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# Liquid-phase microextraction as a sample preparation technique prior to capillary gas chromatographic-determination of benzodiazepines in biological matrices

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**Abstract**

Liquid-phase microextraction (LPME) and gas chromatography were applied to determine diazepam and the main metabolite *N*-desmethyldiazepam in human urine and plasma. The analytes were extracted from 3.0–3.5 ml sample volumes directly into 25 μl of extraction solvent. The microextraction device consisted of a porous hollow fiber of polypropylene attached to two guiding needles inserted through a septum and a 4 ml vial. The hollow fiber filled with extraction solvent was immersed in sample solution. The extraction device was continuously vibrated at 600 rpm for 50 min. An aliquot (1 μl) of the extraction solvent with preconcentrated analytes was injected directly into the capillary gas chromatograph. Thirty samples were extracted simultaneously on the vibrator, providing a high sample capacity. The limits of detection were from 0.020 to 0.115 nmol/ml for diazepam and *N*-desmethyldiazepam in plasma and urine using a nitrogen–phosphorus detector (NPD). 

*Keywords:* Liquid-phase microextraction; Sample preparation; Benzodiazepines

## 1. Introduction

In the analysis of drugs in human urine and plasma liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are the most commonly used techniques for preconcentration and cleanup of samples prior to chromatographic analysis. Common sample volumes are 0.5–5 ml and the target analytes are normally collected in 0.2–10 ml of solvent after extraction in LLE or elution in SPE. These techniques provide analyte enrichment by a factor of 2–10. Evaporation of the solvent to dryness and reconstitution of the dry residue in a smaller solvent volume may achieve further enrichment. In this way target analytes can be preconcentrated up to a factor of 10–20 prior to chromatographic analysis. Evaporation of solvent and reconstitution are time consuming processes, which utilise relatively large amounts of solvents. These processes could be avoided if the sample preparation method delivered the target analytes in a sufficiently small volume of solvent, suitable for direct injection into an analytical instrument.

Solid-phase microextraction (SPME) is a totally

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solvent free sample preparation technique suitable for small sample volumes. The method was originally developed for analysis of aqueous samples in environmental analysis, but has been shown to be suitable for analysis of volatile drugs in biological matrices [1–20]. In spite of its advantage of being a solvent free technique SPME has not been widely implemented in drug analysis. The time to reach equilibrium applied in bioanalysis of drugs is quite long using SPME, in addition the recovery is low [6,7,17,19].

We have recently described a simple in-vial liquid-phase microextraction (LPME) device for drug analysis compatible with capillary gas chromatography (GC), capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) [21]. The target analytes were extracted from the sample matrix and into the small volume of acceptor solution and were preconcentrated by a factor of 30–125. The acceptor solution was directly injected into the analytical instrument. For LPME combined with GC the porous fiber was filled with a suitable organic solvent. For LPME combined with CE and HPLC an organic solvent is immobilised into the pores of the hollow fiber and the internal volume of fiber is filled with aqueous solution in which the analyte is highly soluble. Thirty samples were extracted simultaneously providing a high sample capacity. The solvent consumption was reduced by 99% compared to the traditional methods used for sample preparation.

In this report details on LPME method development prior to GC are discussed. Benzodiazepines are used as model substances and these are determined in human plasma and urine by LPME–GC. The primary goal is to optimise analyte recovery and enrichment by altering the chemical nature of sample matrix and the extraction solvent.

## 2. Experimental

### 2.1. Chemicals

Diazepam and *N*-desmethyldiazepam were obtained from Apothekernes Laboratorium (Oslo, Norway). Prazepam was a gift from the National Institute of Forensic Toxicology (Oslo, Norway). Butyl acetate, hexyl ether and 1-octanol were purchased from Sigma Chemical (St. Louis, MO, USA). Sodium hydrogenphosphat-1-hydrat, sodium hydrogenphosphat-2-hydrat and methanol (MeOH) were from Merck (Darmstadt, Germany). Deionized water was obtained from a Barnstead Easy pure Water System (IA, USA).

