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Abstract

Large quantities of illicit drugs are frequently seized by law enforcement. In such cases, a representative number of samples needs to be quickly examined prior to destruction. No procedure has yet been set up which rapidly provides information regarding the homogeneity of the samples, the presence of controlled substances and the degree of purity.
This study establishes a protocol for fast analysis of cocaine and its most common cutting agent, levamisole, in large seizures. The protocol is based on a hypergeometric sampling approach combined with FTIR spectrometry and Support Vector Machines (SVM) algorithms as analysis methods.
To demonstrate the practical use of this approach, five large cocaine seizures (consisting between 45 and 85 units) were analysed simultaneously with GC-MS, GC-FID and a portable FTIR spectrometer using Attenuated Total Reflectance (ATR) sampling combined with SVM models.
According to the hypergeometric sampling plan of the DWG ENFSI guidelines, the required number of subsamples ranged between 19 and 23.
Considering the identification analyses, the SVM models detected cocaine and levamisole in all subsamples of cases 1 to 5 (100% correct classification), which was confirmed by GC-MS analysis.
Considering the quantification analyses, the SVM models were able to estimate the cocaine and levamisole content in each subsample, compared to GC-FID data.
The developed strategy is easy, cost effective and provides immediate information about both the presence and concentration of cocaine and levamisole. By using this new strategy, the number of confirmation analyses with expensive chromatographic techniques could be significantly reduced.

Introduction

Forensic laboratories often receive large seizures of cocaine which need to be analysed fast prior to destruction. Typically, cocaine seizures are already cut with levamisole when entering Belgium and the average amount of levamisole ranges between 5 and 25 w% for 95% of the samples. Levamisole is not an innocent cutting agent, use of levamisole-laced cocaine is associated with medical harms[1–9]. Moreover, the biotransformation product of levamisole, aminorex, has psychoactive and severe side effects, potentially adding to the cocaine effects[8].

Levamisole is not routinely identified and is rarely quantified by forensic laboratories[10]. In addition to the identification of controlled substances, the degree of purity is also often requested for law enforcement purposes.

In the case of large seizures, chromatographic analysis of the complete seizure is not an option considering high costs and time efficiency. Consequently, sample reduction is desirable. The Drugs Working Group (DWG) of the European Network of Forensic Science Institutes (ENFSI) developed guidelines on representative drug sampling describing three sampling strategies (hypergeometric, binomial and Bayesian) in the case of large seizures of relatively homogeneous material[11]. With the hypergeometric distribution the required number of samples can be calculated in order to declare a certain percentage of the seizure positive for drugs with a certain confidence level[11]. However, the hypergeometric method is quite rigid and still needs a large number of samples to be analysed. For example, for 100 packages, 23 packages have to be sampled (if no negatives in the seizure are assumed) to guarantee with 95% confidence that at least 90% of the packages contains the same substance[11].

In an attempt to further reduce the workload, time and cost of analyses, this paper illustrates one sampling approach for the fast classification and quantification of cocaine and levamisole, by combining Fourier Transform Infrared (FTIR) spectrometry and a chemometric algorithm, called Support Vector Machines (SVM). The applicability of this approach is presented by five case studies of large seizures. Compared to conventional methods, this high throughput approach generates fast, accurate information about the homogeneity of units, the drug presence and purity, with a minimum of confirmation analyses.

Experimental

Cases/sampling

Five cocaine seizures of 2015 were analysed (Table 1). Four cases consisted of rectangular blocks of pressed powder with an average weight of 1 kg per block. One case consisted of oval-shaped blocks wrapped in several layers of plastic (body packages with an average weight of 15 g per package).

For each seizure the number of samples was calculated by using the hypergeometric distribution of the DWG-ENFSI sampling calculator[11] with the following parameters: expected proportions of positives in the population k = 0.90; expected number of negatives in the sample = 0 and a confidence level (1-α) of 0.95. The blocks were randomly sampled on

three locations (± 1 g per sampling). For the body packs, 1 g powder was randomly taken and homogenized.

