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Analysis of lycopene geometrical isomers in biological microsamples by liquid chromatography with coulometric array detection

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Abstract

Methods of analysis for determining low quantities of lycopene *cis–trans* isomers in biological tissues are needed. Development of two liquid chromatography (LC) methods based on the polymeric C_{30} stationary phase equipped with coulometric electrochemical array detection (ED) is described. Separation of 13 lycopene isomers including prolycopene, (a novel tetra-*cis*-lycopene found in *Tangerine* tomatoes) was accomplished with both isocratic and gradient methods using different proportions of methanol, methyl *tert.*-butyl ether, water and 1 *M* ammonium acetate buffer. Carotenoids were detected at potential settings between 200 and 620 mV. Differences in generated current–voltage curves aided in tentative identification of *trans* carotenoid species and select *cis* isomers of lycopene. These methods were successfully applied in the analysis of small quantities of plasma, buccal mucosal cells, prostate and cervical tissues. Limits of detection for *trans*-lycopene by ED were found to be 50 fmol representing a 10- to 100-fold increase over conventional UV–Vis absorbance methods.

Keywords: Geometrical isomers; Coulometric array detection; Lycopene

1. Introduction

The rapid accumulation of observational data in recent years implicating the role of dietary tomato products in protecting against several cancers [1,2] and vascular disease [3] has resulted in the proliferation of experimental research studies on the tomato carotenoid lycopene as the physiologically active agent [4]. The majority of these studies thus far have focused primarily on monitoring circulating levels of lycopene in human plasma while data on tissue deposition and distribution remain limited [5–7]. One reason is that, in order to accurately quantify tissue lycopene concentration, large quantities of tissues from patients are often required limiting the number of possible samples per patient as well as reducing the number of volunteers willing to participate in studies. Another reason is that plasma levels

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of carotenoids are generally considered highly correlated with tissue concentrations [8]. However, Peng et al. [9] recently suggested in their analysis of paired plasma and cervical tissue, from patients with and without cervical cancer, that not all micronutrient concentrations in plasma necessarily reflected a corresponding level in tissue, especially in the case of lycopene. The advantage of accurately detecting and measuring carotenoids in samples of limited size would be twofold, minimizing the degree of invasiveness during tissue biopsies while allowing for increased assays per biopsy.

Electrochemical array detection had been successfully applied to significantly increase sensitivity for both hydrocarbon (β-carotene and α-carotene) and oxygenated (lutein and zeaxanthin) carotenoids [10]. Utilizing a multiple channel array this technique adds a selective dimension beyond previous single-channel liquid chromatography–electrochemical detection (LC–ED) methods. Application of this method to the carotenoid lycopene remains limited, especially with respect to its geometrical isomers (Fig. 1). Consideration of geometrical isomer distribution in human tissues is important in light of reports which highlight a higher ratio of *cis* to *trans* lycopene isomers in human biological fluids and tissues relative to those in the diet [6,11].

While tomatoes represent the most common source of lycopene, some varieties have a special biosynthetic capacity to produce other carotenoids which are either unique or in altered relative abundance. Mutant varieties such as *Tangerine* (*Golden jubilee* and *Tangela*) have the capacity to biosynthesize several *cis* isomers of lycopene which are not commonly found in other varieties [12–14]. In these varieties, the predominant carotenoid is prolycopene, a tetra-*cis* isomer (7,9,7′,9′) of lycopene. Compared to *trans*-lycopene, prolycopene has two hindered *cis* double bonds [15,16] and a shortened chromophore resulting in an absorption maxima shift of 35 nm (435 nm) from *trans*-lycopene (470 nm) [17]. The ability to both biosynthesize and accumulate a unique tetra-*cis*-lycopene isomer make the *Tangerine* tomatoes a potentially important tool for the study of *cis*-lycopene isomer absorption and metabolism in humans.

Past LC–ED reports have either omitted the analysis of lycopene or have been limited to the

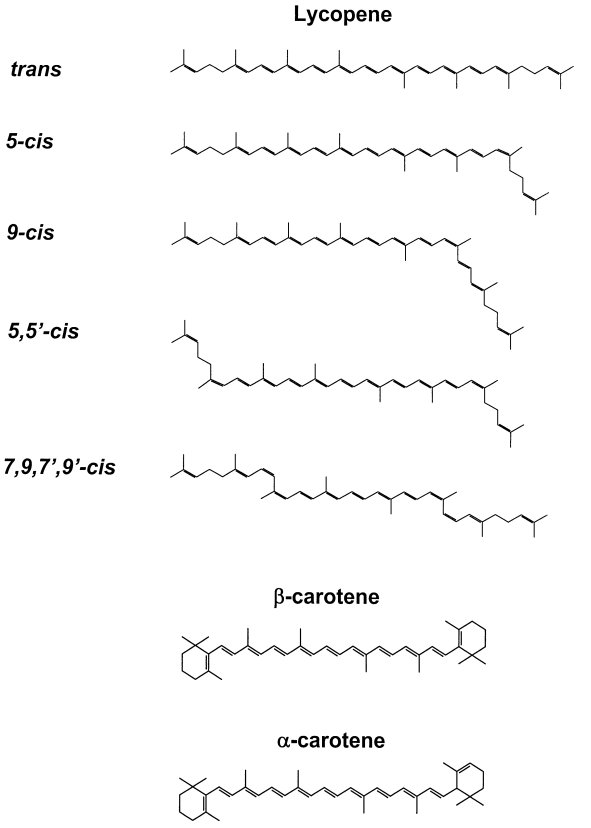

Fig. 1. Structures of three main carotenoid species; β-carotene, α-carotene and lycopene geometrical isomers including prolycopene (7,9,7′,9′-tetra-*cis*-lycopene).

