LSD derivatives. Current legal status, MS spectrum analysis and an attempt to interpret decomposition of fragmentation pathways

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Summary

This article presents a historical overview along with the general characteristics of lysergic acid diethylamide (LSD). The increasingly popular new derivatives of LSD were also included. Their changing legal status over time was presented, followed by an attempt to interpret mass spectrum by analysing the fragmentation pathways of the molecule using 1P-LSD as an example. Attention was paid to the method of sample preparation, as well as appropriately chosen chromatographic conditions.

Key words: LSD, fragmentation, mass spectrum, GC-MS, hallucinogens, NPS

Purpose of the paper

The purpose of this paper is to present selected new LSD derivatives, to observe similarities and differences in the mass spectra obtained, which is directly related to the structure of these substances. Attention was paid to the analysis of chromatographic conditions, including solvent selection when using the gas chromatography-mass spectrometry (GC-MS).

Introduction

Structurally, LSD is derived from lysergic acid (tetracyclic alkaloid), which contains an ergoline skeleton in its structure. Derivatives of this alkaloid, present e.g. in the ergot fungus, parasitised cereals and caused epidemics in the past. Symptoms of poisoning include, for example, mental disorders and painful vasospasm leading to necrosis; in the Middle Ages, this medical condition was called St Anthony's fire (Mirowska-Guzel, Rang (eds), 2014). Even earlier, in 370 BC, Hippocrates provided a description of corn leaf blight, and later described ergot and noted its use to stop postpartum haemorrhage (Schiff, 2006). Thus, ergot has long been the subject of research for the evaluation of its pharmacological properties. Mentions of the psychoactive effects of lysergic acid derivatives also date back to the Renaissance. In the 16th century, the Spanish pioneer ethnographer and missionary, who did his service among the Aztecs, Bernardino de Sahagun, described that the basic ingredient of a stimulant called ololiuqui was grains from the plants Ipomoea violacea and Rivea corymbosa. Crushed and consumed in powder form, they were responsible for euphoria, madness and visions, which was an important part of religious ceremonies of the time (Rostkowska-Nadolska & Machoń, 2009). It was not until several hundred years later, at the beginning of the second half of the 20th century, that research proved that it was LSA amide and lysergic acid hydroxyethylamide that were behind the hallucinogenic effect of the grains (Rostkowska Nadolska & Machoń, 2009). This discovery was closely linked to the events of 1938, when in a chemical laboratory in Basel, at the headquarters of one of the pharmaceutical companies, chemist Albert Hofmann obtained LSD for the first time by chemical synthesis, or more precisely LSD-25, where the number 25 marked the twenty-fifth synthesised substance in the series of lysergic acid derivatives at the time. Shortly afterwards, the chemist shared his observations and reactions after taking LSD, publishing the book *LSD My Problem Child*. In later years, A. Hofmann, who wanted to describe the familiar state of being under the influence of psychedelics from his own experience, was followed by Ernst Junger (author of *Approaches: Drugs and Ecstatic Intoxication*) and Alexander Shulgin, who, together with his wife Ann, published *TiHKAL*, a book presenting research on psychoactive substances from the tryptamine group. There was no longer any doubt that the LSD obtained was a drug with strong hallucinogenic properties.

Current legal status

Analysing statistical data on controlled drugs present on the drug market in Poland, it may be concluded that LSD and its derivatives are second in popularity to, for example, non-fibrous hemp (so-called marihuana), amphetamine, cocaine or MDMA. Another difference is the form of the substance itself, as the most common drugs have a discernible shape: they are plant fragments, clumped substances, powders, tablets. In the case of LSD and its derivatives, the active substance is most often injected into a perforated piece of paper as a carrier for the substance

(so-called blotters, blotting papers) and further distributed on the market in this form. LSD is easy to administer orally, colourless, odourless and tasteless, a typical 'street dose' ranges from 10-300 µg (Szukalski, 1998), while a standard dose inducing an altered state of consciousness oscillates around 75-150 µg (Passie et al., 2008). For comparison, single doses inducing a state of euphoria in humans range from 20-40 mg range from 20-40 mg (amphetamine) (Zajączkowski et al., 2011) or 75-200 mg (MDMA) (Kubica & Gąsiorowski, 2012).

The most commonly tested compound present in blotters was LSD (a controlled substance included in the annexes to the Act of 29 July 2005 on Counteracting Drug Addiction, classified as group I-P, i.e. a substance with no significant indications in medicine). Synthetic hallucinogenic compounds have recently been joined by new LSD derivatives, such as: 1P-LSD, ALD-52, ETH-LAD or AL-LAD. Like LSD, they are sold on the illicit market and come in blotter form.

