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RECENT ADVANCES IN DOPING ANALYSIS (6)

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Elucidation of Carphedon Doping – Designer psychostimulant in Sports

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Introduction

Between March and June 1997, an obviously xenobiotic compound appeared at high concentrations in several cases of routine doping analysis of competition samples at international level (figure skating, athletics). No hint on its origin could be found at the sampling protocols. First attempts to identify this substance failed, because it was contained neither in spectral libraries nor in compound registries. Background information from Peter Hemmersbach (Oslo) made it possible to identify it as the compound "Carphedon", a psycho-stimulant and "stress protector" produced in Russia. The structure of the agent can be interpreted as a phenylethylamine derivative as well as an analogue of pemoline. According to the IOC Medical Code, it can be classified as a related substance of the doping class I.A (stimulants) and has to be considered as a doping agent.

Analytical Findings

This unknown compound produced major signals within several screening procedures. In the screening for nitrogen containing volatile stimulants a so far unknown peak appeared at RT 8.67 min *(fig. 1)*. The EI mass spectrum provides distinct fragment ions and suggests a molecular mass of 218. Nevertheless, there was no usable match in any of the available mass spec libraries: the cholinesterase inhibitor physostigmine was the only promising proposal *(fig. 2)*. At first it seemed to be not completely impossible, because there had been rumours about the misuse of cholinesterase inhibitors (e.g. galanthamine) some years ago. However, it could be excluded by comparison with the authentic compound and, taking the high urine concentration into consideration, for logical reasons because of its toxicity. To get hints on functional groups, the behaviour of the substance under the conditions of some routinely used derivatisation techniques (trifluoroacetylation, trimethylsilylation, methylation) was tested.

Interpretable results were obtained only by TMS derivatisation. Two products were formed, TMS product 1 by addition of one TMS group to the underivatised compound and TMS product 2 by loss of 18 (probably water) from TMS product 1 *(fig. 3)*.
The HPLC screening illustrated the large concentration of this substance, whereas the UV spectrum gave only poor information *(fig. 4)*. The different yields of this compound in both procedures was obviously due to the different solvents used for extraction; ethylacetate which was applied in the sample preparation of the HPLC screening seemed to give a significant higher recovery than diethylether.
In the screening for anabolic steroids and ß2-agonists (combined fraction) a large interfering peak appeared in the RT region of salbutamol *(fig. 5)*. The obtained mass spectrum was identical to that of TMS product 2; under the more drastic conditions of catalysed silylation the loss of water proceeded completely *(fig. 3)*.
In order to gather further qualitative information, the elemental composition of the underivatised compound was determined by HRMS. The calculation based on a presumptive molecular ion of 218, resulted the empirical formula was $C_{12}H_{14}N_2O_2$ *(fig. 6)*.

The analytical findings could be summarised as follows:

- the unknown compound is detectable by GC and HPLC
- it contains at least one silylatable site
- elimination of water may occur under certain conditions
- the molecular weight is probably **218**
- the empirical formula is $\mathbf{C_{12}H_{14}N_2O_2}$

However, spectral library and compound registry searches provided no conclusive information to identify the compound in question.

