ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 985–994

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Improved extraction methods for avoiding the interference of copper in the LC determination of ascorbic acid in multivitamin-mineral tablets

Lill D. Müller *

Municipality of Oslo, Department of Public Health, Division of Food Control, Maridalsveien 3, 0178, Oslo, Norway

Received 27 September 2000; received in revised form 4 January 2001; accepted 7 January 2001

Abstract

A rapid sample extraction procedure for the determination of ascorbic acid (AA) by high performance liquid chromatography (HPLC) in multivitamin-mineral formulations containing interfering copper has been developed. The method takes special precautions to prevent degradation of AA in contact with high concentrations of interfering elements such as copper. Sample preparation involved addition of pyrogallol, citric acid solution and short time extraction (5 min) under an atmosphere of nitrogen to prevent oxidation of AA. Another sample cleanup based on extraction of the multivitamin-mineral tablet with the extraction solution as described above, but with addition of strong cation exchange sorbent, was also developed. The copper and other minerals in the formula were retained on an ion exchange sorbent and thus represses the speed of oxidation of AA. Extracts of multivitamin-mineral tablets were analyzed by ion-pair reversed phase HPLC and UV-diode array detection. Ten multivitamin formulations containing copper were analyzed by the two proposed methods. The analytical results of AA obtained using the simple sample extraction method and the method involving addition of cation exchange were 89–115% and 95–112%, respectively, of declared concentrations. The parameters for the validation of the methods are given.

Keywords: Ascorbic acid; Multivitamin-mineral tablets; Ion-pair reversed phase HPLC; Interference; Copper

1. Introduction

In many procedures for AA determination, too little attention is paid to the instability of the vitamin, which may adversely affect the assays [1]. AA is highly sensitive to heat, alkali, oxygen, light and contact with traces of copper and iron [2,3]. Some investigators have discussed difficulties in quantifying the labile AA in food samples. Metaphosphoric acid, citric acid or citric acid with pyrogallol added are suitable for extraction of AA from food and cause stability of the molecule [2]. Wang et al. have investigated the instability of AA in plasma during sample prepa-

* Tel.: +47-22987456; fax: +47-22987470.
E-mail address: lill.muller@legemiddelverket.no (L.D. Müller).

0731-7085/01/$ - see front matter
PII: S0731-7085(01)00372-7

ration, storage and analysis [1]. Another paper has been published on stabilization of AA, and its quantitative analysis in drug preparation by high-performance liquid chromatography [4]. Several papers have been published on simultaneous analysis of AA and other water soluble vitamins such as niacinamide, pyridoxine, thiamine, and riboflavin in multivitamin-mineral tablets using ion-pair reversed phase liquid chromatography [5–13]. Jenkins [8] reported that addition of magnesium oxide and/or copper gluconate to a multivitamin-mineral formula did not allow complete extraction of the above mentioned water-soluble vitamins. Lam et al. [7] reported that large excess of ferrous fumarate or sulfate in multivitamin-mineral tablets interferes with the assay of AA. The ferrous sulfate or fumarate was removed from the extract of vitamins by addition of cupferron (ammonium salt of *N*-nitrosophenylhydroxyl-amine) to form a water-insoluble complex. However, Lam et al. were not able to assay AA in all the multivitamin-mineral products they investigated [7]. A preliminary study performed by our department strongly supports earlier published observations that previously used methods were not suitable to assay AA in commercial multivitamin-mineral products containing copper. The purpose of the present study was to develop a rapid method for the determination of AA in all sorts of multivitamin-mineral tablets containing copper, iron, and other possible interfering minerals.

2. Experimental

2.1. Reagents

High-quality distilled water was used. Ascorbic acid (AA), pyrogallol ($C_6H_6O_3$), metal salts (Cu, Fe, Mg, Ca, Zn), BDH amberlite IR-120 (Na) 0.300–1.18 mm and citric acid were purchased from Merck (Darmstadt, Germany). 1-Hexanesulfonic acid, sodium salt ($CH_3(CH_2)_5SO_3Na$) was obtained from Aldrich (Gillingham-Dorset), and acetonitrile (HPLC grade) used for HPLC analyses was purchased from Rathbum (Walkerbum, UK).

