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Article

Development and Validation of a Gas Chromatographic Method for the Quantification of Minor Alkaloids in Cocaine

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The quantification of minor alkaloids in seized cocaine can provide information for drug profiling and law enforcement initiatives. This work presents the development and validation of an analytical method to quantitate minor alkaloids in cocaine samples using gas chromatography with flame ionization detector, after derivatization. The method was validated in accordance with the ISO/IEC 17.025:2017 requirements. The calibration was linear (determination coefficient (R^2) ≥ 0.998), ranging from 1.0 to 3,500 mg L^{-1} for all target analytes with suitable selectivity and precision (relative standard deviation lower than 10%). The method showed stability and robustness with respect to analytical parameter variations and presented good accuracy (recovery ranging from 90 to 108%). The method was considered adequate to routine forensic analysis in simultaneous quantification of anhydroecgonine methyl ester, anhydroecgonine, methylecgonine, tropacocaine, norcocaine, *N*-formylcocaine, trimethoxycocaine, ecgonine, benzoylecgonine, *trans*- and *cis*-cinnamoylcocaine. Eleven cocaine samples seized in different Brazilian regions were analyzed and their relative amounts of tropacocaine and trimethoxycocaine with respect to cocaine indicate both the possible varieties of coca leaf used for cocaine production and the likely origin of drug samples (Bolivia/Peru or Colombia). The contents of other minor alkaloids depict aspects of sample history such as purification by oxidation, hydrolysis and dehydration by thermal processes.

Keywords: validation, gas chromatography, cocaine, chemical profiling, minor alkaloids

Introduction

The Brazilian Federal Police (BFP) has been implementing, since 2006, a chemical profiling program for illicit drugs (PeQui project) designed to provide both accredited forensic reports and scientifically based police intelligence/investigative information.[1] The BFP PeQui project mainly deals with samples from international trafficking cocaine seizures, usually involving high amounts (tens to thousands of kg) and high purity (low cut). In this context, the geographic origin of drugs represents one of the most strategic pieces of information for law enforcement agencies.[2]

The article of Broséus *et al.*[3] reviewed the available literature regarding major components found in cocaine samples, suggesting that drug cutting (adulteration) in international trafficking is likely to be done close to the production site, and so could be used as a tool to determine typical *modus operandi* connected with the drug origin. Towards that goal, the PeQui program has implemented a cocaine major alkaloid and pharmaceutical adulterant quantification analysis on a routine basis,[1,4] and a method to identify cocaine sample correlations by occluded solvents.[5]

Besides major components and residual solvents, other alkaloids may be found in small quantities in both the coca leaves and in the final drug product. Their presence and relative amounts will vary with factors such as plant taxonomy, processing steps (extraction, purification, etc.), transportation and storage conditions.[6,7] Some important articles have reported cocaine profiling and origin determination since the 1990's, using different analytical approaches. Ehleringer *et al.*[8,9] have consistently applied minor alkaloid quantification and isotopic ratio analysis

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to indicate probable regions and/or countries of coca leaf cultivation.

Since cocaine minor alkaloid determination has also been used to establish correlations among samples from different seizures,[10-15] it was considered to be the next natural step in the development of the PeQui program, with the aim to complement the analytical tools already available and strength forensic evidences and intelligence data.

In the quantification of cocaine minor alkaloids, gas chromatography (GC) using flame ionization (FID) or mass spectrometry (MS) detection following a derivatization step has been shown to be the analytical technique of choice. The USA Drug Enforcement Administration Agency (DEA) uses GC-FID (chromatographic impurity signature profile analysis, CISPA) in the quantification of the alkaloidal impurities *cis*- and *trans*-cinnamoylcocaine, tropacocaine and trimethoxy-substituted analogs of cocaine. The derivatization step is performed using a *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide/chloroform (MSTFA/$CHCl_3$) 1:1 solution, kept at 75 ºC for 30 min. The CISPA/DEA method utilizes an in house synthetized internal standard (ISTD) derived from cocaine (*p*-fluorococaine) to improve the precision of the quantitative analysis and is performed in a specific GC column (14% cyanopropylphenyl/86% dimethyl polysiloxane phase, e.g., DB-1701).[6] In more recent articles,[11] the DEA/USA reports the quantification of two individual minor alkaloids (tropacocaine, and trimethoxycocaine) and the class of the truxillines to be used as origin indicators in cocaine profiling.

Esseiva *et al.*[12] pointed out that some cocaine minor alkaloids profiling methods in Europe have been moved from GC-FID to GC-MS analysis, due to advances in analytical performance (e.g., resolving power and selectivity). Commercially available *n*-heneicosane was used as ISTD and the derivatization step was performed with the use of a $CHCl_3$/pyridine 5:1 solution and 100 μL of MSTFA that was kept at 80 ºC for 1 h. Quantitative analysis was performed in a 5% phenyl/95% dimethyl polysiloxane phase (e.g., DB-5) GC capillary column and MS analysis.

