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Liquid chromatographic–tandem mass spectrometric method for the plant lignan 7-hydroxymatairesinol and its potential metabolites in human plasma

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Abstract

A HPLC–MS–MS method was developed for the determination of the plant lignan 7-hydroxymatairesinol and its potential metabolites matairesinol, oxomatairesinol, α-conidendrin, 7-hydroxyenterolactone, enterodiol, and enterolactone in human plasma. The method included sample cleanup by solid-phase extraction (SPE) and analysis using a PE Sciex API3000 triple quadrupole mass spectrometer with electrospray ionisation. The lignans were quantified using two deuterated internal standards. They showed good chromatographic linearity, analysis repeatability, and SPE recovery in the presence of plasma. In pooled plasma and in plasma samples collected from two individual subjects lignan glucuronides and sulfates were enzymatically hydrolysed to free lignans and then analysed. All the lignans could be detected in the samples.

Keywords: 7-Hydroxyenterolactone; Mammalian lignans; Enterolactone; Enterodiol

1. Introduction

Lignans are defined as a group of phenolic compounds consisting of phenylpropane dimers linked together by β–β-bonds. Plant lignans are widely distributed in the plant kingdom as secondary plant metabolites. The plant lignan 7-hydroxymatairesinol (HMR) occurs in large amounts in knots of Norway spruce [1]. Also matairesinol (MR), α-conidendrin (CON) [1,2], and 7-oxomatairesinol (OMR) occur in Norway spruce [2] and in some other wood species. MR occurs also in considerable amounts in flaxseed, and in smaller amounts in other seeds, cereals and some berries [3]. Plant lignans are known to be converted to mammalian lignans by intestinal microflora [4]. Known metabolic transformations and possible metabolites of HMR are shown in Fig. 1. MR is known to be converted to the mammalian lignan enterolactone (ENL) both in vivo (in rats) [4,5] and in vitro [6]. Small amounts of the mammalian lignan enterodiol (END) are also formed [5]. Also HMR is converted to ENL and to small amounts of END both in vivo (rats) and in vitro [5–7]. ENL can also be formed from END [4]. 7-Hydroxyenterolactone (HEL) has been shown to be a metabolite of HMR in vitro [6]. MR, OMR, and CON are possible metabolites of HMR and possible

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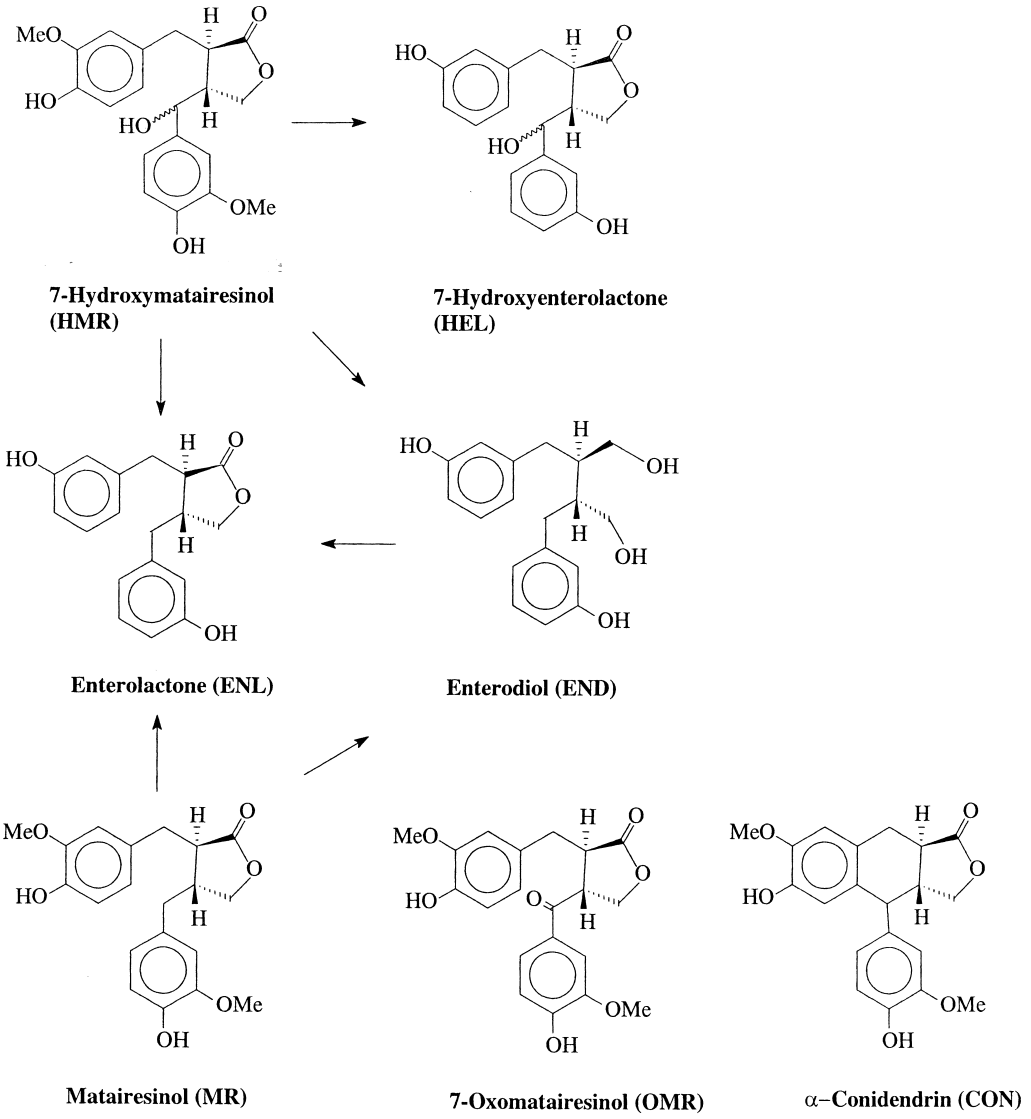

