

An Improved Method of UPLC-PDA-MS/MS Analysis of Lycopene Isomers

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Abstract The aim of the study was to develop a rapid method for analysis of lycopene extracted from tomato puree using UPLC. Further, conditions were optimized to obtain characteristic ions to distinguish lycopene isomers by using APCI-MSMS mode. Chromatographic separation of (all-E)-lycopene and (Z)-lycopene isomers were separated by reversed-phase CSH Phenyl-Hexyl column with short run time and good resolution. Radical cation $[M]^+$ of (all-E)-lycopene and major (Z)-lycopene isomers upon collision-induced dissociation (CID) produced a number of characteristic ions; (13Z)- and (15Z)-lycopene isomers were characterized by the loss of 15 Da (CH_3) to form ions of m/z 521, from the molecular ion m/z 536. In addition, fragmentation patterns of (all-E)-/(Z)-lycopene were also compared with (all-E)- β -carotene with similar molecular mass. Selective CID coupled with UV-Visible spectra was used to differentiate the geometrical isomers of lycopene. The assessment could be helpful in the analysis of carotenoid isomers.

Keywords CSH Phenyl-Hexyl column · UPLC-APCI⁺-MSMS · Collision-induced dissociation

Introduction

Lycopene is a tetra-terpenoid red pigment rich in tomatoes; structurally, it consists of 11 conjugated and 2 non-conjugated

double bonds, which makes them superior antioxidant than β -carotene and α -tocopherol in terms of its singlet oxygen quenching ability (Britton 1995; Rao and Agarwal 2000). Lycopene research has been growing rapidly, and epidemiological studies have demonstrated that the reduction of cardiovascular diseases and prostate cancers is associated with consumption of a tomato-rich diet (Rao and Agarwal 2000; Giovannucci 2005; Beilby et al. 2010; Talvas et al. 2010). Naturally, lycopene is present predominantly in the (all-E) form and readily undergoes isomerization or degradation during processing of tomatoes. However, more than 50 % of lycopene present in human serum and tissues are in (Z) forms (Stahl et al. 1992; Clinton et al. 1996). Several studies have shown the isolation of lycopene from tomatoes by solid-liquid, supercritical fluid (Choksi and Joshi 2007), and pressurized extraction methods (Naviglio et al. 2008). Based on the available literature, to our knowledge, reported extraction procedures were laborious and expensive. Hence, this study reports a simple extraction method to obtain highly pure lycopene with limited steps. Routinely, analyses of lycopene in food and biological samples were done with HPLC-PDA methods (Ferreira et al. 2000; Lyan et al. 2001). Carotenes and major xanthophylls have overlapping absorbance spectra from 445 to 473 nm, which makes their analysis more complex to differentiate carotenoids. Similarly, HPLC separation of (Z)-lycopene isomers also reported to be highly complex, even by using C_{30} chromatography (Emenhiser et al. 1995; Emenhiser et al. 1996). Hence, development of an appropriate method for qualitative and quantitative analysis of lycopene isomers would help in reducing the possibility of interference of carotenoids and their isomers other than the lycopene and ambiguity in the HPLC analysis. The stability studies have shown the isomerization and degradation of (all-E)-lycopene, when exposed to light, oxygen, and high temperatures (Schierle et al. 1997; Anguelova and Warthesen 2000). In addition, they also illustrated that the degradation of lycopene

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under various exposure conditions created the artifacts in the quantification of each carotenoid concentration. Previously, chromatographic separation of carotenoids was based on HPLC analysis by using C₁₈ and C₃₀ columns, which consume more time and solvents (>30 min) (Lin and Chen 2003; Braun et al. 2006; Matsumoto et al. 2007; Bijttebier et al. 2014). Recently, UPLC-MS approaches have been made for the analysis of carotenoids in food and biological samples (Guzman et al. 2012; Delpino-Rius et al. 2014; Chauveau-Duriot et al. 2010). Analysis and spectral confirmation of carotenoids are based on characteristic UV-Visible spectra obtained by diode array detectors and MS data (Romero et al. 2007; Fang et al. 2003; Frenich et al. 2005). Further, tandem mass spectrometry analysis of carotenoids is found to be a more reliable and selective method. Therefore, in the present study, an attempt was made to develop a simple extraction procedure and analytical methods for the determination of lycopene from tomato puree. Investigation was performed by using ACQUITY UPLC coupled with PDA detector, due to its ability to speed the analysis and increase the resolution of chromatographic separation. In addition, mass spectral characteristics and fragmented ions were used as a tool to distinguish lycopene isomers and carotenes with similar molecular mass by using APCI⁺-MSMS.

