1 **Atmospheric pressure chemical ionization tandem mass spectrometry**

2 **(APGC/MS/MS) an alternative to high resolution mass spectrometry**

3 **(HRGC/HRMS) for the determination of dioxins.**

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30 **Abstract**

31 The use of a new atmospheric pressure chemical ionization source for gas

32 chromatography (APGC) coupled to tandem quadrupole mass spectrometer (MS/MS) as

33 an alternative to high-resolution mass spectrometry (HRMS) for the determination of

34 PCDDs/PCDFs is described. The potential of using Atmospheric Pressure Chemical

35 Ionization (APCI) coupled to a tandem quadrupole analyzer has been validated for the

36 identification and quantification of dioxins and furans in different complex matrices.

37 The main advantage of using the APCI source is the soft ionization at atmospheric

38 pressure resulting in very limited fragmentation. APCI mass spectra are dominated by

39 the molecular ion cluster, in contrast with the high energy ionization process under

40 electron ionization (EI). The use of molecular ion as precursor ion in MS/MS enhances

41 selectivity and consequently sensitivity by an increase in signal-to-noise ratios (S/N).

42 For standard solutions of 2,3,7,8-TCDD injecting 10 fg in the splitless mode on a 30m

43 or 60m, 0.25 mm id and 25μm film thickness low polarity capillary columns (DB5MS

44 type) S/N values (greater than 10:1) were routinely obtained. Linearity was achieved in

45 the range ($r^2 > 0.998$) for calibration curves ranging from 100 fg/μL to 1000 pg/μL. The

46 results from a wide variety of complex samples, including certified and standard

47 reference materials and samples from several QA/QC studies which were previously

48 analyzed by EI HRGC/HRMS, were compared with the results from the APGC/MS/MS

49 system. Results between instruments showed good agreement both in individual

50 congeners and toxic equivalence factors (TEQs). The data shows that the use of APGC

51 in combination with MS/MS for the analysis of dioxins has the same potential in term of

52 sensitivity and selectivity as the traditional HRMS instrumentation used for this

53 analysis. The APCI/MS/MS system as being a bench top system is however far more

54 easy to use.

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61   **INTRODUCTION**

62   Within the UNEP program 'Assessment of Existing Capacity and Capacity Building

63   Needs to Analyze POPs in Developing Countries' several activities were undertaken

64   during the period from 2006 to 2010. This program is focused on the development of

65   analytical capacity for the POPs under the Stockholm Convention including several

66   pesticides (DDT, chlordane, toxaphene) and industrial (by)-products (dioxins, PCBs).

67   Recently brominated flame retardants (PBDE) and an organic fluor compound (PFOS)

68   were added to the convention. One of the conclusions of the program was that it is quite

69   a challenge to analyze all POPs in the sample types proposed for the global monitoring

70   program (GMP)[1,2]. This is especially true for developing countries. One of the

71   difficulties to develop a universal method for the Stockholm convention POPs is that

72   both LC and GC have to be used for separation. Although among the newly added

73   compounds to the Stockholm convention, PFOS is routinely analyzed by LC/MS, the

74   most problematic compounds on the Stockholm convention to be analyzed are dioxins.

75   To achieve enough sensitivity and selectivity dioxins are routinely analyzed by using

76   high resolution GC coupled to high resolution MS, instrumentation which is both

77   expensive and complex elaborate to maintain.

78   Dioxins analysis is most commonly carried out using high resolution GC/MS using

79   magnetic sector MS instrumentation operated in EI^+ mode. This technique, even when

80   used at lower ionization energies (~35eV) results in significant fragmentation reducing

81   the intensity of the molecular ion ($M^{\bullet+}$). Typical chemical ionization (CI) produces

82   softer ionization, with an energy transfer that generally is lower and does not exceed

83   5eV. Normally CI mass spectra exhibit less fragment ions than in EI. CI is typically

84   produced under vacuum conditions using an ionization gas which fills the ion source. It

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85   is restricted to specific chemical classes[3,4,5] since it is not as an universal ionization

86   mechanism as EI if no extreme reagents are used. When using CI conditions for the

87   ionization of dioxins often electron capture chemical ionization (NCI) occurs, resulting

88   in loss of intensity of the molecular ion. The importance of an abundant molecular mass

89   peak of the analyte is especially important for the development of MS/MS based

90   methods. The selection of adequate precursor ions and the subsequent application of the

91   MRM mode enhance selectivity and sensitivity, minimizing or even eliminating matrix

92   interferences. When the molecular ion is absent or it has very low abundance, it might

93   be necessary to select a (abundant) fragment ion as precursor ion, with lower m/z and in

94   most cases less compound characteristic. Thus, in addition to the loss of sensitivity, the

95   specificity of the method can also be affected and losing the potential advantages of

96   tandem MS. A soft and universal ionization technique able to provide abundant

97   molecular ions to be used as precursor ions in multi residue GC–MS analysis would be

98   a step forward when using MS/MS. In this way, the low-energy (soft) ionization

99   mechanism occurring in the APGC source generates spectral data typically rich in

100   molecular or <mark>protonated molecule</mark> ion information[6]. Because of the reduced

101   fragmentation when using APCI, the selection of the precursor ion is no longer a

102   compromise between selectivity and sensitivity when developing a MS/MS based

103   method.

