HPLC Method Development and Validation for Simultaneous Quantification of Vitamins C and K₃ in Hard Gelatin Capsules for their Potential Benefit in Postoperative Total Joint Arthroplasty

Yousif Rojeab*, Mohamad Hassoun¹, Ellen Hazelet¹ and Deirdre Myers²

¹Department of Pharmaceutical and Biomedical Sciences
²Department of Pharmacy Practice, College of Pharmacy, Ohio Northern University, Ada, Ohio, USA

Abstract

Efficient, selective, rugged, simple and sensitive isocratic RP-HPLC method for simultaneous quantification of vitamins C and K₃ was developed and validated. This method consisted of UV-detection at 254 nm and separation by a reversed-phase (RP)-C₁₈ column. Mobile phase was composed of 50% methanol, 49% water and 1% glacial acetic acid, at flow rate of 1 mL/min. Injection volume (100 µL) consisted of varied concentrations of both vitamins (0.5 – 50 µg/mL) mixed with vitamin E (internal standard) in 1:1:2 volume ratio. Clear baseline resolution was achieved for all three compounds with retention times of 1.9, 3.3 and 4.3 min for vitamins C, K₃ and E, respectively. The method exhibited excellent linearity over the entire concentration range for both vitamins with R² of ≥ 0.9991. Intra-day (n = 6) accuracy ranged from 92.2 - 102.0% and 99.4 - 106.7% for vitamins C and K₃, respectively, while those for the inter-day assays (n = 12) ranged from 96.9 - 99.1% and 91.7 - 100.4%. This HPLC method was successfully implemented in quantification of vitamins C and K₃ in compounded capsules containing the two ingredients to ensure content uniformity. For vitamin C, content was found to be 101 ± 4% (mean ± standard deviation) of the label claim of 500 mg. As for vitamin K₃ content was found to be 87 ± 6% of the label claim of 5 mg. These capsules were subsequently administered orally in a clinical trial aimed at evaluating any beneficial effect(s) of this vitamin mixture on postoperative total joint arthroplasty.

Keywords: UV-HPLC, Isocratic, Vitamin C, Vitamin K₃, Simultaneous quantification

Introduction

Arthritis comprises more than 100 different rheumatic diseases, the most common of which is osteoarthritis. Common symptoms include pain, stiffness, and swelling in or around the joints. It is estimated that 50 million U.S. adults report doctor-diagnosed arthritis, which, consequently, is America’s leading cause of disability [1]. Total joint Arthroplasty (TJA), also known as total joint replacement, is a highly successful and definitive reconstructive treatment for sufferers of severe arthritis of the major joints. About one million Americans each year elect to undergo TJA to enjoy the benefits of joint pain relief, restoration of joint function and an overall increase in the quality of life.

Despite TJA’s proven clinical success, a small but significant number of patients fall victims to procedure failure. These failures are frequently attributed to a detrimental biological/inflammatory response triggered by the generation of wear micro-particles that dislodge from the prosthetic implant surfaces [2-4]. Particle-induced inflammatory aseptic loosening of total joint prosthetic components remains the greatest threat to the longevity of the artificial device [4-6]. This aseptic loosening occurs when the bone bed adjacent to the implant, and to which the implant is attached, is resorbed leaving a fibrous tissue-filled gap between the bone and prosthetic device. With the loosening of the device, there is a return of pain and loss of function.

It has already been demonstrated that a combination of vitamins C (ascorbic acid) and K (in the form of K₁₂ menadione) in a ratio of 100:1 exhibited anti-tumor activity both in vitro and in vivo [7]. This is due to the effective antioxidant and anti-inflammatory properties of this combination. Therefore, it is suggested that if we could extrapolate those anti-inflammatory effects by administering this vitamin combination to people suffering from joint pain and inflammation due to TJA, we could see potentially significant therapeutic benefits in these situations. For this purpose,
a standard operating procedure (SOP) was developed and implemented by our Pharmacy Skills Lab for the compounding of hard gelatin capsules containing this proprietary ratio of vitamins C and K. Those capsules were to be administered in a clinical trial initiated by Summa Health System and Crystal Clinic Orthopaedic Center in Akron, OH at evaluating the potential for the aforementioned clinical benefits of this vitamin combination.

