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Automation of Solid Phase Extraction Methods using a Robotic X-Y-Z Coordinate Autosampler with Software Control

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Abstract
Solid phase extraction (SPE) is one of the sample preparation methods most widely used by chromatographers, as demonstrated by the numerous published SPE methods found in the literature. Typically, a liquid sample is passed across an adsorbent bed to retain and concentrate target analytes and eliminate interference from the sample matrix. Alternatively, the adsorbent can be used to retain interferences while allowing the target analytes to pass through. Manual SPE cartridge formats can vary from disks through individual cartridges with a range of different volumes to 96-well plates. However, solid phase extraction methods can be tedious and time consuming when performed manually. There is therefore an increasing need for the automation of solid phase extraction methods.

A robotic X-Y-Z coordinate autosampler commonly used for sample introduction in GC or HPLC can be used to perform a wide variety of sample preparation techniques using a single instrument and controlling software. The MAESTRO software allows the user to control the automation of solid phase extraction methods. In addition to ease of use and intuitive windows-based programming, the software includes

tools to optimize method parameters ensuring efficient sequence creation and maximum sample throughput. The sampler can be configured as part of a GC or LC system or as a benchtop workstation.

In this study, we show that existing manual SPE methods can be transferred to standard format automation cartridges and automated using the robotic autosampler in conjunction with the software. Examples of solid phase extraction methods illustrating the conversion from manual to automated methods are shown.

Introduction

Laboratories are constantly faced with demands to reach lower detection limits leading to an increasing need to eliminate interfering compounds from the samples being analyzed. Solid phase extraction (SPE) provides efficient clean-up and concentration of raw samples prior to analysis. Several improvements can be achieved by using SPE including a general cleanup of the baseline for all chromatographic methods, increased selectivity in LC methods by enabling the use of smaller particle size analytical columns, and mitigation of effects such as ionization suppression from matrix components. Performing SPE manually, however, can be tedious and time consuming. There is therefore an increasing need for the automation of SPE methods.

Automated SPE affords the user the following benefits:

- High sample throughput
- Accurate and reproducible results combined with high recovery.
- Improved work conditions for laboratory personnel, less exposure to solvents.
- Easy transfer of existing manual SPE methods using standard format automation cartridges.
- Precise syringe-based control of liquid transfers
- Defined sample preparation duration enabling accurate planning of lab resources.
- Optimal system throughput and best possible utilization of laboratory capacity.

Since the GERSTEL SPE uses standard cartridge sorbents, established manual SPE methods can be directly transferred and automated. The process is conveniently controlled by the MAESTRO software – including such additional sample preparation steps as: dilution, mixing, heating or cooling, and addition of standards or derivatizing agents.

The three SPE procedures used as examples within this study take advantage of the ability of the sorbent bed to retain and concentrate the target analytes and eliminate interference from the sample matrix. Typical steps included in a manual SPE method of this type are:

- Conditioning of the sorbent bed to provide optimized analyte binding.
- Addition of the sample to the conditioned sorbent bed.
- Washing of the sorbent bed to remove interferences from the sorbent matrix, while retaining the analytes of interest.
- Completely eluting the analytes of interest from the sorbent bed in order to acheive maximum analyte recovery.
- Optional solvent evaporation and sample reconstitution, if needed for solvent exchange or sample concentration.

Manual SPE procedures from Macherery-Nagel's SPE Application Guide [1], the Phenomenex Strata X General Procedure Guidelines [2], and an Agilent Application Note [3], were used as examples in this study to illustrate the ease of automating manual SPE procedures.

Experimental

Materials. Two of the manual SPE procedures used as examples in this study were used for the extraction of NSAIDS (naproxen, ketoprofen) of interest to human and veterinary medicine. A third SPE procedure for extraction of benzamidazole fungicides (thiabendazole, carbendazim) is used to monitor levels in foods such as apple juice. All reagents used were reagent grade unless otherwise noted.

1 mg/mL naproxen (cat.#6015083) and ketoprofen (cat.#16423) standards in methanol were obtained from Alltech. Naproxen and ketoprofen samples were prepared as dilutions of the standards in deionized water.

Thiabendazole (cat.#45684) and carbendazim (cat.#45368) were obtained from Sigma-Aldrich. 1 mg/mL thiabendazole and 0.1 mg/mL carbendazim stock solutions were prepared in methanol. Apple juice was obtained from a local market. Fortified deionized water and apple juice samples were prepared by spiking with the appropriate stock standard. All fortified samples, as well as blanks from each matrix, were pre-treated immediately before SPE purification as follows:

- Weigh 10 g of apple juice (or water) sample
- Dilute to 100 mL using deionized water.
- Adjust the pH of the sample to 10 using 1N KOH.

The manual SPE procedure for naproxen specified cartridges with sorbent available in the required autosampler compatible cartridge format. The manual SPE procedures for ketoprofen and the fungicides specified cartridges with sorbents that were not readily available in the autosampler compatible cartridge format required for automation. Therefore, the two different SPE sorbents used (Chromabond "EASY" and Chromabond "HR-CX") were chosen as alternative sorbents to the SPE cartridges listed within the manual SPE procedures. The Chromabond EASY sorbent is a polar modified polystyrene-divinylbenzene copolymer with a weak ion exchanger that is recommended by the manufacturer for drug analysis, it possesses similar characteristics to that of Phenomenex's Strata-X SPE sorbent. The Chromabond HR-CX sorbent is a benzenesulphonic acid modified polymeric cation exchanger that is recommended by the manufacturer for the analysis of fungicides in food products. It has similar characteristics to those of Agilent's SampiQ SCX sorbent. Additional details regarding the specific SPE procedures can be found within the text.

