Supplemental document for identification of illicit drugs and new psychoactive substances by FT-IR ATR technique

ENFSI DWG Quality Assurance Subcommittee
European Project RESPONSE

2016

Co-funded by the Prevention of and Fight against Crime Programme of the European Union

Authors

Gabriella Farkas, István Balatoni
Hungarian Institute for Forensic Sciences, Budapest, Hungary (HIFS)
Mosonyi str. 9., H-1087 Budapest, Hungary
(farkasg@nszkk.gov.hu)
(balatoni.istvan@nszkk.gov.hu)

Reviewers

Tamás Csesztregi, Hungarian Institute for Forensic Sciences, Budapest, Hungary (HIFS)
Ulla-Maija Laakkonen, NBI Forensic laboratory, Vantaa, Finland
Natalie Meert, National Institute for Criminalistics and Criminology, Brussels, Belgium (NICC)

CONTENTS

1 Introduction 3

2 Factors influencing IR spectra of materials 3

2.1 CO_2, water and moisture 3

2.2 Crystal water 5

2.3 Crystallinity, polymorphism 5

2.4 Salts and free-bases 6

2.5 Regio-isomers 7

2.6 Homologues 8

3 Special sample preparation techniques 9

3.1 Extraction methods for tablets and powders 9

3.2 Free-base conversion, extraction 10

3.3 Extraction method for impregnated herbal materials 11

3.4 Alternative methods for analysis of extracts 12

1 Introduction

The FT-IR ATR has become widespread in the recent years because of its simplicity, quickness, the small amount of sample required and the good quality of the spectra. This technique gives the possibility of direct analysis of many different kinds of samples with minimal need of sample preparation. The identification is based on the comparison of the measured spectrum to spectral database using mathematic algorithms. The most frequently applied algorithm is based on the correlation of the spectra of the sample and the reference substances. The library search results show the best matching spectra. The identification highly depends on the quality of the spectra. Since the technique provides analytical information from approximately 10 to 15 micrometers depth of penetration, the measured substances need to contact with the maximum possible surface area of the ATR crystal. For qualitative measurements a spectral range of 650-4000 cm^{-1} or wider and a resolution of 4 cm^{-1} is recommended. In addition to the described settings above, there are also other factors that need to be taken into consideration.

2 Factors influencing IR spectra of materials

2.1 CO_2, water and moisture

The water content of the sample and the presence of atmospheric CO_2 typically cause problems. The range of asymmetric stretching vibration signal of C-O from atmospheric CO_2 is located in the range of 2400-2200 cm^{-1} appearing as two sharp peaks. This occurrence can cause low relative intensity of target signals, which may cause undesired effects on spectral library search. Usually, appearance of CO_2 signal can be traced back to the increase of CO_2 concentration within the beam path during the time between the background and sample measurement (e.g. breathing close to the instrument can cause this effect) (Figure 1).

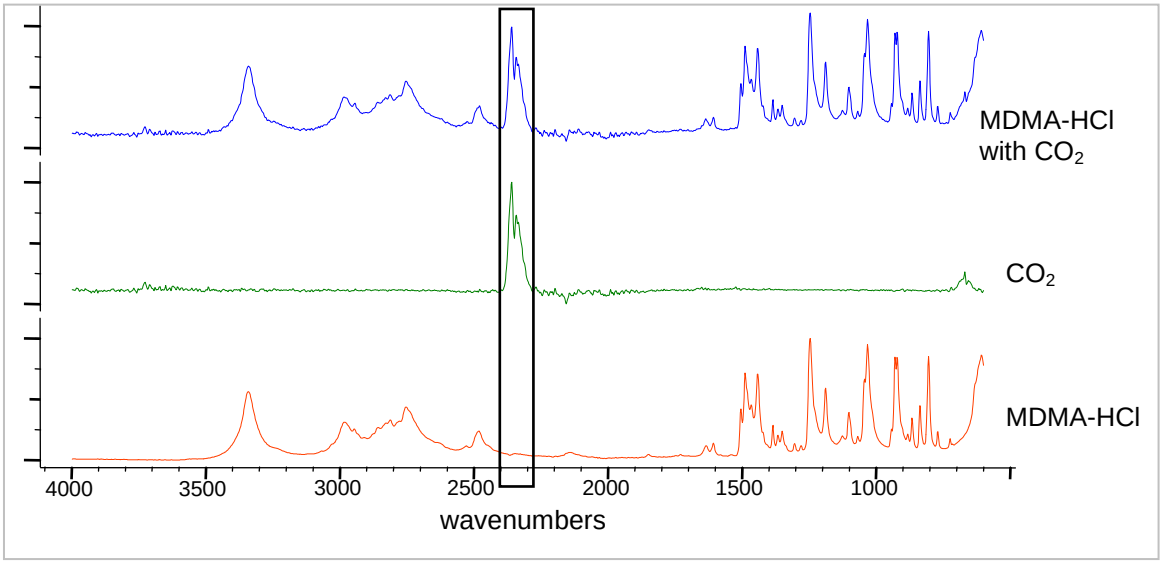


Figure 1.

