QUANTIFICATION OF VERAPAMIL HYDROCHLORIDE IN GASTRIC FLOATING TABLETS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Vu Van Tuan[1], Trinh Nam Trung[2], Nguyen Van Bach[2]

SUMMARY

Objectives: *To establish and validate the methods to assay verapamil hydrochloride (VH) in gastric floating tablets by high performance liquid chromatography (HPLC).* ***Materials and methods:*** *The method was developed according to the monograph of VH extended-release tablets in the USP 43; the method was validated based on the ICH guidelines, including System suitability, selectivity, linear range, repeatability, accuracy, the limit of detection (LOD), the limit of quantification (LOQ).* ***Results:*** *Chromatographic analysis was performed on a Sun Fire™ (RP-C18, 4.6 x 250 mm, 5 µm) with a mixture of acetonitrile and phosphate buffer adjusted to pH 3.6 with o-phosphoric acid (44:56, v/v) as a mobile phase, at a flow rate of 1.0 mL/min, and PDA detection at 278 nm. LOD and LOQ of the method were respectively 0.14 µg/mL and 0.47 µg/mL, the linear range was from 10 to 40 µg/ml with the repeatability and accuracy within the permissible limits.* ***Conclusion:*** *The established HPLC method is appropriate and reliable enough to quantify VH in gastric floating tablets.*

** Keywords: Verapamil hydrochloride; HPLC method; Gastric floating tablets.*

INTRODUCTION

Verapamil hydrochloride is a drug in the 1st generation calcium channel blocker for the treatment of cardiovascular diseases used regularly because of its wide indications. Oral VH is absorbed approximately 90%, but bioavailability is only about 20% because of extensive first-pass metabolism [1, 2]. In order to maintain the drug concentration in the blood at the therapeutic threshold, the gastric floating dosage form is a new direction of interest at present [3]. However, in Vietnam, no quantitative method has been developed and tested for reliability for gastric floating tablets containing VH. This study was conducted in order to contribute to developing a suitable and reliable method for quantification of VH in gastric floating tablets towards application in research and quality control of this new dosage form.

[1]*Dai Nam University*

[2]*Vietnam Military Medical University*

Corresponding author: Vu Van Tuan (vuvantuandkh@gmail.com)

Date received: 05/10/2021

Date accepted: 15/10/2021

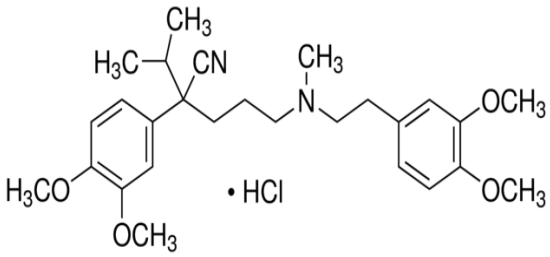

Figure 1: Structural formula of VH.

MATERIALS AND METHODS

1. Materials and equipment

** Materials and chemicals:*

- Standard: VH 100.52% (Calculated on the as is basis, Control Number: 060909), national institute of drug quality control Vietnam.

- Samples: 120 mg VH floating tablets are prepared at the Faculty of Pharmacy (Dai Nam University) by wet granulation method. The formulation includes VH 120 mg; HPMC K4M 58.98 mg; HPMC E6 35 mg; $NaHCO_3$ 29.31 mg; avicel PH-101 50 mg; lactose 98.71 mg; Magnesium stearate 8 mg; PVP K30 10% dissolved in ethanol is sufficient.

- Acetonitrile and methanol (HPLC-Merck), and other chemicals were analytical grades.

** Equipment:*

- HPLC system Alliance Waters 2695D (USA) including 4 solvent channels, autosampler, detector UV2487 and oven, pH meter - Mettler Toledo (Switzerland), analytical balance - Sartorius (readability 0.1 mg).

2. Methods

** Develop quantitative methods:*

The method was developed by referring to the extended-release VH monograph of USP 43 [4], specifically described as follows:

- *Standard solution:* Weigh accurately about 20 mg of VH into a 100 ml volumetric flask and dissolve just enough 100 ml with methanol. Take exactly 1.0 ml of the prepared solution into a 10 ml volumetric flask, dilute to 10.0 mL with methanol, and this find solution was filtered through a 0.45 µm membrane filter to obtain a standard solution with a concentration of about 20 µg/mL.

