

Development and Validation of an ICP-MS Method for Simultaneous Determination of Selected Metals in Electronic Cigarette Aerosol*

by

Shintaro Ohashi

Product Quality Research Center, Japan Tobacco Incorporated, 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa, Japan

SUMMARY

Safety and quality standards for electronic cigarettes (e-cigarettes) have been introduced regionally. In 2016, the U.S. Food and Drug Administration (FDA) issued a rule to regulate e-cigarettes, requiring to report harmful and potentially harmful constituents (PHHCs). In the United Kingdom, the British Standards Institution (BSI) specified the metals to be monitored for e-cigarettes. In this study, a method was developed and validated for the simultaneous determination of 13 metals (Be, Al, Cr, Fe, Co, Ni, Cu, As, Se, Ag, Cd, Sn and Pb) in e-cigarette aerosol. Furthermore, matrix effects of major constituents in the aerosol were investigated using glycerol or 1,2-propylene glycol solutions. E-cigarette aerosol was generated by a rotary smoking machine according to CORESTA Recommended Method N° 81 and collected by an electrostatic precipitator coupled to an impinger containing nitric acid. The collected aerosol was dissolved in nitric acid and an aliquot of this solution was analyzed by inductively coupled plasma mass spectrometry (ICP-MS) equipped with a collision/reaction cell. The linearity of the calibration curve was observed in the range of 0.2 to 100 ng/mL for each analyte; the correlation coefficients were 0.998 or larger, the mean recovery of each standard level ranged from 92.6 to 104.5% and the relative standard deviation amounted to max. 9.5%. Accuracy, repeatability and specificity were validated by spiking three different amounts of analytes into e-cigarette aerosol; the mean recovery of each spiking level ranged from 88.7 to 110.3% with a relative standard deviation amounting to max. 9.2% for all analytes. Background contamination from aerosol generation and collection system existed for some analytes, especially for Al, Fe, Cu and Sn. The potential sources of contamination should be identified and

controlled to reduce the impact of contamination on quantification. In addition, the actual values for samples should be reported with method blank statistics. Increase of the concentrations of glycerol and 1,2-propylene glycol in the prepared sample led to the overestimation of As and Se. The amount of polyols in the collected aerosol should be monitored and controlled for the accurate quantification of As and Se. [Beitr. Tabakforsch. Int. 28 (2018) 2–13]

ZUSAMMENFASSUNG

Regional wurden Sicherheits- und Qualitätsstandards für elektronische Zigaretten (E-Zigaretten) eingeführt. Im Jahr 2016 gab die US-amerikanische Food and Drug Administration (FDA) eine Vorschrift zur Regulierung von E-Zigaretten heraus, mit der eine Meldung schädlicher und potenziell schädlicher Inhaltsstoffe (PHHC) verlangt wird. In Großbritannien legte die British Standards Institution (BSI) die Metalle fest, die bei E-Zigaretten zu überwachen sind. In der vorliegenden Studie wurde ein Verfahren für die gleichzeitige Bestimmung von 13 Metallen (Be, Al, Cr, Fe, Co, Ni, Cu, As, Se, Ag, Cd, Sn und Pb) im E-Zigaretten-Aerosol entwickelt und validiert. Außerdem wurden Matrixeffekte von Hauptbestandteilen im Aerosol mittels Glycerin- oder 1,2-Propylenglykol-Lösung untersucht. Das E-Zigaretten-Aerosol wurde durch eine rotierende Rauchmaschine nach der von der CORESTA empfohlenen Methode Nr. 81 erzeugt und mit einem elektrostatischen Abscheider, der an eine Waschflasche (Impinger) mit Salpetersäure gekoppelt ist, aufgefangen. Das aufgefangene Aerosol wurde in Salpetersäure aufgelöst und ein Teil dieser Lösung wurde mittels Massenspektrometrie mit induktiv gekoppeltem Plasma (ICP-MS) mit einer

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Kollisions-/Reaktionszelle analysiert.

Die Linearität der Kalibrierkurve wurde für jeden Analyten in dem Bereich von 0,2 bis 100 ng/ml beobachtet; die Korrelationskoeffizienten betrugen mindestens 0,998, die mittlere Wiederfindungsrate jeder Standardkonzentration lag zwischen 92,6 und 104,5 % und die relative Standardabweichung betrug max. 9,5 %. Die Genauigkeit, Wiederholbarkeit und Spezifität wurden validiert, indem das E-Zigaretten-Aerosol mit drei verschiedenen Mengen von Analyten angereichert wurde; die mittlere Wiederfindungsrate jeder Anreicherungskonzentration lag zwischen 88,7 und 110,3 % und die relative Standardabweichung betrug max. 9,2 % für alle Analyte. Für einige Analyte, insbesondere für Al, Fe, Cu und Sn, lag eine Hintergrundkontamination aus dem Aerosolerzeugungs- und Auffangsystem vor. Die potenziellen Kontaminationsquellen sollten identifiziert und kontrolliert werden, um die Auswirkungen der Kontamination auf die Quantifizierung zu reduzieren. Darüber hinaus sollten die tatsächlichen Werte für Proben zusammen mit einer Methodenblindwertstatistik berichtet werden. Eine Zunahme der Konzentrationen von Glycerin und 1,2-Propylenglykol in der präparierten Probe führte zur Überschätzung von As und Se. Zur korrekten Quantifizierung von As und Se sollte die Menge von Polyolen im aufgefangenen Aerosol überwacht und kontrolliert werden. [Beitr. Tabakforsch. Int. 28 (2018) 2–13]