104   Atmospheric pressure ionization in GC–MS was first introduced by Horning et al.[7] in

105   the early 1970 using a [63]Ni corona discharge needle to form ions of the target

106   compounds or different reagents[8]. Relatively soon after, this technique was used for the

107   APCI analysis of dioxins by Horning et al.[9], Siegel and McKeown[10] and Mitchum et

108   al.[11] who all used the [63]Ni source to ionize 2,3,7,8-TeCDD at atmospheric conditions.

109   Also, using the [63]Ni source, Mitchum and Stalling[12] were able to analyze all 22 isomeric

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110 tetra-dioxins. The detection limits however were relatively high varying from 60-300

111 ppt and although some selectivity was achieved by using different reagents, APCI at

112 that time was by far less selective and less sensitive than high resolution GC/MS

113 systems which became the preferred methodology for dioxin analysis[13]. Subsequent

114 modifications were described in the 80s[14,15], but the technique was never implemented

115 for common routine analysis.

116 Recently, APCI has made a revival when a new source using nitrogen purge gas was

117 developed and commercialized[16,17,18]. Although not widely applied, it offers attractive

118 analytical capabilities for GC–MS/MS analysis and has been used in different fields,

119 including pesticide residue analysis[19], pharmaceuticals development[20], profiling of

120 phenolic compounds in oil[21] and metabolic profiling[22]. APGC was recently successfully

121 used for the analysis of more than 100 different pesticide residues by Portoles et al.[6]

122 using an improved corona needle discharge source. APGC mass spectra for several of

123 the POPs on the Stockholm convention showed only limited or no fragmentation

124 significantly enhancing the detection limits for compounds such as aldrin, dieldrin and

125 endosulfan when compared to electron ionization (EI) where considerable

126 fragmentation occurred and selection of ions for quantification in the SRM mode is

127 difficult. Especially for POP MS/MS applications it is important to generate as much

128 molecular ion of the most abundant ions of the chlorine cluster for subsequent

129 fragmentation. For isotope dilution quantification using ^{13}C labeled standards, this is

130 preferably the molecular ion.

131 This shows that the use of APGC is a possible way forward in the development of a

132 universal detection system for all Stockholm convention POPs based on mass

133 spectrometry. For dioxins, where high resolution GC/MS systems are often required to

134 avoid inferences and to achieve the extreme low limit of detection (LOD) needed for

135   food and feed[23], air or human samples, APGC could potentially be a very attractive

136   alternative, opening the way for a universal mass detector for all POPs on the

137   Stockholm convention including dioxins.

138   The aim of this paper is to demonstrate the capabilities of APGC/MS/MS (tandem

139   quadrupole) using a APCI based ion source for the determination of dioxins in a variety

140   of samples including environmental, air, human and food sample extracts.

141   **MATERIAL AND METHODS**

142   **PCDD/F standards and Samples**

143   EPA-1613PAR and TF-TCDD-MXB standards solutions containing different mixtures

144   of native PCDD/F congeners, EPA-1613LCS and EPA-1613ISS ^{13}C-labeled PCDD/F

145   standards for sample preparation, as well as calibration standards 1613-EPACSL to

146   1613-EPACS5, with both native and ^{13}C-labeled PCDD/F and TCDD solution TF-

147   TCDD-MXB, were all obtained from Wellington Laboratories (Guelph, Ontario,

148   Canada). Certified reference materials BCR-607 (natural milk powder), BCR-677

149   (sewage sludge), BCR-490 and BCR-615 (fly ash) were acquired from the Institute for

150   Reference Materials and Measurements (IRMM), European Commission-Joint Research

151   Centre (Geel, Belgium). Internal reference materials (spiked feed samples, human blood

152   and naturally contaminated food and feed samples) for routine quality control/quality

153   assurance (QA/QC) in the laboratories and different matrices from international inter-

154   laboratory comparison studies (fish sample from the Interlaboratory Comparison on

155   POPs in Food 2012 (13th Round), Norwegian Institute of Public Health (Oslo, Norway)

156   and PT test samples from proficiency tests organized by the EU-RL for Dioxins and

157   PCBs in Feed and Food) were also used to compare the results on different instruments.

158   Additionally, several procedural blanks have been analyzed both by HRMS and APGC

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159   for all sample types. All blank levels were well below the amounts found in the

160   samples.

Sample Preparation

162   Sample treatment was performed following previously developed and validated

163   methods[24] or standard methods[13] or based on analytical criteria of Commission

164   Regulations (EU) No. 252/2012 and 152/2009 for the official control of food and feed[25].

165   Generally after sample extraction of the organic fraction, fat and polar interfering

166   substances were removed by treating the n-hexane extracts with silica gel modified with

167   sulfuric acid (44%) or gel permeation chromatography. Further sample purification was

168   performed by an automated system (Power Prep™, FMS Inc., Waltham, MA, USA), or

169   a manual clean up using an alumina oxide column eluted with hexane and a

170   hexane/dichloromethane mixture or florisil column eluted with n-heptane and toluene

171   followed by a carbon column eluted with hexane and toluene. The final extracts were

172   either finally analyzed by APGC/MS/MS or both by APGC/MS/MS and HRGC/HRMS.

