

Extraction of Drugs from Animal Feeds Using Accelerated Solvent Extraction (ASE[®])

INTRODUCTION

Maintaining adequate quality control of drug testing and production processes requires the ability to rapidly and efficiently extract prepared animal feed products. Current sample extraction techniques are labor and time intensive, and are often responsible for communication delays between manufacturing and quality control. Automation of the sample extraction process can accelerate the flow of information, free the analyst from the hands-on, repetitive nature of the work, and reduce potential exposure to hazardous solvents.

Accelerated Solvent Extraction (ASE) is an extraction technique developed to speed the extraction process and reduce the total amount of solvent. Conventional liquid solvents are used at elevated temperatures and pressures, which results in increased extraction kinetics. Extraction of sample sizes ranging from 1 to 30 g typically require 12–17 min and 15–50 mL of solvent.

ASE is widely used in the environmental industry to replace time- and solvent-intensive techniques such as Soxhlet and sonication extraction. Many features of the ASE system also make it attractive for use in pharmaceutical laboratories. Users can select organic and aqueous solvents to match the polarity of the extraction fluid to the target analytes. Extractions can be performed at temperatures ranging from ambient to 200 °C. Because the efficiency of a liquid extraction process is directly related to temperature, the user can select the most efficient temperature (maximum temperature below analyte degradation point), thereby reducing the time and the amount of solvent required. Finally, the ability to extract up to 24 samples, unattended, results in a dramatic increase in laboratory efficiency.

This Application Note gives two examples of how ASE can provide extraction efficiencies superior to other techniques. In the first example, ASE is used to extract an antischizophrenic agent from rodent feed used in drug testing. In the second example, ASE is used to extract Lasalocid, a veterinary medicinal added to poultry and cattle feed.

EQUIPMENT

Dionex ASE 200 Accelerated Solvent Extractor equipped with 11-, 22-, or 33-mL cells
Analytical balance
Dionex vials for extract collection (40 mL, P/N 49465; 60 mL, P/N 49466)
Cellulose filter disks (P/N 49458)

REAGENTS

Methanol (HPLC grade or better)
Acetic acid

EXTRACTION CONDITIONS

Rodent Feed:	Antischizophrenic Agent
Extraction Solvent:	Methanol
Temperature:	100 °C
Pressure:	1500 psi (10 MPa)
Heat-up Time:	5 min
Static Time:	5 min
Flush Volume:	60%
Purge Time:	100 s
Static Cycles:	1

Total Extraction Time: 12 min per sample
Total Solvent Use: 30 mL per sample

Poultry and Cattle Feed: Veterinary Medicinal

Extraction Solvent: Methanol + 0.3% Acetic acid
Temperature: 80 °C
Pressure: 1500 psi (10 MPa)
Heat-up Time: 5 min
Static Time: 5 min
Flush Volume: 60%
Purge Time: 100 s
Static Cycles: 1
Total Extraction Time: 12 min per sample
Total Solvent Use: 30 mL per sample

SAMPLE PREPARATION

Weigh and directly add dry, granular feed (1–20 g) to the ASE extraction cells containing a cellulose filter. Samples should be in the ground state (not pelleted). Sand (Fisher Scientific, P/N S23-3) can be used as a dispersant if sample particles tend to clump or adhere firmly.

ANALYTICAL

Feed sample extracts containing the antischizophrenic drug were sent to the manufacturer's facility for quantification. HPLC analysis was performed using an Astec Cyclobond® I 25 cm x 4.6 mm i.d. column at 5 °C and a Brownlee Labs™ Polypore® Phenyl RP (PRP-1) 3 cm x 4.6 mm i.d. pre-column held at ambient temperature. Columns were purchased from Alltech Associates, Inc. An isocratic mobile phase of acetonitrile:triethanolamine (97:3 v/v, pH 4.5) at 1.0 mL/min was used with UV detection at 280 nm.

Lasalocid feed sample extracts were analyzed by HPLC using a Phenomenex Ultracarb 5 ODS 25 cm x 4.6 mm i.d. C18 column with an IPA:water mobile phase (20:80) and UV detection at 248 and 318 nm.

RESULTS AND DISCUSSION

Feed samples were batch-fortified by the manufacturer's facility with an antischizophrenic drug at 10 and 0.2 g/kg. 10-g samples were extracted using ASE at 100 °C with a total extraction time of 12 min per sample and a total solvent volume of 30 mL of methanol per sample. Conventional wrist shaker extraction was performed for 30 min using acetonitrile, followed by filtering and volume adjustment. The method requires a total extraction time of approximately 55 min and uses 100–400 mL of solvent. Extraction of spiked placebo samples (blank samples spiked with the active compound) was performed by first loading the extraction cell and then adding the standard mixture directly to the top of the sample.

Table 1 compares the recovery and reproducibility generated for the target compound using ASE and the wrist shaker extraction method. The recovery of the ASE extractions is higher than the wrist shaker method for the two concentration-level samples. The ASE extracts were shipped to a different location for analysis, which may account for the greater RSD values. Although the higher temperatures used in ASE expedite the extraction process, there was concern that possible co-extractable materials could interfere with the chromatographic analysis of the active compound. Figure 1 compares the chromatographic analysis of ASE-generated extracts with standard and blank runs. No interferences were present in the analysis.

