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TRACE ANALYSIS OF THE ANTIHISTAMINES METHAPYRILENE HYDROCHLORIDE, PYRILAMINE MALEATE AND TRIPROLIDINE HYDROCHLORIDE MONOHYDRATE IN ANIMAL FEED, HUMAN URINE AND WASTEWATER BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND GAS CHROMATOGRAPHY WITH NITROGEN–PHOSPHORUS DETECTION

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SUMMARY

Toxicological evaluation of the antihistamines methapyrilene hydrochloride, pyrilamine maleate, and triprolidine hydrochloride monohydrate using methapyrilene hydrochloride as the positive indicator was investigated as part of a structure–activity relationship study in rats and mice. Prerequisites for the toxicological tests were the development of analytical procedures to certify the dose, homogeneity and stability of the drugs in animal feed and to monitor human urine for possible exposure and to ensure removal of the test agents from wastewater prior to its discharge into the environment. A high-performance liquid chromatographic (HPLC) system was developed using a fluorescence detector for the determination of methapyrilene hydrochloride and pyrilamine maleate in feed at levels as low as 100 ng/g and in human urine as low as 1 ng/g. An HPLC–UV procedure was developed for the determination of triprolidine hydrochloride monohydrate in feed at levels as low as 10 μg/g. Data concerning *p*-values, extraction efficiencies from feed and stability experiments in feed are presented for these antihistamines. A gas chromatographic procedure using a nitrogen–phosphorus detector was also developed for determining the three antihistamines in admixture in wastewater at levels as low as 10 ng/g.

INTRODUCTION

Pyrilamine maleate and triprolidine hydrochloride monohydrate are antihistamines widely used in the treatment of hay fever, urticaria, allergic rhinitis and allergic drug reactions[1]. Methapyrilene hydrochloride was also used for the treatment of these maladies[1] and as a sleep aid[2] until its recent withdrawal from the market because Lijinsky *et al.*[3] demonstrated its carcinogenicity in rats.

Toxicological investigations using methapyrilene hydrochloride and pyrilamine maleate have indicated that they cause unscheduled DNA synthesis in primary cultures

of adult rat hepatocytes[2,4,5] in *in vitro* tests used for the identification of mutagens and/or carcinogens.

After gavage or intramuscular administration of pyrilamine maleate, toxic effects on the early and latter stages of pregnancy were observed in rats with the latter, particularly during parturition, involving both the mother and litter[6]. Lijinsky[7] demonstrated the formation of dialkylnitrosamines by nitrosative dealkylation of tertiary amines at acidic pH. Lijinsky found that methapyrilene and chloropheniramine, which are tertiary amines, react with nitrite at pH 3–4 to form dimethylnitrosamine, which has strong carcinogenic potential. Since pyrilamine and triprolidine have tertiary amine side-chains, they may also yield compounds with carcinogenic potential during normal gastric processes in humans.

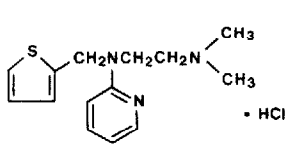
In view of the limited data relating to toxicological effects of pyrilamine maleate and triprolidine hydrochloride monohydrate, these drugs were scheduled for toxicological evaluation at the National Center for Toxicological Research (NCTR) as part of a structure–activity relationship study with several similarly structured antihistamines in rats and mice. Methapyrilene hydrochloride was proposed for use as a positive control in these experiments as it is an established rat carcinogen.

Recently, several high-performance liquid (HPLC) and gas chromatographic (GC) methods have been developed for antihistamine determinations in various substrates and in biological samples[8–16]. However, these methods did not address the specific needs of the proposed toxicological studies, which required quantitation of trace levels of these drugs in animal feed, human urine and wastewater. This paper describes an HPLC procedure using fluorescence detection for the determination of methapyrilene hydrochloride and pyrilamine maleate in animal feed at levels as low as 100 ng/g and in human urine at levels as low as 1 ng/g. HPLC methodology using an ultraviolet (UV) detector for the determination of triprolidine hydrochloride monohydrate in animal feed at levels as low as 10 μg/g and a GC procedure using a nitrogen–phosphorus (N/P) detector for the determination of all three antihistamines in admixture in wastewater at levels as low as 10 ng/g is presented. Data concerning the stability of the test compounds in animal feed, extraction efficiencies from animal feed using various solvents and partition values of all three drugs between dichloromethane and aqueous solutions at various pH values are also reported.