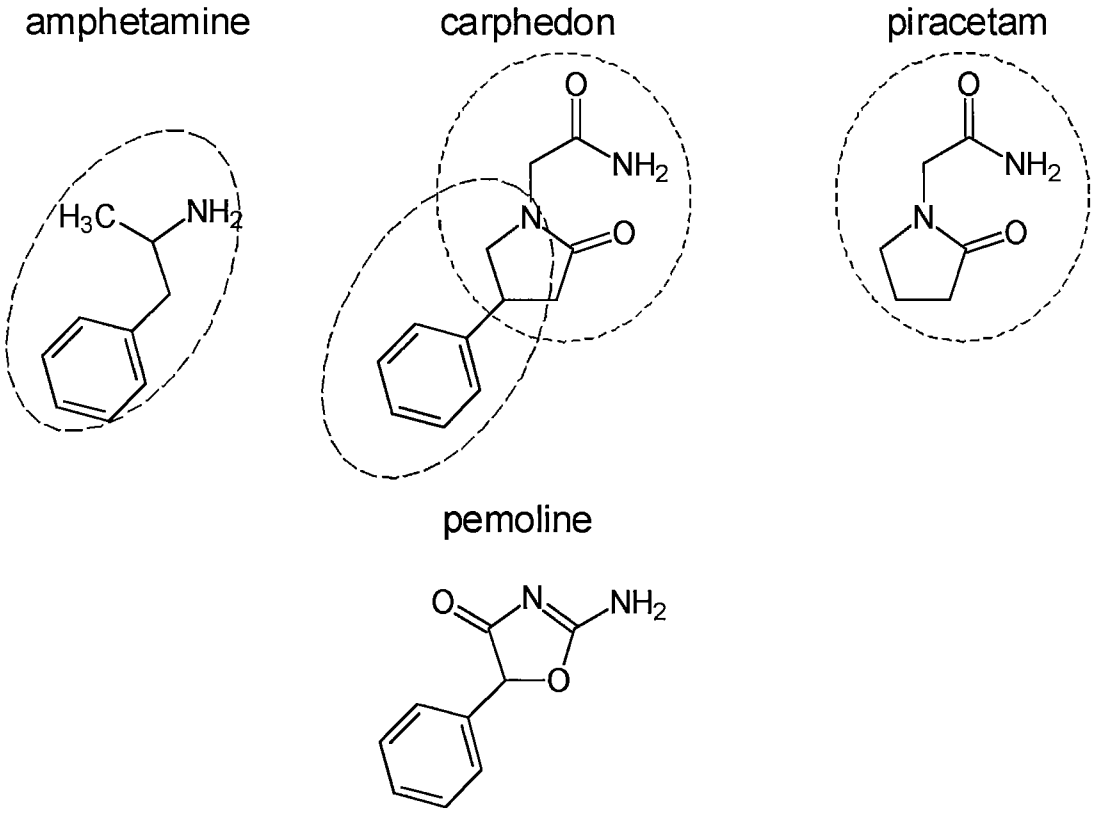
Non-Analytical Findings

At this point it became obvious, that we would need background information to solve this problem. For this reason we contacted Dr. Peter Hemmersbach from Oslo, at that time representative of the IOC accredited laboratories at the Subcommission of Doping. He reported about a meeting of the IOC Medical Commission in February 1997, where colleagues from the Moscow Laboratory had provided some information about a hitherto unknown compound, which had been unofficially produced on small scale at a research institute of cosmic medicine in Russia and could have been possibly misused by athletes [1].

This compound with the name carphedon (phenylpiracetam) was described as a psycho-stimulant and "stress protector". A dose of 100 mg was reported to enhance physical endurance and adaptation as well as resistance against cold.

Interpretation

From then, it was not difficult to identify the unknown compound as being exactly this carphedon *(fig. 7)*. The structure of the agent can be interpreted as a phenylethylamine derivative of the amphetamine type as well as an analogue of pemoline. The intention of the "designer(s)" of this compound could have been to enhance the stimulating effect of the well-known nootropic piracetam (the misuse of which by athletes was shown three years ago [2]) by the introduction of a phenyl group to obtain this phenylethylamine structure.



Consequently, all our confirmed cases of carphedon misuse were reported positive for a primarily unknown substance related to doping class I.A (stimulants) of the IOC Medical Code. Caused by the "bromantane experience" at the Atlanta Games, the involved sports federations hesitated to punish these cases as doping offences as long as carphedon was not explicitly included into the list of examples for the classes of prohibited substances, although the pharmacological and structural characterization as a related substance was recognized.

Summary

The misuse of carphedon could be confirmed in several cases. This elucidation was only possible because this substance produces interfering signals within the commonly used screening procedures and after additional background information could be obtained.
Basing on its structural elements and pharmacological properties, carphedon can be classified as a related substance of the doping class I.A (stimulants) of the IOC Medical Code and has to be considered as a doping agent.
However, the legal uncertainty, remaining as a consequence of the bromantane affair at the Olympic Games in Atlanta 1996, seems to put into question the general acceptance of the *related-substance-concept* when a primarily unknown compound is appearing for the first time.

References

[1] Hemmersbach, P., personal communication

[2] Mueller, R. K.; Grosse, J.; Lang, R.; Thieme, D., Recent Advances in Doping Analysis (3), Cologne, 1996, p. 357-364

