REVIEW

Analysis of vitamin E metabolites in biological specimen

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Vitamin E is known as the most important lipid antioxidant and is widely used to prevent age-associated diseases. Despite increasing knowledge about human vitamin E metabolism, little is known to justify its widespread use. As meta-analyses revealed even harmful effects of high vitamin E doses, a profound understanding of vitamin E metabolism is mandatory. By recent advances in analytical methodology, new metabolites with distinct physicochemical and biological properties were discovered. This review covers current methods to analyze vitamin E metabolites in biological samples. Special emphasis is laid on analytical applications for the identification and quantification of metabolites with a modified hydroxychromanol ring or a truncated side chain.

Keywords:
Analysis / Carboxyhydroxychromanols / MS / Tocopherols / Vitamin E metabolism

1 Introduction

The term vitamin E describes a mixture of isomers that differ by methylation patterns of their hydroxychromanol ring and the number of double bonds in the side chain (α-, β-, γ-, δ-tocopherol and -tocotrienol) and of which α-tocopherol (α-TOH) is assigned the most active vitamer (Fig. 1). By definition, α-TOH prevents the resorption of the fetus in vitamin-E-deficient rats by 100% [1, 2]. Furthermore, symptoms of ataxia in α-TOH transfer protein knockout mice could be impeded by α-TOH administration [3]. The protein is responsible for the transport of α-TOH from the liver to nascent VLDL. Since the discovery of vitamin E by Evans and Bishop [4], α-TOH has been accounted as an antioxidant capable to scavenge reactive oxygen species. Decreased α-TOH levels have been associated with several diseases including cancer, cardiovascular diseases and diabetes [5]. While its role as lipophilic antioxidant *in vitro* is widely accepted, the relevance *in vivo* is still a matter of debate [6–8].

A clear understanding of physiological functions, pharmacokinetics and metabolism of vitamin E is mandatory to establish the requirements of human dietary vitamin E, which were set to 15 mg/day by the Food and Nutrition Board of the Institute of Medicine [9]. In addition, pathophysiological conditions such as cardiovascular diseases, diabetes mellitus or cancer could lead to an increased or even reduced demand of the vitamin. Meta-analyses of human intervention trials revealed an increased mortality risk when high doses of vitamin E were administered, and a recent study suggests that the intake of a combination of 400 IU of vitamin E and 1000 mg vitamin C may diminish the beneficial, anti-diabetic effects of exercise [10–12]. Super nutritional doses of the vitamin should be accounted as pharmacological intervention and investigated as such. Thus, a profound knowledge of the pharmacokinetics and metabolism is mandatory.

Chromatographic methods have been developed to separate vitamin E isomers from other lipids such as triglycerides or phospholipids. Early reports applied TLC followed by the visualization with Emmerie-Engel reagent (2,2′-bipyridyl/$FeCl_3$ in ethanol) to detect tocopherols in biological samples [13]. The development of GC and HPLC allowed separation of vitamers and significantly stimulated vitamin E research. Chromatographic methods for tocopherol isomer analysis are summarized in comprehensive reviews [14, 15]. At present, highly sensitive mass spectroscopic methods detect minute amounts of vitamers in lower nanomolar concentrations in

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Abbreviations: APCI, atmospheric pressure chemical ionization; **CEHC,** carboxyethylhydroxychromanol; **ECD,** electrochemical detection; **ICP-MS,** ion-coupled plasma MS; **5-NGT,** 5-nitro-γ-tocopherol; **α-TOH,** α-tocopherol; **α-TP,** α-tocopheryl phosphate; **α-TQ,** α-tocopherylquinone

different matrices such as human plasma, sunflower oil and milk [16, 17]. A more general approach allows the simultaneous detection of tocopherol isomers and other lipophilic vitamins such as K, D and A [18].

