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Post-mortem toxicological analysis of cocaine: main biological samples and analytical methods

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Post-mortem toxicological analysis of cocaine: main biological samples and analytical methods

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RESUMO

Para a área forense, a cocaína é uma substância comumente analisada em casos de mortes violentas que necessita de provas para determinar o uso de drogas de abuso. A cocaína e seus produtos de biotransforamção podem ser identificados em um cadáver por meio de vários métodos analíticos, como sistemas de detecção por cromatografia. Porém, antes de utilizar a cromatografia é necessário preparar a amostra e, claro, escolher as matrizes biológicas. Pesquisas sobre metodologias analíticas neste campo são facilmente encontradas. No entanto, determinar quais são os mais recomendados para análises toxicológicas de cocaína post mortem não é tão fácil. Portanto, esta revisão de escopo pretende pesquisar os métodos analíticos e amostras biológicas mais utilizadas na análise toxicológica forense post mortem de cocaína e seus produtos de biotransformação. Um total de 21 artigos de 2012 a 2022 foram filtrados de cinco diferentes bases de dados para serem estudados. Os dados coletados indicam que as amostras biológicas mais utilizadas foram sangue e cabelo. A técnica de preparação de amostra mais utilizada foi a extração em fase sólida e o método cromatográfico mais citado foi a cromatografia líquida com espectrometria de massas. A revisão apresenta e debate onde as metodologias validadas estão no espectro de sensibilidade de detecção, por que os limites de quantificação são tão importantes para os métodos e quais as melhores amostras biológicas a serem utilizadas em diferentes casos.

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SUMMARY

Este Trabalho de Conclusão de curso será apresentado na forma de manuscrito que será submetido para publicação na revista Forensic Toxicology, cujas diretrizes e instruções aos autores podem ser encontradas na página https://www.springer.com/journal/11419/submission-guidelines.

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Post-mortem toxicological analysis of cocaine: main biological samples and analytical methods

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ABSTRACT

For the forensic field, cocaine is a common substance analyzed in cases of violent deaths that needs evidence to determine the use of drugs of abuse. Cocaine and its metabolites can be identified in a deceased body through many analytical methods, such as chromatography detection systems. However, prior to utilizing chromatography it is necessary to the sample preparation and, of course, choose the biological matrices. Researches about analytical methodologies in this field are easily found. However, to determine what are the most recommended for post-mortem toxicological cocaine analysis is not so easy. Therefore, this scoping review intends to search for the most used analytical methods and biological samples in the post-mortem forensic toxicological analysis of cocaine and its metabolites. A total of 21 articles from 2012 to 2022 were filtered from five different databases to be studied. The collected data indicate that the most utilized biological sample were blood and hair. The most used sample preparation technique was solid phase extraction and the chromatography method most cited was liquid chromatography with mass spectrometry. The review presents and debates where the validated methodologies are on the detection sensitivity spectrum, why limits of quantification are so important to the methods and what the better biological samples to be utilized in different cases.

Keywords: Cocaine, autopsy, post-mortem, chromatography, extraction methods.

INTRODUCTION

Drugs of abuse are a complex problem on society. Cocaine has a relevance on this issue since the substance has a high socio-economics notability to the world. In 2015 was estimated that 4,5 million of Brazilians consumed cocaine at least once [1] and in 2020, 21,5 million of people around the world consumed this drug, being the continent with the highest prevalence Oceania followed by America. This last one is the biggest producer and distributer of cocaine in the world [2].

The illegal use of cocaine is related not only with overdoses, but also with crimes and violent deaths. Cocaine is related to deaths by traumas, homicides in general and even deaths connected with the drug traffic. Violent deaths have a juridical and police importance and for that the forensic analysis are compulsory, with the toxicological analysis being one of the stages of this process [3].

The post-mortem toxicological analysis of cocaine and its metabolites is greatly used as a tool for identification and characterization of a death related open case. The analysis does not reveal the intent of the case, although it helps to answer some of the questions made during the investigation. The toxicological report and the interpretation of the obtained results are issued after the careful execution of detection, identification, and quantification stages that will indicate toxic substances in the biological samples gathered from the corpse [4].

In post-mortem toxicological analysis there are variables that can change the analytical result and the interpretation. The amount presented in the results is not always the same concentration of analyte at the time of death, this occurs because of the redistribution of the substance in the victim's body. In some tissues the concentration of cocaine will be higher and easier to identify than in others, furthermore there is another factor that can be introduced in a biological matrices reading: specific markers. Detection of these analytes depends on the route of administration and is as important a finding as cocaine itself, some of these analytes are benzoilecgonine (BE), cocaethilene (CE), ecgonil methyl esther (EME) and norcocaine (NC) [5].

For the analysis, biological matrices of the victim, such as blood, hair and urine, are collected by means of necropsy. These matrices go through extraction methods to concentrate the analytes and exclude possible interferences, being, finally, analyzed, by chromatographic techniques coupled to different detectors [6].

Extraction methods utilized in forensics analysis are commonly Liquid-Liquid Extraction (LLE) and Solid Phase Extraction (SPE). However, when it comes to low concentrations of analytes or more specific applications are needed, these classical methods of extractions may not be the best [7]. Extractions methods that have become interesting for chemical analysis, but are still little introduced in forensic field, are microextraction, such as: solid phase microextractions and liquid phase microextractions. The use of these techniques requires low sample/ organic solvents volume and is usually more ecological and faster in terms of the delay in the analysis process [8].

After the extraction techniques, the processed samples were injected into the chromatography system. Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) and Gas Chromatography Mass Spectrometry (GC-MS) are the most applied methods in the forensic area. The results for these methods are well researched and documented, exhibiting great results even on multianalytes screening [9]. The results of the report may present qualitative or quantitative data of the harmful substances the victim ingested before dying, whether or not it was the cause of death [6].

Researches about new development analytical methodologies to identify and quantify drugs of abuse in biological samples are easily found. However, to determinate what are the most used and recommended for the post-mortem toxicological cocaine analysis, as to sample choice, sample preparation technique and chromatography system, it gets lost between all the published information. Therefore, the intent of this review is to exhibit the most common and reliable analytical methods and biological samples used to identify and quantify cocaine in post-mortem forensic toxicology.

