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アプリケーションノート

Application of ACQUITY TQD for the Analysis of Nitrofuran Veterinary Drug Residues in Shrimp

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Abstract

This application note describes the transfer of an existing UPLC method to the tandem quadrupole mass

spectrometer, Waters ACQUITY TQD. Standard solutions spiked into blank matrix extract were used to assess chromatography as there will not be changes to the extraction of nitrofuran metabolites from edible tissues.

Benefits

- Increased speed, while further reducing solvent usage and therefore the costs of solvents and solvent disposal
- Quantifiable detection at twenty times below the EU minimum required performance limit (MPRL) guideline for the four nitrofuran metabolites

Introduction

This application note describes the transfer of an existing UPLC method[1] to the tandem quadrupole mass spectrometer, Waters ACQUITY TQD (Figure 1). Standard solutions spiked into blank matrix extract were used to assess chromatography as there will not be changes to the extraction of nitrofuran metabolites from edible tissues.

The four drugs furazolidone, furaltadone, nitrofurazone, and nitrofurantoin are veterinary drugs that belong to the family of nitrofuran antibiotics and are banned in meat destined for human consumption[2]. These nitrofurans are readily metabolized in animals to undetectable levels, metabolizing to 3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), semicarbazide (SC) and 1-aminohydantoin (AHD), respectively. The structures of the nitrofuran antibiotics and their free metabolites are shown in figure 2. The metabolites are more persistent in edible tissues and can be used to detect use of the parent drug. Proportions of these metabolites exist as protein adducts and the established method of analysis contains acid hydrolysis and derivatization steps prior to extraction[3].

The European Union (EU) has banned the use of these substances, documenting them in Council Regulation 2377/90, annex IV.

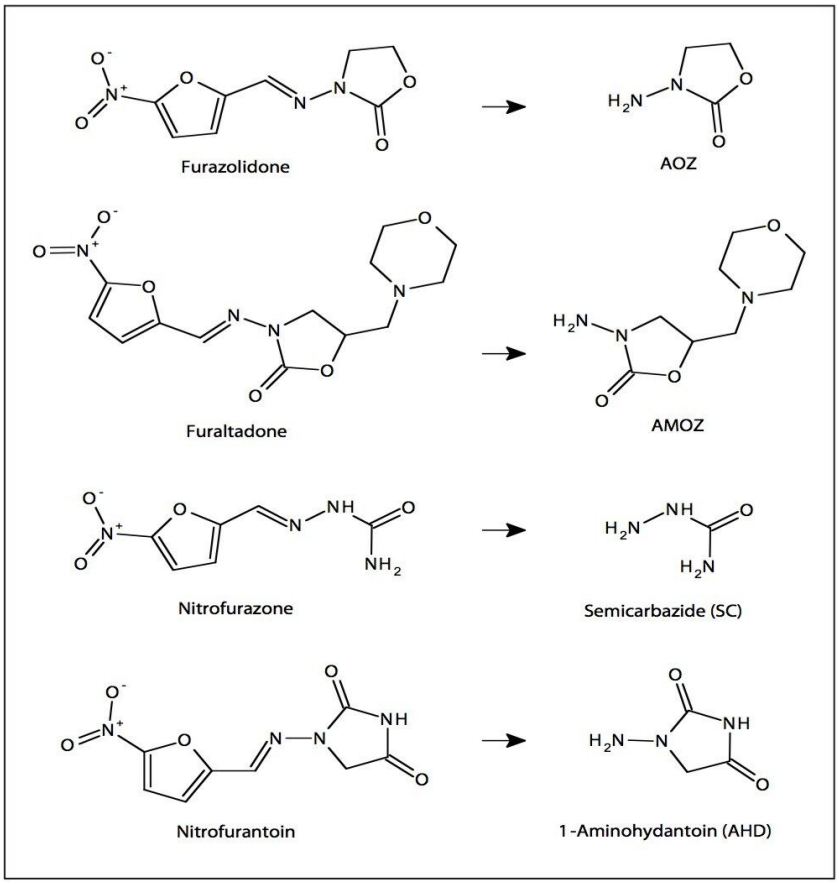
A Minimum Required Performance Limit (MRPL) of 1µg/kg has been set to which laboratories should be able to detect and confirm these compounds. Concerns over nitrofurans are related to potential carcinogenicity (causing cancer) and genotoxicity (causing damage to genetic material in cells) of the drugs and their metabolites as well as the ability to cause allergic reactions[4].

