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Direct determination of monolinuron, linuron and chlorbromuron residues in potato samples by gas chromatography with nitrogen–phosphorus detection☆

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Abstract

A gas chromatography with nitrogen–phosphorus detection direct method for methoxyurea herbicide determination in powdered potato and fresh potato samples has been developed. A previous study of the thermal stability of the phenylurea herbicides seems to confirm that the ones containing the methoxy radical, i.e. monolinuron, linuron and chlorbromuron, were stable. The herbicides were extracted from the sample through liquid–liquid extraction with dichloromethane–light petroleum (1:1), followed by solid-phase extraction in a C_8 cartridge. The recoveries were in the range 84–95% for powdered potato and 86–101% for fresh potato. The RSD values were less than 10%, at 0.1 μg g^{-1} concentration level ($n=4$) for both types of samples. Detection limits of the method were 7.0–30 ng g^{-1} for powdered potato and 6.0–50 ng g^{-1} for fresh potato.

Keywords: Potatoes; Food analysis; Pesticides; Phenylureas

1. Introduction

Phenylureas (PhUs) are the type of herbicides more widely used in pre- and post-emergence. Consequently, they can give rise to residues in crops, soils and surface waters. The widespread agricultural use, toxicity and possible carcinogenicity of the PhU herbicides have stimulated the development of methods for their detection. The herbicidal action of the PhUs is due to inhibition of the Hill reaction in photosynthetic electron transport, interfering with photosynthesis in plants and causing them to starve to death [1].

Monitoring of PhU herbicide residues requires analytical methodologies capable of performing determinations at low concentration levels, such as chromatographic techniques; moreover, when dealing with real samples, sample preparation is necessary because the matrix is usually too complex. In general, a high-performance liquid chromatography (HPLC) technique with different types of detection, such as UV [2–4] and diode array detection (DAD) [5,6], is used; but the sensitivity and separation

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efficiency are poorer than with a gas chromatography (GC) technique. Studies using HPLC have also been done to analyse herbicide stability against time [7]. A widely-used technique nowadays is high-performance liquid chromatography–mass spectrometry (HPLC–MS) [8–11]. GC has also been used, but there are few methods for PhU direct determination due to their assumed thermolability in some solvents at chromatograph temperature [12,13]; consequently most methods involve derivatization. The more usual derivatization reagents are: heptafluorobutyric acid anhydride (HFBA), trimethylanilinium hydroxide (TMAH) or trimethylsulfonium hydroxide (TMSH) and methyl iodide. The derivatized compounds are detected by nitrogen–phosphorus detection (NPD) [14,15], MS [16–18] or electron-capture detection (ECD) [18]. The main disadvantage of the derivatization includes more sample preparation steps and analysis time. There are also some papers where the analyses are based on PhU degradation products at the injector temperature [19].

The most common samples analyzed are water and soil [3,5,9–11]; although PhUs are also studied to a lesser degree in plants and crops [13,20,21].

In summary, thermal instability of PhUs usually prevents their determination by GC. However, GC is commonly preferred over HPLC mainly because of its higher sensitivity and resolution for complex samples and easier identification by MS. So, in this paper, a simple analytical method is proposed for direct determination by GC–NPD of some thermally stable PhU herbicides (linuron, chlorbromuron, and monolinuron) at trace levels in potato samples. These herbicides were extracted from the sample with a dichloromethane–light petroleum mixture and C_8 cartridges were used for clean up.

2. Experimental

2.1. Reagents and standards

HPLC-grade acetonitrile from Romil (Barcelona, Spain), light petroleum (b.p. 40–60 °C) and dichloromethane both from Carlo Erba (Barcelona, Spain), methanol from Romil, potassium chloride from Panreac (Barcelona) and purified water with a Milli-Q system from Millipore (Milford, MA, USA) were used.

Linuron, monolinuron, diuron and neburon (all 99%), chloroxuron (99.7%), monuron (99.9%) and chlorbromuron (99.3%) from Riedel-de Haën (Germany) and fluometuron (99%) from Supelco (Bellefonte, PA, USA) were used. Triphenylphosphate (99%) from Supelco or methylparathion (97%) from Riedel-de Haën was used as internal standard for GC–NPD.

Standard solutions: 1000 μg ml^{-1} stock solutions were prepared by dissolving 25 mg of each PhU herbicide in 25 ml methanol. Standard solutions were stored at −18 °C and new working solutions were prepared every 2 weeks.