METHODS

The method utilized was a scoping review in accordance with the framework outlined by Arksey and O'Malley (2003). The methodological framework has stages to be followed: (1) research the question definition, (2) search for relevant studies in the databases, (3) study selection, (4) charting the data, (5) collating, summarizing, and reporting the results [10].

The question of this research is ‘What are the usual analytical methods and most frequently biological samples used to identify and quantify cocaine in post-mortem forensic toxicology?’. This article also aims to discuss the insurgence of new techniques and advances between the years 2012 and 2022.

To identify potentially relevant studies for inclusion, five databases were selected: PubMed, Embase, Web of Science (WOS), Virtual Health Library (VHL), and Scopus. In every database, descriptors and filters were used to focus the subjects and provide enough material for the research. The results found were exported to EndNote® and the duplicates were automatically deleted.

The proper publications had been found on the databases with the use of three types of descriptors separated into three blocks with the boolean expression AND: Block 1: Cocaine; Block 2: Autopsy OR Autopsies OR Post Mortem Examination OR Post-Mortem Examination OR Post-Mortem Examinations OR Postmortem Examination OR Postmortem Diagnoses OR Postmortem Diagnosis OR Postmortem Examinations; Block 3: Chromatography.

The inclusion criteria to determine which publications would be in the study was: the study must have a post-mortem toxicological analysis of cocaine and its metabolites, with biological samples well determined, concentrations quantified, and has to be a chromatography analysis. Also, the publications must be between the years 2012 and 2022, be an original research article, and also be in English, Portuguese, or Spanish.

The selection of the publications was first by title, followed by abstract, and finally by reading the full text. The title stage was used to delete every article that visibly did not complement the theme of the study. The abstract stage was used to delete articles that did not present post-mortem analysis and the search for any form of cocaine. In the end, the text reading stage excluded articles that did not present enough results and not answered the research question.

After selection stage, the publications had been read and the data inside each one was extracted and exported. The relevant information inside the selected publications was extracted to a table (Appendix 1) that prioritized the analytes, samples and its volumes, extraction method and sortive phase (in case of a SPE), solvent of extraction, detection system, limit of quantification (LOQ), number of real samples and concentrations in real samples. The results were summarized in graphics, tables, or text description. The graphics and analyzes were processed in Microsoft Excel®.

RESULTS

The results found by utilizing the scope review method are seen in Figure 1. Twenty-one articles have been chosen for this review and seven publications were saved to assist on the discussion.

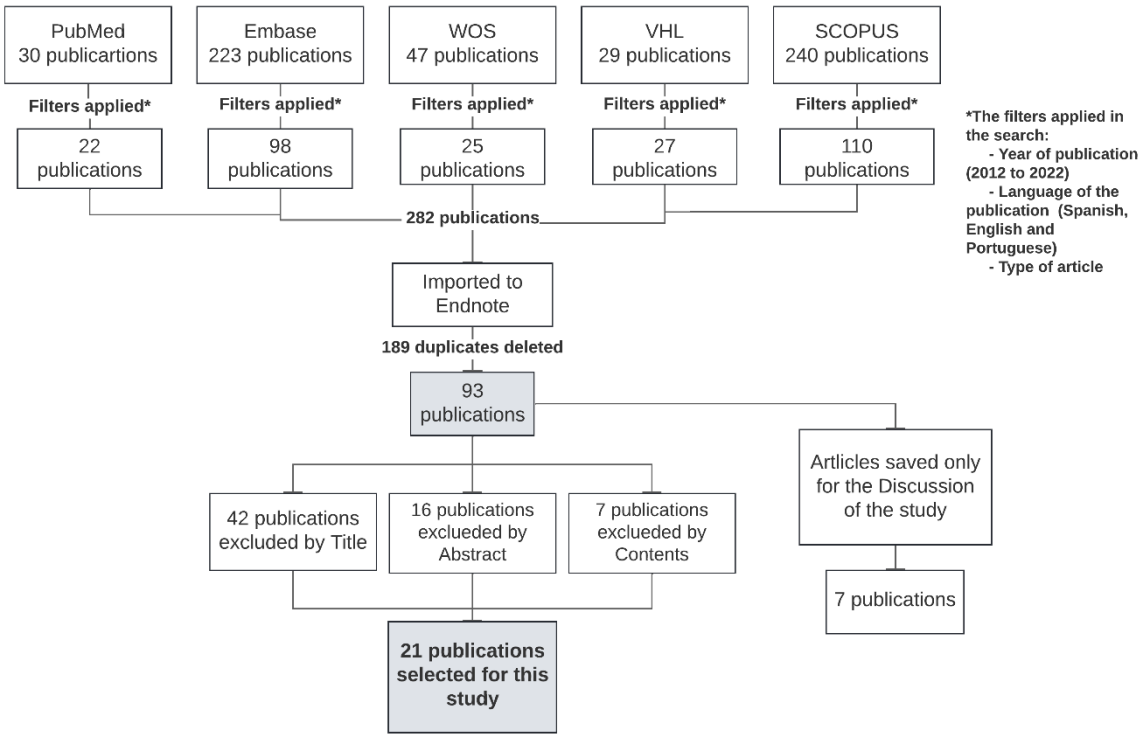

Fig. 1 Methodological flowchart and results findings

The chosen publications were published between the year of 2012 and 2021, the year with the most published articles were 2014 and 2018, both with five articles each. Europe was the region with most published articles, being the countries Italy, Serbia, Germany, Sweden, Switzerland and UK. Brazil had five articles published being the country with most publications in this study, followed by Italy with four articles. Forensic Science International was the journal with the most publications, eight. The impact factor of the journals varied between 0.201 to 4.759 [9,11-30].

