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Plastic antioxidants: A family of cocaine cutting agents analyzed by short column gas chromatography-mass spectrometry

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ABSTRACT

Plastic antioxidants (PAOs), which are used in the industry to prevent degradation caused by thermo-mechanical or thermo-oxidative conditions, have been found in cocaine products seized by the Civil Police of the Federal District, Brazil, since 2019. In this study, a 4-meter short column gas chromatography-mass spectrometry (GC–MS) qualitative method was optimized and validated to detect cocaine, PAOs (antioxidant 168, FOS; antioxidant 1076, NOX; and butylated hydroxytoluene, BHT) and 16 other cutting agents in cocaine base and salt. NOX and FOS are high-boiling-point compounds that are not amenable to the standard GC–MS methods. The method uses a 250 °C split mode injection, final temperature of 280 °C, and a total run time of 16.5 min. PAOs were found in 84.2% of the 38 cocaine base samples and in 21.5% of the 65 cocaine salt samples (mainly NOX); 20 samples that did not contain any cocaine also contained PAOs (30% NOX and 25% FOS). Other cutting agents found in the samples included phenacetin, aminopyrine, and lidocaine in cocaine base; lidocaine, tetracaine, and caffeine in cocaine salt. This is the first report of PAOs detected as cocaine cutting agents and shows another important application of the short column GC–MS method in forensic science that can also be applied in other areas involving these compounds.
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1. Introduction

According to the World Drug Report of the United Nations Office on Drugs and Crime (UNODC), more than 1400 tons of cocaine were seized worldwide in 2019, 83% in the Americas. Brazil is the third in the Americas ranking, with 7% of the global apprehensions, surpassed only by United States of America (18%) and Colombia (34%) [1]. The highest annual prevalence of cocaine use was found in Oceania (2.7% of the population), followed by North America (2.1%), Western and Central Europe (1.4%), and South America (1.0%) [1]. The annual prevalence in Brazil was estimated to be 1.2% in 2015 [2].

Most apprehended cocaine contains diluents and adulterants, collectively called cutting agents [3], which are organic and inorganic substances added along the production chain. What is added to cocaine seems to be related not only to the expected effect the substance might provide, but also to how easily the compound could be obtained, with the profile of cutting agents changing over the years [4,5]. Knowledge of cutting agent profile can provide supporting evidence of common origin or new trends in the illicit drug market and might help to link different seizures [5–7]. Furthermore, cocaine cutting agents can also be toxic and increase the risk of adverse health effects due to synergistic reactions [3,8,9]. A comprehensive method to analyze different cutting agent chemical classes is more likely to be widely adopted and help to increase awareness among legal and health professionals [8].

Since the middle of 2019, the Criminalistics Institute of the Civil Police of the Brazilian Federal District (CI-CP-DF) has been identifying plastic antioxidants (PAOs), pure or as cocaine cutting agents, by Fourier-transformed infrared spectroscopy. In 2020, more than 200 kg of whitish powder containing PAOs was apprehended. PAOs are added to plastics to prevent degradation caused by thermo-mechanical or thermo-oxidative conditions. Primary antioxidants act by donating electron or hydrogen atoms to reduce free radicals, interrupting a chain reaction process that leads to polymer degradation, and secondary antioxidants interrupt the second oxidative cycle by preventing the generation of free radicals [10]. Butylated hydroxytoluene (BHT) and antioxidant 1076 (NOX) are the most used primary antioxidants, while antioxidant 168 (FOS) is one of the most used secondary antioxidants (Table 1). BHT is also used as an antioxidant in the food industry [11].

The polymer industry consumed more than 270,000 tons of PAOs in 2018, a global PAO market valued at more than USD 3 billion [12]. Hindered phenols, like NOX, are effective primary antioxidants at low levels (100–1000 mg/kg) [13], and their maximum allowed concentrations in polymer products and specific migration levels are regulated in the USA [14], European Union [15], and the

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Southern Cone Common Market (Mercosur), which includes Brazil [16].

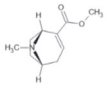
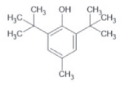
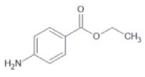
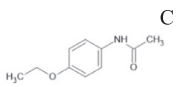
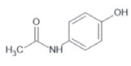
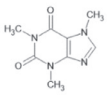
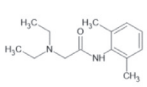
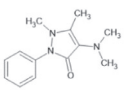
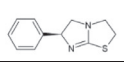
The presence of PAOs in medical products, food packaging and other consumables may be of health concern since they can leach from plastics to food [17]; BHT has been shown to cause lung injury and impaired adipogenesis in mice adipocytes [18]. Analytical methods have been developed for detection of PAOs and their degradants in these products [19]. The American Society for Testing and Materials elected LC-DAD as the method of choice for PAO analysis [20], probably due to the high boiling points of some of their representatives, such as NOX (568.1 ± 45.0 °C) and FOS (594.2 ± 50.0 °C) [21,22].

Most forensic chemistry laboratories rely on GC–MS for their casework due to the method's high sample throughput, reduced sample preparation, and relatively low maintenance cost. The high concentration span of cocaine and cutting agents present in street-level seizures, together with the enormous amount of casework, also favor GC–MS as the method of choice for screening of seized cocaine products.

However, gas chromatography is considered not amenable for analysis of thermally labile and low volatile compounds [23,24]. Nevertheless, Sandra and David [25] obtained good chromatographic peaks of PAO with GC-FID, but oven temperature had to be set at 380 °C, which is unsuitable for the routinely used GC capillary columns in forensic laboratories. Limited work on identification of PAOs has been done using GC–MS [26–29], some using modified hardware to obtain PAO EI-MS spectra [30]. Some authors have been able to analyze both thermally labile [31] and low volatile compounds [32] by reducing column length. Unfortunately, they used nonstandard cold-on-column injection, or a modified GC–MS based on supersonic molecular beams, which limits the adoption of their methods by forensic laboratories.

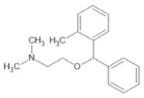
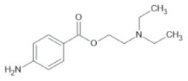
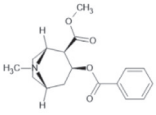
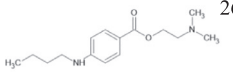
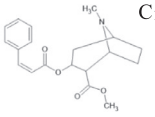
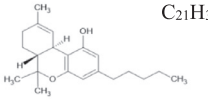
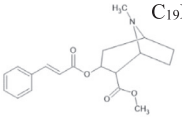
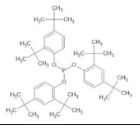
By shortening the analytical column, a proportional reduction in analysis time is obtained, while resolution will only be impacted by the square root of the length reduction ratio. A short column also reduces inlet pressure, which in association with the outlet vacuum promoted by the mass spectrometer led to a higher optimal carrier gas velocity [33–35]. Consequently, higher flow rates

Table 1
Compound name, CAS number, chemical structure, molecular formula, molar mass, major ions (base peak in bold), average retention times (Rt) with their relative standard deviation (RSD,%), and average linear retention index (LRI) for compounds detected by the short column gas chromatography-mass spectrometry method.

