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Review

Chromatography of histamine H_1- and H_2-receptor blockers in biosamples[a]

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CONTENTS

[a] Further references regarding H_2-blockers can be found in the review by Mallikaarjun and Karnes on anti-ulcer compounds on p 407

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LIST OF ABBREVIATIONS

AC	Acetylated, acetate
ACN	Acetonitrile
AMU	Atomic mass unit
B	Blood
CI	Chemical ionization
CT (°C)	Column temperature in degrees centigrade
CU	Clean-up step
EI	Electron-impact ionization
EN	Enzymic cleavage of conjugates
EtAC	Ethyl acetate
EX	Liquid–liquid extraction
FL	Fluorescence detector
FSC	Fused-silica capillary
G	Gastric content
GC	Gas chromatography
HPLC	High-performance liquid chromatography
HPTLC	High-performance thin-layer chromatography
HY	Acid-hydrolysed
ID	Identification (qualitative detection)
INN	International non-proprietary name (WHO)
IR	Infrared
M	mol/l
m/z	mass to charge ratio
ME	Methylated
MeOH	Methanol
MS	Mass spectrometry
NPD	Nitrogen–phosphorus detection
NMR	Nuclear magnetic resonance
P	Plasma
PB (*x.x*)	Phosphate buffer (pH *x.x*)
PC	Precolumn
iPrOH	2-propanol
QU	Quantification
RP	Reversed phase
S	Serum
SIM	Selected-ion monitoring
SP	Solid-phase extraction
T	Tissue
Th. conc.	Therapeutic concentrations could be detected
TEA	Triethylamine
TFA	Trifluoroacetylated

TLC	Thin-layer chromatography
TMS	Trimethylsilylated
U	Urine
UV	Ultraviolet

1. INTRODUCTION

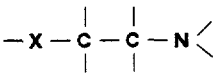
Histamine, the amine of "histos" (Greek word for tissue), is biosynthesized from the amino acid histidine by the L-histidine decarboxylase. It is stored mainly in secretory granules in tissue mast cells and in basophilic granulocytes. It produces its effects through actions on at least two types of receptor, the H_1- and H_2-receptors. Activation of H_1-receptors produces mainly bronchoconstriction, contraction of the gut, vasodilation with increased capillary permeability and stimulation of sensory nerve endings evoking pain and itching. Gastric acid secretion is stimulated by activation of H_2-receptors [1,2]. The described effects of histamine can be antagonized by the following three types of drug: blockers of H_1- and H_2-receptors (see Section 1.1), inhibitors of the L-histidine decarboxylase (*e.g.* tritoqualine) and inhibitors of histamine release from mast cells (*e.g* CI-922, ketotifen, oxatomide or tranilast). In this review, the second and third types are described with the H_1-receptor blockers to symplify matters. Further classifications and the structures of H_1- and H_2-receptor blockers are discussed in Section 1.1.

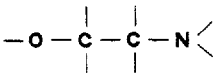
1.1. Classification and chemical structures

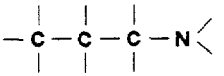
1.1.1. H_1-receptor blockers

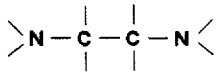
H_1-receptor blockers were first synthesized in 1942 [3]. Since then they have been called "antihistamines". After the discovery of the histamine H_1- and H_2-receptors they were named pharmacologically correctly as "histamine H_1-receptor blockers" or briefly as "H_1-blockers". The name "histamine receptor antagonists" is not correct, because these drugs are antagonists of histamine but not of its receptors. H_1-blockers are today one of the largest groups of drugs widely used as antiallergics. Some of these drugs are used as antiemetics because of their anticholinergic properties or as sedatives because of their depressant effect on the central nervous system. They are also in use for further indications. The usefulness of classic H_1-blockers is limited by side-effects, especially daytime sedation. This side-effect results in a decreased fitness to drive a car [4,5]. The new generation of H_1-blockers (*e.g.* loratanide or terfenadine) lack sedative properties [6].

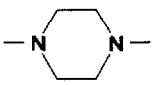
H_1-blockers are structurally similar to histamine, 2-(4-imidazolyl)ethylamine. In contrast to histamine, they have a tertiary amino group linked by a two- or three-atom chain to one or two aromatic rings (**1** in Fig. 1). The X linkage may be an oxygen (alkanolamine antihistamines, **2** in Fig. 1), a carbon (alkylamine antihistamines, **3** in Fig. 1), a nitrogen (ethylenediamine antihistamines, **4** in Fig. 1), a

1 

2 

3 

4 

5 

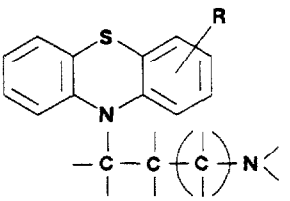
6 

Fig 1. Structures of H_1-receptor blockers (H_1-blockers or antihistamines) general structure (**1**), alkanolamine antihistamines (**2**), alkylamine antihistamines (**3**), ethylenediamine antihistamines (**4**), piperazine antihistamines (**5**), phenothiazine antihistamines (**6**)

nitrogen in a piperazine ring (piperazine antihistamines, **5** in Fig. 1) or a nitrogen in a phenothiazine ring (phenothiazine antihistamines, **6** in Fig. 1). For reasons of space, detailed structures have been omitted, because the chromatography of sixty different H_1-blockers is reviewed in this article. In Table 1 the H_1-blockers are listed according to their chemical class and within the class according to their International Non-Proprietary Names (INN of the World Health Organization) or, if this is not available, to their code names. The analytical methods reviewed herein and the tables with the summarized data are given for each drug. Note that mepyramine is often called pyrylamine in the literature.

TABLE I

LIST OF H_1-RECEPTOR BLOCKERS

The numbers of the structures in Fig. 1, the methods described in this review and the table numbers with the corresponding analytical data are given

*Alkanolamine derivatives (**2** in Fig. 1)*	
Carbinoxamine	GC (Table 6), GC–MS (Table 8)
Chlorbenzoxamine	GC–MS (Table 8)
Chlorphenoxamine	TLC (Table 3), GC (Table 6), GC–MS (Table 8)
Clemastine	GC (Table 6), GC–MS (Table 8)
Diphenhydramine	TLC (Table 3), GC (Table 6), GC–MS (Table 8)
Diphenylpyraline	GC–MS (Table 8)
Doxylamine	HPLC (Table 4), GC (Table 6), GC–MS (Table 8)
Mecloxamine	GC–MS (Table 8)
Medrylamine	GC–MS (Table 8)
Orphenadrine	GC (Table 6), GC–MS (Table 8)
Phenyltoloxamine	GC–MS (Table 8)
*Alkylamine derivatives (**3** in Fig. 1)*	
Azatadine	HPLC (Table 4), GC (Table 6), GC–MS (Table 8)
Azelastine	HPLC (Table 4)
Benzquinamide	GC–MS (Table 8)
Brompheniramine	GC (Table 6), GC–MS (Table 8)
Chlorphen(ir)amine	HPLC (Table 4), GC (Table 6), GC–MS (Table 8)
CI-922	HPLC (Table 4)
Cyproheptadine	HPLC (Table 4), GC (Table 6), GC–MS (Table 8)
Dimetindene	GC–MS (Table 8)
Ketotifen	GC–MS (Table 8)
Loratadine	HPLC (Table 4)
Mebhydroline	HPLC (Table 4), GC–MS (Table 8)
Phenindamine	GC–MS (Table 8)
Pheniramine	GC (Table 6), GC–MS (Table 8)
Pyrrobutamine	GC–MS (Table 8)
Temelastine	HPLC (Table 4)
Terfenadine	TLC (Table 3), GC–MS (Table 8)
Tolpropamine	GC–MS (Table 8)
Tranilast	HPLC (Table 4)
Triprolidine	HPLC (Table 4), GC (Table 6)
*Ethylenediamine derivatives (**4** in Fig. 1)*	
Adeptolon	GC–MS (Table 8)
Antazoline	TLC (Table 3), GC–MS (Table 8)
Bamipine	GC–MS (Table 8)
Chloropyramine	TLC (Table 3), GC–MS (Table 8)
Chlorothen	GC (Table 6)
Clemizole	GC–MS (Table 8)
Histapyrrodine	GC–MS (Table 8)
Mepyramine	TLC (Table 3), HPLC (Table 4), GC (Table 6), GC–MS (Table 8)
Methapyrilene	HPLC (Table 4), GC (Table 6)
(Pyrilamine, see Mepyramine)	

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TABLE 1 *(continued)*

Thenyldiamine	HPLC (Table 4), GC (Table 6)
Tripelenamine	HPLC (Table 4), GC (Table 6), GC–MS (Table 8)
*Piperazine derivatives (**5** in Fig. 1)*	
Buclizine	GC (Table 6), GC–MS (Table 8)
Chlorcyclizine	GC–MS (Table 8)
Cinnarizine	HPLC (Table 4), GC (Table 6), GC–MS (Table 8)
Cyclizine	GC (Table 6), GC–MS (Table 8)
Etodroxizine	GC–MS (Table 8)
Flunarizine	GC (Table 6)
Hydroxyzine	HPLC (Table 4), GC (Table 6), GC–MS (Table 8)
KB-2413	GC (Table 6)
Meclozine	HPLC (Table 4), GC (Table 6), GC–MS (Table 8)
Oxatomide	GC–MS (Table 8)
Revenast	GC (Table 6)
*Phenothiazine derivatives (**6** in Fig. 1)*	
Alimemazine	TLC (Table 3), GC (Table 6), GC–MS (Table 8)
Dimetotiazine	GC–MS (Table 8)
Isothipendyl	GC–MS (Table 8)
Mequitazine	GC–MS (Table 8)
Oxomemazine	GC–MS (Table 8)
Promethazine	TLC (Table 3), HPLC (Table 4), GC (Table 6), GC–MS (Table 8)
Thiethylperazine	GC (Table 6), GC–MS (Table 8)
Triflupromazine	GC (Table 6), GC–MS (Table 8)

