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Intercalibration of chromatographic methods for auxino phytodrugs in *Solanaceae*

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Abstract

Three chromatographic methods are considered for the determination in *Solanaceae* of auxino-similar phytodrugs, so called because their structure resembles an auxine plant hormone. The phytodrugs studied were: 2,4-dichlorophenoxyacetic acid, 2,4-dichlorophenoxypropionic acid, 2,4-dichlorophenoxybutyric acid, 2-methyl-4-chlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, naphthylacetic acid and 2-naphthyloxyacetic acid. Three chromatographic methods, respectively based on ion-interaction HPLC, GC–MS with intra-injector derivatisation and GC–MS with pre-injection derivatisation, were developed, optimised and validated. A comparative discussion of the advantages/disadvantages of the methods suggests a strategy for their preferential use, that is essentially a function of the matrix complexity.

Keywords: *Solanaceae* spp.; Calibration; Vegetables; Validation; Food analysis; Pesticides; Phenoxy acids

1. Introduction

Auxino-similar phytodrugs are recognised as particularly suitable for the treatment of *Solanaceae*. Their structure resembles that of auxine, a plant hormone which controls the growth of stems, roots, flowers and fruits; the most important natural auxine is indole-3-acetic acid that exerts important roles in plant activities, like phototropism, geotropism, apical dominance and fruit growth. Auxino phytodrugs can also improve fruits and vegetables as regards size and colour. Furthermore, auxino-similar phytodrugs guarantee a relatively low environmental impact.

The phytodrugs considered here are: 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4-dichlorophenoxypropionic acid (2,4-DP), 2,4-dichlorophenoxybutyric acid (2,4-DB), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), naphthylacetic acid (NAA) and 2-naphthyloxyacetic acid (BNOA). Structures are presented in Fig. 1.

Generally auxino phytodrugs are mainly determined by HPLC methods with UV [1–7], diode array [3,8], electrochemical [3] and fluorimetric detection after a derivatisation reaction [3,9,10]. An ion interaction RP-HPLC method was reported from this laboratory [11]. Gas chromatographic analysis with mass detection is also used, after derivatisation with pentafluorobenzyl and diazomethane [4,12–17], borum trifluoride [18], trimethyl silyl diazomethane

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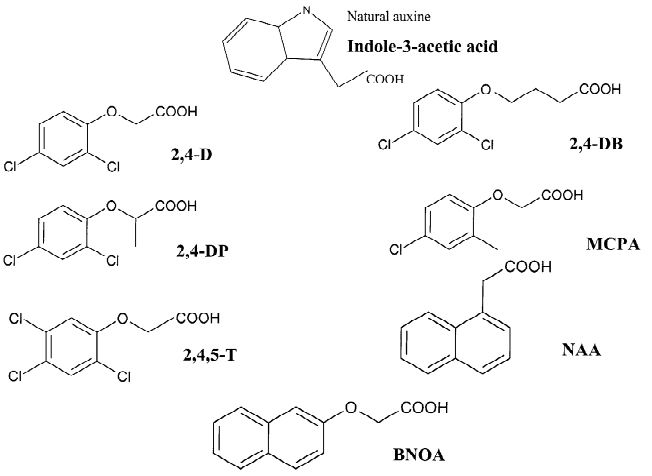

Fig. 1. Auxino hormone and analyte structures.

[19] or associated with intra-injection methylation reaction [20]. For the determination of NAA, methods based on micellar liquid chromatography on a cyano stationary phase [21] and immunoassay [4,13,22,23] are also reported. A simultaneous determination of BNOA and 2,4-D in tomato samples can be found [24]. No other example of multicomponent analysis is known.

Therefore, multiresidue methods for the simultaneous identification and determination of auxino phytodrugs in their mixtures are required. In this paper we develop and compare three chromatographic methods for the simultaneous identification and determination in *Solanaceae* of the most largely used auxino phytodrugs. The methods are an ion-interaction RP-HPLC procedure (modified from Ref. [11]) and two gas chromatographic methods, respectively based on intra-injection and pre-injection derivatisation reactions. The three methods are intercalibrated and the advantages compared and discussed as a function of the sample to be analysed.