Cocaine was the most searched analyte in all the twenty-one articles (100%), benzoilecgonine was the second more present in seventeen publications (81.0%). Cocaethylene was third (52.4%), Ecgonine methyl ester was fourth (38.1%), Norcocaine was fifth (14.3%), anhydroecgonine methyl ester (AEME) and cocaine N-oxide (CNO) only mentioned in one article (4.8%) (Figure 2a) [9,11-30]. Only four articles analyzed

cocaine without any other metabolite included, three of the four articles were a multianalyte screenings [12,17,22,29]. Thirteen articles (62.0%) brought post-mortem investigations in combination with other drugs of abuse and others psychoactive substances, of all these articles only two of them were dated 2014, the others were from 2015 to 2020 [9,12-14,16,17,19,22-24,26-29].

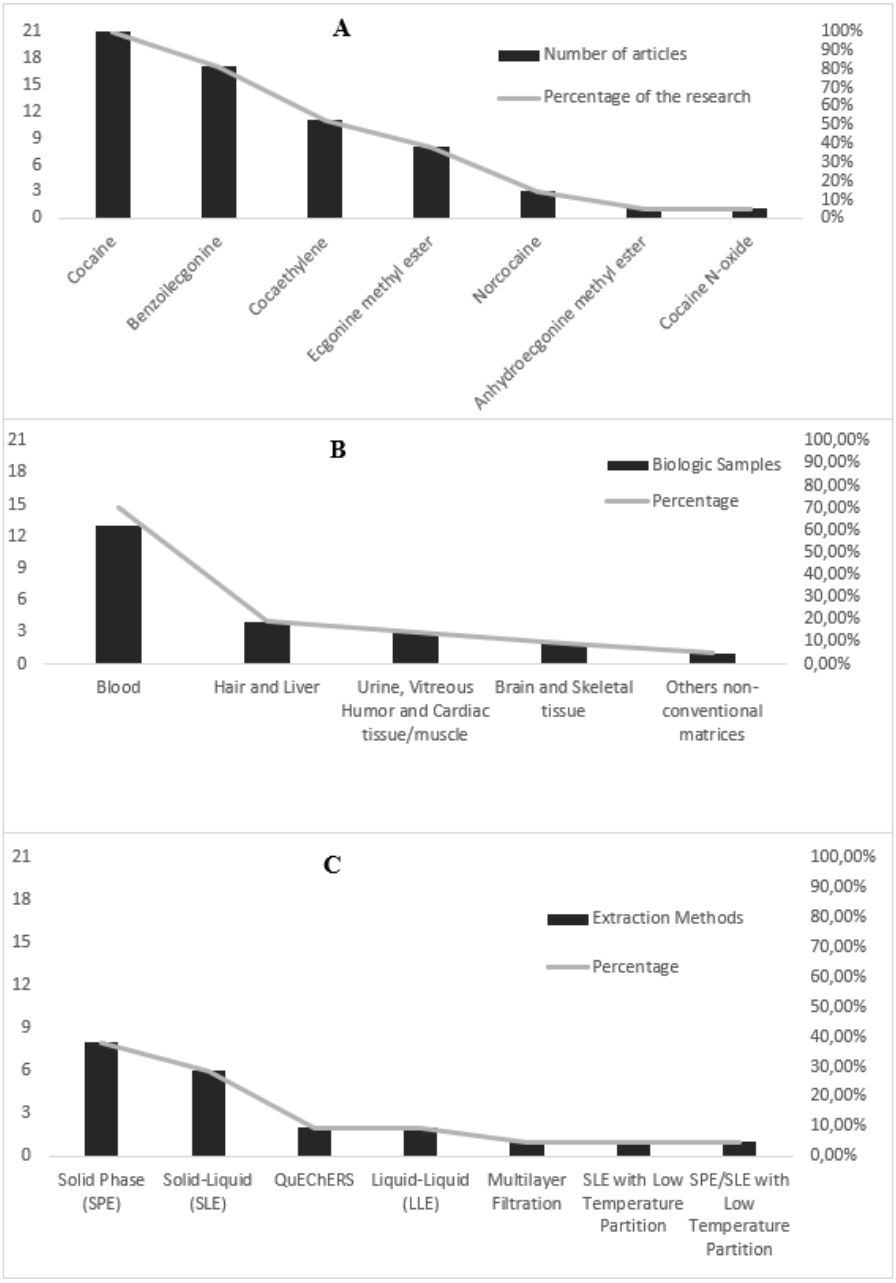

Fig. 2 Synthesis of main results for analytes researched (A), biological samples used (B) and sample preparation methods (C)

The most used biological sample was blood in thirteen articles (62.0%), followed by hair and liver in four articles (19.0%), then urine, humor vitreous and cardiac

tissue/muscle in three (14.3%), brain and skeletal tissue in two (9.5%) and stomach tissue, stomach and brain fluids, lungs, kidney, spleen, plasm, bladder, gallbladder, intestines, fingernails and larvae were mentioned in only one article (4.8%) (Figure 2b) [9,11-30]. Also, adding to the results, seven (33.3%) publications searched for information utilizing more than one biological sample of different types of tissue and fluids, all of the samples, except fingernail, were researched alongside others at least once [9,11-30]. There were few articles (23.8%) that considered the time of death and choice of matrices, almost every article did not comment on the subject, although the studies that accounted the matter were in the majority studies with non-conventional samples, such as skeletal tissue, liver, and others not commonly utilized matrices, which already indicates a choice for samples focused on better research in the area or probable distance of the time of death [11,13,14,27,28].

Initial sample volumes vary between 20µL to 50mL for fluids and 5mg to 5g for tissues. Four articles (19.0%) presented 2mL as the most chosen volume of the raw fluid samples to incorporate into the analysis. For the tissues 2g and 1g appeared each in two publications (9.5%) as the most chosen for tissues (Appendix 1) [9,11-30].

For the sample preparation, the most used was SPE mentioned in eight articles (38.1%) and solid liquid extraction (SLE) was seen in six articles (28.6%). Quick, Easy, Cheap, Effective, Rugged and Safety (QuEChERS), a type of solid phase extraction focused on pesticides, and LLE were utilized in two (9.5%), multilayer filtration and SLE with low temperature partition (SLE-LTP) appeared in one article (4.8%) (Figure 2c). SPE coupled with SLE-LTP was also seen in one article appearing as the unique publication that tried to use two extraction methods associated [9,11-30].

