

ISO Standards for Tea

Intergovernmental Group- on Tea

10th Session

New Dehli, 12-14 May 2010

Andrew Scott, Chairman ISO TC/34 SC 8 Tea



Tea

Over 3 million tonnes produced

In over 30 countries

Providing over 1000 billion cups of tea

International Standards for Tea

International standards are vital to facilitate international trade

To ensure consumer expectations are met

To provide guidance and common understanding of Good Manufacturing practices via compositional specifications

To provide validated methods of analysis

International Standards for Tea

- 1970's - Tea Committee became ISO Working Group 8
 - ISO Technical Committee 34 Sub-committee 8 – Tea
- 1977 - ISO 3720 Black tea standard
 - 8 testing methods to measure basic tea parameters
- 1980 - ISO 1839 Sampling tea
- 1980 - ISO 3103 Preparation of liquor for sensory analysis
- 1982 - Glossary of terms
- 1990 - Instant tea standard
 - 4 supporting test methods

..... why measure polyphenols in tea?

ISO BLACK TEA SPECIFICATION - ISO 3720 1977 (First edition)

“INTRODUCTION

The content of **CAFFEINE** and the content of **POLYPHENOLIC** constituents are important chemical characteristics of black tea, but it has not been possible to include limits for either of them in the specification. In the case of **CAFFEINE**, agreement has not yet been reached on a standard method for the determination.

In the case of **POLYPHENOLIC CONSTITUENTS**, knowledge about test methods is not sufficiently developed to justify the standardization of any one of the methods in existence; moreover, information on the content of these constituents is only available for a few types of tea.”

Tea & International Standards

- 1995 - Measurement of caffeine in tea and instant tea
- 1995 - Measurement of caffeine in tea, instant tea and decaffeinated tea
- 2005 - Measurement of total polyphenols in tea
- 2005 - Measurement of catechins in green (and black) tea

Substances Characteristic of Tea

(15th Meeting ISO/TC 34/SC 8, 1992)

Objectives

Further development of methods for catechins and total polyphenols in green teas

Obtain catechin and total polyphenols data on a range of green teas representative of those in international trade

Examine the applicability of these methods for black and oolong teas to facilitate discrimination from green

Also

Catechin data is needed for the study of the health benefits of tea

Measuring Polyphenols

The methods should be:

- Capable to be used by all scientists in producer & consumer countries
- Produce reproducible results within a laboratory
- Produce reproducible results between laboratories
- A validated method of analysis

Measuring Total Polyphenols

Folin Denis reagent established as phenol reagent to quantify tyrosine in proteins, vanillin in vanilla and method of choice for estimating mono- and poly- phenolic molecules in plant foods.

Issues:

- phenols approximately 50% ionised at pH 9-10
- precipitate with increasing reagent or sodium carbonate concentration or both
- Beer Lambert Law not strictly followed
- saturated sodium carbonate: variation with temperature, equilibration not rapid
- Timing of adding reagents important
- Temperature and other factors affect the blue colour formation
- distilling at high altitude (Kenya tea estate) not equivalent to London / Colworth
- empirical method

First trial evaluated the Folin Denis reagent:

- variability due to making up the reagent

Measuring Total Polyphenols

Folin Ciocalteu reagent similar to Folin Denis reagent and is available commercially

Advantages FC over FD:

- no precipitation except in excess potassium
- more intense and more stable blue colour
- no difficulties in preparation – off the shelf.
- gives less colour in proportions to that of FD for possible interfering reductants [high content of active oxidant in FC promoting more complete oxidation and measurement of more slowly reacting phenolics?]

Measuring Total Polyphenols

Agreed to base test on a method published by Singleton & Rossi (1965) using the Folin-Ciocalteu reagent

Test was developed by a working group of 4 laboratories

Trials:

#1 2 black (Kenya & Argentina) and 1 green (China) teas

#2 2 black (Kenya & Argentina) and 1 green (China) teas

Measuring Total Polyphenols

First International ring trial - 20 participating laboratories:

- showed promising results
- identified that training was required for good precision
- improvements to protocol required
- add a training set of samples

Issues:

- sodium carbonate solution:
 - saturated (Folin Denis) to 20% (Singleton & Rossi 1965)
- high blanks
- mixing of reagents
- timings of additions
- colour development time (FC vs FD)
- age & batch of reagent differences?
- laboratory temperature
- extraction - volume variation / extraction recovery for catechins & flavonoids?