Compound name (CAS number)	Chemical structure, molecular formula, molar mass	Major ions	Rt (min) (RSD,%)	LRI
Nicotine (NIC)[a] (22083-74-5)	 $C_{10}H_{14}N_2$ 162	**84**, 133, 162	1.67 (0.15)[§]	1290
Anhydroecgonine methyl ester (AEME)[b] (43021-26-7)	 $C_{10}H_{15}NO_2$ 181	**152**, 181, 82	1.99 (0.33)[§]	1347
Butylated hydroxytoluene (BHT)[c] (128-37-0)	 $C_{15}H_{24}O$ 220	**205**, 220, 57	2.72 (0.07)[*]	1482
Benzocaine (BZC)[d] (94-09-7)	 $C_9H_{11}NO_2$ 165	**120**, 165, 92	2.89 (0.19)[*]	1509
Phenacetin (PHN)[d] (62-44-2)	 $C_{10}H_{13}NO_2$ 179	**108**, 109, 179	3.59 (0.61)[*]	1640
Acetaminophen (ACT)[d] (103-90-2)	 $C_8H_9NO_2$ 151	**109**, 151, 80	3.70 (1.56)[#]	1652
Caffeine (CAF)[d] (58-08-2)	 $C_8H_{10}N_4O_2$ 194	**194**, 109, 67	4.11 (0.60)[*]	1745
Ketamine (KET)[a] (6740-88-1)	 $C_{13}H_{16}ClNO$ 237	**180**, 182, 152	4.24 (0.51)[§]	1785
Lidocaine (LID)[d] (137-58-6)	 $C_{14}H_{22}N_2O$ 234	**86**, 58, 87	4.53 (0.11)[*]	1835
Aminopyrine (AMN)[d] (58-15-1)	 $C_{13}H_{17}N_3O$ 231	**231**, 56, 97	4.63 (0.11)[*]	1854
Levamisole (LEV)[d] (16595-80-5)	 $C_{11}H_{12}N_2S$ 204	**148**, 204, 203	4.69 (0.03)[#]	1877

(*continued on next page*)

Table 1 (*continued*)

Compound name (CAS number)	Chemical structure, molecular formula, molar mass	Major ions	Rt (min) (RSD,%)	LRI
Orphenadrine (ORP)[d] (341-69-5)	 $C_{18}H_{23}NO$ 269	**58**, 73, 165	4.83 (0.00)[$]	1907
Procaine (PRO)[d] (51-05-8)	 $C_{13}H_{20}N_2O_2$ 236	**86**, 99, 120	5.18 (0.03)[#]	1967
Cocaine (COC) (50-36-2)	 $C_{17}H_{21}NO_4$ 303	**82**, 182, 77	5.89 (0.13)[$]	2148
Tetracaine (TTC)[d] (94-24-6)	 $C_{15}H_{24}N_2O_2$ 264	**58**, 71, 176	6.06 (0.15)[$]	2190
Cis-cinnamoylcocaine (c-CNM)[e] (50763-21-8)	 $C_{19}H_{23}NO_4$ 329	**82**, 182, 96	6.62 (0.15)[$]	2329
Delta 9-Tetrahydro-cannabinol (THC)[a] (1972-08-3)	 $C_{21}H_{30}O_2$ 314	**299**, 231, 314	7.06 (0.00)[$]	2443
Trans-cinnamoylcocaine (t-CNM)[e] (50763-20-7)	 $C_{19}H_{23}NO_4$ 329	**82**, 182, 96	7.13 (0.16)[$]	2463
Antioxidant 168 (FOS)[c] (31570-04-4)	 $C_{42}H_{63}O_3P$ 646	**441**, 57, 147	10.16 (0.05)[*]	3390
Antioxidant 1076 (NOX)[c] (2082-79-3)	 $C_{35}H_{62}O_3$ 530	**530**, 219, 57	10.60 (0.06)[*]	3553

[a] Drug mixed with cocaine. [b] GC artifact. [c] Plastic antioxidant. [d] Cocaine adulterants. [e] Cocaine impurity. [$] $n = 2–66$ (from seized samples). [*] $n = 68$. [#] $n = 26$ (from analytical standards with concentrations between 50 and 1000 µg/mL).

are achieved, decreasing compound residence time in the column, and extending the range of compounds analyzed by GC–MS, including thermally labile and high boiling ones [32].

In this paper, we report the use of a short column method for the detection of the plastic antioxidants BHT, FOS, and NOX together with cocaine and other cutting agents by using a standard GC–MS setup. We also show the prevalence of these compounds in a set of apprehended samples. To the best of the authors' knowledge, this is the first report of PAOs as cocaine cutting agents.

2. Material and methods

2.1. Chemicals and reference materials

Methanol ACS reagent and n-hexane 95% ACS reagent were purchased from Dinâmica (Indaiatuba, SP, Brazil), chloroform ACS reagent from Êxodo (Sumaré, SP, Brazil). BHT (4-methyl-2,6-bis(2-methyl-2-propanyl)phenol), FOS (tris[2,4-bis(2-methyl-2-propanyl) phenyl] phosphite), NOX (octadecyl 3-[4–*hydroxy*-3,5-*bis*(2-*methyl*-2-*propanyl*) *phenyl*]propanoate), acetaminophen (ACT), aminopyrine (AMN), benzocaine (BZC), caffeine (CAF), levamisole HCl (LEV), lidocaine (LID), phenacetin (PHN), procaine HCl (PRO), andcertified reference materials of octadecane (C18) and C7-C40 saturated alkanes standard (1000 µg/mL each component in hexane) (C7-C40 mix) were purchased from Sigma-Aldrich (Santo André, SP, Brazil). Cocaine (COC), tetracaine (TTC), ketamine (KET), dipyrone (DIP), orphenadrine (ORP), *cis* and trans-cinnamoyl cocaine (cis-CNM and trans-CNM), and anhydroecgonine methyl ester (AEME) were characterized from seized samples and used as reference materials for qualitative analyses. Table 1 shows the chemical structure, molecular formula, molar mass and the major ions of all compounds investigated by GC–MS.

2.2. Seized samples

A convenience sample comprised of 138 apprehensions by the CP-DF was analyzed. This sample includes whitish powders, which may contain cocaine in the salt form, and yellowish rocks, which

Table 2
Gas chromatographic parameters used in the traditional (TGCM 1 and 2) and short column (SCM) methods.

