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Structural characterization and thermally induced isomerization investigation of cis- and trans-vitamin K₁ using ion mobility mass spectrometry†

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Since some organic isomers have different activity, choosing of an appropriate quantitative strategy for their reference material (RM) development is critical. Value assignment is the core process during RM production, and recently, mass spectrometry (MS) based quantitative methods are the primary approaches commonly adopted. However, due to the different chemical stability, the results obtained from MS and other non-ionized analysis methods might be inconsistent. For this argument, in this work, cis- and trans-vitamin K₁ was selected as they perform different spatial structure and bio-activity. Initially, liquid chromatography with detection of fluorescence and MS were respectively employed for quantitative comparison, and a 9.73% of isomer quantity difference was observed. Next, ion mobility coupled MS (IM-MS) was adopted for structural investigation of isomers ions. After optimization, isomer mixture was separated in drift tube within several milliseconds. Collision cross-section values calculated using IM-MS matched well with its theoretical value (2.43% of minimum deviation), suggested that the IM-MS method was reliable. Afterwards, by raising the desolvation temperature in ion source, the relative contents of trans-isomer had increased. Tandem mass spectrum of isomers resolved by chromatographic and IM were not identical. All the results indicated that in-source isomerization was occurred on vitamin K₁ isomers. Different thermodynamic stability of isomers might bring uncertainty to quantification results when using MS methods, thus, understanding of such in-source isomerization is conducive to choose an appropriate analytical approach and then develop isomer RM with higher accuracy.

It is clear that evaluation and selection an appropriate analytical strategy is the primary consideration for researchers before value assignment. In most cases, although MS or tandem MS based approaches present a predominant performance compared to other spectral analysis ways, the ionization process before mass spectrometric detection, might be an unfavorable factor to achieve the precise measurement results, especially for isomers with different activity. More specifically, if the chemical structure of target was in-source changed by some external forces, the quantitative result might not reflect the real quantity, namely result in an inaccurate quantification. In some cases, such changes could be ignored while isomers perform the identical function or activity. However, once isomers with different activity or function were generated in ion source, a more gentle alternative analytical approach without isomerization reaction should be considered. Consequently, non-spontaneous conformation alternation induced by analytical instruments should be noticed and need to be avoided as far as possible, particularly in value assignment for RM production.

cis- and trans-vitamin K₁ is an ideal organic compound for such investigation because they perform the different spatial structure and bio-activity for human. In practice, MS and fluorescence based detection methods have been well developed by many groups. Several of these vitamin K-dependent proteins, such as coagulation factors II, VII, IX, X,
protein C, and protein S, play critical roles in hemostasis.\textsuperscript{7–8} Recently, some papers reported that insufficient vitamin K\textsubscript{1} intakes could be a risk factor for Alzheimer disease or may contribute to an acceleration of the progression of Alzheimer\textsuperscript{1}’s disease.\textsuperscript{9,10} It is worth noting that vitamin K\textsubscript{1} synthesized by plants is found exclusively as the biologically active 2\textsuperscript{-}transisoromer. However, foods may contain appreciable quantities of the essentially non-active cis-isomer as a consequence of either its presence in synthetic K\textsubscript{1} used during food supplementation, or photo-isomerization of the trans-isomer during exposure of the food to light.\textsuperscript{11} The planar chemical structures of vitamin K\textsubscript{1} isomers are shown in Fig.1.

High performance liquid chromatography or liquid chromatography-tandem mass spectrometer (LC-MS or LC-MS/MS) analysis using C30 stationary phases is a routine way to identify the vitamin K\textsubscript{1} isomers, which requires approximate 20 minutes per run.\textsuperscript{2,11} Since gasification and ionization were not required, as well as higher sensitivity demanded, LC equipped with fluorescence detector became the favorable platform to analyze the isomers without any structure modification. It is clear that isomer ions detected in MS have the identical mass-to-charge data (m/z). Therefore, the resulting ion chromatograms of isomers could not reflect the in-source isomerization, namely it is not easy to characterize in-source reaction using a conventional MS method. Because of a traveling-wave ion mobility spectrometry (TWIMS) modular was designed between ion source and time-of-flight detector, the coupling of IM to MS (TWIMS-MS) provides a new dimension in the analysis of ion structures.\textsuperscript{12–14} As a powerful complementary analytical approach to nuclear magnetic resonance and X-ray crystallography, more recently, IM-MS has been widely applied in the field of structure characterization, such as in chemical warfare agents,\textsuperscript{15} peptides measurement,\textsuperscript{16} disease research,\textsuperscript{17} drugs\textsuperscript{18} and even protein complex.\textsuperscript{19,20}

