

Original Research

**CALIBRATION PERFORMANCES OF A MULTIRESIDUE PESTICIDES
ANALYSIS IN LEMON AND LETTUCE ON QqQ AND ION TRAP MASS
SPECTROMETERS**

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Abstract

As highly selective and sensitive, mass spectrometry currently stands for the most used analytical technique for both qualitative and quantitative analysis in many fields. One of the most significant factors in analytical calibration and quantification studies is sensitivity, which can be defined as the increment in measured signal per unit change in analyte concentration. Several factors such as the effective sample preparation, mobile phase composition, chromatographic column parameters and mass spectrometer features can affect analyte signal and result in various levels of sensitivity. The aim of this study was to evaluate calibration performances of the pesticides analysis in fruit and vegetable across the two instruments, by comparing the sensitivity on the two most exploited mass analyzers, that is on the linear ion trap and triple quadrupole mass analyzer. The comparison of sensitivity for solvent-based and QuEChERS treated lemon- and lettuce-based pesticides standards demonstrated triple quadrupole as a highly sensitive instrument.

Keywords: fruit, vegetables, mass spectrometry, QuEChERS.

INTRODUCTION

As highly selective and sensitive, mass spectrometry currently stands for the most used analytical technique for both qualitative and quantitative analysis in many fields such as life sciences, forensics, environmental and food safety.

One of the most significant factors in analytical calibration and quantification studies is sensitivity. Sensitivity is defined as the increment in measured signal per unit change in analyte concentration; it can be related to the detection limits, meaning the higher the sensitivity, the lower the detection limits. In practice, across the specified concentration range this parameter can be estimated via the slope of the calibration function.

The basics of sensitivity lay in the in-source ionization efficiency and subsequent transfer efficiency from the atmospheric pressure to the low-pressure region of the mass spectrometer. Several factors such as the effective sample preparation, mobile phase composition,

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chromatographic column parameters and mass spectrometer features can enhance analyte signal when carefully chosen and optimized and can result in a highly sensitive method of analysis [1–3]. In addition to these factors, mass analysis and detection are significant factors in the sensitive analysis. Since the key point of difference between the mass spectrometers is mass analyzer, the improvement in its construction is a promising method for sensitivity enhancement.

Quadrupole tandem mass analyzer (triple quadrupole, QqQ) combines three transmission quadrupoles in a linear configuration, of which the first and the third quadrupole have the role of scanning or filtering device and the central one has the role of a collision cell. Stages of tandem mass analysis within this analyzer (parent ion isolation, parent ion fragmentation, daughter ions isolation and transmission towards the detector) are performed successively in space.

On the contrary, in the ion trap analyzer stages of tandem mass analysis are performed in the same space, successively in time. Since the ion trap works on the principle of filling and emptying the same space with ion packages, the main phenomenon that can affect sensitivity is the space charge effect which arises from the Coulomb interactions between the ions trapped in small space. This phenomenon is circumvented by the accumulation of a limited number of ions, determined in the instrument cycle called the pre-scan.

The aim of this study was to evaluate calibration performances of the analysis across the two instruments, by comparing the sensitivity on the two most exploited mass analyzers, that is on the linear ion trap and triple quadrupole mass analyzer. Performances were evaluated in the simplest MS mode, that is in the MS¹ full scan mode, primarily by the cross-analysis of solvent-based standards of 15 pesticides from different chemical classes. Then, the evaluation was broadened to pesticide standards in lettuce and lemon treated with the QuEChERS sample preparation procedure. These matrices are chosen due to the differences in their composition and appearance; lettuce is a leafy highly pigmented vegetable; lemon is fruit with high amount of water and acid.

MATERIALS AND METHODS

Chemicals

Formic acid (FA) (98%) and 15 high purity pesticide standards (acetamiprid, azoxystrobin, boscalid, buprofezin, cyprodinil, difenoconazole, fenhexamid, kresoxim-methyl, metsulfuron-methyl, propiconazole, pyraclostrobin, pyrimethanil, pyriproxyfen, tebuconazole and trifloxystrobin) were produced by Sigma-Aldrich®, Germany. Ammonium-formate (AMF) (98%), deionized water and HPLC grade ethanol were produced by Carlo Erba, Italy. HPLC grade methanol (MeOH) and acetonitrile (AcN) were produced by J.T. Baker, USA. Prepacked QuEChERS extraction pouches (1 g of NaCl, 4 g of MgSO₄, 1 g of trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate) and dispersion kits (25 mg PSA and 150 mg MgSO₄) were produced by Hillium, USA. Syringe microfilters (Nylon Hydrophilic 0.22 µm) were produced by Membrane Solutions, USA. Fresh lettuce and lemons were purchased in a local supermarket.