quantification of only the *trans* geometrical isomer [7,10,18]. The present study describes two C_{30} LC–ED methods optimized for the separation of *trans* lycopene and several of its *cis* isomers. These methods take into account several distinctive chromatographic and physicochemical properties of lycopene compared to other common dietary carotenoids. Experiments with standard red *Roma*, and orange *Tangerine* tomato extracts as well as iodine-photoisomerized mixture of lycopene isomers were performed to demonstrate selectivity and to assess electrochemical responses of the various geometrical configurations as a function of applied potentials. Small quantities of blood plasma, human prostate, cervical tissue and buccal mucosal cells were subsequently analyzed using this methodology and limits of detection established. These techniques may

be employed to study factors governing the ratio of *cis* to *trans* lycopene isomers in human tissues.

2. Experimental[1]

2.1. Chemicals and standards

Methanol, methyl *tert*.-butyl ether (MTBE), and water (Fisher, Fair Lawn, NJ, USA) were of certified HPLC and ACS grades. A 1.0 *M* ammonium acetate buffer solution (Fluka; Ronkonkoma, NY, USA) was prepared with water and adjusted to pH 4.6 with glacial acetic acid. *trans* standards of lycopene, β-carotene, and α-carotene were obtained from Sigma–Aldrich (St. Louis, MO, USA). Standards were dissolved in methanol–MTBE (1:1) and filtered through a 0.2-μm PTFE filter prior to analysis.

Common red *Roma* tomatoes were obtained from a local grocery. *Tangerine* tomatoes were kindly provided by Dr. David Francis of the Department of Horticulture and Crop Science at The Ohio State University (Columbus, OH, USA). Human blood serum and buccal mucosal cells were collected from healthy student volunteers not under medication which were participating in a research study. All procedures involving sample collection were approved by the Institutional Review Board of The Ohio State University. Human cervical tissue samples were obtained from subjects participating in a research study at the University of California (San Diego, CA, USA). Approval for the collection of the samples was obtained from the committee of Investigation Involving Human Subjects, University of California. Human prostate tissue samples were provided by Dr. Steven Clinton at the Comprehensive Cancer Center, Arthur G. James Cancer Hospital and Solove Research Institute, The Ohio State University.

[1]Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology or Ohio State University, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

2.2. Carotenoid extractions

Approximately 5 g samples of raw tomato were diced and homogenized in 25 ml of methanol with added $CaCO_3$. Carotenoids including lycopene were extracted with three 25-ml portions of a 1:1 solution of acetone–hexane. The filtrates obtained by vacuum filtration were combined in a separatory funnel. Approximately 10 ml of deionized water was added to hasten the phase separation of the hexane layer which was saved. Aliquots of 3 ml were removed and dried under a stream of nitrogen at ambient temperature. Samples were immediately dissolved in methanol–MTBE (1:1) in preparation for LC analysis.

Prostate tissue carotenoids were extracted by the method of Clinton et al. [11]. Buccal mucosal cell collection and extraction was performed by the method of Peng and Peng [19]. Cervical tissue was extracted as described previously [10]. Aliquots of 100 μl of blood plasma were deproteinated with incorporation of 100 μl of ethanol containing a mass fraction of 0.1% butylated hydroxytoluene (BHT). Carotenoids were extracted with two 500-μl portions of acetone–hexane (1:1, v/v) containing a mass fraction of 0.02% BHT. Each portion was vortex-mixed for 30 s, after which the hexane layers were removed and combined. Individual extracts were dried under a stream of nitrogen at ambient temperature and analyzed immediately.

2.3. Instrumentation and chromatography

Separations were achieved using an analytical-scale 250 mm×4.6 mm I.D., 3 μm polymeric C_{30} column prepared at the National Institute of Standards and Technology (Gaithersburg, MD, USA). The synthesis, preparation, and characteristics of the polymeric C_{30} stationary phase for carotenoid analyses have been described previously [20]. Alternatively, the stationary phase is commercially available from Waters/YMC (Milford, MA, USA). Columns were thermostated at 25°C. A guard column (50 mm×4.6 mm I.D.) packed with C_{18} stationary phase (Waters, Milford, MA, USA) was used to protect the analytical column. A Hewlett-Packard Model 1050 solvent delivery system and autosampler (Santa Clara, CA, USA) was used. An ESA Model 5600

Coularray electrochemical detector (Chelmsford, MA, USA) equipped with either four or eight channels in series was used for analysis. The potential settings used in a four-channel configuration from channels 1 to 4 were 220 to 520 mV in 100 mV increments. In an eight-channel configuration cell potentials were set from 200 to 620 mV in 60 mV increments. Analytical cells were cleaned daily by application of elevated (800 mV) and reduced (−600 mV) potentials in 60 s intervals removing compounds found to be unreactive between the applied analytical potentials described above. The chromatographic data were collected and integrated using the ESA Coularray software and data management system.

