APPNOTE

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Safe and Efficient Detection of Chloramphenicol in Food using Automated SPE

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Abstract
Solid Phase Extraction (SPE) using standard cartridges is widely regarded as the method of choice to extract analytes from samples with complex matrices or to extract and concentrate analytes from a wide variety of samples in general. In this paper, an automated SPE system is presented that is based on standard cartridges. It is shown that SPE with standard cartridges is easily and efficiently automated for use in LC/MS-based determination of illegal antibiotics in food products of animal origin. An established manual SPE method was easily transferred to the GERSTEL MultiPurpose Sampler (MPS) using the SPE option under MAESTRO software control. Recovery and precision was improved while significantly reducing the time and effort required for sample preparation.

Introduction
According to the Federal Statistical Office the European Union imported about 6 billion tons of food products of animal origin in 2004. Most of it was meat and meat products as well as fish and fish products. Food that is imported into the European Union (EU) has to meet EU standards of quality. Compliance with EU regulations is routinely monitored. In the past, residues of illegal antibiotics have been found in food products of animal origin like honey, prawn or poultry as well as in animal feed. When nitrofurane antibiotics and the active compound chloramphenicol (CAP) were found it caused real upheaval.

Chloramphenicol (Figure 1) is a broad-spectrum antibiotic that was extracted for the first time in 1947 from the bacteria Streptomyces venezuelae and is now produced synthetically. CAP has excellent antibacterial and pharmacokinetic properties. The application in human medicine and animal health is strongly regulated: The use of CAP in clinical applications is only allowed after careful consideration and only for treatment of contagious diseases like typhus, dysentery, diphtheria or malaria. CAP is commonly used for pets however. Animals that will subsequently be used for food production have not been allowed to be treated with Chloramphenicol within the EU since 1994.

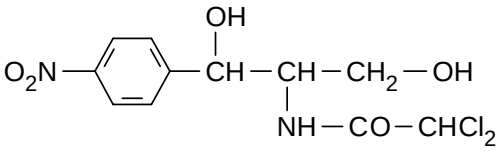

Figure 1: Structure of Chloramphenicol.

According to the Food and Agriculture Organization of the United Nations (FAO) CAP is suspected of causing genetic damage in human cells and of being a carcinogenic. Additionally it may be correlated to irreversible damage of the blood-forming cells of the bone marrow. Up to now no relationship between dosis and effect could be determined which led to the strict prohibition of CAP for the treatment of animals in Europe. Any confirmed positive finding led to the immediate withdrawal of the product from the market.

In literature, radioimmunological and enzymatic methods have been described for determination of chloramphenicol. In practice, only mass spectrometric methods are appropriate. GC/MS in chemical ionization (CI) mode as well as LC/MS enable laboratories to achieve the required low detection limits.

The sensitivity of a method strongly depends on the sample preparation. Even for highly selective LC-MS/MS methods a high matrix

GERSTEL AppNote Nr. 94

background can lead to inadequate quantification. However, this can be handled by spiking the matrix. In addition to liquid-liquid extraction, SPE using cartridges is the sample preparation method of choice when analyzing samples with a high matrix load. For fatty samples or complex finished products with non-polar matrices, the C18 cartridge is the best choice. However this manual procedure is time-consuming and labor-intensive.

The target of this study was to optimize sample preparation for CAP analysis and automate the steps to minimize the time required and improve the quality of results. The GERSTEL MPS autosampler with an automated SPE option as shown in figure 2 was used for this work.

Figure 2: GERSTEL MPS with integrated SPE option, solvent reservoir and injection valve.

The manual SPE method was automated using the GERSTEL MPS SPE option (Figure 3). The automated method gave slightly better results than a highly experienced manual operator.

Figure 3: MPS SPE option with rack for 3 mL standard SPE cartridges (left) and elution unit with cartridge holder (right). The MPS is shown while transporting the cartridge to waste position. 1 mL, 3 mL and 6 mL standard cartridges can be used.

