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DETERMINATION OF VITAMIN C IN MULTIVITAMIN EFFERVESCENT TABLETS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT:

Background*:Multivitamin effervescent tablet is favorite supplement dietary and has been produced by many manufacturers. To verify the amount of vitamin C in multivitamin effervescent tablets is necessary to assure customer's health. But up till now, in present Vietnamese pharmacopoeia, there has not been quantitative method for effervescent tablet yet.* ***Objective:*** *we develop a high-performance liquid chromatography (HPLC) method for determination of vitamin C as main ingredient in multivitamin effervescent tablet with high sensitivity and short analytical*

time. Using HPLC method, we optimize conditions then validate and apply the method for determination of commercial tablets. ***Result****: The optimized conditions included isocratic mobile phase of 5% of methanol and 95% of phosphoric acid solution pH 3.0. Chromatographic system used Phenomenex Lunar* C_{18} *column (250 mm; 4.6 mm; 5 µm) at* 25^0*C, injection volume was 20 µl, flow rate at 1.0 ml/minute, detector UV-Vis at 248 nm. The method enables determination of vitamin C in pharmaceutical preparations within 7 minutes. The assay was validated for vitamin C in the range of 15 – 120 µg/ml. The limit of detection was 0.22 µg/ml, the limit of quantitation was 0.73 µg/ml.* ***Conclusion****: The method was successfully applied to determine 4 products of multivitamin effervescent tablet and can be used in routine analysis.*

Keywords: *Vitamin C, high-performance liquid chromatography, ultra-violet detector, multivitamin effervescent tablet*

I. INTRODUCTION

Vitamin C (L-acid ascorbic), which is chemically named (R)-5-[(S)-1,2-dihydroxyethyl]-3,4-dihydroxy-5H-furan 2-on (Fig.1), contains not less than 99,0% and not more than 100,5% $C_6H_8O_6$

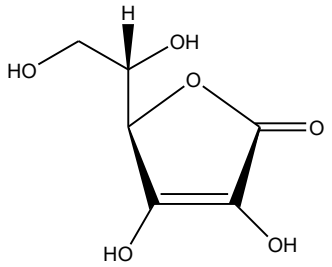

Figure 1: Structure of vitamin C

Vitamin C is distributed all over the body. The biological role of ascorbate is to act as a reducing agent, donating electrons to various enzymatic and a few non-enzymatic reactions. The one- and two-electron oxidized forms of vitamin C, semidehydroascorbic acid and dehydroascorbic acid. Vitamin C is excreted in the urine [5]. Stability of vitamin C depends on many factors. Vitamin C is sensitive to heat, light and oxygen. High humidity could be degrading vitamin C faster [2].

Several techniques have been reported in the literature for the quantitation of vitamin C. In Vietnamese pharmacopoiea IV, US pharmacopoiea 35, British pharmacopoiea 2013 and European pharmacopoiea 7, both material and pharmaceutical products of ascorbic acid were analyzed traditionally by volumetric titration based on reduction-oxidation reactions. This method has its own advantages and acceptable limit of exact, easy to apply but it will be hard to handle when the sample becomes immense. Recently, there have been some scientists who analyzed vitamin C in fruit juice, beverage by high-performance liquid chromatography. This method is extremely quick and efficient, largely automated, accurate and highly reproducible, and it is suitable when samples are large. Thus, the aim of our present study will develop a HPLC method for determination of vitamin C as main ingredient in multivitamin effervescent tablet with high sensitivity and short analytical time. This analysis procedure can contribute a new method to analyze vitamin C in multivitamin effervescent tablets in Vietnamese pharmacopoeia, which is more convenient, accurate and easy to apply by industrial scale.

II. MATERIALS AND METHODS

1 Instrumentation

All experiments were performed on a HPLC system of Dionex Ultimate 3000 (USA) equipped with an auto sampler and Phenomenex Lunar C_{18} column (250 x 4.6mm, 5µm) coupled with temperature control system for column (25^0C) in the Laboratory of analytical chemistry-drug quality control and toxicology department, pharmacy faculty, Can Tho University of Medicine and Pharmacy.