Instruments and instrumental parameters

Samples were analyzed subsequently on two separate LC/MS systems. A sharing point between the systems were the very same chromatography column used for the analytes separation (Hypersil GOLD (C₁₈, 150mm×2.1mm, particle size 3 µm) and the same ionization type (ESI+). 10 µL of the sample was loaded on a column in a partial loop injection mode and eluted with a mixture of: eluent A (solution of 0.10 % of FA and 0.03 % of AMF in water) and eluent B (MeOH) following the gradient: 0–2 min (90 % A), 2–7 min (90–30 % A), 7–30 min (30 % A), 30–35 min (30–90 % A), 35–40 min (90 % A) with flow equal to 300 µL min⁻¹.

Procedures

Stocks preparation

Single-pesticide stock solutions (1 mgmL⁻¹ each) were prepared by dissolving high purity pesticide standards in ethanol. Multi-pesticide solutions were prepared by mixing and diluting single stocks in ethanol.

Sample preparation

One kilogram of fresh lettuce and lemon was cut and homogenized by blending for 5 min. Ten grams of the obtained homogenate of each matrix were extracted with 10 mL of acetonitrile, after which an extraction pouch was added. The mixture was immediately vortexed for one minute and centrifugated (10 min/3000 rpm). A supernatant aliquot was subjected to a dispersive extraction by the addition of one dispersion kit per mL of supernatant. The mixture was vortexed for one minute and centrifugated (10 min/3000 rpm). The supernatant was microfiltered prior to instrumental analysis.

Calibration standards preparation

Solvent-based standard series was prepared by spiking acetonitrile with a multi-pesticide standard solution. For each matrix, standard series with 7 calibration levels was prepared by spiking the obtained blank extracts with multi-pesticides standard solutions.

RESULTS AND DISCUSSION

Calibration performances assessed via pesticides analytical standards

Sensitivity, denoted as parameter a of the calibration function $y = ax + b$, for analysis of pesticide standards on a triple quadrupole is given in Table 1. The comparison of this parameter across analytes (solvent-based standards) revealed the highest sensitivity of the instrument towards buprofezin and cyprodinil ($>200 \times 10^6$ arb. units per µgmL⁻¹). Mid-range sensitivity ($10\text{--}60 \times 10^6$ arb. units per µgmL⁻¹) was achieved for 8 of 15 analytes; the instrument is the least responsive to boscalid, difenoconazole, fenhexamid, kresoxim methyl and metsulfuron methyl with the slopes ranging from approx. 1 to 5×10^6 arb. units per µg mL⁻¹ (Table 1).

Almost the same trend could be observed on the linear ion trap instrument (Table 2). The highest instrumental sensitivity could be observed for buprofezin and cyprodinil, however, the differences between analytes in sensitivity were not as pronounced as on the QqQ instrument. Sensitivity for trifloxystrobin, pyriproxyfen and azoxystrobin was not much lesser than the

sensitivity for buprofezin and cyprodinil. On the QqQ instrument sensitivity for buprofezin and cyprodinil was about 4 times higher than the sensitivity for the following three pesticides, however, on the linear ion trap instrument it is higher by only about 1.5 times. Buprofezin on the QqQ instrument expressed about 3.7 times higher sensitivity than the pyrimethanil, but on the linear ion trap its sensitivity is only about 1.7 times higher than the sensitivity of pyrimethanil. Tebuconazole on the QqQ expressed about 3 times higher sensitivity than acetamiprid, however, on the linear ion trap sensitivity for these analytes are comparable (Tables 1 and 2).

When compared across the instruments, much higher sensitivity could be observed for the instrument with QqQ analyzer. Sensitivity enhancement ranged from 4.28 times for boscalid to 71.24 times for buprofezin. Since the separation parameters (column, flow, mobile phases composition) and the ionization type were the same, these differences can be attributed to the two main features that distinguish the instruments used within this study, that is the ion optics and mass analyzers features.

