

Bioanalysis of antihistamines for clinical or forensic purposes

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Abstract

Antihistamines are a class of drugs that inhibits the action of histamine and are used to alleviate symptoms associated with allergic reactions, but some of them can cause side effects, the most unpleasant and dangerous of which are the sedative effects that may hinder important psychological functions and impair skilled performance. These side effects could decrease safety in certain common and critical tasks, such as driving or operating machinery leading to accidents. Antihistamines can also cause intoxications, sometimes lethal, especially when co-administered with alcohol or other sedative drugs. Thus, the development of analytical methods for their determination in biological fluids is considered to be useful for the investigation of clinical and forensic cases. These methodologies could also be used for pharmacokinetic studies. Several liquid and a few gas chromatographic methods have been

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/bmc.3727

developed for the determination of antihistamines in biological matrices after proper pretreatment procedures. This article reviews the published analytical methodologies that were gathered through the search in PubMed database and the recent developments on isolation or determination of antihistamines in biological materials. Current trends and future perspectives on bioanalysis of antihistamines are also discussed.

Keywords: antihistamines, analysis, chromatography, biological fluids, review

1. Introduction

Antihistamines are a class of drugs that inhibits the action of histamine either by blocking its binding to H₁ histamine receptors, or by inhibiting the enzymatic activity of histidine decarboxylase, an enzyme that catalyzes the transformation of histidine into histamine. Antihistamines are chemically classified into six distinctive chemical groups; alkylamines, ethanolamines, ethylenediamines, piperazines, piperidines and phenothiazines (Table 1) (Jauregui Presa, 1999). The chemical structure of the most commonly used antihistamines are shown in Figure 1.

As classical H₁ receptor antagonists, they can also be classified in three groups that include the first-, second- and third-generation antihistamines (Church and Church, 2013). The first H₁ receptor antagonist, piperoxan (Figure 1), was suggested by Ernest Fourneau and Daniel Bovet in 1933 in their efforts to develop an animal model for anaphylaxis at the Pasteur Institute in Paris (Fourneau and Bovet, 1933). In the following decades, the first-generation of H₁-antihistamines, such as chlorpheniramine, cyclizine, hydroxyzine, meclizine, doxylamine, diphenhydramine, and promethazine were developed. These drugs are still used widely due to their efficacy and their low cost. They show various side-effects (e.g. drowsiness, anxiety, nausea, blurred vision, uncoordination and tremor) due to their lack of selectivity for the H₁ receptor and their easy crossing of the blood-brain barrier (BBB) (Harvey *et al.*, 2006; Church and Church, 2013). Second-generation antihistamines, like

cetirizine, loratadine, rupatadine, mizolastine, and ebastine, are much more selective for peripheral H₁ receptors. They are less selective for the central nervous system H₁ and cholinergic receptors and do not cross easily BBB, reducing this way their sedative side effects, while providing effective relief of allergic conditions (Church and Church, 2013). Third-generation antihistamines are active enantiomers (levocetirizine) or metabolic products (desloratadine, fexofenadine) of second-generation drugs intended to have increased efficacy and fewer adverse reactions (e.g. sedation and psychomotor effects) than the first generation drugs, and are recommended more often by physicians to alleviate the symptoms associated with allergic reactions (Harvey *et al.*, 2006). Indeed, antihistamines do not reverse these types of reactions, as they do not affect the underlying causes of allergy, but they can provide considerable symptom relief. Allergic reactions that can be alleviated from the use of antihistamines include hay fever, allergic rhinitis, atopic eczema, urticaria, allergic conjunctivitis, reactions to insect stings or mild and moderate reactions to food allergens (Harvey *et al.*, 2006; Belsito, 2010; Church and Church, 2013). Antihistamines are also used in therapeutics for treating insomnia and motion sickness. Moreover, they present anticholinergic, antiemetic, antiserotonergic, adrenergic or antiadrenergic, and local anaesthetic actions (Church and Church, 2013).

Antihistamines, and especially the first-generation ones, cause side effects, such as drowsiness, dizziness, nausea, vomiting and confusion. The most unpleasant and dangerous adverse effects for the majority of patients are the sedative effects (Harvey *et al.*, 2006; Church and Church, 2013). These effects hinder important psychological functions and impair skilled performance. This has as a result decreased safety in certain common and critical tasks, such as driving or operating machinery (Nolen, 1997; Jáuregui Presa, 1999; Jáuregui *et al.*, 2006) leading to severe accidents (Barbanoj, 2006). These drugs can also

cause intoxications (Pragst *et al.*, 2006), fatal or not, especially when co-administered with alcohol or other sedative drugs (Zimatkin and Anichtchik, 1999).

A variety of significant interactions may also appear when antihistamines are co-administered with other therapeutic drugs. For example, monoamine oxidase inhibitors prolong and intensify the anticholinergic actions of the antihistamines (Harvey *et al.*, 2006). Moreover, antihistamines may potentiate the sedative activity of barbiturates, alcohol, narcotic analgesics and other sedatives (Zimatkin and Anichtchik, 1999). In recent years it has been discovered that two of the second generation antihistamines (terfenadine and astemizole) may produce life-threatening side effects, like arrhythmia, when co-administered with drugs, like ketoconazole and erythromycin, that inhibit their metabolism (Yap and Camm, 2002; Dávila *et al.*, 2006; Jáuregui *et al.*, 2006).

The therapeutic index of H₁ receptor antagonists is relatively high. Despite that, acute toxicity, especially among children, is almost frequent, while chronic toxicity is rare. Thus, the development of analytical methods for the determination of antihistamines in biological specimens is imperative for the investigation of relative cases. These methods can also be applied for the determination of antihistamines for other clinical or forensic purposes, e.g. to diagnose poisonings (Baker *et al.*, 2003; Pragst *et al.*, 2006), or when suspicions for committing suicide arise (Eckes *et al.*, 2013).

The aim of this article is to review the published analytical methodologies that were gathered through the search in PubMed database and the recent developments in isolation and determination of antihistamines in biological materials. Different analytical techniques have been proposed for pharmacokinetic studies or for the investigation of clinical and forensic cases. These techniques include thin layer chromatography (TLC) (Table 3), gas chromatography (GC) with different detectors like nitrogen phosphorus (NPD), flame ionization (FID), and electron capture (ECD). GC coupled with mass spectrometry (MS)

(Table 4), high-performance liquid chromatography (HPLC) with ultraviolet detection (UV), fluorescence detection (FLD), photodiode array (DAD), LC-MS, and LC-tandem mass spectrometry (MS/MS) (Table 5) have also been proposed. For this review, a systematic approach was used after a detailed search in PubMed using the terms “antihistamines”, “gas chromatography”, “liquid chromatography”, “gc-ms”, “lc-ms”, “tlc” and the name of each antihistamine as keywords. Book chapters relative to the pharmacology of antihistamines were also included. Current trends and future perspectives on bioanalysis of antihistamines are also discussed. To the best of our knowledge, this is the first review on the above subject.

2. Analytical methods for the determination of antihistamines in biological specimens

The most common biological fluids used for the analysis of antihistamines and their metabolites for clinical and forensic toxicology purposes are whole blood, plasma/serum and urine. Blood, plasma and serum can often be interchanged in many methods, while when urine samples are analyzed a hydrolysis step of antihistamines' glucuronides is required before the extraction procedure (Dell and Fiedler, 1977; Maurer and Pfleger, 1988). Immunoassay methods performed for the determination of certain antihistamines are normally applied in plasma or serum (Tables 2, 3 and 5). Plasma is generally a relatively clean biological matrix and was used in the majority of studies for the determination of antihistamines, while urine can be used for screening purposes.

To our knowledge, the already published studies concern the determination of antihistamines in blood (Tables 3-5), plasma (Tables 2-5), serum (Tables 2-5), urine (Tables 3-5), oral fluid (Table 5), tears (Table 5), breast milk (Table 2-5), amniotic fluid or fetal tracheal fluid (Table 4), organ tissues (Tables 3-5) and hair (Table 4). Most of these methods were applied in ante-mortem human specimens (Tables 3-5).

Only a few articles were found concerning the post-mortem forensic detection and determination of antihistamines. In a fatal suicide case, hydroxyzine was determined in

blood, urine, bile and stomach content of post-mortem human samples using a GC-NPD method at 4.18, 1.43, 23.24 and 78.23 $\mu\text{g/mL}$, respectively (Kintz *et al.*, 1990). An LC-MS/MS method was developed for the determination of 18 antihistamines in blood samples of drivers who died in car accidents. In an example of a positive case terfenadine and its metabolite fexofenadine were determined at concentrations 0.3 and 20 $\mu\text{g/mL}$, respectively (Gergov *et al.*, 2001). Pragst *et al.* (2006) reported 68 non-fatal and 55 fatal poisoning cases with diphenhydramine alone or in combination with other drugs. The toxicological analysis was performed by HPLC-DAD and diphenhydramine was detected along with its metabolites, alcohol and/or other drugs like, chloroquine, midazolam, thiopental, pentobarbital and codeine. Diphenhydramine blood concentrations ranged from 0.5 to 8.9 $\mu\text{g/mL}$ in the non-fatal cases and from 0.3 to 119 $\mu\text{g/mL}$ in the fatal cases (Pragst *et al.*, 2006). In another fatal case, chlorpheniramine along with dihydrocodeine was determined in a post-mortem blood sample at a lethal concentration, while the dihydrocodeine concentration was within the therapeutic range. Thus, they concluded that the cause of death was due to chlorpheniramine poisoning (Kinoshita *et al.*, 2012). Twenty suicidal intoxications with diphenhydramine were also investigated by Eckes *et al.* (2013) by analyzing blood and gastric content samples. In most of the cases, diphenhydramine was detected along with other drugs, like amitriptyline, chloroquine, clobutinol, clozapine, doxylamine, ibuprofen, metoprolol, phenobarbital, propranolol, trimipramine or zolpidem, and alcohol. In four cases, diphenhydramine was not considered likely to be the cause of death, while in 11 cases the antihistamine was a contributing agent to death, while five cases were ruled to be monointoxications (Eckes *et al.*, 2013).

In several studies, the determination of antihistamines was performed in experimental animal specimens for pharmacokinetic purposes. The animals used in these studies were dogs, rats, sheep, rabbits, mice and guinea pigs (Tables 3-5).

Details of all the above studies on the determination of antihistamines in biological specimens are given in Tables 2-5.

2.1. Non-chromatographic screening techniques

The available non-chromatographic techniques [radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA)] for the screening of biological fluids (blood/plasma and urine) for the presence of antihistamines are limited (Table 2). Only one ELISA (enzyme-linked immunosorbent assay) (Arnera *et al.*, 1990) and six radioimmunoassay (RIA) (Findlay *et al.*, 1984a; Midha *et al.*, 1984; Radwanski *et al.*, 1987; Hilbert *et al.*, 1987; Hilbert *et al.*, 1988) methods were found concerning the screening and/or the semi-quantification of this category of drugs during the last 30 years. They achieved significant low limits of detection (LODs) and quantification (LOQs). These methods are used to provide an initial screening of biological fluids for the presence of antihistamines and they are usually simple, sensitive, and readily applicable to routine analysis. Most of them were applied during pharmacokinetic studies. They generally require neither extraction of the drug nor expensive instrumentation. To our knowledge, there is no published non-chromatographic screening methodology for the last 30 years concerning the determination of antihistamines in ante- or post-mortem biological specimens.

Findlay *et al.* (1984) developed a sensitive and specific RIA method for the determination of triprolidine in human plasma up to 24 h after its oral administration to healthy subjects at therapeutic doses. The results were confirmed with a semi-quantitative TLC method and the excellent correlation of the results of the two methods confirmed the validity of the RIA procedure for triprolidine analysis in human plasma (Findlay *et al.*, 1984a). In the same year, Midha *et al.* described a simple, precise and sensitive RIA method for the determination of plasma concentrations of chlorpheniramine, and/or its N-dealkylated metabolites up to 96 h after the oral administration of single low doses to healthy volunteers. In this method, 17%

cross-reactivity of the active N-demethyl metabolite was also observed. This RIA method was also compared to an HPLC method and showed a lower LOD (Midha *et al.*, 1984).

Another RIA method was developed by Radwanski *et al.* (1987) for the determination of loratadine and applied to a multiple-dose pharmacokinetic study in human volunteers (Radwanski *et al.*, 1987).

Later, a sensitive and reproducible ELISA method was developed for the measurement of dimethindene in human serum. The method achieved a minimum detectable concentration of 0.1 ng/mL and allowed the determination of the main pharmacokinetic parameters of this drug (Arnera *et al.*, 1990).

2.2.Pretreatment procedures

Prior to chromatographic analysis, the separation of the analytes of interest from biological specimens, through isolation procedures, is usually required. The published chromatographic methods for the determination of antihistamines include mainly liquid-liquid extraction (LLE) and solid-phase extraction (SPE) procedures or direct injection into the chromatographic system (Tables 2-5). Protein precipitation of the samples with methanol (Ming *et al.*, 2007), acetonitrile (Naidong *et al.*, 2002; Gupta *et al.*, 2005; Vlase *et al.*, 2007), HClO₄ 25% (Moncrieff, 1992) or trichloroacetic acid 5-6% (Lange *et al.*, 1968; Shakya *et al.*, 2009; Wichitnithad *et al.*, 2015) have been used prior to LC analysis.