### 2.2. Preparations of standards

Stock standard solution (2 μmol/ml) of diazepam, *N*-desmethyldiazepam and prazepam (internal standard, I.S.) were prepared in methanol. Urine and plasma samples spiked with diazepam (0.5–8.0 nmol/ml) (plasma), *N*-desmethyldiazepam (0.5–8.0 nmol/ml) and prazepam (I.S.) (5 nmol/ml) were prepared freshly prior to analysis from stock solutions.

### 2.3. Sample preparation

An aliquot of urine sample (3500 μl) was placed in a 4 ml vial and prazepam (I.S.)(5 nmol/ml) and 300 μl 0.1 *M* phosphate buffer (pH 7.5) were added. The mixture was agitated for 30 s.

A 3000 μl plasma sample was added to prazepam (I.S.)(5 nmol/ml) and mixed in a 4 ml vial. Then 200 μl methanol was added to the sample to reduce the protein binding of the benzodiazepines. The sample was agitated for 30 s. No further pH control was performed.

### 2.4. Liquid-phase microextraction (LPME)

The LPME device in Fig. 1 consisted of two conventional 0.8 mm O.D. medical syringe needles (guiding needles) inserted through a silicon septum in the screw top (4 ml vial). The two guiding needles were connected by a 6 cm piece of Q 3/2 Accurel KM polypropylene hollow fiber (Akzo Nobel, Wuppertal, Germany). The characteristics of the hollow fiber were as follows; the inner diameter was 600 μm, the thickness of the wall was 200 μm, and the pore size was 0.2 μm. Each piece of fiber was used only once.

The hollow fiber was first dipped in the organic solvent for about 10 s to immobilize solvent in the pores. A 50 μl syringe was used to fill the hollow fibre with 25 μl of the extracting solvent (acceptor

Figure 1

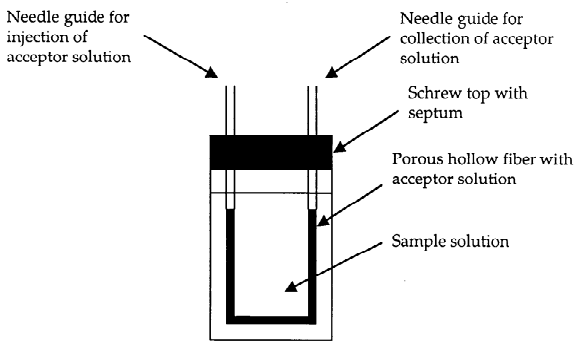


Fig. 1. Disposable LPME device.

solution). For the extraction from urine a mixture of butyl acetate: 1-octanol (1:1, v/v) was used. A mixture of hexyl ether: 1-octanol (1:3, v/v) was utilised as acceptor solution when extracting from plasma.

The hollow fiber with the immobilised extraction solvent was placed into the sample and the extraction unit was vibrated (600 rpm) for 50 min on a Vibramax 100 (Heidolph, Kelheim, Germany). The acceptor solution was collected by application of a small head pressure on one of the guiding needles, transferred to 200 μl inserts and placed in a 2 ml autosampler vial for automated injection into the GC.

### 2.5. Capillary gas chromatographic analysis

The automated injection (1 μl) was performed by a Varian 8200 CX GC Autosampler (Varian, Walnut Creek, CA, USA). The capillary gas chromatograph was equipped with a 30 m×0.25 mm I.D. SPB-1™ (polymethylsilicon) column (0.25 μm film thickness, Supelco, Bellefonte, PA, USA) and a nitrogen–phosphorus detector (NPD). A deactivated fused-silica column (1 m×0.25 mm I.D.) (Supelco, Bellefonte, PA, USA) was used as a retention gap and was coupled between the injection port of the GC and the analytical column. The temperature of the injector and the detector was set at 300°C and 250°C, respectively. Helium was used as the carrier gas at a flow-rate of 2 ml/min (140°C). The detector gases were hydrogen (4 ml/min) and air (175 ml/min). Helium was used as make-up gas at a flow-rate of 26 ml/min. Column temperature programming for urine analysis was: 140°C (initial temperature) held for 1 min, 40°C/min to 230°C, 5°C/min ramp to 250°C, 20°C/min ramp to 300°C (final temperature) with a hold time for 2 min (total 12 min). The temperature programming for the plasma analysis was as outlined for urine analysis except for the omission of the 5°C/min ramp to 250°C. The chromatograms were recorded by Varian Star Chromatography Workstation, version 4.5.

