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# Fishing for a drug: solid-phase microextraction for the assay of clozapine in human plasma

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**Abstract**

Solid-phase microextraction (SPME) was investigated as a sample preparation method for assaying the neuroleptic drug clozapine in human plasma. A mixture of human plasma, water, loxapine (as internal standard) and aqueous NaOH was extracted with a 100-μm polydimethylsiloxane (PDMS) fiber (Supelco). Desorption of the fiber was performed in the injection port of a gas chromatograph at 260°C (HP 5890; 30 m×0.53 mm I.D., 1 μm film capillary; nitrogen–phosphorous selective detection). Fibers were used repeatedly in up to about 75 analyses. The recovery was found to be 3% for clozapine from plasma after 30 min of extraction. However, in spite of the low recovery, the analyte was well separated and the calibration was linear between 100 and 1000 ng/ml. The within-day and between-day precision was consistently about 8 to 15% at concentrations of 200 ng/ml to 1000 ng/ml. No interfering drug was found. The limit of detection was 30 ng/ml. The sample volume was 250 μl. The influence of the concentration of proteins, triglycerides and salt, i.e., changes in the matrix on the peak areas and peak-area ratios was studied. The method is not impaired by physiological changes in the composition of the matrix. Good agreement was found with a liquid–liquid extraction–gas–liquid chromatography (LLE–GLC) standard method and an on-line column-switching high-performance liquid chromatography (HPLC) method for patients' samples and spiked samples, respectively. It is concluded that the method can be used in the therapeutic drug monitoring of clozapine because the therapeutic window of clozapine is from 350 to 600 ng/ml. 

*Keywords:* Solid-phase microextraction; Clozapine

## 1. Introduction

Primarily, solid-phase microextraction (SPME) was used for the analysis of aqueous samples in environmental analytical chemistry. There are only a few publications that describe the use of SPME for assaying drugs in body fluids, i.e., urine, serum or plasma [1–9]. A first attempt to assay psychotropic drugs in human serum or plasma by SPME was published recently [4]. Based on the results of these publications, SPME is regarded as being a potentially suitable method for drug analysis in therapeutic drug monitoring (TDM) and clinical toxicology. The SPME equipment, which dips the fiber into the aqueous sample, looks like equipment used for

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fishing. This is unique and a little bit comical for a sample preparation method. For this reason, we would like to call the method 'fishing for an analyte' or 'fishing for a drug'.

Clozapine is an atypical neuroleptic drug. An 'awakening' of patients who were treatment-resistant to classical neuroleptic drugs was possible with clozapine. Furthermore, in contrast to the typical neuroleptics, no extrapyramidal adverse effects emerge in patients under clozapine therapy. The therapeutic window of clozapine was found to be between 350 and 600 ng/ml [10,11]. Plasma concentrations lower than 350 ng/ml are responsible for a suboptimal therapeutic outcome or even no therapeutic effect (nonresponders). Plasma concentrations higher than 600 ng/ml increase the risk of adverse effects, for instance, seizures. Therefore, treatment with clozapine can be optimized by assaying plasma concentrations. Several methods are known for the quantification of clozapine in human plasma: liquid–liquid extraction–gas–liquid chromatography–selected ion monitoring–mass spectrometry (LLE–GLC–SIM–MS) [12], solid-phase extraction–high-performance liquid chromatography–ultraviolet detection (SPE–HPLC–UV) [13,14], automated column-switching on-line HPLC–UV [15], LLE–megabore capillary–GLC–nitrogen–phosphorus selective detection (NPD) [16] etc. However, the advantages of SPME in sample preparation (solvent-free, easy handling, little equipment required, fast method, easy automation, good linearity) may also be used in the drug analysis of human plasma. SPME is mainly used in concert with GLC. For this reason, a SPME–GLC–NPD method was developed and investigated for the determination of clozapine in human plasma.

## 2. Experimental

### *2.1. Chemicals*

Clozapine (cloz) was kindly donated by Sandoz (Basel, Switzerland). Loxapine (lox), in its succinate form, was purchased from Research Biochemicals International, RBI (Natick, MA, USA). NaCl p.a. was from Laborchemie Apolda (Apolda, Germany). NaOH p.a. and methanol p.a. were purchased from Merck (Darmstadt, Germany). Water was from a 'Milli-Q' system from Millipore (Eschborn, Germany). Drug-free plasma was taken from healthy volunteers.