Experimental

In this study, the focus was on the determination of CAP in prawns. This method can be easily adapted to other food products of animal origin. A 100 g sample of untreated muscle tissue is homogenized using a mixer. 10 mL ethylacetate and Chloramphenicol-d5 (internal standard) are added to a 5 g aliquot of the treated sample. The sample is again mixed using an Ultra Turrax and subsequently centrifuged. The liquid phase is recollected and concentrated in a rotary evaporator. The residue is diluted with methanol/water (1:10) and is then ready for the SPE process.

A standard 3 mL C18 SPE cartridge (M&N C18 endcapped) with 500 mg solid phase was used. In the MPS SPE option, cartridges are capped in such a way that no dead volume is left between the packing and the cap itself. The sample/solvent is loaded onto the cartridge under positive pressure using a syringe, ensuring optimal control over the automated elution steps.

All required steps are selected by mouse-click from a pull-down menu using the PrepBuilder function of the GERSTEL MAESTRO software. The SPE process was set up as followed:

GERSTEL AppNote Nr. 94

1. Conditioning of the SPE cartridge with 4 mL methanol, followed by 4 mL of water
2. Sample introduction (4 mL of the extract)
3. Rinsing the SPE phase with 1 mL water and 4 mL methanol/water mixture (1:10)
4. Elution of the analytes with 3 mL methanol/water mixture (1:1)

These steps lead to an enrichment of the analyte by a factor of 1.3. As a second concentration step the MPS is able to evaporate the solvent using a flow of inert gas while keeping the eluate at a specified temperature. In this study a second concentration factor of 5 (evaporating to 600 µL) and 10 (evaporating to 300µL) were tested.

From a chromatographic point of view Chloramphenicol is not a big challenge. Due to its semi polar character CAP can be separated easily from the matrix using a standard reversed phase (RP) chromatographic column. For this work a Phenomenex maxRP column (250 x 2.1 mm) was used because this column exhibits low bleeding, improving the detection limit of the method. Isocratic elution was performed using a mixture of 0.005 M ammonium formate adjusted with ammonia to pH 8.5 and acetonitrile. The mobile phase was adjusted to give a retention time of about 7.5 min for CAP. The internal standard (CAP- d5) has a slightly shorter retention time due to the isotope effect. The whole chromatographic run including rinsing and equilibration takes at most 25 minutes. During this time the automated SPE (duration about 15 min) for the following sample can be performed in order to maximize throughput. Using this method, 50 analyses can be performed in one day.

For the detection (Figures 4 and 5) an Agilent 1100 MSD 1956B as well as an Agilent 1100 MSD Ultra plus Ion Trap were used. Both systems used identical HPLC methods and columns. The detection was performed in negative ESI mode.

Although the Single-Quad-System in combination with the automated sample preparation achieves an adequate detection limit, the ion trap can go to even lower detection limits. Ion traps can perform MS/MS using the full fragmentation spectrum in contrast to triple quadrupole instruments. This capability can be used to select the best transition in the extracted ion mode. In this case the transitions 321 – 257 amu for CAP and 326 – 262 amu for CAP-d5 were selected (Figure 5).

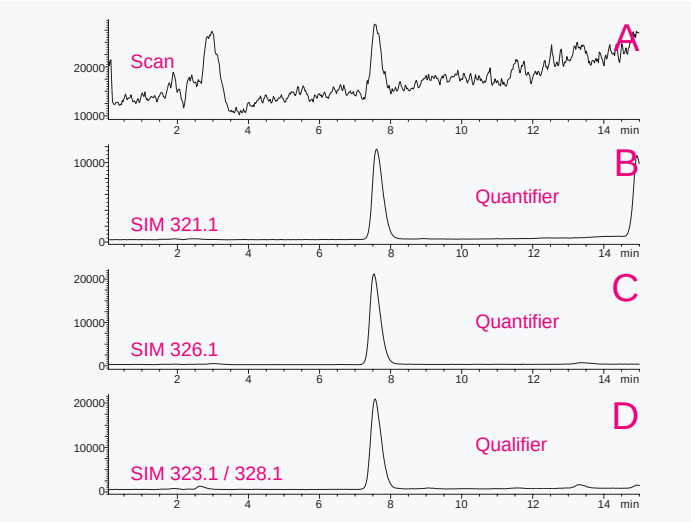

Figure 4: Simultaneously recorded LC/MS chromatogram traces of a prawn sample spiked with 2.0 µg CAP/kg: (A) scan 100–500 amu to check for impurities, (B) SIM at 321.1 amu as quantifier for CAP, (C) SIM at 326.1 amu as quantifier for CAP-d5 and (D) SIM at 323.1 and 328.1 amu as qualifiers for CAP and CAP-d5 respectively. The Agilent MSD 1100 Single Quad is able to achieve a LOD of 0.05 µg/kg for CAP (Signal-to-noise = 10:1).

