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Scout-Multiple Reaction Monitoring: a Liquid Chromatography tandem Mass Spectrometry approach for multi-residue pesticide analysis without time scheduling

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Abstract

In this work, an innovative method is described for multi-residue pesticide analysis by liquid chromatography coupled to targeted mass spectrometry. Called "Scout-MRM, this new acquisition mode lies in the monitoring of complex transitions groups triggered by either endogenous or spiked Scout compounds, hence fully releasing the monitoring of target molecules from time scheduling. As a proof of concept, a Scout-MRM method was built where 5 transitions groups tracking a total of 191 pesticides were successively triggered under the control of 5 spiked-in deuterated pesticides. As expected from its retention time independency, Scout-MRM demonstrates strong detection robustness towards modifications of gradient parameters, as well as easy method transfer between distinct analytical platforms with nearly 100 % recovery after a single run. Finally, Scout-MRM was used for the multi-residue screening and quantification of pesticides in real surface water samples, by applying an external calibration procedure and comparing it with classical scheduled reaction monitoring methods.

Keywords: MRM : Multiple Reaction Monitoring ; sMRM Scheduled Multiple Reaction Monitoring, scout-MRM, retention time independency, multiplexing capability

38

1. Introduction

39

40 In the framework of European environmental legislation, various protocols have been
41 developed for assessing the environmental risk of chemicals. For example, pesticides and
42 biocides undergo rigorous assessment in compliance with Directives 91/414/EEC and
43 98/8/EC. There are more than 1,000 pesticides currently available for use while the number
44 of regulations aimed at protecting human health is increasing. Pesticide testing has become
45 a prerequisite. With hundreds or even thousands of samples to be screened within tight
46 deadlines and according to strict legislation, an efficient solution is required to detect and
47 identify pesticide compounds across multiple classes. Thus, environmental analysis
48 laboratories develop analytical multiplexed methods, known as "multi-residue methods",
49 that allow the simultaneous identification and quantification of many organic
50 contaminants. Due to its sensitivity and specificity, liquid chromatography coupled to mass
51 spectrometry is an appropriate method for detecting organic contaminants and has been
52 widely used for the analysis of pesticide residues [1,2]. Among the different mass
53 spectrometry techniques available, targeted approaches with triple quadrupole analysers
54 in selected reaction monitoring (MRM or MRM) mode are widely used. For each pesticide,
55 2 or 3 MRM transitions are monitored to reduce the risk of false positives [3] (a transition
56 corresponds to a precursor/fragment ion couple): one transition for quantification and two
57 transitions for qualification. Thus, for example, the analysis of 300 compounds requires
58 monitoring from 600 [4] to 900 transitions. As the number of transitions in a method
59 increases, it is necessary to either reduce the dwell times or to increase the cycle time for
60 each scan. It should be remembered that the longer the mass spectrometer spends
61 following a transition, the higher the signal-to-noise ratio. Slow scanning of the triple
62 quadrupole limits the development of highly multiplexed methods. Indeed, to guarantee
63 enough data points per peak (e.g., 15), it is not possible to increase the overall cycle time
64 which is the sum of each dwell time. Thus, instead of programming the recording of a
65 transition throughout the duration of the chromatographic separation, it is monitored only
66 within a time-limited window. This time segmentation can be relatively large and allows
67 monitoring the transitions of several different molecules. However, the strategy most used
68 is to record the specific transitions for a compound only within a window around its
69 retention time. The manufacturers of mass spectrometers have developed their own
70 processes, bearing different names: "Scheduled Multiple Reaction Monitoring" for Sciex
71 (sMRM); [1] "Timed MRM" for Thermo [5]; "Dynamic MRM" for Agilent [6,7]. Whatever the
72 time segment methods used dynamically or not, a retention time must be documented for
73 each pesticide in the method. The major drawback of this procedure is that any
74 unattended shift of retention time may partially or, even worse, totally exclude a
75 compound of interest from its monitoring window, resulting in either a truncated or non-
76 detected peak. [8]. Truncated chromatographic peaks make integration more difficult and
77 the data obtained are of low-quality. Thus, during large analytical campaigns or the transfer
78 of analytical method, the smallest variations in analytical conditions, such as (i) the use of
79 different LC configurations with different solvent delays (e.g., of chromatographic chains),
80 and (ii) differences in applied flow rates, imply readjustments of retention times.

