Printed in the Republic of Korea
ANALYTICAL SCIENCE & TECHNOLOGY
Vol. 33 No. 2, 98-107, 2020
https://doi.org/10.5806/AST.2020.33.2.98

Impurity profiling and chemometric analysis of methamphetamine seizures in Korea

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(Received December 30, 2019; Revised February 27, 2020; Accepted March 16, 2020)

Abstract: Methamphetamine (MA) is currently the most abused illicit drug in Korea. MA is produced by chemical synthesis, and the final target drug that is produced contains small amounts of the precursor chemicals, intermediates, and by-products. To identify and quantify these trace compounds in MA seizures, a practical and feasible approach for conducting chromatographic fingerprinting with a suite of traditional chemometric methods and recently introduced machine learning approaches was examined. This was achieved using gas chromatography (GC) coupled with a flame ionization detector (FID) and mass spectrometry (MS). Following appropriate examination of all the peaks in 71 samples, 166 impurities were selected as the characteristic components. Unsupervised (principal component analysis (PCA), hierarchical cluster analysis (HCA), and K-means clustering) and supervised (partial least squares-discriminant analysis (PLS-DA), orthogonal partial least squares-discriminant analysis (OPLS-DA), support vector machines (SVM), and deep neural network (DNN) with Keras) chemometric techniques were employed for classifying the 71 MA seizures. The results of the PCA, HCA, K-means clustering, PLS-DA, OPLS-DA, SVM, and DNN methods for quality evaluation were in good agreement. However, the tested MA seizures possessed distinct features, such as chirality, cutting agents, and boiling points. The study indicated that the established qualitative and semi-quantitative methods will be practical and useful analytical tools for characterizing trace compounds in illicit MA seizures. Moreover, they will provide a statistical basis for identifying the synthesis route, sources of supply, trafficking routes, and connections between seizures, which will support drug law enforcement agencies in their effort to eliminate organized MA crime

Key words: methamphetamine, impurity profiling, chemometric analysis, GC-FID/MS

1. Introduction

Methamphetamine (MA), more commonly known as “Philopon”, is a highly addictive synthetic substance and the most abused drug in Korea.[1] MA was first synthesized in 1888 by Prof. Nagai Nagayoshi at the

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University of Tokyo in Japan, who had been studying ephedrine extracted from the ephedra plant for its antiasthmatic effect.[2] The precursor compounds for MA can be more easily obtained than those for other clandestinely synthesized drugs. Therefore, cases have occurred in which a drug offender with a certain level of knowledge of chemical synthesis has used a medicinal herb or drug that contains the precursor compounds to produce illicit MA.[3,4] MA abuse is increasing not only in Korea but worldwide and, owing to the easier access and lower cost of MA than other drugs of abuse, the spread of MA has been rapid as an alternative drug to high-cost heroin and cocaine.[5] This rise in the distribution and abuse of MA has become a serious social issue both at home and overseas.[6,7]

Most MA abused in Korea is the illicit MA produced overseas, which is then smuggled into Korea though many countries in Asia. As the clandestine manufacture of MA became a serious problem in China until only a few years ago, it appears that the strict regulations from the Chinese government have reduced the level of illicit MA by a substantial degree.[8] Nonetheless, the total amount of MA smuggled into Korea has not decreased. This can be attributed to two broad reasons. First, to overcome strict regulations, the illicit MA drug cartels in China have moved their home bases to the surrounding countries, including Taiwan, Malaysia, Thailand, Cambodia, Vietnam, and the Philippines.[9-11] Second, as online social networking and international mail make it easier to deliver the product to other countries, the drug trafficking routes of MA have become more diverse than ever in Korea.

If an overseas drug cartel distributes MA in Korea through various routes and the unique properties of the MA produced by that cartel are known, the tracking of the drug criminal organization will be facilitated. Thus, to investigate the connections among drug crime cases and examine the identity and correlations among distributed MA, a scientific technique known as impurity profiling was introduced. The technique analyzes the physical and chemical properties of seized MA using various analytical tools and compares those properties using statistical techniques, thereby supporting drug investigations based on the identified sources of production and supply.