The experts of the Chemistry Section of the Forensic Laboratory of Warsaw Metropolitan Police in the years 2018-2021 carried out dozens of expert analyses, where, in addition to the aforementioned LSD, its derivatives were also the subject of research. The legal status of LSD derivatives was closely linked to the progressive legislative processes towards new psychoactive substances. The first officially reported information on the increased presence of LSD derivatives on the drug market in Poland dates back to 20 February 2019, at which time the Chief Sanitary Inspectorate, through resolutions, recommended the inclusion of ETH-LAD and ALD-52 on the list of new psychoactive substances[1,2]. It was possible to add these substances to the list of new psychoactive substances thanks to the amendment to the Regulation of the Minister of Health on the list of psychotropic substances, narcotic drugs and new psychoactive substances, which became effective on 21 August 2019. As a result of subsequent amendments to the aforementioned Regulation (11 March 2021 and 27 January 2022), other LSD derivatives, i.e. 1P-LSD, AL-LAD and 1cP-LSD, changed their status and became new psychoactive substances. Furthermore, the amendment of 11 March 2021 proved to be crucial in the context of the criminalisation of new LSD derivatives. Amendments were made to Annex 3 of the Regulation "List of New Psychoactive Substances", with the addition of item 7 defining "tryptamine derivatives" as "group VI - NPS". In practice, this means that a new psychoactive substance is any delta-9,10-ergolene-derived compound containing the appropriate structure (Fig. 1), subject to certain assumptions, such as a molecular weight of no more than 500 u or appropriately selected substituents R1-R4. Under the amended legislation, the legislator has specified that:

- "substituent R1 may be a hydrogen atom or a group: alkyl group (up to C3), alkylcarbonyl group (up to C4),
- substituent R2 may be a hydrogen atom or a group: alkyl group (up to C4), allyl or prop-2-in-1-yl group, and
- substituents R3 and R4 may be hydrogen atoms or groups: alkyl group (up to C5), cyclopropyl group, allyl group, 1-hydroxyalkyl group (up to C2).

In addition, the amide nitrogen atom can be part of a ring system: morpholine, pyrrolidine or dimethylazetidine ring system".

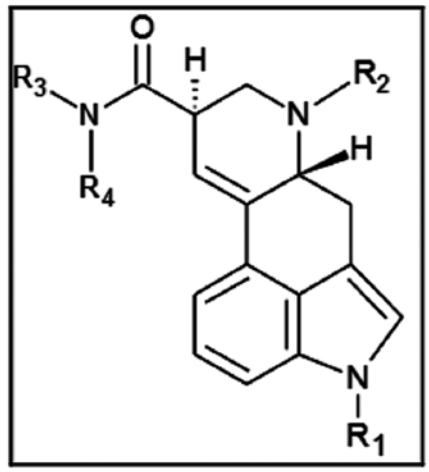

Fig. 1. Structure containing delta-9,10-ergolen

The structural formulae of LSD and nine example derivatives are shown below (Fig. 2). Excluding those already defined by the provisions of the Act on Counteracting Drug Addiction: LSD (group I-P psychotropic substance), 1P-LSD, ALD-52, ETH-LAD and AL-LAD (new psychoactive substances appearing on the NPS list), each of the five compounds shown below meets the definition of a new psychoactive substance. All the compounds shown contain a skeleton derived from delta-9,10-ergolen. In addition:

- 1B-LSD has a substituent R1 which is an alkylcarbonyl (butyryl) group; R2, R3 and R4 are alkyl groups;
- 1P-ETH-LAD has a substituent R1 which is an alkylcarbonyl (propionyl) group; R2, R3 and R4 are alkyl groups;
- ECPLA- has a substituent R4 which is a cyclopropyl group, R1 is a hydrogen atom; R2 and R3 are alkyl groups;

[1] Resolution No. 3/2019 of the Team for the assessment of risks to human health or life associated with the use of new psychoactive substances of 20 February 2019. Chief Sanitary Inspectorate.

[2] Resolution No. 4/2019 of the Team for the assessment of risks to human health or life associated with the use of new psychoactive substances of 20 February 2019. Chief Sanitary Inspectorate.

