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USP ELEMENTAL IMPURITIES: LIMIT TEST FOR METALS IN NICOTINE BY ICP-MS

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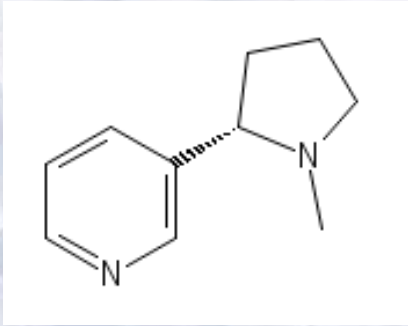
Outline

- Background on USP Nicotine
  - USP Nicotine Monograph
- Method Development
  - Sample preparation
  - Isotopes and internal standards
  - Spectral interferences
- Method Validation
  - Linearity
  - Recoveries
  - Selectivity
  - Drift values



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USP Nicotine

- Designed for pharmaceutical products.
- Limited guidance and regulation for producing e-liquid.
- The USP monograph is considered the gold standard.
- Majority of manufacturers adhere to this testing to ensure quality and consistency of product.
- Good product stewardship.

USP Nicotine Monograph

- Consists of several tests
  - Identification (UV absorption and FTIR)
  - Assay (acid/base titration)
  - Specific Rotation (Polarimetry)
  - Water Determination (Karl Fischer titration)
  - Organic Impurities (HPLC with UV)
  - **Elemental Impurities or Metals (ICP-MS)**
  - Residual Solvents (headspace GC-FID) (not a USP nicotine test)

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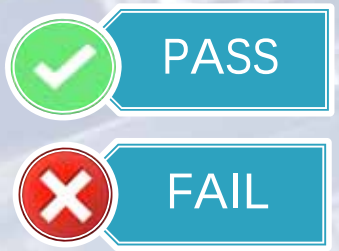
Limit Tests

- Tests that are being used to identify / control impurities
  - Quantitative or semi quantitative tests designed to identify and control small quantities or impurities which are likely to be present in the substances.

- Pass or Fail tests
  - The impurity level in sample solution should not be greater than standard solution.
  - Example: the concentration of lead in USP grade nicotine must be 0.5 µg/g or lower.



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J values – Maximum Concentration

- Metals are placed into three classes, based on toxicity and likelihood of occurrence in product
- Metal toxicity is related to the extent of exposure (three routes of administration: oral, parenteral, inhalational)
- Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance

Permitted inhalation concentrations (J) of elemental impurities

Element	Symbol	Class	[J] Inhalation Conc. (µg/g)
Cadmium	Cd	1	0.2
Lead	Pb	1	0.5
Arsenic	As	1	0.2
Mercury	Hg	1	0.1
Cobalt	Co	2A	0.3
Vanadium	V	2A	0.1
Nickel	Ni	2A	0.5
Lithium	Li	3	2.5
Antimony	Sb	3	2
Barium	Ba	3	30
Molybdenum	Mo	3	1
Copper	Cu	3	3
Tin	Sn	3	6
Chromium	Cr	3	0.3

USP <232> ELEMENTAL IMPURITIES—LIMITS

USP <233> ELEMENTAL IMPURITIES—PROCEDURE

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Method Development

- Sample Preparation
  - Matrix matched standards
  - Dilution in nitric acid
- Instrumental Analysis (ICP-MS)
  - Selection of analyte isotopes
  - Elimination of interferences
    - Kinetic Energy Discrimination (KED)
    - Dynamic Reaction Cell (DRC)
- Data Interpretation and Report Generation (µg/g)

Sample Preparation

Instrumental Analysis

Report

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Sample Preparation

- Sample Preparation
  - Matrix matched standards
  - Avoid contamination (polypropylene containers)
  - No microwave digestion required
  - Samples are diluted in nitric acid and water.

- Advantages
  - More samples can be analyzed in a limited time
  - Improves precision especially for volatile analytes (like Hg) and abundant contaminants (like Cu)
- For Hg only
  - Gold is used as a stabilizer in standards
  - Gold added to samples to match the standard according to USP requirement

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Selection of analyte isotopes

- Choose the most abundant isotope with least interferences
- Some isotopes of different elements overlap
- Primary isotope is used for quantification and secondary is used for confirmation
- Some elements are monoisotopic
- For lead, sum of all isotopes is used

Element	No. Isotopes	Primary	Secondary
Vanadium	2	51	50
Chromium	4	52	53
Cobalt	1	59	-
Nickel	5	60	62
Copper	2	63	65
Lithium	2	7	6
Arsenic	1	75	-
Tin	10	117	118
Cadmium	8	112	111
Antimony	3	121	123
Barium	6	137	135
Molybdenum	7	94	96
Mercury	7	200	202
Lead	4	206/207/208	NA