2.2. Samples

Powdered potato was from Maggi (Barcelona, Spain) and contained 99% dehydrated potato, emulsifier (E-471), stabilizer (E-450a), antioxidant (E-300), preservative (E-223) and seasoning. Fresh potatoes (with approximately 77% water content) were bought at the market (Spain).

2.3. Apparatus and material

The chromatographic system consisted of the following components: a gas chromatograph, Hewlett-Packard 5890 I, equipped with an NPD system and a standard injector. The carrier gas was nitrogen. The operating conditions were: splitless injection through glass-liner; injection volume, 1 μl. Temperature program: injector temperature, 220 °C; detector temperature, 300 °C (NPD); initial oven temperature, 110 °C (2 min); gradient: 5 °C min^{-1} to 140 °C (2 min); 5 °C min^{-1} to 210 °C (2 min); 7 °C min^{-1} to 250 °C (2 min). A semicapillary column HP-5, 5% diphenyl–95% dimethylpolisiloxane (30 m×0.53 mm, 2.65 μm) and data acquisition ChemStation software HP-3365 were used. Other columns HP-1, dimethylsilicone (25 m×0.25 mm, 0.25 μm), and OV-101, polidimethylsiloxane (10 m×0.53 mm, 2.65 μm), were also used for testing.

The GC–MS system used for identification purposes consisted of a gas chromatograph, Hewlett-Packard 5890 II, equipped with a split-splitless

injector and a capillary column VA-5, 5% diphenyl–95% dimethylpolisiloxane (30 m×0.25 mm, 0.25 μm), a mass spectrometer with a quadrupole filter HP 5989 A; and a Wiley library HP 59943 B. The operating conditions were: ionization source temperature, 220 °C; quadrupole filter temperature, 100 °C; ionization energy, 70 eV; the temperature program was the same as the one used in GC–NPD, except that detector temperature was 280 °C; scan mode (20–550 u). An injection volume of 2 μl was used.

A Visiprep vacuum manifold system (Supelco); a vacuum pump (Barna, USA); an ultrasonic bath (P - Selecta, Spain); a centrifuge (Meditronic, P - Selecta) were also used.

Extraction tubes from Pyrex, 40 ml, 9.5×2 cm, and C_8, (500 mg, 6 ml) cartridges from Teknokroma (Barcelona, Spain) were used. Other cartridges, C_{18}, NH_2 and CN (500 mg, 3 ml) from Teknokroma were also used for testing. A 10 μl hypodermic syringe (Hamilton, Reno, NV, USA) with a 70 mm needle was used together with a standard needle microsyringe (51 mm).

2.4. Procedures

2.4.1. Calibration for PhU herbicides

A calibration with the mixture of PhU herbicides in the concentration range 0.10–2.5 μg ml^{-1} was obtained, using 0.5 μg ml^{-1} of methylparathion as internal standard. These solutions were analyzed by GC–NPD four times for each concentration level. A 1 μl volume was injected into the gas chromatograph.

2.4.2. Sample preparation

Three types of potato samples, powdered potato, fresh potato with and without peel, were analyzed. In all cases 2.5 g of sample, contained in a 40 ml extraction tube, was fortified with 0.25–1.6 μg of the herbicides. All were shaken for 1 min by hand and allowed to settle for 1 h. The samples of fresh potatoes were previously washed, wiped dry and triturated.

2.4.3. Liquid–liquid extraction (LLE)

To the spiked potato samples 8 ml of methanol were added and shaken for 30 s using the ultrasonic bath. Then, 8 ml of purified water were added, followed by 0.48 g of KCl (2%) for the powdered potato and 1.2 g (5%) for the fresh potato. Successively, 8 ml of light petroleum–dichloromethane (1:1) were added and shaken for 5 min using the ultrasonic bath. The mixture was centrifuged at 3400 rev./min for 18 min.

An aliquot of the organic phase (5 ml) was collected and evaporated to dryness with argon. The residue was dissolved with 1 ml of methanol.

2.4.4. Clean-up by solid-phase extraction (SPE)

The C_8 cartridge was previously conditioned twice with 2 ml of methanol each time, then 1 ml methanol extract obtained under LLE, was passed through the cartridge and the PhUs were eluted with 1 ml of acetonitrile at 1 ml min^{-1} flow-rate in the Vacuum Manifold System at a pressure of 1 in.Hg (3386.38 Pa). Methylparathion as internal standard (0.5 μg ml^{-1} concentration) was added to the eluate. A 1 μl volume was injected into the gas chromatograph with NPD system.

