

# Trace analysis of lachrymatory agents from self-defence devices

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# A long-time used defence system



# Typical examples of personal defence accessories



# Finding evidence of lacrymatory residues may have important consequences

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## The Stephane Krauth murder case

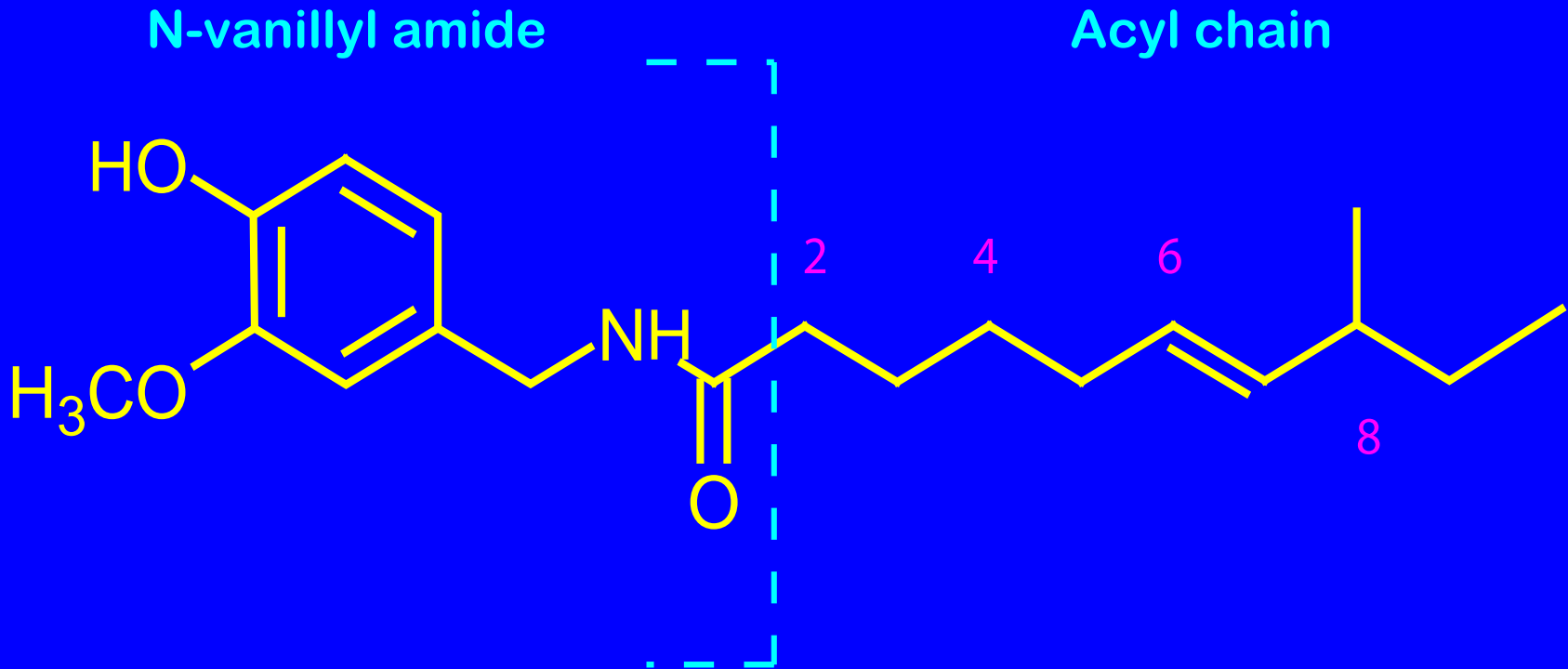
- 22 July 2001, Bitche, (Moselle, France), 16-year-old Karine Schaaf is found dead, next to her damaged mountain bike.
- Headlight fragments and car paint traces orient the IRCGN toward a white Mazda modèle 323, and to his owner Stéphane Krauth, 23 year-old.
- Krauth first claimed that the accident was unintentional. He got into a panic, and burned the victim.
- Trace of lacrymatory residues were found on the bike saddle, thus proving an intentional aggression.
- Friday 22 October 2004, Stephane Krauth was sentenced to 30 year prison, with 20 years of safety period.

# Cayenne or Chili Pepper



**OC : Oleoresin Capsicum**




# Natural capsaicinoids




Carbon number in the acyl chain  
Double bond position, if present  
Position methyl substituent

: 8 à 10  
: 4, 5, 6, 7 ou 8  
: 8, 9 ou 10

# Major natural Capsaicinoids

Name	Structure
Nordihydro-Capsaicine $C_{17}H_{27}NO_3$ 293,199	 <p>The structure shows a benzene ring with a hydroxyl group (HO-) at the 3-position and a methoxy group (H<sub>3</sub>CO-) at the 4-position. A -CH<sub>2</sub>-CH<sub>2</sub>-NH-C(=O)- chain is attached to the 1-position. The chain continues as a saturated heptyl chain ending in an isopropyl group.</p>
Capsaicine $C_{18}H_{27}NO_3$ 305,199	 <p>The structure is similar to Nordihydro-Capsaicine, but the heptyl chain is replaced by an unsaturated chain with a double bond between the 6th and 7th carbons from the amide group, ending in an isopropyl group.</p>
Dihydro-Capsaicine $C_{18}H_{29}NO_3$ 307,25	 <p>The structure is similar to Nordihydro-Capsaicine, but the heptyl chain is replaced by a saturated octyl chain ending in an isopropyl group.</p>

