 doi:10.20944/preprints201911.0390.v1

Technical Note

Validation of a Quantitative Proton Nuclear Magnetic Resonance Spectroscopic Screening Method for Coffee Quality and Authenticity (NMR Coffee Screener)

Alex O. Okaru[1], Andreas Scharinger[2], Tabata Rajcic de Rezende[2], Jan Teipel[2], Thomas Kuballa[2], Stephan G. Walch[2] and Dirk W. Lachenmeier[2,*]

[1] Department of Pharmaceutical Chemistry, University of Nairobi, P.O. Box 19676-00202 Nairobi, Kenya. E-mail: alex.okaru@gmail.com

[2] Chemisches und Veterinäruntersuchungsamt (CVUA) Karlsruhe, Weissenburger Straße 3, 76187 Karlsruhe, Germany; E-mails: andreas.scharinger@cvuaka.bwl.de; tabata.rajcicderezende@cvuaka.bwl.de; jan.teipel@cvuaka.bwl.de; thomas.kuballa@cvuaka.bwl.de; stephan.walch@cvuaka.bwl.de; lachenmeier@web.de

* Correspondence: lachenmeier@web.de; Tel.: +49-721-926-5434

Abstract: Monitoring coffee quality as a means of detecting and preventing economically motivated fraud is an important aspect of international commerce today. Therefore, there is a compelling need for rapid high throughput validated analytical techniques such as quantitative proton NMR spectroscopy for screening and authenticity testing. For this reason, we sought to validate an NMR spectroscopic method for routine screening of coffee for quality and authenticity. A factorial experimental design was used to investigate the influence of NMR device, extraction time and nature of coffee on the content of caffeine, 16-*O*-methylcafestol (OMC), kahweol, furfuryl alcohol and 5-hydroxymethylfurfural (HMF) in coffee. The method was successfully validated for specificity, selectivity, sensitivity and linearity of detector response. The proposed method produced satisfactory precision for all analytes in roasted coffee, except for kahweol in canephora (robusta) coffee. The proposed validated method may be used for routine screening of roasted coffee for quality and authenticity control, as its applicability was demonstrated during the recent OPSON VIII Europol-Interpol operation on coffee fraud control.

Keywords: caffeine; 16-*O*-methylcafestol; kahweol; furfuryl alcohol; tetramethylsilan (TMS); magnetic resonance spectroscopy; validation studies

1. Introduction

Coffee remains a popular beverage worldwide and is typically obtained from the two species *Coffea canephora (*robusta*)* and *Coffea arabica* [1–3]. *Coffea arabica* fetches a higher price in the market owing to its perceived superior organoleptic properties and higher production costs compared to *Coffea canephora* [4]. Consequently, beverage fraud involving complete or partial substitution of arabica with robusta coffee cannot be overruled. On the other hand, the diterpenes cafestol, 16-*O*-methylcafestol (OMC) and kahweol found in the lipid fraction of coffee serve as potential markers for differentiation of *C. canephora* and *C. arabica*. Cafestol is found in both *C. canephora* and *C. arabica* while OMC is specific only to *C. canephora* [5–7]. Kahweol, although present in both types of coffee, is found in significantly higher amounts in *C. arabica.* These differences in the diterpene constituent enable the distinction between the coffees and also enable detection of beverage fraud involving substitution of *C. arabica* with the cheaper *C. canephora* beans using OMC as a marker [8].

A number of analytical techniques such as high performance liquid chromatography [9], gas chromatography with flame ionization detection [10], gas chromatography-mass spectrometry [11,12], proton transfer mass spectrometry [13], nuclear magnetic resonance (NMR) spectroscopy [14], isotope-ratio mass spectrometry [15], near-infrared spectroscopy [16,17], electronic nose [18], flame atomic absorption spectrometry [19] and attenuated total reflectance Fourier transform

 doi:10.20944/preprints201911.0390.v1

infrared spectroscopy [16] among others, have been reported in the literature for quantitative determination of coffee constituents and screening of coffee for adulteration.

