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Direct chiral assay of tramadol and detection of the phase II metabolite *O*-demethyl tramadol glucuronide in human urine using capillary electrophoresis with laser-induced native fluorescence detection

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Abstract

A chiral separation using carboxymethyl-β-cyclodextrin and methyl-β-cyclodextrin for the direct assay of tramadol in human urine by capillary electrophoresis (CE) with laser-induced native fluorescence detection was developed. Furthermore, the phase II metabolite *O*-demethyl tramadol glucuronide was determined from the urine samples and the ratio of the diasteromers was determined. The chiral method was validated. Correlation coefficients were higher than 0.999. Within day variation showed accuracy in the range 96.1–105.8% with a RSD less than 6.00%. Day to day variation present an accuracy ranging from 100.2 to 103.5% with a RSD less than 5.4%. After oral administration of 150 mg tramadol hydrochloride to a healthy volunteer, the urinary excretion was monitored during 24 h. About 11.4% of the dose was excreted as 1*S*,2*S*-tramadol, 16.4% as 1*R*,2*R*-tramadol and 23.7% as *O*-demethyl tramadol glucuronide. The amount of 1*S*,2*S* *O*-demethyl tramadol glucuronide was more than three fold higher as 1*R*,2*R*-*O*-demethyl tramadol glucuronide. The enantiomeric ratio of tramadol and the diastereomeric ratio of *O*-demethyl tramadol glucuronide was deviated from 1.0 showing that a stereoselective metabolism of tramadol occurs. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Tramadol; *O*-Demethyl tramadol glucuronide

1. Introduction

Tramadol hydrochloride 2[(dimethylamino)-methyl]-1(3-methoxyphenyl)cyclohexanol, is a centrally acting analgesic drug with low abuse potential [1,2]. Clinical studies have shown that tramadol does not have a pronounced opioid side effect due to the methoxy group [3]. Tramadol is used as the racemate for therapy. Each enantiomer displays different binding properties for various receptors. 1*R*,2*R*-tramadol preferentially inhibits serotonin reuptake where as 1*S*,2*S*-tramadol mainly inhibits noradrenalin reuptake [4–6]. The 1*R*,2*R*-enantiomer exhibits a ten fold higher analgesic potency than the 1*S*,2*S*-enantiomer [7]. Tramadol is extensively metabolized by *N*- and *O*-demethylation. The phenolic metabolites are

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known to form conjugates to the corresponding glucuronides and sulfates [8]. The determination of tramadol and its metabolites in biological fluids was possible by TLC, GC with nitrogen selective detection, or by GC–MS [8–11]. High performance liquid chromatography with UV and fluorescence detection was also used for the chiral and achiral determination of tramadol and its metabolites [12–15]. The chiral and achiral separation of tramadol and its phase I metabolites with capillary electrophoresis (CE) was also achieved [16–18]. For the first time the achiral direct quantification of tramadol in urine samples using CE with laser induced native fluorescence was described recently [19]. There was no method available for the determination of tramadol in urine samples without extraction or preconcentration. This study describes a sensitive and highly selective CE method with laser-induced native fluorescence detection for the direct chiral assay of tramadol in human urine. Additionally the quantity of the excreted phase II metabolite *O*-demethyl glucuronide was determined and the proportion of the diastereomers was determined.

2. Materials and methods

2.1. Chemicals

Tramadol and *O*-demethyl tramadol were a gift from Grünenthal (Stolberg, Germany). The glucuronide of *O*-demethyl tramadol was synthesized by Bayer AG (Wuppertal, Germany). All other phase I metabolites were synthesized according to literature [20]. *S*(+) Dimetindene, used as internal standard (IS) for the chiral assay of tramadol, was prepared as described previously [21]. Naphazoline nitrate used as I.S. for the quantification of *O*-demethly tramadol glucuronide was a gift form Novartis Pharma (Basel, Switzerland). Carboxymethyl-β-cyclodextrin sodium salt (Carvasol® W7 CM) with an average substitution degree of 5.0 and methyl-β-cyclodextrin with an average substitution degree of 1.8 was donated by Wacker Chemie (Burghausen, Germany).

All chemicals were of analytical grade. Sodium tetraborate (Borax), sodium hydroxide and sodium phosphate were purchased from Merck Eurolab GmbH (Darmstadt, Germany). The water was deionized and double distilled.