Measuring Total Polyphenols

Extraction:

Total Polyphenol & Catechin Analysis

- tea must be finely ground (< 0.5mm)
- small but representative sample of ground tea (0.2g)
- exhaustive double extraction with hot (70°C) 70% methanol give high yield and low catechin degradation
- extract samples are stable at 4°C for 24 after preparation

Note:

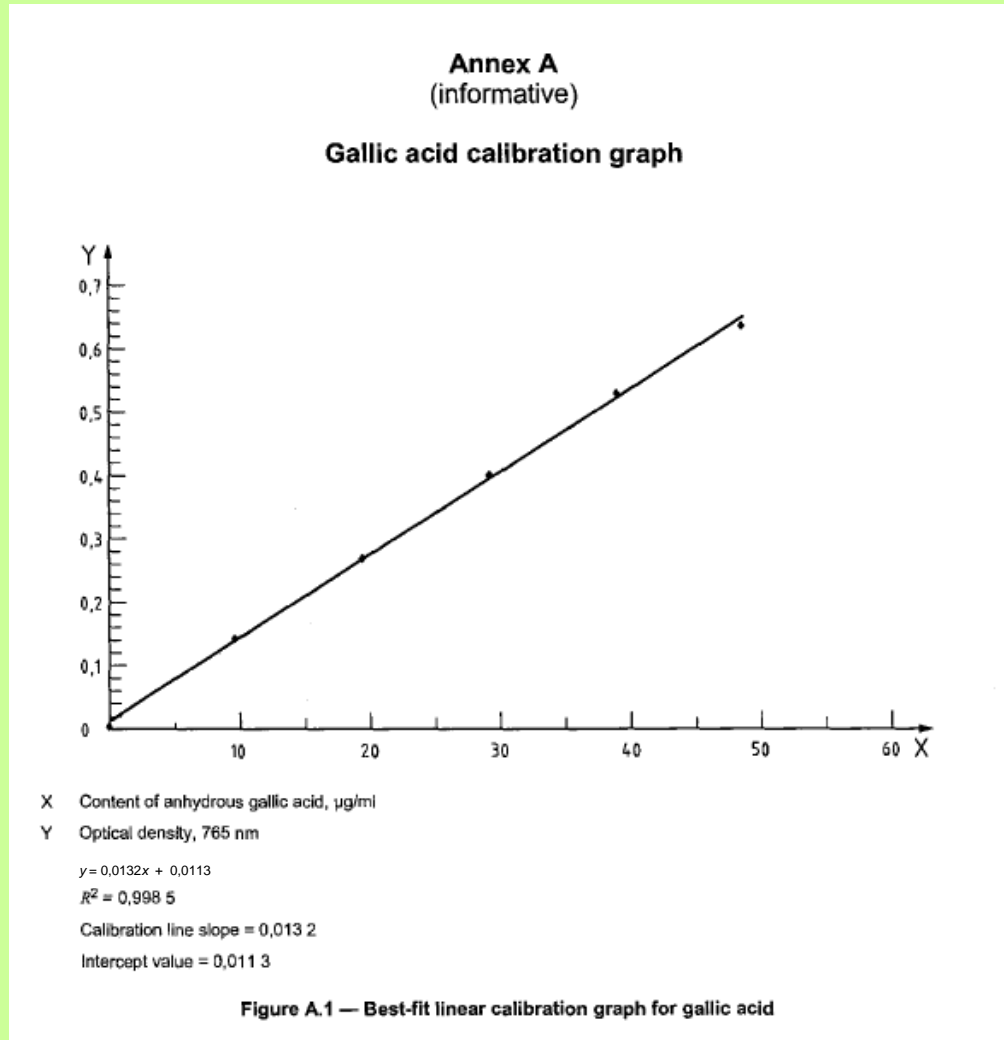
- disposable plastic tubes are easier and more reliable to use than re-useable glassware to eliminate trace contamination etc.

