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Short communication

Analytical procedure for the determination of 1-octacosanol in plasma by solvent extraction and capillary gas chromatography

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Abstract

A simple and rapid method for the determination of 1-octacosanol in plasma, based on an improved Folch extraction technique and capillary gas chromatography, has been developed taking into account the analytical criteria for the pharmacokinetic studies. The procedure was validated in the range nf 50–2000 ng/ml. Despite the complexity of the obtained fingerprints, the efficiency and the separation power of GC allowed the determination of 1-octacosanol in plasma samples. The high recoveries (94.5–98.7%) and precision (1.8–5.8%) obtained are in accordance with the established validation criteria. The validity of this method for pharmacokinetic purposes was shown using an endovenous experiment in animals.

Keywords: 1-Octacosanol

1. Introduction

1-Octacosanol is the main active component of policosanol, a natural mixture of high-molecular-mass alcohols isolated from sugar cane (*Saccharum officinarum* L.) wax, with defined and highly reproducible composition. It is a new natural agent, which has been proved to be effective in the treatment of Type II hypercholesterolemia [1,2].

Among the several analytical procedures that could be used for isolating high-molecular-mass alcohols from plasma, the solvent extraction technique is still a simple and rapid method of choice [3].

In the present work we introduce a methodological improvement of the classical Folch technique [4] for the quantitative isolation of 1-octacosanol in plasma samples. The assayed procedure uses a solvent system consisting of chloroform–methanol (2:1, v/v), that proved to be sufficient to carry out the denaturation of proteins and quantitatively isolate the alcohol being analysed.

In order to reduce possible losses during the manipulation, the developed technique utilizes a one-step withdrawal and pouring of the organic phase from the extraction vessel into a reaction-vial using a thin glass capillary (1.0 mm I.D.).

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The validated procedure, based on the use of a modified Folch technique and quantitative split–splitless capillary gas chromatography, was used for the determination of 1-octacosanol plasma concentrations in an endovenous pharmacokinetic study in animals.

2. Experimental

2.1. Chemicals

Standard solutions of 1-octacosanol (5.0, 50 and 100 μg/ml) and 1-hexacosanol (internal standard, 50 μg/ml) both having 99.0% GC purity (Jannsen-Chimica, Beerse, Belgium), were made up in glass-distilled acetone. Methanol and chloroform were glass distilled prior to their use in the deproteination–extraction process. All solvents were p.a. grade from E. Merck (Darmstadt, Germany). The derivatization reagent, N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), was obtained from Pierce (Rockford, USA).

2.2. Capillary gas chromatography analysis

A GC-14A gas chromatograph (Shimadzu, Japan), with a flame ionization detector (FID) and a split–splitless (SSL), Grob type, injector coupled to a C-R4A, computerized data processor (Shimadzu), was used in this work. The chromatographic separations were done in a fused-silica capillary column SPB-5 (30 m×0.25 mm I.D., 0.5 μm film thickness) from Supelco (Bellefonte, USA). The conditions used for GC analysis consisted of an injector temperature of 320°C, a detector temperature of 320°C, an oven temperature program which went from 150 to 320°C at 10°C/min and was then held for 60 min. The carrier gas (argon) flow-rate was 1.5 ml/min. The hydrogen and air flow-rates for FID were 40 and 400 ml/min, respectively. The SSL injector parameters consisted of a septum purge flow-rate of 5 ml/min and a split-vent flow-rate of 40 ml/min. The injection volume was 1–2 μl with the septum purge and split-vent closed for 30 s. The GC data was obtained by the internal standard method of analysis. The presence of 1-octacosanol in plasma samples from the endovenous assay was confirmed by means of gas chromatography–mass spectrometry (GC–MS) analysis.

2.3. Plasma sample procedure

During a pre-study validation performed in order to demonstrate the gas chromatographic selectivity, potential interferences from the endogenous constituents of rats, swine and Beagle dog plasma samples were not found. Therefore, drug-free Beagle dog plasma samples were chosen for the whole validation procedure.

A 1-ml plasma sample, placed into a rigorously cleaned glass tube (10 ml), was denatured by slowly adding 1 ml of methanol. Afterwards, the mixture was extracted with 2 ml of chloroform by vigorous shaking (5 min). After centrifugation (3000 *g*, 5 min), the organic layer was carefully withdrawn using a thin glass capillary and simultaneously poured into a reaction-vial (as described below). A fixed volume of 1-hexacosanol internal standard solution was added to the extract and the latter was evaporated to dryness at 60°C under nitrogen flow. Finally, the dry extract was derivatized with 50 μl of MSTFA at 60°C for 15 min. After reaching room temperature a 1- or 2-μl aliquot of derivatized sample was injected onto the GC capillary column.

2.4. Withdrawing and pouring of sample extract

In order to reduce possible losses of 1-octacosanol during manipulation of the sample extract, the latter was directly and simultaneously withdrawn from the extraction tube and poured into a reaction-vial (Fig. 1).

