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# Determination of SDZ ICM 567 in blood and muscle microdialysis samples by microbore liquid chromatography with ultraviolet and fluorescence detection

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**Abstract**

Fast, simple and accurate methods for the determination of SDZ ICM 567, the 7-methoxy derivative of tropisetron, in microdialysates have been developed. Sampling by microdialysis from freely moving rats in the portal and jugular vein offers a new technology for pharmacokinetic studies by direct and continuous measurement of unbound drug concentrations with time. SDZ ICM 567 can be identified in small sample volumes of dialysates on a microbore high-performance liquid chromatography column-switching system with ultraviolet detection. In addition, determination of SDZ ICM 567 by fluorimetric detection has been developed for muscle microdialysates from rats. [$^{14}$C]SDZ ICM 567 was used as reference substance for the estimation of the amount of substance transferred through the dialysis membrane. The radioactive measurement (RA) gave the recovery information, whereas the liquid chromatographic method detected the sum of [$^{14}$C]SDZ ICM 567 and dialyzed SDZ ICM 567. © 1997 Elsevier Science B.V.

*Keywords:* N-Methyltropyl-7-methoxyindole-3-carboxylate; Microdialysis; Column switching

## 1. Introduction

Microdialysis sampling in vivo is gaining in importance for the determination of pharmacokinetics [1–6] and metabolism [7–9]. Dialysis in brain became a widely used technique [10–13]. Application to bile [14], blood [15–20] or muscle [21–25] is more recent.

Blood sampling is performed by surgical implantation of a dialysis probe into veins of interest in an experimental animal. Muscle microdialysis is carried out by introducing a probe into the pectoralis muscle of rats. The probe is perfused at a low flow-rate by buffer (e.g., 2 μl/min). Low-molecular-mass compounds can diffuse across the dialysis membrane into the probe. The continuous flow through the probe carries the compounds from the sampling site for analysis. The detection system for the samples can be of a wide range, mainly used is liquid chromatography (LC) with various detection systems ranging from ultraviolet (UV) absorption to mass spectrometry (MS).

Pharmacologically, SDZ ICM 567 (N-methyltropyl-7-methoxyindole-3-carboxylate, I, Fig. 1) is an anxiolytic agent which can be detected in different ways. For common analytical measurements UV

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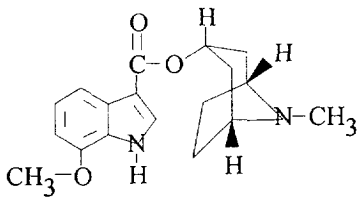


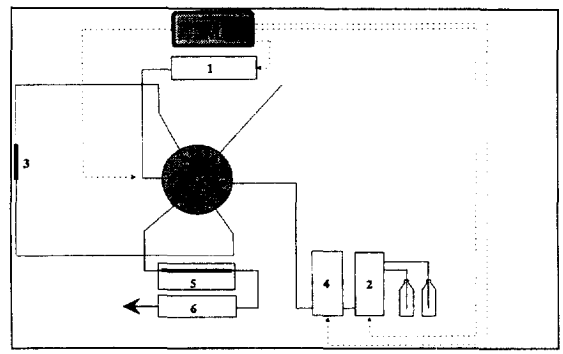
Fig. 1. Structure of I.

detection was used. A fluorimetric determination is applicable and the compound also has electrochemical activity. All the previous methods used standard size LC columns. In the case of the small sample volumes obtained from microdialysis, in the order of 15 to 35 μl, microbore columns were at first tried with UV detection. The concentrations of I expected in muscle dialysates were about ten times lower than those in blood samples. Therefore a ten-fold more sensitive fluorimetric determination has been developed. In this paper a comparison of these LC methods on RP-8 microbore columns is described for the detection of I in blood and muscle microdialysates. These methods were used to determine the pharmacokinetic parameters after oral and i.v. application to rat [26].