Solans *et al.* (2007) subjected plasma samples to enzymatic hydrolysis with β -glucuronidase/arylsulfatase from *Helix pomatia* in order to quantify free and glucuronoconjugated 3-hydroxydesloratadine, a common metabolite of desloratadine and rupatadine. After alkalization of hydrolyzed plasma samples, these two drugs and their common metabolite were extracted with methyl-*tert*-butyl ether and separated with an LC-MS/MS method (Solans *et al.*, 2007).

2.2.1. Liquid-liquid extraction (LLE)

LLE is the most often used extraction method for antihistamines from biological samples, e.g. blood, plasma, serum, urine and saliva, prior to their chromatographic analysis. It has been performed mainly under alkaline conditions (pH 8-11) for specific antihistamines like brompheniramine, chlorpheniramine, hydroxyzine, diphenhydramine, dimethindene, cinnarizine, meclizine, loratadine, desloratadine, rupatadine, oxatomide, and triprolidine, using carbonate, phosphate or borate buffers, KOH or NaOH, NH₃ 25% at various concentrations, sodium carbonate, sodium bicarbonate, TRIS buffer, or ammonium chloride buffer/ammonia 25% (Tables 3-5). Some antihistamines (chlorpheniramine, cinnarizine, cetirizine, dimethindene, ebastine, diphenhydramine, hydroxyzine, loratadine) have been also extracted under acidic conditions (pH 3-6) using concentrated hydrochloric acid, sulfuric acid, or citrate, phosphate, or acetate buffers (Tables 3-5). Meclizine, hydroxyzine, loratadine, and desloratadine have been extracted at physiological pH (Tables 4 and 5).

After the initial alkalization or acidification of the plasma samples, and the hydrolysis or dilution of urine samples, the examined antihistamines were extracted with different organic solvents, as it is shown in Tables 2-5, achieving recoveries higher than 60%. Back-extraction of the analytes into an acidic aqueous phase has been also used to clean-up extracts prior to GC (Table 4) and LC (Table 5) analysis, without any recovery losses.

2.2.2. Solid-phase extraction (SPE)

SPE has been also used to isolate antihistamines and their metabolites from biological matrices (blood, plasma, urine and oral fluid) (Tables 4 and 5). This extraction method usually provides a combination of satisfactory recovery and cleaner extracts compared to LLE (Zwir-Ferenc and Biziuk, 2006).

SPE columns of different polarities have been used for the isolation of certain antihistamines like diphenhydramine, diphenylmethane antihistamines and their analogues, loratadine, desloratadine, triprolidine and cetirizine. The elution of the analytes was achieved by using

polar solvents or their mixtures. In all cases, no interfering peaks related to matrix were found and high recovery percentages were achieved, by both LC and GC methods (Tables 4 and 5). Hindmarsh *et al.* (1977) prepared and used a non-ionic resin column packed with Rephasorb 16-30 Mesh for the isolation of diphenhydramine from urine achieving almost quantitative extraction from the biological matrix (Hindmarsh *et al.*, 1977), while amberlite PAD-2 resin in a glass pipette as solid phase was used for the isolation of cetirizine from urine (Choi *et al.*, 2000). 96-well C₁₈ disk plates with polymer sorbent were alternatively used in LC applications for the isolation of loratadine, desloratadine and cetirizine (Yang *et al.*, 2001; Yang *et al.*, 2003 Song *et al.*, 2005).

Automated SPE systems for online analysis with the use of appropriate columns and gradient elution programs followed by LC-MS/MS analysis have been used for the determination of cetirizine (Song *et al.*, 2005), loratadine and desloratadine (Srinubabu *et al.*, 2007).

Finally, an SPE system using molecular imprinted nano particles (MISPE), combined with a miniaturized homogeneous LLE (MHLLE), for the selective extraction of loratadine from plasma and urine samples was recently described. The proposed method was more sensitive and had wider dynamic linear range and lower LOD than previously reported methods (Ebrahimzadeh *et al.*, 2013).

Generally, SPE offers a faster rate of separation and higher repeatability and accuracy than LLE. Moreover, it is easier to automate, as less manual effort is required. This extraction method usually requires expensive cartridges, while the use of small volumes of solvents and low purity solvents still can give a good separation. Furthermore, no emulsion and cross contamination problems are observed as in case of LLE (Abd-Talib *et al.*, 2014).

2.2.3. Solid-phase micro extraction (SPME)

Nishikawa *et al.* (1997) described a headspace solid-phase micro-extraction (SPME) technique for the isolation of diphenylmethane antihistamines from whole blood and urine

using polydimethylsiloxane-coated fiber and the optimum temperature (98°C) (Nishikawa *et al.*, 1997). Before application of such extraction methods, thermal stability of antihistamines needs to be examined to avoid degradation and poor reproducibility and to increase recovery. This technique was also applied to hair samples from several forensic and clinical cases where the presence of diphenhydramine and other categories of drugs were determined (Sporkert and Pragst, 2000).

2.2.4. Column switching and direct injection

Column switching and direct injection (Yamaguchi *et al.*, 1994; Kim *et al.*, 2005; Emara *et al.*, 2007; Dharuman *et al.*, 2011) have been described in four published articles (Table 5) where HPLC was used to separate antihistamines from matrix constituents and other analytes. In this technique, the sample was absorbed in a pre-column and then analytes were flushed into the analytical column via column-switching or with an on-line pretreatment. These techniques avoid an extraction step -so time is saved- but they require specific instrumentation and are limited to the use of HPLC methods. However, they allow almost quantitative extraction of analytes from biological samples and achieve satisfactory recovery percentages (Emara *et al.*, 2007).

3. Chromatographic techniques

The published methods that are based on TLC, GC, LC or HPLC analysis for the determination of different antihistamines in biological specimens are summarized in Tables 3-5. These methods have been either used for screening purposes or for the identification and quantification of one or more antihistamines, after the necessary sample pretreatment as it was described above.

3.1. Thin Layer Chromatography (TLC)

TLC is the oldest chromatographic technique used for the detection of drugs (Izmailov and Schraiber, 1938; TIAFT, 1992; Fried and Sherma, 1996). Published studies based on TLC

determination of antihistamines (chlorpheniramine, deiphenidramine, hydroxyzine, meclizine, triprolidine, cinnarizine, cetirizine) in biological specimens are limited and rather old (Table 3). TLC methods that detect specific antihistamines in pharmaceutical formulations, alone or in combination with other drugs, have also been described (Fusari *et al.*, 1975; Argekar and Powar, 1999; Makhija and Vavia, 2001; Subramaniyan and Das, 2004; Czerwinska *et al.*, 2013; Lamie and Monir, 2015).

Antihistamines were determined by TLC after previous extraction from biological samples (plasma, serum and urine) with organic solvents (Fike and Sunshine, 1965; Ackermann *et al.*, 1977; Hassan *et al.*, 2002) or after proteolysis in case of plasma or serum (Pandya *et al.*, 1996). Hydrolysis of urine samples with β -glucuronidase (Dell and Fiedler, 1977) can also be performed. Combinations of Dragendorff's, Mandelin's and iodoplatinate spray reagents or fluorescence and ultraviolet detection of the drugs on the TLC plates have been used (Table 3).

Pandya *et al.* (1996) developed a TLC method using high-performance TLC (HPTLC) plates for the determination of cetirizine in plasma. The use of HPTLC plates has the advantage of simultaneous analysis of a large number of samples in a shorter time period, utilizing smaller solvent volumes than HPLC and thus lowering the cost of analysis (Pandya *et al.*, 1996). TLC techniques are obsolete today as methods for detection and determination of drugs and they have been replaced by GC or LC coupled with MS or MS/MS that offer higher sensitivity and reliability. However, TLC can be used on many occasions as a screening technique when other relative methods are not available.

3.2. Gas chromatography (GC)

GC is a very common analytical technique used by toxicological laboratories and is available worldwide. However, as far as the analysis of antihistamines is concerned, published GC methods for the detection of this group of drugs are limited. Most of these methods were

published between the years 1968-1997, while the most recent papers were published in 2006 and 2007. All these methods are presented in Table 4.

The older GC methods used glass or stainless steel coil columns, while the more recent use fused silica capillary columns of ranged polarity (Table 4). In general, capillary columns, due to their longer length, provide much higher separation efficiency than packed columns but are more easily overloaded by too much sample.

Nitrogen, helium or methane has been used as the carrier gas (Table 4). Most of the methods utilized oven temperature programming for the separation of antihistamines, their metabolites and other drugs that could co-exist in the analyzed biological samples.

Traditional detectors, like ECD, FID and NPD, have a limited use today, despite the fact that they gave low LODs and LOQs. When these detectors were used, the number of the determined analytes was generally limited, e.g. one or two antihistamines (Table 4). Only four research teams (O'Brien *et al.*, 1977; Giachetti *et al.*, 1988; Hattori *et al.*, 1992; Nishikawa *et al.*, 1997) managed to determine simultaneously different antihistamines along with other drugs in biological samples using NPD or FID. MS detection by chemical ionization (CI) (Fouda *et al.*, 1978; Kageura *et al.*, 1986; Seno *et al.*, 1993) and electron impact-selected ion monitoring (EI-SIM) (Kintz *et al.*, 1990; Thompson and Leffert, 1980; Seno *et al.*, 1993; Tonn *et al.*, 1993; Martens, 1995; Hasegawa *et al.*, 2006; Pujadas *et al.*, 2007) have also been used for the determination of antihistamines after GC separation. These methods gave satisfactory results concerning the LODs (Table 4). The analyzed antihistamines were screened and quantitated using scan (Maurer and Pflieger, 1988; Seno *et al.*, 1993) or SIM (Kintz *et al.*, 1990; Fouda *et al.*, 1978; Thompson and Leffert, 1980; Kageura *et al.*, 1986; Tonn *et al.*, 1993; Martens, 1995; Hasegawa *et al.*, 2006; Pujadas *et al.*, 2007) mode.

Prior to the GC analysis, a derivatization procedure can be used for the improvement of the chromatographic performance of the analytes of interest. Nevertheless, only four published GC methods (Baltes *et al.*, 1988; Johnson *et al.*, 1994; Maurer and Pfleger, 1988; Hasegawa *et al.*, 2006) for the determination of antihistamines considered derivatization step necessary during their pretreatment procedure. This step is necessary for the antihistamines that possess suitable active functional groups. Derivatization, in general, increases the volatility of analytes, differentiates their chromatographic behavior and enhances the sensitivity of the method (Orata, 2012). Desloratadine, hydroxyzine and cetirizine possess active functional groups (-NH, -OH and -COOH, respectively) and can be derivatized under the appropriate conditions. N-methyl-trimethylsilyltrifluoroacetamide (MSTFA) was used as derivatization reagent in the case of hydroxyzine (that co-existed in oral fluid with other antihistamines and psychoactive drugs) (Hasegawa *et al.*, 2006), while derivatization of cetirizine was achieved by utilizing n-propanol (Baltes *et al.*, 1988). Desloratadine (or descarboethoxyloratadine), a pharmacologically active metabolite of loratadine, was acylated with trifluoroacetic anhydride (TFAA) under appropriate conditions after its extraction from human plasma along with the parent substance, loratadine (Johnson *et al.*, 1994). Brompheniramine was determined in blood after extraction from the biological matrix and oxidation to p-bromophenyl-2-pyridyl ketone (Bruce *et al.*, 1968).

3.3.High-Performance Liquid Chromatography (HPLC)

A large number of HPLC methods concerning the determination of antihistamines in biological matrices can be found in the literature and the majority of them have been published during the last decade. All the reviewed HPLC methods are presented in Table 5.

Most of the published HPLC methods used mainly OctaDecylSilyl (ODS, C₁₈) and C₈ columns to determine one or simultaneously more than one antihistamines in biological samples. Antihistamines have also been determined along with other drugs and metabolites in

biological samples. Cyano/cyanopropyl and amino columns have also been used for the determination of some antihistamines. Various mobile phases were used under gradient or isocratic elution programs. Analytical details of these methods are shown in Table 5.

Choi *et al.* (2000), Gupta *et al.* (2005) and Radler *et al.* (1991) used α_1 -acidglycoprotein chiral columns, in order to separate cetirizine and dimethindene enantiomers, respectively (Table 5), while a β -cyclodextrin filled column was used to separate (*R*)- and (*S*)-enantiomers of chlorpheniramine (Fried *et al.*, 2002). Hydrophilic Interaction Liquid Chromatography (HILIC) was found suitable for the determination of cetirizine in small volumes of human plasma (Li *et al.*, 2004; Song *et al.*, 2005, Apostolou *et al.*, 2008).

