol index from the H-18 singlet.

Table 6. Proton-bookkeeping relations used in this paper (integrals I over the stated limits on a baseline-corrected spectrum)

Quantity	Relation	Assumptions and remarks
Acyl-chain count, N	N = I(2.20–2.45)/2	Two α-protons per acyl chain irrespective of the head group (acid, glyceride, alkyl ester); fatty alcohols and sterols are not counted
Chain-count check	(I(0.78–1.01)/3)/N ≈ 1.00	Deviation reveals differential saturation (short relaxation delays) or fatty-alcohol/sterol contributions to the methyl region
Unsaturated chains, U	U = I(1.90–2.12)/4	Four allylic protons per unsaturated chain; the hydroxyl of partial glycerides at 1.97 ppm is a minor interference
Linolenic chains, Ln	Ln = I(0.95–1.01)/3	Semi-quantitative below ≈ 1 % because the Lorentzian tails of the envelope enter the window
Linoleic chains, L	L = I(2.70–2.86)/2 − 2 Ln	Two bis-allylic protons per linoleic chain, four per linolenic chain
Oleic (monoenoic) chains, O	O = U − L − Ln	Includes palmitoleic
Saturated chains, S	S = N − U	
Glyceride-bound fraction of chains, f_gly	f_gly = 1.5 × I(4.03–4.37)/I(2.20–2.45)	Exact for triglycerides (4 glyceryl CH_2 protons per 3 chains); partial glycerides and the ethyl-ester OCH_2 quartet inflate the numerator
Free (non-glyceride) fraction	1 − f_gly	Includes alkyl esters unless corrected separately
Olefinic consistency	I(5.15–5.45) − I(4.03–4.37)/4 ≈ 2 O + 4 L + 6 Ln	A ratio of measured to predicted olefinic intensity below ≈ 0.95 indicates saturation of the slowly relaxing olefinic and methyl protons
Mean carbon number of the saturated pool, $\bar{n}_S$	$\bar{n}_S$ = [I(1.15–1.45) − 20 O − 14 L − 8 Ln]/(2 S) + 4	Valid when S/N > ≈ 0.4; separates lauric-type pools (12–13) from palmitic/stearic-type pools (16–17)
Double bonds per chain, DB	DB = (O + 2 L + 3 Ln)/N	
Iodine value (free-acid basis)	IV ≈ 253.81 × DB/$\bar{M}$ × 100	$\bar{M}$ = mean chain molar mass; USP window 40–50 for the extract
Acid value (NMR)	AV ≈ 56.11 × (1 − f_gly − f_ester) × N_mmol/g	Equivalent to the ¹H NMR acid-value approach of Skiera et al.; N_mmol/g requires an internal standard
Methyl esters, fatty alcohols, sterols	ME = I(3.67 s)/3; ROH = I(3.64 t)/2; sterol = I(0.68 s)/3	Fatty-alcohol triplet is obliterated by polyethylene glycol excipients; sterol H-18 is at the 0.1–0.3 % level and needs S/N > 1000 on the methyl signal
Ethyl esters	line-shape fit of the 4.12 ppm quartet, or ¹³C (60.1 ppm)	Simple integration is not possible at ≤ 500 MHz because the triglyceride glyceryl lines interleave with the quartet

8.2 Results on the public spectra

Table 7 gives the results for all sixteen public spectra, arranged in order of increasing glyceride-bound fraction, and Figure 3 plots the glyceride-bound fraction against the linoleic chain fraction as a two-ratio identity map. The two reference oils behave as the relations require: olive oil returns 103 % glyceride-bound chains, 77 % oleic, 7.6 % linoleic and 16 % saturated chains with a mean saturated carbon number of 16.9 and an estimated iodine value of 84, and evening primrose oil returns 99 % glyceride-bound chains and an iodine value of 163, with the linoleic count exceeding 80 % because the γ-linolenic chains of that oil contribute four bis-allylic protons each and are counted twice by a relation written for linoleic acid. The retail products divide into three groups. Two products, NR (labeled pure) and NP (labeled with added oil), carry 17 % and 25 % glyceride-bound chains, 30–31 % oleic chains, 65–67 % saturated chains, a mean saturated carbon number of 12.9–13.4 and estimated iodine values of 40–43; every index of NR falls inside the compendial expectation for a lipophilic extract, and NP differs only in a low linoleic fraction (0.9 %) and an anomalous methyl-check ratio. Two products, ET and MDB (both labeled pure), carry 48–54 % glyceride-bound chains but a fatty-acid pattern, 29–31 % oleic, 3.5–3.7 % linoleic, 65–67 % saturated and a mean saturated carbon number of 12.3, that is indistinguishable from that of saw palmetto, or equally from that of a lauric-type oil; half of their acyl chains are bound to glycerol, which places them either at the extreme of the authentic glyceride range reported by Habib and Wyllie or in the territory of admixture with coconut or palm-kernel oil, and the ¹H data alone cannot decide between those readings. The remaining ten products carry 49–74 % glyceride-bound chains with oleic fractions of 42–59 % (GNC, NA, Nat, NW, Sol, TD, CVS, LF, SN) or, in one case (NSI), a linoleic fraction of 26 %; the oleic-rich group is consistent with the olive-oil dilution that the dataset documentation describes, including product NA, which is labeled pure but is spectroscopically a 73 % glyceride material with 49 % oleic chains, and NSI is consistent with a linoleic-rich seed oil such as sunflower or soybean rather than olive oil. Estimated sterol contents from the H-18 singlet lie between 0.1 and 0.6 % w/w for most products, within or above the compendial window, with two products at 0.7–1.3 % and evening primrose oil at 1.1 %; these values carry an uncertainty of perhaps 30 % because of the small size of the signal and the absence of relaxation information, and are reported to show that the index is accessible rather than to characterize the products.

Table 7. Proton-bookkeeping results for the 16 public spectra (ChemoSpec SrE.NMR; ≈ 500 MHz, $CDCl_3$). Sample codes are the dataset labels: pSrE = labeled pure extract, adSrE = labeled as containing added oil

Sample / label	Glyceride-bound chains (%)	Oleic (% chains)	Linoleic (% chains)	Saturated (% chains)	$\bar{n}_S$	Iodine value (est.)	Sterol (% w/w, est.)	CH_3-check ratio	Olefinic consistency
NR / pSrE	17	30.2	4.4	65.4	12.9	43	0.25	0.88	0.82
NP / adSrE	25	31.1	0.9	66.8	13.4	40	0.46	1.08	0.81
ET / pSrE	54	28.8	3.7	67.3	12.3	42	0.27	0.87	0.70
MDB / pSrE	48	31.1	3.5	65.4	12.3	44	0.35	0.88	0.80
CVS / adSrE	49	43.5	6.7	49.7	12.7	61	0.17	0.86	0.84
LF / adSrE	51	47.0	2.8	49.4	13.7	57	1.25	0.97	0.93
SN / adSrE	56	42.3	5.3	52.5	12.7	56	0.32	0.86	0.81
Sol / adSrE	55	47.7	6.9	45.5	13.0	63	0.14	0.85	0.83
TD / adSrE	57	52.5	3.6	42.9	13.0	64	0.58	0.93	0.84
NSI / adSrE	56	27.3	26.1	46.2	13.2	83	0.12	0.89	0.84
NW / adSrE	66	50.6	5.6	43.4	13.0	65	0.09	0.88	0.83
Nat / adSrE	72	50.4	6.3	43.2	13.2	65	0.29	0.92	0.83
NA / pSrE	73	49.4	5.9	44.5	13.0	64	0.52	0.88	0.78
GNC / adSrE	74	59.2	4.9	35.2	14.5	69	0.75	0.92	0.90
TJ / OO	103	76.8	7.6	15.8	16.9	84	0.28	0.86	0.89
SV / EPO	99	0.1	83.7	15.0	11.8	163	1.13	0.87	0.87

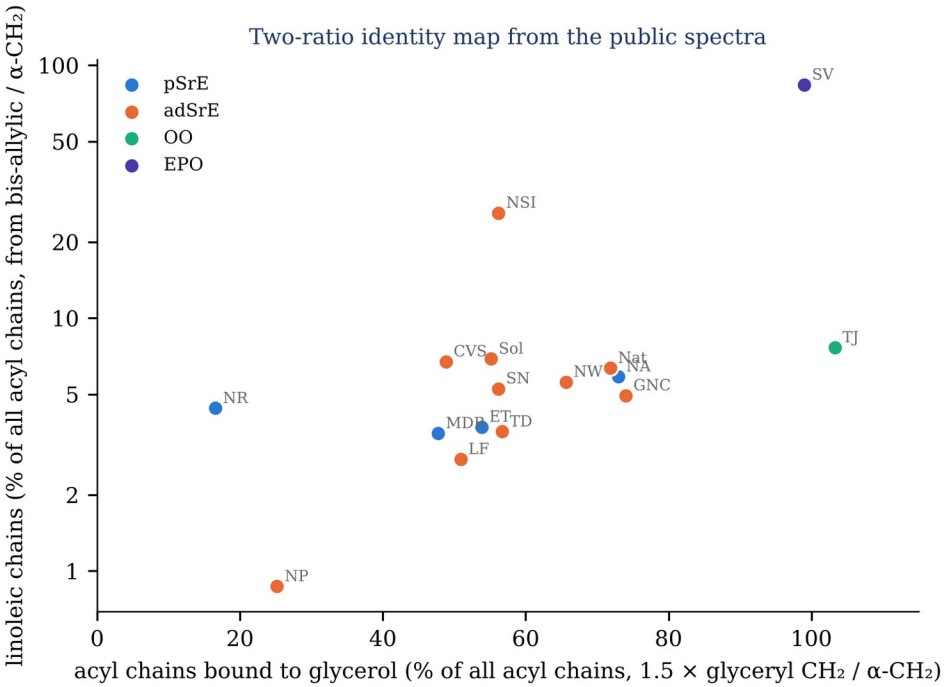

Figure 3. Two-ratio identity map of the sixteen public spectra: the fraction of acyl chains bound to glycerol, from 1.5 × I(4.03–4.37)/I(2.20–2.45), against the linoleic chain fraction from the bis-allylic integral (logarithmic axis). Blue, products labeled pure; orange, products labeled as containing added oil; green, olive oil; violet, evening primrose oil. Only NR and NP lie in the region expected for a free-fatty-acid extract. Data from the ChemoSpec SrE.NMR dataset [33].