Acknowledgement

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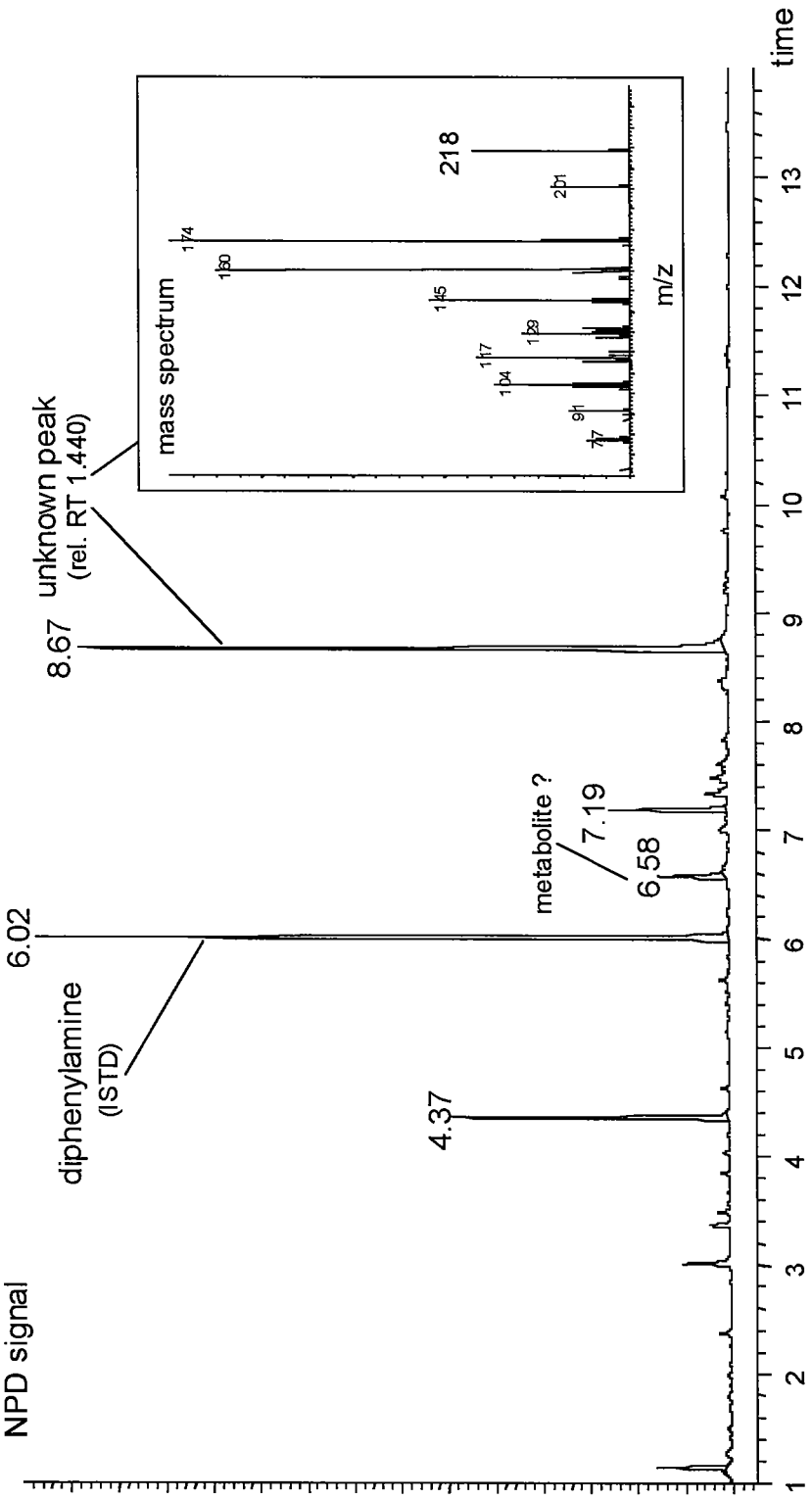


