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Analysis of coffee bean extracts by use of ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry

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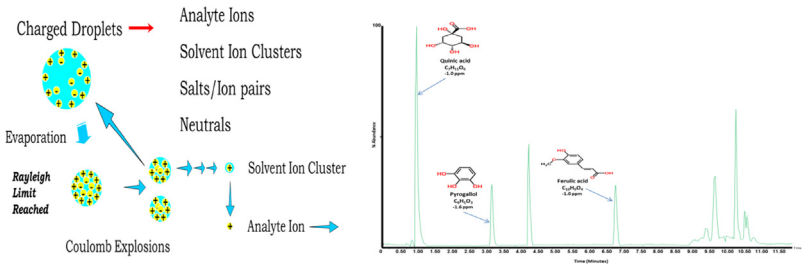
GRAPHICAL ABSTRACT



ABSTRACT

The number of flavour chemicals identified in coffee has reached over 1000 [1,2]. Coffee is one of the world's most popular beverages [3], highly studied for its health-related properties [4–6]. Studies on coffee associated with human health have focused on the negative aspects, such as the toxicity of caffeine [7,8]. Complex chemistry happens during coffee roasting and according to the literature, a number of compounds have been detected and quantified in coffee beans samples by UPLC–Q-TOF/MS [9–12]. The following method offers a simple approach for the qualitative and quantitative analysis of coffee bean extracts using a Waters Acquity G2 UPLC–Q-TOF/MS instrument adapted from the method by Kenny et al., [12]. The following modifications were made:

- The method by Kenny et al. was developed on a triple quadrupole mass spectrometer, the below method was developed on a Q-TOF MS.
- A combination of utilising both base peak index and mass extraction at 0.05 Da allows for a sensitive, quantitative technique amidst poor background noise and poor separation with high mass accuracy (<5 ppm).
- By use of MS^E centroid experiment, greater mass spectral information for metabolite profiling could be obtained.

ARTICLE INFO

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Method details

Liquid chromatography mass spectrometry analysis

The analysis was performed using a Waters Acquity G2 Q-TOF LC–MS instrument. This system is composed of a Waters Acquity UPLC system coupled to a quadrupole time-of flight mass spectrometer. The samples were eluted using a Titan C18 HPLC analytical column (100 mm × 2.1 mm, 1.9 μm) and preceded by a Titan C18 guard cartridge (5 mm × 2.1 mm, 1.9 μm) with the column set to 35 °C. All samples were kept refrigerated to 4 °C in the UPLC autosampler and a 10 μL injection volume was used with a total flow rate of 0.3 mL/min over a total run time of 12 min. All solvents used were LC–MS grade and ultra-pure 18.2 MΩ water was used for each step. Mobile phase A consisted of water + 0.1% formic acid while mobile phase B was acetonitrile + 0.1% formic acid. The following tables contain the gradient details for list of compounds analysed (Tables 1 and 2):

Mass spectrometry detection was conducted through electrospray ionisation using an ms^E centroid experiment in both positive and negative mode and screened in the *m/z* scan range of 50–2000 Da (Table 3) with the analyser set to resolution mode at FWHM. Scanning conditions were set to 1 scan every 0.7 s. Collision energy was set for two functions, function one at low energy with no collision energy applied and function two at high energy using a collision energy ramp from 20 to 75 eV. In negative mode the following MS tune file settings were used: Capillary voltage 3.00 kV, sampling cone 40 V, extraction cone 4.0 V, source temperature 120 °C, desolvation temperature 450 °C, desolvation gas flow 800 L/h, cone gas flow 50 L/h. In positive mode, the following MS tune file settings were used: capillary voltage 3.00 kV, sampling cone 30 V, extraction cone 2.0 V, source temperature 120 °C, desolvation temperature 450 °C, desolvation gas flow 800 L/h, cone gas flow 50 L/h. The accurate mass of the instrument was initially calibrated through direct infusion of a sodium iodide calibrant solution prior to sample analysis. In addition, leucine enkephalin (Leuenk) lockmass solution (2 ng/uL) was infused at 5 μL/min in parallel to the mobile phase flow, scanned and automatically corrected to verify exact mass which ensured high mass accuracy (<5 ppm) throughout the scan range over the course of the submitted sequence. Masslynx v4.1 software was used to control the instrument and also analyse the data.

Sample extraction

Coffee beans (green and roasted) were frozen with liquid nitrogen and ground with a mill. Ground coffee samples (2 g) were extracted with LC grade water at 92 °C (25 mL) then stirred for 6 min at 70–80 °C and placed on ice immediately after in order to cool down rapidly. The samples were centrifuge at 21,481 × *g* for 2 min. After centrifugation the extracts were filtered through a 0.2 μm PVDF membrane. Extracts were poured into 1.5 mL vials and sealed. All other remaining samples and extracts were kept in the freezer at −20 °C.

Table 1
Gradient used for separation and identification of standards reconstituted in water include quinic acid, ferulic acid, pyrogallol, and trigonelline hydrochloride.

Time (min)	Flow rate	%A	%B
Initial	0.3	98.0	2.0
1	0.3	98.0	2.0
2	0.3	90.0	10.0
3	0.3	80.0	20.0
6	0.3	80.0	20.0
7.5	0.3	65.0	35.0
8.5	0.3	10.0	90.0
9.5	0.3	10.0	90.0
12	0.3	98.0	2.0

Table 2
Gradient used for separation and identification of standards reconstituted in MeOH include caffeine, 5-caffeoylquinic acid, vitamin B3, caffeic acid, catechol, and 1,2,4-benzentriol.

Time (min)	Flow rate	%A	%B
Initial	0.3	98.0	2.0
1	0.3	98.0	2.0
2	0.3	90.0	10.0
3	0.3	90.0	10.0
6	0.3	90.0	10.0
7.5	0.3	50.0	50.0
8.5	0.3	10.0	90.0
9.5	0.3	10.0	90.0
12	0.3	98.0	2.0