8.3 Internal consistency checks and evidence of non-quantitative acquisition

Two internal checks in Table 7 show that the public spectra were not acquired under fully relaxed conditions. The methyl-check ratio, the terminal-methyl count divided by the α-methylene count, is 0.85–0.93 for fifteen of the sixteen spectra against the value of 1.00 required by stoichiometry; the olefinic consistency ratio, the measured olefinic integral (corrected for the triglyceride methine) divided by the olefinic intensity predicted from the allylic and bis-allylic integrals, is 0.70–0.93 against the required 1.00. Both deficits fall on the slowly relaxing protons, the terminal methyl and the olefinic methines, relative to the faster-relaxing methylene and α-methylene protons, which is the pattern produced by a relaxation delay short compared with the longest T_1 in the sample, as discussed in Section 5.2. The β-methylene to α-methylene ratio, by contrast, is 1.01–1.11 for all spectra, as expected for two methylene groups of similar relaxation behavior. The practical consequences are that the chain fractions in Table 7 carry a systematic error of the order of 10 % relative (the unsaturated fractions being overestimated and the saturated fractions underestimated because the allylic count is referenced to a depressed α-methylene count only partially), that the glyceride-bound fractions are less affected because the glyceryl and α-methylene protons relax at comparable rates, and that no absolute conclusion about any product should be drawn from these numbers beyond the three-group classification given above. The lesson for the PNA protocol is that the methyl-check ratio must be reported with every quantitative spectrum, and that a fingerprint acquired for throughput cannot afterwards be mined for stoichiometric quantities.

8.4 Uncertainty and detection limits

Under relaxed conditions and at a methyl signal-to-noise ratio of 1 000 or better, the integrals of the major signals are reproducible to about 1 % relative, which propagates to about 1 % absolute in the chain fractions and to about 0.5 % absolute in the glyceride-bound fraction. The detection limit for glyceride-bound chains in a free-fatty-acid matrix is set by the signal-to-noise of the 4.29 ppm doublet of doublets, which carries two protons per triglyceride against two α-methylene protons per chain; at a methyl signal-to-noise of 1 000 a triglyceride contributing 1 % of the chains gives a glyceryl signal with a signal-to-noise of about 5, so that 1–2 % of glyceride-bound chains is the practical limit at 300 MHz and well below the 5–10 % that an authentic extract already contains. The method therefore detects the addition of a triglyceride oil to an extract not at the level of its own detection limit but at the level at which the glyceride-bound fraction leaves the window established on the authentic reference set, which the simulations of Section 9 place at about 5 % w/w for coconut oil if the authentic window is 10 ± 5 %. The sterol H-18 index at 300 MHz has a relative uncertainty of 20–30 % at the compendial level, which suffices to establish presence or absence but not to replace the GC determination; the fatty-alcohol triplet is similar, and is unavailable in the presence of polyethylene glycol. The linolenic estimate from the 0.97 ppm triplet carries a positive bias of about 0.5 % of chains from envelope tails, as the simulations of Section 9 show quantitatively, and should be reported as semi-quantitative.

9. Simulated spectra for extraction-type and adulteration scenarios

9.1 Model construction

The public spectra cover olive-oil and seed-oil dilution but not the cases that matter most, ethanolic extracts with their ethyl esters, lauric-type oils, hydrolyzed oils and designer blends, and no measured spectra of those materials were available. Simulated spectra were therefore constructed, and they are presented with the explicit statement that they are models built from literature chemical shifts and compositions, not measurements. The model represents each acyl chain by its functional-group protons (terminal methyl, methylene envelope, β-methylene, α-methylene, allylic, bis-allylic and olefinic protons, with the α-methylene shift set by the head group as in Table 4), each head group by its own protons (carboxyl, glyceryl, ethyl or methyl), and the fatty alcohols and β-sitosterol by their principal signals; multiplets are rendered as first-order patterns with the coupling constants of Table 4, unresolved multiplets and the methylene envelope as combs of narrow lines, and every line as a Lorentzian of 0.9 Hz full width at 300 MHz and 1.3 Hz at 60 MHz. Compositions are specified as mass fractions of species and converted to proton intensities through the molar masses of the chain population, so that the simulated integrals obey the same stoichiometry as a real spectrum and the Table 6 relations can be applied to them as a self-consistency test. The fatty-acid profiles used are those of Table 2: a typical saw palmetto profile for the extracts, Codex-type profiles for coconut, palm and olive oils, and, for the designer blend, the saw palmetto profile itself, since such a blend is by definition matched to it. Table 8 lists the twelve scenarios; the parameter files are in the companion workbook, and the simulation code is retained so that the scenarios can be re-run with the measured compositions of the reference set when those become available.

Table 8. Composition models used for the simulated spectra (w/w of the lipid; fatty-acid profiles from Table 2)

Code	Scenario	Model composition
A	Hexane/scCO_2-type extract	SPE profile; FFA 88 %, TG 5 %, 1,3-DG 1.5 %, 1,2-DG 1 %, MG 0.5 %, ethyl esters 1.5 %, methyl esters 0.5 %, sterols 0.3 %, fatty alcohols 0.3 %

Code	Scenario	Model composition
B	Ethanolic-type extract	SPE profile; FFA 80 %, TG 7 %, DG 3.5 %, MG 1.5 %, ethyl esters 4.5 %, methyl esters 0.5 %, sterols 0.3 %, fatty alcohols 0.15 %
C	Coconut oil	Codex-type profile; TG 97 %, FFA 1 %, DG 1.6 %, MG 0.2 %, sterols 0.08 %
D	A + 30 % w/w coconut oil	Mixture of A and C
E	Hydrolyzed coconut oil (lauric-type free-fatty-acid blend)	Coconut profile; FFA 97 %, residual glycerides 3 %; no sterols or alcohols
F	A + 50 % w/w hydrolyzed coconut FFA	Mixture of A and E
G	Olive oil	Codex-type profile; TG 96 %, FFA 1 %, DG 2.5 %, sterols 0.15 %
H	Designer free-fatty-acid blend matched to the SPE ratios	SPE profile by construction; FFA 97 %, residual glycerides 3 %; no phytosterols or fatty alcohols
I	A + 30 % w/w olive oil	Mixture of A and G
J	A + 50 % w/w designer blend	Mixture of A and H
K	Palm oil	Codex-type profile; TG 93 %, FFA 3 %, DG 4 %
L	A + 30 % w/w palm oil	Mixture of A and K

9.2 300 MHz renderings and recovered indices

Figure 4 shows the simulated 300 MHz spectrum of scenario A, a hexane or supercritical-CO_2 type extract with 88 % free acids, 8 % glycerides, 2 % alkyl esters and 0.3 % each of sterols and fatty alcohols, with the three diagnostic regions expanded. The spectrum is a fair likeness of the published figures of De Combarieu and co-workers and Perini and co-workers and of the public spectra of Section 7, with the free-acid α-methylene triplet dominant, the glyceride triplet as a weak shoulder 10 Hz upfield, the glyceryl region a cluster of weak doublets of doublets interleaved with the ethyl-ester quartet, and the triglyceride methine as a small signal beside the olefinic multiplet [3,5]. Figure 5 overlays, in the same three regions, the scenarios that represent the authentication problem: the ethanolic-type extract B with 4.5 % ethyl esters, the extract diluted with 30 % coconut oil (D), the extract diluted with 50 % hydrolyzed coconut oil (F), and the extract diluted with 50 % of a designer free-fatty-acid blend matched to its own profile (J). The coconut-oil dilution is unmistakable, the glyceride α-methylene triplet rising to half the intensity of the free-acid triplet, the glyceryl doublets of doublets rising fivefold and the triglyceride methine appearing beside the olefinic signal. The ethanolic extract is distinguished by the stronger ethyl-ester quartet and its interleaving with the triglyceride lines at 4.10–4.16 ppm, a feature that is visible but, as argued in Section 6.3, not integrable at this field. The hydrolyzed-coconut dilution and the designer blend are the sobering cases: trace J coincides with trace A in every region within the line width, and trace F differs only in a smaller olefinic and bis-allylic intensity, because the hydrolyzed lauric oil brings in saturated free acids and nothing else that the spectrum can see.

