Facile and Sensitive Spectrophotometric Determination of Propoxur in Formulations and Environmental Samples

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A facile, rapid, and sensitive spectrophotometric method for the determination of propoxur in insecticidal formulations, fortified water, vegetables, agricultural wastewater, and agricultural soil samples has been elaborated. The proposed method is based on the hydrolysis of propoxur under basic conditions, followed by instantaneous azo coupling of the resulting 2-isopropoxyphenol with the anilines **2a**–**c**. This yielded the orange-red chromophore **3a** (λ_{max} = at 470 nm), the pale-red coupling product **3b** (490 nm), or the red derivative **3c** (478 nm), which are stable for 46 h, 38 h, and 24 h, respectively, and could be readily analyzed spectrophotometrically.

Introduction. – Propoxur (=2-isopropoxyphenyl methyl carbamate) is an insecticide widely used in combating insects in agriculture and households, and public-health pests. It provides control of insects that are resistant to chlorinated hydrocarbons and organo phosphates. In view of its wide application, there is a need to develop new sensitive and reliable methods for the assessment of this insecticide in H_2O and environmental samples. Because of its significance, several methods have been developed for the determination of propoxur: colorimetry [1][2], gas chromatography (GC) [3–6], and high-performance liquid chromatography (HPLC) [7]. However, spectrophotometric techniques are most widely used for the determination of insecticides because these methods are usually inexpensive and straight-forward.

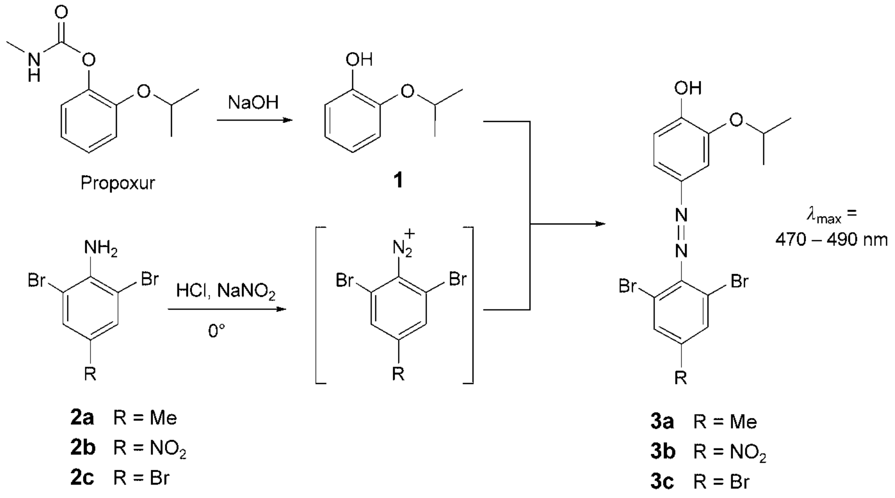
Spectrophotometric techniques for the determination of propoxur are typically based on alkaline hydrolysis, followed by coupling of the resulting phenol derivative with different diazotised reagents such as '3-nitroaniline-4-sulphonic acid' (=4-amino-2-nitrobenzenesulfonic acid) [8], '3-methyl-2-benzothiazolinone hydrazone hydrochloride' [9], '3,5-dibromo-*p*-benzoquinon chlorimine' [10], '*p*-dimethyl-phenylenediamine dihydrochloride' [11], 2-aminobenzophenone [12], '4-amino-antipyrine' [13], and 4-chloroaniline [14]. *Venkateswarlu* and *Seshaia* reported an extraction-based, sensitive spectrophotometric method for the determination of propoxur in the presence of oxidising agent [15]. Some of the above reagents suffer from selectivity, some are not very sensitive, and some require suitable solvents for the extraction of the chromophore, are time consuming, or costly.

In this paper, we describe an alternative spectrophotometric method for the facile, selective, accurate, and inexpensive determination of propoxur formulations, of propoxur in fortified H_2O, vegetables, agricultural wastewater, and agricultural soil samples.

Results and Discussion. – 1. *Method.* Our analytical method is based on alkaline hydrolysis of propoxur, which gives rise to 2-isopropoxyphenol (**1**; *Scheme*). The latter was coupled with diazotised 2,6-dibromo-4-methylbenzenamine (**2a**), 2,6-dibromo-4-nitrobenzenamine (**2b**), or 2,4,6-tribromobenzenamine (**2c**) – readily available commercially or by bromination of the corresponding anilines – and the resulting azo products **3a** – **c** were spectrophotometrically determined in solution.

