

# DEVELOPMENT OF A HPLC AND GC-MS/MS ANALYSIS OF OCHRATOXIN A IN MUSTS AND WINES USING STABLE DILUTION ASSAY

Ochratoxin A (OTA) is a mycotoxin produced by some *Aspergillus* and *Penicillium* strains, present and legislated in different foods. The regulation for wine intake has fixed the maximum levels in wine at 2 µg/L. Some reports refer to OTA contamination in wines up to 15 µg/L, but the majority of the data available are below 1 µg/L. When working at such low concentrations, the problem of the uncertainty of the results becomes decisive towards the implementation of legal limits. A recent study [1] on Portuguese wines showed that the inter-laboratorial standard deviation determined in different samples was ranged from 27 % to 37 %, which could be problematical.

In order to obtain better results, we developed a Stable Isotope Dilution Assay using a deuterated analogue, not only by HPLC-MS/MS as previously described [1], but also by GC-MS/MS.

## SYNTHESIS OF d<sub>4</sub>-OTA

The synthetic pathway developed (figure 1) allowed the insertion of deuterium atoms on the isocoumarine moiety of OTA. Thus two analytical strategies were possible: HPLC-MS/MS as described by [2], but also a GC-MS/MS analysis after hydrolysis and ethyl chloroformate derivatization, which were optimized.

## ANALYSIS

Extraction of OTA was performed as described by the method EN14133, using immuno-affinity cartridge. The methanolic extracts obtained was either directly injected in LC-MS/MS (conditions are given table 1), or hydrolyzed in HCl 37 % for 12h in order to form α-OT.

After elimination of HCl by vacuum evaporation, the extract was derivatized using ethyl chloroformate to give the corresponding ethyl ester of α-OT and d<sub>4</sub>-α-OT.

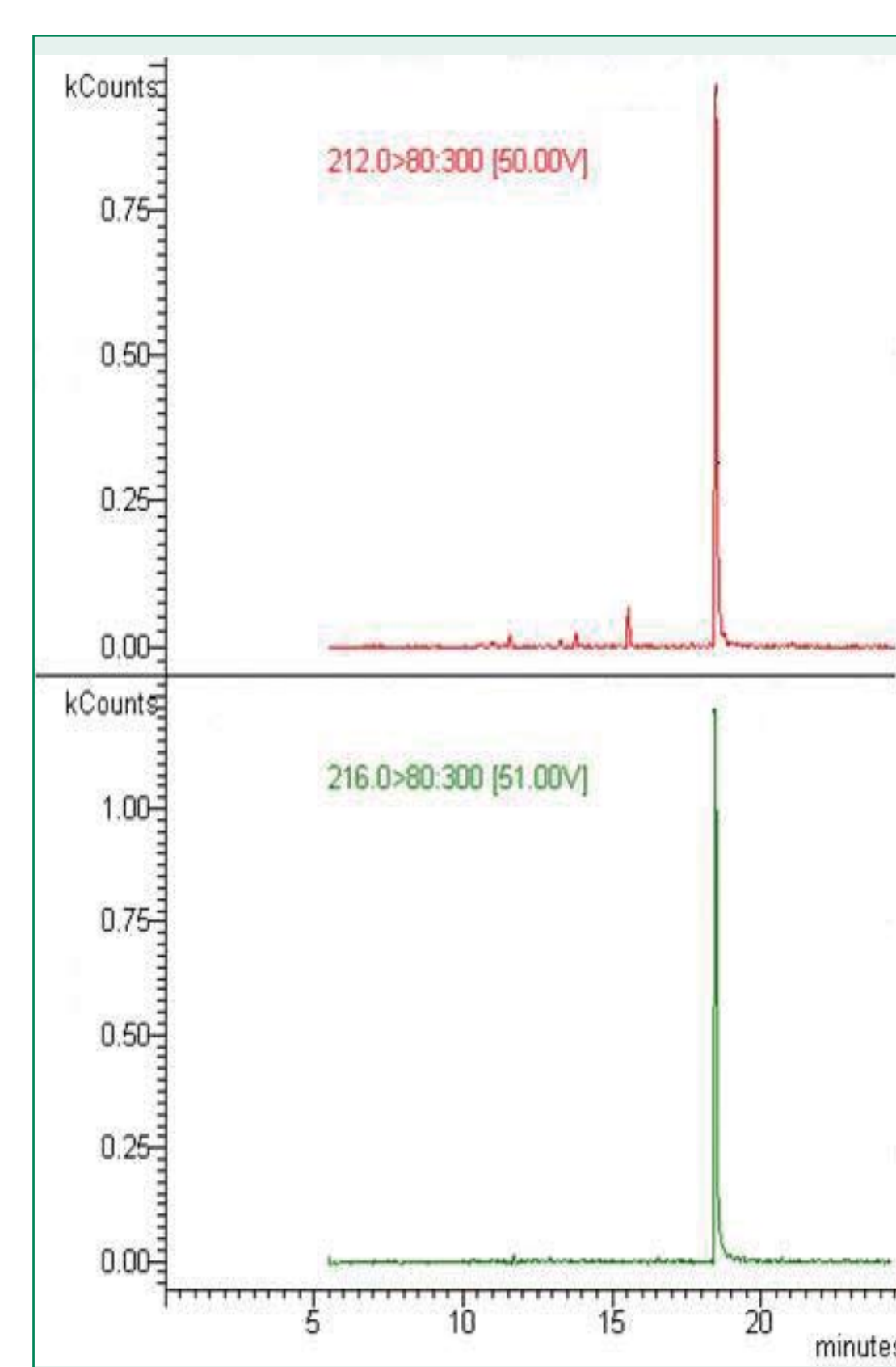
Analysis was then performed by GC-EIMS/MS (conditions are given table 1).