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Instrumental Analysis

175   *APGC/MS/MS conditions*

176   Four different APGC/MS/MS systems were used in this study in four different

177   laboratories at Waters, Manchester, Great Britain; IUPA, Castellon, Spain; EURL for

178   Dioxins and PCBs, Freiburg, Germany and MTM, Örebro, Sweden. The basic set up of

179   the instrument was similar in all cases. The GC system consisted of an Agilent 7890A

180   (Agilent, Palo Alto, CA, USA) equipped with an auto sampler (Agilent 7693). The GC

181   was coupled to a quadrupole mass spectrometer (Xevo TQ-S, Waters Corporation,

182   Manchester, UK), equipped with an APGC ionization source. For dioxin analysis the

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183   GC was equipped with either a silica DB-5MS (UI) capillary column (60 m × 0.25 mm

184   id. × film thickness 0.25 μm) (J&W Scientific, Folsom, CA, USA) or a BPX-5 capillary

185   column (30 m × 0.25 mm i.d.,x 0.25 m) (SGE Analytical Science, Victoria, AUS). The

186   injector was operated in splitless mode, injecting 1 μL at 280 °C at all instruments by

187   pulsed splitless injection using an initial pressure of 240 kPa or injecting 5 ul in the

188   PTV solvent vent mode (EURL, Freiburg). The interface temperature was set to 280-

189   360 °C using N_2 (from gas cylinder, quality ≥ 99.9990%) as make-up gas at 150-370

190   mL/min in the constant flow mode depending on the instrument. As auxiliary gas N_2

191   (from liquid N_2, nitrogen generator or gas cylinder) was used at 250-300 L/h with tube

192   to waste or 200 L/h without tube to waste. The cone gas was used to optimize the

193   ionization, and set at 170-200 L/h when the comparison was made between all four

194   instruments. The APCI corona pin was operated between 1.8 and 2.1 μA. The ionization

195   process occurred within a closed ion volume, which enabled control over the

196   protonation/charge transfer processes.

197   Quantitative analysis was performed in the multiple reaction monitoring mode (MRM)

198   by monitoring two transitions for each of the native PCDD/F congeners and their

199   corresponding ^{13}C-labeled analogues. The molecular ion $[M^{\bullet+}]$ was always selected as

200   precursor ion for all compounds (congeners and ^{13}C analogues) and fragmented by

201   collision in the T-wave collision cell. The data were processed using the quantification

202   application manager TargetLynx^{TM} which automates data acquisition, processing and

203   reporting for quantitative results. It incorporates a range of confirmatory checks that

204   identify samples that fall outside user-specified or regulatory thresholds. A summary of

205   the experimental conditions in the different laboratories is given in **Table 1**. The

206   MS/MS conditions were taken from the literature[26,27] and optimized when needed and

207   are given in **Table 2**.

208

209   **RESULTS AND DISCUSSION**

210   **Ionization optimization**

211   The ionization in the APGC mode was optimized by using high concentration

212   PCDD/PCDF standards (CS5, 200 ng/mL for TCDD) which were injected under full

213   scan conditions. The ionization using APCI under charge-transfer conditions (no H_2O

214   was added to the source) revealed an abundant presence of the molecular ion for all

215   seventeen 2,3,7,8 chlorine substituted dioxins and furans. This is in good agreement

216   with recent publications[6] explaining the ionization mechanism for APCI. The nitrogen

217   plasma (N_2^+ and N_4^+) created by the corona discharge needle ionizes molecules yielding

218   typically $M^{\bullet+}$. The protonated molecule $[M+H]^+$ can be also present in the spectrum due

219   to the presence of water vapor traces in the source, this competing mechanism reduces

220   the intensity of the molecular ion. The ionization under proton-transfer conditions was

221   tested by introducing water as modifier in the APCI source (an uncapped vial with

222   water was placed in a specially designed holder placed in the source door). However,

223   none of the tetra- to octa-chlorinated dioxins or furans showed much protonation, this is

224   exemplified in **Figure 1** which shows the ionization behavior of OCDF with and

225   without water added to the source. It can clearly be seen that ionization which is carried

226   out only with N_2 (charge transfer) is more effective (in terms of response) and the m/z

227   444 precursor resulted in higher spectral abundance without using water as modifier and

228   reducing proton transfer as much as possible. For the continuation of the development

229   of a method for PCDD/DFs analysis charge transfer conditions were used. A simple

230   check to see if protonation occurs is the analysis of phenanthrene and comparing the

231   abundance of m/z 178 (charge transfer) and m/z 179 (protonation), for this relatively

232   easy protonated compound the protonated ion should not exceed 30%.

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234 **Optimization of the cone voltage**

235 The cone voltage was optimized for each PCDD/F by testing values between 20 and

236 70V in order to obtain the best sensitivity. Although no significant differences were

237 observed, slightly higher response factors were obtained at 30V (especially compared

238 with 70V) and thus a cone voltage of 30V was used for most of the experiments chosen.