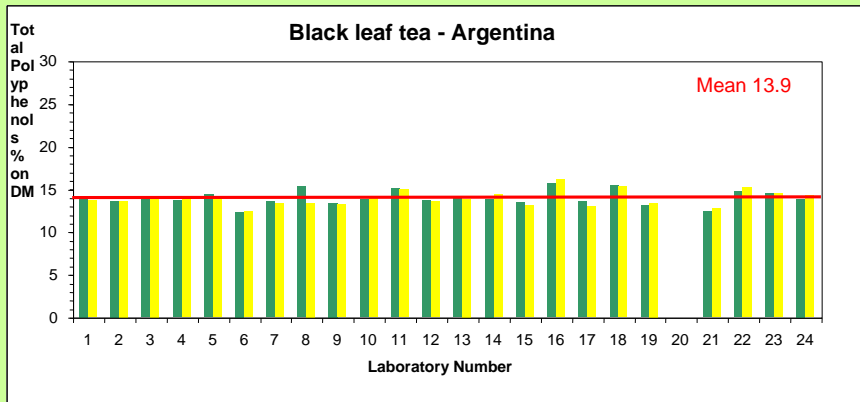
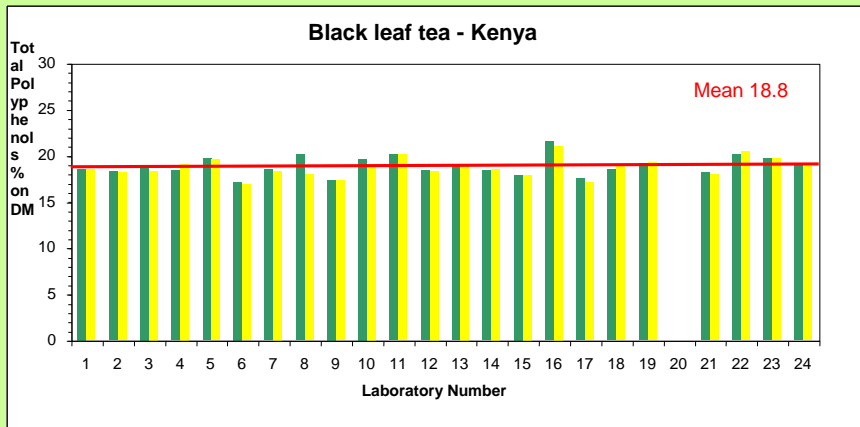
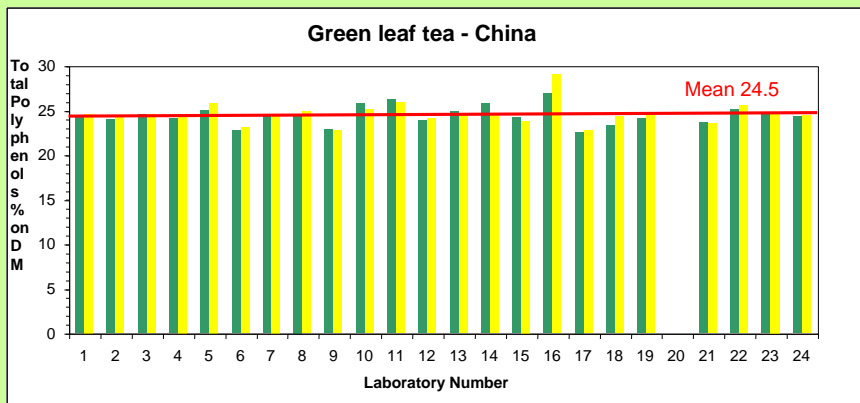
Measuring Total Polyphenols

Second International ring trial - 24 participating laboratories:

- within laboratory repeatability without exception improved
- between laboratory reproducibility values similar to 1st trial

Measuring Total Polyphenols





Measuring Total Polyphenols

Results of interlaboratory test

An interlaboratory test, carried out in 2001 under the auspices of the International Organization for Standardization, gave the statistical results (evaluated in accordance with ISO 5725-2) shown in Table B.1.