Nuclear magnetic resonance spectroscopy in combination with chemometrics has been applied either for routine quality control and/or detection of potentially harmful substances in beverages such alcohol [20], fruit juices [21,22] and coffee [8,23]. NMR spectroscopy may be applied for the quantification of caffeine, OMC, kahweol, 5-hydroxymethylfurfural (HMF) and furfuryl alcohol in coffee [24-26]. For decaffeinated coffee, NMR spectroscopy may be used to determine the residual quantities of caffeine, which would typically be less than 1 g/kg. Furfuryl alcohol and HMF may be used as indicators of the degree of coffee roasting [24]. However, furfuryl alcohol is also of public health significance and therefore may require monitoring using NMR. The International Agency for Research on Cancer (IARC) classifies furfuryl alcohol into Group 2B (possibly carcinogenic) [27]. NMR also offers the advantages of cost-effectiveness especially for screening. Additionally, NMR provides reproducible quantitative data as well as generates unique chemical fingerprints that may be useful for authenticity testing [28,29]. Similar to other analytical techniques, reliable results may only be obtained by use of validated methods. Based on previously published method development and optimization work [14,24-26], the aim of this study was to validate the quantitative NMR spectroscopic method for screening coffee for both quality and authenticity.

2. Materials and Methods

2.1 Chemicals

Reagents and standard compounds were of analytical or HPLC grade. Caffeine, HMF, OMC, kahweol and furfuryl alcohol were purchased from Sigma-Aldrich (Steinheim, Germany). Deuterated chloroform-d_1 (≥99.8% atom % D) and internal reference standard tetramethylsilane (TMS) were from Roth (Karlsruhe, Germany).

2.1.1 Reference standards and preparation of working standard solutions

Primary stock solutions of caffeine, HMF, OMC, kahweol and furfuryl alcohol were prepared in deuterated chloroform solution with 1% TMS. Individual stock solutions were prepared by separately dissolving 5 mg of caffeine, HMF, kahweol and furfuryl alcohol each in 5 mL $CDCl_3$ + TMS. For preparation of OMC stock solution, 10.9 mg of OMC powder was dissolved in 10.9 mL $CDCl_3$ + TMS. Working solutions were obtained by carrying out a 1:2 dilution. The stock solutions were kept in the freezer until use. The guidance concentration and defined working ranges for the working standards are given in Table 1.

Table 1: Working standards used

Substance	Guidance value (mg/kg)	Defined working ranges according to experience (mg/kg)
OMC	< 50 for Arabica	7.5 – 7500
Caffeine	< 1000 for Decaf	7.5 – 7500
Kahweol	< 300 for Robusta	7.5 – 7500
Furfuryl alcohol	-	7.5 – 7500
HMF	-	7.5 – 7500

2.2 Methodology

2.2.1 Samples and sample preparation

Analyte samples weighing 200 mg were dissolved in 1.5 mL of $CDCl_3$ + TMS. The samples were shaken for 10 min or 20 min at 350 rpm on the shaking machine. The solutions were then membrane filtered (0.45µm) before 600 µL of the filtrate was transferred to an NMR tube followed by analysis.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 November 2019 doi:10.20944/preprints201911.0390.v1

2.2.2 NMR analysis

Two 400 MHz (9.4 T) field strength spectrometers were used: an AVANCE 400 Ultra Shield with a 5mm PASEI 1H/D-13C Z-GRD probe, and an Ascend400 with a BBI 400S1 H-BB-D-05 Z (each from Bruker, Rheinstetten, Germany). All samples were measured in 5 mm sample tubes (NMR tube DEU-Quant 5 mm, 7 inch) (Deutero, Kastellaun, Germany). The spectra were automatically acquired at 300.0 K under the control of Sample Track and ICON-NMR (Bruker BioSpin, Rheinstetten, Germany). Detailed information about measurement methodology is available in [26].