### *2.2. Solutions*

A solution (10 μg/ml) of clozapine was prepared daily by mixing 1 ml of a stock solution (100 μg/ml) of clozapine with 9 ml of water. Another solution (20 μg/ml) of clozapine was prepared analogously. The internal standard solution (8 μg/ml) of loxapine was prepared by mixing 800 μl of a stock solution (100 μg/ml) of loxapine with 9.2 ml of water. The solutions were stored at 4°C in the dark. A solution containing 1 *M* NaOH and 6% NaCl was prepared by dissolving 4 g of NaOH and 6 g of NaCl in 100 ml of water. A 50% methanol solution was prepared by adding 50 ml of methanol to 50 ml of water. Plasma was spiked with clozapine solutions of 10 and 20 μg/ml to determine the concentrations of the drug in plasma. To spike plasma, the volume of aqueous solutions added was always held constant at 5% of the volume of the calibration samples.

### *2.3. Sample preparation*

A 50-μl volume of internal standard was added to 250 μl of plasma in a 3-ml glass tube and the mixture was vortex-mixed for 10 s. A 1700-μl volume of water was added to this mixture, which was vortex-mixed for 10 s. Finally, 500 μl of 1 *M* NaOH (6% NaCl) were added and the mixture was again vortex-mixed for 10 s. Approximately 1.5 ml of this mixture was transferred to a 1.5-ml polyethylene tube (Eppendorf, Germany), which was fitted to a shaker (Thermomixer 5436, Eppendorf, Germany). The SPME was carried out by fixing a manual 100-μm polydimethylsiloxane (PDMS) fiber (Supelco, Bellefonte, PA, USA) in the vial and vigorously shaking it at 30°C for 30 min. After extraction, the fiber was washed for 20 s in water and for 20 s in 50% methanol. After 2 min drying in air at ambient temperature, the fiber was ready for injection into the gas chromatograph.

### *2.4. Apparatus and desorption*

A Hewlett-Packard 5890 series II gas chromatograph with a nitrogen–phosphorus-selective detector (NPD) and a split–splitless injector port was used

for the analysis. Separation was obtained with a 30 m×0.53 mm I.D., 1 μm film BPX-5 capillary (SGE, Weiterstadt, Germany) and with $N_2$ (20 ml/min, 150 kPa) as the carrier gas (1 ml/min septum vent). Flow-rates of the detector gases were as follows: air, 100 ml/min; $H_2$, 3 ml/min; auxiliary gas, $N_2$, 6 ml/min. The NPD detector port was maintained at 300°C. The NPD was operated at a baseline of 20 to 25 pA. The injector was operated at 260°C and in the splitless mode during the desorption time. The injection depth for the fiber was 4.4 cm. The run was started after 60 s of desorption and the split was opened. A temperature program was used for the oven and was stopped immediately after reaching the maximal temperature, $T_3$ ($T_1$=160°C, $T_2$=260°C, $T_3$=288°C; $\text{rate}_1$=40°C/min for $T_1$ to $T_2$ and $\text{rate}_2$= 4°C/min for $T_2$ to $T_3$). Hewlett-Packard GC-Chemstation software was used for calculation of retention times, peak widths and peak areas.

### 2.5. Conditions for sample preparation and measurement

Several preliminary experiments were conducted to determine the optimal conditions for extraction and desorption. Extraction was investigated by varying the extraction time from 5 min to 24 h in water and plasma (three measurements in each case). The desorption time was varied from 10 to 90 s (three measurements in each case). The fiber was desorbed for a second time (postdesorption) of 90 s. Therefore, the residue of the first desorption was also analyzed. Two capillaries were tested to optimize the separation and the sensitivity of the method: HP-5: 5% diphenyl- and 95% dimethylpolysiloxane (25 m×0.2 mm I.D.; 0.33 μm film-thickness; Hewlett-Packard, Waldbronn, Germany) and BPX-5: 5% phenyl (equivalent) polysilphenylensiloxane (30 m×0.53 mm I.D.; 1 μm film-thickness; SGE, Weiterstadt, Germany). The photosensitivity of clozapine was tested by exposure of plasma samples to sunlight for 0 to 5 h. Six fibers were used in parallel for six measurements in each case. Special equipment was designed to do this. The equipment consisted of a simple plastic board perforated with 24 holes and was held between two retort stands on top of the shaker. A maximum of 24 fibers could be extracted simultaneously using this method. The diameter of the holes was fitted to the diameter of the manual fiber assemblies (Supelco).

