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Note

Quantitative gas chromatographic–mass spectrometric analysis of acrivastine and a metabolite in human plasma

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Acrivastine, (*E*)-3-{6-[(*E*)-3-(1-pyrrolidinyl)-1-(*p*-tolyl)-1-propenyl]-2-pyridyl}acrylic acid, is a member of a new class of antihistamine drugs with minimal sedative properties and is currently used in human trials. It is an H1-antagonist with a potency similar to triprolidine but with a marked reduction in central nervous system side-effects [1]. The apparent volume of distribution of acrivastine in man [1] is much less than that of triprolidine, suggesting much more restricted tissue distribution for the former compound. In agreement with this proposal is the observation that circulating plasma concentrations of acrivastine are much higher than those of triprolidine after therapeutic oral doses [1,2].

To date, plasma levels of acrivastine have been measured by a sensitive radioimmunoassay (RIA) [2]. Subsequent to the development of this RIA, an active metabolite (I), (*E*)-3-{6-[3-(1-pyrrolidinyl)-1-(*p*-tolyl)-1-propenyl]-2-pyridyl}propionic acid, formed by reduction of the acrylic acid side-chain, was identified in man and determined to be completely cross-reactive with the acrivastine antisera employed in the RIA. To understand drug effects more completely, i.e. the pharmacodynamics and pharmacokinetics of acrivastine, it is important to know plasma levels of both parent drug and active metabolite. The purpose of this paper is to present a gas chromatographic–mass spectrometric (GC–MS) method which can determine acrivastine and metabolite I in human plasma simultaneously and is applicable to pharmacokinetic studies of acrivastine and its metabolite in man.

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EXPERIMENTAL

Instrument conditions

The GC–MS system was a high-resolution mass spectrometer (VG70S) interfaced with a Hewlett Packard 9870 gas chromatograph. The instrument was operated in selected-ion recording (SIR) electron ionization mode (70 eV) at 200°C source temperature. The resolution was set at approximately 3000 (10% valley). Acrivastine and metabolite I were monitored for the reaction product M-69 (*m/z* 351) (viz., neutral loss of pyrrolidine) and the molecular ion (*m/z* 422), respectively. The internal standards for I and acrivastine were also mon-

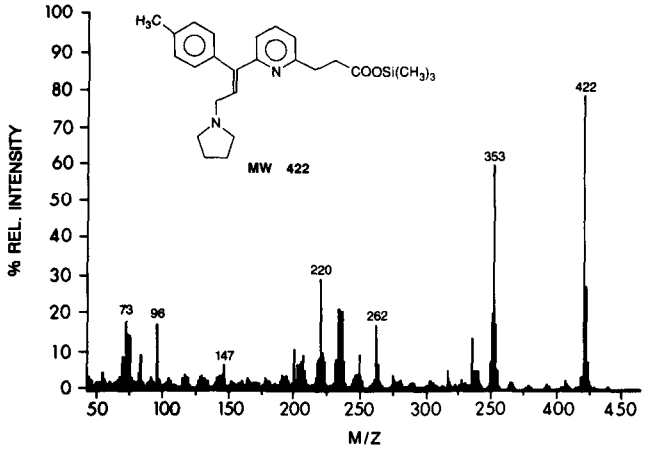

Fig. 1. Electron ionization spectrum of metabolite I.

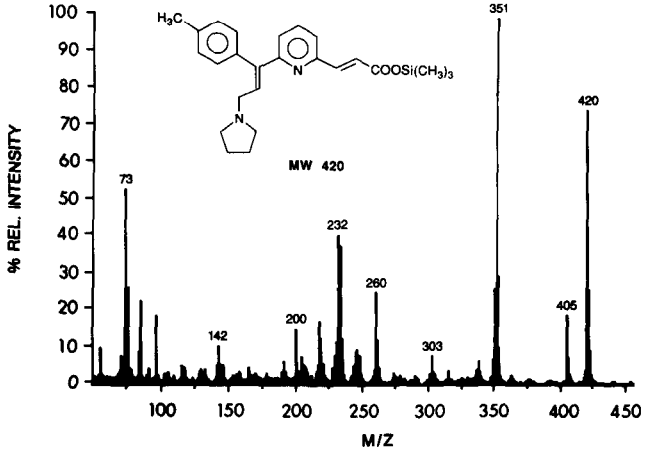

Fig. 2. Electron ionization spectrum of acrivastine.

