An Overview of Official Methods of Analysis

Analytical methods used by enforcement laboratories for the implementation of legislation must be subject to validation procedures, in order to show that the method produces reliable results. These methods need to provide accurate and reproducible results both with and between laboratories within certain minimum performance criteria. This is especially important in view of legal actions and trade specifications, as well as monitoring and risk-assessment studies (Gilbert & Anklam, 2002).

Analytical methods used for dispute, control, inspection, and regulatory purposes are classified as type II and III, respectively, by the Codex Alimentarius Commission (2004).

According to Codex Alimentarius Commission the general criteria for the selection of a method of analysis are:

- Official methods of analysis elaborated by international organization with the characteristics determined in terms of (Codex, 2004; ISO, 1994):
    - *Accuracy*: the closeness of agreement between the reported result and the accepted reference value (certified reference materials);
    - *Applicability*: the analytes, matrices, and concentrations for which a method of analysis may be used satisfactorily to determine compliance with a CODEX standard;
    - *Detection/determination limits*: detection limit is defined as field blank + 3σ, where σ is the standard deviation of the field blank value signal and determination limit is defined as field blank + 6σ or 10σ (IUPAC, 1995; ISO/AOAC/IUPAC, 1995). The determination limit is strictly the lowest concentration of analyte that can be determined with an acceptable level of repeatability precision and trueness;
    - *Linearity*: the ability of a method of analysis, within a certain range, to provide an instrumental response or results proportional to the quality of analyte to be determined in the laboratory sample;
    - *Precision*: repeatability intra-laboratory (within laboratory), reproducibility inter-laboratory (within laboratory and between laboratories): the closeness of agreement between independent test results obtained under stipulated conditions. (*Repeatability*: closeness of agreement between the results of successive measurements of the same measure and carried out in the same conditions of measurement. *Reproducibility*: closeness of agreement between the results of successive measurements of the same measure and carried out in reproducibility conditions: same method on identical test items in different laboratories with different operators using different equipment);
    - *Recovery*: proportion of the amount of analyte present or added to the test material which is extracted and presented for measurement;
    - *Selectivity*: is the extent to which a method can determine particular analyte(s) in mixtures or matrices without interferences from other components;

- *Sensitivity*: change in the response divided by the corresponding change in the concentration of a standard (calibration) curve; i.e., the slope, s_1, of the analytical calibration curve.

Official methods should be those which have been validated in-house and submitted to an interlaboratory collaborative trial at both national and international levels, for which performance characteristics as repeatability (r), reproducibility (R) and Horrat ratio have been established (AOAC, 2002; Codex, 2004) for a matrix or a group of matrices of concern at levels close to the regulatory limits (Gilbert & Anklam, 2002) according to the IUPAC/AOAC/ISO International Harmonized Protocols for Collaborative studies (Thompson & Wood, 1993). Validated methods may subsequently be adopted as official international methods by Codex Alimentarius and AOAC International, or as National and International Standards. Methods that have been validated and adopted by AOAC, Codex, ISO are recognized as being official methods for the purpose of enforcement or international trade purposes.

When official methods are not available, or applicable, a single laboratory validated method may be used so long as it is validated according to an internationally recognized protocol referenced in the harmonized IUPAC Guidelines for Single laboratory Validation of Methods of Analysis; and the use of a method under ISO/IEC 17025:1999 Standard or Principles of Good Laboratory Practice (ISO, 1995).

Although a number of papers have been published on ochratoxin A (OTA) in green, roasted and soluble coffee, and used in survey and research works, three quantitative methods were formally validated through international collaborative trials in the last 40 years through AOAC International.

The AOAC 975.38 validated method dates back to 1975 (Levi, 1975; AOAC, 2000), and only recently two methods for both roasted (Entwisle *et al.*, 2001) and green coffee (Vargas, Santos & Pittet, 2005) have been thoroughly internationally and collaboratively validated using the International Harmonised Protocols (Thompson & Wood, 1993; Horwitz, 1988). Performance characteristics of the methods, in terms of applicability range, recovery rate and RSD_r , RSD_R, Horrat values are shown in Table 2.