Here, comparisons of vitamin K\textsubscript{1} and vitamin K\textsubscript{2} isomers by using HPLC with fluorescence detection and APCI/MS detection were carried out. The vitamin K\textsubscript{1} isomers mixture measured in this work was diluted with hexane from pure material, which would avoid matrix effect derived from sample matrix. Moreover, the effect of desolvation temperature on isomer structure as well as the potential bias in quantitative results were investigated. Compared with column separation, TWIMS could provide the valuable post-source structural information of target ions, which will be the direct evidence for ion shape characterization. The purpose of this study is to identify the possibility of thermal-isomerization on vitamin K\textsubscript{1} when employing MS analytical methods, and whether MS based approach is fit for vitamin K\textsubscript{1} isomer quantification, especially for RM development of vitamin K\textsubscript{1} or other structural analogues isomers.

Materials and methods

Chemicals and reagents

Isomeric mixture of vitamin K\textsubscript{1}, certified reference material of phytomenadione (Std.), formic acid (FA), ammonium formate (AF), urea, dithiothreitol (DTT), iodoacetamide (IAA), sequencing trypsin, bovine serum albumin (BSA) and horse myoglobin (MYO) were all purchased from Sigma-Aldrich (St. Louis, MO). Methanol, hexane and acetonitrile (HPLC-grade) were obtained from Merck (Darmstadt, Germany). The stock and working solution of 2.26 mg L\textsuperscript{-1} and 20.7 µg L\textsuperscript{-1} were prepared by dissolving an accurately weighted quantity of vitamin K\textsubscript{1} in hexane. Std. stock solution (1.31 mg L\textsuperscript{-1}) was prepared and gradient diluted by dissolving an accurately weighted quantity in hexane. Analytical balances (ME614S) and micro balances (SE2) were respectively used for weighting and samples (Sartorius, Germany). For minimizing isomerization and degradation, all solutions were stored in tinfoil covered vials no matter in storage (-20 °C) or experimental environment. Deionized water was obtained from a Millipore Milli-Q system (Bedford, MA).

Isomers separation on the basis of C30 bonded phase chromatography

The liquid chromatography separation of isomers was performed by an ultra-performance liquid chromatography (ACQUITY UPLC, Waters, MA, USA) and YMC Carotenoid C30 column (4.6×250 mm, 3 µm). Fluorescence detector (FLR) and APCI/MS or LCMS tandem mass spectrometry (TWIMS) was designed between ion sourc and desolvation gas temperature were set at 95 °C and 50 L h\textsuperscript{-1}. Cone gas and desolvation gas flow rate were set at 0.6 mL min\textsuperscript{-1} at a flow rate of 0.6 mL min\textsuperscript{-1}. For FLR analysis, isomers were detected with fluorescence after post-column reduction with metallic zinc column (50×4.6 mm, 50–70 µm) (LUBEX, Guangzhou, China), and excitation and emission wavelengths were set at 243 nm and 430 nm. The MS conditions were as follows: positive APCI with the capillary current was 3.5 µA, sampling and extraction cone voltage were 25.0 V and 4.0 V; source temperature was set at 95 °C and 400 °C; cone gas and desolvation gas flow rate were set at 50 L h\textsuperscript{-1} and 500 L h\textsuperscript{-1}.

Statistical analysis

Total area of cis- plus trans-vitamin K\textsubscript{1} was used for vitamin K\textsubscript{1} measurement. Linear correlation coefficients were used to compare vitamin K\textsubscript{1} or trans-vitamin K\textsubscript{1} concentrations, as
Analytical Methods ARTICLE

determined by the LC-FLR and LC-APCI/MS methods. Student’s paired t test was used for characterizing differences in target concentrations as determined by the methods described above. All statistical analyses were performed using Microsoft Excel 2010. Results were considered statistically significant if the observed significance value was less than 0.05 (p < 0.05).

