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

Daniel James O’Driscoll

Alimentary Pharmabiotic Centre, Biosciences Institute, University College Cork, Cork, Ireland

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 MS² centroid experiment, greater mass spectral information for metabolite profiling could be obtained.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/3.0/).
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 x 2.1 mm, 1.9 µm) and preceded by a Titan C18 guard cartridge (5 mm x 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 msÈ 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/µL) 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 centrifuged at 21,481 x 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 |
Stock solution preparation

Two stock solutions were prepared, these included methanol and water depending on the solubility of the compound. All standards were prepared between 1 and 8 mg to a final volume of 10 mL. The methanol stock solution consisted of caffeine, 5-caffeoylquinic acid, vitamin B3, caffeic acid, catechol and 1,2,4-benzenetriol. While the water stock solution consisted of trigonelline hydrochloride, quinic acid, ferulic acid and pyrogallol.

Quantification

Quantification was performed by generation of suitably linear curves for each of the analysed standards (Table 4). All standard curves were created in Microsoft Excel, 2010. For the purpose of this method it was deemed necessary to determine

Table 3
List of compounds (standards) qualitatively and quantitatively analysed in both positive and negative mode ionisation.

| Molecular formula | Monoisotopic mass (Da) | Compounds | Solvent solubility | Polarity (+/−) | % Error ppm |
|-------------------|------------------------|-----------|--------------------|---------------|-------------|
| C₈H₁₀N₄O₂        | 194.080383             | Caffeine  | MeOH               |               | 3.1         |
| C₇H₇NO₂          | 137.047684             | Trigonelline hydrochloride | Water  |               | 5.0         |
| C₁₆H₁₈O₉         | 354.095093             | 5-Caffeoylquinic acid | MeOH       |               | −2.3        |
| C₇H₁₂O₆          | 192.063385             | Quinic acid | Water  |               | −1.0        |
| C₂₁H₂₂O₇        | 328.136298             | Vitamin B3 | MeOH       |               | 4.2         |
| C₁₆H₁₈O₉         | 194.057907             | Ferulic acid  | Water  |               | −1.0        |
| C₆H₅NO₂          | 120.034014             | Vitamin B3 | MeOH       |               | −1.7        |
| C₁₀H₁₀O₄         | 194.057907             | Caffeic acid  | MeOH       |               | 0.9         |
| C₉H₈O₄           | 180.042028             | Quinic acid  | Water  |               | −1.6        |
| C₆H₆O₂           | 126.031693             | Pyrogallol  | Water  |               | −4.0        |

Table 4
R² values for calibration curves (n=8) generated from Waters Acquity G2 UPLC–Q-TOF/MS instrument.

| Compounds                  | Concentration range (mg/mL) | LOD (mg/mL) | LOQ (mg/mL) | R²     | Sample Green Bean (mg/mL) |
|----------------------------|------------------------------|-------------|-------------|--------|---------------------------|
| Caffeine                   | 0.00036–0.00359              | 0.0000119   | 0.0000396   | 0.9967 | 0.00040                   |
| Trigonelline hydrochloride | 0.00047–0.00367              | 0.0000364   | 0.0001215   | 0.9554 | 0.00202                   |
| 5-Caffeoylquinic acid      | 0.00039–0.0028               | 0.000185    | 0.000637    | 0.961  | nd                        |
| Quinic acid                | 0.00026–0.00203              | 0.000020    | 0.000080    | 0.9984 | 0.00143                   |
| Vitamin B3                 | 0.00042–0.00328              | 0.000185    | 0.000617    | 0.9981 | 0.00009                   |
| Ferulic acid               | 0.00038–0.00297              | 0.000020    | 0.000080    | 0.9943 | 0.00100                   |
| Caffeic acid               | 0.00043–0.00336              | 0.000020    | 0.000080    | 0.9974 | 0.000273                  |
| Catechol                   | 0.00015–0.00117              | 0.000020    | 0.000080    | 0.9982 | 0.00066                   |
| Pyrogallol                 | 0.00021–0.00164              | 0.000020    | 0.000080    | 0.9976 | 0.0003                    |
| 1,2,4-Benzenetriol         | 0.0001–0.00781               | 0.000020    | 0.000080    | 0.9966 | 0.00134                   |

Fig. 1. Full scan MS (negative mode) chromatogram conducted in the mass to charge (m/z) range between 50 and 2000 Da displaying elemental composition and error ppm reconstituted in MeOH.
only limit of detection (LOD), limit of quantitation (LOQ) and finally linearity over the range to obtain suitable $R^2$ values. In order to establish LOD and LOQ values, each analyte was determined as concentrations equivalents to three times and 10 times the signal-to-noise ratio of the compounds of interest in the lowest concentration of the calibration curve prepared using the green bean extract. The signal-to-noise ratios were calculated using Waters MassLynx software version 4.1