Method parameter		TGCM 1	TGCM 2	SCM
Injection volume (µL)		1	1	1
Inlet temperature (°C)		280	280	250
Inlet pressure (psi)		10.29	15.99	1.62
Split ratio		20:1	20:1	20:1
Helium gas flow (mL/min)		1	1	4
GC column length (m)		30	30	4
Average gas velocity (cm/s)		37.64	38.87	199.62
Oven temp. program	Initial temp.	100 °C, 1 min	200 °C	50 °C
	Ramp	20 °C/min	15 °C/min	20 °C/min
	Final temp.	312 °C, 4.3 min	300 °C, 1 min	280 °C, 5 min
Total run time (min)		15.9	7.7	16.5
Solvent delay (min)		1.8	2	1
Transfer line, MS source, MS quadrupole temp. (°C)		280, 300, 150	280, 300, 150	280, 300, 150
Scan mass range (*m/z*)		40–55040–750 after 7 min	40–750	40–55040–750 after 9 min

may contain cocaine base derived from coca paste, the most common crack cocaine produced in Brazil [36].

Seized samples were submitted to two preliminary tests as described by UNODC [7]. Solubility test in water was used to differentiate between water-soluble cocaine salt and water-insoluble cocaine base forms, and the Scott test was used as a presumptive indication of the presence of cocaine in the sample.

2.3. GC–MS analyses

Sample analyses were carried out in two different Agilent 7890A gas chromatographs hyphenated to 5975C mass spectrometers (GC–MS) (Agilent Technologies, Santa Clara, CA, USA). Data acquisition and analysis were carried out in Agilent MSD ChemStation and Enhanced ChemStation Data Analysis software [version E.02.02.1431]. Automated Mass Spectral Deconvolution and Identification System (AMDIS) [version 2.73 Build 149.31] and NIST Mass Spectral Search Program (NIST Search) [version 2.3] were used for retention time collection, deconvolution, and identification of the detected analytes. AMDIS parameters were set to *component* mode, *simple* GC–MS data analysis type, and *default* analysis settings. Other mass spectral libraries, including in-house built, were also used to identify the detected analytes.

Samples were analyzed by three different GC methods. Two methods, routinely used in the CI-CP-DF laboratory for sample screening (Traditional GC methods, TGCM), use a 30 m chromatographic column (J&W DB-1 ms Ultra Inert analytical column; 0.25 mm I.D., 0.25 µm film thickness, Agilent, part number 122–0132UI), and differ only by the oven temperature program (7.7 or 15.9 run times) (Table 2). The third method uses a short, 4-meter column, obtained by cutting down the commercial one, which was previously used for the analysis of thermally labile and low volatile compounds by our research group [37].

The three methods shared the following configuration and parameters: ultra-inert split inlet liners (Agilent, part number 5190–2294) with a straight design, glass wool, and a volume of 990 µL, injection volume of 1 µL, a 20:1 split ratio, helium as the carrier gas, full-scan mode acquisition, ionization energy of 70 eV, transfer line, ion source, and quadrupole temperatures set at 280, 300 and 150 °C, respectively. Table 2 shows a comparison of the three method parameters.

2.4. Short column method optimization

During the optimization process, the best solvent that could solubilize all potential components of a seized sample was evaluated. Two 50 mg aliquots of the seized samples were solubilized, one in 1 mL of methanol and the other in 1 mL of chloroform, by 30 s of vortex pulse (Kasvi K45–2820, São José dos Pinhais, PR, Brazil) and 10 min of ultrasonic water bath (SolidSteel SSBu-15 L, Piracicaba, SP, Brazil). For seized samples of less than 200 mg ($n = 6$), two 10 mg aliquots were prepared. Insoluble compounds were decanted by centrifugation (3 min; 10,000 x g), and a 1:10 dilution of the supernatant was prepared in the same solvent for analyses.

Further, the best conditions to reduce sample carry-over at the inlet and at the column were determined, as FOS and NOX are high-boiling-point analytes [21,22] that are retained for a long time in the traditional GC analytical column [26,28]. Five different inlet temperatures (150, 200, 230, 250, and 280 °C) were investigated, and column sample carry-over was challenged by injecting reference materials of BHT, BZC, PHN, ACT, CAF, LID, AMN, LEV, PRO, FOS, and NOX at nine different concentrations (50, 100, 250, 300, 500, 750, 1000, 2500, and 5000 µg/mL) ($n = 26$–68) (same and different days) with three different final temperature hold times (0, 5, and 10 min).

2.5. Short column qualitative method validation

The optimized method was validated according to the International Conference on Harmonisation Validation of Analytical Procedures: Text and Methodology Q2(R1) [38].

Specificity was verified by checking control samples (seized material with no analyte present) for any interference peak eluting at the same retention time as the compounds listed in Table 1.

Limit of detection (LOD) was administratively set at the lowest point of the calibration curve (5 µg/mL), for the purpose of the proposed qualitative method.

Repeatability of retention time was verified by comparing retention times obtained from multiple injections of reference material and seized samples (same and different days) at various analyte concentrations (50 – 5000 µg/mL) and was expressed as percentage relative standard deviation,%RSD.

Linear retention index (LRI), a dimensionless value of a compound retention time on a gas chromatographic column relative to the retention time of a homologous series of n-alkanes run under the same conditions [39], is used to compensate for retention time variations due to major equipment maintenance. LRIs were determined (Eq. (1)) by injecting a working solution of C18 (1000 µg/mL) followed by a working solution of C7-C40 mix (100 µg/mL), prepared in chloroform and hexane, respectively.

$$LRI_x = 100\left(\frac{t_x - t_n}{t_{n+1} - t_n} + n\right) \quad (1)$$

Where LRI_x is the linear retention index of compound *x*, t_x is the retention time of the compound *x*; t_n is the retention time of the