2.2. Apparatus

Briefly, as previously published [19], a modified SpectraPHORESIS 100 CE System (Thermo Separation Products, ThermoQuest Analytische Systeme, Egelsbach, Germany) and fused-silica capillaries (Polymicro Technologies, Phoenix, Arizona, USA) with 363 μm O.D., 50 μm I.D., an effective length of 55 cm and a total length of 75 cm were used. The UV–LIF detector consisted of a frequency doubled argon ion laser (Laxel 95 SHG, Lexel Laser, Polytec GmbH, Waldbronn, Germany) operating at 257 nm with a power of 200 mW. An on column detection window was created by removing a 4 mm section of the polyimide coating on the fused-silica tubing. The resulting fluorescence is imaged onto a spectrograph with an attached intensified CCD-Camera (Flamestar 3 LaVision 2 D Messtechnik GmbH, Göttingen, Germany) in order to achieve wavelength resolution of the emitted light. The fluorescence signal is collected at an angle of 90°to the excitation light with a spherical aluminium mirror (d. 5 cm, F/Number 1.1) to suppress chromatic aberration. The wavelength resolved CE–LIF data were processed with custom algorithms using Mathcad 7 (Mathsoft Inc.).

2.3. Electrophoretic conditions

50 m*M* tetraborate buffer adjusted to pH 10.0 with 5 N NaOH was used. 40 mg/ml carboxymethyl-β-cyclodextrin and 22.5 mg/ml methyl-β-cyclodextrin were added to the run buffer for the chiral assay of tramadol [22,23] 150 m*M* tetraborate buffer adjusted to pH 10.6 with 150 m*M* NaOH was used for the determination of the *O*-demethyl tramadol glucuronide. The applied voltage was +25 kV and the temperature was maintained at 19.0°C. For the separation of the *O*-demethyl tramadol glucuronide diasteromers, 50 m*M* phosphate buffer adjusted to pH 3.0 with phosphoric acid was used. The injection was performed hydrodynamically. Detection was started 6 min after the electrophoretic run. Before every run the capillary was washed with 0.1 N NaOH for 3 min and also with run buffer for 3 min.

2.4. Internal standard solution

The internal standard (IS) for the chiral assay of tramadol stock solution of 100 μg/ml (+)dimetindene in water was prepared weekly and stored at 4°C. A stock solution of 100 μg/ml naphazoline nitrate used as internal standard (IS) for the quantification of *O*-demethyl tramadol glucuronide was also prepared weekly and stored at 4°C.

2.5. Standard solutions

For the chiral assay, stock solutions of 1 mg/ml racemic tramadol were prepared in deionized and double distilled water. Standard solutions ranging from 500 ng to 100 μg/ml were prepared by dilution with blank urine.

For the achiral determination, stock solutions of 1 mg/ml *O*-demethyl tramadol glucuronide were also prepared in deionized and double distilled water. Standard solutions from 100 ng to 50 μg/ml were prepared by dilution with blank urine.

2.6. Urine samples

Pooled blank urine collected from a male healthy volunteer was used to prepare spiked urine samples. A 100 μl volume of the respective standard solution and 25 μl (chiral assay) or 100 μl (achiral determination) were added to 875 (chiral assay) or 800 μl (achiral determination) blank urine in order to achieve a calibration series of 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 μg/ml (chiral assay of tramadol) and 0.01, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 μg/ml (achiral determination of *O*-demethyl tramadol glucuronide), respectively.

2.7. Validation of the chiral assay

2.7.1. Calibration

Daily calibration curves were obtained by analyzing urine samples with known concentrations of the analytes. Linear regression analysis according to the I.S. method was performed by plotting the peak area ratio of 1*S*,2*S*-tramadol or 1*R*,2*R*-tramadol/internal standard against the known concentrations of the samples.

2.7.2. Precision and accuracy

Intra-day variation was assessed by six replicated determinations of four concentrations within the tested range (10, 5 μg/ml, 200, 100 ng/ml). Intra-day accuracy was expressed as the mean value of the results relative to the theoretical values (%). The intra-day precision of the method was expressed as the relative standard deviation (RSD) of the assays prepared for intra-day accuracy. Inter-day variation was determined by analyzing replicates of spiked urine samples with the same concentrations on three separate days. Inter-day accuracy was expressed as the mean of the assays relative to the theoretical values (%). The inter-day precision was expressed as the RSD of the assays made for the inter-day accuracy.

2.7.3. Detectability

The limit of detection (LOD) was defined as the analyte concentration resulting in a S/N-ratio of 3:1. The limit of quantification (LOQ) was defined as the analyte concentration that could be analyzed with acceptable precision and accuracy according to the ICH guidelines [24].

