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Short Communication

Extraction and liquid chromatographic method for the determination of simmondsin in plasma

M. Van Boven[a], P. Daenens[a,*], M. Cokelaere[b], E. Decuypere[c]

[a]*Katholieke Universiteit Leuven, Laboratory of Toxicology, Van Evenstraat 4, B-3000 Leuven, Belgium*
[b]*Katholieke Universiteit Leuven, Campus Kortrijk, Interdisciplinary Research Center, University Campus, B-8500 Kortrijk, Belgium*
[c]*Katholieke Universiteit Leuven, Laboratory for Physiology and Immunology of Domestic Animals, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium*

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Abstract

A solid-phase method for the extraction of simmondsin from plasma was developed along with a liquid chromatographic method for the quantitative measurement of this compound. The extraction of simmondsin is realised with activated carbon. Chromatography is performed on a 25 × 0.46 cm I.D. RP-18, 5-μm HPLC column with a water–methanol (85:15, v/v) mobile phase and ultraviolet absorbance detection at 217 nm. The limit of detection is 100 ng (using 1 ml of plasma). The linear quantitation range is 0.1–200 μg/ml.

1. Introduction

Simmondsin, 2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl-β-D-glucoside (Fig. 1), is the most important glucoside present in jojoba meal. Jojoba meal is obtained after removal of the oil present in the seeds of the jojoba plant (*Simmondsia chinensis*). The oil has important aesthetic and technical qualities that makes it a widespread basic cosmetic ingredient. Because of its economical value, the plant, native to the Sonora Desert (USA), has been cultivated in many arid and semi-arid countries all over the world. Due to its high protein content deoiled jojoba meal has been tried as animal feed. However the meal has been described as being toxic when used as a livestock feed ingredient [1,2]. The effects seem to be especially related to the inhibition of food intake by simmondsin and

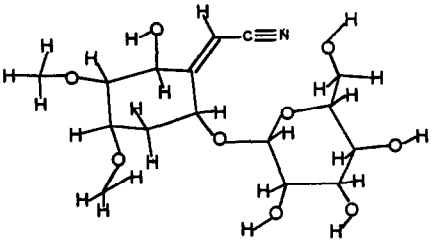

Fig. 1. Structure of simmondsin.

* Corresponding author.

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other related cyanomethylenecyclohexylglycosides [3,4]. The meal contains also simmondsin–ferulates [5,6] which possibly show similar effects as simmondsin. The exact mechanism of action is not yet quite clear. The pharmacological effect may be due to either a random action of simmondsin or to a local action of simmondsin in the gastrointestinal system. Some authors claim that the action is caused by the aglycon formed by enzymatic deconjugation in the gut [1,2,7]. Due to its hydrophillic character a rather poor resorption of simmondsin can be expected following oral intake. In order to study the resorption and elimination of simmondsin from blood following different forms of administration, including intravenous injection, we developed an analytical technique for the determination of this compound in plasma.

2. Experimental

2.1. Reagents and materials

Simmondsin used as reference was isolated from jojoba meal [8]. The internal standard, phenyl-β-D-galactopyranoside, was obtained from Aldrich (Bornem, Belgium). Acetonitrile (Merck, Darmstadt, Germany) was of HPLC grade. All other reagents were of analytical grade. Norit A Supra was obtained from Norit (Amersfoort, Netherlands). For solid-phase extraction (SPE) by bonded silicas the C_2 ethyl, C_{18} octadecyl, cyanopropyl (3 ml) and Certify™ (10 ml) columns were obtained from Analytical International (Harbor City, USA). XAD-2 and XAD-4 were obtained from Supelco Sercolab (Mechelen, Belgium). The Vac Elut vacuum system (Varian, Palo Alto, CA, USA) was used to draw samples through the columns. Ultrafiltration was performed with UFC4LTK25 filters (Millipore, Brussels, Belgium). Iso-disc N-252, 25-mm diameter nylon membrane filters, 0.2-μm particle size were obtained from Supelco Sercolab. Chicken plasma was used throughout this study and obtained from the animalarium of the KU Leuven. Samples were kept in the freezer at −20°C until used.