fig. 1 GC/NPD screening of a sample containing the unknown xenobiotic compound

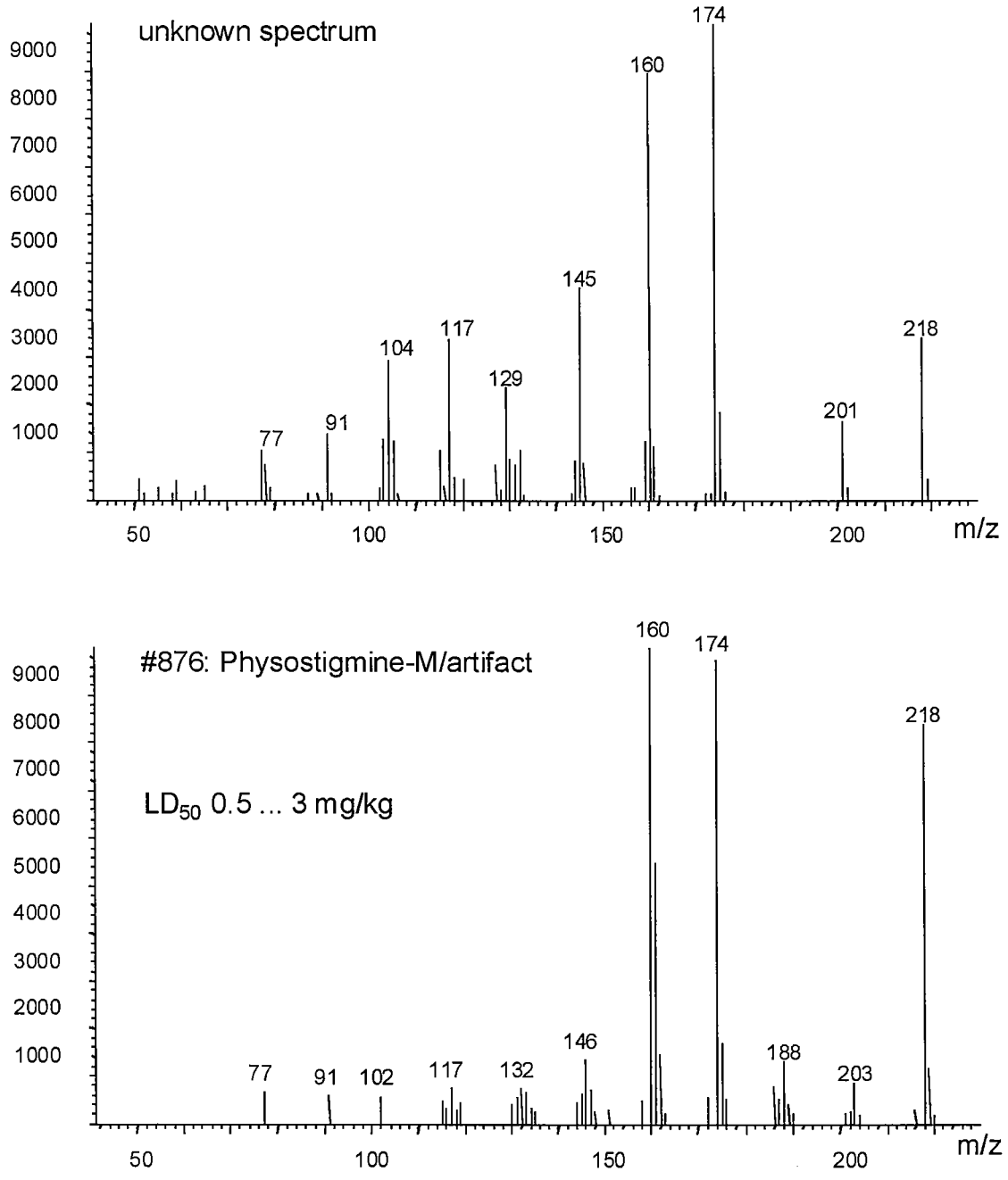


fig. 2 Result of MS library search

