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Short Communication

Quantification of dimethindene in plasma by gas chromatography–mass fragmentography using ammonia chemical ionization

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ABSTRACT

A gas chromatographic–mass fragmentographic method using ammonia chemical ionization for the determination of dimethindene in human plasma is described. The drug was isolated from plasma by liquid–liquid extraction with hexane–2-methylbutanol. Plasma components were separated on a capillary column coated with chemically bonded methyl silicone. For detection of dimethindene, its quasi-molecular ion ($M + H^+$) was mass fragmentographically monitored after chemical ionization with ammonia as reagent gas. Dimethindene was quantified using methaqualone as the internal standard: the quantification limit in plasma was 0.2 ng/ml, the within-run precision was 8.0% and the inter-run precision 5.6%. The plasma concentration–time profile was established after a single dose of 4 mg of dimethindene with an average maximum concentration of 5.5 ng/ml, detectable up to 48 h post application.

INTRODUCTION

Dimethindene, N,N-dimethyl-3-[1-(2-pyridinyl)ethyl)]-1*H*-indene-2-ethanediamine (DIM), is one of the classic H1-receptor antagonists. Its high potency, however, is accompanied by a relatively high sedative side-effect. Maximizing the known antihistaminic effect and minimizing the sedative side-effect is the aim of research groups, and experiments with low doses of DIM in special pharmaceutical formulations are performed. Highly sensitive and specific methods are necessary to quantify DIM in human plasma for pharmacokinetic research.

Up to now only an enzyme-linked immunosorbent assay (ELISA) method has been available to detect DIM in the low nanogram range [1]. Previously published chromatographic methods [2,3] are not sensitive enough.

This paper describes a gas chromatographic–mass fragmentographic technique, using positive chemical ionization with ammonia as the reagent gas, following liquid–liquid extraction with glass-free apparatus.

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EXPERIMENTAL

Reagents

Dimethindene maleate was of pharmaceutical grade (Zyma, Nyon, Switzerland) and methaqualone, the internal standard, was analytical grade (Sigma Chemicals, Unterhaching, Germany), Tris buffer (pH 10.5), *n*-hexane, 2-methylbutan-1-ol, *n*-propanol, toluene and methanol were of analytical grade (Merck, Darmstadt, Germany). For chemical ionization, ammonia of highest purification grade (9.5) was used (L'air Liquide, Bottrop, Germany).

Extraction equipment

Polypropylene tubes (10 ml) with screw caps (Sarstedt, Nümbrecht, Germany) and Eppendorf tubes (2 ml) were used for extraction.

Apparatus

A Hewlett Packard GC 5980, coupled with a mass-selective detector 5971A, and an automatic injector HP 7673A were used. A novel ion source Model G 1072A for chemical ionization mode was installed in the mass-selective detector.

Gas chromatography (GC) was performed on a capillary column (12 m × 0.2 mm I.D.) coated with chemically bonded methyl silicone (HP 1), film thickness 0.33 μm. The chromatographic conditions were: injector temperature, 280°C; initial oven temperature, 100°C for 1.0 min; oven heating, 20°C/min to 300°C, held for 2 min. The injection mode was splitless with a purge at time 0.75 min. The flow-rate of the carrier gas was 1 ml/min.

Mass-selective detection was performed by monitoring the protonated molecular ions of DIM and methaqualone (internal standard) at *m/z* 293 and 251, respectively. The dwell-time was set at 200 ms for each mass. The pressure of the ion source was *ca.* 10 Pa.

Extraction of DIM from plasma

To 2.0 ml of plasma were added 1 ml of distilled water, 10 ng of methaqualone (0.01 ml of a 1.0 mg/l ethanolic solution) and 1.0 ml of Tris buffer. The plasma was vigorously shaken with *n*-hexane–2-methylbutan-1-ol (99:1) for 15 min. After centrifugation for 5 min at 3000 *g*, the organic layer was removed and 0.1 ml propanol was added. The solution was evaporated to dryness by nitrogen in the Pierce Reacti Therm at 40°C. The residue was dissolved in a solution of toluene–methanol (96:4), which was placed into an HP micro vial (silanized) for automatic injection of 1 μl.

The capillary column was deactivated prior to the quantification of DIM by the passage of 2 μl of an extract of tobacco dissolved in 1 ml of ethyl acetate, which was prepared by vortex-mixing about half a cigarette in 10 ml of ethyl acetate for 1 h, centrifugation of the mixture for 5 min at 3000 *g*, and evaporation of the supernatant down to 1 ml. The oven temperature was initially held at 60°C for 2 min, then raised to 300°C at 2°C/min, then held at 300°C for 10 min. All other conditions were the same as described above. The column was then tested by running a mixture of barbitone, phenobarbitone, diphenhydramine and free morphine (20 ng each) dissolved in ethanol under the conditions described for DIM. The test was satisfactory, when the peaks showed no tailing, otherwise the deactivation had to be repeatede.