# Synthetic capsaicinoids

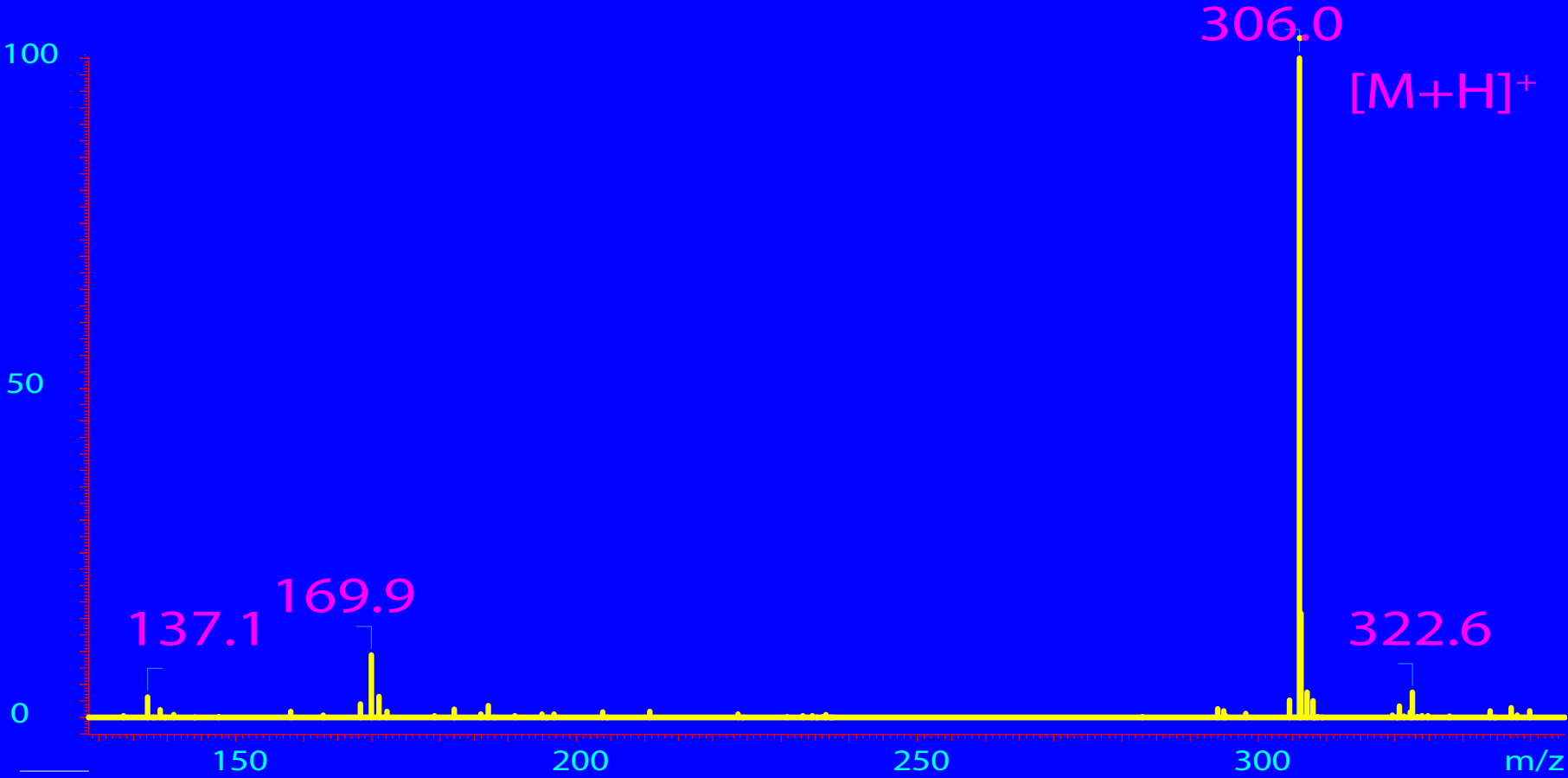
Name	Structure
<p>Nonivamide PAVA Capsaicine synthétique</p> <p><math>C_{17}H_{27}NO_3</math> 293,199</p>	 <p>The chemical structure of Nonivamide (PAVA) consists of a benzene ring substituted with a hydroxyl group (HO-) at the 3-position and a methoxy group (H<sub>3</sub>CO-) at the 4-position. A propyl chain (-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-) is attached to the 1-position of the ring, which is further connected to an amide group (-NH-CO-). The carbonyl oxygen (O) is double-bonded to the nitrogen atom. The nitrogen atom is also bonded to a nonyl chain (a nine-carbon alkyl chain).</p>

Thermolabile molecules, potentially amenable to GC and GC/MS after derivatization.

Easily separated by LC, and detected by APCI<sup>+</sup> with greatly improved speed and sensitivity compared to GC/MS.



# LC-APCI+/MS<sup>1</sup>, Capsaicine



# 'Aerosols' rather than 'gases'

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- **Lacrymatory molecules (CS, OC,..), are solids at ambient temperature.**
- **Self-defence bombs also contain:**
  - **Fixing agents (PEGs)**
  - **A propeller gas : N<sub>2</sub>, freon, light hydrocarbons...**
- **Many lacrymatory molecules (OC, but not CS) and PEGs may remain intact for a long period (8-10 days), following projection, including under severe meteo conditions (wind, rain).**

# LC/MS/MS method used at the IRCGN laboratory

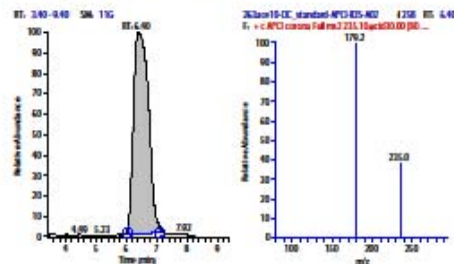
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- **Trace analyses of OC residues is currently done by LC/MS/MS**
  - **Thermo Fisher LCQ advantage**
  - **Water/Methanol gradient**
  - **RPLC, C18, 2 mm i.d., 0.3 mL/min**
  - **APCI<sup>+</sup>; 6 kV, 4  $\mu$ A**
  - **A series of optimized MS/MS segments**

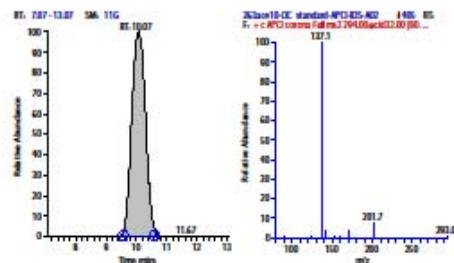
## Recherche de capsaïcinoïdes

Data File: 263ace10-OC\_standard-APCI -IDS -A  
 02  
 ECX  
 Operator:  
 Comments:  
 Acquisition Date: 04/21/10 02:45:25 PM  
 Injection Volume(μl): 5.00  
 Instrument Method: D:\MetAcm\OC\ME268 -APCI2 mm-OC-MS2 -A.meth  
 Current Processing Method: N/A

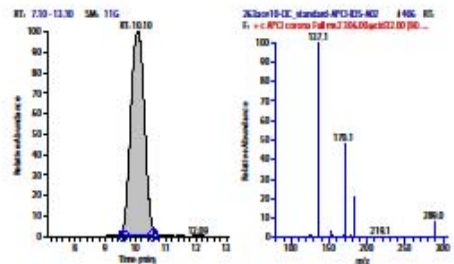
Component Name: Terbutylazine\_D5 (EI)  
 Actual RT(min): 6.40  
 Area(cts-sec): 10763852.37  
 Height: 312333.29  
 Signal To Noise: 801.27