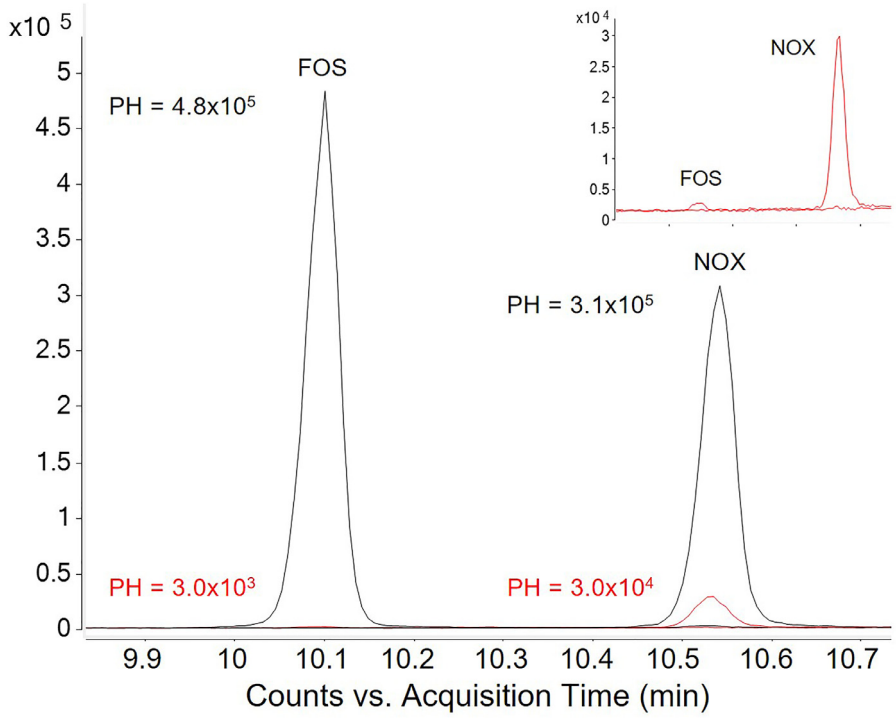


Fig. 1. Antioxidants 168 (FOS) and 1076 (NOX) solvent solubility comparison by total ion chromatogram peak heights (PH). Chloroform (black) and methanol (red) FOS and NOX solutions at 500 µg/mL were analyzed by the short column GC–MS method. Inset figure shows a zoomed scale for methanol solutions. Inlet temperature: 250 °C. Other method parameters as described in Material and Method section.

preceding n-alkane, t_{n+1} is the retention time of the subsequent n-alkane, and n is the number of carbons of the preceding n-alkane.

Peak width, tailing factor, and resolution were estimated by Agilent software. Mass loading was assessed by determining peak tailing [40] of serial dilution solutions of BHT, BZC, PHN, CAF, LID, AMN, FOS, and NOX (50 to 5000 µg/mL) dissolved in chloroform.

2.6. FOS and NOX semi quantitative analysis

FOS and NOX concentration in seized samples were semi quantitatively estimated by comparison with calibration curves at concentrations from 50 to 5000 µg/mL (9 calibration points, $n = 3$).

3. Results and discussion

3.1. Preliminary characterization of seized samples

The Scott colorimetric test showed that 111 samples behaved as positive, 26 as negative, and one as inconclusive. Eight white powder positive samples were found to be false positives for cocaine after GC–MS and FT-IR analyses. Based on GC–MS results, six of the false positive samples contained lidocaine alone (3) or lidocaine mixed with tetracaine and other cutting agents (3), one contained caffeine, orphenadrine and dipyrone, and another caffeine and orphenadrine. Lidocaine and tetracaine are known substances that falsely give a positive color reaction to the Scott test [41]. Caffeine, orphenadrine and dipyrone are active substances in medications for muscle pain and tension relief and are also known to give false positives [42]. Fifteen samples did not contain any analyte under investigation and are not discussed further in the paper. Solubility tests suggested that of the 103 cocaine-containing samples, 65 were cocaine salt and 38 were cocaine base. Physical appearance (whitish powder or yellowish rock) corroborated solubility test results.

3.2. Short column method optimization

NOX is sparingly soluble (0.6 g/100 g) and FOS is practically insoluble in methanol (<0.01 g/100 g), and both are best solubilized in chloroform [43,44]. Cocaine and its adulterants are normally solubilized in methanol for routine GC–MS analysis in the laboratory, but they are also soluble in chloroform, while ACT and PRO are slightly soluble. Fig. 1 shows the chromatograms of FOX and NOX mixtures dissolved in methanol or chloroform, showing a much higher peak when chloroform was used. Chloroform also reduces the presence of diluents not detectable by GC–MS, such as carbohydrates, creatine, boric acid and carbonates/bicarbonates, which minimizes the accumulation of sample residues at the inlet and in the column head. Moreover, chloroform boiling point (61.1 °C), and vapor volume (481 µL versus 955 µL of methanol) are more suitable for the proposed method conditions (1 µL injection, 250 °C inlet temperature, 1.62 psi inlet pressure), which are discussed further. A comparison of analytes other than FOS and NOX diluted in chloroform and in methanol suggested that qualitative differences are negligible or related to sample inhomogeneity (data not shown).

Inlet temperatures at 230 °C or 250 °C and a five-minute hold time at the end of the chromatographic run were sufficient to reduce PAOs' (Figures S1 and S2) and cocaine adulterants' (data not shown) signals to noise levels, eliminating carry-over interferences at any tested analyte concentration level. Therefore, the short column GC–MS method's final configuration was established with a 250 °C injection port temperature and a total run time of 16.5 min. The combination of a short column method with the injection port temperature at 250 °C, carrier gas flow of 4 mL/min, and a 20:1 split injection contributed to a fast and efficient vaporization of the compounds, producing symmetric and narrow peaks, which increased analyte resolution, as shown in Table 3.

Fig. 2A and 2B exemplify the column carry-over of FOS and NOX using the traditional methods, when their ghost peaks were

