

# Automated ion trap LC-MS screening for xenobiotics in vitreous humor

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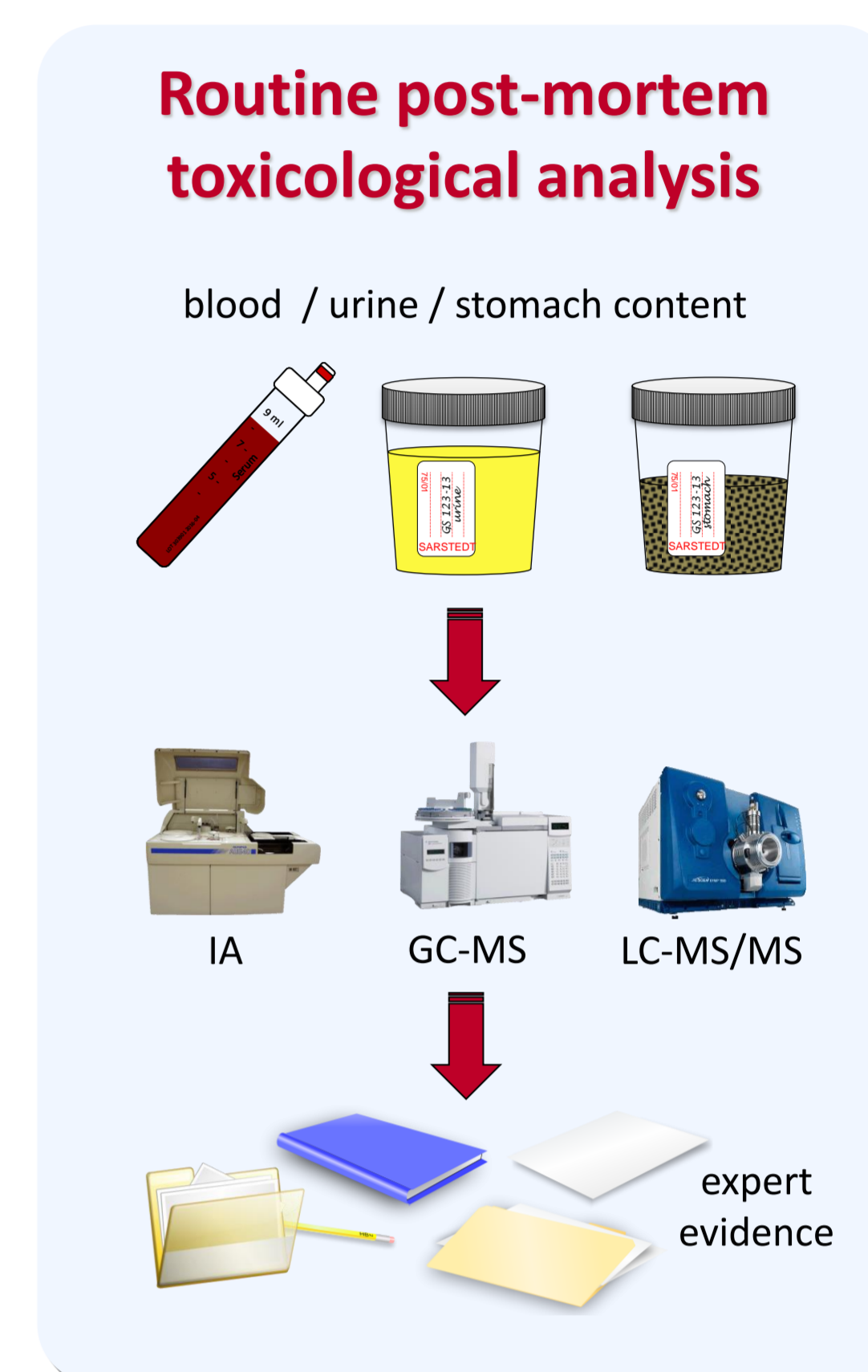
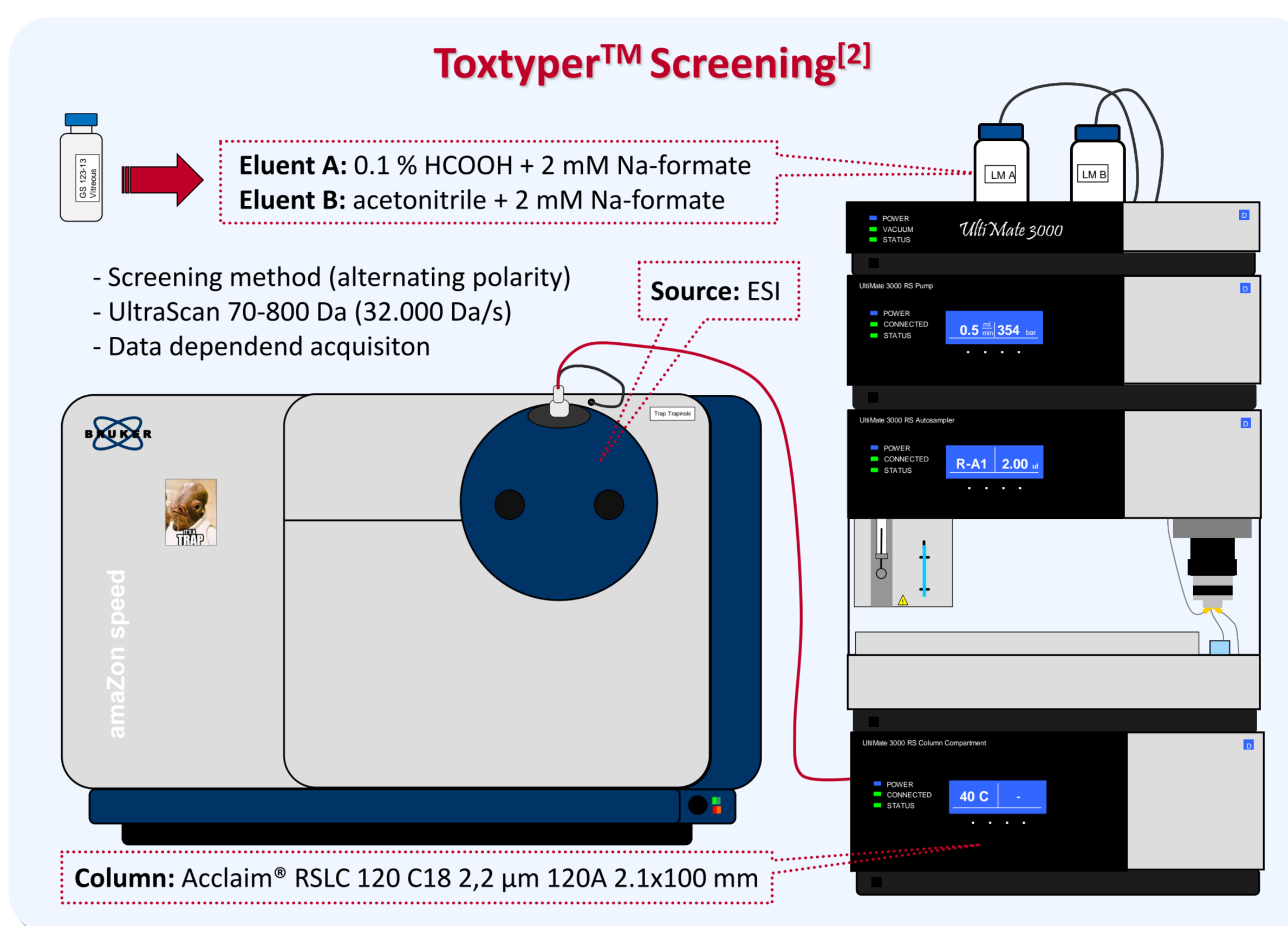
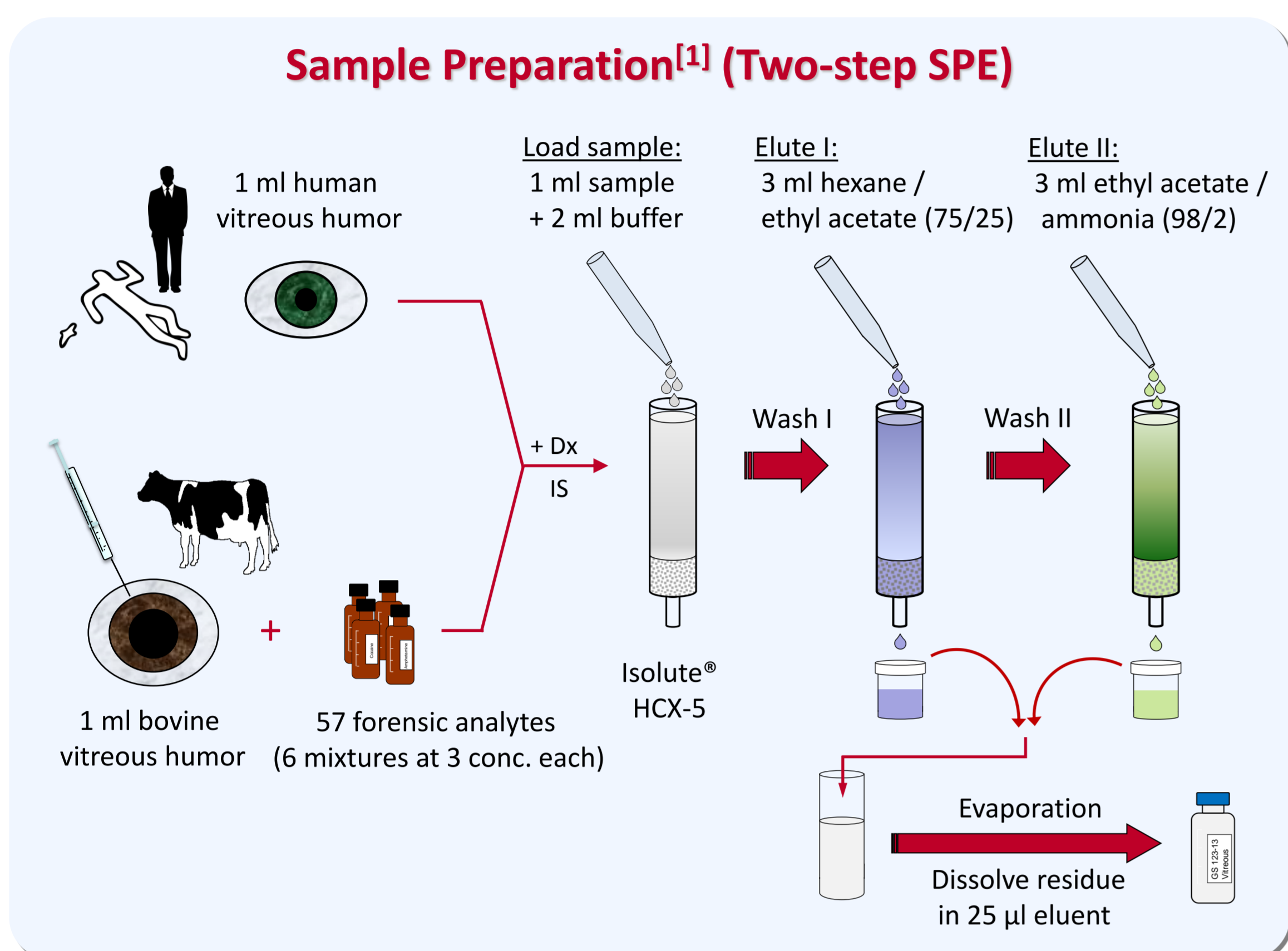