Fig. 1. Structures of analysed lignans. Known metabolic transformations and the possible HMR metabolites and/or ENL precursors 7-oxomatairesinol and α-conidendrin.

precursors to ENL and END; OMR is also a possible metabolite of MR. HEL is both a possible precursor and metabolite of ENL; hydroxylated enterolactones (hydroxylated at the aromatic rings) have been shown to be metabolites of ENL [8]. Many lignans possess interesting biological activities. HMR, END, and ENL are potent antioxidants in vitro [7,9,10]. Furthermore, HMR and ENL have shown chemopreventive properties in dimethylbenz[*a*]-anthracene (DMBA)-induced mammary tumours in rats [7,11–14]. In epidemiological studies, high serum ENL concentrations have been associated with a reduced risk for breast cancer [15–17]. Other population studies show that high serum ENL levels are associated with a reduced risk of coronary heart disease [18,19].

Lignans have previously been analysed in biological samples using, e.g., gas chromatography (GC) [20], high-performance liquid chromatography (HPLC)–UV and HPLC–mass spectrometry (MS)

[21,22], HPLC with coulometric electrode array detection [23,24], GC–MS [25], isotope dilution GC–MS in the selected ion monitoring (SIM) mode [26,27], and time-resolved fluoroimmunoassay (TR-FIA) [17,28,29]. Disadvantages associated with these methods are that the GC and HPLC–UV methods are neither sensitive nor selective, the GC–MS-SIM technique requires time-consuming cleanup, and the TR-FIA method is not very specific and allows determination of only a single compound at a time. HPLC–MS–MS requires no derivatisation and is highly selective and sensitive. A HPLC–MS–MS method using heated nebuliser atmospheric pressure chemical ionisation (HN-APCI) for the determination of isoflavones and some lignans (MR, END, and ENL) in human serum and urine, has been described previously [30]. The aim of this work was to modify and extend this previously developed method. The new method provides better sensitivity and comprises a larger number of lignans, i.e., the plant lignans HMR (two stereoisomers), MR, OMR, and CON, and the plant lignan metabolites HEL, END, and ENL.

2. Experimental

2.1. Equipment

Preparative HPLC separations were carried out with a Merck–Hitachi L-6200 Intelligent HPLC system equipped with an Applied Biosystems 783A UV detector and a Merck–Hitachi D-2500 chromato-integrator. The wavelength of the UV detector was set to 285 nm. The semipreparative reversed-phase (RP) HPLC column used was a 300×7.8 mm Waters Prep Nova-Pak HR C_{18} column with a particle diameter of 6 μm and a pore diameter of 60 Å. The nuclear magnetic resonance (NMR) determinations were conducted using a 500 MHz Jeol NMR spectrometer. The HPLC–MS–MS analyses were conducted with a PE Sciex API3000 triple quadrupole mass spectrometer equipped with an atmospheric pressure ionisation (API) source and a turbo ion spray interface. The analysis data was collected and analysed and the statistics calculated using PE Sciex Analyst software version 1.1.

2.2. Preparation of standards

HMR was isolated as a mixture of two stereoisomers from wood chips of Norway spruce (*Picea abies*) by modification of a method described by Freudenberg and Knof [2]. The purity was over 90%. The two isomers, which differ in the stereochemistry at C-7, HMR1 [(−)-*allo*-HMR)] (minor isomer) and HMR2 [(−)-HMR] (major isomer) with a HMR1/HMR2 ratio of about 5/95 were separated from each other and purified by preparative RP-HPLC. The HMR mixture was first dissolved in methanol–water (30:70, v/v) and filtered and then injected repeatedly in 50–100 μl aliquots to the semipreparative HPLC every 10–12 min. Fractions containing HMR1 and HMR2 were collected in separate vials. Resulting solutions were evaporated to dryness, dissolved in methanol–water (30:70, v/v) and repurified by HPLC in a similar manner. After the second purification cycle HMR1 and HMR2 solutions were evaporated to dryness. Samples were stored at −20 °C or colder. Separation and purification was achieved using an isocratic eluent system. The eluent used was ethanol–water (17:83, v/v) in the first separation and purification cycle, and ethanol–water (18:82, v/v) in the second purification for HMR2 and ethanol–water (19, 81, v/v) for HMR1. The flow-rate was 3 ml/min.

