



# Multiresidue analytical method using dispersive solid-phase extraction and gas chromatography/ion trap mass spectrometry to determine pharmaceuticals in whole blood

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## Abstract

A convenient analytical method for the simultaneous determination of more than 40 pharmaceuticals belonging to various therapeutic categories in whole blood has been developed. Exemplarily, the method was fully validated for eight different pharmaceuticals. The procedure entails addition of acetonitrile, magnesium sulfate and sodium chloride to a small amount of blood, then the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic layer is cleaned up by dispersive solid-phase extraction employing bulk sorbents as well as magnesium sulfate for the removal of residual water. This method was based on the QuEChERS approach developed for pesticide residue analysis in food. Gas chromatography/ion trap mass spectrometry (GC/MS) with electron (EI) and chemical (CI) ionisation was then used for qualitative and quantitative determination of the pharmaceuticals. The dispersive SPE with PSA (sorbent functionalized with primary and secondary amines) was found more suitable than aminopropyl and a styrene-divinylbenzene sorbent for sample clean-up before drug level determination in whole blood and plasma, as it was found that most of endogenous matrix components were removed and the analytes were isolated from spiked samples with recoveries above 80%. Variation coefficients of the repeatability typically smaller than 10% have been achieved for a wide range of the investigated substances. The used analytical conditions allowed to separate successively a variety of drugs and poisons with the typical limit of detection at  $<20 \text{ ng mL}^{-1}$  levels using 1  $\mu\text{L}$  injection of equivalent blood sample in whole blood. The method is simple, rapid, cheap and very effective for therapeutic drug monitoring and forensic chemistry.

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*Keywords:* Dispersive SPE; QuEChERS; Ion trap gas chromatography–mass spectrometry

## 1. Introduction

The determination of plasma levels of various pharmaceuticals has been practised in clinical laboratories for many years by the use of various techniques. The importance of therapeutic drug monitoring in the field of clinical treatments with anticonvulsants, immunosuppressant, chemotherapeutics and other drugs is unchallenged [1–3]. The three mainly used techniques for the determination are immunoassays, HPLC and GC-methods [4,5]. Usually the chromatographic methods need a complex sample preparation procedure. But in combination with mass spectral detection chromatographic methods are still

the first choice for many applications, by reason of their flexibility, selectivity, wide analytical scope, qualitative and quantitative utility and sensitivity.

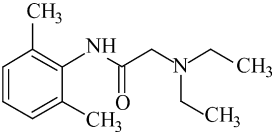
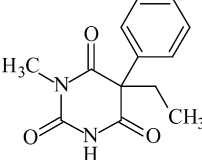
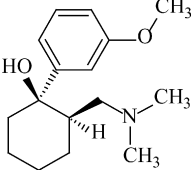
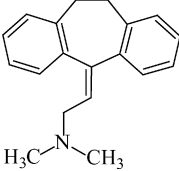
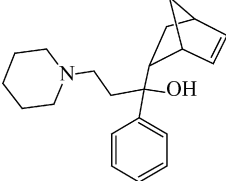
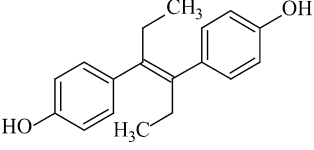
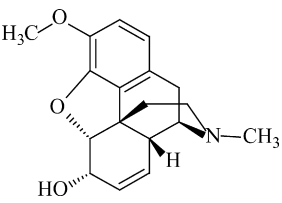
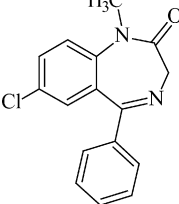
Beside the classic liquid–liquid-extraction (LLE), solid-phase extraction (SPE) has been used in the last few years as a result of its considerable advantages. The SPE often shows better extraction efficiencies than the LLE, especially for polar components or metabolized drugs [6,7]. Due to limitations in the type of sorbents available, for a long time no really fast and easy multiresidue sample preparation was possible. In the last years, after development of polymeric phases, the SPE made up this disadvantage [8]. Nowadays polymeric or mixed-moded sorbents are able to separate acidic, neutral and basic drugs from different biological samples by hydrophobic or ion-exchange interactions. Through this, a multiresidual determination of pharmaceuticals in body specimens is possible.

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In 2003, Anastassiades et al. reported a rapid and inexpensive method to the analysis of pesticides in foods [9–14]. The described method comprises an extraction of the residual pesticides with acetonitrile. Water and proteins are removed from the

raw extract by salting out with sodium chloride and magnesium sulfate. As next step, the dried crude extract is cleaned up by addition of a small amount of SPE sorbent material to an aliquot of the extract. This step is similar to the matrix solid-phase

Table 1  
 Chemical structures and properties of selected pharmaceuticals

| Drug                | Structure   | CAS number | pK <sub>a</sub>   | Log P (o/w) | Therapeutic use                  |
|---------------------|---|------------|---|-------------|----------------------------------|
| Lidocaine           |    | 137-58-6   | 8.0   | 2.48        | Local anaesthetic antiarrhythmic |
| Methylphenobarbital |    | 115-38-8   | 7.8   | 1.92        | Anticonvulsant                   |
| Tramadol            |    | 27203-92-5 | 9.4   | 2.95        | Analgesic                        |
| Amitriptyline       |   | 50-48-6    | 9.4   | 4.61        | Antidepressant                   |
| Biperidene          |  | 514-65-8   | 8.8   | 3.85        | Anticholinergic                  |
| Diethylstilbestrol  |  | 56-53-1    | pK <sub>a</sub> <sup>1</sup> = 9.3<br>pK <sub>a</sub> <sup>2</sup> = 10.0 | 4.77        | Hormone                          |
| Codeine             |  | 76-57-3    | 8.21  | 1.81        | Opioid                           |
| Diazepam            |  | 439-14-5   | 3.4   | 2.99        | Anticonvulsant                   |