Materials and Methods

Chemicals and Reagents

(All-E)-lycopene (>90 %), (all-E)- β -carotene (>99 %), ammonium acetate, toluene, and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (St Louis, MO). UPLC- and MS-grade acetonitrile and methanol were purchased from J.T. Baker (NJ., USA). Acetone, hexane, methanol, and tetrahydrofuran (THF) of HPLC-grade solvents were procured from Merck (Mumbai, India). Mixtures of (all-E) and (Z) isomers of lycopene were prepared by dissolving (all-E)-lycopene (isolated from tomato) in toluene and incubated in a water bath at 37 °C for 24 h under atmospheric oxygen condition with regular shaking (100 rpm). The purity of carotenoid isomers was verified by using characteristic absorption spectra (Emenhiser et al. 1995).

Extraction of Lycopene

Ripened stage tomatoes (Indian hybrid) were chosen randomly from a local supermarket; a known quantity of fruits was taken and washed with deionized water; and only epicarp portions were taken for the preparation of tomato puree using a grinding mixer. Extraction of lycopene was carried out by using the following solvent system: *solvent 1*, methanol/acetone/hexane in the ratio of 25:25:50 v/v/v was used as per the

existing procedures (Lin and Chen 2003; Gutierrez and Luque de Castro 2007). Further, *solvent 1* was modified into *solvent 2*, 25:50:25 and *solvent 3*, 50:25:25 v/v/v ratios, respectively, and compared the yield and purity of lycopene. Based on the analysis of lycopene content in these extracts, *solvent 2* was considered as a better solvent system for isolation. In brief, a portion of the tomato puree ($n=5$) (2.5 g) was mixed with *solvent 2* (25 mL) in the ratio of 1:10 with 0.1 % BHT (w/v) in ethanol added to minimize isomerization/oxidation. The sample extract was vortexed vigorously and kept in the dark at 4 °C for 20 min. Then, top hexane layer was separated by adding equal volume of deionized water in a separating funnel. These procedures were repeated for three times, otherwise until the hexane layer becomes colorless. The pooled hexane layers were collected and evaporated to dryness under N₂ gas, and the residue was redissolved in a known volume of THF (3 mL) for further aliquots. The extracted lycopene was quantified, and its % purity was checked by using UPLC with PDA detector. The peak identity, absorption maxima (λ_{\max}), and characteristics UV-Visible spectra of lycopene isomers were confirmed by UPLC-PDA detector. Extraction and preparation of samples and standards were carried out under dim yellow light to prevent isomerization and degradation.

UPLC Analysis of Lycopene

Chromatographic separation of lycopene was performed by using an ACQUITY UPLC[®] system (Waters Corp., Milford, MA) consisting of binary solvent manager and sample manager, coupled with PDA detector. Lycopene and its isomers were separated by using ACQUITY UPLC[®] CSH Phenyl-Hexyl column (100×2.1 mm; 1.7 μ m) and compared with ACQUITY UPLC[®] BEH C₁₈ (100×2.1 mm; 1.7 μ m) and ACQUITY UPLC[®] BEH shield RP18 columns (100×2.1 mm; 1.7 μ m) (Waters Inc., USA). Mobile phase contained 10 mM ammonium acetate in Milli-Q water (A) and 5 % of THF in acetonitrile (B) with flow rate of 0.4 mL/min and was monitored at 471 nm by using PDA detector. The gradient condition was maintained as follows: 25 % of A and 75 % of B solvents was fixed initially, then increased solvent B to 85 % in 3 min, 90 % in 8 min, and returned to 75 % in 8.2 min. Needle was washed with weak and strong solvents to avoid the cross contamination and analytical error during analysis. The injection volume was 2 μ L, column temperature was set at 45 °C in column heater, and sample manager was maintained at 4 °C. Lycopene was quantified from its peak area by plotting a calibration curve with analytical reference standard. The peak identity of the components was further confirmed by its characteristic UV-Visible spectra recorded with PDA detector. Data acquisition and processing were carried out by using Empower software (Waters, USA)

Method Validation

A stock solution of standard lycopene and β -carotene (1 mg/mL) was prepared in THF separately. Working standard solutions were aliquoted by diluting the stock solution with 50 % THF and 50 % acetonitrile to attain the concentrations ranging from 0.125 to 64 μ g/mL. These stock and working solutions were analyzed immediately or stored at -80 °C. The UPLC method was validated by using various parameters such as LOD (signal-to-noise ratio 3:1) and LOQ (signal-to-noise ratio 10:1), and reproducibility was checked with six replicates where the percent relative standard deviation is less than 5 % and the linear dynamic range of lycopene is greater than 0.998.

