Determination of Carbamic Herbicides by High Performance Liquid Chromatography (HPLC). II. Chlorpropham

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DETERMINATION OF CARBAMIC HERBICIDES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC). II. CHLORPROPHAM.

by

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ABSTRACT

Two simple, precise, and rapid reversed-phase high performance liquid chromatographic procedures, External and Internal standard methods, are described for the determination of chlorpropham in emulsifiable concentrates. Samples are diluted with methanol and 4-nitro-diphenyl ether is added as the internal standard. Calibration and quantitation is made with the use of pure chlorpropham, and results obtained in absolute or relative amounts according to our needs. Linearity in both cases is achieved from 0.1 to 15 µg of chlorpropham, equivalent to 10 µL injections of solutions of this chemical at concentrations of 1×10^{-2} to 1.5 g L^{-1}. Methanol/water (60/40 V/V) at a flow of 2 mL min^{-1} is used as eluent and retention times are approximately 4.4 and 5.9 min respectively for chlorpropham and 4-nitro-diphenyl ether. Detection limit turned out to be 3.9 ng

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of chlorpropham, that is, 10 µL of a solution at 3.9×10^{-4} g L^{-1}.
The Internal standard method improves slightly the confidence limit
and the relative standard deviation with regard to the External
standard method.

INTRODUCTION

Chlorpropham (CIPC), Isopropyl 3-chloro-phenyl-carbamate
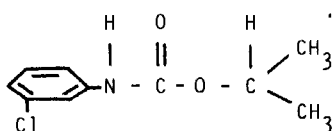
is a pre-emergence non-specific herbicide and inhibitor of sprouting
in ware potatoes.

It presents a low toxicity and is formulated as emulsifiable
concentrate or granules; product and formulated analysis is by
hydrolisis, measuring the carbon dioxide formed or titrating the
liberated 3-chloro-aniline with sodium nitrite (1) and by GLC with
lindane as internal standard (2); a slight decomposition of chlorpropham
occurs with this method. The first two are not specific.

To avoid the above inconveniences, two reversed-phase high
performance liquid chromatographic methods, with external (A) and
internal (B) standards, were developed.

Sparacino and Hines (3) separated some 30 carbamates using both
normal and reverse-phase mode by HPLC, working at the residue level.

MATERIALS AND METHODS

Apparatus

High performance liquid chromatograph Hewlett-Packard 1084 B
equipped with microprocessor, RP-8 chromatographic column and
Millipore filters as described in a previous paper (4).

Reagents

Eluent: Methanol HPLC quality (Merck, Darmstadt)/bidistilled
water (60/40 V/V). Filter through appropriate Millipore filters and
degas under light vacuum.

For method A

1) External standard solution. 0.2 mg mL^{-1} in methanol/water
(65/35 V/V). Weigh approximately 0.050 g of pure chlorpropham (Riedel-
de Haën AG, Seelze-Hannover) into a 50 mL volumetric flask, dissolve
and dilute to volume with methanol/water 65/35. Dilute 10 mL of this
mother solution in a 50 mL volumetric flask with the same solvent.
Filter through appropriate Millipore filter into small vial and cap.

2) 40 % EC solution. Weigh approximately 0.150 g of 40 %
Emulsifiable Concentrate (EC), (Zeltia Agraria, S.A., Porriño, Spain),
into a 50 mL volumetric flask and dilute to volume with methanol/
water 65/35. Dilute 10 mL of this solution in a 50 mL volumetric
flask with the same solvent. Filter through appropriate Millipore
filter into small vial and cap.

3) Working chlorpropham solutions. Weigh 1.1603 g of 40 % EC
into a 50 mL volumetric flask and dilute to volume with methanol.
Dilute 16 mL of this solution in a 25 mL volumetric flask with
methanol (14.852 g L^{-1}). Eleven solutions of decreasing concentration
were prepared, starting from the above and diluting to 1:2 with
methanol.

For method B

1) Internal standard solution. Weigh 1.125 g of 4-nitro-diphenyl
ether (Fluka AG, CH-9470 Buchs, Switzerland) into a 250 mL volumetric
flask, dissolve and dilute to volume with methanol.

2) Mixed standard solution. 0.2 mg mL^{-1} chlorpropham and 0.9 mg
mL^{-1} 4-nitro-diphenyl ether in methanol/water (65/35 V/V). Transfer
10 mL of the mother External standard solution from method A and 10
mL of the above Internal standard solution to a 50 mL volumetric

flask and dilute to volume with methanol/water 65/35. Filter through
appropriate Millipore filter into small vial and cap.