2 Chemical and materials

Methanol was acquired from Baker, USA. Phosphoric acid and Potassium dihydrophosphat was from Merck, Germany. All chemicals and reagents used in this study were at analytical grade.

Vitamin C (L-(+)-Ascorbic acid), purity of 99.5% was used as standard. It was obtained from Institute of drug quality control Ho Chi Minh city, batch: 016 070614, stored at requirement conditions.

We obtained 4 kinds of multivitamin effervescent tablets which contained vitamin C from 4 brand names, we coded them as following names:

X1, product of Viet Nam. Batch used: 680914B, 330914B, 630914B. Labeled claim of vitamin C: 60 mg. Other ingredients: Vitamin B1, B6, B12, Biotin, Acid Folic,...

X2, product of Poland. Batch used: 26408, 53518, 06717. Labeled claim of vitamin C: 60 mg. Other ingredients: Vitamin B1, B5, B6, B12, Vitamin E, Zinc, Selen,...

X3, product of VietNam. Batch used: 1408230, 1405156, 1406250. Labeled claim of vitamin C: 75 mg. Other ingredients: Vitamin B1, B2, B5, B6, B12, PP, B9, B8, Vitamin E, Zinc, Copper,...

X4, product of Germany. Batch used: CM28088, CM26445, CM27221. Labeled claim of vitamin C: 500 mg. Other ingredients: Vitamin B1, B2, B5, B6, B8, B12, PP, Calci, Magie,...

3 Solution preparation

Solvent to dilute samples: Buffer of phosphat pH 3.0, perpared by dissolve 3.4 g of Potassium dihydrophosphat into 900 mL of distiled water, adjust to pH 3.0 by Phosphoric acid 4% and add distiled water to 1000 mL.

Standard solutions: A stock solution of standard vitamin C 300 µg/mL was prepared by dissolving in a 25 mL volumetric flask an accurately weighed amount of approximately 7.5 mg of L-(+)-Ascorbic acid, then filled to assign volume by phosphat buffer pH 3.0. This solution was kept at 5^0C. Subsequent dilutions were performed in order to obtain standard working solutions at the concentration of 60 µg/mL and 30 µg/mL: respectively diluted 2mL and 1mL standard stock solution 300 µg/mL to 10mL in volumetric flasks by phosphat buffer pH 3.0.

Preparation of pharmaceutical sample**:** for each batch, twenty tablets were weighed and ground to a fine powder. An equivalent of the powder corresponding to 60 mg of vitamin C was weighed and disolved in a beaker by 20 mL of phosphat buffer pH 3.0, then adjusted to pH 3.0 by H_3PO_4 4%. This solution was degased and diluted to 100 mL in a volumetric flask (solution A).

Solution used for method development: dilute 1 mL solution A into a volumetric flask of 10 mL. This solution was then filtered through filter paper with 0.45 µm before injection.

Solution used for validation: dilute 0.5 mL solution A into a volumetric flask of 10 mL. The concentration of vitamin C in pharmaceutical sample about 30 µg/mL. This solution was then filtered through filter paper with 0.45 µm before injection.

4 *Optimization of chromatographic conditions*

Different compositions of mobile phases were investigated. Supposing mobile phases:

MeOH : solution of acid H_3PO_4 4%, pH 4.0 , proportion of 5:95 (1)

MeOH : solution of acid H_3PO_4 4%, pH 3.5 , proportion of 5:95 (2)

MeOH : solution of acid H_3PO_4 4%, pH 3.0 , proportion of 5:95 (3)

Injection volume: 20 µL.

Wavelengh of detector: UV-Vis at 248 nm.

Flow rate: 1.0 mL/min.

The optimization was obtained when chromatographic parameters meet requirement such as theoretical plate number (N> 5000), resolution (Rs>1.5), asymetry (0.8 < As< 1.5) and repeatability (RSD of retention times and peak areas were less than 2%).