Determination of intracellular concentrations of tocopherols and tocopherol metabolites or even intracellular distribution of vitamin E needs state-of-the-art analytical approaches. This review focuses on the analysis of vitamin E metabolites that are formed by modifications of the chromanol ring (either by conjugation or by oxidation process), by side chain degradation or by concurrent process (Fig. 1). The metabolic processing of vitamin E was currently discussed in detail [19], but needs further attention as new properties of the metabolites arise from the scientific literature. For example, natural forms of vitamin E were recently shown to be metabolized to long-chain carboxychromanols and their sulfated counterparts. Long-chain carboxychromanols can inhibit cyclooxygenase-2-catalyzed prostaglandin E_2 synthesis in IL-1β-stimulated A549 cells. The cellular inhibition was partially diminished by sesamin, which blocks the metabolism of vitamin E, suggesting that their metabolites may be responsible for the inhibitory effects [20]. One might speculate that tocopherols need to be metabolized to display their "true" vitamin nature as it is known for vitamins A and D. Therefore, more analytical and biochemical research is needed to study these metabolites *in vitro* and *in vivo*. In addition, the discovery of the nitrosylation product of γ-tocopherol as potential marker of inflammation and the phosphate of α-TOH in biological samples and foods require advanced analytical methods.

2 General remarks

The most abundant oxidation products of tocopherols are α-tocopherylquinone (α-TQ), epoxy-α-TQ and 5-nitro-γ-tocopherol (5-NGT). For the analysis of these and other