239 The optimization process is further illustrated in **Figure 2** where the relative response is

240 given as a response surface diagram for the cone gas and the auxiliary gas. A clear

241 optimum and stable region is seen between cone gas flow settings 170-225 L/h and

242 auxiliary gas settings of a larger range (100-200 L/h). Both flows from different

243 directions seem to influence the corona plasma and ion extraction. Too high values of

244 the auxiliary gas flow did result in lower response for the target compounds. When

245 removing the auxiliary to waste tube, lower auxiliary gas flows give better relative

246 response illustrating the complexity of the optimization the different gas flows into the

247 source.

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249 **MRM method**

250 Firstly, product ion scan experiments were performed in order to find selective

251 transitions based on the use of $M^{\bullet+}$ as precursor ion. Different collision energies (10, 20,

252 30, 40 and 50 eV) were tested and the most sensitive transitions were selected for the

253 development of the subsequent MRM method. Collision energies of 30 eV were

254 selected for all the TCDDs and values of 40 eV were selected for the TCDFs. Lower

255 energies led to nearly absence of product ions and too high energies led to excess

256 fragmentation and therefore lower sensitivity At low collision energies the product ion

257 spectrum is dominated by the ^{35}Cl loss, but at the final optimum collision energies the

258 transitions selected corresponded to the loss of $[CO^{35}Cl]$ (**Figure S1**). This fragment is

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259   very specific for dioxins and furans. Collision energies of 30 eV were selected for all

260   the PCDDs and values of 40 eV were selected for the PCDFs (**Table 2**) or 31 eV for

261   both PCDDs and PCDFs. These collision energies are in agreement with GC/MS/MS

262   collision energies using EI ionization[4,5].

263   Both automatic dwell time, set in order to obtain at least 15 points per peak (values

264   ranging from 0.058 to 0.079 s) and a fixed dwell time of 0.1 ms were used in the

265   different instruments tested. In all cases the chromatographic peak shape was considered

266   adequate.

267   **Analytical parameters**

268   The analytical performance was evaluated by calculating the detection limit, linearity,

269   repeatability and reproducibility of the method in four different laboratories. These

270   parameters are compared to the high resolution mass spectrometer, the instrumentation

271   routinely used for the analysis of dioxins using standard method EPA1613 or EN

272   16215. The lowest calibration point for 2,3,7,8-TeCDD in this method contains 500

273   fg/ul (CS1), for the evaluation of high resolution instruments often a solution of 100

274   fg/ul is used at a S/N level > 100. After initial set up and tuning, a dilution of this test

275   solution down to 10 fg was made. Also this solution achieved an S/N values of > 50

276   were achieved and in all four laboratories in most cases when optimum alignment of the

277   corona needle was established in addition to the make-up and auxiliary gas flow

278   conditions (**Figure 3**). Similar results were achieved using toluene or tetradecane as the

279   injection solvent. The ultimate sensitivity was tested by using a mix of different TCDD

280   congeners at concentrations of 2, 5, 10, 25, 50, and 100 fg/ul (TF-TCDD-MXB) of

281   1,3,6,8-TeCDD, 1,3,7,9-TeCDD, 1,3,7,8-TeCDD, 1,4,7,8-TeCDD, 1,2,3,4-TeCDD and

282   2,3,7,8 respectively injected to evaluate the minimum amount of TCDD that could be

283   detected. As can be seen from **Figure 4**, the lowest value which is visible just above

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284 noise level is 2 fg. Also the sensitivity for the other tetra- to octa- substituted PCDD/Fs

285 was good for the 1/10 dilution of calibration solution CSL at 10, 50 and 100 fg/ul for

286 TeCDD   and   TeCDF,   PeCDD,PeCDF,HxCDF,HxCDD,HpCDD,HpCDF   and

287 OCDD/OCDF respectively. This is illustrated in **Figure 5** where the different MRM

288 channels are given for this solution. All these results are impressive and in comparison

289 or even better than routinely achieved with high resolution magnetic sector GC/MS

290 systems.

291

292 The linearity of the method was studied by analyzing the standard solutions (in

293 triplicate) at six concentrations (CSL, CS0.5, CS1-CS4) ranging from 0.1 to 40 ng/mL

294 for the Tetra PCDD/DFs, from 0.5 to 200 ng/ml for the Penta through Hepta PCDD/DFs

295 and from 1.0 to 400 ng/ml for the Octa PCDD/DFs at the four different systems in four

296 different laboratories. The linearity was satisfactory with the correlation coefficients (r^2)

297 larger than 0.998. The RSD of the relative response factors (RRFs) as defined in

298 standard methods EPA 1613 or EU 1948 were also satisfactory and all below 15% as

299 specified in both methods. Based on area the repeatability was within 15% for the

300 injection of 10 fg (n=3-10), and below 10% for all PCDD/DFs for the CSL standard

301 against the corresponding ^{13}C standard (RRF).