To carry out this procedure, the reaction-vial was coupled to the syringe by its needle, then the bent glass capillary was fixed, by its short-end, to it. Afterwards, the large end was carefully introduced into the extraction tube in such a way that it went up to the bottom of the chloroform layer whilst simultaneosly driving a slow air flow, generated by depressing the syringe plunger, through it. In this way, obstruction of the thin capillary by the protein pellet was completely avoided. Forthwith, the organic layer was withdrawn, through the capillary, by

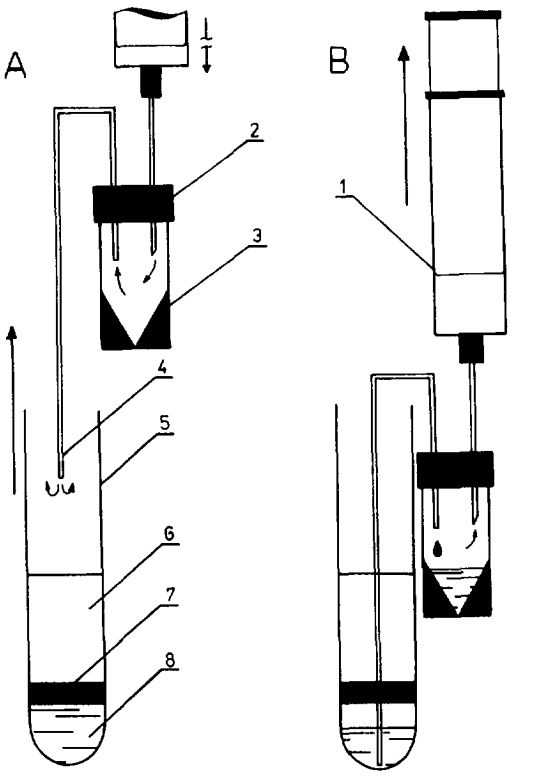

Fig. 1. Arrangement for withdrawing and pouring the organic layer from the extraction tube into the reaction-vial: 1 = hypodermic syringe; 2 = screw-cap with two holes and a silicone seal; 3 = a 5-ml reaction-vial; 4 = glass capillary; 5 = extraction tube; 6 = supernatant phase; 7 = protein pellet; 8 = chloroform layer. (A) The step used to introduce the glass capillary, through the supernatant and protein pellet, right up to the bottom of the organic layer. (B) The step of withdrawing the chloroform layer from the extraction tube and pouring it into the reaction-vial.

moving the syringe plunger up and pouring directly into the reaction-vial.

2.5. Parameters for method validation

For the purpose of carrying out the method validation, six plasma samples spiked with 1-octacosanol concentrations ranging between 50 and 2000 ng/ml (n = 5), were submitted to the procedure described in Section 2.4. Both the theoretical (actual) and the experimental (calculated) concentrations of 1-octacosanol were correlated in order to evaluate the linearity. The accuracy (expressed as the recoveries) of the method was evaluated by assaying plasma spiked with known concentrations of 1-octacosanol. The intra- and inter-run precision data of the assay were calculated by measuring plasma standards in the whole studied range on day one (n = 5) and on the three following days.

2.6. Application of the method

The method described above was used to determine the plasma concentration of 1-octacosanol following intravenous fast infusion of policosanol in rats, swine and Beagle dogs. The samples were taken before starting the infusion of policosanol in the range of 10–240 min.

3. Results and discussion

In principle, the different steps in sample preparation should be as simple as the analytical requirements permit. Taking into account the estimated plasma concentration level of 1-octacosanol and its high hydrophobicity, it may be possible to carry out its quantitative isolation using a few preparation steps and a water-immiscible solvent, such as chloroform [5].

One of the drawbacks found during the pre-study validation were the losses of the analysed alcohol during its withdrawal with Pasteur pipettes and the contamination of the organic extract with the protein pellet. In order to overcome these problems the chloroform layer was transferred by means of the arrangement shown in Fig. 1.

Considering the high molecular mass of 1-octacosanol, its very low abundance with regards to the other constituents in the GC-injected extract and the well-known SSL discrimination features [6], it was necessary to optimize the septum purge split-vent ratio, in order to obtain the most accurate relative response factor (RRF) value between 1-octacosanol and the internal standard, 1-hexacosanol. The oven and injection conditions used allowed us to determine an RRF value of about one (mean ± confidence levels = 1.064 ± 0.099, C.V. = 7.5%, n = 5) and an intra-run RRF of 0.915 ± 0.089, with a C.V. of 7.9% (n = 5). These reliable RRF values, and their corresponding confidence levels, ensure a proper GC sensitivity, necessary for the determi-

nation of the alcohol being analysed. A partial chromatogram from a spiked plasma sample showing the eluting zone of 1-octacosanol, at a concentration of 50.0 ng/ml, the internal standard 1-hexacosanol and cholesterol is shown in Fig. 2.