The effect of NaOH concentration on hydrolysis was studied by varying the amount of base added (1–7 ml of a 2% NaOH solution). We found that 4–6 ml 2% NaOH solution were necessary for complete hydrolysis; thus, a volume of 5 ml was selected for all experiments. Absorbance values were obtained within a pH range of 11–12. Buffer was not required to stabilise the colour. Other alkaline solutions were also tested, but NaOH gave the best results.

Scheme



All diazotizations were carried out at 0–5° in the presence of 0.5–1.5M HCl. In this acidity range, constant absorbances were observed. Above this range, a decrease in the absorbance of **3** was observed. Thus, we performed all reactions in 1M aqueous HCl, which was optimal from both synthetic and spectral points of view. We also studied the effect of reagent concentration (1–6 ml of a 0.2% reagent solution) in 1M HCl. We found that 4 ml of 0.2% reagent solution was sufficient for complete colour development. Higher concentrations did not enhance the absorbance any further, and lower concentrations gave poor results.

Possible effects of non-target species (at µg/ml levels) on the determination of propoxur were studied. Interferences of carbaryl, carbofuran, and other non-target pesticides, if present, could be removed by washing the $CHCl_3$ extracts containing **3** with 0.5–1.0% NaOH solution and with H_2O.

2. *Analytical Data.* Linear calibration graphs for the spectrophotometric determination of **3** were obtained for concentrations in the range of 0.6 to 10 µg/ml. The limit of quantification (LOQ) was determined by taking the ratio of standard deviation (σ)

of the blank with respect to H_2O and the slope (s) of the calibration curve multiplied by a factor of ten. This means that the LOQ was *ca.* 4.9-times the limit of detection (LOD). Naturally, LOQ slightly crosses the limit of the range given by *Lambert–Beer*'s law; however, the LOD was still well below the corresponding lower-limit. The upper range was determined by a plot of absorbance (at λ_{max}) against concentration. The calibration graph had correlation coefficients from $r = 0.999$ to 1.009. The molar absorptivity was 21800–23400 $l\ mol^{-1}\ cm^{-1}$. The optical characteristics of the coloured derivatives **3** are given in *Table 1.*

Table 1. *Optical Characteristics, Precision, and Accuracy of the Spectrophotometric Determination of Propoxur by Means of Chromophores* **3**

Characteristics	**3a**	**3b**	**3c**
Concentration range [µg/ml]	0.4–10.0	0.8–10.0	0.6–10.0
λ_{max} [nm]	470	490	478
Stability [h]	46	24	38
Molar absorptivity [$l\ mol^{-1}\ cm^{-1}$]	$2.28 \cdot 10^4$	$2.34 \cdot 10^4$	$2.18 \cdot 10^4$
Sandell's sensitivity [µg cm^{-2}]	0.013	0.012	0.011
Limit of detection [µg/ml]	0.295	0.672	0.426
Limit of quantification [µg/ml]	1.472	3.361	2.131
Regression[a]) slope b	0.041	0.059	0.053
Regression intercept a	0.087	0.008	0.093
Rel. standard deviation [%][b])	1.74	1.76	1.75
Correlation coefficient (r)	0.999	1.009	0.999
Rel. error [%]	1.32	1.05	1.28

[a]) Regression curve: $y = ax + b$, where x is the concentration of **3** (in µg/ml) and y is the absorbance of **3**. [b]) Determined for $n = 10$.

3. *Propoxur Determinations.* We first tested out method by determining the content of propoxur in commercial insecticidal formulations: *Baygon*® spray (1% content) and *Baygon*® granules (Bait; 2%). When analysing the 1% spray by means of colorimetric analyses of the products **3a**, **3b**, and **3c**, respectively, contents of 0.982 (±0.018), 0.973% (±0.026), and 0.980 (±0.019) of propoxur were obtained; similarly, the 2%-granule baits were found to contain 1.94 (±0.036), 1.93 (±0.08), and 1.90% (±0.09) of insecticide, respectively.

Next, we determined the propoxur content of technical-grade samples. As shown in *Table 2*, our method gave excellent results both with respect to precision (see 'taken' *vs.* 'found') as well as propoxur recovery (typically 99%).

Similar high levels of recovery were obtained for propoxur in both fortified water and vegetable samples, as summarized in *Table 3*. Here, chromophore **3a** turned out to provide the best results, with average recoveries of 95–99% from tap-water, distilled-water, tomato, and potato samples. In case of **3b** and **3c**, somewhat more-fluctuating values in the range of 90–99.5 and 93–98% were found. Generally, the recoveries decreased in the order: tap water > distilled water > potato > tomato.