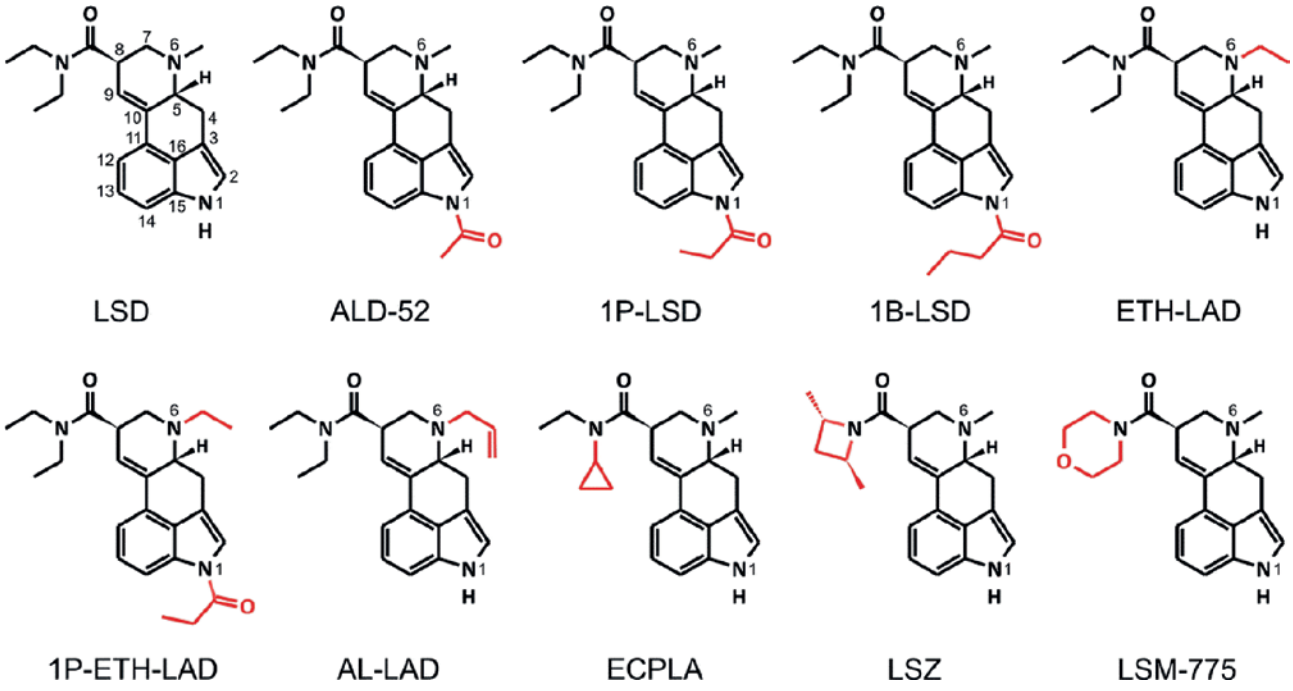

Fig. 2. Structural formula of LSD and its selected derivatives

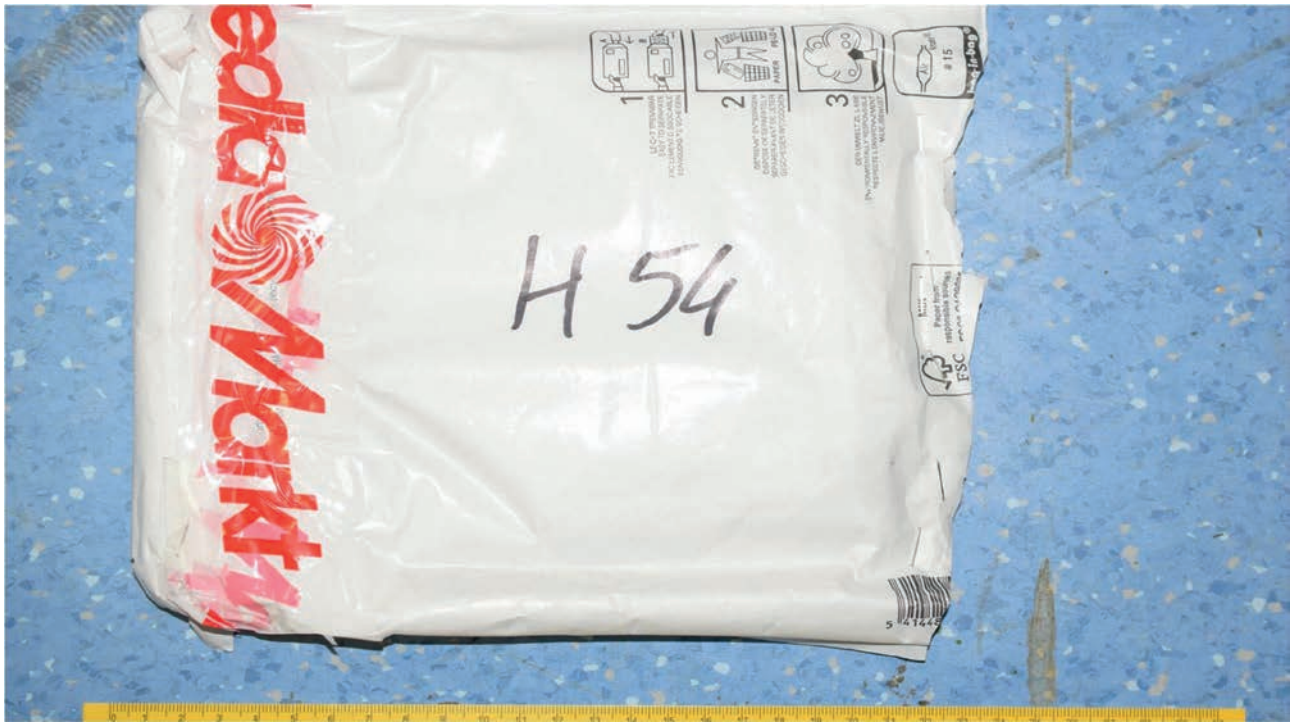

Fig 3. Example of a courier envelope-parcel - external view

Fig. 4. Example of a courier envelope-parcel - contents inside

- LSZ - has an amide nitrogen atom which is part of the dimethylazetidine ring system, R1 is a hydrogen atom, R2 is an alkyl group;
- LSM-775 - has an amide nitrogen atom which is part of a morpholine ring system; R1 is a hydrogen atom, R2 is an alkyl group.

LSD derivatives as evidence

In the second half of 2018, the Forensic Laboratory of Warsaw Metropolitan Police received evidence for a case investigated by the Drug Crime Department of Warsaw Metropolitan Police concerning the possession and attempted distribution of new psychoactive substances, the so-called "legal highs". This material was packed in so-called post safe envelopes, which did not differ in any way from ordinary courier envelope-parcels (Fig. 3, 4).

In the course of the analytical work carried out, it turned out that the evidence in question (nearly 400 traces) consisted of new psychoactive substances, compounds that should have been considered as substitutes and, in smaller quantities, psychotropic substances. Of particular note is the date on which the evidence was secured, i.e. 17 July 2018. Given that the amendment to the Act on Counteracting Drug Addiction (in the wording in force since 21 August 2018) came a little later, it can be presumed that the operational and investigative activities coincided with the sale of substances that were about to turn from uncontrolled goods into controlled goods any moment. The evidence was mainly in the form of powders, as well as powdery, crystalline and clumped substances. There were also tablets and blotters, so called "papers" in the parcel (Figs. 5, 6).