Isomers separation on the basis of travelling wave ion mobility spectrometry

Ion mobility was carried out using a Synapt G2 equipped with TWIMS (Waters, Manchester, UK). In method development, sample was infused into the ion source at a flow rate of 10 μL min⁻¹ along with 50 μL min⁻¹ methanol (0.025% FA and 2.5 mM AF) from UPLC as a makeup solvent. Positive APCI parameters were set as described above. Ions were guided into the ion mobility cell that is flanked by a trap and transfer sub-cell, and the default collision energy of the two sub-cells were set at 6.0 V and 4.0 V to ensure the efficiency of ion transfer. The entrance to the mobility cell is preceded by a helium cell operated at high pressure to facilitate transport of ions into the mobility cell. In order to realize desired separation, key parameters including ion mobility gas flow rate, wave velocity and height were investigated. Data were acquired using Masslynx (V4.1, Waters) and processed using Driftscope (V2.1, Waters) software.

Isomerization investigation

Chromatographic separation of cis-/trans-vitamin K₁ working solution was carried out using C30 column. The liquid chromatography conditions were identical to those described above. Conditions of positive APCI were as described above. Ion mobility parameters including: trap gas flow rate, 5.0 mL min⁻¹; helium gas flow rate, 40 mL min⁻¹; ion mobility gas flow rate, 10 mL min⁻¹; wave velocity, 350 m s⁻¹; and wave height, ramping from 10.0 to 12.0 V. According to the retention time, injection of pure trans-isomer into APCI/IM-MS through valve switching. The effect of desolvation gas temperature on isomers structure was investigated from 300 to 600 °C with increments of 100 °C.

Collision cross-section measurement

Because of the collision cross-section (CCS) could not be calculated directly from TWIMS, a standard curve should be established at first. Clemmer et al. Group²³ have built a standard CCS database of 660 peptides generated by tryptic digestion of 34 common proteins using an ion trap IM-MS technique, which is an extremely useful tool for addressing this issue. In this assay, five peptides of BSA and MYO were selected and used for plotting the calibration curves. Protein enzymatic experiment was referred to our previous work.²⁴ Next, the peptides mixture was infused into APCI and analyzed with specific TWIMS setting. Calibration curves were plotted according to the values obtained from database and experimental results. And then, experimental CCS values of target ions could be calculated based on the different drift time (DT). The mathematical models used here was provided by the study of Smith et al.²⁵ The theoretical CCS values were calculated from the CCS Calculation software provided by Waters (Manchester, UK). The molecular model file was searched and downloaded from PDB website.

Collision-induced dissociation investigation

For the assays of product ions characterization, the working solution was infused into the ion source at a flow rate of 10 μL min⁻¹ along with 50 μL min⁻¹ methanol (0.025% FA and 2.5 mM AF) from UPLC as a makeup solvent. The parameters of TWIMS were identical to those described above. Ion of m/z 451.4 ([M+H]+) was selected and collision-induced dissociation (CID) was carried out in the region of transfer cell located between TWIMS and TOF detector. Tandem mass spectrum were recorded for molecular ions of the cis- and trans-forms of m/z 451.4 exiting from the ion mobility cell. Argon was used as the collision gas at energies of 5, 10, 12.5, 15, 17.5, 20, 25, or 30 eV.

Results and discussion

Isomers measurement

Along with the development of mass spectrometry, in recent decades, some sensitive determination methods for vitamin K₁ or vitamin K homologues have been developed. In practical, however, few reports concerned about the relative amount of cis- or trans-vitamin K₁ measured by different approaches. Here, concentrations of vitamin K₁ ranged from 8.25 to 122.8 µg L⁻¹ (82.5 to 1228 pg per one injection), which including the commonly measuring range for biological or dietary supplement samples, were respectively measured using UPLC-FLR and UPLC-APCI/MS methods with the external standard curves. As shown in Fig.2A, the concentrations measured by the two methods were compared using the paired t test and were not statistically different (p>0.106). Similarly, total concentrations of vitamin K₁ calculated from the two methods were highly correlated (R=0.995). In addition, comparison of trans-isomer was showed in Fig.2B. It is worth noting that the slopes of the two linear equations were different (0.873 of APCI/MS v.s. 0.788 of FLR method), suggested that the trans-isomer amounts measured by the two methods were different. For instance, when 100 pg of vitamin K₁ sample was injected, 87.3 pg and 78.8 pg of trans-vitamin K₁ were respectively obtained, approximate 9.73% of difference, whereas the total amount almost identical. Such finding was interesting.

