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Determination of organic acids in animal feeds using two ion chromatography methods

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Keywords

Dionex ICS-5000+, Dionex IonPac AS11-HC-4µm column, Dionex IonPac ICE-AS6 ion-exclusion column, suppressed conductivity detection

Goal

To quantify organic acids in animal feeds by both anion-exchange and ion-exclusion ion chromatography methods

Introduction

Organic acids and their salts, such as citric acid, malic acid, formic acid, lactic acid, acetic acid, propionic acid, and fumaric acid, are animal feed additives that play an important role in animal feed by improving the animals' performance and reducing the development of pathogenic microorganisms in the intestine. This is especially important for swine husbandry. According to their functional role, the relevant organic acids can be segmented into groups such as: preservatives, acidity regulators, flavoring compounds, silage additives, or other zoo-technical additives.[1] For example, a microencapsulated mixture of citric acid, sorbic acid, and pure botanicals, namely thymol and vanillin, can improve the maturation of the intestinal mucosa and eventually improve the growth of piglets prematurely weaned.[2]

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The most important contribution of organic acids is their antimicrobial and pH-lowering effect. In the feed, as well as in the digestive tract of animals, the non-dissociated acid molecules are responsible for the antimicrobial effect because they diffuse across the cell membrane of pathogens and cause pH imbalances and the inhibition of DNA synthesis of the cell. However, in its dissociated form, the acid is responsible for lowering the pH value and reducing the buffer capacity in the feed. As a result, the growth rate of pathogens is decreased and the reduction of the gastric pH value is accelerated. As a consequence, the protein digestion of piglets with a suboptimally developed enzyme system is improved. Organic acids (OAs) have very different effects depending on their degree of dissociation (pKa value).

Several methods have been used to determine organic acids in animal feeds including HPLC with UV detection. However, several organic acids have poor UV absorption and, therefore, lack sufficient sensitivity for detection. In addition, other UV-absorbing components in animal feed may interfere with the detection of organic acids. In contrast, virtually all carboxylic acids ionize sufficiently, therefore, ion chromatography (IC) with suppressed conductivity detection is the technique of choice to separate and detect a large variety of organic acids.

In this application note, the animal feed sample is extracted with water. The extract is filtered or centrifuged and diluted, if necessary. The amounts and types of OAs from the sample are then determined using two IC methods. Method A is based on anion-exchange separation and method B is based on ion-exclusion separation. The two IC methods were evaluated in terms of separation, calibration, limit of quantification, accuracy, and precision.

Experimental

Equipment and consumables

IC system

- Two Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC systems including*:
  - Dionex ICS-5000⁺ DP Pump module
  - Dionex ICS-5000⁺ EG Eluent Generator module with high-pressure degasser module
  - Dionex ICS-5000⁺ DC Detector/Chromatography module with CD Conductivity Detector
  - Thermo Scientific™ Dionex™ AS-AP Autosampler with sample syringe, 250 µL (P/N 074306) and buffer line 1,200 µL (P/N 074989)

*Either method can be run on a single Dionex ICS-5000⁺ HPIC system with a Thermo Scientific AXP pump to add the external water for the anion-exchange method or the TBAOH regenerant for the ion-exclusion method.

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software, version 7.2 SR4

System A consumables

- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 500 Electrolytic trap column (P/N 075550)
- Thermo Scientific™ Dionex™ AERS 500 suppressor, 2 mm (P/N 082541)

System B consumables

- Thermo Scientific™ Dionex™ ACRS-ICE 500 suppressor, 9 mm (P/N 084715)

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Citric acid monohydrate (Sigma-Aldrich® P/N C7129)
- D-L-Malic acid disodium salt (Sigma-Aldrich P/N M6773-258)
- Formic acid (MP Biomedicals P/N 151162)
- Lactic acid (Fisher Scientific™ A162-500)
- Acetic acid (Sigma-Aldrich P/N A9967)
- Fumaric acid (Fluka® P/N 47900)
- Propionic acid (Fisher Scientific P/N A258-500)
- Succinic acid (Fisher Scientific P/N A294-500)
- Sodium malonate dibasic monohydrate (Sigma-Aldrich P/N M4795)
- Quinic acid (Sigma-Aldrich P/N Q0500)
- Heptafluorobutyric acid (HFBA) (Acros Organics™ P/N 172800250)
- Tetrabutylammonium hydroxide (TBAOH) 55% w/w (Sachem® P/N 355)
- Methanol (CH_3OH), Certified ACS (Fisher Scientific P/N A412)
- Sodium and potassium salts, ACS reagent grade or better, for preparing anion standards

Apparatus and consumables

- Coffee grinder
- Ultrasonic bath
- Centrifuge
- Syringe 3 mL
- Thermo Scientific™ Nalgene™ 0.45 µm PES syringe membrane filter (Fisher Scientific P/N 09740114)
- Sample vial kit, 10 mL, Polystyrene with Caps and Blue Septa (P/N 074228)

Samples

Three animal feed samples (dog, rabbit, and chicken) were obtained from a local animal feed store.