Instrumentation. All analyses were performed using an Agilent 1200 HPLC with a Zorbax Eclipse XDB-C18 RRHT column (4.6 x 50 mm, 1.8 µm), 1315C photodiode array detector (DAD) and GERSTEL MPS 3 autosampler configured with an Active Wash Station. Sample injections were made using a 6 port (0.40 mm) Cheminert C2V injection valve fitted with a 20 µL stainless steel sample loop. HPLC method parameters for each sample type can be found in the references.

All automated SPE PrepSequences were performed using the GERSTEL SPE on an MPS 2XL MultiPurpose Sampler as shown in Figure 1.

Naproxen analysis conditions.

Pump:	Linear gradient, from 95 % to 5 % B in 14 minutes Flowrate = 1.00 mL/min
Mobile Phase:	A - Acetonitrile B – 20 mM potassium phosphate, pH 2.5
Run time:	17 min
Inj. volume:	2.5 µL
Detection:	UV, λ = 230 nm
Col. Temp.:	22°C

Ketoprofen analysis conditions.

Pump:	Linear gradient, from 95 % to 5 % B in 14 minutes Flowrate = 1.00 mL/min
Mobile Phase:	A - Acetonitrile B – 20 mM potassium phosphate, pH 2.5
Run time:	17 min
Inj. volume:	2.5 µL
Detection:	UV, λ = 221 nm
Col. Temp.:	22°C

Thiabendazole and carbendazim analysis conditions.

Pump:	Isocratic, 85 % A: 15 % B Flowrate = 1.00 mL/min
Mobile Phase:	A - 10 mM potassium phosphate, pH 3 B – Acetonitrile
Run time:	3.5 min
Inj. volume:	10 µL
Detection:	UV, λ = 288 nm
Col. Temp.:	25°C

Figure 1. MultiPurpose Sampler MPS XL with GERSTEL SPE option.

Results and Discussion

Automated SPE for the analysis of Naproxen. A graphical representation of the manual SPE procedure for naproxen is shown in Figure 2. The corresponding automated SPE PrepSequence used during testing is shown in Figure 3.

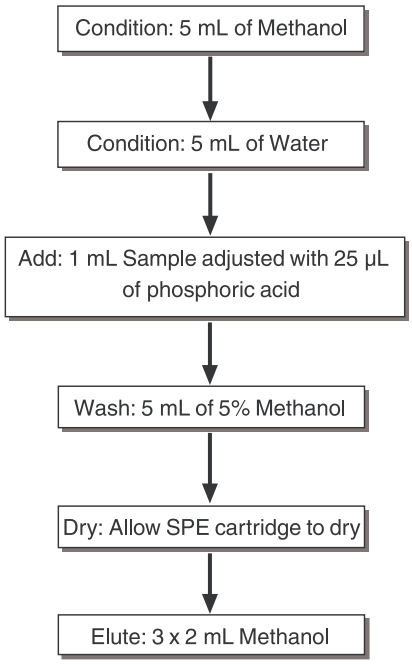

Figure 2. Manual SPE procedure for naproxen.

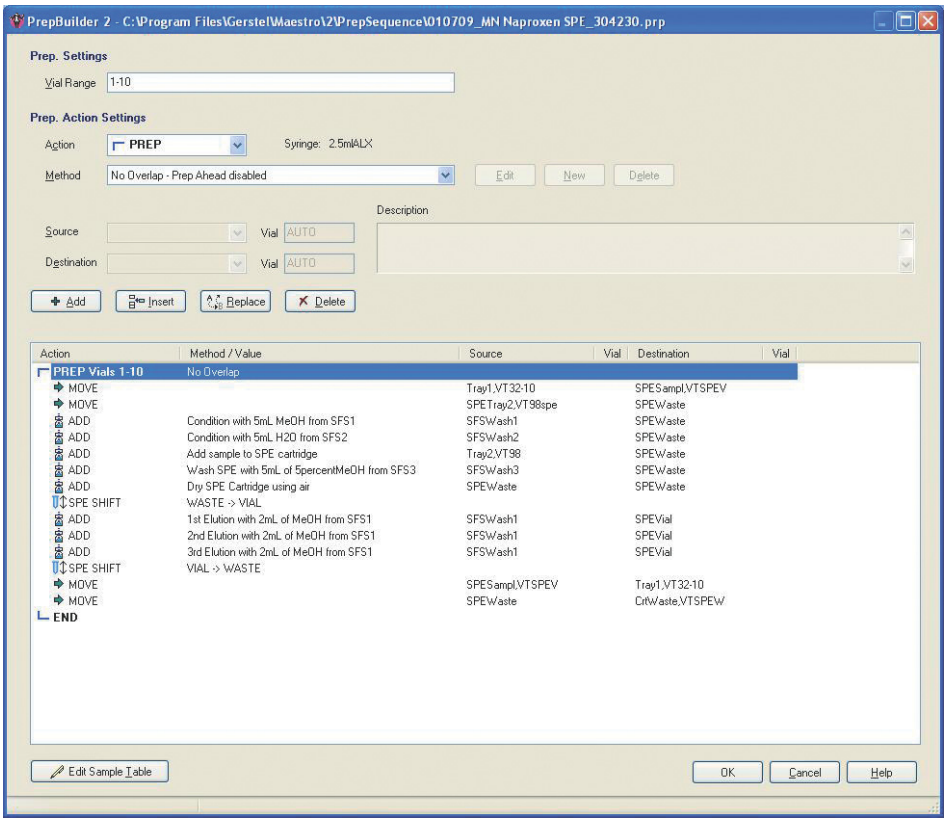

Figure 3. Automated SPE PrepSequence for naproxen.