From Fluka (Switzerland), ENL (used in the analyses) and END were purchased. MR was prepared from HMR as described by Freudenberg and Knof [2] at the Laboratory of Industrial Chemistry at Åbo Akademi University (Turku, Finland). CON was prepared from HMR at Hormos Medical Corp. (Oulu, Finland). OMR, HEL, the internal standards d_6-MR and d_6-ENL, and ENL for preparation of d_6-ENL were prepared in our laboratory. HEL, which was a mixture of two isomers differing in stereochemistry at C-7, was prepared by modification of a method described by Mäkelä et al. [31]. The compound d_6-MR (deuterated at positions C-2, C-2′,C-5, C-5′, C-6, and C-6′) was prepared according to Adlercreutz et al. [25] and d_6-ENL (deuterated at positions C-2, C-2′, C-4, C-4′, C-6, and C-6′) according to Wähälä et al. [32]. ENL was prepared by modification of methods described by Kirk et al. [33] and van Oeveren et al. [34]. The method for preparation of OMR will be published elsewhere.

The compounds were purified using flash chromatography (OMR, HEL, and ENL) or preparative thin-layer chromatography (TLC) (d_6-MR and d_6-ENL).

The purity of the reference compounds was determined in solutions of deuterated acetone by quantitative ^{1}H-NMR using 1,1,2,2-tetrachloroethane (>99.8% purity, Fluka) dissolved in $CDCl_3$ as internal standard. The purities were found to be the following: MR 87% (mean value, n=4, RSD=6.1%); CON 96% (n=3, RSD=4.9%); OMR 79% (n=2); HEL 77%; d_6-MR 91%; d_6-ENL 54%.

2.3. HPLC–MS–MS analyses

The HPLC–MS–MS (electrospray ionization, ESI) analyses were conducted according to a modification of a previously described method [5]. The eluents used were methanol–0.1% HAc–isopropanol (90:9.9:0.1, v/v) (A) and 0.1% HAc–isopropanol (99:1, v/v) (B). A 16 min gradient from 22 to 73% A (corresponding to 20–66% methanol) was used. The final composition was held for 1 min. The total analysis time was 22 min. HPLC-grade methanol and isopropanol and 99–100% acetic acid were purchased from J.T. Baker. The water used in the eluent was purified as described previously [5]. The injection volume was 30 μl. The parent and daughter ion combinations and individual potentials used in the multiple reaction monitoring (MRM) method are shown in Table 1. The dwell times used were 300 ms for HMR and 150 ms for the other compounds.

2.4. Limits of detection (LODs) and linear range

The LOD and the linear range of a compound was determined by injecting solutions of varying concentrations of the pure compound dissolved in methanol–0.1% HAc (10:90, v/v). The LOD was defined as the concentration at which the signal-to-noise (S/N) ratio was about 3. As for HMR, the experiment was done with HMR1 only (the detector responses of HMR1 and HMR2 are similar). The lower limit of the linear range was determined by injection of four solutions at concentrations starting from about 3–4-times the LOD. The upper limit of the linear range was determined by injection of a 10–50 μg/ml solution and three dilutions of this (10×, 100×, and 1000×).

2.5. Standard solutions

The reference compounds were dissolved in methanol at known concentrations. A stock solution was prepared containing the following amounts (ng/ml) of the compounds dissolved in methanol: HMR1 and HMR2 1000, OMR 500, CON 1400, HEL 460,

Table 1
Exact mass, parent and daughter ion combinations, and individual potentials used in LC–MS–MS (MRM) detection of the lignans

Compound	Exact mass	Q1 mass (parent ion)	Q3 mass (daughter ion)	DP[a]	FP[b]	CE[c]	CXP[d]
HMR1 and 2	374.1359	373.00	355.15	−61	−210	−24	−23
HEL	314.1149	313.02	146.92	−51	−170	−30	−9.0
OMR	372.1203	371.03	108.14	−41	−140	−60	−5.0
CON	356.1254	354.97	339.89	−51	−190	−28	−23
MR	358.1410	356.98	82.96	−46	−160	−42	−1.0
END	302.1512	301.02	252.87	−51	−190	−32	−17
ENL	298.1200	297.06	252.96	−46	−160	−28	−15
d_6-MR	364.1788	363.03	83.12	−51	−160	−40	−3.0
d_6-ENL	304.1578	303.10	258.77	−56	−180	−30	−17
MUG	352.0789	350.99	174.84	−26	−90	−34	−11
MUS	294.0980 (K-salt)	254.83 (acid form)	174.88	−31	−130	−24	−11

[a] DP=Declustering potential.
[b] FP=Focusing potential.
[c] CE=Collision energy.
[d] CXP=Collision cell exit potential.

