

## Inhibition of Human Cytochrome P450 Metabolism by Blended Herbal Products and Vitamins

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**ABSTRACT - Purpose.** The use of supplements as herbal and micronutrient natural health products with conventional health products has become increasingly popular. It has been reported that some herbal products can inhibit the activity of cytochrome P450-mediated metabolism and drug disposition. This study was designed to investigate a case report of a severe adverse event to determine the potential interactions of two blended botanical products and vitamins on cytochrome P450-mediated drug metabolism. **Methods.** The effect of extracts from these commercially available herbal formulations, *trans*- $\beta$ -carotene (tBC), multivitamins, and vitamin D<sub>3</sub> supplements on cytochrome P450-mediated drug metabolism of marker substrates was determined *in vitro*. **Results.** The two blended herbal products had a high potential to affect the safety and efficacy of many health products. Some vitamin and tBC containing products also have the potential to affect drug disposition. The tBC content of various products was analyzed and significant discrepancies were found among them and between values indicated on product labels. Product extracts also exhibited a low to moderate capacity to inhibit cytochrome P450 2C9, 2C19 and 3A4-mediated metabolism. **Conclusions.** The findings of this study suggest that these herbal products and most vitamin products may have an inhibitory effect on cytochrome P450 activity that could contribute to development of an adverse event. Further work is warranted to determine how supplementation with these products may affect drug metabolism in an *in vivo* context.

### INTRODUCTION

Natural health products (NHPs), including traditional Chinese medicines, homeopathic remedies, vitamins, minerals and trace elements, herbal remedies, probiotics, amino acids, plant isolates and essential fatty acids are popular complementary or alternative medicine (CAM) therapies. NHPs are frequently used by patients with chronic or recurrent conditions; these patients are also the most likely to be prescribed conventional medications on an ongoing basis (1-5). Although the risk of adverse events (AEs) from drug-NHP interactions is widely reported (6-13), there are relatively few reported NHP-related AEs with the approximately 45,000 NHPs marketed for use in Canada alone (14-15). Currently, AEs associated with NHPs are generally monitored

through a passive surveillance system based entirely on voluntary reporting by health professionals or consumers. There is a staggering under-reporting of potential NHP-related AEs as opposed to their lack of occurrence (14). In the survey of community pharmacists, the findings revealed that almost half (47%) of 132 responding pharmacists had seen a potential NHP-drug interaction, but only two (1.5%) reports were filed (15).

As pharmacists routinely inquire about medication use and recognize AEs, a partnership with community pharmacies was established to conduct a pilot Study Of Natural health product Adverse Reactions (SONAR) to assess the

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feasibility of utilizing active surveillance in participating community pharmacies to identify AEs related to concurrent NHP-prescription drug use. In a preliminary evaluation of the active surveillance case reports, four cases were noted that warranted an in-depth evaluation. The one commonality in these case reports was that all individuals used vitamins concurrently with their health products. Multi-vitamins were identified in 3 cases and a calcium-vitamin D supplement in the fourth. **Case Report:** In the most serious case report, a 38 year old, 140 lb, 5'6" female patient with an aboriginal-Caucasian ethnicity having a hormone disorder experienced an adverse event with fatigue, nervousness, heart palpitation, rash, and muscle twitching. At the onset of the event, the patient had started taking femMED (NRP 410) 4 times daily (Q.I.D., Table 1). Prior to the event, she had been taking a multivitamin with green tea extract twice daily for about 1 year and a 100 mg progesterone cream (compounded in the pharmacy; actual applied dose unknown) once daily for about 5 months. Her naturopath suggested that she may have reacted badly to scullcap and wild yam in the NRP 410 product and was started on Thyrosense (NRP 409), Q.I.D. The adverse events continued and only stopped when all the herbal medications were discontinued at the direction of her physician. She experienced a positive rechallenge. The patient experienced similar AEs when consuming a cup of green tea some time after stopping the herbal medications. NRP 409 and 410 are blended multi-constituent products (Table 2) with limited information on the potential of either the blended product or individual botanical constituents to cause cytochrome P450-mediated interactions. These findings are consistent with our earlier *in vitro* study suggesting that there may be interactions between vitamins and other medicinal products (16).

Vitamins are naturally occurring organic substances that are not synthesized in the body and supplements are taken as a complementary dietary source (17). There are 13 essential vitamins with 3 or more present in multivitamin products. Carotenoid compounds are naturally occurring in orange fruits and vegetables, green vegetables, and in algae. *Trans*- $\beta$ -carotene (tBC), an isoprenoid compound is metabolized by a two step process through retinal to form retinol (vitamin A), and it

can be further oxidized to retinoic acid (18-19). Vitamin D<sub>3</sub> has to be enzymatically transformed into the biologically active form 1,25-dihydroxyvitamin D<sub>3</sub> (DD<sub>3</sub>) (20-21). CYP2C11, 27A1, 2D25, 2R1, 3A4, and 2J3 are known to catalyze vitamin D metabolism (21). tBC and DD<sub>3</sub> can modulate gene expression of *CYP3A* (22-25); tBC can also modulate *MDR1* (24). Expression of CYP3A4 can be induced through different nuclear receptors such as the pregnane X receptor (PXR), the constitutive androstane receptor (CAR) and the vitamin D receptor (VDR) (24). VDR binds to and mediates the effects of the DD<sub>3</sub> to alter gene transcription (22).

In a single-center longitudinal study with 14 healthy Caucasian adult volunteers (7 men, 7 women) self-administered vitamin C 500 mg twice/day for 14 days, no significant effect of vitamin C on CYP3A4 activity was observed (26). However, sex and baseline results were significant predictors of changes in CYP3A4 activity as in men, the mean activity increased by 21.9% (95% confidence interval -3.88-47.6%). The effect in women was not consistent. In a separate 7-day study with 7 healthy male subjects, 1000 mg/day vitamin C significantly decreased the AUC<sub>0-8hr</sub> and C<sub>max</sub> of indinavir IND (27). The C<sub>min</sub> was 32% lower in the presence of vitamin C suggesting that high doses of vitamin C can reduce steady-state indinavir plasma concentrations.

This study was undertaken to evaluate if there was a metabolic mechanism underlying the adverse events noted in the case report. The potential for NRP 409 and 410 to modulate the activity of CYP3A4, a known metabolic enzyme of progesterone, and other P450-mediated metabolism was examined together with extracts from 8 *trans*- $\beta$ -carotene products, 10 multivitamins, and 2 vitamin D<sub>3</sub> supplements (Table 1). Authentic standards of tBC derivatives were also examined by observing their interaction with human cytochrome P450 isozymes.

## METHODS

Human ethics review board approval was obtained in order to collect information on natural health product and drug interactions, and then to conduct interviews with willing individuals and their physician(s).

**Table 1.** Product information taken from the labels of commercially available products including name, unit size, listed weight, measured weight, suggested dose, expiry date (exp), lot number, drug identification number (DIN) or natural product number (NPN) for *trans*- $\beta$ -carotene (tBC), multivitamins, and herbal formulations. NRP, Nutraceutical Research Programme number.