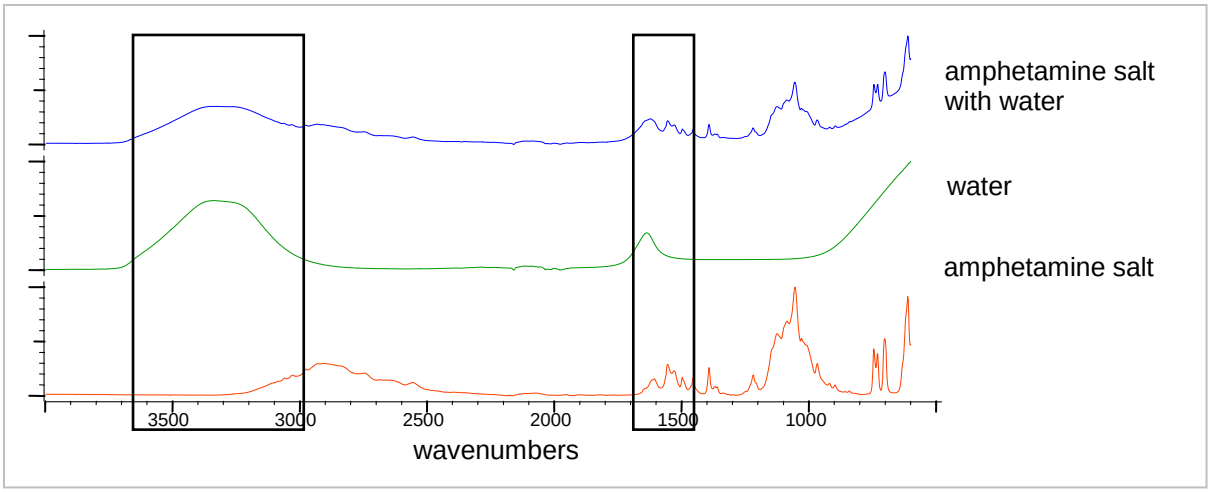


Figure 2.

The stretching vibration signals of O-H of water appear in the range of 3750-2850 cm^{-1} and its bending vibration signals show up from 1750 to 1450 cm^{-1}. These are wide and large intensity bands, overlapping with the vibrations of the target compound (Figure 2).

The moisture content of the sample can induce chemical changes. For instance, hydrogen bonds can be formed between the investigated molecules and the water molecules. This causes band widening in the stretching vibration area of O-H and the signals of C=O and C-N can be significantly shifted to lower wavenumbers.

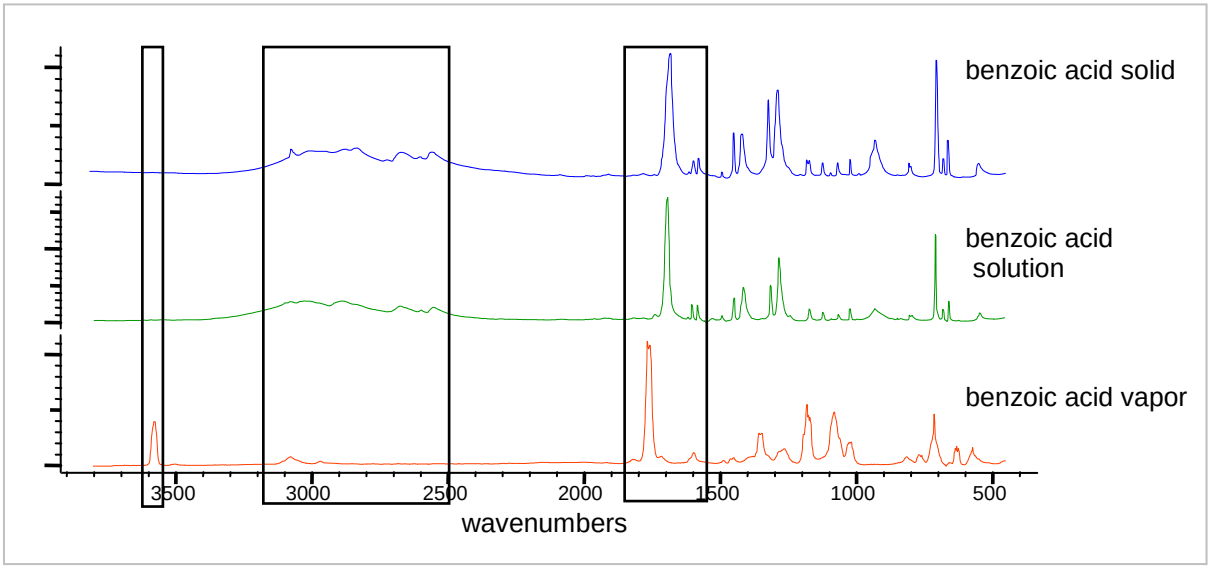
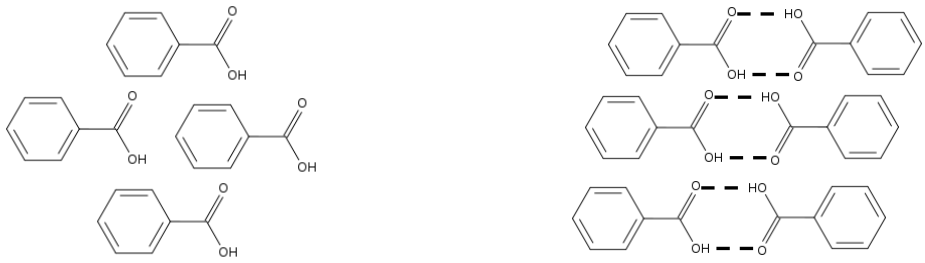


Figure 3.

Figure 3 shows the effect of hydrogen bonds between benzoic acid molecules. In vapor phase, there is no strong secondary interaction between the molecules, they are present in their monomer form (Figure 4). This occurrence is proved by the appearance of a stretching vibration signal of O-H from 3550 to 3600 cm^{-1} and the C=O giving sharp signal at 1760 cm^{-1}.

Thus, due to the intermolecular interaction the O-H band widens and both O-H and C=O signals shift to lower wavenumbers. The solvated molecules are partially in dimer form, whereas all of the monomer signals disappear in solid phase.



monomer dimer

Figure 4.

2.2 Crystal water

If there is water adsorbed physically on the surface, it can be removed by simple physical techniques.