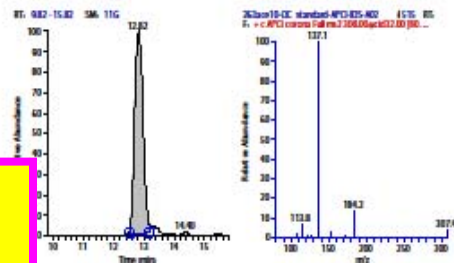
Component Name: Nonivamide  
 Actual RT(min): 10.07  
 Area(cts-sec): 9030266.11  
 Height: 284517.28  
 Signal To Noise: 2650.63



Component Name: Capsaicin  
 Actual RT(min): 10.10  
 Area(cts-sec): 13533379.29  
 Height: 431081.85  
 Signal To Noise: 2591.64



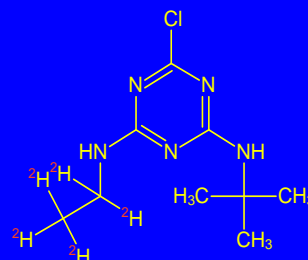
Component Name: Dihydrocapsaicin  
 Actual RT(min): 12.82  
 Area(cts-sec): 2787023.53  
 Height: 144770.07  
 Signal To Noise: 804.99



Typical sample report

# Automatisation

Internal standard : Terbutylazine\_D5

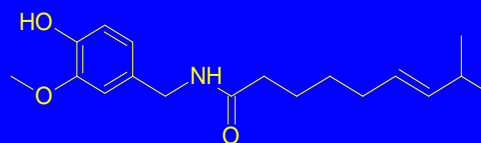


m/z=235.1>cid 30 [m/z 80-295]

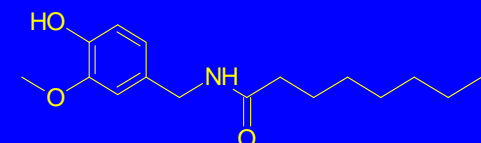
Nonivamide: m/z=294.0>cid 32 [m/z 80-295]



Capsaicin: m/z=306.0>cid 32 [m/z 80-307]



Dihydrocapsaicin: m/z=308.0>cid 32 [m/z 80-309]



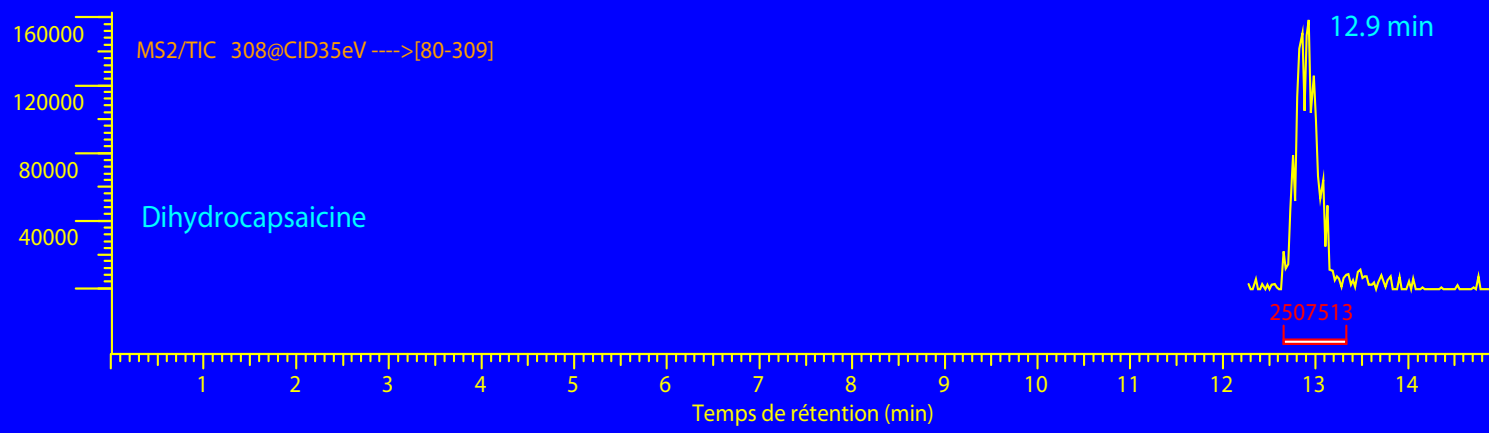
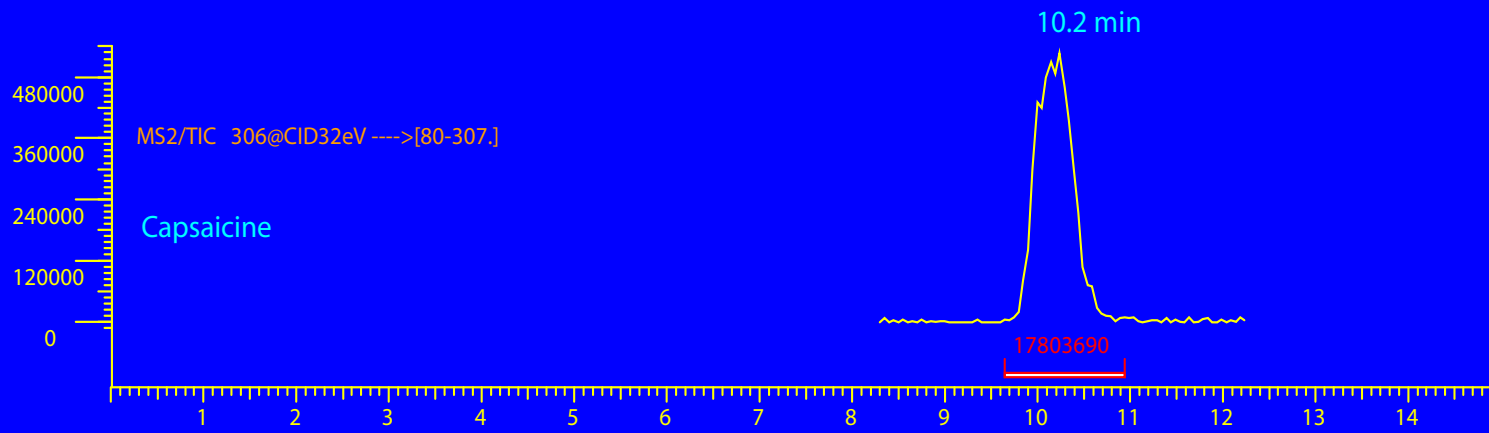
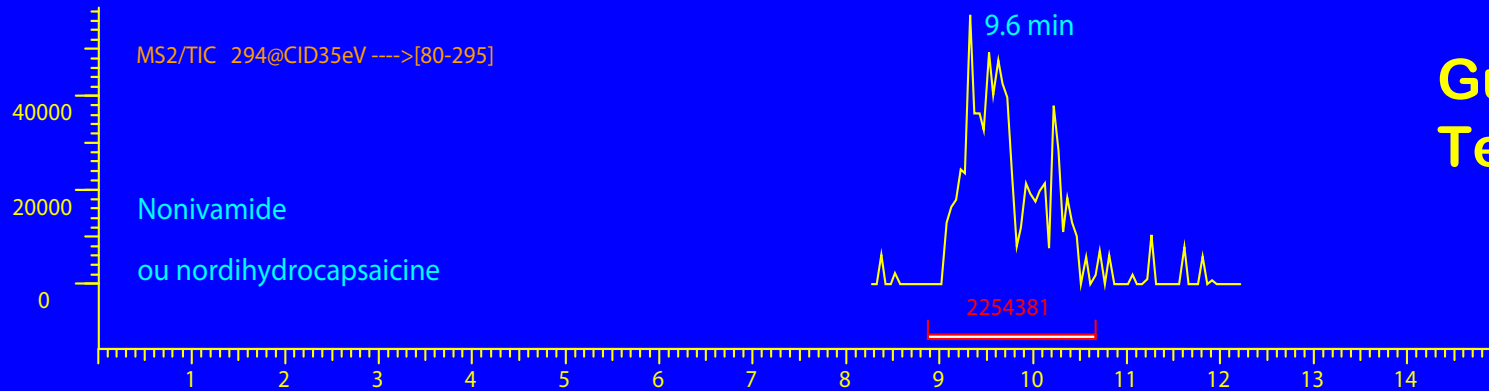
Positive Decision Limit : 50 pg/μL