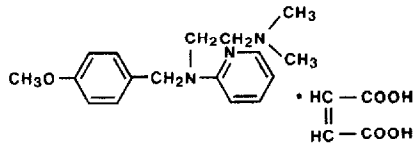
EXPERIMENTAL

Chemicals

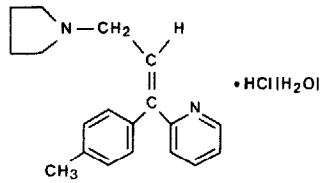
Methapyrilene hydrochloride, 2-[(2-dimethylaminoethyl)-2-thenylamino]pyridine hydrochloride (Abbott Lab., North Chicago, IL, U.S.A.), pyrilamine maleate, 2-[(2-dimethylaminoethyl)(*p*-methoxybenzyl)amino]pyridine maleate (Hexagon Lab., Bronx, NY, U.S.A.), and triprolidine hydrochloride monohydrate, 3-[3-(1-pyrrolidinyl)-1-*p*-tolylpropenyl]pyridine hydrochloride monohydrate (Chemical Dynamics, South Plainfield, NJ, U.S.A.) were used as received. The structures of these compounds are shown in Fig. 1. The test compounds were analyzed by GC with flame-ionization detection (GC–FID) and HPLC, which indicated a purity of essentially 100% for pyrilamine maleate and triprolidine hydrochloride monohydrate and 99.7% for methapyrilene hydrochloride. The methapyrilene hydrochloride contained an unknown impurity which represented 0.3% of the sample assayed. The three test chemicals were converted into



Methapyrilene Hydrochloride



Pyrilamine Maleate



Triprolidine Hydrochloride Monohydrate

Fig. 1. Structures of methapyrilene hydrochloride, pyrilamine maleate and triprolidine hydrochloride monohydrate.

their free amines and electron impact mass spectra were obtained on a Finnigan-MAT (Sunnyvale, CA, U.S.A.) Model 4023 quadrupole mass spectrometer via the solid probe. The INCOS data base library confirmed each compound's spectrum as the most probable structure. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were also found to be consistent with the literature for methapyrilene, pyrilamine and triprolidine structures. The NMR spectra for each compound were obtained using a Bruker Instruments (Billerica, MA, U.S.A.) Model WH-270 spectrometer. All solvents were of UV grade and all reagents were of CP grade.

Extraction of animal feed

Procedure 1: methapyrilene hydrochloride and pyrilamine maleate. A 20-ml volume of 1 *N* hydrochloric acid and 80 ml of methanol were added to a 20-g sample of animal feed (Laboraty Chow, Type 5010M, Ralston Purina, St. Louis, MO, U.S.A.) in a 250-ml erlenmeyer flask fitted with a PTFE-lined screw-cap. The sample was mechanically extracted for 1 h on a reciprocating shaker (Eberbach, Ann Arbon, MI, U.S.A.) at a rate of 200 excursions/min, and allowed to stand for 10 min. The supernatant was decanted into a 50-ml culture tube and centrifuged at 110 *g* for 5 min. (Note: all culture tubes were of borosilicate glass equipped with Teflon-lined screw-caps.)

Procedure 2: triprolidine hydrochloride monohydrate. A 20-ml volume of 2% aqueous ammonia was added to a 5-g sample of animal feed in a 250-ml erlenmeyer flask fitted with a PTFE-lined screw-cap. The container was sealed and incubated in a Freas 260 water-bath (GC/Precision Scientific Group, Chicago, IL, U.S.A.) for 6 h at 37.5°C. The container was allowed to cool (*ca.* 30 min) and after the addition of 80 ml of methanol the sample was handled in the same manner as in procedure 1.

Clean-up of animal feed

A 5-ml aliquot of the supernatant was transferred into a 30-ml culture tube containing 10 ml of 1 *N* hydrochloric acid and 10 ml of dichloromethane and the contents of the tube were shaken vigorously and centrifuged for 2 min at 1000 rpm. The di-

chloromethane layer was withdrawn with a syringe and cannula and discarded. The aqueous layer was extracted with two additional 10-ml portions of dichloromethane which were also discarded. (Note: care was taken not to remove any of the aqueous phase.) A 2-ml volume of 1 *M* dibasic potassium phosphate (pH 9.4) and 1.2 ml of 10 *N* sodium hydroxide solution were added and the aqueous layer was extracted three times with 10-ml portions of dichloromethane which were successively percolated through a plug of anhydrous sodium sulfate (*ca.* 18 mm diameter × 30 mm thick) into a 50-ml round-bottomed flask. The combined extracts were evaporated to dryness at ambient temperature with a rotary evaporator and water pump vacuum. The residue was dissolved in the appropriate solvent and reserved for assay by HPLC.

High-performance liquid chromatography (HPLC)

System A: methapyrilene hydrochloride and pyrilamine maleate. HPLC system A consisted of a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A solvent delivery system, a Rheodyne (Berkeley, CA, U.S.A.) Model 7120 septumless injector, an Altex (Berkeley, CA, U.S.A.) 5-μm Si Ultrasphere column (25 cm × 4.6 mm I.D.), a Farrand Optical (Valhalla, NY, U.S.A.) fluorescence HPLC monitor and a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 7127A strip-chart recorder. The excitation monochromator was set at 310 nm and a 0-52 emission filter with a 10-nm slit program was used (λ_{ex} = 310 nm; λ_{em} = 360 nm for the free amines of methapyrilene and pyrilamine). The mobile phase was 99.5% dichloromethane–0.5% isopropanol containing 0.005 *M* triethylamine (TEA) at a flow-rate of 2.0 ml/min. The retention times (t_R) for methapyrilene and pyrilamine were 7.0 and 8.0 min, respectively. All injection volumes were 50 μl using dichloromethane as the solvent. The standard solutions of methapyrilene hydrochloride (1.140 mg/ml) or pyrilamine maleate (1.410 mg/ml), each equivalent to 1.0 mg/ml of the free amine, were made by evaporating known volumes of each standard in methanol to dryness and dissolving the residues in the appropriate amount of dichloromethane for quantitation of samples.

