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### **Review** article

## Entomotoxicology, experimental set-up and interpretation for forensic toxicologists

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#### Contents

#### ABSTRACT

Forensic entomotoxicology studies the usefulness of insects as alternative toxicological samples. Use of insects as alternative matrix for drug detection is well documented and recommended when conventional matrices such as blood, urine or internal organs are no longer available.

However, several limitations of entomotoxicology have been highlighted, especially concerning interpretation of the drug concentrations in insects on human forensic cases. In addition, the lack of knowledge in pharmacokinetic of drugs in insects, large variability of experimental set-up and toxicological analysis compromise the utility of this science.

This review focuses on the current knowledge of factors influencing drug detection in insects. Reasons for the current limitations, but also recommendations for future research are discussed and proposed in this paper.

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#### 1. Introduction

From 1980 entomologists started to detect drugs in insects, hoping it would become a useful tool in forensic investigations [1,2]. Although this science has permitted to provide answers to real forensic cases in the past decades [3,4], several scientists are still skeptical of the potential value of entomotoxicology in forensic investigations, regarding it as a laboratory curiosity [5] or a scientific imposter [6].

The major interest of entomotoxicology is the determination of drug abuse just before death, especially in skeletonised remains where no tissue or fluids are left. Also in highly decomposed remains, toxicological analyses can be facilitated using insects as less interferences in the analytical run due to matrix decomposi-

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tion are observed [4,7]. Moreover, in a comparative study by Kintz et al. [8] a greater sensitivity was obtained using fly larvae instead of putrefied material. In addition, drug concentrations seem to be more stable in insects, while this is not always the case for several post-mortem tissues [7]. From a pure practical point of view, insects are of interest as they are present in high quantities and their remains (puparia or exuviae) are present for a long time, even when toxicological samples are no longer available [9]. In cases where religious and ethical beliefs result in problems collecting samples for toxicological analysis, insects could be a solution [10].

The use of necrophageous species as matrix for qualitative drug detection is well documented and generally accepted by forensic toxicologists [11]. Many compounds (drugs, metals and pesticides) have been detected in insect tissues in a forensic context (Table 1). Some animal studies have demonstrated a possible correlation between drug concentrations in the substrate and the different developmental stages of insects reared on that substrate [9,11,12]. However, the main problem for entomotoxicologists is interpretation of the results. At this point it is not possible to estimate the cause and circumstances of death from drug concentrations observed in insects reared on the corpse [6]. However, this relationship in human forensic cases is not yet established and still remains a controversy [6,13,14]. According to some authors, it seems hardly expected to find such a quantitative relationship due to a wide array of influencing factors which are largely unexplored and at the moment unpredictable [6].

The aim of this review is to give an overview of the recent knowledge in the field of entomotoxicology. In addition, several pitfalls which do not allow interpretation in forensic investigations at the moment will be discussed.

# 2. Interpretation of current limitations: current knowledge and limitations

#### 2.1. Insects

#### 2.1.1. Life traits and drug pharmacokinetics

Pharmacokinetics of drugs in insects depend on the species, the developmental stage as well as on their feeding activity [4,11].

A part from necrophageous species, bioaccumulation can also occur in parasitoids, predators or omnivorous species [15,16]. However, this drug bioaccumulation will not be similar as these species present different feeding behaviour due to their diet or life history traits [17]. For entomotoxicological investigations, use of necrophageous species belonging to Coleoptera and/or Diptera is recommended as they are the first to colonize the corpse. Necrophageous species are usually very common and abundantly present on the crime scene. Moreover, their biology and development are well-known, as they are already used in forensic entomology to estimate Post-Mortem Interval (PMI) [18].

Not only species is of importance, but also the developmental stage and their activity. After hatching of the eggs, there are 3 larval stages. In the third larval stage, three main activities are observed: feeding, digestion and searching for food [19,20]. Once the maximum larval size is obtained, they stop feeding, walk away from the food source, searching for a place to become a pupa. Differences in drug concentrations are observed in larvae with different feeding activities [20]. Moreover, drug concentrations in puparia are lower then in larvae, but seem to be more reproducible. In highly decomposed bodies, puparia of first generation colonizing insects could be a better 'drug concentration estimator' than larvae from the second generation [21]. However, for forensic entomologists additional quantification of drugs in larvae can be

important to explain alteration of blowfly development and thus PMI estimation.

While there is a difference in drug quantities observed in different larval stages or pupa, there are still a lot of questions unanswered and discussions about drug metabolism, absorption and elimination and thus drug possible drug accumulation and localisation in insects [9,11,17,22–28]. In feeding larvae, drugs could be absorbed across the midgut and distributed into the larvae [29]. However, it is unclear if drugs are stored in haemolymphs, fat bodies or the integument [29,30]. Drugs can be excreted directly via the gut [29] or via malpighian tubules after metabolism. According to Parry et al. [29], drug metabolism in insects occurs in the malpighian tubules via cytochrome P450 and glutathione transferase enzymes. However, drug metabolism in insects is not yet elucidated. Presence of metabolites could result from the action of substrate enzymes [11] and/or larvae metabolism [25,26].

Malpighian tubules do not only result in drug excretion during larval stage, but are still secreting drugs during the post-feeding stage leading to lower drug levels in comparison to actively feeding larvae for most drugs [12,28]. Once insects are in the pupal stage, the malpighian tubules are degraded and the remaining gut content will only be excreted as meconium during emergence of the adult insect [29]. As drug concentrations seem low in adults, most drugs must be excreted during the first two days of adult life [31].

It is clear that drug metabolism and excretion in the different developmental stages of insects should be studied more in detail to get an idea of how and to what extent drugs are incorporated in insect tissues. As a result, entomotoxicologists could determine which insect phase is of most interest to detect drugs, which tissues will lead to highest, most reproducible drug concentrations and thus will be the insect tissues having most change to increase the reliability of forensic entomotoxicological results.

#### 2.1.2. Extrinsic conditions and their influence on insects

The oviposition and development of insects can be affected by bioclimatic factors such as the photoperiod and temperature [32– 37]. This is of major importance for estimation of PMI by forensic entomologists. At first, these environmental factors seem to be less important for drug detection in insects. However, entomotoxicologists will use experimental data -often generated in laboratories under constant light and temperature conditions- for interpretation of real forensic cases. Therefore, entomotoxicologists must be aware of the influence of environmental factors on the insect development.

Constant light increases the variation of the time for an insect to become an adult and significantly delays the development compared to insects reared with cyclic photoperiods [32]. The development can also be accelerated or retarded according to the ambient temperature [36,37]. In addition, rain and thus the humidity influences oviposition and development of blowflies [33]. If entomological experiments are done in the lab, it is therefore important to use a realistic photoperiod 12:12 h (light/ dark), and a humidity and temperature in function of the investigated insect species and the climatic conditions of the region where the forensic case is situated.

Not only the insect development can be changed due to climatic factors, but also the gut motility and thus the absorption and excretion of drugs is dependant on the ambient temperature [29]. Moreover, drug stability in tissues can also be influenced by temperature, humidity and UV-radiation [6]. As a result, environmental factors can have an influence on the final drug concentration present in the analysed insect tissue.