An example of chromatogram obtained for a sample enriched with 5 ppb of OTA and analyzed in GC-MS/MS is shown in figure 2.

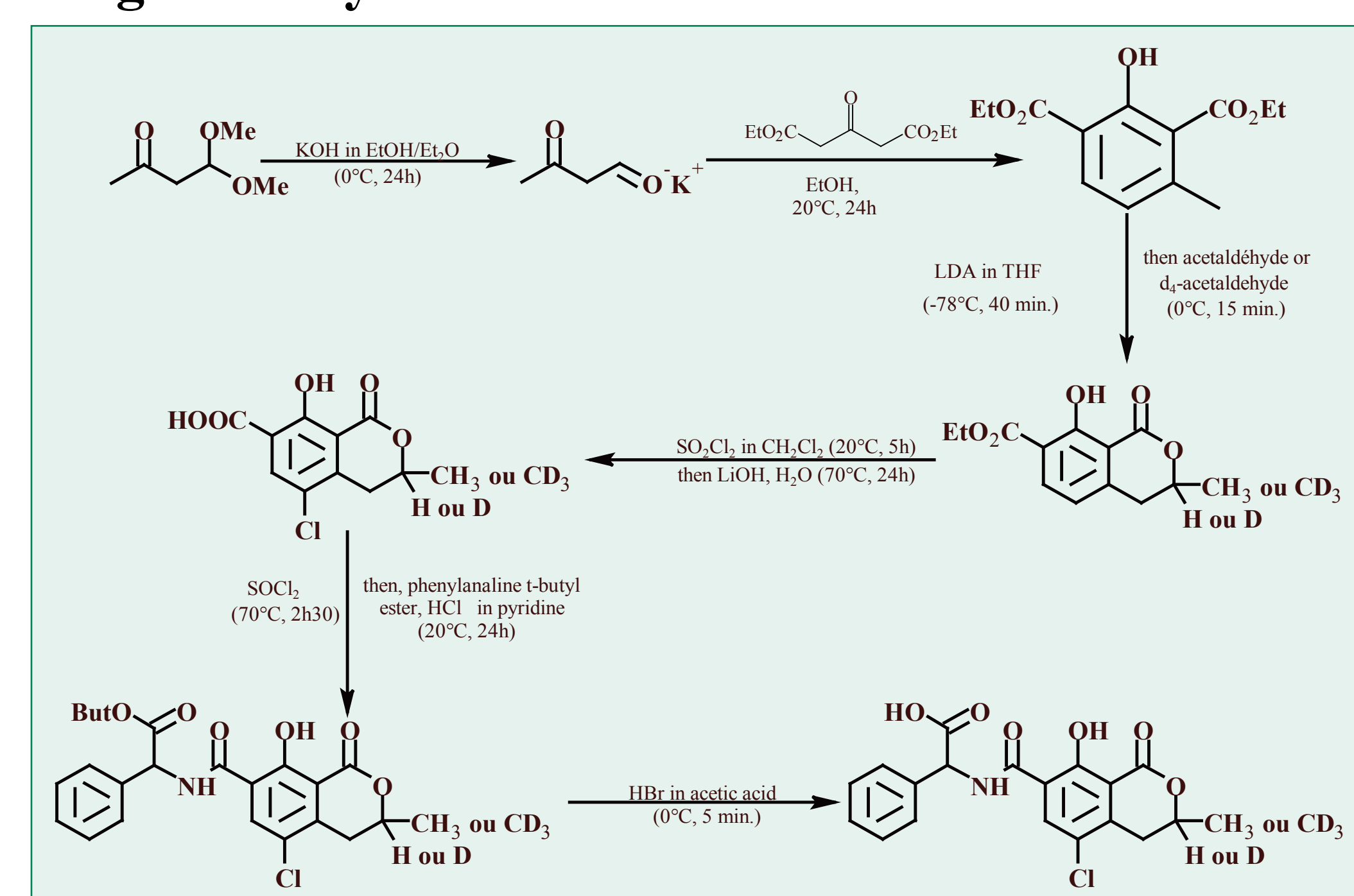
**Figure 2:**  
Example of chromatogram obtained in GC-MS/MS for a wine enriched with 5 ppb of OTA