Detection of antihistamines was accomplished using various detection techniques. UV detection was performed within a wavelength region of 200-280 nm, while 230 and 254 nm was used in DAD. FLD was used directly or after a labeling reaction for the determination of antihistamines and especially those yielding lower LOD and LOQ (Table 5). More than half of the publications presented on Table 5 used MS or MS/MS detection for the identification of antihistamines with significantly low LOQs, as these detectors are used widely for the analysis of drugs in biological samples and offer the advantage of remarkably high sensitivity.

LC-MS and LC-MS/MS systems operating under CI, atmospheric pressure chemical ionization (APCI), atmospheric pressure-electron spray ionization (API-ESI) and EI have been used for the determination of antihistamines, while the majority of the published methods have used electron-spray ionization-positive ion (ESI-PI) mode (Table 5). The combination of HPLC with MS/MS advantages over LC-MS systems providing increased selectivity, specificity and sensitivity of the method. The studied antihistamines were screened and quantitated by using SIM and selected or multiple reaction monitoring (SRM or MRM) mode (Table 5).

Yang *et al.* (2001) evaluated a four-channel multiplexed electron-spray interface on a triple quadrupole mass spectrometer for the comparative validation of different LC-MS/MS methods for the quantitation of loratadine and its metabolite, descarboxyloratadine, in plasma samples from four different species after SPE (Yang *et al.*, 2001).

Apostolou *et al.* (2008) evaluated and compared HILIC-MS/MS and reversed-phase liquid chromatography (RPLC)-MS/MS for the determination of two antihistamines (cetirizine and loratadine) and donepezil in human plasma employing direct injection and LLE, SPE or protein precipitation developed protocols. Direct injection technique combined with HILIC allowed higher sensitivity and resolution than RPLC for the two antihistamines (Apostolou *et al.*, 2008). This technique seems promising and can contribute to broadening the range of antihistamines that can be analyzed with HILIC-MS/MS technique by include those of medium polarity.

3.4. Enantiomeric determination

The availability of analytical methods that can separate enantiomers of a specific drug in some cases can be extremely useful. Pure single enantiomers are supposed to provide safer and more effective alternatives than racemates. In addition to that, almost half of the drugs in use today are chiral (McConathy and Owens, 2003; Nguyen *et al.*, 2006). Moreover, it is well established that the pharmacological activity is mostly restricted to one of the enantiomers and that enantiomers of racemic drugs exhibit different pharmacological profile, while the inactive enantiomer shows undesirable side effects or even toxic (Nguyen *et al.*, 2006). For these reasons, recent regulations and guidelines on the development of new stereoisomeric drugs have orientated research activity towards the development, optimization and validation of chromatographic methods for the separation and determination of the enantiomers, like *R*- and *S*-cetirizine (Baltes *et al.*, 1988; Choi *et al.*, 2000; Strolin Benedetti *et al.*, 2008; Gupta *et al.*, 2005), *R*- and *S*-dimethindene (Radler and Blaschke, 1991) and *R*- and *S*-

chlorpheniramine (Hiep *et al.*, 1998; Fried *et al.*, 2002), that are present in pharmaceutical formulations, as well as in biological samples (Sekhon, 2010). It has to be mentioned here that the development of such kind of methods would be extremely useful when the enantiomers of antihistamines show different pharmacological activity.

4. Discussion and future perspectives

Antihistamines are a group of drugs that is widely used worldwide to control symptoms associated mainly with allergic reactions. The determination of antihistamines in ante- or post-mortem samples is considered to be necessary during the investigation of many clinical or forensic cases, due to the fact that this category of drugs can show synergistic sedative effects with other central nervous system depressants and can impair the skilled performance under normal use. Moreover, their sedative effects can lead to decreased safety in certain common and critical tasks, such as driving or operating machinery (Jáuregui Presa, 1999; Nolen, 1997; Jáuregui *et al.*, 2006).

Chromatographic and immunoassay methods for the measurement of antihistamines in biological specimens published over the last 50 years were reviewed. RIA or ELISA is normally used for screening purposes during antihistamines analysis. LC-MS/MS is generally used today for the determination of antihistamines in biological samples and has replaced the older and rarely used methods like TLC, HPLC-UV or DAD and GC. LC-MS/MS and GC-MS methods seem to achieve higher sensitivity, showing lower LOQs. LLE is extensively used for the isolation of the antihistamines of interest from biological matrices and no significant advantages were observed when SPE or protein precipitation was used. When direct injection or on-line extraction of antihistamines from biological specimens was applied the sensitivity of LC or HILIC methods was increased and a reduced sample preparation time was achieved. However, these procedures require specific and expensive instrumentation that is not available in all laboratories. In the majority of the publications, 1 mL of biological

fluid, and in some cases lower (0.1, 0.3 or 0.5 mL) or larger volumes (2, 4, 5 or 10 mL), were used for the analysis. Most of the articles describe the determination of specific antihistamines alone, while only a few articles describe the determination of antihistamines simultaneously or in combination with other drugs.

The use of GC techniques is limited in the field of antihistamines analysis, although most of them are widely available by most of toxicological laboratories. This is probably due to certain limitations of this technique, as far as antihistamines are concerned, i.e. poor chromatographic behavior or necessity for derivatization.

MS, especially when coupled with GC or LC separation techniques, is a very powerful analytical tool that provides higher sensitivity and specificity for the analysis of clinically relevant analytes in biological fluids (McMaster, 2005; Niwa, 1995; McMaster, 2008) compared to other types of detection (i.e. UV). It offers the advantage of matching a chromatographic peak with a mass spectrum, leading this way to increased certainty that the analyte of interest exists in the analyzed sample. For this reason, this type of detection is considered the standard reference analytical method for a wide range of medicines and drugs and is widely applied in routine analysis of known and/or unknown samples. The technique is used to measure a wide range of compounds, as well as multiple analytes in a single run with relatively low cost per sample. High flexibility is also provided. LC-MS or LC-MS/MS have been increasingly used to develop assays for their detection and determination since they require simple sample preparation and offer short analytical time with high sensitivity and specificity (McMaster, 2005). This is very important considering the fact that some antihistamines have low therapeutic concentrations range (Baselt, 2011) and are usually used concomitantly with other medicinal drugs. On the other hand, limitations of this type of detection are high instrument cost and complexity, which requires extensive training and highly skilled personnel. However, in some cases, like in the case of direct sample injection,

LC technique requires less pretreatment steps than GC. In the latter case, a derivatization process is usually required, when analyzed drugs possess active functional groups, such as -OH, -NH- or -COOH, which increases volatility of analytes (eliminating the presence of polar OH, NH and SH groups), detectability by enhancing sensitivity for the detector, stability of the analytes and reduces adsorption of polar samples on active surfaces of column walls and solid support (Orata, 2012).

In general, GC-MS is a powerful qualitative and quantitative analytical technique involving the separation and detection of gas-phase ions according to their mass-to-charge ratios, which offers the advantage of matching a chromatographic peak with a mass spectrum, leading this way to a safe identification of the analyte. For this reason, as well as due to the development of soft ionization techniques, such as ESI, this technology is widely adopted by highly technical research, clinical, as well as toxicological laboratories in the last decade and applied in routine analysis of a wide range of known and/or unknown samples / small and macro-molecules in biological fluids / specimens. Of interest is the fact that although GC instruments and techniques are generally used worldwide in toxicological and/or clinical laboratories, there is limited bibliography on GC methodologies concerning the determination of antihistamines in biological fluids and post-mortem matrices. The development of specific and sensitive GC-MS methods for the determination of antihistamines in biological specimens would be extremely helpful for all those laboratories that cannot afford more sophisticated and expensive instrumentation. GC-MS/MS and emerging new technologies, such as paper spray MS, may revolutionize antihistamines analysis in the future by providing superior throughput with much less solvent consumption and virtually no sample preparation (Wang *et al.*, 2013).

Blood, plasma, serum and urine remain the leading matrices for the analysis of antihistamines and their metabolites. In some cases, the analysis of alternative matrices like breast milk,

meconium, saliva, hair, tissues or blood spots could be extremely useful for the thorough investigation of specific clinical and forensic cases.

The development of the proper analytical methodology for the investigation of the above cases is extremely challenging, mainly due to the general lack of methods concerning the simultaneous determination of several antihistamines in biological fluids. Furthermore, improvements on the sample pretreatment like automated sample preparation or on-line pretreatment would enable easier and higher quality results. Such improvements will be a significant contribution towards the optimization of the existing analytical methodology for the determination of antihistamines used either in the field of clinical or forensic toxicology. The review of the existing bibliographic data reveals that the most sensitive methodology on antihistamines analysis that is used today often requires sophisticated instrumentation, not always available by common toxicological laboratories.

Acknowledgements

Authors would like to thank Paschalis Tsitsios, Medical Information Expert in Janssen-Cilag, for his scientific support.

Conflict of Interest

The authors declare that they have no conflict of interest.

Figure and Table legends

Figure 1. The chemical structures of the piperoxane (the first antihistamine) and the most commonly used antihistamines.

Table 1. Chemical classification of antihistamines.

Table 2. Summary of studies using immunoassays for screening of antihistamines.

Table 3. Summary of published TLC methods for antihistamines analysis.

Table 4. Summary of published GC methods for antihistamines analysis.

Table 5. Summary of published HPLC methods for antihistamines analysis.

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Table 1. Chemical classification of antihistamines.

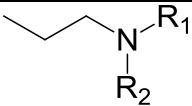
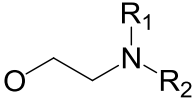
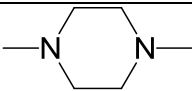
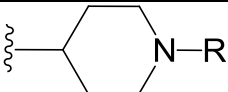
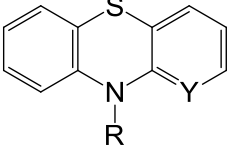
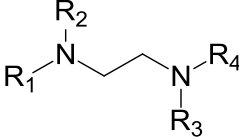
Category	General structure	Substances
Alkylamines		brompheniramine, chlorpheniramine, dexchlorpheniramine, dimethindene, triprolidine, pheniramine, pyrrobutamine
Ethanolamines		diphenhydramine, dimenhydrinate, doxylamine, bromodiphenhydramine, carbinoxamine, trimethobenzamide
Piperazines		cinnarizine, hydroxyzine, oxatomide, cetirizine, levocetirizine, meclozine, buclizine, chlorcyclizine, cyclizine
Piperidines		loratadine, desloratadine, rupatadine, mizolastine, ebastine
Phenothiazines		promethazine, dimethothiazine, trimeprazine
Ethylenediamines		pyrilamine, methapyrilene, mepyramine, halopyramine, antazoline, tripelennamine

Table 2. Summary of studies using immunoassays for screening of antihistamines.

Reference ^a	Drug	Biological fluid	Assay used	Specificities/conclusions
Findlay <i>et al.</i> , 1984a	Tripolidine	Human and rabbit plasma (untreated)	RIA	LOD= 0.1 ng/mL LOL: 0.078-10.0 ng/mL Application to pharmacokinetics of tripolidine in rabbit.
Findlay <i>et al.</i> , 1984b	Tripolidine	Plasma and breast milk (0.2 mL)	RIA	Treatment of milk samples with phosphate buffer and extraction of tripolidine with benzene. LOQ or LOD values are not mentioned.
Midha <i>et al.</i> , 1984	Chlorpheniramine	Plasma (100 μ L)	RIA	LOD= 0.156 ng/mL LOL: 0.156-20 ng/mL Recovery: 95%
Radwanski <i>et al.</i> , 1987	Loratadine	Plasma	RIA	Acidification of plasma samples and extraction of loratadine with cyclohexane. LOQ= 0.3 ng/mL. Application to pharmacokinetic study.
Hilbert <i>et al.</i> , 1987	Loratadine	Human plasma	RIA	Acidification of plasma samples and extraction of loratadine with cyclohexane. LLOQ= 0.3 ng/mL LOL: 0.1-20 ng/mL Application to pharmacokinetic study.
Hilbert <i>et al.</i> , 1988	Loratadine	Plasma or breast milk	RIA	Acidification of plasma/milk samples and extraction of loratadine with cyclohexane. LLOQ= 0.3 ng/mL LOL: 0.1-20 ng/mL Application to pharmacokinetic study. The amount of loratadine excreted into the breast milk was minimal.
Arnera <i>et al.</i> , 1990	Dimethindene	Serum (50 μ L)	ELISA	LOD= 0.1 ng/mL. Application to pharmacokinetic study in healthy human volunteers.

Abbreviations:

ELISA: enzyme-linked immunosorbent assay, LLOQ: lower limit of quantification, LOD: limit of detection, LOL: linearity, LOQ: limit of quantification, RIA: radioimmunoassay.