Because of the importance of the climatic conditions, they should be monitored and noted during the development, sampling

#### Table 1

List of toxic substances detected in different developmental stages of insects.

		Insect species	Developmental stages	Reference
Alcohol	Ethanol	Calliphoridae, Sarcophagidae Phormia regina	L L	[13] [32]
Drugs Antidepressants	Amitryptyline	No specified	L	[6]
Antidepressants	Amitryptymie	Lucilia sericata		
			L	[12]
		Calliphora vicina	С	[64]
			L, P	[28,39]
		Dermestes maculatus	E, F, Pu	[17]
		Megaselia scalaris	E, F, Pu	[17]
	Clomipramine	No specified	L	[6,7]
	ciomprunnie	Lucilia sericata	L	[12]
	Dethionin			
	Dothiepin	No specified	L	[6]
	Fluoxetine	No specified	L	[6]
	nortriptyline	Lucilia sericata	L	[12]
		Dermestes maculatus	E, F, Pu	[17]
		Megaselia scalaris	E, F, Pu	[17]
	Trazodone	Calliphora vicina	L	[39]
	Trimipramine	Calliphora vicina	L	[39]
	Venlafaxine	No specified	L	[6]
arbiturates	Amobarbital	No specified	L	[6]
	Barbiturates	No specified	L	[6]
	Phenobarbital	Cochliomyia macellaria	L	[1]
	i lichodal Dital	5		
		Lucilia sericata	L	[12]
		No specified	L	[6,7]
	Secobarbital	No specified	L	[65]
	Sodium amylobarbitone	Calliphora vicina	L, P	[51]
	Sodium barbitone	Calliphora vicina	L, P	[51]
	Sodium brallobarbitone	Calliphora vicina	L, P	[51]
	Sodium phenobarbitone	Calliphora vicina	L, P	[51]
	Sodium thiopentone	Calliphora vicina	L, P	[51]
enzodiazepines	Alprazolam	No specified	L	[6]
enzodiazepines	Alpiazolalli			
	_	Calliphora vicina	L, P	[26]
	Bromazepam	No specified	L	[6]
		Piophila casei	L, P, A	[40]
	Clonazepam	Calliphora vicina	L, P, A	[26]
	Diazepam	Calliphora vicina	L, P, A	[26]
	Diazepaili		L, F, A	
		Chrysomya albiceps		[27]
		Chrysomya putoria		[27]
	Flunitrazepam	Calliphora vicina	L, P, A	[26]
	Lorazepam	No specified	L	[6]
	I IIIII	Calliphora vicina	L, P, A	[26]
	Nordiazonam		L	
	Nordiazepam	No specified		[6]
		Calliphora vicina	L, P, A	[26]
	Oxazepam	No specified	L	[6,7]
		Calliphora vicina	L, P, A	[26]
	Prazepam	Calliphora vicina	L, P, A	[27]
	Temazepam	Calliphora vicina	L, P, A	[26,39]
	Triazolam	No specified	L	[6]
		Calliphora vicina	L, P, A	[7,26]
		No specified	L	[8]
iscellaneous	Amphetamine	No specified	No specified	[13]
iscenaricous	Amplictamile	Calliphora vicina	L	[51]
	Benzoylecgonine	No specified	L	[4]
	Cocaine	No specified	L	[4,24]
		Lucilia sericata	L	[12]
	Digoxin	No specified	L	[6]
	Meprobamate	No specified	L	[6]
	Nefopam	No specified	L	[6]
	Sodium aminohippurate	Calliphora vicina	L	[51]
	Sodium salicylates	Calliphora vicina	L	[51]
	THC-COOH	No specified	L	[6]
		1		
	11-Hydroxy-THC	No specified	L	[6]
pioids/opiates	Codeine	Lucilia sericata	L, P, A	[11]
		No specified	L	[6,66]
	Methadone	Lucilia sericata	Ĺ	[20]
		Dermestes freshi	L, P, A	
	Morphine			[9,67]
		Thanatophilus sinuatus	L, P, A	[9,67]
		Lucilia sericata	L, P, Pu, A	[9,53]
		Calliphora stygia	L	[42]
		camprora organ		
			L, P, Pu, PP, A	[29]
		Calliphora vicina	L, Pu	[23]
		Calliphora vicina	L, P	[54]
		Protophormia terraenovae	L, P	[54]
		No specified	L	[6,66]
	Opiates	Lucilia sericata	L	[12,14]
	Pholcodine	No specified	L	[6]

		Insect species	Developmental stages	References
	Propoxyphene	No specified	L	[6]
		Calliphora vicina	С	[64]
Phenothiazine	Alimezanine	No specified	L	[6,7]
	Chlorpromazine	No specified	L	[6]
	Cyamezanine	No specified	L	[6]
	Levomepromazine	No specified	L	[6]
		Lucilia sericata	L	[12]
		Piophila casei	L	[40]
	Thioridazine	Lucilia sericata	L	[12]
METALS	Antimony	Calliphora dubia	L, P, Pu, A	[22]
		Lucilia sericata	L	[68]
	Barium	Calliphora dubia	L, P, Pu, A	[22]
		Lucilia sericata	L	[68]
	Cadmium	Lucilia sericata	L, Pu, A	[69]
	Lead	Calliphora dubia	L, P, Pu, A	[22]
		Lucilia sericata	L	[68]
	Mercury	Calliphoridae	L, Pu, A	[31]
PESTICIDES				
organophosphates	Malathion	Chrysomya megacephala	L, P	[62]
			L, PP, P	[70]
			L	[71]
		Chrysomya ruffifacies	L	[71]
	Parathion			
		Diptera	L, P, Pu, A	[41]
		Coleoptera	A	[41]
		Hymenoptera	A	[41]

L, larva; P, pupa; PP, prepupa; Pu, puparia; A, adult; C, crop; F, frass; E, exuvia.

and storage of insects reared experimentally. In real cases, climatic conditions should be investigated via meteorological station to have as much information as possible for insect development. Concerning the influence of environmental factors on the insects gut motility and final absorption of drugs in insect tissues as well as the stability of drugs in those tissues, future research will be necessary to have an idea of the impact of environmental factors on final drug detection.

#### 2.1.3. Insect sampling

For some toxicologists, insect sampling can seem simple; *just take some maggots from the corpse*. However, it is a factor leading to high variability in drug detection.

First of all, sampling of insects (larvae or puparia) can be carried out, around or under the body or body discovery site. When the corpse is highly decomposed, investigators must be aware that the collected insects can originate from a source other than the deceased [38].

In addition, several authors [6,10,39] demonstrate the importance of collecting at different body-sites, as inter-site sampling results in a high variation of drug concentrations. This observation is logic as drugs are distributed in the body according to their physicochemical properties, leading to different drug concentrations in different organs and tissues, and thus also in insects reared on these different substrates. While most investigators [13,14] sample randomly, the best sampling sites for drug detection in insects are the internal organs (e.g. liver), the head-area or muscles in cases where no internal organs are left. However, in the literature, other sampling sites such as the skin surface are observed [15]. In older research or case reports, larvae or puparia are often removed from different areas of the corpse and pooled in one sample [7,8,40], possibly due to problems with the sensitivity of the used analytical methods. However, information concerning drug detection in different body areas as well as inter-insect variation due to differences in developmental stage or activity (see above) will be lost [6,20]. This information may not be of interest for case report interpretation at this moment, but for development of future entomotoxicological knowledge it is of most importance.

