DETERMINATION OF VITAMIN A AND K_3 IN VITAMIN PREMIXES AND MIXED PREMIXES

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Determination of vitamin A

The analytical procedure to be used for the determination of vitamin A in premixes depends on what type of premix (vitamin premix or mixed premix) is being analyzed and what antioxidant (EMQ or BHT) is contained in the premix [1].

For vitamin premixes, the procedure developed by us consists of the following operations:

The premix sample is first hydrolyzed with an alcoholic KOH solution. Since vitamin A is sensitive to light and oxidizing agents, hydrolysis is carried out in a dark flask, under a nitrogen stream and in the presence of hydroquinone as antioxidant and Complexon-III for complexation of the metal salts. Hydrolysis serves the purpose to dissolve the gelatine capsules in which vitamin A ester is introduced into the premix (Mikrovit-A) and to convert the ester into the corresponding alcohol. However, other active agents contained in the premix, as well as various constituents of the carrier (bran) will also hydrolyze.

After hydrolysis, the mixture is filtered and the solution is extracted with petroleum ether in order to separate fat-soluble substances from a great number of compounds converted by hydrolysis into water-soluble potassium salts. The extract is washed with alkali and subsequently with water, to remove final traces of saponaceous compounds.

However, the petroleum ether extract still contains interfering substances. These must be removed by column chromatography, using basic aluminium oxide as adsorbent. The oxide is activated by heating and the required activity is adjusted by subsequent sorption of water. A selective separation of vitamin A from accompanying substances that would interfere with the determination of the vitamin is achieved by appropriate water content of the aluminium oxide and adequate polarity of the successive eluents.

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An aliquot part of the petroleum ether extract is evaporated to a smaller volume and introduced on the column. The eluents are mixtures of petroleum ether with diethyl ether, polarity being a function of the diethyl ether content. Starting with the least polar eluent, impurities are eluted with successively more and more polar eluents, and finally vitamin A is eluted with petroleum ether containing 20% diethyl ether. In the course of these operations, the efficiency of elution and separation are checked by spectrophotometry.

Table 1

Determination of the vitamin A content in vitamin premixes

Sample	Vitamin A content I. U./g		Difference, %
	Added	Found	
Laying Hen Feed No. IV	1600	1580	−1.2
Porkling Feed No. VII/b	800	768	−4.0
Breeder Bull Feed No. XI	500	432	−13.5

Vitamin A purified by the described procedure is then determined using its colour reaction with glycerine dichlorohydrine. An aliquot part of the eluate containing vitamin A is evaporated, dissolved in chloroform and mixed in a 1 : 1 ratio with glycerine dichlorohydrine activated with acetyl chloride. The colour of the solution changes from blue to mauvish pink. Maximum light absorption is measured at 560 nm. A calibration curve prepared with standard vitamin A solutions is used for evaluating the results.

The described method has been applied to a great number of premixes. Among these, we selected three premixes that greatly differ both in composition and vitamin A content to demonstrate how different constituents of vitamin premixes affect the determination of vitamin A.

The results presented in Table 1 for each of these three premixes are mean values of five parallel measurements. Their standard deviation was ±3—4%. The maximum difference between the experimental results and the vitamin A amounts that were originally added to the premix did not exceed —10%. The relatively high negative error found with Breeder Bull Feed No. XI may be explained by the fact that this premix does not contain any antioxidant.

Later, vitamin premixes have been completed with further constituents (e.g. zinc bacithracine, ardinone, dl-methionine), and the grades of some constituents have been changed (e.g. EMQ manufactured by Monsanto has been replaced by EMQ manufactured by Material KTSz). These changes made it necessary to modify our analytical procedure. We succeeded in achieving

satisfactory purification of vitamin A by increasing the activity of the adsorbent and by complementing successive elution stages of the impurities with a more polar eluent.

The results obtained are presented in Table 2.

Table 2

Determination of the vitamin A content in vitamin premixes completed with further constituents

Sample	Vitamin A content I. U./g		Difference, %
	Added	Found	
Poultry Raising No. II	1200	1208	+0.7
		1260	+5.0
Laying Hen Feed No. IV	1600	1560	−2.5
		1670	+4.4
Turkey Starter	1600	1666	+4.1
		1720	+7.5

Mikrovit-A: Nominal value 500,000 I. U./g vitamin A; Found value 555,000 I. U./g vitamin A

Various procedures were tested for determining vitamin A in mixed premixes. We found that the most satisfactory results were obtained by carrying out alkaline hydrolysis in the presence of substantially higher amounts of hydroquinone and of large excesses of sodium sulphide. The role of the latter is to form very poorly soluble sulphides with the metal salts, and thus inactivate these salts which would otherwise enhance the decomposition of vitamin A. To eliminate final traces of interfering metal salts, an acid wash was applied after the alkaline wash following extraction with petroleum ether. Chromatographic separation and determination of vitamin A were then carried out in conformity with the modified procedure as described above.

The analytical results obtained with some mixed premixes are presented in Table 3.

Some of these premixes are originally vitamin premixes (Poultry Raising No. II, Laying Hen Feed No. IV, Turkey Starter) to which mineral constituents in amounts corresponding to the average mineral composition of mixed premixes were added. The samples thus obtained were analyzed as mixed premixes. The sample Mixed Premix No. XV differs from the other analyzed samples by containing the antioxidant BHT (butylhydroxytoluene)

instead of EMQ (ethoxymethylquinoline). A great negative error was found with this premix.