Conditions

System A (ion-exchange system)

Columns:	Thermo Scientific™ Dionex™ IonPac™ AG11-4µm Guard Column, 2 × 50 mm (P/N 078036) Thermo Scientific™ Dionex™ IonPac™ AS11-4µm Analytical Column, 2 × 250 mm (P/N 078035)
Eluent A:	DI Water
Eluent B:	Methanol (CH_3OH)
Eluent Source:	Dionex EGC 500 KOH cartridge with Dionex CR-ATC 500
Flow Rate:	0.35 mL/min
Column Temperature:	45°C
Detector Compartment Temperature:	20°C
Detector Temperature:	35°C
Injection Volume:	5 µL (Full loop)
Detection:	Suppressed conductivity, Dionex AERS 500 suppressor, 2 mm, external water mode, 149 mA, flow rate equal to the eluent flow rate
Run Time:	45 min
Background Conductance:	< 0.4 µS
System Backpressure:	~ 4200 psi

Gradient	*Time (min)*	*KOH (mM)*
	-2	1
	0	1
	10.07	1
	24	15
	24.01	15
	35	27
	40	60
	44	60
	44.01	1
	45	1

Conditions (continued)

System A (ion-exchange system)

Multistep Gradient

Time (min)	*%B*
-2	0
0	0
10.7	8
20	8
25	15
31	15
33	8
33.01	0
45	0

Note: Eluents A (DI water) and B (Methanol) are mixed by the pump and then passed through the EGC KOH cartridge where the KOH gradient is generated

Conditions

System B (ion-exclusion system)

Columns:	Thermo Scientific™ Dionex™ IonPac™ NG1 Guard, 4 × 35 mm (P/N 039567) Thermo Scientific™ Dionex™ IonPac™ ICE-AS6, Analytical, 9 × 250 mm (P/N 079798)
Eluent:	0.32 mM HFBA
Flow Rate:	1 mL/min
Column Temperature:	20°C
Detector Compartment Temperature:	30°C
Detector Temperature:	35°C
Injection Volume:	10 µL (Full loop)
Detection:	Suppressed Conductivity, Dionex ACRS-ICE 500, 9 mm, external mode
Regenerant:	5 mM Tetrabutylammonium hydroxide (flow rate = 2 mL/min)
Run Time:	40 min
Background Conductance:	< 20 µS
System Backpressure:	~ 1100 psi

Preparation of solutions and reagents

Single organic acid standard stock solutions, 2,500 mg/L

Weigh the amount listed in Table 1 into a 100 mL volumetric flask. Dissolve with approximately 80 mL of DI water, mix, and fill to the mark with DI water.

Table 1. Masses of compounds used to prepare 100 mL of 2500 mg/L organic acid standards.

Analyte	Compound	Amount (mg)
Acetic acid	Acetic acid	250.0
Citric acid	Citric acid monohydrate	273.5
Formic acid	Formic acid	250.0
Fumaric acid	Fumaric acid	250.0
Lactic acid	Lactic acid	250.0
Malic acid	D-L-Malic acid disodium salt	332.0
Propionic acid	Propionic acid	250.0
Malonic acid	Malonic acid, disodium salt	355.6
Succinic acid	Succinic acid	250.0
Quinic acid	Quinic acid	250.0

Single anion stock, 1,000 mg/L

Weigh the amount listed in Table 2 into a 100 mL volumetric flask. Dissolve with approximately 80 mL of DI water, mix, and fill to the mark with DI water.

Table 2. Masses of compounds used to prepare 100 mL of 1000 mg/L anion standards.

Analyte	Compound	Amount (mg)
Fluoride	Sodium fluoride (NaF)	221.0
Chloride	Sodium chloride (NaCl)	164.9
Nitrite	Sodium nitrite ($NaNO_2$)	150.0
Bromide	Sodium bromide (NaBr)	128.8
Nitrate	Sodium nitrate ($NaNO_3$)	137.1
Sulfate	Sodium sulfate (Na_2SO_4)	147.9
Phosphate	Potassium phosphate, monobasic (KH_2PO_4)	143.3

System A - Ten mixed organic acid standard solution, 100 mg/L

Accurately pipette 4 mL of each single standard stock solution (Table 1) into a 100 mL volumetric flask and fill up to the mark with DI water. The maximum storage time is 2 months at 4°C.

System B - Nine mixed organic acid standard solution, 100 mg/L

Accurately pipette 4 mL of each single standard stock solution (Table 1, exclude quinic acid) into a 100 mL volumetric flask and fill up to the mark with water.

Working calibration standard solutions

Prepare the calibration standards with DI water according to Table 3. Prepare System A calibration standard (Levels 1–7) using system A mixed standard solution (100 mg/L, 10 OAs). Prepare System B calibration standard (Levels 2–7) using system B mixed standard solution (100 mg/L, 9 OAs).