### 2.6. Quantitation, precision and recovery

The calibration curves were constructed by plotting peak-area ratios ($A_{\text{cloz}}/A_{\text{lox}}$) versus the concentration of clozapine. The concentrations used were 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ng/ml. The limit of detection was estimated at a signal-to-noise ratio of three. The limit of quantification was estimated to be about three times the limit of detection. The precision was determined by the coefficient of variation of the peak-area ratios at four concentrations of clozapine (100, 200, 500 and 1000 ng/ml). For determination of the precision, sufficient quantities of pooled samples at each concentration were prepared and aliquoted for the separate measurements. Six measurements were made for the within-day precision and eight measurements for the between-day precision. Different fibers were always used for the estimation of precision, i.e., precision may be described as '*inter*-fiber' precision. The recovery was determined by comparison of the peak areas of clozapine and loxapine determined using SPME with values obtained for defined amounts of clozapine and loxapine after injection of liquid solutions. The amount injected was comparable with the amount of analytes in the SPME samples after dilution with water and addition of the aqueous NaOH.

### 2.7. Comparison with standard methods

The method was compared with a standard method that is based on LLE and GLC–NPD analysis (spiked samples and patients' samples). This standard method is very similar to a method for the assay of haloperidol which was published recently [17]. However, the concentration of the internal standard solution (chlorhaloperidol) was 10 μg/ml and the final extract of 100 μl was not evaporated. The use of LLE–megabore capillary–GLC–NPD to assay clozapine in human plasma was also described in the literature [16]. In addition, the SPME method was compared with a routine HPLC method [15] that is used in an external laboratory (spiked samples). The results were analyzed by linear regression.

### 2.8. Influence of the matrix

The influence of the matrix on the extraction by SPME was tested by varying the concentrations of proteins, triglycerides and salt. Human plasma spiked with clozapine and loxapine was diluted with zero to nine volumes of water to investigate the influence of proteins on the extraction procedure. The concentration of triglycerides was expected to have an influence on the extraction because most psychopharmacological drugs are highly lipophilic. Therefore, a mixture of an aqueous albumin solution (5% albumin) with three kinds of triglycerides (palmitin-, linole- and stearin-triglycerides) at various concentrations (from 0 to 10 mg/ml triglycerides) was prepared for the tests. The tests were conducted at two concentrations of clozapine (500 and 1000 ng/ml). The concentration of NaCl was varied from 0 to 20% for analysis of the effect of salt.

### 2.9. Lifetime of fibers

The fibers were used repeatedly. The number of applications was not investigated under exactly the same conditions for one fiber, i.e., conditions varied considerably during validation of the method (salt concentration, lipids, temperatures, times of extraction, use of other methods). The number of desorptions was higher than the number of extractions because of postdesorption experiments and the condition of the fibers. However, a curriculum was recorded for every fiber.

## 3. Results and discussion

### 3.1. Extraction, recovery and desorption conditions

Fig. 1 shows the dependence of the peak areas of clozapine and loxapine on the extraction time. Equilibrium was reached after about 720 min of extraction in a mixture of plasma and water (1:7, v/v). As a compromise to speed up the method, an extraction time of 30 min was used for further experiments. An extraction temperature of 30°C was found to be most suitable because the matrix contains proteins that denature at higher temperatures and that clotted around the fiber. The extraction

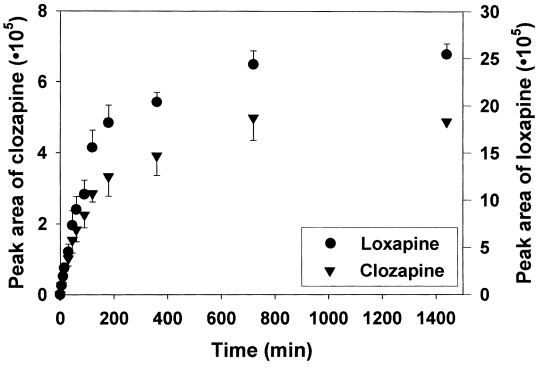


Fig. 1. Dependence of the peak areas of clozapine and loxapine on the extraction time in plasma (mean±S.D., three measurements in each case), with 50 ng/ml of each in the extraction vial and a volume of 1.5 ml.

graph in water was equivalent, but equilibrium was reached after an extraction time of about 180 min (Fig. 2).