The one-step solvent extraction of 1-ml plasma samples with 2 ml of chloroform (5 min vigorous manual shaking), subsequent to denaturation with 1 ml of methanol, was found to achieve high recoveries of 1-octacosanol, with mean values ranging from 94.5 to 98.7% (see Table 1 and Table 2). The method was linear ($y = 0.968x - 0.004$) over the whole assayed concentration range of 50–2000 ng/ml ($r = 0.9992$). At a signal-to-noise ratio of 3:1, the limit of detection for spiked 1-octacosanol plasma samples was 10 ng/ml.

The accuracy, repeatability (intra-run precision) and reproducibility (inter-run precision) of the method were determined in the whole concentration range assayed and are summarized in Table 1 and Table 2.

The method validation results indicate that the performance characteristics of the procedure fulfilled the required considerations for pharmacokinetic studies rather well [7] and that it may be used for that purpose. The procedure was used to evaluate the plasma level of 1-octacosanol, following fast in-

Table 1
Recoveries (accuracy) and intra-run precision of the analytical method for the concentration range 50–2000 ng/ml ($n = 5$)

Concentration		Recovery (%)	Intra-run precision (%)
Spiked (ng/ml)	Found (mean ± S.D.)		
50	48.5 ± 2.4	97.0	4.9
100	98.2 ± 2.2	98.2	2.2
500	480.2 ± 12.0	96.0	2.4
1000	950.2 ± 30.0	95.0	3.1
1500	1480.5 ± 50.0	98.7	3.3
2000	1956.5 ± 32.0	97.8	1.6

Table 2
Recoveries (accuracy) and inter-run precision (over three days) of the analytical method for the concentration range 50–2000 ng/ml

Concentration		Recovery (%)	Inter-run precision (%)
Spiked (ng/ml)	Found (mean ± S.D.)		
50	49.3 ± 2.9	98.6	5.8
100	96.3 ± 2.4	96.3	2.4
500	477.0 ± 13.5	95.4	2.8
1000	945.0 ± 37.4	94.5	3.9
1500	1450.2 ± 56.0	96.9	3.8
2000	1937.5 ± 36.5	96.8	1.8

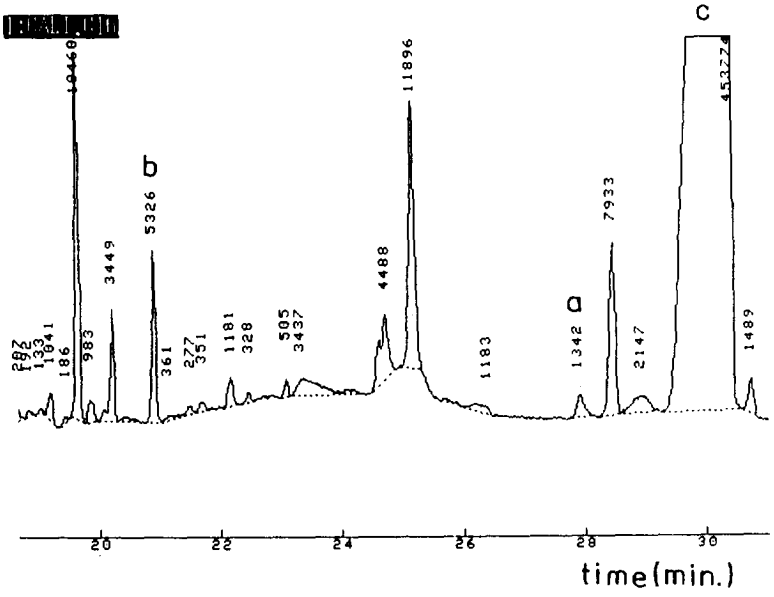

Fig. 2. Partial chromatogram of spiked plasma sample. a=the eluting zone of 1-octacosanol at a concentration of 50 ng/ml; b=the internal standard, 1-hexacosanol; c=cholesterol.

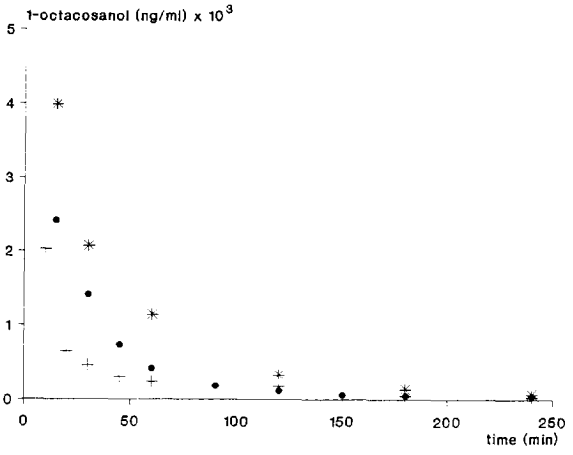

Fig. 3. Mean plasma concentrations of 1-octacosanol vs. time in Beagle dogs (●), swine (+) and rats (*) after an endovenous administration of policosanol (5 mg/kg).

travenous infusion of policosanol in animals. Fig. 3 shows the mean plasma 1-octacosanol concentrations versus time ($n = 4$), in Beagle dogs, swine and rats, respectively.

The results presented were part of an endovenous pharmacokinetic evaluation study [8], that clearly showed that the procedure offers a very simple, rapid and reliable way of carrying out this application.

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