Polytoxicological analysis of urine specimen according to German CTU-criteria: Development of a comprehensive method via solid-phase extraction and LC-MS/MS detection

C. Süßenbach[1], H. R. Wollseifen[2], T. Kretschmer[2], S. Hamm[2], H. Andresen-Streichert[1], T. Kieliba[1]

[1] Institute of Legal Medicine, University Hospital of Cologne [2] Macherey-Nagel GmbH & Co KG, Düren

Aims

According to the German guidelines for proof of abstinence, a defined pattern of analytes has to be tested in urine specimen, including amphetamines, opiates, methadone/EDDP, cocaine/benzoylecgonine, benzodiazepines, and THC-COOH. In addition, the presence or absence of opioids may need to be determined. Currently, a combination of separate GC/MS-, LC-MS/MS-, and immunological methods is used for this purpose. The aim of this project was therefore to develop an appropriate method for determination of all analytes within a single comprehensive work flow.

Methods

For solid-phase extraction (SPE), different sorbents (CHROMABOND® HLB, HR-X, HR-XC, HR-XCW, HR-XA), adsorbent weights (30 mg to 200 mg), and particle sizes (45 µm, 60 µm, 85 µm) were evaluated.

For improved retention and separation via reversed-phase LC-MS/MS, several octadecyl and aryl column materials (NUCLEOSHELL® RP18, RP18plus, Bluebird RP18, PFP, Biphenyl and Phenyl-Hexyl) with different lengths (50 mm, 100 mm, 150 mm), and methanol or acetonitrile as eluents were compared.