2.7.4. Selectivity

Selectivity was investigated by comparing a blank sample with an urine sample spiked with tramadol, the internal standard and the phase I metabolites *N*-demethyl tramadol, *N*,*O*-didemethyl tramadol, *N*,*N*,*O*-tridemethyl tramadol and *O*-demethyl tramadol.

2.8. Application

The method was applied to samples taken from one healthy volunteer. Urine samples were collected during 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–12, 12–24 h after oral administration of 150 mg tramadolhydrochloride (three tablets of Tramal®) each containing 50 mg. The concentration of 1*S*,2*S*- and 1*R*,2*R*-tramadol in the collected intervals were calculated from the calibration equations obtained in Section 2.7.1 and their proportion in the collected intervals was determined. All collected samples were diluted with blank urine because the concentration of the tramadol enantiomers exceeded the calibration range.

2.9. Detection and quantification of O-demethyl tramadol glucuronide and determination of the ratio of the diastereomers

2.9.1. Calibration and detectability

Daily calibration curves were obtained by analyzing urine samples with known concentrations of the analytes. Linear regression analysis according to the I.S. method was performed by plotting the peak area ratio of *O*-demethyl tramadol glucuronide/IS against the known concentrations of the samples. The limit of detection (LOD) was defined as the analyte concentration resulting in a *S/N*-ratio of 3:1.

For *O*-demethyl tramadol glucuronide the amount of the reference compound synthesised by Bayer AG Wuppertal Germany was insufficient for a full validation.

2.9.2. Selectivity

Selectivity was investigated by comparing a blank sample with an urine sample spiked with tramadol, internal standard and the phase I metabolites *N*-demethyl tramadol, *N,O*-didemethyl tramadol, *N,N,O*-tridemethyl tramadol, *O*-demethyl tramadol and *O*-demethyl tramadol glucuronide.

3. Application

The method was applied to some samples taken from the healthy volunteer which were used for the chiral assay. The concentration of *O*-demethyl tramadol glucuronide in the collected intervals was calculated from the calibration equation in Section 2.9.1.

4. Results and discussion

Characteristic fluorescence emission spectra in combination with the retention time allow a better identification of the analytes compared to the migration time alone which would not be possible with filter-based, single-channel fluorescence detectors. To obtain the optimum *S/N*-ratio for the tramadol enantiomers and *O*-demethyl tramadol glucuronide the emitted light was collected in the range from 290 to 320 nm. The main advantage of the method is the capability for the direct determination of the tramadol enantiomers and *O*-demethyl tramadol glucuronide in urine samples due to the extremely high sensitivity. Several other methods have been described in the literature for the chiral assay of tramadol or the achiral determination of the phase II metabolite *O*-demethyl tramadol glucuronide [8–18], but there is no technique available for direct determination of them. Fig. 1 shows an electropherogram of a chiral separation of an urine sample compared to blank urine. No interference of the 1*S*,2*S*- and 1*R*,2*R*-tramadol peaks with peaks of the metabolites was observed. The migration times of 1*S*,2*S*- and 1*R*,2*R*-tramadol and the internal standard were 11.1, 11.5 and 10.7 min. The total analysis time was 13 min. No comigration of endogenous substances with the studied compounds was observed because the emission spectra were recorded and appropriate spectral ranges were individually selected for tramadol and the metabolites in the data processing. The calibration curves of 1*S*,2*S*- and 1*R*,2*R*-tramadol were linear over the whole concentration range. Correlation coefficients were higher than 0.999.

Calibration curve for 1*S*, 2*S* - tramadol: $y = 1.84108 * 10^{-4} x + 0.0068$.

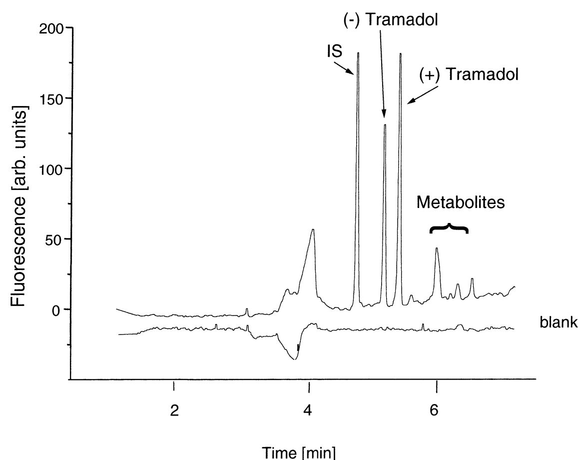

Fig. 1. Chiral separation of an urine sample of a healthy volunteer after oral administration of 150 mg tramadol hydrochloride compared to blank urine. Collection interval: 7–8 h after oral application. Experimental conditions: 50 m*M* borax buffer, pH 10.0, 40 mg/ml carboxymethyl-β-cyclodextrin, 22.5 mg/ml methyl-β-cyclodextrin, applied voltage: +25 kV.