NRP #	Product information	% Recovery**
144	Beta Carotene, unit size: 10 000 IU tBC, listed weight: 6 mg, determined tBC amount: 2.7 mg*, mean unit weight: 1.07 gm, 1 tablet/day, exp. 04/04, lot 40602, DIN 00770795	45.0
145	Beta Carotene, unit size: 10 000 IU tBC, listed weight: 6 mg, determined tBC amount: 4.4 mg, mean unit weight: 1.09 gm, 1 tablet/day, exp. 05/04, lot 8775, DIN 00594628	73.3
146	Beta Carotene, unit size: 10 000 IU tBC, listed weight: 6 mg, determined tBC amount: 4.0 mg, 1 capsule/day, exp. 01/04, lot 8552, DIN 01901680	66.6
147	Beta Carotene, unit size: 10 000 IU tBC, listed weight: 6 mg, determined tBC amount: <0.5 mg, mean unit weight: 0.86 gm, 1 tablet/day, exp. 12/01, lot 1774, DIN 688819	<8.0
148	Beta-Carotene, unit size: 25 000 IU tBC, listed weight: 15 mg, determined tBC amount: 3.9 mg, 1 softgel/day, exp. 08/03, lot 034303, DIN 02030454	26.0
149	Beta Carotene, 25 000 IU tBC, listed weight: 15 mg, mean unit weight: 1.39 gm, determined tBC amount: 4.6 mg, 1 tablet/day, exp. 05/02, lot IE234, DIN 02011433	30.7
157	Beta Carotene, unit size: 25 000 IU tBC, listed weight: 15 mg, determined tBC amount: 13.0 mg, mean unit weight: 599.1 mg, 1 capsule/day, exp. 08/05, lot F03667, DIN 01904388	86.8
395	Vital 1 with green tea extract and chromium, Exact, mean unit weight 1.59 gm, 1 tablet/day, exp. 09/09, lot J0991G2V, NPN 80000345	22.8
396	Vital 1 men's formula, mean unit weight 0.88 gm, 1 tablet/day, exp. 11/10, lot F32165, DIN 02246600	28.6
397	Vital-fem 1, mean unit weight 1.69 gm, 1 tablet/day, exp. 11/09, lot F31748, DIN 02242937	11.4
398	Children's chewable multivitamins, mean unit weight 0.85 gm, 1 tablet/day, exp. 11/10, lot F29352, DIN 02239685	71.7
399	Essentra performa with ginseng, mean unit weight 1.56 gm, 1 tablet/day, exp. 01/09, lot F28949, DIN N/A	20.3
400	Essentra platinum with extra vitamins C and E, mean unit weight 1.27 gm, 2 tablets/day, exp. 11/09, lot F25959, DIN N/A	19.7
401	Essentra elite high potency formula with lutein, mean unit weight 1.65 gm, 1 tablet/day, exp. 10/08, lot F30769, DIN N/A	17.5
402	Select 50+, mean unit weight 1.50 gm, 1 tablet/day, exp. 10/06, lot D14116, DIN N/A	17.8
403	Regular Vita-Vim with green tea & grapeseed phytosome, lycopene and increases Vitamin D, mean unit weight 1.36 gm, 1 caplet/day, exp. 10/08, lot 1331343, DIN N/A	22.2
404	Adult 50+ Vita-Vim with tumeric, lutein, lycopene, oleaselect and Vitamins D and K <sub>1</sub> , mean unit weight 1.82 gm, 1 caplet/day, exp. 11/12, lot 13314333, DIN N/A	14.4
409	ThyroSense, mean unit weight 0.61 gm, 2 capsules/day, exp. 05/09, lot 552527	14.2
410	femMED, mean unit weight 0.61 gm, 2 capsules/day, exp. 02/11, lot 7045	19.2
411	Vitamin D <sub>3</sub> , unit size: 400 IU / 10 mcg, mean unit weight: 0.23 g, 1-2 tablets/day, exp. 07/11, lot 1352814	1.7
412	Vitamin D <sub>3</sub> , unit size: 1000 IU / 25 mcg, mean unit weight: 0.23 gm, 1 tablet/day, exp. 11/11, lot 1415289	2.8

\* The weight of tBC recovered from a product is provided relative to the listed weight of tBC in a single unit of the product. \*\* The weight of the total constituents recovered in the extract is provided relative to the initial amount (30 mg) used to prepare the extract.

### Substrates and Reference Compounds

Thyrosense, femMED, multi-vitamins, *trans*-carotene (tBC), and vitamin D<sub>3</sub> were obtained from local outlets (Table 1). They were selected to give a representation of the local market. These products were assigned unique identifying Nutraceutical

Research Programme (NRP) accession numbers, and product vouchers were stored in the herbarium at the University of Ottawa. Tablets and caplets were finely ground with a mortar and pestle. All products were tested within their expiry date.

**Table 2.** Summary of the components of two blended botanical products (NRP 409) and (NRP 410) listed on the product labels. All amounts are listed in mg. NRP, Nutraceutical Research Programme number. -, absent, x present but the amount not listed.

<b>INGREDIENT</b>	<b>409</b>	<b>410</b>
Ashwagandha ( <i>Withania somnifera</i> ) extract; 1.5% withanolides (5:1 from 750 mg Ashwagandha)	150	-
Guggul ( <i>Commiphora mukul</i> ) extract; 2.5% guggulsterones (3:1 from 360 mg Guggul)	120	-
Saw Palmetto ( <i>Serenoa repens berry</i> )	-	200
Chastetree Berry ( <i>Vitex agnus-castus</i> )	-	100
Milk Thistle ( <i>Silybum marianum seed</i> )	-	50
Scullcap ( <i>Scutellaria lateriflora herb</i> )	-	50
Red Raspberry ( <i>Rubus idaeus leaf extract, 4:1</i> )	-	50
Wild Yam ( <i>Dioscorea composite root</i> )	-	50
Copper	0.500	-
Iodine	0.100	-
L-Tyrosine	500	-
Manganese	0.500	-
Pantothenic acid	100	-
Cellulose	x	x
Microcrystalline cellulose	x	x
Purified water	x	-
Rice starch	x	-
Silicon dioxide	x	-
Vegetable grade magnesium stearate	x	x
Water	-	x
Avg. weight in g ( $\pm$ SD); n =5	0.61 (0.02)	0.61 (0)

**Table 3.** Summary of  $\beta$ -carotene content and excipient components listed on the product labels of the different supplements examined in this study. NRP, Nutraceutical Research Programme number, - absent, x present but the amount not listed.