2. Experimental

2.1. Instrumentation and conditions

A HPLC (Varian, TO, Italy) system equipped with a ProStar 210/215 pump, a ProStar 330 photodiode array detector, autosampler ProStar 410, Rheodyne valve with 100 μl loop and the software Poly-view 2000 was employed. The stationary phase is a Merck (Darmstadt, Germany) Lichrospher RP-100 5 μm endcapped, 250 mm×0.46 mm, coupled with a Merck Lichrocart RP-18 5 μm precolumn.

A GC system (Varian model 3400 MS-Saturn 2) ion trap detector with a conventional split/splitless injector 1077 model was used. The stationary phase was a DB5 HP-5MS 30 m×0.25 mm I.D., 0.25 μm (Agilent, Palo Alto, CA, USA).

The pH measurements were performed with a pH-meter (Crison MO, Italy, model micro-pH 2002) equipped with a combined glass Ag/AgCl electrode. The homogeniser was an Ultra Turrax IKA T25 (Stauten, Germany).

2.2. Reagents

All the analytes (purity >97%) were purchased from Lab Service Analytica (PR, Italy).

Water and acetonitrile were Scharlau HPLC grade. Trimethylhydroxyaniline (TMHA) 0.5 *M* in methanol, anhydrous potassium carbonate, pentyl-, hexyl- and heptylamine were Fluka (Buchs, Switzerland) analytical-grade reagents. Ethyl acetate, acetone, methanol, sodium sulphate and sulphuric acid were Labscan products; hexane and Celite45 were Merck reagents. Ultrapure water was produced from a Milli-Q system (Millipore, Bedford, MA, USA).

The standard solutions of the analytes were prepared at a concentration of 400 mg/l in acetone and

diluted as required with ultrapure water. The solutions were kept at temperatures below −4 °C.

2.3. Procedure

2.3.1. Sample extraction and purification

The extraction process of the analytes from fruits and vegetables was based on acidic–basic extraction. The samples were prepared from the gross sample by the quartature method, homogeneised and purified by a liquid–liquid extraction. Twenty grams of sample were added to 10.0 ml of 0.1 *M* HCl and 100 g of Celite45® and extracted with 150.0 ml of ethyl acetate in an Ultra Turrax homogeniser and the extract was filtered (Buckner filter) on anhydrous sodium sulphate and back-partitioned four times with 20.0 ml of 0.1 *M* NaOH.

The basic portions were combined, acidified with 1.0 *M* HCl to pH values <1 and then extracted with four aliquots (20.0 ml each) of ethyl acetate. The organic phase was filtered on anhydrous sodium sulphate and dried in a Rotavapor at a pressure of 120 mbar and temperature below 40 °C. The residue was recovered with 2.5 ml of acetone, dried and collected as a function of the analytical method to be employed: (i) for method A (ion-interaction (IIR) HPLC) the sample was collected with 1.0 ml of the mobile phase and injected; (ii) for method B (intra-injector derivatization) the residue was collected with 1.0 ml of acetone, transferred into a vial, added to 100 μl of 0.5 *M* TMHA in methanol and injected; (iii) for method C (pre-injection derivatisation with methanol in acidic medium) the residue was recovered with 1.0 ml of methanol, added to 250.0 μl of 18.0 *M* H_2SO_4, digested for 10.0 min and added to 5.0 ml of ultrapure water. After mixing, four extractions (2.0 ml each) of ethyl acetate were performed. The organic fraction was dried under nitrogen, the residue was recovered with 1.0 ml of acetone and injected into the GC–MS system.

2.3.2. Preparation of the mobile phase for the IIR-HPLC method (method A)

The mobile phase was prepared by adding 1.0 *M* orthophosphoric acid of pH 6.4±0.2, to the alkylamine aqueous solution prepared at the concentration required by the experimental design plan. The chromatographic system was conditioned by passing the eluent through the column under isocratic conditions until a stable baseline signal was reached and reproducible retention times were obtained for three subsequent injections (about 1 h, at flow-rate of 1.0 ml/min, was usually required).

