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Journal of Chromatography B, 789 (2003) 115–130
JOURNAL OF CHROMATOGRAPHY B
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Kinetics of kavain and its metabolites after oral application

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Abstract

Kavain metabolism in humans was the target of this current investigation. In the present study a high-performance liquid chromatographic (HPLC–DAD) assay method for the simultaneous determination of kavain and its main metabolites (*p*-hydroxykavain, *p*-hydroxy-5,6-dehydrokavain and *p*-hydroxy-7,8-dihydrokavain) in serum and urine was developed and validated. The metabolites were mainly excreted in the form of their conjugates. All kavain metabolites were detectable in serum and urine, except for *p*-hydroxy-7,8-dihydrokavain, which was found in urine only. Confirmation of the results and identification of the metabolites were performed by LC–MS or LC–MS–MS. Kinetics of kavain and its metabolites in serum were investigated after administration of a single oral dose (800 mg kavain). Within 1 and 4 h after uptake, the serum concentrations ranged between 40 and 10 ng/ml for kavain, 300 and 125 ng/ml for *p*-hydroxykavain, 90 and 40 ng/ml for *o*-desmethyl-hydroxy-5,6-dehydrokavain, and 50 and 30 ng/ml for 5,6-dehydrokavain.

Keywords: Kinetics; Kavain

1. Introduction

Kava extract is a drug used widely in the South Pacific islands. Kavain was originally isolated in the form of its (L)-isomer from the roots of *Piper methysticum* (kava plant) [1]. Kavain, a lactone related to α-pyrone, is synthetically formed as racemate.

Extracts of the root and stem of this shrub, called kava-kava, contain only the L-form. Kavain has distinct anxiolytic, analgesic and muscle relaxation effects [2–5]. Many studies have been done on the clinical and therapeutic effects of kava in different medical areas such as cardiology, gastroenterology, gynaecology and neurology, especially on the interaction between kava and alcohol [4,6–12].

The kava plant contains a complex mixture of substances. However its chemistry and pharmacology have been thoroughly studied [13–19]. The physiological effects of the individual constituents (pyrones and alkaloids) are incompletely understood. Acute kava usage leads to reversible anaesthesia of the mouth and skin, euphoria, sedation, muscle weakness, ataxia and eventually intoxication [20–25].

Previous studies on the pharmacokinetics and pharmacodynamics of the kava extract as well as on kavain showed a maximum plasma concentration of ~18 ng/ml after an oral dose of 200 mg D,L-kavain. The initial resorption time of orally applied kavain in human subjects was ~15 min. The peak plasma concentration is reached in ~1.8 h. Kavain con-

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1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00046-1

centration in blood decreases with a half-life of ~9 h, and the distribution phase lasts 3–5 h [2].

The metabolism of urinary excreted kavain has been studied in rats after intravenous and oral administration [27]. Some of the kavain urinary metabolites were detected and identified as *p*-hydroxybenzoic acid, hippuric acid, 4-hydroxy-6-phenyl-5-hexen-2-one, 4-hydroxy-6-hydroxyphenyl-5-hexen-2-one, and 12-hydroxy-dihydrokavain [26–28]. 12-Hydroxykavain has also been detected in plasma. It has an elimination half-life of 29 h [2]. Due to the lack of sufficient information about the human metabolism of kavain, we performed drug metabolism studies using human Hep-G2 liver cell cultures [29,30] and human individuals. The metabolism of the widely-used phytopharmaceutical kavain performed by the human liver cell-line Hep-G2 results in 13 kavain metabolites [31].

The pharmacokinetics and pharmacodynamics studies in humans were carried out by means of experiments involving self medication. Different oral doses of kavain were administered, and blood and urine samples were collected. The aim of the analyses was to reveal kavain metabolism and kinetics in humans. Furthermore the results of kavain metabolism in humans have to be compared with the results of the in vitro study of the Hep-G2 cells' metabolism [31]. The human samples have been investigated using enzymatic hydrolysis because most of the detected metabolites are conjugated. The pharmacokinetics of kavain (unchanged drug) and its main metabolite *p*-hydroxykavain are discussed.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

All compounds were of analytical grade: methanol, ethanol, acetonitrile, ammonium chloride, dichlormethane, disodium hydrogen phosphate, potassium dihydrogenphosphate, sodium sulfate anhydrous, sodium hydroxide, formic acid, ammonium formate, orthophosphoric acid, and water for chromatography were obtained from E. Merck (Darmstadt, Germany), diethylether (p.a) from Fluka (Neu-Ulm, Germany). D,L-Kavain, *p*-hydroxy-5,6-dehydrokavain, *p*-hydroxykavain, *p*-hydroxy-7,8 dihydrokavain, and 6-phenyl-5-hexen-2,4-dion were provided by Klinge Pharm (Munich, Germany). Brotizolam was obtained from Promochem (Wesel, Germany). β-Glucuronidase and sulfatase from *Helix pomatia* (EC 3.2.1.31, Type H-1) was from Sigma (Deisenhofen, Germany). β-Glucuronidase from *Escherichia coli* (EC 3.2.1.31, Type K-12) was from Roche Diagnostics (Mannheim, Germany).

2.1.2. Preparation of standards

Stock solutions of the reference substances kavain (M-0), *p*-hydroxykavain (M-II), *p*-hydroxy-7,8-dihydrokavain (M-III), *p*-hydroxy-5,6-dehydrokavain (M-IV), and 6-phenyl-5-hexen-2,4-dion (M-V) were prepared in methanol in concentrations of 0.1% (1 mg/ml) and stored at 4 °C.

2.1.3. Internal standards

Brotizolam in methanol 0.01% (100 ng/μl) was used as internal standard for the HPLC and LC–MS analysis.

2.1.4. Buffers

Buffers and solutions were as follows: buffer, pH 9: 1.78 g of disodium hydrogen phosphate (Na_2HPO_4) were dissolved in 100 ml water; buffer, pH 5.5: saturated sodium dihydrogen phosphate (NaH_2PO_4) in water; buffer, pH 7: prepared by mixing 60 ml solution A with 40 ml solution B; solution A: 1.19 g of disodium hydrogen phosphate (Na_2HPO_4) were dissolved in 100 ml water; solution B: 0.91 g of potassium dihydrogen phosphate (KH_2PO_4) were dissolved in 100 ml water.

2.1.5. Biological samples

Blood, serum and urine samples were collected before and after the oral uptake of 800 mg kavain; blood and serum samples have been taken at time intervals of 0.25–4 h (Fig. 10). Urine samples were collected up to 24 h after drug administration. Aliquots of the urine samples were separated and stored at −20 °C. The remaining urine samples were mixed with 24-h urine (Table 6).

This experimental study is based on two self medication trials: kavain was administered in a single oral dose of 6.9 and 7.7 mg/kg.

2.2. Instruments

2.2.1. High performance liquid chromatography (HPLC–DAD)

The analyses were performed with a Waters 2690 separations module with a Water 996 PDA Detector. Separation was carried out with a reversed-phase LiChrospher 60, RP-select B column 250 mm×4.0 mm I.D., particle size 5 μm (Merck, Darmstadt) with isocratic conditions (31% acetonitrile/69% phosphate buffer (w/w), pH 2.3) at a flow rate of 1 ml/min; column temperature was set to 27 °C. Chromatograms were recorded at 190–420 nm with a resolution of 1.2 nm. The reference wavelengths for kavain, *p*-hydroxykavain, *p*-hydroxy-7,8-dihydrokavain and *p*-hydroxy-5,6-dehydrokavain were set to 246, 262, 226 and 226 nm, respectively.