Table 3. Preparation of organic acid calibration standard.

Calibration Standard Level	Volume (mL) of Mixed Standard Solution (100 mg/L) in 20 mL	Concentration of Organic Acids in the Calibration Solution (mg/L)
1	0.2	1
2	0.4	2
3	2	10
4	5	25
5	10	50
6	15	75
7	20	100

System B - IC eluent stock HFBA, 100 mM

Weigh 21.4 g of HFBA into a 1000 mL volumetric flask. Fill with DI water to the mark.

System B - IC eluent HFBA, 0.32 mM

Dilute 3.2 g of HFBA stock (eluent) solution with DI water to the final volume of 1000 mL.

System B - Suppression regenerant (tetrabutylammonium hydroxide, 5 mM)

Pipet 2.29 mL of tetrabutylammonium hydroxide (55% w/w) to a 1000 mL volumetric flask, fill to the mark with DI water.

Sample preparation

1. Grind the animal feed sample with a coffee grinder to a particle size of ≤ 1.0 mm.

2. Accurately weigh 5.00 g ± 10 mg of the prepared sample into a 250 mL conical flask.

3. Add 100 mL DI water to the flask and cap it.

4. Sonicate in ultrasonic bath for 30 min or mix for 60 min on a magnetic stirrer at ambient temperature.

   Note: If the sample contains a high amount of fumaric acid, heat the sample to 60°C to increase solubility.

5. Centrifuge sample extract at 5000 × g for 10 min or filter the sample through folder filter paper.

6. Dilute the sample solution with DI water to the final concentration according to the working range of calibration and filter through a Nalgene 0.45 µm PES syringe membrane filter prior to IC analysis.

Dry matter determination

Weigh an empty dry aluminum dish, weigh approximately 1 g of feed sample into the dish, place dish with wet sample into an oven that has been preheated to 100°C for 24 h. Remove dish with dried samples from oven and record weight.

Results and discussion

Separation

The Dionex IonPac AS11-HC-4µm column is a high resolution, high capacity anion-exchange column. It is specifically designed to provide high resolution of a large number of inorganic anions and organic acid anions from a single sample injection in one gradient run using a hydroxide eluent system.[3] The separation was further optimized with methanol because the solvating power and hydrophobicity of the organic solvent can influence the retention mechanism and improve the resolution of coeluting species. Note that when adding methanol to the eluent stream, the suppressor must be operated in the external water mode. By using a hydroxide gradient, strongly retained trivalent ions, such as phosphate and citrate, are efficiently eluted in the same run, while also providing a baseline resolution of the weakly retained monovalent anions: fluoride, lactate, acetate, and formate.

Figure 1A shows the separation of 10 OAs and seven common anions using system A. Organic acids except fumaric acid were baseline resolved from other OAs and anions. Fumaric acid was not completely resolved from sulfate which may affect the quantification of fumaric acid when the amount of fumaric acid is low relative to sulfate.

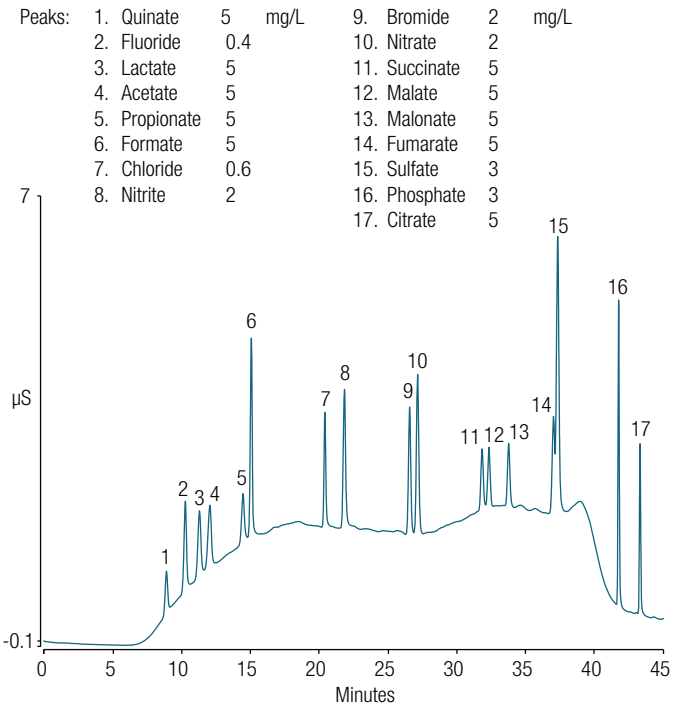

Figure 1A. Separation of an organic acids and inorganic anions standard using system A.