### 2.6. Calculation of enrichment factor and recovery

The enrichment factor ($E_e$) was defined as the ratio between the final analyte concentration $C_a$ in the acceptor phase and initial sample concentration $C_o$ within the sample:

$$E_e = \frac{C_a}{C_o} \quad (1)$$

The recovery ($R$) was defined as the percentage of total amount of analyte, which was transferred to the acceptor phase at the end of the extraction:

$$R = \frac{C_a V_a}{C_o V_s} \cdot 100 \quad (2)$$

where $V_a$ is the acceptor volume and $V_s$ is the sample (donor) volume.

Both enrichment factors and extraction recoveries after LPME of the sample solutions were determined by comparison of peak-heights obtained by injection of standard solutions versus peak-heights obtained from injection of acceptor solution after LPME. Estimation of the recoveries from Eq. (2) were based on an acceptor volume ($V_a$) of 25 μl.

### 2.7. Validation of the method

The calibration graphs for the determination of *N*-desmethyldiazepam in urine and diazepam and *N*-desmethyldiazepam in plasma were based on peak-height measurements versus the peak-height of the I.S. Samples spiked to 1.0, 3.0 and 5.0 nmol/ml of the drugs were analysed for intra- and inter-assay validation data ($n=6$). The limit of detection was determined at a signal-to-noise ratio of 3 ($S/N=3$) and the limit of quantification at a signal-to-noise ratio of 10 ($S/N=10$).

## 3. Results and discussion

### 3.1. Basic principles

The disposable LPME device is illustrated in Fig. 1. The sample solution was filled into a 4 ml sample vial and 25 μl of the acceptor solution was immobilised into the hollow fibre. The analytes of interest were substances of basic nature and the donor solution is adjusted to deionise the analytes and consequently reduce their solubility within the sample solution. The analytes were extracted from an aqueous sample solution through a porous hollow fiber into an organic acceptor phase. During extraction each LPME device was vibrated to promote analyte extraction.

Provided there were high partition coefficients from the sample to the acceptor phase, analyte enrichment occurred due to high volume ratio between the sample and the acceptor solution. Disposable LPME devices eliminated the possibility of carry-over effects and there was no need for regeneration of the porous hollow fiber.

### 3.2. Modification of sample matrix

Diazepam and *N*-desmethyldiazepam are extensively bound to plasma proteins (98–99%) [22] and should be liberated prior to extraction. Decreasing the degree of drug–protein binding can be performed by several actions [23,24]. Both alteration of pH and addition of an organic modifier to plasma may change the chemical structure of the protein so that the drug–protein binding is reduced. The addition of MeOH and 1 *M* HCl in MeOH was tested. The results of these experiments showed that an addition of 200 μl MeOH was sufficient to reduce the degree of drug–protein binding to give acceptable enrichment. An increase in the amount of MeOH added to plasma did not enhance the recovery, but resulted in clogging of endogenous compound around the hollow fiber. The benzodiazepines were best extracted at a pH of 7, and after addition of 200 μl MeOH no modification of the pH was needed.

A third approach to reduce the protein binding is the addition of displacement agents with a higher affinity for the specific protein-binding site than the drug. Addition of the selective displacer 1-octanoic acid (1 m*M*) was evaluated. This displacer has been successfully used in the analysis of benzodiazepines in plasma [25]. However, the addition of 1-octanoic acid (1 m*M*) to plasma resulted in an increased extraction of endogenous compounds and the hollow fiber appeared yellow after extraction. The 1-octanoic acid was therefore not added further, and 200 μl MeOH was the only modifier used in plasma prior to LPME.