5. Method validation

This final method was subsequently validated according to the ICH guideline Q2 (R1) [3] with respect to specificity, linearity range, limit of detection (L.O.D) and limit of quantitation (L.O.Q), accuracy and precision.

6. Method application

The established analytical method was then applied to determine the four batches of vitamin C. Base on the content of vitamin C on Labeled claim, the procedure was then diluted within linearity range.

III. RESULTS AND DISCUSSIONS

1. Optimization of the mobile phase conditions

Vitamin C (ascorbic acid) is a natural water soluble vitamin and also a weak acid which has pKa at first grade equal 4.2 [4]. To Henderson–Hasselbalch's equation at pH = pKa-1, acid form of a weak acid is 10 times more of those at base form. We transfer vitamin C to acid form because at base form (ascorbat) it is easier to be oxidated to dehydroascorbic acid, this will lower concentration of vitamin C, thus lower its absorbance.

UV spectra's of vitamin C:

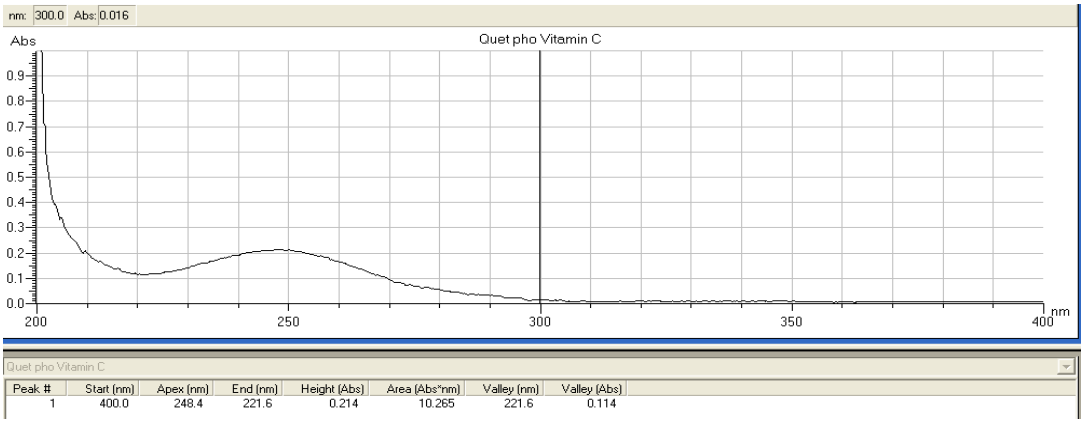

Figure 2: UV spectra's of vitamin C

From the UV absorption spectra, the vitamin C's peak was at 248 nm. Therefore, the maximum number and the height of the peak could be obtained and the baseline of chromatogram was stable at 248 nm. Thus, 248 nm was chosen as detection wavelength.

Supposing mobile phases results: Chromatograms from 3 supposing mobile phases

MeOH : solution of acid H_3PO_4 pH 4.0 , proportion of 5:95 (1)

MeOH : solution of acid H_3PO_4 pH 3.5 , proportion of 5:95 (2)

MeOH : solution of acid H_3PO_4 pH 3.0 , proportion of 5:95 (3)

were presented as following figure 3

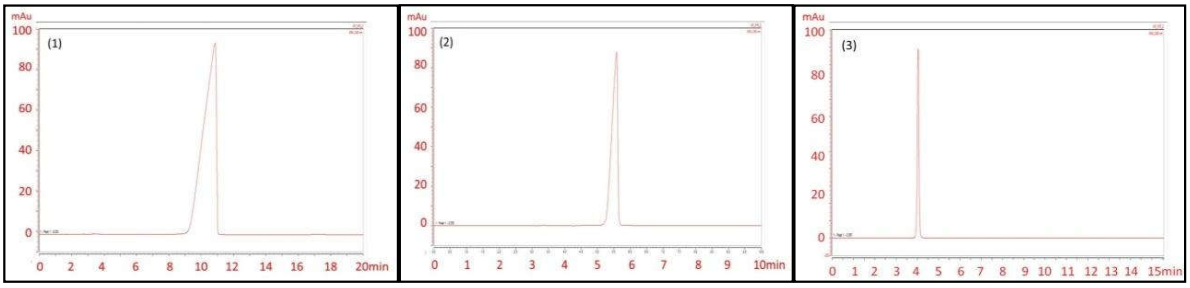

Figure 3: Representative HPLC chromatograms of mobile phase (1), (2), (3)