MR and END 600, and ENL 144. Following dilutions of the standard stock solution were made: 1:5, 1:29, 1:119, 1:479, and 1:1439 (stock solution–methanol, v/v). These dilutions were used for standard additions when accuracy and linearity of the method was tested. For the quantification of lignans in study samples the following dilutions were made: 1:5, 1:29, 1:119, 1:239, and 1:479 (stock solution–methanol, v/v). The concentrations in the most diluted standard sample were (ng/ml): HMR1 and HMR2 2.1, OMR 1.0, CON 2.9, HEL 0.96, MR and END 1.25, and ENL 0.30, which were the quantitation limits of the compounds. The standard solutions were stored at −20 °C.

2.6. Plasma samples

Pooled human plasma donated by the Finnish Red Cross was used for the preparation of plasma blanks, calibration standards, and quality control (QC) samples. The pooled plasma was also used for quantification of lignans, i.e., for determination of basal lignan levels from the general population. In addition, for quantification of lignans, two individual blood samples were collected from two healthy male volunteers. The plasma samples were stored at −20 °C.

2.7. Materials for sample pretreatment

As solvents and in the solid-phase extraction, methanol, acetone (both analytical-reagent grade; Merck), acetic acid (99–100%, J.T. Baker), and distilled water were used. The chemicals used in the enzymatic hydrolysis, i.e., 4-methylumbelliferyl sulfate (MUS) (potassium salt), 4-methylumbelliferyl-β-D-glucuronide (MUG), and β-glucuronidase type H-1, from *Helix pomatia* (β-glucuronidase activity 367 500 units/g, sulfatase activity >10 000 units/g,) were purchased from Sigma. The plasma samples were filtered using 0.8 μm and 0.45 μm Acrodisc syringe filters (Pall Gelman Laboratory, Ann Arbor, MI, USA) and extracted with Oasis HLB 30-mg (Waters, Milford, MA, USA) extraction cartridges by means of a Visiprep vacuum manifold (Supelco, Bellefonte, PA, USA).

2.8. Sample pretreatment

The analytical scheme for quantification of lignans in plasma is shown in Fig. 2. First, a 50-μl methanol solution containing 110–120 ng each of MUS and MUG, which were added as indicators of successful hydrolysis (deconjugation standards), was evaporated to dryness under nitrogen gas at 50 °C in a 6-ml test tube. Then 0.3 ml of a homogenised plasma sample, 1.5 ml 0.1% HAc, and 1 mg of β-glucuronidase/sulfatase freshly dissolved in 0.5 ml 0.1% HAc was added. The samples were incubated at 37 °C in a shaking water bath overnight (approx. 16 h), after which they were centrifuged (10 min, 4000 *g*) and filtered using first a 0.8 μm and then a 0.45 μm syringe filter to efficiently remove all disturbing particles in the plasma. The filtrate was allowed to

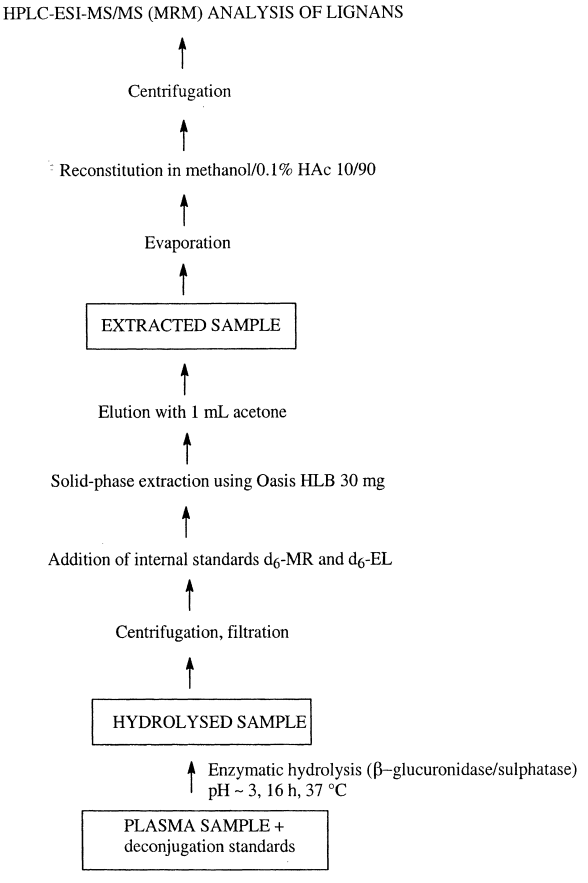

Fig. 2. Analytical scheme for the analysis of lignans in plasma.

drop into test tubes containing the internal standards (323 ng of d_6-MR and 12.5 ng of d_6-ENL). The samples were then subjected to SPE. The extraction column was activated with 1.0 ml of methanol and 1.0 ml of water. The sample (plasma solution) was subjected to the column, washed with 1.0 ml 0.1% HAc and eluted with 1.0 ml of acetone. Finally the samples in acetone were evaporated to dryness with nitrogen at 50 °C. The samples were reconstituted in 200 μl methanol–0.1% HAc (10:90, v/v), transferred to glass vials, and centrifuged at 4000 *g* for 20 min. The samples were stored in a freezer (−20 °C) until LC–MS–MS analysis.