# A dramatic example

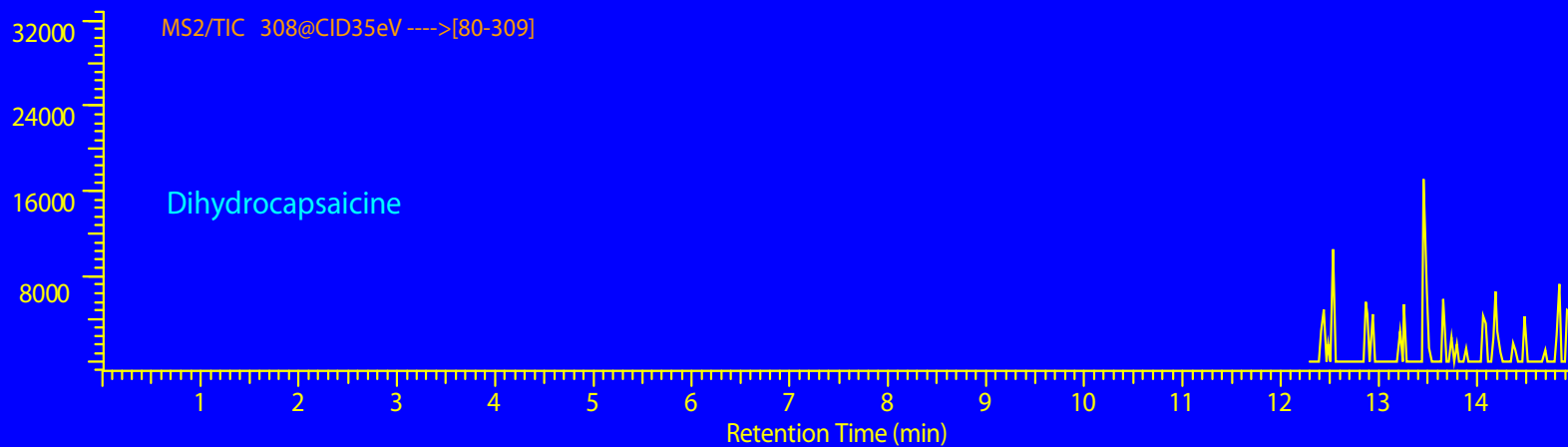
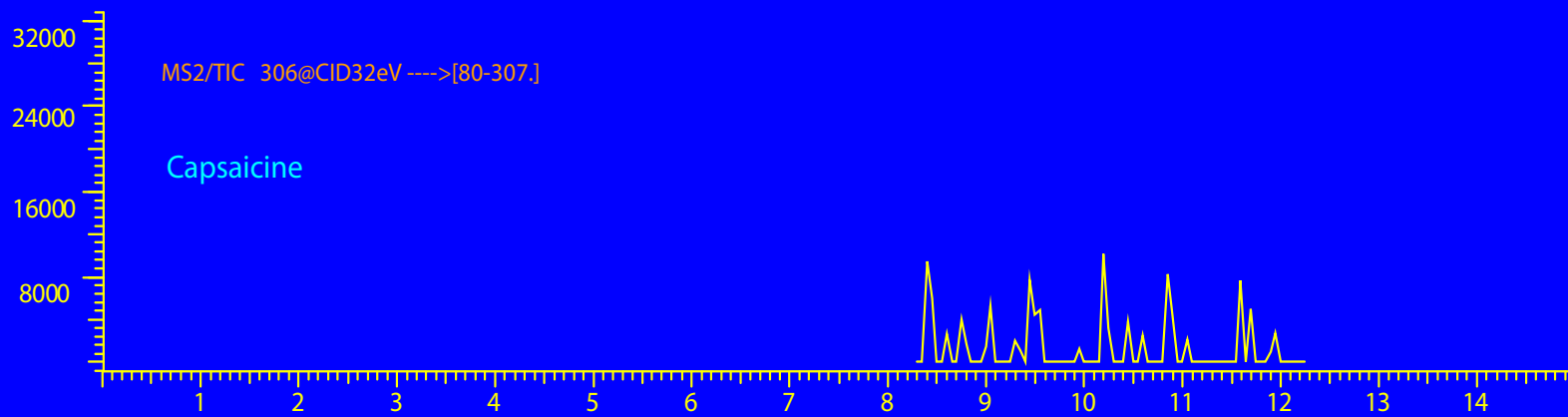
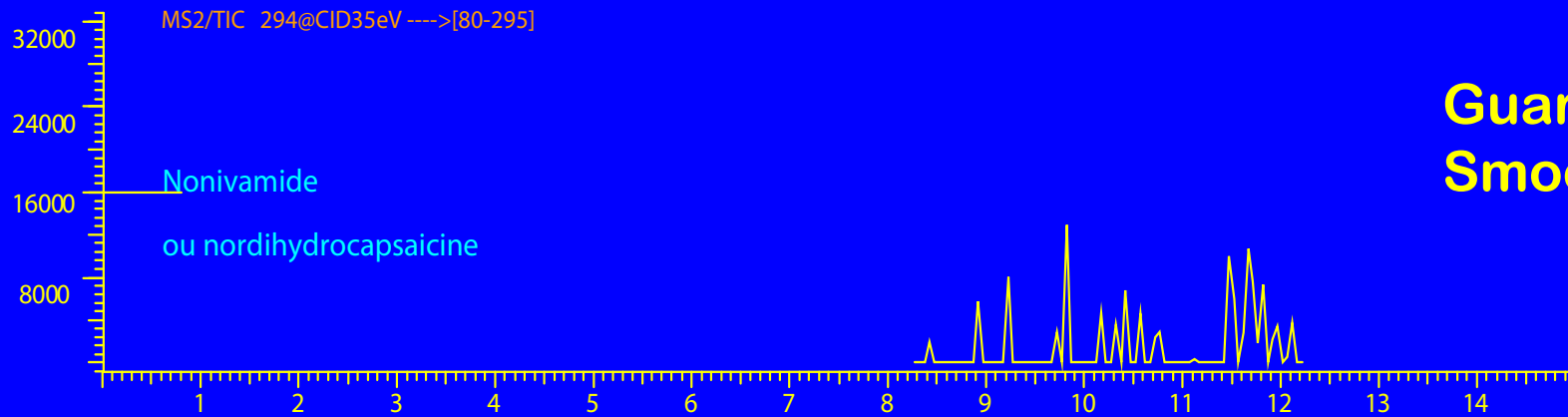
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- **A fight in the street, out of a discotheque, between a person and two disco security guards who used their aerosol bombs.**
- **Becoming blinded and disoriented, the person staggered along a road, and was caught by a passing car. He died later, as a consequence of the accident.**
- **None of the security guards admitted to be responsible. Their jackets were seized and sent to the IRCGN laboratory.**
- **A small piece of the victim cornea was sampled during the post-mortem examination and analyzed.**