Once compounded, an essential quality control test to be performed on these capsules is content uniformity analysis. This quality standard ensures that the finally formed capsules contain the specified amount of each of the two active ingredients. It also ensures consistency of the preparation/compounding procedure and it quantifies any possible batch-to-batch content variation(s). While High Performance Liquid Chromatography (HPLC) analytical methods, in both aqueous and plasma samples, already exist for quantification of vitamins C [8-11] and K [12-14] whether individually or in combination with other components, no method currently exists for simultaneous quantification of these two vitamins in a single, simple and efficient HPLC run. This is due, primarily, to the significant difference in their solubilities and consequently retention times on the HPLC column. While 1 gram of Vitamin C (ascorbic acid) dissolves in about 3 mL water, vitamins K (menadione) and E (α-tocopheryl acetate; used as the internal standard) are practically insoluble in water [15]. The three vitamins’ chemical structures are depicted in Figure 1.

For this reason, a water-soluble form of vitamin K₃ (menadione sodium bisulfite) was utilized in this study. While this form of vitamin K₃ did address the solubility issue of this otherwise lipid-soluble vitamin, still no method currently exists in literature for simultaneous quantification of the two vitamins (C and K₃) in a single, HPLC run. Therefore, the objective of this research was two-fold: first, to develop and validate an isocratic RP-HPLC method for simultaneous quantification of vitamins C and K₃, and second, to apply this method in content uniformity analyses of the different batches that were compounded to contain this vitamin mixture which was subsequently administered orally in a clinical trial aimed at evaluating any beneficial effect(s) of this vitamin mixture on postoperative TJA.

### Experimental

#### Chemicals and reagents

Vitamin C (in the form of sodium ascorbate, USP, pure material) was purchased from Letco Medical (Decator, AL) while Vitamin K₃ (in the form of menadione sodium bisulfite, ≥95%) was supplied by the Apatone Development Center (Akron, OH). Vitamin E (in the form of α-tocopheryl acetate 500 IU/gm; internal standard; I.S.) was obtained from PCCA® (Houston, TX). HPLC-grade water and methanol were purchased from Fisher Scientific (Fair Lawn, NJ) and were used in preparation of the mobile phase, calibration curve solutions, quality control (QC) samples and in preparation of vitamin C- and K₃-containing analytical solutions upon capsule content uniformity analyses. Reagents (glacial acetic acid used for mobile phase preparation and dimethylsulfoxide (DMSO) used for preparation of the I.S. stock solution) were manufactured by EMD® Chemicals and were purchased from Fisher Scientific, USA.

#### HPLC conditions

This RP-HPLC method consisted of UV detection (Waters Model® 2487, Milford, MA) at 254 nm and separation by XTerra® RP-C₁₈ column (4.6 x 150 mm; 5 µm). An in-line degasser/filter (polypropylene, 0.2 µm from Whatman®, Florham Park, NJ) was connected between the mobile phase reservoir and the pump (Waters Binary System Model® 1525). Mobile phase was composed of methanol, water and glacial acetic acid (50:49:1), at a flow rate of 1 mL/min at room temperature of 20°C. Injection volume of 100 µL (via Waters Model® 717 auto-sampler) consisted of varied concentrations of both vitamins (0.5 - 50 µg/mL) mixed with the I.S. in 1:1:2 volume ratio. Total run time of the assay was 6 min. This HPLC system was operated by Breeze™ Software (Waters Corp., Milford, MA, USA).

#### Capsules compounding procedure

Compounding of the vitamin C- and K₃-containing capsules was performed using a semi-automatic ProFill® 3006 bench-top capsule filling machine (Custom Capsules, Pvt. Ltd., India). Briefly, pre-determined weights for vitamins C and K₃ were measured for every batch of 300 capsules (a total of 31 batches were prepared). Then, vitamin K₃ was triturated in a mortar and pestle and combined with pre-ground vitamin C using geometric dilution; a pharmaceutical process implemented to thoroughly mix two ingredients that exist in a given formulation in different proportions where first, the smaller amount, vitamin K₃ is thoroughly mixed with an equal amount of vitamin C. Then, this amount is mixed with an equal amount of the remaining vitamin C and the process is continually repeated until no pure vitamin C is left over. The powder mixture was then poured into a V-type multi-purpose mixer (Gallipot Inc., St. Paul, MN) and rotated for 20 minutes. After that, empty hard gelatin capsules (size 1 white capsules, Gallipot Inc., St. Paul, MN) were loaded onto the filler unit of the capsule filling machine after which capsule caps were removed with the aid of a caps tray. Capsule bodies were evenly filled with the vitamin mixture by hand spreading the powdered material back and forth across the filler unit with occasional tamping to pack the mixture into the capsule bodies. Lastly, the caps tray was placed back on top of the filler unit and capsule locking was achieved by pressing the two trays together. Once formed, capsules were poured onto a clean, dry surface, visually inspected and were subject to weight variation tests where 6 batches of 10 capsules each as well as 10 individual capsules were randomly selected from each batch and weighed on an electronic balance (weight variation allowed was ≤ ± 5% of target value). If more than individual capsule or any