The publications that utilized LLE used a volume of biological samples of 20µL and 500µL, however SLE and SPE also utilized volumes in micrograms (100µL and 85µL), accomplishing satisfactory results. The other articles that utilized SPE and SLE, both required almost an average amount of biological samples, and the QuEChERS both times required volume of 1g and 5g [9,11-30]. Only one article brought LLE in a combination with non-conventional matrices (humor vitreous and plasm), the rest used SPE, SLE or QuEChERS [11-14,16,18,21-23,27-30].

The sortive phase that was included on every analysis made with SPE extraction was a C8 sorbent with a strong cation-exchange (SCX) [9,11,13,15,20,24,25,30]. Dichloromethane incorporated with isopropanol and an ammonium solution appeared in six articles (28.6%) as the solvent for the extraction used in every SPE extraction.

Acetonitrile was seen in the two articles (9.5%) that used LLE and methanol had the major appearance on SLE, five of the six articles used this solvent [9,11-30].

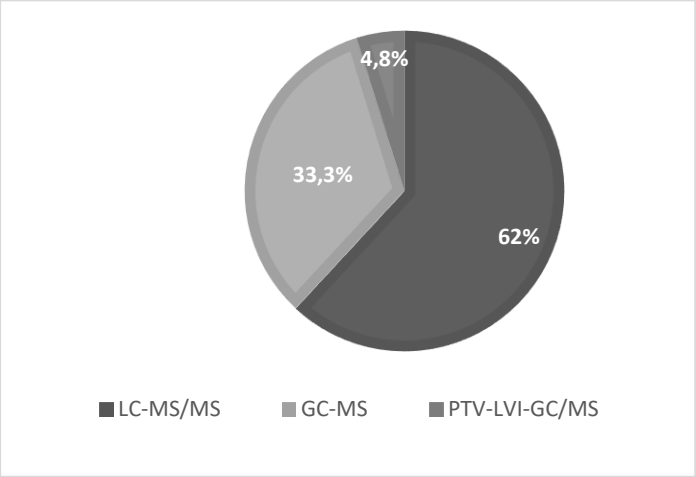

Fig. 3 Synthesis of the main chromatographic techniques applied in the cocaine analysis of the articles reviewed between 2012 and 2021

Liquid chromatography tandem mass spectrometry was the most used, thirteen articles in total (62.0%), followed by gas chromatography mass spectrometry that appeared in seven articles (33.3%%), and gas chromatography-mass spectrometry using large volume injection-programmed temperature vaporization (PTV-LVI-GC/MS), only appeared in one article (4.8%) (Figure 3) [9,11-30]. The study that utilized PTV-LVI-GC/MS had a LOQ of 20ng/mL and real concentration between 40 – 3130ng/mL, it used 1mL of blood and two types of extractions combined d-SPE and SLE- LTP [17].

Table 1 Most used and discussed samples and LOQ results by its analytes

Samples	Analyte	LOQ Variation
Blood	COC	1 - 20ng/mL
	BE	0.5 - 20.9ng/mL
	CE	1 - 10ng/mL
	NC	0.5ng/mL
	EME	2 - 10ng/mL
	AEME	ND
Hair	COC	0.009 - 0.5ng/mg
	BE	0.009 -0.05ng/mg
	CE	0.009 -0.05ng/mg
	NC	0.05ng/mg
	EME	0.009ng/mg
	AEME	0.5ng/mg

Urina	COC	16.95ng/mL
	BE	20.0 ng/mL
	CE	x
	NC	x
	EME	x
	AEME	x
Liver	COC	0.00174 - 0.05ng/mg
	BE	0.00329 - 0.0041ng/mg
	CE	0.00374ng/mg
	NC	x
	EME	0.00617ng/mg
	AEME	x
Vitreous Humor	COC	0.76 - 7.8ng/mL
	BE	2.55ng/mL
	CE	x
	NC	x
	EME	x
	AEME	x
Fingernail	COC	0.01ng/mg
	BE	0.01ng/mg
	CE	0.01ng/mg
	NC	0.01ng/mg
	EME	x
	AEME	x
Skeletal Tissue	COC	0.00047ng/mg
	BE	0.00025ng/mg
	CE	x
	NC	x
	EME	0.00253ng/mg
	AEME	x

The limit of quantification varied between 0.00025ng/mg to 20.9ng/mL on the sixteen articles that presented this particularly result (76.2%). In Table 1 it can be seen the variation was considerable, although, for fluids the results indicate an average value between 0.5 to 20.9ng/mL, for all the samples analyzed, with the highest value of LOQ made with a GC/MS system [15,20]. It can be seen that tissues have lower limits of quantification than fluids, however they needed higher mass of biological samples [9,11-30].

Table 2 Concentrations in the most used and discussed real samples reviewed

Samples	Analytes	Real concentrations
Blood	COC	ND - 4060ng/mL
	BE	ND - 19847ng/mL
	CE	ND - 254ng/mL
	NC	Not confirmed

	EME	ND - 18770ng/mL
	AEME	x
Hair	COC	15.1 - 43.24ng/mg
	BE	0.74 - 22ng/mg
	CE	0.9ng/mg
	NC	1.2ng/mg
	EME	3.31ng/mg
	AEME	5.5ng/mg
Urine	COC	71880 – 200174.90ng/mL
	BE	57453 - 684720ng/mL
	CE	x
	NC	x
	EME	791670ng/mL
	AEME	x
Liver	COC	ND - 21210ng/mL
	BE	ND - 31420ng/mL
	CE	ND
	NC	x
	EME	ND - 71710ng/mL
	AEME	x
Vitreous Humor	COC	ND - 530ng/mL
	BE	11 – 2633.70ng/mL
	CE	ND - 254ng/mL
	NC	x
	EME	x
	AEME	x
Fingernail	COC	6ng/mg
	BE	40ng/mg
	CE	x
	NC	0,25ng/mg
	EME	x
	AEME	x
Skeletal tissue and muscle	COC	ND - 0.38ng/mg
	BE	ND - 0.88ng/mg
	CE	ND - 0.097ng/mg
	NC	x
	EME	ND
	AEME	x

Cocaine concentrations in real samples were the most found (81.0%), BE was found in 14 articles (66.7%) and EME in seven articles (33.3%). These three analytes appeared in almost all concentration detections, and CE was quantified six times (28.6%),

NC two times (9.5%) and AEME appeared only once (4.8%). It is important to addend that CNO was not quantified. The concentration far more than the LOQ variated considerably between not detected and 791670ng/mL [9,11-30]. Overall, almost every article could quantify and analyze cocaine and the metabolites that were to be searched, the non-conventional matrices appeared to have a higher average concentration than conventional matrices (Table 2) [9,11-30].