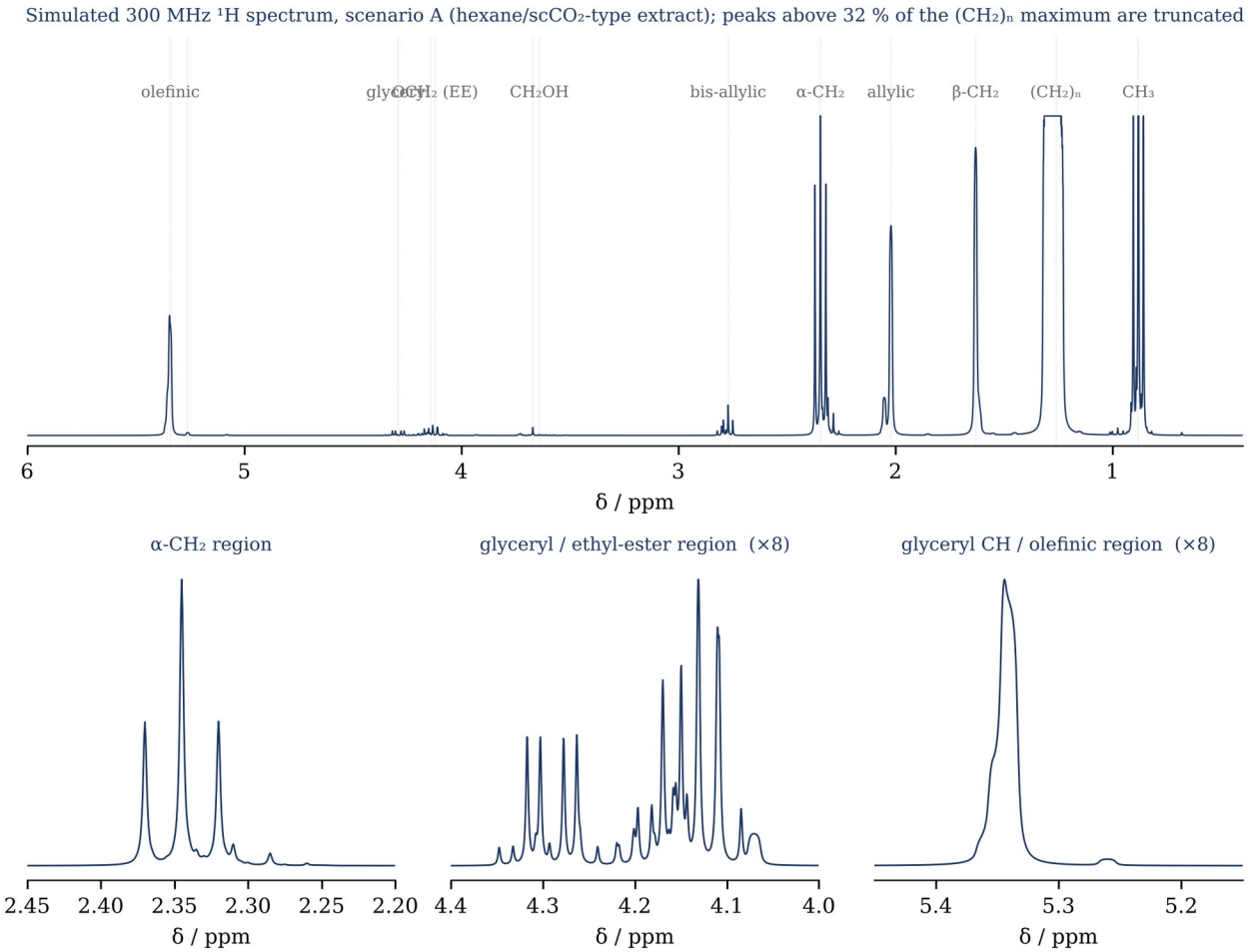

Figure 4. Simulated ¹H NMR spectrum (300 MHz, $CDCl_3$) of scenario A, a hexane/scCO_2-type saw palmetto extract (Table 8), built from the literature chemical shifts of Table 4 with first-order multiplets and 0.9 Hz Lorentzian lines. Top: 0.4–6.0 ppm with intensities above 32 % of the methylene-envelope maximum truncated. Bottom, left to right: the α-methylene region (free-acid triplet at 2.345 ppm, glyceride triplet at 2.31 ppm, methyl- and ethyl-ester triplets at 2.30 and 2.285 ppm); the glyceryl and ethyl-ester region at eight times the scale; the triglyceride methine and olefinic region at eight times the scale. This is a model, not a measured spectrum; the fine structure of the olefinic and allylic multiplets is not modeled.

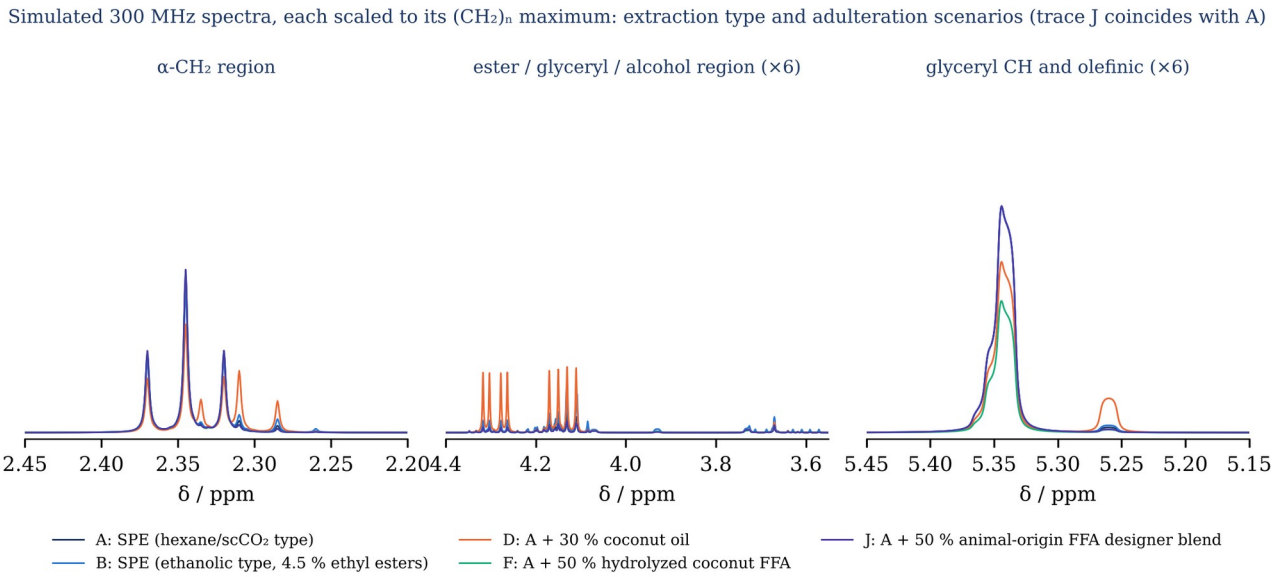

Figure 5. Simulated 300 MHz spectra of five scenarios of Table 8 in the three diagnostic regions, each spectrum scaled to its methylene-envelope maximum: A, hexane/scCO$_2$-type extract (navy); B, ethanolic-type extract with 4.5 % ethyl esters (blue); D, A diluted with 30 % w/w coconut oil (orange); F, A diluted with 50 % w/w hydrolyzed coconut oil (green); J, A diluted with 50 % w/w of a designer free-fatty-acid blend matched to the saw palmetto profile (violet; coincident with A). Intensities above the panel height are truncated. Models, not measurements.

Table 9 compares the indices recovered from the simulated spectra by the Table 6 relations with the model inputs. The recovery test is passed where it should be and fails where the relations predict that it should fail. The glyceride-bound fraction is recovered within 2 % absolute for the triglyceride oils (99.2 % recovered for coconut oil against 99.2 % input; 98.9 against 99.2 for olive oil) and for the mixtures with those oils (39.0 against 37.3 for 30 % coconut oil, 34.0 against 32.2 for 30 % olive oil), and is overestimated by 2.6 % absolute for scenario A and by 6.9 % for the ethanolic scenario B, the excess being the ethyl-ester OCH$_2$ quartet that falls inside the glyceryl integration window; the overestimate is the quantitative form of the warning given in Section 6.3 and is the reason why the ethanolic-type window of Table 15 is set wider. The oleic and saturated chain fractions are recovered within 0.5 % absolute for every scenario, the mean carbon number of the saturated pool within 0.1 for the lauric-type materials (12.9 recovered for 12.86 input in scenario A; 12.4 for 12.46 in coconut oil) and within 0.2 for palm oil (16.0 for 16.1), but is low by 0.7 for olive oil, where saturated chains are only 15 % of the total and the subtraction of the unsaturated contributions dominates the arithmetic; this is the behavior anticipated in Section 8.1 and the reason for the 40 % saturated-fraction threshold. The linolenic fraction is overestimated by about 0.6 % of chains in every scenario because the Lorentzian tails of the envelope enter the 0.95–1.01 ppm window, and the linoleic fraction is correspondingly underestimated by about 1 % of chains through the linolenic correction; both biases are constant across scenarios and could be removed by a tail correction, but it is simpler to regard the two ω-3-dependent quantities as semi-quantitative. The sterol H-18 index has a floor of about 0.7×10^{-3} from the tails of the methyl triplet even when no sterol is present (scenarios E and H), so that the index must be read against that floor, or the singlet fitted, before the absence of sterols is asserted.

