STUDYING TO BUILD THE DETERMINATION PROCESS OF AMMELIDE (AMD) AND DICYANDIAMIDE (DCD) IN ANIMAL FEED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) WITH A DIODE-ARRAY DETECTOR (DAD)

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ABSTRACT

The purpose of this study is to built a process for determining the content of fake protein enhancers such as Ammelide (AMD) and Dicyandiamide (DCD) in animal feed in accordance with the sample preparation procedure combined with a solid phase extraction (SPE) purification process and a high-performance liquid chromatography (HPLC) with a diode-array detector (DAD). The content of AMD and DCD in animal feed samples were determined with quantitative limits of 0.1-1.000 ppm, respectively for both substances, meeting AOAC (Association of Official Analytical Chemists) requirements for method validation and requirements for sensitivity, repeatable, linear intervals to be practically applicable. The procedure of determination has been effectively applied at National Centre for Veterinary Drugs and Bio-Products Control No. 2 to control the content of AMD and DCD in the basis of actual animal feed samples.

Keywords: Fake protein enhancers, AMD, DCD, animal feed, SPE, HPLC-DAD.

1. INTRODUCTION

Researching on determining the content of fake protein enhancers such as Ammelide (AMD) and Dicyandiamide (DCD), has recently become an issue of concern in the food industry. They are characterized by a high content of nitrogen in the molecular formula, so mixed into milk, animal feed to artificially increase the protein content to cope with the product quality controls. Various methods for determining the content of these substances in milk have been developed. Chen *et al*., proposed the process of determining DCD in milk samples by LC-MS/MS using d-SPE and LLE techniques to clean the sample combined with the internal standard to quantify [1]. MacMahona *et al*. published the procedure for determining DCD, AMD and some melamine derivatives in infant food samples by LC-MS/MS method with LOQ from 18-162 ppb (depending on substance) [2]. In addition, some authors published the procedures for determining DCD and AMD by conventional methods such as UV [3], GC/MS [4], ion exchange chromatograph [5] or creating complexes and determining by UV-Vis [6]. In general, the above methods required the use of specific chemicals (using internal standards [1], derivatives [6]) and expensive sample preparation techniques and experienced staffs. However, there has not been any announcement of AMD and DCD analysis methods and regulations on their thresholds in animal feed. This is a very complicated matrix because it is a mixture of

many different components such as proteins, fats, antibiotics, minerals and some other components (existing components are available in natural materials). If these components are not removed before quantifying AMD and DCD, they will cause errors in analysis results. The purpose of this study is to develop a process to identify AMD and DCD according to the sample preparation process combined with the cleaning process by solid phase extraction (SPE) and quantification by HPLC-DAD analysis method, which is popularized in many laboratories in Vietnam.

2. METERIALS AND METHODS

2.1. Materials

2.1.1. Chemicals

Ammelide standard (99%, Dr. Ehrenstofer GmbH), cyanoguanidine (98%, Sigma); ammonia solution: 25%, Merck; acetonitrile (ACN): 99.9%, Fisher; methanol: 99.9%, Fisher; formic acid: 98-100%, Merck; ammonium acetate: 98%, Merck; super clean water: 18 MΩ-cm; trifloroacetic acid (TFA): 99%, acros organic.

2.1.2. Standard solutions, samples and mobile phases

DCD 1,000 ppm stock standard solution: Accurately weighed about 10 ± 0,1 mg of DCD standard in a 10 mL volumetric flask, add 8 mL of H_2O, ultrasonic in 30 minutes, make up to the mark with H_2O. This solution is then stored in a refrigerator at 2-8 °C in a light-free condition.

AMD 1,000 ppm stock standard solution: Accurately weighed about 10 ± 0,1 mg of AMD standard in a 10 mL volumetric flask, add 50 µL of 25% ammonia solution (because ammelide is sparingly soluble in water and easily soluble in mild alkaline solutions, add 8 mL of H_2O, ultrasound in 5 minutes, then make up to the mark with H_2O. This solution is then stored in a refrigerator at 2-8 ^oC in a light-free condition.