2.2. Description of samples

Nine multivitamin-mineral supplement preparations in tablet form and one in capsule form from different manufacturers were analyzed. All supplements contained vitamins A, AA, vitamin B_1, vitamin B_2, vitamin B_6, vitamin D, vitamin E, vitamin B_{12}, niacin, folic acid, biotin and pantothenic acid. The tablets contained copper and one or more of the following elements iron, calcium, phosphorous, iodine, magnesium, zinc, chromium, selenium, molybdenum, manganese and potassium in addition to the previously mentioned vitamins. The content of copper ranged from 0.5 to 4 mg/tablet (Table 3). The content of AA ranged from 30 to 200 mg/tablet (Table 6).

2.3. Preparation of extraction solutions

Extraction solution: Dissolve 1 g of pyrogallol and 19.21 g citric acid in water in a 1-l volumetric flask, corresponding to a 0.100 M solution of citric acid. Protect the volumetric flask from light by wrapping aluminum foil around it.

2.4. Preparation of ion-exchange sorbent

The Na form is obtained from approximately 15 ml of 1 M NaCl per gram resin in a beaker. Place it on a magnetic stirrer for 30 min. Decant the fluid away and repeat the addition of NaCl. Wash the resin twice with 0.1 M citric acid.

2.5. Ion-pair solution (HPLC mobile phase)

The ion-pair solution was prepared according to Perkin Elmer [6]. Dissolve 10 mM (1.88 g) 1-hexanesulfonic acid, sodium salt ($CH_3(CH_2)_5SO_3Na$), 10 ml 100% acetic acid and 1.3 ml triethylamine in water in a 1-l volumetric flask. Filter the ion-pair solution through a Millipore filter HAWPO4700.

2.6. Preparation of standards

Weigh accurately 100 mg AA into a 50-ml volumetric flask, dissolve and dilute to volume with extraction solution. Transfer 2.00, 5.00, and

10.00 ml of the solution into 100-ml volumetric flasks, respectively and dilute to volume with extraction solution. The final concentration of working standards was 40, 100, and 200 μg/ml AA, respectively. Prepare new standard solutions each day of analysis.

2.7. HPLC analyses

Analyses were performed on a Hewlett Packard HPLC system 10 Series equipped with vacuum degasser, gradient pump, auto sampler, column oven and diode array-UV-vis detector monitoring at 275 nm. The signals were recorded on a HP ChemStation for liquid chromatography system. The separation was carried out using a reversed-phase (RP) column (Hypersil BDS-C_{18}, 4×250 mm, 5 μm packing with RP-select B guard column, 10×2 mm, Hewlett Packard) and isocratic elution with ion-pair solution-acetonitrile (98:2 v/v) at a flow-rate of 1.0 ml/min. The temperature of the column oven was 25°C. The chromatography of the sample extract was completed within 4 min. Late eluting substances were flushed out using an acetonitrile-ion-pair (60:40 v/v) solution for 6 min at a flowrate of 1.5 ml/min, prior to reequilibration of the column at an elevated flowrate of 2.1 ml/min with mobile phase for 10 min.

2.8. Calibration graphs

Inject 10 μl of working solution into the chromatograph, and plot peak height vs. concentration by the external standard method.

2.9. Atomic absorption spectrometry

A Varian SpectrAA atomic absorption spectrometer 220 Fast Sequential (flame) was used for determination of copper in prepared samples by the proposed Method 2 (see below).

