

Extraction of Anthelmintic Drugs from a Veterinary Formulation Using Accelerated Solvent Extraction (ASE[®])

INTRODUCTION

Isolation of the active drug from some veterinary formulations can be difficult because the matrix is often complex. These difficulties were traditionally solved by extracting with a wrist-shaker method, sonication, or Soxhlet. Although these techniques produce adequate results, they are very labor intensive and use large amounts of solvent. The time required for these methods can cause bottlenecks in the sample preparation area and solvent use can cost the laboratory thousands of dollars in unwarranted expenses per year.

Accelerated Solvent Extraction (ASE) is an automated extraction technique that uses the same solvents as traditional extraction methods but in significantly smaller amounts and with minimal analyst exposure. ASE achieves equivalent or better results in a fraction of the time by using increased temperature and pressure to enhance the kinetics of the extraction process. This entire process is fully automated and allows the unattended extraction of up to 24 samples.

This application note describes the extraction of the two active species of ivermectin (H2B1a and H2B1b), an anthelmintic drug, from a veterinary formulation containing dried meat products. This formulation is used to treat household cats and dogs for heartworm disease. Figure 1 shows the chemical structure of ivermectin.

EQUIPMENT

Dionex ASE 200 Accelerated Extractor with Solvent Controller (P/N 048765)

11-mL stainless steel extraction cells (P/N 055422)

Dionex cellulose filters (P/N 049458)

Dionex collection vials, 40 mL (P/N 048783)

Analytical balance (accurate nearest 0.0001 g or better)

Sand (Ottawa Standard, Fisher Scientific, Cat. No. S23-3)

Alumina cartridge 5 mL (Fisher)

PTFE syringe Filter, 0.2 μm (Fisher Scientific)

Tyler 10 sieve (Fisher Scientific)

REAGENTS

Hydromatrix[™] (Varian Associates)

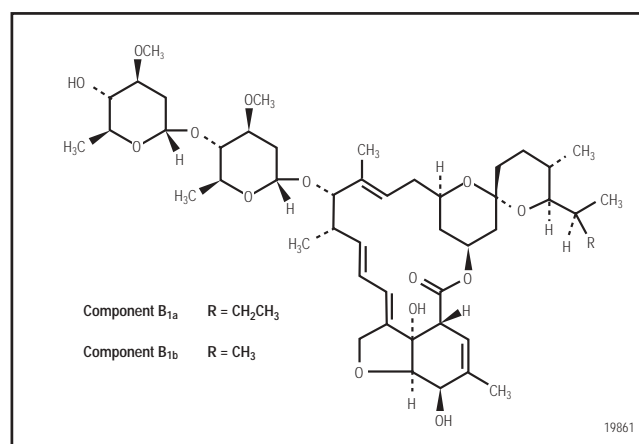


Figure 1. Chemical structure of ivermectin.

SOLVENTS

Methanol

Water

(All solvents are pesticide-grade or equivalent and available from Fisher Scientific.)

EXTRACTION CONDITIONS

Solvent: 95% methanol, 5% water

Temperature: 120 °C

Pressure: 1500 psi

Heatup time: 6 min

Static time: 10 min

Static cycles: 1

Flush: 30%

Purge: 60 s

SAMPLE PREPARATION

The tablet should be finely ground using a food processor or coffee grinder to a powder that can pass through a Tyler 10 sieve. Accurately weigh out approximately 0.5–1.5 g of the powder and blend with 1.5 g of Hydromatrix using a mortar and pestle. Transfer the mixture to an 11-mL stainless steel extraction cell containing a cellulose filter. Top off any dead space in the cell with Ottawa sand. Prepare any other tablet samples and load them into extraction cells.

EXTRACTION PROCEDURE

Place the cells onto the ASE 200. Label the appropriate number of collection vials and place these into the carousel. Set up the method suggested above and begin the extraction. When the extraction is complete, the extract can then be diluted to the desired volume and passed through a 5-mL alumina cartridge. Finally, filter the extract into an HPLC vial through a 0.2- μ m PFTE filter and analyze using HPLC.¹

RESULTS AND DISCUSSION

Sample preparation is critical to good recoveries. Grind the samples to a uniform particle size to ensure proper permeation of the solvent into the matrix. The sample extracts may be somewhat cloudy due to the extractions of fats and other coextractables, so it is important to pass the extracts through a short column of alumina.

Table 1. ASE Recovery of Ivermectin from Spiked Placebo Samples

Target* %	Set 1	Set 2	Set 3	Set 4	Mean	% RSD
50	98.4	103.9	97.3	100.0	99.9	2.9
75	99.2	102.0	98.7	98.7	99.7	1.6
100	102.3	99.9	100.4	100.6	100.8	1.0
125	103.3	101.2	103.3	102.1	102.1	1.3
150	100.9	98.2	99.6	100.1	100.1	1.6

* Shows a range of 50–150% of the target concentration (0.9 μ g/mL)

Table 1 shows the results of extracting placebo preparations spiked with a varying range of ivermectin concentrations (50–150%). The average recovery for all concentration levels was 100.5%.

Table 2 shows the precision of the ASE method. Six preparations from one lot of HEARTGARD® Chewables for Cats were extracted and analyzed. The average percent recovery for these six preparations was 102.3% with an RSD of 0.90%. These recovery and precision values are as good or better than observed with traditional extraction techniques.

Table 2. ASE Method Precision Summary: Extraction of Ivermectin from HEARTGARD Chewables for Cats

Preparation	% Recovery
1	101.3
2	103.5
3	101.6
4	102.4
5	101.6
6	103.2
Mean	102.3
% RSD	0.90

CONCLUSIONS

These results confirm that ASE is comparable to traditional extraction methods for the difficult extractions of active drugs from veterinary formulations. Traditional extraction methods usually take from one to several hours for each sample and require large amounts of solvent. With ASE, the extraction time is cut to approximately 15 min per sample and uses only 25–30 mL of solvent. In addition, the ASE 200 can extract up to 24 samples, sequentially, without user intervention.

ACKNOWLEDGEMENTS

We would like to acknowledge the work of Andreas Abend and his colleagues at Merck & Co. Inc.

REFERENCES

1. Abend, A. M.; Chung L.; McCollum, D. G.; Wuelfing, W. P. "Development and Validation of an Automated Extraction Method (Accelerated Solvent Extraction®) and a Reverse-Phase HPLC Analysis Method for Assay of Ivermectin in a Meat-Based Chewable Formulation." *Pharm and Bio Med Analysis* **2003**, *31*, 1177–1183.



HEARTGARD is a registered trademark of Merial.
ASE is a registered trademarks of Dionex Corporation.

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

Dionex Corporation
Salt Lake City Technical Center
1515 West 2200 South, Suite A
Salt Lake City, UT
84119-1484
(801) 972-9292

Dionex U.S. Regional Offices
Sunnyvale, CA (408) 737-8522
Westmont, IL (630) 789-3660
Houston, TX (281) 847-5652
Atlanta, GA (770) 432-8100
Marlton, NJ (856) 596-06009

Dionex International Subsidiaries
Austria (01) 616 51 25 Belgium (03) 353 42 94 Canada (905) 844-9650 China (852) 2428 3282 Denmark 36 36 90 90
France 01 39 30 01 10 Germany 06126-991-0 Italy (06) 66 51 50 52 Japan (06) 6885-1213 Korea 82 2 2653 2580
The Netherlands (0161) 43 43 03 Switzerland (062) 205 99 66 United Kingdom (01276) 691722
* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.