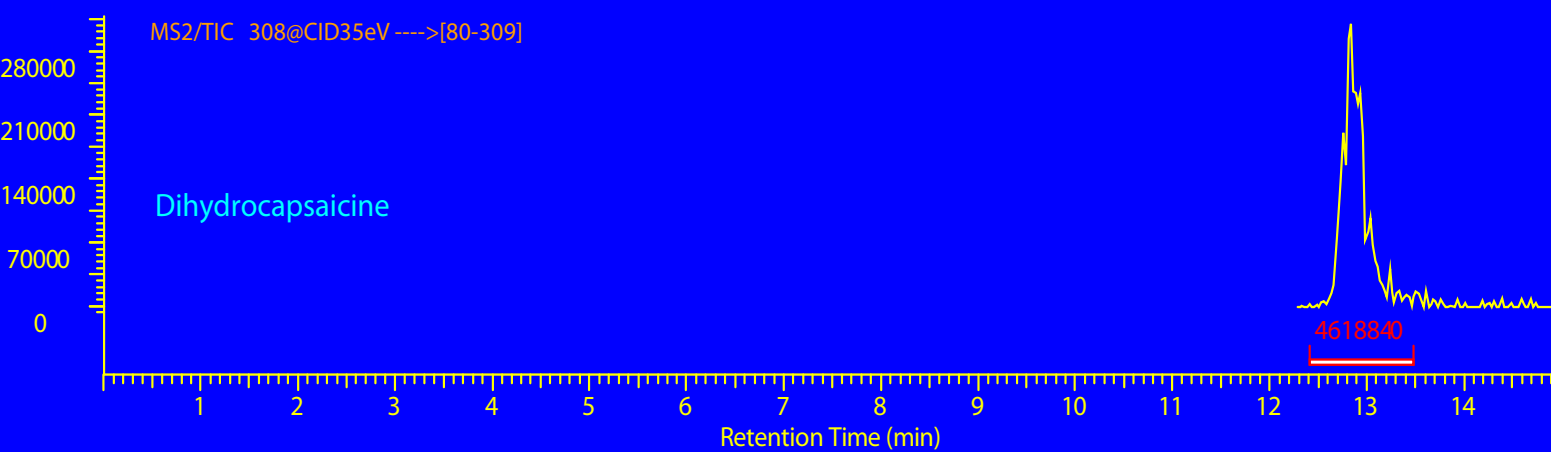
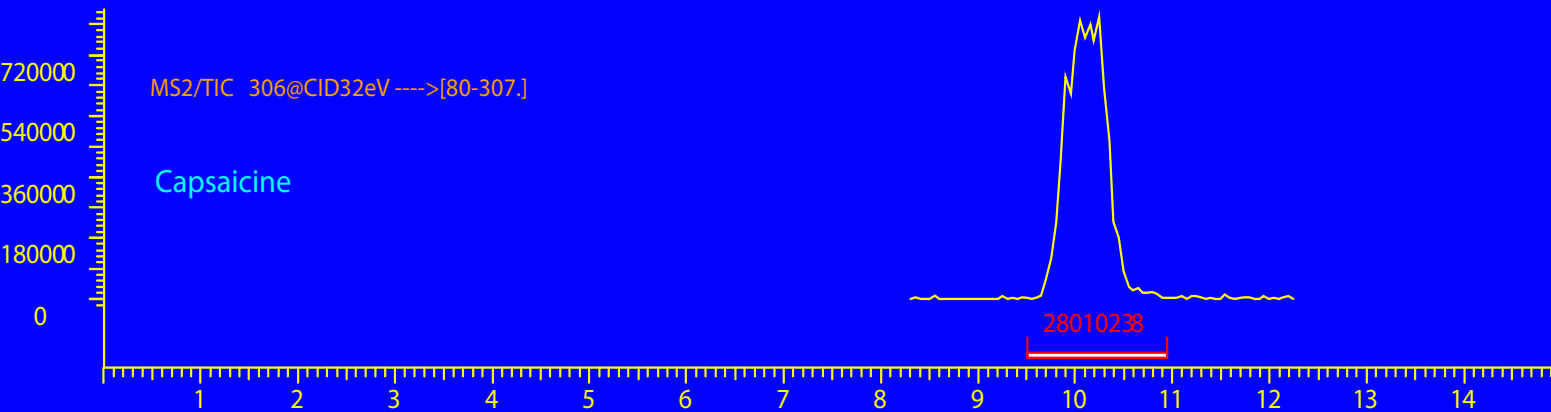
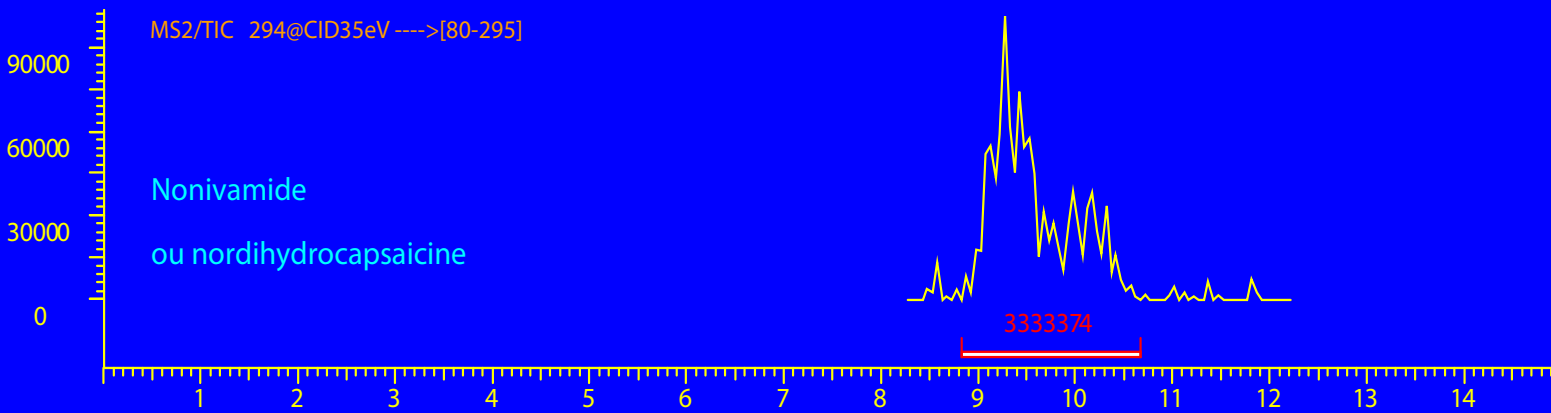
# Guard 1 jacket Textil fabric