2.2.2. Liquid chromatography–mass spectrometry (LC–MS)

The following instrumentation was used: an API 365 triple-quadrupole mass spectrometer from Applied-Biosystems/Sciex (Langen, Germany) and a Pentium PC with Analyst 1.1 software. Gradient elution was achieved using two LC10AD pumps (Shimadzu, Duisburg, Germany) and a reversed-phase column (polar-RP hexyl-propyl, polar end-capped), 2 mm I.D.×150 mm, 3-μm particle size (Phenomenex, Aschaffenburg, Germany) with a C_{18} (2)-guard cartridge (2 mm I.D.×4 mm) (Phenomenex).

This HPLC system was coupled without split to the API 365 using a Turbo IonSpray™ source with heated nitrogen (3 l/min, 400 °C) as turbo-gas (for drying of the spray). Deionized water (<0.1 μS from a cartridge-deionizer; Memtech, Moorenweis, Germany), gradient grade acetonitrile, 25% aqueous ammonia and formic acid (analytical grade, Merck) were used as HPLC solvents or for dissolving drug standards. For the HPLC, the following gradient was used with solvent A (10 m*M* ammonium formate, 0.1% formic acid, pH 3) and solvent B (acetonitrile/water, 95:5, v/v; containing 0.1% formic acid and 1 m*M* ammonium formate) using a total flow rate of 0.25 ml/min: 0–1 min: 5% B; 1–5 min: 5–30% B linear; 5–15 min: 30–70% B linear; 15–19 min: 70–95% B linear; 19–22 min: 95% B; 22–24 min: 95–5% B linear; 24–28 min: 5% B (for equilibration). For the ESI/CID-spectra of reference compounds a RP-C18 column was used (XTerra C18, 100×2 mm, 3.5 μm particle size) with the same solvent B (acetonitrile). Ionization was performed using a needle voltage of 5250 V (positive mode). Aliquots (10 μl) of urine extract (dissolved in HPLC-solvents A:B, 80:20, v/v) were injected. For the acquisition of reference spectra, 200 ng of the reference compounds (kavain and four metabolites) had been used previously with the API 365 in the single-quadrupole mode (Q1-scan) with a scan-range of 50–550 amu using a looped experiment with orifice-voltage switching (20, 50 and 80 V) between each scan, a dwell-time of 0.2 ms and a step-size of 0.1 amu [32]. This caused a total scan-time for the looped experiment of 6 s. Mass resolution had been set to 0.7+0.1 amu (peak width at half height) using polypropyleneglycol for mass axis calibration.

2.3. HPLC method validation

Determination of kavain and its metabolites was carried out by HPLC–DAD. The analytical procedure was proofed and validated for the kinetics study. The UV spectra for kavain and the known metabolites were compared with available reference substances. The HPLC results for kavain were confirmed with LC–MS and/or LC–MS–MS. The following validation categories were discussed for the HPLC–DAD method.

2.3.1. Linearity

Detector response linearity studies were performed by preparing six duplicate calibrations covering the range of 5–100 ng/ml for kavain (M-0) and 50–600 ng/ml for *p*-hydroxykavain (M-II), *p*-hydroxy-7,8-dihydrokavain (M-III) and *p*-hydroxy-5,6-dehydrokavain (M-IV). Linear regression lines were obtained by plotting the peak area ratios: target peak areas divided by internal standard peak areas.

2.3.2. Precision

In order to evaluate the repeatability (within-day precision) and reproducibility (between-days precision) of the method, replicate analyses (n=6) of plasma spiked with kavain and *p*-hydroxykavain (main metabolite) at concentrations of 5 and 40, and 50 and 500 ng/ml, respectively, were carried out.

Table 1
Within-day precision ($n=6$)

Substance	Suspected amount (ng/ml)	Mean detected amount (ng/ml)	SD (±)	C.V. (%)	Mean recovery (%)
D,L-Kavain	5	4.85	0.37	7.69	97
	40	36.27	0.48	1.31	91
p-Hydroxykavain	50	48	0.84	1.74	96
	500	480	10.5	2.2	95

2.3.3. Recovery

The absolute recoveries of the kavain and *p*-hydroxykavain were determined by comparing the peak areas of spiked plasma samples and reference samples. The reference samples were injected directly into the HPLC.

2.3.4. Limit of detection

The limit of detection (LOD) for kavain was determined as the lowest concentration giving a response. LOD was 1 ng/ml serum in spiked serum sample for kavain (signal-to-noise ratio of 3). The limit of quantitation (lowest concentration of the calibration curve) was 5 ng/ml in spiked serum.

2.4. Sample preparation and extraction procedure

Samples (1 ml) of blood, serum and urine were mixed with 100 μl buffer (pH 7) and 200 U β-glucuronidase from *E. coli*. The samples were incubated for 5 min at room temperature.

Samples (1 ml) of blood, serum and urine were mixed with 100 μl buffer (pH 5.5) and 200 U β-glucuronidase and sulfatase from *H. pomatia*. The samples were incubated in a water bath at 45 °C for 24 h.

The hydrolysed samples were mixed with 10 μl (0.01% brotizolam) as internal standard and 2 ml buffer (pH 9). The samples were extracted with 3 ml of dichlormethane:diethylether (7:3, v/v) [33], vortexed for 5 min and centrifuged at 3000 rpm for 10 min. The aqueous layer was discarded. The organic phase was transferred into a high recovery glass vial and evaporated to dryness (N_2 stream). The extract residues were reconstituted in 50 μl methanol, and 10 μl were analysed by HPLC–DAD and LC–MS.

Blood, serum and urine samples were extracted without enzymatic treatment for determination of the metabolites in their free form.

3. Results and discussion

3.1. Evaluation and validation of the analytical methods

A method for the determination of kavain and its metabolite *p*-hydroxykavain has been evaluated and validated. The limit of detection was 1 ng/ml for kavain using HPLC–DAD. The repeatability (within-day precision) and reproducibility (between-day precision) were proven, as shown in Tables 1 and 2. The values for the coefficient of variation (C.V.) ranged from 1.17 to 7.69% for kavain and from 0.96 to 2.2% for *p*-hydroxykavain and the mean recoveries ranged between 91 and 97%.

The linearity tests for kavain and its metabolites using their maximal absorption wavelength (246 nm for kavain, 262 nm for *p*-hydroxykavain, 226 nm for *p*-hydroxy-7,8-dihydrokavain and *p*-hydroxy-5,6-de-

Table 2
Between-day precision ($n=6$)

Substance	Suspected amount (ng/ml)	Mean detected amount (ng/ml)	SD (±)	C.V. (%)	Mean recovery (%)
D,L-Kavain	5	4.50	0.19	4.11	91
	40	38.60	0.45	1.17	96
p-Hydroxykavain	50	48	0.92	1.90	97
	500	483	4.65	0.96	94