Equipment used and measuring conditions

Chemical analysis of the blotters was carried out using gas chromatography coupled with a Shimadzu GC-MS mass spectrometer equipped with SemiVolatiles column, approximately 30 m long and with an inner diameter of 0.25 mm, with a film thickness of 0.25 µm. The following measurement method was used:

a. temperature program: 100˚C-1.00 min; 100˚C ÷ 290˚C x 20˚/min-10.00 min; 290˚C-27.00 min;
b. temperature of injection chamber: 250˚C;
c. injection: 1µl;
d. injection type: split 20:1;
e. detector: MS;
f. temperature of the detector: 230˚C;
g. mass range: 40÷500 m/z;
h. carrier gas: helium.

Samples were analysed as methanol and acetonitrile extracts. The tests performed excluded the presence of LSD in the secured blotters. As a result of the activities carried out, the secured evidence, which consisted of "papers", revealed the presence of LSD derivatives such as: ETH-LAD, AL-LAD, ALD-52 and 1P-LSD. Each derivative differed slightly in retention time (18.33 min, 19.83 min, 22.42 min, 24.58 min, respectively) and had a different mass spectrum, but

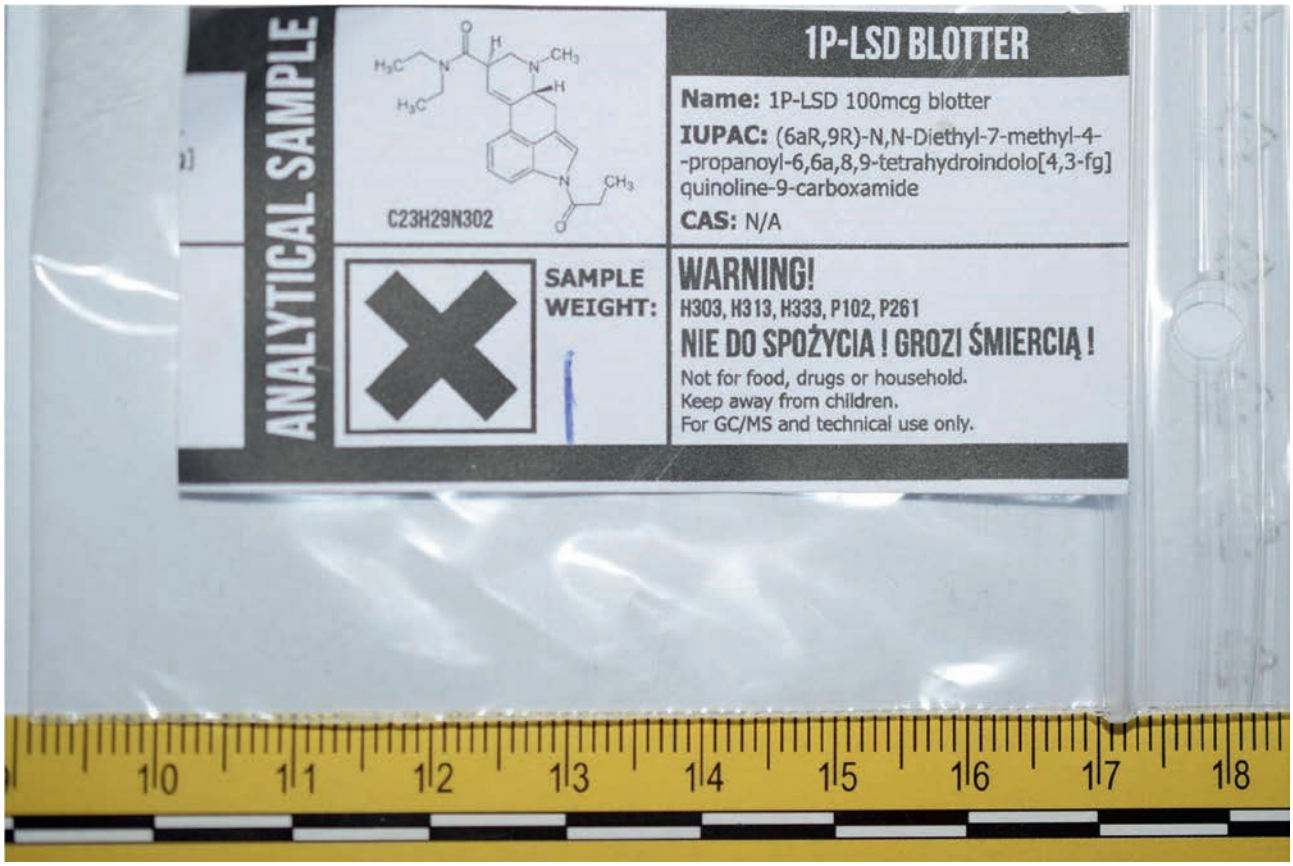

Fig. 5. Foil packaging containing blotters - note the professional label showing the description of the substance together with the structural formula

the 'core' of the mass spectrum (in the form of high-intensity ions) remained identical for all the derivatives analysed, with slight differences observed only in a certain area of the spectrum. 1P-LSD was classified categorically based on retention time and mass spectrum (Forensic Laboratory of Warsaw Metropolitan Police had a reference substance for this substance). In the course of the work carried out, an attempt to evaporate the previously added solvent and then analyse under infrared (ATR) conditions was abandoned due to the form of the material, i.e. blotters - paper fragments. For the remaining substances, i.e. ETH-LAD, ALD-52 and AL-LAD, only mass spectra were used, which were matched with spectra from libraries (NIST and SWGDRUGS) integrated into the analysis programme. By analysing the mass spectra of these derivatives, it was noticed that a certain area of these spectra is the same. An attempt was made to interpret the mass spectra resulting from the decomposition of molecules in MS using the example of a selected derivative, i.e. 1P-LSD.