Reference ^a	Drugs	Specimen	Detection method	Extraction method	Plates	Mobile phase	LOD/LOQ (ng/mL)	Recovery (%)	Concentration range (ng/mL)
Fike and Sunshine, 1965	Chlorpheniramine, diphenhydramine, hydroxyzine, meclizine, triprolidine and 20 more antihistamines	Urine, blood or homogenized tissue	UV 254 nm, spray with Dragendorff's and Mandelin's reagents	LLE (alkalinization, chloroform)	Silica gel G (glass plates)	cyclohexane-benzene - diethylamine (75:15:10)	LOD= about 0.001 ppm for a 10 gram of sample	NS	NS
Lange <i>et al.</i> , 1968	Chlorpheniramine and other alkylamines	Capillary blood (0.1 mL)	Fluorescence, excitation 546 nm and 525 nm, emission 570 nm (spray with Dragendorff's reagent)	50 μ L 10% aq. trichloroacetic acid solution	Silica gel G	ammoniacal chloroform-ether (1:1)	LOD= 0.5	99	NS
DeAngelis <i>et al.</i> , 1977	Tripolidine	Human and rat plasma (1 mL)	Fluorescence excitation 300 nm, emission 405 nm, spray with 2M aq. ammonium bisulfate	Dichloroethane, centrifugation	Silicoplate-22, 0.25 mm	methanol-ammonium hydroxide-chloroform (10:1:89)	LOD= 0.8 LOQ= 0.4 ng spotted on the plate	83 (human plasma)	1-35
Hindmarsh <i>et al.</i> , 1977	Diphenhydramine (and methaqualone)	Rat and human urine (5 mL)	spray with 5% H ₂ SO ₄ , iodoplatinate spray reagent, Dragendorff's reagent	LLE / SPE	Silica gel GF (300 μ)	chloroform-methanol (90:10) and then chloroform	LLOD = 150 (ng)	98.3 (n-butyl chloride as the extraction solvent)	NS

								t)	
Dell and Fiedler, 1977	Diphenhydramine, cinnarizine (orphenadrine, diphenylpyralin) and metabolites	Human, dog and rabbit urine (10 mL)		acidification, hydrolysis with β -glucuronidase, extraction with chloroform	Kieselgel HF-plates	chloroform-n-heptane (7:3)	NS	NS	NS
Ackermann <i>et al.</i> , 1977	Hydroxyzine	Animal liver	spray with Dragendorff's reagents	LLE (alkalinization, chloroform)	Kieselgel G 0.25 mm	chloroform-aceton (1:1) with conc. NH_3 -wasser (1:1)	LOD= 0.01 ppm LOQ= 0.05 ppm liver, kidney, muscular substance	80-100 (liver, kidney)	100-2,000
		Milk					LOD= 0.1 ppm LOQ= 0.5 ppm (milk)	70-100 (muscular substance) 40-100 (milk)	
Findlay <i>et al.</i> , 1984a	Tripolidine	Plasma	Spray with 2M ammonium bisulfate / fluorescence excitation 300nm, cut-off filter 400 nm	Extraction with benzene	Silica gel GF ₂₅₄ 0.25 mm	chloroform-methanol-ammonium hydroxide (91:8:1)	NS	95%	1-8
Wood <i>et al.</i> , 1987	¹⁴ C-cetirizine	Plasma, urine	UV 254 nm	NS	Kieselgel F ₂₅₄	chloroform-methanol-ammonium	NS	NS	NS

						a (85:15)			
Pandya <i>et al.</i> , 1996	Cetirizine	Plasma (1 mL)	Dual wavelen gth scanner	LLE (proteolys is of plasma proteins with 0.35% pepsin, phosphate buffer pH 5, chloroform)	Silica gel 60 F ₂₅₄ (HPT LC)	chloroform- methanol (75:25:2)	LOD= 50	98	50-5,000
Hassan <i>et al.</i> , 2002	Cinnarizine	Rat serum (1 mL)	UV 250 nm	LLE (acidificat ion, chloroform)	What man K6 F ₂₅₄ silica gel	benzene - methanol-formic acid (80:17:3)	LLOD = 16	98.6	4,000- 30,000

Table 3. Summary of published TLC methods for antihistamines analysis.

Abbreviations:

HPTLC: high-performance thin-layer chromatography, LLE: liquid-liquid extraction, LLOD: lower limit of detection, LOD: limit of detection, LOQ: limit of quantification, NS: not stated, TLC: thin-layer chromatography, UV: ultraviolet.

Reference ^a	Drugs	Biological fluid	Extraction method	Column	Detection method	LOD/LOQ (ng/mL)	Recovery (%)	Concentration range (ng/mL)	Internal standard
Bruce <i>et al.</i> , 1968	Brompheniramine	Blood, urine (5 mL)	LLE (alkalinization, ether, oxidation of drugs, isooctane)	stainless steel packed with SE-30	Tritium foil ECD	NS	99-100	NS	Chlorpheniramine
Barnhart and Johnson, 1970	Chlorpheniramine	Plasma, serum (2 mL)	LLE (alkalinization, ether, acetic anhydride, 0.1 M pH 5.5 citrate-phosphate buffer, washing of aq. layer with ether, evaporation, 2N KOH and 1% KMnO ₄ , isooctane)	silanized glass column packed with 3% SP-2250	ECD	NS	NS	NS	Brompheniramine
Townley <i>et al.</i> , 1970	Chlorpheniramine	Plasma (5 mL)	LLE (acidification, diethyl ether, back extraction with diethyl ether after alkalization, n-heptane)	glass U column packed with 0.1 % Ucon oil	FID	NS	NS	2.5-1,000	Brompheniramine
van der Kleijn <i>et al.</i> , 1971	Hydroxyzine (barbiturates, benzodiazepines)	Blood, urine (1-2 mL)	LLE (alkalinization, ether)	stainless steel column on the FID and borosilicate glass column on the ECD: packed with was 3% OV-17	FID/ECD	NS	NS	NS	Griseofulvin
Bilzer and Gundert-Remy, 1973	Diphenhydramine	Plasma (2 mL)	LLE (alkalinization, n-heptane)	glass column packed with 3% OV-17	Nitrogen selective detector	NS	50-60	10-100	Orphenadrine
Hanna and Tang,	Chlorpheniramine	Plasma (2	LLE (alkalinization,	stainless steel column packed	FID	NS	>100	100-700	

1974		mL)	chloroform)	with 5% SE-30					
Baugh and Calvert, 1976	Diphenhydramine	Plasma (3 mL)	LLE (alkalinization, n-heptane)	glass column packed with KOH 2%, Carbowax 5%	Alkali flame ionization detector	NS	NS	10-67	Orphenadrine
Hindmarsh <i>et al.</i> , 1977	Diphenhydramine in combination with methaqualone	Rat and human urine	LLE (alkalinization, extraction with organic solvents) or SPE (nonionic resin SPE, alkalinization, n-butyl-chloride)	stainless steel column packed with 3% OV-17	Hydrogen FID	NS	98.3 (extraction with n-butyl chloride)	NS	(each drug may serve as an internal standard for the other)
O'Brien <i>et al.</i> , 1977	Chlorpheniramine	Plasma (5 mL)	LLE (ether, back-wash into acid, final basic extraction into hexane)	glass column packed with 2% OV-101	Nitrogen sensitive thermionic detector	LOD= 1	82	15-100	Pyrilamine
Fouda <i>et al.</i> , 1978	Meclizine	Human plasma (4 mL)	LLE (physiological pH, toluene, alkaline pH, diethyl ether, residues dissolved in methanol)	glass column packed with 3% OV-17	MS (CI)	LLOQ= 5	60 (at 100 ng/mL)	5-200	[² H ₅]-meclizine
Morris <i>et al.</i> , 1979	Cinnarizine	Plasma (1 mL)	LLE (acidification, chloroform)	borosilicate glass column packed with 2.5% OV-17	nitrogen selective FID	LOD= 10	60	10-5,000	Thioridazine (external standard)
Thompson and Leffert, 1980	Chlorpheniramine	Serum (1 mL)	LLE (alkalinization, n-hexane, 0.1N HCl, back extraction with ethyl acetate after alkalinization)	glass column packed with 3% OV-22	MS (EI)	LOD= 1-2	NS	0-160	Chlorpheniramine-d ₄
Ali, 1981	Chlorpheniramine	Urine (5 mL)	LLE (alkalinization, chloroform or ether)	glass column packed with 3% OV-17	FID	NS	>90	500-4,800	Brompheniramine

Werme ille and Huber, 1982	Dimethinde ne	Seru m, urine (5 mL)	LLE (25% NH ₃ , pentane)	glass column packed with 10% Apiezon L- 2% KOH	FID	LOD= 10 LOQ= 4	~100 (urin e)	10-200 (both tissues)	Docosane
Woeste nborgh s <i>et al.</i> , 1982	Cinnarizine (and flunarizine)	Hum an/do g plas ma (2 mL), urine , milk (1 mL)	LLE (alkalinization, back-extraction into H ₂ SO ₄ , re- extraction into heptane- isoamyl alcohol)	glass column packed with 3% OV-17	Nitro gen- select ive therm ionic detect or	LOD= 0.5	91- 94	0.5-250	R 13415
Aberne thy and Greenb latt, 1983	Diphenhydr amine	Plas ma (0.25 -5 mL)	LLE [alkalinization, hexane:isoamyl alcohol (98:2), alkaline pH, toluene:isomyl alcohol (85:15)]	glass column packed with 3% SP-2250	NPD	LOD= 1	97.3 (plas ma at 25 ng/m L)	1-300	Orphenad rine
Meathe rall, 1984	Diphenhydr amine	Seru m (1 mL)	LLE (alkalinization, hexane)	007 bonded methyl silicone wide-bore fused-silica capillary column	NPD- FID	LOD= 0.9	98- 106	not linear below 30 ng/mL	Orphenad rine
Yoo and Axelso n, 1986	Diphenhydr amine	Shee p plas ma (0.3- 0.5 mL)	LLE (alkalinization, 0.05M Et ₃ N in CH ₂ Cl ₂)	5% phenylmet hyl silicone capillary column	NPD	LOQ= 2	103. 9	4-640	Orphenad rine
Kageur a <i>et al.</i> , 1986	Hydroxyzin e and changed compounds	Urine (5 mL)	LLE (CH ₂ Cl ₂)	glass coil column packed with 4% Dexsil 300GC	MS (CI)	NS	NS	NS	NS
Masum oto <i>et al.</i> , 1986	Chlorphenir amine (in combinatio n with	Plas ma (2	LLE (alkalinization, 10% solution of n-	50% phenylmet hyl silicone fused	NPD	LOD= 0.3	~100	3.0-11.9	Pyrilamin e

1986	codeine)	mL)	butanol in toluene)	capillary column					
Baltes <i>et al.</i> , 1988	Cetirizine	Plasma (1 mL)	LLE (citrate buffer 1M, pH5, chloroform, derivatization with n-propanol)	methylsilicone fused silica column	NPD/FID	LOD= 20	89	100-1,000	ucb 26294
Giachetti <i>et al.</i> , 1988	Diphenhydramine, meclizine, cinnarizine, chlor-/brom-pheniramine and 7 more antihistamines	Plasma (1 mL)	LLE [alkalinization, chloroform: dichloromethane: methanol, (80:20:2, v/v)]	silica capillary column	NPD	LOD= 1	96.6-98.3	5-250	7-methylmianserine
Maurer and Pfleger, 1988	Diphenhydramine and 11 more alkanolamine antihistamines and their metabolites	Urine (10 mL)	LLE [hydrolysis, alkalinization, dichloromethane:2-propanol: ethyl acetate (1:1:3), acetylation of extract]	HP methylsilicone capillary column	MS	NS	NS	NS	NS
Dostal and Schwetz, 1989	Diphenhydramine	Rat milk	LLE [alkalinization, hexane:isoamyl alcohol (98:2), then again alkaline pH, toluene:isomyl alcohol (85:15)]	glass column packed with 3% SP-2250	NPD	LOQ= 200 LOD= 20	57	200-50,000	Orphenadrine
		Rat plasma (1 mL)					52		
Kintz <i>et al.</i> , 1990	Hydroxyzine	Plasma (1 mL), whole blood, urine, bile, stomach content	LLE [alkalinization, n-heptane:isoamyl alcohol (98.5:1.5, v/v)]	glass column packed with Alltach 3% OV-17 MS: fused silica capillary column SGE, BP-1 (methylmethylsiloxane)	NPD (+MS for confirmation –EI, ITD)	LLOD= 0.8 LOQ= 0.8	81.3 (plasma)	10-500	Acepromazine

		(post mortem biological samples)		)					
Hattori <i>et al.</i> , 1992	Diphenhydramine and 10 more antihistamines	Whole blood (1 mL)	SPE Sep-Pak C ₁₈ , chloroform:methanol (8:2)	DB-1 non-polar fused-silica capillary column	Surface Ionization detection (SID) and NPD	LOD= 2-5 pg (ca. 6-20 fmol) on-column (100 - 250 pg/ml body fluid)	103	10-50 pmol/mL	
		Urine (1 mL)					117		
Seno <i>et al.</i> , 1993	15 diphenylmethane antihistamines and their analogues	Whole blood, urine (1 mL)	SPE [Sep-Pak C ₁₈ , chloroform:methanol (9:1)]	DB-1 fused silica capillary column and DB-17 fused silica capillary column	MS (PIEI, PICI, NICI)	LOD= 20-50 ng (PIEI), 100-200 ng (PICI), 500-100 ng (NICI)	>60-90 49.4 (for meclizine)		
Tonn <i>et al.</i> , 1993	Diphenhydramine, [² H ₁₀]-diphenhydramine	Fetal or maternal plasma, amniotic fluid, fetal tracheal fluid of pregnant ewes (0.1-1 mL)	LLE (alkaline pH, 0.05M Et ₃ N in 2% isopropanol:98% hexane)	DB-1701 silica capillary column	MSD (EI)	LOD= 0.5 in all matrices LOQ= 2.0 in all matrices	>97	2-200	Orphenadrine
Johnson <i>et al.</i> ,	Loratadine, descarboeth	Plasma	LLE [neutral pH,	RTX 20 fused-silica	NPD	LOQ= 0.1	~100 %	0.1-30	SCH 37370