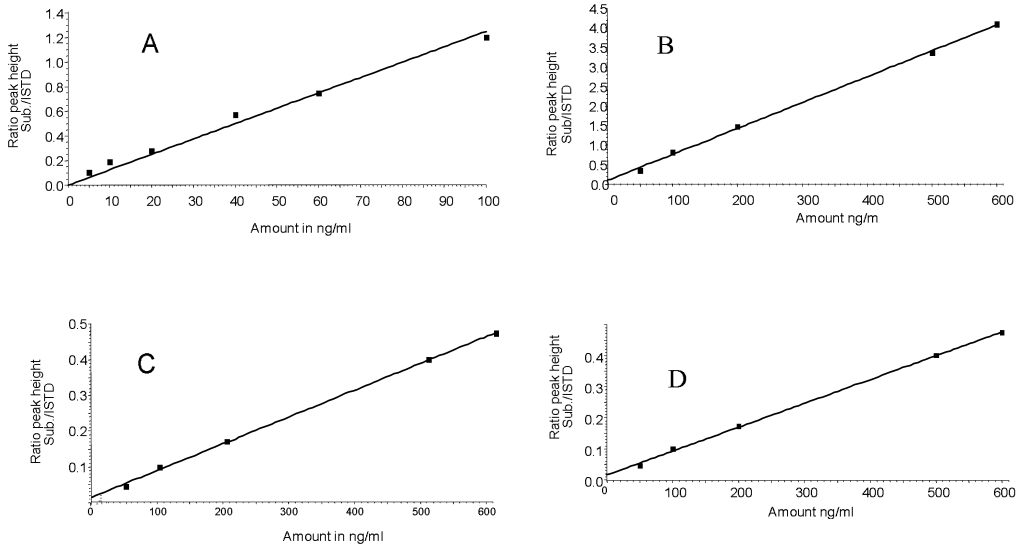

Fig. 1. Calibration curves of kavain and its metabolites in serum: (A) kavain, 5–100 ng/ml; (B) *p*-hydroxykavain, 50–600 ng/ml; (C) *p*-hydroxy-7,8-dihydrokavain, 50–600 ng/ml; (D) *p*-hydroxy-5,6-dehydrokavain, 50–600 ng/ml.

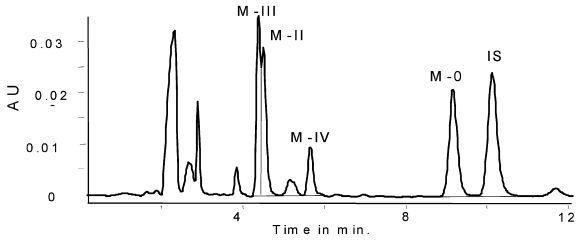

Fig. 2. HPLC–DAD chromatogram. Mixture of kavain and its metabolites in methanol at a concentration of 100 ng/ml: kavain (M-0), *p*-hydroxykavain (M-II), *p*-hydroxy-7,8-dihydrokavain (M-III), *p*-hydroxy-5,6-dehydrokavain (M-IV) and brotizolam (I.S.).

hydrokavain) were investigated. Due to the blood concentrations, the calibrators for kavain ranged between 5 and 100 ng/ml and for the metabolites between 50 and 600 ng/ml (Fig. 1).

Kavain and its metabolites were analysed by HPLC–DAD and LC–MS–MS to determine their retention times (Fig. 2 and Table 3) and UV spectra (Figs. 3 and 4) or mass spectra (Figs. 5–9). LC–MS spectra for kavain and its available metabolites were previously unpublished [32]. Therefore, in-source fragmentation of protonated molecules by electrospray-ionisation with collision induced dissociation (ESI-CID) was used with voltage switching (20, 50, 80 V orifice-voltage (OR)). For kavain the protonated molecule (MH^+) showed only low abundance (m/z 231), whereas for the metabolites base peak intensity was obtained for the protonated molecules with low orifice. At higher voltages all compounds showed rich ESI-CID-mass spectra with characteristic fragment ions.

3.2. Biotransformation of kavain

The main metabolic pathway of kavain (M-0) is hydroxylation at C-12 of the aromatic ring [2,26–28,34]. The hydroxylated metabolites excreted mainly in form of their conjugates [2,27]. After the administration of a single oral dose of 200 mg kavain to a human, *p*-hydroxykavain in its sulfate form was detected in plasma samples. It reached its maximum 1.7 h after uptake and showed mean elimination half-life of 29 h.

In urine, depending to the kavain administered, 0.3% was found as dehydrokavain, 1.0% as free *p*-hydroxykavain and 18% as conjugated *p*-hydroxykavain (~85% sulfate and 15% glucuronide) [2]. As shown in our study, in blood and serum, *p*-hydroxykavain can not only be found in its sulfate form (III) but also in its free (I) and glucuronide (II) forms. About 10% was present as free form (Fig. 10). The

Table 3
LC–MS (ESI-CID) analyses of 24-h urine after kavain oral uptake

Substance	RT* of reference compounds (min)	RT* of urinary metabolites (min)	Prominent fragments in decreasing order of magnitude
p-Hydroxy-7,8-dihydrokavain	8.68	10.83	203, 171, 133, 107, 231, 77 (*m*/*z* 249)
p-Hydroxykavain	8.78	10.93	215, 197, 169, 141, 131, 115, 109, 91, 77 (*m*/*z* 247)
p-Hydroxy-5,6-dehydrokavain	10.16	11.43	217, 185, 147, 140, 139, 128, 115, 91, 77 (*m*/*z* 245)
Kavain (unchanged)	13.50	13.84	185, 153, 128, 115, 91, 77 (*m*/*z* 231)
5,6-Dehydrokavain	–[a]	14.44	103, 69, 115, 131, 141, 152, 186, 201 (*m*/*z* 229)
6-Phenyl-5-hexen-2,4-dione	18.12	–[b]	131, 128, 103, 91, 77 (*m*/*z* 189)

[a] No reference compound available.
[b] Detected in urine by using a gradient LC system.
* RT = retention time.

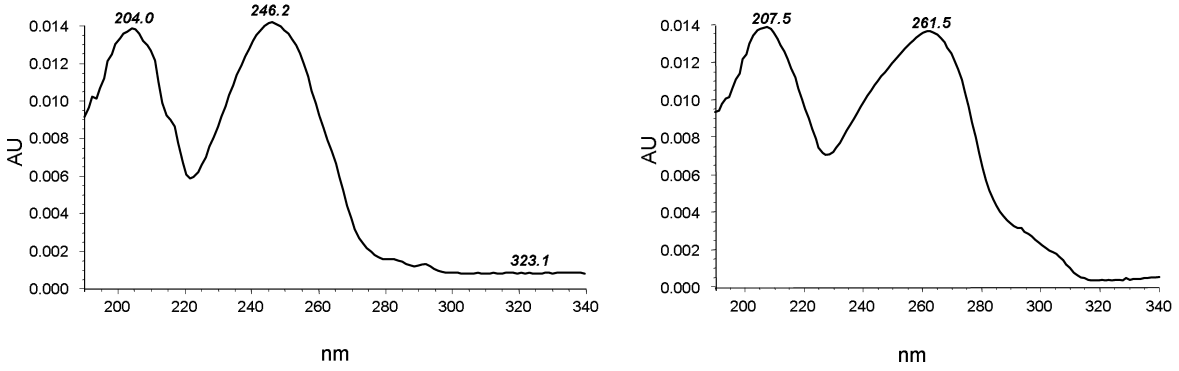

Fig. 3. UV spectrum of M-0 (left) and M-II (right).

forms appeared in blood after ~0.25 h and reached their maximum 0.75 h after oral uptake. *p*-Hydroxykavain (M-II) is furthermore metabolised to *p*-hydroxy-7,8-dihydrokavain (M-III). It was not found in blood or serum but only in the urine 8 h after uptake in the form of its conjugates glucuronide and sulfate (Fig. 11). The chemical structure of this metabolite presents a strong non-polar molecule, which could

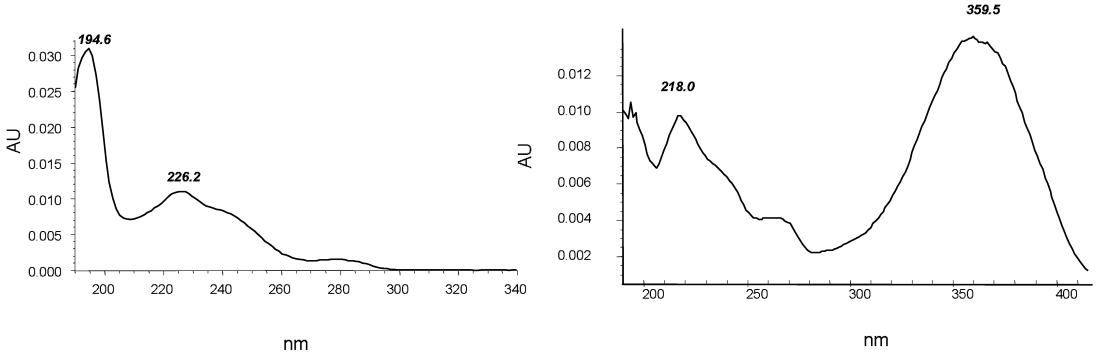

Fig. 4. UV spectrum of M-III (left) and M-IV (right).