**Table 1: Analytical conditions used for HPLC-MS/MS and GC-MS/MS**

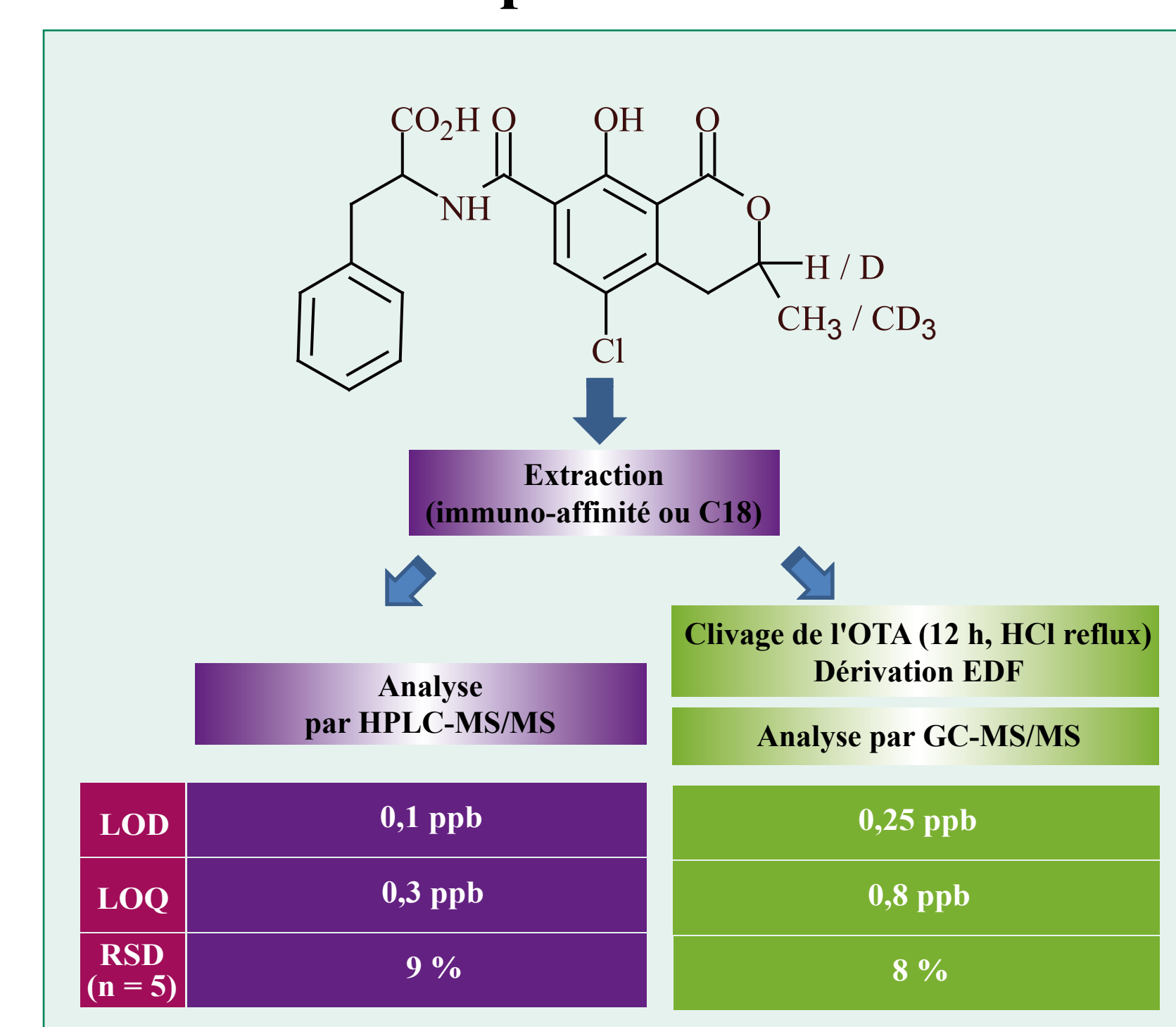
	HPLC-MS/MS	GC-MS/MS
Column type	LiChrospher 100-RP-18	DB5-MS
Ionisation type	ESI +	EI
Parent ion for natural OTA (deuterated)	m/z = 404 (408)	m/z = 212 (166)
Daughter ion for natural OTA (deuterated)	m/z = 358 (362)	m/z = 216 (169)



**Figure 1: Synthesis of deuterated and natural OTA**



**Figure 3: Analytical process and performances**



## PERFORMANCE OF THE METHOD

Repeatability was estimated on wines samples without OTA contamination but enriched with 5 ppb of OTA (5 repetitions of the entire analytical process). The values obtained are satisfactory even if they have to be confirmed at lower levels (figure 3). LODs and LOQs are quite satisfactory and amelioration is under study in GC-MS/MS, using HS-SPME on the final extract.

### References:

[1] N. Ratola et al., 2006. *Talanta* 70(4), 720-31

[2] M. Lindenmeier et al., 2004. *J. Chromatogr. A*, 1023, 57-66

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