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Review

Bioanalysis of anti-ulcer agents[a]

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CONTENTS

1. INTRODUCTION

The role of endogenous histamine in the hypersecretion of gastric acid, and subsequently in peptic ulcers, has led to the development of H_2-receptor- or histamine-blocking agents through systematic modification of the histamine molecule. These H_2-receptor blockers are the drugs most commonly prescribed for the treatment of peptic ulcer disease. Cimetidine and ranitidine are the better known agents in this class. Cimetidine, however, has been proven to competitively inhibit the metabolism of drugs that undergo oxidative metabolism possibly leading to serious side-effects due to the accumulation of these drugs. The newer

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[a] Further references regarding H_2-blockers can be found in the review of Maurer on histamine blockers on p. 369.

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drugs in this class are not only more potent than cimetidine, but also do not suffer from the above problem [1].

A more recent class of anti-ulcer compounds are the gastric acid (proton pump) inhibitors, of which omeprazole is a prototype drug. It is effective in the inhibition of both basal and stimulated gastric acid secretion. Pirenzepine, an M_1-muscarinic receptor blocker, is another drug that has proven to be effective in the treatment of peptic ulcers. Muscarinic blockers act upon several systems that regulate both acid production and the protective mucosal barrier. Other anti-ulcer drugs which possess different modes of pharmacological action are carbenoxolone sodium (a steroid-like mineralocorticoid) and prostaglandins E_2 and I_2 [1].

Liquid and gas chromatographic methods of analysis of human samples of all the classes of anti-ulcer drugs except the prostaglandins are discussed in this review. An excellent review of such techniques has been published in 1985 [2] and therefore we have restricted the present article to analytical methodology published after 1985. Wherever applicable, analysis of the metabolites of the above drugs will be considered. Details of all the liquid chromatographic methods are presented in Table 1 and gas chromatographic techniques are presented in Table 2.

2 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

High-performance liquid chromatography (HPLC) is the primary method which has been used for the bioanalysis of anti-ulcer agents. Although there are a variety of separation techniques used for the various types of agents, detection of these compounds with HPLC systems has been accomplished with ultraviolet (UV) absorbance. A few of the compounds have been shown to fluoresce and therefore provide better detectability. The lack of extensive conjugation in most of the structures, however, prevents strong fluorescence in most cases.

2.1. H_2-Receptor antagonists

2.1.1. Cimetidine

Rustum and Hoffman [3] have reported a reversed-phase method for the simultaneous analysis of cimetidine (Fig. 1a) and its major metabolites, cimetidine sulfoxide and hydroxymethylcimetidine. They utilised a simple, single-step, organic liquid phase extraction procedure for human plasma and whole blood samples. The evaporation step was eliminated from the procedure, thereby reducing manipulative errors and the sample preparation time to 5 min. Extraction efficiency ranged from 80 to 85%. The method utilised a short 6-cm column resulting in a very rapid analysis with all the analytes eluting in 7 min (Fig. 2).

2.1.2. Ranitidine

Several reversed-phase methods have been developed for the analysis of ran-

TABLE I

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS

ACN = acetonitrile, CI = chemical ionization, em = emission wavelength, ex = excitation wavelength, FL = fluorescence, HAc = acetic acid, NaOAc = sodium acetate, NH_4OAc = ammonium acetate, TEA = triethylamine.

