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Modified Multi Target Screening (MTS) with QTrap 3200 and methanol as eluent with Luna PFP column

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Abstract

Aim: To switch our Multi-Target Screening (MTS) procedure for 700 drugs from acetonitrile to methanol with a new column to save costs.

Methods: For LC a new column was used, Phenomenex Luna PFP (2) (150 x 2 mm, 5 µm), two different gradient HPLC systems (Agilent 1100 or Shimadzu) and a Qtrap 3200 mass spectrometer were available for determination of retention times. Gradient elution was performed with methanol using formic acid (0.2 %, solvent A) and methanol with 0.2 % formic acid (solvent B). Overall run-time was 18 min.

Results: Retention times of 621 compounds were compared with the two LC-MS/MS systems, a Multi-Target-Screening procedure as used before with Restek Allure PPF column was set-up with a new scheduled MRM catalogue (new retention times for the MRM-survey scans with scheduled MRM) with a time window of 60 s for each compound.
A new internal standard mixture was applied for quality control of the procedure.

Discussion: The Multi Target Screening with QTrap 3200 has been adapted to a new column and methanol as eluent with advantages over the previously published method. The reproducibility of retention times has been tested using one type of column from different batches, and was found to be better than with Restek Allure PFP columns. Costs per sample were reduced by use of methanol instead of acetonitrile for gradient elution and solvent flow could be kept constant, whereas in the previous procedure it was raised from 0.3 to 1 mL/min flow rate.

Conclusions: Application of the new MTS procedure to urine, serum and blood samples showed similar results as obtained previously with the Restek Allure PFP column. Limits of identification for selected compounds are still under investigation. The integration of a new mixture of internal standards makes it possible to control the efficiency of sample preparation steps (precipitation/dilution, extraction) for use with biological samples.

1. Introduction

Until 2009, a Multi Target Screening (MTS) had been developed with LC-Qtrap-MS/MS with library searching based on the "Weinmann" MS/MS library for compound identification using a Restek Allure PFP column and gradient elution with acetonitrile [1, 2, 3]. Extracts of serum

or blood or diluted urine samples are injected in the LC-MS/MS system and multiple-reaction monitoring of 700 compounds is used for compound detection. In case of a signal in MRM above a set threshold, an enhanced product ion scan is triggered, and the resulting MS/MS-spectrum is searched in a library with 1253 compounds. The data acquisition and data analysis is automated, data analysis is routinely performed with Cliquid 2.0 or 3.0 software package. Acquisition and analysis are recommended to be performed on different computers, since work on data analysis (with Cliquid) and control of the results (hits) with Analyst still uses some resources and time of a chemist. For the first system we used a Phenomenex Allure PFP column, with elevated flow of acetonitrile, which raised in price during the automobile crisis. Methanol is cheaper, and therefore, we changed the system to a methanol based LC-system, and had to exchange the chromatographic column, since methanol could not elute all compounds from the Allure PFP column.

2. Material and Methods

Columns used were different batches of Phenomenex Luna PFP (2) (150 x 2 mm, 5 µm), two gradient HPLC systems (Agilent 1100 or Shimadzu) and a Qtrap 3200 mass spectrometer were available for determination of retention times. Gradient elution was performed with methanol using formic acid (0.2 %, solvent A) and methanol with 0.2 % formic acid (solvent B). Overall run-time was 18 min.
The LC-system based on this new column and the internal standard mixture was developed in a collaboration with T. Grobosch and C. Köhler (BBGes), who use this system for their screening procedure based on automated SPE and QToF technology (see oral presentation V-17, Mosbach 2011 [4]).
The different LC-systems had different dead volumes. The Agilent system has a larger pulse damper and a larger gradient mixer; volumes are larger than used in the mixer of the Shimadzu system. Fig. 1 shows the parts built in an Agilent system and Fig. 2 the mixer of the Shimadzu pump system. Therefore, a shift of retention time can be explained by slower gradient generation from the pump system.