Urine is a matrix with extensive individual variations in e.g. pH and content of salt. The addition of 300 μl 0.1 *M* phosphate buffer (pH 7.5) was found to ensure a pH of about 7 in the different urine samples tested.

The addition of salt to the urine matrix may decrease the solubility of the target analytes and therefore increase the enrichment of analytes [10,26,27]. The effect of salt addition depends upon the analyte extracted rather than on the type of extraction. In these experiments with LPME the addition of sodium chloride gave no further enrichment of diazepam and *N*-desmethyldiazepam and was therefore omitted in the final method.

### 3.3. Selection of acceptor solution

Selection of extraction solvent is of major importance in LPME as in LLE. The water immiscible organic solvent used as acceptor solution immobilised in the hollow fiber should be selected to provide high solubility of target analytes (good extraction solvent) and be compatible with direct injection into the capillary GC column. For practical reasons it is an additional advantage to select a solvent of relatively low volatility to prevent evaporation during extraction and transfer to autosampler vials. Because the hollow fiber was made of polypropylene the solvent should have a matching polarity to effectively wet the walls of the pores of the hollow fiber to prevent leakage during extraction and enhance contact between the extraction solvent and the sample.

Due to previous experience 1-octanol [5,9,28] was investigated along with several other organic solvents. 1-octanol is favorable, as log *P* values are available for a lot of analytes [29,30].

In the LPME extraction from urine 1-octanol, ethyl acetate and butyl acetate were tried as acceptor solvents alone or in mixtures with 1-octanol (Table

Table 1
Extraction recoveries of *N*-desmethyldiazepam from urine, and diazepam and *N*-desmethyldiazepam in plasma with different organic solvents as acceptor phase

| Matrix | Solvent/mixture | Extraction recovery (%) |
|---|---|---|
| Urine | 1-octanol | 28 |
| | butyl acetate: 1-octanol (1:1, v/v) | 69 |
| | ethyl acetate: 1-octanol (1:1, v/v) | 46 |
| Plasma[a] | hexyl ether: | 62 D |
| | 1-octanol (1:3, v/v) | 41 ND |
| | hexyl ether: | 60 D |
| | 1-octanol (3:1, v/v) | 43 ND |
| | hexyl ether: | 68 D |
| | 1-octanol (1:1, v/v) | 50 ND |

[a] D: Diazepam; ND: *N*-desmethyl-diazepam.

1). The solvents investigated were immiscible with urine, had relative low volatility and therefore a minimum of evaporation during extraction. Based on these experiments, a mixture of butyl acetate and 1-octanol 1:1 (v/v) was selected as acceptor phase for the microextraction from urine.

During the LPME extraction from plasma loss of acceptor solution was observed when the mixture of butyl acetate and 1-octanol (1:1) was used as acceptor. Different mixtures of 1-octanol and hexyl ether (1:3, 3:1, 1:1 (v/v)) (Table 1) were therefore tested as acceptor solutions. The 1:3 (v/v) composition of hexyl ether and 1-octanol was selected as acceptor phase in the microextraction from plasma as it showed slightly better $E_e$ than the other mixtures.

### 3.4. Optimisation of extraction

During LPME extraction the device was vibrated to replenish donor phase around the hollow fiber and the acceptor phase inside the hollow fiber. Fig. 2 shows enrichment factors obtained of analytes by LPME versus the extraction time. Fifty min was selected as the optimal extraction time for both urine and plasma. At this extraction time the plateau of equilibrium is reached, providing stability and constant extraction conditions for the samples.