Table 1
Results of the interday validation: Chiral determination of (−)1*S*,2*S*-tramadol in human urine

		Day 1 (100 ng)		Day 2 (100 ng)		Day 3 (100 ng)
		area/area I.S.		area/area I.S.		area/area I.S.
	1	0.0236	1	0.0257	1	0.0266
	2	0.0269	2	0.0239	2	0.0232
	3	0.0257	3	0.0268	3	0.0258
	4	0.0235	4	0.0249	4	0.0244
	5	0.0254	5	0.0264	5	0.0251
	6	0.0268	6	0.0256	6	0.0261
MW		0.0253		0.0255		0.0252
av. accuracy (%)		**100.5**		**101.5**		**99.9**
precision (%)		**6.00**		**4.18**		**4.95**
		Day 1 (200 ng)		Day 2 (200 ng)		Day 3 (200 ng)
		area/area I.S.		area/area I.S.		area/area I.S.
	1	0.0464	1	0.0415	1	0.0461
	2	0.0457	2	0.0430	2	0.0468
	3	0.0449	3	0.0409	3	0.0460
	4	0.0446	4	0.0439	4	0.0473
	5	0.0415	5	0.0433	5	0.0412
	6	0.0412	6	0.0408	6	0.0471
MW		0.0441		0.0422		0.0458
av. accuracy (%)		**101.3**		**96.1**		**105.8**
precision (%)		**4.99**		**3.15**		**5.00**
		Day 1 (5 μg)		Day 2 (5 μg)		Day 3 (5 μg)
		area/area I.S.		area/area I.S.		area/area I.S.
	1	0.8590	1	1.0048	1	1.0013
	2	0.9493	2	0.9441	2	0.9937
	3	0.8651	3	0.9078	3	0.9648
	4	0.9619	4	0.8774	4	0.9220
	5	0.8910	5	0.9198	5	0.9289
	6	0.9020	6	0.9192	6	0.9279
MW		0.9047		0.9288		0.9564
av. accuracy (%)		**97.5**		**100.2**		**103.2**
precision (%)		**4.72**		**4.64**		**3.69**
		Day 1 (10 μg)		Day 2 (10 μg)		Day 3 (10 μg)
		area/area I.S.		area/area I.S.		area/area I.S.
	1	1.8453	1	1.9652	1	1.9591
	2	1.8970	2	1.7704	2	2.0247
	3	1.9073	3	1.8284	3	1.8866
	4	1.8494	4	1.6977	4	1.8923
	5	1.8550	5	1.6652	5	1.8837
	6	1.7896	6	1.7780	6	1.8521
MW		1.8573		1.7841		1.9164
av. accuracy (%)		**100.5**		**99.5**		**103.7**
precision (%)		**2.27**		**5.96**		**3.32**

Table 2
Results of the interday validation: Chiral determination of (+)1*R*,2*R*-tramadol in human urine