## Introduction

Screening for xenobiotics is a crucial part of post-mortem toxicological analysis. Urine and/or blood are the matrices of choice for systematic toxicological analysis but the sample volume that can be preserved during autopsy is sometimes very limited. In such cases, analyzing tissue samples like liver can be a suitable but laborious option. Vitreous humor is an easy-to-handle body fluid only little affected by putrefaction and therefore a smart alternative to urine or blood. Due to the blood-retinal barrier, interpretation of quantitative results in vitreous humor is quite difficult but is a suitable matrix for qualitative screening analysis.

When analyzing body fluids, liquid chromatography - mass spectrometry has become the method of choice for a wide range of analytical questions. In this project, a previously developed automated LC-MS<sup>n</sup> approach was evaluated for the detection of drugs and drugs of abuse in vitreous humor to extend its application to post-mortem analysis.

## Experimental



## Results

### Evaluating detection limits in bovine vitreous humor

In total, 57 substances distributed to six mixtures were analyzed at three different concentrations (low, med, high). Medium concentration levels were adjusted to vitreous humor concentrations reported in the literature and cut-offs of other established screening methods<sup>[1]</sup>.

Approximately 94 % of the compounds could be detected and identified correctly at each concentration level investigated. Mirtazepine could only be detected at medium (10 ng/ml) and high concentration levels (20 ng/ml) while tramadol and olanzapine could only be identified correctly at high concentration levels (25 and 150 ng/ml, respectively). LSD was the only compound that could not be detected at all, probably due to its' sensitivity to light.