In the development of this work, a pragmatic approach has been taken by choosing among the available options in the literature those that would be fit for purpose of the Brazilian Federal Police lab. It took into consideration that the use of a GC-FID equipment leads to sturdier calibration curves as compared to GC-MS. Also, the employment of DB-1 chromatographic columns for development and routine analysis, pyridine/MSTFA in the derivatization step and *n*-heneicosane as internal standard aimed to combine and improve the main features of the reference methods (Table S1, Supplementary Information (SI) section).

It was also considered important to quantitate the largest number possible of minor alkaloids in cocaine samples, since they are known to bring relevant forensic and law enforcement information.[11] Such method has the potential to be used in important police intelligence applications such as seizure correlations, coca leaf origin inference and characterization of the physicochemical history of the samples (e.g., cocaine hydrolysis, oxidation, extraction steps, acid-base reactions, etc.). The minor analytes quantified in this study are shown in Scheme 1.

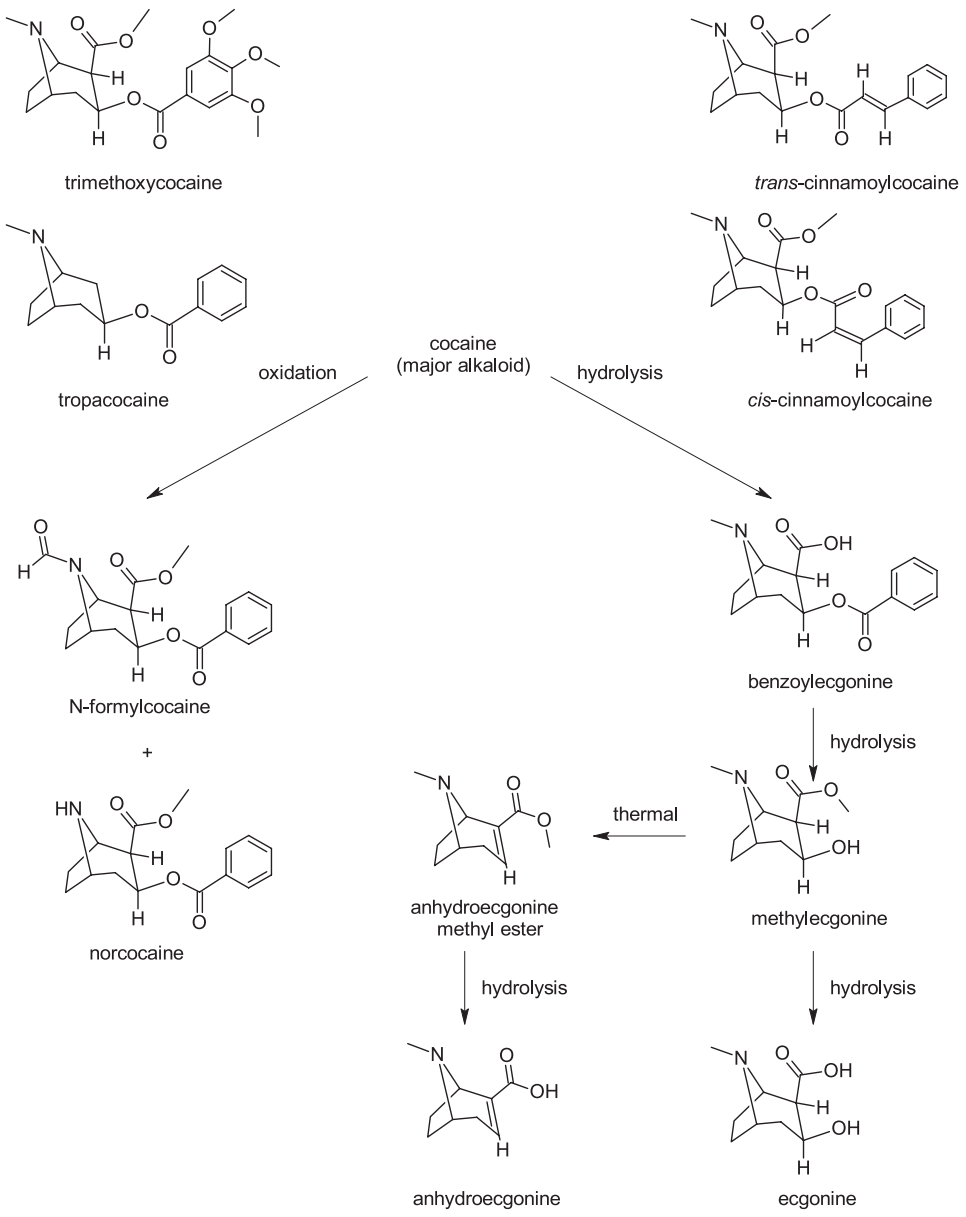
As the proposed method to quantify minor alkaloids in cocaine samples does not use the same experimental conditions as those previously published in the specialized literature, it was considered necessary to fully validate the process to meet the BPF lab quality assurance requirements. For the first time in the specialized literature, all the relevant validation parameters to quantify minor alkaloids in cocaine were studied and published (specificity/selectivity, linearity, accuracy, precision (repeatability and intermediate precision) and robustness), as well the data was analyzed by suitable statistical tests.

