

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

Jingli Hu, and Jeff Rohrer, Thermo Fisher Scientific, Sunnyvale, CA

ABSTRACT

Purpose: 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 by improving the animals' performance and reducing the development of pathogenic micro-organisms in the intestine. This study is to quantify organic acids in animal feeds by both anion-exchange and ion-exclusion ion chromatography methods.

Methods: Three animal feed samples were analyzed in this study. The organic acids were extracted from animal feed with water and then determined using two ion chromatography (IC) methods with suppressed conductivity detection. Method 1 is an anion-exchange method using a Thermo Scientific™ Dionex™ IonPac™ AS11-HC-4 µm column, which is ideal for separating a wide range of organic acids and inorganic anions. Method 2 is an ion-exclusion method using a Thermo Scientific™ Dionex™ IonPac™ ICE-AS6 column, which is ideal for measuring organic acids without interference from inorganic anions. Both methods were validated in terms of linearity, sensitivity, selectivity, accuracy, and precision.

Results: The limits of quantification (LOQs) ranged from 5.66–13.4 mg/kg for Method 1 and 9.49–26.2 mg/kg for Method 2. While either method is appropriate for determining organic acids in animal feed, some feed contains malonic acid, which is better separated from citric acid using Method 1. Additionally, some feed contains a large amount of sulfate, which is better separated from fumaric acid using Method 2.

INTRODUCTION

Organic acids are important components of animal feed. 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. For example, microencapsulated mixture of citric acid, sorbic acid (OA) and pure botanicals, namely thymol and vanillin, (PB) can improve the maturation of the intestinal mucosa and eventually improving the growth of piglets prematurely weaned.² 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 suboptimal developed enzyme system is improved. Organic acids 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, IC with suppressed conductivity detection is the technique of choice to separate and detect a large variety of organic acids.

MATERIALS AND METHODS

Sample Preparation

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

Grind the animal feed sample with a coffee grinder to a particle size of less than or equal to 1.0 mm. Accurately weigh 5.00 g ± 0.01 mg of the prepared sample into a 250 mL conical flask. Add 100 mL DI water to the flask and cap it.

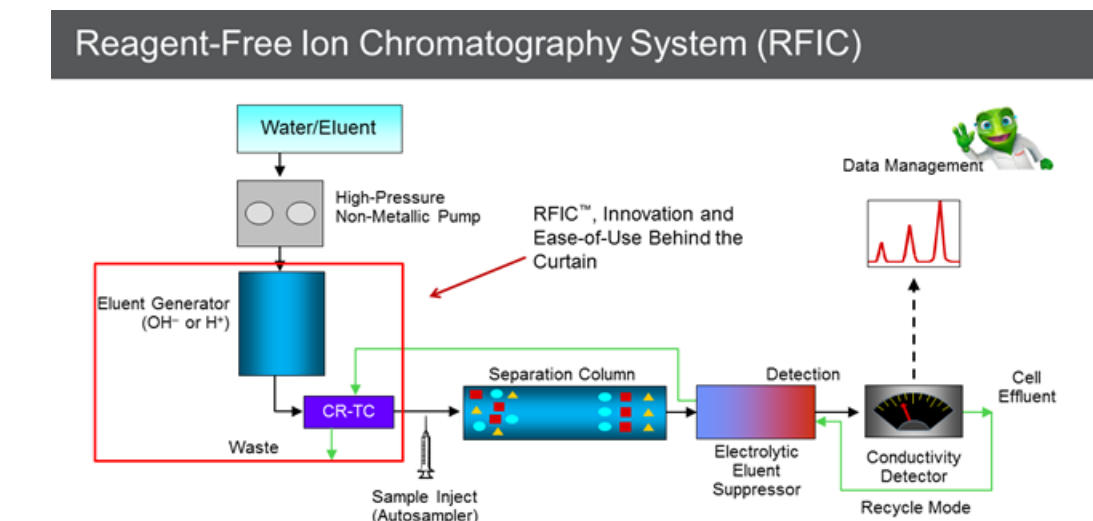
Sonicate in ultrasonic bath for 30 min or mix for 60 min on a magnetic stirrer at ambient temperature. Note: If the sample contains a high amount of fumaric acid, heat the sample to 60 °C to increase solubility.

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

Data Analysis : By Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2 SR4.

