A novel front-end automation approach for sample introduction, cleanup and collection

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Overview

- A standalone solid-phase extraction system and a multipurpose autosampler is combined to obtain a flexible front-end system.
- The potential of the system is shown by analyzing standard solutions in LC, online SPE and the new inject-extract-collect approach.
- Good overall system performance, linearity and repeatability is obtained.
- The presented automated approach is capable of injecting crude samples and collecting purified extracts to be further processed by other analysis and detection techniques including LC-MS parallel processing.

Introduction

Over the past few years special tools have been developed to enhance the performance of mass spec instruments. New ion-source and linear trap technology improved sensitivity and decreased the limits of detection. In order to take full advantage of these new developments, the improvement of front-end systems have become of equal importance. Separation performance is increased and or analysis time reduced by means of UHPLC. Additionally, online solid-phase extraction systems are adapted to automate sample preparation and reduce matrix effects. In this poster a novel automated approach is shown for injecting crude samples and collecting the purified extracts in order to be further processed by any other analysis and detection technique e.g. multiple (LC-)MS systems for parallel processing.

Experimental conditions

Instruments and settings

SP1240 | Xprezzo | Integrity

Pump | Extraction | Autosampler

LC conditions

Mobile phase:	isocratic 35/65 ACN/H_2O at 1 mL/min
Column:	Inertsil ODS-3 4.6 x 50 mm, 3 µm
Injection:	2 µL Carbamazepine solution in 10/90 ACN/H_2O
Wash:	250 µL 35/65 ACN/H_2O

Online Extraction conditions

Cartridge:	HySphere C18HD 10x2 mm (7 µm)
Condition:	1 mL ACN at 5 mL/min
Equilibrate:	1 mL 5/95 ACN/H_2O at 5 mL/min
Sample load*:	1 mL 5/95 ACN/H_2O at 2 mL/min
Wash:	1 mL 5/95 ACN/H_2O at 5 mL/min
Elution*:	3 min with LC mobile phase

** Injection and analysis conditions as for LC*

Extract collection conditions

Cartridge:	HySphere C18HD 10x2 mm (7 µm)
Condition:	1 mL ACN at 5 mL/min
Equilibrate:	1 mL 5/95 ACN/H_2O at 5 mL/min
Sample load*:	1 mL 5/95 ACN/H_2O at 2 mL/min
Wash:	1 mL 5/95 ACN/H_2O at 5 mL/min
Drying:	4 mL Air at 4 mL/min
Elution:	35/65 ACN/H_2O at 1 mL/min (variable volume)
Collection:	4 mL Air at 4 mL/min

** Injection 100 µL, Carbamazepine solution in 10/90 ACN/H2O*

Collected extracts are subsequently analyzed utilizing LC (5 µL injections)

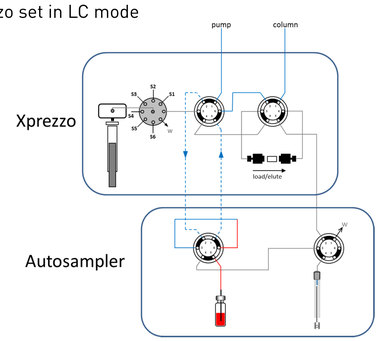
Detection conditions

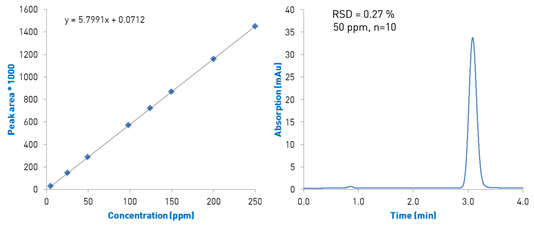
UV detection: Jasco UV-2075 with TP cell, 285 nm

Results and Discussion

Standard LC analysis

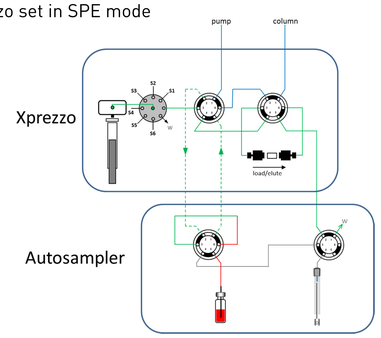
Xprezzo set in LC mode

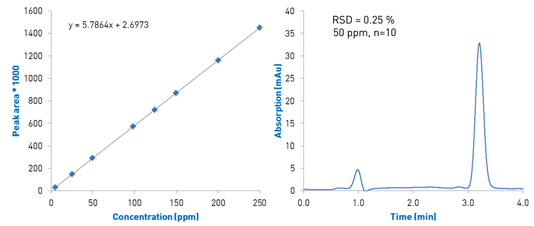




Online SPE analysis

Xprezzo set in SPE mode

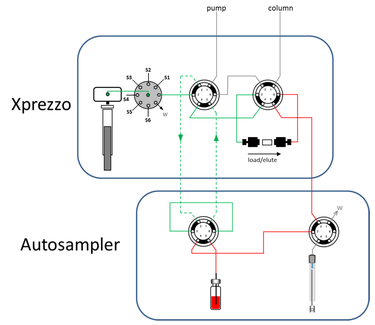




- As shown good linearity and repeatability is obtained for both the standard LC runs and the online SPE determination of Carbamazepine test solutions.
- Additionally, there is no statistical difference found between the LC and online SPE calibration curves indicating that there is no analyte loss for online SPE, i.e., the total amount of analyte injected is detected.

