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In vitro metabolic studies of AM2233 and JWH-210, novel synthetic cannabinoids

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Abstract

The smoking of “herbal mixtures” as an alternative to the cannabis-based products has become an issue not only in the social context, but also in sport drug testing where reliable detection methods are demanded by antidoping laboratories. These herbal blends are legally sold via the Internet in many countries, and their composition is changed from time to time in response to the legislation bans.
We performed an *in vitro* study on the metabolism of AM2233, 1-[(*N*-methylpiperidin-2-yl)methyl]-3-(2-iodobenzoyl)indole, and JWH-210, 4-ethylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone, which were isolated using preparative liquid chromatography from the smoking mixtures sold in Russia. After incubation of pure fractions with human liver microsomes (HLM) as well as with CYP450 isoenzymes 3A4 and 2B6, the metabolic pathways were identified by means of liquid chromatography coupled to tandem mass spectrometry with electrospray ionization in positive mode. It was found that in case of AM2233 the *in vitro* reactions mainly include monohydroxylation and *N*-demethylation, while JWH-210 formed a variety of products such as monohydroxy, dihydroxy, despentylhydroxy, oxo (or epoxy) and oxohydroxy metabolites. The HLM were found to be superior over the other two isoenzymes for generation of the metabolites of these cannabimimetics.

Introduction

Recently, synthetic cannabinoids have become a great concern due to their widespread abuse in many countries. Since the variety of these compounds is enormous and the data on their biotransformation are very limited, toxicological screening for synthetic cannabinoids is problematic, to say the least. When possibility to perform an excretion study is questionable, an *in vitro* simulated metabolism may be considered as a reasonable alternative. By the moment of preparation of this manuscript, no data was available on the metabolism of AM2233, and only one report dealt with detection of urinary metabolites of JWH-210 [1]. Therefore, the aim of the present study was to investigate the metabolism of two novel cannabimimetics, AM2233 and JWH-210, using an *in vitro* approach.

Experimental

At the time when this study was initiated, both AM2233 and JWH-210 were legal in Russia, and respective smoking mixtures were bought via the Internet. The identity was tested by gas chromatography – mass spectrometric analysis of organic extract prepared as follows (data not shown). One hundred mg of each raw plant material was extracted with 5ml of methanol under sonication for 10 min and centrifuged. Ten µl of each extract was further purified by high performance liquid chromatography (HPLC) to isolate fractions corresponding to the main component. After eluent evaporation, every fraction was reconstituted 50 µl of methanol. An 1100 HPLC system (Agilent, Waldbronn, Germany) was employed to collect fractions. *In vitro* experiments were done using human liver microsomes (HLM), and 3A4 and 2B6 enzymes received from BD Gentest (Woburn, MA, USA). Ten µL aliquots of purified fractions were incubated with HLM or 3A4/2B6 enzymes according to the manufacturer’s protocol. Solid phase extraction was then used to remove the MS incompatible components of the reaction mixture. The eluant was evaporated and reconstituted in 200 µL of methanol/water (60/40) for subsequent LC-MS/MS analysis on an Acquity LC (Waters, Milford, MA, USA) coupled to a triple quadrupole mass spectrometer TSQ Vantage (ThermoFisher Scientific, San Jose, CA, USA).

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Waters Acquity BEH C18 column (100 mm × 2.1 mm, particle size 1.7 µm) maintained at 60°C was used for separation. The mobile phase was composed of 0.1% of formic acid in water and 0.1% of formic acid in methanol. The heated electrospray (ESI) ion source was used for ionization. Positive ions were detected in the fullscan, MS/MS (product ion scan) and selected reaction monitoring (SRM) modes. The collision gas pressure was 1.5 mTorr (argon 99.9995%). The vaporizer and capillary temperatures were set at 370 and 300°C, respectively, with a spray voltage 4000 V.