	Day 1 (100 ng)	Day 2 (100 ng)	Day 3 (100 ng)
	area/area I.S.	area/area I.S.	area/area I.S.
1	0.0239	0.0249	0.0244
2	0.0238	0.0239	0.0253
3	0.0234	0.0246	0.0255
4	0.0235	0.0252	0.0249
5	0.0249	0.0246	0.0256
6	0.0249	0.0247	0.0264
MW	0.0241	0.0247	0.0254
av. accuracy (%)	**97.3**	**100.0**	**103.8**
precision (%)	**2.79**	**1.75**	**2.67**
	Day 1 (200 ng)	Day 2 (200 ng)	Day 3 (200 ng)
	area/area I.S.	area/area I.S.	area/area I.S.
1	0.0453	0.0463	0.0426
2	0.0420	0.0436	0.0438
3	0.0435	0.0405	0.0459
4	0.0466	0.0458	0.0458
5	0.0414	0.0435	0.0420
6	0.0457	0.0425	0.0458
MW	0.0441	0.0437	0.0443
av. accuracy (%)	**102.7**	**101.6**	**103.3**
precision (%)	**4.79**	**4.90**	**3.97**
	Day 1 (5 μg)	Day 2 (5 μg)	Day 3 (5 μg)
	area/area I.S.	area/area I.S.	area/area I.S.
1	0.9074	1.0045	1.0143
2	0.9572	0.9498	1.0082
3	0.9232	0.9161	0.9827
4	0.9924	0.9130	0.9454
5	0.9068	0.9944	0.9481
6	0.9639	0.9477	0.9635
MW	0.9418	0.9543	0.9770
av. accuracy (%)	**101.2**	**102.5**	**105.0**
precision (%)	**3.69**	**4.02**	**3.04**
	Day 1 (10 μg)	Day 2 (10 μg)	Day 3 (10 μg)
	area/area I.S.	area/area I.S.	area/area I.S.
1	1.9443	2.0269	2.0160
2	1.9507	1.8487	2.0150
3	1.9826	1.8796	1.8985
4	1.9967	1.7487	1.9422
5	1.9832	1.7670	1.9439
6	1.8268	1.8505	1.9575
MW	1.9474	1.8536	1.9622
av. accuracy (%)	**104.9**	**99.9**	**105.7**
precision (%)	**3.21**	**5.35**	**2.34**

Calibration curve for 1*R*, 2*R* - tramadol: $y = 1.84993*10^{-4}x + 0.0061$.

The results obtained from the inter- and intra-day precision and accuracy studies are listed in Tables 1–4. For all samples, inter- and intra-day precision was better than 90%. The inter- and intra-day

Table 3
Results of the intra-day validation: Chiral determination of (−)1*S*,2*S*-tramadol in human urine

Day 1–3 (100 ng)		area/area I.S.	Day 1–3 (200 ng)	area/area I.S.
	1	0.0236	1	0.0464
	2	0.0269	2	0.0457
	3	0.0257	3	0.0449
	4	0.0235	4	0.0446
	5	0.0254	5	0.0415
	6	0.0268	6	0.0412
	7	0.0257	7	0.0415
	8	0.0239	8	0.0430
	9	0.0268	9	0.0409
	10	0.0249	10	0.0439
	11	0.0264	11	0.0433
	12	0.0256	12	0.0408
	13	0.0266	13	0.0461
	14	0.0232	14	0.0469
	15	0.0258	15	0.0460
	16	0.0244	16	0.0473
	17	0.0251	17	0.0412
	18	0.0261	18	0.0471
MW		0.0254		0.0440
av. accuracy (%)		**101.0**		**101.0**
precision (%)		**4.82**		**5.40**

Day 1–3 (5 μg)		area/area I.S.	Day 1–3 (10 μg)	area/area I.S.
	1	0.8590	1	1.8452
	2	0.9493	2	1.8970
	3	0.8651	3	1.9073
	4	0.9619	4	1.8494
	5	0.8910	5	1.8550
	6	0.9020	6	1.7896
	7	1.0048	7	1.9652
	8	0.9441	8	1.7704
	9	0.9078	9	1.8284
	10	0.8774	10	1.6977
	11	0.9189	11	1.6652
	12	0.9192	12	1.7780
	13	1.0013	13	1.9591
	14	0.9937	14	2.0247
	15	0.9648	15	1.8866
	16	0.9220	16	1.8923
	17	0.9289	17	1.8837
	18	0.9279	18	1.8521
MW		0.9300		1.8526
av. accuracy (%)		**100.3**		**100.3**
rel. standard deviation (%)		**4.71**		**4.87**

Table 4
Results of the intra-day validation: Chiral determination of (+)1*R*,2*R*-tramadol in human urine