RESUME

Les normes de sécurité et de qualité applicables aux cigarettes électroniques (e-cigarettes) ont été introduites au niveau régional. En 2016, la Food and Drug Administration (FDA) américaine a émis une règle relative aux e-cigarettes, qui exige la communication des composants nocifs et potentiellement nocifs (HPHC en anglais). Au Royaume-Uni, la British Standards Institution (BSI) a précisé les métaux devant être contrôlés sur les e-cigarettes. Dans le cadre de la présente étude, une méthode de détermination simultanée de 13 métaux (Be, Al, Cr, Fe, Co, Ni, Cu, As, Se, Ag, Cd, Sn et Pb) présents dans l'aérosol des e-cigarettes fut mise au point et validée. Par ailleurs, l'effet de matrice des principaux composants de l'aérosol fut étudié à l'aide de solutions contenant du glycérol ou du 1,2-propyléneglycol.

L'aérosol de la cigarette électronique fut produit à l'aide d'une machine à fumer rotative, suivant la méthode n°81 recommandée par le CORESTA et fut prélevé par un précipitateur électrostatique couplé à un épurateur contenant de l'acide nitrique. L'aérosol prélevé fut dissout dans l'acide nitrique et une aliquote de cette solution fut analysée par un spectromètre de masse à couplage inductif (ICP-MS) équipé d'une cellule de collision/réaction.

La linéarité de la courbe de calibration fut observée dans une plage allant de 0,2 à 100 ng/ml pour chaque analyte; les coefficients de corrélation s'élèveront à 0,998 voire plus, la récupération moyenne de chaque niveau normalisé oscilla entre 92,6 et 104,5% et l'écart-type relatif ne dépassa pas 9,5%. La précision, la répétabilité et la spécificité furent validées en ajoutant trois quantités différentes d'analytes à l'aérosol de l'e-cigarette; la récupération moyenne pour chaque niveau ajouté oscilla entre 88,7 et 110,3% tandis

que l'écart-type relatif ne dépassa pas 9,2% pour la totalité des analytes. Une contamination de base imputable au système de production et de prélèvement de l'aérosol fut observée pour certains analytes, notamment l'aluminium, le fer, le cuivre et l'étain. Les sources potentielles de contamination devraient être identifiées et maîtrisées afin de réduire l'incidence de la contamination sur la quantification. En outre, les valeurs réelles des échantillons devraient être accompagnées des statistiques de l'essai à blanc. L'augmentation des concentrations de glycérol et de 1,2-propyléneglycol dans l'échantillon préparé causa une surestimation de l'arsenic et du sélénium. La quantité de polyols dans l'aérosol prélevé devrait être contrôlée et maîtrisée pour une quantification précise de l'arsenic et du sélénium. [Beitr. Tabakforsch. Int. 28 (2018) 2–13]

INTRODUCTION

Electronic cigarettes (e-cigarettes) have gained a global market share since their introduction into the European and American market in 2006 and 2007, respectively (1, 2). An e-cigarette is a battery-powered device to generate aerosol by heating a mixture, which typically contains humectants such as glycerol and/or 1,2-propylene glycol, water, nicotine and flavors (3–5). Basically, an e-cigarette consists of a battery, an atomizer and a cartridge, although the design is variable (6). A rechargeable e-cigarette has either a pre-filled or refillable cartridge while a disposable one has a prefilled cartridge with a non-rechargeable battery. Device properties and performance vary among brands in pressure drop, the airflow rate required to generate aerosol, aerosol density and the total number of puffs per cartridge (7). To date, concerns about the safety and quality of e-cigarettes have been raised. Since e-cigarettes contain metal components, which come in contact with the liquid, contamination of the aerosol with metals can occur. Several studies have shown the presence of metals in e-cigarette aerosol as well as variability in metal concentrations (8–12). WILLIAMS *et al.* found small particles composed of metals and silicates which were present in the aerosol of tested e-cigarettes (13).

Safety and quality standards for e-cigarettes have been introduced regionally. In 2016, the U.S. Food and Drug Administration (FDA) issued the Deeming Rule expanding the regulatory authority to cover all tobacco products, including e-cigarettes (14). The FDA requires e-cigarette manufacturers to report the quantities of harmful and potentially harmful constituents (PHPCs) in the liquid and aerosol, which was generated under a range of conditions of use, including both non-intense (e.g., lower levels of exposure) and intense (e.g., higher levels of exposure). In the United Kingdom, the British Standards Institution (BSI) published the standard for vaping products in 2015, which specified the metals to be monitored in the liquid and aerosol of e-cigarettes (15). A study for a simultaneous determination method for the metals specified in the HPHC list and the BSI standard has not been reported previously while determination methods for some of the metals were validated and reported (8, 11, 16).

Inductively coupled plasma mass spectrometry (ICP-MS) has been accepted as an analytical technique for simul-

taneous multi-element determinations at trace levels. However, the technique may suffer from spectral and non-spectral interferences (17, 18). Spectral interferences are caused by polyatomic, isobaric and doubly charged ions with the same nominal mass-to-charge ratio as the analyte of interest. Non-spectral interferences, referred to as matrix effects or matrix-induced signal variations, are highly dependent on sample matrices and instrumental conditions. Since matrix effects of e-cigarette aerosol in ICP-MS have rarely been studied, it is important to evaluate the impact on accuracy and precision.