A waiting time of 5 min for temperature equilibration was used for every measurement. All spectra were acquired at 300 K. The NMR spectra were acquired using a Bruker pulse program (zg30) with 64 scans (NS) and 2 prior dummy scans (DS) with a relaxation delay (D1) of 30 s and an acquisition time of 7.97 s. The time domain was set to 131072 with a spectral width of 20.5503 (8223.68 Hz) for UlraShield and 20.5617 (8223.69 Hz) for Ascend. The size of the real spectrum (SI) was 262144. The receiver gain was set to 45.2. All spectra were recorded with the basopt mode. The acquisition parameters were constant for all spectra for PULCON measurement according to Lachenmeier et al. [26]. The FID was multiplied with exponential window function, and spectra were automatically phased, baseline-corrected using TopSpin version 3.2 and 3.5 (Bruker Biospin, Rheinstetten, Germany).

2.2.3 Experimental design

A factorial experimental design was adopted for the validation studies. For this purpose, six matrix calibration series, each consisting of two blanks and ten samples with increasing amounts namely 1, 5, 10, 25, 50, 100, 250, 500, 750 and 1000 mg/L of analyte were prepared. The factorial design was employed for the investigation of the influence of the three experimental factors, NMR spectrometer type, coffee type and shaking time (see Table 2). Each measurement series corresponds to a different combination of factor characteristics (see Table S1 for full design).

Table 2. Factorial experimental design used

Array	Factor 1: NMR device	Factor 2: Coffee type	Factor 3: Shaking Time (min)
1	Ultrashield/Ascend	100 % Arabica decaffeinated	20
2	Ultrashield/Ascend	100 % Robusta	20
3	Ultrashield/Ascend	Green coffee	20
4	Ultrashield/Ascend	100 % Arabica decaffeinated	10
5	Ultrashield/Ascend	100 % Robusta	10
6	Ultrashield/Ascend	Green coffee	10

2.2.4 Preparation of working and test solutions

Stock solutions (1000 and 5000 mg/L) of each of the analytes comprising caffeine, HMF, OMC, kahweol and furfuryl alcohol were used to prepare 1, 5, 10, 25, 50, 75, 125, 250, 500, 750, 1000 mg/L working solutions. Additionally, two blanks were made for each of the measurement series. Separate test solutions were prepared for the three matrices (100% Arabica decaffeinated, 100% Robusta coffee and green coffee). The dilution matrix to achieve the desired concentration is shown in Table 3.

 doi:10.20944/preprints201911.0390.v1

Therefore for the six matrix calibration series, a total of 72 test solutions were prepared. However, since all samples were run in two instruments (Ultrashield/Ascend), 144 measurement results were obtained (or 120 without the blank values).

Table 3: Dilution matrix

Desired calibration Concentration (mg/L)	Dilution of stock solution (1000 mg/L)	Desired Final concentration (mg/L)	Volume per stock solution (µL)	Volume of all analytes (µL)	Volume of $CDCl_3$ (µL)
0 (Blank, 2x)	0	0	-	-	1500
1	1:1000	1	1.5	7.5	1492.5
5	1:200	5	7.5	37.5	1462.5
10	1:100	10	15	75	1425
25	1:40	25	37.5	187.5	1312.5
50	1:20	50	75	375	1125
100	1:10	100	150	750	750
250	1:20	250	75	375	1125
500	1:10	500	150	750	750
750	1:6.66	750	225	1125	375
1000	1:5	1000	300	1500	-

2.3 Validation studies

Three different coffee matrices spanning the broadest possible spectrum of different coffee constituents were used during validation. These consisted of decaffeinated coffee (Decaf. Arabica, matrix 1), robusta coffee (matrix 2), and a raw coffee (green coffee, matrix 3). For the preparation of the spiked matrix samples, each pure substance was weighed before being dissolved in $CDCl_3$ and TMS solution (usually in 5 - 10 mL). Subsequently, the test samples were spiked in the specified concentration range (see Supplementary Table S1). A control was also run after a series of measurements in order to ascertain that analyses were properly performed so that test results obtained could be considered reliable.