3. Results and discussion

3.1. Preliminary studies

Different temperature programs for separation and identification of the PhUs were studied. To minimize the degradation of the PhU herbicides an optimum temperature program with low ramps was used together with different-needle microsyringes (51 and 70 mm). Several columns of different polarity (HP-1, OV-101, HP-5) were tested; the best efficiency was for the HP-5 column. Chlorbromuron, fluometuron, linuron, monolinuron and monuron herbicides showed a good resolution. However, chloroxuron, diuron and neburon showed a poor sensitivity and thermal instability. Furthermore, diuron and neburon overlapped with monolinuron in the optimum conditions.

In this study, triphenylphosphate and methylparathion were tested as internal standards (I.S.). We chose methylparathion because among the studied PhU herbicides it appears at a mid-way position in the chromatogram without overlapping

with the herbicide peaks, thus, the total analysis time was shortened. We could have used triphenylphosphate as an alternative I.S., since methylparathion can be employed as an insecticide for potatoes; nevertheless, in our study, no peak for methylparathion appeared in the potato sample.

3.2. Stability studies

Since the aim of this paper is to develop a direct GC method, a brief study of thermal stability was carried out, working with standard solutions at 1.5 μg ml^{-1} concentration level, in order to confirm some aspects of the literature.

Decreasing injector temperature from 280 to 220 °C the stability of PhUs increases as was indicated by the increase of the relative peak areas and their reproducibility. Consequently, an injector temperature of 220 °C was selected. An improvement in the reproducibility of the areas was also observed after changing the standard needle microsyringe (51 mm) for a long needle (70 mm) microsyringe [22]. The optimal temperature gradient program has been indicated in the Experimental section. Fig. 1 shows the chromatogram obtained by GC–NPD using this temperature program at 1.5 μg ml^{-1} herbicide concentration. The total analysis time was of 29.2 min approximately, where the retention times for the herbicides and I.S. studied were the following: fluometuron, 19.5 min; monolinuron, 22.2 min; monuron, 24.4 min; I.S. (methylparathion), 25.4 min; linuron, 26.9 min and chlorbromuron, 29.2 min. Keeping the standard solution at room temperature, degradation products for monuron and fluometuron appeared after 4 days but no additional peaks were observed for the other PhUs at least in the following 2 weeks.

The confirmation of the PhU herbicides and their degradation products was carried out by GC–MS. Scan mode was used for all the compounds. Fluometuron, monolinuron, monuron, linuron, chlorbromuron and methylparathion were confirmed by