For MA impurity profiling, several analytical methods have been developed, such as gas chromatography-flame ionization detection (GC-FID), gas chromatography-mass spectrometry (GC-MS), GC-FID/MS, capillary electrophoresis, high-performance liquid chromatography (HPLC), and LC-MS.[12-20] The most commonly used analytic tool for drug impurity profiling is chromatography.[21] Depending on the detector attached to the GC, the data of various properties can be obtained. To illustrate, comparing the chromatograms of an identical substance from FID and MS shows that the GC-MS chromatogram exhibits higher compound-dependency than the GC-FID chromatogram. FID is a universal detector of volatile hydrocarbon compounds and has an outstanding level of sensitivity.[22,23] The application of GC-FID/MS in impurity profiling is anticipated to be more useful than FID or MS alone in profiling as it encompasses the benefits of FID and outstanding ability of MS in the identification of components.[13-15]

The MA smuggled into Korea is a synthetic substance that is produced by numerous synthetic pathways and distributed through illegal means. This MA includes the precursors, intermediates, and by-products involved in the synthetic process up to the final product. The illicit MA does not adhere to normal quality control; thus, it may contain impurities with substantial health hazards. The content of the intermediates and impurities vary according to the method of synthesis and whether the manufacturing process included an incomplete reaction or purification. The MA production methods are broadly divided into two: i) synthesis using *l*-ephedrine or *d*-pseudoephedrine as the source material; ii) synthesis using phenyl-2-propanone as the source material. The main impurities in MA include dimethylamphetamine, N-formylamphetamine, N-formylmethamphetamine, methylephedrine, ephedrine, and chloroephedrine.[24,25] Among various compounds, route specific impurities have been identified. For example, α-benzyl-N-methylphenethylamine produced by the Leuckart route, (1S,2S)-1-methylamino-1-

phenyl-2-chloropropane and N-methyl-1-{4-[2-(methylamino) propyl]phenyl}-1-phenylpropane-2-amine by the Emde route, and 1,3-dimethyl-2-phenylnaphthalene and 1-benzyl-3-methylnaphthalene by the Nagai route.[26-30] For the impurity profiling data obtained from such analyses, different statistical techniques can be applied to conduct a chemometric analysis.[31-36]

For MA impurity profiling, this study carried out GC-FID/MS, in which each component in a trace amount of impurities was identified through GC-MS, after which 166 characteristic impurity compounds were selected. Using the chromatograms produced by GC-FID, semi-quantification analysis was carried out. For chemometric analysis, unsupervised techniques (principal component analysis (PCA), hierarchical clustering analysis (HCA), and K-means clustering) and supervised techniques (partial least squares-discriminant analysis (PLS-DA), orthogonal partial least squares-discriminant analysis (OPLS-DA), support vector machine (SVM), and deep neural network (DNN) models) were applied. The diverse statistical techniques in this study showed that the results were in good agreement and the utility of the qualification and semi-quantification methods was verified. The trace compounds in the impurities contained in MA seizures were identified and the statistical technique to elucidate the correlations among the synthesis route, supply source, trafficking route, and drug seizures was defined.

2. Experimental Procedures

2.1. Reagents and apparatus

The standard materials, *d*-MA, *l*-MA, *d,l*-MA, and *d,l*-amphetamine, were purchased from Cerilliant (Austin, TX, USA) and nonacosane, used as the internal standard (IS) material, was purchased from Sigma-Aldrich (St. Louis, MO, USA). The standard materials for the main impurities in MA, N-formylmethamphetamine, N-formylephedrine, N-acetylamphetamine, N-acetylephedrine, N,O-diacetylephedrine, cis-3,4-dimethyl-5-phenyl-2- oxazolidinone, trans-3,4-dimethyl-5-phenyl-2-oxazolidinone, chloroephedrine, cis-/trans-1,2-dimethyl-3-phenylaziridine, and (1S,2S)-1-methylamino-1-phenyl-2-chloropropane, were each produced according to the respective synthetic procedures by the Daegu Center, Korea Basic Science Institute. In addition, *l*-ephedrine (99 %), *d*-pseudoephedrine (99 %), thionyl chloride (≥99 %), (S)-(-)-N-(trifluoroacetyl)prolyl chloride, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl were purchased from Sigma-Aldrich. The solvents, ethyl acetate and hexane, were of HPLC grade and purchased from J.T. Baker/Avantor (Center Valley, PA, USA) and distilled water (LiChrosolv grade) was purchased from Merck (Darmstadt, Germany). All other reagents were of ACS grade and the polypropylene centrifuge tubes (5.0 mL) were purchased from Eppendorf (Hamburg, Germany).