itored for the molecular ion (*m/z* 429) and M-69 ion (*m/z* 358), respectively (Figs. 1 and 2). Acrivastine ions (*m/z* 422 and 429) and I ions (*m/z* 351 and 358) were divided into two groups. Ions were monitored by voltage scanning and the two groups were selected by switching the magnetic field. The GC capillary column was a DB-5 (15 m×0.25 mm I.D.) from J&W Scientific (Folsom, CA, U.S.A.) and it was directly inserted to the mass spectrometer ion source. The gas chromatograph was programmed starting at 225°C with a 4-min hold to 300°C at the rate of 8°C min^{-1}. The samples were injected using a J&W on-column injector.

Internal standards

The stable isotope analogues (perdeuterated in the tolyl group) of I and acrivastine were used as internal standards. They were synthesized at Burroughs Wellcome, using perdeuterated toluylaldehyde purchased from Cambridge Isotope Laboratories (Woburn, MA, U.S.A.) Purity of the final products was determined to be 99% and confirmed by 300-MHz ^1H NMR and GC–MS. All seven protons of the tolyl groups of I and acrivastine were replaced by deuterium.

Materials

Acrivastine and I were synthesized by the Wellcome Research Laboratories of Wellcome Foundation (Dartford, U.K.).

All solvents were HPLC grade. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Pierce (Rockford, IL, U.S.A.). C_{18} Sep-Pak cartridges were obtained from Waters Assoc. (Milford, MA, U.S.A.). Human plasma samples had been stored at −70°C. Prior to this analysis, the sum of acrivastine and I present in the samples were analyzed by RIA according to the published method [2].

Plasma standard curve preparation

Stock solutions of acrivastine, I and their respective deuterated analogues were prepared by accurately weighing 2–5 mg of compounds into a 10-ml volumetric flask and then brought to final volume with water. Once prepared a given stock solution was sealed and stored at 4°C in the dark. Under these conditions of storage all stock solutions were stable at least four months. Standard curve samples were prepared by serial dilution over a concentration range of 0–256 ng free base equivalents per ml of a normal human plasma. They were extracted according to the method described in the following section and analyzed by GC–MS.

Plasma sample clean-up

Plasma standard and human plasma samples for analysis were prepared by solid-phase extraction on a Sep-Pak C_{18} cartridge. As necessary, plasma samples were clarified by centrifugation and/or filtration. Prior to use, all Sep-Pak

cartridges were washed with 5 ml of methanol followed by 10 ml of water. Samples were prepared for analysis by mixing 0.5 ml each of plasma and internal standard solution which contained 200 ng each of deuterated acrivastine and deuterated I per ml water. A 1-ml aliquot of the mixture was loaded into a 3-ml syringe attached to a Sep-Pak cartridge. This solution was passed through the cartridge and then washed with 3 ml of 30% methanol in water. The drug and internal standards were eluted from the cartridge in 2 ml of methanol and then evaporated to dryness under a stream of nitrogen at 50°C. The residue was washed with two 3-ml portions of hexane and dried under nitrogen without heating. The residue was reconstituted in 400 μl of methanol by vortexing and sonication and then transferred to a 1-ml Reactivial (Pierce). This solution was again evaporated to dryness under a stream of nitrogen without heating in preparation for derivatization.

Derivatization

Dried residues were reacted with 50 μl of a BSTFA–acetonitrile–dichloromethane (2:1:0.1) mixture for 15 min at 100°C. For GC–MS analyses, approximately 1.5 μl were injected onto the GC column using an on-column injector.

RESULTS AND DISCUSSION

Plasma standard curve and precision

Standard curves for determining plasma concentrations of drugs were generated on different dates and their values were compared to determine the reproducibility of the method. Analysis of all standard curves showed very reproducible, biphasic linear regression lines. For acrivastine, 0–250 and 0–30 ng, the linear regression equations $y=0.0067x-0.016$ and $y=0.0058x-0.00073$ were used, respectively. The coefficients of variation observed for 4–250 ng were 4.6–14.6% and for 2 ng it was 34.4%. For I, 0–250 and 0–30 ng, the linear regression equations $y=0.0055x-0.0093$ and $y=0.0049x-0.0011$ were used respectively. The coefficients of variation observed for 2–250 ng were 4.2–17.8%. The practical sensitivity limit of the assay for both compounds was approximately 2 ng per ml plasma. Although the RIA was more sensitive (0.1 ng/ml), it could not distinguish the individual amounts of acrivastine and I.

