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Botanical Identification of Saw Palmetto Extract

Saw palmetto extract is a popular ingredient in the dietary supplements field used for normalizing prostate function and relieving lower urinary tract symptoms (e.g., inability to void urine) related to benign prostatic hyperplasia. The 2015 HerbalGram Herb Market Report ranked saw palmetto products among the 20 top-selling herbal supplements in both mainstream and natural retail outlets in the United States.[ref 1]

Unfortunately, due to the price increase of saw palmetto oil in the past five years, unscrupulous suppliers have taken advantage of these analytical challenges to pass other vegetable oils off as saw palmetto extracts entirely and/or to dilute saw palmetto extracts with these lower-cost vegetable oils.

Reports of the addition of undeclared vegetable oils (e.g., palm oil, canola oil, or coconut oil) to saw palmetto extracts for financial gain appeared in the early 2000s. Since these vegetable oils contain some of the same components as ripe saw palmetto berries, the detection of this type of adulteration is not always straightforward. ref 1 However, each oil product has a unique and distinctive fatty acids profile, and can be detected by Gas Chromatographic (GC) method. The fatty acids profile in saw palmetto extract can be used as one of the effective ways of identification. If the concentration of lauric acid in relation to the concentration of respective fatty acid falls into the range demonstrated in Table 3 in the attached testing method, then the product can be identified as saw palmetto extract.

This attached GC-FID detection method can be used to identify fatty acids composition of oil products. The fatty acids profile in saw palmetto extract is very unique and distinctive, thus it can be used as one of the effective ways of identification. This method can be used as qualitative identification as well as quantitative analysis.

Ref 1 http://www.naturalmedicinejournal.com/community-news/botanical-adulterants-program-publishes-bulletin-adulteration-saw-palmetto

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Botanical Identification of Saw Palmetto Extract by Fatty Acids using GC-FID

1. OBJECTIVE

Establish the identification method of saw palmetto extract by fatty acids profile.

2. STANDARDS

Methyl Caprylate，CAS:106-70-7

Methly Caproate，CAS:111-11-5

Methyl Caprate，CAS:110-42-9

Methyl Laurate，CAS:111-82-0

Methyl Myristate，CAS:124-10-7

Methyl Palmitate，CAS:112-39-0

Methyl Palmitoleate，CAS:112-25-8

Methyl Stearate，CAS:112-61-8

Methyl Oleate，CAS:112-62-9

Methyl Linoleate，CAS:112-63-0

Methyl Linolenate，CAS:301-00-8

Nonadecane，CAS:629-92-5

3. REAGENTS

4.1 Methanol (Chromatographic grade)

4.2 Hexanes (Analytical grade)

4.3 Concentrated Sulfuric Acid (Analytical grade)

4.4 Sodium chloride (Analytical grade)

4. EQUIPMENT

4.1 GC-FID

4.2 Analytical balance (accurately 0.01mg)

4.3 Volumetric glass： saponification flask, 50mL graduated flask

5. PREPARATION OF SOLUTIONS

5.1 Internal standard solution:

12mg/mL of nonadecane in hexanes

5.2 Standard stock solution:

Dissolve quantities of Methyl Caprylate, Methly Caproate, Methyl Caprate, Methyl Laurate，Methyl Myristate, Methyl Palmitate, Methyl Palmitoleate, Methyl Stearate, Methyl Oleate, Methyl Linoleate, and Methyl Linolenate in hexanes to obtain concentrations of each methyl ester as given in Table 1.

Table 1: Concentration of Each Methyl Ester

Methyl ester	Concentration (mg/mL)
Methyl Laurate	5
Methyl Oleate	5
Methyl Myristate	2
Methyl Palmitate	2
Methyl Linoleate	1
Methly Caproate	0.4
Methyl Caprylate	0.4
Methyl Caprate	0.4
Methyl Palmitoleate	0.4
Methyl Stearate	0.4
Methyl Linolenate	0.4

5.3 Standard solution

Transfer 1.0mL of the *Internal standard solution* to 5mL of the *Standard stock solution*.

5.4 Sample solution

Accurately weighed quantity of about 100mg sample to saponification flask，add 10.0mL of a solution of sulfuric acid in methanol (10 in 90)， reflux in water (oil) both for two hours，shaking from time to time. Cool the flask to room temperature. Add 1.0mL *Internal standard solution*，10mL water，2g sodium chloride and 5mL hexane. Shake well，allow the layers to separate completely，and use the hexanes layer.

6. CHROMATOGRAPHIC CONDITION

Column：HP-INNOWAX (30m×0.25mm×0.25μm)

Injector temperature：250℃

Detector temperature：300℃

Carrier gas flowing rate：1mL/min

Injection Volume: 1μL

Column temperature: Initially at 120℃, hold 3min, then to rise to 220℃ at the rate of 50℃ /min, hold 12 min.

7. SYSTEM SUITABILITY

7.1 Sample: *Standard solution*

See table 2 for the relative retention times.