Calibration standards were analyzed using the SPE procedure. A calibration curve generated from 0.5, 1, 5, and 10 µg/mL naproxen standards prepared in water, resulted in a correlation coefficient of r^2=0.999 (Figure 4).

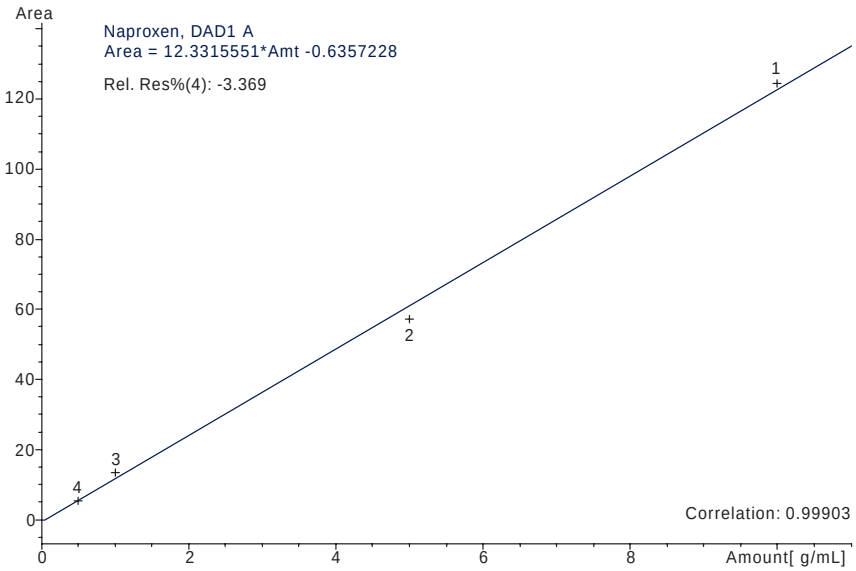

Figure 4. Naproxen calibration curve .

Recovery was calculated using the following procedure: A sample fortified with naproxen was extracted using the automated solid phase procedure and the analysis result compared to that of an unextracted naproxen standard which was prepared at the equivalent final concentration. The comparison is shown in Figure 5. Recovery was calculated as the ratio between the resulting peak areas. Table 1 shows percent recovery and precision data.