2.10. Checking the interference of Cu, Fe, Mg, Ca, and Zn

Weigh 1.26 mg $CuSO_4$ (equivalent to 0.5 mg Cu), 6.3 mg $CuSO_4$ (equivalent to 2.5 mg Cu^{2+}), 12.6 mg $CuSO_4$ (equivalent to 5 mg Cu^{2+}), 99.5 mg $FeSO_4 \cdot 7H_2O$ (equivalent to 20 mg Fe^{2+}), 1.01 g $MgSO_4 \cdot 7H_2O$ (equivalent to 100 mg Mg^{2+}), 0.55g $CaCl_2$ (equivalent to 200 mg Ca^{2+}), 65.9 mg $MnSO_4$ (equivalent to 24 mg Mn^{2+}) and 65.9 mg $ZnSO_4 \cdot 7H_2O$ (equivalent to 15 mg Zn), respectively into 250-ml Erlenmeyer flasks containing 60.0 mg of AA. After addition of 100 ml water, stopper the flask securely. Place the Erlenmeyer flask on a magnetic stirrer for 5 min. Transfer the sample to an amber HPLC vial and analyze immediately by HPLC. Weigh 1.26 mg $CuSO_4$ and 6.3 mg $CuSO_4$, respectively into a 250-ml Erlenmeyer flask containing 60.0 mg of AA, 99.5 mg $FeSO_4 \cdot 7H_2O$, 1.01 g $MgSO_4 \cdot 7H_2O$, 0.55 g $CaCl_2$, 65.9 mg $MnSO_4$ and 65.9 mg $ZnSO_4 \cdot 7H_2O$. Prepare the two samples as described above.

2.11. Checking the stability of AA in an extract

Prepare an extract of multivitamin-mineral tablet according to sample extraction procedure method 1 and 2, respectively. Transfer the sample to an amber HPLC vial, stopper and store in the autosampler at 22°C (room temperature). Analyze at intervals (five times) over a 2-h period.

2.12. Sample extraction procedure I (preliminary study)

The sample preparation was performed as described by Perkin Elmer [6]. Add 10 ml of 1% ammonia in DMSO to the ground powder of multivitamin tablet and sonicate it in an ultrasonic bath for 2 min. Add 90 ml of 2% acetic acid in water and stir for 1 min with a magnetic stirring bar. Filter the extract immediately through a 0.45-μm membrane filter, transfer into an amber vial and analyze immediately by HPLC.

2.13. Sample extraction procedure II (preliminary study)

The sample preparation was performed as generally described by Ivanovic et al. [9]. Ground a multivitamin tablet in a mortar and quantitatively transfer the entire mass into an Erlenmeyer flask.

Add 100 ml 0.01 M HCl to the flask, place the Erlenmeyer flask in an ultrasonic bath for 10 min, centrifuge and filter for 1 min instead of 10 min through a Costar (Corning Costar Corp., Cambridge, MA) Spin-X centrifuge filter unit with a 0.45-μm nylon membrane. Transfer the sample to an amber HPLC vial and analyze by HPLC immediately.

2.14. Sample extraction procedure (method 1)

A multivitamin-mineral tablet: Ground one multivitamin-mineral tablet carefully in a mortar, quantitatively transfer the entire mass into an Erlenmeyer flask and add 100 ml extraction solution. Flush the flask with nitrogen and stopper securely. Protect the Erlenmeyer flask from light by wrapping aluminum foil around it and then place it on a magnetic stirrer for 5 min. Based on label declarations, appropriate dilutions are made with the extraction solution. Filter the sample through a Costar (Corning Costar Corp., Cambridge, MA) Spin-X centrifuge filter unit with a 0.45-μm nylon membrane for 1 min. Transfer the sample to an amber HPLC vial and analyze by HPLC immediately.

A multivitamin-mineral capsule: Cut the capsule in pieces with a pair of scissors, and transfer the entire content quantitatively into a 250-ml Erlenmeyer flask with 20 ml hexane. Place the Erlenmeyer flask on a magnetic stirrer for 30 s, and add 100 ml extraction solution. Stir the Erlenmeyer flask on a magnetic stirrer for 5 min. After extraction, filter the bottom-layer water phase of the sample through a Costar Spin-X centrifuge filter unit and prepare the sample as further described in the extraction procedure for multivitamin-mineral tablet described above.