## 2. Experimental

### 2.1. Apparatus

The column-switching method used two pumps (Fig. 2): a Beckmann programmable solvent pump Model 126 (pump 1) and an auxiliary pump, a Beckmann programmable solvent pump Model 116 (pump 2) (Beckmann Instruments, San Ramon, CA, USA). The switching valves were electrical valves Type E-C6W from Valco (Schenkon, Switzerland). The injection was carried out by an autosampler, Model Gina 160 (Gynkothek, Munich, Germany) equipped with a 100 μl syringe. The sample injection volume was 25 μl. The vials consisted of polypropylene with a glass insert. The column was thermostatically controlled at 70°C by an oven from Hengeller Analytic (Riehen, Switzerland). The UV detector was from Linear Instruments (Reno, NV, USA) Model UVIS 204, with the standard UV cell, path length 6 mm, volume 9 μl. The fluorescence




M PC-Controller
A VALCO Electrical Valve Typ E-C6W
1 Pump 1: Beckman Programmable Solvent Pump 126
2 Pump 2: Beckman Programmable Solvent Pump 116
3 Micro-Precolumn Stagroma 2 x 10 mm
4 Autosampler Gynkothek Model Gina 160
5 Analytical Column Stagroma C8, Spherisorb 5μm, 2x 100 mm
6 UV-Detector Linear UVIS 204

Fig. 2. Column-switching scheme for blood microdialysis.

detector used was from Waters 470 (Milford, MA, USA). All the capillaries were of polyether ether ketone (PEEK) material with an I.D. 0.18 mm. The integration of the chromatograms was carried out with the Access Chrom Data Acquisition System from Perkin-Elmer–Nelson Systems (Cuppertino, CA, USA) on a Microvax via an A/D interface, Type 941.

### 2.2. Chemicals and reagents

Acetonitrile and methanol were of HPLC-grade from Rathburn (Walkerburn, UK). Water was purified by ion-exchange through Waters Milli-Q-cartridges in the laboratory. Octanesulfonic acid sodium salt monohydrate (OSS) (No. 74885) was from Fluka (Buchs, Switzerland), sodium acetate (No. 6265) and 2-propanol (No. 9634) for the washing phases were from Merck (Darmstadt, Germany).

The reagents for the dialysis buffer, $Na_2HPO_4 \cdot 2H_2O$ (No. 6580) and $KH_2PO$ (No. 6404) were both from Merck. Methoxyflurane (Methofane) for the anaesthesia was from Pitman-Moore (Mundelein, IL, USA). I and the reference substance [$^{14}$C]I were prepared at Sandoz Pharma (Basel, Switzerland).

For dialysis two buffer solutions were prepared. Buffer 1 (0.02 *M* $Na_2HPO_4$ buffer) consists of 3.6 g

$Na_2HPO_4 \cdot 2H_2O$ and 6 g NaCl diluted in 1000 ml of water and buffer 2 (0.02 *M* $KH_2PO_4$ buffer) consists of 2.7 g $KH_2PO_4$ and 6 g NaCl in 1000 ml of water. Buffer 1 is adjusted by buffer 2 up to a pH of 7.4. The buffer is stable following storage at 4°C for at least 1 month.

Working standard solutions were prepared by dilution of 1 mg I with dialysis buffer in a volumetric flask to 10 ml and further dilution of this stock solution with buffer to give concentrations ranging from 0.01 to 100 ng/ml. The dilutions are stable at 4°C for at least one week. The standard solution of [$^{14}$C]I was prepared by dissolving 1 mg [$^{14}$C]I in 10 ml of water with 0.01% ethanol. This stock solution was diluted with dialysis buffer to give a concentration of 50 ng/ml (radioactivity of 34 000 dpm/μl) and was used as reference.

For precleaning the vein and muscle microdialysates a 0.01 *M* sodium acetate solution (1.36 g $NaCH_3COO \cdot 3H_2O$ in 1000 ml water) as aqueous washing phase [A] and acetonitrile–2-propanol–methanol (33:33:33, v/v/v) as organic washing phase [B] were used. The mobile phase [C] was prepared by mixing 20 parts 1% octanesulfonic acid in water and 80 parts acetonitrile (v/v).

### 2.3. Chromatographic conditions and switching parameters for blood microdialysates

An RP-8 analytical column from Stagroma (Wallisellen, Switzerland) 5 μm, 100×2 mm (Serial No. 280489 14) was used. The empty pre-column, 10×2 mm, was also from Stagroma and was manually filled with Perisorb RP18, 30–40 μm. The mobile phase (C) was applied by the main pump 1 (Fig. 2). The aqueous washing phase (A) was pumped by pump 2. All eluents were degassed with helium for 10 min. The flow-rate of the mobile phase was 0.2 ml/min, the flow-rate of the washing phase 0.5 ml/min. 25 μl microdialysate was transferred to the pre-column and cleaned of biological interferences; after 3.0 min I is transferred to the analytical column. The switching conditions in detail are presented in Table 1.

The settings of the UV detector were: $\lambda$=290 nm, range: 0.001, rise time: 4.0. The total run time was 11 min, the retention time of the substance 8.2±0.05 min.