With the first mobile phase condition, isocratic of MeOH:H_3PO4 pH 4.0 (5:95, v/v), peak from vitamin C had rather much retention time (about 10 minutes) and broaden peak. Thus, this was not ideal mobile phase. Trying the second mobile phase, isocratic of MeOH:H_3PO_4 pH 3.5 (5:95, v/v), peak from vitamin C had good improvement about retention time but the shape of peak was still broaden and asymetry was out of limit. So we went to the next mobile phase. The third mobile phase by isocratic of MeOH:H_3PO_4 4%, pH 3.0 (5:95, v/v) gave good parameters for vitamin C peak in standard solution. All retention time, shape, asymetry... of peak were in the limit. Thus, it was chosen as optimum chromatography condition. Besides that, to a research of Uprety et al. (1963) [8], in pH range of 2.0-8.0 vitamin C was most stable at pH 3.0. When added weighed powder of commercial tablet to buffer pH 3.0 we could recognize that pH of solution rose and when this solution was injected to optimized chromatographic condition, the RSD was out of acceptant limit (RSD > 2%). We adjusted pH of sample solution to pH 3.0 by H_3PO_4 acid before filled enough volume to volumetric flask, this made RSD of all peak' parameters under 2%.

Compared to some previous research of vitamin C [1], [6], [7], [9], this mobile phase was better and more simple because it did not contain salt forms, which could lead to deposition in column and machinery.

From above results, the best chromatographic condition was:

- HPLC system of Dionex Ultimate 3000.
- Mobile phase: MeOH : solution of acid H_3PO_4 pH 3.0 , proportion of 5:95.
- Column: Phenomenex Luna C_{18} (250 mm; 4,6 mm; 5 µm)
- Injection volume: 20 µL
- Flow rate: 1.0 mL/min.
- Column temparature: 25^0C
- Wavelengh of detector: 248 nm

2. Method validation

2.1 System suitability

System suitability was tested by performing six replicate injections and determining theoretical plate number (N), resolution (Rs), symmetrical factor (As) and repeatability (RSD of retention times and peak areas) for the analytes of interest. The %RSD values of peak area and retention time for all peaks were less than 2% indicating the precise analysis of vitamin C by this system. All the results showed that the proposed method met the requirements.

Table 1. Result from system suitability of standard and sample solution (n=6)

	T_R	S	Rs	N	A_S
Vitamin C from standard solution					
Average	4.05	62.15	4.40	13781	0.96
RSD%	0.14	1.67	1.76	1.25	1.82
Vitamin C from tablet X1					
Average	4.03	60.25	9.72	12248	0.98
RSD%	0.1	1.39	1.17	1.01	1.71

T_R: retention time. S: peak area. Rs: resolution. N: theoretical plate number. As: symmetrical factor.

2.2. Specificity (selectivity)

The selectivity was tested by applying the HPLC method to analyze pharmaceutical sample. It was evaluated by comparing the retention time of standard compound with that of the respective peaks obtained by analyzing samples. The HPLC method was able to discriminate vitamin C from the other constituents of the material (other vitamin, ingredient, etc..). There was no interference with the peaks of vitamin C in pharmaceutical sample (shown in Fig. 4).