<b>Ingredient</b>	<b>NRP 144§</b>	<b>NRP 145§</b>	<b>NRP 146§</b>	<b>NRP 147§</b>	<b>NRP 148</b>	<b>NRP 149§</b>	<b>NRP 157</b>
<b>MEDICINAL</b>							
$\beta$ -carotene	10,000 IU	10,000 IU	10,000 IU	10,000 IU	25,000 IU	25,000 IU	25,000 IU
<b>NON-MEDICINAL</b>							
Beeswax					-		x
Gelatin					x		x
Glycerin					x		x
Hydrogenated soybean oil					-		x
Purified water					x		x
Soybean oil					-		x
Soy lecithin					-		x
Vegetable oil shortening					-		x
Avg. weight in mg ( $\pm$ SD); n =5	440 (0)	450 (0.01)	380 (0.01)	290 (0)	270 (0.01)	760 (0.01)	250 (0)

§ no excipients were reported on the package.

**Table 4.** Summary of vitamin, mineral, and excipient components listed on the product labels of the multivitamin supplements (Nutraceutical Research Programme numbers 395 to 404). All amounts are listed in mg unless otherwise indicated. -, absent; x, present but amount not listed. Supplemental information is appended at the end.

<b>Ingredient</b>	<b>395</b>	<b>396</b>	<b>397</b>	<b>398</b>	<b>399</b>	<b>400</b>	<b>401</b>	<b>402</b>	<b>403§</b>	<b>404§</b>
<b>VITAMIN</b>										
Vitamin A	-	4000 IU	4000 IU	2500 IU	1000 IU	500 IU	1000 IU	1000 IU	3000 IU	2000 IU
Vitamin B <sub>1</sub>	1.9	2.25	1.5	1.05	4.5	1.15	2.25	2.25	2.5	2.25
Vitamin B <sub>2</sub>	2.1	2.55	1.7	1.2	5.1	1.6	3.2	3.2	2.5	3.2
Vitamin B <sub>6</sub>	2.5	3	2	1.05	6	5	8	8	25	8
Vitamin B <sub>12</sub>	0.0075	0.009	0.006	0.0045	0.02	0.0125	0.025	0.025	0.025	0.025
Vitamin C	60	90	60	60	120	125	90	90	150	90
Vitamin D <sub>3</sub>	400 IU	400 IU	400 IU	400 IU	400 IU	200 IU	400 IU	600 IU	600 IU	800 IU
Vitamin E	30 IU	45 IU	30 IU	15 IU	60 IU	100 IU	75 IU	75 IU	15 IU	40 IU
B-Carotene	2500 IU	1000 IU	4050 IU	-	2000 IU	5000 IU	3000 IU	3000 IU	2000 IU	6000 IU
Biotin	-	-	-	-	0.04	0.0225	0.045	0.045	0.015	0.045
Folic Acid	0.4	0.4	0.4	0.3	0.6	0.35	0.6	0.6	0.4	0.6
Niacinamide	25	20	20	13.5	15	7.5	15	15	-	35
Pantothenic Acid	12.5	10	10	-	10	5	10	10	-	10
Vitamin K Ave	-	-	-	-	-	-	-	0.025	-	0.08
Vitamin/mg unit weight	2.01	3.69	5.68	1.98	2.40	3.94	3.05	3.18	3.84	5.96
<b>MINERAL</b>										
Calcium	300	-	450	-	162	87.5	200	200	150	200
Chlorine	-	34	-	-	80	-	-	-	-	-
Chromium	0.15	0.15	-	-	0.025	0.0125	0.1	0.1	0.001	0.1
Copper	2	2	-	-	1.4	-	2	1	1	2
Iodine	-	0.15	-	-	0.15	0.075	0.15	0.15	0.1	0.1
Iron	14	-	27	-	8	5	4	4	4	-
Lutein	-	-	-	-	-	0.25	0.25	-	0.3*	0.3*
Magnesium	50	100	-	-	50	2.5	50	50	75	50
Manganese	2	3.3	-	-	4	-	5	5	-	5
Molybedum	-	0.0417	-	-	0.05	0.0125	0.025	0.045	-	0.025
Nickel	-	-	-	-	0.005	0.0025	0.005	-	-	0.005
Phosphorus	-	-	-	-	125	62.5	125	-	-	125
Potassium	-	0.0375	-	-	72	20	80	80	-	80
Selenium	0.07	0.0875	-	-	0.055	0.0125	0.025	0.055	0.001	0.025
Silicon	-	-	-	-	0.01	0.005	0.01	-	-	0.001
Tin	-	-	-	-	0.01	0.05	0.01	-	-	0.001
Vanadium	-	-	-	-	0.001	0.005	0.001	-	-	0.001
Zinc	0.015	15	15	-	10	7.5	15	7.5	10	15
Avg. weight (mg ± SD); n=5	1509 (0.01)	880 (0.02)	1690 (0.01)	850 (0.02)	1560 (0.02)	1270 (0)	1650 (0.01)	1500 (0.01)	1306 (0.02)	1820 (0.01)

§ no excipients were reported on the package. \* from *Tagetes erecta*, flower. \*\* Derived from 75 mg of a 25:1 standardized extract containing 36% EGCG

Authentic tBC, nictotinamide adenine dinucleotide phosphate reduced form (NADPH), retinol (ROL), retinal (RAL), retinoic acid (RTE),

tranylcypromine, quinidine, bifonazole, and verapamil were purchased from Sigma-Aldrich (Oakville, ON, Canada). 3-Cyano-7-

ethoxycoumarin (CEC), dibenzylfluorescein (DBF), 7-methoxy-4-(trifluoromethyl)-coumarin (MFC), AMMC, microsomes derived from Baculovirus infected insect cells expressing CYP2C19, 2D6, 3A4, 3A7 or 19 were purchased from BD Biosciences (Mississauga, ON, Canada). Ketoconazole was purchased from Calbiochem (Gibbstown, NJ, USA). All other chemicals and solvents were of analytical grade.

For the tBC studies, extracts were prepared from 100 mg/mL ground tBC material in acetonitrile, ethanol, methanol, or water by sonication and vortexing for 1 min. The extract was separated from the undissolved material by centrifugation for 18 min at 13,000 rpm. For the multi-vitamin and herbal formulation studies, a similar procedure was followed, but the extracts were prepared from 30 mg/ml in methanol. For the vitamin D<sub>3</sub> studies, the extracts were prepared from 50 mg/ml in methanol. Liquid tBC capsules were emptied into a 1.5 mL microfuge tube and 3 volumes of 55% ethanol was added. The solution was vortexed and centrifuged for 18 min at 13,000 rpm. All samples were stored at -20°C, kept protected from light, and were freshly prepared daily.

#### Carotene Biomarker Analysis

Ground tBC tablets (100 mg) were mixed thoroughly with 10 mL of butanol, centrifuged, and filtered. The process was repeated once more. A 200  $\mu$ L was withdrawn from this combined butanol stock solution and diluted with 2 mL of butanol for a total volume of 2.2 mL. After a brief mixing, and centrifugation at 3,500 x g for 15 min, 20  $\mu$ L of the clear solution was analyzed for tBC content.

Gelatin capsules containing tBC were weighed (approx. 380 mg) and a careful incision was made in each to avoid the loss of material. The capsules were placed in a container with 15 mL butanol, and then stirred for 15 min at a moderate speed. The liquid content was decanted into a centrifuge tube, and the residue was re-extracted with the same volume of butanol. The combined butanol (dark red and heavily turbid) extract was vortexed briefly, centrifuged at 3,500 x g for 30 min, and 200  $\mu$ L of the clear solution was diluted with 2 mL butanol and analyzed.