### 2.4. Chromatographic conditions and switching parameters for the muscle microdialysis

The two six-port automated switching valves (Fig. 3) allow the flow to be directed in the forward-flush mode or in the back-flush mode from the pre-column to the waste and in the back-flush mode from the pre-column to the analytical column.

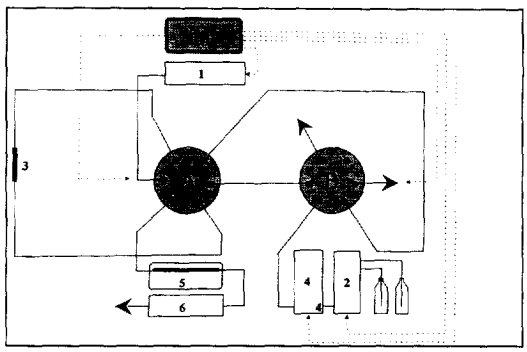
Pump 1 is used to deliver isocratically the mobile phase (C) to the analytical column at a flow-rate of 0.2 ml/min. Pump 2 is used to deliver either the aqueous buffer, phase A or the organic solvent mixture, phase B.

The program stored in the Analog Interface Module (Beckman System Gold) is started after injection of a 25 μl sample of microdialysate from pectoralis muscle as shown in Table 2. By pumping aqueous phase to the pre-column, I is retained at the head of the pre-column. The free polar endogenous com-

Table 1
Time program and switching parameters for the vein microdialysates

| Time (min) | Flow-rate (ml/min) | Phase A[a] (%) | Phase C[b] (%) | Comments |
|---|---|---|---|---|
| 0.0 | 0.5 | 100 | 0 | Injection of the microdialysate and washing off by-products |
| 3.0 | 0.2 | 0 | 100 | Switching valve is switched on; desorption of SDZ ICM 567 and transfer to the analytical column |
| 8.0 | 0.5 | 100 | 0 | End of the transfer and preconditioning of the pre-column for the next run |
| 11.0 | 0.5 | 100 | 0 | End of the time program |

[a] Mobile phase A: sodium acetate trihydrate, 2.72 g in 2000 g bidistilled water, pH 7.4.
[b] Mobile phase C: acetontrile–21.4 m*M* OSS (80:20, v/v).




**M** PC-Controller
**A, B** VALCO Electrical Valves Typ E-C6W
*1* Pump 1: Beckman Programmable Solvent Pump 126
**2** Pump 2: Beckman Programmable Solvent Pump 16
**3** Micro-Precolumn Stagroma 2 x10 mm
**4** Autosampler Gynkothek Model Gina 160
**5** Analytical Column Stagroma C8 Spherisorb 5 μm, 2x 100 mm
*6* Fluorescence Detector Waters Model 470

Fig. 3. Column-switching scheme for muscle microdialysis.

ponents from the microdialysates were washed off in the back-flush mode to avoid interferences before elution of the analyte. To prevent peak broadening, I is eluted also in the back-flush mode from the pre-column to the analytical column by pumping mobile phase C. After elution and separation, the intrinsic fluorescence of I is detected. The excitation and emission wavelengths of the detector were set to 292 nm and 355 nm, respectively.

### 2.5. Calculation of results in biological samples

For the calculation of the recovery from the microdialysis probe [$^{14}$C]I is added to the buffer as a reference at a concentration of 50 ng/ml [$C_{in}$]. The recovery is calculated according to the following equation:

$$\text{Recovery} = \frac{[C_{in}] - [C_{out}]}{[C_{in}]} \quad (1)$$

where [$C_{in}$] = concentration of [$^{14}$C]I added to perfusate (solution entering the probe) and [$C_{out}$] = concentration of [$^{14}$C]I in the dialysate (solution leaving the probe).

The unlabelled drug, I was applied i.v. or orally and samples were collected over 30-min periods. The volume was divided into two parts, 20 μl for the RA measurement of radioactivity for the determination of recovery, and 35 μl for the high-performance liquid chromatography (HPLC) measurement. The HPLC method detected both, cold and hot I [$C_{HPLC}$]. Therefore a calculation of free I in blood (Eq. (2)) [$C_{free}$] after oral or i.v. application is possible:

$$[C_{free}] = ([C_{HPLC}] - [C_{out}]) \times \text{recovery} \quad (2)$$

The total concentration [$C_{total}$] is calculated in the following way:

