Methodology

2.1 Samples

Samples were collected from retail outlets in the north of England and via the internet in February 2021. In accordance with the FSA's request the turmeric containing supplements (n = 15), ground/powdered turmeric (n = 10) and raw/fresh turmeric (n = 5) were purchased from a mixture of providers, for example, retail outlets and the internet. Sample details were recorded and the associated packaging material was photographed.

2.2 Sample preparation for trace element analysis

2.2.1 Fresh turmeric

The fresh turmeric samples were washed with Millipore water, chopped with ceramic knife on a plastic board, then blended using a Buchi B400 Blender fitted with ceramic blades. 20 g of each homogenised samples was transferred to an acid cleaned vessel prior to trace element extraction and analysis.

2.2.2 Ground turmeric

The ground turmeric samples were mixed in the container in which they were received. Replicate aliquots were removed directly for trace element analysis.

2.2.3 Turmeric supplements

Tablets – a portion of a tablet, an entire tablet or a number of intact tablets (depending on tablet mass) were removed directly for trace element analysis. Capsules - an entire capsule was removed directly for trace element analysis.

2.3 Trace element analysis

2.3.1 Sample extraction

Three sub-samples (3 x 0.5-1.0 g) of each turmeric survey sample were placed directly into TFM microwave digestion tubes and digested in a mixture of nitric acid and hydrochloric acid using a high-pressure microwave digestion system (Milestone UltraWave).

2.3.2 Inductively coupled plasma (ICP) – mass spectrometry (MS) analysis

Samples were analysed on an Agilent 7700x ICP – mass spectrometer fitted with an ASX-500 auto-sampler and a standard front end, consisting of nickel cones, a glass double-pass spray chamber and a glass concentric nebuliser. The analysis was conducted with a collision cell

operated in both “no gas” and helium mode, the latter to remove any interferences. The instrument was operated in general purpose mode with carrier gas flow 1.0 L/minute, RF power 1550 W, sampling depth 10 mm, peri-pump speed 0.1 rps. Concentrations of the following elements were determined: Ag - silver, Al - aluminium, As - arsenic, Ba - barium, Be - beryllium, Bi - bismuth, Ca - calcium, Cd - cadmium, Ce - cerium, Co - cobalt, Cr - chromium, Cs - caesium, Cu - copper, Dy - dysprosium, Er - erbium, Eu - europium, Fe - iron, Ga - gallium, Gd - gadolinium, Ge - germanium, Hg - mercury, Ho - holmium, K - potassium, La - lanthanum, Lu - lutetium, Mg - magnesium, Mn - manganese, Mo - molybdenum, Na - sodium, Nd - neodymium, Ni - nickel, P - phosphorus, Pb - lead, Pr - praseodymium, Rb - rubidium, S - sulphur, Sb - antimony, Se - selenium, Sm - samarium, Sn - tin, Sr - strontium, Tb - terbium, Tl - thallium, Tm - thulium, U - uranium, V - vanadium, Yb - ytterbium, Zn - zinc, Hf - Hafnium, Ir - Iridium, Nb - Niobium, Os - Osmium, Pd - Palladium, Pt - Platinum, Re - Rhenium, Ru - Ruthenium, Sc - Scandium, Ta - Tantalum, Te - Tellurium, Th - Thorium, Ti - Titanium, W - Tungsten, Y - Yttrium. Quality checks included blanks, spikes, and certified reference materials (CRMs). The methodology used is accredited to ISO17025.

2.4 Method development and validation – curcumin in turmeric containing supplements, ground/powdered turmeric and raw/fresh turmeric

Methodology was identified which allowed for the determination of the curcuminoids: curcumin, bisdemethoxycurcumin (BDMC) and demethoxycurcumin (DMC) (Figure 1) [3]. This methodology was established in-house with additional confirmatory analytical capability using Time-of-Flight (TOF) mass spectrometry (MS). In short, the method involved: extraction of the dried sample 0.5 g with tetrahydrofuran (10 mL) by shaking overnight at ambient temperature. The samples were then diluted 1,000 fold with methanol before a portion (1 mL) was evaporated to dryness under nitrogen. The residue was over-spiked and reconstituted in methanol to a total volume of 1mL prior to analysis by reverse phase HPLC using an ACE3 C18-AR 2.1 mm id x 100 mm, LC column with diode array (DAD - the quantitation wavelength was 425 nm) and TOF-MS detection. The mobile phase was 1.275% formic acid and methanol. The limit of quantification (LOQ) was determined using the measured concentration for the lowest calibration standard equivalent to the 1,000 fold diluted sample (0.00005 g/mL).
Replicate (n = 7 at each concentration) spiked samples following dilution (spiked to achieve concentrations of 0.45, 4.5 and 9 µg/mL in the solution equivalent to 9,000, 90,000 and 180,000 mg/kg in the samples) were prepared using lyophilised root ginger as a blank matrix. The method validation was performed in replicate (n=3) and the method performance characteristics determined.

2.5 Sample preparation for curcumin, bisdemethoxycurcumin and demethoxycurcumin analysis

2.5.1 Fresh turmeric

The fresh turmeric samples were washed with Millipore water, chopped with ceramic knife on a plastic board, then blended using a Buchi B400 blender fitted with ceramic blades. The blended sample was lyophilised using an Edwards pirani freeze dryer. The samples were mixed, and replicate aliquots were taken for analysis.