1.1.2. H_2-receptor blockers

After the discovery of the H_2-receptors in 1972 [7] in the clinical use of the H_2-receptor blocker cimetidine was approved in 1977 by the U.S. Food and Drug Administration. It has since been widely used for therapy of gastric and duodenal ulcer diseases and other gastric hypersecretory states [2]. Other H_2-blockers have been produced and are in use or in clinical research The structures of the H_2-blockers reviewed in this article are shown in Fig. 2. Although the "classical" H_2-blocker cimetidine retaines the imidazole ring of histamine this is not essential for the effect. The only essential is a basic or basic substituted aromatic ring, as shown in Fig. 2 on the left side of the structures. The side-chain is longer than in histamine. Instead of the primary amino group of histamine, H_2-blockers contain relatively polar groups, usually unprotonated at physiological pH [8]. In Table 2 the H_2-blockers are listed according to their chemical class and within the class according to their INN or, if this is not available, to their code names. The analytical methods reviewed herein and the tables in which the data are summarized are given for each drug, together with the appropriate structure number in Fig. 2.

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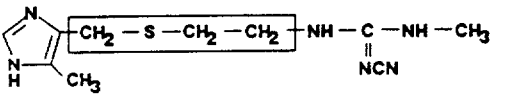

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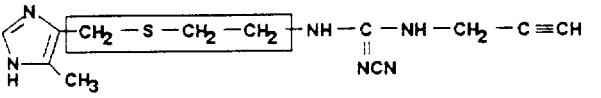

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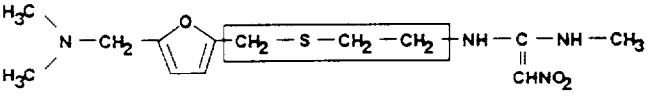

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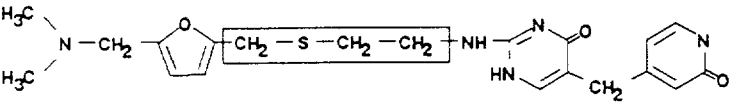

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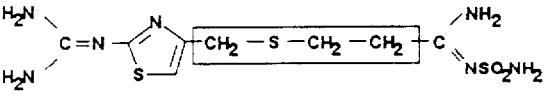

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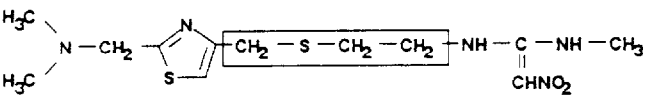

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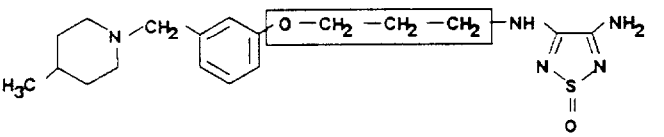

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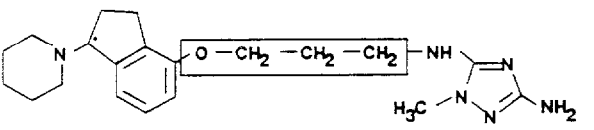

Fig 2

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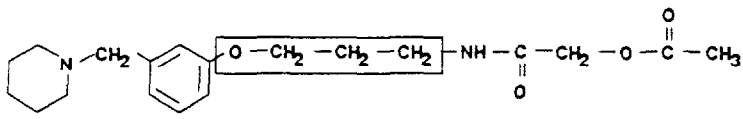

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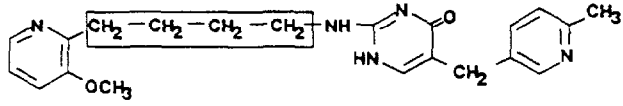

Fig 2 Structures of H_2-receptor blockers (H_2-blockers) cimetidine (**1**), etintidine (**2**), ranitidine (**3**), SKF-93574 (**4**), famotidine (**5**), nizatidine (**6**), ORF-17910 (**7**), RGW-2568 (**8**), roxatidine (TZU-0460) (**9**), icotidine (SKF-93319) (H_1 and H_2blocker) (**10**)

1.2. Biomedical importance of the procedures reviewed

Chromatographic procedures for the determination of H_1- and H_2-receptor blockers in biosamples are necessary for pharmacokinetic studies, including the identification of metabolites, for monitoring the compliance of patients resistant to treatment, and finally for several problems in clinical and forensic toxicology.

TABLE 2

LIST OF H_2-RECEPTOR BLOCKERS

The numbers of the structures in Fig 2, the methods described in this review and the table numbers with the corresponding analytical data are given

Imidazole derivatives	
Cimetidine (**1** in Fig 2)	HPLC (Table 5)
Entintidine (**2** in Fig. 2)	HPLC (Table 5)
Furan derivatives	
Ranitidine (**3** in Fig. 2)	HPLC (Table 5)
SKF-93574 (**4** in Fig 2)	HPLC (Table 5)
Thiazole derivatives	
Famotidine (**5** in Fig 2)	HPLC (Table 5)
Nizatidine (**6** in Fig 2)	HPLC (Table 5)
Arylether derivatives	
ORF-17910 (**7** in Fig. 2)	HPLC (Table 5)
RGW-2568 (**8** in Fig 2)	HPLC (Table 5)
Roxatidine (TZU-0460) (**9** in Fig 2)	HPLC (Table 5), GC (Table 7), GC–MS (Table 9)
Pyridine derivative	
Icotidine (SKF-93319) (**10** in Fig 2) (H_1- and H_2-blocker)	HPLC (Table 5)

1.2.1. Pharmacokinetic studies

Quantification of drugs and at least their pharmacologically active metabolites in plasma is essential for pharmacokinetic studies Chromatographic methods are the procedures of choice because the parent compound and its metabolites can be separately determined, in contrast to immunoassays and radioactivity measurements.

Despite the widespread use of H_1-blockers for almost 50 years, little was known about their clinical pharmacokinetics until quite recently [9]. Many of these drugs were put onto the market before there was much interest in pharmacokinetic studies. Furthermore, the concentrations of these lipophilic drugs in biosamples are very low, and only modern chromatographic techniques have the sensitivity and specifity required for their quantification. Since H_1-blockers are lipophilic, they can be determined by gas chromatography (GC) with or without mass spectrometry (MS). Because of their aromatic rings, they can also be determined by high-performance liquid chromatography (HPLC) with ultraviolet detection (UV). HPLC techniques were most frequently used for quantification of H_1-blockers in biosamples in the period covered by this review. However, in some cases GC procedures were more sensitive than HPLC procedures [10–13]. If GC procedures described earlier had been approved [14], they were used for actual pharmacokinetic studies [15,16]. Finally, a new GC assay was described for the quantification of twelve H_1-blockers in plasma [17]

In contrast to the H_1-blockers, H_2-blockers are relatively polar and hydrophilic molecules [1,8]. Most of them cannot be determined by GC, at least not with the required sensitivity, so HPLC is the method of choice. Only roxatidine (**9** in Fig. 2), which lacks a polar group, was determined by GC [18] and GC–MS [19].

1 2.2. Identification of metabolites

For the identification of metabolites in urine or fungal cultures [20], chromatographic separation methods were coupled off-line or on-line with spectroscopic techniques. Off-line coupling was performed using thin-layer chromatography (TLC) [21] or HPLC [19,20,22,23]. As usual, GC was coupled on-line with the MS [19,24–30], a method that is preferable in cases where the GC volatility of the metabolites is sufficient after derivatization and the mass spectral information is also sufficient.

1.2.3. Monitoring patient compliance

Therapeutic drug monitoring of histamine receptor blockers is unusual because the margin of therapeutic safety of these drugs is relatively large. However, the compliance of patients resistant to treatment should be monitored by determination of the plasma concentrations. Procedures described for pharmacokinetic or toxicological purposes can be applied. In many cases it is sufficient to detect the H_1-blockers in urine using TLC, GC or GC–MS. In any case of doubt the TLC and GC results should be confirmed, preferably by GC–MS [24–27].

1.2.4 Clinical and forensic toxicology

H_2-blockers do not lead to severe clinical symptoms when taken in overdose [31,32]. However, H_1-blockers are often, alone or in combination with other drugs and/or ethanol, the cause of intoxication [33,34] or the cause of decreased fitness to drive a car [4,5]. Because H_1-blockers used in ointments can be absorbed transdermally [35,36], intoxications have been observed when large areas of skin, especially of infants and children, were embrocated [37–39]. When addicted patients, therapeutically treated with H_1-blockers, are toxicologically monitored, H_1-blockers must be differentiated from drugs taken addictively. Therefore, qualitative and quantitative determination of H_1-blockers is necessary for many toxicological purposes. Immunoassays are not commercially available, because therapeutic drug monitoring is not routinely performed and, therefore, the market is too small. As described in Section 1.2.1, quantification can be performed using HPLC, GC or GC–MS To simplify the selection of the most appropriate procedure in emergency cases, the procedures are listed according to the methods, the category and finally to the INN of the drugs (Tables 3–9). Further details are discussed in Section 3.