Human plasma sample analysis

For routine clinical sample analysis, in addition to a plasma standard curve for each set (0–24 h) of samples, control spiked samples were inserted at the end of the set. The duplicate control spiked samples were prepared in normal plasma and their spiked drug concentration levels were 5, 10, 25, 50, 100 and 200 ng/ml each for I and acrivastine. The results from five different preparations on different dates are presented in Table I.

TABLE I

CONTROL RECOVERY EXPERIMENTS

Five different controls on different dates.

Added (ng)	Found (mean) (ng)	Recovery (%)	S.D. of found	Coefficient of variation (%)
5	5.7	114	0.2	3.6
10	10	100	1.3	12.3
25	26.6	106	2.7	10.1
50	47.4	95	1.7	3.6
100	100.7	101	4.5	4.5
200	192.3	96	6.5	3.4

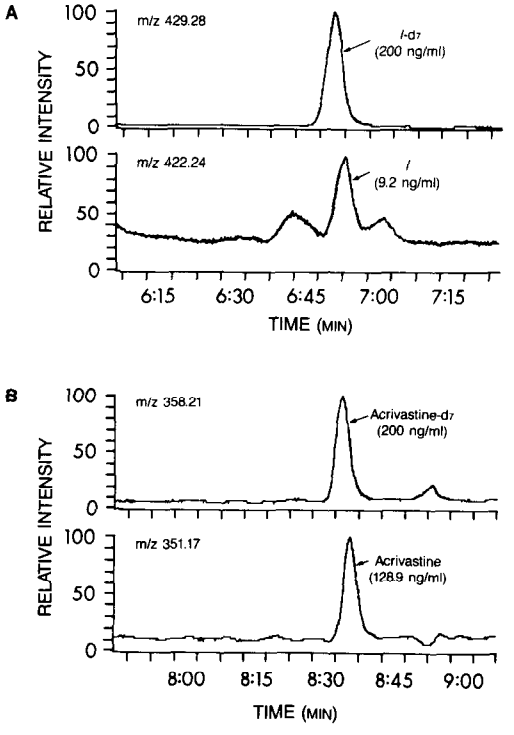

Fig. 3. SIR profile of a plasma sample from patient B. A patient was given acrivastine (a 16-mg single oral dose) and a plasma sample was obtained at 0.5 h after dosing. A, Metabolite I; B, acrivastine.

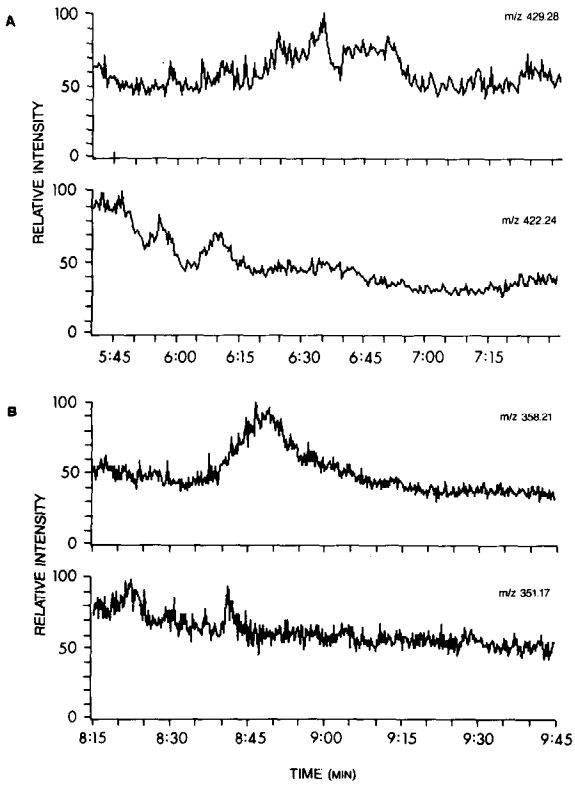

Fig. 4. SIR profile of blank normal plasma. A normal human plasma sample was processed as described in the Experimental section and analyzed by the GC–MS method. The signals were normalized. Actual signal levels were approximately 100 times less than those of the baseline of Fig. 3.