$$[C_{total}] = [C_{free}] \times 5 \quad (3)$$

Table 2
Time program and switching parameters for the muscle microdialysates

| Time (min) | Flow-rate (ml/min) | Phase A[a] (%) | Phase B[b] (%) | Phase C[c] (%) | Comments |
|---|---|---|---|---|---|
| 0.0 | 0.5 | 100 | 0 | 100 | Injection of microdialysate and aqueous front washing |
| 2.0 | 0.5 | 100 | 0 | 100 | Back-flush washing |
| 3.0 | 0.2 | 0 | 0 | 100 | Back-flush transfer into analytical column |
| 7.0 | 1.0 | 0 | 100 | 100 | End of transfer and forward-flush organic solvent pre-column washing |
| 10.0 | 2.0 | 100 | 0 | 100 | Reconditioning of pre-column by aqueous front washing for next injection |
| 20.0 | 1.0 | 100 | 0 | 100 | End of time program |

[a] Mobile phase A: sodium acetate trihydrate, 2.72 g in 2000 g bidistilled water, pH 7.4.
[b] Mobile phase B: methanol–aceonitrile–2-propanol (33:33:33, v/v/v).
[c] Mobile phase C: acetontrile–21.4 m*M* OSS (80:20, v/v).

The factor 5 depends on the protein binding, which is 20% for I, as described above.

For each analytical sequence a calibration curve was calculated from the areas obtained for the calibration standards. The models used were as follows:

$$F_i = a + bX_i \quad (4)$$

$$V_i = (P1)^2(F_i)^{P2} \quad (5)$$

The regression parameters $a$ and $b$ as well as the variance parameters $P1$ and $P2$ were determined by the program ELSFIT [27] in order to minimize the objective function OBJels:

$$\text{OBJels} = \sum[(Y_i - F_i)^2(1/V_i) + \ln V_i] \quad (6)$$

where $X_i$ and $Y_i$ are the concentration and peak area of particular calibration standards, respectively, and $\Sigma[\,]$ is the sum of the function over all calibration standards.

## 3. Microdialysis

### 3.1. Blood microdialysis and sample preparation

For the determination of the bioavailability and the first-pass-effect of I the microdialysis method was tested in freely moving rats. Dialysis buffer is perfused through probes implanted into the portal and jugular vein. The microdialysis cellulose acetate membranes of the cylindrical probes made of soft cannulae have a length of 10 mm, 0.5 mm O.D. and a molecular mass cut-off of 20 000. This specification allows only free I and other low-molecular-mass compounds to diffuse into the buffer. Substances bound to proteins, proteins and other large molecules are not able to pass the membrane. Earlier investigations have shown that the free fraction of I in blood is 20%, is independent of the concentration.

Male Wistar rats (Hanover, 139 KV), weighing 250–300 g, were used in this study. After anaesthesia with methoxyflurane, microdialysis probes were implanted into the portal and the right jugular veins. Each rat was used in two experiments at the first and the third day after surgery. For the experiment the rat was placed in a cage. The probes were perfused at a flow-rate of 2 μl/min with buffer containing 50 ng/ml [$^{14}$C]I as reference. From the outlets of the probes samples were collected in 30-min periods over 4 h after drug administration. On the first day the drug was administered orally (3 mg/kg) and on the third day intravenously (1.5 mg/kg). The samples were stored at −20°C until analysis. Table 3 shows the results obtained after an i.v. administration.

### 3.2. Muscle microdialysis and sample preparation

Microdialysis sampling from muscle tissue has been described in detail previously [26]. In brief, a

Table 3
Sample table for a pharmakokinetic study of I after i.v. application containing the sampling time, $ICM_{HPLC}$, $ICM_{free}$ and $ICM_{total}$ concentrations (all corrected for recovery) in portal and jugular vein

| Sampling time (min) | Portal vein | | | Jugular vein | | |
|---|---|---|---|---|---|---|
| | $ICM_{HPLC}$ (ng/ml) | $ICM_{free}$ (ng/ml) | $ICM_{total}$ (ng/ml) | $ICM_{HPLC}$ (ng/ml) | $ICM_{free}$ (ng/ml) | $ICM_{total}$ (ng/ml) |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 24.2 | 54.73 | 237.94 | 18.6 | 36.82 | 204.58 |
| 60 | 13.4 | 30.30 | 131.75 | 13.6 | 26.93 | 149.59 |
| 90 | 9.2 | 20.81 | 90.46 | 12.2 | 24.15 | 134.19 |
| 120 | 5.9 | 13.34 | 58.01 | 9.0 | 17.82 | 98.99 |
| 150 | 4.9 | 11.08 | 48.18 | 7.1 | 14.06 | 78.09 |
| 180 | 9.2 | 20.81 | 90.46 | 5.3 | 10.59 | 58.29 |
| 210 | 0 | 0 | 0 | 2.7 | 5.35 | 29.70 |
| 240 | 0 | 0 | 0 | 2.9 | 5.74 | 31.90 |