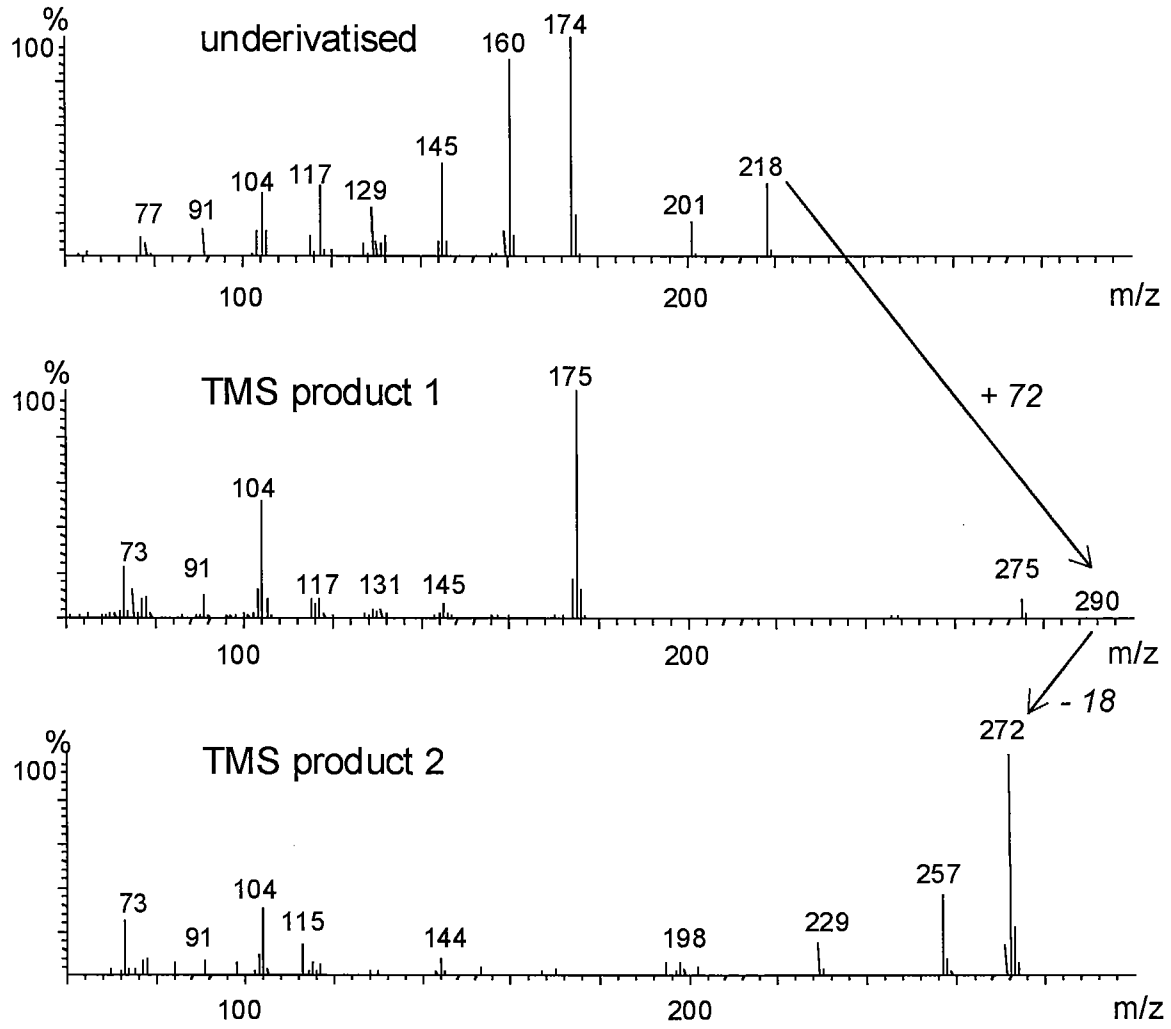


fig. 3 Mass spectra of the unknown xenobiotic substance: underivatised compound and two products of trimethylsilylation