However, before quantification in plasma (see QU in ID/QU column in Tables 3–9) the drugs, which are usually unknown, must first be identified (see ID). It is preferable to use urine for the screening because the concentrations of the drugs are much higher in urine than in plasma. Because more than sixty H_1-blockers are in use, and each of them may produce several metabolites, identification using chromatographic procedures without highly specific detection (such as MS) is very troublesome. Finally, the H_1-blockers must be differentiated from thousands of other drugs, poisons or endogenous biomolecules, because any chromatographic zone or peak may represent a potential poison. The efficiency for these purposes of the described TLC, HPLC, GC or GC–MS procedures is discussed in Section 3.

1.3 Choice of the references

The reviewed references were selected by on-line searching in the Medline database on CD-ROM (Silver Platter, Boston, London, Amsterdam, 1985–1989) and in the Chemical Abstracts Services The period from January 1985 to September 1989 was covered. Furthermore, additional references cited in the bibliography sections of the *Journal of Chromatography* from 1985 to August 1989 were used. Papers from 1984 were considered, if they were not reviewed by Schwarz *et al.* in 1985 [40]. In accordance with the aims of this review volume, methods for the determination of histamine receptor blockers in drug preparations were not considered.

2 SAMPLE PREPARATION

Appropriate preparation of samples is an important prerequisite for chromatography in biosamples It involves isolation and if necessary cleavage of conjugates and/or derivatization of the drugs and their metabolites. Isolation was performed by liquid–liquid extraction at a pH at which the analyte is unionized (see EX in Work-up column in Tables 3–9) or by solid-phase extraction (see SP) followed by clean-up steps (see CU) and concentration of the extract. In my experience, solid-phase extraction is preferable if particular substances have to be selectively isolated in series from relatively homogeneous samples, such as plasma samples, in pharmacokinetic studies. Universal liquid–liquid extraction procedures are preferable for "general unknown analysis" procedures in emergency cases [24–27,41–50] because substances with very different physicochemical properties must be isolated from heterogeneous matrices.

Conjugates can be cleaved by gentle but time-consuming enzymic hydrolysis (see EN) in metabolic studies. In toxicological analysis it is preferable to cleave the conjugates by rapid acid hydrolysis (see HY). However, the possible formation of artifacts during this procedure must be monitored. Derivatization steps are necessary if relatively polar compounds are to be determined by GC or GC–MS. In the reviewed papers, the following procedures were used. propionylation, acetylation (AC), methylation (ME), trimethylsilylation (TMS) and trifluoroacetylation (TFA) (see Work-up column in Tables 3–9). Acetylation has been approved for the identification of metabolites [24–27,41–54] It leads to stable derivatives with good GC properties. The acetylation mixture can be evaporated before analysis so that the resolving power of capillary columns does not decrease, in contrast to TMS, for example The mass spectra of acetyl derivatives can easily be interpreted, but TMS derivatives often produce an intense peak at m/z 73 (C_3H_9Si) and no further characteristic fragment ions. The molecular mass does not increase very much, in contrast to what happens with TMS or TFA, so that compounds with relatively high molecular mass and several derivatizable groups can be measured with low-priced mass-selective detectors with a mass range up to only 650 a.m.u.

Derivatization for HPLC procedures using UV or fluorescence detection are required if there are no UV-absorbing or fluorescent structures [55,56] in the molecule.

2.1. Plasma

H_1- and H_2-blockers are drugs with alkaline pK_a values, and therefore the isolation steps in all the reviewed papers were performed at an alkaline pH usually after addition of aqueous sodium or potassium hydroxide. Diethyl ether, ethyl acetate, dichloromethane, chloroform or alkanes were used as extractants in most cases. Clean-up steps were performed in some procedures (see CU in Work-

up column in Tables 3–9). However, it should be checked if these relatively complicated and time-consuming steps are really necessary for the aim of the study.

2.2. Urine

Most of the lipophilic H_1-blockers are excreted in urine in a metabolized and conjugated form, especially in the late phase of excretion Therefore, the conjugates must be cleaved by enzymic or acid hydrolysis before isolation. Polar metabolites (*e g.* hydroxy- and N- or O-desalkyl metabolites), which should be identified or quantified in urine, must be derivatized if a GC procedure such as GC–MS is to be used (see EN, HY, AC, TMS, ME, TFA in Work-up column in Tables 3–9).

The relatively polar H_2-blockers are excreted in urine almost unchanged. Therefore, the isolation procedure for plasma can also be used for urine.

2.3. Other biosamples

Saliva samples [35] and gastric contents [57] can be worked up like plasma samples. When bile samples are extracted at a basic pH, interfering peaks appear. One or two clean-up steps (CU) are necessary to eliminate the endogenous biomolecules [58,59]. Tissue samples (*e g.* brain) are first homogenized and then the matrix is removed by extraction [60]. Milk samples should be centrifuged before extraction to remove the fatty layer [18,61]. When fungal cultures are worked up, the fungal cells are pelleted by centrifugation before conventional extraction [20,22,62].

3 CHROMATOGRAPHY

Chromatographic procedures for the determination of histamine H_1- and H_2-receptor blockers in biosamples published in the past five years are reviewed here according to the techniques used. The principal information on each procedure is summarized in Tables 3–9 to simplify the rapid selecton of a suitable method.

The procedures are listed according to the drug names (INN or code names) If metabolites were determined, "metabolite(s)" is given in the Drug column. Entries in the ID/QU column specify whether the drugs were identified (ID) or quantified (QU). Qualitative detection procedures are subsumed under ID. The kind of biosample used is given in the Sample column (P = plasma, S = serum, B = blood, U = urine, G = gastric contents, T = tissue). The sample preparation discussed in Section 2 is concisely summarized in the Work-up column. The principal information on the stationary and mobile phase, as well as the detection mode and the analytical detection limit, are given. For reasons of space, not all parameters of the analytical quality control are listed. Unfortunately, these data, which are essential for quantification in plasma, were reported incompletely or

not at all in some papers, especially in some pharmacokinetic papers (*e.g.* refs. 11, 12, 15, 16, 23 and 63–68). This is in accordance with the report published in 1988 by Eggers and Bircher [69]. However, independent interpretation of pharmacokinetic results, for example, is difficult or even impossible, when the quality criteria are not sufficiently documented.

For toxicological screening procedures in urine, the quality criteria of the parent compound are of limited value if the amounts of metabolites are much higher in urine than those of the parent drug and the metabolites are detected by the procedure. Most of the toxicologically relevant drugs (*e.g.* H_1-blockers) are lipophilic substances that undergo extensive metabolism. Because pure samples of the metabolites are not usually available, it is necessary to control the quality of the screening procedures using urine samples from volunteers or in-patients treated with a known dose of the drug [24–28,70]. The procedure should be sufficiently sensitive to detect therapeutic concentrations at least over a 12–24 h period after ingestion (see Th. conc. in the Det. limit column in Tables 3–9). In my experience, procedures are suitable for toxicological purposes if this criterion is met

3.1. Thin-layer chromatography

3.1.1. H_1-receptor blockers

Only one TLC procedure for the detection of a few H_1-blockers in urine (Table 3) was published in the period covered [70]. This relatively simple procedure is claimed to allow monitoring of patient compliance in a pharmacy laboratory. However, it is very difficult to detect the prescribed drug if other or additional drugs were taken. Neither the sample preparation (extraction with dichloromethane or diethyl ether at an alkaline pH), nor the chromatographic separation (very similiar R_F values of different drugs and suspected metabolites) is of sufficient selectivity and specifity, and neither is the chemical detection (Dragendorff's reagent). This simple procedure can be recommended only for toxicological screenings, if positive results are confirmed especially by GC–MS [24–27].

TLC is still used for separation of metabolites prior to their identification using MS, nuclear magnetic resonance (NMR), infrared (IR) and/or UV spectroscopy [21].