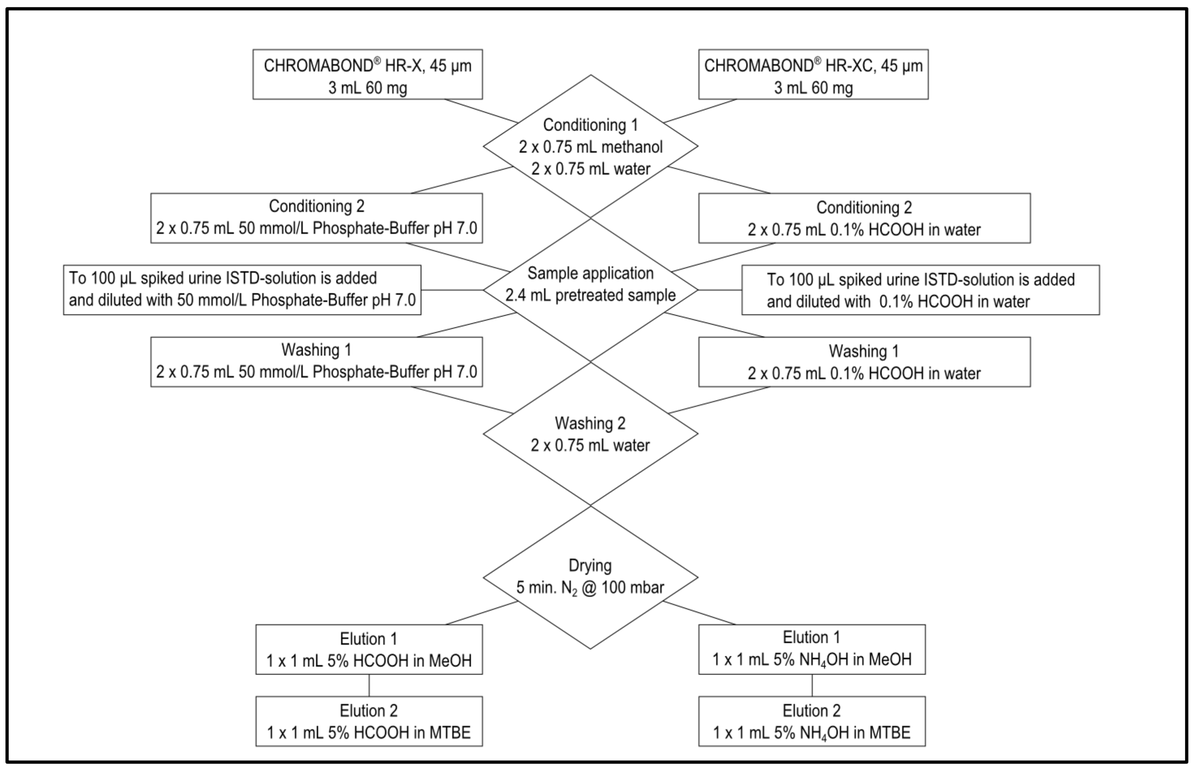

Figure 3: Protocol for comparison of HR-XC and HR-X SPE materials

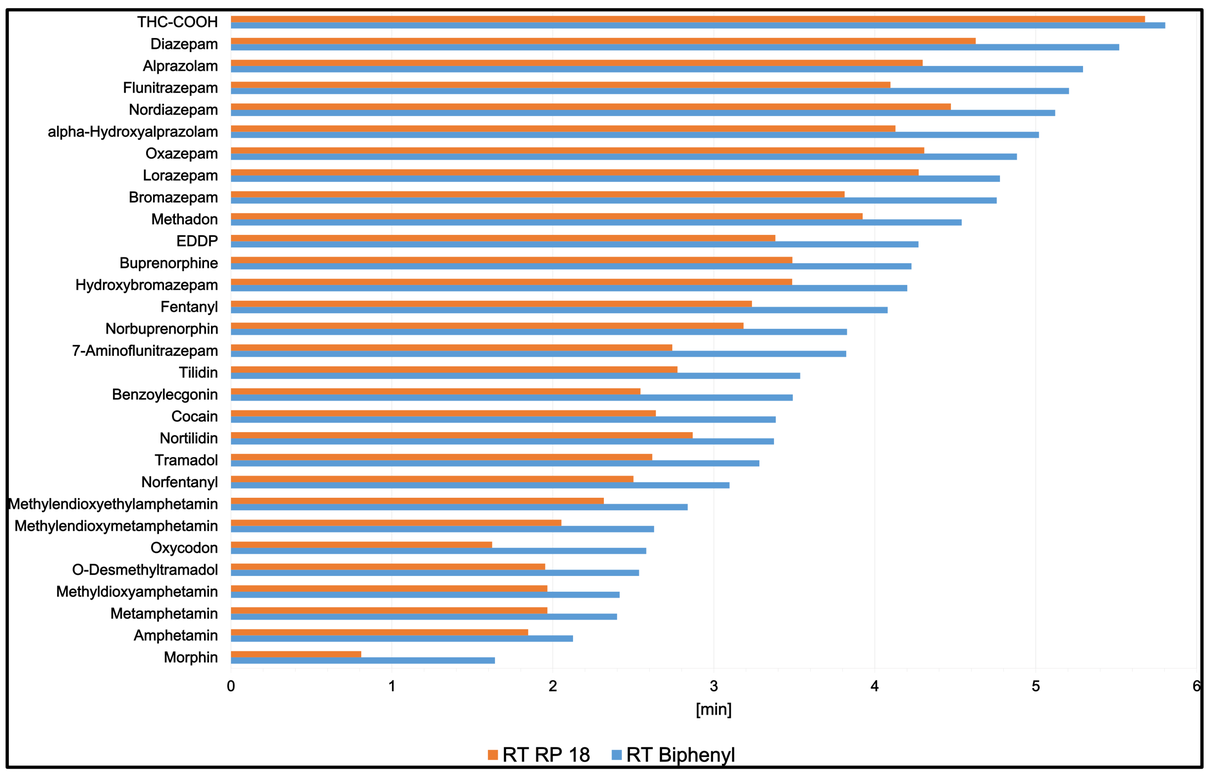

Figure 1: Retention times using the NUCLEOSHELL® RP18, 2.7 µm, and Biphenyl, 2.7 µm

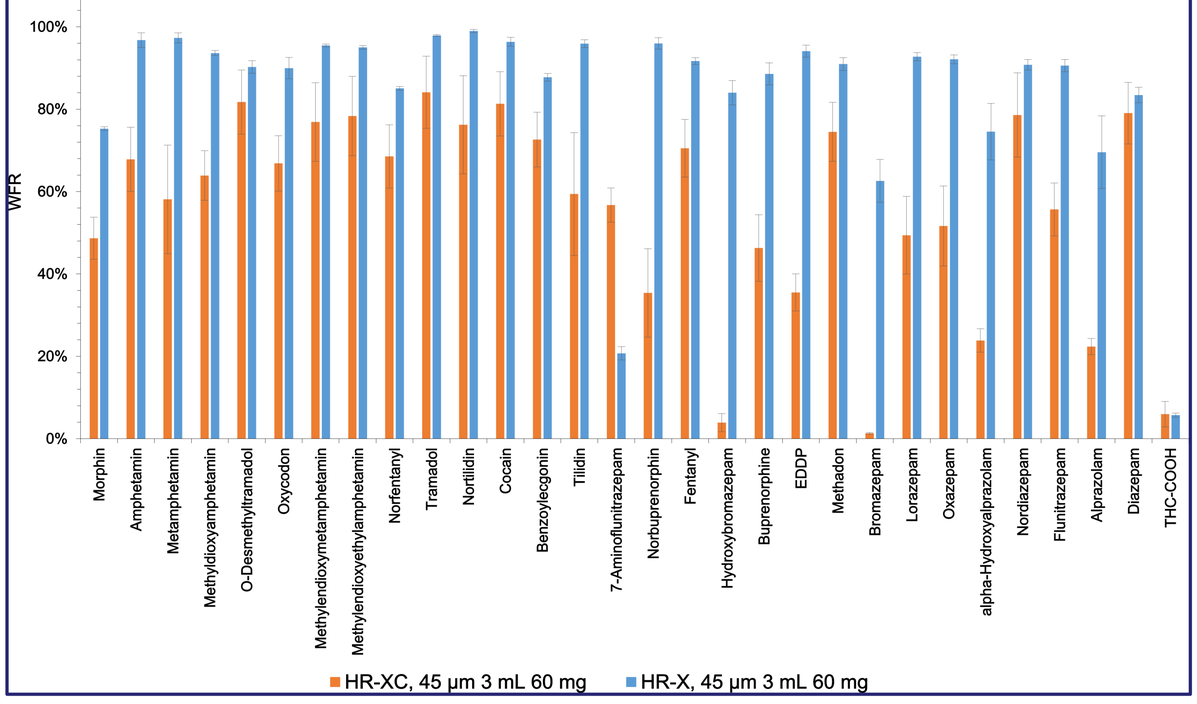

Figure 4: Comparison of the recovery rates of the two most efficient SPE materials