DISCUSSION

The cocaine metabolites are an important path to finding real results of the drug in the deceased body. The main metabolites have a higher plasma half-life than cocaine which can indicate not only the use of the drug, once was metabolized by the body, but good comparative results with the possibility of more real numbers to the concentration of the drug prior to death [31]. BE and EME are the major biomarkers for cocaine formed by an enzymatic hydrolysis, meaning almost every case with assumption of cocaine consumption they must be found. CE is a result of the use of alcohol alongside cocaine and NC is an active metabolite [14,18]. Also, there are other metabolites less researched, such as AEME or even CNO, a product of cocaine once it goes through pyrolysis and a new biomarker that show interest in the identification of cocaine use by hair samples, respectively [18,23]. Therefore, research for cocaine and at least one metabolite is recommended, although not observed in four works [12,17,22,29].

In a lot of publications, it is seen that cocaine does not have the bigger concentrations which indicates that the analytes such as BE, EME and CE may be of importance, once the body goes through metabolization of the drug itself, for the quantification, history of drug use of the victim and to the case. However, of all the articles analyzed cocaine presented the bigger concentrations on hair and brain samples when compared to the concentrations of the biomarkers [13,14,16,18], this is due to the metabolization in both organs, the keratinization, growth and external contamination of the hair that occurs once the use of cocaine is made regularly increases COC concentrations to other metabolites, and for the brain, the blood-brain barrier difficults the passage of the biomarkers, allowing cocaine to have higher concentrations [32,14].

Cocaine and its metabolites are analytes of interest when it comes to post-mortem analysis, however as it could be seen in this review, toxicological analysis usually tends to search for more drugs of abuse and psychoactive substances altogether. When

more than one drug is searched, the toxicology analysis can elucidate a better view of the case and contribute with fully results for the investigation. Furthermore, with just one analytical method it is possible to look for many compounds and save time and cost. In the recent years it is became more common, as the chromatography instruments are more modern and the detectors are more sensitive [9,31], however techniques as this one means the chromatography systems in use has to be well calibrated to avoid saturation and cross reactivity causing false results [9]. Throughout the ten years of the scope review analyses, there is an inclination to the thought that new published articles have more analytes to identify in the same method than old publications from 2012 to 2014.

The information about collection of samples and time of death was not informed by many researches considered in this review. Although this information should be considered when it comes to choosing the sample and search for analytes with the most precise concentration prior to death [5]. Cocaine has a high redistribution of concentrations in the cadaver, the movement of the substance in the victim's body after death is rapid. Understanding the cocaine redistribution process means defining which biological matrices to use in every criminal investigation. For each individual case, in some tissues the concentration of cocaine and metabolites will be higher and easier to identify than in others [5].

Non-conventional biological samples are important to the post-mortem toxicology once it shows solid results on cases where the victim has been exposed for a significant period of time to the environment. In these cases, an extreme redistribution occurs on the system and interferes with the results as the corpse decompose [27]. It can be seen in the review that non-conventional samples do have great concentration results after the analysis, however the difference when compared to concentrations of conventional samples analyzed alongside is minimal, which indicates that blood and the others conventional samples are resourceful matrices and shows solid results when collection of these samples is possible [9,11-31].

Non-conventional samples also have hardships to be encountered along the analysis, these samples are of difficult collection, some need clean up procedures and have a higher consume of time and cost than more conventional samples. Also, more research is needed to provide better understandings of the results readings and also for hair there is a vast external contamination problem that can modify and cause false results, proving the necessity of more publications in this area [5,14,23,32]. Guidelines may help when it comes to collection of these samples, the International Association of Forensic

Toxicologists, for example, created an international guideline to help not only collection of the matter but also for understanding of when to use each sample [33].

Hair, for example, a non-conventional sample, has been researched on the past ten years more than urine. Hair has a lot of benefits, as its window of time when it comes to real quantification of concentrations of substances prior to death, the facility to extract the sample and its storage [23]. Blood is the most used sample in the articles selected to this review study. It is the conventional sample and less expansive biological matter to utilize, express great concentrations of the drugs and substances consumed before death and today on the literature is the specimen with the largest studies and information [9]. The redistribution, especially on peripheral blood, is lower than in many other organs and tissues, so the drug concentrations are usually close to the concentrations before the death, causing the interpretation of the result to be easier and a golden method to choose from [11,25].

The sample preparations used in the toxicologic analysis are an important stage of the process, it will concentrate the analytes and purge interferents. It is a special part of the forensic analyst day-to-day and represented the most error prone part. These methods, either being SPE or LLE, are constantly evolving in researches to improve the forensic field, presenting better results on each analysis made and with solutions to a few problems the extractions have, such as elevated solvent consumption, increased cost for the procedures and complex matrice applications in the extractions [34].

In this review, SPE was utilized the most and the solvent and sortive phase was the same for every article mentioned was similar always using dichloromethane and ammonia as the solvent and the C8/SCX as sortive phase, this information proves that it is a validated methodology with reliable results. The C8 sorbent with a strong cation-exchange is proved to accomplish promising results and a better extraction of cocaine and metabolites, due to their physical chemical properties [6].

GC-MS is brought as a good system for detection of drugs since it presents satisfactory results and has a good cost-benefit, with easier methods to prepare the samples and an easier understanding of the mechanism of the system. However, when it comes to sensitivity and selectivity it is not the best equipment [34]. The limitations this system has is due to the fact that gas chromatography decrease the possibility to be used with good results on multianalyte detections [35]. In this review, the LOQs for COC and BE with higher results, were made by a GC-MS system and were a blood analysis, 20 and 20.9ng/mL, respectively, [17,20]. This is a satisfactory result that can identify low levels of

cocaine, however, in this review LC-MS/MS has been utilized more, and has great new studies proving this technology can be even more preferable as it appears to overcome the difficulties GC-MS has. The utilization of MS/MS generates even more sensitive and selective results, with far rapid analyses [36].