The recoveries of clozapine and loxapine were low, being 3.2 and 19.4% after 30 min of extraction with a new fiber (number of extractions and desorptions <25), respectively. However, the recoveries decreased even further in old fibers, i.e., after multiple extractions and desorptions (>50), the values were 1.4% for clozapine and 7.6% for loxapine after 30 min of extraction, and 7.1% for clozapine and 40.7% for loxapine at equilibrium. However, it is important to emphasize that the ratio of the recoveries in new and old fibers remained

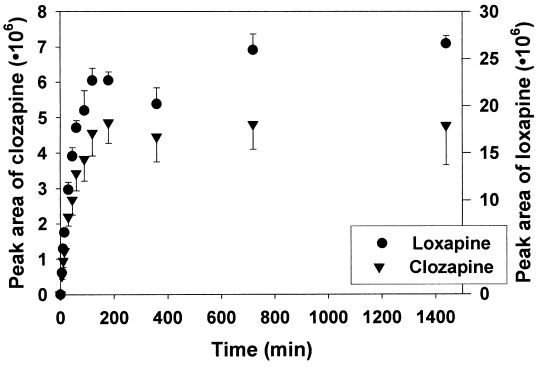


Fig. 2. Dependence of the peak areas of clozapine and loxapine on the extraction time in water (mean±S.D., three measurements in each case), with 400 ng/ml of each in the extraction vial and a volume of 1.5 ml.

constant. The recoveries of the extraction from water were considerably higher after 30 min of extraction and rather similar in equilibrium, i.e., 4.1% for clozapine and 26.4% for loxapine after 30 min of extraction, and 9.1% for clozapine and 54.0% for loxapine at equilibrium. The high protein binding of both drugs appears to be the main limiting factor inhibiting a more rapid extraction in plasma. This was found also for tricyclic antidepressant drugs [4]. However, whether the low recovery lowers the quality and applicability of the method requires further investigation in experiments involving changes in the matrix. It was shown that this is not the case for the present method (see below). Furthermore, a low recovery does not necessarily imply insufficient precision of a method. This is the case for instance when using a method with a well-chosen internal standard, as in the present case. Changes in the recovery due to changes in the matrix, changes in the fiber or due to small deviations from the standard procedure (extraction time, properties of the fiber, etc.) are compensated for by the internal standard. The similar behaviors of the analyte and internal standard regarding extraction time and the constant ratio of recoveries in new and old fibers are two examples of the capacity of the internal standard to compensate for differences in the recovery of the analyte, i.e., the extraction of internal standard is not finished after, say, 5 min and the analyte needs hours or vice versa. Taking into account this equivalent behavior during sample preparation, extraction and desorption, the numerical differences in recovery between cloz and lox are of no concern. However, the precision will be investigated in detail below.

The distribution constants, $K_{fw}$, of clozapine and loxapine between the fiber (the coating) and water were calculated using the known amount of drug extracted in equilibrium and the volume of the sample (1.5 ml) and of the coating ($6.6 \cdot 10^{-4}$ ml [4]). Values of $K_{fw}=227$ for clozapine and $K_{fw}=2671$ for loxapine resulted. Both values indicate a distribution that is highly in favor of the coating of the fiber. This was expected because of the known lipophilicity of psychotropic drugs. However, the considerable difference between clozapine and loxapine (a factor of ten) was not expected. Thus, this difference is explained by the protic N–H–hydrogen atom that occurs in clozapine but not in loxapine. In spite of the lipophilicity of both compounds, this difference in the chemical structure is thought to account for the higher hydrophilicity of clozapine. Thus, although loxapine is rated as a good internal standard for clozapine, *N*-propylnorclozapine [12] is expected to provide an even better performance. However, *N*-propylnorclozapine is not commercially available.

At a temperature of 260°C, the optimal desorption time for clozapine was found to be 60 s. In this time, nearly 100% of the analyte is desorbed and the fiber can be used in subsequent extractions. Fig. 3 shows the desorption and postdesorption graph of clozapine. The graph for loxapine is similar (data not shown). The exposure time to sunlight had no influence on the peak area of clozapine. The peak-area ratio ($A_{cloz}/A_{lox}$) was also constant after various exposure times. Therefore, photosensitivity, which is suspected for several tricyclic antipsychotic drugs [18], does not impair the method.