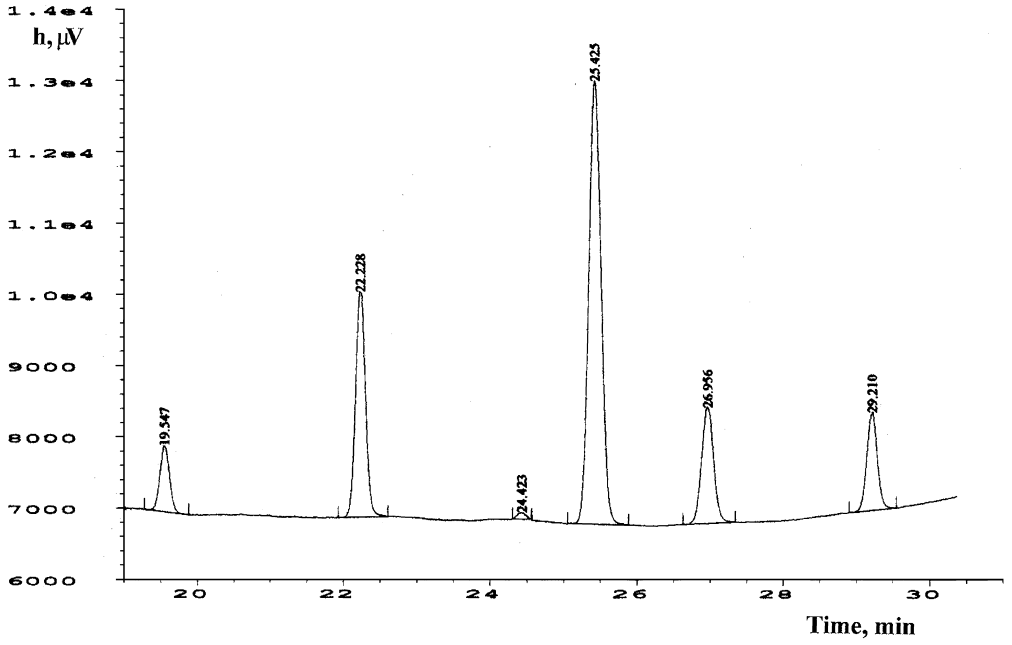


Fig. 1. Chromatogram of the mixture of PhU herbicides by GC–NPD. Column HP-5 (30 m×0.53 mm×2.65 μm); flow-rate, 28.3 ml min^{-1}; injection volume, 1 μl; [Herbicides], 1.5 μg ml^{-1}; [$I.S._{Methylparathion}$], 2 μg ml^{-1}. Temperature program: $T_{injector}$: 220 °C, $T_{detector}$: 300 °C; $T_{oven\ initial}$: 110 °C (2 min.) Gradient: 5 °C/min–140 °C (2 min); 5 °C/min–210 °C (2 min); 7 °C/min–250 °C (2 min). Elution order, fluometuron, monolinuron, monuron, I.S., linuron, chlorbromuron.

comparison of their spectra with the library MS spectra. Fluometuron and monuron were further confirmed by the presence of their degradation products (Fig. 2), both of which were derivatives of methyl esters of carbamic acid, since in a methanolic medium the cleavage of the urea bridge by thermal degradation is favoured [12]. Furthermore, those degradation products were also identified as metabolites of fluometuron and monuron, because they had a peak at m/z ratio of 187 and 185. These ratios belong to the isocyanate of fluometuron and monuron, respectively.

From this study, we can conclude that PhU herbicides monolinuron, linuron and chlorbromuron, which contain a methoxy radical, Table 1, are thermally stable enough to allow their direct de-