The analysis standard solution is diluted from stock standard solution with a mixture of solvent ACN - ammonium acetate 10 mM (50:50, v:v).

Ammonium acetate solution 10 mM: Accurately weighed about 393.3 mg of ammonium acetate (99%, Merck) into Becher 500 mL, dissolved with 500 mL H_2O.

Sample extract solution ACN:H_2O *(50:50, v:v)*: Dissolved 50 mL of ACN solution into 50 mL H_2O, mixed thoroughly with the vortex.

Ammonium acetate soluble solution 10 mM - ACN (50:50, v:v): Dissolved 50 mL of 10 mM ammonium acetate into 50 mL of ACN, mixed thoroughly with the vortex.

TFA solution 0.1%: Dissolved 100 µL of 99% TFA solution in 100 mL H_2O, mixed thoroughly with the vortex.

Ammonia solution 5% in MeOH: Dissolved 20 mL of 25% ammonia solution into 80 mL MeOH, mixed thoroughly with the vortex.

Mobile phase: Mobile phase A: ammonium acetate 10 mM, adjusted to pH 6.5 with 0.1% formic acid solution; Mobile phase B: ACN.

2.1.3. Blank sample and standard spiked sample

Blank sample: The composition of blank sample was similar to the real sample but does not contain analyte or analyte less than the quantitative level of the method being applied. The used blank sample was the "Asian piglet concentrate-feed form" from the Asian company.

Standard spiked sample: Blank sample was added to a quantity of standard AMD, DCD solution with known concentrations, then mixed well and dried at a temperature of about 60 ºC in 8 hours.

2.2. Methods

2.2.1. Analytical process

The analytical procedure was based on analytical procedures (FDA LIB 4422, CLG Me.01,..) [7, 8] and studies by Shen *et al.* [9], Fu & Schreiber [10], Turowski Maciej [11], Krunve *et al.* [12, 13]: Static phase with Hilic separation column: Inertsil, 5 µm (pore size 100 Å), 4.6 × 250 mm; Flow rate: 0.3-0.5 mL/minute; pH 6-7; Mobile phase solvent (ACN): 50-70; Device used to survey results: HPLC-DAD: wavelength 210 nm; Mobile phase A: amonium acetate 10 mM; Mobile phase B: ACN.

2.2.2. Sample processing procedure

Samples of animal feed were ground and homogenized by IKA homogenizer. Weighed 1-2 ± 0.5 g of homogenized sample into a 50 mL centrifuge tube, added exactly 25 mL of ACN:H_2O extraction solution (50:50, v:v). Shaked well with Vortex (2500 rpm) in 30 minutes, then centrifuged the entire extract solution (6000 rpm, 4 °C) in 10 minutes. The resulting extract is filtered through a membrane filter (0.45 µm - 25 mm) [7, 8]. Took exactly 20 mL of solution after filtration to clean with solid phase extraction (SPE) with an extraction solvent of ACN:H_2O (50:50, v:v), SPE SCX (cation extraction) extraction column 500 mg/3 mL [7, 14, 15]. The solution obtained after cleaning by SPE will be concentrated with nitrogen gas and redissolved with 2 mL of dissolved solution. Injected then into the HPLC system.

2.2.3. Optimization of the HPLC conditions

2.2.3.1. Selection of static phase

Based on previous studies on AMD and DCD content determination methods [16-19], the water-based interaction chromatography technique (Hilic) was selected.

2.2.3.2. Investigation of the flow rate, composition of mobile phase and pH

Standard solutions with concentrations of 50 ppb (AMD) and 50 ppb (DCD) were used to investigate the effects of flow rate, mobile phase composition and pH. Investigation of the optimal condition of the mobile phase component was conducted on HPLC-DAD: 210 nm wavelength with the following parameters: Flow rate: 0.3-0.5 (low - high); Isocratic running mode; Mobile phase A (ammonium acetate 10 mM): 20-60 (low - high); Mobile phase B (ACN): 80-40 (low - high) and pH: 6-7 (low - high);

Based on the research of Srinubabu *et al.* [20], the experimental model of 2^k was selected and arranged according to Table 1.