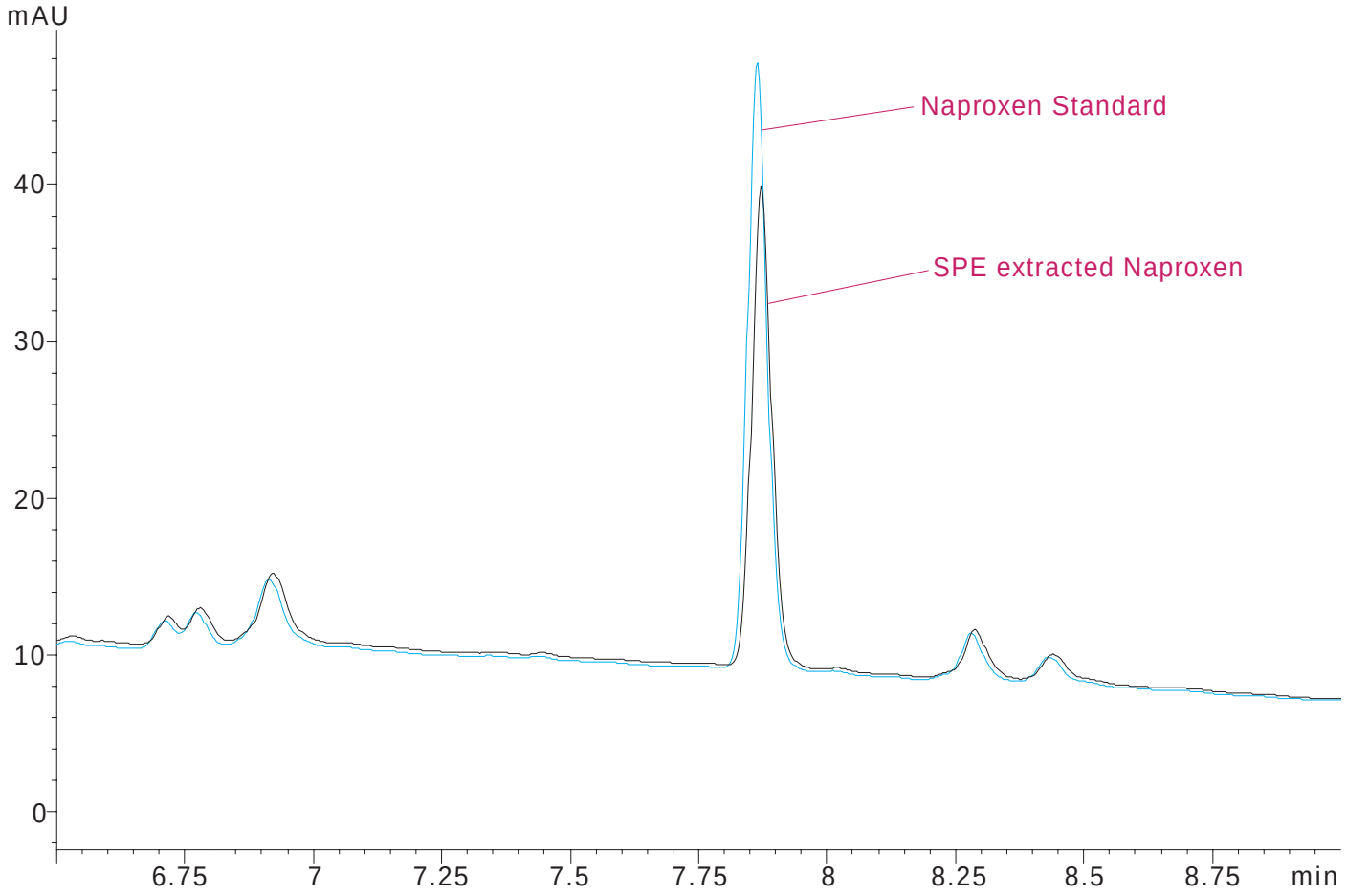

Figure 5. Overlay chromatograms of a sample fortified with naproxen and extracted using the automated SPE procedure and of a naproxen standard prepared at the equivalent final concentration.

Table 1. Percent recovery and precision results for naproxen.

Naproxen	Standard [Area]	SPE Sample [Area]
	106	83.8572
		84.3103
		86.1401
		88.7493
		81.1417
		67.6187
mean		82.0
% CV		9.11
% Recovery		77

Automated SPE for the Analysis of Ketoprofen. A graphical representation of the manual SPE procedure for ketoprofen is shown in Figure 6. The corresponding automated SPE PrepSequence used during testing is shown in Figure 7. Following elution, all ketoprofen samples were concentrated by evaporating to dryness under a stream of nitrogen and then reconstituted using 500 µL of methanol.

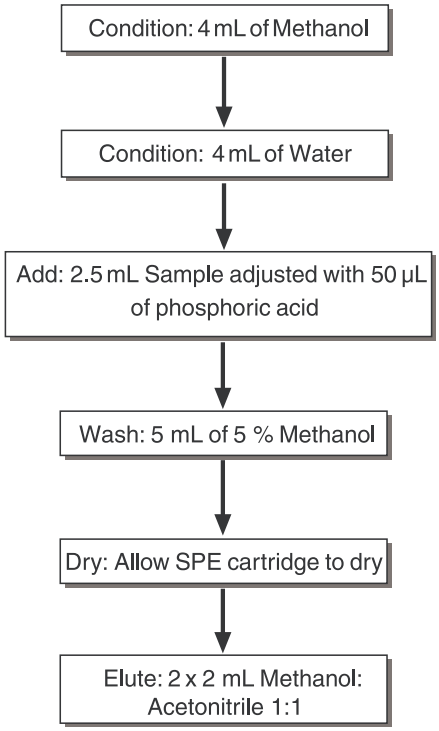

Figure 6. Manual SPE procedure for ketoprofen.

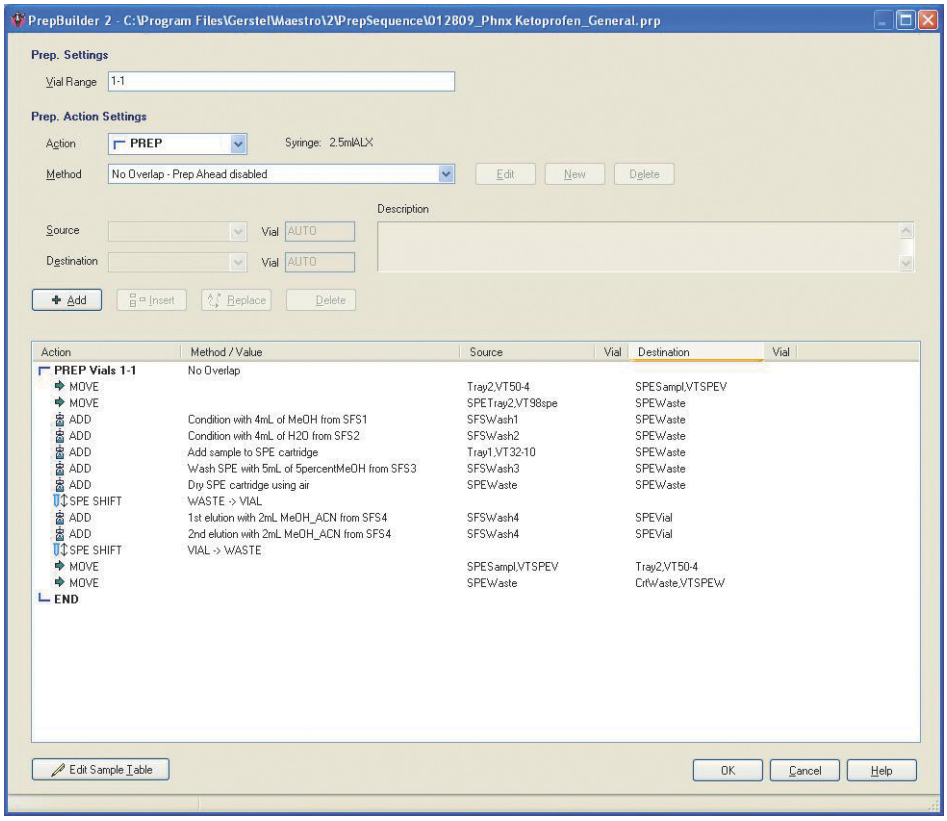

Figure 7. Automated SPE PrepSequence for ketoprofen.