Table 9. Indices recovered from the simulated 300 MHz spectra by the Table 6 relations, compared with the model input

Code	Glyceride-bound chains, model (%)	Glyceride-bound chains, recovered (%)	Oleic (% chains)	Linoleic (% chains)	Saturated (% chains)	$\bar{n}_S$	Sterol H-18/CH$_3$ (×1000)
A	8.2	10.8	33.8	4.4	60.5	12.9	2.3

Code	Glyceride-bound chains, model (%)	Glyceride-bound chains, recovered (%)	Oleic (% chains)	Linoleic (% chains)	Saturated (% chains)	$\bar{n}_S$	Sterol H-18/CH_3 (×1000)
B	12.6	19.5	33.9	4.4	60.3	12.9	2.3
C	99.2	99.2	7.3	0.8	91.2	12.4	1.1
D	37.3	39.0	25.4	3.2	70.3	12.7	1.9
E	3.1	3.5	7.3	0.8	91.2	12.4	0.7
F	5.5	6.9	19.6	2.5	77.0	12.6	1.4
G	99.2	98.9	74.9	8.4	15.1	15.6	1.7
H	3.0	3.4	33.7	4.5	60.4	12.8	0.7
I	32.2	34.0	44.6	5.4	48.5	13.1	2.1
J	5.5	7.0	33.8	4.5	60.4	12.8	1.5
K	97.3	97.5	40.3	8.7	50.0	16.0	1.1
L	32.3	34.2	35.5	5.6	57.6	13.6	1.9

9.3 The hydrolyzed-oil and designer-blend limit

Scenarios F and J quantify the limit stated qualitatively in Section 4.2. A 50 % dilution of the extract with hydrolyzed coconut oil lowers the glyceride-bound fraction from 10.8 to 6.9 % (the authentic glycerides are diluted), lowers the oleic fraction from 33.8 to 19.6 % and the linoleic fraction from 4.4 to 2.5 %, raises the saturated fraction from 60 to 77 %, leaves the chain-length index unchanged at 12.6–12.9, and halves the sterol index. The unsaturation changes are large enough to be caught by the oleic window of Table 15, but only because the adulterant was taken as crude hydrolyzed coconut oil; a blend in which part of the lauric-type acid was replaced by oleic acid from any source would restore the oleic fraction, and scenario J shows the end point of that reasoning. A 50 % dilution with a free-fatty-acid blend matched to the extract's own profile changes no chain fraction by more than 0.1 % absolute, lowers the glyceride-bound fraction from 10.8 to 7.0 %, and lowers the sterol and fatty-alcohol indices by half; the first of these is within the natural range and the second is at the detection limit of the 300 MHz experiment and unavailable in the presence of polyethylene glycol. The ¹H spectrum of such a blend is, for practical purposes, the spectrum of the extract. The conclusion is not that NMR is useless against designer blends but that its contribution in that case is confined to the minor constituents and to the trans-olefinic and allylic signals at high field, and that the decision must be carried by stable-isotope ratio analysis, which Perini and co-workers showed to be decisive because the $\delta^{18}O$, $\delta^{2}H$ and $\delta^{13}C$ values of animal fats and of oleochemical fatty acids are far from those of the authentic extract, and by GC determination of the sterols and fatty alcohols that the blend lacks [5,7,10].

9.4 Spike response of the glyceride ratio

Table 10 follows three integral ratios through a simulated spike series of coconut oil, and of hydrolyzed coconut oil, into the scenario-A extract. The glyceryl-to-α-methylene ratio responds linearly to coconut oil, from 0.072 at 0 % to 0.104 at 5 %, 0.136 at 10 %, 0.198 at 20 % and 0.259 at 30 %, with a slope of 0.0062 per per cent of adulterant that is reproduced in the glyceride-bound fraction (10.8, 15.6, 20.3, 29.7 and 38.8 %). Against an authentic window of 10 ± 5 % glyceride-bound chains, which is the provisional window of Table 15 for hexane and CO_2 extracts, 5 % coconut oil reaches the edge of the window and 10 % is clearly outside it; the practical detection limit for a triglyceride adulterant is therefore set by the natural variability of the authentic glyceride content, not by the instrument, and will be established only when the reference set has been measured. The bis-allylic and olefinic ratios fall with coconut oil in exactly the way they fall with hydrolyzed coconut oil, which shows that unsaturation alone cannot tell a triglyceride adulterant from its hydrolyzed form; only the glyceryl ratio does, and for the hydrolyzed form the glyceryl ratio moves in the wrong direction. The series will be re-run with measured spectra of gravimetric spikes in version 1.1.

Table 10. Simulated spike response (300 MHz) of three integral ratios to coconut oil and to hydrolyzed coconut oil added to the scenario-A extract

Adulterant (% w/w)	Coconut oil: glyceryl/α-CH_2	Coconut oil: glyceride-bound chains (%)	Coconut oil: bis-allylic/α-CH_2	Coconut oil: olefinic/α-CH_2	Hydrolyzed coconut: glyceryl/α-CH_2	Hydrolyzed coconut: bis-allylic/α-CH_2	Hydrolyzed coconut: olefinic/α-CH_2
0	0.072	10.8	0.0715	0.454	0.072	0.0715	0.454
5	0.104	15.6	0.0687	0.435	0.069	0.0686	0.434
10	0.136	20.4	0.0660	0.416	0.066	0.0657	0.414
20	0.198	29.7	0.0606	0.379	0.061	0.0601	0.375
30	0.260	39.0	0.0553	0.342	0.056	0.0547	0.337
50	0.380	56.9	0.0451	0.270	0.046	0.0443	0.264
75	0.524	78.5	0.0327	0.184	0.034	0.0322	0.180
100	0.662	99.2	0.0208	0.101	0.024	0.0210	0.100

10. Field strength and benchtop (60 MHz) feasibility

Table 11 converts the chemical-shift separations of the diagnostic signal pairs into hertz at the proton frequencies of the instruments in common use and compares them with the separation needed to resolve the multiplets concerned. The arithmetic is unforgiving at 60 MHz. The free-acid and glyceride α-methylene triplets, 0.035 ppm apart, are separated by 2.1 Hz against a coupling constant of 7.5 Hz, so that the two patterns collapse into one and the α-methylene region can be integrated only as a block; the triglyceride methine and the olefinic multiplet, 0.08 ppm apart, are 4.8 Hz apart and merge; the sterol H-18 singlet at 0.68 ppm lies 5 Hz from the outer line of the terminal-methyl triplet and cannot be seen; and the ethyl-ester quartet spans 3.94–4.30 ppm, the whole glyceryl region. At 300 MHz the α-methylene pair is resolved at 10.5 Hz (visibly, though not to baseline), the methine and olefinic signals by 24 Hz, the sterol singlet by 60 Hz and the fatty-alcohol triplet from the methyl-ester singlet by 9 Hz; the ethyl-ester quartet remains interleaved with the triglyceride lines at every field below about 800 MHz. The glyceryl region itself, however, survives the move to low field. Its lines spread into a comb between 3.95 and 4.45 ppm, but the comb remains isolated from the α-methylene block above 2.5 ppm and from the olefinic block above 5.1 ppm, so that the one measurement on which triglyceride adulteration turns, the glyceryl-to-α-methylene ratio, is available at 60 MHz, as are the allylic and bis-allylic integrals and hence the unsaturation. Figure 6 shows the simulated 60 MHz rendering of scenarios A, B and D; the coconut-oil dilution is evident in the glyceryl comb and in the extra lines within the α-methylene block, whereas the ethanolic extract differs from the hexane extract only in the intensity of the comb. The chain-length index is not reliable at 60 MHz because the envelope, β-methylene and terminal-methyl signals overlap, and the sterol and fatty-alcohol indices are lost. Second-order effects, which the first-order rendering of Figure 6 ignores, make the real 60 MHz spectrum less tidy than the figure: the two glyceryl methylene protons of each sn-1,3 position, 9 Hz apart at 60 MHz with an 11.9 Hz geminal coupling, form a strongly coupled AB system, and the integral of the region is conserved although its appearance is not.

Table 11. Separation of diagnostic signal pairs expressed in hertz at the ¹H frequencies of common instruments, compared with the approximate separation needed for baseline resolution of the multiplets

Signal pair	Δδ (ppm)	60 MHz	300 MHz	400 MHz	500 MHz	600 MHz	Needed (Hz)
Free-acid α-CH_2 2.345 vs glyceride α-CH_2 2.31 (two 7.5 Hz triplets)	0.035	2.1	10.5	14.0	17.5	21.0	≈ 15
Triglyceride glyceryl CH_2 4.14 vs ethyl-ester OCH_2 4.12 (dd vs 7.1 Hz quartet)	0.020	1.2	6.0	8.0	10.0	12.0	≈ 20
Triglyceride CH 5.26 vs olefinic 5.34	0.080	4.8	24.0	32.0	40.0	48.0	≈ 15
Fatty-alcohol CH_2OH 3.64 (t) vs methyl-ester OCH_3 3.67 (s)	0.030	1.8	9.0	12.0	15.0	18.0	≈ 12
Sterol H-18 0.68 (s) vs terminal CH_3 0.88 (t)	0.200	12.0	60.0	80.0	100.0	120.0	≈ 12

Signal pair	Δδ (ppm)	60 MHz	300 MHz	400 MHz	500 MHz	600 MHz	Needed (Hz)
1,2-Diglyceride CH 5.08 vs triglyceride CH 5.26	0.180	10.8	54.0	72.0	90.0	108.0	≈ 10
Linolenic ω-CH_3 0.97 (t) vs terminal CH_3 0.88 (t)	0.090	5.4	27.0	36.0	45.0	54.0	≈ 14