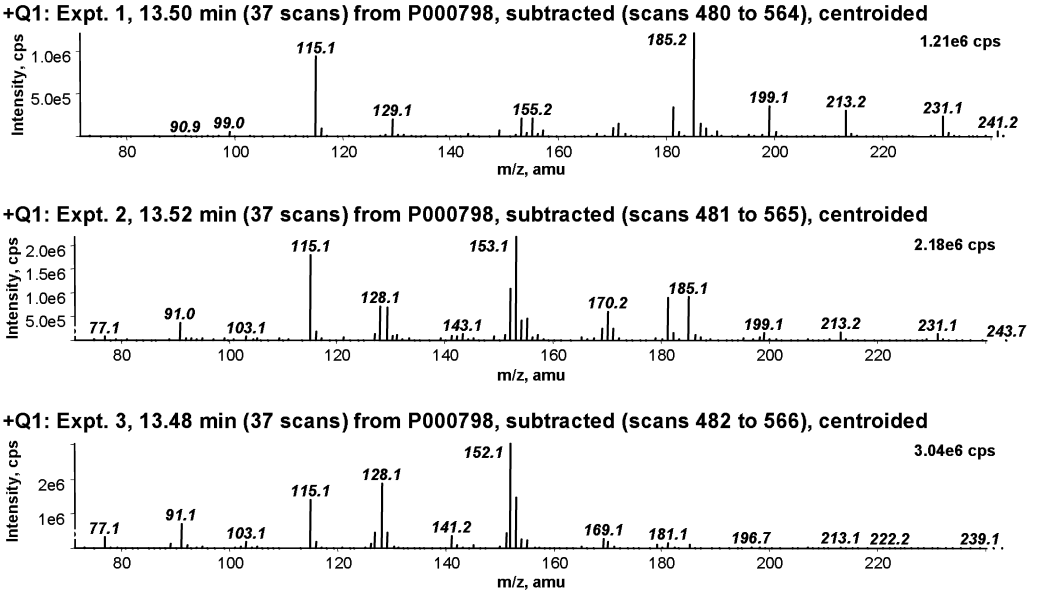

Fig. 5. ESI-CID mass spectra of kavain (M-0). Reference substance: orifice-voltages 20, 50 and 80 V, respectively (from top to bottom). Protonated molecule (m/z 231) and characteristic fragment ions (m/z 185, 153, 128, 115, 91, 77).

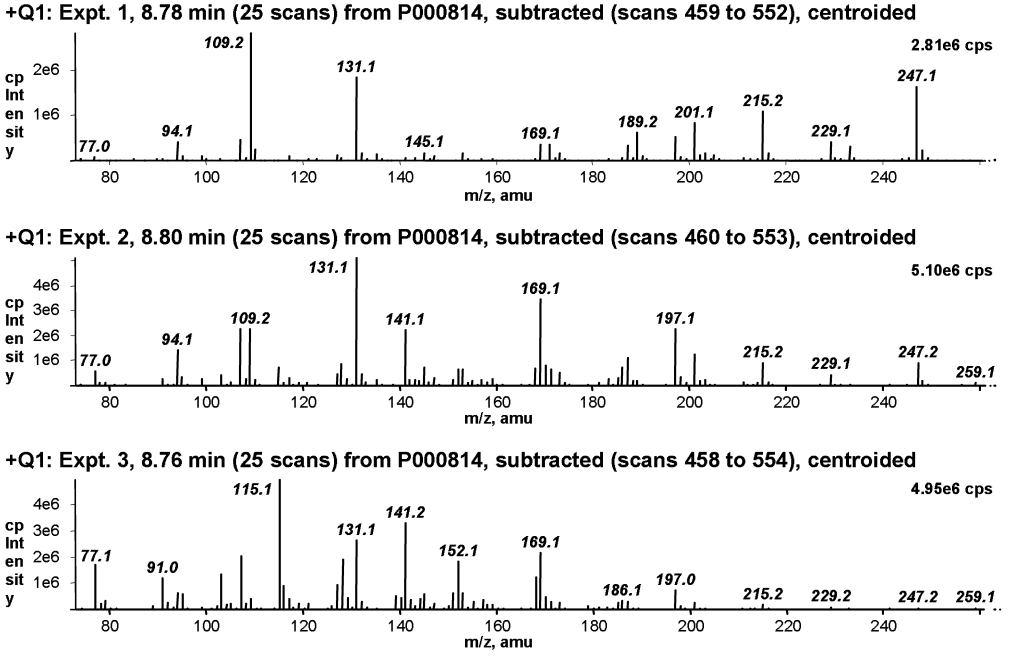

Fig. 6. ESI-CID mass spectra of *p*-hydroxykavain (M-II). Reference substance: orifice-voltages 20, 50 and 80 V, respectively (from top to bottom). Protonated molecule (m/z 247) and characteristic fragment ions (m/z 215, 197, 169, 141, 131, 115, 109, 91, 77).

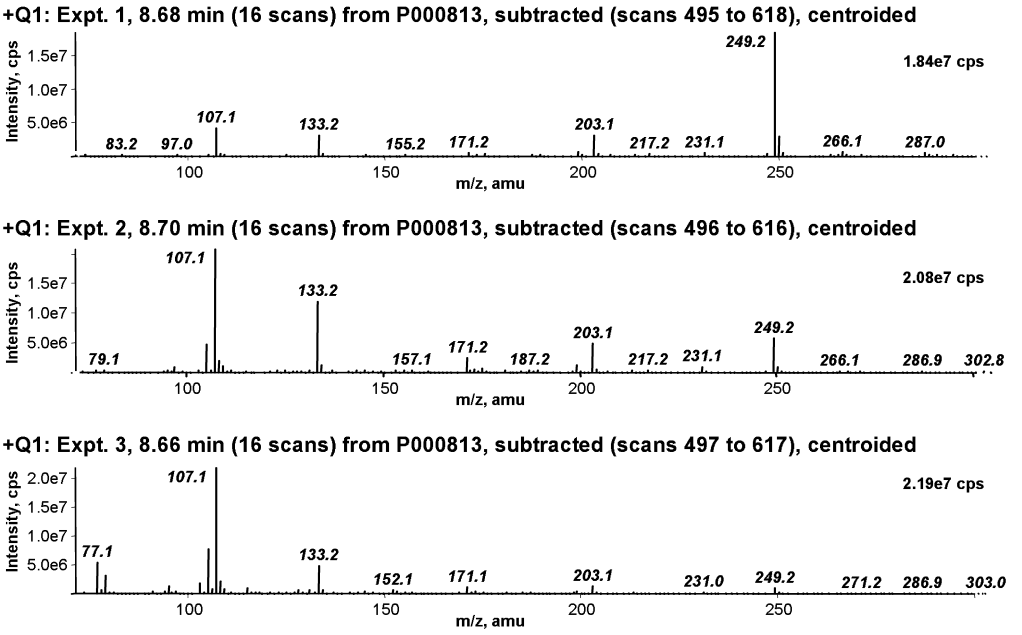

Fig. 7. ESI-CID mass spectra of *p*-hydroxy-7,8-dihydro-kavain (M-III). Reference substance: orifice-voltages 20, 50 and 80 V, respectively (from top to bottom). Protonated molecule (*m*/*z* 249) and characteristic fragment ions (*m*/*z* 203, 171, 133, 107, 77).