The purpose of this study was to develop and validate a method for the simultaneous determination of 13 metals (Be, Al, Cr, Fe, Co, Ni, Cu, As, Se, Ag, Cd, Sn and Pb) in e-cigarette aerosol. Furthermore, matrix effects in the presence of glycerol or 1,2-propylene glycol, which account for most of the aerosol, were investigated.

MATERIALS AND METHODS

Reagents and electronic cigarettes

Ultrapure nitric acid (60–62% w/w, 1.38 g/cm³) and single-element standards of Be, Sc and Y in nitric acid (1000 mg/L) were purchased from Kanto Chemical (Tokyo, Japan). Multi-element standard XSTC-622B of Ag, Al, As, B, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Ge, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, Sb, Se, Si, Sn, Sr, Ti, V, W, Zn and Zr in nitric acid (10 mg/L) was purchased from SPEX CertiPrep (Metuchen, NJ, USA). Tuning solution for ICP-MS (Li, Y, Ce, Tl, and Co in nitric acid, 10 ng/mL) was purchased from Agilent Technologies (Santa Clara, CA, USA). Glycerol (> 99% w/w) and 1,2-propylene glycol (> 99% w/w) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

One commercially available e-cigarette was used for the validation study due to the lack of a reference e-cigarette. It had a rechargeable battery and a disposable cartomizer prefilled with a mixture of nicotine, water, glycerol, 1,2-propylene glycol and flavoring.

Standard preparation

The secondary internal standard solution (100 µg/mL) was prepared by transferring the primary standards of Sc and Y into a volumetric flask and filling up with 5% v/v nitric acid in ultrapure water (resistivity > 18.2 MΩ·cm). Nine standards plus a blank (0, 0.2, 0.5, 1, 2, 5, 10, 20, 50 and 100 ng/mL), containing 10 ng/mL of Sc and Y, were prepared by diluting commercially available standards in 5% v/v nitric acid. The flasks were made of polypropylene or polyethylene and were cleaned before use by soaking and consecutive rinsing with acetone, concentrated nitric acid and ultrapure water. Disposable polypropylene pipette tips were rinsed with 5% v/v nitric acid.

Aerosol generation and collection

Aerosol generation and collection were performed under ISO 3402 testing conditions (temperature 22 ± 2 °C, relative humidity 60 ± 5%) (19). An e-cigarette was smoked on

a modified 20-port rotary smoking machine RM20H from Borgwaldt KC (Hamburg, Germany). It was connected to a pump system with a mass flow controller in order to generate aerosol while stopping the motion of a rotating metal ring on a metal plate, on which metal particles can be generated by attrition as the puff number greatly increases. Aerosol was generated according to CORESTA Recommended Method N° 81 (puff volume: 55 ± 0.3 mL, puff duration: 3 ± 0.1 s, puff interval: 30 s, puff profile: rectangular shape) (20). Aerosol of 50 puffs was collected based on Health Canada Test Method T-109 (21). An electrostatic precipitator with a tungsten electrode tip (Borgwaldt KC, Hamburg, Germany) was used to trap aerosol in a glass tube. A glass impinger containing 30 mL of 5% v/v nitric acid, which was placed between an electrostatic precipitator and the pump system, was utilized to trap gaseous phase metals. All the batteries were fully charged before use. Method blanks were prepared in the same procedure as e-cigarette samples but with no e-cigarette in the sample holder.

Sample preparation

The collected aerosol was prepared and analyzed by modifying Standard Method 3125 developed by AMERICAN PUBLIC HEALTH ASSOCIATION (22). The condensate trapped inside the glass tube was dissolved in 5% v/v nitric acid, and then 0.1 mL of internal standard stock solution was added. This solution was diluted and made up to 100 mL with nitric acid prior to instrumental analysis. The impinger solution was prepared and analyzed in the same way as the collected aerosol.

ICP-MS analysis

The metals were analyzed by an Agilent 7500cx ICP-MS equipped with a collision/reaction cell (Santa Clara, CA, USA). The operating conditions were optimized as shown in Table 1. To minimize oxide and double charged ratio, the

Table 1. Operating conditions for the ICP-MS.

Parameter	Setting
RF power	1500 W
Plasma gas flow rate	15 L/min
Auxiliary gas flow rate	Ar 0.58 L/min
Carrier gas flow rate	Ar 0.25 L/min
Peristaltic pump speed	0.1 rpm
Peristaltic pump tubing	1.02 mm inner diameter
Sampling cone	Pt sampling cone, 10 mm insert
Skimmer cone	Pt skimmer cone with Cu base
Sampling depth	12 mm
Cell gas flow rate	He 5 mL/min: ²⁷ Al, ⁵² Cr, ⁵⁹ Co, ⁶⁰ Ni, ⁶³ Cu, ⁷⁵ As, ¹¹¹ Cd, ²⁰⁸ Pb, ⁴⁵ Sc, ⁸⁹ Y H ₂ 4 mL/min: ⁵⁶ Fe, ⁷⁸ Se, ¹⁰⁷ Ag, ¹¹⁸ Sn, ⁴⁵ Sc, ⁸⁹ Y
Dwell time per isotope	0.33 s: ⁹ Be, ²⁷ Al, ⁵² Cr, ⁵⁶ Fe, ⁵⁹ Co, ⁶⁰ Ni, ⁶³ Cu, ¹⁰⁷ Ag, ¹¹¹ Cd, ¹¹⁸ Sn, ²⁰⁸ Pb, ⁴⁵ Sc, ⁸⁹ Y 2 s: ⁷⁵ As, ⁷⁸ Se
Data point	3 points per peak
Repetition	3 times

following parameters were tuned in standard (no gas) mode with 1 ng/mL tuning solution: radio frequency (RF) power, carrier and auxiliary gas flow rate, sampling depth and sample uptake rate. For each analyte, appropriate isotope, internal standard (Sc or Y) and cell gas (no gas, He or H₂) mode were selected by comparing the measured counts between 5 ng/mL multi-element standard and blank. P/A factors were tuned before analysis to have a linear response over a wide range of analyte concentrations.