A set of 11 real case cocaine samples seized in different Brazilian regions were analyzed with the developed BFP cocaine minor alkaloids method. As a local preliminary analysis and following the classic literature guidance, the relative amounts of tropacocaine (TRO) and trimetoxycocaine (TRI) were used to indicate the possible varieties of coca leaf used for cocaine production and to also indicate the origin of the drug samples (Bolivia/Peru or Colombia).[11] The same samples were analyzed by the DEA/USA Cocaine Signature Program[6] and the origin assignment results were compared with the GC-FID quantitative results of TRO and TRI obtained locally. *N*-Formylcocaine (NFC), norcocaine (NOR) and *cis/trans*-cinnamoylcocaine (*c*CC/*t*CC) content were used to indicate purification process by oxidation.[7] Ecgonine (EC), benzoylecgonine (BE) and methylecognine (ME) are indicators of the degree of sample hydrolysis[7] and, finally, the dehydration products anhydroecgonine (AE) and anhydroecgonine methyl ester (AEME) were used to indicate that samples could be submitted to thermal process.[16]

Experimental

Standards and chemicals

Anhydroecgonine.HCl (95.9%), benzoylecgonine.$4H_2O$ (79.4%), ecgonine.HCl (99.3%), *N*-formylcocaine (96.3%), methylecgonine.HCl (92.0%), norcocaine.HCl (90.9%), 3,4,5-trimethoxycocaine.HCl (94.0%), tropacocaine.HCl (99.7%) were purchased from NMI (Lindfield, Australia);



Scheme 1. Cocaine minor alkaloids analyzed in this work.

benzoylecgonine (99.9%) and methylecgonine (83.0%) were purchased from Lipomed (Arlesheim, Switzerland); *trans*-cinnamoylcocaine (99.8%) was provided by the DEA (Dulles, USA, Special Testing and Research Laboratory (STRL)) and anhydroecgonine methyl ester (99.3%) was provided from the Federal University of Rio Grande do Sul (UFRGS, Porto Alegre, Brazil). A high purity hydrochloride cocaine control sample (CCS) seized by the BFP (Brasília, Brazil) was homogenized and used as a working control in some experiments. Cocaine (COC) and *cis/trans*-cinnamoylcocaine (*c*CC/*t*CC) contents in CCS are 93.22, 0.75 and 0.96%, respectively. MSTFA (*N*-methyl-*N*-TMS-trifluoroacetamide) was purchased from Sigma-Aldrich (St. Louis, USA). The analytical stock solutions of each analyte were prepared by dilution of reference materials with chloroform ($CHCl_3$) high performance liquid chromatography (HPLC) grade (Tedia Brazil, Rio de Janeiro, Brazil).

The internal standard solution was prepared with *n*-heneicosane (Acros Organics, New Jersey, USA) at 0.3 g L^{-1}, dissolved in a 5:1 (v/v) mixture of $CHCl_3$/pyridine (Vetec, Duque de Caxias, Brazil) and kept under refrigeration.

Instrumental

The GC analyses were conducted in an Agilent Technologies 6890N gas chromatograph with a flame

ionization detector (GC-FID), and an Agilent Technologies 7693A Series auto sampler, according to the following conditions: injection volume: 1.0 μL; split ratio: 50:1; chromatographic column: DB1MS methyl siloxane, 35 m × 200 μm (inner diameter) × 0.33 μm film thickness; oven temperature program: 160 °C for 1 min, 4.0 °C min^{-1} to 200 °C, 6.0 °C min^{-1} to 275 °C for 6.5 min; injection port temperature: 230 °C; FID temperature: 320 °C; carrier gas flow rate: 1.2 mL min^{-1} (helium).

Qualitative GC-MS analysis were carried out on the same equipment, in a parallel injector coupled with a mass spectrometer Agilent 5973 Inert (70 eV), using the same chromatographic conditions.

Infrared spectroscopy (FTIR/ATR-Nicolet iS10 model, equipped with a SMART iTR accessory) and qualitative spot tests analyses were used to distinguish cocaine base from cocaine hydrochloride samples.

Sample and standards preparation

The cocaine samples were analyzed by weighing approximately 10 ± 0.5 mg of the homogenized powder, in crimp top 2 mL glass vials. Each sample was analyzed in triplicate. To the samples were added 500 μL (Gilson Pipetman P1000) of the internal standard solution and 100 μL (Gilson Pipetman P100) of MSTFA. The vial was sealed and the solution was heated at 80 ºC in a thermoblock (Boekel Scientific) for 1 h prior to direct injection into the chromatographic system. The internal standard solution was also used both to dissolve the minor alkaloid reference materials and to dilute the stock solutions used in the preparation of the analytical curves.