Drug		Column, mobile phase and detection	Sample extraction and matrix	Linear range	Limit of detection	References
H_2-Antagonists						
1	BMY 26517	ODS-3 RAC, 5 μm, 100 mm × 9 4 mm, CH_3OH–H_2O (45 55) + 0 31% HAc, 1 5 ml/min, FL ex 370 nm, em 410 nm	Liquid–liquid, plasma	4.3–429 0 ng/ml	0 4 ng/ml	20
2.	Cimetidine	Styrene divinyl benzene polymeric, 10 μm, 60 mm × 4 6 mm, ACN–20 m*M* potassium phosphate (12 5 87.5) pH 9 5, 1 0 ml/min; UV 210 nm	Liquid–liquid, plasma, whole blood	0.04–100 μg/ml	0 04 μg/ml	3
3.	Etintidine	Bondapak C_{18}, ACN–0 02 *M* NaOAc-85%	Liquid–liquid,		Not reported	16,17
		H_3PO_4 (100 1000 1, v/v)	plasma	0 1-40 0 μg/ml		
		ACN–0 02 *M* NaOAc-CH_3OH-85% H_3PO_4 (80:1000 25 0 065, v/v), UV 229 nm	urine	0 4–400 0 μg/ml		
4	Famotidine	Altex RP-8, 5 μm, 250 mm × 4 6 mm; ACN–0 019 *M* phosphoric acid (90 10), UV 267 nm	Solid phase (Baker-10), Bond-Elut, silica,			12
			plasma	5 0–50 0 ng/ml	5 0 ng/ml	
			urine	0 5–30 0 μg/ml	500 ng/ml	
5	Famotidine	Spherisorb ODS II, 5 μm, 250 mm × 5 mm, ACN–H_2O dodecanesulfonic acid (830 170 0 18·0 2), 1 0 ml/min, UV 267 nm	Solid phase, Baker silica,		10 0 ng/ml	13
			plasma	0–200 0 ng/ml		
			urine	5 0- 25 0 μg/ml		
6	Famotidine	Alltech CN, 5 μm, 150 mm × 4 6 mm, ACN–10 m*M* NaH_2PO_4 (8 92), 1 0 ml/min, UV 267 nm	Liquid–liquid, urine	0 4–25 0 μg/ml	70 0 ng/ml	14
7	Nizatidine and metabolites	C_8, 6 μm, 250 mm × 4 6 mm, (ACN + 1% TEA)–0 02 *M* NH_4OAc (81 5 18 5), 1 0 ml/min; UV 313 nm	Liquid–liquid,		10 0 ng/ml	15
			plasma	20 0–200 0 ng/ml		
			urine	0 1–3 0 μg/ml		

(Continued on p 410)

Table 1 (*continued*)

Drug		Column, mobile phase and detection	Sample extraction and matrix	Linear range	Limit of detection	References
8.	OPC-12759	YMC Pack A-303 ODS, 10 μm, 250 mm × 4 6 mm, ACN–tetrahydrofuran–HAc–H_2O (32 3·1 64), 1 2 ml/min, FL ex 330 nm, em 375 nm	Liquid–liquid, plasma urine	 0 01–10 0 μg/ml 0 5–500 0 μg/ml	Not reported	21
9	Ranitidine	Zorbax CN, 6 μm, 150 mm × 4.6 mm, ACN 0 05 *M* KH_2PO_4 (15 85) with 0 005 *M* octanesulfonic acid, 2 0 ml/min, UV 318 nm	Liquid–liquid, plasma urine	 0.5 ng/ml–10 μg/ml, 5-500 μg/ml	1 0 ng/ml	7
10	Ranitidine	PRP-1, 10 μm, 60 mm × 4 6 mm, ACN (5 m*M* KH_2PO_4 + 5 m*M* sodium pentanesulfonate) (18 82) pH 10, 1 0 ml/min, UV 314 nm	Liquid–liquid, plasma, whole blood	7 0 ng/ml 30 0 μg/ml	0 7 ng/ml	8
11	Ranitidine	Spherisorb phenyl, 5 μm, 100 mm × 4 6 mm, ACN–0 02 *M* phosphate (89 11 for plasma) (88 12) for urine) pH 3, 1 0 ml/min, UV 228 nm	Automated solid phase (AASP), cyano, plasma, urine, peritoneal dialysate	1 9–687 2 ng/ml	2 0 ng/ml	9
12	Ranitidine	Spherisorb ODS II, 5 μm, 250 mm × 4.6 mm, ACN–CH_3OH–(0 01 *M* octanesulphonate in 0 05 *M* ammonium acetate) (60 30 5) pH 3 8, 1 2 ml/min, UV 330 nm	Liquid-liquid plasma direct injection, urine	 0–200 0 ng/ml 0 5–2 5 μg/ml	10 0 ng/ml	10
13	Ranitidine and metabolites (N-, S-oxides and desmethyl)	Partisil SCX, 10 μm, 260 mm × 4 6 mm, ACN–tetrahydrofuran 0 1 *M* sodium acetate (36 7 5 56.5) pH 5, 1 9 ml/min, UV 320 nm	Liquid–liquid; plasma urine	 0 04–4 0 μg/ml 0–350 0 μg/ml	 5, 10, 15 and 4 ng/ml 0 15, 0 15, 0 2 and 0 15 μg/ml	11
14	Ranitidine and metabolites	Hypersil ODS, 5 μm, 200 mm × 2 mm, ACN–water (50 50), ACN-0 05 *M* ammonium acetate (60.40), ACN-0 05 *M* ammonium acetate gradient; variable flow-rate, quadrople mass spectroscopy, CI mode	Liquid–liquid, urine	0–40 μg/ml	5.0 ng on column	4

