EVALUATION OF SAMPLE PREPARATION TECHNIQUES ADEQUATE FOR MULTIVARIATE ALGORITHMS APPLIED FOR THE IDENTIFICATION AND CLASSIFICATION OF AMPHETAMINE ANALOGUES WITH GC-FTIR

Mirela PRAISLER, Inge DIRINCK, Willy LAMBERT,
Andreas DE LEENHEER, Luc MASSART

Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, University of Ghent, Ghent, Belgium

ABSTRACT: Sample preparation techniques were evaluated for street samples as part of an optimization strategy in forming the GC-FTIR knowledge base of an expert system based on Soft Independent Modelling of Class Analogy (SIMCA). The spectra of the nonderivatized amphetamines have larger discrimination power than those of their HFB-derivatives. Significant changes in band profiles are more valuable information for the knowledge base of the expert system, than an increased number of common absorption bands with profiles less sensitive to small molecular structural changes.

INTRODUCTION

Amphetamines are characterized by a basic skeleton containing an aromatic ring, linked by an aliphatic side chain, with one or two carbon atoms, to an amino group. One factor influencing the pharmacological activity of amphetamines is the substitution pattern of the aromatic ring (Figure 1).

The phenyl nonsubstituted amphetamine analogues, like amphetamine (AMP) and methamphetamine (MAMP), have mainly CNS stimulating and anorexinogenic
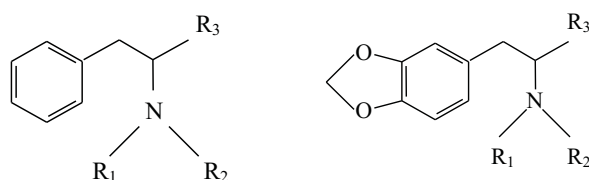

Fig. 1. Molecular structure of the main amphetamine analogues.

properties. The phenyl disubstituted amphetamine analogues, like 3,4-methylenedioxymethamphetamine (MDMA or Ecstasy) or 2,5-dimethoxyamphetamine, possess hallucinogenic and mood-modifying properties in addition to the CNS stimulation [4].

If various tests screening for the main stimulant amphetamines are available, the number of tests screening for hallucinogenic amphetamines is very limited, probably because the latter drugs of abuse do not present medical interest. As a response to this analytical need, we have built an expert system [2, 3, 5, 7] assessing simultaneously the structural similarity of an unknown with the class of phenyl disubstituted hallucinogenic amphetamines or with the class of stimulant amphetamines. The system was built using an in-house made vapor-phase FTIR library developed at the Laboratory of Toxicology, University of Ghent [1]. The similarity between the vapor-phase FTIR spectra of a class of structurally-related compounds was modeled using principal component analysis (PCA) [2, 3, 6], and the class identity was assigned on basis of Soft Independent Modeling of Class Analogy (SIMCA) [6]. The test confirms the structural similarity of the unknown found in illicit tablets or powders with the structural prototypes within limits set on basis of structure-activity relationships corroborated with data about abuse liability and health hazard. The system, as any screening test, is not expected to positively identify amphetamines, but to determine the possibility that members of particular types of amphetamines may be present and lead to more confirmation testing.

EXPERIMENTAL

A Perking Elmer (Buckinghamshire, UK). Autosystem GC was interfaced with a light pipe GC-IR System 2000 and connected to a FTIR System 2000 with a mid-infrared source and a medium band liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. Temperature-programmed separations were carried out on a Hewlett-Packard (Palo Alto, CA, USA) Ultra-1 methylsilicone capillary column (25 m x 0.32 mm i.d., 0.52 μm film thickness). Real time spectra were obtained by addition of two scans, with a spectral resolution of 8 cm^{-1} and 32 background scans. The scan range was from 4000 to 580 cm^{-1}. Chromatograms were calculated by the Gram-Schmidt vector orthogonalization method. Methanolic stock solutions (1.0 mg/ml) of the reference standards were injected into the GC-FTIR system. Gram-Schmidt reconstruction was performed using 10 basis vectors throughout the run. Baseline correction was performed on the reconstructed Gram-Schmidt chromatogram (GS) and low-noise vapor-phase FTIR spectra were generated after co-addition. The obtained reference vapor-phase FTIR spectra were normalized and stored, at 5 cm-1 intervals, in a computer-based library.