UPLC-MS Conditions

Qualitative analysis of carotenoids was done by using Waters Xevo TQD mass spectrometer interfaced with the ACQUITY UPLC[®] system via an APCI source operated in positive ion mode. An aliquot (2 μ L) of lycopene sample containing \sim 40 μ g/mL was injected onto the column. The gradient conditions used are the same as mentioned in the section UPLC analysis. Instrument was conditioned by the following parameters: corona voltage 0.9 kV, cone 35 V, RF 2.50 V, extractor 3.00 V, source temperature 150 °C, probe temperature 450 °C, cone gas flow 50 L/h, desolvation gas flow 900 L/h. Mass spectra of (all-E)-/(Z)-lycopene and (all-E)- β -carotene

were acquired with an m/z 50–1000 scan range. The MS identity of standard (all-E)-lycopene and (all-E)- β -carotene was compared with the extracted sample. Data were processed with Mass Lynx 4.1 software (Waters, USA).

MS/MS Analysis

Argon was used as collision gas, and collision-induced dissociation energies were acquired from 10 to 40 eV. The instrumental operating parameters for the tandem quadrupole were the same as mentioned in the MS conditions. Positive ion collision-induced dissociation of ions were recorded and compared for the molecular ions of (all-E)-/(Z)-lycopene and (all-E)- β -carotene. Different collision-induced dissociation energies were used, 10, 15, 18, 20, 30, and 40 eV, to check the fragmentation pattern. Tandem mass spectrometry (MS/MS) analysis was repeated three times to check the reproducibility.

Results and Discussion

In the present study, attempt was made to obtain natural lycopene to substitute commercial or synthetic. Among the three different solvent systems used for the extraction of lycopene content (mg/100 g wet weight), the quantitative results demonstrated that *solvent 2*, methanol/acetone/hexane (25:50:25, v/v/v) (9.01 ± 0.65), was a better solvent system when

Fig. 1 A typical UPLC UV-Visible chromatographic profile of analytical standard and lycopene extracted from tomato puree. 1, Lycopene epoxide; 2, (13Z)-lycopene; 3, (15Z)-lycopene; 4, (all-E)-lycopene; and 5, (all-E)- β -carotene (1.81 %) (a). UV-Visible overlaid spectra (b) and their mass spectra (c)

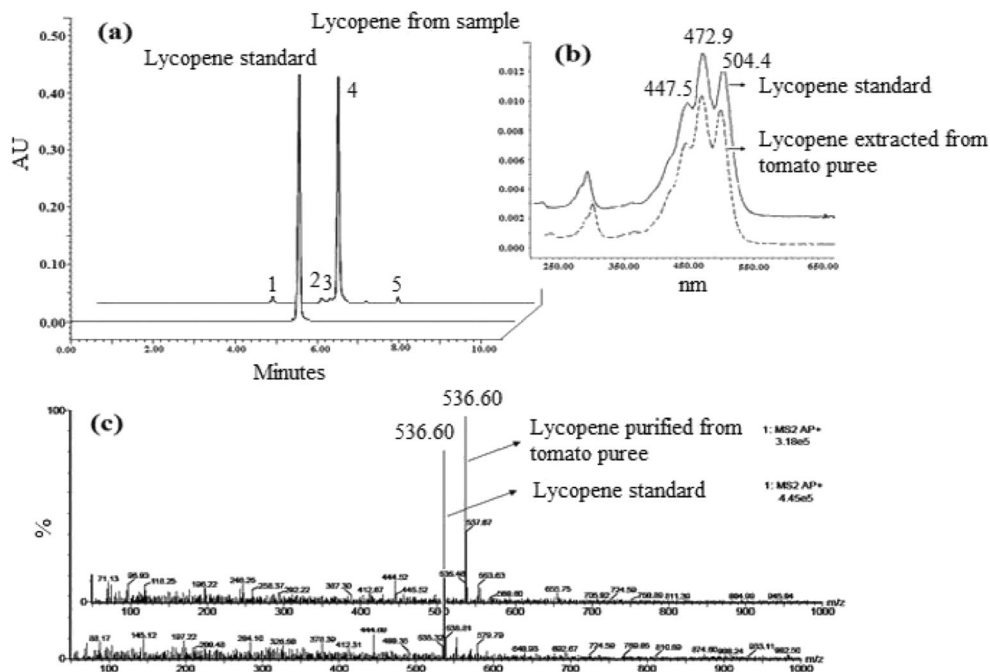


Table 1 Statistical parameters of (all-E)-lycopene by using UPLC-PDA analysis

Concentration of std ($\mu\text{g/mL}$)	Concentration of std (μM)	Nominal concentration injected on column (pmol)	Measured concentration (mean \pm SD pmol)	Accuracy (% deviation)	Intermediate precision (RSD %)
1.00	1.86	3.7	4.1 \pm 0.3	15.23	0.82
2.00	3.72	7.4	6.7 \pm 0.7	-8.63	0.61
4.00	7.45	14.9	11.0 \pm 3.9	-26.22	0.83
8.00	14.90	29.8	21.1 \pm 6.5	-29.20	1.20
16.00	29.80	59.6	49.2 \pm 9.2	-17.30	0.45
32.00	59.60	119.2	115.4 \pm 5.0	-3.37	0.48
64.00	119.20	238.4	240.2 \pm 1.0	0.72	0.31