For preparation of QC samples and standards for the calibration curve, 200 μl of each standard solution and the same amount of internal standards as above (dissolved in 50 μl methanol) was transferred to 6-ml test tubes. The solvent was evaporated to dryness under nitrogen gas at 50 °C. Pooled plasma (0.3 ml) and 0.1% HAc (1.7 ml) were mixed and then centrifuged and filtered as described above. The plasma solution was transferred to the 6-ml test tube containing the standards and mixed well. The samples were then subjected to SPE as described above.

2.9. Quantitation

For END and ENL the internal standard was d_6-ENL, while for the other compounds the internal standard was d_6-MR. The QC samples consisted of two parallel samples each of: (1) the stock standard solution (high-concentration level, 144–1000 ng/ml); (2) the 1:29 dilution (middle-concentration level, 4.8–33 ng/ml); (3) the 1:479 dilution of the stock solution (low-concentration level, 0.3–2.1 ng/ml). The calculated concentration of a lignan in the study samples was considered as reliable when it was within 20% of the theoretical concentration (accuracy 80–120%) in four of the six QC samples and if at least one sample on each QC level was within this limit. The absence of MUS and MUG peaks was an indication of successful enzymatic hydrolysis.

2.10. Validation

The linearity of the calibration curve was determined in three parallel samples which were analysed twice. The extraction recovery was determined by comparing the linearity of the calibration curve of an analyte in the presence of plasma with the linearity in the absence of plasma. The analysis repeatability was determined in six parallel plasma samples spiked with: (1) the stock standard solution; (2) the 1:29 dilution, and (3) the 1:1439 dilution of the stock solution (0.10–0.97 ng/ml).

In order to check whether the enzymatic hydrolysis possibly could have any effect on the linearity, analysis repeatability or extraction recovery of this type of compounds, a set of unhydrolysed and hydrolysed plasma samples were spiked with d_6-ENL, and 323 ng of d_6-MR was added as internal standard. The effect of hydrolysis was determined by comparing the linearity of the calibration curve of the analyte in hydrolysed plasma samples with the linearity in unhydrolysed samples. The analysis repeatability was determined as in the other validation experiment.

3. Results

3.1. LODs, linear range, and validation

The LODs and linear range of the lignans in the absence of plasma are presented in Table 2. The linearity of the calibration curve of the lignans is represented by the *k* (slope) and *r* (linear regression coefficient) values. These values are presented in Table 3 both in the presence and absence of plasma. The SPE recoveries expressed as the ratio between *k* in the presence and absence of matrix are presented

Table 2
LODs and linear range of the lignans in the absence of plasma

Compound	LOD		Linear range (ng/ml)
	pg	pg/ml	
HMR1	8.0	267	0.60–6500
HEL isomer 2	0.27	9.0	0.20–4000
OMR	0.37	12	0.30–3000
CON	0.37	12	0.20–4700
MR	0.63	21	0.67–4600
END	0.08	2.7	0.20–4800
ENL	0.004	0.13	0.00067–15 000

Table 3
Calibration curve slope and linear regression coefficient of the lignans in the presence and absence of plasma

Compound	k[a], mean		% SPE recovery	RSD (%)[b] of k		r[c], mean	
	Plasma			Plasma		Plasma	
	Abs.[d]	Pres.[e]		Abs.	Pres.	Abs.	Pres.
HMR2	0.729	0.474	65	6.2	17	0.9935	0.9946
HMR1	0.850	0.590	69	5.8	18	0.9934	0.9960
HEL, both isomers	2.94	2.98	101	7.9	18	0.9860	0.9943
OMR	0.762	0.644	84	6.7	17	0.9925	0.9899
CON	0.781	0.655	84	8.0	16	0.9896	0.9938
MR	0.583	0.522	89	4.6	15	0.9950	0.9917
END	0.0338	0.0292	86	3.7	6.5	0.9945	0.9981
ENL	0.0868	0.0870	100	3.8	6.7	0.9915	0.9895

The mean values were calculated from three parallel samples analysed in duplicate.
[a] k=Calibration curve slope.
[b] RSD=Relative standard deviation.
[c] r=Linear regression coefficient of the calibration curve.
[d] In the absence of plasma.
[e] In the presence of plasma.

in Table 3. Table 4 shows analysis repeatabilities of the lignans in the presence of plasma.

The experiment for checking the effect of enzymatic hydrolysis using d_6-ENL as model compound showed that hydrolysis had a negligible effect on the linearities, analysis repeatabilities or extraction recoveries. The calibration curve linearities and the r values were very similar in unhydrolysed and in hydrolysed plasma samples. The mean k value of hydrolysed samples was 98% of the value of the unhydrolysed samples, and the r value was 0.99 of both. Also the repeatability of hydrolysed samples was good, the RSDs being <10% at all concentration levels.

Table 4
Analysis repeatability of the lignans in the presence of plasma, RSD (%) of six parallel samples except if otherwise noted

Compound	Concentration level		
	Low	Middle	High
HMR2	19.7	10.3	9.9
HMR1	6.6[a]	12.2	8.1
HEL, both isomers	14.9	15.0	6.1
OMR	7.1[a]	11.7	8.4
CON	14.4[a]	15.8	7.3
MR	7.9[a]	13.3	7.9
END	17.3	16.4	15.7
ENL	19.6[a]	10.5[a]	9.0

[a] Five parallel samples.