At the moment, standards and guidelines for insect sampling in forensic entomotoxicology are published by Amendt et al. [18] and Carvahlo et al. [10]. Unfortunately, the minimum number of specimens that should be sampled is not mentioned and the differentiation between insect activities and maturity is not specified. Only Tracqui et al. [6] describes sampling of minimal 30 specimens of the same stage and activity from each location. This is maybe not so important for real cases, but is certainly of interest for entomotoxicological experiments to ensure a good statistical analysis. In Fig. 1, a modified sampling protocol based on the ones from Amendt et al. [18] and Carvahlo et al. [10] is shown. The adaptations focus on the amount of specimens sampled, the sampling frequency and information concerning the insect activity. The sampling in experimental set-up can occur between different developmental stages [9,12] or at regular time intervals [11]. In the same interval time, different developmental stages or activities are observed, resulting in irreproducible quantitative results [20]. Therefore, sampling more frequently at onset of each developmental phase is recommended. We also suggest keeping larvae in the feeding phase alive during several hours before killing them, to be sure that drugs present in their digestive tract are completely excreted. These additional parameters are of most interest for research purposes and less for case report interpretation at this moment.

#### 2.2. Analytical procedures

Reporting reliable analytical data should be the backbone of forensic research. Entomotoxicological investigators need results that are valid, reproducible and comparable to draw scientifically correct conclusions from their experiments. This is of major importance as these results will be used by others for case-report interpretation. Therefore, international agreement concerning proper insect killing methods, storage, sample handling and analysis of samples are needed. In addition, the entomotoxicologist should pay attention to validation requirements.

When reviewing the literature, the conclusion rises that there is no recommended standard protocol for killing insects, for their

Case N° [	Case N° Localisation Date/time					
Victime	Victime Age (y) Sex Weight (Kg)					
Decompositio	Decomposition stage Estimated PMI					
Traces :	Traces : Syringes (Illegal) substances Prescription					
Cause of dea	ath					
Temperature	Temperature (°C) Climatic conditions					
Site description						
Sample N°	Location *	Species	Developmental stages ** N			
L	- B - M - O -		L D D D EP D A D			
L	_ B _ W _ O _		L D P D EP D A D			
L	□ B □ M □ O □		LO PO EPO AO			
L	- B - M - O -		LO PO EPO AO			
L	- B - M - O -					
L	B B M B O B		LO PO EPO AO			
L	□ B □ M □ O □		LO PO EPO AO			
L	- B - M - O -		LO PO EPO AO			
L	- B - M - O -		L D P D EP D A D			
L	B B M B O B		L D P D EP D A D			
L	B B M B O B		L D P D EP D A D			

\* L, liver; B, brain; M, muscle; O, others

\*\* L, larvae; P, pupae; EP, empty pupae; A, adults

**Fig. 1.** Protocol sheet of samples for entomotoxicological analysis. (\*) L, liver; B, brain; M, muscle; O, others. (\*\*) L, larvae; P, pupae; EP, empty pupae; A, adults.

storage, and for decontamination of the samples. These steps, however, can influence the result as drug stability or contamination from the food source will alter the analytical result and thus the final conclusion.

Specimens such as larvae or adults are killed either by freezing [6,11,12,14,26,34] or boiling [22]. Thereafter, they are stored in alcohol (70%) [22] or at -20 °C [6,11,12,14,26,34]. Pupae, on the other hand, are stored under dry conditions at 2-6 °C. At the moment, we would recommend storage under dry conditions at -20 °C for all samples to ensure drug stability and to diminish possible drug extraction from the matrix when storing in alcohol. However, future research must explore drug stability using different storage methods, but also under realistic forensic circumstances. Another important step before analysis is the decontamination of insects. Higher drug concentrations were observed for unwashed specimens than washed ones due to surface contamination [39]. Insect specimens should be washed using deionized water, a physiological NaCl solution (0.9%) [11] or using methanol [9]. However, no study has yet evaluated the efficiency of those washing steps.

Once the specimens are killed and decontaminated, the samples will be prepared for analysis. The sample preparation depends on the nature of the insect tissue and on the drug of interest. Because insect specimens are solid, they will first be macerated and homogenized [41,42], or digested using strong acids, bases or enzymes [22,43], or pulverized by grinding [11,14,35]. To our experience, use of a Precellys 48 homogenizer (Bertin Technologies, Montigny-Le-Bretonneux, France) leads to pulverization of a single specimen in a separated vial, avoiding cross contamination between different samples [20]. Once the samples are pulverized, drugs are extracted from the matrix. Optimal extraction techniques

lead to enhanced sensitivity of the method, but will also remove potential interfering matrix compounds, resulting in enhanced selectivity and a more reproducible method independent of variations in the sample matrix. Table 2 summarizes the detected substances, and their sample preparation/analysis observed in the literature. It is clear that entomotoxicologists still use classic extraction techniques such as protein precipitation, liquid-liquid extraction (LLE) or solid phase extraction (SPE). It is not the scope of this review to discuss the pros and cons of the different extraction techniques in detail. For this, we refer to other publications [44]. However, the aim of the final extraction technique is extracting the compounds of interest in a straightforward way, resulting in the required sensitivity, without matrix effects and with an extract that is compatible with the final analytical procedure.

Several analytical drug detection/quantification procedures have been used for the analysis of insect tissues (Table 2). The choice of technique depends on the physicochemical properties of the drugs of interest and of course on the required selectivity and sensitivity. In light of recent developments in the field of entomotoxicology, it is important to have very sensitive methods to ensure analysis of single specimens from different locations of the body for case report interpretation (see above). However, the observed techniques used in previous entomotoxicological research and case reports are not always sensitive enough and most of the time they require pools of specimens to detect any present drug. While in the past techniques such as MS/MS were not available in most laboratories, today these techniques can lead to a sensitivity which permits analysis of one single specimen [20,25,26] and will hopefully result in new insights in this field as well as better drug detection in forensic investigations. Another observation is that previously techniques that were not specific, e.g. immunoassays, were often applied to draw conclusions. Again use of MS or MS/MS methods, properly validated, can lead to more selective and specific drug detection leading to more reliable results.

Not only the choice and development of the applied technique is important to ensure a reliable drug detection and quantification; a validation according to international standards [45-47] should be obtained to ensure the validity of the results and thus the final conclusion/interpretation. It seems very plausible that a method should be 'fit for purpose'; however, when reviewing entomotoxicological publications, the methods tend to lack proper validation. Most validation guidelines include selectivity, sensitivity, calibration, accuracy, precision, and stability as fundamental validation parameters. Although some publications discuss precision and bias of the method, only a few studies have measured the extraction recovery, matrix effects or stability of the samples [11,20,26]. For methods using LC-MS, evaluation of matrix effects is obligatory as co-eluting compounds can influence the ionization of the compounds of interest, influencing sensitivity, and quantification of the target analyte. During development of the method, the extraction technique should be evaluated to guarantee reproducible results and adequate sensitivity. However, a problem for determination of the extraction recovery in insect tissues is that often spiking drugs to blank larvae or pupae does not resemble "real cases" as the drugs will not be incorporated in the tissue but just spiked 'onto' the matrix. Stability is another important parameter that should be evaluated more in detail during the whole entomological study ranging from the killing procedure, to the storage and processing of the sample to ensure proper results.