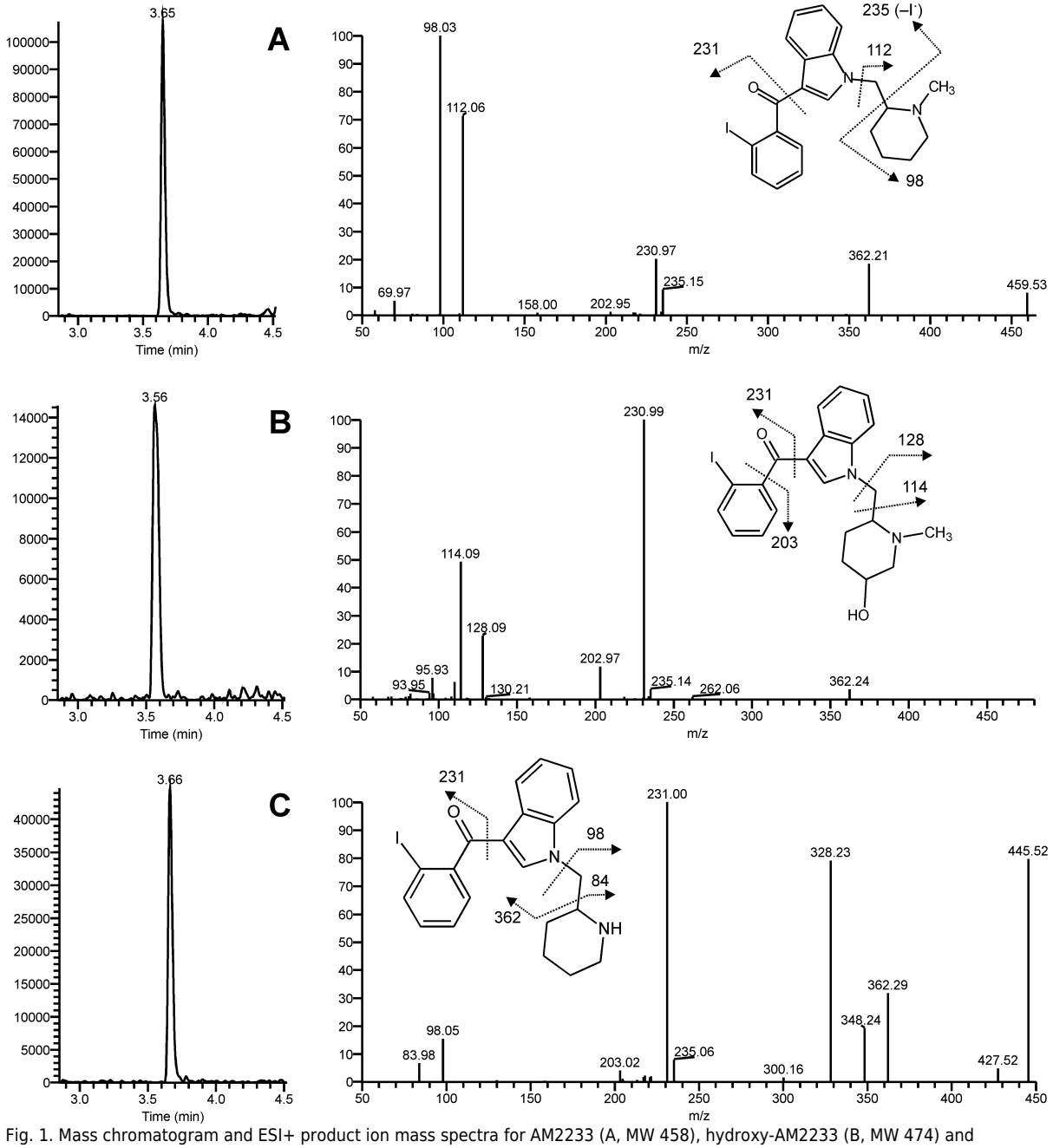

Fig. 1. Mass chromatogram and ESI+ product ion mass spectra for AM2233 (A, MW 458), hydroxy-AM2233 (B, MW 474) and N-desmethyl-AM2233 (C, MW 444) produced at 20, 25 and 15 eV, respectively.

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Results and Discussion

A preliminary GC-MS analysis of the extracts prepared from smoking mixtures has shown that the material containing AM2233 was relatively pure; however HPLC cleanup was undertaken to exclude potential side reactions and loss of enzyme activity. The second smoking mixture contained JWH-210, JWH-018 and AM-2201, and therefore HPLC cleanup was indispensable. In all cases mass spectral identification of smoking mixture ingredients was done using a freeware Cayman Spectral Library [2]. Interestingly, AM2233 turned out to be a relatively polar compound as under the conditions of reversed-phase LC it elutes earlier than any cannabimimetic we analyzed so far. After incubation of purified cannabimimetics it was found that AM2233 is relatively stable metabolically, perhaps due to its polarity, and undergoes hydroxylation and *N*-demethylation only (Fig.1). Contrarily, JWH-210 formed multiple products such as monohydroxy, dihydroxy (Fig.2), despentylhydroxy, oxo and oxohydroxy (Fig.3) metabolites.

