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Short communication

Simultaneous quantitation of lauric acid and ethyl laureate in *Sabal serrulata* by capillary gas chromatography and derivatisation with trimethyl sulphoniumhydroxide

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Abstract

In order to standardise *Sabal serrulata* ethanolic extract preparations the free fatty acids and fatty acid ethyl esters were analysed by capillary gas chromatography. Lauric acid and ethyl laureate, constituting the major proportion, were used as marker substances and were simultaneously quantitated. The free fatty acids were selectively derivatised by "dry" trimethyl sulphoniumhydroxide. The method was successfully validated according to up-to-date guidelines.

1. Introduction

Sabal serrulata Rohm. [*Serenoa repens* (Batr.) Small] (Palmae) is a tropical palm tree the berries of which are used for the preparation of an ethanolic extract [1]. This extract is used in several countries for the treatment of benign prostrate hyperplasia and prostatitis. The major constituents of the ethanolic *Sabal* extract are free fatty acids (FFAs) and their corresponding ethyl esters (FAEs), triglycerides, fatty alcohols, phytosterols and some triterpenes [1]. Since the FFAs and FAEs have recently been found to be responsible for the main biochemical activity of *Sabal* extracts, i.e. inhibition of testosteron 5-α-reductase, it is important to quantitate them with a validated method, in order to standardise the extract preparation. Especially lauric, linoleic and linolenic acid inhibit the 5-α-reductase [2].

We developed a GC method with flame ionization detection (FID) to simultaneously quantitate the FFAs, derivatised to fatty acid methyl esters (FAMEs), and the ethyl esters present in the ethanolic extract. None of the currently applied derivatisation techniques could be easily applied (e.g. 5% H_2SO_4 in methanol, BF_3 methanol, . . .) [3], because they convert not only the FFAs but also the FAEs to FAMEs. However, derivatisation could be performed with trimethyl sulphoniumhydroxide (TMSH) as described below.

2. Experimental

2.1. Chemicals

All solvents were HPLC grade from Labscan (N.V. de Bournonville, Belgium). The free fatty acids were obtained from Sigma (Bornem, Belgium). Ethyl undecanoate (EtC_{11}) and undecanoic acid (C_{11}) were from Sigma (99+%

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capillary GC). Trimethyl sulphoniumhydroxide was obtained from Macherey-Nagel (0.2 *M* TMSH in methanol) (Filterservice, Eupen, Belgium). Extrelut 3 pre-packed columns for extraction were from Merck (Overijse, Belgium).

2.2. Instrumentation

A Hewlett-Packard 5890 GC–FID with HP GC Chemstation software was used for data acquisition and integration. A split injection technique was applied (split ratio, 1:50). A 50 m × 0.25 mm I.D., 0.39 mm O.D., 0.20 μm film thickness cyanopropyl column CP-Sil 88 (Chrompack, Antwerp, Belgium), was connected to a nonpolar retention gap by a quick seal connector. The following temperature programme was used: 6 min 80°C, in 24 min to 200°C, which was maintained for 6 min. The injector temperature was 250°C, the FID detector temperature was 270°C.

2.3. Sample and sample preparation

Sabal serrulata extract was obtained from Madaus s.a. (Köln, Germany). A 4-ml volume of *Sabal* extract was adjusted to pH 3 by addition of 0.1 *M* hydrochloric acid; 1 ml acetone–water (9:1) containing the internal standards was added, and diluted with water to 10 ml. A 3-ml volume of this sample solution was brought on an Extrelut column. Extrelut separates the substances according to their partition between the eluent, which has to be immiscible with water, and the solid Extrelut phase. After 20 min the FFAs and FAEs were eluted from the column with three 15-ml volumes of chloroform–hexane (8:2). The organic solvents were evaporated under vacuum. The residue was dissolved in 1 ml dichloromethane; 100 μl of this solution was derivatised with 100 μl TMSH of which the solvent was previously evaporated. A 1-μl aliquot of the latter solution was injected onto the GC system.

3. Results and discussion

3.1. Derivatisation with TMSH

TMSH was used as derivatising agent for FFA to FAME. The advantage of derivatisation with TMSH is that the reaction occurs almost immediately at room temperature or in the GC injector [4]. The commercially available TMSH is a 0.2 *M* solution in methanol which rapidly converts FFA into FAME. Depending on the amount of TMSH added to a solution of FAE, transesterification of FAE to FAME was observed. However, if the methanol solvent is evaporated under a nitrogen stream, the "dry" TMSH is still active as a methylation agent for FFA, but the formation of FAME from FAE does not take place. A typical chromatogram of the fatty acid containing fraction of *Sabal serrulata* is given in Fig. 1. The peaks appear in pairs: the first is the methyl ester, the second is the ethyl ester of the fatty acid.

3.2. Quantitation of lauric acid and ethyl laureate and method validation

Undecanoic acid and ethyl undecanoate were added to the sample as internal standards. We calculated the amount of methyl laureate (MeC12) and ethyl laureate (EtC12) using the relative response factor. The method used for quantitation of lauric acid and ethyl laureate was validated as described below.