Table 1 Linear regression parameters ($y = ax + b$, conc. range $0.01 - 15.00 \mu\text{g mL}^{-1}$) for the analysis of solvent-based, lemon-based and lettuce-based pesticides standards on a triple quadrupole instrument

	a ($\times 10^6$)	b ($\times 10^6$)	R ²	a ($\times 10^6$)	b ($\times 10^6$)	R ²	a ($\times 10^6$)	b ($\times 10^6$)	R ²
	Solvent			Lemon			Lettuce		
Acetamiprid	13.51	11.04	0.9924	11.17	9.04	0.9881	9.76	12.20	0.9648
Azoxystrobin	50.78	-3.57	0.9995	32.20	15.34	0.9931	17.76	16.43	0.9699
Boscalid	1.07	1.67	0.9977	0.89	0.22	0.9990	0.58	0.14	0.9985
Buprofezin	219.41	2.19	0.9999	69.23	48.51	0.9799	118.17	94.73	0.9748
Cyprodinil	213.60	15.60	0.9999	68.28	60.18	0.9790	97.04	90.62	0.9660
Difenoconazole	5.17	0.10	0.9985	17.40	3.97	0.9991	13.24	1.32	0.9958
Fenhexamid	5.05	-0.65	0.9992	6.19	22.7	0.9923	3.00	-0.16	0.9992
Kresoxim methyl	2.32	0.05	0.9999	3.18	0.23	0.9999	1.37	0.22	0.9958
Metsulfuron methyl	2.60	2.29	0.9981	1.34	2.32	0.9988	1.08	2.95	0.9721
Propiconazole	33.96	0.91	1.0000	23.76	3.84	0.9983	22.22	5.04	0.9979
Pyraclostrobin	22.80	-0.14	1.0000	16.61	2.55	0.9993	12.49	5.41	0.9905
Pyrimethanil	58.54	45.89	0.9907	31.86	49.00	0.9947	36.10	51.75	0.9943
Pyriproxyfen	40.34	-20.23	0.9410	24.84	-19.68	0.9702	36.70	-34.50	0.9570
Tebuconazole	38.83	-0.04	1.0000	22.70	3.57	0.9987	23.19	6.18	0.9965
Trifloxystrobin	46.46	4.43	0.9998	53.91	17.42	0.9970	32.00	18.97	0.9848

Calibration performances assessed via matrix-based pesticides standards

In QuEChERS treated lettuce analyzed on the QqQ instrument the highest sensitivity was achieved for buprofezin (118×10^6 arb. units per $\mu\text{g mL}^{-1}$); the sensitivity for the following cyprodinil was about 1.2 times lower (Table 1). A mid-range sensitivity ($9.8 - 36 \times 10^6$ arb. units per $\mu\text{g mL}^{-1}$) was achieved for 9 of 15 analytes, while the instrument had lower sensitivity ($0.5 - 3.0 \times 10^6$ arb. units per $\mu\text{g mL}^{-1}$) for 4 of 15 analytes (Table 1). The lowest sensitivity of 0.58×10^6 arb. units per $\mu\text{g mL}^{-1}$ was achieved for boscalid.

The highest sensitivity in lettuce extracts analyzed on the linear ion trap instrument of 2.34×10^6 arb. units per $\mu\text{g mL}^{-1}$ was achieved for cyprodinil (Table 2) and it was 41 times

lower than the corresponding one on the QqQ instrument. Almost equal sensitivity was achieved for azoxystrobin, buprofezin, pyriproxyfen and trifloxystrobin (Table 2). The lowest sensitivity of 0.02×10^6 arb. units per $\mu\text{g mL}^{-1}$ was achieved for metsulfuron methyl and it was 54 times lower than the sensitivity for this pesticide on the QqQ instrument. In general, sensitivity on the QqQ was higher than the sensitivity on the linear trap from 5.27 times (boscalid) to 68 times (buprofezin).

In QuEChERS treated lemon analyzed on the QqQ instrument the highest sensitivity was achieved for buprofezin and cyprodinil ($\approx 69 \times 10^6$ arb. units per $\mu\text{g mL}^{-1}$) (Table 1). A mid-range sensitivity ($11\text{--}54 \times 10^6$ arb. units per $\mu\text{g mL}^{-1}$) was achieved for 9 of 15 analytes. Lower sensitivity ($0.9\text{--}6.2 \times 10^6$ arb. units per $\mu\text{g mL}^{-1}$) was achieved for 4 of 15 analytes (Table 1). The lowest sensitivity of 0.89×10^6 arb. units per $\mu\text{g mL}^{-1}$ was achieved for boscalid.