The introduction of the ACQUITY TQD (Figure 1) allows scientists to perform nitrofuran metabolite analysis while harnessing all the benefits that this new instrument brings to the laboratory.

The IntelliStart technology in this instrument is designed to reduce the burden of complicated operation, time-intensive troubleshooting, and upkeep. Its small footprint will give any laboratory an advantage as this powerful tool removes the need for larger instrumentation.

This note describes an extended nitrofuran method, for four residues and their associated internal standards, which exceeds the requirements for analysis of these regulated compounds.

Figure 1. ACQUITY TQD featuring the TQ detector.



Experimental

Solvent solutions were produced in 1:4 methanol: water (v/v) containing the four derivatized metabolites. These solutions were further diluted in extracted blank matrix to form a calibration curve over the range 0.05 to 5 ng/mL. Deuterated internal standards, 2-NP-AOZ-D_4, 2-NP-AMOZ-D_5, 2-NP-SC-$^{13}C_{11}{}^{5}N_2$, and 2-ANP-HD$^{13}C_3$ were also added. The chromatography was assessed for each compound at each level. All concentrations reported in this application note are comparable to the nitrofuran antibiotics (pre-extraction) as correction factors, were applied to the volumes of derivatized metabolites. These correction factors also took into account the 2x concentration step during sample extraction. This means that the 0.1 ng/mL solution injected is directly comparable to shrimp containing 0.05 µg/kg.

UPLC Conditions

LC System:	ACQUITY UPLC System

Column:	ACQUITY UPLC BEH C_{18} Column 2.1 x 100 mm, 1.7 µm
Column temp.:	40 ˚C
Flow rate:	450 µL/min
Mobile phase A:	1:4 methanol:water (v/v) + 0.5 mM ammonium acetate
Mobile phase B:	9:1 methanol:water (v/v) + 0.5 mM ammonium acetate

MS Conditions

MS System:	ACQUITY TQ detector
Ionization mode:	ESI positive
Capillary voltage:	1 KV
Cone voltage:	See table 1
Desolvation gas:	Nitrogen, 1000 L/Hr, 450 ˚C
Cone gas:	Nitrogen, 5 L/Hr
Source temp.:	120 ˚C
Acquisition:	Multiple Reaction Monitoring (MRM)
Collision gas:	Argon at 3.5 x 10^{-3} mBar

All compounds were optimized and two Multiple Reaction Monitoring (MRM) transitions were obtained for each derivatized metabolite and one for each internal standard. These two MRM transitions were monitored for each derivatized metabolite in accordance with European Union guidelines5. The primary transition is used for quantification and the secondary transition is used for confirmation purposes. These

MRM transitions are listed in table 1, along with their respective cone voltages and collision energies.

Acquisition and Processing Methods

Waters MassLynx Software v4.1 was used for data acquisition and the TargetLynx application manager was used for data processing.

Metabolite	MRM Transition	Cone Voltage (V)	Collision energy (eV)
2-NP-AOZ	236.0>134.0	35	14
	236.0>104.0		20
2-NP-AMOZ	335.1>291.2	31	12
	335.1>100.0		26
2-NP-SCA	209.0>166.1	25	8
	209.0>192.1		10
2-NP-AHD	249.0>133.9	32	10
	249.0>104.0		20
2-NP-AOZ-D_4	240.0>134.0	35	13
2-NP-AMOZ-D_5	340.1>296.2	30	12
2-NP-SCA-$^{13}C_1$ $^{15}N_2$	212.0>168.0	30	10
2-NP-AHD-$^{13}C_3$	252.0>134.0	30	11

Table 1. MRM transitions for the derivatized metabolites and their internal standards.

Results and Discussion

All nitrofurans were separated successfully. Figure 3 shows the total ion chromatogram (TIC) for all derivatized metabolites. Only one time window was required for the eight compounds being analyzed. A dwell time of 0.025 seconds was used giving approximately 14 data points across each peak.