System B: triprolidine hydrochloride monohydrate. HPLC system B consisted of a Waters Model 6000A solvent delivery system, a Rheodyne Model 7120 septumless injector, an Altex 5-μm Ultrasphere ODS column (25 cm × 4.6 mm I.D.) and a Waters Model 440 ultraviolet detector set at 254 nm. The mobile phase was 90% methanol–10% 0.01 *M* monobasic potassium phosphate buffer containing 0.005 *M* tetramethylammonium hydrogen chloride (the buffer was adjusted to pH 7.0 with 10 *N* sodium hydroxide at a flow-rate of 1.0 ml/min. The retention time (t_R) for triprolidine was 6.5 min. All injection volumes were 10 μl using methanol as the solvent. The standard solution of triprolidine hydrochloride monohydrate (1.196 mg/ml), equivalent to 1.0 mg/ml of the free amine, in methanol was diluted to the appropriate volume with methanol for use in quantitation of samples.

Gas chromatography

A Tracor (Austin, TX, U.S.A.) Model 560 instrument equipped with a Tracor Model 702 sensitized rubidium-bead nitrogen–phosphorus detector and a 180 cm × 2 mm I.D. glass column containing 3% (w/w) SP2100 DB (80–100 mesh), conditioned at 260°C overnight before use, was operated at 200°C with a helium carrier gas flow-rate of 25 ml/min and the injection port and detector were operated at 220 and 280°C, respectively. The retention times for methapyrilene, pyrilamine and triprolidine were 3.0,

6.8 and 7.4 min, respectively. Atrazine was used as a reference to monitor the performance of the detector bead. The antihistamines gave a linear response over the concentration range 2–600 ng, which was the concentration range of the assays. Peak heights were used for quantitation of samples. All injection volumes were 4 μl using methanol as the solvent.

Preparation of dosed feed for stability experiments

The three antihistamines were dosed separately into the feed. Batches of animal feed (2 kg) (Laboratory Chow, Type 5010M) spiked with 0, 114 or 2280 ppm of methapyrilene hydrochloride, 0, 141 or 2820 ppm of pyrilamine maleate or 0, 120 or 2400 ppm of triprolidine hydrochloride monohydrate to yield the equivalent of 0, 100 or 2000 ppm of the free base, were prepared by adding the appropriate amount of the test chemical in 50 ml of 95% ethanol to the feed through the intensifier bar of the blender with an additional 50 ml of 95% ethanol added throught the bar as a rinse. The feed was mixed in a Model LV Twin-Shell laboratory blender (Patterson-Kelly, East Stroudsburg, PA, U.S.A.) with the shell of the blender operated at 20 rpm during the 40–min mixing process. The intensifier bar was operated at 3300 rpm during the first 35 min of the mixing and then turned off. At the end of the blending process, each batch was transferred to a stainless-steel pan and dried for 1 h in an autoclave set at ambient temperature under 300 mmHg pressure. Each batch was then divided into 500- and 1500-g portions and reserved for the experiments.

Stability experiments

The 500-g portion of each batch of dosed feed was placed in a crystallizing dish (*ca.* 19 cm diameter × 10 cm deep) and allowed to stand in the open vessel in a fume hood under tungsten-lamp lighting at ambient temperature. These portions were used for short-term stability tests to simulate animal test conditions. Duplicate 20-g samples of feed were taken from each dish immediately, and 1, 2, 4, 8 and 16 days later for analysis of methapyrilene, pyrilamine or triprolidine by the appropriate HPLC procedure. The feed was thoroughly mixed just before removal of each sample. The 1500-g portion of the batch was sealed in amber-glass bottles and stored in a light-free cabinet at ambient temperature, and used for long-term stability tests under simulated storage conditions. Duplicate 20-g samples of feed were taken from each bottle immediately, and 1, 2, 4, 8 and 16 weeks later, and assayed by the appropriate HPLC procedure.

Extraction of human urine and wastewater

Fifty millilitres of human urine (pH 6.8) or wastewater (pH 8.0) were pipetted into a 70-ml culture tube equipped with PTFE-lined screw-caps, 0.5 ml of 0.1 *N* sodium hydroxide solution and 20 ml of dichloromethane were added and the tube was vigorously shaken and centrifuged for 2 min at 500 rpm. The dichloromethane layer was removed by syringe and cannula and percolated through a plug of anhydrous sodium sulphate, and collected in a 100-ml round-bottomed flask containing one drop of diethylene glycol (used as a keeper). The extraction was repeated with two additional 20-ml portions of dichloromethane and the combined extract (60 ml) evaporated to dryness as described for the extraction and clean-up of animal feed. The residue (equivalent to 50 g of human urine or wastewater) was subjected to analysis by HPLC system A or GC with an N/P detector.