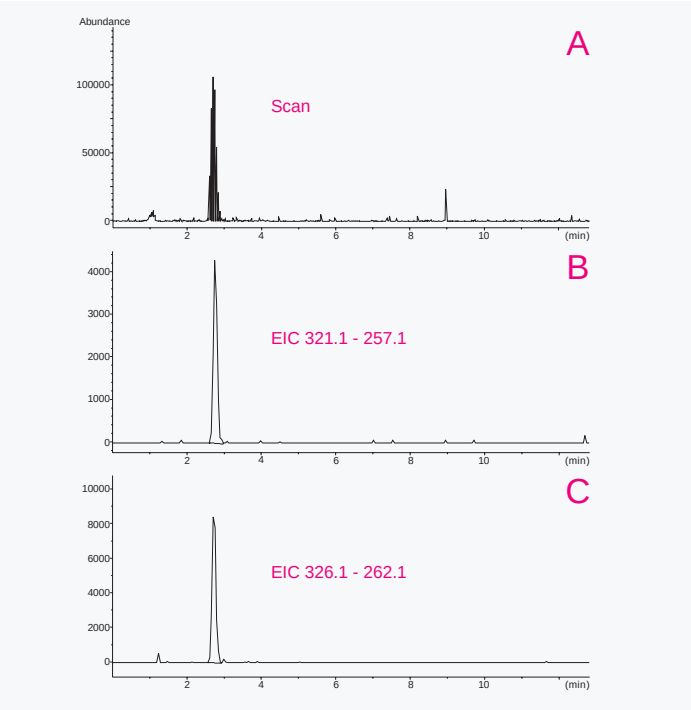

Figure 5: LC/MSMS chromatograms of CAP and CAP-d5 using the Agilent 1100 MSD Ultra plus Ion Trap. (A) Scan, (B) extracted ion chromatogram for the 321.1 – 257.1 amu transition (CAP) and (C) the transition of 326.1 – 262.1 amu (CAP-d5).

GERSTEL AppNote Nr. 94

Results and Discussion

Evaporative concentration of the eluate by a factor of 10 after the SPE process enables the analyst to achieve a very good LOD of 0.01 µg/kg for CAP using the ion trap system. The injected amount at this concentration is equivalent to 1 pg of CAP (Figure 6). Despite concentrating the eluate by a factor of 10 no significant interference from the accompanying matrix is observed in the quantification thanks to the SPE cleanup. This was tested in further experiments using more complex matrices (e.g. prepared prawn with spices and garlic oil). No significant differences were observed compared to the results from pure prawn samples.

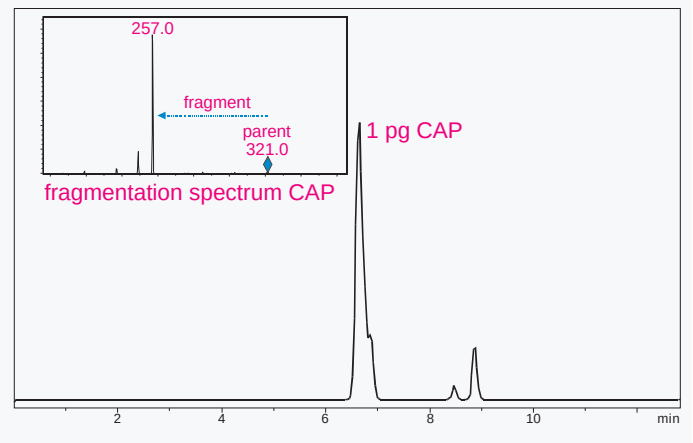

Figure 6: Chromatogram of the 321.0 –257.1 transition; detection of 0.01 µg/kg CAP in prawns.