#### 2.3. Experimental set-up

The major drawback of the field of entomotoxicology at the moment is the lack of interpretation of detected drug concentrations. While this problem will not be resolved in the near future, entomotoxicologists try to establish a relationship between drug

#### Table 2

Comparison of sample preparation and analytical procedures for each toxic substance.

Instand based Animal series         Extraction           Animal based Animal	Toxic substances	Sample preparation		Analytical procedures	References
Think Drag(13.2)(13.2)Anitypy fun (nortypy fun)Rasic conditions (amount) by drood in account (nortypy fun)(2.5)(2.5)Anitypy fun (nortypy fun)Rasic conditions (amount) by drood in account (nortypy fun)(2.5)(2.5)Anitypy fun (nortypy fun)Rasic conditions (amount) by drood in account (nortypy fun)(2.5)(2.5)Anitypy fun (nortypy fun)Rasic conditions (by drood in account (nortypy fun)(2.5)(2.5)Anitypy fun (nortypy fun)Rasic conditions (by drood in account (nortypy fun)(2.5)(2.5)Anitypy fun (nortypy fun)(2.5)(2.5)(2.5)(2.5)Anitypy fun (nortypy fun)(2.5)(2.5)(2.5)(2.5)Anitypy fun (nortypy fun)(2.5)(2.5)(2.5)(2.5)Anitypy fun (nortypy fun)(2.5)(2.5)(2.5)(2.5)(2.5)Anitypy fun (nortypy fun)(2.5)(2.5)(2.5)(2.5)(2.5)Barbanchild(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)Barbanchild(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)Sodiam harbinoh(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)Barbanchild(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)Sodiam Arbinoharbinone(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5)(2.5) <t< th=""><th></th><th>Digestion</th><th>Extraction</th><th></th><th></th></t<>		Digestion	Extraction		
Drogs         Andidepresents         CC-MS         [12,30]           Andidepresents         Basic conditions (anononion hydrodol)         I.E.         III.C	Alcohol				
Amitry prime (norty pry line)         Basic conditions (amonoium hydroxide)         UE         GC-MS         [13]           Amitry prime (norty pry line)         Basic conditions (amonoium hydroxide)         IE         GC-MS         [24]           Comingramine         III         III         [26]         [27]         [26]           Comingramine         IIII         IIII         [26]         [27]         [26]           Trazodone         IIIII         IIIII         [26]         [27]         [26]         [27]           Trazodone         IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Ethanol			HS GC-FID	[13,32]
Amitrypytyline (nortryptyline)         LE         C.C.MS         [12]           Basic conditions (atmoniun hydroxide)         HRC-UV         [64]           LE         HRC-UV (larve)         [17]           *actide conditions (hydrochic acid)         LE         HRC-UV (larve)         [17]           Tranzdone         HRC-UV (larve)         [18]         [17]           Tranzdone         HRC-UV (larve)         [18]           Tranzdone         HRC-UV (larve)         [19]           Branzohne         LE         HRC-UV (larve)         [19]           Solium findome         LE         HRC-UV (larve)         [19]           Branzohne         LE         HRC-UV (larve)         [19]           Solium findome         LE         CC-MS         [11]           Solium indomanione         LE         CC-MS         [12]           Solium indomanione         Sig         III PC-UV         [3]           Solium phonionione         Sig         III PC-UV         [3]	Drugs				
Lie Conditions (amonium hydroide) *acidic conditions (amonium hydroide) *acidic conditions (hydrochloric acid) Lie C-MS [17] Lie Convignamine Lie Lie Lie C-MS [17] Lie Convignamine Lie Lie Lie Lie Lie Lie Lie Lie Lie Li					
Basic conditions (amountin hydroxie)         LE         C-MS         [17]           Acide conditions (hydrochoic acid)         LE         HRC-1V (Larva)         [28]           Consipamine         LE         C-MS         [17]           Tranohoe         LE         C-MS         [17]           Tranohoe         LE         C-MS         [39]           Franchoe         LE         C-MS         [3]           Solum mylobarbitora         Secolarbial         GC-MS         [17]           Solum balobarbitone         SE         CC-MS         [3]           Solum balobarbitone         SE         HEC-UV         [3]<	Amitryptyline (nortryptyline)				
+acidic conditions (hydrochoire acid) Factors (pupper) Fa					
$ \begin{array}{c c c c c c } \begin{tabular}{c c c c c } \begin{tabular}{c c c c c c } \begin{tabular}{c c c c c c c } \begin{tabular}{c c c c c c c c c c c c c c c c c c c $			LLE	GC-MS	[17]
ClampiannineHRC by CompanyPIRC by Company <td></td> <td></td> <td>LLE</td> <td>HPLC-UV (larvae)</td> <td>[28]</td>			LLE	HPLC-UV (larvae)	[28]
Transpanie     LLE     CC-MS     [12]       Transpanie     LLE     HTC-UV     [39]       Barbinization     CC-MS     [11]       Socium anylobarbinal     LLE     CC-MS     [12]       Socium anylobarbinal     LLE     CC-MS     [11]       Socium anylobarbina     SLE     CC-MS     [12]       Socium barbinal     SLE     CC-MS     [12]       Socium barbina     SLE     HTC-UV     [51]       Socium barbina     La     HTC-UV     [51]       Huritazzata     La     HTC-UV     [51]				GC-MS (pupae)	
Transdone Transpranine         LLE         HRC-UV         139           Transpranine         LLE         HRC-UV         139           Babbiturates	Clomipramine				[7]
Tringramine BabrituresLEHC-UV[39]Phenobarbital			LLE	GC-MS	[12]
Tringramine BabrituresLEHC-UV[39]Phenobarbital	Trazodone		LLE	HPLC-UV	[39]
Barbinates         GC-MS         HPLC           Phenobarbital         HPLC         71           Scobarbital         LEE         GC-MS         12]           Scobarbital         SLE         HPLC-UV         51]           Sodium anylobarbitone         SLE         HPLC-UV         51]           Sodium barbitone         SLE         HPLC-UV         51]           Sodium phenobarbitone         SLE         HPLC-UV         51]           Benzoalizepines (alprazolam, chazepam, ordizepam, paraepam, norazepam, nozaepam, nozaepam, nozaepam, nozaepam, nozaepam, nozaepam, nozaepam, nozaepam         L2         SPE           Ternazepan         HPLC         7]         1]         1]         1]           Miscellanceous         HPLC         7]         1]         1]           Miscellanceous         HPLC         7]         1]         1]           Miscellanceous         HPLC         1]         1]         1] </td <td></td> <td></td> <td></td> <td></td> <td></td>					
Pienobarbital     CGMS     [1]       Secobarbital     EE     CGMS     [2]       Sodium anylobarbitone     SLE     HPLC-1V     [5]       Sodium brabitone     SLE     HPLC-1V     [5]       Sodium brabarbitone     SLE     HPLC-1V     [5]       Benzodiazepines (altrazolam, diazepan, diazepan     [1]     [1]       Immazepan     Immazepan, Immazepan, Immazepan, Citazepan, diazepan, diazep					[]
secharbial         μEc         GC-MS         [2]           Sodium anylobarbitone         SEE         HPL-CV         [5]           Sodium harbitone         SEE         HPL-CV         [5]           Sodium barbitone         SEE         HPL-CV         [5]           Sodium barbitone         SEE         HPL-CV         [5]           Sodium barbitone         SEE         HPL-CV         [5]           Sodium phenobarbitone         SEE         HPL-CV         [5]           Sodium phenobarbitone         SEE         HPL-CV         [5]           Benzodiazepines         (1) Precipitation         [1]         [2]           Benzodiazepines (alprazolam, coazepam, orazepam, orazepam         [1]         [2]         [2]           Bronazepam         (1) Precipitation         [2]         [2]         [2]           Triazolam         HPL         [2]         [2]         [2]           Marcellaneous         HPL         [2]         [3]         [3]           Gozare         ER         HPL-CV         [3]         [3]           Gozare         ER         HPL-CV         [3]         [3]           Merbalize				GC-MS	[1]
SecobarbitalLEBGC-MS[12]Solium anylobarbinoneSEGC-MS[65]Solium barbinoneSEHPLC-UV[51]Solium barbinoneSEHPLC-UV[51]Solium barbinoneSEHPLC-UV[51]Solium barbinoneSEHPLC-UV[51]Solium barbinoneSEHPLC-UV[51]Solium barbinoneSEHPLC-UV[51]BenzoalizerpinesSEHPLC-UV[51]BenzoalizerpinesIPrecipitationIC-MS/MS[26]flumitrazerpan, lorazepan, norazepan,	i nenobul brui				