Test Method(s) Thermo Scientific Dionex ICS-5000* HPIC system 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
- Dionex AS-AP Autosampler with sample syringe, 250 µL (P/N 074306) and buffer line 1200 µL (P/N 074989)



Chromatographic Conditions

	Method 1 (Ion Exchange System)	Method 2 (Ion Exclusion System)
Columns:	Dionex IonPac AG11-4µm Guard Column, 2 x 50 mm (P/N 078036) Dionex IonPac AS11-4µm Analytical Column, 2 x 250 mm (P/N 078036)	Dionex IonPac NG1 Guard, 4 x 35 mm (P/N 039567) Dionex IonPac ICE AS6, 9 x 250 mm Analytical , 9 x 250 mm (P/N 079798)
Eluent A:	DI Water (Gradient)	0.32 mM HFBA (Isocratic)
Eluent B:	Methanol (CH ₃ OH)	
Eluent Source:	Thermo Scientific Dionex EGC 500 KOH Eluent Generator Cartridge with Thermo Scientific™ Dionex™ CR-ATC 500 Continuously Regenerated Anion Trap Column	Manual Preparation
Flow Rate:	0.35 mL/min	1 mL/min
Column Temperature:	45 °C	20 °C
Detector Compartment Temperature:	20 °C	30 °C
Detector Temperature:	35 °C	35 °C
Injection Volume:	5 µL, (Full loop)	10 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	Suppressed Conductivity, Dionex ACRS-ICE 500 , 9 mm, external mode
Run time:	45 min	40 min
Background Conductance:	< 0.4 µS	< 20 µS
System backpressure:	~ 4200 psi	~ 1100 psi

Method 1 KOH Gradient		Method 1 Pump Gradient		Multi-Step Gradient	
Time (min)	KOH (mM)	Time (min)	B (%)	Time (min)	B (%)
-2	1	-2	0	-2	0
0	1	0	0	0	0
10.07	1	10.7	8	10.7	8
24	15	20	8	20	8
24.01	15	25	15	25	15
35	27	31	15	31	15
40	60	33	8	33	8
44	60	33.01	8	33.01	8
44.01	1	45	0	45	0
45	1				

RESULTS

Separation of organic acid standards

Figure 1 shows the separation of 10 organic acids and seven common anions using Method 1. Organic acids, except fumaric acid, were baseline resolved from other organic acids 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 2 shows the separation of 9 organic acids using Method 2. Quinic acid was not included here because it coelutes with malic acid. The coelution of quinic and malic acid may affect the quantification of malic in feed sample using Method 2 if quinic acid is present at a high amount. Citrate and malonate were not completely separated using Method 2 as shown in Figure 2, which may affect the quantification of citrate and malonate using Method 2.

Figure 1. Separation of organic acids and inorganic anions standard using Method 1.