Table 1
Mass–charge ratio utilised for detection in methods B and C

Analyte	*m/z* (1)	*m/z* (2)	*m/z* (3)
2,4-D	199	175	234
2,4-DP	162	248	189
2,4-DB	101	59	–
2,4,5-T	233	235	270
MCPA	141	155	214
NAA	141	200	115
BNOA	216	–	–

2.3.3. Methods B and C—experimental conditions

The oven temperature started at 60 °C, after 3 min it reached 150 °C at a rate of 20 °C/min and 210 °C in 3 min; then with a rate of 20 °C/min the temperature reached 280 °C, where it remained for 5 min. The ion source and the transfer line were set at 280 °C. The injector was at a temperature of 250 °C and remained in the splitless mode for the first 0.75 s then it turned to split mode. The ionisation mode was electron impact (EI) and detection was performed by MS with scans of mass ranging between 57 and 273 u and a s/scan ratio of 0.6. The identification and the quantification of the analytes were performed in ion extraction mode extracting the characteristic *m/z* ratios from the US National Institute of Standards and Technology (NIST) spectra database, and are summarised in Table 1.

3. Results and discussion

3.1. Method optimisation

3.1.1. Method A—IIR-HPLC method

The IIR-HPLC technique is a development of the conventional RP-HPLC in which a suitable ion interaction reagent (IIR) is added to the mobile phase. The technique is very versatile since retention depends on many factors, that, on the other hand, require accurate tuning. Previous studies [25,26] evidenced that when alkylammonium orthophosphate

Table 2
Experiments of the fractional design (N, alkyl chain length; C_{ACN}, acetonitrile concentration; F, mobile phase flow-rate; C_{IIR}, ion interaction reagent concentration)

	Variables			
	N	C_{ACN}	F	$C_{IIR} = NC_{ACN}F$
I CP	0	0	0	0
II CP	0	0	0	0
1	−	−	−	−
2	+	−	−	+
3	−	+	−	+
4	+	+	−	−
5	−	−	+	+
6	+	−	+	−
7	−	+	+	−
8	+	+	+	+
III CP	0	0	0	0

salts are the IIRs, the retention mechanism is mainly affected by the following experimental factors: the alkyl chain length (N) of the alkylammonium ion of the IIR, the concentration (C_{IIR}) of the IIR and the acetonitrile percentage (C_{ACN}).

To simultaneously optimise the three variables considered and to take into account their possible interactions, a multivariate analysis of the experimental design was used. Beside the mentioned variables (N, C_{IIR} and C_{ACN}), also the elution flow-rate F was considered (even if its effect is predictable) in order to obtain from the chemometric treatment the complete set of chromatographic conditions able to maximise the resolution of the analytes. A *fractional factorial* 2^{4-1} *design* was chosen to treat the four variables involved [27], the extreme values (indicated with − and +) of the variables being: N (−)=5, N (+)=7; C_{ACN} (−)=26%, C_{ACN} (+)=30%; F (−)=0.8 ml/min, F (+)=1.2 ml/min; C_{IIR} (−)=2.0 mM, C_{IIR} (+)=8.0 mM. The experiments of the fractional design are reported in Table 2, where 0 represents the central point (CP) and the response is the retention time of the seven analytes.

Table 3 reports, for the analytes considered, the effect of the factors (N, C_{ACN}, C_{IIR} and F) and of their interactions. The relevant effects at the 95% confidence level are reported in bold. The table shows that the most important effects are due (besides that expected of the flow-rate) to the alkyl chain length N and to the concentration C_{ACN} of the organic modifier. The IIR concentration plays a relevant effect only for NAA.

The retention times calculated for each analyte by the model fit well the experimental data for all the analytes. A grid search algorithm [28] spanning the domain of the experimental factors allowed to find the experimental conditions that provide the simulated best resolution of the analyte mixture.