![Figure 1: Chemical structures of (a) vitamin C (ascorbic acid), (b) vitamin K₃ (menadione) and (c) vitamin E (α-tocopheryl acetate; internal standard).](image-url)
one batch of 10 capsules fell out of this range, all 300 capsules in
that batch were weighed, individually, and those out of range
were rejected. All finished capsules were then kept refrigerated
at 2-5°C in properly labeled amber vials from which 5 capsules
were randomly selected from each batch for subsequent content
uniformity analysis within a few months (preliminary stability
data has indicated no significant degradation of either vitamin at
this storage temperature for up to 10 months).

Preparation of calibration curves and content uniformity analyses

Stock solutions of vitamins C, K₃, and I.S. were prepared by
dissolving 100 mg of the vitamin in 1 mL water, mobile phase
and DMSO, respectively (due to their different solubilities [15]),
aided by vortexing until complete dissolution. For vitamins C
and K₃, proper serial dilution scheme, with the mobile phase,
was followed to prepare the working solutions and obtain the
final concentrations for the 5-point calibration curves of 0.5,
1, 5, 10 and 50 µg/mL for each vitamin. As for the I.S., serial
dilution with the mobile phase was followed to achieve a final
concentration of 62.5 µg/mL (this value was chosen because
UV absorbance for the I.S. at this concentration under the HPLC
conditions applied (*wavelength of 254 nm*) resulted in peak
height comparable to those of the mid-range concentrations of
vitamins C and K₃, within the calibration curve which was deemed
appropriate). Since this method was designed for simultaneous
quantification of vitamins C and K₃ in a single run, calibration
curve samples consisted of "paired" concentrations consisting
of the lowest concentration of 0.5 µg/mL of each vitamin for
the first point. The next point on the calibration curve consisted
of a final concentration of 1 µg/mL of each vitamin and so on.
These final concentrations were obtained after accounting for
the dilution factor involved upon mixing the two vitamins and
the I.S. in 1:1:2 volume ratio. Below is a summary of the final
concentrations of calibration standards used in quantification
of vitamins C and K₃ (Table 1).

For quantification of vitamins C and K₃ in capsules from the
different batches, 100 mg of the capsule content was weighed
dissolved in 1 mL water and properly diluted with the mobile
phase to within the range of the calibration curve, mixed with the
I.S. and injected (a dilution factor of 1/2000 was appropriate for
this purpose and was corrected for upon calculating the content
% for each vitamin in the capsule). All calibration, batch and QC
samples were prepared in medical-grade polypropylene micro-
centrifuge tubes (Axygen Scientific Inc., Union City, CA) at room
temperature of ~20°C.

Method validation

The method was validated for selectivity, linearity, Limits of
Detection (LOD) and quantification (LOQ), accuracy, precision
and ruggedness. For purposes of evaluating those validation
parameters, peak height ratios of the analyte (vitamin C or K₃)
normalized by that of the I.S. were used. Linear regression
analysis was followed where those ratios were plotted against
the corresponding analyte concentration and a straight-line
equation of the general formula: \( Y = mx + b \) was generated for
each calibration curve where \( Y \): analyte/I.S. peak ratio; \( m \): slope
of the line; \( x \): analyte concentration and \( b \): \( y \)-intercept. Intra-day
(\( n = 6 \)) and inter-day (\( n = 18 \)) calibration curves were constructed
over a period of 5 months and it was from those preparations
that the linearity represented by correlation coefficient (\( R^2 \)), was
established over the calibration curve range (final concentrations
of 0.5, 1, 5, 10 and 50 µg/mL for each vitamin).