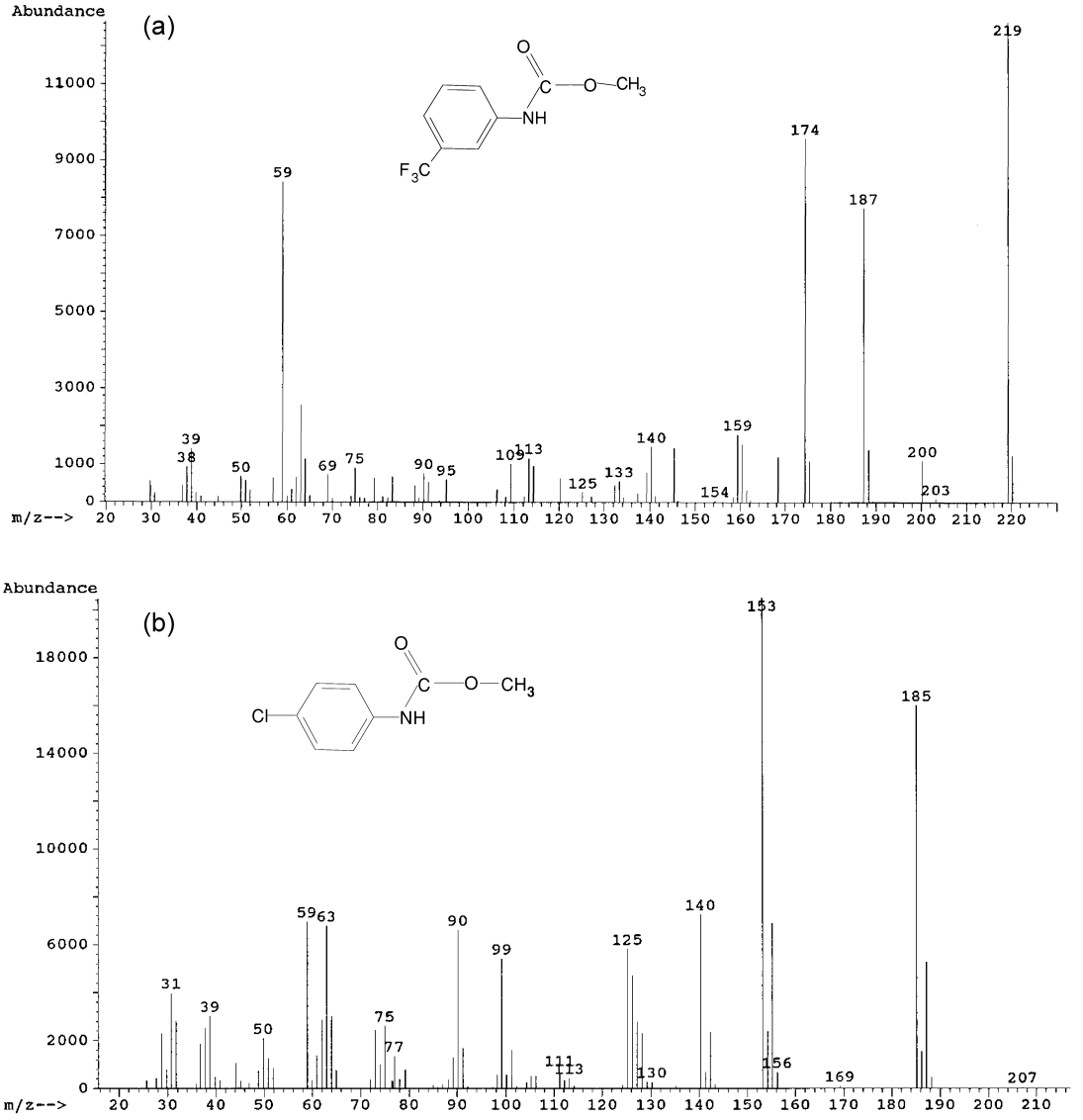
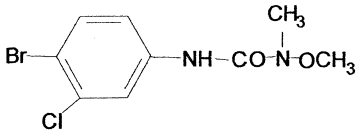
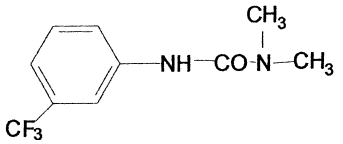
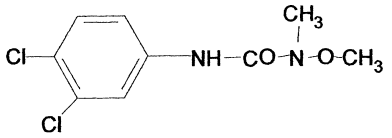
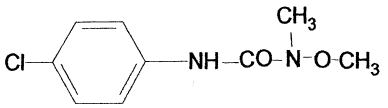
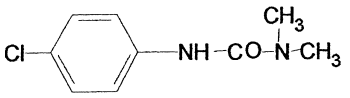


Fig. 2. Experimental MS spectra of metabolites. (a) Fluometuron metabolite (3-trifluomethylphenylcarbamic acid, methyl ester). (b) Monuron metabolite (4-chlorphenylcarbamic acid, methyl ester).

Table 1
Names and chemical structures of PhU herbicides used in this study

Common name	Molecular structure	IUPAC name
Chlorbromuron		3-(4-Bromo-3-chlorophenyl)-1-methoxy-1-methylurea
Fluometuron		3-(Trifluoromethylphenyl)-1,1-dimethylurea
Linuron		3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea
Monolinuron		3-(4-Chlorophenyl)-1-methoxy-1-methylurea
Monuron		3-(4-Chlorophenyl)-1,1-dimethylurea

termination by GC. However, fluometuron and monuron have a methyl radical, which makes them thermally unstable, according to the studies of Berrada et al. [12].

3.3. Analytical characteristics of PhU herbicides in standard solutions

A study of the analytical characteristics of the

Table 2
Analytical characteristics for PhU herbicides by GC–NPD

Sample	Herbicide	Linear regression	Sensitivity (s) (μV g ng^{-1})	LOD[a] (ng g^{-1})	LOQ[b] (ng g^{-1})
Standard	Monolinuron	$y=0.4698x-0.0282$	1.47	16	82
	Linuron	$y=0.3027x-0.0161$	0.81	30	149
	Chlorbromuron	$y=0.1932x-0.0155$	0.67	36	181
Powdered potato	Monolinuron	$y=0.3165x-0.0011$	3	7	37
	Linuron	$y=0.1362x-0.0066$	0.90	21	104
	Chlorbromuron	$y=0.0895x+0.0006$	0.64	29	146
Fresh potato with peel	Monolinuron	$y=0.5338x-0.0317$	3	6	32
	Linuron	$y=0.212x-0.0108$	1	19	96
	Chlorbromuron	$y=0.1121x-0.0005$	0.4	47	234
Fresh potato without peel	Monolinuron	$y=0.2875x-0.0033$	2	10	51
	Linuron	$y=0.1223x-0.0077$	0.8	24	122
	Chlorbromuron	$y=0.0668x+0.0002$	0.4	45	224