Calibration standards were analyzed using the SPE procedure. A calibration curve generated from 0.2, 1, 2, 10, and 20 µg/mL ketoprofen standards prepared in water resulted in a correlation coefficient of r^2=0.997 (Figure 8). All ketoprofen samples were fortified with 10 µL of 50 µg/mL naproxen as an internal standard.

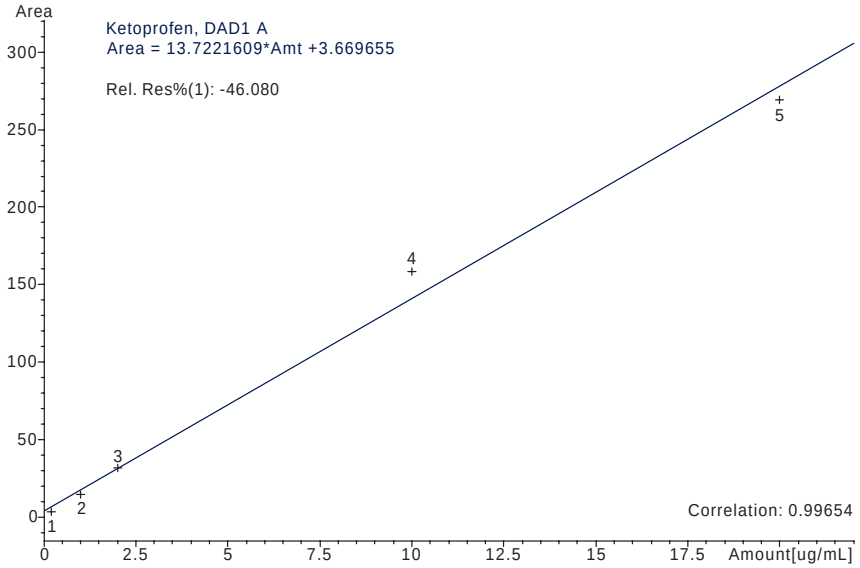

Figure 8. Ketoprofen calibration curve.

Recovery of ketoprofen was calculated using the following procedure: A sample fortified with ketoprofen was extracted using the automated solid phase procedure and the analysis result compared to that of an unextracted ketoprofen standard which was prepared at the equivalent final concentration. The comparison is shown in Figure 9. Recovery was calculated as the ratio between the resulting peak areas. Table 2 shows percent recovery and precision data.

