New Automated Screening System for the Determination of Basic Compounds in Urine by On-Line Extraction-HPLC-DAD

Thomas Grobosch, Lena Schönberg, Dagmar Lampe, Charlotte Kloft

Abstract

A new automated, qualitative screening HPLC method for the identification of basic compounds in urine has been established. A 1-ml volume of urine was automatedly extracted on-line and separated on two coupled strong cation-exchange columns under isocratic conditions. The use of the photodiode-array detector (DAD, λ=190-370 nm) gave access to a library of more than 2600 toxicologically relevant compounds. The validated method is reliable, simple and in addition it has been successfully proven in the analysis of real biological specimens for the routine use as an additional tool in systematic toxicological analysis and drugs of abuse confirmation analysis.

1. Introduction

Systematic toxicological analysis (STA) based on GC, HPLC and immunological methods is usually performed in plasma/serum and urine. However, some compounds such as psilocin, scopolamine and morphine with short half-lives, are difficult to detect with common STA screening methods and require specialised analytical methods. The Remedi^{TM}-HS (Bio-Rad, Munich, Germany) presents such a specialised system for the analysis of basic compounds (e.g. alkaloids). However, it will be taken out of service at the end of 2008.

The aim of this study was to develop a chromatographic screening method for toxicological analysis in urine with main focus on basic compounds, taking advantage of the larger time window of detection in urine compared to blood. Furthermore, as urine presents the matrix of choice for drugs of abuse (DOA) analysis, it was investigated if the method is suitable for this field of application. A HPLC-system with a DAD was chosen to access a commercially available spectra library with >2600 spectra [3] and to allow the identification of toxicologically relevant metabolites by comparing their spectra to those of the parent compound.

The utility of the developed method for STA and DOA analysis is discussed in the following and illustrated with example chromatograms of both, the developed system and the Remedi^{TM}-HS.

2. Material and Method

2.1 Urine samples

Urine samples were sent to our laboratory from hospital emergency rooms, psychiatric units and substance abuse clinics for analysis. The samples were delivered in monovettes and stored at 5-8°C until they were analysed.

2.2 Sample preparation

The urine samples were centrifuged for 5 min at 15 000 x g, 1.0 mL of each sample was transferred to a 2.0-mL polypropylene cup, diluted with 500 µL internal standard solution, vortexed for 10 s and centrifuged again for 5 min at 15 000 x g. The samples were placed into the auto sampler. The injection volume was 1.0 mL.

2.3. Extraction and analytical procedure

Prepared urine samples were extracted by automated on-line extraction and separated on two coupled HPLC columns under isocratic conditions. The mobile phase consisted of 0.05 *M* potassium dihydrogen phosphate buffer (pH 2.3) and acetonitrile/water (90/10, v/v). Peak identification was carried out by chromatographic data and spectra comparison with > 2600 spectra. Criteria for positive peak identification were a 99.9% agreement between the obtained and the library spectrum (similarity ≥ 0.999) and a maximum deviation of the relative retention time of ± 5%.

2.4 Validation

The method was validated by an exemplary performance control test, which consisted of codeine **1**, EDDP **2**, morphine **3**, scopolamine **4**, MDA **5** and the internal standard. Recovery was > 73% and intra-assay precision ranged from 0.4-7.2%. Linearity was obtained from 0.10(0.25)-15.0 µg/mL for **1**,**2**,**3** and **4** and 0.10-5.0 µg/mL for **5** ($R^2 \geq 0.993$). The LLOQ was 0.10 µg/mL for **1,2,3,5** and 0.25 µg/mL (S/N>3) for **4**. All stock solutions showed stability over an observed time period of 28 days. The detailed method and validation data has been published elsewhere [5].

3. Results and Discussion

The developed system has proven to be an adequate alternative to the Remedi^{TM}-HS system. In comparison to the Remedi^{TM}-HS the new system led to a five times lower limit of detection for benzoylecgonine and therefore replaced time and work intensive methods in many cases. The new system has been applied to over 700 cases of clinical toxicological investigations including drugs of abuse confirmation screenings. The examples of different intoxications (Jimson weed,

magic mushrooms, amphetamine, cocaine) illustrate the applicability of the new tool in the field of clinical toxicology.