Inorganic anions and carbonate can interfere when determining OAs in animal feed using anion-exchange chromatography. Inorganic anion interferences can be easily eliminated using ion-exclusion chromatography because they are excluded from the column (i.e., not retained). The Dionex IonPac ICE-AS6 ion-exclusion column is designed for the determination of aliphatic OAs and alcohols in complex or high-ionic strength samples, including food and beverage products, biological samples, fermentation processes, industrial process liquors, and waste waters.[4]

Figure 1B shows the separation of nine OAs using system B. Quinic acid was not included here because it coelutes with malic acid. The coelution of quinic and malic acids may affect the quantification of malic acid in feed samples using system B if quinic acid is present at a high amount. Citrate and malonate were not completely separated using system B, as shown in Figure 1B, which may affect the quantification of citrate and malonate using system B.

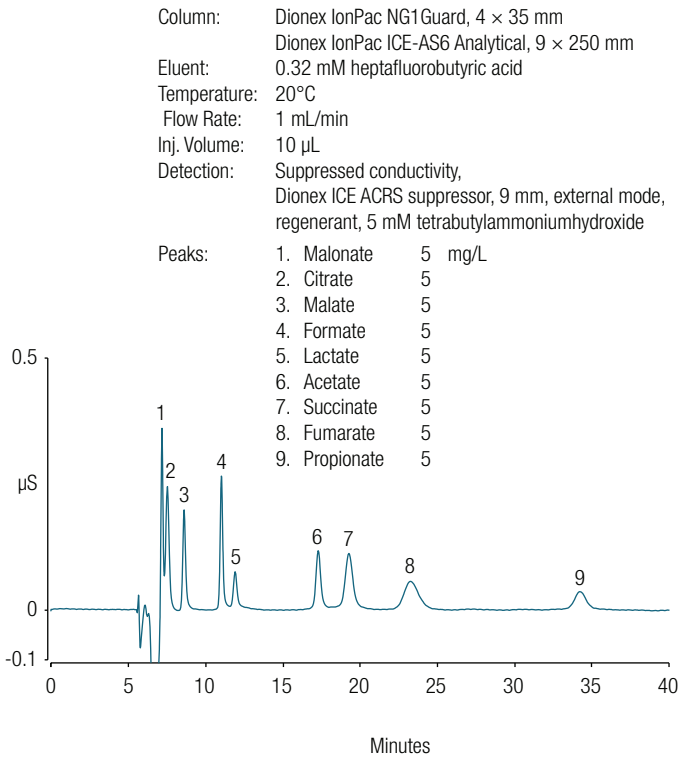

Figure 1B. Separation of an organic acids standard using system B.

Method linear calibration ranges, limit of detection (LOD), and limit of quantitation (LOQ)

Calibration curves with seven concentration levels ranging from 1 to 100 mg/L were constructed for the 10 OAs: acetic acid, citric acid, formic acid, fumaric acid, lactic acid, malic acid, propionic acid, malonic acid, succinic acid, and quinic acid using system A. Due to the incomplete dissociation of these weak carboxylic acids, the calibration curves show deviation from linearity in the selected calibration ranges. Therefore, the calibration plots of peak area versus concentration were fit using a quadratic regression function. Figure 2A shows an example of the citric acid calibration curve using system A. Table 4A summarizes the calibration, LOD, and LOQ of OAs using system A.

Calibration curves with six concentration levels ranging from 2 to 100 mg/L were constructed for nine organic acids including acetic acid, citric acid, formic acid, fumaric acid, lactic acid, malic acid, propionic acid, malonic acid, and succinic acid using system B. The calibration plots of peak area versus concentration were fit using a linear regression function. Figure 2B shows an example citric acid calibration curve using system B. Table 4B summarizes the calibration, LOD, and LOQ of organic acids using system B.

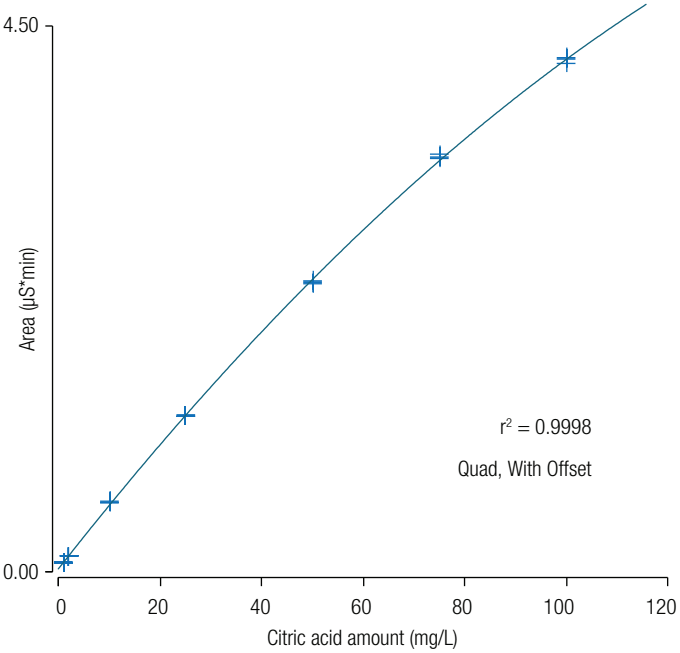

Figure 2A. Citric acid calibration curve using system A.