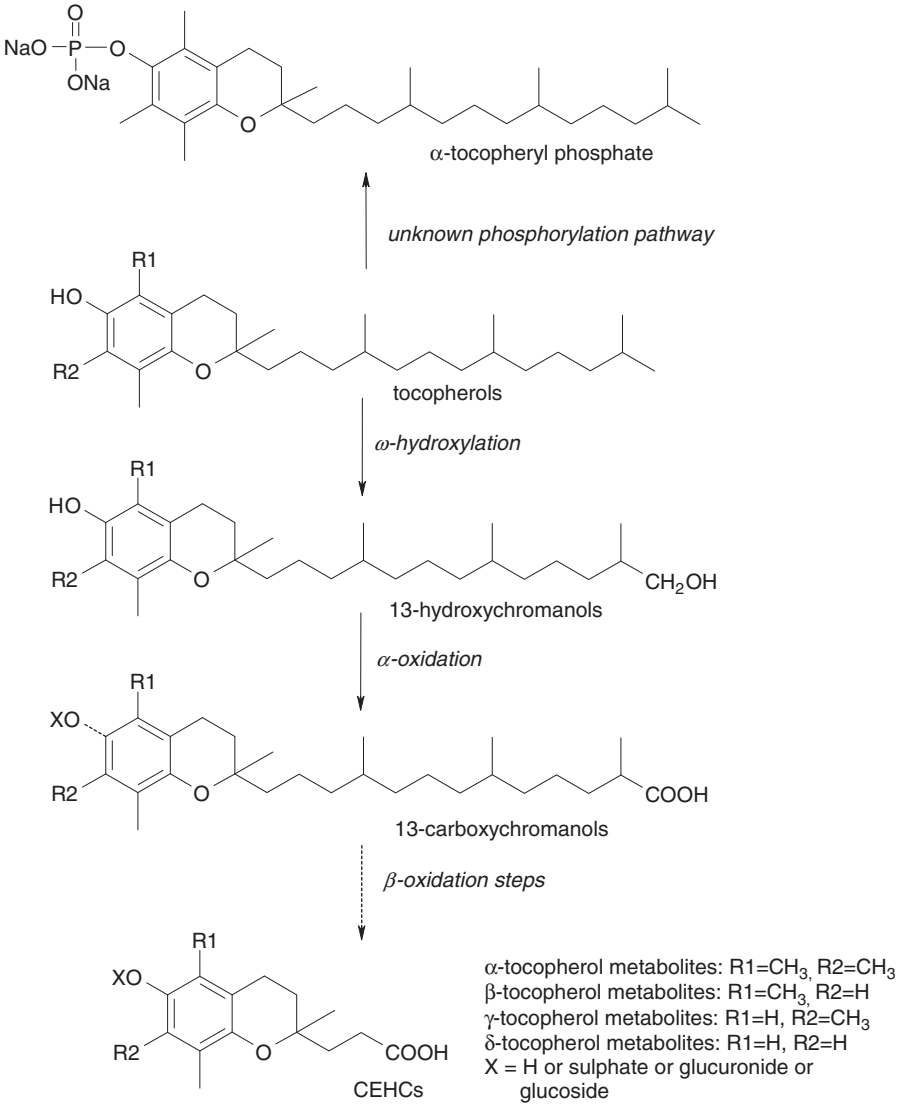

Figure 1. Vitamin E metabolites derived from side-chain truncation and conjugation.

metabolites, all workup procedures must be carried out under inert gas atmosphere to avoid artificial tocopherylquinone formation.

Typically, simultaneous separation and detection of these metabolites and tocopherols can be achieved, whereas metabolites that emerge from detoxification or side-chain degradation are more hydrophilic and thus different chromatographic conditions and detection methods must be used.

3 Analysis of tocopherol metabolites with intact side chain

3.1 α-TQ

α-TQ is formed by two electron oxidants such as HOCl and $ONOO^-$, whereas isomeric 5,6-epoxy-α-TQ and 2,3-epoxy-α-TQ are generated following exposure to radical oxidants [21]. Interestingly, α-TQ has been found to be the main oxidation product in human plasma (1% of α-TOH) and atherosclerotic plaques (11% of α-TOH) [21–23].

Tocopherylquinones were extracted under inert gas atmosphere with organic solvents and trimethylsilyl derivatized with *N,O*-(bistrimethylsilyl)trifluoroacetamide/trimethylchlorosilane (10:1 v/v) or a mixture of *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide/*N*-trimethylsilyl-imidazole for gas chromatographic separation [21, 24, 25] (Table 1). Trimethylsilyl derivatives were characterized and quantified by GC-MS. A different approach used post-column derivatization in a photoreactor or on a Zn column to investigate tocopherylquinones by FL or UV detection [23]. Finally, atmospheric pressure chemical ionization MS (APCI-MS) was used for simultaneous detection of tocopherol isomers and tocopherylquinone in human plasma [16]. Modern mass spectroscopic methods have the advantage to circumvent laborious de-conjugation and derivatization procedures. Despite initial investigations, LC-MS is less time and cost consuming than the procedures described earlier.

In human intervention trials, vitamin E was believed to act as an antioxidant; however, scare analytical proofs were established so far [26]. Future trials are recommended to determine oxidation products such as α-TQ to balance the amount of oxidative stress.

3.2 5-NGT

Due to structural differences between α- and γ-tocopherol (methylation at position 5), the latter efficiently scavenges reactive nitrogen species forming 5-NGT [27]. Elevated levels of the nitration product of γ-tocopherol were detected in states of acute and chronic inflammatory stress, *i.e.* in plasma of patients with coronary heart disease [28], in smokers [29] or in brain tissue of patients with Alzheimer's disease [30]. 5-NGT is extracted from plasma or tissue samples by organic solvents such as hexane. It is separated from other tocopherol isomers by RP-HPLC and detected by MS with APCI [29] and ESI [28], respectively, or by coulometric detection [30–32] (Table 1). Both detection methods allow sub-picomolar metabolite quantification.

Side-chain degradation products of 5-NGT, namely 5-nitro-γ-carboxyethylhydroxychromanol (CEHC), could not be detected in urine and cell culture experiments, presumably due to a diminished cellular uptake of 5-NGT [33].

3.3 α-Tocopheryl phosphate

α-Tocopheryl phosphate (α-TP), a redox silent form of α-TOH, occurs naturally in animal tissue (liver, fat), foodstuff such as chocolate and cheese [34] and human adipose tissues [35]. α-TP effectively inhibits cell proliferation, downregulates CD36 transcription and reduces atherosclerotic plaque formation [36, 37]. It is assumed to be a signaling molecule transmitting the nonantioxidative effects of α-TOH [38]. However, the author likes to point out that no physiological role has been assigned to α-TP *in vivo*. Since its discovery in 2003, no human study has been conducted and thus the relevance for human is still missing.