1994	oxyloratadine	(1 mL)	hexane:methyl- <i>tert</i> -butyl ether (50:50, v/v)] or [alkaline pH, methyl- <i>tert</i> -butyl ether:toluene (15:85), TFAA derivatization)	capillary column containing 20% phenyl methyl bonded silicone					and SCH 40129
Martens, 1995	Loratadine	Serum (1 mL)	LLE [alkalinization, n-hexane:n-amyl alcohol (95:5, v/v)]	HP Capillary column	MS (EI)	LOQ= 1.5 LOD= 0.5	70	2-60	Etoloxamine
	Pheniramine	LOQ= 0.6 LOD= 2				6-600			
Nishikawa <i>et al.</i> , 1997	Diphenhydramine and 12 more diphenylmethane antihistamines and their analogues	Whole blood (0.5 mL)	SPME [polydimethylsiloxane-coated fibers, 10N NaOH for alkalization of samples]	DB-1 fused-silica capillary column	FID	LOD= 76-473	(SPME) 0.3-7.21	350-5,600	Orphenadrine
		Urine (0.5 mL)				LOD= 62 (Diphenhydramine)	1.92-75.4	180-2,800 (Diphenhydramine) 350-5,700 (Triprolidine) 140-2,200 (Chlorpheniramine)	
Sporke <i>et al.</i> and Pragst,	Diphenhydramine	Hair	HS-SPME (hydrolysis of hair matrix by 4%)	HP capillary column	GC-MS	LOD= 0.05 ng/mg	0.7-1.7	0.5-10 ng/mg	Ethylbenzhydramine

2000			NaOH NaOH + 0.5 g Na ₂ SO ₄)	containing 95% dimethylsiloxane /5% diphenylsiloxane		LOQ= 0.15 ng/mg			
Hasegawa <i>et al.</i> , 2006	Diphenhydramine	Human plasma (0.1 mL)	SPE (MonoTip C ₁₈ tips with C ₁₈ -bonded monolithic silica gel), elution with methanol by 5 aspirating/ dispensing cycles (after alkalization)	DB-1MS fused silica capillary column	MS (EI)	LOD= 3	91-100	10-5,000	Cyproheptadine
	Triprolidine					LOD= 10	86-96	20-2,000	
	Chlorpheniramine					LOD= 2	89-100	5-2,000	
	7 more antihistamines						73.8-105		
Pujadas <i>et al.</i> , 2007	Loratadine	Oral fluid	SPE [Bond Elut Certify columns, acidification, dichloromethane:isopropanol:ammonium (80:20:2, v/v/v), derivatization with MSTFA)	Ultra1 methylsilicone capillary column	MS (EI)	LOD= 2.9 ng/mL	67-78		d3-morphine (d3-Morphine-bis-O-TMS)
	Hydroxyzine (hydroxyzine-O-TMS)					LOQ= 8.8			
	Diphenhydramine					LOD= 0.9	70-99		
	Psychoactive drugs					LOQ= 2.5			
						LOD= 5.4	74-96		
						LOQ= 16.3			

Abbreviations: CI: chemical ionization, EI: electron impact, ECD: electron capture detector, FID: flame ionization detector, HS-SPME: headspace solid-phase microextraction, LLE: liquid-liquid extraction, LOD: limit of detection, LOQ: limit of quantification, MS: mass spectrometry, MSTFA: N-methyl-N-trimethylsilyltrifluoroacetamine, NPD: nitrogen phosphorus detector, NS: not stated, SPE: solid-phase extraction.

Table 4. Summary of published GC methods for antihistamines analysis.

Table 5. Summary of published HPLC methods.

Reference ^a	Drugs	Tissue	Extraction method	Column	Mobile phase	Detection method	LOD/LOQ (ng/mL)	Recovery (%)	Concentration range (ng/mL)	Internal standard
Athar et al., 1979	Chlorpheniramine (and two metabolites)	Plasma, saliva (1 mL)	LLE (alkalinization, diethyl ether or hexane, dilute acid)	μ Bondapak C ₁₈	ACN/phosphate (20:80), flow-rate: 2 mL/min	UV 254	LOQ=2	85	3.52-25.16	Brompheniramine
		Urine (1 mL)						98	100-1500	
Hundt et al., 1980	Cinnarizine	Plasma (5 mL)	LLE (alkalinization, diethyl ether)	Spherisorb 5 C ₁₈	MeOH:0.05M NH ₄ H ₂ PO ₄ , flow rate: 1.0 mL/min	UV 258	LOD=2	97.7-104	20-100	Chlorbenzoxamine
Nitsche and Mascher, 1982	Cinnarizine	Plasma (1 mL)	LLE (acidification, carbon tetrachloride)	LiChrosorb column RP-8	MeOH-acetate buffer (85:15), flow-rate: 2.0 mL/min	UV 250	LOD=2	101.5	20-220	DPA 14
Puttemans et al., 1984	Cinnarizine	Whole blood, plasma (4 mL)	LLE [phosphate buffer, pH 3, chloroform-hexane (2:3)]	Ultrasphere RP-18	MeOH-phosphate buffer (90:10), 1.5 mL/min	UV 254	LOD=2	97	4-200 (plasma)	Meclozine
Midha et al., 1984	Chlorpheniramine	Plasma (2 mL)	LLE (alkalinization, 3% isopropyl alcohol in pentane)	Spherisorb 5 μ m cyano column	0.05M CH ₃ COONH ₄ -ACN (30:70), flow rate: 3 mL/min	UV 229	LOQ=2.5	78	5-25	Prochlorperazine
Chovan et al., 1985	Meclozine	Rat/dog plasma	LLE (physiological pH, toluene, alkalinization, diethyl ether)	Novapak C ₁₈	A: 8 mM SDS in water with 0.5% (v/v) glacial CH ₃ COOH B: 40 mM SDS in water with 0.5% (v/v) glacial CH ₃ COOH, isocratic 78% B:22%	UV 232	NS	NS	NS	NS

					A, flow rate: 1.2 mL/min					
Miyamoto, 1987	Chlorpheniramine	Plasma (1 mL)	SPE (stainless-steel hypodermic needle, 23 mm x 0.5 mm, filled with 3.0 g of Extrelut, alkalization, carbon tetrachloride, derivatization with BCF)	Zorbax C ₁₈	MeOH-water (84:16, v/v), flow-rate: 1.7 mL/min	FLD (excitation 350 nm, emission 500 nm)	LOD= 0.1	86.7-95.8	0-40	
Radwanski et al., 1987	Desloratadine	Plasma	LLE alkalization, dichloromethane, derivatization with NBD-Cl	5 μ DuPont Zorbax CN	toluene-ethyl acetate (64:4, v/v), flow-rate: 1.0 mL/min	FLD (excitation 465 nm, emission 550 nm)	LOQ= 0.6	NS	NS	NS
Hilbert et al., 1987; Hilbert et al., 1988	Desloratadine	Plasma	LLE (alkalinization, dichloromethane, derivatization with NBD-Cl/ (milk samples) filtration of extract through glass wool before derivatization)	5 μ DuPont Zorbax CN	toluene-ethyl acetate (96:4, v/v), flow-rate: 1.0 mL/min	FLD (excitation 465 nm, emission 550 nm)	LOQ= 0.6	NS	0.6-24	NS
		Breast milk					LOQ= 1			
Fujii et al., 1990	Oxatomide	Serum (300 μ L)		Deproteinization: ISRP column, separation: C ₁₈	Deproteinization: 0.01M CH ₃ COONH ₄ , analytical separation: 0.01M CH ₃ COONH ₄ -MeOH (20:80)	FLD (excitation 280 nm, emission 309 nm)	LOD= 0.5	>98	Linear up to 50 ng/mL	
Rosset and	Cetirizine	Urine (0.5)	LLE (acidifica	Chromapak 50DS-2	5 mM aq.	(PDA) UV 230	LOD= 20	78.8	100-10000	UCB J028

Lefebvre, 1991		mL)	tion, chloroform)	(C ₁₈)	tetrabutylammonium phosphate-MeOH-THF (33:65:2), flow-rate: 0.1 mL/min					
Radler and Blaskhe, 1991	Dimethindene	Urine (4 mL)	LLE (alkalinization, twice n-hexane)	Semipreparative column: LiChrospher® 60CN Analytical column: LiChrospher® 60CN Chiral column: α ₁ -acid glycoprotein cartridge	Semipreparative and analytical column: 0.02M KH ₂ PO ₄ -ACN-water (60:32:8, v/v), flow-rate: 2.5 mL/min or 1 mL/min Chiral column: 0.02M phosphate buffer (pH 7.0) cont. 5% i-PrOH, flow-rate: 0.3 mL/min	UV 254 MS (CI)	NS	98-100	3-600	Fendiline
	N-demethyl dimethindene							49-60		
Moncrieff, 1992	Cetirizine	Serum (100 μL)	PP with HClO ₄ 25%, centrifugation	Spherisorb S5 C ₁₈ -2	ACN-MeOH-0.05 M phosphate buffer (33:9:58), flow-rate: 2 mL/min	UV 211	LOD=20	NS	10-100	
Benvvenuti et al., 1992	Oxatomide	Plasma (1 mL)	LLE [alkalinization, n-hexane-isoamyl alcohol (98.5:1.5)]	HS-5 C ₈	acetate buffer-ACN (45:55), flow-rate: 1.5 mL/min	FLD (excitation 280 nm and emission 310 nm)	LOD=0.2	~100	0.5-20	Verapamil
		Urine (diluted in water)					LOD=1		1-20	
Choll et al., 1993	Dimethindene	Urine (1 mL)	on-line SPE: LiChroCART (4 mm x 4.0 mm I.D., 5 μm) or LiChrospher 100 RP-18	Suplex pKb- 100	(A) 0.01M CH ₃ COONa and 0.04M (NH ₄) ₂ SO ₄ -ACN (90:10),	UV 258	LOQ=5 pmol/mL	>100%	4.9-49.0 pmol/mL	

					(B) 0.05M CH ₃ CO ONH ₄ , (C) MeOH- water (90:10)					
Yama guchi <i>et al.</i> , 1994	Chlorpheni ramine	Hum an seru m (500 μL)	LLE (alkaliniz ation, diethyl ether)	Cl and C2 columns: Inertsil C ₁₈ -2 column switching	For C1: 20 mM KH ₂ PO ₄ -ACN (70:30) containi ng 5 mM SOS, for C2: 20 mM KH ₂ PO ₄ -ACN (70:30), flow- rate: 1 mL/min	UV 210	LOD= 0.2 LLOQ= 0.5	88.9- 107.4	0.5-50	
Hole man and Danie lson, 1994	Brompheni ramine	Hum an urine		Asahipak ODP-50 C ₁₈	ACN- 0.025 M borate buffer, pH 9.0 (40:60), flow- rate: 0.8 mL/min	HPLC- CL and UV (214 and 254 nm)	LOD= 90-210 (CL) 70-130 (UV 214nm) 200- 2,400 (UV 254 nm)		500- 2,200	N,N- dimethyl benzena mine
	Chlorpheni ramine								300- 1,500	
	Diphenhyd ramine								500- 7,500	
	Phenirami ne									
Puigd emon t <i>et al.</i> , 1994	Ebastine	Dog plas ma (0.5 mL)	IS, 1 mL CH ₃ CO ONa buffer (0.2 M, pH 4.5), centrifug ation, supernata nt removed and submitte d to SPE	LiChrosph er 100 RP- C ₁₈	ACN- 60% CH ₃ CO ONH ₄ (0.2 M, pH 4.5) cont. 0.1 % Et ₃ N, flow- rate: 1 mL/min	UV 257	NS	NS	5-500	LAS Z- 120
Musc ara and De Nucci , 1995	Cetirizine	Plas ma (1 mL)	LLE (acidifica tion, ethyl acetate, centrifug ation, re- extractio n of org. phase with 0.25 M H ₃ PO ₄)	LiChrosor b 5 RP-C ₁₈	0.01 M phospha te buffer- ACN (55:45, v/v)	UV 230	LOD= 5	75	20-800	P265
Hiep <i>et al.</i> , 19 98	Chlorpheni ramine (CPAM), its	Urine (1 mL)	LLE (alkaliniz ation, diethyl	achiral Nucleosil cyanoprop yl column	hexane- ethanol- diethyla mine	UV 262	LOQ= 35	51-82	0-1000	Bromphe niramine