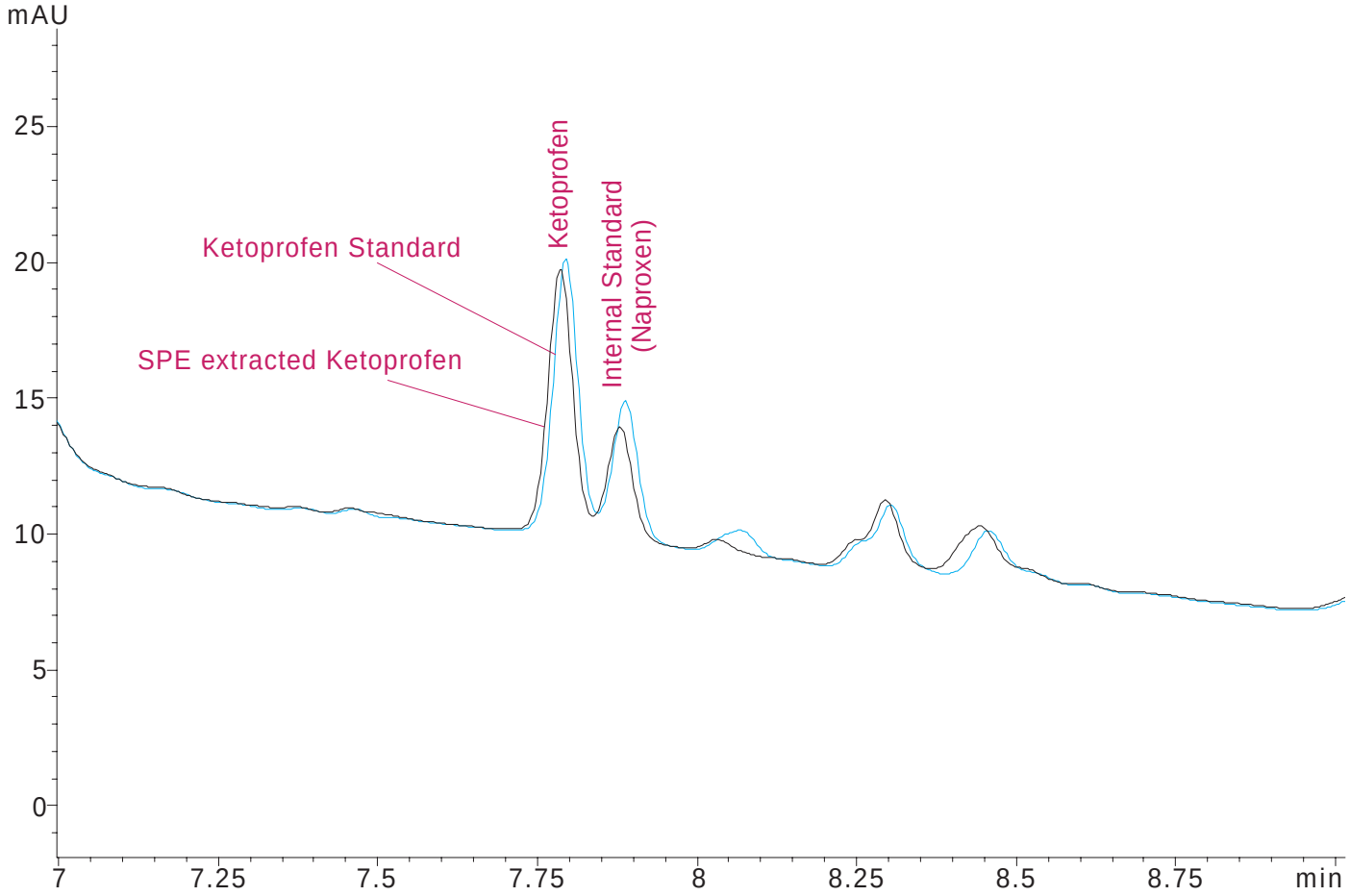

Figure 9. Overlay chromatograms of a sample fortified with ketoprofen and extracted using the automated SPE procedure and of a ketoprofen standard prepared at the equivalent final concentration.

Table 2. Percent recovery and precision results for ketoprofen.

Ketoprofen	Standard [Area]	SPE Sample [Area]
	31.27931	27.20211
	25.94999	24.90162
	28.73183	19.25798
		25.95632
		23.12750
		21.01649
mean	28.7	23.6
% CV		13
% Recovery		82

Automated SPE for the Analysis of Thiabendazole and Carbendazim. A graphical representation of the manual SPE procedure for thiabendazole and carbendazim is shown in Figure 10. The corresponding automated SPE PrepSequence used during testing is shown in Figure 11. Following elution, all thiabendazole/carbendazim samples were concentrated by evaporating to dryness under a stream of nitrogen and then reconstituting using 1 mL of mobile phase.

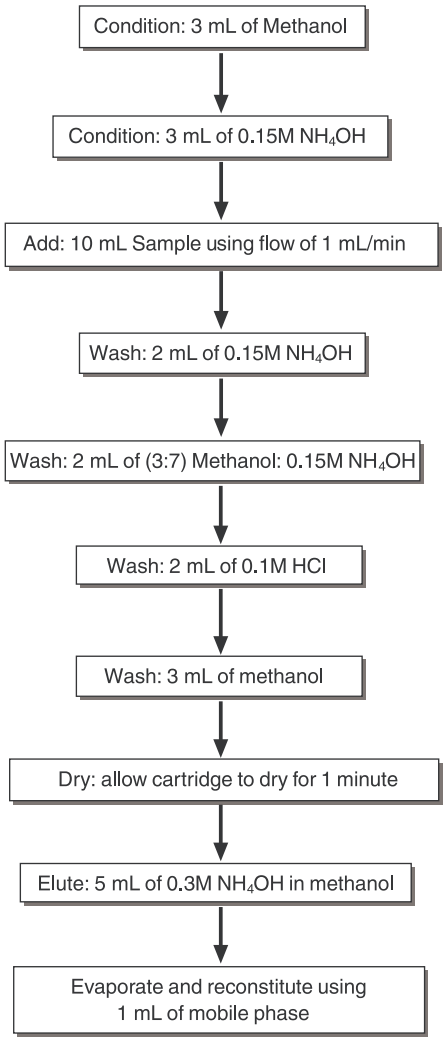

Figure 10. Manual SPE procedure for thiabendazole and carbendazim.