The method was validated exemplarily for these substances.

dispersion, where matrix is homogenized with bulk sorbents. The authors named this new method QuEChERS, which is the abbreviation of Quick, Easy, Cheap, Effective, Rugged and Safe [9]. The authors reported outstanding recoveries for different pesticide classes [9]. In 2005, Posnyiak et al. reported a method for the determination of sulfonamides in chicken muscles and

used a dispersive solid-phase extraction of the sulfonamides as sample clean-up step [15]. A similar approach for the determination of antibiotics in bovine kidney tissue is described in [16].

This paper describes the extension of the QuEChERS approach to determination of pharmaceuticals and toxins in whole blood. Similar to the original QuEChERS approach a

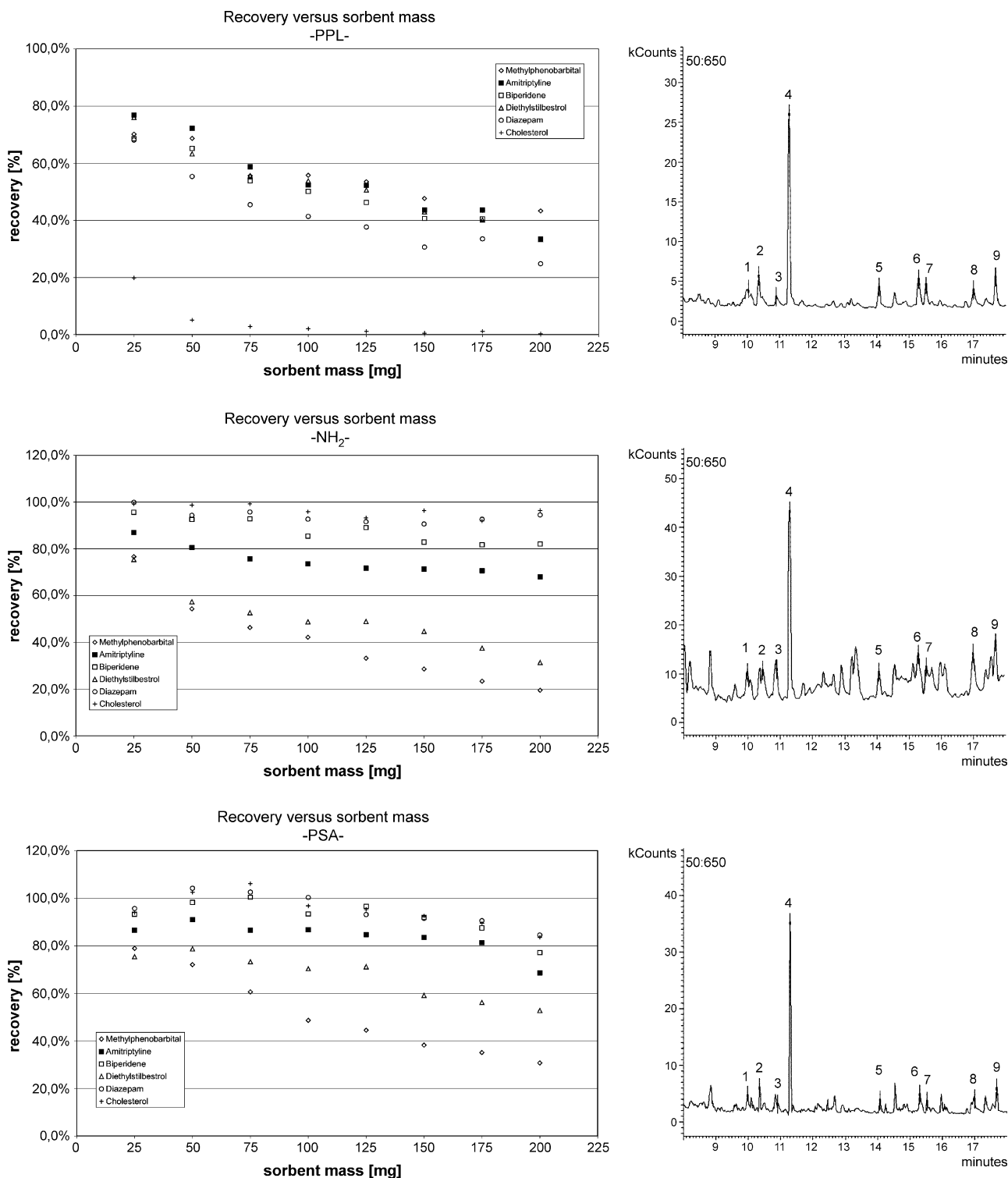


Fig. 1. Recovery versus used sorbent mass. Also typical chromatograms in EI-Mode obtained with 25 mg sorbent and analyte concentration of 100 ng mL<sup>-1</sup> are shown. 1 = Lidocaine, 2 = methylphenobarbital, 3 = tramadol, 4 = triphenylmethane (I.S.), 5 = amiripryline, 6 = biperidene, 7 = diethylstilbestrol, 8 = codeine, 9 = diazepam.

very easy, cheap and fast approach for the analysis of several drugs and poisons with no need of expensive equipment for analysis is presented. The recovery data for more than 40 different drugs and poisons from spiked whole blood samples were

evaluated using GC/MS (ion trap in both EI and CI modes). Finally the validation and the resulting data of eight pharmaceuticals with different chemical and pharmaceutical properties were conducted.

