

# ISO 18447:2021-06 (E)

## Tea - Determination of theaflavins in black tea - Method using high performance liquid chromatography

---

<b>Contents</b>	<b>Page</b>
Foreword.....	iv
<b>1 Scope</b> .....	<b>1</b>
<b>2 Normative references</b> .....	<b>1</b>
<b>3 Terms and definitions</b> .....	<b>1</b>
<b>4 Principle</b> .....	<b>1</b>
<b>5 Reagents</b> .....	<b>1</b>
5.1 General.....	1
5.2 HPLC mobile phases.....	2
5.3 Stock standard solutions.....	2
5.3.1 General.....	2
5.3.2 Preparation of individual theaflavin stock standard solutions.....	3
5.4 Mixed standard solutions.....	3
5.5 Caffeine standard — Preparation of caffeine stock solution, corresponding to 2,00 mg/ml.....	3
<b>6 Apparatus</b> .....	<b>4</b>
<b>7 Sampling</b> .....	<b>5</b>
<b>8 Preparation of test samples</b> .....	<b>5</b>
<b>9 Procedure</b> .....	<b>5</b>
9.1 General.....	5
9.2 Determination of dry matter content.....	5
9.3 Test portion.....	6
9.3.1 Instant tea.....	6
9.3.2 Leaf tea.....	6
9.4 Extraction of theaflavins.....	6
9.4.1 Instant tea.....	6
9.4.2 Leaf tea.....	6
9.5 Dilution.....	6
9.6 Determination.....	7
9.6.1 General.....	7
9.6.2 Adjustment of the apparatus.....	7
9.6.3 HPLC analysis.....	7
9.6.4 Identification.....	7
<b>10 Calculation</b> .....	<b>8</b>
10.1 General.....	8
10.2 Quantitation using theaflavin standards.....	8
10.3 Quantitation using a caffeine standard and theaflavin RRFs.....	9
<b>11 Precision</b> .....	<b>10</b>
11.1 Interlaboratory test.....	10
11.2 Repeatability.....	10
11.3 Reproducibility.....	10
<b>12 Test report</b> .....	<b>10</b>
<b>Annex A (informative) Results of interlaboratory tests</b> .....	<b>12</b>
<b>Annex B (informative) Assessment of the purity of standards used in the RRF work</b> .....	<b>13</b>
<b>Annex C (informative) Typical HPLC chromatograms</b> .....	<b>14</b>
<b>Bibliography</b> .....	<b>19</b>