The highest sensitivity in lemon extracts analyzed on the linear ion trap instrument of 1.13×10^6 arb. units per $\mu\text{g mL}^{-1}$ was achieved for cyprodinil (Table 2) and it was 60 times lower than the corresponding one on the QqQ instrument. On the linear trap, almost equal sensitivity was achieved for azoxystrobin, pyriproxyfen and trifloxystrobin, while on the QqQ sensitivity for these pesticides decreased as follows trifloxystrobin > azoxystrobin > pyriproxyfen. The lowest sensitivity of 0.01×10^6 arb. units per $\mu\text{g mL}^{-1}$ was achieved for metsulfuron methyl and it was 134 times lower than the sensitivity for this pesticide on the QqQ instrument. In general, the sensitivity on the QqQ was higher than the sensitivity on the linear trap from 15 times (boscalid) to 138 times (pyraclostrobin).

Table 2 Linear regression parameters ($y = ax + b$, conc. range $0.01\text{--}15.00 \mu\text{g mL}^{-1}$) for the analysis of solvent-based, lemon-based and lettuce-based pesticides standards on a linear ion trap instrument

	a ($\times 10^6$)	b ($\times 10^6$)	R ²	a ($\times 10^6$)	b ($\times 10^6$)	R ²	a ($\times 10^6$)	b ($\times 10^6$)	R ²
	Solvent			Lemon			Lettuce		
Acetamiprid	0.71	0.52	0.9816	0.20	0.17	0.9767	0.43	0.36	0.9698
Azoxystrobin	2.34	2.06	0.9803	0.62	0.60	0.9647	1.19	1.46	0.9528
Boscalid	0.25	0.65	0.9713	0.06	0.39	0.9997	0.11	0.61	0.9945
Buprofezin	3.08	2.27	0.9838	0.81	0.43	0.9876	1.73	1.36	0.9739
Cyprodinil	4.21	2.86	0.9909	1.13	0.98	0.9736	2.34	2.70	0.9748
Difenoconazole	1.13	-0.09	0.9986	0.30	0.04	0.9990	0.57	0.004	0.9945
Fenhexamid	0.55	0.10	0.9986	0.14	0.03	0.9991	0.27	0.10	0.9930
Kresoxim methyl	0.19	0.02	0.9979	0.05	0.01	0.9974	0.09	0.01	0.9944
Metsulfuron methyl	0.06	0.18	0.9638	0.01	0.09	0.9859	0.02	0.15	0.9726
Propiconazole	0.79	0.13	0.9991	0.21	0.07	0.9957	0.40	0.10	0.9965
Pyraclostrobin	0.63	0.36	0.9893	0.12	0.07	0.9930	0.28	0.14	0.9883
Pyrimethanil	1.82	1.14	0.9935	0.37	0.14	0.9961	0.90	0.54	0.9924
Pyriproxyfen	2.75	1.02	0.9944	0.68	0.29	0.9925	1.33	0.77	0.9882
Tebuconazole	0.75	0.05	0.9998	0.20	0.03	0.9991	0.36	0.09	0.9962
Trifloxystrobin	2.70	0.63	0.9980	0.70	0.29	0.9946	1.33	0.68	0.9908

On the QqQ instruments, in all tested matrices correlation coefficient (R^2) was close to 1; it ranged from 0.9410 to 1.000 (solvent-based standards), from 0.9702 to 0.9999 (lemon-based standards) and from 0.9570 to 0.9992 (lettuce-based standards). On the linear ion trap instrument the conclusions were similar. Correlation coefficient ranged from 0.9638 to 0.9998

(solvent-based standards), from 0.9647 to 0.9991 (lemon-based standards) and from 0.9528 to 0.9965 (lettuce-based standards). Deviation from the back-calculated concentration was in case of each linear regression for each analyte on both instruments less than 20%. Evaluation results for both calibration parameters suggest that, under the tested conditions, reliable analytes quantification could be performed.

CONCLUSION

Calibration performances of analysis on the linear ion trap and triple quadrupole mass analyzers was compared. A batch of solvent-based and QuEChERS treated lettuce- and lemon-based standards of 15 pesticides from 9 chemical classes was analyzed, with both analyzers operating in full scan MS¹ mode. Sensitivity, correlation coefficients and deviation from the back-calculated concentration were estimated from the calculated linear regression. A much higher sensitivity was observed in analysis on the triple quadrupole; the sensitivity for boscalid in solvent was about 4 times higher and for buprofezin 71 times higher on the triple quadrupole than on the linear trap. The same trend in sensitivity could be observed in fruit and vegetables extracts. Shifting from linear trap to triple quadrupole, sensitivity enhancement ranged from about 5 to even 138 times. The other two calibration parameters on both instruments were acceptable, demonstrating the potential for reliable analytes quantification in actual samples analysis.

In conclusion, triple quadrupole instrument has provided much higher sensitivity in multiresidue pesticides analysis. Such instruments should be implemented when highly sensitive analysis is required.

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