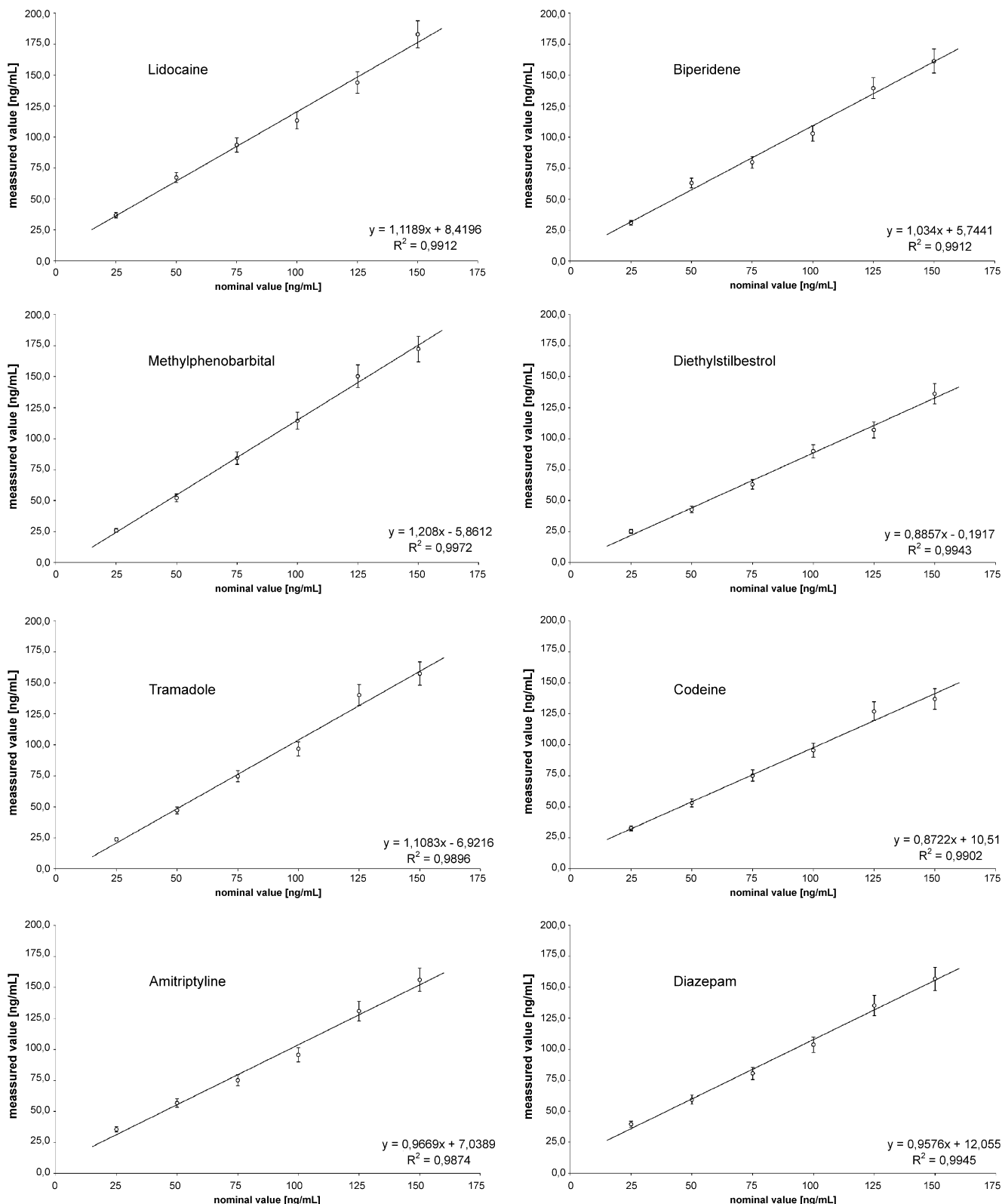


Fig. 2. Methodical deviation between instrument linearity (calibration data from matrix-matched standards) and recovery at different levels.

## 2. Experimental

### 2.1. Materials

Acetonitrile (MeCN), *tert*-butylmethylether (TBME) and methanol (MeOH) were purchased from Acros Organics (Geel, Belgium) and VWR Prolab (Leuven, Belgium) in HPLC grade.

Magnesium sulfate and sodium chloride in powder form and analytical grade were obtained from Grüssing, Germany.

The solid-phase extraction sorbents Bondesil NH<sub>2</sub> (amino-propyl), PSA (primary and secondary amine) and PPL (styrene-divinylbenzene) in powder form with a particle size of 40 μm were obtained from Varian (Harbor City, USA). C18 (octadecylsilane), alumina and silica gel were obtained from Sigma Aldrich (Steinheim, Germany).

Drugs were obtained from Synopharm (Barsbüttel, Germany), Sigma Aldrich (Seelze, Germany) and Caesar and Lorentz (Hilden, Germany). The internal standard (I.S.) triphenylmethane (TPM) was purchased from Fluka (Buchs, Switzerland).