The problem appeared similar to the problem encountered when vitamin premixes with changed compositions were analyzed. We therefore studied the

Table 3

Determination of the vitamin A content in mixed premixes

Sample	Vitamin A content I.U/g		Difference, %
	Added	Found	
Poultry Raising No. II	1200	1154	−3.8
(made up to mixed)		1230	+2.5
Laying Hen Feed No. IV	1600	1380	−13.7
(made up to mixed)		1445	−9.7
Turkey Starter	1600	1545	−3.4
(made up to mixed)		1523	−4.8
Bari Laying Hen Mixed Premix	266.6	242.0	−9.3
		235.6	−11.6
Mixed Premix No. XV	1000	766	−23.0
(with BHT)		725	−27.5

Mikrovit-A: Nominal value 500,000 I. U./g vitamin A; Found value 555,000 I. U./g vitamin A

effect of BHT on the individual stages of the analytical procedure. After appropriate preliminary experiments made with stock solutions, we modified the conditions of chromatographic purification, viz. activity of the adsorbent and polarity of the successive eluents. In this way satisfactory selectivity was obtained. The modified procedure allows vitamin A determination in mixed premixes containing BHT with an error below −15%, standard deviation not exceeding ±5%.

Determination of vitamin K_3

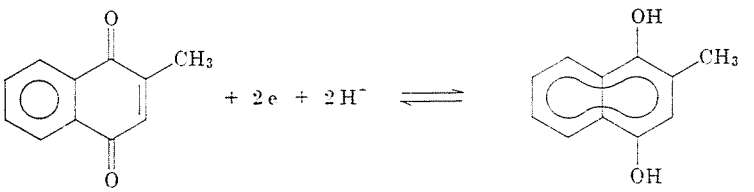
We developed a polarographic and a spectrophotometric procedure for determining vitamin K_3 in premixes [1].

The preliminary operations are as follows: the weighed sample (its weight depending on the vitamin K_3 content of the premix) is spread in dry state on a glass filter. Since vitamin K_3 is contained in the premix in the form of menadion-bisulphite-sodium, a compound readily soluble in water, it is

extracted from the sample by adding small portions of water heated to ~40 °C. The aqueous filtrate is made alkaline by adding NaOH, and menadion released from the bisulphite compound is extracted with petroleum ether. The extract is washed with distilled water till it is free of alkali. An aliquot part is evaporated to dryness and the residue is dissolved in ethanol.

Polarographic determination

The solution of menadion in ethanol is polarographed in the presence of an ethanolic ammonium chloride—ammonium hydroxide buffer solution serving as supporting electrolyte. To eliminate oxygen a nitrogen stream is passed through the solution. Menadion gives a two-electron cathodic reduction wave (I). This is measured with a large-surface mercury anode in the 0 . . . —0.55 V potential range. When the above supporting electrolite is used, the half-wave potential of menadion is $E_{1/2} = -0.24$ V.



(I)

The measured data are evaluated by standard addition.

Fig. 1 shows a polarogram of menadion, taken under the above-listed conditions with a Radiometer polarograph PO—4.

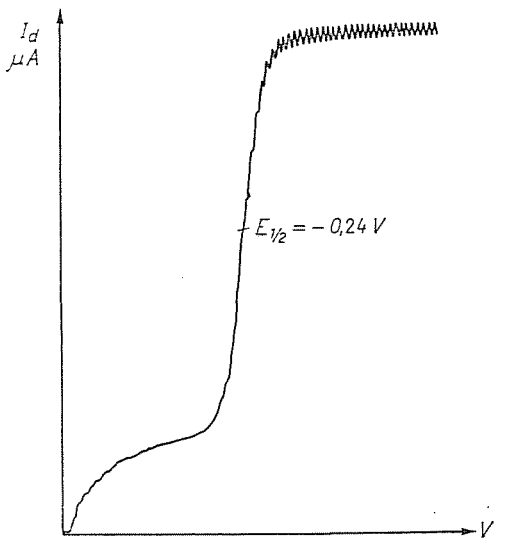


Fig. 1. Polarogram of vitamin K_3. Supporting electrolyte: ammonium chloride — ammonium hydroxide in ethanol

Spectrophotometric determination

This procedure is based on the colour reaction with 2,4-dinitrophenylhydrazine. Hydrochloric 2,4-dinitrophenylhydrazine is added from a pipette to the ethanolic menadion solution and the mixture is heated to 60 °C in a water bath for 25 minutes. After cooling, the solution is made alkaline with ammonia and diluted with ethanol. The light absorption of the green solution containing the dinitrophenylhydrazone of menadion is measured at 635 nm against pure ethanol. The results are evaluated by means of a calibration curve prepared with standard menadion solutions. The menadion content of the standard solutions is checked spectrophotometrically in the UV range.

Both the polarographic and spectrophotometric determinations of menadion are suited for analyzing premix samples containing 1—2 mg/g menadion bisulphite-sodium.

The procedures were checked by adding 1.0 and 2.0 mg/g menadion-bisulphite-sodium, resp., to a premix model substance not containing vitamin K_3. The vitamin K_3 values obtained with both procedures deviated by maximum —10% from the added amount.

Summary

The A vitamin content of vitamin premixes was determined by colour reaction with glycerine dichlorohydrine. After hydrolysis, the mixture was extracted with petroleum ether and purified with aluminium oxide by column chromatography. The method was suitable for determining the A vitamin content of mixed premixes by varying adequately the experimental conditions of hydrolysis and column chromatographic purification.

Two methods have been elaborated to determine the K_3 vitamin content of premixes.

* K_3-bisulphite-sodium is extracted from the premix with water and by alkalising this extract K_3-vitamin is formed, which is determined by polarography or by colour reaction with 2,4-dinitro-phenyl-hydrazine spectrophotometrically.

References

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