The chemical structure of Lasalocid is shown in Figure 2. This compound is currently licensed for veterinary use as an antibacterial (coccidiostat) in poultry and a growth promoter in cattle. The conventional method for the extraction of Lasalocid is either soaking the sample overnight or sonication for 30 min in 100–200 mL methanol. The ASE method requires only 12 min and 30 mL methanol containing 0.3% (v/v) acetic acid per sample. The results shown in Table 2 summarize extractions performed for poultry and cattle feed containing differing amounts of Lasalocid. Comparison of the average and standard deviations indicate that the two techniques generate equivalent results, with ASE recovery values between 96–105%, relative to the conventional method.

Table 1 Recovery of an Antischizophrenic Drug from 10 g of Rodent Feed using ASE and Wrist Shaker Extraction Methods

Extraction Method	Feed Level (% RSD)*	
	0.2 g/kg	10 g/kg
ASE (placebo spikes)	0.199 (4.1)	10.1 (4.2)
ASE	0.185 (4.2)	9.68 (4.4)
Wrist Shaker	0.170 (1.3)	9.43 (1.3)

*n=10

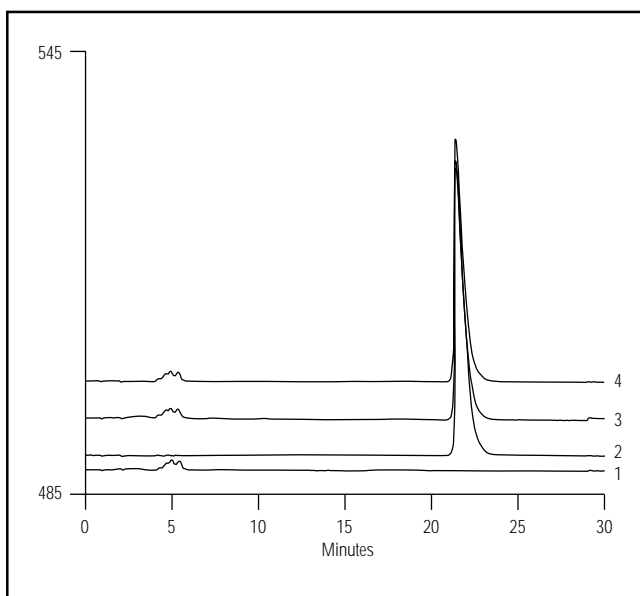


Figure 1. HPLC analysis of feed standards and extracts. (1) ASE extraction blank, (2) drug standard, (3) ASE extract of spiked standard, (4) ASE extraction of drug containing feed sample.

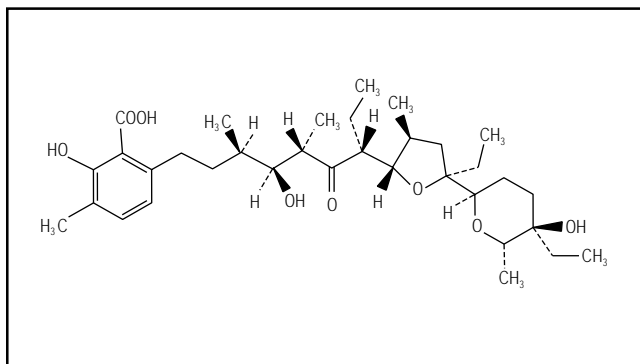


Figure 2. Lasalocid chemical structure.

Table 2 Lasalocid Recovered from 10-g Samples of Poultry and Cattle Feed Taken from 12 Individual Lots by ASE and Sonication Extraction Methods

Sample #	Lasalocid Recovered (ppm)	
	ASE	Sonication
1	80.0	77.5
2	82.2	78.4
3	81.4	79.4
4	82.0	78.7
5	89.5	78.2
6	85.5	81.2
7	136.0	130.3
8	138.3	140.8
9	136.3	141.1
10	135.2	136.8
11	133.8	135.8
12	138.0	133.7

CONCLUSION

Accelerated solvent extraction takes advantage of enhanced solubilization kinetics that occur at temperatures higher than are commonly used to perform liquid solvent extractions. As the efficiency of the extraction process improves, less solvent and time are required to complete the process. Because reducing solvent consumption and increasing sample throughput are important concerns to laboratories, ASE offers significant advantages for both production and research labs.

In this Application Note, ASE was compared to conventional solvent extractions of compounds added to animal feeds. ASE provides results comparable to conventional extraction methods while reducing the time and volume of extraction solvent typically associated with these analyses. Chromatographic profiles indicate ASE-generated extracts are nearly identical in composition to those generated by conventional techniques. In addition to savings in time and solvent consumption, ASE technology is automated to increase laboratory productivity.

LIST OF SUPPLIERS

Fisher Scientific, 711 Forbes Avenue, Pittsburgh, Pennsylvania, 15219-4785, USA. Tel.: 1-800-766-7000

Alltech Associates, Inc., 2051 Waukegan Road, Deerfield, Illinois, 60015, USA. Tel.: 1-800-255-8324

Phenomenex, 2320 W. 205th Street, Torrance, California, 90501, USA. Tel.: 1-310-212-0555



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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) 596-0600

Dionex International Subsidiaries
Austria (01) 616 51 25 *Belgium* (015) 203800 *Canada* (905) 844-9650 *France* 01 39 30 01 10 *Germany* 06126-991-0
Italy (06) 66 51 50 52 *Japan* (06) 6885-1213 *The Netherlands* (0161) 434303 *Switzerland* (062) 205 99 66 *United Kingdom* (01276) 691722
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