- *Test solution*: Weigh 20 tablets, calculate average weight, grind to fine powder, accurately weigh powder corresponding to about 20 mg VH into a 100 mL volumetric flask, add 50 mL of methanol, shake sonicate for 30 min, to stabilize to room temperature, add enough methanol to 100.0 mL, filter through filter paper, discard the first 20 mL of filtrate. Transfer 1.0 mL of the filtrate to a 10 mL volumetric flask, add to volume with methanol, mix well, filter through a 0.45 µm membrane filter to obtain the test solution.

- *Chromatic conditions:*

+ Mobile phase: Acetonitrile: buffer KH_2PO_4 0.05M pH 3.6 (44: 56, v/v)

+ SunFireTM column (4.6 x 250 mm; 5 µm).

+ Detector 2998 PDA set at 278 nm.

+ Flow rate: 1.0 mL/min.

+ Injection volume: 20 µl.

- *Calculate:* The ratio of the VH content of the test tablet to the amount stated on the label (P) is calculated according to the following formula:

$$P = \frac{S_t.C_s.m_{20}}{20.S_s.m_t.120}.100\ (\%)$$

Where: S_t, S_s is the area of the peak VH on the chromatogram of the standard and test solution, respectively (µV.s); C_s is the actual concentration of the standard solution for injection chromatography (µg/ml); m_t is the weight of powder weighed (g); m_{20} is the weight of 20 random tablets for quantification (g).

** Method validation:*

The process of validating the quantitative method was carried out according to the guidelines of ICH [5]:

- *Systematic suitability:* Dissolve the standard VH in methanol to obtain a concentration of 20 µg/mL, perform chromatography 6 times with the same standard solution.

- *Specificity*: Prepare test solutions, standard solutions as described in the analytical method. The placebo sample was prepared as a test sample but replaced the powder with a proportional mixture of excipients according to the formulation.

- *Linear range:* Dissolve exactly 20 mg of the standard in just enough 100.0 ml of methanol to obtain a stock standard solution with a concentration of about 200 µg/ml. From the stock standard solutions, dilute with the same solvent to a series of standard solutions with concentrations of 10, 15, 20, 25, 30, 40 µg/mL. Analyze a series of standard solutions according to the developed method. VH peak area was used as a response signal to prepare the calibration curve.

- *Repeatability:* Conduct analysis of 6 test solutions prepared in parallel according to the developed method. Determine the relative standard deviation (RSD) of the analytical results.

- *Accuracy:* Standard addition test solutions are prepared by accurately weighing a quantity of powder corresponding to approximately 10 mg VH (9 samples) into a 100 ml volumetric flask, adding 6.0 respectively; 10.0; 14.0 mg VH standard and 50 ml methanol, sonicated for 15 min, add enough methanol to make up the volume. Filter, take 1.0 mL of the filtrate and dilute to 10.0 mL with methanol. Perform chromatography of the 9 standard addition test solutions, the standard solutions, and the test solutions. From the analysis results, calculate the recovery of the method at 3 concentrations of 80, 100, and 120% of the quantitative concentration.

- *Limit of detection (LOD) and limit of quantification (LOQ):* Record the noise signal of the placebo sample several times, calculate the standard deviation (SD). LOD = 3SD/standard curve slope; LOQ = 10SD/standard curve slope.

RESULTS AND DISCUSSION

1. System compatibility

The VH peak areas determined from 6 repeated injections of the VH standard solution with a concentration of 20.31 µg/mL are presented in table 1.

Table 1: Results for 6 times analysis with a standard solution.

No	Retention time t_R (min)	Peak area (µV.s)	Asymmetry factor	Number of theoretical plates
1	5.168	302293	1.28	11781
2	5.142	296075	1.26	11322
3	5.121	301500	1.27	11712
4	5.106	291912	1.26	11630
5	5.092	301613	1.27	11856
6	5.063	301164	1.27	11938
Average	5.11	299092	1.26	11706
RSD (%)	0.72	1.397	0.593	1.85

The results of table 1 show that the average retention time of VH is 5.11 minutes with an average peak area of 299092 µV.s; the asymmetry coefficient is 1.26, and the theoretical number of disks is about 11700. The RSD of the retention time is 0.72 (less than 1%). The RSD of peak area, asymmetry, and number of theoretical plates are all not more than 2%. This result meets the requirements of ICH [5], proving that the chromatographic system used is suitable and stable for quantitative analysis of VH.

2. Specificity

The chromatograms of the placebo solution, the standard solution, and the test solution are shown in figure 2.