2.5.2 Ground turmeric

The ground turmeric samples were mixed in the container in which they were received. Replicate aliquots were taken for analysis.

2.5.3 Turmeric supplements

Tablets – Five tablets (of each type) were ground using a pestle and mortar. Replicate aliquots were taken for analysis. Capsules - Five capsule were emptied, then mixed. Replicate aliquots were taken for analysis.

2.6 Curcumin, bisdemethoxycurcumin and demethoxycurcumin analysis

2.6.1 Sample extraction

Duplicate aliquots (0.5 g) of each prepared sample were placed in amber vials (12 mL) and capped. Tetrahydrofuran (10 mL) was added, sonicated (10 mins) then shaken overnight at ambient temperature. The samples were allowed to settle (2 hrs, 4°C) then diluted 1,000 fold with methanol before a portion (1 mL) was aliquoted to a 2 mL vial and capped.

2.6.2 Standard preparation

Standards were prepared using lyophilised ginger extracted using the above procedure. A portion of the extract (1 mL) was evaporated to dryness under nitrogen. The residue was over-spiked with curcumin, bisdemethoxycurcumin and demethoxycurcumin at levels equivalent to 0, 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 µg/mL and reconstituted in methanol to a total volume of 1mL.

2.6.3 LC-TOF-MS analysis

Portions of the solvent extracts of the samples and standards were analysed using an Agilent 1200 liquid chromatograph coupled with an Agilent 6230 TOF. Chromatographic separation was achieved on an ACE3 C18-AR 2.1 mm id x 100 mm held at 60oC. The mobile phases comprised of water containing 1.275% formic acid (A) and methanol (B). The gradient started at 20% B and changed to 100% B at 15 minutes before returning to 20% B at 20 minutes. The flow rate was 0.4 mL/minute and the injection volume was 10 µL. The MS was operated in positive ESI mode with monitoring from 50 – 1600 m/z. Spectral data were acquired by diode array and the quantitation wavelength was 425 nm.

2.7 Method development – piperine in turmeric containing supplements

Methodology was identified in house which allowed for the determination of piperine. This methodology was established in-house using Time-of-Flight (TOF) mass spectrometry (MS). In short, the method involved: extraction of the dried sample 0.5 g with tetrahydrofuran (10 mL) by shaking overnight at ambient temperature. The samples were then diluted 1,000 fold with methanol before a portion (1 mL) was evaporated to dryness under nitrogen. The residue was over-spiked and reconstituted in methanol (1 ml) prior to analysis by reverse phase HPLC using an ACE3 C18-AR 2.1 mm id x 100 mm, LC column with TOF-MS detection. The mobile phase was 1.275% formic acid and methanol. The limit of quantification (LOQ) was determined using the measured concentration for the lowest calibration standard equivalent to the 1,000 fold diluted sample (0.00005 g/mL).

Replicate (n = 7 at each concentration) spiked samples following dilution (spiked to achieve concentrations of 0.045 and 0.9 µg/mL in the solution equivalent to 900 and 18,000 mg/kg in the samples) were prepared using lyophilised root ginger as a blank matrix. The method validation was performed in replicate (n = 3) and the method performance characteristics determined.

2.8 Sample preparation for piperine analysis

2.8.1 Fresh turmeric

The fresh turmeric samples were washed with Millipore water, chopped with ceramic knife on a plastic board, then blended using a Buchi B400 blender fitted with ceramic blades. The blended sample was lyophilised using an Edwards pirani freeze dryer. The samples were mixed, and replicate aliquots were taken for analysis.

2.8.2 Ground turmeric

The ground turmeric samples were mixed in the container in which they were received. Replicate aliquots were taken for analysis.

2.8.3 Turmeric supplements

Tablets – Five tablets (of each type) were ground using a pestle and mortar. Replicate aliquots were taken for analysis. Capsules - Five capsule were emptied, then mixed. Replicate aliquots were taken for analysis.

2.9 Piperine analysis

2.9.1 Sample extraction

Duplicate aliquots (0.5 g) of each prepared sample were placed in amber vials (12 mL) and capped. Tetrahydrofuran (10 mL) was added, sonicated (10 minutes) then shaken overnight at ambient temperature. The samples were allowed to settle (2 hrs, 4°C) then diluted 1,000 fold with methanol before a portion (1 mL) was aliquoted to a 2 mL vial and capped.

2.9.2 Standard preparation

Standards were prepared using lyophilised ginger extracted using the above procedure. A portion of the extract (1 mL) was evaporated to dryness under nitrogen. The residue was over-spiked with piperine at levels equivalent to 0, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1 µg/mL and reconstituted in methanol to a total volume of 1 mL.

2.9.3 LC-TOF-MS analysis

Portions of the solvent extracts were analysed using an Agilent 1200 liquid chromatograph coupled with an Agilent 6230 TOF. Sample extracts were diluted as required. Chromatographic separation was achieved on an ACE3 C18-AR 2.1 mm id x 100 mm held at 60oC. The mobile phases comprised of water containing 1.275% formic acid (A) and methanol (B). The gradient started at 20% B and changed to 100% B at 15 minutes before returning to 20% B at 20 minutes. The flow rate was 0.4 mL/minute and the injection volume was 10 µL. The MS was operated in positive ESI mode with monitoring from 50 – 1600 m/z. Quantitation was carried out using m/z 286.1438 ± 100 ppm.