81 As a consequence, a new mode of multiplexed targeted analysis, called "Scout-MRM", in
82 the context of multi pesticide residue monitoring has been reported. This method, which

83 does not require retention time setting, solves the limitation affecting large multi pesticide
84 assays regarding chromatography reproducibility and offers plug and play implementation
85 between laboratories.

86

87 **2. Materials and Methods**

88 2.1 Chemicals and reagents

89 All the reagents were of analytical grade. Water, methanol and acetonitrile (LC-Ms grade)
90 were purchased from Fisher Scientific (Strasbourg, France). Acetone, formic acid (FA) and
91 formate ammonium (LC-MS grade) was obtained from Sigma-Aldrich (St Quentin-Fallavier,
92 France).

93 Over 191 pesticides of different classes were used to develop the Scout-MRM method. All
94 the substances were certified for internal calibration or as external calibration reference
95 materials and were provided by Sciex (Toronto, Canada), in its iDQuant™ Standards Kit for
96 Pesticide Analysis. As scout standard, five deuterated pesticides ([D₄]-Cyromazine, [D₆]-
97 Fenitrothion, [D₆]-Linuron [D₅]-Simazine and [D₉]-Tebuconazole) were purchased from Dr.
98 Ehrenstorfer (Augsburg, Germany) and Sigma Aldrich (St Quentin-Fallavier, France). These
99 pesticides were then formulated by Anaquant (Lyon-France) and coated on READYBEADS™.
100 This technology is used to obtain the fast and reproducible preparation of standard
101 solutions and, in particular, it avoids storage, weighing and stability issues.

102 To perform the comparison between Scout-MRM and sMRM reference method, a mixture
103 of 17 pesticides in acetone was used to establish the calibration curves (acetochlore,
104 atrazine, azoxystrobin, chlorpyrifos methyl, chlortoluron, 3,4-dichloroaniline, 3-(3,4-
105 dichlorophenyl)-1-methylurea (DCPMU), diflufenicanil, diuron, isoproturon, linuron,
106 metolachlore, norflurazon, norflurazon desmethyl, procymidone, simazine, tebuconazole).
107 Depending on the pesticide, concentrations of stock solutions ranged from 200 to 8000
108 ng/mL. Nine calibration standard mixtures were prepared following initial 1/100 dilution in
109 water containing 10% acetonitrile and serial dilutions in water (See supplementary table 3)
110 External calibration was used. The Low Limit of Quantitation (LLOQ) ranged from 0.002 to
111 0.8 ng/mL. A coated READYBEADS™ was placed in each standard vial during the dilution
112 process and the final volume of the solvent was 1 mL.

113

114 2.2 Sample extraction

115 Ten water samples (named real sample) were collected from rivers (3 up or downstream
116 massif Central site, Beaujolais Veneyard downstream), stored at 4°C and filtered within 24
117 hours with 0.7 µm GF/F glass fibre membranes (Millipore). Pesticide concentrations in
118 water samples were determined after solid phase extraction (SPE) followed by liquid
119 chromatography coupled with tandem mass spectrometry (LC-MS/MS). The SPE extraction
120 was carried out with 6 mL/200 mg Oasis HLB cartridges (Waters) conditioned with 3 mL of
121 methanol, 3 mL of acetonitrile and 3 mL of ultrapure water. 250 mL of filtered water was
122 extracted at a flowrate of 10mL/min. Elution was performed with 6 mL of acetonitrile at a
123 flowrate of 1 mL/min. Organic extract was gently evaporated under nitrogen and finally
124 diluted in 250 µL of water/acetonitrile (80/20, v/v) containing deuterated diuron D₆, used as

125 the injection internal standard (IS). The concentration factor was 1000. Each sample was
126 split into two vials. The first vial was used for pesticide determination with the sMRM
127 reference method and the second was diluted 10-fold with ultra-pure water and a one
128 scouts standard READYBEADS™ was added in each vial before analysis with Scout-MRM
129 method.