The conditions are: heptylamine 2.0 mM, pH 7.0, flow-rate 0.8 ml/min and acetonitrile percentage in the mobile phase 26.0%. The simulated retention times calculated by the regression models reported in Table 3 and the optimised experimental setting provide the chromatogram reported in Fig. 2, (UV detection at 210 nm). Unfortunately, in these conditions 2,4-D and MCPA co-elute but, due to their very different absorbance spectra, their identification can be easily performed by diode array detection.

Table 3
Effect of the factors for the phytodrugs considered

	MCPA	2,4-DP	2,4-D	NAA	2,4,5-T	BNOA	2,4-DB
Average	**7.05**	**8.94**	**7.37**	**3.16**	**12.52**	**8.88**	**25.36**
N	**3.60**	**4.39**	**3.85**	**1.99**	**7.26**	**5.00**	**9.71**
C_{ACN}	−1.07	−2.33	−1.03	**−0.78**	**−2.68**	−0.76	−6.91
NC_{ACN}	2.21	2.30	**2.35**	**1.52**	**4.27**	**3.08**	3.48
F	**−4.67**	**−5.73**	**−4.90**	**−2.04**	**−9.32**	**−5.68**	**−16.13**
NF	−0.52	−0.11	−0.69	−0.14	−1.20	−0.47	−0.09
$C_{ACN}F$	2.04	**4.04**	**2.28**	**1.78**	**5.12**	**2.30**	**9.19**
C_{IIR}	0.17	0.96	0.13	**−1.31**	0.29	0.32	−0.69

Analytes: 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2,4-dichlorophenoxypropionic acid (2,4-DP), 2,4-dichlorophenoxyacetic acid (2,4-D), naphthylacetic acid (NAA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2-naphthyloxyacetic acid (BNOA) and 2,4-dichlorophenoxybutyric acid (2,4-DB). N, alkyl chain length; C_{ACN}, acetonitrile concentration; F, mobile phase flow-rate; C_{IIR}, ion interaction reagent concentration.