In general, a bias quantitative result achieved from intra or inter measurement methods is common, especially for matrix based samples. The co-eluting compounds can positively or negatively affect the analyte signal within LC-MS or MS/MS analyses. Apparently, such bias was not caused by matrix effect in this assay. One explanation is that the compound structure might be changed during the ionization process. Therefore, separation and identification of vitamin K₁ isomer ions after ion source was the key step for this speculcation.

Development of IM-MS method and isomerization reaction

During the liquid chromatographic separation, the isomers were distinguished by column stationary phase according to molecular polarity; but for ion mobility technology, the target ions in the gas phase were differed by their shapes and conformations. As shown in Fig.3A, a baseline separation of cis-
and trans-isomer mixture was observed with Carotenoid C30 column. Chromatographic peaks of retention time of 13.5 min and 15.6 min were observed respectively corresponding to trans- and cis-vitamin K₁. As previously described, working solution was infused into APCI/IM-MS to facilitate tuning the relevant ions signals. Avoiding ions fragmentation and improving the ion mobility resolution were the main principles during method development. Compared with classic quadrupole TOF mass spectrometry, vacuum in ion mobility sub-cells need to be adjusted to avoid ion fragmentation by controlling the flow rate of helium and nitrogen. For this purpose, parameters of ion mobility cell have been optimized. Afterwards, as shown in Fig. 3B and 3C, separation of isomers was achieved, and the main parameters were listed in Table 1. Apparently, one entire analysis run usually needs approximate 20 minutes for chromatographic separation, but ion mobility separation only needs several milliseconds.

Ions of m/z 451.4 were selected and separated with the use of positive APCI/IM-MS, meanwhile, two peaks of m/z 451.4 were observed at different drift time. When using C30 column, the correspondence for chromatographic retention times and isomer peaks was clear according to many reports. But for ion mobility separation, the spinning isomer ions were moved forward in drift tube under the forces of electric and reversed ion mobility gas. Since the trans-form is nearly planar as well as cis-isomer is three-dimensional, and the smaller structure would spend less time to pass through the drift tube, it can be expected that peak a (a1 plus a2) and b (b1 plus b2) were respectively corresponding to trans- and cis-vitamin K₁. As described above, the comparison results suggested that thermal isomerization might be occurred during ionization process. For further investigation, according to the retention times, isomerically pure isomer was injected into APCI/MS only. The desolvation temperature used in this assay varied from 300 °C to 600 °C with the increment of 100 °C, and the peak area percentages of trans-vitamin K₁ under each temperature were detected and calculated by integration of their ion mobility spectrum.

As shown in Fig. 4A, peaks a and b appeared when extracting ion mobility spectrum of m/z 451.4. The percentages of trans-isomer (peak a) were slightly as desolvation temperature was elevated (Fig. 4B). For vitamin K₁ structure, two main groups were located on each side of the double bond, namely 1, 4-naphthoquinone and hydrophobic alkyl chain. Apparently, compared to trans-isomer, the main chemical groups in cis-isomer performed a bigger steric hindrance as they have a shorter spatial distance. Thus, the thermodynamic stability of them are different. Thermodynamically favored cis-vitamin K₁ had more energy than trans-isomer and performed a poor stability. Therefore, isomerization reaction on cis-isomer would be occurred much easier than did on trans-isomer when they under a high temperature surrounding, such as the ionization environment. Our previous work showed that although a lower desolvation temperature needed in electrospray ionization, a similar percentage variation on isomers had also been observed (Fig.S1). Meanwhile, for APCI ion source, the potential impact factors including of capillary current and source temperature, have been investigated. The results suggested that the relative percentages of isomer had not changed significantly (Fig.S2).