Recovery experiments

Triplicate 5-g samples of animal feed were spiked with 1 ml of methanol containing enough methapyrilene hydrochloride and pyrilamine maleate to give sample levels equivalent to 0, 0.1, 1, 10, 100 and 1000 ppm of each free amine. Feed samples were also spiked in an identical manner with triprolidine hydrochloride monohydrate to give sample levels equivalent to 0, 10, 100 and 1000 ppm of the free amine. The samples were allowed to stand for 16 h at ambient temperature before extraction, partitioning clean-up, and analysis by the appropriate HPLC system.

Triplicate 50-ml samples of human urine in 70-ml culture tubes were similiarly spiked with 0, 1, 10, 100, 500 and 1000 ng/ml levels of the free bases of the test compounds. The tubes were sealed, vigorously shaken for 1 min and allowed to stand for 16 h in the refrigerator at 5°C before extraction with dichloromethane and analysis by HPLC system A.

Triplicate 50-ml samples of wastewater in 70-ml culture tubes were similiarly spiked with 0, 10, 100 and 1000 ng/ml levels of the free bases of the test compounds. The tubes were sealed, vigorously shaken for 1 min and allowed to stand for 16 h in a refrigerator at 5°C before extraction with dichloromethane and analysis by GC with N/P detection.

RESULTS AND DISCUSSION

HPLC, utilizing a fluorescence or UV detector, and GC, using a sensitized rubidium-bead N/P detector, were used to determine the optimal detection parameters for determination of the three antihistamines scheduled for toxicological evaluation. After the instrument requirements for detection of these antihistamines had been addressed, efficient extraction techniques were sought for recovering the test chemicals from animal feed. Recoveries of methapyrilene hydrochloride or pyrilamine maleate were determined for feed dosed at levels of 0, 0.1, 1, 10, 100 and 1000 ppm based on the free amine, using 1 *N* hydrochloric acid to deactivate the feed followed by extraction with methanol, which was the procedure that we employed for other similiarly structured antihistamines[17,18]. The percent recoveries for methapyrilene and pyrilamine were 93.3 ± 1.7 and 94.1 ± 0.6% at the 100 ppm level and 98.0 ± 1.0 and 96.7 ± 1.5% for the 1000 ppm level. However, this extraction system was inefficient for recovering triprolidine from feed after an 8-day contact time (81% at the 100 ppm level). Several attempts were made to improve the recovery using various solvent systems, and these systems and recoveries are listed in Table I. After employing deactivation of the dosed feed with 2% aqueous ammonia at 37.5°C for 6 h, followed by extraction with methanol, excellent recoveries (92%) of the triprolidine were obtained from the animal feed dosed at the 100 ppm level. The model of acid treatment followed by extraction with methanol yielded excellent recoveries for methapyrilene and pyrilamine from dosed animal feed, whereas triprolidine required the treatment mentioned previously in order to obtain good recoveries; these extraction systems were chosen for incorporation into the analytical procedure.

Determination of partition values was also necessary in the development of sample clean-up procedures for animal feed extracts, as the extracts contain co-extractives that reduce the sensitivity of the method and interfere in the analysis. The test chemical's acidic, basic and partition distribution properties between dichloromethane and

TABLE I

TRIPROLIDINE HYDROCHLORIDE MONOHYDRATE RECOVERED (AS ITS FREE AMINE) FROM ANIMAL FEED USING FOUR EXTRACTION SOLVENT SYSTEMS

Five grams of animal feed and 100 ml of solvent were used in all tests.

Solvent	*Contact time (h)**	*Recovery (%)***
Methanol (80 ml)–1 *N* HCl (20 ml)	0.25	81
Acetone (90 ml)–2% NH_4OH (10 ml)	16	58
Dichloromethane (90 ml)–10% NH_4OH (10 ml)	16	62
Methanol (80 ml)–2% NH_4OH (20 ml)	16	87
Methanol (80 ml)–2% NH_4OH (20 ml)***	16	92

* Time elapsed between spiking and addition of extraction solvent.

** Assays performed by HPLC system B.

*** 20 ml of 2% aqueous ammonium hydroxide were added to the animal feed and the sample was then incubated at 37.5°C for 6 h, followed by mechanical extraction of the sample with 80 ml of methanol.

aqueous solutions were investigated and are shown in Table II. The partition values for the three antihistamines were zero at pH 1 or less, and after adjusting the aqueous phase with 10 *N* sodium hydroxide solution to pH 7 or greater, the partition values were 1.0. A large percentage of the interfering components and co-extractives could be removed from the extracts of animal feed in this manner, thereby extending the life of the HPLC and GC columns and improving the accuracy of the analytical method.