### 3.2. Matrix effects

Fig. 4 shows the influence of the concentration of proteins on the extraction. The concentration of proteins was changed by dilution of samples with water. In spite of the decreased concentration of analytes in the diluted sample, the peak areas of clozapine and loxapine remained almost constant for the entire series of measurements. This also applied to the peak-area ratios (data not shown). The peak areas increased if the concentration of analyte was held constant with decreased concentration of pro-

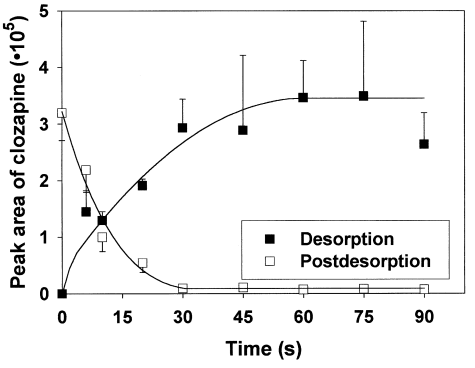


Fig. 3. Dependence of the peak area of clozapine on the desorption time (mean±S.D., three measurements in each case), with $T=260$°C and 500 ng/ml clozapine in the sample.

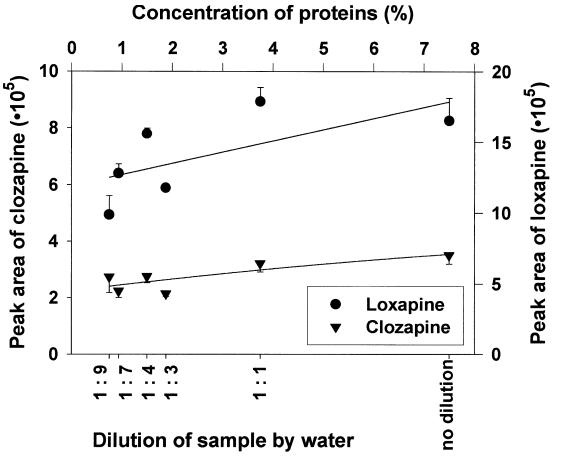


Fig. 4. Dependence of the peak areas of clozapine and loxapine on the dilution of the sample with water (mean±S.D., three measurements in each case), with 500 ng/ml each in the sample without dilution.

teins (data not shown). This effect of plasma proteins has been discussed in detail in a previous publication [4]. Because of these results, the plasma samples were diluted with seven volumes of water in further experiments. This will decrease the influence of changes in the matrix on the method. Furthermore, the lifetime of the fiber will be improved because clotting of proteins on the fiber during extraction, and burning in of contaminations into the polymer film during desorption, will be lowered.

Fig. 5 shows the relationship between the concentration of triglycerides and the peak areas of clozapine and loxapine. With increased concentrations of triglycerides, the peak areas of clozapine and loxapine decreased (cloz: $r=0.411$, $P=0.09$; lox: $r=0.612$, $P=0.007$; linear model). This is explained by the increased lipophilicity of the matrix, which decreases the extraction of lipophilic drugs. However, due to the increased viscosity of the matrix, this effect may also be explained, at least in part, by the decreased diffusion of the analytes. The peak-area ratio ($A_{cloz}/A_{lox}$) increased only very slightly on increasing the concentration of triglycerides (Fig. 6). Furthermore, it should be recognized that the physiological concentration of triglycerides is between about 2.4 and 4.2 mg/ml in human plasma. Therefore, it is concluded that this physiological variability of the matrix (including highly lipemic plasma) cannot impair the method because these small differences in $A_{cloz}/A_{lox}$ are within the precision of the assay (see below).

Fig. 7 shows the influence of added salt on the matrix. Peak areas of clozapine and loxapine did not change in the range from 0 to 8% NaCl. The peak areas decreased at higher concentrations of NaCl (8 to 20%). An empirical model $y=[-b_0x/(b_1-x)]+b_2$, where $y$=peak area and $x$=concentration of salt, was fitted to the data and revealed an adequate goodness of fitting (cloz: $r=0.990$, $P=0.029$; lox: $r=0.981$, $P=0.057$; software, SPSS). The decrease in the peak areas may be explained by the increased viscosity of the sample at higher concentrations of salt. This effect may overcome an increase in peak

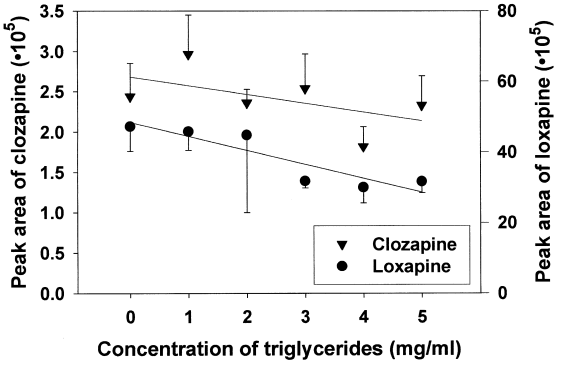


Fig. 5. Dependence of the peak areas of clozapine and loxapine on the concentration of triglycerides (mean±S.D., three measurements in each case), using the SPME standard method and with 500 ng/ml clozapine in the sample.