The training data matrix representing the knowledge-base of the expert system contained the training sets consisting of spectra of stimulant amphetamine analogues (class code M), of hallucinogenic amphetamine analogues (class code T), of their HFB-derivatives (class code DM and DT, respectively), and of counter-examples (class code N).

Each of the five classes was modeled using principal component analysis (PCA). Data were mean-centered and scaled using the variables' standard deviation. The validation method was full cross-validation. A number of three principal components (PCs) were used to model each class. Then SIMCA classification was run, with a 5% significance level, for the entire database (spectral library). The knowledge base of the system was optimized by an iterative process enhancing the model true positive rates and maximizing the total correct classification rate. PCA and SIMCA were performed using the software package the Unscrambler® (Camo AS, Sweden).

RESULTS AND DISCUSSION

We have explored the classification results obtained with two types of sample preparations, both yielding analytical advantages potentially relevant to the selectivity of the expert system. The derivatization procedure using heptafluorobutyric anhydride (HFBA) yields HFB-derivatives displaying increased infrared sensitivity and additional absorption bands, associated with the chemical groups added to the molecular structure (R_1 = -CO-$(CF_2)_2$-CF_3). On the other hand, the spectra of the nonderivatized analogues are more selective, FTIR spectra of lower weight molecules being more sensitive to small changes in the molecular structure.

The relevance of these advantages was explored using SIMCA classification. In its optimized form, the expert system classified 81.13% of the 159 tested compounds with a significance level of 5%, and the total correct classification rate was 93.93%. The best selectivity in discriminating among phenyl nonsubstituted amphetamine analogues, 3,4-methylenedioxy- amphetamine analogues, and nonamphetamine compounds was obtained with nonderivatized samples. The classification yielded 96.30% true positive (nonderivatized) amphetamines, and only 85.71% true positive derivatives. The results are due to the fact that the spectra of nonderivatized samples (Figure 2) exhibit a much larger number of strongly discriminating absorptions.

The discrimination power [6] of a wavenumber k distinguishing between model M and T may be quantitatively determined as:

$$P_d = \frac{S_M(T,k)^2 + S_T(T,k)^2}{S_M(M,k)^2 + S_T(T,k)^2},$$

where S_M (T, k) is the standard deviation for variable (wavenumber) k when fitting the spectrum of a sample from the training set of the model M (stimulant amphetamines), onto model T (hallucinogenic amphetamines). The spectral windows with the highest discrimination power (Figure 3) differentiating the stimulant amphetamines from the hallucinogenic amphetamines are 3100-3000 cm^{-1}, where the absorption bands associated with the aryl-CH stretching vibrations appear.

The overtone and combinations bands around 1945 and 1795, as well as the 750–650 cm^{-1} region where the absorption bands associated with the aryl-CH out-of-plane
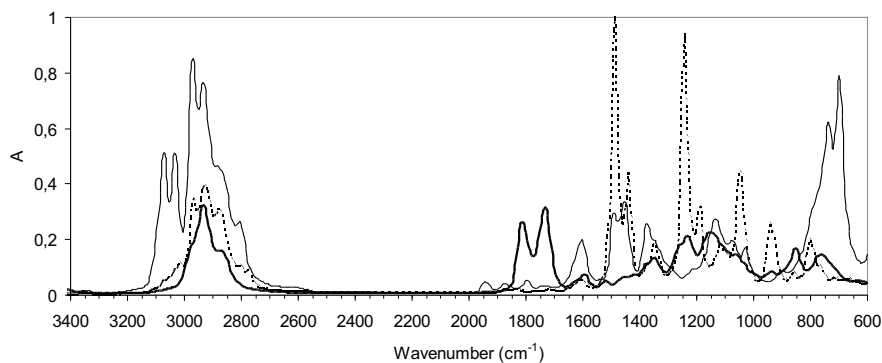
Fig. 2. Mean spectra of stimulant amphetamines (———), of hallucinogenic amphetamines (- - -), and of counterparts (———).