Regression analysis

The concentration of the analyte was determined using weighted least squares linear regression (23): $y = ax + b$, where y is the ratio of the analyte response to the internal standard response, a is the slope of the line, x is the concentration of analyte, b is the y-intercept of the line. The weighting factor was chosen based on the plot of analyte concentration versus standard deviation of analyte-to-internal standard response ratios.

Method validation

The developed method was validated based on the INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH) guideline Q2(R1) and the EURACHEM/CITAC guide (24, 25). The evaluated validation parameters were specificity, linearity, range, accuracy, precision (repeatability) and limits of detection and quantification.

RESULTS

Linearity and range

Linearity was evaluated by analyzing standards with nine different concentrations (0.2, 0.5, 1, 2, 5, 10, 20, 50 and 100 ng/mL) in triplicate. Figure 1 shows regression parameters such as the correlation coefficient, slope and y-intercept of the calibration curve and residual sum of squares. Each correlation coefficient was 0.998 or larger. The mean recovery of each standard level for all analytes ranged from 92.6 to 104.5% with a relative standard deviation between 0.3 to 9.5% (Table 2).

Limits of detection and quantification

The limit of detection (LOD) and quantification (LOQ) were determined based on the standard deviation of ten independent measurements of the lowest standard level ($LOD = 3.3 \sigma/a$, $LOQ = 10 \sigma/a$). The results showed each LOQ was below the lowest concentration of standards (Table 3).

Accuracy and precision

Accuracy and repeatability were evaluated by spiking and recovery tests due to no reference e-cigarettes being available. The collected aerosol was split into two portions, and a defined amount of analytes was spiked into one

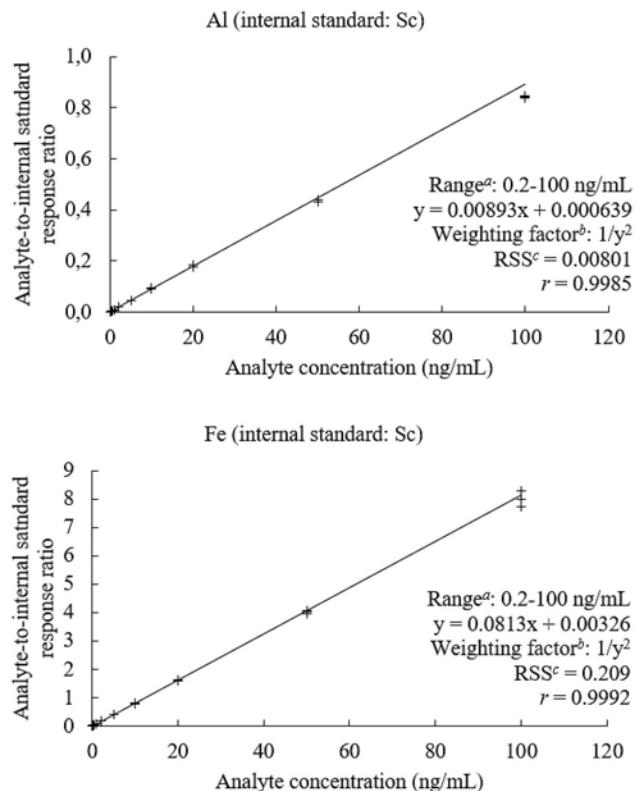
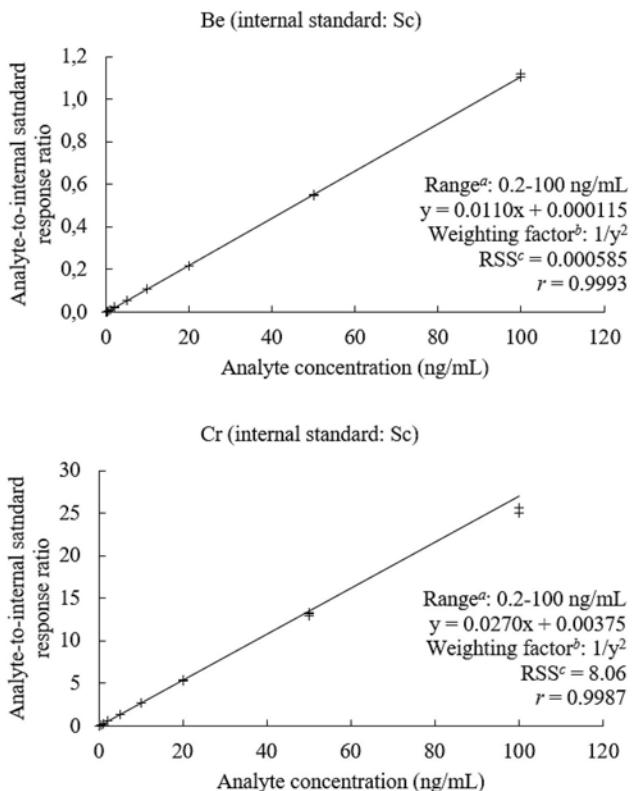


Figure 1. Calibration curve and regression parameters for each analyte. Nine levels of calibration standards (0.2, 0.5, 1, 2, 5, 10, 20, 50 and 100 ng/mL for each analyte) were analyzed in three replicates. The most appropriate linear regression model of three (weighting factor: 1, 1/y and 1/y²) was chosen based on the plot of analyte concentration (x) versus standard deviation of analyte-to-internal standard response (y). RSS: residual sum of squares.