Linearity

To examine the linearity six dichloromethane solutions containing the internal standards (C_{11} = 1.030 mg/ml; EtC_{11} = 1.128 mg/ml), lauric acid (0.409–2.570 mg/ml) and ethyl laureate (0.433–1.924 mg/ml), were injected twice a day. The calibration curve, area C_{12}/C_{11} vs. concentration C_{12}/C_{11}, was calculated according to the least squares method ($y = a + bx$). The results of the evaluation of the linearity are presented in Table 1.

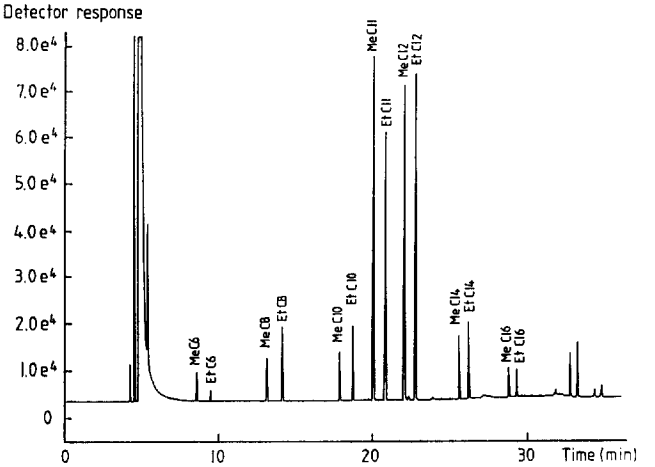

Fig. 1. Fatty acid containing fraction of *Sabal serrulata* [Me (methyl)/Et (ethyl) ester of C6: caproic acid, C8: caprilic acid, C10: capric acid, C11: undecanoic acid, C12: lauric acid, C14: myristic acid, C16: palmitic acid].

Table 1
Linearity

Parameter	MeC12/MeC11	EtC12/EtC11
$a \pm s_a$ [a]	0.0085 ± 0.0082	0.0059 ± 0.0051
$b \pm s_b$ [b]	0.9774 ± 0.0052	0.9798 ± 0.0045
r [c]	0.9999	0.9999
t_a [d]	$1.044 < 2.228$	$1.171 < 2.228$
t_b [e]	$189.805 > 2.228$	$218.693 > 2.228$
ANOVA[f]	NS	NS

[a] Standard error of a.
[b] Standard error of b.
[c] Correlation coefficient.
[d] Test on the significance of the slope.
[e] Test on the inclusion of the point (0,0).
[f] Analysis of variance according to Ref. [5].

Sensitivity and detection limit

The sensitivity is defined by the slope of the calibration curve [6]. The detection limit was calculated according to Kaiser [7]. The sensitivity and detection limit are given in Table 2.

Table 2
Sensitivity, detection limit

	MeC12	EtC12
Sensitivity	0.9774	0.9798
c_1(mg/ml)[a]	0.0501	0.0387

[a] Detection limit.

Precision

Lauric acid and ethyl laureate were determined in six different samples of *Sabal* extract. This was repeated on three different days to examine the intermediate precision [8] in addition to the repeatability [9]. A single factor (ANOVA, $\alpha = 0.05$) showed that there was no significant difference between the results on different days for MeC12 and EtC12. The results are summarised in Table 3.

Accuracy

Accuracy can be examined by comparison with another method, or by the standard addition method when dealing with plant preparations.

Table 3
Precision

Parameter	Me C12	Et C12
Conc. in Sabal extr. (mg/ml)	0.4942	0.7431
Repeatability (%)	2.05	4.26
Intermediate precision (%)	5.86	5.80
ANOVA[a]		
F	3.445	0.131
$F_{critical}$	3.682	3.739
p-value	0.059	0.878

[a] Analysis of variance according to Ref. [5].

Therefore, we preferred to determine the accuracy by the standard addition method. Known amounts of lauric acid (0.096–0.386 mg) and ethyl laureate (0.128–0.512 mg) were added to the sample solution at four different concentration levels in duplo. The slope of the least squares curve, i.e. amount found vs. amount added of the compound to be determined, expresses the recovery [10]. The recovery of MeC12 was 104.0%, the recovery of EtC12 was 101.9%. Undecanonic acid and ethyl undecanoate, express a similar behaviour in the sample clean-up stage and in the GC injector as lauric acid and ethyl laureate, which is reflected by the satisfactory recovery.

4. Conclusion

Free fatty acids can be derivatised to FAME, using "dry" TMSH, without conversion of the FAEs to FAMEs. The developed method can be used to quantitate in one single run lauric acid and ethyl laureate, which are used as marker substances for the preparation of *Sabal serrulata* ethanolic extracts. The method was thoroughly validated. The method is used to follow the concentration of lauric acid and ethyl laureate in stability studies.

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