In some cases, the samples contain water molecules as a part of the crystal structure of the material. Crystal water is usually formed during the crystallization of the substance. In some crystalline substances, adsorbed moisture can be transformed to crystal water.

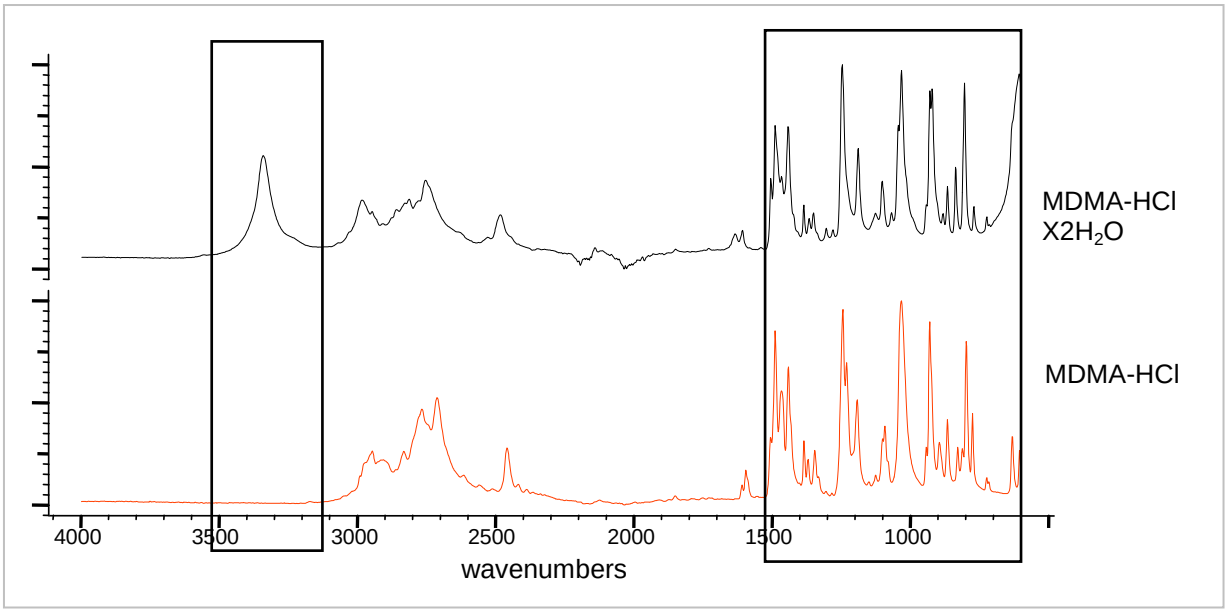


Figure 5.

An example for the crystal water effect is shown in Figure 5. The band having a typical shape in the range of 3500-3100 cm^{-1} is from the O-H stretching vibration of the water. The crystal water has influence on vibrations of the molecules. Usually slight differences can be seen in the fingerprint region. The shift and intensity changes of signals associating with N-H and C=O vibrations are often visible in the spectra.

2.3 Crystallinity, polymorphism

The IR technique gives possibility for measurement of solid, liquid and gas phase samples. In case of solid components, the crystallinity of the material has only a slight effect on the quality of the spectrum. According to general experience, a higher

degree of crystallinity causes sharper bands. However, this effect does not exclude reliable measurement of amorphous materials.
Polymorphism is the appearance of chemically equivalent materials in different crystal structures. The crystal classes are not distinguishable using the described approach, but x-ray diffraction techniques give possibility for the investigation of this phenomenon.

2.4 Salts and free-bases

Significant differences occur between the spectra of molecules in salt and free-base forms.
Stretching vibration of N-H bond in primary and secondary amines has bands in the range of about 3400-3000 cm^{-1}. Primary amines have medium intensity bands in the range of 3400-3380 cm^{-1} and 3345-3325 cm^{-1}. Low intensity signals of secondary amines appear in the range of about 3360-3310 cm^{-1}.
Typical absorption range of N-H bond bending vibration of primary amines is 1650-1590 cm^{-1} with strong bands. Medium bands appear by N-H bond bending vibration of secondary amines in the range of about 1650-1550 cm^{-1}.
In the case of primary amines, the stretching vibration of C-N bonds has medium/strong bands in the range of 1080-1040 cm^{-1}. The bands of secondary amines are in range of 1180-1140 cm^{-1}.
The ranges of bands and their intensities can be slightly different between various salt forms. This region of the spectra often contains overlapping bands associated with the vibrational transitions of C-H and C-C bonds (Figure 6).