flexible microdialysis probe was introduced into the jugular vein and a similar flexible microdialysis probe was implanted into the pectoralis muscle. Before the beginning of analysis, the probes were abundantly equilibrated by perfusion with phosphate buffer at a flow-rate of 2 μl/min for at least 30 min. Thereafter, the perfusion medium was rapidly replaced by a solution containing [$^{14}$C]I as the reference compound to permit the determination of the efficiency of the probes. After 15 min, an i.v. bolus of 1.5 mg/kg I was injected via the femoral vein. Microdialysis sampling from muscle was carried out for 2.5 h in 30-min collection periods.

## 4. Results and discussion

### *4.1. Chromatography and specificity for venous dialysates*

As the microdialysis perfusion flow-rate is typically only 2 μl/min, the sample volumes are very small and the development of a very sensitive method was necessary. Therefore, we tried to use a microbore LC column, micro pre-columns and a micro UV cell. The micro UV cell did not give appropriate results at a flow-rate range higher than 250 μl/min. A normal UV cell gave better results using a flow-rate of 300 μl/min.

Initially, an isocratic method with liquid extraction had been evaluated and as the detection limit was low enough for a pharmacokinetic study, we analyzed some samples. However, biological interferences in the blank samples made this method unsuitable for routine work. Therefore, further investigations were undertaken using a column-switching system. This technique allows an on-line cleaning of the perfusates on the pre-column, which leads to sharp substance peaks without interferences by a blank. Furthermore, this column-switching technique is also applicable to plasma samples, simplifying the precleaning procedure and reducing the analysis time enormously.

Fig. 4 shows the chromatograms obtained with an I standard solution (100 ng/ml) (A), a microdialysis blank perfusate from a jugular vein (B) and a microdialysis sample (C) of the jugular vein 30 min after application of 1.5 mg/kg i.v. I. This typical chromatogram shows no interferences after the venous dialysis and precleaning the samples by a column-switching system.

Fig. 5 displays the pharmacokinetic profile of [$C_{free}$] and [$C_{total}$] drug in the jugular and portal veins after an i.v. application of 1.5 mg/kg I. The concentrations are in good agreement to values

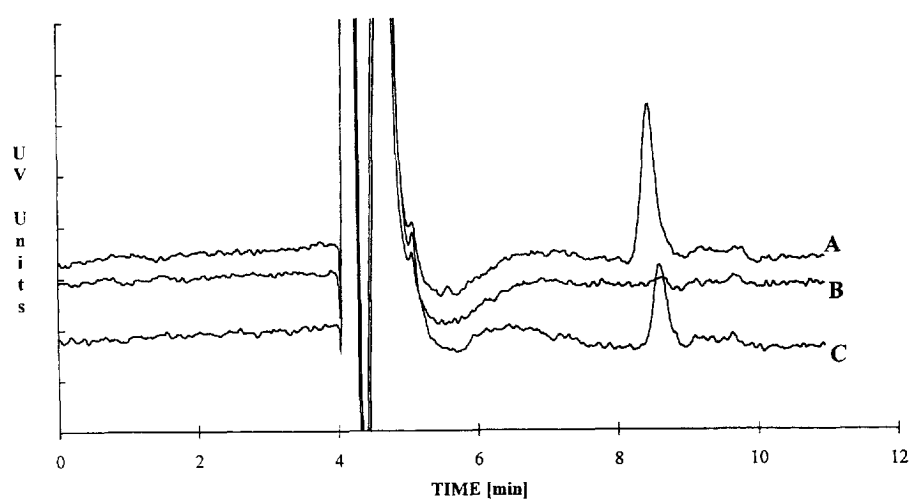



Fig. 4. Chromatogram of (A) a I standard containing 50 ng/ml, (B) a microdialysis blank and (C) a microdialysis sample of the jugular vein after 30 min i.v. application containing 25 ng/ml I.