#### HPLC Analysis of $\beta$ -carotene

An Agilent Model 1100 Series equipped with a photodiode array detector and a degasser was used to separate, identify and quantify  $\beta$ -carotenes in tablets and capsules. Separation was carried out on a 5  $\mu$ m Phenomenex Primesphere C-18 reversed phase HPLC column (250 mm x 4.6 mm ID) protected with a C-18 guard column. The mobile phase consisted of solvent A: acetonitrile:methanol:dichloromethane (60:30:10 v/v/v) and solvent B: acetonitrile:methanol:dichloromethane (50:30:20 v/v/v) at a flow rate of 1 mL per min. The sample injection volume was 20  $\mu$ L, and components were eluted starting with 100% solvent A and reaching 100% solvent B in 20 min. The spectra were run at 450 nm. Under these conditions  $\beta$ -carotene and  $\alpha$ -carotene had a retention time of 15.6 min and 16.2 min (small peak), respectively. Concentration of tBC was calculated by comparison to a calibration curve of authentic material.

#### Cytochrome P450 Assay

Aliquots of extract solutions were screened for their ability to inhibit CYP2C9, 2C19, 2D6, 3A4, 3A5 or 3A7 marker substrates using an *in vitro* fluorometric assay in clear-bottom, opaque-welled microtiter plates (96 well, Corning Costar, model # CSOO-3632, Corning, NY). For the tBC and multi-vitamin/vitamin D<sub>3</sub>/herbal formulation studies, 3  $\mu$ L and 2  $\mu$ L of extract were tested, respectively. The assay procedure was reported previously (28-29). All control, control-blank, test and test-blank wells were balanced to contain an equal volume of methanol or water present in the extracts; no well contained more than 1% of the solvent vehicle. All wells had 0.6 mM NADPH; substrate (0.12  $\mu$ M AMMC – 2D6; 25  $\mu$ M CEC – 2C19; 1  $\mu$ M DBF – 3A4/5/7, 19; 100  $\mu$ M MFC – 2C9) with or without test sample. However, only 0.3 mM NADPH was required to maintain linear reaction conditions in the CYP2D6 assay. Control and test wells contained active isozyme (70 nM 2C9, 20 nM 2C19, 10 nM 2D6, 3A4/5/7, 19) in phosphate buffer (KH<sub>2</sub>PO<sub>4</sub>, pH 7.4, 0.2 M), whereas isozyme denatured by boiling for 15 min was added to the control-blank and test-blank wells. The positive inhibitors used were 75  $\mu$ M tranylecypromine (CYP2C19), 2  $\mu$ M quinidine (CYP2D6), 1  $\mu$ M ketoconazole (CYP3A4, 3A7), and 0.4  $\mu$ M bifonazole (CYP19). All isozymes were stored at -80°C until used and

were not subjected to more than 2 freeze-thaw cycles. Isozymes were rapidly thawed in a 37°C sand bath and mixed gently with the substrate solution prior to addition. The microwell plates were incubated 20-60 min at 37°C depending upon the isozyme, and the final fluorescence was measured using a Cytofluor 4000 Fluorescence Measurement System.

All samples were prepared in triplicate with the resultant percent inhibition calculations based on the mathematical combinations for the differences in fluorescence between the test/test-blank wells and the mean difference between each control and blank well. Controls were run with every assay. Each assay was repeated at least once. As a cell-free assay where a 20-25% coefficient of variation may occur, negative values indicating a high level of substrate biotransformation are not indicative of induction but represent inherent biological variation of the assay. All assays were performed with in-date material under gold fluorescent lighting or reduced lighting.

## RESULTS

### Samples and Constituents

Twenty one vitamin samples were examined in this study (Table 1). The active medicinal ingredients and non-medicinal excipients for the tBC, multivitamin and vitamin D<sub>3</sub> products are listed in Tables 3 to 5, respectively. There are differences in the content of medicinal constituents and excipients between the vitamin products. The percent recovery of the total non-soluble suspended solid constituents

in the extracts of all products was examined (Table 1). Total percent recovery for the 7 different tBC products (NRP 144-149, 157) was not determined as HPLC analysis for the total amount of tBC present was undertaken. These extracts had levels 13.2% to 92.0% less tBC than the amount specified on the product labels (Table 1). Most of the tBC products contained between 4 to 5 mg tBC per tablet, except for NRP 144 and 147 which contained much less tBC. NRP 157 had the highest amount of tBC detected with 13 mg tBC per tablet. The percent recovery from the multivitamin samples (NRPs 395 to 404) ranged from 11.4% to 28.6% for most products except the chewable vitamin NRP 398 which had a 71.7% recovery. The lowest recoveries were with the vitamin D<sub>3</sub> products NRP 411 and 412 at 1.7 to 2.8%. NRP 409 and NRP 410 had an average recovery of 14.2 and 19.2%, respectively.

### *trans*-β-Carotene

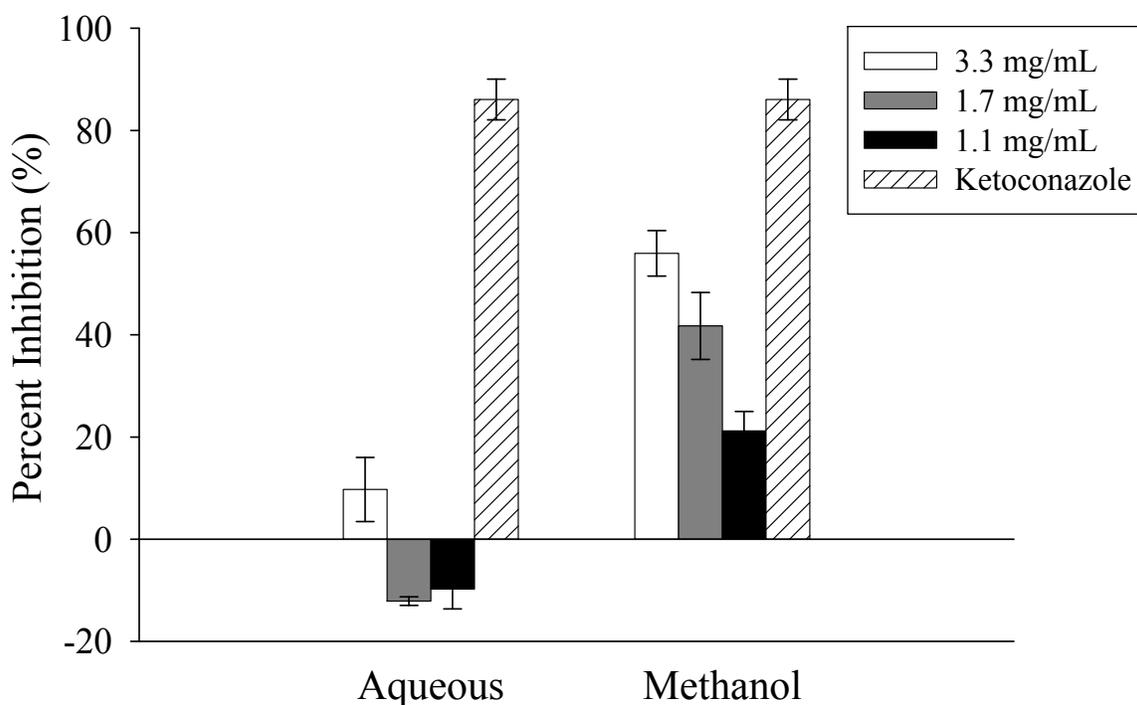
The potential for tBC product extracts to affect cytochrome P450-mediated metabolism of marker substrate by three different isozymes (CYP3A4, 2C19, and 2C9) *in vitro* was examined in two stages. In the first stage the tBC products underwent an initial screening for CYP inhibition using 100 mg/ml stock extracts. The tBC extracts were more inhibitory towards CYP2C9 and 2C19 with ranges of 65.2% to 86.2% inhibition (Table 6). The tBC extracts were less inhibitory towards CYP3A4 and inhibited with ranges 12.6% to 73.3% of CYP3A4 activity.