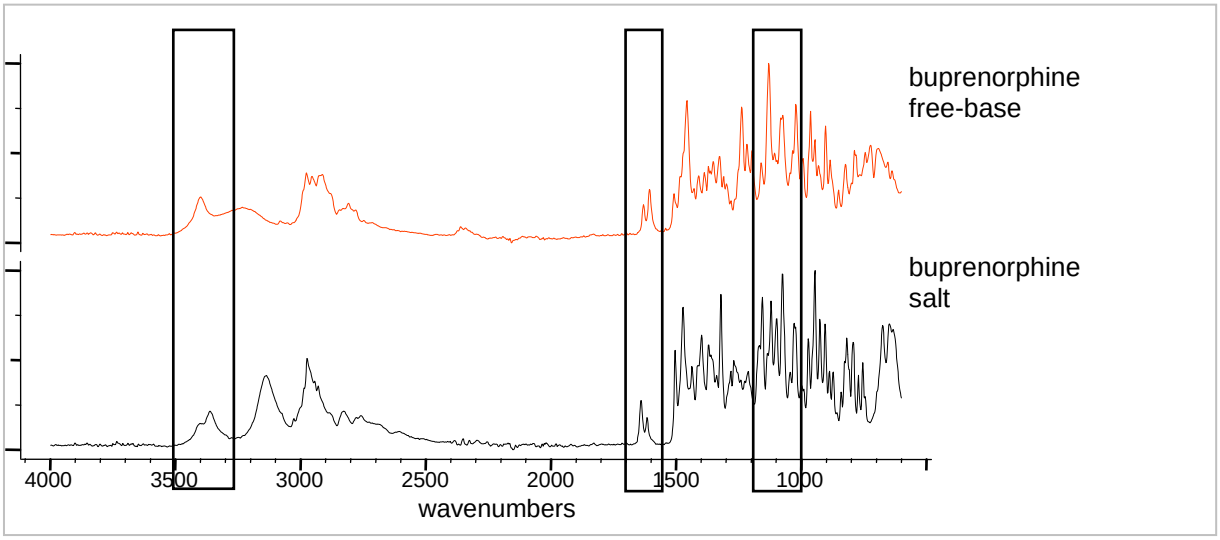


Figure 6.

Beside the effects mentioned above, the vibration bands of polyatomic anions can also appear in the spectra of salts. The most common polyatomic ions and their characteristic bands are listed in Figure 7.

CO_3^{2-}	1450-1410, 880-800 cm^{-1}
SO_4^{2-}	1130-1080, 680-610 cm^{-1}
NO_3^{-}	1410-1340, 860-800 cm^{-1}
PO_4^{3-}	1100-950 cm^{-1}
NH_4^{+}	3350-3030, 1485-1390 cm^{-1}

Figure 7. (Based on Peter Larkin: Infrared and Raman spectroscopy, 2011)

Two different amphetamine salts are compared in Figure 8. Beside the changes mentioned above, an intensive signal of S=O bond shows up in the spectrum of amphetamine sulphate.

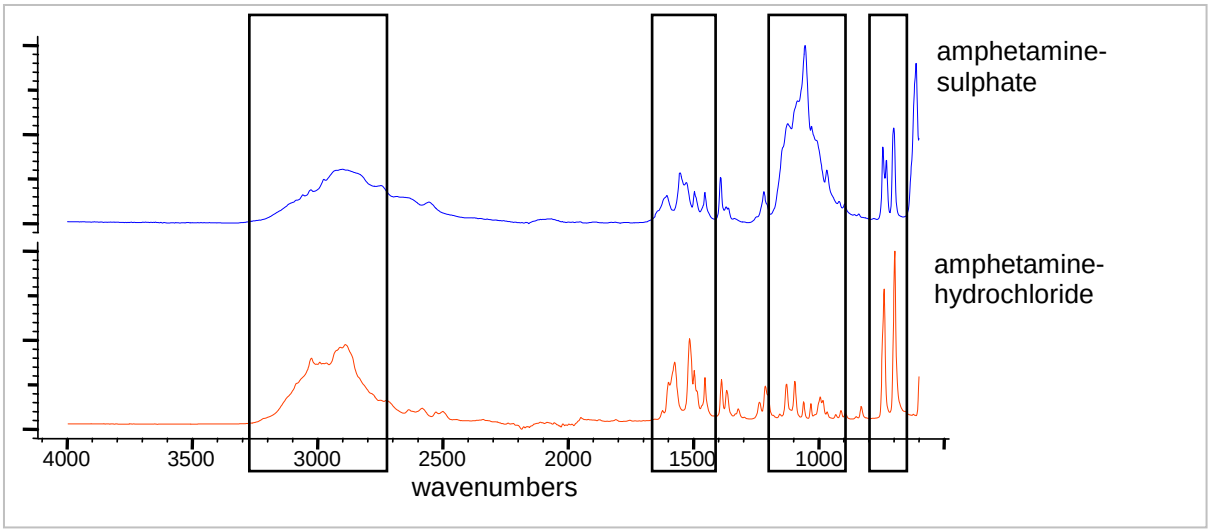


Figure 8.

2.5 Regio-isomers

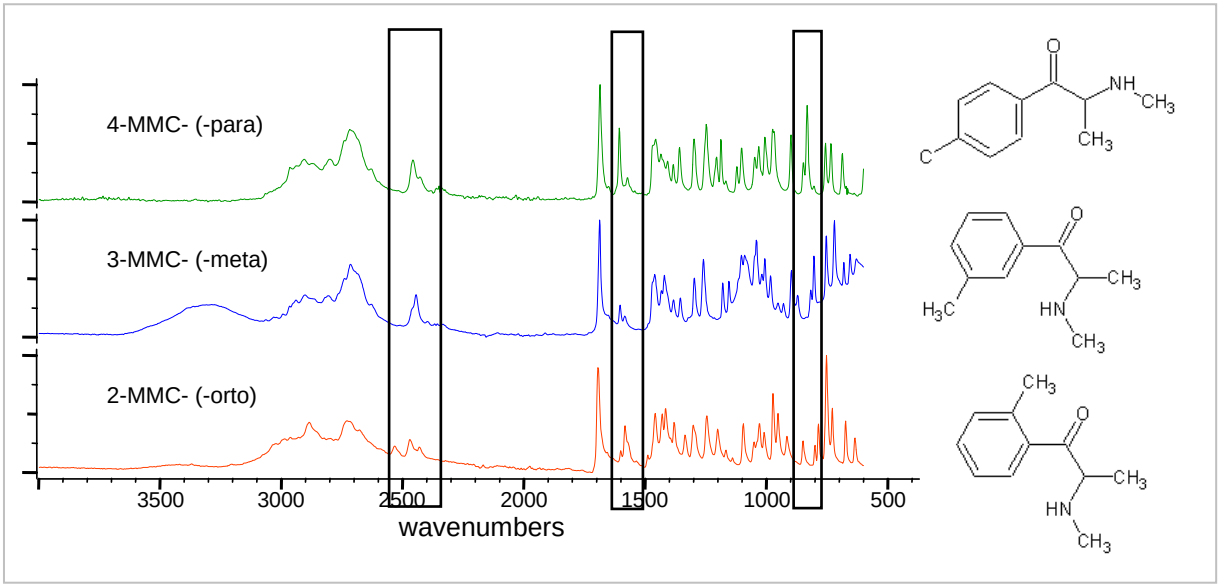


Figure 9.

