

Determination of choline chloride content



Chinese scientists Chang Biying, Song Rong, Ding Yong Sheng and Liew Chee Yong* discuss the adulteration of choline chloride products which has required the development of a new method of assay.



Choline chloride or trimethyl (2-hydroxyethyl) ammonium chloride is an essential feed ingredient for optimum animal growth. Choline is classified as a vitamin and belongs to the group of water soluble B-vitamins. Choline has three essential metabolic functions:

- i) It is a key constituent of phosphatidylcholine and phosphocholine and represents about 70 – 80 % of total phospholipids in the body.
- ii) It plays a vital role in fat transport and metabolism, thus preventing abnormal fat accumulation in the liver. It is also important in the establishment and maintenance of cell membrane structure.
- iii) Choline is a precursor to acetylcholine in impulse transmission of all nerves.
- iv) Choline is a source of free methyl groups, essential for numerous biological functions.

Natural feed components contain certain quantities of choline but cannot satisfy the animal needs for rapid growth. Hence, choline chloride products have become the most direct and economic way of choline supplementation.

In recent years, fraudulence in choline chloride products has affected the feed industry. One reason for this is the low selectivity of the current national

method (HG2941-1999) for choline determination on feed grade choline chloride products in China. Unlawful parties add cheap inorganic chloride, organic hydrochloride or other chloride substances into the final choline chloride products that interfere with the analysis and produce misleading results.

The China National Centre for Quality Supervision and Test of Feed and The Ministry of Agriculture accepted the need for resetting a new national standard to replace the current standard for choline determination. For feed grade products, the most commonly used methods are AgNO₃ titration (commonly known as Volhard titration), the perchloric acid method (Silver titration), the sodium tetraphenylborate method, and the Reinecke Salt Colour Comparison method.

The Volhard and perchloric acid methods are of poor selectivity and are not specific for choline. The sodium tetraphenylborate acid method is of higher selectivity but the analysis result can be influenced by the presence of some cations in the product i.e. K⁺ and NH₄⁺.

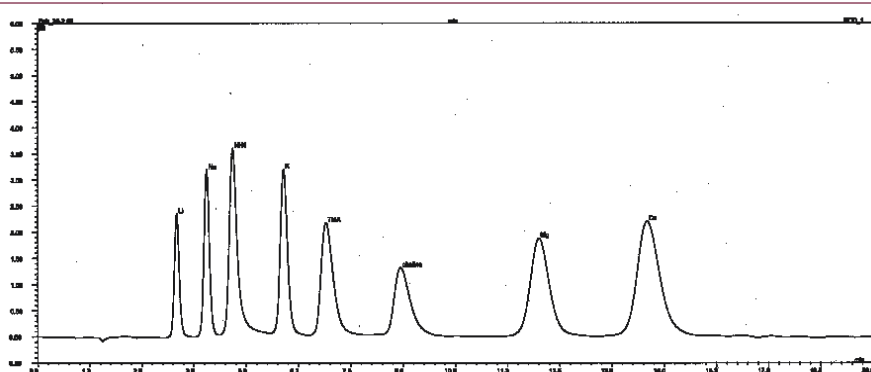
The Reinecke Salt method is highly selective for choline chloride. The USA National Feed Ingredients Association (NFIA) has set the Reinecke Colour Comparison method as the



Table 1: Accuracy test.

Carrier	Choline chloride inclusion rate (%)	No of tests	Result (% choline chloride)	Recovery (%)	CV (%)
Liquid	75	3	74.57	99.40	0.26
	70	3	9.65	99.50	
Corn cob	50	5	50.42	100.8	0.74
Rice husk	60	4	59.58	99.30	0.62
Bran	50	5	50.02	100	1.20
Anhydrous silicic acid	50	5	50.44	100.9	0.74

Figure 1: Separation result of the six commonly found cations in choline chloride and trimethylamines-ammonium using cation exchange chromatography.



Ion chromatography

Column: 4mm x 250 mm Carboxylic/Phosphoric acid cation exchange resin
 Granularity: 8.5 µm
 Column temperature: 30°C
 Flow: 17.5 mmol/L sulfuric acid solution
 Flow speed: 1 mL/min
 Detector: Electric conductance detector with recycled mode function suppressor

◁ standard for choline determination in choline chloride vegetable and premix products.