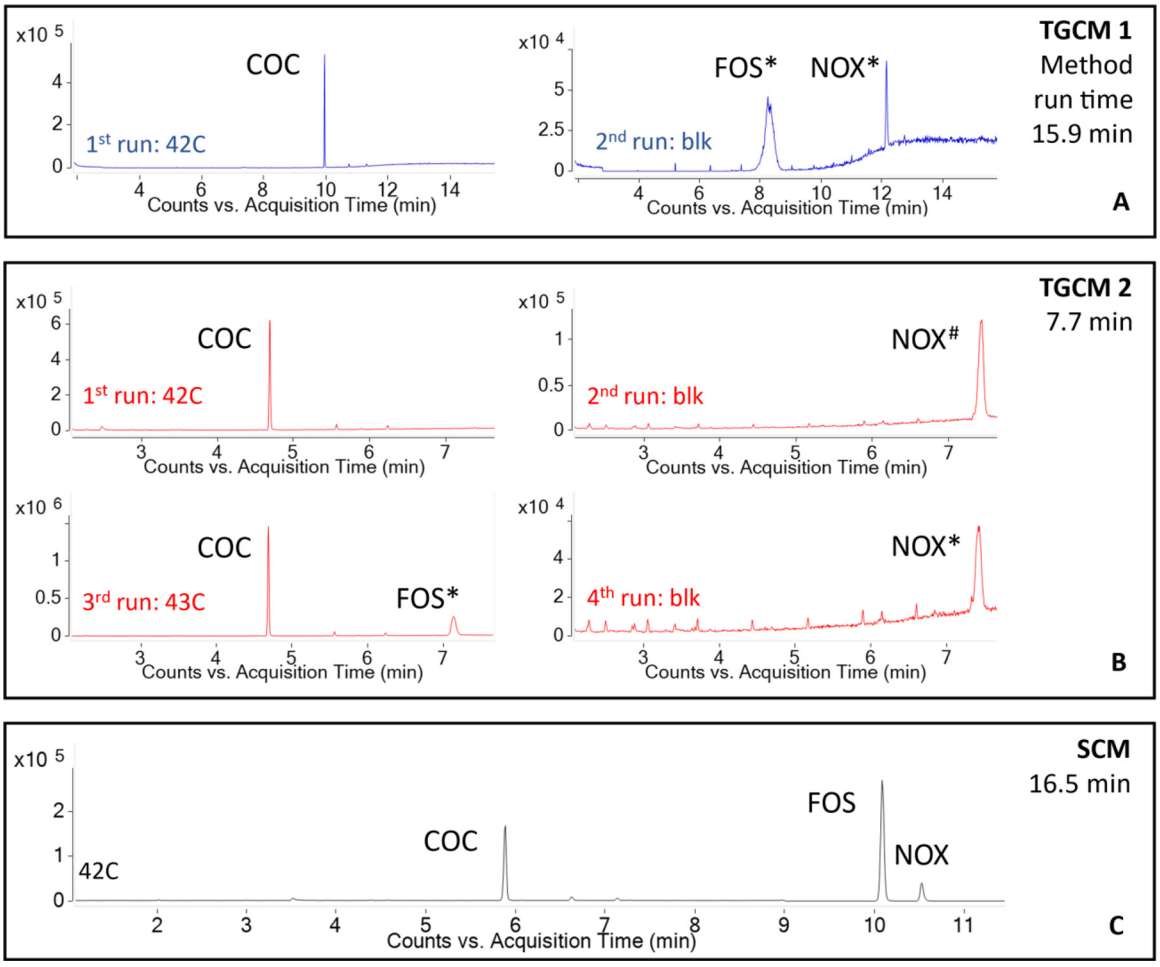


Fig. 2. Column carry-over of antioxidants 168 (FOS) and 1076 (NOX) in the 30 m long analytical column. With (A) traditional method 1 (TGCM 1) and (B) traditional method 2 (TGCM 2), FOS and NOX from seized sample 42C are retained for one to three subsequent runs. When analyzed by the 4 m short column method (SCM) (C), analytes were all detected in less than 11 min. Sample injection sequence: 39C, blank (blk), 42C, blk, 43C, blk. FOS and NOX from samples 39C (#) and 42C (*) are presented in the total ion chromatograms.

Table 3
Inlet temperature influence on chromatographic parameters of a 4 m column GC–MS method for FOS and NOX analysis.

Analyte	Inlet temperature (°C)	Peak width (min)	Tailing	Resolution
FOS	150	0.091	2.26	
NOX		0.163	2.81	2.24
FOS	200	0.052	1.14	
NOX		0.061	1.92	4.65
FOS	230	0.046	1.03	
NOX		0.049	1.15	5.52
FOS	250	0.045	1.09	
NOX		0.048	1.39	5.61
FOS	280	0.045	1.09	
NOX		0.049	1.12	5.59

FOS: antioxidant 168, NOX: antioxidant 1076.

observed after more than 22 and 28 min of sample injection, respectively. FOS and NOX remained in the column for one (TGCM 1) to three (TGCM 2) subsequent runs, contaminating either the next blank (TGCM 1) or the next sample and subsequent blank (TGCM 2).

In the short column, analytes have a lower residence time and elute at lower temperature, allowing FOS and NOX to be seen together with cocaine in a single run (Fig. 2C). Additionally, the vacuum at the outlet of the GC–MS column allows the inlet to be set to a sub-atmospheric pressure (0.11 atmospheric pressure at 50 °C, the initial oven temperature), which creates a low-pressure condition in the column, enhancing carrier gas diffusivity, and further increasing the optimum carrier gas velocity (199.62 cm/s at 50 °C) [33,45]. These special conditions reduced FOS volatilization temperature to 254 °C and NOX to 265 °C.

All detected compounds presented peak resolution (R_s) higher than 1.5 (complete baseline separation), except for the analyte pairs PHN/ACT (Rs=0.55), and AMN/LEV (R_s=0.85), whose identifications were achieved by mass spectral deconvolution done by AMDIS.

3.3. Short column qualitative method validation

Interference peaks were not observed when control samples were analyzed by the optimized short column method, indicating good selectivity. FOS and NOX detection limits were administratively established at 5 µg/mL, the lowest concentration tested. At this concentration, FOS and NOX signal to noise ratios (s/n) were 12 and 37, respectively. The other analytes had higher s/n, but their detection limits were administratively established at the same concentration level since analytes at this concentration will account for 0.1% of the sample mass. Seized samples were 1:10 diluted for analysis in a 20:1 split mode injection and the short column method was optimized in full-scan mode; therefore, there is room for sensitivity increase for a quantitative method optimized in the selected ion monitoring mode in the future.