Day 1–3 (100 ng)		Day 1–3 (200 ng)	
	area/area I.S.		area/area I.S.
1	0.0239	1	0.0453
2	0.0238	2	0.0420
3	0.0234	3	0.0435
4	0.0235	4	0.0466
5	0.0249	5	0.0415
6	0.0249	6	0.0457
7	0.0249	7	0.0463
8	0.0239	8	0.0436
9	0.0246	9	0.0405
10	0.0252	10	0.0458
11	0.0246	11	0.0435
12	0.0247	12	0.0425
13	0.0244	13	0.0426
14	0.0253	14	0.0438
15	0.0255	15	0.0459
16	0.0249	16	0.0458
17	0.0256	17	0.0420
18	0.0264	18	0.0458
MW	0.0247		0.0440
av accuracy (%)	**100.5**		**102.4**
precision (%)	**3.15**		**4.32**
Day 1–3 (5 μg)		Day 1–3 (10 μg)	
	area/area I.S.		area/area I.S.
1	0.9074	1	1.9443
2	0.9572	2	1.9507
3	0.9232	3	1.9826
4	0.9924	4	1.9967
5	0.9068	5	1.9832
6	0.9639	6	1.8268
7	1.0045	7	2.0269
8	0.9498	8	1.8487
9	0.9161	9	1.8796
10	0.9130	10	1.7487
11	0.9944	11	1.7670
12	0.9477	12	1.8505
13	1.0143	13	2.0160
14	1.0082	14	2.0150
15	0.9827	15	1.8985
16	0.9454	16	1.9422
17	0.9481	17	1.9439
18	0.9635	18	1.9575
MW	0.9577		1.9210
av. accuracy (%)	**102.9**		**105.5**
precision (%)	**3.73**		**4.39**

accuracy ranged between 90 and 110%. These results meet the requirements for the validation of biological samples [24].

The limit of detection (LOD) (*S*/*N*-ratio=3:1) was determined to be 50 ng/ml for each enantiomer and the limit of quantification was determined to be 100 ng/ml. The concentration of 1*S*,2*S*-tramadol and 1*R*,2*R*-tramadol in the different collection intervals are shown in Fig. 2 and the cumulative excretion is given is Fig. 3. After oral administration 11.4% was excreted as 1*S*,2*S*-tramadol and 16.4% as 1*R*,2*R*-tramadol. The stereoselective excretion of tramadol was transformed into the relative ratios of the enantiomers. The investigation showed in the first 2 h as well as between 12 and 24 h after oral administration a time dependent increase of the excretion of the 1*R*,2*R*-tramadol shown in Table 5.

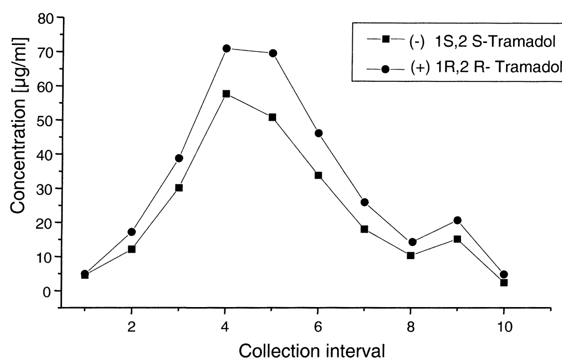

Fig. 2. Concentration of 1*S*,2*S*-tramadol and 1*R*,2*R*-tramadol in the different collection intervals.

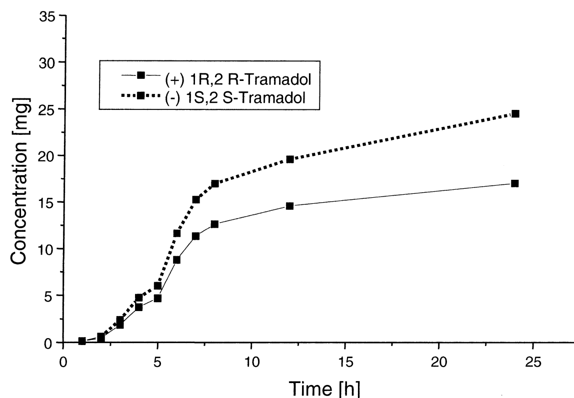

Fig. 3. Cumulative excretion of 1*S*,2*S*-tramadol and 1*R*,2*R*-tramadol as a function of time.

Table 5
Percentage of the tramadol enantiomers ($n=6$)

Collection interval [h]	Percentage of the enantiomers [%]	
	1*S*,2*S*-tramadol	1*R*,2*R*-tramadol
0–1	48.5	51.5
1–2	41.4	58.6
2–3	43.7	56.3
3–4	44.8	55.2
4–5	42.2	57.8
5–6	42.3	57.7
6–7	42.0	58.0
7–8	41.1	58.9
8–12	42.3	57.7
12–24	33.3	66.7

This is in good agreement with the results of previous investigations [12,16], which also showed a higher excretion of the 1*R*,2*R*-enantiomer.