HPLC system A, using fluorescence detection, was developed for the analysis of dosed animal feed for levels of methapyrilene and pyrilamine below 10 ppm, because the GC method with N/P detection was not adequate for those levels in feed. Various solvent systems were evaluated to determine optimal conditions for HPLC separation and the fluorescence response for the antihistamines (Table III). Although dichloromethane gave the highest relative fluorescence intensity for the two antihistamines, the

TABLE II

PARTITION VALUES FOR THE THREE ANTIHISTAMINES BETWEEN DICHLOROMETHANE AND AQUEOUS SOLUTIONS AT VARIOUS pH VALUES (1.00 = 100% EXTRACTION INTO ORGANIC PHASE)

pH	*Methapyrilene hydrochloride*	*Pyrilamine maleate*	*Triprolidine hydrochloride monohydrate*
0	0.00	0.00	0.00
1	0.00	0.00	0.00
3	0.02	0.02	0.04
5	0.72	0.75	0.78
7	1.00	1.00	1.00
9	1.00	1.00	1.00
11	1.00	1.00	1.00

TABLE III

SPECTROFLUORIMETRIC INVESTIGATION OF THE ANTIHISTAMINES (λ_{ex} 310 nm, λ_{em} 360 nm)

Solvent	*Relative intensity of antihistamine solution (10 μg/ml)*	
	Methapyrilene	*Pyrilamine*
Dichloromethane	31	42
2-Propanol	11	13
Methanol	2.5	3.1
Methanol–buffer (9:1, v/v; 0.01 *M* KH_2PO_4, pH 7)	3.2	3.9
Dichloromethane–2-propanol (99.5:0.5, v/v; 0.005 *M* triethylamine)*	28	37
Acetonitrile	15	15
1 *N* Sulphuric Acid	0.6	0.2

* This solvent system was chosen for incorporation into the analytical procedure.

mobile phase system of dichloromethane–2-propanol (99.5:0.5, v/v) containing 0.005 *M* triethylamine provided the best separation and excellent sensitivity. After the HPLC system A parameters had been developed for quantitating the two antihistamines in animal feed, recovery experiments were initiated to ascertain the accuracy and precision of the analytical procedure (Table IV).

TABLE IV

RESULTS OF ANALYSIS OF ANIMAL FEED SPIKED WITH METHAPYRILENE HYDROCHLORIDE AND PYRILAMINE MALEATE USING HPLC SYSTEM A WITH FLUORESCENCE DETECTION

*Amount added (μg/g)**	*Antihistamines recovered ($\bar{x}$ ± SD)***			
	Methapyrilene		*Pyrilamine*	
	μg/g	*%*	*μg/g*	*%*
Control	0.050 ± 0.010	—	0.050 ± 0.010	—
0.1	0.080 ± 0.010	80.0 ± 10.0	0.070 ± 0.010	70.0 ± 10.0
1.0	0.846 ± 0.021	84.6 ± 2.1	0.822 ± 0.017	82.2 ± 1.7
10	9.31 ± 0.30	93.1 ± 3.0	9.90 ± 0.4	99.0 ± 4.0
100	93.3 ± 1.7	93.3 ± 1.7	94.1 ± 0.6	94.1 ± 0.6
1000	980 ± 10	98.0 ± 1.0	967 ± 15	96.7 ± 1.5

* To 20 g of animal feed; based on the free amine.

** Means and standard deviations of triplicate analyses; spiked samples are corrected for background of control samples.

The recoveries of antihistamines increased with the concentration of the antihistamines added. The background for the unspiked control animal feed was equivalent to 0.050 ppm of both methapyrilene and pyrilamine. Typical HPLC traces of a mixture of methapyrilene and pyrilamine standards, and for animal feed extracts unspiked and spiked with the two antihistamines analysed using HPLC system A, are shown in Fig. 2.

Recovery experiments were also performed with triprolidine hydrochloride monohydrate after development of a procedure for its extraction from animal feed with