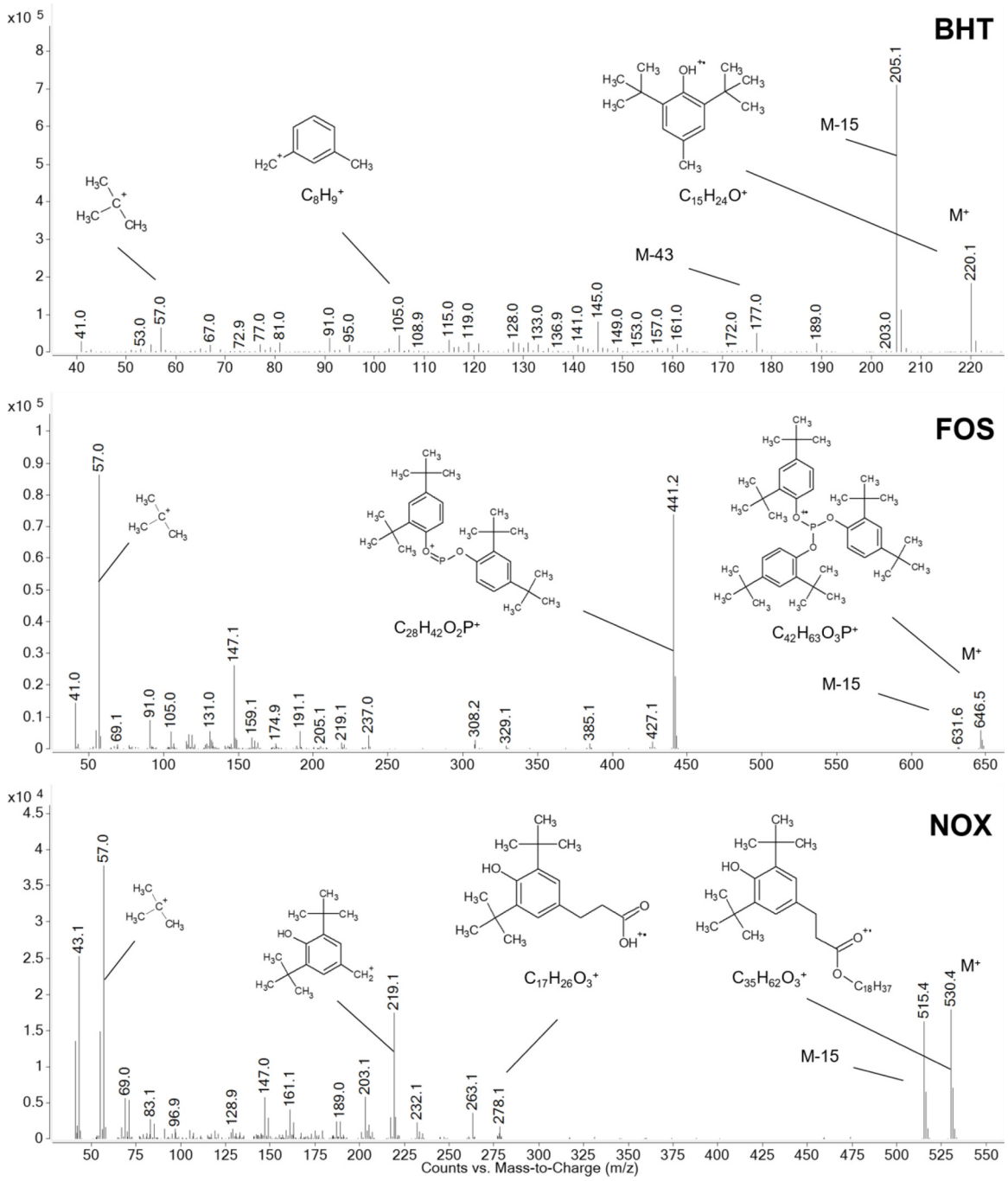


Fig. 3. Electron ionization mass spectra of the plastic antioxidants BHT butylated hydroxytoluene, FOS antioxidant 168, and NOX antioxidant 1076.

The impact of analyte concentration on retention time was also evaluated and variation was negligible. ACT had the highest variation (RSD of 1.56%), while BHT, FOS, and NOX had an RSD of 0.05–0.06% across the whole concentration range (Table 1).

Retention time repeatability in the optimized short column method was impacted after major maintenance when liner and column were changed. C7-C40 n-alkanes mix was run in each batch and used for LRI computation. The mix was spiked with C18 to facilitate the identification of the other n-alkanes present in the mix (Fig. S3). LRI values were very similar before and after maintenance (Fig. S4) and were used to minimize the observed variations in the retention times, improving the ability to correctly identify the compounds. Average retention times and LRI of BHT, FOS, and NOX in the short column method were determined as 2.72 min (1482); 10.16 min (3390); and 10.60 min (3553) (Table 1).

Mass overloading started at sample concentration of 750 µg/mL for BZC, PHN, CAF, and LID, and of 2500 µg/mL for AMN and BHT. It was not observed for FOS and NOX even at the highest concentrations tested (5000 µg/mL).

3.4. PAO electron ionization mass spectra

The electron ionization mass spectra of the three PAOs and their major ions are shown in Fig. 3. BHT, FOS, and NOX are 1,3-di–tert-butylbenzene derivatives and share some similarities in their fragmentation pathways. A loss of a methyl radical from one