302

303 An important criterion for the unequivocal identification of the PCDD/F congeners is

304 the ion abundance ratio between the two monitored product ions, resulting from two

305 different precursor ions. For quality control the ion abundance ratios can be compared

306 with calculated or measured values. The calculated ratio depends on the relative

307 abundance of the two selected precursor ions of the molecular ion $[M^{\bullet +}]$ and the

308 probability of the loss of $[CO^{35}Cl]$ or $[CO^{37}Cl]$ for formation of each product ion. It is

309 only comparable with the measured ratios, if identical collision energy and collision gas

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310 pressure is applied for both transitions. The measured ion abundance ratios in

311 calibration standards and sample extracts matched the calculated values within the QC

312 limits of ± 15 %, as derived from EPA 1613 for HRMS.

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314 Besides the use of the signal-to-noise for the calculation of the limit of quantification

315 (LOQ) these ion abundance ratios in combination with the relative response factors

316 from calibration can be used as criteria to check the reliability of the results in the low

317 concentration range. Based on maximum deviations of ± 15 % of the calculated value

318 for ion abundance ratios and deviations of ≤ 30 % of the relative response factor of the

319 mean value (with a CV ≤ 20% for the complete calibration) LOQs for 2,3,7,8-TCDD

320 and 2,3,7,8-TCDF were obtained in the range of 10 – 30 fg on column for calibration

321 standards.

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323 **Comparison APGC and high resolution GC/MS**

324 *Quality controls, certified reference materials*

325 In order to test the capabilities of the developed method using GC-(APCI) MS/MS,

326 several samples previously analyzed by HRMS were injected in the new system.

327 Sample extracts from existing samples which previously had been run on a high

328 resolution system were re-injected on the APGC/MS/MS system in three different

329 laboratories, the EURL for Dioxins and PCBs in Germany, CSIC and IUPA in Spain

330 and MTM in Sweden. A summary of the results based on TEQ[28] are given in **Figure 6**

331 where the results of the different laboratories are given over a wide concentration range

332 and a variety of different samples. The correlation between the results is very good and

333 the relative difference $\frac{X_{apgc} - X_{hrms}}{X_{hrms}}$ between the APGC results and the HRMS was less

334 than 7% for all samples given in **Table 3**, when the APGC runs passed all QA/QC

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335   criteria in terms of chromatographic separation, linearity, S/N ratio and ion abundance

336   ratio of selected transitions. In some cases loss of chromatography was seen which

337   affected the results of the individual isomers. Also for some of the samples run on the

338   shorter 30m BPX-5 an overestimation of 1,2,3,7,8,9-HxCDF was seen. This is however

339   not specific for the APGC but has also been seen for HRMS[29]. On average the results

340   for the individual congeners compared well with the HRMS results accept for levels just

341   above the detection limit. These isomers however contribute very little to the total TEQ,

342   and although the relative difference could be larger than 25%, this was not reflected in

343   the total TEQ.   More detailed congener specific data is given in the supplemental

344   information where a comparison of results of analysis with APGC-MS/MS with results

345   of GC-HRMS QC-charts for mixed animal fat, fish oil and hen's eggs sample is

346   specified (**Figure S2**).

347

348   **CONCLUSION**

349   The results of the APGC system are impressive and comparable with HRMs both in

350   selectivity but also in sensitivity (10 fg, S/N < 50). Sensitivity of conventional

351   GC/MS/MS systems has been lower than traditional HRMS. For monitoring purposes of

352   all the POPs on the Stockholm Convention in complex samples such as air, human

353   blood or milk included in UNEP global monitoring program this is a big step forward

354   when the most difficult compound class can be analyzed on the same instrumentation,

355   including difficult pesticides, brominated flame retardant but also persistent fluor

356   compounds including PFOS connecting the instrument to (UP)LC.

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359　**ACKNOWLEDGEMENT**

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366　*Table 1. Experimental APGC/MS/MS conditions used by the different laboratories after*

367　*optimization.*

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Instrumentation GC conditions	
Column:	DB-5MS 60 m x 0.25 mm, 0.25 μm BPx-5 30m x 0.25 mm, 0.25 μm
Carrier gas:	Helium at 1.4-2 mL/min
Injector mode:	Pulsed Splitless, 240- 450 kPa (1-2 mins) MMI Solvent Vent (50 kPa, 10 ml/min, 0.5 min)
Injector liner:	Single gooseneck splitless liner, 4mm Deactivated dimpled splittless liner, 2 mm
Column pneumatics:	Constant flow
Injection volume (μL):	1 ul splitless 5 ul MMI
Injector temperature (°C):	Splitless 280 ^{0}C MMI PTV mode (100 ^{0}C, 0.5 min; 340 ^{0}C, 20 min)
MS Conditions	
MS system:	Xevo TQ-S
Ionisation:	APGC with Dry N_2
Corona current:	1.5-2.1 μA
Source offset:	60-70 V
Cone Voltage:	30, 35 or 70 V
Source temperature:	150^{0}C
Cone gas flow:	170-200 L/h
Acquisition:	MRM
Collision gas:	Argon at 3.5-6.2 10^{-3} mbar
GC interface	280-360 ^{0}C
Aux gas flow	250-300 L/h 200 L/h (without tube to waste)
Make up gas	150-370 ml/min

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377   *Table 2. Multiple Reaction Monitoring (MRM) conditions for MS/MS method.*