Separations were achieved using both an isocratic and gradient elution with a binary mobile phase of different concentrations of methanol–MTBE–ammonium acetate. Isocratic conditions were set at volume fractions 45% of reservoir A (methanol–MTBE–1 mol/l ammonium acetate solution, 95:3:2, v/v) and 55% of reservoir B (methanol–MTBE–1 mol/l ammonium acetate solution, 25:73:2, v/v) with a chromatographic run time of 50 min for all analyses. Solvent gradient conditions were used in analysis of specific geometrical isomers (prolycopene) originating from *Tangerine* tomato. Solvent and gradient conditions are described in Table 1. Tentative peak identifications of chromatographic separations were made by evaluation of current–voltage curves (CVCs). These identifications were confirmed by injection of authentic standards for *trans* α- and β-carotenes, and lycopene. Tentative identification of lycopene isomers was aided by comparison to previous LC C_{30} separations [11,21] as well as spectral data gained from an in-line photodiode array detector.

Table 1
C_{30} LC gradient conditions for lycopene isomer separation

Time (min)	Flow-rate (ml/min)	% Solvent A	% Solvent B
0.0	1.0	100	0
40	1.0	15	85
50	1.0	0	100

Binary mobile phase consists of methanol, methyl *tert*.-butyl ether, water, and 1.0 mol/l ammonium acetate in differing concentrations. Solvent A=88:5:5:2; solvent B=28:70:0:2.

2.4. Lycopene calibration plots

Standard calibration plots were prepared for both isocratic four-channel and gradient eight-channel LC methods. Stock solutions of *trans*-lycopene were prepared in hexane between 0.5 and 5.0 μmol/l. Final stock concentrations were determined using the Beer–Lambert relation with the molar absorptivity of lycopene in hexane (ϵ=184 000) [17,22]. The hexane stock solution was volumetrically diluted in series with methanol–MTBE (1:1, v/v). Aliquots of 20 μl each of these standard solutions were injected onto the LC. Calibration plots were constructed by plotting peak area versus lycopene concentration.

2.5. Iodine isomerization

A *trans*-lycopene standard was isomerized by the method of Zeichmeister [23]. Lycopene crystals were dissolved in hexane to which iodine was added at ≈1.0% of the carotenoid mass. The solution was then exposed to ambient artificial laboratory light for 30 min. The solvent was evaporated under a stream of nitrogen, and the resulting mixture of isomers were stored at −20°C until analysis.

2.6. Limit of detection

Detection limits for the electrochemical detector were established using *trans*-lycopene as a standard. Four 10-fold serial dilutions were prepared for each standard from a $1.72 \cdot 10^{-6}$ mol/l stock solution. Further five- and twofold dilutions were conducted to determine the detection limit, defined as a response three times the peak-to-peak noise level. Chromatograms were obtained with 20 μl injections of each solution.

3. Results and discussion

3.1. Standards and current–voltage curves

The separation of major carotenes including α-carotene, β-carotene and iodine photoisomerized lycopene on a polymeric C_{30} column with a four-channel electrochemical array is shown in Fig. 2. Detection of 13 lycopene isomers including *trans*-

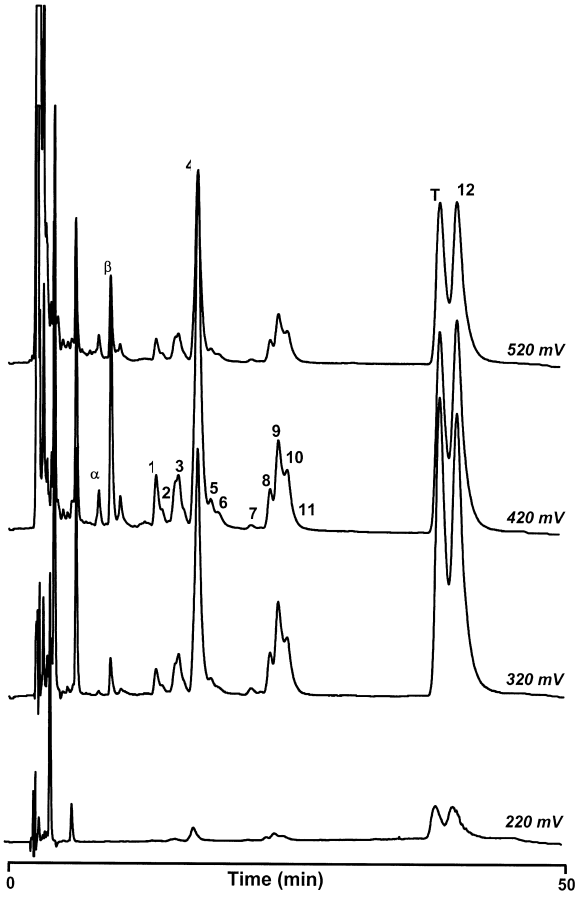

Fig. 2. Isocratic separation of iodine isomerized lycopene on a four-channel coulometric array detector (100 nA full scale). Dominant response channel for all lycopene isomers is channel 2 (320 mV). Peak identifications: T=*trans*-lycopene; 1–12= predominant *cis*-lycopene isomers in order of elution.