### 2.2. Standard solutions

All mentioned drugs and toxic substances were dissolved in MeCN. These stock solutions were used as standards for the experiments and stored at –20 °C. A solution of 1 μg mL<sup>-1</sup> TPM in MeCN was used as I.S. in the experiments.

The full validation was performed with eight different pharmaceuticals (lidocaine, methylphenobarbital, tramadole, amitriptyline, biperidene, diethylstilbestrol, codeine and diazepam). Table 1 shows those substances and their chemical properties.

### 2.3. Sample preparation equipment

A refrigerated centrifuge type Verifuge 3.0 (Heraeus, Germany) and a mixing apparatus Vortex Genie 2 (Scientific Apparatus, USA) were used for separating and mixing steps.

### 2.4. Sample preparation

For our experiments, we used whole blood from healthy and adult pigs. The blood was obtained from a local butcher right after slaughtering and was deep-frozen until analysis. **Two hundred fifty milligrams** (±5%) sodium chloride and 500 mg (±5%) magnesium sulfate were filled into a 15 mL centrifuge tube and 2000 μL MeCN and 10 μL I.S. solution were added. Then, 1000 μL of the whole blood sample was added. The mixture was shaken vigorously and vortexed for 1 min. The mixture was centrifuged for 5 min at 4000 revolutions per minute (rpm). An aliquot of 1000 μL extract was transferred into a microcentrifuge tube, which contained 25 mg (±3 mg) Bondesil PSA and 25 mg (±3 mg) magnesium sulfate for a final drying step. The suspension was vortexed for 1 min and then centrifuged for 3 min at 4000 rpm. The supernatant was separated from the solids and transferred into an autosampler vial. The time effort is approximately 30 min for eight different samples.

### 2.5. GC/MS system

A Varian ion trap Saturn 2100 with MS<sup>n</sup>-option and multi-CI-option was coupled with a Varian 3800 gas chromatograph equipped with a Varian 8400 autosampler. The system was equipped with a 1177 splitt/splittless injector and a 1079 programmable temperature vaporization (PTV) injector. Varian's MS workstation software Version 6.6 was used for instrument control and data analysis. Varian VF-5ms and Varian VF-Xms capillary columns of 30 m, 0.25 mm I.D. and 0.25 μm film thickness were employed throughout the studies. The chromatographic conditions for GC-method 1 at the VF-5 ms column were as following: helium (99.999%) constant flow of 1.0 mL min<sup>-1</sup>, inlet temperature 250 °C, injection volume 1 μL (splittless). MS transfer line temperature 270 °C, ion trap temperature 200 °C. Temperature program of 50 °C for 1 min, then a ramp of 30 °C min<sup>-1</sup> to 200 °C followed by a 5 °C min<sup>-1</sup> ramp to 280 °C and a ramp of 40 °C min<sup>-1</sup> to 300 °C (held for 5 min). The chromatographic conditions the method 2 at the VF-Xms column were as following: helium constant flow of 1.0 mL min<sup>-1</sup>, inlet temperature at the moment of injection 65 °C and injection volume 2 μL. Starting in splitless mode, one minute after injection the split valve was set to a ratio of 1:100. Two minutes after injection the split was switched to 1:25. The temperature program of the injector was 65 °C for 1 min, then a ramp of 200 °C min<sup>-1</sup> to 250 °C (held for the whole run). The temperature program of the chromatographic system was 65 °C for 1 min, then a ramp of 30 °C min<sup>-1</sup> to 200 °C followed by 5 °C min<sup>-1</sup> ramp to 280 °C and a ramp of 40 °C min<sup>-1</sup> to 300 °C (held for 5 min). MS transfer line temperature 270 °C, ion trap temperature 200 °C.

The ion trap was operated in full scan mode detecting ions between 50 *m/z* and 650 *m/z*. Ionisation was performed with electron ionisation for method development and chemical ionisation with liquid MeOH for quantitative determinations. The CI storage level was 19.0 *m/z*, maximum ionisation time 2000 μs, maximum reaction time 40 ms and the target TIC 5000 counts. The ejection amplitude was set to 19 V.

## 3. Results and discussion

### 3.1. Sample clean-up development

Among the solid-phase materials tested were one polymeric sorbent (Bondesil PPL) and two sorbents with weak anion exchange and polar capabilities (Bondesil NH<sub>2</sub> and PSA). Furthermore, we tested very polar sorbents as silica and alumina and apolar sorbents as C18. Various solvents (MeOH, TBME, chloroform) were tested. On the basis of these preliminary investigations, we extracted the drugs with MeCN and the polymeric sorbent Bondesil PPL and the two modified silica sorbents Bondesil NH<sub>2</sub> and PSA. The Bondesil PPL material is a styrene-divinylbenzene (PS-DVB) polymer with very hydrophobic properties. It is designed for retaining very polar substances from aqueous samples and gives very clean extracts [17,18]. In consequence of its specific properties PPL retains sugars, fatty acids, sterols and the pharmaceuticals independent of their structure. In our studies primarily cholesterol was retained by PPL.