Data analysis

Integration of the selected peak areas were performed using software MSD ChemStation-Enhanced Data Analysis,[17] from Agilent Technologies. Quantitative statistical data analysis was performed in Microsoft Excel 2019.[18] All results are expressed as non-derivatized analytes.

Validation methodology and acceptance criteria

The following analytical parameters were investigated during method validation: selectivity, linearity, limits of detection and quantification, precision (repeatability and intermediate precision), accuracy and robustness. The BFP quality management system acceptance criteria are presented Table 1.

Table 1. Figures of merit and acceptance criteria

Figure of merit	Quality management system acceptance criteria
Selectivity	chromatographic peak resolution (R_S): $R_S \geq 2$ the derivatization blank must not present any extraneous peak coeluting with the analytes or the internal standard
Linearity	coefficient of determination (R^2) ≥ 0.998
Precision (repeatability)	relative standard deviation (RSD) among replicates lower than 5%
Intermediate precision	relative standard deviation (RSD) among replicates lower than 10%
Robustness	relative error lower than 10%
Accuracy	recovery between 90 and 110% of target concentration
Stability	relative error lower than 5%

Results and Discussion

Selectivity

Figure 1 presents a typical GC-FID of a cocaine hydrochloride control sample (CCS). Minor cocaine alkaloid peaks are separated enough to assure resolution (R_S) above the acceptance criteria (equation 1).

$$R_S = 2 \times \frac{t_{r_1} - t_{r_2}}{w_{b_1} - w_{b_2}} \quad (1)$$

where t_{r_1} and t_{r_2} represent, respectively, the retention times of analyte 1 and 2, and w_{b_1} and w_{b_2} stand for analyte 1 and 2 peak widths, respectively.

When the pharmaceutical products typically used to adulterate cocaine were added to the sample, it was noted that only phenacetin and ecgonine-2TMS (TMS: trimethylsilyl derivatized analyte) peaks have resolution below the acceptance criteria (R_S = 1.2). As phenacetin is previously quantified throughout the major component methodology in PeQui program prior minor alkaloid analysis, the integration performance can be evaluated if necessary. Table S2 (SI section) shows the results to selectivity to all peaks integrated.

Analytical curves, range and linearity

Method linearity and linear range were assessed by performing triplicates at seven reference material concentration levels. The analytical curves were built with the help of two stock solutions (SS): SS1 containing the alkaloids typically found at low concentrations (anhydroecgonine methyl ester, anhydroecgonine