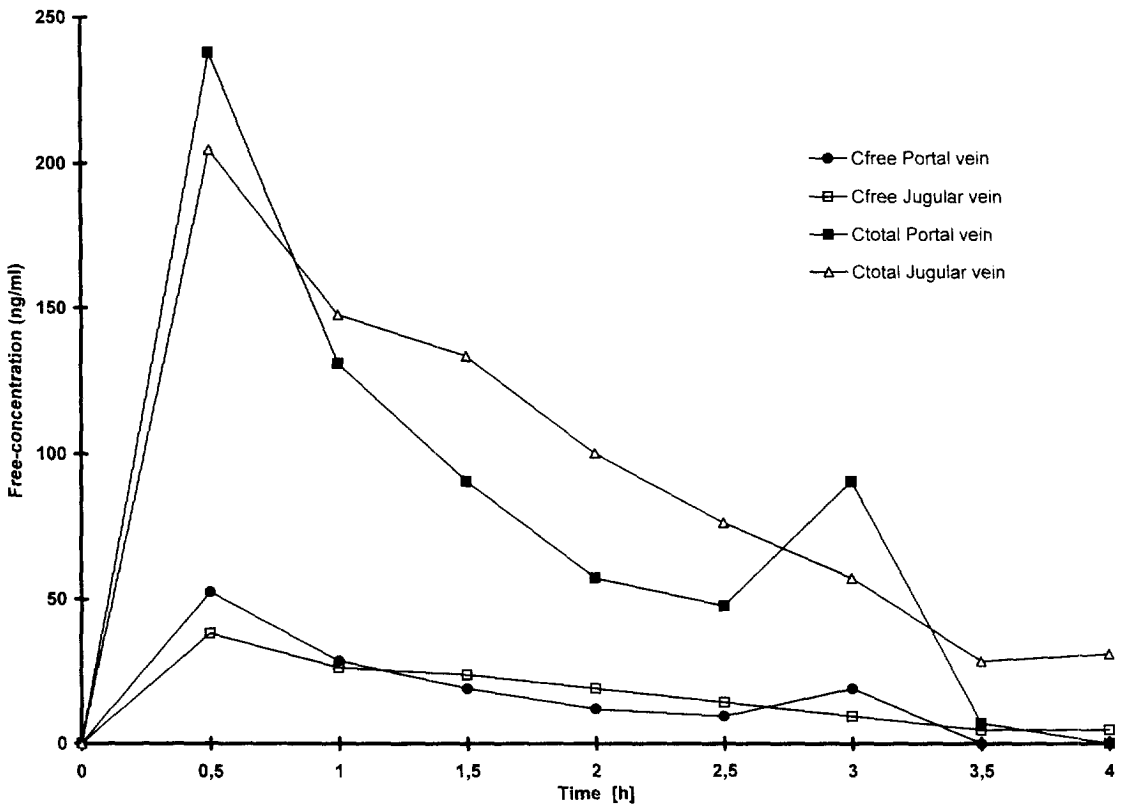



Fig. 5. Pharmacokinetic profiles of the free and the total concentrations of I (corrected for recovery) in portal and jugular vein after i.v. application (1.5 mg/kg rat).

measured with conventional techniques. Thus, the new sampling technology in combination with this analytical method allows a fast and accurate determination of I in the blood of live animals.

The advantage of this sampling method is to avoid blood loss, and as the animals are free moving, not anaesthetized, the conditions for pharmacokinetic studies are more comparable to normal conditions than with other techniques. Disadvantages are possible blood clotting problems for the semi-permeable dialysis membrane and the very difficult operation, which takes a lot of sensitivity as the portal vein has only an I.D. of 2–3 mm and a high flow-rate. The high pressure in this vessel, its location and the small I.D. require a highly developed surgical technique. The jugular vein has an I.D. of only 2 mm but is easier to handle due to its better accessibility and the lower flow-rate.

### *4.2. Chromatography and specificity for muscle*

Fig. 6 shows a chromatogram of a microdialysate from the pectoralis muscle of a rat. At the retention time of I the chromatogram of blank (Fig. 6A) was free of interferences by non-protein bound endogenous compounds (see also Table 3). Fig. 6B and Fig. 6C show chromatograms of muscle microdialysates 2.5 h and 0.5 h after administration of an i.v. bolus of 1.5 mg/kg I, respectively. The retention time was 9.05 min ($n=8$, C.V. = 1.3%). The chromatograms are reproducible (isocratic mode), and no endogenous compounds interfere with this assay. The limit of quantification (LOQ) of I using fluorescence detection was 1.6 ng/ml (25 pg absolute) (Table 5).