GC-MS was brought by one of the authors as a simple and robust technique, that has a large sum of problems with sensibility, however is an equipment far utilized by the scientists and analysts. PTV-LVI-GC/MS was documented as an option that would help GC-MS become more sensitive and precise [17]. Although PTV-LVI-GC/MS improves the sensibility of the gas chromatography the results did not present any difference to the GC-MS analysis and did not surpass the liquid chromatography, the quantified LOQ was 20ng/mL and all of the LC-MS findings were far lower.

LOQs affect directly the drug concentrations found, if the limit is not lower enough the quantification of the drug may not be a reliable source of information causing a false result on the analysis [37]. The LOQs defined in ANSI/ASB Standard 036 (2019) must be identified with validated methods, for example, in forensic toxicology the LOQ cut-off must be ≤10 ng/mL, following the instructions on the guideline [37]. The LOQs brought by the publications allow get good results of the concentrations in real samples quantified, some could not quantify the samples, however there were innumerous real cases and victims in all the articles and overall, the results were validated at the end of the publication.

The publications were able to quantify almost every analyte of interest searched in all the samples, and of the publications that showed “not detected” results, only two were not able to quantify any analyte of interest searched [28,29]. The variations of the concentrations found in this review may be explained by the nature of the case the publications bring, there are innumerous cases and causes of death, redistribution and how much of the drug the victim consumed prior to death are factors that can cause disparity in the numbers [9,11-30].

The toxic effects resulted by consuming cocaine already can be seen with blood concentrations between 250ng/mL to 500ng/mL [14], there were publications that brought far higher real concentrations results than these ones. Wherefore, applied on real cases, a result like this would indicate a consumption of the drug probably of importance to the unfolding of a forensic case.

CONCLUSION

Cocaine and the metabolites such as EME, NC, BE and CE have an important role in the forensic toxicologic post-mortem analysis, is a wide spread drug of abuse that is connected with multiples types of cases from violent deaths. The most utilized biological sample was blood and hair, with a reliable result and good quantification of the biomarkers as well. Non-conventional biological samples are of interest and should be more documented. The most used type of extraction was yet classic SPE with analysis by LC-MS/MS. Most of the studies reviewed were about method validations, which indicates the preoccupation with precise results. Another critical point is the correlation of cocaine concentration and the time of death, which are fell explored in researches. Current chromatographic methods have allowed obtaining adequate LOQs and quantification in biological samples. Therefore, it is observed that future advances should focus on miniaturized and automated sample preparation, with adequate cost-benefit and a better understanding of post-mortem redistribution.

DECLARATIONS

Conflict of interest The authors declare that there is no conflict of interests.

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APPENDIX

Appendix 1 Results table of the twenty-one articles reviewed

Appendix 1 – Scope Review of important information about the toxicologic analysis										
Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; BE; EME; CE	**Dried Blood Spots (DBS);**	**85µL (Blood)**	**SPE**	**C8 sorbent and a strong cation-exchange (SCX) sorbent (200mg)**	**Dichloromethane-isopropanol (8:2 v/v) with 2% ammonia solution**	**LC-MS/MS**	**10ng/mL for all 4 analytes**	**45 with 8 positives**	**COC:49 - 2260ng/mL BE:338 – 4470ng/mL EME: Not quantificated – 910ng/mL**	Moretti et al., 2018 (Italy)
EME; COC; BE; CE; Opioids; Analgesics; Benzodiazepines; Amphetamines and methylphenidate; antidepressants; Imidazopiridynes; Barbiturates.	**Whole Blood**	**1mL and 0.1mL (dilution test)**	**SPE**	**C8 phase and an ion exchange (BCX) phase bonded to the same particle (200mg/3mL)**	**Fraction A: Hexane and ethyl acetate (v/v) Fraction B: Dichloromethane/isopropanol/ammonium hydroxide (78:20:2, v/v)**	**LC–MS/MS**	**1ng/mL for COC, BE and CE; 2ng/mL for EME**	**More than 1000 with 452 positives**	**In 10 positive cases COC: not detected – 11ng/mL BE: not detected – 34ng/mL EME: not detected – 52ng/mL CE: not detected**	Ahmed; Al-Asmari, 2020 (Saudi Arabia)

Appendix 1 – Scope Review of important information about the toxicologic analysis										
Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; BE	**Cardiac Blood; Left Cardiac Blood; Femoral Arterial Blood; Femoral Venous Blood; Urine; VH; Cerebrospinal Fluid; Brain Accumbens Nucleus; Brain Ventral Tegmental Area; Liver**	**2mL**	**SPE**	**C8/SCX mixed-phase columns**	**Dichloromethane-isopropanol-ammonia 78:20:2 and ethyl acetate-ammonia 98:2**	**GC-MS**	**VH: COC - 0.76ng/mL BE - 2.55ng/mL** **Blood: COC - 5.03ng/mL BE - 14.29ng/mL** **Urine: COC - 16.95ng/mL BE - 20.00ng/mL** **AN: COC - 2.78ng/g BE - 14.29ng/g** **VTA: COC - 3.44ng/g BE - 12.35ng/g** **Liver: COC - 1.74ng/g BE - 4.10ng/g** **CSF: COC - 0.62ng/mL BE -2.55ng/mL**	**-**	**In 1 positive case** **FVB: COC - 3,210.60ng/mL BE - 19847.00ng/mL** **FAB: COC - 970.4ng/mL BE - 3031.70ng/mL** **RCB: COC - 1111.50ng/mL BE - 3458.90ng/mL** **LCB: COC - 1635.90ng/mL BE - 3116.40ng/mL** **VH: COC - 230.8ng/mL BE - 2633.70ng/mL** **U: COC - 200174.90 ng/mL BE -57453.00ng/mL** **CSF: COC - 537.4ng/mL BE - 3132.50ng/mL** **VTA: COC - 2673.10 ng/g BE - 2842.90ng/g** **AN: COC - 2,384.60ng/g BE - 1652.30ng/g** **LIVER: COC - 1.9ng/g BE - 973.90ng/g**	Alvear et al., 2014 (Chile)