Recovery and repeatability of manual SPE depends mainly on the human factor. The experience, knowledge and diligence of each individual laboratory technician has a big influence on the quality of results. Even minor errors can lead to major fluctuation in both recovery and repeatability. The use of the GERSTEL MPS with integrated SPE option shows that no fluctuations are observed when automating these steps. The excellent repeatability of the whole method (including extraction, sample preparation and LC/MS analysis) is visualized by a signal overlay of Chloramphenicol traces from six different samples (prawn samples spiked with 2.0 µg/kg Chloramphenicol each) (Figure 7). The recovery and repeatability is nevertheless generally good. For CAP a standard deviation of 2.0 % for the automated and 2.2 % for the manual approach using a highly experienced technician was achieved. The recovery was 92.1 % (MPS) and 89.6 % (manual) respectively as can be seen in figure 8.

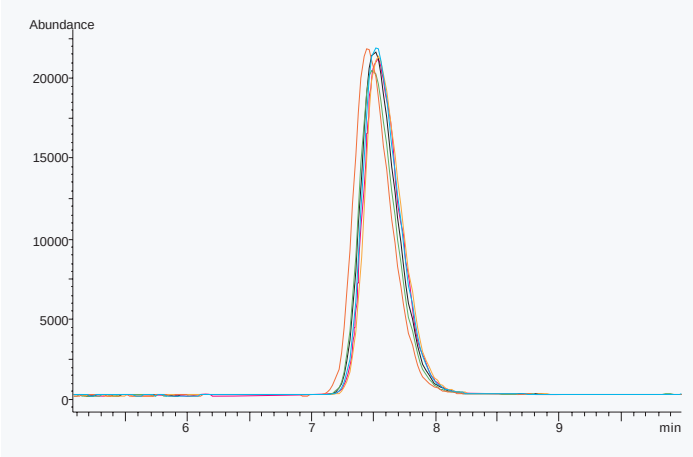

Figure 7: Overlay of CAP traces from six different samples.

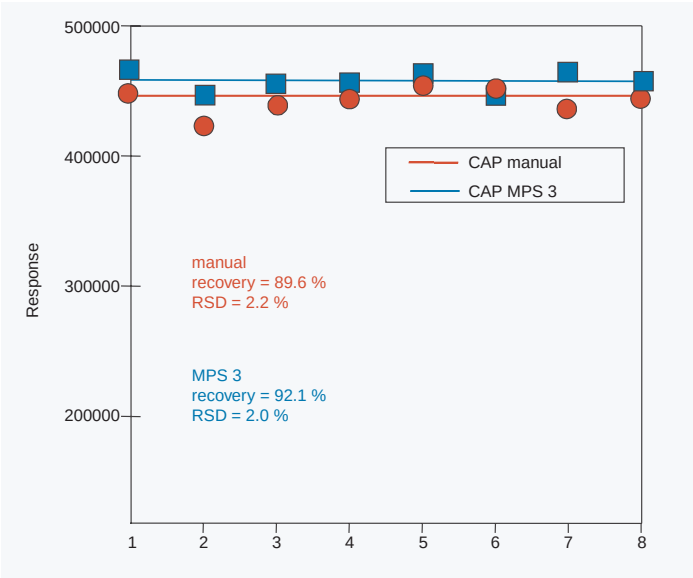

Figure 8: Recovery and repeatability for the determination of Chloramphenicol in prawns with manual and automated sample preparation.

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Conclusions

This example shows that the use of SPE for sample preparation enables sensitive detection of pharmaceutical residues even in complex matrices. Sample throughput can be significantly improved when automating the sample preparation steps using the GERSTEL MPS with integrated SPE module while maintaining or improving recovery and repeatability compared with the manual method. SPE sample preparation using the MPS easily accomplished reaching the MDL (Minimum Detection Limit) for Chloramphenicol of 0.3 µg/kg mandated by the EU. Using automated evaporative concentration and Ion Trap detection the LOD can be lowered to 0.01 µg/kg. Despite the concentration steps no significant matrix effect is observed thanks to the SPE sample preparation.