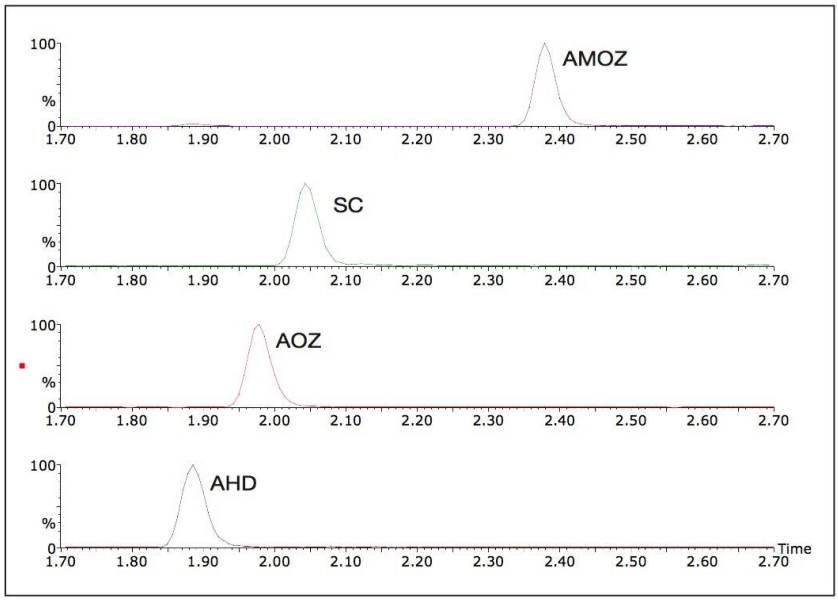
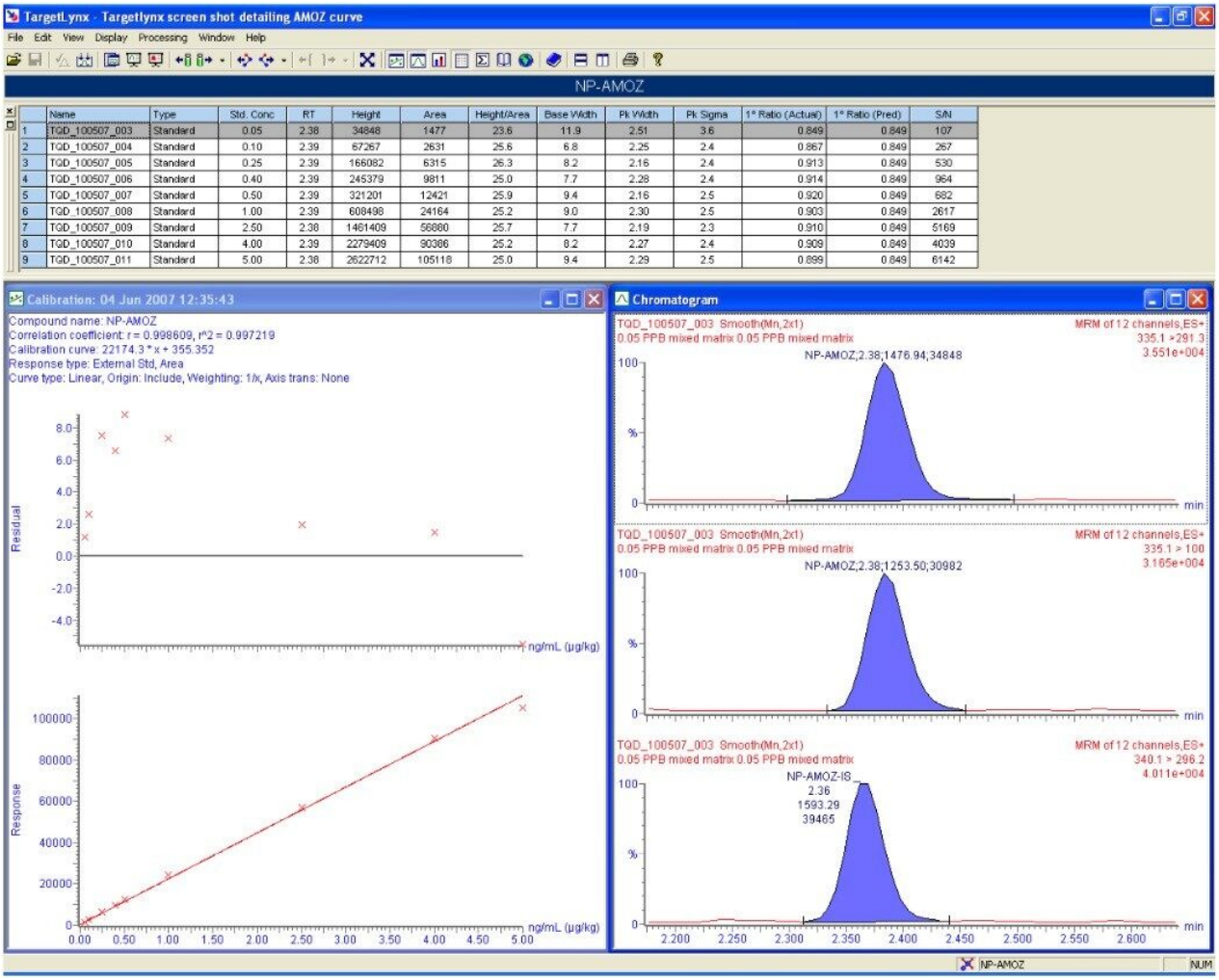