Evaluating the influence of factors based on the result of comparing the ability of separation, retention time, peak area and analyte stability from the obtained results.

2.2.4. Investigation of SPE extraction

2.2.4.1. Selection of extraction solvent

The extraction solvent, ACN:H_2O (50:50, v:v), was selected according to the references TCVN 9048-2012 [14], FDA LIB 4422 [15], CLG - Melamine 1.0 [7].

Table 1. Experimental arrangement for investigating the optimal condition of mobile phase composition for HPLC

No.	% ACN	Flow rate	pH of mobile phase
1	50	0.3	6
2	70	0.3	6
3	50	0.5	6
4	70	0.5	6
5	50	0.3	7
6	70	0.3	7
7	50	0.5	7
8	70	0.5	7
9	60	0.4	6.5
10	60	0.4	6.5
11	60	0.4	6.5

2.2.4.2. Investigation of SPE extraction procedure

The SPE extraction procedure was proposed according to the Phenomenex instructions, including the following steps: Activated the column: added 5 mL MeOH and 5 mL H_2O, respectively; Added sample: took exactly 20 mL of sample into the column so that the flow rate of the sample through the column is 2-4 drops/10s; Dried the column: used a vacuum pump to dry the solution contained in the column; Washed impurities: added 5 mL H_2O and 5 mL 0,1% TFA, respectively; Dried the column: used a vacuum pump to dry the solution contained in the column; Recovery of analyte: Added 8-10 mL of 5%/MeOH ammonium acetate eluent to the column, dripping speed of 2-4 drops/10 seconds. Because the amount of elution solvent will determine the recovery of elution solvent volumes at 3 levels of 6 mL, 8 mL and 10 mL, with the analyte (AMD and DCD) content at two levels of 5 ppb and 120 ppb will be surveyed; Dried the column: used a vacuum pump to dry the solution contained in the column; All eluents were evaporated in a boiling pot (45-55 °C) combined with blowing nitrogen, then redissolved with 1 mL 10 mM ammonium acetate - ACN (50-50) and then, injected into the HPLC system.

The results of the survey were evaluated based on the comparison of the results of the analyte content obtained and the theoretical concentration.

2.2.5. Appraisal method

2.2.5.1. Specificity

Following the guidelines of the European Analytical Society, the HPLC-DAD is acceptable to confirm a positive sample [21].

2.2.5.2. Investigation of LOD, LOQ

LOD detection limit (qualitative limit) is determined according to the method evaluation guidelines of the National Institute for Food Control [22], LOQ was determined by the following formula:

$$LOQ = 3 - 10 \times C_{LOD} \quad (1)$$

LOD and LOQ of the device were determined as follows: Standard solution with concentration of about 100 ppb or less, injected this solution into DAD; Diluted the concentration of the above solution until a signal of the peak that met the signal / noise requirements (S/N) ≥ 3 - 10 (for substances classified as toxin) and S/N ≥ 3 (for substances not classified as toxic), according to SANCO/825/00 rev.8.1 16/11/2010 [23]; Calculated to determine the LOD, LOQ of the device.

The LOD and LOQ of the method were as follows: From the LOD of the device, the amount of standard solution added to the blank sample was calculated so that 1 g of the standard spiked sample contained was equal to the LOD of the device; Homogenized the standard spiked sample according to ISO Guide 35:2017 [24] and EC 657/2002 [25]; Processed sample and injected into the chromatographic system to determine S/N; Increased or decreased the amount of standard solution added to the blank sample according to the results of S/N until it complied with the requirements for the determination of LOD and LOQ according to EC 657/2002 [25].

2.2.5.3. Investigating linear intervals

Standard solution with concentration ranged from 1 - 1,000 ppb, then injected into HPLC system with injection procedure from low to high concentration solution. The standard solution was treated the same as the sample solution. The calibration curve was investigated on the DAD at 210 nm.