The regio-isomers of substances containing substituted aromatic rings can be distinguished by IR spectroscopy. Figure 9 shows an example of three regio-isomers of substituted cathinone derivatives. The pattern of the stretching vibration band of C-H bonds in the range of 2600-2400 cm^{-1} and the stretching vibration aromatic ring in the range of 1650-1400 cm^{-1} are conspicuously different among the three isomers. The range of bending vibration signals of C-H bonds gives possibility for the isomer identification. In the case of a disubstituted benzene ring, in the range of 735-770 cm^{-1} the orto-, around 690 cm^{-1} and 750-810 cm^{-1} the meta-, and in the range of 810-840 cm^{-1} the para- positions are presumable. The possible conjugation in the molecules can influence on the signal ranges (Figure 10).

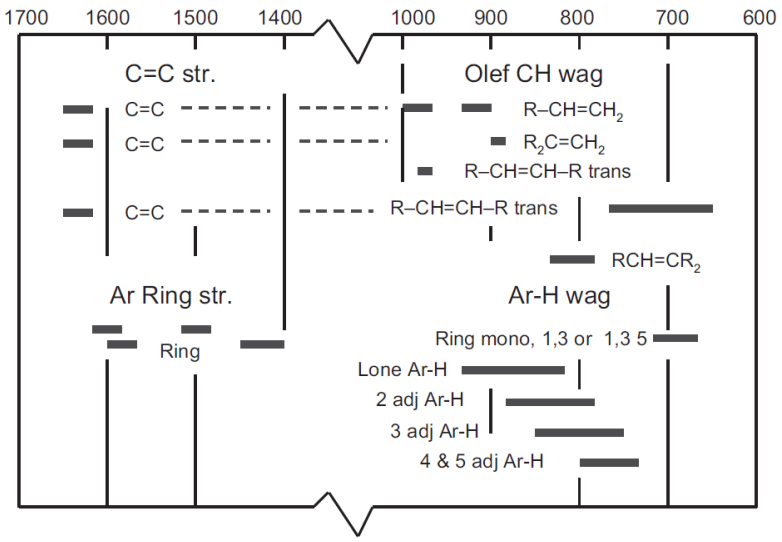


Figure 10.*

2.6 Homologues

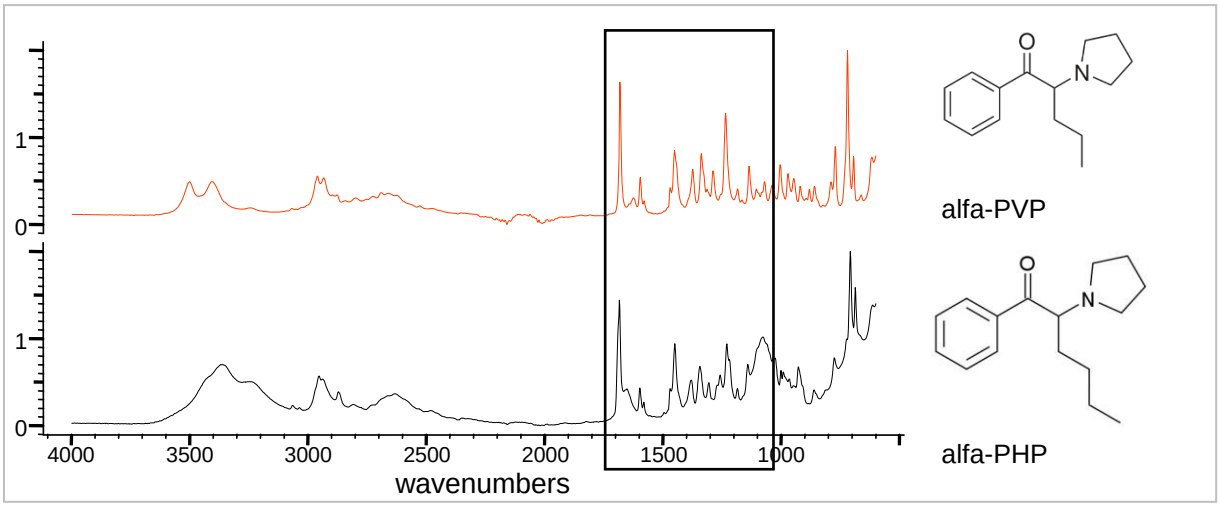


Figure 11.

Only slight differences occur in the IR spectra of homologue molecules. The homologues differ only the number of $–CH_2–$ groups in their alkyl chain moiety,

* Peter Larkin: Infrared and Raman spectroscopy Publishing year or other information?

therefore the stretching vibrations show undesired similarity in the spectral range used for the identification (above 1000 cm^{-1}) (Figure 11). In this case the identification of the components is not possible with absolute certainty by IR spectrometry. (However, homologues usually can be clearly distinguished by mass spectroscopy.)

3 Special sample preparation techniques

3.1 Extraction methods for tablets and powders

Pure seized materials are unusual in the daily routine. In most cases, presence of other materials in addition to the active ingredients may disturb the identification. Degree of the disturbance is related to the additives – active compounds concentration ratio. Usually, additives (adulterants and cutting agents) are simple organic molecules, such as lactose, caffeine, ascorbic acid, carbohydrates and etc. If the amount of additives reaches a critical concentration level, undesirable overlapping may make the bands of active component unassignable.
Extraction with solvents - based on solubility differences - can provide a solution to this problem in the case of tablets and mixture of powders.
A number of amine-type drugs are spread in the illicit market as salts (i.e. amphetamines, cathinone derivatives). These have as good water solubility as many additives. However, their solubility is different in alcohols, esters, ketones, ethers or hydrocarbons.