Figure 3. TIC showing the four nitrofuran metabolites in shrimp matrix at 1 ng/mL (equivalent to 2µg/kg in shrimp).

The limit of detection achieved for all components of the derivatized metabolite mixture was less than 0.05 ng/mL (equivalent to 0.025 µg/kg in shrimp). This is forty times below the EU minimum required performance limit (MPRL) guideline. The 0.05 ng/mL matrix-matched standard for 2-NP-AMOZ is illustrated in Figure 4.



	Name	Type	Std. Conc	RT	Height	Area	Height/Area	Base Width	Pk Width	Pk Sigma	1° Ratio (Actual)	1° Ratio (Pred)	S/N
1	TQD_100507_003	Standard	0.05	2.38	34848	1477	23.6	11.9	2.51	3.6	0.849	0.849	107
2	TQD_100507_004	Standard	0.10	2.39	67267	2631	25.6	6.8	2.25	2.4	0.867	0.849	267
3	TQD_100507_005	Standard	0.25	2.39	166082	6315	26.3	8.2	2.16	2.4	0.913	0.849	530
4	TQD_100507_006	Standard	0.40	2.39	245379	9811	25.0	7.7	2.28	2.4	0.914	0.849	964
5	TQD_100507_007	Standard	0.50	2.39	321201	12421	25.9	9.4	2.16	2.5	0.920	0.849	682
6	TQD_100507_008	Standard	1.00	2.39	608498	24164	25.2	9.0	2.30	2.5	0.903	0.849	2617
7	TQD_100507_009	Standard	2.50	2.38	1461409	56880	25.7	7.7	2.19	2.3	0.910	0.849	5169
8	TQD_100507_010	Standard	4.00	2.39	2279409	90386	25.2	8.2	2.27	2.4	0.909	0.849	4039
9	TQD_100507_011	Standard	5.00	2.38	2622712	105118	25.0	9.4	2.29	2.5	0.899	0.849	6142

Figure 4. TargetLynx screen shot showing response for 2-NP-AMOZ at 0.05 ng/mL (0.025 µg/kg in shrimp).

A calibration curve was spiked over the range of 0.05-5 ng/mL which gave a good linear response (r2=0.9972). Good peak shape was achieved for both analyte and internal standard. The lowest quantifiable level achieved by the TQD in this analysis was 0.05 ng/mL. This level gave an acceptable S:N of 5:1 or greater for all four nitrofuran metabolites in shrimp with 50µL injection volume. This level is twenty times lower than the MPRL set by the EU.

A run of 50 samples in matrix at a concentration of 5 ng/mL was injected. This was used to assess robustness of ACQUITY TQD response over a four hour run. Injections of 10 µL were performed as a limited amount of sample was present. Figure 5 shows a plot of injection number vs. response ratio (analyte peak area/internal standard peak area). There is no noticeable change in response for any compound over the course of the run.