2.3.1 Selectivity

The selectivity of each analyte was established by checking the analyte specific NMR parameters namely coupling constant, multiplicity and chemical shift. For this purpose, all analytes were mixed in a solution without matrix. To achieve this, 100 µL of each of the five analytes were pipetted into a NMR tube followed by addition of 500 µL $CDCl_3$ before NMR analysis (desired concentration 500 mg/l).

Furthermore, all analytes in a solution were mixed with 100% Arabica decaffeinated coffee in order to check possible matrix disturbances. This was achieved by pipetting 150 µL of each of the five analytes into a NMR tube followed by addition of 750 µL $CDCl_3$ before adding 200 mg of coffee sample (desired concentration 100 mg/l). The solution was shaken for 20 min at 350 rpm on the shaker, then membrane filtered and used directly for NMR measurement. For comparison, an NMR spectrum of a coffee sample (without analytes) was also acquired. The coffee sample was prepared by dissolving 200 mg coffee sample in 1.5 mL $CDCl_3$, shaken for 20 min at 350 rpm on the shaker, membrane filtered and used for NMR analysis.

2.3.2 Detection and quantification limits

 doi:10.20944/preprints201911.0390.v1

In order to determine the detection limit, 9 spiking levels of different concentrations were added to decaffeinated Arabica coffee (Matrix 1), processed, measured and evaluated. The detection and determination limits were determined in the lower working range according to DIN 32645.

2.3.3 Precision and recovery

For the determination of the measurement uncertainties and the recoveries, 9 spiking levels at different concentrations were added to the 3 matrices and processed. The measurement uncertainty was determined with the aid of ANOVA Design Expert Software V.7.0.

2.4 Data analysis and quality control

Peak areas in the 1D-proton NMR spectra were evaluated with the help of a compiled MatLab script. The peak areas were determined using a line fitting algorithm. Quantification was done using the eretic factor, which was previously determined using a quant reference (for details see [26]). At the end of each measurement series, a control solution was measured as a safeguard. The assignment of the signal patterns and the determination of the exact position of the signals were performed by the analysis of a 2D-JRES-NMR spectrum. Note: due to the restricted solubility of caffeine in $CDCl_3$, an empirical factor of 6 for recalculation has to be used, as determined based on HPLC measurements using the German reference procedure.

2.5 Method performance

The method was assessed for performance by calculating the standard deviation of the intra-laboratory reproducibility, recovery, robustness, limits of measurements and the total uncertainty of the measurements as a function of concentration.

3. Results and Discussion

3.1. Validation

3.1.1 Specificity and selectivity

The use of working reference standards enabled accurate assignment of chemical shifts. The chemical shifts of the analytes in the different matrices are shown in Table 4. OMC presented a slight offset in the integration range that led to a too high integral. However, this problem was circumvented by integrating the range next to OMC (3.04 ppm - 3.10 ppm) and subtracting it from the sum of the integral OMC.

Table 4: Characteristic signals of the constituents in coffee and their ranges

Analyte	Integration range (ppm)
OMC	3.185 – 3.125
Caffeine	3.44 – 3.38
Kahweol	5.925 – 5.85
Furfuryl alcohol	7.411 – 7.39
HMF	9.69 – 9.67

3.1.2 Analytical limits

The detection and quantification limits are given in Table 5 together with the linearity ranges. The limits of measurements were adjudged fit for purpose.

Table 5: Limits of detection and quantification of analytes determined

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Analyte	Detection limit (mg/kg)	Determination limit (mg/kg)	Concentration range for determination of limit (mg/kg)
OMC	2.5	7.4	7.5 – 187.5
Caffeine	15.7	43.1	7.5 – 187.5
Kahweol	186.0	501.4	187.5 – 1875.0
Furfuryl alcohol	11.6	39.4	7.5 – 75
HMF	6.3	22.9	7.5 – 75

3.1.3 Precision

The recoveries of the different analytes in various matrices are shown in Table 6. Although, the recoveries in a majority of the matrices used were within limits, green coffee gave poor unsatisfactory recoveries for caffeine, OMC and kahweol. Moreover, the recovery of kahweol from robusta coffee was out of specifications too (see table 6).