The extraction recoveries found were 69% and 50% for *N*-desmethyldiazepam in urine and plasma and the corresponding enrichment factors $E_e$ were

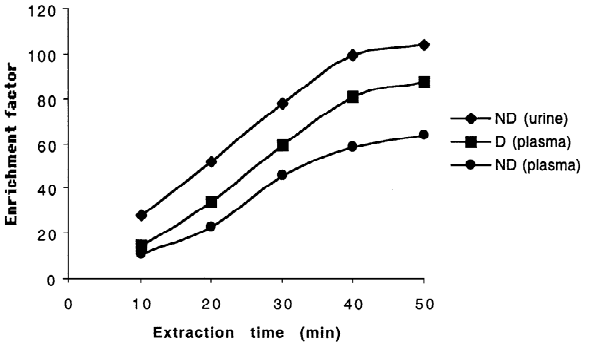


Fig. 2. Enrichment factors obtained by LPME of diazepam (■ D) and *N*-desmethyldiazepam (● ND) in plasma and *N*-desmethyldiazepam (♦ ND) in urine.

104 and 64. For diazepam in plasma the recovery was 68% and the enrichment factor $E_e$ was 88.

The extraction recovery obtained in plasma was lower than in urine when the analytes were extracted for the same time. This can be explained by plasma containing endogenous compounds that exert a higher degree of viscosity than urine matrix. In addition the degree of drug–protein binding has to be considered.

Compared to different LLE and SPE methods [31–34] the LPME method gave a satisfactory sensitivity and a much better enrichment of the analytes of interest. In addition the consumption of solvents per analysis was reduced by up to 200 times.

Other comparable membrane methods like supported liquid membrane extraction (SLM) and microporous membrane liquid–liquid extraction (MMLLE) typically give enrichment factors of 30–70 in biomedical applications [35–38]. The advantage of LPME compared to SLME or MMLLE is the elimination of memory effects, since each fiber is only used once. However an I.S. should be added in LPME to compensate for small variations in fiber dimensions.

### 3.5. Capillary GC analysis

A satisfactory separation of the compounds was achieved on a polymethylsilicon capillary column within 9 and 12 min for urine and plasma analysis, respectively.

The deactivated retention gap of 1 m was sufficient to provide reconcentration of the analytes by solvent effect and reduce the peak broadening. Up to five analyses could be performed per hour for urine and plasma samples.

Chromatograms of drug-free urine sample and a urine sample spiked with 5 nmol/ml of *N*-desmethyldiazepam and prazepam (I.S.) are shown in Fig. 3. Presented in Fig. 4 are the chromatograms of drug-free plasma and plasma spiked with 5 nmol/ml diazepam, *N*-desmethyldiazepam and prazepam (I.S.). The extracts are remarkably clean and no interfering peaks were detected in either drug-free plasma or urine samples.

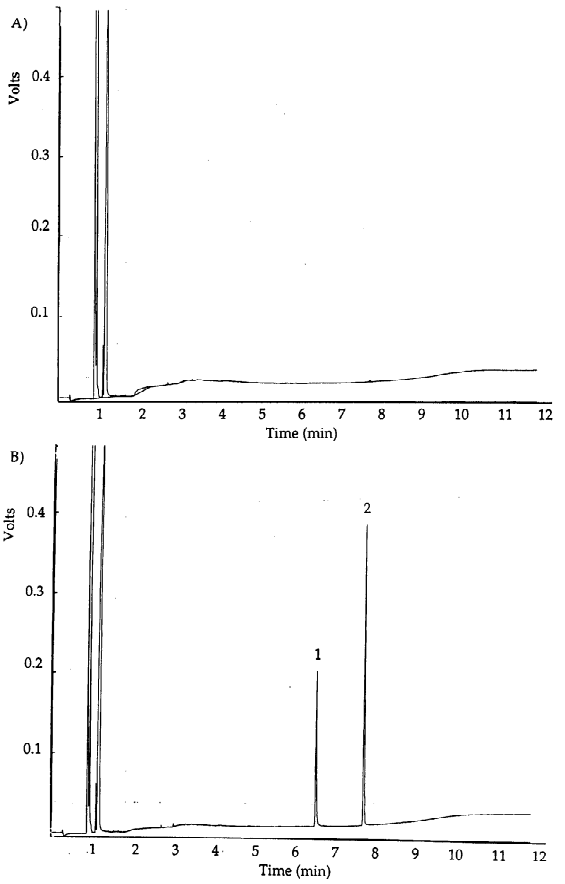


Fig. 3. Chromatograms of (A) drug-free urine sample and (B) a urine sample spiked with 5 nmol/ml *N*-desmethyldiazepam and prazepam (5 nmol/ml, I.S.). Peaks: 1 = *N*-desmethyldiazepam, 2 = prazepam.