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Selection of Internal Standards (IS)

- IS corrects for the loss of analyte during sample preparation or instrument drift.
- IS should not be present in the sample
- IS should have similar characteristics to analyte (close mass)
- IS should be added to all standards and samples

Element	Mass	Sc 45	Ge 74	Y 89	In 115	Bi 209
Lithium	7	×				
Vanadium	51	×				
Chromium	52	×				
Cobalt	59	×				
Nickel	60	×				
Copper	63		×			
Arsenic	75		×			
Molybdenum	94			×		
Tin	117			×		
Cadmium	112				×	
Antimony	121				×	
Barium	137				×	
Mercury	200					×
Lead	207					×

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ICP–MS Interferences

Polyatomic Ions

- By-product of plasma reaction
- Maximum effect on period 4 elements

- Arsenic (As)
- Cobalt (Co)
- Vanadium(V)
- Nickel (Ni)
- Copper (Cu)
- Chromium (Cr)
- Lithium (Li)
- Tin (Sn)
- Lead (Pb)
- Cadmium (Cd)
- Mercury (Hg)
- Antimony (Sb)
- Barium (Ba)
- Molybdenum (Mo)

Analyte Isotope	Interfering ion
$^{51}V^{+}$	$^{35}Cl^{16}O^{+}$
$^{52}Cr^{+}$	$^{36}Ar^{16}O^{+}$, $^{40}Ar^{12}C^{+}$
$^{59}Co^{+}$	$^{40}Ar^{18}OH^{+}$
$^{60}Ni^{+}$	$^{44}Ca^{16}O^{+}$
$^{63}Cu^{+}$	$^{40}Ar^{23}Na^{+}$
$^{75}As^{+}$	$^{40}Ar^{35}Cl^{+}$
$^{56}Fe^{+}$	$^{40}Ar^{16}O^{+}$
$^{66}Zn^{+}$	$^{34}S^{16}O_2^{+}$
$^{78}Se^{+}$	$^{40}Ar^{38}Ar^{+}$
$^{72}Ge^{+}$	$^{40}Ar^{16}O_2^{+}$

Matrix interferences

- Origination from the matrix of sample

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Kinetic Energy Discrimination (KED)

- An inert gas is used in a collision cell
- Both analytes and polyatomic interferences collide with He
- Polyatomic ions have larger collision cross section, they undergo more collisions and lose more kinetic energy
- By setting a voltage barrier between the collision cell and the quadrupole, interferences are removed

- Arsenic (As)
- Cobalt (Co)
- Vanadium(V)
- Nickel (Ni)
- Copper (Cu)
- Chromium (Cr)
- Lithium (Li)
- Tin (Sn)
- Lead (Pb)
- Cadmium (Cd)
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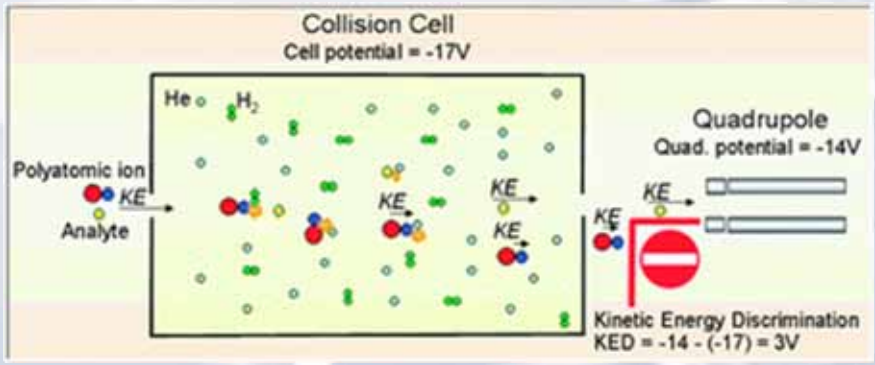
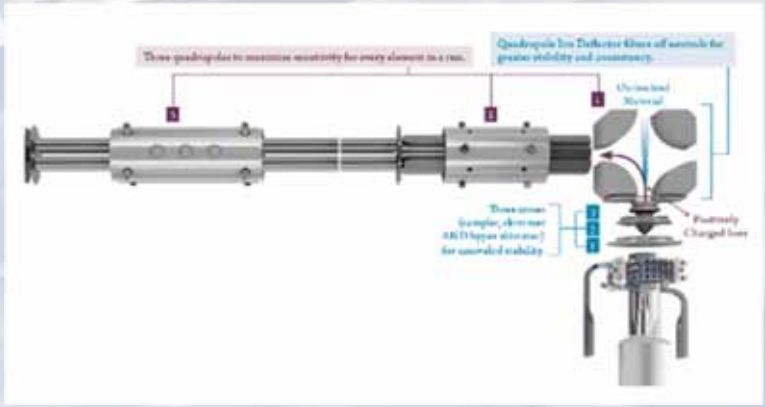
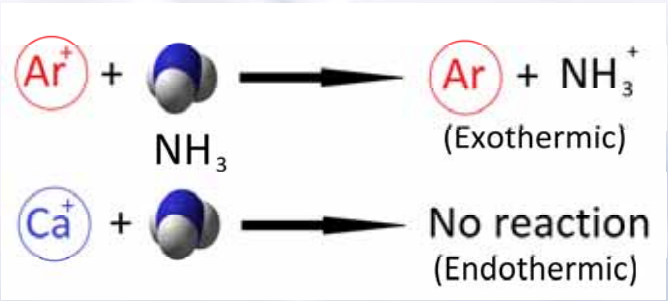