Often, the cutting agents of tablets - e.g. di- or polysaccharides – are slightly soluble or insoluble in alcoholic solvents. Addition of a little amount of solvent to a powder mixture or tablet can enrich the active compounds in the liquid phase. Samples taken from the liquid phase can be spread directly onto the ATR crystal. As the solvent evaporates, a film-layer is formed on the ATR surface.

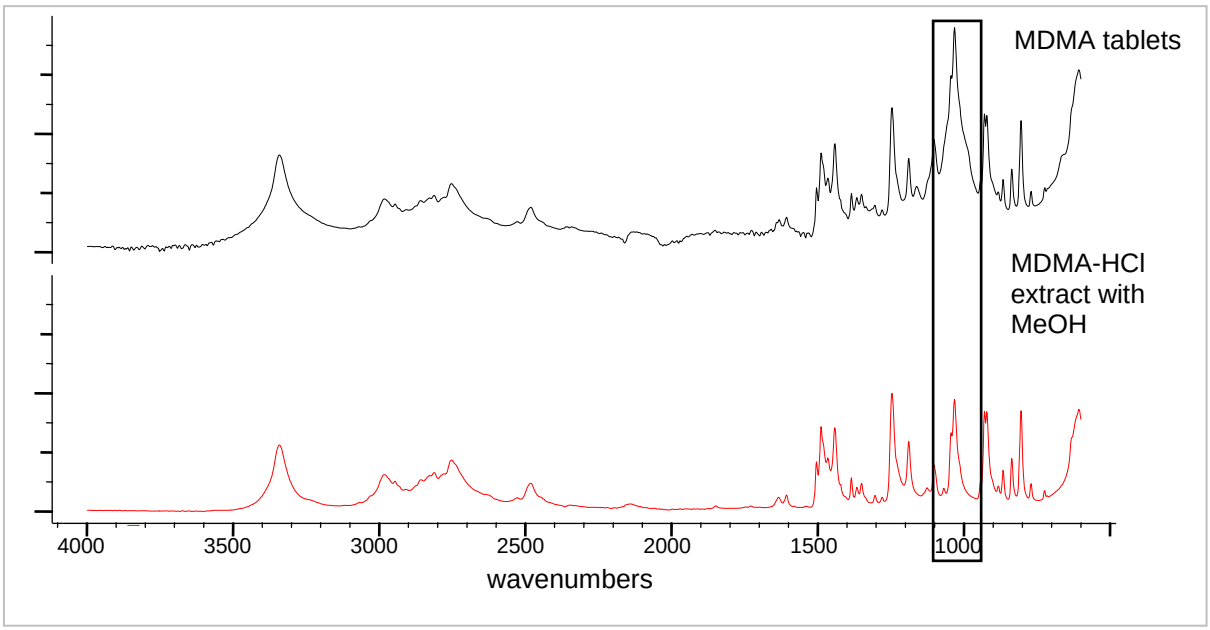


Figure 12.

Spectra of MDMA-tablet and the extract of the tablet are presented in Figure 12. The signal of cellulose from 990 to 1100 cm^{-1} appears with significant relative intensity

overlapping a part with the characteristic bands of MDMA, enhancing the uncertainty of the identification. Using an alcoholic extraction technique, MDMA is significantly enriched in the liquid phase compared to other ingredients of the tablet.

The caffeine is a typical adulterant in amphetamine powders. Its solubility in alcoholic solvents is comparable to the solubility of amphetamine salt, and thus the alcoholic extraction method is inefficient in this case.
However, caffeine is soluble in halogenated hydrocarbons, while amphetamine salt is not. That means, the extraction of caffeine with e.g. chloroform is possible from the powder enriching the relative concentration of the amphetamine salt in the extraction residue, which can be treated as a solid phase sample.

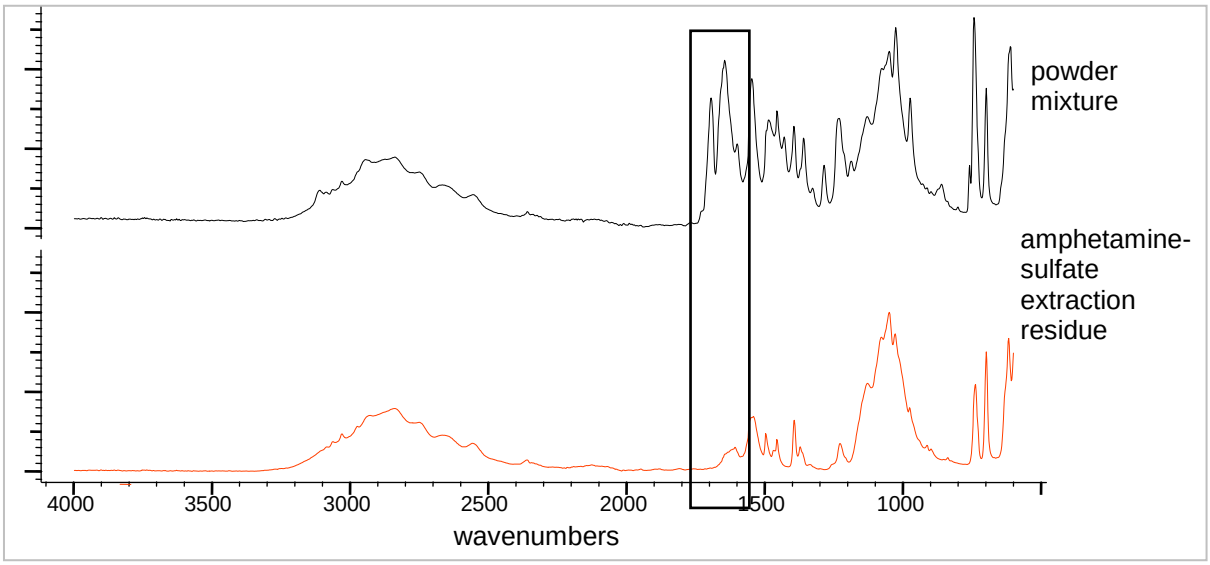


Figure 13.