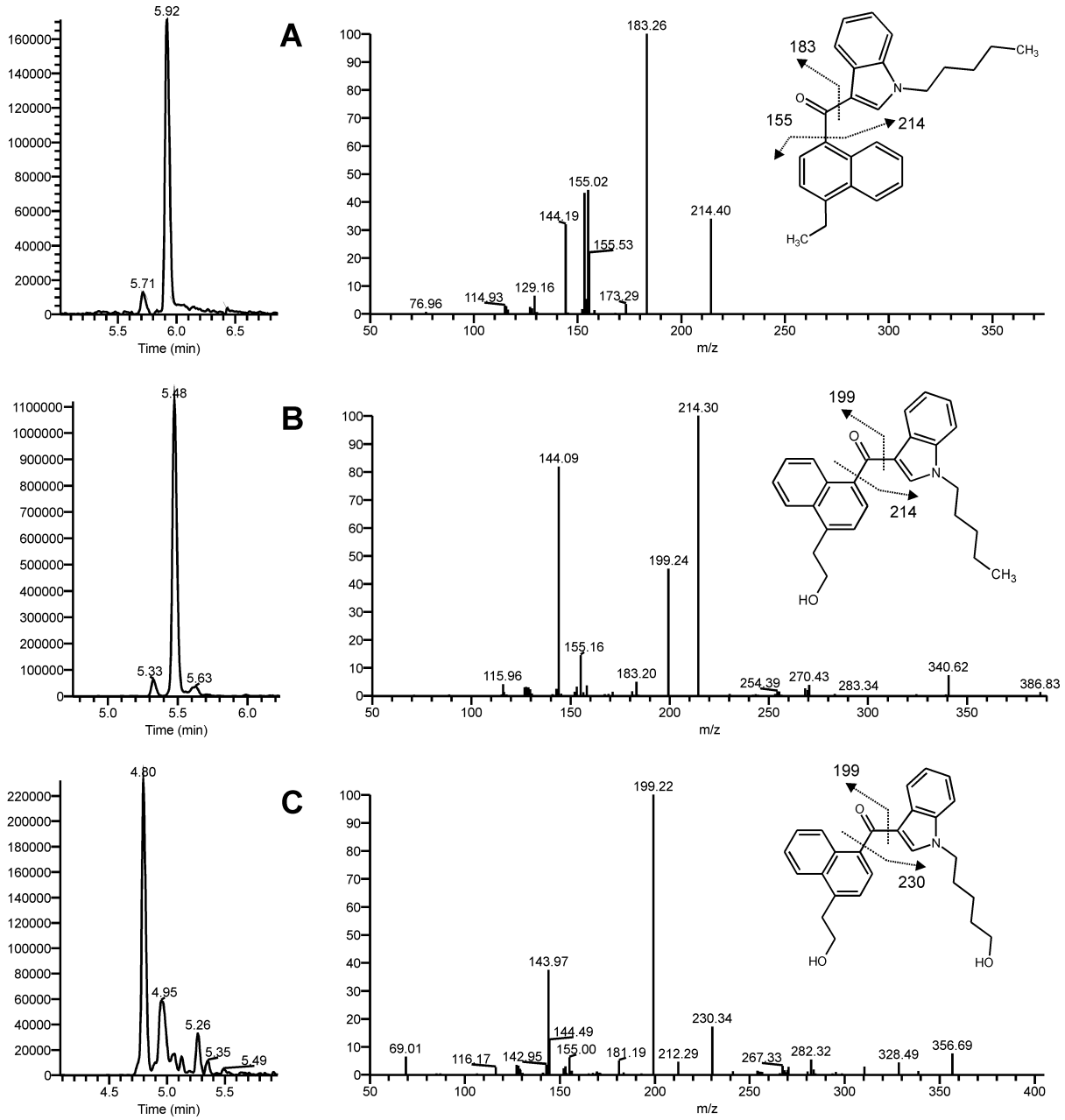

Fig. 2. Mass chromatogram and ESI+ product ion mass spectra for JWH-210 (A, MW 369), hydroxy-JWH-210 (B, MW 385) and dihydroxy-JWH-210 (C, MW 401) produced at 25 eV.