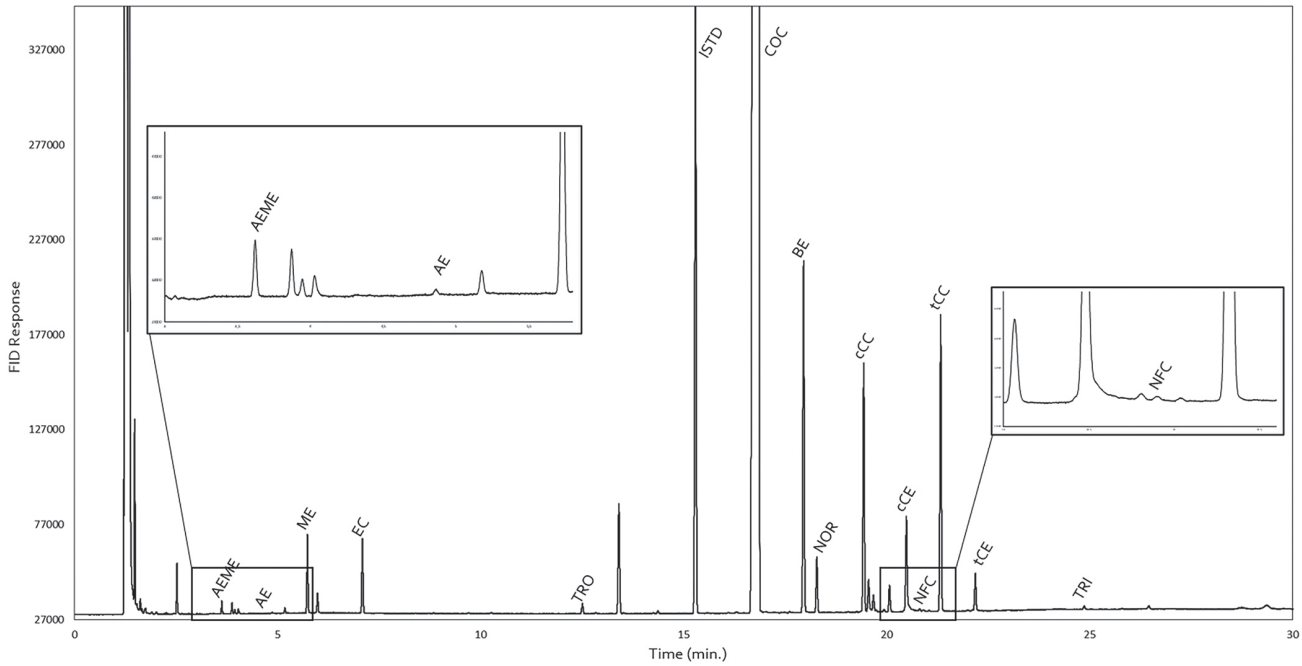


Figure 1. Typical GC-FID chromatogram of a derivatized hydrochloride cocaine sample, where: AEME: anhydroecgonine methyl ester; AE: anhydroecgonine-TMS; ME: methylecgonine-TMS; EC: ecgonine-2TMS; TRO: tropacocaine; ISTD: internal standard (*n*-heneicosane); COC: cocaine; BE: benzoylecgonine-*N*-TMS; NOR: norcocaine; *c*CC: *cis*-cinnamoylcocaine; *c*CE: *cis*-cinnamoylecgonine-TMS (not quantified); *t*CC: *trans*-cinnamoylcocaine; *t*CE: *trans*-cinnamoylecgonine-TMS (not quantified); NFC: *N*-formylcocaine; TRI: trimethoxycocaine.

methylecgonine, tropacocaine, norcocaine, *N*-formylcocaine and trimethoxycocaine), and SS2 containing the alkaloids usually found at high concentration (ecgonine, benzoylecgonine and *trans*-cinnamoylcocaine). All curves and their corresponding data are available in Tables S3 and S4 (SI section). Dimensionless alkaloid to ISTD concentration and chromatographic area ratios were employed, respectively, as dependent and independent variables. Table 2 presents the linear range, regression and determination coefficients for all analytes herein studied. Due to their chemical similarity, the analytical curve for *trans*-cinnamoylcocaine was used to quantify the isomer *cis*-cinnamoylcocaine. Calibration plots for all analytical curves are available in the Figure S1 (SI section).

As it may be observed from the data, results proved to be linear over the entire concentration range studied and the values of R^2 were higher than 0.999 in all cases.

The analysis of variance (ANOVA) with F = MSReg/MSRes (MSReg: regression mean square; MSRes: residues mean square) reached values all above the critical F at 5% confidence level, indicating that, at 95% confidence level, the null hypothesis (Ho: a = 0) is rejected for the slope of the regression curves (Table S5, SI section).[19] Grubbs' test for outliers showed no discrepant

Table 2. Analytical curve[a] parameters for cocaine minor alkaloids

Compound	Intercept	Slope	Linear range / (mg L^{-1})	Determination coefficient (R^2)
Anhydroecgonine methyl ester	0.00032	0.48175	1-91	0.9997
Anhydroecgonine	–0.00005	0.83883	1-80	0.9998
Methylecgonine	–0.00007	0.75284	1-77	0.9997
Tropacocaine	0.00019	0.70819	1-90	0.9997
Norcocaine	0.00003	0.70323	1-84	0.9997
N-Formylcocaine	–0.00212	0.68640	1-94	0.9992
Trimethoxycocaine	–0.00035	0.49022	1-90	0.9997
Ecgonine[b]	0.00109	0.99408	2-580	0.9999
Benzoylecgonine[b]	0.00271	0.69710	15-3,569	0.9999
trans-Cinnamoylcocaine[b]	0.00171	0.66110	5-1,266	0.9999

[a]$y = ax + b$ where y = analyte/internal standard area ratio and x = analyte/internal standard concentration ratio; [b]results for weighted analytical curves.

data with G minimum and G maximum < $G_{95\%}$ (critical value at 95% confidence level) for all analytes (Tables S6 and S7, SI section).[20]

Cochran's tests showed heteroscedasticity in data for ecgonine, benzoylecgonine and *trans*-cinnamoylcocaine, for the chosen concentration range (Table S8, SI section). Residue plots reflect that tendency (Figure S2, SI section), as the standard deviation of analyte to internal standard relative area increases with alkaloid concentration.

Therefore, a weighted least squares linear regression was best fit for the analytical curves of ecgonine, benzoylecgonine and *trans*-cinnamoylcocaine. In this type of model, calibration points with higher experimental standard deviations have less weight in the positioning of the final regression straight line.[21,22]

Limits of detection and quantification

Both detection (LOD) and quantification limits (LOQ) were statistically estimated from regression data, according to the following equations.[22]

$$LOD = 3 \times \frac{s_b}{a} \quad (2)$$

$$LOQ = 10 \times \frac{s_b}{a} \quad (3)$$

where a stands for the slope and s_b represents the intercept (b) standard deviation of the regression straight line.

The observed ranges were from 0.1 to 1.0 mg L^{-1} for LOD and from 0.3 to 3.0 mg L^{-1} for LOQ. Table 3 presents the corresponding experimental values for all analytes which are expressed both as solution concentration values (mg L^{-1}) and as sample weight basis (mg kg^{-1}).