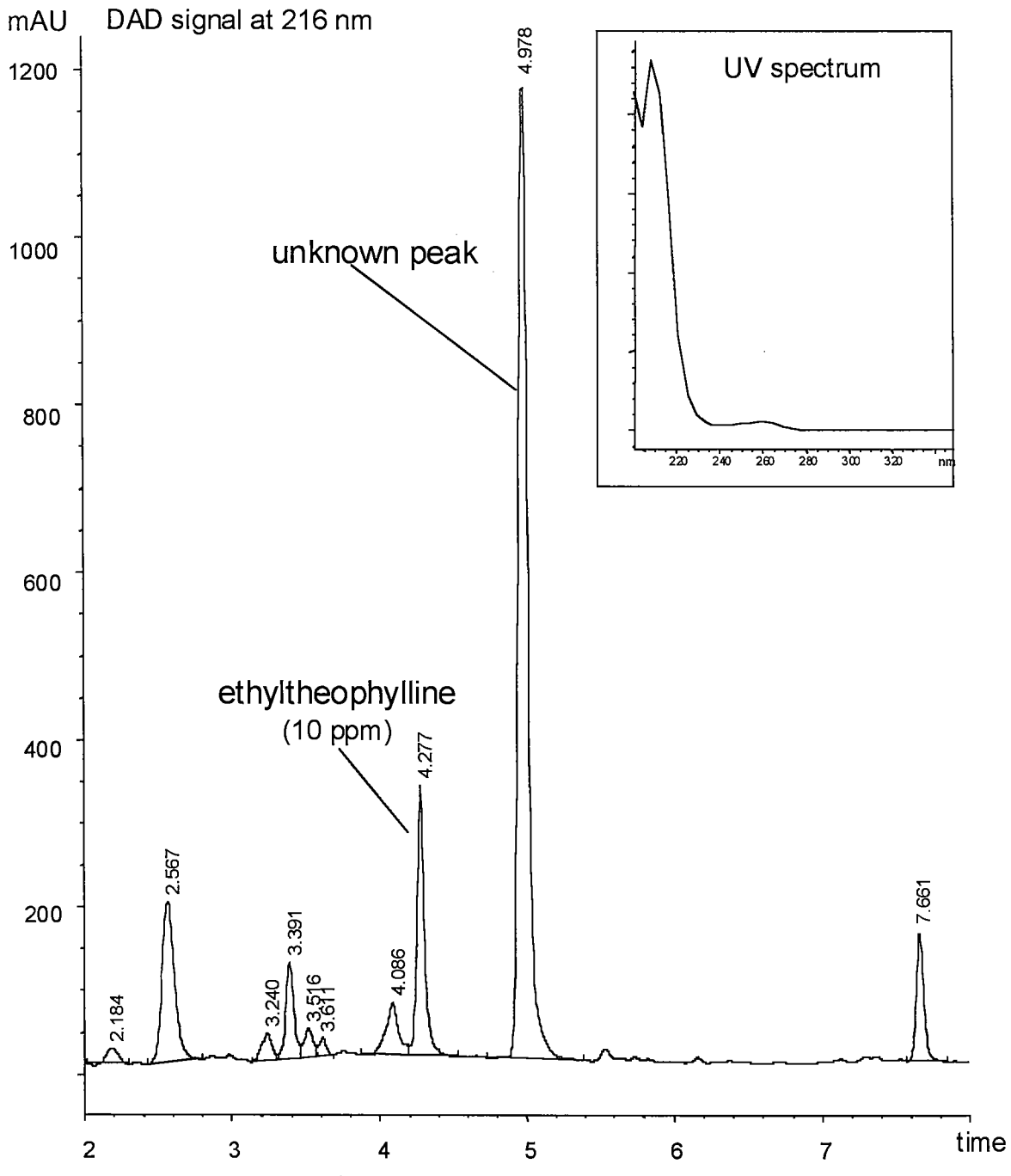


fig. 4 HPLC screening with the dominating signal of the unknown substance at RT 4.98 min

