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Note

Determination of crotamiton in plasma and urine by high-performance liquid chromatography

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Crotamiton (crotonyl-N-ethyl-O-toluidine) is a scabicide, insecticide and antipruritic agent [1] Pure crotamiton is a mixture of *trans* and *cis* isomers (Fig 1), in approximate proportions of 95 5 (with a limit of 85 15) This paper describes a method for determining crotamiton in human plasma and urine by quantifying the *trans* isomer after separation of the two isomers and expressing the results as crotamiton

EXPERIMENTAL

Solvents and reagents

Crotamiton was supplied by Laboratories Ciba-Geigy (Huningue, France). The *trans* and *cis* isomers of crotamiton and N-ethyl-N-propionyl-O-toluidine (internal standard) were supplied by Ciba-Geigy (Basle, Switzerland) (Fig 1) The solvents and reagents used were acetonitrile (Baker, Deventer, The Netherlands), methanol (Prolabo, Paris, France), *n*-heptane (Merck, Darmstadt, F R G), isoamyl alcohol (Merck), pH 12 buffer (Titrisol) (Merck) and deionized water Potassium dihydrogenphosphate solution (0 01 *M*) was prepared by dissolving 2.72 g of potassium dihydrogenphosphate (Merck) in water and diluting to a final volume of 2000 ml

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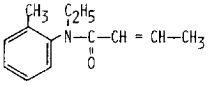
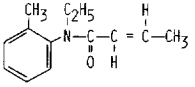


I



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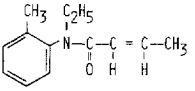

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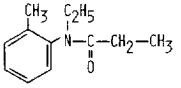

IV

Fig 1 Structures of crotamiton (I), *trans* isomer (II), *cis* isomer (III) and internal standard (IV)

Chromatography

Chromatography was performed on a system equipped with a Waters M510 solvent-delivery pump and Waters Wisp M710B automatic sampler

The column was connected to a Kratos 783 variable-wavelength UV detector set at 220 nm A Hewlett-Packard 3388 integrator recorded the chromatograms and calculated the peak heights A Hypersil ODS (5 μm) chromatographic column (20 cm$\times$4 6 mm I D) (No 79916 OD, Option 574) was supplied by Hewlett-Packard and the compounds were eluted at a flow-rate of 1 5 ml/min using degassed acetonitrile–0 01 *M* potassium dihydrogenphosphate (45 55, v/v) as the mobile phase at laboratory temperature

Preparation of plasma calibration standards

Aliquots of crotamiton standard solutions and a constant amount of internal standard were added to 1 ml of plasma Calibration standards for plasma were in the range corresponding to plasma concentrations between 43 3 and 1919 n*M*

Preparation of urine calibration standards

Aliquots of crotamiton standard solutions were added with a constant amount of internal standard to 150 μl of urine. Calibration standards for urine were in the range corresponding to urine concentrations between 0 32 and 12.8 μM

Extraction procedure for plasma and urine

An internal standard solution (2 44 nmol in 40 μl) prepared in methanol was introduced into a 10-ml glass tube and evaporated to dryness under a stream of nitrogen at room temperature only if urine was to be extracted After the addition of 1 ml of plasma or 150 μl of urine, 1 ml of undiluted pH 12 buffer and 4 ml of *n*-heptane containing 1% isoamyl alcohol, the tube was shaken for 20 min at 300 rpm and centrifuged for 5 min at 2500 *g* The organic phase was transferred into a 5-ml glass tube and evaporated to dryness under a stream of nitrogen at room temperature The residue was dissolved in 500 μl of mobile phase and 75 or 65 μl were injected on to the column for plasma or urine extracts, respectively

Calibration graphs for plasma and urine

These were obtained by plotting the peak-height ratio of crotamiton to the internal standard versus crotamiton concentration, and their equations were calculated by using weighted linear least-squares regression with a weighting factor of $1/(\text{concentration})^2$

In plasma, the linear calibration range was 43 3–1919 n*M*, which corresponds to the regression equation $y = 0\ 00102x + 0\ 00312$, where x is concentration in M and y is the peak-height ratio of *trans*-crotamiton to the internal standard

In urine, the linear calibration range was 0 32–12 8 μM which corresponds to the regression equation $y = 0\ 1643x + 0\ 0061$, where x is concentration in μM and y is the peak-height ratio of *trans*-crotamiton to the internal standard