Here, it is worth noting that no matter what ratio of cis/trans-isomer injected into the ion source, a constant ratio of

![Fig.2 Comparison of the (A) vitamin K1 and (B) trans-vitamin K1 concentrations measured by the UPLC-APCI/MS method and UPLC with fluorescence detection after post-column reduction method (n=3)](image)

![Fig.3 The total ion chromatogram of liquid separation (A) and ion mobility spectrum (B and C) of vitamin K₁ isomers using different analysis approach.](image)
cis-trans-compound could be obtained under a specific temperature setting. As depicted in Fig. 3B, IM spectrum generated from trans-vitamin K\textsubscript{1} (13.5 min) and cis-vitamin K\textsubscript{1} (15.6 min) also including another isomer at same retention times, suggested that both isomers had isomerized in the ion source to get similar ratios of trans-vitamin K\textsubscript{1} (a1, a2) and cis-vitamin K\textsubscript{1} (b1, b2). The thermal isomerization might be the major reason to explain it. Although such structural variation could reached equilibrium, the quantification results of isomers would be incorrect. For instance, when carrying out quantitative research of vitamin K\textsubscript{1} isomers using APCI/MS or tandem MS approaches, thermally induced isomerization, which is an important factor that could affect the accuracy of the quantitative results, should be carefully considered.

**CCS calculation**

Calculation of molecular cross-section value is another significant advantage of IM-MS technology. Through the comparison of CCS values between experimental results and theoretical values, the developed IM-MS method in single lab could be evaluated objectively. Because different ion mobility settings corresponding to the different pressures filled in ion mobility cell, a calibration curve should be established when changing ion mobility parameters. Ions fragmentation might happen when they passing through a higher vacuum to a lower environment (namely from quadrupole to ion mobility cell). The flow rate of helium in sub-cell, which was designed for preventing ion fragmentation, was set at 40 mL min\textsuperscript{-1} here; meanwhile, 10 mL min\textsuperscript{-1} of IMS gas flow rate was adopted. The vacuum were respectively corresponding to 0.972 mbar and 0.727 mbar, which was an acceptable pressure combination. Next, calibration curves were generated. Five selected peptides were adopted for plotting the calibration curves, and the regression equations were y=8E-38 x\textsuperscript{1.7114} (R\textsuperscript{2}=0.9682) for BSA peptides and y=1E-31 x\textsuperscript{1.4323} (R\textsuperscript{2}=0.9915) for MYO peptides. The details were shown in the ESI Fig. S3 and S4.† All the data related to CCS values were listed in Table 1, and they were consistent with the isomers structural features (Theoretical CCS value of trans-vitamin K\textsubscript{1} was not given because the structure file was not available in PDB). The deviation of CCS values obtained from two curves were about

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**Table 1 Collision Cross-Section (CCS) values and TWIMS parameters**

<table>
<thead>
<tr>
<th>Isomer structure</th>
<th>Helium cell gas flow rate (mL/min)</th>
<th>IMS gas flow rate (mL/min)</th>
<th>Wave velocity (m/s) and height (V)</th>
<th>experimental CCS (±SD) (Å\textsuperscript{2})</th>
<th>theoretical CCS (Å\textsuperscript{2})</th>
<th>ΔCCS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis-</td>
<td>40</td>
<td>10</td>
<td>350, 10-12</td>
<td>172.91±0.89\textsuperscript{1}</td>
<td>177.21</td>
<td>2.43%</td>
</tr>
<tr>
<td>Trans-</td>
<td></td>
<td></td>
<td></td>
<td>171.73±1.06\textsuperscript{2}</td>
<td></td>
<td>3.09%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>188.92±0.84\textsuperscript{2}</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>190.89±1.01\textsuperscript{2}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The classical equation for CCS calculation was applied in Driftscope software with modifications as described by Smith et al.\textsuperscript{25}

\[
\Omega = \frac{(18\pi)^{\frac{1}{2}} ze}{16 (K_b T)^{\frac{1}{2}}} \left[ \frac{1}{m_1} + \frac{1}{m_N} \right]^{\frac{1}{2}} \frac{760}{P} \frac{T}{273.2} \frac{L}{N_D} \frac{1}{P} \Delta E
\]

Ω, the CCS of an analyte ion; \(z\), the number of charges on the analyte ion; \(e\), the charge on an electron; \(K_b\), Boltzmann constant; \(T\), temperature (K); \(m_1\), molecular mass of the analyte ion; \(m_N\), molecular mass of the IMS gas; \(N\), the number density of the IMS gas; \(k\), the mobility of analyte ion. \textsuperscript{2}Calibrated by using BSA peptides. \textsuperscript{3}Calibrated by using MYO peptides.