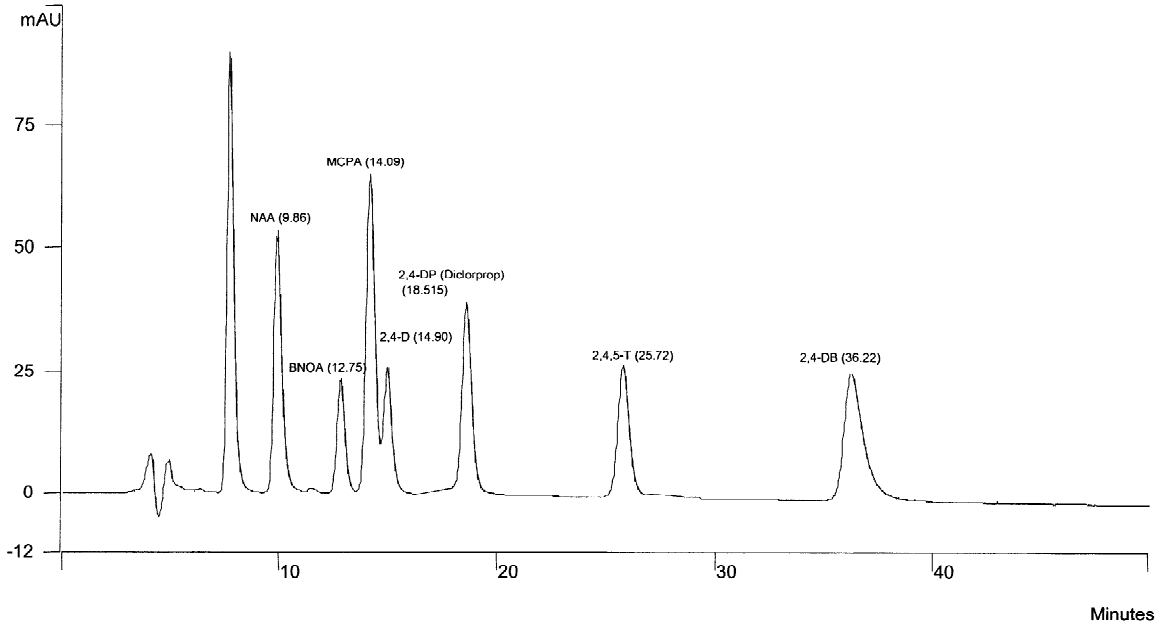

Fig. 2. IIR-HPLC separation of the mixture of the analytes (0.1 mg/l each). Mobile phase, heptylamine 2.0 m*M*, pH 7.0; flow-rate, 0.8 ml/min; ACN% in the mobile phase 26.0%. The stationary phase is a Merck Lichrospher RP-100 5 μm endcapped, 250 mm×0.46 mm, coupled with a Merck Lichrocart RP-18 5 μm precolumn. UV detection at 220 nm. Injection loop is 100 μl.

3.1.2. Method B—intra-injection THMA derivatisation method

Hydrophilic molecules, like the phytodrugs studied here, must undergo a derivatisation process prior to gas chromatographic analysis. Intra-injector derivatisation is a widely used technique in the GC analysis of fatty acids and it is generally based on the esterification reaction with TMHA as methylating agent. To increase the efficiency of the methylation reaction, the temperature of the injector and the stability of the reagents have been carefully controlled and the optimised conditions correspond to the use of 1.00 ml of the standard solutions (concentration 1.0 mg/l of each analyte in acetone) added to 100 μl of TMHA.

As concerns temperature, a maximum of sensitivity is observed in the range between 220 and 280 °C. To guarantee reproducibility of the procedure and to avoid (or minimise) the reaction of the methylating agent with acetone used as the solvent, a strong excess of methylating agent must be added. Since methylated standards (except that for BNOA) are not commercially available, the methylation reaction efficiency is evaluated through the quantification performed by the IIR-HPLC method of the unreacted fraction. For all the analytes, percentages greater than 90% have been obtained except for BNOA, for which the conversion is evaluated with respect to the certified methylated standard and percentages greater than 99% are obtained.

For a tomato sample spiked with a mixture of the phytodrugs considered, it is possible to obtain in a running time of about 35 min the separation of all the seven analytes.

3.1.3. Method C—pre-injection derivation method

The analytes were methylated in a mixture of aqueous sulfuric acid and methanol, the methylated products were extracted in hexane and dried under nitrogen flow; the residue was collected in acetone and injected into the gas-chromatographic system, operating under the same chromatographic conditions optimised for method B. The efficiency of the derivatisation process was evaluated by determining, in the aqueous fraction and by the IIR-HPLC method, the unreacted fraction; this result was always lower than the detection limits of the analytes. Also this method allows to obtain the separation of all the

analytes in a chromatographic run of about 35 min associated to a relatively low matrix effect, essentially due to the further derivatisation step; but, on the other hand, the average recovery is lower.

3.2. Validation of the methods

The experimental error was analysed as a function of the concentration involved. Methods A and B prove to be sufficiently homoscedastic at the 95% confidence level, through the maximum variance and the Bartlett and Cochran tests [29]. On the contrary, method C shows a significant increase of the experimental error with the increase of concentration. To take this behaviour into account, when building the calibration curve for method C, a weighted least square fitting was employed, in which the weights introduced were proportional to the inverse of the experimental errors estimated for each concentration.

In order to compare the performances of the three methods developed, mixtures containing the seven analytes were prepared and analysed. Due to the different sensitivities of the three methods, for HPLC and intra-injector GC method the concentration ranged between 0.5 and 1.5 mg/l and for the pre-derivatisation GC method between 2.0 and 10.0 mg/l. The solutions were pretreated according to the optimised procedure reported in Section 2. Six different concentration levels were considered and three replicates were performed for each concentration.