Therefore, the characteristic bands of extraction residue associated with caffeine from 1600 to 1800 cm^{-1} have significantly lower intensity than in the spectrum of the original powder mixture (Figure 13).

3.2 Free-base conversion, extraction

Unknown salt forms with unavailable reference spectra make the identification unsuccessful. In these cases, the free-base conversion may give a solution. The conversion from salts to free-base can be carried out using a strong base, for instance NaOH or NH_4OH solution. After addition of the strong base solution to the salt, the free-base can be extracted with non-polar organic solvent. The organic phase can be spread onto the ATR surface and after the evaporation of the solvent, the active component is detectable.

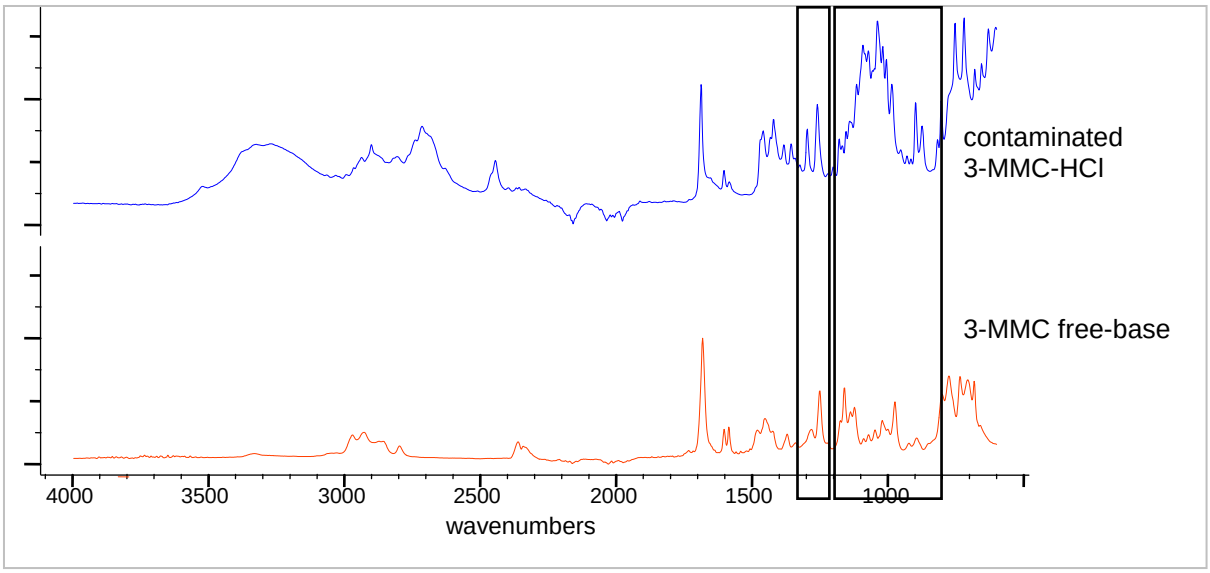


Figure 14.

This method is also recommended in the case of salt mixtures or presence of undesirable ingredients or impurities which cannot be transferred to the organic phase during the process (Figure 14).

3.3 Extraction method for impregnated herbal materials

The easiest way to isolate the components impregnated onto the surface of plant materials is extraction with solvent and residue formation by the evaporation of the solvent. The procedure is simple. After the addition of solvent to the plant material, a few drops of solution can be taken as a sample. After spreading the solution onto the ATR surface and the evaporation of the volatile solvent, the residue remains as a film layer, which is directly measurable. Some instruments have a feature which allows real time display of the spectrum during the evaporation.

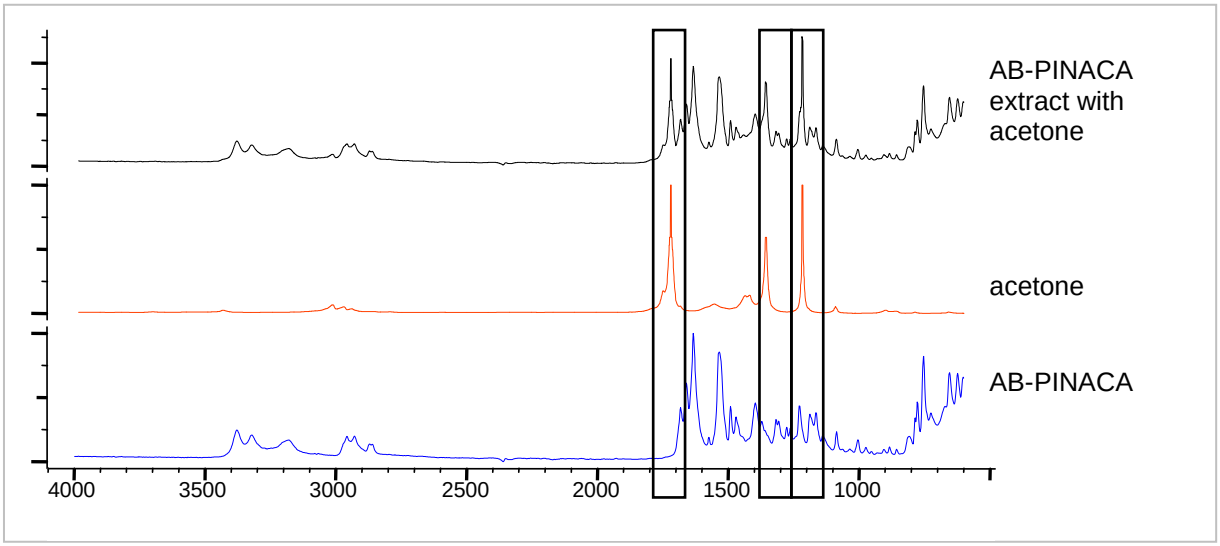


Figure 15.