Table 6: Recovery of coffee constituents from different matrices

Matrix	Recovery (%)				
	Caffeine	OMC	Kahweol	Furfuryl alcohol	HMF
Decaf. Arabica	101	97	95	97	102
Robusta	102	101	74*	99	101
Green coffee	137*	54*	188*	93	107

* Outside of specification. Specification: 90-110%

The coefficient of variation (CV) was used as criterion for evaluating the precision of the proposed NMR method. The acceptance criterion for precision was a CV of less than 15%. Apart from kahweol, the precision of all the other analytes was found being within the limits of acceptance in all matrices. The analytes, caffeine, OMC, furfuryl alcohol and HMF present in roasted coffee (Arabica and Robusta) can therefore be determined with sufficient precision and accuracy by using the proposed NMR method. However, kahweol may not be quantified with adequate precision in robusta due to its low content in this matrix. In addition to the out-of-specification recoveries, the precision of all the analytes for green coffee were unsuitable (Table 7). Further work, potentially by improving the extraction appears to be necessary for green coffee.

Table 7: Precision of coffee constituents

Matrix	Precision (CV)				
	Caffeine	OMC	Kahweol	Furfuryl alcohol	HMF
Decaf. Arabica	8.1	6.5	22.2*	6.1	8.3
Robusta	7.4	7.8	32.7*	5.8	6.9
Green coffee	104*	188*	570*	25*	27*

* Outside of specification. Specification <15%. CV - coefficient of variation

3.1.4 Linearity of detector response

The linearity was established in the concentration ranges (working range) listed in Table 8. The linearity was determined in matrix 1 (see Annex 4). Since the coefficients of determination (R^2) were all >0.99 over the concentration ranges examined, the method may be considered to be fit-for-purpose.

Table 8: Linear concentration range of the coffee analytes

Analyte	Linearity (mg/kg)	Coefficient of determination (R^2)

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OMC	7.5 – 5625	1.0000
Caffeine	7.5 – 5625	1.0000
Kahweol	7.5 – 5625	0.9949
Furfuryl alcohol	7.5 – 5625	1.0000
HMF	7.5 – 5625	0.9997

3.1.5 Effect of matrix

ANOVA revealed that the models are significant for all analytes and can be evaluated. For all analytes it was shown that the instrument used (i.e. NMR spectrometer type) has no significant influence on the analytical results. The measurements can thus be performed on both spectrometers. Similarly, the extraction time had no significant influence. If the results are viewed manually, the extraction time of 20 minutes seems adequate, but not statistically significant, to achieve better results and was thus defined as a setting.

However the influence of variety of coffee was found to be statistically significant especially with green coffee, which had a significantly greater dispersion. Roasting was found to have no influence on the determinations since similar recoveries were obtained for the analytes. The method can therefore only be considered successfully validated for the determination of OMC, caffeine, kahweol, furfuryl alcohol and HMF in samples of roasted coffee. Measurements of green coffee shall be considered as indicative only.

4 Conclusions

The proposed NMR spectroscopic method gave satisfactory validation results for specificity, selectivity and linearity. All analytes examined gave satisfactory recoveries except caffeine, OMC and Kahweol in green coffee and kahweol in robusta coffee (due to its very low content in this matrix). The analytical limits were found to be adequate for routine NMR measurements for the analytes. Importantly, the proton NMR spectroscopic method was found to be suitable for unambiguously coffee screening and authenticity testing. Additionally, the method may be adopted for routine quantitation of furfuryl alcohol in coffee in analytical laboratories. The applicability of the method was further proven during the recent OPSON VIII Europol-Interpol operation, in which more than 150 roasted coffee samples were analyzed using the validated NMR procedure within the 2-week operation period. In this sample, 3 cases of substantial admixture of robusta into coffee claimed as 100% arabica could be determined.

Author Contributions: Conceptualization, D.W.L.; methodology, A.S., J.T., T.K. and D.W.L.; formal analysis, J.T., T.R.D.R and AS.; resources, S.G.W. and D.W.L.; data curation, A.S., J.T., T.R.D.R, T.K.; writing—original draft preparation, A.O.O and D.W.L.; writing—review and editing, D.W.L. and A.O.; supervision, D.W.L. and S.G.W.