The procedure was applied to the quantitative measurement of I in microdialysates from pectoralis muscle of rats with fluorescence detection. Fig. 7

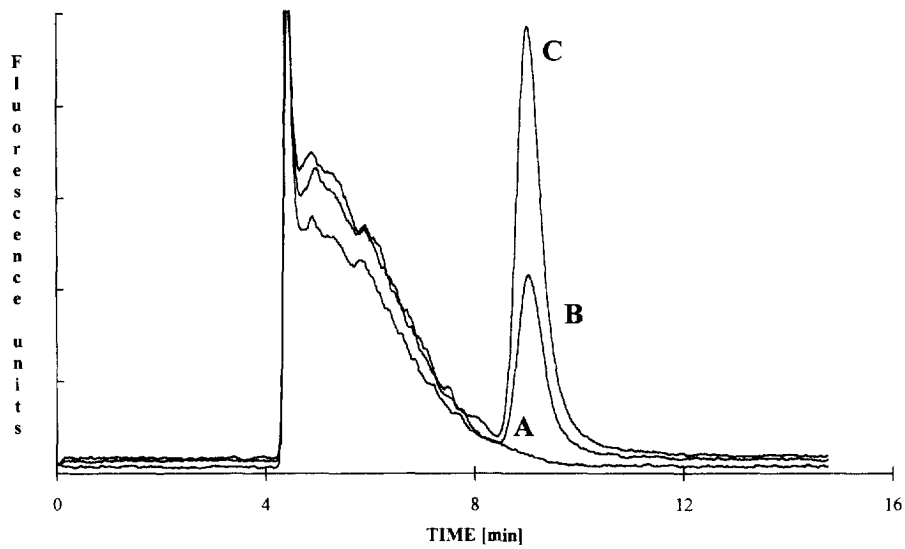



Fig. 6. Chromatogram of (A) blank muscle microdialysate from rat, (B) muscle dialysate 150 min after an i.v. bolus of 1.5 mg/kg of I ($C_{out}$ 32 ng/ml, uncorrected for recovery) and (C) muscle dialysate from rat 30 min after oral administration of 3 mg/kg I ($C_{out}$ 75 ng/ml, uncorrected for recovery).

shows the free-concentration (corrected for in vivo recovery) versus time in muscle obtained from three rats after administration of an i.v. bolus of 1.5 mg/kg I via the femoral vein. It shows a wide inter-individual variation, particularly during its distribution from blood to muscle.

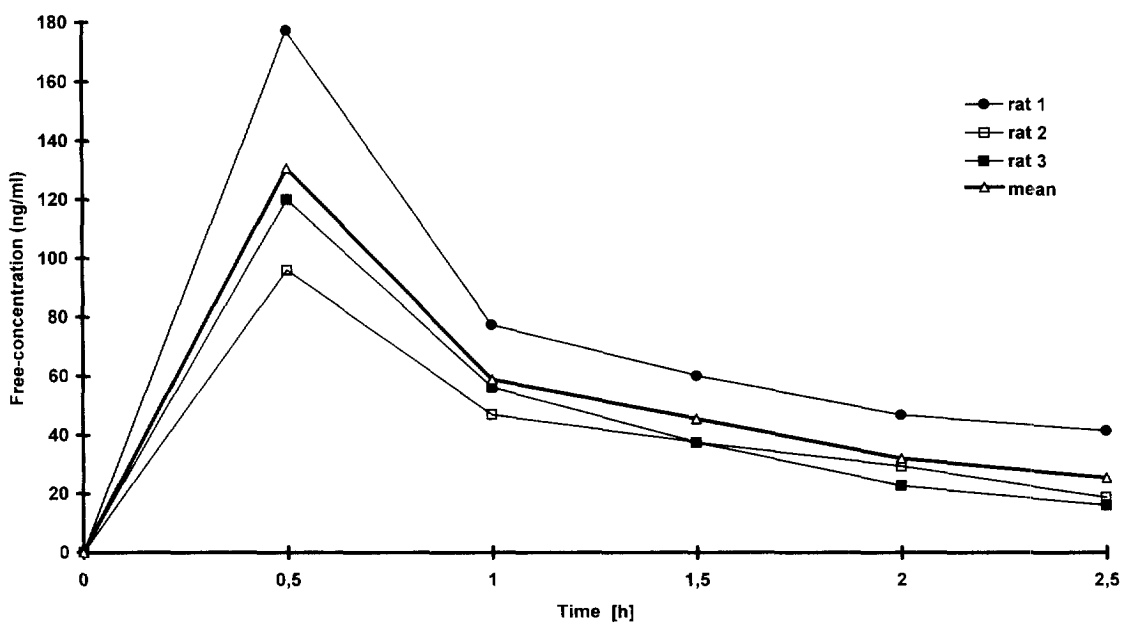



Fig. 7. Free concentration (corrected for in vivo recovery) versus time profile of I in muscle microdialysate from three rats after an i.v. bolus of 1.5 mg/kg.