3.2. Selectivity and accuracy of the method

Fig. 3 shows extracted ion MRM chromatograms of the lignans in a low-concentration QC plasma sample (1:1439 dilution of the stock standard solution, concentration range 0.10–0.97 ng/ml). Fig. 4 shows extracted ion MRM chromatograms of the lignans in a spiked high-concentration pooled plasma sample. The relative retention times of the analytes were stable throughout the experiment. They were (relative to d_6-MR): HMR2 0.67, HMR1 0.71, HEL, isomer 1 0.84, HEL, isomer 2 0.88, OMR 0.90, CON 0.92, MR 1.01, END 1.07, d_6-ENL 1.11, and ENL 1.12. The enzymatic hydrolysis seemed to be successful as no MUS or MUG peaks could be detected in the hydrolysed samples.

3.3. Lignans in plasma

In pooled unhydrolysed plasma all the analysed lignans except HMR and OMR could be detected, but the amounts were below the quantitation limit. When the pooled plasma was enzymatically hydrolysed, quantifiable amounts of END and ENL could

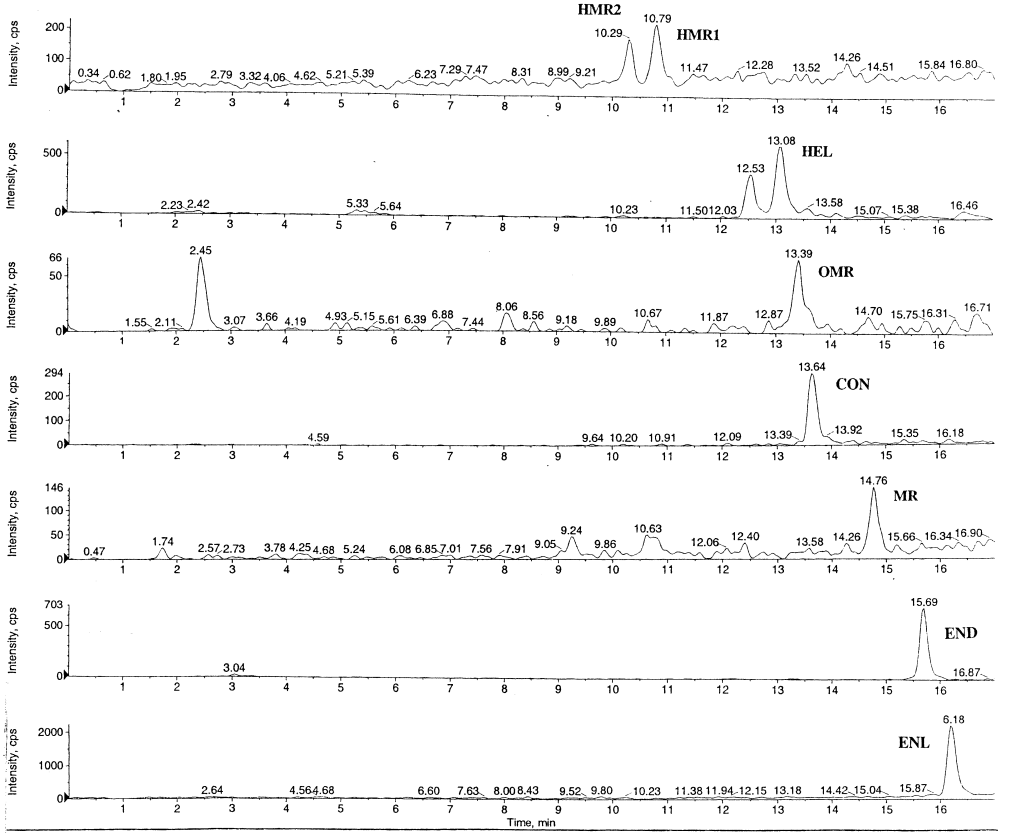

Fig. 3. Typical extracted ion MRM chromatograms of the lignans in a spiked low-concentration plasma sample (1:1439 dilution).

be detected. The average concentration of END was 1.9 ng/ml (6.3±0.91 n*M*, *n*=6) and of ENL 9.0 ng/ml (30±6.7 n*M*, *n*=5).

As in the pooled plasma, all the lignans could be detected in the blood collected from the two individuals. Fig. 5 shows extracted ion MRM chromatograms of the lignans in the sample of one of the subjects. The HMRs, ENL, and MR could be detected in quantifiable amounts in the blood of both individuals. The concentration of the HMRs was 9.0 and 15.4 ng/ml (24 and 41 n*M*), the HMR2 isomer showing a higher concentration than HMR1 (HMR2/HMR1 ratio 76/24 in both individuals). The concentration of MR was 1.3 and 1.8 ng/ml (3.6 and 5.0 n*M*). END could be quantified in the blood of one of the subjects; the concentration was 1.8 ng/ml (6.0 n*M*). The concentration of ENL was 14.8 and 6.5 ng/ml (50 and 22 n*M*).