Appendix 1 – Scope Review of important information about the toxicologic analysis										
Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; Acetaminophen; Alprazolam; Amlodipine; Atropine; Chlorpromazine; Clonazepam; Clonidine; Codeine; Diazepam; Flunitrazepam; Heroin; Ketamine; Methampheta mine; Morphine; Nicotine; Nordiazepam; Norketamine; Olanzapine; Oxazepam; Pethidine; Pheniramnei; Timolol; Zolpidem	**Vitreous Humor; Whole Blood; Plasma**	**20 µL**	**LLE**	**-**	**Acetonitrile; Formic acid 0.1%; Homatropine 500ng/mL; Sulphadimethoxine 10 ng/mL**	**ESI-LC-MS/MS**	**7.8ng/mL**	**40**	**In 10 positive cases COC: Not detected CE: not detected – 254ng/mL**	Arora et al., 2015 (India)

Appendix 1 – Scope Review of important information about the toxicologic analysis										
Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; BE; Morphine, Benzodiazepines; Amphetamines;	**Hair; Larvae; Cardiac Tissiue**	**Larvae: 360 mg** **Cardiac Tissue: 5mL** **Hair: 50mL**	**SPE**	**C8/SCX mixed-phase columns**	**Dichloromethane/methanol 8/2; 2% ammonia (v:v)**	**GC/MS**	**-**	**1**	**Larvae: COC -5.35ng/mg** **BE -17.32ng/mg** **Cardiac Tissue: COC -328ng/mL** **BE – 509ng/mL** **Hair: COC-43.24ng/mg** **BE - 0.74ng/mg**	Basilicata et al., 2019 (Italy)

Appendix 1 – Scope Review of important information about the toxicologic analysis										
Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; BE; EME; levamisole	**Blood; Urine; Liver with gall bladder; Kidney; bladder Brain; Heart; Lung; Spleen; Stomach; Small and large intestines**	**Fluids: 2 mL Tissues: 2g**	**-**	**-**	**-**	**ESI-LC-MS/MS**	**-**	**1**	**Blood: COC – 4060ng/mL BE – 10420ng/mL EME - 18770mg/mL Urine: COC – 71880ng/mL BE - 684720ng/mL EME - 791670ng/mL Liver and gall bladder: COC - 21210ng/mL BE - 31430ng/mL Eme - 71710ng/mL Kidney and Bladder: COC - 24930ng/mL BE - 16000ng/mL EME - 30910ng/mL Brain: COC - 18.95ng/mg BE - 1.95ng/mg EME - 11.37ng/mg Heart, Lung and Spleen: COC – 9210ng/mL BE – 8430ng/mL EME – 15960ng/mL Stomach: COC – 4460ng/mL BE – 6350ng/mL EME – 12500ng/mL Small and large intestines: COC – 6110ng/mL BE – 27760ng/mL EME – 39160ng/mL**	Brajković et al., 2016 (Serbia)

Appendix 1 – Scope Review of important information about the toxicologic analysis										
Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; BE	**Whole Blood**	**2 mL**	**SPE**	**C8 sorbent and a strong cation-exchange (SCX) sorbent**	**Dichloromethane:isopropanol:ammonia (78:20:2 v/v/v); ethyl acetate:ammonia (98:2 v/v)**	**GC-MS**	**COC: 10.9ng/mL BE: 20.6ng/mL**	**2,353 with 341 positives**	**In 8 positive cases COC: 87 - 7500ng/mL**	Bravo et al., 2012 (Chile)
COC; BE; CE; EME; Opioids; Amphetamines; Benzodiazepines	**Hair**	**20 mg**	**SLE**	**-**	**Methanol/acetonitrile/2 mM ammonium formate (25:25:50, v/v/v)**	**LC–QTOF-MS**	**COC; BE; CE; EME: 0.009ng/mg**	**90**	**In 1 positive case COC: 27 ng/mg BE: 22 ng/mg EME: 0.16 ng/mg**	Broecker et al., 2018 (Germany)
COC; Pesticides; Benzodiazepies; Amphetamines; antidepressants; anticonvulsivants.	**Blood**	**1mL**	**d-SPE and SLE- LTP**	**-**	**ACN and EtOAc**	**PTV-LVI-GC–MS**	**20ng/mL**	**10**	**COC: 40 – 3130ng/mL**	Ferrari Júnior et al., 2018 (Brazil)

Appendix 1 – Scope Review of important information about the toxicologic analysis										
Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; BE; CE; NC; AEME	**Blood and hair**	**Blood: 500 µL** **Hair: 45 mg/50mg**	**Blood: LLE** **Hair: SLE**	**-**	**Blood: (ACN)/MeOH (80:20/v:v)** **Hair: (1 mM of Ammonium formate in water with 0.1% formic acid**	**UPLC-ESI-MS/ MS**	**Blood: COC and CE - 1.0 ng/mL** **BE and NC - 0.5 ng/mL** **AEME - n/d** **Hair: COC and AEME - 0.5 ng/mg** **BE, CE and NC - 0.05 ng/mg**	**105**	**Mean Values for hair:** **COC - 15.1ng/mg** **BE - 3.1ng/mg** **CE - 0.9ng/mg** **NC - 1.2ng/mg** **AEME - 5.5ng/mg** **Mean Values for blood:** **COC - 290ng/mL** **BE - 607ng/mL** **NC – not confirmed**	Fonseca Pego et al., 2018 (Brazil)