4. *Statistical Analysis.* The performance of the new method was compared statistically in terms of *Student*'s test (*t*-values) and the variance ratio of the '*F*-test'. These tests showed no significant difference in accuracy and precision between the proposed methods and reference methods [6][15]. The low values of the relative

Table 2. *Recovery of Propoxur in Technical-Grade Samples Determined Spectrophotometrically* via *Chromophores* **3**

	3a		**3b**		**3c**	
Taken [µg/ml]	Found [µg/ml^{-1}]	Recovery [%]	Found [µg/ml^{-1}]	Recovery [%]	Found [µg/ml]	Recovery [%]
3.50	3.49	99.85	3.48	99.68	3.48	99.45
3.60	3.55	98.77	3.54	98.50	3.50	97.22
3.70	3.69	99.78	3.67	99.21	3.68	99.65
3.80	3.76	99.07	3.78	99.52	3.75	98.68
3.90	3.89	99.89	3.88	99.64	3.82	97.94
4.00	3.99	99.80	3.97	99.30	3.98	99.72
		99.52 (0.48)[a])		**99.30** (1.02)[a])		**98.77** (0.44)[a])

[a]) Average and, in parentheses, standard deviation.

Table 3. *Average Recovery* (in %) *of Propoxur from Fortified-Water and Vegetable Samples as Determined Spectrophotometrically from Compounds* **3**

	Fortification [µg/ml]	Tap water		Distilled water		Tomato		Potato	
		Recovery	SD[a])	Recovery	SD	Recovery	SD	Recovery	SD
3a	0.4–2.0	98.48	0.59	97.44	0.31	96.79	1.24	97.64	0.38
3b	0.6–2.2	97.52	1.69	97.03	1.59	95.47	3.52	96.85	1.32
3c	0.8–2.4	96.23	1.42	96.15	3.06	95.50	1.77	96.50	2.94

[a]) Standard deviation.

standard deviation further reflect the high precision of our method. Theoretical *t*-values at the 95% confidence level were found to be 2.75. A comparison of the results of our experiments with similar, known procedures is given in *Table 4*. As can be seen, our method gave satisfactory results for the determination of propoxur in water, vegetables, and agricultural soil.

Conclusions. – Our analytical method for the determination of the insecticide propoxur in a variety of samples (water, vegetables, soil) was found to be facile, rapid, inexpensive, and accurate. The coupling reagents **2a–c** are commercially available or can be readily prepared in one step. The proposed methods do not require any *special* solvent for the extraction of the chromophores **3**, and the latter are quite stable relative to other species reported in the literature [10–15].

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Experimental Part

General. All chemicals were of anal. grade, and doubly-distilled H_2O was used throughout the experiments. Propoxur stock soln. (1000 µg/ml) was prepared from 0.10 g of pure insecticide dissolved in 100 ml of carbonyl free MeOH. The stock soln. was progressively diluted with the solvent to obtain standard solns. of desired concentration. Compounds **2a** or **2c** (0.2 g each), dissolved in MeOH (5 ml), and compound **2b** in acetone

Table 4. *Comparison of Different Methods for Propoxur Determination*. Recovery in %, amounts of insecticide in μg; values in parentheses represent statistical *F*- and/or *t*-values.