$n=4$; y, relative area; x, analyte concentration.
[a] Limit of detection ($2N/s$).
[b] Limit of quantitation ($10N/s$).

method for PhU determination in standard solutions was carried out by GC–NPD. The linear concentration ranges studied were 0.1–2.5 μg ml^{-1} for monolinuron and linuron, 0.25–2.5 μg ml^{-1} for chlorbromuron. The regression coefficients were from 0.997 to 0.999 for monolinuron and chlorbromuron, respectively. Table 2 shows linear regression, sensitivity, limit of detection (LOD, two times the noise to sensitivity ratio, $2N/s$) and limit of quantitation (LOQ, $10N/s$). The LOD was in the range 16–36 ng ml^{-1} for monolinuron and chlorbromuron, respectively. The reproducibility studies carried out for four different samples showed that linuron and monolinuron yielded the most reproducible results. The RSD values were less than 10% at all the concentration ranges studied.

3.4. Determination of PhU herbicides from potato sample: recovery studies

The determination of PhU herbicides in powdered and fresh potato samples was carried out. First, the optimal conditions for determination of PhU herbicides from powdered potato samples were established. A blank of the sample was prepared. It was observed that some peaks coming from the sample matrix were eluted at retention times of 20.2, 26.8 and 30.8; but these peaks belong neither to the studied PhU herbicides, nor to the internal standard (methylparathion). No significant difference was observed for I.S. areas in the presence of the sample. Thus, a recovery study of the monolinuron, linuron and chlorbromuron herbicides was carried out from the powdered potato sample. To optimize the process of sample preparation and clean up, LLE and SPE techniques were used. The sample was spiked with PhU standard solution as indicated in Procedure.

The variables studied are summarized in Table 3. Optimal conditions were chosen from recovery and RSD values. The sample amount chosen favours the pre-concentration of the herbicides. The optimal conditions for LLE were selected taking into account a suitable polarity for the mass transfer of the herbicides from the aqueous phase to the organic phase, showed by a clean interphase.

The SPE was carried out to clean the sample, avoiding dirt in the injections and so minimizing herbicide degradation. Before carrying out the SPE, the extract of the organic phase obtained by LLE was evaporated and the solid residue was dissolved in methanol. The optimal conditions for SPE were selected taking into account the best retention of the

Table 3
Optimization of variables for PhU herbicide determination in potato samples

	Variable	Studied range	Optimal conditions			
			Measures	Recovery (%)	Herbicide	RSD (%)[c]
	Sample mass (g)	1–3	2.5	71–82	Monolinuron and chlorbromuron	8
LLE[d]	Organic phase solvent[a]		Dichloromethane/ light petroleum	77–88	Linuron and monolinuron	2
	Dichloromethane–light petroleum ratio	1/1–2/1	1/1	95–104	Linuron and chlorbromuron	3
	Methanol–water ratio	0.5/1–2/1	1/1	78–109	Linuron and chlorbromuron	6
	Ratio of organic–aqueous phase solvents	1/5–2.5/3.5	2/4	85–106	Monolinuron and chlorbromuron	9
	KCl (%)	2–10	2[b]	92–101	Linuron and chlorbromuron	6
	Shaking time (min)	2–10	5	86–111	Chlorbromuron and monolinuron	7
	Centrifugation time (min)	10–18	18	80–87	Linuron and chlorbromuron	5
SPE[e]	Methanol as residue solvent (ml)	0.5–2	1	73–89	Linuron and chlorbromuron	4
	Stationary phase	C_8, C_{18}, NH_2, CN	C_8	91–102	Linuron and monolinuron	5
	Drying time of cartridge (min)	1–7	4	82–93	Linuron and chlorbromuron	6
	Eluent volume (acetonitrile) (ml)	0.5–1	1	84–95	Linuron and chlorbromuron	4

[a] Dichloromethane–light petroleum (1:1), hexane–light petroleum (1:1), chloroform–light petroleum (1:1), cyclohexane–light petroleum (1:1), light petroleum and dichloromethane.
[b] For fresh potato, 5% of KCl.
[c] RSD, relative standard deviation, $n=4$ (the highest value obtained).
[d] LLE, liquid–liquid extraction.
[e] SPE, solid-phase extraction.

herbicides in the cartridge, more precise RSD% values and a shorter total analysis time.