2.15. Sample extraction procedure (method 2)

Weigh an amount of ground multivitamin-mineral tablet equivalent to 1/6 of the fill and transfer it into an Erlenmeyer flask. Add 6 g of ion exchange-resin BDH Amberlite IR-120 (Na) and 100 ml of extraction solution. Prepare the sample as further described in the extraction procedure for multivitamin-mineral tablet (method 1).

3. Results and discussion

3.1. Preliminary study of the extraction procedure of AA

An examination of recent literature has revealed that investigators had problems with excepients in vitamin formulations when analyzing AA. They were not able to analyze AA in all sorts of multivitamin tablets containing interfering minerals [7,8]. In our preliminary study, five different multivitamin-mineral formulation tablets were extracted for AA according to two earlier established extraction procedures [6,9]. The extracts of the tablets were analyzed for AA according to our modified version of a HPLC method described by Perkin Elmer [6]. The assay values of AA in multivitamin formula containing only vitamins agreed well with the label claim of the product. However, the amount of AA found in some multivitamin-mineral formulations containing minerals including copper was distinctly lower than the label claim of the products (Table 1). The two earlier established extraction methods (sample extraction procedures I and II) were unsuitable for extraction of AA in some of the multivitamin-mineral formulations.

3.2. The influence of Cu on the assay of AA

It has been reported that traces of copper catalyze the oxidation of AA [2]. One multivitamin-mineral formula may contain 0.5–4 mg copper, and the presence of copper in multivitamin-mineral tablets seemed to interfere with the quantification of AA. Takenaka et al [14] reported that vitamin B_{12} was destroyed in contact with substantial amounts of vitamin C in the presence of copper. The destruction of B_{12} was assumed to be related to radical generation by AA in the presence of copper [14]. In this investigation we have studied the effect of Cu, Fe, Mg, Ca, Zn, and Mn on the recovery of AA (Table 2). The amounts of each element corresponding to the label claim of multivitamin-mineral formula was added separately to 60 mg AA in 100 ml water. Only copper interferes greatly with the assay of AA. Lam et al. [7] has reported that Fe^{2+} influenced the recovery

of AA. Fe^{3+} has, however, a dramatic effect on oxidation of AA because Fe^{3+} has a high reduction potential (0.771) in the electrochemical series [15]. The effect of the concentration of copper was examined. With a concentration of 0.5 mg Cu, AA decreases slightly. With increasing concentrations of copper, the concentration of AA decreases markedly (Table 2). The effect of elements such as Fe, Mg, Ca, Zn, and Mn on the recovery of AA in contact with copper was examined. The recovery of AA in contact with varied concentrations of copper (0.5 and 2.5 mg) and the other elements was decreased markedly compared to the recovery of AA in contact with only copper present.

However, the AA concentrations in the sample containing all the elements except copper showed no apparent degradation (Table 2). This indicates that the stability of AA in contact with copper is likely to be influenced by the total concentrations of ions in the sample.

3.3. Procedure of extraction method 1

In this study we have examined a simple extraction procedure for AA in food samples proposed by Vanderslice and Higgs [16] which was modified for determination of AA in multi-vitamin-mineral formulas. AA in the multivitamin-mineral tablets was extracted with a solution of citric acid and pyrogallol as described in method 1. Citric acid possibly formed stable complexes with Cu, Fe and

Table 1
Comparison of values obtained for %AA of declared concentrations found in some formulations by preliminary extraction methods I and II and the proposed methods 1 and 2

Preparation	Prelimin. method 1[a]		Prelimin. method II[a]		New method 1		New method 2	
	Found %	R.S.D. %	Found %	R.S.D. %	Found %	R.S.D. %	Found %	R.S.D. %
Omnimin Plus	64	8.5	80	6.2	98	4.5	95	5.8
Multi med Mg	39	7.5	75.3	5.8	103	4.0	108	6.3
Vitaplex kosttilsk	28	8.7	65	6.4	93	5.9	98	6.8
Urte+Total vit. min	46	7.2	62	5.9	103	2.9	98	5.3
Ferrosan Pluss	53	7.1	66	5.5	111	4.4	96	5.7

[a] Preliminary methods I and II were described by Perkin Elmer [6] and Ivanovic et al. [9], respectively.