4. Discussion

4.1. LODs, linear range, and sensitivity

Table 2 shows that the LODs are low compared to those achieved in previously published methods, especially that of ENL (4 fg/injection). E.g., using HPLC with coulometric array detection, the LODs of MR, END, and ENL were 6.5, 5.8, and 6.2 pg/injection, respectively [24], and the TR-FIA method required an amount of 2.1 pg ENL [28], which means an approximately 500-times lower sensitivity than with our method. The LODs obtained in this work were also much lower (e.g., for ENL 1000 times lower) than those obtained (with matrix present) in the previously published HPLC–MS–MS method [30]. Matrix only cannot explain differences of that size. The differences are also due to the

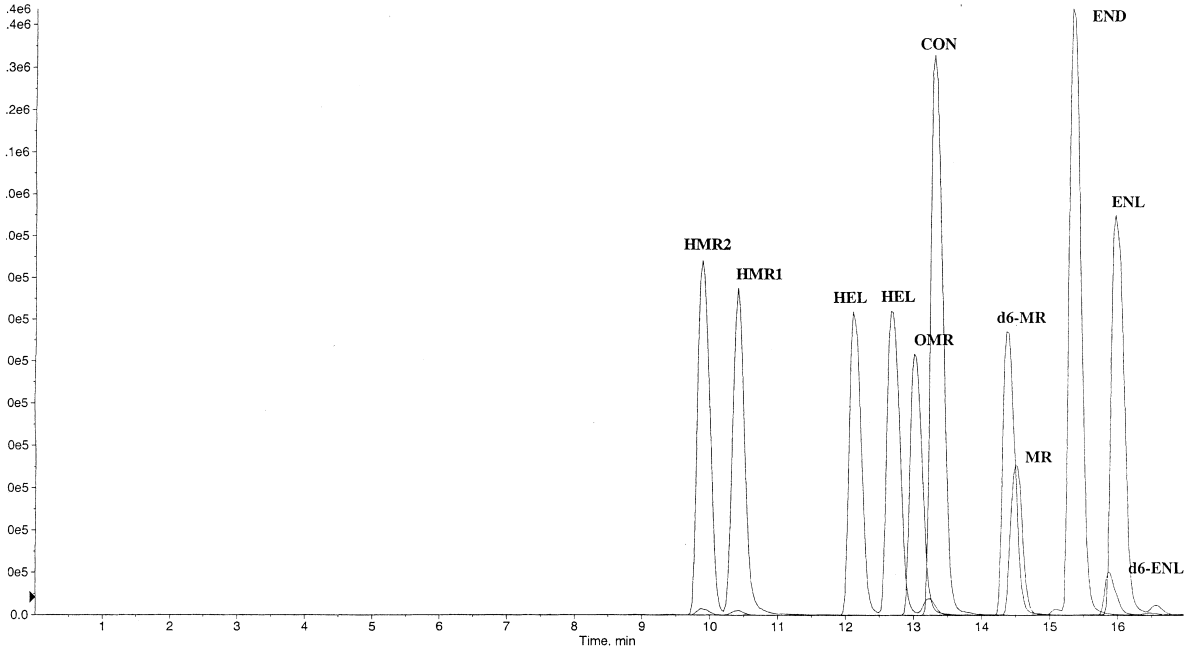

Fig. 4. Typical extracted ion MRM chromatogram of a spiked high-concentration plasma sample.

instrument model and the ionisation method. We noticed that electrospray ionisation gave better sensitivity for lignans than HN-APCI. In this work LODs and linear ranges were not determined in the presence of plasma because of interfering lignan background in the plasma blank. This will be discussed below.

4.2. Validation

Table 3 shows that the presence of plasma usually caused a lowering of the calibration curve slope, which reflects SPE loss. All the other compounds except the HMRs showed good or acceptable SPE recoveries. The HMRs were the most polar compounds and it is possible that they may partly be eluted in the wash step. As expected, the variation of k is bigger in the presence of plasma, but still acceptable (RSD$<$20%). In the absence of plasma, all the compounds show a very small k variation in the six parallels; the RSD being lower than 10%. The calibration curve linearities in the presence of plasma were good ($r \geq 0.99$). All the compounds showed an acceptable analysis repeatability (RSD$<$20%) at all the concentration levels (Table 4). As expected, most of the compounds showed a better repeatability at the highest concentration level than at the low- and middle-concentration levels.

4.3. Selectivity and accuracy of the method

The selectivity of the method is demonstrated by the absence of disturbing peaks in the vicinity of the analytes and generally very small peaks besides the analyte peaks (Fig. 3). The concentrations of the analytes in this sample was at a level of 2–14-times higher (for ENL 450-times higher) than the lower limit of the linear range (determined in methanol–0.1% HAc, see Table 2).

4.4. Lignan background

Water used in the eluent seemed to contain traces of lignans no matter which purification system had been used. The lignans seemed to accumulate in the HPLC column during the column equilibration time and to elute when the gradient increased. This caused big peaks especially of ENL in the solvent blank.