Sample	Propoxur added [μg]	**3a**		**3b**		**3c**		'4-Amino-antipyrine' [15] Recovery	GC [6] Recovery
		Found[a])	Recovery	Found[a])	Recovery	Found[a])	Recovery		
Baygon bait (2%)	–	1.94 ± 0.07	97.00 ± 2.85	1.93 ± 0.08	96.50 ± 3.42	1.90 ± 0.09	95.00 ± 4.21	94.50 ± 3.85	98.50 ± 0.90
(*F*, *t*)			(2.1, 0.7)		(2.3, 1.1)		(2.6, 1.0)	(2.3, 0.89)	(2.4, 1.0)
Tap water	5.0	4.92	98.48 ± 1.65	4.88	97.52 ± 1.12	4.81	96.23 ± 1.12	95.45 ± 4.92	98.00 ± 1.65
(*F*, *t*)			(2.36, 0.68)		(2.48, 0.64)		(2.33, 0.7)	(2.40, 0.9)	(2.35, 0.8)
Distilled water	6.0	5.86	97.44 ± 1.7	5.82	97.03 ± 1.8	5.80	96.15 ± .1.9	97.67 ± 2.11	98.40 ± 1.48
(*F*, *t*)			(1.76, 0.69)		(1.79, 0.58)		(1.65, 0.69)	(1.87, 0.71)	(1.78, 0.64)
Tomato	4.0	3.86	96.79 ± 1.5	3.81	95.43 ± 1.4	3.81	95.50 ± 1.6	–	–
(*t*)			(1.12)		(1.1)		(0.98)		
Potato	4.0	3.89	97.64 ± 1.2	3.86	96.85 ± 1.3	3.85	96.50 ± 1.5	–	–
(*t*)			(0.84)		(1.12)		(0.97)		
Wastewater[b])	10.0	9.75	97.50 ± 1.01	9.50	95.60 ± 1.2	9.60	96.00 ± 1.3	–	–
(*t*)			(0.98)		(0.79)		(0.87)		
Wastewater[c])	10.0	9.75	97.50 ± 0.9	9.50	95.60 ± 1.3	9.50	95.80 ± 0.6	–	–
(*t*)			(1.08)		(0.98)		(0.96)		
Soil[b])	15.0	14.56	96.70 ± 1.2	14.58	97.00 ± 1.1	14.53	96.10 ± 1.5	–	–
(*t*)			(1.2)		(1.10)		(0.99)		
Soil[b])	15.0	14.36	95.80 ± 1.6	14.58	97.00 ± 1.2	14.53	96.10 ± 1.4	–	–
(*t*)			(1.12)		(0.94)		(1.09)		

[a]) Averaged values for $n = 10$. [b]) Agricultural samples collected from S.V. Agricultural Research Institute, Tirupati, A.P., India. [c]) Agricultural samples collected from Perur, Chandragiri Mandal, Chittur District, A.P., India.

(5 ml), were diluted with 100 ml of H_2O. For hydrolysis and diazotisations, 2% aq. NaOH, and 0.3% $NaNO_2$ in 1M HCl, were used, respectively. Absorbance measurements were performed on a *Hitachi U-2001* spectrophotometer in a 1-cm quartz cell at r.t.

Coupling Reagents. All reagents **2** are commercially available (*Aldrich* or *Fluka*). They can also be readily prepared by bromination of the corresponding anilines. General procedure for the synthesis of *2,6-dibromo-4-methylbenzenamine* (**2a**): To a soln. of 4-methylbenzeneamine (2 g) in 1:1 HCl/H_2O (25 ml), a soln. of Br_2 (2 ml) in AcOH (5 ml) was added dropwise while stirring, the temp. being kept below 38°. After 3 h, the precipitate was filtered, washed with H_2O, and dried at 40° to afford **2a** (80%). M.p. 75–77°. Compounds **2b** and **2c** were prepared analogously, and recrystallized from aq. MeOH.

Sample Preparation. a) The fortified H_2O samples containing 0.4–2.4 µg/ml of propoxur (*Table 3*) were extracted with $CHCl_3$, the combined org. extracts were washed with 0.1M K_2CO_3 soln. to break any emulsion formed during the extraction, and dried (Na_2SO_4). Finally, the solvent was evaporated, and the dry residue was dissolved in MeOH from which the amount of insecticide was determined spectrophotometrically. *b*) In the case of vegetables, 50 g of fresh vegetable or fruit was uniformly mixed with MeOH (10 ml) containing propoxur (10 µg), and left standing for 24 h. This material was extracted according to a procedure described in [9]. The extracts were evaporated on a steam bath, and the residue was dissolved in MeOH and analyzed spectrophotometrically. *c*) Agricultural wastewater samples (10 ml) were extracted with $CHCl_3$ (2 × 10 ml). The org. extract was evaporated to dryness at $51 \pm 6°$ under reduced pressure, the residue was dissolved in 50% aq. MeOH (25 ml), and suitable aliquots were analysed spectrophotometrically. *d*) Different soil samples (10 g) were treated and analysed as outlined earlier [13].

General Procedure for the Determination of Propoxur. Propoxur test soln. (20 ml) was hydrolyzed for 5 min with 2% NaOH soln. (5 ml) at r.t. The diazonium salts of compounds **2** were prepared by mixing a 0.3% soln. of $NaNO_2$ (2 ml) with a 0.2% soln of **2** (4 ml) and 1M aq. HCl (2 ml). Coupling between diazotised **2** and the hydrolysate at 0° yielded the chromophores **3**.

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