Tabel 1 (*continued*)

Drug		Column mobile phase and detection	Sample extraction and matrix	Linear range	Limit of detection	References
15	RG W-2568	Bonded phenyl, 5 µm, 150 mm × 4 5 mm, ACN–CH_3OH–0 02 *M* Na_2HPO_4 (24.16 60) + 0 01% TEA pH 3, 1 5 ml/min, UV 214 nm	Liquid–liquid, plasma	Low curve 0–20 0 µg/ml,	10 0 ng/ml	18
			urine	high curve 20 00–500 0 µg/ml	1 0 µg/ml	
16	SK&F 93574	Ultrasphere™ ODS, 5 µm, 150 mm × 4 6 mm, 40°C, 0 01 *M* KH_2PO_4–CH_3OH–ACN (70 20·10) pH 4, 1 0 ml/min, UV 229 nm	Solid phase C_{18} Bond-Elut, plasma, urine	Not reported	20 0 ng/ml	19
Proton pump inhibitors						
	Omeprazole and metabolites (sulphone and sulphide)	C_8, 5 µm, 150 mm × 4 6 mm, CH_3OH–ACN 0 025 *M* phosphate buffer (40 8.52) pH 7 4, 1 1 ml/min; UV 302 nm	Liquid–liquid, plasma	5 0–200 0 ng/ml	5 0, 10 0, 7 5 ng/ml	22
Muscarinic receptor antagonists						
	Pirenzepine	Ultrasphere silica, 5 µm, 250 mm × 4 6 mm, Porasil silica, 10 µm, 300 mm × 4 6 mm, 0 014 *M* TEA–ACN (40·60) pH 10 5, Nucleosil SA, 10 µm, 250 mm × 4 6 mm, 0 1% TEA–ACN (45 55) pH 9, 1 5 ml/min, UV 280 nm	Liquid liquid; plasma	5 0–100 0 ng/ml	2 5 ng/ml	23

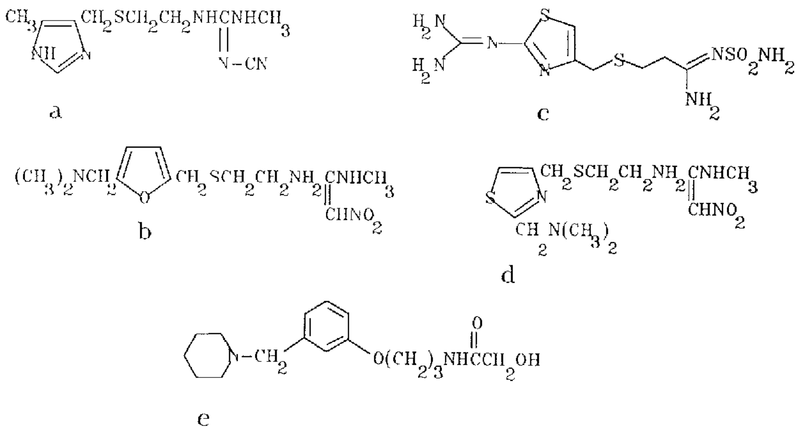

Fig 1 Structures of H_2-receptor antagonists (a) Cimetidine, (b) ranitidine, (c) famotidine, (d) nizatidine, (e) roxatidine