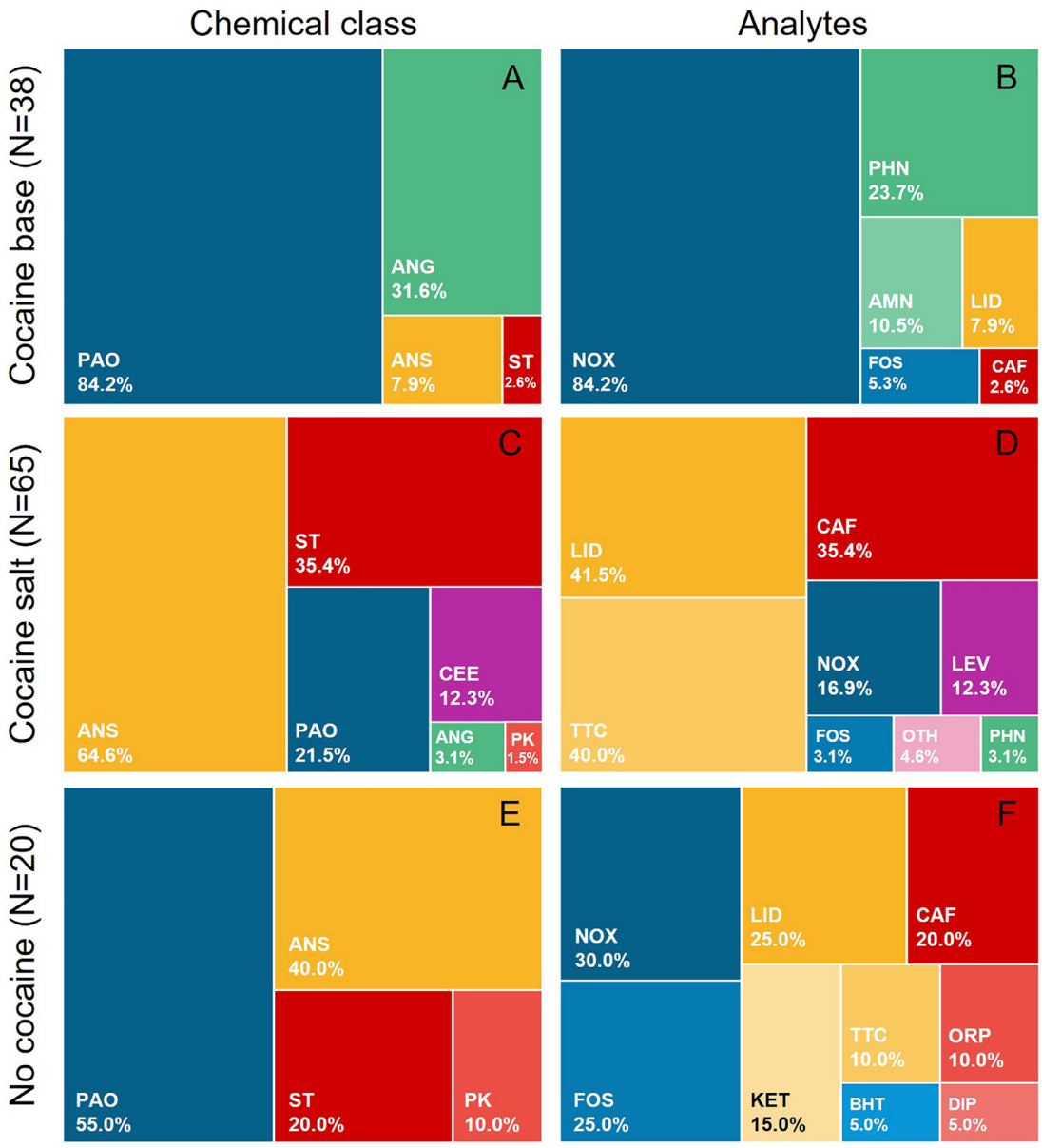


Fig. 4. Distribution of cocaine cutting agents (CCA) detected in samples seized by the Civil Police of the Brazilian Federal District. Percentages are related to the number of samples in each group. Cocaine base (A) CCA chemical classes, and (B) analytes. Cocaine salt (C) CCA chemical classes, and (D) analytes. Samples without cocaine (E) CCA chemical classes, and (F) analytes. PAO plastic antioxidants, ANG analgesics, ANS anesthetics, ST stimulants, CEE cocaine effect enhancer, PK pain killer, NOX antioxidant 1076, FOS antioxidant 168, PHN phenacetin, AMN aminopyrine, LID lidocaine, CAF caffeine, TTC tetracaine, LEV levamisole, BHT butylated hydroxytoluene, KET ketamine, ORP orphenadrine, DIP dipyrone, OTH others.

of their tert–butyl groups leads to $[M\text{-}CH_3]^+$ ions (*m/z* 205, 631, and 515, respectively). Their EI-MS also share a tert–butyl cation (*m/z* 57).

In the BHT fragmentation, loss of 43 Da ($C_3H_7\bullet$) from the molecular ion, presumed to involve migration of a CH_2 group from the tert–butyl group to the oxygen or ring, yields a *m/z* 177 fragment. Ion series *m/z* 51, 65, 77, and 91 strongly indicate the presence of a benzyl instead of a benzoyl group in the *m/z* 105 fragment, therefore a dimethylbenzene ion.

The FOS mass spectrum is dominated by the *m/z* 441 fragment formed by the loss of one of its monomers linked by the phosphorous atom, while the NOX *m/z* 278 fragment is formed by the classic McLafferty rearrangement, with the loss of the alkyl portion of the ester and hydrogen migration from the alkyl group to the carboxyl moiety.

3.5. Analyses of seized samples

At least one compound was detected in the 123 seized samples (65 cocaine salt samples, 38 cocaine base samples, and 20 samples with no detected cocaine) analyzed by the optimized short column method; up to 7 analytes were detected in a single sample and cutting agent diversity was greater in cocaine salt (10 different substances) than in cocaine base (6) (Tables S1 to S3). Fig. 4 shows the distribution of the cutting agents detected in the seized samples. PAOs were by far the most common chemical class detected in cocaine base samples, with 84.2% of the seized samples containing NOX, the third most common chemical class detected in cocaine salt samples (21.5%) and the most common cutting agent detected in samples without cocaine (55.0%). Analgesics (31.6%; PHN and AMN) were the second most prevalent adulterant class in co-

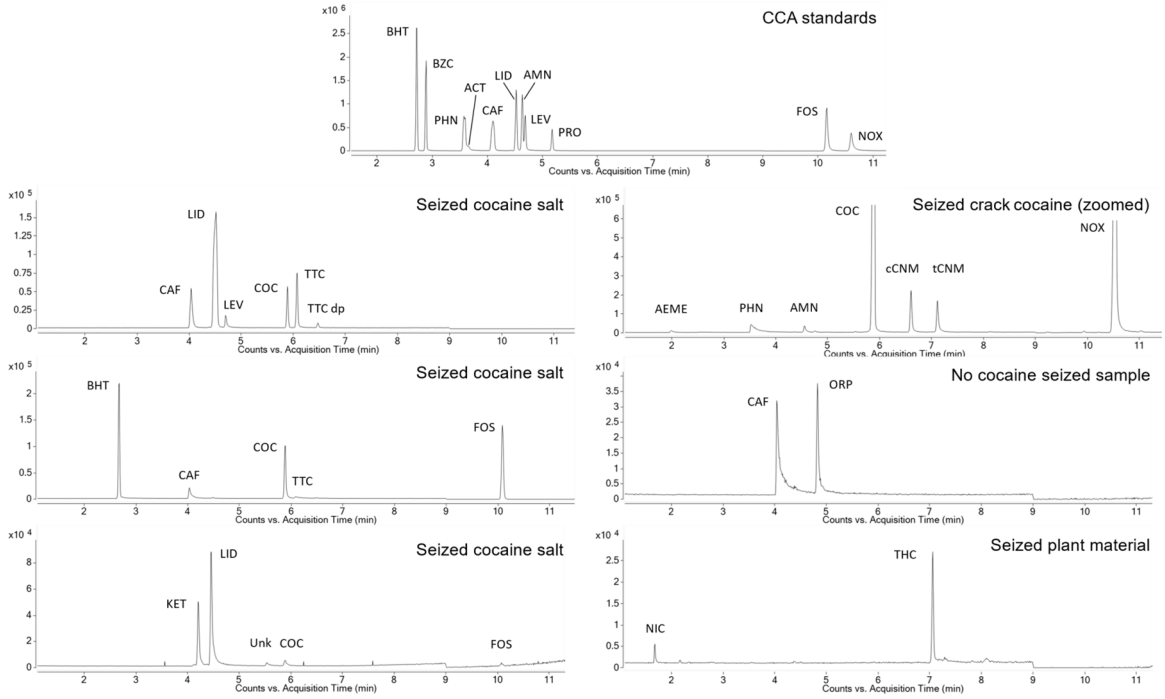


Fig. 5. Examples of total ion chromatograms (TIC) of cocaine cutting agents (CCA) standards, seized samples containing cocaine salt, cocaine base, or no cocaine together with CCA, and a seized sample of plant material with nicotine (NIC) and tetrahydrocannabinol (THC) which sometimes is used as a substrate to smoke crack cocaine. TIC were obtained by the optimized short column GC–MS method. Inlet temperature: 250 °C. Other method parameters as described in Material and Method section. BHT: butylated hydroxytoluene, BZC: benzocaine, PHN: phenacetin, ACT: acetaminophen, CAF: caffeine, LID: lidocaine, AMN: aminopyrine, LEV: levamisole, PRO: procaine, FOS: antioxidant 168, NOX: antioxidant 1076, COC: cocaine, TTC: tetracaine, TTC dp: TTC degradation product, KET: ketamine, AEME: anhydroecgonine methyl ester, c/t-CNM: *cis*/trans cinnamoylcocaine, ORP: orphenadrine.

caine base samples, while anesthetics LID and TTC were present in more than 60% of cocaine salt samples, followed by the stimulant CAF (35.4%). AMN (10.5%) was only observed in cocaine base samples, while TTC (40.0%) and LEV (12.3%) were only detected in cocaine salt samples (Fig. 4). GC–MS chromatograms of seized samples containing at least one compound listed in Table 1 are shown in Fig. 5.