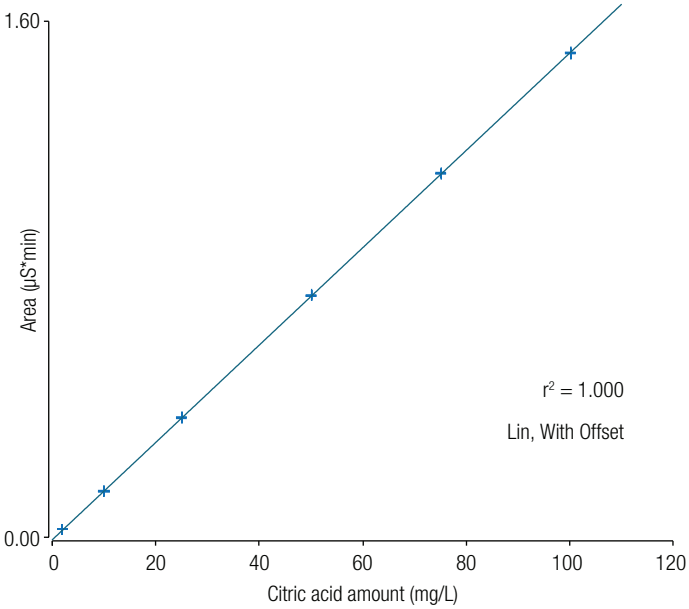

Figure 2B. Citric acid calibration curve using system B.

Table 4A. Calibration, LOD, and LOQ of organic acids using system A.

Analyte	Range (mg/L)	Coefficient of Determination (r^2) Quadratic Fitting	LOD (mg/L) in Extract Solution	LOQ (mg/L) in Extract Solution	LOD (mg/kg) in Feed	LOQ (mg/kg) in Feed
Acetic acid	1–100	0.9997	0.12	0.41	2.44	8.14
Citric acid	1–100	0.9998	0.08	0.28	1.70	5.66
Formic acid	1–100	0.9998	0.20	0.67	4.02	13.4
Fumaric acid	1–100	0.9998	0.13	0.44	2.61	8.70
Lactic acid	1–100	0.9997	0.17	0.58	3.48	11.6
Malic acid	1–100	0.9999	0.10	0.35	2.08	6.94
Malonic acid	1–100	0.9999	0.12	0.40	2.39	7.98
Propionic acid	1–100	0.9997	0.12	0.39	2.33	7.77
Succinic acid	1–100	0.9999	0.14	0.48	2.85	9.51
Quinic acid	1–100	0.9999	0.22	0.72	4.31	14.4

Table 4B. Calibration, LOD, and LOQ of organic acids using system B.

Analyte	Range (mg/L)	Coefficient of Determination (r^2) Linear Fitting	LOD (mg/L)	LOQ (mg/L)	LOD (mg/kg) in Feed	LOQ (mg/kg) in Feed
Acetic acid	2–100	1.0000	0.16	0.52	3.15	10.5
Citric acid	2–100	1.0000	0.26	0.85	5.13	17.1
Formic acid	2–100	1.0000	0.28	0.92	5.50	18.3
Fumaric acid	2–100	1.0000	0.30	1.00	6.00	20.0
Lactic acid	2–100	1.0000	0.24	0.80	4.79	16.0
Malic acid	2–100	1.0000	0.14	0.47	2.85	9.49
Malonic acid	2–100	1.0000	0.36	1.20	7.18	23.9
Propionic acid	2–100	1.0000	0.39	1.31	7.86	26.2
Succinic acid	2–100	1.0000	0.28	0.95	5.69	19.0

To determine the limit of detection (LOD) and limit of quantification (LOQ), the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute, but close to the peak of interest. The LOD and LOQ were then calculated from the average peak height of three injections of standard mixture (0.25, 0.5, or 1 ppm). The LOD and LOQ in the feed sample were calculated on the basis of the sample weight (5 g) and extraction volume (100 mL).

Analysis of animal feed samples

Three animal feed samples were extracted and analyzed using the two IC methods described above. The concentrations of OAs were accurately quantified from their respective calibration curves. The concentrations of all the anions were estimated using the anion mixture. Tables 5A and 5B summarize the results of OAs using systems A and B, respectively. In dog feed, citric, malic, and lactic acids are the three major OAs. In rabbit feed, citric, malic, and malonic acids are the three major OAs. In chicken, citric acid and malic acid are the two major OAs.

Table 5A. Amounts of organic acids in animal feed determined using system A (g/kg dry feed sample).