In most cases, analyses of tocopherols from complex matrices such as plants or animal tissue start with an alkaline hydrolysis (2 M sodium or potassium hydroxide) of triglycerides to liberate tocopherols. Under these conditions, one would expect hydrolysis of α-TP; however, the molecule is converted into its water-soluble disodium or dipotassium salt which is surprisingly stable. Of note, α-TP thus escapes classical sample workups. Gianello *et al.* described an extraction procedure where the alkaline solution is acidified and α-TP is extracted with hexane [34]. To estimate the physicochemical properties of α-TP, we calculated the pK_a values *via* ACD/pKa software [39]. The program calculated two pK_a values, 6.07 and 1.64, which are in accordance to values of structural related phosphates published previously [40]. According to these calculations, we expect the disodium salt of α-TP to occur at physiological pH, the monoprotonated form at pH below 6.07 and diprotonated α-TP at pH lower than 1.64 (unpublished results).

As α-TP lack redox activity and the inertness of the phosphorylated phenolic group to chemical derivatization, electrochemical detection (ECD) or GC-MS is not applicable. Fluorescence detection of α-TP was used in several studies with a detection limit of 10 ng/mL [34, 36, 41] (Table 1). In addition, MS and MS/MS with ESI were used to elucidate the identity of the molecule in biological samples.

4 Analysis of tocopherol metabolites with shortened side chain

Previous studies have shown that tocopherols and tocotrienols are metabolized *via* an initial ϖ-hydroxylation through CYP3A4 and/or CYP4F2 leading to 13-hydroxychromanols (13-OH) and α-oxidation of the 13-carbon

Table 1. Methods to analyze oxidation products of vitamin E

Metabolites	Matrices	Stationary phase, mobile phase, detection	Detection limits	Linearity	Refs.
5-NGT	Human plasma *in vitro* and *in vivo*	Waters symmetry LC-18 column (3.5 μm, 4.6 × 75 mm), MeOH 100%, MS (APCI(−))	20 fmol (o.c.)	0.1–3.0 pmol	[29]
	Human plasma, atherosclerotic plaque	Agilent LiChrosphere C18 column (5 μm, 4 × 125 mm), gradient MeOH/ethyl acetate, UV and MS/MS (ESI(−))	5 pmol (o.c.)	0–500 pmol	[28]
	LPS-stimulated primary rat astrocytes, brain tissue (Alzheimer patients)	Toso-Haas ODS-80TM (5 μm, 4.6 × 250 mm), ACN/MeOH (83:12 v/v, 0.2% acetic acid, 30 mM Li-acetate), ECD	0.5 pmol (o.c.)	n.d.	[30, 32]
	Rat plasma	Supelco Supelcosil LC-18-DB (3 μm, 4.6 × 150 mm), MeOH/Li-acetate (0.5 M, pH 4.75) 95:5 v/v, ECD	10 fmol (o.c.)	0.25–250 pmol	[31]
α-TQ	Human plasma	Knauer LiChrosorb-100 RP-18 (10 μm, 250 × 4.6 mm), MeOH, PR (Ex254) FL (Ex294, Ex331) or Zn-column reduction FL	250 or 100 pg (o.c.)	0.5–50 ng/50 μL	[23]
	Standards	Restek Rtx-5MS (0.25 μm, 30 m × 0.25 mm), silylation, GC-MS	1–10 ng (o.c.)	n.d.	[25]
	Human plasma, fish oil	Varian A2014250X020 propyl-amine column (3 μm, 250 × 2 mm), gradient hexane and hexane/1,4 dioxane (1:1 v/v), MS (APCI(+))	9 nM	0.01–29 μmol/L	[16]
α-TQ and epoxy-α-TQ isomers	Atherosclerotic plaque	J&W Scientific DB-5ms (30 m × 0.25 mm), silylation, stable-isotope dilution GC-MS	10–25 fmol (o.c.)	0.025–2000 pmol	[21, 24]
α-Tocopheronolacton (Simon metabolite)	Human urine	–, –, CID-MS	n.d.	n.d.	[61]