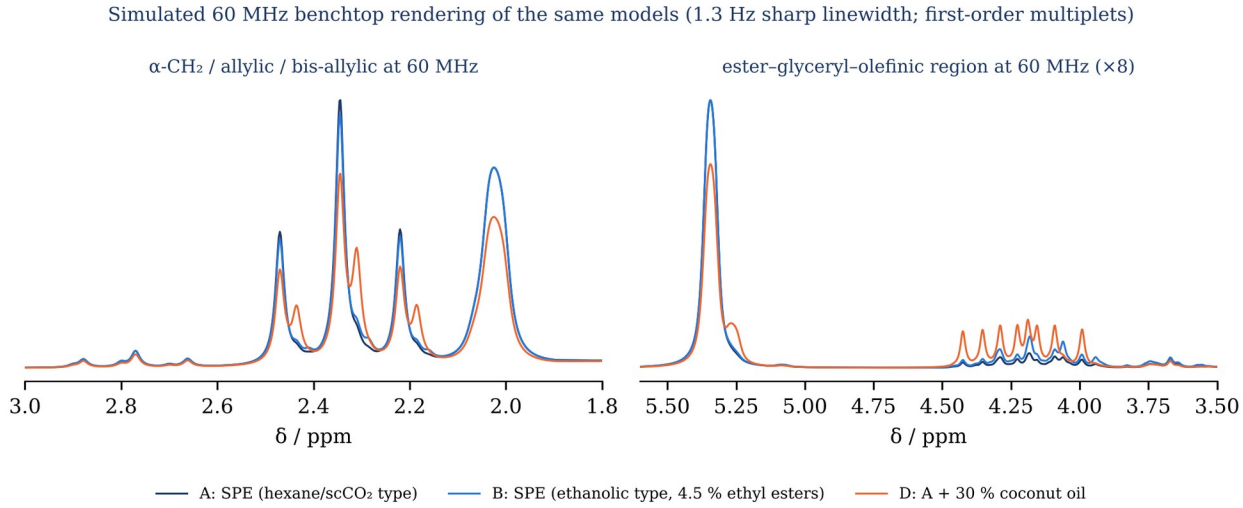

Figure 6. Simulated 60 MHz rendering of scenarios A (hexane/$scCO_2$-type extract, navy), B (ethanolic type, blue) and D (A with 30 % coconut oil, orange), with 1.3 Hz Lorentzian lines and first-order multiplets; second-order distortion of the glyceryl AB systems is not modeled. Left: the α-methylene, allylic and bis-allylic block; right: the ester–glyceryl–olefinic region at eight times the scale. The glyceryl comb between 3.95 and 4.45 ppm remains isolated and integrable at 60 MHz; the free-acid/glyceride α-methylene distinction does not survive.

The benchtop literature on edible oils supports this assessment. Parker and co-workers showed on a 60 MHz instrument that hazelnut oil in olive oil could be detected at about 13 % by a peak ratio and 11 % by whole-spectrum chemometrics, better than infrared spectroscopy in specificity, with the glyceryl and olefinic signals resolved at that field [48]; Gouilleux and co-workers recovered the classification of six oils at 43 MHz by ultrafast two-dimensional acquisition where one-dimensional low-field spectra overlapped [49]; and Jakes and co-workers built a one-class "authentic region" model for beef against horse meat from 60 MHz spectra of chloroform extracts, which is the statistical form most appropriate to a benchtop saw palmetto screen [50]. For PNA the division of labor follows directly: the AI-60 or Spinsolve can carry a glyceride-fraction and unsaturation screen of incoming lots, flagging any sample with a glyceryl ratio above the authentic window for 300 MHz confirmation; the MVX300 carries the full bookkeeping, the sterol and fatty-alcohol indices, the ^{13}C carbonyl speciation and the chemometric membership test; and the designer-blend question is referred out to isotope-ratio mass spectrometry.

11. Metabolomic fingerprinting and chemometrics

11.1 Published NMR chemometric studies of saw palmetto

Table 12 summarizes the three primary NMR fingerprinting studies, the BAPP evaluation, and the treatment of the public data in this paper. Booker and co-workers, working at 500 MHz on thirty-four products from nine countries, bucketed the spectra at 0.04 ppm over 0–10 ppm with the solvent regions excluded, applied peak-range normalization and principal component analysis, and found that products made by different extraction processes could be separated whereas products made by similar processes could not; the loadings

were dominated by the olefinic region, which they attributed to oleic acid, and by the 4.1–4.2 ppm ester region, in which caproic acid ethyl ester was identified against a standard, and the outliers were products with added olive or soybean oil, a product in polyethylene glycol, and one showing an aldehyde signal [11]. De Combarieu and co-workers, at 400 MHz on twenty-six industrial batches of the three extract types, used full-resolution data (12 900 points per spectrum over 0–6 ppm) aligned by correlation-optimized warping, normalized to the maximum peak and mean-centered; their first three components explained 44.8, 31.5 and 7.5 % of the variance, the hexane and CO_2 batches were superimposable, and the ethanolic batches were displaced along the first component by a loading that they read as more monounsaturated chains and more sn-1,3-diglycerides and less linoleic acid, by less than the batch-to-batch spread; their conclusion was phytochemical equivalence of the three extract types [3,51]. Perini and co-workers then used the same NMR protocol on thirty authenticated extracts and nine commercial extracts and found all nine outside the authentic cluster in the first three components, with the NMR result fully consistent with the isotopic and phytochemical results [5]. The BAPP guidance document, reviewing these studies, located the discriminating regions at 4.1–4.2 and 5.3–5.5 ppm, proposed the triglyceride glyceryl signals as a visual marker, and noted that the method requires an expensive instrument and a reference library that most quality-control laboratories do not have [6,10]. Three features of these studies deserve comment. The first is that each used a reference set of real, documented batches, which is the only basis on which a fingerprint can be interpreted; the second is that the discriminating loadings were, in every case, the speciation and unsaturation signals that Sections 6 and 8 treat quantitatively, so that the chemometric and the bookkeeping readings of the spectrum are the same reading in different clothes; and the third is that all three studies used unsupervised PCA and visual cluster membership rather than a validated classifier, which is adequate for exploration but not for a reportable authenticity decision.

Table 12. Published ¹H NMR fingerprinting and chemometric studies of saw palmetto extracts and products

Study	Instrument and sample	Samples	Data treatment	Principal findings
Booker et al. 2014	500 MHz (Bruker Avance); $CDCl_3$ for soft-gel fills, CD_3OD for powders; 30° pulse, 1 s relaxation delay, 256 scans; diffusion-edited acquisition with ethanol/water suppression	34 products by NMR (57 products from nine countries in total; 46 by GC; 26 by both)	0.04-ppm buckets over 0–10 ppm (251 buckets, solvent regions excluded); peak-range normalization; PCA in SIMCA 13	Products made by different extraction methods were separated; products made by similar methods were not. Loadings dominated by the olefinic region (5.3–5.5 ppm) and the ester region (4.1–4.2 ppm) where caproic acid ethyl ester was identified. Outliers contained added olive or soybean oil; one hexane extract in polyethylene glycol gave a large 3.62–3.64 ppm singlet
De Combarieu et al. 2015	Agilent 400-MR DD2 (399.78 MHz); ≈ 10 mg in 0.75 mL $CDCl_3$; 45° pulse, 1 s relaxation delay, 128 scans, 303 K	26 industrial batches: 7 ethanolic, 9 $scCO_2$, 10 hexane	Full-resolution data (26 × 12 900 points, 0–6 ppm) aligned by correlation-optimized warping, normalized to the maximum peak, mean-centered; PCA in MATLAB (PC1 44.8 %, PC2 31.5 %, PC3 7.5 %)	$scCO_2$ and hexane extracts were superimposable; ethanolic batches differed slightly (more monounsaturated acids and sn-1,3-diglycerides, less linoleic acid) but by less than batch-to-batch variability; the three extract types were judged phytochemically equivalent
Perini et al. 2018	As De Combarieu 2015	30 authenticated extracts (2009–2017) and 9 commercial extracts	PCA of the NMR data (three components shown), alongside PCA of $\delta^{18}O/\delta^{2}H$, fatty-acid ratios, and sterol/triglyceride profiles	All nine commercial samples fell outside the authentic cluster; three were vegetable-oil blends (coconut or babassu), six were designer blends of animal-origin fatty acids that passed the USP ratio test

Study	Instrument and sample	Samples	Data treatment	Principal findings
Gafner 2019 (BAPP Laboratory Guidance Document) and Gafner et al. 2023	Evaluation of the published methods	—	—	The α/α′ and β glyceryl proton signals of triglycerides (ca. 4.20 and 5.25 ppm) are much weaker in saw palmetto extract than in vegetable-oil adulterants and are proposed as a visual adulteration marker; the 4.1–4.2 and 5.3–5.5 ppm regions drive the PCA clustering; a vegetable oil, possibly coconut oil, was identified by ¹H NMR in a commercial sample
This paper (public ChemoSpec SrE.NMR spectra)	≈ 500 MHz (inferred); $CDCl_3$; acquisition parameters undocumented	14 retail products and two reference oils	0.02-ppm buckets over 0.5–6.0 ppm, total-area normalization, mean-centering, Pareto scaling; PCA; proton bookkeeping	See Sections 7, 8 and 11