# Guard 2 jacket Smooth leather



# Victim cornea





# Would the analysis be feasible using a single analyzer LC/MS ?

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- **Advantages of a single analyzer LC/MS**

- Low Cost
- Simple spectra with clear sample molecular information
- No preliminary optimization for finding relevant MS/MS transitions (source and collision cell fragmentation potentials)

- **Inconvenients**

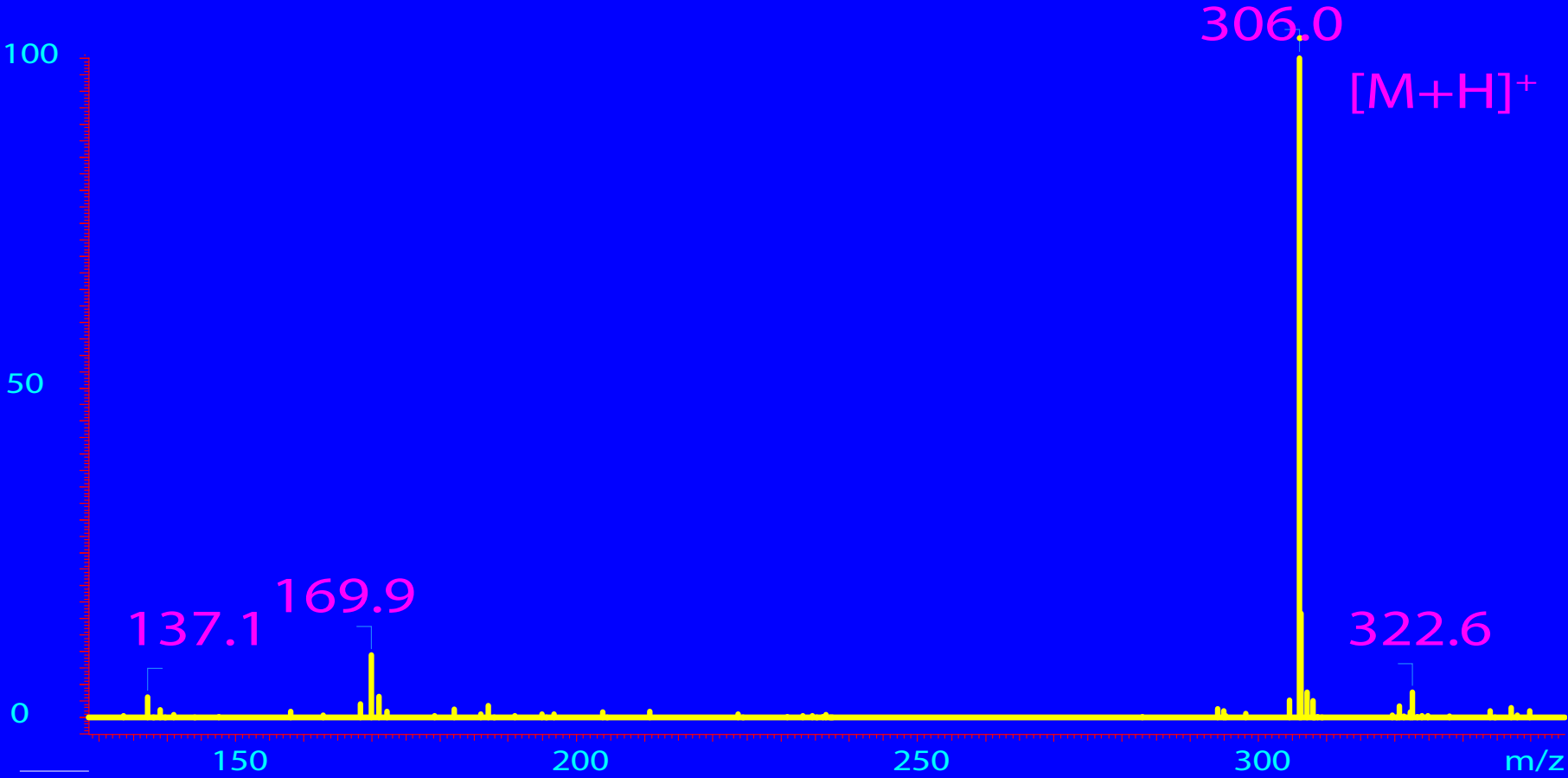
- Poor selectivity in case of complex mixtures
  - Quadripole low resolution gives mass accuracies  $\pm 0.1$  Da/z
- Background contamination in full scan mode