The AOAC 975.38 method for the determination of OTA in green coffee (Levi, 1975; AOAC, 2000) is a TLC based method. It is not sufficiently sensitive (with a detection limit of 20 μg/kg), and does not comply with international guidelines practiced by some markets or trade specifications *(see: 'Overview of sampling of ochratoxin A in coffee' [.pdf], found in the Support Documentation area of this Section)*. In addition, it does not have associated performance data.

The European Union has adopted the method's performance criteria approach for mycotoxin analytical methods where recovery rate, RSD_r and RSD_R, per range of contamination (Table 1). The AOAC official method for roasted coffee (Entwisle *et al.*, 2001) has been adopted as a CEN method EN 14132:2003 'Foodstuffs - Determination of ochratoxin A in barley and roasted coffee'.

In line with the method performance criteria approach (CEN, 1999), the European Union has laid down provisions for the methods of analysis for official control of the levels of ochratoxin A in foodstuffs including coffee, meaning that European laboratories for enforcement, control and inspection shall select the validated method of choice as long as the method meets the performance characteristics for ochratoxin A. Recovery of the method must be reported at all times (EC, 2002; EC, 2005).

At the time of writing, no official screening method for ochratoxin A in coffee (be it ELISA, Fluormetry, Flow Lateral Device, or Chemioluminescence) has been reported on the AOAC website.

***Table 1**: Method's Performance Criteria for ochratoxin A Analytical Methods, adopted by CEN (CEN, 1999)*

Level (µg/kg)	Recovery (%)	RSD_r	RSD_R
< 1	50 to 120	40	60
1 – 10	70 to 110	20	30

When instrumentation such as High Performance Liquid Chromatography (HPLC) is not available, and official methods cannot be employed, Thin Layer Chromatography (TLC) associated with clean-up techniques (e.g. immunoaffinity column clean-up) is a robust technique providing good separation and low detection limits (Santos & Vargas, 2002).

The estimation of measurement uncertainty has been part of some European Union regulations. Analytical results, reported corrected or uncorrected by recovery, must be reported as X +/- U, where X is the analytical result and U is the expanded uncertainty (EC, 2005).

CODEX (Codex, 2003) recommends that the measurement uncertainty of an analytical result may be estimated in a number of procedures, notably those described by ISO GUM (ISO, 1995) and EURACHEM/CITAC (EURACHEM/CITAC, 2000). These documents recommend procedures based on a component-by-component approach, method validation data (reproducibility determined during validation of analytical methods), internal quality control data (treatment of the recovery data, when the method becomes a routine method of the laboratory) and proficiency test data. An estimation of the measurement uncertainty using the ISO component-by-component approach is not necessary if other forms of data are available and used to estimate the uncertainty. In many cases the overall uncertainty can be determined by an inter-laboratory (collaborative) study by a number of laboratories and a number of measurement uncertainty.

Table 2: *Official methods for ochratoxin A analysis in coffee*

					Method performance				
Year	**Matrix**	**Cleanup**	**Detection / Quantification**	**Confirmation**	**LD/LQ (μg/kg)**	**Recovery (%)**	**Relative standard deviation (%)**	**Range of applicability (μg/kg)**	**Reference**
1980	Green coffee	CC: Celite / bicarbonate	TLC visual	NH_3, $AlCL_3$, $NaHC0_3$,	20/	60-86	21-32	41-230	Levi 1975, AOAC 975.38, AOAC 2000
2001	Roasted coffee	Phenyl silane / IAC	HPLC	Not given	0.1/	85 (65-97)	RSR_r: 6 RSD_R:13 RSR_r: 2-27 RSD_R:14-71	3.5 0.1-5.4	Entwisle *et al.* 2001, AOAC 2000.09, CEN EN 14132:2003
2005	Green coffee	IAC	HPLC	Not given	0.1/	85 (65-97)	RSR_r: 7.42 RSD_R:16.34 RSR_r: 9-16 RSD_R:20.-29	4.48 2.60, 6.32, 12.89	Vargas *et al.* 2005, AOAC 2004.10

Key:

CC: Column Chromatography; HPLC: High Performance Liquid Chromatography; IAC: Immunoaffinity Column; TLC: Thin Layer Chromatography

References:

AOAC - Association of Official Analytical Chemist. 2000. *Natural Toxins. Official Methods of AOAC International*. Volume 2, Chapter 49, 1-64. 17th edition. (Edited by William Horwitz).