Analysis of cocaine seizures

ATR-FTIR spectra were acquired in absorbance mode (4000-500 cm^{-1}, 24 scans, resolution of 4 cm^{-1}), using a portable FTIR spectrometer with a single reflection diamond crystal ATR accessory with pressure applicator (Bruker Corporation, Ettlingen, Germany)[12]. Accredited GC-MS and GC-FID analysis (Agilent Technologies, Santa Clara, CA, USA) were used as reference methods for the identification and quantification of the powders[12].

Chemometric analysis

Full ATR-FTIR spectra were pre-processed using standard normal variate[13]. Four SVM models were developed in PLS Toolbox (Eigenvector Research Inc., Manson, WA, USA). SVM Discriminant Analysis (SVM-DA)[14–16] was used to build two classification models, one for cocaine and one for levamisole detection. SVM Regression (SVMR)[14,15] was used to build two quantification models, one for cocaine and one for levamisole.

For the cocaine classification model, the calibration dataset included 515 spectra of 481 authentic street samples (377 with cocaine and 104 without cocaine) and 34 reference materials[12].

For the cocaine quantification model, the calibration set consisted only of the 377 cocaine hydrochloride containing samples seized during the period from January 2013 to July 2015 (4 to 99 w%)[12].

The classification dataset for levamisole consisted of 385 spectra (249 street samples with levamisole, 136 samples and reference material without levamisole).

The quantification dataset for levamisole contained 249 spectra of representative samples containing levamisole hydrochloride (5 to 78 w%; median 10 w% ± 12), seized during the period from January 2013 to July 2015.

In order to determine the model parameters, including the number of support vectors (SVs), double cross-validations (with five random subsets and one iteration) were performed[12]. The parameters of the SVM-DA calibration models were the following: for cocaine: SVs = 16 and cost = 0.32 and for levamisole: SVs = 15 and cost = 0.32.

The parameters of the SVMR calibration models were the following: for cocaine SVs = 237, cost = 0.03 and epsilon = 0.10 and for levamisole: SVs = 244, cost = 0.03 and epsilon = 0.01.

The root mean squared error of prediction (RMSEP) and correlation coefficients (R^2) were used to evaluate the agreement between SVMR and GC-FID results[12].

Results and discussion

Representative sampling of five large seizures was performed using the hypergeometric approach. Table 1 describes each case in terms of the number of seized samples (N samples) and the required number of subsamples (N subsamples).

Cocaine and levamisole were identified in all subsamples of cases 1 to 5 with SVM-DA, which was in agreement with the GC-MS results. Based on these results it can be concluded that 100% of the subsamples were correctly classified with the SVM-DA models.

Tables 1 and 2 show an overview of the quantitative results (GC-FID and SVMR) for cocaine and levamisole, respectively. For each case, the mean and standard deviation (SD) are presented. For cases 1 to 5, 19 to 23 subsamples were analysed. The mean purity of cocaine ranged between 56 and 96 w%, determined by GC-FID. With the SVMR model, the cocaine content was predicted between 63 and 88 w% (Table 1). The mean GC-FID concentrations of levamisole ranged between <1 and 46 w%, compared to 8 and 30 w% predicted with the SVMR model (Table 2). Figure 1 and 2 illustrate the individual results for each case.
As shown in these figures, GC-FID and SVMR showed a high correlation (for cocaine: R^2 = 0.88 and for levamisole: R^2 = 0.91).
For cases 1 to 3, a good agreement between the GC-FID values and the SVMR values was observed (RMSEP ≤ 5% for cocaine and ≤ 6% for levamisole), no substantial differences were found (Figure 1 and 2; Table 1 and 2).
For case 4, SVMR showed two subgroups within the seizure, which was confirmed by GC-FID (RMSEP 4% for cocaine and ≤ 5% for levamisole). The cocaine and levamisole content differed in 2 out of the 21 subsamples. For cocaine, the GC-FID concentrations ranged from 72 to 80 w% for 19 samples and from 93 to 98 w% for the other two subsamples. With SVMR analysis, the same two groups could be distinguished (cocaine concentrations ranging from 74 to 83 w% (n=19) versus 87 to 90 w% (n=2)). Also the levamisole concentrations related in the same way from 13 to 21 w% (n=19) versus <1 w% (n=2)) for GC-FID and from 12 to 18 w% (n=19) versus 8 w% (n=2) for SVMR.
Concerning the two subsamples, the levamisole concentration predicted with the SVMR model was around 8 w% for both samples, while less than 1 w% levamisole was measured with GC-FID. This can be explained by the fact that not enough samples were present in the calibration dataset between 1 and 10 w%. As previously described, the SVM-DA model detected levamisole in these two samples, despite of the very low concentration of levamisole.
For case 5, GC-FID cocaine concentrations ranged between 55 and 57 w%, while the SVMR cocaine concentrations ranged from 59 to 69 w% (Table 1 and Figure 1). For levamisole, GC-FID concentrations ranged from 45 to 48 w%, compared to SVMR (20 to 38 w%)(Table 2 and Figure 2). Summarized, the SVMR results showed a higher variation in levamisole content between the subsamples (RMSEP of 15% for levamisole compared to 8% for cocaine). This discrepancy could imply that there were two subgroups of samples. However, when considering the GC-FID results, all subsamples had similar concentrations.
A possible explanation for this discrepancy could be the intrinsic heterogeneity of the mother sample (body pack). To verify whether the sampling procedure influenced the results, 9 additional body packs from the same seizure were sampled (homogenisation of the complete body pack instead of a 1 g sample). After complete homogenisation, the results of these 9 additional analyses were almost the same as the initial 23 subsamples: with SVMR (cocaine: 62 ± 2 w% and levamisole: 27 ± 1 w%) compared to GC-FID (cocaine: 56 ± 1 w% and levamisole: 47 ± 1 w%). No improvement in the predicted levamisole concentrations was