3.2. High-performance liquid chromatography

3.2.1. H_1-receptor blockers

Because of their widespread application, HPLC techniques were most frequently used for quantification of H_1-blockers in biosamples in the period covered. As shown in Table 4 most of the papers describe reversed-phase chromatography on 5-μm octadecyl or cyano columns at ambient temperature with isocratic elution and UV detection. If the column was heated, the column temper-

TABLE 3

THIN-LAYER CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF H_1-RECEPTOR BLOCKERS IN BIOSAMPLES

Drug	ID/QU	Sample	Work-up	Stationary phase	Mobile phase	Detection	Det. limit	Ref.
Alimemazine metabolites	–[a]	U	EN, EX	Silica gel	(I) Benzene–acetone–MeOH (7·2·1); (II) chloroform–MeOH (9·1); (III) chloroform–MeOH acetone–H_2O (64 27 5 4)	$HCHO/H_2SO_4$		21
Antazoline	ID	U	EX	Silica gel (HPTLC)	Toluene–iPrOH–ammonia (25%) (3;6 1)	Dragendorff	Th. conc	70
Chloropyramine	ID	U	EX	Silica gel (HPTLC)	Toluene–iPrOH–ammonia (25%) (3 6 1)	Dragendorff	Th. conc	70
Chlorphenoxamine	ID	U	EX	Silica gel (HPTLC)	Toluene–iPrOH–ammonia (25%) (3 6 1)	Dragendorff	Th. conc	70
Diphenhydramine	ID	U	EX	Silica gel (HPTLC)	Toluene–iPrOH ammonia (25%) (3 6 1)	Dragendorff	Th. conc	70
Mepyramine	ID	U	EX	Silica gel (HPTLC)	Toluene–iPrOH ammonia (25%) (3 6 1)	Dragendorff	Th. conc	70
Promethazine	ID	U	EX	Silica gel (HPTLC)	Toluene iPrOH–ammonia (25%) (3 6 1)	Dragendorff	Th. conc	70
(Pyrilamine, see Mepyramine)								
Terfenadine	ID	U	EX	Silica gel (HPTLC)	Toluene–iPrOH–ammonia (25%) (3,6,1)	Dragendorff	Th. conc	70

[a] The compounds were separated for spectroscopic identification

ature (CT) is given in Table 4. In some cases the analytical column was protected with a precolumn (see PC in Table 4). Column-switching techniques have been applied if peaks of biomolecules interfered with the determination [61,71]. The mobile phase was usually a mixture of phosphate buffer (PB) with variable pH and an organic modifier such as methanol (MeOH) or acetonitrile (ACN). Because basic drugs often give poor peak shapes on reversed-phase columns, minor amounts of an amine such as triethylamine (TEA), *n*-nonylamine or dibutylamine were added (see Mobile phase column). Gradient elution was used to prevent contamination of later samples [72] or to improve the separation if several metabolites were to be detected [23,29,73]. For the determination of very low concentrations (< 1 ng/ml), electrochemical [71] or fluorescence detection without [74,75] or after derivatization [55,56] was preferred.

Most of the described HPLC procedures can be used to quantify H_1-blockers in biosamples for pharmacokinetic and toxicological purposes, because the criteria of quality control and the detection limits are sufficient. Gill and Wanogho [76] described an HPLC system for the separation of some H_1-blockers and antidepressants. This study was performed using solutions of pure substances. Although the aim of the study was the identification of unknown compounds and the quantification of specific drugs in (forensic) biosamples, corresponding studies were not performed. Neither sample preparation procedures nor data on the selectivity, specifity and sensitivity are given. In my experience, unknown compounds cannot be identified using such a simple chromatographic procedure, because thousands of toxicologically relevant substances and metabolites have to be differentiated. However, the combination of HPLC with photodiode array detectors allow a more selective detection. If a suitable UV spectral library is available, unknown drugs can be identified in biosamples using computer library searches [77–79] Unfortunately (in contrast to MS), UV spectra of metabolites, endogenous biomolecules and impurities can only be recorded using pure reference substances.

3.2.2. H_2-receptor blockers

HPLC is the method of choice for the chromatographic determination of H_2-blockers in biosamples, because they are relatively polar and hydrophilic compounds. The chromatographic conditions applied are rather variable (Table 5) in contrast to those used for H_1-blockers Silica or reversed-phase C_{18}, C_8 or CN columns were used with isocratic elution and UV detection. If the column was heated the column temperature (CT) is given in Table 5 In some cases the analytical column was protected with a precolumn (see PC in Table 5). Some of the H_2-blockers were eluted by a mixture of phosphate buffer (PB) and an organic modifier such as methanol (MeOH) or acetonitrile (ACN). Ammonia, sodium acetate or phosphoric acid was also used as the aqueous component. Typical high-performance liquid chromatograms of extracts from drug-free samples (A, D), samples spiked with ranitidine (B, E), and samples after treatment with 10

TABLE 4

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF H_1-RECEPTOR BLOCKERS IN BIOSAMPLES

Drug	ID/QU	Sample	Work-up	Stationary phase	Mobile phase	Detection	Det. limit	Ref.
Azatadine	QU	U	Alkal HY, EN, EX, CU	μBondapak CN (10 μm), PC silica Guard-Pak	0.05 *M* PB (5.75)–ACN (20:9), 2 ml/min	UV 214 nm		63
Azatadine	–[a]	–[a]	–[a]	Hypersil ODS (5 μm)	ACN–PB (3:0) (3:7), + 0.06% nonylamine, 2 ml/min	UV 230 nm		76
Azelastine and metabolite	QU	P	EX, CU	Ultrasphere ODS RP (5 μm), CT (°C) 30	Tetrahydrofuran–H_2O (30:70) + 0.3% TEA	FL 213 nm	0.33 ng/ml	74
Chlorphen(ir)amine	QU	P	EX	Spherisorb CN (5 μm)	0.05 *M* NH_4AC–ACN (30:70), 3 ml/min	UV 229 nm	0.156 ng/ml	87
Chlorphen(ir)amine	QU	S	EX	Supelco LC-8-DB (3 μm)	0.02 *M* PB (2.0)–ACN (85:15) + 0.2% TEA, 1.5 ml/min	UV 262 nm		64
Chlorphen(ir)amine	QU	B	SP[b]	Zorbax ODS; CT (°C) 50	MeOH–H_2O (84:16), 1.7 ml/min	FL 350 nm	0.1 ng/ml	55
Chlorphen(ir)amine	QU	P, U	EX	Ultrasphere Si (5 μm)	7.5 m*M* PB (7.0)–ACN (40:60), 1 ml/min	UV 200 nm	0.7 ng/ml P, 100 ng/ml U	88
Chlorphen(ir)amine and metabolites	QU	T	EX	Alltech C_{18}	ACN–PB (2.5) (1:1), 1 ml/min	UV 254 nm	2000 ng/ml	36
CI-922	QU	P	EX	Biosil ODS (5 μm)	ACN–H_2O–CH_3COOH (50:50:1), 1 ml/min	UV 340 nm	20 ng/ml	89
CI-922	QU	P	SP, CU	Bio-Rad C_{18} (5 μm)	ACN–H_2O–CH_3COOH (45:55:1), 1 ml/min	UV 340 nm	25 ng/ml	90
Cinnarizine	QU	B, P	EX	Ultrasphere RP-18 (5 μm), PC do (10 μm)	MeOH–PB (7:0) (9:1), 1.5 ml/min	UV 254 nm	2 ng/ml	91
Cyproheptadine and metabolites	QU	T	EX, CU	Partisil 10/25 ODS	MeOH–0.03 *M* KH_2PO_4 (60:40), 1.4 ml/min	UV 210 nm	500 ng/g, 200 ng/g for metabolites	92
Cyproheptadine	–[a]	–[a]	–[a]	Hypersil ODS (5 μm)	ACN–PB (3:0) (3:7), + 0.06% nonylamine, 2 ml/min	UV 230 nm		76

Deptropine	–[a]	–[a]	–[a]	Hypersil ODS (5 μm)	ACN–PB (3·0) (3·7), + 0 06% nonylamine, 2 ml/min	UV 230 nm		76
Doxylamine and metabolites	ID	U	HY, EX	Supelco LC-CN (5 μm), PC ditto (50 μm)	Gradient. (A) MeOH–0.01 *M* PB (7 3) (5:95), (B) MeOH–0 01 *M* PB (7.3) (95 5), 1 ml/min	UV 254 nm		29,73
Hydroxyzine	QU	S	EX, CU	Waters CN RP	ACN–0 075 *M* PB (3 0) (27 73) + 0 02 *M* dibutylamine, 1 ml/min	UV 229 nm	3 ng/ml	93, 94
Loratadine metabolite	QU	P, breast milk	EX, NBD-Cl	Zorbax CN (5 μm)	Toluene–EtAC (96 4), 1 ml/min	FL 465 nm	0.6 ng/ml	56, 95, 96
Mebhydroline	–[a]	–[a]	–[a]	Hypersil ODS (5 μm)	ACN–PB (3 0) (3 7), + 0.06% nonylamine, 2 ml/min	UV 230 nm		76
Meclozine	QU	P	EX	Novapak C_{18} (4 μm); PC Corasil	Gradient. (A) 8 m*M* $NaC_{12}H_{23}SO_4$ + 0.5% CH_3COOH (pH 4.3); (B) 40 m*M* $NaC_{12}H_{23}SO_4$ + 0 5% CH_3COOH–ACN (20:80), 1 2 ml/min	UV 232 nm	25 ng/ml	72
Meclozine	QU	P, U	EX	Ultrasphere RP-8 (5 μm)	ACN–H_2O (30.70), 2 5 ml/min	UV 230 nm	20 ng/ml	97
Mepyramine	QU	U	EX	Ultrasphere Si (5 μm)	CH_2Cl_2–iPrOH + 5 m*M* TEA (995 5), 2 ml/min	FL 310 nm	1 ng/g	75
Mepyramine and metabolites	–[c]	Fungal cultures	EX	Ultrasphere CN (5 μm)	0 01 *M* PB (7.0)–ACN (60·40), 2 ml/min	UV 254 nm		22
Mepyramine and metabolites	ID[c]	P, U, CU	EN, EX,	Supelco C_{18} (5 μm); PC Corasil C_{18} (40 μm)	Gradient. MeOH–0 01 *M* PB (7 0) (10:90) to (100 0), 1 ml/min	UV 254 nm		23
Mepyramine and metabolites	QU	P, U	EN, EX	Supelco CN (5 μm), PC Pelliguard LC-CN (50 μm)	Gradient· MeOH–0.01 *M* PB (7.0) (5·95) to (95:5), 1 ml/min	UV 254 nm, radioactivity measurement		23