2.2. Apparatus and conditions

A Model L-6200 high-performance liquid chromatograph (Merck-Hitachi, Darmstadt, Germany) was used, equipped with a variable wavelength L-4250 UV-Vis detector (Merck-Hitachi) operated at 217 nm. The samples were applied by a Rheodyne Model 7125 (Berkeley, CA, USA) sample-loop injector with a volume of 200 μl. Separation was performed on a 250 × 46 mm I.D. RP-18 endcapped (5 μm) column using water–methanol (85:15, v/v) as eluent. The column was protected by a 4 × 4 mm I.D. RP-18 endcapped (5 μm) guard column (Merck). After injecting twenty biological extracts the guard column has to be replaced.

2.3. Preparation of standard solutions

A stock solution of simmondsin was prepared by dissolving 100 mg in methanol (100 ml). This solution was kept in the freezer (−20°C). Working solutions were prepared at 10 ng/μl and 100 ng/μl in methanol. Phenyl-β-D-galactopyranoside (100 mg) was dissolved in 100 ml of methanol; a dilution in water was prepared to obtain a working solution of 2 μg/ml. Plasma samples were spiked with simmondsin, dissolved in methanol 10 ng/μl and 100 ng/μl, to obtain concentrations of 0.1 to 8 μg/ml.

2.4. Deproteinization

To 1-ml volumes of plasma, 5 ml of acetonitrile were added. After vortex-mixing for 1 min, the mixtures were centrifuged at 2000 *g* for 10 min and the supernatants were transferred to conical 10-ml tubes. The solvent was evaporated under a stream of nitrogen and the residue reconstituted in 1 ml of water by sonication. The latter samples were used for further extraction as described below. Alternatively, the deproteinization procedure was done with methanol in the same way.

2.5. Extraction

To the 1 ml deproteinized spiked plasma samples (0.5, 2.0 and 8.0 μg/ml, see above), 1

ml of internal standard solution (2 μg/ml) and 1 ml of activated carbon suspension (2% in water) were added. After vortex-mixing the mixtures for one minute, the carbon was separated by centrifugation at 4000 *g* for 5 min. The supernatant was examined for the presence of non-absorbed simmondsin and internal standard. The isolated activated carbon fractions were rinsed twice with 2 ml of water. Each wash was submitted to HPLC analysis for simmondsin and internal standard. Finally, in separate experiments, simmondsin and internal standard were eluted from the activated carbon with 4 ml of one of the following solvents: ether, acetone, toluene, ethylacetate, isopropanol, methanol and acetonitrile. After centrifugation at 4000 *g* for 5 min, the organic solvent was filtered over a nylon membrane filter and evaporated under a stream of nitrogen. The residue was dissolved in 100 μl of HPLC mobile phase and a 25 μl aliquot was injected onto the HPLC system.

3. Results and discussion

3.1. Deproteinization

Neither the ultrafiltrates nor the reconstituted aqueous solutions from acetonitrile-deproteinized plasma samples containing simmondsin concentrations below 10 μg/ml were suitable for direct injection due to the many interfering peaks from endogenous compounds. In order to allow analysis of these samples, a preliminary extraction with activated carbon was necessary.

3.2. Extraction.

Isolation of simmondsin from plasma

Solvent–solvent extraction from spiked plasma samples did not result in any significant recovery of simmondsin due to the hydrophillic character of the molecule. Solid-phase extraction of simmondsin from plasma with C_2, C_{18}, cyanopropyl and Certify columns failed to extract simmondsin on a quantitative basis, or the compound eluted partially from the cartridges during the washing procedure. The extraction procedure with XAD-2 and XAD-4 failed to isolate simmondsin from deproteinized plasma on a quantitative basis; rinsing the columns with water resulted in the loss of part of the adsorbed simmondsin. The extracts obtained by direct extraction of blank plasma samples with activated carbon gave chromatograms with interfering peaks for both simmondsin and the internal standard. For that reason extractions with active carbon were done on deproteinized plasma samples. This procedure allows the extraction of simmondsin from aqueous solutions on a quantitative basis. Simmondsin was neither detected in the samples after the extraction nor in the water wash of the isolated carbon fractions. The described internal standard behaved in the same way as simmondsin.