**Table 5.** Summary of the components of the vitamin D<sub>3</sub> supplements (NRP 411 and 412) listed on the product labels. All amounts are listed in mg unless otherwise indicated. -, absent; x, present. NRP, Nutraceutical Research Programme number.

Ingredient	NRP 411	NRP 412
Vitamin D <sub>3</sub> (Cholecalciferol)	10 mcg / 400 IU	25 mcg / 1000 IU
<b>NON-MEDICINAL</b>		
Cellulose	x	x
Dicalcium phosphate	x	x
Modified cellulose gum	x	-
Vegetable magnesium stearate	x	x
Avg. weight in mg (± SD); n =5	230 (0.01)	230 (0.01)

**Table 6.** The percent inhibition of the cytochrome P450-mediated metabolism of substrates by various *trans*- $\beta$ -carotene product extracts (100 mg/ml stock extracts). Mean  $\pm$  SD of at least two separate tests. NRP, Nutraceutical Research Programme number. ND, not determined.

NRP #	2C9	2C19	3A4
144	74.6 $\pm$ 6.2	80.7 $\pm$ 7.5	59.3 $\pm$ 1.5
145	ND	79.4 $\pm$ 4.8	ND
146 (aqueous layer)	80.4 $\pm$ 2.2	69.0 $\pm$ 0.7	73.1 $\pm$ 4.7
146 (oily layer)	77.0 $\pm$ 4.9	65.2 $\pm$ 1.3	45.8 $\pm$ 1.7
147	70.4 $\pm$ 0.7	85.4 $\pm$ 2.7	73.3 $\pm$ 3.7
148 (aqueous layer)	77.6 $\pm$ 0.5	86.2 $\pm$ 3.1	34.6 $\pm$ 3.6
148 (oily layer)	73.8 $\pm$ 1.8	69.2 $\pm$ 2.3	47.0 $\pm$ 1.4
149	66.4 $\pm$ 0.6	85.1 $\pm$ 7.0	59.3 $\pm$ 2.6
157	ND	ND	12.6 $\pm$ 2.6



**Figure 1.** Effect of aqueous and methanolic extracts from *trans*- $\beta$ -carotene (NRP 157) on the cytochrome P450 3A7-mediated metabolism of dibenzylfluorescein relative to ketoconazole (0.5  $\mu$ g/ml). Mean  $\pm$  SD of at least two separate tests. NRP, Nutraceutical Research Programme number.

There was no discernable relationship between tBC content (Table 1) and the inhibitory effect. In fact, NRP 147, whose tBC content was less than 500  $\mu$ g per tablet was found to be highly inhibitory (70-85%) towards the three isozymes. There was little variability in the ability of either layer to inhibit CYP-mediated metabolism.

In the second study, the effect of aqueous and methanolic extracts of NRP 157 on the *in vitro*

metabolism of DBF by CYP3A4, 3A5 and 3A7 was examined. Neither aqueous and methanol extracts at the three concentrations inhibited CYP3A4 or CYP3A5 metabolism (results not shown). The methanol extracts had a dose-dependant inhibitory effect on CYP 3A7 (Figure 1). Only the highest concentration aqueous extract had an inhibitory effect.

The potential for authentic tBC and 3 derivative standards to mediate biotransformation was examined in four isozymes (Table 7). The 4 compounds had similar inhibitory marked activity towards CYP2C9 and 2C19 with less affect on CYP3A4. All 4 had markedly less activity towards CYP2D6-mediated metabolism. The retinoic acid had the least activity in this *in vitro* assay.

Degradation products of retinol were not detected in NRP 157. At times a small shoulder appeared which could be attributed to *cis*- $\beta$ -carotene and/or  $\alpha$ -carotene (results not shown). There was initially a considerable variation in the values of  $\beta$ -carotene in NRP 156 as the tablets had large number of flakes requiring the samples to be finely ground. There was also variation in capsules, but within acceptable limits.

### Multivitamins and Vitamin D<sub>3</sub>

A comparative evaluation of aqueous and methanolic extracts found that the methanolic extracts consistently resulted in the highest inhibitory effects. The 10 multivitamins and the 2

vitamin D<sub>3</sub> products were examined for their ability to inhibit CYP2C19-, 2D6-, 3A4-, 3A7-, and 19-mediated metabolism using methanolic extracts (Table 8). Of the 12 extracts examined, 9 had a negative inhibitory effect towards CYP2C19 activity. Three products, NRP 395, 396, and 399 had an inhibitory range of 43% to 77.2 %. Most of the extracts had a weak or negative inhibitory effect towards CYP2D6 activity. The most inhibitory product was NRP 295 which inhibited 25.2% of CYP2D6 activity. Similar results were observed for CYP3A4 and 3A7 and inhibition from the products ranged from 3.7% to 69.4%. The extracts had a weak or negative inhibitory effect towards CYP19 activity which ranged from -8.2% to 23.7%. Three of the multivitamin products, NRP 395, 396, and 399 were generally more inhibitory towards the CYPs isozymes. Both NRP 395 and 399 contained the herbal components, green tea and ginseng, respectively (Table 4). The vitamin D<sub>3</sub> products had a very weak or negative inhibitory effect towards all 5 CYP isozymes examined.

**Table 7.** The percent inhibition of cytochrome P450-mediated metabolism by *trans*- $\beta$ -carotene, retinol, retinal, and retinoic acid in 100% ethanol (1 mg/ml stock extracts). Mean  $\pm$  SD of at least two separate tests. NRP, Nutraceutical Research Programme number.