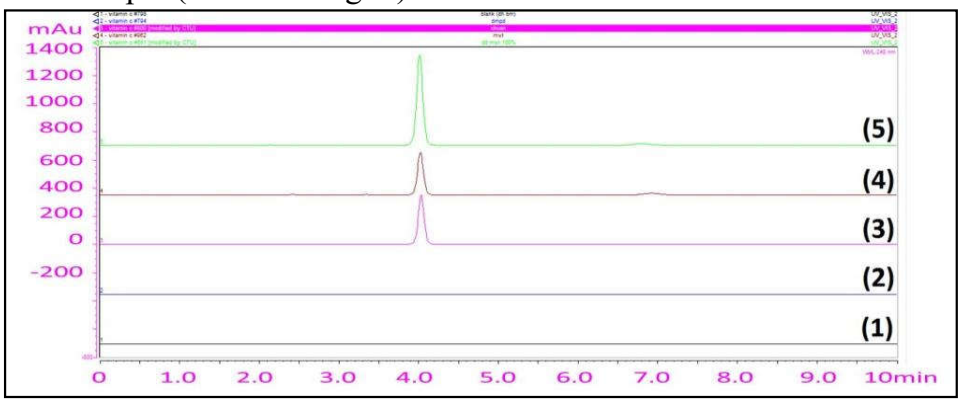

Figure 4: Representative HPLC chromatograms of standards and pharmaceutical sample at 248 nm. (1) mobile phase, (2) dissolving solvent, (3) vitamin C standards, (4) pharmaceutical sample, (5) sample spiked with vitamin C.

2.3 Precision

Intra-day precision was calculated from six replicate injections in the same day while inter-day precision was based on six injections on three consecutive days. The relative standard deviation (RSD) values for both intra-day and inter-day precision were below 2%. illustrating the good precision of the development.

Table 2. Result from precision of 4 products

Product	RSD	
	Intra-day precision	Inter-day precision
X1	0.64% 0.64%	0.75%

Product	RSD	
	Intra-day precision	Inter-day precision
	0.92%	
X2	1.32% 0.86% 0.65%	0.98%
X3	1.99% 1.96% 1.82%	1.92%
X4	0.73% 1.63% 1.88%	1.49%

2.4 Accuracy (Recovery)

Accuracy was calculated as the percentage recovery of a known amount of standard added to the sample. Standard vitamin C solution was added to commercial sample solution and analyzed by the proposed method. As can be seen from the following results, the proposed method was accurate over the investigated concentration range since the difference of tolerance limits of RSD did not exceed the acceptance limit.

Table 3. Accuracy results of vitamin C in tablets

Product	Amount standard added to sample					
	80%		100%		120%	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
X1	100.90%	1.11%	98.62%	0.26%	101.52%	0.20%
X2	99.83%	1.10%	101.15%	0.32%	100.42%	0.88%
X3	100.03%	1.24%	100.13%	0.79%	100.64%	1.14%
X4	99.66%	1.48%	100.06%	0.74%	99.48%	1.25%

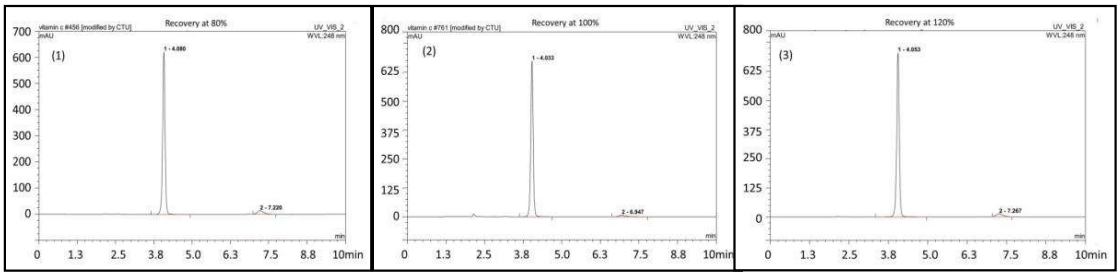

Figure 5: Representative HPLC chromatograms of vitamin C accuracy: recovery at 80, 100 and 120%, respectively (1), (2), (3)

2.5 Limit of detection - Limit of quantitation

Signals from diluted standard solution (S) and blank solution (N) were collected to make S/N ratio. The limit of detection (LOD) was tested till S/N ratio reached 3/1 then S/N at 10/1 was the limit of quantitation (LOQ).

