

VITAMIN C,
DERIVATIVES
AND
IMPURITIES
OF CITREX

8.0

TNO Chemistry*Memorandum*

Nederlandse Organisatie voor
toegepast-natuurwetenschappelijk
onderzoek / Netherlands Organisation
for Applied Scientific Research



To
E. Mandersloot, Citrex Nederland B.V.

From
Dr. W.H.J. Vaes

Subject
Results of preliminary analysis of Vi-care products

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Introduction

Herewith we present the results of the research that you have ordered of 3 samples Vi-care solution. The results are approved by me, undersigned. With these results, the first phase of the research as offered to you on December 1, 2001, is completed.

Contents

The following data are mentioned successively:

- sample identification
- used analytical methods
- results of analysis
- remarks concerning the results of analysis
- example chromatogram of the analysis of anions

Results

Our findings show that the content of total vitamin C determined by us is lower than the quantity that is dosed in Vi-care according to your specification. The determined quantities of dehydroascorbic acid are very low. This indicates anyhow that the product is stable.

In the analysis of anions through anionchromatography, all three (chloride, citrate, lactate) of the dosed ingredients were determined. The measured contents fairly match the quantity that is dosed according to your specification. Furthermore, through anionchromatography the total content of vitamin C and vitamin C-oxidation products after complete oxidation is determined as oxalate. The conclusion (after conversion to molar units) can be drawn that, besides the before mentioned ascorbic acid and dehydroascorbic acid, no proof has come up from the analysis of the presence of other degradation products of vitamin C.

In addition, in the three different products a total of 7 impurities were found with low content 0,2-0,7 g/kg (< 0,1 mass-percent of the product). This equals 1-3% of the specified quantity of the active component (vitamin C). One of the impurities is, based on the retention time, identified as phosphate. The concentration of phosphate in the product varies from <0,1-0,4 g/kg. The other impurities could not be identified. Regarding the other impurities, we can almost certainly state that they are not likely to be one of the following short chain of organic acids or inorganic anions: glycol acid, acetic acid, propionic acid, glutaric acid, amber acid, malic acid, maleic acid, malonic acid, fumaric acid, fluoride, chlorite, bromate, nitrite, chloride, bromide, nitrate, selenite,

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Date
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or iodide. The identity of the impurities can possibly be investigated in the next phase of the research, Phase 2, by means of techniques such as mass-spectrometry.

For your information, an example of a chromatogram of the anion analysis of your product Vi-care can be found on page 3, together with a blank.

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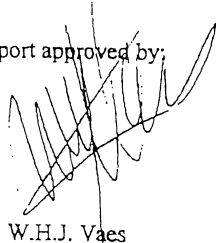
Conclusion

Summarised, we can conclude that the product, as delivered to us, shows a lower content of vitamin C than we expected on the basis of your specification. Furthermore, a small amount of impurities were found in your product at a concentration higher than 0,1% of the active component.

Regulatory Issues

1. The content is significantly lower than the claim of 25g/kg; this could possibly be resolved by adjusting the claim, e.g. 21g/kg.
2. There are no indications for the presence of degradation products of vitamin C. In relation to vitamin C, 5 compounds in contents of > 1% were found. From a purely legal perspective, these impurities should become known. In consultation with the CTB, Citrex can of course point out the nature of the raw materials.
3. A logical next step would be that the CTB would ask to have the same analysis conducted under GLP as a foundation of the composition. It is possible to also do the regulatory required validation (specificity, variability, reproducibility).
Validation of the method for the product should contain 2 methods: vitamin C, and anions analysis.

Report approved by:


Dr. W.H.J. Vaes

Sample identification				Date
TNO sample code	Your sample code	Production date	Matrix	14 February 2002
0932/02/1497	batch 1237	15-11-2000	Vi-care solution	Our reference
0932/02/1498	batch 1387	11-11-2001	Vi-care solution	DAM 02-0906/VAW
0932/02/1502	Citrex B Liq.	Unknown	Vi-care solution	Page
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Used methods		
DAM/VIT/034	vitamin C in fluids	HPLC-fluorescence
VIT/063	ascorbyl-2-phosphate in food/premixes/feed	HPLC-UV
not standardised	anions using anion chromatography	Dionex-conductivity

Dates of analysis	
Vitamin C in fluids	21-12-2001
Ascorbyl-2-phosphate in food/premixes/feed	21-12-2001
Anions using anion chromatography	10-01-2002

TNO sample code	0932/02/1497	0932/02/1498	0932/02/1502
Analyte			
Total vitamin C	21.5 g/kg	20.2 g/kg	22.2 g/kg
Dehydroascorbic acid	0.52 g/kg	0.86 g/kg	0.58 g/kg
Ascorbic acid (calculated)	21.0 g/kg	19.3 g/kg	21.6 g/kg
Ascorbyl-2-monophosphate	<50 mg/kg	<50 mg/kg	<50 mg/kg
Lactate	34.8 g/kg	29.9 g/kg	34.1 g/kg
Chloride	10.9 g/kg	10.0 g/kg	10.8 g/kg
Oxalate	8.1 g/kg	7.0 g/kg	8.8 g/kg
Citrate	18.6 g/kg	13.7 g/kg	11.6 g/kg
Impurity 1	0.3 g/kg (1 %)	0.3 g/kg (1 %)	0.3 g/kg (1 %)
Impurity 2	<0.1 g/kg	<0.1 g/kg	0.3 g/kg (1 %)
Impurity 3	<0.1 g/kg	<0.1 g/kg	0.4 g/kg (1 %)
Impurity 4	0.7 g/kg (3%)	<0.1 g/kg	0.5 g/kg (2 %)
Impurity 5	0.6 g/kg (2 %)	0.4 g/kg (1 %)	0.5 g/kg (2 %)
Impurity 6	0.4 g/kg (2 %)	0.3 g/kg (1 %)	0.5 g/kg (2 %)
Phosphate	0.4 g/kg (2 %)	<0.1 g/kg	0.2 g/kg (1 %)

Remarks concerning the analysis results

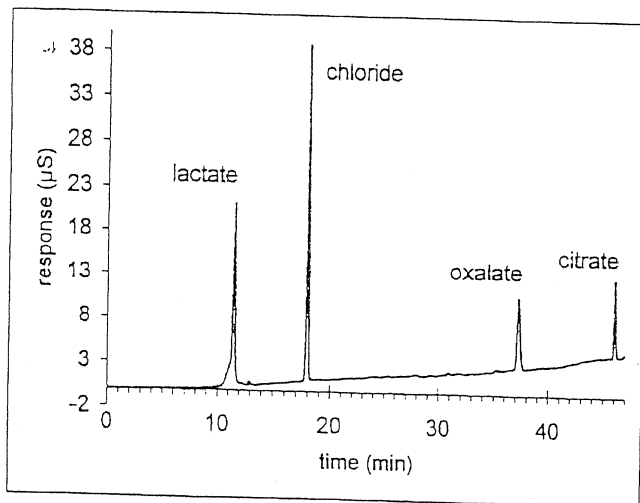
- To quantify the impurities, the response of lactate was used. This response is an average response for a small singly charged organic anion.
- The content of the impurities and phosphate is indicated within brackets as percentage of the labelclaim of the active ingredient, i.e. vitamin C (25 g/kg).

Example chromatogram of the anion chromatographic analysis of sample 0932/02/1498 (batch 1387).

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Example chromatogram of the anion chromatographic analysis of demineralised water with 10mM NaHO (blank).