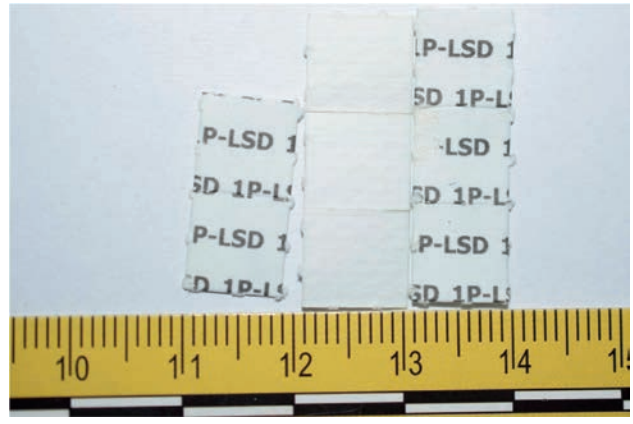

Fig. 6. Examples of perforated blotters

Interpretation of decomposition paths in the mass spectra of selected LSD derivatives

Monitoring popular websites and dark net forums dealing with the distribution of new drugs, one gets the impression that new LSD derivatives such as: 1P-LSD, ETH-LAD or ALD-52 are becoming increasingly popular. The compounds described in this publication are structurally related to LSD derivatives formed by the addition of appropriate functional groups to the 'matrix' (which is LSD), resulting in a different molecular weight of the compound.