The chromatograms of an extract of a plasma sample collected from a patient (patient B) 0.5 h after an oral dose of 16 mg acrivastine syrup and a normal human plasma blank are presented in Figs. 3 and 4. Results from analysis of the 0–24 h human plasma samples after a single oral dose of 16 mg acrivastine are shown in Fig. 5. The method was also compared with the RIA data which were obtained three years ago. There is a very close correlation between the sum of I and acrivastine values obtained by GC–MS and RIA results. A comparison of the two methods is presented in Table II and shows approximately 10% difference in their values for the 1-h time points (peak concentration) and 0.5-h time points. We believe the overall comparisons are very reasonable because the two different analytical methods were performed three years apart.

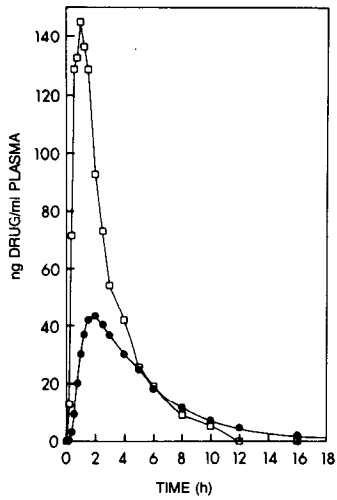

Fig. 5. Concentration–time plot for patient B given acrivastine (a 16-mg single oral dose). (●) Acrivastine; (□) metabolite I.

Comments on the method

Acrivastine and I are stable compounds. Water or methanol solutions of these compounds stored under laboratory conditions (25 °C and light) for four months showed no significant decomposition. However, trimethylsilyl (TMS) derivatives of pure drugs or plasma sample extracts were stable only for 48 h, after which time the sensitivity of TMS derivatives decreased to approximately 50%, although the relative ratios of internal standards to analytes remained the same. It is important that samples with low drug concentrations be analyzed on the same day they are derivatized. Since internal standards have seven deuterium atoms in the tolyl group, internal standards and drugs are separated approximately 2 s in GC retention time. Such separation did not change the relative ratios of ion intensities of internal standards and analytes in the ion source under present extraction and instrument conditions.

TMS derivatives of these compounds gave the best results with an on-column injector. The TMS derivatives were extremely sensitive to any trace amount of acidic compounds left on the column. If the column was previously used for the analysis of acidic compounds, it was essential to pretreat the column with triethylamine prior to analyses of acrivastine and I TMS derivatives. With all previously mentioned precautions, the method performed very reliably with numerous repeated analyses.

Currently we are using this method to determine levels of acrivastine and metabolite I in human plasma samples from human clinical studies. No serious interference was encountered for metabolite I in clinical samples; however, one

TABLE II

COMPARISON OF DRUG CONCENTRATION LEVEL BY GC-MS AND RIA

Patient A given 16 mg acrivastine in a syrup (a single oral dose).

Sampling time (h)	Concentration (ng/ml)			
	GC-MS			RIA (total)
	Acrivastine	I	Total	
0.00	0.0	0.0	0.0	0.0
0.17	0.0	0.0	0.0	1.5
0.33	30.9	4.0	34.9	36.0
0.50	95.1	5.1	100.2	89.0
0.75	170.5	13.4	183.9	192.0
1.00	227.1	24.0	251.1	232.0
1.25	194.1	30.5	224.6	165.0
1.50	190.9	34.8	225.7	207.0
2.00	148.5	34.8	183.3	160.0
2.50	121.8	34.8	156.6	109.0
4.00	68.3	28.3	96.6	76.0
5.00	51.1	24.0	75.0	63.0
6.00	32.6	17.7	50.3	40.0
8.00	15.6	12.3	27.8	22.0
10.00	7.3	6.8	14.2	8.3
12.00	4.6	4.7	9.3	4.0
16.00	0.0	2.1	2.1	2.0
24.00	0.0	0.0	0.0	0.0

patient gave a serious interference for acrivastine at low levels (0–5 ng/ml). We did not investigate the nature of this interference since it was not a consistent problem.

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