Table 2
The influence of Cu^{2+}, Fe^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+} and Zn^{2+} on the recovery of AA

AA (mg)	Cu^{2+}	Fe^{2+}	Mg^{2+}	Ca^{2+}	Mn^{2+}	Zn^{2+}	Found AA (mg)	Recovery%[a]	R.S.D.%
60		20					58	96.7	2.3
60			100				58.9	98.2	2.5
60				200			58.2	97	2.1
60					24		55.7	92.8	1.9
60						15	57.8	96.3	2.6
60		20	100	200	24	15	56.2	93.7	2.8
60	0.5						54.8	91.3	0.8
60	0.5	20	100	200	24	15	26.8	44.7	4.1
60	2.5						26.6	44.3	8.7
60	2.5	20	100	200	24	15	5.3	8.8	13.2
60	5.0						15.2	25.3	11.4

[a] Average of three determinations.

Table 3
The label claims of copper, other minerals and the analysis results of copper in extracts from formulations obtained by the proposed method 2

Preparation	Cu mg	Fe mg	Mg mg	Ca mg	Mn mg	Zn mg	Cu (mg/l)[a] method 2	R.S.D.%[b]
Omnimin Plus	4	18	150	200	5	25	0.19	4.7
Multi med Mg	2	14	100		2.5	15	0.08	4
Vitamineraler	2	15				10	0.17	3.9
Vitaplexkosttilsk	2	14	75		2.5	15	0.16	4.2
Urte+Total vit. min.	1.25	12	50	100	2.1	10	0.12	4.4
Ferrosan Pluss	2	14	75		2.5	15	0.25	4.1
Ferrosan Daglig	2	14	75		2.5	15	0.23	4.6
Collett Kostpluss	1.5	10			1	10	0.22	3.8
Spektro Multi VitaMin	0.5	5	75	200	1.6	4	0.25	4.5
Scanasan	0.8	6			1.3	5	0.34	4.3

[a] The analysis results were obtained using flame atomic absorption spectrometry.
[b] Average of three determinations.

other complex binding metals and thus stabilized AA [16]. It has been shown that AA in an oxygen saturated solution in the presence of a Cu solution is more stable at pH 3–4.5 than at pH 6–7 [17]. Pyrogallol (in the extraction solution) was added to the sample, and the mixture was extracted under nitrogen on a magnetic stirrer to prevent oxidation of AA [16]. The optimal extraction time of the formulation on the magnetic stirrer was 5 min. The solubility of AA was good.

3.4. Procedure of extraction method 2

Strong cation exchange sorbent was added to the sample of multivitamin-mineral tablet to prevent AA been in contact with a high concentration of copper (described in sample preparation, method 2). Copper and other minerals as Ca, Mg, Fe in the sample solution will be retained on a strong cation exchanger sorbent (Amberlite IR-120).

The high amount of elements in one multivitamin-mineral tablets exceeded the capacity of 6 g of sorbent. The capacity was estimated by determining the concentration of copper in 10 different prepared samples by flame atomic absorption spectrometry (Table 3). The study showed that the capacity of 6 g of sorbent was great enough to retain only 1/6 of the content of the elements in most tablets. This amount was sufficient to remove 90% or more of the copper content from the multivitamin-mineral tablet. The content of copper in the prepared samples were then below 0.3 mg/l (Table 3).

3.5. HPLC method

The extracts of the tablets were analyzed according to our modified version of a HPLC method described by Perkin Elmer [6]. In contrast to some previously published HPLC methods their method completely separated niacin and AA on ion-pair reverse phased HPLC [18]. Perkin Elmers HPLC method used an elution system consisting of 15% methanol and 85% ion-pair solution [6]. In order to obtain a retention time (2.2 min) of AA as long as possible, the mobile phase was only 2% v/v acetonitrile in the ion-pair solution, and the length of the HPLC column RP-18 was 25 cm. In order to evaluate the interference from niacin, niacin and AA were analyzed under the established chromatographic conditions. The AA and niacin were baseline separated and were eluted at 2.2 and 2.7 min, respectively. The peak identity was confirmed by comparing the absorption spectrum of the peak with the spectrum of an AA standard in the HPLC library. The purity index of the peak was greater than 940, thus indicating that there was no coelution and that the peak was pure.