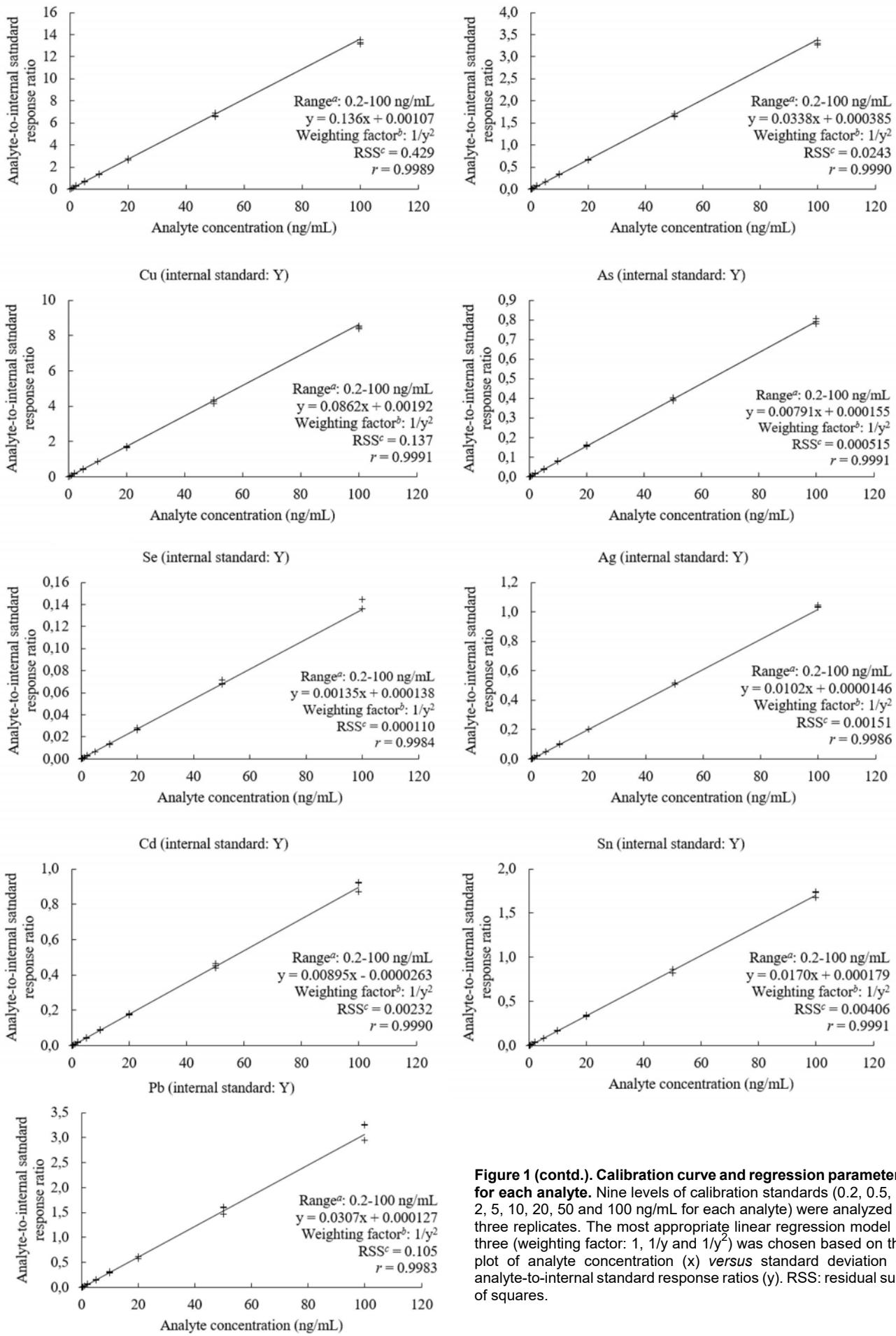


Figure 1 (contd.). Calibration curve and regression parameters for each analyte. Nine levels of calibration standards (0.2, 0.5, 1, 2, 5, 10, 20, 50 and 100 ng/mL for each analyte) were analyzed in three replicates. The most appropriate linear regression model of three (weighting factor: 1, $1/y$ and $1/y^2$) was chosen based on the plot of analyte concentration (x) versus standard deviation of analyte-to-internal standard response ratios (y). RSS: residual sum of squares.

Table 2. Percent recovery for each concentration of calibration standards.

