# Determination of cholesterol and cholesteryl esters in *Xenopus laevis* embryos through direct flow injection electrospray ionisation mass spectrometry

**R. t'Kindt[a], M. Storme[a], N. Willemarck[b], K. Vleminckx[b], F. Van Roy[c], J. Swinnen[d] and J. Van Bocxlaer[a]**

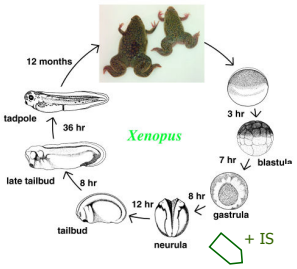
[a]Laboratory of Medical Biochemistry and Clinical Analysis, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium
[b]Department for Molecular Biomedical Research, VIB, Technologiepark 927, B-9052 Ghent, Belgium
[c]Department of Molecular Biology, Ghent University, Technologiepark 927, B-9052 Ghent, Belgium
[d]Laboratory of Experimental Medicine and Endocrinology, Faculty of Medicine, Catholic University of Leuven, Leuven, Belgium

## Introduction

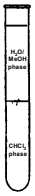
Cholesterol is an important structural component of animal cell membranes, especially in the liquid-ordered membrane microdomains, the so-called lipid rafts. Cholesteryl ester (CE) is the major transport and storage form of cholesterol. In the perspective of a biological question, a method for the quantification of cholesterol and CE is being developed. Cholesterol quantification is established in *Xenopus laevis* embryos in the early neurula stage together with the identification of the most important CE.

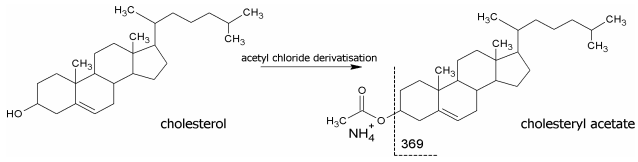
## Cholesterol quantification

### Experimental

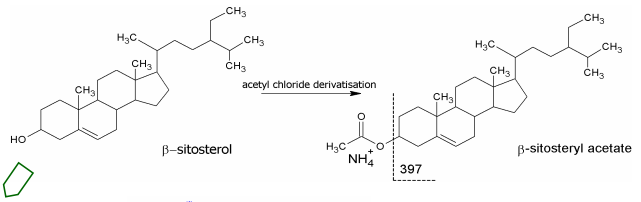



**Bligh & Dyer extraction**
Recover chloroform phase, evaporate under $N_2$

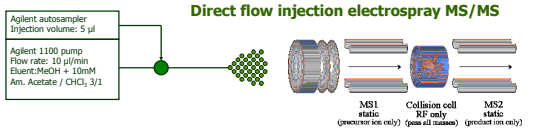


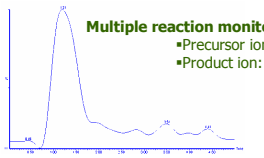




Acetylation
➢ No interference with CE




**Direct flow injection electrospray MS/MS**



**Multiple reaction monitoring**
- Precursor ion: *m/z* 446.4
- Product ion: *m/z* 369.3

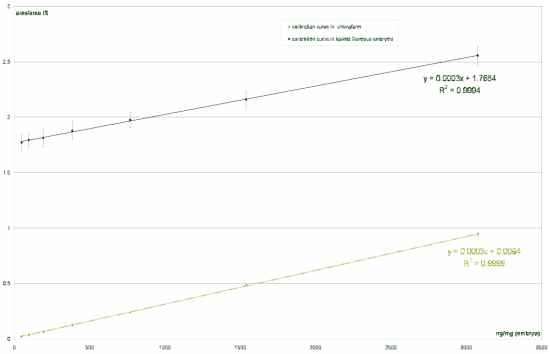
**Data processing**
Quanlynx®

### Results

Neutral free sterols are very poorly ionisable through electrospray ionisation (ESI). Cholesterol is hereby acetylated through a derivatisation step with acetyl chloride, resulting in an ionisable cholesteryl acetate (CE 2:0) by means of ammonium adduct formation. This is also compatible with the identification, and later on quantification, of CE. A fragment of *m/z* 369.3 upon collision-induced fragmentation is generated. Quantification of cholesterol can thus be accomplished using a multiple reaction monitoring (MRM) transition of *m/z* 446.4 to *m/z* 369.3. This derivatisation also allows us to analyse both cholesterol and CE in a parallel way.

Quantification of cholesterol was performed using direct flow injection analysis of the *Xenopus* embryo extract. The method has a high throughput, a runtime of only 5 minutes is needed. β-Sitosterol, a typical plant steroid, was used as an internal standard. Blank *Xenopus* extracts did not show any interfering mass. As cholesterol is an endogenous compound, detection limit was approximated through spiking of β-sitosterol to *Xenopus* embryo extract. Using only 5 *Xenopus* embryos, a calibration curve of cholesterol (spiked to 5 WT *Xenopus* embryos) is established between 96 and 3000 ng/mg embryonic material.

| detection limit (ng/mg embryo) (ß-sitosterol) | repeatibility (overall; n=50) | LLOQ (ng/mg embryo) (cholesterol) | precision | accuracy | cholesterol content (µg/mg embryo) | variance analytical (n=9) | variance biological (n=3) |
|---|---|---|---|---|---|---|---|
| 17.40 | 11.23% | 96.15 | 4.42% | 105.30% | 5.54 ± 0.14 | 7.60% | 10.59% |



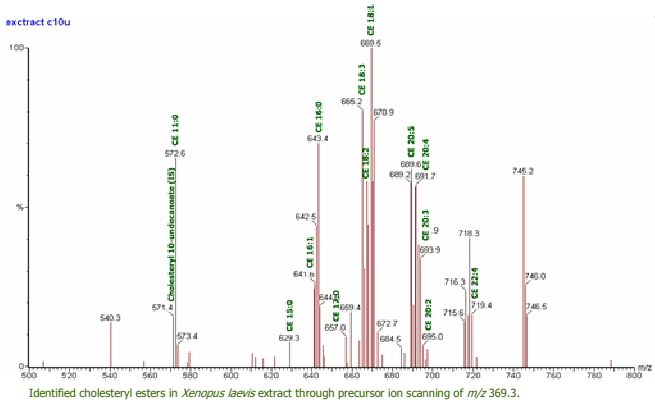

Calibration curve for cholesterol concentration in pure chloroform and in spiked *Xenopus* extracts, n = 3. The intercept equals the cholesterol concentration in *Xenopus* embryo.

## Cholesteryl ester identification

In the perspective of a metabolic profiling method for cholesterol and its esters, a precursor ion scan method was developed. All precursor ions with the specific fragment of *m/z* 369.3 are scanned in the range from *m/z* 500 to 800. Cholesteryl 10-undecanoate is used as an internal standard to quantify the CE in a later stadium. Derivatisation did not interfere with CE identification. Most of the detected peaks can be adressed to common CE (see in figure).

## Conclusion

- Cholesterol can be quantified in a fast and reproducible way in *Xenopus* embryos. Notwithstanding the high concentration present in these embryos, a LLOQ of 96.15 ng/mg embryo is feasible.
- The mean cholesterol concentration in the early neurula stage is 5.54 ng/mg embryo, with a biological variance of 10.59%.
- Cholesteryl esters can be identified in the same samples. Quantification of these compounds will be established soon.




Identified cholesteryl esters in *Xenopus laevis* extract through precursor ion scanning of *m/z* 369.3.

@ ruben.tkindt@ugent.be