A response factor (F) was calculated as a function of the peak area of 2,4-dichlorobenzoic used as the internal standard:

$$F = \frac{A_{\mathrm{PA}}}{A_{\mathrm{I.S.}}} \cdot \frac{C_{\mathrm{I.S.}}}{C_{\mathrm{PA}}}$$

where A_{PA} and C_{PA} are, respectively, the area and the concentration of the active molecule, while $A_{\mathrm{I.S.}}$ and $C_{\mathrm{I.S.}}$ are the peak area and the concentration of the internal standard. The F values for the first two methods are practically constant in the range of concentration considered, while the method based on GC with pre-injection derivatisation provides values of F which changed along the concentration range. In this last case the necessity of a non-linear calibration curve is identified and no internal standard with the same dependence signal/concentration as the one exhibited by the analyte can be found. This suggested that calibration models be built directly based on the peak areas, without employing the internal standard peak area.

For the first two methods the calibration models are linear models, while for method C a quadratic model is calculated with the weighted least square algorithm:

$$y' = a + bx + cx^2$$

(y' being the peak area of the analyte).

All the calibration models provide a good fitting to the experimental data, with $R^2 > 0.99$ for all the analytes in method A and $R^2 > 0.97$ for all the analytes for the methods B and C. The Mandel test [30] shows good linearity in the concentration range between 0.1 and 1.5 mg/l for the HPLC and for the intra-injector method. For method C only a narrow region at the lowest concentration provides satisfactory linear models but the range depends on the specific analyte.

The limits of detection (LODs) are evaluated as $3s_{\mathrm{b}}/b$ where s_{b} is the uncertainty of a blank peak and b is the slope of the calibration curve.

The limits of quantification (LOQs) are evaluated as $10s_{\mathrm{b}}/b$. Both values of LOD and LOQ are reported in Table 4. The LODs and LOQs of the pre-injection GC method are always much larger (about 10 times or more) than those of the other two methods.

By spiking a tomato sample used as the reference without contamination of phenoxyacid phytodrugs three times with three different concentration levels, the recovery yield was evaluated; the concentration ranged between 0.05 and 0.30 mg/l for the HPLC method and the intra-injector method and between 0.30 and 0.90 mg/l for the pre-injection method. The full extraction process was performed and the extract analysed by the three methods. The results show the absence of any correlation between recovery and concentration. The average recovery yields (summarised in the Table 5) for the three methods were all greater than 80% with a maximum standard deviation of 20%.

In order to evaluate the matrix effect and possible synergic effects, an extract of tomato was spiked with a mixture of the seven analytes considered at

Table 4
LOD and LOQ values for the three methods and the maximum admitted concentration

	Maximum admitted concentration (mg/kg)	IIR-HPLC (mg/l)		GC-intra-injector (mg/l)		GC-pre-injection (mg/l)	
		LOD	LOQ	LOD	LOQ	LOD	LOQ
MCPA	0.10	0.03	0.09	0.02	0.07	0.1	0.3
2,4-DP	0.05	0.02	0.06	0.02	0.06	0.1	0.3
2,4-D	0.10	0.05	0.10	0.02	0.07	0.1	0.4
NAA	0.01	0.02	0.04	0.03	0.09	0.1	0.4
2,4,5-T	0.05	0.03	0.09	0.02	0.06	0.1	0.3
BNOA	0.05	0.02	0.06	0.03	0.09	0.2	0.6
2,4-DB	0.05	0.02	0.07	0.02	0.07	0.1	0.3

Abbreviations as in Table 3.

Table 5
Recovery yields (percentage values) for the three methods. Abbreviations as in Table 3

	IIR-HPLC	GC-intra-injector	GC-pre-injection
MCPA	92±7	88±8	93±15
2,4-DP	91±7	84±8	90±18
2,4-D	85±5	81±7	96±19
NAA	96±3	87±11	93±17
2,4,5-T	87±9	80±8	99±11
BNOA	89±14	83±11	96±16
2,4-DB	89±13	80±12	95±12

three different concentration levels and the spiked samples were analysed with the three methods. All the procedures were repeated three times and a *t*-test was performed. The *t*-value calculated as $t = |x - \bar{x}|/s\sqrt{n}$ indicates that only for the GC method B the deviations were statistically significant and that matrix effect was present; in this case the external calibration plot cannot be used but it is necessary to use the standard addition method.