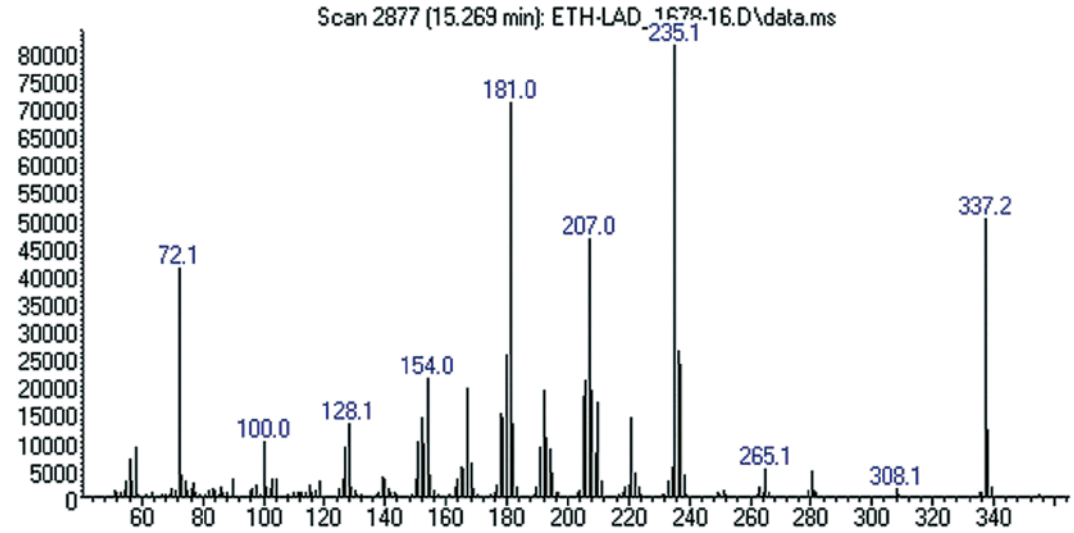

Fig. 7. Mass spectrum of ETH-LAD

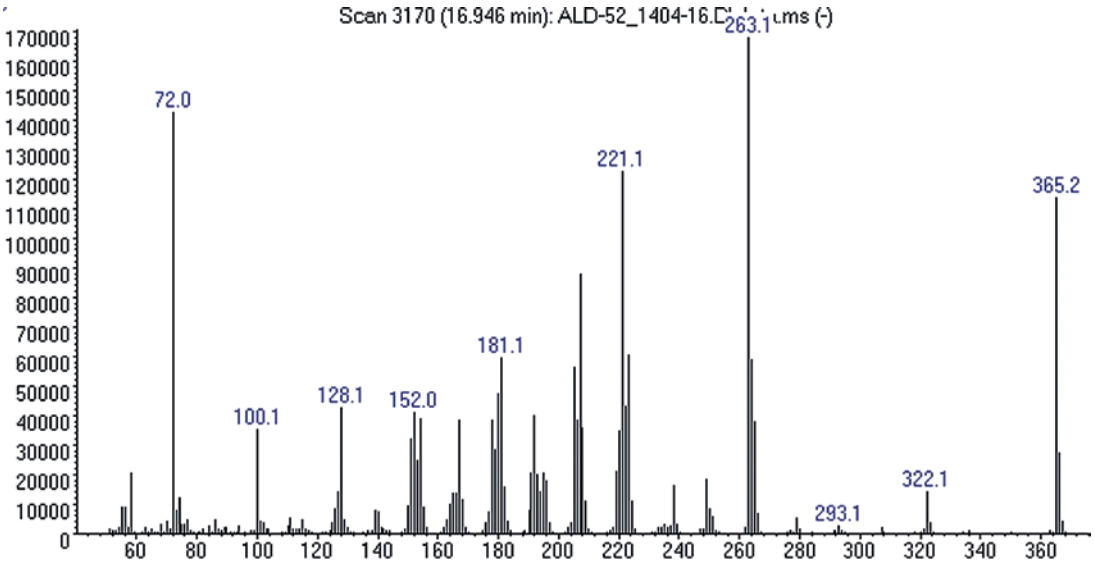

Fig. 8. Mass spectrum of ALD-52

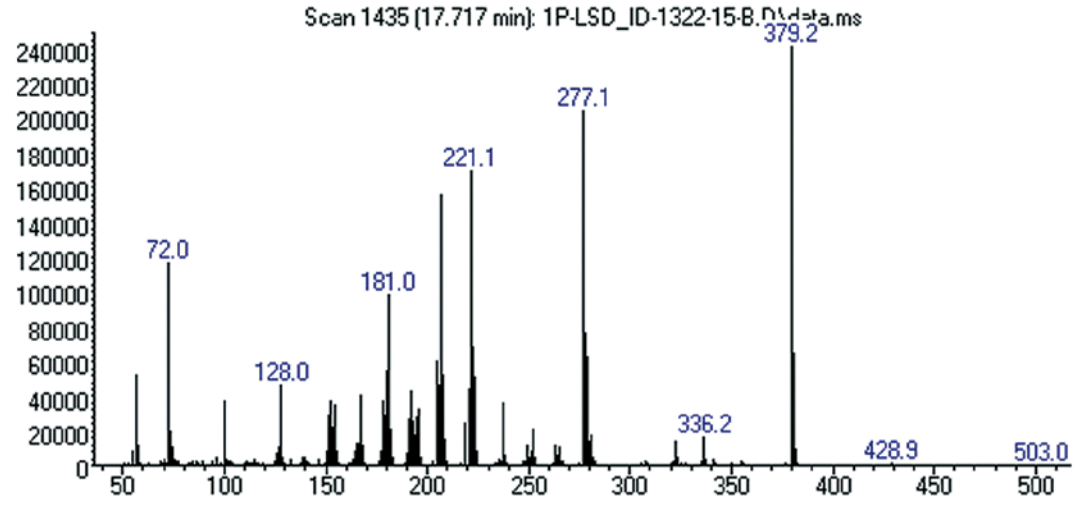

Fig. 9. Mass spectrum of 1P-LSD

As can be seen from the mass spectra of ALD-52, 1P-LSD, ETH LAD presented above, ions with m/z values of 72, 100, 154, 181 and 221 occur each time in the analysed spectra. It can therefore be assumed that these ions relate to the fragmentation of structure such as ergoline (Fig. 10), which is a component of LSD as well as its derivatives, although the vast majority of naturally occurring ergolines have an additional double bond at position 8,9 (delta 8,9-ergolene) or at position 9,10 (delta 9,10-ergolene).

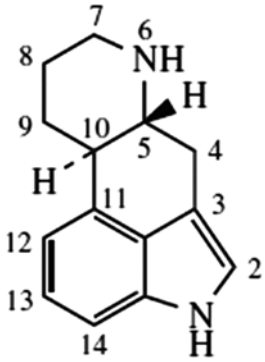

Fig. 10. Structural formula of ergoline

Other m/z values not recurring in the analysed spectra, on the other hand, should be attributed to core-added structures differing, for example, by the presence of an additional methyl group, which is probably the case for 1P-LSD and 1B-LSD, (379, 393) (Figs. 9, 11).

The interpretation of the mass spectrum of 1P-LSD began with an analysis of the structure of amide bond and two ethyl groups attached to it. The presence in the spectrum of an iminium ion (m/z 72) and a fragment having an oxygen atom with a positive charge (m/z 100) can be explained by assuming fragmentation based on alpha cleavage (this process involves compounds containing carbonyl groups, such as amides). **[A]** (Fig. 12).

The presence of the 336 ion can be attributed to processes associated with the retro-Diels-Alder reaction, which is characteristic of a structure containing a cyclohexene ring.

When 1P-LSD is fragmented, there is a loss of N-methylmethimine (CH2=N-CH3) **[B1]**.

The main difference in the structure of LSD and 1P-LSD is the presence of a propionyl group attached to nitrogen contained in indole. The molecular ion, whose value directly illustrates molecular mass (using electron ionisation), present in LSD gives an image of the ion at m/z 323, whereas 1P-LSD gives an image of the ion at m/z 379. This difference (m/z 56) is due to the molecular mass of the attached propionyl group, whose 'separation' can be observed and associated with the presence of the 322 ion **[B2].**

On the other hand, the appearance of the ion at m/z 278 in the mass spectrum is probably related to the separation of the N,N-diethylformamide group. Further fragmentation of this residue may give a signal at m/z 263, and this may indicate the absence of a methyl group **[B3, C].**

Another likely fragmentation pathway is the loss of N,N-diethylacrylamide. The presence of the ion at m/z 252 can be attributed to this process. The resulting structure probably undergoes further fragmentation processes, which may be evidenced by the presence of ions at m/z 237, 181, 154, 128, as well as 196 and 167 **[B4, C].**

