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THIN-LAYER CHROMATOGRAPHIC DETERMINATION OF THE β-FLUOROETHYL ESTER OF XENYL ACETIC ACID (M 2060) IN THE TECHNICAL PRODUCT AND IN 2% LIQUID FORMULATIONS*

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SUMMARY

Two methods, based on preliminary thin-layer chromatography, have been developed for determining the β-fluoroethyl ester of xenyl acetic acid in the technical product and the 2% liquid formulation.

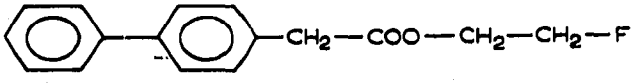
The acaricide, or a convenient amount of the formulation, is dissolved in acetone and the solution is spotted on a thin-layer chromatographic plate.

After development and identification by means of a UV lamp, the M 2060 area is isolated, detached and eluted. In the case of the determination of the active ingredient in the technical product, hydrolysis is conducted on the eluate residue, followed by titration of the resulting xenyl acetic acid.

In the liquid formulation, dehalogenation of the eluate residue is carried out with sodium biphenyl and fluorine is determined colorimetrically, by means of cerium nitrate and alizarin complexone, under special conditions.

INTRODUCTION

The β-fluoroethyl ester of xenyl acetic acid (M 2060)** is a compound that was discovered by the Agricultural Research Institute of the Montecatini Edison Compa-



ny, and is characterized by an extremely high level of activity against the eggs of mites even when used in mid-winter treatments[1,2].

This is the outstanding property which distinguishes M 2060 from any other acaricide on the market. In view of its effectiveness against pests and its high toxicity for warm-blooded animals, this product was formulated for winter use only, and for

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** Italian patent No. 710,046, granted June 27, 1966. Proposed common name (ISO) for the active ingredient: fluenethyl.

simultaneous control of the hibernating stages of aphids, scales, Psyllae and mites, and consists of 2% active ingredient in mineral oil.

The method, developed for the determination of the active ingredient in the technical product and the formulation, is based on a combination of thin-layer chromatography with a final determination procedure which is both specific and accurate. This combination has already been successfully adopted for the analysis of the most varied types of product[3–6] and has often permitted analytical difficulties, arising from the presence of products with very similar physico-chemical properties, to be overcome.

In the technical product, M 2060 is separated by thin-layer chromatography, eluted and hydrolyzed. The xenyl acetic acid formed is titrated with alkali, according to a method already used for organic acid esters[7–9].

In the case of the 2% liquid formulation, the evaluation procedure of the active ingredient had to be modified, due to the fact that a thorough separation of the acaricide from formulants is not practicable by means of thin-layer chromatography and this causes interference in the final volumetric titration. To obviate this, the determination of M 2060 *via* its fluorine is carried out after chromatography.

The compound is dehalogenated with sodium biphenyl[10] and the resulting fluoride is treated with cerium nitrate and alizarin complexone, in order to obtain a coloured compound which can be subjected to photometric determination[11,12].

It was thought less advisable to use the colorimetric method for the determination of the active ingredient in the technical product because this procedure provides less accurate and precise results than those obtained by volumetric titration, and this may have some bearing on the analysis of highly purified products.

EXPERIMENTAL

Determination of M 2060 in the technical product

Of thoroughly homogenized sample 2.5 g is weighed accurately into a 50 ml volumetric flask, dissolved in acetone and diluted to volume. With a controlled pipette, 1 ml of acetone solution is applied as a uniform thin streak (14 cm long and 3 cm from bottom edge) to a glass plate (20 × 20 cm) covered with a 1 mm layer of Silica Gel $HF_{254+366}$. The outside of the tip of the pipette is rinsed with a few drops of acetone by means of a glass capillary.

After the solvent has completely evaporated, the chromatogram is developed with *n*-hexane–ethyl acetate (9:1), in a saturated chamber, until the solvent front reaches 3 cm from the upper scored line. This operation is repeated four times. When the plate is completely dry, it is exposed to UV light in order to visualize the area containing the M 2060 (R_F = 0.45). This area is marked, allowing a safety margin depending on the presence of other compounds and the silica gel from around the zone considered is completely removed with a microscope slide. The M 2060 area is then scraped off and transferred quantitatively onto a 10 G 4 Jena glass crucible mounted on a vacuum assembly, and eluted with 30–40 ml acetone.