130

131 2.3 LC-MS/MS analysis

132 2.3.1 LC-MS/MS in University of Lyon

133 The Scout-MRM method was performed on a 1290 HPLC system (Agilent Technologies)
134 coupled with a QTRAP 6500® from SCIEX (Concord, Canada) equipped with a Turbo V™
135 source (SCIEX). Instrument control, data acquisition and processing were performed using
136 modified Analyst 1.6.2 software®. Implementing the "Scout-MRM" tool in the Analyst®
137 1.6.2 software required computer developments by David Cox at the Sciex R&D laboratory.
138 Chromatographic separations were performed on a 3.5µm BEH C₁₈, 100 x2.1mm (Waters,
139 Milford, MA, USA), at ambient temperature. The flow rate was to set at 300 µl/min and an
140 elution gradient was used. The mobile phase consisted of (A) water containing 0.05 %
141 formic acid and 2 mM ammonium formate and methanol containing 0.05% formic acid,
142 2mM formate ammonium. The gradient started with 10% of B. This was held for the first 2
143 min. Then, the amount of solvent B was increased to 70% within 15 min. Afterwards,
144 solvent B was increased again to 100% within 0.1 min. This was held for 3 min. Afterwards
145 solvent B was decreased to 100% within 0.1 min and held for 5 min. before the next
146 injection. The sample injection volume was 1 µL and the run time of the assay was 25 min.
147 MS analysis was carried out in positive ionization mode using an ion spray voltage of 5500
148 V. The curtain gas (nitrogen) and the nebulizer (nitrogen) flows were set at 50 and 70 psi
149 respectively. The Turbo V™ ion source was set at 550 °C with the auxiliary gas flow
150 (nitrogen) set at 60 psi.

151

152 2.3.2. LC-MS/MS in Toronto (Sciex)

153 The Scout-MRM method was performed on an HPLC Model- LC 20 AT Prominence,
154 (Shimadzu, Japan) coupled with a QTRAP 5500® from SCIEX (Concord, Canada) equipped
155 with a Turbo V™ source (SCIEX). Instrument control, data acquisition and processing were
156 performed using modified Analyst 1.6.2 software®. Implementing the "Scout-MRM" tool in
157 the Analyst® 1.6.2 software required computer developments by David Cox at the Sciex
158 R&D laboratory. Chromatographic separations were performed on 3 µm X Bridge C₁₈, 100 x
159 2.1mm (Waters, Milford, MA, USA), operated at 50°C. The flow rate was to set at 600
160 µl/min. An elution gradient was used. The mobile phase consisted of (A) water containing
161 0.05 % formic acid and 2 mM ammonium formate and methanol containing 0.05% formic
162 acid, 2mM formate ammonium. The gradient started with 5% of B. This was held for the
163 first min. Then, the amount of solvent B was increased to 95% within 25 min. This was held
164 for 5 min. Afterwards solvent B was decreased to 100% within 1 min and held for 5 min.
165 before the next injection. The sample injection volume was 10 µL and the run time of the
166 assay was 36 min. MS analysis was carried out in positive ionization mode using an ion
167 spray voltage of 5500 V. The curtain gas (nitrogen) and the nebulizer (nitrogen) flows were

168 set at 25 and 30 psi, respectively. The Turbo V™ ion source was set at 550°C with the
169 auxiliary gas flow (nitrogen) set at 70 psi.

170

171 2.3.3. LC-MS/MS sMRM method in INRAE (ex IRSTEA)

172 Liquid chromatography was performed on a Shimadzu series Nexera X2 UHPLC system
173 (Marne-la-Vallée, France). Pesticides were separated on an Acquity HSS T3 1.8 µm, 2.1 ×
174 100 mm Waters column (Milford, MA, USA). The column temperature was set at 30°C +/-
175 0.5°C. The injection volume was 20 µL. The chromatographic separation of analytes was
176 carried out with acetonitrile and ultrapure water, both with formic acid (0.1%) in an
177 analytical gradient (from 0 to 90% acetonitrile) of 15 min at 0.6 mL/min. The UHPLC system
178 was coupled to a triple-quadrupole mass spectrometer (MS/MS) API 4000 from Sciex
179 (Concord, Canada). The electrospray ion source (Turbo-Ionspray from AB Sciex) was set to
180 positive mode at 600°C. The ion spray voltage was +5500 V. Nitrogen was used as curtain
181 and collision gas, while air was used as nebulizer and drying gas. The pesticides analysed
182 were identified and confirmed by their specific retention times, two characteristic
183 precursor-product ion transitions (quantifier and qualifier), and specific ratios of the
184 intensities of the product ions, in compliance with European Commission Decision
185 2002/657/EC (European_Commission, 2002). Quantification was performed with
186 deuterated diuron d6. Within-laboratory validation was performed to evaluate the
187 analytical performances of the SPE-UHPLC-MS/MS protocol according to the following
188 criteria: linearity, limit of quantification (LOQ), precision and trueness, inspired from
189 reference standards (AFNOR, 2009; SANCO/12495/2011, 2012). Quantification limits were
190 between 5 and 50 ng/L depending on the pesticide and recoveries ranged from 67 % to 107
191 %. Analytical uncertainties were determined using the within-laboratory reproducibility
192 relative standard deviation. An expanded coverage factor of k=2 was used to calculate the
193 expanded uncertainty with a confidence interval of 95% (AFNOR, 2003;
194 International_Organization_for_Standardization, 2012). Uncertainties were evaluated
195 between 30% and 60% on the whole range of the method's application.