(Continued on p 386)

TABLE 3 *(continued)*

Drug	ID/QU	Sample	Work-up	Stationary phase	Mobile phase	Detection	Det. limit	Ref
Methapyrilene	QU	U	EX	Ultrasphere Si (5 μm)	CH_2Cl_2–iPrOH + 5 m*M* TEA (995·5) 2 ml/min	FL 310 nm	1 ng/g	75
Methapyrilene and metabolites	QU	Fungal cultures	EX	Ultrasphere CN (5 μm)	0.01 *M* PB (7 0)–ACN (60·40), 2 ml/min	UV 254 nm		62
Promethazine	QU	P	EX	Micropak CN (10 μm)[d]; PC ditto, pressure col ditto (5 μm)	ACN–0 01 *M* $NH_4H_2PO_4$ (55 45)	Electro-chemical detection	0 1 ng/ml	71
(Pyrilamine see Mepyramine)								
Temelastine	QU	P, U, bile	(EN), EX, CU	Zorbax CN (5 μm), CT 35°C	MeOH–H_2O–H_3PO_4 (500·500·0 56)	UV 229 nm	50 ng/ml	58
Thenyldiamine and metabolites	QU	Fungal cultures	EX	Ultrasphere CN (5 μm)	0 01 *M* PB (7.0)–ACN (60 40), 2 ml/min	UV 254 nm		62
Tranilast	QU	P	EX	Radial Pak C_{18} (10 μm)	0 01 *M* PB (3 5) ACN (4 3), 1 5 ml/min	UV 280 nm	500 ng/ml	98
Tripelenamine	QU	Bovine P, milk	P· PC, milk: EX	Techsphere CN (3 μm)[d], PC Corasil RP 18 (37 50 μm)	ACN–0 05 *M* acetate buffer (7.2) (70 30), 0 9 ml/min, PC H_2O, 0.9 ml/min	UV 246 nm	2 ng/ml	61
Tripelenamine and metabolites	QU	Fungal cultures	EX	Ultrasphere CN (5 μm)	0 01 *M* PB (7 0)–ACN (60 40), 2 ml/min	UV 254 nm		62
Triprolidine	QU	P, U	EX, CU	Bondapak RP-18	ACN–0 075 *M* PB (2 5)–diethylamine (25 75:0 03), 1 ml/min	UV 229 nm	3 ng/ml	99
Tripolidine and metabolites	—[c]	Fungal cultures	EX	Ultrasphere CN (5 μm)	0 01 *M* PB (7 0)–ACN (60 40), 2 ml/min	UV 254 nm		20
Tripolidine	—[a]	—[a]	—[a]	Hypersil ODS (5 μm)	ACN–PB (3 0) (3 7), + 0.06% nonylamine, 2 ml/min	UV 230 nm		76

[a] A system is described only for separation of some antidepressants and antihistamines Its applicability for the qualitative and quantitative determination in biosamples is only discussed

[b] Benzyl chloroformate derivatization

[c] The compounds were separated for spectroscopic identification

[d] Column-switching technique

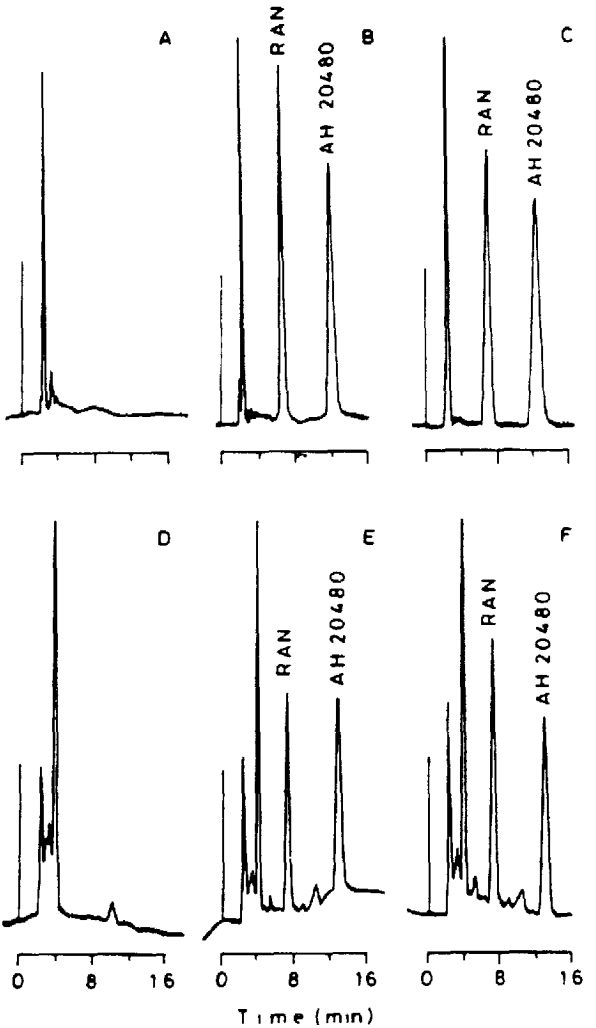

Fig. 3 Typical high-performance liquid chromatograms of extracts from drug-free samples (A, D), samples spiked with ranitidine (B, E) and samples after treatment with 10 mg/kg ranitidine (C, F) [60] A, B and C were rat plasma samples and D, E and F were rat brain samples (Details of the method are summarized in Table 5)

mg/kg ranitidine (C, F) are shown in Fig. 3 [60]. A, B and C were rat plasma samples and D, E and F were rat brain samples.

The described HPLC procedures allow either precise quantification of H_2-blockers in biosamples or separation prior to spectroscopic identification. However, it is incomprehensible why editors of scientific journals accept papers in which basic chromatographic data, such as detection mode or stationary and mobile phase, are not given [67,80]. The lack of quality control data has already been criticized in Section 3.

TABLE 5

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF H_2-RECEPTOR BLOCKERS IN BIOSAMPLES

Drug	ID/QU	Sample	Work-up	Stationary phase	Mobile phase	Detection	Det. limit	Ref.
Cimetidine[a]	QU	P	EX	Spherisorb Si (5 μm)	ACN–H_2O–ammonia (95:5:0.2), 1 ml/min	UV 228 nm	100 ng/ml	100
Cimetidine	QU	P, U	SP	Sepralyte RP-18 (3 μm), CT 50°C	(0.1 *M* $NaClO_4$, 0.01 *M* H_3PO_4)–ACN (92:8), 1.5 ml/min	UV 228 nm	100 ng/ml P, 10 000 ng/ml U	101
Cimetidine	QU, bile	P, U,	EX, CU	LiChrosorb RP-8 (10 μm), CT 40°C	ACN–H_2O–0.033 *M* PB (4:9) (40:39:1), 1 ml/min	UV 228 nm	50 ng/ml	59
Etintidine	QU	P	EX	μBondapak C_{18}	0.02 *M* NaAC–ACN–H_3PO_4 (1000:100:1), 2 ml/min	UV 229 nm		65
Etintidine	QU	U	EX	μBondapak C_{18}	0.02 *M* NaAC–ACN–MeOH–H_3PO_4 (1000:80:25:0.0651), 2 ml/min	UV 229 nm		65
Etintidine	QU	P	EX	2 × Brownlee Si (10 μm)	ACN–MeOH–H_2O–NH_4OH (1000:65:20:2), 2 ml/min	UV 229 nm		66
Famotidine	QU	P, U	SP	Altex RP-8 (5 μm), PC ditto (10 μm)	0.019 *M* H_3PO_4–ACN (90:10)	UV 254 nm	5 ng/ml P, 500 ng/ml U	102
Famotidine	QU	U	EX	Econosphere CN (5 μm)	10 m*M* NaH_2PO_4–ACN (92:8), 1 ml/min	UV 267 nm	70 ng/ml	103
Icotidine (SKF-93319)	QU	P	EX, CU	Lichrosorb Si 60 (5 μm)	ACN–MeOH–H_2O–ammonia (260.80:10.1.5), 2 ml/min	UV 229 nm	100 ng/ml	104
Nizatidine	QU	P	?	Zorbax C_8	0.02 *M* NH_4AC–ACN–TEA (815:183:2)	?		67