Calibration

A calibration curve was established by analysing spiked plasma samples containing 0.1, 0.2, 0.5, 1.0, 2.0, 4.0 and 8.0 ng of DIM per ml of plasma. Each sample was analysed in triplicate. The linear regression was calculated as $y = 0.01425x - 0.00075$, and the correlation coefficient was $r = 0.9995$.

RESULTS AND DISCUSSION

Gas chromatography

DIM tends to exhibit slight tailing during GC because of its pyridinyl stucture. With the HP 1 capillary column it was well separated under the described conditions and in the range of detection limit. However, prior deactivation of the column by injection of tobacco extract onto the column was necessary. Tobacco contains numerous

polar compounds that occupy all the active sites of the stationary phase as well as the capillary. Combined with the low rate of heating, this results in an increase of the peak response by at least a factor of 2. This step had to be repeated whenever any changes were made to the gas chromatograph, *e.g.* replacement of the injector insert line or cutting the column end at the injector site.

Mass-selective detection

The weak protonisation of DIM by ammonia chemical ionization was the key making possible the detection of picogram amounts of DIM in plasma extracts. Initial monitoring of the most intense ion m/z 58 of DIM in electron-impact (EI) mode failed to reach this because the background was too high. Fig. 1 compares the chemical ionization (CI) and EI mass spectra of DIM. Fig. 2 shows the mass fragmentograms of a blank plasma extract and an authentic plasma extract containing 0.53 ng of DIM per ml of plasma.

Extraction

The use of a non-polar solvent mixed with small portion of a higher alcohol leads to a rather selective and exhaustive extraction of DIM from

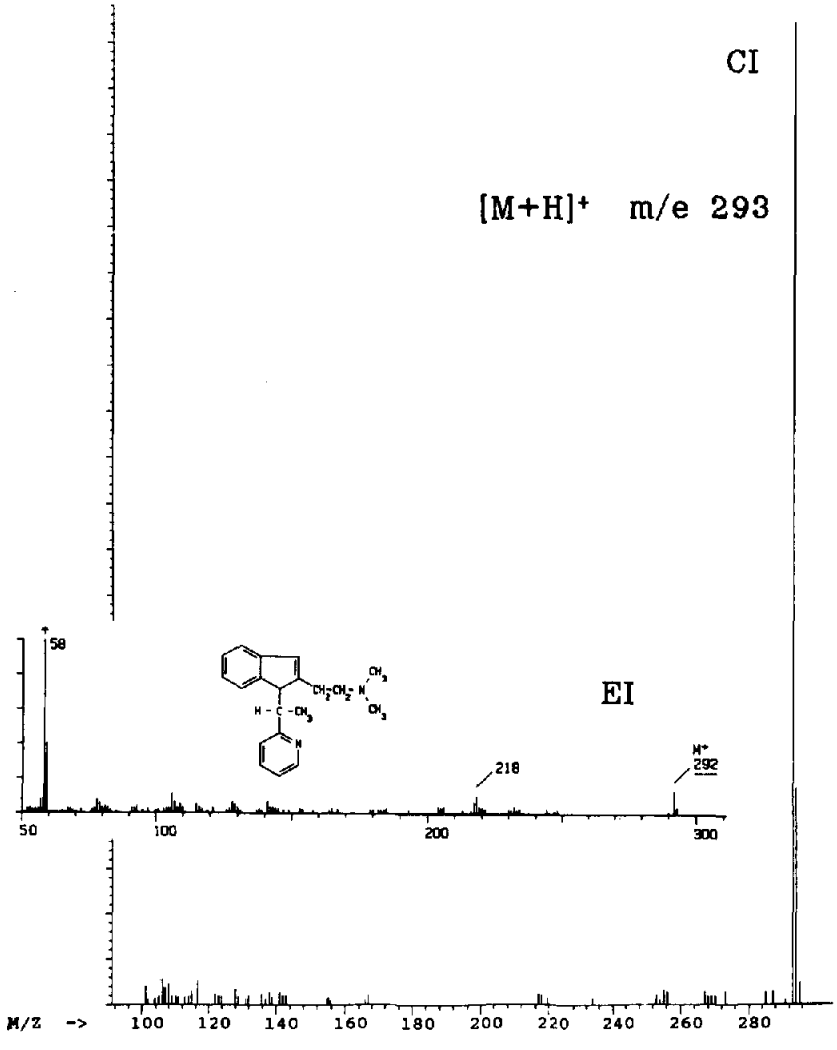

Fig. 1. Electron impact (EI) [5] and ammonia chemical ionization (CI) mass spectra of dimethindene.