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Fig.4 Effect of desolvation gas temperature on the abundances of cis- (peak b) and trans-isomers (peak a) of vitamin K\textsubscript{1} vitamin K\textsubscript{1} (b1, b2). The thermal isomerization might be the major reason to explain it. Although such structural variation could reached equilibrium, the quantification results of isomers would be incorrect. For instance, when carrying out quantitative research of vitamin K\textsubscript{1} isomers using APCI/MS or tandem MS approaches, thermally induced isomerization, which is an important factor that could affect the accuracy of the quantitative results, should be carefully considered.
2.43% and 3.09%, suggested that the IM-MS method developed in this paper was convinced.

**Tandem mass spectrum resolved by liquid chromatographic and ion mobility**

Compared with conventional MS or MS/MS analysis, TWIMS could provide ion structure information in gas phase rapidly since isomers can be separated by ion mobility in milliseconds timescale. In addition, since in-source isomerization occurred prior to IM separation, CID occurred on transfer cell could provide an opportunity to determine if isomers can be distinguished based on their fragmentation patterns. As depicted in Fig. 5, no matter what fragmentation energy was used, the product ions of cis- and trans-isomers of vitamin K$_1$ were similar. As discussed above, since in-source isomerization was occurred after chromatographic separation, each ion chromatogram peak resolved by C30 column should contained a little portion of another isomer. Such post-column reaction might be the main reason for producing similar MS/MS spectrum. Therefore, the conventional tandem mass spectrum could not be used for isomers identification. Instead, when ion mobility function was operated, some differences were observed.

Meanwhile, the product ions in different drift time were extracted (IM-MS/MS). Although both isomers generated product ions of identical masses, the relative abundances of these ions varied considerably. When 5 eV of fragmentation energy was used, the base peak in the tandem mass spectrum of cis-vitamin K$_1$ corresponding to an ion of m/z 128.1, which was formed by the loss of a terminal C$_{9}$H$_{19}$ group. But for trans-isomer, the base peak was the molecular ion of m/z 451.4. Meanwhile, trans-isomers have a much more stability than cis-isomers under each CID energy, these results were also in accordance with their structural characteristics. Meanwhile, for both isomers, naphthoquinone ions of m/z 187.1 in IM-MS/MS spectrum fragmented much more extensively than did in MS/MS mode. It indicated that although the helium gas filled in front of IM cell could maintain ions integrity, the different vacuum among each section still little unfavorable for ion transfer. Tandem mass spectrum of IM was helpful for isomer structure investigation. It was hard for people to distinguish the isomers through conventional APCI-MS/MS method without chromatographic separation. Moreover, the comparison between product ions obtained from the two methods is another proof for in-source isomerization.

**Fig.5 Positive APCI-MS/MS (up) and APCI-IM-MS/MS (down) CID spectrum of the [M+H]$^+$ ions of m/z 451.4 corresponding to cis-vitamin K$_1$ and trans-vitamin K$_1$**

**Conclusions**

As a powerful analytical tool, TWIMS was applied for isomerization investigation of vitamin K$_1$ in this paper. A series assays were carried out on the basis of TWIMS, and the results suggested that in-source isomerization was occurred in positive APCI ionization process. Although cis/trans isomerization could not be eliminated, this temperature dependence suggested that lower desolvation temperature can minimize cis/trans isomerization. Such conclusion does not make much sense for total amount quantification, because vitamin K$_1$ measured using the two methods had matched well. But for vitamin K$_1$ isomers, since thermally induced
isomerization was occurred easily while using MS method, the quantification results of isomer were inaccurate.

It is actually a harsh process when solution-state molecular converting into gas-state ion, and the gaseous ions of vitamin K\textsubscript{i} isomers are somewhat structural changed. Since no harsh environment during fluorescence detection process, it will be the better analytical method for quantification of vitamin K\textsubscript{i} geometrical isomers, until thermal-isomerization can be eliminated during ionization process. Reference material is the source of quantity value transfer widely used in most quantitative assays. Therefore, choosing of a reliable and stable analytical approach is critical for its production. However, if stable isotope labeled vitamin K\textsubscript{i} isomer reference material was produced using fluorescence method and used as internal standard, the measurement results obtained from MS will be reliable.

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Reference

Description:
Vitamin K$_1$ isomers separation on the basis of APCI/ion mobility mass spectrometry