CID, collision-induced dissociation; Ex, excitation; FL, fluorescence; MeOH, methanol; n.d., no data; o.c., on column.

atom results in 13-carboxychromanols (13-COOH) (Fig. 1) [42, 43]. Subsequent β-oxidation steps lead to carboxychromanols with shortened side chain. The main urinary vitamin E metabolite found in human is the conjugated form of CEHC [43, 44]. In addition, long-chain metabolites were identified *in vitro* and *in vivo* [45, 46]. New properties were assigned to vitamin E metabolites that differ from the mother compounds (tocopherols). Wechter *et al.* identified γ-CEHC as endogenous natriuretic factor, the activity of which is mediated by an inhibition of the 70 pS K^+ channel in the apical membrane of the thick ascending limb of the kidney [47]. Furthermore, antiproliferative effects were shown for γ-CEHC in PC-3 prostate cancer cells and for long-chain metabolites (α- and δ-13-COOH) in murine glioma C6 cells [48, 49]. α- and γ-CEHC reduce the inflammatory response in TNF-α or bacterial lipopolysaccharide-treated rat aortic endothelial cells and mouse microglial cultures through inhibiting prostaglandin E_2 and nitric oxide production [50]. Jiang *et al.* showed that γ-13-COOH is a potent inhibitor of cyclooxygenases 1 and 2 *via* interacting with the substrate-binding site of the enzyme [20].

4.1 Long-chain metabolites

Long-, medium- and short-chain metabolites of vitamin E were found as phase II 6-*O*-chromanol derivatives, primarily as sulfates, glucuronides and glucosides, respectively, or as nonconjugated carboxychromanols. Quantitative extraction of the metabolites from biological samples affords deconjugation by sulfatase/glucuronidase treatment or by chemical hydrolysis.

Long-chain chromanols are not excreted into urine and were found in rat plasma, liver and in cell culture media of human lung epithelial A549 cells. Jiang and Freiser identified free and sulfated conjugates of γ- and δ-13-COOH, γ- and δ-11-COOH, and γ- and δ-9-COOH [46, 51, 52] (Table 2). The authors proved the sulfate-conjugation by ion-coupled plasma MS (ICP-MS), as classical MS cannot easily distinguish between phosphate and sulfate conjugates due to identical masses of $-OPO_3H_2$ and $-OSO_3H$ residues [53]. Both sulfated and free carboxychromanols were separated by reversed-phase chromatography and quantified simultaneously by fluorescence detection. Alternatively, silylation of free carboxychromanols make them applicable for gas chromatographic separation [42]. Similar to α-TP analysis, ECD or derivatization of sulfated conjugates is not applicable. Again, MS is the method of choice when investigating these metabolites or conjugates thereof.

4.2 Medium-chain metabolites

Medium-chain metabolites, such as 7- and 5-carboxychromanols, are short-living intermediates and usually not found in significant amounts in plasma or urine. α-7-COOH and α-5-COOH (CMBHC) were identified in the supernatant media of α-TOH-treated liver HepG2 cells, and ataxia with isolated vitamin E deficiency patients receiving 800 mg (*RRR*)-α-TOH-secreted α-5-COOH in urine [43, 54] (Table 2). The structures of both the metabolites were elucidated by GC-MS.

4.3 Short-chain metabolites

3′-Carboxychromanols (CEHCs) were the first tocopherol metabolites reported in animals and human urine [47, 55, 56]. While most of γ-tocopherol is rapidly eliminated *via* urinary excretion of γ-CEHC and unknown pathways [57], only 0.1–5% of α-TOH is excreted as α-CEHC depending on α-TOH plasma level and sample workup [56, 58, 59].