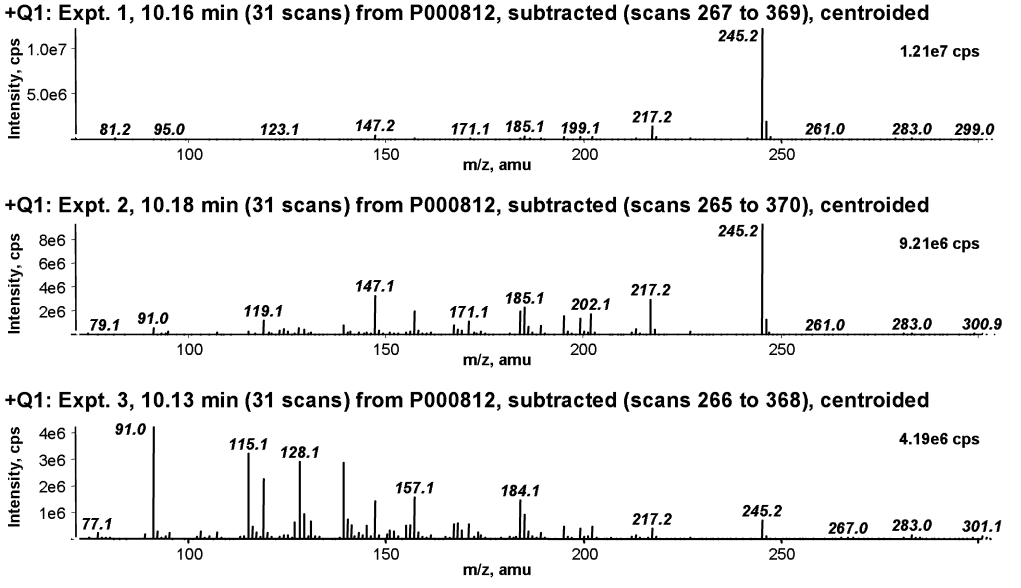

Fig. 8. ESI-CID mass spectra of *p*-hydroxy-5,6-dehydro-kavain (M-IV). Reference substance: orifice-voltages 20, 50 and 80 V, respectively (from top to bottom). Protonated molecule (*m*/*z* 245) and characteristic fragment ions (*m*/*z* 217, 185, 147, 140–139, 128, 115, 91, 77).

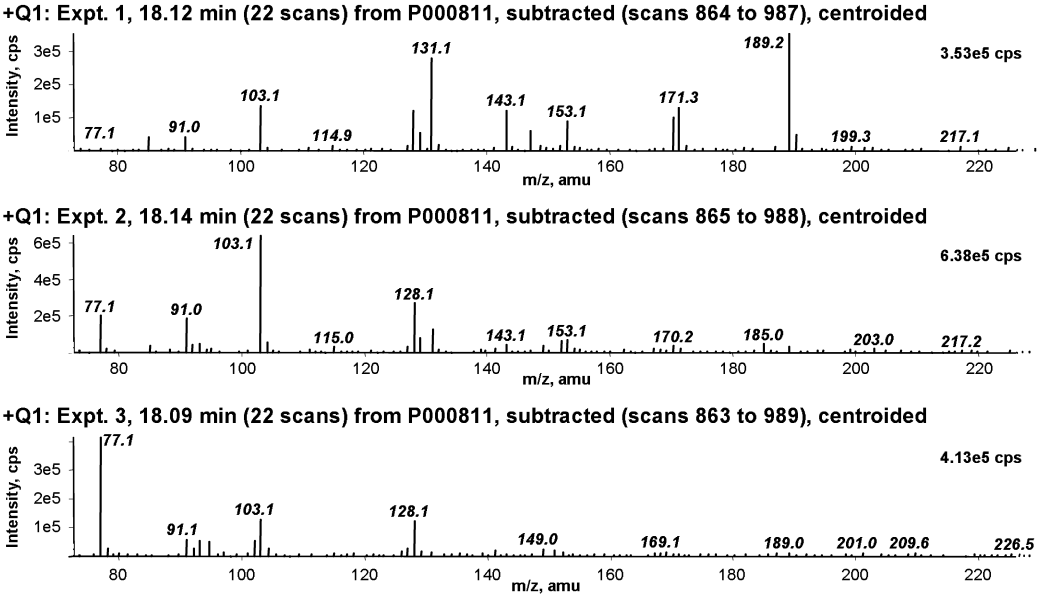

Fig. 9. ESI-CID mass spectra of 6-phenyl-5-hexen-2,4-dion (M-V). Reference substance: orifice-voltages 20, 50 and 80 V, respectively (from top to bottom). Protonated molecule (m/z 189) and characteristic fragment ions (m/z 131, 128, 103, 91, 77).

lead to a delay in the elimination rate observed in our study.

In addition opening of the kavain lactone ring, desmethylation of the 4-methoxy group of the lactone ring system, decarboxylation and oxidation yielded the metabolite 6-phenyl-5-hexen-2,4-dione (Fig. 12). This metabolite was detected in the 24-h urine by LC–MS using a gradient system.

Furthermore kavain is metabolised by a dehydration process at the position 5,6 to give its analogue 5,6-dehydrokavain (M-I) (Fig. 13). Previously dehydrokavain was only reported as urinary metabolite [2] and as the kava plant lactone desmethoxyyangonin [28]. This metabolite could be detected up to 4 h in the blood, serum and urine. Its UV spectrum is similar to *p*-hydroxykavain (Fig. 14) and has a retention time close to kavain. It was identified by a product ion scan in the triple-quadrupole mode (Fig. 15).

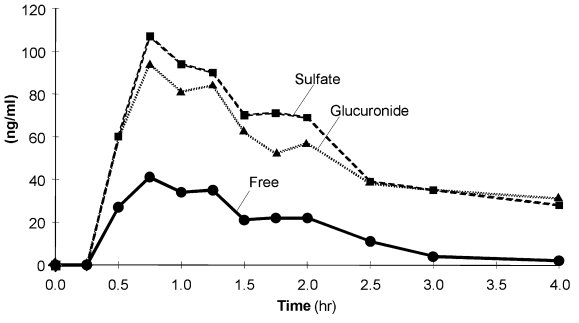

Fig. 10. Non-compartmental analysis of *p*-hydroxykavain in serum. Serum sample after kavain oral uptake (800 mg).

5,6-Dehydrokavain is further hydroxylated at the C-12 of the phenyl ring to produce *p*-hydroxy-5,6-dehydrokavain (M-IV). This metabolite was detected at concentrations near the LOD in blood and urine in the first few hours after kavain uptake. M-IV might be metabolised by desmethylation of the 4-methoxy group of the lactone ring to give *o*-desmethyl-hydroxy-5,6-dehydrokavain (M-VI). M-VI was detected in blood, serum and urine with early retention time using HPLC–DAD. Its chromophore is similar to *p*-hydroxykavain (Fig. 16).