observed. However, the variability in the quantitative SVMR results of the subsamples (expressed as SD) was significantly lower than in the previous experiments. This means that there was an influence of the sample homogeneity on the dispersion, but not on the estimated concentrations.

Subsequently, a limitation of the calibration models could be a possible explanation. It should be pointed out that a 50:50 w% cocaine levamisole mixture, the composition of the case 5, was not present in the calibration dataset. The typical range of levamisole in cocaine samples is between 5 and 25 w% for 95% of the samples. Consequently, the calibration models were not fitted and there was no extra data available for optimisation. When extending the calibration models with the spectra of the 9 additional body packages, predictions for cocaine (57 ± 3 w%) and levamisole (39 ± 3 w%) were improved and the concentrations were in agreement with GC-FID. It should be emphasized that the models can be updated each time new samples, which are outside the current concentration range, appear.

Overall, it was observed that most of the predicted SVMR values were situated within the measurement uncertainty range (6.4% relatively) of the GC-FID method. Therefore, the differences between GC-FID and SVMR are practically insignificant. Moreover, considering the typical heterogeneity of a cocaine sample (approximately 10% sampling RSD at a 1 gram level)[17], the obtained quantitative results can be considered fit for purpose.

For cases 1 to 3, the subsamples were homogenous and representative for the whole seizure according to the SVM data, which was in accordance with the chromatographic data. Based on the results of cases 1 to 3, the number of samples could be reduced to one confirmation analysis by GC-MS and GC-FID. This would have been sufficient to undoubtedly identify and quantify the controlled substance, cocaine, since the same conclusions with less workload, time and costs would be reached. For cases 4 to 5, the subsamples were heterogeneous according to the SVM data and two subgroups were identified. For case 4, the SVM data were in agreement with the chromatographic results, in contrast to case 5. Therefore, two confirmation analyses (1 subsample of each subgroup) by GC-MS and GC-FID should be performed for cases 4 and 5. An important advantage of the proposed approach is the possibility of on-site sampling and measuring since the spectrometer with SVM models is portable. For example, in case 4 when noticing heterogeneity of the samples, the number of subsamples could be increased.

Another advantage is that in the case of inconclusive SVM results more confirmation analyses can be performed.

Conclusion

The ATR-FTIR technique combined with SVM provided immediate, reliable information about the homogeneity of units and the drug presence and purity. It allowed to successfully reduce the number of samples needed for confirmation analysis. Nevertheless, the proposed strategy maintains a sufficiently high confidence for conclusions in court. Moreover, the detection and quantification of levamisole, the most important cutting agent in cocaine street samples, can also be of interest for forensic and/or health purposes.