A typical workup protocol consists of enzymatic or chemical hydrolysis of the conjugates, acidification and extraction with organic solvents. In case of α-CEHC, procedures need to be carried out under inert gas atmosphere to prevent oxidative formation of α-tocopheronolactone (Simon metabolite), a metabolite discussed as a marker of oxidative stress [56]. Omitting harsh workup procedures, Pope *et al.* were able to detect minute amounts of α-tocopheronolactone in human urine [60, 61].

Enzymatic cleavage of α-CEHC conjugates was accomplished with the use of β-glucuronidase type H-1 from *Helix pomatia* with glucuronidase and sulfatase activity [62–64], with β-glucuronidase type IX-A from *Escherichia coli* with glucuronidase activity only [65], or with sulfatase type H-1 [63]. Li *et al.* observed that pre-treatment of rat urine with β-glucuronidase (with sulfatase activity) or sulfatase H-1 liberated only small amounts of α-CEHC, whereas hydrolysis with 6N HCl under reducing conditions (ascorbate) increased the amount of α-CEHC by 50-fold [63]. A less-elaborate approach used 3 N methanolic HCl for hydrolysis and esterification of conjugates. The corresponding CEHC methylesters were detected by HPLC-ECD [66].

CEHCs have been detected by GC-MS, HPLC-ECD and LC-MS. For GC-MS analysis, the *O*-silylation of the phenol and the carboxy group is mandatory. For this purpose, a mixture of *N,O*-(bistrimethylsilyl)trifluoroacetamide and trimethylchlorosilane (99:1 v/v) in anhydrous pyridine was used in most of the studies [43, 57, 67]. ECD is highly selective and sensitive; however, metabolite fractions need to be confirmed by fully characterized standards. In addition, conjugates are not prone to ECD. A most promising approach for the simultaneous detection of free and conjugated long- and short-chain metabolites is ESI-MS or MS/MS with excellent detection levels (20–80 fmol) [46, 52, 64, 68]. The method allows the identification of new metabolites without laborious de-conjugation and derivatization procedures.

As γ-CEHC acts as a natriuretic hormone, some efforts were made to investigate the properties of the stereoisomers

Table 2. Methods to analyze vitamin E metabolites

Metabolites	Matrices	Stationary phase, mobile phase, detection	Detection limits	Linearity	Refs.
α-TP	Liver and adipose tissue, foodstuff	–, direct injection, MS (ESI(−))	n.d.	n.d.	[35]
	Rat liver, 3T3-L1 adipocytes	(i) Phenomenex Luna C8 (5 μm, 4 × 150 mm), gradient isopropanol (0.2% phosphoric acid) and water, FL (Ex297; Em319), (ii) column see (i), gradient ACN/H_2O (75:25 v/v 0.1% formic acid) and isopropanol (0.1% formic acid), MS (ESI(−)), (iii) Waters C18 bridged ethane-linked hybrid (1.7 μm, 2.1 × 50 mm), MeOH (pH 9.5), MS/MS (ESI (+))	(i)10 ng/mL (ii) n.d. (iii) n.d.	(i) n.d. n.d. n.d.	[34]
	Rabbit serum, THP-1 cells	Phenomenex Luna C8 (5 μm, 4 × 150 mm), gradient isopropanol (0.2% phosphoric acid) and water, FL (Ex297; Em319) UV (286 nm)	10 ng/mL	n.d.	[36, 41]
γ- and α-13-OH, γ- and α-13-COOH, X = H	Rat and human liver microsomes	Hewlett Packard HP-1 methylsiloxane capillary column (30 m × 0.25 mm), silylation, GC-MS (SIM)	n.d.	n.d.	[42]
γ-13-OH, δ-13-OH, γ- and δ-13-COOH, X = H 13-COOH metabolite from γ-tocotrienol	Rat plasma and liver, A549 cells	(i) Supelco Supelcosil LC-18-DB (5 μm, 4.6 × 150 mm), gradient ACN/10 mM ammonium acetate pH 4.5 (35:65 v/v) and ACN/10 mM ammonium acetate pH 4.5 (96:4 v/v), FL (Ex292; Em327), (ii) column see (i), gradient see (i), MS (ESI(−)), (iii) DB-1 capillary column (15 m), silylation, GC-MS	50 nM	50 nM–5 μM	[20, 46, 51, 52]
13-OH and 13-COOH metabolites of all tocopherol and tocotrienol isomers, respectively	A549 and HepG2 cells	Hewlett Packard HP-1 methylsiloxane capillary column (0.25 mm × 30 m), silylation, GC-MS (SIM)	n.d.	n.d.	[73]
γ- and δ-13-COOH, X = sulfate	A549 cells	(i) 13-COOH, X = H, MS (ESI(–)), (ii) –, direct injection, ^{34}S and ^{31}P ICP-MS	(i), (ii), n.d.	n.d.	[46]
γ- and δ-11-COOH, and γ- and δ-9-COOH, X = H and sulfate	Rat plasma, A549 cells				[51, 52]
α-7-COOH, X = H	HepG2 cells	Merck RP-18 end-capped (250 × 4 mm), tetramethyl-ammoniumhydroxide (20% in water)/water/CAN (25:625:350 v/v/v) and 0.02% EDTA, ECD	1 nM	n.d.	[43]
			n.d.	n.d.	[54]