itidine (Fig. 1b). One such technique developed by Lant *et al.* [4] used mass spectroscopy (MS) for the detection of the drug and its metabolites in urine samples. In this method the sample eluate from the column was introduced into the mass spectrometer via a direct liquid introduction (DLI) probe, thereby overcoming thermal degradation of the analytes which was a problem in earlier HPLC-MS methods [5,6].

Mullersman and Derendorf [7] have utilized an ion-pair approach for the analysis of this drug in plasma and urine. The method enabled the detection of nanogram levels of ranitidine with a simple extraction technique and was used to study the partition of ranitidine into red blood cells. In another method, rapid analysis of ranitidine in whole blood and plasma was achieved by using a short 6-cm polymeric reversed-phase column [8]. The sensitivity of this method was

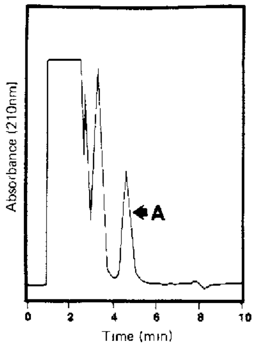

Fig 2 Typical chromatogram of extract of whole blood from a patient treated with cimetidine Peak A = cimetidine Concentration found was 3.5 μg/ml (From ref. 3 with permission.)

comparable to that of the previous method and the internal standard was readily available. Extraction efficiency ranged from 90 to 96%.

A method designed to reduce sample processing time and variability was the automated solid-phase extraction HPLC method by Karnes *et al.* [9]. In this method, sample extraction was accomplished on the AASP (Advanced Automated Sample Preparation) system, utilizing a cyanopropyl bonded-phase cartridge, followed by on-line elution of the analyte with the mobile phase. The use of solid-phase extraction resulted in very high recoveries over the range of concentrations analyzed in plasma, urine and peritoneal dialysate (Fig. 3). Sample processing time, which is a very important factor in the analysis of large numbers of samples, was considerably decreased. High reproducibility was achieved by reduction of errors due to sample manipulations.

Direct injection of urine samples for the HPLC determination of ranitidine was accomplished by Salem *et al.* [10], thereby reducing sample preparation time significantly. The method was also applicable to plasma samples if preceded by liquid–liquid extraction. A new approach to the separation of ranitidine and its N-oxide, S-oxide and desmethyl metabolites from plasma and urine involved the use of ion-exchange chromatography [11] A systematic study of solvent systems for the liquid–liquid extraction scheme was also presented. This extraction scheme was convenient as it did not involve an evaporation step.

2.1.3. Famotidine

The first of the three methods that have been published since 1985 for this relatively new H_2-antagonist (Fig. 1c) was a solid-phase extraction method for plasma and urine samples [12]. This method utilized the Baker-10 extraction system with Bond Elut silica columns. Since famotidine is poorly soluble in most organic solvents, the use of solid-phase extraction was indicated and a mean recovery of 90.3% was obtained from plasma This was higher than that obtained in comparison to liquid–liquid extraction methods. The authors state that the use of silica and C_8 columns in tandem resulted in extremely clean backgrounds (Fig. 4). The method posseses all the advantages that normally accompany solid-phase extraction techniques.

Kroemer and Klotz [13] have published an HPLC method for the analysis of famotidine in plasma and urine using a solid-phase extraction procedure based on a modification of earlier work [12] This method, unlike the original work, included an internal standard, which resulted in increased reproducibility of data. The previous method [12] used peak heights and areas of standards for calibration.