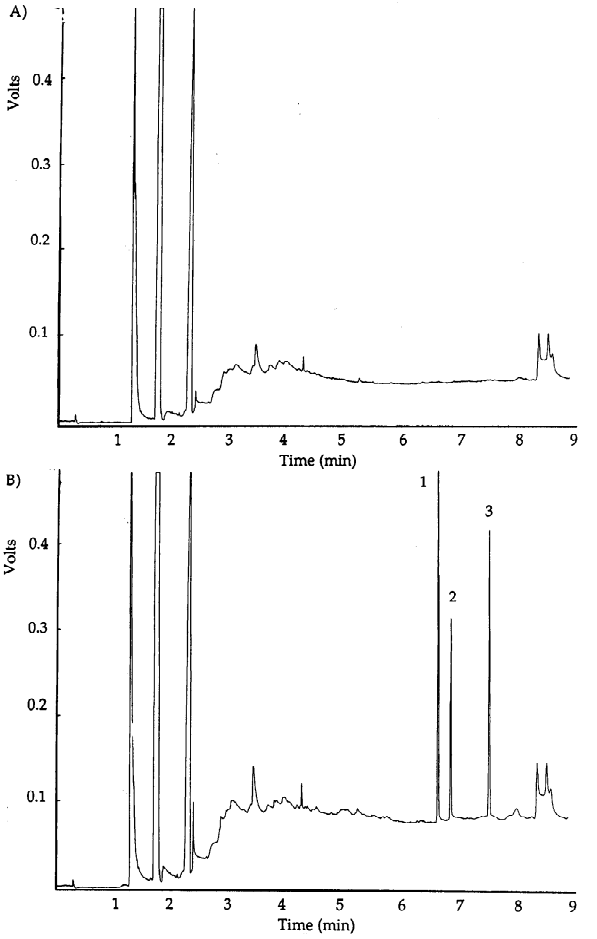


Fig. 4. Chromatograms of (A) drug-free plasma sample and (B) a plasma sample spiked with 5 nmol/ml diazepam *N*-desmethyldiazepam and prazepam (5 nmol/ml, I.S.). Peaks: 1 = diazepam, 2 = *N*-desmethyldiazepam, 3 = prazepam.

### *3.6. Validation of the method*

The calibration graphs were linear in the concentration range 0.5–8.0 nmol/ml of diazepam and *N*-desmethyldiazepam, with correlation coefficients $r = 0.996$ or better. The limit of detection at a signal-to-noise ratio of 3 ($S/N = 3$) was 0.020 nmol/ml and 0.115 nmol/ml for *N*-desmethyldiazepam in urine and plasma, respectively, and 0.025 nmol/ml for diazepam in plasma. The limit of quantification at a signal-to-noise ratio of 10 ($S/N = 10$) was 0.070 nmol/ml and 380 nmol/ml for *N*-desmethyldiazepam in urine and plasma, respectively, and

Table 2
Intra- and inter-assay variations in urine after LPME, expressed as the mean of parallel samples±standard deviation (SD), relative standard deviation (RSD) and bias

| Drug | Concentration added (nmol/ml) | Measured concentration (mean±SD) (nmol/ml) | RSD (%) | Bias (%) |
|---|---|---|---|---|
| *Intra-assay (n=6)* | | | | |
| *N*-desmethyldiazepam | 1.00 | 0.91±0.04 | 4.4 | −9.0 |
| | 3.00 | 2.63±0.09 | 3.5 | −12.3 |
| | 5.00 | 5.01±0.23 | 4.7 | +0.3 |
| *Inter-assay (n=6)* | | | | |
| *N*-desmethyldiazepam | 1.00 | 0.93±0.05 | 5.4 | −7.0 |
| | 3.00 | 2.53±0.10 | 3.9 | −15.6 |
| | 5.00 | 4.55±0.30 | 6.6 | −9.0 |