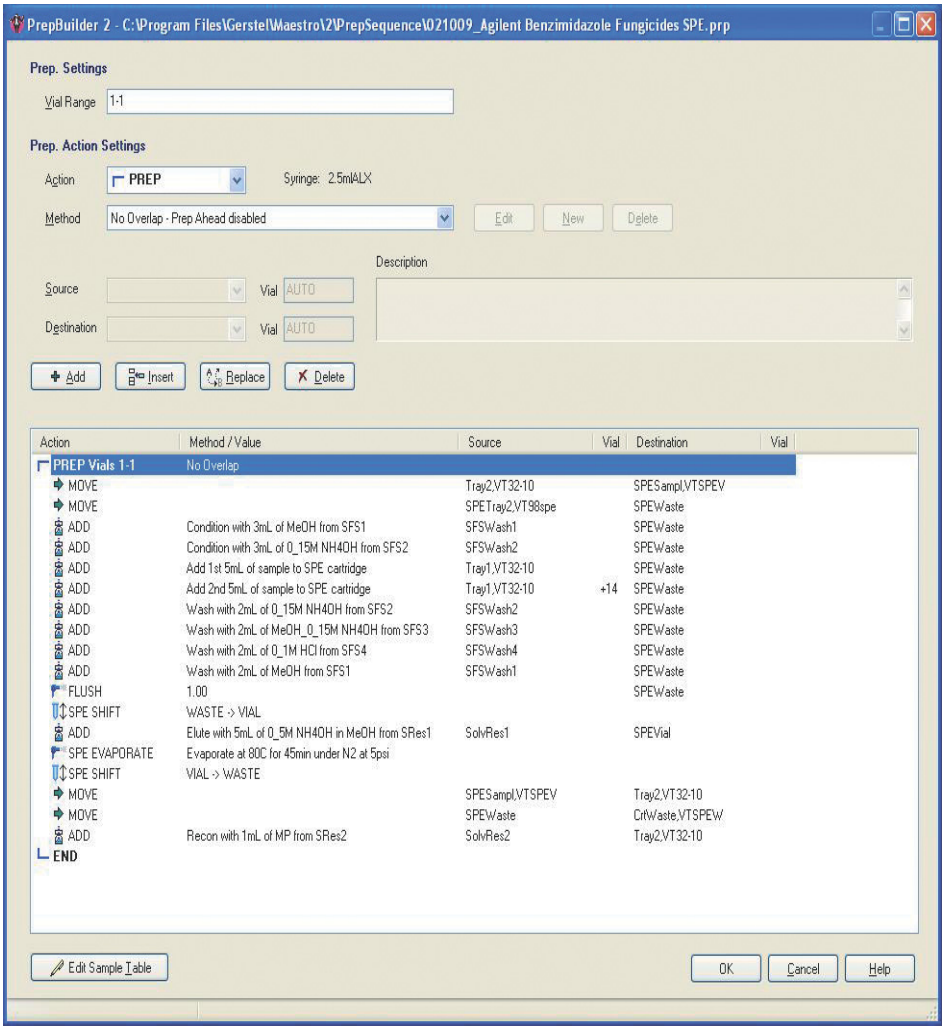

Figure 11. Automated SPE PrepSequence for thiabendazole and carbendazim.

Calibration curves were generated from standards containing 10, 25, 50, and 100 ng/mL of both thiabendazole and carbendazim prepared in mobile phase. Resulting correlation coefficients were r^2=0.999 for thiabendazole (Figure 12) and r^2=0.999 for carbendazim (Figure 13).

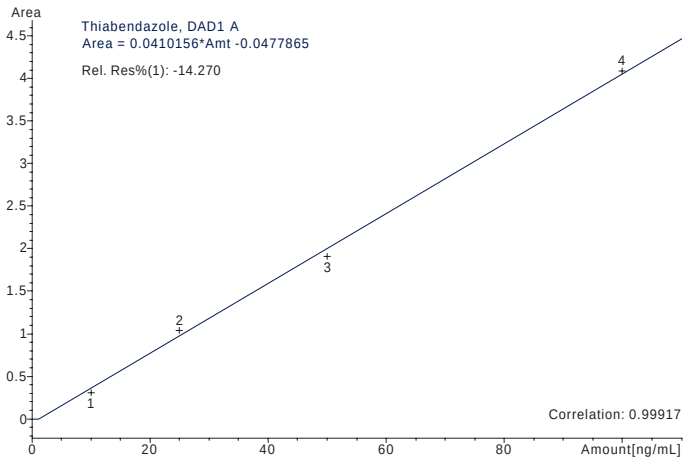

Figure 12. Thiabendazole calibration curve.

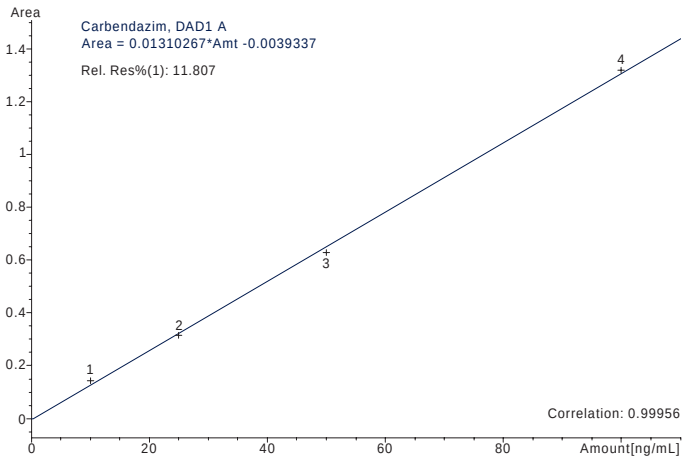

Figure 13. Carbendazim calibration curve.

Recovery of thiabendazole and carbendazim was calculated using the following procedure: A sample of apple juice fortified with thiabendazole and carbendazim was extracted using the automated solid phase procedure and the analysis result compared to that of an unextracted standard containing thiabendazole and carbendazim at the equivalent final concentrations. The comparison is shown in Figure 14. Recovery was calculated as the ratio between the resulting peak areas. Table 3 shows percent recovery and precision data for both compounds.