The maximal serum concentration of M-VI was observed ~2 h after oral uptake (Fig. 17). It seems not to be conjugated.

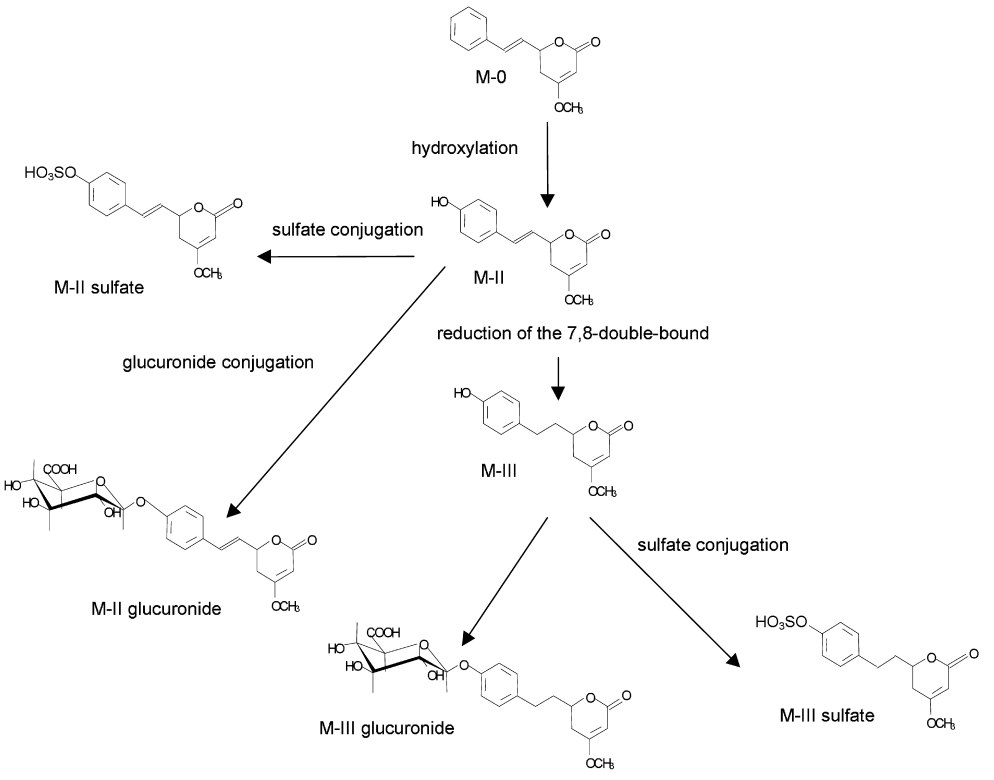

Fig. 11. Metabolic pathway of kavain (metabolites detected in urine part 1): kavain (M-0), *p*-hydroxykavain (M-II) and *p*-hydroxy-7,8-dihydrokavain (M-III).

All metabolites were measured by LC–MS with ESI-CID. A summary of LC–ESI-CID–MS and MS–MS is given in Table 3.

3.3. Kinetics of p-hydroxykavain

The pharmaceutical preparation of kavain used in this study is marked under the trade name Neuronika® (Klinge Pharma, Munich, Germany).

Our study is based on kavain self medication. Four Neuronika® capsules of 800 mg kavain were given in oral doses. Blood, serum and urine were collected (see Section 2.1.5).

Serum concentrations ranged between 10 and 40 ng/ml for kavain (M-0), 30 and 50 ng/ml for 5,6-dehydrokavain (M-I), 125 and 300 ng/ml for *p*-hydroxykavain (M-II), and 40 and 90 ng/ml for *o*-desmethyl-hydroxy-5,6-dehydrokavain (M-VI) (Fig. 18). *p*-Hydroxykavain (M-II) was selected for the kinetics study after oral kavain dose.

3.3.1. Serum concentration of time courses

The time courses of the free and conjugated forms of M-II were analysed by non-compartmental analysis using WinNonLin 3.4 (Pharsight, USA). The results are documented in Table 4. The kinetic data in Tables 4 and 5 are based on kavain oral dose.

All three forms of M-II start to appear in serum after a rather short lag time of 0.25 h, peaking after 30 min at 0.75 h, which indicates a high metabolic

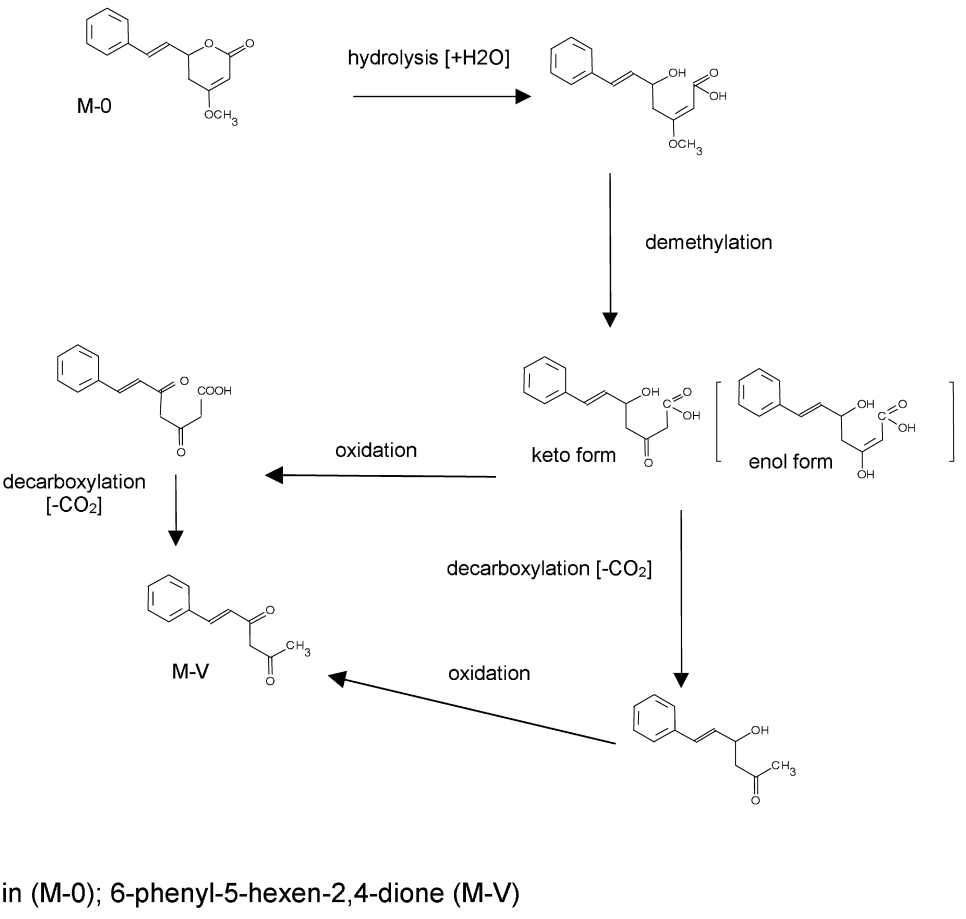

Fig. 12. Metabolic pathway of kavain (metabolites detected in urine part 2): kavain (M-0) and 6-phenyl-5-hexen-2,4-dione (M-V).

turnover. Glucuronide (II) and sulfate (III) metabolite concentrations are both 2–2.5 fold higher in serum than that of the free form (I). Relatively high concentrations of the kavain-metabolites indicate, that the period of 4 h between kavain uptake and the last sample collection seems to be too short to access the complete kinetic time course as there are relatively high concentrations left at this time (Fig. 10).