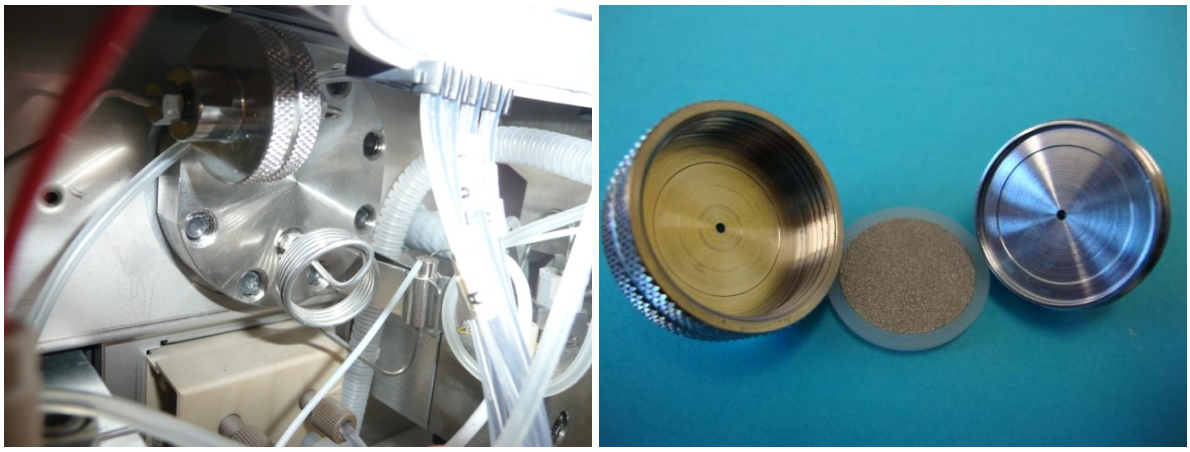

Fig. 1. Parts of the Agilent system: pulse damper (coil of stainless steel tubing) and mixer, i.e., filter cartridge (dead volume from pumps to injector: approx. 0.5 mL). Dead volume in detail: Y-Connector 3 µL, capillary: 2 µL, pulse damper: 80 µL/100 bar, mixer: 200 µL, purge valve 80 µL, loop 20 µL, and tubing (depending on length and diameter). Sum: approx. 400 µL.

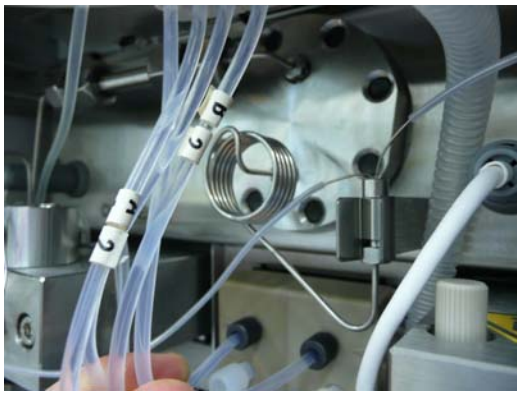

Fig. 2. *Left:* Parts of a standard Agilent system: mixer is the packed steel column (built in horizontally on the left), which was exchanged in our system by the round filter based mixer (see Fig. 1). The column-type mixer has an even larger dead volume (approx. 600 µL). *Right*: Shimadzu mixer and tubing (from mixer to column): dead volume approx. 100 µL

3. Results and Discussion

The dead volumes of the HPLC-devices are critical for the speed of gradient mixing. Since the method runs with gradient elution, and Agilent systems have a larger dead volume, a shift of retention times of approx. 1 minute was observed for the Agilent system compared to the Shimadzu system.

The retention times of up to now 700 compounds were determined - 621 of which were compared on two instruments - and the Multi-Target-Screening procedure as used before with Restek Allure PPF column was set-up with a Phenomenex PFP column and a new scheduled MRM catalogue (new retention times for the MRM-survey scans with scheduled MRM) with a time window of 60 s for each compound. In previous experiments different batches of the column showed good reproducibility of retention times for a system suitability test mixture applied. Furthermore peak shapes with Phenomenex PFP columns were better than with Allure PFP (Restek), especially with the highly lipophilic compounds – such as THC - which had shown peak broadening with the former system.

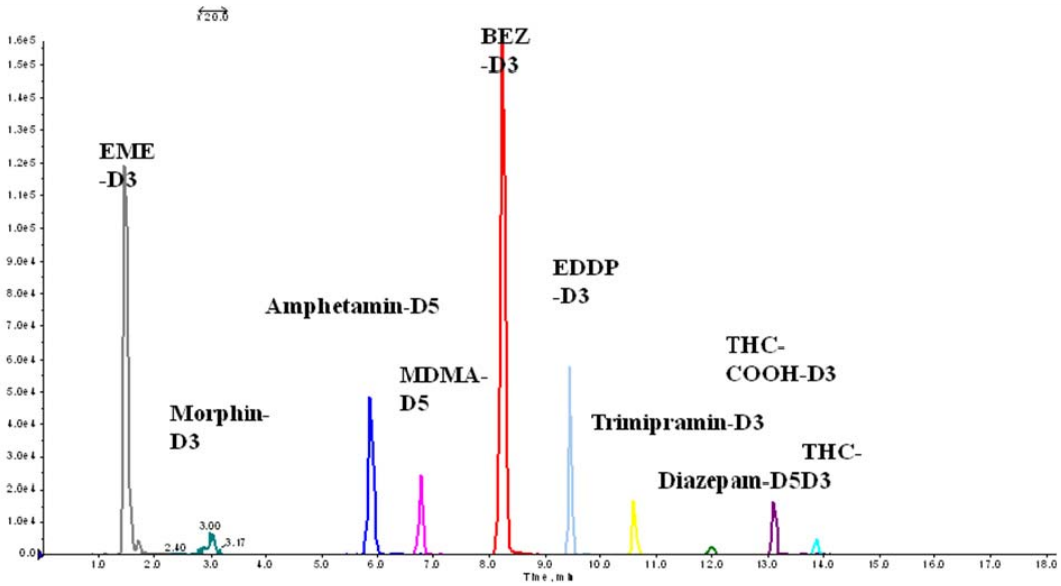

Fig. 3. A new internal standard mixture was applied for quality control of the procedure.