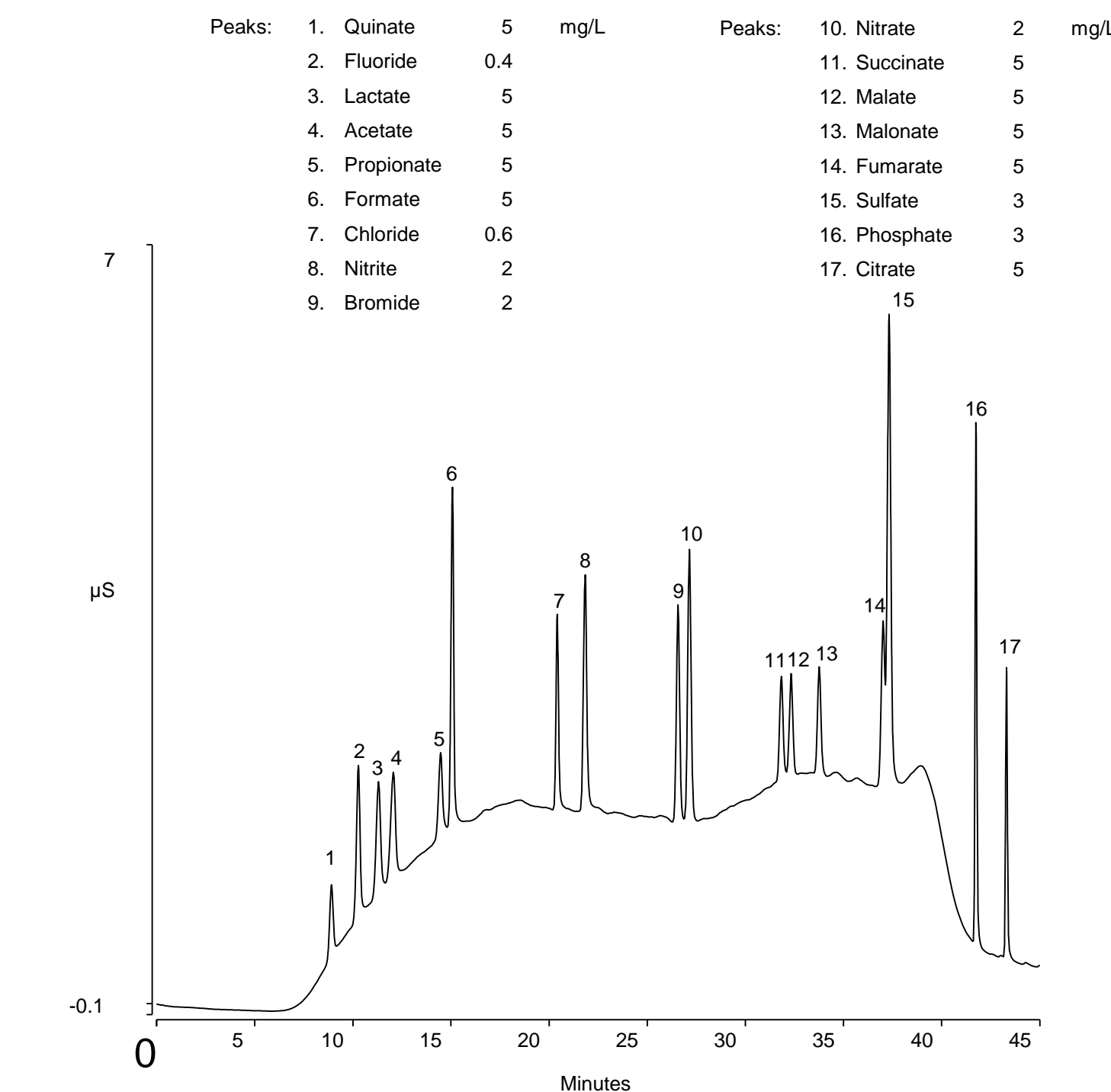
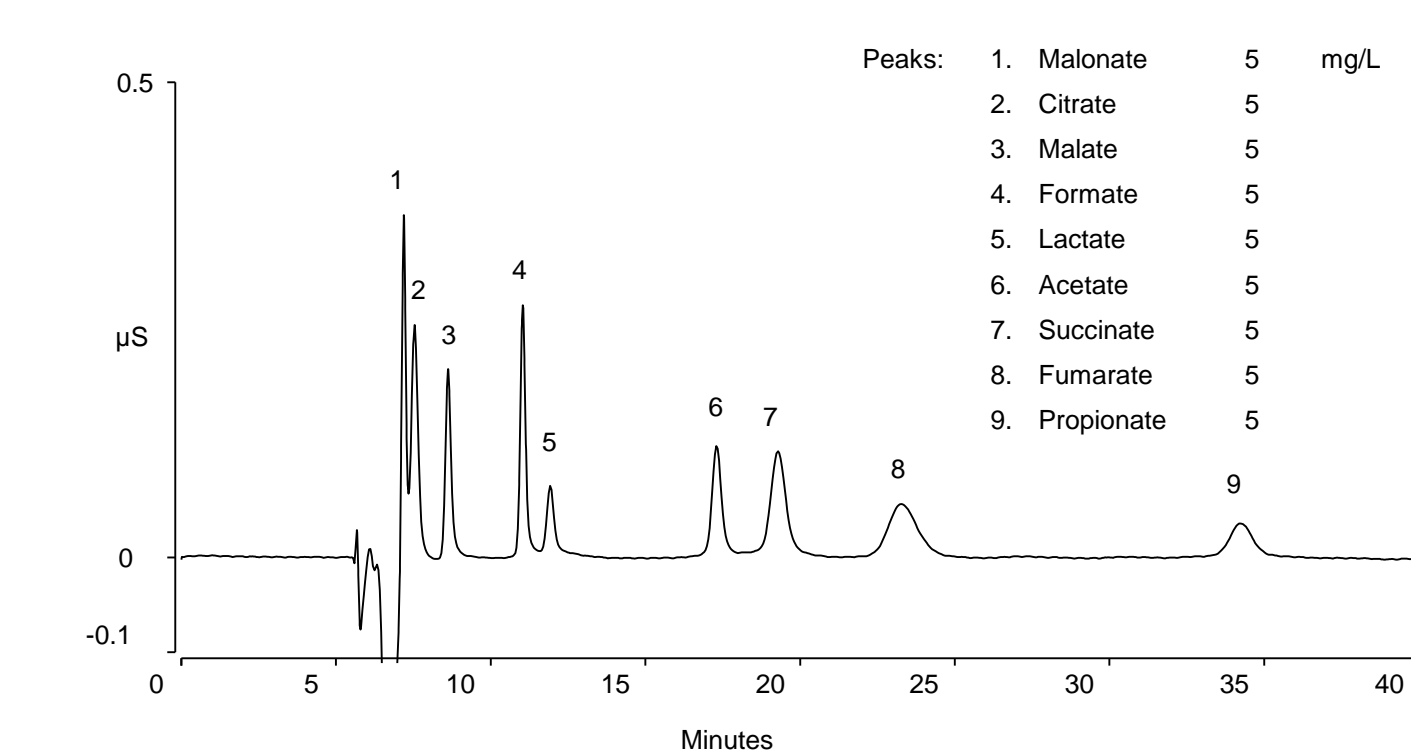