The half-lives of terminal slope phase were reached from 0.7 to 1.9 h, indicating that at least the bound forms may be found in serum up to ~10 h after oral dosage of kavain. The areas under the concentration–time curves reflect the fact already mentioned: high amounts of metabolite are present in the glucuronide and in the sulfate conjugated forms (~45% each) whereas the free form only represents 10%.

Because of the sparse data set one and two compartment models could not be discriminated. It is suspected that a two compartment model will be more appropriate if concentrations are measured over at least 10 h. In any case a more complete study of the kavain kinetics should include simultaneous measurements of kavain and the metabolites in blood and serum as well as urinary excretion of these compounds over 24 h or longer.

3.3.2. Blood concentration of time courses

The data more or less reflect the fact that blood concentrations are ~50% of the serum data, which

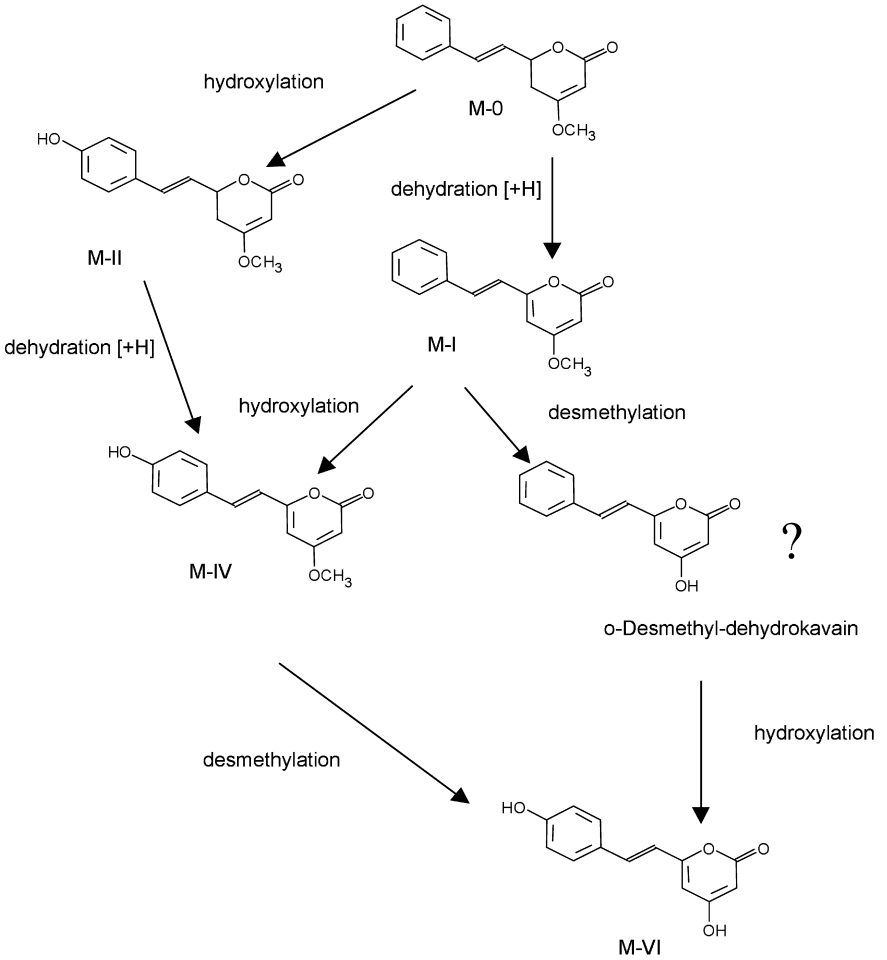

Fig. 13. Metabolic pathway of kavain (metabolites detected in blood): kavain (M-0), 5,6-dehydrokavain (M-I), *p*-hydroxykavain (M-II), and *o*-desmethyl-hydroxy-5,6-dehydrokavain (M-VI).

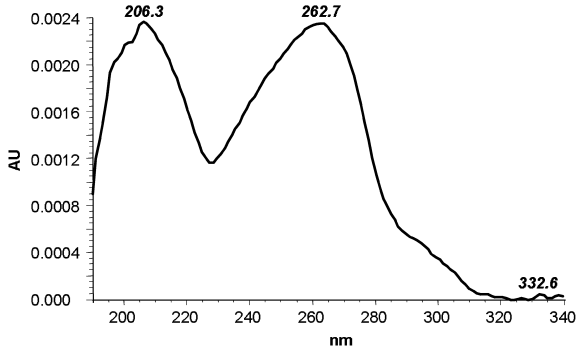

Fig. 14. UV spectrum of M-I.

correspond to various evaluations of serum to blood ratios in the next section, as shown in Table 5, which gives the results of non-compartmental analysis of blood concentration time courses.

3.3.3. Renal excretion data of p-hydroxykavain

While renal excretion of free *p*-hydroxykavain is in the same order of magnitude as creatinin clearance (~7.2 l/h), glucuronide and sulfate metabolites exhibit very high clearance values. Excretion via urine seems to continue beyond 24 h as indicated by the relatively high amounts found during the last sample

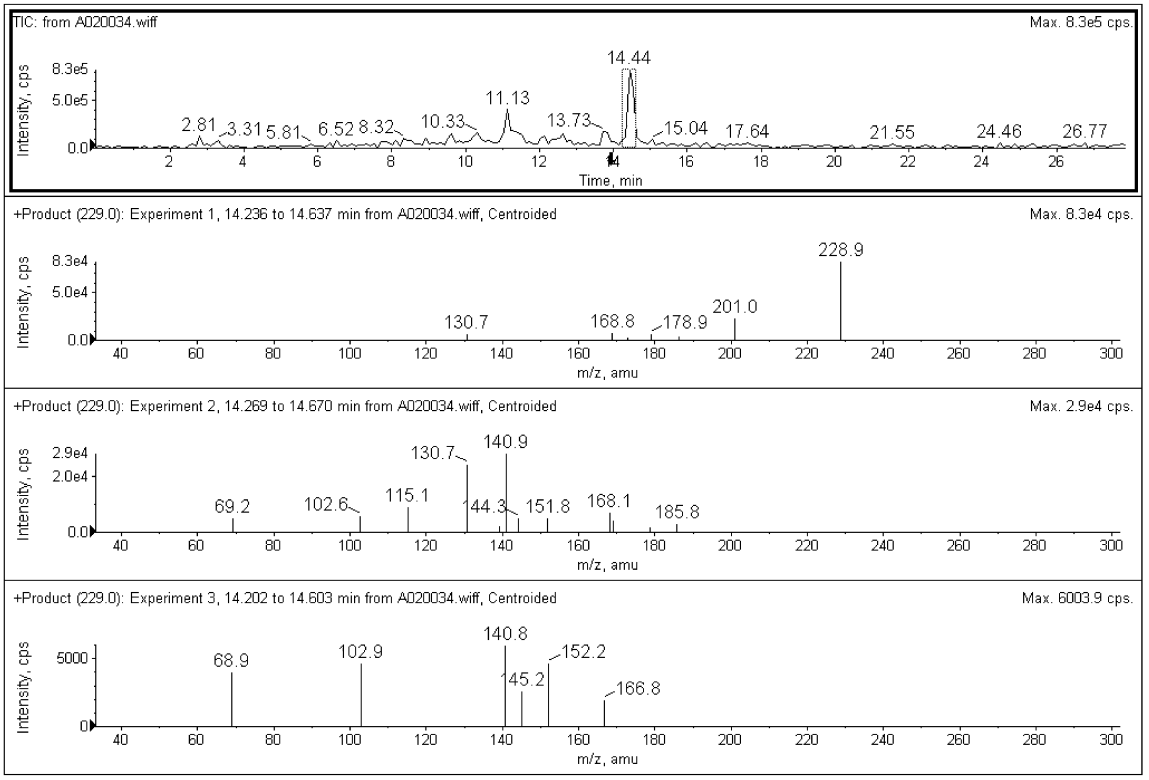

Fig. 15. LC–MS–MS of M-I (RT 14.44 min) in urine after kavain oral uptake (24-h urine).

period. From the total amount in all three forms excreted (38.4 mg/ml over 24 h) it can be deduced that 4.8% of the kavain dose of 800 mg appears as *p*-hydroxykavain in urine during 24 h (Table 6).