Values are means \pm SEM of six samples

compared to *solvent 1* (25:25:50, v/v/v) (7.23 \pm 0.36) and *solvent 3* (50:25:25, v/v/v) (6.22 \pm 0.47 mg), respectively (Choksi and Joshi 2007; Gutierrez and Luque de Castro 2007; Cucu et al. 2012). The modified *solvent 2* ratio was more suitable to obtain 93 % pure lycopene from total carotenoids with limited processing steps. The UPLC profile of carotenoids extracted from tomato puree and standard lycopene is shown in Fig. 1. Among dietary sources, geometrical isomers of carotenoids either exist naturally or are formed during food processing. Existing liquid chromatographic methods used routinely for the separation of carotenoid isomers were carried out by using C₃₀ stationary phase. However, acyclic carotenoids actively retain on the HPLC stationary phase when compared to the other cyclic carotenoids (Emenhiser et al. 1996). The UPLC examinations have shown increased resolution in shorter run times which were further evaluated and confirmed by intermediate precision (Fig. 1 and Table 1). The stationary phases with C₁₈ and C₃₀ columns employed generally under gradient conditions are laborious and prolong the HPLC analysis time; in contrast, CSH Phenyl-Hexyl column has performed better and is relatively simple with high resolution. Further, UPLC analysis of lycopene using BEH C₁₈ and BEH shield RP18 columns extended the retention time with lesser resolution, when compared with CSH Phenyl-Hexyl column. The UPLC columns are packed with porous sub-2- μm particles, which results in increased interaction between stationary

phase and analytes with an improved chromatographic separation as outcome (Nguyen et al. 2006). In the present study, separation of (all-E)-lycopene on CSH Phenyl-Hexyl column was achieved within shorter run time at 5.56 min, whereas others have shown elution of (all-E)-lycopene on C₃₀ column at 47.3 min, (Lin and Chen 2003), 20 min (Braun et al. 2006), 65.5 min (Matsumoto et al. 2007) by HPLC and between 11 and 16 min in different UPLC stationary phases (Bijttebier et al. 2014), respectively. The LOD and LOQ of standard lycopene analyzed were 0.436 and 0.931 pmol on CSH Phenyl-Hexyl column under standardized conditions, and % RSD ($n=6$) was 3.01 and 4.81 for LOD and LOQ, respectively. The lycopene standard curve has shown least-squares linear regression analysis of the data, providing an excellent coefficient of determination value ($r=0.999$ and $r^2=0.998$) with slope and intercept $y=3.228894e+004x-4.944146e+004$. The gradient elution of analytes performed better with excellent resolution and sensitivity when the column was maintained at 45 °C compared to below 40 °C. The UPLC eluted peaks consist of the following order: (1) lycopene epoxide (1.85 %) (data not shown), (2) (13Z)-lycopene (1.70 %), (3) (15Z)-lycopene (0.60 %), (4) (all-E)-lycopene (93.99 %), and (5) (all-E)- β -carotene (1.81 %). The peak identity of the each component was further confirmed by its retention time and characteristic UV-Visible absorption spectra of reference analytical standards. For (Z)-lycopene isomers, absorption spectra

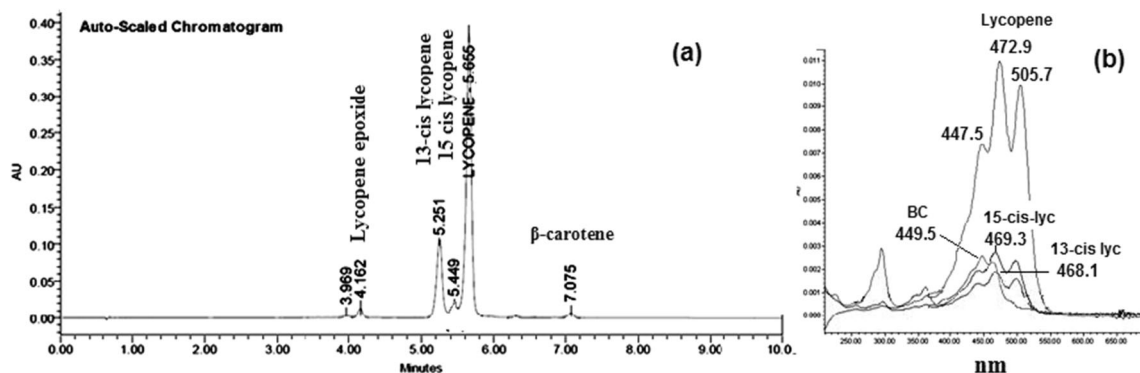


Fig. 2 A typical UPLC profile of isomerized lycopene (a) their overlaid spectra (b)