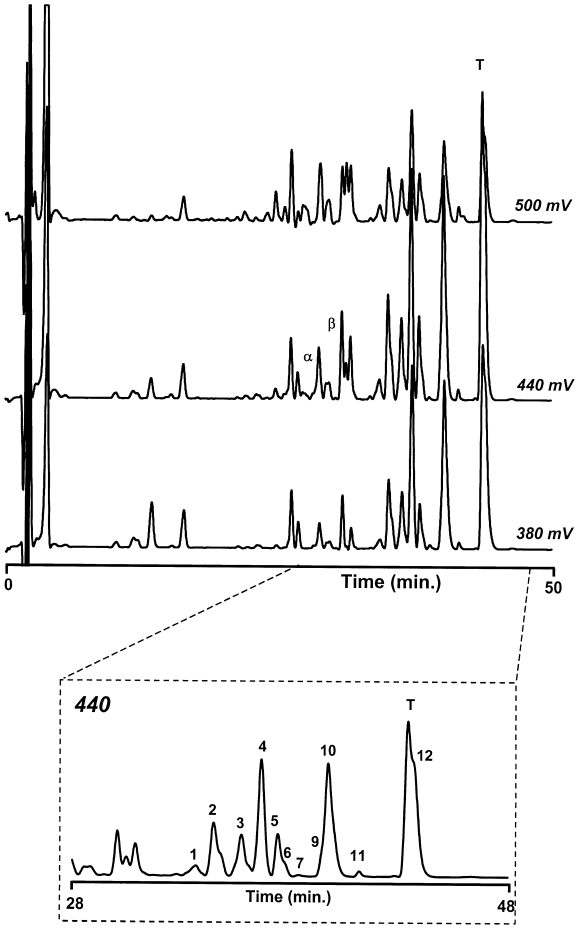

Fig. 3. Gradient separation of iodine isomerized lycopene on an eight-channel coulometric array detector. Channels 4 (380 mV), 5 (440 mV), and 6 (500 mV) depicted (100 nA full scale). Dominant response channel for all lycopene isomers is channel 5. Peak identifications: α=α-carotene; β=β-carotene; T=*trans*-lycopene; 1–12=predominant *cis*-lycopene isomers in order of elution. Inset depicts expanded view of lycopene isomer separation between 28 and 48 min.

lycopene was achieved with the four-channel array, and isomers were tentatively identified by comparison of parallel photodiode array spectra with published absorption maxima for *trans*-carotenoids and lycopene isomers [17,22]. Baseline resolution of α- and β-carotenes also was achieved within 10 min. This isocratic method demonstrates excellent resolution of late-eluting lycopene isomers including the *trans* species from isomer 12, tentatively identified as 5-*cis*-lycopene using previously published spectra [22].

Fig. 3 shows separation of 12 lycopene isomers by gradient elution on an eight-channel electrochemical array. This gradient was designed specifically to resolve prolycopene (7,9,7′,9′-tetra-*cis*-lycopene) which under isocratic conditions was found to co-elute completely with β-carotene. This method can be effectively applied for the separation of lycopene isomers from food samples rich in carotenoids such as tomatoes (Fig. 4a and b). Fig. 5a shows the CVC of *trans*-lycopene compared with those of α-carotene and β-carotene gathered from the four-channel array. Dominant response channels for the two types of carotenes were observed to be different, specifically

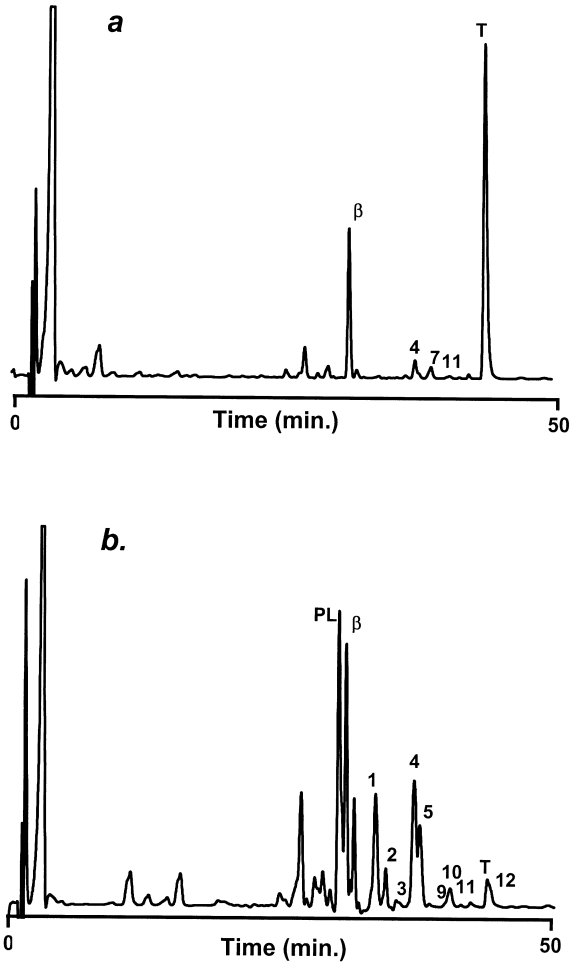

Fig. 4. Gradient LC–ED analysis of (a) *Roma* tomato extract and (b) *Tangerine* tomato extract. Depicted in dominant response channel 5 (440 mV), 100 nA full scale. Lycopene isomers are identified by number in order of elution. *trans*-Lycopene and prolycopene are denoted by T and PL, respectively.