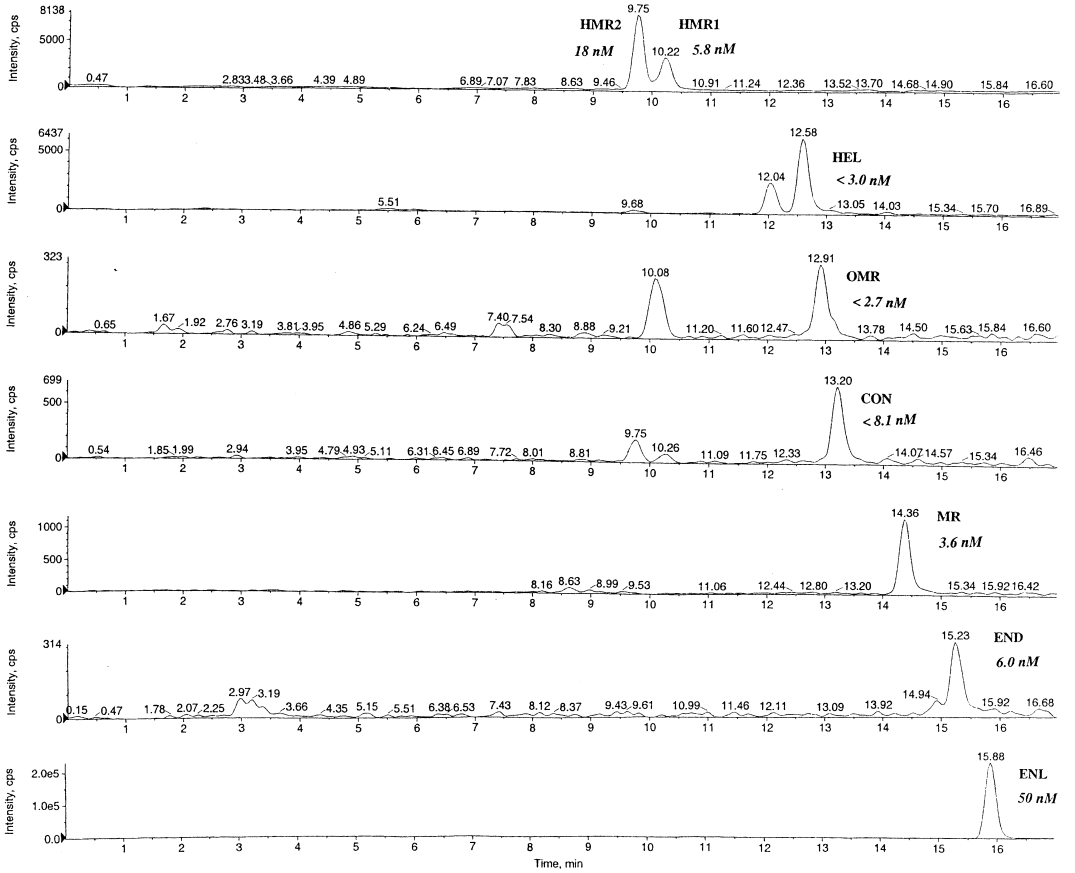

Fig. 5. Extracted ion MRM chromatograms of the lignans in one of the individual hydrolysed plasma samples.

Because of this the water used in the eluent had to be further purified using RP-18 material. The procedure has been described previously [5]. The reagent blank was lignan free. The plasma blank contained lignans which were detectable even without hydrolysis. The possibility to find lignan-free plasma blanks is probably quite small.

4.5. Lignans in hydrolysed plasma

The increased concentration of lignans after hydrolysis of plasma is expected, as lignans have been shown to be almost exclusively in conjugated form (as glucuronides and sulfates) in human serum and urine [25,27,35]. To our knowledge, no investigations have been published previously on the detection of HMR, HEL, CON, and OMR in human body fluids. HEL has been analysed in human urine, but the compound could not be detected [8].

The high background of lignans in hydrolysed plasma samples made it impossible to use hydrolysed plasma as matrix in the standard and QC samples. Real study samples, on the other hand, should preferably be hydrolysed in order to determine the lignans as quantitatively as possible. However, as pointed out before, our results indicate that hydrolysis of plasma does not affect the chromatographic linearity, SPE recovery, or analysis repeatability of the lignans.

The measured ENL concentrations in the blood collected from the two individuals (50 and 22 n*M*)

fall within the average ENL serum level of Finnish men, 0–95.6 n*M* (n=1168) determined by Kilkkinen et al. [29].

5. Conclusions

The described method makes it possible to determine a large number of lignans, i.e., five plant lignans and three mammalian lignans, at low concentration levels in human plasma. Several lignans could be detected in human plasma which, to our knowledge, have not been detected in human body fluids before, i.e., 7-hydroxymatairesinol, α-conidendrin, 7-hydroxyenterolactone, and 7-oxomatairesinol. The method requires no time-consuming cleanup steps and provides improved sensitivity compared to previously published methods.

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