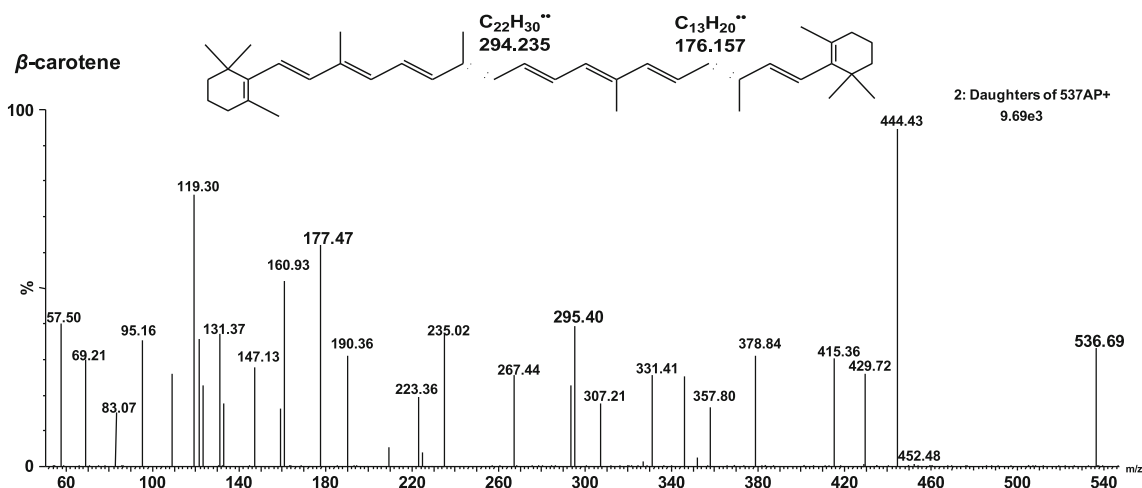


Fig. 4 Positive ion APCI tandem mass spectra and their fragmentation pattern of (all-E)- β -carotene (refer Fig. 3 and Table 1; for comparison of fragmentation pattern of (all-E)-/(Z)-lycopene and (all-E)- β -carotene)

and λ_{\max} were comparable with the previous report (Emenhiser et al. 1995) (Fig. 1). In the present study, isomerization of lycopene generated 25 % of (13Z)-lycopene and 2 % of (15Z)-lycopene isomers from (all-E)-lycopene (Fig. 2). The (all-E) to (Z) isomerization could be probably due to the formation of a (Z) C=C bond in the central positions of the chain. This interaction may take place by the hydrophobic interaction which affects the lateral methyl group. It is reported that (13Z)-lycopene is one of the most stable isomers and is present abundant in nature (Ramirez et al. 2010). Many reports have also shown that the (Z)-isomers of lycopene could be more bioavailable and play an important role in biological functions than (all-E)-lycopene (Böhm and Bitsch 1999; Boileau et al. 1999).

There are several reports on LC-MS and MS/MS using atmospheric chemical ionization (APCI) and electrospray ionization (ESI) methods for carotenoid analysis (Fang et al. 2003; Van Breemen et al. 1995; Lacker et al. 1999). The current study compared the different ionization methods for

analysis of (all-E)- and (Z)-lycopene isomers. Among three different modes of mass spectral analysis, APCI⁺ showed better ionization (Fig. 1) when compared to APCI^{ve} and ESI⁺ (data not shown). Further, MS/MS analysis of both standard and purified lycopene showed a similar pattern of results. Among different collision energies used, 18 eV produced the characteristic molecular ions and its % abundance for (all-E)-/(Z)-lycopene and (all-E)- β -carotene. The molecular ion of (all-E)-lycopene detected at m/z 467 (Fig. 3c) formed by the elimination of a terminal isoprene group was comparable with the APCI^{ve} ionization mode reported by others (Fang et al. 2003; Van Breemen et al. 2012; Kopec et al. 2010). Further, [M-69]⁻ fragment ion was also reported by using fast atom bombardment tandem mass spectrometry (Van Breemen et al. 1995).

The (all-E)-lycopene and (Z)-lycopene isomers may be distinguished based on the relative abundances of fragmented ions of m/z 521, for (Z)-lycopene was confirmed by “electrospray ionization” mobility studies (Dong et al.