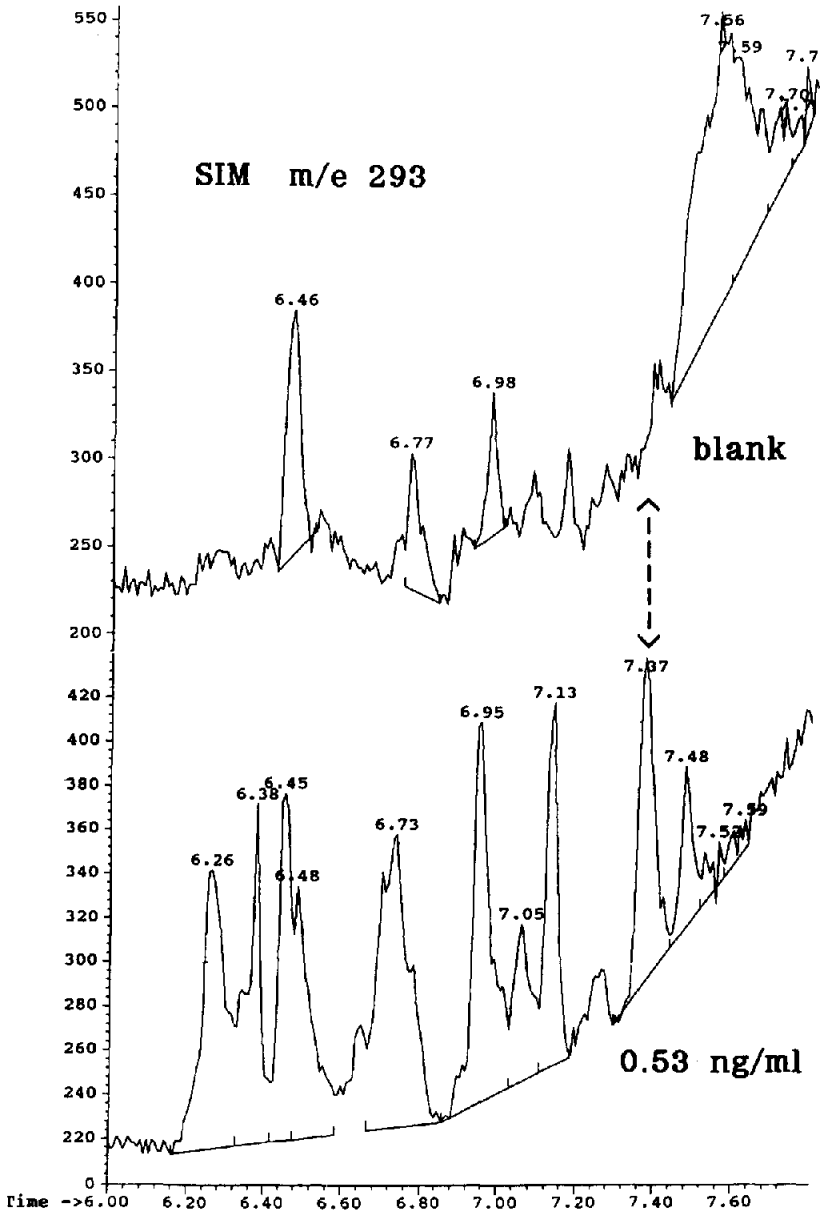

Fig. 2. Single-ion monitoring with m/z 293 (protonated dimethindene) from blank and authentic plasma extracts.

plasma, and avoids the coextraction of many endogenous and other exogenous substances, *e.g.* caffeine [3].

Considerable improvement in the extraction yield and the detection limit was brought about by using polypropylene tubes for liquid–liquid extraction as well as for evaporation of the organic layer. DIM is unavoidably adsorbed on silanized glass surfaces. This phenomenon was also described by Wermeille *et al.* [1].

Quality control

The extraction recovery of DIM from plasma at the 2.5 ng/ml level was 93%. The within-run

TABLE I

VALIDATION TEST BY BLIND ANALYSIS

Sample No.	Spiked concentration (ng/ml)	Found concentration (ng/ml)
1	3.41	3.12
2	1.40	1.47
3	8.5	11.2
4	0.0	0
5	2.75	2.05
6	0.70	0.93
7	2.08	1.83
8	5.68	6.94

precision of the method was 8.0% at 1.0 ng/ml. The precision from day to day was calculated as 5.6% at 2.5 ng/ml.

