Update of Qualitative Analysis Considerations in GC-MS(/MS)

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Definitions

- ***Indication*** = result of a screening method (*i.e.* "presumed" positive or negative)

- ***Determination*** = result from an analytical quantitative method (*e.g.* GC/PFPD, LC/UV)

- ***Identification*** = qualitative result from a highly selective method (*e.g.* GC-MS, LC-MS^n)

- ***Confirmation*** = result from 2 or more independent analyses in agreement (ideally, one of which uses a different chemical mechanism or approach)

Old School Approach

0) Screening: Field testing (*e.g.*, bioassay or immunoassay) – narrow scope

1) Quantification: Official determinative method (*e.g.* GC-NPD/ECD) – inefficient

2) Identification/Confirmation: Qualitative analysis using GC-/MS(-MS) – wasteful

New School Approach

A) Screening/Identification: Rapid testing by MS-based method – broad scope; (non?)targeted identification?

B) Quantification/Confirmation: Official determinative method (GC-MS/MS?) – targeted positives or elucidative approach(es)

- Rely on confirmation to eliminate false positives, but presumptive positives must be within reason

Factors to Consider in Identification

- Chromatographic t_R and peak shape
- Adequate S/N ratio
- Multiple detector and elemental information
- Characterization of blanks and carry-over
- Presence of molecular ion
- Isotope pattern and nitrogen rule
- Comparison with reference standard
- MS fragmentation pattern makes sense
- Result makes analytical sense

USDA-FSIS GC-MS/MS Identification Criteria

1. Retention time (t_R) is within ± 0.1 min of average t_R and peak shape matches that of reference std
2. t_R and peak shape of qualifier ion(s) matches those of the quantification ion
3. 2 qualifier ions ≤|20%| or 1 qualifier ion ≤|10%| of avg. ion ratio from contemporaneous reference stds
4. Absence of positive findings in known blanks
5. Signal > ½ "tolerance" calibration standards in matrix
6. Rate of false positives ≤5% (and false negatives <10%)
7. The ion transitions used make structural sense

Ion Ratio Criteria in 2002/657/EC (EU)

Rel. Abundance *vs.* Base Peak	Acceptable Diff. vs. Ref. API-MS
>50%	±20% RSD
>20-50%	±25% RSD
>10-20%	±30% RSD
≤10%	±50% RSD

Ref. Ratio	EU Range*	FSIS (1 ion)	(2 ions)
70%	56% – 84%	60% – 80%	50% – 90%
24%	18% – 30%	14% – 34%	4% – 44%
12%	8.4% – 15.6%	3% – 23%	>0% – 33%
4%	2% – 6%	>0% – 14%	>0% – 24%

* 2 ion transitions needed to achieve 3 ident. points in MS/MS

Guidelines in SANCO/12571/2013

Rel. Abundance *vs.* Base Peak	Acceptable Diff. vs. Ref. EI-MS (≥3* ions)	MS/MS (≥2 ions)
>50%	±10% RSD	±30% RSD
>20-50%	±15% RSD	±30% RSD
>10-20%	±20% RSD	±30% RSD
≤10%	±50% RSD	±30% RSD

Ref. Ratio	EI-MS Range*	MS/MS
70%	63 – 77%	49 – 91%
24%	20.4 – 27.6%	16.8 – 31.2%
12%	9.6 – 14.4%	8.4 – 15.6%
4%	2 – 6%	2.8 – 6.2%

* ≥2 ions in high resolution MS with mass accuracy ≤5 ppm

Bottom Line

There are many complicated opinions of "good enough" criteria to meet MS-based identification standards

But they are all based on generalizations, not scientific assessments at all actual conditions

The bottom line is rates of false pos/neg

If analytical conditions shown to meet <5% false results in extensive validation (multi-matrix, multi-level, blind), then it should be acceptable

Rely on Orthogonal Confirmation Methods