Table 2 Tandem mass spectra of APCI⁺ product ions and their relative abundance at 18-eV collision-induced dissociation

Carotenoids	Other significant ions and their relative % abundance
(13Z)-lycopene	69.3 (32 %) , 81.1 (100 %), 93.3 (55 %), 105.2 (28 %), 120.9 (45 %), 145.4 (30 %), 171.3 (60 %), 195.4 (35 %), 209.4 (15 %), 223.4 (30 %), 247.4 (17 %), 269.3 (18 %) , 305.4 (11 %), 375.3 (10 %), 387.4 (8 %) , 411.6 (5 %), 444.2 (8 %), 467.3 (5 %) , 521.2 (10 %) , 536.6 (35 %)
(15Z)-lycopene	69.3 (100 %) , 81.3 (80 %), 95.3 (52 %), 107.3 (85 %), 133.3 (30 %), 145.2 (75 %), 159.4 (30 %), 173.2 (42 %), 197.4 (20 %), 221.1 (18 %), 269.2 (12 %) , 327.7 (8 %), 350.6 (6 %), 375.6 (18 %), 399.4 (5 %), 427.5 (5 %) , 444.6 (18 %), 467.5 (15 %) , 521.8 (5 %) , 536.9 (10 %)
(all-E)-lycopene	57.1 (9 %), 69.3 (100 %) , 81.2 (95 %), 93.1 (38 %), 107.2 (58 %), 133.3 (42 %), 173.3 (40 %), 197.3 (22 %), 223.3 (15 %), 237.3 (8 %), 269.4 (29 %) , 301.4 (5 %), 333.3 (4 %), 375.5 (5 %), 444.5 (9 %), 467.5 (12 %) , 536.6 (12 %)
(all-E)- β -carotene	57.5 (40 %), 69.2 (30 %), 83.1 (15 %), 95.2 (35 %), 119.3 (75 %), 131.4 (37 %), 147.1 (28 %), 160.9 (52 %), 177.5 (62 %) , 190.4 (30 %), 223.4 (18 %), 235.0 (36 %), 267.4 (25 %), 295.4 (40 %) , 307.2 (19 %), 331.4 (25 %), 357.8 (18 %), 378.8 (31 %), 415.4 (30 %), 429.7 (26 %), 444.4 (91 %), 536.7 (32 %)

Bold fragment m/z values are used to distinguish (Z)-lycopene isomers and (all-E)- β -carotene from (all-E)-lycopene and underlined values to distinguish (Z)-isomers from (all-E)- β -carotene

2010). The $[M]^+$ ions of (Z)-lycopene fragment may be formed during collision-induced dissociation of ions of m/z 521, whereas (all-E)-lycopene either does not form or produces them at much lower relative abundance (Dong et al. 2010). The present study also reports the detection of m/z 521 in MS/MS spectrum of (Z)-lycopenes with a loss of 15 Da (methyl group) (Fig. 3a, b) and was absent or present at very lower abundance in (all-E)-lycopene (Fig. 3c). Further, (all-E)-lycopene could be distinguished from all-(E)- β -carotene by using MS/MS, and the base peak of (all-E)- β -carotene was observed at m/z 444 (Kaufmann et al. 1996; Huo et al. 2011). The relative abundance of m/z 444 and absence of m/z 467 can be used to distinguish (all-E)- β -carotene from (all-E)-lycopene (Fig. 4). Other major/significant ions formed are mentioned in the Table 2. Each experiment was repeated three times on each day to check the reproducibility to acquire characteristic MS/MS patterns among the lycopene isomers and (all-E)- β -carotene. It was observed that there was discrepancy in the presence of m/z 521, was rather present in (all-E)-lycopene, and the reason may be due to switching of (all-E) to (Z)-lycopene under high probe temperature; hence, a more sensitive technique like ion mobility studies may help in further differentiation of (all-E)- and (Z)-lycopene. The spectral mass characteristic patterns among the structural isomers were found to be consistent. In addition, this technique could be helpful in the simultaneous determination of other hydroxy and epoxy carotenoids with selective CID experiments as studied by HPLC-DAD-MS (ESI⁺) analysis (Crupi et al. 2010). A variety of carotenoid geometrical isomers exist in nature, and the separation of isomers with high resolution and their mass spectral characteristics are warranted (Bijttebier et al. 2013).

Conclusion

The assessment facilitated the simple method of lycopene isolation, selective analysis for characterization of similar types of carotenoids with lower LOD. The analysis of geometric isomers of dietary carotenoids in biological samples using C₃₀ columns requires longer run time in contrast with CSH Phenyl-Hexyl column. The advantage of the developed method is to reduce run time and cost of analysis. The fragmentation patterns were investigated that are typically used for chromatographic analysis of biological samples in contrast to the fragmentation obtained by direct infusion. Even though, APCI⁺-MS/MS conditions were optimized to obtain characteristic product ions and their relative abundance of (13Z)- and (15Z)-lycopene, differentiation of geometrical isomers by ion mobility technique is deserved for future study. In addition, the developed conditions could help to distinguish cyclic and acyclic carotenoids of similar molecular mass and circumvent interference and ambiguity.