# Increased possibilities using ACD Intellitarget

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- **Increased selectivity due to additional search criteria**
  - Besides extracting current profiles within a target window of  $\pm 0.1$  Da/z
  - Retention parameters with a preset window (ex: 0.5 min)
- **IntelliTarget checks for**
  - $^{13}\text{C}/^{12}\text{C}$  ration for the  $(\text{M}+\text{H}^+) + 1$
  - Coherence with the target chemical formulae
  - Automatically assign masses and labels protonated molecular ions (in cas of  $\text{APCI}^+$  or  $\text{ESI}^+$ )

# LC-APCI+/MS<sup>1</sup>, Capsaicine



# List of targets for ACD IntelliTarget

**Target Ions**

▼	Reference Mass	Formula	Mass Delta	Ion Mode	Label	Mode	RT	RT Window
1	293.1991	C17H27NO3	0	+H	Nonivamide	Parent	10.07	0.5
2	293.1991	C17H27NO3	0	+H	Nordihydrocapsaicine	Parent	9.40	0.5
3	305.1991	C18H27NO3	0	+H	Capsaicine	Parent	10.10	0.5
4	307.2147	C18H29NO3	0	+H	Dihydrocapsaicine	Parent	13.00	0.5
5	321.2304	C19H31NO3	0	+H	Homodihydrocapsaicine	Parent	15.50	0.5
6	319.2147	C19H29NO3	0	+H	Homocapsaicine	Parent	13.35	0.5

Load Save Import... Add Delete Clear

Use Label Descriptor Only  
 Overwrite Manually Created Labels  
 Overwrite Automatically Created Labels

OK Cancel Help

IntelliTarget Parameters Manager

Parameter	Value
<b>12C/13C Error (%)</b>	
Value	30
<b>Additional M Ion Correction (%)</b>	
Value	5
<b>Apply Ion Presence Detection for</b>	
Apply	True
Molecular Ions	True
Adduct Ions	True
Primary Fragment Ions	True
Multimer Ions	True
Unconfirmed 12C Ions	False
Unconfirmed 13C Ions	False
Unassigned Ions	False
Label Ions with Status Not Found	True
<b>Baseline Correction</b>	
Baseline Correction	True
Box Half Width (Scans)	5
Noise Factor	1
<b>Calculate the Following Mass Values</b>	
Peak Top	True
Peak Top Window Type	% of Peak FWHM
Peak Top Window Value	50
Left Shoulder	False
Exclude the First Scan	True
Right Shoulder	False
Exclude the Last Scan	True
Entire Peak	False
Exclude the First and Last Scans	True

Expand All

Collapse All

Restore Default

Restore Section Defaults

Restore All Defaults

Load...

Save...