2.2.5.4. Investigating repeatability and recovery

Based on the guidance of ISO Guide 35:2017 [24] and EC 657/2002 [25], standard spiked sample with known concentration of AMD and DCD standard solution created to investigate the repeatability and recovery. The concentration of standard solution added to animal feed samples is shown in Table 2 and Table 3.

Table 2. Concentration of standard solution added to sample to investigate repeatability and recovery of HPLC - DAD

Substances	Concentration (ppm)		
	Sample 1	Sample 2	Sample 3
AMD	10	50	100
DCD	10	500	100

Table 3. Concentration added to sample for SPE extraction volume survey

Substances	Concentration (ppb)	
	Sample 4 (SPE)	Sample 5 (SPE)
AMD	5	120
DCD	5	120

The standard spiked sample was calibrated to determine repeatability at a concentration of 150 ppb of AMD and DCD (3 times). The recovery, the repeatability and the accuracy of the method were evaluated based on a comparison of the obtained results and the theoretical concentration of the analyte. The evaluation was based on the guidance of AOAC Appendix F [26, 27].

3. RESULTS AND DISCUSSION

3.1. Optimized the HPLC mobile phase conditions

Based on previous studies [12, 13], three factors influenced the analysis process were mobile phase solvent (ACN) (50-70), flow rate (TDD) (0.3-0.5 mL/minute) and pH mobile phase (pH 6-7) selected.

Table 4. Results of optimization of HPLC mobile phase conditions

No.	% ACN	Flow rate (TDD)	pH mobile phase	Retention time		Peak area	
				AMD	DCD	AMD	DCD
1	50	0.3	6	8.90	10.3	7,902.09	16,867.43
2	70	0.3	6	9.34	10.4	8,644.51	18,938.03
3	50	0.5	6	5.29	6.21	4,770.924	10,227.53
4	70	0.5	6	5.10	6.18	5,133.33	10,323.73
5	50	0.3	7	8.84	10.4	7,968.61	11,273.2
6	70	0.3	7	9.37	10.4	8,654.31	17,328.3
7	50	0.5	7	5.285	6.20	4,810.89	10,246.23
8	70	0.5	7	5.558	6.20	5,081.43	10,638.37
9	60	0.4	6.5	6.63	7.71	6,837.4	14,879.7
10	60	0.4	6.5	6.631	7.70	5,902.57	13,886.85
11	60	0.4	6.5	6.631	7.70	6,905.8	14,922.05

The results in Table 4 showed that the pH mobile phase varied from 6-7, the peak areas of AMD and DCD were not significantly changed. Increasing the amount of ACN from 50% to 70%, the substances were in the column longer. At the high flow rate, the substances output faster, the peak parameters were better than at the low flow rate. According to the survey, the results were stable at a flow rate of 0.4 mL/minute and 60% ACN.

The data in Figure 1 and Table 5 showed that the flow rate of the mobile phase significantly affected to the AMD analysis by HPLC. Similarly, results of DCD were shown in Figure 2 and Table 5.