Table 2  
Recovery of the dispersive solid-phase extraction, chromatographic and mass spectral data obtained by the two GC/MS methods for all tested pharmaceuticals as well as selected ions for quantification and qualification

| Drug data               |                | Recovery data<br>Spiking level: 100 ng mL <sup>-1</sup> (n = 3) |              | GC data              |              |              | MS Data [m/z]<br>(relative intensity) |                |              |              | Chemical ionisation reactant: liquid MeOH |                |                |              |
|-------------------------|----------------|---|--------------|----------------------|--------------|--------------|---------------------------------------|----------------|--------------|--------------|---|----------------|----------------|--------------|
| Name                    | M <sub>R</sub> | Recovery (%)  | RSD (%)      | Retention time [min] |              | RTT          | Electronic ionisation                 |                |              |              | Base peak                                 |                | Prominent ions |              |
|                         |                |   |              | VF-5 ms              | VF-X ms      |              | Base peak                             | Prominent ions |              |              | Base peak                                 | Prominent ions |                |              |
| Acetaminophen           | 151            | 90.7  | 10.1         | 8.19                 | 8.24         | 0.72         | 108.9                                 | 150.8 (42.2)   | 80.0 (41.7)  | 80.9 (20.6)  | 152.0                                     | 152.9 (9.6)    | 61.0 (1.7)     | 110.0 (1.4)  |
| Amitriptyline           | 277            | 95.6  | 5.8          | 14.11                | 14.12        | 1.24         | 58.0                                  | 202.3 (4.6)    | 59.0 (4.4)   | 55.0 (2.5)   | 278.2                                     | 58.0 (60.3)    | 279.1 (23.1)   | 233.0 (4.9)  |
| Articaine               | 284            | 98.1  | 11.2         | 12.40                | 12.34        | 1.09         | 85.9                                  | 170.9 (23.0)   | 138.8 (10.7) | 165.9 (10.7) | 285.1                                     | 85.9 (69.6)    | 286.0 (15.9)   | 55.8 (7.2)   |
| Atropine                | 289            | 101.7   | 8.9          | 14.18                | 14.38        | 1.25         | 124.0                                 | 82.0 (26.7)    | 93.9 (20.7)  | 83.0 (16.9)  | 124.0                                     | 290.3 (21.5)   | 124.9 (9.6)    | 67.0 (6.4)   |
| Benzocaine              | 265            | 99.2  | 6.3          | 7.46                 | 7.45         | 0.66         | 119.9                                 | 165.0 (40.2)   | 64.9 (27.5)  | 91.9 (25.6)  | 165.9                                     | 64.9 (11.6)    | 166.9 (9.9)    | 120.0 (6.8)  |
| Biperidene              | 311            | 102.9   | 7.9          | 15.33                | 15.42        | 1.35         | 98.0                                  | 218.2 (18.2)   | 54.9 (8.7)   | 77.0 (8.8)   | 312.2                                     | 97.9 (34.8)    | 313.3 (22.7)   | 294.3 (5.4)  |
| Caffeine                | 194            | 87.1  | 12.3         | 9.65                 | 9.85         | 0.85         | 194.0                                 | 54.9 (38.2)    | 67.0 (28.4)  | 108.8 (26.9) | 195.0                                     | 54.9 (19.4)    | 67.0 (18.3)    | 196.0 (9.9)  |
| Cholesterol             | 386            | <sup>a</sup>  | <sup>a</sup> | 27.31                | 27.16        | 2.39         | 301.3                                 | 213.3 (67.2)   | 386.3 (63.9) | 368.5 (66.6) | 369.4                                     | 370.3 (27.3)   | 54.9 (7.5)     | 243.2 (4.6)  |
| Codeine                 | 299            | 95.7  | 9.8          | 17.03                | 17.50        | 1.54         | 299.0                                 | 162.1 (43.7)   | 229.1 (27.4) | 214.2 (28.2) | 282.1                                     | 300.2 (32.7)   | 283.1 (19.1)   | 301.1 (7.0)  |
| Diazepam                | 284            | 103.7   | 6.2          | 17.74                | 18.21        | 1.60         | 256.0                                 | 283.2 (78.1)   | 257.3 (53.1) | 221.2 (45.5) | 285.1                                     | 287.1 (34.8)   | 286.0 (18.1)   | 288.1 (6.5)  |
| Dienestrol              | 266            | 92.5  | 11.3         | 15.81                | 16.14        | 1.42         | 266.1                                 | 251.0 (73.2)   | 237.2 (57.0) | 77.0 (33.9)  | 267.1                                     | 173.0 (22.9)   | 286.0 (18.1)   | 121.0 (6.1)  |
| Diethylstilbestrol      | 268            | 89.9  | 15.7         | 15.57                | 15.85        | 1.40         | 268.0                                 | 145.1 (74.5)   | 239.2 (91.9) | 107.1 (44.2) | 268.9                                     | 134.9 (38.2)   | 270.0 (19.7)   | 174.9 (20.5) |
| Diphenhydramine         | 255            | 98.9  | 7.0          | 9.91                 | 9.75         | 0.86         | 58.0                                  | 165.1 (15.2)   | 167.0 (6.5)  | 157.0 (5.7)  | 58.0                                      | 167.0 (29.4)   | 168.0 (4.3)    | 59.0 (3.0)   |
| Doxepine                | 279            | 97.8  | 8.2          | 14.54                | 14.67        | 1.29         | 58.0                                  | 165.2 (3.3)    | 59.0 (3.5)   | 178.1 (2.9)  | 58.0                                      | 280.3 (35.0)   | 281.3 (7.5)    | 59.0 (3.7)   |
| Escitalopram            | 324            | 93.5  | 12.4         | 17.09                | 17.21        | 1.52         | 58.0                                  | 238.1 (6.2)    | 324.2 (6.3)  | 325.2 (3.1)  | 58.0                                      | 325.3 (20.7)   | 58.9 (3.8)     | 26.3 (4.4)   |
| Estradiol               | 272            | 85.0  | 14.1         | 20.08                | 20.80        | 1.83         | 272.2                                 | 213.1 (49.3)   | 171.9 (34.2) | 160.0 (29.1) | 273.1                                     | 255.2 (93.6)   | 256.3 (18.1)   | 274.0 (18.1) |
| Imipramine              | 280            | 98.1  | 5.2          | 14.46                | 14.54        | 1.28         | 243.3                                 | 235.2 (86.0)   | 59.0 (84.2)  | 193.2 (38.1) | 281.3                                     | 86.0 (96.6)    | 58.0 (80.5)    | 282.2 (20.7) |
| Lidocaine               | 234            | 103.1   | 11.2         | 10.02                | 9.87         | 0.87         | 86.0                                  | 57.9 (19.0)    | 87.0 (13.4)  | 77.0 (8.0)   | 235.1                                     | 236.0 (15.3)   | 86.0 (8.3)     | 88.8 (6.7)   |