Analyte	Level 1 ^a		Level 2		Level 3 ^a		Level 4		Level 5		Level 6		Level 7		Level 8		Level 9	
	Recovery ^b (%)	RSD (%)	Recovery ^b (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Be	1001	8	1000	12	998	21	1004	7	996	10	997	3	995	15	1001	16	1010	5
Al	1009	53	993	95	1014	66	1020	39	1027	25	1045	27	1016	14	980	13	938	19
Cr	991	8	1014	23	1008	6	1014	4	1012	9	1013	4	1018	4	989	7	950	7
Fe	989	9	1004	31	1024	27	1016	9	1017	15	1014	9	1018	13	1012	6	926	30
Co	994	8	1004	14	1017	14	1011	13	1011	8	999	4	1000	8	987	9	980	16
Ni	991	6	1011	14	1023	6	1001	13	1009	5	1001	7	1001	4	982	9	985	9
Cu	999	6	992	12	1013	6	1011	9	1008	8	1003	7	1002	4	986	9	989	9
As	1008	12	983	23	1003	20	998	10	993	8	1003	22	1013	9	999	9	1006	7
Se	1002	3	1001	10	996	23	996	14	991	7	992	11	1004	7	1011	7	1010	10
Ag	1006	8	993	11	986	17	995	19	1003	15	999	11	1006	9	1005	13	1012	20
Cd	997	12	1010	20	1005	21	993	28	1001	20	989	13	1003	16	999	14	1008	7
Sn	989	10	1032	36	998	30	1004	8	994	9	989	4	996	7	1000	8	1005	11
Pb	1005	6	992	8	1007	14	984	11	983	14	992	8	998	6	1012	3	1032	10

^a Nine levels of calibration standards (0.2, 0.5, 1, 2, 5, 10, 20, 50 and 100 ng/mL for each analyte) were individually prepared on four days.^b Percent recovery was calculated by comparing the expected concentration with the measured concentration.

Table 3. Limits of detection (LOD) and quantification (LOQ) for each analyte.

Analyte	LOD ^a (ng/mL)	LOQ ^a (ng/mL)
Be	91	277
Al	539	1630
Cr	165	500
Fe	146	443
Co	97	295
Ni	164	496
Cu	99	300
As	197	596
Se	544	1649
Ag	137	415
Cd	315	953
Sn	253	766
Pb	245	743

^a LOD and LOQ were determined based on the slope and the standard deviation of the analyte-to-internal standard response ratios, which were obtained by 10 measurements of the lowest standard level (0.2 ng/mL): LOD = 3.3 σ/a, LOQ = 10 σ/a.

half. Three different amounts of analytes were added into aerosol collected by an electrostatic precipitator and impinger solution in triplicate, respectively (0.1, 0.5 and 2 µg of each analyte per 50-puff aerosol). Percent recovery was calculated by comparing the spiked sample values with the unspiked sample values. For all analytes, the mean recoveries of three spiking levels ranged from 88.7 to 110.3% (Table 4). Each relative standard deviation was below 9.2% and slightly decreased for increasing spiking amounts.

Blank test

To measure background contamination during smoke runs and sample preparations, each method blank was individually prepared in the same procedure as e-cigarette samples. As shown in Table 5, Cr, Co, As, Se and Cd were not detected in any method blanks. Be, Ni and Pb were detected in some method blanks but not quantified. The maximum concentrations of Al, Fe, Cu and Sn were above the lowest standard level.

Matrix effects

Matrix effects in the presence of glycerol or 1,2-propylene glycol were investigated by recovery and spike tests. Three levels of analytes were added into 0.1, 0.2 and 0.3% w/v of glycol or 1,2-propylene glycol solution in triplicate, respectively (0.1, 0.5 and 2 µg of each analyte per 100 mL of each solution). With the exception of As and Se, the mean recoveries of three spiking levels were between 85.5 to 108.8% and each relative standard deviation was within 6.9% (Tables 6, 7). While the mean recoveries ranged from 98.3 to 106.1% for As and from 89.8 to 117.8% for Se at the lowest level of these polyol solutions, the mean recoveries were between 114.5 and 124.9% for As and between 126.1 and 144.8% for Se at the highest level.

Table 4. Results of spike and recovery test using electronic cigarette aerosol.

Analyte	Amount spiked (ng/mL)	Electrostatic precipitator ^a		Impinger solution	
		Recovery (%) ^b	RSD (%)	Recovery (%)	RSD (%)
Be	1	973	10	989	9
	5	942	9	973	5
	20	943	6	979	4
Al	1	990	62	1034	14
	5	971	23	955	48
	20	992	11	951	14
Cr	1	1044	10	1072	5
	5	1018	8	1063	8
	20	1024	10	1056	3
Fe	1	994	6	1056	8
	5	998	2	1039	5
	20	995	3	1047	4
Co	1	1008	4	993	2
	5	998	8	978	7
	20	1008	7	983	3
Ni	1	1004	23	1007	5
	5	1000	5	978	9
	20	1006	6	987	5
Cu	1	986	32	1004	10
	5	995	15	986	6
	20	1006	3	996	7
As	1	1000	15	975	14
	5	1018	5	958	9
	20	1034	2	983	6
Se	1	954	92	924	17
	5	1046	21	927	9
	20	1086	9	946	3
Ag	1	927	19	887	14
	5	916	1	902	4
	20	925	6	901	3
Cd	1	1010	14	1046	28
	5	993	7	1011	8
	20	987	3	1012	5
Sn	1	994	10	1011	7
	5	973	2	1011	5
	20	979	4	1009	7
Pb	1	1036	7	1103	14
	5	1025	2	1084	21
	20	1024	5	1082	4

^a Aerosol of 50 puffs was collected by an electrostatic precipitator coupled to impinger containing nitric acid.

^b Percent recovery was calculated by comparing the spiked sample value with the unspiked sample value (n = 3).

Table 5. Background contamination during aerosol collection and sample preparation. Each blank sample was individually prepared in the same manner as electronic cigarette sample (50 puffs).