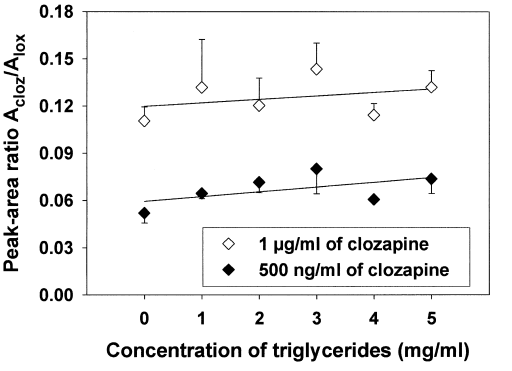


Fig. 6. Dependence of the peak-area ratio, $A_{cloz}/A_{lox}$, at two concentrations of clozapine (500 ng/ml and 1 µg/ml) on the concentration of triglycerides (mean±S.D., three measurements in each case) using the SPME standard method.

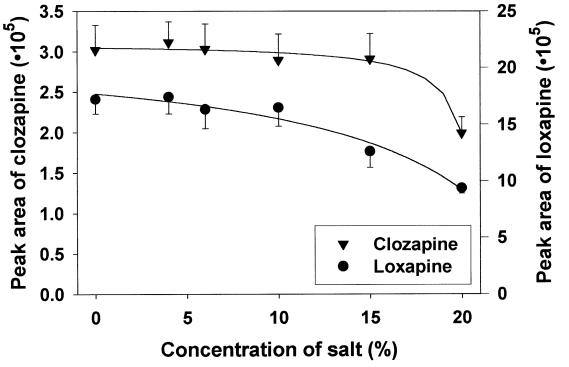


Fig. 7. Dependence of the peak areas of clozapine and loxapine on the concentration of salt (mean±S.D., three measurements in each case) with 500 ng/ml of each in the sample.

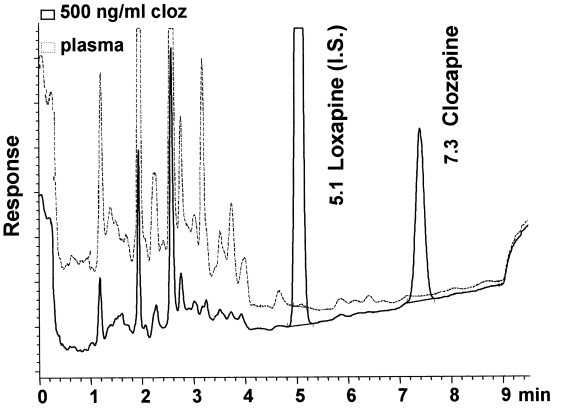


Fig. 8. Typical chromatograms of drug-free plasma and plasma spiked with 500 ng/ml of clozapine.

areas due to the increased polarity that was expected initially for the lipophilic drugs investigated. Peak-area ratios did not change with increased concentrations of salt (data not shown).

In summary, differences in the matrix influence the peak areas of clozapine and loxapine to some degree. However, the peak-area ratio, $A_{cloz}/A_{lox}$ remains constant within the range of the precision of the method. Because of the low recovery of the method, it is important to clarify that differences in the matrix do not influence the results. The choice of internal standard is crucial for the development of a SPME method for human plasma. Loxapine was chosen as an internal standard and was found to be suitable for assaying clozapine because of its very similar chemical structure and physicochemical properties to those of clozapine. In comparison to other areas of chemical analysis, it is easy to find a suitable internal standard in drug analysis.

### *3.3. Chromatographic conditions and selectivity*

Two capillaries were tested during optimization of the separation and sensitivity of the method. The peak areas of the 0.2-mm-I.D. capillary were too small for the anticipated sensitivity. Therefore, a megabore capillary was tested. Peak areas increased considerably and were regarded as being large enough for the anticipated detection limit. Fig. 8 depicts typical chromatograms of drug-free plasma and plasma spiked with 500 ng/ml of clozapine. No interfering peaks appeared in the chromatograms of drug-free plasma from healthy volunteers. Signals from the matrix were mainly in the range $t_R<4$ min. A very minor peak at $t_R=5.1$ min appeared and partly interfered with loxapine (I.S.). However, this peak did not affect the identification and quantitation because the peak area of loxapine was very high in comparison with this interference. The method was tested for selectivity with regard to possible interferences from several classes of psychotropic drugs, metabolites of clozapine, the matrix (plasma) and contaminant of the fiber. No interferences with other drugs, desmethylclozapine (Table 1), with the matrix or with contaminants of the fiber were found. A particular problem is the thermal degradation of clozapine-*N*-oxide to clozapine during injection in