The most recently published method provided lower detection limits than the earlier method in urine [14]. Results of a study on the effect of solvents (used for reconstitution of evaporated samples) and the salts present in water, on peak distortion and peak height were also presented The authors also reported the absence of interferences from several other drugs in the determination of famotidine by this method

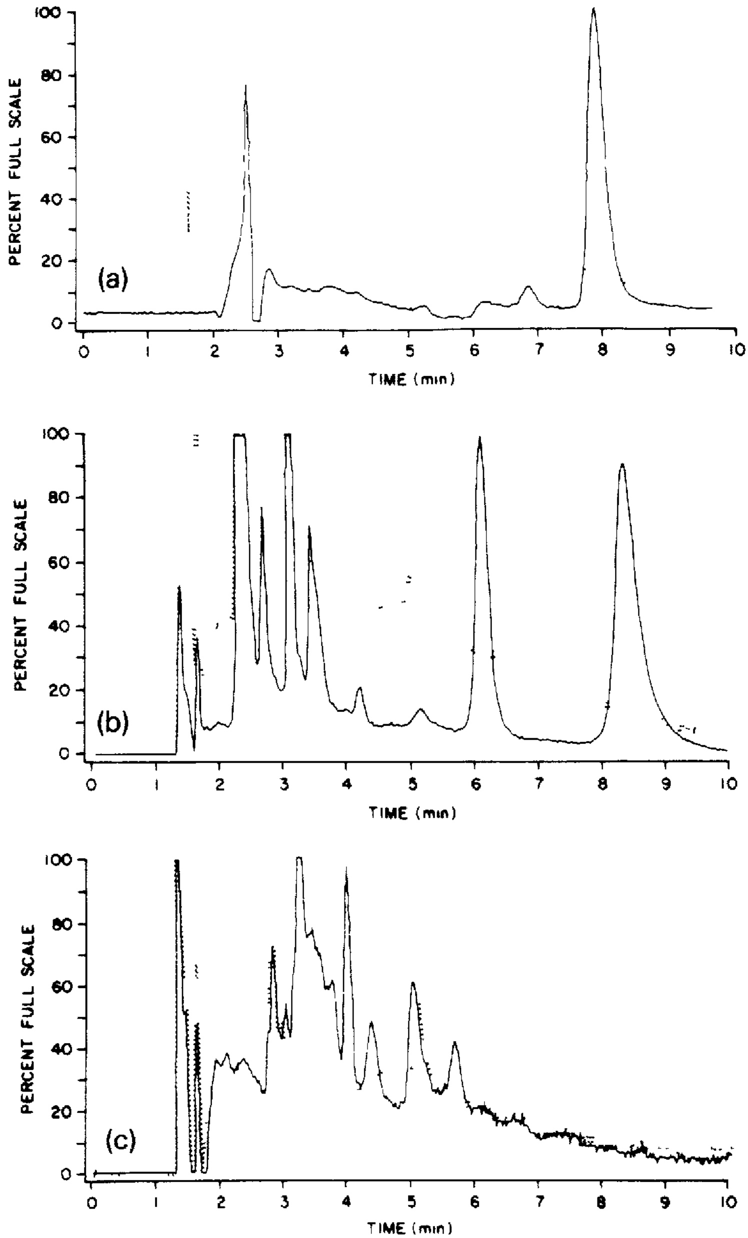

Fig 3 (a) Chromatogram of blank plasma (broken line, normalised at 4 3 10^{-5} a u.f s) and plasma spiked with 10 ng/ml ranitidine and internal standard (solid line, normalised at 7.6 10^{-4} a.u.f s.), retention times 6 9 min for ranitidine and 8 min for internal standard (b) Chromatogram of blank urine (broken line, normalised at 1 0 10^{-8} a u f s.) and urine spiked with 50 ng/ml ranitidine and internal standard (solid line, normalised at 4 78 10^{-4} a u f s), retention times 6 2 min for ranitidine and 8 4 min for the internal standard. (c) Chromatogram of blank peritoneal dialysate (broken line, normalised at 1 02 10^{-4} a u.f.s.) and dialysate from a patient receiving ranitidine which was analysed to contain 15 6 ng/ml (solid line, normalised at 9.8 10^{-5} a u.f s); retention time· 5 8 min for ranitidine (From ref 9 with permission.)