11.2 Preprocessing: alignment, bucketing, normalization, centering and scaling

The preprocessing of lipid ¹H spectra for chemometrics is simpler than that of aqueous metabolomic spectra because the chemical shifts are insensitive to pH, but it is not trivial. Small shift variations arise from concentration, temperature and the water content of the solvent, and Defernez and Colquhoun showed that such variations degrade principal component models by generating spurious loadings at peak flanks [52]. Alignment is therefore the first step: correlation-optimized warping, as used by De Combarieu and co-workers, or interval-correlation shifting (icoshift), which aligns segments of full-resolution spectra against a reference by fast Fourier correlation and is the more convenient tool when the spectra are to be kept at full resolution [51,53]. Bucketing at 0.02–0.04 ppm then reduces the data to a few hundred variables, suppresses residual misalignment and, for lipid spectra whose information is carried by a dozen signal groups, loses nothing of consequence; full-resolution data, as De Combarieu and co-workers argued, keep the loadings interpretable as spectra, and either approach is acceptable provided the alignment is adequate [3]. Normalization corrects for the concentration of the sample and is a row operation; scaling adjusts the weight of each variable and is a column operation, and Craig and co-workers showed that the two interact, constant-sum normalization in particular introducing correlations between variables that can masquerade as chemistry [54]. For saw palmetto spectra, in which the methylene envelope carries half the total intensity, normalization to the total area is dominated by the envelope and normalization to the maximum peak by the same signal; normalization to the α-methylene integral, which counts chains, is chemically the most defensible choice and is recommended for the PNA protocol, although total-area normalization was used for the public data in this paper for comparability with the literature. Mean-centering is applied in every case. For scaling, van den Berg and co-workers compared centering, autoscaling, Pareto, range, vast, level, log and power transforms and showed that Pareto scaling, which divides each variable by the square root of its standard deviation, reduces the dominance of the largest signals while retaining a measure of their original weight and amplifying noise less than autoscaling [55]; it is the PNA standard for chemometric work and was used here. The solvent region (7.1–7.4 ppm), the reference region (−0.1 to 0.1 ppm) and, for capsule contents, the polyethylene glycol singlet are excluded before any model is built.

11.3 Unsupervised analysis of the public spectra

The sixteen public spectra were bucketed at 0.02 ppm over 0.5–6.0 ppm (275 buckets), normalized to total area, mean-centered and Pareto-scaled, and decomposed by singular value decomposition. With all sixteen spectra the first component (56.4 % of the variance) is an unsaturation axis dominated by the evening primrose oil, whose bis-allylic, allylic and olefinic buckets load positively against the methylene envelope,

and the second component (24.8 %) is the free-acid/glyceride axis; a single extreme reference sample thus dictates the model, a familiar lesson about outliers in small sets. With the two reference oils removed the fourteen retail products give a more informative model, shown in Figure 7, whose first three components explain 43.3, 26.7 and 14.5 % of the variance. The first component loads negatively on the 1.27 ppm envelope bucket, the 2.35 ppm free-acid α-methylene bucket, the 0.89 ppm methyl bucket and the 1.65 ppm β-methylene bucket, and positively on the 2.31 ppm glyceride α-methylene bucket, the 2.01–2.05 ppm allylic buckets, the 5.33 ppm olefinic bucket and the glyceryl buckets; it is the glyceride-bound-fraction axis of Section 8, and it orders the products exactly as Table 7 does, with NR and NP at the free-acid end and GNC, NA and Nat at the glyceride end. The second component loads negatively on the 1.25 ppm bucket and the 0.87 ppm methyl bucket and positively on the 1.31–1.37 ppm buckets and the allylic and bis-allylic buckets; it describes the shape of the methylene envelope, narrow and centered at 1.25–1.26 ppm for lauric-dominated chain pools and broadened toward 1.3 ppm for long unsaturated chains, and it separates ET and MDB, the two glyceride-rich products whose fatty-acid pattern is lauric-type, from NSI, the linoleic-rich product. Average-linkage hierarchical clustering on the correlation distance of the same matrix isolates only NSI and the evening primrose oil at the two-cluster level. The dataset labels do not separate in either model, which is unsurprising given that product NA, labeled pure, is spectroscopically a glyceride oil. The exercise shows that with fourteen samples of unknown provenance a principal component model can describe the chemistry, through loadings that are interpretable as the signals of Table 4, but can neither validate the labels nor support a classification, and that any supervised model built on such a set would be an exercise in fitting labels of unknown truth.

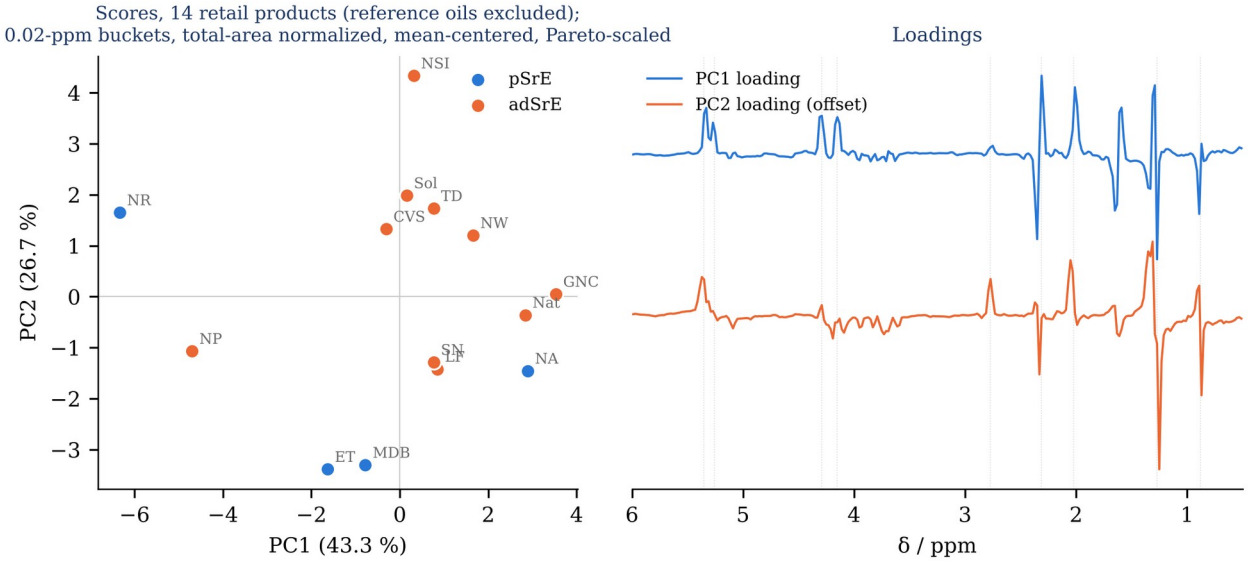

Figure 7. Principal component analysis of the fourteen retail-product spectra of the ChemoSpec SrE.NMR dataset (reference oils excluded): 0.02-ppm buckets over 0.5–6.0 ppm, total-area normalization, mean-centering, Pareto scaling. Left: scores on the first two components (43.3 and 26.7 % of the variance), blue for products labeled pure, orange for products labeled as containing added oil. Right: loadings of the first two components plotted against chemical shift (second component offset). The first component is the free-acid/glyceride axis; the second describes the shape of the methylene envelope. Data from [33].

11.4 Supervised and one-class models for authentication

When a documented reference set exists the natural temptation is a two-class discriminant model, authentic against adulterated, built by partial least squares discriminant analysis or its orthogonalized form, which removes from the spectral matrix the variation uncorrelated with class membership so that the predictive component is interpretable [56,57]. Two warnings apply. Westerhuis and co-workers showed that PLS-DA models on small, high-dimensional sets overfit readily, that the apparent separation in a score plot is not evidence of a real class difference, and that cross-model validation with permutation testing is needed before any classification rate is reported [58]. Rodionova, Titova and Pomerantsev argued, more fundamentally, that discriminant analysis is the wrong statistical form for authentication, because a discriminant model can only assign a sample to one of the classes it was trained on, and the population of adulterants is open-ended; an authentic saw palmetto model trained against coconut oil and olive oil says nothing useful about a sample adulterated with a designer blend or with an oil it has never seen [59]. The appropriate form is a one-class model, soft independent modeling of class analogy or its data-driven variant, in which the authentic reference set defines an acceptance region in the space of its own principal components and a test sample is accepted or rejected on its distance from that region, with the type I and type II errors set explicitly; Jakes and co-workers used exactly this form, an authentic region at $p = 0.001$, for a 60 MHz meat-authentication screen [50,59]. For saw palmetto the authentic set must span the three extraction types, which differ systematically (Section 11.1), either as three sub-models or as one model with extraction type as a known factor; it must span producers, harvest years and ripeness, since Section 3.1 shows that these move the oleic/lauric balance; and it should contain not fewer than thirty batches, which is what Perini and co-workers used and what a six-component model with reasonable confidence limits requires [5]. The bookkeeping indices of Section 8 may themselves be used as the variables of such a model, a six- or eight-variable set with direct chemical meaning, in place of or alongside the bucketed spectra; a model on indices is less sensitive to alignment and baseline artifacts and its acceptance region can be stated in chemical terms, which is the form in which an acceptance criterion can be written into a specification.