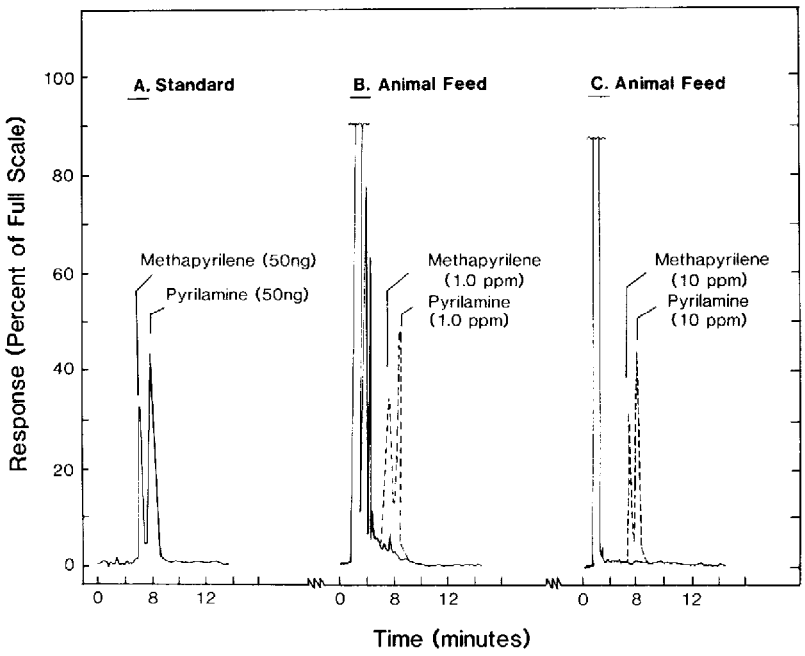


Fig. 2. System A HPLC traces. A is a standard containing 1 μg/ml each of methapyrilene and pyrilamine in dichloromethane. In B and C, the solid lines represent unspiked cleaned-up extracts of animal feed (the injections for B and C represent 50 and 5 mg-equiv. of sample, respectively); the broken lines (superimposed) illustrate the responses for the two antihistamines in amounts equivalent to 1.0 and 10 μg/g (ppm) levels of their free bases (in the sample). Extracts were spiked with a mixture of the two antihistamines after partitioning clean-up. All injection volumes were 50 μl using dichloromethane as the solvent. In all chromatograms the fluorescence detector was set a 0.1 × full-scale.

subsequent analysis using HPLC system B (Table V). The recoveries again increased with concentration, but the sensitivity was only 10 ppm. For control animal feed the background was equivalent to 2.0 ppm of triprolidine, but the precision of HPLC system B was good since the recovery was 89.1 ± 1.6% at the 10 ppm level. Typical system

TABLE V

RESULTS OF ANALYSIS OF ANIMAL FEED SPIKED WITH TRIPROLIDINE HYDROCHLORIDE MONOHYDRATE USING HPLC SYSTEM B WITH UV DETECTION

*Amount added (μg/g)**	*Amount of antihistamine recovered ($\bar{x}$ ± SD)***		
	*Equivalent amount of sample injected (mg)****	*Triprolidine*	
		μg/g	*%*
Control	250	2.00 ± 0.5	—
10	250	8.91 ± 0.16	89.1 ± 1.6
100	20	97.0 ± 1.4	97.0 ± 1.4
1000	10	980 ± 10	98.0 ± 1.0

* To 5 g of animal feed; based on the free amine.

** Means and standard deviations of triplicate analyses; results are corrected for background of control samples.

*** Injected in 10 μl of methanol.

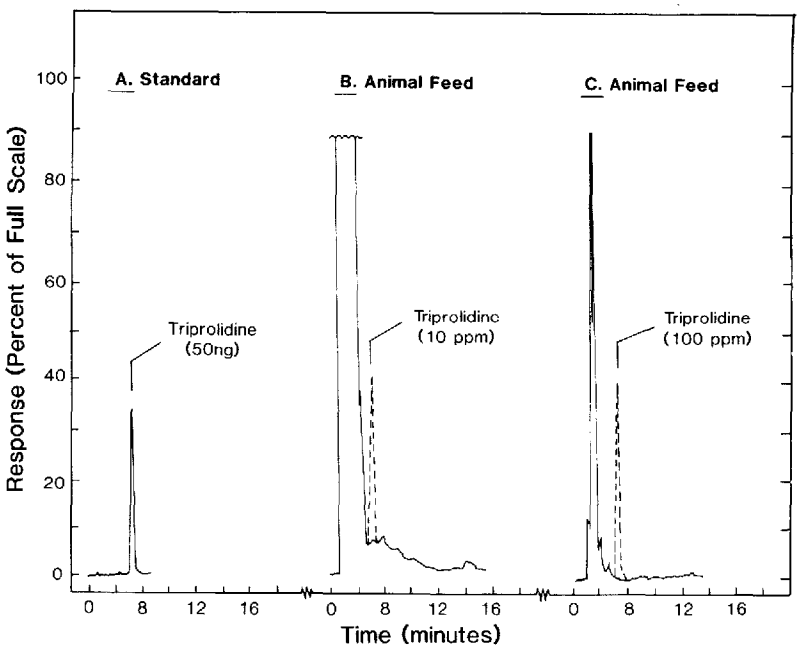


Fig. 3. System B HPLC traces. A is a standard of triprolidine containing 5 μg/ml in methanol. In B and C, the solid lines represent unspiked cleaned-up extracts of animal feed (the injections for B and C represent 5 and 0.5 mg-equiv. of sample, respectively); the broken lines (superimposed) illustrate responses for triprolidine in amounts equivalent to 10 and 100 μg/g of the free base (in the sample). Extracts were spiked after partitioning clean-up. All injection volumes were 10 μl using methanol as the solvent. In all chromatograms the UV detector was set at 0.02 a.u.f.s.