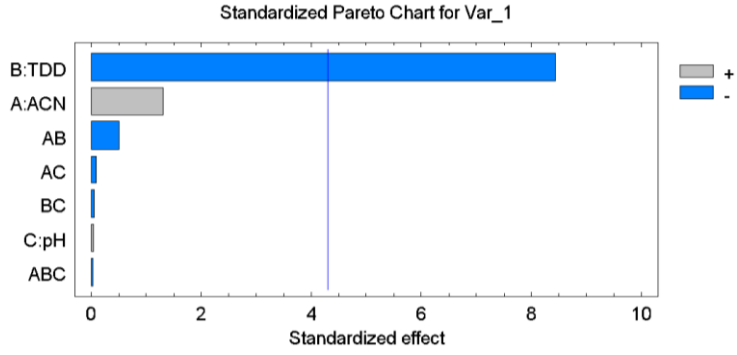

Figure 1. Pareto frequency chart of AMD effects

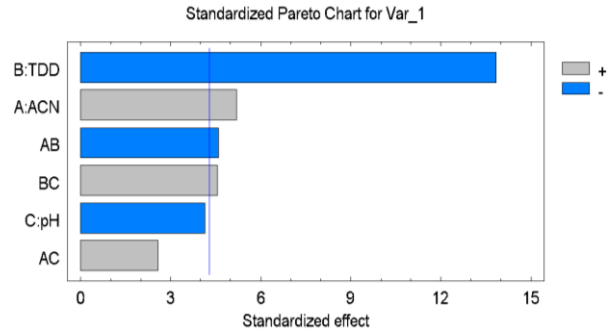

Figure 2. Pareto frequency chart of DCD effects

Table 5. Variant values of AMD and DCD

Substances	DCD		AMD	
Source	F-Ratio	P-Value	F-Ratio	P-Value
A: % ACN	27.00	0.0351	1.69	0.3232
B: flow rate	191.99	0.0052	71.15	0.0138
C: pH	17.18	0.0536	0.00	0.9713
AB	21.22	0.0440	0.25	0.6657
AC	6.67	0.1229	0.01	0.9339
BC	20.67	0.0451	0.00	0.9607
ABC			0.00	0.9843
Lack-of-fit	8.11	0.1098	0.04	0.8667
R-squared (%)	93.9871		97.2903	
R-squared (adjusted for Degree of freedom) (%)	84.9678		90.9678	
Standard Error of Est,	586.137		560.514	
Mean absolute error	599.492		127.002	

Factors with a P-Value < 0.05 significantly affected the analysis results (peak area). Accordingly, the variant values in Table 5 showed: For AMD: flow rate of the mobile phase (P-Value = 0.0138) significantly effected on the analysis results; For DCD: % ACN (P-Value = 0,0351) and the mobile phase flow rate (P-Value = 0.052) have a significant influence on the analysis results. Lack-of-fit parameters with P-Value = 0.1098 (> 0.05) and R-squared > 90% at $\alpha = 95\%$ showed that 2^k model was suitable for experimental design.

For AMD, the prediction equation:

Area = 8,600.55 + 66,8136*%ACN – 12,746.3*TDD + 116.262*pH – 42.2666*%ACN*TDD – 0.198763*%ACN*pH + 306.751*TDD*pH - 8.78914*%ACN*TDD*pH (2)

For DCD, the prediction equation:

Area = 97,598.0 – 206.027*%ACN – 93,914.8*TDD – 15,675.6*pH - 954.671*%ACN*TDD + 107.011*%ACN*pH + 18,843.3*TDD*pH (3)

Where:

TDD: Flow rate of mobile phase (mL/minute)

pH: pH of mobile phase

The results in Table 6 showed the optimization of parameters of HPLC technique for AMD and DCD analysis.

Table 6. Results of optimization of HPLC specifications for AMD and DCD

Substances			DCD	AMD
Factors	Low	High	Optimal values	Optimal values
ACN	50.0	70.0	60.7355	61.3798
Flow rate	0.3	0.5	0.387795	0.378455
pH	6.0	7.0	6.51702	6.51686

To simply the installation of device, the following parameters were proposed: %ACN: 60%; Flow rate: 0.4 mL/minute: pH: 6.5. Ammonium acetate 10 mM - ACN (50:50, v:v) was chosen as the solvent, pH 6.5. AMD standard solutions (50 ppm) and DCD (50 ppm) were used to verify optimum results of HPLC. The verification results were shown in Table 7.

Table 7. Compared results after optimization

Values	Peak area of DCD			Peak area of AMD		
The predicted optimal value	5,905.82			14,922.05		
The actual optimal value	5,878.58	5,778.81	5,818.74	14,915.4	13,254.6	14,275.8
T_{stat}	2.775					
$T_{critical}$ ($\alpha = 0.05$)	4.302					

The statistical results showed that there was almost no difference ($t_{critical} > t_{stat}$; $\alpha = 0.05$) between the actual value and the predicted optimal value. Therefore, the selected parameters can be applied in real sample.