Nonacosane, as the internal standard material, was dissolved in hexane and diluted to the final concentration of 10 μg/mL by the addition of ethyl acetate. The IS solutions were placed in sealed containers to prevent leaks and stored at -20 °C for subsequent use.

2.2. MA seizures

MA seizures were obtained from Narcotics Departments at the District Prosecutors' Offices and, among them, 71 samples were used in subsequent analyses. To evaluate the performance of the classification models, the dataset was split into k number of training and testing subsets for K-fold cross-validation with repetitive assessments and the data sets were composed based on the features and the output from the 71 samples applied to the classification models.

2.3. Instrumental analysis

For MA impurity profiling, the 7890 Series Gas Chromatograph-Flame Ionization Detector/5975C Mass Selective Detector (Agilent, Santa Clara, CA, USA) was used and the 7683B series dual injector was used as the automated sample injector. The analytical column was the DB-5MS (30 m × 0.32 mm I.D., 1.0 μm film thickness, Agilent). The flow rate of the carrier gas (He) was 4.0 mL/min and the FID flow rate was set to 5.0 mL/min. The GC column oven temperature was maintained at 40 °C

for 1 min, then increased to 100 °C at the rate of 5 °C/min, and up to 300 °C at 10 °C/min, after which the temperature was maintained for 4 min. The temperatures of the injector and detector were set to 250 °C and 280 °C, respectively. A 2 μL sample was injected in the splitless mode (purge-on time, 1.0 min). At the detection condition for each component, hydrogen gas (30 mL/min) and air (300 mL/min) were used at the set temperature of 300 °C for FID and, for MS, the analyses involved the scan mode (*m/z* 40–500) at the electron ionization mode (70 eV).

2.4. Sample preparation

Each MA seizure (50 mg) was weighed, placed in a centrifuge tube, and dissolved in 2 mL of potassium phosphate buffer (0.1 M, pH 7.0), after which the pH was adjusted by the addition of 0.25 mL of 10 % Na_2CO_3 solution. To this, 0.5 mL of ethyl acetate that contained 10 μg/mL nonacosane (IS) was added, followed by 10 min of shaking for extraction and 10 min of ultracentrifugation at 20,000 *g*. Afterward, 2 μL of the supernatant was injected into the GC-FID/MS for analysis.

2.5. Impurity profiling

For impurity profiling, 71 MA seizure samples were analyzed. The peaks on each of the chromatograms were compared to select 166 compounds that were common across the samples or representative of the properties of MA. The selected compounds were defined as impurities.

These impurities were α-benzyl-N-methylphenethylamine, Leuckart route-specific; (1S,2S)-1-methylamino-1-phenyl-2-chloropropane and 1-dimethylamino-1-phenyl-2-chloropropane, specific to the metal-catalyzed hydrogenation method; diastereomers of N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine and N-methyl-1-{4-[2-(methylamino) propyl]phenyl}-1-phenylpropane-2-amine, Emde route-specific; and 1,3-dimethyl-2-phenylnaphthalene and 1-benzyl-3-methylnaphthalene, Nagai route-specific.

2.6. Chemometric analysis

For chemometric analyses, unsupervised techniques (PCA, HCA, and K-means clustering) and supervised techniques (PLS-DA, OPLS-DA, SVM, and DNN) were used for the prediction models. To develop the classification model for the statistical analyses of unsupervised techniques, PCA and HCA were carried out using SIMCA (ver. 13.0.3.0, Umetrics AB, Umeå, Sweden) and Scikit-learn and Matplotlib libraries based on Python 3.8.0 (https://www.python.org) were used for K-means clustering. The models based on PCA allowed the visualization of 166 impurity compounds by converting them to two-dimensional data through dimensionality reduction and, for the cluster distance estimation in HCA, Ward's linkage method was used. The data with reduced dimensions after PCA were used for the K-means clustering. The supervised classification model is a statistical method in which, from the multivariate observed values in several known groups, a model is built for each group to represent the characteristics of the group, Then, to which group a new observed value should be assigned is determined. In this study, the 166 route-specific impurity compounds selected by analyzing 71 samples were used to create three groups. To develop a supervised model, SIMCA was used to carry out PLS-DA and OPLS-DA. Then, SVM was carried out using Python-based Scikit-learn and Matplotlib libraries and Python TensorFlow-based Keras was used to carry out DNN. For the boundary type in SVM, a linear structure was applied and the cost (C), a variable that regulates overfitting among data, was set to 10. The DNN was composed of one input layer, three hidden layers, and one output layer. The activation function in the input layer used the rectified linear unit, whereas that in the output layer used softmax. The settings were epochs = 1,000 and batch size = 10 and, for the loss function and optimizer function, categorical cross-entropy and Adam were used.