In China, the Reinecke salt colour comparison method (GB/T 17481-1998) is also the national standard for checking choline content in the feed premix. However, due to high variation, this method is only fit for checking low choline content i.e. to ppm level. For higher concentrations (50 - 75%), this method is unsatisfactory.

We have developed a method, the Reinecke Salt Gravimetric Method for regular, basic laboratory quality checking of choline chloride contents. To meet new challenges in the future market, we suggest the use of the more scientific and high precision chromatography method as the final confirmation method. We have conducted a series of tests to confirm the precision, reliability and consistency of these methods

including the use of real and fraudulent products.

Reinecke Salt Gravimetric Method

Changes from the standard Reinecke Colour Comparison method are slight, and involve water extraction temperature (fixed at 70 ± 3°C.), removal of possible contaminants such as trimethylamines and some primary and secondary amines, NaOH dose (3 ml of NaOH), boiling time (a boiling time of 5 min), and pH conditions (a pH of 5-6). Other parameters in these experiments remain the same as before. Full details of the method can be obtained from the senior author.

Ion chromatography

In order to obtain good separation from impurities and possible adulterants, cation exchange chromatography with conductivity

detector is recommended, with conditions as per table.

Separation result

Figure 1 shows separation result of the six commonly found cations in choline chloride and trimethylamines-ammonium using cation exchange chromatography.

Accuracy of the method

For the accuracy test, we firstly incorporated different concentrations of choline chloride (75% (50%) and a fixed amount of TMA-HCL into the liquid formulation and the corn cob carrier. The recovery of choline was determined and the variations calculated. The same test procedures were repeated for different choline chloride carriers i.e. rice husk, rice bran and anhydrous silicic acid. See table 1.

Results indicate that irrespective of types of carriers or in the original formulation, the Reinecke method demonstrates a high level of accuracy. With normal concentrations of 50 - 75 %, the method showed excellent average recovery (100.1%) with a test error of 0.71%.

Laboratory comparison test

We performed several tests to reconfirm the validity of Reinecke salt gravimetric method. See table 2.

Results again clearly demonstrated that the Reinecke Salt Gravimetric Method is highly accurate (average recovery of about 100%) and precise (CV value of about 0.44%)

Comparison

A comparison was conducted using the above two methods to determine the choline chloride content of a 50 % choline chloride ▷

Table 2: Laboratory test comparison on existing choline chloride products in the market using the Reinecke Salt Gravimetric Method.

Carrier	Choline chloride inclusion rate (%)	No of test	Test results (% choline chloride)		Recovery(%)		CV (%)	
			Centre	Feed lab	Centre	Feed lab	Centre	Feed lab
Rice husk	60	3	59.64	59.94	99.4	99.9	0.95	0.31
Wheat	60	4	60.14	60.27	100.2	100.4	0.34	0.12
Anhydrous silicic acid	50	4	49.89	50.00	99.8	100.0	0.57	0.34

◁ feed grade product. See table 3.

The Reinecke Salt Gravimetric method is of higher accuracy and precision as compared to the Reinecke Salt Colour Comparison Method and is therefore more suitable for the determination of choline chloride products.

Recovery and Precision

We used different carrier materials i.e. pure corn cob, rice husk, wheat and silica and incorporated a fixed quantity of choline chloride. We then made an extraction, filtration, dilution and analyzed using ion chromatography method. See table 4.

The results demonstrate the accuracy and precision of the ion chromatography method. Average recovery of the different carriers is about 100.7 % with an average CV value of 0.88%.

Comparison

For test comparison between the Reinecke Salt Gravimetric Method, the Ion Chromatography Method and the Perchloric Acid Titration Method, we collected 12 choline chloride samples available in the market. See table 5.

For good quality choline products, the variation of results between these three methods of analysis is very small (less than 3%). These three methods were able to fulfil the basic requirements on accuracy for determination of choline content. For fraudulent products (samples 8-12), both Reinecke Salt Gravimetric and Ion Chromatography methods remained accurate and able to distinguish the real from the fraudulent. However, the Perchloric Acid Titration method was not able to do so.

Analysis results from Europe

Based on the proposed Reinecke Salt Gravimetric method, UCB's laboratory in Belgium conducted an analysis on six choline chloride samples collected from the market. See table 6.