| Memantine               | 179            | 103.5   | 7.2          | 5.81                 | <sup>b</sup> | <sup>b</sup> | 122.0                                 | 108.0 (95.0)   | 107.0 (16.7) | 92.9 (12.8)  | <sup>b</sup>                              | <sup>b</sup>   | <sup>b</sup>   | <sup>b</sup> |
| Methylphenobarbital     | 246            | 94.3  | 13.1         | 10.40                | 10.50        | 0.93         | 218.0                                 | 117.0 (22.2)   | 146.0 (10.5) | 160.0 (5.7)  | 246.9                                     | 87.8 (29.6)    | 248.0 (15.4)   | 88.8 (8.2)   |
| Nicotine                | 162            | 99.2  | 11.1         | 6.17                 | <sup>b</sup> | <sup>b</sup> | 84.0                                  | 133.0 (29.9)   | 163.0 (19.0) | 161.0 (17.3) | <sup>b</sup>                              | <sup>b</sup>   | <sup>b</sup>   | <sup>b</sup> |
| Papaverine              | 339            | 90.2  | 18.9         | 23.30                | 23.91        | 2.11         | 324.2                                 | 338.3 (89.5)   | 339.3 (42.0) | 308.2 (25.6) | 340.3                                     | 341.3 (22.0)   | 342.3 (3.1)    | 54.8 (1.0)   |
| Paroxetine              | 329            | 94.1  | 7.3          | 19.78                | 20.29        | 1.79         | 192.1                                 | 70.7 (46.2)    | 329.2 (44.3) | 138.0 (38.9) | 330.3                                     | 331.3 (20.9)   | 70.0 (2.7)     | 68.0 (2.6)   |
| Phenobarbital           | 232            | 101.2   | 13.3         | 11.14                | 11.46        | 1.01         | 204.1                                 | 117.0 (17.2)   | 161.0 (14.2) | 205.0 (11.8) | 233.0                                     | 234.2 (14.3)   | 62.9 (2.5)     | 235.1 (1.6)  |
| Phenytioine             | 252            | 95.9  | 8.2          | 16.25                | –            | –            | 180.0                                 | 209.0 (73.3)   | 223.0 (59.8) | 103.9 (49.4) | 253.1                                     | 254.1 (17.1)   | 174.9 (7.3)    | 93.8 (1.3)   |
| Pridinol                | 295            | 101.3   | 9.8          | 15.71                | 15.92        | 1.29         | 98.0                                  | 77.0 (19.6)    | 105.0 (18.4) | 84.0 (14.5)  | 98.0                                      | 296.3 (79.5)   | 297.2 (16.7)   | 54.9 (6.5)   |
| Prilocaine              | 220            | 98.6  | 7.4          | 9.60                 | 9.49         | 0.84         | 85.9                                  | 77.0 (7.6)     | 105.9 (7.2)  | 86.9 (6.2)   | 221.0                                     | 85.9 (26.9)    | 221.9 (14.1)   | 55.8 (5.2)   |
| Procaine                | 236            | 94.3  | 7.9          | 11.85                | 12.00        | 1.06         | 86.0                                  | 119.9 (27.7)   | 98.9 (26.3)  | 64.9 (17.0)  | 237.2                                     | 99.9 (29.7)    | 238.1 (14.8)   | 55.9 (9.9)   |
| Scopolamine             | 303            | 94.8  | 13.0         | 15.97                | 16.35        | 1.44         | 93.8                                  | 137.9 (67.1)   | 108.0 (38.2) | 153.9 (28.8) | 137.9                                     | 304.3 (40.4)   | 156.0 (12.5)   | 138.9 (8.9)  |
| Sparteine               | 234            | 98.4  | 12.2         | 9.42                 | 9.31         | 0.83         | 235.2                                 | 137.0 (80.6)   | 98.0 (56.9)  | 234.1 (37.2) | 235.2                                     | 233.3 (28.5)   | 236.2 (16.0)   | 234.3 (15.0) |
| Strychnine              | 334            | 86.0  | 15.1         | 29.21                | 29.92        | 2.64         | 334.3                                 | 335.3 (25.7)   | 333.5 (16.0) | 130.0 (14.9) | 335.4                                     | 336.5 (23.5)   | 337.4 (3.0)    | 333.5 (2.2)  |
| Testosterone propionate | 344            | 91.0  | 14.3         | 23.34                | 23.81        | 2.10         | 123.9                                 | 146.9 (65.2)   | 228.2 (50.7) | 56.8 (46.0)  | 345.3                                     | 271.2 (27.7)   | 346.2 (23.1)   | 56.8 (13.8)  |
| Theobromine             | 180            | 90.8  | 11.9         | 9.90                 | 10.22        | 0.90         | 180.0                                 | 67.0 (35.8)    | 54.9 (35.2)  | 108.8 (20.6) | 181.1                                     | 67.0 (8.4)     | 54.9 (8.9)     | 182.1 (8.9)  |
| Theophylline            | 180            | 86.2  | 13.3         | 11.11                | 11.59        | 1.02         | 180.0                                 | 94.8 (34.7)    | 52.9 (23.2)  | 67.9 (23.1)  | 181.0                                     | 182.1 (9.2)    | 67.9 (2.8)     | 67.0 (2.0)   |
| Thiopental              | 242            | 103.2   | 9.1          | 9.90                 | 10.32        | 0.91         | 156.9                                 | 172.8 (75.5)   | 171.9 (63.6) | 68.8 (11.9)  | 243.0                                     | 172.9 (18.8)   | 243.9 (13.4)   | 54.9 (4.8)   |
| Tiamulin                | 493            | 56.0  | 17.8         | 30.90                | –            | –            | 86.0                                  | 58.0 (7.2)     | 86.9 (5.8)   | 119.0 (4.0)  | 492.5                                     | 192.2 (41.5)   | 439.5 (30.6)   | 494.5 (10.2) |
| Tiapride                | 328            | 73.9  | 13.1         | 23.14                | 23.89        | 2.11         | 86.0                                  | 58.0 (91.1)    | 86.9 (6.0)   | 199.0 (3.8)  | 329.5                                     | 330.3 (18.5)   | 58.0 (10.1)    | 331.3 (6.8)  |
| Tramadol                | 263            | 92.3  | 11.2         | 10.94                | 10.89        | 0.96         | 58.0                                  | 77.0 (8.0)     | 262.8 (5.4)  | 55.0 (13.6)  | 58.0                                      | 264.0 (14.1)   | 55.8 (12.3)    | 58.9 (4.5)   |
| Triphenylmethane        | 244            | <sup>a</sup>  | <sup>a</sup> | 11.34                | 11.36        | 1.00         | 244.0                                 | 165.2 (59.5)   | 167.1 (47.7) | 243.5 (35.2) | 167.0                                     | 168.0 (13.9)   | 64.9 (1.1)     | –            |
| Tropine                 | 141            | 102.1   | 7.4          | 5.53                 | <sup>b</sup> | <sup>b</sup> | 82.0                                  | 96.0 (70.7)    | 83.0 (49.3)  | 124.0 (24.0) | <sup>b</sup>                              | <sup>b</sup>   | <sup>b</sup>   | <sup>b</sup> |
| Xylazine                | 220            | 92.1  | 7.2          | 11.31                | 11.56        | 1.02         | 205.1                                 | 220.1 (49.2)   | 177.1 (31.4) | 146.1 (24.6) | 329.5                                     | 330.3 (18.5)   | 58.0 (10.1)    | 331.3 (6.8)  |