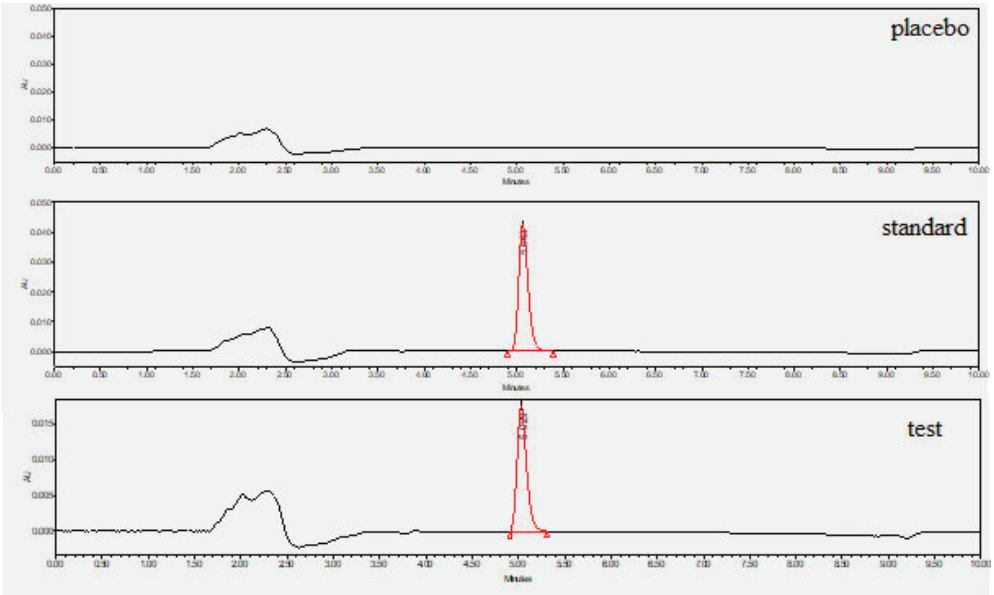

Figure 2: Chromatograms of standard, test solutions, and placebo.

The results of figure 2 show that the main peak in the standard solution chromatogram and the test solution chromatogram have the same retention time at 5.11 minutes. On the chromatogram of the placebo solution, no peak was observed around the time of 5.11 minutes. On the test solution chromatogram, the scanning spectrum at the top and foot of the peak has the same shape as the scanned VH spectrum in the standard solution chromatogram. This result proves that the method has been developed specifically for VH analysis, the presence of excipients in the samples does not affect the analytical results.

3. Linear range

The peak areas of the series of standard solutions with concentrations from 10.15 to 40.61 µg/mL are shown in table 2 and figure 3.

Table 2: Peak area of the VH standard solution series.

No	1	2	3	4	5	6
Concentration (µg/mL)	10.15	15.23	20.31	25.38	30.46	40.61
Peak area (µV.s)	149159	228581	301462	376100	456675	605692

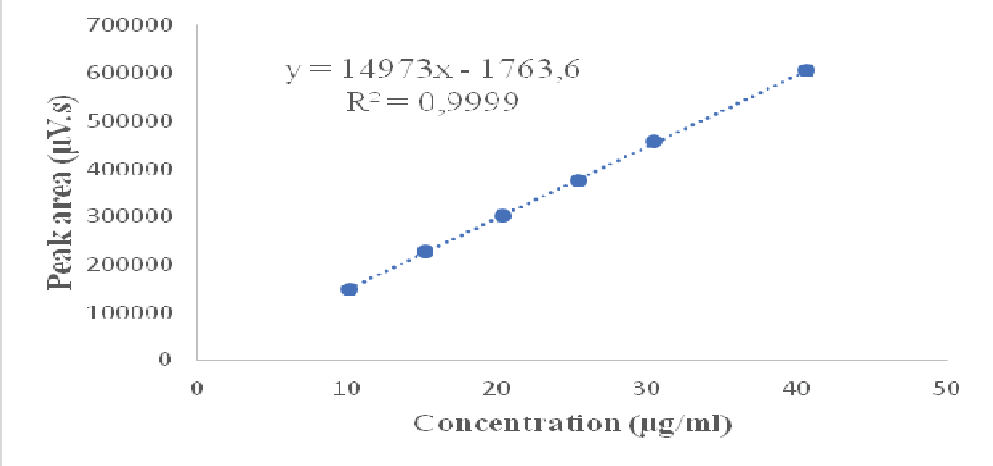

Figure 3: Graph shows the dependence of peak area on VH concentration.