Nizatidine	QU	P	EX	Alltech RoSil 5 NH_2	Ethylene dichloride–MeOH–diethylamine (97 3 0 2)	UV 332 nnm	5 ng/ml	105
ORF-17910	QU	P	EX	Partisil 10, ODS-3	0 02 *M* PB (3 5)–MeOH–ACN (5 1 1), 2 ml/min	UV 229 nm	40 ng/ml	60
Ranitidine	QU	S	EX, CU	Zorbax, trimethylsilane RP, PC· Vydac RP (40 μm)	ACN–0 03 *M* PB (3 0) (16 84), 1 ml/min	UV 307 nm	3 ng/ml	106
Ranitidine	QU	P	EX	?	?	?	10 ng/ml	80
Ranitidine	QU	P, T (brain)	EX	μBondapak C_{18}	0 075 *M* PB–ACN (85 15), 1 5 ml/min	UV 313 nm	10 ng/ml P, 25 ng/ml T	60
Ranitidine	QU	B, P	EX	PRP-1 (10 μm)	ACN- 5 m*M* K_2HO_4/$NaC_5H_{11}SO_4$ (18 82), 1 ml/min	UV 314 nm	0 7 ng/ml	107
Ranitidine	QU	P	EX	Alltech RoSil 5 NH_2	Ethylene dichloride–MeOH-diethylamine (97 3 0.2)	UV 332 nm	5 ng/ml	105
RGW-2568	QU	P, U	EX, CU	Bonded phenyl (5 μm)	0 02 *M* PB (3 0)–MeOH–ACN (60 16 24, P), (60.20:20, U), 1 5 ml/min	UV 214 nm	10 ng/ml P, 200 ng/ml U	108
Roxatidine	QU	P	SP	Nucleosil C_{18} (5 μm)	ACN–PB (5 4) (5·50), 1 ml/min	UV 198 nm	5 ng/ml	109
Roxatidine metabolite	ID[b]	U	EN, EX + SP	ODS (5 μm)	MeOH–H_2O -TEA–CH_3COOH (250 750.5 2 5), 1 ml/min	UV 275 nm		19
SKF-93574	QU	P	SP	Ultrasphere ODS	0.01 *M* PB (4 0)–MeOH–ACN (70 20 10), 1 ml/min (P, U), 0 05 *M* PB (4 0)–MeOH–ACN (80 40 10), 1 5 ml/min (bile)	UV 229 nm	20 ng/ml P	110

[a] Simultaneous determination of cimetidine and antipyrine is described

[b] The compounds were separated for spectroscopic identification

3.3. Gas chromatography

Gas chromatography, especially with the modern fused-silica capillary columns (FSC), is still an important method in biomedicine (Tables 6 and 7). Few authors used packed columns [15,16,28,68]. GC is suitable for quantification as well as for identification of drugs in biosamples if these are sufficiently volatile. However, the specificity of the identification of unknown drugs and metabolites depends on the specificity of the detector. In analytical toxicology *ca.* 8000 drugs or poisons may appear in the biosamples [53,81] and each compound may form several metabolites. Endogenous biomolecules must be separated and differentiated. In my experience it is not sufficient to employ GC with only nitrogen–phosphorus detection (NPD) for this purpose instead of MS (see Detection column in Tables 6 and 7). Even if two different capillary columns are used [57,68,82,83], identification only via the relative retention time or the retention index is very questionable If further chromatographic or immunological detection procedures are performed [81], some of the compounds may be identified, but not in the short time necessary in emergency cases. Finally, all chromatographic spots or peaks have to be identified because any of them may represent a potential poison.

3.3.1. H_1-receptor blockers

H_1-blockers can be determined by GC because of their lipophilic properties. If the GC procedures described earlier had been approved [14], they were used for actual pharmacokinetic studies [15,16]. In some cases the GC procedures were more sensitive than HPLC procedures [10–13]. Typical chromatograms of such a GC procedure are shown in Fig. 4 [13] Chromatogram A represents that of the blank plasma sample and B that of a plasma sample taken 1 h after oral application of 20 mg of revenast.

Giachetti *et al.* [17] described an assay for the quantification of twelve H_1-blockers in plasma. However, some interfering peaks appeared in the blank plasma extract. It is stated by the authors that many (but not which) drugs could be detected at a concentration lower than 1 ng/ml.

Fretthold *et al.* [68], Taylor *et al.* [84], Watts and Simonick [83], Turcant *et al.* [82] and Manca *et al.* [57] have all described toxicological screening procedures for the identification of H_1-blockers and other drugs. However, interferences were tested only for 20–100 substances. The forthcoming handbook and computer library of Pfleger *et al.* [53,54], as well as the handbook of de Zeeuw *et al.* [85], include retention indices of 4200 toxicologically relevant substances, metabolites and biomolecules On the basis of this collection a lot of interferences must be considered. The notion that a lot of analyses have been carried out with the published procedures is not convincing, because no external quality control, *e.g* by GC–MS, was performed

TABLE 6

GAS CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF H_1-RECEPTOR BLOCKERS IN BIOSAMPLES

Drug	ID/QU	Sample	Work-Up	Stationary phase	Mobile phase	Detection	Det. limit	Ref.
Alimemazine and metabolites	ID, QU	P	EX	FSC Ultra-1 and FSC CP-Sil CB[a], CT (°C) 50 170/30, 170 280/4	Helium, 1 3 ml/min	NPD	100 ng/ml	82
Azatadine	ID, QU	P	EX	FSC Ultra-1 and FSC CP-Sil CB[a], CT (°C) 50–170/30, 170–280/4	Helium, 1 3 ml/min	NPD	100 ng/ml	82
Brompheniramine	QU	P	EX	FSC CP-Sil 5, CT (°C)· 140–260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Brompheniramine	ID, QU	P	EX	FSC Ultra-1 and FSC CP-Sil CB[a], CT (°C) 50–170/30, 170–280/4	Helium, 1 3 ml/min	NPD	100 ng/ml	82
Brompheniramine	ID	B	EX, CU	FSC Ultra-1 and FSC HP-17[a], CT (°C) 110–280/7	Helium	NPD	100 ng/ml	83
Buclizine	ID	B	EX, CU	FSC Ultra-1 and FSC HP-17[a], CT (°C) 110 280/7	Helium	NPD	100 ng/ml	83
Carbinoxamine	QU	P	EX	FSC CP-Sil 5; CT (°C) 140–260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Chlorothen	ID	U	HY, EX	FSC DB-1701; CT (°C) 200–280/20	Helium	NPD		111
Chlorphen(ir)amine	QU	P	EX	FSC CP-Sil 5, CT (°C) 140–260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Chlorphen(ir)amine	QU	S	EX	DB-2250, CT (°C) 170–270/8	Nitrogen, 30 ml/min	NPD		68
Chlorphen(ir)amine	ID	S, U	EX	FSC BP-1 and FSC DB-1701[a], CT (°C) 170 270/10	Helium, 1 7 ml/min	NPD		68
Chlorphen(ir)amine and metabolite	ID	B	EX, CU	FSC Ultra-1 and FSC HP-17[a], CT (°C) 110–280/7	Helium	NPD	100 ng/ml	83
Chlorphen(ir)amine	ID	U	EX	FSC DB-1701, CT (°C) 185–265/8	Helium, 3 2 ml/min	NPD		84
Chlorphen(ir)amine	ID	P, U, G	EX	FSC DB-1 and FSC DB-17[a], CT (°C) 120–280/8	Helium, 1 5 ml/min, 2 3 ml/min resp	NPD		57
Chlorphenoxamine	QU	P	EX	3% OV-17	?	NPD	10 ng/ml	28
Chlorphenoxamine	ID, QU	P	EX	FSC Ultra-1 and FSC CP-Sil CB[a], CT (°C) 50–170/30, 170–280/4	Helium, 1 3 ml/min	NPD	100 ng/ml	82
Cinnarizine	QU	P	EX	FSC CP-Sil 5, CT (°C) 140–260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Clemastine	ID, QU	P	EX	FSC Ultra-1 and FSC CP-Sil CB[a], CT (°C): 50 170/30, 170–280/4	Helium 1 3 ml/min	NPD	100 ng/ml	82
Clemastine	ID	P, U, G	EX	FSC DB-1 and FSC DB-17[a]; CT (°C) 120 280/8	Helium, 1 5 ml/min, 2 3 ml/min resp	NPD		57

(Continued on p. 392)