Funding: This research received no external funding.

Acknowledgments: Cornelia Ritter is thanked for excellent technical assistance.

Conflicts of Interest: The authors declare no conflict of interest.

 doi:10.20944/preprints201911.0390.v1

Annex

Table S1. Raw results of method validation for coffee using an factorial experimental design

Run	Factor 1 Spiked Concentration (mg/kg)	Factor 2 NMR type	Factor 3 Extraction time (min)	Factor 4 Coffee type	Response 1 Caffeine (mg/kg)	Response 2 OMC (mg/kg)	Response 3 Kahweol (mg/kg)	Response 4 Furfuryl alcohol (mg/kg)	Response 5 HMF (mg/kg)
1	7.5	Ascend	10	Decaf. Arabica	10.5	17	162.5	n.d.	7
2	37.5	Ascend	10	Decaf. Arabica	42.5	30	n.d.	13.5	35
3	75	Ascend	10	Decaf. Arabica	83.5	60	324.5	64.5	85
4	187.5	Ascend	10	Decaf. Arabica	203.5	190	n.d.	177.5	206
5	375	Ascend	10	Decaf. Arabica	400.5	372	n.d.	361.5	412
6	750	Ascend	10	Decaf. Arabica	786.5	767	260.5	720.5	813
7	1875	Ascend	10	Decaf. Arabica	1924.5	1855	1726.5	1788.5	1854
8	3750	Ascend	10	Decaf. Arabica	3827.5	3705	3103.5	3587.5	3691
9	5625	Ascend	10	Decaf. Arabica	5765.5	5594	5073.5	5405.5	5581
10	7500	Ascend	10	Decaf. Arabica	7517.5	7294	6419.5	7057.5	7282
11	7.5	Ascend	10	Canephora	n.d.	n.d.	n.d.	13	14.5
12	37.5	Ascend	10	Canephora	n.d.	n.d.	36.5	24	59.5
13	75	Ascend	10	Canephora	85.5	76	61.5	78	84.5
14	187.5	Ascend	10	Canephora	335.5	254	103.5	200	220.5
15	375	Ascend	10	Canephora	431.5	384	220.5	391	443.5
16	750	Ascend	10	Canephora	762.5	772	306.5	751	839.5
17	1875	Ascend	10	Canephora	1765.5	1809	1734.5	1836	1884.5
18	3750	Ascend	10	Canephora	3783.5	3691	3405.5	3667	3746.5
19	5625	Ascend	10	Canephora	5614.5	5544	5183.5	5446	5596.5
20	7500	Ascend	10	Canephora	7615.5	7437	6929.5	7302	7472.5
21	7.5	Ascend	20	Decaf. Arabica	7	10.5	255.5	14	12.5
22	37.5	Ascend	20	Decaf. Arabica	30	34.5	272.5	43	44.5
23	75	Ascend	20	Decaf. Arabica	73	65.5	250.5	76	82.5
24	187.5	Ascend	20	Decaf. Arabica	174	159.5	104.5	162	184.5
25	375	Ascend	20	Decaf. Arabica	378	359.5	332.5	359	401.5
26	750	Ascend	20	Decaf. Arabica	743	716.5	652.5	691	775.5
27	1875	Ascend	20	Decaf. Arabica	1852	1801.5	2002.5	1741	1790.5
28	3750	Ascend	20	Decaf. Arabica	3643	3561.5	3579.5	3450	3525.5
29	5625	Ascend	20	Decaf. Arabica	5471	5336.5	5244.5	5184	5301.5
30	7500	Ascend	20	Decaf. Arabica	7264	7088.5	6793.5	6869	7073.5
31	7.5	Ascend	20	Canephora	n.d.	n.d.	83	2.5	5
32	37.5	Ascend	20	Canephora	n.d.	n.d.	45	25.5	31
33	75	Ascend	20	Canephora	213.5	169	26	79.5	67
34	187.5	Ascend	20	Canephora	249.5	227	70	178.5	182
35	375	Ascend	20	Canephora	404.5	358	211	352.5	386
36	750	Ascend	20	Canephora	759.5	753	335	704.5	772
37	1875	Ascend	20	Canephora	1742.5	1749	1696	1723.5	1738
38	3750	Ascend	20	Canephora	3467.5	3477	3355	3451.5	3518
39	5625	Ascend	20	Canephora	5391.5	5320	5084	5200.5	5311
40	7500	Ascend	20	Canephora	6956.5	6969	6696	6896.5	7040
41	7.5	UltraShield	10	Decaf. Arabica	16.5	n.d.	270.5	5.5	3
42	37.5	UltraShield	10	Decaf. Arabica	49.5	22	n.d.	28.5	38
43	75	UltraShield	10	Decaf. Arabica	89.5	79	169.5	75.5	73
44	187.5	UltraShield	10	Decaf. Arabica	207.5	195	n.d.	181.5	198
45	375	UltraShield	10	Decaf. Arabica	429.5	400	n.d.	381.5	439
46	750	UltraShield	10	Decaf. Arabica	814.5	775	214.5	755.5	847
47	1875	UltraShield	10	Decaf. Arabica	2034.5	1935	1897.5	1892.5	1935