These results further confirm the high selectivity and accuracy of the Reinecke Salt Gravimetric and the Ion Chromatography methods. These two methods are satisfactory to distinguish any possible product fraudulence.

Table 3: Comparison between Reinecke Salt Gravimetric method and Reinecke Colour Method.

Replication	Reinecke Salt Gravimetric (% Choline chloride)	Reinecke Salt Colour Comparison (% Choline chloride)
1	50.26	45.77
2	50.23	51.41
3	51.09	48.40
4	50.23	51.78
5	50.29	52.96
Average	50.42	50.06
Average Recovery (%)	100.8	100.1
CV (%)	0.74	5.85

Table 4: The recovery of choline in different carriers using Ion Chromatography Method.

Carrier	Choline chloride inclusion rate (g)	Replication	Analyzed content (g choline chloride)	Recovery (%)	CV (%)
Corn cob	0.246	5	0.2436	99.04	0.55
Rice Husk	0.25	6	0.2503	100.1	0.92
Wheat	0.25	5	0.2528	101.1	1.25
Silica	0.20	5	0.2048	102.4	0.81

Table 5: Comparison results of the different methods.

Sample	Choline chloride content (%)		
	Reinecke salt	Ion chromatography	Perchloric acid titration
1	61.37	62.19	61.92
2	50.88	50.67	50.83
3	50.84	50.79	50.06
4	50.81	50.52	-
5	50.66	49.25	52.28
6	45.90	46.16	52.63
7	46.12	44.77	49.64
8	11.69	11.84	50.05
9	9.00	8.88	55.26
10	8.74	8.37	52.98
11	0	0	67.71
12	0	0	23.6

Conclusion

For the estimation of the choline chloride content in a commercial sample choline chloride, the Volhard method (also known as Mohr method) is a reliable and fast method to estimate the content. But only if the product is not (fraudulent) mixed with other salts, like sodium chloride, calcium chloride and the like. So a guarantee of the supplier that the chloride content has a 1 : 1 relationship with the choline

chloride content should be given.

If there are doubts about the reliability of the supplier, an additional test can be done to assure the product contains the indicated amount of choline chloride. In that case, we suggest the use of the two most suitable method of analysis for the determination of choline chloride content in choline chloride feed grade products.

The Reinecke salt gravimetric method is proposed as the regular

Table 6: Analysis results from Europe.

Sample	Choline chloride content (%)			Other cations ((g/g)					
	Volhardt	Reinecke	Ion chromatography	Na	NH ₄ ⁺	K	TMA	Ca	Mg
1	52.20	51.30	50.80	50 (0.03)	—	1760 (0.6)	370 (0.06)	600 (0.4)	180 (0.21)
2	49.20	46.90	44.40	—	—	—	17135 (4.0)	—	285 (0.3)
3	52.20	16.90	13.40	5400 (3.3)	28800 (22.3)	2700 (1.0)	22000 (5.1)	650 (0.4)	147 (0.2)
4	56.80	5.80	6.00	48000 (29.1)	100 (0.1)	2800 (1.0)	—	17000 (11.9)	6500 (7.5)
5	54.70	6.90	5.80	16900 (10.3)	—	—	7500 (1.7)	73000 (51.1)	700 (0.8)
6	58.40	24.50	24.00	41500 (25.2)	200 (0.2)	—	370 (0.08)	5300 (3.7)	—

◁ method and ion chromatography method as the final confirmation method. Both methods exhibit high levels of selectivity and can accurately and precisely distinguish any product adulteration.

Moreover, the operation procedures are simple and easy to conduct. This is of especially true for the Reinecke salt gravimetric method where no sophisticated apparatus in the lab is needed, and

yet it meets the needs of the choline chloride users and quality control people in the laboratory.

Therefore, the Reinecke salt gravimetric method should be the method for choline chloride determination if they are doubt about the quality of the product. The ion chromatography method, is more specific for the detection of choline in choline chloride products. Ion chromatography is able to meet

whatsoever new challenges that may arise in the near future and should be treated as the final confirmation method. ■

** Prof Chang Biying and Song Rong, China National Centre for Quality Supervision & Test of Feed; Ding Yong Sheng, China Science Institute Biological & Environmental Research Department, and Liew Chee Yong, UCB Asia Pacific. Contact Prof Chang on e-mail chbiying@hotmail.com or Mr Liew on cyliew@ucb-group.com*