Table 2. Continued

Metabolites	Matrices	Stationary phase, mobile phase, detection	Detection limits	Linearity	Refs.
α-5-COOH (CMBHC), X = H	Human plasma (AVED patient)	DB-5MS capillary column (0.25 μm, 0.25 mm × 30 m), silylation, GC-MS			
α- and γ-5-COOH, X = H	HepG2 cells	Hewlett Packard HP-1 methylsiloxane capillary column (0.25 mm × 30 m), silylation, GC-MS (SIM)	n.d.	n.d.	[74]
α- and γ-3-COOH (CEHC), X = H	Human urine	Sepserv Ultrasep ES100 RP-8, gradient ACN and McIlvaine buffer (0.01 M citric acid, 0.02 M di-ammonium hydrogen phosphate, pH 4.15) both containing 1% Li-perchlorate, ECD	10 fmol (o.c.)	0.2–20 pmol	[75]
	Human plasma and urine	Hewlett Packard HP-1 methylsiloxane capillary column (0.33 μm, 0.2 mm × 25 m), silylation, GC-MS (SIM)	2–5 nM	0–200 nM	[57]
	Human plasma (renal failure patients)	Eka Nobel Kromasil 100 C8 (5 μm, 150 × 4.6 mm), ACN/5 mM tetrabutylammoniumbromide 0.02% EDTA (pH 4.5)(35:65 v/v), ECD	n.d.	n.d.	[76]
	Human plasma and urine, rat liver, lung and kidney	Waters Symmetry Shield RP-18 (3.5 μm, 3 × 150 mm), gradient MeOH/water (0.05% acetic acid), MS (ESI(−))	0.08 pmol 20 fmol (o.c.)	0.025–20 pmol	[64, 68, 77]
	Rat urine	GL Science ODS-3 (250 × 2.1 mm), ACN/50 mM Na-perchlorate (pH 3.6) (35:65 v/v), ECD	n.d.	0.01–0.05 μg/mL	[66, 78]
	HepG2 cells				[74]
α-, δ-, and γ-3-COOH, X = H	HepG2 cells, human serum				[43, 65]
γ-3-COOH, X = H	Rat urine and bile	ODS-3, ACN/water (45:55 v/v) containing 50 mM Na-perchlorate, ECD (CEHC methylester)	n.d.	0.01–0.05 μg/mL	[66]
α-3-COOH, X = H	Human urine				[54]
R,S-γ-3-COOH, X = H	Synthetic	Regis *S, S*–Whelk-O 1 (4.6 × 250 mm), hexane/2-propanol/acetic acid (80:20:0.5 v/v/v), UV (295 nm)	n.d.	n.d.	[69]
	Rat plasma	column switching: TSKgel Super-Phenyl (100 × 4.6 mm),TSKgel ODS-80Ts (250 × 4.6 mm), Chiralcel OD-RH (150 × 4.6 mm), DBD-PZ derivatization, FL (Ex450; Em560)	n.d.	n.d.	[71, 72]
α-3-COOH, X = glucuronide	Human urine	–, –, CID-MS	n.d.	n.d.	[61]
α-3-COOH, X = sulfate	Rat urine	Phenomenex Luna C18 (5 μm, 250 × 10 mm), MeOH/water (50:50 v/v), MS (ESI(−))	n.d.	n.d.	[63]
	Human urine				[61]
γ-3-COOH, X = β-D-glucoside	Mouse urine	Waters Acquity BEH C18 (1.7 μm, 50 × 2.1 mm), gradient water/ACN both cont. (0.1% formic acid), UPLC-TOFMS (+)	n.d.	n.d.	[62]