3. Results and Discussion

3.1. Impurity analysis

In chemometric analyses, the first step is the screening of peaks that represent the characteristics

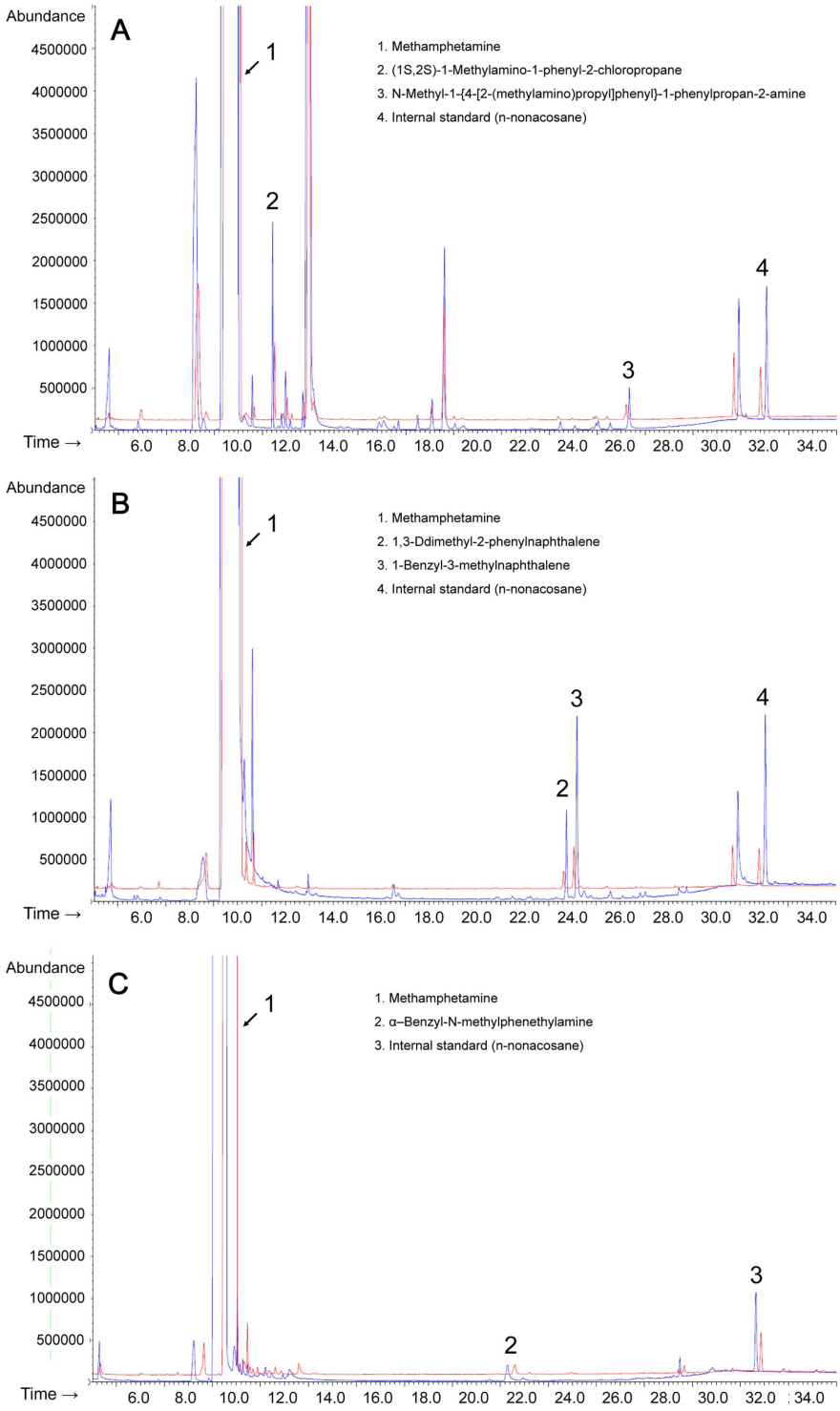

Fig. 1. Representative GC-MS TIC (blue) and GC-FID (red) chromatograms of seized MA via (A) Emde, (B) Nagai and (C) Leuckart routes.