The most frequent error is caused by the remaining solvent. For instance, in Figure 15 the characteristic bands of acetone are visible in the first spectrum.

During the measurement of samples prepared with this method, differences can be observed between the spectra of evaporated sample and the reference solid powder (Figure 16). Applying the same extraction procedure for the powder reference material, equivalent spectra can be obtained.

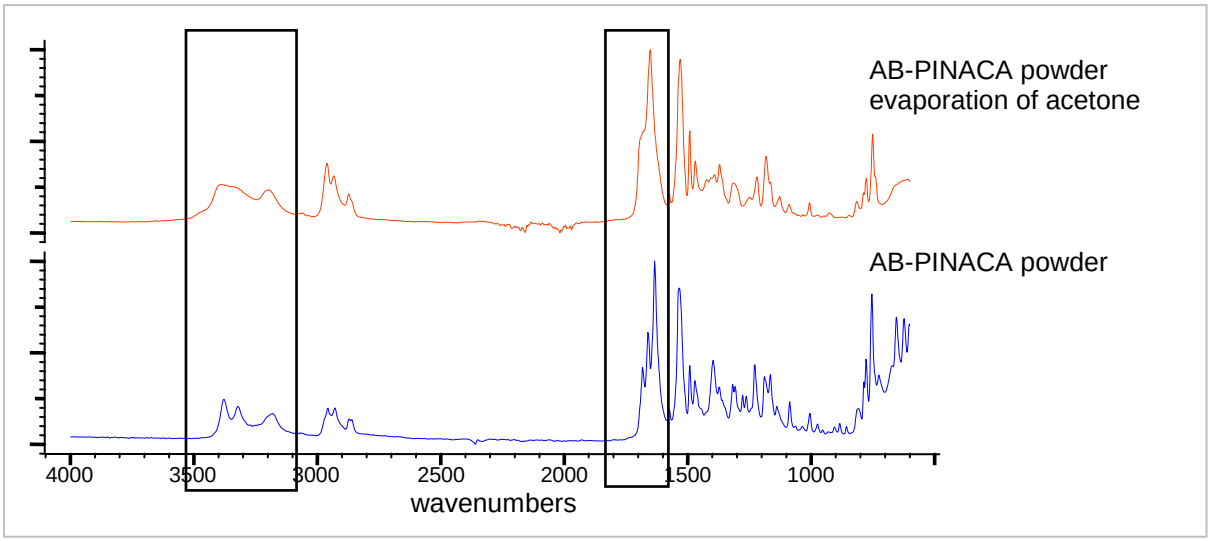


Figure 16.

In the case of impregnated plant materials, the components originated from the plant preparation – such as glycerol – can interfere with the identification. The extraction of glycerol can be prevented by using hexane extraction. (Figure 17).

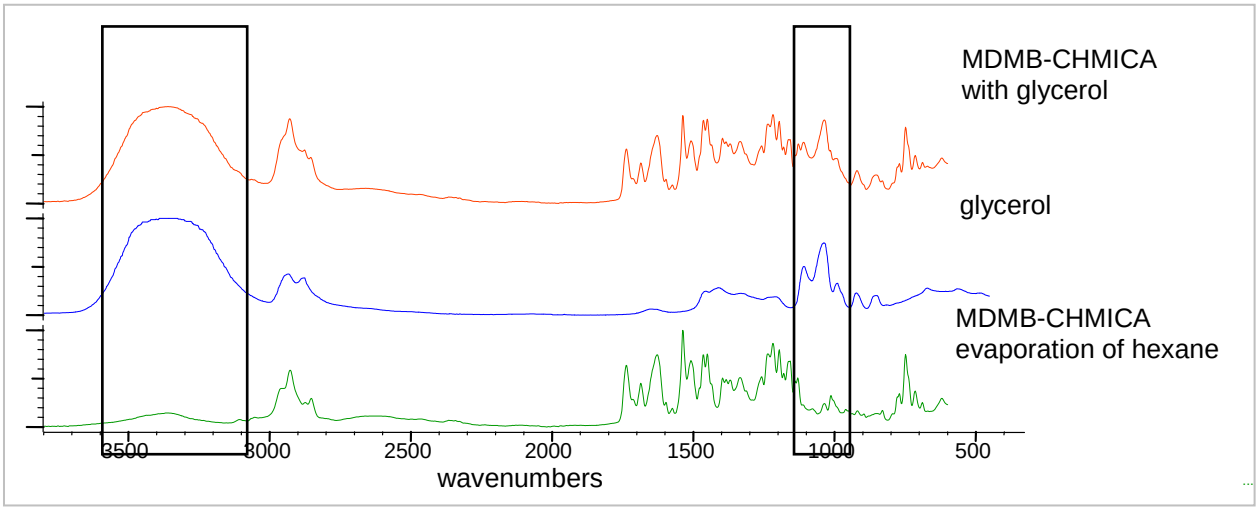


Figure 17.

3.4 Alternative methods for analysis of extracts

Commonly used solvents (i.e. acetone) cannot be used for every type of ATR unit. Incompatible solvents must be checked in the reference manual of the ATR unit. However, a sample solution can be impregnated onto the surface of fine crystalline NaCl or KBr support, which can then be treated as a routine powder sample.