This is the first study to detect PAOs in cocaine-based products, but various studies conducted in Brazil and other countries analyzed seized samples of cocaine and its cutting agents. Most of the seized samples of cocaine base included in this work are derived from coca paste, therefore, impurities and cutting agents detected in Brazilian crack cocaine reflected more closely those detected in coca paste than those commonly found in cocaine salt samples, which in turn is the starting material for production of crack cocaine in Europe and the USA [36,46].

Grobério et al. [47] and Maldaner et al. [48] observed a similar difference in adulterant frequency between cocaine salt and cocaine base samples to what was found in this study. Grobério et al. [47] analyzed 1085 cocaine samples seized in seven Brazilian states between 2009 and 2013, 805 classified as cocaine base and 280 as cocaine salt. Samples were drawn from large seizures ($>$ 5 kg), with individual samples weighing more than 0.5 kg and having a high cocaine content, suggesting that any adulteration found in the samples have been added in the upper levels of the drug trafficking chain. PHN and AMN were the major adulterants in cocaine base (41.1 and 13.6%, respectively), while LEV (7.9%) was the prevalent one in cocaine salt samples.

In the study conducted by Maldaner et al. [48], 642 street level cocaine samples seized in five Brazilian states between 2011 and 2014 were analyzed. PHN was present in 54% of 411 cocaine base samples, while CAF (58%) and LID (37%) were the most common adulterants in the 65 cocaine salt samples. Cocaine concentrations in the cocaine base samples were high (average content of 66%) and similar to seizures from international trafficking, while cocaine salt samples had lower cocaine content, suggesting that a significant cutting process took place before seizures.

Grobério and Maldaner's works indicate that PHN and AMN are adulterants added to cocaine base in the upper levels of the drug trafficking chain (wholesale market), while LID and CAF might be added to cocaine salt at lower distribution levels (retail market). Samples without cocaine analyzed in the present work seem to corroborate this hypothesis, since CAF and LID are amongst the most common adulterants detected in the samples, while PHN and AMN were not detected. These samples were apprehended in clandestine laboratories and most likely would be added to cocaine salt before going to the street market.

PAO pure samples have been apprehended in Brazilian clandestine drug laboratories, which may suggest that FOS and NOX are added at the lower distribution levels close to the retail market. However, most of the cocaine base samples also contained those compounds, which contradicts this hypothesis, although they were also detected in salt cocaine products. As PAO is a newly detected family of cocaine cutting agents, other studies are needed to better understand at which point they are added in the cocaine drug trafficking chain.

The possibility that the PAOs found in cocaine seized samples are contaminants that have migrated from the plastic material used in the packaging was considered. BHT, FOS, and NOX are minor components of plastic material, normally present at levels lower than 1% (w/w) [14–16]. In this study, FOS was detected in 10 and NOX in 49 of the 138 analyzed samples, with median concentrations estimated as 49 and 84 g/kg, respectively (Fig. 6), which is at least 5 times higher than the expected concentration in the plastic material itself. Therefore, our results indicate that PAOs were intentionally added to the seized cocaine samples.

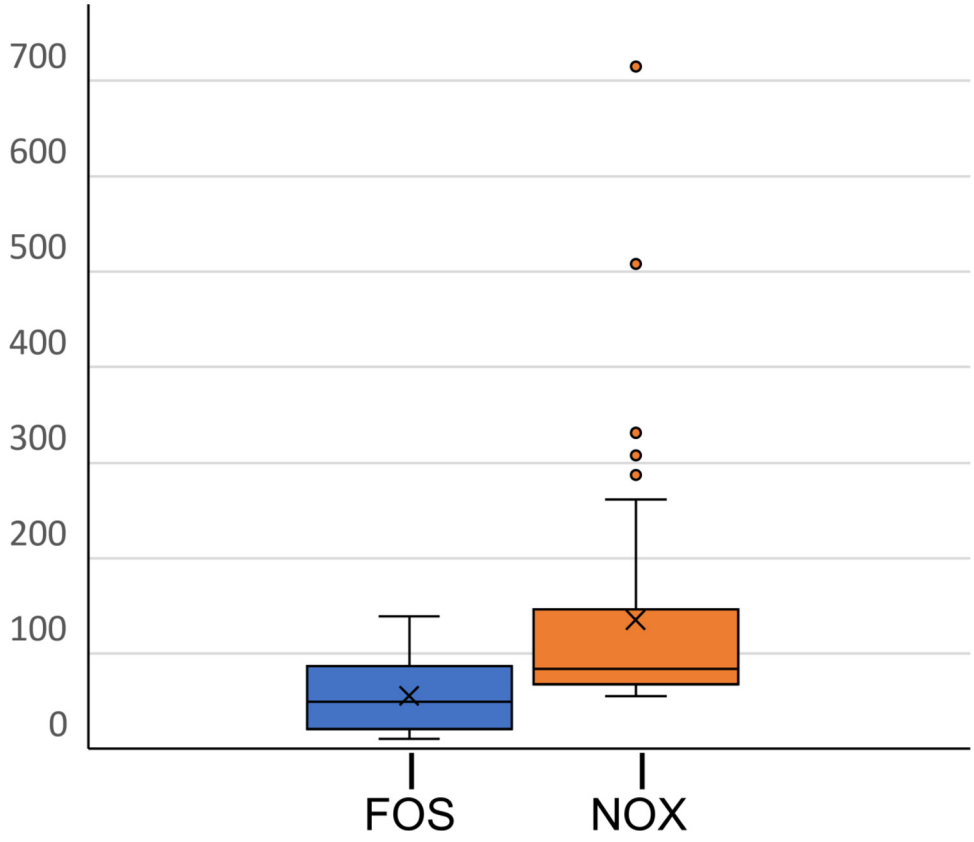


Fig. 6. Estimated concentrations (g/kg) of FOS and NOX detected in seized samples.

4. Conclusions

A short column qualitative GC–MS method was optimized and validated for the detection of the high-boiling-point compounds FOS and NOX, BHT, cocaine, and another 16 cutting agents commonly found in seized cocaine samples. PAOs were detected in 84.2% of the cocaine base, 21.5% of the cocaine salt and 55.0% of the samples with no detected cocaine. BHT, FOS, and NOX mass spectra presented intense molecular ions and a specific mass fragmentation pattern, which makes it easier to obtain good matches with commercial and in-house databases. To the best of our knowledge, this is the first report of plastic antioxidants in seized cocaine products.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.463170.

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