Precision and accuracy

Precision was assessed by means of repeatability and intermediate precision experiments. The equipment repeatability was established with six (n = 6) sequential injections of the same sample solution in a preassigned GC-FID equipment and in the same day. The method repeatability was established with six (n = 6) independent analysis of one homogeneous sample in the same day. Intermediate precision (IP) was evaluated with six (n = 6) independent analysis in two different modes (IP 1 and IP 2, as in Table 4). Mode IP 1 was performed by the same analyst on different days and IP 2 by different analysts on different days (Table 4).

Accuracy was estimated by recovery assays. The percentage of recovery (R) was established from triplicates (n = 3) of cocaine samples fortified with standard working solutions at three concentrations level (high, medium, low) within each analytical curve range (Table 4).

As it can observed from Table 4, the repeatability values (relative standard deviation (RSD), in percentage) of the analytes present in the cocaine control sample ranged between 0.1 and 2.8% and the values for the intermediate precision ranged between 1.2 and 9.5%.

For recovery, values ranged between 90 and 108% for most analytes (Table 4) indicating that the proposed method is also accurate. Only norcocaine showed results somewhat smaller than the ordinary acceptance criteria (R, between 60 and 85%), but this isolated result did not invalidate the method and can be understood as the methodology becomes less accurate when the results are closer to the limit of quantification (1.5 mg L^{-1} for norcocaine). Also, it is important to keep in mind that norcocaine and *N*-formylcocaine are analytes that indicate the utilization, or

Table 3. Limits of detection and quantification for cocaine minor alkaloids

Compound	Solution		Sample[a]	
	LOD / (mg L^{-1})	LOQ / (mg L^{-1})	LOD / (mg kg^{-1})	LOQ / (mg kg^{-1})
Anhydroecgonine methyl ester	0.5	1.8	31	103
Anhydroecgonine	0.4	1.4	24	79
Methylecgonine	0.5	1.5	27	91
Tropacocaine	0.5	1.6	28	94
Norcocaine	0.4	1.5	26	87
N-Formylcocaine	0.6	1.9	34	112
Trimethoxycocaine	0.8	2.8	49	162
Ecgonine	0.1	0.3	5	17
Benzoylecgonine	1.0	3.0	53	176
trans-Cinnamoylcocaine	0.6	2.0	45	150

[a]Typical sample weight (10.25 mg). LOD: limit of detection; LOQ: limit of quantification.

Table 4. Results for precision and accuracy/recovery

Analyte	Repeatability (RSD) / %		Intermediate precision (RSD) / %		Recovery / %		
	Equipment	Method	IP 1	IP 2	High	Medium	Low
Anhydroecgonine methyl ester	2.8	2.4	1.8	3.4	96	99	99
Anhydroecgonine	2.7	2.8	1.5	4.6	98	97	99
Methylecgonine	0.1	0.9	1.5	7.9	98	98	96
Tropacocaine	0.9	0.9	2.0	3.4	96	98	90
Norcocaine	0.5	0.8	7.5	9.5	85	76	60
N-Formylcocaine[a]	–	–	–	–	98	105	98
Trimethoxycocaine	1.5	0.8	2.1	2.3	99	97	101
Ecgonine	0.7	1.0	1.2	1.9	100	102	106
Benzoylecgonine	0.8	1.0	1.3	2.2	101	104	108
trans-Cinnamoylcocaine	0.2	0.7	1.5	2.4	103	108	106

[a]Analyte < LOQ in control sample. IP 1: different days/same analyst; IP 2: different days/different analysts; RSD: relative standard deviation.

not, of oxidation steps to purify cocaine, and the accuracy of their contents is not critical to this data analysis.

Robustness

Method robustness was evaluated by the Youden's test,[23,24] a set of experiments designed to compare a reference condition with slightly perturbed versions of it. In the standard procedure, seven chosen key method parameters (i.e., carrier gas flow-rate, split ratio, injector and oven temperatures and injection volume in the GC-FID analysis; time and temperature in the derivatization step), were evaluated at two intensity levels (high and low, as compared with the reference values) (Table S9, SI section). The resulting saturated fractional factorial experimental design (2^{7-4}_{III}) led to eight independent experiments which are performed to determine the influence of each parameter in the final result.[19]

The highest result obtained in the Youden's test experiments conducted with cocaine control sample is 3.5% in the anhydroecgonine content, when the carrier gas flow rate was altered and so the procedure can be considered robust regarding all analytes quantified (Table S10, SI section).

Stability

Stability was assessed using the derivatized control sample (CCS), which was injected in the GC-FID system in triplicate after each storage condition. Two storage regimes were studied: room temperature for 24 h, 48 h and 4 days and freeze/thaw cycles at −20 ºC for 24 h, 4 days and 7 days.