	CYP2C9	CYP2C19	CYP2D6	CYP3A4
<i>trans</i> - $\beta$ -carotene	66.0 $\pm$ 4.2	68.2 $\pm$ 3.3	9.8 $\pm$ 1.2	38.1 $\pm$ 2.8
retinol	76.7 $\pm$ 0.4	78.5 $\pm$ 5.6	2.4 $\pm$ 1.3	77.1 $\pm$ 9.9
retinal	84.0 $\pm$ 5.0	79.2 $\pm$ 1.5	21.2 $\pm$ 1.9	25.9 $\pm$ 11.6
retinoic acid	57.6 $\pm$ 3.3	45.7 $\pm$ 9.6	7.6 $\pm$ 0.6	27.2 $\pm$ 1.1

**Table 8.** The percent inhibition of the cytochrome P450-mediated metabolism of substrates by various multivitamin (30 mg ground material/ml) and vitamin D<sub>3</sub> products (50 mg ground material/ml) stock methanol extracts respectively). Mean  $\pm$  SD of at least two separate tests. NRP, Nutraceutical Research Programme number.

NRP #	2C19	2D6	3A4	3A7	19
395	43.8 $\pm$ 2.9	25.2 $\pm$ 1.3	46.7 $\pm$ 5.8	69.4 $\pm$ 3.2	23.7 $\pm$ 0.7
396	77.2 $\pm$ 2.2	-4.6 $\pm$ 1.1	50.6 $\pm$ 7.1	37.6 $\pm$ 1.1	20.9 $\pm$ 0.4
397	-7.4 $\pm$ 12.3	-15.3 $\pm$ 3.8	20.5 $\pm$ 8.5	25.1 $\pm$ 5.7	7.9 $\pm$ 4.4
398	-14.0 $\pm$ 1.2	-15.1 $\pm$ 5.5	12.9 $\pm$ 4.1	18.3 $\pm$ 1.5	-0.9 $\pm$ 4.5
399	55.8 $\pm$ 7.8	0.5 $\pm$ 1.5	29.5 $\pm$ 7.0	35.9 $\pm$ 14.9	22.2 $\pm$ 0.1
400	-20.2 $\pm$ 15.0	-11.8 $\pm$ 2.5	12.7 $\pm$ 7.4	18.6 $\pm$ 0.1	0.9 $\pm$ 2.5
401	-29.7 $\pm$ 13.7	-9.3 $\pm$ 0.3	10.4 $\pm$ 8.6	14.0 $\pm$ 1.9	-8.2 $\pm$ 4.7
402	-23.4 $\pm$ 3.0	-8.9 $\pm$ 2.2	20.8 $\pm$ 2.5	22.5 $\pm$ 4.9	7.7 $\pm$ 2.0
403	-11.8 $\pm$ 12.2	17.8 $\pm$ 1.1	13.2 $\pm$ 7.7	29.4 $\pm$ 0.4	12.1 $\pm$ 3.8
404	-0.3 $\pm$ 5.8	9.3 $\pm$ 0.2	23.7 $\pm$ 4.9	18.3 $\pm$ 0.1	3.2 $\pm$ 2.2
411	-2.2 $\pm$ 1.5	-9.6 $\pm$ 0.2	6.0 $\pm$ 2.0	5.7 $\pm$ 0.1	9.1 $\pm$ 0.6
412	10.7 $\pm$ 0.4	-5.1 $\pm$ 3.5	12.5 $\pm$ 1.3	3.7 $\pm$ 3.9	13.1 $\pm$ 3.1
<b>Positive Inhibitor</b>	91.2 $\pm$ 0.7	88.7 $\pm$ 0.2	89.5 $\pm$ 0.2	54.7 $\pm$ 0.0	74.1 $\pm$ 2.6

### Herbal Formulations

Two herbal formulation products associated with the case report, NRP 409 and NRP 410 were examined for their ability to inhibit CYP2C19-, 2D6-, 3A4-, 3A7-, and 19-mediated metabolism using aqueous and methanolic extracts. Marked differences in inhibition towards the different isozymes were observed, as well as the inhibitory potential between aqueous and methanolic extracts. The methanolic extract was more inhibitory than the aqueous extract for NRP 409 for the 5 isozymes examined (Figure 2A). CYP2D6-mediated metabolism was the least inhibited. The methanolic extract inhibited all of the other isozymes with greater than 62.6% inhibition. The activities of CYP2C19, 3A4 and 3A7 were inhibited by the methanolic extract by greater than 94.1% relative to the vehicle control. The aqueous extract inhibited the majority of the isozymes with approximately half the potency as the methanolic extract. The greatest variance was observed with the aqueous extract inhibition of 21.5% as compared to methanolic 95.0% inhibition of CYP3A4 activity.

As with NRP 409, the methanolic extract of NRP 410 was more inhibitory than the aqueous extract for all of the isozymes except with CYP2D6 (Figure 2B). The difference in CYP2D6 inhibition was substantial, as the aqueous extract inhibited 75.8% of CYP2D6 activity, but the methanolic extract inhibited only 1.1%. A great difference in inhibition between the extracts was also observed for CYP3A4, as the aqueous extract had no inhibition towards the isozyme, but the methanolic extract completely inhibited the isozyme relative to the vehicle control. Both extracts affected CYP19-mediated metabolism with similar weak potencies.

### DISCUSSION

In this study, the effect of an extract of a constant weight of ground material to inhibit biotransformation was compared between commercially available products. The findings demonstrated that the inhibitory values for NRP 147 with very low levels of tBC were similar to those with the highest content, NRP 157. Even in studies where the same amount of tBC was constant between samples (data not reported), the inhibitory potential could not be correlated to the tBC content of the samples. It is also noteworthy that in the soft gel products (NRP 146 and 148) marked inhibitory

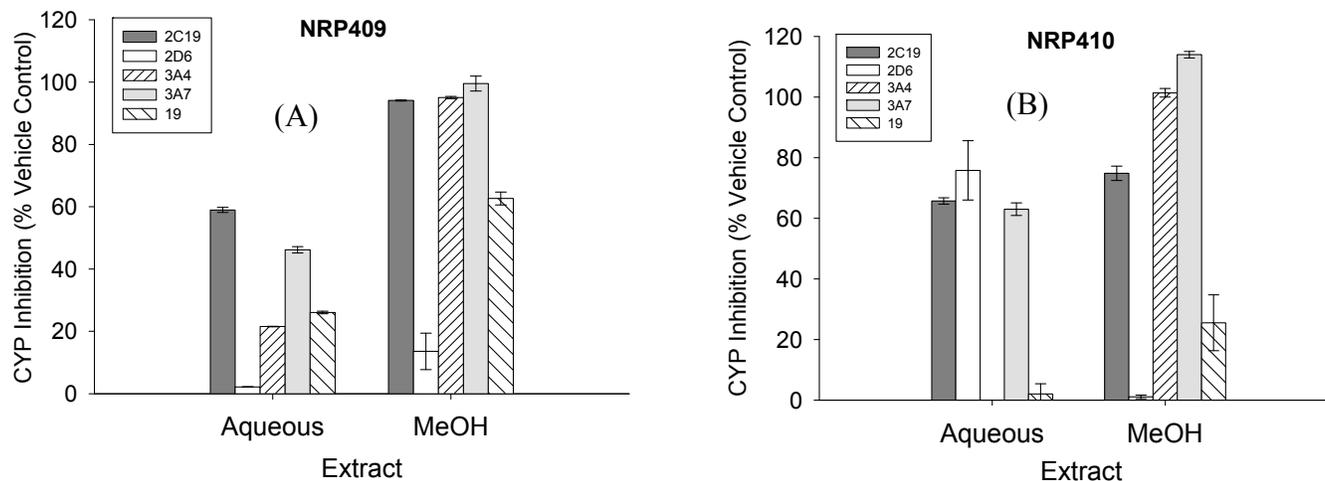
activity was present in the two distinct layers obtained following centrifugation. These findings suggest that the inhibitory potential of these samples resides in multiple substances which could include potential degradative products or excipients. The inhibitory potential of the vitamins is modest. The potential of the different vitamin products examined in this study to affect CYP-mediated metabolism is consistent with our earlier study (16) and that of other NHPs (30-34).