Table B.1 — Precision data

Sample identification	Green leaf tea	Black leaf tea	Black leaf tea
	A	B	C
Number of participating laboratories	23	23	23
Number of accepted test results	20	20	20
Mean total polyphenol content, % (mass fraction), on a dry matter basis	24,35	18,81	13,95
Repeatability standard deviation, s_r	0,332	0,218	0,214
Repeatability coefficient of variation, %	1,36	1,16	1,53
Repeatability limit, r ($= 2,8 s_r$)	0,93	0,61	0,60
Reproducibility standard deviation, s_R	1,129	1,186	1,029
Reproducibility coefficient of variation, %	4,64	6,31	7,38
Reproducibility limit, R ($= 2,8 s_R$)	3,16	3,32	2,88

Measuring Total Polyphenols

Total extraction of polyphenols:

ISO Black tea – 14.63% on dry matter basis (54 samples)
 Green tea – 17.29% on dry matter basis (102 samples)

University of Braunschweig study

Black teas:	UK	14.92%
	Intl A	14.61%
	Intl B	15.14%
	USA	15.29%

UK Tea Trade database:

557 mg/l polyphenols in a brewed cup of tea (71 samples)



Determination of substances characteristic of green and black tea —

Part 1: Content of total polyphenols in
tea — Colorimetric method using
Folin-Ciocalteu reagent

ICS 67.140.10

Measuring Catechins

The method should be:

- Capable to be used by all scientists in producer & consumer countries
- Fit for the purpose
- Produce reproducible results within a laboratory
- Produce reproducible results between laboratories

Measuring Catechins

Uses HPLC to separate the constituents and measures the UV absorbance of the individually separated catechins

Samples – Stage 1 instant teas – 1 green & 1 black

- Stage 2: 2 green teas [China sm lf & China bold lf]
2 black teas [Darjeeling Snowview & Lingia]

Remove all variation other than laboratory testing:

- the same columns used (Unilever prep)

[Phenomenex Luna® 5µm Phenyl-Hexyl 250x4.6mm with Phenomenex Security Guard® 4x3mm Phenyl-Hexyl guard]

- pure catechin standards used (Unilever)

- tea samples ground to powder and mixed to homogeneity

Measuring Catechins

Extraction - same as Total Polyphenol Analysis

HPLC conditions

- HPLC with Luna[®] Phenyl-Hexyl column improves resolution of catechins compared with C18
- UV wavelength detection (278 nm)
- Temperature control to 35°C to maintain resolution and prevent drifting peaks
- Solvent gradient 2% acetic acid - acetonitrile with EDTA; Acetonitrile 9-32% over 15mins
- Samples for analysis are diluted in stabilising solution containing EDTA and ascorbate in acetonitrile
- Oxidation (metal catalysed or otherwise) must be avoided for reproducible results in dilute solutions

Measuring Catechins

Test was developed by a working group of 4 laboratories

#1 International ring trial - 17 laboratories participated :

16 results were returned

Issues:

- adequate within laboratory - variability between laboratories
- recalculation of data using consensus relative response factors improved repeatability
- RRF's viable approach for quantitation of catechins
[pure catechins – not readily available, impure and very costly]
- variability most likely due to degradation during chromatography (metal contamination) -> include EDTA in mobile phase

Measuring Catechins

#2 International ring trial - 17 laboratories participated:

14 results were returned

Statistical methods: Cochran test (duplicates), Dixon test (between laboratories), Friedman test (consistent highs/lows)

Relative response factor relative to caffeine std (more convenient retention time than gallic acid)