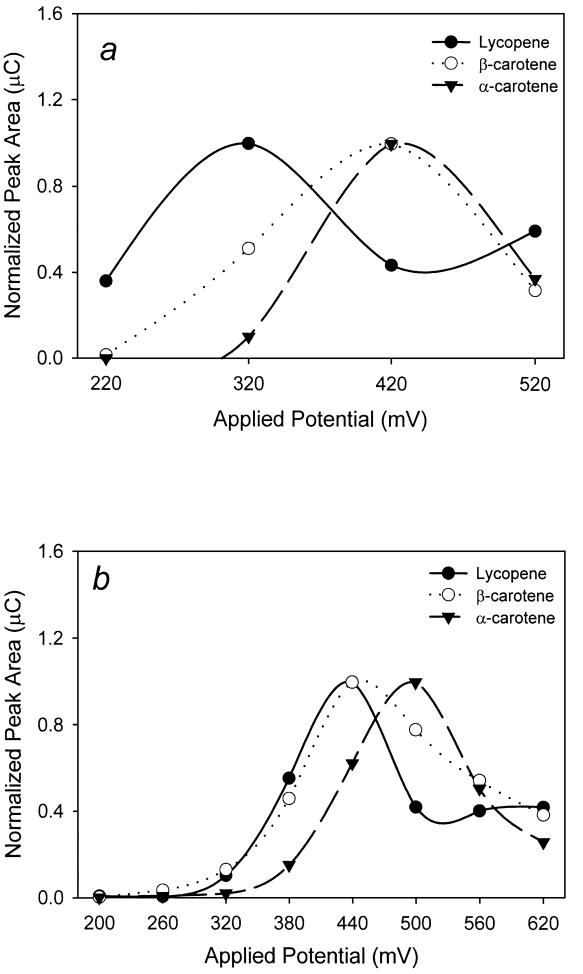

Fig. 5. Generated current voltage curves (CVCs) for *trans*-lycopene, α-carotene, and β-carotene by (a) isocratic elution by four-channel and (b) gradient elution by eight-channel coulometric array detection. Data were generated by analysis of authentic standards.

420 mV (channel 3) for α-carotene and β-carotene, and 320 mV (channel 2) for *trans*-lycopene. These differences allow for the use of CVCs in differentiating lycopene from other carotenoids in more complex samples in a manner similar to that described previously [10]. CVCs of the same carotenoids generated from the eight-channel array can be seen in Fig. 5b. *trans*-Lycopene was found to respond dominantly at 440 mV (channel 5). As with the four-channel array α-carotene maintained a higher dominant response channel (500 mV, channel 6) than lycopene. Observed differences in dominant response channels for lycopene between the two methods were expected as both the number of cells and applied potential differed between methods (as did solvent composition at the point of elution, which may affect both response and optimal oxidation potentials). In previous reports with coulometric LC–ED we have shown that α-carotene responds dominantly at slightly higher potential than β-carotene [10]. The dominant oxidation of β-carotene at lower potential was explained by the extension of the conjugated π

electron structure. The dominant oxidation of lycopene at these lower potentials may be related to the similar π electron structure. Differences between β-carotene and lycopene may arise from the acyclic structure of lycopene as well as the two additional non-conjugated double bonds at the 1 and 1′ positions.

Fig. 6a shows CVCs of predominant *cis*-lycopene isomers obtained after iodine isomerization and detection on the four-channel array. Of the 12 identified isomers all were found to behave similarly to the *trans* molecule, showing a dominant response on channel 2 (320 mV). These results differ from those observed in a similar study with β-carotene where the 9-*cis* and 13-*cis* isomers maintained a higher dominant potential while the early eluting 15-*cis* β-carotene molecule behaved much like the *trans* species [10]. The less discriminant nature of a four-channel system may allow for subtle difference in dominant response channels between lycopene geometrical isomers to go undetected. While expansion of the system to eight channels did not allow for full differentiation between all isomer species (Fig. 6b) it was capable of resolving the difference between prolycopene and other isomers including *trans*-lycopene (Fig. 6c). This early eluting poly-*cis* form of lycopene has a distinct CVC different from *trans*-lycopene with a higher dominant response at 500 mV (channel 6).

Calibration curves with *trans*-lycopene were used to establish detection limits and dynamic range of the detector. Detector response maintained linearity over the concentration range of 10 μmol/l to 1 nmol/l for both four ($y=53.55x+0.746$; $R^2=0.9997$) and eight ($y=31.55x+0.596$; $R^2=0.9991$) channel arrays. Detection limits for *trans*-lycopene were similar between four- and eight-channel arrays, determined to be 50 fmol on-column defined by a signal-to-noise ratio of 3:1, representing a 10–100-fold increase in sensitivity from conventional LC–UV–Vis absorbance methods. These findings are in accordance with those previously determined in our laboratory for β-carotene [10].