Animal Feed	Rep	Citric Acid	Malic Acid	Formic Acid	Lactic Acid	Acetic Acid	Fumaric Acid	Propionic Acid	Malonic Acid	Succinic Acid	Quinic Acid
Dog	1	1.19	0.966	0.108	1.99	0.671	0.0221	0.108	0.834	0.336	0.0846
	2	1.42	1.12	0.120	2.25	0.797	0.0264	0.115	0.938	0.374	0.0878
	3	1.42	1.11	0.127	2.28	0.807	0.0246	0.118	0.933	0.371	0.088
	CV %	9.93	8.05	8.30	7.43	9.96	9.05	4.37	6.51	5.93	9.93
Rabbit	1	3.56	6.52	0.0717	0.588	0.437	0.0981	0.0499	5.07	0.630	0.135
	2	3.60	6.60	0.0731	0.610	0.420	0.0920	0.0554	4.98	0.667	0.128
	3	3.82	6.75	0.0820	0.585	0.462	0.108	0.0487	5.10	0.623	0.135
	CV %	3.76	1.78	7.40	2.26	4.88	8.10	6.95	1.28	3.70	3.23
Chicken	1	5.60	0.919	0.0341	0.0690	0.120	0.0504	N/A	0.158	0.0989	0.0434
	2	5.27	0.871	0.0318	0.0752	0.111	0.0557	N/A	0.164	0.0974	0.0435
	3	5.71	1.00	0.0325	0.0760	0.108	0.0592	N/A	0.169	0.0995	0.0373
	CV %	4.19	7.18	3.57	5.17	5.59	8.07	N/A	3.26	1.12	8.59

Table 5B. Amounts of organic acids in animal feed determined using system B (g/kg dry feed sample).

Animal Feed	Rep	Citric Acid	Malic Acid	Formic Acid	Lactic Acid	Acetic Acid	Fumaric Acid	Propionic Acid	Malonic Acid	Succinic Acid	Quinic Acid
Dog	1	1.26	0.908	0.0946	2.43	0.614	0.0400	0.108	0.802	0.322	0.0846
	2	1.45	1.03	0.108	2.82	0.732	0.0426	0.121	0.870	0.376	0.0878
	3	1.42	1.00	0.106	2.80	0.713	0.0417	0.107	0.869	0.362	0.088
		CV %	7.4	6.37	7.09	8.19	9.27	3.15	7.02	4.59	7.98
Rabbit	1	3.93	6.60	0.0865	0.660	0.358	0.135	0.0481	4.34	0.688	0.135
	2	3.95	6.70	0.0835	0.674	0.371	0.133	0.0439	4.41	0.708	0.128
	3	4.16	6.35	0.0824	0.675	0.376	0.136	0.0479	4.04	0.721	0.135
		CV %	3.26	2.79	2.53	1.28	2.52	0.95	5.07	4.58	2.36
Chicken	1	5.73	0.788	0.0315	0.0765	0.139	0.0715	ND	ND	0.101	0.0434
	2	5.24	0.658	0.0304	0.0705	0.118	0.0627	ND	ND	0.118	0.0435
	3	5.88	0.778	0.0320	0.0759	0.123	0.0694	ND	ND	0.106	0.0373
		CV %	5.98	9.76	2.54	4.39	8.7	6.8	N/A	N/A	8.16

Fumarate and sulfate were not completely resolved using system A, as shown in Figure 1A. Therefore, the result using system B is more reliable. Tables 5A and 5B show that the fumaric acid result obtained from method A is lower than the result obtained from method B. This is due to the fact that fumaric acid was not completely separated from sulfate using system A, as shown in Figures 3A and 4A.

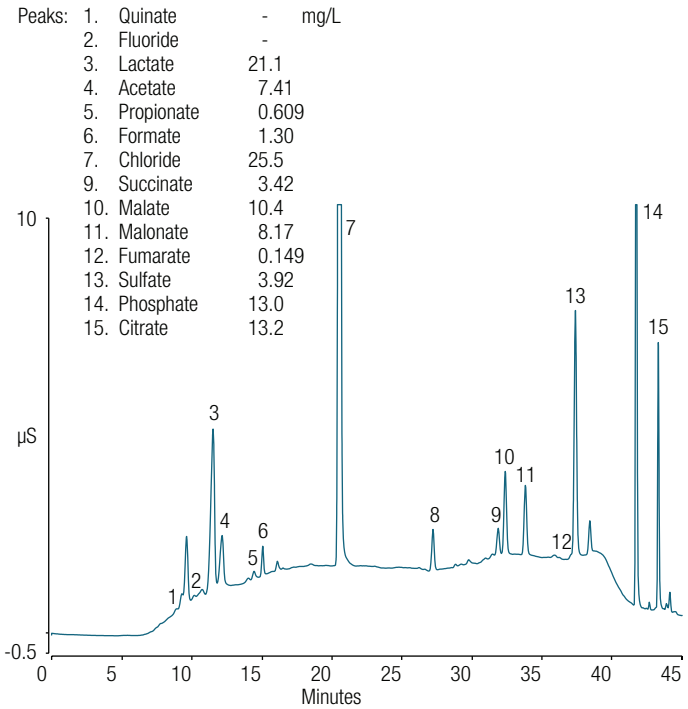

Figure 3A. Separation of dog feed extract (5-fold diluted) using system A.