<sup>a</sup> Recovery of cholesterol and triphenylmethane (I.S.) not tested.

<sup>b</sup> Signal appears before solvent cut.

The Bondesil NH2 material is a modified silica with an aminopropyl group retaining sugars and other polar substances through hydrogen bonding and acidic compounds like free fatty acids through anion exchange. In comparison to the other used sorbents, Bondesil NH2 results in unclean extracts and was found not suitable for the experiments.

The Bondesil PSA material is a modified silica functionalized with ethylenediamine groups. Through this PSA is very similar to the NH2 material, but has a higher ionic capacity than NH2. Through the bidentate structure, PSA has a high chelating effect. As a result of the secondary and primary amino groups the retention of free fatty acids and other polar matrix compounds is very strong. PSA also gives very clean extracts with a lower retention of cholesterol than PPL (Fig. 1).

Fig. 1 shows the recoveries depending on sorbent types and used sorbent masses and typical chromatograms with electronic ionisation and 25 mg sorbent used in each case. Using the basic materials Bondesil NH2 and PSA, the recoveries of the acidic analytes declined rapidly. Also the recovery of neutral and basic analytes is fading with the use of higher sorbent masses. By the use of the polymeric sorbent Bondesil PPL, all analytes were removed from the extract independent on their chemical properties, as expected. Therefore, we used the Bondesil PSA for further experiments, because the material is able to remove inorganic ions and sugars very effectively. For the experiments 25 mg of the sorbent were used.

### 3.2. Qualitative results

To demonstrate the multiresidual ability of the introduced method, we tested the total recoveries of more than 40 different drugs and poisons at fixed level of  $100 \text{ ng mL}^{-1}$ . The results of threefold measurements and all chromatographic and mass spectral data are shown in Table 2. Mass spectral data are listed according to the European Commission Decision 2002/657/EC [19].

### 3.3. Method validation

The suitability of the method was tested with eight different pharmaceuticals.

For verification of selectivity, the extracts from ten different analyte free blood samples were analysed.