vibrations are found, are a function of the substitution pattern of the phenyl ring. Although the overtone and combination bands are so weak, they contribute to the discrimination as much as the strong aryl-CH out-of-plane bands, showing that the intensity of an absorption is less important for classification (discrimination or recognition) purposes than the stability or the specificity of the band. The discriminating spectral windows are wider than those differentiating stimulant amphetamines from nonamphetamines (1850–1650 cm^{-1}). As a result, no false negative was encountered in the case of the nonderivatized amphetamine analogues. On the other hand, the inactive
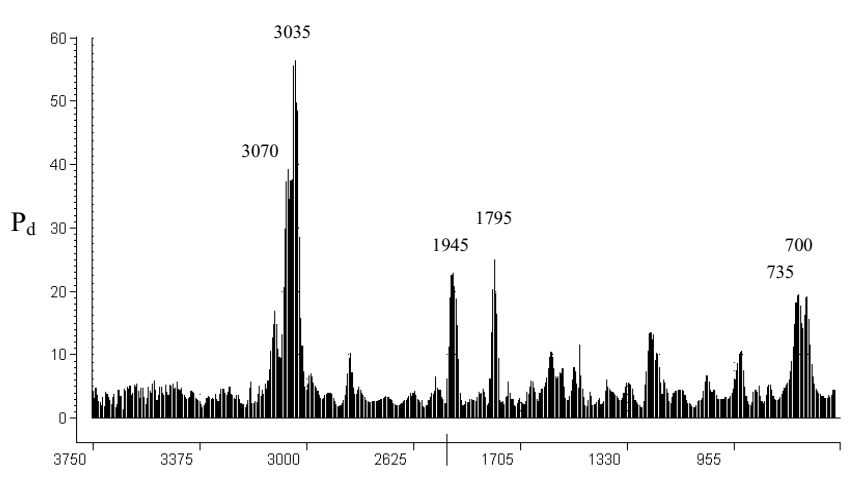

Fig. 3. Discrimination power of the absorptions differentiating stimulants from halucinogens.

precursor safrole was identified as (the only) false T positive. While the M model yielded a correct classification, the T model is thus specific, but less selective.
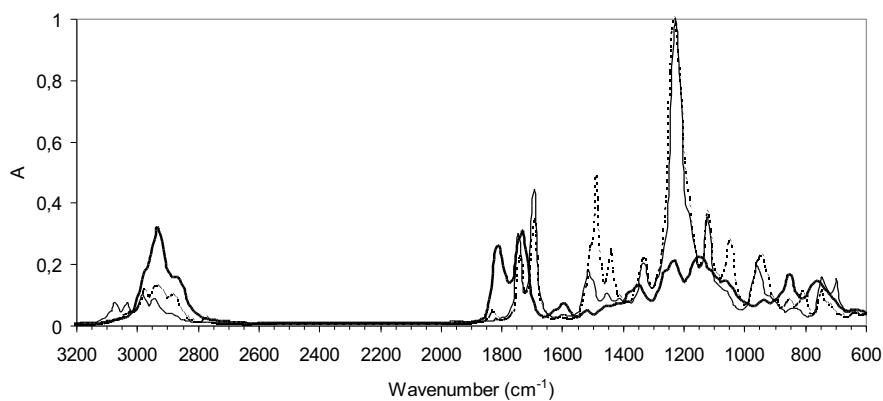

Figure 4. Mean spectra of the HFB–derivatives of stimulant amphetamines (——), of hallucinogenic amphetamines (– – –), and of randomly selected counterparts (——).
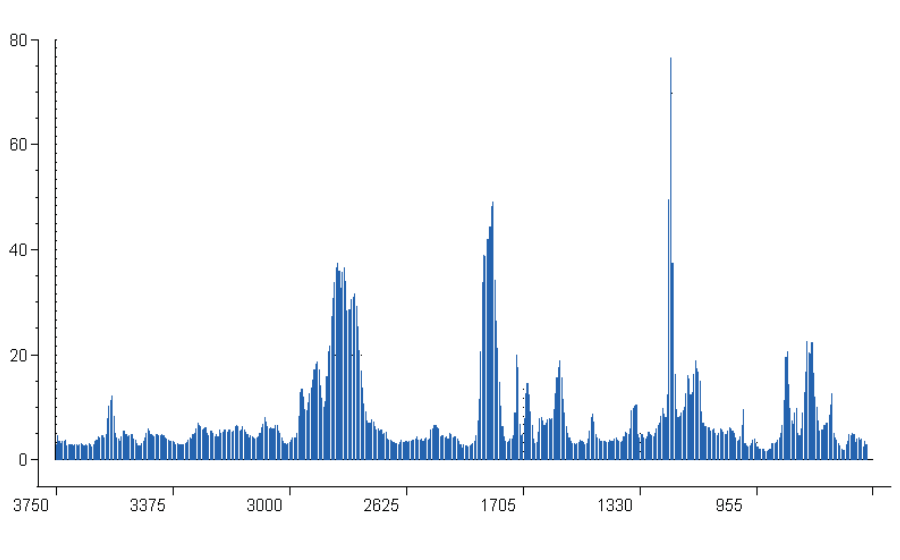

Fig. 5. Discrimination power of the absorptions differentiating the HFB-derivatives of stimulants from counterparts.