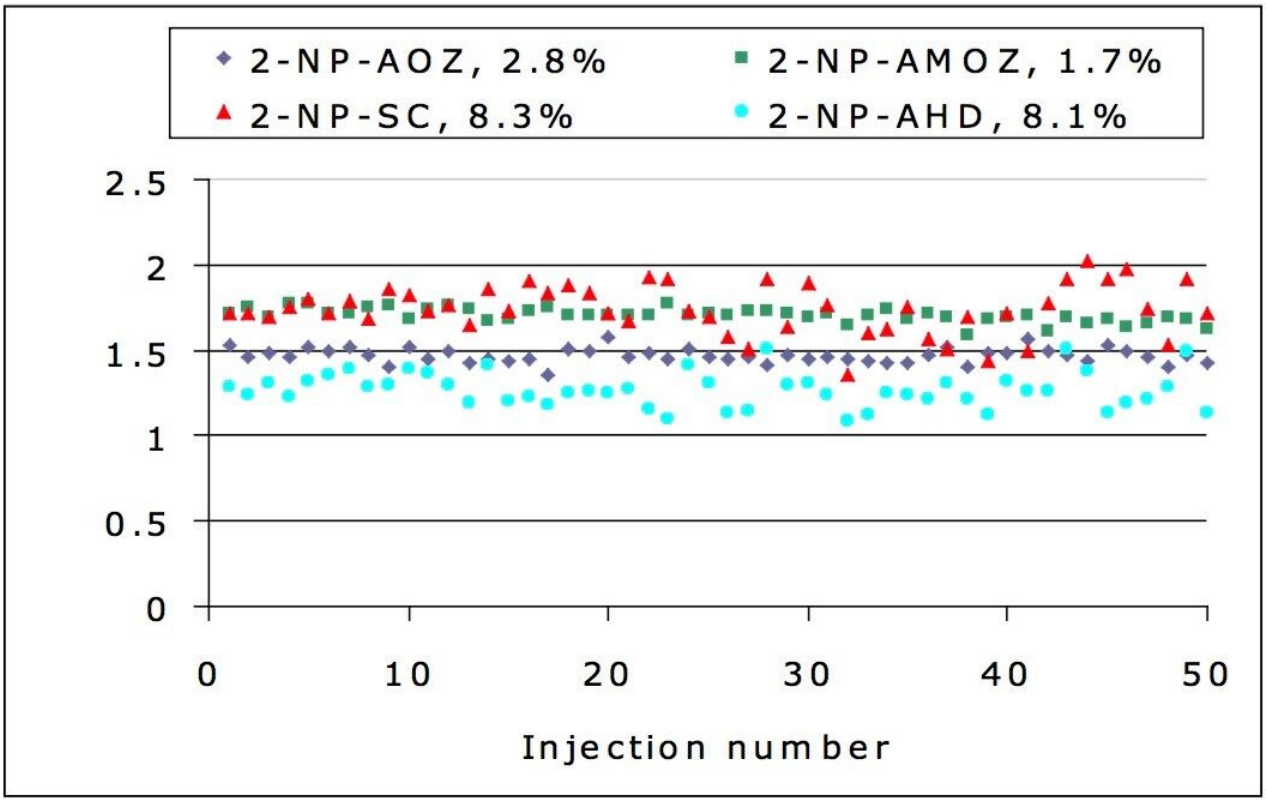


Figure 5. Plot showing peak area over 50 injections in shrimp. Extracts were injected at a concentration of 5 ng/mL. The percent relative standard deviation (%RSD) is shown in the graph header.

Figure 6 illustrates the robustness of the ion ratios over the same 50 injections. This plot shows that there is no significant change in ion ratio for any compound over the course of the run. A stable ion ratio is fundamental when confirming the identity of compounds.