of a given material on the chromatogram. A more accurate result, based on analogy, can be obtained from statistical analyses with a larger number of components that specify the pattern of each sample. Thus, to identify as many characteristic materials as possible, this study optimized the data extraction method and used a device that consisted of GC with attached MS and FID detectors to examine peak components, and the chromatograms obtained from FID with least variability to the impurity profiling were applied. For the selection of impurity compounds that displayed unique properties, the results of the analysis of 71 MA samples were each checked manually for whether they matched the peak components and each sample was compared with the detected peak to select common or distinct peaks as the impurity.

The production method-specific impurities were α-benzyl-N-methylphenethylamine by the Leuckart route, (1S,2S)-1-methylamino-1-phenyl-2-chloropropane and N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropane-2-amine by the Emde route, and 1,3-dimethyl-2-phenylnaphthalene and 1-benzyl-3-methylnaphthalene by the Nagai route. In addition, with the starting materials ephedrine, pseudoephedrine, and methylephedrine, 1,3-dimethyl-2-phenylnaphthalene, 1-benzyl-3-methylnaphthalene, α-benzyl-N-methylphenethylamine, the diastereomers of N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine, (1S,2S)-1-methylamino-1-phenyl-2-chloropropane, and N-methyl-1-4-[2-(methylamino)propyl]phenyl-1-phenylpropane-2-amine were identified. These impurities were closely associated with the production method and the previously determined specific impurities led to the division of 166 impurity compounds into three groups depending on the impurity.

Fig. 1 shows the GC-MS and GC-FID chromatograms that display MA impurity compounds, for which the chromatographic impurity profile that represent the MA seizures in each group corresponding to the three production methods are indicated.

3.2. Unsupervised clustering analysis

For unsupervised statistical analyses, PCA, HCA, and K-means clustering were carried out. PCA ran for 166 impurity compounds from 71 MA seizure samples. The SIMCA program was used and, across all compounds obtained via the covariance eigenvalue decomposition, the correlation between the principal component PC-1 with the largest dispersion and principal component PC-2 with the largest dispersion among those orthogonal to the first data is indicated in *Fig.* 2. PCA was used to generate mutually independent principal components through the mathematical linear combination of original variables and, based on this, the observed values located close to the space with the reduced dimension were shown to exhibit similar properties. HCA was also carried out using the SIMCA program and, by grouping close individuals and expressing them as a dendrogram, the results of HCA can be intuitively examined (*Fig.* 3). HCA was suitable in cases with a small number of analytical samples and an increase in sample number reduced the intuitiveness and halved the utility of the analysis. Next, for the quantified values of impurity compounds of MA seizure samples, K-means clustering

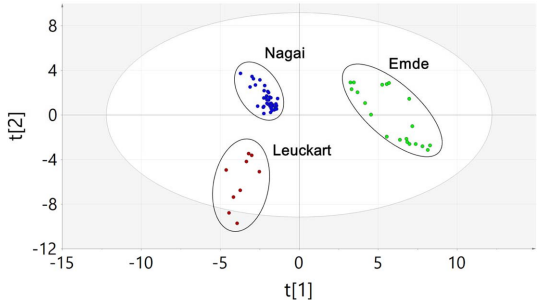

Fig. 2. PCA with PC-1 and 2 for impurities in MA samples.

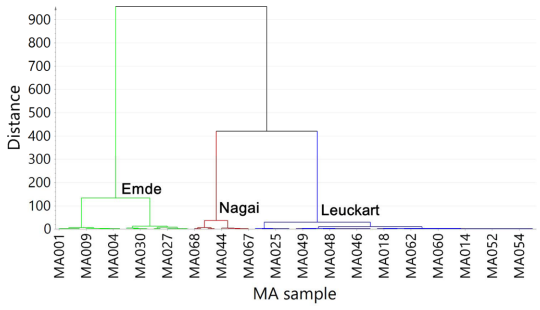

Fig. 3. Dendrogram of HCA for impurities in MA samples.