A calibration graph was established every day and its validity was checked by analysis, in duplicate, of samples spiked with a low and a high amount of crotamiton If the duplicate spiked samples gave results outside the accuracy range 90–110%, a new calibration graph had to be prepared

RESULTS AND DISCUSSION

Selectivity

At the beginning of the development of the HPLC method, concentrated solutions of crotamiton were injected Crotamiton yielded two chromatographic peaks

Investigations were made to identify these two peaks The first peak was identified as the *trans* and the second as the *cis* isomer of crotamiton (Fig 2) In the actual HPLC method, only the *trans* isomer, which is well separated from the *cis* isomer, has been quantified with calibration solutions of crotamiton containing ca 95% of the *trans* and 5% of the *cis* isomer

We preferred to use crotamiton active ingredient as a reference, because of the contamination of the *trans* with the *cis* isomer (Fig 2) and the need, when

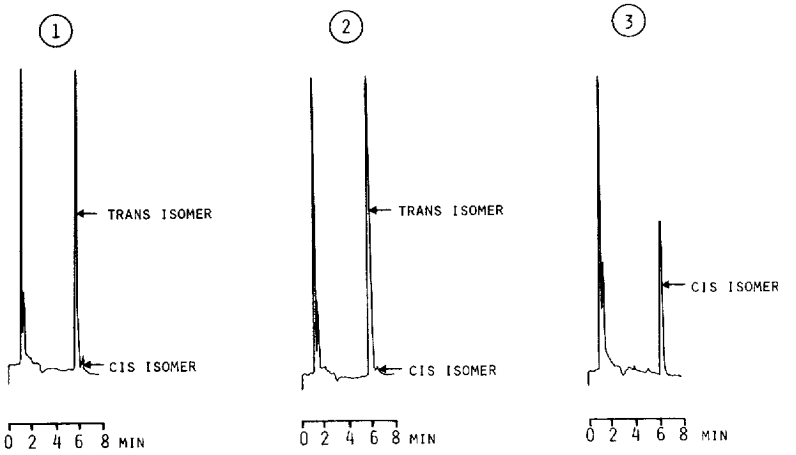

Fig 2 Examples of chromatograms 1, Injection of a pure solution containing 0 115 nmol (23 4 ng) of crotamiton, 2, injection of a pure solution containing 0 096 nmol (19 5 ng) of the *trans* isomer, 3, injection of a pure solution containing 0 070 nmol (14 5 ng) of the *cis* isomer Detector sensitivity, 10^{-3} a u f s , integrator attenuation, 5 (320 μV/cm)

using the *trans* isomer as a reference, to determine the relative amounts of the two isomers in the active ingredient.

Plasma and urine interference

The *trans* isomer of crotamiton and the internal standard are conveniently separated from plasma and urine components Plasma and urine from different volunteers were used to check the separation (Figs 3 and 4)

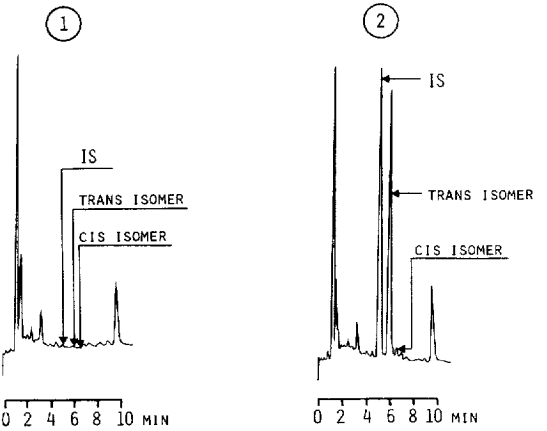

Fig 3 Examples of chromatograms 1, Human plasma blank (extract of 1 ml of plasma), 2, the same plasma spiked to produce 767 n*M* (156 ng/ml) crotamiton and 2 44 μM (467 ng/ml) internal standard (IS) Detector sensitivity, 10^{-3} a u f s , integrator attenuation, 5 (320 μV/cm)