Elution of simmondsin from activated carbon

Both simmondsin and internal standard could be recovered quantitatively from the activated carbon by washing with 4 ml of acetonitrile, acetone or *n*-propanol. In addition, when applied to deproteinized blank plasma samples, the use of acetone resulted in chromatograms without any interfering peaks from endogenous compounds.

3.3. Calibration

The above obtained results allowed to construct calibration graphs in the following way: to spiked plasma samples (1 ml) containing 0.1, 0.2, 0.5, 1.0, 2.0, 4.0 and 8.0 μg/ml of simmondsin, 1 ml of internal standard solution (2.0 μg/ml) was added along with 1 ml of activated carbon suspension (2% in water). After vortex-mixing the mixture for one minute, the carbon was separated by centrifugation at 4000 *g* for 5 min. The supernatant was discarded. The isolated activated carbon fraction was rinsed twice with 2 ml of water. Finally simmondsin was eluted from the activated carbon with 4 ml of acetone. After centrifugation at 4000 *g* for 5 min, the organic solvent was filtered over a nylon membrane filter and evaporated under a stream of nitrogen. The residue was dissolved in 100 μl of the eluent used for HPLC and 25 μl was injected. The ratios of the areas of simmondsin to the areas of the internal standard were plotted

against the concentration of simmondsin and linear regression analysis was applied to the calibration graphs obtained. The curves were linear ($y = 0.05 + 0.16 \times$, $r = 0.997$) and reproducible. The obtained chromatograms showed good separation between the standard and the internal standard. Typical chromatograms from a blank and spiked plasma sample along with a chromatogram of a mixture of simmondsin and internal standard are represented in Fig. 2. The method has successfully been applied in a kinetic study in chickens; no interference of metabolites or decomposition products were observed.

3.4. Reproducibility

The precision and accuracy of the assay (within-day analysis) for simmondsin was evaluated by assaying 5 replicates of plasma samples with three different concentrations (0.5, 2.0 and 8.0 μg/ml). The results are shown in Table 1. The relative standard deviation (R.S.D.) varied from 6.4 to 2.1% over the concentration range 0.5–8.0 μg/ml.

3.5. Recovery and sensitivity

The recovery of simmondsin by the described extraction procedure was determined for concentrations of 0.5, 2.0 and 8.0 μg/ml plasma, by comparing peak areas after extraction with peak areas of standard solutions. The recoveries from aqueous solutions were quantitative. The recoveries from plasma, using acetone as a solvent, were 73 ± 6% for all concentrations. The described method allows the detection of simmondsin in plasma down to 100 ng/ml. For concentrations higher than 10 μg/ml, the residues obtained after the acetonitrile deproteinization step and reconstitution in HPLC solvent can be injected without further extraction with activated carbon.

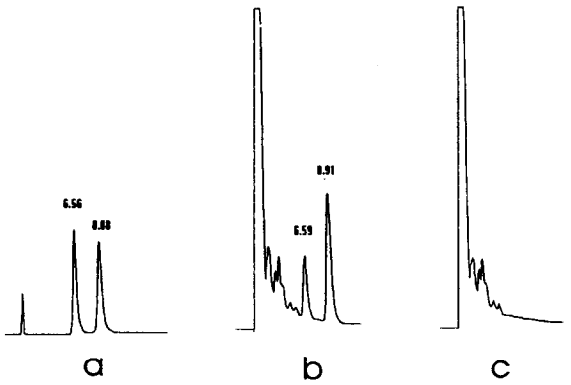

Fig. 2. (a) Chromatogram of simmondsin (2 μg/ml) and internal standard (2 μg/ml); t_R of simmondsin and of internal standard are 6.56 and 8.88 min, respectively. (b) Typical plasma extract containing simmondsin at 2.6 μg/ml. (c) Blank plasma chromatogram.

Table 1
Precision and accuracy of the assay ($n = 5$)

Concentration (μg/ml)		R.S.D.
Added	Found	(%)
0.5	0.55	6.4
2.0	1.9	5.8
8.0	7.9	2.1

4. Conclusions

A sensitive and selective method has been developed for the determination of simmondsin in plasma samples. This method allows detection down to 100 ng/ml and its applicability is being studied in animal experiments following different forms of administration of simmondsin.

5. Acknowledgement

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