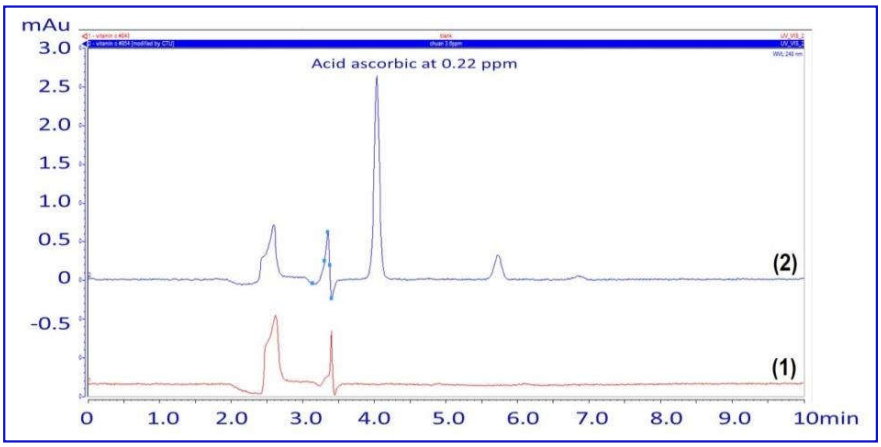

Figure 6: Chromatogram from blank solution (1) and standard solution of vitamin C at 0.22 µg/mL (2)

Figure 6 showed the limit of detection was 0.22 µg/mL. Therefore, the limit of quantitation was 0.73 µg/mL (LOD x 3.3).

2.5 Linearity of concentration

Five sets of concentrations at 15 µg/mL. 30 µg/mL. 60 µg/mL. 90 µg/mL and 120 µg/mL of standard vitamin C solution were made and the peaks area were plotted to obtain the linear line. The five-point line was found to be linear (Table 4) and gave good correlation coefficient.

Table 4. Peak area of standard vitamin C solution at 5 concentrations

Concentration (µg/mL)	S (mAu.min)
15.35	16.89
30.70	32.94
61.41	65.45
92.11	97.36
122.81	131.30

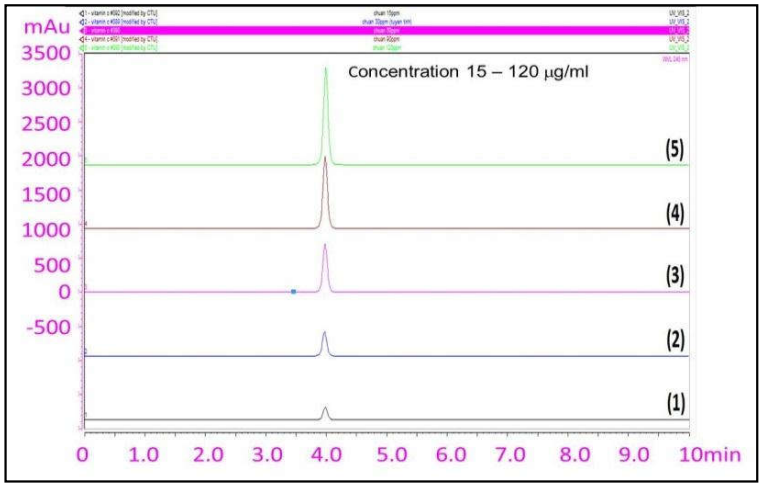

Figure 7: Representative HPLC chromatograms of vitamin C in rage of concentration 15 – 120 µg/ml

3. Method application

The optimized proposed HPLC method was applied to the determination of vitamin C in 4 commercial effevescent tablets. Standard solution was used at concentration of 30 µg/mL, sample solutions from tablets were diluted to the same concentration of standard solution.