196

197 **3. Results and discussion**

198 3.1 Principle of Scout-MRM

199 Scout MRM is a new targeted Multiple Reaction Monitoring mode enabling the
200 deployment of large multiplexed assay. The major breakthrough relies on the fact that it
201 releases the acquisition from scheduling retention windows. As already described in the
202 context of relative peptide quantification [9,10], scout molecules are compounds
203 dispatched along the chromatogram, which individually controls the triggering of a
204 transition group. Hence, each transition group is framed by two scouts. Once the signal
205 intensity of a given scout exceeds a user-defined threshold, it triggers the monitoring of a
206 transition group while the previous one is stopped and so on (Figure 1). Two major
207 advantages arise from this innovative process: i) the easiness of building a multiplexed
208 assay by simply positioning the target molecules between two scouts; ii) the release of the
209 acquisition from the retention time dimension which makes any unattended retention time

210 shift with no consequence An advantage of not using time segmentation is the possibility
211 of using the real multiplexing capabilities of MRM methods to their maximum.

212 To illustrate the benefits of the approach, a proof of concept has been carried where a
213 scout-MRM assay was developed targeting 191 pesticides. Five labelled pesticides were
214 used as spiked-in scout compounds and judiciously selected in order to dispatch the 191
215 pesticides targets into groups of approximately the same number of transitions. In their
216 order of use, these scouts were: Cyromazine-D4; Simazine-D5; Linuron-D6; Féntrothion-
217 D6; and Tebuconazole-D9. At the beginning of the run, the mass spectrometer was
218 scheduled to follow the most intense transition of Cyromazine-D4 (Scout 1). When the two
219 most intense transitions of Cyromazine-D4 were detected above a user-predefined
220 threshold, then the monitoring of all the transitions of group A was triggered (Group A, list
221 of pesticides in supplementary Table 1). Group A is thus defined as the set of transitions
222 tracking 42 pesticides eluted between Cyromazine-D4 (Scout 1) and Simazine-D5 (Scout 2).
223 Once the transition signals of Simazine-D5 reach triggering defined threshold, group A
224 monitoring is stopped while the one of Group B transitions is initiated with Linuron-D6 as
225 third scout, and so on. This triggering process continued throughout the chromatographic
226 elution of the pesticides with Scout 4 (Féntrothion-D6) and 5 (Tebuconazole-D9). Since
227 "Scout-MRM" acquisition is a succession of MRM experiments triggered only by signals of
228 the scout whose thresholds are user-defined, pesticides transitions can be arranged in a
229 completely random manner within a group.

230 The only constraint to consider during a Scout-MRM based assay is the upper limit of
231 concurrent transitions positioned within the same group in such a way that the duty cycle
232 compulsorily ensures the required minimum of data points per peak. If additional
233 pesticides were to be included in the Scout-MRM test, then it would be sufficient simply to
234 add new deuterated scout pesticides.

235

236 3.2 Performance of Scout-MRM under different chromatographic conditions

237 As described above, Scout-MRM approach developed here, differs from conventional
238 methods in which a scheduled time windows or retention time are fixed. In conventional
239 methods this implies not only detailed knowledge requirements of the expected retention
240 time for all the pesticides monitored but also stable and reproducible liquid
241 chromatography conditions. This is no longer an issue with this approach, as shown in
242 Figure 2. In this case, no retention time is programmed. This presents an advantage
243 because we no longer have to take into account any changes in retention time for pesticide
244 detection. The isocratic step of the LC method was deliberately extended by 5 minutes or
245 the gradient slope was decreased (Figure 2 A and B). In this case as shown, all the
246 pesticides were detected (100%). The detection repeatability of Scout-MRM was however
247 compared to Scheduled-MRM on the same instrument by calculating the coefficient of
248 variation (CV) of triplicate injections. Identical ranges of CVs were obtained for both modes
249 of acquisition (supplementary figure 1).