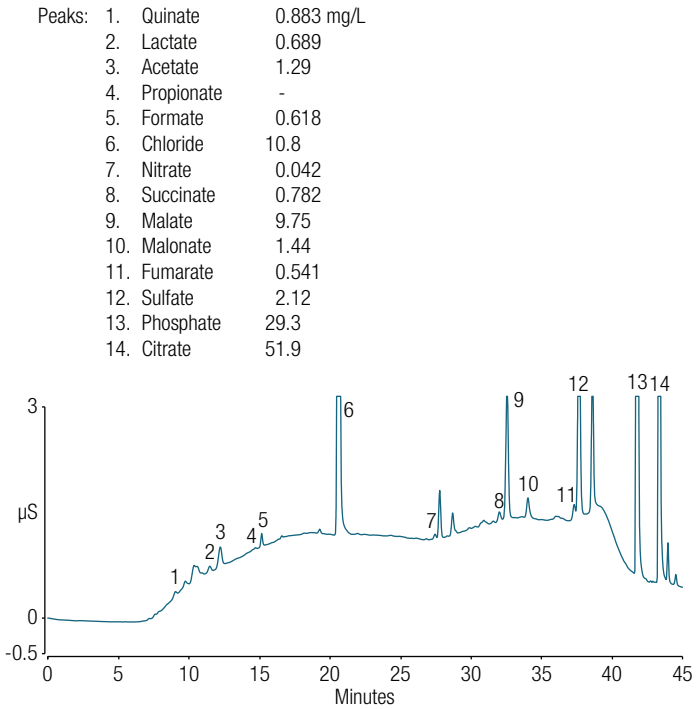

Figure 4A. Separation of chicken feed extract (5-fold diluted) using system A.

Citric and malonic acids were not completely resolved using system B, as shown in Figure 1B. Therefore, the result using system A is more reliable with respect to citrate and malonate. Tables 5A and 5B show that the citric and malonic acids in dog and rabbit feed samples are still accurately determined using system B even though they were not completely resolved. However, the amount of malonic acid in the chicken feed sample is very low relative to citric acid, which affects the detection of malonic acid using system B, as shown in Figure 4B.

Malic and quinic acids completely coelute in system B, therefore, quinic acid is not included in the system B analysis. However, the amount of quinic acid is very low in feed samples according to the results obtained from method A. As a result, malic acid was still accurately measured using system B.

Method accuracy

The accuracies of the system A and system B methods were evaluated by determining recoveries of malic, lactic, and acetic acids in spiked animal feed samples. (Tables 6A and 6B). Recoveries were calculated from the difference in response between the spiked and unspiked samples. The recovery for three OAs ranged from 90% to 105%, indicating both methods can accurately determine OAs in animal feed samples.

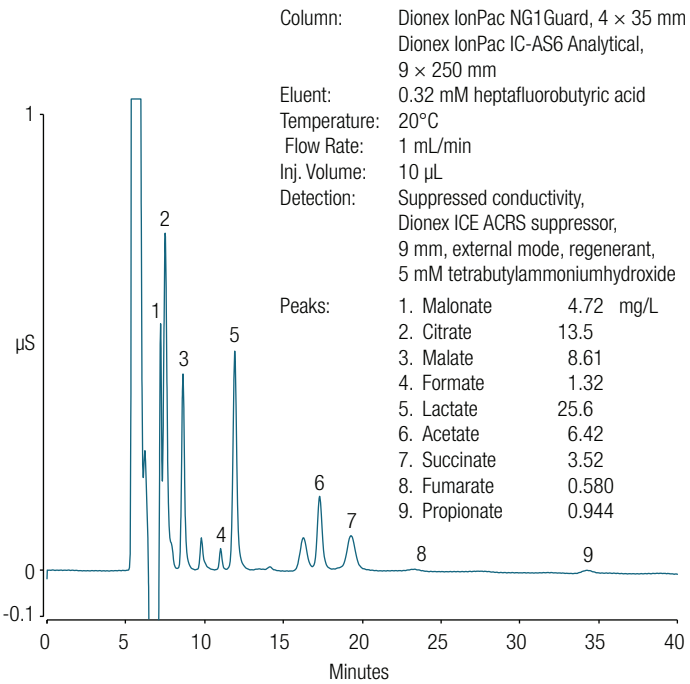

Figure 3B. Separation of dog feed extract (5-fold diluted) using system B.