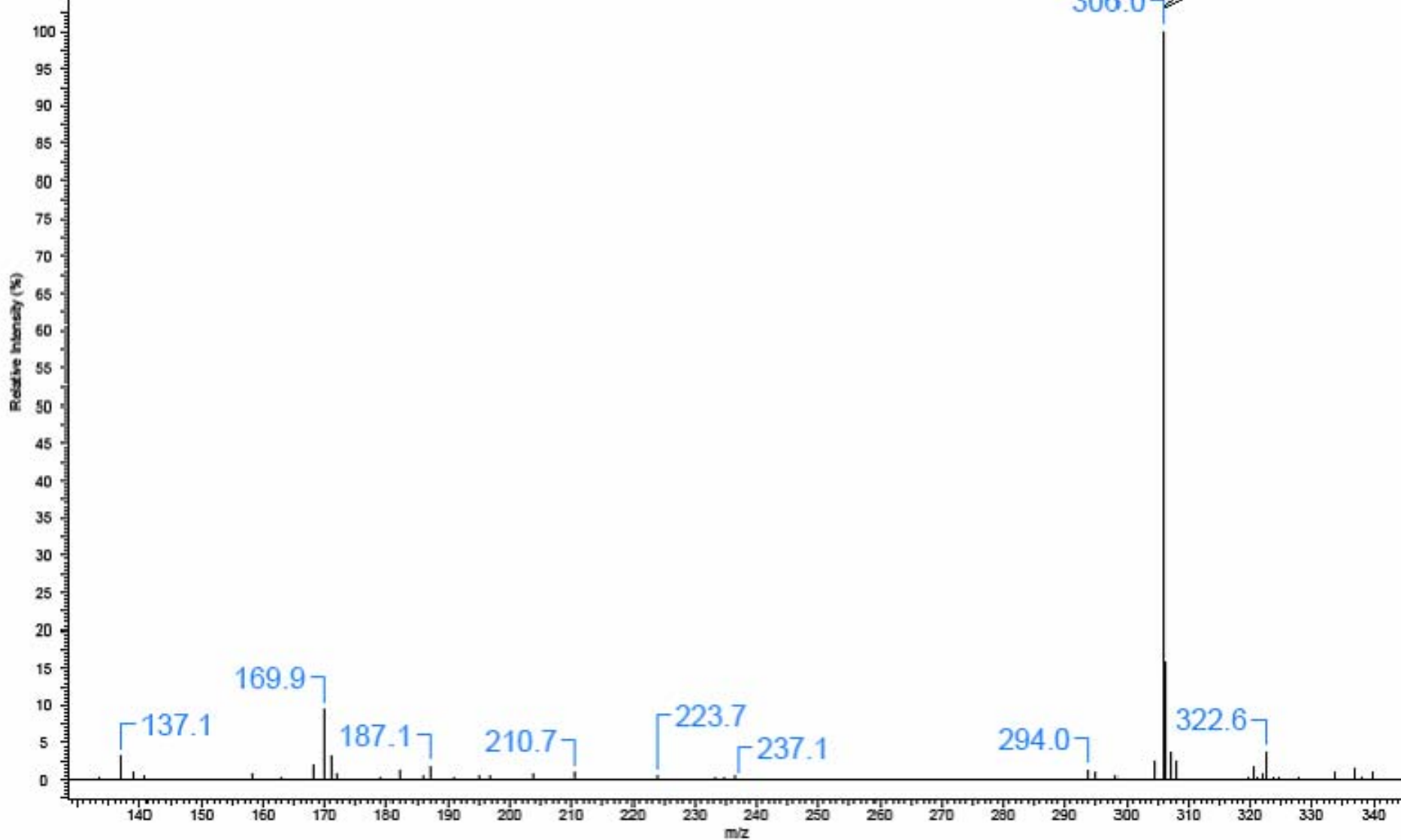
OK



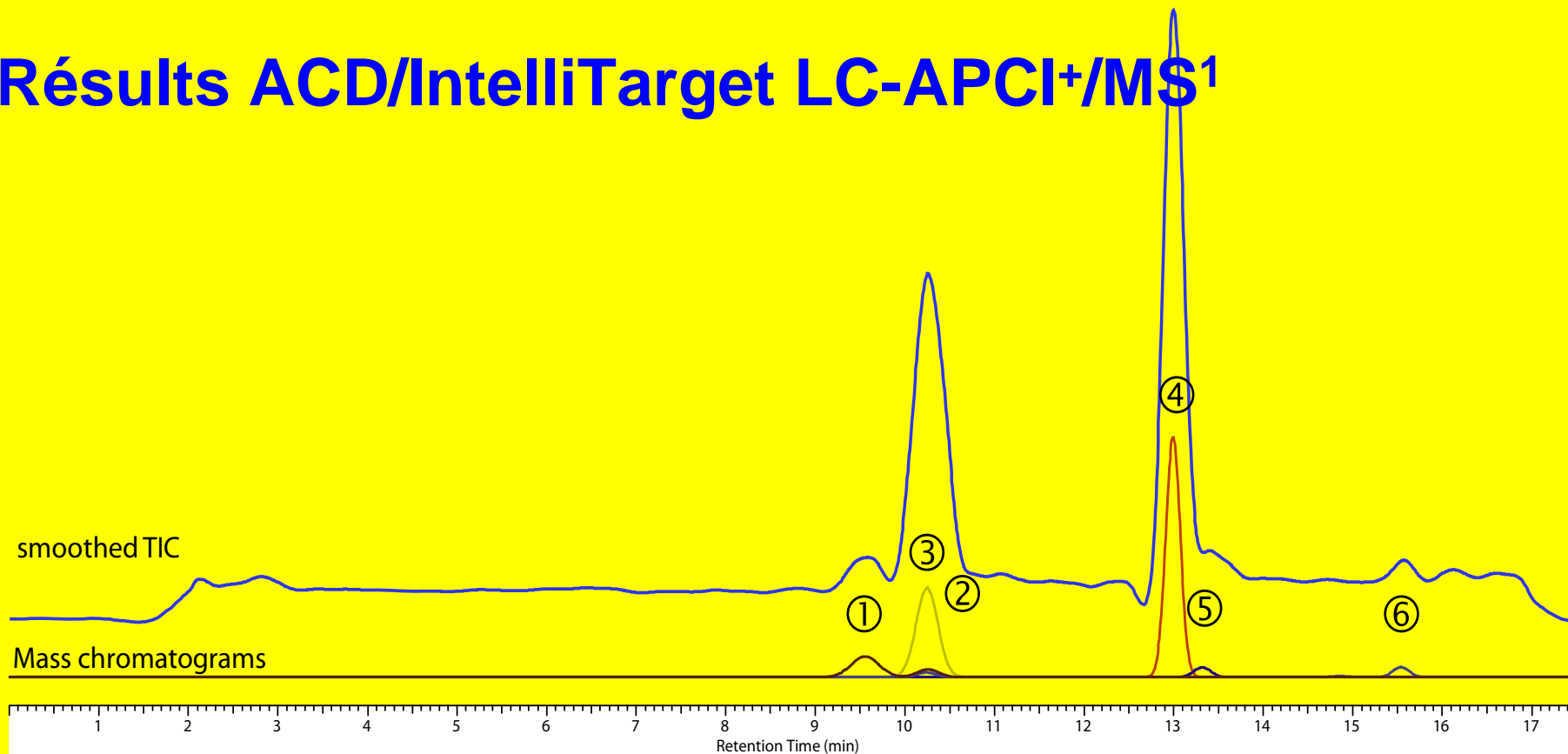
Cancel



Help



# Résultats ACD/IntelliTarget LC-APCI+/MS<sup>1</sup>



No.	m/z	tR (min)	Label	Notation	Class	CHNO	12C/13C	Peak Top Mass	Mass Delta	Target Status
1	294.206	9.552	Nordihydrocapsaicine	[M+H] <sup>+</sup>	Good	Pass	Pass	294.07	0.136	Found
2	306.206	10.248	Capsaicine	[M+H] <sup>+</sup>	Good	Pass	Pass	306.036	0.17	Found
3	294.206	10.255	Nonivamide	[M+H] <sup>+</sup>	Good	Pass	Pass	294.136	0.07	Found
4	308.222	12.993	Dihydrocapsaicine	[M+H] <sup>+</sup>	Good	Pass	Pass	308.077	0.145	Found
5	320.222	13.32	Homocapsaicine	[M+H] <sup>+</sup>	Good	Pass	Pass	320.089	0.133	Found
6	322.238	15.536	Homodihydrocapsaicine	[M+H] <sup>+</sup>	Good	Pass	Pass	322.089	0.149	Found

# Conclusions

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- **ACD IntelliTarget module can be applied to rapidly identify targeted molecules in complex mixtures, following a well defined and validated analytical protocol.**
- **It is well adapted to rapid and automated analyses of large series of samples.**
- **It improves and increases the selectivity performance of simple and low-cost LC/MS instruments.**





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## Recherche rapide de molécules cibles dans des mélanges séparés par couplage LC/MS<sup>1</sup> à simple analyseur

### RÉSUMÉ

Plusieurs molécules lacrymogènes caractéristiques contenues dans des bombes aérosols d'auto-défense ont été retrouvées dans des extraits d'échantillons analysés par LC/APCI<sup>+</sup>/MS à basse résolution, en mode de balayages complets (MS<sup>1</sup>). La recherche ciblée de ces molécules a été obtenue en dépouillant les données expérimentales au moyen du logiciel IntelliTarget (ACD/labs), conçu pour assister la recherche rapide et automatisée de molécules potentiellement présentes dans des mélanges complexes. Le dépouillement rétrospectif des spectres de masse bruts a permis de valider la présence des molécules ciblées - la capsaïcine et plusieurs de ses homologues - sans procéder à une confirmation par LC/MS<sup>2</sup> sur un autre instrument, ou par une seconde analyse LC/MS<sup>1</sup> dans des conditions chromatographiques différentes.

### MOTS-CLÉS

Mots clés : Analyse ciblée, LC/APCI<sup>+</sup>/MS à basse résolution, molécules lacrymogènes, capsaïcine, aérosol poivré

Target molecule analysis of complex mixtures from LC/MS<sup>1</sup> data acquired on a single analyzer instrument