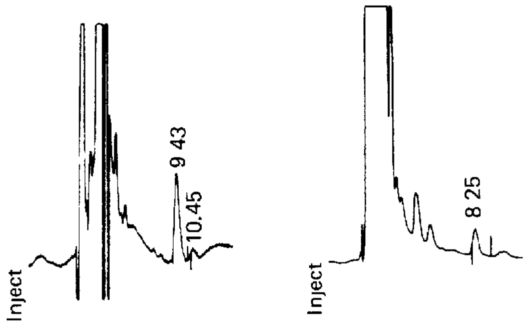

Fig 4 Chromatograms of famotidine in (left) plasma of a normal subject dosed with 10 mg of famotodine, 7 h post-dose, 13 6 ng/ml, and (right) urine of normal subject dosed with 20 mg of famotidine, 12–24 h collection post-dose, 0 68 μg/ml (From ref 12 with permission)

2.1.4. Nizatidine

Nizatidine (Fig. 1d) was analysed in plasma, saliva and urine samples by a reversed-phase method [15]. The method was sufficient to detect nizatidine at nanogram levels Two nizatidine metabolites were separated in urine by this method following the administration of ^{14}C-labelled nizatidine.

2.1.5. Etintidine

Huang *et al.* [16,17] have developed an HPLC method for the analysis of etintidine in plasma and urine, which has been described briefly in their papers on the pharmacokinetics of this drug. Reversed-phase HPLC with liquid–liquid extraction of the samples was employed in their method.

2.1 6. Investigational drugs

All the drugs in this class are presently in various stages of clinical investigation. A rapid HPLC method with a run time of 10 min for the analysis of RG W-2568 (Fig 5a) in plasma and urine has been published by Miksic *et al.* [18]. Solid-phase extraction was used for the analysis of SK&F 93574 (Fig. 5b) in plasma, urine and bile [19] and separation was achieved by reversed-phase chromatography (Fig. 6a) The mean recovery of SK&F 93574 from all the matrices was approximately 90%. A reversed-phase HPLC method for the analysis of BMY 26517 (Fig. 5c) in plasma has been reported [20] Fluorescence detection was used to achieve a very sensitive method (Fig. 6b). The extraction efficiency of this liquid–liquid extraction procedure was 98%. Fluorescence detection has also been used for the analysis of OPC-12759 (Fig. 5d) in plasma and urine [21] The method is very sensitive and rapid with the analyte eluting in 8 min from urine and 6 min from plasma. Recovery from plasma (82%) was lower than that from urine (97%) when liquid–liquid extraction was employed.

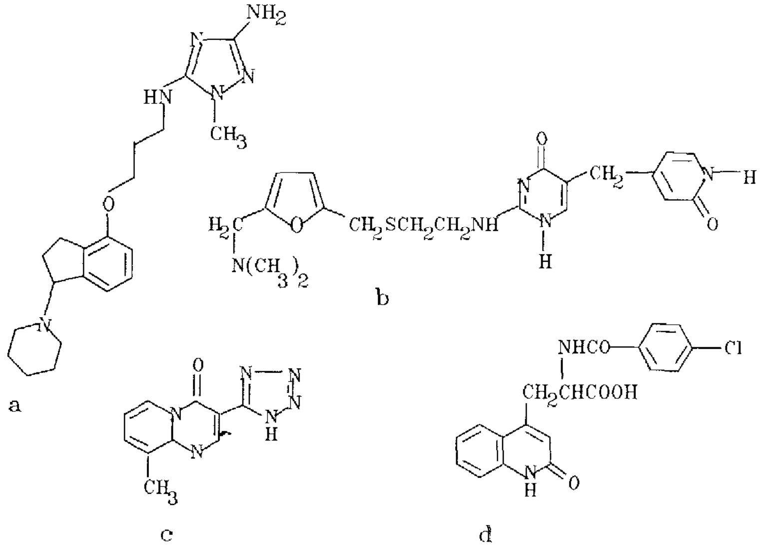

Fig 5 Structures of investigational H_2-receptor antagonists (a) RG W-2568, (b) SK&F 93574, (c) BMY 26517, (d) OPC-12759