Table 1
Retention times of potential interfering drugs

| Drug or metabolite | Retention time (min) |
|---|---|
| Amitriptyline | 3.1 |
| Nortriptyline | 3.1 |
| Desipramine | 3.3 |
| Oxazepam | 3.7 |
| Clomipramine | 3.9 |
| Lorazepam | 4.0 |
| Levomepromazine | 4.5 |
| **Loxapine** | **5.1** |
| Olanzapine | 6.1 |
| **Clozapine** | **7.3** |
| Desmethylclozapine | 7.9 |
| Haloperidol | 8.1 |
| Reduced haloperidol | 8.6 |

GC. This may result in an overestimation of clozapine in samples from patients [19]. The injection of a liquid solution of clozapine-*N*-oxide (3 μl of a 25-ng/μl solution) confirmed this potential interference. However, the amount of clozapine formed was small in this liquid injection and no clozapine was detected in chromatograms of SPME extracts of 500 ng/ml of clozapine-*N*-oxide in plasma. Therefore, the method is selective, as required for TDM.

### *3.4. Calibration and precision*

Calibrations were found to be linear between 100 and 1000 ng/ml. Calibration curves were typically described by the formula $y=Bx+A$, where $y$ is the peak-area ratio, $A_{cloz}/A_{lox}$, $x$ is the concentration of clozapine, and A and B are the intercept and slope, respectively. Typical results for $A$, $B$ and $r$ of linear regression were −0.0051, 0.000134 ml/ng and 0.987, respectively. The limits of detection and determination were 30 and 100 ng/ml, respectively. The method is regarded as being suitable for TDM because the therapeutic window of clozapine is between 350 and 600 ng/ml. Precision was determined as the percentage coefficient of variation of six measurements in each case at four concentrations (100, 200, 500 and 1000 ng/ml). The within-day precisions were 10.0, 7.9, 9.7 and 14.5%, respectively. The results of the between-day precision (eight measurements in each case) were 22.6, 12.7, 7.9 and 10.6%, respectively. The between-day precision of 22.6% at 100 ng/ml may be regarded as an additional indication of the limit of determination of 100 ng/ml. It should be emphasized that the precision was determined using different fibers, i.e., this is an *inter*-fiber precision. Sometimes, absolute peak areas varied considerably between fibers, depending mainly on the number of previous applications of a fiber (up to about 100 applications!). The older the fiber, the lower the absolute peak areas were found to be. However, the peak-area ratios remained relatively constant. This issue concerning other influences has been discussed previously, for example, extraction time. Nevertheless, in spite of the considerable compensation for differences in fibers by the internal standard, the precision is expected to be slightly better if only one fiber is used for analyses (*intra*-fiber precision). The precisions found for other clozapine assay methods have been reported in the literature to be within the concentration range of about 50 to 1000 ng/ml: < 6% [12], 1.7 to 6.3% [13], 1.5 to 4.5% (within-day) and 1.3 to 9.4% (between-day) [14], 2.9 to 8.9% (within-day) and 6.3 to 7.5% (between-day) [15], and 1 to 3.8% [16]. Therefore, possible advantages of SPME as a sample preparation method are at the cost of a slightly lower precision. Nevertheless, the precision is sufficient for an application in TDM.

### *3.5. Comparison with standard methods and assay of patients' samples*

The method was compared with a standard method of the same laboratory that is based on traditional LLE and GLC–NPD analysis. A different capillary and a different internal standard were used. Eight identical samples, between 100 and 1000 ng/ml, were measured with both methods and the results were analyzed by linear regression. Very good agreement was found, as indicated by a correlation coefficient $r=0.9810$, slope $B=0.9618$ and intercept $A=14.4$ ng/ml (linear model). Very good agreement was also found when comparing the results ($n=16$) with those obtained using the routine HPLC method of an external laboratory [15]:$r=0.976$, $B=1.00$, $A=18.2$ ng/ml (Fig. 9). Finally, several samples from patients of the local psychiatric clinic ($n=26$) were analyzed simultaneously by SPME–GLC–NPD and

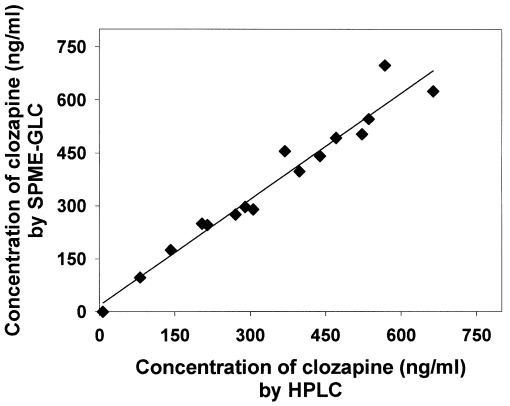


Fig. 9. Comparison of the SPME–GLC method with a HPLC standard method (spiked samples).