0.080 nmol/ml for diazepam in plasma. The intra- and inter-assay for extraction from urine and plasma are shown in Tables 2 and 3. The RSD and bias for intra- and inter-assay in urine were 6.6% and 15.6%, respectively. The corresponding RSD and bias in plasma were 12.3% and 11.7%.

## 4. Conclusions

The potential for liquid-phase microextraction (LPME) has been demonstrated as a sample preparation technique prior to GC for drug analysis in biological matrices. Diazepam and *N*-desmethyldiazepam in human urine and plasma were used as model compounds. LPME provided extracts with highly enriched analytes and excellent clean up of endogenous compounds. Solvent consumption was greatly reduced compared to traditional LLE. Furthermore there was no need for evaporation of solvent and reconstitution of analytes prior to injection into the GC. Disposable extraction units eliminated the possibility of carry-over and the need of regeneration of the fiber, as the costs of the extraction unit were low. Only the porous polypropylene hollow fiber had to be replaced for the

Table 3
Intra- and inter-assay variations in plasma after LPME, expressed as the mean of parallel samples±standard deviation (SD), relative standard deviation (RSD) and bias

| Drug | Concentration added (nmol/ml) | Measured concentration (mean±SD) (nmol/ml) | RSD (%) | Bias (%) |
|---|---|---|---|---|
| *Intra-assay (n=6)* | | | | |
| Diazepam | 1.00 | 0.97±0.10 | 10.4 | −3.0 |
| | 3.00 | 2.75±0.15 | 5.4 | −8.3 |
| | 5.00 | 4.87±0.15 | 3.0 | −2.6 |
| *N*-desmethyldiazepam | 1.00 | 0.94±0.07 | 7.3 | −6.0 |
| | 3.00 | 2.65±0.10 | 3.9 | −11.7 |
| | 5.00 | 4.57±0.12 | 2.7 | −8.6 |
| *Inter-assay (n=6)* | | | | |
| Diazepam | 1.00 | 1.01±0.11 | 10.9 | +1.0 |
| | 3.00 | 2.91±0.26 | 8.9 | −3.0 |
| | 5.00 | 4.88±0.37 | 7.6 | −2.0 |
| *N*-desmethyldiazepam | 1.00 | 1.06±0.12 | 11.3 | +6.0 |
| | 3.00 | 2.68±0.33 | 12.3 | −10.7 |
| | 5.00 | 4.76±0.49 | 10.3 | −4.8 |

next extraction. Thirty samples were extracted simultaneously.