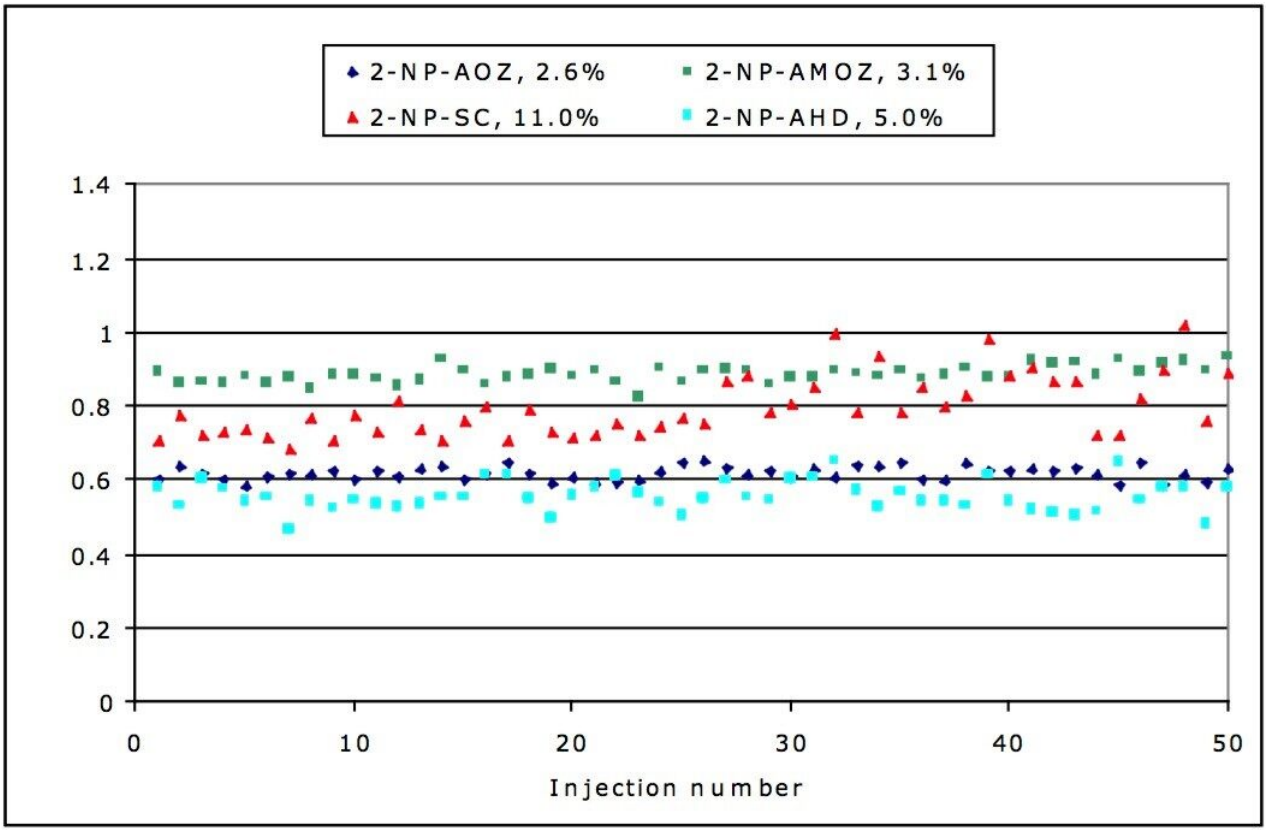


Figure 6. Plot showing peak area over 50 injections in shrimp. Extracts were injected at a concentration of 5 ng/mL. All injections lie within the ±20% boundary required for confirmation with the average difference shown in the header of the graph.

Conclusion

The established UPLC method for the determination of nitrofuran veterinary drug residues, which are hazardous to human health, has been successfully transferred to ACQUITY TQD.

The ACQUITY TQD provides sensitivity that allows quantifiable detection at twenty times below the EU minimum required performance limit (MPRL) guideline for the four nitrofuran metabolites.

Robustness of the ACQUITY TQD was proven with a 50 injection sequence that did not show a major change (<20%) in response ratio with relative standard deviations being 8.3% or less for all compounds.

Confirmation was achieved using a secondary MRM transition over a 50 injection sequence, where the variance was 11% or less in all cases showing it to be robust.

This multiple nitrofuran method obsoletes the use of several single nitrofuran methods where repeat analysis is required.

The method allows the determination of multiple contaminants per sample which enables a complete picture to be obtained of exposure to these compounds from the human diet.

The benefits of UPLC for a revenue conscious laboratory are shown with increased speed, while further reducing solvent usage and therefore the costs of solvents and solvent disposal.

Acknowledgements

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References

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