Table 2: Relative retention time of each methyl ester

Methyl Ester	Relative Retention Time
Mehtyl caproate	0.39
Mehtyl caprylate	0.56
Mehtyl caprate	0.76
Mehtyl laurate	0.94
Nonadecane	1.0
Mehtyl myristate	1.1
Mehtyl palmitate	1.3
Mehtyl palmitoleate	1.35
Mehtyl stearate	1.65
Mehtyl oleate	1.7
Mehtyl linoleate	1.8
Mehtyl linolenate	2.0

7.2 Sample testing

Accurately inject the *Standard solution* for three times when the chromatographic system is stabilized under the condition. The RSD should be not more than 2.0%. Separately inject equal volumes of standard solution and sample solution to the chromatograph，record the chromatograms，and measure the peak responses of fatty acid esters. Calculate the percentage of each fatty acid in the portion of the Saw Palmetto taken by the formula:

$$content(\%) = 500(C/W)(Ru/Rs)(Ma/Me)$$

In which:

C—the concentration of the respective methyl ester in the standard slution(mg/mL)

W—the weight of the sample(mg)

Ru—the rations of the responses of the relevent methyl ester peak and the internal standard peak obtained from the sample solution

Rs—the rations of the responses of the relevent methyl ester peak and the internal standard peak obtained from the standard solution

Ma—molecular weight of the relevant fatty acid

Me—molecular weight of the methyl ester of the relevant fatty acid

8. IDENTIFICATION

The retention times of the 11 major peaks of the sample solution correspond to those in the chromatogram of the standard solution. The ranges for ratios of the concentration of lauric acid to the concentration of the respective fatty acid are in table 3:

Table 3: Ranges for ratios of the concentration of lauric acid to that of respective fatty acid.

Caproic	Caprylic	Capric	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic
8.5～24	8.5～17.5	9.0～16	2.2～2.8	2.8～3.9	14～26	0.60～1.65	5.0～16	31.5～55

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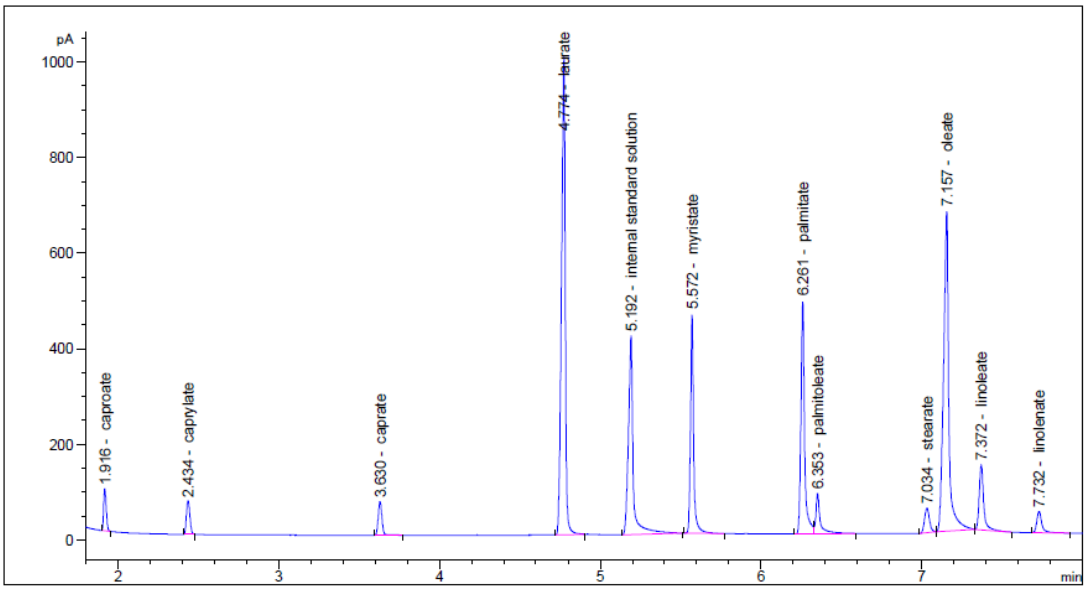
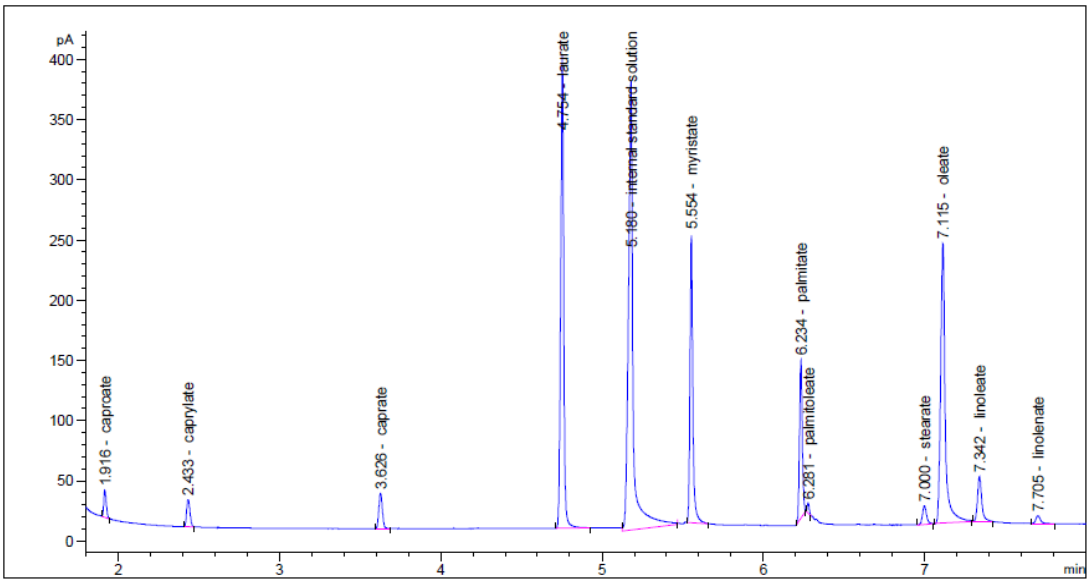
9. The chromatogram of fatty acids standards



10. The chromatogram of saw palmetto oil



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