## References


[1] M. Chiarotti, R. Marsili, J. Microcol. Sep. 6 (1994) 577.
[2] T. Kumazawa, X.-P. Lee, M.-C. Tsai, H. Seno, A. Ishii, K. Sato, Jpn. J. Forensic Toxicol. 13 (1995) 25.
[3] K. Ameno, C. Fuke, S. Ameno, H. Kinoshita, I. Ijiri, Can. Soc. Forensic Sci. 29 (1996) 43.
[4] A. Ishii, H. Seno, T. Kumazawa, K. Watanabe, H. Hattori, O. Suzuki, Chromatographia 43 (1996) 331.
[5] M. Krogh, H. Grefslie, K.E. Rasmussen, J. Chromatogr. B 689 (1997) 357.
[6] H.G. Ugland, M. Krogh, K.E. Rasmussen, J. Chromatogr. B 701 (1997) 29.
[7] H.G. Ugland, M. Krogh, K.E. Rasmussen, J. Pharm. Biomed. Anal. 19 (1999) 463.
[8] S. Ulrich, J. Martens, J. Chromatogr. B 696 (1997) 217.
[9] K. Johansen Reubsaet, H.R. Norli, P. Hemmersbach, K.E. Rasmussen, J. Pharm. Biomed. Anal. 18 (1998) 667.
[10] B.J. Hall, J.S. Brodbelt, J. Chromatogr. A 777 (1997) 275.
[11] M. Nishikawa, H. Seno, A. Ishii, O. Suzuki, T. Kumazawa, K. Watanabe, H. Hattori, J. Chromatogr. Sci. 35 (1997) 275.
[12] L. Junting, C. Peng, O. Suzuki, Forensic Sci. Int. 97 (1998) 93.
[13] S.W. Myung, H.K. Min, S. Kim, M. Kim, J.B. Cho, T.J. Kim, J. Chromatogr. B 716 (1998) 359.
[14] A. Namera, T. Watanabe, M. Yashiki, Y. Iwasaki, T. Kojima, J. Anal. Toxicol. 22 (1998) 396.
[15] E.H.M. Koster, N.S.K. Hofman, G.J. De Jong, Chromatographia 47 (1998) 678.
[16] F. Guan, H. Seno, A. Ishii, K. Watanabe, T. Kumazawa, H. Hattori, J. Anal. Toxicol. 23 (1999) 54.
[17] Y. Luo, L. Pan, J. Pawliszyn, J. Microcol. Sep. 10 (1998) 193.
[18] S. Strano-Rossi, M. Chiarotti, J. Anal. Toxicol. 23 (1999) 7.
[19] S. Ulrich, S. Kruggel, H. Weigmann, C. Hiemke, J. Chromatogr. B 731 (1999) 231.
[20] H. Kataoka, S. Narimatsu, H.L. Lord, J. Pawliszyn, Anal. Chem. 71 (1999) 4237.
[21] K.E. Rasmussen, S. Pedersen-Bjergaard, M. Krogh, H.G. Ugland, T. Grønhaug, J. Chromatogr. A 873 (2000) 3.
[22] W.M. Meylan, P.H. Howard, J. Pharm. Sci. 84 (1995) 83.
[23] S.R. Corporation, http://esc-plaza.syrres.com/interkow/kowdemo.htm
[24] Martindale: The Complete Drug Reference, 32nd ed, Pharmaceutical Press, London, 1999.
[25] K.-G. Wahlund, T. Arvidsson, J. Chromatogr. 282 (1983) 527.
[26] J. Blanchard, J. Chromatogr. 226 (1981) 455.
[27] N.C. van de Merbel, J.M. Teule, H. Lingeman, U.A.T. Brinkman, J. Pharm. Biomed. Anal. 10 (1992) 225.
[28] S. Li, S.G. Weber, Anal. Chem. 69 (1997) 1217.
[29] R. Eisert, J. Pawliszyn, Critical Reviews in Analytical Chemistry 27 (1997) 103.
[30] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
[31] H. Gjerde, E. Dahlin, A.S. Christophersen, J. Pharm. Biomed. Anal. 10 (1992) 317.
[32] A.J.H. Louter, E. Bosma, J.C.A. Schipperen, J.J. Vreuls, U. Brinkman, J. Chromatogr. B 689 (1997) 35.
[33] P. Lillsunde, T. Seppala, J. Chromatogr. B 533 (1990) 97.
[34] H.T. Karnes, L.A. Beightol, R.J. Serafin, D. Farthing, J. Chromatogr. 424 (1988) 398.
[35] J.Å. Jonsson, L. Mathiasson, Trends Anal. Chem. 18 (1999) 318.
[36] J.Å. Jonsson, L. Mathiasson, Trends Anal. Chem. 18 (1999) 325.
[37] Y. Shen, J.Å. Jonsson, L. Mathiasson, Anal. Chem. 18 (1998) 946.
[38] J.Å. Jonsson, M. Andersson, C. Melander, J. Norberg, E. Thordarson, L. Mathiasson, J. Chromatogr. A 870 (2000) 151.