Socium aprilowitabilitionSECAMS(SiSodium phythabilitoneSEHPLC-UV(SiSodium phythabilitoneSEHPLC-UV(SiSodium phenobaritioneSEHPLC-UV(SiSodium phenobaritioneSEHPLC-UV(SiSodium phenobaritioneSEHPLC-UV(SiSodium phenobaritioneSEHPLC-UV(SiBenzodiazepines (alprazolan, clonazepam, nordiazepam, onzepam, on			LIF		
Sodium anylobarbinone     SE     HPLC-UV     [51]       Sodium barbinone     SE     HPLC-UV     [51]       Sodium thopentone     SE     HPLC-UV     [51]       Benzodiazepines     SE     HPLC-UV     [51]       Benzodiazepines     SE     HPLC-UV     [51]       Imutinzzepam, lonzepam, noxazepam, oxazepam, prazepam, triazolam)     ILP     (2) SPE     [51]       Benzodiazepines (Lonzepam, noxazepam, oxazepam, rinazolam)     ILE     GC-MS     [7]       Boxazepam     ILE     GC-MS     [7]       Tranzepam     HPLC     [7]     [7]       Benzoylecgonine     SE     HPLC-UV     [5]       Codien     RA     [6]     [2]       Metadone     Enzymatic conditions (β-glucornidae)     [2]     [2] <td< td=""><td>Secobarbital</td><td></td><td></td><td></td><td></td></td<>	Secobarbital				
Soldium barbinoneSLEHPLC-UV[51]Soldium phenobarbinoneSLEHPLC-UV[51]Soldium phenobarbinoneSLEHPLC-UV[51]Soldium phenobarbinoneSLEHPLC-UV[51]Benzodiazepines (upzaolan, clonazepam, diazepam, funitrazepam, ternazepam, nordiazepam, oxazepam, funitrazepam, ternazepam, nordiazepam, oxazepam, munitrazepam, ternazepam, nordiazepam, oxazepam, imagemam(1) PrecipitationLC-MS/MS[26]Benzodiazepines (upzaolan, clonazepam, diazepam, funitrazepam, ternazepam, ternazepam, tazolan(1) PrecipitationLC-MS/MS[40]Bromazepam(1) Storatube(1) Trenazepam[40][27][40][27]OxazepamLLEHPLC[27] <td></td> <td></td> <td></td> <td></td> <td></td>					
Softium phonbarbitoneSLEHPLC-UV[51]Softium phonbarbitoneSLEHPLC-UV[51]Softium phonbarbitoneSLEHPLC-UV[51]Softium phonbarbitoneSLEHPLC-UV[51]Benzodiazepines (dapzaolan, clonazepan, diazepan, nurizzepam, norazepan, nordiazepan, oxazepan, prazepam, tenazepam, triazolam)(1) PrecipitationLC-MS/MS[26]Precodiazepines (dapzaolan, clonazepan, oxazepan, nurizzepam, tenazepam, triazolam)(2) SPE (3) LLE[27]Benzodiazepines (dapzaolan, clonazepam, oxazepan, tenazepam(1) Precipitation[27]Diazepam(1) Precipitation[27][27]OxazepamHPLC[27][27]TenazepamHPLC[27][27]TenazepamHPLC[27][27]TenazepamHPLC[27][27]TenazepamHPLC[27][27]TenazepamHPLC[27][27]TenazepamHPLC[27][28]MiscellaneousHPLC[27][28]MiscellaneousHPLC[28][28]MiscellaneousSEEGC-MS[31]BenzoylecgonineLIERA, GC-MS[41]CocineSLEHPLC-UV[51]CodeineEnzymatic conditions (β-glucoronidae)ILEILEMiscellaneousEnzymatic conditions (β-glucoronidae)ILEILEMorbhineEnzymatic conditions (β-glucoronidae)ILEILC-MSMorbhineEnzymatic conditions (β-glucoronidae					
Solium phenobaritomeSLEHPLC-UV[51]Solium thoopentoneSLEHPLC-UV[51]Benzodiazepines (alprazolan, clonazepam, diazepam, oxazepam, nordiazepam, oxazepam, nordiazepam, oxazepam, nordiazepam, oxazepam, nordiazepam, oxazepam, triazolam)(1) PrecipitationLC-MS/MS[26]prazepam, tenazepam, triazolam)(2) SFE(3) UE(4) Toxitube(4) Toxitube(5) Toxitube					
Solium intogenomeSLEHPLC-UV[51]Benzodiazepines(1) PrecipitationLC-MS/MS[26]Brunzdiazepines (diprazolam, clonazepam, diazepam, triazolam)(2) SPE(3) LLE(3) LLEprazepam, trinazepam, triazolam)(2) SPE(3) LLE(4) Toxitube(40)Bromazepam, triazolam)(2) SPE(3) LLE(4) Toxitube(40)DiazepamLLEGC-MS(2) SPE(3) LLE(4) Toxitube(4) ToxitubeDiazepamLLEGC-MS(3) LLE(4) Toxitube(4) Toxitube(4) Toxitube(4) ToxitubeDiazepamLLEHPLC(7) Toxitube(4) Toxitube(5) Toxitube <t< td=""><td></td><td></td><td></td><td></td><td></td></t<>					
Benzodiazepines     LC-MS/MS     [26]       Benzodiazepines (dirzepam, nordiazepam, oxazepam, funitriazepam, iorazepam, nordiazepam, oxazepam, prazepam, temazepam, tirazolam)     (1) Precipitation     LC-MS/MS     [26]       (2) SPF (3) LLE     (2) SPF (3) LLE     (2) SPF (3) LLE     (4)       Bromazepam, temazepam, tirazolam)     (2) SPF (3) LLE     (4)       Bromazepam     (4) Toxitube     (40)       Diazepam     (1) Precipitation     (2) SPF (3) LLE       Bromazepam     (1) Precipitation     (40)       Diazepam     (1) Precipitation     (2) SPF (3) LLE       Natarepam     (1) Precipitation     (40)       Trazolam     (1) Precipitation     (2) SPF (3) LLE       Miscellaneous     (1) Precipitation     (2) SPF (1) SP       Miscellaneous     (1) Precipitation     (1) Precipitation       Benzoylecgonine     (1) LLE     (1) Precipitation       Cocaine     (1) LLE     (2) SPE (2) C-MS     (2) SPE (2) C-MS       Softiom amminohippurate     (2) SPE (2) Codinies     (2) SPE (2) C-MS     (2) SPE (2) C-MS       Morphine     Enzymatic conditions (DiThioThreito)     (2) C-MS     (4) LLE       Morphine     Enzymatic conditions (DiThioThreito)     (2) C-MS     (3) C-MS       Propoxyphene     SLE     (2) C-MS       Precotitazine     (2) C-					
funitrazepam, norażepam, oxazepam, prazepam, temazepam, temazepam, temazepam, temazepam, temazepam, temazepam, temazepam         (2) SPE           gromazepam         (2) SPE           Bromazepam         (2) SPE           Bromazepam         (2) SPE           Bromazepam         (2) SPE           Okazepam         (2) SPE           Diazepam         (2) SPE           Okazepam         (2) SPE           Triazolam         HPLC           Miscellancous         HPLC           Amphetamine         SPE           Groam         LE           Amphetamine         LE           Benzoylecgonine         LE           Cocaine         LE           Golding Amminohippurate         SPE           Opioids/opiates         SPE           Codeine         Enzymatic conditions (β-glucoronidase)           Merhadone         IE           Morphine         Enzymatic conditions (DiThioThreitol)           Propoxyphene         FRA           Phenothiazine         SE           Alumemazine         LE           Le         CC-MS           Le         CC-MS           Throidazine         LE           Metalone         CC-MS	Benzodiazepines				
C) SPE         (3) LLE         (4) Toxitube           Bromazepam         HPLC         (4)           Diazepam         LLE         GC-MS         [27]           Oxazepam         HPLC         (7)           Triazolam         HPLC         [39]           Triazolam         HPLC         [39]           Triazolam         HPLC         [39]           Miscellanceous         HPLC         [31]           Amphetamine         SPE         GC-MS         [31]           Benzoylecgonine         LLE         HPLC-UV         [51]           Gocaine         LLE         HPLC-UV         [51]           Sodium amminohippurate         SPE         GC-MS         [4]           Opioids/opiates         GC-MS         [2]         [2]           Godeine         Enzymatic conditions (β-glucoronidae)         LLE         LC-MS         [6]           Morphine         Enzymatic conditions (DTThioThreito)         RIA         [2]         [2]           Propoxyphene         Fnort         KIA         [2]         [2]           Propoxyphene         SLE         LC-MS         [2]         [2]           Propoxyphene         SLE         CASI         [3]     <	flunitrazepam, lorazepam, nordiazepam, oxazepam,		(1) Precipitation	LC–MS/MS	[26]
Bromazepam     HPL     W       Bromazepam     LE     HPLC     V       Diazepam     HPLC     V     71       Oxazepam     HPLC     V     39       Tranzolam     HPLC     V     39       Tranzolam     HPLC     V     39       Miscellancous     HPLC     V     39       Miscelancous     LE     HPLC-UV     13       Gocaine     LLE     HPLC-UV     13       Benzoylecgonine     LLE     RA, CC-MS     14       Cocaine     LLE     RA, CC-MS     14       Cocaine     LLE     CC-MS     12       Sodium amminohipipurate     SPE     CC-MS     13       Opioids/opiates     SEE     CC-MS     14       Codeine     RIA, CC-MS     14       Methadone     Enzymatic conditions (β-glucoronidaso)     LE     C-MS     14       Morphine     Enzymatic conditions (DThioThreiot)     LE     LC-MS     66       Morphine     Enzymatic conditions (DThioThreiot)     LE     LC-MS     66       Methadone     LC-US     KIA     14       Morphine     LC-MS     66     12       Propoxyphene     RIA     12     14       Nimemazin	prazepani, temazepani, triazolani)		(2) SPE		