Compound	Precursor Ion	Product Ion	Collision Energy (eV)	Precursor Ion	Product Ion	Collision Energy (eV)
TCDF	304	241	40	306	243	40
[13]C TCDF	316	252	40	318	254	40
TCDD	320	257	30	322	259	30
[13]C TCDD	332	268	30	334	270	30
PCDF	338	275	40	340	277	40
[13]C PCDF	350	286	40	352	288	40
PCDD	354	291	30	356	293	30
[13]C PCDD	366	302	30	368	304	30
HxCDF	374	311	40	376	313	40
[13]C HxCDF	386	322	40	388	324	40
HxCDD	390	327	30	392	329	30
[13]C HxCDD	402	338	30	404	340	30
HpCDF	408	345	40	410	347	40
[13]C HpCDF	420	356	40	422	358	40
HpCDD	424	361	30	426	363	30
[13]C HpCDD	436	372	30	438	374	30
OCDF	442	379	40	444	381	40
[13]C OCDD	470	406	30	472	408	30
OCDD	458	395	30	460	397	30

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7　　　384　　*Table 3. Comparison of results obtained for a variety of samples analyzed both by*
8　　　385　　*APGC/MS/MS and HRGC/HRMS. Data are given on WHO-PCDD/DF TEQ per*
9　　　386　　*sample.*
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	APGC	HRMS	
EURL	0.85	0.83	2%
(pg/g lipids)	0.69	0.72	-3%
	1.24	1.31	-6%
	1.07	1.14	-7%
	1.86	1.89	-2%
	3.46	3.39	2%
MTM	6.1	5.8	5%
(pg/PUF)	13.9	14.0	-1%
	45.6	47.7	-5%
	63.8	62.0	3%
	172	168	3%
	17.3	16.2	7%
CSIC/IUPA	2.19	2.12	3%
(pg/g)	0.40	0.41	-2%
	0.62	0.59	4%
	238	228	4%
	3640	3470	5%
	96.2	89.4	7%

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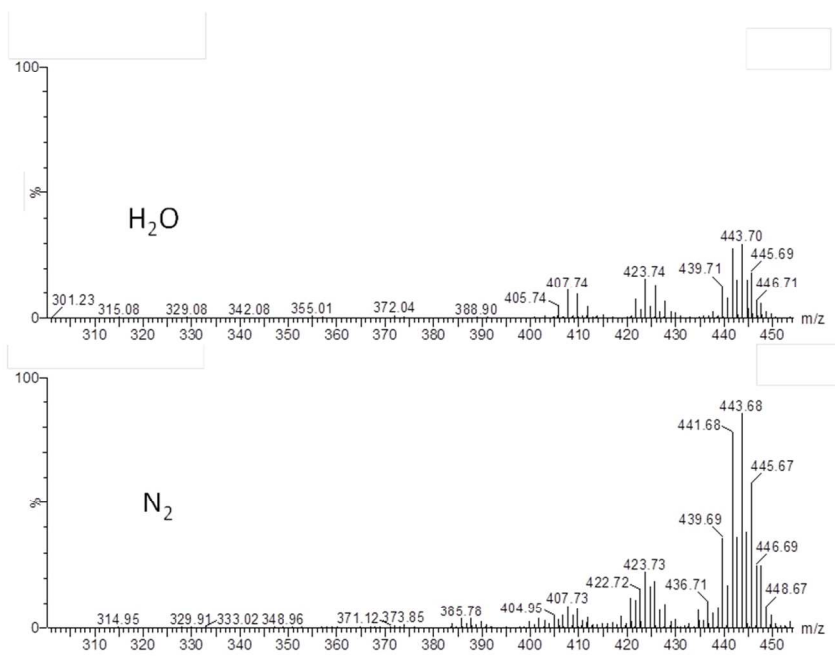[29] D. Fraisse, O. Paisse, L. Nguyen Hong, and M. F. Gonnord. *Fresenius J Anal Chem* **1994** 348:154-158

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Figure 1. Comparison of the ionization characteristics of OCDF in the APGC source with (enhancing protonation) and without water (enhancing charge transfer) in the source.
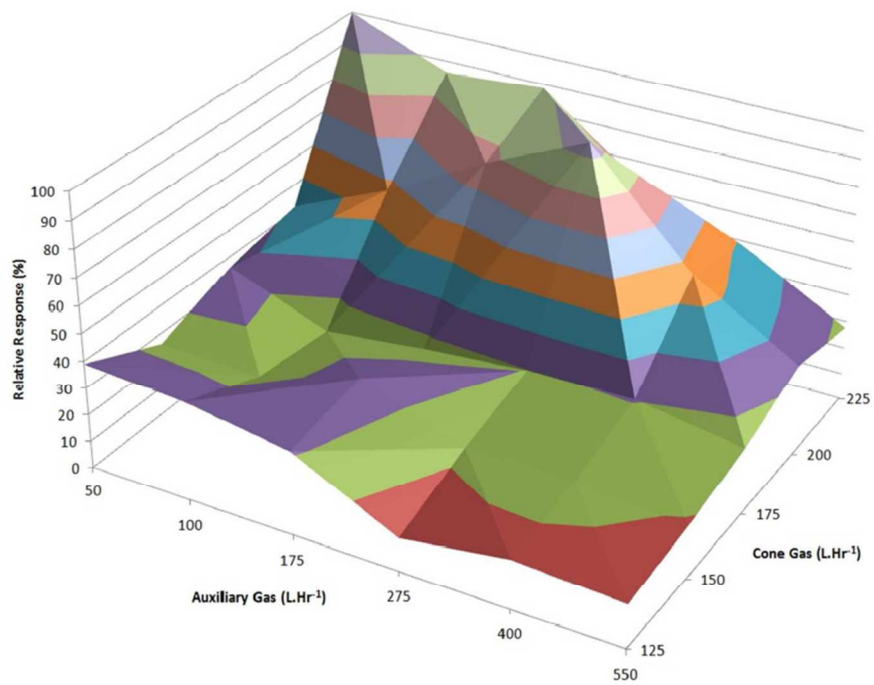254x190mm (96 x 96 DPI)