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In the latter case, fragmentation pattern suggests that the 30 *Da* increase corresponds to oxohydroxy rather than carboxy metabolite. Interesting to note that 2B6 enzyme selectively produced a monohydroxy metabolite of JWH-210 corresponding to the peak with RT=5.33 min in Fig. 2B, and not the base peak at RT=5.48 min. As noticed for other synthetic cannabinoids of aminoalkylindole family, the presence of ion at *m/z* 144 is consistent with an intact indole, *m/z* 214 – intact *N*-pentyl indole, and *m/z* 230 – hydroxylated *N*-pentyl indole [1].

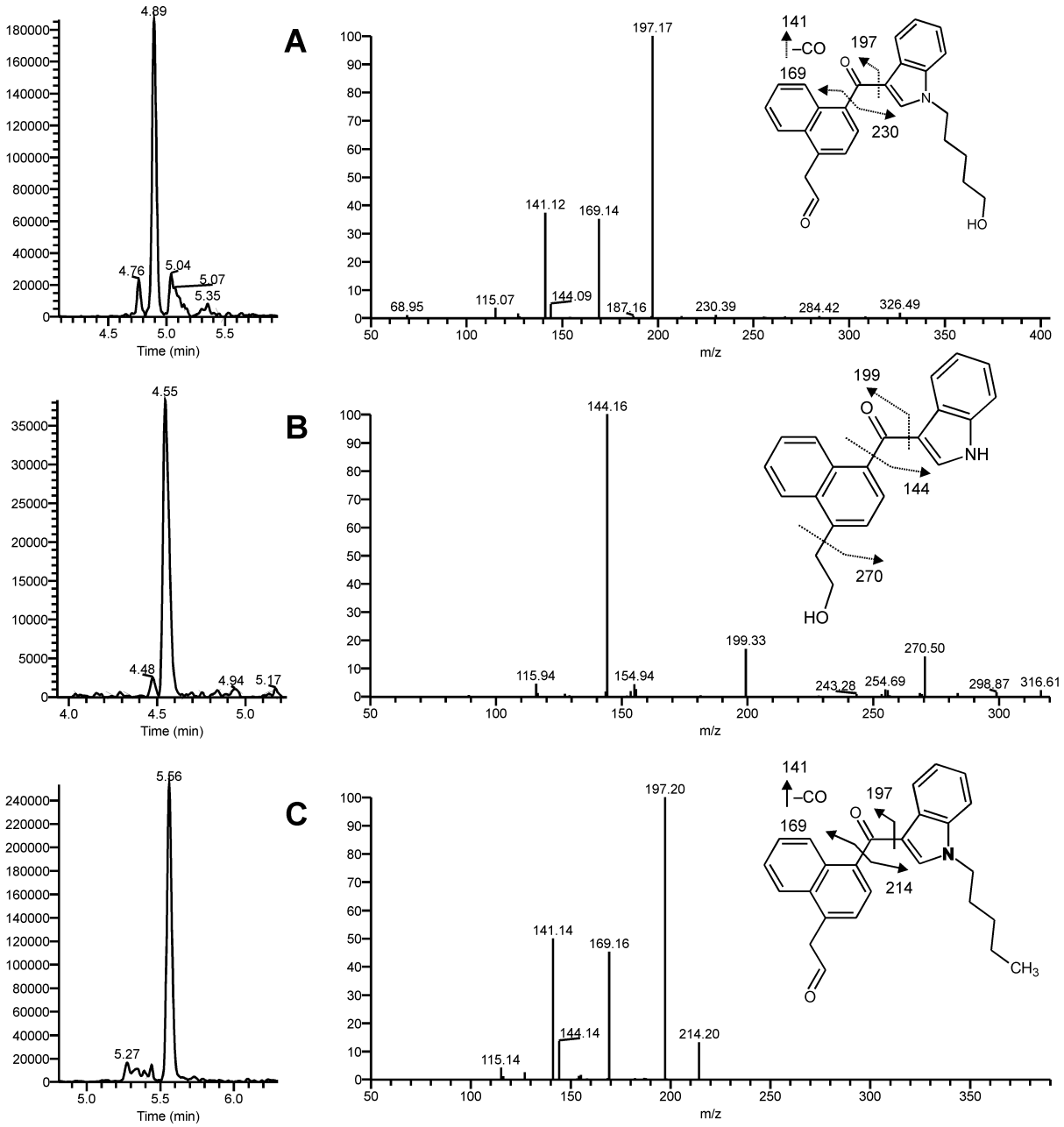

Fig. 3. Mass chromatogram and ESI+ product ion mass spectra for oxohydroxy-JWH-210 (A, MW 399), N-despentylhydroxy-JWH-210 (B, MW 315) and oxo-JWH-210 (C, MW 383) produced at 25, 20 and 25 eV, respectively.

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Positions of hydroxyls as shown in Fig.1-3 are tentative, and only demonstrate the part of molecule they are most likely located in (indole, side chain at indole nitrogen, or substituent at carbonyl group). The use of different enzyme preparations has showed that the best metabolic activity is achieved when human liver microsomes are utilized (Fig.4): so, 2B6 showed no activity towards AM2233.