2.2. Proton pump inhibitors (omeprazole)

A highly sensitive reversed-phase HPLC method for the analysis of omeprazole (Fig. 7) and its metabolites was published in 1988 by Amantea and Narang [22]. Absolute recoveries of omeprazole, its sulphone and sulphide metabolites were 96, 42 and 96%, respectively. Despite the low recovery of the sulphone metabolite the detection limits of the method were adequate.

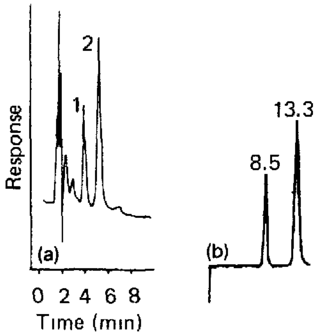

Fig 6. (a) Chromatogram of plasma extract from a human volunteer following intravenous infusion of SK&F 93574 at a dose of 35 μg/kg Peaks 1 = SK&F 93574, 2 = 93924 (internal standard) (from ref 19 with permission) (b) Chromatogram of human plasma spiked with 125 ng/ml internal standard (retention time = 13 3 min) and 74 ng/ml BMY 26517 (retention time = 8 5 min) (from ref 20 with permission)

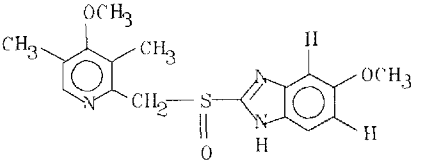

Fig. 7. Structure of the proton pump inhibitor omeprazole

2.3. Muscarinic receptor antagonists (pirenzepine)

Meineke *et al.* [23] have reported two liquid chromatographic approaches for the analysis of this drug (Fig 8a) in plasma. The first used a normal-phase silica column and the second utilized an ion-exchange column. Samples were extracted from plasma by liquid–liquid extraction. The extraction efficiency was determined to be 80%, although it was reported that this could be increased to 95% by extracting twice. Intermittent purge steps were reported to be essential for the silica columns as prolonged use of these columns at the high pH of the mobile phase (10.5) resulted in below average column life. The chromatographic behaviour of the drug and the internal standard on the ion-exchange column was of interest, because of the apparent non-uniform mechanism of separation.

3 GAS CHROMATOGRAPHY

3.1. H_2-Receptor antagonists (roxatidine)

A highly sensitive method for the analysis of roxatidine (Fig. 1e) in human plasma, urine and milk by capillary gas chromatography has been reported [24]. In this procedure, the extracted roxatidine was esterified with propionic anhydride to facilitate volatilization prior to analysis. High extraction recoveries were obtained and roxatidine eluted at 6.3 min under the conditions of chromatography (Fig. 9).

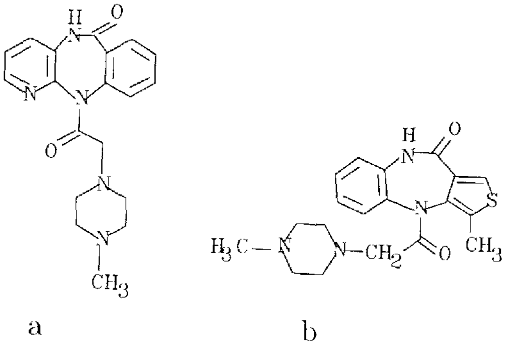

Fig 8 Structures of muscarinic receptor antagonists (a) Pirenzipine, (b) telenzepine