In future, the strategy can be used for sampling and analysis of other controlled substances.

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TABLES

Table 1: Type of samples, number of samples, number of subsamples and quantitative results of cocaine (cases 1 to 5).

Case	**Type**	**N samples**	**N subsamples**		**GC-FID**	**SVMR**
					mean ± SD (w%)	mean ± SD (w%)
1	block	50	19		77 ± 2	80 ± 3
2	block	45	20		82 ± 2	80 ± 3
3	block	50	19		70 ± 2	75 ± 2
4*	block	62	21	19	76 ± 2	78 ± 2
				2	96 ± 3**	88 ± 2**
5	body pack	85	23		56 ± 1	63 ± 2

(N samples = number of seized samples; N subsamples = number of samples for sampling using the hypergeometric approach; *Case 4 had two subgroups; **SD of only two samples)

Table 2: Type of samples, number of samples, number of subsamples and quantitative results of levamisole (cases 1 to 5).

Case	Type	N samples	N subsamples		GC-FID	SVMR
					mean ± SD (w%)	mean ± SD (w%)
1	block	50	19		16 ± 1	12 ± 1
2	block	45	20		10 ± 2	11 ± 1
3	block	50	19		20 ± 2	15 ± 1
4*	block	62	21	19	18 ± 2	14 ± 2
				2	<1**	8**
5	body pack	85	23		46 ± 1	32 ± 4

(N samples = number of seized samples, N subsamples = number of samples for sampling using the hypergeometric approach; *Case 4 had two subgroups; **SDs were 0)