The limit of quantification was 0.2 ng of DIM per ml of plasma, with a peak signal-to-noise ratio greater than 2 and a precision of 13.7%. The absolute detection limit for DIM was 20 pg on the column, with a signal-to-noise ratio greater than 2.

The method was validated by a blind analysis of human serum samples, which were prepared by the Zyma Research Labs.The results obtained are shown in Table I. The linear regression of

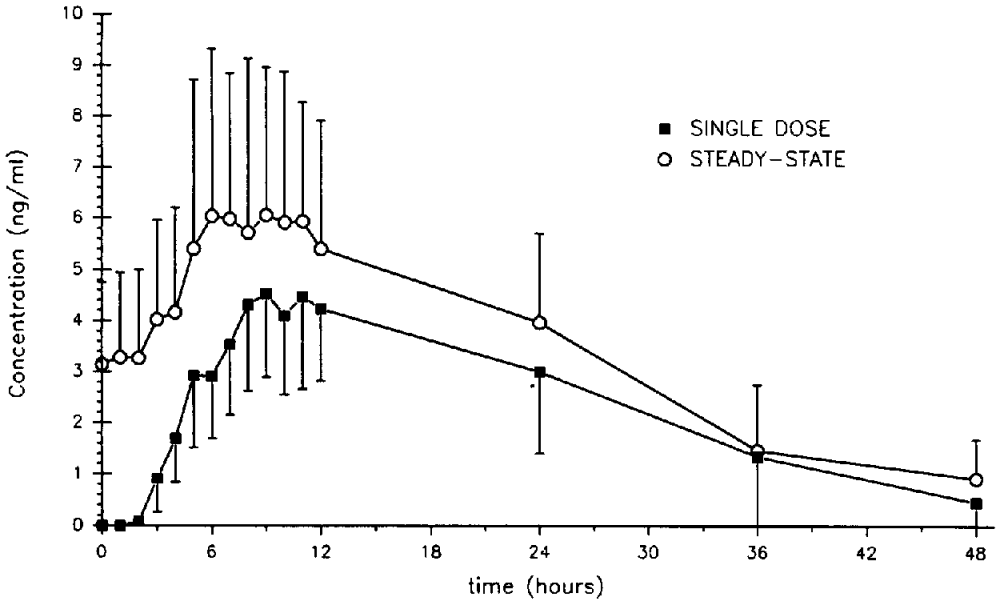

Fig. 3. Plasma concentration–time profiles of dimethindene after a single dose (■) and after a one-week treatment (○) with 4 mg of Fenistil OAD (n = 12).

TABLE II

MAIN PHARMACOKINETIC PARAMETERS OBTAINED WITH A ONCE-A-DAY 4 mg FORMULATION OF DIM MALEATE

	t_{max} (h)	C_{max} (ng/ml)	$t_{1/2el}$ (h)	$AUC_{0-\infty}$ (h · ng/ml)	$C_{min-216h}$ (ng/ml)	AUC_{0-24} (h · ng/ml)	AUC_{ss}/AUC_{sd}	Accumulation ratio
Single dose	12.3 ± 5.6	5.5 ± 1.6	11.1 ± 4.2	130.3 ± 55.1				
Steady state	9.0 ± 5.2	7.5 ± 3.0			4.0 ± 1.7	115.1 ± 48.1		
Parameters of comparison							0.98 ± 0.5	1.27 ± 0.54

these values was $y = -0.602 + 1.32x$, with a correlation coefficient of $r = 0.986$.

Applicability of the method

A total of 384 plasma samples drawn from volunteers after a single oral dose, as well as after a one-week treatment of 4 mg of DIM maleate in a new slow-release formulation (Fenistil OAD = once a day), were analysed. The average plasma concentration–time curves are shown in Fig.3.

The main pharmacokinetic data are listed in Table II. Comparison of these data with those obtained with a solution indicates a similar AUC but a larger half-life of elimination, a longer time to reach maximum concentration and a lower peak level [4]. These results are consistent with a slow-release formulation.

After 200 analytical runs the sensitivity decreased suddenly, and the ion source had to be decontaminated. It is notable that the reagent gas supply could not be interrupted between runs, but this served to increase the precision of the method.

With the method and equipment described, it was possible to analyse large series of plasma samples containing DIM down to the subnanogram range.

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