3.2 Muscarinic receptor antagonists (telenzepine)

A solid-phase extraction technique in conjunction with a gas chromatographic–mass spectrometric method of analysis for telezepine in serum (Fig. 8b) has been reported by Sturm and Junker [25]. Derivatization of the analyte to a methyl ester through a benzodiazepino–benzimidazole rearrangement was necessary, because the unmodified analyte provided a non-linear response and severe adsorption at lower levels. Extraction efficiency as determined by using ^{3}H-labelled analyte was 84.5%, evaluated at 20 ng/ml (Table 2).

4 SUMMARY

In reviewing the analytical methods for the analysis of anti-ulcer drugs we observed an increase in the utilization of solid-phase extraction techniques, though the traditional liquid–liquid methods are still predominant. Liquid chromatographic techniques are employed more than gas chromatographic methods which reflects the general trend in chromatographic analysis for analytes in the

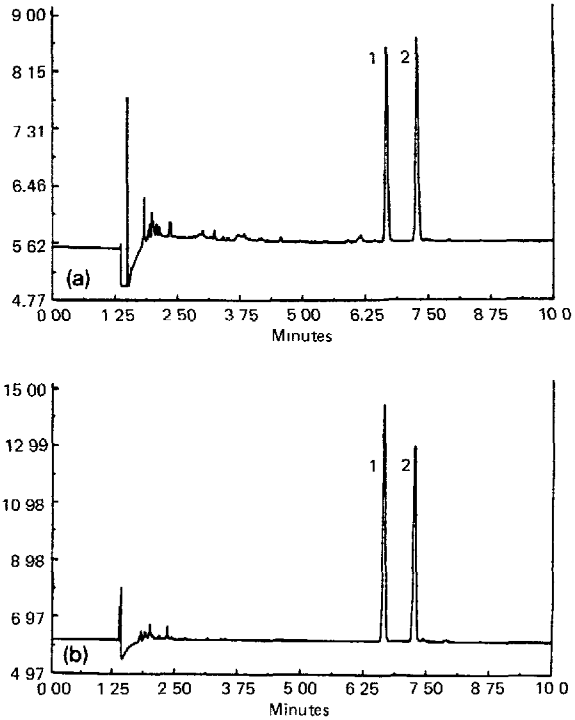

Fig 9 (a) Chromatogram of extract of plasma sample from a volunteer, 4 h after administration of roxatidine acetate (150 mg). (b) Chromatogram of extract of 0–12 h urine sample from day 2 of a multiple-dose study (150 mg roxatidine acetate per day) (From ref 24 with permission)

TABLE 2

GAS CHROMATOGRAPHIC TECHNIQUES

Drug	Column, mobile phase and detection	Sample extraction and matrix	Linear range	Limit of detection	Reference
H_2-Antagonists					
Roxatidine	Fused-silica capillary, 25 m × 0.31 mm, 0.52-μm cross-linked methyl silicone coat, carrier helium, 28 cm/s, nitrogen-selective detector	Liquid–liquid, plasma, urine, milk		 5.0 ng/ml 0.4 μg/ml 5.0 ng/ml	24
Muscarinic receptor antagonists					
Telenzepine	Glass column with 3% OV-17, carrier helium, 15 ml/min, mass spectroscopy, electron impact	Solid-phase, Sep-Pak C_{18}, serum	2.0–40.0 ng/ml	2.0 ng/ml	25

nanogram range. We foresee a continued increase in the use of solid-phase extraction methodology (automated or otherwise) due to the potential for dramatic decreases in extraction times, cost and significant enhancement of extraction efficiency. Because the therapeutic concentrations of these drugs tend to be in the low nanogram range in plasma and the current trend in drug development is toward more potent agents, we anticipate the application of more sensitive liquid chromatographic detection techniques such as electrochemical and chemiluminescence detection to overcome the limitations of currently used technology.

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