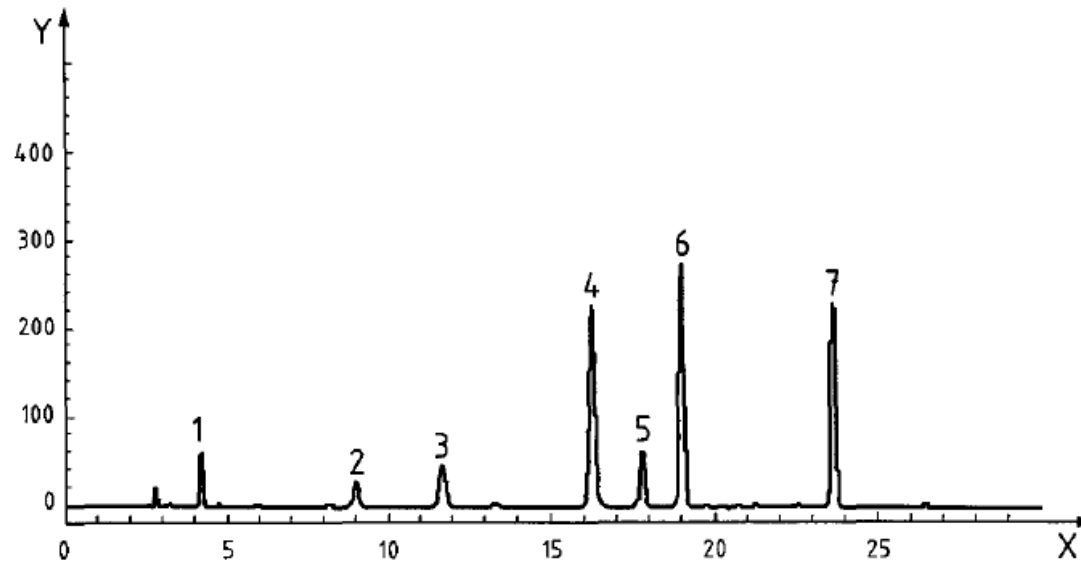
Standard purity assessments [HPLC, NMR, moisture]

Measuring Catechins

Comparison of statistical analysis results on recalculated RRF's vs Laboratory's calibrations:

- total catechin values lower as a consequence of using standard RRF's expressed on dry matter basis
- an improvement on repeatability values with RRF recalculated data set
- a marginal improvement in reproducibility in 3 out of the 4 teas with the RRF re-calculated data set.