3.3. Intercalibration of the methods

The method intercalibration was performed by comparing the results obtained with the three methods in the analysis of a sample of tomato spiked with a mixture of the analytes.

Since the comparison must be done for the same concentrations of the analytes, the less sensitive method (the pre-injector one) leads to a constraint on the concentrations used, that range between 0.3 and 0.6 mg/l.

The tomato sample was homogenised, the mixture of the analytes added to it, extracted, purified and the recovery evaluated. Ten replicates were performed. The results given as the average values $\bar{x}$ are reported in Table 6 together with the estimated

Table 6
Intercalibration parameters for the three methods: the average values $\bar{x}$, the estimated standard deviation *s*, the relative standard deviation (RSD, given as $(s/\bar{x}) \cdot 100$) and the percentual error $e\% = ((|\bar{x}| - \mu)/t) \cdot 100$. In parentheses are reported the standard deviation when the average $\bar{x}$ corresponds to the true value

	IIR-HPLC				GC-intra-injector				GC-pre-injection			
	$\bar{x}$ (mg/l)	*s*	RSD (%)	*e*%	$\bar{x}$ (mg/l)	*s*	RSD (%)	*e*%	$\bar{x}$ (mg/l)	*s*	RSD (%)	*e*%
MCPA	0.37	0.02	4.88	3.39	0.35	0.02	5.28	3.39	0.39	0.04	10.36	1.80
2,4-DP	0.35	0.01	3.58	1.70	0.34	0.02	5.92	1.70	0.36	0.03	7.85	4.65
2,4-D	0.57	0.02	2.94	0.70	0.57	0.03	5.73	0.70	0.59	0.04	7.55	2.78
NAA	0.45	0.01	2.29	(0.01)	0.46	0.02	5.14	(0.01)	0.46	0.06	12.45	2.22
2,4,5-T	0.41	0.02	5.55	(0.01)	0.42	0.02	4.82	(0.01)	0.43	0.06	14.91	4.87
BNOA	0.81	0.06	7.84	2.53	0.79	0.09	11.14	2.53	0.78	0.11	14.52	1.26
2,4-DB	0.34	0.04	11.25	6.85	0.39	0.04	9.43	6.85	0.37	0.04	10.53	1.37

standard deviation s and the percent variation coefficient C.V.% (given as $(s/\bar{x})\cdot 100$).

The same table shows the percentual error for the three methods: $e\% = ((|x| - \mu)/t)\cdot 100$, where μ represents the true value (the added amount).

It can be noticed that the error is always lower than 7% and that the error for the pre-injector method is on average the greatest. Also, the reproducibility of the inter-injector method is generally lower than that of the pre-injection method.

In order to compare the results of the three methods, the Friedman test [31] was employed; this non-parametric test is able to treat and compare k blocks of data simultaneously. According to the Friedman test, the data of the n samples are ordered in a matrix with k columns and n rows. A rank is then assigned in every row, giving the lowest rank to the lowest value, and progressively increasing the rank as the value increases. In the present instance the ranks 1, 2 and 3 were assigned to the concentration values obtained with the three methods. Then a χ^2 value was calculated as:

$$\chi^2 = \frac{12}{nk(k+1)} \sum_{j=1}^{k} R_j^2 - 3n(k+1)$$

where R_j is the sum of the ranks of the jth columns. The calculated value was compared with the value of χ^2 distribution for 9 degrees of freedom. As χ^2 (calculated) $< \chi^2$ ($\alpha = 0.05$) for all the analytes, it can be concluded that the results obtained with the three methods do not differ statistically from each other at a significance level $\alpha = 0.05$.

Nevertheless, a method can be preferred to the other two as a function of the sample to be analysed.