Analyte	Blank sample	Concentration (ng/mL)								
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Be	Electrostatic precipitator	< 0.0277 ^a								
	Impinger solution	ND ^b	ND	< 0.0277 ^a	< 0.0277 ^a	< 0.0277 ^a	< 0.0277 ^a	ND ^b	ND ^b	< 0.0277 ^a
Al	Electrostatic precipitator	648	441	657	650	600	994	478	593	601
	Impinger solution	154	135	128	108	140	140	127	121	209
Cr	Electrostatic precipitator	ND								
	Impinger solution	ND								
Fe	Electrostatic precipitator	0.166 ^c	0.112 ^c	0.149 ^c	0.238	0.0890 ^c	0.164 ^c	0.0827 ^c	0.111 ^c	0.136 ^c
	Impinger solution	329	0.166 ^c	0.194 ^c	0.190 ^c	0.180 ^c	458	211	0.141 ^c	310
Co	Electrostatic precipitator	ND								
	Impinger solution	ND								
Ni	Electrostatic precipitator	ND								
	Impinger solution	< 0.0496 ^a	ND	ND	< 0.0496 ^a	ND	< 0.0496 ^a	ND	ND	< 0.0496 ^a
Cu	Electrostatic precipitator	ND	ND	ND	ND	< 0.0300 ^a	ND	< 0.0300 ^a	ND	ND
	Impinger solution	< 0.0300 ^a	ND	ND	< 0.0300 ^a	ND	< 0.0300 ^a	ND	ND	< 0.0300 ^a
As	Electrostatic precipitator	ND								
	Impinger solution	ND								
Se	Electrostatic precipitator	ND								
	Impinger solution	ND								
Ag	Electrostatic precipitator	0.0484 ^c	0.0445 ^c	0.0428 ^c	< 0.0415 ^a					
	Impinger solution	< 0.0415 ^a								
Cd	Electrostatic precipitator	ND								
	Impinger solution	ND								
Sn	Electrostatic precipitator	< 0.0766 ^a	< 0.0766 ^a	< 0.0766 ^a	0.0885 ^c	0.105 ^c	0.0940 ^c	0.0487 ^c	0.159 ^c	0.104 ^c
	Impinger solution	213	0.142 ^c	0.128 ^c	0.134 ^c	208	0.190 ^c	0.150 ^c	0.132 ^c	0.176 ^c
Pb	Electrostatic precipitator	ND								
	Impinger solution	< 0.0743 ^a	ND	< 0.0743 ^a	ND	ND	ND	ND	< 0.0743 ^a	< 0.0743 ^a

^a The value was detected but not quantified.

^b ND = The value was not detected.

^c The value was over the limit of quantification but below the lowest concentration of calibration standards.

Table 6. Results of spike and recovery test using three levels of glycerol solution (0.1, 0.2 and 0.3 % w/v). Percent recovery was calculated by comparing the spiked sample value with the unspiked sample value (n = 3).

Analyte	Amount spiked (ng/mL)	Glycerol solution					
		0.1 % (w/v)		0.2 % (w/v)		0.3 % (w/v)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Be	1	958	10	924	13	1034	14
	5	958	2	911	6	1022	5
	20	962	5	916	3	1038	4
Al	1	1088	10	1034	25	1067	1
	5	1070	16	1035	13	1018	13
	20	1037	5	1019	11	1006	21
Cr	1	1003	6	1009	4	996	2
	5	985	5	1001	9	982	6
	20	968	9	974	3	974	1
Fe	1	929	29	930	4	930	10
	5	932	10	923	3	921	9
	20	929	3	913	5	922	3
Co	1	1005	15	1042	9	1068	12
	5	991	5	1039	3	1052	1
	20	985	4	1016	8	1050	3
Ni	1	1010	11	1036	6	1077	8
	5	994	5	1038	6	1060	6
	20	982	2	1017	10	1052	3
Cu	1	998	14	1035	2	1076	13
	5	991	6	1040	2	1052	3
	20	979	7	1021	11	1046	1
As	1	983	16	991	53	1145	19
	5	1024	3	1092	3	1165	21
	20	1030	6	1102	15	1189	5
Se	1	898	169	1039	110	1261	41
	5	1078	33	1181	25	1305	11
	20	1108	5	1214	11	1320	8
Ag	1	975	12	898	12	922	12
	5	1016	4	922	11	899	2
	20	1030	5	925	12	902	4
Cd	1	968	10	982	17	963	48
	5	986	6	985	4	958	13
	20	980	7	981	10	961	10
Sn	1	947	21	988	4	969	10
	5	958	7	959	8	944	7
	20	970	7	954	11	947	1
Pb	1	996	21	978	6	983	6
	5	984	5	974	5	960	11
	20	979	10	983	17	983	6

DISCUSSION

The primary purpose of this study was to develop and validate a method for the simultaneous determination of 13 metals in e-cigarette aerosol, which were specified in the HPHC list and the BSI standard. The developed method was validated using one commercially available e-cigarette since analyte-certified reference e-cigarettes or typical analyte-free matrices were not available. Validation parameters were evaluated based on the ICH guideline and the

EURACHEM/CITAC guide (24, 25). Furthermore, matrix effects of major constituents in the aerosol were investigated using glycerol or 1,2-propylene glycol solutions. Initially, linearity was evaluated by analyzing multi-element standards with 0.2 to 100 ng/mL of each analyte. Since the previous studies demonstrated the levels of some metals in e-cigarette aerosol varied among and within brands as well as product performance (7, 9, 10), a wide range of analyte concentrations was applied. For all analytes, the correlation coefficient of the calibration curve

Table 7. Results of spike and recovery test using three levels of 1,2-propylene glycol solution (0.1, 0.2 and 0.3 % w/v). Percent recovery was calculated by comparing the spiked sample value with the unspiked sample value (n = 3).