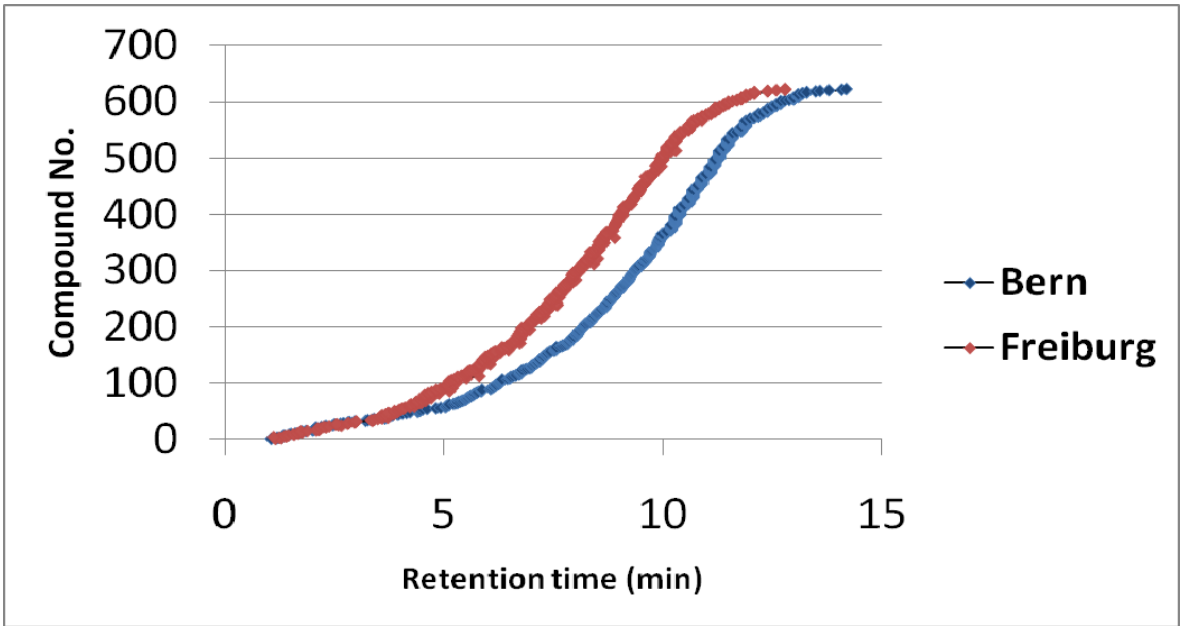

Fig. 4. Comparison of retention times of 621 compounds run at Freiburg with Shimadzu and at Bern with Agilent LC-system. Shift of retention time during gradient elution is obvious due to the larger dead volume of the Agilent system.

4. Conclusion

The new MTS procedure showed good robustness on different instruments concerning retention times and batch-to-batch reproducibility of columns used. Application of the new MTS procedure to urine, serum and blood samples (results not shown here) showed similar results as obtained previously with the Restek Allure PFP column. Limits of identification for selected compounds are still under investigation. The integration of a new mixture of internal standards makes it possible to control the efficiency of sample preparation steps (precipitation/dilution, extraction) for use with biological samples. New compounds – such as designer drugs - will be integrated in the MTS procedure.

5. References

[1] Dresen S, Gergov M, Politi L, Halter C, Weinmann W. ESI-MS/MS library of 1,253 compounds for application in forensic and clinical toxicology. Anal Bioanal Chem. 2009, 395(8): 2521-6.

[2] Dresen S, Ferreirós N, Gnann H, Zimmermann R, Weinmann W. Detection and identification of 700 drugs by multi-target screening with a 3200 Q TRAP((R)) LC-MS/MS system and library searching. Anal Bioanal Chem. 2010, 396(7): 2425-34.

[3] Oberacher H, Weinmann W, Dresen S., Quality evaluation of tandem mass spectral libraries. Anal Bioanal Chem. 2011, 400(8): 2641-8.

[4] Köhler C, Grobosch T, Binscheck T, Surmann P. Development of a systematic toxicological screening method using an automated on-line SPE-LC-QqTOF System (XLC-QqTOF). Oral presentation at GTFCH-Symposium Mosbach 2011, V 17.