12. An authentication decision logic combining NMR with orthogonal methods

Table 13 sets each documented adulterant class against what the ¹H spectrum can show at 300 MHz and at 60 MHz and against the orthogonal method that settles the question, and the tiered logic that follows from it is set out here in prose. The first tier is documentary and organoleptic: the declared extraction type, the declared carriers and excipients, and the color and odor of the material, which the BAPP documents treat as the first line of defense and which determine how the NMR result is to be read, since a glyceride content that is damning for a hexane extract may be ordinary for a CO_2 extract or a declared softgel blend [4,10]. The second tier is the NMR screen, on the benchtop instrument or at 300 MHz in fingerprint mode: the glyceryl-to-α-methylene ratio, the allylic and bis-allylic ratios, the presence of a polyethylene glycol singlet, methyl-ester singlet or aldehyde signals, and a visual comparison of the α-methylene and glyceryl regions with the reference spectra of the same extraction type. A sample that is a triglyceride oil, or that contains tens of per cent of one, fails here and needs no further NMR work beyond quantification of the glyceride fraction for the report. The third tier, for samples that pass the screen, is the quantitative 300 MHz bookkeeping of Section 8 under relaxed conditions, with the methyl-check ratio reported, followed by comparison of the chain fractions, chain-length index, iodine value, sterol and fatty-alcohol indices and alkyl-ester indices with the acceptance windows of the reference set, and by the one-class membership test of Section 11.4 against the bucketed reference spectra; a ¹³C carbonyl spectrum is added whenever the glyceride fraction or

the ethyl-ester content is near a boundary, because the carbonyl region speciates the chains without the overlaps of the proton spectrum (Section 6.6). The fourth tier is orthogonal and is mandatory for any sample that passes the third, because passing the NMR tiers excludes triglyceride adulteration and gross profile deviation but does not exclude hydrolyzed lauric oils or designer free-fatty-acid blends: the compendial GC-FID fatty-acid ratios (or the GC/MS selected-ion-monitoring variant developed for supplements [60]), the AOAC GC determination of sterols and the GC determination of fatty alcohols, which designer blends lack, and stable-isotope ratio analysis, bulk $\delta^{18}O$ and $\delta^{2}H$ at a minimum and compound-specific $\delta^{13}C$ and $\delta^{2}H$ of the individual acids where the stakes justify it [1,5,7,32]. DNA barcoding has no role for the extract and is reserved for raw material [20]. The logic is deliberately asymmetric: NMR can condemn a sample on its own, but it cannot on its own certify one, and a laboratory report that rests an authenticity claim on ¹H NMR alone would not survive the Perini designer-blend precedent in a dispute.

Table 13. Adulterant classes documented for saw palmetto extract and the capability of ¹H NMR and of the orthogonal methods to reveal them

Adulterant class	Documented by	¹H NMR (300 MHz) evidence	Benchtop 60 MHz	Orthogonal method that settles the question
Long-chain triglyceride oils (olive, canola, sunflower, palm, peanut, soybean)	BAPP bulletin; Booker 2014; Gafner 2023	Glyceryl CH_2/CH signals, glyceride α-CH_2 at 2.31 ppm, oleic or linoleic excess, $\tilde{n}_s$ increase (palm); detectable at a few per cent	Glyceryl region integral (4.0–4.45 ppm) and olefinic/bis-allylic ratios; chain-length index unreliable	GC-FID fatty-acid ratios (USP); sterol profile
Lauric-type triglyceride oils (coconut, palm kernel, babassu)	Perini 2018; BAPP bulletin	Glyceryl signals and glyceride α-CH_2 (clear); fatty-acid profile nearly silent except lower oleic/linoleic	Glyceryl region integral	GC ratios (caprylic/capric excess); sterols; $\delta^{18}O/\delta^{2}H$
Hydrolyzed lauric-type oils (free-fatty-acid blends)	Inferred from the designer-blend reports; not separately documented	Only dilution of oleic/linoleic, sterols and fatty alcohols; no glyceride signature. Not reliably detectable alone	Not detectable	Isotope ratios ($\delta^{18}O$, $\delta^{2}H$, $\delta^{13}C$); sterol and fatty-alcohol content by GC
Designer free-fatty-acid blends matched to the USP ratios (animal-origin fatty acids)	Perini 2018; Perini 2019; BAPP bulletin v3	Spectrum indistinguishable from authentic extract in the major signals; absence of phytosterol H-18 and fatty-alcohol signals, and trans-olefinic/allylic signals (≥ 500 MHz) are the only leads	Not detectable	Bulk and compound-specific $\delta^{18}O$, $\delta^{2}H$, $\delta^{13}C$ (decisive); GC sterols (cholesterol vs phytosterols)
Whole or partial substitution with exhausted berry powder or related palms (raw material)	BAPP bulletin	Not applicable to the extract	—	DNA mini-barcode; microscopy
Excipient carry-over in dosage forms (PEG, glycerin, carrier oils, emulsifiers)	Booker 2014; Cartwright 2023	PEG singlet 3.64 ppm; glycerin and emulsifiers in the 3.5–4.3 ppm region; carrier oils as glycerides	PEG singlet visible	Label review; LC-MS

A quantitative addendum to the logic concerns the form in which NMR results are reported. The glyceride-bound fraction and the chain fractions are stoichiometric quantities with stated integration limits and a stated relaxation condition, and should be reported as such, with the methyl-check ratio, in the same way that an acid value or an iodine value is reported; they are then directly comparable between laboratories, which a principal component score is not. The membership statistic from the one-class model should be reported with its confidence level and with the identity and size of the reference set, and the extraction type assumed in the comparison should be stated, because, as De Combarieu and co-workers showed, the ethanolic extract is displaced from the hexane and CO_2 extracts in the fingerprint by a real, if small, compositional difference [3].

13. Proposed PNA protocol and reference-set plan

Table 14 sets out the acquisition and processing protocol proposed for the Varian Mercury-MVX 300 (299.99 MHz ¹H, 5 mm ATB probe) and for the 60 MHz benchtop instruments, in two modes: a fingerprint mode for the chemometric comparison and a quantitative mode for the bookkeeping, the latter conditioned on a measured T_1 set and verified by the methyl-check ratio. The quantities to be reported are listed in the last row of the table, and Table 15 gives the provisional acceptance windows derived from the literature compositions of Section 3, the compendial windows of Table 1 and the simulations of Section 9. The windows are provisional in the strict sense: they are the author's reading of what the literature implies an authentic lipophilic extract should show, and they are to be replaced, index by index, by the mean and tolerance interval measured on the reference set.

Table 14. Proposed PNA acquisition and processing protocol for saw palmetto extracts (to be validated on the reference set of Section 13)

Step	300 MHz (Varian Mercury-MVX 300, 5 mm ATB probe)	60 MHz benchtop (4IR AI-60 or Magritek Spinsolve)
Sample	40–60 mg extract (softgel fill or neat extract) in 0.7 mL $CDCl_3$ containing 0.03 % TMS; optional internal standard (e.g., 1,2,4,5-tetrachloro-3-nitrobenzene or dimethyl sulfone) for absolute quantitation; capsule shells and PEG matrices removed or recorded	100–150 mg in 0.7 mL $CDCl_3$; 5 mm tube; same internal standard
Temperature	298 K; equilibrate 5 min	Magnet temperature (typically 299–301 K); equilibrate 10 min
Pulse program and flip angle	Single-pulse, 30° flip angle (or 90° with adequate delay)	Single-pulse, 90°
Relaxation delay	Fingerprint mode: 2 s. Quantitative mode: acquisition time 4 s + d1 ≥ 20 s (90°) or ≥ 8 s (30°), set from measured T_1 of the olefinic, methyl and α-CH_2 protons	Quantitative mode: d1 ≥ 15 s; T_1 values at 1.4 T are shorter but must be measured
Scans and spectral width	64–128 scans (fingerprint) or 32 scans (quantitative); 16 ppm; 64 k points	32–256 scans; 20 ppm
Processing	Zero-fill to 128 k, exponential 0.3 Hz, automatic phase (checked), polynomial baseline through signal-free windows, TMS referencing	Exponential 0.5–1 Hz; same baseline strategy
Integration	Fixed limits of Table 6; additional 3.67 (OCH_3), 3.64 (CH_2OH), 0.68 (H-18) windows; ethyl esters by line-shape fit	Glyceryl region 3.95–4.45 ppm; α-CH_2 2.1–2.5 ppm as a block; olefinic 5.1–5.6 ppm
Chemometric export	0.02-ppm buckets 0.5–6.0 ppm after icoshift or correlation-optimized-warping alignment; total-area normalization; mean-centering; Pareto scaling	0.05-ppm buckets 0.5–6.0 ppm; same treatment
Reporting	f_gly, free-acid fraction, oleic/linoleic/saturated chain fractions, $\bar{n}_s$, iodine value, sterol H-18 index, fatty-alcohol index, ME/EE indices; membership statistics against the authentic set	f_gly and unsaturation ratios only

Table 15. Provisional NMR acceptance windows for lipophilic saw palmetto extracts, derived from the literature compositions and the simulations; to be replaced by values measured on the authentic reference set

Index	Provisional window (hexane / $scCO_2$ types)	Provisional window (ethanolic type)	Basis
Glyceride-bound chains, f_gly	≤ 20 %	≤ 30 %	Marti 2019 (TG 5–6 %; glycerides highest in $scCO_2$/ethanolic extracts); Habib & Wyllie 2004 glyceride range 7–52 % as the upper envelope of authentic variability
Oleic chains	27–37 % of chains	27–37 %	USP lauric/oleic 0.60–1.15 with lauric 27–33 %

Index	Provisional window (hexane / scCO$_2$ types)	Provisional window (ethanolic type)	Basis
Linoleic chains	3–7 % of chains	2–6 %	USP lauric/linoleic 5.0–16; De Combarieu 2015 (lower linoleic in ethanolic batches)
Saturated chains	56–66 % of chains	56–66 %	Balance of the profile
$\bar{n}_S$	12.3–13.4	12.3–13.4	Lauric-dominated pool; rises above 13.5 with palm oil or animal-fat triglycerides
Iodine value (NMR estimate)	38–52	38–52	USP 40–50
Sterol H-18 index	present; ≈ 0.1–0.3 % of the methyl integral (≈ 0.2–0.5 % w/w sterols)	as left	USP 0.2–0.5 %; public spectra
Fatty-alcohol CH_2OH triplet	present (0.15–0.35 % w/w) unless PEG present	weak (0.01–0.15 % w/w)	USP
Methyl-ester OCH_3	< 2 % of chains	< 2 %	Marti 2019 (≈ 2 % methyl + ethyl esters)
Ethyl-ester OCH_2	< 2 % of chains	2–6 %	Booker 2014; EMA assessment report