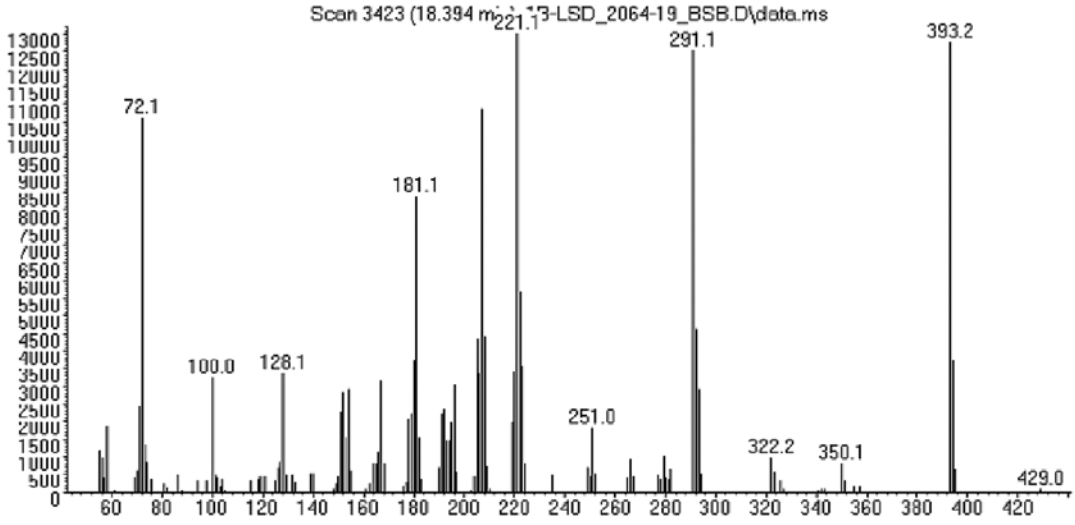

Fig. 11. Mass spectrum of 1B-LSD, molecular ion 393

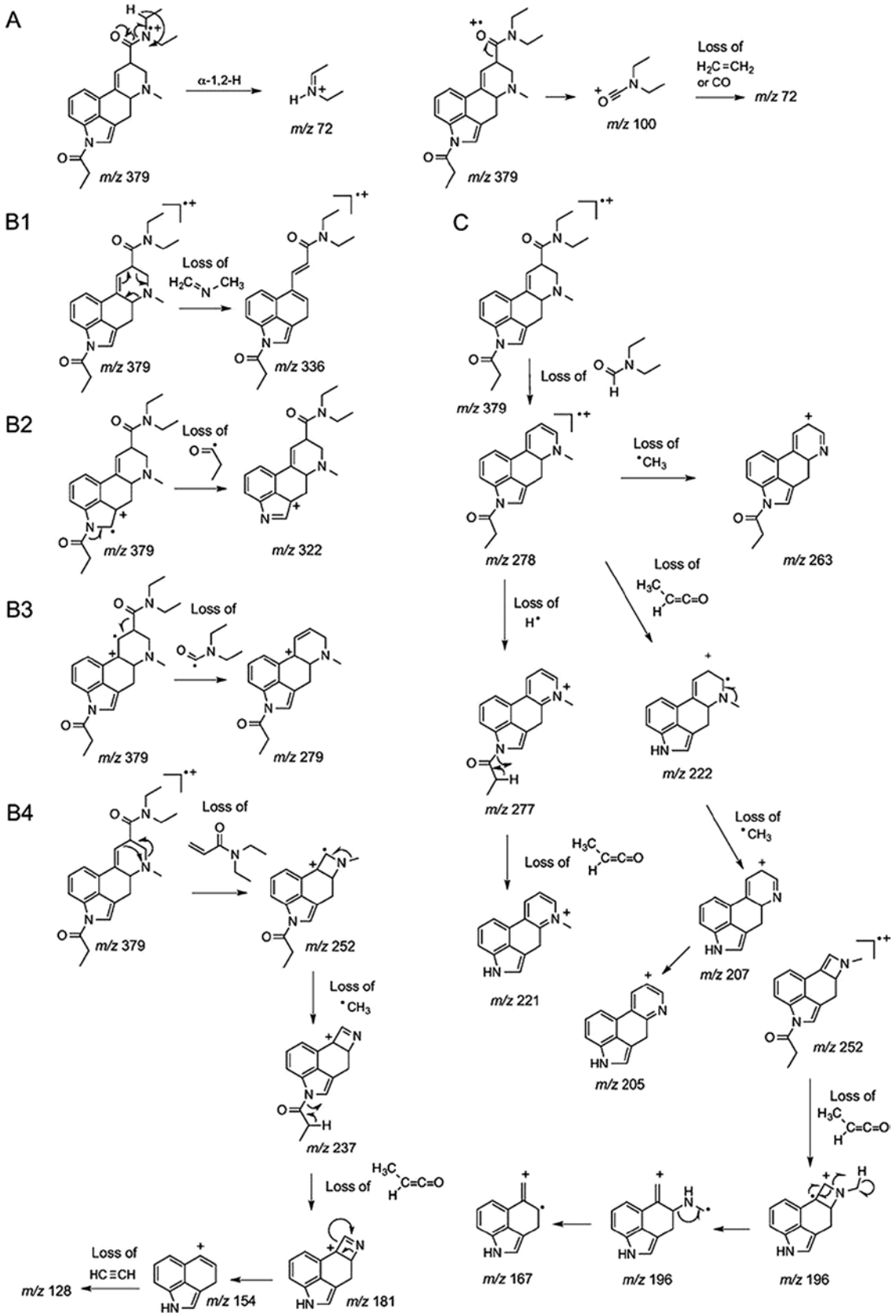

Fig. 12. Probable structures obtained by fragmentation in the EI source

Results of the analysis with the use of methanol and acetonitrile

Chromatographic analysis of 1P-LSD was performed by extracting the test blotters separately in methanol and acetonitrile. A certain limitation of the use of a low molecular weight alcohol - in this case methanol - is its susceptibility to a transesterification reaction, which can result in false positives towards the presence of LSD and 1P-LSD. For the purposes of this publication, blotters containing 1P-LSD were experimentally tested using methanol and acetonitrile (as two separate extracts). Although the mass spectrum obtained and peak integration present in the chromatogram due to the use of methanol is not problematic, extraction with acetonitrile should be considered. The results of the analysis of methanol extracts indicate the possibility of transesterification in the sample, which leads to false positives (presence of LSD and 1P-LSD).

Integration of peaks (determination of retention times) for the analysis conditions thus selected (described above) occurred at about 18-25 min. also for the other derivatives tested.

Interestingly, transesterification probably occurs after prolonged exposure of the blotter to methanol as a solvent (time is required for the transesterification process to occur), which was not observed with acetonitrile.

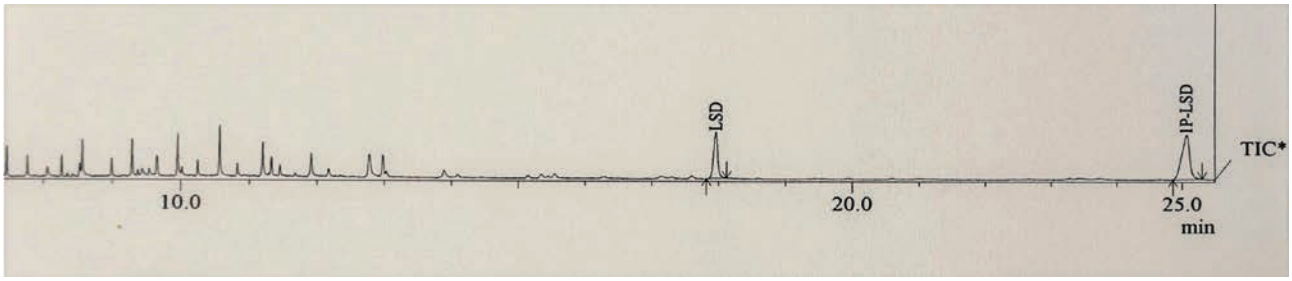

Fig. 13. Excerpt from a chromatogram showing a false positive - presence of LSD and expected 1P-LSD

Summary and conclusions

For proper mass spectrum analysis or attempts to identify potential fragmentation pathways, the selection of a uniform ionisation technique is crucial. Electron Ionisation (EI) was used both in the course of the tests performed and in the interpretation of spectra from libraries.

LSD and its derivatives are characterised by very similar mass spectra, and some of them, such as AL-LAD and ETH-LAD, even have an identical image in a certain area of spectrum.