O-demethyl tramadol glucuronide is the main phase II metabolite of tramadol. Due to their pronounced hydrophilicity it is complicated to extract glucuronides. With our method a direct achiral determination using CE with LIF detection in the deep UV is possible. Fig. 4 shows the electropherogram of an urine sample compared to blank urine. No interference with endogenous compounds or tramadol and its metabolites was observed, however, no separation of diastereomers was achieved. The calibration curve was linear over the entire range tested. The correlation coefficient was better than 0.999; $y=2.03*10^{-3}x+0.03488$.

The LOD was determined to be 10 ng/ml. The concentration of the analyte in the different collection intervals taken from one healthy volunteer is shown in Fig. 5. In Fig. 6 the cumulative excretion as a function of time is given. 23.5% of the administrated dose was found as *O*-demethyl tramadol glucuronide.

The *O*-demethyl tramadol glucuronide diastereomers were separated in phosphate buffer pH 3.0 without chiral selectors. They were identified by spiking the sample with the pure diastereomers

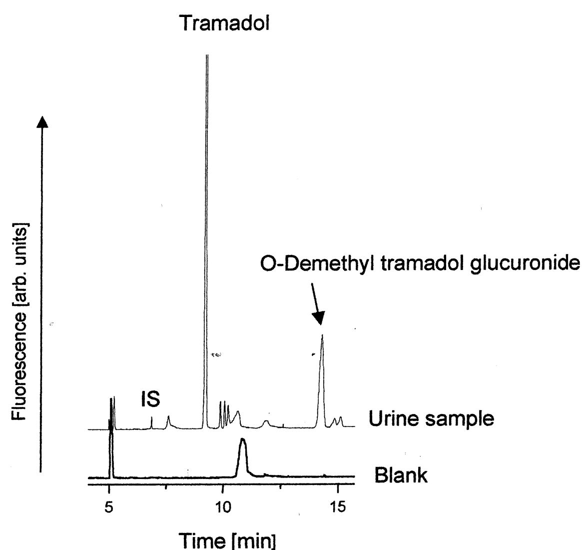

Fig. 4. Urine sample of a healthy volunteer after oral administration of 150 mg tramadol hydrochloride. Collection interval: 7–8 h after oral application. Electrophoretic conditions: 150 m*M* borax buffer, pH 10.6, applied voltage: +20 kV.

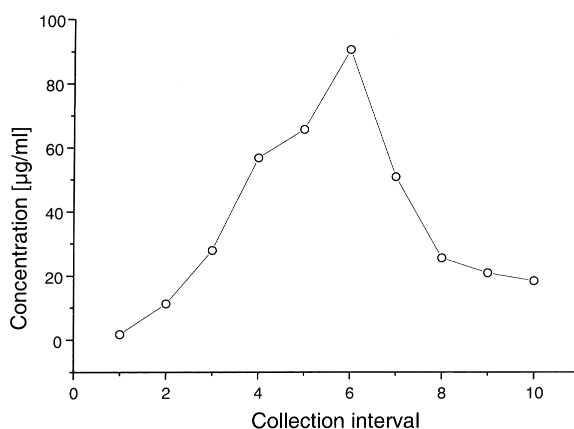

Fig. 5. Concentration of *O*-demethyl tramadol glucuronide in the different collection intervals.

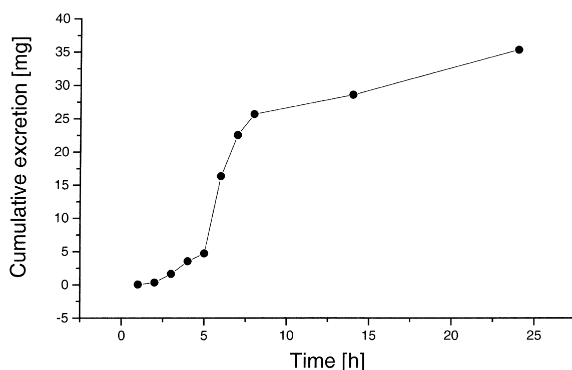

Fig. 6. Cumulative excretion of *O*-demethyl tramadol glucuronide as a function of time.