Figure 2. Separation of organic acids standards using Method 2.



Method Linear Calibration Ranges, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

Tables 1 and 2 summarize the Calibration, LOD, and LOQ of organic acids using Method 1 and Method 2, respectively. Quinic acid was not included in Method 2 because it coelutes with malic acid.

Table 1. Calibration, LOD, and LOQ of organic acids using Method 1.

Analyte	Range (mg/L)	Coefficient of Determination (r ²)	LOD (µg/k) in feed	LOQ (mg/k) in feed
Acetic acid	1-100	0.9997	2.44	8.14
Citric acid	1-100	0.9998	1.70	5.66
Formic acid	1-100	0.9998	4.02	13.4
Fumaric acid	1-100	0.9998	2.61	8.70
Lactic acid	1-100	0.9997	3.48	11.6
Malic acid	1-100	0.9999	2.08	6.94
Malonic acid	1-100	0.9999	2.39	7.98
Propionic acid	1-100	0.9997	2.33	7.77
Succinic acid	1-100	0.9999	2.85	9.51
Quinic acid	1-100	0.9999	4.31	14.4

Table 2. Calibration, LOD, and LOQ of organic acids using Method 2.

Analyte	Range (mg/L)	Linearity (r ²) Linear fitting	LOD (mg/kg) in feed	LOQ (mg/kg) in feed
Acetic acid	2-100	1.0000	3.15	10.5
Citric acid	2-100	1.0000	5.13	17.1
Formic acid	2-100	1.0000	5.50	18.3
Fumaric acid	2-100	1.0000	6.00	20.0
Lactic acid	2-100	1.0000	4.79	16.0
Malic acid	2-100	1.0000	2.85	9.49
Malonic acid	2-100	1.0000	7.18	23.9
Propionic acid	2-100	1.0000	7.86	26.2
Succinic acid	2-100	1.0000	5.69	19.0

Analysis of Animal Feed Samples

Three animal feed samples were extracted and analyzed by the two ion chromatography methods described above. Table 3 summarizes the results of organic acids using Method 1 and Method 2. The accuracies of the Method 1 and Method 2 were evaluated by determining recoveries of malic, lactic, and acetic acid in spiked animal feed samples. The recovery for three organic acids ranged from 90–105%, indicating both methods can accurately determine organic acids in animal feed samples. Method precision was evaluated through triplicate whole-procedure analysis from sample extraction to IC analysis. The CV of three replicate analyses ranged from 1–10%. Figure 3 and Figure 4 show the chromatograms of dog food extract using Methods 1 and 2, respectively.

Table 3. Amount of organic acid in animal feed (g/kg dry feed sample).