Peak areas from standard and sample solutions were collected and the amount of vitamin C in tablets were calculated by this formula:

$$X = \frac{St \times mtb \times D \times 100}{1,0617 \times P \times 1000} \text{ (mg)}$$

With:

X: average amount of vitamin C in 1 tablet (mg)

St: peak area of vitamin C in sample solution (mAU.min)

mtb: average mass of 1 tablet (g)

D: degree of dilution

P: amount of powder taken from tablet (g)

The results of the tablets are summarized in Table 5.

Table 5. Assay results from 4 commercial tablets

Product		**X1**	**X2**	**X3**	**X4**
Labeled claim of vitamin C		60 mg	60 mg	75 mg	500 mg
Assay results	Batch 1	56.66 mg	65.71 mg	69.81 mg	503.11 mg
	Batch 2	56.02 mg	63.54 mg	69.59 mg	501.51 mg
	Batch 3	56.61 mg	64.54 mg	68.67 mg	498.43 mg
	Average	56.43 mg	64.60 mg	69.36 mg	501.02 mg
	Average RSD	1.18%	0.75%	1.87%	1.39%

IV CONCLUSIONS

In conclusion, the presented HPLC method is novel, simple, selective, cost-effective, reproducible, and can be reliably used by almost every drug laboratory. The method enables determination of vitamin C in pharmaceutical preparations within 7 minutes. With isocratic mobile phase of MeOH:H_3PO_4 pH 3.0 (5:95, v/v), using Phenomenex Lunar C_{18} column (250 mm; 4.6 mm; 5 µm). This method could be implemented in laboratories that require time and labor saving. Finally, the method was applied to the analysis for four products of multivitamin effervescent tablets.

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ENDOVASCULAR INTERVENTION FOR ACUTE ISCHEMIC STROKE: A REVIEW OF RECENT TRIAL IN CANTHO UNIVERSITY OF MEDICINE AND PHARMACY

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ABSTRACT

Introduction: *Acute cerebral ischemic stroke is a severe condition with high rate of mortality and morbidity. Treatment for ischemic stroke could be intravenous thrombolysis or catheter based revascularization. In many institutes, intravenous thrombolysis was used regularly for the ischemic stroke patients who come to the hospital within 3 hours since the onset. In other big institutes, endovascular revascularization including mechanical thrombectomy with or without intra-arterial thrombolysis, angioplasty with or without stenting has been increasingly used as one of the treatments for cerebral thromboembolism. In this article, we are reporting our outcomes of endovascular intervention as a treatment for ischemic stroke with catheter based thrombectomy, angioplasty and stenting.* ***Method and subjects:*** *All cerebral ischemic stroke patients who come to Can Tho University of Medicine and Pharmacy Hospital within 8 hours from the first symptom would be included. National institute of health stroke (NIHSS) scale were used for stroke grading which should be 10 or above. Diagnoses were confirmed by plain computed tomography (CT) of brain and cerebral computed tomography angiography (CTA) or magnetic resonance imaging (MRI) of the brain. Routine biochemical pre-op investigations were conducted and the patients were screened for other comorbidities. Cerebral angiography would be indicated when CT angiography showed occlusion at carotid artery, M1 middle cerebral arteries and basilar arteries. Vascularization procedure would be performed with penumbra thrombus aspiration system. In cases that needed angioplasty and stenting, gateway and saphire balloons were used for vessel dilation. Wingspan and carotid wallstent were used for intra and extracranial stenoses respectively. Post-procedure cares were conducted in combination with anesthesiology department, endovascular intervention team and medical physicians for at least 2 weeks.* ***Results:*** *A total of 28 patients were recruited from 1st January 2016 to 30th of March 2017 (15 months). Male and female ratio were 1.54:1(17/11). Ages ranged from 38 to 90 (mean 64). NIHSS score ranged from 10-24. 100% (28/28) of patients had comorbidity with hypertension (23/28), diabetes (6/28), atrial fibrillation (13/28), mitral valve stenosis and insufficiency (1/28). 22/28 patients had*