Minor alkaloid concentrations from each stability experiment were compared with those from freshly prepared samples and did not exhibit analyte degradation higher than 10%.

Real case samples

Once the quantitative method was fully validated, a set of 11 real case samples, seized by BFP throughout the country, were analyzed to provide information concerning cocaine minor alkaloids chemical profiling. Tables 5 and 6 compiles the results for five cocaine base samples and six cocaine hydrochloride samples, respectively. Those samples were also processed by the DEA Cocaine Signature Program,[6] where they were also analyzed by isotope ratio mass spectrometry and had truxillines alkaloids content determined, as part of the assignment of the geographical origin of the coca leaf extracted to provide the seized cocaine sample.

As a local preliminary analysis, the relative amounts to cocaine (COC) of tropacocaine (TRO) and trimethoxycocaine (TRI) can be used to indicate both the possible varieties of coca leaf used for cocaine production and the possible origin of the drug samples (Bolivia/Peru or Colombia).[11] The TRO/COC and TRI/COC ratio is higher than 0.15% only for samples HCl-1 and HCl-4 (Table 6), which indicate the variety *Erythroxylum novogranatense* var. *novogranatense* typically of Colombian origin. All the other cocaine base samples (Table 5) and the rest of the hydrochloride samples (Table 6) present a TRO/COC ratio lower than 0.15% and TRI/COC ratio lower than 0.35%, which indicate the variety *Erythroxylum coca* var. *coca*, typically cultivated both in Bolivia and Peru. The comparison with the DEA results shows that only one sample was misclassified (sample Base-2, classified by

Table 5. Real case cocaine base samples

	Sample				
	Base-1	Base-2	Base-3	Base-4	Base-5
	Minor alkaloids / (mg kg^{-1})				
AEME	1900	1964	2543	3659	1727
AE	1301	119	216	349	114
ME	140	144	138	169	215
EC	27416	3612	4853	7123	5764
TRO	1012	1224	1143	1298	396
BE	75001	22482	37113	54048	24863
NOR	< LOQ	274	259	288	130
*c*CC	10752	61877	54622	34256	22197
*t*CC	5665	35231	36371	21495	25182
NFC	754	241	244	313	313
TRI	< LOQ	2325	1600	907	265
	Major alkaloids / %				
Cocaine[a]	72.9	81.7	79.8	78.5	85.9
*c*CC + *t*CC	1.6	9.7	9.1	5.6	4.7
	Origin				
Coca leaf variety	ECVC	ECVC	ECVC	ECVC	ECVC
Typical producing country[b]	Bolivia or Peru	Bolivia or Peru	Bolivia or Peru	Bolivia or Peru	Bolivia or Peru
STRL/DEA results[c]	Peru	Colombia	Peru	Peru	Bolivia
Seizure state	SP	MS	MS	MS	MT

[a]BFP results;[4] [b]based on TRO/cocaine and TRI/cocaine ratio; [c]DEA, Special Testing and Research Laboratory (STRL) results.[11] AEME: anhydroecgonine methyl ester; AE: anhydroecgonine; ME: methylecgonine; EC: ecgonine; TRO: tropacocaine; BE: benzoylecgonine; NOR: norcocaine; *c*CC: *cis*-cinnamoylcocaine; *t*CC: *trans*-cinnamoylcocaine; NFC: *N*-formylcocaine; TRI: trimethoxycocaine; LOQ: limit of quantification; ECVC: *Erythroxylum coca* var. *coca*; SP: São Paulo State; MS: Mato Grosso do Sul State; MT: Mato Grosso State.

Table 6. Real case cocaine hydrochloride samples

	Sample					
	HCl-1	HCl-2	HCl-3	HCl-4	HCl-5	HCl-6
	Minor alkaloids / (mg kg^{-1})					
AEME	584	485	152	284	285	< LOQ
AE	502	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
ME	14695	2266	197	2102	464	346
EC	12329	1925	178	537	330	190
TRO	5985	398	1058	2753	1263	1186
BE	11097	12687	648	1079	2524	672
NOR	11611	2034	35931	235	17717	18732
*c*CC	2626	8819	266	361	289	306
*t*CC	3294	11029	< LOQ	1021	< LOQ	1603
NFC	364	170	365	< LOQ	234	357
TRI	1646	232	874	3641	1321	1352
	Major alkaloids / %					
Cocaine[a]	70.8	93.2	91.9	91.4	89.1	89.2
*c*CC + *t*CC	0.6	2.0	0.0	0.1	0.0	0.2
	Origin					
Coca leaf variety	ENVN	ECVC	ECVC	ENVN	ECVC	ECVC
Typical producing country[b]	Colombia	Bolivia or Peru	Bolivia or Peru	Colombia	Bolivia or Peru	Bolivia or Peru
STRL/DEA results[c]	Colombia	Peru	Peru	Colombia	Bolivia	Bolivia
Seizure state	PR	DF	SP	SP	SP	SP