J. Anal. At. Spectrom., 2009,24, 1406



Dynamic Reaction Cell (DRC)

- A highly reactive gas is used in the cell
- Usually the interference will react with DRC gas and the interference is removed



- Arsenic (As)
- Cobalt (Co)
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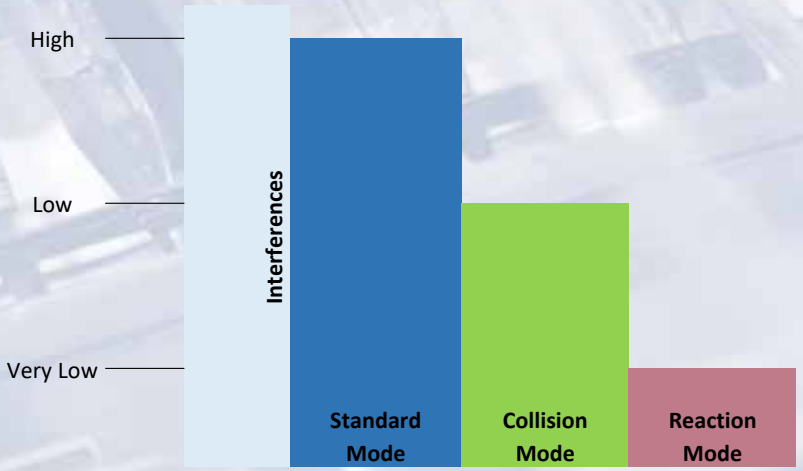

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Method Validation

- The described was validated, as described by the International Conference of Harmonization (ICH) and the United States Food and Drug Administration (FDA) and United States Pharmacopeia (USP) guidelines:
  - Linearity
  - Selectivity
  - Accuracy
  - Precision – Method/Instrument
  - Intermediate Precision
  - Range
  - Robustness (Drift and standard stability)
  - Limit of Detection (LOD) / Limit of Quantitation (LOQ)

Linearity, Range, Drift, LOQ

- Calibration curve
  - Include 50% J, 100% J and 150% J for all elements
  - 50% J and 150% J standards are used as check standards
  - Separate set of standards used for Hg containing gold as stabilizer