The study of authentic reference compounds on their inhibitory effect on CYP2C9-, CYP2C19-, CYP2D6-, and CYP3A4-mediated suggests that the derivatives have a higher inhibitory effect on the metabolism of these isozymes. The low recovery of tBC in the commercial products was unexpected, as was the subsequent determination that retinoic acid derivatives were not detected.

The AEs, noted in the case report where the female patient with hormone disorder treated with a topical progesterone cream and a multivitamin product after supplementing her therapy with NRP 410, could be attributed to a number of factors such as; adulteration with a therapeutic product, an allergic response to one of the components, an interaction with one or more of the components or concomitant medications or non-medicinal excipients, or an unrelated cause of event mistakenly associated with these medications.

The rechallenge response can eliminate the last factor that this was a mistaken association. An allergy seems unlikely as the response was noted separately with green tea as the components in this tea are less likely to be presented in either of the 2 NHPs associated with this report. As the original product lot could not be determined and no original material was available, the potential for adulteration cannot be unequivocally excluded. This left our examination to determine the potential risk of an interaction.

As noted above, the only commonality in the evaluation of the 4 case reports was vitamins. This reflects the common use of these products and cannot be taken as a causal factor without further evaluation; nor does this observation preclude the involvement of other medicinal products. The findings reported here would suggest that there is a real but low to modest potential for vitamins to be the main source of this AE.



**Figure 2.** Effect of aqueous and methanolic extracts from (A) ThyroSense (NRP 409) and (B) femMED (NRP 410) on cytochrome P450 2C19-, 2D6-, 3A4-, 3A7-, and 19-mediated metabolism. Mean  $\pm$  SD of at least two separate tests. NRP. Nutraceutical Research Programme number.

In addition to the presence of one or more vitamins in these products, it was evident from the product labels than many of these products also contained a wide range of minerals and excipients. Some products also contained a natural health product. The excipients were presumably included as inert vehicles to facilitate formulation; however, the inertness is now being questioned as some recent studies have indicated that some excipients can markedly affect drug transport and disposition (35-38). Ren et al (35) examined the effects of 22 common excipients on cytochrome P450 3A4 and found that 15 of 22 (68.2%) tested excipients could inhibit the activity of CYP3A4 more than 50% in vitro, particularly the surfactants and polymers. Ren et al (36) examined four common nonionic surfactants polysorbate 20, polyoxyl 35 castor oil, polyoxyl 40 stearate and poloxamer 188, on cytochrome P450 3A. All surfactants inhibited midazolam 1'-hydroxylation in a concentration-dependent manner and presented a mixed competitive inhibitory model. Zhu et al. (37) investigated the effects of polyoxyethylene 40 stearate on the activity of P-glycoprotein (P-gp) and six major cytochrome P450 (CYP) isoforms. The stearate inhibited P-gp mediated efflux in a concentration-dependent manner and CYP2C9 and CYP2C19 inhibition was found to be clinically significant. Mudra and Borchardt (38) investigated the effects of polyethylene glycol (PEG) 400 (2% or 20%, v/v), D-alpha-tocopheryl polyethylene glycol-

1000 succinate (TPGS; 100  $\mu$ g/mL), Cremophor EL (47.5  $\mu$ g/mL), and polysorbate (Tween) 80 (25  $\mu$ g/mL) solubilizing agents on P450 (CYP) 3A and P-glycoprotein (P-gp) in the rat intestinal mucosa. Only Tween 80 increased paracellular absorption. Rat intestinal CYP3A was significantly inhibited by PEG-400. Cremophor and TPGS increased the fraction of norverapamil in plasma, consistent with excipient-mediated inhibition of P-gp. Further studies are required to determine the extent, if any, to which excipients in the products examined in this study contributed to the inhibitory potential, but the possibility remains that there is a potential for interaction.

As the progesterone cream was compounded in a local pharmacy, there is no information on how it was compounded which would affect bioavailability (39) and thus the potential for interaction. Progesterone is a 3A4 substrate where the oxidative biotransformation occurs through hydroxylation at the C16 position (40). Hence, any interaction which could affect the disposition of progesterone could increase the risk of an AE.

The results of this study clearly demonstrate that there is a potential for both NRP 409 and 410 to affect P450-mediated metabolism. None of the botanical materials in NHP 409 and 410 are known stimulants that could produce these AEs. Skullcap and Withania have sedative/adaptogen properties; Chasteberry, Saw palmetto, and wild yam are reportedly hormone modulating products; milk

thistle is taken for liver ailments and raspberry is used as an antioxidant. Guggul which is commonly used for cholesterol management was initiated. The *in vitro* vitamin and NHP 409 and 410 data obtained with this study suggests that there is a potential risk for an interaction with progesterone. However, the extended therapeutic period and use of at least 3 health products confounds whether a single product was responsible for the interaction, such as NRP 410 which was the last product introduced or the interaction was a result of a sufficiently high loading effect. While the case reports and the *in vitro* analysis strongly suggest that a potential for interactions and affection of CYP450 enzymes is present, a clinical study would be necessary to determine the pharmacoepidemiologic impact of these findings.

Vitamin use, like that of other NHPs is not always disclosed to the health care professional. As some vitamins can have toxicity or cause serious adverse events through interaction their concurrent use with other therapeutic products should be monitored. With the exception of the two herbal products NRP 409 and 410, all of the NHPs tested had a low to moderate potential to affect CYP-mediated metabolism. These two herbals had a strong inhibitory effect. The screening clearly demonstrated the *in vitro* potential for these products to affect CYP-mediated metabolism is variable and may be affected by the sum of their components.

Generally, the inhibitory or stimulatory effect of a product is proportional to concentration. This can then be related to the dose and rate of uptake. In this study it was noted that the products with the highest vitamin content did not have the correspondingly highest inhibitory activity. The multi-component nature of the medicinal and non-medicinal components in these products makes it difficult to clearly rationalize effect on this basis. It may be that the event occurred as a response to the total xenobiotic loading rather than due to a single product.