[a]BFP results;[4] [b]based on TRO/cocaine and TRI/cocaine ratio; [c]DEA, Special Testing and Research Laboratory (STRL) results.[11] AEME: anhydroecgonine methyl ester; AE: anhydroecgonine; ME: methylecgonine; EC: ecgonine; TRO: tropacocaine; BE: benzoylecgonine; NOR: norcocaine; *c*CC: *cis*-cinnamoylcocaine; *t*CC: *trans*-cinnamoylcocaine; NFC: *N*-formylcocaine; TRI: trimethoxycocaine; LOQ: limit of quantification; ENVN: *Erythroxylum novogranatense* var. *novogranatense*; ECVC: *Erythroxylum coca* var. *coca*; PR: Paraná State; DF: Federal District; SP: São Paulo State.

the DEA CSP as Colombian and as Bolivian/Peruvian by TRO/COC and TRI/COC ratio) and the other 10 samples were correctly classified (Tables 5 and 6). According to specialized literature,[11] a good accurateness in coca leaf origin determination requires the addition of independent physicochemical techniques (such as isotope ratio mass spectrometry, truxilline quantification, etc.) to the profiling protocol. Also, local changes in coca leaf extraction procedures and purification processes must be taken into consideration,[25] in order to add assertiveness to law enforcement demands. Consistent seized cocaine origin information is undeniably useful for drug offer reduction initiatives within Brazil.

Besides origin information provided by TRO and TRI quantification, other minor alkaloids can also provide valuable information concerning seized cocaine. In cocaine base samples (Table 5), dehydrated alkaloids AEME and AE, as well the hydrolyzed alkaloids BE and EC, exhibit concentration from 3 to 24 higher than in cocaine hydrochloride samples (Table 6), confirming the tendency of base cocaine to be more prone to deteriorate than cocaine in salt form.[7,16] Within cocaine hydrochloride set of samples, HCl-1 and HCl-2 present the higher content of BE and AEME, since they are the older samples in this group, but, even so, the alkaloids formed after hydrolysis have 3 to 65 times lower concentration in those cocaine salt samples if compared to the average concentration in cocaine base samples.

The contents of alkaloids NFC, NOR, *t*CC and *c*CC are chemical indicators for the utilization of oxidizing purification process during cocaine refining.[7] Cocaine base samples (Table 5) showed higher *t*CC+*c*CC and lower NOR concentration as compared to cocaine hydrochloride ones, consistent with low rates of purification by oxidation. Base-1 displayed the highest concentration of NFC and the lowest *t*CC+*c*CC, which is consistent with some purification by oxidation particularly in this sample. On the other side, cocaine hydrochloride samples (Table 6) present the opposite behavior, i.e., high NOR and low *t*CC+*c*CC concentrations, indicating substantial rates of purification by oxidation. The exception is the sample HCl-4, presenting relatively low NOR, NFC and *t*CC+*c*CC concentrations. This apparent contradiction could be related to an alternative purification process which would selectively remove NOR and NFC more efficiently. Occluded solvent analysis shall be conducted to investigate this unexpected situation and provide more information in a near future.

Regarding the Brazilian States where those samples were seized, it was observed for cocaine base that Mato Grosso and Mato Grosso do Sul samples indicate mostly Bolivian or Peruvian origin. This result is somehow expected since the two states share more than 2,400 km of borders with Bolivia and Paraguay. São Paulo and Paraná states present seizures of more variable indicative origins. This could be explained by the relative proximity of these states to Mato Grosso do Sul at the west side of the country and the presence of very busy ports and airports connecting their localities to neighboring countries in South America (e.g., Colombia).

Conclusions

This work presented the development and validation of an analytical method to identify and quantitate minor alkaloids in cocaine samples seized by the Brazilian Federal Police, by GC-FID after derivatization with MSTFA/pyridine. The main advantages of this method when compared to previously published work were the utilization of relative less expensive procedures to sample preparation and the use of GC-FID to provide stable and robust quantitative results.

The method was fully validated according to ISO/IEC 17.025:2017[26] normative of the forensic laboratories quality management system. It presents suitable selectivity and precision and the analytical curves proved to be linear within the working range of all analytes. The method was shown to be stable, robust and to have good accuracy.

The validated method was considered fit for purpose to routine forensic analysis and was applied to quantitate minor alkaloids in a set of 11 real case cocaine samples seized in different Brazilian regions. The cocaine minor alkaloid contents were used to compare different samples, focusing on purification process by oxidation, degree of sample hydrolysis and dehydration by thermal process.

Finally, the relative amounts of tropacocaine and trimetoxycocaine with respect to cocaine indicated both the possible varieties of coca leaf used for cocaine production and the origin of the drug samples (Bolivia/Peru or Colombia). Most samples seemed to originate from the extraction of ECVC coca leaves, from both Bolivia and Peru. Method results have been compared with the DEA reference lab and only one sample out of eleven was misclassified.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

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