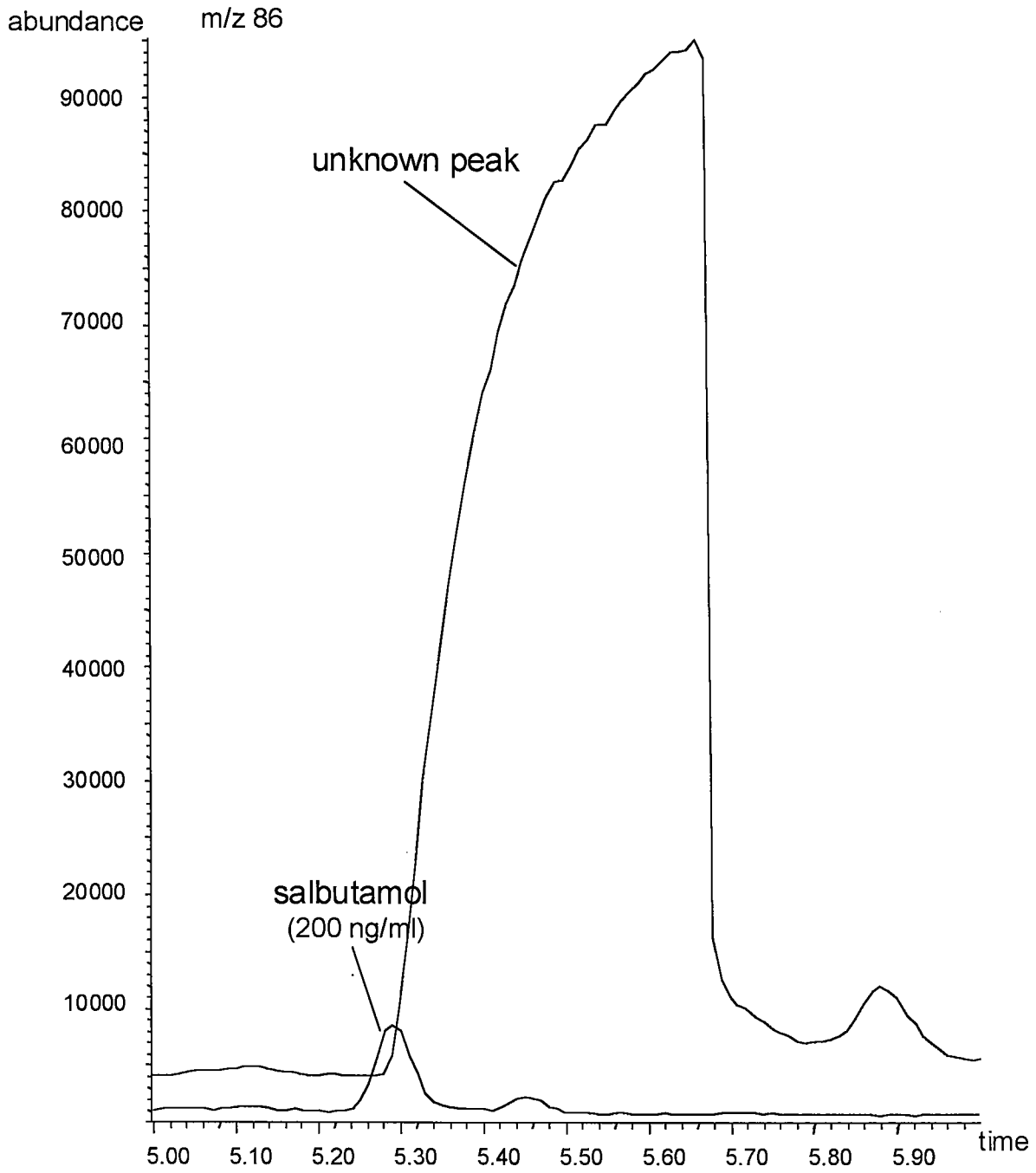
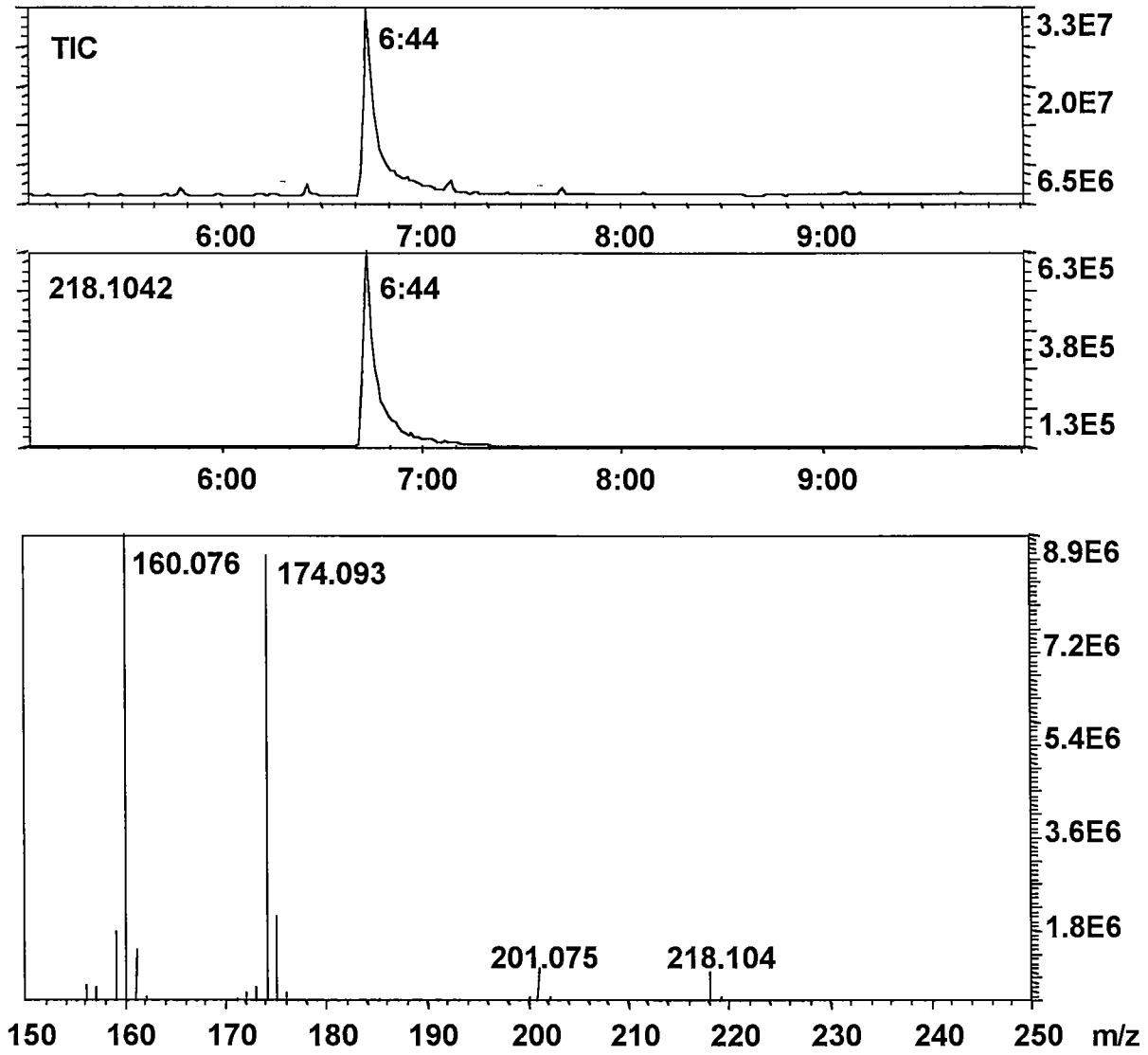


fig. 5 Combined fraction of screening for anabolics showing the signal of the unknown substance interfering with the retention region of salbutamol