The linearity of the assay was proven according to regression line by the method of least squares and expressed by the coefficient of determination ( $R^2$ ). Mathematically the linearity of the assay was checked by comparing a linear curve fit and a non-linear curve fit with a Mandel test according to DIN 38402 [20]. For all pharmaceuticals, a linear curve fit results in the best fitting. Six-point matrix-matched calibration curves were evaluated by spiking of analyte free blood samples with increasing amounts of each of the model substances. Calibration curves were obtained for each model substance by plotting the recorded peak area (ion counts) of the quantifier ions versus the corresponding concentrations of the analytes in concentrations between 25 and  $250 \text{ ng mL}^{-1}$ . We observed linearity in the whole range. All values of the correlation factors  $R^2$  of the calibration curves are all higher than 0.99.

Precision of the instrument and repeatability (*precision of the method*) were measured with analyte free blood samples which were spiked with model substances to a concentration of  $100 \text{ ng mL}^{-1}$ . The spiked samples were analysed and precision of the instrument and repeatability were calculated as relative standard deviation (RSD). The variation coefficients of the instrument precision were smaller than 8% in all cases. The RSD of inter-day repeatability tests were generally below 20%.

The recovery (*trueness*) of the method was also measured in blank blood samples which were spiked with the same levels as used for determination of linearity. The spiked blood samples were analysed and the recoveries were calculated by comparison of the measured concentration to the spiked samples. Fig. 2 shows the plots of calibration curves versus recoveries at the tested levels. This test is an excellent indicator for systematic errors. Obviously no methodical deviation could be detected.

The limit of detection (LOD, CCB), the limit of decision ( $CC\alpha$ ) and the limit of quantification (LOQ) were determined by using the calibration curve procedure according to DIN 32645 [15]. Only  $1 \mu\text{L}$  of equivalent sample was injected into the GC/MS. A lower limit of detection could be achieved by using a large volume injection (LVI) in combination with the programmable temperature vaporation injector or retention gaps. The limit of detection was calculated with a statistical certainty of  $1 - \beta$  ( $\beta = 0.05$ ), the limit of decision was calculated with a certainty of  $1 - \alpha$  ( $\alpha = 0.05$ ). The limit of quantification

Table 3  
Validation data of the eight model substances

| Name                | Instrument precision<br>( $n = 6$ ) RSD (%) | Method precision<br>( $n = 6$ ) RSD (%) | $R^2$  | LOD (CCB)<br>( $\text{ng mL}^{-1}$ ) | LOQ<br>( $\text{ng mL}^{-1}$ ) | Recovery (%) |
|---------------------|---|---|--------|--------------------------------------|--------------------------------|--------------|
| Lidocaine           | 5.0   | 6.6                                     | 0.9912 | 14.4                                 | 24.0                           | 103.1        |
| Methylphenobarbital | 3.5   | 10.4                                    | 0.9972 | 8.3                                  | 21.5                           | 94.3         |
| Tramadole           | 7.4   | 12.0                                    | 0.9981 | 5.6                                  | 11.3                           | 92.3         |
| Biperidene          | 4.3   | 10.3                                    | 0.9913 | 12.2                                 | 29.8                           | 102.9        |
| Amitriptyline       | 3.3   | 6.8                                     | 0.9928 | 11.1                                 | 27.5                           | 95.6         |
| Diethylstilbestrol  | 4.7   | 16.1                                    | 0.9943 | 17.2                                 | 39.0                           | 89.9         |
| Codeine             | 6.3   | 13.3                                    | 0.9931 | 12.9                                 | 28.1                           | 95.7         |
| Diazepam            | 7.3   | 6.4                                     | 0.9945 | 11.7                                 | 28.7                           | 103.7        |

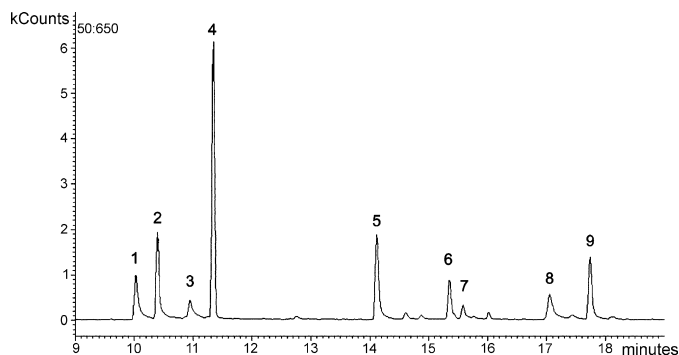


Fig. 3. Typical chromatogram in CI mode with methanol as reactant obtained at an analyte concentration of  $100 \text{ ng mL}^{-1}$  in whole blood after clean-up with the described method and 25 mg used sorbent mass. 1 = Lidocaine, 2 = methylphenobarbital, 3 = tramadole, 4 = triphenylmethane (I.S.), 5 = amitriptyline, 6 = biperidene, 7 = diethylstilbestrol, 8 = codeine, 9 = diazepam.

was calculated with  $1 - \beta$  ( $\beta = 0.05$ ) and a relative uncertainty of 33.3% according to DIN 32645 [21]. All values mentioned above are shown in Table 3. A typical chromatogram with chemical ionisation with liquid methanol as reactant is shown in Fig. 3.

#### 4. Conclusion

A simple, rapid and inexpensive extraction and sample clean-up using the QuEChERS approach has been developed. The method has high throughput capabilities and could be used for detection and quantification of pharmaceuticals and several toxins. Since this method affords no special equipments for sample clean-up, needs only minimum amounts of solvents and minimal time, it should be very useful for analytical chemists in clinical laboratories and forensic scientists.

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