TABLE 6 *(continued)*

Drug	ID/QU	Sample	Work-Up	Stationary phase	Mobile phase	Detection	Det. limit	Ref
Cyclizine	QU	P	EX	FSC CP-Sil 5, CT (°C) 140–260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Cyclizine	QU	B, U	EX	FSC SPB-5, CT (°C) 180–220/20	Nitrogen, 1 ml/min	NPD	1 ng/ml	10
Cyclizine	ID	U	EX	FSC DB-1701, CT (°C) 185–265/8	Helium, 3 2 ml/min	NPD		84
Cyproheptadine	ID, QU	P	EX	FSC Ultra-1 and FSC CP-Sil CB[a]; CT (°C) 50–170/30, 170–280/4	Helium, 1 3 ml/min	NPD	100 ng/ml	82
Diphenhydramine	QU	P	EX	FSC CP-Sil 5, CT (°C) 140–260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Diphenhydramine	QU	P	EX, CU	FSC HP-17, CT (°C) 190–240/2/5	Helium, 1 ml/min	NPD		11, 12
Diphenhydramine	QU	S	EX	DB-2250; CT (°C) 170- 270/8	Nitrogen, 30 ml/min	NPD		68
Diphenhydramine	QU	P	EX	3% SP-2250 on 80/100 Supelcoport; CT (°C) 230	Helium, 30 ml/min	NPD		15, 16
Diphenhydramine	ID, QU	P	EX	FSC Ultra-1 and FSC CP-Sil CB[a], CT (°C), 50–170/30, 170–280/4	Helium, 1 3 ml/min	NPD	100 ng/ml	82
Diphenhydramine and metabolites	ID	S, U	EX	FSC BP-1 and FSC DB-1701[a], CT (°C) 170–270/10	Helium, 1 7 ml/min	NPD		68
Diphenhydramine	ID	B	EX, CU	FSC Ultra-1 and FSC HP-17[a], CT (°C)· 110 280/7	Helium	NPD	100 ng/ml	83
Diphenhydramine	ID	U	EX	FSC DB-1701; CT (°C) 185–265/8	Helium, 3.2 ml/min	NPD		84
Doxylamine	QU	P	EX	3% SP-2250 on 80/100 Supelcoport, CT (°C)· 230	Helium, 30 ml/min	NPD	1 ng/ml	14, 15, 16
Doxylamine	ID, QU	P	EX	FSC Ultra-1 and FSC CP-Sil CB[a], CT (°C) 50–170/30, 170–280/4	Helium, 1 3 ml/min	NPD	100 ng/ml	82
Doxylamine and metabolites	ID	U	HY, EX	FSC DB-1701, CT (°C): 200–280/20	Helium, 1 ml/min	NPD		73
Doxylamine	ID	S, U	EX	FSC BP-1 and FSC DB-1701[a], CT (°C) 170–270/10	Helium, 1 7 ml/min	NPD		68
Doxylamine	ID	B	EX, CU	FSC Ultra-1 and FSC HP-17[a]; CT (°C) 110- 280/7	Helium	NPD	100 ng/ml	83
Doxylamine and metabolites	ID	U	HY, EX	FSC DB-1701, CT (°C) 200–280/20	Helium	NPD		111
Flunarizine	QU	P	EX, CU	FSC DB-1, CT (°C) 90-265/30	Helium, 4 5 ml/min	NPD	0.25 ng/ml	112
Flunarizine	QU	P	EX	FSC CP-Sil 5; CT (°C) 140–260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Hydroxyzine	ID	B	EX, CU	FSC Ultra-1 and FSC HP-17[a], CT (°C)· 110–280/7	Helium	NPD	100 ng/ml	83

KB-2413	QU	P	EX, CU	FSC OV-1701; CT (°C): 250	Helium, 1 4 ml/min	NPD	1 ng/ml	113
Meclozine	QU	P	EX	FSC CP-Sil 5; CT (°C): 140- 260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Mepyramine	QU	P	EX	FSC CP-Sil 5; CT (°C) 140–260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Mepyramine	ID	B	EX, CU	FSC Ultra-1 and FSC HP-17[a], CT (°C): 110–280/7	Helium	NPD	100 ng/ml	83
Mepyramine	ID	U	HY, EX	FSC DB-1701, CT (°C) 200–280/20	Helium	NPD		111
Methapyrilene	ID	S, U	EX	FSC BP-1 and FSC DB-1701[a], CT (°C)· 170–270/10	Helium, 1 7 ml/min	NPD		68
Methapyrilene	ID	U	HY, EX	FSC DB-1701, CT (°C) 200–280/20	Helium	NPD		111
Orphenadrine	ID	U	EX	FSC DB-1701, CT (°C) 185–265/8	Helium, 3 2 ml/min	NPD		84
Pheniramine	QU	P	EX	FSC CP-Sil 5; CT (°C). 140–260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Pheniramine	ID	P, U, G	EX	FSC DB-1 and FSC DB-17[a], CT (°C): 120–280/8	Helium, 1 5 ml/min, 2 3 ml/min resp	NPD		57
Promethazine	QU	P	EX	FSC CP-Sil 5, CT (°C) 140 260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Promethazine	ID, QU	P	EX	FSC Ultra-1 and FSC CP-Sil CB[a], CT (°C) 50–170/30, 180- 280/4	Helium, 1 3 ml/min	NPD	100 ng/ml	82
(Pyrilamine, see Mepyramine)								
Revenast	QU	P	SP	FSC Methylsilicone, CT (°C)· 50–330/25	Helium	NPD	2 ng/ml	13
Thenyldiamine	ID	U	HY, EX	FSC DB-1701, CT (°C) 200–280/20	Helium	NPD		111
Thiethylperazine	ID	P, U, G,	EX	FSC DB-1 and FSC DB-17[a], CT (°C) 120–280/8	Helium, 1 5 ml/min, 2 3 ml/min resp.	NPD		57
Triflupromazine	ID	B	EX, CU	FSC Ultra-1 and FSC HP-17[a], CT (°C) 110–280/7	Helium	NPD	100 ng/ml	83
Tripelenamine	QU	P	EX	FSC CP-Sil 5, CT (°C) 140–260/20	Helium, 3 5 ml/min	NPD	1 ng/ml (?)	17
Tripelenamine	ID	S, U	EX	FSC BP-1 and FSC DB-1701[a], CT (°C) 170–270/10	Helium, 1.7 ml/min	NPD		68
Tripelenamine	ID	U	HY, EX	FSC DB-1701, CT (°C) 200–280/20	Helium	NPD		111
Triprolidine	ID, QU	P	EX	FSC Ultra-1 and FSC CP-Sil CB[a], CT (°C). 50- 170/30, 170–280/4	Helium, 1 3 ml/min	NPD	100 ng/ml	82
Triprolidine	ID	U	HY, EX	FSC DB-1701; CT (°C) 200–280/20	Helium	NPD		111

[a] Dual capillary system

TABLE 7

GAS CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF H_2-RECEPTOR BLOCKERS IN BIOSAMPLES

Drug	ID/QU	Sample	Work-Up	Stationary phase	Mobile phase	Detection	Det. limit	Ref
Roxatidine and metabolite	QU	P, U, milk	EX, propinoyl	FSC methylsilicone, CT (°C) 280	Helium	NPD	5 ng/ml P, milk, 1000 ng/ml U	18

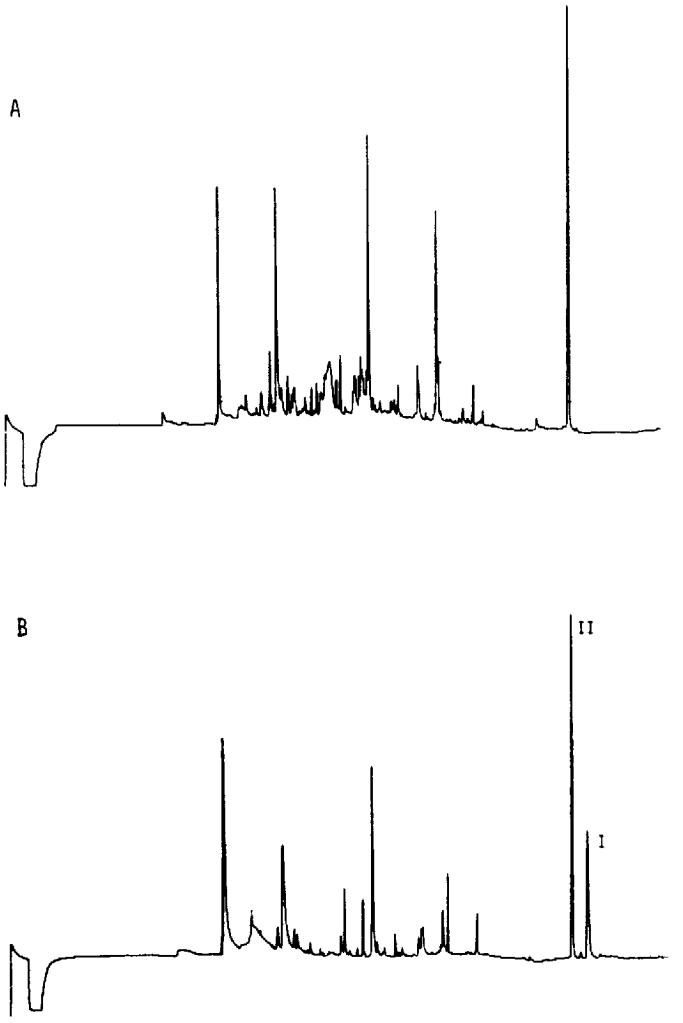

Fig. 4. Gas chromatograms (NPD) of (A) blank plasma and (B) a plasma sample taken 1 h after oral application of 20 mg of revenast [13] (Details of the method are summarized in Table 6.)

3.3.2. H_2-receptor blockers

Roxatidine, which lacks a polar group, is the only H_2-blocker that was determined by GC [18]. The extract was propionylated to improve the GC characteristics of desacetylroxatidine and to distinguish this metabolite from the parent compound.