It can be concluded on the basis of our investigations that the ion-pair reversed phase HPLC method is robust, because slight variations (5%) in eluent composition, temperature and flow rate have little or no effects on the results. The stability of the column was evaluated and the retention time of a standard solution of AA in 40 sample injections (after washing with the eluent for 6 min) was calculated. The mean of the observed retention time was 2.22 min $\pm$ 0.5 R.S.D.%.

3.6. Calibration curve

The maximum absorptivity of AA in the mobile phase occurs at 244 nm. A monitoring wavelength of 275 nm was chosen, and the calibration curves were linear up to at least 200 μg/ml. The linear regression was height $= 0.975332 \times \text{Amt} - 8.4390538$. A correlation coefficient of 0.999, the standard deviation of the slope (S_a) 0.0075048 and the standard deviation of the intercept (S_b) 0.43025013 were obtained for the HPLC method.

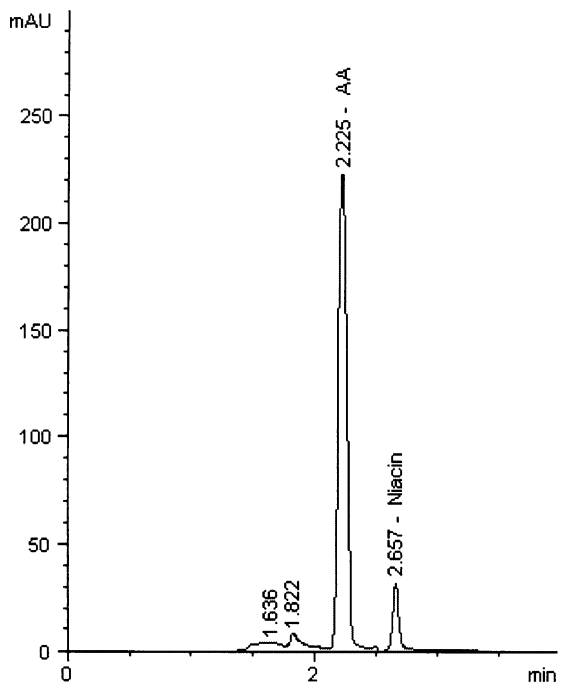

Fig. 1. Typical chromatogram of multivitamin-mineral tablet (Vitaplex) extract.

The calibration curves show intercepts at the *Y*-axis corresponding to a value of 8.65 μg/ml. The lower limit of quantitation (LOQ) 10 μg/ml was therefore determined by the lowest standard possible to be included in the standard curve. LOQ corresponding to 53 times the chromatographic noise.

A typical chromatogram obtained from an extract of vitamin-mineral formulation is shown in Fig. 1.

3.7. Recovery and precision of the proposed methods

Recovery data for method 1 on vitamin-mineral formulations 'Vitaplex kosttilsk' and 'Multi med Mg' were found by adding known amounts, i.e. 50, 100, and 150 mg AA, respectively, to the multivitamin-mineral tablet. However, recovery data for method 2 on vitamin-mineral formulation 'Vitaplex kosttilsk' were found by adding 10 and 50 mg AA, respectively, to amounts corresponding to 1/6 of the weight of one multivitamin-mineral tablet. These multivitamin-mineral tablets were chosen because of the high contents of copper and other minerals especially. The average recovery value (%) for AA and relative standard deviation in formulations 'Vitaplex kosttilsk.' and 'Multi med Mg' analyzed by the proposed methods 1 and 2 are shown in Table 4. The results showed that the proposed methods were accurate.