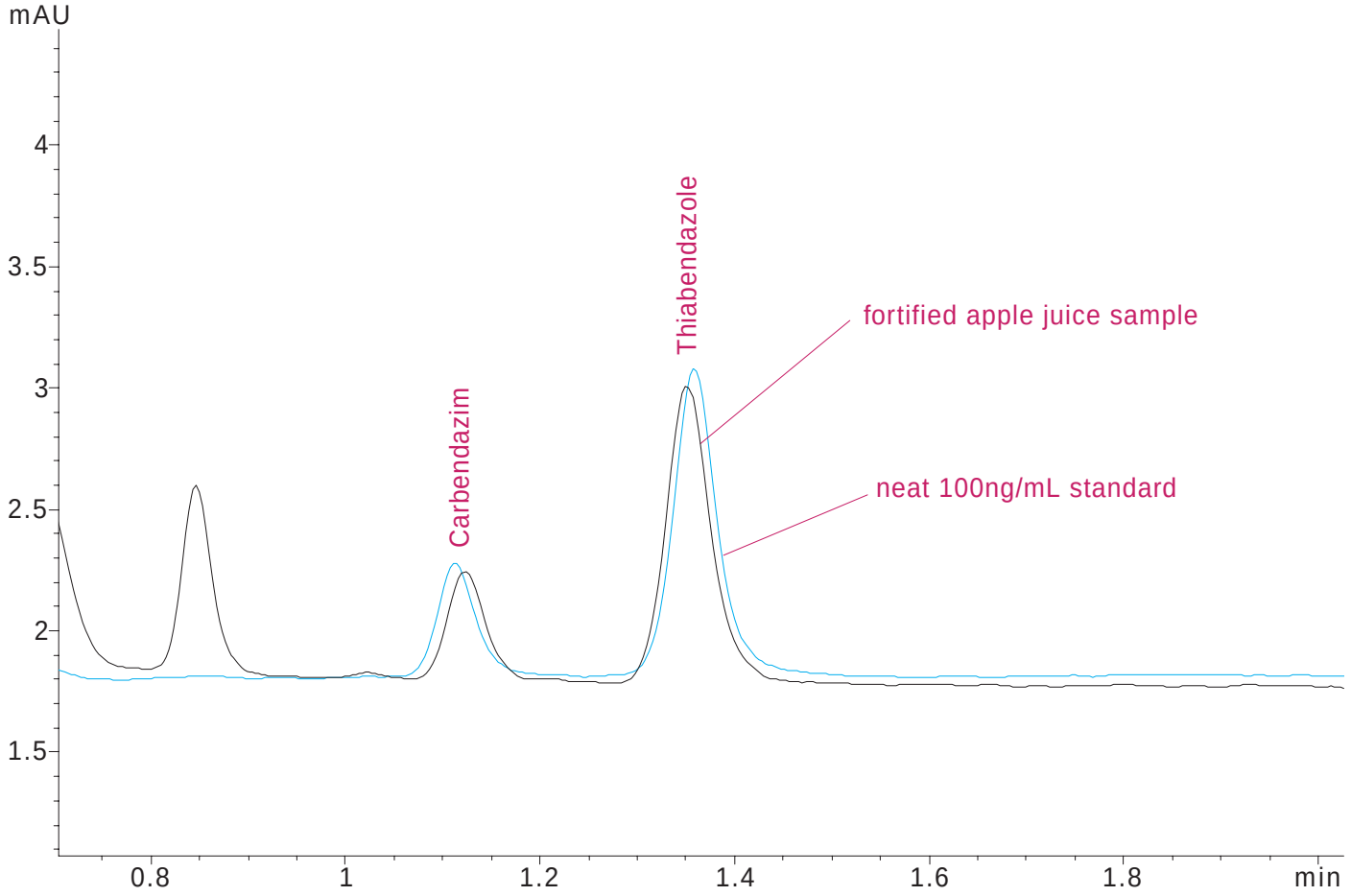

Figure 14. Overlay chromatograms of an apple juice sample fortified with thiabendazole and carbendazim and extracted using the automated SPE procedure and of a standard prepared at the equivalent final concentrations.

Table 3. Percent recovery and precision results for thiabendazole and carbendazim.

	Thiabendazole		Carbendazim	
	Standard [Area]	SPE Sample [Area]	Standard [Area]	SPE Sample [Area]
	4.08946	4.05923	1.31824	1.19061
		4.11309		1.11645
		3.93372		1.20139
mean		4.04		1.17
% CV		2.28		3.95
% Recovery		99		89

Conclusions

As a result of this study, we were able to demonstrate:

- The easy transfer of existing manual SPE methods using standard format automation cartridges.
- Basic equivalence was obtained for two different SPE sorbents from different manufacturers using alternative sorbents available in standard format automation cartridges
- Good quantitative methods could be produced when automating a manual SPE procedure using the GERSTEL SPE standard format cartridge.

The automation of manual solid phase extraction procedures using the MultiPurpose Sampler (MPS) with MAESTRO software, affords the user the following benefits:

- Easy transfer of existing manual SPE methods using standard format automation cartridges.
- Improved work conditions for laboratory personnel, less exposure to solvents.
- Elimination of tedious manual sample preparation steps.
- When configured with a GC or LC system, the ability to inject the resulting extract immediately after preparation.
- Precise syringe-based control of liquid transfers
- Optimal system throughput and best possible utilization of laboratory capacity.
- Accurate and reproducible results combined with high recovery.

References

[1] Solid Phase Extraction Application Guide, Application # 304230, Macherey-Nagel, www.mn-net.com

[2] Phenomenex, Strata X General Procedure Guidelines, www.phenomenex.com

[3] Agilent Technologies, Application Note – Determination of Benzimidazole Fungicides in Apple Juice by SampliQ Polymer SCX Solid-Phase Extraction with High-Performance Liquid Chromatography, Chenhao Zhai and Yun Zou, Agilent Technologies Co., Ltd., China, Carol Ball, Agilent Technologies, Inc. USA., www.agilent.com/chem

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