250 To assess Scout-MRM robustness, the portability of the assays between two distinct sites
251 and experimental set-up was evaluated. Indeed, the comparison of the same analysis (set
252 of samples) at two different laboratories has been performed. For this purpose, one Excel
253 file was sent containing the MRM transitions and their Scout-MRM group affiliation was

254 sent to Sciex Research Laboratory in Toronto. While the chemistry of the C₁₈ stationary
255 phase was identical, the particle sizes, flow rate, elution gradient and temperature were
256 different. Despite these substantial modifications, 92% of the pesticides were detected as
257 illustrated in figure 2A. The remaining 8% corresponded to group changes probably due to
258 a modification of chromatographic selectivity. Indeed, if the order of elution of the
259 compounds varies to cause a peak inversion between a "Scout" and a compound of
260 interest, the latter will not be followed at the right time and cannot be detected. A second
261 analysis to identify undetected pesticides by positioning them in adjacent groups ensures
262 that they are detected correctly. Peak intensities were different since electrospray
263 ionisation [11] depending on mobile phase composition and / or comparisons were
264 performed on different generations of mass spectrometers (API 6500 QTRAP vs API5500
265 QTRAP).

266 This result unequivocally demonstrate the capacity of Scout-MRM to facilitate the sharing
267 and implementation of ready-to-use-assay following a plug-and-play philosophy.

268

269 3.3 Applications and comparison of Scout-MRM vs MRM

270 Having preliminarily demonstrated the detection repeatability and robustness towards
271 radical change of chromatographic conditions of Scout-MRM mode, Scout-MRM was
272 ultimately compared to conventional MRM and scheduled MRM towards pesticides
273 evaluation in river water samples. The goal was here not to deliver accurate evaluation of
274 surface water contamination but to evaluate whether different instruments and distinct
275 modes of acquisition might provide reliable quantification results. Again, distinct
276 experimental set-up were used during the study, in particular an older generation
277 instrument at the INRAE lab (API 4000 triple quadrupole instrument) on which
278 measurements were carried out routinely in MRM mode. Although only 16 pesticides
279 frequently detected in surface waters were subjected to analysis in the INRAE study (sMRM
280 reference method on an API4000), the method containing all pesticides was applied.

281 In the INRAE study only 9 out of the 17 targeted pesticides were detected and quantified.
282 The Scout-MRM method was used for the acquisition but only the concentrations of the 9
283 pesticides measured by INRAE were measured precisely and reported in table 1. With our
284 more sensitive API 6500 QTRAP, more pesticides were detected but not quantified (more
285 than 65, see Supplementary Table 2). As shown in Table 1, the results are similar except for
286 atrazine, whose detection was about between -28% and -55% lower. This result can be
287 explained by the matrix effect, which was different due to sample dilution. Indeed, as API
288 6500 QTRAP is more sensitive, the water samples must be diluted by 10 before analysis.
289 This was supported by the fact that the comparison between Scout-MRM and sMRM on
290 API 6500 QTRAP gave approximatively the same concentrations for atrazine and all the
291 other pesticides.

292

293 **4. Conclusion**

294

295 The present proof-of-concept study was intended to illustrate how Scout-MRM may
296 advantageously replace conventional time-scheduled acquisition mode for implementing
297 multiresidue pesticide assay. Scout-MRM mode has been originally conceived to fully
298 release a highly multiplexed assay from the absolute retention time of the respective
299 targeted compounds. Thus, 100% recovery were obtained for a 191-plex pesticide panel
300 despite radical modifications of the chromatographic parameters between runs. Even more
301 impressive than expected, 92% recovery of the 191-plex pesticide panel was obtained
302 across a first run analysis after a simple mail transfer of Scout-MRM method to a distinct
303 lab and, again, despite substantial differences in the chromatographic set-up. This supports
304 the idea that Scout-MRM is the fit-for-purpose tool for the dissemination and
305 implementation of ready-to-use methods, especially between different laboratories.
306 Finally, the fact that any unattended retention shift does not translate into a false negative
307 detection strengthens the confidence level of pesticide assays and the resulting results. It
308 should also be noted that routine laboratories running high-throughput pesticide assay are
309 used to introduce few internal standards in each run. This function can be here
310 advantageously fulfilled by the deuterated scout pesticides, hence without any radical
311 change in the procedure. The Scout-MRM provisional software patch is available for
312 academic research on request from SCIEX (please contact yves.leblanc@sciex.com) and will
313 be integrated automatically in the MS controlling software instruments.