Bromazepam     HPLC     (40)       Diazepam     LE     GC-MS     (27)       Diazepam     HPLC-UV     (7)       Temazepam     LE     HPLC-UV     [39]       Triazolm     HPLC     [7]       Miscellaneous     F     (7)       Miscellaneous     SPE     GC-MS     [3]       Miscellaneous     LE     HPLC-UV     [5]       Miscellaneous     LE     HPLC-UV     [5]       Monazepam     LE     HPLC-UV     [5]       Benzoylecgonine     LE     HPLC-UV     [5]       Cocane     RIA, CC-MS     [4]       Sodium amminohippurate     SPE     GC-MS     [4]       Opicids/opiates     Enzymatic conditions (β-glucoronidase)     LE     HPLC-UV     [5]       Morphine     Enzymatic conditions (β-glucoronidase)     LE     LC-MS     [6]       Morphine     Enzymatic conditions (β-glucoronidase)     LE     LC-MS     [6]       Morphine     Enzymatic conditions (β-glucoronidase)     LE     LC-MS     [2]       Morphine     Enzymatic conditions (β-glucoronidase)     LE     LC-MS     [3]       Morphine     Enzymatic conditions (β-glucoronidase)     LE     LC-MS     [4]       Misethadone     LC-MS			. ,		
DiazepamLLECC-MS[27]OxazepamHPLCHPLC[7]TemazepamLLEHPLC-UV[3]TinazolamLLEHPLC-UV[3]MiscellaneousLLEHPLC-UV[5]BenzoylecgonineLLERIA, CC-MS[1]GocaineLLERIA, CC-MS[4]CocaineLLERIA, CC-MS[4]CocaineLLERIA, CC-MS[4]CocaineLLERIA, CC-MS[4]CocaineLLERIA, CC-MS[4]CocaineLLERIA, CC-MS[4]CocaineLLERIA, CC-MS[4]CocaineLLERIA, CC-MS[4]CodelineEnzymatic conditions (β-glucoronidase)LLE[C-MS[6]MethadoneLLELC-MS[6][1]MorphineEnzymatic conditions (DiThioThreito)LLE[C-MS[6]MorphineEnzymatic conditions (DiThioThreito)LLE[C-MS[6]MiscaineEnzymatic conditions (DiThioThreito)LLE[2][3]PropoxypheneEnzymatic conditions (DiThioThreito)LLE[4][4]PhenothiazineEnzymatic conditions (DiThioThreito)[2][4][3]MiscaineEnzymatic conditions (DiThioThreito)[2][4][3]HendotiazineEnzymatic conditions (DiThioThreito)[4][4][4]LevomepromazineEnzymatic conditions (Nitric acid)[4][4][4]Hendotiaz	Bromazenam		(I) Iokitube	НЫС	[40]
NazepamHPLCHPLC(7)TemazepamHPLC139TriazolamHPLC7.8MiscellaneousKCC-MS131MiscellaneousLEGC-MS131BenzoylecgonineLERA, CC-MS41CocaineLERA, CC-MS41CocaineLERA, CC-MS41CocaineLERA, CC-MS41CocaineLECC-MS121Sodium amminohippurateSEHPLC-UV51Opioids/opiatesLECC-MS121CodeineEnzymatic conditions (β-glucoronidas)LEC-MS66MethadoneEnzymatic conditions (DThioThreito)RA191191MorphineEnzymatic conditions (DThioThreito)RA141,53194,93PropoxypheneFraposity Conditions (DThioThreito)LEC-MS161PropoxypheneFraposity Conditions (Nitric acid)LEC-MS194,153PropoxypheneLECC-MS114,53144,53PropoxypheneLECC-MS114,153114,153PropoxypheneLECC-MS114,153114,153PropoxypheneLECC-MS114,153114,153PropoxypheneLECC-MS114,153114,153PropoxypheneLECC-MS114,153114,153LevonepromazineLECC-MS114,153114,153HaltionAcidic conditions (Nitric acid)LECC-MS114,153			LLE		
TemazepamLLEHPLC-UV[39]TriazolamHPLC-UV[37]MiscellaneousSPEC-MS[13]AmphetamineSPEC-MS[13]BenzoylecgonineLLEHPLC-UV[51]CocaineLLERIA, CC-MS[4]CocaineLLERIA, CC-MS[4]CocaineSPECC-MS[12]Journa maninohipurateSPECC-MS[2]Opioids/opiatesSPECC-MS[2]CodeineEnzymatic conditions (β-glucoronidas)LLELC-MS[1]MethadoneLLELC-MS/MS[20][2]MorphineEnzymatic conditions (β-glucoronidas)LLELC-MS/MS[2]MorphineEnzymatic conditions (β-glucoronidas)LLELC-MS[2]MethadoneLLELC-MS/MS[2][2]MorphineEnzymatic conditions (β-glucoronidas)LLELC-MS[2]MorphineEnzymatic conditions (β-glucoronidas)LLE[2][3]PropoxypheneRLA[3][4][4][4]PhenothiazineLC-MS[4][4][4]LevomepromazineLLLE[4][4][4][4]LevomepromazineLLELLE[2][4][4][4]LevomepromazineLLELLE[2][4][4][4]LevomepromazineLLE[4][4][4][4][4]LevomepromazineLLE[4	-		222		
Triazolam     HPLC     [7,8]       Miscellaneous     SPE     CC-MS     [1]       Amphetamine     SPE     CC-MS     [4]       Benzoylecgonine     LLE     RIA, CC-MS     [4]       Cocaine     LLE     RIA, CC-MS     [4]       Cocaine     LLE     RIA, CC-MS     [4]       Sodium amminohippurate     SPE     CC-MS     [24]       Opioids/opiates     SPE     CC-MS     [1]       Coderine     LLE     LC-MS     [1]       Methadone     LLE     LC-MS/MS     [26]       Morphine     Enzymatic conditions (β-glucoronidase)     LLE     LC-MS     [66]       Morphine     Enzymatic conditions (DiThioThreitol)     RIA     [23]     [66]       Propoxyphene     Free prizeitation     EIA     [24]     [24]       Propoxyphene     SLE     CC-NS     [41]       Phonothiazine     LLE     CC-MS     [41]       ILe     CC-MS     [41]     [41]       Adimenszine     HPLC     [42]     [41]       Levomepromazine     LLE     LLE     [41]       Lead, barium, antimony     Acidic conditions (Nitric acid)     LLE     [40]       Hetcl     CC-MS     [12]       Lead, bar			LIF		
Miscellaneous         SPE         CC-MS         [13]           Amphetamine         SPE         CC-MS         [13]           Benzoylecgonine         LLE         HPLC-UV         [51]           Benzoylecgonine         LLE         RIA, CC-MS         [4]           Cocaine         LLE         RIA, CC-MS         [12]           Sodium amminohippurate         SPE         CC-MS         [24]           Sodium amminohippurate         SPE         CC-MS         [24]           Opioids/opiates         LLE         LC-MS         [51]           Opioids/opiates         LLE         LC-MS         [66]           Methadone         LLE         LC-MS         [66]           Morphine         Enzymatic conditions (β-glucoronidase)         LLE         LC-MS         [66]           Morphine         Enzymatic conditions (DiThioThreitol)         RIA         [9]         [14]           Propoxyphene         EL         LC-MS         [66]         [24]           Propoxyphene         SE         CC-MS         [20]           Phenothiazine         LLE         LLE         [24]         [23]           Rtaine         LLE         CC-MS         [21]         [24]         [24] <td></td> <td></td> <td>ELL</td> <td></td> <td></td>			ELL		
AmphetamineSPE LE LE HPLC-UV[13] [51]BenzoylecgonineLE LEHPLC-UV[51]CocaineLE LERA, GC-MS[4]CocaineLERA, GC-MS[4]Sodium amminohippurateSPE Sodium amminohippurateGC-MS[24]Sodium amminohippurateSEHPLC-UV[51]Opicids/opiatesEnzymatic conditions (β-glucoronidase)LELC-MS[66]MethadoneEnzymatic conditions (β-glucoronidase)LELC-MS/MS[66]MorphineEnzymatic conditions (DThioThreitol)RA[9]MorphineEnzymatic conditions (DThioThreitol)LELC-MS[66]PropoxypheneFor SoliaLEC-CAPD[61]PropoxypheneFor SoliaLECC-MS[7]PhenothiazineLECC-MS[1][1]AlimemazineLECC-MS[1][1]LevomepromazineLECC-MS[1][1]IdaditionAcidic conditions (Nitric acid)LECC-MS[1]PhetotiazineLECC-MS[1][1]LevomepromazineLECC-MS[1][1]LevomepromazineLECC-MS[2][2]PhetotiazineLECC-MS[2][2]MetalsLECC-MS[2][2]MetalsCC-MS[2][2][2]MetalsLECC-MS[2][2]MetalsLECC-MS[				TH LC	[7,0]
$\begin{array}{cccc} & \label{eq:basic} & \$			SDE	CC-MS	[13]
BenzoylecgonineLLERIA, CC-MS(4)CocaineLLERIA, CC-MS(4)CocaineLLERIA, CC-MS(4)LLERC-MS(12)SpecCC-MS(24)SpecCC-MS(24)Opioids/opiatesLLELC-WS(51)CodeineLLELC-MS(66)MethadoneLLELC-MS/MS(20)MorphineEnzymatic conditions (β-glucoronidase)LLELC-MS/MS(20)MorphineEnzymatic conditions (DiThioThreitol)RIA(9)(20)MorphineEnzymatic conditions (DiThioThreitol)RIA(23)(24)MorphineEnzymatic conditions (DiThioThreitol)RIA(24)(24)MorphineEnzymatic conditions (DiThioThreitol)RIA(24)(24)MorphineEnzymatic conditions (DiThioThreitol)RIA(24)(24)MorphineEnzymatic conditions (DiThioThreitol)RIA(24)(24)MorphineEnzymatic conditions (DiThioThreitol)RIA(24)(24)PreposypheneSESECC-NPD(64)(24)PhenothiazineLERIA(21)(24)(24)AlimemazineLLECC-MS(12)(24)(24)Levon promazineLLECC-MS(12)(24)(24)MetalsLLECC-MS(22)(24)(24)MetalsLLECC-MS(22)(24)(24)MetalsLLE <td< td=""><td>Amplictamine</td><td></td><td></td><td></td><td></td></td<>	Amplictamine				
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Malathion         SPE         GC-ECD         [62]           LLE         GC-MS         [70,71]		Acıdic conditions (Nitric acid)		ICP-MS	[22,68]
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	materion				
	Parathion		LLE	LC-DAD	[70,71]