The solvent is allowed to remain in contact with the adsorbent for a few minutes before filtering. It is filtered, under vacuum, directly into a 100 ml Kjeldahl flask. After each wash with acetone, the vacuum is released and the silica gel in the crucible is carefully stirred with a glass rod. Using a rotating evaporator, the filtrate

is cautiously evaporated at 35° (maximum) nearly to dryness; 20 ml methanol is added followed by 10 ml methanolic 2 *N* KOH. A few glass beads are introduced and the flask is placed under a condenser and gently refluxed for 1 h. The solution is transferred to a 400 ml beaker, washed with up to 150 ml water and concentrated to about 30 ml; a further 100 ml water is added and the solution is boiled again to 50 ml in order to eliminate all the methanol. It is then transferred to a 250 ml separatory funnel, washed with 50 ml water, neutralized to phenolphthalein with HCl and 5 ml excess is added.

The hydrolysate is extracted with three 50 ml portions of ethyl ether, after stirring each time for 1 min, and allowing the phases to separate thoroughly. The ether extracts are combined and washed with three 10 ml portions of a saturated solution of NaCl, and filtered through a small cotton plug into a 300 ml erlenmeyer flask. The separatory funnel and the cotton are washed with a further 50 ml ether. The solvent is evaporated to dryness by means of the rotating evaporator and the last traces of HCl are removed by a current of air.

Acetone (14 ml) and CO_2-free water (6 ml) are added to dissolve the residue, 2 further drops of phenolphthalein are added and the solution is finally titrated with 0.01 *N* NaOH solution.

Determination of M 2060 in the liquid formulation

Of the homogenized formulation 1.25 g is weighed into an acid weighing bottle, transferred to a 25 ml volumetric flask, dissolved in acetone and diluted to volume. 1 ml of the acetone solution is applied to the thin-layer plate, by means of a controlled pipette, in a uniform thin streak 11 cm long, 3 cm from the right side of the plate and 3 cm from its bottom edge. A glass capillary is used to rinse the outside of the tip of the pipette with a few drops of acetone. On the left of the streak, 2 cm from the edge of the plate, a small amount (0.15 ml) of sample solution with the addition of 1 mg M 2060 is spotted; the purpose of this is to permit better detection of the M 2060 area.

Chromatographic development, as described for the determination of the active ingredient in the technical product, is then carried out. The area corresponding to the M 2060 is marked and eluted with acetone. Using the rotating evaporator, the filtrate is carefully evaporated at 35° (maximum) to dryness. 10 ml toluene and the contents of a sodium biphenyl bottle (South-Western Analytical Chemicals) are added to the residue with stirring. After 2 min the excess reagent is destroyed with 2 ml water. The mixture is cooled to room temperature, and the contents of the Kjeldahl flask are transferred to a separatory funnel and extracted with four 20 ml portions of water. The water extracts are collected in a 100 ml volumetric flask, neutralized first with conc. HCl, then with dilute acid, using 2 drops of phenolphthalein as indicator. The solution is diluted to volume, stirred and filtered through a Whatman filter paper No. 42 into a dry erlenmeyer flask. 25 ml of filtrate is transferred to a 50 ml volumetric flask. 0.1 *N* NaOH is first added and then 0.1 *N* HCl dropwise, for decoloration of the indicator. 2 ml pH 4 buffer solution is added; (60 g sodium acetate trihydrate in 500 ml water; 115 ml glacial acetic acid; diluted to 1 l), then 5 ml 0.001 *M* cerium nitrate solution (0.4342 g cerous nitrate hexahydrate in water, diluted to 1 l), 10 ml acetonitrile and 5 ml 0.001 *M* alizarin complexone solution (77 mg of 1,2-di-