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48	3750	UltraShield	10	Decaf. Arabica	4048.5	3916	3509.5	3801.5	3908
49	5625	UltraShield	10	Decaf. Arabica	6040.5	5837	5357.5	5682.5	5836
50	7500	UltraShield	10	Decaf. Arabica	7713.5	7457	6550.5	7265.5	7445
51	7.5	UltraShield	10	Canephora	n.d.	n.d.	n.d.	7	10.5
52	37.5	UltraShield	10	Canephora	n.d.	n.d.	n.d.	28	46.5
53	75	UltraShield	10	Canephora	3	39	64.5	77	87.5
54	187.5	UltraShield	10	Canephora	334	251	77.5	209	217.5
55	375	UltraShield	10	Canephora	379	370	255.5	418	439.5
56	750	UltraShield	10	Canephora	798	802	370.5	788	882.5
57	1875	UltraShield	10	Canephora	1892	1921	1803.5	1961	2007
58	3750	UltraShield	10	Canephora	4037	3947	3690.5	3890	3983
59	5625	UltraShield	10	Canephora	5974	5872	5492.5	5788	5928
60	7500	UltraShield	10	Canephora	8185	7993	7384.5	7790	7977
61	7.5	UltraShield	20	Decaf. Arabica	18	n.d.	414	12.5	10
62	37.5	UltraShield	20	Decaf. Arabica	39	17.5	243	37.5	32
63	75	UltraShield	20	Decaf. Arabica	90	79.5	374	74.5	80
64	187.5	UltraShield	20	Decaf. Arabica	193	163.5	102	180.5	197
65	375	UltraShield	20	Decaf. Arabica	417	401.5	509	376.5	438
66	750	UltraShield	20	Decaf. Arabica	811	765.5	871	750.5	834
67	1875	UltraShield	20	Decaf. Arabica	1957	1887	2024	1833.5	1874
68	3750	UltraShield	20	Decaf. Arabica	3893	3768	3733	3670.5	3758
69	5625	UltraShield	20	Decaf. Arabica	5839	5664	5573	5493.5	5656
70	7500	UltraShield	20	Decaf. Arabica	7769	7521	7172	7336.5	7539
71	7.5	UltraShield	20	Canephora	n.d.	n.d.	13.5	21	n.d.
72	37.5	UltraShield	20	Canephora	n.d.	4	n.d.	27	33.5
73	75	UltraShield	20	Canephora	237	180	65.5	81	77.5
74	187.5	UltraShield	20	Canephora	281	262	123.5	203	208.5
75	375	UltraShield	20	Canephora	421	377	190.5	380	409.5
76	750	UltraShield	20	Canephora	857	809	394.5	748	848.5
77	1875	UltraShield	20	Canephora	1974	1949	1835.5	1902	1931
78	3750	UltraShield	20	Canephora	3868	3841	3700.5	3789	3896
79	5625	UltraShield	20	Canephora	6024	5901	5622.5	5734	5866
80	7500	UltraShield	20	Canephora	7695	7662	7384.5	7581	7766
81	7.5	Ascend	10	Green coffee	n.d.	n.d.	n.d.	n.d.	9
82	37.5	Ascend	10	Green coffee	41	46	87	24	48
83	75	Ascend	10	Green coffee	75	78	34	57	80
84	187.5	Ascend	10	Green coffee	199	183	83	167	205
85	375	Ascend	10	Green coffee	371	359	n.d.	369	414
86	750	Ascend	10	Green coffee	776	726	112	714	809
87	1875	Ascend	10	Green coffee	1946	1819	1384	1816	1873
88	3750	Ascend	10	Green coffee	3765	3517	2964	3533	3560
89	5625	Ascend	10	Green coffee	3782	3668	2004	3649	3773
90	7500	Ascend	10	Green coffee	5111	4966	3165	4875	5060
91	7.5	Ascend	20	Green coffee	45.5	n.d.	194.5	11	14
92	37.5	Ascend	20	Green coffee	57.5	29	27.5	33	44
93	75	Ascend	20	Green coffee	n.d.	n.d.	n.d.	46	43
94	187.5	Ascend	20	Green coffee	223.5	198	104.5	176	211
95	375	Ascend	20	Green coffee	411.5	363	222.5	367	425
96	750	Ascend	20	Green coffee	830.5	730	384.5	728	848
97	1875	Ascend	20	Green coffee	1956.5	1829	1344.5	1822	1896
98	3750	Ascend	20	Green coffee	3805.5	3613	3454.5	3527	3650
99	5625	Ascend	20	Green coffee	3858.5	3738	1813.5	3731	3852
100	7500	Ascend	20	Green coffee	7916.5	7596	6483.5	7429	7708
101	7.5	UltraShield	10	Green coffee	n.d.	n.d.	n.d.	n.d.	6.5