In this method, selectivity was defined according to Dadgar
D and Burnett PE [16], as the ability of the analytical method to
accurately differentiate the analyte from other components in
the mixture. This was achieved by evaluating the chromatograms
for each one of the 3 components (vitamins C, K₃ and the I.S.)
individually and comparing those to chromatograms containing
all 3 components together. LOD and LOQ were calculated to
account for the signal-to-noise ratio [17] where LOD = 2 (H/h)
and LOQ = 10 (H/h), where H is the peak height corresponding to
the analyte; h is the absolute value of the largest noise fluctuation
from the baseline of the chromatogram of a blank solution (in our
case the mobile phase). The Upper Limit of Quantification (ULQ)
was determined by injecting increasingly higher concentrations
of each vitamin until signal saturation was observed (i.e., no more
proportional increase in the peak height upon increasing the
analyte’s concentration).

Accuracy of an analytical procedure expresses the closeness
of agreement between the value which is accepted as either
a conventional true value or an accepted reference value and
the values found (measured). Precision, on the other hand, of
an analytical procedure expresses the closeness of agreement
(degree of scatter) between a series of measurements obtained
from multiple sampling of the same homogenous sample under
the prescribed conditions [18]. For accuracy and precision
evaluations, independent runs (from freshly prepared stock
solutions for each vitamin) consisting of three concentrations
each: 2 (low), 8 (medium) and 40 (high) µg/mL within the
range of our calibration curve (0.5 - 50 µg/mL) were prepared
and injected upon mixing with the I.S. These injections were
considered QC standards and were used to access the intra-
(\( n = 6 \)) and inter-day (\( n = 12 \)) precision and accuracy.

Finally, ruggedness of an analytical procedure is the degree of
reproducibility of results obtained by analysis of the same sample
under a variety of normal test conditions, i.e., different analysts,
laboratories, instruments, reagents, assay temperatures, small
variations in mobile phase, etc. [17]. Ruggedness of our method
was evaluated where the 18 calibration curves were prepared by
2 analysts in our lab (12 prepared by one and 6 by the other), and
data generated was comparatively evaluated.

Results

Selectivity

Clear base-line resolution over the entire range of
concentrations encountered for vitamins C, K₃ and the I.S.
with no peak interference was achieved as evidenced by the chromatograms (Figures 2 and 3a-c). The retention times for vitamins C, K₃, and the I.S. averaged (mean ± standard deviation) 1.9 ± 0.003, 3.26 ± 0.05, and 4.30 ± 0.01 min, respectively, based

![Figure 2: Authentic chromatogram corresponding to vitamins C (retention time 1.9 min), K₃ (retention time 3.5 min) and E (I.S.; retention time 4.3 min) with clear baseline resolution. The peaks represent 3 concentrations (0.5, 1, and 5 µg/mL) of vitamins C and K₃ and that of the I.S. at 62.5 µg/mL.](image)

![Figure 3a: Overlay of the HPLC chromatograms from all 31 batches analyzed demonstrating high consistency in vitamin C content. Peaks for vitamin K₃ and the internal standard look much smaller than those in figure 1 because of the auto scale adjustment to the relative sizes of the peaks within the chromatogram.](image)

![Figure 3b: Up-close depiction of vitamin C peaks in figure 3a.](image)
on the intra-day variability (n = 30 each). As for the inter-day variability, corresponding values were 1.94 ± 0.01, 3.45 ± 0.08, and 4.33 ± 0.03 min, respectively, for the 3 vitamins (n = 90 each) (Table 2). A chromatographic run time of 5 min was sufficient for complete elution of all three compounds.

**Linearity, calibration, range, and limits of detection and quantification**

LOD and LOQ were found to be 0.008 and 0.040 µg/mL, and 0.009 and 0.046 µg/mL for vitamins C and K₃, respectively. ULQ was determined to be 500 µg/mL for vitamin C and 1500 µg/mL for vitamin K₃. The method exhibited excellent linearity over the entire concentration range for both vitamins within the calibration curve range (0.5 - 50 µg/mL) with R² values of 0.9991 and 0.9995 for vitamin C and 0.9994 and 0.9993 for vitamin K₃ from intra- (n = 6) and inter-day (n = 18) assays, respectively. Average linear regression equations for vitamins C and K₃ are provided in Table 3.

**Accuracy and precision**

For the intra-day assays (n = 6), accuracy ranged from 92.24 – 101.97% and 99.39 – 106.70% for vitamins C and K₃, respectively, while those for the inter-day assays (n = 12) ranged from 96.88 – 99.08% and 91.67 - 100.38% for the two vitamins, respectively (Table 4). The precision of the method, as represented by C.V. (coefficient of variation; also known as relative standard deviation; defined as (standard deviation / mean) x 100%) was calculated to be between 2.55 - 4.66% and 2.02 - 9.88% for the intra-day assays for vitamins C and vitamin K₃, respectively. Corresponding values for the inter-day assays were 5.41 - 9.26% and 2.12 - 5.78% for the two vitamins, respectively (Table 4).