	monodesmethylmetabolite (DCPM)		ether)	connected to an amylase Chiralpak AD [®] column via an EPS 130 HP2P automatic switching valve	[(96:4:0.05) for CPAM and (85:15:0.1) for DCPM], flow rate: 0.5 mL/min					
Kumar <i>et al.</i> , 1998	Diphenhydramine, <i>N</i> -Oxide metabolite and their deuterium-labeled analogues	Sheep ovine plasma (1 mL)	LLE (alkalinization, ethyl acetate with 0.05M (0.72%, v/v) thiethylamine)	HP YMC amino column (normal phase)	gradient ACN-2mM CH ₃ COONH ₄ (1% glacial CH ₃ COOH)	MS/MS (ESI) (PI) MRM (<i>m/z</i> 256->167 for Diphenhydramine)	LOQ= 0.2 (DPHM, [² H ₁₀]-DPHM)	69-79.9	0.4-100.0 ng/mL (N-oxides)	Orphenadrine
		Urine (1 mL)					LOQ= 0.4 (N-oxides)	71.3-82.3	0.2-250.0 ng/mL (parent amines)	
Choll <i>et al.</i> , 1998	Dimethidene	Tears (4 µL)	0.01M HCl- <i>n</i> -propanol (95:5)	CN column Nucleosil CC-5-CN (reversed-phase mode)	0.05 M (NH ₄) ₃ PO ₄ buffer pH 5.5-ACN (60:40), 1.2 mL/min, isocratic	UV 258	LOD= 2.4 LOQ= 12.0	NS	12-1000	
Mack <i>et al.</i> , 1999	Cetirizine	Plasma (0.5 mL)	LLE with dichloromethane	RP-Nucleosil 100-3 C ₁₈	MeOH-30 mM KH ₂ PO ₄ (pH 6.8), gradient, flow rate: 0.5-0.7 mL/min	UV 232	LOQ= 10.09	NS	10-1100	Diazepam
Zaate <i>et al.</i> , 1999	Chlorpheniramine	Serum (2 mL)	LLE (alkalinization, diethyl ether)	LiChrosphere RP C ₁₈	ACN-MeOH-0.075 M NH ₄ H ₂ PO ₄ (23:9:68), flow-rate: 1.2 mL/min	UV 269	LOD= 0.5	86	2.5-15	Brompheniramine
Celma <i>et al.</i> , 2000	Chlorpheniramine (and paracetamol)	Plasma (0.5 mL)	LLE (alkalinization, diethyl ether)	Kromasil C ₁₈	water/ACN (80:20), 0.5% HCOOH, 1 mM PFPA, flow-rate: 1 mL (isocratic)	MS/MS (API) MRM (<i>m/z</i> 275 for chlorpheniramine)	LOQ= 0.2	75-79 (chlorpheniramine)	0.2-50	2-acetamidophenol
Zaate	Cetirizine	Serum	LLE	RP	13 mM	UV 230	LOD=	96.5	10-500	Diazepam

r et al., 2000		m (1 mL)	with dichloro methane	Novapak C ₁₈	H ₃ PO ₄ -ACN (61:39)		5			m
Choi et al., 2000	Cetirizine (+), (-) enantiomers	Urine (5 mL)	SPE amberlite PAD-2 resin in a pipette, acidification, ethyl acetate	chiral stationary phase α ₁ -acidglycoprotein column	10mM phosphate buffer (pH 7.0)-ACN (95:5), flow-rate: 0.9 mL/min	UV 230	LOD=400	(+)-CTZ higher extraction than that of (-)-CTZ	2.5-200	Roxatidine
Gergov et al., 2001	18 antihistamine drugs	Blood (1 mL)	LLE (alkalinization, butyl acetate for the basic drugs or acidification, CH ₂ Cl ₂ -MeOH, 95:5 for the acidic drugs)	Genesis C ₁₈	ACN-CH ₃ COONH ₄ buffer gradient ACN and isocratic, flow-rate: 0.2 mL/min	MS/MS (ESI) MRM	LOQ=0.5-10	43-113 (basic drugs) 23-66 (acidic drugs)		Dibenzepine
Kunicki, 2001	Loratadine	Plasma	LLE 2-methylbutane: hexane (2:1)	Supercosil C ₁₈ -DB	ACN-water-0.5M KH ₂ PO ₄ :H ₃ PO ₄ (440:480:80:1, v/v), isocratic, flow-rate: 1.8 mL/min	UV 200	LOD=0.175 (serum) LOQ=0.5	71	0.5-50	Diazepam
Sutherland et al., 2001	Loratadine	Plasma (1 mL)	LLE (alkalinization, toluene, back extraction of desloratadine into 2% HCOOH)	Phenomenex Luna® C ₁₈ (2)	ACN-0.1% HCOOH, gradient, flow-rate: 0.3 mL/min	MS/MS (ESI) MRM (m/z 82.7 →336.8 for loratadine, m/z 309.9 →259.0 for desloratadine)	LLOQ=0.10	61	0.10-20.2	Fluspirilene
	Desloratadine (active metabolite)							100%	0.10-20.3	Fluoxetine
Matsuda et al., 2001	Ebastine and 2 metabolites	Plasma (1 mL)	MeOH-ACN (1:1), SPE [Bond Elut C ₁₈ cartridge, MeOH-50 mM	cyano column	ACN-MeOH-0.012M CH ₃ COONH ₄ (20:30:48), flow-rate: 1.2 mL/min	UV 254	LOQ=3	NS	3-1000	Flunarizine dihydrochloride

			phosphate buffer pH 2.5 (9:1)]							
Yang <i>et al.</i> , 2001	Loratadine, desloratadine (active metabolite)	Plasma (rat, rabbit, mouse, dog) (500 µL)	SPE: 96-well SPE C ₁₈ , 2mM CH ₃ COONH ₄ , 0.1% CH ₃ COOH, 0.1% HCOOH in 50:50 ACN in MeOH plates washed (then) with (2mM CH ₃ COONH ₄ , 0.1% CH ₃ COOH, 0.1% HCOOH in water)	four DBS-C ₈ columns	MeOH-25mM HCOONH ₄ pH 3.5 (85:15), isocratic, flow-rate: 0.8 mL/min	MS/MS (ESI)* * multiple x ed MRM (m/z 383 → 337 for loratadine, m/z 311 → 259 for desloratadine)	LOQ=1		1-1000	² H ₄ -loratadine, ² H ₄ -desloratadine
de Jager <i>et al.</i> , 2002	Cetirizine	Plasma (100 µL)	Extractionless method	Phenomenex Luna® C ₈ (2)	ACN-0.2% HCOOH (44:56)	MS/MS (ESI) SRM	LLOQ=0.5	92	0.5-808	Oxybutylin
Eriksen <i>et al.</i> , 2002	Cetirizine	Plasma (250 µL)	SPE (Oasis HLB cartridges, methanol)	XTerra MS C ₁₈	ACN-0.1% HCOOH, fast gradient (from 5-100%), flow rate: 0.4 mL/min	MS/MS (ESI) MRM (m/z 389.3 → 201.1 for cetirizine, 375.3 → 201.1 for hydroxyzine)	LOQ=5	89.7-97.7	5-500	Hydroxyzine
Fried <i>et al.</i> , 2002	Chlorpheniramine (R and S enantiomers) and metabolites	Plasma (1 mL)	LLE (alkalinization, ether)	CYCLOBOND I 2000™ column (β-cyclodextrin)	DEA (0.25%, pH 4.4)-MeOH-ACN [85:7.5:7.5, (v/v/v)], flow-rate: 0.5 mL/min	MS (ESI, API) SIM (m/z 275.1 for chlorpheniramine)	LLOD=0.13 LLOQ=0.25	80-84	0.1-50.00	
Hassan <i>et al.</i> , 2002	Cinnarizine, metabolites	Serum (rat, 1 mL)	LLE (acidification, chloroform)	Bondapack C ₁₈	MeOH-acetate buffer pH 4 (70:30), flow	MS (EI)	LOD=10 ng/µL	99.6	300-10,000	Sulphanilamide

					rate: 1.5 mL/min					
Takagaki <i>et al.</i> , 2002	Chlorpheniramine	Plasma (0.5 mL)	LLE (alkalinization, diethyl ether)	Develosil PhA	20 mM CH ₃ COONH ₄ -ACN-MeOH, gradient, flow-rate: 0.3 mL/min	MS (ESI) SIM (<i>m/z</i> 275)	LLOQ= 0.52	NS	0.52–20.8	AD-5423
Naidong <i>et al.</i> , 2002	Loratadine and other drugs	Plasma (200 µL)	PP with ACN / LLE (alkalinization, methyl- <i>tert</i> -butyl ether or hexane) / SPE (Certify mixed mode or Bond Elut C18 or Oasis HLB SPE, 2% NH ₄ OH in ACN or methanol)	Inertsil C ₁₈ -3 or Betasil silica column	ACN-water-formic acid, gradient elution, flow-rate: 0.5 mL/min	MS/MS (ESI) MRM (<i>m/z</i> 383 → 337 for loratadine)			0.050–100	
Naragalli <i>et al.</i> , 2003	Cetirizine hydrochloride (and paracetamol)	Plasma	LLE (diethyl ether)	CLC C ₁₈	ACN-water (55:45), flow rate: 0.8 mL/min isocratic elution	UV 230	LOD= 248 LOQ= 550	93–100	1.5–55,000 for cetirizine	Nimesulide
Yang <i>et al.</i> , 2003	Desloratadine	Plasma	TOMTE C [®] extractor and C ₁₈ SD 96-well extraction disk plates, 2 mM CH ₃ COONH ₄ , 0.1% CH ₃ COOH, 0.1% HCOOH in 50:50 (v/v) ACN in MeOH	BDS-C ₈	A: (20:80) ACN/water in 2 mM CH ₃ COONH ₄ , 0.1% CH ₃ COOH, 0.1% HCOOH and B: (85:15) MeOH/25 mM HCOONH ₄ , pH 3.5, flow rate: 0.250 mL/min	MS/MS (ESI) SRM (<i>m/z</i> 311.2 → 259.1)	LLOQ= 0.025	45–50	0.025–10 (for both)	[² H ₄]-desloratadine
	3-OH-desloratadine (metabolite)							55–59.6		[² H ₄]-3-OH-desloratadine

Yin <i>et al.</i> , 2003	Loratadine	Human plasma (1 mL)	LLE (alkalinization, toluene)	Waters Symmetry C ₁₈	MeOH-ACN-0.05M KH ₂ PO ₄ (pH 2.0), gradient, flow-rate: 1.2 mL/min	FLD (excitation 290 nm and emission 480 nm)	LLOQ= 0.5	80-82	0.5–16	Propranolol hydrochloride
	Desloratadine							67-70		
Naidong <i>et al.</i> , 2003	Loratadine	Plasma	LLE (alkalinization, hexane)	Betasil silica	ACN-water-TFA (90:10:0.1), isocratic, flow-rate: 0.5 mL/min	MS/MS (ESI) MRM (<i>m/z</i> 383 → 337 for loratadine and 311 → 259 for desloratadine)	LLOQ= 0.010	41-68	0.010-1	Loratadine- <i>d</i> ₃
	Desloratadine						LLOQ= 0.025		0.025-2.5	desloratadine- <i>d</i> ₃
Salem <i>et al.</i> , 2004	Loratadine	Plasma (1 mL)	LLE [alkalinization, isooctane-isoamyl alcohol (95:5, v/v)]	narrow-bore Zorbax SB-phenyl 5	ACN-0.2% HCOOH (68:32, v/v), flow-rate: 0.2 mL/min	MS/MS (ESI-IT) SRM (<i>m/z</i> 337 for loratadine)	LLOQ= 0.10	94-98	0.10-10.0	Itraconazole
Amini and Ahmadiani, 2004	Loratadine	Plasma (250 µL)	LLE (alkalinization, <i>n</i> -butyl alcohol- <i>n</i> -hexane (2:98), back-extraction into diluted HClO ₄)	Shimpack CLC-C ₈	ACN-20 mM NaH ₂ PO ₄ triethylamine (43:57:0.02), isocratic, flow-rate: 1.0 mL/min	FLD (excitation 290 nm and emission 460 nm)	LOD= 0.2 LOQ= 0.62	84	0.62–20	Diazepam
Liu <i>et al.</i> , 2004	Desloratadine	Dog plasma (1 mL)	LLE [alkalinization, toluene- <i>i</i> -PrOH (95:5, v/v)]	Hypersil CN column	MeOH-ACN-KH ₂ PO ₄ buffer pH 5.5, 0.01M (35:35:30), flow-rate: 0.8 mL/min	UV 241	LOD= 5	78.8-82	5.0-800	N-methyl-desloratadine
Chen <i>et al.</i> , 2004a	Loratadine	Plasma (0.5 mL)	LLE [alkalinization, hexane-methyl- <i>tert</i> -butyl (50:50, v/v)]	C ₁₈ -3 reverse column	ACN-CH ₃ COONH ₄ (pH 4.0, 0.02M) gradient, flow-rate: 0.2 mL/min	RP HPLC-MS (ESI) SIM	LOQ= 0.2	80-85	0.2-100	SCH 37370
Chen <i>et al.</i> ,	Chlorpheniramine	Plasma	LLE [alkalinization]	Diamonsil C ₁₈	MeOH-water-	MS/MS (APCI)	LLOQ= 0.2	77 (chlorpheniramine)	0.2–40.0	Diphenhydramine

