Mixed-mode solid-phase extraction for the screening of drugs in systematic toxicological analysis

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SUMMARY

The isolation of a large variety of drugs from biological specimens is being considered a fundamental but difficult task when screening for potentially harmful substances. The latter, which is also called systematic toxicological analysis (STA), applies to all areas of toxicology, such as forensic, clinical, workplace, human performance, and environmental toxicology. Other areas in which screening analyses are often performed are doping analyses and residue analyses in food products. In this thesis it has been attempted to develop simple and useful methods that are generally applicable to the pretreatment of biological samples in STA by focusing on the potentials of solid-phase extraction (SPE) for the isolation of drugs and other toxicologically relevant substances.

Chapter 1 introduces the demands and problems of biological sample preparation for drug screening in STA, and outlines the aims and scope of this thesis. The largest challenge is to find a method that is capable to deal with the entire scope of toxicologically relevant drugs, regardless whether they are acidic, basic, neutral, non-polar, or polar substances.

In *Chapter 2* the basic theories and the strategies of SPE and the developments and trends of SPE in drug screening are reviewed. Prior to SPE biological samples need to be pretreated. Different samples and different detection techniques may need different pretreatment methods varying from simple to complex. The common methods are dilution, precipitation, hydrolysis, sonication, enzymic digestions, or the combination of these methods. When dealing with SPE, each step must be optimized carefully, since many factors (such as the properties of sorbents, wash solvents and eluents, pH of sample and column system and flow-rates of samples and eluents passing through the column) may influence the final results. From reviewing the literature, it was assumed that mixed-mode SPE columns, containing hydrophobic and cation exchange functional groups may be a proper compromise for drug screening. Finally, new developments in SPE materials and methodology are addressed as well as the trends to automate SPE for drug screening.

Chapter 3 gives a profile of the potentials of the mixed-mode Bond Elut Certify columns by extracting six selected drugs from plasma. The high recoveries (86-112%) indicate that the mixed-mode SPE column which exhibits a unique hydrophobic-cation exchange extraction mechanism may be a good choice for the extraction of a wide variety of drugs from biological matrices.

Chapter 4 shows the development of a one-column two-fraction SPE procedure for drug screening. A broad selection of drugs was extracted from plasma by using a single Bond Elut Certify column. According to the physical-chemical properties, various classes of drugs were extracted and separated into two fractions. The acidic, neutral and weakly basic drugs were present in the first fraction (acetone-chloroform), while the basic drugs

were present in the second fraction (ammoniated ethyl acetate). Some weakly basic drugs with a pK_a close to the pH of the extraction system (3.3) appeared in both fractions. The recoveries of 25 drugs ranged from 82 to 105%, with relative standard deviations less than 10%. The extraction of seven drugs from urine also showed good recoveries (97-104%) and precision (RSD 1.6-5.7%).

Chapter 5 deals with solid-phase extraction of drugs from whole blood. Four blood pretreatment methods were tested. Sonication/dilution was found to be the best one. The extraction procedure developed for plasma was successfully used for whole blood with for 15 drugs recoveries higher than 81% with relative standard deviation of 8.2% or less. The extraction procedure developed for Bond Elut Certify columns was found to work equally well on Clean Screen DAU columns, another commercial brand of mixed-mode bonded silica columns, which contain similar functional groups as the former.

Chapter 6 evaluates the use of Bond Elut Certify columns for the extraction of morphine from whole blood. The extraction procedure was optimized by monitoring tritiated morphine in each step. In the final procedure more than 80% of tritiated morphine was recovered. The results show that the recoveries of morphine were independent of the concentration in the range of 5-4000 ng/mL.

Chapter 7 shows that the extraction of various drugs from liver is more difficult than from biological fluids. Because of the complexity of the liver matrix, two different enzymic digestion methods may be required and the extraction should be performed on two Bond Elut Certify columns, separately: acidic and neutral drugs on the one hand, basic drugs on the other hand.

The investigations described in *Chapter 8* demonstrate the potentials of Bond Elut Certify columns for the extraction of basic drugs from biological fluids at low concentrations. By using these columns along with our extraction procedure the basic drugs at low levels (100-200 ng/mL) could be quantitatively extracted from biological fluids, and subsequently analyzed by GC-NPD.

Chapter 9 evaluates the reusability of Bond Elut Certify columns for the extraction of drugs from plasma. The recovery studies show that these columns can be reused after regenerating the functional groups of the column sorbents. However, the extraction potentials of these bonded silica columns decrease with the number of reuses. It is recommended, therefore, to reuse Bond Elut Certify columns only once or twice.

A lot-to-lot reproducibility study (*Chapter 10*) demonstrates that the two commercial mixed-mode SPE columns, Bond Elut Certify and Clean Screen DAU, are suitable for routine drug screening in STA. Both brands provided satisfactory recoveries (>81%) and precisions (SD<9.2). The 95% confidence intervals of the means obtained by ANOVA show that there are no significant differences between the tested lots, neither for Bond Elut Certify, nor for Clean Screen DAU columns. However, there was a slight

difference in the recoveries of the two brands that was significant at the 95% level.

The last two Chapters represent attempts to automate our SPE procedure for the extraction of drugs, using an ASPEC system. The initial study was based on the build-in operation program of the ASPEC system, and a semi-automated procedure was developed for use with 1 mL Clean Screen DAU columns (*Chapter 11*). Although the ASPEC system can work in either a batch or a sequential mode, only the latter was found suitable when dealing with larger numbers of samples. In *Chapter 12* a fully automated SPE procedure is described using 3 mL Bond Elut Certify columns. The extraction process, from column preconditioning to the addition of the chromatographic standard solution, performed fully automatically after we modified the software of ASPEC system. The sample and the eluent flow-rates were optimized. The results show that the automated procedure offers satisfactory recovery (>82%) with very good reproducibility (RSD<4.6%).

Furthermore, it is important to note that the developed concept appears to be independent on the brand of the columns, but that it can be generally applied to SPE columns that provide a suitable mix of hydrophobic and cation exchange interactions.

Finally, it can be concluded that in sample preparation science, liquid-liquid extraction is no longer the only means to isolate drugs from biological matrices for drug screening in STA. This thesis demonstrates that this task can be accomplished by mixed-mode solid-phase extraction, not only manually but also automatically. It can be expected that mixed-mode solid-phase extraction will play a very important role in drug screening in the near future.