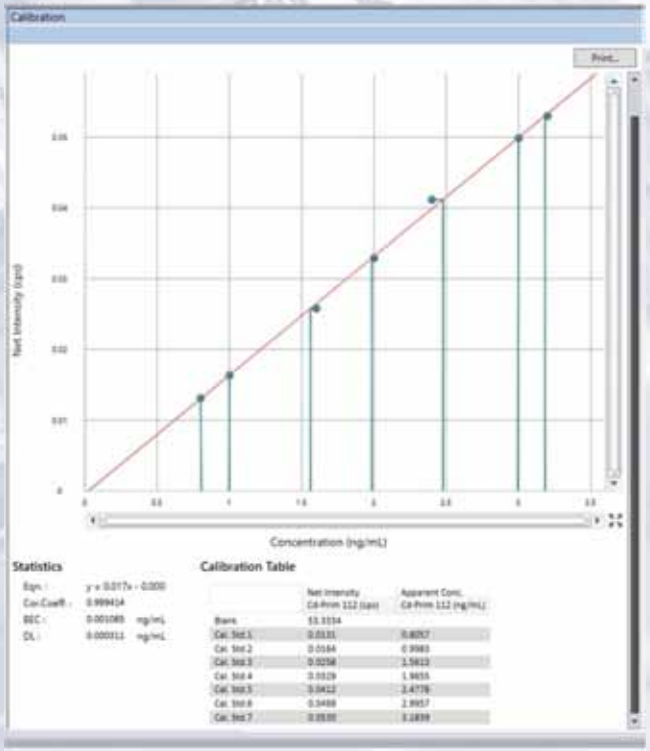
Standard	1	2	3	4	5	6	7
Metal	Concentration (ng/mL)						
Cd	0.8	1	1.6	2	2.4	3	3.2
Pb	2	2.5	4	5	6	7.5	8
As	0.8	1	1.6	2	2.4	3	3.2
Hg	0.4	0.5	0.8	1	1.2	1.5	1.6
Co	1.2	1.5	2.4	3	3.6	4.5	4.8
V	0.4	0.5	0.8	1	1.2	1.5	1.6
Ni	2	2.5	4	5	6	7.5	8
Li	10	12.5	20	25	30	37.5	40
Sb	8	10	16	20	24	30	32
Ba	120	150	240	300	360	450	480
Mo	4	5	8	10	12	15	16
Cu	12	15	24	30	36	45	48
Sn	24	30	48	60	72	90	96
Cr	1.2	1.5	2.4	3	3.6	4.5	4.8
Hg	0.4	0.5	0.8	1	1.2	1.5	1.6
J Value *:	-	50%	-	100%	-	150%	-

Linearity, Range, Drift, LOQ

- Curve Acceptance Criteria
  - Each calibration curve consists of six standards
  - Regression $\geq$ 0.995
  - Residuals within 15% of theoretical values
  - QC within $\pm$ 15% of theoretical value

- Drift
  - The drift for Std 2 within 20% for each target element compared to injection of matrix blank

Cd Calibration Curve



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Accuracy

- Samples were spiked at three concentration levels for each metal across the calibration curve (50% J, 100% J and 150% J) and recoveries were calculated
- Each recovery at each level for all elements must be 70-150% (USP requirement)
- Recovery were within 100 ± 20%
- The accuracy experiment was repeated on two (2) additional days.

Cd Recoveries (Day1)

	Blank	50%J Recovery		100% J Recovery		150% J Recovery	
Replicate	Conc. (ng/mL)	Conc. (ng/mL)	% Rec	Conc. (ng/mL)	% Rec	Conc. (ng/mL)	% Rec
Day 1 - Analyst I							
1	0.011	0.919	90.9	1.891	94.0	2.813	93.4
2	-	0.936	92.6	1.877	93.3	2.743	91.1
3	-	0.939	92.9	1.861	92.5	2.800	93.0
4	-	0.932	92.1	1.886	93.8	2.813	93.4
5	-	0.977	96.6	1.830	91.0	2.732	90.7
6	-	-	-	1.917	95.3	-	-
Average	**0.011**	**0.941**	**93.0**	**1.877**	**93.3**	**2.780**	**92.3**
SD	**-**	**0.022**		**0.029**		**0.039**	
RSD	**-**	**2.3**		**1.6**		**1.4**	

Selectivity (System Suitability)

- Samples varied by source (supply origin, lot, etc.)
- Samples were spiked at 100% J value for each metal, and recoveries were calculated
- Each recovery was within 100 + 20%

Class 1 Selectivity Recoveries

	Lead		Arsenic		Mercury		Cadmium	
Replicate	Conc. (ng/mL)	Rec (%)	Conc. (ng/mL)	Rec (%)	Conc. (ng/mL)	Rec (%)	Conc. (ng/mL)	Rec (%)
Nicotine sample #1								
Blank	ND	-	0.247	-	0.107	-	0.011	
1	4.510	90.2	2.359	105.6	1.091	98.4	1.789	88.9
2	4.459	89.2	2.248	100.0	1.082	97.4	1.883	93.6
3	4.498	90.0	2.256	100.5	1.091	98.3	1.821	90.5
4	4.408	88.2	2.244	99.8	1.133	102.5	1.731	86.0
5	4.482	89.6	2.383	106.8	1.082	97.5	1.794	89.2
Average	**4.471**	**89.4**	**2.298**	**102.5**	**1.096**	**98.8**	**1.804**	**89.6**
SD	**0.040**		**0.067**		**0.021**		**0.055**	
RSD	**0.9**		**2.9**		**1.9**		**3.1**	

Summary

- A method was developed and validated for detection of heavy metals in pure nicotine products
- KED and DRC were employed to remove interferences
- The method has high selectivity and accuracy

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Thank you for your time and attention.

For further information

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