The fragmentation of substances under study was found to be directly linked with the retro-Diels-Alder reaction, i.e. decomposition, which is characteristic of LSD derivatives (presence of a cyclohexene ring with six different substituents). In addition, the 'nitrogen rule' is followed, where LSD and the derivatives presented have an odd number of nitrogen atoms (3), thus their molecular weights are characterised by an odd number, e.g. (LSD-323), ETH-LAD (337), AL-LAD (349), ALD-52 (365), 1P-LSD (379). Fragmentation of a segment containing an amide group with two ethyl groups is likely to give the spectrum the presence of an ion at a value reduced by 100 (102) from the mass of the molecular ion.

Fragmentation derived from delta-9,10 ergolene (a constituent structure of LSD and its derivatives) was also observed to occur in an analogous manner. By analysing the structural formulae of LSD derivatives, it was found that they contain groups, e.g. methyl, ethyl, allyl, propionyl, butyryl, which are added (as a homologous series) to N-1 (indole nitrogen) and N-6. When interpreting the mass spectrum in a certain section of it, differences in m/z 12, 14 were observed (which also directly translates into m/z values corresponding to molecular ions).

Furthermore, what deserves attention are the conditions for the analysis of so-called blotters, such as split, concentration of the sample (relatively small amount of solvent to the surface of a single blotter), and, in the case of work with methanol, performing the test without undue delay (potential risk of transesterification process). It is worth mentioning that in the case of an adequately unconcentrated sample (which may result, for example, in a weak or small peak - a shape that is difficult to integrate successfully) one should perform noise analysis or monitor selected ions (SIM mode).

Sources of figures:

Fig. 1-6, 10, 13: author

Fig. 12: Brandt, S.D, Kavanagh, P.V, Westphal, F., Stratford, A., Elliott, S.E, Hoang, K., Wallach, J., Halberstadt, A.L. (2016).

Fig. 7-9, 11: https://www.policija.si/eng/about-the-police/organization/general-police-directorate/national-forensic-laboratory/project-response

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Translation GTC AMG sp. z o.o.