	Method 1			Method 2		
	Dog	Rabbit	Chicken	Dog	Rabbit	Chicken
Acetic acid	0.76	0.44	0.11	0.69	0.37	0.13
Citric acid	1.34	3.66	5.53	1.38	4.01	5.62
Formic acid	0.12	0.08	0.03	0.10	0.08	0.03
Fumaric acid	0.02	0.10	0.06	0.04	0.13	0.07
Lactic acid	2.17	0.59	0.07	2.68	0.67	0.07
Malic acid	1.07	6.62	0.93	0.98	6.55	0.74
Malonic acid	0.90	5.05	0.16	0.85	4.26	N/A
Propionic acid	0.11	0.05	N/A	0.11	0.05	N/A
Succinic acid	0.36	0.64	0.10	0.35	0.71	0.11
Quinic acid	0.09	0.13	0.04			

Figure 3. Separation of dog food extract (5 fold diluted) using Method 1.

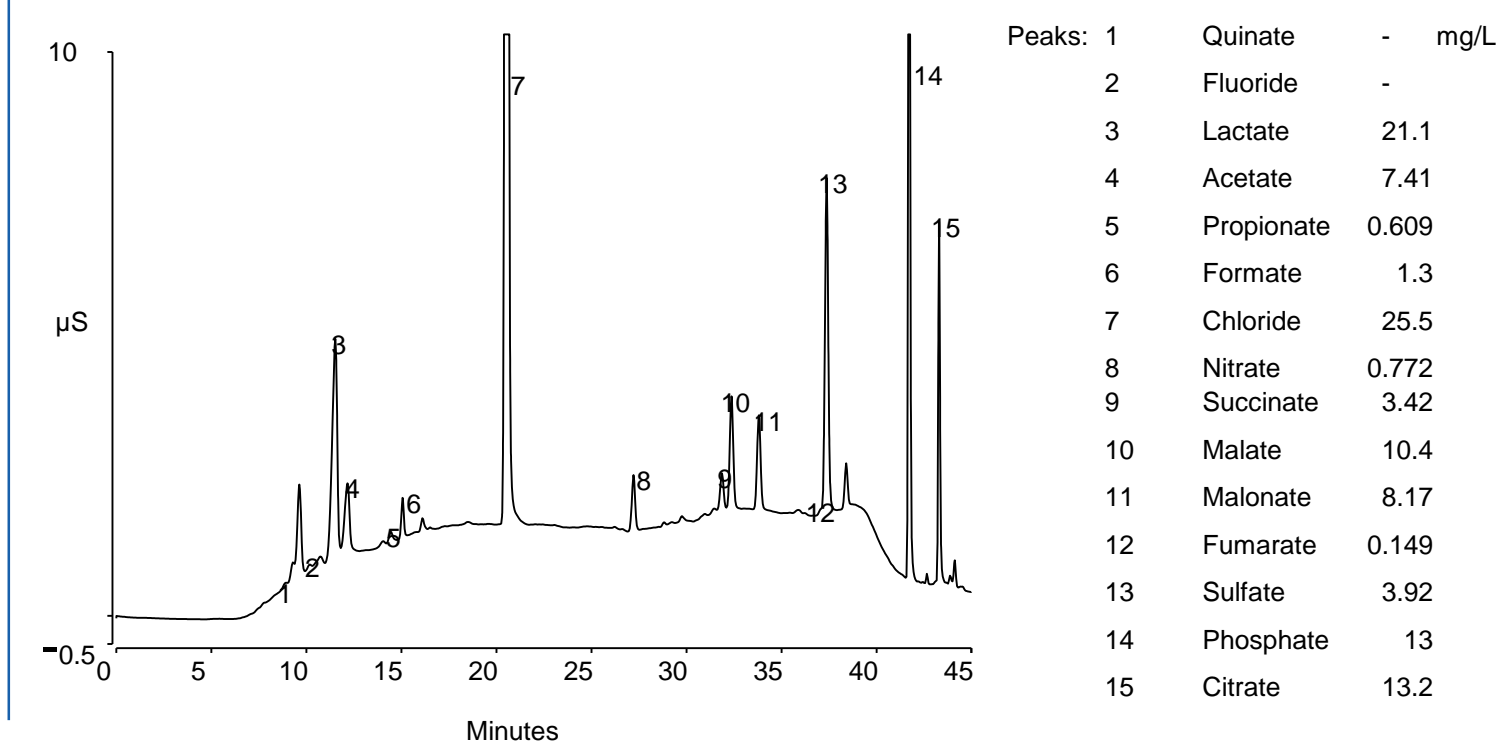
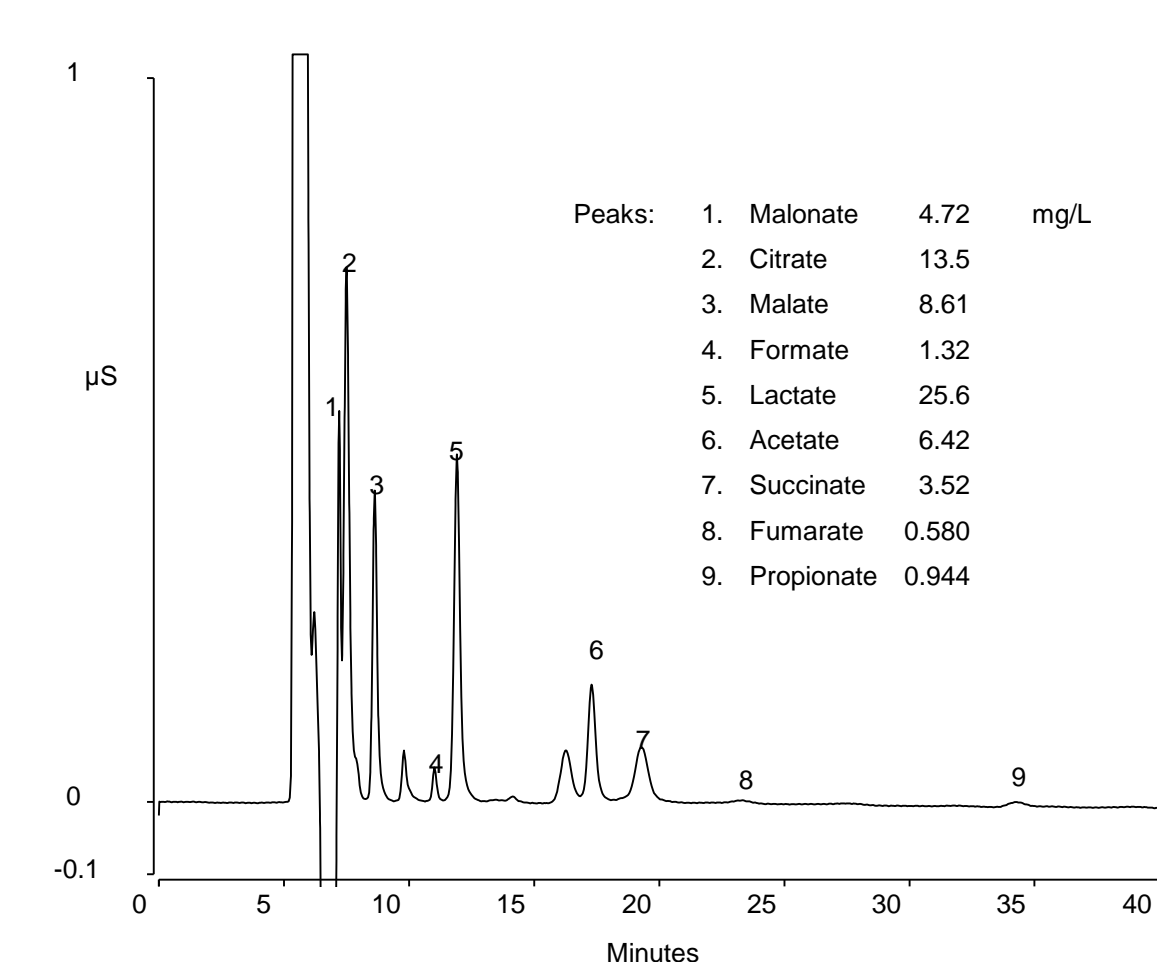


Figure 4. Separation of dog food extract (5-fold diluted) using Method 2.



CONCLUSIONS

- This study demonstrated that organic acids can be determined accurately in animal feed samples using two ion chromatography methods.
- Method 1 is ion exchange method using a Dionex IonPac AS11HC-4 µm column, which is ideal for separating a wide range of organic acids and inorganic anions present in samples. Method 1 uses hydroxide as an eluent, which can take advantage of Dionex EGC 500 KOH Eluent Generator Cartridge to generate consistent KOH eluent automatically using DI water.
- Method 2 is ion exclusion method using a Dionex ICE-AS6 column, which is ideal for measuring organic acids without interference from inorganic ions.

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TRADEMARKS/LICENSING

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