2004 b	(and pseudoephedrine)	(0.5 mL)	ation, n-hexane-dichloromethane-2-propanol (2:1:0.1)]		HCOOH (70:30:1), flow-rate: 0.65 mL/min	SRM (m/z 275 $\rightarrow$ 230 for chlorpheniramine and 256 $\rightarrow$ 167 for diphenhydramine)	(chlorpheniramine)	heniramine)	(chlorpheniramine)	e
Li <i>et al.</i> , 2004	Cetirizine (and isoniazide)	Plasma (100 μ L)	SPE (Waters MCX m -Elute plate, ACN-5% conc. NH ₄ OH)	Betasil silica	ACN-water-HCOOH (85:15:0.1, v/v), isocratic, flow-rate: 0.5 mL/min (direct injection of eluents)	HILIC-MS/MS (ESI) MRM (m/z 275 $\rightarrow$ 230 for chlorpheniramine and 256 $\rightarrow$ 167 for diphenhydramine)	LLOQ=1	NS	1–1000	Cetirizine- d_8
Pého <i>et al.</i> , 2004	Hydroxyzine	Plasma (1 mL)	LLE [alkalinization, hexane-isoamylalcohol (99:1)]	Spherisorb S5 C ₈	ACN-phosphate buffer (45:55), flow rate: 2 mL/min	RP-HPLC-UV 235	LOD=10 LLOQ=20	~90	20–1500	Clothiapine
Kang <i>et al.</i> , 2004	Ebastine and 3 metabolites	Plasma (1 mL)	LLE [acidification, diethylether-dichloromethane (1:1)]	RP-Luna C ₁₈	ACN-5mM CH ₃ COONH ₄ (50:50%), isocratic, flow-rate: 0.2 mL/min	MS/MS (ESI) MRM (m/z 470 $\rightarrow$ 167 for ebastine)	LOD=0.2 (ebastine)	88–105	0.5–10 (ebastine)	Terfenadine
Song <i>et al.</i> , 2005	Cetirizine	Plasma (0.1 mL)	automated SPE [96-well plate on polymer sorbent (Strata X), ACN]	Betasil silica column	ACN-H ₂ O-CH ₃ COOH-TFA (93:7:1:0.025) flow-rate: 0.5 mL/min	HILIC-MS/MS (ESI) MRM (m/z 389 $\rightarrow$ 201 for cetirizine)	LLOQ=1.00	85–88	1.00–1,000	Cetirizine- d_8
Kim <i>et al.</i> , 2005	Cetirizine	Plasma (1 mL)	LLE with dichloromethane	C ₈ SG 80 for selective absorption of CTZ in plasma,	mobile phase 1 [ACN-20mM phosphate buffer	Semi-microbore column switching	LOD=2	>80	10–400	Methylparaben

				C_{18} UG 120V for concentration of CTZ as an intermediate column, C_{18} UG 120V column as a separation column	(pH 2.8) (15:85)], flow-rate: 0.5 mL/min mobile phase 2 [ACN-20mM phosphate buffer (pH 2.8) (35:65)], flow-rate: 0.1 mL/min	HPLC-UV 235				
Gupta <i>et al.</i> , 2005	Cetirizine enantiomers (R, S)	Guinea pig plasma (50 μ L)	LLE (plasma/brain: precipitation with ACN, centrifugation)	AGP* *a1-acid glycoprotein	6.5% ACN in 10mM ammonium acetate, pH 7.0, flow-rate: 0.9 mL/min	MS/MS (ESI) MRM (m/z 389.1 $\rightarrow$ 200.9 for cetirizine)	LLOQ= 0.25	NS	0.25–5000	ucb 20028
		brain tissue					LLOQ= 2.5 ng/g		2.5–250 ng/g	
		microdialysis samples					LLOQ= 0.25		0.25–50 (for both enantiomers)	
Sun <i>et al.</i> , 2005	Loratadine (and pseudoephedrine)	Plasma (1 mL)	LLE (alkalinization, ethyl ether)	C_{18} (Shim-pack)	ACN-water (80:20), containing 0.1% glacial acetic acid, gradient, flow-rate: 0.2 mL/min	MS (ESI) SIM (m/z 383 for loratadine)	LOQ= 0.010	83-93	0.05-10.00	Diazepam
Pragst <i>et al.</i> , 2006	Diphenhydramine and metabolites	Blood (0.5 mL)	LLE (Tris 0.2 M, pH 9.0, CH_2Cl_2)	RP8ec column	ACN:0.1 M phosphate buffer pH 2.3 in (37:63, v/v), isocratic, flow-rate: 1 mL/min	DAD	LOD= 300	NS	NS	5-(4-methylphenyl)-5-phenylhydantoin
Shen <i>et al.</i> , 2006	Loratadine	Plasma (0.25 mL)	96-well SPE Plates (acidification)	Waters Acquity C_{18}	Mobile phase A: 10mM HCOON	MS/MS (ESI) MRM	LLOQ= 0.025	36.1-41.5	0.025-10	$^2H_4$ -desloratadine

	3-hydroxydesloratadine	mL)	tion, MeOH-ACN-NH ₃ (45:45:10:4, v:v:v:v)		H ₄ with 0.2% HCOOH and mobile phase B: HCOONH ₄ in MeOH with 0.2% HCOOH, gradient	(m/z 311 → 259 for desloratadine)		52.2-55		² H ₄ -3-hydroxydesloratadine
Ren <i>et al.</i> , 2006	Chlorpheniramine	Plasma		Zorbax SB-C ₁₈	MeOH-water-HCOOH (80: 20: 0.5, v/v), isocratic, flow rate of 0.5 mL/min	MS/MS (ESI)	LLOQ= 0.05	NS	0.05-20	Diphenhydramine
	Ephedrine					SRM (m/z 275 → 230 for chlorpheniramine and 256 → 167 for diphenhydramine)			0.5-200	
Miyauchi <i>et al.</i> , 2006	Diphenhydramine	Serum (0.5 mL)	SPE (acidification, Oasis MCX, ACN, ACN-25% NH ₃ , glycerol-water (1:1))	Symmetry C ₁₈	MeOH-10 mM CH ₃ COONH ₄ , gradient, flow-rate: 0.2 mL/min	MS (ESI)	LOD= 0.5	83.2	NS	Ethinamide
	Hydroxyzine					SIM (m/z 256 for diphenhydramine and 375 for hydroxyzine)	LOD= 1	93.4		
	various sedative-hypnotics									
Sora <i>et al.</i> , 2007	Loratadine	Plasma (1 mL)	LLE (alkalinization, diethyl ether)	Purospher® STAR RP-18	15 mM SDS+0.2% H ₃ PO ₄ -ACN (49:51), isocratic, flow-rate: 1 mL/min	IPLC/FID (excitation 280 nm, emission 440 nm)	LLOQ= 0.3	75.7	0.5–20	8-chloroazatadine
	Desloratadine						LLOQ= 0.3	72.2	0.5–20	
Solans <i>et al.</i> , 2007	Rupatadine, desloratadine, 3-OH-desloratadine	Plasma (0.5 mL)	LLE (enzymatic hydrolysis with β-glycuronidase arylsulfatase from <i>Helix pomatia</i> , alkalization,	Colombus C ₁₈	0.2M CH ₃ COONH ₄ pH 4.5-ACN, gradient, flow-rate: 1 mL/min	MS/MS (CI) MRM (m/z 416.2 → 282.2 for rupertadine and 311.2 → 259.2 for	LOQ= 0.2 for all analytes		0.20-40	Clomipramine

			methyl- tert-butyl ether)			deslorat adine)				
Vlase <i>et al.</i> , 2007	Loratadine , desloratadi ne	Plas ma (0.3 mL)	PP with ACN	Zorbax SB-C ₁₈	0.4% HCOOH - ACN (92:8), isocratic , flow- rate:1 mL/min	MS/MS (ESPI) MRM (m/z 383 ->337 for loratadi ne and 311 ->259, 294, 282 for deslorat adine)	LOQ= 0.52 (for both)	~100	0.52– 52.3	Metoclo pramide
Srinu babu <i>et al.</i> , 2007	Loratadine	Plas ma (0.5 mL)	On-line coupling extractio n with Cyclone P 50 mm× 0.5mm, 50 µm HTLC column, 50% ACN in 10mM HCOON H ₄	Zorbax XDB C ₁₈	ACN- 0.5% HCOOH (10:90), gradient, flow- rate: 2.0 mL/min	High- through put LC– MS/MS (ESI) SRM (m/z 383.2 - >337.28 for loratadi ne,311. 6 ->259.0 for deslorat adine and 389.1 ->201.1 for cetirizin e)		73.2	0.409– 20.310	Cetirizin e
	Desloratad ine							57.1	0.403– 17.751	
El- Enan y <i>et al.</i> , 2007	Desloratad ine	Plas ma (1 mL)	SPE (Sep- Pak® C ₁₈ , 4% NH ₄ OH in MeOH- ACN- water (45:45:1 0)	Hibar®, LiChrosor b®, cyanoprop yl column, Hibar®, LiChrosor b® RP-18 pre-packed column	ACN- water (60:40), flow- rate: 1 mL/min	HPLC- UV (375 nm), fluoresc ence (reactio ns with appropri ate reagents I,II, III, IV)	LOD= 0.112 (I), 0.004 (II), 0.72 (III), 0.290 (IV) LOQ= 0.340 (I), 0.012 (II), 0.522 (III), 0.890 (IV)	99.5- 99.7	(I) 0.5- 6.0 µg/mL (II) 0.02- 0.4 µg/mL (III) 1- 10 µg/mL (IV) 1- 30 µg/mL	
Shen <i>et al.</i> , 2007	Desloratad ine (and	Plas ma (50 µL)	Micro- SPE (C ₈) (micro- SPE tips,	Betasil Silica-100	25mM CH ₃ CO ONH ₄ in 1%	ANP LC- MS/MS (ESI)	LOQ= 0.10	80 (four aspirate /dispens	0.10-40	² H ₄ - deslorata dine

	pseudoephedrine)		3% NH ₄ OH in MeOH)		HCOOH -25mM CH ₃ COONH ₄ in MeOH (15:85, v/v), isocratic, flow-rate: 0.3 mL/min	MRM (m/z 311 → 259 for desloratadine)		e cycles)		
Kowalski and Plenis, 2007	Cetirizine	Plasma (0.5 mL)	LLE with dichloromethane	Nucleosil-100 C ₁₈	ACN-water (60:40, v/v), flow rate: 1 mL/min	UV 227	LOD= 5 LOQ= 10	94	10-1000	Indometacin
Ming et al., 2007	Cetirizine	Plasma (200 µL)	PP	LiChrospher C ₈ column	water with 0.13% HCOOH -MeOH, gradient, flow-rate: 0.1 mL/min	MS/MS (ESI) SRM (m/z 389.07 → 200.91)	LOQ= 1.0	90-94	1.0-400	Phenylalanine
Hamad et al., 2007	Hydroxyzine	Serum (200 µL)	LLE with dichloromethane, labeling reaction	LiChrosorb Si 60	ACN-water (90:10, v/v) containing 0.02% Et ₃ N and 0.03% CH ₃ COOH, isocratic, flow-rate: 1.0 mL/min	FLD (excitation 320 nm, emission 410 nm)	LOD= 10 LOQ= 25	95	25-2000	Haloperidol
	Cetirizine						LOD= 5 LOQ= 25	75	25-2000	
Emara et al., 2007	Loratadine	Serum (250 µL)	Direct injection	a short protein-coated RP8 silica column, analytical column: Shim-pack cyanopropyl	Phosphate-buffered saline (pH 7.4), flow-rate: 1 mL/min <i>Analytical mobile phase:</i> ACN-12 mM CH ₃ COONH ₄ (45: 55), flow-rate: 1.5 mL/min	UV 243	LOD= 0.045 LOQ= 0.15	96-98	10-1000	
	Desloratadine							95-97		
del	Chlorpheniramine	Urine	SPE	Sunfire C ₈	Formate	MS/MS	LOD=	104.7	10-500	