The evaluation of the analysed samples demonstrated, that the developed analytical database represents a reliable method for the identification of basic substances. A detailed report will be given subsequently [6]. In the following figures are example chromatograms of two intoxication cases (Fig. 1 and 2) and two drugs of abuse confirmation cases (DOA, Fig. 3 and 4) are shown.

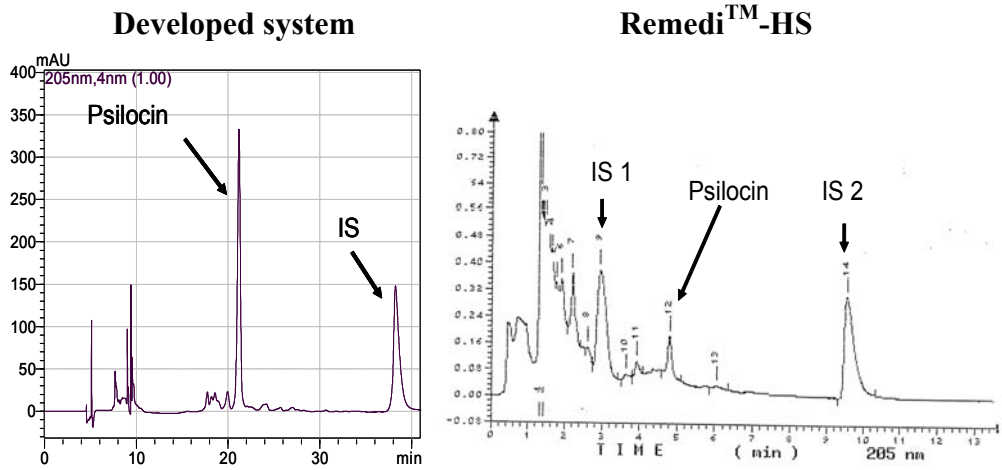

Fig. 1: *Psilocin intoxication, female patient, (*1998), creatinine = 0.98 g/L*

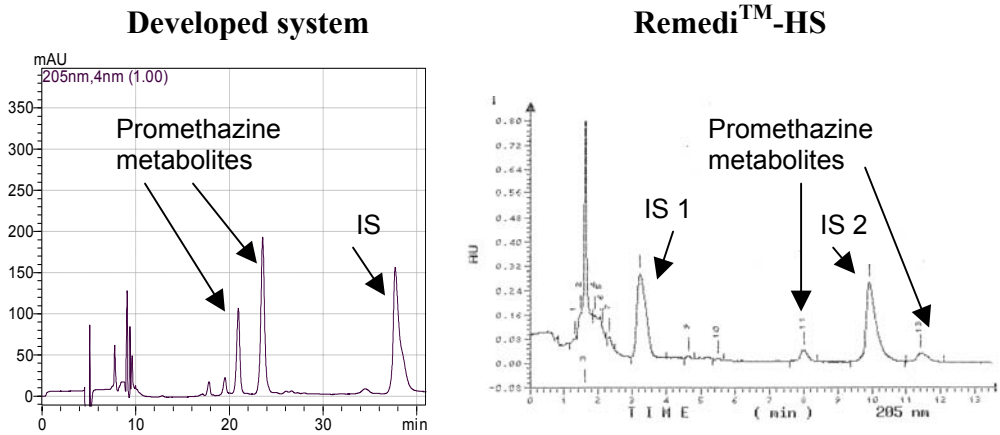

Fig. 2: *Promethazine ingestion, female patient, (*1966), creatinine = 0.87 g/L*

Developed system **Remedi^{TM}-HS**

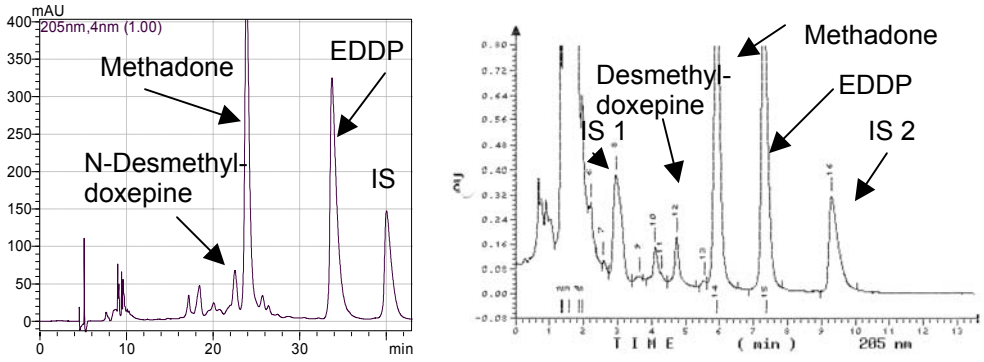

Fig. 3: *Methadone substitution, male patient, creatinine = 1.18 g/L*

Developed system **Remedi^{TM}-HS**

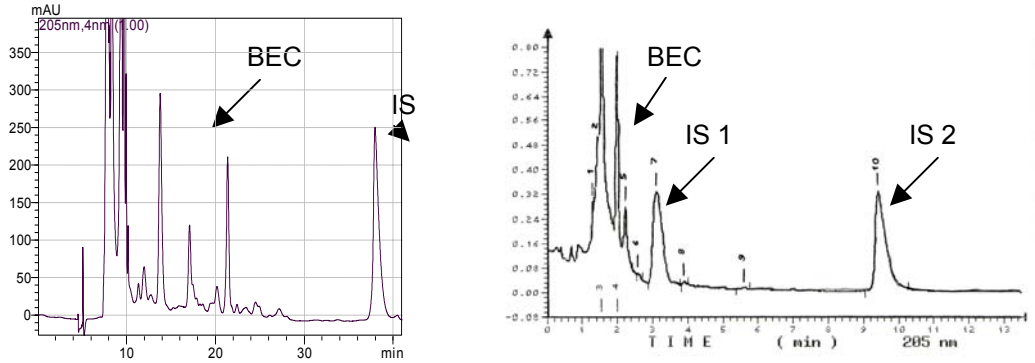

Fig. 4: *Cocaine abuse, male patient, creatinine = 0.45 g/L*

As can be seen from the Figures 1-4, the compared methods demonstrated the same final analysis results and thus can be used for the same fields of application. In cases of STA, the HPLC-UV method is well suited as a complementary method to other techniques within the rational chemical-analytical approach of general unknown screening in order to identify as many xenobiotics as possible. According to N. Sadeg et al., who described a 12 months` experience of toxicological screening with the Remedi^{TM}-HS in a general hospital in France [4], it can be also stated for the developed method, that this technique presents a valuable tool for additional urine screening within STA. The shorter analysis time of the Remedi^{TM}-HS (approx. 20 min versus 41 min (developed method)) may be an advantage for fast diagnosis in intoxication cases but the better quality of chromatography in combination with the higher sensitivity of the new system offers