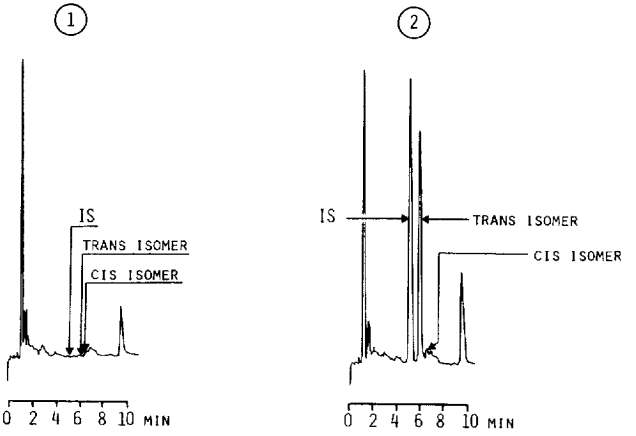

Fig 4 Examples of chromatograms 1, Human urine blank (extract of 150 μl of urine), 2, the same urine spiked to produce 5 12 μM (1 04 μg/ml) crotamiton and 16 3 μM (3 12 μg/ml) internal standard Detector sensitivity, 10^{-3} a u f s , integrator attenuation, 5 (320 μV/cm)

TABLE I

WITHIN-DAY PRECISION AND ACCURACY OF CROTAMITON DETERMINATION IN SPIKED PLASMA SAMPLES

Concentration added (nM)	Concentration found (mean ± S D , n=6) (nM)	Accuracy (%)
43 3	45 5 ± 1 7	105 2
76 7	74 6 ± 2 8	97 3
153	148 ± 3 0	96 8
307	294 ± 19	95 7
615	646 ± 38	105 0
1919	1909 ± 130	99 5
	Mean	99 9
	C V (%)	6 1

Within-day accuracy

Human plasma and urine samples containing crotamiton at different concentrations were analysed repeatedly for each concentration on the same day The results are shown in Table I for plasma and in Table II for urine

Limits of quantitation in plasma and urine

The estimated limits of quantitation were 43.3 nM for crotamiton in plasma with a coefficient of variation of 3.7% and a mean accuracy of 105 2%, and 0 32 μM for crotamiton in urine with a coefficient of variation of 10 9% and a mean accuracy of 95 8%

TABLE II

WITHIN-DAY PRECISION AND ACCURACY OF CROTAMITON DETERMINATION IN SPIKED URINE SAMPLES

Concentration added (μM)	Concentration found (mean ± S D , $n=6$) (μM)	Accuracy (%)
0 32	0 31 ± 0 03	95 8
1 02	1 07 ± 0 09	105 3
4 09	4 10 ± 0 23	100 1
12 80	13 0 ± 0 4	101 3
		Mean 100 6
		C V (%) 7 7

Stability

The reference methanolic solutions of crotamiton and the internal standard solution should be prepared every 2 months and stored at 5 °C Crotamiton, when dissolved in the mobile phase, was stable for 24 h at room temperature and at 5 °C At room temperature, crotamiton was stable for 24 h in spiked plasma samples. After three cycles of thawing and freezing, no decrease in the recovery of crotamiton was recorded. In spiked plasma samples stored at −20 °C, crotamiton was stable for 9 months

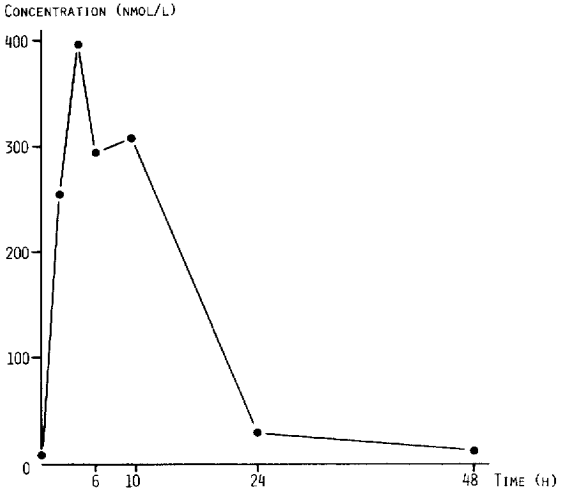

Fig 5 Mean plasma concentrations of crotamiton (nmol/l) obtained for three healthy subjects after a topical application of 18 g of Eurax lotion containing 10% of crotamiton

Application

This method was applied to determine the plasma concentrations and the urinary excretion of crotamiton after a topical application to three volunteers of 18 g of Eurax lotion containing 10% of crotamiton Fig 5 shows the curve of the mean plasma concentrations of crotamiton Urinary excretion as a percentage of the dose was less than 1% These results demonstrate that crotamiton reached the systemic circulation at a low level

CONCLUSION

This HPLC method permits the separation of the *trans* from the *cis* isomer of crotamiton The determination in plasma and urine is carried out on the *trans* isomer expressed as crotamiton

REFERENCE

1 Merck Index, Merck, Rahway, NJ, 10th ed , 1983, p 372