Mar Rami rez-Fernandez <i>et al.</i> , 2007	ramine, other drugs and metabolites	(500 μ L)	(Oasis MCX, 5% NH ₃ in MeOH)	column	buffer-MeOH-CAN, gradient, 0.3 mL/min	(ESI) MRM (<i>m/z</i> 275.20 - >230.10, 167.11)	0.0125 LOQ=10			
Morita <i>et al.</i> , 2008	Levocetirizine	Plasma (200 μ L)	LLE with cold dichloromethane	Polaris C ₁₈	ACN-water-HCOOH (80:19.9:0.10), flow-rate: 0.2 mL/min	MS/MS (ESI) SRM (<i>m/z</i> 389 ->201)	LOQ=0.5	87.9	0.5-500.0	Fexofenadine
She <i>et al.</i> , 2008	Oxatomide	Plasma (0.5 mL)	LLE (alkalinization, ethyl ether)	VP-ODS C ₁₈	MeOH-CH ₃ COONH ₄ (10mM, pH 4.0) (85:15), isocratic, flow-rate: 0.2 mL/min	HPLC-MS (ESI) SIM (<i>m/z</i> 427.10)	LLOD=0.2 LLOQ=0.5	>90	0.5-500	Flunarizine hydrochloride
Tian <i>et al.</i> , 2008	Rupatadine	Plasma (1 mL)	LLE [alkalinization, cyclohexanediethyl ether (50:50, v/v)]	Shim-pack VP-ODS C ₁₈	MeOH-CH ₃ COONH ₄ (5mM, pH 2.2) (50:50) isocratic, flow-rate: 0.2 mL/min	HPLC-MS/MS (ESI) MRM (<i>m/z</i> 416 ->309)	LLOD=0.05 LLOQ=0.1	80-87	0.1-100	Ebastem
Ismail <i>et al.</i> , 2008	Chlorpheniramine	Plasma (500 μ L)	LLE (alkalinization, methyl- <i>tert</i> -butyl ether)	Betasil Diol-100	2mM HCOONH ₄ in MeOH-ACN (20:80), isocratic, flow-rate: 0.35 mL/min	MS/MS (ESI and APCI) MRM (<i>m/z</i> 275 ->230 for chlorpheniramine and 256 ->167 for diphenhydramine)	LLOQ=0.2	NS	0.2-100	Diphenhydramine or chlorpheniramine- <i>d</i> ₆
Strolin Benedetti <i>et al.</i> , 2008	Levocetirizine, dexchlorphenirizine	Plasma Urine	¹⁴ C-enantiomers / cetirizine	analytical chiral radio column Ultron ES-OVM	K ₂ HPO ₄ buffer 20 mM (pH 6.6)-ACN (88.5:11.5), isocratic, flow	UV	LOQ=20 LOQ=100		200-5,000	

					rate: 1 mL/min					
Wen <i>et al.</i> , 2009	Rupatadine	Plasma (0.5 mL)	LLE [methyl <i>tert</i> -butyl ether- <i>n</i> -hexane (1:1, v/v)]	Ultimate™ AQ-C ₁₈	MeOH-water (80:20) cont. 0.0005 % HCOOH, flow-rate: 0.3 mL/min	MS/MS (ESI) MRM (<i>m/z</i> 416 → 309 for rupatadine, 311 → 259 for desloratadine, 256 → 167 for diphenhydramine)	LLOQ= 0.1	78-81	0.1–50	Diphenhydramine
	Desloratadine							75-81	0.1–20	
Shakya <i>et al.</i> , 2009	Tripolidine	Plasma (200 µL)	direct PP	C ₁₈ ACE®	water-MeOH-HCOOH (55:45:0.5), isocratic, flow-rate: 0.3 mL/min	ESI-ion trap-MS/MS SIM (<i>m/z</i> 279.1 → 208.1)	LLOQ= 0.2	93.6	0.2–20.0	Gabapentin
Moreno <i>et al.</i> , 2010	Chlorpheniramine	Plasma (300 µL)	LLE (diethyl ether–dichloromethane 80:20, v/v)	Phenomenex Gemini® C ₁₈	MeOH-2.5 mM NH ₄ OH, gradient, flow-rate: 1.0 mL/min	MS/MS (ESI) MRM (<i>m/z</i> 275.2 → 230.2 for chlorpheniramine and 319.2 → 274.1 for bromopheniramine)	LLOQ= 0.05	79-87	0.05–10	Bromopheniramine
Lou <i>et al.</i> , 2010 (122)	Chlorpheniramine in combination with other drugs	Plasma (50 µL)	extraction by vortex-mixing for 20 seconds	YMC-ODS-AQ C ₁₈	0.3% (v/v) CH ₃ COOH-MeOH, gradient, flow rate: 0.30 mL/min	MS/MS (ESI) SRM (<i>m/z</i> 275.0 → 229.9 for chlorpheniramine and 256 → 167 for diphenhydramine)	LLOQ= 0.1 (chlorpheniramine)	92-106 (chlorpheniramine)	0.1–50 (chlorpheniramine)	Diphenhydramine

Patel <i>et al.</i> , 2010	Loratadine	Plasma (500 μ L)	LLE (alkalinization, ethyl acetate in hexane)	Betabasic cyano column	MeOH-water-1.0M $F_3COO NH_4$, flow-rate: 0.5 mL/min	MS/MS (ESI) MRM (m/z 383.3 $\rightarrow$ 337.1 for loratadine and 311.2 $\rightarrow$ 259.2 for desloratadine)	LLOQ= 0.05	89.6-90.7	0.05-15.00	Desipramine
	Desloratadine (active metabolite)							37.5-42.5		
Li <i>et al.</i> , 2010	Cinnarizine	Blood of dogs (0.4 mL)	LLE (MeOH, cyclohexane)	Microsphere C_{18}		UV 253		83-89	5.0-10,000	Diazepam
Hu <i>et al.</i> , 2011	Chlorpheniramine and three other drugs	Beagle dog plasma (1 mL)	LLE [alkalinization, diethyl ether-dichloromethane (60:40)]	RP Phenomenex Luna C_{18}	HCOOH-10 mM CH_3COONH_4 -MeOH (0.2:62:38), flow-rate: 0.2 mL/min	MS/MS (ESI) SRM (m/z 275.1 $\rightarrow$ 230.1)	LLOQ= 0.2	86-90	0.2-40	Lidocaine
Wang <i>et al.</i> , 2011	Mecizine	Plasma (100 μ L)	LLE with ACN containing 2 mM CH_3COONH_4 and 0.2% HCOOH	Zorbax SB- C_{18}	ACN-0.2% HCOOH cont. 2mM amino acetate, gradient, flow-rate: 0.35 mL/min	MS/MS (ESI) MRM (m/z 405 $\rightarrow$ 203)	LLOQ= 0.5	86.8-113.7	0.5-200	Flunarizine
Dharuman <i>et al.</i> , 2011	Cetirizine (and ambroxol)	Plasma (250 μ L)	LLE CH_2Cl_2 : Et_2O (80:20, v/v)	1. Supelcosil T M LC ABZ multifunction column for selective absorption of analytes in plasma 2. Kromasil C_8 micro bore analytical column used for concentration of analytes 3. A Kromasil C_{18} analytical	1. ACN-phosph. buffer pH 3.5 (20:80, v/v), flow-rate: 0.5 mL/min 2. ACN-phosph. buffer pH 3.5 (20:80, v/v), flow-rate: 0.5 mL/min 3. ACN- Et_3N (0.5%) in phosph. buffer (pH 3.5,	UV 230 (column switching, PDA detector)	LLOQ= 2	93	2-450	Nebivolol
		Urine (500 μ L)	LLE with diethyl ether				LLOQ= 1		1-500	

				column used as a separation column	20 mM) (55:45, v/v), flow-rate: 0.4 mL/min					
Ebrahimzadeh <i>et al.</i> , 2012	Chlorpheniramine (and dextromethorphan)	Plasma (2 mL)	fiber liquid phase microextraction (HF-LPME)	Capital HPLC column ODS-H C ₁₈	KH ₂ PO ₄ (pH=2.5, 0.01M)-ACN-MeOH (60:24:16), 1 mL/min isocratic	UV 220	LOD=3	~80 (chlorpheniramine)	0.01-1,000	
Hasnain <i>et al.</i> , 2013	Desloratadine	Plasma (0.5 mL)	SPE [Oasis HLB cartridges, NH ₃ :MeOH (3:97)]	hypurity Advance	A: ACN-MeOH (40:60) and B: MeOH-water (90:10) [90:10], flow-rate: 1 mL/min	MS/MS (ESI) MRM (m/z 311.03 → 259.1 for desloratadine)	LLOQ=0.1	74.6	0.1-11	Desloratadine-d ₅
	3-OH desloratadine							69.3		
Ebrahimzadeh <i>et al.</i> , 2013	Loratadine	Human plasma (10 mL), urine	MISPE-MHLE (1-decanol, SDS as surfactant)	ODS-H C ₁₈	KH ₂ PO ₄ (0.01 M, pH 2.8)-ACN (30:70, v/v), isocratic, flow-rate: 1 mL/min	PDA 254	LOD=200	90	1-1,000	
Wichitnithad <i>et al.</i> , 2015	Levocetirizine	Human plasma	PP (6% trichloroacetic acid in water)	Altima C ₁₈	ACN:10 M ammonium formate pH 3.5 (80:20, v/v), isocratic, flow-rate: 1 mL/min	MS/MS MRM (m/z 389 → 201 for levocetirizine and 375 → 201 for hydroxyzine)	LLOQ=1.0	59 (for levocetirizine) 60 (for hydroxyzine)	1-500	Hydroxyzine

Abbreviations:

ACN: acetonitrile, ANP: aqueous normal phase), CL: chemiluminescence, DEA: diethylamine, EI: electron impact, ESI: electron spray ionization, FLD: fluorescence detection, HILIC: hydrophilic interaction chromatography, HPLC: high-performance liquid chromatography, i-PrOH: isopropanol, LLOD: lower limit of detection, LLOQ: lower limit of quantification, LOD: limit of detection, LOQ: limit of quantification, MeOH: methanol, MRM: multiple reaction monitoring, MS: mass spectrometry, MS/MS: tandem mass spectrometry, NS: not stated, PDA: photodiode array, PP: protein precipitation, SDS = sodium dodecyl sulfate, SIM: single ion monitoring, SRM: selected reaction monitoring, UV: ultraviolet.

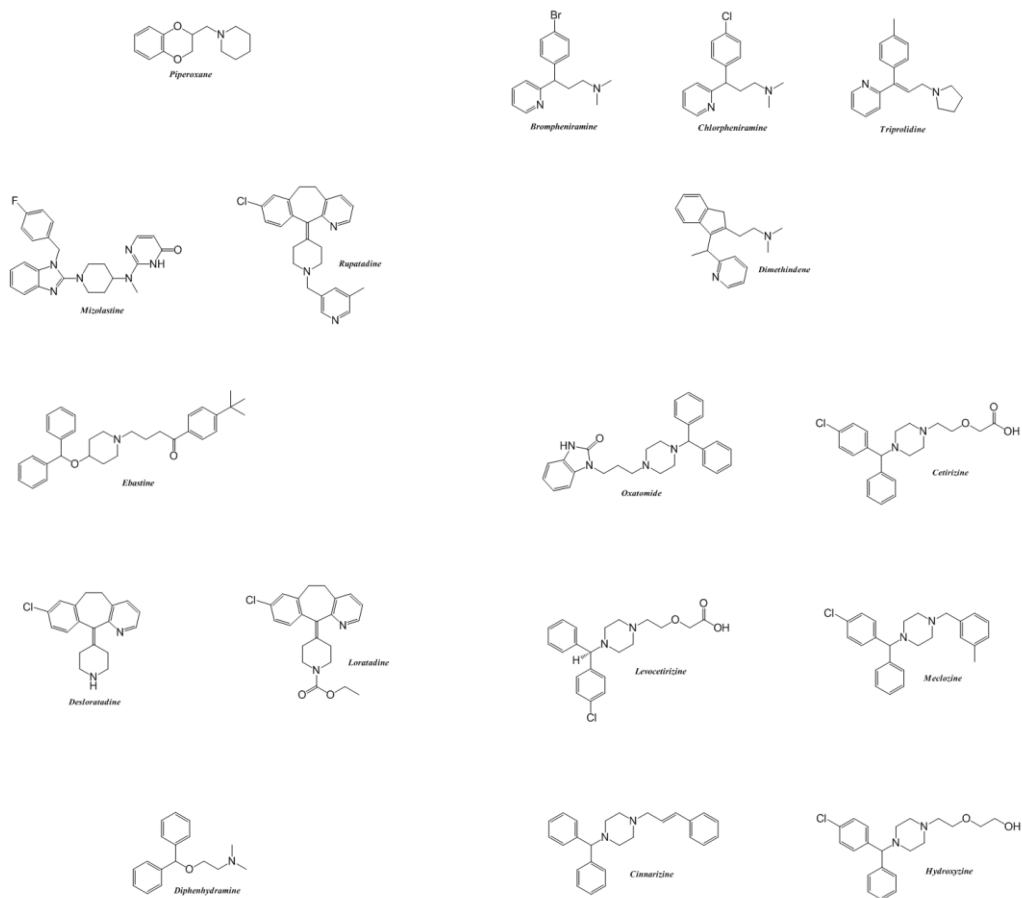


Figure 1. The chemical structures of the piperoxane (the first antihistamine) and the most commonly used antihistamines.