AVED, ataxia with isolated vitamin E deficiency; CID, collision-induced dissociation; Em, emission; Ex, excitation; FL, fluorescence; MeOH, methanol; n.d., no data; o.c., on column; SIM, single ion monitoring; TOFMS, time-of-flight MS.

R-γ-CEHC and *S*-γ-CEHC. Both enantiomers were synthesized and separated by chiral chromatography to show that only the *S*-isomers were able to inhibit the 70 pS K^+ channels in low nanomolar range [69, 70]. Takata *et al.* were able to separate a mixture of *R*- and *S*-γ-CEHC by chiral chromatography and detect the *S*-enantiomer in rat plasma after administration of a γ-tocopherol or γ-tocotrienol prodrug [71, 72].

Finally, a recent metabolome analysis of pregnane-X-receptor-activated mice revealed a new metabolite, identified as the glucoside of α-CEHC [62]. The metabolite was found by a *de novo* approach where urine and plasma were analyzed by principal component analysis to determine which ions contribute most to the difference between control and pregnenolone-16α-carbonitrile-treated mice. In the future, metabolomics can be used as a powerful analytic tool for the identification of new vitamin E metabolites and maybe more interestingly, the quantification of metabolites in general after vitamin E supplementation.

5 Summary and future perspectives

In the last decade, several new vitamin E metabolites were identified by advanced analytical approaches and were found to have properties very distinct from their precursors. Analytical approaches made use of GC-MS, HPLC-ECD or fluorescence detection. The use of MS brought new insights into the distribution of conjugated metabolites in plasma and urine. However, less is known about tissue distribution in liver or fat. Pharmacokinetics of tocopherols are well studied in rats and mice; however, a full balancing of vitamin E in humans has not been conducted.

MS has developed into an important tool in nutrition research. Its use in vitamin E research will unravel the bioavailability and bio-efficacy of tocopherols in human. MS methods circumvent painstaking hydrolysis and derivatization procedures and are able to characterize new metabolites *via* MS/MS. In addition, LC-MS is time and cost efficient, which makes it suitable for future intervention trials.

Finally, metabolomic approaches that use a combination of ultra-performance liquid chromatography combined with TOF-MS are superior in analyte separation and spectral quality to conventional LC-MS. Using these techniques, comparatively small pilot trials could investigate the global impact of vitamin E administration.

The metabolites used in text were automatically created by AutoNom 2000 software according to IUPAC rules [79] and are as follows]: α-13-OH, 2-(13-hydroxy-4,8,12-trimethyl-tridecyl)-2,5,7,8-tetramethyl-chroman-6-ol; α-13-COOH, 13-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-2,6,10-trimethyl-tridecanoic acid; α-11-COOH 11-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-4,8-dimethyl-undecanoic acid; α-9-COOH, 9-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-2,6-dimethyl-nonanoic acid; α-7-COOH, 7-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-4-methyl-heptanoic acid; α-5-COOH/α-CMBHC, 5-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-2-methyl-pentanoic acid; α-3-COOH/α-CEHC, 3-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-propionic acid).

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