3) Mixed 40 % EC solution. Transfer 10 mL of the 40 % EC
solution from method A, at a concentration of 0.150 g 50 mL^{-1},
and 10 mL of the above Internal standard solution to a 50 mL
volumetric flask and dilute to volume with methanol/water 65/35.
Filter through appropriate Millipore filter into small vial and
cap.

4) Mixed working chlorpropham solutions. Weigh 1.1343 g of
40 % EC into a 50 mL volumetric flask and dilute to volume with
methanol. Dilute 16 mL of this solution in a 25 mL volumetric flask
with methanol (14.519 g L^{-1}). Prepare eleven solutions of decreasing
concentration, starting from the above and diluting always to 1:2
with methanol, in 25 mL volumetric flasks. Add to every flask 5 mL
of the Internal standard solution and dilute to volume with methanol.

Chromatographic conditions

Eluent: Methanol/water (60/40 V/V), flow 2 mL min^{-1}, column
temp. 40ºC, methanol temp. 45ºC, water temp. 80ºC, variable wavelength
detector vis.-UV, wavelength 240 nm vs. 430 nm, inject. vol. 10 µL,
attn. 2^8.

Calibration and quantitation

Inject 10 µL aliquots of every standard solution, for every
method, into chromatograph until variation in standard peak areas
is less than 1 %. Adjust detector sensitivity in order to obtain
peak heights ca 60-80 % full scale. Calibrate and inject 10 µL of
the samples to be analyzed. Results will be obtained in absolute or
relative amounts, according to our needs, by automatic integration
of the peak areas.

RESULTS AND DISCUSSION

Some previous tests were made to find out how many analyses
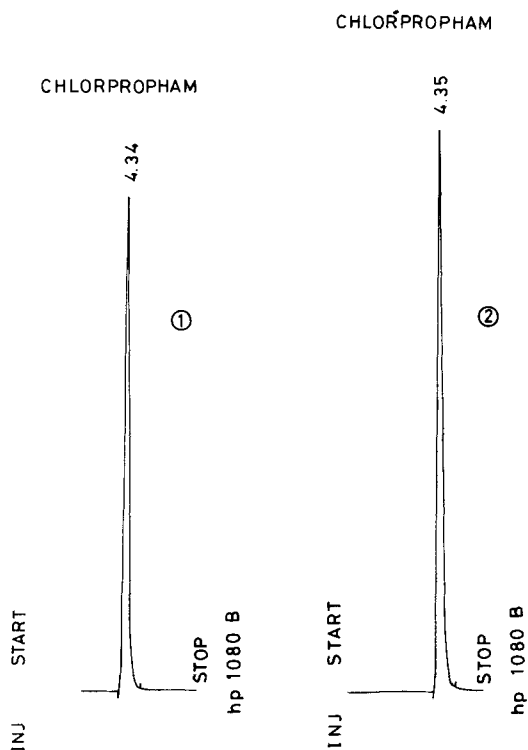should be made for every sample and how many injections for every

Figure 1. Chromatography of pure ① and 40 % EC
chlorpropham ② with CH_3OH/H_2O (60/40
V/V) as eluent.

analysis, in every method, in order to maintain a precision lower
than 1 % (P = 0.01). One analysis and one injection are enough for
the Internal standard method B. One analysis but three injections
are necessary for the External standard method A.

The chromatography of pure and 40 % EC of clorpropham, with
External standard, is shown in Figure 1. Retention time is approximately
the same in both cases: 4.34 and 4.35 min. The concentration of active
ingredient in the EC labelled 40 %, mean of 6 determinations, is
41.9 ± 0.13, s = 0.127, S_r = 0.303.

Scan for chlorpropham was done and it presents a maximum of
absorbance at 240 nm and a shoulder at about 280 nm.

Table I. μg of chlorpropham injected and found and its correlation
   coefficient. Accuracy % and precision %.
   ESTD method A.

Correlation coefficient	injected μg	found μg	accuracy %	precision % [a]
	62.229	36.042	42.08	0.56
	31.115	27.404	11.93	0.68
	15.557	16.092	-3.44	1.29
	7.779	8.032	-3.25	0.13
	3.889	4.078	-4.86	0.71
	1.945	1.994	-2.52	0.31
0.9999	0.972	0.994	-2.26	0.77
	0.486	0.493	-1.44	1.02
	0.243	0.240	1.23	1.05
	0.122	0.117	4.10	1.71
	0.060	0.048	20.00	3.18

[a] Standard relative deviation of three determinations

$$\text{accuracy } \% = \frac{\text{injected } \mu g - \text{found } \mu g}{\text{injected } \mu g} \times 100$$