The results of figure 3 and table 2 show that the standard curve has the equation y = 14973x - 1763.6; it has the coefficient R^2 = 0.9999 in the range from 0.99 to 1. This confirms that there is a close linear dependence between the peak area and the concentration of VH in the solution in the concentration range from 10.15 to 40.61 µg/mL. The degree of influence (F) of the cut-off coefficient was 0.59% (not more than 2%). Therefore, the developed method can be used for the quantification of VH by comparing the peak areas of the test solutions and the standard solutions whose concentrations are in the linear range.

4. Accuracy

The area of the VH peak on the test solution chromatogram was 304088 µV.s. The area of the VH peak on the standard solution chromatogram was 301620 µV.s. The results of the standard addition test solutions analysis are shown in table 3.

Table 3: Results determine recovery rate at 3 different concentrations.

No	Added (mg)	Peak area (µV.s)	Found (mg)	Recovery (%)	Average (%)	RSD (%)
1	6.11	242894	6.06	99.14	99.07	0.91
2	6.11	242717	6.11	99.93		
3	6.11	243318	6.00	98.13		
4	10.18	303528	10.11	99.32	99.77	0.91
5	10.18	305543	10.10	99.17		
6	10.18	305340	10.26	100.81		
7	14.26	366083	14.41	101.07	100.94	0.60
8	14.26	367097	14.30	100.29		
9	14.26	369178	14.47	101.48		

The recovery rate at different concentrations from 98.13 to 101.48 was in the range of 98 - 102% compared to the standard amount added; RSD obtained from 0.60 to 0.91% were not more than 2%, proving that the developed method ensures accuracy in the range of 80 -120% of the quantitative concentration required by ICH [5].

5. Repeatability

The VH standard solution at a concentration of 20.31 µg/mL has a measuring signal of 301620 µV.s. The peak areas of the six test solutions carried out in parallel are shown in table 4.

Table 4: Quantitative results of 6 test samples.

No	Weight of materials (mg)	Peak area (µV.s)	Recovery ratio (%)	Statistic
1	68.1	301663	101.96	Average recovery ratio = 102.57% RSD = 1.01%
2	68.8	305020	102.04	
3	67.5	304957	103.99	
4	68.5	301112	101.18	
5	68.9	307762	102.81	
6	67.6	303739	103.42	

The results of table 4 show that: The RSD of the quantitative results of 6 parallel samples was 1.01%, this value was less than the acceptance limit of 2%. This proves that the method ensures the repeatability requirement according to the guidelines of ICH [5].

6. Limit of detection (LOD) and limit of quantification (LOQ)

On the chromatogram of the blank sample, the background signal was measured in the period from 4.8 min to 5.4 min. The results are recorded in table 5.

Table 5: Results of determination of LOD and LOQ.

No	Background noise (µV.s)
1	2905
2	4316
3	3450
Mean	3557
SD	711.6

Table 5 data shows that: The standard deviation of the signal measured on the placebo background at the retention time position of VH was 711.6 µV.s. From that, the limit of detection (LOD) and limit of quantification (LOQ) of the method with VH were 0.14 µg/mL and 0.47 µg/mL, respectively.

CONCLUSION

The study has developed and validated a method to quantify VH in gastric floating tablets by HPLC as follows: Standard VH or powder are dissolved in methanol to obtain a concentration of about 20 µg/mL, perform chromatography and compare the peak areas obtained. Chromatographic conditions include SunFireTM column (4.6 x 250 mm; 5 µm), ultraviolet detector at 278 nm, mobile phase consisting of acetonitrile and phosphate buffer pH 3.6 ratio 44:56 (v/v), flow rate: 1.0 mL/min, sample injection volume 20 µL at room temperature. The method is also fully validated according to the ICH guidelines on validating analytical methods [5]. The validation results show that a reliable method can be applied to evaluate the quality of gastric floating tablets containing VH.

REFERENCES

1. Ministry of Health. Vietnam's National Pharmacopoeia. Medical Publisher 2017; 435-436.

2. Sweetman S. C. et al. Martindale 36th, RPS Publishing, 2009:1421.

3. Shaha S. H. et al. An overview of a gastro-retentive floating drug delivery system. Asian Journal of Pharmaceutical Sciences 2009; 4(1):65-80.

4. The United States Pharmacopeial Convention, USP 43: VH Extended-Release Tablets 2020.

5. International Conference on Harmonisation, ICH Q2(R1). Validation of Analytical Procedures: Text and Methodology 2015.