Mass	%RA	PPM	mDa	Calc. Mass	DBE	C	H	N	O
218,1043	5,4	5,8	1,3	218,1055	7	12	14	2	2
		-12,6	-2,8	218,1015	3	7	14	4	4
201,0752	6,1	-1,3	-0,3	201,0750	4	7	11	3	4
		12	2,4	201,0776	8,5	10	9	4	1
		18,7	3,8	201,0790	8	12	11	1	2
174,0932	92,1	-7,4	-1,3	174,0919	6,5	11	12	1	1
		-15,1	-2,6	174,0905	7	9	10	4	
160,0767	100	-2,9	-0,5	160,0762	6,5	10	10	1	1
		-11,3	-1,8	160,0749	7	8	8	4	
		-19,7	-3,2	160,0736	2	7	12	4	

fig. 6 HRMS voltage scan at 3k resolution and table of calculations for the determination of the elemental composition

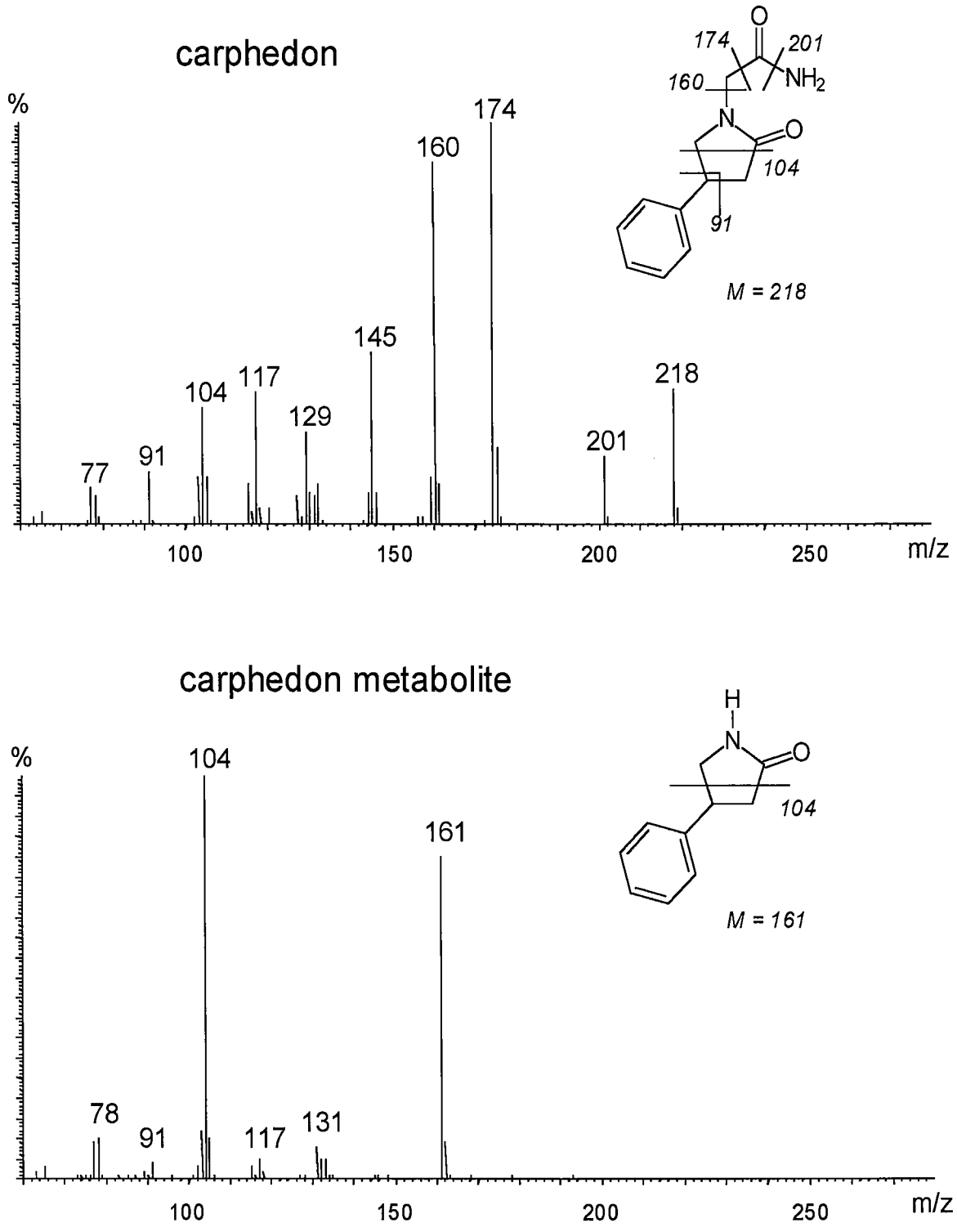


fig. 7 Mass spectra of carphedon and its metabolite (or GC artifact)