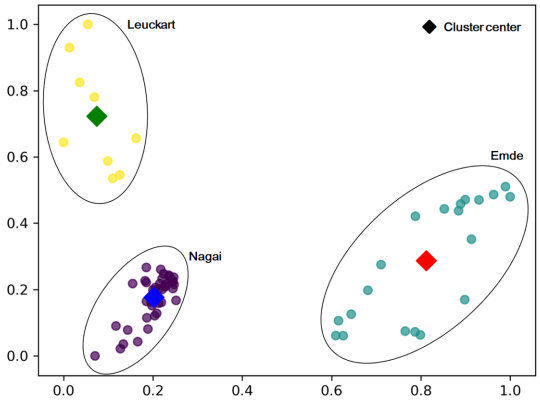

Fig. 4. K-means clustering for impurities in MA samples.

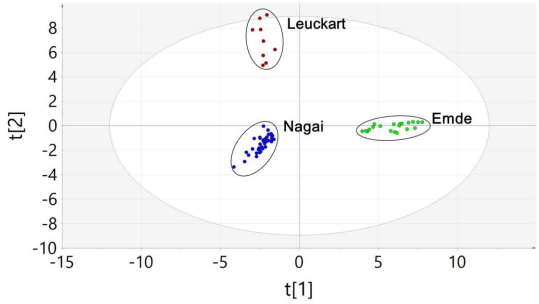

Fig. 5. PLS-DA with results of the classification of MA samples ($R^2X = 0.246$, $R^2Y = 0.942$, $Q^2 = 0.920$).

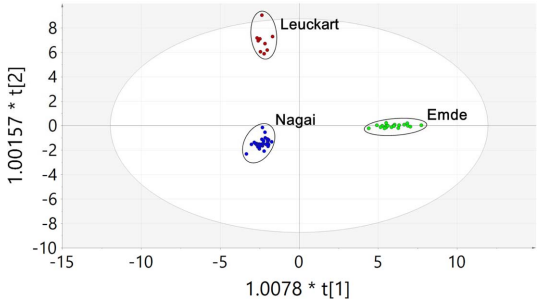

Fig. 6. OPLS-DA with results of the classification of MA samples ($R^2X = 0.361$, $R^2Y = 0.979$, $Q^2 = 0.964$).

and visualization were carried out in the Scikit-learn and Matplotlib libraries for Python. As a non-hierarchical clustering analysis, K-means clustering uses an individual located furthest from the cluster center at the onset as the initial point and, by assigning this data point to the point closest to the cluster center and estimating the mean value of the assigned data point, the center is reset. This process is repeated until the data point displays no further change. Here, K indicates the number of clusters, which is usually set to 3–10. The quantified values of impurity compounds of 71 MA seizure samples were used as the input and the analysis was carried out at K = 3, the result of which is presented in *Fig.* 4. The advantages of K-means clustering are the rapid calculations and utility to classify the clusters based on a large dataset.

3.3. Supervised classification analysis

To develop a classification model for MA impurity profiling that exhibits a high level of similarity to the set classification model, PLS-DA and OPLS-DA were carried out using the SIMCA program and machine learning SVM was carried out using Python-based Scikit-learn libraries. For DNN, TensorFlow-based Keras was used.

For the quantified values of 166 impurity compounds of 71 MA seizure samples, PLS-DA and OPLS-DA results are presented in *Fig.* 5 and *Fig.* 6, respectively. PLS-DA is advantageous for the identification of new variables through the simultaneous consideration of the independent and dependent variables and deduction of a correlation based on the identified variables. OPLS-DA is a highly useful method in the search for variables that contribute to the construction and determination of prediction models. It is an analytic tool that is suitable for the search for variables that display cluster differences and optimization of the visualized result of each data point that contributes to the determination by assigning the dependent variables of a set based on the classification process. The results of PLS-DA and OPLS-DA showed that the data can be categorized into three groups and the variable importance in the projection (VIP) score of OPLS-DA showed specific impurity candidates (*Fig.* 7). Among the impurities with a significant VIP score ≥1, those specific to the production method were (1S,2S)-1-methylamino-1-phenyl-2-chloropropane and N-methyl-1-4-[2-(methylamino)propyl]phenyl-1-phenyl propane-2-amine by the Emde route, 3-dimethyl-2-phenyl naphthalene and 1-benzyl-3-methylnaphthalene by the Nagai route, and α-benzyl-N-methylphenethylamine by

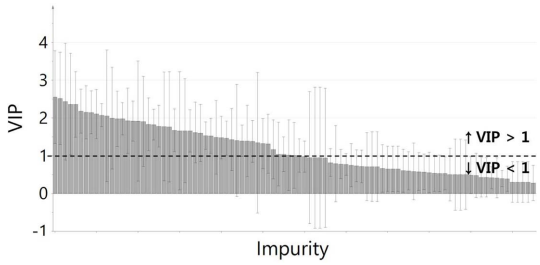

Fig. 7. OPLS-VIP for the variables of MA data set.