The advantages of an electrochemical array over conventional single-channel electrochemical detectors is illustrated by the ability to discriminate between different carotenoid species including select geometrical isomers by comparison of their respec-

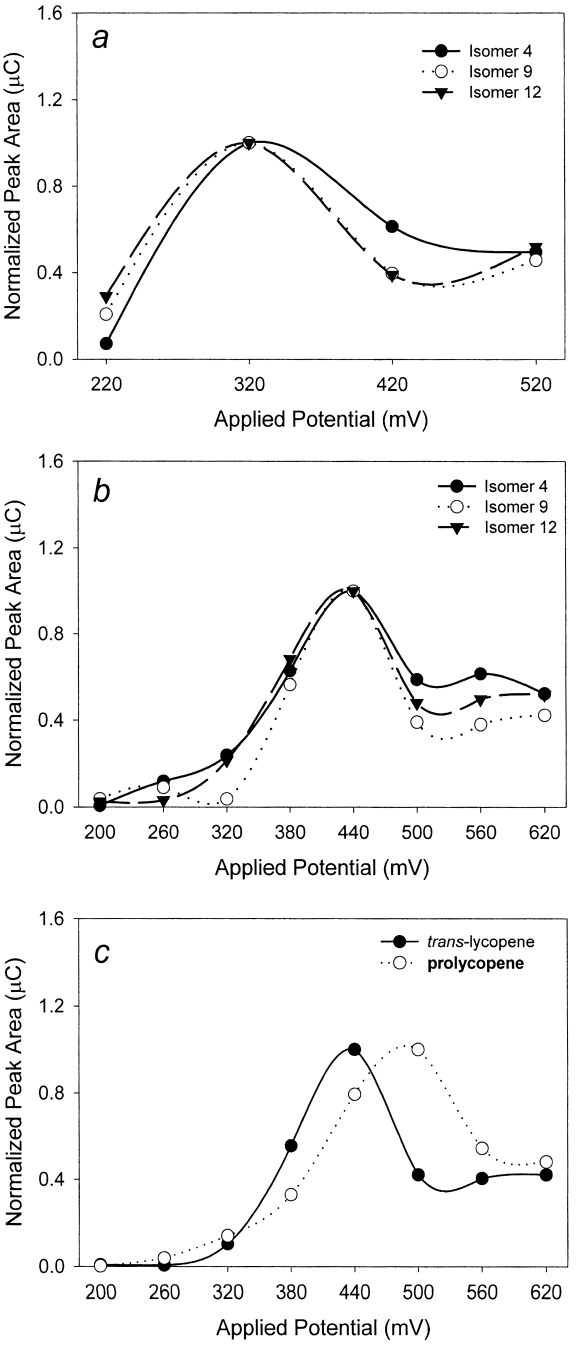

Fig. 6. Current voltage curves (CVCs) for *cis*-lycopene isomers. (a) *cis*-Lycopenes 4, 9 and 12 generated by isocratic elution from four-channel; (b) *cis*-lycopenes 4, 9 and 12 generated from gradient elution by eight-channel coulometric array detection and (c) prolycopene from *Tangerine* tomato. All isomers with the exception of prolycopene were derived from iodine isomerization of *trans*-lycopene standard.

tive CVCs. This information is useful for identification purposes especially when sample size is limited and trace quantities of carotenoids are to be measured, making in-line spectral analysis difficult to obtain.

3.2. Application to biological samples

Versatility of both the isocratic and gradient LC–ED methods was demonstrated by application to a variety of biological fluids and tissues. Samples analyzed include human plasma, buccal mucosal cells, prostate and cervical tissue biopsies. These biological samples represent areas of recent interest with regard to carotenoid bioavailability and disease prevention [4].

3.2.1. Isocratic analysis

Fig. 7a shows the separation of 13 geometric isomers of lycopene from a 50 μl extract of human blood plasma by the isocratic four-channel method. The chromatogram from the dominant response channel 2 is shown, corresponding to 320 mV applied potential. Identification of *trans*-lycopene was made by comparison to an authentic standard. Isomers were tentatively identified by comparison to previous C_{30} separations conducted in our laboratory [21] as well as preliminary in-line photodiode array data. The abundance of *cis*-lycopene isomers in human plasma, approximately 60% of the total lycopene found in extracts, is consistent with previous reports [11].

Chromatographic separation of 11 geometric isomers of lycopene from a sample of human prostate tissue is illustrated in Fig. 7b. It is important to note that this analysis represents an effective 10- to 100-fold reduction of sample size requirement from previous prostate tissue assays conducted in our laboratory [11] further demonstrating the advantages of LC–ED analysis over conventional techniques. Depicted is the dominant response channel 2 corresponding to 320 mV applied potential. Again, the abundance of *cis*-lycopene isomers observed is similar to that noted in samples of greater mass analyzed previously by conventional UV–Vis absorbance methods [11], and accounts for approximately 75% of the total lycopene.