Analyte	Amount spiked (ng/mL)	1,2-Propylene glycol solution					
		0.1 % w/v		0.2 % w/v		0.3 % w/v	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Be	1	1000	4	963	15	1049	4
	5	995	6	945	3	1047	2
	20	997	4	958	14	1049	9
Al	1	1050	21	1063	11	1008	69
	5	1065	7	1021	10	998	13
	20	1044	15	1018	1	982	3
Cr	1	995	5	1009	2	1028	10
	5	979	3	991	3	997	2
	20	965	9	988	4	992	10
Fe	1	968	15	948	2	973	11
	5	938	0	930	6	945	5
	20	948	4	930	4	946	2
Co	1	999	9	1046	10	1079	7
	5	985	8	1024	4	1063	9
	20	986	6	1026	6	1053	9
Ni	1	999	10	1043	9	1058	4
	5	984	8	1021	2	1061	5
	20	988	4	1031	1	1057	12
Cu	1	1000	19	1035	4	1073	9
	5	982	6	1032	15	1064	9
	20	982	5	1026	2	1058	12
As	1	1061	5	1142	15	1247	3
	5	1046	5	1125	14	1249	9
	20	1050	7	1132	1	1242	16
Se	1	1178	20	1274	33	1432	20
	5	1157	1	1283	4	1448	14
	20	1156	6	1289	11	1444	6
Ag	1	1019	7	899	16	868	12
	5	1001	6	908	1	855	9
	20	1012	14	923	8	858	3
Cd	1	978	17	992	1	968	28
	5	961	5	974	6	965	10
	20	984	8	989	10	978	6
Sn	1	986	19	976	13	968	10
	5	960	3	953	4	950	7
	20	967	8	959	5	960	4
Pb	1	988	13	980	26	989	13
	5	948	2	960	10	978	15
	20	971	5	974	1	1004	22

was 0.998 or larger, the mean recovery of each standard level ranged from 92.6 to 104.5% and the relative standard deviation was within 9.5% (Figure 1, Table 2). Thus, the linear relationship of analyte concentration *versus* analyte-to-internal standard response ratio was verified in the range specified for each analyte.

For the e-cigarette employed for this validation study, the mass-percent composition of major constituents in the aerosol was determined by GC-FID (Gas Chromatography - Flame Ionization Detection). The aerosol consisted of

2.0% of nicotine, 14.2% of water, 20.0% of glycerol, 63.4% of 1,2-propylene glycol and 0.5% of other constituents, which were presumed to be flavor constituents. TAYYARAH *et al.* reported nicotine, water, humectants involving glycerol and/or 1,2-propylene glycol and flavors in the aerosol of 5 different e-cigarettes ranged from 1–2%, 10–19%, 70–85%, and 3–11%, respectively (4). By comparison, the tested e-cigarette was considered to be similar in chemical composition to these e-cigarettes. Accuracy, repeatability and specificity were evaluated by

spiking three different amounts of analytes into aerosol collected by electrostatic precipitator and impinger solution, respectively (0.1, 0.5 and 2.0 µg of each analyte per 50-puff aerosol). For all analytes, the mean recovery of each spiking level ranged from 88.7 to 110.3% with a relative standard deviation amounted to max. 9.2% (Table 4). From the results, accuracy, repeatability and specificity were considered acceptable.

To measure background contamination during smoke run and sample preparation, method blanks were prepared and analyzed in 10 replicates. The results showed the amounts of Al, Fe, Cu and Sn varied and the maximum concentrations were above the lowest standard level, whereas the other analytes were undetectable or unquantifiable (Table 5). OTTE *et al.* reported background contamination was observed for linear and rotary smoking machines coupled with an electrostatic precipitator and/or impinger solution, showing background levels of contaminants varied among and within the tested aerosol generation and collection systems (26). In several studies, quartz fiber or mixed cellulose ester membrane filter pads were used as an alternative approach for aerosol collection (10, 11, 27). However, the presence of metals in the filter method blank was observed. Therefore, the potential sources of contamination should be identified and controlled to reduce the impact of contamination on quantification. In addition, in order to quantitatively estimate the extent of background contamination, method blanks should be prepared and analyzed with every batch of samples.

Matrix effects in the presence of 0.1, 0.2 and 0.3% w/v glycerol or 1,2-propylene glycol, corresponding to the amounts of polyols in the tested e-cigarette aerosol per approximately 48, 97 and 145 puffs, were investigated. The results showed that the increase of the concentrations of polyols in the prepared sample led to the overestimation of Se and As while the mean recoveries of the tested e-cigarette aerosol per 50 puffs ranged from 92.4 to 108.6% (Tables 6, 7). By comparing the plot of analyte concentration versus response between solutions with and without polyols, the signal enhancement of As and Se in the presence of polyols was found (data not shown). LARSEN *et al.* reported the addition of carbon as methanol into aqueous solutions in combination with increased plasma power input enhanced the signal intensities of As and Se species in ICP-MS (28). Thus, the amount of polyols in the prepared sample should be monitored and controlled.

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Corresponding author:

Shintaro Ohashi
Product Quality Research Center
Japan Tobacco Incorporated
6-2, Umegaoka, Aoba-ku
Yokohama, Kanagawa, Japan
E-mail: shintaro.a.ohashi@jt.com