FIGURES

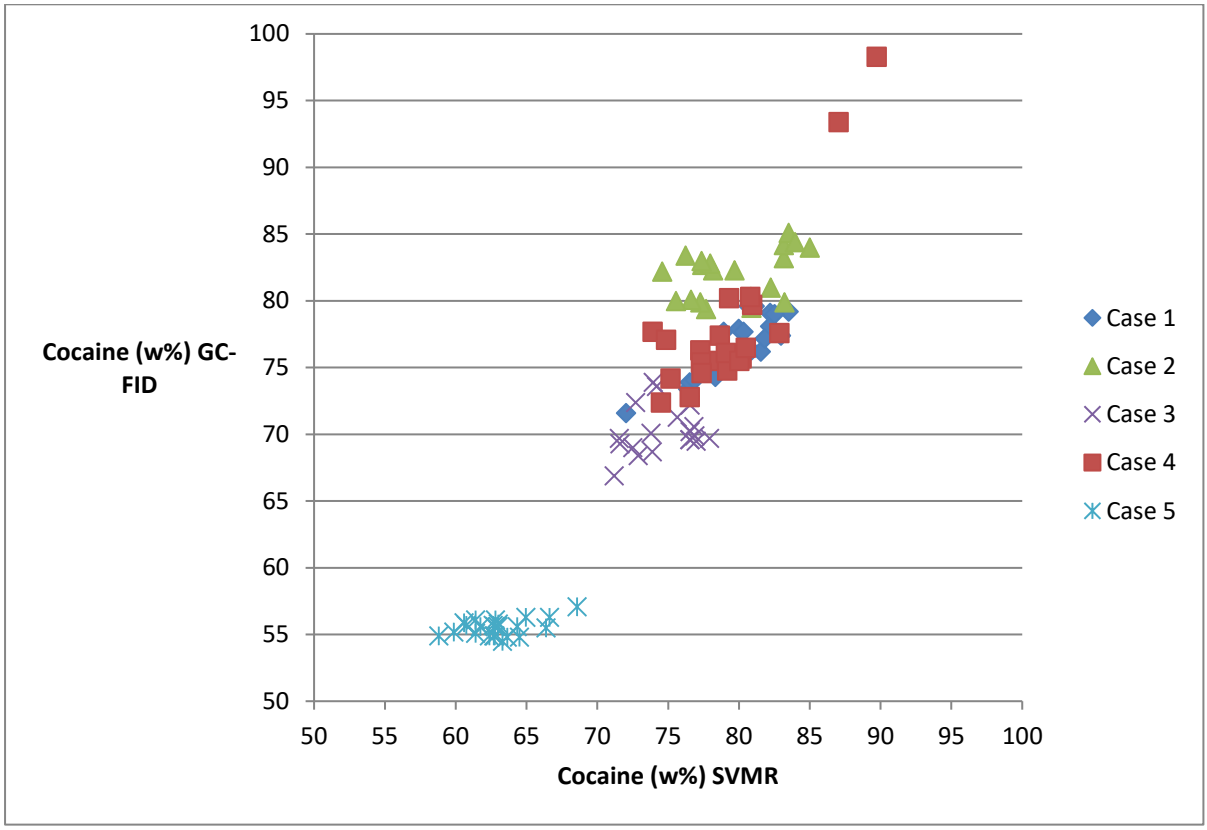

Figure 1: Quantitative results of the five cases with cocaine concentrations measured with GC-FID (y-axis) and predicted by SVMR (x-axis).

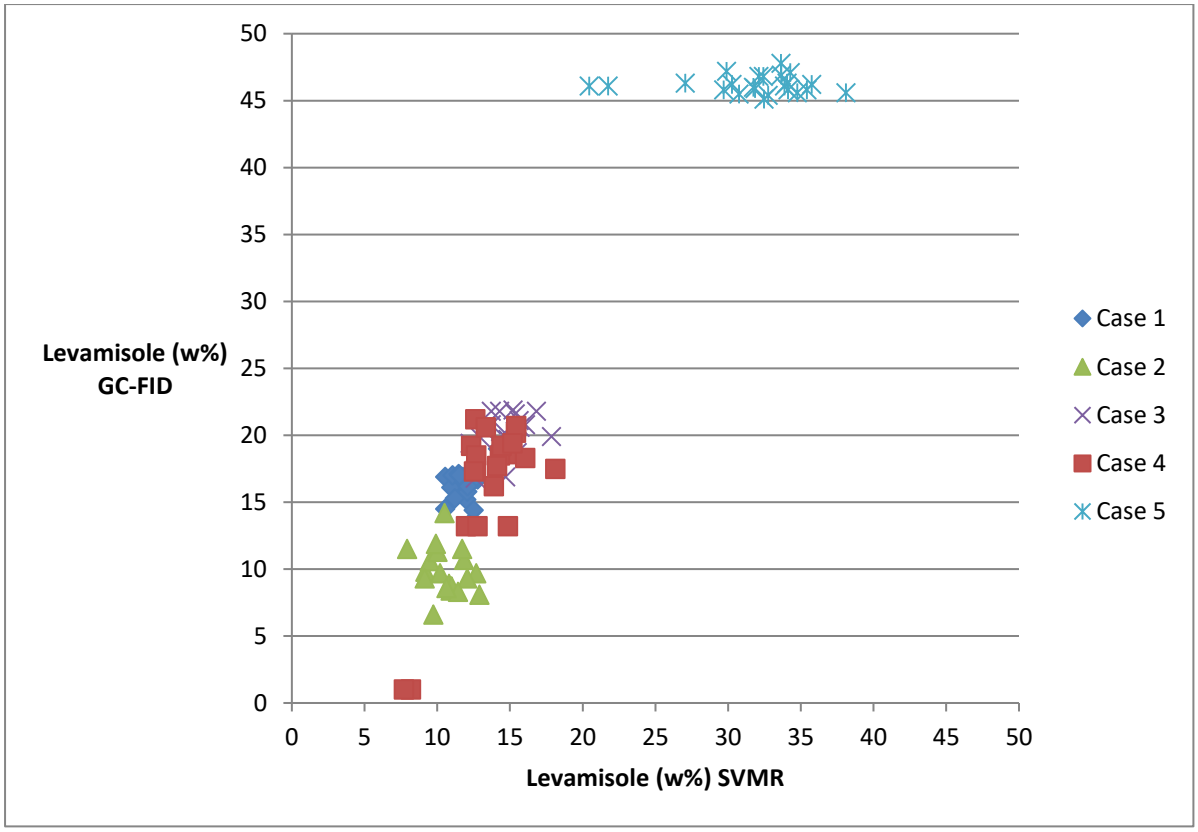

Figure 2: Quantitative results of the five cases with levamisole concentrations measured with GC-FID (y-axis) and predicted by SVMR (x-axis).