 doi:10.20944/preprints201911.0390.v1

102	37.5	UltraShield	10	Green coffee	n.d.	n.d.	1113.4	n.d.	n.d.
103	75	UltraShield	10	Green coffee	80	7.5	29	73.5	73.5
104	187.5	UltraShield	10	Green coffee	298.8	212.8	1433.3	191.5	197.3
105	375	UltraShield	10	Green coffee	n.d.	n.d.	899	n.d.	n.d.
106	750	UltraShield	10	Green coffee	809	723.5	148	740.5	855.5
107	1875	UltraShield	10	Green coffee	1996	1809.1	2977.2	1775.1	1781.5
108	3750	UltraShield	10	Green coffee	3035.7	2822.2	4075	2756.1	2825.1
109	5625	UltraShield	10	Green coffee	3217.6	3045.9	2941.5	3039.2	3112.8
110	7500	UltraShield	10	Green coffee	5369.3	5148.2	4154.7	5046.5	5221.8
111	7.5	UltraShield	20	Green coffee	31.5	n.d.	242.5	9.5	7.5
112	37.5	UltraShield	20	Green coffee	56.5	44	92.5	21.5	56.5
113	75	UltraShield	20	Green coffee	n.d.	1	n.d.	35.5	55.5
114	187.5	UltraShield	20	Green coffee	229.5	168	44.5	185.5	219.5
115	375	UltraShield	20	Green coffee	438.5	387	224.5	388.5	461.5
116	750	UltraShield	20	Green coffee	849.5	780	360.5	748.5	882.5
117	1875	UltraShield	20	Green coffee	2146.5	2003	1566.5	1992.5	2086
118	3750	UltraShield	20	Green coffee	4040.5	3825	3667.5	3734.5	3878
119	5625	UltraShield	20	Green coffee	4065.5	3955	1908.5	3924.5	4061
120	7500	UltraShield	20	Green coffee	8393.5	8102	6879.5	7840.5	8140

n.d., not detectable (response <0).

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