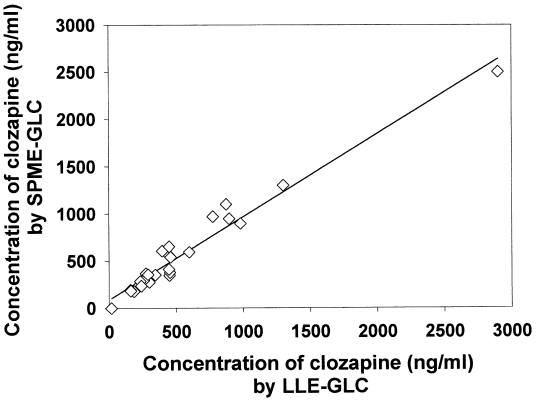


Fig. 10. Comparison of the SPME–GLC method with a LLE–GLC standard method (patients' samples).

LLE–GLC–NPD (Fig. 10). Very good agreement was found between the methods, as indicated by $r=0.979$, $B=0.879$ and $A=90.1$ ng/ml. This also applies to the data analysis without the value $>2000$ ng/ml, which was considerably higher than the calibration range of both methods: $r=0.923$, $B=1.034$ and $A=26.2$ ng/ml.

### 3.6. Lifetime of fibers

The number of repeated applications of a fiber was between about 50 and 100 times (Table 2). This gives an indication of the high chemical and thermal stability of the polydimethylsiloxane film. The washing step is crucial to prolong the lifetime of the fiber. The performance of fibers decreased after about 50 extractions, i.e., peak areas decreased by a factor of about two. Thus, as shown above, the recovery decreased in old fibers and the sensitivity also decreased. If the peak area of the internal standard drops to half of that of a new fiber, this may serve as a signal to discard a fiber after repeated applications. However, it is possible to increase the extraction time to maintain a constant sensitivity in extractions with older fibers. It should be mentioned that the present validation of the method was conducted under extreme (bad) conditions, therefore, sometimes using old fibers. It is expected that the precision of the method may be slightly improved if fibers are not used for more than 50 repeated extractions. Besides its simplicity, the cost-saving of SPME is mainly determined by the repeated use of fibers.

Table 2
Lifetime of the fibers

| Fiber | Number of conditions | Number of extractions | | Number of injections |
|---|---|---|---|---|
| | | From plasma | From water | |
| 1 | 0 | 60 | 5 | 87 |
| 2[a] | 5 | 27 | 3 | 58 |
| 3[a] | 4 | 27 | 4 | 57 |
| 4 | 4 | 46 | 5 | 73 |
| 5 | 4 | 103 | 12 | 141 |
| 6[b] | 4 | 20 | 5 | 39 |
| 7 | 3 | 71 | 5 | 88 |
| 8 | 1 | 84 | 4 | 99 |
| 9 | 1 | 43 | 0 | 49 |

[a] Fibers were tested at extremely high extraction temperatures (50°C).
[b] Fiber was broken.

## 4. Conclusions

SPME is anticipated as being an effective and robust method for the TDM of selected drugs. Sample preparation by SPME represents a cost-saving in comparison to three-step LLE and SPE because considerably less equipment, glassware, chemicals and time are needed. It may be similar to a simple one-step LLE or even automated column-switching on-line HPLC. Criteria for determining if analysis of a drug by SPME would be favorable are that (1) it should have a concentration that is not too low in serum or plasma and (2) a high retention index, to get a good separation from matrix peaks. These two criteria are fulfilled by clozapine. Because the therapeutic window of clozapine is between 350 and 600 ng/ml and its quantification limit is 100 ng/ml, the method is sensitive enough for TDM. The retention index is high enough in comparison to interferences by the matrix. In spite of low recoveries, which are caused by protein binding, suitable methods for the quantitative analysis of other drugs may also be developed in the future. Indeed, the influence of the matrix has to be investigated in more detail than in sample preparation methods with higher recoveries, for example, LLE.

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