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Figure 2. Response surface plot showing auxiliary gas (LHr-1) versus cone gas (LHr-1) optimization for PeCDD.
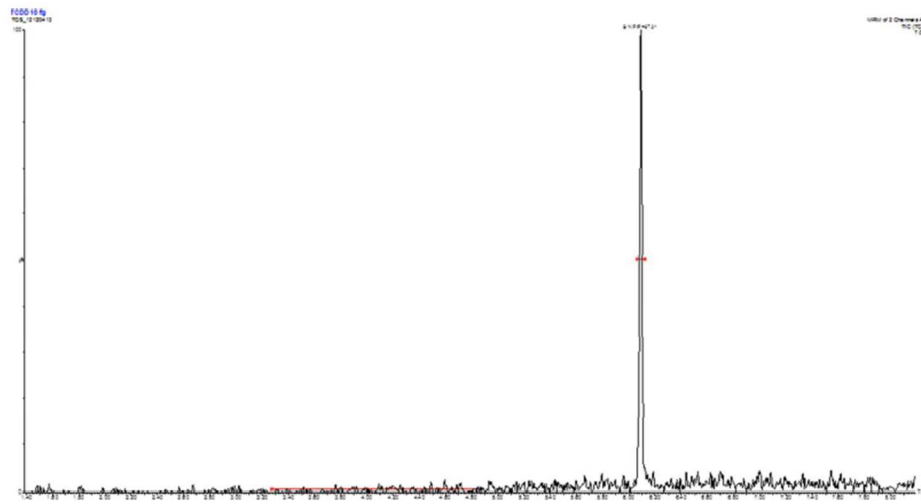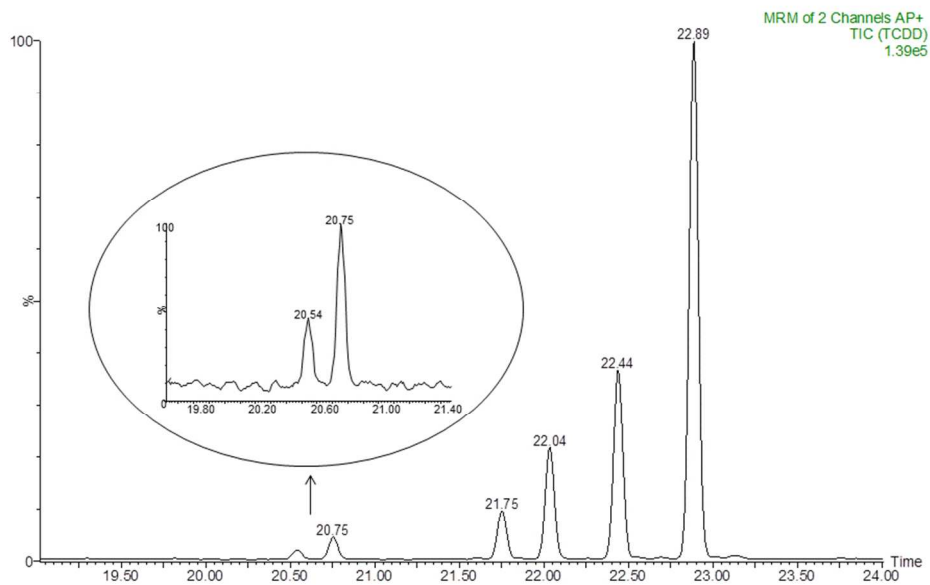240x168mm (96 x 96 DPI)

Figure 3. Injection of 1 ul of a 10 fg/ul solution of TCDD on a BPx-5 30m x 0.25 mm, 0.25 µm column using the APGC conditions in Table 2.
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Figure 4. Sensitivity test after injection of 1 ul of a test solution containing 2-100 pg/ul of different TCDD isomers on a 60 m DB-5MS column (0.25 mm id x 0.25 um).
254x190mm (96 x 96 DPI)