novel possibilities in urine screening of xenobiotics. Thus, 41 min for analysis is still acceptable and means that measurement of approximately 32 samples per 24 h is possible.

Both methods should be run as part of a complex analysis strategy within STA in acute intoxication cases which take about 1-2 h. In cases of DOA, immunological pre-screening should be performed. If the immunoassay result cannot be verified by the HPLC-UV method, a more sensitive method (e.g. GC-MS [2]) must be considered.

4. Conclusions

The developed system proved to be an adequate alternative to the Remedi^{TM}-HS system [1]. In comparison to the Remedi^{TM}-HS the new system led to a five times lower limit of detection for benzoylecgonine and therefore has replaced time and work intensive methods in many cases. Furthermore, the developed system offers the advantages of common HPLC equipment, laboratory material and modern computer software. The new system has been applied to over 700 cases of clinical toxicological investigations including drugs of abuse, confirmation screenings (amphetamines, cocaine/BEC, EDDP/methadone and opiates (morphine, codeine)) [6].

5. References

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Dr. rer. nat.Thomas Grobosch,
PD Dr. med. Dagmar Lampe
BBGes
Institute of Clinical Toxicology
and Poison Control Centre
Oranienburgerstr. 285
D-13437 Berlin
grobosch.tox@bbges.de
Lampe.tox@bbges.de

Dr. Lena Schönberg
Department of Clinical Pharmacy
Institute of Pharmacy
Freie Universitaet Berlin
Kelchstr. 31
D-12169 Berlin
L.Schoenberg@gmx.de

Prof. Dr. Charlotte Kloft
Department of Clinical Pharmacy
Faculty of Pharmacy,
Martin-Luther-Universitaet Halle-Wittenberg
Wolfgang-Langenbeck-Strasse 4
D-06120 Halle-Wittenberg
charlotte.kloft@pharmazie.uni-halle.de