TABLE VI

RESULTS OF STABILITY STUDIES WITH TRIPROLIDINE HYDROCHLORIDE MONOHYDRATE IN ANIMAL FEED SPIKED AT TWO LEVELS

The assays were performed with HPLC system B using a UV detector set at 254 nm.

Sampling intervals	*Antihistamine recovered (μg/g)**		
	Control (none added)	*100 μg/g added*	*2000 μg/g added*
*Short-term study***			
(Days)	2.0	100	2000
0	2.1	97	1990
1	2.1	97	1980
4	1.9	95	1950
8	2.2	93	1910
16	2.0	93	1910
*Long-term study****			
(Weeks)			
0	2.0	100	2000
1	2.2	98	1980
2	2.4	95	1970
4	2.2	98	1970
8	2.0	98	1980
16	2.0	85	1910

* Means of duplicate assays; corrected for background of control samples and recovery. Dose levels based on the free amine.

** Open container, incandescent lighting and ambient temperature.

*** Sealed container, light-free cabinet and ambient temperature.

B HPLC traces for the analysis of animal feed containing triprolidine are shown in Fig. 3.

Stability studies for the three antihistamines in animal feed were determined to ensure proper dose concentrations during the toxicological experiments with test animals. Results of the short- and long-term stability tests for triprolidine in animal feed are presented in Table VI. The 100 and 2000 ppm triprolidine dosed samples showed a 7% and 5% reduction over the short-term study, and a 15% and 5% decrease in recoverable residues for the long-term study. Methapyrilene and pyrilamine were similarly stable.

Recovery experiments were performed on the analysis of human urine for methapyrilene and pyrilamine using HPLC system A (Table VII). The recoveries for the 1 ppb dose level were 60.6% for methapyrilene and 70.0% for pyrilamine and those at the 1000 ppb level were 84.5% for methapyrilene and 86.6% for pyrilamine. The background for control samples of human urine using HPLC system A was equivalent to 0.20 ± 0.10 ppb of each of the two antihistamines. Typical system A HPLC traces of human urine unspiked and spiked with methapyrilene and pyrilamine are shown in Fig. 4. Recovery experiments on the analysis of triprolidine in samples of human urine were not performed, because the determination of this antihistamine in this substrate was not required.

TABLE VII

RESULTS OF ANALYSIS OF HUMAN URINE SPIKED WITH TWO ANTIHISTAMINES USING HPLC SYSTEM A

*Amount added (ng/g)**	*Equivalent amount of sample/injection (mg)*	*Antihistamines recovered ($x \pm SD$)***			
		Methapyrilene		*Pyrilamine*	
		ng/g	*%*	*ng/g*	*%*
Control	2.5	0.200 ± 0.10	—	0.200 ± 0.10	—
1	2.5	0.606 ± 0.06	60.6 ± 6.0	0.700 ± 0.10	70.0 ± 10.0
10	2.5	6.15 ± 0.06	61.5 ± 0.6	7.43 ± 0.09	74.3 ± 0.90
100	2.5	76.8 ± 0.40	76.8 ± 0.4	76.9 ± 0.9	76.9 ± 0.9
500	1.0	391 ± 0.6	78.5 ± 0.12	398 ± 4.0	79.6 ± 0.8
1000	0.5	845 ± 7.0	84.5 ± 0.7	866 ± 16	86.6 ± 1.6

* To 50 ml of sample; based on the free amine.

** Means and standard deviations from triplicate assays; spiked sample corrected for background of control samples.

Data from recovery experiments on GC analysis with N/P detection of a mixture of the three antihistamines in wastewater at levels as low as 10 ppb are shown in Table VIII. The background of unspiked wastewater was equivalent to 0.080, 0.160 and 0.160 ppb of methapyrilene, pyrilamine and triprolidine, respectively. An analytical procedure using GC analysis with N/P detection of a mixture of the three antihistamines in wastewater was developed to reduce the development time for the analytical methods. Typical gas chromatograms of a standard mixture of the three antihistamine unspiked and spiked in wastewater are shown in Fig. 5.

TABLE VIII

RESULTS OF ANALYSIS OF WASTEWATER SPIKED WITH A MIXTURE OF THE THREE ANTIHISTAMINE SALTS USING GC WITH N/P DETECTION

*Amount added (ng/g)**	*Equivalent amount of sample/injection (mg)*	*Antihistamines recovered (x ± SD)***					
		Methapyrilene		*Pyrilamine*		*Triprolidine*	
		ng/g	*%*	*ng/g*	*%*	*ng/g*	*%*
0	100	0.080 ± 0.20	—	0.160 ± 0.03	—	0.160 ± 0.050	—
10	100	9.72 ± 0.20	97.2 ± 2.0	8.52 ± 0.45	85.2 ± 4.5	85.2 ± 0.40	76.8 ± 4.0
100	50	101 ± 2.0	101 ± 2.0	102 ± 3.0	93.1 ± 5.0	93.1 ± 5.0	93.1 ± 5.0
1000	50	950 ± 10	95 ± 1.0	930 ± 20	93.0 ± 2.0	910 ± 16	91.0 ± 1.6

* Per 50-ml sample; based on the free amine.