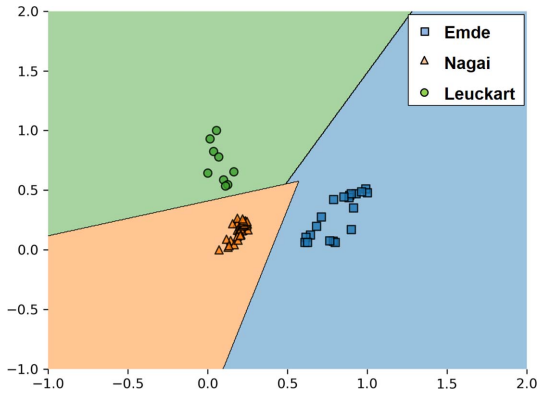

Fig. 8. SVM with results of the classification of MA samples ($R^2 = 1$).

Model: "sequential_7"

Layer (type)	Output Shape	Param #
dense_25 (Dense)	(None, 60)	10020
dense_26 (Dense)	(None, 60)	3660
dense_27 (Dense)	(None, 60)	3660
dense_28 (Dense)	(None, 3)	183

Total params: 17,523
Trainable params: 17,523
Non-trainable params: 0

DNN Mean accuracy: 94.29

DNN Mean loss: 23.50

Fig. 9. DNN with results of the classification of MA samples.

the Leuckart route. These results supported the results of impurity profiling. Furthermore, SVM was carried out, as a method that used to be applied prior to deep learning and would ensure significant results based on the amount of data. SVM visualization is presented in *Fig*. 8. Using OPLS-DA visualization, the SVM result showed that the samples were divided among the three production methods without an overlap. The result of DNN using TensorFlow-based Keras is presented in *Fig*. 9, in which the accuracy of the analytical model is indicated. The DNN result ran the calculations by incorporating characteristics such as sample number and training frequency.[37,38]

Table 1. K-fold cross-validation testing accuracy (%) from four different classifier

Classifier	Accuracy (%)
PLS-DA (k = 7)	92.0
OPLS-DA (k = 7)	96.4
SVM (k = 7)	100
DNN	94.3

PLS-DA: partial least squares-discriminant analysis; OPLS-DA: orthogonal partial least squares-discriminant analysis; SVM: support vector machines; DNN: deep neural network.

3.4. Comparison of accuracy among classification models

To verify the accuracy (%) of the supervised classification models, cross-validation was carried out to classify the models. The cross-validation ran 7 times for 7 groups, which were randomly categorized for the 71 sets of MA data that constituted the classification model. SVM exhibited the highest accuracy for the impurity profiling for MA seizures. The results of the cross-validation are shown in *Table* 1. The accuracies were: PLS-DA 92.0 %; OPLS-DA 96.4 %; SVM 100 %; and DNN 94.3%. The comparative analysis of the cross-validation results showed that SVM, with the highest accuracy, was the most suitable classification model for MA impurity profiling.

4. Conclusions

This study analyzed the impurities in the smuggled or distributed MA seizures and, using six methods, conducted a chemometric analysis. Specific impurities were selected to determine the prediction model and the analyses were carried out using unsupervised

clustering and supervised classification models. PCA was used to characterize the unique properties of each sample and HCA was used to realize a dendrogram of the hierarchical structure for the intuitive division of clusters. Furthermore, four classification models were applied to the result of MA impurity profiling to obtain data based on the predictive values of K-fold cross-validation. The comparison and review of these values showed that SVM had the highest accuracy among the models; thus, SVM is the suitable model for MA impurity profiling. The results of this study are anticipated to assist in the investigation of drug offenders who distribute MA from overseas in Korea. To optimize the analytical models, further studies should continue to focus on the discovery of specific MA impurities and acquire additional MA seizure samples.

Acknowledgements

This work was partially supported by the Bio & Medical Technology Development Program of the National Research Foundation of Korea (NRF) and funded by the Korean government (MSIT) (NRF-2015M3A9E1028325).

Supplemental Material

The Python script for generating the models is available at https://github.com/paxus11.

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