To check its validity, the proposed method was applied for monolinuron, linuron and chlorbromuron determination in fresh potato samples with and without peel. Blanks of the samples of fresh potato with and without peel were prepared. It was also observed that there was no appreciable interference in the zone where the PhU herbicides and internal standard elute.

The main problem was the difficulty in separating the organic and the aqueous phases to carry out the LLE. So, some variables of the LLE method were optimized. Taking into account that the water content of the fresh potato was about 77%, the methanol–water ratio was optimized. Tests from absence of water to the presence of 8 ml were carried out. The optimal methanol–water ratio was 1:1 (8 ml water), with recoveries between 93 and 107% (monolinuron and chlorbromuron, respectively). However, 5 ml could not be extracted from the organic phase and so, another significant variable (% KCl) in the phase separation was modified. From 2 to 10% of KCl was studied, 5% being the optimal amount, with recovery values between 86 and 101% for linuron and chlorbromuron, respectively. Fig. 3a and b show the chromatograms of powdered potato for the blank and the fortified sample with the PhU herbicides, which shows the pattern of this type of samples.

3.5. Influence of the herbicide concentration in the recovery studies: analytical characteristics

Recovery studies for PhU herbicides in powdered potato and fresh potato (with and without peel) were carried out by GC–NPD. For the analysis, the samples were fortified to three concentration levels: 0.10, 0.30 and 0.64 μg g^{-1}. The recoveries for all samples were in general from 74 to 105% for linuron and chlorbromuron, respectively, except for 0.1 μg g^{-1} concentration level for powdered potato and fresh potato (from 75 to 116%). The RSD% values for $n=4$ were in general lower than 10%, except for linuron at the lowest concentration level of 0.1 μg