**Application**

This method was applied for simultaneous quantification of vitamins C and K₃ to ensure content uniformity in capsules compounded to contain the two active ingredients in the predetermined weights. Thirty-one batches were analyzed, in triplicates, for vitamins C and K₃ content. A pre-determined range of 85 - 115% of the label claim was considered acceptable as per USP [19]. Batch preparations were carried out over a period of over 1 year. For vitamin C, content was found to be 101 ± 4% (mean ± standard deviation) of the label claim of 500 mg (range: 89.1 - 114.9%). As for vitamin K₃, content was found to be 87 ± 6% (mean ± standard deviation) of the label claim of 5 mg (range: 64* - 103%) (Figure 4).

This value for vitamin C content (%) was exceptionally high since the next highest was only 109%; closer to the collective range for all batches analyzed.

---

**Table 2:** Intra- and inter-day variability in retention times for all 3 components (vitamins C, K₃ and the internal standard (I.S.)) within the HPLC chromatogram.

<table>
<thead>
<tr>
<th>Component</th>
<th>Intra-day (n = 30)</th>
<th>Inter-day (n = 90)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rₜ (min)</td>
<td>C.V. (%)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1.94 ± 0.003</td>
<td>0.16</td>
</tr>
<tr>
<td>Vitamin K₃</td>
<td>3.26 ± 0.05</td>
<td>1.42</td>
</tr>
<tr>
<td>I.S.</td>
<td>4.30 ± 0.01</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*Rₜ:* Retention time; represented as mean ± standard deviation

*C.V. (%): Coefficient of variation percent = (mean / standard deviation) x 100%

---

**Table 3:** Linear regression equations for the two analytes, vitamins C and K₃.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Intra-day assays</th>
<th>Inter-day assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>$y = (0.772 ± 0.038) x - (0.60 ± 0.112)$</td>
<td>$y = (0.833 ± 0.065) x - (0.462 ± 0.196)$</td>
</tr>
<tr>
<td>Vitamin K₃</td>
<td>$y = (0.409 ± 0.012) x + (0.221 ± 0.016)$</td>
<td>$y = (0.401 ± 0.013) x + (0.235 ± 0.0678)$</td>
</tr>
</tbody>
</table>

*y*-variable in these expressions represents vitamin C or K₃ peak height over that of the I.S., while the x-variable represents vitamin’s concentration in µg/mL.
Table 4: Intra- and inter-day accuracy and precision for vitamins C and K₃

<table>
<thead>
<tr>
<th></th>
<th>Intra-day</th>
<th>Measured Conc. (µg/mL)</th>
<th>Accuracy (%)</th>
<th>C.V. (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>2</td>
<td>2.04 ± 0.05</td>
<td>101.97</td>
<td>2.55</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7.38 ± 0.20</td>
<td>92.24</td>
<td>2.65</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>40.32 ± 1.88</td>
<td>100.80</td>
<td>4.66</td>
<td>6</td>
</tr>
<tr>
<td>Vitamin K₃</td>
<td>2</td>
<td>2.13 ± 0.21</td>
<td>106.70</td>
<td>9.88</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8.28 ± 0.17</td>
<td>103.51</td>
<td>2.02</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>39.76 ± 1.18</td>
<td>99.39</td>
<td>2.98</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Inter-day</th>
<th>Measured Conc. (µg/mL)</th>
<th>Accuracy (%)</th>
<th>C.V. (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>2</td>
<td>1.96 ± 0.18</td>
<td>97.81</td>
<td>9.26</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7.75 ± 0.67</td>
<td>96.88</td>
<td>8.66</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>39.63 ± 2.14</td>
<td>99.08</td>
<td>5.41</td>
<td>12</td>
</tr>
<tr>
<td>Vitamin K₃</td>
<td>2</td>
<td>1.83 ± 0.11</td>
<td>91.67</td>
<td>5.78</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8.03 ± 0.17</td>
<td>100.38</td>
<td>2.12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>37.59 ± 2.08</td>
<td>93.98</td>
<td>5.53</td>
<td>12</td>
</tr>
</tbody>
</table>

†Defined as (measured conc./ theoretical conc.) x 100%
‡C.V. (%): Coefficient of variation percent; a representative of method precision, calculated as (mean / standard deviation) x 100%
§Number of replicates

Figure 4: Average content (%) for vitamins C and K₃ upon analysis of compounded capsules. Pre-determined acceptable range for content (100 ± 15%) is represented by dashed lines (error bars denote standard deviation).