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Conflict of Interest Bangalore Prabhashankar Arathi declares that she has no conflict of interest. Poorigali Raghavendra Rao Sowmya declares that she has no conflict of interest. Kariyappa Vija declares that he has no conflict of interest. Pullancheri Dilshad declares that she has no conflict of interest. Bhattacharya Saikat declares that he has no conflict of interest. Vaidyanathan Gopal declares that he has no conflict of interest. Rangaswamy Lakshminarayana declares that he has no conflict of interest. This article does not contain any studies with human or animal subjects.

References

- Angelova T, Warthesen J (2000) Lycopene stability in tomato powders. *J Food Sci* 65:67–70
- Beilby J, Ambrosini GL, Rossi E, de Klerk NH, Musk AW (2010) Serum levels of folate, lycopene, β -carotene, retinol and vitamin-E and prostate cancer risk. *Eur J Clin Nutr* 64:1235–1238
- Bijttebier SKA, Hondt ED, Hermans N, Apers S, Voorspoels S (2013) Unravelling ionization and fragmentation pathways of carotenoids using orbitrap technology: a first step towards identification of unknowns. *J Mass Spec* 48:740–754
- Bijttebier S, Hondt ED, Hermans N, Apers S, Voorspoels S (2014) Ultra high performance liquid chromatography versus high performance liquid chromatography: Stationary phase selectivity for generic carotenoid screening. *J Chromatogr A* 1332:46–56
- Böhm V, Bitsch R (1999) Intestinal absorption of lycopene from different matrices and interactions to other carotenoids, the lipid status, and the antioxidant capacity of human plasma. *Eur J Nutrition* 38(3): 118–125
- Boileau AC, Merchen NR, Wasson K, Atkinson CA, Erdman JJW (1999) *Cis*-lycopene is more bioavailable than *trans*-lycopene in vitro and in vivo in lymph-cannulated ferrets. *J Nutr* 129:1176–1181
- Braun CL, Jackson HL, Lockwood SF, Nadolski G (2006) Purification of synthetic all-E lycopene (Ψ , Ψ -carotene-16, 16'-diol). *J Chromatogr B* 834:208–212
- Britton G (1995) Structure and properties of carotenoids in relation to function. *FASEB J* 9(15):1551–1558
- Chauveau Duriot B, Doreau M, Noziere P, Graulet B (2010) Simultaneous quantification of carotenoids, retinol, and tocopherols in forages, bovine plasma, and milk: validation of a novel UPLC method. *Analyt and Bioanalyt Chem* 392:777–790
- Choksi PM, Joshi VY (2007) A review on lycopene-extraction, purification, stability and applications. *Int J Food Proper* 10:289–298
- Clinton SK, Emehiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ (1996) *Cis-trans* lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer Epid Biomar Prevention* 10:823–833
- Crupi P, Milella RA, Antonacci D (2010) Simultaneous HPLC-DAD-MS (ESI⁺) determination of structural and geometrical isomers of carotenoids in mature grapes. *J Mass Spec* 45:971–980
- Cucu T, Huvaere K, Anne Van Den Bergh M, Vinkx C, Loco JV (2012) A simple and fast HPLC method to determine lycopene in foods. *Food Analyt Method* 5:1221–1228
- Delpino Rius A, Eras J, Marsol-Vall A, Vilaro F, Balcells M, Canela-Garayoa R (2014) Ultra performance liquid chromatography