314

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316 The authors thank David Cox from SCIEX for developing the Scout-MRM software patch and
317 Matthieu le Dréau from INRAE for pesticide quantification.

318

319

Figure caption

320

321 Figure 1 : Principe of scout-MRM acquisition mode

322 Figure 2: Example of 3 chromatograms in different LC conditions. Extraction of 5 pesticides of Scout-
323 MRM group D (Flufenacet, Fenbuconazole, Picoxystrobin, Dimosystrobin and Benalaxyl). (A) LC-
324 MS/MS analysis in Toronto with different LC conditions (different LC and MS systems; different
325 gradient), (B) and (C) same LC-MS/MS systems with different isocratic steps. Pesticides concentration
326 was 1 ng/mL.

327

328

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- 371

Fig. 1

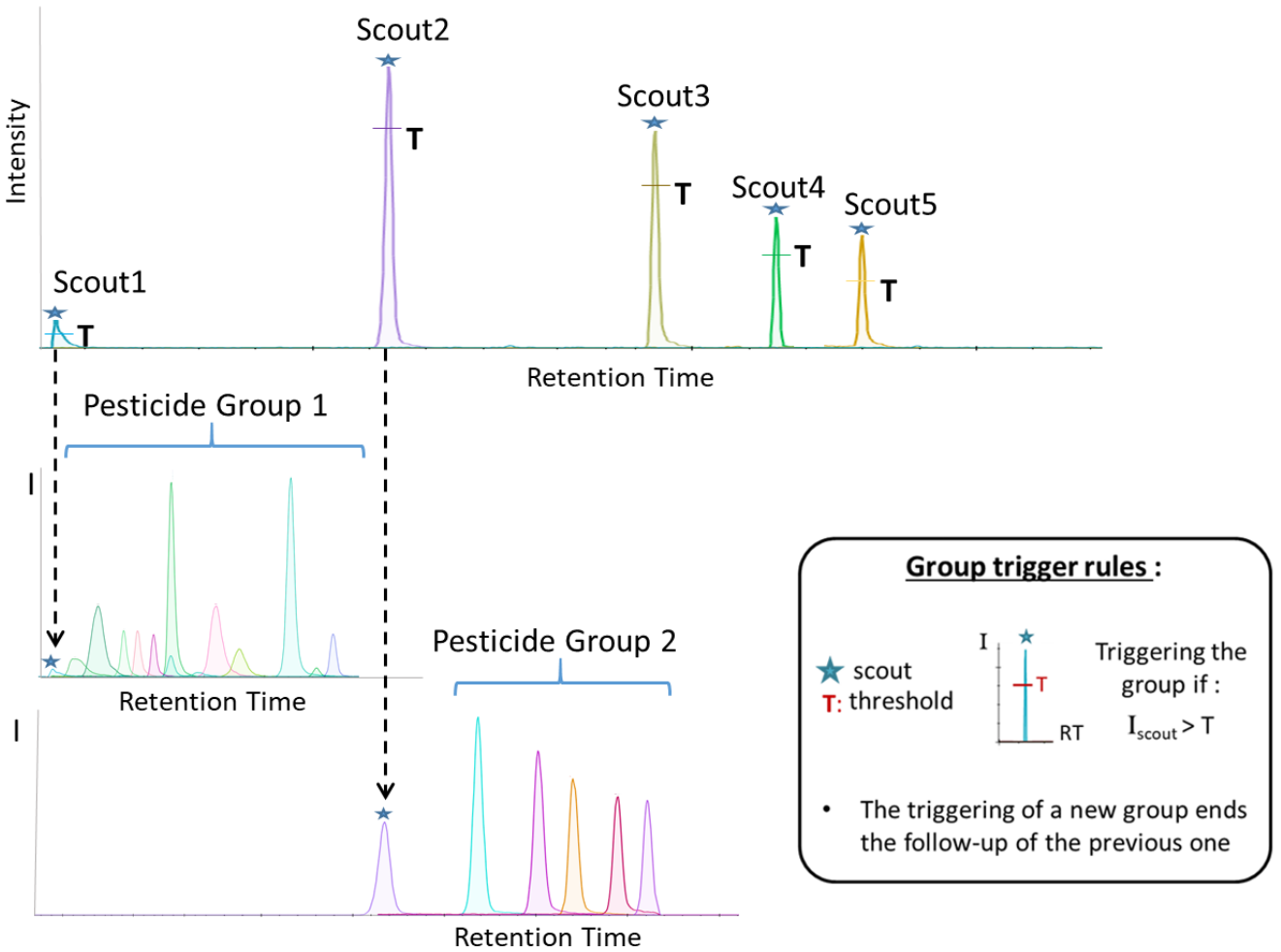


Fig. 2

★ Scout

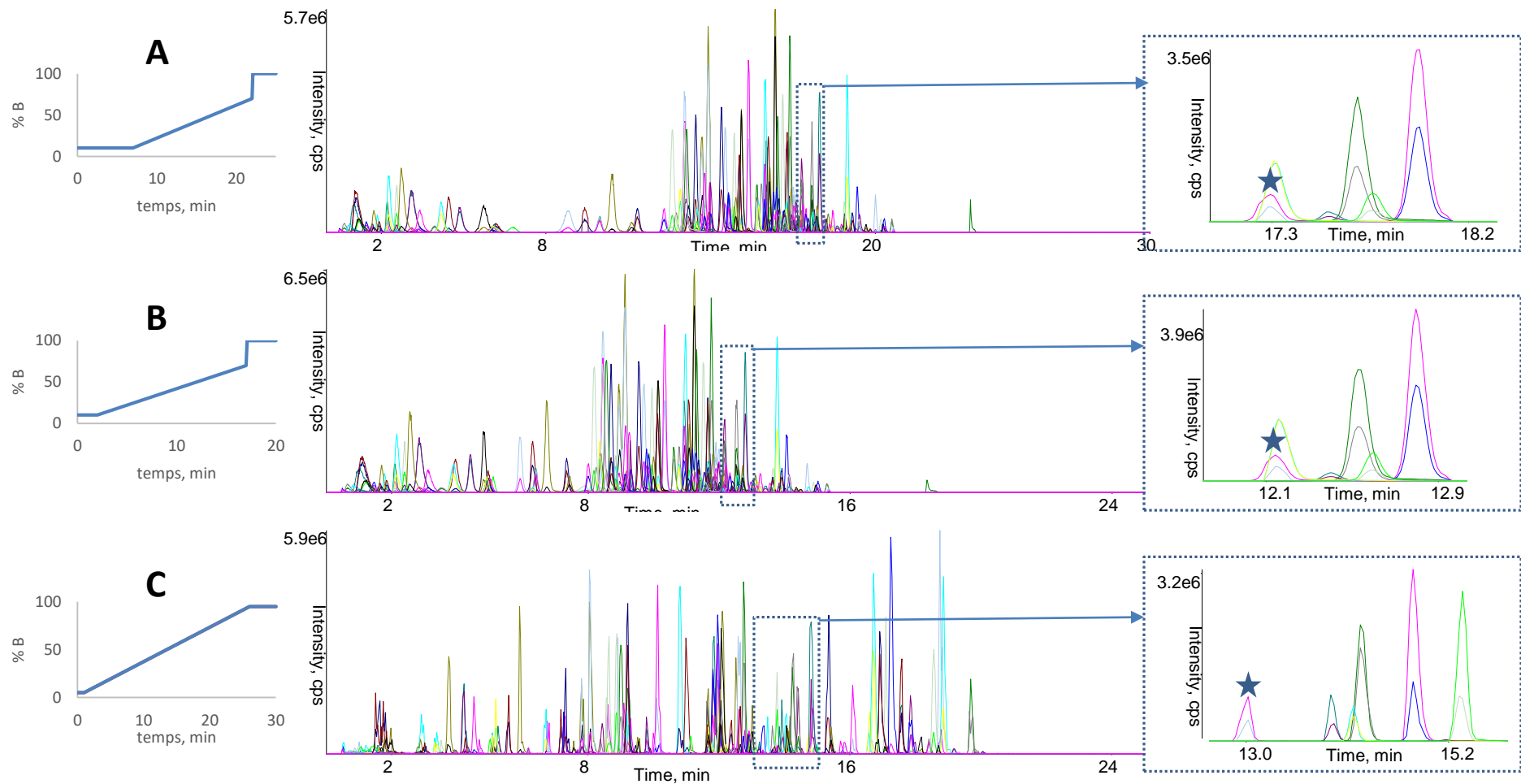


Table 1: Limit of Quantification and pesticide measurements in river water samples using a reference method (A) on a API4000 triple quadrupole mass spectrometer (Irstea Lab), and (B) Scout-MRM and (C) sMRM approach on an hybrid quadrupole ion trap API6500 mass spectrometer (ISA lab). () in brackets error calculation between method A and B; in square brackets error calculation between method B and C.