3.2. Investigation of sample cleaning procedure by SPE

Table 8 and Table 9 showed the survey results of AMD and DCD contents at different elution solvent volumes.

Table 8. Survey results of AMD content at different elution solvent volumes

AMD (5 ppb)				AMD (120 ppb)		
The volume of eluting solvent	6 mL	8 mL	10 mL	6 mL	8 mL	10 mL
AMD content detected (ppb)	2.0559	4.1396	3.8067	101.0632	113.3745	121.5610
	1.6520	4.4079	4.3899	103.5953	112.0980	116.3981
	2.0453	4.3627	4.3631	102.5272	118.2784	121.3872

Table 9. Survey results of DCD content at different elution solvent volumes

DCD 5 ppb				DCD 120 ppb		
The volume of eluting solvent	6 mL	8 mL	10 mL	6 mL	8 mL	10 mL
DCD content detected (ppb)	2.3425	4.5084	5.5057	82.2926	119.6097	129.3401
	2.2589	4.7848	4.0333	107.1688	102.9985	107.8945
	3.0764	4.8819	5.5259	71.6622	103.1706	108.7572

Table 10. Results of the recovery survey

The volume of eluting solvent	AMD (5 ppb)			AMD (120 ppb)		
	6 mL	8 mL	10 mL	6 mL	8 mL	10 mL
Recovery	38.35 %	87.31 %	78.66 %	85.3 %	95.5 %	78.7 %
	DCD (5 ppb)			DCD (120 ppb)		
Recovery	50.95 %	95.2 %	92.97 %	72.53%	90.5 %	96.11 %

The analytical results in Table 10 showed that the amount of elution solvent was 6 mL, the recovery efficiency varied between 38% and 85%. At the volumes of solvent respectively 8 mL and 10 mL, the results showed that there was not significant difference. Therefore, the recommended elution volume was 8 mL.

3.3. Validation of analytical methods

3.3.1. Specificity / selection

The results of determination of AMD and DCD content of animal feed samples "Concentrated feed for pigs from training - finishing" from Asian company were determined that AMD and DCD content were negative.

3.3.2. The limit of detection (LOD) and the limit of quantitation (LOQ)

The results of the limit of detection (LOD) and the limit of quantitation (LOQ) for AMD and DCD were presented in Table 11.

Table 11. LOD, LOQ survey results for LC-DAD

Substances		Concentration (ppm)	Ratio S/N	Number of injections
AMD	LOD	0.05	5.01	7
	LOQ	0.1	14.18	
DCD	LOD	0.05	3.25	
	LOQ	0.1	10.16	

LOD and LOQ of AMD and DCD of LC-DAD method were 0.05 and 0.1 ppm, respectively. These were similar to these of the previous published with another methods [2, 3].

3.3.3. Results of investigating linear intervals

Results of investigating linear intervals for HPLC-DAD in Table 12 showed that AMD and DCD have linear range of 0.1-50 ppm.

Table 12. Investigating linear intervals AMD and DCD

Substances	Concentration of standard solution (ppm)	Correlation coefficients
DCD	0.1	0.9998
	0.5	
	1	
	5.0	
	10	
	50	
AMD	0.1	0.9999
	0.5	
	1	
	5.0	
	10	
	50	

3.3.4. Accuracy, repeatability and recovery

Table 13 and Table 14 showed the results of the investigation of repeatability and recovery of standard spiked animal feed samples. According to the AOAC (app-f) documentation of the validity of the method, the results were on completely responsive. Recoveries ranged from 92.4% to 98.2% that were relatively higher than those in previous studies (84.6%-96.8% - DCD analysis with HPLC-UV) [3], (61.4%-117.2% - AMD analysis with GC-MS/MS) [4].

