1 February 2022

CHECKLIST FOR PROTOCOL OF ANALYSIS (POA)
HEAVY METAL TESTING (As, Cd, Hg, Pb) IN TRADITIONAL PRODUCTS

NO.	INFORMATION/PARAMETER REQUIRED	AVAILABILITY
1	Specification of testing	
2	List of all apparatus & equipment used	
3	List of all chemicals & reagents used	
4	Sampling procedure	
5	Step-by-step preparation of standards/ solutions used	
6	Preparation of Calibration Curve & QC Check and their acceptance criteria of Calibration Curve & QC Check ($r^2 > 0.995$, etc)	
7	Detailed procedure for sample digestion Microwave digestor / Heating mantle / Trace metal digestor / Hot Block Digestor / Ashing / No digestion needed	
8	Detailed test procedure for heavy metal analysis Atomic Absorption Spectrometer (AAS) / Flow Injection Analysis System (FIAS) / Hydride Generation Atomic Absorption Spectrometer (HGAAS) / Inductive Coupled Plasma Emission Spectrometer (ICPOES) / Inductive Coupled Plasma Mass Spectrometer (ICPMS)	

1 February 2022

CHECKLIST FOR ANALYTICAL METHOD VALIDATION (AMV)
HEAVY METAL TEST (As, Cd, Hg, Pb) IN TRADITIONAL PRODUCTS

<table>
<tr><th>PARAMETER</th><th>NO.</th><th>INFORMATION/DOCUMENTS REQUIRED</th><th>AVAILABILITY</th></tr>
<tr><td rowspan="2">General</td><td>1</td><td>List of samples / matrix to be validated</td><td></td></tr>
<tr><td>2</td><td>Validation must be done for all dosage forms/ matrix applied</td><td></td></tr>
<tr><td rowspan="3">Linearity</td><td>1</td><td>Testing method</td><td></td></tr>
<tr><td>2</td><td>Linearity graph starts at LOQ concentration and shall cover the concentration of the specification. Minimum of 6 readings.</td><td></td></tr>
<tr><td>3</td><td>Acceptance criteria: $r^2 > 0.995$</td><td></td></tr>
<tr><td rowspan="2">Accuracy/ Trueness</td><td>1</td><td>☐ Comparison with certified reference material. Dosage form of CRM must be similar to sample tested. Minimum of 10 readings. % recovery shall be as table A.
OR
☐ Spiking with a known concentration of standard in the same sample matrices. Minimum of 10 readings. % recovery shall be as table A.
OR
☐ Comparison with a known standard method. Samples were spiked with standard and tested using laboratory method and standard method. Minimum of 10 readings. Both method shall have equal % recovery compare to table A.
<table><tr><th>Spiking concentration</th><th>% recovery</th></tr><tr><td>≤ 1ppb</td><td>50 – 120%</td></tr><tr><td>> 1 ppb to 10 ppb</td><td>70 – 110%</td></tr><tr><td>≥ 10 ppb</td><td>80 – 110%</td></tr></table>Table A. Acceptance criteria
Sources: Official Journal of the European Communities (2002/657/EC)</td><td></td></tr>
<tr><td>2</td><td>The concentration of spiked samples should cover NPRA concentration limit to prove that the method capable of detecting high concentration levels.</td><td></td></tr>
<tr><td>Precision</td><td>1</td><td>☐ 3 different samples containing heavy metal of interest having same dosage form were tested with the method. Minimum 10 duplicate readings by either two different analyst or two different calibration curves or two different days. Acceptance criteria shall be as Table B
OR
☐ 1 sample containing heavy metal of interest were spiked at three different concentrations were tested with the method. Minimum 10 duplicate readings by either two different analyst or two different calibration curves or two different days. Acceptance criteria shall be as Table B</td><td></td></tr>
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<tr><th>Analyte / Spiking concentration</th><th>Acceptable %RSD</th></tr>
<tr><td>≥ 100 ppm</td><td>5%</td></tr>
<tr><td>10 ppm</td><td>7%</td></tr>
<tr><td>1 ppm</td><td>11%</td></tr>
<tr><td>100 ppb</td><td>15%</td></tr>
<tr><td>10 ppb</td><td>21%</td></tr>
<tr><td>1 ppb</td><td>30%</td></tr>
<tr><td>0.1 ppb</td><td>43%</td></tr>
</table>
Table B. Acceptance criteria
Sources: AOAC 1989 Guidelines for collaborative study procedure to validate characteristics of a method of analysis
</td><td></td></tr>
<tr><td rowspan="2">LOD</td><td>1</td><td>☐ Base on signal to noise ratio. Minimum of 10 readings.
OR
☐ Base on linearity study. Linearity graph included. Minimum of 10 readings.</td><td></td></tr>
<tr><td>2</td><td>Acceptance criteria: %RSD < 10%</td><td></td></tr>
<tr><td rowspan="2">LOQ</td><td>1</td><td>☐ Base on signal to noise ratio. Predetermined LOQ were confirmed. Minimum of 10 readings.
OR
☐ Base on linearity study. Linearity graph included. Predetermined LOQ were confirmed. Minimum of 10 readings.</td><td></td></tr>
<tr><td>2</td><td>☐ Acceptance criteria: %RSD < 10%</td><td></td></tr>
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