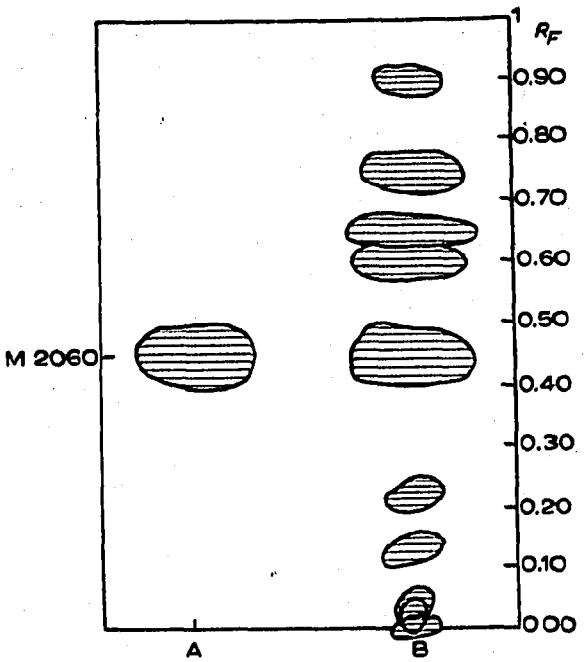

Fig. 1. Thin-layer chromatography of M 2060. Silica Gel $HF_{254+366}$, 1 mm thick. Solvent system: *n*-hexane–ethyl acetate (90:10); No. 5 migrations. Identification by UV (= 366 nm). A = pure M 2060; B = pure M 2060 plus the impurities contained in the technical product.

TABLE I

RESULTS OF M 2060 DETERMINATIONS ON SAMPLES OBTAINED BY ADDING VARYING AMOUNTS OF DIFFERENT IMPURITIES IN THE TECHNICAL PRODUCT TO KNOWN AMOUNTS OF PURE PRODUCT

M 2060 expected (mg)	*% calc.*	*M 2060 found (mg)*	*% found*
28.0	55.5	27.2	54.0
28.0	55.5	27.3	54.1
28.9	54.5	29.4	55.5
28.9	54.5	29.3	55.3

TABLE II

RESULTS OF M 2060 DETERMINATIONS ON ARTIFICIALLY-DECOMPOSED TECHNICAL PRODUCT

Method 1: thin-layer chromatography, hydrolysis and volumetric titration (%)	*Method 2: thin-layer chromatography, dehalogenation and fluorine determination (%)*
52.9	53.0
52.8	52.6
53.1	52.6
52.6	51.9
	51.7
	52.2

TABLE III

RESULTS OF M 2060 DETERMINATIONS ON SAMPLES OF PURE AND TECHNICAL PRODUCTS, ACCORDING TO THE CHROMATOGRAPHIC–VOLUMETRIC METHOD

	Pure M 2060 (%)	*Technical M 2060 (%)*
	100.7	95.6
	100.1	95.4
	100.5	94.7
	100.8	94.7
	100.1	95.7
	100.7	95.5
	100.2	94.2
		94.5
		94.8
		94.9
		95.0
		95.5
		95.5
		94.9
Mean	100.4%	95.1%
Standard deviation	0.29	0.47
Mean deviation	0.26	0.41

hydroxyanthraquinone-3-methylamine N,N-diacetic acid dissolved in 0.04 ml ammonium hydroxide 22° Be and 6 ml water; 0.03 ml glacial acetic acid; diluted to 200 ml. Store in dark). The reactants are mixed by swirling and diluted to 50 ml with water. After 60 min, the absorbance at 617 nm is measured against a reagent blank, in 1 cm cuvettes, and the amount of fluorine is determined from a calibration curve obtained by adding the reagents to known amounts of NaF solutions. Beer's law is obeyed up to a concentration of 25 μg fluorine in 50 ml.

TABLE IV

RESULTS OF M 2060 DETERMINATIONS ON A SAMPLE OF A 2% LIQUID FORMULATION, ACCORDING TO THE CHROMATOGRAPHIC–COLORIMETRIC METHOD

	Analysis	*% M 2060*
	1	1.78
	2	1.75
	3	1.78
	4	1.68
	5	1.76
	6	1.73
	7	1.74
	8	1.73
	9	1.73
	10	1.69
	11	1.71
Mean		1.73
Standard deviation		0.033
Mean deviation		0.024

RESULTS AND DISCUSSION

Of the large number of solvent mixtures tested, *n*-hexane–ethyl acetate gave the best separation of M 2060. The impurities normally present in the technical product do not interfere, as they are easily separated by thin-layer chromatography (Fig. 1).

Table I shows the results obtained by volumetric determinations on M 2060 samples to which the impurities present in the technical product had been expressly added.

Both methods, volumetric and colorimetric, can be used for the determination of the active ingredient in the technical product. However, since the latter shows a slightly lower degree of reproducibility, preference should be given to the former.

Table II gives the results obtained by analysing, according to the methods proposed, a sample of thermally-decomposed technical material.

Tables III and IV give the results of M 2060 determinations on samples of pure and technical products and a 2% liquid formulation.

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