Table 13. Concentration added to the sample to investigate recovery, repeatability

Substances	Concentration (ppm)		
	Sample 1	Sample 2	Sample 3
AMD	1	5	10
DCD	1	5	10

Table 14. Accuracy, repeatability and recovery

Substances	Level spiked (ppm)	Assay concentration (ppm)				Results		AOAC requirements	
		Sample 1	Sample 2	Sample 3	Average	Recovery %	RSD (%)	Recovery %	RSD (%)
DCD	1	0.92	0.96	0.95	0.94	94.33	5.43	60 - 115	< 21
	5	4.87	4.93	4.94	4.91	98.2	0.77		
	10	9.21	9.29	9.23	9.24	92.4	0.45	80 – 110	< 15
AMD	1	0.93	0.89	0.97	0.93	93.0	4.30	60 - 115	< 21
	5	4.83	4.81	4.84	4.83	96.5	0.23		
	10	9.11	9.35	9.26	92.4	92.4	1.31	80 – 110	< 15

4. CONCLUSION

The study has established a process to identify AMD and DCD according to the sample preparation process combined with SPE technique and quantification by HPLC-DAD. This proposed analytical procedure included sample pretreatment using SPE SCX (cation extraction) extraction column 500 mg/3 mL with an extraction solvent of ACN:H_2O (50:50, v:v) at elution volume of 8 mL, followed by pre-concentration using nitrogen gas and analysis with HPLC-DAD. Static phase with Hilic separation column: Inertsil, 5 µm (pore size 100 Å), 4.6 × 250 mm; Flow rate: 0.4 mL/minute; pH 6.5; Mobile phase solvent (ACN): 60%; Wavelength 210 nm. The method is capable of quantifying AMD and DCD content in real animal feed samples with quantitative limits of 0.1-1.000 ppm, respectively, for both substances, meeting the requirements of AOAC for method validation. The method has been effectively applied at the National Centre for Veterinary Drugs and Bio-Products Control No.2 to control AMD and DCD content in animal feed sample background. The method satisfies the requirements of sensitivity, repeatability, linear intervals to be practical in application. In the future, this method may be proposed to further research and develop into a Vietnamese Standard. However, more research is needed to assess the factors that influence the analysis process from more feed sample bases to expand the applicability of the method to serve the needs of analysis as well as contribute to the analysis, support the quality management of animal feed, contribute to ensuring food safety and hygiene.

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TÓM TẮT

NGHIÊN CỨU XÂY DỰNG QUY TRÌNH XÁC ĐỊNH HÀM LƯỢNG AMMELIDE (AMD) VÀ DICYANDIAMIDE (DCD) TRONG THỨC ĂN CHĂN NUÔI BẰNG SẮC KÝ LỎNG HIỆU NĂNG CAO ĐẦU DÒ DAD (HPLC-DAD)

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Mục đích của nghiên cứu này là xây dựng quy trình xác định hàm lượng các các chất tăng đạm giả Ammelide (AMD) và Dicyandiamide (DCD) trong thức ăn chăn nuôi (TACN), theo quy trình chuẩn bị mẫu kết hợp với quá trình làm sạch bằng kỹ thuật chiết pha rắn (SPE) và định lượng bằng phương pháp phân tích sắc ký lỏng hiệu năng cao đầu dò DAD (HPLC-DAD). Hàm lượng AMD và DCD trong mẫu TACN được xác định với giới hạn định lượng LOD và LOQ của AMD, DCD có nồng độ là 0.05 và 0.1 ppm, đáp ứng được yêu cầu của AOAC (Association of Official Analytical Chemists) về thẩm định phương pháp và về độ nhạy, độ lặp lại, khoảng tuyến tính để có thể ứng dụng vào thực tế. Quy trình xác định đã được áp dụng hiệu quả tại Trung tâm Kiểm nghiệm Thuốc Thú y TW II để kiểm soát hàm lượng AMD và DCD trong nền mẫu TACN thực tế.

Từ khóa: Chất tăng đạm giả, AMD, DCD, thức ăn chăn nuôi, SPE, HPLC-DAD.