(CL chemiluminescence, DAD diode array detector, ECD electron capture detection, EIA enzyme immunoassay, FID flame ionization detector, FPIA fluorescence polarisation immunoassay, GC gas chromatography, HS headspace, HPLC High performance liquid chromatography, ICP inductively coupled plasma, LC liquid chromatography, LLE liquidliquid extraction, MS mass spectrometry, NPD nitrogen-phosphorus detection, RIA radioimmunoassay, SLE supported liquid extraction, SPE solid phase extraction, UV ultraviolet). <sup>\*</sup>Data coming from Tracqui et al. [4] are not mentioned because analytical methods (GC-MS and LC-MS) were not specified for each compound. concentrations in insects and their substrates via experiments. Interpretation of these experiments are not only complicated by differences in insect species, developmental stages, activities and analytical problems as discussed above, but is also due to different experimental conditions.

Artificial foodstuff [48–51] or post-mortem spiked animal organs [9,11,22,23,25,26,32,39,42,52] are mostly used to rear insects. These substrates are widely used, as they are easy to prepare and have a low cost. Unfortunately, problems during preparation and storage such as insufficient drug homogeneity, nutritional requirements or water can occur [48]. These parameters should be monitored during the experiments and substrates with the propensity to lose water should not be frozen prior to use but stored at 4 °C [48]. One of the most important drawbacks of this type of substrate is the fact that the spiked drugs are not metabolized by a living system. This is important for interpretation as in real case scenario's the drug availability will be altered and the produced metabolites will also be consumed by the insect [50,52].