Typical HPLC chromatograms



Key

X Retention time, min

Y Response, mAU

1 gallic acid

2 EGC

3 catechin

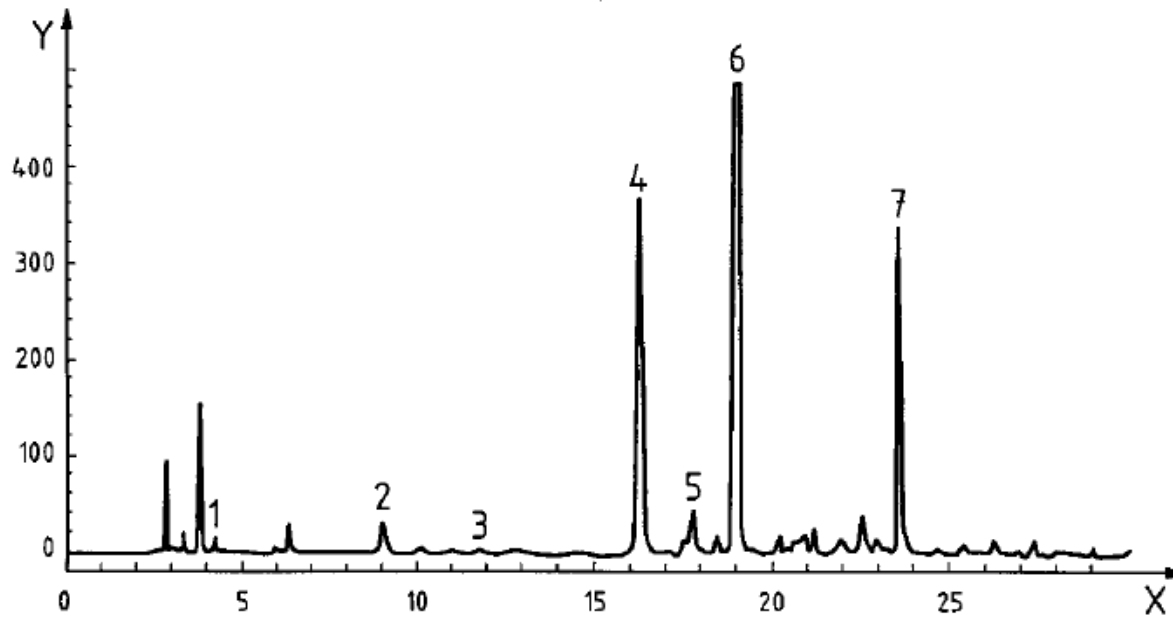
4 caffeine

5 epicatechin

6 EGCG

7 ECG

Figure C.1 — Mixed catechin standard B



Key

X Retention time, min

Y Response, mAU

1 gallic acid

2 EGC

3 catechin

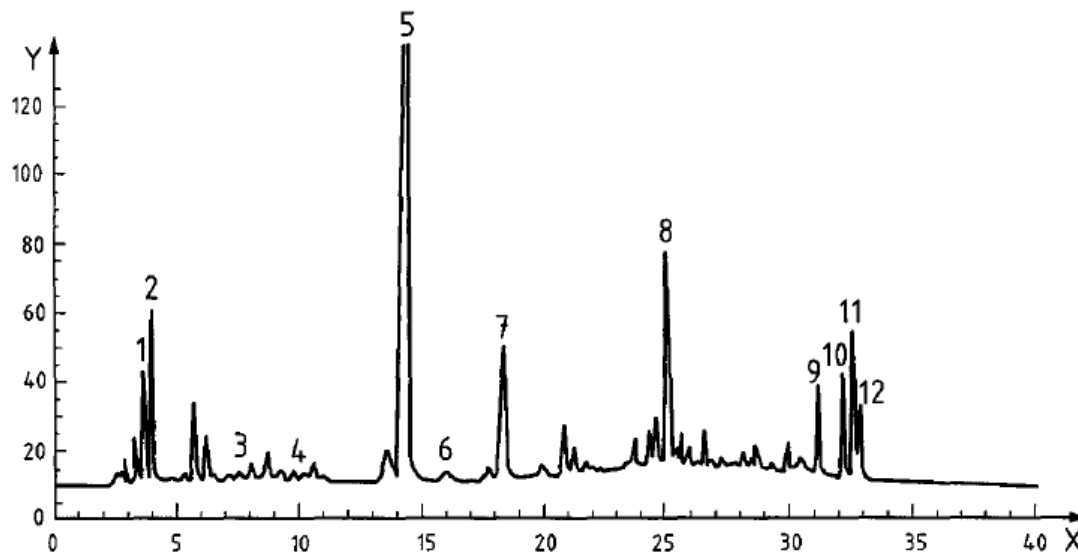
4 caffeine

5 epicatechin

6 EGCG

7 ECG

Figure C.2 — Green leaf tea extract



Key

X Retention time, min

Y Response, mAU

1 theogallin

2 gallic acid

3 EGC

4 catechin

5 caffeine

6 epicatechin

7 EGCG

8 ECG

9 TF

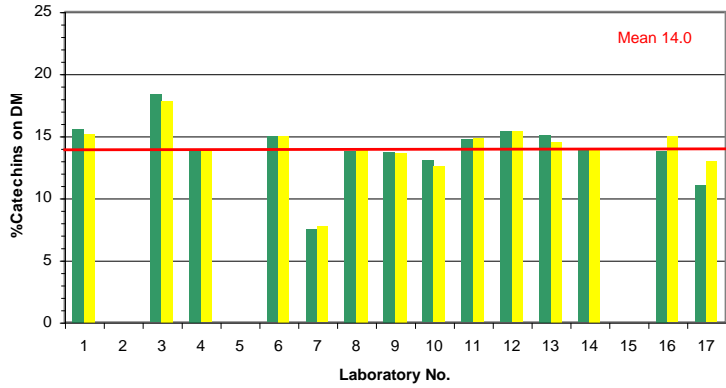
10 TFDG

11 TF-3-MG

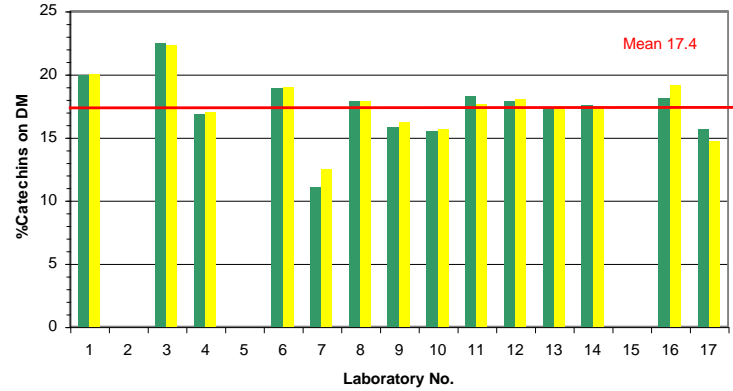
12 TF-3-MG

Figure C.4 — Black leaf tea extract

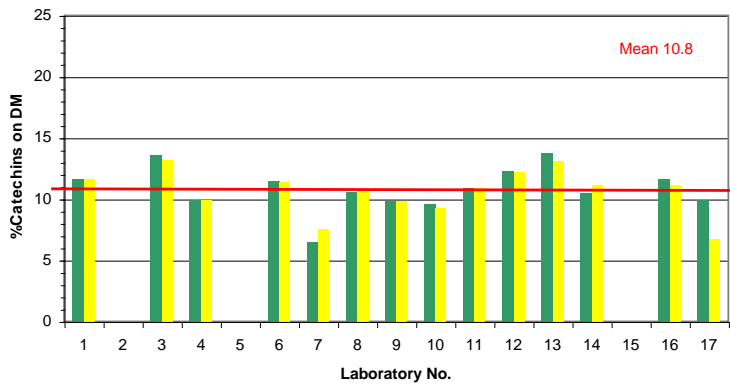
Green Leaf Tea - Sample 1



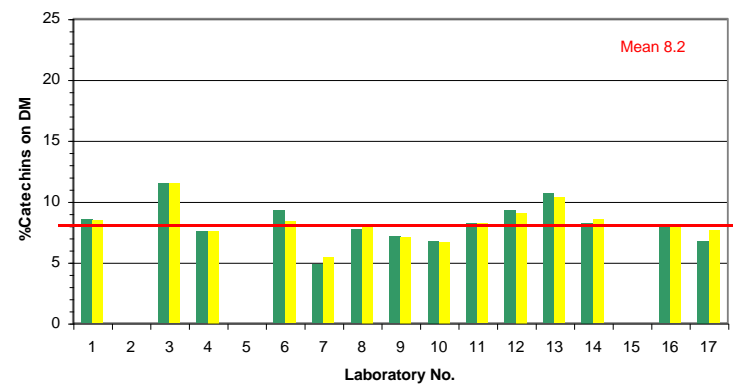
Green Leaf Tea - Sample 2



Black Leaf Tea - Sample 3



Black Leaf Tea - Sample 4



Measuring Catechins

9.3 Quantitation using a caffeine standard and catechin Relative Response Factors (RRFs)

9.3.1 The RRF values (measured with respect to caffeine) for gallic acid and the individual catechins obtained from the international interlaboratory test^[3] are given in Table 4. These consensus values, obtained on standards of known purity and expressed