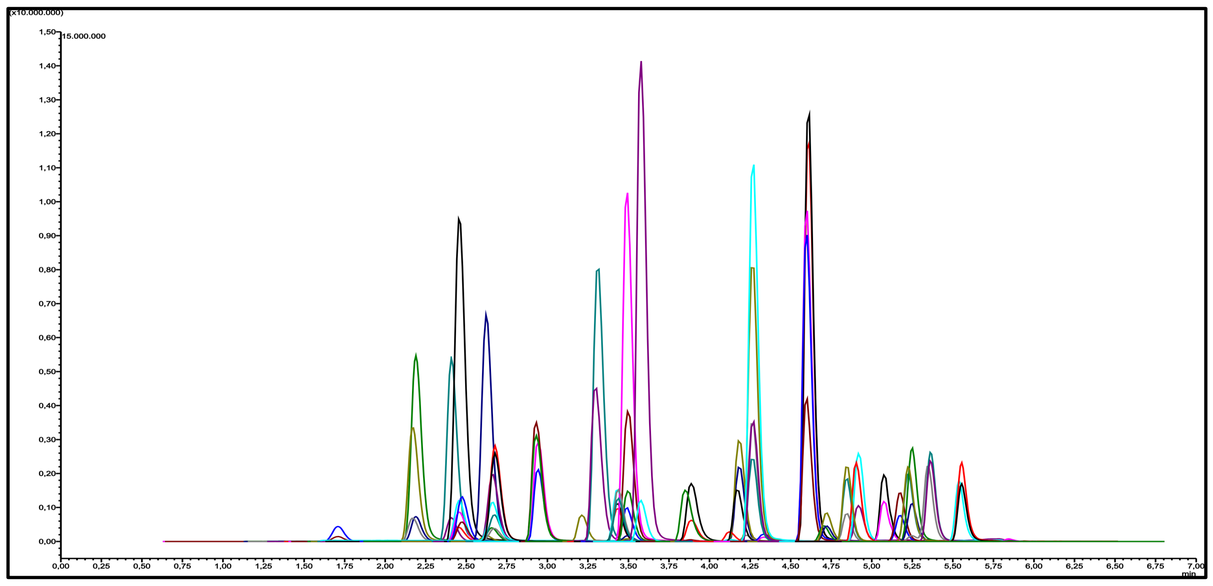

Figure 2: Chomatogram of the analytes using the NUCLEOSHELL ® Biphenyl, 2.7 µm, 100 mm

Results and Discussion I

After first experiments, comparison of the “top two“ chromatographic columns (RP18 and Biphenyl, Figure 1) showed a clear benefit of the biphenyl material. An exemplary chromatogram of 53 analytes including 23 internal standards is shown in Figure 2.

LC separation was by 0.1% formic acid in water (eluent A), and 0.1% formic acid in methanol (eluent B). Gradient elution was performed utilizing the following profile: 0 min, 10% B, held for 0 min; increased to 95% B within 5.0 min, held for 2.0 min. With a constant flow rate of 0.4 mL/min and a column oven temperature of 30° C, a total run time of 15 minutes was obtained.

Results and Discussion II

Different SPE sorbents were compared with different procedures. The protocol (Figure 3) and the recovery rates (Figure 4) for the SPE materials HR-XC and HR-X (45 µm, 3 mL, 60 mg) are displayed.

In the optimized method, SPE is carried out with a hydrophobic polystyrene-divinylbenzene copolymer material (HR-X, 45 µm), using methanol with 5% formic acid and MTBE (tert-butyl methyl ether) with 5% formic acid as extraction eluent. A NUCLEOSHELL® Biphenyl chromatographic column (EC 100/2 mm, 2.7 µm), with a methanol-water gradient was used.

Deuterated internal standards were implemented and the required sensitivity was accomplished with a sample volume of 100 µL urine.

Recovery of at least 50% was reached for all parameters – with exception of 7-aminoflunitrazepam and THC-COOH.

As expected, achieving a good detection of THC-COOH was difficult, but analysis of 7-aminoflunitrazepam and bromazepam was also challenging.

Conclusion

By comparing different chromatographic columns, SPE materials and solvents, we eventually developed a satisfactory method that allows determination of thirty analytes in a single analytical run and fulfills the requirements for the limits of quantification (according to German guidelines).

Few optimization steps have to be conducted and the final method will be fully validated according to the GTFCh guidelines.