Separation of nine geometric isomers of lycopene in buccal mucosal cells was achieved (Fig. 7c). Cell pellets collected for this study contained an average of 34.7±16.5 μg protein (mean±standard error for $n=40$) as determined by the Bradford method (Bio-Rad Labs., Hercules, CA, USA). Peng and Peng [19] showed a correlation between plasma and buccal cell carotenoids. Buccal cells represent a non-invasive source of human tissue in which carotenoid deposi-

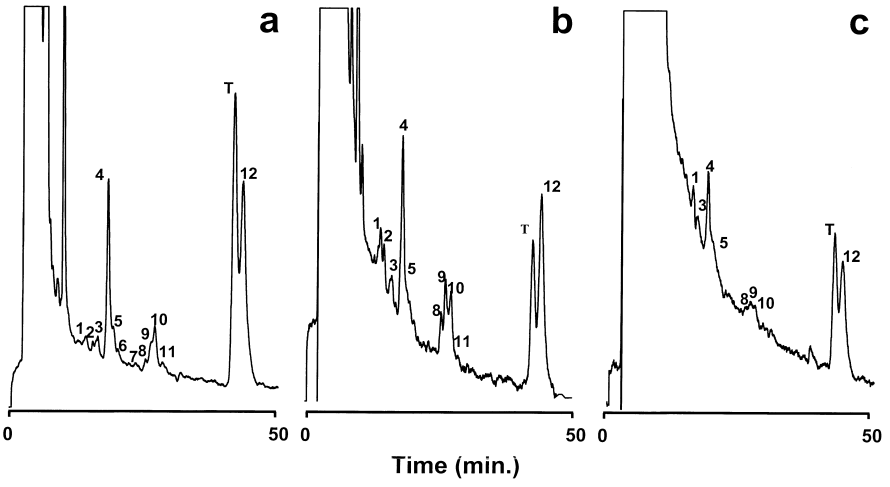

Fig. 7. Representative isocratic four-channel LC–ED C_{30} chromatograms. Channel 2 (320 mV) is depicted for (a) 50-μl human plasma (20 nA full scale); (b) 10-mg prostate tissue biopsy sample (10 nA full scale); (c) buccal mucosal cells (5 nA full scale). Peak identifications: T=*trans*-lycopene; 1–12=*cis*-lycopene isomers.

tion may be monitored. A similar geometrical isomer distribution between these biological samples would make them valuable targets as representative non-invasive samples for future human intervention trials. To date such a correlation has not been established for any carotenoid geometrical isomers including lycopene.

3.2.2. Gradient analysis

Plasma was separated from raw blood collected from patients consuming sauce produced from either *Roma* or *Tangerine* tomatoes as part of a separate investigation. Separation of *cis*-lycopene isomers by gradient LC–ED from blood plasma of patients consuming red *Roma* tomato is shown in Fig. 8a. Similar lycopene isomer profiles were noted between samples separated by the isocratic method (Fig. 7a). The enhanced capability of gradient elution is noted in the resolution of prolycopene from β-carotene in the *Tangerine* tomato (Fig. 4b). This method allowed for resolution of this isomer in the blood plasma of patients consuming prolycopene from sauce produced from *Tangerine* tomato (Fig. 8b). Two unidentified peaks were noted in the same plasma. In-line photodiode array detection revealed the UV–Vis absorbance spectra of these peaks to be similar to that of *trans*-lycopene pointing to the possibility that these may also be minor lycopene isomers formed from degradative processes during food preparation, digestion and/or absorption and distribution through the body. This gradient methodology when coupled to the generated CVC for prolycopene offers a selective and sensitive method for the continued study of the relationship between *cis* to *trans* isomer ratio both in the diet and human biological samples.

The analysis of a 10 mg biopsy of cervical tissue was accomplished by the LC–ED gradient method (Fig. 9). We have previously reported the successful analysis of predominant cervical tissue carotenoids with exception of lycopene by LC–ED [10]. Ten *cis*-lycopene isomers were detected showing that as with other biological samples *cis* to *trans* isomer ratio in cervical tissue may be as high as other biological samples such as plasma, representing approximately 60% of the total lycopene concen-