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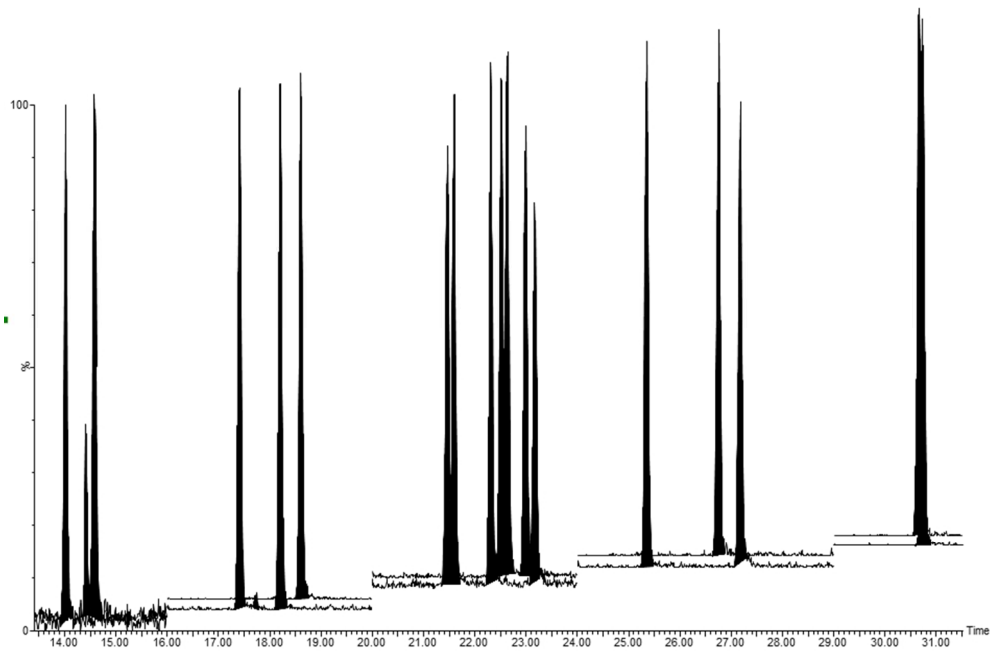46
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Figure 5. TeCDD and TeCDF at 10 fg, PeCDD, PeCDF, HxCDF, HxCDD, HpCDD, and HpCDF at 50fg and OCDD and OCDF at 100 fg, 1ul injected see Table 2 for MRM conditions.
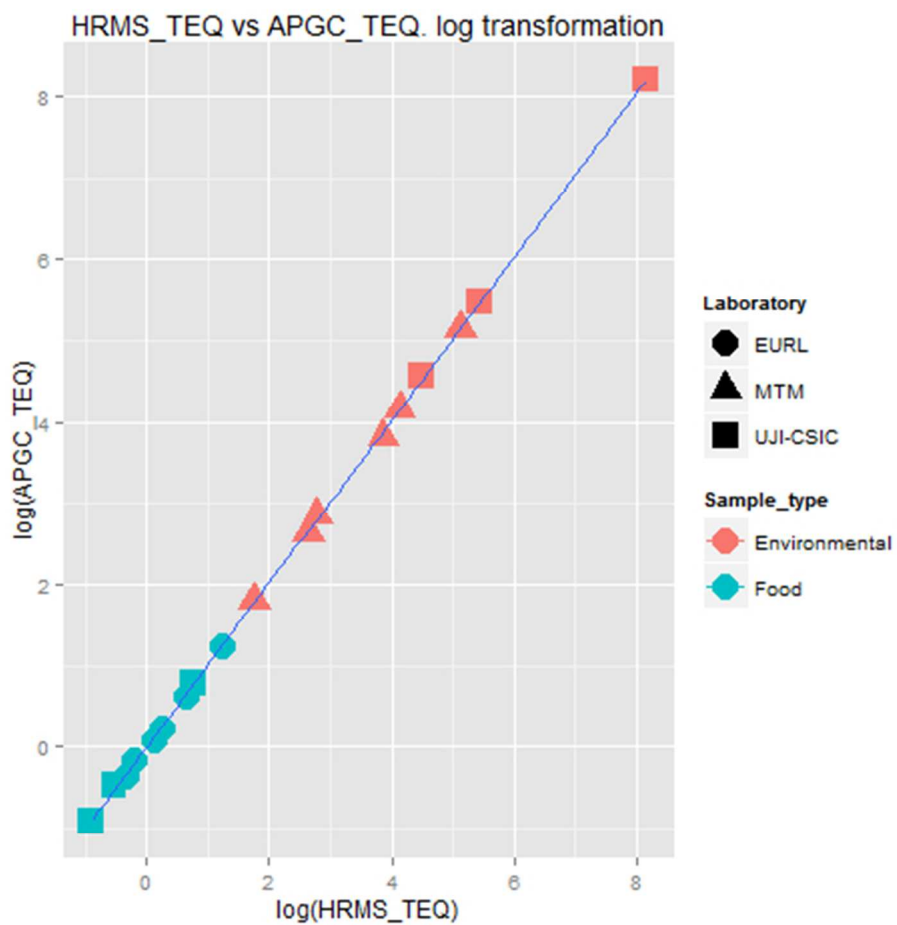254x190mm (96 x 96 DPI)

Figure 6. Comparrison of APGC results and HRMS for different samples as described in the method and material section in three different laboratories.
127x127mm (96 x 96 DPI)