Limit of detection was set to the lowest concentration level that could be identified correctly in duplicate determination.

| Limit of Detection | Substances   |
|--------------------|--|
| 5.0 ng/ml:         | Buprenorphine, Carbamazepine, Cocaine, Diazepam, Melperone, Methadone, Metoprolol, Phenazone, Propranolol, Venlafaxine, Zolpidem   |
| 10 ng/ml:          | Acebutolol, Amitriptyline, Bisoprolol, Codeine, Fentanyl, Flunitrazepam, Methamphetamine, Mianserine, Mirtazepine, Moclobemide, Phencyclidine, Trimipramine, Sulpiride, Warfarin |
| 12,5 ng/ml:        | 6-MAM, Atenolol, Clozapine, Diltiazem, MDE, MDMA, Midazolam, Morphine, Nortriptyline, Oxycodone  |
| 25 ng/ml:          | Amphetamine, Fluoxetine, Fluvoxamine, Risperidone, Sotalol, Tramadol   |
| 50 ng/ml:          | Carvedilol, Chloroquine, Citalopram, Doxepin, Paroxetine, Verapamil, Zopiclon  |
| 150 ng/ml:         | BE, Dixyazine, Olanzapine, Phenytoin, Sildenafil   |

### Real samples

The results from real cases were in good agreement with the findings from routine post-mortem analysis. In vitreous humor of 24 autopsy cases (c1 - c24) 76 % of the active agent consumed by the deceased could be identified in accordance to routine post-mortem analysis. The almost ubiquitous compounds caffeine, nicotine and their metabolites as well as alcohol and its metabolite ethylglucuronide were excluded from this evaluation. So in six cases (25 %) screening results from vitreous humor perfectly matched the results of post mortem analysis

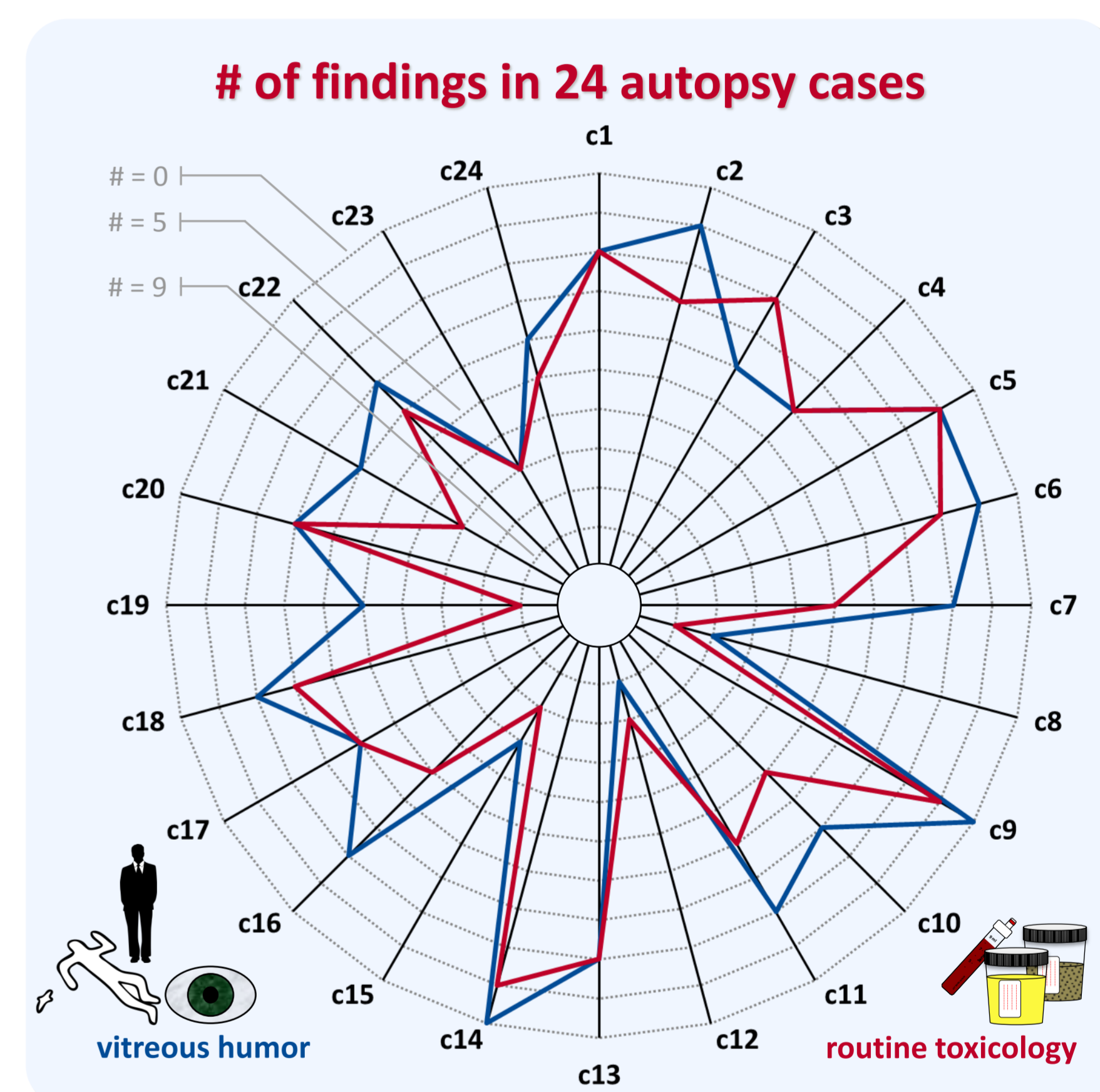
In cases c2, c6 and c24 paracetamol and/or ibuprofen were the only compounds not detected in vitreous humor. It is known, that compounds with high polarity (e.g. paracetamol or oxazepam) and/or a high plasma protein binding rate (e.g. ibuprofen) only poorly pass the blood-retinal barrier and therefore are not necessarily detectable in vitreous humor by a general screening approach.