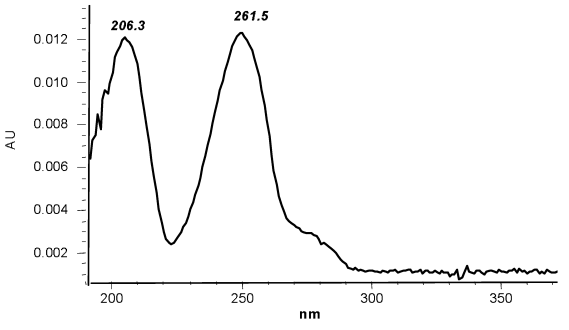

Fig. 16. UV spectrum of M-VI.

4. Conclusion

The self medication study on kavain metabolism proved that kavain metabolised through different metabolic pathways. Unchanged kavain (M-0) could not be detected in the urine, but was detected at low concentrations in blood and serum. *p*-Hydroxykavain (M-II) is the main kavain metabolite. It was found in blood and urine in its free and conjugate forms (glucuronide and sulfate).

The data of *p*-hydroxykavain in the blood samples indicated that blood concentration is ~50% of the serum data. *p*-Hydroxy-7,8-dihydrokavain (M-III) was not detected in blood and serum, but only in urine; it presents a non-polar molecule, which might lead to retardation of its elimination.

5,6-Dehydrokavain (M-I) is a stable kavain human metabolite. *o*-Desmethyl-hydroxy-5,6-dehydrokavain (M-VI) is thought to be a new kavain metabolite.

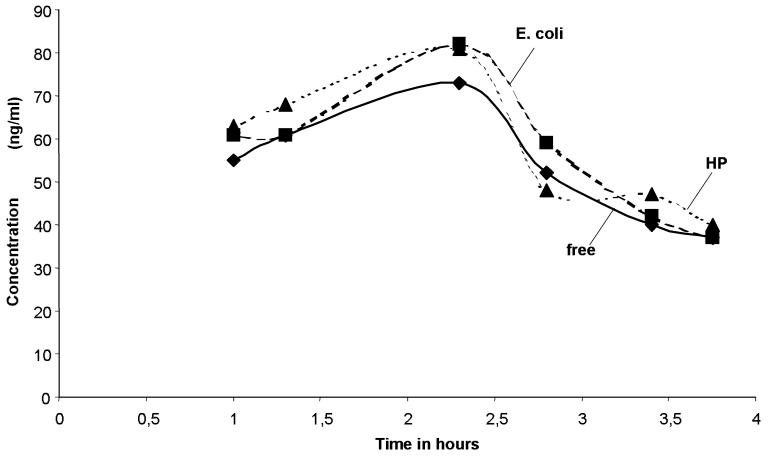

Fig. 17. M-VI in serum with and without enzymatic hydrolysis.

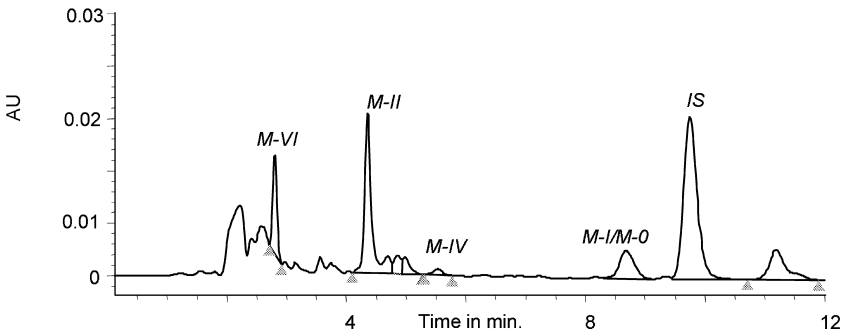

Fig. 18. HPLC chromatogram of serum sample 45 min after kavain oral uptake: dehydrokavain/kavain (M-I/M-0), *p*-hydroxykavain (M-II), *p*-hydroxy-5,6-dehydrokavain (M-IV), *o*-desmethyl-hydroxy-5,6-dehydrokavain (M-VI) and brotizolam (I.S.).

Table 4
p-Hydroxykavain in serum (free and conjugated forms)

Parameters	Unit	I	II	III
T_{lag}	h	0.25	0.25	0.25
T_{max}	h	0.75	0.75	0.75
C_{max}	ng/ml	41.0	94.0	107.0
C_{last}	ng/ml	2.0	31.0	28.0
λ_z	1/h	0.98	0.369	0.45
T_{50}	h	0.71	1.88	1.55
AUC_{inf}	ng/ml per h	64.80	274.15	271.44
V_{zF}	l	12.64×10^3	7.91×10^3	6.61×10^3
MRT	h	1.57	3.15	2.72

AUC_{inf}, area under concentration–time curve (extrapolated to infinity); C_{last}, concentration at T_{last}; C_{max}, maximal concentration; I, free form; II, free plus glucuronide form; III, free plus glucuronide plus sulfate form; λ_z, rate constant of terminal slope; MRT: mean residence time; T_{lag}, lag time before onset of kinetics; T_{max}, time of maximal concentration; T_{50}, half-life during terminal slope; V_{zF}, apparent volume of distribution.

Table 5
p-Hydroxykavain in blood (free and conjugated forms)

Parameters	Unit	I	II	III
T_{lag}	h	0.25	0.25	0.25
T_{max}	h	0.75	0.75	0.75
C_{max}	ng/ml	20.0	53.0	62.0
C_{last}	ng/ml	4.0	11.0	13.0
λ_z	1/h	0.49	0.51	0.50
T_{50}	h	1.40	1.28	1.37
AUC_{inf}	ng/ml per h	33.96	112.34	145.23
V_{zF}	l	47.70×10^3	13.17×10^3	10.90×10^3
MRT	h	2.74	2.26	2.53

AUC_{inf}, area under concentration–time curve (extrapolated to infinity); C_{last}, concentration at T_{last}; C_{max}, maximal concentration; I, free form; II, free plus glucuronide form; III, free plus glucuronide plus sulfate form; λ_z, rate constant of terminal slope; MRT, mean residence time; T_{lag}, lag time before onset of kinetics; T_{max}, time of maximal concentration; T_{50}, half-life during terminal slope; V_{zF}, apparent volume of distribution.

Table 6
Urinary excretion of *p*-hydroxykavain after kavain oral uptake

Time (h)	Unit	I	II	III
0–2	mg/l	91	1404	5954
2–4	mg/l	88	1043	4360
4–8	mg/l	82.5	1750	7690
8–18	mg/l	53.9	2765	9952
18–24	mg/l	18	787	2377
0–24	mg/l	334	7749	30 333
Cl renal	l/h	0.98	0.70	209

Cl renal, renal clearance; I, free form; II, free plus glucuronide form; III, free plus glucuronide plus sulfate form.

The presented study on kavain and its metabolites might help to answer certain forensic questions such as driving under the influence of kavain.

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