	LOQ	56_01	56_02	56_03	56_04	56_05	56_06	56_09	56_11	56_13	56_14	
Irstea – MRM (Method A)	Atrazine	0.20	2.4	2.3	1.1	1.0	3.0	2.7	2.0	2.3	2.0	2.0
	Azoxystrobin	0.50	2.3			5.6	6.3	6.1				
	Chlortoluron	1.00					25.3					
	3-(3,4-dichlorophenyl) diuron	1.00							4.9			
		0.50					8.2	5.3	11.3	8.7	6.3	8.0
	isoproturon	1.00				9.2						
	metolachlore	1.00	17.2	6.6	25.5	5.6			6.1			
	norflurazon	0.50							9.3	9.8	9.2	8.6
	simazine	0.20	1.2				2.7	1.6	4.7	6.3	4.6	4.8
	tebuconazole	0.50					5.7	9.6	20.4	15.9		16.2
ISA – Scout (Method B)	LOQ	56_01	56_02	56_03	56_04	56_05	56_06	56_09	56_11	56_13	56_14	
	Atrazine	0.04	1.7 (-29)	1.7 (-28)	0.5 (-55)	0.6 (-39)	1.7 (-44)	1.7 (-35)	1.2 (-43)	1.6 (-33)	1.2 (-40)	1.2(-39)
	Azoxystrobin	0.005	2.1 (-9)			4.9 (-12)	5.0 (-21)	5.2 (-14)				
	Chlortoluron	0.05					23.5 (-7)					
	3-(3,4-dichlorophenyl) diuron	0.01							4.8 (-1.5)			
		0.10					7.0 (-14)	4.6 (-13)	9.4 (-17)	8.6(-1)	6.4(1)	7.8(-3)
	isoproturon	0.01				8.4 (-8)						
	metolachlore	0.05	14.8 (-14)	6.8 (-2)	19.1 (-25)	5.9 (5)			6.0 (-2)			
	norflurazon	0.05				0.3			8.5 (-9)	10.7 (9)	10.4 (13)	9.6 (12)
	simazine	0.02	1.3 (2)				2.5(-7)	1.7 (3)	4.4 (-6)	6.7 (8)	4.8 (5)	5.0 (6)
tebuconazole	0.05					4.8 (-15)	8.6 (-10)	17.7 (-13)	16.8 (6)		19.3 (20)	
ISA – sMRM (Method C)	LOQ	56_01	56_02	56_03	56_04	56_05	56_06	56_09	56_11	56_13	56_14	
	Atrazine	0.04	1.7 [3]	1.6 [6]	0.5 [0]	0.6 [-4]	1.6 [7]	1.6 [11]	1.2 [0]	1.6 [-1]	1.3 [10]	1.1 [7]
	Azoxystrobin	0.005	2.1 [2]			3.8 [28]	5.2 [-3]	5.3 [-3]				
	Chlortoluron	0.05					22.6 [4]					
	3-(3,4-dichlorophenyl) diuron	0.01							5.1 [-5]			
		0.10					7.2 [-2]	4.8 [-4]	9.5[-1]	8.9 [-3]	6.7[-5]	8.2 [-5]
	isoproturon	0.01				7.9 [7]						
	metolachlore	0.05	14.5 [2]	6.7 [1]	18.2 [5]	5.8 [1]			6.1 [-2]			
	norflurazon	0.05							8.8 [-4]	10.7 [-1]	11.0 [-5]	9.7 [-1]
	simazine	0.02	1.2 [7]				2.3 [10]	1.4 [20]	4.2 [5]	6.4 [5]	4.5 [6]	4.7 [7]
tebuconazole	0.05					5.1 [-6]	8.9 [-3]	19.0 [-7]	18.7 [-10]		20.6 [-6]	