AOAC - Association of Official Analytical Chemist. 2002. *AOAC Official Methods of Analysis. Appendix G: Guidelines for Collaborative Study Procedures to validate characteristics of a method of analysis*. 12 p.

CEN - European Committee for Standardization. 1999. *CEN Report: Food Analysis. Biotoxins: Criteria of analytical methods of mycotoxins*. Brussels. CR 13505:1999 E. 8p.

CODEX - Codex Alimentarius Commission. 2003. Codex committee on methods of analysis and sampling. ALINORM 03/23: Proposed draft guidelines on measurement uncertainty, Appendix V and Proposed draft guidelines for evaluating acceptable methods of analysis, Appendix VII.

CODEX - Codex Alimentarius Commission. 2004. Joint FAO/WHO Food Standard Programme. *Procedural Manual*. 14th Edition. Rome.

EC - European Commission. 2002. Commission Regulation (EC) No. 472/2002 of 12 March 2002, Brussels, Belgium. *Official Journal of the European Communities L 75/18-20*

EC - European Commission. 2005. Commission Directive 2005/5/EC of 26 January 2005 amending Directive 2002/26/EC as regards sampling methods and methods of analysis for the official control of the levels of ochratoxin A in certain foodstuffs. *Official Journal of the European Union L27/38, published in 29 January 2005.*

Entwisle, A.C., Williams, A.C., Mann, P.J., Russell, J., Slack, P.T., Gilbert, J. 2001. Combined phenyl silane and immunoaffinity column clean-up HPLC for the determination of ochratoxin A in roasted coffee: Collaborative study. *Journal of AOAC International*, 84: 444-450.

EURACHEM/CITAC. 2000. *Guide: Quantifying Uncertainty in Analytical Measurement*. Second edition. (Editors: Ellison, S.L.R., Rosslein, M., Williams, A.).

Gilbert, J., Anklam, E. 2002. Validation of analytical methods for determining mycotoxins in foodstuffs. *Trends in Analytical Chemistry*, 21 (67): 468-486.

Horwitz, W. 1988. IUPAC - Protocol for the Design, Conduct and Interpretation of Collaborative Studies. *Pure & Appl. Chem.* 60 (6): 855-864.

ISO 5725. 1994. *Accuracy (trueness and precision) of measurement methods and results, parts 1-6*. International Organization for Standardization, Geneva.

ISO GUM. 1995. *Guide to the Expression of Uncertainty in Measurement (revised edition)*. International Organisation for Standardization: Geneva.

ISO / AOAC / IUPAC. 1995. *International harmonised guidelines for internal quality control in analytical chemistry laboratories*. (Edited by Thompson, M., & Wood, R.) *Pure Appl. Chem.,* 67: 649-666.

IUPAC - International Union of Pure and Applied Chemistry. 1995. IUPAC Harmonized Protocols for the adoption of standardised analytical methods and for the presentation of their performance characteristics. *Pure and Applied Chemistry*, 62: 149-162.

Levi, C.P. 1975. Collaborative study of a method for the determination of ochratoxin A in green coffee. *Journal of Association of Official Analytical Chemist*, 58 (2): 258-262.

Santos E.A., & Vargas E.A. 2002. Immunoaffinity column clean-up and thin layer chromatography for determination of ochratoxin A in green coffee. *Food Additives & Contaminants*, 19 (5): 447-456.

Thompson, M., & Wood, R. 1993. International protocol for proficiency testing of (chemical) analytical laboratories. *J. Assoc. Off. Anal. Chem*. 76 (4): 926-940.

Vargas, E.A., Santos, E.A., Pittet, A. 2005. Determination of ochratoxin A in green coffee by immunoaffinity column clean-up and HPLC. *Journal of AOAC International*