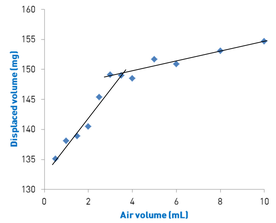
Extract collection

Extraction of Carbamazepine is performed as done for online SPE. After extraction the cartridge (and flow path) is dried with air.
Next, a precisely metered volume of desorption liquid is flushed over the SPE cartridge for elution. The complete extract is then transferred towards a sealed empty vial by means of air.
Finally, the extract is analyzed by means of standard LC (same system).



Investigation of drying step

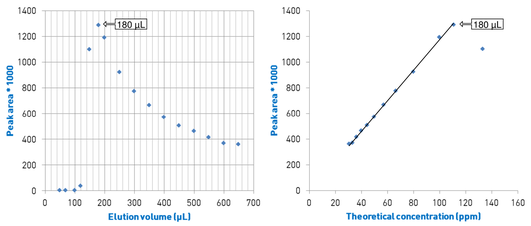
The collection flow path is filled with 5% ACN solution (i.e. green and red flow path displayed above without the 200 µL loop).
Next various volumes of air are dispensed at 4 mL/min to empty/dry the flow path. The displaced volume is collected in a vial and measured by weighing.



- An air volume of 4 mL is chosen for drying the flow path before elution and also for displacing the extraction volume into a sealed empty vial.
- Larger volumes of air require more time than the 60 s currently used and give hardly better results with respect to drying and extract displacement.

Investigation of elution volume

100 µL Carbamazepine solution (200 ppm) is injected and extracted. Next, various elution volumes (35% ACN) are used and collected into a sealed empty vial. Finally, LC analysis of the extracts is performed.

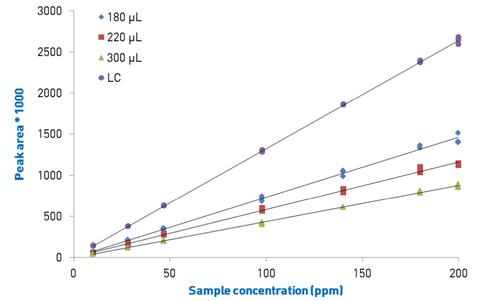


- Elution volumes smaller than 180 µL (35% ACN) are insufficient to desorb Carbamazepine from the C18 SPE cartridge.
- Larger elution volumes just dilute the extract obtained.
- The precision of a 180, 220 and 300 µL elution volume turned out to be 0.9, 0.8 and 0.5% (RSD, n=10), respectively, which equals a standard deviation of 1.5 µL independent of the used elution volume.

Inject, extract and collect approach

Calibration curve comparison

A series of standard solutions is prepared and either analyzed by injecting 5 µL directly (LC) or by injecting 100 µL and utilizing the inject, extract and collect procedure with 3 different elution volumes. The complete measurement is repeated 3 times for each concentration. 5 µL of the extract is analyzed with LC.



- As shown good linearity is obtained for the complete inject, extract and collect procedure.
- The slopes of the calibration curves reflect the dilution factors that occur when injecting 100 µL sample and collecting 180, 220 and 300 µL extract.
- The measured dilution factors 1.77, 2.29 and 3.04 are very close to the theoretical ones (1.8, 2.2 and 3.0, respectively) indicating a good overall performance of the system.

Method repeatability

The relative standard deviation of the inject, extract and collect procedure is calculated at 7 concentrations and the 3 different elution volumes.
It was found that the repeatability depends on the analyte concentration used and the elution volume. The RSD values obtained vary between 0.8 and 4.9%.

The overall repeatability per utilized elution volume is calculated by dividing the peak area obtained by the Carbamazepine concentration in the original sample and calculating the RSD of the 21 data points.

Overall method repeatability (n=21)

	180 µL	220 µL	300 µL	LC
Average	7286	5872	4307	13334
Standard deviation	268.2	185.7	123.0	251.5
Relative standard deviation [%]	3.68	3.16	2.85	1.89

- As shown good method repeatability is obtained for the complete inject, extract and collect procedure.

Conclusion

- A novel front-end system is shown that is capable of automated sample injection, solid-phase extraction and extract collection in sealed vials.
- Analysis methodology can be easily selected by means of the touchscreen of the Xprezzo. The combination with a multipurpose autosampler for extract collection emphasizes the system potential.
- Both the LC and online SPE mode show good linearity and repeatability.
- For extract collection the system flow path is dried with air. A compromise is made between drying time and the residual liquid inside the flow path.
- For Carbamazepine on a C18HD cartridge an elution volume of 180 µL was found to be most suitable.
- A good overall performance is shown for the complete inject, extract and collect approach, i.e., no analyte loss and linearity / repeatability that can measure up with default LC analysis.

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