Table 4
Intra- and inter-assay variation of I in dialysis buffer determined with HPLC–UV detection

| Spiked concentration (ng/ml) | Measured concentration (ng/ml, $n=8$) (Mean±S.D.) | Intra-day precision ($n=8$) C.V. (%) | Inter-day precision ($n=8$) C.V. (%) |
|---|---|---|---|
| 50 | 49.55±1.25 | 7.9 | 2.6 |
| 25 | 26.18±2.5 | 12.1 | 4.1 |
| 10 | 9.85±0.7 | 3.2 | 9.1 |
| 5 | 5.28±0.9 | 7.9 | 10.1 |

### 4.3. Precision and reproducibility in buffer

For the validation of the method the intra- and inter-day precision were determined. Table 4 presents the variability of four concentrations. The within-day coefficient of variation was between 3.2–12.1%, the between-day precision varied between 2.6–10.1%.

Based on the assessment of data (accuracy, reproducibility, precision) the LOQ was expressed as the least concentration for which the precision C.V. is below 20% was set to 5 ng/ml (125 pg/injected sample).

### 4.4. Linearity and accuracy in venous dialysates

The linearity of this method was tested over a range from 2–1000 ng/ml. A linear regression analysis using the peak area versus the concentration was performed on each day. The correlation coefficient was 0.997, the limit of detection (LOD) 3 ng/ml (75 pg absolute, signal-to-noise ratio=3).

A daily analysis of duplicate standards at concentrations of 3, 5, 10, 25, 50 and 100 ng/ml, and two reference-solutions (25 ng/ml) at the beginning and the end of the run were used to assess the linearity of the method. The daily mean of the references was 23.3 to 27.3 ng/ml. This shows an accuracy of the reference from 93.1–109%. This results support a linear concentration–response relationship.

Validation of results of the venous microdialysates was obtained analogous to the method used for microdialysates in muscle.

### 4.5. Linearity and accuracy in muscle microdialysates

The calibration curve was linear from 0.8 to 1000 ng/ml and the mean of correlation coefficient ($r^2$) was greater than 0.997 ($n=20$, C.V.=0.25%). Within-day and day-to-day precision are summarized in Table 5. The within-day precision was assessed by carrying four replicate calibration standards (perfusion of the microdialysis probes) of seven concentrations (200, 100, 50, 25, 12.5, 6.25, 1.56 ng/ml) through the entire procedure in one day. Between-day reproducibility was obtained by using the same standards for analysis over a period of 4 weeks. All coefficients of variation of within-day and day-to-day

Table 5
Accuracy and precision of I using fluorescence detection

| Spiked concentration (ng/ml) | Measured concentration (ng/ml, $n=4$) | | Within-day precision ($n=4$) C.V. (%) | Day-to-day precision (4 weeks, $n=2$) C.V. (%) |
|---|---|---|---|---|
| | Mean±S.D. | C.V. (%) | | |
| 200 | 199.0±8.1 | 4.0 | 4.1 | 4.5 |
| 100 | 98.4±3.9 | 4.0 | 4.0 | 4.5 |
| 50 | 44.2±5.3 | 10.7 | 12.1 | 9.0 |
| 25 | 20.7±0.5 | 2.0 | 2.4 | 3.2 |
| 12.50 | 8.8±0.9 | 7.9 | 11.2 | 10.1 |
| 6.25 | 4.4±0.4 | 5.9 | 8.4 | 9.8 |
| 1.56 | 1.2±0.1 | 2.6 | 3.7 | 8.8 |

precision were ranging from 2.4 to 12.1% and from 3.2 to 10.1%, respectively (Table 5).

## 5. Conclusions

Column-switching LC methods allow the accurate and rapid determination of I in microdialysis perfusates. The use of a microbore column (2 mm) provides better detection limits than those obtained with methods using standard sized columns, while requiring significantly less sample. The limits of quantification for both detection methods (125 pg absolute for the UV determination and 25 pg absolute for the fluorescence detection) provide a sensitive mean for quantification of microdialysates. The dialysis technique based on UV or on fluorescence detection of I offers a new technology for pharmacokinetic studies with sufficient reproducibility, sensitivity and accuracy. More than 1400 samples have been analyzed by the present method.

Further investigations will be performed for an on-line analysis method for a direct and rapid evaluation of perfusion experiments. Although this column-switching LC method has been developed for microdialysis sampling from tissues and/or blood, we have no doubt of its adaptability for plasma.

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