on an anhydrous standard basis, enable quantitation to be achieved with reference to the caffeine standard. Comparison of results obtained using either catechin standards or a caffeine standard with catechin RRFs is given in Annex E.

Table 4 — Consensus Relative Response Factors

Component	Relative Response Factor (RRF) with respect to caffeine (calculated on standard dry matter basis)
Gallic acid	0,84
(-)-Epigallocatechin (EGC)	11,24
(+)-Catechin (+C)	3,58
(-)-Epicatechin (EC)	3,67
(-)-Epigallocatechin gallate (EGCG)	1,72
(-)-Epicatechin gallate (ECG)	1,42

Measuring Catechins

Table A.1 — Precision data (no EDTA)

Sample Identification	Sample 1 Green leaf tea	Sample 2 Green leaf tea	Sample 3 Black leaf tea	Sample 4 Black leaf tea
Number of participating laboratories	14	14	14	14
Number of accepted test results	9	10	11	10
Mean total catechin content, % (mass fraction), on dry matter basis	12,30	15,70	9,53	7,19
Repeatability standard deviation, s_r	0,194	0,163	0,221	0,095
Repeatability coefficient of variation, %	1,58	1,04	2,32	1,32
Repeatability limit, r ($= 2,8 s_r$)	0,54	0,46	0,62	0,27
Reproducibility standard deviation, s_R	0,888	1,664	1,066	0,925
Reproducibility coefficient of variation, %	7,22	10,60	11,19	12,87
Reproducibility limit, R ($= 2,8 s_R$)	2,49	4,66	2,98	2,59