The findings of this study suggest that some vitamins and tBC containing products examined under our conditions have the potential to affect drug metabolism and disposition. The blended herbal products NRP 409 and 410, however, have the highest potential to affect the safety and efficacy of health products. Further studies are warranted to

determine whether these *in vitro* effects have a clinical significance.

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**Supplemental information for Table 4.** Summary of vitamin, mineral, and excipient components listed on the product labels of the multivitamin supplements (Nutraceutical Research Programme numbers 395 to 404). All amounts are listed in mg unless otherwise indicated. -, absent; x, present but amount not listed.

<b>Ingredient</b>	<b>395</b>	<b>396</b>	<b>397</b>	<b>398</b>	<b>399</b>	<b>400</b>	<b>401</b>	<b>402</b>	<b>403§</b>	<b>404§</b>
<b>OTHER</b>										
Green tea leaves ( <i>Camellia sinensis</i> ) **	1.875	-	-	-	-	-	-	-	-	-
Ginseng ( <i>Panax ginseng</i> ) (root)	-	-	-	-	50	-	-	-	-	-
Citrus bioflavonoids ( <i>Citrus limon</i> , rind)	-	-	-	-	-	-	-	-	2	2
Lycopene	-	-	-	-	-	-	-	-	0.3	0.3
Digestive enzymes	-	-	-	-	-	-	-	-	4	2
Peppermint extract ( <i>Mentha piperita</i> , leaves)	-	-	-	-	-	-	-	-	2	-
Green tea phytosome ( <i>Camellia sinensis</i> , leaf; <i>Glycine max</i> , soybean)	-	-	-	-	-	-	-	-	1	-
Grape seed phytosome ( <i>Vitis vinifera</i> , seeds; <i>Glycine max</i> , soybean)	-	-	-	-	-	-	-	-	1	-
Natural mixed vitamin E tocopherols	-	-	-	-	-	-	-	-	-	10 IU
Oleselect ( <i>Olea europaea</i> , fruit) (standardized to 30% phenols)	-	-	-	-	-	-	-	-	-	4
Turmeric extract ( <i>Curcuma longa</i> , stalk)	-	-	-	-	-	-	-	-	-	2
<b>NON-MEDICINAL</b>										
Acacia gum	x	-	-	-	-	-	-	x	-	-
Acetylated monoglycerides	x	-	-	-	-	-	-	-	-	-
Ascorbyl palmitate	x	-	-	-	-	-	-	x	-	-

Aspartame	-	-	-	X	-	-	-	-
BHT	-	-	-	-	-	-	-	X
Calcium cyclamate	-	-	-	X	-	-	-	-
Calcium phosphate dibasic	X	X	-	-	-	-	-	-
Calcium silicate	-	X	-	-	-	X	X	-
Cellulose	-	-	X	-	-	-	-	-
Citric acid	-	-	-	X	-	-	-	X
Colloidal silicon dioxide	-	-	-	-	X	X	X	-
Corn starch	X	-	-	-	-	-	-	X
Croscarmellose sodium	X	X	X	-	X	X	X	X
Crospovidone	X	-	-	-	-	-	-	X
Dextrose monohydrate	X	-	-	-	-	-	-	-
Dicalcium phosphate dl-alpha-tocopheryl acetate	-	-	-	-	-	-	-	X
EDTA	-	-	X	-	-	-	-	-
Ethanol	X	-	-	-	-	-	-	-
Gelatin	X	-	-	-	X	X	X	X
Hydrogenated soybean oil	X	-	-	-	-	-	-	X
Hydrolyzed polyvinyl alcohol	-	-	-	-	X	-	-	-
Hydroxypropyl methylcellulose	-	X	-	-	X	-	-	-
Hydroxypropylcellulose	X	-	-	-	-	-	-	-
Hydroxypropyl methylcellulose	-	-	X	-	-	-	-	-
Hypromellose	X	-	-	-	-	-	-	X
Isopropyl alcohol	X	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	X
Magnesium stearate	X	X	X	X	X	X	X	X
Malic acid	-	-	-	X	-	-	-	-
Maltodextrin	X	-	-	-	-	-	-	X
Medium chain triglycerides	-	-	-	-	-	-	-	X
Methylcellulose	X	-	-	-	-	-	-	X
Methyl paraben	-	-	X	-	-	-	-	-
Microcrystalline cellulose	X	X	-	-	X	X	X	X
Mineral oil	-	-	-	-	-	-	-	X
Modified food starch	-	-	-	-	-	-	-	X
Monoester: propylene glycol and glycerol	-	-	-	X	-	-	-	-
Natural and artificial flavours	-	-	-	X	-	-	-	-
Partially	-	-	-	-	-	X	X	-

hydrolyzed polyvinyl alcohol								
Polydextrose	x	-	-	-	-	-	-	x
Polyethylene glycol	x	x	x	-	x	x	x	-
Polysorbate	-	-	-	-	-	-	-	x
Povidone	x	x	x	x	x	-	-	-
Propyl paraben	-	-	x	-	-	-	-	-
Purified water	x	-	-	-	-	-	-	-
Shellac glaze	x	-	-	-	-	-	-	-
Silicon dioxide	x	x	x	-	-	-	-	x
Sodium ascorbate	x	-	-	-	-	-	-	x
Sodium benzoate	-	-	-	-	-	-	-	x
Sodium citrate	-	-	-	-	-	-	-	x
Sodium lauryl sulfate	-	-	-	-	-	-	-	x
Sorbic acid	-	-	-	-	-	-	-	x
Sorbitol	-	-	-	x	-	-	-	-
Soya lecithin	-	-	-	-	-	-	x	-
Stearic acid	x	-	x	-	-	-	-	x
Sucrose	x	-	-	-	-	-	-	x
Sugar	-	x	x	x	x	-	-	-
Talc	-	-	-	-	x	x	x	x
Tartrazine	-	-	-	-	-	x	-	-
Titanium dioxide	x	x	x	-	x	x	x	x
Triacetin	x	-	-	-	-	-	-	-
Tricalcium phosphate	x	-	-	-	-	-	-	-
Triethyl citrate	-	-	-	-	-	-	-	x
Allura red AC aluminum lake	-	-	-	-	x	-	-	-
D&C yellow No. 10	x	x	-	-	-	-	-	-
FD&C blue No. 1	x	-	-	-	-	-	-	-
FD&C blue No. 2	-	-	-	x	-	-	-	-
FD&C red No. 40	x	-	-	-	-	-	-	-
FD&C red No. 3	-	-	-	x	-	-	-	-
FD&C yellow No. 6	-	x	-	x	-	-	-	x
Indigo carmine aluminum lake	-	-	-	-	x	-	-	-
Red iron oxide	-	-	x	-	-	-	-	-
Sunset Yellow FCF aluminum lake	-	-	-	-	x	x	x	-
Yellow iron oxide	-	-	x	-	-	-	-	-

§ no excipients were reported on the package.

\* from *Tagetes erecta*, flower

\*\* Derived from 75 mg of a 25:1 standardized extract containing 36% EGCG.