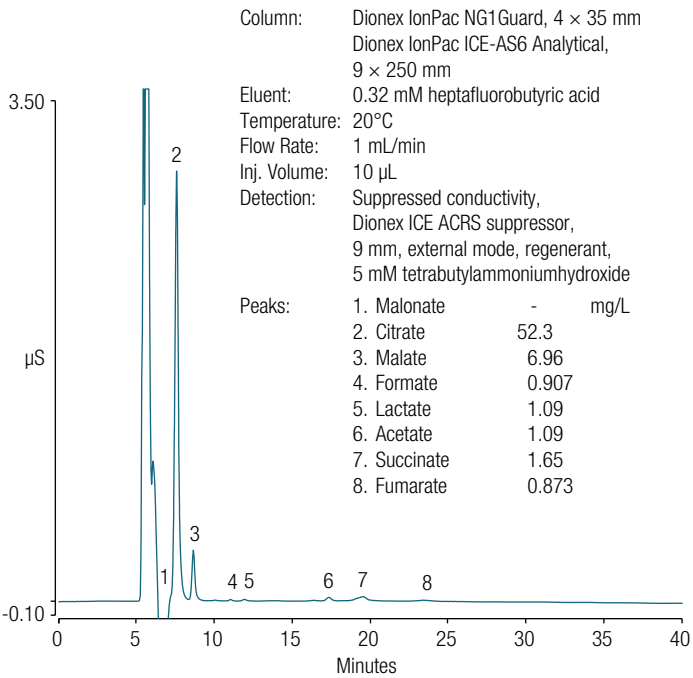

Figure 4B. Separation of chicken feed extract (5-fold diluted) using system B.

Table 6A. Recoveries of malic acid, lactic acid, and acetic acid spiked in feed extracts using system A.

Feed Sample	Analyte	Rep 1			Rep 2			Rep 3		
	Acid	Found (mg/L)	Added (mg/L)	Recovery (%)	Found (mg/L)	Added (mg/L)	Recovery (%)	Found (mg/L)	Added (µg/L)	Recovery (%)
Dog 5-fold diluted	Malic	8.98	14.2	93.4	10.4	14.2	101	10.3	14.2	92.1
	Lactic	18.4	24.7	94.3	21.1	24.7	102	21.4	24.7	92.2
	Acetic	6.24	6.01	96.0	7.41	6.01	100	7.50	6.01	91.3
Rabbit 5-fold diluted	Malic	61.4	47.4	101	59.1	47.4	99.4	61.3	47.4	101
	Lactic	3.96	5.92	99.6	3.51	5.92	101	3.59	5.91	92.9
	Acetic	3.93	3.00	99.8	3.78	3.00	96.3	3.91	3.00	91.3
Chicken	Malic	39.1	28.5	93.9	43.7	28.5	96.9	45.1	28.5	99.2
	Lactic	1.74	2.96	103	1.63	2.96	92.4	1.86	2.96	104
	Acetic	5.79	5.01	96.2	6.10	5.01	98.8	6.70	5.01	101

Table 6B. Recoveries of malic acid, lactic acid, and acetic acid spiked in feed extracts using system B.

Feed Sample	Analyte	Rep 1			Rep 2			Rep 3		
	Acid	Found (mg/L)	Added (mg/L)	Recovery (%)	Found (mg/L)	Added (mg/L)	Recovery (%)	Found (mg/L)	Added (µg/L)	Recovery (%)
Dog 5-fold diluted	Malic	7.02	14.2	99.6	8.63	14.2	98.9	8.49	14.2	96.9
	Lactic	21.9	24.7	99.0	25.6	24.7	93.0	25.4	24.7	92.4
	Acetic	5.12	6.01	99.0	6.43	6.01	93.7	6.11	6.01	94.2
Rabbit 5-fold diluted	Malic	59.0	47.4	99.5	58.8	47.4	99.7	59.2	47.4	100
	Lactic	5.90	5.92	93.2	6.09	5.92	92.3	6.08	5.91	90.5
	Acetic	3.33	3.00	100	3.44	3.00	93.4	3.36	3.00	98.0
Chicken	Malic	29.6	28.5	99.1	34.7	28.5	95.9	33.2	28.5	98.7
	Lactic	2.95	2.96	95.6	3.36	2.96	92.3	3.25	2.96	99.6
	Acetic	4.31	5.01	98.7	6.07	5.01	98.3	5.52	5.01	99.2

Method precision

Method precision was evaluated through triplicate whole-procedure analysis from sample extraction to IC analysis. As shown in Tables 5A and 5B, the CV of three replicate analysis range from 1% to 10%.

Conclusion

This study demonstrated that OAs can be determined accurately in animal feed samples using two IC methods. Method A is an ion-exchange method using a Dionex IonPac AS11-HC-4 µm column, which is ideal for separating a wide range of OAs and inorganic anions present in samples. Method B is ion-exclusion method using a Dionex IonPac ICE-AS6 column, which is ideal for measuring OAs without interference from inorganic ions. Method A uses a hydroxide as eluent, which allows the use of the Dionex EGC 500 KOH eluent generation cartridge to generate consistent KOH eluent automatically using DI water.

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