**Base peak index (BPI) full scan MS chromatographic profiles**

**Discussion of figures**

Figs. 1 and 2 represent total ion count chromatograms generated from the analysis of methanol in both positive and negative mode mass spectrometry while Figs. 3 and 4 were analysed in water. From the peaks obtained it can be seen that some compounds ionise in both positive and negative polarity, labelled peaks represent best ionisation. In Fig. 1, peak at retention time 1.40 represents vitamin B3, in Fig. 2 the peaks at retention time 4.49 and 7.71 represent both 5-caffeoylquinic acid and caffeic acid, respectively. For confidentiality reasons, peak at retention time 4.30 in Fig. 3, peak at retention time 1.56 in Figs. 1 and 2 and finally peaks at retention times 4.27 and 6.46 cannot be disclosed.

---

Fig. 2. Full scan MS (positive mode) chromatogram conducted in the mass to charge ($m/z$) range between 50 and 2000 Da displaying elemental composition and error ppm reconstituted in MeOH.

Fig. 3. Full scan MS (negative mode) chromatogram conducted in the mass to charge ($m/z$) range between 50 and 2000 Da displaying elemental composition and error ppm reconstituted in water.
Acknowledgements

The publication has emanated from research supported in part by a research grant from Science Foundation Ireland (SFI) under Grant Number SFI/12/RC/2273. Funding under the higher education authorities (HEA’s) programme for research in third-level institutions (PRTLI) and co-funded under the European Regional Development Fund. The author would also like to acknowledge both Camille Delebecque and Sophie Deterre of the Afineur coffee company. MethodsX thanks the reviewers of this article (Giovanni Caprioli and a second reviewer who would like to remain anonymous) for taking the time to provide valuable feedback.

References

[1] I. Flament, C. Chevalier, Analysis of volatile constituents of coffee aroma, Chem. Ind. (1988) 592–596.
[2] T. Shibamoto, An overview of coffee aroma and flavor chemistry, in: Proceedings of the 14th International Scientific Colloquium on Coffee, San Francisco, 1991, Association Scientifique Internationale du Cafe, Paris, France, 1992, pp. 107–116.
[3] Production and consumption, Trouble brewing: the changing face of coffee production, 2006 http://pubs.wri.org/pubscontent_text.cfm?ContentID=1445 (accessed 4.11.08).
[4] B. Schilter, C. Cavin, A. Tritscher, A. Constable, in: Coffee, R.J. Clarke, O.G. Vizthum (Eds.), Health Effects and Safety Considerations, Blackwell Science, Oxford, UK, 2001, pp. 165–183.
[5] R.S. Sandler, Diet and cancer, Nutr. Cancer 4 (1983) 273–279.
[6] G. Caprioli, S. Logrippe, M.G. Cahill, K.J. James, High-performance liquid chromatography LTQ-Orbitrap mass spectrometry method for tomatidine and non-target metabolites quantification in organic and normal tomatoes, Int. J. Food Sci. Nutr. 65 (4) (2014) 465–469.
[7] J.A. Carrillo, J. Benitez, CYP1A2 activity, gender and smoking, as variables influencing the toxicity of caffeine, Br. J. Clin. Pharmacol. 41 (1996) 605–608.
[8] K.W. Derlet, J.C. Tseng, T.E. Albertson, Potentiation of cocaine and d-amphetamine toxicity with caffeine, Am. J. Emerg. Med. 10 (1992) 211–216.
[9] D. Perrone, C.M. Donangelo, A. Farah, Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography–mass spectrometry, Food Chem. 110 (2008) 1030–1035.
[10] X. Goxiang, Y. Mao, Y. Yan, N. Mingming, S. Hua, M. Huang, Z. Aihua, Xiaojiao Z., C. Tianlu, J. Wei, Characterization of Pu-erh tea using chemical and metabolic profiling approaches, J. Agric. Food Chem. 57 (2009) 3046–3054.
[11] R. Hertz-Schünemann, R. Dorfner, C. Veretzian, T. Streibel, R. Zimmermann, On-line process monitoring of coffee roasting by resonant laser ionisation time-of-flight mass spectrometry: bridging the gap from industrial batch roasting to flavour formation inside an individual coffee bean, J. Mass Spectrom. 48 (2013) 1253–1265.
[12] O. Kenny, T.J. Smyth, C.M. Hewage, N.P. Brunton, Antioxidant properties and quantitative UPLC–MS/MS analysis of phenolic compounds in dandelion (Taraxacum officinale) root extracts, Free Radic. Antioxid. 4 (January–June (1)) (2014) 55.