Appendix 1 – Scope Review of important information about the toxicologic analysis										
Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; BE; CE; Benzodiazepines; Analgesics; Antidepressants; Anesthesics; Amphetamines; Opioids; Antihistamines; Ephedrine; Antipsychotics; Anticonvulsants; Carbamates	**Blood and Urine**	**100 µL**	**Multi-layer filtration (Replacement spe)**	**-**	**Acetonitrile fortified with IS**	**LC–MS-MS**	**For blood: COC, BE and CE: 10ng/mL** ***Urine was qualitative**	**52**	**-**	Gevorkyan et al., 2020 (USA)
COC; BE	**Blood**	**-**	**SPE**	**C8 sorbent and a strong cation-exchange (SCX) sorbent**	**-**	**GC–MS**	**20ng/mL for both subtances**	**Over 50,000 with 132 positives**	**COC: 20ng/mL – 1250ng/mL** **BE: 200ng/mL – 2960ng/mL**	Jones; Holmgren, 2014 (Sweden)

Appendix 1 – Scope Review of important information about the toxicologic analysis										
Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; BE; NC; CE	**Fingernail**	**5mg**	**SLE**	**-**	**MeOH**	**LC–ESI-MS/MS**	**0.01ng/mg for all four analytes**	**1**	**COC - ≈ 6ng/mg NC - ≈ 0.25ng/mg BE - ≈ 40ng/mg** ***Better results were seen in the dorsal scraping of the nail**	Madry et al., 2014 (Switzerland)
COC	**Liver**	**2 g**	**SLE–LTP**	**-**	**Pure acetonitrile or a solution consisting of acetonitrile/ethyl acetate 87.5:12.5%, v/v**	**GC–MS**	**50.0 ng/g**	**8**	**COC: 0.3335ng/mg - 5.969ng/mg**	Magalhães et al., 2013 (Brazil)
CNO; COC; BE; CE; NC; opioids; benzodiazepines; amines	**Hair**	**10mg**	**SLE**	**-**	**2 : 1 water : methanol with 0.1% formic acid extraction**	**LC–MS-MS**	**-**	**10**	**Only CNO was analised** **No real [ ]**	Marsh et al., 2014 (USA)

Appendix 1 – Scope Review of important information about the toxicologic analysis										
Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; BE; CE; EME; Benzodiazepines; opioids; amphetamines; antidepressants; dibenzothiazepines; Butyrophenones;	**Blood; DBS**	**85 µL**	**SPE**	**C8 sorbent and a strong cation-exchange (SCX) sorbent**	**Dichloromethane–isopropanol mixture (8:2 v/v) with 2% ammonia solution**	**LC–MS/ MS**	**-**	**20**	**DBS:** **COC – 1357ng/mL – 45.7ng/mL** **BE – 3750ng/mL – 105ng/mL** **EME – 3410ng/mL – 59.6ng/mL** **CE – 108ng/mL – 70.7ng/mL** **Blood:** **COC – 1350ng/mL – 40ng/mL** **BE – 4170ng/mL – 87.1ng/mL** **EME – 3120ng/mL – 52ng/mL** **CE – 104ng/mL – 81ng/mL**	Moretti et al., 2021 (Italy)

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Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; CE; EME; BE; Opioids; Amphetamines	**DBS**	**100 µL**	**SLE**	**-**	**Methanol/0.1 % formic acid**	**UHPLC–MS/MS**	**2ng/mL for all four analytes**	**10 with 4 positives**	**COC: 2.4ng/mL BE: 37ng/mL CE: n/d EME: n/d**	Odoardi et al., 2014 (Italy)
COC; BE; EME; Benzodiazepines; Opioids; Amphetamines; Antidepressants	**Skeletal Tissue (fresh and 1 year burial)**	**1g**	**SLE**	**-**	**H2O: MeOH 80:20 v/v**	**UHPLC–MS/MS**	**COC: 0.00047 ng/mg EME: 0.00253 ng/mg BE: 0.00025 ng/mg**	**10 from 6 individuals**	**COC: 0.00374ng/mg - <LOQ BE: 0.00131ng/mg - <LOQ EME: n/d**	Orfanidis et al., 2018 (Greece)
COC; EME; CE; BE; Benzodiazepines; Opioids; Amphetamines; Antidepressants; Cannabinoids; etc	**Liver**	**1g**	**QuEChERS (6 different protocols**	**-**	**Protocol B: chloroform–methanol (2:1, v/v) Protocol C: diethyl ether-dichloromethane (1:1, v/v)**	**UHPLC–MS/MS**	**EME: 0.00617 ng/mg BE: 0.00329 ng/mg COC: 0.00350 ng/mg CE: 0.00374 ng/mg**	**14**	**None of the four analytes of interest were detected in the real samples extracted with protocol D1**	Orfanidis et al., 2020 (Greece)

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Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; Amytriptiline; Carbaryl; Diazepam; Diazion; Heptachlor; Malathion Permethrin; Phenobarbital; Prochloraz; Tramadol	**Stomach contents**	**5g or 5mL**	**QuEChERS**	**-**	**Acetonitrile**	**GC–MS**	**0.2 mg/kg**	**16**	**COC: n/d**	Peres et al., 2019 (Brazil)

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Analytes	**Samples**	**Sample Volume**	**Extraction Method**	**Sortive Phase (Mass)**	**Solvent of Extraction**	**Detection System**	**LOQ**	**Number of real Samples**	**Real Concentrations**	**Reference (Country)**
COC; BE; CE	**Skeletal muscle; Femoral blood; Cardiac blood; Vitreous humour; Cardiac muscle**	**0.5 g**	**SPE**	**Non-polar (C8) and strong cation exchange retention**	**-**	**GC–ion trap-MS/MS**	**-**	**9**	**SM:** **COC – n/d – 0.38ng/mg** **BE - n/d – 0.88ng/mg** **CE – n/d – 0.097ng/mg** **FB (cases 5, 6 and 8: No sample for analysis):** **COC – n/d - 170ng/mL** **BE – n/d - 880ng/mL** **CE – n/d - 52ng/mL** **CB:** **COC – n/d - 160ng/mL** **BE - n/d - 710ng/mL** **CE – n/d - 55ng/mL** **VH:** **COC – 530ng/mL – 4ng/mL** **BE –960ng/mL – 11ng/mL** **CE – n/d - 180ng/mL** **CM:** **COC – n/d - 0.73ng/mg** **BE – n/d – 1.5ng/mg** **CE – n/d – 0.21ng/mg**	Rees et al., 2013 (UK; Brazil)