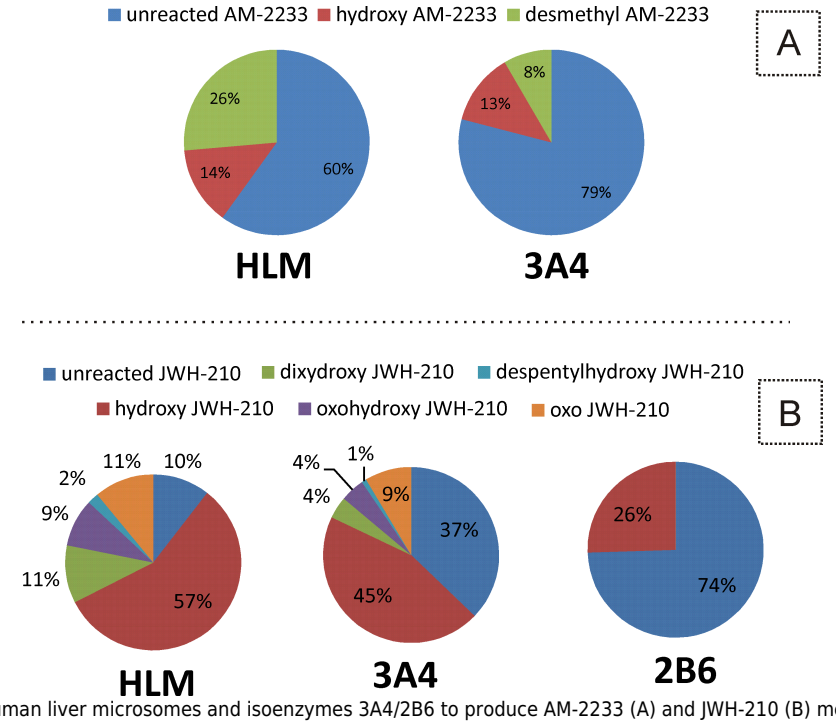

Fig. 4. Activity of human liver microsomes and isoenzymes 3A4/2B6 to produce AM-2233 (A) and JWH-210 (B) metabolites.

Conclusions

A metabolism of two novel synthetic cannabinoids AM2233 and JWH-210 isolated from smoking mixtures bought via the Internet was studied *in vitro*. Probably due to its relatively high polarity, AM2233 demonstrated only moderate biotransformation leading to monohydroxy-AM2233 and *N*-desmethyl-AM2233. In contrast, JWH-210 produced multiple metabolites such as monohydroxy-, dihydroxy-, despentylhydroxy-, oxo- and oxohydroxy-JWH-210. Of course, an *in vivo* metabolism will not strictly follow these findings but the data reported herein may help expand screenings procedures in antidoping laboratories.

References

[1] Hutter M, Broecker S, Kneisel S, Auwärter V. (2012) Identification of the major urinary metabolites in man of seven synthetic cannabinoids of the aminoalkylindole type present as adulterants in “herbal mixtures” using LC-MS/MS techniques. *J Mass Spectrom.* **47**(1), 54-65.
[2] Cayman Spectral Library (CSL). https://www.caymanchem.com/app/template/SpectralLibrary.vm (access date 01.09.2012)