3.4. Gas chromatography–mass spectrometry

GC–MS is the method of choice for the specific identification of unknown

TABLE 8

GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC METHODS FOR THE DETERMINATION OF H_1-RECEPTOR BLOCKERS IN BIOSAMPLES

Drug	ID/QU	Sample	Work-Up	Stationary phase	Mobile phase	Detection	Det. limit	Ref
Adeptolon and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		26
Alimemazine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th. conc	27
Antazoline and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		26
Azatadine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		25
Bamipine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc.	26
Benzquinamide and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100 310/30	Helium, 1 ml/min	EI-MS		25
Brompheniramine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100 310/30	Helium, 1 ml/min	EI-MS	Th conc	25
Buclizine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100 310/30	Helium, 1 ml/min	EI-MS	Th conc	26
Carbinoxamine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	24
Chlorbenzoxamine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	24
Chlorcyclizine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C), 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	26
Chloropyramine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		26
Chlorphen(ir)amine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone; CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	25
Chlorphenoxamine	QU	P, saliva	EX	FSC OV-101; CT (°C) 230	Helium, 10 ml/min	SIM	2 5 ng/ml	35

Chlorphenoxamine and metabolites	ID	U	EN, EX, ME + AC	FSC SE 54, CT (°C), 70–300/15	?	EI/CI-MS	Th conc.	28
Chlorphenoxamine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		24
Cinnarizine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	26
Clemastine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		24
Clemizole and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	26
Cyclizine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	26
Cyproheptadine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	25
Dimetindene and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	25
Dimetotiazine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		27
Diphenhydramine	QU	P, saliva	EX	FSC OV-101, CT (°C) 230	Helium, 10 ml/min	SIM	2.5 ng/ml	35
Diphenhydramine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	24
Diphenylpyraline and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium 1 ml/min	EI-MS	Th conc	24
Doxylamine and metabolites	ID	U	HY, EX	FSC DB-5, CT (°C) 60–240/15	Helium, 1 ml/min	CI-MS		73
Doxylamine and metabolites	ID	U	EN, EX, AC	FSC DB-5, CT (°C), 100–310/30	Helium	CI-MS		29
Doxylamine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C), 100–310/30	Helium, 1 ml/min	EI-MS	Th. conc	24
Etodroxizine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	26
Histapyrrodine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		26

(Continued on p 398)

TABLE 8 *(continued)*

Drug	ID/QU	Sample	Work-Up	Stationary phase	Mobile phase	Detection	Det. limit	Ref
Hydroxyzine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th. conc.	26
Isothipendyl and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th. conc.	27
Ketotifen and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th. conc.	25
Mebhydroline and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		25
Mecloxamine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		24
Meclozine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th. conc.	26
Medrylamine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		24
Mepyramine and metabolites	ID	U	EN, EX, TMS	3% OV-11 on 100/200 mesh Gas Chrom Q, CT (°C) 190–250/10	Methane	CI-MS		30
Mepyramine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th. conc.	26
Mequitazine	QU	P, U	EX, CU	FSC CP-Sil 5, CT (°C) 210–310/10	Helium, 1.2 ml/min	SIM	0.5 ng/ml	86
Mequitazine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th. conc.	27
Orphenadrine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th. conc.	24
Oxatomide and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		26

Oxomemazine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100-310/30	Helium, 1 ml/min	EI-MS	Th conc	27
Phenindamine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		25
Pheniramine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	25
Phenyltoloxamine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	24
Promethazine	QU	P	EX	3% Sp 2100, CT (°C) ?	Helium, 20 ml/min	SIM	0 5 ng/ml	71
Promethazine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc.	27
(Pyrilamine, see Mepyramine)								
Pyrrobutamine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100 310/30	Helium, 1 ml/min	EI-MS		25
Terfenadine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	25
Thiethylperazine and metabolites	ID	U	HY, EX, AC	1 SC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		27
Tolpropamine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS		25
Triflupromazine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	27
Tripelenamine and metabolites	ID	U	EN, EX, TMS	3% OV-11 on 100/120 mesh Gas Chrom Q, CT (°C) 190 250/10	Methane	CI-MS		30
Tripelenamine and metabolites	ID	U	HY, EX, AC	FSC methylsilicone, CT (°C) 100–310/30	Helium, 1 ml/min	EI-MS	Th conc	26

TABLE 9

GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC METHODS FOR THE DETERMINATION OF H_2-RECEPTOR BLOCKERS IN BIOSAMPLES

Drug	ID/QU	Sample	Work-Up	Stationary phase	Mobile phase	Detection	Det. limit	Ref
Roxatidine metabolites	ID	U	EN, EX + SP, TMS, ME, TFA	2% OV-1 on 80/100 mesh uniport HP, CT (°C), 140–252/5	Nitrogen, 30 ml/min	EI-MS		19

substances and their metabolites in biosamples [51–54]. It is also suitable for sensitive quantification [35,71,86] (see ID/QU column in Tables 8 and 9).

3.4.1 H_1-receptor blockers

Some H_1-blockers in plasma and saliva were quantified [35,71,86]. Studies on the metabolism of some H_1-blockers were performed using GC–MS [29,30,73]. Identification of 50 H_1-blockers and their metabolites in urine was described [24–27]. These procedures allow rapid and specific detection and differentiation of therapeutic concentrations of alkanolamine, alkylamine, ethylenediamine, piperazine and phenothiazine antihistamines (H_1-blockers). The ion chromatograms shown in Fig. 5 may indicate alkylamine antihistamines and/or their metabolites in urine samples after acid hydrolysis and acetylation [25]. The positive signals represent the dehydrated N-desalkyldihydroxy metabolite of terfenadine (1), the dehydrated and actylated N-desalkyl metabolite (2) and dehydrated terfenadine (3). In contrast to ion fragmentography, ion chromatography is based on the full-scan mode and, therefore, the specific identification is carried out by

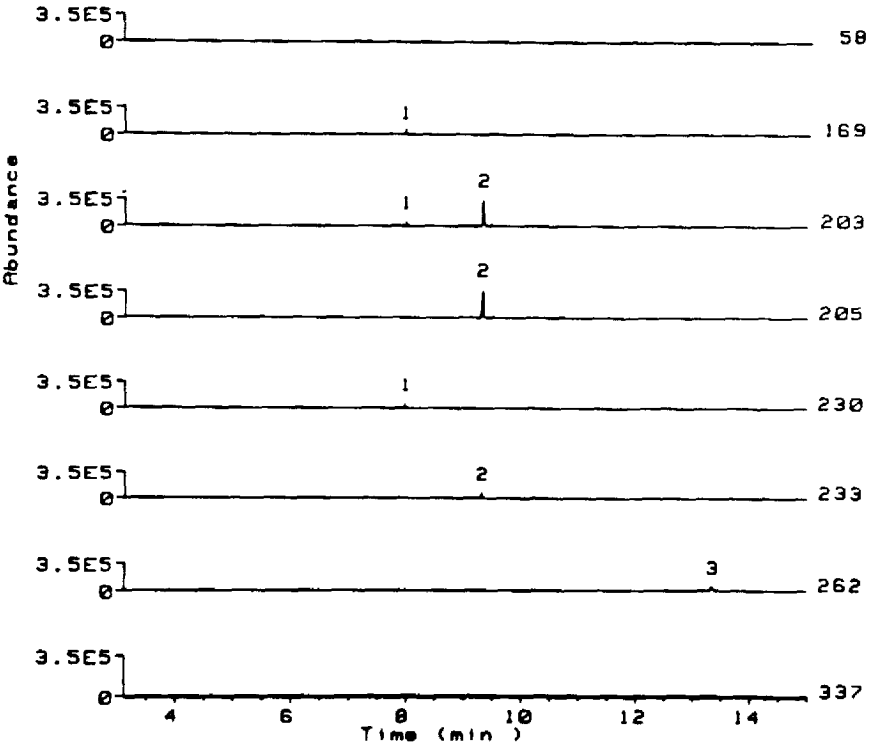

Fig. 5. Ion chromatograms, the ions of which may indicate alkylamine antihistamines and/or their metabolites in urine samples after acid hydrolysis and acetylation [25]. The positive signals represent the dehydrated N-desalkyldihydroxy metabolite of terfenadine (1), the dehydrated and acetylated N-desalkyl metabolite (2) and dehydrated terfenadine (3). (Details of the method are summarized in Table 8.)

comparison of the peak underlying full mass spectra with reference spectra published in the corresponding papers. Because of mass spectral identification, interferences by other drugs are improbable. These methods have the further advantage that they can be integrated in a general screening procedure for several catagories of drugs (general unknown analysis) [51,53,54] It allows simultaneous detection of butyrophenones [41], analgesics [42], opioids [43], antidepressants [44], neuroleptics [45], antiparkinsonians [46], β-blockers [47], benzodiazepines [48], antiarrhythmics [49], laxatives [50] and their metabolites by searching for fragment ions typical for these drugs in the stored spectra. In my experience, such an extensive systematic toxicological analysis procedure cannot be performed by a combination of several relatively unspecific chromatographic procedures [81].

3.4 2. H_2-receptor blockers

Again, roxatidine is the only H_2-blocker that was determined by GC–MS [19]. Its metabolites were derivatized by trimethylsilylation, methylation and trifluoroacetylation to improve their GC characteristics.

4 SUMMARY

This paper reviews thin-layer chromatographic, high-performance liquid chromatographic, gas chromatographic and gas chromatographic–mass spectrometric procedures for the identification and quantification of sixty histamine H_1- and ten H_2-receptor blockers in biosamples, published from 1984 to 1989. The biomedical importance of the published procedures and consequences for their choice, the sample preparation and the chromatography itself are discussed. The fundamental information about the biosample assayed, work-up, stationary and mobile phase, detection mode and sensitivity of each procedure is summarized in seven tables. They are arranged according to the chromatographic method and the category of histamine receptor blockers Examples of typical chromatographic separations are presented in three figures

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