The reference set is the whole of the matter, and its design is proposed as follows. Authentic extracts of each of the three types should be obtained with documented provenance from not fewer than three primary extractors, in not fewer than thirty batches spanning at least two harvest years, with the NIST extract SRM 3251 included as an anchor for the sterol and carotenoid values even though its fatty-acid values have been withdrawn [25,26]. An adulterant library should be assembled from retail oils of certified type (coconut, palm kernel, babassu where obtainable, palm, olive, canola, sunflower, soybean and medium-chain-triglyceride oil), from rendered animal fats (lard, tallow, chicken fat), from oleochemical fatty acids (lauric, myristic, oleic, and a commercial coconut fatty-acid distillate), and from two materials prepared in the laboratory, a hydrolyzed coconut oil made by saponification and acidification, and a designer blend of free fatty acids formulated to the USP lauric-acid ratios. Gravimetric spike series at 2, 5, 10, 20, 30 and 50 % w/w of each adulterant into an authentic hexane extract and an authentic CO_2 extract would then provide the calibration data for the glyceride ratio, the unsaturation ratios and the chain-length index, and the sensitivity and specificity of the one-class model at each level. Every spectrum would be acquired in both modes at 300 MHz and in fingerprint mode at 60 MHz, with ^{13}C carbonyl spectra on the authentic set and on the 50 % spikes and ^{31}P phosphitylation spectra on a subset to establish the sterol and fatty-alcohol contents independently of the ^{1}H indices; GC fatty-acid, sterol and fatty-alcohol data on the authentic set would be obtained from the suppliers' certificates or commissioned, and bulk isotope ratios on a subset would be commissioned from a stable-isotope laboratory. The data would be assembled into the PNA X-matrix pipeline, modeled by the methods of Section 11, and reported as version 1.1 of this paper, in which the public and simulated figures are replaced by measured ones and the provisional windows by measured ones.

14. Critical assessment

The case for ^{1}H NMR in saw palmetto authentication is strong on one point and must be stated with its limits on every other. The strong point is speciation: the extract is a free-fatty-acid material and every oil and fat is a triglyceride material, the α-methylene shift and the glyceryl signals report the difference without derivatization, and a few per cent of any triglyceride adulterant moves the glyceride-bound fraction out of an authentic window that, on the literature, is narrow for hexane and CO_2 extracts. Nothing in the compendial GC method sees this, and the BAPP documents have already identified the triglyceride signals as the practical marker [6,10]. The limits are the following.

The literature base is thin and not independent. Of the three primary NMR studies, two come from the same industrial group working on its own products and its own reference collection, and the conclusion of phytochemical equivalence between extract types rests on twenty-six batches from one producer [3,5]; the third is a survey of retail products of unknown provenance in which extraction type was inferred after the fact [11]. No independent laboratory has published a reference set, no interlaboratory study exists, and the Laboratory Guidance Document's observation that the instrument and the reference library are beyond most quality-control laboratories remains true [10]. The public spectra used here are of retail products whose labels are demonstrably unreliable (a product labeled pure is a glyceride oil) and whose acquisition parameters are unknown, and the internal checks show them to have been acquired under non-quantitative conditions; the bookkeeping results on them illustrate the method and nothing more. The simulated spectra are first-order Lorentzian models with assumed compositions; they reproduce the stoichiometry of the problem but not the line shapes, the second-order effects at 60 MHz, or the real variability of the materials.

The natural variability of the glyceride content is the largest open question. If the 7–52 % glyceride range that the European assessment report quotes from Habib and Wyllie describes authentic extracts rather than the commercial market, the glyceride marker loses most of its power, because a 50 % glyceride extract and a 1:1 blend of extract with coconut oil would then be indistinguishable on that axis [9,21]. The LC-HRMS comparison of Marti and co-workers, which places triglycerides at 5–6 % in hexane and CO_2 extracts of a single fruit batch, and the pharmacopoeial description of the hexane extract as 92 % fatty acids free or esterified, suggest a narrower authentic range, but the point will be settled only by the reference set, and the provisional window of Table 15 should not be used in a report before then [2,8]. The two glyceride-rich products in the public set that carry a saw-palmetto-type fatty-acid profile, ET and MDB, are exactly the ambiguous case, and the ^{1}H spectrum cannot resolve it; a GC sterol and fatty-alcohol determination, which would be diluted by coconut oil and not by a glyceride-rich authentic extract, can.

The minor-constituent indices are at the edge of the method. The sterol H-18 singlet and the fatty-alcohol triplet are at 0.1–0.3 % of the methyl intensity, the H-18 index has a floor from the methyl triplet tails that must be subtracted or fitted, the fatty-alcohol triplet is lost in polyethylene glycol formulations, and the ethyl-ester quartet cannot be integrated at any field the laboratory will use; the ^{31}P phosphitylation method and the ^{13}C carbonyl region are the remedies, at the cost of derivatization or acquisition time, and GC remains the reference. The linolenic and linoleic estimates carry a known bias from envelope tails. None of these limitations is fatal, but each must be stated in a report.

The designer blend is the case that defines what NMR cannot do. Section 9 shows that a free-fatty-acid blend matched to the extract's profile is spectroscopically identical to the extract in every major signal, and Perini and co-workers found six such blends among nine commercial samples [5]. A laboratory that certifies authenticity on ^{1}H NMR alone is exposed to exactly this fraud, and an opposing expert would need no more than the Perini paper to make the point. The isotope ratios are decisive because the $\delta^{18}O$ of the authentic extract is extraordinary, far above that of any plant oil, and because $\delta^{13}C$ and $\delta^{2}H$ separate animal fat and oleochemical acids from the palm [5,7]; the honest position is that NMR condemns and isotope-ratio analysis and GC certify, and that a PNA offering should be structured as a screen and quantification service that refers the certification step out, or that partners with an isotope laboratory, rather than as a stand-alone authentication.

On the chemometric side, the published work and the demonstration here are unsupervised and descriptive. Principal component score plots on fourteen or twenty-six samples are exploratory, their apparent clusters are not classification results, and no saw palmetto NMR classifier with validated sensitivity and specificity exists in the literature. The correct statistical form is a one-class model on a documented authentic set, with

extraction type as a known factor, validated by cross-validation and permutation and reported with error rates [58,59]; building it is the work of version 1.1 and cannot be anticipated. Finally, compliance with an NMR window is not the same as authenticity, and non-compliance is not the same as fraud: an ethanolic extract legitimately carries more glycerides and ethyl esters than a hexane extract, a softgel may legitimately contain a declared carrier oil, and the method's output must always be read against the declared extraction type and formulation. With those qualifications, the method is ready to be built, and the measured study proposed in Section 13 is the next step.

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Summary

Saw palmetto extract is a free-fatty-acid lipid, roughly 85 % free acids with lauric and oleic acids at about 30 % each, a few per cent of glycerides and alkyl esters, 0.2–0.5 % phytosterols and 0.15–0.35 % long-chain fatty alcohols, specified by the pharmacopoeias through a total fatty-acid content, lauric-acid ratios and small sterol and alcohol windows. Its price, supply volatility and simple composition have attracted dilution with vegetable oils, substitution with lauric-type oils, and designer free-fatty-acid blends that pass the compendial GC ratios, six of nine commercial extracts in the only forensic study being such blends of animal-origin fatty acids. ¹H NMR in $CDCl_3$ sees what the GC method destroys: the α-methylene shift (2.345 ppm for free acids, 2.31 ppm for glycerides, 2.28–2.30 ppm for alkyl esters) and the glyceryl signals at 4.14, 4.29 and 5.26 ppm report the speciation of the chains directly, and a set of bookkeeping relations referenced to the α-methylene integral returns the glyceride-bound fraction, the oleic, linoleic and saturated chain fractions, the mean carbon number of the saturated pool, an iodine value and semi-quantitative sterol and fatty-alcohol indices from one spectrum. Applied to sixteen public ≈ 500 MHz spectra, the relations identify two retail products with the expected free-acid profile, a group of oleic-rich glyceride products consistent with olive-oil dilution, one linoleic-rich seed-oil blend and two lauric-type glyceride-rich products that the spectrum cannot resolve, and they expose, through the methyl-check and olefinic-consistency ratios, that the public spectra were not acquired under relaxed conditions. Simulations of twelve scenarios reproduce the stoichiometry, place the practical detection limit for a triglyceride adulterant at the natural variability of the authentic glyceride content (about 5–10 % w/w against a provisional 10 ± 5 % window), and show that hydrolyzed lauric oils and designer blends are spectroscopically silent in every major signal, so that stable-isotope ratio analysis and GC sterol and fatty-alcohol determination remain indispensable for certification. A 60 MHz benchtop screen retains the glyceryl ratio and the unsaturation ratios but loses the free-acid/glyceride α-methylene distinction, the sterol and alcohol indices and the chain-length index. The chemometric workflow of alignment, 0.02-ppm bucketing, chain-count normalization, mean-centering, Pareto scaling, principal component analysis and one-class membership testing is set out and demonstrated, with the warning that the published studies are unsupervised and that no validated classifier exists. A PNA protocol for the MVX300 and the benchtop instruments, provisional acceptance windows and a reference-set and spike-series plan are proposed for version 1.1, in which measured PNA spectra will replace the public and simulated data used here.