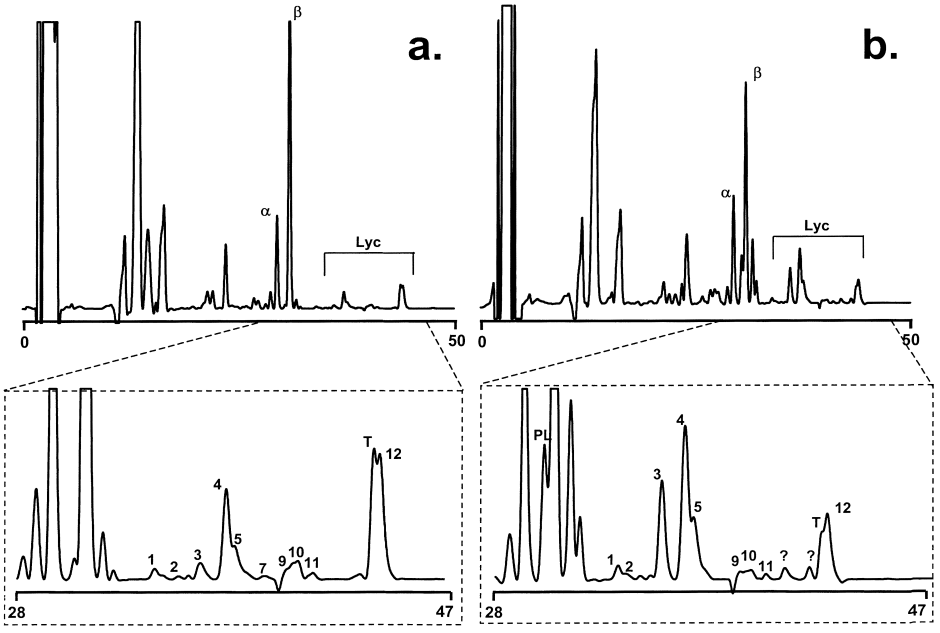

Fig. 8. Representative lycopene isomer separation by gradient C_{30} LC–ED from 100 μl human plasma of patients consuming sauce produced from (a) *Roma* and (b) *Tangerine* variety tomatoes. Detection was performed on an eight-channel coulometric array detector. Channel 5 (440 mV) is depicted for both samples (20 nA full scale). Peak identifications: α=α-carotene; β=β-carotene; T=*trans*-lycopene; PL=prolycopene. Inset highlights *cis*-lycopene separation labeled 1–12.

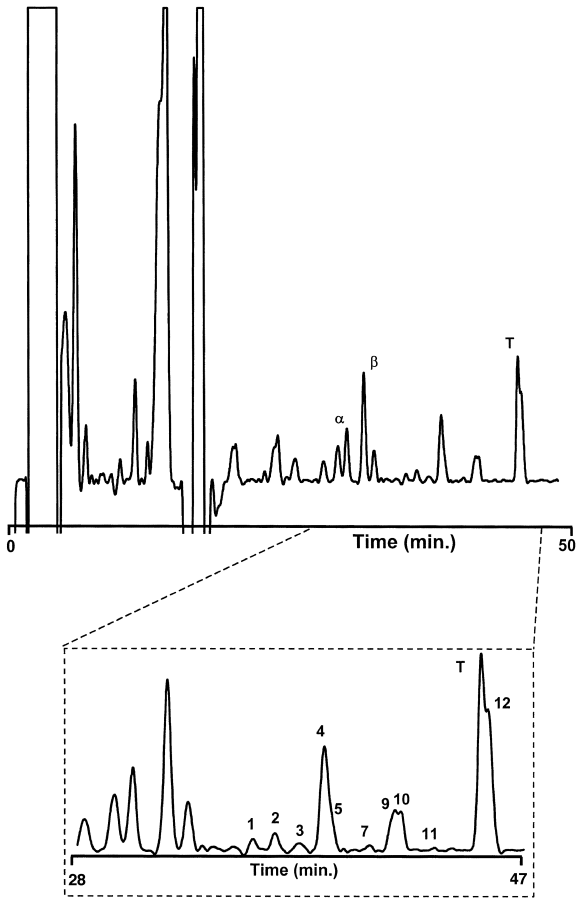

Fig. 9. Gradient C_{30} LC–ED chromatography of an extract from 10 mg of human cervical tissue detected on an eight-channel coulometric array detector. Channel 5 (440 mV) is depicted (10 nA full scale). Peak identifications: α=α-carotene; β=β-carotene; T=*trans*-lycopene. Inset highlights *cis*-lycopene separation labeled 1–12.

tration. The use of reduced sample sizes without loss in sensitivity may expand the capabilities of current experimental protocols.

As with previous LC–ED reports a number of electrochemically active compounds beyond the carotenoids of interest can be found in the biological samples. Significant amounts of lipophilic compounds such as tocopherols have been identified in these samples (data not shown). Also enzymatic treatment is used in the extraction of select biologicals such as prostate and cervical tissue. A number of electrochemically active contaminants have been noted through control experiments with these commercial enzyme preparations, and must therefore be considered in designing LC methodologies to ensure minimal interference with analyte measurement.

3.3. Precision and recovery

Intra-day variability expressed as relative standard deviation (RSD) was determined for both isocratic and gradient plasma lycopene analysis. Aliquots of 100 μl of pooled plasma samples were extracted in triplicate and analyzed by both isocratic and gradient methods described previously. Intra-day variability for lycopene was found to be 7.7 and 9.2% for isocratic and gradient methods, respectively. Mean recovery of lycopene from plasma was determined by spiking pooled plasma with 100 pmol of *trans*-lycopene and found to be greater than 90% for both methods. The small size of available human tissue biopsy samples combined with the nature of tissue procurement procedures made assessment of recovery from cervical, prostate and buccal mucosal cell samples difficult.

C_{30} columns employed in this and other studies conducted in our laboratory have maintained comparable life times to that of typical reverse phase columns. In cases were cleaning of the stationary phase is required we have utilized a simple procedure based on methanol, acetonitrile and tetrahydrofuran (THF). A step gradient is initiated whereby each of these solvents washes the column for 15 min with the exception of 100% THF which washes for 30 min. Following this procedure the column is reequilibrated with 100% methanol prior to further analysis.

ED has proven to be a useful means of detection in microanalytical applications. Detection limits were determined to be 50 fmol per 20 μl injection for both four-channel isocratic and eight-channel gradient methods. These represent a 10- to 100-fold improvement relative to conventional UV–Vis absorbance detection methods. The selectivity of these methods toward different carotenoid species is similar to that demonstrated by previous studies with other carotenoids in our laboratory [10]. Development of these lycopene microanalytical techniques combined with the potential of prolycopene rich *Tangerine* tomatoes will allow us to expand our understanding of factors

associated with lycopene distribution, metabolism and in vivo isomerization.

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