Measuring Catechins

Table A.2 — Precision data – EDTA included in mobile phase

Sample identification	Sample 1 Green leaf tea	Sample 2 Green leaf tea	Sample 3 Black leaf tea	Sample 4 Black leaf tea
Number of participating laboratories	11	11	11	11
Number of accepted test results	9	9	9	9
Mean total catechin content, % (mass fraction), on dry matter basis	12,14	14,78	8,93	6,81
Repeatability standard deviation, s_r	0,21	0,43	0,17	0,19
Repeatability coefficient of variation, %	1,75	2,93	1,87	2,75
Repeatability limit, $r (= 2,8 s_r)$	0,59	1,21	0,47	0,52
Reproducibility standard deviation, s_R	1,21	1,33	0,67	0,58
Reproducibility coefficient of variation, %	10,00	8,97	7,52	8,48
Reproducibility limit, $R (= 2,8 s_R)$	3,40	3,71	1,88	1,62
NOTE The black leaf teas, samples 3 and 4, are both lightly fermented Darjeeling, and therefore still have a reasonable catechin content. More extensively fermented black teas will have lower catechin contents, and would therefore be expected to have a negative effect on the precision data.				

Measuring Catechins

Total catechin content:

ISO	Black tea 1	–	8.93% on dry matter basis
	Black tea 2	–	6.81% on dry matter basis
	Green tea 1	–	12.14% on dry matter basis
	Green tea 2	–	14.78% on dry matter basis

Within laboratory repeatability of the analysis is good (9)

Between laboratory reproducibility has larger variation but this is to measure 5 constituents (9)

Measuring Catechins

Conclusions:

- method adequately repeatable within laboratory
- largest contribution to total variance comes from between laboratories [analysis of 7 constituents]
- recalculation of data using consensus RRF's improved the repeatability but only a marginal improvement on reproducibility
- variance from some laboratories - degradation of catechins - take precautions [EDTA already added to mobile phase]



202830

BRITISH STANDARD

BS ISO
14502-2:2005

Determination of substances characteristic of green and black tea —

**Part 2: Content of catechins in green
tea — Method using high-performance
liquid chromatography**

ICS 67.140.10

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Conclusions

Two Internationally validated methods analysis for measuring (i) total polyphenols and (ii) catechins in leaf or instant tea are now available.

These methods can be adapted to measure polyphenol & catechins in tea brews

Recommendation

Commend these robust methods of analysis for routine use in the measurement of polyphenols in tea.

Tea trade & academics in each country goes about collecting data on teas available in the trade to generate typical values of polyphenols in tea

Agree with regulatory authorities the appropriate use of typical polyphenol content in teas with consumers (marketing, on pack etc)

References

Singleton, V.L., Orthofer,R. and Lamuela-Raventos, R.M. *Analysis of total polyphenolics and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent.* In: *Methods in Enzymology. Oxidants and Antioxidants, Part A*,Lester Packer, ed. (1999), 299, pp. 152-178.

ISO/TC 34/SC 8 – Tea N513, *Report from the project leader of the ISO ring test on ISO/CD 14502-2 (Tea – Methods for determination of substances characteristic of green and black tea – Part 2: Determination of catechins in green tea – Method using high performance liquid chromatography).*

Current Projects

Publication of polyphenol data in green and black tea

Green tea specification

Black tea specification revision

White Tea specification

Theanine measurement in tea

Acknowledgements

Dr Peter Collier ex-Chairman ISO TC 34/SC 8 - Tea

Clive Dacombe - Unilever

Professor Ulrich Engelhardt – University of Braunschweig

Katie Donnelly – Tetley

Dr Andrew Lea – RSSL

Dr Conrad Astill - Unilever

Dr Jacek Obuchowicz