3.4. Application to a real sample

The methodologies developed in this work have been applied in the analysis of fruits and vegetables which are generally treated with auxino-phytodrugs. In particular the *Solanaceae* are more frequently suspected for phenoxyacid contamination.

The analysis of two samples of tomatoes produced in Sicily shows the presence of BNOA (2-naphthoxyacetic acid) at levels corresponding to the maximum admitted concentration. In turn, a courgette sample suspected for contamination by phenoxyacid phytodrugs, shows the absence of phenoxyacid phytodrugs when analysed with both IIR-HPLC and GC–MS methods (Fig. 3).

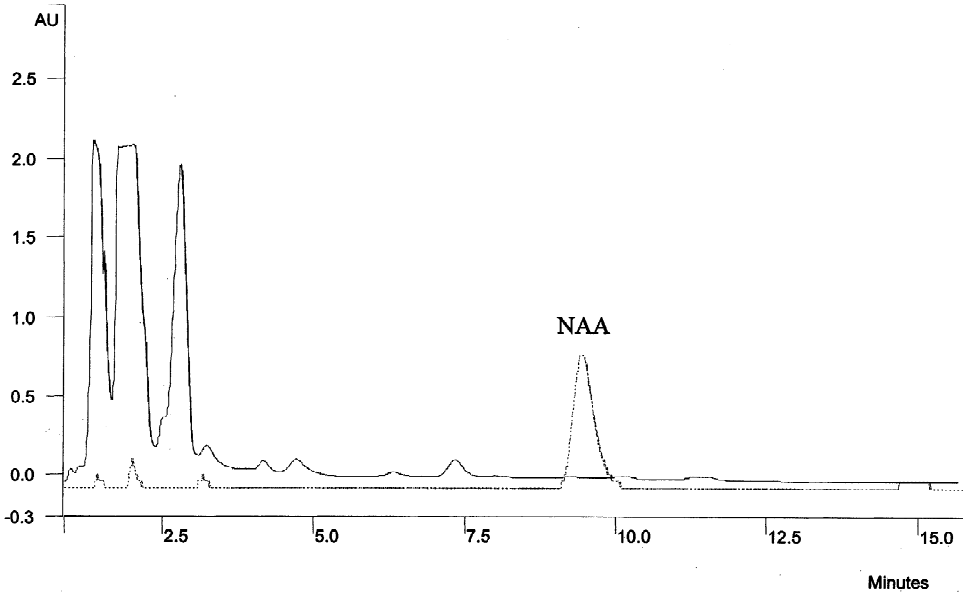

Fig. 3. HPLC chromatograms of a courgette suspected for NAA contamination (continuous line) and of the standard NAA (at LOQ level) (dotted line). Conditions as in Fig. 2.

When other vegetables must be analysed, a test for the validity of recovery and matrix effect for the new matrix must be performed and if the test is negative a new evaluation is necessary. In the case of the courgette sample the test of the validation parameters was positive and the recovery percentages previously evaluated for the tomato matrix could be used.

4. Conclusions

In order to compare an IIR-HPLC method, that does not require any pretreatment, and two GC–MS methods that, respectively, require intra-injector and pre-injection derivatisation, the analysis of seven auxino-pesticides was performed. Table 4 reports, for each analyte, the maximum concentration admitted in vegetables by the EEC regulations (91/414/CE, 2000/60/CE). It can be noticed that the GC–MS pre-injection derivatisation method is characterised by LOQs too high with respect to the threshold concentration values, so that the method can be employed only with a previous pre-concentration step. On the other hand, this method offers the advantages of good applicability, when used in the analysis of complex matrices, due to the supplementary extraction and purification steps required.

The intra-injector methodology presents the advantages of good sensitivity and selectivity due to the use of the ion-extraction mode in mass spectrometry.

On the basis of the results collected, the first step of the analysis could be the extraction of the analytes and a first evaluation by GC–MS (with pre-injection derivatisation); if a greater sensitivity is required or the matrix effect is relevant, the intra-injector method must be used. If the matrix effect is negligible, the IIR-HPLC method can be advantageously used, since it does not require any derivatisation process.

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