The method 1 reproducibility was evaluated by performing eight repetitive analyses within 24 h of three different multivitamin-mineral tablets: 'Vitaplex kosttilsk.', 'Multi med Mg' and 2 tbl. 'Urte + Total vit. min.' containing, respectively 60, 60, and 90 mg AA, respectively. The mean result and standard deviation of the assay were 56 ± 3, 62 ± 23 92.8 ± 2.5, respectively. The method 2 reproducibility was evaluated by performing eight repetitive analyses within 24 h of one multivitamin-mineral tablet ('Vitaplex kosttilsk.') containing 60 mg AA. The mean value and standard deviation was 58.5 ± 4.3. The results show that the described methods are reproducible.

Table 4
Recoveries of AA from spiked multivitamin-mineral formulations obtained by the proposed methods 1 and 2

Preparation	Method	Added (mg)	Found (mg)	Rec.[a]	R.S.D.%
Vitaplex kosttilsk	Method 1	0	56	–	–
Vitaplex kosttilsk	Method 1	50	106.5	100	3.8
Vitaplex kosttilsk	Method 1	100	149.2	96	3.6
Vitaplex kosttilsk	Method 1	150	190.5	93	1.7
Multi med Mg	Method 1	0	62	–	–
Multi med Mg	Method 1	50	105.4	94	0.8
Multi med Mg	Method 1	100	148.8	92	3.7
Multi med Mg	Method 1	150	204.2	96	3.2
Vitaplex kosttilsk	Method 2	0	9.75	–	–
Vitaplex kosttilsk	Method 2	10	19.3	98	5.3
Vitaplex kosttilsk	Method 2	50	58	97	2.9

[a] Average of three determinations.

Table 5
Stability study of AA in extracts of Vitaplex multivitamin-mineral tablet

Time (min)	AA mg (method 1)	R.S.D.%[a]	AA mg (method 2)	R.S.D.%[a]
0	56.1	5.9	58.5	6.8
30	50.7	6.3	58.6	7.0
60	45.9	6.1	54.4	6.9
90	41.4	6.0	51.0	6.6
120	37.5	6.1	47.3	6.7

[a] Average of three determinations.

3.8. Study of the stability of AA in the extract of a multivitamin-mineral tablet

The stability of AA in the tablet extracts from both extraction method 1 and 2 was determined by making five consecutive injections of, respectively, two vitamin-mineral tablet ('Vitaplex kostilsk.') extracts over a 2-h period (Table 5). The experimental conditions of temperature, photochemical effects, solvent effects and ionic strength were constant during the stability study. The results from extraction method 1 showed an approximately 10% decrease in the content of AA in the sample after each period of 30 min. It was therefore important to analyze the sample of mineral-vitamin tablet by HPLC immediately after preparation. The results from extraction method 2 showed no decrease in the content of AA in a period of 30 min and the sample of mineral-vitamin tablet should therefore be analyzed by HPLC during the 30-min period after preparation. The results from extraction methods 1 and 2 showed a relative standard deviation of $< 7\%$ for AA in the whole 2-h period.

3.9. Quantification of AA in the formulations

As shown in Table 6, the amount of AA per tablet or capsule varied between 20 and 200 mg. The analytical results of AA in multivitamin-mineral tablets containing copper, analyzed by methods 1 and 2 were found to be 89–115% and 95–112%, respectively, compared to the declared values.

Table 6
Analytical results of AA, %AA of declared concentrations in extracts from formulations obtained by the proposed methods 1 and 2

Preparation	Label mg AA	Method 1 AA mg	Method 1 % AA	R.S.D.%[a]	Method 2 AA mg	Method 2 % AA	R.S.D.%[a]
Omnimin Plus	200	195	98	4.5	189.1	95	5.8
Multi med Mg	60	62	103	4.0	64.8	108	6.3
Vitamineraler	60	64.8	108	4.2	57.3	95	5.6
Vitaplex kosttilsk	60	56	93	5.9	58.5	98	6.8
Urte+Total vit. min.	45	46.4	103	2.9	44	98	5.3
Ferrosan Pluss	90	100	111	4.4	86	96	5.7
Ferrosan Daglig	60	69	115	5.2	67	112	6.2
Collett Kostpluss	60	57	95	4.2	64	107	5.7
Spektro Multi VitaMin	100	89	89	4.9	94.7	95	6.2
Scanasan	30	27	90	5.4	31	103	6.5

[a] Average of three determinations.