Olanzapine was the only compound not detectable in vitreous humor in cases c9 and c22. Serum concentrations of olanzapine were 94 and 190 ng/ml, respectively. Nevertheless, sample c15 shows that olanzapine is detectable in vitreous humor although only identified in urine and stomach content of the deceased.

Sample c15 illustrates, that also the ingestion of multiple drugs can be detected easily in vitreous humor. Promethazine could not be identified in vitreous humor but serum levels at the time of death turned out to be below 10 ng/ml.

Although Norbuprenorphine and THC-COOH could be detected in some of the cases investigated, a targeted LC-MS approach seems mandatory for the detection of low dose drugs like buprenorphine (C<sub>SERUM</sub> < 0.8 ng/ml in c10) and other selected analytes like THC and GHB.

| Case | Substances in Vitreous Humor   | Substances in Routine Toxicology   |
|------|--|--|
| c10  | Diazepam, Nordazepam, Temazepam, Sertraline, Lorazepam   | Diazepam, Nordazepam, Temazepam, Sertraline, Lorazepam, Oxazepam   |
| c11  | Citalopram, Methadone + EDDP   | Citalopram, Methadone + EDDP, THC-Metabolites, GHB (endogenous)  |
| c15  | Trimipramine, Zopiclon, Quetiapine, 7-Amiflunitrazepam, Oxazepam, Olanzapine, Desmethylvenlafaxine | Trimipramine + M., Zopiclon, Quetiapine + M., Flunitrazepam + M., Oxazepam, Olanzapine, Venlafaxine + M., Promethazine |



In some cases the intake of a substance could neither be confirmed in the corresponding body fluids nor by the case history:

In case c3 a male person (age 21) died in hospital one day after a cardiac seizure. This could explain the detection of the antiarrhythmic flecainide in vitreous humor but there were no additional findings that would have confirmed the intake of sildenafil.

In case c12, zopiclon was detected in vitreous humor besides various others drugs but not identified during routine analysis. According to the police record, the deceased was under treatment and self medication with unspecified narcotics and analgesics.

For final method evaluation a confirmatory analysis to determine vitreous humor concentrations of at least the most common substances found in post-mortem analysis is mandatory.

## Conclusion

The applied screening approach is a suitable tool for the detection of xenobiotics in vitreous humor. Besides the known physiological limitations of the matrix itself, the obtained limits of detection seem to be adequate for forensic casework. The easy handling of vitreous humor when compared to sample preparation of tissue samples combined with the LC-MS<sup>n</sup> analysis and automated data evaluation of the Toxytper present a time- and cost-effective screening alternative for post-mortem cases if no urine and/or only a limited volume of blood is available.

Due to the lack of human samples acquirable under controlled conditions, analysis of fortified bovine vitreous humor and further comparison of urine/blood and vitreous humor findings in post-mortem cases are necessary before finally implementing the method in routine post-mortem analysis.

## References

- [1] Pelander et al.: Vitreous Humor as an Alternative Matrix for Comprehensive Drug Screening in Postmortem Toxicology by LC-TOF-MS, *JAT* 2010 34(6): 312-8
- [2] Huppertz et al.: Ein automatisiertes MS<sup>n</sup>-basiertes Screening Verfahren für die klinische und forensische Toxikologie, *Toxichem Krimtech* 2013(80): 299-303