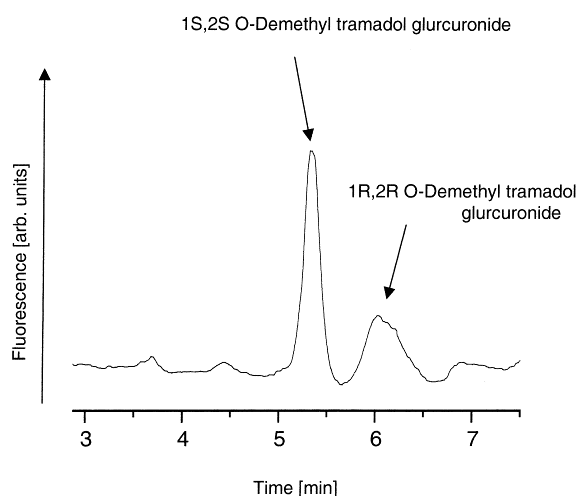

Fig. 7. Diastereomeric separation of 1*R*,2*R*- and 1*S*,2*S*-*O*-demethyl tramadol glucuronide. Electrophoretic conditions: 50 m*M* phosphate buffer, pH 3.0, applied voltage: 25 kV.

1*R*,2*R*-*O*-demethyl tramadol glucuronide and 1*S*,2*S*-*O*-demethyl tramadol glucuronide, respectively. Fig. 7 shows a representative electropherogram of the diastereomeric separation. The ratio of the diastereomers was expressed as a percentage of the peak area of each diastereomer with regard to the whole peak area of both diastereomers. A four fold higher excretion of 1*S*,2*S*-*O*-demethyl tramadol glucuronide was observed. This is in good agreement with previous studies [25]. The ratio of the diastereomers is shown in Fig. 8.

5. Conclusion

Capillary electrophoresis with laser induced native fluorescence detection (CE–LINF) using a frequency doubled argon ion laser allows a direct chiral determination of tramadol and also for the *O*-demethyl

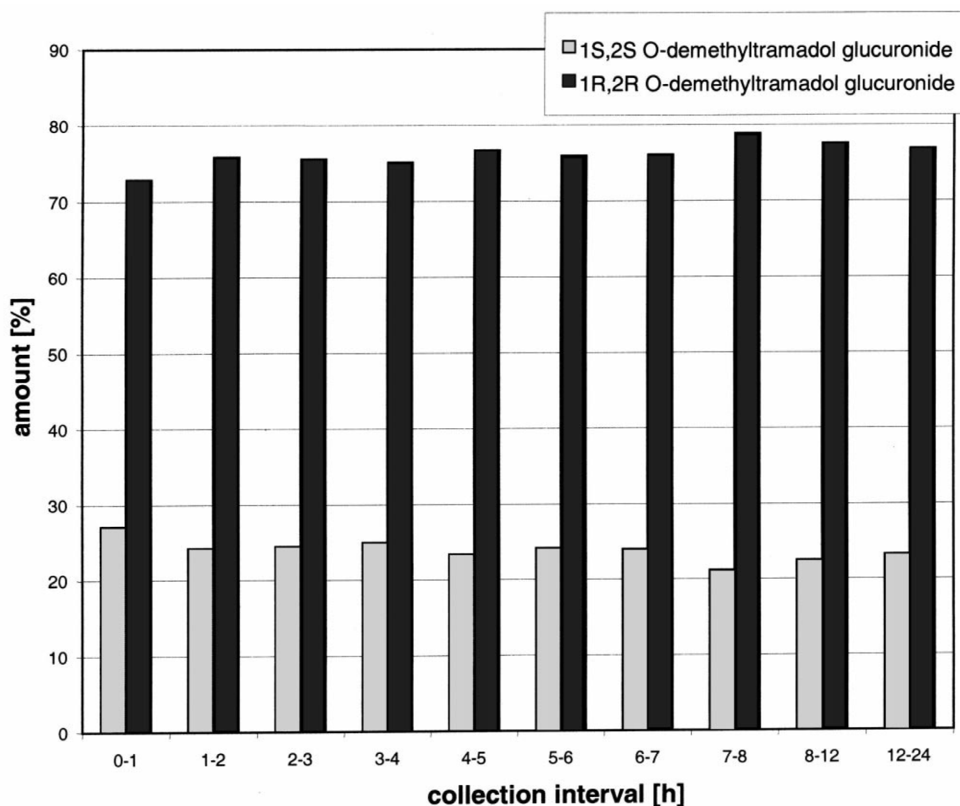

Fig. 8. Ratio of diastereomers. *O*-demethyl tramadol glucuronides in human urine after oral application of 150 mg tramadol hydrochloride.

tramadol glucuronide. Especially, the direct quantification of the hydrophilic glucuronide is advantageous to other methods. CE–LINF detection is a rapid, specific, sensitive and accurate method with great potential for sensitive assays of fluorescent drugs in biological samples.

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