4. Conclusion

The two published extraction methods used in preliminary investigations do not take any special precautions in order to prevent degradation of AA in contact with copper. The extraction solution as described in sample extraction procedure I was not suitable to assay AA in multivitamin-mineral tablets containing copper because of the great instability of AA in basic ammonia solution. The other sample extraction procedure II described an extraction time that was too long (20 min). Our two proposed sample extraction methods described for analyzing AA in multivitamin-mineral tablets containing an interfering element such as copper without the loss of AA during preparation of the samples. The methods were accurate and reliable for analyzing AA. The major modification of the proposed sample extraction method 1 was the addition of an ion exchange-resin to the sample as described in sample extraction method 2. The investigations indicated that this did not improve the precision of the assay of AA. The loss of AA during the extraction of multivitamin-mineral tablet in 5 min as described in the proposed sample extraction method 1 was relatively small. Method 1 has the simplest sample preparation procedure involving use of the whole tablet. The author therefore proposes this method for routine determinations of AA in multivitamin-mineral tablets.

Acknowledgements

Appreciation is due to the Department of Public Health for samples and facilities provided, Sverre H. Omang for running the Atomic Absorptions measurements and Astri Rogstad and Sverre H. Omang for valuable review of the manuscript.

References

[1] S. Wang, I. Schram, R. Sund, Eur. J. Pharm. Sci 3 (4) (1995) 231–239.
[2] L.M. Nollet, Food Analysis by HPLC, Marcel Dekker, New York, 1992, p. 345.
[3] M. Windholz, L.Y. Stroumtsos, M.N. Fertig, The Merck Index, Merck & Co, Rahway, USA, 1976, pp. 110–111.
[4] S. Yamauchi, N. Noriyuki, T. Kinoshita, T. Hanai, Yakuzaigaku 53 (3) (1993) 169–175.
[5] Hewlett Packard, HPLC for Food Analysis, Hewlett-Packard Company, Germany, 1996, pp. 38–39.
[6] Perkin Elmer Co, Food Analysis by HPLC an Overview, LC-300, Perkin Elmer, Norwalk, 1993, pp. 25–28.
[7] F. Lam, I.J. Holcomb, A.J. Fusari, J Assoc. Off. Anal. Chem. 67 (5) (1984) 1007–1011.
[8] C. Jenkins, Pharm. Technol. 6 (1982) 53–59.
[9] D. Ivanovic, A. Popovic, D. Radulovic, M. Medenica, J. Pharm. Biomed. Anal. 18 (1999) 999–1004.
[10] I. Papadoyannis, G. Tsioni, V. Samanidou, J. Liquid Chromatogr. Relat. Technol. 20 (19) (1997) 3203–3231.
[11] M. Amin, J. Reusch, Analyst 112 (7) (1987) 989–991.
[12] M. Gennaro, J. Chromatogr. Sci. 29 (9) (1991) 410–415.
[13] J.D. Felicio de Souza, E. Goncalez, An. Assoc. Bras. Quim. 45 (4) (1996) 152–154.

[14] S. Takenaka, S. Sugiyama, F. Watanabe, K. Abe, Y. Tamura, Y. Nakano, Biotechnol. Biochem. 61 (12) (1997) 2137–2139.
[15] D.R. Lide, H.P.R. Frederikse, CRC Handbook of Chemistry and Physics, Section 8 Table 2, Florida, 1996, pp. 8–26.
[16] J.T. Vanderslice, D.J. Higgs, J. Chromatogr. Sci. 22 (1984) 485–489.
[17] T.E. Furia, Handbook of Food Additives, The Chemical Rubber Co, Cleveland, 1968, p. 113.
[18] W.G. Chase, A.M. Soliman, J. Micronutrient Anal. 7 (1990) 15–25.