The discrimination between the HFB-derivatives of the stimulants and the rest of the compounds is mainly ensured by the band at 1230 cm^{-1} (Figures 4 and 5), associated with the stretching vibrations of the C-F bond. This absorption is very selective in discriminating the HFB-derivatives of stimulant amphetamines from those of nonamphetamine compounds, even when the latter have very similar molecular structures. For example, the derivatives of sympathomimetic amines such as ephedrine-HFB, pseudoephedrine-HFB, N-methylephedrine-HFB, or p-hydroxyephedrine-HFB, are classified as negatives, in the same way as their nonderivatized counterparts are. The band also discriminates the HFB-derivatives of the stimulant amphetamines from other type of derivatives of the amphetamines, such as those obtained with pentafluoropropionyl anhydride (PFPA, R_1 = -CO-CF$_2$-CF$_3$).

On the other hand, derivatization with HFBA changes the molecular size of the amphetamine analogues (and thus the optically active vibrations) to the extent that a significant part of the spectral information about the substitution pattern of the phenyl ring is lost. The C-F discriminating band is very similar in the spectra of the HFB-derivatives of the stimulant and of the hallucinogenic amphetamines. The effect of the substitution of the phenyl ring in the spectra of the HFB-derivatives is only a shift of 5 cm^{-1}, the HFB-derivatives of stimulant amphetamines absorbing at 1230 cm^{-1}, while the HFB-derivatives of hallucinogenic amphetamines absorb at 1235 cm^{-1}. In addition, there are no discriminating intensity variations, as this band is the strongest (A = 1) in the spectra of both DM and DT derivatives. As a result, the spectral window actually used for the recognition of these compounds is very narrow (Figure 5). Because the similarity of the spectra of the HFB-derivatives of amphetamine analogues, as one single class, is relatively too high, slightly atypical absorptions (band shifts) lead to the classification of true HFB-derivatives of amphetamine analogues as negatives. The correct classification rate for HFB-derivatives of amphetamines was lower, in our trials, than the rate of their nonderivatized counterparts only because of false negatives (derivatized amphetamine analogues classified as negatives). While in the case of the nonderivatized amphetamine analogues no false negative was encountered, the DM and DT models are more selective than specific. False negatives were identified, 1-phenyl-2-butanamine-HFB among the HFB-derivatives of phenyl nonsubstituted (stimulant) amphetamines, and 2,5-dimethoxyamphetamine-HFB among the tested phenyl disubstituted (hallucinogenic) amphetamine analogues.

CONCLUSIONS

Sample preparation techniques were evaluated as part of an optimization strategy in forming the GC-FTIR knowledge base of an expert system based on Soft Independent Modeling of Class Analogy (SIMCA). The system assigns an unknown the identity of one of the modeled classes by seeking a pattern-to-pattern match, as opposed to the identification of an individual compound performed using a peak-to-peak match. The

system discriminates the class of amphetamines from the other toxicologically relevant compounds, and differentiates between stimulant amphetamine analogues and hallucinogenic amphetamine analogues. Its output narrows considerably the structure elucidation process, and indicates the most probable type of activity and of toxicity of the unknown.

The FTIR spectral models and their associated discrimination power seem to be strongly and positively influenced by vibrational spectra fuzziness, i.e. the wavenumber variations of the maximum absorption of a band (associated with the vibrations of a given chemical group/structural unit) when the molecular chemical environment changes. The lower specificity of the models built with the spectra of the derivatized amphetamine analogues is the effect of two factors. First, from the spectroscopic point of view, derivatization increases the size of the amphetamine analogues to such an extent that IR spectra become significantly less sensitive to small changes in the molecular structure. The loss in band parameter variation makes interclass separation more difficult. Secondly, from the computational point of view, spectra normalization is a pre-processing technique that counteracts the advantage of derivatized analogues (enhanced IR absorptions). In conclusion, when spectra are normalized, the most reliable classification is obtained with the spectra of amphetamine analogues rather than with those of their derivatives.

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