This value for vitamin K₃ content (%) was exceptionally low and was regarded as an outlier since the next lowest was only 76%; closer to the collective range for all batches analyzed. The vitamin K₃ batch with only 64% of label claim was deemed unacceptable for inclusion in the clinical trial.

Discussion

The method described in this work was custom developed for simultaneous quantification of vitamins C and K₃ in a single HPLC run. In our case, hard gelatin capsules were purposely compounded to contain the two agents in a proprietary ratio. These capsules were subsequently administered orally to evaluate any potential therapeutic benefit(s) of this vitamin mixture on postoperative TJA. A key feature of this method was its simplicity, and hence, applicability whether in academic or industrial labs for similar sort of analyses. Efficiency was another feature of this method; while sample run time was set at 6 min, complete separation and base-line resolution for all three compounds (Vitamins C, K₃, and E) was achieved within 5 min indicative of high cost effectiveness ratio. Even when other groups reported methods for simultaneous quantification of hydrophilic vitamins such as vitamin C in presence of lipophilic ones such as vitamins A (in the form of β-carotene) and E, much longer run times of 15 and 25 min were necessary for complete elution [20,21]. Owing to the short retention time of our method, we were able to analyze large numbers of batches on any given day in a relatively short amount of time.

While acceptable range for accuracy should be within ± 15% of the expected true value [22], our method demonstrated high accuracy indicative of method reliability, at least within the concentration ranges selected, the selection of which was based on the expected vitamins' concentration encountered in the compounded capsules. Likewise, small variations in the intra- and inter-day assays for QC samples established the method's repeatability and high intermediate precision [18]. High sensitivity, represented by a 2-digit nano-gram per mL range for the LOQ, was another characteristic of this method although the lowest quantifiable concentration measured and analyzed in capsules was more than 10 times the LOQ for either vitamin. High sensitivity is attributed, at least in part, to the selection of a detection UV wavelength of 254 nm, which was in close proximity to maximum UV absorption for vitamin C of 245 nm [23] and that for vitamin K₃ of 252 nm [24], under acidic pH.

Though its robustness has not been thoroughly evaluated, this method demonstrated high degree of ruggedness [17] owing to the fact the calibration curves/QC samples for both vitamins were prepared by two analysts in our lab and data were pooled together. It is worth mentioning that all calibration curve points/QC samples were accounted for during the method development/validation (no points were dropped).

In this method, vitamin E was used as the internal standard. However, vitamin E is a common ingredient in multi-vitamin containing products so with slight modification, such as setting UV absorbance wavelength at 300 nm which has been shown to allow for maximum absorbance for vitamin E [21], this method could be effectively utilized for quantification of those three vitamins in a single, simple run. This method was successfully applied in simultaneous quantification of vitamins C and K₃ to ensure content uniformity in capsules compounded to contain a mixture of the two active ingredients and for the vast majority of batches, capsules' content fell within the pre-determined accepted range of 100 ± 15% of the label claim. It is this method's overall reliability that gave us confidence in our capsule compounding
procedure and in administering those capsules, except for those that failed the content uniformity testing, in the clinical trial for which they were prepared.

Conclusion

An efficient, selective, rugged, simple and sensitive isocratic RP-HPLC method for simultaneous quantification of vitamins C and K$_3$ was developed and validated. This HPLC method was successfully implemented in quantification of vitamins C and K$_3$ in compounded capsules containing the two ingredients to ensure content uniformity. These capsules were compounded for oral administration in clinical trial aimed at evaluating any beneficial effect(s) of this vitamin mixture on postoperative TJA.

Acknowledgement

We would like to acknowledge the contributions of the multiple PharmD students at The College of Pharmacy at Ohio Northern University who assisted in the compounding of the vitamin C- and K$_3$-containing hard gelatin capsules and for performing the necessary weight variation tests.

References

1. Arthritis: Meeting the Challenge of Living Well. CDC (Centers for Disease Control and Prevention). Atlanta, GA. 2012.