   The eleven working clorpropham solutions from method A were
analyzed. Table I summarizes injected and found μg of chlorpropham
as well as accuracy, precision and calculated correlation coefficient
for those numbers in brackets, in which accuracy and precision are
smaller than 5 %. From this Table it is possible to reach the conclusion
that Beer's law is followed, and the optimum working space is found,
with a correlation coefficient of 0.9999, between 0.122 and 15.557 μg
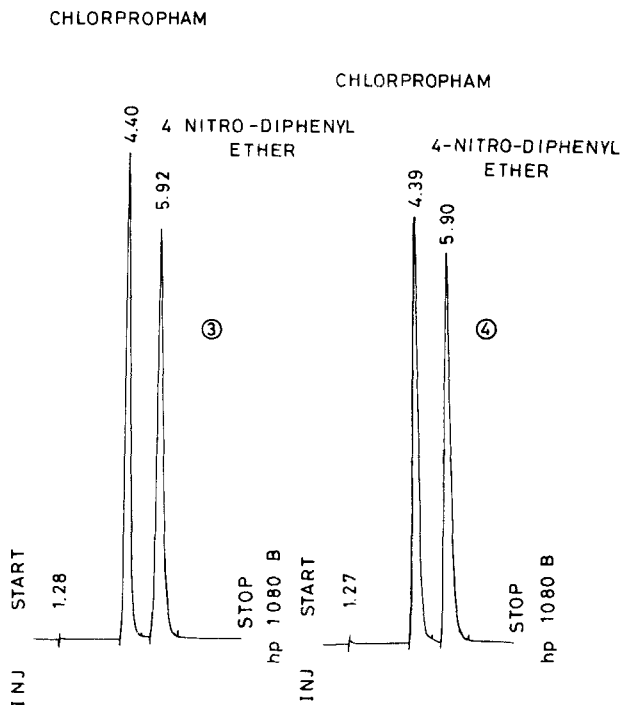of chlorpropham.

CHLORPROPHAM

Figure 2. Chromatography of pure ③ and 40 % E C chlorpropham ④ with CH_3OH H_2O (60 /40 V/V) as eluent and 4-nitro-diphenyl ether as internal standard.

In spite of the very good repeatability of the automatic variable volume injector, the introduction of an internal standard, 4-nitro-diphenyl ether, improves the results.

The chromatography of pure and 40 % EC of chlorpropham, with internal standard, is shown in Figure 2. Retention times are aproximately the same in both cases: 4.40-5.92 and 4.39-5.90 min. The concentration of active ingredient in the EC labelled 40 %, mean of 6 determinations, is 41.3 $\pm$ 0.08, s = 0.079, S_r = 0.191.

The eleven Mixed working chlorpropham solutions from method B were analyzed. Table II shows injected and found µg of chlorpropham

Table II. μg of chlorpropham injected and found and its correlation
         coefficient. Accuracy % and precision %.
         ISTD method B.

Correlation coefficient	injected μg	found μg	accuracy %	precision % [a]
	59.935	30.592	48.96	2.15
	29.967	25.704	14.23	0.71
	14.984	15.140	-1.04	0.23
	7.492	7.820	-4.38	0.30
	3.746	3.850	-2.78	0.27
0.9999	1.873	1.920	-2.51	0.21
	0.936	0.961	-2.67	1.04
	0.468	0.491	-4.91	2.60
	0.234	0.238	-1.71	2.52
	0.117	0.119	-1.71	4.37
	0.059	0.058	1.69	11.31

[a] Standard relative deviation of three determinations

$$\text{accuracy } \% = \frac{\text{injected } \mu g - \text{found } \mu g}{\text{injected } \mu g} \times 100$$

as well as accuracy, precision, and calculated correlation coefficient
for those numbers in brackets in which accuracy and precision are
smaller than 5 %. From this Table it is possible to reach the conclu-
sion that, although Beer's law is followed from 0.059 to 14.984 μg,
the optimum working space, with a correlation coefficient of 0.9999,
is only found between 0.117 and 14.984 μg of chlorpropham.

       Lower concentrations of the working chlorpropham solutions
from method A were employed using a signal to noise ratio of 2:1,
to find the detection limit which turned out to be 3.9 ng of

chlorpropham, equivalent to 10 μL of a solution of this chemical at a concentration of 3.9×10^{-4} g L^{-1}.

In conclusion, both methods work satisfactorily in the analysis of EC of chlorpropham, the Internal standard one improves slightly the confidence limit and the relative standard deviation.

Linearity in both cases is achieved from about 0.1 to 15 μg of chlorpropham, equivalent to 10 μL injections of this chemical at concentrations of 1×10^{-2} to 1.5 g L^{-1}.

<div align="center">REFERENCES</div>

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