- analysis to study the changes in the carotenoid profile of commercial monovarietal fruit juices. *J Chromatogr A* 1131:90–99
- Dong L, Shion H, Davis RG, Penak BT, Perez JC, van Breemen RB (2010) Collision cross-section determination and tandem mass spectrometric analysis of isomeric carotenoids using electrospray ion mobility time-of-flight mass spectrometry. *Analyt Chem* 82:9014–9021
- Emenhiser C, Simunovic N, Sander LC, Schwartz SJ (1996) Separation of geometrical carotenoid isomers in biological extracts using a polymeric C₃₀ column in reversed-phase liquid chromatography. *J Agric Food Chem* 44:3887–3893
- Emenhiser C, Sander LC, Schwartz SJ (1995) Capability of a polymeric C₃₀ stationary phase to resolve *cis-trans* carotenoid isomers in reversed-phase liquid chromatography. *J Chromatogr A* 707:205–216
- Fang LQ, Pajkovic N, Wang Y, Gu CG, van Breemen RB (2003) Quantitative analysis of lycopene isomers in human plasma using high-performance liquid chromatography-tandem mass spectrometry. *Analyt Chem* 75:812–817
- Ferreira AL, Yeum KJ, Liu C, Smith D, Krinsky NI, Wang XD (2000) Tissue distribution of lycopene in ferrets and rats after lycopene supplementation. *J Nutr* 130:1256–1260
- French AG, Torres MEH, Vega AB, Vidal JLVM, Bolanos PP (2005) Determination of ascorbic acid and carotenoids in food commodities by liquid chromatography with mass spectrometry detection. *J Agric Food Chem* 53:7371–7376
- Giovannucci E (2005) Tomato products, lycopene, and prostate cancer: a review of the epidemiological literature. *J Nutr* 135:20305–20315
- Gutierrez MJR, Luque de Castro MD (2007) Lycopene: The need for better methods for characterization and determination. *Trends in Analyt Chem* 26:163–170
- Guzman I, Yousef GG, Brown AF (2012) Simultaneous extraction and quantitation of carotenoids, chlorophylls, and tocopherols in Brassica vegetables. *J Agric Food Chem* 60:7238–7244
- Huo MM, Liu WL, Zhen ZR, Zhang W, Li AH, Xu DP (2011) Effect of end groups on the Raman spectra of lycopene and β -carotene under high pressure. *Molecu* 16:1973–1980
- Kaufmann R, Wingerath T, Kirsch D, Stahl W, Sies H (1996) Analysis of carotenoids and carotenol fatty acid esters by Matrix-Assisted Laser Desorption Ionization (MALDI) and MALDI-post-source-decay mass spectrometry. *Analyt Chem* 238:117–128
- Kopec RE, Riedl KM, Harrison EH, Curley RW Jr, Hruszkewycz DP, Clinton SK (2010) Identification and quantification of apolycopenals in fruits, vegetables and human plasma. *J Agric Food Chem* 58:3290–3296
- Lacker T, Strohschein S, Albert K (1999) Separation and identification of various carotenoids by C₃₀ reversed-phase high-performance liquid chromatography coupled to UV and atmospheric pressure chemical ionization mass spectrometric detection. *J Chromatogr* 85(4):37–44
- Lin CH, Chen BH (2003) Determination of carotenoids in tomato juice by liquid chromatography. *J Chromatogr A* 1012:103–109
- Lyan B, Braesco VA, Cardinault N, Tyssandier V, Borel P, Gouabau MCA (2001) Simple method for clinical determination of 13 carotenoids in human plasma using an isocratic high-performance liquid chromatographic method. *J Chromatogr B* 751:297–303
- Matsumoto H, Ikoma Y, Kato M, Kuniga T, Nakajima N, Yoshida T (2007) Quantification of carotenoids in citrus fruit by LC-MS and comparison of patterns of seasonal changes for carotenoids among citrus varieties. *J Agric Food Chem* 55:2356–2368
- Naviglio D, Caruso T, Iannece P, Arago NA, Sanini A (2008) Characterization of high purity lycopene from tomato wastes using a new pressurized extraction approach. *J Agric Food Chem* 56:6227–6231
- Nguyen DTT, Guillaume D, Rudaz S, Veuthey JL (2006) Fast analysis in liquid chromatography using small particle size and high pressure. *J Separation Science* 29:1836–1848
- Ramirez MRL, Cortes S, Mendez MP, Blanchd G (2010) *Trans-cis* isomerisation of the carotenoids lycopene upon complexation with cholesteric polyester carriers investigated by Raman spectroscopy and density functional theory. *J Raman Spectr* 44:1170–1177
- Rao AV, Agarwal S (2000) Role of antioxidant lycopene in cancer and heart disease. *J American College Nutr* 19:563–569
- Romero MG, Roman DA, Carretero AS, Gutierrez AF (2007) Analytical determination of antioxidants in tomato: typical components of the Mediterranean diet. *J Separ Science* 30:452–461
- Schierle J, Bretzel W, Buhler I, Faccin N, Hess D, Steiner K (1997) Content and isomeric ratio of lycopene in food and human blood plasma. *Food Chem* 59:459–465
- Stahl W, Schwarz W, Sundquist AR, Sies H (1992) *Cis-trans* isomers of lycopene and β -carotene in human serum and tissues. *Archives of Biophy and Biochem* 294:173–177
- Talvas J, Veyrat CC, Guy L, Rambeau M, Lyan B, Quinard RM (2010) Differential effects of lycopene consumed in tomato paste and lycopene in the form of a purified extract on target genes of cancer prostatic cells. *American J Clinical Nutr* 91:1716–1724
- Van Breemen RB, Dong L, Pajkovic ND (2012) Atmospheric pressure chemical ionization tandem mass spectrometry of carotenoids. *Intl J Mass Spec* 312:163–172
- Van Breemen RB, Schmitz HH, Schwartz SJ (1995) Fast atom bombardment tandem mass spectrometry of arotenoids. *J Agric Food Chem* 43:384–389