** Means and standard deviations from triplicate assays; spiked samples are corrected for background of control samples.

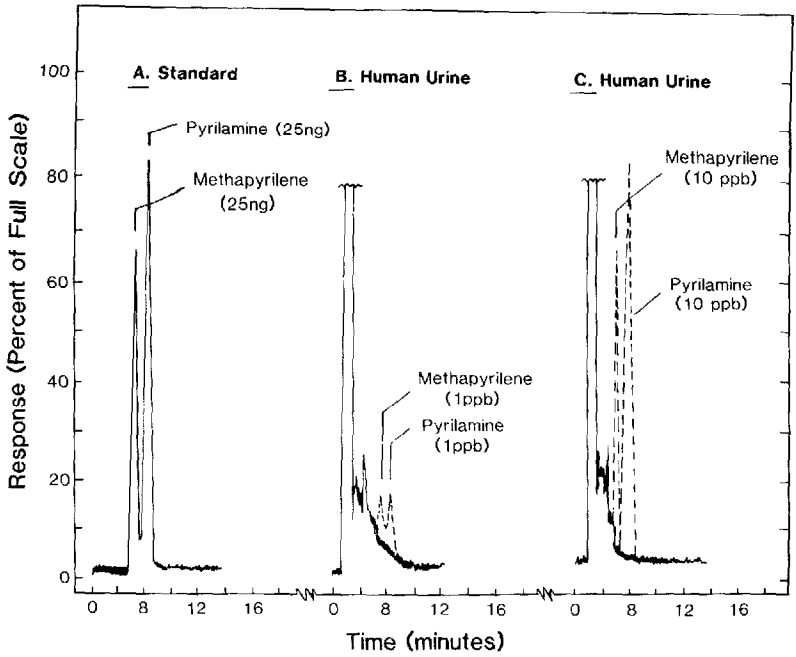


Fig. 4. System A HPLC traces. A is a standard containing 0.5 μg/ml each of methapyrilene and pyrilamine in dichloromethane. In B and C, the solid lines represent unspiked partitioned extracts of human urine (the injections for B and C represent 2.5 ml-equiv. of sample); the broken lines (superimposed) illustrate responses for methapyrilene and pyrilamine in amounts equivalent to 1 and 10 ng/ml (ppb) of their free bases (in the sample). Extracts were spiked after partitioning clean-up. All injection volumes were 50 μl using dichloromethane as the solvent. In all chromatograms the fluorescence detector was set a 0.03 × full-scale.

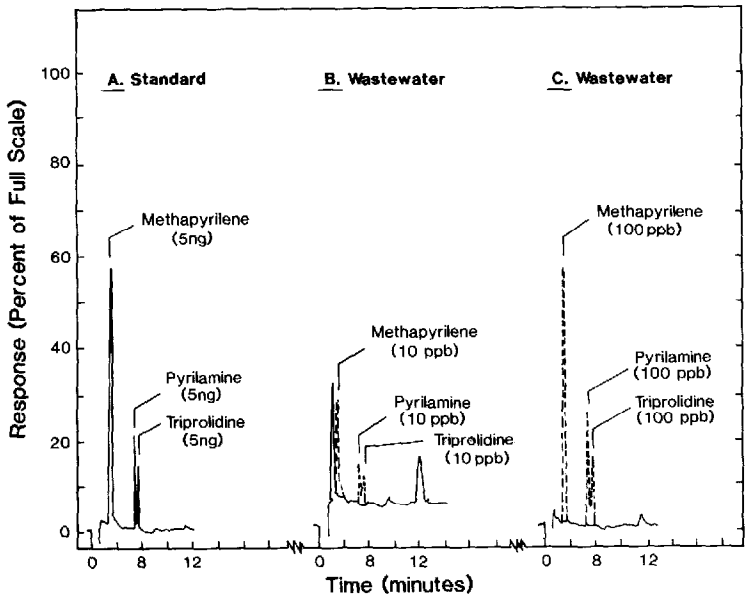


Fig. 5. Gas chromatograms. A is a standard containing 2.5 μg/ml of each of the three antihistamines. In B and C, the solid lines illustrate unspiked partitioned extracts of wastewater (the injections for B and C represent 100 and 50 μl-equiv. of sample); the broken lines (superimposed) illustrate responses for methapyrilene, pyrilamine and triprolidine in amounts equivalent to 10 and 100 ng/ml of their free base (in the sample). Extracts were spiked after partitioning clean-up. All injection volumes were 2 μl using methanol as the solvent. Full-scale response for A and C was $8 \cdot 10^{-11}$ A and that for B was $2 \cdot 10^{-11}$ A.

The analytical procedures reported here have allowed the toxicological evaluation of methapyrilene hydrochloride, pyrilamine maleate and triprolidine hydrochloride monohydrate in these laboratories, which would not have been possible otherwise.

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