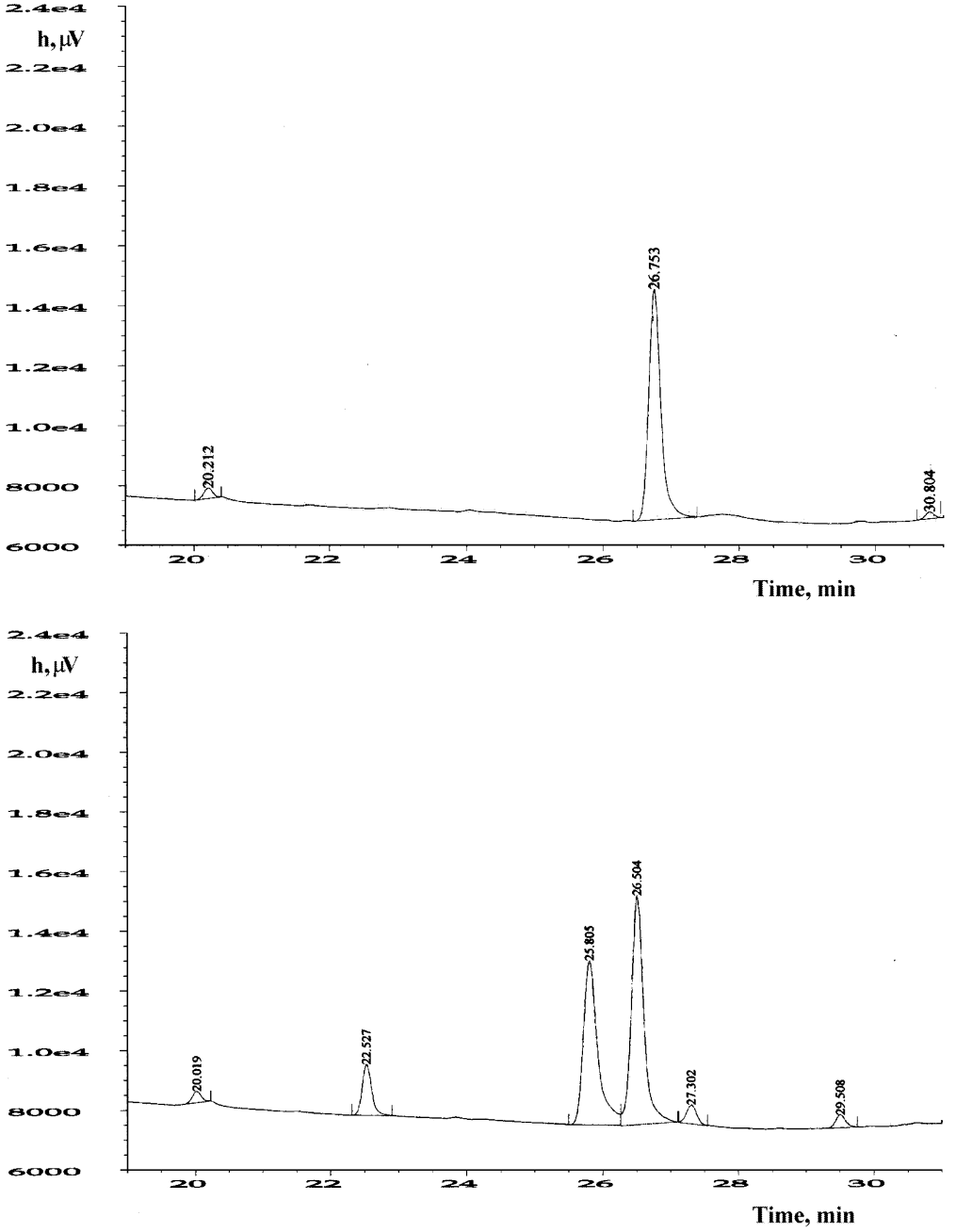


Fig. 3. Chromatograms of powdered potato by GC–NPD. (a) Blank, (b) 1.6 μg herbicide fortified sample. Conditions: column HP-5 (30 m×0.53 mm×2.65 μm); flow-rate, 28.3 ml min^{-1}; injection volume, 1 μl; m_{potato}, 2.5 g; [$I.S._{Methylparathion}$], 0.5 μg ml^{-1}. Temperature program: $T_{injector}$: 220 °C; $T_{detector}$: 300 °C; $T_{oven\ initial}$: 110 °C (2 min) Gradient: 5 °C/min–140 °C (2 min); 5 °C/min–210 °C (2 min); 7 °C/min–250 °C (2 min).

Table 4
Recovery studies of PhU herbicides in potato samples at three concentration levels

Herbicide	C ($\mu g\ g^{-1}$)	Powdered potato		Fresh potato			
		Recovery (%)	RSD (%, $n=4$)	With peel		Without peel	
				Recovery (%)	RSD (%, $n=4$)	Recovery (%)	RSD (%, $n=4$)
Monolinuron	0.10	84	10	91	8	88	9
Linuron		75	6	86	15	116	14
Chlorbromuron		116	10	84	2	112	6
Monolinuron	0.30	92	5	98	4	77	8
Linuron		93	9	82	6	74	5
Chlorbromuron		97	8	105	5	84	9
Monolinuron	0.64	93	3	93	5	87	3
Linuron		77	7	88	6	83	3
Chlorbromuron		93	7	99	7	96	9

g^{-1}. With respect to sample type there were significant differences in the recovery values. The results are shown in Table 4.

The analytical characteristics, linear regression obtained using three concentration levels of enrichment, sensitivity, LOD and LOQ are shown in Table 2. The slopes of the regression lines are different depending on the samples. The LOD values were in the range 6–47 ng g^{-1}, lower than 100–200 ng g^{-1}, which are the levels established by the European directive. The regression coefficients were from 0.995 to 0.999. These LOQ were lower than those stated in the literature (Mattern et al. [13]) for direct determination of linuron in potato sample (0.2 ppm) by GC–MS.

4. Conclusions

The PhU herbicides containing a methoxy radical (monolinuron, linuron and chlorbromuron) were thermostable and could be directly detected by GC–NPD. However, the PhU herbicides with a methyl radical (fluometuron and monuron) were thermally unstable.

The proposed GC–NPD method for the determination of monolinuron, linuron and chlorbromuron in powdered potato and fresh potato samples is direct; it involves the use of a HP-5 universal column and a long needle microsyringe (70 mm) for injection, using a standard injector. Thermal degradation was minimized and/or avoided by using a temperature gradient program of weak ramps. The method allows determination at the lowest trace concentration levels established by the European directive, 0.2 mg kg^{-1} for monolinuron and 0.1 mg kg^{-1} for linuron.

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