The way to get around the major drawback of post-mortem spiked substrates is the use of live-animal models, in which drugs are given ante-mortem orally or via injection. Use of live-animals gives a realistic view because the drug is metabolized. However, it is not easy to set-up statistical valid tests. In addition, experiments must be approved by an animal ethics committee [33] and the delay of acceptation can be very long. The animal is mostly sacrificed 30 min [10] after drug administration and the whole animal [9,53–55], a single organ (mostly the liver) or the muscle [14,32,56-62] are thereafter exposed to insect larvae under laboratory conditions. If the whole animal is used to rear larvae, the process is more realistic. but interpretation of the experiment is more complex due to the movement of larvae on the cadaver. Because larvae wander on the cadaver and the accurate location of feeding is not known the link between the drug concentration in actual feeding place and the insect is not possible. In addition, post-mortem redistribution may increase interpretation problems. When a single organ or muscle tissue is used, results obtained will vary according to the different type of tissue [55]. The choice of animal species will also have implications on the degree of information obtained from the antemortem experiment. Most of authors use small animals such as domestic rabbits [9,27,53,54,56-61] or rats [62], if possible with the same weight and sex [10] to avoid variability from these parameters. Because these animal models have limited use as drug distribution and metabolism will be different compared to humans [63], some researchers use pigs [32,35]. However we must keep in mind that an animal model never guarantees a simulation of human drug overdose due to pharmacokinetic differences [52]. Moreover, certainly if larger animals are used, the number of replicates can be very low. Three replicates is the minimum for statistical analysis [10,32,62].

Before entomo(toxico)logical experimental results can be used for case-report interpretation, possible differences in protocol such as method of injection/oral overdose, time before analysis (postmortem redistribution), drug stability, bacterial metabolism must be studied to understand their influence on the obtained results [6,63]. In addition, more research concerning the comparison of spiked foodstuff versus live-animal models should be conducted and therefore experimental protocols should be harmonized as much as possible [48].

#### 2.4. Interpretation in forensic cases

At the moment, there is general agreement in the forensic toxicology community that drug concentrations in insects cannot be interpreted. While entomotoxicologists try to find a relationship between drug concentrations in the substrate and the reared insects using experiments with spiked artificial foodstuff or animal models, collection of insect specimens from human post-mortem case reports are the most interesting. These insects are reared in realistic circumstances. However, there are several drawbacks. First of all, it is difficult and time-consuming to obtain a human case report database for research; e.g. Tracqui et al. [6] analyzed 29 necropsies in 15 years. Some researchers use human tissues [4,6–8,13,17,24,34] such as muscle [9,64] or liver [12,14] to rear larvae experimentally, however, this is ethically questionable. Moreover, determination of drug concentrations in decomposing tissues is not obvious and even impossible in skeletonised remains. Even though drug concentrations are determined, post-mortem drug redistribution and drug stability are not always well known, and complicate interpretation [6]. Unless enormous advances occur concerning the knowledge of factors influencing drug concentrations in insects, it is almost of no interest to have a toxicological investigation linking drug concentration to possible effect in practical casework [6]. However, for skeletonised remains, insects can be the last resort for a toxicological finding that can give information concerning possible drug use before death. Moreover, drug detection in insects can help the entomologist to determine the PMI, as insect development will be changed by drugs present in the corpse.

#### 3. Conclusion

In the latest years more research has been conducted in the field of entomotoxicology, trying to find a relationship between drug concentrations in the substrate and insects reared on that substrate, and to increase the knowledge of insect development. A literature review demonstrates that drug detection and analytical quantification in insects is not a problem. Although the recent progress in this science, interpretation of entomotoxicological results from human cases is not yet established nor expected. The aim to establish a link between substrate and insect, and thus toxicological interpretation, is perhaps a utopia due to several unknown and complicating factors. However, when reviewing the current studies, one can not deny the fact that maybe this current limitation is partially due to insufficient knowledge of insect development and activity, of proper use and validation of analytical procedures and lack of a general consensus concerning experimental set-up and sampling. While some problems can be resolved by a standardization of methods, procedures, and validation protocols, others need further research. Thus, research in the near future should focus on physiological process during the feeding stage, drug metabolism and accumulation-excretion mechanisms in insects, but also on drug redistribution and post-mortem drug stability. After studying these parameters, a long-term comparative study could be applied on insects reared on human cases. At the moment, detection of drug in insects is certainly possible. While it can not yet be used for toxicological interpretation in most cases, when only skeletonised remains are left, puparia can be the only hope for a toxicologist to have some information concerning drug use prior to death. In addition, detection of drugs in insect tissues by the analytical toxicologist can aid the determination of the PMI by the entomologist. Hopefully, the importance of this science will increase when validated experimental protocols and methods will be systematically applied in each entomotoxicological analysis.

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